

SEED 2009

## Day 5: Gel Purification

**Learning Objective:** This lab will teach you how DNA of a target length is recovered after separation by gel electrophoresis

**Background:** Last week you saw how DNA of different lengths can be separated using gel electrophoresis. This is an important process in molecular biology not only because it can be used for characterization but also because it can be used to selectively recover the desired fraction of DNA. This separation gives a higher degree of certainty of the product in the cloning process as constructs of the wrong length are removed and have no chance of providing erroneous results. However, this benefit comes with a drawback, decreased DNA product; as a result of inefficiencies in the purification process, some of the desired DNA is also lost.

**The exercise:** You will recover and purify the two DNA parts which were separated last week using gel electrophoresis, the plasmid pSB4A5 and the LacZa-GFP PCR product. You will quantify the amounts of these DNA products after purification so that you can move forward with the cloning process.

**Materials:** Agarose Gel Slices with DNA  
Qiagen Gel Extraction Spin Column and Buffers  
Microcentrifuge  
1.5 mL Microcentrifuge Tubes  
Deionized water  
UV Cuvettes  
Spectrophotometer

### Protocol

1. Weigh the piece of gel. (Hint: don't remove the gel from the tube, use an empty tube to tare the scale)
2. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100  $\mu$ l). For example, add 300  $\mu$ l of Buffer QG to each 100 mg of gel. The maximum amount of gel slice per QIAquick column is 400 mg; for gel slices >400 mg use more than one QIAquick column.
3. Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation. **IMPORTANT:** Solubilize agarose completely.

4. 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  $\mu$ l of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow. The adsorption of DNA to the QIAquick membrane is efficient only at pH  $\leq$ 7.5. Buffer QG contains a pH indicator which is yellow at pH  $\leq$ 7.5 and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.
5. Add 1 gel volume of isopropanol to the sample and mix. For example, if the agarose gel slice is 100 mg, add 100  $\mu$ l isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield. Do not centrifuge the sample at this stage.
6. Place a QIAquick spin column in a provided 2 ml collection tube.
7. To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min. The maximum volume of the column reservoir is 800  $\mu$ l. For sample volumes of more than 800  $\mu$ l, simply load and spin again.
8. Discard flow-through and place QIAquick column back in the same collection tube. Collection tubes are reused to reduce plastic waste.
9. Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min. This step will remove all traces of agarose.
10. To wash, add 0.75 ml of Buffer PE to QIAquick column and centrifuge for 1 min. Note: If the DNA will be used for salt-sensitive applications, such as blunt-end ligation and direct sequencing, let the column stand 2–5 min after addition of Buffer PE, before centrifuging.
11. Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at 17,900  $\times$  g (13,000 rpm). **IMPORTANT:** Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.
12. Place QIAquick column into a clean, labeled 1.5 ml microcentrifuge tube.
13. To elute DNA, add 50  $\mu$ l of water (pH 7.0–8.5) to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge for 1 min. **IMPORTANT:** Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA.

14. Elute a second time as in step 13 using 50 more uL of water.
15. Pipette 70 uL of your gel purification products into separate, labeled UV microcuvettes. In another UV microcuvette, prepare a blank with 70 uL of deionized water.
16. Blank the Spectrophotometer using the water-filled cuvette
17. Record the absorbance of each of the samples at 230, 260, and 280 nm.
18. Pipette the DNA back from the cuvette in the microfuge tube. Store the microcentrifuge tube in a freezer at -20C.