DNA tagmentation for Omni ATAC-seq (for NGI user)

Currently, the Omni ATACseq protocol at NGI is only applicable for 50,000 of freshly isolated/harvested cells. We recommend that the tagmentation reaction should be performed in batches of not more than 8 samples at the time. The main parts of the preparation are divided into the two steps:

1. Cell preparation

2. Tagmentation reaction

1. Equipment

Instrument / equipment	Application
Thermal Mixer	Enzymatic incubations
4°C centrifuge for 1.5 ml tubes	Pelleting cells
Centrifuge for 1.5 ml tubes	Column based DNA purification

2. Reagents

Kit/ Reagent	Cat number / Supplier	Storage conditions
Digitonin	Supplied by NGI	-20 °C
Tween-20	11332465001 / Sigma	4 °C
NP40	85125 / Fisher Scientific	RT
1x PBS	E404-100TABS / VWR	4 °C
Tagment DNA Enzyme and Buffer	Supplied by NGI	-20 °C
Zymo DNA Clean & Concentrator TM-5	BioSite-D4011 / Nordic Biosite	RT

3. Protocol

3.1 Cell Preparation

Before you start the protocol please make sure,

- Check that the cell viability is not less than 90%.

- Resuspend the harvested cell in PBS. Make sure that all traces of culture media have been removed from the harvested cell.

- Make sure to not overload or underload the cells in the tagmentation reaction. Count the cells either manually or automatically (with a cell counter).

3.1.1 Preparation of Buffers

3.1.1.1 ATAC-RSB buffer

Prepare ATAC-RSB buffer as follows and keep on ice

Reagents	Stock concentration	Target concentration	Volume (µl)
Tris HCl, pH 7.4	1 M	10 mM	500
NaCl	5 M	10 mM	100
$MgCl_2$	1 M	3 mM	150
RNase-free H_2O			49,250
Total volume			50,000

3.1.1.2 Lysis buffer

Prepare lysis buffer as follows and keep on ice

Reagents	Stock concentration	Target concentration	Volume for 1 rxn (µl)	Volume for 10 rxn (µl)
NP-40	10 % (100X)	0.1 % (1X)	0.5	5
Tween 20	10 % (100X)	0.1 % (1X)	0.5	5
Digitonin	1 % (100X)	0.01 % (1X)	0.5	5
ATAC-RSB buffer			48.5	485
Total volume			50	500

3.1.1.1 Wash buffer

Prepare wash buffer as follows and keep on ice

Reagents	Stock concentration	Target concentration	Volume for 1 rxn (µl)	Volume for 10 rxn (µl)
Tween 20	10 % (100X)	0.1 % (1X)	10	100
ATAC-RSB buffer			990	9,900
Total volume			1000	10,000

3.1.2 Cell Lysis

Before starting, make sure the centrifuge has been cooled down to 4°C. Lyse the cells by doing the following steps,

1. Aliquot 50,000 viable cells into a new 1.5 ml Eppendorf LoBind tube.
2. Centrifuge the cells at 500 x g for 10 min at 4°C.

   IMPORTANT: be sure you place the samples in the centrifuge in a way that you will know where the pellet will be. A pellet will not always be visible. Centrifugation speed might be adjusted according to the cell type.
3. Remove all supernatants. Use a P1000 followed by a P10 pipette to make sure that all the supernatant is removed.
4. Re-suspend the cell pellet in a 50 µl cold Lysis buffer, pipetting up and down 3 times.
5. Incubate on ice for 3 min.
6. Add 1 ml of cold Wash buffer.
7. Mix by inverting the tube at least 3 times.
8. Pellet the cell nuclei at 500 x g for 10 min at 4°C

   IMPORTANT: be sure you place the samples in the centrifuge in a way that you will know where the pellet will be. A pellet will not always be visible. Centrifugation speed might be adjusted according to the cell type.
9. Remove all supernatants. Use a P1000 followed by a P10 pipette to make sure that all the supernatant is removed.
10. Place the samples on ice, and **immediately** continue the Tagmentation reaction.

3.2 Tagmentation Reaction

Before you start

- Defrost the Tagmentation Buffer on ice.
- Pre-heat the Thermal Mixer to 37 °C.
- **Prepare the Tagmentation master mix in a chemical hood (!) Be aware that the TD buffer contains Formamide.**

Hazard statements	Formamide
H351	Suspected of causing cancer.
H360FD	May damage fertility. May damage the unborn child.
H373	May cause damage to organs (blood, cardiovascular system) through prolonged or repeated exposure.

3.2.1 Tagmentation

Before starting the reaction,

- Make sure you have enough of the tagmentation buffer (limiting factor of the kit).
- Pre-heat the Thermal Mixer to 37°C.
- If the samples arrived in 2ml tubes, transfer the samples in the Master Mix to a new 1.5 ml reaction tube.
- Defrost the Tagmentation Buffer on ice.

Set up the tagmentation master mix as follows,

IMPORTANT:
Prepare the Tagmentation master mix in a chemical hood. TD buffer contains Formamide.
Since the TDE1 enzyme is not stable in the reaction buffer, the tagmentation master mix should be prepared just before use.

Reagent	Volume for 1 rxn (µl)	Volume for 10 rxn (µl)
2x TD buffer	25	250
TDE1 enzyme	2.5	25
1x PBS	16.5	165
1% Digitonin (100x)	0.5	5
10% Tween-20 (100x)	0.5	5
RNase-free H_2O	5	50
Total volume	50	50

1. Resuspended the pellet (prepared in section 3.1.2) in 50 µl Tagmentation master mix

2. Incubate samples for 30 min at 37˚C in the Thermal Mixer with a mixing speed of 1000 rpm.

3. **Immediately** continue to step 3.2.2

3.2.2 Cleanup of tagmented DNA

Before starting please make sure,

- Ethanol has been added to the **DNA Wash Buffer concentrate**.
- Prepare the needed amount of Zymo-Spin™ IC-XL Columns and collection tube
- In a 1.5 ml Eppendorf LoBind tube, add **250 µl of ChIP DNA Binding Buffer** to the tagmented DNA and mix thoroughly by pipetting.

Clean the tagmented DNA by following these steps,

1. Transfer mixture to a Zymo-Spin™ IC-XL Column in a collection tube.
2. Centrifuge for 30 seconds at 13,000 g and discard the flow-through.
3. Add **200 µl DNA Wash Buffer** to the column. Centrifuge for 1 minute at 13,000 g.
4. Repeat the step above for a total of 2 wash steps.
5. Add **15 µl DNA Elution Buffer** directly to the column matrix and incubate at room temperature for one minute.

   IMPORTANT: Ensure that the elution buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA.
6. Transfer the column to a 1.5 ml Eppendorf tube.
7. Centrifuge for 30 seconds at 13,000 g to elute the DNA.
8. When working with multiple samples, transfer the eluted DNA to an Eppendorf TwinTec 96 plate.
9. Transfer the eluted DNA to an Eppendorf TwinTec 96 plate supplied by NGI.

Make sure the aluminium seal and NGI barcode labels are properly attached. Contact the project coordinators at NGI to arrange shipment of the tagmented DNA.

Protocol adapted from:

Corces, M., Trevino, A., Hamilton, E. et al. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat Methods 14, 959–962 (2017). https://doi.org/10.1038/nmeth.4396 (https://www.nature.com/articles/nmeth.4396#citeas)