GEL EXTRACTION KIT

Aim: To get back the DNA purified thanks to the electrophoresis on agarose gel.

Materials:

- Molecular biology equipment: 55 °C water baths or dry block, pipette set, ice bucket, electrophoresis tank and power supply, imaging system, precision balance, microcentrifuge or vacuum manifold, UV face mask, spectrophotometer, UV quartz cuvette
- UV table, with long wavelength position
- Scalpel, and blade
- Gel extraction Kit from QIAGEN
- Isopropanol

Protocol:

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.

2. Weigh the gel slice in a colorless tube. Add 3 volumes Buffer QG to 1 volume gel (100 mg gel ~ 100 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 (or until the gel slice has completely dissolved). Vortex the tube every 2– 3 min to help dissolve gel. After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose). If the color 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 of isopropanol to the sample and mix.

5. Place a QIAquick spin column in a provided 2 ml collection tube or into a vacuum manifold. To bind DNA, apply the sample to the QIAquick column and centrifuge for 1 min at 13000 rpm or apply vacuum to the manifold until all the samples have passed through the column. Discard flow-through and place the QIAquick column back into the same tube. For sample volumes of >800 µl, load and spin/apply vacuum again.

6. If DNA will subsequently be used for sequencing, *in vitro* transcription, or microinjection, add 500 µl Buffer QG to the QIAquick column and centrifuge for 1 min or apply vacuum. Discard flow- through and place the QIAquick column back into the same tube.

7. To wash, add 750 µl Buffer PE to QIAquick column and centrifuge for 1 min or apply vacuum. Discard flow-through and place the QIAquick column back into the same tube.

8. *Note: If the DNA will be used for salt-sensitive applications (e.g., sequencing, blunt-ended ligation), let the column stand 2–5 min after addition of Buffer PE. Centrifuge the QIAquick column in the provided 2 ml collection tube for 1 min to remove residual wash buffer.*

9. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.

10. To elute DNA, add 50 µl Buffer EB, TE 0.1 (Tris-Cl 10 mM, pH 8.5, 0.1 mM EDTA) or water to the center of the QIAquick membrane and centrifuge the column for 1 min. For increased DNA concentration, add 30 µl Buffer EB to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge for 1 min. After the addition of Buffer EB to the QIAquick membrane, increasing the incubation time to up to 4 min can increase the yield of purified DNA.

11. If purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

12. Check amount of recovered DNA either by gel electrophoresis, and imaging or with a spectrophotometer and UV quartz cuvette.