INTEGRA

User guide for 10x Genomics GEM-X-APEX (Flex v2) on ASSIST PLUS

Equipment and consumables list

Check	PN	Description		Quantity
		Equipment	**Used in Steps**	
	4505	ASSIST PLUS base unit	All	1
	4222	Communication module	All	2
	4211	Charging/communication stand for 1 pipette	All	1
	4633	12 channel 300 µl VIAFLO pipette	Fixation/Hybridization/Barcoding/Wash	1
	4510	Slanted plate holder	Fixation/Hybridization/Barcoding/Wash	1
	4547	Dual reservoir adapter	Fixation/Hybridization/Barcoding/Wash	1
	4551	Labware pedestal	Fixation/Hybridization/Barcoding/Wash	1
	4532	5-1250 µl D-ONE Single channel pipetting module	Normalization/Post Gem	1
	4535	Tip deck for D-ONE pipetting module	Normalization/Post Gem	1
	4540	1.5/2.0ml Tube rack	Post Gem	1
	6250	96 Cooling block	Post Gem	1
	4900	MAG module	Post Gem DNA Cleanup	1
	4906	96 PCR adapter for MAG module	Post Gem DNA Cleanup	1
	4561	4 Position slider base	All, 48 Samples only	1
	4565	Slider for 0.2 ml strip tubes	All, 48 Samples only	1
	4547	Slider for 25 ml reservoir	All, 48 Samples only	1
	4562	Slider for 1.5/2.0 ml tubes	All, 48 Samples only	1
		Consumables		
	6535	300 µl GRIPTIPS, sterile, filter, low retention	Fixation/Hybridization/Barcoding/Wash	
	6565	125 µl GRIPTIPS, sterile filter, low retention	Normalization/Post Gem	
	6545	1250 µl GRIPTIPS, sterile filter, low retention	Normalization/Post Gem	
	4381, 4382, 4383	25 ml SureFlo reservoirs, polystyrene	Fixation/Hybridization/Barcoding/Wash	
	4391, 4392, 4393, 4322	100 ml SureFlo reservoirs, polystyrene	Fixation/Hybridization/Barcoding/Wash	
	4316, 4317	25 ml Reservoirs, polypropylene	Fixation/Hybridization/Barcoding/Wash	
		Other Reagents and Supplies not provided by INTEGRA		
	P-96-450V-C	Axygen Corning 500 µl V-Bottom Plate		
		10X Genomics GEM-X Flex v2 Reagent Kits for 96 Reactions		

Important Notes

- These instructions are for using VIALAB programs that were developed for processing of 96 samples. If running fewer than 96 samples, modifications are required. Refer to messages in 48 sample programs for guidance.
- In the dual reservoir adapter, always use INTEGRA SureFlo™ polystyrene 25 ml reservoirs for clean reagents.
- In the dual reservoir adapter, always use INTEGRA polypropylene (non-SureFlo) 25 ml reservoirs for collecting cell pools.
- For collecting waste in the dual reservoir adapter, either polystyrene or polypropylene reservoirs may be used.
- Refer to 10X Genomics Flex Apex protocol for proper sample and reagent handling details.

Program: 0-1_Fixation (10 min, 192 tips)

In this step 180 µl supernatant is aspirated off wells. 100 µl fixative is dispensed into 96 wells. 12 channel 300 µl VIAFLO multichannel pipette and 300 µl sterile, filter, low retention GRIPTIPS are used (**Figure 1**).

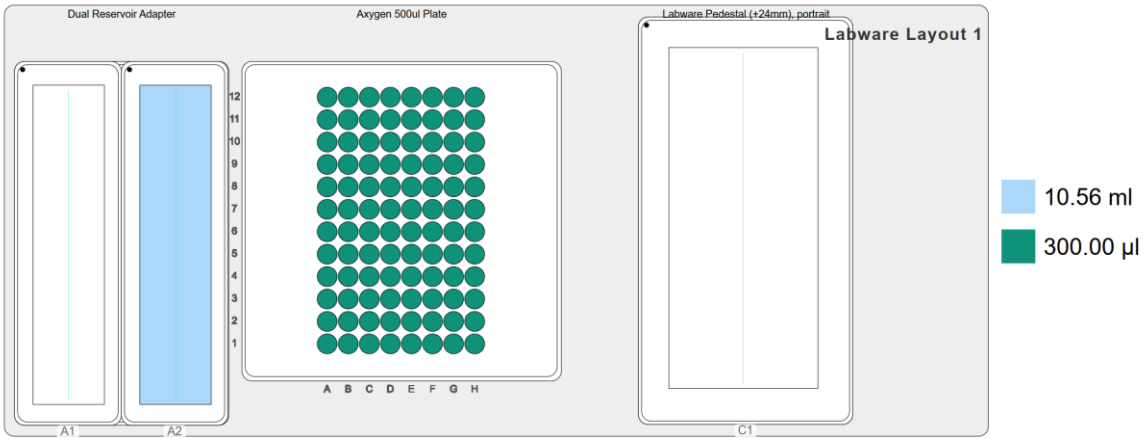

Figure 1: Deck setup for fixation. **Position A:** Dual reservoir adapter - INTEGRA Polypropylene (Non-SureFlo) 25 ml reservoir (left), INTEGRA SureFlo™ Polystyrene 25ml reservoir (right), **Position B:** Axygen plate on stanted plate holder at 20° containing samples, **Position C:** 100 ml INTEGRA reservoir on labware pedestal.

