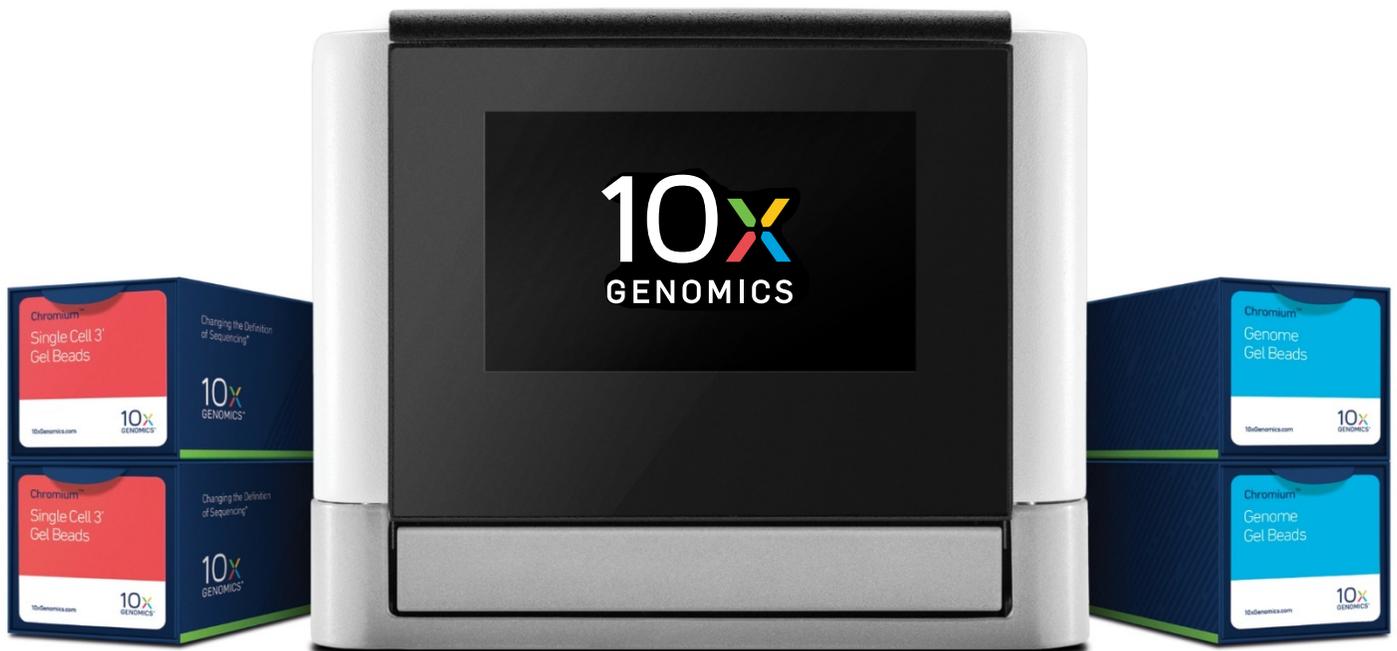


Chromium

Controller Training Kit User Guide



Available for use only with the indicated Chromium Controller (PN-120223;120246) or Chromium Single Cell Controller (PN-120263; 120212).



Notices

Manual Part Number

CG00021 Rev F

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Introduction

Chromium Controller Training Kit

Chromium Training Kit Components

Chromium Accessories

Additional Kits, Reagents & Equipment



Chromium Controller Training Kit

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

- How to mix sample and Master Mix
- How to prepare Gel Beads
- How to load a Chromium Chip with the Reaction Mix, Gel Beads, and Partitioning Oil
- How to load a Chromium Chip into the Chromium Controller (or Chromium Single Cell Controller) and run the Controller
- How to inspect the resulting Gel Bead-in-EMulsion (GEMs) in the Chromium Chip
- How to transfer the GEMs to a PCR plate and seal in preparation for thermal cycling
- How to process the GEMs immediately after collection

For guidance on other items, 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* (CG00025) or the *Chromium Single Cell Controller* (CG00051).
- For guidance on preparing a sample that can be quantitated and moved through library generation and sequencing, refer to the applicable Sample Preparation Demonstrated Protocols and to the *Chromium Single Cell 3' v2 Reagent Kits User Guide* (CG00052), the *Chromium Single Cell V(D)J Reagents Kits User Guide* (CG000086), the *Chromium Genome v2 Reagent Kits User Guide* (CG00043), and the *Exome Demonstrated Protocol* (CG000059).
- For guidance on application-specific training, including the different volumes recommended for use in different applications, refer to the *Chromium Single Cell 3' v2 Reagent Kits User Guide*, the *Chromium Single Cell V(D)J Reagents Kits User Guide*, the *Chromium Genome v2 Reagent Kits User Guide*, and the *Exome Demonstrated Protocol*.

