Low input long-read DNA isolation for Nanopore sequencing

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Abstract

DNA isolation from gram negative bacteria with chemical SDS lysis (Phenol) for sequencing on Nanopore. Fast low input cell culture (5ml) with max output (2-3 µg) and longer reads (30kb+) .

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Before start

Prepare buffer (taken from http://bit.ly/2FfjomS @Josh Quick)

TLB:

10 mM Tris-Cl, pH 8.0

25 mM EDTA, pH 8.0

0.5% (w/v) SDS

20 µg/ml Qiagen RNase A (add fresh just before use)

Materials

1X PBS (Phosphate-buffered saline) by Contributed by users

Proteinase K 17916 by Life Technologies

Buffered Phenol Chloroform Isoamyl alcohol (P:C:I) ((25:24:1, saturated with 10 mM Tris, pH 8.0 and 1 mM EDTA Sigma P2069 by Sigma

Ethanol (100%, Molecular Biology Grade) BP2818500 by Fisher Scientific

100ml Sodium acetate, pH5.2 [3M] R010 by G-Biosciences

1 x TE Buffer 12090015 by Thermo Fisher Scientific

Protocol

Harvest bacterial cells

Step 1.

5 ml bacterial overnight cellculture OD600 (0.8) spin at 4500-5000 x g , 4°C for 15 min.

AMOUNT
5 ml Additional info: bacterial overnight cellculture OD600 (0.8)

Lysis

Step 2.

Resuspend by pipette mixing in 200 µl sterile PBS.

AMOUNT
200 µl Additional info: 1X PBS

REAGENTS
1X PBS (Phosphate-buffered saline) by Contributed by users

Lysis

Step 3.

Add 2.5 ml TLB and vortex at full speed for 10 seconds.

AMOUNT
2500 µl Additional info: TLB

Lysis

Step 4.

Incubate at 37°C for 1 hour.

TEMPERATURE
37 °C Additional info:

Lysis

Step 5.

Add 25 µl Qiagen Proteinase K or other stock solution to a final concentration of 200 µg/ml.

AMOUNT
25 µl Additional info: Proteinase K

REAGENTS
Proteinase K 17916 by Life Technologies

Lysis

Step 6.

Mix by pippetting with 1000 µl blue tips 4-5 times.

Lysis

Step 7.

Incubate at 50°C for 2 hours, mix every 30 minutes by slowly rotating end-over-end 3 times.

TEMPERATURE
50 °C Additional info:

Phenol

Step 8.

Add 2.5 ml Buffered Phenol Chloroform Isoamyl alcohol and mix slowly by rotating end-over-end until mixure becomes milky. Incubate 10 min on a Hula mixer.

AMOUNT
2500 µl Additional info: Buffered Phenol Chloroform Isoamyl alcohol

Phenol

Step 9.

Spin at 5000 x g for 15 min

Phenol

Step 10.

Remove the aqueous phases and transfer it into a new tube.

Phenol

Step 11.

Repeat step 7-9

Ethanol precipitation

Step 12.

add 5 ml ice-cold 100% Ethanol and 250 µl 3M Sodium Acetate. Mix by slowly rotating end-over-end.

AMOUNT
5 ml Additional info: 100% ice-cold Ethanol
AMOUNT
250 µl Additional info: 3M Sodium Acetat
REAGENTS
Ethanol (100%, Molecular Biology Grade) BP2818500 by Fisher Scientific
100ml Sodium acetate, pH5.2 [3M] R010 by G-Biosciences

Ethanol precipitation

Step 13.

Incubate at -20°C for 10 min.

TEMPERATURE

-20 °C Additional info:

Ethanol precipitation

Step 14.

Spin at 5000 x g at 4°C for 30 min.

DNA

Step 15.

Add 200 µl 1 x TE

AMOUNT

200 µl Additional info: 1 x TE Buffer

REAGENTS

1 x TE Buffer 12090015 by Thermo Fisher Scientific

DNA

Step 16.

Incubate at Roomtemperatur over night (ca. 12 h).