Crayfish Extraction with NucleoSpin® Plant II Kit

Material:

- Tweezers
- 20ml Tubes (white lid)
- Spattle
- Pestle
- 1.5ml Tube (2x times the extraction amount)
- Beaker (MiliQ)
- Beaker (EtOH)
- Beaker (Javel)
- Beaker (for flow-through waste)

Procedure:

- **Preparation**:
  - Check if PL1 buffer is cloudy, if yes put it in a beaker with warm water to let it dissolve
  - Do aliquots of each solution with the amount you need for the extraction in a 20ml Tube (PL1, PC, PW1, PW2-buffers), in 1.5ml Tube(PE-buffer) and in a 0.5ml Tube (RNase A)
  - Clean pestles and tweezers first with Javel then with 70%EtOH and with MiliQ and let it dry *(can be done between the first incubation time)*
  - Preheat heating block at 55°C
  - **Incubate PE buffer at 65°C (Elution buffer has to be at 65°C for the last step!)**

- **Extraction:**
  - Take sample from the tube and put it in a new 1.5ml Tube (If sample size is too big, cut it in this size ▭)
  - Incubate samples at **55°C** for **20min** with open lid and cover tubes with a tissue
  - Put a spatula tip of quartz into the tube
  - Homogenize tissue very well with a clean pestle (approx. 30 times)
  - Pipette **400ul PL1 Buffer** along the pestle
  - Add **10ul** of **RNase A** and vortex tube very well

- Incubate tube for **30min** at **65°C** and **400rpm**, invert tubes few times between the incubation
- Place Nucleo Spin Filter (violet ring) into a new collection tube (2ml)
- Mix lysate by pipetting up and down and then load lysate onto the column. Be sure that you load everything
- Centrifuge tube at **11`000 x g** for **5min**. During Centrifugation prepare 1.5ml Tubes for the next step
- Transfer flow-through into a new 1.5ml Tube and throw away column (If not all liquid has passed the filter, repeat centrifugation step)
- Add **450ul PC Buffer** and vortex well
- Place NucleoSpin Plant II Column (green ring) into a new Collection Tube (2ml) and load **max. 650ul** of the **sample** and Centrifuge at **11`000 x g** for **1min** (If there is more left then repeat this step) -> Discard flow-through
- Add **400ul PW1 Buffer** to the column and centrifuge for **1min** at **11`000xg** -> Discard flow-through
- Add **650ul PW2 Buffer** to the column and centrifuge for **1min** at **11`000xg** -> discard flow-through
- Add **250ul PW2 Buffer** to the column and centrifuge for **2min** at **11`000xg** -> discard flow-through
- During centrifugation time prepare 1.5ml Tube for the next step
- Place column into a 1.5ml Tube
- Pipette preheated **50ul PE Buffer (65°C)** onto the membrane and incubate tube for **5min** at **65°C**
- Centrifuge tube for **1min** at **11`000 x g**
- Repeat this step with another **50ul PE Buffer (65°C)**
- Store DNA at -20°C

- **Cleaning:**
  - Clean pestle with a brush and water and rinse ones with MiliQ before you let it dry
  - Clean Tweezer first with Javel, then with 70%EtOH and with MiliQ