NucleoSpin Plasmid QuickPure : Isolation of high-copy plasmid DNA from E. coli

This protocol is for a preparation of up to 15µg of high-copy plasmid or DNA using the Macherey-Nagel Nucleospin plasmid QuickPure Kit.

Procedure

1.Use 1-5mL (we used 1.5mL) of a saturated E.Coli LB culture, pellet cells in a standard benchtop microcentrifuge for 30 s at 11,000 x g. Discard the supernatant and remove as much of the liquid as possible.

2. Add 250 µL Buffer A1. Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Buffer A2!

3. Check Buffer A2 for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30 – 40 °C until precipitate is dissolved completely. Cool buffer down to room temperature (18 – 25 °C).

4. Add 250 µL Buffer A2. Mix gently by inverting the tube 6 – 8 times. Do not vortex to avoid shearing of genomic DNA. Incubate at room temperature for up to 5 min or until lysate appears clear.

5. Add 300 µL Buffer A3. Mix thoroughly by inverting the tube 6 – 8 times. Do not vortex to avoid shearing of genomic DNA!

6. Centrifuge for 5 min at 11,000 x g at room temperature. Repeat this step in case the supernatant is not clear!

7. Place a NucleoSpin Plasmid Column in a Collection Tube (2 mL) and decant the supernatant from step 3 or pipette a maximum

of 750 µL of the supernatant onto the column. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the NucleoSpin Plasmid Column back into the collection tube.

8. Add 600 µL Buffer A4 (supplemented with ethanol). Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the NucleoSpin Plasmid Column back into the empty collection tube.

9. Centrifuge for 2 min at 11,000 x g and discard the collection tube.

10. Place the NucleoSpin Plasmid Column in a 1.5 mL tube and add 50 µL Buffer AE. Incubate for 1 min at room temperature. Centrifuge for 1 min at 11,000 x g.

This protocol is extracted from "Plasmid DNA purification user manual" by **Macherey-Nagel**