



User Guide | CG000766 | Rev C

GEM-X

Training Reagent Kits

For use with:

GEM-X Training Reagents, Gel Beads and Chip Kit

16 rxns PN-1000745

Chromium GEM-X Single Cell 3' Chip Kit

4 chips PN-1000690

GEM-X OCM Training Reagents, Gel Beads and Chip Kit

32 rxns PN-1000773

GEM-X OCM 3' Chip Kit v4 4-plex

2 chips PN-1000747

Take 1 minute to evaluate this protocol. Scan this code or [click here](#).



Notices

Document Number

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Document Revision Summary

Document Number

CG000766 | Rev C

Title

GEM-X Training Reagent Kits

Revision

Rev B to Rev C

Revision Date

November 14, 2024

Description of Changes

Updated to include GEM-X OCM Training guidance and information throughout:

- [Training Objective on page 6](#)
- [GEM-X Training Reagent Kits on page 7](#)
- [Tips & Best Practices](#)
 - [50% Glycerol Solution for Addition to Unused Chip Wells on page 11](#)
 - [GEM-X OCM Chip Handling on page 12](#)
- [GEM-X OCM Training](#)
 - [Get Started on page 28](#)
 - [Step 1: Assemble GEM-X OCM Chip on page 29](#)
 - [Multiplexing Guidelines on page 31](#)
 - [Step 2: Load GEM-X OCM Chip on page 33](#)
 - [Step 3: Run the Chromium X Series Instrument on page 35](#)
 - [Step 4: Transfer GEMs on page 36](#)
 - [Step 5: Process Collected GEMs on page 38](#)
- [Troubleshooting](#)
 - [GEM Generation & Barcoding - GEM-X OCM on page 43](#)
- Updated for general minor consistency of language and terms throughout

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Introduction

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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 GEM-X and GEM-X OCM Chip with the Reaction Mix, Gel Beads, and Partitioning Oil
- Loading a GEM-X and GEM-X OCM Chip on a compatible Chromium X Series instrument and running it. For guidance on compatible instruments, consult the Chromium X Series Instrument User Guide with Readiness Test (CG000396).
- Inspecting the resulting Gel Beads-in-emulsion (GEMs)
- Transferring the GEMs in preparation for thermal cycling
- Processing GEMs immediately after collection

Note that this document provides training guidance for both GEM-X and GEM-X OCM workflows.

GEM-X workflow uses the GEM-X chip where up to 8 samples can be loaded and 8 emulsions generated (see [GEM-X Training on page 18](#)). GEM-X OCM workflow uses the OCM chip, where up to 4 samples can be multiplexed per emulsion and up to 2 emulsions generated from 8 samples (see [GEM-X OCM Training on page 27](#)).

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

- For guidance on qualifying the Chromium X Series instrument, 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.

GEM-X Training Reagent Kits

Refer to SDS for handling and disposal information

GEM-X Training Reagents, Gel Beads and Chip Kits, 32 rxns PN-1000745


GEM-X
Training Reagents and Gel Bead Kit
 16 rxns, PN-1000744
Shipped at ambient temperature
Store at 4°C

	#	PN
GEM-X Training Gel Beads	4	2001250
Training Master Mix	2	220086




Chromium GEM-X Single Cell 3' Chip Kit v4, 4 chips PN-1000690

Chromium
Partitioning Oil B
Shipped at ambient temperature
Store at ambient temperature


	#	PN
 Partitioning Oil B	4	2001213

Chromium
Recovery Agent
Shipped at ambient temperature
Store at ambient temperature

	#	PN
 Recovery Agent	4	220016

Chromium GEM-X
3' Chip & Gaskets
Shipped at ambient temperature
Store at ambient temperature

	#	PN
GEM-X 3' Chip	4	2001097
X/iX Chip Gasket, 2-pack	2	3000656




GEM-X OCM Chip Training Reagent Kits


Refer to SDS for handling and disposal information

GEM-X OCM Training Reagents, Gel Beads and Chip Kits, 32 rxns PN-1000773

GEM-X Training Reagents and Gel Bead Kit 16 rxns, PN-1000744 <i>Shipped at ambient temperature</i> <i>Store at 4°C</i>		
	#	PN
GEM-X Training Gel Beads	4	2001250
Training Master Mix	2	220086
10x GENOMICS		

GEM-X OCM 3' Chip Kit v4 4-plex, 2 chips PN-1000747

Chromium Partitioning Oil B <i>Shipped at ambient temperature</i> <i>Store at ambient temperature</i>		
	#	PN
 Partitioning Oil B	4	2001213

Chromium Recovery Agent <i>Shipped at ambient temperature</i> <i>Store at ambient temperature</i>		
	#	PN
 Recovery Agent	4	220016

GEM-X 3' OCM Chip & Gaskets <i>Shipped at ambient temperature</i> <i>Store at ambient temperature</i>		
	#	PN
GEM-X 3' OCM Chip	2	2001099
X/iX Chip Gasket, 2-pack	1	3000656
10x GENOMICS		

Note that reagents are provided in excess of the OCM chips provided.

10x Genomics Accessories

Product	Part Number (Kit)	Part Number (Item)
10x Vortex Adapter	120251	330002
10x Magnetic Separator B*	1000709 (Chromium X/iX Series Accessory Kit)/	2001212
Chromium X/iX Chip Holder (also referred to as Chromium X Series Chip Holder)	1000821 (Chromium X Series Accessory Kit)/ 1000707 (GEM-X Transition Kit)	3000598

**10x Magnetic Separator (PN-230003) is untested but interchangeable with the 10x Magnetic Separator B (PN-2001212).*

Third-Party Items

Successful execution of this workflow requires third-party reagents, kits, and equipment in addition to those provided by 10x Genomics. All third-party reagents and consumables should be obtained prior to starting this workflow.

Refer to the relevant Protocol Planner for a detailed list of the following third-party items:

- Additional reagents, kits, and equipment
- Recommended pipette tips
- Recommended thermal cyclers



10x Genomics has tested all items listed in the Protocol Planner. These items perform optimally with the assay. Substituting materials may adversely affect assay performance.



Tips & Best Practices



Icons



Tips & Best Practices section for additional guidance



Signifies critical step requiring accurate execution



Troubleshooting section for additional guidance



Version specific protocol updates



GEM-X OCM specific protocol updates



GEM-X specific protocol updates

Emulsion-safe Plastics

- Use validated emulsion-safe plastic consumables when handling GEMs as some plastics can destabilize GEMs.
- Consult relevant Protocol Planner for a detailed list of plastics and other consumables.