Chromium Training Kit Components

CRITICAL!

Parts from Chromium Training Kits are **NOT** interchangeable with parts from Chromium Single Cell 3' v2 Reagent Kits, Single Cell V(D)J Reagent Kits, or Chromium Genome v2 Reagents Kits, despite the same or similar names.

Product	Description	#	Part Number
Chromium Training Chip Kit (store at ambient temperature)			1000179 (America & Asia Pacific) 120244 (Europe, Middle East & Africa)
	<div style="border: 1px solid gray; padding: 5px; font-size: small;"> Some Chromium Training Chip Kits may include 5 Partitioning Oil tubes (PN-220088) </div>	Training Chips	1
Gaskets		1	370017
Partitioning Oil		6	220088
 Recovery Agent		6	220016
Chromium Training Reagents and Gel Bead Kit (store at 4°C)			120238
<div style="border: 1px solid gray; padding: 5px; font-size: small;"> PN-220021 may be stored at 4°C along with other reagents of this kit even though PN-220021 tube indicates storage at -20°C </div>	Training Gel Beads	6	220085
	 Training Master Mix	3	220086
	 Surrogate Fluid	2	220021
	 Training Sample	1	220087

Chromium Accessories

Product	Description	Part Number
10x Vortex Adapter	<p>The 10x Vortex Adapter attaches to the top of a standard laboratory vortexer and enables the use of the 10x Vortex Clip and to vortex Gel Bead Strips.</p> 	330002
10x Vortex Clip	<p>The 10x Vortex Clip coupled with the 10x Vortex Adapter enables users to vortex 8-tube strips with ease.</p> 	230002
10x Chip Holder	<p>The 10x Chip Holder encases the Chromium Chips and holds them in the correct position in the Chromium Controller. The 10x Gasket fits over the top of the 10x Chip Holder before inserting the assembly in the Chromium Controller.</p> <p>The 10x Chip Holder lid also conveniently flips over to become a stand, holding the Chromium Chip at the ideal 45° angle for removing GEMs from the Recovery Wells after a Chromium Controller run.</p> <p>Squeeze the black sliders on the back side of the 10x Chip Holder together to unlock the lid and return the 10x Chip Holder to a flat position.</p> 	330019
10x Magnetic Separator	<p>The 10x Magnetic Separator offers two positions of the magnets relative to the 8-tube strip inserted, depending on its orientation. Simply flip the 10x Magnetic Separator over to switch between the magnets being High or Low.</p>	230003

Additional Kits, Reagents & Equipment

Product	Description	Part Number
USA Scientific	TempAssure PCR 8-tube strip* (<i>alternate to Eppendorf product</i>)	1402-4700
Eppendorf	twin.tec 96-Well PCR Plate* Semi-skirted	951020362
	twin.tec 96-Well PCR Plate* Divisible, unskirted	0030133374
	twin.tec 96-Well PCR Plate* Unskirted	0030133390
	PCR Tubes 0.2 mL 8-tube strips* (<i>alternate to USA Scientific product</i>)	0030124359 (OUS) 951010022 (US)
Bio-Rad	PX1 PCR Plate Sealer**	1814000
Rainin	Tips LTS 200UL Filter RT-L200FLR	17007961
	Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805
	Pierceable Foil Heat Seal	1814040
VWR	Vortex Mixer*	10153-838
	Divided Polystyrene Reservoirs**	41428-958

*No substitutions are allowed. Items have been validated by 10x Genomics and are required for 10x workflows, training and system operations. **Substituting materials may adversely affect system performance and are not supported.

Recommended Thermal Cyclers

Thermal cyclers used with the Chromium Genome and Chromium Single Cell Protocols must support uniform heating of 125 μ l and 100 μ l emulsion volumes, respectively. Thermal cyclers recommended for use are:

- Bio-Rad C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module (PN-1851197)
- Eppendorf MasterCycler Pro (PN North America 950030010, International 6321 000.019)
- Thermo Fisher Veriti 96-Well Thermal Cycler (PN-4375786)

Controller Training Protocol



Training Step 1

1. Getting Started!

Equilibrate to room temperature before use:

Item	Part Number	Storage Location
Training Gel Beads <i>Equilibrate to room temperature 30 min before loading the Training Chip</i>	220085	4°C

Place on ice:

Item	Part Number	Storage Location
 Training Master Mix <i>One tube sufficient for 16 samples</i>	220086	4°C
 Training Sample <i>One tube sufficient for 48 samples</i>	220087	4°C
Chilled Metal Block	-	-

Obtain:

Item	Part Number	Storage Location
Partitioning Oil	220017 or 220088	Ambient temperature
Surrogate Fluid	220021	4°C
Training Chip(s)	2000204 (America & Asia Pacific) 230026 (Europe, Middle East & Africa)	Ambient temperature
10x Gasket(s)	370017	Ambient temperature
10x Chip Holder	330019	Ambient temperature

Note on 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

Alternatively, 50% glycerol solution can be made from a stock solution of glycerol as follows:

- Mix an equal volume of water and 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.

Training Step 2

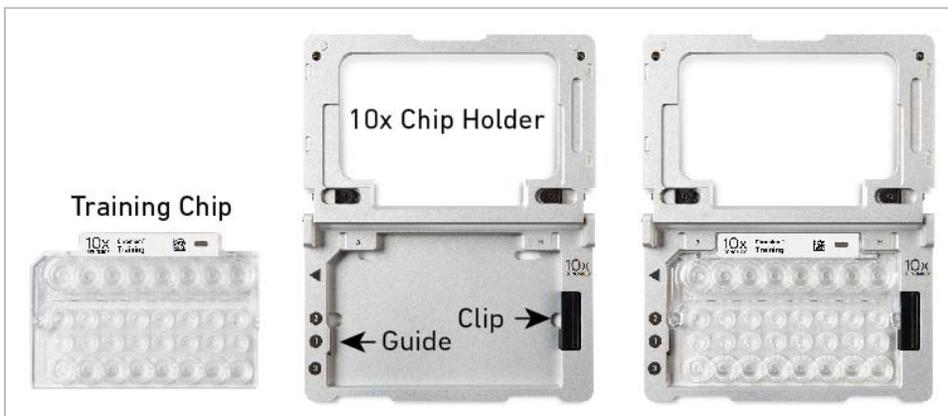
2. Assemble & Load a Chip and Chip Holder

2.1. Assemble a Training Chip and a 10x Chip Holder

NOTE

Always handle the Chromium Chip by its edges and avoid touching its bottom surface. Once the chip is in the holder, keep the assembly horizontal at all times to avoid wetting the 10x Gasket with Partitioning Oil.

- Align the notch on the upper left corner of the Training Chip with the notch on the 10x Chip Holder and insert the left-hand side of the Training Chip under the guide.
- Depress the right-hand side of the Training Chip until the spring-loaded clip engages the Training Chip. Close the hinged lid of the 10x Chip Holder.



- Close the hinged lid of the 10x Chip Holder. After loading the Training Chip, the 10x Chip Holder should lay flat on the bench top with the lid closed.
- Position the assembly so that the Partitioning Oil wells (row labeled 3) are toward you and identify the rows labeled 1, 2 and 3 for correct addition of the reagents.

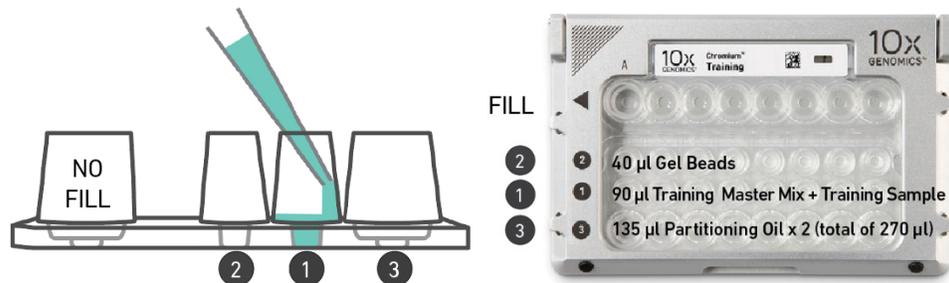


2.2. Load a Training Chip

The following instructions are similar to those for loading a Chromium Single Cell A Chip. However, refer to the Chromium Single Cell 3' v2 Reagent Kits User Guide or the Chromium Single Cell V(D)J Reagent Kits User Guide for specific instructions on handling cell samples and specific application volumes. Furthermore, refer to the Chromium Genome v2 Reagent Kits User Guide or the Exome Demonstrated Protocol for specific instructions on loading a Chromium Genome Chip since several differences apply.

NOTE

Dispense against the side of the well, as illustrated below. Avoid contact with the bottom of the well. Bubble formation is normal and does not affect performance.



