Mar 28, 2025

CycloneSEQ library construction from DNA of isolated bacteria

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzk3k2vx1/v1

Wang Mengmeng[1], Hewei Liang[1], Tao Zeng[1], Junyi Chen[1], Yuanqiang Zou[1], Liang Xiao[1]
[1]BGI

GigaScience Press BGI

Mengmeng Wang
BGI

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzk3k2vx1/v1

Protocol Citation: Wang Mengmeng, Hewei Liang, Tao Zeng, Junyi Chen, Yuanqiang Zou, Liang Xiao 2025. CycloneSEQ library construction from DNA of isolated bacteria. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.rm7vzk3k2vx1/v1**

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

Created: November 21, 2024

Last Modified: March 28, 2025

Protocol Integer ID: 112526

Keywords: genomics, long-read sequencing, microbiology, CycloneSEQ, cycloneseq library construction from dna, isolated bacteria cycloneseq, cycloneseq library construction, cycloneseq library protocol, research this cycloneseq library protocol, cycloneseq platform, assembly of circular complete genome, circular complete genome, read sequencing, sequencing platform, genome, bacterial strain, mixed microbial community, bacteria, dna

Funders Acknowledgements:
The Shenzhen Municipal Government of China
Grant ID: XMHT20220104017, CXB201108250097A

Abstract

CycloneSEQ is a novel long-read sequencing platform developed by BGI-Research This CycloneSEQ library protocol is tailored for the assembly of circular complete genomes from mixed microbial communities. DNA is extracted from bacterial strains and after construction the resulting libraries can be sequenced on the CycloneSEQ platform.

Troubleshooting

CycloneSEQ library construction from DNA of isolated bacteria

50m

1 Approximately 2 mL of liquid medium with 10^8 or 10^9 CFU (colony forming units) of bacterial cells are extracted using a DNA extraction Kit for high-throughput applications.

2 CycloneSEQ library preparation and sequencing follows the manufacturer's guidelines. Each sample, containing 2 µg of input DNA (≥21 ng/µL), is diluted with nuclease-free water to 192 µL, then mixed with 14 µL each of DNA repair buffers 1 and 2, 12 µL of DNA repair enzyme 1, and 8 µL µL of DNA repair enzyme 2.

3 The mixtures are then incubated in a thermocycler at 20 °C for 00:10:00, 65 °C for 00:10:00, and held at 4 °C.

20m

4 After incubation, the mixtures are purified with 1.0x DNA clean beads and eluted with 240 µL of nuclease-free water.

5 The end-repaired samples are then mixed with 10 µL of sequencing adaptors, 100 µL of 4x ligation buffer, 40 µL of DNA ligase, and 10 µL of nuclease-free water, and incubated at 25 °C for 00:30:00.

30m

6 The ligated products are purified again with 1.0x DNA clean beads, resuspended with long fragment wash buffer, and recovered into 42 µL of elution buffer.

7 The resulting libraries are quantified using a Qubit fluorometer and sequenced on the CycloneSEQ WuTong02 platform according to the manufacturers sequencing protocols.