General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Keep all enzymes and reagent 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.
- Limit Partitioning Oil exposure to air 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.

50% Glycerol Solution for Addition to Unused Chip Wells

- Purchase 50% glycerol solution from Ricca Chemical Company, Glycerin (glycerol), 50% (v/v) Aqueous Solution, PN-3290-32.

OR

- Prepare 50% glycerol solution:
 - Mix an equal volume of water and $\geq 99\%$ glycerol, Molecular Biology Grade.
 - Filter through a 0.2 μm filter.

- Store at **-20°C** in 1-ml LoBind tubes. 50% glycerol solution should be equilibrated to room temperature before use.
- **GEM-X workflow:** 50% glycerol solution should be loaded to all unused wells in row 1, 2, and 3.
- **GEM-X OCM workflow:** If loading only one set (each set = 4 samples), 50% glycerol solution should be added to each unused well in row 1, 2, and 3 of the second set.

Pipette Calibration

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

GEM-X 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.
- Fill all unused input wells in rows labeled 1, 2, and 3 on a chip with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the wells in the top 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 wetting failures.
- Minimize the distance that a loaded chip is moved to reach the compatible Chromium X series instrument.
- 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.

GEM-X OCM Chip Handling

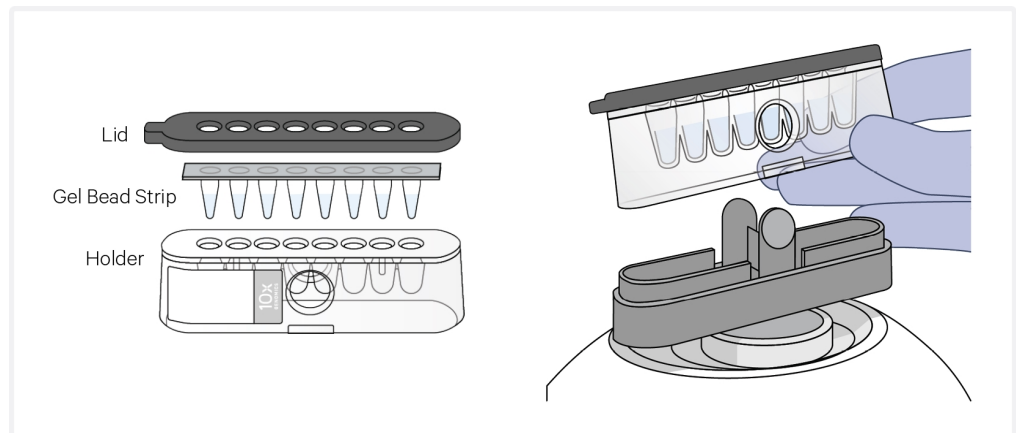
- Minimize exposure of reagents, chips, and gaskets to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that

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.
- When running 4 samples (loading only one multiplexing set), fill the unused wells of the other set in rows labeled 1, 2, and 3 with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the wells in the top 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 wetting failures.
- Minimize the distance that a loaded chip is moved to reach the compatible Chromium X series instrument.
- 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. DO NOT pick up the assembled chip holder by the holder lid as this could accidentally lead to spillage or dropping of the chip holder.

Gel Bead Handling

- Use one tube of Gel Beads per sample. 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. Training Gel Bead storage is different from Gel Beads used in a real experiment.
- 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.
- 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.



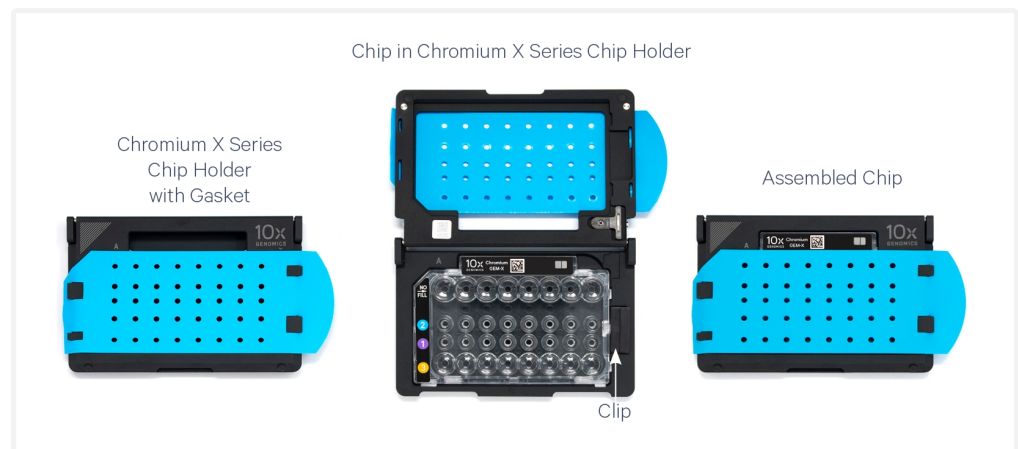
GEM-X Chip & Holder Assembly with Gasket

- 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 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.

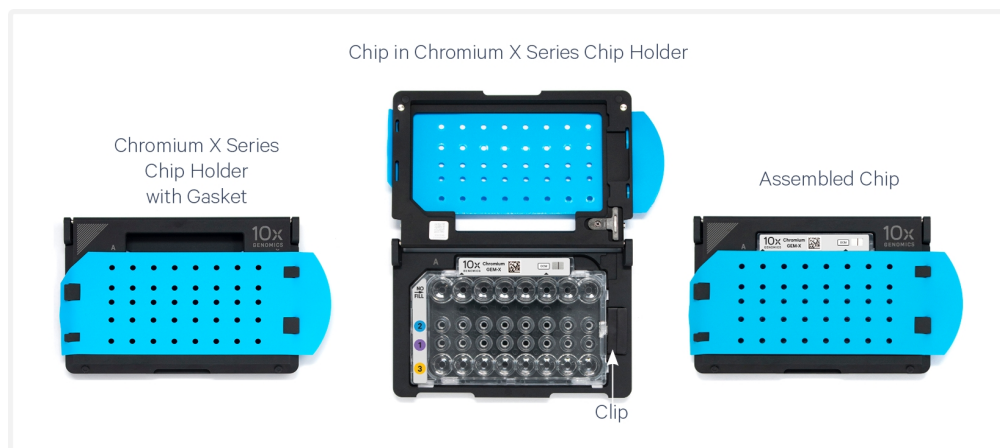
One of the rows of the gasket will not align with the GEM-X chip wells. This is expected and will not impact the assay.

