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SAFE Hi-C: A Simplified, Amplification-free and Economically Efficient High-throughput Chromosome Conformation Capturing Method

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Abstract

PCR amplification of Hi-C libraries introduces unusable duplicates and results in a biased representation of chromatin interactions. We present a simplified, fast, and economically efficient Hi-C library preparation procedure that generates sufficient non-amplified ligation products for deep sequencing. Comprehensive analysis of the resulting data indicates that amplification-free Hi-C preserves higher complexity of chromatin interaction and lowers sequencing depth dramatically for the same number of unique paired reads. With amplification bias avoided, our method may produce a chromatin interaction network more faithfully reflecting the real three-dimensional genomic architecture.

Introduction

Hi-C compares the number of ligation events between each fragment pair in a large population of cells and thereby allows the identification of various genome structural features including compartments, topologically associating domains (TADs), and loops[1-5]. PCR amplification is a default step in Hi-C-related experiments[6] to guarantee sequencing primer addition and to produce a sufficient amount of DNA for sequencing, especially for Hi-C experiments involving single or low cell numbers[7-18]. Though universal primers are used, PCR amplification introduces duplicates and may skew Hi-C library composition, which may not be fully corrected by normalization methods[19-21].

Efficient recovery of enough ligated fragments for direct high-throughput sequencing is important for accurate depicting the three dimensional genome architecture and the understanding of its functional role in transcription regulation, replication, genome stability and other critical biological activities related to chromatin. We present SAFE Hi-C, a *s*implified, *a*mplification-*f*ree, and economically *e*fficient process, in which paired reads generated by independent ligation events are saved.

Reagents

- Schneider’s Medium (Gibco, 21720024)
- FBS (Sigma, F7524)
- Penicillin/Streptomycin P/S (Sigma, P0781)

- formaldehyde solution (Sigma, F8775)
- Glycine (sigma, G8898)
- protease inhibitors (Sigma, P8340)
- IGEPAL® CA-630 (Sigma, I8896)
- Triton X-100 (Sigma, 93443)
- Sodium dodecyl sulfate (SDS) (Sigma, L3771)
- DpnII restriction enzyme (NEB, R0147)
- biotin–dATP (Thermo Fisher, 19524016)
- dCTP, dGTP, and dTTP (Thermo Fisher, R0151, 10219012, R0161)
- DNA Polymerase I Large (Klenow) Fragment (NEB, M0210)
- T4 DNA ligase buffer with 10 mM ATP (NEB, B0202)
- BSA (Thermo Fisher, AM2616)
- T4 DNA Ligase (NEB, M0202)
- Proteinase K (TRANSGEN, GE201)
- RNase A (TRANSGEN, GE101)
- Streptavidin Dynabeads (NEB, S1420S)
- T4 PNK (NEB, M0201)
- T4 DNA polymerase I (NEB, M0203)
- Klenow exo minus (NEB, M0212)
- 1X Quick ligation reaction buffer (NEB, B6058)
- Full Y-Adaptor (Vazyme, N802)
- NEB DNA Quick ligase (NEB, M2200)
- Lysis Buffer (10 mM Tris-HCl pH8.0, 10 mM NaCl, 0.2% Igepal CA630, 1X protease inhibitors)
- 1X Tween Wash Buffer (1X TWB, 5 mM Tris-HCl pH 7.5, 0.5 mM EDTA, 1 M NaCl, 0.05% Tween-20)
- 2X Binding Buffer (2X BB, 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 2M NaCl)

Equipment

· Covaris LE220

· DynaMag™-2 Magnet (Thermo Fisher, 12321D)

Procedure

Cell Culture

S2 cells were cultured in Schneider's Medium (Gibco, 21720024) supplemented with 10% heat inactivated FBS (Sigma, F7524) and 1% Penicillin/Streptomycin P/S (Sigma, P0781) at 27°C.

K562 cells were incubated in 1× RPMI1640 media supplemented with 10% FBS at 37°C with 5% CO2.