- Load 10.56 ml fixative in reservoir A2.
- Run program "**0-1_Fixation_SPH.iaa"**. When prompted, remove slanted plate holder from Deck B and replace plate on Deck B.

Program: 0-2_Additive C quench (21 min, 288 tips)

In this step 200 µl Additive C is added to the samples/fixative. The operator centrifuges the plate off the deck of the ASSIST PLUS. After centrifugation, replace the plate on the deck on the slanted plate holder at 20°.

Fixative/Additive C is removed from the plate and 200 µl Quenching Buffer is added. 12 channel 300 µl VIAFLO multichannel pipette and 300 µl sterile, filter, low retention GRIPTIPS are used (**Figure 2**).

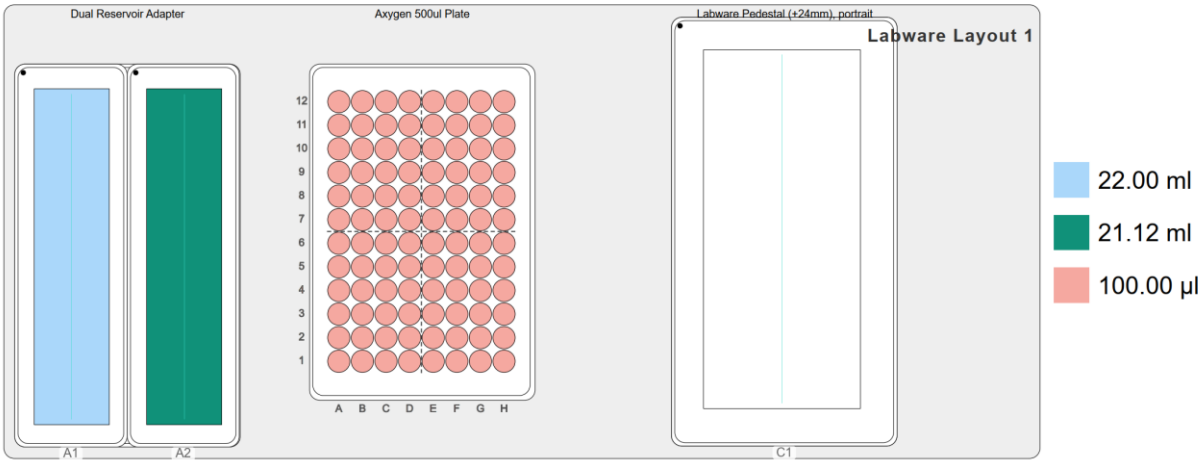

Figure 2: Deck setup for addition of Additive C and Quenching Bufffer. **Position A:** Dual reservoir adapter - INTEGRA Polypropylene (Non-SureFlo) 25 ml reservoir (left), INTEGRA SureFlo™ Polystyrene 25 ml reservoir (right), **Position B:** Axygen plate containing samples/fixative, **Position C:** 100 ml INTEGRA reservoir on labware pedestal.

- Load 22 ml Additive C in reservoir A1.
- Run program **0-2_Additive C_Quench_SPH.iaa**. When instructed on the pipette, centrifuge the plate. Replace the plate on the slanted plate holder at 20°. Load 21.12 ml Quenching Buffer in a clean reservoir on A2. Continue the program.

Program: 0-3_Storage (10 min, 192 tips)

In this step 20 µl Enhancer is dispensed into plate followed by dispensing of 55 µl Gycerol. 12 channel 300 µl VIAFLO multichannel pipette and 300 µl sterile, filter, low retention GRIPTIPS are used (**Figure 3**).

Note: This program is written assuming a sample volume of 200 µl. If sample volume deviates from this, adjustments to the program will be required.

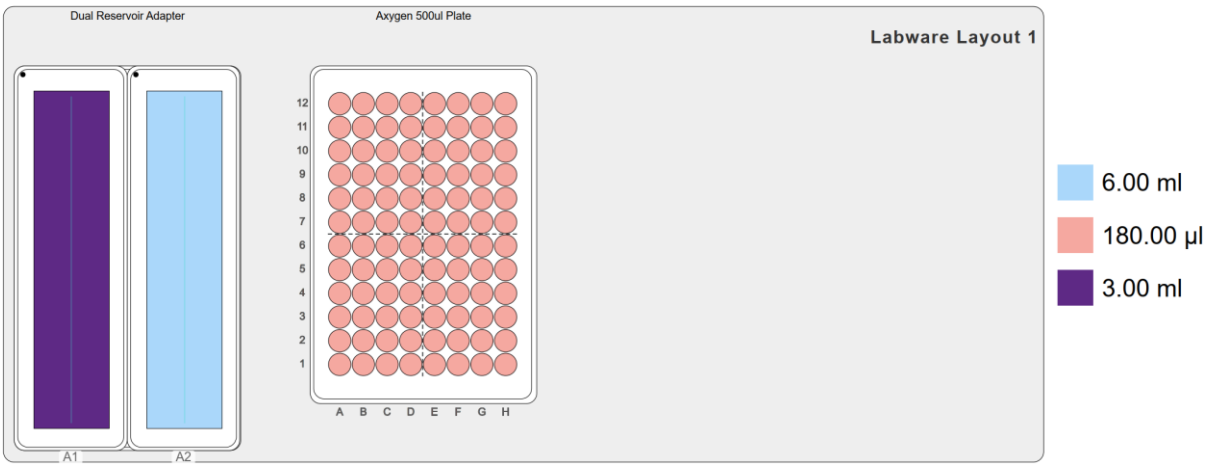

Figure 3: Deck setup for storage. **Position A:** Dual reservoir adapter - INTEGRA SureFlo™ Polystyrene 25 ml reservoir (both) **Position B:** Axygen plate containing samples/fixative.