Generic images representative of the GEM-X and GEM-X OCM Chip are shown below. The Chromium X/iX Chip Holder is also referred to as Chromium X Series Chip holder.

GEM-X Chip



GEM-X OCM Chip



GEM-X and GEM-X OCM 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.
- After an instrument run, to troubleshoot any potential clogs and failures, it is recommended to photograph the GEMs in the chip recovery wells and the pipette tips during GEM retrieval (or the tubes after GEM transfer).

Chromium X Series Instrument Firmware

- **GEM-X 3' chips:** To run the GEM-X 3' chips in the Chromium X/iX instrument, firmware version 2.0.0 or higher is required; however the most updated version is recommended for best performance.

To run the GEM-X 3' chips in the Chromium Xo instrument, use the most updated firmware version.

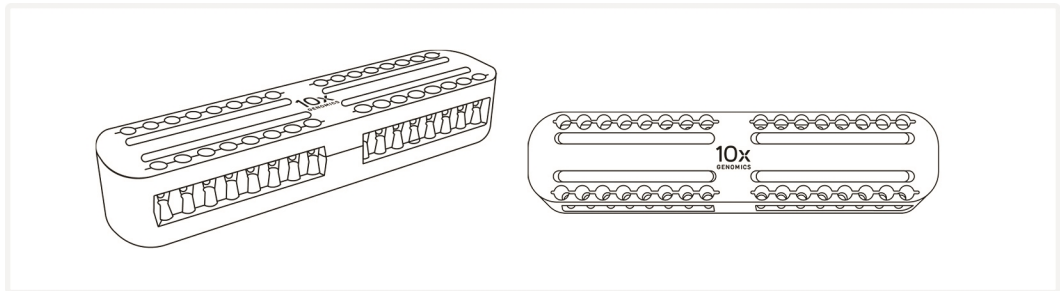
- **GEM-X 3' OCM chips:** To run the GEM-X 3' OCM chips in the Chromium X instrument, firmware version 2.0.0 or higher is required; however the most updated version is recommended for best performance.

To run the GEM-X 3' OCM chips in the Chromium Xo instrument, use the most updated firmware version.

- The current firmware version of the instrument will be displayed on the instrument homescreen.
- Consult the Chromium X Series Instrument User Guide with Readiness Test (CG000396) for detailed instructions on updating the firmware.

10x Magnetic Separator B

- Images below are illustrative - actual appearance of magnetic separator may vary. Guidance applies to all 10x Magnetic Separators.
- 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.
- If using MicroAmp 8-Tube Strips, use only the high position (magnet•**High**) throughout the protocol.



GEM-X Training

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Get Started

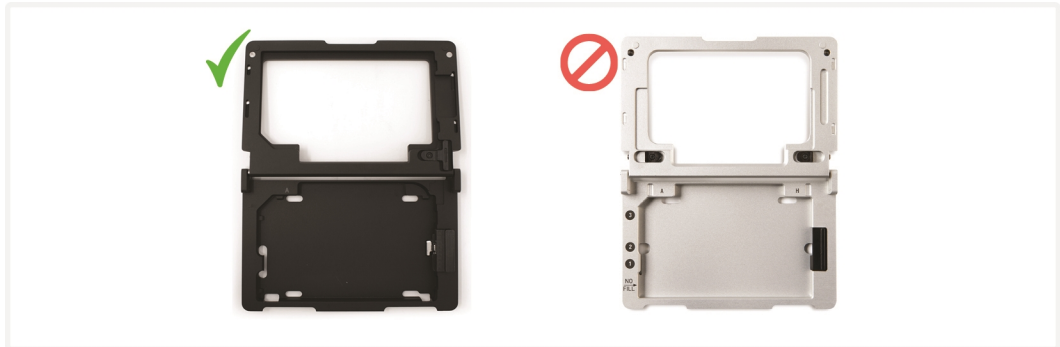
Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
<input type="checkbox"/>	GEM-X Training Gel Beads	2001250	Equilibrate to room temperature 30 min before loading the chip.	4°C
Place on Ice				
<input type="checkbox"/>	Training Master Mix	220086	One tube is sufficient for 16 samples.	4°C
Obtain				
<input type="checkbox"/>	1X PBS <i>Used as sample</i>	-	-	Ambient
<input type="checkbox"/>	Partitioning Oil B	2001213	—	Ambient
<input type="checkbox"/>	50% glycerol solution	220021	—	4°C
<input type="checkbox"/>	GEM-X 3' Chip*	1000690	See Tips & Best Practices.	Ambient
<input type="checkbox"/>	X/iX Chip Gasket	3000656	See Tips & Best Practices.	Ambient
<input type="checkbox"/>	Chromium X/iX or Chromium X Series Chip Holder	3000598	See Tips & Best Practices.	Ambient

*Note that although the training reagents have only been tested with the GEM-X 3' Chip, the run performance is applicable to other GEM-X assays as well.



Step 1: Assemble GEM-X Chip

The GEM-X chip is only compatible with Chromium X/iX Chip Holder (also referred to as Chromium X Series Chip Holder; PN-3000598). DO NOT use any other holder.



TIPS

See [Tips & Best Practices on page 10](#) 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.



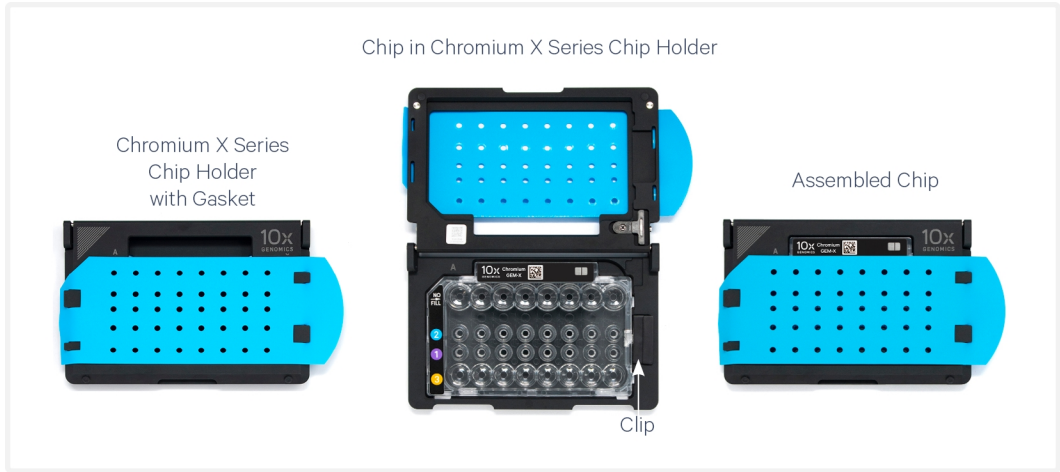
- Remove the chip from the sealed bag. Use the chip within ≤ 24 h.
- Align notch on the chip (upper left corner) and the open holder with the gasket attached.
- Slide the chip to the left until the guide on the holder is inserted into the chip. Depress the right hand side of the chip until the spring-loaded clip engages.

