## 5.3 NucleoSpin® Plasmid QuickPure: Isolation of high-copy plasmid DNA from *E. coli*

**Before starting the preparation:**

- Check if Wash Buffer AQ was prepared according to section 3.

**1 Cultivate and harvest bacterial cells**

Use **1 – 3 ml** 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.

**11,000 x *g***
**30 s**

**2 Cell lysis**

**+ 250 µL A1**

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!

**Resuspend**

*Attention: 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).*

**+ 250 µL A2**

**Mix**

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.

**RT**
**5 min**

**+ 300 µL A3**

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

**Mix**

**3 Clarification of lysate**

Centrifuge for **5 min** at **11,000 x *g*** at room temperature.

**11,000 x *g***
**5 min**

**4 Bind DNA**

Place a NucleoSpin® Plasmid QuickPure 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 QuickPure Column back into the collection tube.

**Load supernatant**

**11,000 x *g***
**1 min**

Repeat this step to load the remaining lysate.

**5 Wash silica membrane**

Add **450 µL Buffer AQ** (supplemented with ethanol, see section 3). Centrifuge for **3 min** at **11,000 x *g***.

**+ 450 µL AQ**

Very carefully discard the collection tube and the flow-through and make sure the spin cup outlet does not touch the wash buffer surface. Otherwise repeat the centrifugation step.

**11,000 x *g***
**3 min**

**6 Dry silica membrane**

The drying of the NucleoSpin® Plasmid QuickPure Column is performed by the 3 min centrifugation in step 5.

**7 Elute DNA**

Place the NucleoSpin® Plasmid QuickPure Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL Buffer AE**. Incubate for **1 min** at **room temperature**. Centrifuge for **1 min** at **11,000 x *g***.

**+ 50 µL AE**

**RT**
**1 min**

**11,000 x *g***
**1 min**

*Note: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) see section 2.4.*