CG000210 Rev F

USER GUIDE

Chromium Next GEM Training Kit

FOR USE WITH

Chromium Next GEM Training Reagents, Gel Beads & Chip Kits, 48 rxns PN-1000143

Next GEM reagents are specific to Next GEM products and should not be used interchangeably with non-Next GEM reagents.

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Notices

Document Number

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Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

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Document Revision Summary

Document Number	CG000210
Title	Chromium Next GEM Training Kit User Guide
Revision	Rev E to Rev F
Revision Date	January 2023

Specific Changes:

- Updated schematic in step 1.2c to match the loading description (page 17).

General Changes:

- Updated for general minor consistency of language and terms throughout.

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Objective

The purpose of this User Guide is to train new users on:

- Mixing sample and Master Mix
- Preparing Gel Beads
- Loading a Chromium Next GEM Training Chip with the Reaction Mix, Gel Beads, and Partitioning Oil
- Loading a Chromium Next GEM Training Chip into the Chromium Controller (or Chromium Single Cell Controller) and run the Controller
- Inspecting the resulting Gel Bead-in-emulsion (GEMs) in the chip
- Transferring the GEMs in preparation for thermal cycling
- Processing GEMs immediately after collection

For additional guidance, refer to the User Guides cited below:

- For guidance on qualifying the Chromium Controller or Chromium Single Cell Controller, refer to the Chromium Controller Specifications (CG00020) or the Chromium Single Cell Controller Specifications (CG00050), and the Chromium Controller Readiness Test User Guide with Chromium Next GEM Test Chip (CG000222).
- For guidance on sample preparation for library construction and sequencing, refer to the applicable Demonstrated Protocol and User Guide available at the 10x Genomics Support website.

Chromium Next GEM Training Reagent Kits

Chromium Next GEM Training Reagents, Gel Beads & Chip Kit, 48 rxns PN-1000143

Chromium Next GEM Training Reagents & Gel Bead Kit, 48 rxns PN-1000144 (store at 4°C)

Chromium
Next GEM
Training Reagents &
Gel Bead Kit

	#	PN
Chromium Next GEM Training Gel Beads	6	2000200
Training Master Mix	3	220086
Surrogate Fluid	2	220021
Training Sample	1	220087

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Chromium Next GEM Training Chip Kit, 48 rxns PN-1000145 (store at ambient temperature)

Chromium
Partitioning Oil

	#	PN
Partitioning Oil	6	2000190

Chromium
Recovery Agent

	#	PN
Recovery Agent	6	220016

Chromium
Chips & Gaskets

	#	PN
Chromium Next GEM Training Chip	6	2000201
Gaskets, 6-pack	1	370017

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Chromium Accessories

Product	PN (Orderable)	PN (Item)
10x Vortex Adapter	120251	330002
10x Magnetic Separator	120250	230003
Chromium Next GEM Secondary Holder	1000195	3000332

Recommended Thermal Cyclers

The table below lists the thermal cyclers that have been validated by 10x Genomics for all currently available Chromium Single Cell and Visium Spatial protocols.

Supplier	Description	Part Number (US)
Bio-Rad	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
Analytik Jena†	Biometra TAdvanced 96 SG*	846-x-070-241 *(x = 2 for 230 V; 4 for 115 V; 5 for 100 V, 50-60 Hz)*
Eppendorf‡	Mastercycler X50s*	6311000010
	Mastercycler Pro *(discontinued)*	North America 950030010 International 6321 000.019
Thermo Fisher Scientific	Veriti 96-Well Thermal Cycler *(discontinued)*	4375786

For select instruments, ramp rates should be adjusted for all steps as described below:

†*Analytik Jena Biometra TAdvanced 96 SG: 2°C/sec for both heating and cooling*

‡*Eppendorf Mastercycler X50s: 3°C/sec heating and 2°C/sec cooling*

**Not validated for Visium Spatial protocols for FFPE*

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the 10x workflows, training, and system operations. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment, such as water baths, centrifuges, vortex mixers, pH meters, freezers, etc.

