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Chapter title

ATAC-see: A Tn5 transposase-mediated assay for detection of chromatin accessibility with imaging

Summary/Abstract

Assay of transposase-accessible chromatin with visualization (ATAC-see), a transposase-mediated imaging technology that enables direct imaging of the accessible genome in situ and deep sequencing to reveal the identity of the imaged elements. Here we image spatial organization of the accessible genome in HT1080 cells with this method.

Keywords

ATAC-see, Tn5 transposase; chromatin accessibility; in situ imaging, epigenetics, 3D genome organization

1 Introduction

Eukaryotic genomes are extensively compacted in their genome to form euchromatin and heterochromatin[1,2]. Euchromatin has active regulatory elements whose access control the gene activity, whereas heterochromatin is mostly inactive and contains low gene activity[1,2]. These accessible elements comprise approximately 2-3% of the genome[3-5] in any given cell types that include enhancers, promoters, and other regulatory sequences critical for development processes and disease progression[6,7]. Nuclear architecture and 3D genome organization are tightly linked to gene expression, replication and DNA repair[6-8]. We previously reported ATAC-see[9], where hyperactive Tn5 transposase loaded with fluorescence dye labelled DNA adaptors selectively inserts the adaptors into accessible chromatin loci within fixed cells. The covalently inserted fluorophores at open chromatin sites genome-wide allows us to image the open chromatin sites in the intact cells. Thus, ATAC-see decodes molecular accessibility of chromatin by detecting inserted fluorophores. After imaging the spatial organization of the accessible genome in 3D, the inserted adaptors still allow deep sequencing to map open chromatin sites on the same sample, in the identical manner of ATAC-seq. Here, we image spatial organization of the accessible genome in HT1080 cells with this method.

2 Materials

All solutions are prepared with ultrapure water (18 MΩ-cm at 25 °C). Prepare and store reagents at room temperature (unless indicated otherwise).

2.1 Hyperactive Tn5 production

1). pTXB1-Tn5 plasmid.

2). T7 Express LysY/Iq *E. coli* strain.

3). LB medium: For each 950 mL of MilliQ H2O, add 10 g of Tryptone, 10 g of Sodium Chloride (NaCl) and 5 g of Yeast Extract, mix until powder is dissolved. Adjust the pH solution to ~7.0 using sodium hydroxide (NaOH) and make the final volume up to 1000 mL by adding MilliQ H2O water. Autoclave using liquid cycle.

4). Isopropyl β-d-1-thiogalactopyranoside (IPTG): Dissolve 2.38 g of IPTG in 8 mL of sterilized double-distilled water (ddH_2O) to make 1M IPTG stock solution. Filter with a 0.22 μm filter and store aliquots at -20 °C.

5). Proteinase inhibitor: Proteinase inhibitor tablet is dissolved in sterilized ddH_2O to make 50X stock. Make aliquots and store at -20 °C.

6). Chitin resin.

7). Dithiothreitol (DTT): Dissolve 1.54 g of Dithiothreitol (DTT) in 10 mL of sterilized ddH_2O to make 1M stock. Filter with a 0.22 μm filter and store the aliquots at -20 °C.

8). Bradford assay kit.

9). Ultracel 30-K column.

10). NuPAGE Novex 4-12% Bis-Tris gel.

11). Coomassie Brilliant Blue R-250: Add 100 mL of glacial acetic acid to 400 mL of sterilized ddH_2O. Dissolve 0.5 g of Coomassie R-250 dye in 500 mL methanol. Mix the acetic acid and methanol solutions and filter through a Whatman No. 1 filter to remove any particulate matter.

2.2 Tn5 transposase assembly

1). DNA adaptor oligos were synthesized at Integrated DNA technologies (IDT) with following sequences (*see* **Note 1 & 2**):

Tn5MErev, 5'-[phos]CTGTCTCTTATACACATCT-3';

Tn5ME-A-ATTO590:

5′-/ATTO590/TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′;

Tn5ME-B-ATTO590:

5′-/ATTO590/GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′.

2). Tn5 transposases were produced according to Picelli et al[10].

3). 2X dialysis buffer (DB)[9,10]: To make 10 ml of 2x DB buffer,

mix 1 ml of 1M HEPES-KOH (pH 7.2), 400 µl of 5M NaCl, 40 µl of 0.5M EDTA, 20 µl ml of 1M DTT, 20 µl ml of Triton X-100, 2 ml of glycerol and 6.52 ml of sterilized ddH_2O. Make aliquots and store at -20 °C.