Cell Crosslinking

1. 30 million Drosophila S2 cells or 250 thousand human K562 cells are collected by centrifugation at 300xG for 5 minutes.
2. Resuspend cells in fresh medium. Add freshly made formaldehyde solution (sigma, F8775) to a final concentration of 1%, v/v. Incubate at room temperature for 10 minutes with mixing.
3. Add 1/10 volume of 2.5 M glycine to quench the reaction. Incubate at room temperature for 5 minutes.
4. Centrifuge for 5 minutes at 300xG at 4°C and discard supernatant.
5. Resuspend cells in 1 mL of cold 1X PBS and spin for 5 minutes at 300xG at 4°C.
6. Discard supernatant. The cell pellets can store at -80°C or proceed to the rest of the protocol.

Lysis and Restriction Digest

1. Crosslinked cells were resuspended in 500 µL of ice-cold Hi-C lysis buffer (10 mM Tris-HCl pH8.0, 10 mM NaCl, 0.2% Igepal CA630, 1X protease inhibitors (Sigma, P8340)) and rotated at 4°C for 30

minutes.

2. Nuclei were pelleted at 4 °C for 5 minutes at 2,500xG, and the supernatant was discarded.

3. Pelleted nuclei were washed once with 500 µL of ice-cold Hi-C lysis buffer.

4. Remove supernatant and resuspend pellet with 100 µL of 0.5% SDS. Incubate at 62°C for 10 minutes.

5. Add 285 µL of water and 50 µL of 10% Triton X-100 (Sigma, 93443) to quench the SDS. Mix well, avoiding excessive foaming. Incubate at 37°C for 15 minutes.

6. Add 50 µL of NEB Buffer 3.1 and 20 µL of 10 U/µL DpnII restriction enzyme (NEB, R0147).

7. The sample was rotated at 37°C for 4 h.

8. DpnII was then heat inactivated at 65°C for 20 minutes with no shaking or rotation.

Marking of DNA Ends, Proximity Ligation, and Crosslink Reversal

1. To fill-in the restriction fragment overhangs and mark the DNA ends with biotin, 52 µL of incorporation master mix was then added:

37.5 µL of 0.4 mM biotin–dATP (Thermo Fisher, 19524016);

4.5 µL of dCTP, dGTP, and dTTP mix at 10 mM each (Thermo Fisher, R0151, 10219012, R0161);

and 10 µL of 5 U/µL DNA Polymerase I Large (Klenow) Fragment (NEB, M0210).

2. The reactions were then rotated at 37°C for 45 minutes.

3. Add 948 µL of ligation master mix:

150 µL of 10× NEB T4 DNA ligase buffer with 10 mM ATP (NEB, B0202),

125 µL of 10% Triton X-100,

3 µL of 50 mg/mL BSA (Thermo Fisher, AM2616),

10 µL of 400 U/µL T4 DNA Ligase (NEB, M0202),

and 660 µL of water.

4. The reactions were then rotated at 16 °C for 4 h and room temperature for 1 h.

5. 45 µL of 10% SDS and 55 µL of 20 mg/mL Proteinase K (TRANSGEN, GE201) were added. Incubate

at 55°C for at least 2 hours (overnight recommended).

6. Add 20 µL RNase A (TRANSGEN, GE101) and incubate at 37°C for 30 minutes.

7. DNA was purified by phenol:chloroform:isoamyl alcohol (25:24:1) extraction. Dissolve DNA pellet in 100µL of 1X Tris buffer (10 mM Tris-HCl, pH 8)

DNA shearing

1 Shear DNA to a

Instrument: Covaris LE220 (Covaris, Woburn, MA)

Volume of Library: 100µL in a Covaris microTUBE

Fill Level: 10

Duty Cycle: 15

PIP: 500

Time: 58 seconds

Biotin Pull-Down and add Full Y-Adaptor

1. Transfer 100 µL of Streptavidin Dynabeads (NEB, S1420S) into a 1.5 mL low-bind tube. Separate on a magnet and discard the solution.

2. Wash the beads with 200 µL 1X Tween Wash Buffer (1X TWB, 5 mM Tris-HCl pH 7.5, 0.5 mM EDTA, 1 M NaCl, 0.05% Tween-20). Separate on a magnet and discard the solution.

3. Resuspend the beads with 100 µL 2X Binding Buffer (2X BB, 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 2M NaCl).

