firefly®+ technical note

Agilent SureSelect Max DNA Library Preparation with Enzymatic Fragmentation & Target Enrichment using Fast Hybridization

This technical note provides supporting information for the firefly+ protocols listed below. These protocols are based on the manual Agilent SureSelect Max protocols and are available to download from the firefly community. Here, we outline protocol run times, parts required and provide details on the steps performed in each of the firefly+ protocols.

firefly+ protocols

Protocol name	firefly+ run time
SureSelect Max DNA Library Preparation	~3 hours 50mins (96 sample run)
SureSelect Max Target Enrichment part 1	~4 hours 35mins (8 pool run)
SureSelect Max Target Enrichment part 2	~1 hour 12mins (8 pool run)

Table 1. firefly+ protocol names and run times

Figure 1. SureSelect Max Library Preparation and Target Enrichment workflow on firefly+

Reagents

Protocol	Part name	Part number
SureSelect Max DNA Library Preparation	SureSelect Max Enzymatic Fragmentation Library Prep Kit	G9660A, G9660B
	SureSelect Max Adaptors and UDI Primers Kit for ILM	G9668A, G9668B, G9668C, G9668D
	SureSelect Max Purification Beads	G9962A (5 mL), G9962B (30 mL)
SureSelect Max Target Enrichment	SureSelect Max Fast Hyb Kit	G9689A, G9689B
	SureSelect Max Blockers and Primers Kit for ILM	G9699A, G9699B
	SureSelect Max Purification Beads	G9962A (5 mL), G9962B (30 mL)
	SureSelect XT HS Probe	As required

Table 2. Agilent kits used in the DNA Library Preparation with Enzymatic Fragmentation and Target Enrichment with Fast Hybridization workflows

sptlabtech

Overview

SPT Labtech firefly+ protocols have been developed to run Agilent SureSelect Max DNA Library Preparation with Enzymatic Fragmentation and SureSelect Max Target Enrichment using Fast Hybridization. The protocols run in 96 well plate format and make use of the on-deck thermocycler (ODTC) on firefly+ to allow walkaway workflows. Variables allow a user to run from 1 to 12 columns of library preparation and 1 to 2 columns of pre-capture pools or individual libraries through Target Enrichment.

Protocol highlights

- Full walkaway library preparation protocol
- Protocol variables provide flexibility to process from 1 to 12 columns of library preparation in a 96 well plate format
- Target Enrichment is split over 2 walkaway protocols with a split prior to the amplification of captured libraries step
- Protocol variables provide the flexibility to run either 1 to 2 columns of pools or samples through the target enrichment workflow following on from library preparation
- The on-deck thermocycler (ODTC) on firefly+ allows all incubation and thermocycling steps to take place on the firefly+ - including probe hybridization and hot wash steps

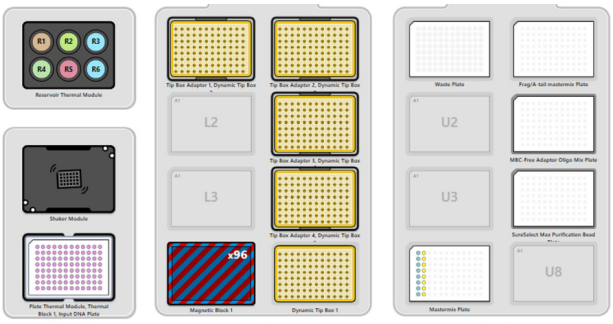

Figure 2. firefly starting deck layout for a 96-sample library preparation run. Deck Layout – Basement: Reservoirs R1 – SureSelect Max Purification Beads, R2 – Nuclease Free Water, R3+R6 - 70% Ethanol, R4 - Amplification Master Mix, R5 - Ligation Master Mix, Plate Thermal Module - 96 sample Thermo Adapter Block and Input DNA Plate. Lower Deck: L1, L5, L6, L7 - 100µL 96 format ATL tips on ATL 35-125µL Tip Stands, L8 - 100µL 96 format ATL tips, L4 – Alpaqua Magnum FLX 96 sample Magnetic Block. Upper Deck: U1 - Waste Plate, U4 Mastermix Plate, U5 Frag/Atail mastermix Plate, U6 – MBC Free Adapter Oligo Mix Plate, U7 – SureSelect Max Purification Bead Plate

Protocol overview

SureSelect Max DNA Library Preparation

The firefly+ protocol is based on the manual workflow outlined in the Agilent SureSelect Max DNA Library Preparation with Enzymatic Fragmentation For illumina Platform NGS Protocol (Version A0 September 2024).

