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Hi-C library preparation for the Lateolabrax maculatus genome

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We use this protocol and it's working

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Abstract

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

Materials

STEP MATERIALS

- PBS **Invitrogen - Thermo Fisher**
- 37% formaldehyde **Merck MilliporeSigma (Sigma-Aldrich)**
- glycine **Merck MilliporeSigma (Sigma-Aldrich)**
- 10 mM Tris-HCl **Merck MilliporeSigma (Sigma-Aldrich)**
- 10 mM NaCl **Beyotime**
- 1× PMSF **Merck MilliporeSigma (Sigma-Aldrich)**
- enzyme (Mbo I) **New England Biolabs**
- 10 mM dCTP, 10 mM dGTP, 10 mM dTTP **Invitrogen - Thermo Fisher**
- 5U/ µl DNA Polymerase I, Large (Klenow) Fragment **New England Biolabs**
- biotinylated residue (0.4 mM biotin-14-dATP) **Invitrogen - Thermo Fisher**
- 10X NEB T4 DNA ligase buffer **New England Biolabs**
- 10% Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich)**
- 10 mg/ml BSA **New England Biolabs**
- T4 DNA ligase **New England Biolabs**
- 10 mg/ml proteinase K **New England Biolabs**
- 1% SDS **Ambion**
- MPS **Invitrogen - Thermo Fisher**
- 70% ethanol **Sinopharm Chemical Reagent Co.**
- Dynabeads MyOne Streptavidin T1 **Invitrogen - Thermo Fisher**
- 10X NEB T4 DNA ligase buffer **New England Biolabs**
- 10 mM ATP **New England Biolabs**
- 25 mM dNTP mix **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 **New England Biolabs**
- 10X NEBuffer 2 **New England Biolabs**
- 10 mM dATP **Invitrogen - Thermo Fisher**
- 5 U/µl NEB Klenow exo minus **New England Biolabs**
- 10X T4 PNK Reaction Buffer **New England Biolabs**
- 100mM ATP **FERMENTAS Inc.**

600 U/ul T4 DNA Ligase **New England Biolabs**

50% PEG8000 **RIGAKU**

PBS **Invitrogen - Thermo Fisher**

37% formaldehyde **Merck MilliporeSigma (Sigma-Aldrich)**

glycine **Merck MilliporeSigma (Sigma-Aldrich)**

10 mM Tris-HCl **Merck MilliporeSigma (Sigma-Aldrich)**

10 mM NaCl **Beyotime**

1× PMSF **Merck MilliporeSigma (Sigma-Aldrich)**

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T4 DNA ligase **New England Biolabs**

10 mg/ml proteinase K **New England Biolabs**

1% SDS **Ambion**

MPS **Invitrogen - Thermo Fisher**

70% ethanol **Sinopharm Chemical Reagent Co.**

Dynabeads MyOne Streptavidin T1 **Invitrogen - Thermo Fisher**

10X NEB T4 DNA ligase buffer **New England Biolabs**

10 mM ATP **New England Biolabs**

25 mM dNTP mix **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 **New England Biolabs**

