Mini-Prep

For this project the HiYield Plasmid Mini DNA Kit from the company “SGL” was used.

Part 1 (day 1)

- Select 12 clones of the 24h incubated E. coli plate from the E. coli-transformation.
- Prepare an eprouvette containing 2mL selective medium (depending on the resistance of the plasmid) for each selected clone.
- Prepare an Agar-Plate with the correct selective agar.
- Label the eprouvettes with the numbers of the clones, divide the Agar-Plate in sections and label them according to the number of clones this will serve as master plate.
- Use sterile tips to take some bacterial material of the selected clones of the E. coli plate, draw a line in the correct section of the master plate and put the tip in the correct eprouvette.
- Incubate for 24h, 37°C, shaking.

Part 2 (day 2)

- Wrap the master plate in parafilm and store it at 4°C
- Transfer 1mL of the E. coli cells of the eprouvette in a 1,5 mL reaction tube.
- Centrifuge: 15,000 x g, 1min.
- Discard the supernatant.
- Repeat Step 8-10 until all the E. coli culture is in the reaction tube.
- Add 200µL PD1 Buffer (make sure the RNAse A was added)
- Resuspend the cells by vortexing or pipetting up and down.
- Add 200µL PD2 Buffer + mix gently by inverting 10 times (do not vortex to avoid shearing the genomic DNA)
- Incubate at room temperature for at least 2min but do not exceed 5min.
- Add 300µL PD3 Buffer + immediately mix gently by inverting 10 times (do not vortex to avoid shearing the genomic DNA)
- Centrifuge: 15,000 x g, 3min
- Place a PD column in a 2mL Collection Tube.
- Add the supernatant of step 17.
- Centrifuge: 15,000 x g, 1min
- Discard the flow-through.
- Place the PD column back in the 2mL Collection Tube.
- Add 400µL W1 Buffer into the PD Column.
- Centrifuge: 15,000 x g, 1min
- Discard the flow-through
- Place the PD column back in the 2mL Collection Tube.
- Add 600µL Wash Buffer into the PD Column.
- Centrifuge: 15,000 x g, 1min
- Discard the flow-through.
- Place the PD column back in the 2mL Collection Tube.
- Centrifuge: 15,000 x g, 3min to dry the column matrix.
- Transfer the column into a 1,5mL reaction tube.
- Add 50µL of Elution Buffer in the centre of the column matrix.

- Incubate 2min at room temperature.
- Centrifuge: 15,000x g, 2min to elute the DNA.
- Measure the DNA concentration via NanoDrop and store at -20°C