firefly®
user guide

For instruments with serial numbers between FF1013 – FF2002

Thank you!

Thank you for purchasing firefly® - the all-in-one liquid handling platform for genomics by SPT Labtech.

Our ongoing commitment is to help you get the best results, so we designed and manufactured this instrument with you in mind. Our revolutionary firefly platform accelerates
genomic research by bringing together multiple technologies within a single compact design for more efficient library and sample preparation workflows.

This user guide is your ready reference to the care and operation of your firefly and should be stored in a convenient place. With the proper handling and maintenance, as detailed in this guide, your instrument will provide years of efficient and reliable service.

We truly appreciate your business and the trust you have placed in us. If you have any questions, comments, or suggestions regarding your firefly, please feel free to contact us by email at: fireflysupport@sptlabtech.com

Specifications

Key concepts

firefly is a benchtop instrument optimized for performing genomics liquid handling workflows. There are genomics and non-genomics variants, and a choice of 3 or 6 dispensing heads.

It features a 384 air displacement pipetting head and positive displacement dispense heads, with reagent temperature control from an optional reservoir thermal module, to provide flexible, high performance approaches to a range of applications such as NGS library prep and PCR set up.

A two tier moving deck system, with an additional basement level optionally equipped with a plate shaker and plate thermal module, enables firefly to carry out multiple protocol steps with fewer user interactions.

firefly is available with an optional vertical laminar flow module with HEPA filter.

The optional firefly+ module expands firefly's plate handling capability, enabling longer and more complex protocols to be run without user interactions. firefly+ can include a thermocycler, to more fully support NGS applications.

Safety

Read these safety instructions before operating firefly.

firefly is designed and engineered with your safety in mind. If you use firefly in a manner not specified by SPT Labtech, the protection provided may be impaired.

Do not try to operate the instrument or carry out maintenance tasks unless you have been trained to do so.

Do not operate firefly if the instrument is visibly damaged unless a reliance service engineer has confirmed that it is safe to do so.

Read the safety instructions in the Inheco On Deck Thermocycler manual, if you have firefly+ with this option.

Read the safety instructions in the CAS vertical laminar flow module manual, if one is fitted.

Hazard summary

Caution

DANGER: automatically controlled moving machinery

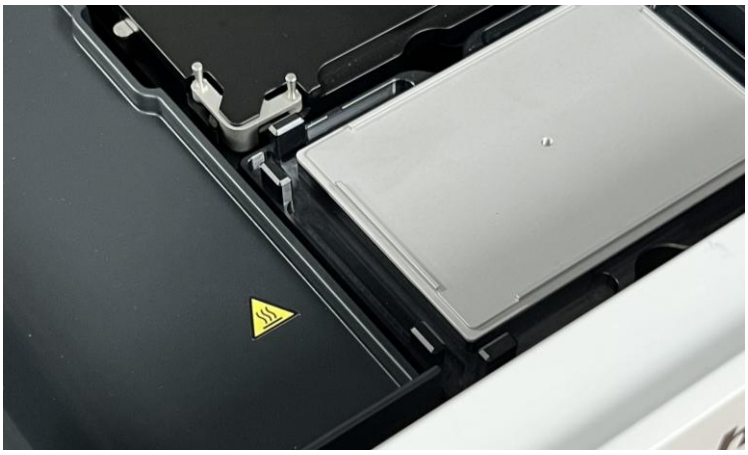
Caution

DANGER: hot surfaces

Warning symbols on firefly

Take care when accessing the basement of a Genomics instrument if either of the thermal modules have been used for heating, as they will remain hot for several minutes.

Take notice of the warning triangles alerting you to parts which may be hot.



The plate thermal module is on the left (shown above) and the reservoir thermal module is on the right (shown below).

If the thermal modules have been used for cooling, do not access them immediately. Wait several minutes before accessing them; this allows them to warm and avoids the possibility of cold burns.

Handle thermal blocks with care as residual heat/cold will take time to stabilize back to safe temperatures after use.

Warning symbols on firefly+

Take notice of the Inheco Thermal Cycler warnings symbols for hot surfaces, if you have used the thermal cycler for heating, and for crushing hazards, should you put your hand in the lid while it is closing.

Warning

The thermal cycler is independently powered so switching off firefly does not stop the thermal cycler operating.

Accessing firefly

To access firefly:

- If you are loading materials using 'Setup & Loading', select 'Get Access'. The software will control moving the heads and decks to positions which give you easy access to the area you need, then stop the instrument.
- If you reach a 'user interaction' step in a protocol, once the instructions are shown on screen, you can open the instrument. Heads and decks may be moved to safe positions before the door is opened but this depends on the protocol design, so be cautious when accessing firefly.
- If you need to access firefly immediately, slide up the door. The door is interlocked so firefly stops as soon as it is opened.

Warning

Take care as heads and decks may not be in positions which give you easy access.

Note

You can move both heads back and forwards but not up or down after triggering the door interlock. The decks will always slide freely when the door is open.

To access firefly when it is unpowered, you can open the door and move the decks and heads manually.

Accessing firefly+

To access firefly+ shelves, open the door at the front of the unit, to load your materials.

If you need to access the thermal cycler e.g., for cleaning, you will need to reach in through firefly's door.

- Remove all labware from the decks.
- Ensure you have switched off firefly and the thermal cycler.
- Move the decks right, away from firefly+
- Move the heads to the back, to give yourself as much space as possible

You should then be able to reach round behind the firefly+ shelves to the thermal cycler.

Warning

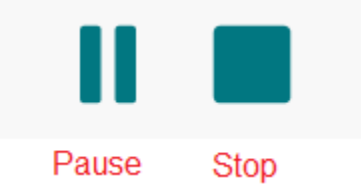
You should load firefly+ before you start executing your protocol, so no grippers are moving.

If you need to access firefly+ immediately, while a protocol is running, open the door. The door is interlocked so firefly stops as soon as it is opened.

Stopping firefly

Stop

Use the Pause or Stop buttons if you need to stop firefly while running a protocol outside of a user interaction step.

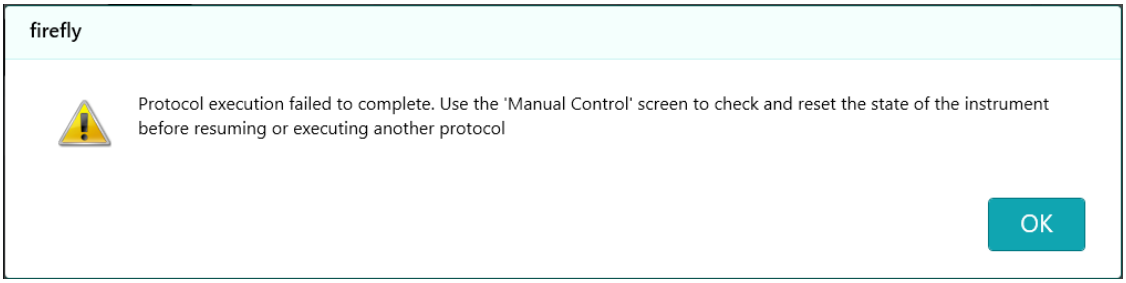


Pause enables you to continue running your protocol, by pressing the button again. Stop ends protocol execution as well as stopping the instrument.

Important

firefly will not stop immediately you click Stop or Pause, it will finish the current protocol step first.

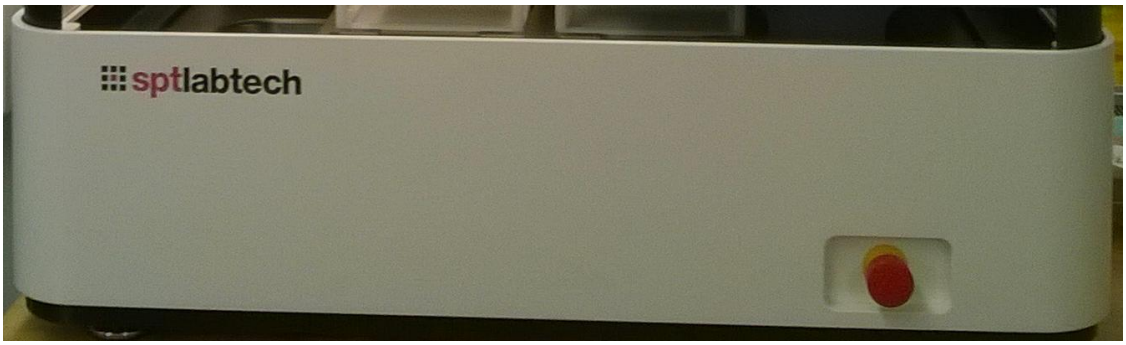
If you use Stop, firefly will remind you to check and reset your instrument before further use.



If your instrument is fitted with the optional vertical laminar flow module, use Stop on the module's touchscreen to stop the fans.

Emergency stop

firefly has an e-stop button to stop the instrument immediately, if this would be safer than opening the door in an emergency.



Tip

Don't use the emergency stop if you want to stop the instrument but there is no danger. As firefly will stop instantly, you may damage or waste your reagents.

You can open the door, e.g., to remove materials, after you have used the e-stop.

Warning

Take care as heads and decks may not be in positions which give you easy access.

Important

You can move both heads back and forwards but not up or down after triggering the e-stop.

Important

firefly emergency stop does not stop the vertical laminar flow module operating. Use Stop on the module's screen.

After you have used the emergency stop button, you will need to twist it clockwise to unlock it before you can restart.

Warning

Do not do this until you are sure that firefly is in a safe condition.

firefly will power up when you reset the e-stop, but you will need to manually restart your protocol.

Stopping firefly+

All the stop functions will also stop firefly+ movements. If the grippers are holding a plate when you stop the instrument, they will remain gripped and not drop the plate.

Warning

If firefly+ is fitted with the on deck thermal cycler, this will not stop operating when you stop firefly+ as it is independently powered. Follow the shutdown instructions in the Inheco On Deck Thermocycler manual.

Reagent safety

Caution

For fire safety, the maximum recommended quantity of 80% ethanol to use in firefly is 200mL.

Caution

firefly's enclosure does not provide protection from biological or chemical hazards.

You must carry out risk assessments for the reagents you plan to use, and take appropriate safety measures.

If you are using a firefly+ instrument, you must carry out a risk assessment for use of the storage shelves and of your incubation protocols using the ODTC.

If you have a vertical laminar flow module fitted, remember that this will flow air towards the operator.

Moving firefly safely

Moving firefly after commissioning onsite could be dangerous and should be undertaken by trained reliance service engineers. Contact reliance for assistance if you need to move your instrument.

Caution

firefly weighs 122.5kg and moving it safely will require careful planning and 4 people to carry out the lift. Incorrect lifting may in the worst-case cause injury or death.

Caution

firefly+ must be detached from firefly before any moves as the joint between them is not structurally sound enough and would form a hinge. Contact reliance for assistance.

Important

Moving firefly could affect alignment, impacting its performance. Contact reliance for assistance if you need to move your instrument.

Ergonomics

firefly's touchscreen is mounted on a rotating bevel, so you can adjust its viewing angle to be comfortable in use. It cannot be removed from the instrument and is primarily intended to be used for running protocols. Use the Bluetooth keyboard and mouse if needed.

When you are designing protocols, you will find it easier to use a freestanding PC or laptop, and install the standalone firefly app.

EMC (electromagnetic compatibility)

No specific EMC precautions are required when operating firefly or firefly+.

Important

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Noise levels

firefly and firefly+ airborne noise emissions do not exceed 70 dB(A).

Vertical laminar flow module noise emissions do not exceed 60 dB(A).

firefly technology

Hardware options

firefly instruments variants are available which use either ATL (Auto Tip Load) or EZL (EZ-Load) pipette tip technologies.

ATL instruments can be supplied with a firefly+ module for additional plate handling capabilities, and optional thermocycler capability.

	Dispense heads	Shaker module	Thermal modules	firefly+
Genomics	6	Yes	Yes	Optional
Genomics	3	Yes	Yes	Optional
Non-genomics	6	No	No	Optional
Non-genomics	3	No	No	Optional

EZL firefly instruments are available in the following configurations:

	Dispense heads	Shaker module	Thermal modules
Genomics	6	Yes	Yes
Genomics	3	Yes	Yes
Non-genomics	6	No	No
Non-genomics	3	No	No

Illustrations in this manual show a variety of instrument types and configurations.

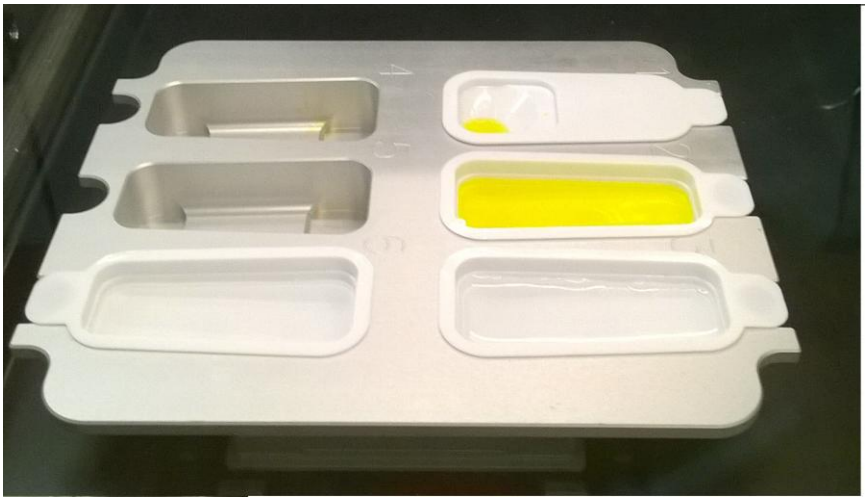
Dispensing

firefly syringes can be synchronized for high throughput dispensing or utilized independently for reagent flexibility. The positive displacement syringes dispense volumes from 200nL to 3960µL. Each 4mL syringe can be used to multi-dispense smaller volumes for faster dispensing to a 96 or 384 well plate. The non-contact dispensing mechanism enables syringes to be used for multiple operations per reagent.

The syringe dispensing heads can dispense to plates located in deck positions 6 and 7, on both the upper and lower decks.

The syringes are filled from 6 reservoirs, located in a reservoir tray in the basement.

There are multiple reservoir and reservoir tray types available, to meet different dispensing needs.



Pipetting

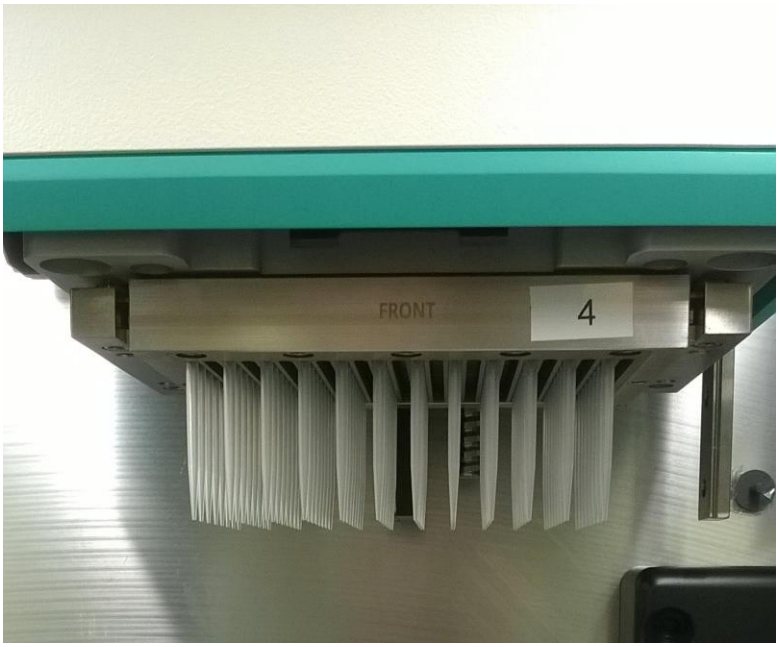
firefly can pipette volumes from 0.5µL to 125µL. The pipetting head has tip options to operate in several configurations: 384 and 96 tips for full plates, or columns of 16 (384 format) and 8 (96 format) tips, for working with partial plates or working across plates in processes such as serial dilutions.

The pipetting head can access all deck positions when using arrays of tips and columns of tips. There are spatial limitations associated with the movement of the pipetting head when relatively tall labware surrounds the pipetting location, but firefly software will warn you of these during the protocol design process.

There are two types of pipetting head.

EZL head

The EZL head uses a stainless steel cassette - visible above the tips in the illustration below - to hold pipette tips. Tips can be loaded as either a complete set of 96 or 384, or tip strips can be used for partial plate pipetting.

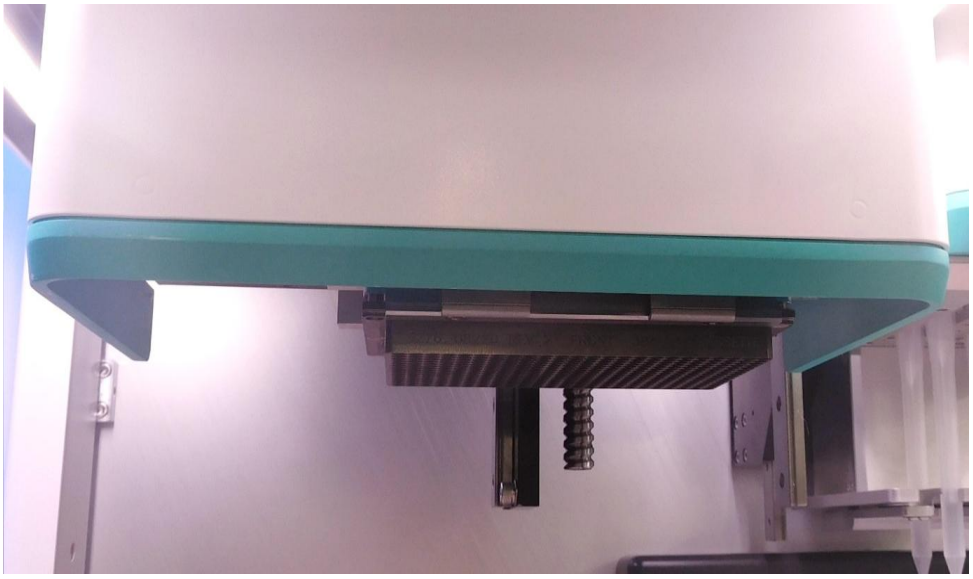


To prepare EZ-load tips for loading, you put them into a tip cassette which you place on a tip stand on the lower deck, and firefly software controls loading the tips to the pipette head. Tip removal is also automated: you simply need to unload the tip stands of used tips and then remove the tip cassette from the stand.

EZ-load firefly is supplied with 5 tip stands and 5 tip cassettes as standard. Additional tip stands, tip cassettes and strip tip adaptors are available from SPT Labtech.

ATL head

The ATL head tips are directly loaded from the tip box, with the quantity specified in the firefly protocol. You can use one or more columns of 96 or 384 tips.

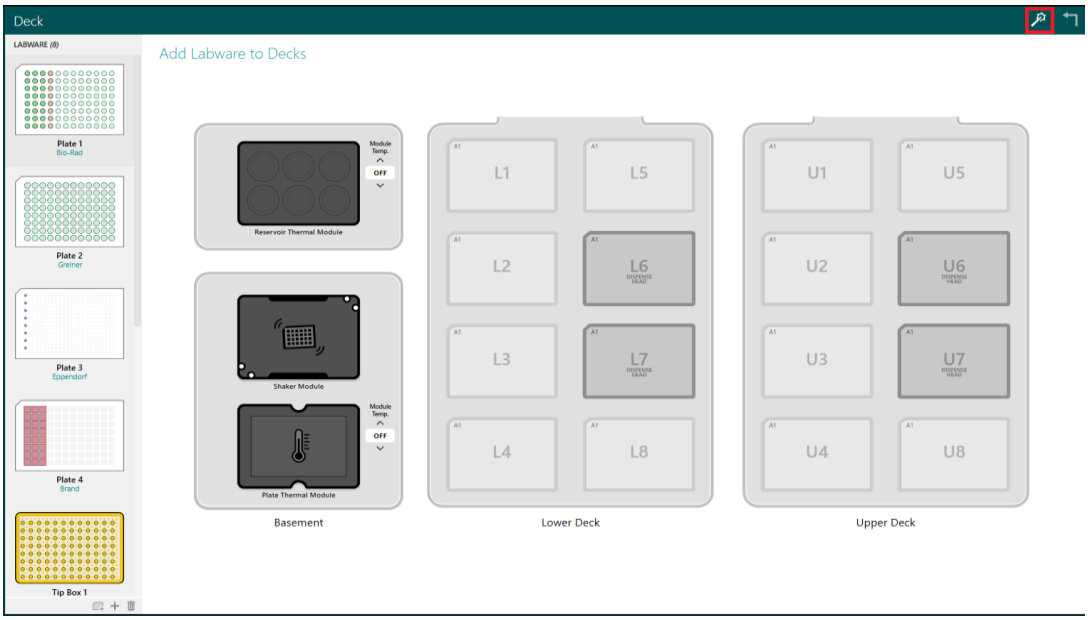


firefly loads clean tips automatically. To load ATL tips, you optionally place the tip box on a tip box adapter (supplied with firefly) and firefly software controls loading the tips to the pipette head. Tip unloading is also software controlled; you simply load an empty tip box into which the used tips are unloaded, ready for removal and disposal.

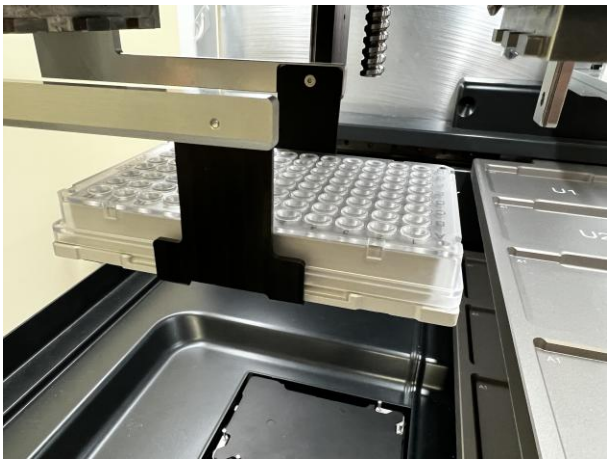
Decks and grippers

firefly has two movable decks, each with space for 8 plates. The top deck can be used for items with a maximum height of 49.7mm. The bottom deck has a maximum height limit of 93mm so deep items such as tip sets must be loaded on the bottom deck.

The 'Auto Fill Deck' function (highlighted) in firefly software will determine a correct loading location for all the items used in a protocol.



The pipetting head incorporates grippers to move labware between decks and on and off accessories such as magnetic blocks. Grippers can also be used to stack items e.g., a plate and a thermal block, and then move them together as shown below. On a Genomics instrument, grippers move labware on and off the plate thermal module and shaker module.



The gripper can move labware when tips are loaded onto the pipetting head as it reaches down below the tip level, or tips can be unloaded before working with the gripper.

Plate heating and cooling (Genomics option)

The plate thermal module provides plate heating and cooling. Temperatures are selectable from -20°C to 99°C in steps of 0.1°C. The nominal regulation accuracy is better than ±0.1°C with a uniformity of temperature distribution better than ±0.7°C at 4°C across the cooling surface.

Note

Plate thermal module ramping times are rapid, with a cooling/heating speed 12°C / min above RT, but the module is designed for incubations only. PCR should be carried out off-line using an external thermocycler or the firefly+ ODTC.

Thermal block

The plate thermal module is used with a thermal block, an adapter which is placed on top of the plate thermal module beneath the plate. Remove it from the plate thermal module with the plate and it will continue to provide passive heating or cooling: it will slow down the rate of temperature change of the plate contents to approximately 1°C per minute.

There are specific thermal blocks for 96 and 384 well plates. A block for 96 well PCR plates is supplied as standard. You can purchase additional blocks:

- 3276-01065 THERMAL ADAPTER FOR PCR PLATE (96 WELL)
- 3276-01066 THERMAL ADAPTER FOR PCR PLATE (384 WELL)

as you can concurrently use multiple blocks on an instrument.

Plate shaking (Genomics option)

The shaker module mixes at speeds between 200 - 3000 rpm. The shaker can be used with all firefly plate types, including deep well plates although there is a height restriction meaning that decks could not be moved with some deep blocks on the shaker.

Reagent cooling and heating (Genomics option)

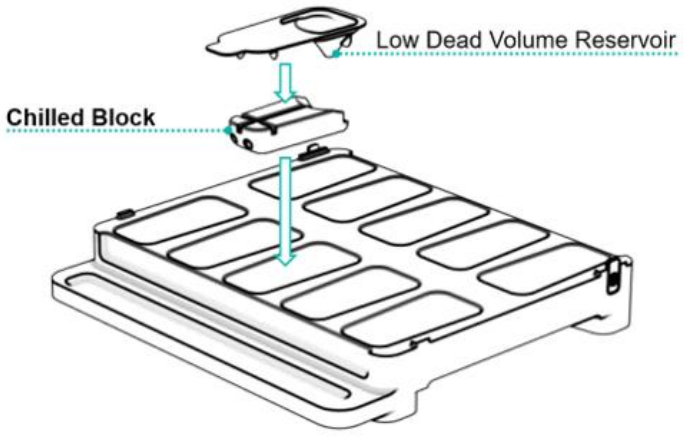
The dispense head reservoirs can be cooled by using the optional reservoir thermal module. This has the same heating and cooling properties as the plate thermal module. Temperatures are selectable from -20°C to 99°C in steps of 0.1°C. All reservoirs are cooled or heated to the same temperature. If using the reservoir thermal module, you replace the standard plastic reservoir tray with an aluminum reservoir tray.

If you wish to use high volume reservoirs, you will need to use the specific reservoir tray (shown below) and the inserts for standard or low volume reservoirs. Lids are available for high volume reservoirs, to reduce evaporation.

Passive reagent cooling (standard reservoir tray)

To cool a single reagent, you could use a LDV reservoir and an LDV reservoir cooling block. This is a passive cooling system, so you will not be able to maintain reagent temperature or control it precisely.

1. Put the LDV reservoir cooling block in a freezer for 2 hours.
2. Place the chilled block in the reservoir tray beneath the LDV reservoir.



Note

Plate thermal module ramping times are rapid, with a cooling/heating speed 12°C / min above RT, but the module is designed for incubations only. PCR should be carried out off-line using an external thermocycler or the firefly+ ODTC. As the chilled block is not an asset in firefly software, you will need to use user interactions or your method documents to include instructions to use it.

Passive reagent cooling (HV reservoir tray)

To cool a single reagent, use a standard reservoir and insert or a LDV reservoir and insert. The aluminum inserts are shown below.

This is a passive cooling system, so you will not be able to maintain reagent temperature or control it precisely.

1. Put the insert in a freezer for 2 hours.
2. Place the insert in the HV reservoir tray beneath the reservoir.

You could also use a pre-chilled insert in addition to the thermal module, e.g., if one material needs to be at a lower temperature than the rest.

Labware barcodes

firefly software enables you to capture barcodes from your plates and reagents, and from firefly reservoirs, syringes and tips, using a handheld barcode reader. You can optionally include or omit the use of barcodes in the protocols you design on an item by item basis e.g., you could just record sample barcodes.

firefly software will prompt you to scan or type in barcodes when loading materials to run a protocol.

> **Important**
>
> The recommended barcode reader is the Zebra DS2208.

Item	Permitted barcode data
Tip box	Barcode format is fixed and is the same as the Tip box part number. An exception is made for empty ATL tip-boxes: it would accept any part-number of a non-empty tip-box with compatible tips.
Plate	firefly provides a custom format for plate barcodes, which is a reg-ex string. If the reg-ex field is left blank when desigining the protocol, any non-empty text would be treated as valid barcode.
Syringe	Syringes barcodes are restricted to part-number followed by “-LOT“ and 5 digits.
Reservoir	Reservoirs can utilize free-format barcodes (same as plates).
Reagent	Reagents can utilize free-format barcodes (same as plates).

Item	Permitted barcode data
Syringe/Reservoir/Reagent	Combined prompt allows a single scan to be applied to all syringes which use the same reservoir and reagent.

Magnetic blocks

You can use a variety of Alpaqua and Permagen magnetic blocks with firefly, for use with 96 and 384 well plates.

Before you can use a magnetic block, you must download the definition from Community Labware. If you need a specific type which is not available, contact reliance for advice.

You will also need to download the stack definition for the magnetic block with the plate which you want to use, otherwise it will not be available when you are designing your protocols.

firefly+

firefly+ extends firefly's capabilities with a choice of integrated thermocycler modules and additional storage capability for plates, lids, tips and thermal blocks. It is only available for use with the firefly ATL head variant.

It includes:

- an optional Inheco On Deck Thermal Cycler in 96- or 384-well format (if you don't require this, a drip tray is installed in the base of firefly+)
- 15 x medium shelves (max. labware height 66mm - ideal for additional tip boxes)
- 9 x small shelves (max. labware height 27mm - ideal for additional plates and lids)

The shelves are arranged to hold SLAS/ANSI (SBS) labware within the height limit of the particular shelf, as shown above.

Important

Do not use firefly+ for storing filled trough plates as the movement of the firefly+ grippers is

likely to spill the contents.

On Deck Thermal Cycler (ODTC)

The Inheco On Deck Thermal Cycler XL is a firefly+ option which enables you to carry out incubation steps in PCR, NGS and gene analysis protocols without requiring a separate instrument.

Warning

Do not attempt to use the ODTC standalone. It must always be used within a firefly protocol.

Details of the ODTC and instructions for use are in the Inheco On Deck Thermocycler manual.

Important

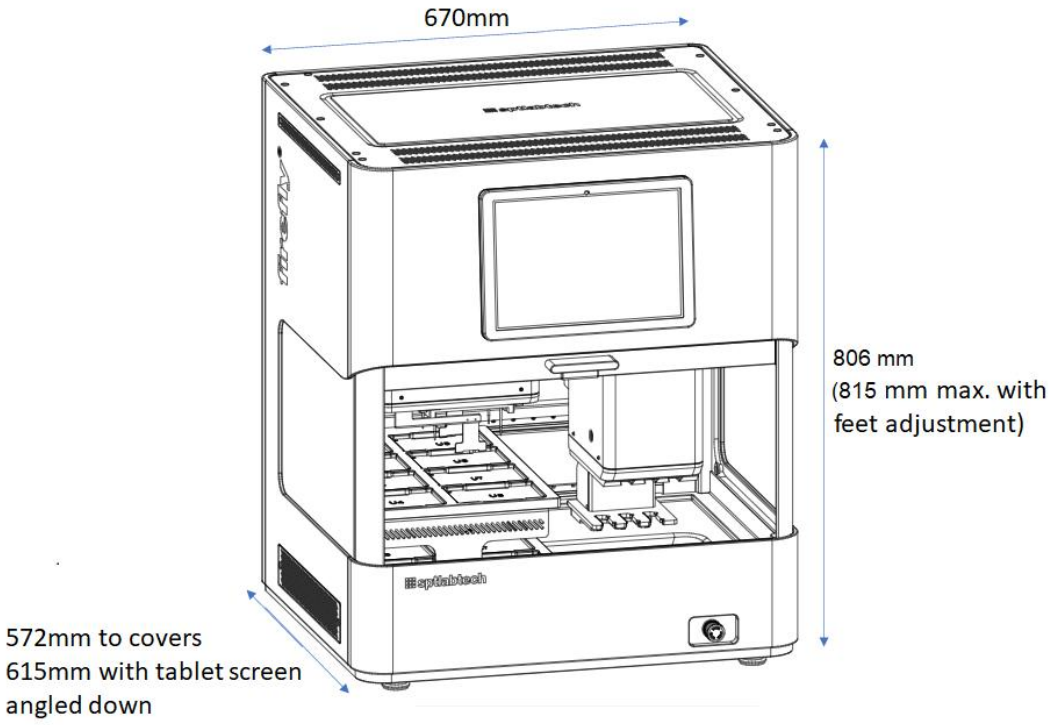
You can only use skirted PCR plates with the On Deck Thermal Cycler and the firefly+ grippers.

Installation

Pre-installation requirements

Space

firefly's dimensions are W 670mm x D 572mm x H 806mm



You should allow space close to the instrument for your keyboard and mouse. It is advisable to have a small gap of 152mm at each side of the instrument and 76mm behind the instrument to allow for ventilation and for service access.

firefly weighs approximately 122.5kg so it requires a sturdy lab bench. It needs to be kept stable so do not use a bench with wheels or castors, or one which is prone to rocking. The bench must be flat and level. Suitable designs are shown below.

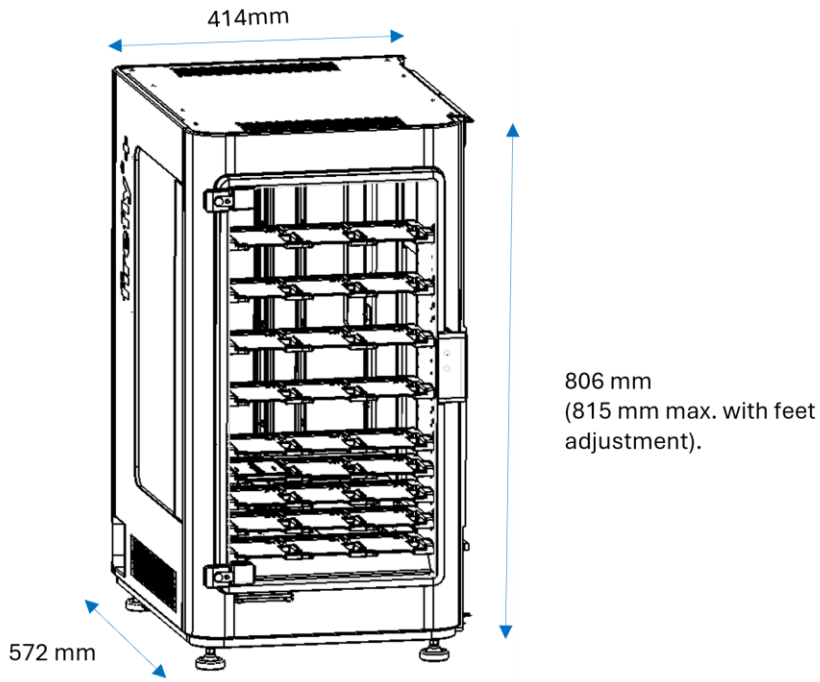
Do not use bench types such as these.

firefly fitted with the optional vertical laminar flow module is W 670mm x D 572mm x H 1062mm and weighs approximately 142kg.

As firefly may induce some vibrations in the bench, consider what equipment shares the same bench as the firefly. Any equipment sensitive to vibrations such as balances or microscopes should not be placed next to firefly. Conversely equipment that also generates vibrations, such as a centrifuge, should not be placed close to the firefly as this may impact performance.

Space - firefly+

firefly+ dimensions are W 414mm x D 572mm x H 806mm



The front door swings open fully, to give access to the shelves for loading and unloading labware, so do not place other items in front of the unit.

The Inheco ODTC control box can sit adjacent to or above/below firefly+, and can be placed horizontally or vertically (256.5mm x 414.5mm x 58mm).

Also allow air space of 152mm to the side of firefly+ and 76mm behind, for ventilation and service access. Do not position the unit against a wall or the side of a cabinet.

firefly and firefly+ total weight is 170kg. The dimensions of the total unit are W 1090.5 mm x D 572 mm x H 806 mm. The firefly with the firefly+ is designed to be used on a laboratory bench which must be strong, stable, flat and level. The bench must be able to comfortably support the mass of the firefly with the firefly+ unit and have enough space to be situated (see above dimensions). The bench should be stable, not fitted with castors and not prone to rocking as the firefly has several moving masses in the form of its deck plates, plus dispense and pipetting heads which are regularly moving during normal operation. firefly with firefly+ has 8 feet which all should be firmly located on the work surface.

Environmental requirements

firefly / firefly+ is designed for use in a standard laboratory environment:

- temperature 15 – 32°C
- relative humidity 30 – 80%.

Cleaning

Maintaining cleanliness within both the firefly instrument and its housing facility promotes the reliability of automated workflows and contributes to improved reproducibility and robustness of the workflow outputs.

> **Note**
>
> The firefly instrument is enclosed in panels and operates with the interlocked door closed during protocol execution, which contributes to maintaining a clean environment for automated workflows.

To further protect the interior of the instrument from contamination and accumulation of dust, you should:

- keep firefly's door closed when the instrument is on standby or turned off
- minimize physical contact with the interior of the instrument and use appropriate PPE, such as disposable laboratory gloves and lab coats
- always use clean labware and accessories which are free of dust and residues
- regularly clean the firefly facility, including washing the floors and wiping the benches

If your instrument is fitted with firefly+, you should additionally:

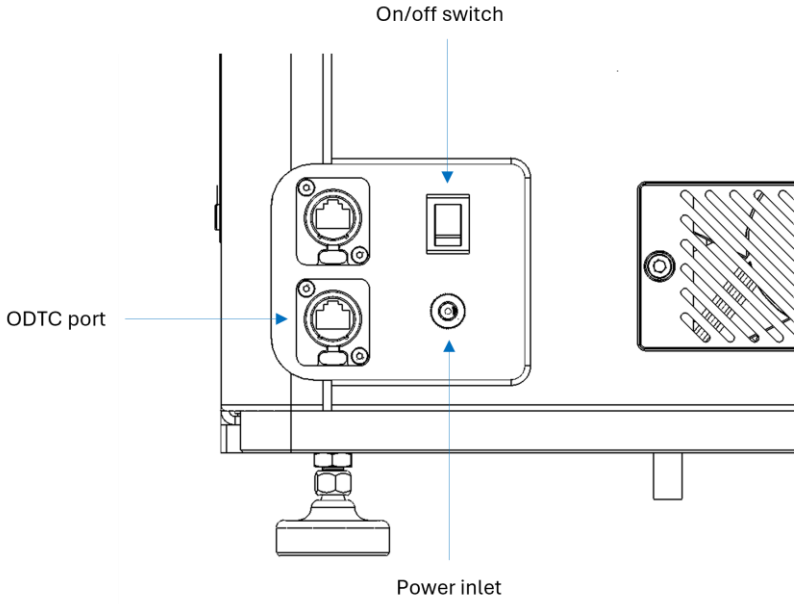
- keep firefly+'s door closed when the instrument is on standby or turned off
- not load firefly+ with troughs or any plates with full wells which could easily spill
- not put used plate lids directly on the shelves; condensate may drip from the undersides onto other shelves or plates. Always put them on a waste plate.

If your instrument is fitted with a vertical laminar flow module you should follow the care instructions in the CAS vertical laminar flow module manual.

Power

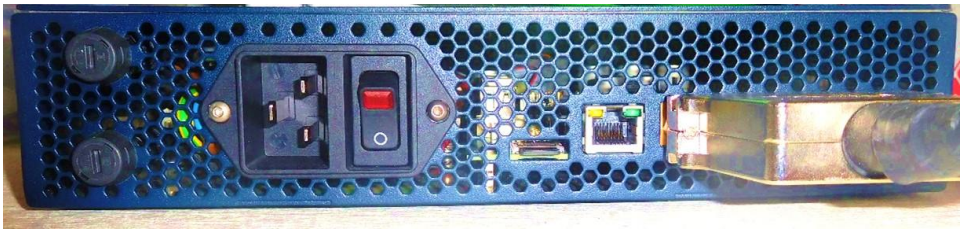
firefly requires one mains power connection. It is supplied with an appropriate lead for the country of sale.

firefly+ requires an additional mains power connection for its 24V 2.5A power supply. It is supplied with an appropriate power supply and lead for the country of sale.



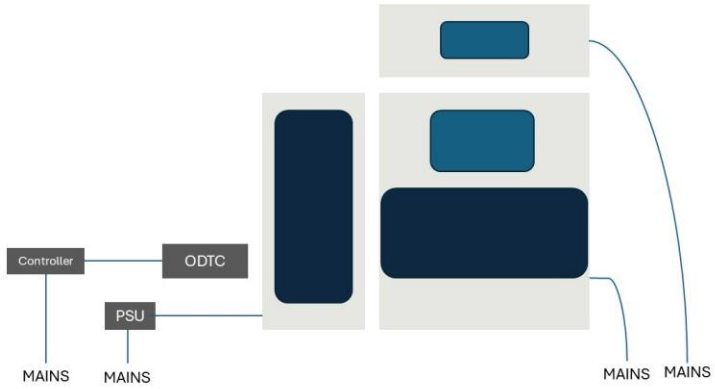
It also requires a data connection to the ODTC, which is set up when reliance install the instrument.

The ODTC (shown below) requires a separate mains power connection, 1250 W input, to the thermal cycler controller, which then provides a 24V supply to the thermal cycler unit within firefly+. It is supplied with an appropriate lead for the country of sale.



firefly supplied with a vertical laminar flow module requires an additional mains power connection.

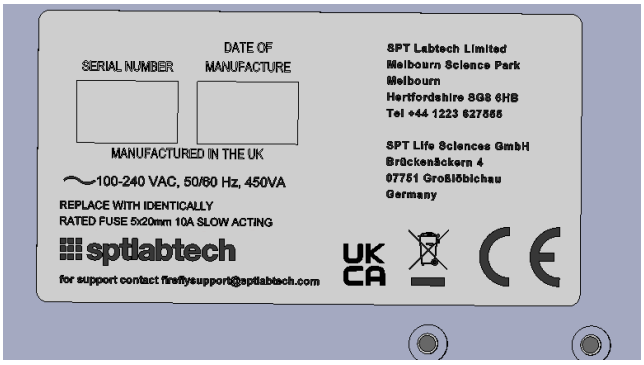
The mains power connection requirements of firefly with all the optional modules are shown diagrammatically below.



Reliance will discuss power requirements with you as part of the pre-installation process e.g., the correct positioning of the power supply units, the number of mains sockets required or if it is safe to use adapter blocks.

Fuses

There are two 5x20mm 10A T (Anti Surge) Ceramic Cartridge Fuses in the IEC inlet.



If at any time you believe a fuse has blown, contact reliance who will investigate what caused the failure. Do not just change the fuse.

The vertical laminar flow module fuse is detailed in the CAS vertical laminar flow module manual.

Network access

The firefly tablet requires internet access via wi-fi to access cloud storage and download labware and protocols.

Installing firefly

firefly is shipped in two crates. Do not open them when they are delivered: reliance service engineers will install and set up firefly.

For the installation, reliance engineers will require:

- use of a pallet truck or similar with a rating above 250kg, for moving the cases
- a clear route to the laboratory where firefly is to be installed

User training

reliance will run initial firefly training sessions when they install your instrument. These will include basic instructions for using the hardware and software to run a protocol, and other instrument related tasks such as calibration.

reliance application scientists will contact you after the installation to arrange training in protocol creation and using firefly for application specific tasks.

User account creation

firefly software is configured with an Admin account, user name 'Admin', which you use to create your users' accounts.

Installing firefly software (standalone)

firefly software is open license so you can install it on any Windows 10 or 11 device, including Macs running Parallels Desktop. This enables you to author protocols off-line and use cloud storage or your network to make them available to the firefly instrument.

Important

Protocol design is much easier on a laptop or desktop PC than on the firefly tablet, so SPT Labtech recommends this approach.

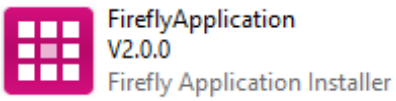
System requirements

The minimum hardware/software requirements are:

- 8GB RAM (16GB recommended)
- at least 10 GB of free disk space. Storage of databases, protocols and log files will use up free disk space, so it is recommended that a much greater amount of free disk space is available
- Windows 10 (22H2 or later) / Windows 11 (22H2 or later)
- Screen resolution of at least 1920x1080

Installation

To install firefly, download the latest installer from SPT Labtech's downloads area (you may be sent a link to this from your installation service engineer) and double-click the firefly application installer file.

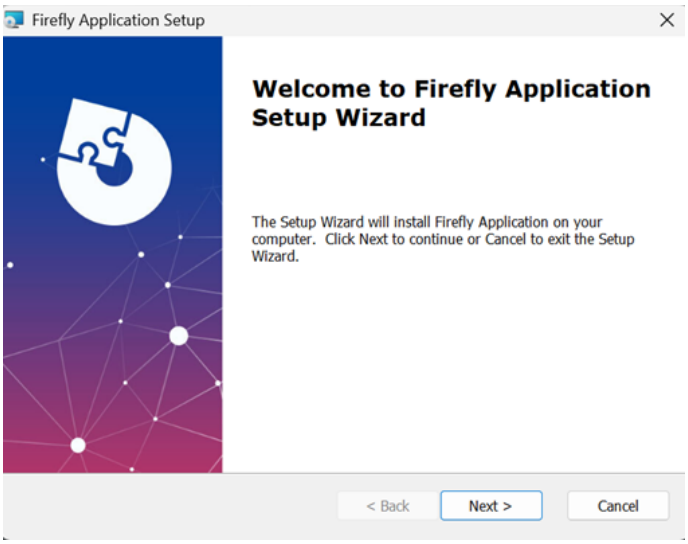


The firefly installer will also install prerequisites it has discovered that are not currently installed on the target machine. The prerequisites are:

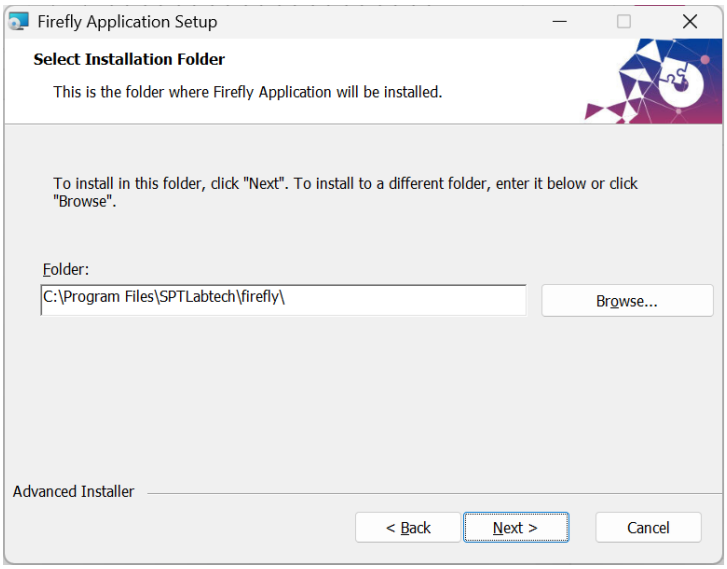
- The appropriate version of the .NET desktop runtime (e.g., v9.0.2 64-bit)
- The appropriate version of the .NET hosting bundle (e.g., v9.0.2 64-bit)
- The appropriate version of the .NET runtime (e.g., v9.0.2 64-bit)

If the installer detects it needs to install any of the prerequisites above, it will do so before installing the firefly software.

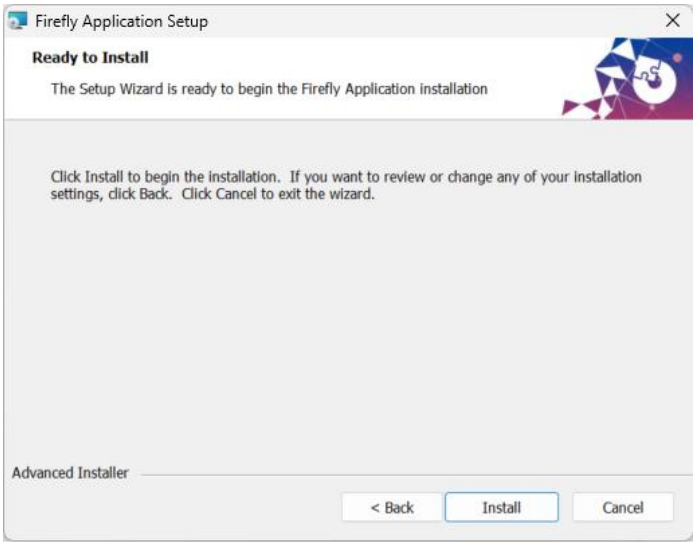
Installation will then continue to the main firefly software:

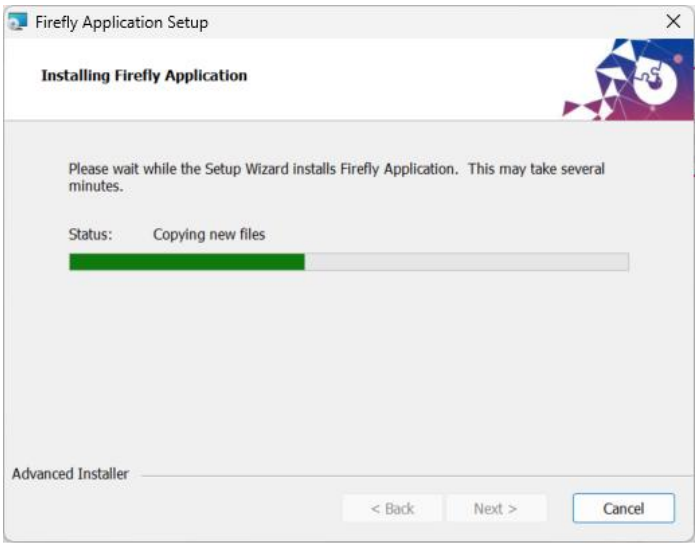


SPT Labtech recommends you leave the default settings for the installation folder:

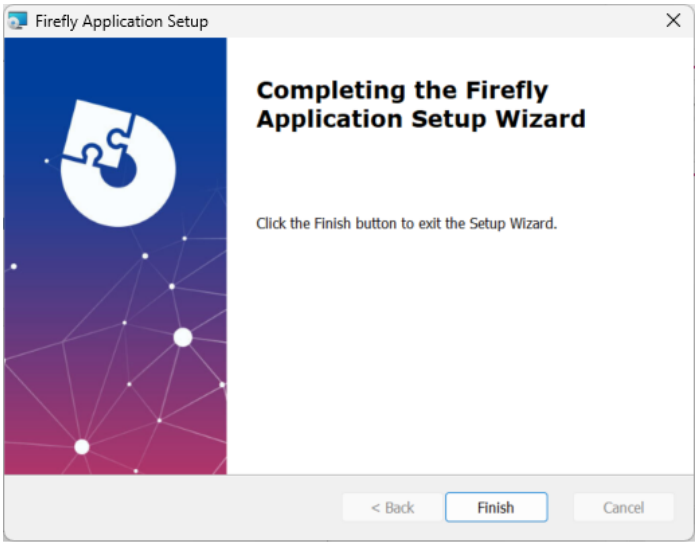


Click **Next >** and then **Install** to start the installation, which may take a couple of minutes.





Once the software has been installed, you should see the following dialog:

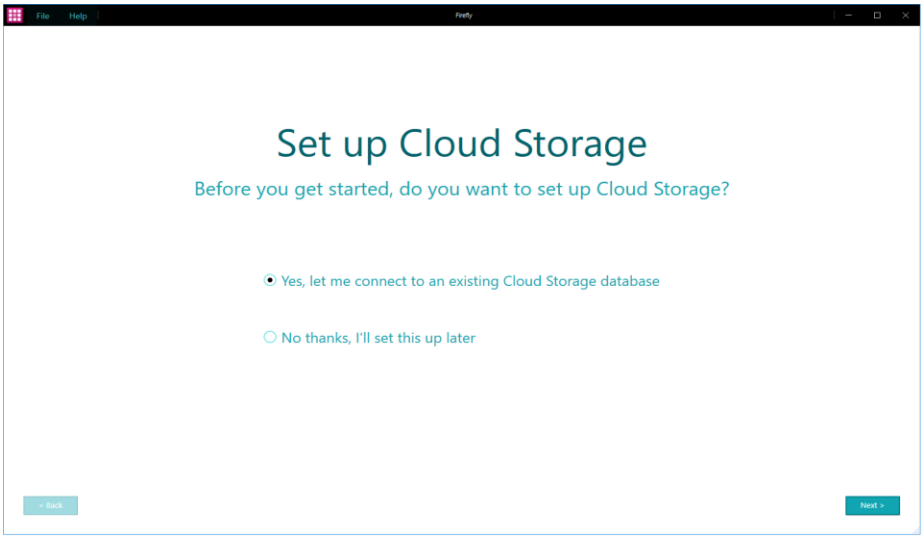


Once installed, the firefly software will be accessible from the Start menu or from a desktop shortcut.

Please contact reliance if you have any problems with installing the firefly app.

Connecting to cloud storage

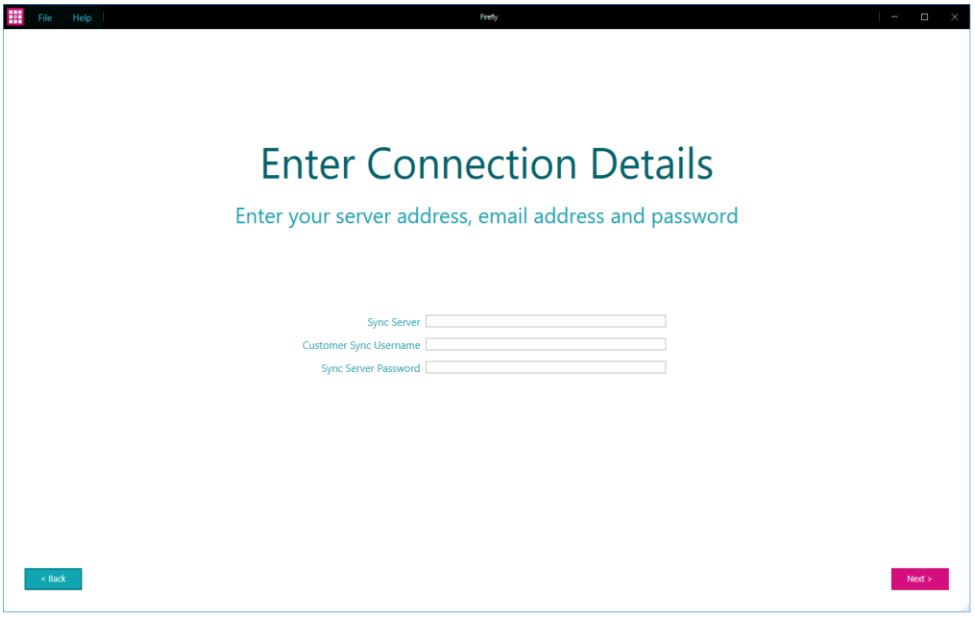
The first time you run firefly, you will be asked whether you wish to set up cloud storage. If you wish to do so, click Next.



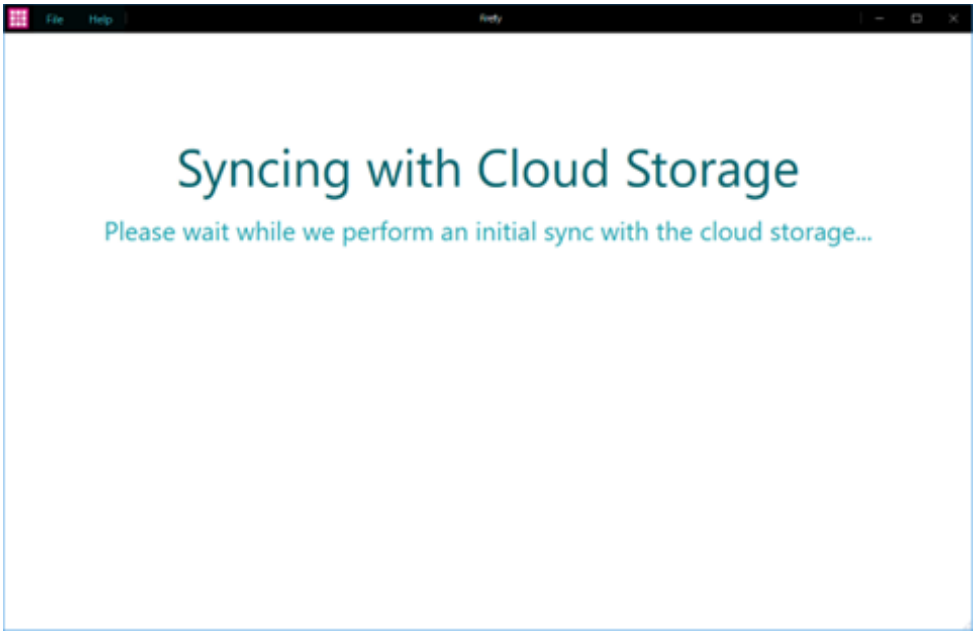
You will then be asked to provide details of the remote “Sync” server from where data from firefly will be synchronized or shared with others connected to the same server.

- Sync Server: the host name of the server from which you wish to synchronize your firefly data
- Customer Sync Username: the username/email address with which you are registered with firefly
- Sync Server Password: the password for your firefly registration

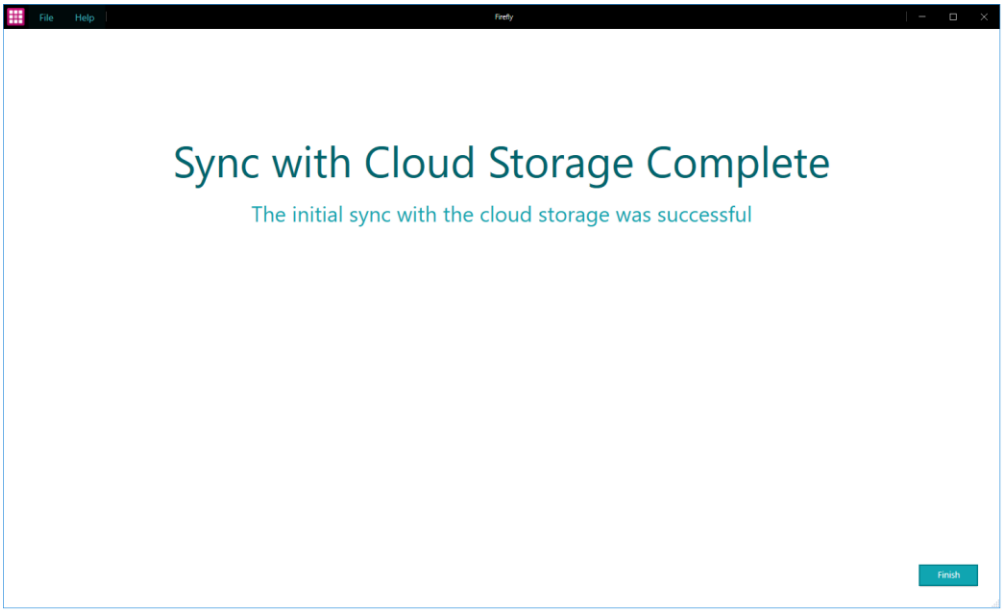
You should ask your firefly administrator for this information as reliance will have organized cloud storage access when setting up your firefly instrument.



Once you have provided the correct credentials, the firefly software will perform an initial synchronization with the server which may take a few minutes.



Once the sync is complete, you will be notified with the following screen:



You can then log in to firefly software as you would on the instrument's tablet.

Connecting standalone

If you don't want to set up cloud storage, you can select the second option “No thanks, I’ll set this up later.” Selecting this option will take you straight to the log-in screen. Any data you create using firefly will be saved locally to your machine and you'll need to use a USB stick or local network drives to transfer your protocols to your firefly instrument.

Installing INHECO 'Script Editor 3' software for the firefly+ thermal cycler

The Inheco On Deck Thermal Cycler (ODTC) uses Inheco software to design incubation protocols. If your instrument includes firefly+ with a thermal cycler, your reliance service engineer will install Inheco's 'Script Editor 3' software on the computer you're going to use to design protocols.

Consumables

firefly requires instrument-specific tips, syringes and reservoirs for correct operation. You must always use the correct consumables, which are available from SPT Labtech.

EZL firefly is supplied with a starter kit containing:

- 1 x Standard Reservoirs (pack of 50)
- 1 x LDV Reservoirs (pack of 25)
- 1 x Standard Syringes (tray of 10)
- 1 x ULR Syringes (tray of 10)
- 2 x Racks of 125µL 384 Filter Tips
- 2 x Racks of 125µL 96 Filter Tips
- 2 x Racks of 50µL 384 Filter Tips
- 2 x Racks of 50µL 96 Filter Tips

ATL firefly is supplied with a starter kit containing:

- 1 x Standard Reservoirs (pack of 50)
- 1 x LDV Reservoirs (pack of 25)
- 1 x Standard Syringes (tray of 10)
- 1 x ULR Syringes (tray of 10)
- 2 x Racks of 50µL 96 Filter Tips
- 2 x Racks of 125µL 96 Filter Tips
- 2 x Racks of 50µL 384 Filter Tips
- 2 x Racks of 125µL 384 Filter Tips

EZL tips

Disposable pipetting tips are available in a variety of sizes for working with different plate types. Use the part numbers to order the correct variants.

The EZL plastic tip collar is colored to reflect the tip volume.

- Yellow = 125µL
- Orange = 50µL

	96 array	384 array	8 strips (96 format)	16 strips (384 format)
125µL filtered	125-096-FF-FS	125-384-EZ-FS	125-008-EZ-FS	125-016-EZ-FS
125µL non-filtered	125-096-FF-S	125-384-EZ-S	125-008-EZ-S	125-016-EZ-S
50µL filtered	050-096-FF-FS	050-384-EZ-FS	050-008-EZ-FS	050-016-EZ-FS
50µL non-filtered	050-096-FF-S	050-384-EZ-S	050-008-EZ-S	050-016-EZ-S

Note that a filtered version has lower capacity because the filter takes up space (i.e.,125µl filtered is actually 100µl, 50µl filtered is actually 35µl).

All the tip types listed above are sterile. Non-sterile options of some tip types are available – please enquire.

ATL tips

Disposable ATL pipetting tips are available in the following sizes. Use the part numbers to order the correct variants.

The plate on which the firefly+ tips sit in their tip box will be colored to reflect the tip volume.

- Yellow = 125µL
- Orange = 50µL

	96 array	384 array
125µL filtered	125-96-FF-AL-FS	125-384-AL-FS
125µL non-filtered	125-96-FF-AL-S	125-384-AL-S
50µL filtered	050-96-FF-AL-FS	050-384-AL-FS
50µL non-filtered	050-96-FF-AL-S	050-384-AL-S

Note that a filtered version has lower capacity because the filter takes up space (i.e.,125µl filtered is actually 100µl, 50µl filtered is actually 35µl).

All the tip types listed above are sterile. Non-sterile options of some tip types are available – please enquire.

Tip box adapters

Tip box adapters are required to keep ATL tip boxes rigid while tips are loaded onto the pipetting head. You can load 1-3 columns of tips without an adapter; more than that will require one. firefly software will remind you to use the adapter when required, when designing protocols.

ATL instruments are supplied with tip box adapters; you can purchase more if you find you need them.

Syringes

Dispensing syringes are available in one size only, which is suitable for use with 96 or 384 well microplates. Packs contain 100 syringes. Ultra Low Retention (ULR) syringes feature an external hydrophobic/ oleophobic coating to dispense concentrated detergent and protein solutions, or genomics Master Mixes. All syringes are disposable.

The syringe range available is:

- Sterile-ULR (4150-07209)
- Sterile-Standard (4150-07201)
- Non-Sterile-ULR (4150-07208)
- Non-Sterile Standard (4150-07200).

Reservoirs

Reservoirs are available in three sizes: high volume (in packs of 24), standard (in packs of 50) and low dead volume (LDV), in packs of 25. Lids are available for use with high volume reservoirs, to reduce evaporation; their use is optional.

You will need the high volume reservoir tray (see below, the empty tray is on the right) to use high volume reservoirs. This is available in the high volume reservoir kit, which also includes the inserts needed to use standard and LDV reservoirs with high volume reservoirs.

High volume reservoirs have 45mL capacity and 500µL recoverable dead volume.
Standard reservoirs have 10mL capacity and fill level indicators for 5mL and 10mL. The dead volume is 200µL, which is recoverable.
Low dead volume reservoirs have 1.5mL capacity and 30µL recoverable dead volume.

Reservoirs are disposable but can be washed after use to allow a number of re-uses if you wish.

The reservoir range available is:

- Sterile LDV (4150-07203)
- Sterile Standard (4150-07204)
- Sterile High Volume (4150-07304)
- Sterile High Volume with Lids (4150-07305)
- Non-Sterile LDV (4150-07202)
- Non-Sterile Standard (4150-07103)
- Non-Sterile High Volume (4150-07300)
- Non-Sterile High Volume Lid (4150-07303)

Note

Sterile HV reservoirs and lids take on a slightly yellow discoloration as a consequence of the sterilization process.

Plates

firefly is compatible with skirted 96 and 384 plates including deep well blocks and 1x12 and 1x24 column troughs, for use with strip tips.

Important

Only use the exact plate types specified in a protocol as the movements of the firefly heads will be correct for those plates. If you need to use alternatives, you will need to revise the plate settings in the protocol first.

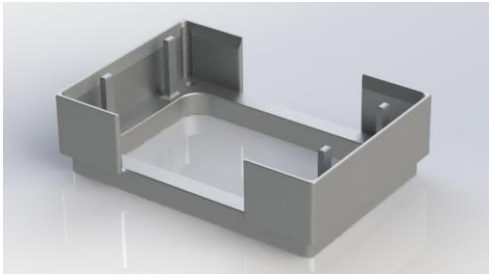
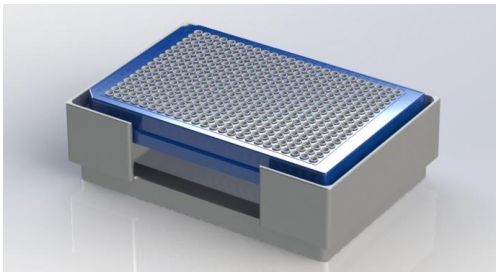
If you cannot find the plates you want to use when you try to swap the plate type, check if they are available through Community Labware.

If they are not, and you need to use a specific non-standard type e.g., 1x1 trough plates or 48 well plates, contact reliance for advice.

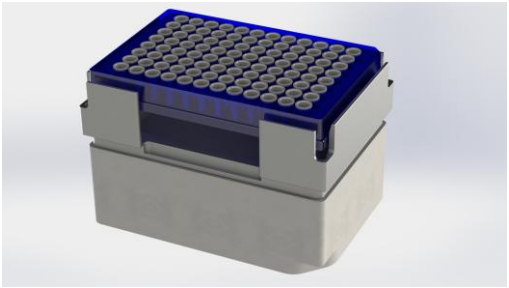
Plate risers

To avoid collisions between the dispense head and labware of differing heights, you may need to use a riser under your plate. A universal riser is supplied with your instrument.

There are two types of risers, the universal riser (below) is for use on the upper deck.

For the lower deck you would need to use the taller lower deck riser (below), if you are using EZL tips.

You cannot use these lower deck risers with ATL tips; there is a specific variant for ATL instruments.

You can purchase additional risers from SPT Labtech.

Plate lids

firefly is compatible with some types of plate lid, which you can download from Community Labware.

> **Important**
>
> Do not substitute alternative lids for the type specified in a protocol as firefly's grippers may not move correctly to pick the lid up.

If you need to use an alternative, check if it is available through Community Labware, download it and modify your protocol to use your preferred lids.

If you cannot find the lid you want on Community and you need the specific type, contact reliance for advice.

> **Important**
>
> To not substitute alternative lids for the type specified in a protocol as firefly's grippers may not move correctly to pick the lid up.

Plate seals

You can use plates with pre-pierced foil seals with firefly.

> **Important**
>
> It is critical that the foil is completely pierced before the plate is loaded onto the firefly deck.

Pierce the foil by pushing a clean plate or 8-strip tube into the foil seal to ensure the foil has been pierced completely. Piercing the foil using a pipette tip is not recommended since it does not create an opening in the foil that the pipetting head tips can freely move though.

Starting firefly

Turn on the instrument, using the on/off button located on the right-hand side of firefly.

After a few moments, the instrument lights will come on, showing that it is ready for use, and the firefly tablet will start up.

If your instrument has the optional laminar flow module:

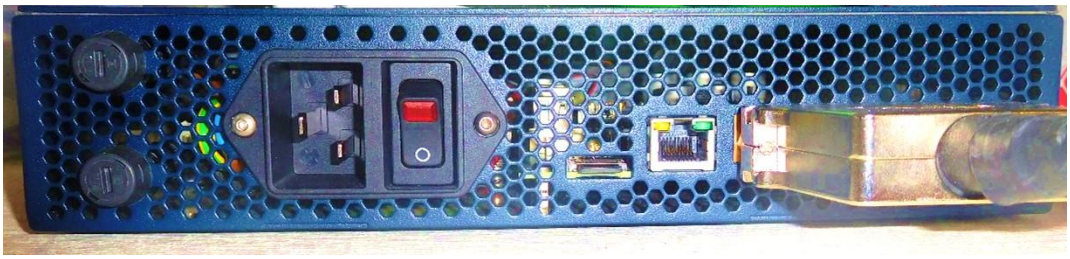
1. Switch on the unit at the rear before you switch on firefly. The display at the front will be illuminated.
2. Press the fan icon on the control panel – this will start the integral fan. The main display will show `AIRFLOW STABILISING` for 60 seconds and the audible alarm will sound.
3. You can use the Alarm Mute button to silence the alarm.
4. Once the airflow is settled the display will show a safe ‘green’ filter status.

Tip

You don't need to switch on firefly until the filter shows the 'green' status, as only then is it ready to use.

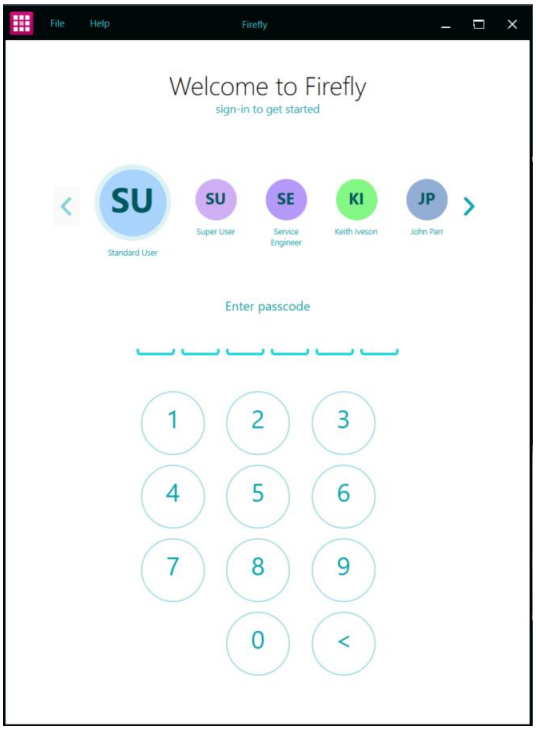
If your instrument has firefly+, it will power up with the main instrument.

If it has the ODTC, you will need to switch it on, using the on / off switch on the control unit, which is outside of firefly+.



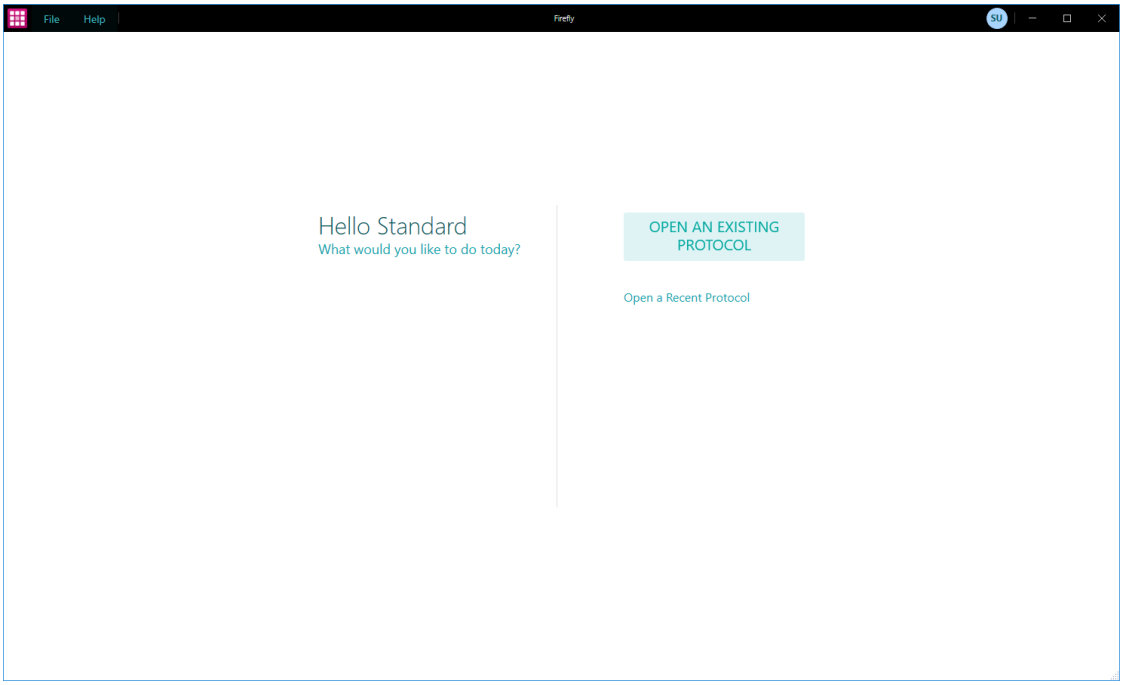
Log in to Windows on the firefly tablet.