The firefly+ protocol (SureSelect Max DNA Library Preparation) automates following steps from chapter 2 of the manual protocol, after which an optional QC can be performed:

- **Step 2.** Fragment, end-repair, and 3'-dA-tail the DNA (Frag/A-Tail)
- **Step 3.** Ligate the adaptor
- **Step 4.** Purify libraries using magnetic purification beads
- **Step 5.** Amplify and index the libraries
- **Step 6.** Purify amplified libraries using magnetic purification beads

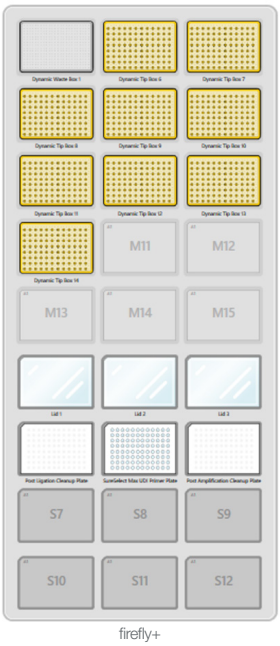

Figure 3. firefly+ starting layout for a 96-sample library preparation run. Deck Layout M1 – Empty ATL tip box, M2-M10 100µL 96 format ATL tips, S1-S3 Plate Lids, S4 – Post Ligation Cleanup Plate, S5 – SureSelect Max UDI Primer Plate, S6 – Post Amplification Cleanup Plate

SureSelect Max Target Enrichment

The firefly+ protocols are based on the manual workflow outlined in the Agilent SureSelect Max Target Enrichment using Fast Hybridization For NGS using the illumina Platform Protocol (Version A0 September 2024). These protocols support both the post-capture pooling workflow and the pre-capture pooling workflow.

The first firefly+ protocol (SureSelect Max Target Enrichment part 1) automates following steps from chapter 2 of the manual protocol:

- **Step 2.** Hybridize libraries to the SureSelect probe
- **Step 3.** Prepare streptavidin beads and buffers for capture
- **Step 4.** Capture the hybridized libraries

The second firefly+ protocol (SureSelect Max Target Enrichment part 2) follows on and automates the following steps from chapter 2 of the manual protocol:

- **Step 5.** Amplify the captured libraries
- **Step 6.** Purify the final libraries using magnetic purification beads

QC and quantification of the final libraries can then be performed.

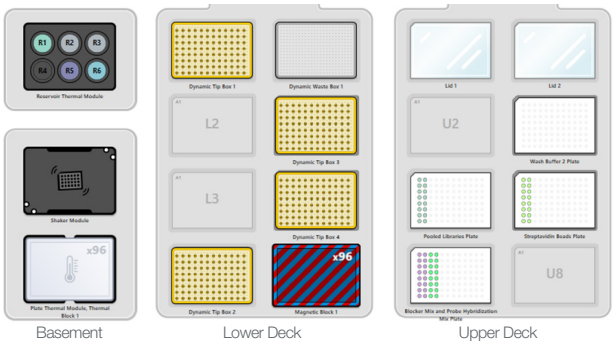

Figure 4. firefly starting deck layout for a 16 sample Target Enrichment run (Part 1). Deck Layout – Basement: Reservoirs R1 – SureSelect Bead Binding Buffer, R2+R3 – Wash Buffer 2, R5 – Nuclease-Free Water, R6 – Wash Buffer 1. Lower Deck: L1+L4+L6+L7 - 100µL 96 format ATL tips, L4 – Empty ATL tip box, L8 - Alpaqua Magnum FLX 96 sample Magnetic Block. Upper Deck: U1+U5 – Plate Lids, U3 – Pooled Libraries Plate, U4 – Blocker Mix and Probe Hybridisation Mix Plate, U6 – Wash Buffer 2 Plate, U7 – Streptavidin Beads Plate

