Plasmid Extraction (miniPrep)

Taken from Qiagen's miniPrep protocol from miniPrep kit

PROTOCOL:

We used a Qiagen miniPrep kit.

Since we are working with a low copy plasmid/phagemid we prepared 4 ml of culture.

1. Take 4 ml of culture, centrifuge at 8000 rpm 16°C for 5min
2. Discard the supernatant and keep the bacterial pellet
3. Resuspend the pellet in 250µl of buffer P1 and transfer to a microcentrifuge tube. Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
4. Add 250µl of buffer P2, mix by inverting gently the tube 4-6 times. Wait for 2 minutes.
5. Add 250µl of N3 buffer in order to stop the lysis. Mix directly by inverting gently the tube 4-6 times. The solution should become cloudy.
6. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge. A compact white pellet will form.
7. Transfer the 800 µl of the supernatant from step 6 to a QIAprep 2.0 spin column by pipetting.
8. Centrifuge for 1 min at 13,000 rpm. Discard the flow-through.
9. Wash the QIAprep 2.0 spin column by adding 500µl of Buffer PB and centrifuging for 1min at 13,000 rpm. Discard the flow-through.
   Note: We are using XL-1 Blue and DH5®α strains. Host strains, such as XL-1 Blue and DH5®α, do not require this additional wash step, but we still did it.
10. Wash QIAprep 2.0 spin column by adding 750µl Buffer PE and centrifuging for 1min at 13,000 rpm.
11. Discard the flow-through, and centrifuge at full speed for an additional 1 min to remove residual wash buffer.
12. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) to the center of each QIAprep 2.0 spin column, let stand for 1 min and centrifuge for 1 min at 13,000 rpm.