Plasmid Extraction

Aim

To extract plasmid from bacteria.

Procedure

Before using this protocol:
Add provided RNase A solution to Buffer P1 and store at 2-8 °C
Add ethanol (96-100 %) to Buffer PE (See instructions on the bottle)
Optional: Add LyseBlue reagent to Buffer P1, 1:1000.

1. Pellet 1-5 ml bacterial overnight culture by centrifugation at > 8000 rpm for 3 min at 15-25 °C
2. Resuspend pelleted bacterial cells in 250 μl Buffer P1 and transfer to an Eppendorf tube
3. Add 250 μl Buffer P2. Mix thoroughly and gently the tube until the solution becomes clear.

   Caution! This reaction must not proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.
4. Add 350 μl Buffer N3, mix immediately and thoroughly by inverting the tube. If using LyseBlue reagent, the solution will become colorless.
5. Centrifuge for 10 min at 13 000 rpm.
6. Transfer the supernatant to the QIAprep spin column by pipetting.
7. Wash the spin column by adding 500 μl Buffer PB. Centrifuge for 30-60 sec, 13 000 rpm. Discard the flow-through.
8. Wash by adding 750 μl to the spin column. Centrifuge for 30-60 sec, 13 000 rpm and discard the flow-through. Spin for 1 min to remove residual wash. buffer and discard the flow-through
9. Place the spin column in a clean Eppendorf tube. Elute the DNA by adding 30 μl EB buffer or ddH_2O. Centrifuge for 1 min, 13 000 rpm

Note!

This protocol was originally distributed from QIAGEN and has been modified with the aim of achieving a higher yield.

Sources

https://www.qiagen.com/ie/resources/resourcedetail?id=89bfa021-7310-4c0f-90e0-6a9c84f66cee&lang=en (retrieved 04.10.2016)