Select the firefly app icon and log in to the firefly software by selecting your user account icon and entering your passcode.

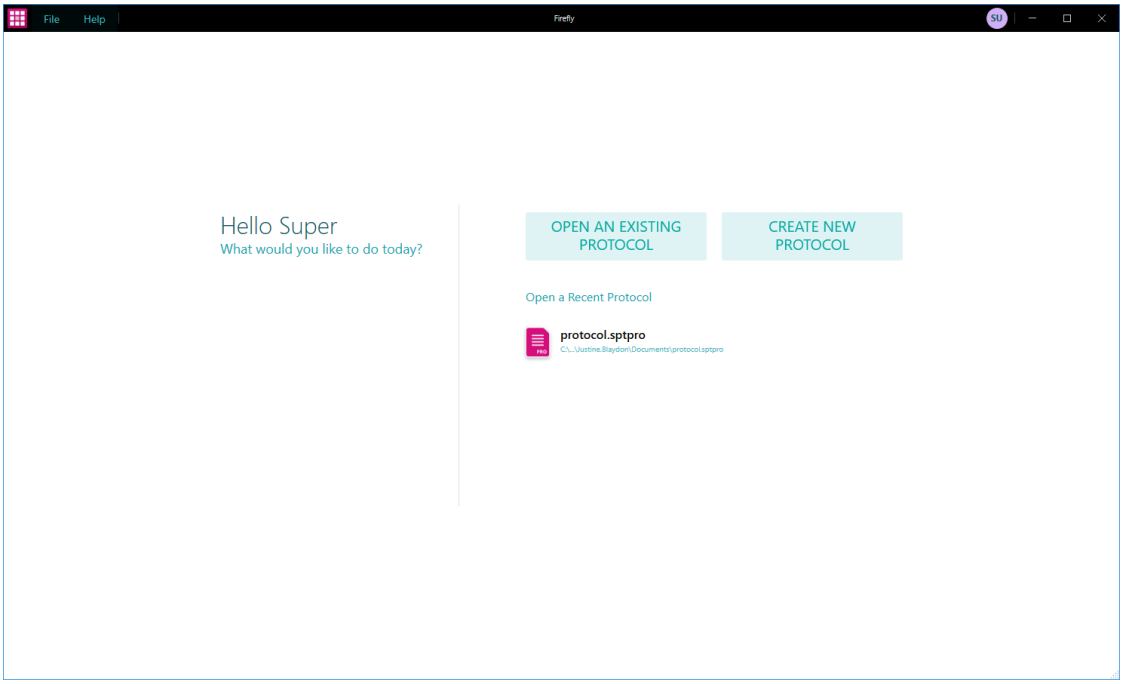


What you will see next depends on your user level.

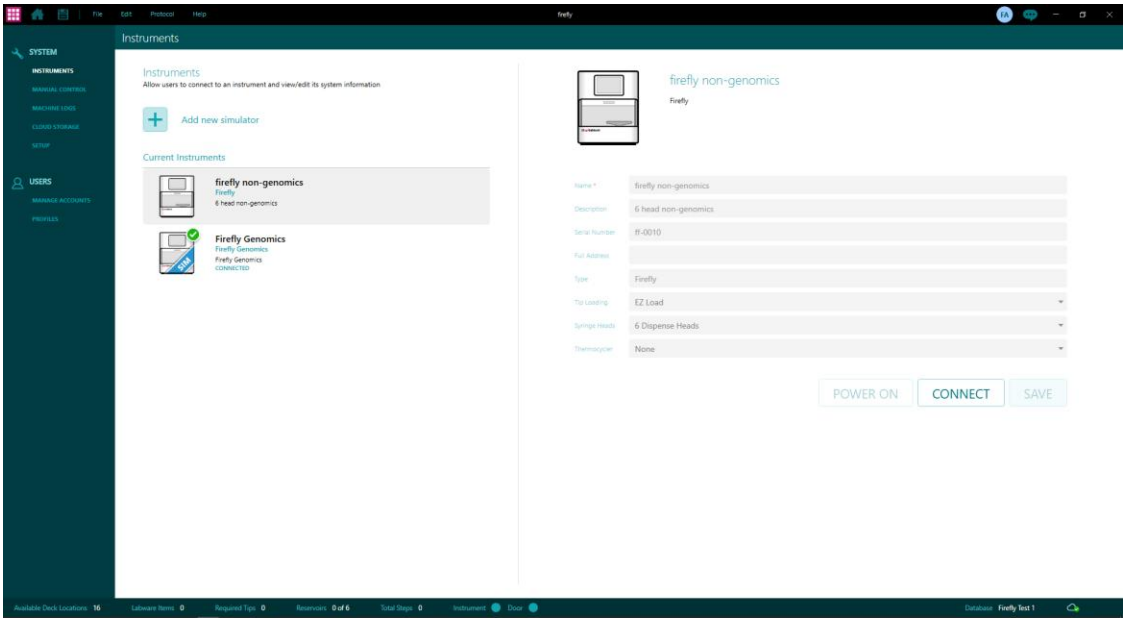
If you have standard access, you will be able to open and run existing protocols. If you have previously run protocols on your instrument, they will be listed under 'Open a Recent Protocol'. You must select a protocol to run.



If you are a super user, you will be able to download protocols from the cloud, or write your own, and you will see create and open options on the opening screen.

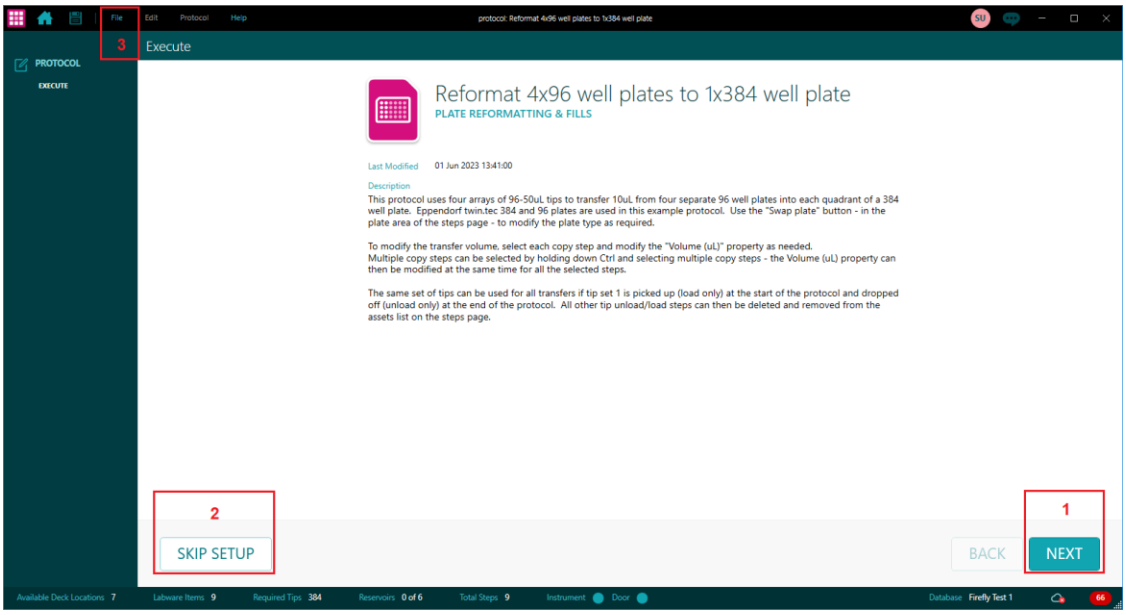


If you are an administrator, you will see system administration options.



Software overview: standard user

This is firefly's opening screen if you are logged on as a standard user. It shows the description of the protocol which you selected when you logged on.



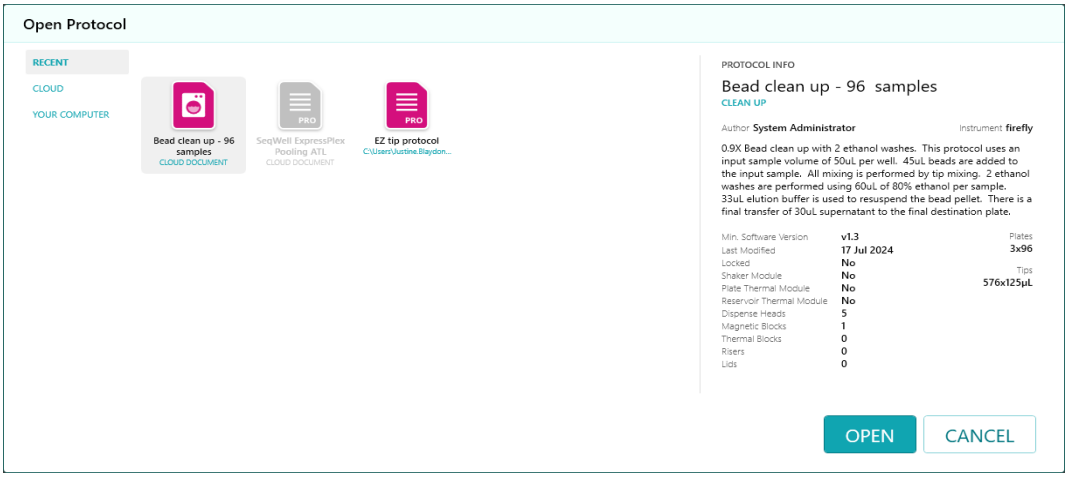
You have three main options available:

1. Select 'Next' to start firefly set up
2. Select 'Skip Setup' if you are confident loading firefly without guidance and have been given permissions in the software to use this feature.
3. Select 'File' on the menu if you need to open a different protocol

Running a protocol - standard user

Open a protocol

If you need to run a different protocol to the one you initially selected, use the 'File' menu, 'Open', 'Open recent' or 'Open from your computer' to select it. If you have cloud access and use 'Open', this will show you all protocols shared within your organization, unless they are in a private folder.



Cloud protocols also show basic information about the protocol, including the minimum hardware specification and software version it can be run with. This is useful if you have multiple, different firefly instruments.

PROTOCOL INFO

Bead clean up - 96 samples

CLEAN UP

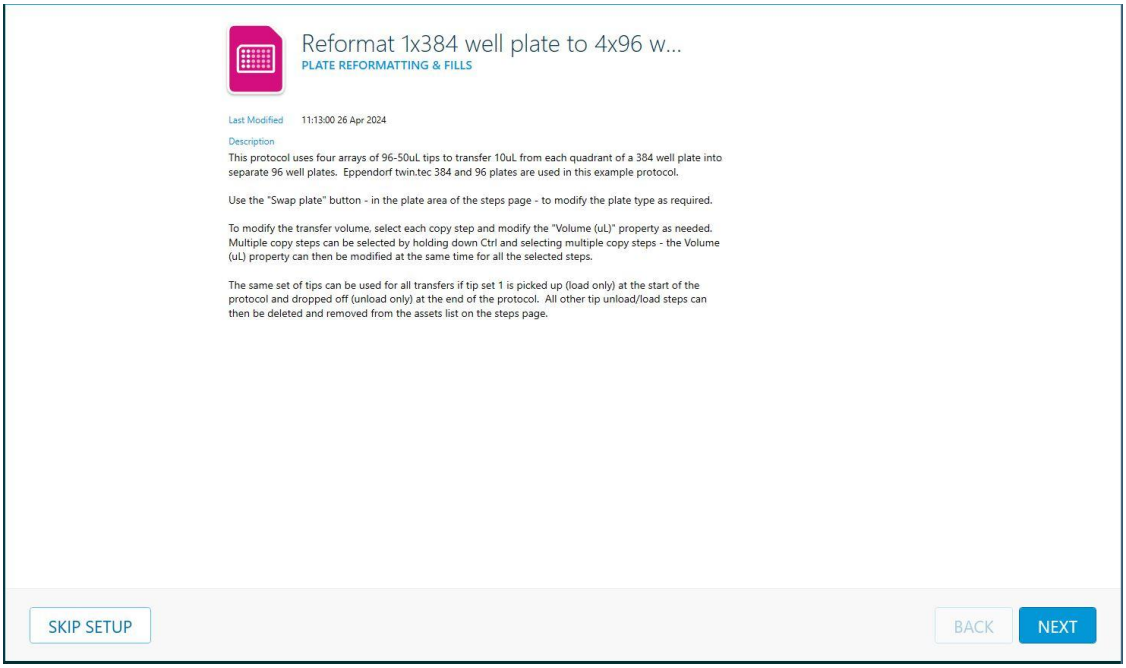
Author **System Administrator** Instrument **firefly**

0.9X Bead clean up with 2 ethanol washes. This protocol uses an input sample volume of 50uL per well. 45uL beads are added to the input sample. All mixing is performed by tip mixing. 2 ethanol washes are performed using 60uL of 80% ethanol per sample. 33uL elution buffer is used to resuspend the bead pellet. There is a final transfer of 30uL supernatant to the final destination plate.

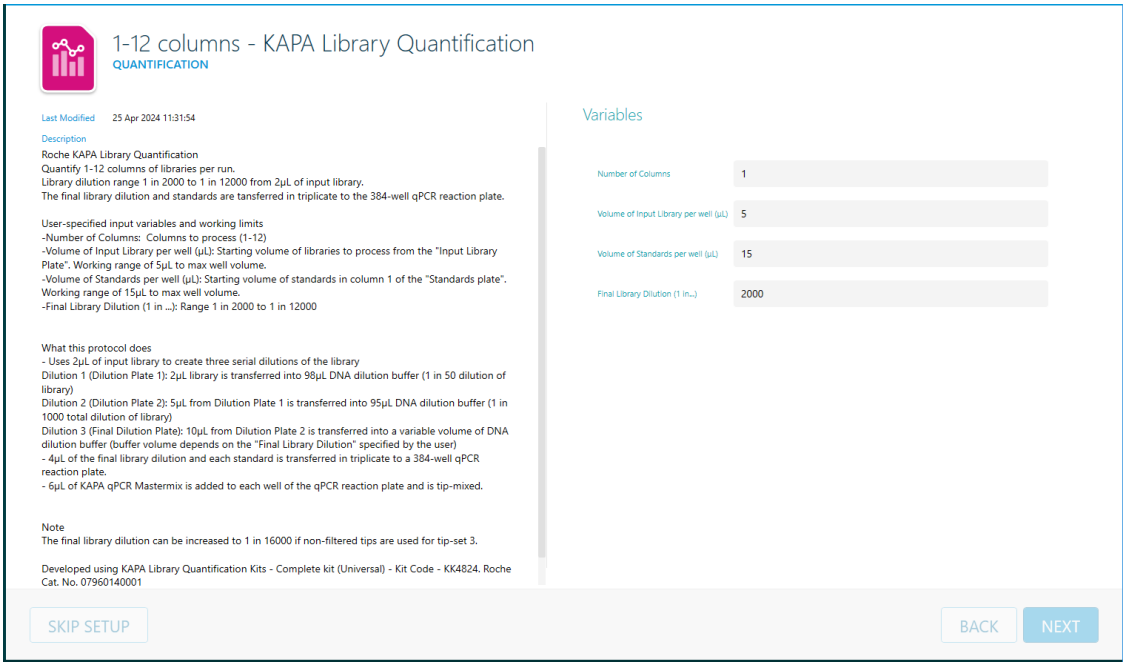
Min. Software Version	**v1.3**	Plates	**3x96**
Last Modified	**17 Jul 2024**		
Locked	**No**	Tips	**576x125µL**
Shaker Module	**No**		
Plate Thermal Module	**No**		
Reservoir Thermal Module	**No**		
Dispense Heads	**5**		
Magnetic Blocks	**1**		
Thermal Blocks	**0**		
Risers	**0**		
Lids	**0**		

Reviewing a protocol

On opening a protocol, you will see its description.



If your protocol uses variables, you now specify them e.g., the quantity of tip strips to be used.

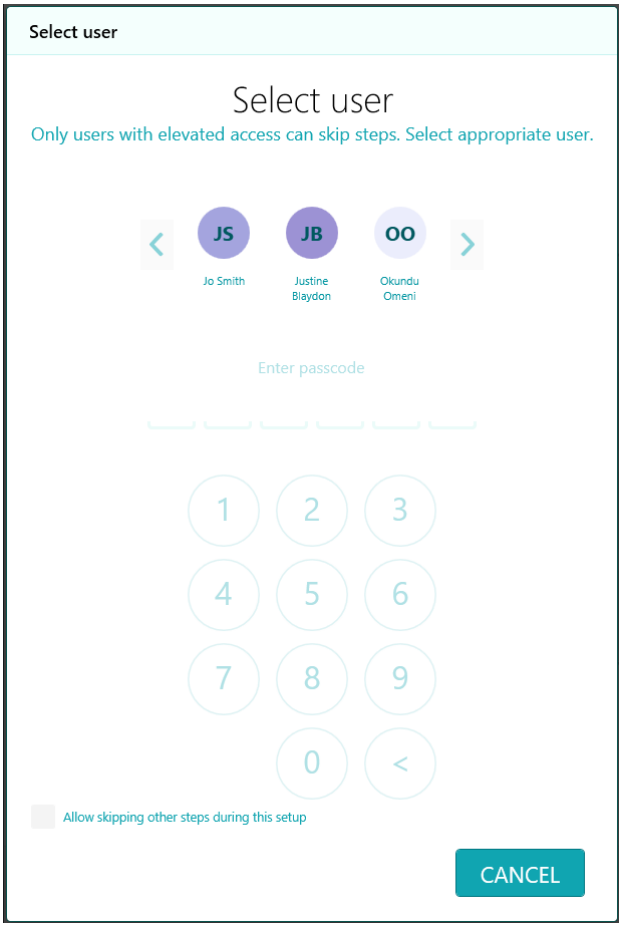


From here you can start to set up your protocol by selecting 'Next'.

Using Skip Setup

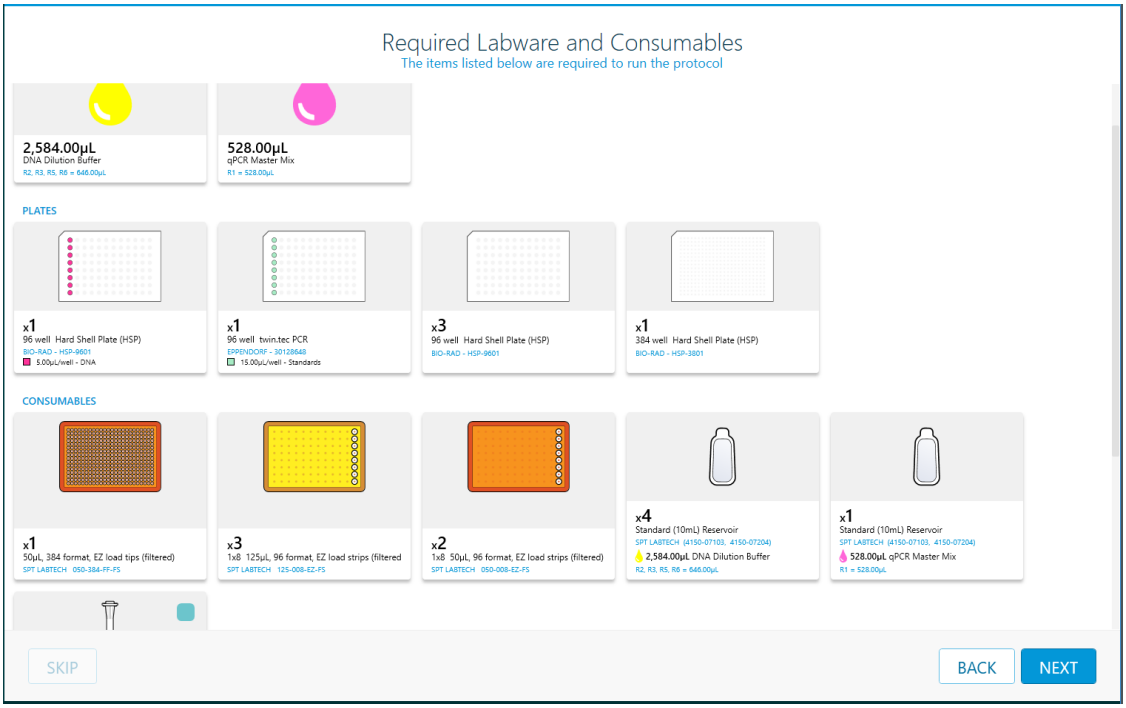
If you are familiar with the protocol and running firefly, you may be permitted to use 'Skip Setup'. This will bypass the 'Setup and Loading wizard' to go straight to the Execute screen.

If you do not have default permission to skip setup, you will need to provide an authorization passcode e.g., a supervisor's, to indicate that they have approved your actions before you can skip the setup steps when executing a particular protocol. Ticking 'Allow skipping other steps during this setup' permits the same authorization to be used more than once, but only while setting up the current protocol.

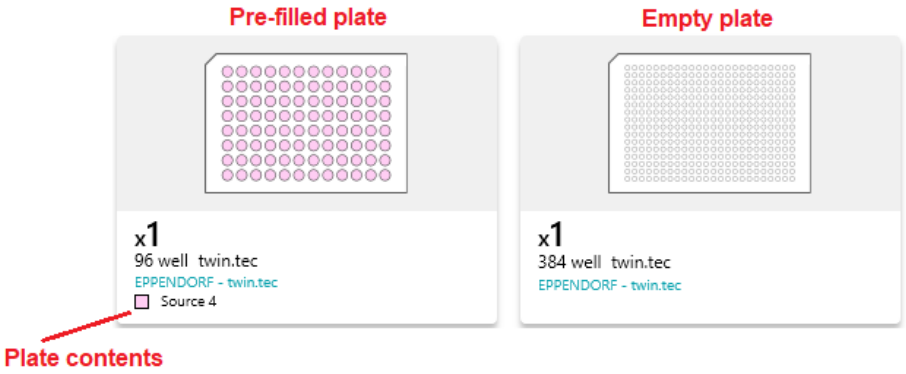


Checking required labware & consumables

The 'Labware and Consumables' screen shows you all the materials firefly needs to run your protocol (you may need to scroll down to see everything), including quantities and types of all reservoirs and syringes used. Check the labware carefully e.g., if tips are sterile or filtered.



Colored squares below the plate indicate that it is filled before it is loaded. You will need to prepare these plates before starting your protocol on firefly.



Note

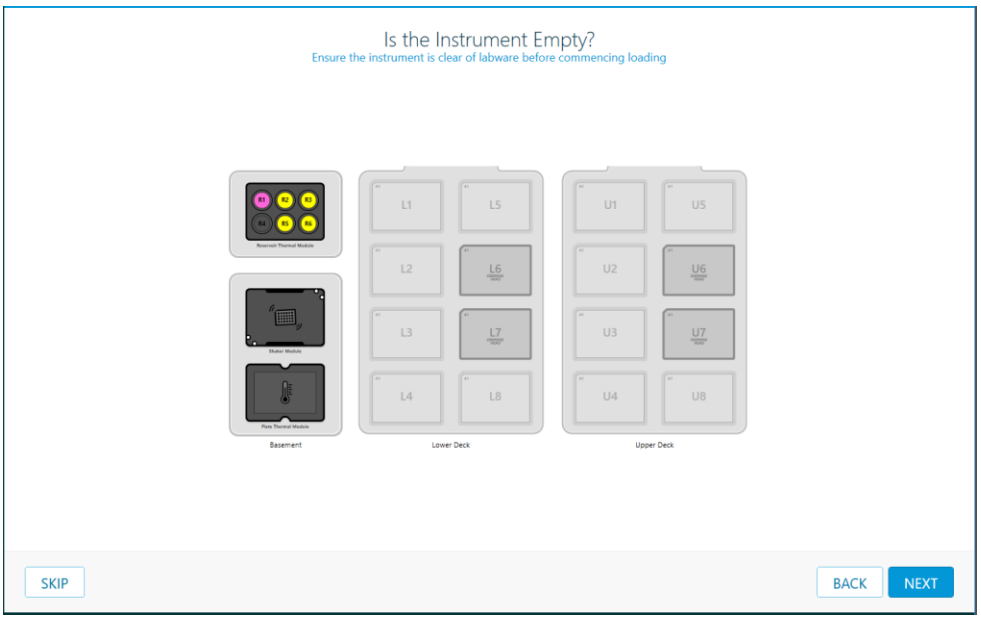
This screen does not show you the materials and equipment you will need for the steps which are not performed on firefly.

Before you start you should review the SOP for the protocol to see what additional equipment you will need, particularly as you may need to have other equipment ready for

immediate transfer of plates e.g., for PCR. When you have your materials ready, click 'Next'.

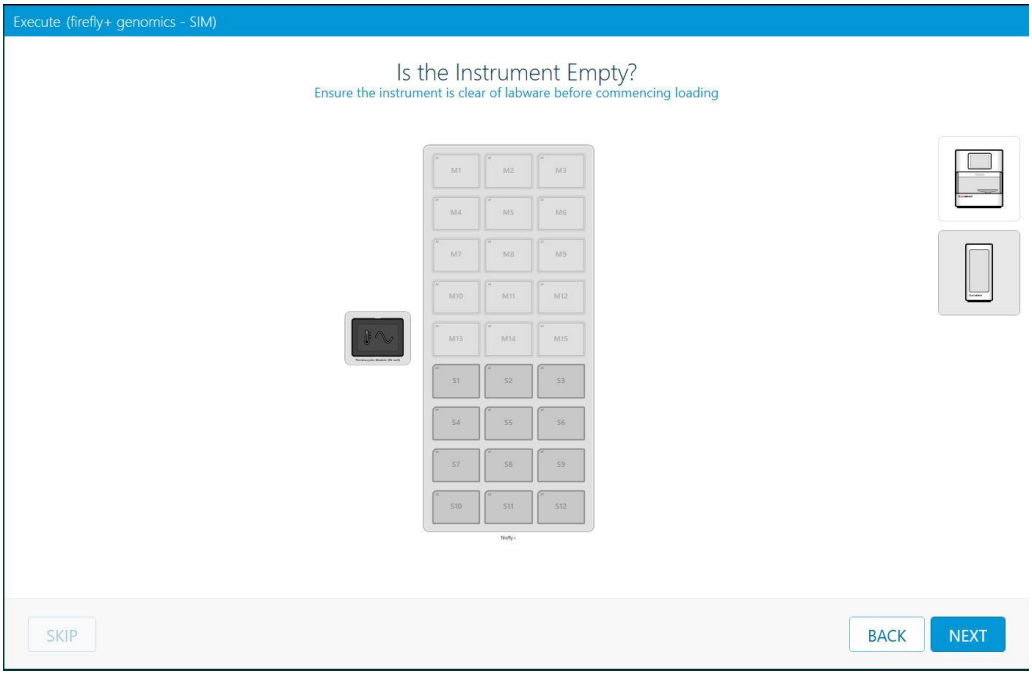
Preparing firefly

The first screen reminds you to empty firefly of any labware from a previous protocol. Click 'Next' when it is empty.



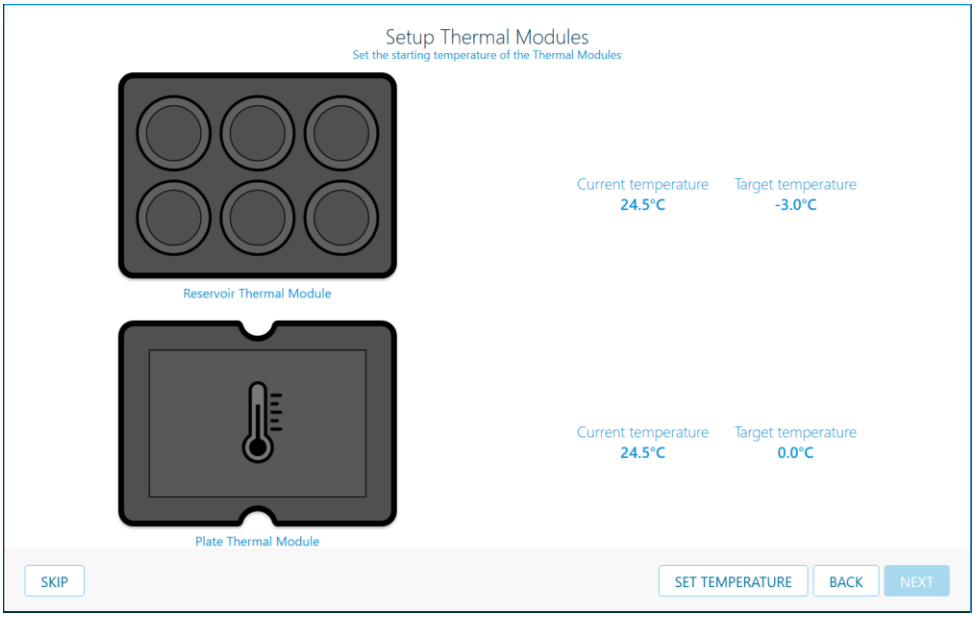
The screen will show your firefly's configuration; the number of dispense heads will be correct and the shaker and thermal modules will be greyed-out on non-genomics instruments.

If your instrument has firefly+ this is also shown; toggle between the views using the icons on the right.

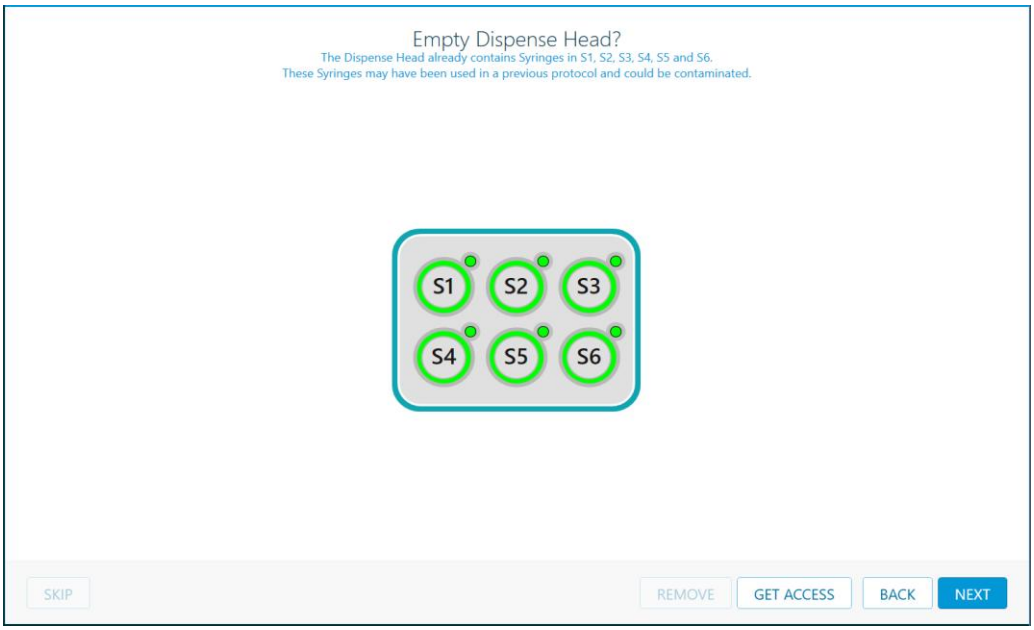


If your protocol has an initial temperature setpoint for either the plate or the reservoir thermal blocks, you will start heating or cooling them as part of set up. Use 'Set temperature' to begin heating or cooling. 'Next' is not enabled until the block reaches the set temperature.

If the protocol uses the ODTC for an incubate step, it will be initialized automatically.



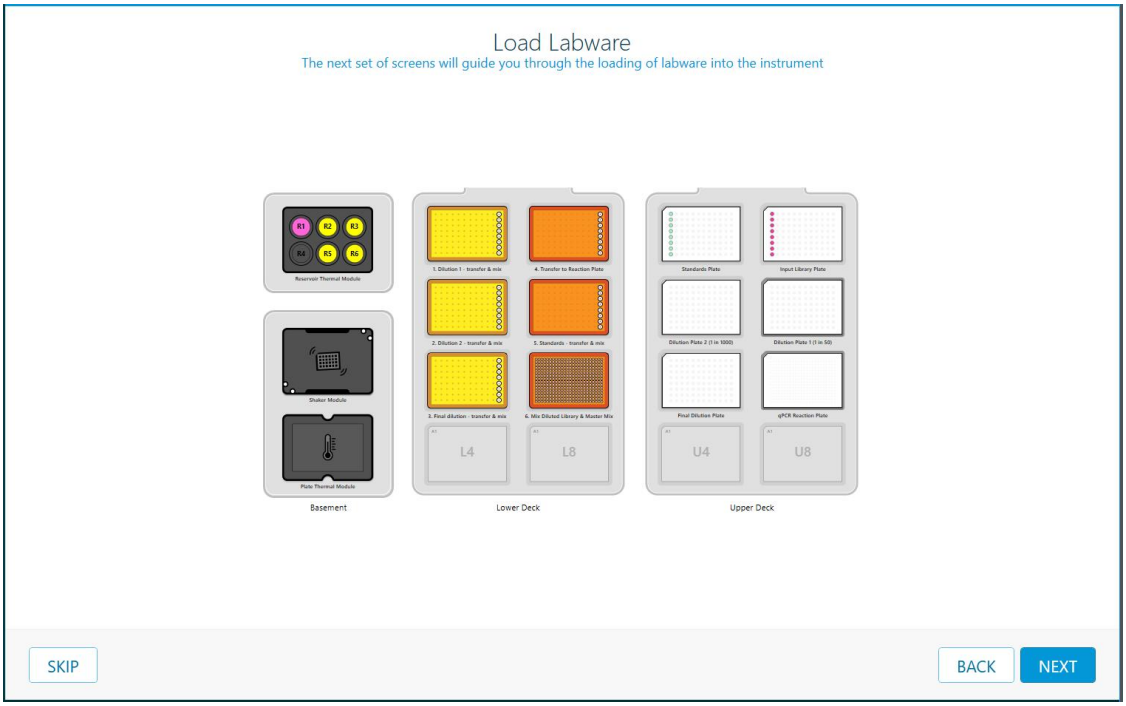
If the protocol requires the dispense head and it already contains syringes from a previous protocol, the next screen will prompt you to remove them. You do not have to do this e.g., if you are running the same protocol again and your lab procedures do not require you to change the syringes.



Loading firefly

The next screen starts the instrument set up process, by showing the completed deck set-up. firefly will guide you through loading each item in the following screens. Each step shows one loading operation and has options for 'Skip', Get Access', 'Back' and 'Next'. When the buttons are greyed out, firefly is operating and not ready for user access.

You can select 'Skip' to move through some or all of the sections of the loading sequence more quickly if you are an experienced firefly user and you have permission to do so.

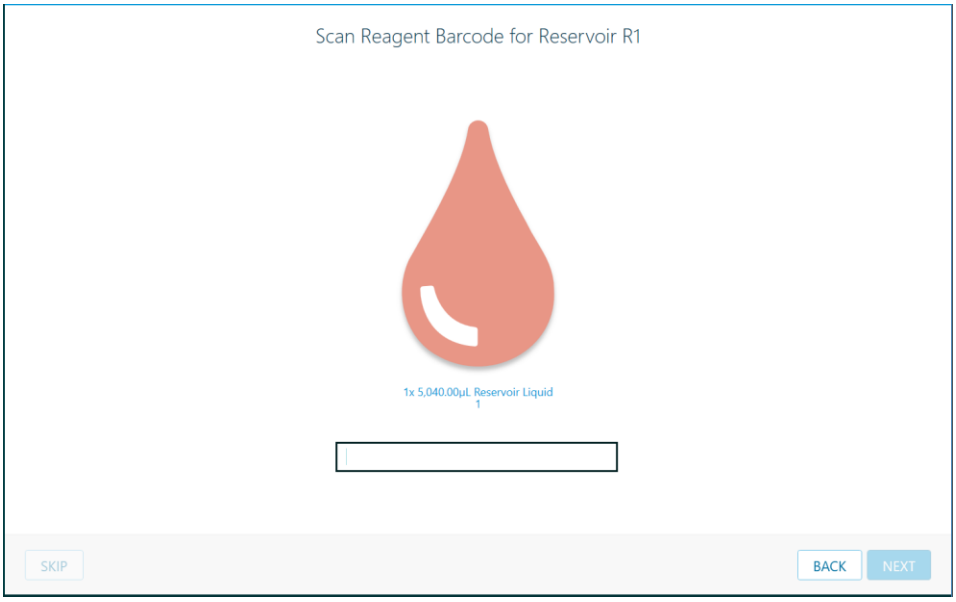


Ensure you have all your items ready e.g., cold plates or reservoir inserts are chilled, tip sets or strips are loaded into tip cassettes and syringes are assembled, then click 'Next'.

Follow the on-screen instructions for loading each item and the instructions below for physical loading.

1. Select 'Get Access', unless you are loading syringes in which case follow the specific instructions below. Wait for firefly heads and decks to move to safe positions before you open the door.

2. You may need to scan barcodes on reagents, tip sets, plates, syringes or reservoirs.

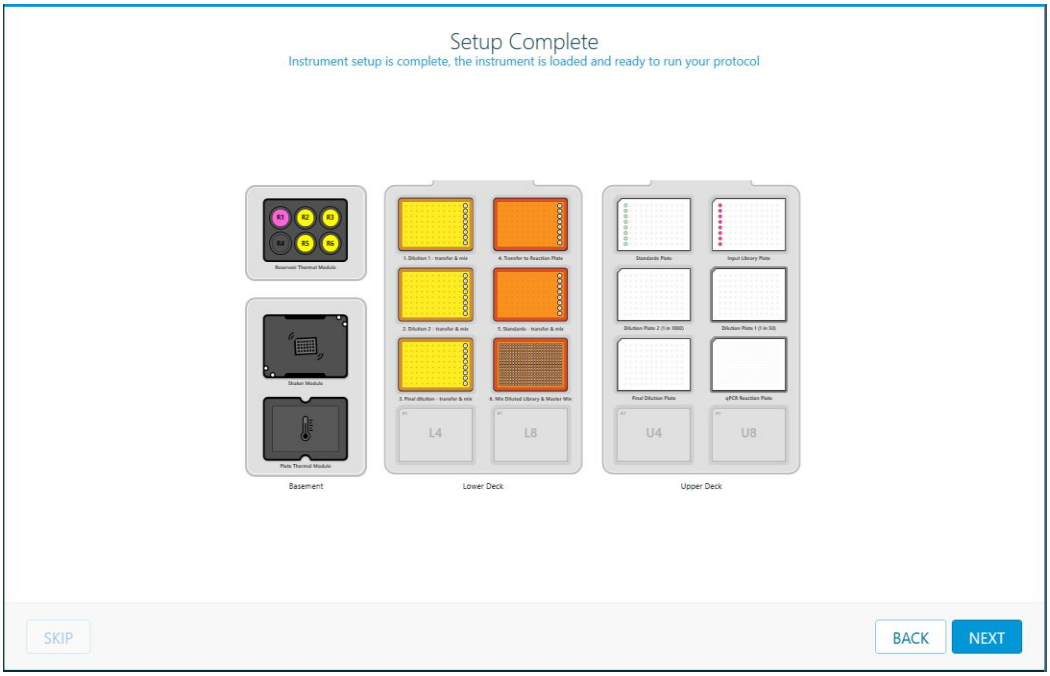


3. Open the door.
4. Load your materials (see specific instructions below).

> **Important**
>
> Be sure to put each item on the deck location specified. firefly cannot validate that you have done this correctly so you must follow the instructions exactly or your protocol will fail.

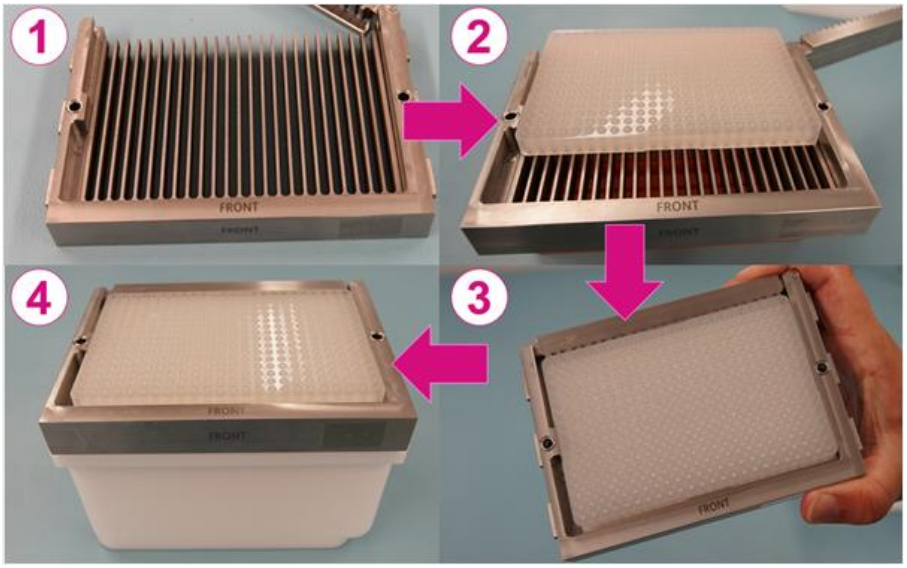
5. Close the door.
6. Click 'Next' on the wizard, or 'Back' if you are loading materials in a different order to that suggested and repeat the loading process for the next item.

When all materials and consumables are loaded, you will see 'Setup complete'.



Loading EZL tips

To load a full array of 96 or 384 tips:



1. Open the tip cassette.
2. Carefully slide the tips into the tip cassette. For 96 tips, the A1 tip must be at the open end of the hinge for the tips to fit securely in the cassette. As the tips are being

positioned into the cassette, lift the collar holding the tips together just enough to clear the grilles of the cassette.

Important

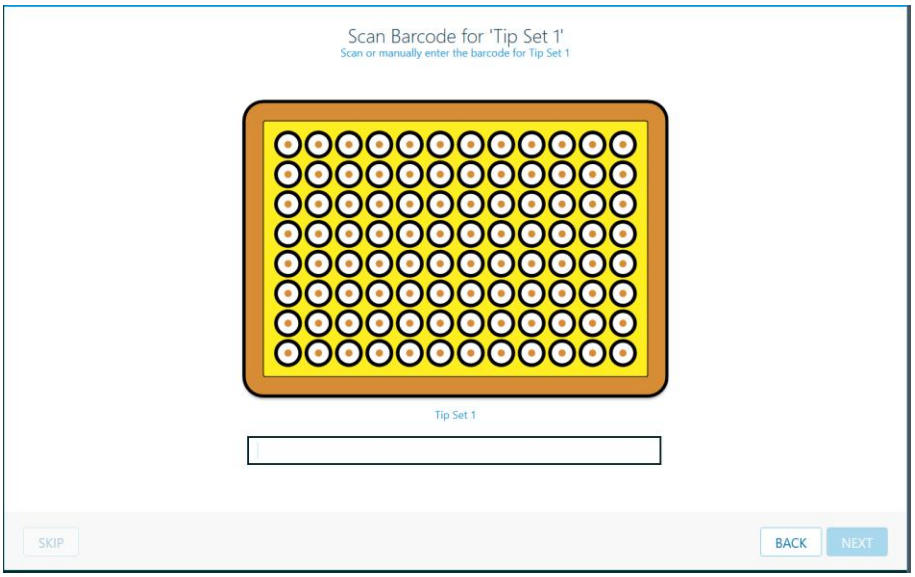
Avoid dropping the tips into the closed cassette from above the cassette, as this action may cause the tips to touch and contaminate the cassette and/or tips

3. Close the gate. Don't tip the cassette upside down - the tips will fall out.
4. Place the tip cassette into the tip stand.

Important

The tip stand must be clean and free of dust before tips are loaded into it. Failure to ensure this may result in dust and debris being transferred to the tips

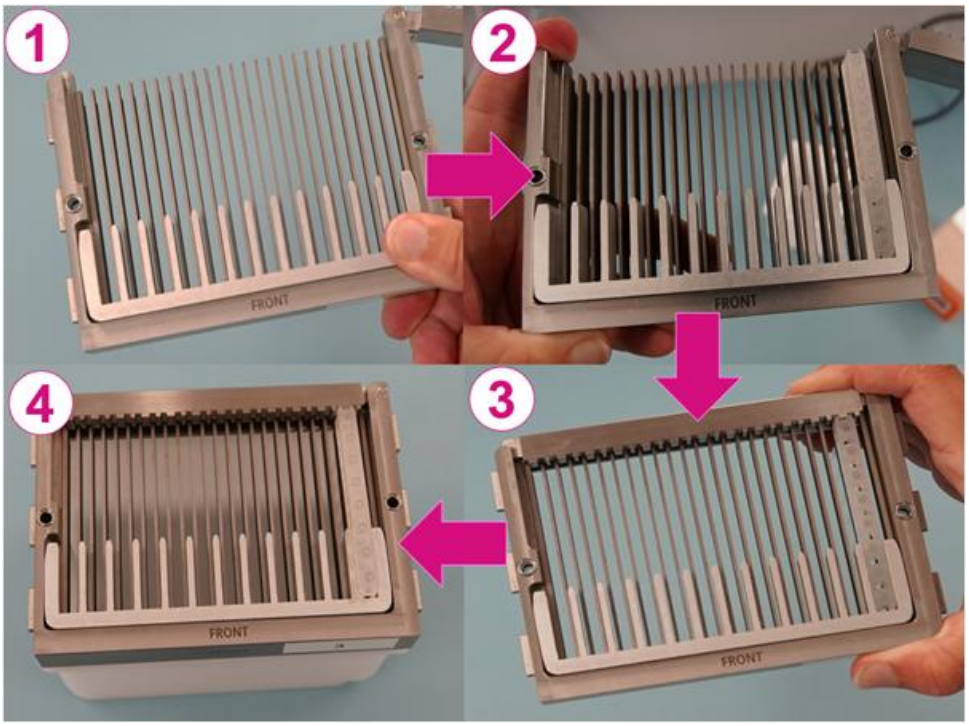
5. Scan the barcode, if required.



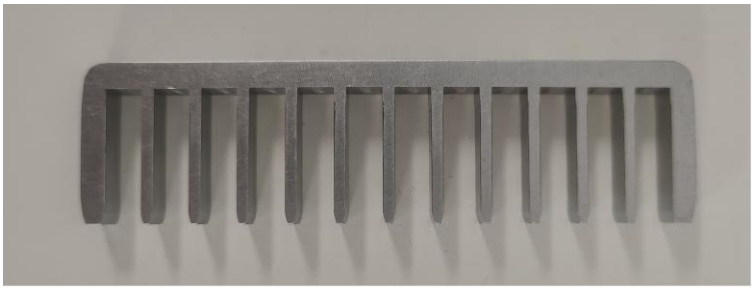
6. Place the tip set on the lower deck in the position shown on the Load Tips screen.

The word "FRONT" is present on each cassette, and it must be facing you when the tip set is loaded onto the deck.

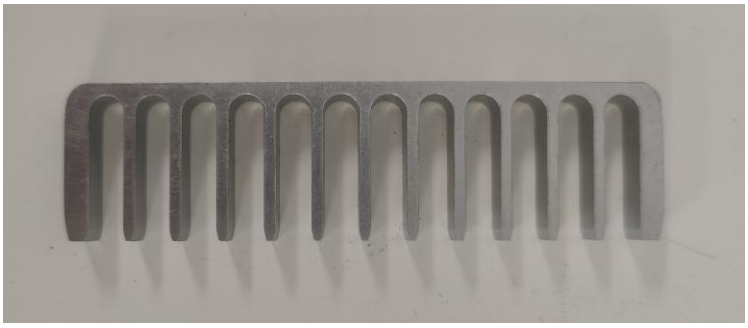
To load one or more tip strips (all sizes):



1. Select the correct strip tip adaptor (16 or 8) for your plate and tips. For 96 format tips, use the 8 adaptor below.



For 384 format tips, use the 16 adaptor below.



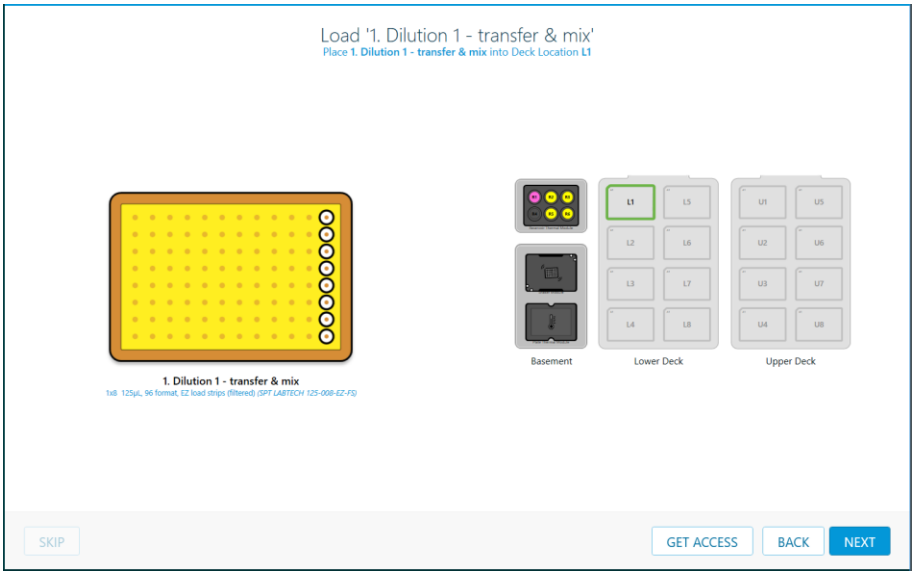
Place the strip tips adaptor on the tip cassette, to keep the strip tips in position.

2. Slide the tip strip into the tip adaptor, so that it is at the far right position (column 12 for 96 wells).
3. If required, add additional strip tips, working from the right. Close the tip cassette gate. Don't tip the cassette upside down - the tips will fall out.
4. Place the tip cassette into the tip stand.

Important

The tip stand must be clean and free of dust before tips are loaded into it. Failure to ensure this may result in dust and debris being transferred to the tips

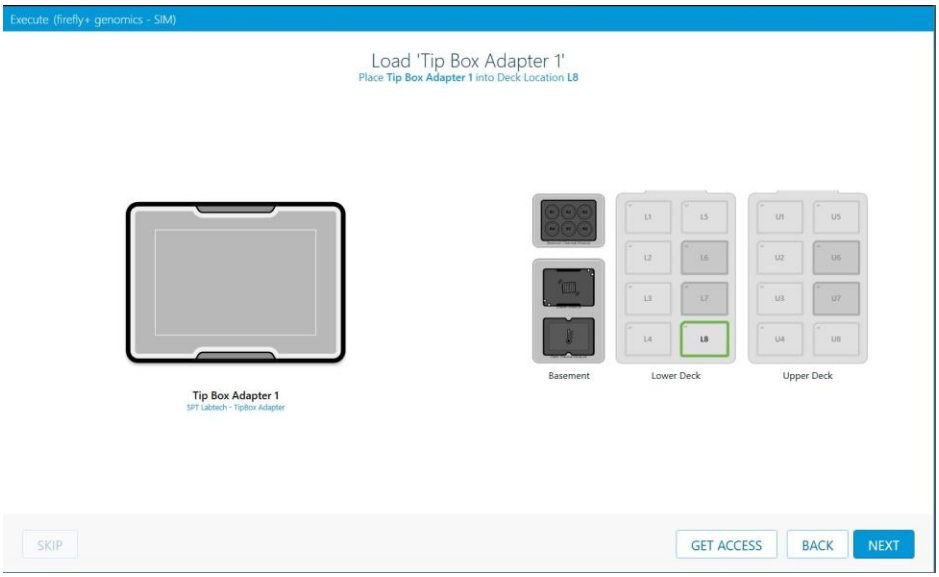
5. Place the tip set on the lower deck in the position shown on the Load Tips screen.



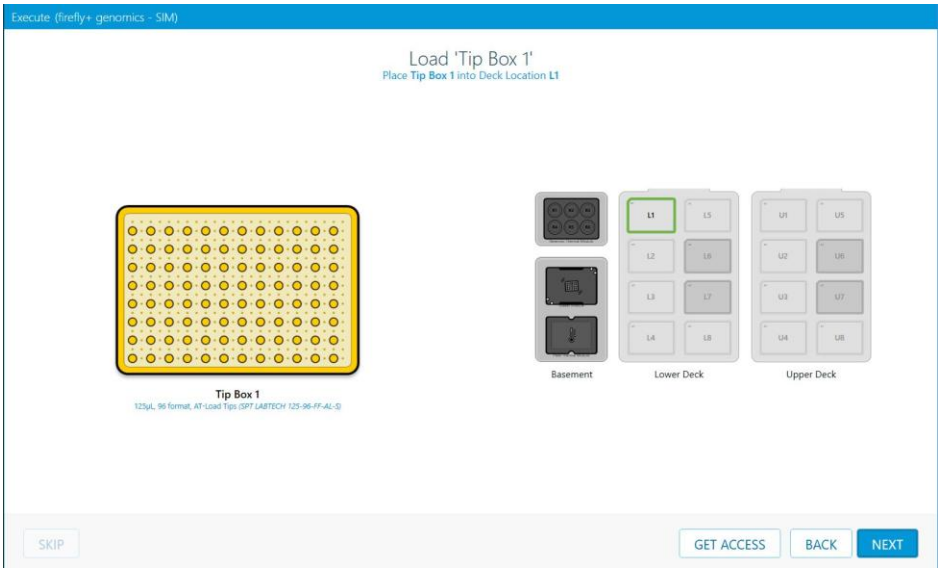
The word "FRONT" is present on each cassette, and it must be facing you when the tip set is loaded onto the deck.

Loading ATL tips

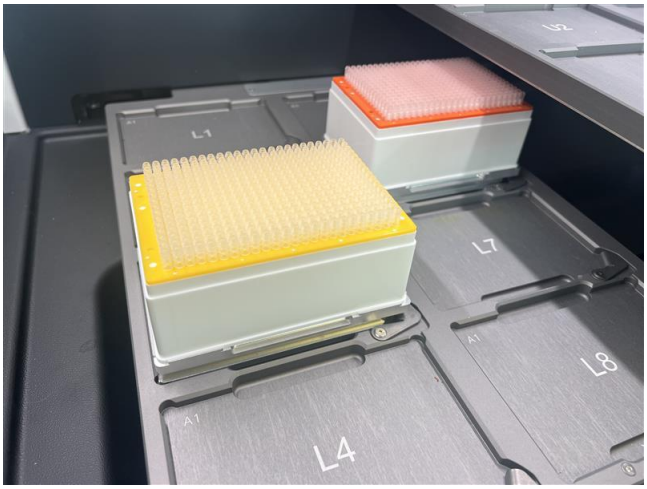
If your protocol requires the use of a tip box adapter, place this in the position shown in the set up instructions.



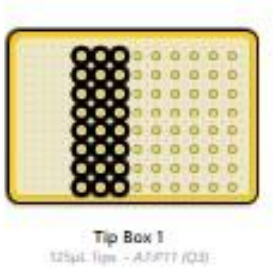
To load an ATL tip box, remove the lid and place the tip box in the position shown on the Load Tips screen.



You may load the ATL tip box directly on the tip box adapter.



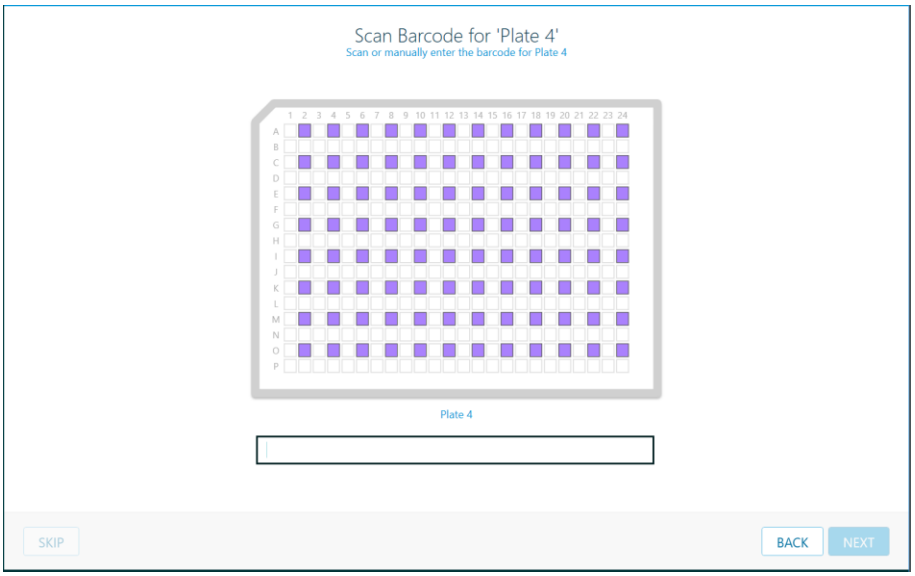
You can load a full tip box when you only need to use e.g., 3 columns, as only the correct number of columns will be loaded on the pipetting head. In the protocol step below, the columns of tips shown with heavy outlines will be loaded, the remaining tips (shown by small circles) will be left in the box.



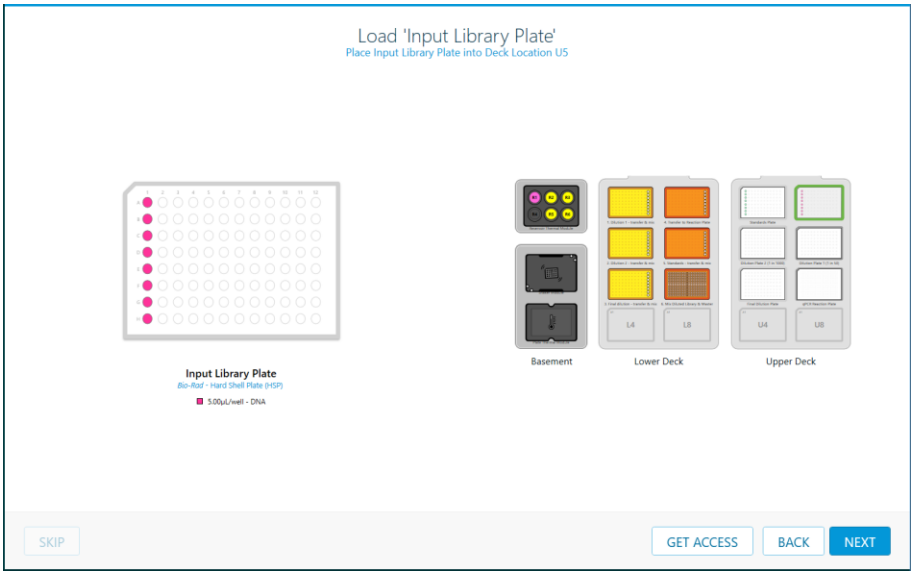
Partial boxes of tips can be used in other protocols.

Loading plates, risers, lids, thermal blocks and magnetic blocks

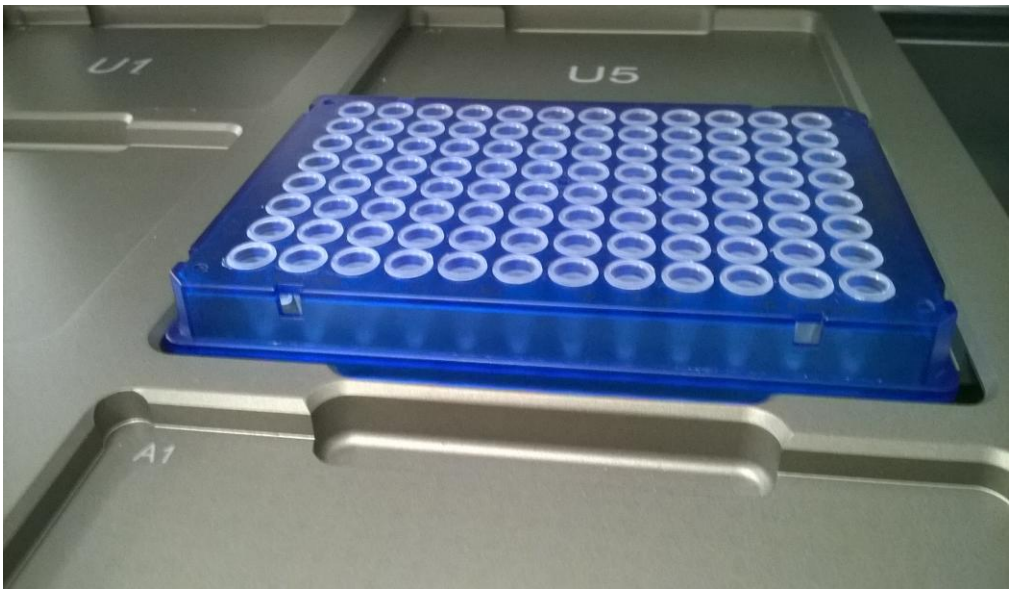
1. Scan the plate barcode if required.



2. Load plates, risers, lids, magnetic blocks or thermal blocks in the upper or lower deck positions specified in firefly setup. The deck locations are labelled.



For plates, A1 is positioned at the back left corner: this is labelled on each deck position.



Warning

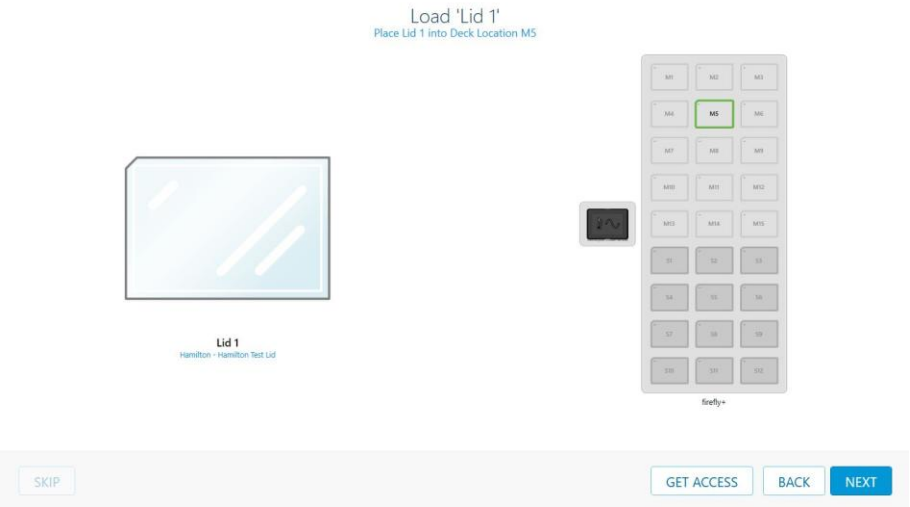
If you are loading plates containing reagents, you may wish to use lids or non-pierced seals while loading but you must:

- Remove non-pierced seals before starting protocol execution
- Manually remove lids, or include a Move or Take Off step in the protocol to remove the lids

3. Ensure your plates are seated firmly in position and flat. There is a plate clamp at the bottom right corner which engages after loading to keep the plate stationary but for it to operate correctly, the plate must be positioned correctly on the deck.

Loading firefly+

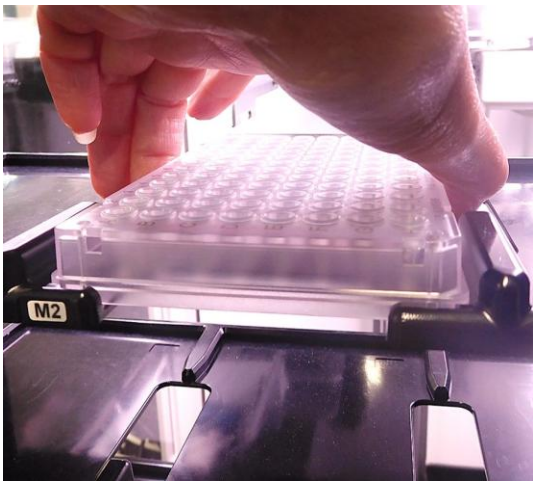
firefly software directs you to load plates and other consumables on the firefly+ shelves when loading the firefly decks.



Open the door to access firefly+ shelves.

Warning

To reduce contamination risk, do not touch the top surfaces of labware as you load the shelves. Hold labware by the sides as you load firefly+.



Important

firefly software cannot validate that the item in a shelf location is correct when running a

protocol. Be careful and check that you have placed labware in the position shown on screen.

Removing syringes

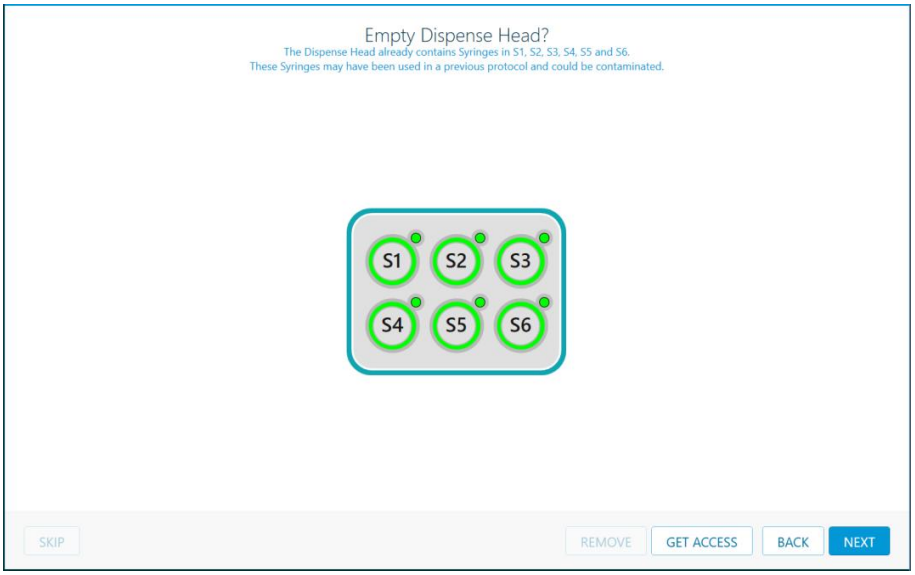
If firefly has previously run a protocol, it may have syringes loaded from that protocol. firefly software detects this and prompts you to remove them.

You will need an empty reservoir for each syringe, to purge them of any remaining liquid before removing the syringes.

However, if you are running the same protocol repeatedly, you may not need to replace the syringes to avoid contamination, in which case click 'Next' and continue, reusing the same syringes.

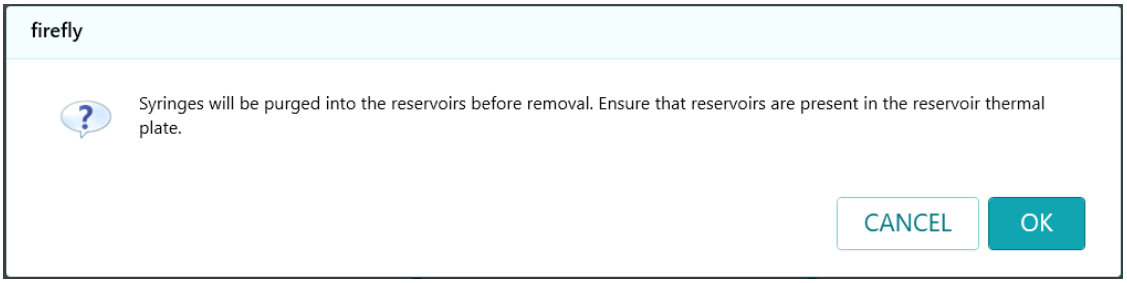
To remove syringes:

1. Use 'Get access' to safely access the dispense head, and open the doors. Load empty reservoirs for waste liquid.



2. Click the Remove button to dispense all liquid in the syringe and home the piston rods to allow safe removal of the syringe.

3. firefly shows a final popup for you to confirm that the reservoirs are ready for use in purging.



firefly then purges the syringes, ready for removal.

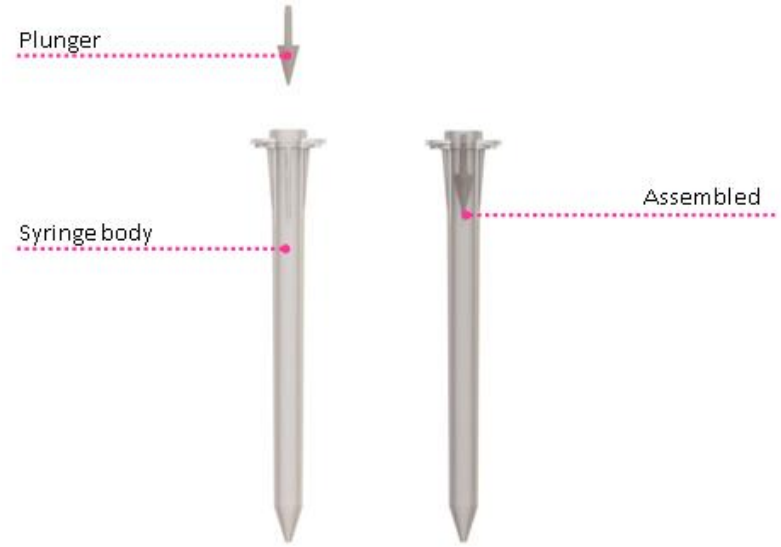
Important

Don't try and remove the syringe without using the Remove function first; if the piston rod is still engaged, you will damage your instrument

4. Remove syringes by rotating them clockwise, which will free them from the track. Remove the liquid waste. When you have removed all the syringes, the 'Remove' function will no longer be available (greyed out button).
5. Select 'Next' to continue.

Assembling syringes

Before you can load syringes, you will need to assemble them:



1. Place **one** plunger in the syringe, point down, as shown above.

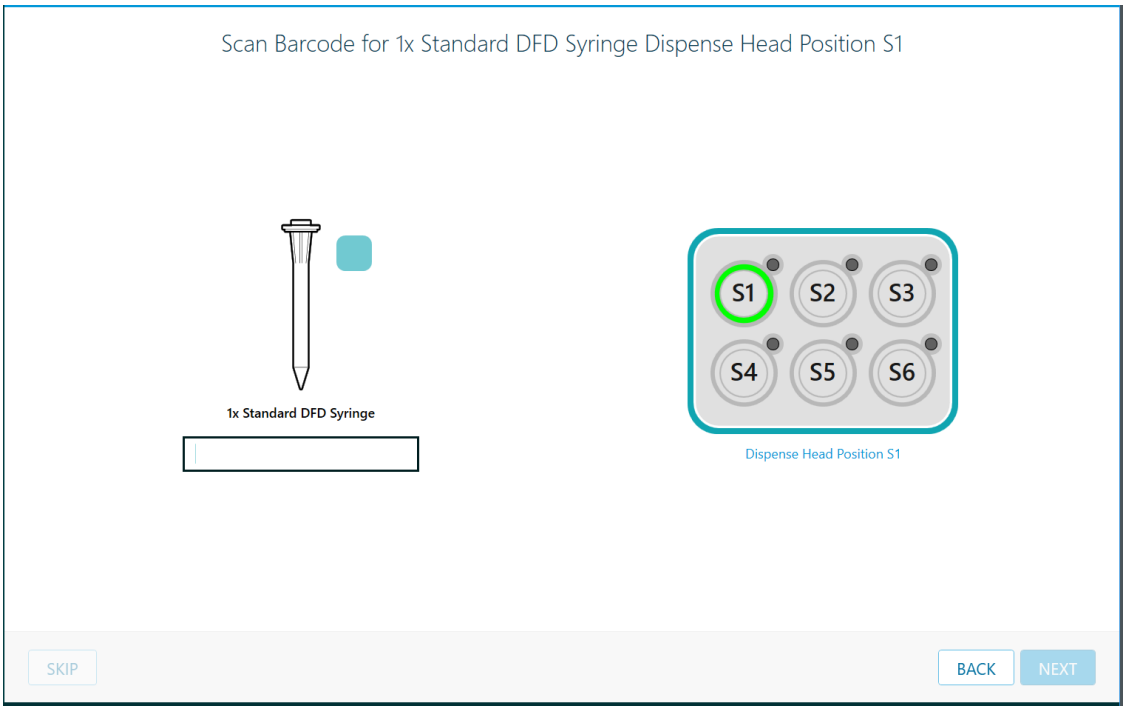
Warning

Make sure you only place one plunger in the syringe. If you insert two, you will break firefly, and need a service engineer visit to get it working again.