- Load 3 ml Enhancer in reservoir A1.
- Load 6 ml Glycerol in A2
- Run program **0-3_Storage.iaa**.

Program: 0-4_Post storage processing (10 min, 120 tips)

In this step supernatant is aspirated off cells followed by dispense of 200 µl Quenching Buffer B. 12 channel 300 µl VIAFLO multichannel pipette and 300 µl sterile, filter, low retention GRIPTIPS are used (**Figure 4**).

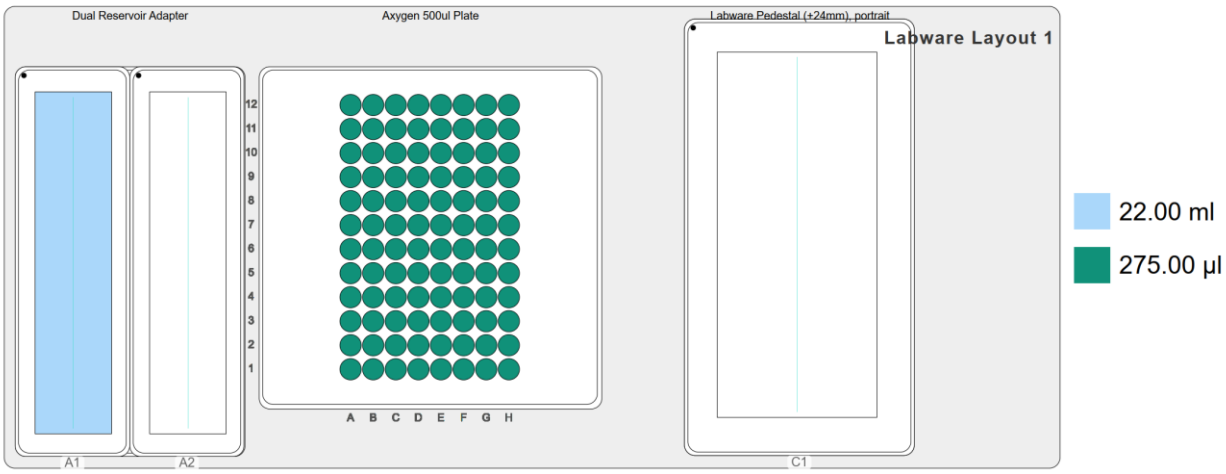

Figure 4: Deck setup for post storage processing. **Position A:** Dual reservoir adapter - INTEGRA SureFlo™ Polystyrene 25ml reservoir (left), INTEGRA Polypropylene (Non-SureFlo) 25ml reservoir (right), **Position B:** Axygen plate containing samples/fixative on slanted plate holder set at 20°, **Position C:** 100 ml INTEGRA reservoir on labware pedestal

- Load 22 ml Quencher in reservoir A1.
- Run program **0-4_Post Storage Processing SPH.iaa**. When indicated, remove plate from slanted plate holder and place plate on Deck B. Resume program.

Program: 1-1_Normalization (74 min, 96 tips)

In this step normalizes samples to desired number of cells. Use the Worklist Template "10X Flex_v2_GEM_X_Custom_Normalization_Template v4.xltm" to automatically calculate transfer volumes for each sample based upon cell concentrations and desired number of cells per sample. 5-1250 µl D-ONE single channel pipetting module, tip deck for D-ONE pipetting module and 125 µl and 1250 µl sterile, filter, low retention GRIPTIPS are used (**Figure 5**).

