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Long-read single-cell genome, transcriptome and open chromatin profiling links genotype to phenotypes. V.1

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Alexandra Pančíková[1], Ruben Cools[1], Marios Eftychiou[1], Margo Aertgeerts[1], joris Vande Velde[1], Heidi Segers[2], Jan Cools[1], Luuk Harbers[1], Jonas Demeulemeester[1]

[1]VIB Center for Cancer Biology, VIB, Leuven, Belgium;
[2]Paediatric Oncology, Department of Oncology, KU Leuven, Leuven, Belgium

joris Vande Velde
VIB

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We use this protocol and it's working

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Abstract

The 10X Multiome ATAC + Gene Expression assay permits capture of both RNA expression and epigenomic profiles from the same nuclei for a deeper understanding of cell type or state gene regulation. Here we present a modified version of the published 10X protocol: SPLONGGET ! SPLONGGET captures whole genome, epigenome and open chromatin info at single cell level. It leverages 10X Genomics barcoding, modified library preparation, and fragment-length independent sequencing to unlock comprehensive single-cell multiomics.

Attachments

PDF

CG000338_ChromiumNex...

4.8MB

Guidelines

Notes before start: Use wide bore tips for all steps! Work on ice (4°C) at all times when extracting nuclei.

Nuclei Isolation

1 Nuclei Isolation is done according to the **10xgenomics protocol GC000365: Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing.**

- Resuspend nuclei in a minimum 50 µL of 1X Diluted Nuclei Buffer (20X nuclei buffer, PN2000207, provided by 10x Genomics), volume depends on target concentration. 1X Diluted Nuclei Buffer is prepared following the user guide **Chromium Next GEM Single Cell Multiome ATAC + Gene Expression (CG000338 Rev D)**.

- Count nuclei and dilute nuclei stock to be in the 970 - 3230 nuclei per µL range in 5 µL total volume using the dilution table below of the single cell Multiome ATAC + Gene Expression user guide_CG000338 Rev F. ⇒ **Do note targeting less than 3000 nuclei can result in ATAC / Whole Genome and cDNA PCR failure**

Nuclei Concentration Guidelines

Based on the Targeted Nuclei Recovery, resuspend the nuclei in Diluted Nuclei Buffer to get corresponding Nuclei Stock Concentrations (see Table). This enables pipetting volumes of the Nuclei Stock for Transposition (step 1.1) to be 2-5 µl. Higher Nuclei Stock Concentrations will result in lower pipetting volumes that may increase nuclei input variability.

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/µl)
3,000	970-2,420
4,000	1,290-3,230
5,000	1,610-4,030
6,000	1,940-4,840
7,000	2,260-5,650
8,000	2,580-6,450
9,000	2,900-7,260
10,000	3,230-8,060

Calculate volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of 5 µl

$$\text{Volume of Nuclei Stock (µl)} = \frac{\text{Targeted Nuclei Recovery x 1.61 (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/ µl)}}$$

Volume of Diluted Nuclei Buffer* (µl) = **5 µl** - volume of Nuclei Stock (µl)
*Use ONLY Diluted Nuclei Buffer (Dilute 20X Nuclei Buffer (PN-2000207) 1:20 in nuclease-free water)

Example Calculation

Targeted Nuclei Recovery = 4000 nuclei
Nuclei Stock Concentration = 2500 nuclei/µl
Recovery efficiency factor 1.61

$$\text{Volume of Nuclei Stock (µl)} = \frac{\text{Targeted Nuclei Recovery x 1.61 (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/µl)}} = \frac{4000 \times 1.61}{2500} = \mathbf{2.58\ µl}$$

Volume of Diluted Nuclei Buffer = 5 µl - 2.58 µl = **2.42 µl**

Chromium Next GEM Long Read Single Cell Multiome ATAC / Whole Genome + Gene Expression

2 **The first three sections are followed exactly as described in the Single Cell Multiome ATAC + Gene Expression User Guide (CG000338 Rev F):**
Section 1: Transposition (p27 to p30) ,
Section 2: GEM Generation & Barcoding (p31 to p37)
Section 3: Post GEM Incubation Cleanup (p38 to p42) are performed exactly as described in the User Guide: Chromium Next GEM Single Cell Multiome ATAC + Gene Expression - CG000338 Rev F

Note: In Section 3 you obtained 46 µl purified Sample to start with the modified Pre-Amplification PCR step

Pre-Amplification PCR

3 **GET SPLONGGET!**

Equilibrate Following Reagents to Room Temperature:

- Pre-AMP primers [10X: 2000271]
- SPRIselect Reagent [Beckman Coulter: B23318]

Place on Ice

- LongAmp® Hot Start *Taq* 2X Master Mix [NEB: M0533]

Prepare/Obtain

- Buffer EB [Qiagen: 19086]
- 80% Ethanol in Nuclease Free H20 - 1mL per Sample
- Magnetic Separator for PCR strips

4 **Prepare Pre-Amplification Mix + PCR**

- Prepare Pre-Amplification Mix on ice!

