Gel extraction with Quiagen QIAquick

Notes:

- All centrifugation steps are carried out at 13 000 rpm.

For sequencing (more thorough washing)

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.
   - ➢ How to cut DNA out of gel - using UV imaging in the gel room in floor 3 - remember lab coat, gloves and UV shield!
     1. Make sure you have images your gel
     2. Weigh your tubes without gel
     3. Clean cast and scalpel with ethanol
     4. Put gel on cast
     5. Put on UV shields + turn the blinds + turn off the lights
     6. Cut the gel with 4 incisions as close as possible to the DNA
     7. Image after cutting to see that you have everything
     8. Weigh your tubes with gel
2. Add 3 volumes Buffer QG to 1 volume gel (1 mg gel ≈ 1 µL). The maximum amount of gel per spin column is 400 mg. For > 2% agarose gels, add 6 volumes Buffer QG
3. Incubate at 50 °C for 10 min. Vortex the tube every 2-3 min to help dissolve the gel.
   - ➢ After the gel slice has dissolved completely, check that the colour of the mixture is yellow (similar to buffer QG without dissolved agarose. If the colour of the mixture is orange or violet, add 10 µL 3 M sodium acetate, pH 5.0, and mix. The mixture turns yellow.
4. Add 1 gel volume isopropanol to the sample and mix
5. Place a QIAquick spin column in a provided 2 mL collection tube. To bind DNA, apply the sample to the QUIquick column and centrifuge for 1 min. Discard flow-through and place the QIAquick column back into the same tube. For sample volumes > 800 µL, load and spin again.
6. Add 500 µL BUffer QG to the QIAquick column and centrifuge for 1 min. Discard flow-through and place the QUIAquick column back into the same tube.
7. To wash, add 750 µL Buffer PE to the QIAquick column and let the column stand for 2–5 min. Then centrifuge for 1 min. Discard flow-through and place the QUIAquick column back into the same tube.
8. Place the QIAquick column into a clean 1.5 mL microcentrifuge tube.
9. Add 30 µL Buffer EB to the center of the QIAquick membrane, let the column stand for 4 min, and then centrifuge for 1 min.