User Guide | CG000427 | Rev C

Chromium Next GEM Single Cell HT Training Reagent Kits

For use with:

Chromium Next GEM HT Training Reagents, Gel Beads and Chip Kits
32 rxns PN-1000386

Notices

Document Number

CG000427 | Rev C

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
CG000427 | Rev C

Title
Chromium Next GEM Single Cell HT Training Reagent Kits

Revision
Rev B to Rev C

Revision Date
January 06, 2022

Specific Changes
Added additional recommended pipette tips on page 10.

General Changes
Updated for general minor consistency of language and terms throughout.

Table of Contents

Introduction

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 HT Training Chip with the Reaction Mix, Gel Beads, and Partitioning Oil
- Loading a Chromium Next GEM HT Training Chip into the Chromium X and run the Chromium X
- Inspecting the resulting Gel Beads-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 X, refer to the Chromium X Series Specifications (CG000415).
- 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 Single Cell HT Training Reagent Kits

Refer to SDS for handling and disposal information

Chromium Next GEM HT Training Reagents, Gel Beads and Chip Kits, 32 rxns PN-1000386

Chromium Next GEM
HT Training Reagents and Gel Bead Kit
32 rxns, PN-1000384
Store at 4°C

	#	PN
Chromium Next GEM HT Training Gel Beads	2	2000466
Training Master Mix	4	220086
Surrogate Fluid	2	220021
Training Sample	2	220087

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PN-220086, PN-220021, and PN-220087 may be stored at 4°C along with other reagents in the kit, even though tubes indicate storage at −20°C.

Chromium Next GEM Training Chip Kit, 32 rxns PN-1000385

Chromium
Partitioning Oil
Store at ambient temperature

	#	PN
Partitioning Oil	2	220088

Chromium
Recovery Agent
Store at ambient temperature

	#	PN
Recovery Agent	2	2000434

Chromium Next GEM
Training Chips & Gaskets
Store at ambient temperature

	#	PN
Chromium Next GEM HT Training Chip	2	2000467
Chip Gaskets, HT, 2-pack	1	3000656

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10x Genomics Accessories

Product	Part Number	Part Number (Item)
10x Vortex Adapter	120251	330002
10x Magnetic Separator HT	1000394	2000431
Chromium X Chip Holder	1000393	3000598

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for 10x Genomics 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		
Choose either Eppendorf, USA Scientific, or Thermo Fisher Scientific PCR 8-tube strips		
Eppendorf	PCR Tubes 0.2 ml 8-tube strips	951010022
	DNA LoBind Tubes, 1.5 ml	022431021
	DNA LoBind Tubes, 2.0 ml	022431048
USA Scientific	TempAssure PCR 8-tube strip *(alternate to Eppendorf or Thermo Fisher Scientific product)*	1402-4700
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml *(alternate to Eppendorf or USA Scientific product)*	N8010580
	MicroAmp 8 -Cap Strip, clear	N8010535
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water Low TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	AM9937 12090-015
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
Ricca Chemical Company	Glycerin (glycerol), 50% (v/v) Aqueous Solution	3290-32
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
Eppendorf	Eppendorf ThermoMixer C (when using 8 rxn reagent kits)	5382000023
	Eppendorf SmartBlock 1.5 ml, Thermoblock for 24 reaction vessel *(alternatively, use a temperature-controlled Heat Block)*	5360000038

Recommended Pipette Tips

10x Genomics recommends using only validated emulsion-safe pipette tips for all Single Cell protocols. Rainin pipette tips have been extensively validated by 10x Genomics and are highly recommended for all single cell assays. If Rainin tips are unavailable, any of the listed alternate pipette tips validated by 10x Genomics may be used.

