Modified Qiagen QIAprep Spin Miniprep

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Abstract

Modified to try to get increased yields.

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Protocol

Lysis

Step 1.
Resuspend pelleted bacterial cells in 250µl Buffer P1 and transfer to a micro-centrifuge tube.
Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

Lysis

Step 2.
Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.
Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.
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.

AMOUNT
250 µl Additional info:

REAGENTS
Buffer P2 by Contributed by users

Lysis

Step 3.
Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.
To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g. ≥5 ml) may require inverting up to 10 times. 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 sus- pension indicates that the SDS has been effectively precipitated.

AMOUNT

350 µl Additional info:

REAGENTS

Buffer N3 by Contributed by users

Lysis

Step 4.

Centrifuge for 10 min at 13,000 rpm (17,900 x *g*) in a table-top microcentrifuge. A compact white pellet will form.

DURATION

00:10:00

Binding

Step 5.

Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.

ANNOTATIONS

Alan Cone 10 Jul 2015

Roughly about 850 uL.

Binding

Step 6.

Centrifuge for 30–60 s. Place flow through back into the spin column.

DURATION

00:01:00

Binding

Step 7.

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

DURATION

00:01:00

Wash

Step 8.

Wash the QIAprep spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through. This step is necessary to remove trace nuclease activity when using endA+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5®α do not require this additional wash step.

AMOUNT

500 µl Additional info:

REAGENTS

Buffer PB by Contributed by users

DURATION

00:01:00

Wash

Step 9.

Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.

AMOUNT

750 µl Additional info:

REAGENTS

Buffer PE by Contributed by users

DURATION

00:01:00

Wash

Step 10.

Discard the flow-through, and centrifuge at full speed for an additional 1 min to remove residual wash buffer.

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.

DURATION

00:01:00

ANNOTATIONS

Emily Hsiue 02 Jul 2017

Spin 3-5 minutes

Elution

Step 11.

Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 20 µl water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

DURATION

00:01:00

Elution

Step 12.

Elute DNA, add 30 µl water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

DURATION

00:01:00