firefly® technical note
GEM-X 5’ Gene Expression v3 Library Prep

This technical note provides supporting information for automating 10X Genomics' GEM-X 5’ Gene Expression v3 (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.

This method was developed with an EZ-load 6 head genomics (v1.5.4 software) firefly using 16 samples, Biorad HSP-9601 PCR plates and the Alpaqua Magnum FLX magnet. Use of alternative firefly configurations or labware may require further optimization.

Workflow overview

firefly protocols

Protocol number	Protocol name	Estimated run time (minutes)
Protocol 1 of 6	5.1 GEX Fragmentation, End Repair & A-tailing	5
Protocol 2 of 6	5.2 GEX Post Frag, ER & AT - Double Sided SPRIselect	40
Protocol 3 of 6	5.3 GEX Adapter Ligation	5
Protocol 4 of 6	5.4 GEX Post Ligation Cleanup - SPRIselect	35
Protocol 5 of 6	5.5 GEX Sample Index PCR	5
Protocol 6 of 6	5.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect	40

Table 1. Protocols & estimated run times used in 10x Genomics GEM-X 5’ Gene Expression v3 Library Prep on firefly.

Input variables

Protocol number	Protocol name	Variable ID	Default Value
Protocol 1 of 6	5.1 GEX Fragmentation, End Repair & A-tailing	Number of Samples	16
Protocol 2 of 6	5.2 GEX Post Frag, ER & AT - Double Sided SPRIselect	Number of Samples	16
Protocol 3 of 6	5.3 GEX Adapter Ligation	Number of Samples	16
Protocol 3 of 6	5.3 GEX Adapter Ligation	Ligation Mix Overage %	0.5
Protocol 4 of 6	5.4 GEX Post Ligation Cleanup - SPRIselect	Number of Samples	16
Protocol 5 of 6	5.5 GEX Sample Index PCR	Number of Samples	16
Protocol 5 of 6	5.5 GEX Sample Index PCR	Dual Index TT Starting Column	1
Protocol 6 of 6	5.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect	Number of Samples	16

Table 2. Variables used in 10x Genomics GEM-X 5’ Gene Expression v3 Library Prep on firefly.

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Consumables

Supplier	Part Name	Part Number	Number Required / Run - 16 Samples
SPT Labtech	EZ-Load Strip Tips, 100µl, with Filters, Sterile, 8 Tips Per Strip	125-008-EZ-FS	12
SPT Labtech	Universal Tip Loading Cassette	FFY-A-01-EZL-SL-5	5
SPT Labtech	Universal Tip Stand	3276-08075	5
SPT Labtech	Microplate Riser	3276-01838	1
SPT Labtech	dragonfly® discovery Sterile LDV Reservoirs - sterile	4150-07203	3
SPT Labtech	dragonfly® discovery Sterile Reservoirs - sterile	4150-07204	12
SPT Labtech	dragonfly® discovery Sterile Syringes - sterile	4150-07201	9
SPT Labtech	dragonfly® discovery Sterile, Ultra Low Retention Syringes - sterile	4150-07209	6
SPT Labtech	Strip Tip Insert - 8 Channel Offset	FFY-A-01-EZL-096-SC-8	5
SPT Labtech	Thermal Adapter for PCR Plate, 96	3276-01065	1
Biorad	Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted	HSP-9601	3
Fisher scientific	Fisherbrand™ 96-Well DeepWell™ Polypropylene Microplates	12-566-611	3

Table 3. Consumables & labware required for 10x Genomics GEM-X 5' Gene Expression v3 Library Prep on firefly.

Reagents

Protocol	Protocol Name	Reagent	Reservoir Type / dead volume (µL)	Total Volume (µL)
1 of 6	5.1 GEX Fragmentation, End Repair & A-tailing	Fragmentation Mix	LDV; 75	715
2 of 6	5.2 GEX Post Frag, ER & AT - Double Sided SPRIselect	80% EtOH	STD; 240	2640
		Butter EB	STD, 240	1056
		SPRIselect Reagent	STD, 240	880
3 of 6	5.3 GEX Adapter Ligation	Adapter Ligation Mix	LDV; 75	875
4 of 6	5.4 GEX Post Ligation Cleanup - SPRIselect	80% EtOH	STD; 240	2640
		Butter EB	STD, 240	736
		SPRIselect Reagent	STD, 240	1520
5 of 6	5.5 GEX Sample Index PCR	Library Amp Mix	LDV; 75	875
6 of 6	5.6 Post Sample Index PCR Double Sided Size Selection – SPRIselect	80% EtOH	STD; 240	2640
		Butter EB	STD, 240	736
		SPRIselect Reagent	STD, 240	1520

Table 4. Reagent volumes required for 16 samples using 10x Genomics GEM-X 5' Gene Expression v3 Library Prep on firefly.

