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Post GEM–RT Cleanup and cDNA Amplification

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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.

After the GEMs Generation and Barcoding, GEMs are broken and pooled fractions are recovered. Silane magnetic beads are used to purify the first-strand cDNA from the post GEM-RT reaction mixture, which includes leftover biochemical reagents and primers. Barcoded, full-length cDNA is amplified via PCR to generate sufficient mass for library construction.

This protocol details the post GEM-RT cleanup and cDNA amplification, cleanup - SPRIselect and quantification.

Attachments

DOCX

snRNAseq_ProtocolsIO..

66KB

Image Attribution

Nayra Cardenes, PhD

Materials

- Dynabeads MyOne Silane **10x Genomics Catalog #**2000048
- Reducing Agent B **10x Genomics Catalog #**2000087
- cDNA Primers (2000089)
- Dynabeads MyOne Silane **10x Genomics Catalog #**2000048
- Amp Mix (2000047/2000103)
- Cleanup Buffer (2000088)

Dynabeads Cleanup Mix:

	A	B	C	D
	Reagents	1X (µl)	4X+10% (µl)	8X+10% (µl)
	Cleanup Buffer	182	801	1602
	Dynabeads MyOne SILANE	8	35	70
	Reducing Agent B	5	22	44
	Nuclease-free Water	5	22	44
	Total	200	880	1760

Elution Solution I:

	A	B	C
	Reagents	1X (µl)	10X (µl)
	Buffer EB	98	980
	10% Tween 20	1	10
	Reducing Agent B	1	10
	Total	100	1000

cDNA Amplification Reaction Mix:

	A	B	C	D
	Reagents	1X (µl)	4X+10% (µl)	8X+10% (µl)
	Amp Mix	50	220	440
	cDNA Primers	15	66	132
	Total	65	286	572

Safety warnings

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Before start

- **Equilibrate to Room temperature (RT) –** Reducing Agent B (2000087), cDNA Primers (2000089) and Dynabeads MyOne SILANE (2000048)
- **Place on ice –** Amp Mix (2000047/2000103)
- **Thaw at 65°C-** Cleanup Buffer (2000088)

Dynabeads

29m

1 Add 125 µL Recovery Agent to each sample at Room temperature .

> **Note**
>
> DO NOT pipette mix or vortex the biphasic mixture and wait 00:02:00 .

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

> **Note**
>
> 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 2.

2 Slowly remove and discard 125 µL Recovery Agent/Partitioning Oil (pink) from the bottom of the tube.

> **Note**
>
> DO NOT aspirate any aqueous sample.

3 Prepare Dynabeads Cleanup Mix:

	A	B	C	D
	Reagents	1X (µl)	4X+10% (µl)	8X+10% (µl)
	Cleanup Buffer	182	801	1602
	Dynabeads MyOne SILANE	8	35	70
	Reducing Agent B	5	22	44
	Nuclease-free Water	5	22	44
	Total	200	880	1760

Calculations for Dynabeads Cleanup Mix preparation.

4 Vortex and add 200 µL to each sample. Pipette mix 10x (pipette set to 200 µl).

5 Incubate 00:10:00 at Room temperature . 15m

Note

Keep caps open.

Pipette mix again at ~ 00:05:00 after start of incubation to resuspend settled beads.

6 Prepare Elution Solution I. Vortex and centrifuge briefly.

Elution Solution I

	A	B	C
	Reagents	1X (µl)	10X (µl)
	Buffer EB	98	980
	10% Tween 20	1	10
	Reducing Agent B	1	10
	Total	100	1000

Calculations for Elution Solution I preparation.

7 At the end of 00:10:00 incubation, place on a 10x Magnetic Separator. High position (magnet.High) until the solution clears. 10m

8 Remove the supernatant (aqueous phase and Recovery Agent).

9 Add 300 µL 80% ethanol to the pellet while on the magnet. Wait 00:00:30 . 30s

10 Remove the ethanol.

11 Add 200 µL 80% ethanol to pellet. Wait 00:00:30 . 30s

12 Remove the ethanol.

13 Centrifuge briefly. Place on the magnet. Low.

14 Remove remaining ethanol. Air dry for 00:01:00 . 1m

15 Remove from the magnet. Immediately add 35.5 µL Elution Solution I.

16 Pipette mix (pipette set to 30 µl) without introducing bubbles.

17 Incubate 00:02:00 at Room temperature . 2m

18 Place on the magnet. Low until the solution clears.

19 Transfer 35 µL sample to a new tube strip.

cDNA amplification 3d

20 Prepare cDNA Amplification Mix On ice . Add reagents in the order listed. Vortex and centrifuge briefly.

cDNA Amplification Reaction Mix

	A	B	C	D
	Reagents	1X (µl)	4X+10% (µl)	8X+10% (µl)
	Amp Mix	50	220	440
	cDNA Primers	15	66	132

	A	B	C	D
	Total	65	286	572

Calculations for cDNA Amplification Reaction Mix preparation.

21 Add 65 µL cDNA Amplification Reaction Mix to 35 µL sample.

22 Pipette mix 15x (pipette set to 90 µl). Centrifuge briefly.

23 Incubate in a thermal cycler with the following protocol:

	A	B	C
	Lid Temperature	Reaction Volume	Run Time
	105°C	100 µl	~30-45 min
	Step	Temperature	Time
	1	98°C	3 min
	2	98°C	15 sec
	3	63°C	20 sec
	4	72°C	1 min
	5	Go to Step 2 – 11 cycles	
	6	72°C	1 min
	7	4°C	Hold

Thermocycler protocol.

24 Store at 4 °C for up to 72:00:00 or -20 °C for ≤1 week or proceed to the next step. 3d

cDNA Cleanup – SPRIselect: 3d 0h 9m 30s

25 Vortex to resuspend the SPRIselect reagent. Add 60 µL SPRIselect reagent (0.6X) to each sample and pipette mix 15x (pipette set to 150 µl).

26 Incubate 00:05:00 at Room temperature . 5m

27 Place on the magnet. High until the solution clears.

28 Remove the supernatant.

29 Add 200 µL 80% ethanol to the pellet. Wait 00:00:30 . 30s

30 Remove the ethanol.

31 Repeat steps 29 and 30 for a total of 2 washes.

32 Centrifuge briefly and place on the magnet. Low.

33 Remove any remaining ethanol. Air dry for 00:02:00 . 2m

> **Note**
>
> DO NOT exceed 2 min as this will decrease elution efficiency.

34 Remove from magnet. Add 40.5 µL Buffer EB. Pipette mix 15x.

35 Incubate for 00:02:00 at Room temperature . 2m

36 Place the tube strip on the magnet. High until the solution clears.

37 Transfer 40 µL sample to a new tube strip.

38 Store at 4 °C for up to 72:00:00 or at -20 °C for up to 4 weeks, or proceed to the next step.

3d

cDNA Quantification

39 Run 1 µL sample (Dilution Factor 1:10) on an Agilent Bioanalyzer High Sensitivity chip.

Protocol references

https://cdn.10xgenomics.com/image/upload/v1660261285/support-documents/CG000505_Chromium_Nuclei_Isolation_Kit_UG_RevA.pdf