Supplier	Description	Part Number (US)
Plastics		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips DNA LoBind Tubes, 1.5 ml DNA LoBind Tubes, 2.0 ml	951010022 022431021 022431048
USA Scientific	TempAssure PCR 8-tube strip	1402-4700
Rainin	Tips LTS W-O 200UL Filter RT-L200WFLR Tips LTS 20UL Filter RT-L10FLR Tips LTS 200UL Filter RT-L200FLR Tips LTS 1ML Filter RT-L1000FLR	30389241 30389226 30389240 30389213
Equipment		
VWR	Vortex Mixer Divided Polystyrene Reservoirs	10153-838 41428-958
Thermo Fisher Scientific	MYFUGE 12 Mini Centrifuge (alternatively, use any equivalent mini centrifuge)	C1012
Rainin	Pipet-Lite LTS Pipette L-2XLS Pipet-Lite LTS Pipette L-10XLS Pipet-Lite LTS Pipette L-20XLS Pipet-Lite LTS Pipette L-100XLS Pipet-Lite LTS Pipette L-200XLS Pipet-Lite LTS Pipette L-1000XLS Pipet-Lite Multi Pipette L8-10XLS Pipet-Lite Multi Pipette L8-20XLS Pipet-Lite Multi Pipette L8-50XLS Pipet-Lite Multi Pipette L8-200XLS	17014393 17014388 17014392 17014384 17014391 17014382 17013802 17013803 17013804 17013805

Choose either Eppendorf or USA Scientific PCR 8-tube strips.

Tips & Best Practices

Icons

			
Tips & Best Practices section includes additional guidance	Signifies critical step requiring accurate execution	Troubleshooting section includes additional guidance	Next GEM specific protocol step updates

Emulsion-safe Plastics

- Use 10x Genomics validated emulsion-safe plastic consumables when handling GEMs as some plastics can destabilize GEMs.

General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Calculate reagent volumes with 10% excess of 1 rxn values.
- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.

Surrogate Fluid

- Surrogate Fluid is glycerol in a ~50% volume/volume aqueous solution.
- 50% glycerol solution can be purchased: Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32

  OR

- Prepare 50% glycerol solution:

  i. Mix an equal volume of water and 99% Glycerol, Molecular Biology Grade.

  ii. Filter through a 0.2-µm filter.

  iii. Store at –20°C in 1-ml LoBind tubes. 50% glycerol solution should be equilibrated to room temperature before use.

Pipette Calibration

- Follow manufacturer's calibration and maintenance schedules.
- Pipette accuracy is particularly important when using SPRIselect reagents.

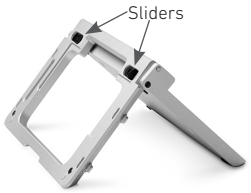
Chromium Next GEM Chip Handling

- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- After removing the chip from the sealed bag, use in ≤ 24 h.
- Execute steps without pause or delay, unless indicated. When multiple chips are to be used, load, run, and collect the content from one chip before loading the next.
- Fill all unused input wells in rows labeled 1, 2, and 3 on a chip with an appropriate volume of Surrogate Fluid before loading the used wells. DO NOT add Surrogate Fluid to the wells in the bottom NO FILL row.
- Avoid contacting the bottom surface of the chip with gloved hands and other surfaces. Frictional charging can lead to inadequate priming of the channels, potentially leading to either clogs or wetting failures.
- Minimize the distance that a loaded chip is moved to reach the Chromium Controller.
- Keep the chip horizontal to prevent wetting the gasket with oil, which depletes the input volume and may adversely affect the quality of the resulting emulsion.

Chromium Next GEM Secondary Holders

Next GEM

- Chromium Next GEM Secondary Holders encase Chromium Next GEM Chips.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal recovery well content removal.
- Squeeze the black sliders on the back side of the holder together to unlock the lid and return the holder to a flat position.

