

Lab 4: Visualization of PCR product and gel extraction/purification

The objective of this lab is to isolate and purify the DNA replicated in the PCR reactions set up in Lab 3. First, the DNA is separated by size and visualized using agarose gel electrophoresis. Next, the DNA, which should appear as a discrete “band”, is extracted from the soluble gel with a razor blade, dissolved, bound to a QIAGEN column, and washed with ethanol before eluting in water. The purified PCR product will be used in future labs to generate your DNA sequence.

What is agarose gel electrophoresis?

Agarose gel electrophoresis is a technique that allows us to separate fragments of DNA by size. DNA is negatively charged (because of the phosphate backbone). As you run your gel, the electrical current repels negatively charged ions towards the positive pole (think “run to red”), and pulls positively charged ions towards the negative pole. The pores in the agarose gel impede the large DNA fragments more than the small fragments, so small fragments migrate faster.

What is an agarose gel? Agarose is a chain of sugar molecules extracted from some seaweeds. An agarose gel is created by mixing it with a water-based buffer and heating the mixture. The buffer contains salts for conduction of current. When cooled, the buffer and agarose mixture forms a solid, Jell-O-like substance. During electrophoresis the gel is immersed in the same buffer with which the gel was made.

Size standard: In order to determine the size of your PCR product, you need a size standard. It contains DNA fragments of known size. You can compare the position of your PCR fragment on the gel to the size standard. We often refer to the size standard as the “ladder”.

DNA stain: You will not be able to see your DNA unless you stain it. The most common DNA stain is ethidium bromide (EtBr). However, EtBr is a nasty carcinogen that inserts itself between nucleotides, and can cause mutations. EtBr becomes visible when exposed to UV light, which can also cause mutations and cancer. Therefore, be careful and **wear gloves when handling the gel.**

Materials

Agarose, 1X TBE, 6X blue loading dye, Ethidium bromide (mutagen), PCR products and negative and positive controls, electrophoresis gel mold, combs, rig, and power supply
Pipettor with tips, UV safety goggles, UV box.

Methods

Your TA poured the gel so you don't need to sit around while it sets.

1. Add 10 uL 6X blue loading dye to 50 uL PCR product.
2. Load all 60 uL into gel well very carefully with help of your TA!
3. Put top on gel rig and turn on voltage at 125-150 volts.
4. Let gel run until DNA has migrated 2/3 of the way to the next row of wells.
5. Remove gel and place on UV box to identify region with DNA copy. Wear UV goggles. IDEALLY, the PCR product will show up as a single bright glowing band.

Gel extraction

This procedure uses a commercial kit from QIAGEN, Inc. As in the DNA extraction in Lab 2, the DNA is selectively bound to a silica-gel membrane in a spin column. The binding step relies on the presence of chaotropic salts. After the salts are washed out with the ethanol buffer and water is added, the DNA will come into aqueous solution and elute out of the spin column with water. The result is high-copy pure PCR products in solution ready for DNA sequencing. **You must wear gloves!**

Materials

Buffer QG (contains the chaotropic salt guanidine thiocyanate, nasty stuff), buffer PE (contains ethanol), distilled H₂O, DNA fragment in gel, razor blade, (2) 1.5 mL microcentrifuge tube, QIAquick spin column in 2 mL collection tube, microcentrifuge.

Methods

1. Cut the DNA fragment from the agarose gel with a clean razor blade. Cut closely and cut away extra agarose. Place gel slice in 1.5 mL microcentrifuge tube.
2. Add 500 uL of the yellow Buffer QG to gel in microcentrifuge tube.
3. Incubate 10 min (or until gel slice dissolved) in heat block at 50°C. Gently mix the contents of the tube.
4. Place the DNA sample in a QIAquick spin column with 2 mL collection tube and centrifuge for 1 min at 13,000 rpm. Use a pipette with clean tip to load the column. The maximum volume of the spin column is 800 uL. Do not overload the column. If you have a large amount of sample, centrifuge half of the sample, then discard flow-through, reload and spin again. You do not need a new collection tube.
5. Discard flow-through and place spin column back in collection tube.
6. Add additional 500 uL of yellow Buffer QG to spin column and centrifuge for 1 min at 13,000 rpm. This will remove the last traces of agarose gel.
7. Add 750 uL of Buffer PE to spin column. Centrifuge for 1 min at 13,000 rpm.
8. Discard flow-through and centrifuge for an additional 1 min at 13,000 rpm. This will remove residual ethanol.
9. Place spin column into a new 1.5 mL microcentrifuge tube.
10. Elute DNA by adding 30 uL distilled H₂O to the center of the spin column membrane and let the column stand for 5 min. Centrifuge at 13,000 rpm for 1 min.
11. The liquid at the bottom of this 1.5 mL microcentrifuge tube is your purified PCR product. Please label this tube. It will be stored in the freezer and used for a PCR-based DNA sequencing reaction using fluorescent dyes.