	A	B
	Reagent	**µl / Reaction**
	Post GEM Cleanup Sample	46
	LongAmp Hot Start Taq 2X Master Mix	50
	Pre-Amp Primers	4
	Total Volume	**100 µl**

- Pipette mix and centrifuge briefly to collect reaction at the bottom, before incubating the plate in a thermocycler at:

	A	B	C
	Lid Temperature Time	**Reaction Volume**	**Run Time**
	105 °C	100 µl	30 min
	Step	**Temperature**	**Time**
	1.	65°C	5 min

	A	B	C
	2.	95°C	3 min
	3.	95°C	30 sec
	4.	63°C	6 min Go to step 3 repeat 6X (Total 7 cycles)
	5.	4°C	Hold (Max 18 hours)

The table recommends a starting point for cycle number optimization for based on Targeted Nuclei Recovery.

5 Pre-Amplification (1.6X) SPRI Cleanup

5.1 Vortex the SPRIselect reagent until fully resuspended. Add **160 µl (1.6X)** SPRIselect reagent
to each sample. Pipette mix thoroughly

5.2 Incubate **5 min** at room temperature

5.3 Centrifuge briefly. Place on the magnet pos High until the solution clears

5.4 Remove the supernatant

5.5 Add 300 µl 80% ethanol to the pellet. Wait **30 sec** and **discard all ethanol**

5.6 Add 200 µl 80% ethanol to the pellet. Wait **30 sec** and **discard all ethanol**

5.7 **After the last ethanol removal - Centrifuge briefly** and Place on the magnet pos Low

5.8 Remove any remaining ethanol

5.9 Remove the tube strip from the magnet. Immediately add **100 µl Buffer EB**

5.10 Gently pipette mix (pipette set to 90 µl) without introducing bubbles

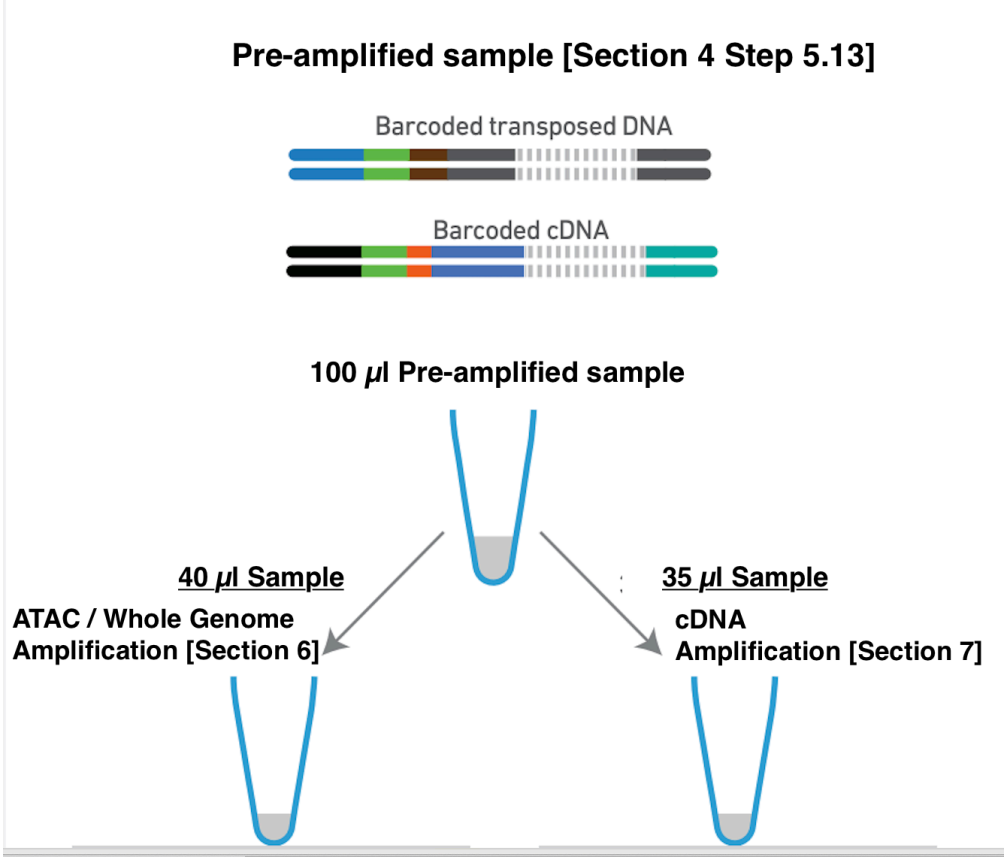
5.11 Incubate **5 min** at room temperature