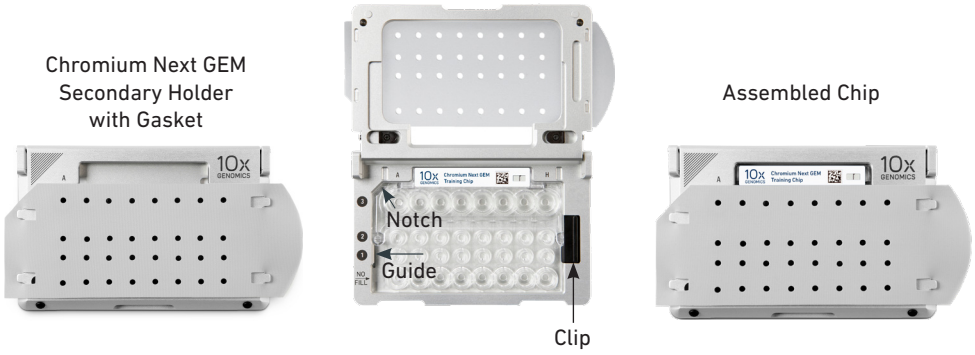


Chromium Next GEM Chip & Holder Assembly with Gasket

Next GEM

- Close the holder lid. Attach the gasket by holding the tongue (curved end, to the right) and hook the gasket on the left-hand tabs of the holder. Gently pull the gasket toward the right and hook it on the two right-hand tabs.
- DO NOT touch the smooth side of the gasket.
- Open the chip holder.
- Align notch on the chip (upper left corner) and the open holder with the gasket attached.
- Slide the chip to the left until the chip is inserted under the guide on the holder. Depress the right hand side of the chip until the spring-loaded clip engages.
- Keep the assembled unit with the attached gasket until ready for dispensing reagents into the wells.

Chip in Chromium Next GEM Secondary Holder



Chromium Next GEM Chip Loading

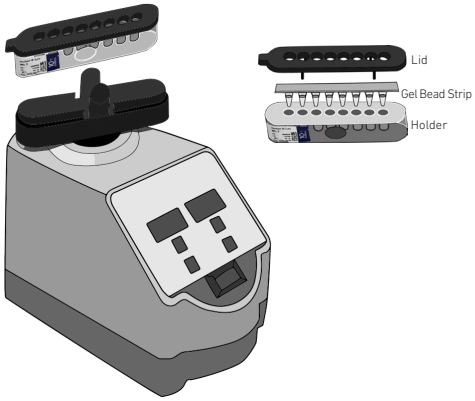
Next GEM

- Place the assembled chip and holder flat (gasket attached) on the bench with the lid open.
- Dispense at the bottom of the wells without introducing bubbles.
- When dispensing Gel Beads into the chip, wait for the remainder to drain into the bottom of the pipette tips and dispense again to ensure complete transfer.
- Refer to Load Chromium Next GEM Training Chip for specific instructions.

Gel Bead Handling

Next GEM

- Use one tube of Gel Beads per sample. DO NOT puncture the foil seals of tubes not used at the time.
- Equilibrate the Gel Beads strip to room temperature before use.
- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec.**
- Centrifuge the Gel Bead strip for ~**5 sec**. Confirm there are no bubbles at the bottom of the tubes and the liquid levels look even. Place the Gel Bead strip back in the holder and secure the holder lid.



- If the required volume of beads cannot be recovered, place the pipette tips against the sidewalls and slowly dispense the Gel Beads back into the tubes. DO NOT introduce bubbles into the tubes and verify that the pipette tips contain no leftover Gel Beads. Withdraw the full volume of beads again by pipetting slowly.