Supplier	Description	Part Number (US)
Recommended Pipettes & Pipette tips		
Rainin	**Pipettes** Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805
	Pipet-Lite Multi Pipette L8-10XLS+	17013802
	Pipet-Lite Multi Pipette L8-20XLS+	17013803
	Pipet-Lite LTS Pipette L-2XLS+	17014393
	Pipet-Lite LTS Pipette L-10XLS+	17014388
	Pipet-Lite LTS Pipette L-20XLS+	17014392
	Pipet-Lite LTS Pipette L-100XLS+	17014384
	Pipet-Lite LTS Pipette L-200XLS+	17014391
	Pipet-Lite LTS Pipette L-1000XLS+	17014382
	Pipette Tips Tips LTS 200UL Filter RT-L200FLR	30389240
	Tips LTS 1ML Filter RT-L1000FLR	30389213
	Tips LTS 20UL Filter RT-L10FLR	30389226
Alternate Recommendations *(If Rainin pipette tips are unavailable, any of the listed pipette tips may be used)*		
Eppendorf	**Pipettes** Eppendorf Research Plus, 8-channel, epT.I.P.S. Box, 0.5 – 10 µl	3125000010
	Eppendorf Research Plus, 8-channel, epT.I.P.S. Box, 10 – 100 µl	3125000036
	Eppendorf Research Plus, 8-channel, epT.I.P.S. Box, 100 – 300 µl	3125000052
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 0.1 – 2.5 µl	3123000012
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 0.5 – 10 µl	3123000020
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 2 – 20 µl	3123000039
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 2 – 200 µl	3123000055

Supplier	Description	Part Number (US)
	Eppendorf Research Plus, 1-channel, epT.I.P.S.® Box, 100 – 1000 µl	3123000063
	Pipette Tips (compatible with Eppendorf pipettes only) ep Dualfilter T.I.P.S., 2-20 µl	0030078535
	ep Dualfilter T.I.P.S., 2-200 µl	0030078551
	ep Dualfilter T.I.P.S., 2-1,000 µl	0030078578
Labcon*	ZAP SLIK 20 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1143-965-008
	ZAP SLIK 200 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1144-965-008
	ZAP SLIK 1200 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1145-965-008
Biotix*	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 0.1-20 µl	63300931
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 200 µl	63300001
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 1200 µl	63300004

*Compatible with Rainin pipettes

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Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Next GEM High Throughput (HT) specific protocol step updates

Emulsion-safe Plastics

Use 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.
- Keep all enzymes and Master Mixes on ice during setup and use. Promptly move reagents back to the recommended storage.
- Calculate reagent volumes with 10% excess of 1 reaction values.
- Cover Partitioning Oil tubes and reservoirs to minimize evaporation.
- If using multiple chips, use separate reagent reservoirs for each chip during loading.
- Thoroughly mix samples with the beads during bead-based cleanup steps.

Surrogate Fluid

- Surrogate Fluid is glycerol in a ~50% volume/volume aqueous solution.
- Surrogate Fluid is provided in the Chromium Next GEM HT Training Kit, but is not provided with 10x Genomics Single Cell assay kits. 50% glycerol must be purchased or prepared when running 10x Genomics Single Cell assays.
- 50% glycerol solution can be purchased: Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32

OR

- Prepare a 50% glycerol solution:
  i. Mix an equal volume of water at 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

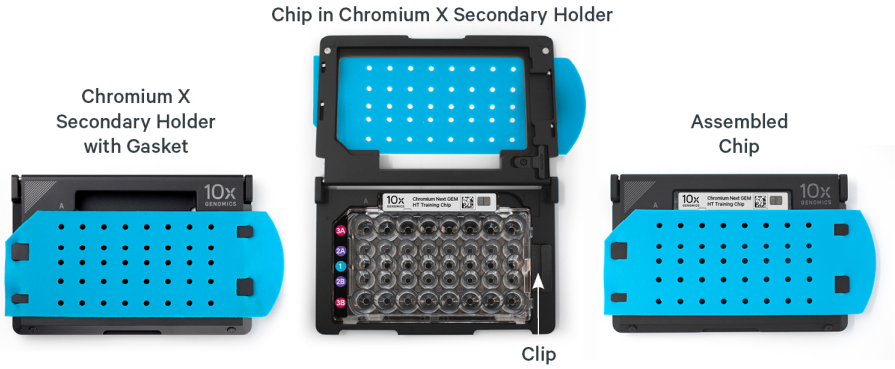
- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- After removing the chip from the sealed bag, use in **≤24 h**.
- Execute steps without pause or delay, unless indicated. When using multiple chips, load, run, and collect the content from one chip before loading the next.
- Only even number of reactions can be run on the chip. Refer to 1.1 Load Chromium Next GEM Chip HT Training Chip on page 26 for specific instructions.
- Fill all unused paired input wells on a chip with an appropriate volume of 50% glycerol solution before loading the used wells.
- 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 X.
- 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 X Chip Holders

