1. **Preparation of cells or nuclei from 2.1. fresh or 2.2 frozen cells**

2.1 Make a single cell suspension of your cells of interest. For adherent cultured cells, use Accutase to release cells from dish. Stop the reaction using full culture media.

2.2 If cells are frozen, quick-thaw tube in hand or by dipping in 37°C water bath, transfer cells into a larger tube and let cells recover in warm media (containing FBS) for around 30 min. Incrementally double the volume of warm media until you have a total volume of x-x ml (30-40?)

2.3. Spin at 500g for 5 min RT and aspirate media. Wash cells by gently resuspending them in PBS and spin down at 500g for 5 min

2.4. Discard PBS, resuspend pellet in 100 µl PBS.

2.5. Fix cell suspension by adding 100 µl 2% Paraformaldehyde (PFA) resulting in a 1% PFA concentration for 10 min at room temperature.

2.6. Quench fixation by adding 200 µl 0.25M Glycine to a final concentration of 0.125 M.

2.7. Spin the fixed cells at 500g for 10 min, and remove the supernatant.

2.8. Resuspend the cell pellet in 500 µl PBS, continue to counting.

Note: The protocol can be paused here. Fixed cell suspensions are stable for up to 2 weeks at 4°C.

1. **Count cells**

Count cells under microscope using Trypan blue staining (1:1).

* 1. It’s desired to have round shaped cell-like trypan blue positive or negative cells. Cells dissociated from frozen cryopreserved tissue may look dead or permeabilized from freeze/thaw. If > 90% cells are trypan blue positive: skip any additional permeabilization step.
  2. If large amounts of debris are present, filter again through 40 µm strainer.
  3. In case of 10-80% dead cells: Make sure your cells are viable! We recommend viability above 90% and preferably around. If you are using cells directly from culture without fluorescence activated cell sorting enrichment for viable cells, you should try to clean up dead cells by one or more of the following: i). For samples with 5-15% dead cells, treat cells in culture medium with DNase (Worthington cat# LS002007) at a final concentration of 200 U/ml. DNase needs divalent cations so treat cells in culture media that lacks EDTA. Treat for 30 minutes at 37°C. Then proceed to washing. Wash thoroughly with PBS to remove DNase prior to proceeding to ATAC-seq transposition reaction. ii). For samples with more than 15-20% dead cells, separate viable cells over ficoll. Make sure ficoll and centrifuge are at room temp and that the brake has been switched to off. Exact conditions are dependent on cell type and cell number. A standard spin is for 25 minutes at 400 RCF with no brake. Prior to ficoll, it may help to treat cells with DNase as above. iii). If viability is still a problem, either sort or use a magnetic bead depletion based on Annexin V.

Desired amount of cells: 50,000 cells starting material for each bulk ATAC sample.

NOTE: at this point you could stain and sort your cells.

**4.** **Permeabilization** (skip if > 90% of cells are trypan blue positive)

4.1. Spin each sample for 5 min at 500g

4.2. Add 100 uL of **RSBT** to pelleted cells. Resuspend by pipetting 5 times. Incubate 2-5 min on ice, then add 1 ml of **wash buffer**, then spin cells down for 5 min at 500 g.

Optional: take 5 uL of sample after 2 – 5 min lysis and mix with 5 uL of Trypan blue in a separate 1.5 mL tube. Check under microscope. All cells should be trypan blue positive but still round shaped, not fuzzy.

**5.** **Transposition of cells**

5.1. Remove supernatant with a p200. Proceed with caution as pellet may be fragile at this step

5.2. Add 100 µL of **ATAC Reaction Mix** for every 100,000 cells per sample. Resuspend gently up and down with pipette for 6 intervals to fully mix

5.3. Incubate reaction for 30 min in a thermoshaker at 37°C

5.4.1 purify tagmented DNA using the Qiagen MinElute kit, elute in 20 ul EB buffer.

*5.4.2 Quench ATAC reaction by adding 8 µL of 0.5M EDTA for every 100,000 cells. Place on ice until ready to sort if you sort at this step. If you sort here, perform a MinElute DNA purification after you are done and elute in 20 ul EB buffer*

NOTE: at this point you could stain and sort your cells.

**6.** **Reverse x-linking**

6.1. add 20 ul **2x reverse x-linking buffer (add fresh Proteinase K)** to each sample

6.2. Incubate tubes at 65°C overnight (at least 4 h).

6.3. inactivate the PK at 85°C for 5 min

6.4. perform MinElute DNA cleanup, elute in 10 ul EB and continue with regular library preparation.

Note: During the 65°C incubation, evaporation from the well may occur. We recommend using a PCR machine with a heated lid for the incubation to reduce evaporation. If after all evaporation is observed, use the reverse X-link buffer to refill the wells to the original volume.

**Reagent List**

**ATAC-RSB-T**

|  |  |  |
| --- | --- | --- |
| Name | Amount | Final Conc. |
| RSB | 978 uL |  |
| 10 % Tween20 | 10 uL | 0.1 % |
| 10% NP40 | 10 uL | 0.1 % |
| 5% Digitonin | 2 ul | 0.01 % |
| Total | 1 **mL** |  |

**ATAC-Wash buffer**

|  |  |  |
| --- | --- | --- |
| Name | Amount | Final Conc. |
| RSB | 990 uL |  |
| 10 % Tween20 | 10 uL | 0.1 % |
| Total | 1 **mL** |  |

**ATAC Reaction Mix, per 50,000 nuclei**

|  |  |
| --- | --- |
| Name | Amount |
| 2xTD Buffer | 25 uL |
| H2O | 5 uL |
| PBS | 16.5 uL |
| 1% Digitonin | 0.5 uL |
| 10% Tween | 0.5 uL |
| Tn5 | 2.5 uL |
| Total | 50 uL |

Can be prepared ahead of time:

**ATAC-RSB** \*store -20°C

|  |  |  |
| --- | --- | --- |
| Name | Amount | Final Conc. |
| 1M Tris-HCl pH 7.4 | 500 uL | 10mM |
| 5M NaCl | 100 uL | 10mM |
| 1M MgCl2 | 150 uL | 3mM |
| H2O | 49.25 **mL** | NA |
| Total | 50 **mL** |  |

**2x TD Buffer** \*store -20°C

|  |  |  |
| --- | --- | --- |
| Name | Amount | Final Conc. |
| 1M tris HCl pH 7.6 | 4 **mL** | 20mM |
| 1M MgCl2 | 2 **mL** | 10mM |
| Dimethyl Formamide | 40 **mL** | 20% |
| H2O | 174 **mL** | NA |
| Total | 200 **mL** |  |

**2 X Reverse X-Link Buffer** \*store RT

|  |  |  |
| --- | --- | --- |
| Name | Amount | Final Conc. |
| Tris HCl pH 8.0 (1M stock) | 1 ml | 100mM |
| Tween20 (10% stock) | 1ml | 1% |
| Igepal CA-630 (10% stock) | 1 ml | 1% |
| H2O | 7 ml |  |
| Total | 10 **mL** |  |

**2 X Reverse X-Link Buffer** \*prepare fresh per sample

|  |  |
| --- | --- |
| Name | Amount |
| 2 X Reverse x-link buffer | 100 ul |
| Proteinase K (Ambion, AM2546) | 1:100 |
| Total | 100 ul |