Training Step 1

Chip Assembly & Loading

1.1 Assemble Chromium Next GEM Training Chip

1.2 Load Chromium Next GEM Training Chip

1

1.0 Chip Assembly & Loading

Next GEM

GET STARTED!				
Action	**Item**	**10x PN**	**Preparation & Handling**	**Storage**
Equilibrate to Room Temperature	**Chromium Next GEM Training Gel Beads**	2000200	Equilibrate to room temperature 30 min before loading the Training Chip.	4°C
Place on Ice	**Training Master Mix**	220086	One tube is sufficient for 16 samples.	4°C
	Training Sample	220087	One tube is sufficient for 48 samples.	4°C
Obtain	**Partitioning Oil**	2000190	-	Ambient
	Surrogate Fluid	220021	-	4°C
	Chromium Next GEM Training Chip(s)	2000201	See Tips & Best Practices.	Ambient
	10x Gasket	370017	See Tips & Best Practices.	Ambient
	Chromium Next GEM Secondary Holder	3000332	See Tips & Best Practices.	Ambient

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for this protocol.

1.1 Assemble Chromium Next GEM Training Chip

Next GEM

TIPS

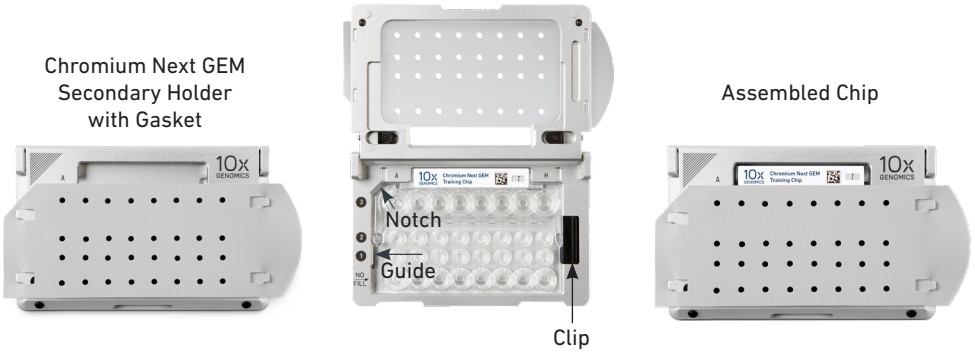
Assemble Chromium Next GEM Chip

See Tips & Best Practices for chip handling instructions.

- Close the holder lid. Attach the gasket by holding the tongue (curved end, to the right) and hook the gasket on the left-hand tabs of the holder. Gently pull the gasket toward the right and hook it on the two right-hand tabs.
- DO NOT touch the smooth side of the gasket.
- Open the chip holder.
- Remove the chip from the sealed bag. Use the chip within ≤ 24 h.
- Align notch on the chip (upper left corner) and the open holder with the gasket attached.
- Slide the chip to the left until the chip is inserted under the guide on the holder. Depress the right hand side of the chip until the spring-loaded clip engages.
- Keep the assembled unit with the attached gasket open until ready for and while dispensing reagents into the wells. DO NOT touch the smooth side of the gasket. After loading reagents, close the chip holder. DO NOT press down on the top of the gasket.

For GEM generation, load the indicated reagents only in the specified rows, starting from row labeled 1, followed by rows labeled 2 and 3. DO NOT load reagents in the bottom row labeled NO FILL. See step 1.2 for details.

Chip in Chromium Next GEM Secondary Holder



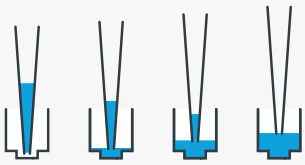
1.2 Load Chromium Next GEM Training Chip

After removing chip from the sealed bag, use in ≤24 h.

Open the lid (gasket attached) of the assembled chip and lay flat for loading.

When loading the chip, raising and depressing the pipette plunger should each take ~5 sec.

When dispensing, raise the pipette tips at the same rate as the liquid is rising, keeping the tips slightly submerged.