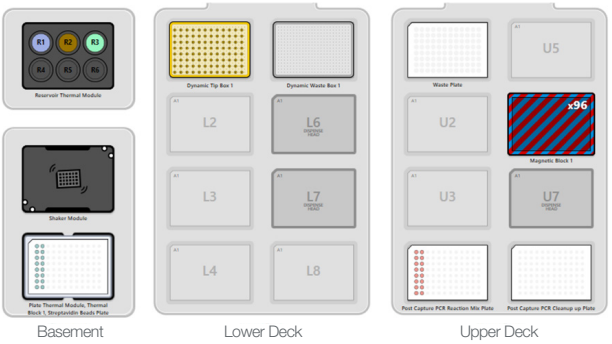

Figure 6. firefly starting deck layout for a 16 sample Target Enrichment run (Part 2). Deck Layout – Basement: Reservoirs R1 – 70% Ethanol, R2 – SureSelect Max Purification Beads, R3 – Low TE Buffer, Plate thermal Module - 96 sample Thermo Adapter Block and Streptavidin Beads Plate. Lower Deck: L1 - 100µL 96 format ATL tips, L4 – Empty ATL Tip Box. Upper Deck: U1 – Waste Plate, U4 – Post Capture PCR Reaction Mix Plate, U6 - Alpaqua Magnum FLX 96 sample Magnetic Block, U8 – Post Capture PCR Cleanup Plate.

Figure 5. firefly+ starting layout for a 16 sample Target Enrichment run (Part 1). Deck Layout M1 - 100µL 96 format ATL tips, M15 – Waste Plate, S3+S4+S5 – Plate Lids on Plates

Figure 7. firefly+ starting layout for a 16 sample Target Enrichment run (Part 2). Deck Layout – S1 – Plate Lid.

Variables

SureSelect Max DNA Library Preparation

Users can select to run this protocol with the following variables:

- Number of Columns – range 1 to 12
- UDI starting column – range 1 to 12
- ODTC Data files
  - Frag A-Tail (10-minute Fragmentation duration)– this file can be changed to adjust fragmentation duration based on DNA quality and NGS read length required.
  - PCR (7 cycles)– this file can be changed to adjust the number of PCR cycles

SureSelect Max Target Enrichment part 1

Users can select to run this protocol with the following variables:

- Number of Columns – range 1 to 2
- ODTC Data files
  - Hybridization program - is set for the Hybridisation program listed in Table 9 of the manual. The 1 minute incubation temperature for Segment 4 has been optimized and is set to 66˚C rather than 68˚C as stated in the manual protocol.

SureSelect Max Target Enrichment part 2

Users can select to run this protocol with the following variables:

- Number of Columns – range 1 to 2
- Waste starting column – range 1 to 12
- ODTC Data Files
  - Post Capture PCR (10 cycles) – this file can be changed to adjust the numbers of PCR cycles

Reagent volumes

SureSelect Max DNA Library Preparation

The volumes of each reagent that are needed to run the SureSelect Max DNA Library Preparation and SureSelect Max Target Enrichment protocols on firefly+ are dependent on number of samples being processed. The required volumes of these reagents are shown in the EXECUTE section of the firefly software.