CRITICAL!

The order in which the wells of the Training Chips are loaded is critical for optimal performance. Always load the rows in the labeled order: 1 followed by 2, then 3.

- a) If processing fewer than 8 samples per Training Chip, first add the following volumes of Surrogate Fluid to each unused well:
 - i. **90 µl** in the row labeled 1
 - ii. **40 µl** in the row labeled 2
 - iii. **270 µl** in the row labeled 3

CRITICAL!

Do not add Surrogate Fluid to Recovery Wells (row labeled ◀). Do not use Partitioning Oil or any other solution as a substitute for Surrogate Fluid.

- b) Vortex the Training Master Mix at full speed for 15 sec, centrifuge briefly and place on a chilled metal block resting on ice.
- c) Add **98 µl** Training Master Mix to each well of the 8-tube strip on a chilled metal block resting on ice.
- d) Slowly add **2 µl** Training Sample into each well of the tube strip containing Master Mix.

NOTE

Pipette the sample very slowly into the Master Mix. It should take 5 sec to raise and 5 sec to depress the pipette plunger. The total volume of the combined Training Master Mix and Training Sample is 100 µl in each well.

- e) With a pipette set to 90 µl, gently pipette mix 5 times while keeping the tube strip in a chilled metal block resting on ice.
- f) Using a **narrow-bore** pipette tip, slowly transfer **90 µl** Training Master Mix + Training Sample **against the side of each well in the row labeled 1**.

CRITICAL!

Pipette slowly. Raising and depressing the pipette plunger should each take 2 sec.

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- g) Snap the Training Gel Bead Strip into a 10x Vortex Adapter. Vortex at full speed for **30 sec.**

CRITICAL!

A 30 sec wait while vortexing the Training Gel Bead Strip is required to ensure proper priming of the Master Mix containing cells in the Training Chip. Then, immediately load the Training Gel Beads.

- h) Remove the Training Gel Bead Strip from the 10x Vortex Adapter and flick the Training Gel Bead Strip in a sharp, downward motion to ensure maximum Gel Bead recovery. Confirm that there are no bubbles at the bottom of the tubes and that liquid levels are uniform.

CRITICAL!

Collection of the entire volume of Training Gel Beads at the bottom of the strip tube is required to ensure the full volume of beads is transferred to the Training Chip.

NOTE

Pipette Training Gel Beads slowly as they have a viscosity similar to high-concentration glycerol.

- i) Set a pipette to 40 μ l and, without engaging the plunger, puncture the foil seal on the Gel Bead Strip. The pipette tips should extend no more than 2 mm below the seal.



- j) Once the holes are formed, raise the pipette tips above the seal and engage the plunger.
- k) Lower the tips to the bottom of the wells and widen the opening by gently rocking the tips back and forth, keeping the plunger engaged.

NOTE

Widening the foil seal opening allows the pipette tips to reach the bottom of the Gel Bead Strip wells. This is important for recovering the full volume of Gel Beads required for optimal performance.

- l) With the pipette tips still in the Gel Bead Strip, very slowly aspirate the required volume of Gel Beads. After aspiration stops, leave the pipette tips in the wells for an additional 5 sec to allow pressure to equilibrate.



NOTE

If the full required volume of beads cannot be recovered, place the pipette tips against the sidewalls of the Gel Bead Strip wells and slowly dispense the Gel Beads back into the strip. Take care not to introduce bubbles into the wells and verify that the pipette tips contain no leftover Gel Beads. Attempt to withdraw the full volume of beads again by pipetting slowly.

- m) Slowly dispense the Training Gel Beads **against the side of each well in the row labeled 2.**

CRITICAL!

Confirm that the pipette tips do not contain leftover Gel Beads. If necessary, wait for the remaining Gel Beads to drain into the bottom of the pipette tips and dispense into the wells.

- n) Pipette a total volume of **270 µl** of Partitioning Oil **against the side of each the well in the row labeled 3 by pipetting two aliquots of 135 µl** from a reagent reservoir. Do not add Partitioning Oil to any unused input wells that already contain Surrogate Fluid.

CRITICAL!

