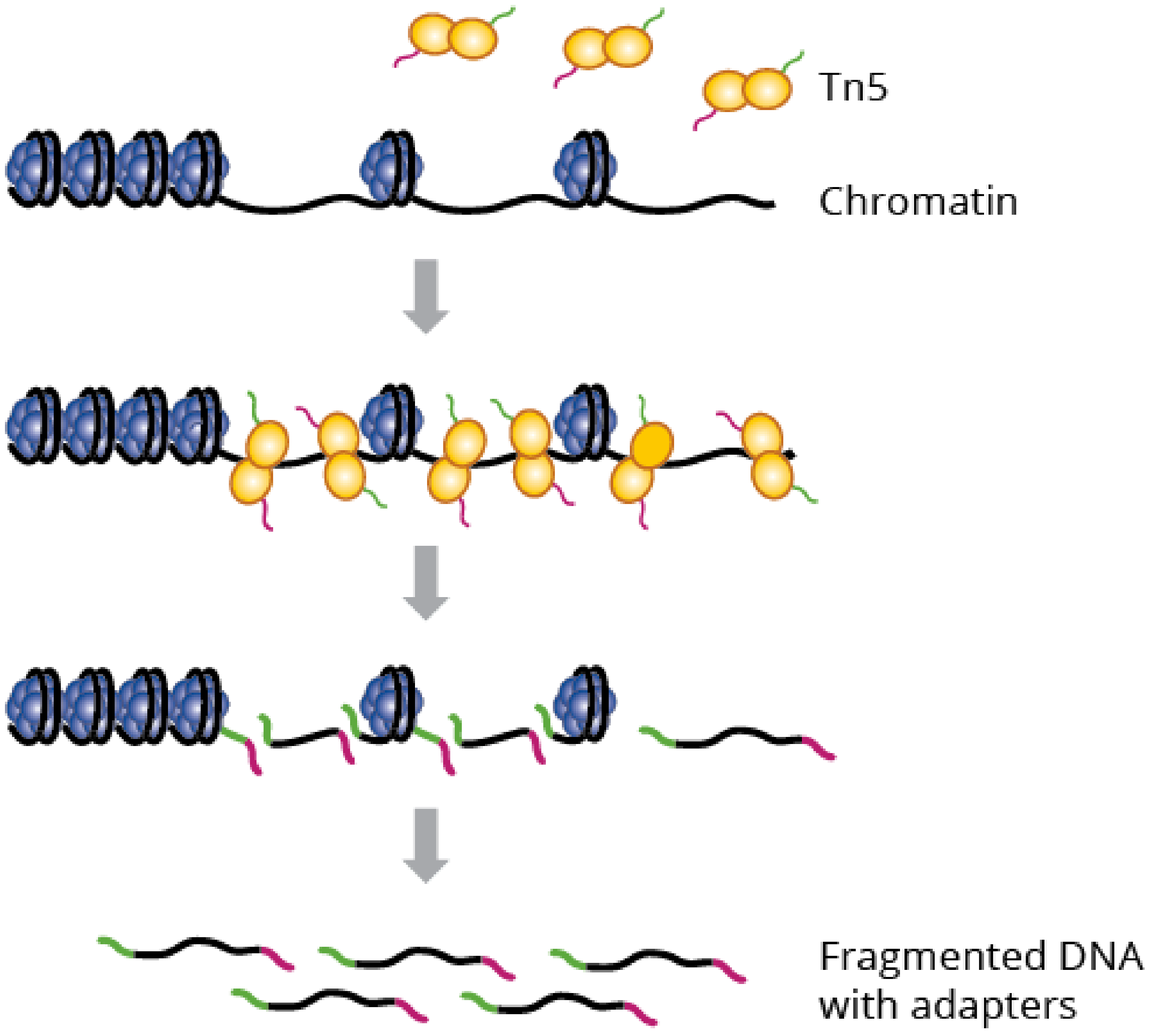
Getting started with scATACseq

EMBL course: Attacking open chromatin with ATAC sequencing

April 2024

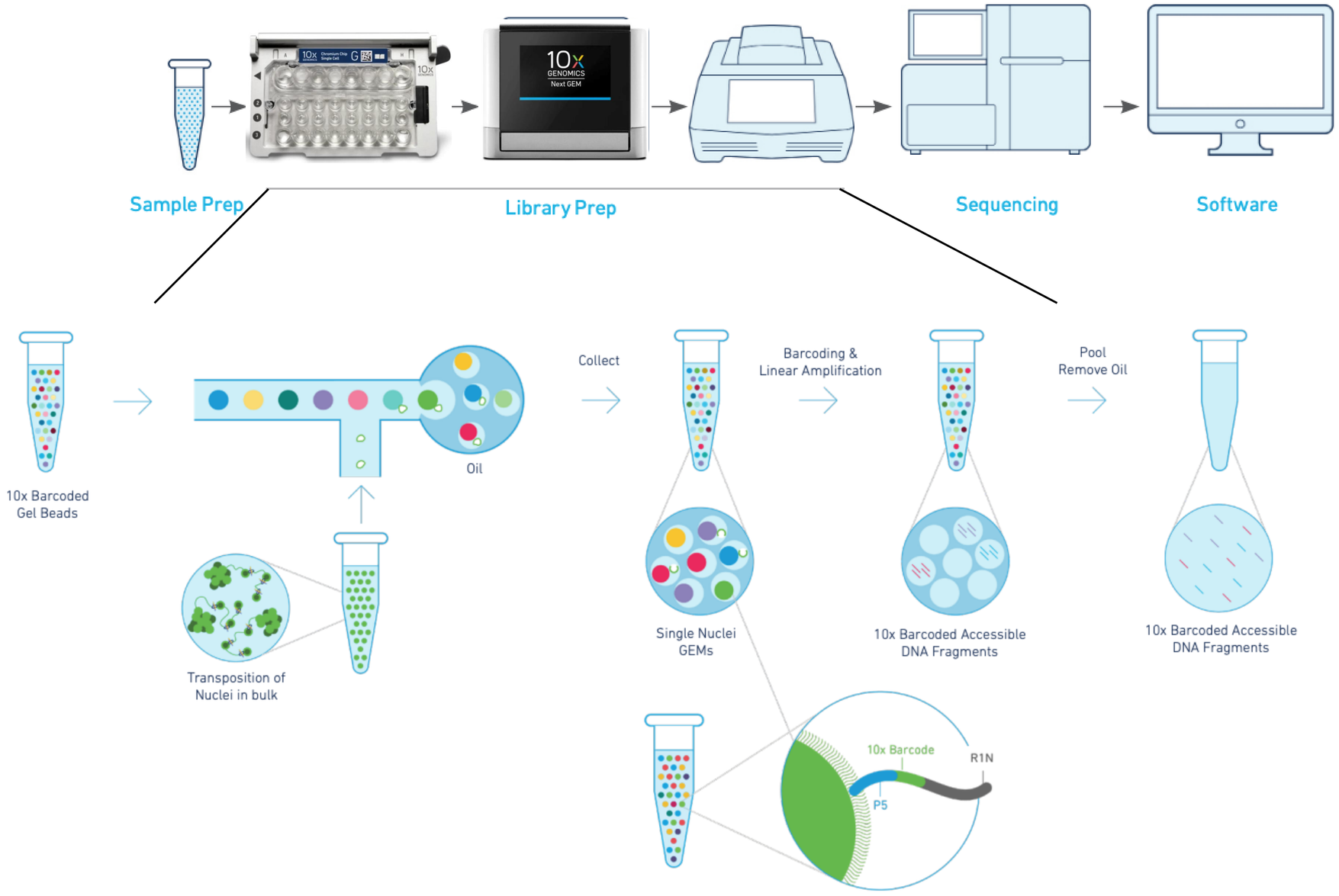
Introduction to 10x scATACseq

the basics - key steps in ATACseq



10x technology

overview



Practical day1

overview

	Steps		Timing	Stop & Store
4 h	**Nuclei Isolation**			
	Dependent on Cell Type		~1-2 h	
	Step 1 – Transposition			
	1.1	Prepare Transposition Mix	10 min	
	1.2	Isothermal Incubation	30 min	
	Step 2 – GEM Generation & Barcoding			
	2.1	Prepare Master Mix	10 min	
	2.2	Load Chromium Next GEM Chip H	10 min	
	2.3	Run the Chromium Controller	18 min	
	2.4	Transfer GEMs	3 min	
	2.5	GEM Incubation	45 min	STOP 15°C ≤18 h or –20°C ≤ 1 week

