Cleanup of a PCR Amplification Product

"Cleanup" means to purify the DNA in the completed PCR reaction by removal of remaining dNTPs, primers, Taq, and Mg ion. These components were all required for the PCR reaction, but now we consider them to be "contaminants" or "impurities" (How fickel!) because they could interfere with subsequent manipulations such as DNA sequencing or restriction digests. If the only thing you want to do with the PCR reaction is to run it on an agarose gel, then cleanup is not necessary.

The QIAquick system is a commercial kit (Qiagen Corp.) that is based on the selective binding properties of a proprietary silica-gel membrane. The membrane is incorporated in a disposable plastic "spin column" that can be inserted into the top of a standard microfuge tube. Fluids are drawn through the membrane by gravity when the column is spun in a centrifuge and the fluid collects in the microfuge tube. This procedure purifies single- or double-stranded DNA fragments from PCR and other enzymatic reactions. Fragments ranging from 100 bp to 10 kb can be purified from primers, nucleotides, polymerases, and salts using QIAquick spin columns in a microcentrifuge. Up to 10 µg of DNA can bind to the membrane in each QIAquick column. List price of this kit is $2 per sample.

In Step 3 of the following procedure, high molecular weight DNA (your PCR product) binds to the silica-gel membrane in the presence of high salt concentration and low pH, while the contaminants pass through the column. The binding buffer PB provides the optimal salt concentration and pH for adsorption of HMW DNA to the QIAquick membrane. The binding of nucleic acids to the membrane surface also requires a high concentration of a "chaotropic agent" such as guanidine hydrochloride. (What the heck is a chaotropic agent?) DNA binding is also affected by pH. During the DNA binding step, unwanted contaminants such as primers, salts, enzymes, and unincorporated nucleotides do not bind to the silica membrane, but flow through the column.

Then in Step 5 the membrane is thoroughly washed with the ethanol-containing Buffer PE to remove any remaining contaminants. Any residual buffer PE, which may interfere with subsequent enzymatic reactions, is removed by an additional centrifugation step.

The DNA is removed from (eluted from) the membrane with low concentration Tris buffer (EB), or with water, in Step 8. DNA elution from the membrane is strongly dependent on the salt concentration and pH of the elution buffer. Contrary to DNA binding, elution is most efficient under basic conditions and low salt concentrations.

DNA % recovery depends on the following three factors: the volume of elution buffer, how the buffer is applied to the column, and the incubation time of the buffer on the column. The point to keep in mind is that there is a compromise between % recovery and DNA concentration. DNA is eluted in a final volume of 30 to 200 µl. 100–200 µl of elution buffer completely covers the QIAquick membrane, ensuring maximum yield, even when not applied directly to the center of the membrane. Elution with ≤50 µl requires the buffer to be added
directly to the center of the membrane, and if elution is done with the minimum
recommended volume of 30 µl; an additional 1 min incubation (Step 8) is required for maximum
recovery. DNA will be up to 1.7 times more concentrated if the QIAquick column is incubated
for 1 min with 30 µl of elution buffer, than if it is eluted in 50 µl without incubation.

In the following procedure you need to be attentive to where the DNA is at the end of each
centrifugation; on the membrane? or in the eluate?

Refer to the QIAquick product literature on the Qiagen web site for additional details.
**QIAquick PCR Purification Kit Procedure**

**WEAR GLOVES AND GOGGLES THROUGHOUT THIS PROCEDURE**

**REAGENTS**

PB (or PBI) High salt, low pH, binding buffer used in Step 1.

- PB contains a pH indicating dye that assures that you have the correct pH for DNA binding.
- PB and PBI contain chaotropic salts (probably guanidine) and should be considered a hazardous chemical reagent.

PE Wash buffer used in Step 5. PE contains ethanol added just before use.

EB Elution Buffer used in Step 8 as an alternative to water.

1. Add 5 volumes of Buffer PB (or PBI), NOT Buffer PE (!), to the PCR sample and mix/spin.
   
   i.e. Add 100 uL PB to 20 uL of PCR rx.

   If we are using PBI instead of PB, the color of the pH indicator in the buffer should remain YELLOW. If the color turns orange to violet, then you must adjust the pH down by adding 10 uL of 3 M Sodium Acetate.

2. Place a QIAquick spin column in a 2 ml collection tube.

3. To bind DNA, apply the entire sample directly to the QIAquick column with a P-200 micropipette and then centrifuge for 30–60 sec. at maximum speed.

4. Discard the flow-through fluid by pipetting it into the waste container provided for that purpose. Place the QIAquick column (with bound DNA) back into the same collection tube.

5. To wash the membrane, add 0.75 ml Buffer PE (NOT Buffer PBI!) to the QIAquick column and centrifuge for 30–60 s. at maximum speed.

6. Discard the flow-through fluid again as in Step 4. Place the QIAquick column (with bound DNA) back in the same tube. Centrifuge the column for an additional 1 min. at maximum speed.

   IMPORTANT: Residual ethanol from Buffer PE will not be completely removed from the membrane unless the flow-through from Step 5 is discarded before this additional centrifugation.
7. Place the QIAquick column (with bound DNA) in a CLEAN 1.5 ml microcentrifuge tube.

8. To elute the DNA from the membrane, add 50 µl of nuclease-free (NF) water or EB to the center of the QIAquick membrane and centrifuge the column for 1 min. at maximum speed.

IMPORTANT: Ensure that the NF water is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The typical eluate volume recovered is 45–48 µl.