firefly® technical note

# SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian

## Overview

- This technical note describes how to run the Takara Bio SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian workflow (Cat. Nos. 634485, 634486, 634487 & 634488) on SPT Labtech's firefly.
- The kit enables generation of strand-specific, Illumina-compatible RNA-seq libraries from picogram to low-nanogram inputs of mammalian total RNA or intact cells. The workflow combines first-strand cDNA synthesis, addition of Illumina adapters and barcodes, NucleoMag bead-based purification, ribosomal cDNA depletion using ZapR v3 and R-Probes v3, and final library amplification.
- This automated method was developed for 96 samples using 96-well Bio-Rad Hard Shell PCR plates, Abgene 0.8ml Deep Well plate (AB0765) and 2 magnets: Alpaqua 96 magnum FLX and the Permagen 96 LE Magnet. Alternative labware may require optimisation. The protocol has been configured for use with firefly genomics configuration.
- Instrument configuration: firefly 6 Head, genomics
- Development software version: v1.8
- Deviation(s) from published method:
  - Volumes loaded into reservoirs are adjusted to account for dead volume and automated pipetting.
  - Ethanol Additions during bead clean-up have been reduced to allow for single transfer removal of ethanol with 125ul tips.
  - The underlying biochemistry (master mix compositions and cycling programs) follows the Takara Bio user manual. Users must refer to the manual for all thermocycler programs and detailed incubation steps.

## firefly protocols

| Protocol number | Protocol name | firefly run time (minutes) |
|---|---|---|
| 1 of 5 | First-Strand cDNA Synthesis | 10 |
| 2 of 5 | PCR 1—Addition of Illumina Adapters and Indexes | 7 |
| 3 of 5 | Purification of RNA-Seq Library with NucleoMag Beads and ZapR v3 / R-Probes v3 | 89 |
| 4 of 5 | PCR 2—Final RNA-Seq Library Amplification | 5 |
| 5 of 5 | Purification of Final RNA-Seq Library Using NucleoMag Beads | 89 |

Table 1. Overview of protocols to process 96 samples SMARTER STRANDED TOTAL RNA-SEQ KIT V3 - PICO INPUT MAMMALIAN on SPT Labtech's firefly. Times may vary based on user.

## Input variables

| Input Variable | Interval | Minimum | Maximum |
|---|---|---|---|
| Number of Columns | 1 | 1 | 12 |
| Initial total RNA Volume (µl) | 1 | 1 | 8 |

Table 2. General variables for using Smarter Stranded Total Rna-Seq Kit V3 - Pico Input Mammalian



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# Master Mix Calculations

| Protocol name | Reagent | Total Required Volume (µL) for 96 samples |
|---|---|---|
| First-Strand cDNA Synthesis | Fragmentation Master Mix (SMART Pico Oligos Mix v3 + 5X First-Strand Buffer+ Nuclease-Free Water) | 762-1530* |
| First-Strand cDNA Synthesis | First-Strand Master Mix (SMART UMI-TSO Mix v3 + RNase Inhibitor + SMARTScribe II Reverse Transcriptase) | 762 |
| PCR 1—Addition of Illumina Adapters and Indexes | PCR 1 Master Mix (Nuclease-free water + SeqAmp CB PCR Buffer (2X) + SeqAmp DNA Polymerase) | 2928 |
| Purification of RNA-Seq Library with NucleoMag Beads and ZapR v3 / R-Probes v3 | ZapR Master Mix (Nuclease-free water + 10X ZapR Buffer + ZapR v3 + R-Probes v3) | 2352 |
| PCR 2—Final RNA-Seq Library Amplification | PCR 2 Master Mix (Nuclease-free water + SeqAmp CB PCR Buffer (2X) + PCR2 Primers v3 + SeqAmp DNA Polymerase) | 7920 |

Table 3. Master Mix volumes for processing 96 samples using SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian on SPT Labtech's firefly liquid handler. Values include additional volume to account for reservoir dead volume and automated pipetting. For volumes required for alternative sample counts, please contact SPT Labtech.
*Fragmentation Master Mix volume will vary depending on initial RNA volume.