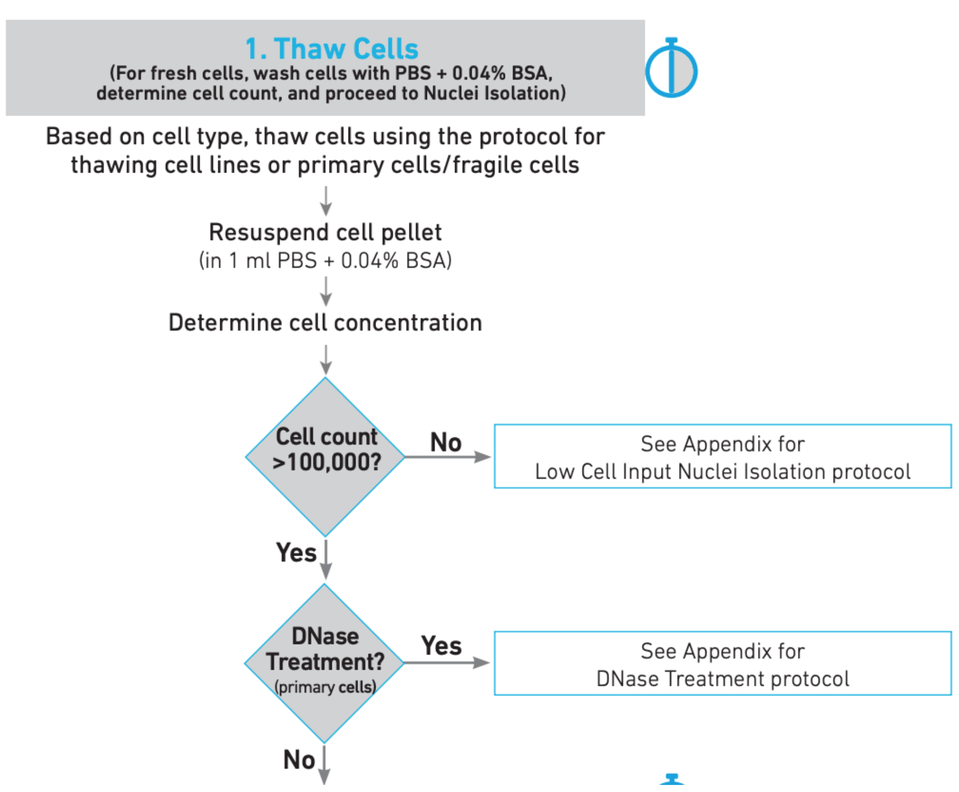
Practical day2

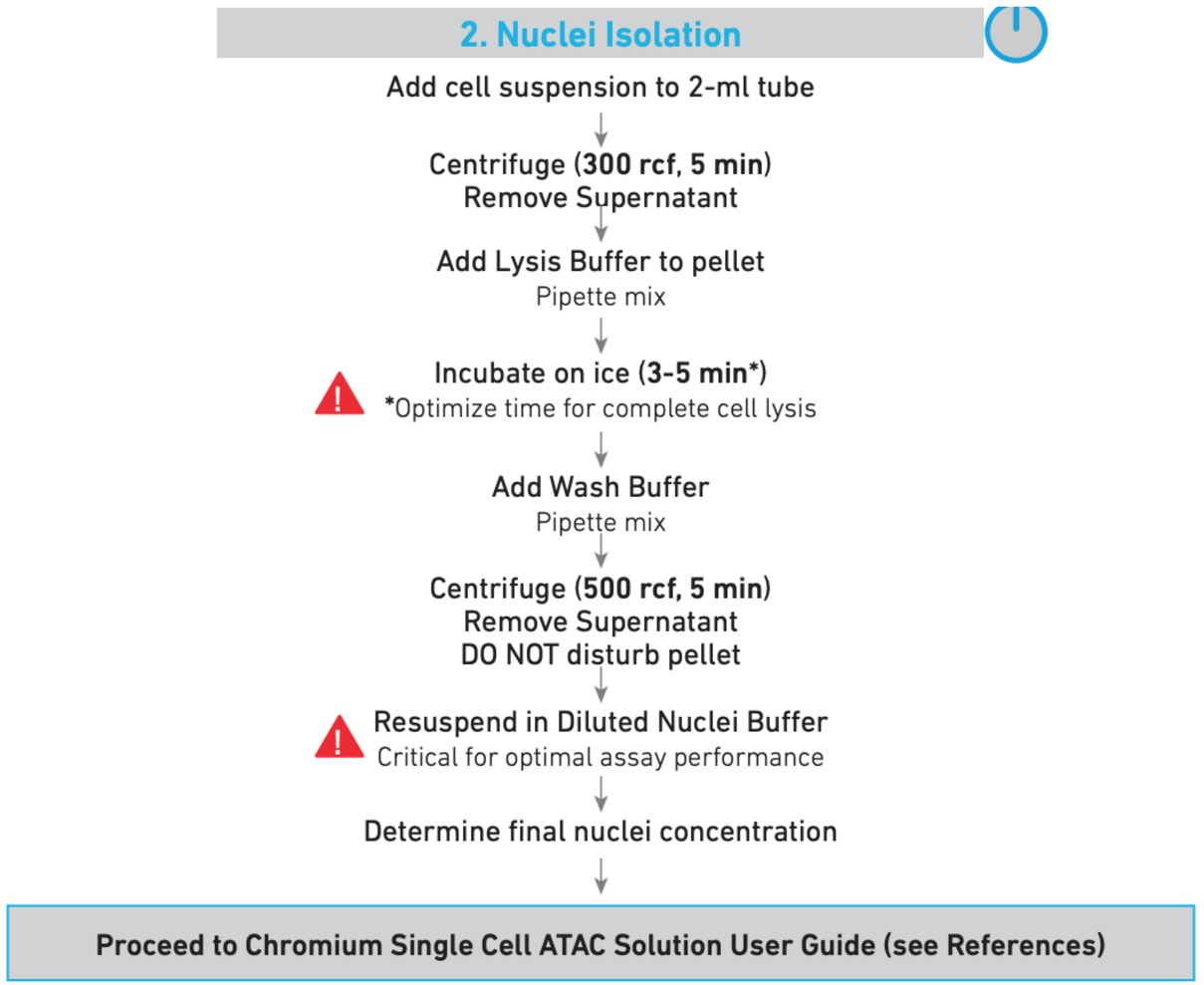
overview

3 h	**Step 3 – Post GEM Incubation Cleanup**			
	3.1	Post GEM Incubation Cleanup – Dynabeads	35 min	
	3.2	Post GEM Incubation Cleanup – SPRIselect	15 min	STOP 4°C ≤ 72 h or –20°C ≤ 2 weeks
	Step 4 – Library Construction			
	4.1	Sample Index PCR	40 min	
	4.2	Post Sample Index Double Sided Size Selection – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C long-term
	4.3	Post Library Construction QC	60 min	