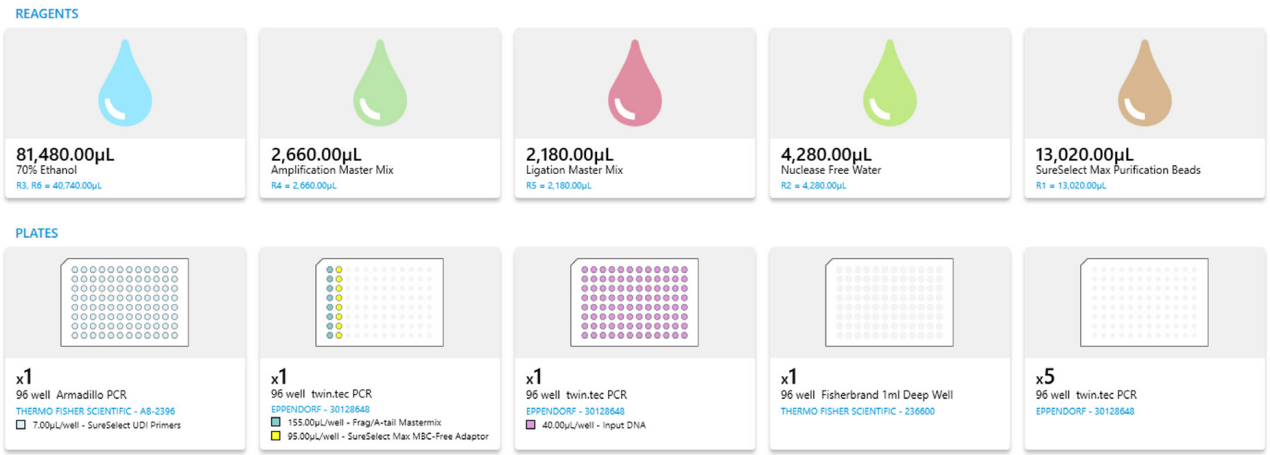

Figure 8. Example of the Reagents and Plates required for a 12-column library preparation run

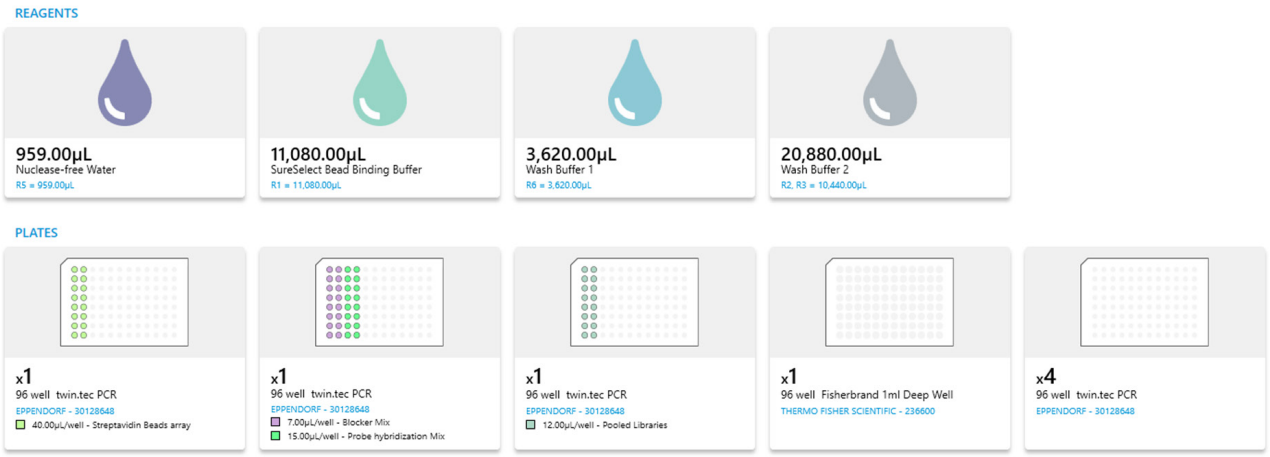

Figure 9. Example Reagents and Plates required for a 2-column Target Enrichment run (part 1)