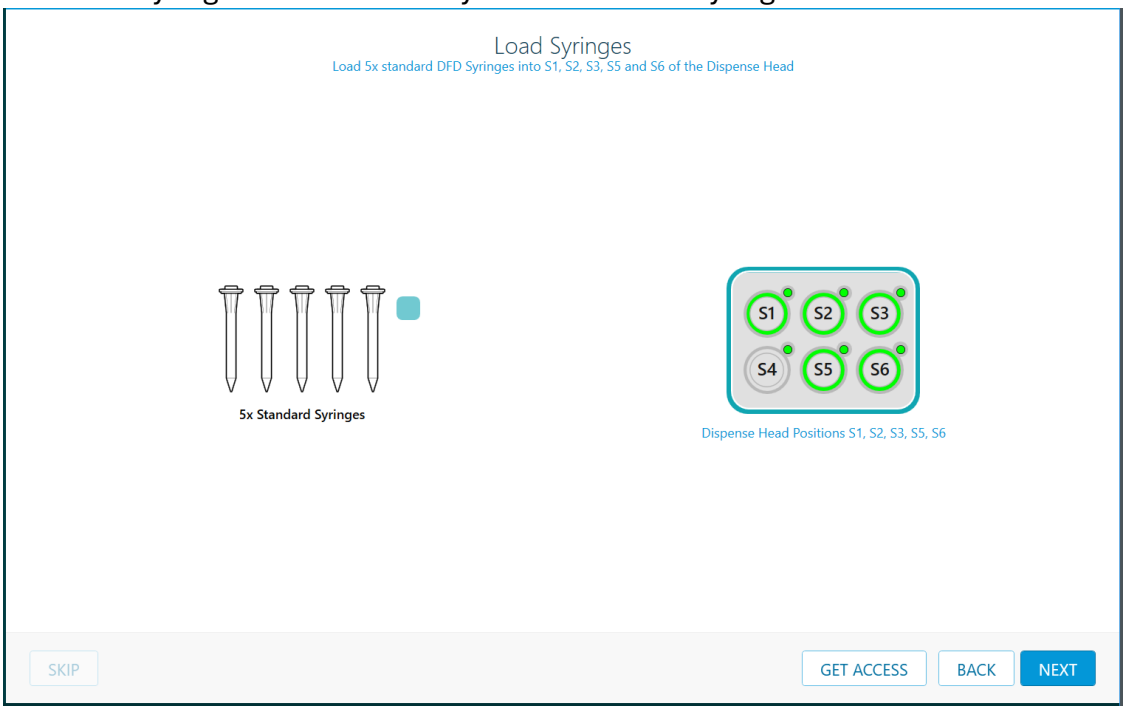
2. Gently press it far enough into the syringe that it is completely enclosed. It doesn't matter whether it's straight, or how deep in the syringe it is when you load it.

Loading syringes

Before you load a syringe, you may need to scan its barcode.



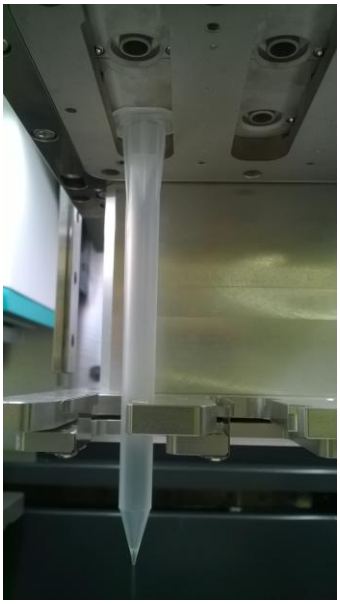
The Load Syringes screen directs you to load each syringe in turn.



1. Load syringes into the positions highlighted on the setup page. The dispense head has three tracks. 3 head instruments have one syringe in each track, 6 head instruments have two. Position S1 is the syringe furthest back and furthest left, as you look at the dispense head, circled in the illustration below.

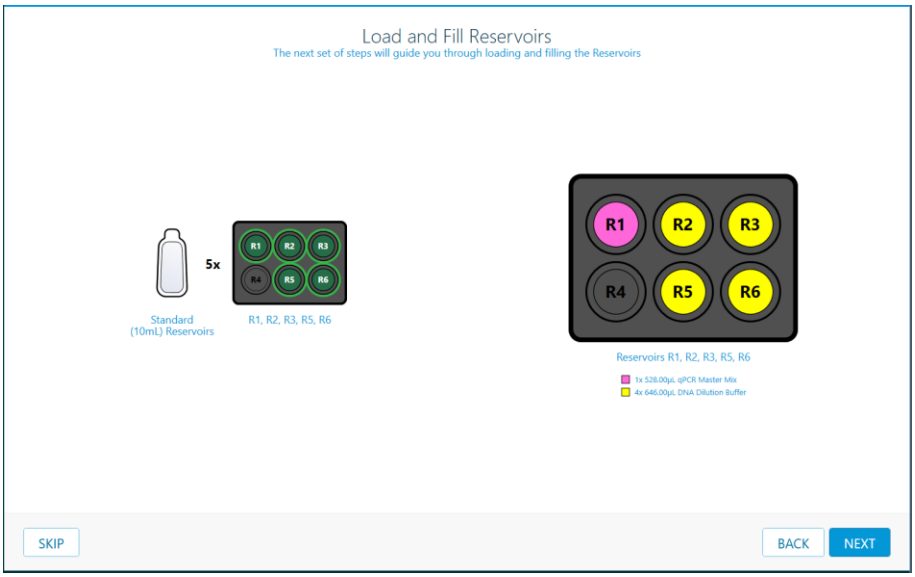
2. Slide each syringe into the correct track.
3. For a back row syringe, slide the syringe until it stops, then push upwards and rotate 90 degrees counterclockwise.

   For a front row syringe, push upwards while sliding the syringe into the track; when it pushes into a hole, then rotate counterclockwise.



Loading reagent reservoirs

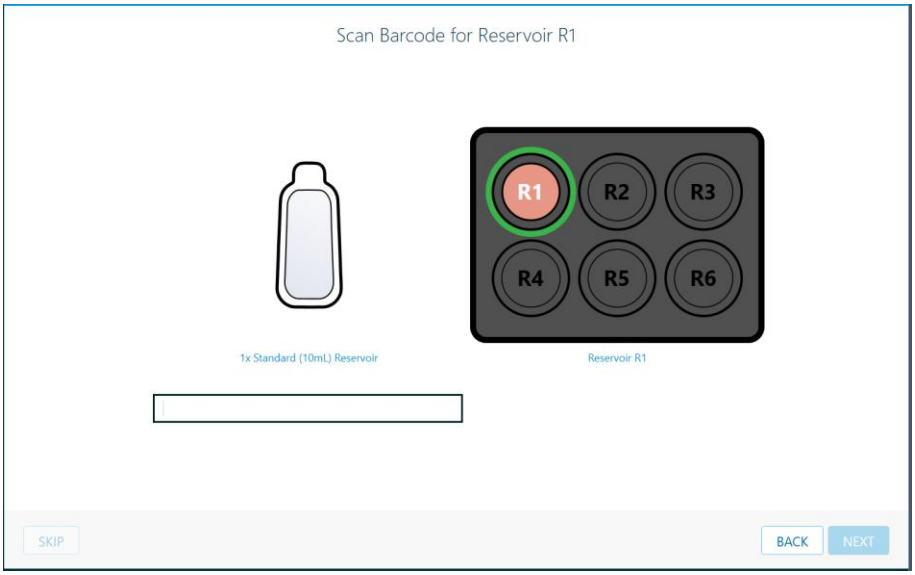
The initial Load Reservoirs screen is an overview of all reservoirs required. Only R1 - R3 are used for a 3 head instrument.



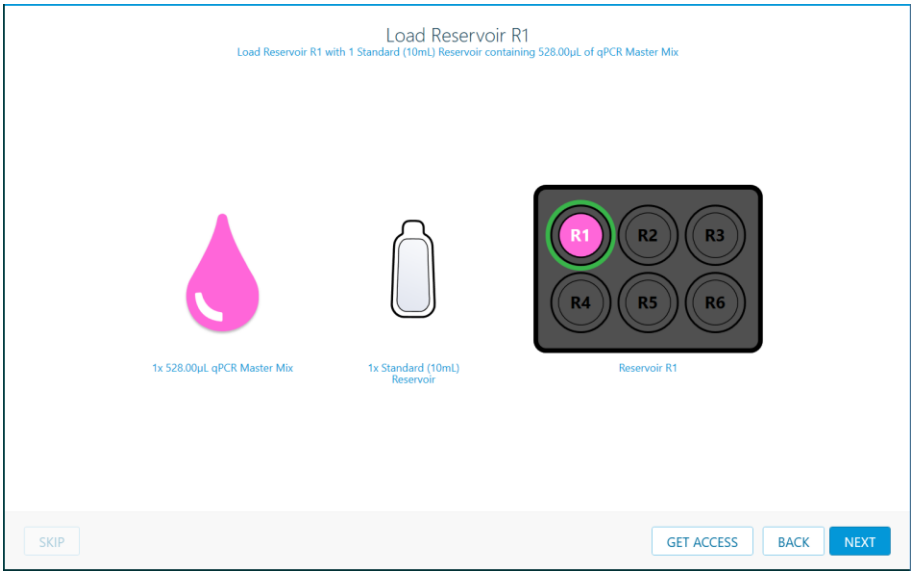
Ensure you have the correct tray for your reservoirs. If your protocol requires high volume reservoirs, you will need to use the correct tray, and the correct inserts for any standard or LDV reservoirs.

After the overview screen, you will see the instructions for each reagent and reservoir in turn.

Scan the reagent and reservoir barcodes as directed, if they are used.



The detailed instructions specify the liquid to be loaded in each reservoir. and the volume required.



To load the reservoir loading tray:

1. Remove the reservoir loading tray, if there is one loaded in the instrument.
2. Place either a high volume, a standard or a 1.5mL Low Dead Volume (LDV) reservoir in each position as specified in the loading screen. Use 'Next' to see the details of all reservoirs to be loaded.

3. Fill each reservoir according to the instructions in the wizard. Any inaccuracy in the volume that is loaded into the reservoirs could cause a problem with the dispense. To avoid issues, use a calibrated pipette and good pipetting technique when loading reservoirs with liquid. The volume includes the calculated dead volumes in the reservoir and the syringe, in addition to the volumes dispensed. 10mL reservoirs have molded 5mL and 10mL (the maximum fill) level marks as a guide.

4. If you are using high volume reservoirs, you may also be using lids to lessen evaporation. Make sure you have put the lids on the correct way round, with the hole in the lid facing the center of the tray, as shown below.

Important

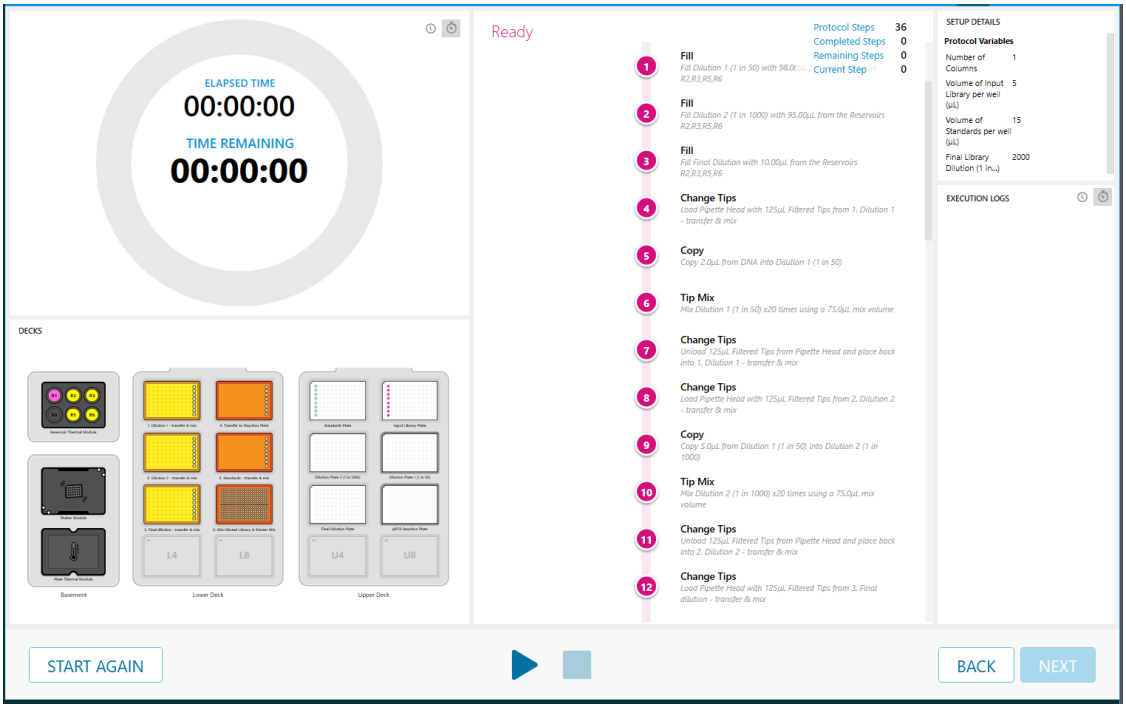
firefly syringes will crash into the reservoir lids if the holes are not correctly aligned with the tip positions.

5. Carefully move the reservoir tray, with the filled reservoirs, back to firefly.

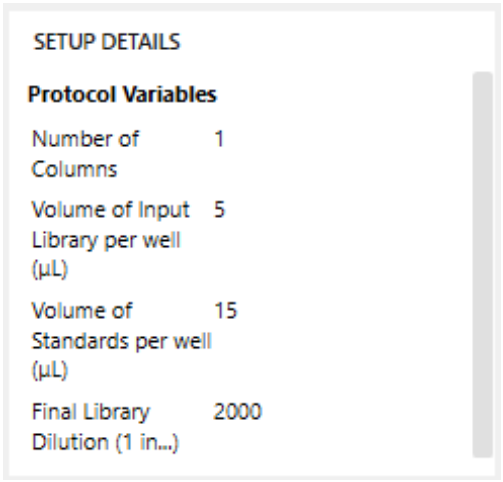
6. Use the guide slots to reposition it in the instrument. You should hear a click when the tray reaches its correct position.

Running your protocol

After checking your protocol and loading its materials, select 'Next' to run the protocol.

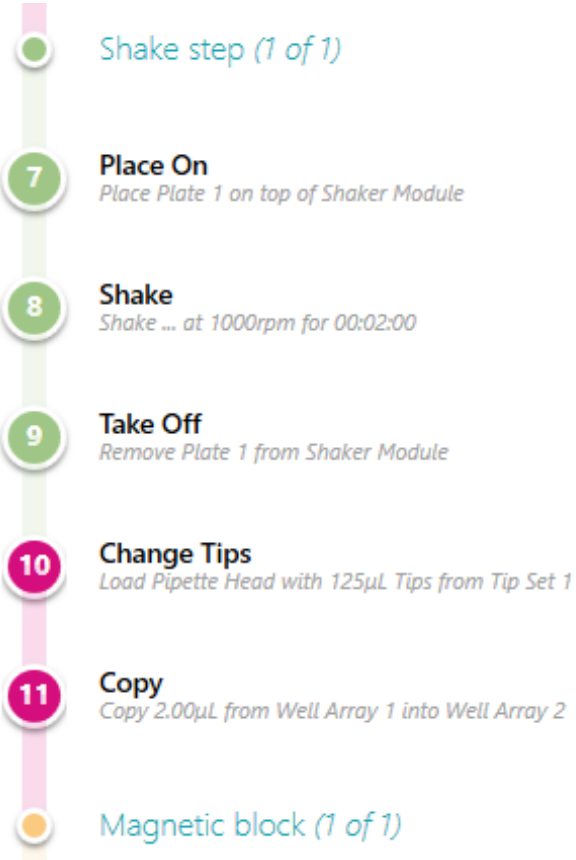


This default screen shows the setup details (top right) - these are the scanned barcodes, and the variable values used. If no variables or barcodes were used, this section is omitted.



It shows the current status ('Ready') and the steps in the currently loaded protocol. If the protocol includes Notes these will show on a yellow background. Read these before starting the protocol, as they contain guidance on the process.

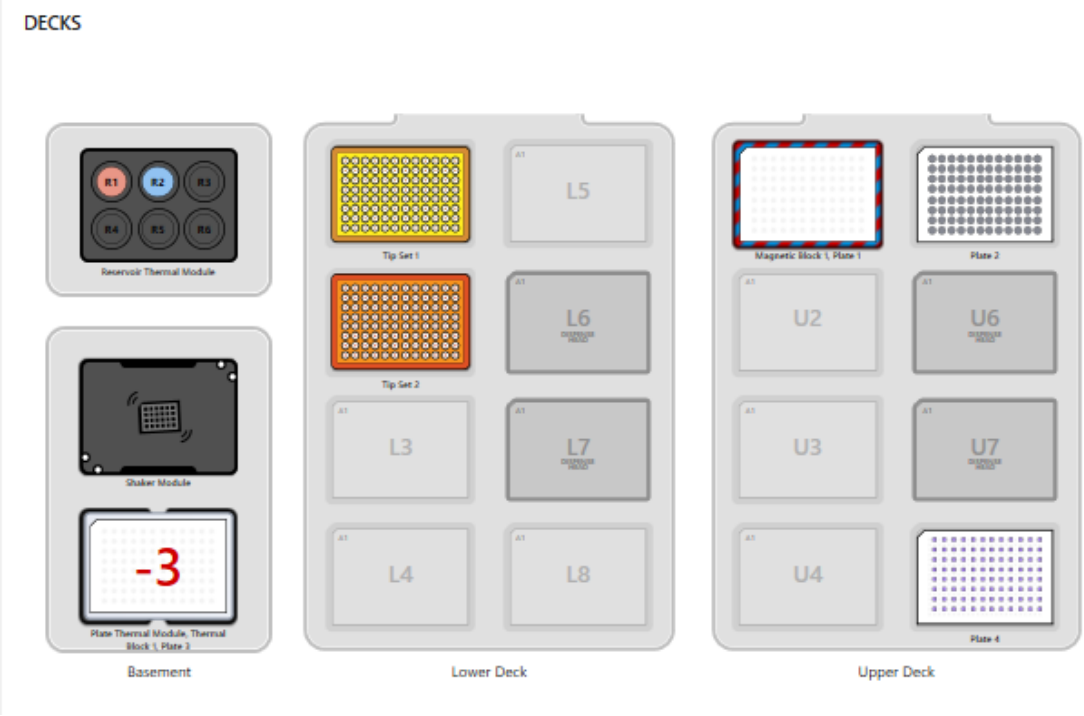
If the protocol includes Groups, they will each be shown in a different color. So, in the protocol below, there are 2 grouped steps: 'Shake step' is shown in green and 'Magnetic block' in orange. If these steps are repeated later in the protocol, the same colors will be used for easy reference.



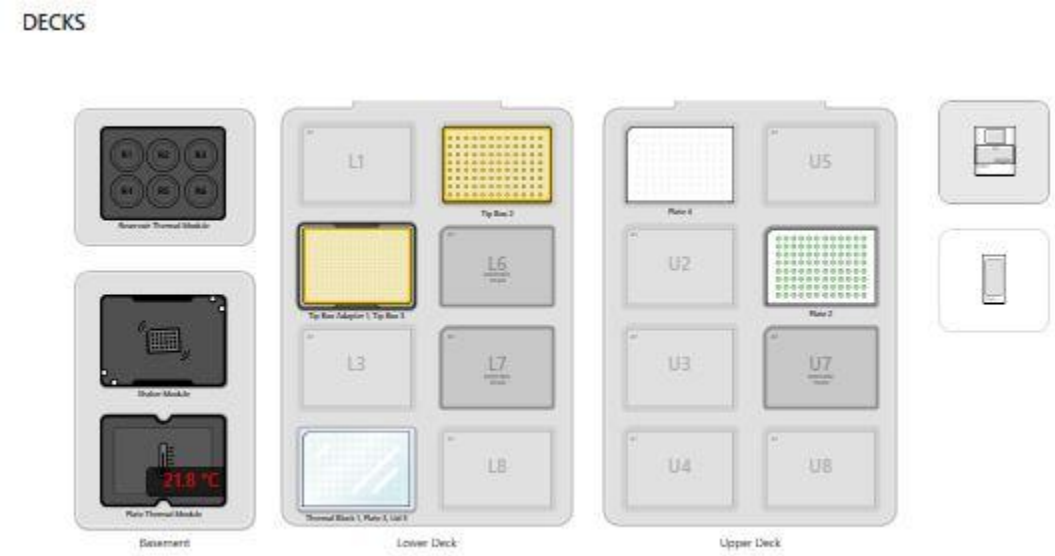
Select the Start button to run the protocol.

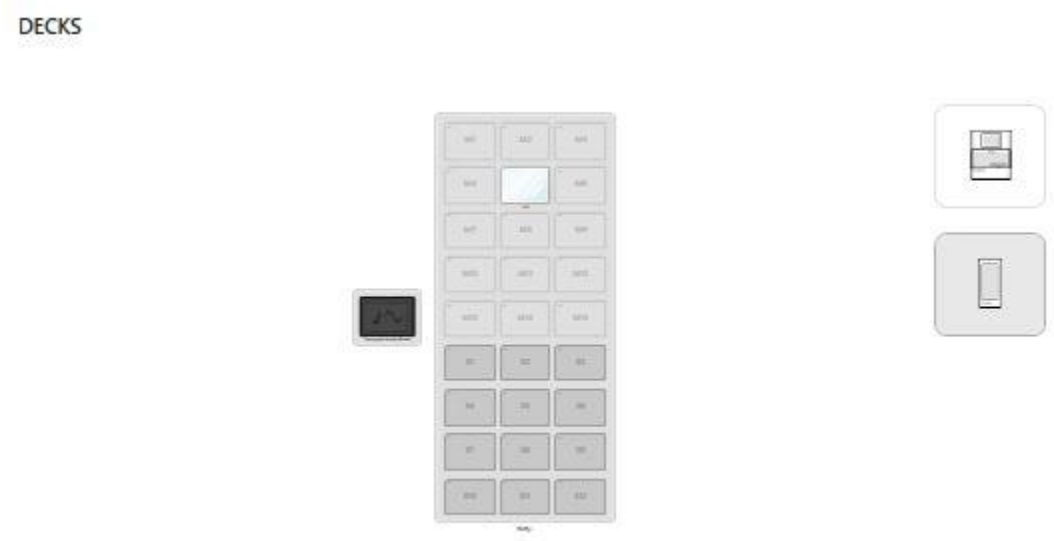
The screen will be updated with the elapsed time and the overall time remaining.

If you are using the thermal modules, the current temperature is shown.



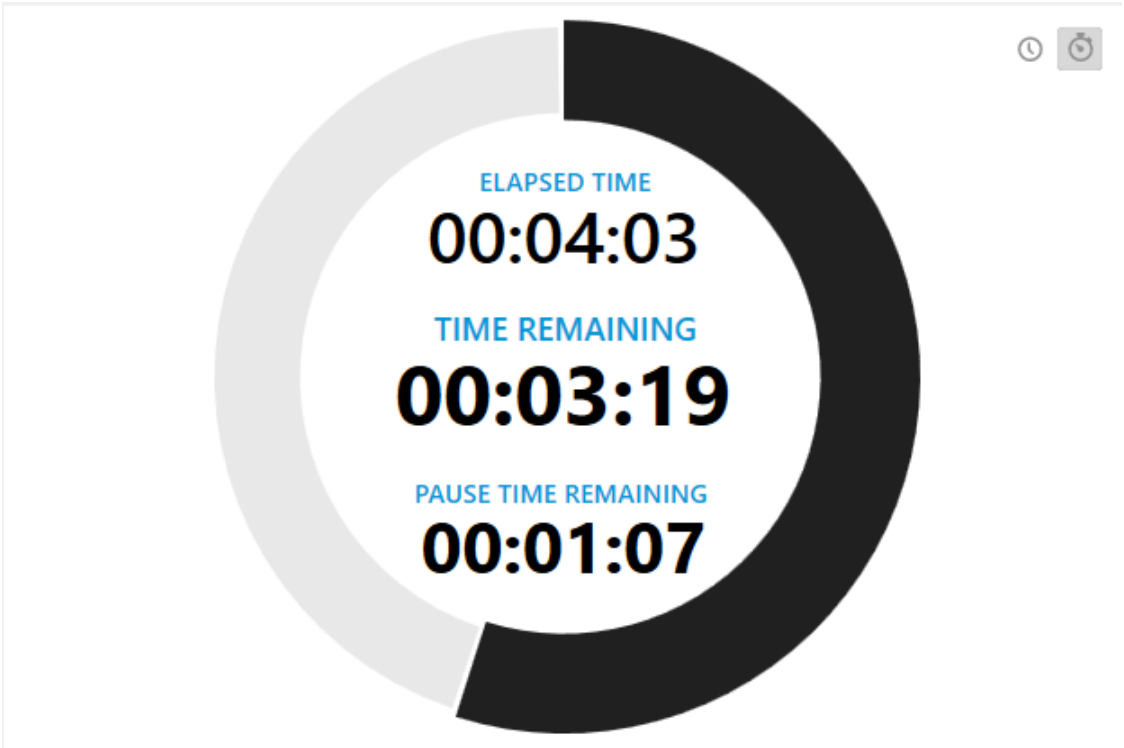
If you are using firefly+ you will see this view of the decks. Use the small icons on the right to toggle between the views.



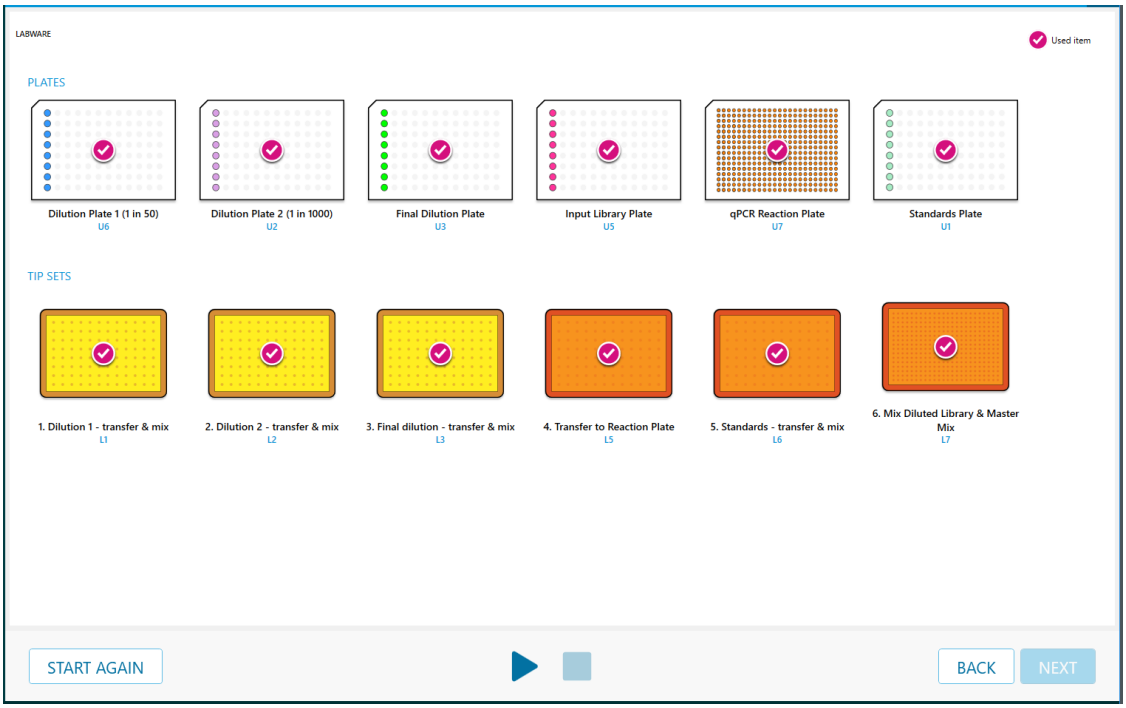


It will also start to generate the execution log: you can select this to display elapsed time (stopwatch) or actual time (clock).

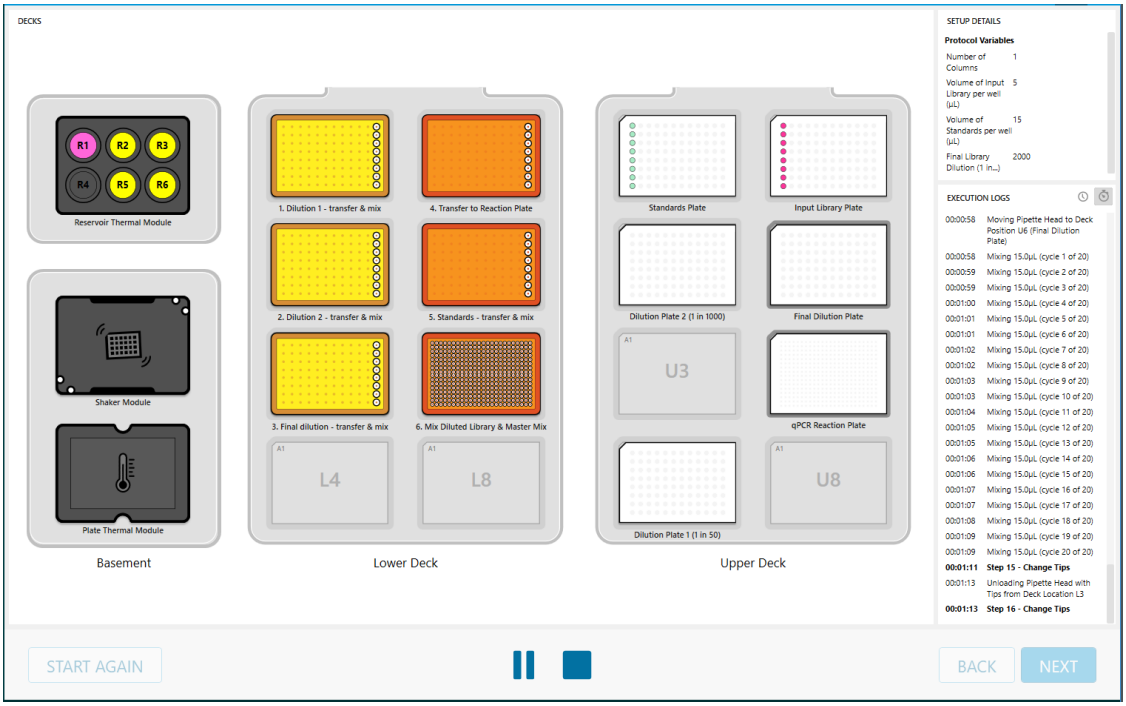
If your protocol includes a pause, the pause time remaining will be shown with the overall time remaining. You can select to display progress as a circle (shown below) or as a bar. User interaction steps are shown by pink lines.



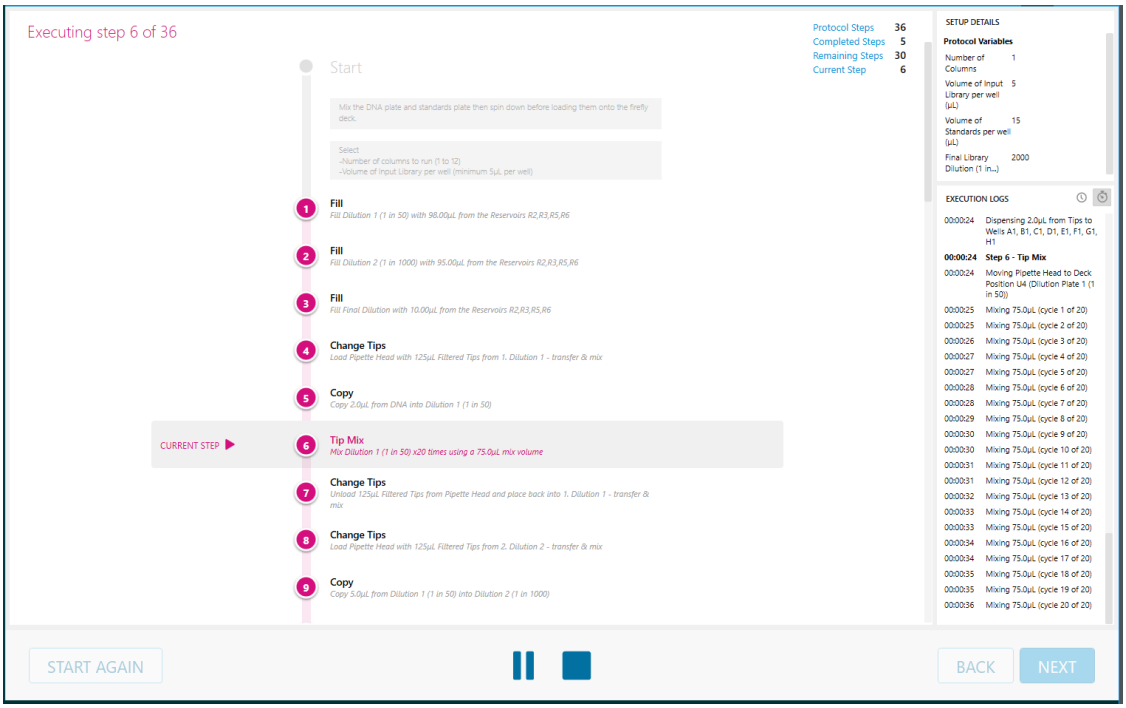
While your protocol is running, you can select the view you find most useful, e.g., the labware usage



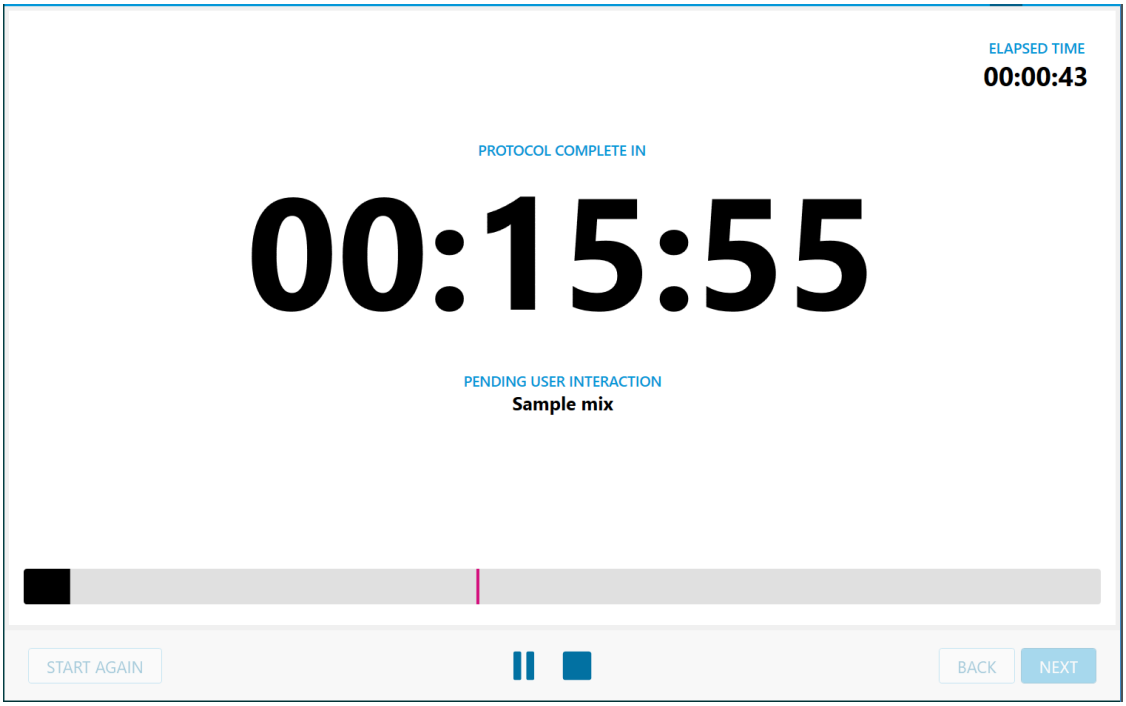
or the decks:



Alternately, you may prefer a simple step by step view, to monitor progress.



You can also select to display the time remaining to completion; useful if you are monitoring several instruments at once. This view includes a notification of the next user interaction, so you can prepare for it if necessary. Pink lines on the black progress bar indicate where user interactions take place.



If firefly cannot complete the protocol, you will get feedback on the step which failed - shown as red text in the Execution Logs.

You can pause a protocol at any stage, using the 'Pause' button on the Execute screen.

If you need to end a protocol for non-urgent reasons e.g., you become aware of a problem with the reagents, use the 'Stop' button on the Execute screen.

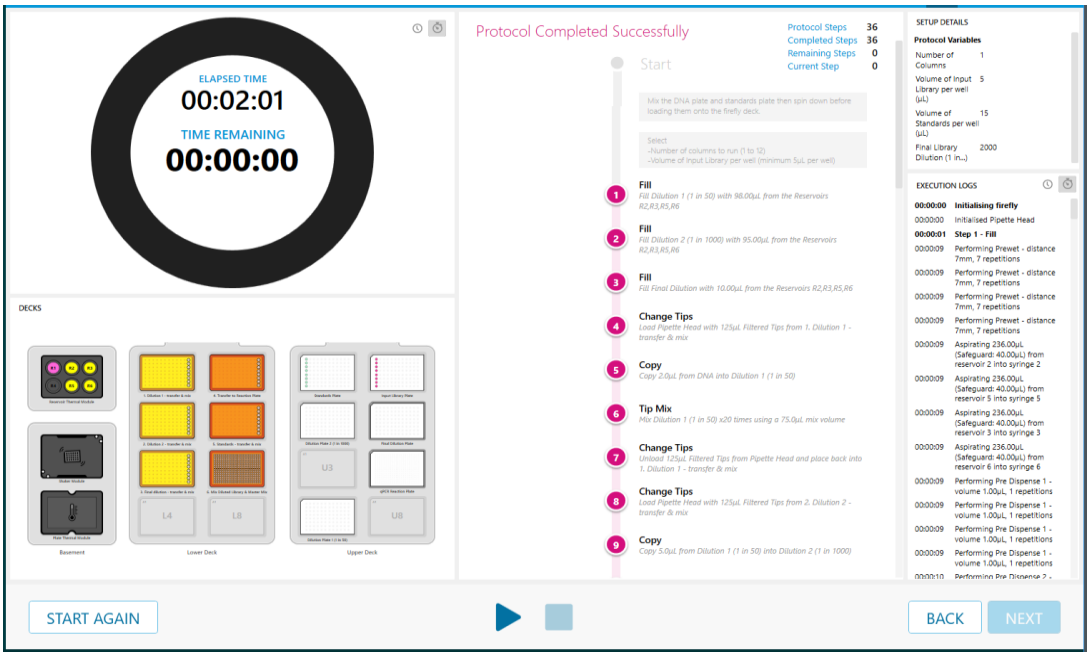
Important

Stopping the firefly protocol will not stop the ODTC running, if there is an incubation step in the protocol. You will need to use the manual controls to stop the ODTC protocol.

Important

Use the emergency stop if you need to stop firefly instantly for safety reasons

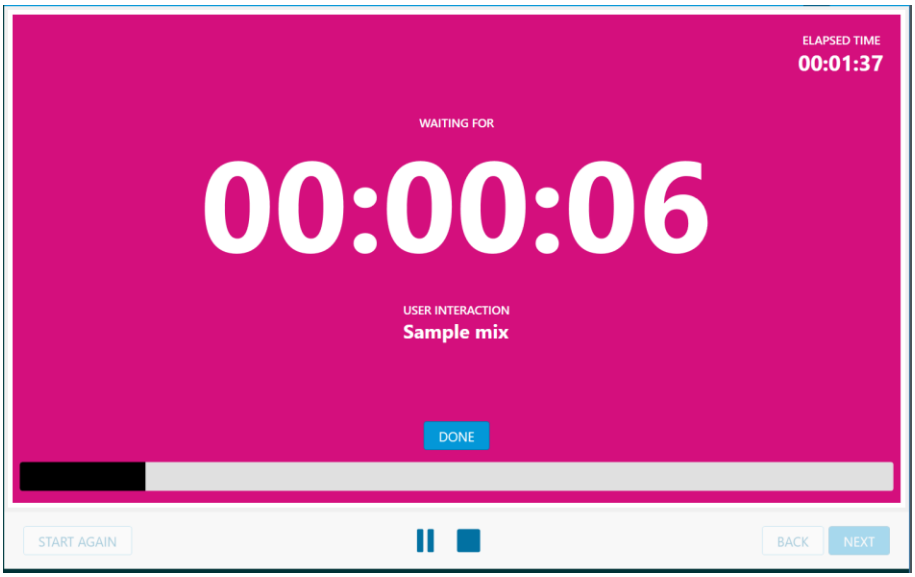
firefly will chime when the protocol is finished. Ensure that the volume on the tablet is turned up in order to hear this chime.



Use 'Start again' to restart the same protocol.

Carrying out user interactions

firefly shows this screen when a user interaction is needed.



Once you see this screen you can access firefly and carry out the user interaction instruction. At this point, the heads and decks may have been moved to give you safe access to the area you require e.g., to the upper deck to remove a plate. However this is not always practical i.e., if the head must remain in a plate, so be careful when you access firefly.

The black progress bar indicates overall progress executing the protocol. Pink lines on the bar indicate future user interactions.

Important

Do not click 'Done' on this form immediately: complete the required actions first. Clicking 'Done' dismisses the form and continues the protocol.

Open firefly's door and complete the user action. Close the door before clicking 'Done' on the user interaction pop up form. The door indicator will be blue if the door is properly closed.

If you click 'Done' before the door is properly closed, the protocol execution will stop.

If the user action involves instruments other than firefly, complete the specified task before clicking 'Done' as the firefly software will continue the protocol at that point, expecting all previous steps to be complete.

Unloading firefly

At the end of a protocol, you will need to clear all labware and consumables from firefly, ready for the next user. If you are not running another protocol immediately, you should shut down firefly.

On opening the door, the decks can be moved freely and all labware and consumables can be accessed and removed for disposal or storage according to your SOPs. Syringes can be left on the firefly and used again in the next run or replaced with new syringes if required during the set up and loading of the next protocol.

If your instrument is fitted with a vertical laminar flow module, leave this running for a few moments after you have emptied the instrument, to ensure that it is flushed free of aerosols.

Tip

You can switch off firefly immediately after running the protocol, and leave the laminar flow module running. Remember to switch it off when it has flushed the instrument.

Unloading firefly+

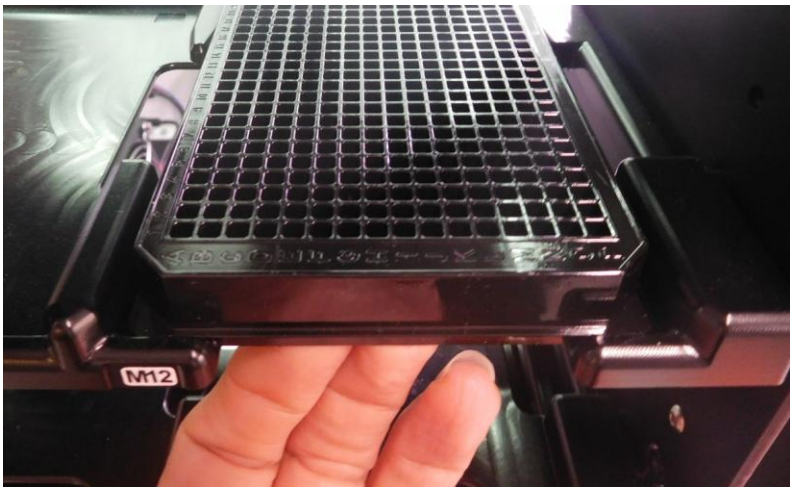
Open the door to remove labware and consumables from firefly+.

Warning

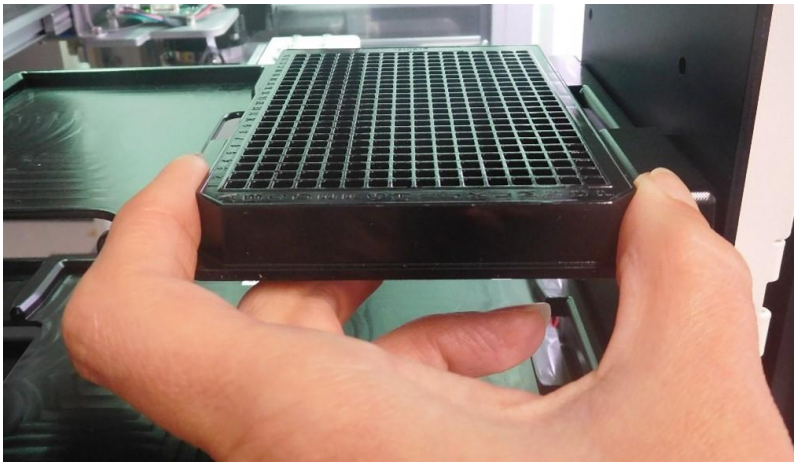
To reduce contamination risk, do not touch the top surfaces of labware as you unload the shelves.

Use the notches in the shelves to assist with removing labware.

- With a finger, use the notch to hook the labware from underneath to lift it and bring it forward onto the front lip of the shelf.



- Then pick up the labware with your index finger and thumb holding the left and right sides.



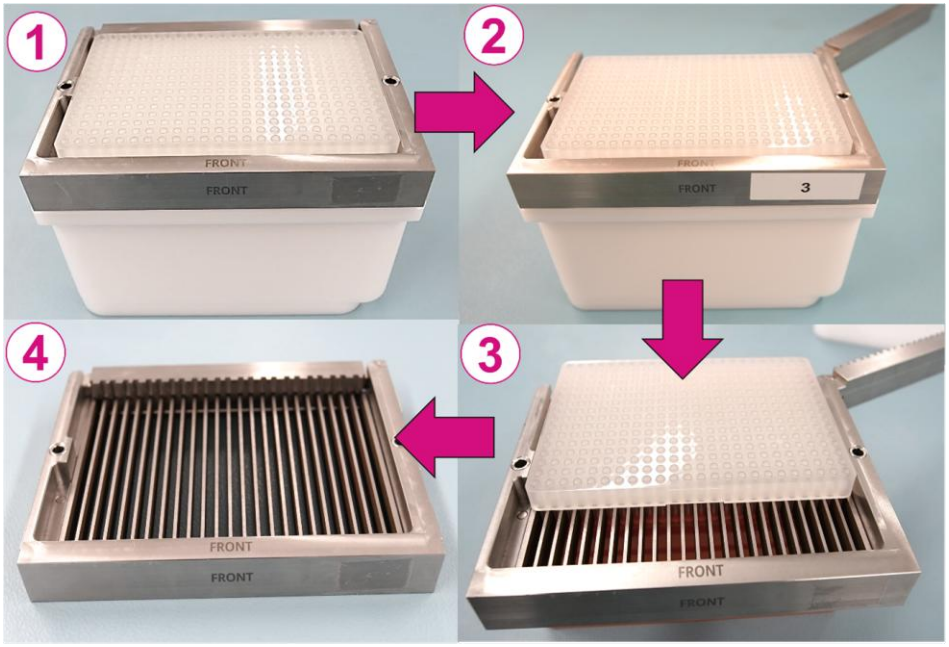
Disassembling EZL tip sets

Unload the full cassette from the tip stand, being careful of the used tips, and open the hinged gate.

Slide the tip array or strip tips out of the cassette from the open side. It is important to reduce contact of the tips with the cassette at this point, to keep it clean. Tip cassettes are considered as part of the pipetting head mechanism and are not expected to come in contact with working surfaces of the tips or samples.

Important

Cleaning tip cassettes with cleaning solutions is not recommended, and should not be required. If in exceptional circumstances it is unavoidable, follow these instructions carefully.



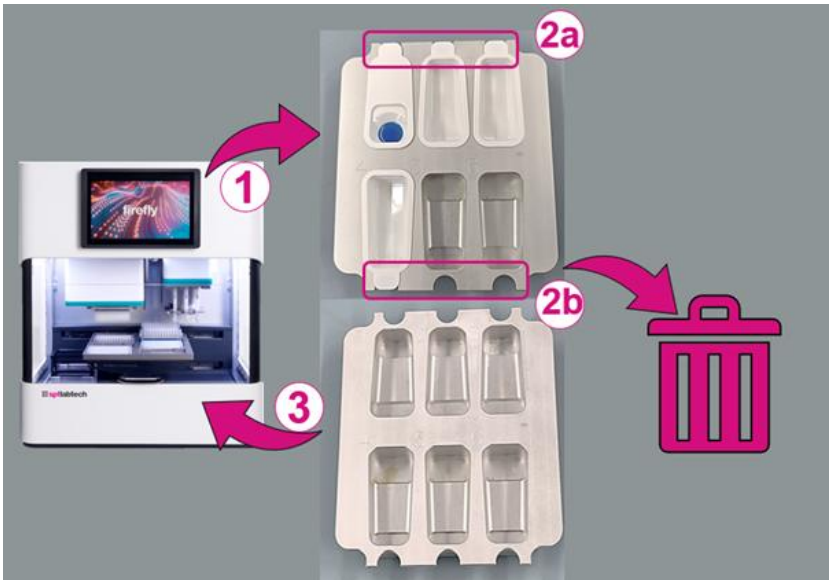
Dispose of used tips appropriately.

Close the gate of the cassette.

Clean the tip stand.

Unloading reservoirs

This is shown with the standard reservoir tray, but the process is the same for the high volume tray.



1. Remove the reservoir tray from the firefly and set it on a flat benchtop.
2. Unload all reservoirs and empty them of the dead volumes of reagent, according to your SOPs.

   a. There is a small indentation to make it easy to pick up the reservoirs.

   b. Ensure reservoirs are disposed of appropriately, or wash any which you are going to reuse.

3. Clean the reservoir tray and inserts if you have spilled any reagents, then replace it in the instrument.

Shutting down firefly

As part of SPT Labtech's ongoing sustainability efforts, we encourage all users to switch off firefly when not in use. This simple step helps lower energy consumption and contributes to collective carbon-reduction goals. firefly is designed for safe and efficient shutdown.

To shut down firefly, you must use the Shutdown function on the Manual Controls screen.

Caution

Always use Shutdown in software if you want to switch off firefly.

Do not switch off the instrument when the software is running.

After shutting down the software, switch off firefly using the switch on the side.

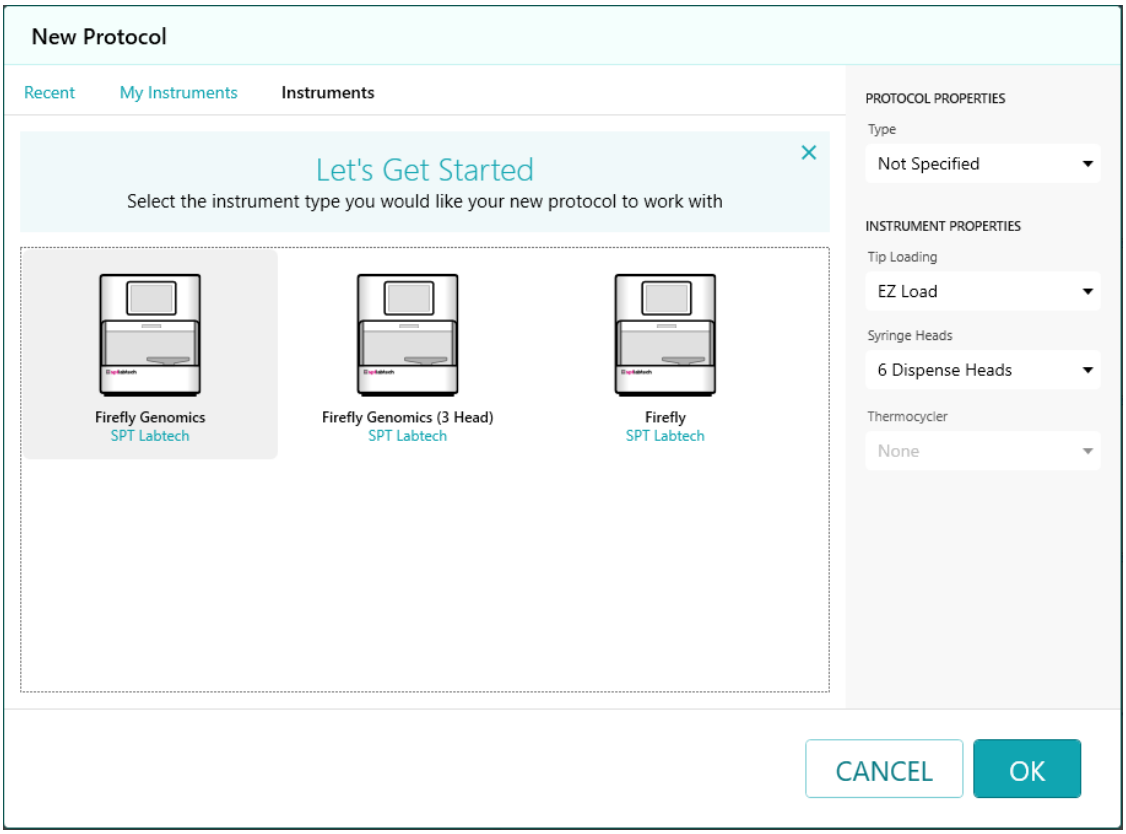
Tip

Always switch off firefly when it is not in use, following the shutdown instructions.

firefly is designed for this mode of use; it does not need to be kept powered.

Software overview: superuser

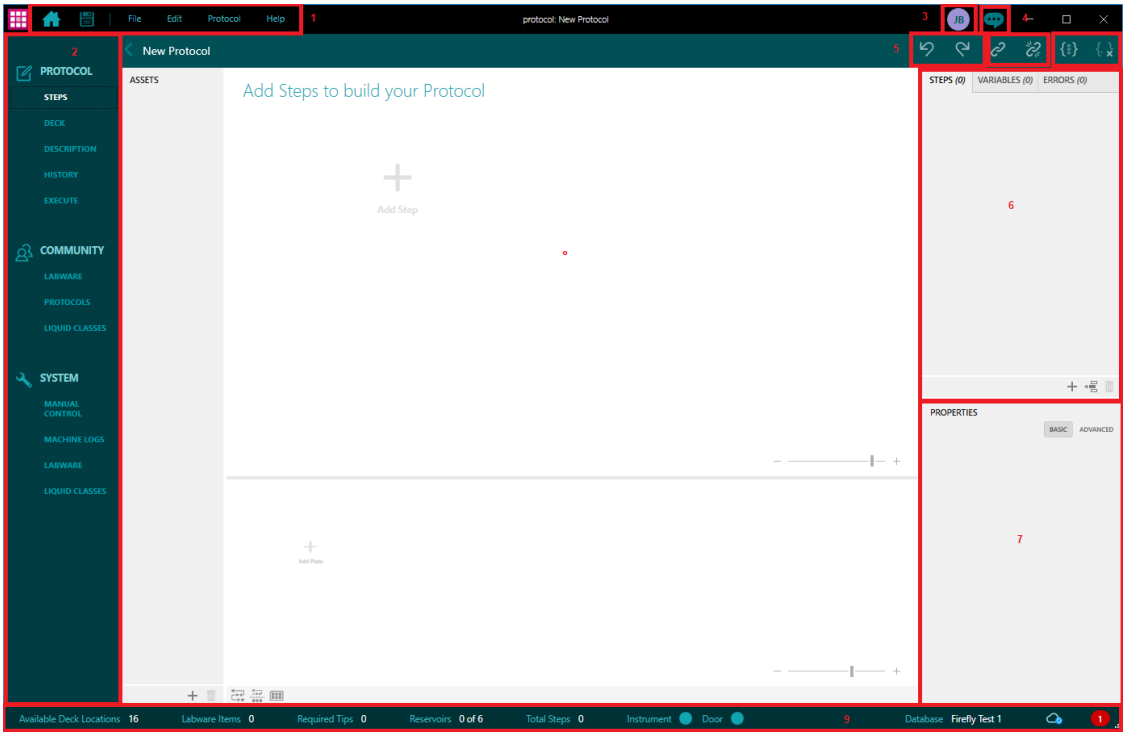
If you select to design a protocol, you will see a pop-up asking you to select the instrument type for the protocol to run on.



Select standard or Genomics firefly and the number of syringe heads; the correct firefly layout will then be shown in the protocol development tools. You do not have to specify a type.

If you have a simulated instrument running, its type will be shown with a green tick. You can design for any other firefly specification, but you will not be able to run your protocol in simulation without changing the simulated instrument.

This is firefly's opening screen, if you selected to design a protocol.



The numbered areas are:

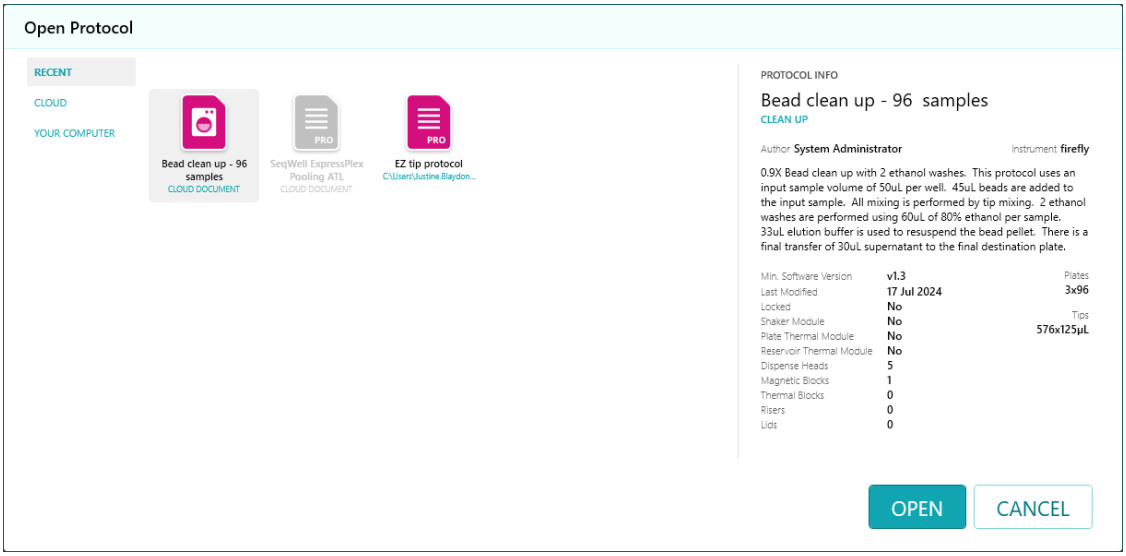
1. is the Menu bar, to open or edit protocols, which is always available
2. is the Navigation pane, giving access to all firefly functions, which is always available
3. shows the logged in user; right click this control to edit your profile e.g., change your password
4. is the control for the firefly feedback hub
5. these are the controls which are specific to the design view: from left to right they are Undo/Redo, Link and Group
6. is the overview area, with tabs for steps, errors and variables. It is specific to the design view.
7. is the properties panel, which is specific to the design view
8. is the main design area

9. is the Status bar, which shows you the state of the instrument, and its current activity

Running a protocol - super user

Selecting a protocol

Use the 'File' menu, 'Open', 'Open recent' or 'Open from your computer' if you already have the protocol you wish to run. If you have cloud access and use 'Open', this will show you all protocols shared within your organization.

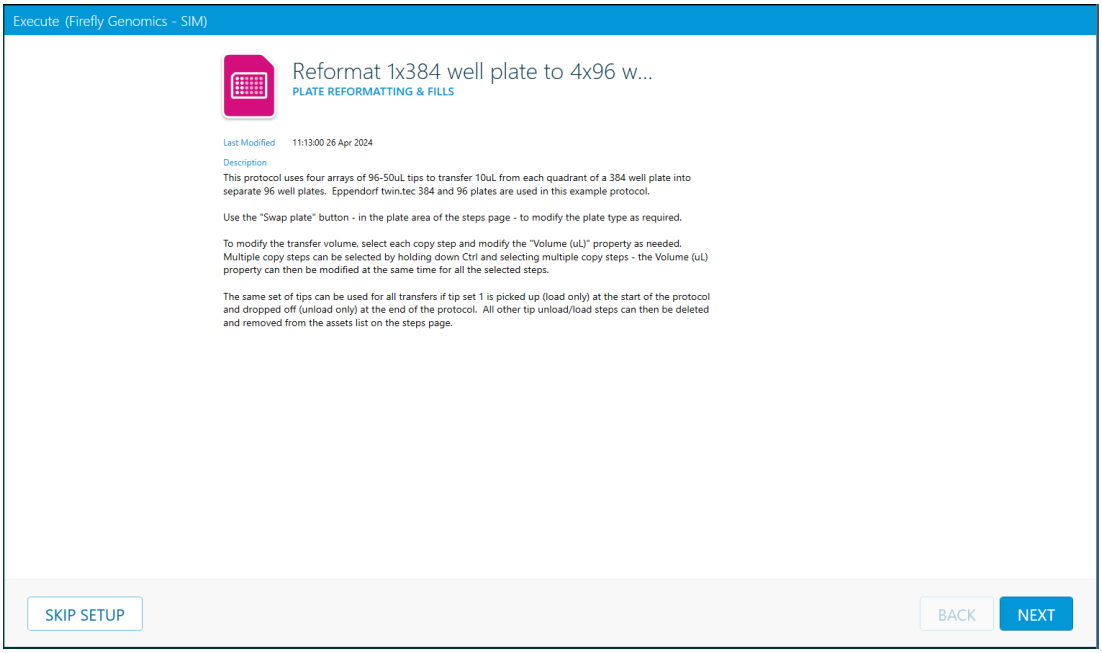


If you do not have the protocol you need, select 'Protocols' from 'Community' to view protocols available for download.

Reviewing a protocol

Use the Description screen to review the designer's description. If you have selected to run a protocol, this is the opening screen.

If you are running a protocol on a simulated instrument, the top bar is blue, on an actual instrument it is green.



If the protocol requires you to set variables, they will be shown here.

Select 'Next' to see the labware and reagents required, or 'Skip Setup' if you are experienced running the protocol and do not need the set up guide. If you select it, you will need to enter your passcode, to show you have permission to use it (see Using Skip Setup if you do not have this permission yourself).

Checking required materials

To check the labware and reagents required, use the 'Required Labware and Consumables' screen (you may need to scroll down to see everything). You can update the choice of reservoirs, syringes or tips e.g., if you are experimenting with modifications to a protocol.

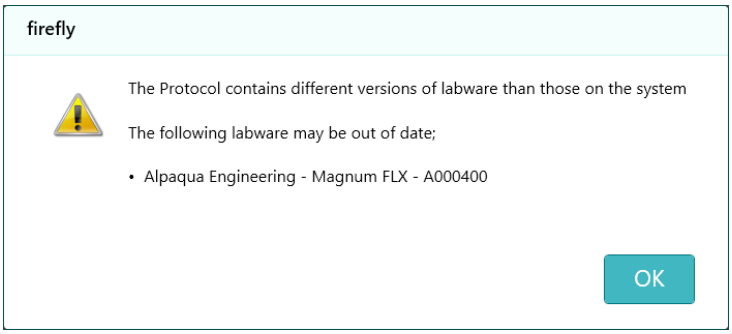
> **Note**
>
> This will not show you the materials and equipment you will need for the steps which are not performed on firefly.

Before you start you should review the SOP for the protocol to see what additional equipment you will need, particularly as you may need to have other equipment ready for immediate transfer of plates e.g., for PCR.

Differing labware versions

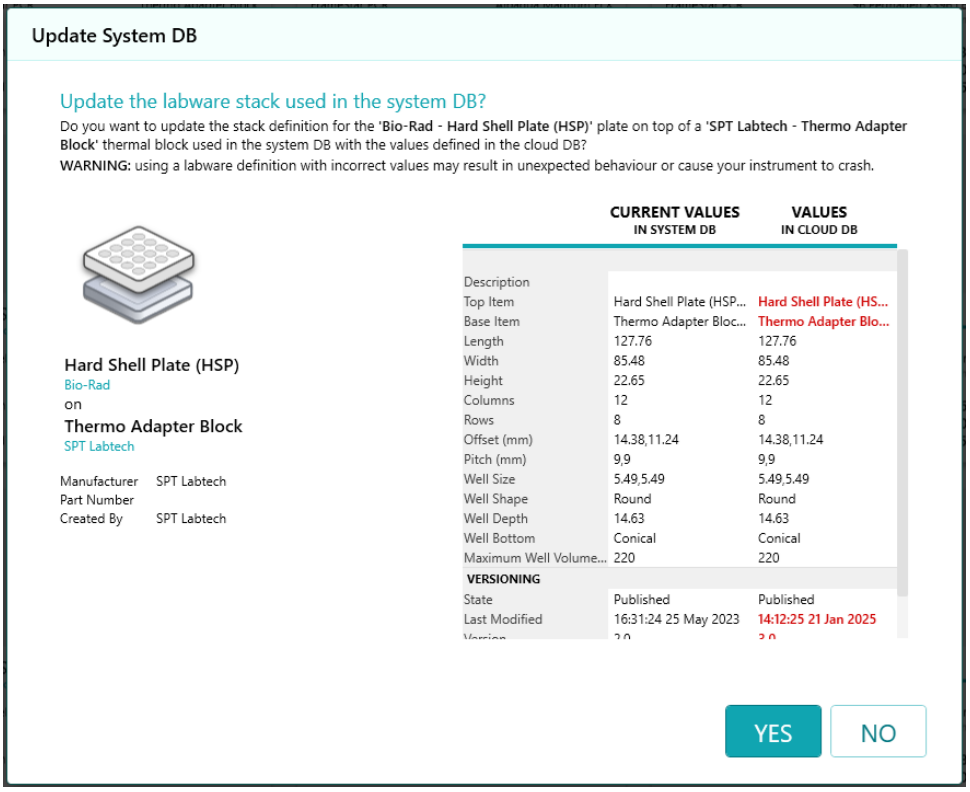
If you open a protocol which was created a while back, you may be alerted that the labware definitions used are out of date.

Similarly, if you download a protocol which has newer labware definitions than those in your local database, you will see a warning that your local labware definitions need updating.



Clicking 'OK' does not update your definitions so you do not need to do anything if your protocol has been running with no problems and you are using existing labware. The protocol will still run.

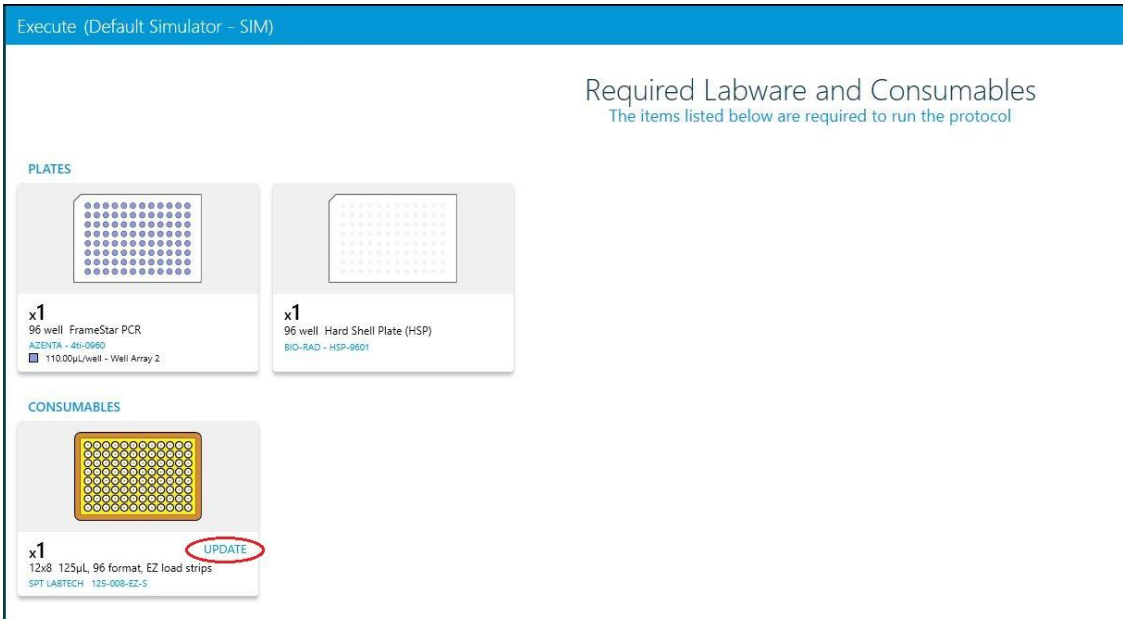
However, if you have new labware you may wish to update to the latest definitions which will be dimensionally accurate if there have been changes. firefly shows you the differences between the labware versions, to assist you in choosing whether to update.



firefly setup

firefly software includes set up guides for loading labware and consumables to run a protocol. The 'Skip' option allows you to load firefly without using the guide, simply using this view as an instruction, if you are an experienced user. You can also use 'Skip' to bypass any steps which are not required, which is useful when testing a newly designed protocol with a simulated instrument.

1. Ensure you have all your items ready e.g., cold plates are chilled, syringes are assembled, before starting to load firefly. If you have permission set, you can revise the labware to be used. On the 'Required Labware and Consumables' screen, you will see 'update' if you can revise that specific consumable e.g., to replace 12 tip strips with a full tip set or to use a different reservoir type. Note that you cannot swap plate types, or any labware which is not labelled 'update'.



2. Select 'Next' to start the set up guide. If firefly has already run a protocol, it starts by verifying that the instrument is empty of labware.

   If either of the thermal modules are required, the next step shows the appropriate module(s), click 'Set Temperature' to begin heating or cooling.

   If the dispense head is required, it will then prompt you to remove any used syringes.

   Each subsequent step shows a specific operation and has options for 'Skip', Get Access', 'Back' and 'Next'. Buttons are greyed out when an option is not available.

3. Follow the on-screen instructions for loading each item and the standard user instructions for physical loading.

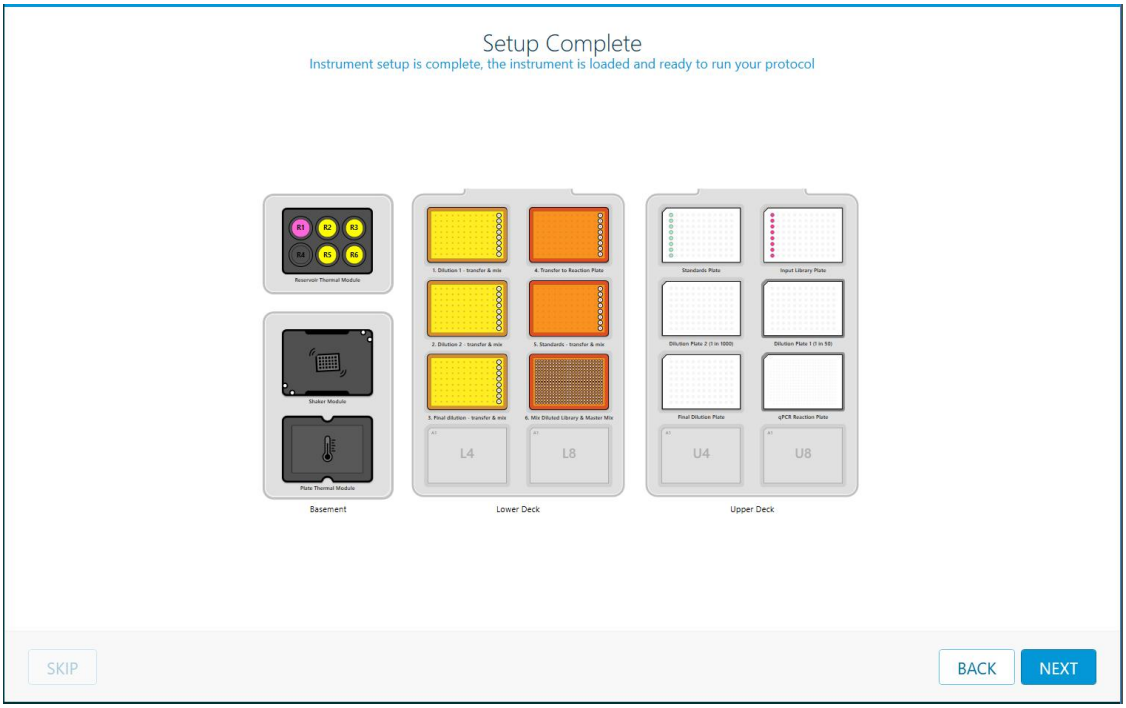
   1. You may need to scan barcodes for plates, tip-boxes, reservoirs, reagents or syringes before you load them.
   2. Select 'Get Access', unless you are loading syringes in which case follow the loading instructions.
   3. Open the door.
   4. Load your materials (see specific instructions below).

Important

Be sure to put each item on the deck location specified. firefly cannot validate that you have done this correctly so you must follow the instructions exactly or your protocol will fail.

