firefly® technical note

10x Genomics GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads

This technical note provides supporting information for automating 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads (CG001702 | 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 for 48 samples.

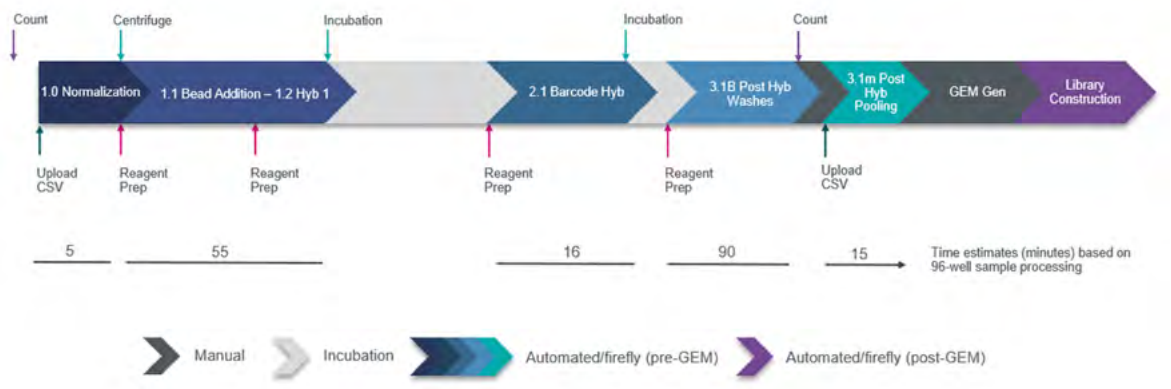


Figure 1. Workflow diagram for Flex v2 (Apex) with Concanavalin A Magnetic Beads on firefly.

firefly protocols

Protocol number	Protocol name	Run time (minutes)
Protocol 1 of 6	1.0 10x Flex Apex - Normalization	5
Protocol 2 of 6	1.1 10x Flex Apex - Magnetic Bead Binding	45
Protocol 3 of 6	1.2 10x Flex Apex - Probe Hybridization	12
Protocol 4 of 6	2.1 10x Flex Apex - Barcode Oligo Hybridization	16
Protocol 5 of 6	3.1b 10x Flex Apex - Post Hybridization Washes	90
Protocol 6 of 6	3.1j 10x Flex Apex - Sample Pooling	15

Table 1. protocols & run times used in 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads on firefly (48 samples).

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Input variables

Protocol number	Protocol name	Variable ID	Default Value
Protocol 1 of 6	1.0 10x Flex Apex - Normalization	Cherry pick CSV	File path
Protocol 2 of 6	1.1 10x Flex Apex - Magnetic Bead Binding	Number of Samples	48
		Input Sample Volume (µL)	180
		Diluted Bead Buffer Syringe Count	1
		Diluted Bead Buffer Volume (µL)	165
		Target Remaining Supernatant (µL)	15
Protocol 3 of 6	1.2 10x Flex Apex - Probe Hyb	Hyb 1 Syringe count	2
		Number of Samples	48
		Sample Input Volume (µL)	180
		Probe Hyb Mix Volume (µL)	50
Protocol 4 of 6	2.1 10x Flex Apex - Barcode Hyb	Hyb 2 Syringe count	2
		Number of Samples	48
		Barcode Hyb Mix Volume (µL)	40
		Post-Hyb Buffer B Volume (µL)	130
		Post-Hyb Buffer B Syringe Count	4
		Barcode Oligo Volume (µL)	10
Protocol 5 of 6	3.1B 10x Flex Apex - Post Hyb Washes	Number of Samples	48
		Post-Hyb Wash Buffer B Volume (µL)	180
		Post-Hyb Wash Buffer B Syringe Count	4
		Post-Hyb Resuspension Buffer B Volume (µL)	100
		Post-Hyb Resuspension Buffer B Syringe Count	2
Protocol 6 of 6	3.1m 10x Flex Apex - Sample Pooling	Number of Samples	48
		Pooling CSV	File path

Table 2. Variables used in 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads on firefly (48 samples). Static variables, including those defined as algebraic expressions, are not shown.