5.12 Centrifuge briefly. Place on the magnet pos High until the solution clears.

5.13 Transfer 100 µl Pre-AMP sample to a new tube strip

5.14 **Safe Stop:** Store at 4°C for up to 72 h or at −20°C for long term storage, or proceed to the next
step

5.15



⇒ Store remaining pre-amplifcation product at -20°C

ATAC / Whole Genome Amplification

6 **Equilibrate Following Reagents to Room Temperature:**

- Sample Index Plate N, Set A [10X: 300427]
- SPRIselect Reagent [Beckman Coulter: B23318]

Place on Ice

- LongAmp® Hot Start *Taq* 2X Master Mix [NEB: M0533]
- SI-PCR Primer B [10X:2000128]

Prepare/Obtain

- Buffer EB [Qiagen: 19086]
- 80% Ethanol in Nuclease Free H20 - 1mL per Sample
- Magnetic Separator for PCR strips

7 **ATAC / Whole Genome Amplification + Sample index**

- Prepare Sample Index PCR Mix on ice!

	A	B
	Reagent	**ul / Reaction**
	Pre-amplified sample	40
	LongAmp Hot Start Taq 2X Master Mix	50
	SI-PCR Primer B	7,5
	Total Volume	**97,5 µl**

- Pipette mix and centrifuge briefly to collect reaction at the bottom.
- **Add 2,5 µl of an individual Sample Index N, Set A** to each sample. [Choose the appropriate sample index sets to ensure no sample indices overlap in a multiplexed sequencing run] ⇒ **Total Volume of ATAC / Whole Genome PCR reaction = 100 µl**
- Pipette mix and centrifuge briefly to collect reaction at the bottom, before incubating the plate in a thermocycler at:

	A	B	C
	Lid Temperature	**Reaction Volume**	**Run Time**
	105°C	100 µl	59 min
	Step	**Temperature**	**Time**
	1.	95°C	1 min 30 sec
	2.	95°C	30 sec
	3.	65°C	6 min Go to step 2 repeat 7X (Total 8 cycles)
	4.	65°C	5 min
	5.	4°C	Hold (Max 72 hours)

The optimal number of cycles is a trade-off between generating sufficient final mass for library construction and minimizing PCR amplification artifacts. The number of cDNA cycles should also be reduced if large numbers of nuclei are sampled.

8 Post ATAC / Whole Genome amplification (1.6X) SPRI Cleanup

8.1 Vortex the SPRIselect reagent until fully resuspended. Add **160 µl (1.6X)** SPRIselect reagent
to each sample. Pipette mix thoroughly

8.2 Incubate **5 min** at room temperature

8.3 Centrifuge briefly. Place on the magnet pos High until the solution clears

8.4 Remove the supernatant

8.5 Add 300 µl 80% ethanol to the pellet. Wait **30 sec** and **discard all ethanol**

8.6 Add 200 µl 80% ethanol to the pellet. Wait **30 sec** and **discard all ethanol**

8.7 **After the last ethanol removal - Centrifuge briefly** and Place on the magnet pos Low

