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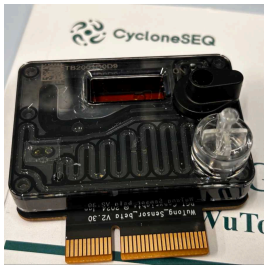
CycloneSEQ based Pore-C Library Preparation and Sequencing Protocol



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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is designed for Pore-C library preparation and sequencing of extracted animal DNA on the CycloneSEQ sequencing platform.

Troubleshooting

Tissue Fixation & Crosslinking

1 Homogenization & Washing:
- Using 100 mg of fresh tissue per sample, homogenize in 2 mL 1x PBS using a sterile pestle.
- Centrifuge at 2,000xg for 5min at 4°C. Discard supernatant.

2 Fixation:
- Resuspend pellet in 10 mL 1% formaldehyde in 1x PBS
- Incubate 10 min at RT with gentle rotation.

3 Quenching
- Add 527 µL 2.5 M glycine.
- Incubate 5 min at RT, then incubate 10 min on ice.
- Centrifuge at 2,000xg for 10 min at 4°C. Discard the supernatant.

Nuclei isolation & Digestion

4 Lysis:
- Resuspend pellet in 550 µL ice-cold lysis buffer (*10 mM Tris-HCl pH 8.0, 10 mM NaCl, 0.2% Igepal-CA630, 50 µL of protease inhibitor cocktail)*
- Incubate 15 min on ice.
- Centrifuge at 2,000xg for 10 min at 4°C. Discard supernatant.

5 Digestion Prep:
- Resuspend pellet in 200 µL ice-cold 1.5× digestion buffer.
- Centrifuge at 2,000×g for 10 min at 4°C.
- Resuspend pellet in 300 µL 1.5× digestion buffer.
- Add 33.5 µL 1% SDS.

6 Digestion:
- Incubate in thermomixer: 65°C, 300 rpm, 10 min.
- Add 37.5 µL 10% Triton X-100 (to quench SDS).
- Add 45 µL NlaIII (10 U/µL) + 34 µL nuclease-free water.
- Rotate 18 hr at 37°C.
- Heat-inactivate: 65°C for 20 min.

Proximity Ligation

7 Ligation Master Mix:
Prepare *550 µL* mix per sample:
- 100 µL 10× T4 DNA ligase buffer (with ATP)
- 10 µL BSA (10 mg/mL)

- 50 µL T4 DNA ligase (400 U/µL)
- 390 µL nuclease-free water

8 Ligation:
- Add master mix to sample.
- Rotate 5 hr at RT.

Crosslink Reversal & DNA Purification

9 Protein Digestion:
- Add master mix:
  (1) 100 µL 10% SDS
  (2) 500 µL 20% Tween-20
  (3) 100 µL Proteinase K (20 mg/mL)
  (4) 300 µL nuclease-free water
- Incubate 56°C for 18 hr.

10 DNA Extraction:
- Add equal volume phenol:chloroform:isoamyl alcohol (25:24:1).
- Centrifuge 12,000×g for 15 min at 4°C.
- Transfer aqueous phase to new tube.

11 Precipitation:
- Add 0.7× volume isopropanol.
- Incubate -20°C for 1 hr.
- Centrifuge 12,000×g for 30 min at 4°C.
- Wash pellet with 70% ethanol. Air-dry.

Library Construction

12 Size Selection & Bead Cleanup:
- Resuspend DNA in 50 µL nuclease-free water.
- Perform size selection >1.5 kb using DNA Clean Beads per manufacturer's instructions.

13 CycloneSEQ Library Prep:
- Construct library using CycloneSEQ Long-Read WGS Kit (refer to separate protocol).
- Quantify with Qubit fluorometer.

Sequencing

14 Run Sequencing:
- Load library onto CycloneSEQ WuTong02 platform.
- Follow manufacturer's run parameters.