Reagent volumes

The reagent volumes required to run 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads 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 6

1.0 10x Flex Apex Normalization

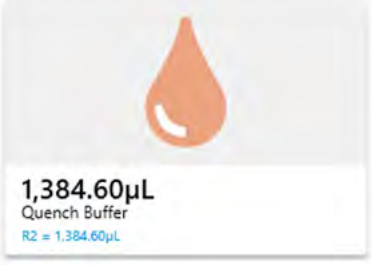


Figure 2. 1.0 10x Flex Apex - Normalization Quench Buffer reagent volumes dependent on input sample concentrations and target input cell numbers

Protocol 2 of 6
1.1 10x Flex Apex Magnetic Bead Binding



Figure 3. 1.1 10x Flex Apex – Magnetic Bead Binding minimum required reagent volumes

Protocol 3 of 6
1.2 10x Flex Apex 1st Probe Hyb



Figure 4. 1.2 10x Flex Apex - Probe Hyb minimum required reagent volumes

Protocol 4 of 6
2.1 10x Flex Apex Barcode Hyb

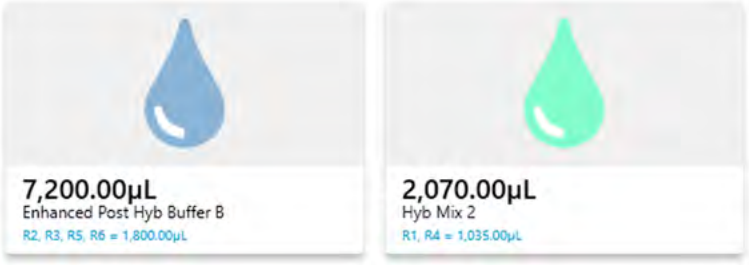


Figure 5. 2.1 10x Flex Apex - Barcode Hyb minimum required reagent volumes

Protocol 5 of 6
3.1B 10x Flex Apex Post Hyb Washes

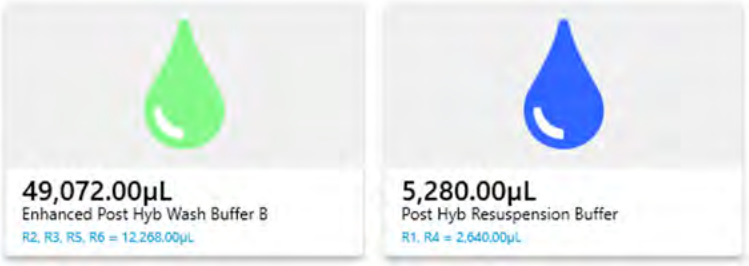


Figure 6. 3.1B 10x Flex Apex - Post Hyb Washes minimum required reagent volumes

Consumables

Supplier	Part Name	Part Number	Number Required
SPT Labtech	Thermo Adapter Block	3276-01065	1
SPT Labtech	ATL 35-125uL Tip Stand	3276-08335 (REV 2)	7
SPT Labtech	firefly® HV Reservoir Tray	3276-01928	1
SPT Labtech	dragonfly® discovery Sterile Reservoirs	4150-07204	13
SPT Labtech	dragonfly® discovery Sterile HV Reservoirs with lids	4150-07300	4
SPT Labtech	dragonfly® discovery Sterile Syringes	4150-07201	10
SPT Labtech	dragonfly® discovery Sterile, Ultra Low Retention Syringes	4150-07209	6
SPT Labtech	Empty Tip Rack	n/a	1
SPT Labtech	AT-Load firefly® Pipette Tips, 100 µL with filters, Sterile 96 Tips Per Rack	125-096-FF-AL-FS	7
Hamilton	PCR ComfortLid	814300	1
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1
Thermo Fisher Scientific	Abgene 0.8mL Deep Well	AB0765	3
Bio-Rad	Hard Shell Plate (HSP)	HSP-9601	3

Table 3. Consumables & labware required for 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads on firefly (48 samples).

Protocol overview

This method was developed with a 6 head Genomics firefly (v2.2 software) using 96 samples starting from fixed samples resuspended in Quench Buffer, Bio-Rad Hard Shell 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 - 96 samples.