# Consumables and Accessories

| Pipette Head Consumable Type | Product Number | Protocol 1 | Protocol 2 | Protocol 3 | Protocol 4 | Protocol 5 |
|---|---|---|---|---|---|---|
| 50 µL Tips (Filtered) | 050-096-FF-FS | — | 1× | — | — | 2× |
| 125 µL Tips (Filtered) | 125-096-FF-FS | — | — | 8× | — | 6× |

Table 4. This table outlines the Pipette Head consumables required for each protocol, including product numbers and quantities based on the number of columns in use. *$x$ represents the number of columns.

| Consumable Type | Product Number | Protocol 1 | Protocol 2 | Protocol 3 | Protocol 4 | Protocol 5 |
|---|---|---|---|---|---|---|
| Syringes (Ultra-low Retention) | 4150-07208 | 1× | — | 2× | — | 1× |
| Syringes (Standard) | 4150-07200 | 1× | 1× | 3× | 1× | 4× |
| Reservoirs (Standard) | 4150-07103 | 2× | 1× | 4× | 1× | 5× |
| Reservoirs (Low Dead Volume) | 4150-07202 | 1× | — | 1× | — | — |

Table 5. This table outlines the Dispense Head consumables required for each protocol, with quantities adjusted for fewer columns.
* The quantity of 4150-07103 reservoirs and 4150-07202 reservoirs may vary for protocols using fewer than 12 columns.

# Workflow overview

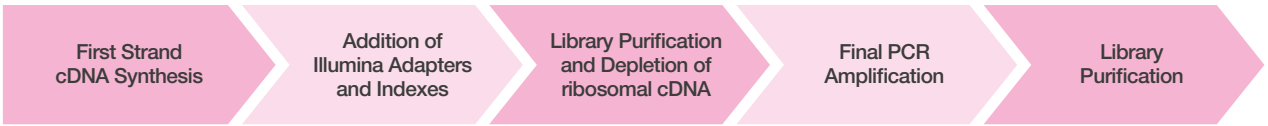



Figure 1. Overview of the steps performed by firefly to automate the SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian workflow.

# Workflow details

**Step names and protocol descriptions correspond to those in the SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian User Manual. Users must refer to the Takara Bio manual for full thermocycler programs, incubation conditions, and safe stopping points.**

## Protocol 1 of 5 – 4.a First-Strand cDNA Synthesis

This protocol adds fragmentation master mix to the RNA input plate. The plate should then be Sealed, mixed and moved to a thermocycler to run the Fragmentation program. The firefly then adds the First-Strand Master Mix to the RNA input plate. The plate should then be Sealed, mixed and moved to a thermocycler to run the First Strand synthesis Program. The resulting first-strand cDNA is carried forward into PCR 1.

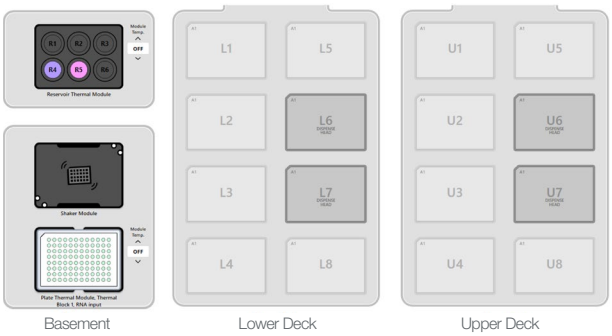



Figure 2. firefly deck layout for Protocol 1 – First-Strand cDNA Synthesis.

## Protocol 2 of 5 – PCR 1—Addition of Illumina Adapters and Indexes

This protocol adds Illumina adapters and barcodes to first-strand cDNA using a limited-cycle PCR. firefly dispenses PCR 1 Master Mix and SMARTer RNA Unique Dual Index primers. Thermocycler conditions, including the number of cycles for different input types (regular versus FFPE RNA), follow the SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian User Manual.

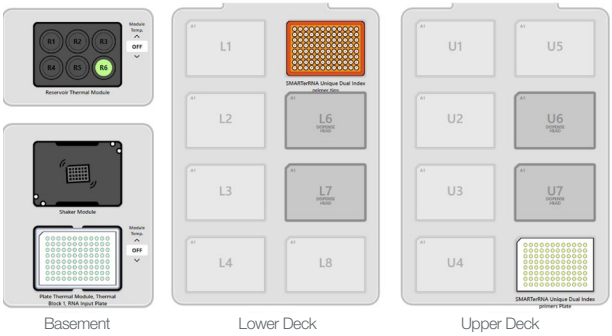



Figure 3. firefly deck layout for Protocol 2 – PCR 1: Addition of Illumina Adapters and Indexes.

