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

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Qiagen QIAprep Spin Miniprep for cosmids from metagenomic library

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ABSTRACT

Taken from QIAprep Miniprep Handbook (see attachment). Isolating cosmids from metagenomic clones for sequencing or transformations.

ATTACHMENTS

HB-1206-007_HB_QIAprep_Miniprep_1220_WW.pdf

GUIDELINES

All centrifugation steps are carried out at 14000 rpm (~16873 x *g*)) in an Eppendorf 5418 table-top microcentrifuge. Volumes of Buffers P1, P2, and N3 have been doubled as we are using a 10 ml culture volume.

MATERIALS

QIAprep Spin Miniprep Kit (Qiagen, 27104/27106)

Tetracycline hydrochloride (Sigma Aldrich, T3383-25G)

LB Broth contents:

- 4 g NaCl
- 4 g Tryptone
- 2 g Yeast Extract
- dH2O to 400 mL

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BEFORE START INSTRUCTIONS

- Add the provided RNase A solution to Buffer P1 before use. Use 1 vial RNase A (centrifuge briefly before use) per bottle Buffer P1 for a final concentration of 100 µg/ml. Mix and store at 2–8°C.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Check Buffers P2 and N3 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer P2 vigorously.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO2 in the air.
- **Optional**: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use 1 vial LyseBlue reagent per bottle Buffer P1 for a final dilution of 1:1000 (e.g., 10 µl LyseBlue into 10 ml Buffer P1). LyseBlue provides visual identification of optimum buffer mixing, thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris.

Grow bacterial culture overnight

1 Make up 15 mg/mL tetracycline hydrochloride in water.

2 Dilute 1/1000 in LB broth (final conc. 15 ug/mL).

3 Aliquot 10 mL tetracycline LB into 50 mL Falcon tubes.

4 Using a sterile inoculation loop, scrape the top of the glycerol stock and inoculate in tetracycline LB.

5 Grow for up to 12-16 hours at 37°C and shaking at 180-200 rpm.

Note

Growth for more than 16 h is not recommended because cells begin to lyse and plasmid DNA yields may be reduced.

Harvest cells

6 Centrifuge at 4500 rpm (4347 x *g*) in the Eppendorf 5920 R centrifuge.

7 Pour off supernatant.

Isolate cosmid

8 Re-suspend pelleted bacterial cells in 500 µL Buffer P1 and transfer to a 2 mL micro-centrifuge tube.

Expected result

No cell clumps should be visible after resuspension of the pellet.

9 Add 500 µL Buffer P2 and mix thoroughly by inverting the tube 4–6 times. Do not allow the lysis reaction to proceed for more than 5 min.

Note

Do not vortex, as this will result in shearing of genomic DNA.

Expected result

The solution should become viscous and slightly clear.

If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

10 Add 700 µL Buffer N3 and mix immediately and thoroughly by inverting the tube up to 10 times.

Expected result

The solution should become cloudy.

If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

11 Centrifuge for 10 min.

Expected result

A compact white pellet will form.

12 Apply 800 µL of the supernatant from step 10 to the QIAprep 2.0 Spin Column by pipetting.

13 Centrifuge for 30-60 s. Discard the flow through.

14 Repeat steps 11 and 12 with the remaining supernatant, carefully avoiding the pellet.

15 Wash the QIAprep 2.0 Spin Column by adding 500 µL Buffer PB and centrifuging for 30–60 s. Discard the flow-through.

Note

This step is necessary to remove trace nuclease activity as the host strain is HB101.
DH5α does not require this additional wash step.

16 Wash the QIAprep 2.0 Spin Column by adding 750 µL Buffer PE and centrifuging for 30–60 s.

17 Discard the flow through

18 Centrifuge at full speed for an additional 1 min to remove residual wash buffer.

Note

Important: Residual wash buffer will not be completely removed unless the flow through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

19 Place the QIAprep 2.0 Spin Column in a sterile 1.5 ml microcentrifuge tube.

20 Heat Buffer EB or water at 70°C in a block heater

Note

This is necessary as the metagenomic cosmids >10 kb

21 To elute DNA, add 50 uL pre-heated buffer EB or water to the centre of each QIAprep 2.0 Spin Column

Note

If water is used for elution, make sure that its pH is between 7.0 and 8.5. Elution efficiency is dependent on pH, and the maximum elution efficiency is achieved within this range. A pH <7.0 can decrease yield.

Store DNA at −30 to −15°C when eluted with water, as DNA may degrade in the absence of a buffering agent.

22 Let stand for 60 s

23 Centrifuge for 60 s

24 Measure the concentration of dsDNA in 2 µL of sample using the NanoDrop spectrophotometer

Note

Blank with the solution (EB buffer or water) used to elute DNA

Expected result

A 10 ml overnight LB culture typically yields 5-10 ug DNA