Jun 10, 2025

CycloneSEQ long-read WGS library preparation and sequencing

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.n92ldnk2nv5b/v1

Qunfei Guo[1], youliang Pan[1], Wei Dai[1], Wei Jiang[1]
[1]BGI

Guo qunfei
BGI

DOI: **https://dx.doi.org/10.17504/protocols.io.n92ldnk2nv5b/v1**

Protocol Citation: Qunfei Guo, youliang Pan, Wei Dai, Wei Jiang 2025. CycloneSEQ long-read WGS library preparation and sequencing. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.n92ldnk2nv5b/v1**

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

Created: June 07, 2025

Last Modified: June 10, 2025

Protocol Integer ID: 219759

Keywords: CycloneSeq, Long-read sequencing, Genomics, WGS, sequencing cycloneseq, libraries on the cycloneseq platform, cycloneseq platform, cycloneseq, sequencing platform, genome, sequencing, read wgs library preparation, extracting dna, dna from animal tissue, wg, dna, read

Funders Acknowledgements:
Nation Key R&D Program of China
Grant ID: 2024TFC3406300

Abstract

CycloneSEQ is a novel long-read sequencing platform developed by BGI-Research. After extracting DNA from animal tissue, we use this protocol to prepare and sequence long-read whole-genome (WGS) libraries on the CycloneSEQ platform.

Troubleshooting

DNA Repair

1 Sample Transfer and Dilution

- Transfer 2 µg of DNA sample (concentration ≥23 ng/µL) to a sterile tube.
- Add nuclease-free water to adjust the total volume to 44 µL.

2 Repair Mix Preparation

- Add the following reagents to the diluted DNA:
  （1）6 µL DNA repair buffer 1
  （2）3 µL DNA repair buffer 2
  （3）3 µL DNA repair enzyme 1
  （4）4 µL DNA repair enzyme 2
- Mix thoroughly by gentle pipetting.

3 Incubation

- Place in a pre-programmed thermocycler and run:
  （1）20°C for 10 minutes
  （2）65°C for 10 minutes
  （3）Hold at 4°C (until purification).

Purification of Repaired DNA

4 Bead Cleanup:

- Add 1.0x volume DNA clean beads to the reaction mix.
- Incubate for 5 minutes at room temperature.
- Pellet beads on a magnetic stand. Discard supernatant.

5 Elution

- Wash beads twice with 80% ethanol. Air-dry completely.
- Resuspend beads in 60 µL nuclease-free water.
- Transfer supernatant (repaired DNA) to a new tube.

Adaptor Ligation

6 Ligation Mix Preparation:

Combine:

- Purified DNA (60 µL)
- 10 µL Sequencing adaptors
- 25 µL 4x ligation buffer
- 10 µL DNA ligase
- 2.5 µL nuclease-free water

Mix gently by pipetting.

7 Incubation

- Incubate at 25°C for 30 minutes.

Purification of Ligated Products

8 Bead cleanup:

- Add 1.0x volume DNA clean beads.
- Incubate 5 minutes at RT. Pellet on magnetic stand. Discard supernatant.

9 Wash and Elution:

- Resuspend beads in 500 µL long fragment wash buffer (gentle pipetting).
- Pellet beads, discard supernatant. Air-dry briefly.
- Elute DNA in 17 µL elution buffer. Transfer to a clean tube.

Library Quantification and Sequencing

10 Quantification:

- Measure library concentration using a Qubit fluorometer.

11 Sequencing:

- Load Prepared library onto the CycloneSEQ WuTong02 platform.
- Run sequencing according to manufacturer specifications.