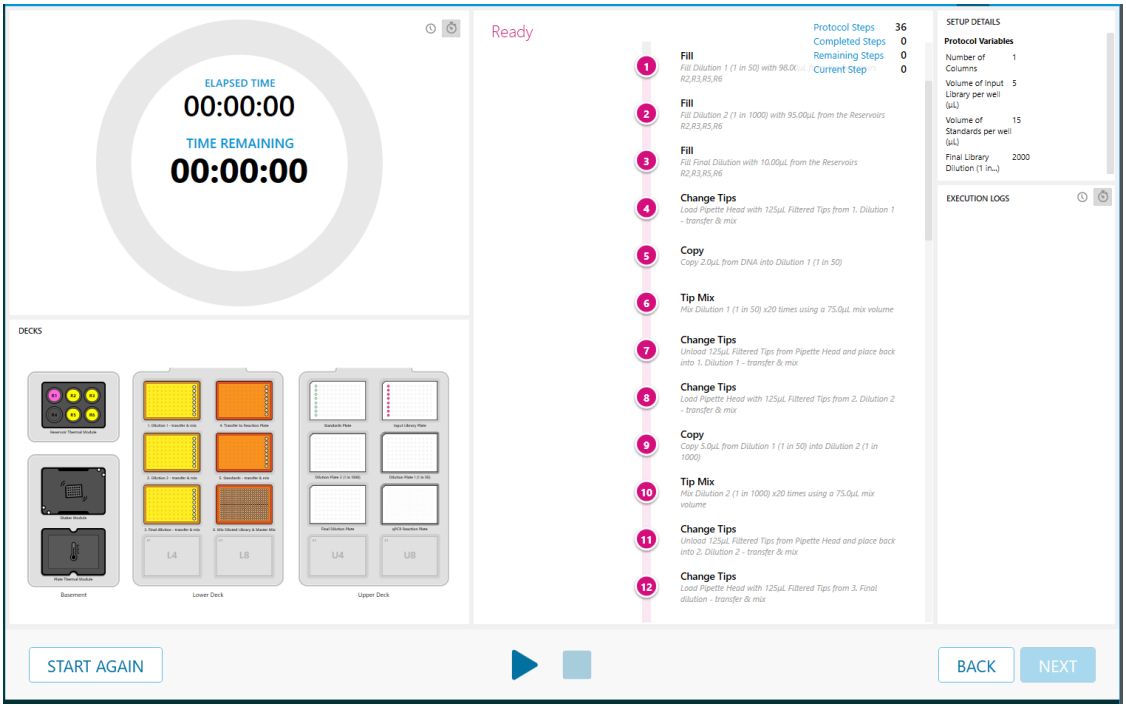
4. Close the door.
5. Click 'Next' on the wizard, or 'Back' if you are loading materials in a different order to that suggested and repeat the loading process for the next item.

When all materials and consumables are loaded, the wizard will show 'Setup complete'.



Execute

After checking your protocol and loading its materials, select 'Next' to run the protocol.



See the standard user instructions for a detailed description of this screen, and the options available. Follow the instructions for carrying out user interactions.

Select the Start button to run the protocol.

If firefly cannot complete the protocol, you will get feedback on the step which failed - shown as red text in the Execution Logs.

You can pause a protocol at any stage, using the 'Pause' button on the Execute screen.

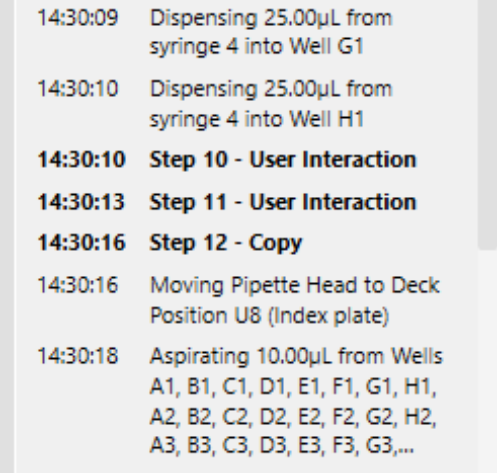
If you need to end a protocol for non-urgent reasons e.g., you become aware of a problem with the reagents, use the 'Stop' button on the Execute screen.

> **Important**
>
> Use the emergency stop if you need to stop firefly instantly for safety reasons.

firefly will chime when the protocol is finished. Ensure that the volume on the tablet is turned up in order to hear this chime.

Execution Log

While a protocol is running, firefly generates the Execution Log.



If you want a copy of the execution log for your records, use the Execution History Report function, which creates a PDF of the execution log.

Unloading firefly

At the end of a protocol, you will need to clear all labware and consumables from firefly, ready for the next user.

On opening the door, the decks can be moved freely and all labware and consumables can be accessed and removed for disposal or storage according to your SOPs. Syringes can be left on the firefly and used again in the next run or replaced with new syringes if required during the set up and loading of the next protocol. You can also access manual controls to remove the syringes at any time.

Designing protocols

Editing a protocol

It is often quicker and easier to edit a downloaded protocol than to design one from scratch. Downloaded protocols are not locked, so you can change labware and reagents, add, re-order or remove steps to meet your process requirements.

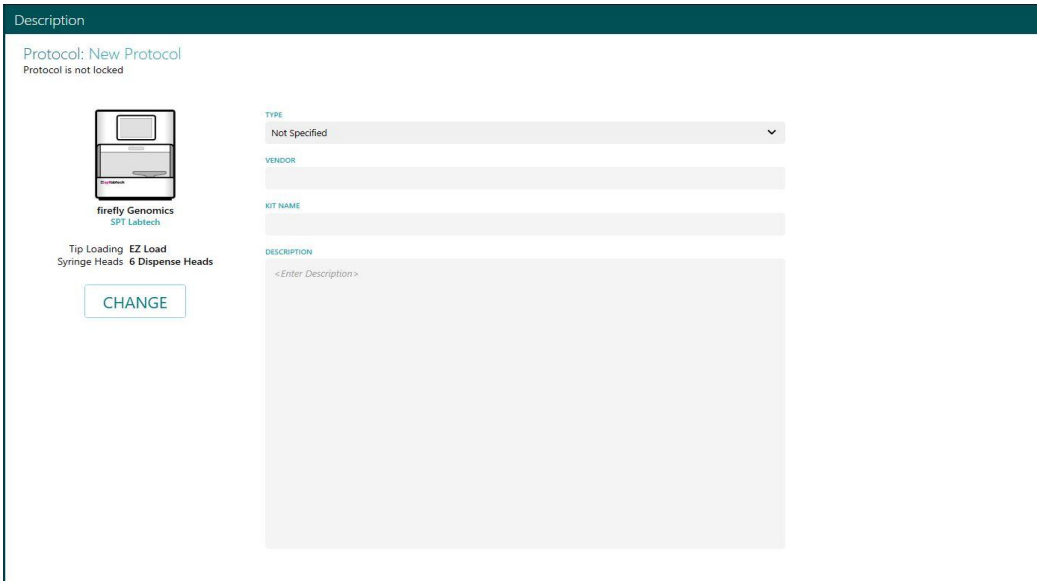
You may wish to use 'Save As' and rename your protocol, if you want to keep your downloaded copy unchanged (or you could always download it again).

If you make a mistake when editing, you can use the undo and redo functions; these will work for multiple steps.

Select an item or a step to see its properties. Some are only visible if you select 'Advanced'. Many properties are default values, set within firefly software, but you will need to set e.g., aspirate and dispense volumes. If you forget to set a property, firefly will warn you that it is missing.

Changing the target instrument

All firefly protocols are designed for a target instrument. If you need to change this, open Description, and click the Change button.



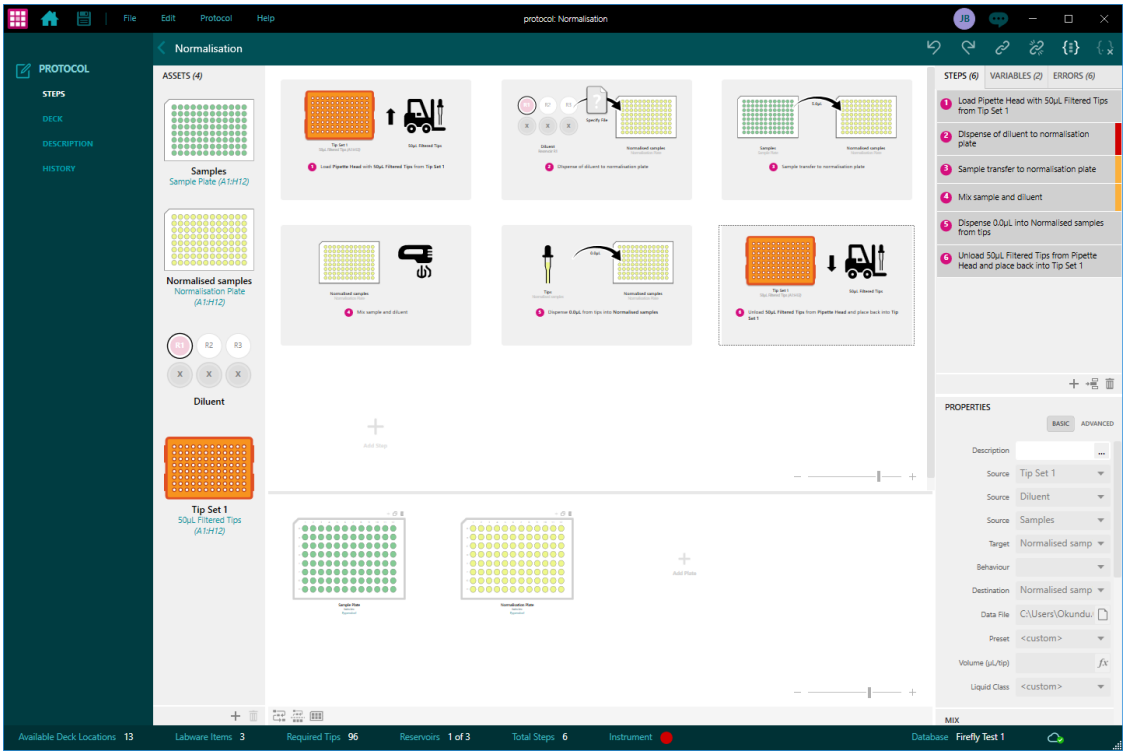
This opens the popup form to select an instrument type. Note that if you select a type which cannot support steps in your protocol, you will need to revise it to clear the errors.

Opening multiple protocols

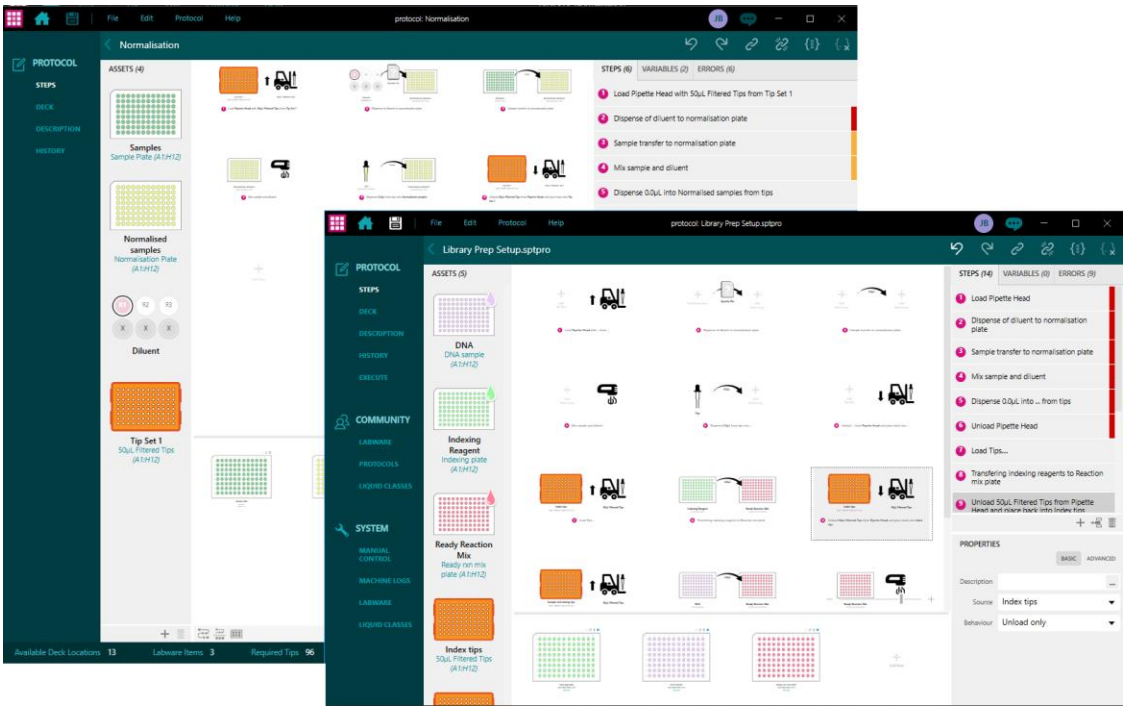
If you want to work from an existing protocol without editing it e.g., to see how to create a particular sequence, you can:

1. Open the protocol you wish to edit, or set up firefly to create a new protocol.
2. Open another copy of the firefly software from the desktop shortcut.
3. Log in again. You will only see the option to load an existing protocol, not to design. Select the protocol you want to use as a template for your protocol.

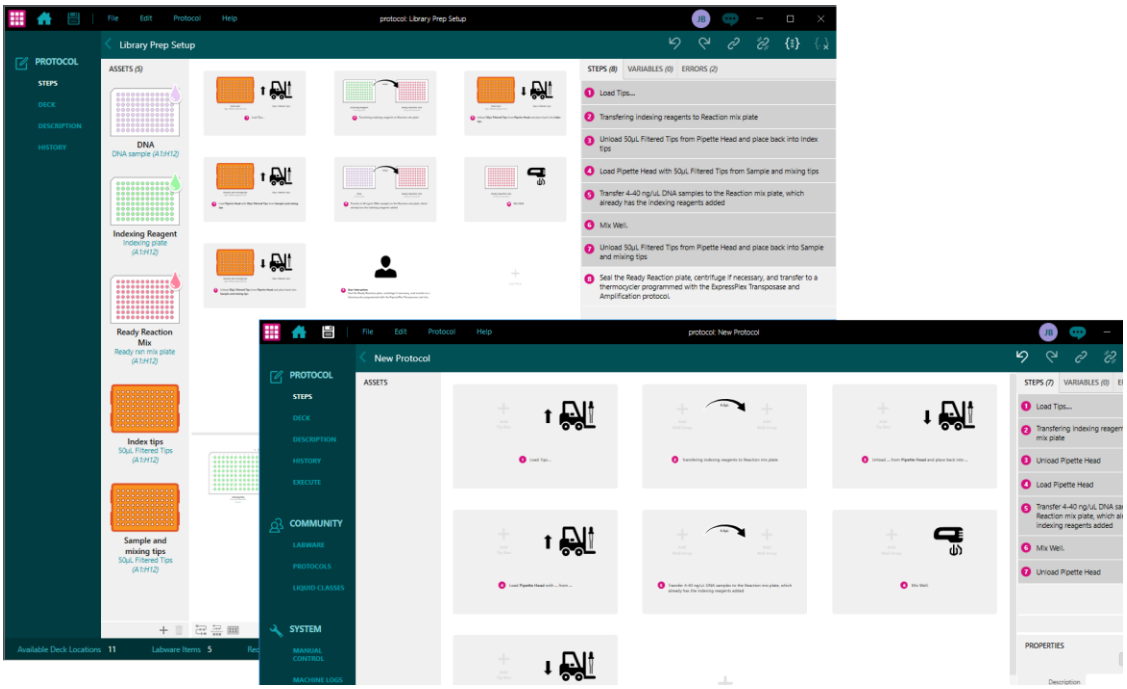
   You'll notice that you will only see the Protocol functions in the second firefly window; this is to prevent conflicts so e.g., only the first copy opened can run a protocol (even in simulation) or send feedback.
4. You can now use the second protocol as a prompt, when designing your protocol.



5. You can select steps (the steps with grey backgrounds above) in the second protocol, copy them and paste them into your protocol if you want to use the sequence. You can add the steps to an existing protocol:



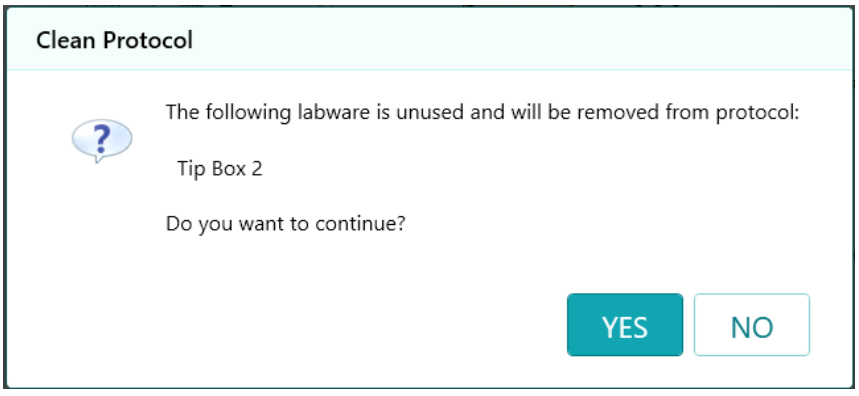
or use them to start a new protocol from scratch.



Note that only the protocol steps are copied, not the assets. You can see this most clearly in the example above, where there are no plates or tip boxes as there are in the original. You'll need to add these.

Using Clean to remove unwanted labware

If you are modifying an existing protocol, you may find that you have more labware specified than you are sure your protocol steps require. To remove labware which is not used in any step, select 'Clean' on the 'Protocol' menu.

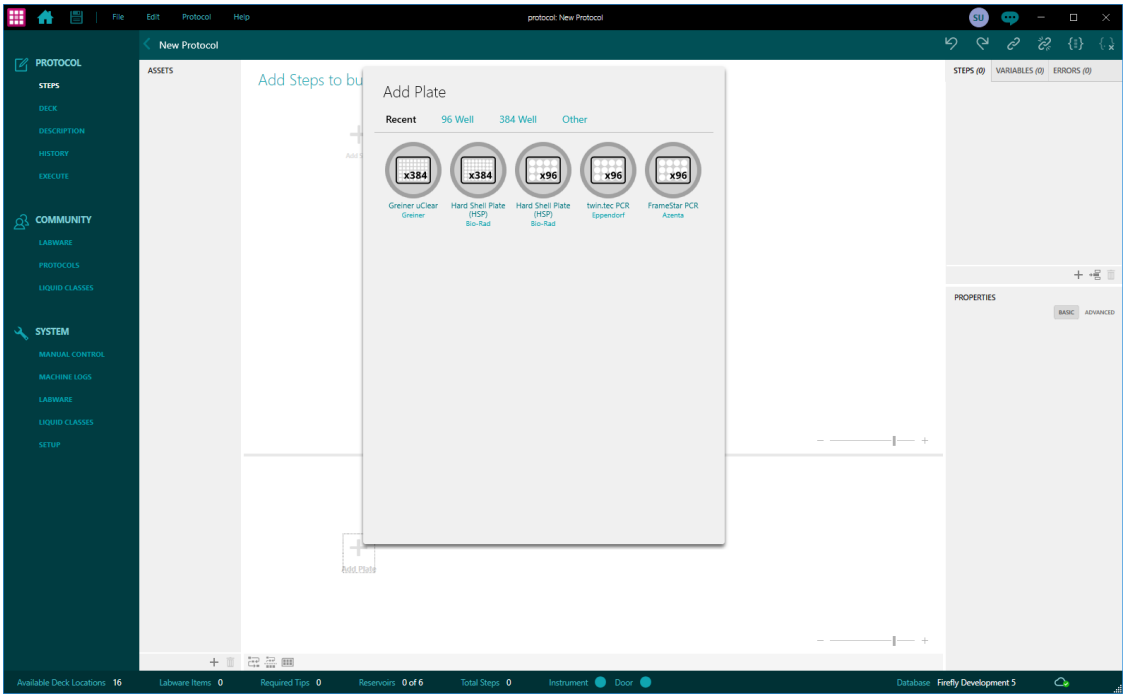


Clean identifies and lists unused labware, which you can then choose to remove from your protocol. You can run it at any point in protocol design, but it is most useful to run before you set up the decks.

Editing plates

Click the Add Plate '+' icon in the Plates area, to add a plate to a protocol.

Select the plate you want from the pop-up form; there are tabs for 96 and 384 well plates. The 'Other' tab includes troughs.

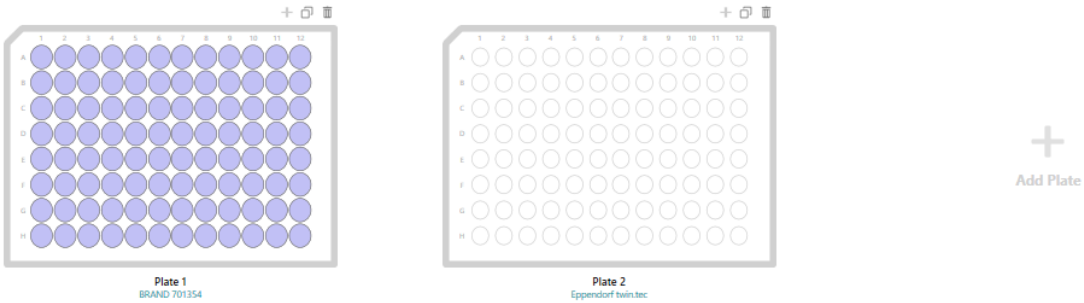


You can only use plates which you have already installed, so if you cannot see the plate you want, you need to look in the 'Community' 'Labware' section and download the plate before using it in a protocol.

All plate types, including troughs, can be used with the pipette and dispense heads.

> **Important**
>
> Trough plates cannot be used with the shaker, the plate thermal module or the ODTC.

Using the mouse to click and drag across, select the well array in the plate which you will use in the protocol. In the plates shown below, the left one has all wells selected as a well array, the right has none.



Then click the small '+' above the plate, to add the well array to the protocol assets. You can create multiple well arrays in the same plate, using the same method.

You cannot make a plate an asset, only a well array so if you want to use the whole plate, drag to select all the wells then right click on the plate and use 'Add to assets'.

To swap plate types in a protocol, click the 'Swap Plate' icon - the central one - above the plate you wish to swap out. If you had well arrays defined in that plate, they will be carried over to the new plate.

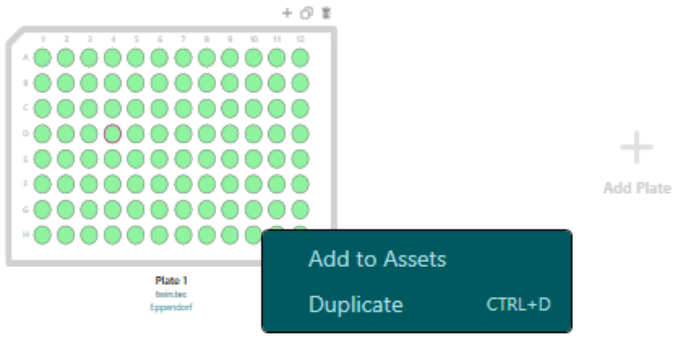
If your protocol uses multiple plates of the type you are swapping, firefly gives you the option to select to swap all plates to your new type.

To delete a plate, click the bin icon above the plate. If the plate was still in use in a protocol, deleting it will cause errors, so check the Errors list on the right for any steps which need correcting.

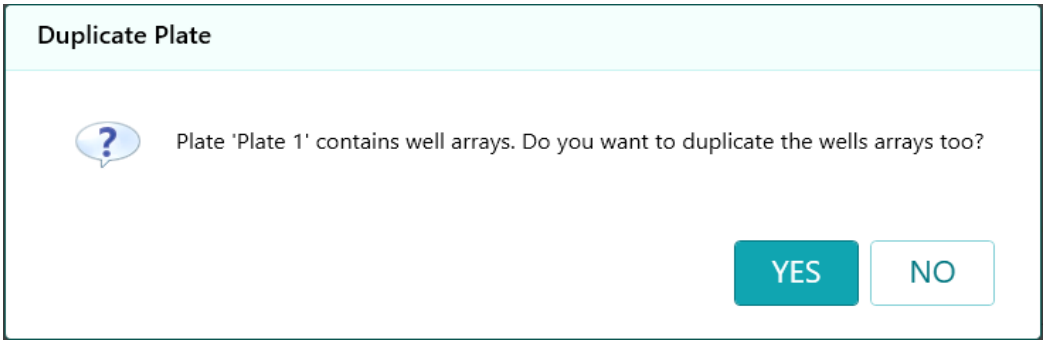
Property	Form	Notes
Name	Free text	
Type	[auto filled, not editable]	Plate manufacturer and name
Type	[auto filled, not editable]	Plate format e.g., 96 well
Max Well Volume	[auto filled, not editable]	
Dead Volume	[auto filled, not editable]	
Deck location	Select from dropdown list	If you select a location here, the plate will be placed there when you open the 'Deck' view.
Barcode - enable prompt	Tick box	
Format	Regex	Use a regex to define the form of your barcode data

Copying plates

To copy a plate, select it in the Plates area and either use Ctrl-D or select Duplicate in the right mouse button menu.



If you had defined one or more well arrays in the plate, duplicate offers you the option to also copy them.



Editing assets

To add any asset other than a well array, click the '+' icon on the Assets area, and select the item you want from the pop up menu.

Check 'Available Deck Locations' on the Status bar to make sure you are not adding more plates than there are deck locations. Reservoirs, the shaker module and the thermal modules do not take up deck locations.

If you need to use more plates, create user interaction steps to remove used items and add new ones at an appropriate point later in the protocol.

To delete any asset including a well array, select it and then click the bin icon for the Assets area. Check the Errors list on the right of the screen in case deleting the asset has caused a problem in the loaded protocol.

Editing well arrays

To adjust the wells used in a well array, use the 'Wells' section of Properties, identifying your selection by rows and columns i.e., A1:E7. If you increase the number of wells, check that you still have sufficient volumes of reagents, and sufficient tips loaded.

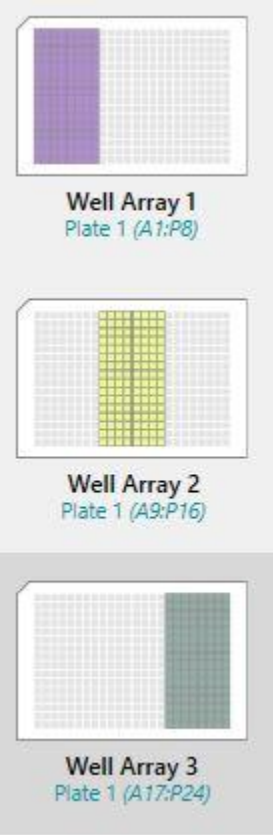
If you are using an array of 96 tips to pipette between a 96 well plate and 384 well plate, a tiling pattern can be used to define the well array asset for the 384 well plate. In this way a single quadrant of the 384 well plate can be defined as a well array asset.

Property	Form	Notes
Name	Free text	
Colour	Select from pop-up form	Use to differentiate reagents
Starting volume per well (µL)	Numeric / Variable	0.00 to maximum value for the well type
Maximum volume per	[auto filled, not editable]	Defined by the plate type selected

Property	Form	Notes
well (µL)		
Dead volume per well (µL)	[auto filled, not editable]	Defined by the plate type selected
Array		
Plate	Select from dropdown list	Select from list of existing plate identifiers
Wells	A1:H12 format	An array can be anything from a single well to a full plate
Pattern	Select from form	All possible patterns are shown as unit cells.

Right click on a well array in the Assets panel to access the Split by Columns function, which divides the well array.

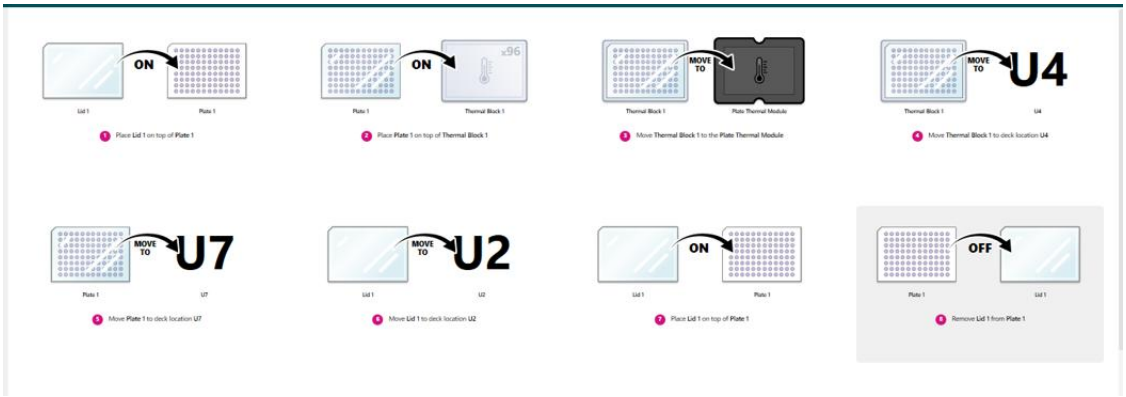
For ease of use, you can add a whole plate well array, then use this function to split it into multiple smaller arrays without needing to define them individually.



You can then choose to rename the new arrays or assign them distinct colors.

Editing lids

You can either load plates which are lidded or load lids ready for use in a later protocol step. When you remove a lid, it will be placed in an empty deck location, so every time you place a lid on a plate you will free up a deck location.



Check 'Available Deck Locations' on the Status bar to check you have enough free space, as you use lids and plates. If you need to use more lids, create user interaction steps to remove used items and add new ones at an appropriate point later in the protocol.

When placing a lid on a plate, you are creating a stack. You can only use combinations of lids and plates which have a stack definition. Use Community to download stack definitions. If you cannot find what you need, contact reliance. You can continue designing with an undefined stack, but you cannot run the protocol.

firefly software will warn you if you try to use a dispensing or pipetting step without removing a plate's lid first.

Important

If you use Auto Deck Fill when you have an undefined stack in your protocol, collision detection would work based on the wrong stack definition so shouldn't be trusted until this error is removed.

Download the stack definition before using Auto Deck Fill.

Note

If your protocol places used lids on the shelves of firefly+, any condensate may drip and contaminate shelves or other labware. To avoid this, when you remove a lid from a plate, move it to a waste plate.

Property	Form	Notes
Name	Free text	
Type	Select from dropdown list	
Deck location	Select from dropdown list	If you select a location here, the lid will be placed there when you open the 'Deck' view.

Editing EZL tip sets

You will need to load sufficient tip sets for the quantity of tips you will use. Check the Errors list to see if you need to adjust the quantities. As tip sets can only be loaded on the lower deck, you may not be able to load all the tips you will need at the start of the protocol. Remove used tip sets and replenish tips in a user interaction step, ensuring that the tip type of the fresh tips matches those of the tips that have been removed.

Property	Form	Notes
Name	Free text	
Format	Select from dropdown list	96 or 384 tip set
Tip type	Select from dropdown list	
Use strips	Tick box	
Strips	Numeric / Variable	Quantity of strips
Working volume (µL)	[Auto filled]	This is defined by the tip type
Deck location	Dropdown list	
Barcode - enable prompt	Tick box	This is set up for scanning the barcode on the box

Note

If you are using 16 tip strips, these have a gap between loaded strips and cannot be loaded as adjacent strips due to the strip tip adapter arrangement. Therefore, you cannot address adjacent columns of a 384-well plate simultaneously as the EZL tip strips will have gaps when loaded into the adapter.

Editing ATL tip boxes

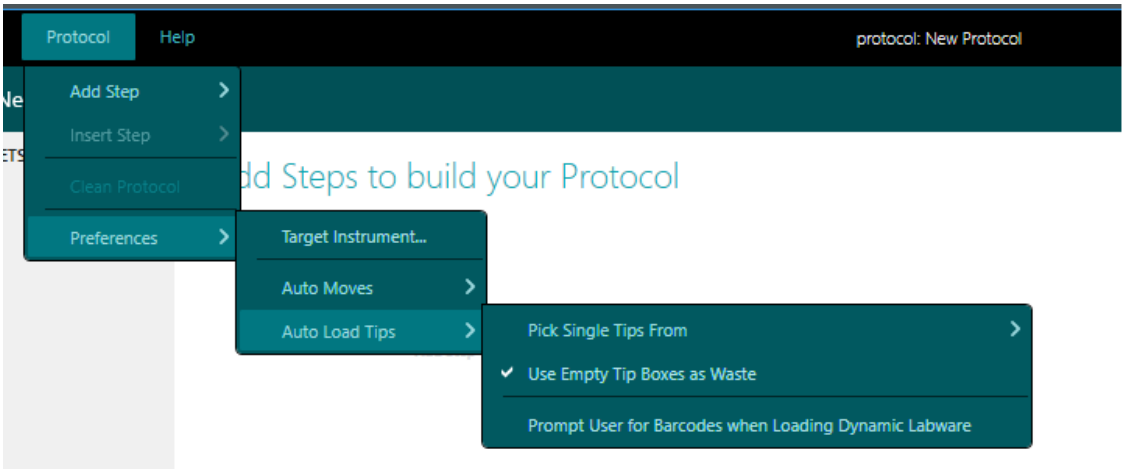
You will need to load sufficient tip boxes for the quantity of tips you will use. Check the Errors list to see if you need to adjust the quantities.

If you are using Auto Load, do not select tip boxes. The Auto Load process will identify the tip boxes required, and will list them in the Required Labware and Consumables screen when you execute the protocol.

You can load several columns of ATL tips only, not the entire 12 or 24 columns. This enables you to carry out multiple pipetting steps with fresh tips for each, while still using the same tip box.

> **Note**
>
> Auto Load does not work with partially filled tip boxes, you will need to use manual Load and Unload steps with partial tip boxes. The tips must fill from the H12 position back, so they are all on the right of the tip box.

Select the Use Empty Tip Boxes as Waste from the Protocol menu to return waste tips to the empty box(es) already loaded. This saves space on firefly's decks, by not loading a dedicated waste tip box.



If you do not choose this option, you will need to load an empty waste tip box for your protocol, to avoid unloading used tips back into a tip box which still contains unused tips.

You can place ATL tip boxes on upper or lower deck locations. There is no upper limit on quantity of tip boxes in use in one protocol.

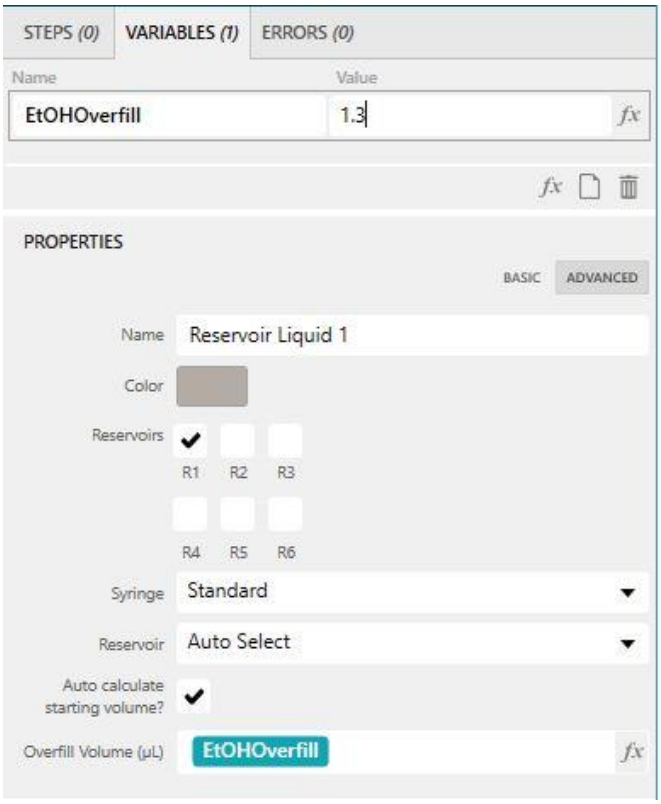
Property	Form	Notes
Name	Free text	
Type	Select from dropdown list	96 or 384 tips of differing types
Available columns	Numeric / Variable	Quantity of columns in the tip box
Working volume (µL)	[Auto filled]	This is defined by the tip type
Deck location	Dropdown list	You can use the upper or lower deck
Barcode - enable prompt	Tick box	This is set up for scanning the barcode on the box

If you want to scan tip box barcodes, and are using Auto Load, select the 'Prompt user for barcodes when loading dynamic labware' option, under Protocol, Preferences (as shown above).

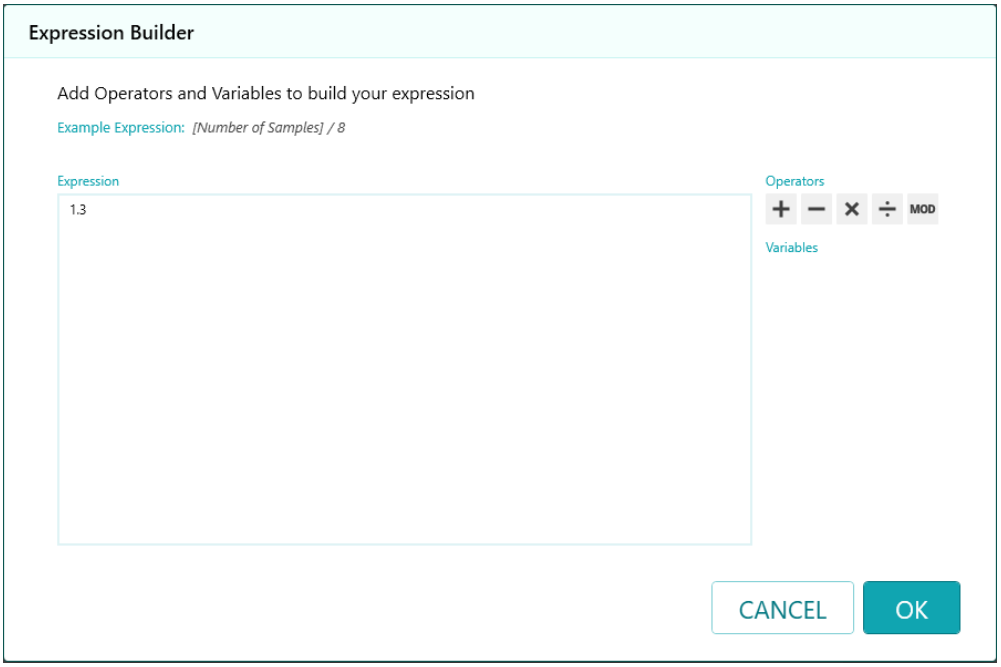
Using variables

Wherever you see the variable symbol fx for a property, you can choose to use a variable set when the protocol is executed, in place of an absolute value.

1. Select the Variables tab in the right panel.
2. Click on Create Expression Variable fx to create a new variable whose value you will define using firefly software. If you want to use values defined elsewhere, select to create a File Path Variable
3. Name your variable.
4. You can set an initial value for your variable, as shown below. If you do this, users executing the protocol will see this value but will have the option to modify it, if they have permission (basic users do not, by default).

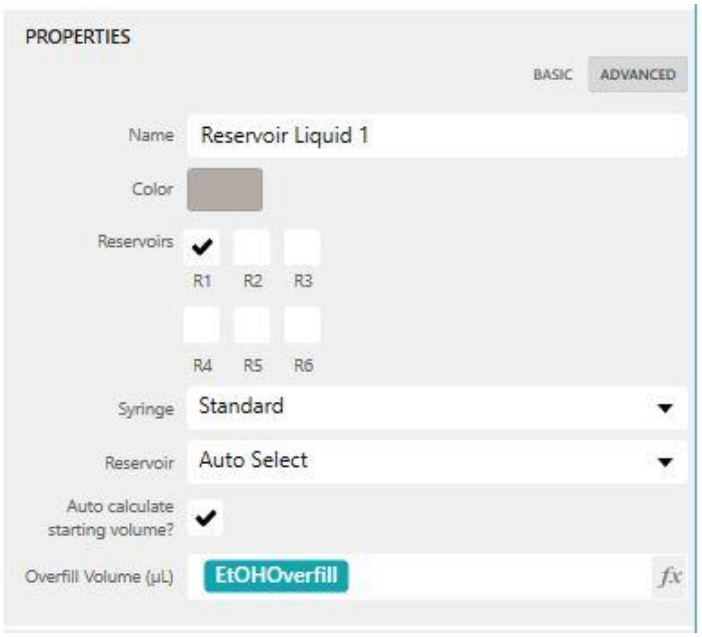


If you select the fx next to the Value column, you will open the Expression Builder, to define your variable.



Having defined your variable, you can use it repeatedly in the current protocol (it will not be visible to use in other protocols).

To use the variable, select fx next to the Property you want to set, and select the named variable as shown below.



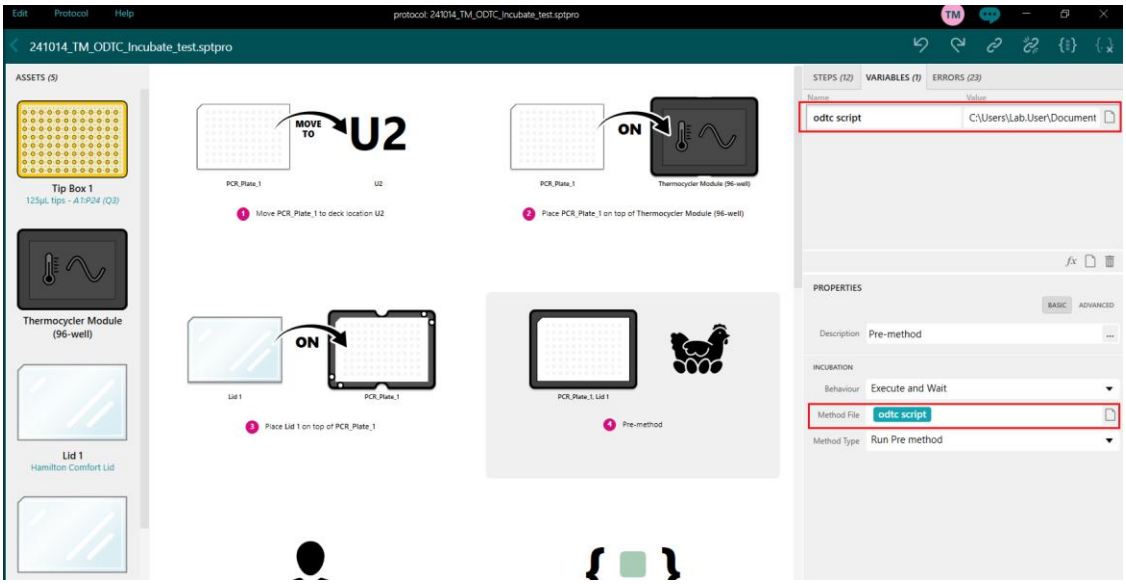
Variables are always shown with a teal background in protocols.

You can use variables to set aspirate, dispense and mix volumes, in addition to starting and overfill volumes.

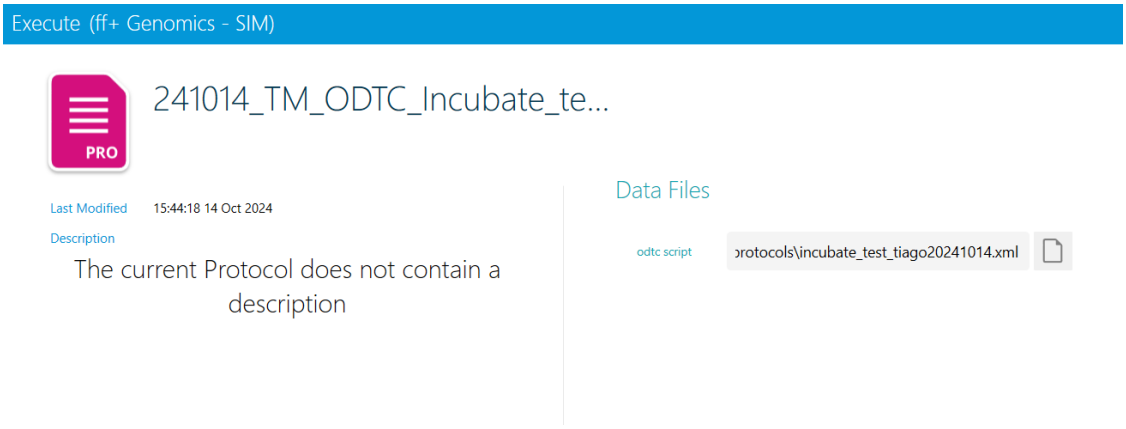
Using file path variables

You can use a file path variable to have variant files for File Fill, File Dispense or Incubate steps. Using this you can design e.g., variations of your PCR script such as 5 cycles, 10 cycles and 18 cycles, and choose the one to run in the Deck Loading Wizard, before you execute the firefly protocol. You can use this to test e.g. different ODTC protocols within the same firefly protocol instead of needing to write multiple variants of the firefly protocol, as shown in the example below.

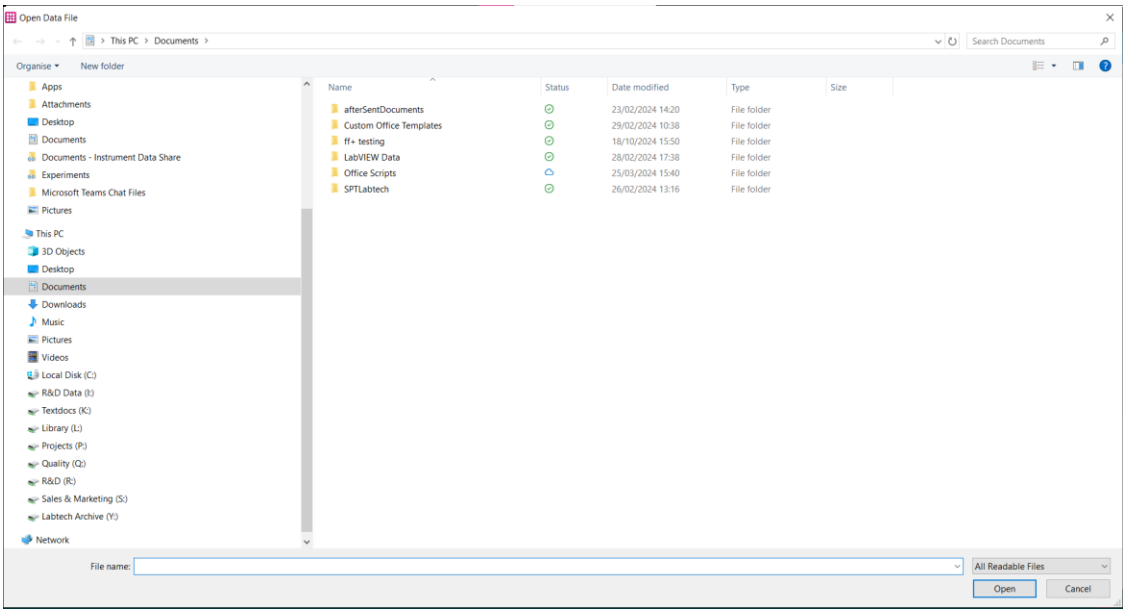
When designing the protocol, associate one of your PCR scripts with a variable.



When you execute the protocol, because you had used a variable, not a fixed file name, you can use the Data Files option to select a different script, by clicking the File Path icon 



This opens the file path browser so you can select an alternative file at the point of use.

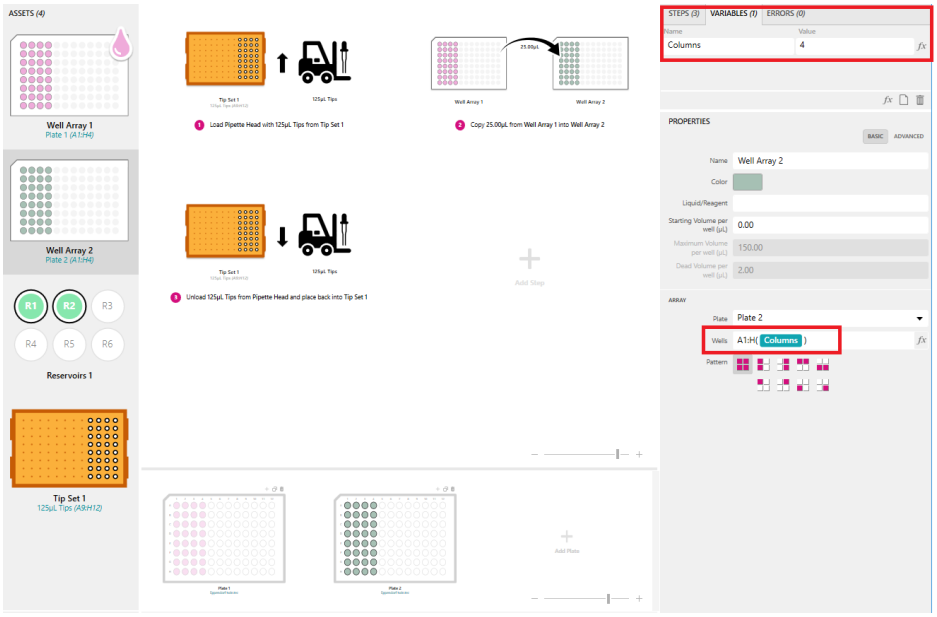


Designing a scalable protocol

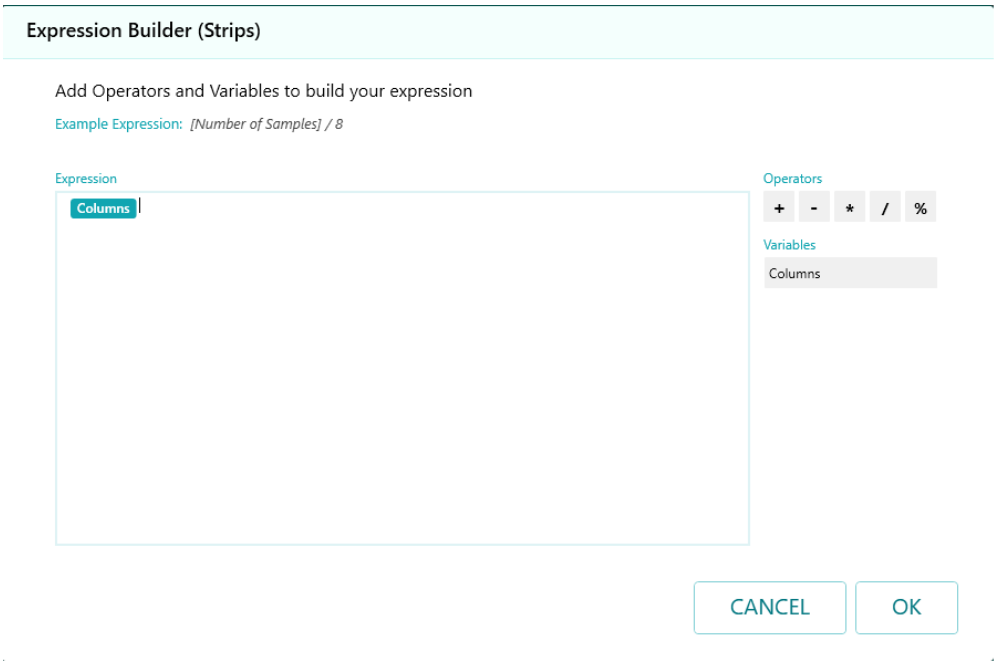
You can make your protocol scalable by using a variable size well array, number of strip tips or volume of reagents.

Scaling EZL strip tips usage

1. Select the Variables tab and create a column variable with an initial value, as shown.

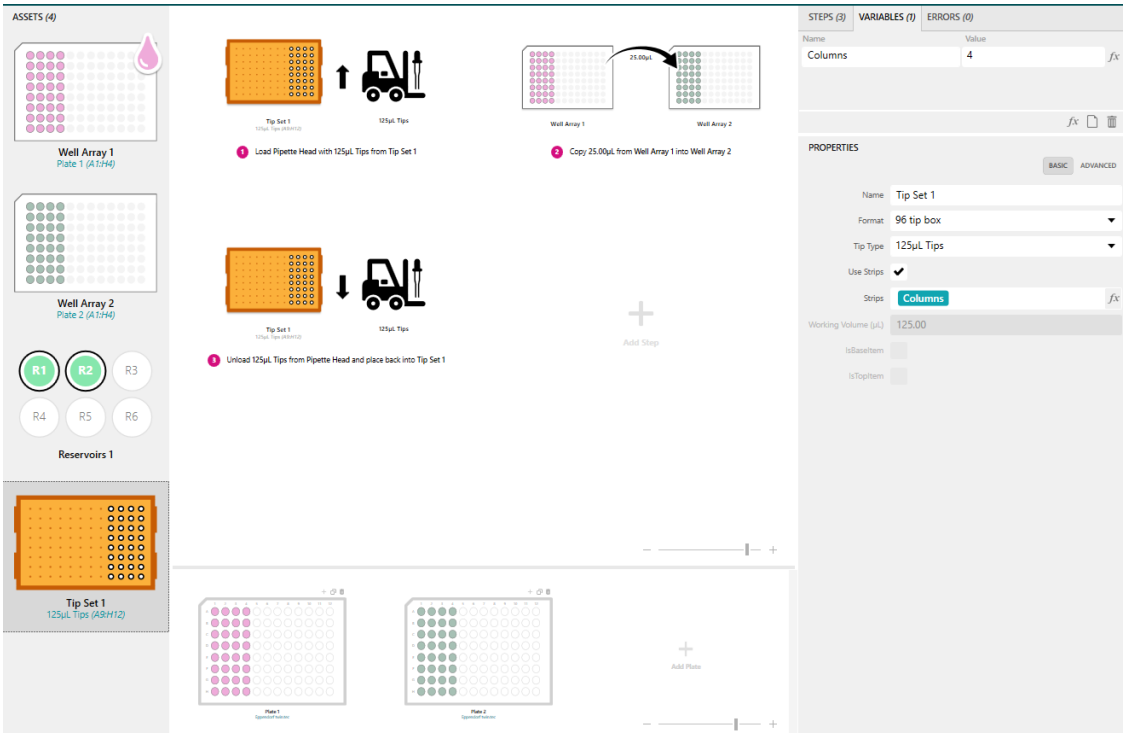


2. Select the 'fx' button on the tip set properties to open the Expression Builder (Strips).



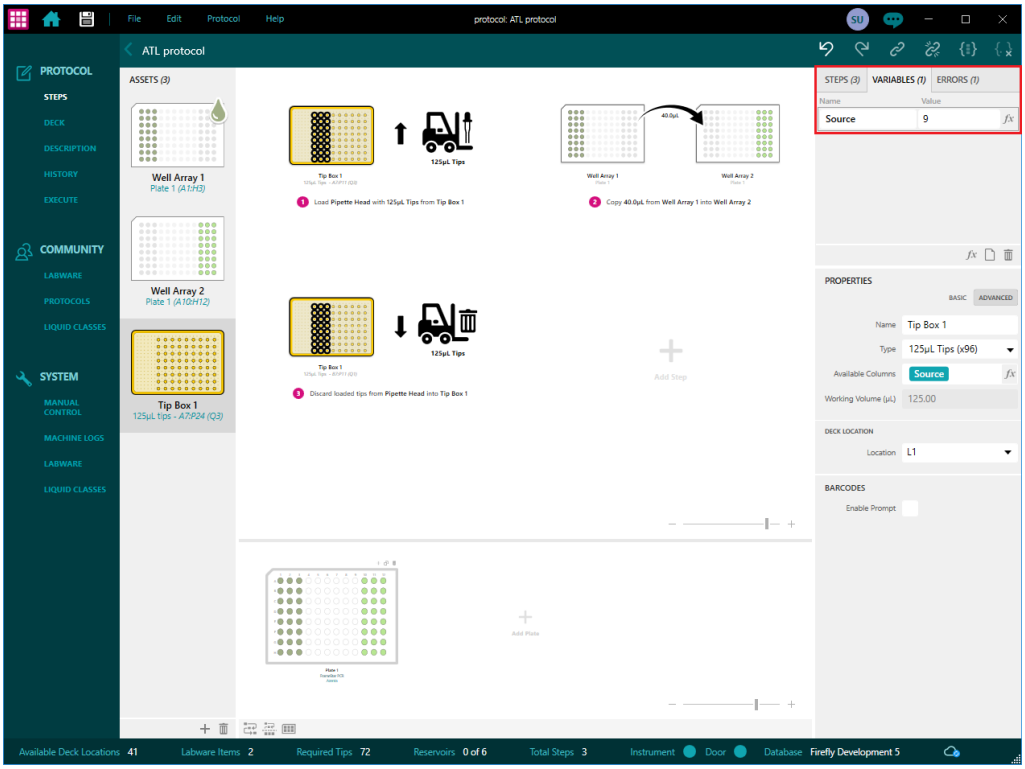
3. Create an expression which replaces a fixed number of strip tips with your variable. This can be very simple, like the example shown above which just sets the number of strip tips used to match the number of well array columns.

   Your variable now appears in the Strips property for the tip set.

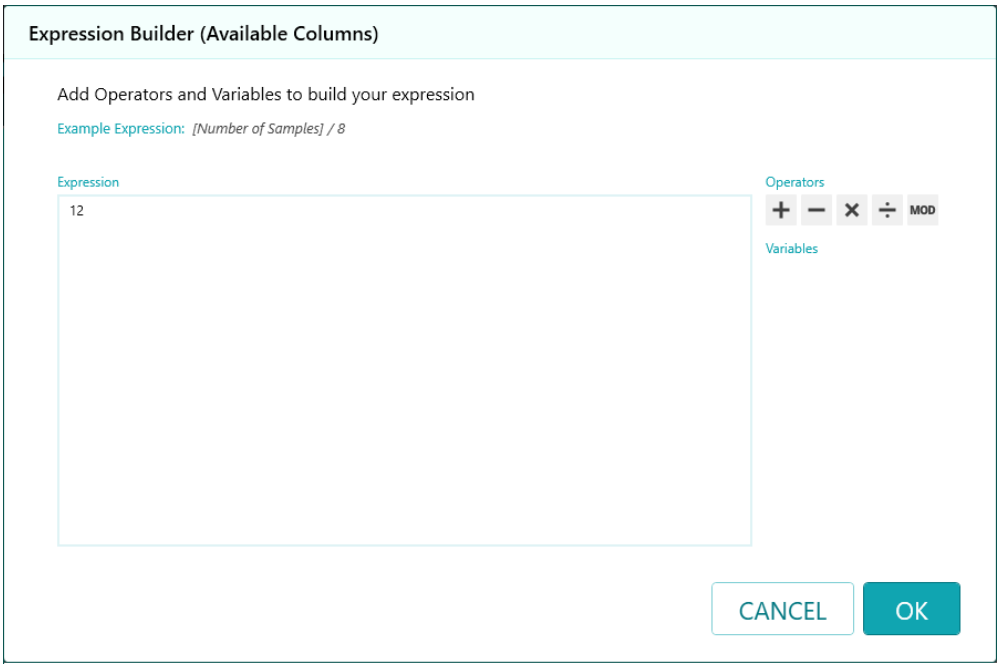


Scaling ATL column usage

1. Select the Variables tab and create a variable for the number of columns to use, with an initial value, as shown.

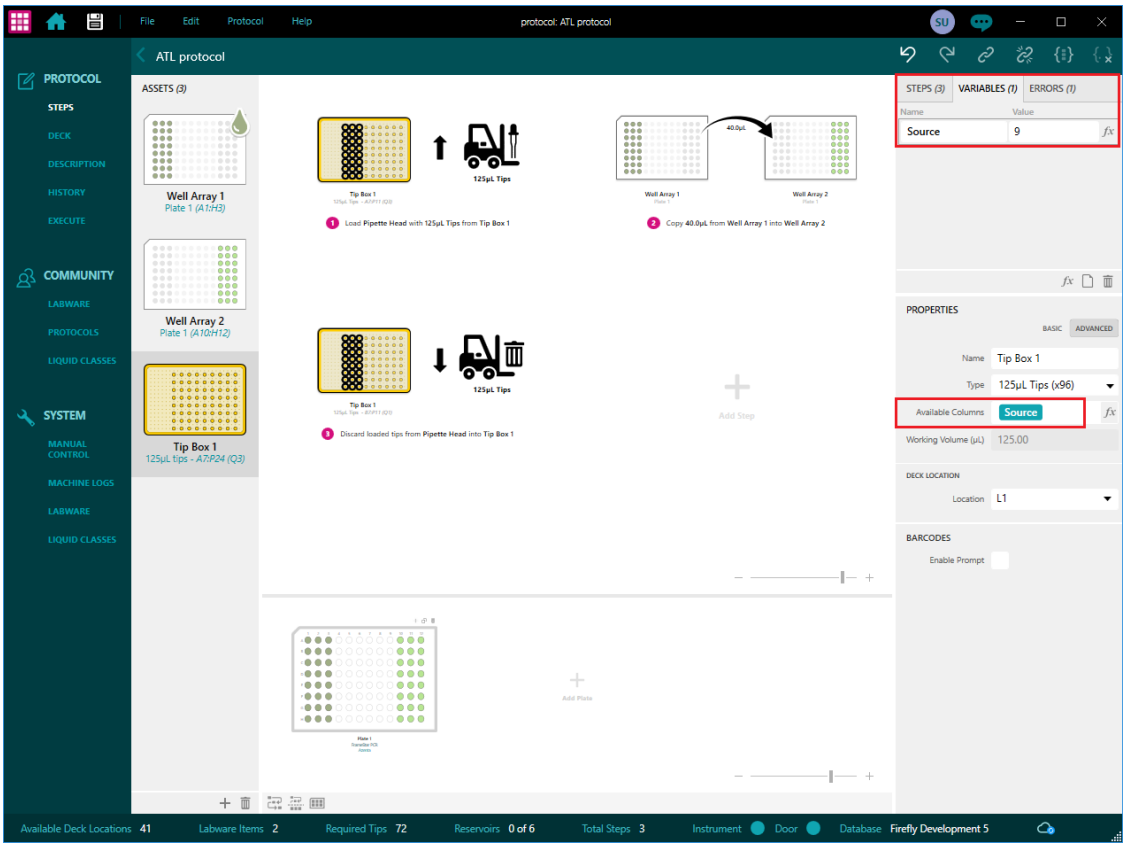


2. Select the 'fx' button on the tip set properties to open the Expression Builder (Available Columns). The default value is 12 for a 96 tip box or 24 for a 384 tip box.



3. Create an expression which replaces a fixed number of columns with your variable, or with a mathematical expression utilizing variables.

   Your variable now appears in the Available Columns property for the tip box.



Note

If you set your variable to 4 or more columns, you will see an error in your protocol, that the tip box must be placed on a tip box adapter before the tips can be loaded. Because of this change in behavior, you may find it easier to create and save multiple protocol variants for different column numbers.

Scaling well arrays

1. Select the 'fx' button on the well array properties to open the Expression Builder (Wells)

2. Create an expression which replaces fixed values in a well array with your variables. This can be very simple, like the example shown above which just allows you to vary the number of columns when running the protocol.
Your variable now appears in the Wells property for the well array.

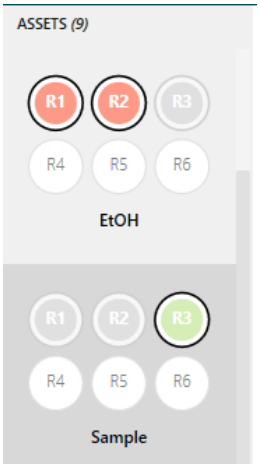
Editing reservoirs

When editing the reagents to be dispensed in a protocol, you will need to define Reservoirs for the materials. You can use the 'auto' dropdown to select a suitable reservoir based on the dispense volume or select the specific type.

You may find it more convenient to use multiple reservoirs for the same material if you are dispensing to a large quantity of wells, as parallel dispensing is faster. However, you will need more of your reagent, as you will always need the same additional volumes for the excess aspirate volume in the syringe and for the dead volume in the reservoir, irrespective of your dispense volume.

	LDV reservoir	**Standard reservoir**	Large reservoir
Safeguard volume - volume that needs to remain in the tips during a dispense	40µL	40µL	40µL
Reservoir dead volume	30µL	195µL	500µL
Total	70µL	235µL	540µL

To add a new reagent, you will need to add a new Reservoirs Asset, then use the Reservoirs Property to select which Reservoir to use. If you select a reservoir which had been in use, it will be reassigned, and you will generate an error that the previous reagent is no longer loaded. Color coding your materials will make it easier to keep track of what is in use, particularly in long protocols.



The Assets shown above includes two reagents in reservoirs, the first uses two, the second uses one.

You can select to require barcodes for the reagents, the syringes or the reservoirs used. You will need to specify the barcode format for reagents or reservoirs.

You do not need to specify the volume of reagent in a reservoir, it can be calculated by the firefly software and then displayed in the setup instructions.

Use the Overfill volume if you know that you will want an excess of reagent in the reservoir e.g., because it will evaporate during protocol execution.

Property	Form	Notes
Name	Free text	
Colour	Select from pop-up form	Use to differentiate reagents
Reservoirs	Select one or more from the 6 positions	Unavailable reservoirs are grey, not white
Syringe	Select from dropdown list	Standard or ULR (volume is the same)
Reservoir	Select from dropdown list	Standard, high volume, LDV or auto
Auto calculate starting volume?	Tick box	
Starting volume (µL)	Numeric / Variable	0.00 to maximum value for the reservoir type. Only shown if auto calculate has not been ticked.
Overfill volume (µL)	Numeric / Variable	
Maximum volume (µL)	[auto filled, not editable]	This is set when the reservoir type is selected
Barcodes		
Reagent barcode		
Enable prompt	Tick box	
Format	Regex	Use a regex to define the form of your barcode data
Reservoir barcodes		
Enable prompt	Tick box	
Combine prompts	Tick box	Show barcode fields together in protocol setup
Format	Regex	Use a regex to define the form of your barcode data

Property	Form	Notes
Syringe barcodes		
Enable prompt	Tick box	This is set up for scanning the barcode on the box
Combine prompts	Tick box	Show barcode fields together in protocol setup

Editing magnetic blocks

Magnetic blocks are simple, passive devices which require no configuration. You can use multiple magnetic blocks in a protocol.

You can only use magnetic block types which you have already installed, so if you cannot see the one you want to use, you look in the 'Community', 'Labware' section and download it.

Property	Form
Name	Free text
Type	Select type from dropdown list
Deck location	Select from dropdown list

Editing risers

Risers are simple, passive devices which require no configuration. They are used to lift shallow plates so that heads dispensing to them do not collide with taller labware in adjacent deck locations.

You can use multiple risers in a protocol. There are specific types for upper and lower deck use.

Note

EZL lower deck risers are not compatible with the height of ATL tip boxes so do not use them on an ATL instrument.

You can only use riser types which you have already installed, so if you cannot see the one you want to use, look in the 'Community', 'Labware' section and download it.

Property	Form
Name	Free text

Property	Form
Type	Select type from dropdown list; check it is correct for the deck
Deck location	Select from dropdown list

Editing thermal blocks

Thermal blocks are used in conjunction with the plate thermal module, there are designs for use with 96 or 384 well plates. After they have been heated or cooled to the required temperature while located on the plate thermal module, they can be moved with a plate to e.g., the dispense head, to keep the plate warm or cool for longer.

You can use multiple thermal blocks simultaneously on firefly e.g., to cool both a source and destination plate.

Property	Form
Name	Free text
Type	Select type from dropdown list
Location	Select from dropdown list

Editing tip box adapters (ATL instruments only)

If you are going to load more than 3 columns of tips from an ATL tip box, it must be placed on a tip box adaptor, to support it. You can load the tip box and adapter together, at the start of the protocol, or move the tip box onto it later. You can use multiple tip box adapters simultaneously on firefly.

Tip box adapters can only be placed on firefly's lower deck.

You do not need to use a tip box adapter when unloading ATL tips.

Property	Form
Name	Free text
Type	Select type from dropdown list
Location	Select from dropdown list

Using regexes to define barcode data

Depending on your plate and reagent suppliers, you will need firefly to recognize different strings of data as correct, when you read the item barcodes e.g., 05427ES-021 or STBB0728K9. You can also define your own data formats for reservoirs. If you are using SPT Labtech consumables, the correct barcode format is preloaded.

To ensure that the correct items are loaded in firefly, you will need to define a regular expression (a 'regex') which corresponds to the expected combination of characters in the consumables barcodes. Write this in the Barcode Format field.

If the barcode then scanned does not match the pattern, it will not be accepted and may mean that you have picked up the wrong plate type or wrong reagent when running the protocol.

To use barcode data, you will need to define a regular expression (a 'regex') which corresponds to the expected combination of characters. If the barcode then scanned does not match the pattern, it will not be accepted and may mean that you have picked up the wrong plate type or wrong reagent when running the protocol.

You do not have to use this functionality, to use the barcode reader. You can just record the barcode e.g., if you use reagents from multiple suppliers, and want to keep that flexibility. To do this, click the 'Barcode - Enable prompt' option but leave the Format field empty.

If you have not tried designing a regex before, there is a practical tutorial at https://regexone.com/lesson/introduction_abcs.

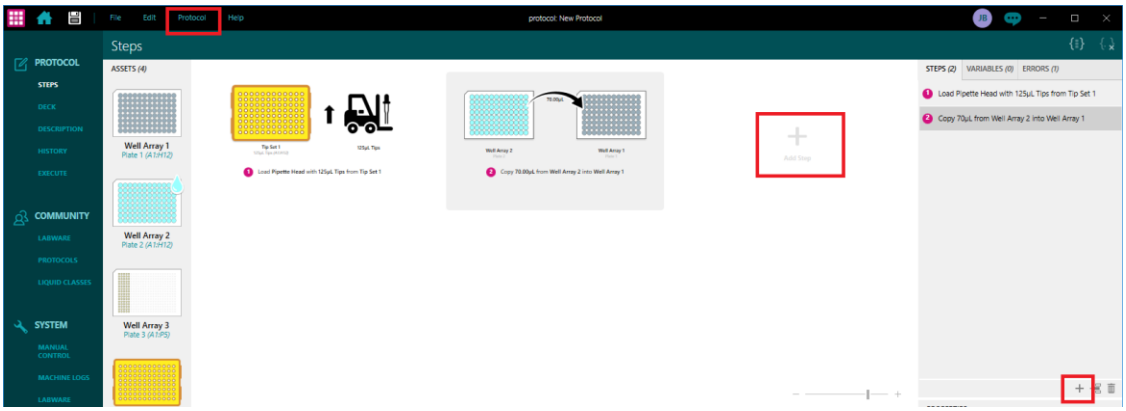
You can use the https://regex101.com/ website to test your regex and confirm it meets your barcode format and parameters.

Editing steps

When editing protocol steps, check the Errors panel on the right of the screen, in case your changes cause problems elsewhere in the protocol which will need you to make changes to other steps e.g., to load larger reagent volumes.

Adding steps

To add a step at the end of a protocol, click the Add step '+' icon, use the Protocol menu item or click the '+' icon below the Steps list on the right of the screen.



Select the action you want to add from the 'Add Step' menu or 'Add Step' pop-up form.

You can copy and paste one or more steps should you need them repeatedly in a protocol.

Inserting steps

To insert a step part way through a protocol:

1. Select the step which will immediately follow the new addition.
2. Right click and select 'Insert' from the popup menu to bring up the protocol steps menu, or select the main Protocol menu in the toolbar, then select 'Insert Step' from the dropdown menu.
3. Select the specific function you wish to add from the insert menu.

Alternatively, you can use the insert function on the Steps panel. Again, select the step which will immediately follow the new addition, then use the Insert function.

Grouping steps

If you have a long protocol, or one where the same steps and methods are used repeatedly, you can group them into a single Group. Select the steps, then click Group, on the left.

You will then see a single icon for all the grouped steps, which you can move, cut, copy and paste to use repeatedly in your protocol.

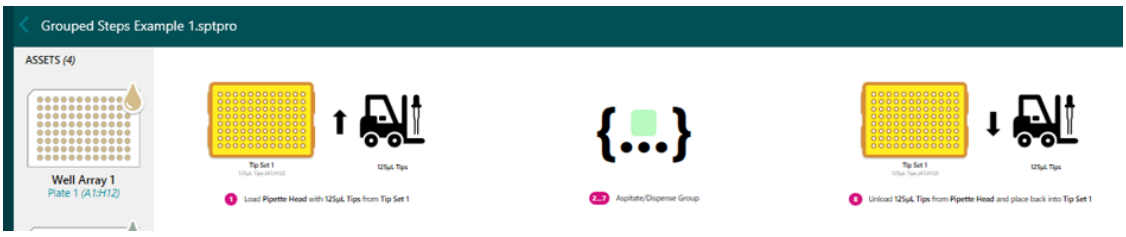
Give your group a descriptive name as you will not immediately see the steps contained in the group in the Steps view in Execute.

You will see the individual steps when you execute the protocol.

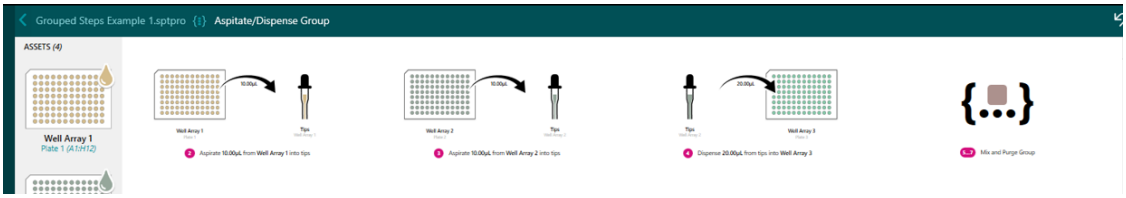
Property	Form	Notes
Description	Free text	Use this to give your group a meaningful name
Colour	Select from pop-up form	Use to identify and differentiate groups
Cycles	Numeric	1 or more iterations of the grouped steps

You can ungroup them again by clicking the Ungroup icon on the right. You can do this to see the steps contained in the group.

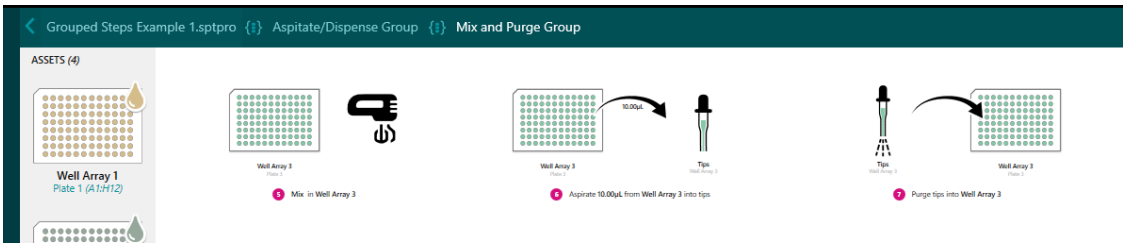
You do not need to use Ungroup to edit the steps. Double-click the group icon.