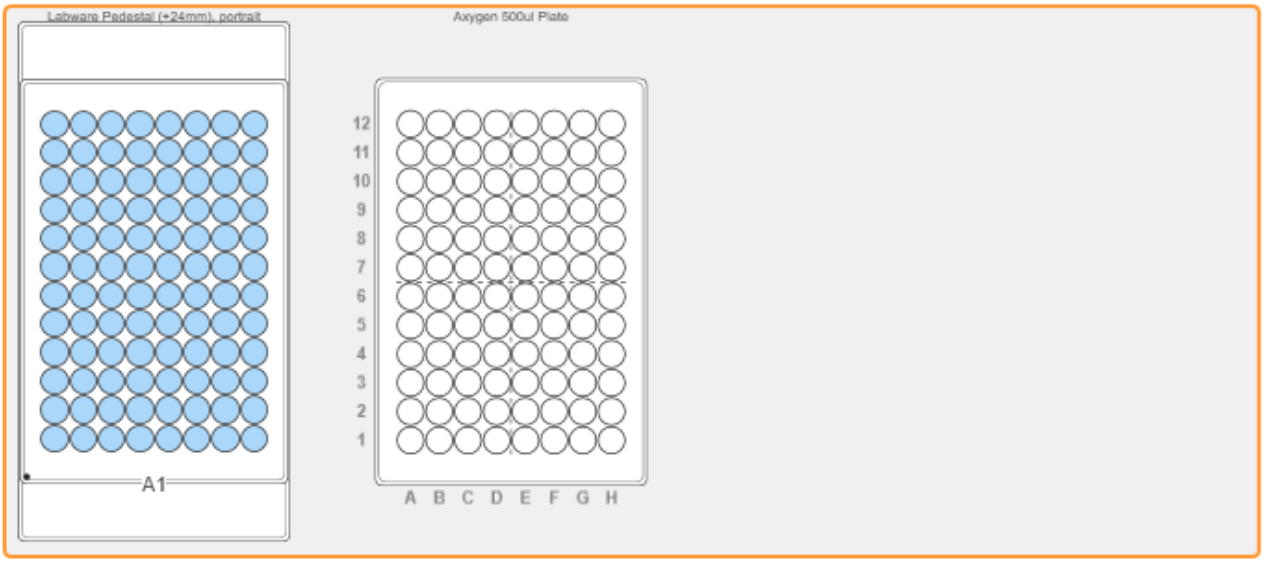

Figure 5: Deck setup for normalization. **Position A:** Labware pedestal with sample plate, **Position B:** Clean Axygen plate.

Worklist instructions:

1. Open the file "10X Flex_APEX_ -Custom_Normalization_Template v4.xltm". In the "Sample Raw Data" Worksheet, complete the Sample ID, Concentration (cells/µl), the target number of cells, and the starting volume for each sample. The transfer volume will be calculated automatically.
2. Navigate to the "Worklist Template" Worksheet (**Figure 5**). Check that data has populated the sheet correctly. If running fewer than 96 samples, remove any data from unused cells. The default values for Source Mix Volume (125), Source SBO (0.5) and Source Mix Cycles (5) have been pre-populated. Scroll to the right and click the orange button to Generate a Worklist for import into VIALAB. Save the generated worklist in a file on your computer where you can easily retrieve it.

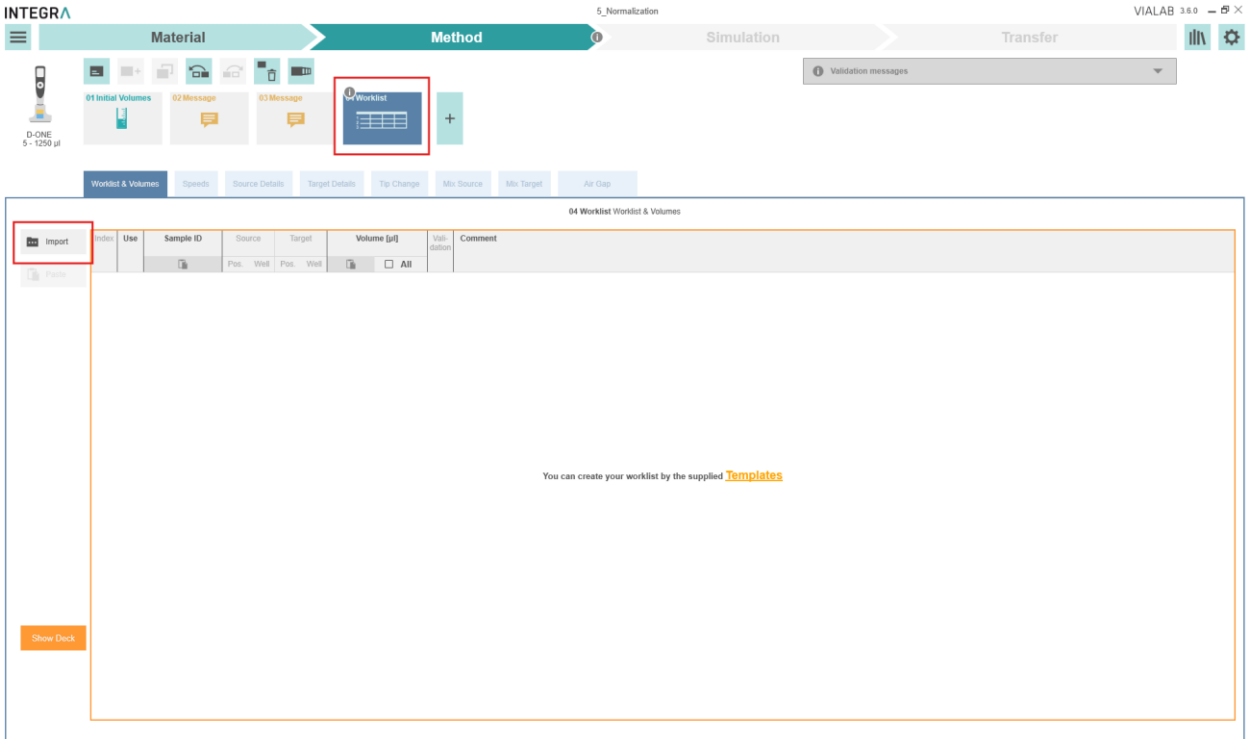

Figure 6: Worklist upload.

3. Close the Template without saving it so it reverts to its original format.
4. Open the VIALAB program **'1-1_Normalization.iaa'**. On step 4 of the protocol, click "Import" and navigate to the .csv file saved in step 2. Select "Open" to upload the file.
5. Run program **1-1_Normalization.iaa**.