2.3 Tn5 tagmentation

1). 1% Formaldehyde: Dilute 16 times formaldehyde solution (16% stock) in the sterilized PBS to make 1% formaldehyde (*see* **Note 3**).

2). Phosphate-buffered saline (PBS) solution: Add PBS tablet in ddH_2O. Mix the solution completely to dissolve all insoluble matter and sterilize the PBS solution with autoclaving at 115°C for 15 mins.

3). 2×Tagment DNA (TD) buffer[11]: To make 10 ml 2x TD buffer, mix 200 µl of 1M Tris-HCl (pH 7.6), 200 µl of 0.5M MgCl2, 2 ml of *N,N*-Dimethylformamide (DMF) and 7.6 ml of sterilized ddH_2O. Make aliquots and store at -20 °C.

4). Lysis buffer: To make 10 ml lysis buffer, mix 100 µl of 1M Tris-HCl (pH 7.4), 20 µl of 5M NaCl, 30 µl of 1M MgCl2, 10 µl of IGEPAL CA-630 and 9.84 ml of sterilized dH_2O. Store the solution at +4 °C (*see* **Note 4**).

5). Glass coverslips for cell culture: Place the cover slips in a loosely covered glass beaker containing 1M HCl for 4 hours at 50 ºC. After cooling down to room temperature, rinse the coverslips with ddH_2O. Next, three washes with 50% EtOH,

70% EtOH, 95% EtOH respectively for 30 mins. Store coverslip in absolute EtOH until further use.

6). Washing buffer: To make 1L wash buffer, mix 1ml of 10% sodium dodecyl sulfate (SDS), 100 ml of 0.5M EDTA, with 899 ml of sterilized PBS (*see* **Note 5**).

2.4 Immunostaining of mitochondria and nuclear lamina

1). Primary antibodies dilution (1:100): Dilute the antibodies 100 times (rabbit anti-Lamin B1 antibody and mouse anti-mitochondria antibody) with antibody dilution reagent (00-3218, ThermoFisher Scientific) (*see* **Note 6**).

2). VECTASHIELD Antifade Mounting Medium with 4′,6-diamidino-2-phenylindole (DAPI).

3). Secondary antibodies dilution (1:500): Dilute the antibodies 500 times (goat anti-rabbit-ATTO488 and goat anti-mouse-Atto647N with antibody dilution reagent (*see* **Note 6**).

4). Washing buffer (1L): Mix 0.5 ml of Tween-20 in the 1000 ml of sterilized PBS.

2.5 Cell culture

HT1080 cells were cultured in DMEM/F-12, GlutaMAX™ supplement, 10% fetal bovine serum [12], and 1% Pen/Strep with SecureSlip cell culture system.

2.6. Equipment

1). Cell culture incubator

2). Confocal microscopy

3). Humidity chamber box

4.) Sonicator

5). Heat block

3 Methods

Step 1: Hyperactive Tn5 production

Hyperactive Tn5 was produced as previously described[9,10]. pTXB1-Tn5 plasmid (60240, Addgene) was introduced into T7 Express LysY/Iq *E. coli* strain. Overnight cultured *E. coli* (10 ml) was inoculated to LB medium (500 ml) at 37°C for 1.5 hours. [13]hours. When the cell density (OD600) becomes 0.9, then Tn5 protein was induced by adding 0.25 mM IPTG for 4 hours. *E. coli* pellet was resuspended in lysis buffer (20 mM HEPES-KOH pH 7.2, 0.8 M NaCl, 1mM EDTA, 10% glycerol, 0.2% triton X-100, complete proteinase inhibitor) followed by mild sonication to lyse the cells. Chitin resin (10 ml) was added to the supernatant and incubated for 1 hour at 4°C with slow rotation. Unbound resin was washed by the lysis buffer extensively. Next, lysis buffer containing 100 mM DTT was added to the bound resin and stored in 4°C. After 48 hours, protein was eluted by gravity flow and collected in the 1 ml fractions. Each fraction (1 ul) was added to detergent compatible Bradford assay[13] and peaked fractions were pooled and dialyzed against 2X dialysis buffer (100 mM HEPE-KOH pH7.2, 0.2 M NaCl, 0.2 mM EDTA, 2 mM DTT, 0.2% triton X-100, 20% glycerol). Dialyzed Tn5 protein was concentrated by using Ultracel 30-K column, and the quantity of Tn5 was measured by Bradford assay and visualized on NuPAGE Novex 4-12% Bis-Tris gel followed by Coomassie blue staining[14].

