

Omni-ATAC-seq: Improved ATAC-seq protocol

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Abstract

We present Omni-ATAC, an improved ATAC-seq protocol for chromatin accessibility profiling that works universally across multiple cell types and cell contexts with significant improvement of signal-to-background ratio and information content. The Omni-ATAC protocol enables chromatin accessibility profiling from material that was previously deemed intractable including frozen tissue samples.

Introduction

This ATAC-seq protocol outperforms the standard ATAC-seq protocol across all cell types and cell contexts tested. The modifications (i) lead to a strong reduction in the percent of reads mapping to mitochondrial DNA, (ii) increase the signal-to-background ratio, and (iii) increase the library complexity.

Reagents

Detergents - All detergents are resuspended as 100x stock solutions

Digitonin - (Promega cat# G9441) Digitonin is supplied at 2% in DMSO. Dilute 1:1 with water to make a 1% (100x) stock solution. Avoid more than 5 freeze thaw cycles. Can be kept at -20°C for up to 6 months.

Tween-20 - (Sigma/Roche cat# 11332465001) Tween-20 is supplied at 10%. Use at this concentration (100x stock). Store at 4°C.

NP40 - (Sigma/Roche cat# 11332473001) NP40 is supplied at 10%. Use at this concentration (100x stock). Store at 4°C.

DNase - (Worthington cat# LS002007) resuspended in Hanks balanced salt solution (HBSS) at a final concentration of 200 U/ml

Ficoll - (GE cat# 17-1440-02)

1M Tris-HCl pH 7.5 - (Invitrogen #15567-027)

5M NaCl - (Ambion/Thermo #AM9759)

1M MgCl₂ - (Ambion/Thermo #AM9530G)

Nextera® DNA Sample Preparation Kit - (Illumina #FC-121-1030)

Zymo DNA Clean and Concentrator-5 Kit - (Zymo #D4014)

NEBNext® High-Fidelity 2X PCR Master Mix - (NEB #M0541L)

SYBR® Gold Nucleic Acid Gel Stain - (Thermo #S-11494)

ATAC-seq adapters

KAPA Library Quantification kit - (KAPA #KK4854)

Equipment

Thermoshaker, Thermocycler, centrifuge, qPCR machine

Procedure

Prior to Transposition:

Make sure your cells are viable! We recommend viability above 90% and preferably around 95%. 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:

1. 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.
2. For samples with more than 15-20% dead cells, separate viable cells over ficoll (GE cat# 17-1440-02). 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.
3. If viability is still a problem, either sort or use a magnetic bead depletion based on Annexin V (Miltenyi cat# 130-090-201).

Buffers and Reagents:

ATAC-Resuspension Buffer (RSB)

For 50 ml ATAC-RSB, combine 500 ul 1M Tris-HCl pH 7.4, 100 ul 5M NaCl, 150 ul 1M MgCl₂, and 49.25 ml sterile water.

Omni-ATAC: Optimized Transposition reaction

1. Pellet 50,000 viable cells at 500 RCF at 4°C for 5 min in a fixed angle centrifuge.
2. Add 50 ul cold ATAC-Resuspension Buffer (RSB) containing 0.1% NP40, 0.1% Tween-20, and 0.01% Digitonin and pipette up and down 3 times.
3. Incubate on ice for 3 minutes.
4. Wash out lysis with 1 ml of cold ATAC-RSB containing 0.1% Tween-20 but NO NP40 or digitonin and invert tube 3 times to mix
5. Pellet nuclei at 500 RCF for 10 min at 4°C in a fixed angle centrifuge.
6. Aspirate all supernatant, carefully avoiding visible cell pellet, using two pipetting steps (aspirate down to 100 ul with a p1000 pipette and remove final 100 ul with a p200 pipette).
7. Resuspend cell pellet in 50 ul of transposition mixture by pipetting up and down 6 times. Transposition mix = (25 ul 2x TD buffer, 2.5 ul transposase (100nM final), 16.5 ul PBS, 0.5 ul 1% digitonin, 0.5 ul 10% Tween-20, 5 ul H₂O)
8. Incubate reaction at 37°C for 30 minutes in a thermomixer with 1000 RPM mixing.

Pre-amplification of transposed fragments:

1. Cleanup reaction with a Zymo DNA Clean and Concentrator-5 Kit (cat# D4014). Make sure to use a different kit for pre- and post-amplification so as to not cross contaminate post-amplification product into pre-amplification samples. **NOTE:** If you don't have time or don't feel like doing the cleanup immediately following transposition, resuspend the ATAC reaction in 250 ul (5 volumes) of DNA Binding Buffer and store at -20°C. The DNA is stable for at least 2 weeks in this buffer at -20°C. Allow to warm back to room temperature and mix thoroughly before loading onto the column.
2. Elute DNA in 21 ul elution buffer and store at -20°C until ready to amplify. This

elution typically results in ~20 ul of product. Use all 20 ul of product in the following PCR.