Sample prep

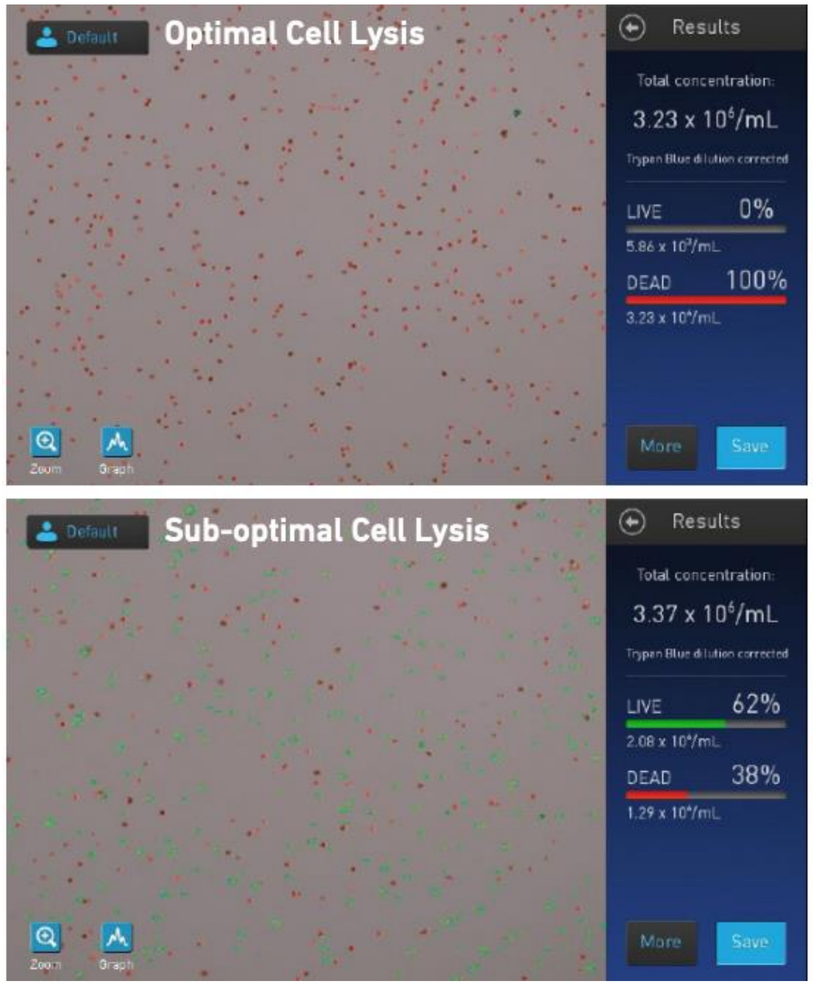
most important step

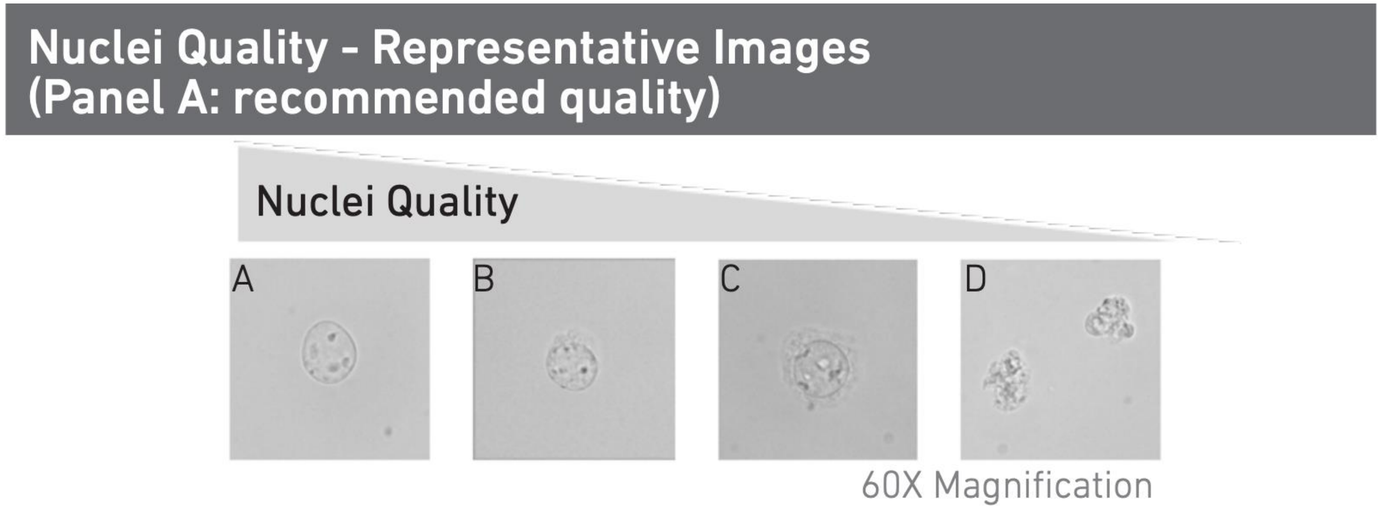




Sample Prep

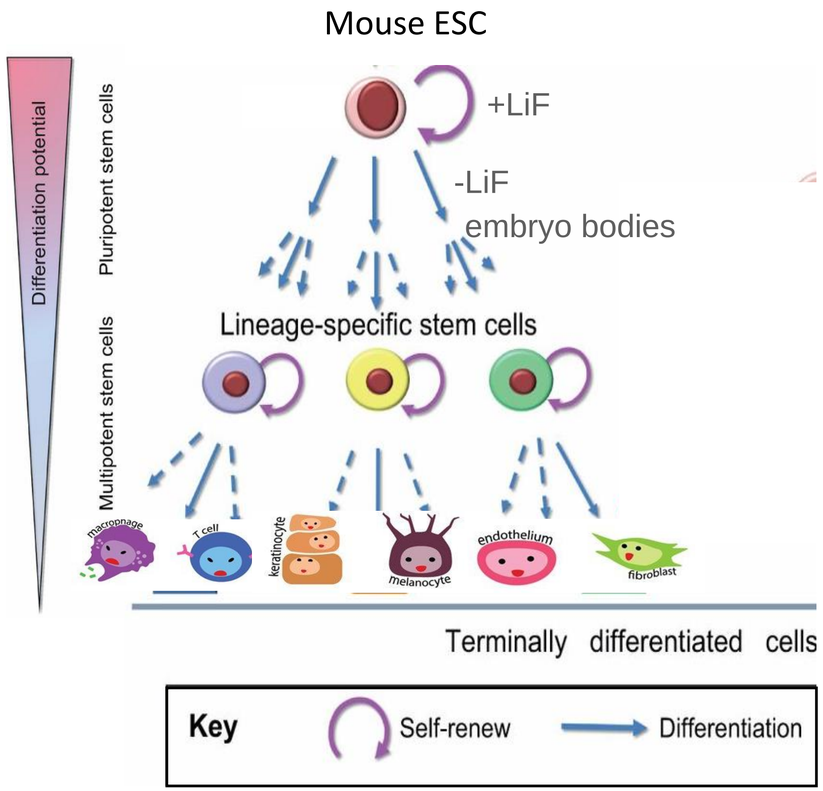
trash in - trash out

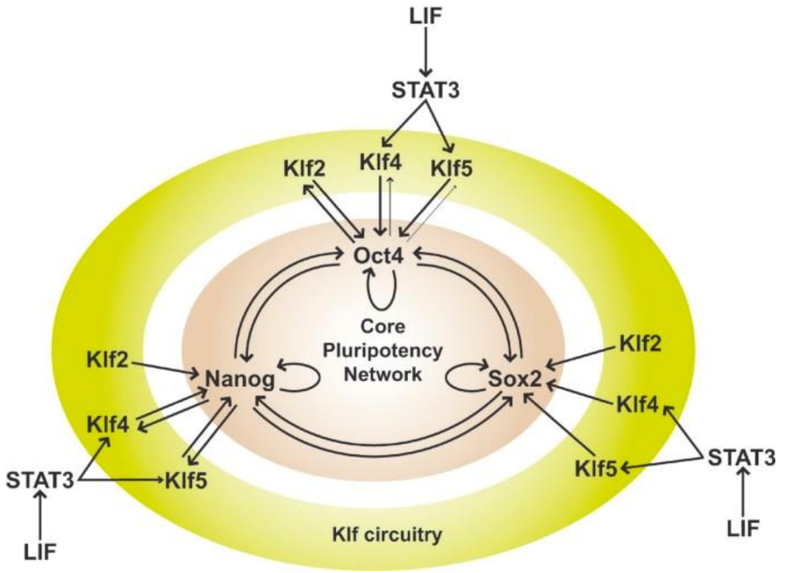




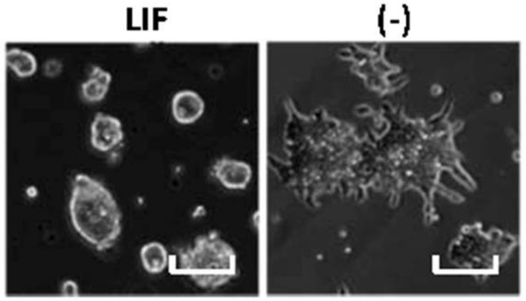
Let’s start

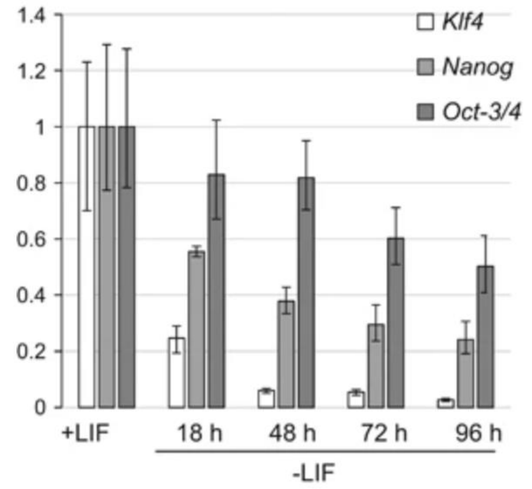
- 9 groups, 9x2 samples (replicates)
- samples are 1 x10^6 cells already in suspension
- an equal mixture of undifferentiated mESCs and mESCs cultured without LIF (induction of differentiation)





Bourillot PY & Savatier P, 2010





Mix of 1 x 10^6 cells:

- mESCs + LiF (pluripotent)
- mESCs -LiF (differentiating)

2. Nuclei Isolation

If using fresh cells, perform 1-2 washes with PBS + 0.04% BSA and determine cell count, before proceeding to step 2a. Nuclei may be isolated from 100,000-1,000,000 cells using this protocol. Viability >70% is recommended before starting nuclei isolation.