Protocol 1 of 6
1.1 10x Flex Apex Normalization

This protocol performs Step 1.0 Normalization of 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads (CG001702 | Rev A)

Prior to executing this protocol:

- **1.1a** Aliquot the desired number of fixed cells/nuclei resuspended in Quench Buffer into each well of a PCR plate, max = 180 µL

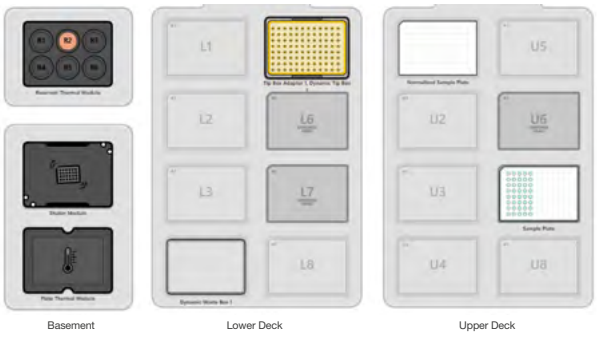


Figure 7. 1.0 10x Flex Apex - Normalization deck layout

Protocol 3 of 6
1.2 10x Flex Apex - Probe Hyb

This protocol performs Step 1.2 Probe Hybridization of 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads (CG001702 | Rev A)

Prior to executing this protocol:

- **1.2b** Prepare Probe Hyb Mix at room temperature

Notes User Interaction 1: 1.2h - 1.2i Firmly seal the plate & incubate for 16–24 h at 42°C.

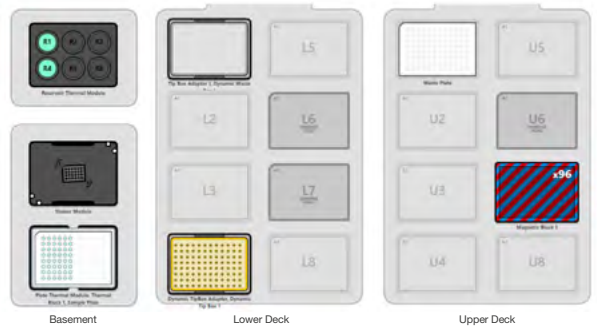


Figure 9. 1.2 10x Flex Apex - Probe Hyb deck layout

Protocol 2 of 6
1.1 10x Flex Apex - Magnetic Bead Binding

This protocol performs Step 1.1 Magnetic Bead Binding of 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads (CG001702 | Rev A

Prior to executing this protocol:

- **1.1a** Aliquot desired number of fixed cells/nuclei resuspended in Quench Buffer into each well of PCR plate, max = 180 µl
- **1.1b** Centrifuge the fixed cells/nuclei in the plate at 850 rcf for 5 min at 4°C
- Prepare Diluted Bead Buffer

Notes When cell input is >250,000, supernatant removal aspiration heights may need to be decreased

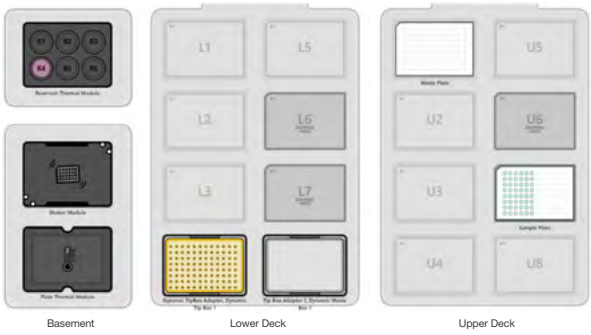


Figure 8. 1.1 10x Flex Apex – Magnetic Bead Binding deck layout

Protocol 4 of 6
2.1 10x Flex Apex - Barcode Hyb

This protocol performs Step 2.1 Barcode Oligo Hybridization of 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads protocol (CG001702 | Rev A)

Prior to executing this protocol:

- **2.1b** Prepare Post-Hyb Wash Buffer B
- **2.1c** Prepare Barcode Oligo Hyb Mix
- **2.1d** Incubate Barcode Oligo Hyb Mix at 42° for at least 5 min
- **2.1e** Remove plate from overnight incubation. Keep at room temperature for 10 min before proceeding to next step.
- **2.1k** Retrieve thawed Barcoding Oligo plates from ice. Vortex and centrifuge at 850 rcf for 5 min.

