SQK-MAP005 protocol for library preparation for Nanopore sequencing

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Abstract

This protocol describes the library preparation for Nanopore sequencing according to the SQK-MAP005 protocol.

It accompanies the *GigaScience* publication:

Benjamin Istace, et al. (2017) De novo assembly and population genomic survey of natural yeast isolates with the Oxford Nanopore MinION sequencer. *GigaScience...*

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Protocol

DNA fragmentation

Step 1.

Fragment DNA (2µg for a 8Kb or 6 to 10µg for a 20Kb size) by using Covaris gTube

DNA repair

Step 2.

Perform PreCR treatment (NEB PreCR® Repair Mix) of fragmented DNA

Clean Up

Step 3.

The DNA repair reaction was cleaned up with Agencourt AMPure XP beads (1x)

End Repair

Step 4.

DNA fragments were End-repaired by using NEBNext® End Repair Module

Clean Up

Step 5.

The end-repair reaction was cleaned up with Agencourt AMPure XP beads (1x)

dA-tailing

Step 6.

DNA fragments were dA-tailed by using the NEBNext® dA-Tailing Module

Clean Up

Step 7.

The dA-tail reaction was cleaned up with AMPure beads (1x)

Adaptors ligation

Step 8.

Nanopore adaptors were ligated to the dA-tailed DNA fragment by using NEB Blunt/TA Ligase Master Mix

Clean Up

Step 9.

The ligation reaction was cleaned up with His-Tag Dynabeads (1x)