a. Add 100,000-1,000,000 cells to a 2-ml microcentrifuge tube. Centrifuge at **300 rcf** for **5 min** at **4°C**.

b. Remove **ALL** the supernatant without disrupting the cell pellet.

c. Add **100 µl** chilled Lysis Buffer. Pipette mix 10x.

d. Incubate for **3-5 min*** on ice.
*Cryopreserved PBMCs were incubated for **3 min**
*Cryopreserved cell lines were incubated for **5 min**

Optimize incubation time based on cell type. Sub-optimal or prolonged lysis times can both alter assay performance. Assess lysis efficacy via the Countess II FL Automated Cell Counter/microscopy (see Results).

e. Add **1 ml** chilled Wash Buffer to the lysed cells. Pipette mix 5x.

f. Centrifuge at **500 rcf** for **5 min** at **4°C**.

g. Remove the supernatant without disrupting the nuclei pellet.

h. Based on cell concentration step 2a and assuming ~50% nuclei loss during cell lysis, resuspend in chilled Diluted Nuclei Buffer. See Nuclei Stock Concentration Table and Example Calculation in Appendix. Maintain on ice.

The resuspension in Diluted Nuclei Buffer is critical for optimal Single Cell ATAC assay performance. The composition of the Tris-based Diluted Nuclei Buffer, including Magnesium concentration, has been optimized for the Transposition and Barcoding steps in the Single Cell ATAC protocol. Suspension of nuclei in a different buffer may not be compatible.

i. **OPTIONAL** If cell debris and large clumps are observed, pass through a cell strainer. For low volume, use a **40 µm** Flowmi Cell Strainer to minimize volume loss.

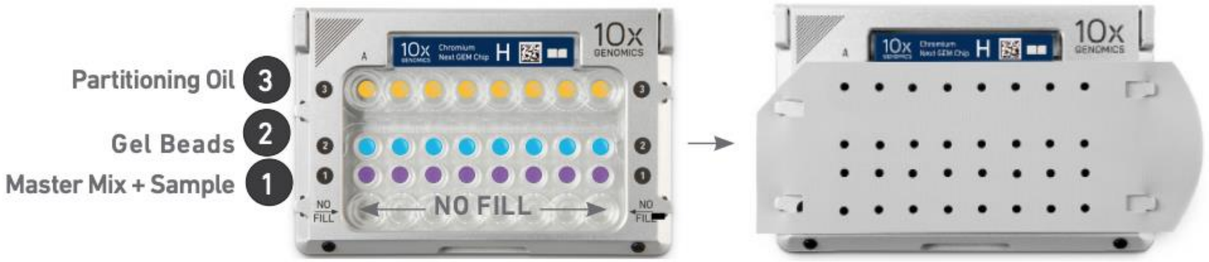
j. Determine the nuclei concentration using a Countess II FL Automated Cell Counter (see Appendix) or a hemocytometer.

k. Proceed **immediately** to Chromium Single Cell ATAC Solution User Guide (see References).