8.8 Remove any remaining ethanol

8.9 Remove the tube strip from the magnet. Immediately add **32 µl Buffer EB**

8.10 Gently pipette mix (pipette set to 90 µl) without introducing bubbles

8.11 Incubate **10 min** at room temperature

8.12 Centrifuge briefly. Place on the magnet pos Low until the solution clears.

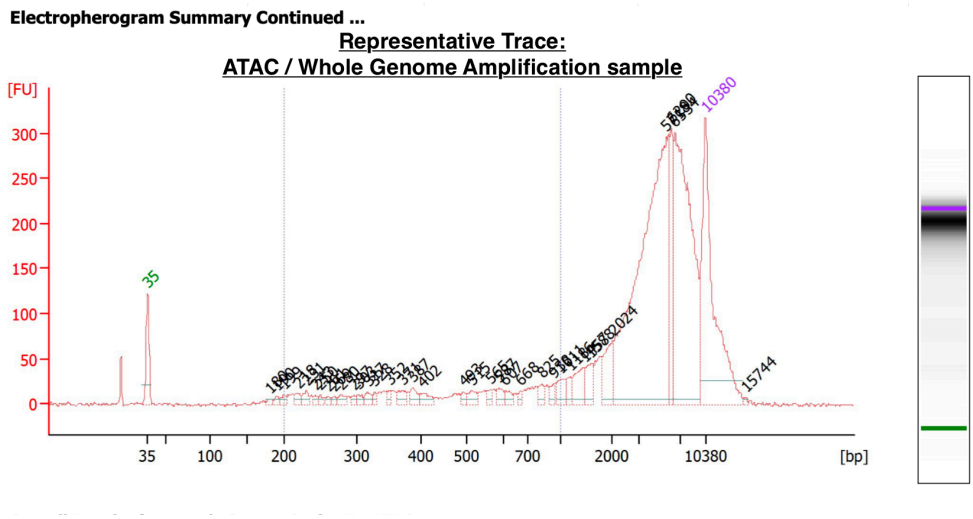
8.13 Transfer 32 µl ATAC / Whole Genome sample to a new tube strip

8.14 Measure sample Concentration on Qubit.

8.15 Store at 4°C for up to 72 h or at −20°C for long term storage until Oxford Nanopore Ligation sequencing DNA V14 (SQK-LSK114).

9 **Post ATAC / Whole Genome Amplification QC**

- Run 1 µl sample **(max 5ng)** on the Agilent Bioanalyzer High Sensitivity DNA chip to determine fragment size.



- Alternate QC methods like Agilent TapeSation or LabChip can be used as well.

cDNA Amplication

10 **Equilibrate Following Reagents to Room Temperature:**

- cDNA primers [10X: 2000089]
- SPRIselect Reagent [Beckman Coulter: B23318]

Place on Ice

- Amp Mix [10X: 2000047/2000103]

Prepare/Obtain

- Buffer EB [Qiagen: 19086]
- 80% Ethanol in Nuclease Free H20 - 1mL per Sample
- Magnetic Separator for PCR strips

11 **cDNA Amplification**

- Prepare cDNA amplification PCR Mix on ice!

	A	B
	Reagent	**µl / Reaction**
	Pre-amplified sample	35
	Amp Mix	50
	cDNA Primers	15
	Total Volume	**100 µl**

- Pipette mix and centrifuge briefly to collect reaction at the bottom, before incubating the plate in a thermocycler at:

	A	B	C
	Lid Temperature	**Reaction Volume**	**Run Time**
	105°C	100 µl	59 min
	Step	**Temperature**	**Time**
	1.	98°C	3 min
	2.	98°C	15 sec
	3.	63°C	20 sec
	4.	72°C	1 min Go to step 2 repeat 7X (Total 8 cycles)
	5.	72°C	2 min
	6.	4°C	Hold (Max 72 hours)

12 Post cDNA amplification (0.6X) SPRI Cleanup

12.1 Vortex to resuspend the SPRIselect reagent. Add **60 µl SPRIselect reagent (0.6X)** to each sample and pipette mix thoroughly

12.2 Incubate **5 min** at room temperature

12.3 Place on the magnet pos High until the solution clears.

12.4 Remove the supernatant

12.5 Add 200 µl 80% ethanol to the pellet. Wait **30 sec** and **discard all ethanol**

12.6 Add 200 µl 80% ethanol to the pellet. Wait **30 sec** and **discard all ethanol**

12.7 **After the last ethanol removal - Centrifuge briefly** and Place on the magnet pos Low

