Nov 13, 2024

CycloneSEQ library construction from single bacteria

DOI
https://dx.doi.org/10.17504/protocols.io.4r3l2989qv1y/v1

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

[1]BGI Research

GigaScience Press BGI

Mengmeng Wang
BGI

DOI: https://dx.doi.org/10.17504/protocols.io.4r3l2989qv1y/v1

Protocol Citation: Wang Mengmeng, Liang Hewei, Tao Zeng, Chen Junyi, Zou Yuanqiang, Xiao Liang 2024. CycloneSEQ library construction from single bacteria. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l2989qv1y/v1

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

Created: November 12, 2024

Last Modified: November 13, 2024

Protocol Integer ID: 111943

Keywords: cycloneseq library construction for single bacteria, cycloneseq library construction from single bacteria, quality complete bacterial genome, complete bacterial genome, cycloneseq library construction, bacterial draft assembly, gaps in bacterial draft assembly, cycloneseq platform, single bacteria, dnqseq platform, hybrid assembly method, assembly, read sequencing, bacteria, genome, sequencing

Abstract

In a recent study, we constructed several high-quality complete bacterial genomes using a hybrid assembly method, which combines long-read sequencing from the CycloneSEQ platform and short-read sequencing from the DNQSEQ platform to fill gaps in bacterial draft assemblies.
The workflow of CycloneSEQ library construction for single bacteria can be found in the source paper and the following protocol.

1 Approximately 2 mL of liquid medium with 10^8 or 10^9 cfu of bacterial cells was extracted using the DNA Kit for high-throughput applications.

2 The CycloneSEQ library preparation and sequencing followed the manufacturer's guidelines. Each sample, containing 2 µg of input DNA (≥21 ng/µL), was 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 of DNA repair enzyme 2.

3 The mixtures were 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 were purified with 1.0x DNA clean beads and eluted with 240 µL of nuclease-free water.

5 The end-repaired samples were 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 minutes. 30m

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

7 The libraries were quantified using a Qubit fluorometer and sequenced on the CycloneSEQ WuTong02 platform according to the protocol.