10x Chip GEM formation

g. Attach 10x Gasket

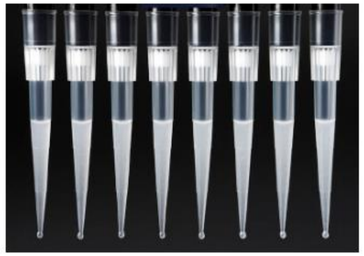
Align the notch with the top left-hand corner. Ensure the gasket holes are aligned with the wells. Avoid touching the smooth surface.

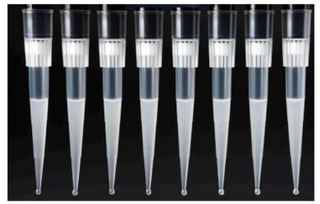
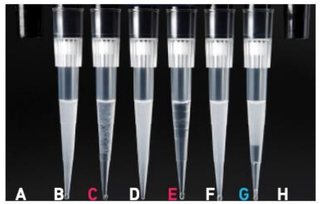


Keep horizontal to avoid wetting the gasket. DO NOT press down on the gasket.

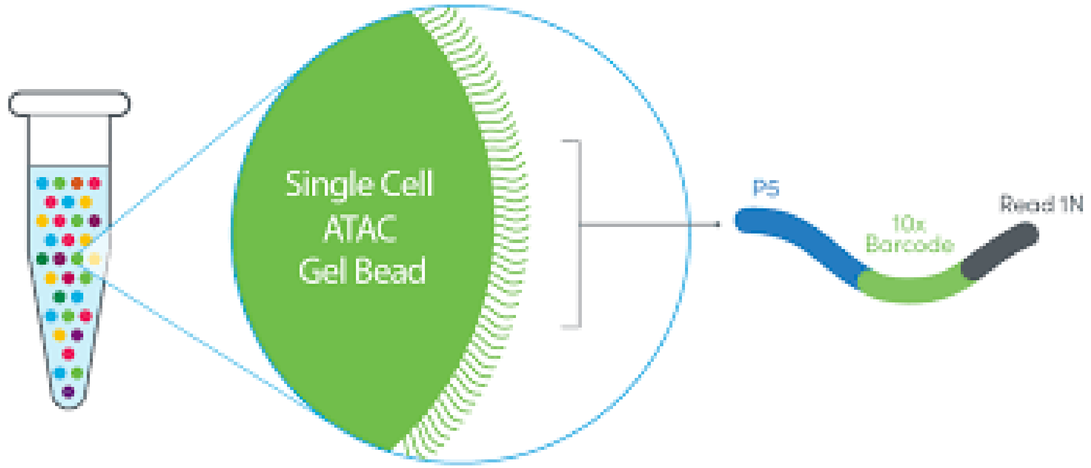
Transfer GEMs

GEMs



STEP	NORMAL	REAGENT CLOGS & WETTING FAILURES
2.4 d After Chip H is removed from the Controller and the wells are exposed	 All 8 recovery wells (row labeled 3) are similar in volume and opacity.	 Recovery well G indicates a reagent clog. Recovery well C and E indicate a wetting failure. Recovery wells B, D, and F are normal. Wells A and H contain 50% Glycerol Solution.
2.4 f Transfer GEMs from Chip H Row Labeled 3	All liquid levels are similar in volume and opacity without air trapped in the pipette tips. 	Pipette tips C and E indicate a wetting failure. Pipette tip C contains partially emulsified GEMs. Emulsion is absent in pipette tip E. Pipette tip G indicates a reagent clog. 