This opens the steps within the group.

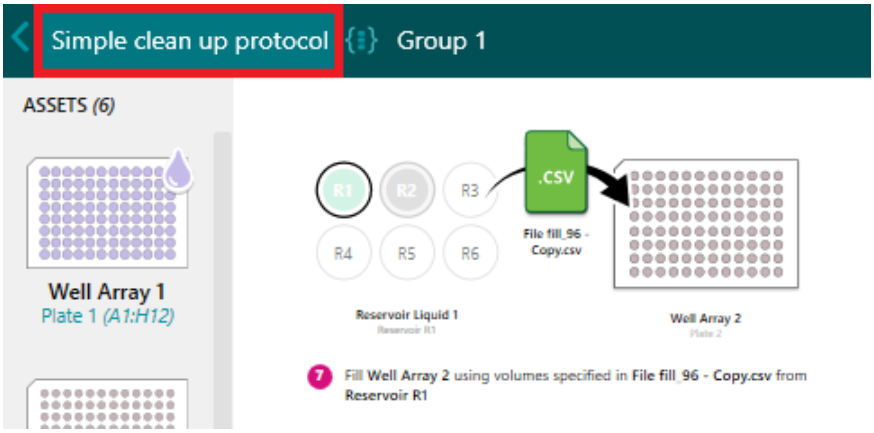


If you have nested groups, you could then open the nested group in the same way (you can only have one group level at a time open).



The breadcrumbs bar will always show you which group you have open and where it sits within the protocol.

To exit a group and return to an 'outer' level, click the level you want to move to in the breadcrumbs bar (highlighted in red - this will exit to the main protocol level).



Repeating steps

To repeat a step exactly, right click on it and select Copy from the right click menu, or use the Ctrl-C shortcut. Right click again and select Paste (or use the Ctrl-V shortcut) and the step will be added to the end of the protocol, with all Properties unchanged.

To repeat a sequence of steps, make them into a group and set the group's Cycles property to the number of repetitions you require.

To repeat a sequence at intervals throughout your protocol, create a Group for those steps and use Copy and Paste to use them repeatedly.

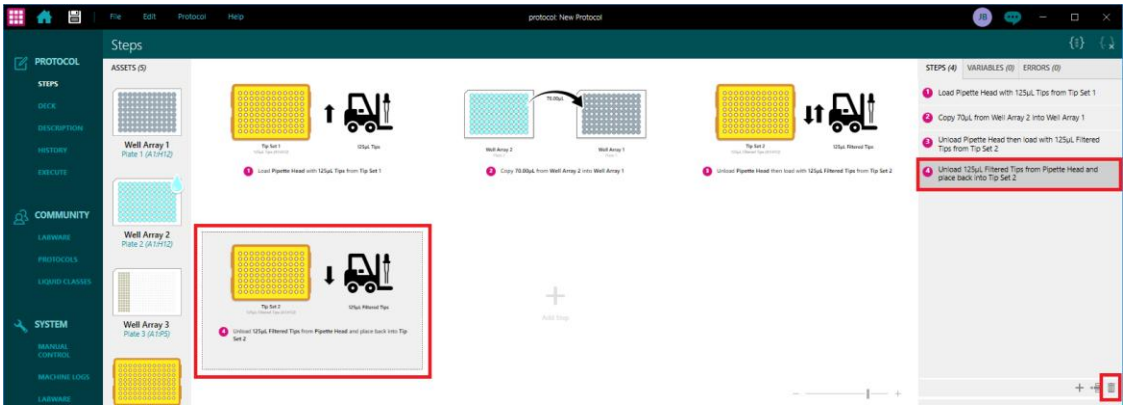
Re-ordering steps

To change the order in which steps execute, select the step you want to move, and drag it to its correct position. You may find it useful to adjust the Steps zoom level first, to get the view you need.

You may find it easiest to use Group before moving multiple steps, and then ungroup them after using drag and drop.

Deleting steps

To delete a step, either select it in the Steps or the Steps list at the right of the screen, then click on the bin below the Steps list, use the keyboard Delete key or Delete in the right click menu.



Pipetting Head: Load / Change EZL tips

Load / Change tips enables you to switch to clean pipetting tips. You will need to include this function twice in any protocol which will carry out pipetting operations, to load tips at the start and to unload them at the end of the protocol. Add a Tip Set as an asset, and then drag it to the Load / Change tips step.

You can use multiple tip sets in a protocol, to keep using new tips, but you will need to load sufficient tip sets for each change of tips.

If you need more tip sets than you have space to load at the start of the protocol, use User Interactions to remove used tip sets and replenish new ones. A maximum of 8 tip sets can be defined and used in a protocol. For this reason, ensure that the fresh tips which are loaded when the tips are replenished are of the same type as the tips that are removed from the deck.

> **Note**
>
> EZL tip sets can only be placed on firefly lower deck positions.

At the end of a protocol, you must unload the final set of tips. The protocol will not execute if tips remain on the pipetting head at the end of the protocol.

Property	Form	Notes
Description	Free text	
Source	Auto filled from the drag and drop operation or select from dropdown list	
Behaviour	Select from dropdown list	Options are: Unload then load, Load only, Unload only

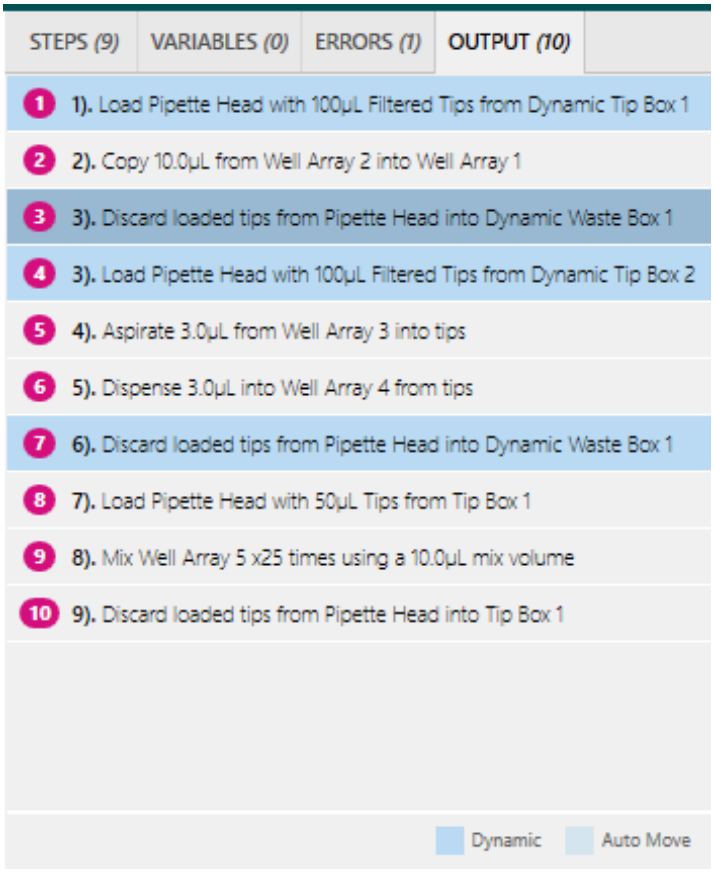
Use the 'Load only' behavior when there are no tips on the head i.e., the first tip loading operation, and 'Unload only' at the end of the protocol. The Load/Change tips icon is modified, depending on the behavior selected; this makes it easier to keep track of what tips are available.

Pipetting Head: Auto Load Tips

The Auto Load Tips function is only available for ATL head firefly.

> **Note**
>
> You must turn on Auto Moves to enable Auto Load Tips.

Using Auto Load Tips is the simplest way to load tips when designing your protocols, and its use is strongly recommended. You only need to include one Auto Load step when you want to change tips, and firefly software will handle the discarding of used tips and the loading of clean tips. This makes it much easier to follow the flow of processes in a protocol, as you do not see the tip management steps until the protocol is executing. If you want to see all the steps in your protocol when designing, look at the Output panel.

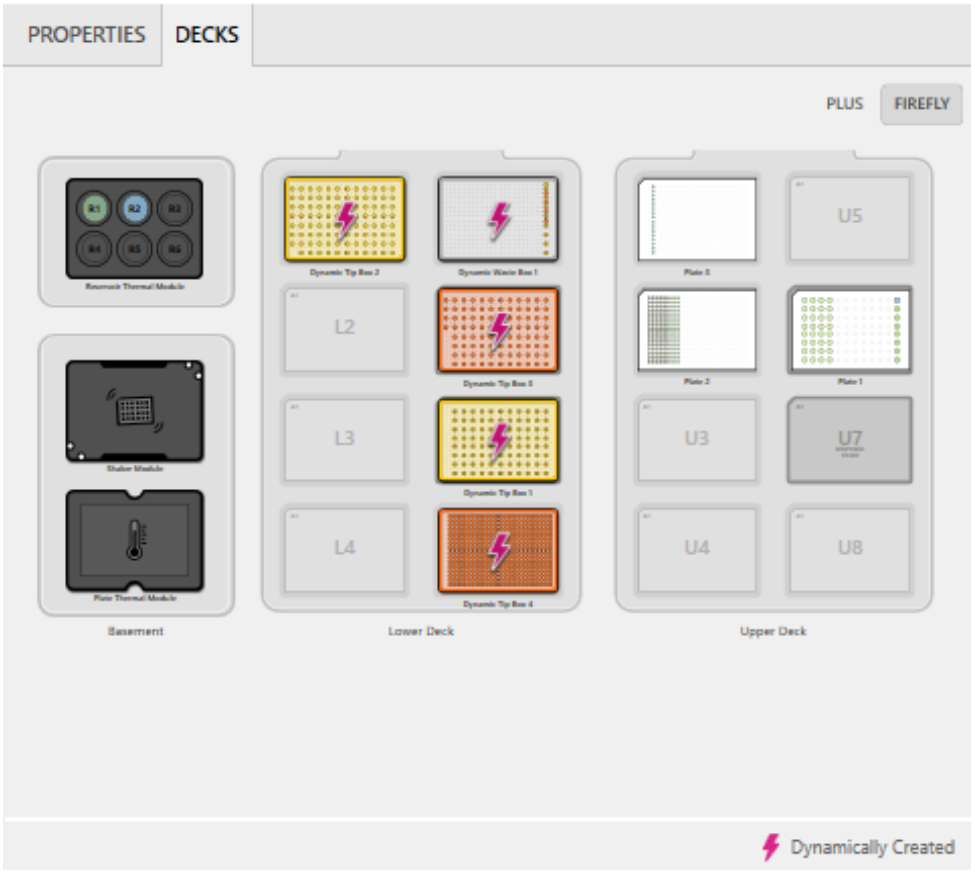


This shows all the steps that will execute, including the Auto Load tip loading and discarding, and any other Auto Move steps which are used in the protocol.

You do not load tip boxes as assets when using Auto Tip Load, and any tip boxes which you have loaded will be ignored. Instead firefly created 'dynamic' tip boxes, as required for the protocol steps after the tips are loaded. It also manages use of tip box adapters.

Property	Form	Notes
Description	Free text	
Behaviour	Select from dropdown list	Use the Discard Loaded Tips option if you want to remove auto loaded tips then switch to using Load Tips i.e. because you want to use a part used tip box.
Tips	Select from dropdown list	By default firefly will select a suitable size of filtered tip to use, but if you prefer to use unfiltered tips, you can select these instead.

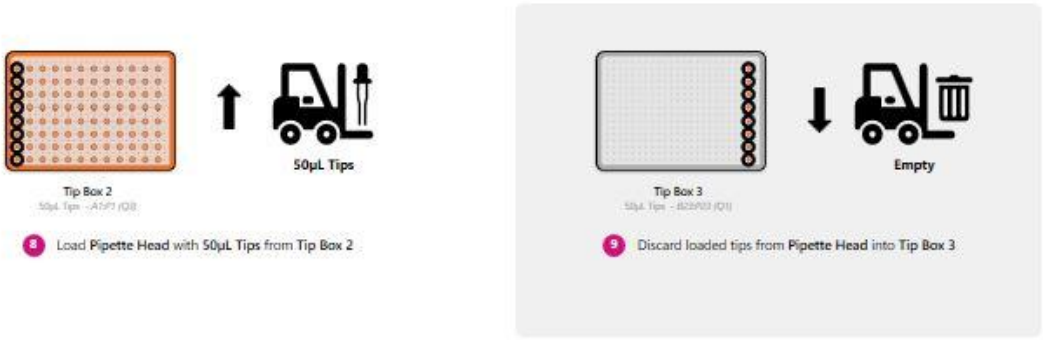
To see the what labware the Auto Load steps have dynamically created, use the Decks tab, behind the Properties tab. This will show you both the labware you have placed on the deck and the labware required by the auto load operations.



Pipetting Head: Load / Discard ATL tips

Load / Change tips enables you to switch to clean pipetting tips. You will need to include this function twice in any protocol which will carry out pipetting operations, to load tips at the start and to unload them at the end of the protocol. Add a Tip Box as an asset, and then drag it to the Load / Discard tips step.

You can use e.g. 3 columns from a tip box, then use the same tip box at a later point in the protocol to load fresh tips, so a single tip box may provide the tips for multiple protocol steps. You will also need to have loaded an empty tip box for unloading the used tips, as you might otherwise risk contaminating unused tips if you unloaded into a partly filled box of new tips (it is possible, but firefly software will generate a warning if you do this). You can select Protocol, Preferences and tick 'Use empty tip boxes as waste' to save having to load an empty tip box.



There are some limitations when loading and unloading ATL tips:

- The pipetting head cannot pick up or hold different tip types at the same time.
- The pipetting head cannot pick up tips that have different tiling patterns at the same time.
- When you discard tips, you must maintain the same tiling pattern, so you cannot e.g., discard 4 sets of 96 tips into a 384 tip box.

Important

You must have placed the tip box on a tip box adapter before you can load more than 3 columns of tips at once, as the decks are not rigid enough to cope with the loading force when picking up more tips.

If you need more tip sets than you have space to load at the start of the protocol, use User Interactions to remove used tip sets and replenish new ones.

You can use up to 8 tip boxes in a protocol, or 24 with firefly+.

You will need to remove the empty tip box from the tip box adapter, and place the replacement tip box on the adapter, using Move and Place On / Take Off steps, if you are working with i.e. full plates.

At the end of a protocol, you must unload the final set of tips. The protocol will not execute if tips remain on the pipetting head at the end of the protocol.

Property	Form	Notes
Description	Free text	
Behaviour	Select from dropdown list	Options are: Load tips, Discard tips
Tips		
Picking method	Select from dropdown list	Options are: Columns, Rows or Single Tip
Tip Box	Auto filled from the drag and drop operation or select from dropdown list	
Columns	Numeric / Variable	See Scaling ATL column usage for using variables
Discard offset	Numeric / Variable	If you are unloading waste tips to the same tip box, adding an offset will position them further from the remaining clean tips.

Consolidating part used tip boxes

You can use Load and Discard steps with no other processes, to combine partly used boxes of tips, or to arrange tips in a certain layout. This saves wasting tips and enables you to control how they are tiled. It also enables you to use Auto Load in your protocols, which requires full tip boxes.

It may also be useful if you want to run a protocol on an ATL head firefly which was originally written for an EZL firefly using strip tips, as there is no direct equivalent available. First design and run the protocol to arrange the tips e.g., to have a single strip of tips in the tip box. Then run the main protocol with that pre-prepared tip box.

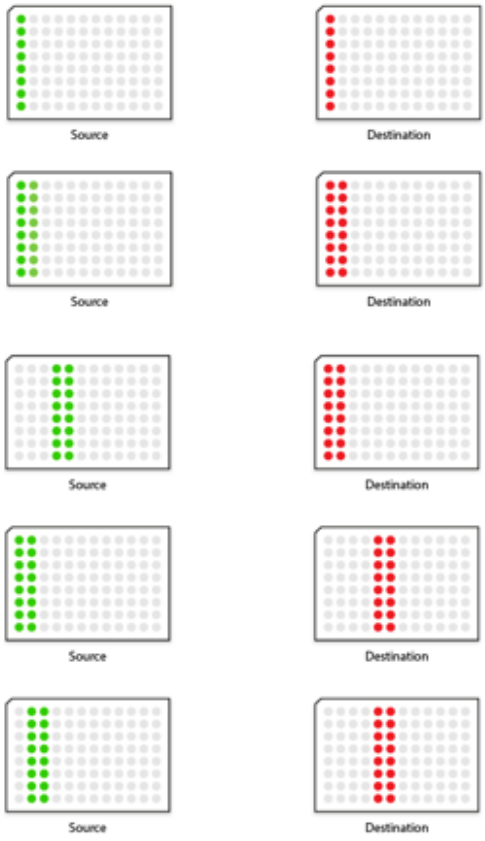
Tip

You do not have to consolidate tip boxes if it would be more convenient to use the partially

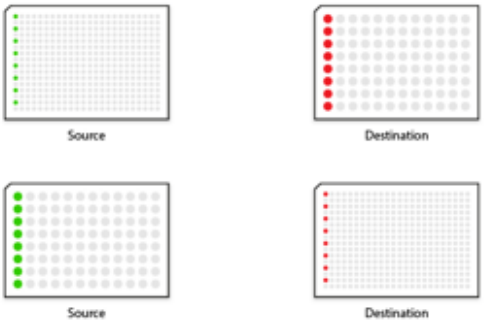
filled ones in protocols with standard Load and Discard tip steps.

Pipetting Head: Copy

Copy combines aspirate and dispense pipetting functions to copy a well array to a second well array, which can be in the same plate or in a second plate. The well arrays must be the same size and shape as shown below.



Copy between plate formats



You can copy between 96 and 384 well plates, if you use an appropriate tiling pattern in the 384 well plate.

If you select a liquid class, other properties will automatically be set to suitable values, though you can edit these if you wish.

Property	Form	Notes
Description	Free text	
Source	Auto filled from the drag and drop operation, but can be edited from dropdown list	The location from which liquid is aspirated.
Destination	Auto filled from the drag and drop operation, but can be edited from dropdown list	The location to which liquid is dispensed.
Volume (µL/tip)	Numeric / Variable	The volume of liquid transferred per well, from the source wells to the destination wells.
Liquid class	Select from dropdown list	Select from all installed liquid classes
Aspirate		
Speed (µL/s)	Numeric	The speed at which liquid is aspirated into the tip.
Blowout volume (µL)	Numeric	A volume of air that is aspirated before the liquid.
Air transport volume (µL)	Numeric	A volume of air that is aspirated after the liquid.
Pause (s)	Numeric	Delay immediately after liquid is aspirated into the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the aspiration.
Tip move speed (mm/s)	Numeric	Speed at which the tips move to the start height for the aspiration.
Keep tips in wells	Tick box	Keeps the tips at the aspirate end height after liquid has been aspirated into the tips. No tip touch will be performed.
Extraction speed (mm/s)	Numeric	Speed at which the tips move upwards after liquid has been aspirated into the tips.
Overshoots		
Enable liquid	Tick box	Primes the position of the piston, which is

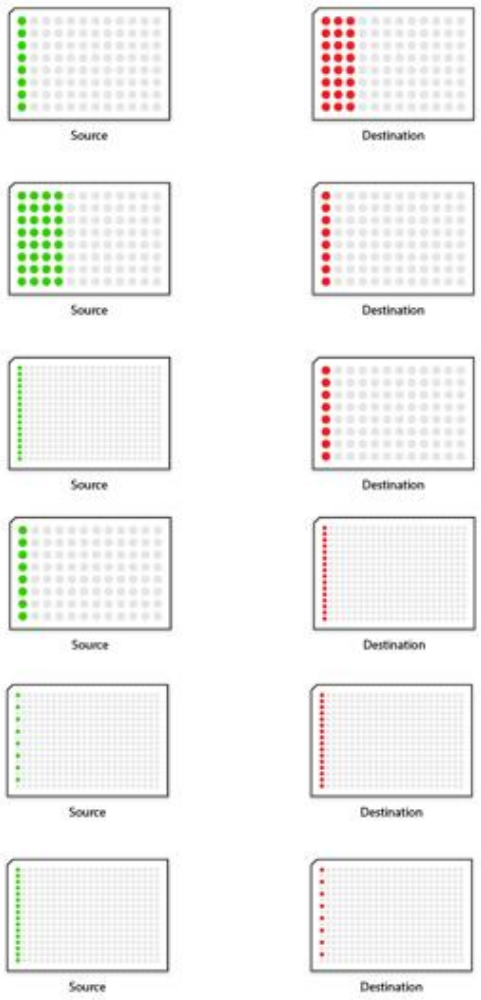
Property	Form	Notes
overshoot		necessary for accurate aspiration.
Enable blowout overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Enable transport overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Tip tracking		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid for the aspiration.
Tip liquid offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Start height (%)	Numeric	Height in the well at which the aspirate will start when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the aspirate will finish when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the aspiration. Specify the side of the well or surface of the liquid.
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.
Dwell period (ms)	Numeric	Duration for which the tips will remain at the tip touch position
Force	Select from dropdown list	The speed at which the tip touch is performed.
Dispense		
Speed (µL/s)	Numeric	The speed at which liquid is dispensed from the

Property	Form	Notes
		tips.
Blowout volume (µL)	Numeric	Volume of air to dispense from the tips after the liquid.
Air transport volume (µL)	Numeric	Volume of air to dispense from the tips before the liquid.
Pause (s)	Numeric	Delay immediately after liquid is dispensed from the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the dispense.
Tip move speed (mm/s)	Numeric	Speed at which the tips move to the dispense start height.
Keep tips in wells	Tick box	Keeps the tips at the dispense end height after liquid has been dispensed from the tips. No tip touch will be performed.
Extraction speed	Numeric	Speed at which the tips move upwards after liquid has been dispensed from the tips.
Tip tracking		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid for the dispense
Tip liquid offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Start height (%)	Numeric	Height in the well at which the dispense will start when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the dispense will finish when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the dispense. Specify the side of the well or surface of the liquid.

Property	Form	Notes
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.
Dwell period (ms)	Numeric	Duration for which the tips will remain at the tip touch position.
Force	Select from dropdown list	The speed at which the tip touch is performed.

Pipetting Head: Aspirate

Aspirate is a simple function to aspirate reagent from a plate well into a pipette tip. You can aspirate from a single plate or perform multiple aspirate steps into the same tips from different wells, so long as there is sufficient volume. You can use the aspirate and dispense functions to pipette between well arrays which are not possible when using Copy.



firefly software will warn you in the Errors panel if you have selected volumes which are incompatible with the tips or wells used.

You can adjust multiple parameters for this step, but the pre-set default values should work for most reagents. If you select a liquid class, other properties will automatically be set to suitable values, though you can edit these if you wish. Use the Advanced properties if you have reagents which are particularly demanding, or for which you already know the optimal pipetting parameters in detail.

Property	Form	Notes
Description	Free text	
Source	Auto filled from the drag and drop operation, but can be edited from	The location from which liquid is aspirated.

Property	Form	Notes
	dropdown list	
Volume (µL/tip)	Numeric / Variable	The volume of liquid transferred per well, from the source wells to the destination wells.
Liquid class	Select from dropdown list	Select from all installed liquid classes
Aspirate		
Speed (µL/s)	Numeric	The speed at which liquid is aspirated into the tip.
Blowout volume (µL)	Numeric	A volume of air that is aspirated before the liquid.
Air transport volume (µL)	Numeric	A volume of air that is aspirated after the liquid.
Pause (s)	Numeric	Delay immediately after liquid is aspirated into the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the aspiration.
Tip move speed (mm/s)	Numeric	Speed at which the tips move to the start height for the aspiration.
Keep tips in wells	Tick box	Keeps the tips at the aspirate end height after liquid has been aspirated into the tips. No tip touch will be performed.
Extraction speed (mm/s)	Numeric	Speed at which the tips move upwards after liquid has been aspirated into the tips.
Overshoots		
Enable liquid overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Enable blowout overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Enable transport overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Tip tracking		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid for the aspiration.

Property	Form	Notes
Tip liquid offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Start height (%)	Numeric	Height in the well at which the aspirate will start when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the aspirate will finish when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the aspiration. Specify the side of the well or surface of the liquid.
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.
Dwell period (ms)	Numeric	Duration for which the tips will remain at the tip touch position
Force	Select from dropdown list	The speed at which the tip touch is performed.

Tip tracking is a facility to move the tip lower into the well as more liquid is aspirated. By keeping the tip a set distance below the surface of the liquid, it is possible to ensure that the sample is aspirated from e.g., a particular layer, if the well contents have been separated.

Pipetting Head: Dispense

Dispense is a simple function to pipette from a tip into a well array.

You do not need to select a source; firefly will use the reagent which has already been aspirated into the pipette head. Selecting a dispense step without a previous aspirate step will cause an error in the protocol.

You can adjust multiple parameters for this step, but the pre-set default values should work for most reagents. If you select a liquid class, other properties will automatically be set to suitable values, though you can edit these if you wish. Use the Advanced properties if you have reagents which are particularly demanding, or for which you already know the optimal dispense parameters in detail.

Property	Form	Notes
Description	Free text	
Destination	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Volume (µL/tip)	Numeric / Variable	The volume of liquid to dispense.
Liquid class	Select from dropdown list	Select from all installed liquid classes
Dispense		
Speed (µL/s)	Numeric	The speed at which liquid is dispensed from the tips.
Blowout volume (µL)	Numeric	Volume of air to dispense from the tips after the liquid.
Air transport volume (µL)	Numeric	Volume of air to dispense from the tips before the liquid.
Pause (s)	Numeric	Delay immediately after liquid is dispensed from the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the dispense.
Tip move speed (mm/s)	Numeric	Speed at which the tips move to the dispense start height.
Keep tips in wells	Tick box	Keeps the tips at the dispense end height after liquid has been dispensed from the tips. No tip

Property	Form	Notes
		touch will be performed.
Extraction speed	Numeric	Speed at which the tips move upwards after liquid has been dispensed from the tips.
Tip tracking		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid for the dispense
Tip liquid offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Start height (%)	Numeric	Height in the well at which the dispense will start when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the dispense will finish when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the dispense. Specify the side of the well or surface of the liquid.
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.
Dwell period (ms)	Numeric	Duration for which the tips will remain at the tip touch position.
Force	Select from dropdown list	The speed at which the tip touch is performed.

Pipetting Head: Mix

Mix aspirates then dispenses repeatedly, to mix the contents of a well. You can set the aspirate and dispense heights within the well to enhance the mixing of reagents in layers.

This step includes an additional aspirate and dispense at the end of the mix, this can be performed at a different speed or height in order to ensure clean tips and prevent tips dripping or being dirty when leaving the plate.

You can adjust multiple parameters for this step, but the pre-set default values should work for most reagents.

Property	Form	Notes
Description	Free text	
Target	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Keep tips in wells	Tick box	Keeps the tips at the dispense end height after liquid has been dispensed from the tips. No tip touch will be performed.
Extraction speed (mm/s)	Numeric	Speed at which the tips move downwards to the mix start height and upwards on completion of the mix.
Pre mix		
Blowout volume (µL)	Numeric	Volume of air aspirated into the tip before the mix.
Mix		
Mix volume (µL/tip)	Numeric / Variable	Volume to aspirate and dispense for the mix.
Mix cycles	Numeric / Variable	Number of times the mix volume is aspirated then dispensed.
Tracking	Tick box	Automated tip tracking
Aspirate height (%)	Numeric	Height from the top of the well that the mix aspiration will start. The well graphic updates with your changed value.
Dispense height (%)	Numeric	Height from the top of the well that the mix dispense will start. The well graphic updates with your changed value.

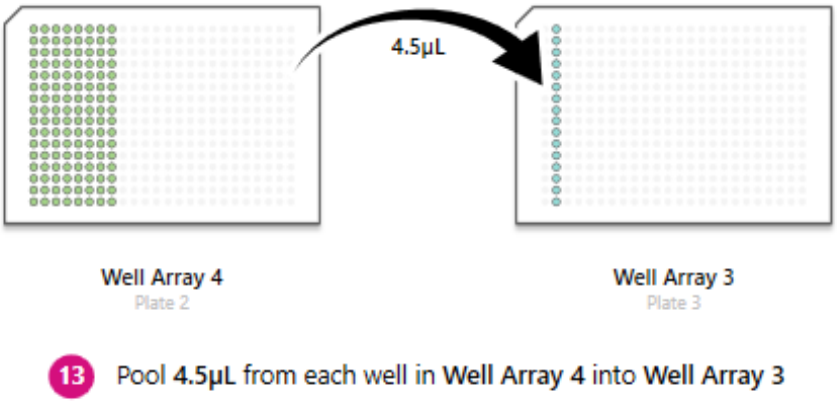
Property	Form	Notes
Aspirate		
Speed (µL/s)	Numeric	Speed at which liquid is aspirated into the tips.
Pause (s)	Numeric	Delay immediately after liquid is aspirated into the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the aspiration.
Dispense		
Speed (µL/s)	Numeric	Speed at which liquid is dispensed from the tips.
Pause (s)	Numeric	Delay immediately after liquid has been dispensed from the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the dispense.
Post Mix		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid for the final aspiration and/or dispense after the mix.
Tip liquid offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Aspirate		
Enable	Tick box	The post mix aspirate is optional.
Volume (µL/tip)	Numeric / Variable	Volume of liquid transferred per well.
Speed (µL/s)	Numeric	The speed at which liquid is aspirated into the tip.
Air transport volume (µL)	Numeric	A volume of air that is aspirated after the liquid.
Pause (s)	Numeric	Delay immediately after liquid is aspirated into the tips.
Start height (%)	Numeric	Height in the well at which the aspirate will start when auto tracking is not used. The well graphic updates with your changed value. Not available when auto tracking is used.

Property	Form	Notes
End height (%)	Numeric	Height in the well at which the aspirate will finish when auto tracking is not used. The well graphic updates with your changed value. Not available when auto tracking is used.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the aspiration.
Dispense		
Enable	Tick box	The post mix dispense is optional
Volume (µL/tip)	Numeric / Variable	Volume of liquid to dispense
Speed (µL/s)	Numeric	Speed at which liquid is dispensed from the tips
Air transport volume (µL)	Numeric	Volume of air to dispense from the tips before the liquid.
Blowout volume (µL)	Numeric	Volume of air to dispense from the tips after the liquid
Pause (s)	Numeric	Delay immediately after liquid is dispensed from the tips.
Start height (%)	Numeric	Height in the well at which the dispense will start when auto tracking is not used. The well graphic updates with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the dispense will finish when auto tracking is not use. The well graphic updates with your changed value. Not available when auto tracking is used.
Tip offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the dispense. Specify the side of the well or surface of the liquid.
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.

Property	Form	Notes
Dwell period (ms)	Numeric	Duration for which the tips will remain at the tip touch position.
Force	Select from dropdown list	The speed at which the tip touch is performed.

Pipetting Head: Pool

Pool pipettes the contents of multiple columns of wells into a single well (you will need to define a well array for this), column or row. firefly will warn you if you exceed the available well volume in the destination plate.



You can pool between different plate formats, if compatible tips are used.

Tip

It is much easier to design protocols incorporating pooling steps, if you are using the Auto Load tips function.

You can adjust multiple parameters for this step, but the pre-set default values should work for most reagents. If you select a liquid class, other properties will automatically be set to suitable values, though you can edit these if you wish. Use the Advanced properties if you have reagents which are particularly demanding, or for which you already know the optimal pipetting parameters in detail.

Property	Form	Notes
Description	Free text	

Property	Form	Notes
Source	Auto filled from the drag and drop operation, but can be edited from dropdown list	The location from which liquid is aspirated.
Destination	Auto filled from the drag and drop operation, but can be edited from dropdown list	The location to which liquid is dispensed.
Volume (µL/tip)	Numeric / Variable	The volume of liquid transferred per well, from the source wells to the destination wells.
Liquid class	Select from dropdown list	Select from all installed liquid classes
Aspirate		
Speed (µL/s)	Numeric	The speed at which liquid is aspirated into the tip.
Blowout volume (µL)	Numeric	A volume of air that is aspirated before the liquid.
Air transport volume (µL)	Numeric	A volume of air that is aspirated after the liquid.
Pause (s)	Numeric	Delay immediately after liquid is aspirated into the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the aspiration.
Tip move speed (mm/s)	Numeric	Speed at which the tips move to the start height for the aspiration.
Keep tips in wells	Tick box	Keeps the tips at the aspirate end height after liquid has been aspirated into the tips. No tip touch will be performed.
Extraction speed (mm/s)	Numeric	Speed at which the tips move upwards after liquid has been aspirated into the tips.
Overshoots		
Enable liquid overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Enable blowout overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.

Property	Form	Notes
Enable transport overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Tip tracking		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid for the aspiration.
Tip liquid offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Start height (%)	Numeric	Height in the well at which the aspirate will start when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the aspirate will finish when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the aspiration. Specify the side of the well or surface of the liquid.
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.
Dwell period (ms)	Numeric	Duration for which the tips will remain at the tip touch position
Force	Select from dropdown list	The speed at which the tip touch is performed.
Dispense		
Speed (µL/s)	Numeric	The speed at which liquid is dispensed from the tips.
Blowout volume (µL)	Numeric	Volume of air to dispense from the tips after the liquid.
Air transport	Numeric	Volume of air to dispense from the tips before

Property	Form	Notes
volume (µL)		the liquid.
Pause (s)	Numeric	Delay immediately after liquid is dispensed from the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the dispense.
Tip move speed (mm/s)	Numeric	Speed at which the tips move to the dispense start height.
Keep tips in wells	Tick box	Keeps the tips at the dispense end height after liquid has been dispensed from the tips. No tip touch will be performed.
Extraction speed	Numeric	Speed at which the tips move upwards after liquid has been dispensed from the tips.
Tip tracking		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid for the dispense
Tip liquid offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Start height (%)	Numeric	Height in the well at which the dispense will start when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the dispense will finish when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the dispense. Specify the side of the well or surface of the liquid.
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.
Dwell period	Numeric	Duration for which the tips will remain at the tip

Property	Form	Notes
(ms)		touch position.
Force	Select from dropdown list	The speed at which the tip touch is performed.

Note

The same tips are used throughout the pooling step. If this is undesirable, do not use the pool function, use separate copy steps with tip changes between each one.

Pipetting Head: Cherry Pick

The cherry pick step defines a collection of transfers from a well in the source well array to a well in the destination well array. The volumes of these transfers may be different for each individual transfer. Each source and destination well can only be mapped once per cherry pick step.

The transfers can be defined in a cherry pick CSV file, or directly in the cherry pick table. If you use a CSV file, the format is:

source well, destination well, volume

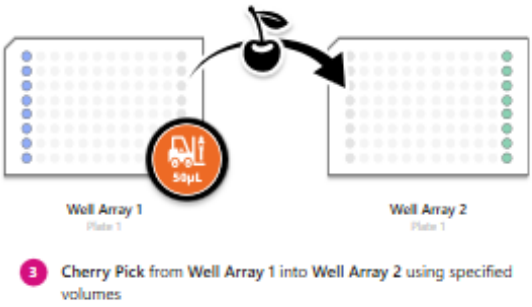
so the following instruction would take the contents of one plate column and reorder it in another.

```
A1,H12,1
B1,G12,2
C1,F12,3
D1,E12,4
E1,D12,5
F1,C12,6
G1,B12,3
H1,A12,3
```

You do not need to specify or identify the plates in the CSV file.

The cherry-pick properties allow you to select whether a new tip is used for each transfer, or the same tip is re-used across all transfers in the step.

- If you select Change Tip between transfers, the cherry-pick step will use a new tip for each transfer. In this mode the picking up/discarding of tips is done automatically as part of the cherry-pick step.

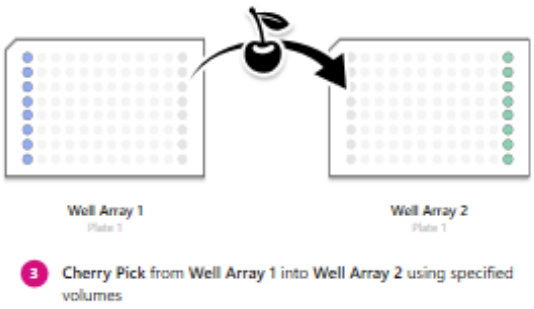


  - You must have turned on Auto Move to use this facility. Each single transfer is a Copy operation and there are multiple moves and loading and unloading

steps required for each one i.e. a cherry pick step with 6 transfers is 24 steps long when executing.

- You do not need to include tip loading and unloading step for the tips required, it is added automatically.

- If you don't tick Change Tip between transfers, the cherry-pick step will use the same tip in the head for all liquid transfers.



- You will need to include a tip loading step before the cherry pick step.

> **Tip**
>
> Using Auto Load is strongly recommended, as it will simplify tip management.

You can adjust multiple parameters for this step, but the pre-set default values should work for most reagents. Use the Advanced properties if you have reagents which are particularly demanding, or for which you already know the optimal pipetting parameters in detail.

Property	Form	Notes
Description	Free text	
Source	Auto filled from the drag and drop operation, but can be edited from dropdown list	The location from which liquid is aspirated.
Destination	Auto filled from the drag and drop operation, but can be edited from dropdown list	The location to which liquid is dispensed.

Property	Form	Notes
Change Tip between transfers	Tick box	See notes above.
Tips	Select from dropdown list	Select from all tip types.
Aspirate		
Speed (µL/s)	Numeric	The speed at which liquid is aspirated into the tip.
Blowout volume (µL)	Numeric	A volume of air that is aspirated before the liquid.
Air transport volume (µL)	Numeric	A volume of air that is aspirated after the liquid.
Pause (s)	Numeric	Delay immediately after liquid is aspirated into the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the aspiration.
Tip move speed (mm/s)	Numeric	Speed at which the tips move to the start height for the aspiration.
Keep tips in wells	Tick box	Keeps the tips at the aspirate end height after liquid has been aspirated into the tips. No tip touch will be performed.
Extraction speed (mm/s)	Numeric	Speed at which the tips move upwards after liquid has been aspirated into the tips.
Overshoots		
Enable liquid overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Enable blowout overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Enable transport overshoot	Tick box	Primes the position of the piston, which is necessary for accurate aspiration.
Tip tracking		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid for the aspiration.
Tip liquid offset	Numeric	Distance of the tips below the liquid surface

Property	Form	Notes
(mm)		when auto tracking is used. Set to zero if auto tracking is not used.
Start height (%)	Numeric	Height in the well at which the aspirate will start when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the aspirate will finish when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the aspiration. Specify the side of the well or surface of the liquid.
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.
Dwell period (ms)	Numeric	Duration for which the tips will remain at the tip touch position
Force	Select from dropdown list	The speed at which the tip touch is performed.
Dispense		
Speed (µL/s)	Numeric	The speed at which liquid is dispensed from the tips.
Blowout volume (µL)	Numeric	Volume of air to dispense from the tips after the liquid.
Air transport volume (µL)	Numeric	Volume of air to dispense from the tips before the liquid.
Pause (s)	Numeric	Delay immediately after liquid is dispensed from the tips.
Tip offset (mm)	Numeric	The distance the tip is offset from the center of the well for the dispense.
Tip move speed (mm/s)	Numeric	Speed at which the tips move to the dispense start height.

Property	Form	Notes
Keep tips in wells	Tick box	Keeps the tips at the dispense end height after liquid has been dispensed from the tips. No tip touch will be performed.
Extraction speed	Numeric	Speed at which the tips move upwards after liquid has been dispensed from the tips.
Tip tracking		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid for the dispense
Tip liquid offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Start height (%)	Numeric	Height in the well at which the dispense will start when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the dispense will finish when auto tracking is not used. The well graphic and the height in mm update with your changed value. Not available when auto tracking is used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the dispense. Specify the side of the well or surface of the liquid.
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.
Dwell period (ms)	Numeric	Duration for which the tips will remain at the tip touch position.
Force	Select from dropdown list	The speed at which the tip touch is performed.
Transfer		
Data file	Select using the popup form	The list of transfers. If you specify this, the transfers will populate the transfer rows below.
Source /	Define the transfer by	You can manually specify Destination and

Property	Form	Notes
Destination / Volume (ml)	double clicking on the volume or the destination	Volume in any order. The Destination must be a well address in the Destination array. firefly will warn you if the total transfer volumes are greater than the source well volumes.

Pipetting Head: Purge

Purge is used to empty remaining reagent from tips e.g., before they are unloaded.

Use the Advanced Tip Tracking and Tip Touch functions if you want to be sure the tips are clean after purging, with no droplets on the tips.

Property	Form	Notes
Description	Free text	
Destination	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Purge		
Purge speed (µL/s)	Numeric	Speed at which liquid is purged from the tips
Pause (s)	Numeric	
Tip tracking		
Auto tracking	Tick box	Automated z-movement to maintain tips beneath the liquid.
Tip liquid offset (mm)	Numeric	Distance of the tips below the liquid surface when auto tracking is used. Set to zero if auto tracking is not used.
Start height (%)	Numeric	Height in the well at which the purge will start when auto tracking is not used. The well graphic updates with your changed value. Not available when auto tracking is used.
End height (%)	Numeric	Height in the well at which the purge will finish when auto tracking is not used. The well graphic updates with your changed value. Not available when auto tracking is used.
Tip touch		
Method	Select from dropdown list	Position the tips will touch in the well after the purge. Specify the side of the well or surface of the liquid.
Height (%)	Numeric	Height from the top of the well at which the tip touch is performed. The well graphic updates with your changed value.
Dwell period	Numeric	Duration for which the tips will remain at the

Property	Form	Notes
(ms)		tip touch position.
Force	Select from dropdown list	The speed at which the tip touch is performed.

Syringe Head: Aspirate

Aspirate is a simple function to aspirate reagent from a reservoir into a syringe.

You can adjust multiple parameters for this step, but the pre-set default values should work for most reagents. Use the Advanced properties if you have reagents which are particularly demanding, or for which you already know the optimal dispense parameters in detail.

Property	Form	Notes
Description	Free text	
Source	[auto filled from the drag and drop operation, not editable]	
Volume (µL)	Numeric / variable	Volume of liquid aspirated.
Preset	Select from dropdown list	Options for specific aspirate tasks
Aspirate		
Speed (mm/s)	Numeric	Aspirate speed of the liquid
Safeguard volume (µL)	Numeric	Additional volume aspirated into the syringe which remains in the syringe after the dispense. When using separate Dispense Head Aspirate and Dispense steps do not alter the default 40µL safeguard volume for the Aspirate to avoid a volume mismatch software error with the Dispense step.
Reservoir extraction speed (mm/s)	Numeric	
Pre wet		
Enable	Tick box	Aspirate then dispense liquid into the syringe to wet the syringe body and plunger. It is required before the initial aspiration of liquid into the syringe.

Property	Form	Notes
Repetitions	Numeric	Number of aspirate/dispense cycles to perform for the pre-wet.
Speed (mm/s)	Numeric	The speed the piston rod moves the plunger up during the prewet. This will change the speed the liquid is aspirated into the syringe.
Pause (ms)	Numeric	Time delay between each aspirate-dispense cycle of the pre-wet. It is not applied after the final repetition of the pre-wet.
Shot distance (mm)	Numeric	The distance the piston rod is forced down by the solenoid for the shot of the pre-wet. Must be less than the up-down distance.
Shot duration (ms)	Numeric	Duration the solenoid is energized to execute the shot of the pre-wet.
Updown distance (mm)	Numeric	Distance the plunger moves up/down for the pre-wet.
Pre dispense 1		
Enable	Tick box	Priming dispense(s) performed prior to dispensing to the destination plate.
Volume (µL)	Numeric	Volume to dispense per repetition of the pre-dispense.
Repetitions	Numeric	Number of times to dispense the pre-dispense volume.
Suckback threshold (µL)	Numeric	The maximum dispense volume to which suckback is applied. For solenoid dispenses only.
Pre Dispense pause (ms)	Numeric	The pause before each pre-dispense repetition. For solenoid dispenses only.
Post Dispense pause (ms)	Numeric	The pause after each pre-dispense repetition. For solenoid dispenses only.
Pre dispense 2		
Enable	Tick box	Priming dispense(s) performed prior to dispensing to the destination plate.
Volume (µL)	Numeric	Volume to dispense per repetition of the pre-dispense.
Repetitions	Numeric	Number of times to dispense the pre-dispense volume.
Suckback	Numeric	The maximum dispense volume to which suckback

Property	Form	Notes
threshold (µL)		is applied. For solenoid dispenses only.
Pre Dispense pause (ms)	Numeric	The pause before each pre-dispense repetition. For solenoid dispenses only.
Post Dispense pause (ms)	Numeric	The pause after each pre-dispense repetition. For solenoid dispenses only.

Syringe Head: Dispense

Dispense is a simple function to dispense from one or more syringes into a well array. Select a reservoir for the source; firefly understands to use the syringe filled from that reservoir, and if there are multiple reservoirs filled with the same reagent, to use all the syringes for faster parallel dispensing.

You can adjust multiple parameters for this step, but the pre-set default values should work for most reagents. Use the Advanced properties if you have reagents which are particularly demanding, or for which you already know the optimal dispense parameters in detail.

Property	Form	Notes
Description	Free text	
Source	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Destination	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Volume (µL)	Numeric / Variable	
Preset	Select from dropdown list	Options for specific tasks
Dispense		
Syringe speed (mm/s)	Numeric	The speed at which the piston rod moves downwards for solenoid dispenses.
Syringe ramp	Select from dropdown list	Acceleration profile of the piston rod for a solenoid dispense.
Max Shot Volume (µL)	Numeric	Maximum volume of a droplet used during a solenoid dispense.
Single Shot Min Volume (µL)	Numeric	Minimum volume of a droplet used during a solenoid dispense.

Property	Form	Notes
Multi Shot Min Volume (µL)	Numeric	Minimum volume of a droplet used during a multi shot solenoid dispense
Pre Dispense Pause (ms)	Numeric	The pause before each dispense. For solenoid dispenses only.
Post Dispense Pause (ms)	Numeric	The pause after each dispense. For solenoid dispenses only.
Syringe Z Offset (mm)	Numeric	Z offset when putting syringe into well automatically does an up-down move in between dispenses.
Syringe Offset (mm)	Numeric	The distance the syringe is offset from the center of the well for the dispense.
Direct drive dispense		
Move Speed (mm/s)	Numeric	The speed at which the piston rod moves downwards for direct drive dispenses
Inter Dispense pause (ms)	Numeric	
Threshold (µL)	Numeric	The volume above which direct drive dispensing is enabled
Auto Finishing Shot	Tick box	This step is optional, though included by default for direct drive dispensing.
Finishing Shot Volume (µL)	Numeric	
Finishing Shot Upper Threshold (µL)	Numeric	

Syringe Head: Fill

Fill is a single step function to aspirate from a reservoir and dispense into a well array. Drag and drop the required assets onto the step and check there are no volumetric errors.

You can adjust multiple aspirate and dispense parameters for this step, but the pre-set default values should work for most reagents.

Property	Form	Notes
Description	Free text	
Source	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Destination	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Volume (µL)	Numeric / Variable	Volume of liquid aspirated.
Preset	Select from dropdown list	Options for specific tasks
Aspirate		
Speed (mm/s)	Numeric	Aspirate speed of the liquid
Safeguard volume (µL)	Numeric	Additional volume aspirated into the syringe which remains in the syringe after the dispense.
Reservoir extraction speed (mm/s)	Numeric	
Pre wet		
Enable	Tick box	Aspirate then dispense liquid into the syringe to wet the syringe body and plunger. It is required before the initial aspiration of liquid into the syringe.
Repetitions	Numeric	Number of aspirate/dispense cycles to perform for the pre-wet.
Speed (mm/s)	Numeric	The speed the piston rod moves the plunger up during the prewet. This will change the speed the liquid is aspirated into the syringe.

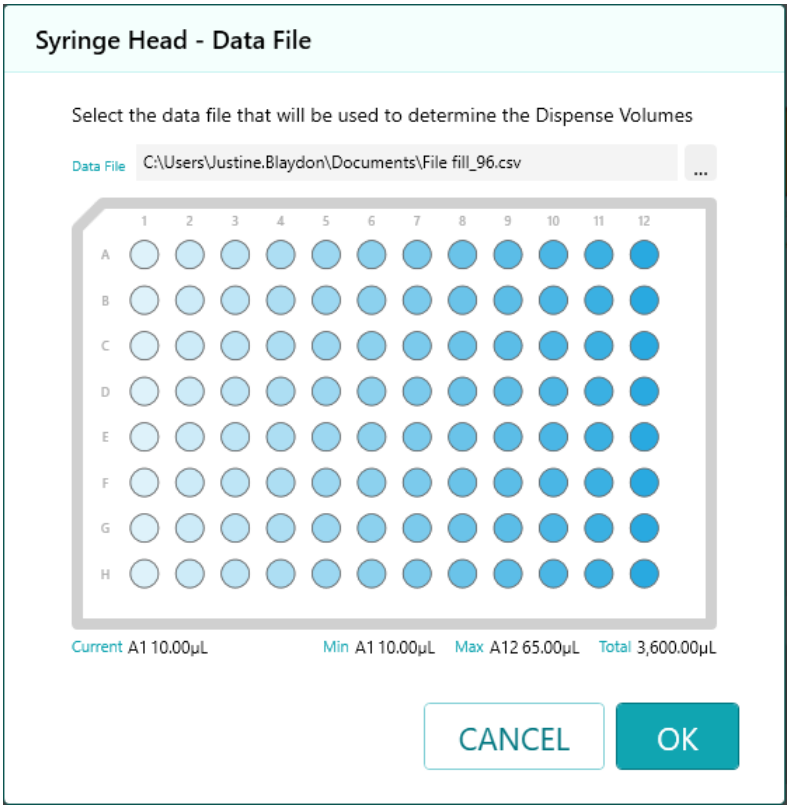
Property	Form	Notes
Pause (ms)	Numeric	Time delay between each aspirate-dispense cycle of the pre-wet. It is not applied after the final repetition of the pre-wet.
Shot distance (mm)	Numeric	The distance the piston rod is forced down by the solenoid for the shot of the pre-wet. Must be less than the up-down distance.
Shot duration (ms)	Numeric	Duration the solenoid is energized to execute the shot of the pre-wet.
Updown distance (mm)	Numeric	Distance the plunger moves up/down for the pre-wet.
Pre dispense 1		
Enable	Tick box	Priming dispense(s) performed prior to dispensing to the destination plate.
Volume (µL)	Numeric	Volume to dispense per repetition of the pre-dispense.
Repetitions	Numeric	Number of times to dispense the pre-dispense volume.
Suckback threshold (µL)	Numeric	The maximum dispense volume to which suckback is applied. For solenoid dispenses only.
Pre Dispense pause (ms)	Numeric	The pause before each pre-dispense repetition. For solenoid dispenses only.
Post Dispense pause (ms)	Numeric	The pause after each pre-dispense repetition. For solenoid dispenses only.
Pre dispense 2		
Enable	Tick box	Priming dispense(s) performed prior to dispensing to the destination plate.
Volume (µL)	Numeric	Volume to dispense per repetition of the pre-dispense.
Repetitions	Numeric	Number of times to dispense the pre-dispense volume.
Suckback threshold (µL)	Numeric	The maximum dispense volume to which suckback is applied. For solenoid dispenses only.
Pre Dispense pause (ms)	Numeric	The pause before each pre-dispense repetition. For solenoid dispenses only.

Property	Form	Notes
Post Dispense pause (ms)	Numeric	The pause after each pre-dispense repetition. For solenoid dispenses only.
Dispense		
Syringe speed (mm/s)	Numeric	The speed at which the piston rod moves downwards for solenoid dispenses.
Syringe ramp	Select from dropdown list	Acceleration profile of the piston rod for a solenoid dispense.
Max Shot Volume (µL)	Numeric	Maximum volume of a droplet used during a solenoid dispense.
Single Shot Min Volume (µL)	Numeric	Minimum volume of a droplet used during a solenoid dispense.
Multi Shot Min Volume (µL)	Numeric	Minimum volume of a droplet used during a multi shot solenoid dispense
Pre Dispense Pause (ms)	Numeric	The pause before each dispense. For solenoid dispenses only.
Post Dispense Pause (ms)	Numeric	The pause after each dispense. For solenoid dispenses only.
Syringe Z Offset (mm)	Numeric	Z offset when putting syringe into well automatically does an up-down move in between dispenses.
Syringe Offset (mm)	Numeric	The distance the syringe is offset from the center of the well for the dispense.
Direct drive dispense		
Move Speed (mm/s)	Numeric	The speed at which the piston rod moves downwards for direct drive dispenses.
Inter Dispense pause (ms)	Numeric	
Threshold (µL)	Numeric	The volume above which direct drive dispensing is enabled.
Auto Finishing Shot	Tick box	This step is optional, though included by default for direct drive dispensing.
Finishing Shot Volume (µL)	Numeric	
Finishing Shot Upper	Numeric	

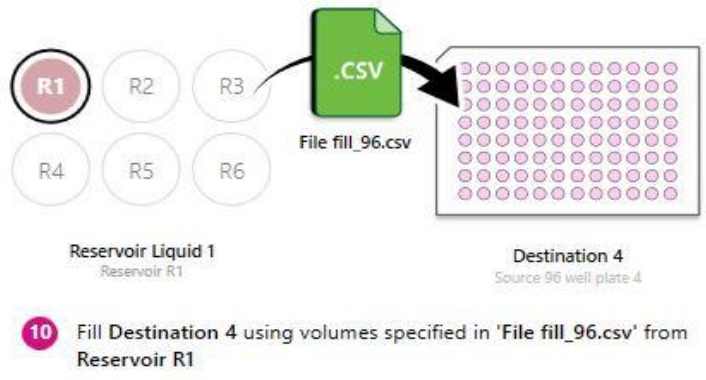
Property	Form	Notes
Threshold (µL)		

Syringe Head: File Fill

File Fill is a variation of the Fill function which aspirates from a reservoir and dispenses variable volumes across the plate which are inputted from a CSV file identified in the Properties. Drag and drop the required assets onto the step and check there are no volumetric errors. When you have selected the CSV file, a popup appears to show you the dispense pattern from the file data.



Once you have chosen a file, the file name is shown in the protocol step.



You can adjust multiple aspirate and dispense parameters for this step, but the pre-set default values should work for most reagents.

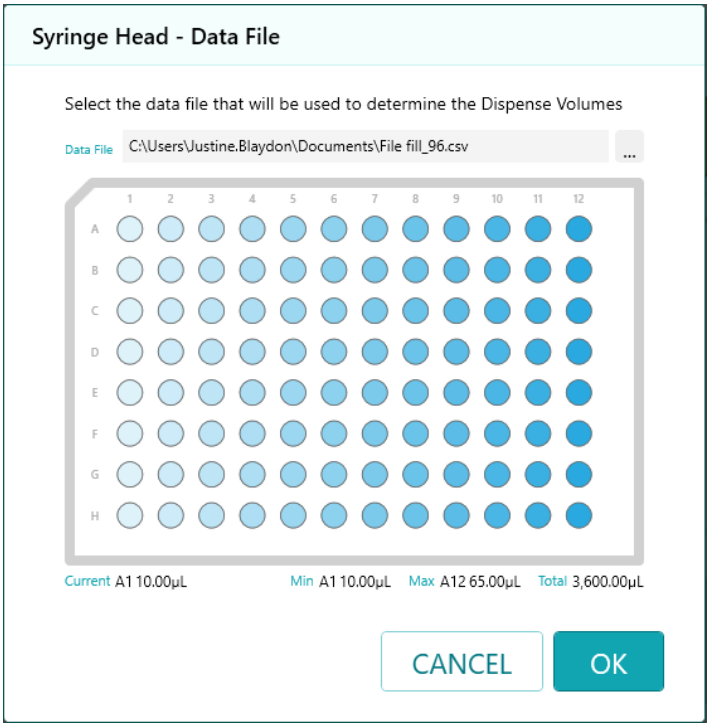
Property	Form	Notes
Description	Free text	
Source	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Destination	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Data File	Select using the pop up form	This is the file defining the dispense volumes.
Preset	Select from dropdown list	Options for specific tasks
Aspirate		
Speed (mm/s)	Numeric	Aspirate speed of the liquid
Safeguard volume (µL)	Numeric	Additional volume aspirated into the syringe which remains in the syringe after the dispense.
Reservoir extraction speed (mm/s)	Numeric	
Pre wet		
Enable	Tick box	Aspirate then dispense liquid into the syringe to wet the syringe body and plunger. It is required before the initial aspiration of liquid into the syringe.
Repetitions	Numeric	Number of aspirate/dispense cycles to perform for the pre-wet.
Speed (mm/s)	Numeric	The speed the piston rod moves the plunger up during the prewet. This will change the speed the liquid is aspirated into the syringe.
Pause (ms)	Numeric	Time delay between each aspirate-dispense cycle of the pre-wet. It is not applied after the final repetition of the pre-wet.

Property	Form	Notes
Shot distance (mm)	Numeric	The distance the piston rod is forced down by the solenoid for the shot of the pre-wet. Must be less than the up-down distance.
Shot duration (ms)	Numeric	Duration the solenoid is energized to execute the shot of the pre-wet.
Updown distance (mm)	Numeric	Distance the plunger moves up/down for the pre-wet.
Pre dispense 1		
Enable	Tick box	Priming dispense(s) performed prior to dispensing to the destination plate.
Volume (µL)	Numeric	Volume to dispense per repetition of the pre-dispense.
Repetitions	Numeric	Number of times to dispense the pre-dispense volume.
Suckback threshold (µL)	Numeric	The maximum dispense volume to which suckback is applied. For solenoid dispenses only.
Pre Dispense pause (ms)	Numeric	The pause before each pre-dispense repetition. For solenoid dispenses only.
Post Dispense pause (ms)	Numeric	The pause after each pre-dispense repetition. For solenoid dispenses only.
Pre dispense 2		
Enable	Tick box	Priming dispense(s) performed prior to dispensing to the destination plate.
Volume (µL)	Numeric	Volume to dispense per repetition of the pre-dispense.
Repetitions	Numeric	Number of times to dispense the pre-dispense volume.
Suckback threshold (µL)	Numeric	The maximum dispense volume to which suckback is applied. For solenoid dispenses only.
Pre Dispense pause (ms)	Numeric	The pause before each pre-dispense repetition. For solenoid dispenses only.
Post Dispense pause (ms)	Numeric	The pause after each pre-dispense repetition. For solenoid dispenses only.
Dispense		

Property	Form	Notes
Syringe speed (mm/s)	Numeric	The speed at which the piston rod moves downwards for solenoid dispenses.
Syringe ramp	Select from dropdown list	Acceleration profile of the piston rod for a solenoid dispense.
Max Shot Volume (µL)	Numeric	Maximum volume of a droplet used during a solenoid dispense.
Single Shot Min Volume (µL)	Numeric	Minimum volume of a droplet used during a solenoid dispense.
Multi Shot Min Volume (µL)	Numeric	Minimum volume of a droplet used during a multi shot solenoid dispense
Pre Dispense Pause (ms)	Numeric	The pause before each dispense. For solenoid dispenses only.
Post Dispense Pause (ms)	Numeric	The pause after each dispense. For solenoid dispenses only.
Syringe Z Offset (mm)	Numeric	Z offset when putting syringe into well automatically does an up-down move in between dispenses.
Syringe Offset (mm)	Numeric	The distance the syringe is offset from the center of the well for the dispense.
Direct drive dispense		
Move Speed (mm/s)	Numeric	The speed at which the piston rod moves downwards for direct drive dispenses.
Inter Dispense pause (ms)	Numeric	
Threshold (µL)	Numeric	The volume above which direct drive dispensing is enabled.
Auto Finishing Shot	Tick box	This step is optional, though included by default for direct drive dispensing.
Finishing Shot Volume (µL)	Numeric	
Finishing Shot Upper Threshold (µL)	Numeric	

Syringe Head: File Dispense

File Dispense is a variation of the Dispense function which dispenses variable volumes across the plate which are inputted from a CSV file identified in the Properties. Drag and drop the required assets onto the step and check there are no volumetric errors. When you have selected the CSV file, a popup appears to show you the dispense pattern from the file data.



Once you have chosen a CSV file, the file name is shown in the protocol step.

You can adjust multiple dispense parameters for this step, but the pre-set default values should work for most reagents.

Property	Form	Notes
Description	Free text	
Source	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Destination	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Data File	Select using the pop up form	This is the file defining the dispense

Property	Form	Notes
		volumes.
Preset	Select from dropdown list	Options for specific tasks
Dispense		
Syringe speed (mm/s)	Numeric	The speed at which the piston rod moves downwards for solenoid dispenses.
Syringe ramp	Select from dropdown list	Acceleration profile of the piston rod for a solenoid dispense.
Max Shot Volume (µL)	Numeric	Maximum volume of a droplet used during a solenoid dispense.
Single Shot Min Volume (µL)	Numeric	Minimum volume of a droplet used during a solenoid dispense.
Multi Shot Min Volume (µL)	Numeric	Minimum volume of a droplet used during a multi shot solenoid dispense
Pre Dispense Pause (ms)	Numeric	The pause before each dispense. For solenoid dispenses only.
Post Dispense Pause (ms)	Numeric	The pause after each dispense. For solenoid dispenses only.
Syringe Z Offset (mm)	Numeric	Z offset when putting syringe into well automatically does an up-down move in between dispenses.
Syringe Offset (mm)	Numeric	The distance the syringe is offset from the center of the well for the dispense.
Direct drive dispense		
Move Speed (mm/s)	Numeric	The speed at which the piston rod moves downwards for direct drive dispenses
Inter Dispense pause (ms)	Numeric	
Threshold (µL)	Numeric	The volume above which direct drive dispensing is enabled
Auto Finishing Shot	Tick box	This step is optional, though included by default for direct drive dispensing.
Finishing Shot	Numeric	

Property	Form	Notes
Volume (µL)		
Finishing Shot Upper Threshold (µL)	Numeric	

Syringe Head: Purge

Purge is used to empty the remaining reagent from syringes. You must do this before they are removed.

The pre-set default values should work for most reagents. Use the Advanced properties if you have reagents which are particularly demanding.

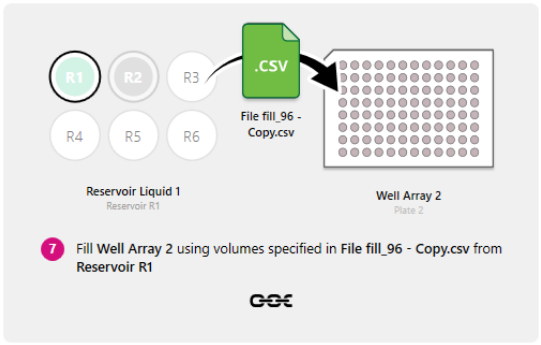
Property	Form	Notes
Description	Free text	
Final Shot Duration (ms)	Numeric	Duration the solenoid is energized.
Source	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Speed (mm/s)	Numeric	

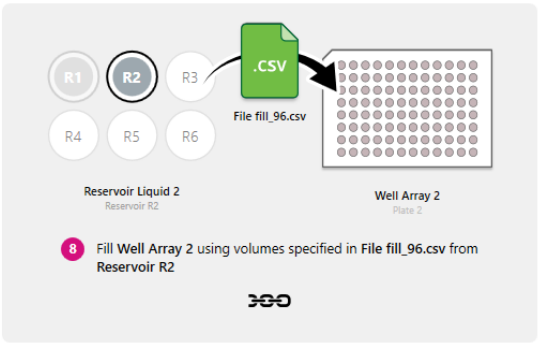
Linked dispensing

If you want to use multiple reagents with one plate, you can perform Aspirate, Dispense, File Fill, File Dispense or Purge steps concurrently.

1. Create consecutive steps, two or more of the same type. You can aspirate or purge using different reservoirs, but you must fill or dispense into the same plate.
2. Select (shift + click) the steps you want to link. When you have done this, the Link function is enabled (on the left)

3. Click Link and the steps will be linked. Linked steps show a chain-link icon below the text.





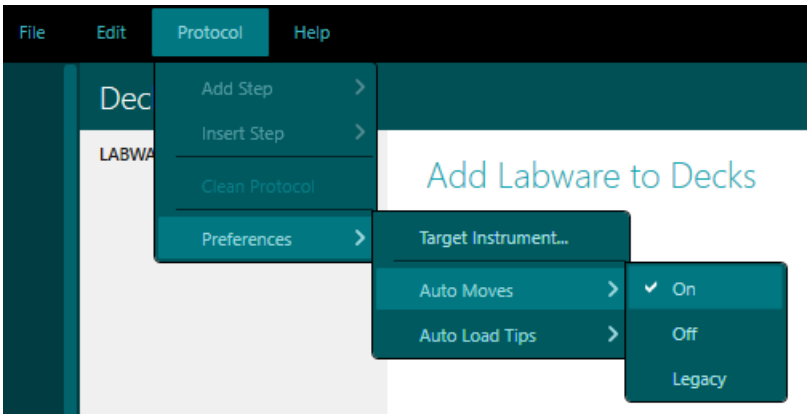
The steps will be performed concurrently not consecutively, speeding up protocol completion.

To unlink steps, select one or more of the linked steps and the Unlink function (on the right) will be enabled. Click this to unlink.

Auto Move

Using Auto Move removes the need to add any labware movement steps, when designing protocols, both saving effort and making protocol files considerably easier to view and understand, as the only steps shown are the functional ones e.g. mixing, chilling, dispensing etc..

To turn on Auto Moves, use the Protocol menu item.



Tip

For designing new protocols, setting Auto Moves to On is recommended. If you turn it off, you will have to specify every labware movement required for your protocol, which will quickly become very complex.

You can turn on Auto Move and still continue to use specific Move, Place On and Take Off steps in your protocols, e.g. because you want to position a plate on the deck so it can be easily removed in a subsequent User Interaction. Auto Move will not alter your deliberately added moves.

The Auto Move algorithm takes into consideration all the requirements for using the labware in subsequent steps e.g. that a tip box will need to be on a tip box adapter before tips can be loaded. In general, it uses restrictions designed to ensure that any auto-moves carried out cannot alter the function (or meaning) of your protocol design.

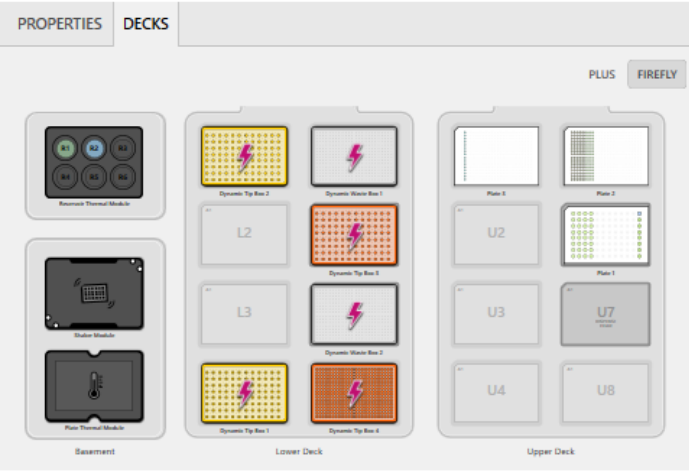
Auto-moves must follow these rules:

- Auto-moves can only break or form stacks that do not affect protocol function.
    - Auto-moves can take an item off or put an item onto a riser (it will not stack incompatible items)
    - Auto-moves can take a tip box off or put a tip box on to a tip box adapter
    - No other labware stacks may be altered by auto-moves (except to take an item off a stack in a specific Take Off step)
- Auto-moves follow all other physical restrictions on labware in firefly
- Auto-moves can move entire labware stacks to any empty deck or firefly+ location
- Auto-moves cannot take items off or put items on to the plate thermal module or shaker.
- For take-off and move steps which specifically reference an item which is on the thermal module or shaker, auto-moves is allowed to remove the specified item (and those above it).

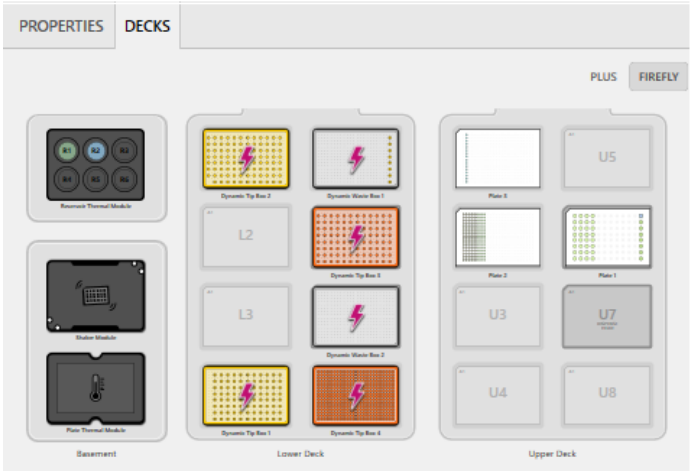
Note

Auto-moves cannot move items which are not compatible with firefly's gripper (e.g. EZL tip boxes, lower deck risers, tip box adapters, etc.). You will need to create specific Move steps to reposition any of these items.

Use the Decks tab (behind Properties) if you want to see the results of auto moves. You will see the deck change from step to step in your protocol if you have auto moves enabled.



step 1



step 9

Auto Move: Stack Definitions

Some of the auto-moves which may be considered will result in the formation of stacks.

All stacks require a stack definition to be available for each pair of items involved. This allows firefly to calculate the necessary combined heights and offsets to allow the grippers to correctly perform the place-on/take-off operations.

The auto-moves algorithm will avoid creating any moves which result in stacks for which a stack definition is not already installed in the firefly app or in the protocol.

If you realise that a particular stack is unavailable and that it is hindering auto-moves within your protocol, you can install the stack definition from Community to allow auto-moves to begin using moves which form this stack.

If a particular stack is essential for a particular step (e.g. a tip box requiring placement on top of a tip box adapter) a validation error will be displayed indicating you must install the specified stack.

Auto Move: Legacy mode

If you are using a protocol designed for firefly v1.8 or v1.9 software, you should set Auto Move to 'Legacy' as this will be compatible with the extent of auto-moves implemented in earlier versions of the software.