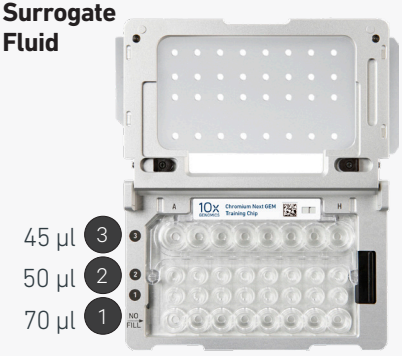
a. Add Surrogate Fluid to each unused well

(if processing <8 samples/chip)

- **70 µl** in each unused well in row labeled **1**
- **50 µl** in each unused well in row labeled **2**
- **45 µl** in each unused well in row labeled **3**

DO NOT add Surrogate Fluid to the bottom row of NO FILL wells.
DO NOT use any substitute for Surrogate Fluid.

Surrogate Fluid



b. Prepare Training Master Mix + Training Sample

- Vortex the Training Master Mix 15 sec, centrifuge briefly and place on ice. Add **73 µl** Training Master Mix to each well of the 8-tube strip on ice. Slowly add **2 µl** Training Sample into each well of the tube strip containing Master Mix.

c. Load Row Labeled 1

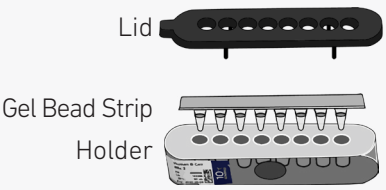
- Gently pipette mix the Training Master Mix + Training Sample
- Using the same pipette tip, dispense **70 µl** Training Master Mix + Training Sample into the bottom center of each well in **row labeled 1** without introducing bubbles.

Training Master Mix + Training Sample

d. Prepare Gel Beads

- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec.**
- Centrifuge the Gel Bead strip for **~5 sec.**
- Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even.
- Place the Gel Bead strip back in the holder. Secure the holder lid.

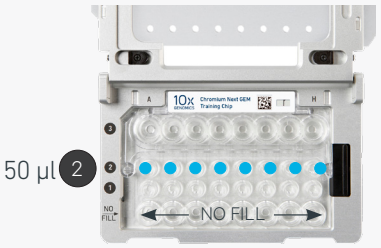
Prep Gel Beads



e. Load Row Labeled 2

- Puncture the foil seal of the Gel Bead tubes.
- Slowly aspirate **50 µl** Gel Beads.
- Dispense into the wells in **row labeled 2** without introducing bubbles.
- Wait **30 sec.**

Gel Beads

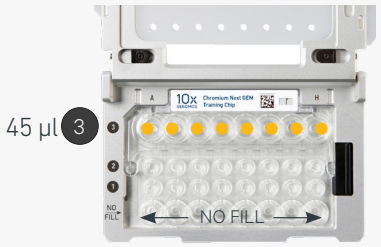


f. Load Row Labeled 3

- Dispense **45 µl** Partitioning Oil into the wells in **row labeled 3** from a reagent reservoir.

Failure to add Partitioning Oil to the top row labeled 3 will prevent GEM generation and can damage the Chromium Controller.

Partitioning Oil



g. Prepare for Run

- Close the lid (gasket already attached). DO NOT touch the smooth side of the gasket. DO NOT press down on the top of the gasket.

  Run the chip in the Chromium Controller or X/iX immediately after loading the Partitioning Oil.

Training Step 2

Run the Chromium Controller

2.1 Run the Chromium Controller

2.1 Run the Chromium Controller

Next GEM

a. Press the eject button on the touchscreen of the Chromium Controller to eject the tray.

b. Place the assembled chip with the gasket in the tray, ensuring that the chip stays horizontal. Press the button to retract the tray.

c. Confirm the Chromium Training program on screen. Press the play button.

d. At the completion of the run (**~18 min**), the Chromium Controller will chime. **Immediately** proceed to the next step.

Firmware Version 4.0 or higher is required in the Chromium Controller or the Chromium Single Cell Controller used for this protocol.