A generic image representative of the GEM-X 3' Chip is shown on the next page.

- Keep the assembled unit with the attached gasket open until ready for and while dispensing reagents into the wells.
- 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 top row labeled NO FILL. See step 1.2 for details.





Step 2: Load GEM-X 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.
- Ensure that the Gel Beads are properly thawed and ready to use.
- 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.

Color Legend

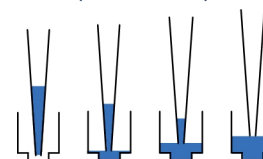
- 2: Gel beads
- 1: Sample
- 3: Oil

The Chromium X/iX (X Series) Chip Holder, X/iX Chip Gasket, and GEM-X chip images shown below are representative and do not show the specific color & label. Chip holder and gasket should be black and blue in color, respectively. Refer to [Assemble GEM-X Chip on page 1](#) for details.

GEM-X 3' Chip, gasket attached
Representative chip image



Pipette technique



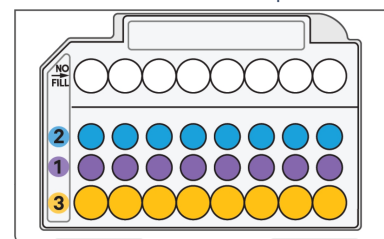
a. If loading less than 8 samples/chip, add 50% glycerol solution to each unused well in row 1, 2, and 3

- **60 µl** in each unused well in row labeled **1**
- **60 µl** in each unused well in row labeled **2**
- **250 µl** in each unused well in row labeled **3**

DO NOT add 50% glycerol solution to the wells in top row labeled **NO FILL**.

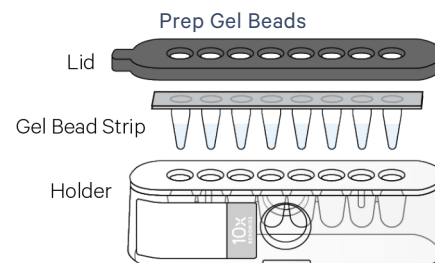
DO NOT use any substitute for 50% glycerol solution.

Glycerol
in GEM-X 3' Chip



b. 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 & the liquid levels are even.
- Place the Gel Bead strip back in the holder. Secure the holder lid.



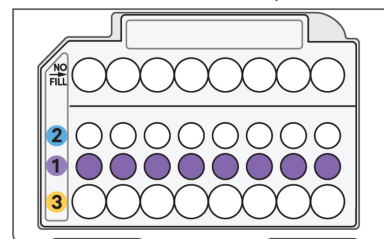
c. Prepare Training Master Mix + 1X PBS (Sample)

- Vortex the Training Master Mix 15 sec, centrifuge briefly and place on ice.
- Add **63 μ l** Training Master Mix to each well of the 8-tube strip on ice.
- Add **2 μ l** 1X PBS into the Master Mix. *Total of 65 μ l in each tube.*

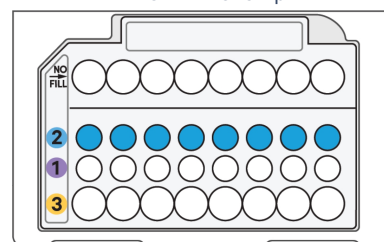
Prep Training Mater Mix & 1X PBS

**d. Load Row Labeled 1**

- Gently pipette mix the Master Mix + 1X PBS.
- Using a multichannel pipette (P100/200), dispense **60 μ l** Master Mix + 1X PBS into the bottom center of each well in **row labeled 1** without introducing bubbles.
- Wait **30 sec.**

60 μ l Master Mix + 1X PBS
in GEM-X 3' Chip**e. Load Row Labeled 2**

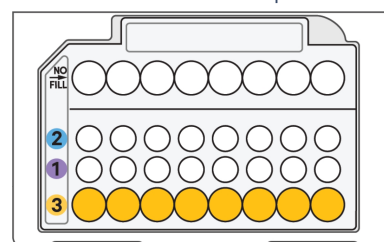
- Puncture the foil seal of the Gel Bead tubes.
- Using a multichannel pipette (P100/200), slowly aspirate **60 μ l** Gel Beads.
- Dispense into the bottom center of each well in **row labeled 2** without introducing bubbles.
- Wait **30 sec.**

60 μ l Gel Beads
in GEM-X 3' Chip**f. Load Row Labeled 3**

- Using a multichannel pipette (p100/200), dispense **250 μ l** Partitioning Oil B into the wells in **row labeled 3** by pipetting two aliquots of **125 μ l** from a reagent reservoir.

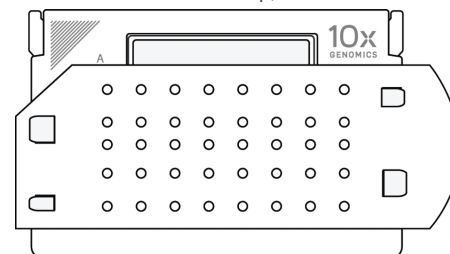


Failure to add Partitioning Oil B to the row labeled 3 will prevent GEM generation and can damage the instrument.

250 μ l Partitioning Oil B
in GEM-X 3' Chip**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.
- Keep the chip horizontal and be careful when moving/setting down the chip to avoid wetting the gasket with oil or spilling oil over the outside of the wells.*


GEM-X 3' Chip, closed





*Run the chip in the Chromium X Series instrument **immediately** after loading the Partitioning Oil B.*

*If the chip was tilted, oil spillage may appear as fluid between the chip and surface of the chip holder. It is recommended to proceed with the run in such cases. If the recovered emulsion volume appears normal, proceed with the rest of the protocol. If enough oil is spilled out of the well, it can result in <80 μ l recovered emulsion volume.


Step 3: Run the Chromium X Series Instrument

-  Firmware version 2.0.0 or higher is required for Chromium X/Xi instrument used for this protocol, however the most updated firmware version is recommended for best performance. For Chromium Xo instrument, use the most updated firmware version.

