This protocol is based on the Qiagen Miniprep instruction manual.
This document is version 1.02: last updated 8.1.11.

∅∅∅ DNA Purification (Miniprep)

This protocol will purify DNA based on the QIAgen miniprep system. Bacteria must be first pelleted, then washed and lysed in accordance with the instructions. The supernatant will then be added to a column, where the DNA will be filtered, washed, then eluded in 30-50µL of water.

Solutions
Cell solution
Buffer P2 (at 4°C)
Buffer N3
Buffer PB
Buffer PE
Buffer EB

Materials
100,1000µl Pipettes
Centrifuge tubes
Spin columns with inserts

You will also need access to a
Centrifuge (rotor at 4°C)
Vortex

Procedure

1. Transfer the **bacterial grown solution** into **1.5mL centrifuge tubes** with a **1000µL pipette**. An autopipette can also be used.

2. Pellet the solution in the **centrifuge** at **8000 rpm** for **1 minute.** Discard the supernatant.

3. Add **250µL Buffer P1** to each centrifuge tube and **vortex** until pellet disappears and bacteria are completely resuspended.

4. Add **250µL Buffer P2.** Mix *immediately* by inverting the tube. *Do not vortex*!

5. Add **350µL Buffer N3.** Mix *immediately* by inverting the tube. *Do not vortex*! Try to avoid the formation of chunks, as buffer N3 will trigger the formation of a precipitate.

6. Centrifuge the tube for **10 minutes** at **13 000 rpm**. A white pellet will form.

7. Label a clean, new Qiagen spin column. Pour the supernatant from the centrifuge tube into the top of the column. Remove any remaining supernatant with a **100µL pipette** pipette into the column.

8. Place the column in a flow-through container. Centrifuge at **13 000 rpm** for **1 minute.** Decant the flow-through.

9. Add **500µL Buffer PB** with a **1000µL pipette.** Centrifuge at **13 000 rpm** for **1 minute.** Decant the flow-through.

10. Add **750µL Buffer PE** with a **1000µL pipette.** Centrifuge at **13 000 rpm** for **1 minute.** Decant the flow-through.

11. Centrifuge at **13 000 rpm** for **1 minute.** This will remove any residual buffer in the column. It is not necessary to decant the flow-through, as the column collection tube will be discarded.

12. Remove the column from its collector and insert into a fresh, labeled **1.5mL centrifuge tube.**

13. Add **30-50µL Buffer EB** to the center of the column's filter with a **100µL pipette**. Ensure the buffer is absorbed by the filter.

14. Centrifuge at **13 000 rpm** for **5 minutes.** The flowthrough will contain the desired DNA.