CELL GROWTH MUST BE IN EXOPNENTIAL PHASE
> 10,000 CELLS COUNTED ON BIORAD.
USE OWN MEDIUM.

Protocol for ATAC-seq method (tested for cell line GM12878 and HPC cells)

21. 09. 2015

Cell harvesting and counting

Cells were grown in suspension 10 mL RPMI medium, resuspend several times with the long pipette.

- Take out 5 mL cell suspension from T25 flask, transfer into 15 mL falcon tube
- Centrifuge cell suspension at 1000 rpm/500 x g, 5 min, 4°C
- Take out 4 mL supernatant and resuspend the cells in 1 mL remaining medium
- Use BioRad cell counter /Trypan Blue to count the cells
- If BioRad not accurate enough: mix 90 µl Trypan Blue with 10 µl cell suspension, count using Neubauer chamber
- Dilute the corresponding amount of cells into 1 mL fresh medium to obtain 50'000 cells

Library preparation (w/o lysis step)

- Centrifuge 50,000 cells 5 min at 500 × g, 4°C
- Remove and discard supernatant. Wash cells once with 50 µl of cold PBS buffer, transfer cell suspension into PCR tubes
- Centrifuge 5 min at 500 × g, 4°C
- Remove and discard supernatant, **DRY PELLET important!**
- Make sure the cell pellet is placed on ice
- To make the transposition reaction mix, combine the following (master mix can be prepared):
    - 25 µl TD (Tagment DNA Buffer)
    - 2.5 µl TDE1 (Amplicon Tagment Mix)
    - 22 µl nuclease-free H2O
    - 0.5 µl 1% Digitonin (solved in DMSO) → DUE TO AVOIDING MITOCH. DNA.
- Resuspend nuclei pellet in the transposition reaction mix, 5x pipetting up and down
- Incubate the transposition reaction at 37°C for 30 min
- Purify using a Qiagen MinElute PCR Purification Kit
- Elute transposed DNA in 10 µl elution buffer (**Buffer EB** from the MinElute kit consisting of 10 mM Tris·Cl, pH 8)
- Store purified DNA at −20°C if necessary

PCR amplification

To amplify transposed DNA fragments, combine the following in a 0.2 ml PCR tube

- 10 µl transposed DNA
- 10 µl nuclease-free H2O
- 2.5 µl of 25 µM Nextera Indexing Primer 1
- 2.5 µl of 25 µM Nextera Indexing Primer 2
- 25 µl of NEBNext High-Fidelity 2x PCR Master Mix

For sample 1: White lid S502 (120 µL) ID 2012939-S502 / Orange lid N703 (80 µL) ID 2023118-N703

For sample 2: White lid S503 (120 µL) ID 2014025-S503 / Orange lid N705 (80 µL) ID 2026886-N705

For sample 3: White lid S505 (120 μL) ID 2010309-S505 / Orange lid N706 (80 μL) ID 2024194-N706

Thermal cycle

- 1 cycle: 5 min @72°C
    - 30 sec @98°C
- 5 cycles: 10 sec @98°C
  30 sec @63°C
  1 min @72°C
- Hold @4°C

qPCR check

Prepare qPCR Master Mix as the following (conc. for 4x master mix):

- 14.4 μl nuclease-free H2O
- 3.6 μl SYBR Green (10x) (dilute SYBR Green 100x as the following: 900 μl H2O, 100 μl SYBR Green (100x))
- 20 μl NEBNext High-Fidelity 2x PCR Master Mix

To each well, add 9.5 μl Master Mix

Add

- 5 μl of previously amplified DNA
- 0.25 μl Nextera PCR primer 1
- 0.25 μl Nextera PCR primer 2

Using a qPCR cycler, do as the following

- 1 cycle: 30 sec @98°C
- 25 cycles: 10 sec @98°C
  30 sec @63°C
  1 min @72°C

According to the standard protocol 1/3 of the height should be the number of additional cycles needed, if # of additional cycles >12, then the quality of the library cannot be guaranteed.

Run additional cycles

- 1 cycle: 30 sec @98°C
- N cycles: 10 sec @98°C
  30 sec @63°C
  1 min @72°C
- Hold @4°C

SPRI beads cleanup (aims to take all DNA products, no size selection)

DNA cleanup with SPRI magnetic beads 1.2: 1 (1.2x volume of beads : 1 volume of PCR product)

For 45 µl PCR product mix, add 54 µl Magnetic beads

Always prepare 1 mL fresh 80% EtOH

At RT!

- Pipette up and down 20x with 70 µl volume, avoid bubbles and always homogenize the suspension during the 10 min incubation time
- Put the tubes on magnet, wait until the beads are separated from the solution (ca. 5 min)
- Discard supernatant, try to take out all liquid!
- Add 150 µl of 80% EtOH to the beads (tubes remain on the magnet!), incubate for 30 sec at RT
- Repeat washing procedure with 80% EtOH
- Take out all supernatant EtOH, first with 200 µl pipette, then with 10 µl pipette, let tubes drying on magnet for 5 min, take off the tubes, let it drying for 3 min
- Elute PCR products in 15 µl elution buffer (add 17 µl!), pipette 15x up and down
- Put the tubes on magnet, wait until the beads are separated from the solution (ca. 5 min)
- Take out 15 µl out of each tube

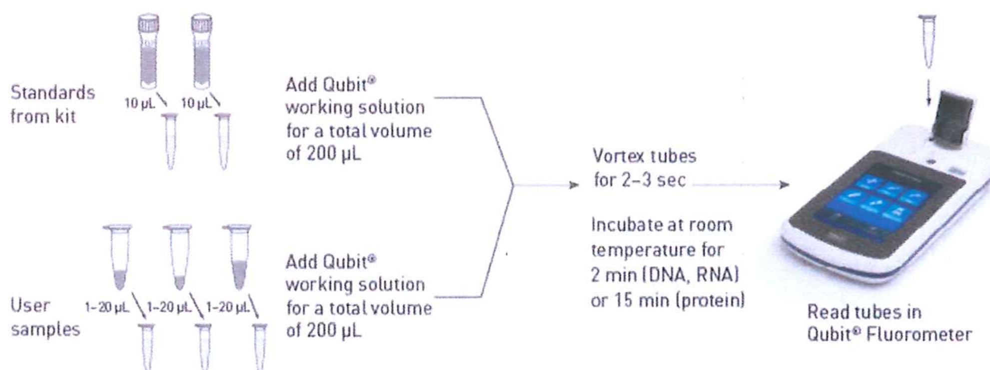
QC using Qubit

Prepare MasterMix:

For 1 sample: 199 µl buffer, 1 µl dye

Use 2 DNA standards (**190 µl premix, 10 µl DNA**), for DNA samples (199 µl premix, 1 µl DNA)

Sample 1: xxx ng/µl Sample 2: xxx ng/µl Sample 3: xxx ng/µl



Pooling of the samples

Measure DNA conc. of samples with Qubit (Replicate A, B, C), take the sample with the highest conc. as $c_{(highest)}$:

$c_{(highest)}/c_{(sample)} \times 2$ µl

or

$c_{(highest)}/c_{(sample)} \times 3\ \mu l$

Pool the samples and store the rest at -20°C, use Eppendorf tubes of the gene core facility

QC using Bioanalyzer

Use 1 µl of purified product for a Bioanalyzer