



firefly® technical note

SMART-Seq® mRNA LP kit LibraryPrep

This technical note provides supporting information for automating TAKARA - SMART-Seq® mRNA LP (Cat. Nos. 634768, 634769, & 634771 - 100422) on SPT Labtech firefly liquid handler. These protocols 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 protocol

firefly protocols

Protocol number	Protocol name	Estimated run time (minutes)
Protocol 1 of 7	1.V.B RNACellsNonCSSBuffer	7
Protocol 2 of 7	2.V.C FS cDNA Synth	5
Protocol 3 of 7	3.V.D cDNA Amp by LD-PCR	5
Protocol 4 of 7	4.V.E Purification Amp cDNA	35
Protocol 5 of 7	5.VI.A cDNA Dil-Library Prep	5
Protocol 6 of 7	6.VI.B. Library Amp	5
Protocol 7 of 7	7.VI.C Pool Purify AmpLib	35

Table 1. protocols & estimated run times used in TAKARA - SMART-Seq® mRNA LP on firefly.

Input variables

Protocol number	Protocol name	Variable ID	Default Value
Protocol 1 of 7	1.V.B RNACellsNonCSSBuffer	Number of Columns	12
Protocol 1 of 7	1.V.B RNACellsNonCSSBuffer	Lysis buffer volume (uL)	1
Protocol 1 of 7	1.V.B RNACellsNonCSSBuffer	Sample Starting Column (1-12)	1
Protocol 1 of 7	1.V.B RNACellsNonCSSBuffer	3' SMART-Seq CDS Primer II A volume (uL)	2
Protocol 2 of 7	2.V.C FS cDNA Synth	Number of Columns	12
		RT Master mix (uL)	7.5
		Sample Starting Column	1
Protocol 3 of 7	3.V.D cDNA Amp by LD-PCR	Number of Columns	12
		PCR Master Mix (uL)	30
		Sample Starting Column	1

Protocol number	Protocol name	Variable ID	Default Value
Protocol 4 of 7	4.V.E Purification Amp cDNA	Sample volume per well (μL)	50
		Bead volume per well (μL)	50
		Final sample volume per well (μL)	15
		Number of Columns	12
		First column Clean up plate	1
		First column Final plate	1
Protocol 5 of 7	5.VI.A cDNA Dil-Library Prep	Number of Columns	12
		Stem-Loop Adapters volume (μL)	4
Protocol 5 of 7	5.VI.A cDNA Dil-Library Prep	Sample Starting Column	1
		Library Prep Master Mix volume (μL)	10.5
Protocol 6 of 7	6.VI.B. Library Amp	Number of Columns	12
		Sample Starting Volume (μL)	22.5
		Library Amplification Master Mix (μL)	25.5
		Index starting volume (μL)	1
		Index transfer volume (μL)	2
		Sample Starting Column	1
Protocol 7 of 7	7.VI.C Pool Purify AmpLib	Starting Index Column	1
		Sample volume per well (μL)	50
		Bead volume per well (μL)	40
		Final sample volume per well (μL)	25
		Number of Columns	12
		First column Clean up plate	1
		First column Final plate	1

Table 2. Variables used in TAKARA - SMART-Seq® mRNA LP on firefly. Static variables, including those defined as algebraic expressions, are not shown.

Reagent volumes

The reagent volumes required to run TAKARA - SMART-Seq® mRNA LP on SPT Labtech firefly depend on the number of samples being processed. Default required minimum volumes for these reagents, based on the number of samples shown in the Input variables table, are shown below and in the EXECUTE section of the firefly software.