Protocol Overview

Protocol 1 of 6

5.1 GEX Fragmentation, End Repair & A-tailing

Protocol 1 of 6: 5.1 Fragmentation, End Repair & A-tailing

This protocol performs step 5.1 of 10x Genomics' GEM-X 5' Gene Expression v3 (CG000770/CG000733 | Rev A)

Prior to executing this protocol:

- **5.1a.** Prepare thermal cycler
- **5.1b.** Vortex Fragmentation Buffer, verify there is no precipitate
- **5.1c.** Prepare Fragmentation Mix on ice

This protocol is compatible with 8 - 48 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update Fragmentation Master Mix reservoir asset definition.

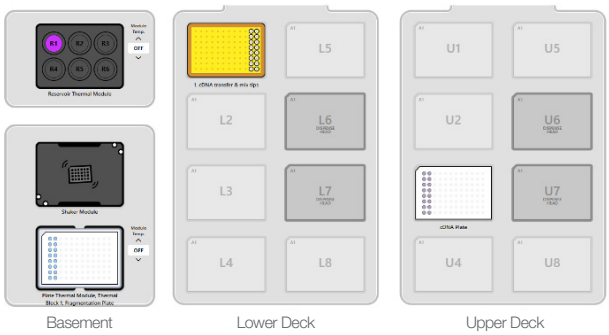

Figure 1. 5.1 GEX Fragmentation, End Repair & A-tailing deck layout.

Protocol 2 of 6

5.2 GEX Post Frag, ER & AT - Double Sided SPRIselect

Protocol 2 of 6: 5.2 GEX Fragmentation, End Repair & A-tailing Double Sided - SPRIselect

This protocol performs step 5.2 of 10x Genomics' GEM-X 5' Gene Expression v3 (CG000770/CG000733 | Rev A)

Prior to executing this protocol:

- **5.2a** Vortex to resuspend the SPRIselect reagent

Bead binding incubation steps have been increased from standard 10x protocol to maximize recovery as this protocol is fully walk-away.

This protocol is compatible with 8 - 48 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

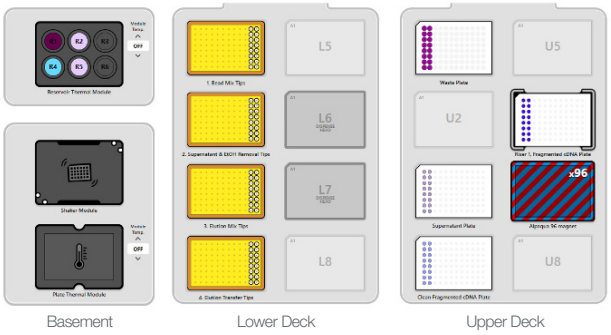

Figure 2. 5.2 GEX Post Frag, ER & AT - Double Sided SPRIselect deck layout.

Protocol 3 of 6

5.3 GEX Adapter Ligation

Protocol 3 of 6: 5.3 GEX Adapter Ligation

This protocol performs step 5.3 of 10x Genomics' GEM-X 5' Gene Expression v3 (CG000770/CG000733 | Rev A)

Prior to executing this protocol:

- **5.3a** Prepare Ligation Mix

This protocol is compatible with 8 - 48 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update Ligation Master Mix Aspiration step.

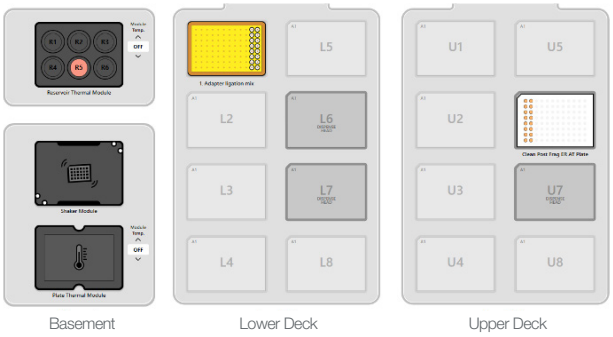

Figure 3. 5.3 GEX Adapter Ligation deck layout.