3. Amplify for 5 cycles using NEBNext 2x MasterMix. Each reaction should contain 2.5 ul of 25 uM i5 primer, 2.5 ul of 25 uM i7 primer, 25 ul 2x NEBNext master mix, and 20 ul transposed/cleaned-up sample. Thermocycler conditions (72°C for 5 min, 98°C for 30 sec, followed by 5 cycles of [98°C for 10 sec, 63°C for 30 sec, 72°C for 1 min] then hold at 4°C.
4. After amplification is complete, remove tubes from thermocycler and store on ice. Proceed to *“qPCR amplification to determine additional cycles”* immediately.

qPCR amplification to determine additional cycles:

Using 5 ul (10%) of the pre-amplified mixture, run a 15 ul qPCR to determine the number of additional cycles needed. The conditions for this 15 ul volume PCR are:

3.76 ul sterile water, 0.5 ul 25 uM i5 primer, 0.5 ul 25 uM i7 primer, 0.24 ul 25x SYBR Gold (in DMSO), 5 ul 2x NEBNext master mix, 5 ul pre-amplified sample.

Cycling conditions for this PCR are: 98°C for 30 sec, followed by 20 cycles of [98°C for 10 sec, 63°C for 30 sec, 72°C for 1 min]

After qPCR amplification, manually assess the amplification profiles and determine the required number of additional cycles to amplify. See Buenrostro et al 2015 (PMID: 25559105) for a detailed explanation of how to properly amplify ATAC-seq libraries.

Using the Omni-ATAC protocol, we find that the number of cycles of amplification required is very low. Many libraries show sufficient amplification after the 5 pre-amplification cycles. Some libraries are even “over-amplified” at this point but it is important that all libraries undergo these 5 pre-amplification cycles in order to add on the appropriate Illumina adapter sequences.

Final amplification and cleanup:

1. Using the remainder of the pre-amplified DNA, run the required number of additional cycles. Place the pre-amplified tubes (now containing 45 ul) back into the

thermocycler without addition of any more reagents.

2. Purify the final PCR reaction using a Zymo DNA Clean and Concentrator-5 Kit (cat# D4014) and elute in 20 ul H₂O.

Quantify library concentration using the KAPA Library Quantification Kit

The KAPA Library Quantification kit (cat# KK4854) comes with standards that range from 20 pM to 0.002 pM. Most ATAC-seq libraries are between 4 nM and 20 nM if amplified correctly.

1. Samples should be diluted 1000 fold to fall within the concentration range of the standards. To do this, first dilute 40x by adding 0.5 ul library to 19.5 ul sterile water. Next, dilute 4 ul of this 40x diluted mixture into 96 ul sterile water for a 25x dilution. This gives a cumulative 1000x dilution.
2. Each qPCR reaction will be a 10 ul total volume. When first opening the kit, add the primer to the enzyme mix. This will make the mix 5 parts enzyme mix and 1 part primer mix. For each 10 ul reaction, you will add 6 ul of this mix (effectively adding 5 ul enzyme mix and 1 ul of primer mix).
3. Run all samples in technical duplicate using 2 ul of library / standard / no template control per 10 ul reaction.
4. Run reactions according to the following thermal profile: 95°C for 5min followed by 35 cycles of [95°C for 30 sec, 60°C for 45 sec]

Figures

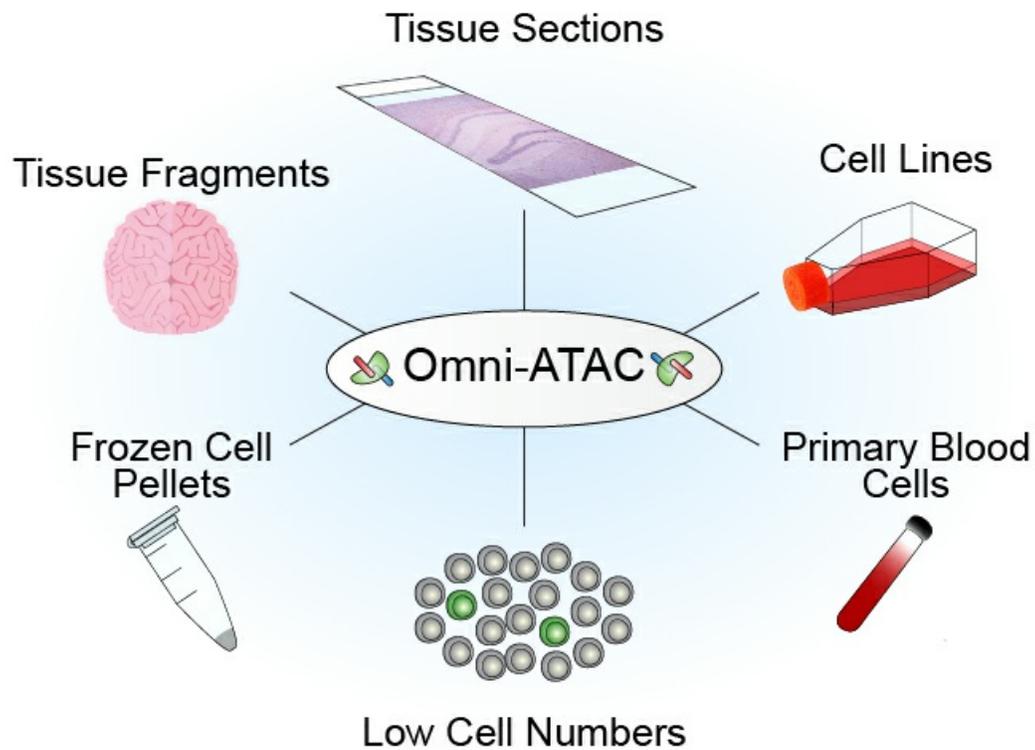


Figure 1

Omni-ATAC applications Omni-ATAC provides improved data quality across multiple applications

An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues

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