

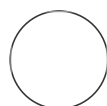


APR 10, 2023

Isolation of bacterial DNA with Gentra Puregene kit (modified protocol)

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ABSTRACT

The Qiagen protocol for purification of genomic DNA from gram-positive bacterial cultures using Yeast/Bact. Kit ([Gentra Puregene Handbook - QIAGEN](#)) was modified to aid in the isolation of bacterial DNA from heterogeneous, low volume, low OD cultures

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.5qpvorwo7v4o/v1

Protocol Citation: o.pogoutse, Megan Frederickson 2023. Isolation of bacterial DNA with Gentra Puregene kit (modified protocol). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.5qpvorwo7v4o/v1>

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

Created: Mar 02, 2023

Last Modified: Apr 10, 2023

PROTOCOL integer ID:
77988

Cell lysis

1 transfer culture to 1.5ml microfuge tube

- 2 centrifuge for 10min at 12000rpm to pellet cells
- 3 decant or aspirate off supernatant
- 4 add 1 ml sterile PBS to pellet and vortex briefly to resuspend cells
- 5 centrifuge for 10 min at 12000rpm
- 6 decant of supernatant
- 7 repeat steps 4-6 one more time
- 8 resuspend pellet in 50ul PBS buffer
- 9 add 2ul of MetaPolyzyme solution (5mg/ml) (Sigma#MAC4L)

- 10 incubate overnight at 35C
- 11 add 310ul lysis solution (Qiagen #158113) to each tube. Pipette up and down gently to resuspend cells
- 12 heat samples to 80°C for 5-10 minutes to complete the lysis
**stop step: lysed cells are stable at room temperature

RNase treatment

- 13 add 1.2ul of 10mg/ml RNase A per tube
- 14 invert tube gently to mix
- 15 incubate at 37°C for 30-60 minutes

Protein precipitation

- 16 cool sample to room temperature

- 17 add 105ul of protein precipitation solution (Qiagen #158123)
- 18 invert tube gently several times to mix
- 19 place on ice for 30 minutes
- 20 centrifuge at 12000rpm for 3 minutes.
(The precipitated protein should form a tight pellet).
- 21 transfer supernatant to a fresh sterile tube (by pipette)

DNA precipitation

- 22 add 1-2ul of glycogen (20mg/ml) (Thermofisher #R0561) to the supernatant and mix it by pipetting up and down
- 23 add 400ul of 100% isopropanol
- 24 invert tube gently 50 times to mix
(optionally: incubate at RT for 30 min to improve DNA yield)

- 25 pellet at 13000rpm for 3 minutes
- 26 carefully decant off isopropanol (the pellet might be loose)
- 27 add 500ul of 70% ethanol (cold), and invert tube a few times to wash the DNA pellet
- 28 centrifuge at 13000rpm for 3 minutes
- 29 pour off ethanol carefully – pellet may be loose
- 30 air dry pellet for 15-30 minutes
- 31 rehydrate DNA in ~35-50ul of 10mM Tris buffer (pH7.5-8.0)
(adjust for large or smaller pellet)