Protocol 1 of 7

1.V.B RNACellsNonCSSBuffer

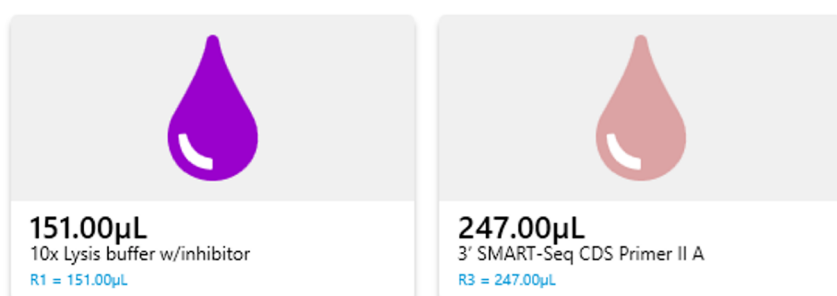


Figure 1. 1.V.B RNACellsNonCSSBuffer minimum required reagent volumes

Protocol 2 of 7

2.V.C FS cDNA Synth

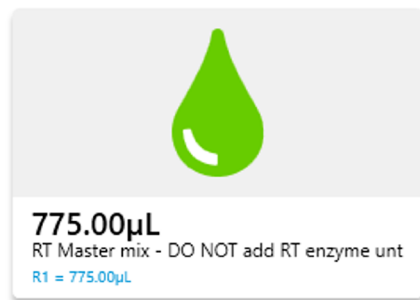


Figure 2. 2.V.C FS cDNA Synth minimum required reagent volumes

Protocol 3 of 7

3.V.D cDNA Amp by LD-PCR

REAGENTS



Figure 3. 3.V.D cDNA Amp by LD-PCR minimum required reagent volumes

Protocol 4 of 7

4.V.E Purification Amp cDNA

REAGENTS



Figure 4. 4.V.E Purification Amp cDNA minimum required reagent volumes

Protocol 5 of 7

5.VI.A cDNA Dil-Library Prep

REAGENTS

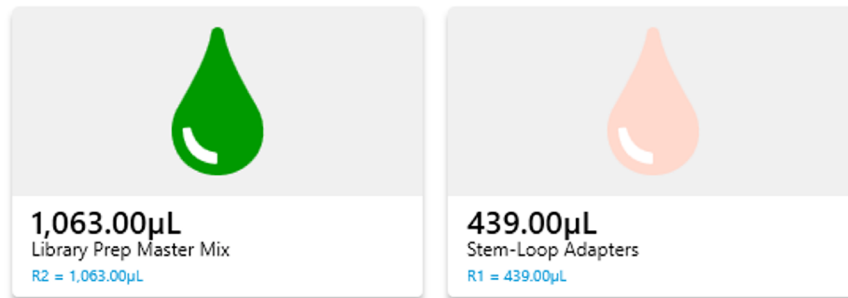


Figure 5. 5.VI.A cDNA Dil-Library Prep minimum required reagent volumes

Protocol 6 of 7

6.VI.B. Library Amp

REAGENTS

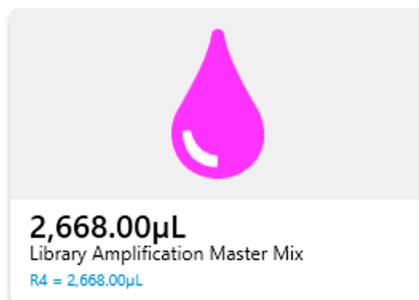


Figure 6. 6.VI.B. Library Amp minimum required reagent volumes

Protocol 7 of 7

7.VI.C Pool Purify AmpLib

REAGENTS



Figure 7. 7.VI.C Pool Purify AmpLib minimum required reagent volumes

Consumables

Supplier	Part Name	Part Number	Number Required
SPT Labtech	40mm Upper Deck Riser	3276-01838	1
SPT Labtech	Thermo Adapter Block	3276-01065	1
SPT Labtech	dragonfly® discovery Sterile LDV Reservoirs	4150-07203	5
SPT Labtech	dragonfly® Sterile Standard Reservoirs	4150-07202	9
SPT Labtech	dragonfly® discovery Sterile Syringes	4150-07201	10
SPT Labtech	dragonfly® discovery Sterile, Ultra Low Retention Syringes	4150-07209	4
SPT Labtech	firefly® Tips, 100µl, with Filters, Sterile	125-96-FF-FS	6
SPT Labtech	firefly® Tips, 35µl, with Filters, Sterile	050-96-FF-FS	7
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1
Bio-Rad	Hard Shell Plate (HSP)	HSP-9601	3
Revvity	StorPlate 2mL Deep Well V-bottom	6008880	2

Table 3. Consumables & labware required for TAKARA - SMART-Seq® mRNA LP on firefly.

Protocol overview

TAKARA - SMART-Seq® mRNA LP kit - For Research Use Only.

Not for use in diagnostic procedures. - Cat. Nos. 634768, 634769, & 634771 (100422)

This protocol was developed on a firefly 6 head genomics EZL and v1.8.6 software.

Use of alternative labware may require additional optimization.

Protocols:

1. V.B RNACellsNonCSSBuffer OR 1.V.B RNACellsNonCSSBuffer-noNorm
2. V.C FS cDNA Synth
3. V.D cDNA Amp by LD-PCR
4. V.E Purification Amp cDNA
5. VI.A cDNA Dil-Library Prep
6. VI.B. Library Amp
7. VI.C Pool Purify AmpLib

Note(s):

Update 3' SMART-Seq CDS Primer II A. variable in protocol 1 using Section V.D, Table 4

These protocols must be ran consecutively as as ethanol volumes have been reduced to 60 uL (from the original 200 uL), storage at 4C or 20C may result in adapter carry-over.

Some incubation times are modified from the manual due to optimization

Update Number of Columns (1-12) variable accordingly. Default settings expect strip tips – if full arrays are going to be used then the tip box assets need to have “Use Strips” ticked OFF.

Protocol 1 of 7

1.V.B RNACellsNonCSSBuffer

Note(s):

3' SMART-Seq CDS Primer II A. variable – Update accordingly using Section V.D, Table 4.