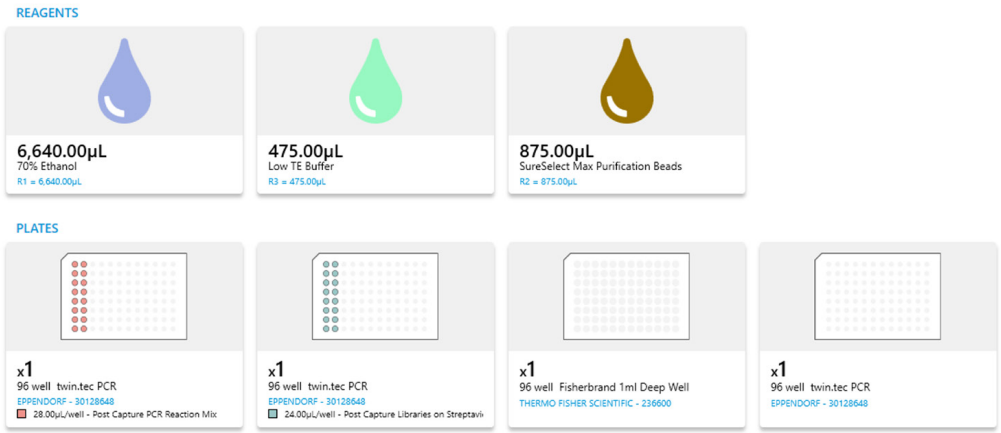

Figure 10. Example Reagents and Plates required for a 2-column Target Enrichment run (part 2)

Consumables

SureSelect Max DNA Library Preparation

Supplier	Part name	Part number	Number required	Note
SPT Labtech	Standard Syringe	4150-07200	6	
SPT Labtech	High volume reservoir	4150-07300	6 (total)	Reservoir types needed are dependent on the number of columns processed
	Standard reservoir	4150-07103		
	Low dead volume (LDV) reservoir	4150-07202		
SPT Labtech	100µL, 96 format, AT-Load Tips (filtered)	125-96-FF-AL-FS	3 to 15 boxes	Number required depends on the number of columns processed
Eppendorf	96 well twin.tec PCR plate	30128648	7	
Thermo Fisher Scientific	96 well Fisherbrand 1mL Deep Well	236600	1	Waste plate
Hamilton	PCR ComfortLid	814300	3	

Table 3. Consumables required for a SureSelect Max DNA Library Preparation run on firefly+

Agilent SureSelect Max Target Enrichment

Supplier	Part name	Part number	Number required	Note
SPT Labtech	Standard Syringe	4150-07200	8	
SPT Labtech	High volume reservoir	4150-07300	8 (total)	Reservoir types needed are dependent on the number of columns processed
	Standard reservoir	4150-07103		
	Low dead volume (LDV) reservoir	4150-07202		
SPT Labtech	100µL, 96 format, AT-Load Tips (filtered)	125-96-FF-AL-FS	5 to 7 boxes	Number required depends on the number of columns processed
Eppendorf	96 well twin.tec PCR plate	30128648	9	
Thermo Fisher Scientific	96 well Fisherbrand 1mL Deep Well	236600	1	Waste plate. Same waste plate can be used for Part 1 and Part 2 of the protocol
Hamilton	PCR ComfortLid	814300	6	

Table 4. Consumables required for a SureSelect Max Target Enrichment run on firefly+

Parts required

SureSelect Max DNA Library Preparation

Supplier	Part name	Part number	Number required	Note
SPT Labtech	ATL 35-125µL Tip Stand	3276-08335 (REV 2)	0 to 4	Number required depends on the number of columns processed
SPT Labtech	96 Thermo Adapter Block	3276-01065	1	
SPT Labtech	firefly reservoir tray v2	3276-01928	1	Required for runs of 3+ columns
SPT Labtech	Standard reservoir puck for firefly reservoir tray v2	3276-01897	0 to 4	Number required depends on the number of columns processed
SPT Labtech	LDV puck for firefly reservoir tray v2	3276-01898	0 to 4	Number required depends on the number of columns processed
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1	

Table 5. Parts required for a SureSelect Max DNA Library Preparation run on firefly+

SureSelect Max Target Enrichment

Supplier	Part name	Part number	Number required	Note
SPT Labtech	96 Thermo Adapter Block	3276-01065	1	
SPT Labtech	firefly reservoir tray v2	3276-01928	1	Required for 2 column runs
SPT Labtech	Standard reservoir puck for firefly reservoir tray v2	3276-01897	0 to 1	Number required depends on the number of columns processed
SPT Labtech	LDV puck for firefly reservoir tray v2	3276-01898	0 to 1	Number required depends on the number of columns processed
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1	

Table 6. Parts required for a SureSelect Max Target Enrichment run on firefly+