- Chromium X Chip Holders encase Chromium Next GEM Chips used for the HT (high throughput) assay.
- The holder lid flips over to become a stand, holding the chip at 45 degrees for optimal recovery well content removal.
- Squeeze the slider 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

- 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 guide on the holder is inserted into the chip. 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.



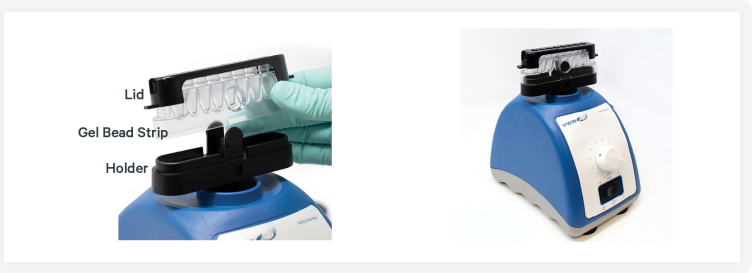
Chromium Next GEM Chip Loading

- 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.

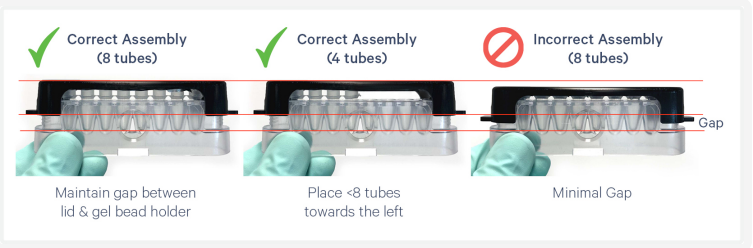
- Refer to 1.1 Load Chromium Next GEM Chip HT Training Chip on page 26 for specific instructions.

Gel Bead Handling

- Use one tube of Gel Beads per sample **pair**. DO NOT puncture the foil seals of tubes not used at the time.
- After removing the Gel Bead strip from the packaging, equilibrate the Gel Bead strip to **room temperature** for at least **30 min** before use.
- Store unused Gel Beads at **−80°C** and avoid more than 12 freeze-thaw cycles. DO NOT store Gel Beads at **−20°C**.
- Snap the tube strip holder with the Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**.



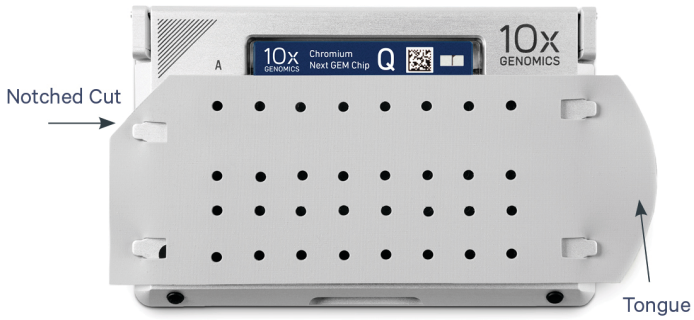
- Centrifuge the Gel Bead strip for **~5 sec** after removing from the holder. Confirm there are no bubbles at the bottom of tubes and the liquid levels look even. Place Gel Bead strip back in the holder and secure the holder lid.
- Ensure that the gel bead strip is positioned with one tube in the left-most position (do not center the strip if using fewer than 8 tubes). Gently depress the lid until light resistance is met. DO NOT attempt to further depress the lid, even if it may be angled with respect to the strip holder.



