SQK-MAP006 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-MAP006 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 FFPE treatment (NEBNext® FFPE DNA 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

dA-tailing

Step 5.
DNA fragments were dA-tailed by using the NEBNext® dA-Tailing Module

Clean Up

Step 6.

The End-Prep reaction was cleaned up with AMPure beads (1x)

Adaptors ligation

Step 7.
Nanopore adaptors were ligated to the dA-tailed DNA fragment by using NEB Blunt/TA Ligase Master Mix

Clean Up

Step 8.

The ligation reaction was cleaned up with MyOne C1-beads (1x)