Training Step 3

Collect GEMs

3.1 Transfer GEMs

3.1 Transfer GEMs

Next GEM

a. Place a tube strip on ice.

b. Press the eject button of the Controller and remove the chip.

c. Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.

d. Visually compare the remaining volume in rows labeled 1-2. Abnormally high volume in one well relative to other wells may indicate a clog.

e. Slowly aspirate **80 µl** GEMs from the lowest points of the recovery wells in the top row labeled 3 without creating a seal between the tips and the bottom of the wells.

f. Withdraw pipette tips from the wells. GEMs should appear opaque and uniform across all channels. Excess Partitioning Oil (clear) in the pipette tips indicates a potential clog.

g. Over the course of **~20 sec**, dispense GEMs into the tube strip on ice with the pipette tips against the sidewalls of the tubes.

Incomplete recovery of GEMs will impact performance. Confirm the pipette tips do not contain residual GEMs. If residual GEMs are present, wait for remaining GEMs to drain into the bottom of the pipette tips and dispense into the tubes.

h. If multiple chips are run back-to-back, cap/cover the GEM-containing tube strip and place on ice for no more than 1 h.

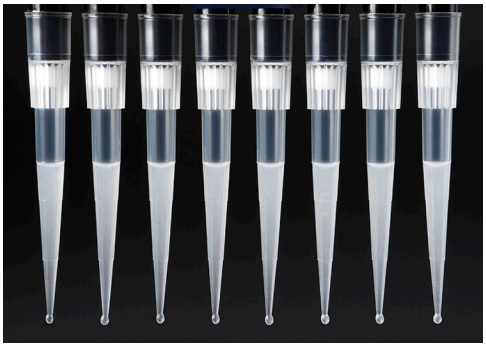
i. Discard the used Chromium Next GEM Training Chip. Push the black sliding latches on the back of the Chromium Next GEM Secondary Holder toward the middle to release the lock and close the lid.

j. This training protocol does not simulate the RT incubation step and proceeds directly to post GEM processing.

Expose Wells at 45 Degrees

Transfer GEMs

GEMs



Training Step 4

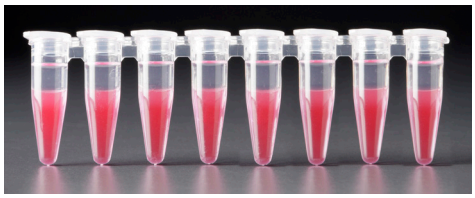
Post GEM Collection Processing

4.1 Process Collected GEMs

4.1 Process Collected GEMs

a. Add **125 µl** Recovery Agent to each sample at room temperature. DO NOT pipette mix or vortex the biphasic mixture. Wait **2 min**.

Biphasic Mixture



The resulting biphasic mixture contains Recovery Agent/Partitioning Oil (pink) and aqueous phase (clear), with no persisting emulsion (opaque).

If biphasic separation is incomplete:
Firmly secure the cap on the tube strip, ensuring that no liquid is trapped between the cap and the tube rim. Mix by inverting the capped tube strip 5x and centrifuge briefly.
DO NOT invert without firmly securing the caps.

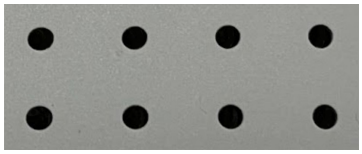
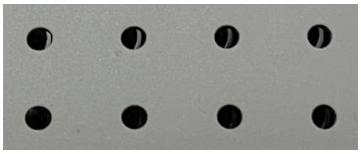
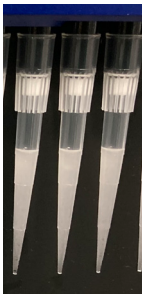
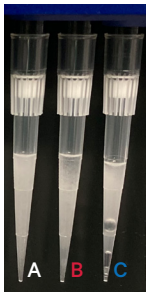
A smaller aqueous phase volume indicates a clog during GEM generation.

b. This concludes the Training Kit protocol.
This training protocol does not proceed with cDNA amplification or other steps found in other User Guides.