- 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.

10x Gasket Attachment



- **Before reagents are loaded,** 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.
- Keep the assembled unit with the gasket attached until ready for dispensing reagents into the wells.
- After loading reagents, DO NOT press down on the top of the gasket. Keep the assembly horizontal to avoid wetting the gasket with Partitioning Oil.

10x Magnetic Separator HT

- Offers two positions of the magnets (high and low) relative to a tube, depending on its orientation. Flip the magnetic separator over to switch between high (magnet•**High**) or low (magnet•**Low**) positions.
- The 10x Magnetic Separator HT can accommodate four 8-Tube Strips
- If using MicroAmp 8-Tube Strips, use the high position (magnet•**High**) only throughout the protocol.

10x Magnetic Separator HT

High

Low

Step 1:

Training Step 1

1

1.0 Get Started

Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
☐	**Chromium Next GEM HT Training Gel Beads**	2000466	Equilibrate to room temperature 30 min before loading the 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**	220088	—	Ambient
☐	**Surrogate Fluid**	220021	—	4°C
☐	**Chromium Next GEM HT Training Chip(s)**	2000467	See Tips & Best Practices.	Ambient
☐	**10x Gasket, HT**	3000656	See Tips & Best Practices.	Ambient
☐	**Chromium X Chip Holder**	3000598	See Tips & Best Practices.	Ambient

Assemble Chromium Next GEM HT Training Chip

TIPS See Tips & Best Practices on page 12 for chip handling instructions.

a. 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.

b. DO NOT touch the smooth side of the gasket.

c. Open the chip holder.

d. Remove the chip from the sealed bag. Use the chip within ≤ **24 h**.

e. Align notch on the chip (upper left corner) and the open holder with the gasket attached.

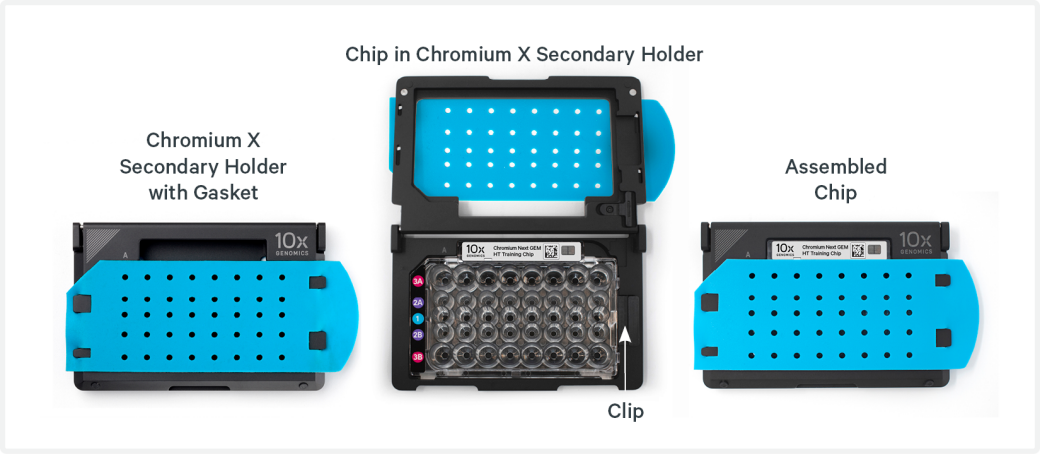
f. Slide the chip to the left until the guide on the holder is inserted into the chip. Depress the right hand side of the chip until the spring-loaded clip engages.

g. Keep the assembled unit with the attached gasket open until ready for and while dispensing reagents into the wells.

h. DO NOT touch the smooth side of the gasket.

i. 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 2A & 2B and 3A & 3B.



