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

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

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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

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 80ºC 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 37ºC 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)