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

- a. Press the eject button on the instrument 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. Confirm program on the screen. Press the play button.
-  d. At completion of the run (~**6 min**), the instrument will chime. **Immediately** proceed to the next step.

Step 4: Transfer GEMs

- a. Place a tube strip on ice.
- b. Press the eject button of the Chromium instrument 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. Be careful when opening the chip holder at 45 degrees to avoid wetting the gasket with oil or spilling oil.
-  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. Take a picture of the chip.
- e. Slowly aspirate **80 µl** GEMs from the lowest points of the recovery wells in the top NO FILL row 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.

Take a picture of the GEMs in the pipette tips and/or the tube strip.

-  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.



Step 5: Process Collected GEMs



- a.** Add **125 μ l** 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.

GEM-X OCM Training

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Step 4: Transfer GEMs	36
Step 5: Process Collected GEMs	38

Get Started

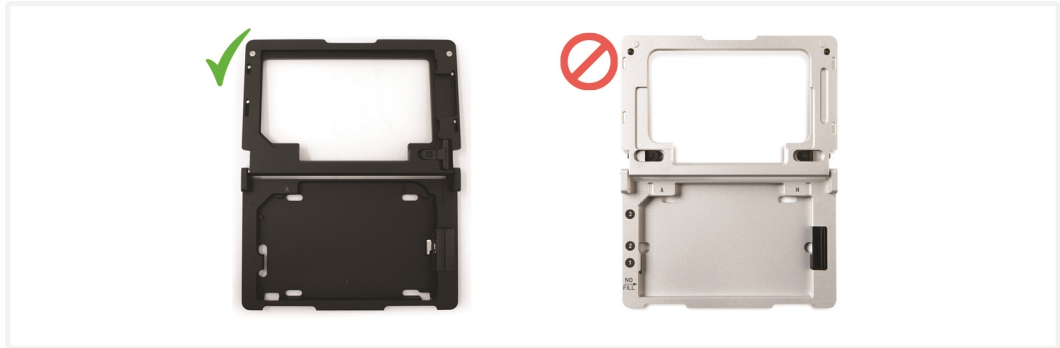
Action	Item	10x PN	Preparation & Handling	Storage
Equilibrate to Room Temperature				
<input type="checkbox"/>	GEM-X Training Gel Beads	2001250	Equilibrate to room temperature 30 min before loading the chip.	4°C
Place on Ice				
<input type="checkbox"/>	Training Master Mix	220086	One tube is sufficient for 16 samples.	4°C
Obtain				
<input type="checkbox"/>	1X PBS <i>Used as sample</i>	-	-	Ambient
<input type="checkbox"/>	Partitioning Oil B	2001213	—	Ambient
<input type="checkbox"/>	50% glycerol solution	220021	—	4°C
<input type="checkbox"/>	GEM-X 3' OCM Chip*	2001097	See Tips & Best Practices.	Ambient
<input type="checkbox"/>	X/iX Chip Gasket	3000656	See Tips & Best Practices.	Ambient
<input type="checkbox"/>	Chromium X/iX or Chromium X Series Chip Holder	3000598	See Tips & Best Practices.	Ambient

*Note that although the training reagents have only been tested with the GEM-X 3' OCM Chip, the run performance is applicable to other GEM-X OCM assays as well.



Step 1: Assemble GEM-X OCM Chip

The GEM-X OCM chip is only compatible with Chromium X/iX Chip Holder (also referred to as Chromium X Series Chip Holder; PN-3000598). DO NOT use any other holder.



See [Tips & Best Practices on page 10](#) 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.



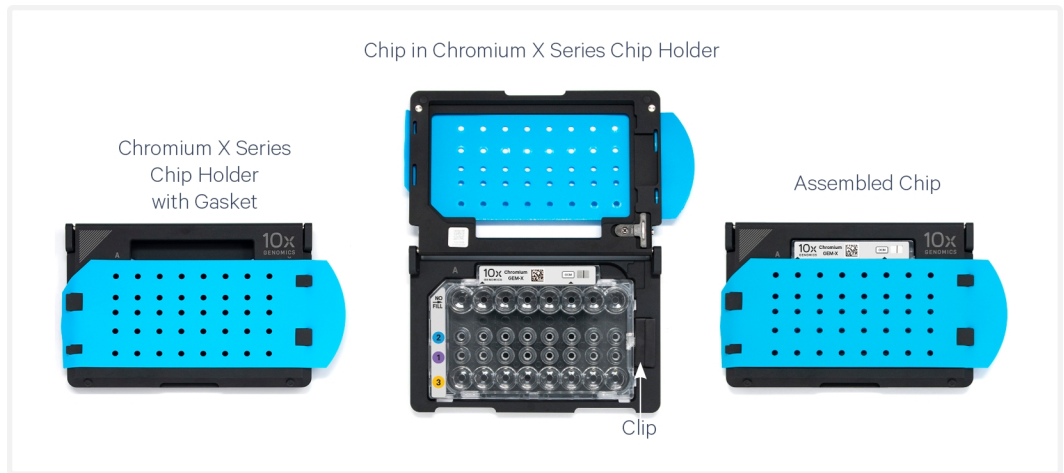
- Remove the chip from the sealed bag. Use the chip within \leq **24 h**.
- Align notch on the chip (upper left corner) and the open holder with the gasket attached.
- Slide the chip to the left until the guide on the holder is inserted into the chip. Depress the right hand side of the chip until the spring-loaded clip engages.

A generic image representative of the GEM-X 3' OCM Chip is shown below.

- Keep the assembled unit with the attached gasket open until ready for and while dispensing reagents into the wells.
- 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 top row labeled NO FILL. See step 2 for details.