Program: 1-2_Probe hybridization (9 min, 192 tips)

In this step supernatant is aspirated off cells followed by dispense of Hybridization Mix. 12 channel 300 µl VIAFLO multichannel pipette and 300 µl sterile, filter, low retention GRIPTIPS are used (**Figure 6**).

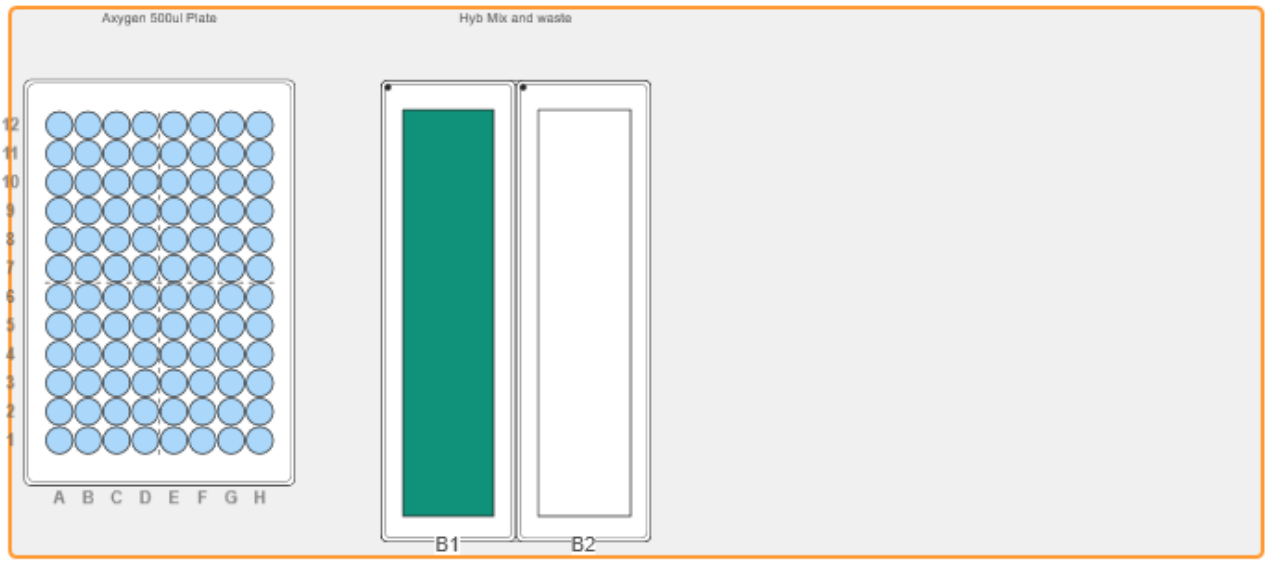

Figure 7: Deck setup for programs for probe hybridization and post hybridization wash. **Position A:** Axygen plate containing samples, **Position B:** Dual Reservoir Adapter - INTEGRA SureFlo™ Polystyrene 25 ml reservoir (left), INTEGRA Polypropylene (Non-SureFlo) 25 ml reservoir (right).

- Load 5.28 ml Hybridization Mix in reservoir B1.
- Run program **1-2_Post Hybridization.iaa**.

Program: 2-1_Post hybridization wash (8 min, 96 tips)

In this step 200 µl Post Hyb WB is dispensed into sample wells. 12 channel 300 µl VIAFLO multichannel pipette and 300 µl sterile, filter, low retention GRIPTIPS are used (see **Figure 7**).

- Load 21 ml Post-Hyb WB into a clean reservoir on B1.
- Run program **2-1_Post Hyb Wash.iaa**

Program: 2-2_Barcoding (12 min, 288 tips)

In this step 250 µl supernatant is removed from sample wells. 40 µl Oligo-Hybridization Mix is added to wells followed by addition of 10 µl barcodes. 12 channel 300 µl VIAFLO multichannel pipette and 300 µl sterile, filter, low retention GRIPTIPS are used (**Figure 7**).