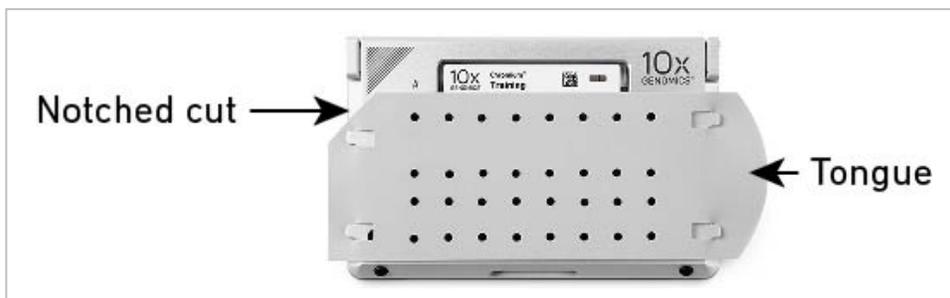
Failure to add Partitioning Oil can damage the Chromium Controller or the Single Cell Chromium Controller.

Training Step 3

3. Attach a Gasket & Run the Chromium Controller

3.1. Attach a 10x Gasket

- a) Position the assembly so the Partitioning Oil wells (row labeled 3) are toward you. Attach the 10x Gasket. The notched cut should be at the top left corner. Holding the 10x Gasket by the tongue (curved end, to the right) and hook it on the left-hand tabs of the 10x Chip Holder. Gently pull the 10x Gasket toward the right and hook it on the two right-hand tabs. Ensure the 10x Gasket holes are aligned with the wells. Avoid touching the smooth side of the 10x Gasket and do not press down on the top of the 10x Gasket after attachment. Keep the assembly horizontal to avoid wetting the 10x Gasket with Partitioning Oil.



NOTE

For optimal results, the loaded Training Chip should be run on the Chromium Controller ≤ 2 min after loading. Training Chips run on the Chromium Controller > 20 min after loading may result in decreased application performance.

3.2. Run the Chromium Controller

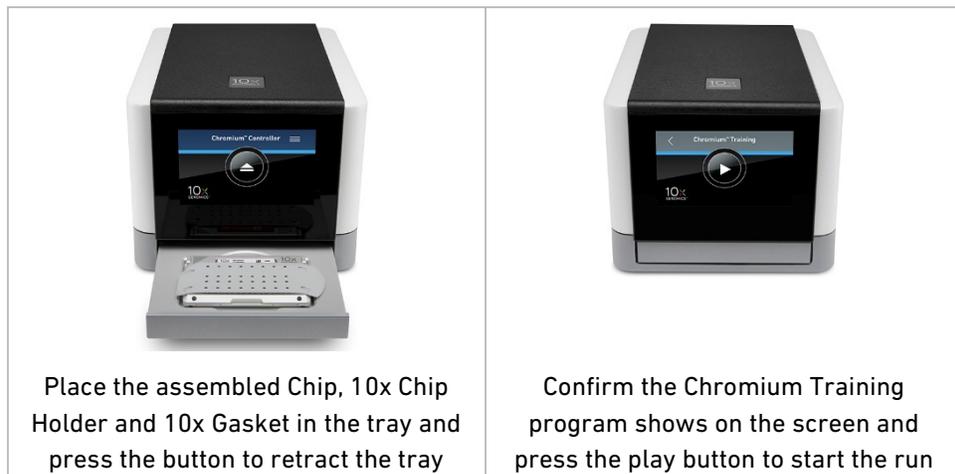
NOTE

The same instructions apply to the Chromium Single Cell Controller.

- a) Touch the touchscreen to wake up the Chromium Controller and access options.
- b) Press the button on the touchscreen of the Chromium Controller to eject the tray.

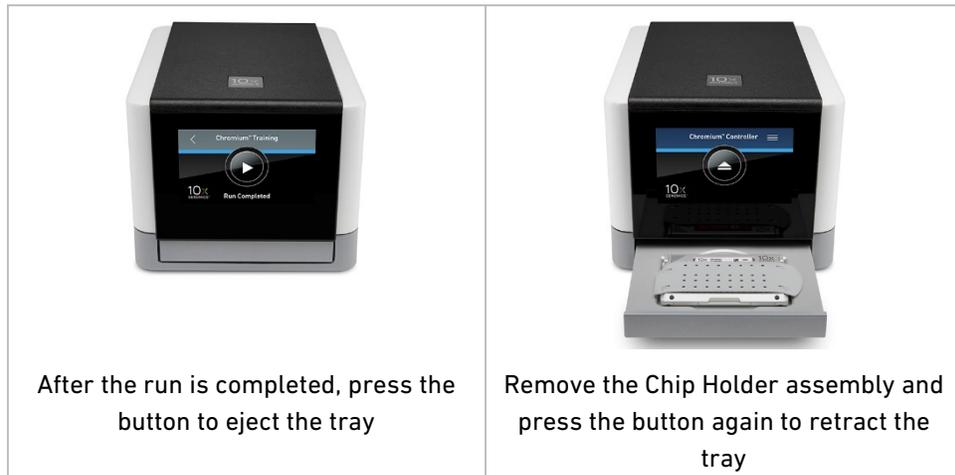


- c) Place the Training Chip assembled in 10x Chip Holder with a 10x Gasket installed on the tray (align the diagonal etchings on the top left corner of the 10x Chip Holder and the back left corner of the tray).
- d) Press the button to retract the tray. Confirm the Chromium Training program shows on screen and press the play button to begin the run.