Multiplexing Guidelines

Read these guidelines before loading the GEM-X OCM Chip

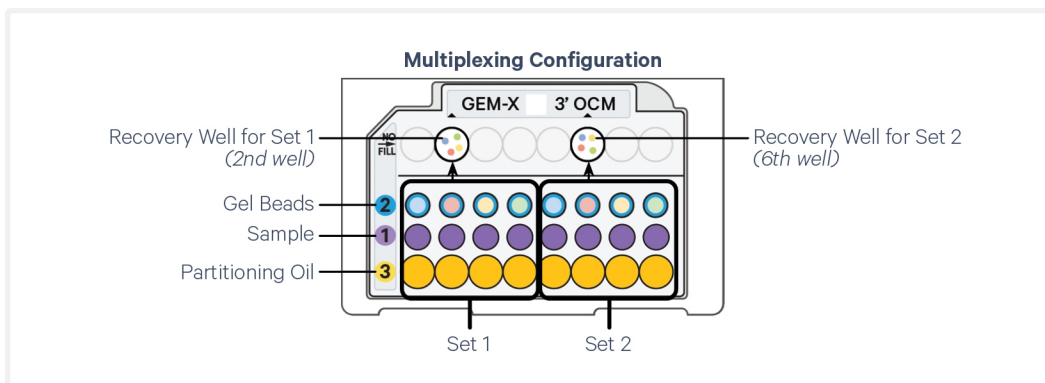
GEM-X OCM chip configuration

The GEM-X OCM chip is subdivided into two multiplexing sets, each set configured to multiplex up to 4 samples by co-partitioning them with corresponding gel beads to generate a pool of GEMs in a single recovery well (wells 2 and 6 in top row).

When running 4 samples (loading only one multiplexing set), fill the unused wells of the other set in rows labeled 1, 2, and 3 with an appropriate volume of 50% glycerol solution before loading the used wells. DO NOT add glycerol to the wells in the top NO FILL row. During training, one or both sets should be fully loaded.

GEM-X OCM Gel Beads are color-coded. To mimic these beads, label the training beads with a marker and load them according to the directions below.

GEM-X 3' OCM Chip



Chip loading overview

- **Row labeled 1:** 4 samples can be loaded in 4 consecutive wells of each set, with the option of loading just one set (4 samples) or both sets (8 samples). If only one multiplexing set is being used, the wells in the second set should be loaded with 50% glycerol.
- **Row labeled 2:** For the 4 samples loaded in a set, the 4 corresponding consecutive wells are loaded with gel beads, where the blue gel bead is loaded in the first well of the set.



Note the position of the sample and the corresponding gel bead color loaded on the chip. This information will be required during data analysis for a real experiment.

- **Row labeled 3**: Partitioning Oil B is loaded in the corresponding wells of the set.

Follow the step-by-step chip loading instructions provided in step 2.



Step 2: Load GEM-X OCM 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.
- Ensure that the Gel Beads are properly thawed and ready to use.
- When loading the chip, raising and depressing the pipette plunger should each take **~5 sec**.

Color Legend

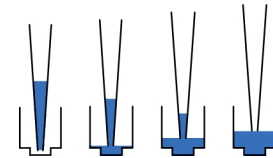
- 2: Gel beads (4 colors - blue, red, yellow, & green)
- 1: Sample
- 3: Oil

The Chromium X/iX (X Series) Chip Holder, X/iX Chip Gasket, and GEM-X chip images shown below are representative and do not show the specific color & label. Chip holder and gasket should be black and blue in color, respectively. Refer to [Step 1: Assemble GEM-X OCM Chip on page 29](#) for details.

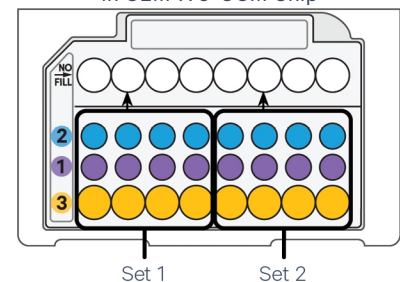
GEM-X 3' OCM Chip, gasket attached
Representative chip image



Pipette technique



Glycerol
in GEM-X 3' OCM Chip



a. If loading only one set (each set = 4 samples), add 50% glycerol solution to each unused well in row 1, 2, and 3 of the second set

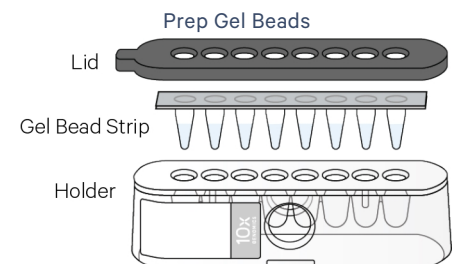
- **15 µl** in each unused well in row labeled **1**
- **18 µl** in each unused well in row labeled **2**
- **70 µl** in each unused well in row labeled **3**

DO NOT add 50% glycerol solution to the wells in top row labeled NO FILL.

DO NOT use any substitute for 50% glycerol solution.

b. 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 & the liquid levels are even.
- Place the Gel Bead strip back in the holder. Secure the holder lid.



c. Prepare Training Master Mix + 1X PBS (Sample)

- Vortex the Training Master Mix 15 sec, centrifuge briefly and place on ice.
- Add **15 µl** Training Master Mix to each well of the 8-tube strip on ice.
- Add **2 µl** 1X PBS into the Master Mix. *Total of 17 µl in each tube.*

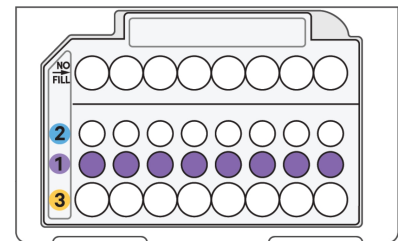
Prep Training Master Mix & 1X PBS



d. Load Row Labeled 1

- Using a multichannel pipette (P20), gently pipette mix the Master Mix + 1X PBS.
- Using the same pipette tip, dispense **15 µl** Master Mix + 1X PBS into the bottom center of each well in **row labeled 1** without introducing bubbles.
- Wait **30 sec.**

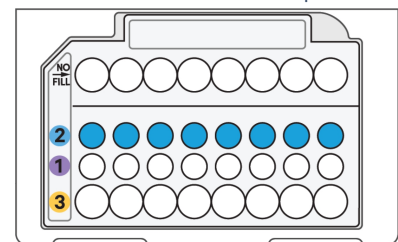
15 µl Master Mix + 1X PBS in GEM-X 3' OCM Chip



e. Load Row Labeled 2

- Puncture the foil seal of the Gel Bead tubes.
- Using a multichannel pipette (P20), slowly aspirate **18 µl** Gel Beads.
- Dispense into the bottom center of each well in **row labeled 2** without introducing bubbles.
- Wait **30 sec.**

18 µl Gel Beads in GEM-X 3' OCM Chip



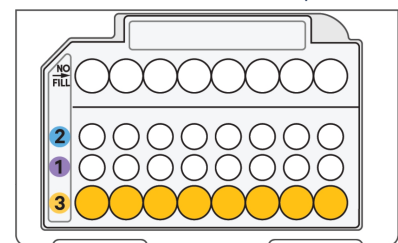
f. Load Row Labeled 3

- Using a multichannel pipette (P100/200), dispense **70 µl** Partitioning Oil B into the wells in **row labeled 3**.