Sample Loading Guidelines

Read these guidelines before loading Chromium Next GEM HT Chips

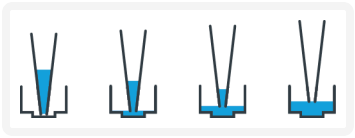
- Up to 16 independent samples can be run on the chip.
- Only even number of reactions can be run on the chip.
- Corresponding wells in rows 2A & 2B of the chip should both be either loaded with independent samples/mock-sample or with glycerol.
- **For even number of samples**:
  Load independent samples (Training Master Mix + Training Sample) in pairs in rows 2A & 2B.
  See Example 1 below.
- **For odd number of samples** (applicable when running an odd number of samples on Chip M and Chip N):
  Load the unpaired sample in a well in row 2A and a mock-sample (Master Mix + Water) in the corresponding well in row 2B. Additionally, add Partitioning Oil to the corresponding well in row 3B.
  See Example 2 below. Example 2 features Chip M - an odd number of samples will not be loaded to the HT Training Chip.
- Follow the step-by-step chip loading instructions provided in step 1.2.

Sample loading configuration examples

1.1 HT Load Chromium Next GEM Chip HT Training Chip

TIPS

- 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 loading less than 16 samples/chip)

- **130 µl** in each unused well in row labeled 1
- **140 µl** in each unused well in rows labeled 2A & 2B
- **140 µl** in each unused well in rows labeled 3A & 3B

DO NOT use any substitute for Surrogate Fluid.
For odd number of samples, if a sample is loaded in a well in row 2A, load a mock-sample (Training Master Mix + Water) and NOT Surrogate Fluid in the corresponding well in row 2B. Additionally, add Partitioning Oil to the corresponding well in row 3B.

b. Prepare Gel Beads

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

c. Prepare Training Master Mix + Training Sample

- Vortex the Training Master Mix **15 sec**, centrifuge briefly and place on ice.
- Add **146 µl** Training Master Mix to each well of the 8-tube strip on ice.
- Slowly add **4 µl** Training Sample into each well of the tube strip containing Training Master Mix.

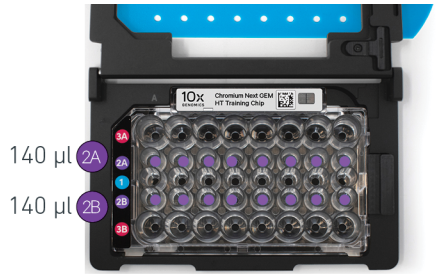
d. Load Row Labeled 1

- Puncture the foil seal of the Gel Bead tubes. Slowly aspirate **130 µl** Gel Beads.
- Dispense into the wells in **row labeled 1** without introducing bubbles.
- Wait **30 sec**.

e. Load Rows Labeled 2A, 2B

ONLY even number of reactions should be run on the chip. See Sample Loading Guidelines for more information and examples.

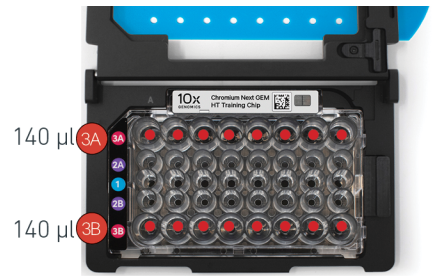
- Up to 16 independent samples can be run on the chip. Sample inputs for 2A should be equal to 2B (e.g. if processing only 8 samples, run 4 in 2A and 4 in 2B)
- **First, process up to 8 samples:** Gently pipette mix the Training Master Mix + Training Sample (prepared at step 1.2c) using a multichannel pipette. Using the same pipette tips, dispense **140 µl** Training Master Mix + Training Sample into the bottom center of wells in **row labeled 2A**.
- **Next, process up to 8 additional samples:** Gently pipette mix the Training Master Mix + Training Sample (prepared at step 1.2c) using a multichannel pipette. Using the same pipette tips, dispense **140 µl** Training Master Mix + Training Sample into the bottom center of wells in **row labeled 2B**.
- Wait **30 sec**.



f. Load Rows Labeled 3A, 3B

- Dispense **140 µl** Partitioning Oil into the wells in **rows labeled 3A & 3B** from a reagent reservoir.