Notes
User Interaction 1: 2.1l - 2.1m Firmly seal the plate & incubate Hyb Reaction at 42°C 2 hours

This method was developed with a 6 head Genomics firefly (v2.2 software) firefly using 96 samples starting from fixed samples resuspended in Quench Buffer, Bio-Rad Hard Shell 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 - 96 samples.

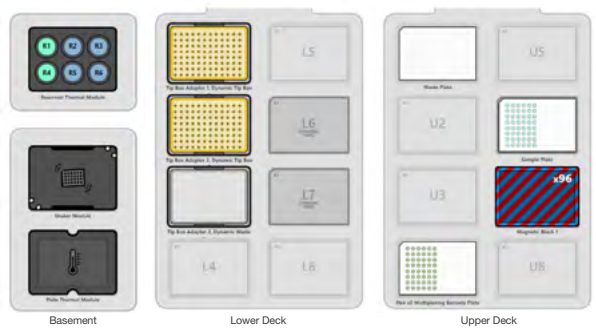


Figure 10. 2.1 10x Flex Apex - Barcode Hyb deck layout

Protocol 5 of 6
3.1b 10x Flex Apex - Post Hyb Washes

This protocol performs Step 3.1b Post-Hyb Buffer B Washes of 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads (CG001702 | Rev A)

Prior to executing this protocol:

- **3.1a** Remove plates from the thermomixer/oven/thermal cycler after Barcode Oligo Hybridization. Keep at room temperature for 10 min before proceeding to next step.
- **3.1b** Retrieve Post-Hyb Wash Buffer B prepared in step b of Barcode Oligo Hybridization
- **3.1c** Prepare Post-Hyb Resuspension Buffer B. Maintain at 4°C.

Notes

- Do not load Post-Hyb Resuspension Buffer B to reservoir(s) until prompted
- Supernatant removal step volumes account for incubation evaporation
- HV reservoirs with lids required to reduce Enhanced Buffer B evaporation, overfill set to 15% as default

User Interaction 1: load pre-chilled Post-Hyb Resuspension Buffer B to appropriate reservoir(s)

User Interaction 2: Store samples or proceed to pooling

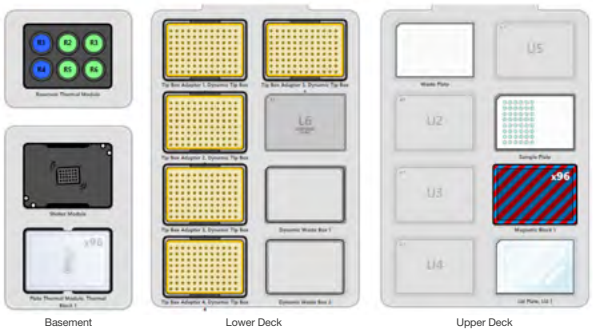


Figure 11. 3.1B 10x Flex Apex Post Hyb Washes deck layout

Protocol 6 of 6
3.1m 10x Flex Apex – Sample Pooling

This protocol performs Step 3.1m Sample Pooling of 10X Genomics' GEM-X Flex v2 (Apex) with Concanavalin A Magnetic Beads (CG001702 | Rev A)

Prior to executing this protocol:

- Determine cell concentration of the sample using an automated cell counter or a hemocytometer (optional)
- Calculate pool volumes - upload CSV

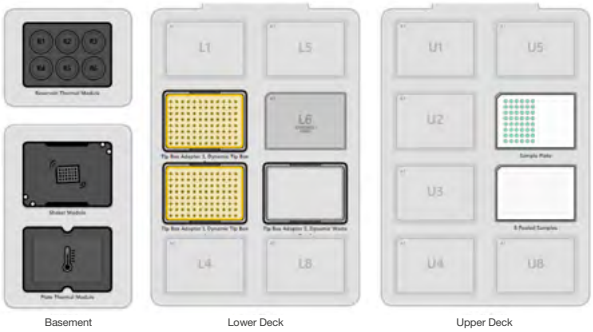


Figure 12. 3.1m 10x Flex Apex - Sample Pooling deck layout

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