4. Transfer 100 µL sonicated DNA to the beads resuspended by 2X BB

5. Incubate at room temperature for 15 minutes with rotation to bind biotinylated DNA

6. Separate on a magnet and discard the solution.

7. Wash the beads by adding 200 µL of 1X TWB. Heat the tubes on a Thermomixer at 55°C for 2 minutes. Reclaim the beads using a magnet. Discard supernatant.

8. Resuspend beads with 200 µL 1X NEB T4 DNA ligase buffer (NEB, B0202) and transfer to a new 1.5 mL low-bind tube. Separate on magnet and remove the supernatant.

9. Repairing ends of sheared DNA with 100 µL master mix:

10 µL 10X NEB T4 DNA ligase buffer with 10 mM ATP

2 µL of 25 mM dNTP mix

5 µL of 10 U/µL NEB T4 PNK (NEB, M0201)

4 µL of 3 U/µL NEB T4 DNA polymerase I (NEB, M0203)

1 µL of 5 U/µL NEB DNA polymerase I, Large (Klenow) Fragment (NEB, M0210)

78 µL water

10. Incubate at room temperature for 30 minutes.

11. Separate on magnet and remove the supernatant.

12. Add 200 µL 1X TWB to resuspend the beads and incubate on 55°C for 2 minutes. Separate on magnet and remove the supernatant.

13. Add 200 µL 1X NEBuffer 2 to resuspend the beads and transfer to a new 1.5 mL low-bind tube. Separate on magnet and remove the supernatant.

14. Resuspend beads in 100 µL of dATP attachment master mix:

10 µL of 10X NEBuffer 2

5 µL of 10 mM dATP

5 µL of 5U/µL NEB Klenow exo minus (NEB, M0212)

80 µL water

15. Incubate at 37 °C for 30 minutes

16. Separate on magnet and remove the supernatant

17. Add 200 µL 1X TWB to resuspend the beads and incubate on 55°C for 2 minutes. Separate on magnet and remove the supernatant.

18. Add 200 µL 1X Quick ligation reaction buffer (NEB, B6058) to resuspend the beads and transfer to a new 1.5 mL low-bind tube. Separate on magnet and remove the supernatant.

19. Resuspend the beads with 50 µL 1X NEB Quick ligation reaction buffer

20. Add 3 µL Full Y-Adaptor (Vazyme, N802). Mix thoroughly.

21. Add 2 µL of NEB DNA Quick ligase (NEB, M2200).

22. Incubate at room temperature for 30 minutes on rotator.

23. Separate on magnet and remove the supernatant.

Final amplification-free library for sequencing

1. Add 200 µL 1X TWB to resuspend the beads and incubate on 55°C for 2 minutes. Separate on magnet and remove the supernatant.

2. Add 200 µL 0.8X PCR Buffer to resuspend the beads and transfer to a new 1.5 mL low-bind tube. Separate on magnet and remove the supernatant.

3. Resuspend the beads in 100 µL 0.8× PCR Buffer and incubated at 98°C for 10 minutes.

4. Put the tube on wet ice immediately and incubate for 2 minutes.

5. The supernatant was recovered, quantified, and used for direct sequencing on the Illumina HiSeq X10 platform

Troubleshooting

Lysis and Restriction Digest

· Before restriction enzyme digestion, take a small amount of sample and reverse crosslink to check the quality of DNA by gel electrophoresis.

· After restriction digestion, take another small amount of sample and reverse crosslink to check if DNA is digested by comparing with DNA before digestion.

DNA shearing

· For different sonication instrument, parameters shall be optimized.

· Low-bind Microtubes are highly recommended through the whole process to minimize the loss of DNA.

Time Taken

· Cell crosslinking: 0.5 h

· Lysis and Restriction Digestion: 3 h

· Marking of DNA Ends, Proximity Ligation, and Crosslink Reversal: 3h

· Biotin Pull-Down, Full Y-Adaptor addition, final library strip-off: 2h

Anticipated Results

By avoiding amplification, SAFE Hi-C can be used to improve the quality of Hi-C analysis as well as to save time, reagents and reduce cost.

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Amplification-free library preparation with SAFE Hi-C uses ligation products for deep sequencing to improve traditional Hi-C analysis

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