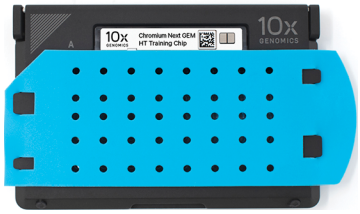
Failure to add Partitioning Oil to the rows labeled 3A and 3B will prevent GEM generation and can damage Chromium X.



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 Chromium X **immediately** after loading the Partitioning Oil. ONLY even number of reactions should be run on the chip. See Sample Loading Guidelines on page 25 for more information & examples.*



Step 2:

Training Step 2

2

2.0 Run Chromium X

Consult the Chromium X Series (X/iX) User Guide (CG000396) for detailed instrument operation instructions and follow the Chromium X touchscreen prompts for execution.

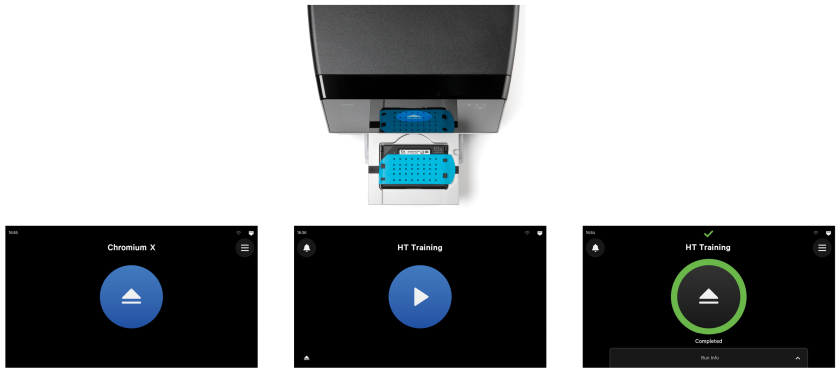
a. Press the eject button on the Chromium X to eject the tray. If the eject button is not touched within **1 min**, tray will close automatically. System requires a few seconds before the tray can be ejected again.

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. Press the play button.

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

Chromium X



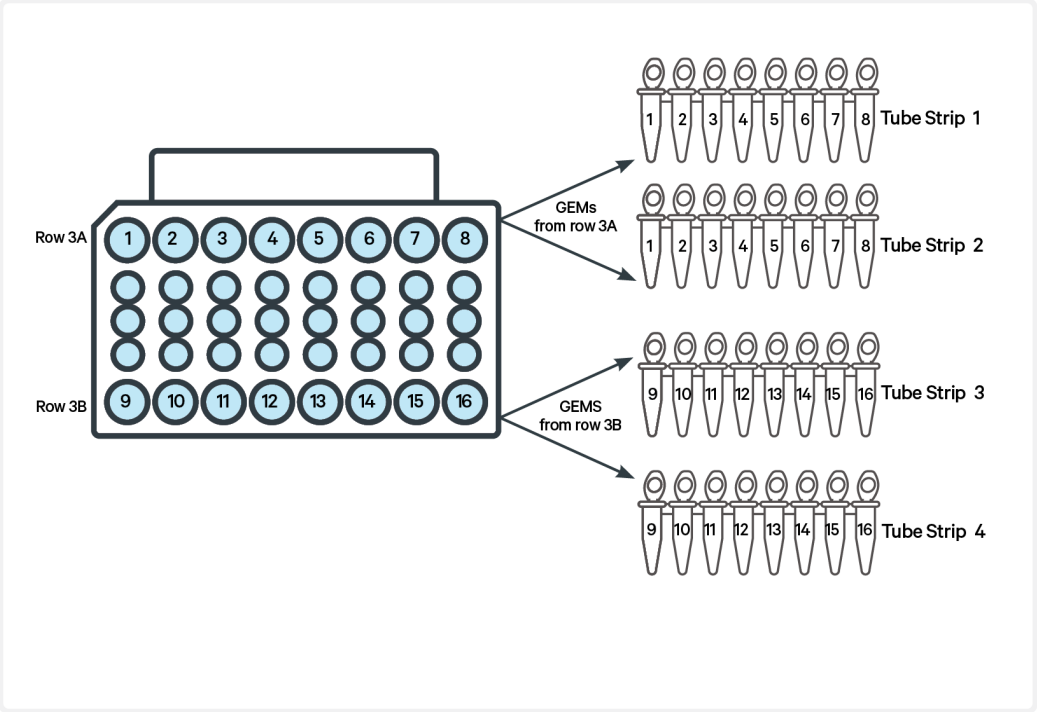
Step 3:

Training Step 3

3

GEM Transfer Overview

For a sample loaded in a well in either row 2A or 2B of the chip, GEMs are retrieved from the corresponding well in row 3A and 3B and transferred to two tubes. The example below shows transfer of GEMs generated from 16 samples. GEMs from the chip are transferred to four tube strips, where the GEMs generated from each sample are transferred to 2 corresponding tubes in the indicated tube strips.



3.0 HT Transfer GEMs

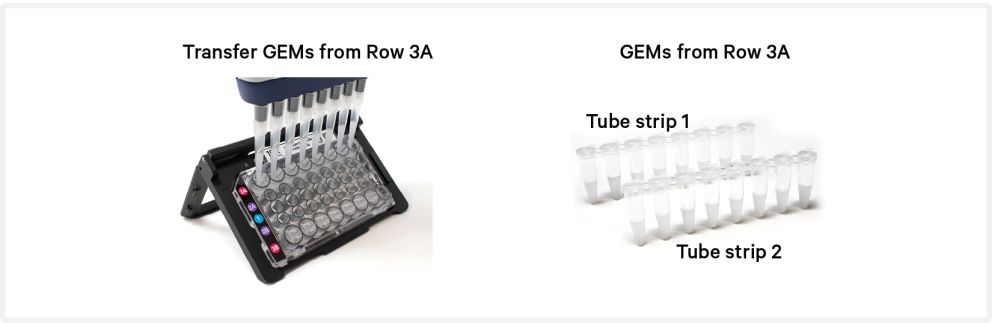
a. Label four tube strips and place on ice.

b. Press the eject button of Chromium X and remove the chip.

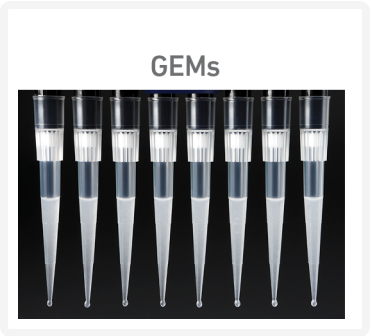
c. Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees.

d. Check the volume in rows labeled 1, 2A, and 2B. Abnormally high volume relative to other wells indicates a clog. Significant volume of non-sample fluid is expected in rows 2A and 2B after a successful run and does not indicate a sample clog.

e. Retrieve GEMs from row labeled 3A: Slowly aspirate **70 µl** GEMs from the lowest points of the recovery wells in the top row labeled 3A without creating a seal between the tips and the bottom of the wells.



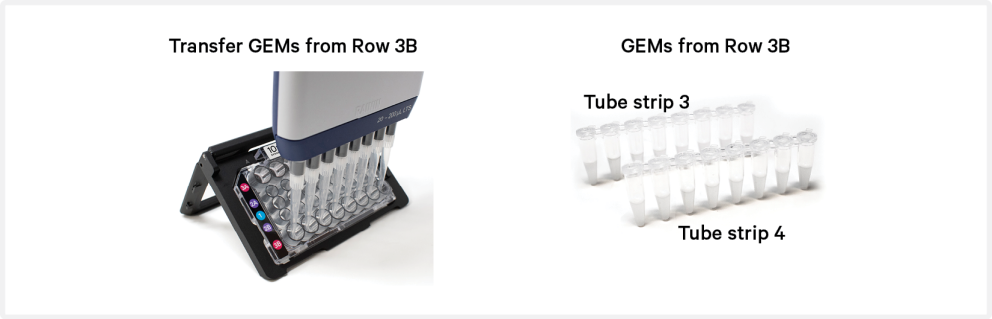
f. Withdraw pipette tips from the wells. GEMs should appear opaque and uniform across all channels. Excess Partitioning Oil (clear) in the pipette tips indicates a potential clog.