Step 2: Tn5 transposome assembly

1). Oligonucleotides (Tn5ME-A-ATTO590, Tn5ME-B-ATTO590, Tn5MErev) were resuspended in the TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) to a final concentration of 100 µM each.

2). Equimolar amounts of Tn5MErev/Tn5ME-A-ATTO590 and Tn5MErev/Tn5ME-B-ATTO590 were mixed in separate 200 µl PCR tubes.

3). These two tubes of oligos mixtures were denatured on a thermocycler for 5 min at 95°C and cooled down slowly on the thermocycler by turning off the thermocycler.

4). The Tn5 transposome was assembled with the following components: 0.25 vol Tn5MErev/Tn5ME-A-ATTO590 + Tn5MErev/Tn5ME-B-ATTO590 (final concentration of each double strand oligo is now 50 µM each), 0.4 vol glycerol (100% solution), 0.12 vol 2X dialysis buffer (100 mM HEPES-KOH at pH 7.2, 0.2 M NaCl, 0.2 mM EDTA, 2 mM DTT, 0.2% Triton X-100, 20% glycerol, 0.1 vol SL-Tn5 (50 µM) and 0.13 vol of sterilized ddH_2O.

5). The reagents were mixed gently, and the solution was incubated for 1 hour at 25 ºC. Afterward annealing, the Tn5 assembly is stored at -20 ºC.

Step 3: Slide preparation and fixation

First, precleaned glass coverslips were placed in the 6 well cell cultures plate. Then, HT1080 cells were grown on precleaned glass coverslip until 80-90% confluent, fixed with 1% formaldehyde[15] (Sigma-Aldrich) for 10 min at room temperature and quenched with 0.125 M glycine for 5 min at room temperature.

Step 4: ATAC-see

1). Glass coverslip with fixed HT1080 cells were permeabilized with lysis buffer (10 mM Tris-Cl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% IGEPAL CA-630) for 10 min.

2). Pre-mixed (50 µl) transposase reaction solution (2.5 µl 2mM ATTO-Tn5, 25 µl 2X TD buffer, 22.5 µl water) was added onto the slide, and the cells on the slide were incubated at 37°C for 30 min.

3). After Tn5 tagmentation, the cells were washed with washing buffer (0.01% SDS, 50 mM EDTA in PBS) three times at 55 ºC for 15 mins each.

Step 5: Immuno-staining after ATAC-see

1). Cells were blocked with antibody dilution reagent for 1 hour at room temperature.

2.) Primary antibodies (rabbit anti-LaminB1, ab16048, Abcam and mouse anti-mitochondria, ab3298, Abcam) were diluted (1:100) in the antibody dilution reagent and incubated overnight at 4 °C.

3). After washing with washing buffer (containing 0.05% Tween-20 in PBS) for 3 times 10 min each, slides were incubated with secondary antibodies (goat anti-rabbit-ATTO488,18772-1ML-F, Sigma-Aldrich and goat anti-mouse-Atto647N, 50185-1ML-F, Sigma-Aldrich) diluted to 1:500, for 45 min at room temperature.

4). Finally, slides were washed with washing buffer, 3 times for 10 min each, mounted using Vectashield with DAPI (H-1200, Vector labs) and imaged with confocal microscopy.

4 Notes

1.) The modification with fluorescent dyes on Tn5 adaptors could be other dyes instead of Atto590 or other big molecules e.g, Biotin. However, the modification must be on the 5’ end of oligos.

2). There could be free DNA oligos in the assembled ATTO-Tn5, which could potentially introduce some unspecific signal for ATAC-see. To remove the free oligos, HPLC purification could be used to remove the free DNA oligos.

3.) Paraformaldehyde is a potential carcinogen so, all formaldehyde work must be conducted in a properly operating fume hood.

4). The concentration of IGEPAL CA-630 in lysis buffer in the Tn5 tagmentation could be cell type specific, which could refer to the concentration used in ATAC-seq for different cell lines.

5.) Wear a mask while preparing a solution containing sodium dodecyl sulfate (SDS).

6). Alternative to antibody dilution reagent (00-3218, ThermoFisher Scientific), we can also use freshly prepared antibody dilution buffer: 0.05 g bovine serum albumin (BSA) dissolve in 5 ml of sterilized PBS. The solution is filter sterilize with 0.22 μm filter.

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