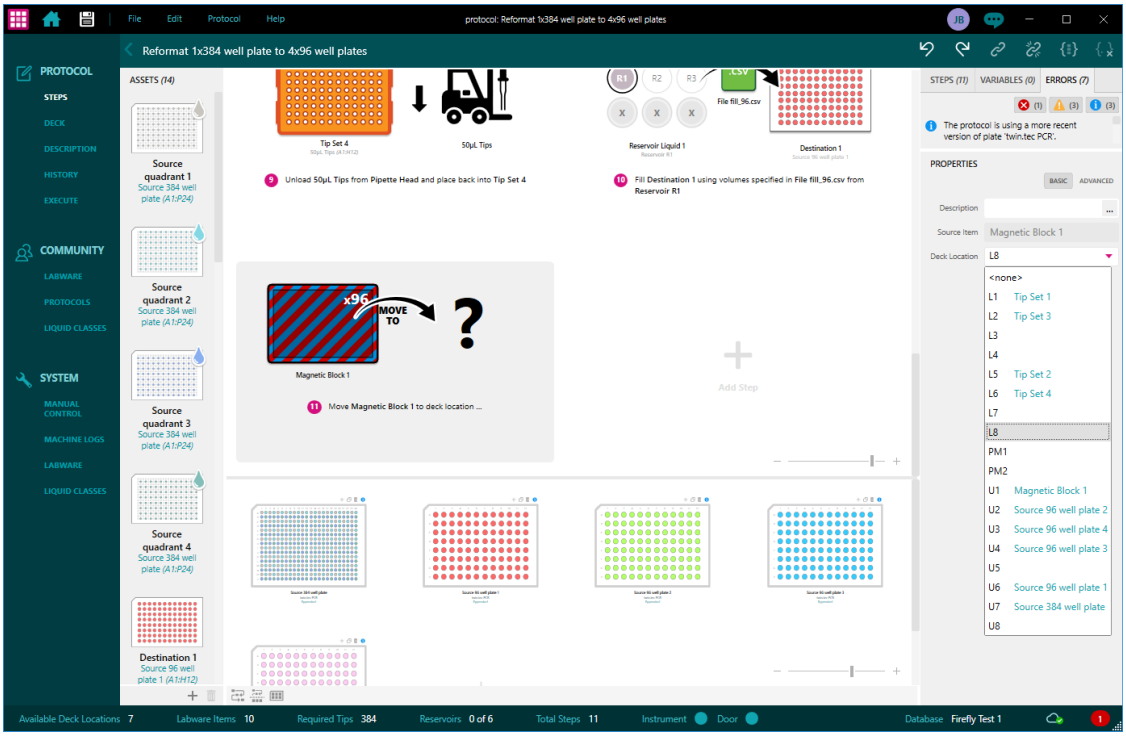
Other: Move

Move is a versatile function which you can use to:

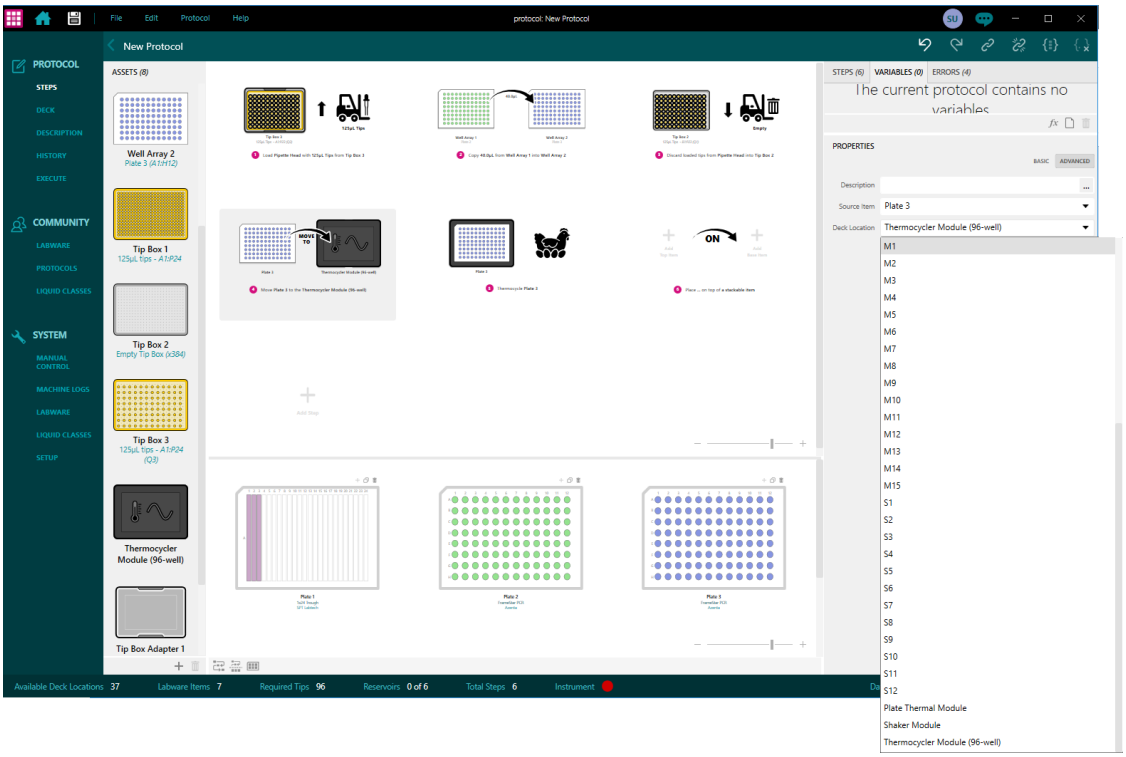
- reposition assets on the same deck
- move assets between decks
- move assets between firefly and firefly+
- move assets on and off the shaker and the plate thermal module
- move assets on and off the thermal cycler
- create stacks (the same as Place On)
- remove assets from stacks (the same as Take Off)

Property	Form	Notes
Description	Free text	
Source Item	Auto filled from the drag and drop operation, but can be edited from dropdown list	
Deck Location	Select from dropdown list, including firefly+ locations if applicable	

Moves are validated so that you do not damage firefly or your labware e.g., you cannot move an EZL tip set to a location on the upper deck as it is too tall. When you select to move an asset, the software shows you a dropdown list of possible destination locations, and which of them are already occupied.



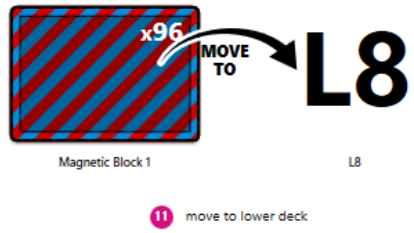
For firefly+ instruments, this is modified to include all firefly+ locations, plus the thermal cycler as possible destinations.



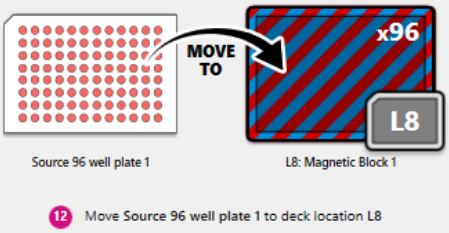
Possible locations for labware items are shown below:

	Shaker Module	Plate Thermal Module	Reservoir Thermal Module	ODTC Module	Lower Deck	Upper Deck	firefly+ Expansion Module (M1-M15)	firefly+ Expansion Module (S1-S12)	firefly+ ODTC
Lids	Y	Y	-	Y	Y	Y	Y	Y	Y
Plates	Y	Y	-	Y	Y	Y	Y	Y	Y
Magnetic Blocks	-	-	-	-	Y	Y	-	-	-
Thermal Blocks	Y	Y	-	-	Y	Y	-	-	-
Risers	-	-	-	-	Y	Y	-	-	-
EZ Tip Boxes	-	-	-	-	Y	-	-	-	-
ATL Tip Boxes	-	-	-	-	Y	Y	Y	-	-
LV Tip Boxes	-	-	-	-	-	-	-	-	-
Tip Box Adapters	-	-	-	-	Y	-	-	-	-
Cassettes	-	-	-	-	Y	-	-	-	-
Cassette Stands	-	-	-	-	Y	-	-	-	-

You cannot choose to move an asset to a deck location which is occupied unless the two assets can form a stack e.g., a 96 well plate on a 96 well magnetic block.



11 move to lower deck



12 Move Source 96 well plate 1 to deck location L8

If you create an undefined stack, the software will raise an error telling you that you need to download the stack definition. You do not have to do this immediately; you can continue designing but you will not be able to run the protocol without it.

firefly may need to use an intermediate location e.g. for firefly+ grippers to set down a plate which is then picked up by firefly grippers, but you do not need to specify this in your move instructions. firefly software will warn you if you design a move which is not possible with the currently empty deck locations.

firefly+ grippers move labware between the shelves, to and from firefly, and on and off the On Deck Thermal Cycler, if fitted. Possible moves are listed below. firefly+ grippers only

have limited place on / take off capabilities so you will need to use firefly deck locations and firefly grippers to create stacks.

Step	Item Being Moved	Destination Item	ODTC	firefy+ (M1-M3)	firefy+ (M4-M15)	firefy+ (S1-S12)	Lower Deck (L2, L3)	Upper Deck (U2, U3)
MOVE	PLATE	-	YES	YES	YES	YES	YES	YES
MOVE	LID	-	YES	YES	YES	YES	YES	YES
MOVE	PLATE+LID	-	YES	YES	YES	YES	YES	YES
MOVE	TIPBOX	-	NO	YES	YES	NO	YES	YES
PLACE ON	LID	PLATE	YES	NO	NO	NO	NO	NO
PLACE ON	PLATE	MAG BLK	NO	NO	NO	NO	NO	NO
PLACE ON	PLATE	THRM BLK	NO	NO	NO	NO	NO	NO
PLACE ON	PLATE	RISER	NO	NO	NO	NO	NO	NO
PLACE ON	TIPBOX	TB ADAPTER	NO	NO	NO	NO	NO	NO
TAKE OFF	LID	PLATE	YES	NO	NO	NO	YES	YES
TAKE OFF	PLATE	MAG BLK	NO	NO	NO	NO	NO	NO
TAKE OFF	PLATE	THRM BLK	NO	NO	NO	NO	NO	NO
TAKE OFF	PLATE	RISER	NO	NO	NO	NO	NO	NO
TAKE OFF	TIPBOX	TB ADAPTER	NO	NO	NO	NO	YES	NO

*All info in the table assumes adequate deck clearance for the step to be performed

Other: Place on

Place on is a simple function to instruct the grippers to pick up one item and place it on another. Drag and drop Assets to the top and bottom item positions: if you attempt something which is not possible e.g., making the plate thermal module a top item, the software will not let you drop the item. Allowed combinations are:

- plates can go on the decks, firefly+ shelves, risers, thermal block, magnetic block, the shaker and the thermal cycler
- lids can go on the decks or plates; clean, unused lids can also go on firefly+ shelves. Used lids can be placed on a waste plate, or directly on the shelves if you do not have concerns about contamination.

Note

firefly+ grippers cannot place a lid on a plate on any of the firefly+ shelves, so if you want to put a lid on a waste plate to prevent contamination, you will need to do this while both the lid and the plate are in firefly deck locations.

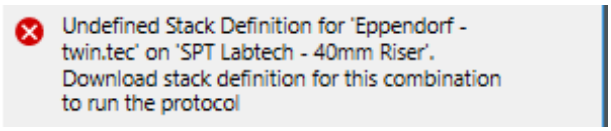
- magnetic blocks can only go onto the decks, not the firefly+ shelves
- thermal blocks can go onto the decks, firefly+ shelves or the plate thermal module
- ATL tip boxes can be placed on tip box adapters, or directly on decks or shelves

Property	Form
Description	Free text
Top item	Auto filled from the drag and drop operation, but can be edited from dropdown list
Base item	Auto filled from the drag and drop operation, but can be edited from dropdown list

Note

You cannot place a trough plate on the shaker, the plate thermal module or the ODTC, if fitted.

When you use Place on, you create a stack. Check the Errors panel: if you have created an undefined stack, you will need to download the definition from Community. You do not have to do this immediately; you can continue designing but you will not be able to run the protocol without it.



Other: Take off

Take off is a simple function to instruct the grippers to pick up a stacked item and remove it. Drag and drop an Asset to the bottom item position and firefly software will automatically identify the item directly above it, to be removed. If the stack is plate thermal module - thermal block - plate, if you select the module as the base item, it will remove both the block and the plate as a small stack.

Property	Form
Description	Free text
Top item	Auto filled from the drag and drop operation, but can be edited from dropdown list
Base item	Auto filled from the drag and drop operation, but can be edited from dropdown list
Takeoff location	Set automatically if Auto Moves is enabled

Other: Heat / Cool

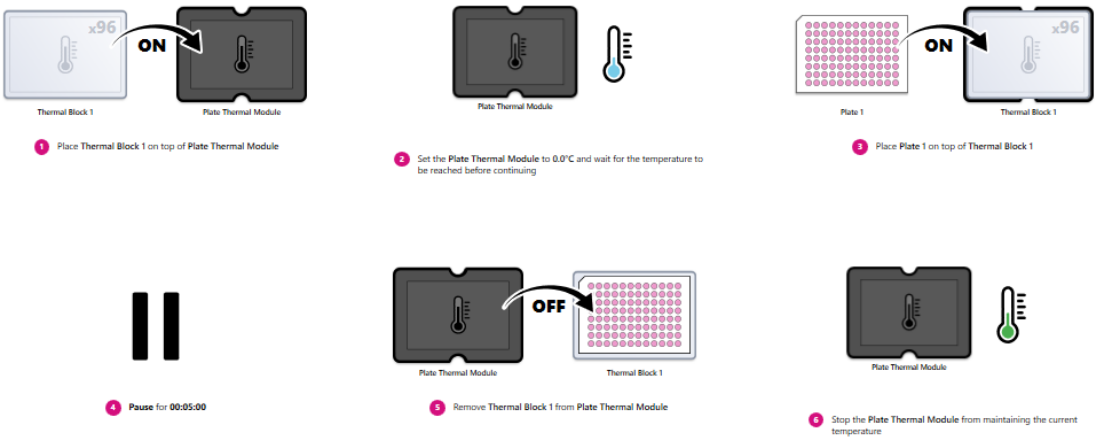
Use Heat / Cool to control the warming or cooling of the thermal blocks during protocol execution.

Use Properties to set the temperature and behavior:

- Start and continue' to have the protocol continue with the next step while the thermal module gets to temperature. If you do this, be aware that depending on your protocol design, the thermal module may be used before your set temperature is reached. You could use a pause to delay using the plate thermal module or aspirating from the reagent reservoirs if temperature is process critical, while enabling other actions e.g., tip loading or user interactions to take place.
- 'Start and wait' means that firefly will do nothing until the temperature setpoint is reached. Heating or cooling rates are dependent on the plate types and reagents used but for example a 96 well plate filled with 50µL water is cooled from 24°C to 4°C in about 5 minutes.

When you run your protocol, the software will prompt you to start heating or cooling the thermal modules(s) in use as part of the protocol setup. If you know that they will not be needed until late in a long protocol, you may decide not to start them immediately.

Use a Move or Place On step to put a thermal block on the plate thermal module so that it is heated or cooled before you use another Move or Place On to put the plate on the thermal block. Alternatively make a stack with the plate and thermal block and place them on at the same time but then the thermal block will be heating or cooling while the plate does.



If you have the plate thermal module behavior as 'Start and continue', your plate will be kept at the set temperature while you carry out subsequent steps. You can use a Pause

step if you want to continue heating or cooling for a defined period, or if you don't need any concurrent actions.

Use another Heat / Cool step if you want to modify the temperature while the plate is on the plate thermal module.

Use Move or Take Off to remove the plate, or select the thermal block to remove and the plate together as a stacked object.

Property	Form	Notes
Description	Free text	
Thermal module	Select from dropdown list	plate thermal module, reservoir thermal module
Behaviour	Select from dropdown list	Start & wait, Start & continue, Stop
Temperature	Numeric	-20°C to 99°C in steps of 0.1°C

Use a Heat / Cool step with 'Stop' behavior to stop the plate thermal module from heating or cooling once you finish using it. Do not set the temperature. This will minimize the heat emissions within firefly and help to keep the internal temperature stable. If you have heated the plate thermal module, this will cool it as soon as possible, making user access to the basement safer.

Other: Shake

To use the Shaker Module, you must first use Move or Place On to move the plate to it.

> **Note**
>
> You cannot place trough plates on the shaker.

Use Shake to control the plate shaking process by setting the behavior properties:

- Choose 'Start and continue' to have the protocol continue with the next step while the Shaker is shaking,
    - If you want shaking to continue for the duration of the subsequent step or steps and only stop when that is complete, set the Duration to a much longer period than those steps will require, then include a Shake step with Stop as the behavior after the last of those steps.
- Choose 'Start and wait', to have firefly do nothing until the shake duration is completed.

Then use Move or Take Off to remove the plate from the shaker module.

Property	Form	Notes
Description	Free text	
Behaviour	Select from dropdown list	Start & wait, Start & continue, Stop
Speed	Numeric	Possible values are 200 - 3000rpm
Duration (hours, minutes, seconds)	hours:minutes:seconds	Possible values are 1 second - 23 hours, 59 mins, 59 seconds

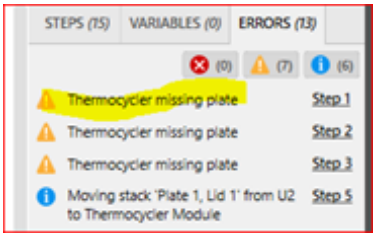
Other: Incubate

This is only enabled on firefly+ instruments with a thermal cycler.

To use the thermal cycler, you will need to write both a pre- method and a main method, using the Inheco ODTC Script Editor 3. You can choose whether to run these separately, or consecutively, in the same Incubate step. The pre- method sets the conditions for the main method, most obviously the starting temperature for the main method.

You can also download an ODTC protocol as part of a Community firefly protocol. It will be downloaded to the ODTC unit and all previous methods are deleted from the unit.

You can run the ODTC without loading a plate; the software will warn you but the protocol will run.



You will need to run a drying protocol with no plate loaded, if there is condensation in the ODTC from the previous protocol.

Use Behaviour to define what firefly does while the thermal cycler method runs:

- Open lid opens the ODTC lid. You need a step with this behavior before you load a plate.
- Close lid is an optional step, as you can run an ODTC protocol with the lid open.
- Choose 'Execute and continue' to have the firefly protocol continue with the next step while the thermal cycler runs.
- 'Execute and wait' means that firefly will do nothing until the thermal cycler method has completed.
- 'Stop' stops the incubation protocol.

Property	Form	Notes
Description	Free text	
Behaviour	Select from dropdown list	Stop, Open lid, Close lid, Execute & continue, Execute & wait
Method File	Select using the pop up form	This is the file defining the thermal cycle steps.
Method Type	Select from dropdown list	Run pre method, Run main method or Run both. If you select to run both, you will need a method file which contains both pre and main method instructions.

Note

If you have an ODTC hold step in an ODTC protocol and use a 'Start and wait' property in the incubate step, the firefly protocol will only wait for the ODTC protocol steps. It does not continue to wait during the ODTC Hold command.

Other: User interaction

Create a User interaction step whenever you need the user to add or remove materials from firefly, to remove the plate for processing on a different instrument e.g., a thermocycler or any other manual task.

When the protocol runs, the Description will be shown to the user as instructions. When they click Done, they will be confirming that they have completed all the manual tasks, and that firefly should continue running the protocol.

Use the Rest state property to enable safe access to firefly. As it is not possible to easily access all areas of the instrument at the same time, setting this property will ensure decks and heads are moved clear of the labware requiring user interaction.

- If you need to access both the reservoirs and the upper deck, create two consecutive user interaction steps, one for each area, setting the rest state for each area in turn.
- If you do not want the heads moved, or any disruption to firefly hardware during the user interaction, select 'Do nothing'. This may make access more awkward, and you might want to warn the user about that in the User Interaction instructions.

Property	Form	Notes
Description	Free text	Use for the user instructions
Rest state	Select from dropdown list	List of possible instrument areas to access. Home will return both heads and decks to their home positions, also the firefly+ grippers if the instrument has them.

Other: Script

Script is a simple function to call a batch script (.bat file), PowerShell script (.ps1 file) or executable file (.exe). Designing and coding scripts is outside the scope of this manual, but the capability is there if your lab requires it.

> **Note**
>
> Your system administrator will need to enable the 'Can execute protocols containing script steps' privilege for super users and / or users, to allow them to run protocols which include scripts.

A simple example script might be to send firefly messages to Microsoft Teams (you would need to set up a firefly Incoming Webhook to use the notifier script). You could use it as the final step in a protocol, to notify that the running protocol is about to complete.

```
# Replace with your actual Teams Incoming Webhook URL
$teamsWebhookUrl = 'https://outlook.office.com/webhook/your-webhook-url-goes-here'

# Build the payload with a simple fixed message
$payload = @{
    title = "Lab Notification"
    text  = "☑ Firefly protocol is complete."
} | ConvertTo-Json -Depth 3

# Send the message to Teams
try {
    Invoke-RestMethod -Uri $teamsWebhookUrl -Method Post -ContentType 'application/json' -Body $payload
    Write-Host "Firefly completion message sent to Teams successfully."
} catch {
    Write-Error "Failed to send message to Teams: $_"
}
```

Property	Form	Notes
Description	Free text	
Script File	Select using the pop up form	This is the script file.
Abort Protocol on Script Failure	Tick box	

Important

You use the script function at your own risk.

firefly does not have any way of checking whether a script is valid and will execute correctly. Only include scripts in your protocol if you have verified that they run correctly, as firefly cannot handle errors generated by scripts.

Other: Lights

firefly is fitted with LED lights. These make user interactions or monitoring the protocol easier as firefly's cabinet blocks the room light.

Property	Form	Notes
Description	Free text	
Is enabled	Tick box	The default selection is that white lights are on
Color	Select from pop-up form	Select the color lighting. The selected color will be shown on the icon in the protocol.

You may wish to turn the lights off for all or part of your protocol, if you have sensitive materials. Alternatively, you may find that you can specify a color for the lights (like a red light in a photographic darkroom) which is safe for your reagents or process.

Note

If firefly goes into error, the lights will return to the default error state, e.g., they will be turned on as white lights, irrespective of the previous setting in the protocol.

Other: Tidy

During protocol execution, the decks may gradually fill up with items that are no longer required. Keeping these items on the decks can slow down protocol execution, particularly if you have enabled auto moves. For firefly+ instruments, run Tidy to move items no longer required from the decks (primarily plates and tip boxes) to the firefly+ shelves, and free up deck locations. Tidy will relocate items which are not used as part of any further steps in the protocol, with some restrictions:

- They must be compatible with firefly+ so Tidy would not move e.g., a magnetic block to the shelves.
- They must not be part of a stack which contains items which are not compatible with firefly+.
- They must not be part of a stack which contains other items which will still be used.

Property	Form	Notes
Description	Free text	
Items to tidy Tip Boxes	Tick box	This is the default selection
Items to tidy Plates	Tick box	You may prefer not to tick this if there are finished plates on the deck, which you would rather remove manually, as firefly cannot differentiate why a plate is no longer required.

Tip

As running Tidy requires the grippers to be in constant use, it would be best to include it in a protocol immediately after transferring the active plate to e.g. the thermal module, and selecting 'Start & Continue' behavior.

Other: Notes

Notes appear in protocol design views as Post-it notes. Use them to give information to the user that does not interrupt the firefly workflow.

When executing a protocol, notes are shown with a yellow background in the Steps.

Property	Form	Notes
Description	Free text	This is the text of the note

Other: Pause

Pause will put firefly into an inactive state e.g., while a plate is cooling on the plate thermal module.

Property	Form	Notes
Description	Free text	Use to describe purpose of the pause
Duration (hours, minutes, seconds)	hours:minutes:seconds	1 second - 23 hours, 59 mins, 59 seconds
Rest State	Select from dropdown list	List of possible instrument areas to access. Home will return both heads and decks to their home positions, and home the firefly+ grippers if applicable.

You can use the rest state to move heads away from plates e.g., by homing them, if you are concerned that reagents might drip into the plates during the pause.

You do not need to use Pause for a user interaction i.e., to remove the plate from firefly, as the instrument will pause until the user clicks 'Done' on the user interaction instruction to confirm that they have completed it.

Designing a complete protocol

To design a protocol from scratch, you will need to:

- define the plates and well arrays to be used
- define the assets needed for the protocol
- define the sequence of protocol steps
- set asset or step properties where necessary
- position the assets on the deck

The important points to remember are:

- firefly can only dispense into one plate or carry out one pipetting task at a time, so you cannot design a protocol with concurrent pipetting and dispensing steps
- firefly and firefly+ grippers can continue to move items during a shake, heat/cool or incubate step, if you have selected 'Start and continue' behavior. The grippers cannot operate during dispensing, pipetting, loading or unloading steps as the heads will require access to the decks.
- firefly protocols must be linear, the protocol design doesn't allow for conditional actions. Once a protocol is started, firefly will run it to the end unless the user intervenes and cancels it. If you want to automate a process which has different steps depending on an assay result, you will have to stop at the decision point.
- There are limitations on where labware can be placed to allow the dispensing and pipetting heads to access plates. Using the Deck wizard will ensure that you place your labware correctly.

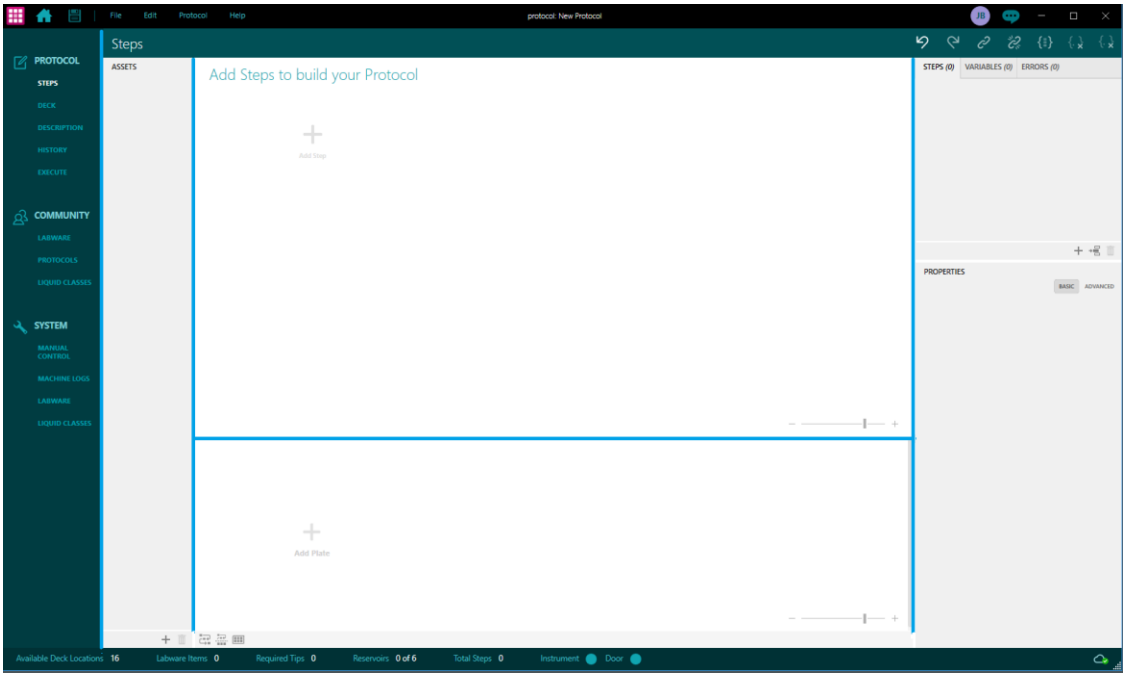
You may find it helpful to design the main workflow of your protocol in a charting tool or sketch it by hand, before starting to build it up in firefly software.

firefly software is very versatile and allows you to use any design approach you wish when you start to build your protocol on the Design screen. You can start by defining the protocol steps, then add the required assets or start with the assets and then the steps to process them. There are no constraints on the order in which you build up a protocol.

Use Notes to organize long protocols into sections, you can also use Group to hide the details of completed process sequences and clarify the Steps view.

You can choose to view Plates, Steps or both when designing. Click the icon for the option which you find most useful.

You can also resize all the Design areas to show whatever is most convenient for your task by dragging the frame edges e.g., to maximize the Steps area as shown below. Blue lines mark the draggable frame edges.



The Editing a protocol section of this manual describes how to add and use plates, assets and protocol steps in detail. When building them into a new protocol, there are various standard sequences for using them.

Pipetting

You will need to include these elements to carry out any pipetting task.

1. Loading tip sets or tip boxes and plates, using 'Setup & loading'.
2. A 'load tips' step to load the pipette head with tips.
3. A pipetting task (copy, mix or aspirate then dispense).

4. A 'load tips' step to remove the used tips, using the 'unload only' option. You can use the same tips repeatedly, or change them at whatever points in the protocol are appropriate. At the end of the protocol, you must remove the last set of tips before you run another protocol or shutdown firefly. If there is liquid remaining in the tips, the software will prompt you to purge the tips before unloading.

5. A User interaction for unloading the plates and the used tips, unless they are to be unloaded after the protocol completes.

The pipette head requires enough space to access well arrays e.g., it cannot dispense into a deck location which has a tip stand to the left of it. Use the Auto Deck Fill to ensure that you place your labware appropriately.

Dispensing

You will need to include these elements to carry out any dispensing task.

1. Loading reservoirs, syringes and a destination plate using 'Setup & loading'.

2. Either a fill step or separate aspirate and dispense steps. If there is liquid remaining in the syringes, the software will prompt you to purge the syringes before unloading.

If you will only be dispensing one or two reagents, and have plenty of material, you could use more than one reservoir for each and link the dispenses: firefly will then dispense from multiple syringes in parallel and complete the step faster. This could be useful for larger volume dispense steps. The downside would be a larger reagent dead volume is needed, but this is recoverable at the end of the protocol.

Heating or chilling

Plates

You will usually need to include these elements to use the plate thermal module.

1. A 'Start and wait' or 'Start and continue' step to start the plate thermal module heating or chilling, and set the required temperature. Using 'Start and continue' enables this to happen while other protocol steps are progressing.

2. A 'Move' or 'Place on' step to put a thermal block on the plate thermal module, unless you had loaded it there at the start of the protocol.

3. A 'Move' or 'Place on' step to put the plate on the thermal block, unless you had loaded the plate in that position at the start of the protocol.

4. One or more steps which will be carried out while the plate is being heated or cooled. This could be a pipetting step (which can be carried out while one of the plates is on the plate thermal module) or any other action not involving the plate.
5. A 'Move' or 'Take off' step to remove the plate from the plate thermal module.
6. A 'Stop' step, to turn off heating or chilling, once it is not needed.

Only the plate on the plate thermal module has active temperature control. You could have multiple thermal blocks to keep multiple plates chilled or heated, moving them around together and taking turns returning them to the plate thermal module for further heating or cooling. The thermal block will slow down the rate of temperature change of the plate contents to approximately 1°C per minute, and so can keep the plate temperature below 10°C for 5 minutes after you have cooled it on the plate thermal module to 4°C.

Reservoirs

You will need these elements to heat or cool reagents in reservoirs. A 'Start and wait' or 'Start and continue' step to start the reservoir thermal module heating or chilling. Make this one of the first steps of the protocol if you want to load reagents and maintain their correct temperature, or set the reservoir thermal plate temperature using the Decks screen. Use a 'Stop' step to end heating or cooling if it is no longer needed.

Incubate, using the thermal cycler

You will need these elements to use the ODTC. Before you incorporate an 'Incubate' step in your firefly protocol, you must have created the pre method and main method XML files in the ScriptEditor 3 software.

You can use lidded or unlidded plates on the ODTC. You can run an incubation step with the ODTC lid open or closed.

Note

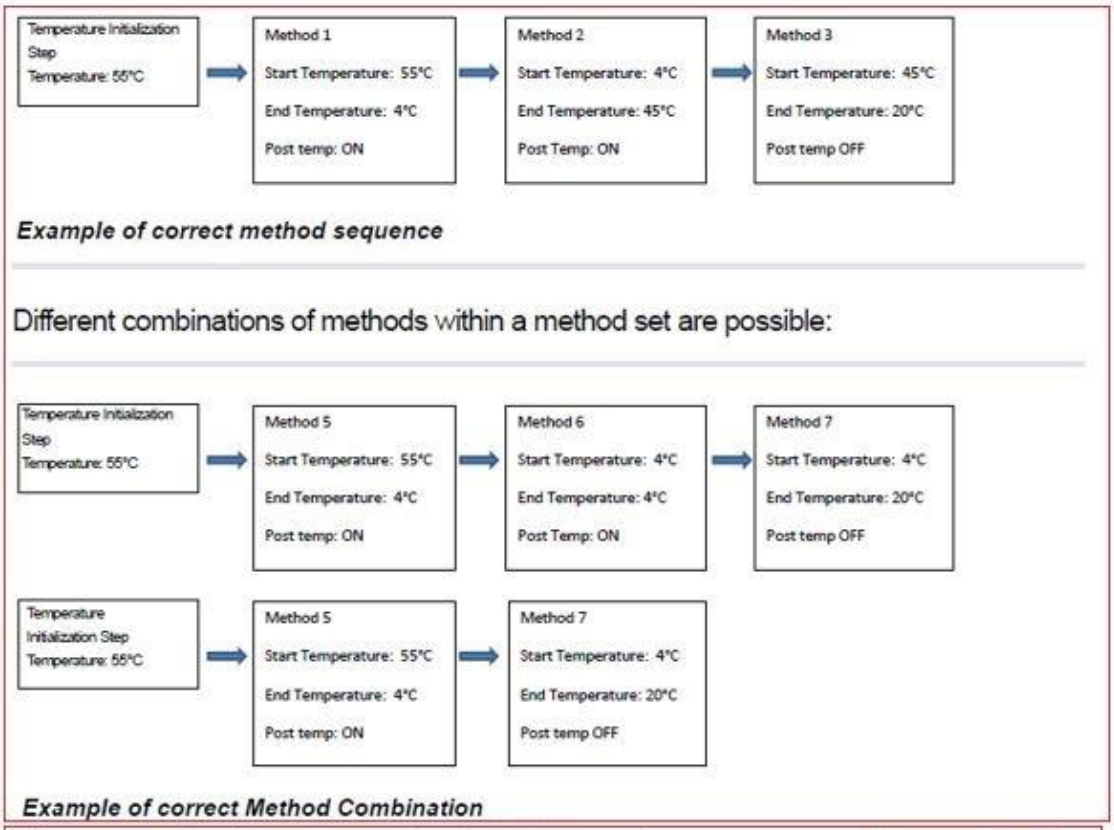
The height of the plate or plate + lid stack must not exceed the ODTC clearance:

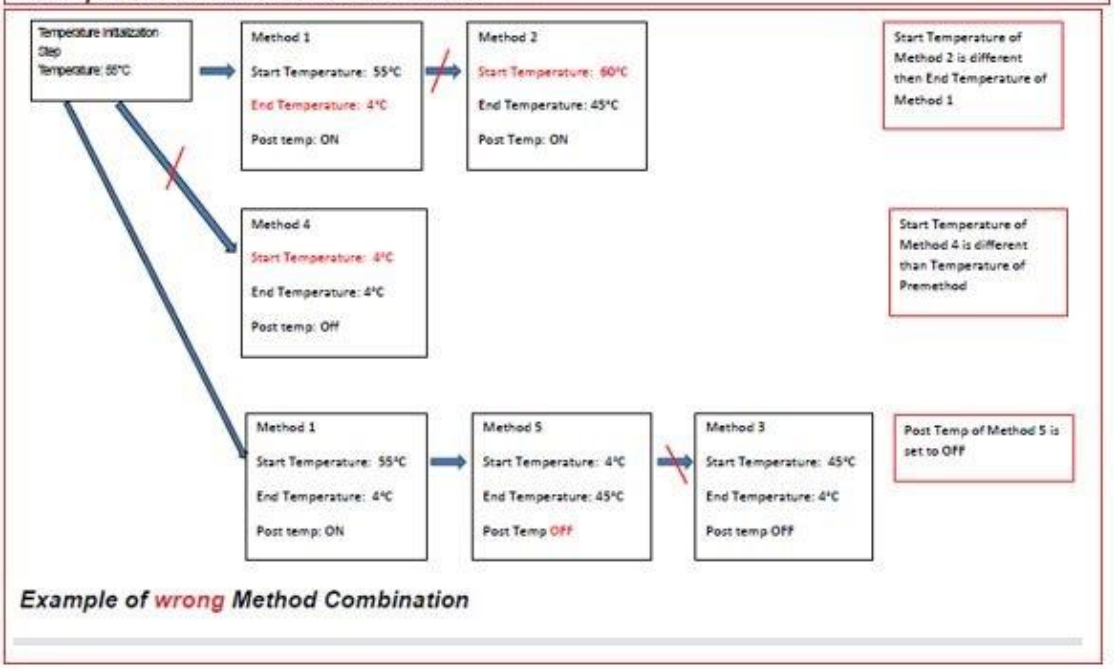
- 28.0mm for 384 plates
- 32.0mm for 96 plates

1. Use an 'Incubate' step with Open lid behavior.
2. Use a 'Move' step to transfer the plate to the thermal cycler.
3. Use an 'Incubate' step with Close lid behavior, optionally.

4. Use an 'Incubate' step to run the pre- method, or, alternatively use the 'Run both' Method Type to run the whole protocol. The purpose of the pre- method is to heat or cool the ODTC to the required temperature, so you may wish to run this using 'execute and wait' behavior, before loading the plate.

5. Use a second 'Incubate' step to run the main method. You can run multiple main methods sequentially, but to do this you must set the Post Heating property to true for all but the last method.





6. Use an 'Incubate' step with Open lid behavior, if you had closed the lid previously.
7. Use another 'Move' step to remove the plate.

If you are going to remove a lid from a plate after PCR, you may want to transfer it to a waste plate, to keep the firefly+ shelves clean.

> **Important**
>
> Do not leave a plate in the thermal cycler for hours after completing a PCR step, as this causes condensation to build up in the thermal cycler. SPT Labtech recommends moving the PCR plate to firefly's thermal module, if you want to keep it cool until you are ready to remove it from the instrument.

Using hold temperature

You can utilize the ODTC's indefinite temperature hold function within a firefly protocol to keep a plate at a fixed temperature. This may be useful if you want to incubate successive plates at the same temperature, as loading and unloading plates does not stop an indefinite hold.

However, if you use this, when the firefly protocol completes, the ODTC will continue to run and hold the temperature, even after the plate has been removed from the ODTC.

So that it is not forgotten about, design the firefly protocol so that:

- If you are using the temperature hold in the middle of the firefly protocol, include a subsequent Incubate step with Behaviour set to Stop. This will cancel the indefinite hold.
- If you are using an indefinite hold at the end of the firefly protocol so that you can leave firefly running and come back for the plate at a convenient time, design your protocol with a User Interaction step to retrieve the plate. After that, include another Incubate step with Behaviour set to Stop as the last step. This way it will be clear that firefly and the ODTC are still running, and that the plate has been kept at a controlled temperature but needs to be unloaded.

Designing User interactions

When you design a user interaction, you are giving the firefly user instructions which will be displayed as a message on the screen so they must be clear and in the correct order.

You may want to include user interactions for unloading reagents and consumables used in your protocol, or you may want to complete the protocol and then use your lab procedures for emptying and cleaning firefly.

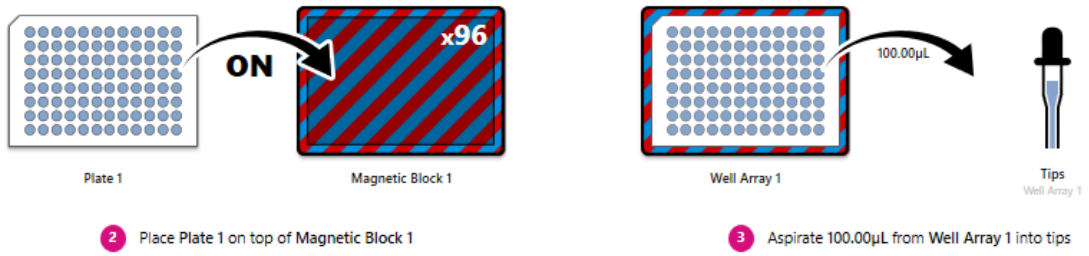
- Use the correct Rest state to enable the user to access specific areas safely.
- Use the unloading instructions as a prompt for your user interactions and SOPs.
- Write a series of short user interaction steps rather than one detailed complex instruction, if the user has multiple tasks to carry out. That way they can confirm when they have finished each one.
- If the user is going to need labware or reagents which are not loaded in firefly, create a User interaction early in the protocol to confirm that those items are available and ready for use. This is particularly important if there are time critical steps in the protocol which must not be delayed.
- Think ahead: if the plate will need to be transferred to another instrument, does it need time to set up or to reach a temperature setpoint? If so, you should have a User interaction to get that instrument ready at an earlier stage in the protocol, so the user is preparing it while firefly is performing other steps.
- You could give the user conditional instructions, e.g., the first user interaction is to perform an assay and the second is to use the results to determine how much diluent to add to a sample.
- If there is anything particularly complex, the User interaction could direct the user to another method document in the lab, to display charts, diagrams or more detailed instructions than can be shown on firefly's tablet screen.

Stacking assets

You use the pipetting head grippers to stack plates on top of other assets, using 'Move' or Place on' to create a stack, and 'Move' or 'Take off' to separate the items. Some items, like the plate thermal module, can only be base items.

> **Note**
>
> To use the ODTC, you must have a stack definition for the ODTC with each of the PCR plate types you wish to use. Use Community to download stack definitions. If you cannot find what you need, contact reliance.

To move stacked assets together, drag and drop the bottom item from Assets to Steps, when designing. The graphic will show that the two assets are moved together.



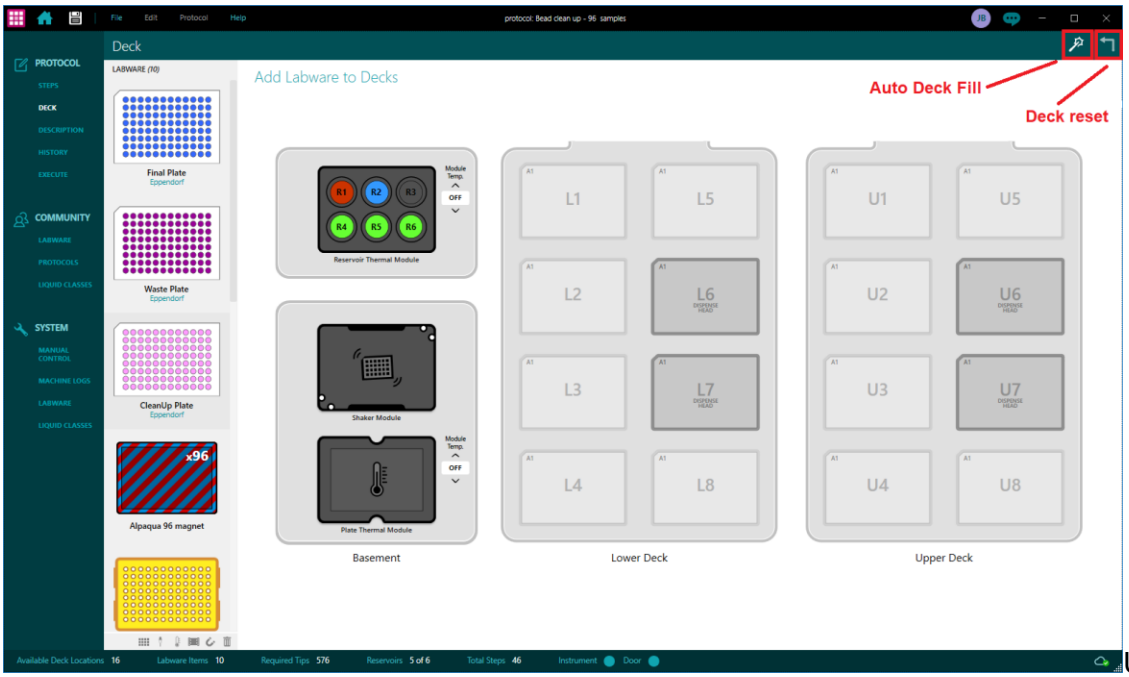
Using stacks, you can keep a plate on a magnetic block or thermal block while pipetting or dispensing.

You can only use combinations of lids and plates or plates and base items which have a stack definition. Use Community to download stack definitions. If you cannot find what you need, contact reliance.

Setting up the Decks

Once you have finalized the labware your protocol will require, open the Deck screen to set up loading firefly.

You can set the initial temperature of the reservoir or plate thermal blocks.



Use the Auto Deck Fill (highlighted above) to automatically select the layout for your assets.

Important

If you use Auto Deck Fill when you have an undefined stack in your protocol, collision detection would work based on the wrong stack definition so shouldn't be trusted until this error is removed.

Download the stack definition before using Auto Deck Fill.

You can drag and drop your assets onto deck locations. If you do this, check the Errors panel on the Steps view afterwards, in case you need to revise your layout. There is a Reset Decks function which empties the entire deck, to start again if you need to. To remove a single asset, right click on it and select 'Remove'. You can then drag it to a new location.

If you are not using Auto Deck fill, the allowed positions for labware loading are shown below.

EZL head

Labware Item	Shaker Module	Thermal Plate Module	Lower Deck	Upper Deck	Total ↓	Notes
Lids	1	1	8	8	**18**	
Plates	1	1	8	8	**18**	
Magnetic Blocks	-	-	8	8	**16**	
Thermal Blocks	1	1	8	8	**18**	
Risers	-	-	8	8	**16**	Lower Deck riser can not go on Upper Deck
EZ-Load Tip Boxes	-	-	8	-	**8**	

ATL head

Labware Item	Shaker Module	Thermal Plate Module	Lower Deck	Upper Deck	Total	Notes
Lids	1	1	8	8	**18**	
Plates	1	1	8	8	**18**	
Magnetic Blocks	-	-	8	8	**16**	
Thermal Blocks	1	1	8	8	**18**	
Risers	-	-	8	8	**16**	Lower Deck riser can not go on Upper Deck
AT-Load Tip Boxes	-	-	8	8	**31**	
Tip Box Adapters	-	-	8	-	**8**	

Using firefly+

The Auto Deck Fill function will use the shelves in firefly+ to place suitable labware. There are height restrictions (shown in mm below) for the labware which you can place on each shelf type.

The overall allowed storage positions are:

Labware Item	Shaker Module	Thermal Plate Module	Lower Deck	Upper Deck	Firefly+	ODTC	Total	Notes
Lids	1	1	8	8	27	1	**46**	
Plates	1	1	8	8	27	1	**46**	
Magnetic Blocks	-	-	8	8	-	-	**16**	
Thermal Blocks	1	1	8	8	-	-	**18**	
Risers	-	-	8	8	-	-	**16**	Lower Deck riser can not go on Upper Deck
AT-Load Tip Boxes	-	-	8	8	15	-	**31**	
Tip Box Adapters	-	-	8	-	-	-	**8**	

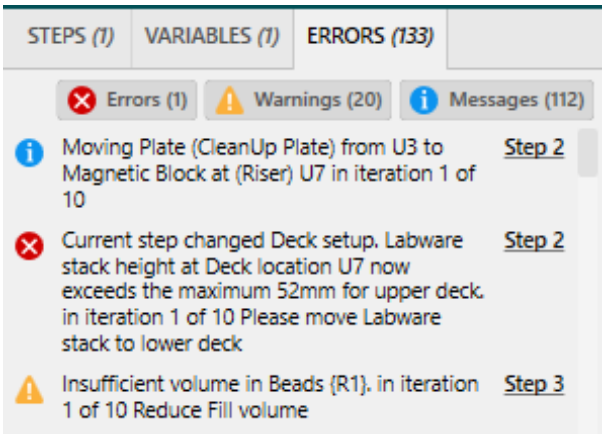
If the shelves are in use, the firefly+ grippers need a clear deck location to place labware, before it is used in a protocol. This can be U2, U3, L2 or L3. You may need to modify your deck layout manually to clear one of these deck locations if you see error messages when you attempt to run your protocol.

Ideally try and keep all 4 of these locations (or as many as are practical for your materials) empty, when setting up the decks for your protocol, as it will make protocol execution more efficient.

Getting feedback from the Errors panel

Check the Errors panel as you are designing your protocol: it will notify you of volumetric inconsistencies or missing steps such as using tips without a prior Load Tips step. There are 3 levels of severity:

1. Errors are the most serious problems i.e., when you have not defined a step correctly. If there are Errors, your protocol cannot be executed.
2. Warnings will not prevent firefly executing your protocol, but they alert you to significant problems i.e., when cross contamination may occur.
3. Information messages alert you to minor issues or possible improvements.



Everywhere you see '{xxx}' there will be the name of an asset, a plate, a well volume or other specific item in the actual error messages.

Clicking on the associated step number will highlight the step in the step area.

Resolve all errors to enable the protocol to run.

Errors

The {item} is not present on the targeted instrument [shown if protocol requires modules which are not present on the instrument]

The protocol makes use of hardware that is not present on your instrument. Affected steps will need to be updated and/or replaced in order to run the protocol.

The protocol makes use of deck location {xxx} that is not present on your instrument. Remove references to invalid deck locations to run the protocol.

Dynamic labware {dynamicitem.labware.Id} could not be placed. Ensure there is space on the relevant deck.

Protocol contains labware that has been allocated an initial deck location not present on

Errors

the targeted instrument

Well arrays

{wellArrayType} well array not set.

Destination well array not set.

Well selection mismatch. Source and Destination well arrays should be equal in size.

Invalid volume at well {xxx}. Specified volume must be at least zero.

Invalid volume at well {xxx}. Specified volume must be less than {xxx}µL maximum well volume.

The starting volume of {wellArray.Id} is invalid. Specified volume must be at least 0µL.

Source and Destination well arrays contain overlapping wells. Source and destination well arrays cannot share the same wells.

Tips & pipetting

Source tip set has not been specified

Incompatible pitch between loaded tips and {xxx} well array. Change {xxx} well array.

Tip Set '{xxx}' must be on the lower deck when loading

Tip Set '{xxx}' must be on the lower deck when unloading

Insufficient tips loaded in head. Check that the loaded tip box contains enough tips to perform {xxx}.

Tips have not been loaded into head. Make sure tips are loaded before performing {xxx}.

Cannot perform tip unload. The head does not contain tips.

Cannot perform tip load. {xxx} must be unloaded first.

Cannot perform tip unload. The head contains {xxx} not {xxx}.

Insufficient volume in tips. Reduce {xxx} volume.

Incompatible pitch between loaded tips and {xxx} well array. Change {wellArrayType} tiling pattern or use 384-well compatible tip set

Incompatible pitch between loaded tips and {xxx} well array. 96-well compatible tip set required.

Incompatible pitch between loaded tips and {xxx} well array. Avoid using strips or change tiling patterns.

When Pipetting to the lower deck, the plate should be on a lower deck riser to give the greatest flexibility.

Surrounding Deck locations should be clear of Tip Sets when Pipetting to a regular plate on a Magnetic Block on the lower deck. Suggestion: Move labware to upper deck if possible

Surrounding Deck locations should be clear of Tip Sets when Pipetting to a tall plate on a

Errors

Magnetic Block. Suggestion: To allow more Tip Set locations, locate labware in first or last row

Pipetting head can not hold more than a single row of tips. Rearrange well region into columns, or reduce well region to a single row.

Insufficient transfer volume. Specified volume must be at least {minimum Allowed Volume}µL.

{xxx} volume exceeds tip capacity of {xxx}µL. Change tip type or reduce {xxx} volume. Adjusting Blowout, Transport and Overshoot volumes can also reduce required tip capacity.

Insufficient safeguard volume. Specified volume must be at least 0µL.

The {step xxx} {speed} is out of range and would cause protocol execution to fail

Invalid number of mix cycles. Specified value must be greater than 0.

Tips contain {liquidVolume}µL of liquid and {airVolume}µL of air aspirated by step {xxx}. Empty tips before unloading.

Tips contain {liquidVolume}µL of liquid and {airVolume}µL of air aspirated by steps {xxx}. Empty tips before unloading.

Tips contain {liquidVolume}µL of liquid aspirated by step {xxx}. Empty tips before unloading.

Tips contain {liquidVolume}µL of liquid aspirated by steps {xxx}. Empty tips before unloading.

Tips contain {airVolume}µL of air aspirated by step {xxx}. Empty tips before unloading.

Tips contain {airVolume}µL of air aspirated by steps {xxx}. Empty tips before unloading.

The tip head will contain tips at the end of execution. Add a Tip Change step to the end of the protocol to unload.

Tip Box '{xxx}' must be on the lower deck when loading.

Tip Box '{xxx}' must be on the lower deck when unloading.

{TipType} cannot be loaded from a 96-well tip box.

{TipType} cannot be discarded to a 384-well tip box.

Syringes & dispensing

{xxx} and {xxx} share {syringes}. Reservoirs cannot be shared across assets

Source reservoir not set.

No reservoirs selected in source reservoir.

Maximum reservoir capacity has been exceeded in {syringeArray}. Change reservoir type or reduce the dispense volume

Errors

Maximum reservoir capacity has been exceeded in {syringeArray}. Increase the number of reservoirs, change the reservoir type or reduce the dispense volume

Maximum Syringe volume of {Volume}µL exceeded in {source}. Reduce {step} volume, change the reservoir type, or increase the number of syringes used.

Maximum Syringe volume of {Volume}µL exceeded in {source}. Reduce {step} volume or change the reservoir type.

Maximum reservoir capacity exceeded. Reduce {step} volume.

Maximum reservoir capacity exceeded. Reduce {step} volume or change the reservoir type.

Maximum Syringe volume of {xxx}µL exceeded in {source}. Reduce {step xxx} volume or increase the number of syringes used.

Insufficient volume in {syringesUsed} to perform this dispense. Ensure a dispense head aspirate step has been performed to all the Syringes used.

Maximum Syringe volume of {xxx}µL exceeded in {source}. Reduce {xxx} volume.

Maximum volume {xxx}µL exceeded in {syringeArray xxx}. Reduce {xxx} volume.

Transfer volume exceeds the maximum allowed. Specified volume must be <= {maxVolume}µL which is the maximum syringe volume.

Transfer volume + safeguard volume exceeds the maximum allowed. Specified volume must be <= {maxVolume}µL which is the maximum syringe volume

The liquid dispense will also contain safeguard liquid. Check liquid and safeguard volumes.

The {zeroSpeed.StepRef} {zeroSpeed.SpeedName} is set to zero and would cause protocol execution to fail.

Destination has not been specified.

Number of columns in Destination: {xxx}, does not match the number of columns in Data File: {xxx}.

Number of rows in Destination: {xxx}, does not match the number of rows in Data File: {xxx}.

Destination Plate Id: {xxx}, does not match the Plate Id in Data File: {xxx}.

CSV file not specified or does not exist. Enter the file path in the Data File property.

CSV file does not exist. Check the file path in the Data File property.

All volumes in Data File are zero.

No Dispense Pattern found. Please check that volume is not Zero.

Insufficient transfer volume in Data File for one or more selected wells. Specified volume must be at least Single Shot Min Volume ({xxx}µL)

A multi-column transfer between a {source} and a {destination} is not supported.

Errors

No volume to dispense. Ensure at least one well has a non-zero dispense volume specified or remove step if not needed.

Volume is below the Single Shot Min Volume ({xxx}µL).

Single Shot Min Volume must be at least equal to Multi Shot Min Volume.

Multi Shot Min Volume must be less than or equal to half of Max Shot Volume.

Syringes contain liquid from a previous aspiration. Dispense all liquid before re-aspirating.

Required syringes are not present on the targeted instrument

Can't execute step: No empty locations found to move Plate {destinationPlate.Id} for Dispense", suggestion:"At least one deck location needs to be empty to allow auto moves for Dispense Head steps"

Pause

Pause duration must be greater than 0.

Thermal Modules

Thermal Module not set.

Stop behaviour used before Start and Continue.

{xxx} Temperature must be >= -20°C.

{xxx} Temperature must be <= 99°C".

Shaker

Shaker missing plate.

Shake speed must be 200 or more.

Shake speed must be 3000 or less.

Shake duration must be greater than 0.

Trough plates cannot be shaken.

ODTC

An Incubate Pre method has already been run.

An Incubate Pre method was run on a different plate.

An Incubate Pre method must be run before a Main method.

{xxx} well Plates cannot be placed on the thermocycler.

Cannot place {Lid} on top of {Plate 1} whilst {Plate 1} is on the Thermocycler.", suggestion: "Remove {Plate 1} from the Thermocycler before placing labware on top of it.

Stacks

Top and Base Items not on same stack.

Base item is empty. Add a stackable item (e.g., Magnetic Block, Thermal Plate).

Errors

Top item is empty. Add a top item (e.g., Plate).

{xxx} already has an item on top of it.

{xxx} plate '{xxx}' covered by lid '{xxx}'. Remove lid from plate before performing {xxx}

Collision detection

Moving {srcLabware} from {srcLoc} will collide with {dstLabware} at {dstLoc}.

If {prevCollidingSyringes} not attached, the Dispense head back frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

The Dispense head back frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

If {prevCollidingSyringes} not attached, the Dispense head front frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

The Dispense head front frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

If {prevCollidingSyringes} not attached, the Dispense head left frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

The Dispense head left frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

If {prevCollidingSyringes} not attached, the Dispense head back frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

The Dispense head back frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

If {prevCollidingSyringes} not attached, the Dispense head front frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

The Dispense head front frame will collide with labware in deck location {collisionLoc.Location}", suggestion: $"Move labware from {collisionLoc.Location}.

Tips loaded in head will collide with {xxx} plate. Match loaded tips with number of wells on plate.

"Pipetting Head would collide with labware in deck location {xxx}", suggestion: "Use a riser or move labware to the first Column".

Pipetting Head would collide with labware in deck location {xxx} (collision margin: {clearance} > 0)", suggestion: "Use a riser or move labware from {xxx}".

Errors

Cannot load tips: The Pipette Head will collide with labware at {adjacentLeft} with height {xxx}mm whilst attempting tip load from tip box at {tipBoxLocation} at height of {xxx} mm

Cannot load tips: The Pipette Head will collide with labware at {adjacentLeft} with height {xxx}mm whilst attempting tip load from tip box at {tipBoxLocation} at height of {xxx} mm

Cannot discard tips: The Pipette Head Cover will collide with labware at {xxx} (with height {xxx}mm) whilst attempting tip load from tip box at {tipBoxLocation} at height of {xxx}mm

Cannot discard tips: The Pipette Head Cover will collide with labware at {xxx} (with height {xxx}mm) whilst attempting tip load from tip box at {tipBoxLocation} at height of {xxx}mm

Cannot load tips: The Head Gripper will collide with labware at {adjacentLeft} with height {xxx}mm whilst attempting tip load from tip box at {tipBoxLocation} at height of {xxx} mm

Cannot discard tips: The Head Gripper will collide with labware at {xxx} (with height {xxx}mm) whilst attempting tip load from tip box at {tipBoxLocation} at height of {xxx}mm

Height restrictions

Current step changed Deck setup. {Labware stack/Labware} height at Deck location {xxx} now exceeds the maximum {xxx}mm for lower deck.

Current step changed Deck setup. {Labware stack/Labware} height at Deck location {xxx} now exceeds the maximum {xxx}mm for upper deck.

{Labware stack/Labware} height at Deck location {xxx} exceeds the maximum {xxx}mm for lower deck

{Labware stack/Labware} height at Deck location {xxx} exceeds the maximum {xxx}mm for upper deck

{Labware stack/Labware} height at Deck location {xxx} exceeds the maximum {xxx}mm for upper deck. Suggestion: {Labware stack/Labware} cannot be used on firefly instrument.

The Syringe Z-Offset of {xxx}mm is greater than the maximum allowed of {xxx}mm due to hardware limits. Decrease the Z-Offset.

firefly+

Cannot take '{topItem}' off '{lowerItem} in location {xxx} directly

Cannot place '{topItem}' on top of '{topItemInDestination} in location {xxx} directly from {xxx}

{topItem}' is incompatible with location {xxx}

Error or warning depending on asset

Insufficient transfer volume in Data File for one or more selected wells. Specified volume must be at least {minimumAllowedVolume}µL.

Warnings

The Syringes ({xxx}) - IF ATTACHED) will collide with labware in deck location {xxx}. Switch to top Row Syringes or move labware from {xxx}.

The Syringes ({xxx}) - IF ATTACHED) will collide with labware in deck location {xxx}. Switch to bottom Row Syringes or move labware from {xxx}.

The Syringes ({xxx}) - IF ATTACHED) will collide with labware in deck location {xxx}. Switch to left Column Syringes or move labware from {xxx}.

The Syringes ({xxx}) - IF ATTACHED) will collide with labware in deck location {xxx}. Switch to top-left Row Syringes or move labware from {xxx}.

The Syringes ({xxx}) - IF ATTACHED) will collide with labware in deck location {xxx}. Switch to bottom-left Row Syringes or move labware from {xxx}.

Please ensure syringes are removed before running protocol to avoid collision with the {xxx} at Deck location {xxx}

Insufficient volume in {syringeArray xxx}. Reduce {xxx} volume.

Insufficient transfer volume. Specified volume must be at least {minimumAllowedVolume}µL.

Volumes used in this step exceed the maximum Source well volume of {xxx}µL. Reduce {step xxx} volume or change Source plate.

Volumes used in this step exceed the maximum Destination well volume of {xxx}µL. Reduce {step xxx} volume or change Destination plate.

The starting volume of {xxx} is greater than the maximum well volume of {well Array Maximum Allowed Volume}µL".

Cannot Perform Left Or Right Tip Touch for Plate {xxx}.

Excessive tips used with source well array to perform {xxx}. Consider alternate tiling pattern.

Excessive tips used with source well array to perform {xxx}. Contamination may occur.

Excessive tips used with {wellArrayType} well array to perform {xxx}. Contamination may occur. Tips and Well Array's dimensions should ideally match.

Start Well Height set to >100% and would be clipped at 100% to allow dispense at well bottom.

Start Well Height set to >110% and would be clipped to 110% to avoid a crash. If Aspirate plate stack is not sprung please set to <=100% to avoid a crash.

End Well Height set to >100% and would be clipped at 100% to allow dispense at well bottom.

End Well Height set to >110% and would be clipped to 110% to avoid a crash. If Aspirate plate stack is not sprung please set to <=100% to avoid a crash.

Warnings

Insufficient mix volume. Specified volume must be at least {minimumAllowedVolume}µL.

Insufficient post mix aspirate volume. Specified volume (or blowout) must be at least {minimumAllowedVolume}µL.

Insufficient post mix dispense volume. Specified volume (or air-gaps) must be at least {minimumAllowedVolume}µL.

The dispense blowout volume will also contain liquid. Check liquid volumes and air-gaps.

The liquid dispense will also contain air. Check liquid volumes and air-gaps.

The dispense air transport volume will also contain liquid. Check liquid volumes and air-gaps.

Recommended reservoir capacity has been exceeded in {syringeArray}. Change reservoir type or reduce the dispense volume.

Recommended reservoir capacity has been exceeded in {syringeArray}. Increase the number of reservoirs, change the reservoir type or reduce the dispense volume.

Recommended reservoir capacity of {Volume}µL exceeded. Reduce {step} volume.

Recommended reservoir capacity of {Volume}µL exceeded. Reduce {step} volume or change the reservoir type.

{syringes} will contain up to {maxLiquidVolume}µL of liquid plus {maxSafeguardVolume}µL safeguard volume after the step is complete. Check liquid and safeguard volumes are correct and perform manual purge after execution.

{syringes} contain up to {maxLiquidVolume}µL of liquid volume. Check liquid volumes are correct or add dispense step to purge.

{syringes} contain up to {maxLiquidVolume}µL of safeguard volume. Perform manual purge after execution.

User Interaction does not contain a message. Enter a message to direct/instruct the user.

Plate {xxx} has no Volumetric data so will ignore AutoTracking and pipette from bottom of the well.

The {labwareType} '{labwareName}' has not been approved. Using an unapproved {labwareType} may cause your instrument to crash.

The protocol is using an out-of-date definition of {labwareType} '{labwareName}'. Consider updating the Protocol to use the latest version of the {labwareType} from the local DB.

The protocol is using an unapproved stack '{stackName}'. Using an unapproved stack may cause your instrument to crash.

Insufficient liquid volume in {source} to perform {xxx}. Reduce {xxx} volume or increase liquid volume in {source}.

Insufficient liquid volume in Source wells to perform {xxx}. Reduce {xxx} volume or increase

Warnings

liquid volume in Source wells by selecting the well array asset in the assets list and modifying the “Starting Volume per well (µL)” property.

Unnecessary purge. Syringes do not contain any liquid.

Unnecessary purge. Tips do not contain any liquid or air-gaps.

On completion of this {xxx} step the volume remaining in the Source wells will be below the dead volume of {xxx}µL. Reduce the {xxx} volume or increase liquid volume in Source wells by selecting the well array asset in the assets list and modifying the “Starting Volume per well (µL)” property.

On completion of this {step} step the volume remaining in {syringeArray} will be below the dead volume of {Volume}µL. Reduce {Title} volume.

A lid or a film must be placed on top of a plate on the thermocycler.

An Incubate Pre method has no corresponding Main method.

Warning or Information

Moving {labware} from {location} to {destination location}.

Information

Start Well Height set to >100%. If Aspirate plate stack is not sprung please set to <=100% to avoid a crash.

End Well Height set to >100%. If Aspirate plate stack is not sprung please set to <=100% to avoid a crash.

Tips have been used in a previous step. Possible contamination risk

Tips contain liquid from previous step. Possible contamination risk

{xxx Thermal Module} missing thermal block.

{xxx Thermal Module} missing plate.

{volumePerTip}µL per tip will be transferred from the Trough plate.

{volumePerTip}µL per tip will be transferred to the Trough plate.

Failed to check reservoir volumes. Check Instrument connection.

Failed to check reservoir volumes. Check Machine Logs.

Reservoir volume cannot be checked due to errors in DFD related steps. Correct the reported errors.

The {labwareType} '{labwareName}' is not installed in the local DB and can only be used with the current protocol.

Information

The plate {plateName} in this protocol is not installed in the system DB and is only available for use within the current protocol. Add to system DB.

The protocol is using a different stack definition for {stackName} than is saved in the system DB. Update protocol.

The protocol is using a more recent definition of {labwareType} '{labwareName}'. Consider updating the local DB to the version of the {labwareType} used with the Protocol.

The protocol is using a more recent version of {xxx}. Update system DB.

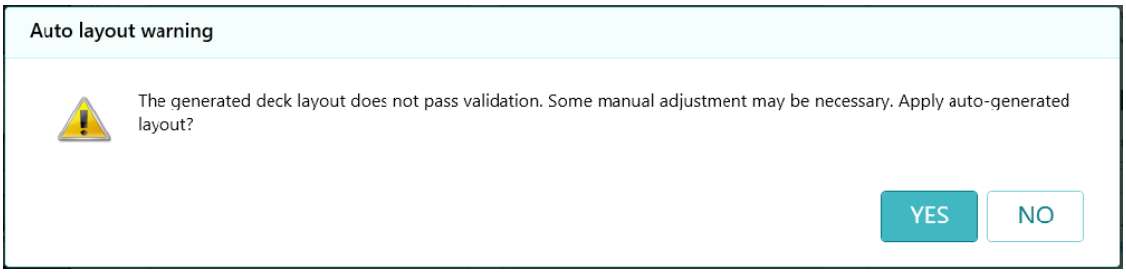
Undefined Stack Definition for '{topItem}' on '{baseItem}'. Collisions my occur and/or aspirate and dispense actions may be performed at incorrect heights.

The system DB contains a different, but unapproved, stack definition for {stackName}.

The system DB contains a more recent version of {xxx}. Update protocol.

Collision detection

As you design your protocol, firefly will warn you about collisions, where labware will obstruct the movement of the pipetting head e.g., if you are loading tips from a tip box when there is a plate on the thermal module. The error message will suggest modifications to solve the collision issue, but you may not want to implement them. If you use Auto Fill Decks while you have an unsolved collision error, you will be warned that you need to place your labware manually.



As you cannot execute a protocol which has errors, you can try and solve the collision problem by:

- manually dragging and repositioning the labware which will collide, particularly if you have empty positions available on the decks
- revise the order of protocol steps other than the suggested modification if you have scope to do this
- use the deck appropriate riser to lift the lowest plate and so raise the working height of the head, avoiding collisions

Keep checking the Errors panel as you make changes, to see whether you have resolved the error.

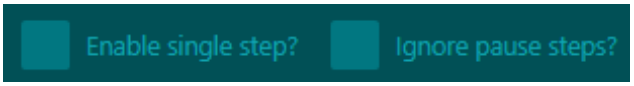
Testing your protocol in simulation

If you are using a standalone installation of firefly software, it will include a simulated firefly instrument which is started and connected automatically when you open the firefly app. Use the simulated firefly to test run your protocol before you transfer it to your lab instrument.

To test a protocol design using the simulated instrument, select 'Execute' as you would on the instrument. Execute is not enabled if a protocol has errors, you will need to address those first.

> **Note**
>
> Superusers do not have permission to use development functions by default. firefly administrators will need to add this permission to their profile, or create an additional profile type which has this permission.

Before you start running a protocol, you can enable one or both of the development tools:

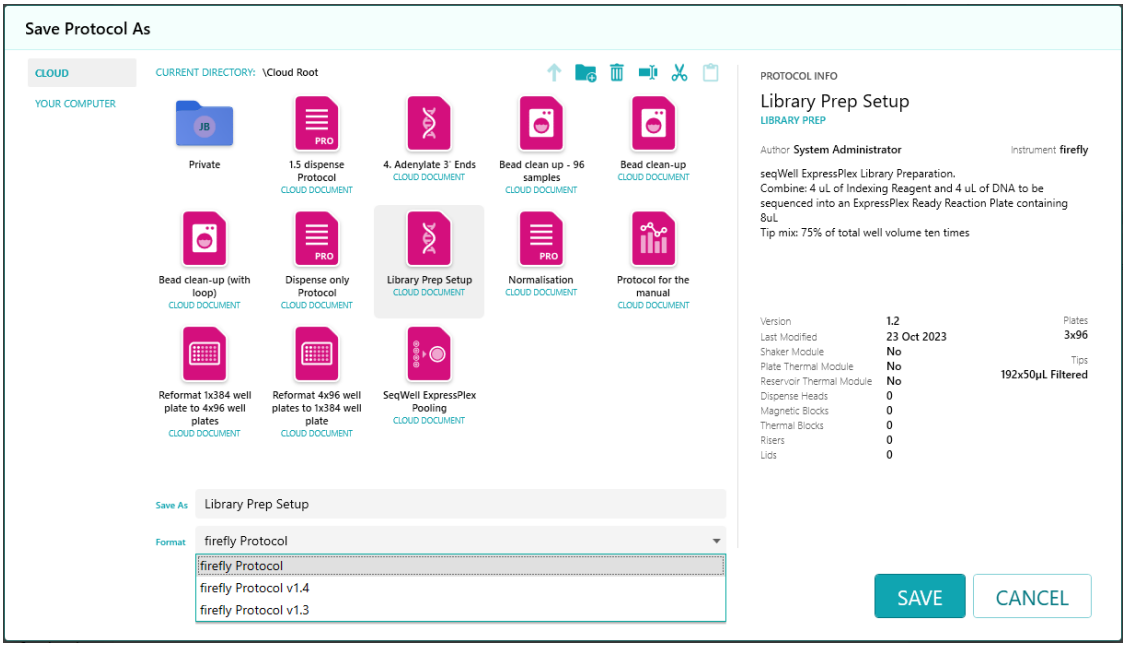


- Use 'Enable single step' to work through a protocol one step at a time; this is useful if you want to review the logs at each point before continuing
- Use 'Ignore pause steps' to speed up protocol test runs

If your protocol cannot complete, you will get feedback on the step which failed - shown as red text in the Execution Logs.

Saving your protocol

Use File, Save, Save As or Save as Local Document, to save your protocol.



The save locations available will depend on your network configuration, and may include Cloud storage in addition to Your Computer. Save as Local Document will not use cloud storage locations. Use a private folder if you know your protocol is not ready to be shared yet. Use a folder with 'read only' permissions for other users to ensure your protocol cannot be modified or deleted.

You have the option of saving the protocol in a format suitable for earlier versions of firefly software, if these are in use in your lab. firefly will warn you if saving in an older format will result in a loss of functionality e.g., older versions of the software did not support the use of plate lids.

Version control

firefly automatically timestamps and versions all protocols when you save them.

Version	1.2
Last Modified	23 Oct 2023
Shaker Module	No
Plate Thermal Module	No
Reservoir Thermal Module	No
Dispense Heads	0
Magnetic Blocks	0
Thermal Blocks	0
Risers	0
Lids	0

Plates
3x96

Tips
192x50µL Filtered

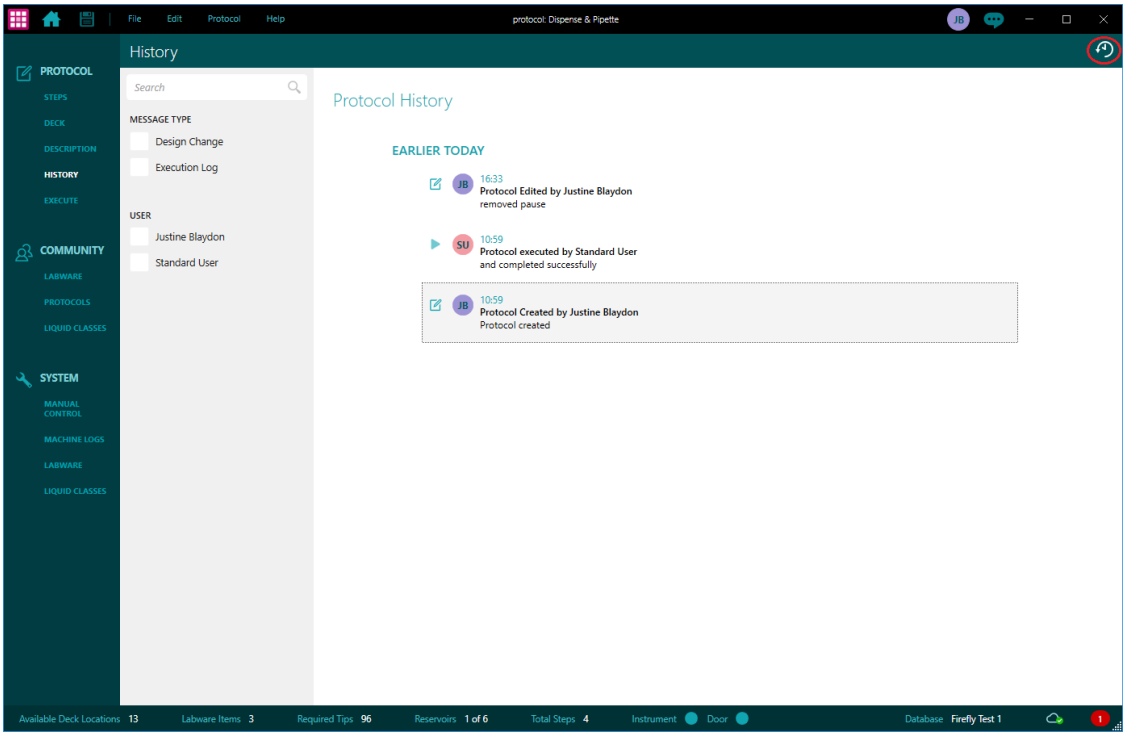
Rollback to a previous version

Note

Superusers do not have permission to roll back protocols by default. firefly administrators will need to add this permission to their profile, or create an additional profile type which has this permission.

If you have made changes to a protocol which you subsequently decide are not helpful, you do not need to remove them all by hand if you have rollback permission.

