firefly®+ technical note

10 Genomics' GEM-X 5’ Gene Expression Library Construction

This technical note provides supporting information for automating 10X Genomics' GEM-X 5’ Gene Expression Library Construction (CG000770/CG000733 | Rev A) 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 1	10x GEX 5’ Library Construction	182

Table 1. protocols & estimated run times used in GEM-X 5’ Gene Expression Library Construction on firefly.

Input variables

Protocol number	Protocol name	Variable ID	Default Value
Protocol 1 of 1	10x GEX 5’ Library Construction	cDNA Start Column	1
Protocol 1 of 1	10x GEX 5’ Library Construction	Number of Columns	1
Protocol 1 of 1	10x GEX 5’ Library Construction	Index Start Column	1
Protocol 1 of 1	10x GEX 5’ Library Construction	Index PCR File - ODTC	C:\10x 5’ GEXIndex PCR\10x GEM PCR-14 Cycles-50-250ng cDNA.xml
Protocol 1 of 1	10x GEX 5’ Library Construction	Ligation File - ODTC	C:\10x 5’ GEX\10x GEM Adapter Ligation.xml
Protocol 1 of 1	10x GEX 5’ Library Construction	Frag File - ODTC	C:\10x 5’ GEX\10x GEM Frag, ER, AT.xml

Table 2. Variables used in GEM-X 5’ Gene Expression Library Construction on firefly. Static variables, including those defined as algebraic expressions, are not shown.

sptlabtech

Reagent volumes

The reagent volumes required to run GEM-X 5’ Gene Expression Library Construction 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 1

10x GEX 5’ Library Construction

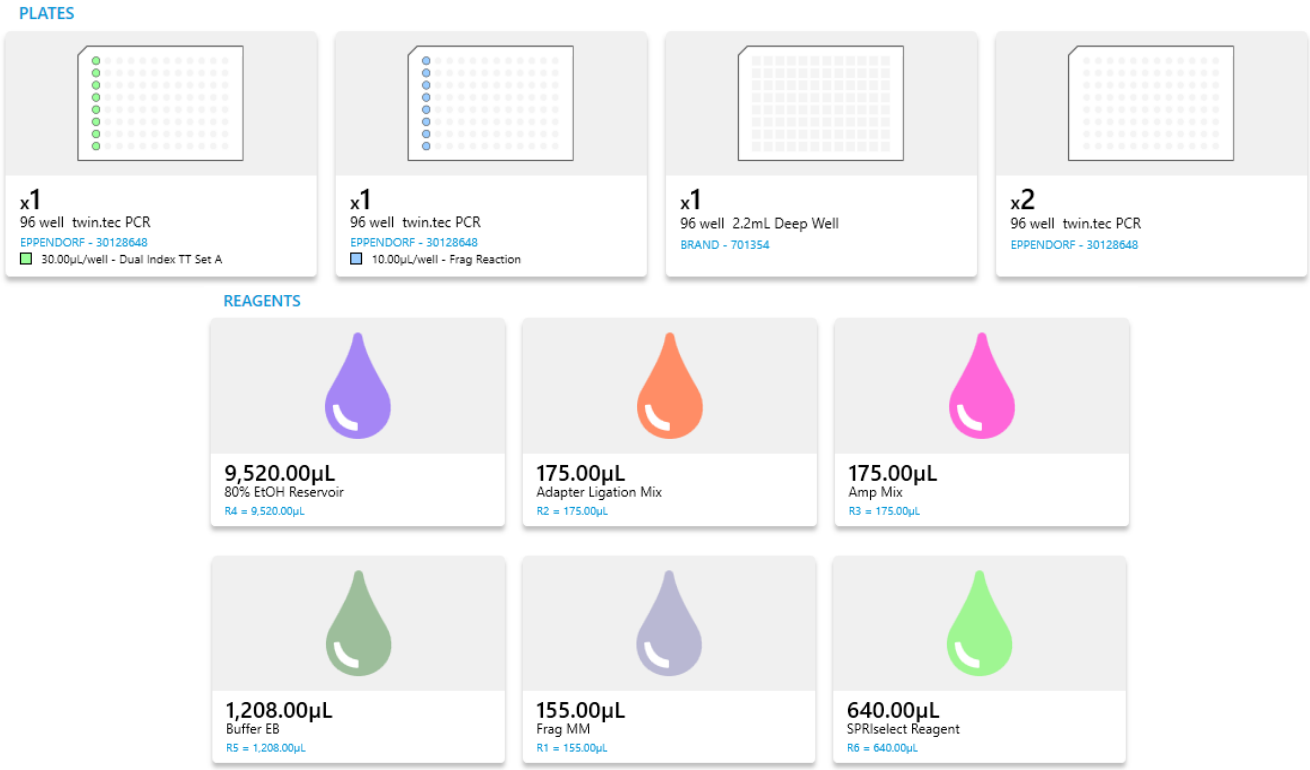

Figure 1. 10x GEX 5’ Library Construction minimum required reagent volumes.