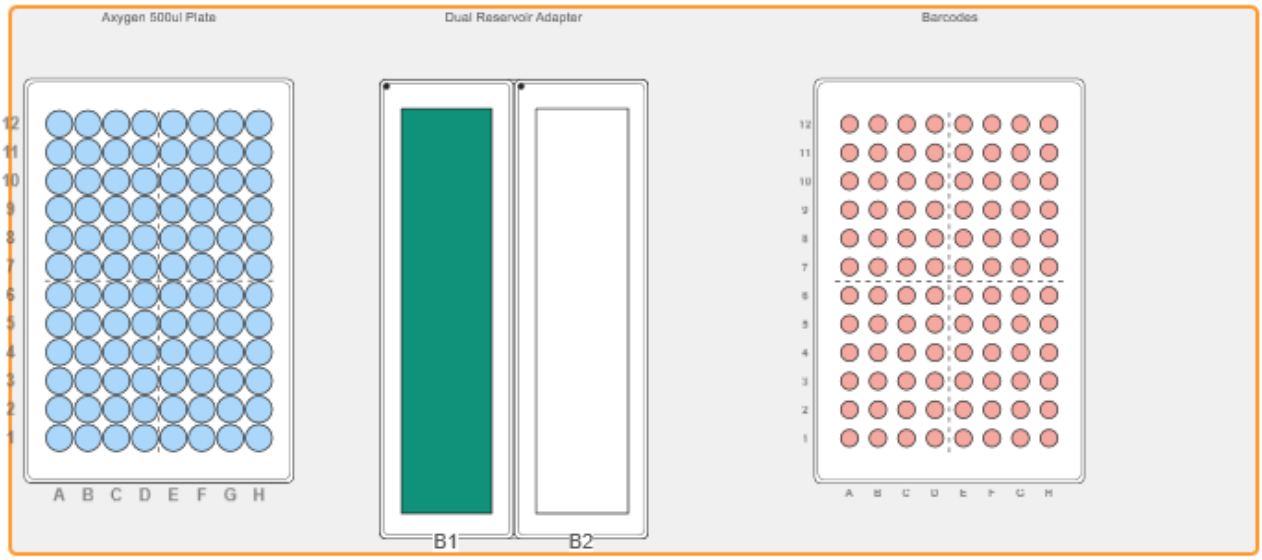

Figure 8: Deck setup for barcoding. **Position A:** Axygen plate containing samples, **Position B:** Dual Reservoir Adapter - INTEGRA SureFlo™ Polystyrene 25 ml reservoir (left), INTEGRA Polypropylene (Non-SureFlo) 25 ml reservoir (right), **Position C:** Barcode plate.

- Load 4.25 ml Oligo-Hybridization Mix in reservoir B1. Place empty reservoir in B2.
- Run program **2-2_Barcoding.iaa**

Program: 3-0_Pre-Wash Pooling (19 min, 216 tips)

In this step 150 µl Post-Hyb Wash Buffer B is added to sample plate. Samples are pooled in a reservoir followed by manual transfer to a 15 or 50 ml conical tube containing 100ul Post-Hyb Wash Buffer B per sample. 200 µl Post-Hyb Wash Buffer B is added to the sample plate. The rinsate is then pooled in a reservoir. Manually add the rinsate to the pooled samples in the 50 ml conical tube. 12 channel 300 µl VIAFLO multichannel pipette and 300 µl sterile, filter, low retention GRIPTIPS are used (**Figure 8**).

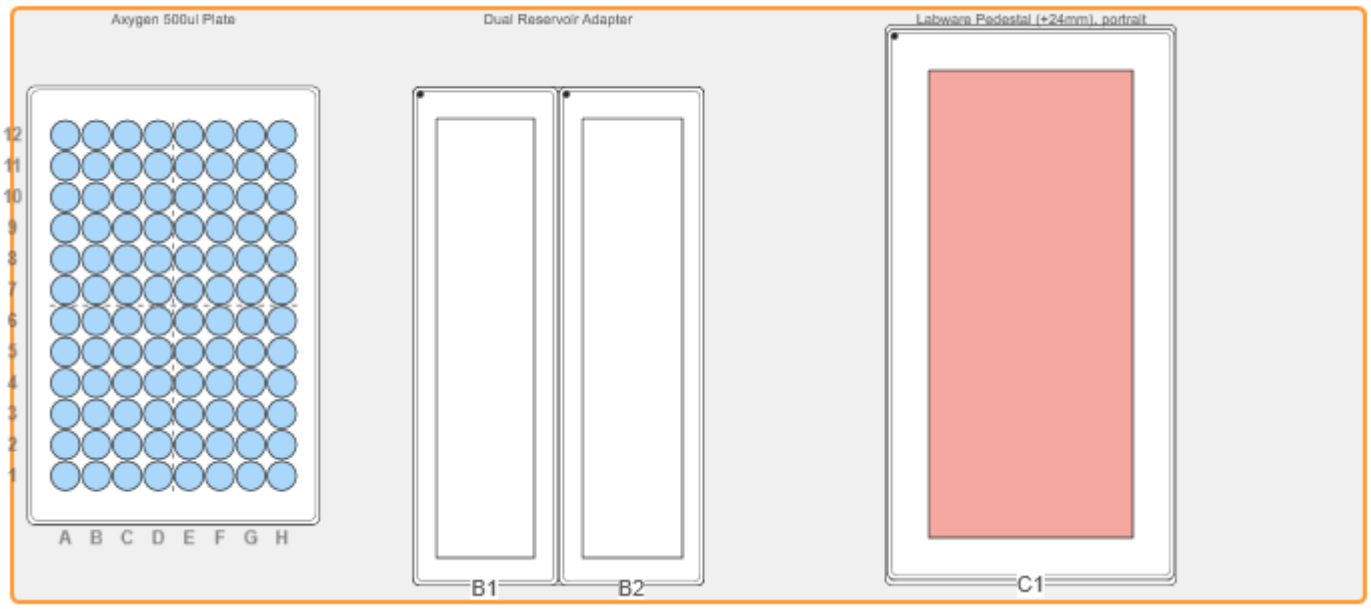

Figure 9: Deck setup for pooling. **Position A:** Axygen plate containing samples, **Position B:** INTEGRA Polypropylene (Non-SureFlo) 25 ml reservoir (right), **Position C:** 100 ml reservoir on Labware pedestal.

- Place a clean polypropylene reservoir on Deck B2.
- Load 35 ml Post-Hyb Wash Buffer B in reservoir on Deck C.
- Run program **3-0_Pre-Wash Pooling.iaa**

Perform the steps in section 4.0-5.2 manually.

