Endotoxin-free Midi-Prep: Date:

- Exclusively use disposable plastic pipettes!!!
- Set centrifuge to 4°C.
- Measure OD of a 1:10 dilution of the bacteria culture (reference: DYT): λ = 600 nm (Spectrometer at Tobi's bench)
  Volume to be used: V [ml] = $400/OD_{600}$
- Fill bacteria culture into 50 mL falcons -> gloves
- Centrifuge at 5000 g, 4°C for 10 minutes (Centrifuge at PCR machine)
- Discard supernatant.
- Resuspend pellet completely in **8 mL RES-EF + RNAse A** buffer (in freezer besides cold room) -> 8 mL for all falcons
- Vortex the cells until no clumps remain in the suspension.
- Check LYS-EF buffer for precipitated SDS: if white precipitate is visible, warm buffer for several minutes at 30-40°C until precipitate is dissolved completely, cool buffer down to room temperature.
- Ad **8 mL LYS-EF** buffer to the suspension. Mix by gently inverting the tube 5 x. DO NOT VORTEX!
- **Incubate for 5 minutes** at room temperature.
- Place column into a 250 Erlenmeyer flask with the help of a "plastic washer", equilibrate column: apply **15 mL EQU-EF** buffer onto the rim of the column filter, make sure to wet the entire filter. Allow the column to empty by gravity flow.
- Add **8 mL NEU-EF** buffer to the suspension and immediately mix the lysate by inverting the tube 10-15 times. DO NOT VORTEX! **Incubate on ice for 5 minutes.**
- Invert the tube 3 times directly before applying the lysate to the column filter.
- **1st wash**: Apply **5 mL FIL-EF** buffer to the the rim of the column filter. Allow the column to empty by gravity flow.
- Discard column filter.
- **2nd wash**: Wash with **35 mL ENDO-EF** buffer. Allow the column to empty by gravity flow.
- **3rd wash**: Wash with **15 mL WASH-EF** buffer. Allow the column to empty by gravity flow.
- **Elution**: Elute DNA with **5 mL ELU-EF buffer**. Collect eluate in a 50 mL falcon.
- **Precipitate** DNA: Add **0.7 volumes isopropanol** (3.5 mL for 5 mL eluate), vortex well and let the mixture sit for 2 minutes.
- Remove plunger from a 30 mL Syringe and attach "NucleoBond" Finalizer to the outlet. Fill mixture into syringe, insert plunger and press the mixture **slowly** through finalizer using constant force!
- Discard flow-through.
- Wash precipitate: Remove finalizer from syringe, pull out plunger and reattach finalizer to syringe outlet. Fill **2 mL endotoxin-free 70% EtOH** into syringe, insert plunger and press ethanol **slowly** through finalizer.
- Discard flow-trough.
- Dry filter membrane:
  - Remove finalizer from syringe, pull and press plunger several times with appropriate force. Pull out the plunger and reattach finalizer.

- Press air through finalizer with appropriate force while touching a tissue with the tip of the finalizer (soak up EtOH). Repeat this step 5 times. Attention: Never pull the plunger back if the finalizer is attached to the outlet!!!

➔ **Elute** DNA: Pull out plunger of a 1 mL syringe and attach finalizer to its outlet. Pipette **700 µL TE-EF** buffer into syringe. Place finalizer over a fresh collection tube (1.5 mL), elute DNA **carefully** by inserting plunger.

➔ Remove finalizer from syringe, pull plunger and reattach finalizer to outlet. **Transfer the 1st elute back** into syringe and elute into the same eppi a second time.

➔ Remove finalizer from syringe, pull out plunger to aspirate air, teattach finalizer and press air out again to force as much eluate as possible.

➔ Meassure **DNA concentration** with Nano-Drop.