Hi-C library preparation for the Lateolabrax maculatus genome

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Abstract

This protocol is used to clarity the process of Hi-C library preparation for L.maculatus genome.

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Protocol

Sample preparation

Step 1.

1) Blood sample was centrifuged at 2500g for 5min at 4℃,collect precipitated cell;

2) Add 1ml PBS to resuspended blood cell.

REAGENTS
PBS by Invitrogen - Thermo Fisher

Formaldehyde fixation

Step 2.

1) 37% formaldehyde was added with the final concentration of 1 % and the reaction was stopped with glycine after standing for 10 min at room temperature.

2) The formaldehyde fixed powder was then re-suspended in nuclei isolation buffer (10 mM Tris-HCl pH 8.0 , 10 mM NaCl, 1× PMSF).

REAGENTS
37% formaldehyde by Sigma
glycine by Sigma
10 mM Tris-HCl by Sigma
10 mM NaCl by Beyotime
1× PMSF by Sigma

Enzyme digestion

Step 3.

Add enzyme (Mbo I) to digest the DNA.

REAGENTS

enzyme (Mbo I) by New England Biolabs

DNA fragment end reparation

Step 4.

Add 10 mM dCTP, 10 mM dGTP, 10 mM dTTP,5U/ µl DNA Polymerase I, Large (Klenow) Fragment using a biotinylated residue (0.4 mM biotin-14-dATP).

REAGENTS

10 mM dCTP, 10 mM dGTP, 10 mM dTTP by Invitrogen - Thermo Fisher

5U/ µl DNA Polymerase I, Large (Klenow) Fragment by New England Biolabs

biotinylated residue (0.4 mM biotin-14-dATP) by Invitrogen - Thermo Fisher

In situ ligation

Step 5.

Add 10X NEB T4 DNA ligase buffer , 10% Triton X-100,10 mg/ml BSA , T4 DNA ligase.

REAGENTS

10X NEB T4 DNA ligase buffer by New England Biolabs

10% Triton X-100 by Sigma

10 mg/ml BSA by New England Biolabs

T4 DNA ligase by New England Biolabs

Reverse-crosslinked

Step 6.

Add 10 mg/ml proteinase K and 1% SDS to the tube and incubate at 56℃ for overnight and purified ,put the Reverse-crosslinked DNA liquid into three tube equally, add 1.5× volumes of AMpure XP mixture to each tube, vortex and spin down briefly, incubate for 10 min at room temperature, place on the MPS for 5 min at room temperature, discard supernatant, wash the beads twice with 1 ml of freshly made 70% ethanol, air-dry the beads completely and re-suspend the beads in 30 µl of ddH2O.

REAGENTS

10 mg/ml proteinase K by New England Biolabs

1% SDS by Ambion

MPS by Invitrogen - Thermo Fisher

70% ethanol by Sinopharm Chemical Reagent Co.

Biotin-containing fragments capture

Step 7.

Shear20 mg of DNA and capturing the biotin-containing fragments on streptavidin-coated beads using Dynabeads MyOne Streptavidin T1.

REAGENTS

Dynabeads MyOne Streptavidin T1 by Invitrogen - Thermo Fisher

DNA fragment end repairation

Step 8.

Add 10X NEB T4 DNA ligase buffer with 10 mM ATP, 25 mM dNTP mix, 10 U/µl NEB T4 PNK, 3 U/µl NEB T4 DNA polymerase I , 5 U/µl NEB DNA polymerase I, Large (Klenow) Fragment.

REAGENTS

- 10X NEB T4 DNA ligase buffer by New England Biolabs
- 10 mM ATP by New England Biolabs
- 25 mM dNTP mix by Enzymatics
- 10 U/µl NEB T4 PNK, 3 U/µl NEB T4 DNA polymerase I , 5 U/µl NEB DNA polymerase I, Large (Klenow) Fragment by New England Biolabs

DNA fragment adenylation

Step 9.

Add 10X NEBuffer 2, 10 mM dATP, 5 U/µl NEB Klenow exo minus.

REAGENTS

- 10X NEBuffer 2 by New England Biolabs
- 10 mM dATP by Invitrogen - Thermo Fisher
- 5 U/µl NEB Klenow exo minus by New England Biolabs

Adaptor ligation

Step 10.

Add 10X T4 PNK Reaction Buffer, 100mM ATP, 600 U/ul T4 DNA Ligase, 50% PEG8000 , 50 uM Ad153 barcode oligo_2B mix (BGI, Shenzhen, China), and followed by PCR (95℃ 3 min.; [98℃ 20 sec., 60℃ 15 sec., 72℃ 15 sec.] (8 cycles); 72℃ 10 min.

REAGENTS

- 10X T4 PNK Reaction Buffer by New England Biolabs
- 100mM ATP by FERMENTAS Inc.
- 600 U/ul T4 DNA Ligase by New England Biolabs
- 50% PEG8000 by RIGAKU