Jun 18, 2024

GEM Generation and Barcoding

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.5jyl82d16l2w/v1

Heidi Monroe[1], Nayra Cardenes[2], Melanie Königshoff[2], koenigshoffm[2], Robert Lafyatis[1]

[1]University of Pittsburgh;
[2]Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, School of Medicine, University of Pittsburgh

TriState SenNet | Cellular Senescence Net… | 1 more workspace

Nayra Cardenes
Divisioin of Pulmonary, Allergy, Critical Care and Sleep Med...

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5jyl82d16l2w/v1

Protocol Citation: Heidi Monroe, Nayra Cardenes, Melanie Königshoff, koenigshoffm, Robert Lafyatis 2024. GEM Generation and Barcoding. **protocols.io https://dx.doi.org/10.17504/protocols.io.5jyl82d16l2w/v1**

Protocol status: Working

Created: June 12, 2024

Last Modified: June 18, 2024

Protocol Integer ID: 101652

Keywords: Chromium Next GEM Chip, Barcoding, Cell suspension, PCR, SenNet, TriState, snRNAseq, scRNAseq

Funders Acknowledgement:
TriState SenNET (Lung and Heart) Tissue Map and Atlas consortium - NIA
Grant ID: U54AG075931

Abstract

The Chromium Single Cell Gene Expression Solution upgrades short read sequencers to deliver a scalable microfluidic platform for 3' digital gene expression by profiling 500-10,000 individual cells per sample.

A pool of ~3,500,000 10x Barcodes are sampled separately to index each cell's transcriptome. It is done by partitioning thousands of cells (or nuclei) into nanoliter-scale Gel Beads-in-emulsion (GEMs), where all generated cDNA share a common 10x Barcode. Dual Indexed libraries are generated and sequenced from the cDNA and 10x Barcodes are used to associate individual reads back to the individual partitions.

This protocol outlines the process for generating Gel Beads-in-emulsion (GEMs) by combining barcoded Single Cell 3' v3.1 Gel Beads, a Master Mix containing cells, and Partitioning Oil onto Chromium Next GEM Chip G. To achieve single cell resolution, cells are delivered at a limiting dilution, such that the majority (~90-99%) of generated GEMs contain no cell, while the remainder largely contain a single cell.

This protocol details the GEM Generation and Barcoding procedures.

Attachments

DOCX

snRNAseq_ProtocolsIO..

66KB

Image Attribution

Nayra Cardenes, PhD

Materials

- Single Cell 3’ v3.1 Gel Beads (2000164)
- RT Reagent B (2000165)
- Template Switch Oligo (3000228)
- RT Enzyme C (20000085/2000102) and Cells suspension

Master Mix:

	A	B	C	D
	Reagents	1X (µl)	4X+10% (µl)	8X+10% (µl)
	RT Reagent B	18.8	82.7	165.4
	Template Switch Oligo	2.4	10.6	21.1
	Reducing Agent B	2	8.8	17.6
	RT Enzyme C	8.7	38.3	76.6
	Total	31.9	140.4	280.7

Safety warnings

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Before start

- **Equilibrate to room temperature (RT)** - Single Cell 3’ v3.1 Gel Beads (2000164), RT Reagent B (2000165) and Template Switch Oligo (3000228)
- **Place on ice** – RT Enzyme C (20000085/2000102) and Cells suspension
- **Prepare Master Mix** - Prepare On ice .

GEM Generation and Barcoding

4d 0h 19m 25s

1 Prepare Master Mix On ice . Pipette mix 15× and centrifuge briefly.

Master Mix:

	A	B	C	D
	Reagents	1X (µl)	4X+10% (µl)	8X+10% (µl)
	RT Reagent B	18.8	82.7	165.4
	Template Switch Oligo	2.4	10.6	21.1
	Reducing Agent B	2	8.8	17.6
	RT Enzyme C	8.7	38.3	76.6
	Total	31.9	140.4	280.7

2 Add 31.9 µL master mix into each tube of a PCR 8-tube strip On ice .

3 **Assemble Chromium Next GEM Chip**

3.1 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.

3.2 Remove the chip from the sealed bag, and use it within ≤ 24:00:00 . 1d

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

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

3.5 Keep the assembled unit open until all reagents have been loaded. Then close the chip holder.

4 **Load Chromium NextGEM Chip G**

4.1 Add 50% glycerol solution to each unused well (if processing <8 samples/chip)

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

5 **Prepare Master Mix + Cell suspension:**

5.1 Refer to the Cell Suspension Volume Calculator Table (refer to Chromium Next GEM Single Cell 3' user Guide).

5.2 Add the appropriate volume of nuclease-free water to master mix. Pipette mix 5×. Add corresponding volume of single cell suspension to master mix. Total of 75 µL in each tube.

5.3 Gently pipette mix the cell suspension before adding to the master mix.

6 **Load Row Labeled 1**

6.1 Gently pipette mix the Master Mix + Cell Suspension.

6.2 Using the same pipette tip, dispense 70 µL master mix + cell suspension into the bottom center of each well in row labeled 1 without introducing bubbles.

7 **Prepare Gel Beads**

7.1 Snap the tube strip holder with the Gel Bead strip into a 10× Vortex Adapter. Vortex 00:00:30 . 30s

7.2 Centrifuge the Gel Bead strip for ~ 00:00:05 . 5s

7.3 Confirm there are no bubbles at the bottom of the tubes and the liquid levels are even.

7.4 Place the Gel Bead strip back in the holder. Secure the holder lid.

8 **Load Row Labeled 2**

8.1 Puncture the foil seal of the Gel Bead tubes.

8.2 Slowly aspirate 50 µL Gel Beads.

8.3 Dispense into the wells in row labeled 2 without introducing bubbles.

8.4 Wait 00:00:30 . 30s

9 **Load Row Labeled 3**

9.1 Dispense 45 µL partitioning oil into the wells in row labeled 3 from a reagent reservoir and close lid.

Note

- Failure to add Partitioning Oil to the top row labeled 3 will prevent GEM generation and can damage the Chromium Controller or X/iX.
- Run the chip in the Chromium Controller or X/iX immediately after loading the Partitioning Oil.

10 **Run the Chromium Controller or X/iX**

10.1 Press the eject button on the controller to eject the tray.

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

10.3 Press the play button.

10.4 At completion of the run (~ 00:18:00), the Controller will chime. Immediately proceed to the next step. 18m

11 **Transfer GEMs**

11.1 Place a tube strip On ice .

11.2 Press the eject button of the controller.

11.3 Discard the gasket. Open the chip holder. Fold the lid back until it clicks to expose the wells at 45 degrees
Visually compare the remaining volume in rows labeled 1-2. Abnormally high volume in one well relative to other wells may indicate a clog.

11.4 Slowly aspirate 100 µL GEMs from the lowest points of the recovery wells in the top row labeled 3 without creating a seal between the tips and the bottom of the wells.

11.5 Over the course of ~ 00:00:20 , dispense GEMs into the tube strip on ice with the pipette tips against the sidewalls of the tubes. 20s

12 **GEM-RT Incubation**

12.1 Incubate in a thermal cycler with the following protocol:

	A	B	C
	Lid Temperature	Reaction Volume	Run Time
	53°C	125 µl	~55 min
	Step	Temperature	Time
	1	53°C	45 min
	2	85°C	5 min
	3	4°C	Hold

Thermocycler protocol.

12.2 Store at 4 °C for up to 72:00:00 or at -20 °C for up to a week, or proceed to the next step. 3d