10X NEBuffer 2 **New England Biolabs**

10 mM dATP **Invitrogen - Thermo Fisher**

5 U/µl NEB Klenow exo minus **New England Biolabs**

10X T4 PNK Reaction Buffer **New England Biolabs**

100mM ATP **FERMENTAS Inc.**

600 U/ul T4 DNA Ligase **New England Biolabs**

50% PEG8000 **RIGAKU**

Protocol materials

- 10 mM NaCl **Beyotime**
- PBS **Invitrogen - Thermo Fisher**
- 37% formaldehyde **Merck MilliporeSigma (Sigma-Aldrich)**
- 10X NEBuffer 2 **New England Biolabs**
- 10 mM dATP **Invitrogen - Thermo Fisher**
- 5 U/µl NEB Klenow exo minus **New England Biolabs**
- 5U/ µl DNA Polymerase I, Large (Klenow) Fragment **New England Biolabs**
- 1% SDS **Ambion**
- MPS **Invitrogen - Thermo Fisher**
- 25 mM dNTP mix **Enzymatics**
- 600 U/ul T4 DNA Ligase **New England Biolabs**
- 10 mg/ml BSA **New England Biolabs**
- 1× PMSF **Merck MilliporeSigma (Sigma-Aldrich)**
- 10 mg/ml proteinase K **New England Biolabs**
- 10X NEB T4 DNA ligase buffer **New England Biolabs**
- biotinylated residue (0.4 mM biotin-14-dATP) **Invitrogen - Thermo Fisher**
- 10 mM Tris-HCl **Merck MilliporeSigma (Sigma-Aldrich)**
- 10 U/µl NEB T4 PNK, 3 U/µl NEB T4 DNA polymerase I , 5 U/µl NEB DNA polymerase I, Large (Klenow) Fragment **New England Biolabs**
- 100mM ATP **FERMENTAS Inc.**
- 10% Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich)**
- T4 DNA ligase **New England Biolabs**
- 50% PEG8000 **RIGAKU**
- 10 mM ATP **New England Biolabs**
- 70% ethanol **Sinopharm Chemical Reagent Co.**
- 10X NEB T4 DNA ligase buffer **New England Biolabs**
- enzyme (Mbo I) **New England Biolabs**
- glycine **Merck MilliporeSigma (Sigma-Aldrich)**
- 10 mM dCTP, 10 mM dGTP, 10 mM dTTP **Invitrogen - Thermo Fisher**
- Dynabeads MyOne Streptavidin T1 **Invitrogen - Thermo Fisher**

10X T4 PNK Reaction Buffer **New England Biolabs**

Sample preparation

1 1) Blood sample was centrifuged at 2500g for 5min at 4°C,collect precipitated cell;
2) Add 1ml PBS to resuspended blood cell.

PBS **Invitrogen - Thermo Fisher**

Formaldehyde fixation

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).

37% formaldehyde **Merck MilliporeSigma (Sigma-Aldrich)**

glycine **Merck MilliporeSigma (Sigma-Aldrich)**

10 mM Tris-HCl **Merck MilliporeSigma (Sigma-Aldrich)**

10 mM NaCl **Beyotime**

1× PMSF **Merck MilliporeSigma (Sigma-Aldrich)**

Enzyme digestion

3 Add enzyme (Mbo I) to digest the DNA.

enzyme (Mbo I) **New England Biolabs**

DNA fragment end reparation

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).

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

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

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

In situ ligation

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

10X NEB T4 DNA ligase buffer **New England Biolabs**

10% Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich)**

10 mg/ml BSA **New England Biolabs**

T4 DNA ligase **New England Biolabs**

Reverse-crosslinked

6 Add 10 mg/ml proteinase K and 1% SDS to the tube and incubate at 56°C 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.

10 mg/ml proteinase K **New England Biolabs**

1% SDS **Ambion**

MPS **Invitrogen - Thermo Fisher**

70% ethanol **Sinopharm Chemical Reagent Co.**

Biotin-containing fragments capture

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

Dynabeads MyOne Streptavidin T1 **Invitrogen - Thermo Fisher**

DNA fragment end repairation

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.

10X NEB T4 DNA ligase buffer **New England Biolabs**

10 mM ATP **New England Biolabs**

25 mM dNTP mix **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 **New England Biolabs**

DNA fragment adenylation

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

10X NEBuffer 2 **New England Biolabs**

10 mM dATP **Invitrogen - Thermo Fisher**

5 U/µl NEB Klenow exo minus **New England Biolabs**

Adaptor ligation

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°C 3 min.; [98°C 20 sec., 60°C 15 sec., 72°C 15 sec.] (8 cycles); 72°C 10 min.

10X T4 PNK Reaction Buffer **New England Biolabs**

100mM ATP **FERMENTAS Inc.**

600 U/ul T4 DNA Ligase **New England Biolabs**

50% PEG8000 **RIGAKU**