Program: 5-2_Pre-amplification (3 min, 4 tips)

In this step Pre-amp mix is added to 4 sample pools in strip tube. Modify the number of samples as needed by adjusting the target locations. 5-1250 µl D-ONE single channel pipetting module, tip deck for D-ONE pipetting module and 125 µl and 1250 µl sterile, filter, low retention GRIPTIPS are used (**Figure 9**).

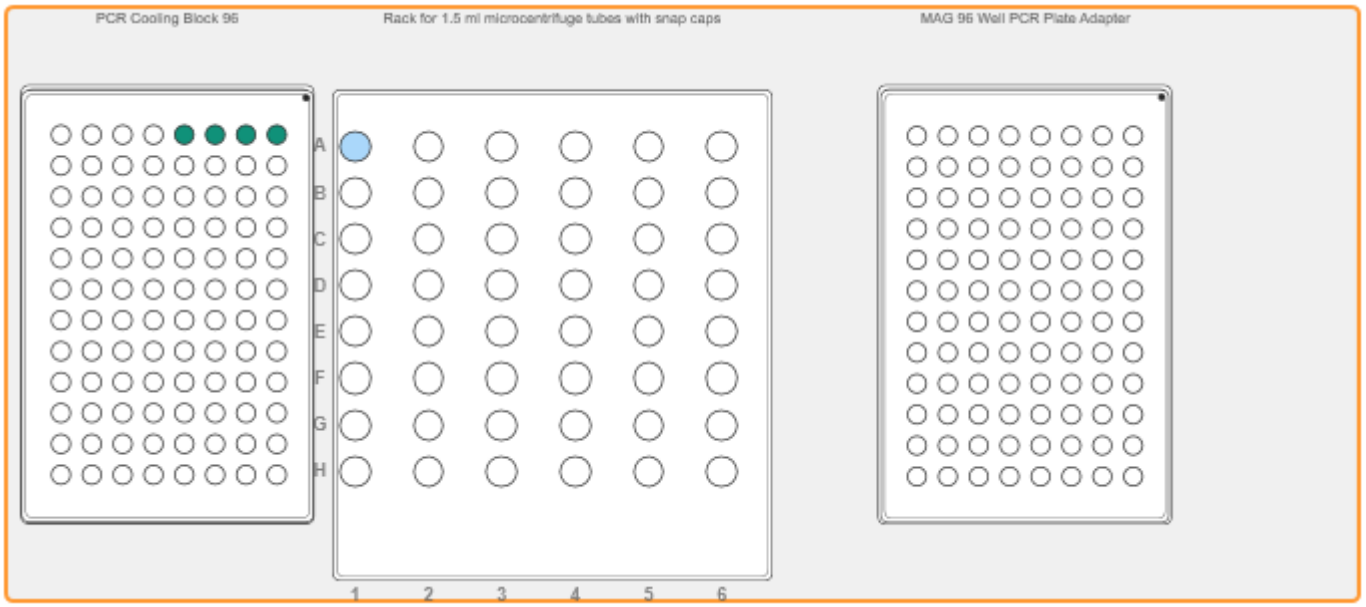

Figure 10: Deck setup for pre-amplification. **Position A:** PCR cooling block with strip tubes containing split pools in A1-D-1 (upper right), **Position B:** Tube rack, **Position C:** MAG Module.

- Place 1.5 ml microcentrifuge tube containing Pre-Amp mix on Deck B, A1.
- Run Program **5-2 Pre-Amplification**. Transfer strip tube to thermocycler.

Program: 5-3_DNA cleanup (43 min, 38 tips)

In this step magnetic bead cleanup after amplification for 4 GEM pools is performed. 5-1250 µl D-ONE single channel pipetting module, tip deck for D-ONE pipetting module and 125 µl and 1250 µl sterile, filter, low retention GRIPTIPS are used (**Figure 10**).

Note: This program has not been validated. Contact INTEGRA Biosciences for more information.

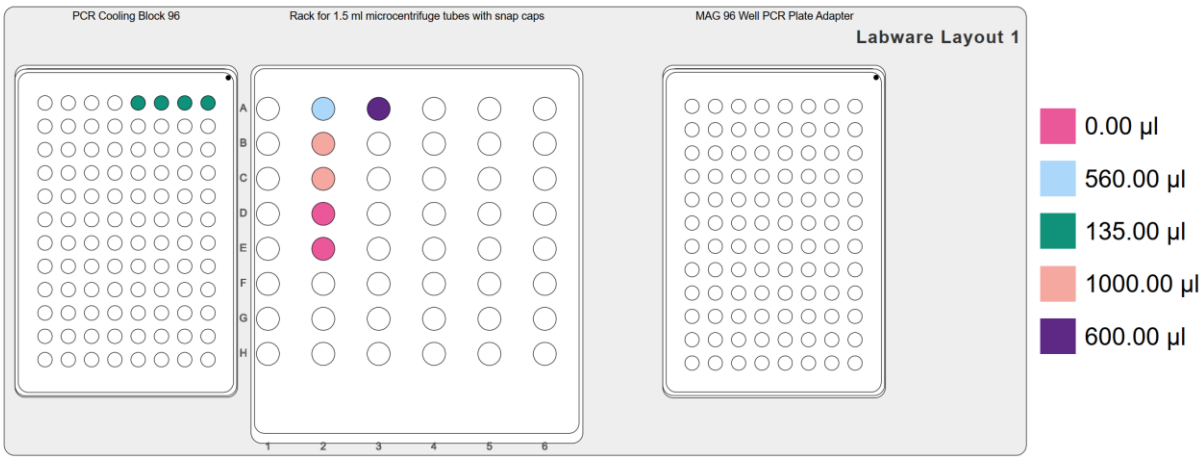

Figure 10: Deck setup for programs for DNA cleanup. **Position A:** PCR cooling block with strip tubes containing split pools in A1-D-1 (upper right), **Position B:** Tube rack, **Position C:** MAG Module with clean Lo-Bind Twin Tec Eppendorf plate.