## Protocol 3 of 5 – Purification of RNA-Seq Library with NucleoMag Beads and ZapR v3 / R-Probes v3

This protocol automates purification of the RNA-seq library using NucleoMag beads followed by depletion of ribosomal cDNA with ZapR v3 and R-Probes v3. firefly performs bead binding, ethanol washes, and elution steps, then dispenses the ZapR Master Mix. The ribosomal cDNA depletion reaction is run on a thermal cycler according to the Takara Bio user manual before proceeding to PCR 2.

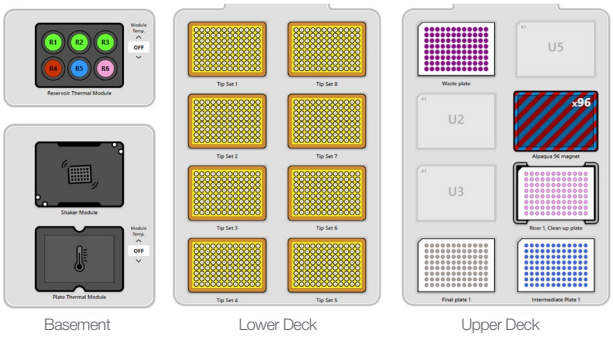



Figure 4. firefly deck layout for Protocol 3 – Purification of RNA-Seq Library with NucleoMag Beads and ZapR v3 / R-Probes v3.

## Protocol 4 of 5 – PCR 2—Final RNA-Seq Library Amplification

This protocol carries out final amplification of RNA-seq libraries that have undergone ribosomal cDNA depletion. firefly dispenses PCR 2 Master Mix to each sample. PCR cycling conditions, including the number of cycles recommended for different input ranges, must be taken from the SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian User Manual.

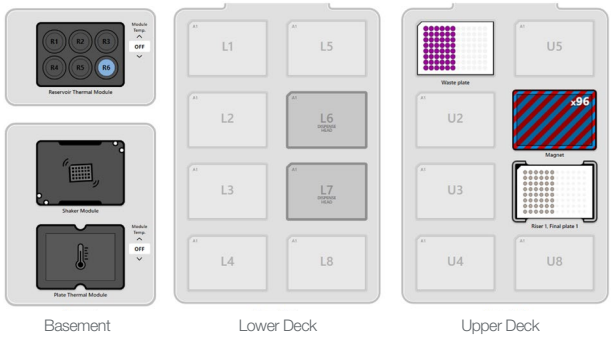



Figure 5. firefly deck layout for Protocol 4 – PCR 2: Final RNA-Seq Library Amplification.

## Protocol 5 of 5 – Purification of Final RNA-Seq Library Using NucleoMag Beads

This protocol automates the final purification of amplified RNA-seq libraries with NucleoMag beads, ethanol washes, and elution in Tris Buffer or nuclease-free water, yielding the final sequencing-ready libraries. Bead handling and wash steps are automated on firefly, while incubation and magnet separation times follow the guidance from the Takara Bio user manual.

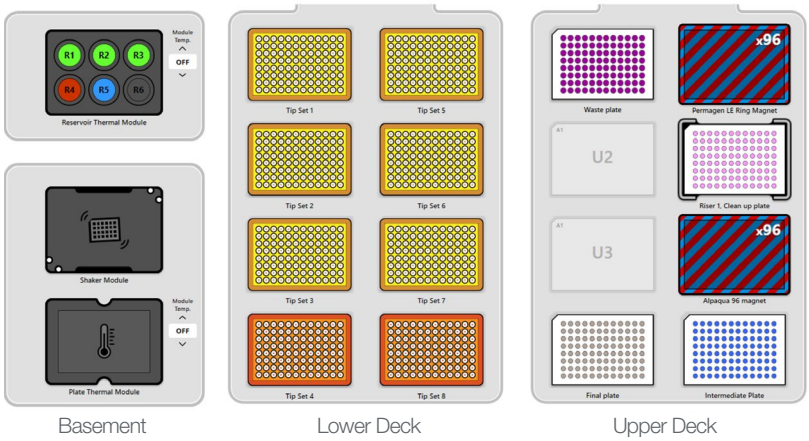



Figure 6. firefly deck layout for Protocol 5 – Purification of Final RNA-Seq Library Using NucleoMag Beads.

For questions regarding SPT Labtech's firefly, contact our support team through **fireflysupport@sptlabtech.com**

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