Failure to add Partitioning Oil B to the row labeled 3 will prevent GEM generation and can damage the instrument.

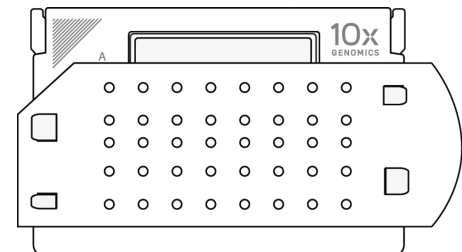
70 µl Partitioning Oil B in GEM-X 3' OCM Chip



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.
- Keep the chip horizontal and be careful when moving/setting down the chip to avoid wetting the gasket with oil or spilling oil over the outside of the wells.*

GEM-X 3' OCM Chip, closed



*Run the chip in the Chromium X Series instrument **immediately** after loading the Partitioning Oil B.*

Step 3: Run the Chromium X Series Instrument



Firmware version 2.0.0 or higher is required for Chromium X instrument used for this protocol, however the most updated firmware version is recommended for best performance. For Chromium Xo instrument, use the most updated firmware version.

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


- a. Press the eject button on the instrument 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. Confirm GEM-X OCM program on the screen. Press the play button.
- d. At completion of the run (~**4 min**), the instrument will chime.
Immediately proceed to the next step.

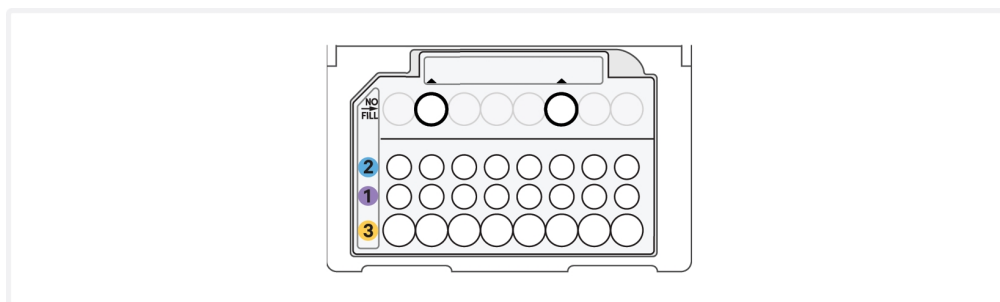


Step 4: Transfer GEMs

- a. Place a tube strip on ice.
- b. Press the eject button of the Chromium instrument and remove the chip.
- c. Discard the gasket. Open the chip holder. Inspect and photograph GEMs while the chip is still horizontal (see [GEM Generation & Barcoding - GEM-X OCM on page 43](#)).



- d. Fold the lid back until it clicks to expose the wells at 45 degrees. Be careful when opening the chip holder at 45 degrees to avoid wetting the gasket with oil or spilling oil.
- e.  Visually compare the remaining volume in rows labeled 1. An abnormally high volume in one well relative to other wells may indicate a clog. Photograph the GEM(s) in the chip.
- f. Using a single channel pipette, slowly aspirate **80 μ l** GEMs from the lowest points of the **second** and **sixth** recovery wells in the top NO FILL row without creating a seal between the tips and the bottom of the wells.



- g. Withdraw pipette tips from the wells. GEMs should appear opaque and uniform across both the recovery wells. Excess Partitioning Oil (clear) in the pipette tips indicates a potential clog. Photograph GEMs in the pipette tip against a dark background.



See [GEM Generation & Barcoding - GEM-X OCM](#) on page 43 for additional guidance for clogs/emulsion failures.



- h.** Over the course of **~20 sec**, dispense GEMs retrieved from recovery wells 2 and 6 into two separate consecutive tubes of a tube strip on ice with the pipette tip against the sidewall of the tube.

Step 5: Process Collected GEMs



- a. Add **125 μ l** 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.

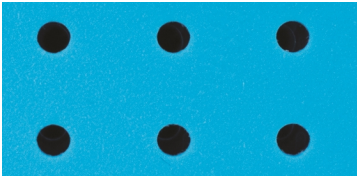
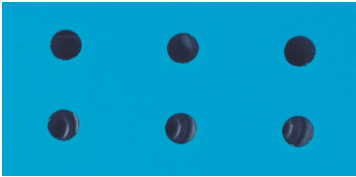

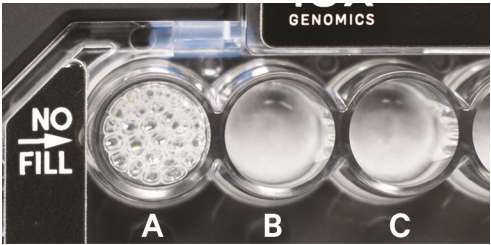
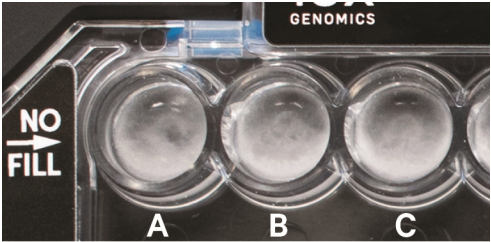
- b. This concludes the Training Kit protocol. This training protocol does not proceed with cDNA amplification or other steps found in other User Guides.

Troubleshooting



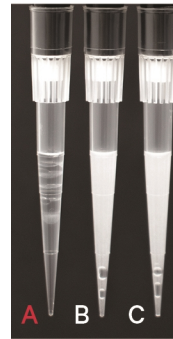
GEM Generation & Barcoding - GEM-X	40
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GEM Generation & Barcoding - GEM-X

STEP	Normal	Impacted
<p>Load Chromium GEM-X Chip</p>		
	<p>Gasket holes are aligned with the sample and gel bead wells.</p>	<p>Gasket holes are misaligned with the gel bead wells. Open and close the chip holder slowly once. <i>One of the rows of Chromium X/iX Chip Gasket will not align with the GEM-X chip. This is normal and will not impact the assay.</i></p>
<p>After Chip is removed from the instrument and the wells are exposed.</p>		
	<p>All recovery wells are similar in volume and opacity.</p>	<p>Recovery well A indicates a wetting failure. Not all wetting failures may present themselves with excess bubbles (foam).</p>
		 <p>Recovery wells A-C show normal emulsions. Not all normal emulsions may have small (in size and number) bubbles in the recovery wells.</p> <p>Inspecting emulsions in pipette tips is recommended for diagnosing emulsion failures.</p>

STEP	Normal	Impacted
------	--------	----------

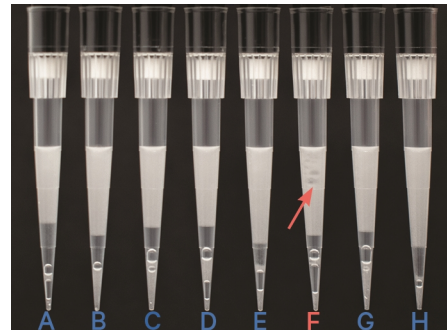
Transfer GEMs from Chip



- Wetting failure
- Normal emulsion

All liquid levels are similar in volume and opacity without air trapped in the pipette tips.