- Centrifuge samples for 30 seconds and place sample strip tube on Deck A, A1-H1

- Place clean strip tube on Deck A, A3-H3
- Place 1.5 ml microcentrifuge tube containing 560µl beads on Deck B, A2.
- Place 1.5 ml microcentrifuge tubes containing 1 ml ethanol each on Deck B, B2 and C2.
- Place empty 1.5 ml microcentrifuge tubes on Deck B, D2-F2.
- Place 1.5 ml microcentrifuge tube containing 600 µl Elution buffer on Deck B, A3.
- Run Program **5-3_DNA Cleanup.iaa**.

Program: 6-1_Sample index PCR (7 min, 12 tips)

In this step, addition of PCR Mix and Indexes to GEMs based on target cell numbers, number of pooled samples and index plate is performed. Refer to Custom Worklist Template **10X FLEX APEX Sample Index Template.** 5-1250 µl D-ONE single channel pipetting module, tip deck for D-ONE pipetting module and 125 µl and 1250 µl sterile, filter, low retention GRIPTIPS are used (**Figure 11**).

Note: This program has not been validated. Contact INTEGRA Biosciences for more information.

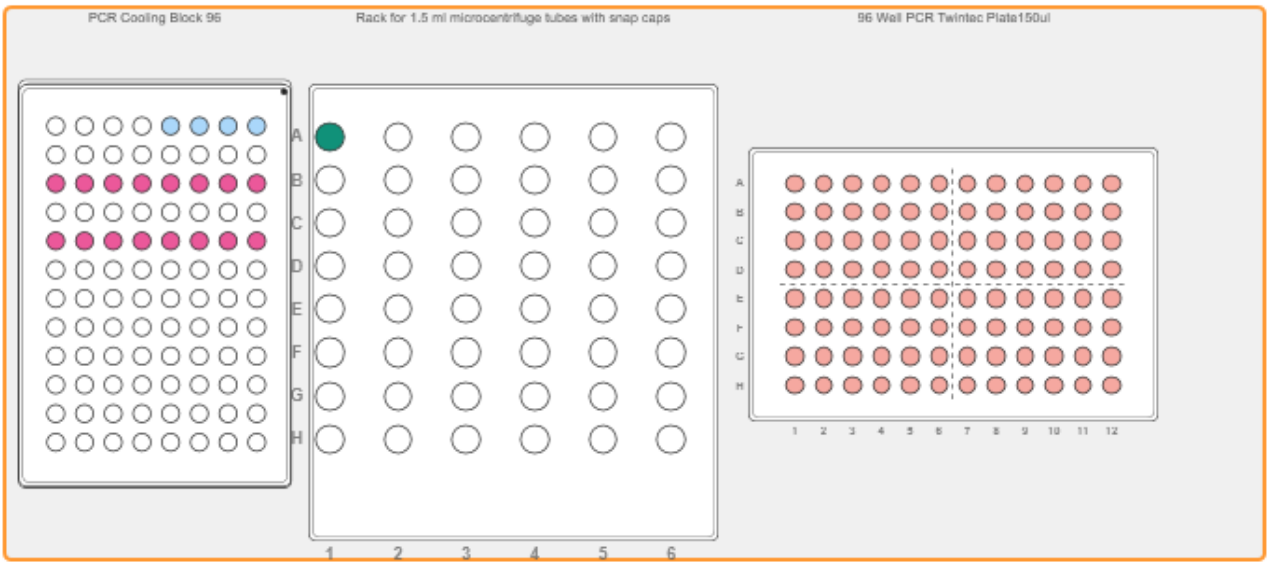

Figure 11: Deck setup for sample index PCR. **Position A:** Strip tubes holding purified GEM pools (column 1), empty strip tubes, the number of strip tubes used is based on the number of individual samples requiring indexes (columns 3, 5, 7), **Position B:** Index master mix (well A1), **Position C:** Index plate.

Worklist instructions:

- Open the file "10X FLEX APEX Sample Index Template Step 6.xltm".
- Start on Worksheet entitled "Raw Data".
- In cell B2 enter "Yes" or "No" based on the question "Are you targeting more than 320,000 cells".
- In cell E2, enter the number of sample pools in column 1.
- In cell H2, enter the well ID of the starting well of the indexes you are using. The indexes will be transferred to the samples column-wise.
- Once data is entered in the "Raw Data" worksheet, the Sample Transfer Template and PCR Mix Transfer Template will be automatically populated. Navigate to the Sample Transfer Template and press the button that says "Generate txt File to Upload into VIALAB". Two txt files will be generated. Upload the text files into each of the Worklist Method steps in program 6_1. Check data for accuracy. Modify pipetting parameters as desired.

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Associated documents

CG000833	Plate-based Sample Fixation for GEM-X Flex v2 User Guide
CG000834	10X Genomics GEM-X APEX (Flex v2) Instructions for Use