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

h. Using the same pipette tips, slowly aspirate remaining **70 µl** GEMs from the wells in the top row labeled 3A and dispense in second tube strip as described above.

i. Retrieve from row labeled 3B: Slowly aspirate **70 µl** GEMs from the lowest points of the recovery wells in the bottom row labeled 3B without creating a seal between the tips and the bottom of the wells.



j. Repeat steps f and g, dispensing GEMs into third tube strip on ice with the pipette tips against the sidewalls of the tubes.

k. Using the same pipette tips, slowly aspirate remaining **70 µl** GEMs from the wells in the bottom row labeled 3B and dispense in fourth tube strip as described above.

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

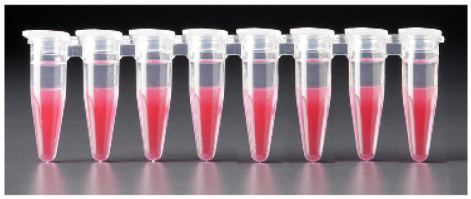
Step 4:

Training Step 4

4

4.0 Process Collected GEMs

Biphasic Mixture



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

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

If biphasic separation is incomplete:

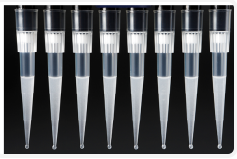
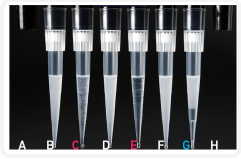
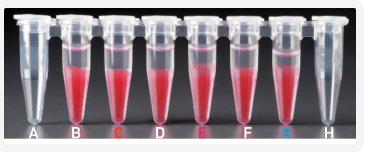
- Firmly secure the cap on the tube strip, ensuring that no liquid is trapped between the cap and the tube rim.
- Mix by inverting the capped tube strip 5x, centrifuge briefly, and proceed to step b. DO NOT invert without firmly securing the caps.

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

GEMs

Step	Normal	Reagent Clogs & Wetting Failures
3.0d **After HT Training Chip is removed from Chromium X and the wells are exposed**	 All 16 recovery wells (rows 3A, 3B) are similar in volume and opacity.	 Recovery well G indicates a reagent clog. Recovery well C and E indicate a wetting failure. Wells A, H, I & P contain Surrogate Fluid.
3.0f **Transfer GEMs**	 All liquid levels are similar in volume and opacity without air trapped in the pipette tips.	 Pipette tips C and E indicate a wetting failure. Pipette tip C contains partially emulsified GEMs. Emulsion is absent in pipette tip E. Pipette tip G indicates a reagent clog.
4.0a **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).

If a channel clogs or wetting failure occurs during GEM generation, it is recommended that the sample be remade. If any of the listed issues occur, take a picture and send it to support@10xgenomics.com for further assistance.

Chromium X Series Errors

The Chromium X touchscreen will guide the user through recoverable errors. If the error continues, or if the instrument has seen critical or intermediate errors, email support@10xgenomics.com with the displayed error code. Support will request a troubleshooting package. Upload pertinent logs to 10x Genomics by navigating to the Logs menu option on screen.

There are two types of errors:

Critical Errors – When the instrument has seen a critical error, the run will immediately abort. Do not proceed with any further runs. Contact support@10xgenomics.com with the error code.

a. System Error

b. Pressure Error

c. Chip Error

d. Run Error

e. Temperature Error

f. Software Error

User Recoverable Errors – Follow error handling instructions through the touchscreen and continue the run.

a. Gasket Error

b. Tray Error

c. Chip Error

d. Unsupported Chip Error

e. Network Error

f. Update Error

Consult the Chromium X Series (X/iX) User Guide (CG000396) for additional information and follow the Chromium X touchscreen prompts for execution. The Chromium X touchscreen will guide the user through recoverable errors.