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- e) At the completion of the run (~6 min), the Chromium Controller will chime. Press the button to eject the empty tray. Proceed immediately to the next step.



Training Step 4

4. Collect GEMs

NOTE

The following instructions are similar to those for collecting GEMs from a Chromium Single Cell A Chip. However, refer to the Chromium Single Cell 3' v2 Reagent Kits User Guide, the Chromium Single Cell V(D)J Reagent Kits User Guide, the Chromium Genome v2 Reagent Kits User Guide, or the Exome Demonstrated Protocol for specific instructions since several differences apply.

4.1. Transfer GEMs to a 96-well PCR Plate

- Maintain an Eppendorf twin.tec 96-well PCR plate for GEM transfer on a chilled metal block resting on ice.
- Remove and discard the 10x Gasket from the Training Chip. Press the button to retract the empty tray (or the tray will automatically close after 40 sec).
- Open the 10x Chip Holder and fold the lid back until it clicks to expose the wells at a 45-degree angle.
- Check for volume uniformity in the Gel Bead, Sample, and Partitioning Oil wells remaining in the Training Chip.



NOTE

Abnormally high volume in any of the wells may indicate that a clog occurred during GEM generation. See Practical Tips for more information.

- Slowly aspirate **80 μ l** GEMs from the lowest points of the Recovery Wells (row labeled ◀) without creating a seal between the tips and the bottom of the wells. Avoid introducing air bubbles.

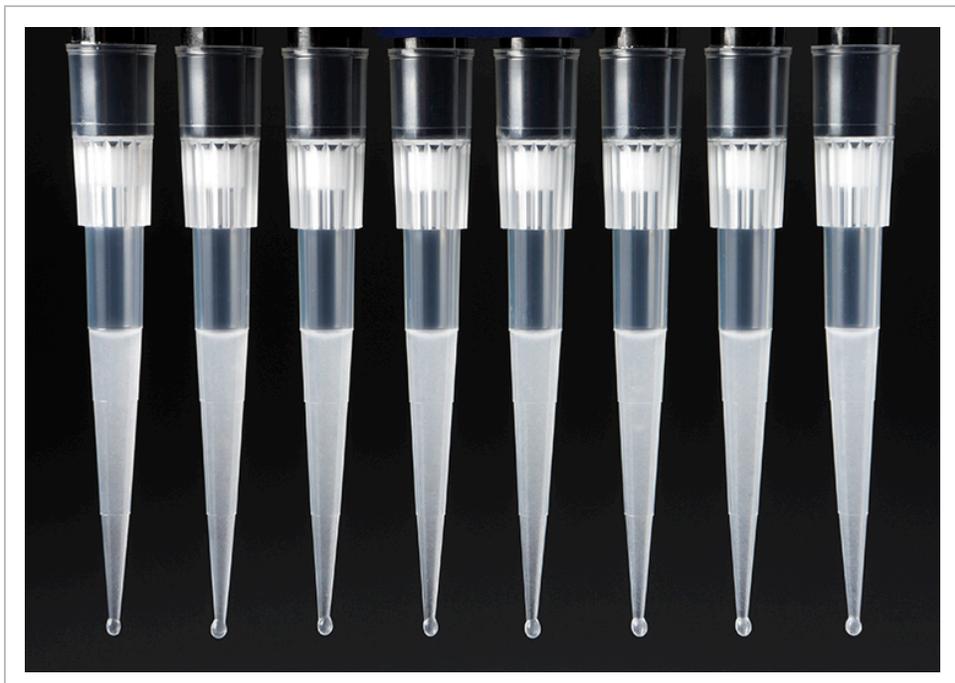
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NOTE

Pipette GEMs slowly as they have a high viscosity. If a tip aspirates excessive air the sample may be compromised.

- f) Withdraw pipette tips from the wells and verify that there is no air in the tips. GEMs should appear opaque and uniform across all channels.



NOTE

The presence of excess Partitioning Oil (clear) indicates a potential clog during GEM generation. See Practical Tips for more information.