Day2 practical part



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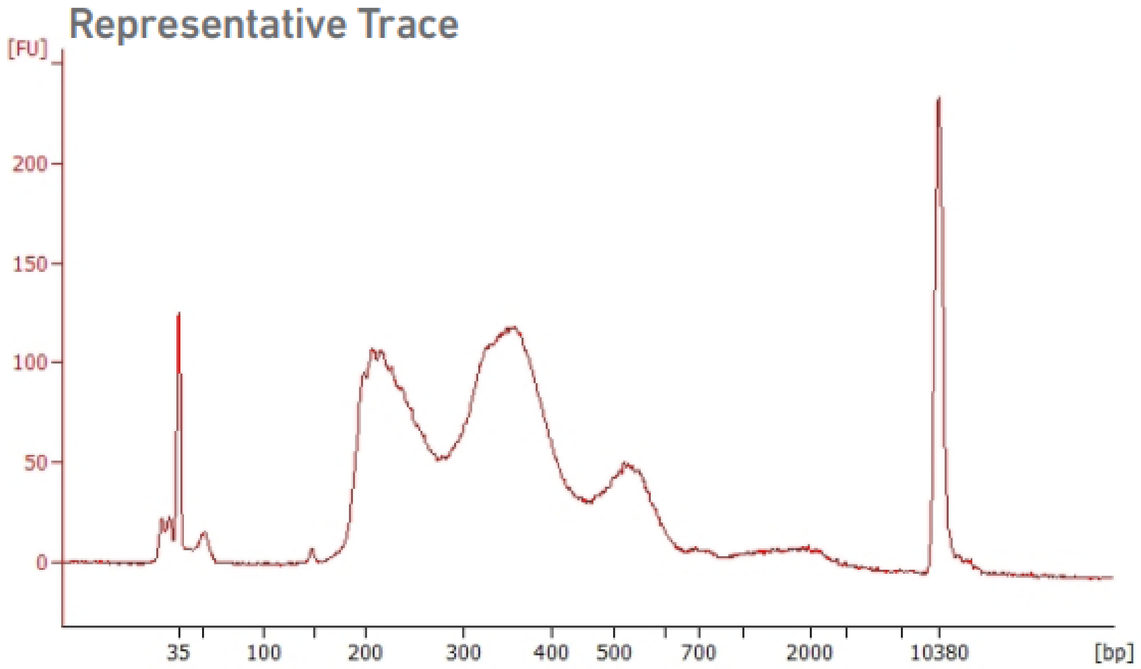
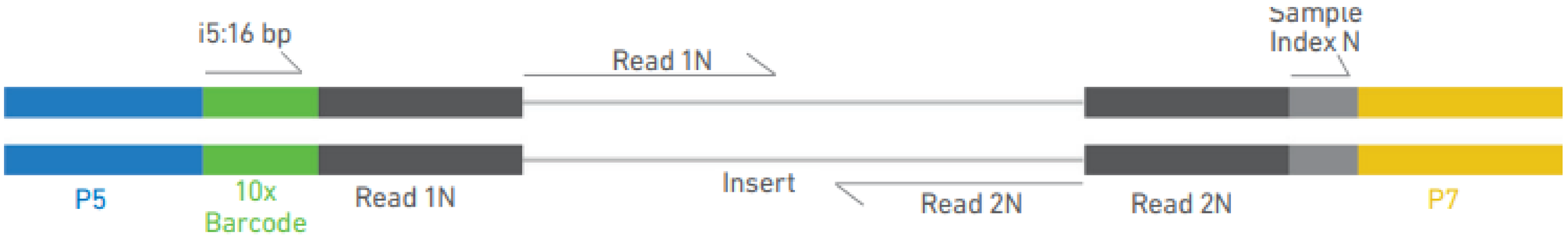
QC and sequencing





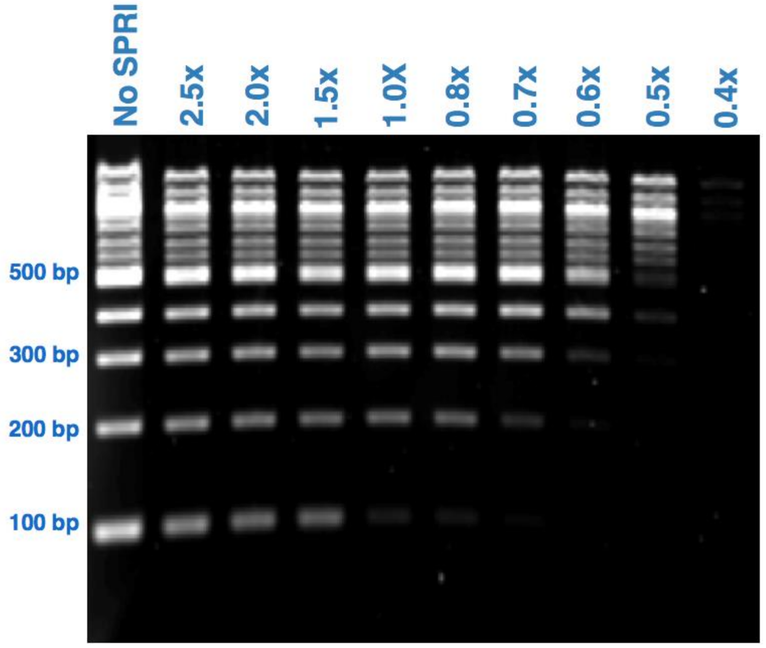
Beads

- Dynabeads: The use of DynaBeads MyOne Silane beads is critical for removing contaminants post GEM incubation and the cleanup of the product

The Dynabeads MyOne™ SILANE are monosized ferrimagnetic beads are 1 µm in diameter, and are composed of highly cross-linked polystyrene with evenly distributed magnetic material. The beads are further coated, enclosing the iron oxide inside the beads and presenting a bead surface with optimized silica-like chemistry. The increased magnetic strength of these beads ensure rapid magnetic mobility and efficient isolation of DNA.

- SPRI beads: used for removing certain size of nucleic acid based on the ratio of salt (NaCL) to DNA

Solid Phase Reversible Immobilisation beads



Each bead is made of polystyrene surrounded by a layer of magnetite, which is coated with carboxyl molecules. It is these that reversibly bind DNA in the presence of the “crowding agent” polyethylene glycol (PEG) and salt (20% PEG, 2.5M NaCl is the magic mix).