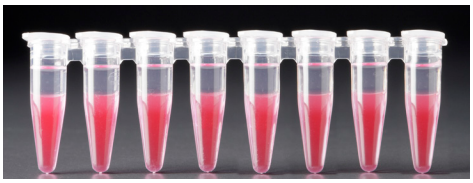
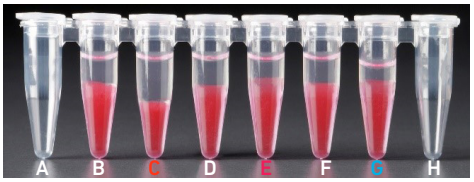
Troubleshooting

5

GEM Generation

STEP	NORMAL	IMPACTED
1.2 Load Training Chip	 Gasket holes are aligned with the sample and gel bead wells.	 **Misaligned gasket holes & chip wells** Gasket holes are misaligned with the gel bead wells. Open and close the chip holder slowly once.
3.1 d After Training Chip is removed from the Controller and the wells are exposed	 All 8 recovery wells are similar in volume and opacity.	 Recovery well G indicates a reagent clog. Recovery well C and E indicate a wetting failure. Recovery wells B, D, and F are normal. Wells A and H contain Surrogate Fluid.
3.1 f Transfer GEMs from Training Chip Row Labeled 3	 All liquid levels are similar in volume and opacity without air trapped in the pipette tips.	 Adequate emulsion volume (no clog or wetting failure) Wetting failure Low emulsion volume (clog) Pipette tip A shows normal GEM generation, pipette tip B indicates a wetting failure, and pipette tip C shows a clog and wetting failure.

GEM Generation

STEP	NORMAL	IMPACTED
4.1 a After transfer of the GEMs + Recovery Agent	 All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/Partitioning Oil (pink).	 Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear). Tube C and E indicate a wetting failure has occurred. There is an abnormal volume of Recovery Agent/Partitioning Oil (pink).

Chromium Controller Errors

If the Chromium Controller or the Chromium Single Cell Controller fails to start, an error tone will sound and one of the following error messages will be displayed:

a. **Chip not read – Try again:** Eject the tray, remove and/or reposition the Chromium Next GEM Secondary Holder assembly and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

b. **Check gasket:** Eject the tray by pressing the eject button to check that the 10x Gasket is correctly installed on the Chromium Next GEM Chip. If the error message persists, contact support@10xgenomics.com for further assistance.

c. **Error Detected: Row _ Pressure:**

   i. If this message is received within a few seconds of starting a run, eject the tray by pressing the eject button and check for dirt or deposits on the 10x Gasket. If dirt is observed, replace with a new 10x Gasket, open and close the lid to ensure the gasket is properly aligned and try again. If the error message is still received after trying this more than twice, contact support@10xgenomics.com for further assistance.

   ii. If this message is received after a few minutes into the run, the Chromium Next GEM Chip must be discarded. **Do not try running this Chromium Next GEM Chip again as this may damage the Chromium Controller.**

d. **Invalid Chip CRC Value:** This indicates that a Chromium Next GEM Chip has been used with an older firmware version. The chip must be discarded. Contact support@10xgenomics.com for further assistance.

e. **Chip Holder Not Present:** Open the controller drawer and check if chip holder is present. Insert chip properly into chip holder and retry.

f. **Unauthorized Chip:** This indicates that an incompatible non-Next GEM chip has been used with an instrument that only can run Next GEM assays. Use only Chromium Controller (PN-120223;120246) or Chromium Single Cell Controller (PN-120263;120212) to run that chip or chip must be discarded. Contact support@10xgenomics.com for further assistance.

g. **Endpoint Reached Early:** If this message is received, contact support@10xgenomics.com for further assistance.