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- g) Over the course of ~20 sec, dispense the GEMs into the Eppendorf twin-tec 96-well PCR plate (on a chilled metal block resting on ice) with the pipette tips **against the side walls of the wells**. Keep the tips above the liquid level to minimize GEMs lost on the outside of the tips.



NOTE

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 plate.

- h) Check the volume uniformity of the GEMs and the Partitioning Oil in the PCR plate. The GEMs appear cloudy (opaque) and settle on top of the wells. The Partitioning Oil is clear and settles on the bottom of the wells.

NOTE

A clog occurred if the Partitioning Oil volume in one or more wells is increased compared to other wells. See Practical Tips for more information.

- i) Discard the used Training Chip. Push the black sliding latches on the back of the 10x Chip Holder toward the middle to release the lock and close the lid.

4.2. Seal the GEM Plate

- a) Set the Bio-Rad PX1 Plate Sealer to **185°C** for **6 sec**.
- b) Place the GEM plate in the PX1 Plate Sealer plate block. Load the block and plate into the sealer tray.
- c) Place the pierceable foil heat seal on the plate with the red stripe facing up.

CRITICAL!

Proper positioning of the foil seal with the red stripe up is critical. If positioned incorrectly, the foil seal can stick to the plate sealer, damage the sealer, and/or render the plate unusable. The GEMs cannot be transferred from the plate due to the potential of sample loss. These GEMs will be unusable.

- d) Once the plate is sealed and ejected, promptly remove the plate from the plate block and store the plate at **4°C overnight**, if needed, or proceed directly to Post GEM Collection Processing.

Training Step 5

5. Post GEM Collection Processing

- a) Label an 8-tube strip to track the orientation of the samples transferred from the PCR plate.
- b) Remove the foil seal by placing the 96-well plate in a rack to stabilize it and peel the foil seal off the PCR plate.

NOTE

Piercing the foil seal with pipette tips is not recommended, as contact with the GEMs may cause adherence to the pipette tip resulting in unrecoverable GEMs.

- c) For each Training Chip processed (i.e. each set of 8 samples), pour the contents of a single 2.0 ml Recovery Agent tube into the 8-channel portion of an 8+4 divided reagent reservoir.
- d) Add **125 µl** Recovery Agent to each well containing post thermal cycled GEMs. **Wait 60 sec** before moving the entire volume in multiple transfers to a tube strip. Retrieve as much aqueous solution as possible from the bottom of the wells.

NOTE

After transferring the initial volume to a tube strip, aqueous phase recovery can be maximized by lightly sealing the plate with a Microseal® 'B' Adhesive Seal and spinning in a plate centrifuge at 1200 rpm for 30 sec. The collected volumes can be combined with those previously transferred into the tube strip.

- e) After collection into the tube strip, check the biphasic mixture contains distinct Recovery Agent/Partitioning Oil (pink) and aqueous phases (clear) with no persisting emulsion.



NOTE

A decrease in the aqueous phase indicates that a clog occurred during GEM generation. Samples from clogged channels might be compromised. See Practical Tips for more information.

Chromium Controller Errors

6. Chromium Controller Errors

If the Chromium 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 10x Chip 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 there is a 10x Gasket on the Chromium Chip. In the case the 10x Gasket installation was forgotten, install and try again. In the case a 10x Gasket was already installed, remove, reapply, and try again. If the error message is still received after trying either of these more than twice, contact support@10xgenomics.com for further assistance.
- c) **Pressure not at Setpoint:**
 - 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 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 Chip must be discarded. **Do not try running this Chromium Chip again as this may damage the Chromium Controller.**
- d) **CAUTION: Chip Holder not Present:** Eject the tray by pressing the eject button to check there is a 10x Chip Holder encasing the Chromium Chip. In the case the 10x Chip Holder was forgotten, install with a 10x Gasket in place, and try again. If the error message is still received after a 10x Chip Holder is confirmed as in place, contact support@10xgenomics.com for further assistance.
- e) **Invalid Chip CRC Value:** This indicates the Chromium Chip has encountered an error, should not be run, and must be discarded. Contact support@10xgenomics.com for further assistance.

Practical Tips

7. Failure Modes during GEM Generation

NOTE

The following are failure modes that may be encountered when running the Chromium Training Chip and the Chromium Single Cell A Chip. Refer to the Chromium Genome Reagent Kits User Guide for specifics on failure modes that may be encountered when running Chromium Genome Chips.

Reagent Clogs

GEM reagents are manufactured in a cleanroom environment to minimize the level of particles and fibers that could clog microfluidic channels during GEM generation and therefore reduce technical performance.