12.8 Remove any remaining ethanol

12.9 Remove the tube strip from the magnet. Immediately add **32 µl Buffer EB**

12.10 Gently pipette mix (pipette set to 90 µl) without introducing bubbles

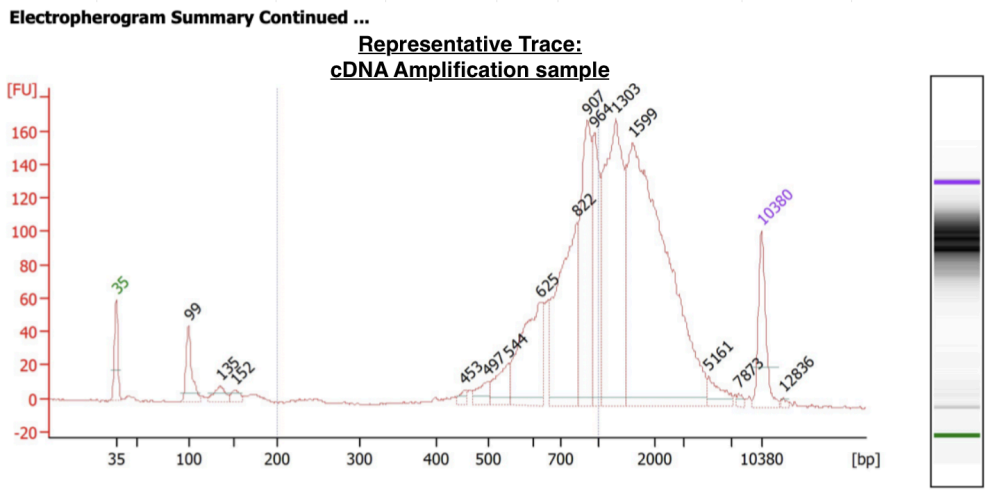
12.11 Incubate **5 min** at room temperature

12.12 Centrifuge briefly. Place on the magnet pos Low until the solution clears.

12.13 Transfer 32 µl cDNA sample to a new tube strip

13 **Post cDNA Amplification QC**

- Run 1 µl sample **(max 5 ng)** on the Agilent Bioanalyzer High Sensitivity DNA chip to determine fragment size.



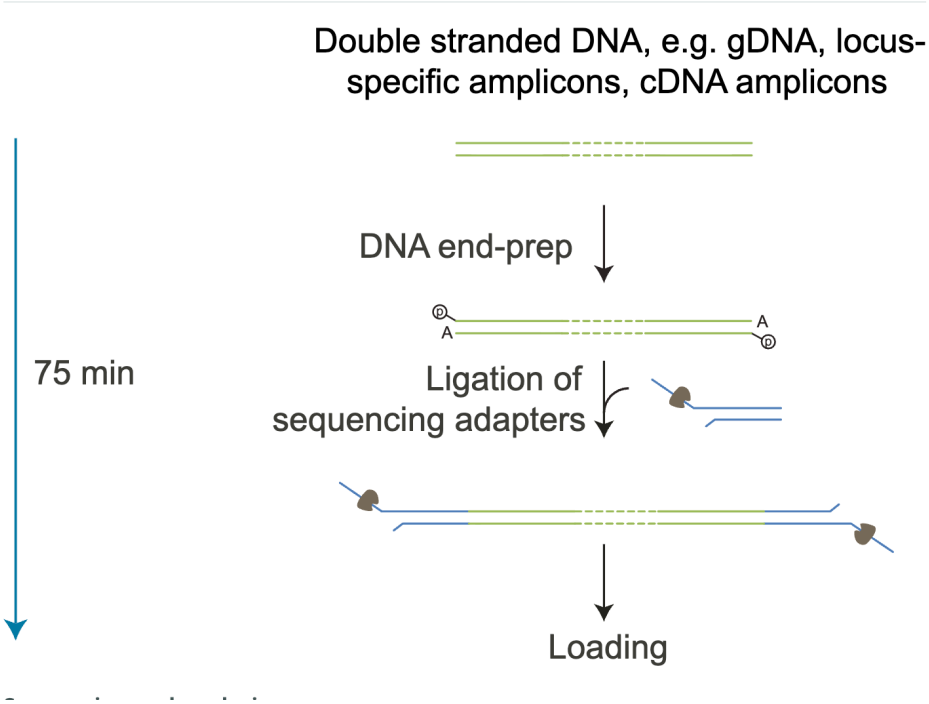
Oxford Nanopore (ONT) Library preparation and Sequencing

14

- **ONT Library Preparation**

Both ATAC / Whole Genome and non fragmented cDNA samples are library prepped exactly following **Oxford Nanopore Ligation sequencing amplicons V14 (SQK-LSK114**) protocol:

- LSK114 Library Input amounts were adjusted to 150 fmol
- Short Fragment Buffer (ONT, SFB) was used during library preparation wash steps to retain all fragment sizes



https://nanoporetech.com/document/ligation-sequencing-amplicons-sqk-lsk114

- **ONT sequencing**

Each LSK114 library is sequenced separately on minimal 1 Oxford Nanopore PromethION FlowCell [FLO-PRO114M] (or latest PromethION FlowCell version).

- **Expected Yield**

With a yield of ~80-100M reads per flow cell, we typically achieve a coverage of ~4000 transcripts per cell for a library containing ~3,000 cells