Protocol 4 of 6

5.4 GEX Post Ligation Cleanup - SPRIselect

Protocol 4 of 6: 5.4 GEX Post Ligation Cleanup - SPRIselect

This protocol performs step 5.4 of 10x Genomics' GEM-X 5' Gene Expression v3 (CG000770/CG000733 | Rev A)

Prior to executing this protocol:

- **5.2a** Vortex to resuspend the SPRIselect reagent

Bead binding incubation steps have been increased from standard 10x protocol to maximize recovery as this protocol is fully walk-away.

This protocol is compatible with 8 - 48 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

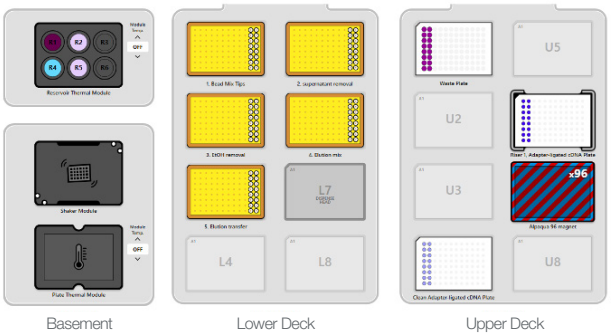

Figure 4. 5.4 GEX Post Ligation Cleanup - SPRIselect deck layout.

Protocol 5 of 6

5.5 GEX Sample Index PCR

Protocol 5 of 6: 5.5 GEX Sample Index PCR

This protocol performs step 5.5 of 10x Genomics' GEM-X 5' Gene Expression v3 (CG000770/CG000733 | Rev A)

Prior to executing this protocol:

- **5.5a** Record the 10x sample index name used
- **5.5b** Prepare (Library) Amp Mix
- **5.5d** Program thermal cycler

This protocol is compatible with 8 - 48 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update (Library) Amp Mix reservoir type.

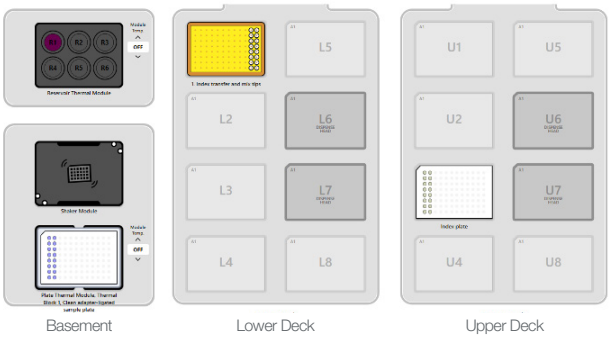

Figure 5. 5.5 GEX Sample Index PCR deck layout.

Protocol 6 of 6

5.6 Post Sample Index PCR Double Sided Size Selection - SPRIselect

Protocol 6 of 6: 5.6 Post Sample Index PCR Double Sided Size Selection - SPRIselect

This protocol performs step 5.6 of 10x Genomics' GEM-X 5' Gene Expression v3 (CG000770/CG000733 | Rev A)

Prior to executing this protocol:

- **5.2a** Vortex to resuspend the SPRIselect reagent

Bead binding incubation steps have been increased from standard 10x protocol to maximize recovery as this protocol is fully walk-away.

This protocol is compatible with 8 - 48 samples as written and has been updated to v1.8.6 firefly software. To process > 48 samples, update EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

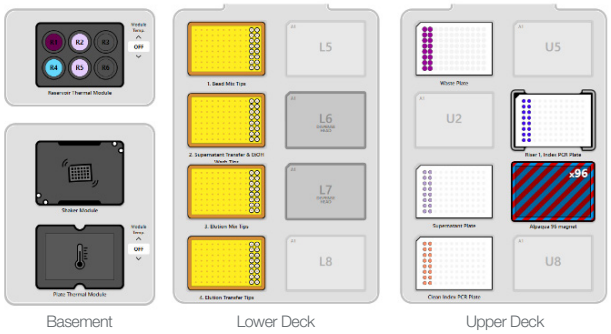

Figure 6. 5.6 Post Sample Index PCR Double Sided Size Selection - SPRIselect deck layout.

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