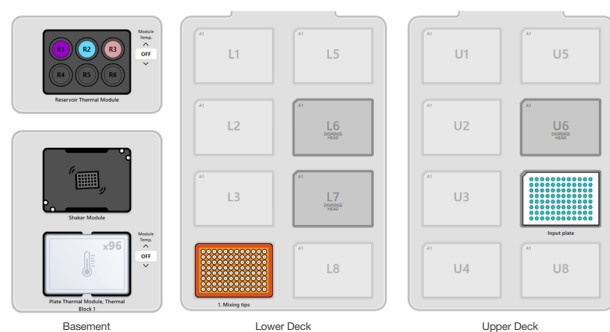


Figure 8. V.B RNACellsNonCSSBuffer deck layout

Protocol 2 of 7

2.V.C FS cDNA Synth

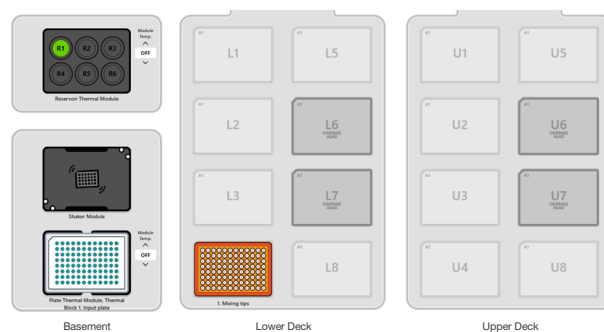


Figure 9. V.C FS cDNA Synth deck layout

3.V.D cDNA Amp by LD-PCR

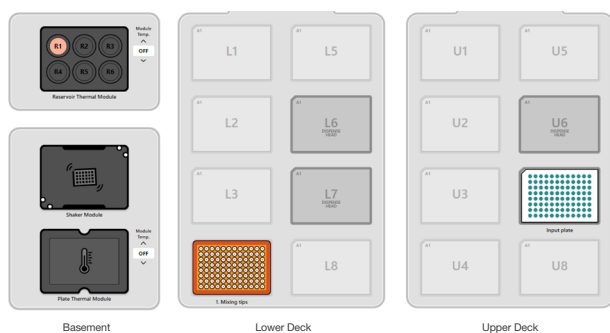


Figure 10. V.D cDNA Amp by LD-PCR deck layout

4.V.E Purification Amp cDNA

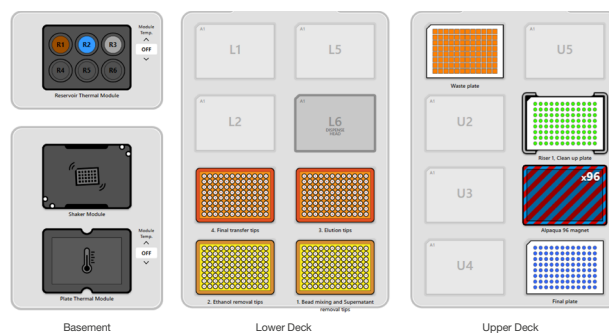


Figure 11. V.E Purification Amp cDNA deck layout

5.VI.A cDNA Dil-Library Prep

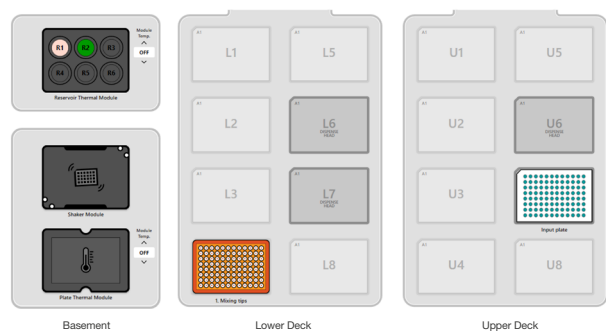


Figure 12. VI.A cDNA Dil-Library Prep deck layout

6.VI.B. Library Amp

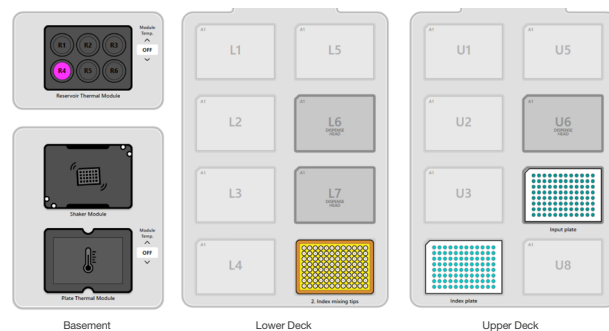


Figure 13. VI.B. Library Amp deck layout

7.VI.C Pool Purify Amp Lib

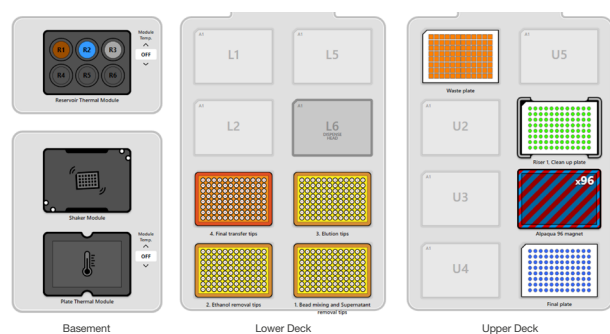


Figure 14. VI.C Pool Purify AmpLib deck layout