Open History for the protocol. If you have permission to rollback versions, the icon (circled in red) will appear white, as it does below.

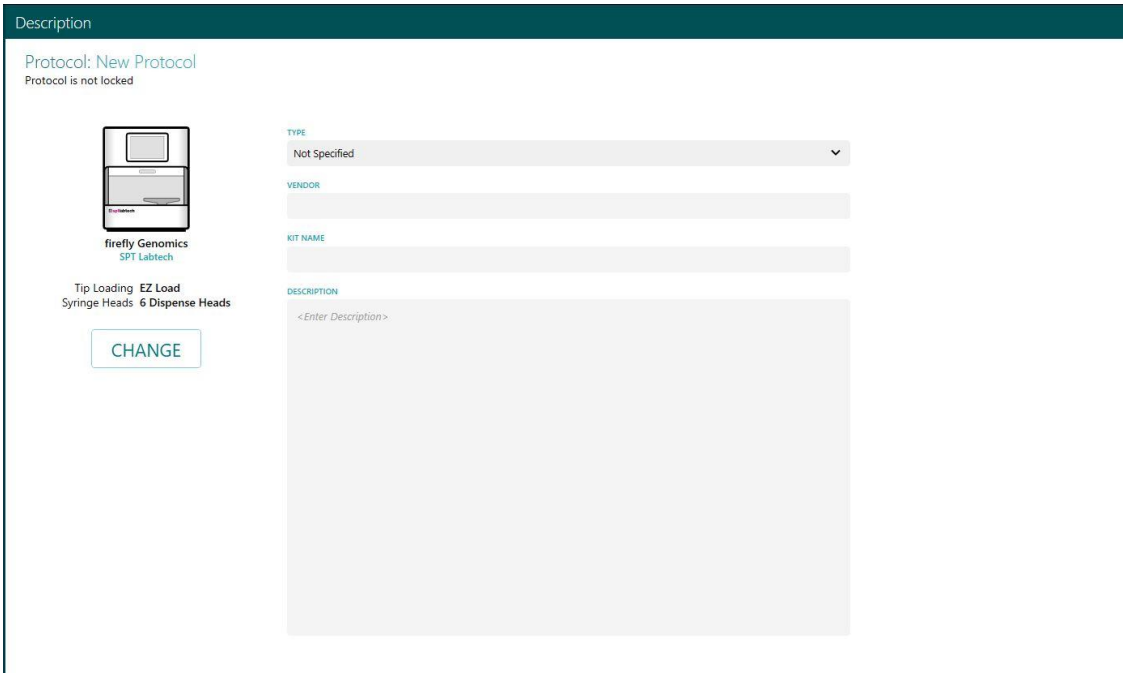


Select the version you wish to revert to - it could be any number of versions back - and click the rollback icon. The software will ask you to confirm the action.

When you return to the Steps view, you will immediately see the form of the protocol which you selected to revert to.

Description

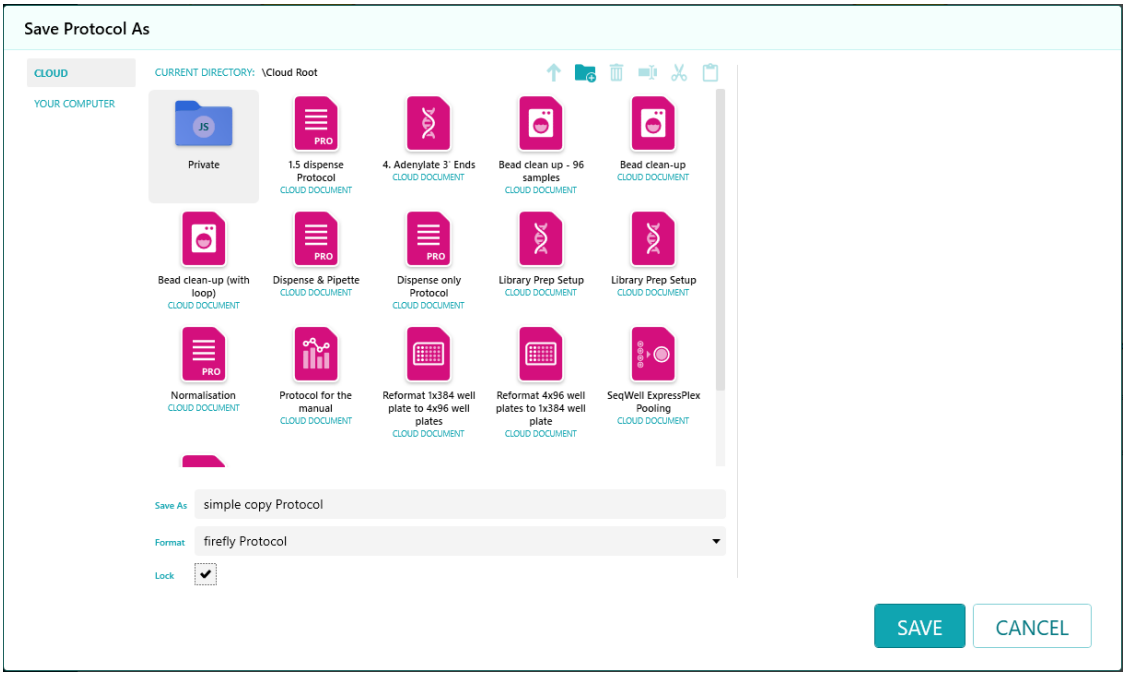
After saving your protocol, use Description to classify it and describe it. This step is optional but will help you manage your protocols once you have a large library or are sharing with other users.



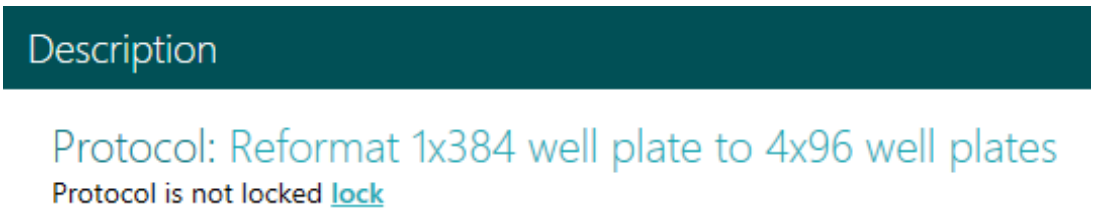
- Select a protocol type e.g., clean up from the dropdown list in Type.
- Select a Vendor or Kit Name, if the protocol relates to a specific assay.
- Write as detailed a description as you feel will be useful in Description. If you are planning to share the protocol, it will be helpful to other users to do this.
- It would also be useful to include details of other materials required for the protocol in the description, as 'Labware & Consumables' will only show materials loaded in firefly.

Locking a protocol

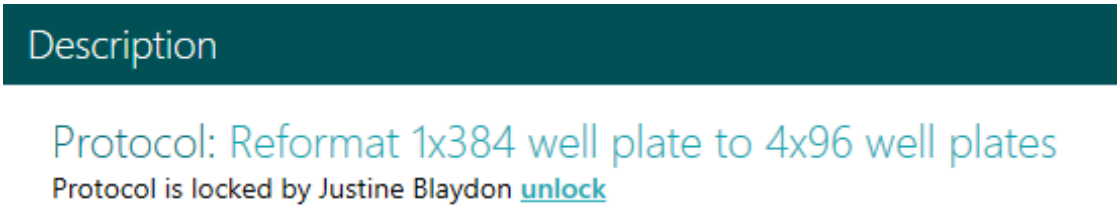
You can also lock a protocol when saving it, by ticking 'Lock'. All users who have permission to design protocols can also lock and unlock their own protocols. You can lock a protocol to ensure there are no further edits, and that other users cannot delete it.



After saving, select the protocol's Description. Under the protocol name is the current locked / not locked status, and the option to lock an unlocked protocol, if you did not do so when saving.



Click 'Lock' and the screen updates the lock status,



If you now try and edit the steps or deck layout, it is not possible, preventing any accidental or unplanned changes.

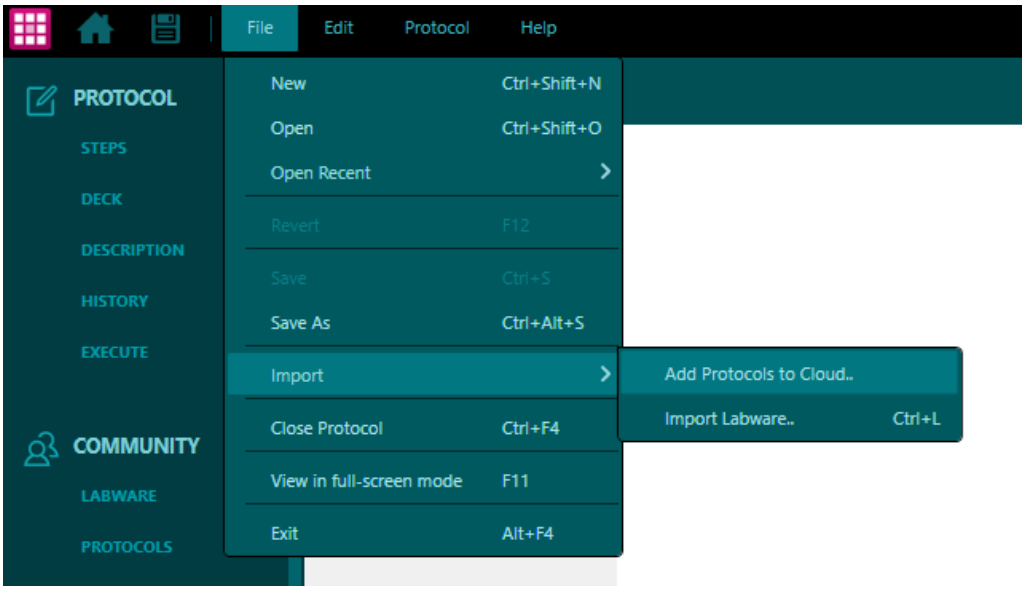
firefly software includes an additional 'Lock protocols' permission, which gives a user permission to lock or unlock any protocols, irrespective of author. If your lab uses this functionality, then other users may unlock and edit your protocol, or delete it.

Note

No user profile has permission to 'lock protocols' by default. firefly administrators will need to add this permission to their profile, or create an additional profile type which has this permission e.g., a QA function or a system administrator.

Add Protocols to Cloud

If you have designed protocols which you then want to share within your organization, select File on the main menu bar, then Import, Add protocols to Cloud.



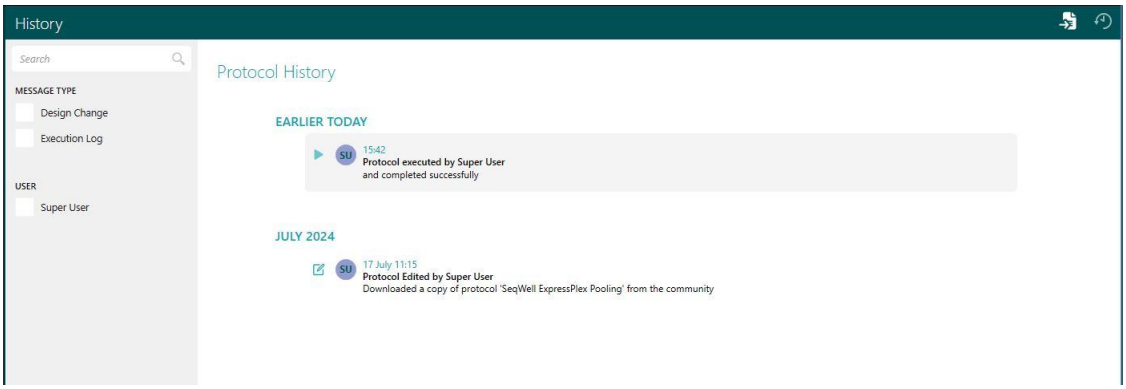
This enables you to upload your locally stored protocols to your shared Cloud storage space, where other users can download them onto other firefly instruments.

> **Note**
>
> This function does not add your protocols to Community, so they will not be visible outside of your organization (or whatever part of it is using the Cloud storage defined on your firefly instruments).

History

You can see how often your protocol has been run, and whether it completed successfully, in the History view. The data includes runs on a simulated firefly. This data may be useful before finalizing a protocol. It does not give you the details of why or how a protocol failed.

If your protocol is stored on the Cloud, history will also show when the design was updated and saved, and the user's comments when they saved the change (note, there is no check whether users have documented the change; if they have not, all you will see is that the protocol was modified, the time and the user).



You can filter this view, to exclude either protocol runs or design changes, or to select particular users or search terms.

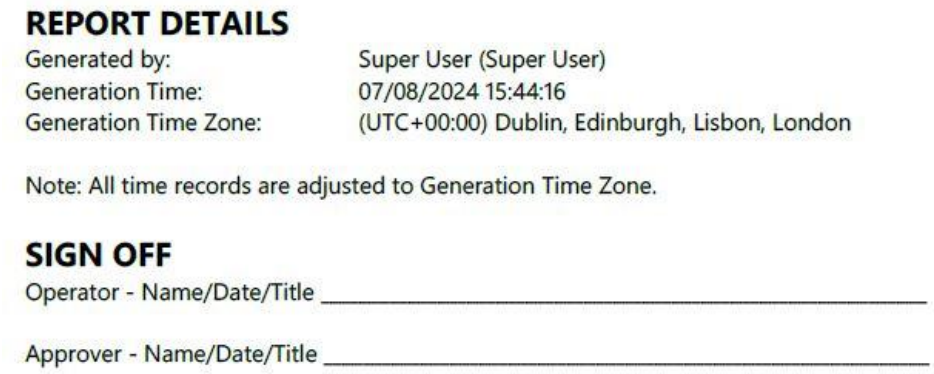
Execution History Report

You can generate an Execution History Report for a specific protocol execution by selecting in the protocol history then clicking the Export Execution Report button at the top right 

A pop-up form allows you to name the PDF file and specify the save location. The execution report contains full details of the protocol (including the protocol script), the instrument

used, the labware, and each timestamped step in the execution. It also includes all warnings or error messages.

The report includes details of when it was printed, and by whom, and includes space for sign off by both the operator and an approval signatory.



REPORT DETAILS

Generated by: Super User (Super User)
Generation Time: 07/08/2024 15:44:16
Generation Time Zone: (UTC+00:00) Dublin, Edinburgh, Lisbon, London

Note: All time records are adjusted to Generation Time Zone.

SIGN OFF

Operator - Name/Date/Title _____

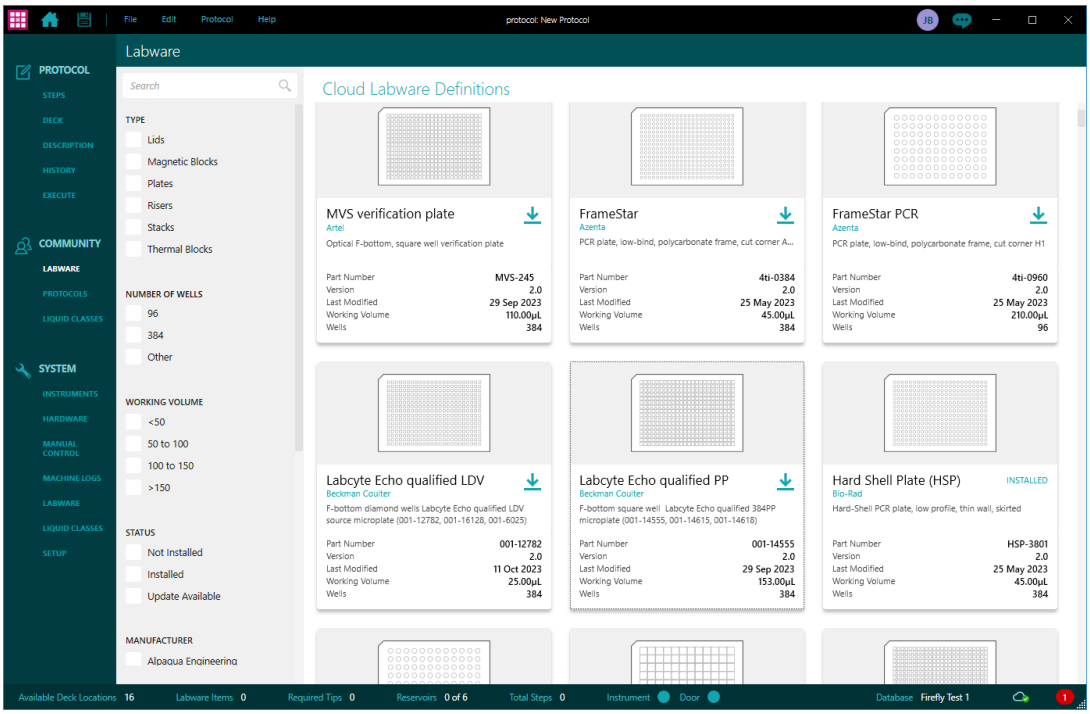
Approver - Name/Date/Title _____

Reports can be printed multiple times, at any time.

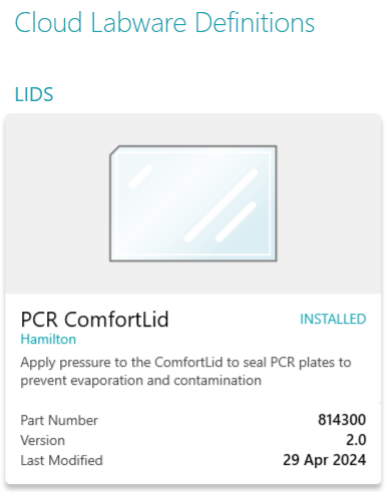
Community

Labware

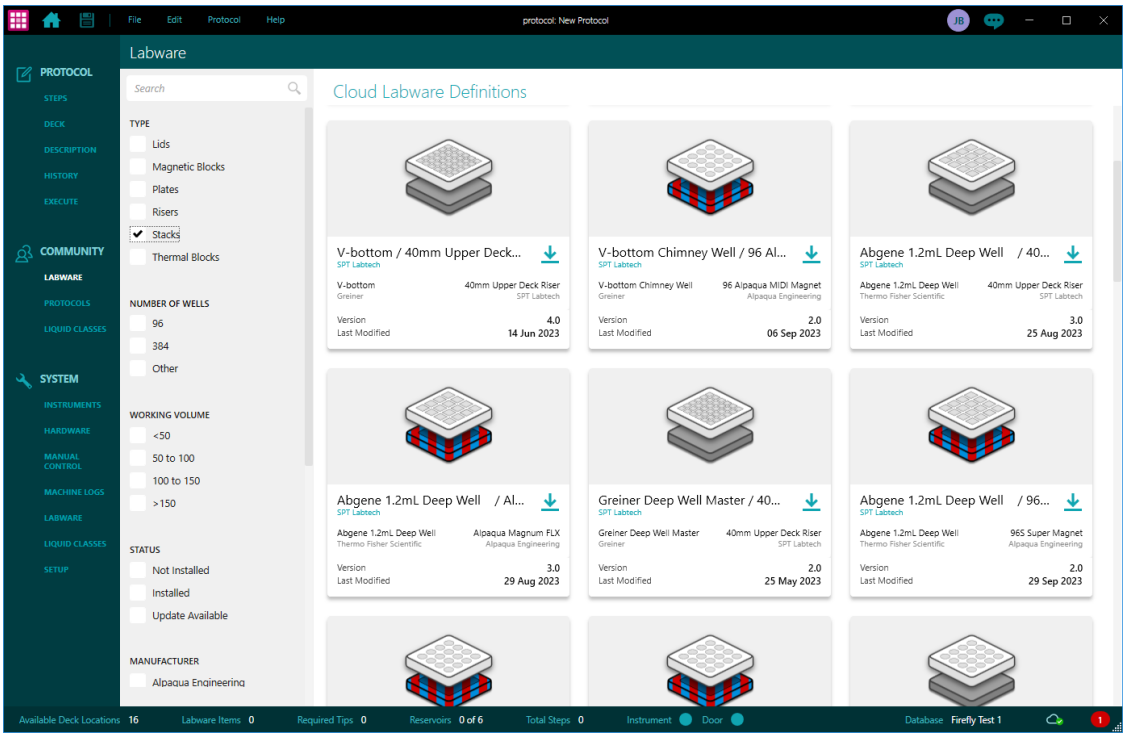
If the labware you want to use is not immediately available when designing firefly protocols, check for it in 'Community', 'Labware'. It includes a search facility to e.g., define plate properties.



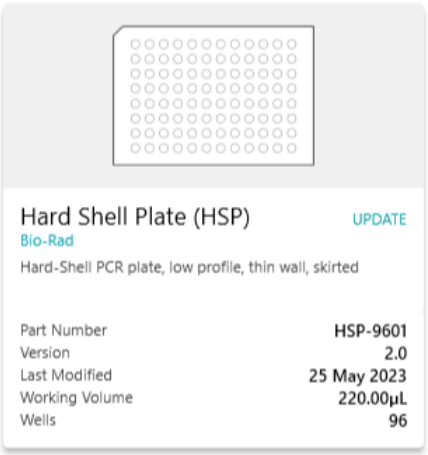
When you have found the labware you want, click the blue arrow to download it. The download arrow will be replaced by the word 'Installed'. It will then be available to use in protocols.



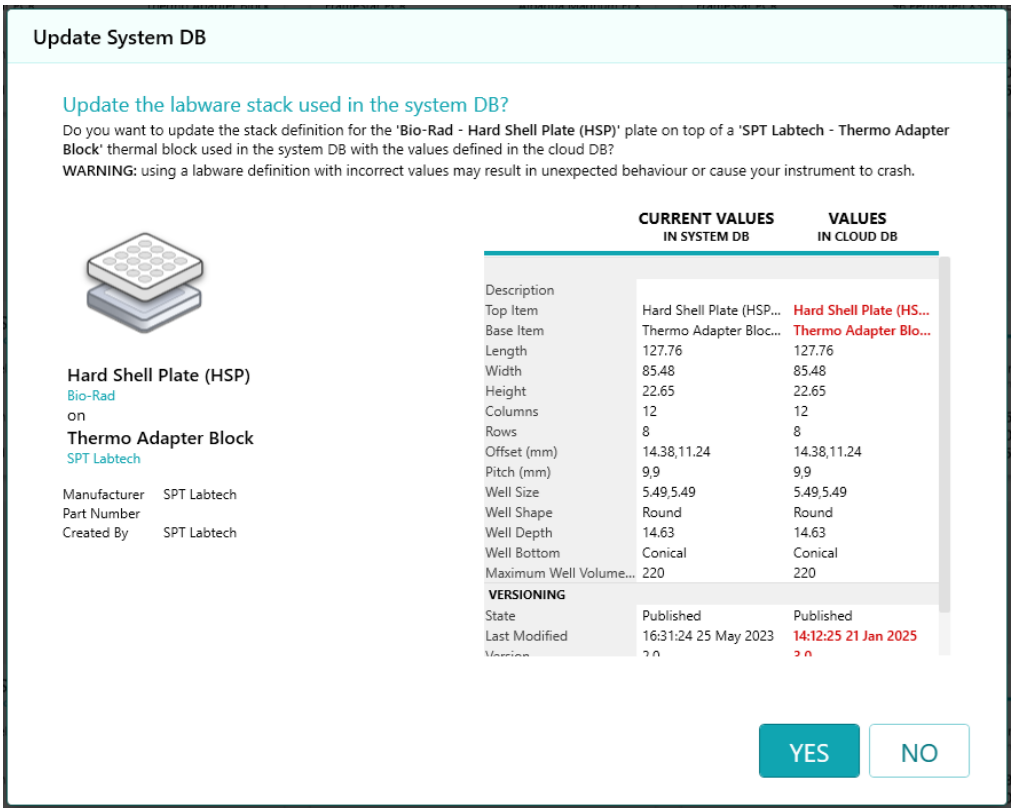
You will also need to download definitions for any stacks which you wish to use in your protocols. You will not be able to run a protocol unless you have downloaded definitions for the stacks used. If you cannot find what you need, contact reliance.



If you have previously installed labware or stacks and the definition has since been updated e.g., to take account of manufacturer's changes, you will see 'Update' instead of 'Installed'.



If you are using existing labware supplies, you may prefer not to update definitions. If you have new labware, using the new definitions will be more accurate. firefly displays the differences between the installed details and the new definition, so you can check what would be more appropriate to use.



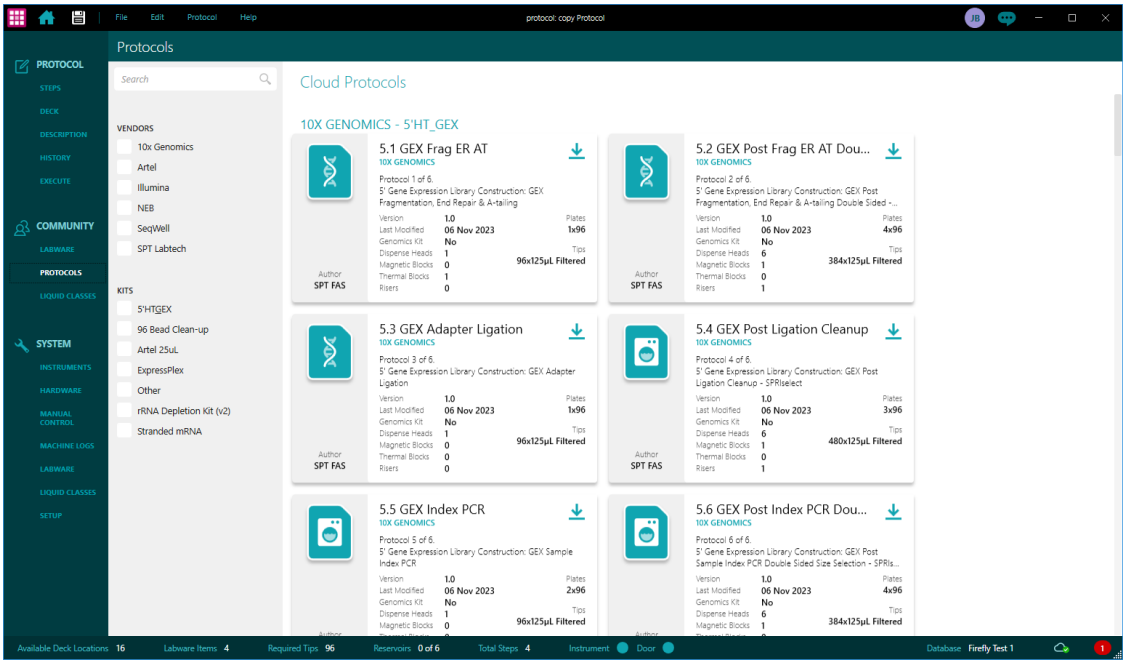
Tip

Do not attempt to use any labware which does not appear in 'Labware', unless SPT Labtech have provided you with the labware definition to use.

If you open a protocol which uses labware which is not installed on your system, it will be downloaded automatically.

Protocols

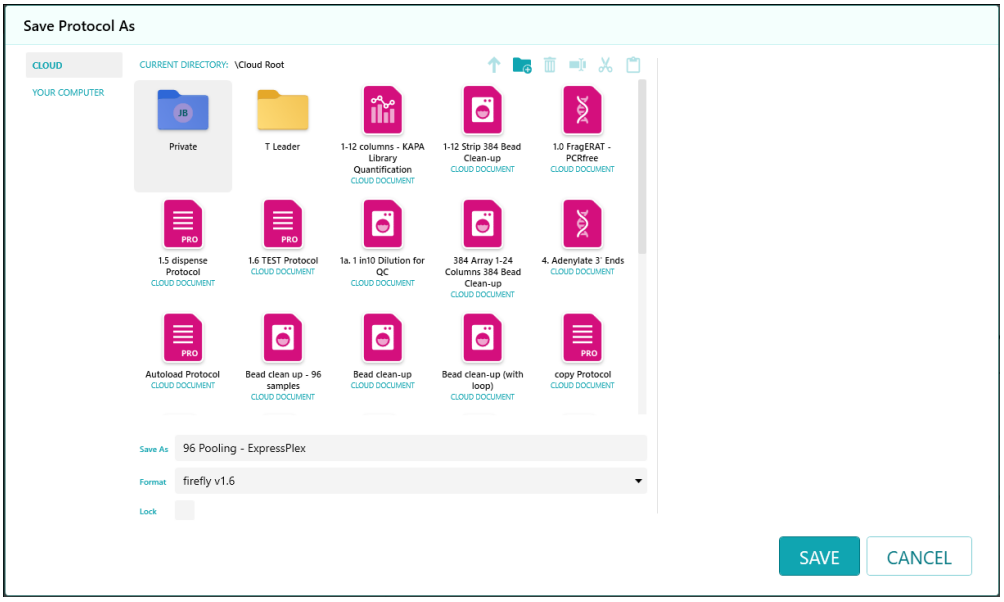
You can download firefly protocols from the Cloud based firefly Community.



There are various options to simplify searching for protocols:

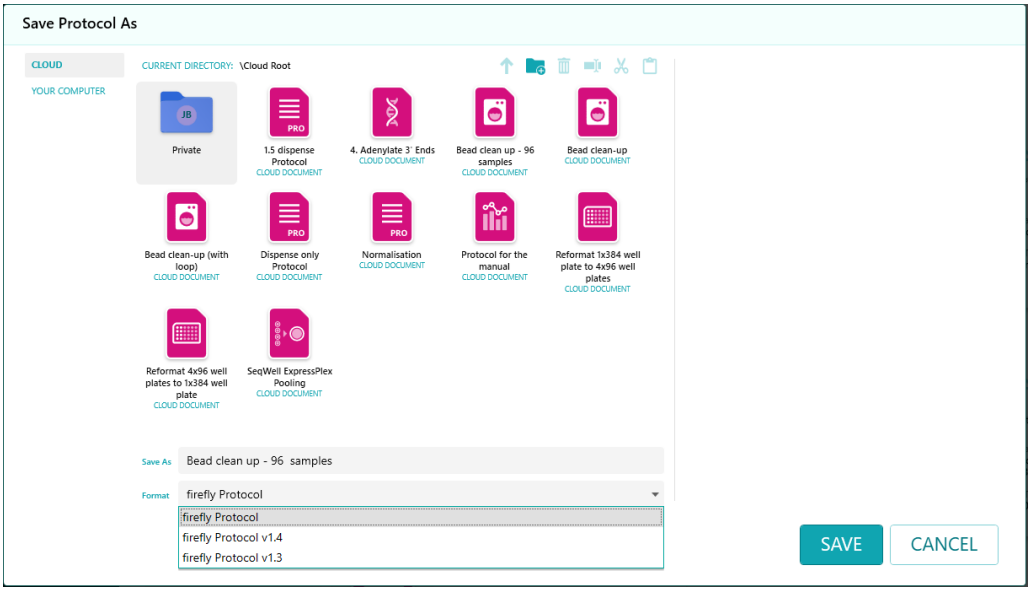
- free text search - this searches the names of available protocols, and will return all partial matches
- vendors - use this search if you want to run a protocol using a specific company's products
- kits - use this search if you want to run a specific assay on firefly. Note that you may need to download multiple protocols to perform the complete assay.

When you have found the one you need, download a local copy, by clicking on the arrow. This will open the Save Protocol As form which enables you to store your copy in a Cloud location or on your local PC or networked drives. You can lock the protocol to prevent further editing.



The download arrow is replaced by the word 'Installed' for protocols which you have already downloaded. If a protocol has been updated after you downloaded it, you will see 'Update'. All protocols are versioned and show their last modified date, so you can decide whether to download the update or retain your current version e.g., because you are using older reagent kits.

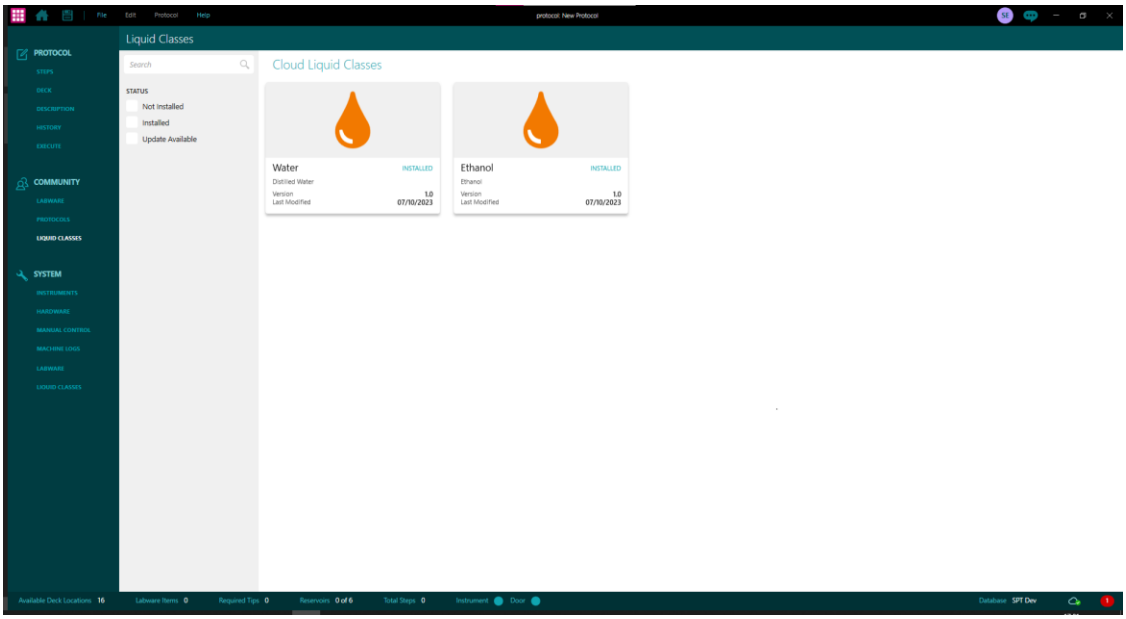
You can also select to download the protocol formatted for an older version of firefly software, if this is what your instrument is running.



If you cannot find the exact protocol you need, you can either design it from scratch yourself, or you can download the closest match and use the 'Design' tools to edit it before you use it.

Liquid classes

You can also download liquid classes from the Cloud based firefly Community. These are optional; you can design and execute firefly protocols without using them, but they are a convenience as they characterize commonly used solvents and serums.



Search for liquid classes by name. When you have found the liquid class you want, click the blue arrow to download it. The download arrow will be replaced by the word 'Installed'. It will then be available to use in protocols. If you cannot find the liquid class you wanted, you can define it.

If you had previously installed a liquid class and the definition has since been updated, you will see 'Update' instead of 'Installed'. Updating is optional; if your current definition is performing well, you may not wish to revise it. You can check the Last Modified date to see what would be more appropriate.

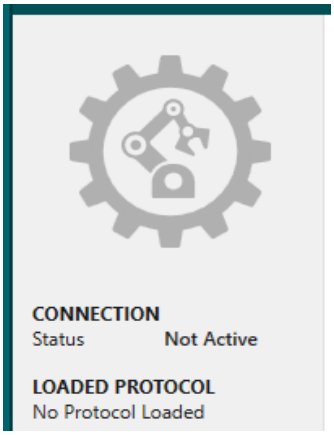
System

The System Instruments view is firefly's opening screen if you are logged on an administrator.

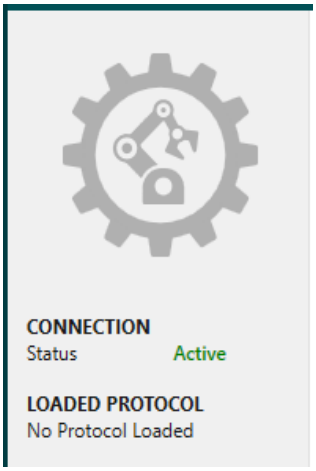
Automation

firefly software includes an API to enable the instrument to be incorporated in a laboratory automation system. This is described in the firefly API Guide and the firefly Integrators' Manual.

The Automation screen shows whether the instrument is controlled locally



or is running under API control.



Note

When running under automated control, firefly will still require user interactions e.g., to

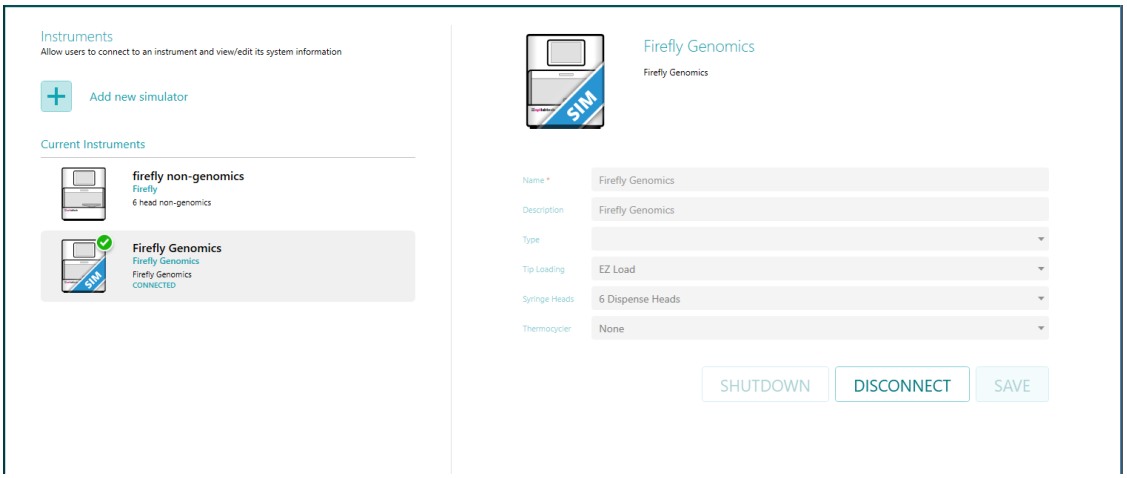
load and unload syringes. For all manual steps, follow the methods described in this manual.

Instruments

The instruments screen shows the details of any simulated firefly instruments which have been set up. As standard, it is only available to administrators.

You should not need to make any changes unless you want to switch the type of simulated instrument but if there is a problem with it, you may need to check it.

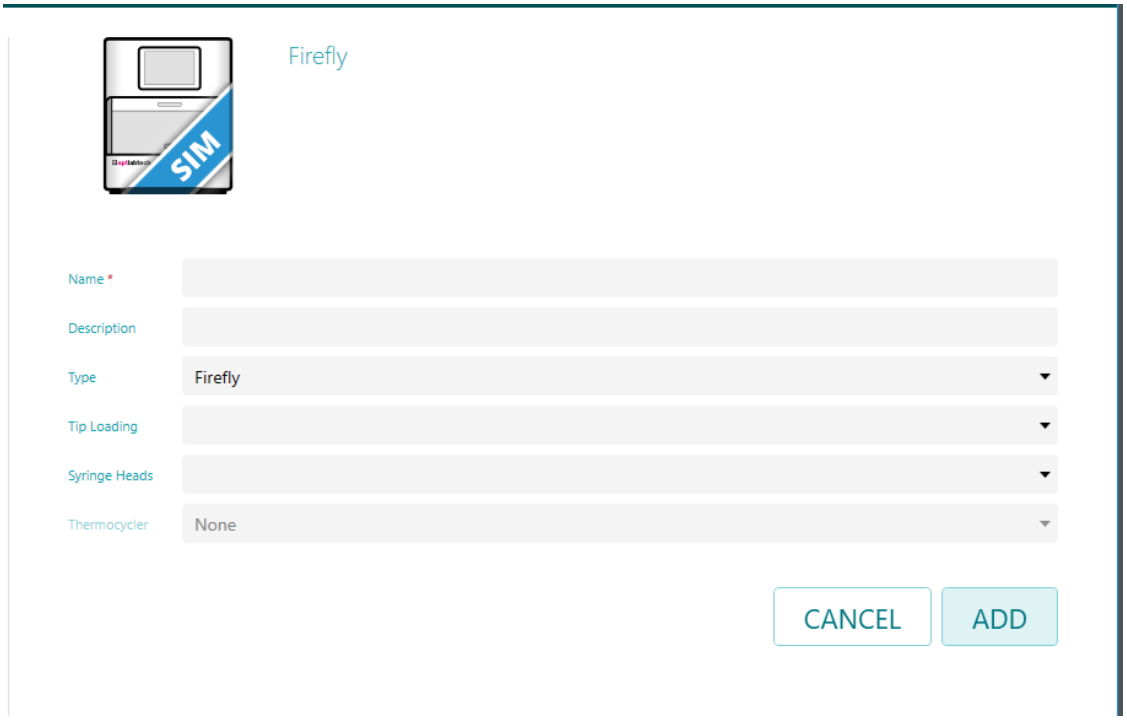
1. Open System, Instruments, and select the instrument in use.



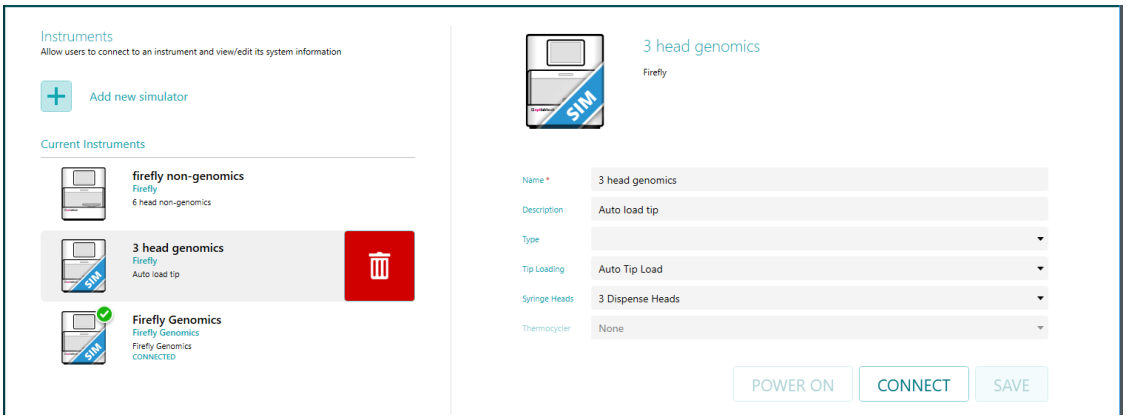
2. Click Disconnect, if you want to alter the simulation in any way e.g., the number of heads.
3. After editing, click Save, then Connect to restart the simulation.
4. Once the Connect button is renamed to Disconnect, the instrument can be used.

You can also add a new simulated instrument:

1. Click + Add new instrument.
2. Select the type of instrument to add.
3. Name the instrument (mandatory) and specify any further details e.g., the tip loading type, if this will be helpful to your protocol development.



4. Select Add. The new simulated instrument is added to your Current Instruments list.



The instrument in use does not change when you create a new instrument.

Note

This function only adds new simulated instruments. Service engineers add new physical instruments using the commissioning functions.

To swap simulated instruments,

1. If there is a currently connected instrument, select it, then select Disconnect.
2. Select the new instrument in 'Current Systems', then select Connect.

When the system is running, it will show a green tick symbol and is ready to be used.

To swap from a simulated to a physical instrument, select Setup, which will disconnect the simulated instrument in use.

Manual controls

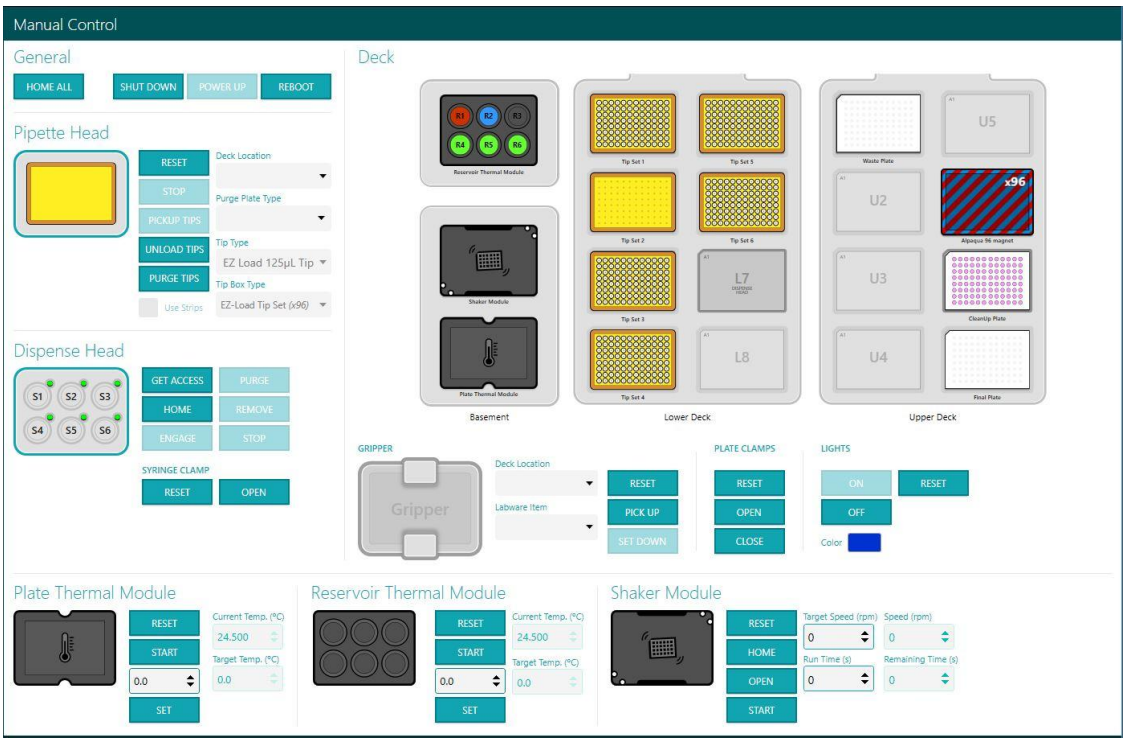
You can directly control the separate firefly modules using the Manual Controls. You may need to do this to reset firefly after stopping a protocol: specific instructions for resetting are shown for the firefly modules you would need to use to do this.

Warning

Do not operate firefly using the manual controls unless you have been trained to do so.

A brief overview of these controls is included here for reference. The appearance will be specific to your instrument type e.g., only 3 dispense heads are shown for 3 head instruments, with the other 3 greyed-out.

If you access the manual controls during protocol execution e.g., after you have stopped firefly to resolve a problem, they will show the current arrangement of labware.



You can remove labware and reservoirs as you would normally, but you will need to purge and unload any syringes or pipette tips that were in use when execution stopped, and you may also need to reset other modules.

Caution

Take care accessing firefly after a protocol has failed to complete; there may be hot surfaces or awkward access. Home the grippers to access the decks and reservoirs.

General

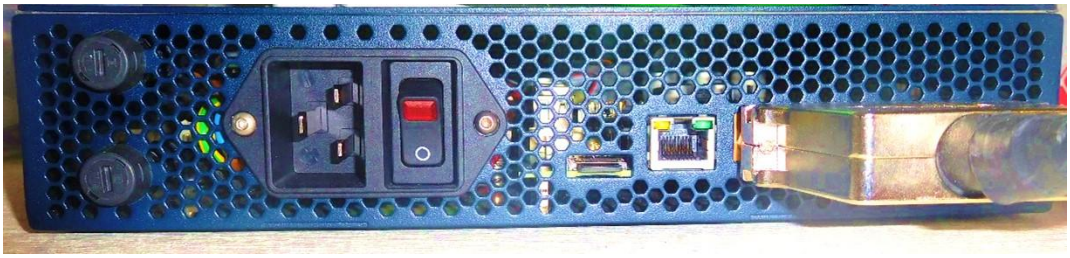
Control	Function
Home all	Moves lower deck to left position and upper deck to right position and opens the plate clamps. This also homes the pipette head and dispense head. If you have firefly+, Home All will home the grippers.
Shutdown	Shut down the firefly instrument
Power up	Start the firefly instrument
Reboot	Turns off the power from the boards. This does not power off the embedded PC.

Important

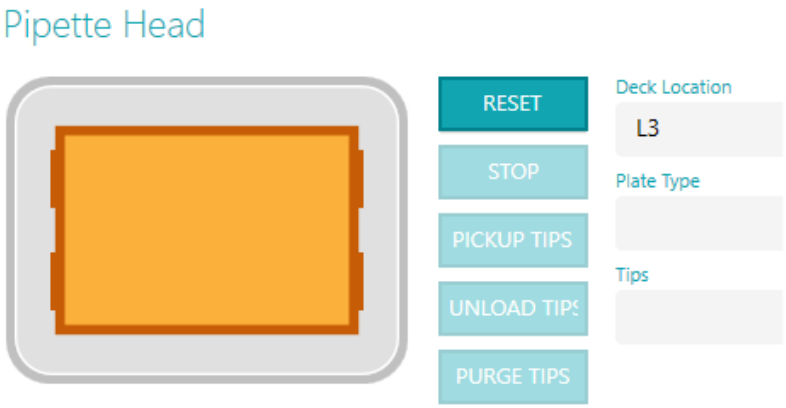
firefly Shutdown does not shut down the ODTC as it has its own power supply.

Important

firefly reboot does not reboot the ODTC, if fitted. To reboot it you need to physically switch off the control unit and switch it on again.



Pipette Head



Empty loaded tips:

1. Select a deck location from the dropdown list. Make sure there is a plate in that location, which you can dispense the liquid into.
2. Select the plate type from the dropdown list.
3. Select the tip type present on the pipette head from the list.
4. Click 'Purge tips' to empty them.

> **Note**
>
> You cannot purge to a plate on a stack.

Unload tips:

1. Select the deck location from the drop-down menu that contains the tip stand you will unload tips into.
2. Click the unload tips button.

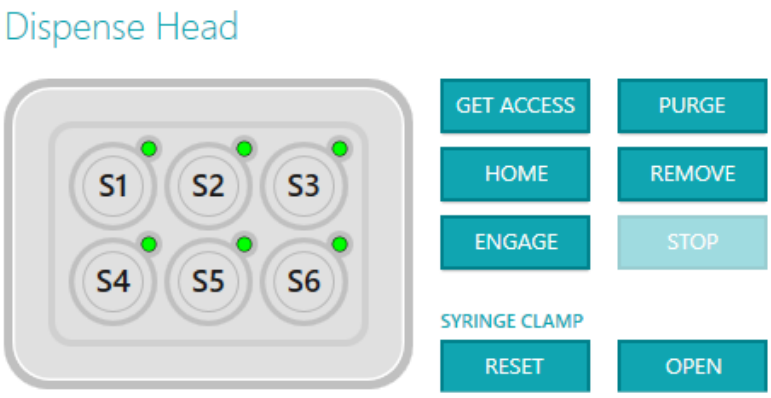
Pick up tips:

1. Select the deck location of the tip set to pick up, from the drop-down menu.
2. Select the correct tip type from the drop-down menu.
3. Click the pickup tips button.

Control	Function
Reset	Resets all parameters to default and initializes to ready the pipette head to perform a new function.
Stop	Cancels the current action of the pipette head.
Pickup Tips	Picks up tips from a specified deck location.
Unload Tips	Unloads tips to a specified deck location.
Purge Tips	Expels remaining liquid from all tips.

Dispense Head

Only S1 to S3 are enabled on a 3 head firefly.



Purge syringes:

1. Select the syringes to purge by selecting the syringe locations on the dispense head graphic. The location will turn green when selected.
2. Ensure reservoirs have been loaded into the reservoir tray. Click the purge button. This will purge all liquid from the syringes into the reservoirs.

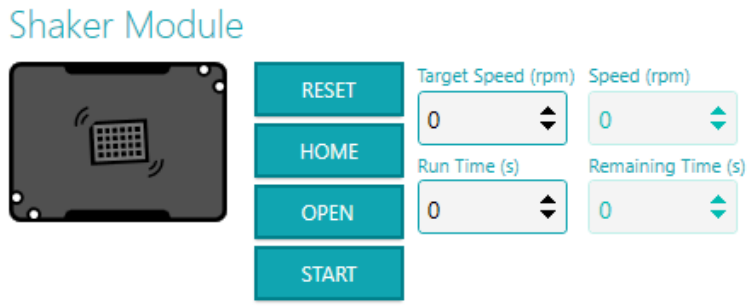
Control	Function
Get Access	Moves dispense head to the front of the firefly and opens syringe clamps to allow user to get access to the dispense head.
Home	This performs the remove function on selected syringes and then returns the head to the default location.
Engage	To be used with a new syringe. Moves dispense head to just above the reservoirs, then will drive the piston rod down to engage the plunger of the selected syringe.
Purge	Expels liquid from the syringes back into the reservoir.
Remove	Expels liquid from the syringes back into the reservoir then withdraws the plunger from the syringe. The syringe clamp opens to enable the selected syringe(s) to be removed.
Stop	Cancels the current action of the dispense head.

Syringe Clamp

Control	Function
Reset	Resets syringe clamps to default open position.
Open/Close	Controls if the syringe clamps are open or closed.

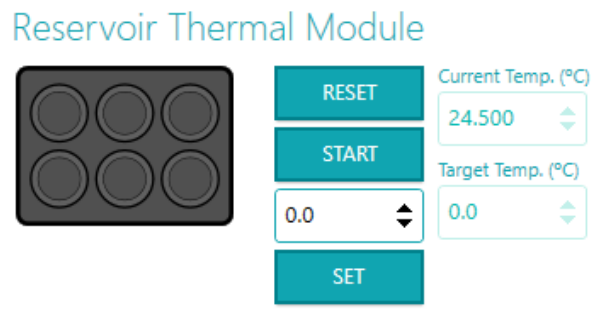
Process Modules (Genomics instruments only)

Shaker Module



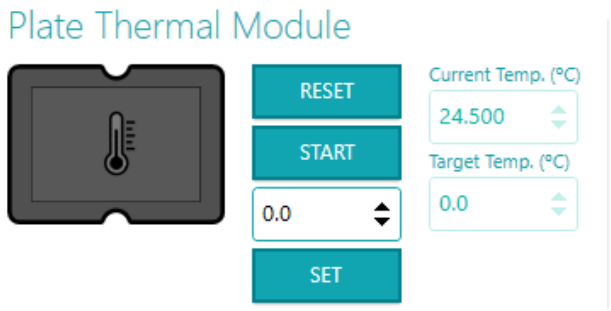
Control	Function
Reset	Resets all parameters to default to ready the module to perform a new function.
Home	Ensures shaker module is in correct position to receive a plate from the deck.
Open/Close	Controls if the plate clamps on the shaker module are open or closed
Start	Starts the shaker module for the specified speed and time set.

Reservoir thermal module



Control	Function
Reset	Resets all parameters to default to ready the module to perform a new function.
Start / Stop	Turns on or off the thermal module so that it can reach target temperature.
Set	Sets a temperature that the thermal module will adjust to over time.

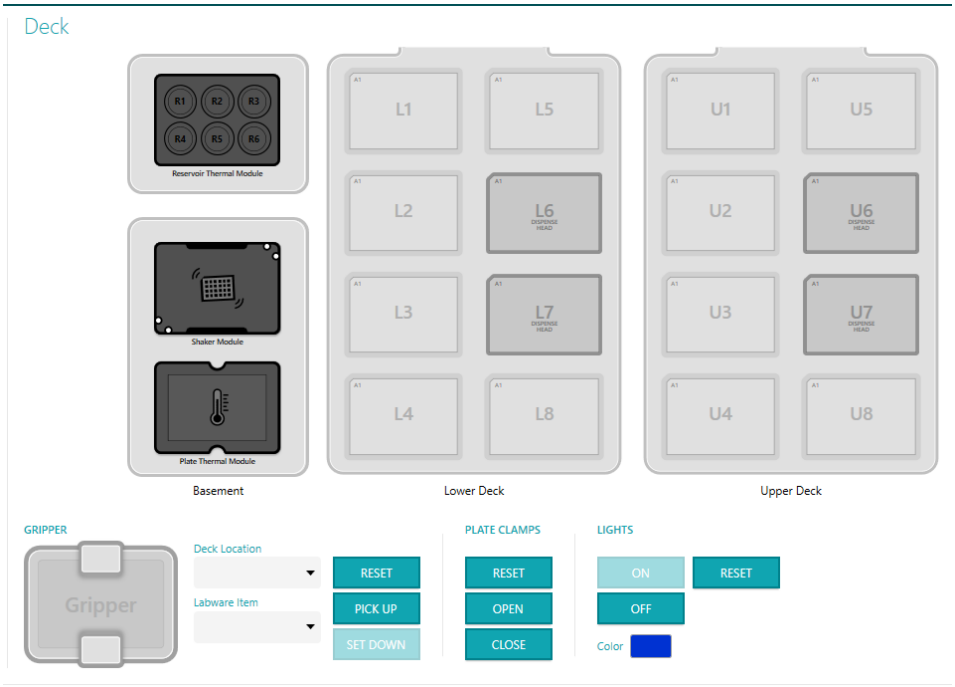
Plate thermal module



Control	Function
Reset	Resets all parameters to default to ready the module to perform a new function.
Start / Stop	Turns on or off the thermal module so that it can reach target temperature.
Set	Sets a temperature that the thermal module will adjust to over time.

Deck

The deck functions relate to repositioning labware e.g., to work around a problem during protocol execution. You can use the grippers to reposition labware, then 'Apply Changes' to update firefly.



Warning

Collision validation is not currently supported on the manual control page, so you must be careful when moving items around. firefly will not warn you that labware will collide nor will it stop the head moving.

Gripper

Control	Function
Reset	Moves grippers back to default position within the pipette head casing.
Pick up	Picks up specified labware from specified deck location
Set down	Puts down labware on specified deck location

Plate Clamps

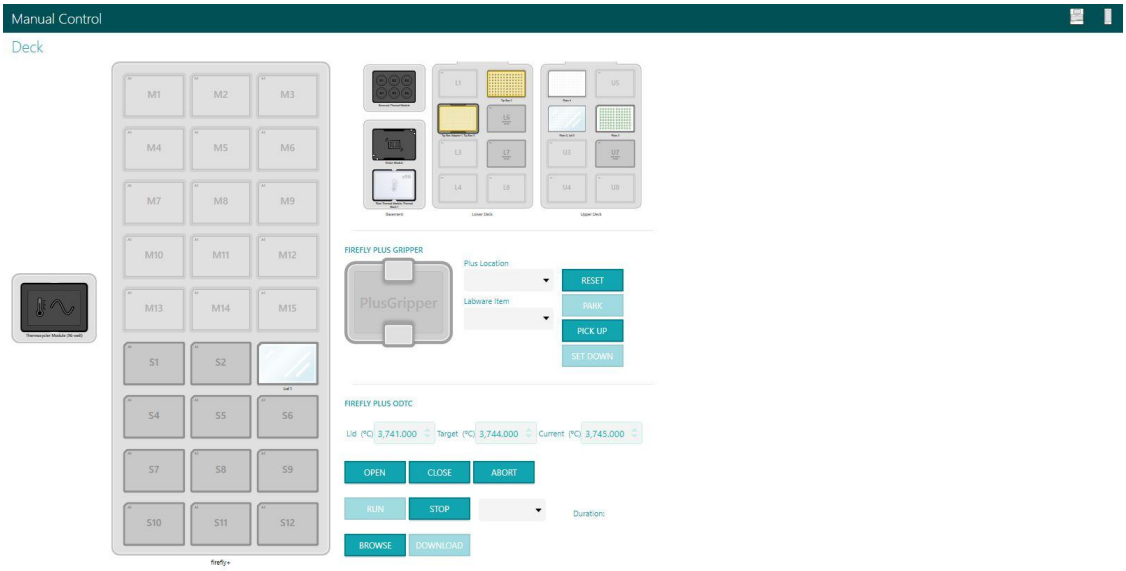
Control	Function
Reset	Resets the plate clamps in case of an error and closes clamps on both decks.
Open	Opens the plate clamps on both decks
Close	Closes the plate clamps on both decks

Lights

Control	
On	Turns the LED lights on
Off	Turns the lights off
Reset	Reverts the lights to the previously set color
Color	Select the color for the lights

Manual controls firefly+

If you are using firefly+, use the  icons to switch between the firefly deck controls and firefly+ shelves and gripper.



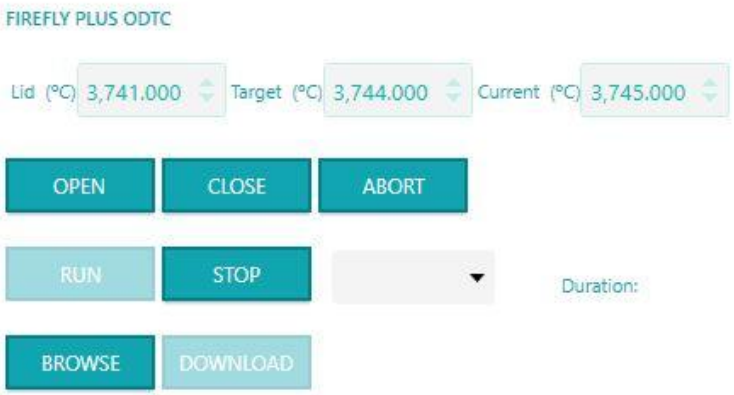
The deck controls give you access to the firefly+ gripper and the ODTC. It can be used to run the ODTC without having created a firefly protocol around the Inheco protocol.

Gripper

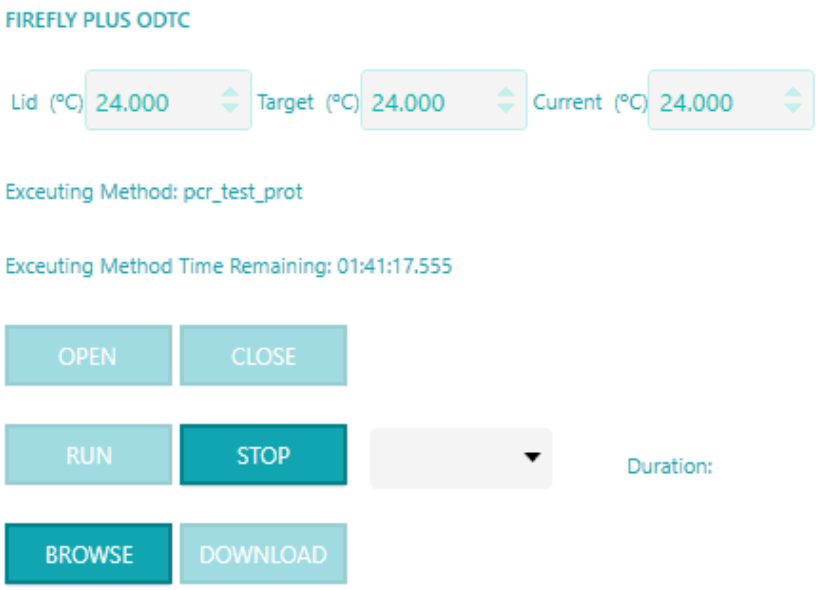
Control	Function
Reset	Stops the current command, after warning the user that any plate held will be dropped
Park	
Pick up	Picks up specified labware from specified deck or shelf location
Set down	Puts down labware on specified deck or shelf location

ODTC

If you are viewing the manual controls for a simulated instrument, they will show implausibly high values for the lid, target and current temperatures. This is intentional: to readily differentiate actual and simulated instruments.



If an ODTC protocol is running, details will be shown here, regardless of whether there is a firefly protocol running. Use the Stop button here if you want to end the protocol.

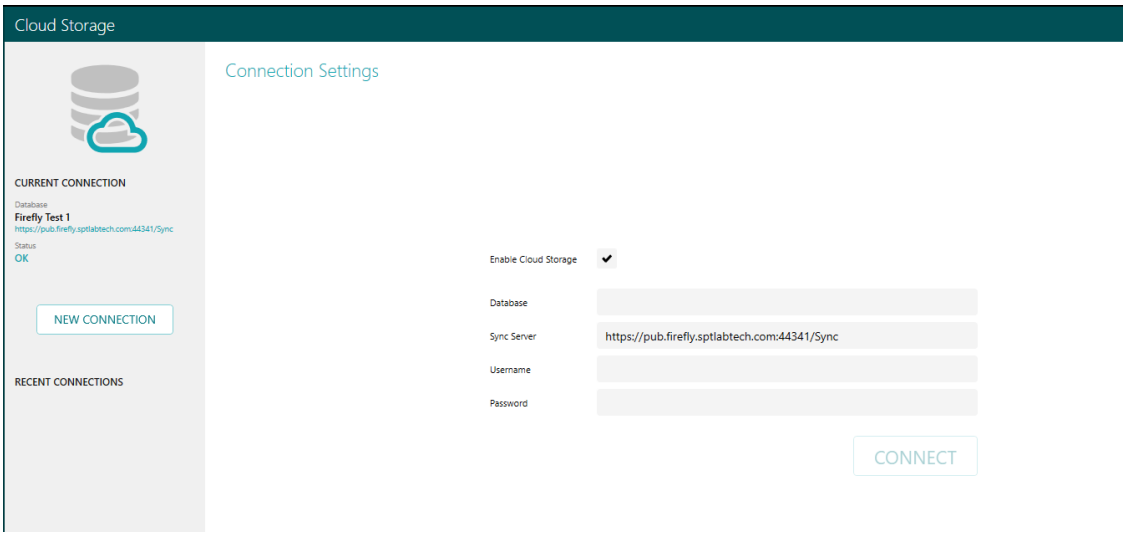


Control	Function
Open	Opens the lid
Close	Closes the lid

Control	Function
Abort	Stops the current protocol running. The Inheco On Deck Thermocycler manual describes the difference between stopping and aborting a protocol.
Run	Start running the selected protocol. Use the dropdown selector to run only the pre method or main method from a protocol.
Stop	Stop running the current protocol.
Browse	Select and open an ODTC protocol
Download	Download a copy of the current protocol XML file

Cloud storage

Cloud storage shows the details of your company's cloud storage connection. As standard, it is only available to administrators.



You can enable or disable cloud storage; be aware that doing this will limit users' ability to access protocols.

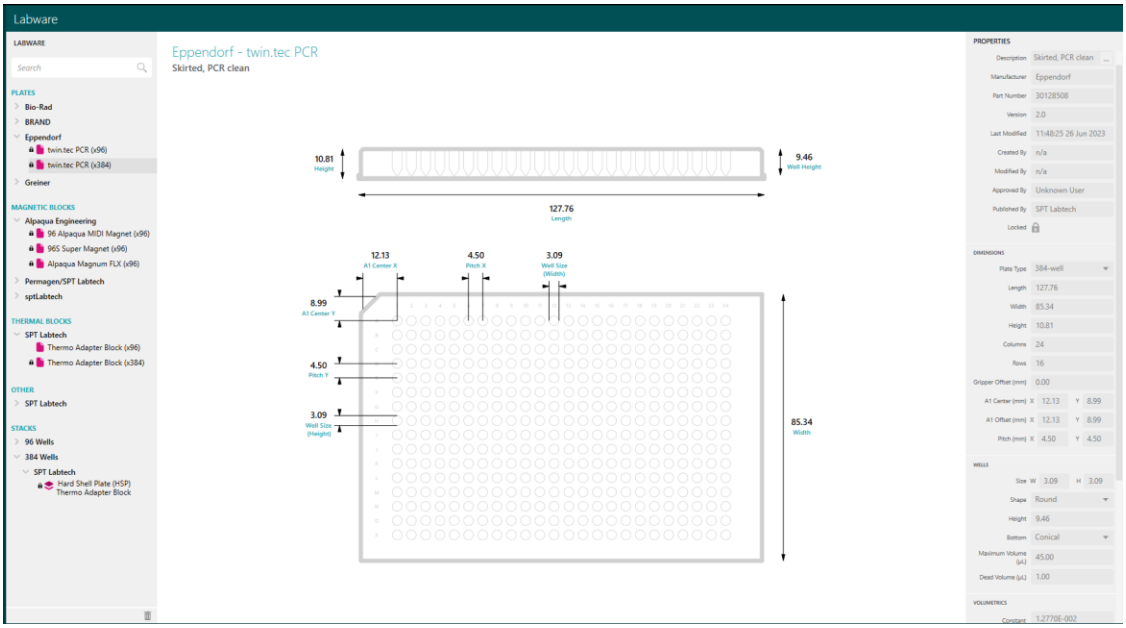
SPT Labtech will work with you to set up cloud storage for your firefly users when setting up your instrument. You can revise your connection settings to use a new cloud Sync Server, or verify your current connection status.

Labware

The Labware screen shows the detailed specifications of all labware and stacks which are available to you to use in protocol design. If you want to use an item which is not listed, you will need to download it from Community.

The padlock symbol shows that the labware's specification has been verified and approved. It is then locked, to ensure no inadvertent changes 

You can also check which version of labware is in use, although you will need to use Community to check for updates to labware specifications.

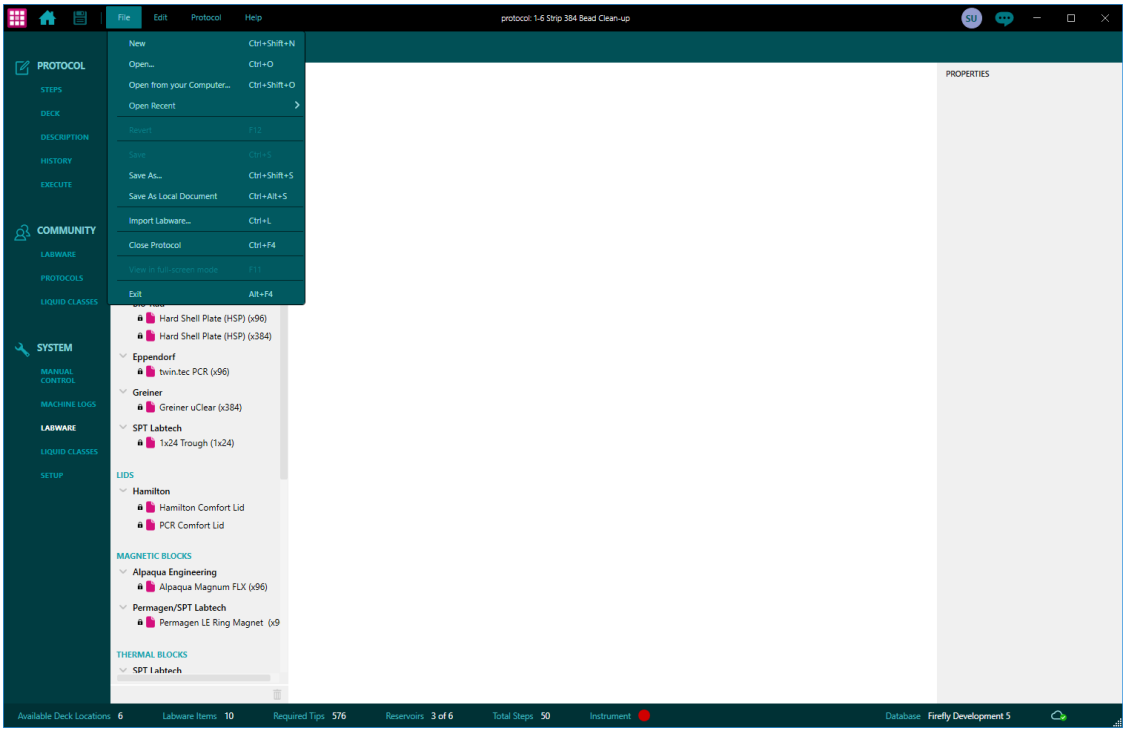


If SPT Labtech application scientists have designed a protocol for your lab, which is not available for download from Community, it is possible that it will include unapproved labware (the symbol is an open padlock), which is only for use in that specific protocol.

If you have permission, you can delete labware. Right click the item, then Delete. You can delete both locked and unlocked labware.

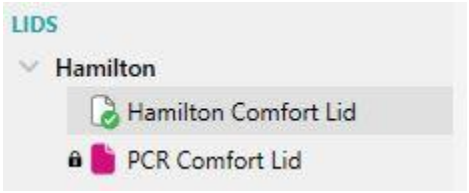
Import labware

If SPT Labtech application scientists have designed a labware item for your lab, which is not available for download from Community, you will need to import it. On the Labware screen, select Import Labware from the File menu.



A popup form opens for you to select the labware file e.g., from Downloads. It will have a .sptlab file extension. Select the item, then Open, to import. A second popup form confirms the labware to be added.

Imported labware (the Hamilton Comfort Lid) is differentiated from labware downloaded from Community (the PCR Comfort Lid).



Liquid classes

Liquid classes enable you to define aspirate and dispense settings for liquids which you frequently use in protocols. This enables you to design protocols more efficiently, as once you select the liquid class, the pipette head aspirate or dispense properties are automatically set to the appropriate values for that liquid. The dispense head uses presets instead of liquid classes.

You can also download liquid classes from Community.

