DNA Purification (gel extraction)

First, perform gel electrophoresis (gel electrophoresis protocol)

Cutting the gel

- A quick picture can be taken of the gel. Let it stay in the tray as to not destroy it.
- To cut the gel turn off the light and close the curtains.
- Clean a scalpel using ethanol.
- Place gel on UV plate and turn on the UV light to see the bands. Remember to wear safety visor.
- Cut across and then excise bands one at a time. Make sure to get entire band but not too much extra gel. Turn off UV light as soon as possible to avoid damage to DNA.
- Collect gel in 2.0 mL microtubes.
- Remember to weigh the tubes following band excision.

DNA purification:

Get the QIA DNA gel purification kit from gel room.

Protocol can be done with either vaccum or centrifugation.

Vacuum protocol:

It is important that vacuum are closed on tubes soon as they run dry, that all unused vacuum spaces are closed when running, and that vacuum is opened before turning off the vacuum suction.

- For 100 mg gel material add 300 µL **QG buffer** (max 400 mg gel/column – more can be used).
- Incubate in 10 min at 50°C. Vortex every 2-3 min (not necessarily required).
    - Check if color is yellow, if not add 10 µL 3 M sodium acetate (pH 5.0) and mix.
- Place QUI quick spin column in vacuum manifold and wash (Check if new 1x or 2x).
    - 1 mL 0.1 M HCl (located next too)
    - 3 x 1 mL MQ H_2O
    - 0.5 mL **PB**
    - For each apply and apply vacuum to clean.

- Apply gel mixture to manifold and vacuum. **Multiple tubes can be combined in one if same DNA**.
- Apply 750 µL **PE** and vacuum.
- Put spin column in 2 mL tube and centrifuge for 2 min to remove residual buffer.
- Place spin column in clean micro centrifuge tube.
- Add 50 µL **EB** (drops in center) and let it stand for 1 min, then centrifuge for 1 min.
- Optional: Repeat with 30 µL **EB** (to get all?).

Centrifuge protocol:

1. Add equal amount of SPW buffer: 100 µl for 100 µg gel.
2. Incubate 7 min at 60ºC, or until fully dissolved. Vortex every 1-2 min (not necessarily required).
    - Check if color is yellow, if not add 10 µL 3 M sodium acetate (pH 5.0) and mix.
3. Transfer max 700 µl to spin column, centrifuge at 10,000 g 1 min. Discard flow through, reuse collection tube.
4. Repeat step 3 until all is transferred.
5. Add 700 SPW buffer, spin 1 min at 10,000 g.
6. Add 300 µl DNA binding buffer with ethanol. Spin 2 min at max (>13,000 g). Discard flow through.
7. Repeat step 6
8. Spin 1 minutes to dry column and remove ethanol.
9. Place in new, 1,5 eppendorf tube for collection
10. Add 30-50 µl elution buffer to the center of the spin column, depending on band strength. Use more buffer when more DNA is present in gel excisions. Let sit 2 minutes before spinning 1 min at max
11. Store purified DNA at -20 ºC