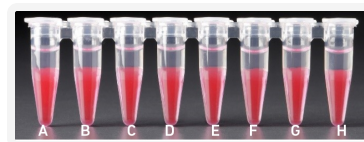
Pipette tip A shows wetting failure. Pipette tips B-C show uniform emulsions & slightly low volumes. (~95 µl)
 Most wetting failures will not impact emulsion volumes of other sample run on the chip.
 Occasionally, wetting failures may impact the emulsion volumes recovered from other lanes. This is expected and does not indicate an emulsion failure if the samples are uniform in volume and opacity (B-C).



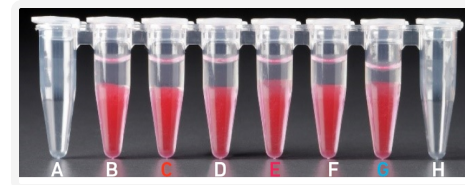
In pipette tips A-E & G-H, a portion of the emulsion displays separate layers of oil, which indicates clog.
 Pipette tip F indicates a clog (separate oil layers) and wetting failure (non-uniform emulsion).

Emulsion failures are not expected to be observed across the entire chip. The above images are for illustrative purposes to show a range in emulsion failures. A clog is not expected to impact other lanes on the chip.

After transfer of the GEMs + Recovery Agent



All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/Partitioning Oil (pink).

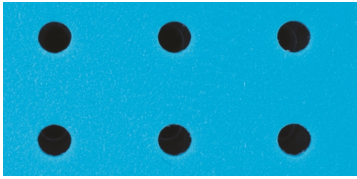
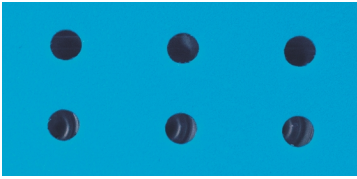
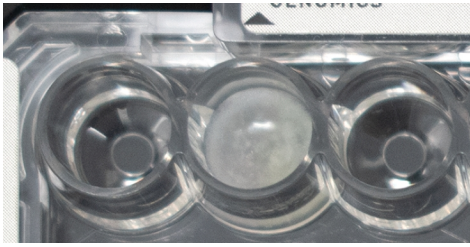
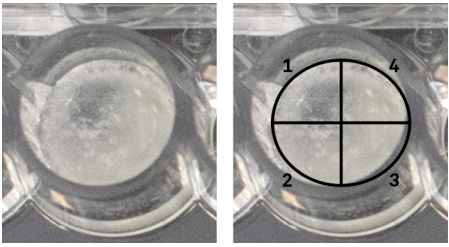
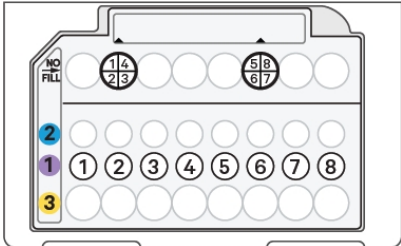
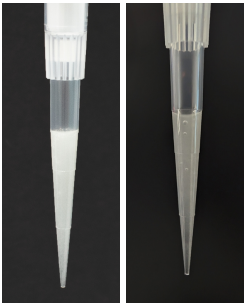
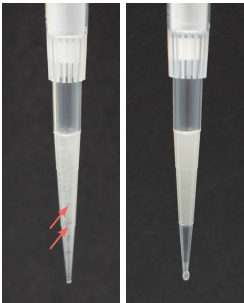


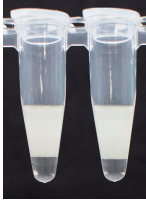
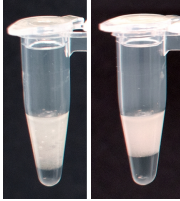
Tube G indicates a reagent clog has occurred. There is a decreased volume of aqueous layer (clear).
 Tube C and E indicate a wetting failure has occurred. There is an abnormal volume of Recovery Agent/Partitioning Oil (pink).



If any of the listed issues occur, take a picture and send it to support@10xgenomics.com for further assistance.

GEM Generation & Barcoding - GEM-X OCM

STEP	Normal	Impacted
<p>Load Chromium GEM-X Chip</p>		
	<p>Gasket holes are aligned with the sample and gel bead wells.</p>	<p>Gasket holes are misaligned with the gel bead wells. Open and close the chip holder slowly once. One of the gasket rows (second from top) will not align with the GEM-X chip. This is normal & will not impact the assay.</p>
<p>After Chip is removed from the instrument and the wells are exposed</p>		
	<p>Recovery well shows consistent volume and opacity.</p>	 <p>The quadrant numbers correlate with the sample well numbers.</p>
	<p>Inspecting emulsions in chip wells & pipette tips is recommended for diagnosing failures. Occurrence of small bubbles in the emulsion is normal & does not indicate failure.</p>	
<p>Transfer GEMs from Chip</p>		
	<p>Consistent volume, opacity, no air trapped. Emulsion density (oil settling) differences seen only if recovery is delayed; likely no impact on assay.</p>	<p>First pipette tip shows wetting failure. Second pipette tip shows a clog.</p>

STEP	Normal	Impacted
After transfer of the GEMs to tubes		
	GEMs transferred immediately to the tubes. Over time some settling is normal.	GEMs transferred immediately to the tubes. First tube shows a wetting failure. Second tube shows a clog. If excess oil is observed immediately after emulsion recovery, it would likely indicate a clog.

 After GEM generation, it is strongly recommended to always take a picture of:

- Emulsion(s) in the chip (horizontal and at 45 degrees)
- Emulsion(s) in the pipette tip/tube
- The chip after emulsions have been recovered

Contact 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 Instrument User Guide with Readiness Test (CG000396) for additional information and follow the instrument touchscreen prompts for execution. The instrument touchscreen will guide the user through recoverable errors.