To avoid clogs, it is also important for users to minimize exposure of reagents, chips, and gaskets to sources of particles and fibers such as open reagent reservoirs, laboratory wipes, frequently opened flip-cap tubes, clothing that easily sheds fibers, and dusty surfaces.

There are several ways to identify if a clog has occurred as outlined below. If any of the following occur, take a picture and send it to support@10xgenomics.com for further assistance. If a channel clogs during GEM generation, it is recommended that the sample be remade.

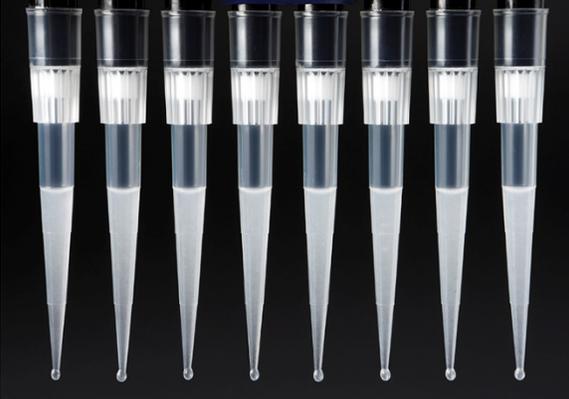
Wetting Failures

Once reagents are added to the Chromium Chip wells, they immediately flow into and prime the microfluidic channels on the chip. Incorrect priming can result in wetting failures, in which polydisperse, millimeter-scale droplets are formed instead of a uniform GEM.

To minimize the occurrence of wetting failures, it is critical to add reagents in the stipulated order and to wait 30 sec between addition of Master Mix and addition of Gel Beads.

There are several ways to identify if a wetting failure has occurred as outlined below. If any of the following occur, take a picture and send it to support@10xgenomics.com for further assistance. If a wetting failure occurs during GEM generation, it is recommended that the sample be remade.

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Normal Operations	Reagent Clogs or Wetting Failures
 <p>After the Chromium Training Chip is removed from the Chromium Controller and the wells exposed:</p> <p>All Recovery Well levels are similar in volume and opacity.</p>	 <p>After the Chromium Training Chip is removed from the Chromium Controller and the wells exposed:</p> <p>Recovery Well G indicates a reagent clog has occurred in this channel.</p> <p>Recovery Wells C and E indicate a wetting failure has occurred in these channels. There is an absence of emulsion.</p> <p>Recovery Wells B, D, and F are normal.</p> <p>Note Surrogate Fluid was entered into wells A and H.</p>
 <p>A B C D E F G H</p>	 <p>A B C D E F G H</p>
<p>After aspirating the GEMs from the Chromium Training Chip Recovery Wells:</p> <p>All liquid levels are similar in volume and opacity. There is also no air in the pipette tips.</p>	<p>After aspirating the GEMs from the Chromium Training Chip Recovery Wells:</p> <p>Pipette tip G indicates a reagent clog has occurred in this channel. There is excess Partitioning Oil (clear) and air in the pipette tip. In some reagent clog cases, there is only 5-10 µl excess Partitioning Oil (and no air) in the pipette tip.</p> <p>Pipette tips C and E indicate indicate a wetting failure has occurred in these channels. Pipette tip C contains partially emulsified GEMs. Emulsion is absent in pipette tip E.</p> <p>Pipette tips B, D, and F are normal.</p> <p>Pipette tips A and H are missing.</p>

CONTROLLER TRAINING PROTOCOL

Normal Operations	Reagent Clogs or Wetting Failures
 <p data-bbox="248 464 751 489">A B C D E F G H</p> <p data-bbox="204 512 776 575">After transfer of the GEMs + Recovery Agent to a tube strip:</p> <p data-bbox="204 594 787 695">All liquid levels are similar in the aqueous sample volume (clear) and Recovery Agent/Partitioning Oil (pink).</p>	 <p data-bbox="872 464 1375 489">A B C D E F G H</p> <p data-bbox="824 512 1401 575">After transfer of the GEMs + Recovery Agent to a tube strip:</p> <p data-bbox="824 594 1404 730">Tube G indicates a reagent clog has occurred in this channel. There is a decreased volume of aqueous sample (clear) when compared to normal channels B, D, and F.</p> <p data-bbox="824 749 1404 924">Tubes C and E indicate a wetting failure has occurred in these channels. There can be an abnormal volume of Recovery Agent/Partitioning Oil (pink) when compared to normal channels B, D, and F.</p> <p data-bbox="824 942 1118 972">Tubes A and H are empty.</p>