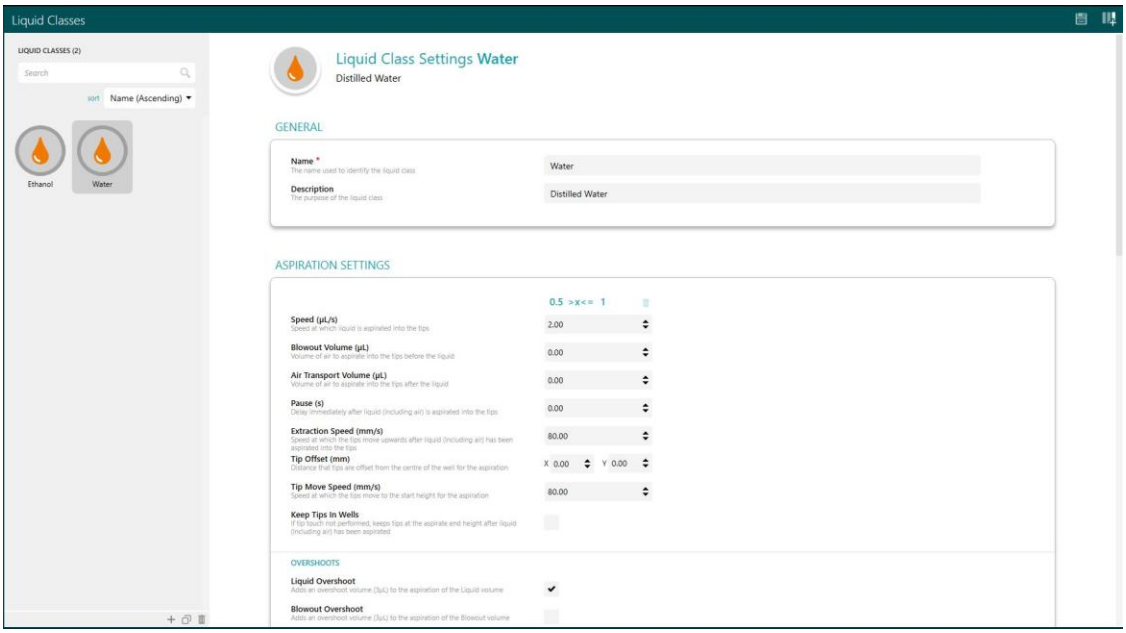
Using liquid classes is entirely optional, firefly software always allows you to set protocol step parameters manually.

Create liquid class

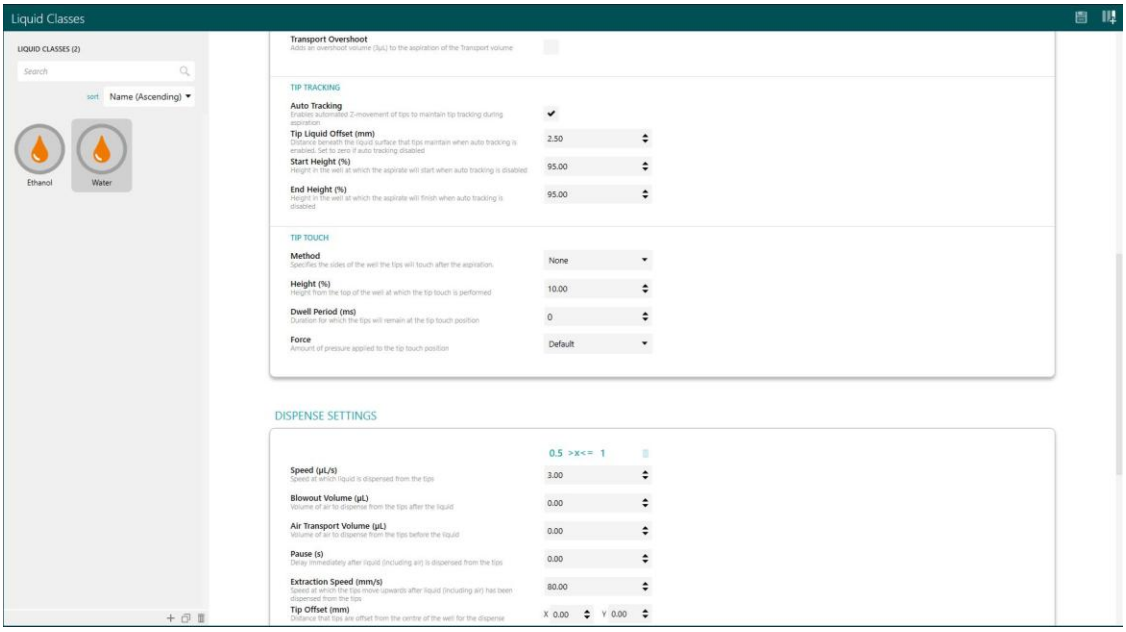
To create a new liquid class, click on the '+' on the Liquid Classes form.

Use the copy function (middle button) to copy an existing liquid class and open it for editing.

Name your liquid class and optionally add a description.

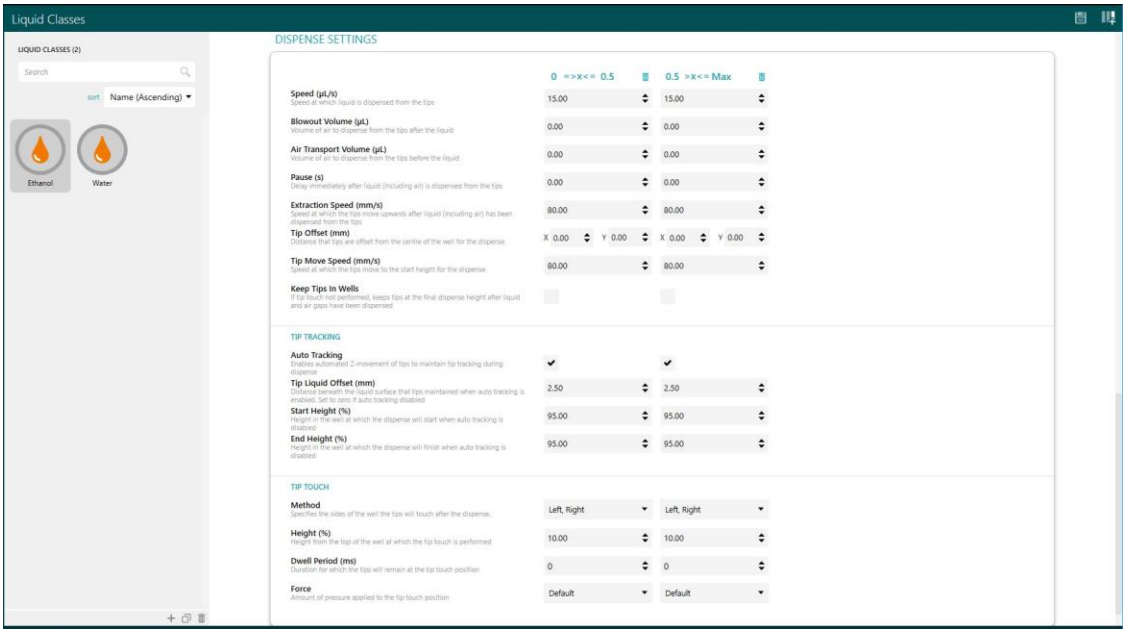


Set aspirate and dispense parameters for pipetting. These may be based on your experience with other liquid handling instruments. The parameters have brief descriptions. You can also deselect certain features such as tip tracking, if you do not want to use them.



If you need to vary your settings with increasing volumes, you can define multiple sets of parameters for specific volume ranges, using 'Add Volume Range' (at the top right, next to Save).

If you do this, firefly will automatically select the correct parameters for the volumes in your protocol pipetting step.

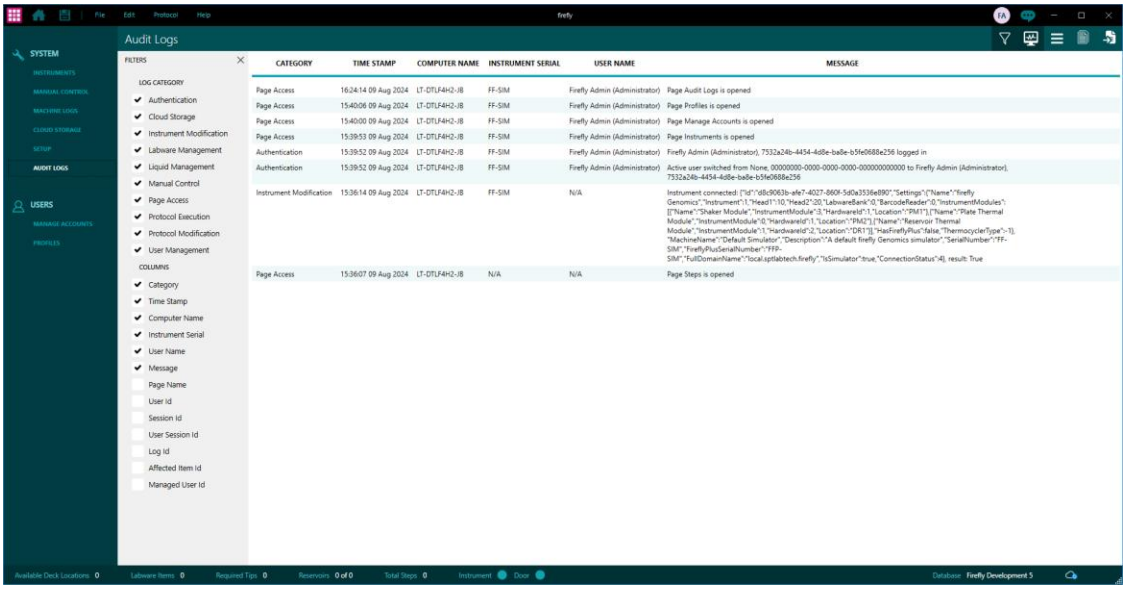


Save your liquid class (Save icon at top right) to make it available for use.

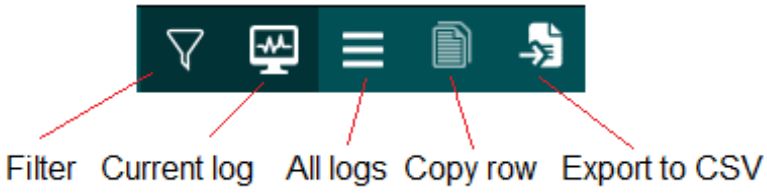
Audit Logs

The audit log records all actions which occur on the firefly instrument, real or simulated. It can be used to support compliance activities, as it includes failed actions e.g., login, which may be of concern, as all events are time and date stamped, and show the logged in user.

By default, viewing the audit log is an administrator permission, but other groups i.e., QA could be set up with permission to view it.



As every action of instrument and user is recorded, there are functions to narrow the scope of data.



There are numerous filters, which can be used in combination, to view specific events or event types.

The current log enables you to view data relating to the current session of firefly use.

All logs loads all audit trail data for the firefly. You can define specific users, time periods or other filters to screen the data.

Copy row enables you to copy one or more selected rows of the audit log to the clipboard. You can also use the right mouse button menu to access copy functions.

To copy all log data, use the Export to CSV option. You can select the export location.

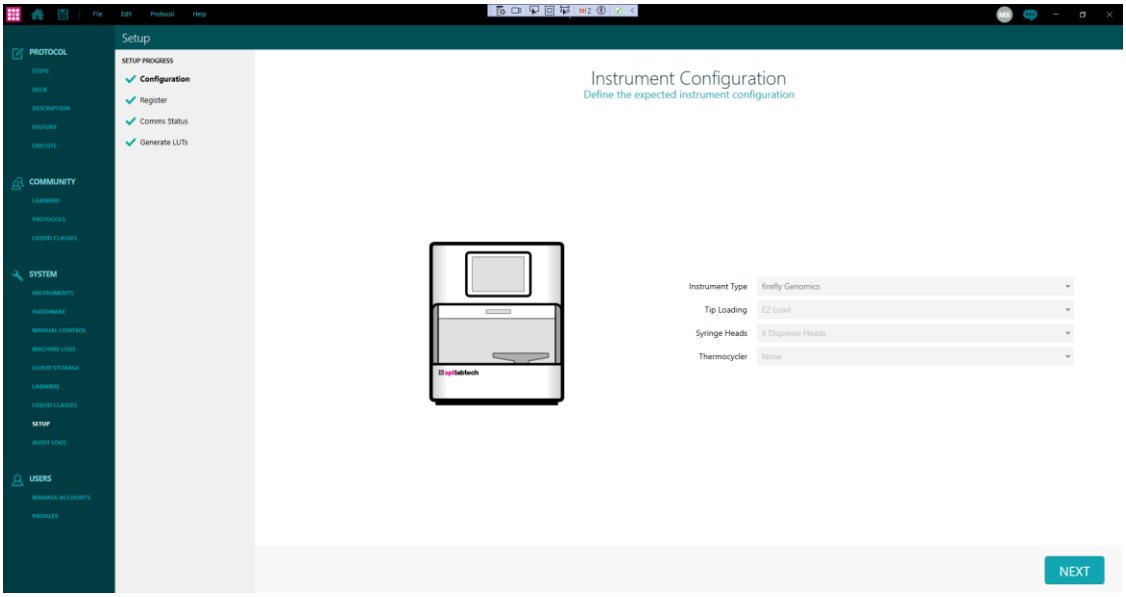
Instrument

Setup

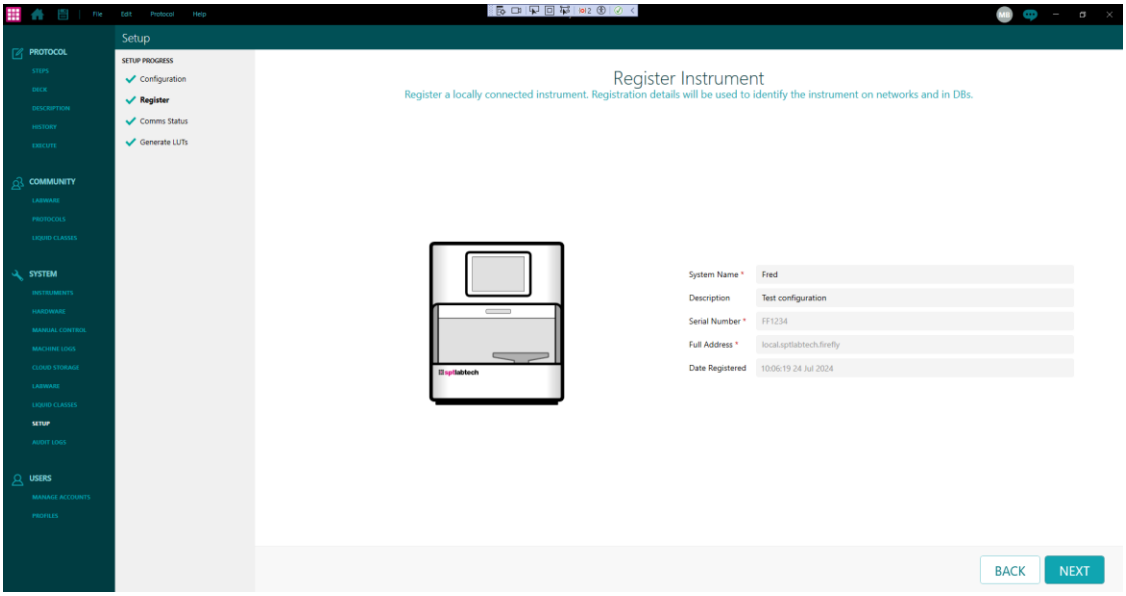
Setup contains a restricted form of the wizard to guide the user through setting up a new physical firefly instrument. You should not need to access these functions unless requested by a reliance service engineer, or in response to error messages generated by your instrument.

> **Note**
>
> SPT Labtech does not recommend that Administrators should enable Setup access for users as standard. This privilege should be limited to users with knowledge of liquid handling automation.

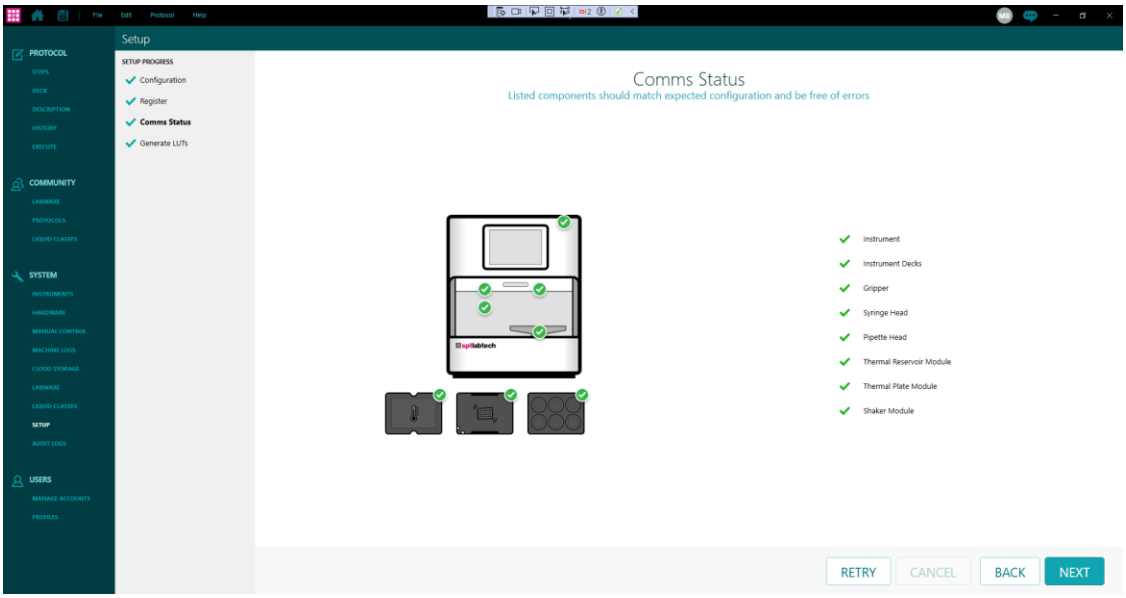
The first screen shows the instrument configuration. This screen is read-only. Select Next to continue.



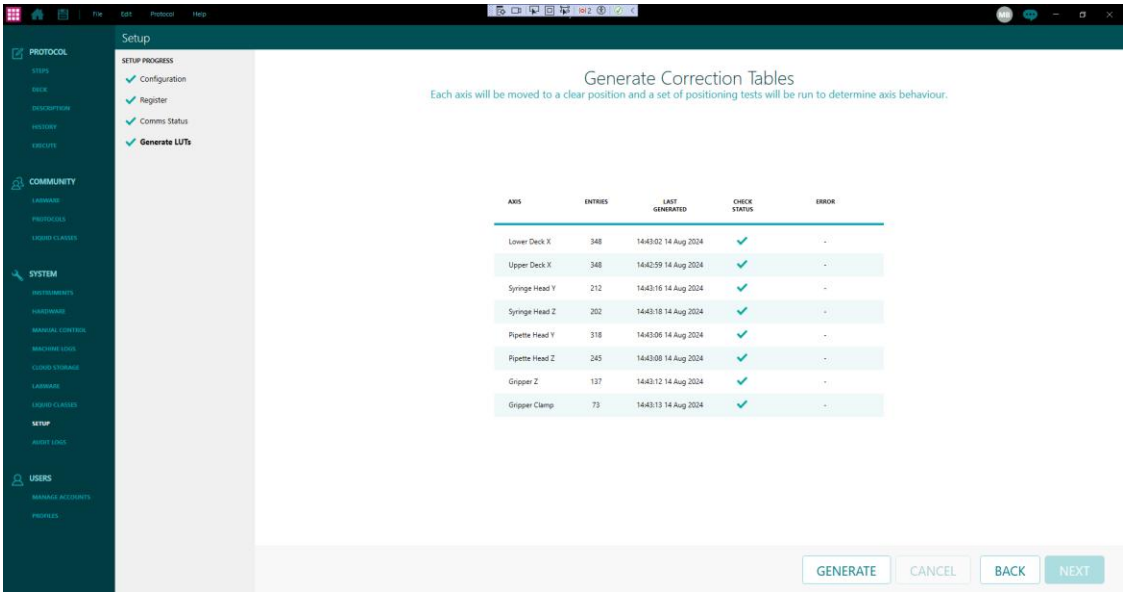
The next screen shows the instrument registration details. You can change the instrument name or description. If you do, when you save the change, your firefly will reboot.



The third screen shows the comms status of firefly's internal connections. If there is a problem with any of these, an x will be shown in place of the tick. If you see this, contact reliance. Only use the Retry function if advised to do so.

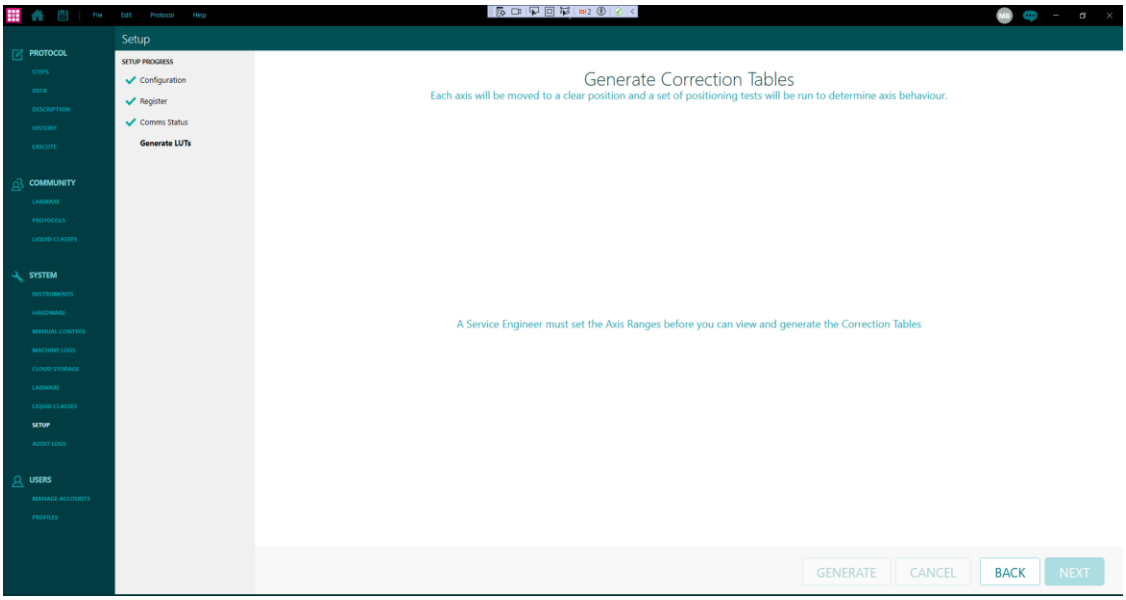


The final screen shows the Look Up Tables (LUTs) / Correction Tables. These are generated by performing test moves for the heads, the grippers and the decks, to confirm their positional accuracy.



Important

If you see the screen below, for the Generate LUTs option, your instrument has not been fully set up. Contact reliance for assistance. Do not try to use the instrument.

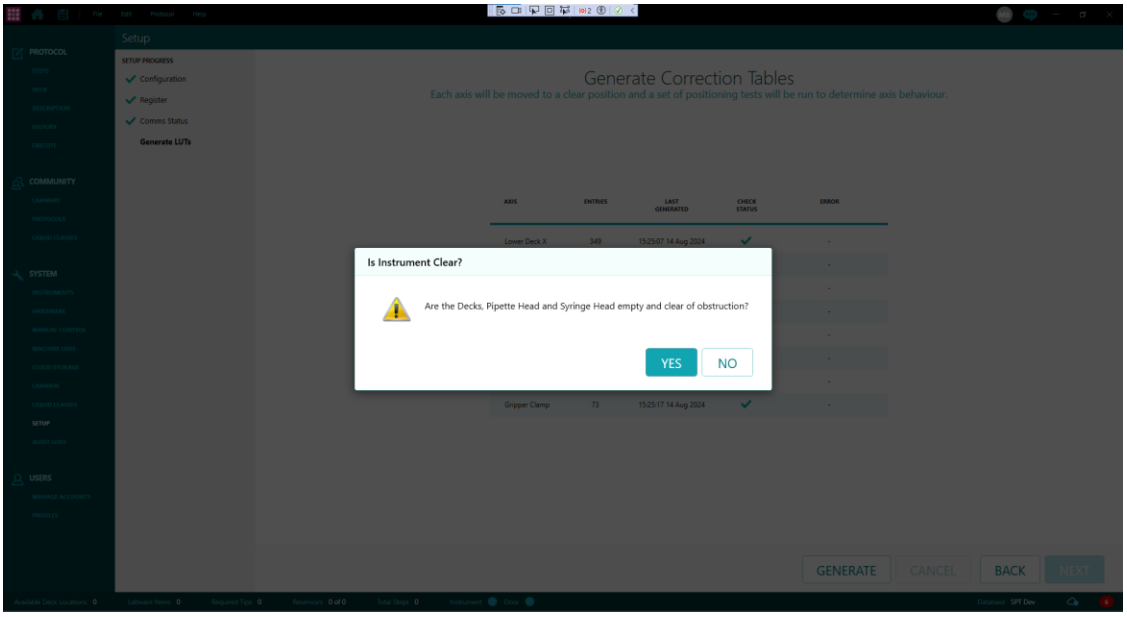


If all the positional checks were last run successfully, you will see something like this. Take no further action if all the checks were successful.

AXIS	ENTRIES	LAST GENERATED	CHECK STATUS	ERROR
Lower Deck X	348	14:43:02 14 Aug 2024	✓	-
Upper Deck X	348	14:42:59 14 Aug 2024	✓	-
Syringe Head Y	212	14:43:16 14 Aug 2024	✓	-
Syringe Head Z	202	14:43:18 14 Aug 2024	✓	-
Pipette Head Y	318	14:43:06 14 Aug 2024	✓	-
Pipette Head Z	245	14:43:08 14 Aug 2024	✓	-
Gripper Z	137	14:43:12 14 Aug 2024	✓	-
Gripper Clamp	73	14:43:13 14 Aug 2024	✓	-

If any of the axes are in error, there will be an X for the check status for that axis, and error information. In this case, reliance service engineers may advise you to regenerate the LUTs.

1. Select Generate.
2. Check the instrument is clear of labware, and select Ok, when prompted.



Caution

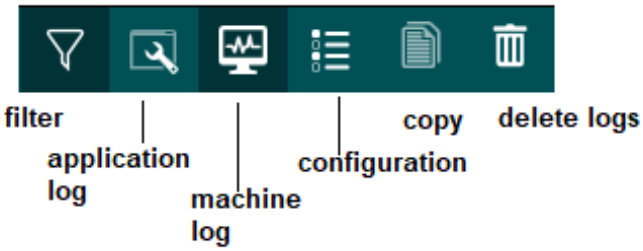
If firefly is not clear of labware, the moving heads and decks could collide with it and be damaged. Do not run Generate Correction Tables until the instrument is clear.

3. While the checks are running, you will see a busy symbol.
4. When the LUTs have been regenerated, you will see the record of the positioning tests which have just been run.

Machine Logs

As standard, firefly Super Users and Administrators can access Machine Logs but there is no need for any users to check the logs routinely. You may be asked to check the logs by reliance service engineers if you have problems with your instrument.

There are multiple log views available from the controls on the top menu.



The machine log records events in hardware, the application log records events in software.

Copy enables you to copy selected log lines to the clipboard.

Managing user accounts

firefly software is very flexible and allows you to set up whatever combination of user permissions will be useful for your laboratory team. The sets of permissions are called profiles, and you can revise them at any time, if you find that your first arrangement is not working well in practice.

firefly software includes three standard profiles: standard user, super user and administrator, but if you prefer you can create your own specific set of profiles to suit your company's roles.

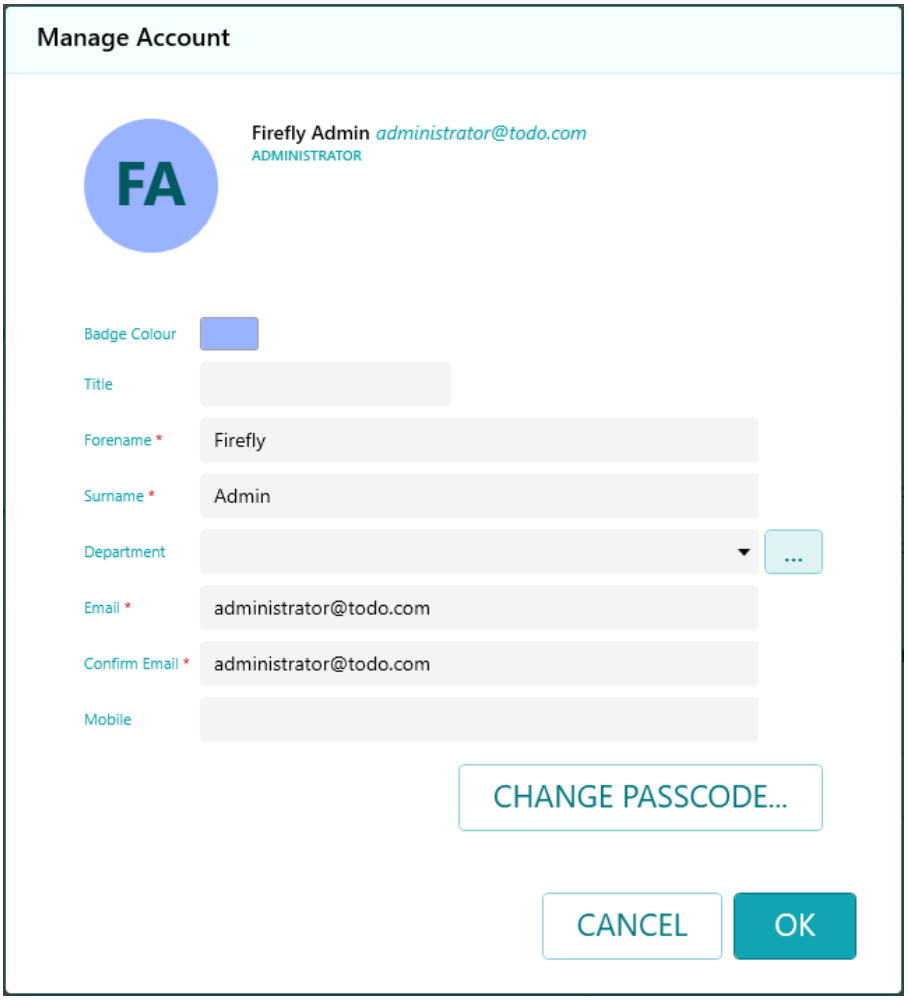
As standard, only administrators can access the profile and user management functions (though all users can edit details of their own accounts).

Managing your own account

Click on the user account button (circled in red) to access your account details. This function is available to all users.

Select 'Manage Account' from the menu, to update your details or change your passcode.

If you wish, you can also select to change your badge color from that which firefly software set automatically.



Select your department from the dropdown list. If it is not shown correctly, click the '...' button to revise or add information. When you first log in, your administrator will have set an initial passcode, which you must change immediately, using Change Passcode.

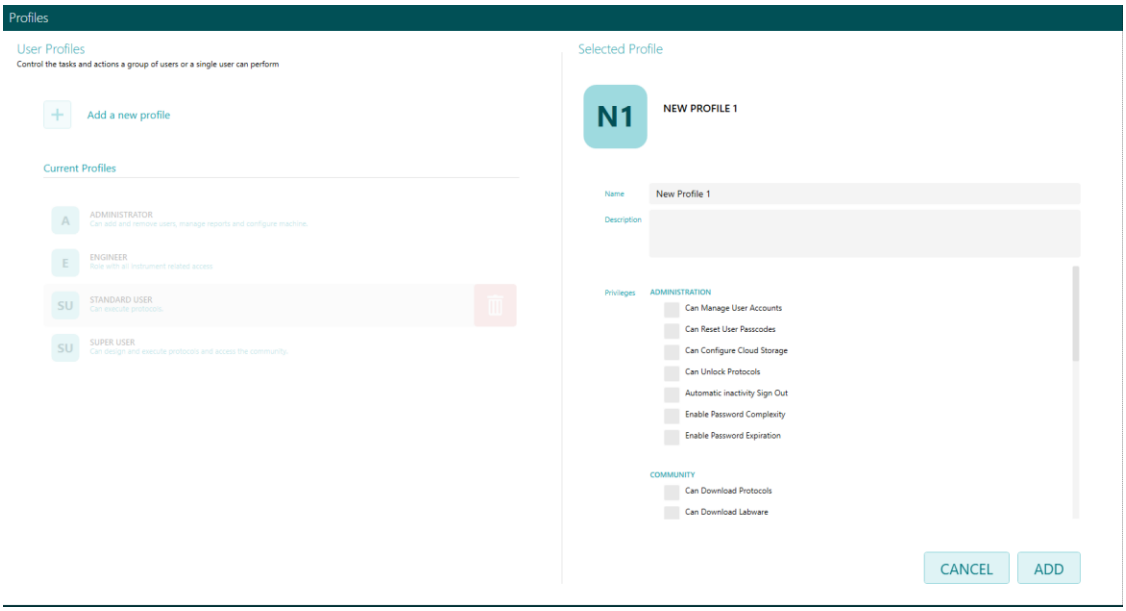
Your organization may implement a requirement for passcode complexity. This will require that:

- no digit can be repeated more than 3 times consecutively in your code
- no digit can be repeated more than 4 times in the code
- there must be at least 3 different digits

Your organization may also require you to change your password at set intervals (90 days is the default).

Creating or modifying profiles

You need to define profiles, using 'Users', 'Profiles' before you can create user accounts, unless you will be using the standard profiles.



You can modify the existing profiles, or use 'Add a new profile' if you want completely different roles.

Tick or untick Privileges to create appropriate profiles for the responsibilities of staff in your laboratory, then 'Save' when you are done. Use the specific privileges to create roles required to meet regulatory requirements e.g., you may want to restrict the privilege of locking / unlocking all protocols to a system administrator or QA function.

Administration Privileges	
Can manage use accounts	This is an administrator function by default.
Can reset user passwords	This is an administrator function by default.
Can configure cloud storage	This is an administrator function by default.
Can unlock protocols	All users can lock or unlock protocols which they have created, this privilege is to lock or unlock protocols created by others. It may be useful as an approval function for a team leader or QA role.

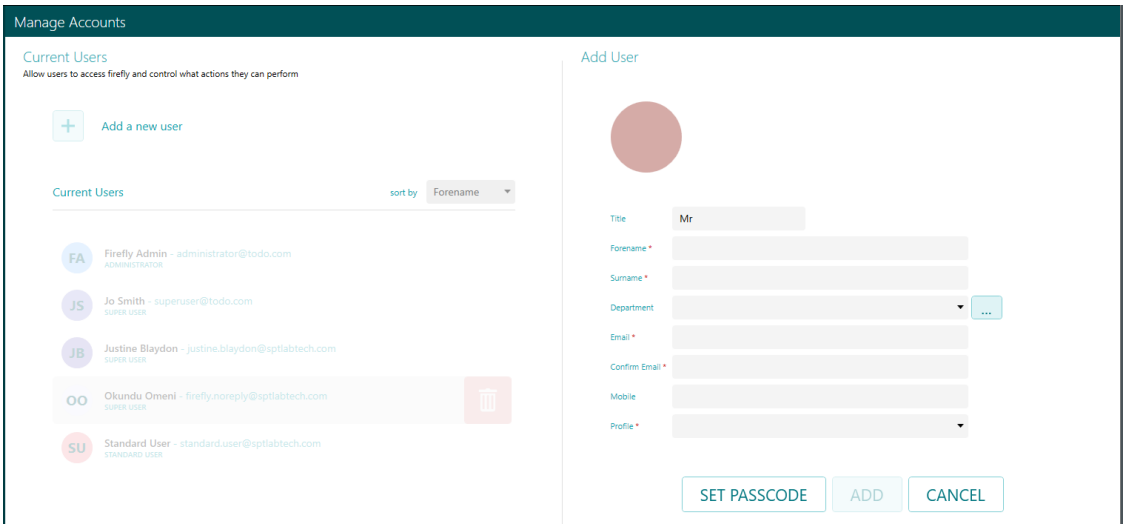
Administration Privileges	
Automatic inactivity sign out	This is not enabled by default, but may be appropriate for regulated labs.
Enable password complexity	This is not enabled by default, but may be appropriate for regulated labs.
Enable password expiration	This is not enabled by default, but may be appropriate for regulated labs. The default is expiry after 90 days.
Automation Privileges	
Can enable automation.	This enables low level control of firefly instruments via the firefly API, and is ordinarily reserved for service engineers.
Community Privileges	
Can download protocols	This is a super user function by default.
Can download labware	This is a super user function by default.
Can download liquid classes	This is a super user function by default.
Instrument Privileges	
Can access machine logs	This gives access to the machine logs which may be useful to super users, admin users such as lab managers, or site engineers.
Can control instrument hardware directly	This enables low level control of firefly instruments, and is ordinarily reserved for service engineers.
Can configure instrument	This is an administrator function by default, to set up a simulated firefly.
Can control instrument manually	This gives access to the manual controls. It is only appropriate for users who have been trained in using these functions by reliance.
Can access instrument setup	This gives limited access to the setup functions. It is not ordinarily required.
Can access audit logs	Access to the audit log is not enabled by default, but may be appropriate for admin or QA functions in regulated labs.
Can manage liquid classes	This is a super user function by default.
Labware Privileges	
Can manage labware	This is a super user function by default.

Administration Privileges	
Protocol Privileges	
Can override execution setup	This is a super user function by default.
Can design protocols	This is a super user function by default.
Can rollback protocols	This enables users to revert to an earlier version of a protocol. It is not enabled by default.
Can execute protocols	This is a user and super user function by default.
Can execute protocols in debug mode	This privilege allows users to execute a protocol one step at a time, or to ignore pauses, both of which may be useful in protocol development.
Can adjust protocol variables	This is a user and super user function by default, to set protocol variables before execution.
Can execute protocols containing script steps.	This is a service engineer function by default.
Can update labware during protocol execution	This is a super user function by default, to update certain consumables.
Can digitally sign execution reports	Digitally signing execution reports is not enabled by default, but may be appropriate for admin or QA functions in regulated labs.

Name the new profiles; it would be useful to give them descriptive names e.g., 'Senior Technician' or to match the terms used in your SOPs.

Adding user accounts

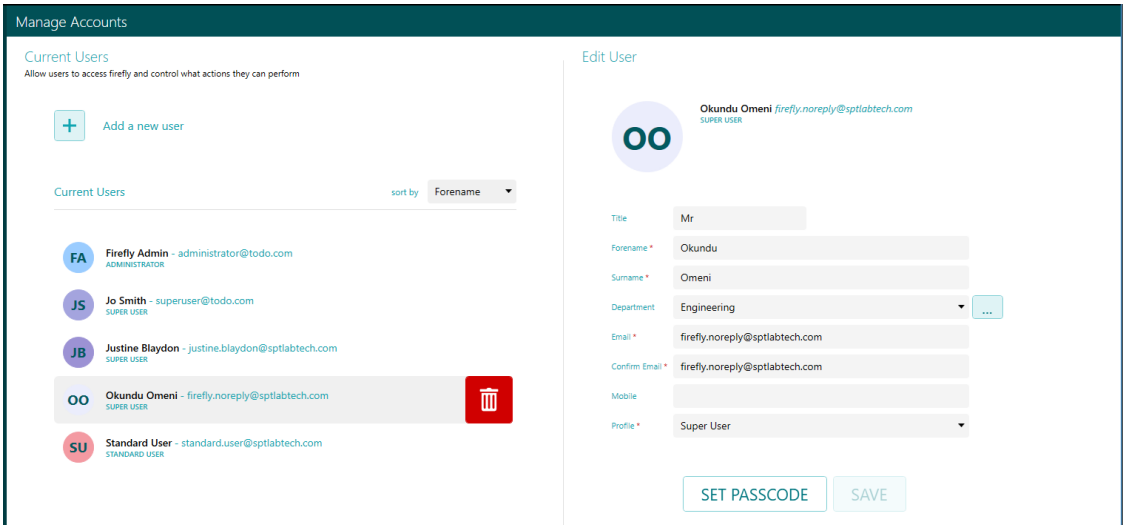
Use 'Users', 'Manage Accounts', 'Add a new user' to create specific users' accounts, and link them to one of your predefined profiles. You must fill in the starred fields for each new user and set an initial passcode for them. Use '...' if you need to create new department details for them.



Updating or deleting user accounts

Users are all able to reset their passwords and update all their details except for their profile.

Administrators can also edit users' details, and additionally revise their profile.



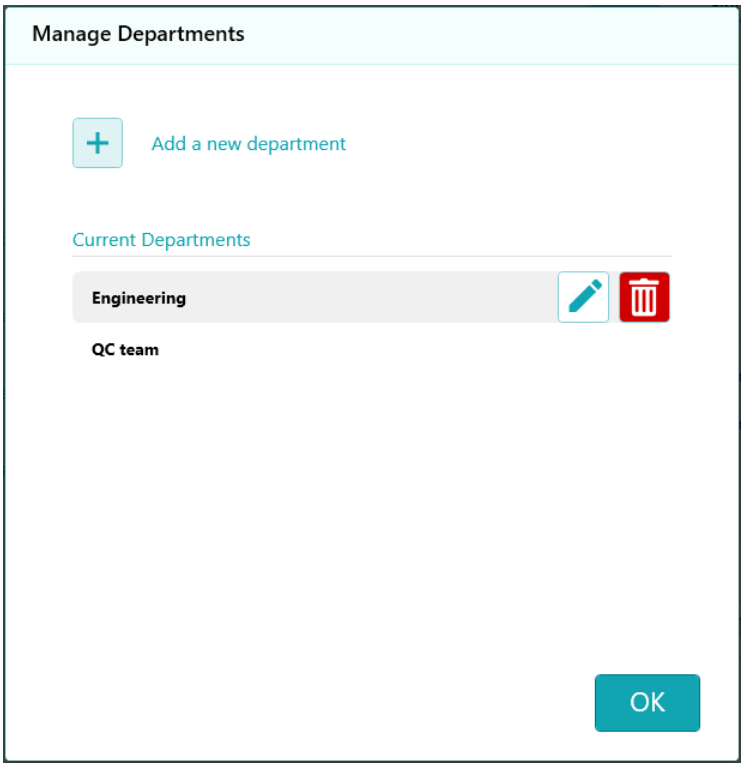
To delete a user, click the red bin next to their name on the 'Managing Accounts' view.

Important

Make sure that the user has moved any private protocols or other firefly data into public folders before their account is deleted as otherwise no one will be able to access them.

Managing departments

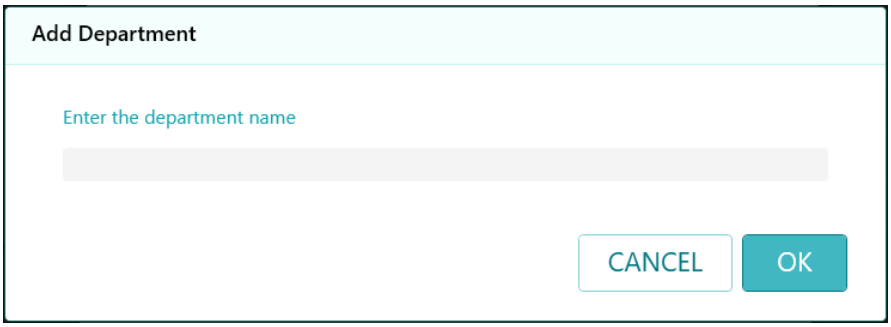
Click the '...' button on the Manage Account popup or Manage Accounts form, to edit departments.



- Select the pencil button to edit a department's name
- Select the waste bin button to delete a department
- Select the + button to add a new department

Adding a department

Use the Add Department popup form to add a new department name.



Deleting profiles

You will need to use 'Managing Accounts' to reassign or delete users assigned to a profile before you will be able to delete it.

To delete a profile, click the red bin next to it in the 'Profiles' view. firefly will not allow you to delete the profile if it is still in use.

Error handling

firefly displays error messages in red in the execution log, if there is a problem with your protocol or the instrument.

Error Message	Response
Validation	
This would display the first validation error as the reason for not executing the protocol	Make sure the Protocol has no errors before trying to execute it
Tip change errors	
Pipette Head failed to load Tips.	This is usually because tips are already loaded
Pipette Head failed to unload Tips.	This is usually because no tips are currently loaded
Pipette head errors	
Failed to set Pipette Head to volume mode.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to set Pipette Head aspirate speed.	Pipette head error. If this persists, try power cycling your instrument.
Failed to aspirate Blowout Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to aspirate Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to set Pipette Head dispense speed.	Pipette head error. If this persists, try power cycling your instrument.
Failed to dispense Air Transport Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to dispense Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to dispense Blowout Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.

Error Message	Response
Failed to aspirate Mix Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to set Pipette Head dispense speed.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to dispense Mix Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to set Pipette Head Post Mix aspirate speed.	Pipette head error. If this persists, try power cycling your instrument.
Failed to aspirate Post Mix Aspirate Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to dispense Post Mix Aspirate Overshoot Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to aspirate Post Mix Aspirate Air Transport Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to set Pipette Head Post Mix dispense speed.	Pipette head error. If this persists, try power cycling your instrument.
Failed to dispense Post Mix Air Transport Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to dispense Post Mix Dispense Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Failed to dispense Post Mix Blowout Volume.	Pipette head error. It may need purging and resetting. You will need access to the manual controls to do this.
Firefly HvHead Error[Aspirate]	Pipette head error during aspirate. May need purging and resetting. You will need access to the manual controls to do this.
Firefly HvHead Error[Dispense]	Pipette head error during dispense. May need purging and resetting. You will need access to the manual controls to do this.

Error Message	Response
Dispense head errors	
Dispense run cancelled	Dispense head error. Purge syringes of any liquids before continuing. You will need access to the manual controls to do this.
Stopping dispense run, due to problem	Dispense head error. Purge syringes of any liquids before continuing. You will need access to the manual controls to do this.
Take off	
Can't move item: {item name} off stack. No empty locations found	All deck positions are occupied. Was a user interaction to remove a plate missed?

Not all errors will lead to an error message; some are managed in the software e.g., if you accidentally loaded a 384 ATL tip box when the protocol required a 96 tip array, the head will pick up tips leaving column 24 behind.

If ATL tips have not loaded properly, use the manual controls to unload them to an empty tip box. Check they are undamaged. If they are fine, restart your protocol and try to load them again. If they are damaged or not straight, unload them to any suitably sized holding container, e.g. a trough plate, if they will not unload to a tip box, and contact reliance for assistance.

If you cannot unload the tips at all, perhaps because there is a problem with the hooks holding the ATL tip box adapter, contact reliance for assistance.

If your protocol failed to complete because the tablet lost communication with the firefly control boards, use the recovery wizard.

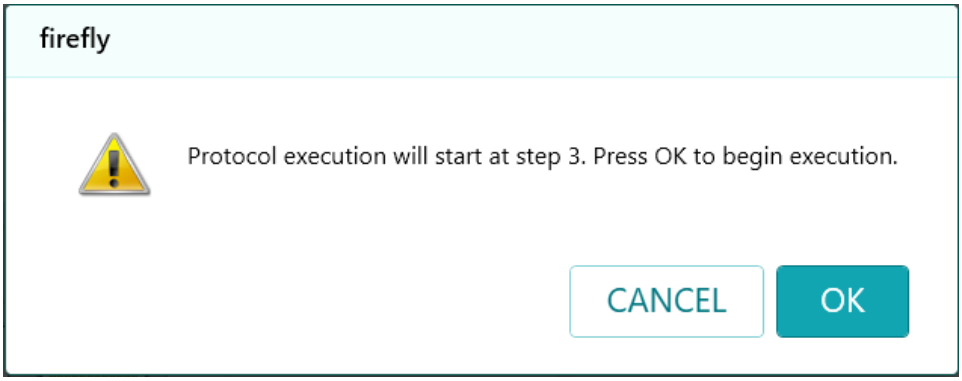
Contact reliance if you have a problem with your firefly or firefly+ instrument, as it has no user-repairable parts.

If the vertical laminar flow module displays a 'Low Airflow' alarm message, contact reliance.

Restarting a protocol from a specific step

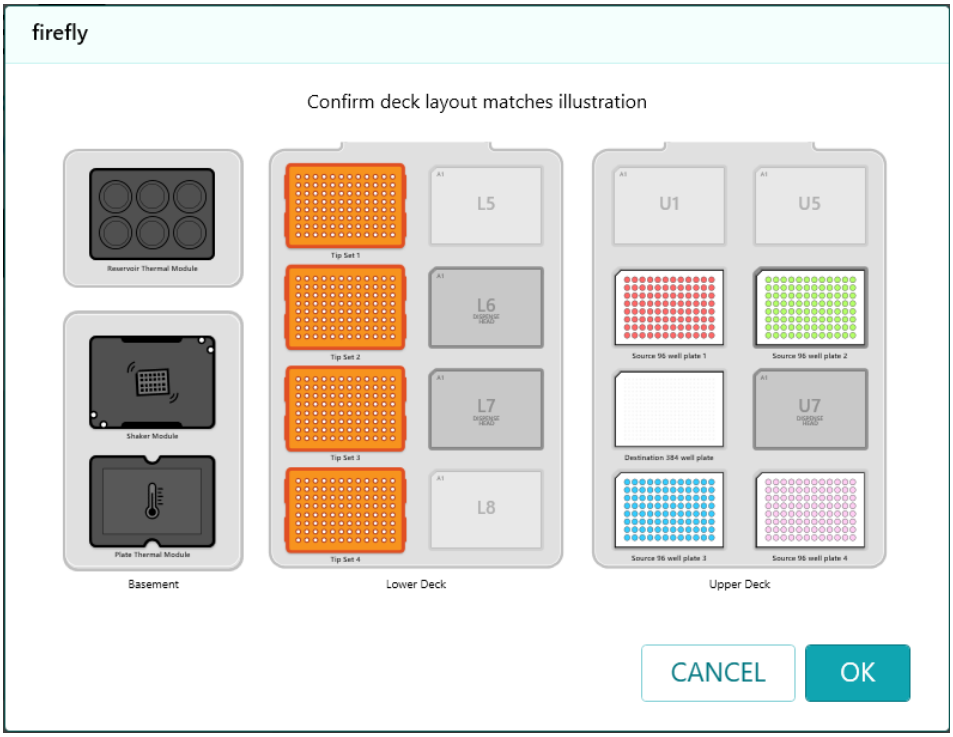
If you have had to stop a protocol, you may want to restart from a specific step partway through, if you have permission to do so.

Select the step from the dropdown list. firefly will ask you to confirm your choice.



Before you select Start, check that you are re-starting at a sensible point e.g., you are not about to dispense when there is no liquid aspirated into the syringes.

firefly will ask you to confirm the deck layout before allowing you to restart the protocol.



Check that the illustration is correct before restarting, or your protocol will fail again.

Note

If you had run an ODTC pre-method before the protocol stopped, you will need to re-run it before you can run the main method. Restart your firefly protocol so that running the ODTC pre-method is the first step.

Restarting after a protocol failed to complete

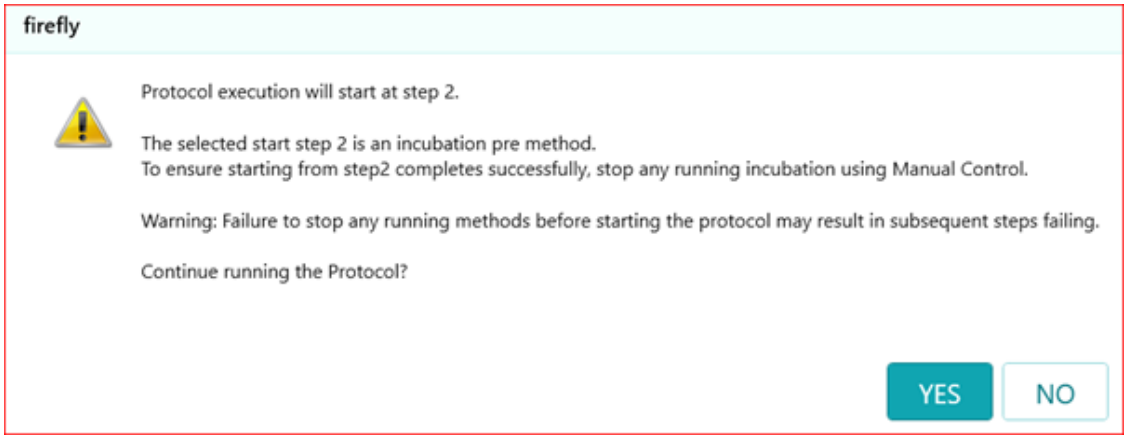
If your protocol failed to complete for any reason, before you try and run it again, you should:

- Check the Execution Log for the cause of the problem. If necessary, modify your protocol design or deck layout before you run again.
- Use the Manual Controls to clear firefly's decks and purge and remove tips and syringes.
- Use the Manual Controls to 'Home' firefly, and firefly+. This ensures that the instrument is in the correct state to restart.

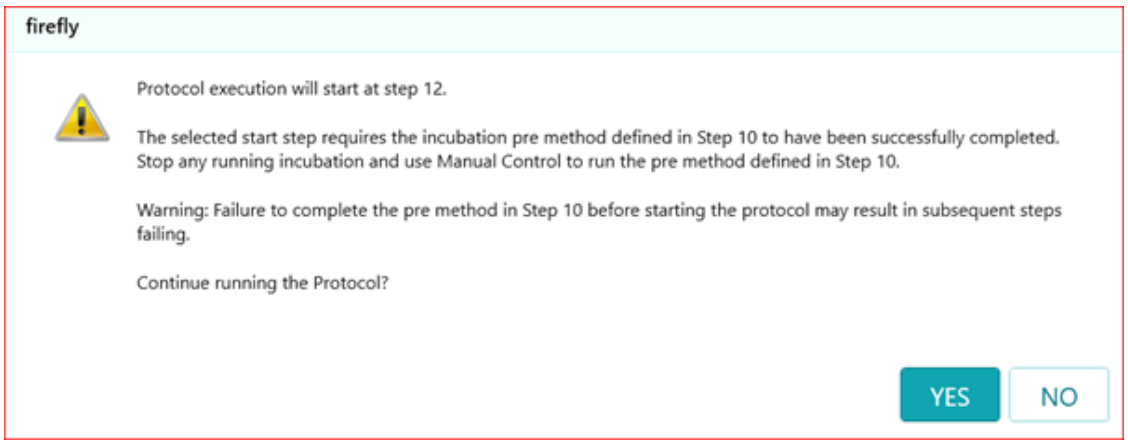
- Follow the instructions for restarting from a specific step.

> **Important**
>
> If you had run an ODTC pre-method before the protocol stopped, you will need to re-run it before you can run the main method. Restart your firefly protocol so that running the ODTC pre-method is the first step.

- If your protocol utilized the ODTC, you will see the following prompts for restarting a protocol.
    - If execution restarts with a Pre method running, you will see guidance for ending it and restarting:



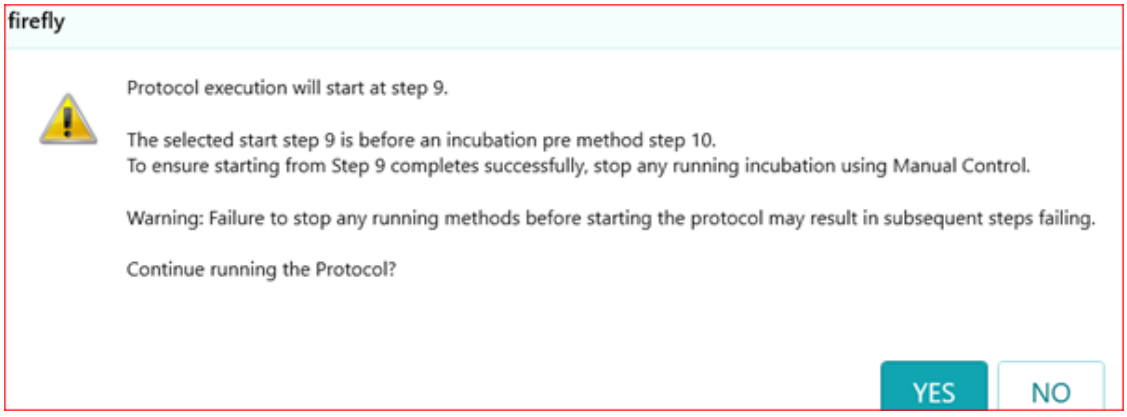
    - If it starts after the Pre method, but before the Main method, you will see:



- Once the ODTC is idle and ready to run, the start requirements, i.e. block temperature, are compared. The state of the hardware is compared to the subsequent main method incubation starting requirements. If they do not match the protocol will not start e.g. because the Pre method has not been run as instructed.

- The state of the ODTC is also checked, in case it has been switched off i.e. when clearing up the failed protocol, and is not ready and initialized.

- You will then be ready to rerun the ODTC protocol.

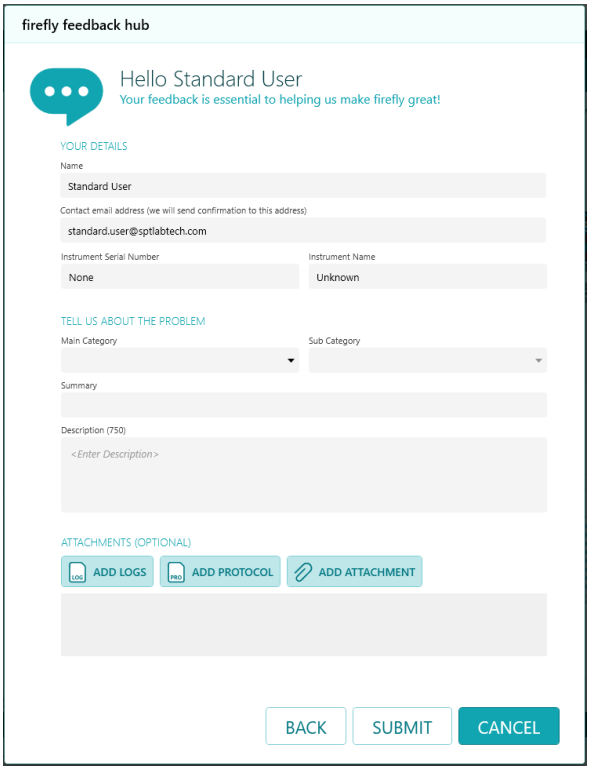


Using the firefly feedback hub

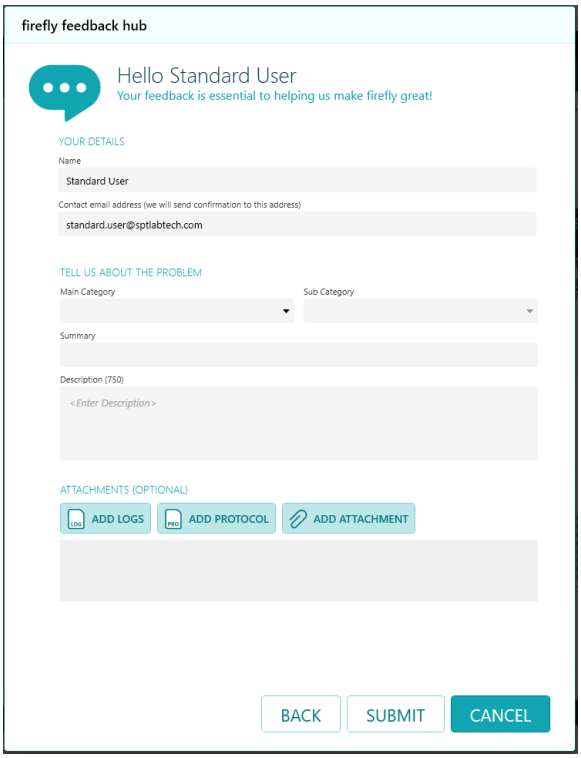
To report a problem with firefly, open the firefly feedback hub from the control on the menu bar (circled in red). This function is available to all user account types.

Use the opening form to select your feedback type.

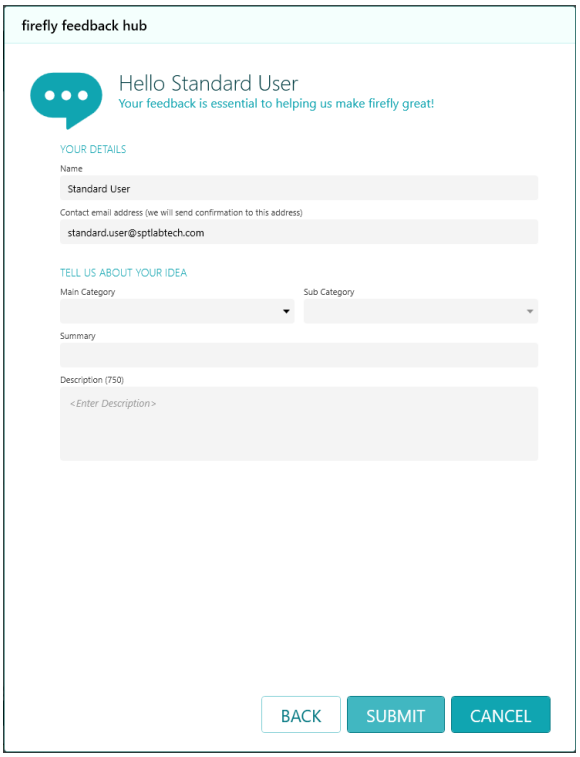
If you have a problem operating your instrument, use the Service Request option to request help from reliance service engineers. It is useful to send the protocol which was in use when the problem occurred and any other information which may be useful such as photos of the state of the machine, logs or screenshots.



The process is similar to report a software bug:



You can also make a more general request for additional firefly functionality, hardware or software.



Care and maintenance

Preventing condensation in the ODTC

If you have used the ODTC to cool a plate as part of your PCR protocol, it is likely that there will be condensation in the unit. To minimize this, design your protocol to avoid prolonged periods of cooling, particularly if these are just holding the plate at a specified temperature after the PCR steps have completed. Instead, when PCR is complete, use Move to place the plate on the plate thermal module until you are ready to unload it.

Cleaning

Regular scheduled cleaning of firefly surfaces and components helps to sustain consistent instrument performance. The frequency of regular (maintenance) cleaning can vary depending on several factors, including your specific laboratory protocols, the types of liquids and samples being handled, existing cleaning requirements of your facility, and the overall usage of your firefly instrument.

You should inspect your instrument regularly for stains, dust or debris accumulation and adjust your regular cleaning interval based on your workflow requirements, usage of the instrument and existing cleaning procedures in your facility.

Cleaning materials

Low-lint paper tissue or cloths are recommended for cleaning the firefly instrument and accessories. Avoid using abrasive materials.

The preferred cleaning solutions for the firefly instrument and accessories are 70% alcohol (e.g., ethanol or isopropanol) and distilled water.

Corrosive cleaning solutions, such as bleach or acid-based solutions (e.g., LookOut DNA Erase), may lead to corrosion of instrument components. Such solutions should be avoided and only used with care if crucial for the workflow. If the use of such solutions is deemed essential, spray the cleaning solution onto a tissue or cloth first and then wipe the surfaces as needed. This operation must always be followed by wiping the surfaces with a tissue soaked in 70% alcohol solution.

Disinfectants and detergents (e.g., Chemgene HDL4L, RNase Zap) should be used sparingly and only if crucial for the workflow. If the use of such solutions is deemed essential, spray the cleaning solution onto a tissue or cloth first and then wipe the surfaces as needed. This operation must always be followed by wiping the surfaces with a tissue soaked in 70% alcohol solution.

It is recommended that cleaning solutions with less than 1% of sodium hydroxide are used.

Routine cleaning

For general cleaning of a clean, dry instrument:

1. Switch off firefly, and disconnect the power cable.
2. Gently wipe the exterior of the instrument with a clean lint-free cloth or cleanser wipe. You can use dilute (70% v/v) ethanol to moisten the cloth, or as a spray. If necessary, the exterior surfaces of the firefly instrument can be cleaned with tissue soaked in disinfectant or detergent, followed by tissue soaked in 70% alcohol.
3. Clean the decks, inner surfaces of the instrument panels and the pipetting and dispense head covers with tissue soaked in disinfectant or detergent, followed by tissue soaked in 70% alcohol.

Tip

Avoid spraying cleaning solutions directly into the firefly instrument. It is preferable to spray the cleaning solutions onto a low-lint paper tissue or cloth first and then wipe the surfaces inside the instrument.

Warning

Avoid cleaning the tablet with solvent or cleanser that may damage the screen surface.

Turn it off and gently wipe it, using a soft, dry, lint-free cloth.

Cleaning the vertical laminar flow module

Follow the cleaning instructions in the CAS vertical laminar flow module manual.

Cleaning firefly+

Clean the shelves and interior and exterior surfaces

Cleaning the ODTC

Follow the instructions in the Inheco On Deck Thermocycler manual.

Cleaning assets

To clean tip stands, reservoirs or reservoir plates, select cleaning agents compatible with your reagents which will not damage the asset material.

Asset	Material
Tip stand	Acetal copolymer (Polyoxymethylene)
Reservoirs and lids	Polypropylene
Reservoir plate for use without thermal module	Ertalyte (Polyethylene terephthalate)
Reservoir plate for use with thermal module	Clear anodized aluminum
Reservoir plate and inserts for use with HV reservoirs	Clear anodized aluminum

Cleaning EZL firefly tip cassettes

Warning

Only in exceptional circumstances, such as a major spill of biological material onto the cassette, should cleaning of cassettes with cleaning solutions be considered.

If there is an unavoidable need for cleaning a cassette, avoid using corrosive substances if possible. If cleaning with a disinfectant or detergent is considered necessary due to the nature of the spilled liquid or workflow, always follow it with 70% alcohol. It is preferable to opt for carefully cleaning the cassettes with 70% alcohol alone.

Tip

Take particular care to ensure that cassettes are not left exposed to cleaning liquid residue long-term, as this may cause corrosion.

As cassettes contain inaccessible areas, such as hinges and grilles, it is difficult to ensure that all cleaning liquid residue is removed, and the cassette is dry. Rinsing out the cleaning solutions with 70% alcohol may help remove the cleaning solutions, but do not leave a cassette soaking in any solutions for longer than 30 seconds.

Using a drying cabinet may facilitate drying cassettes, but take care when removing them from the heated drying cabinets as they can get hot.

Warning

If you clean cassettes with harsh reagents and go against the cleaning instructions above, SPT Labtech may not be willing to replace damaged cassettes free of charge.

Cleaning EZL firefly tip stands

Tip stands can be cleaned after and/or before protocol runs to safeguard against contamination.

Spray cleaning solutions directly into tip stands and then wipe dry with a tissue or cloth. Alternatively, spray cleaning solutions onto a tissue or cloth, then wipe your tip stands with the soaked tissue or cloth.

If cleaning tip stands with a corrosive, disinfectant, or detergent cleaning solutions is required, it must be followed by cleaning them with 70% alcohol.

Store tip cassettes separately from tip stands immediately after tip stand cleaning, to allow cleaning solutions to drain or evaporate.

EZL pipette head cleaning

You will need to define an appropriate cleaning regime for the pipetting head, so that it does not become contaminated by reagents or damaged by harsh solvents or cleaners. Pipetting head gaskets ensure a tight seal between the pipetting head and the tips. You should inspect the gaskets regularly for dust and residue build-up, as this can interfere with pipetting accuracy and precision.

If you see dust or residue build-up, gently wipe the gaskets with a dry lint-free cloth.

Important

Avoid putting pressure on the gaskets during wiping, as this may damage them or dislodge them from the pipetting head.

If wiping the gaskets with a dry lint-free cloth does not remove the residue, a small amount of distilled water or 70% alcohol solution can be applied to the lint-free cloth.

Proper usage of tips will prevent contamination. Avoid touching the bottom of plates while aspirating or dispensing to prevent liquid from reversing flow and contaminating the head.

Clearing up spillages (firefly and firefly+)

Clean up all spillages inside the instrument as soon as possible.

If you have a spillage e.g., from very full reservoirs:

1. Stop firefly before you do anything else
2. Switch off

If the spillage is small and easily contained:

1. Mop up the liquid, using appropriate PPE for the reagents in use on firefly.
2. Select cleaning agents which are compatible with the spilled material and will not react with it. Minor spills can be cleaned with a low-lint paper tissue or cloth soaked in 70% alcohol. If cleaning with a disinfectant or detergent is deemed necessary due to the nature of the spilled liquid or workflow, wipe the spill with tissue soaked in desired cleaning solution, followed by tissue soaked in 70% alcohol.
3. Using appropriate PPE for both the reagents and the cleaning agent, clean where the spillage occurred.

Spill trays are located at the bottom of the interior of the firefly instrument and will contain spills and prevent damage to the electrical components underneath. If the spillage is a larger volume and you suspect that it may have contaminated the instrument basement, mop up the liquid you can easily reach and contact reliance for assistance.

Routine preventative maintenance

Proper instrument care and maintenance is an important part of user safety. It can help to prolong the equipment's life and have a positive impact on your results as well.

Follow the periodic maintenance instructions in the Inheco On Deck Thermocycler manual, if you have firefly+ with this option.

Follow the periodic maintenance instructions in the CAS vertical laminar flow module manual, if one is fitted.

Software updates

reliance will inform you when firefly software updates are ready. Updates will not be installed automatically.

Servicing requirements

firefly has no user-serviceable parts. Contact reliance to arrange maintenance for your instrument.

> **Important**
>
> Yearly servicing is recommended.

Putting firefly into storage

If you need to put firefly into storage, clean it thoroughly first.

Plan to move it according to the handling instructions, and store in a clean location which meets firefly environmental conditions.

Contact Support

reliance

reliance is your support partner throughout the life of your instrument, minimizing downtime, maintaining optimal performance, and giving you absolute confidence to assure research success. SPT Labtech products are renowned for their industry-leading reliability and efficiency. With our reliance service, you have access to a dedicated support team to safeguard your investment and secure your productivity.

To request support from reliance, please use the firefly feedback hub or the contact information below:

Tel: +1 (855) 601-5867 (USA)

+44 (0)1223 627500 (UK/Europe)

Email: fireflysupport@sptlabtech.com

Web: sptlabtech.com/support

Compliance information

firefly is designed and manufactured in the United Kingdom by:

SPT Labtech Ltd

Melbourn Science Park

Melbourn

Hertfordshire

SG8 6HB

EC declaration CE of conformity

Product	firefly

firefly is a benchtop instrument optimised for performing genomics liquid handling workflows.

There are eight versions; firefly 3 and firefly 6 differ only in the number of dispense syringes, both are available in genomics or non-genomics variants. Genomics variants have shaking and temperature control modules in the base, non-genomics variants do not.

Each variant has a 384 channel pipetting head, which can be of EZ Load or Auto Tip Load type, and two moving decks. The decks each have 8 pockets suitable for SBS plates. firefly is controlled by an attached touchscreen.

Serial number: FF1013 onward

Manufacturer: SPT Labtech Ltd
Address: Melbourn Science Park
Cambridge Road
Melbourn
Royston
Hertfordshire SG8 6HB

We hereby declare that the product above complies with all relevant provisions of the following directives:

Machinery	**Base Directive 2006/42/EC**
EMC	**Base Directive 2014/30/EU**
RoHS2	**Base Directive 2011/65/EU amended by directives 2015/863 and 2017/2102**
CE marking	**Base Directive 93/68/EEC** [CE Marking]

The product has been designed and manufactured in accordance with harmonised standards:

EN 55011:2016
EN 55032:2015

EN 61000-3-2:2014
EN 61000-3-3:2013
EN 61326-1:2013

EN 13849-1:2015

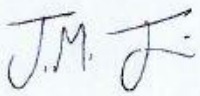
The product has been designed and manufactured in accordance with the following technical standards and specifications:

EN 61010-1:2010/A1:2019

A technical file is retained at the manufacturer's address.

Name and address of the person who compiled the Technical File:

Andrew Holford
SPT Life Sciences GmbH
Brückenäckern 4,
07751 Großlöbichau,
Germany

Signed	
Name	Joby Jenkins
Position	CTO
Date	31st April 2025
Signed at	SPT Labtech Ltd Melbourn Science Park Melbourn Hertfordshire SG8 6HB

EC declaration CE of conformity

Product	Firefly+

Firefly+ is an expansion module that is designed to be coupled to a firefly instrument to increase capacity and enhance functionality.

It is fitted with a four-axis robot, an on deck thermal cycler, and racking that holds labware

Serial number: FFP-0001 onward

Manufacturer	SPT Labtech Ltd
Address	Melbourn Science Park Cambridge Road Melbourn Royston Hertfordshire SG8 6HB

We hereby declare that the product above complies with all relevant provisions of the following directives:

Machinery	**Base Directive 2006/42/EC**
EMC	**Base Directive 2014/30/EU**
RoHS2	**Base Directive 2011/65/EU amended by directives 2015/863 and 2017/2102**
CE marking	**Base Directive 93/68/EEC** [CE Marking]

The product has been designed and manufactured in accordance with harmonised standards:

EN 55011:2016
EN 55032:2015

EN 61000-3-2:2014
EN 61000-3-3:2013
EN 61326-1:2013

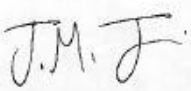
The product has been designed and manufactured in accordance with the following technical standards and specifications:

EN 61010-1:2010/A1:2019

A technical file is retained at the manufacturer's address.

Name and address of the person who compiled the Technical File:

Ian Burrell
SPT Life Sciences GmbH
Brückenäckern 4,
07751 Großlöbichau,
Germany

Signed	
Name	Joby Jenkins
Position	CTO
Date	19th December 2024
Signed at	SPT Labtech Ltd Melbourn Science Park Melbourn Hertfordshire SG8 6HB