Consumables

Supplier	Part Name	Part Number	Number Required
SPT Labtech	dragonfly® discovery Sterile LDV Reservoirs	4150-07203	3
SPT Labtech	dragonfly® discovery Sterile, Ultra Low Retention Syringes	4150-07209	3
SPT Labtech	AT-Load firefly® Pipette Tips, 100 µL with filters, Sterile 96 Tips Per Rack	125-096-FF-AL-FS	3
SPT Labtech	dragonfly® discovery Sterile Reservoirs	4150-07204	3
SPT Labtech	dragonfly® discovery Sterile Syringes	4150-07201	3
Brand	2.2mL Deep Well	701354	1
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1
Eppendorf	twin.tec PCR	30128648	2
Hamilton	PCR ComfortLid	814300	1

Table 3. Consumables & labware required for GEM-X 5’ Gene Expression Library Construction on firefly.

Protocol overview

Protocol 1 of 1

10x GEX 5’ Library Construction

This protocol performs step 5 of 10X Genomics’ GEM-X 5’ Gene Expression v3 (CG000770/CG000733 | Rev A)

Prior to or during execution of this protocol:
Vortex to resuspend the SPRIselect reagent

- **5.1b.** Vortex Fragmentation Buffer, verify there is no precipitate
- **5.2c.** Prepare Fragmentation Mix on ice
- **5.3a** Prepare Ligation Mix
- **5.5a** Record the 10x sample index name used
- **5.5b** Prepare (Library) Amp Mix

This protocol requires user interventions:

Step 6: Load Fragmentation Mix

Step 22: Load SPRIselect Reagent, 80% EtOH and Buffer EB to appropriate

Step 74: Load Ligation Mix

Step 87: Load SPRIselect Reagent, 80% EtOH and Buffer EB to appropriate reservoirs

Step 131: Load Dual Index Plate to S10; Confirm Final Libraries plate loaded to S12

Step 132: Load Amp Mix

Step 145: Load SPRIselect Reagent, 80% EtOH and Buffer EB to appropriate reservoirs

Notes: This method was developed with a 6 head Genomics firefly+ (v2.2 software) firefly using 8 samples, Eppendorf Twin.Tec PCR plates and the Alpaqua Magnum FLX magnet. Use of alternative firefly configurations or labware may require further optimization.

This protocol is compatible with 8 - 24 samples as written. To process > 24 samples, additional modifications may be required.

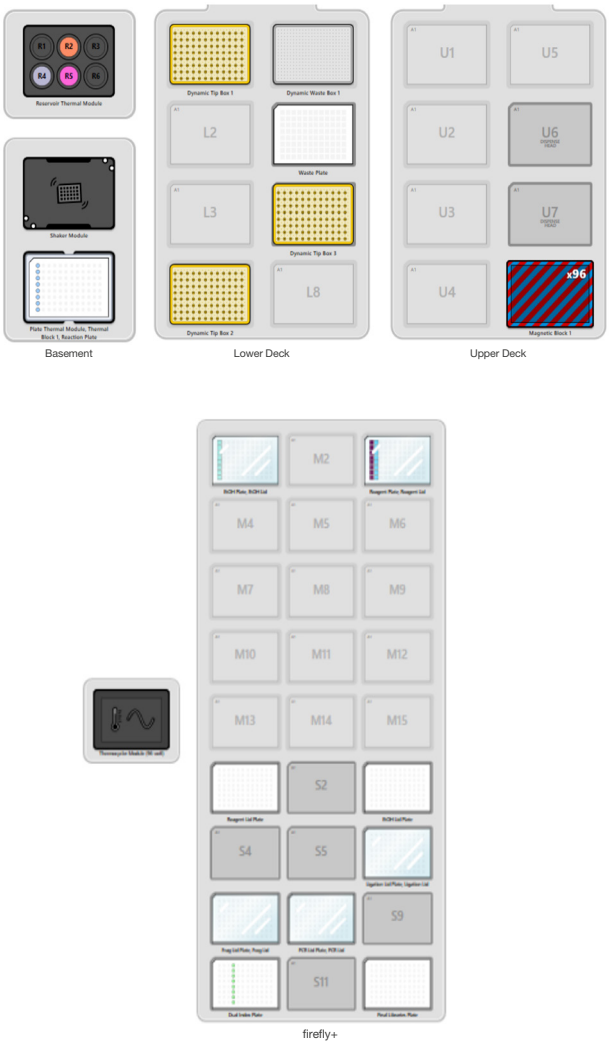

Figure 2. 10x GEX 5’ Library Construction deck layout.