7.13 Experimental Microbial Genetics

Fall 2008

QIAquick PCR & Enzyme Purification Kit Protocol

This protocol is designed to purify single- or double-stranded 100bp-10kb DNA fragments from PCR and other enzymatic reactions. You should read through the QIAquick Spin Handbook posted at the 7.13 Stellar website for in depth information about this protocol.

Important points before starting

■ The yellow color of Buffer PBI indicates a pH ≤7.5.
■ Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
■ All centrifugation steps are carried out at 13,000 rpm (~17,900 x *g*) in a conventional table-top microcentrifuge.

1. Add 5 volumes of Buffer PBI to 1 volume of the PCR/Enzyme reaction and mix (it is not necessary to remove mineral oil or kerosene).
For example, add 500 µl of Buffer PB to 100 µl PCR sample (not including oil).

2. Check that the color of the mixture is yellow (similar to Buffer PBI without the PCR sample).
If the color of the mixture is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.

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

4. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60 s.

5. Discard flow-through. Place the QIAquick column back into the same tube.
Collection tubes are re-used to reduce plastic waste.

6. To wash, add 0.75 ml Buffer PE to the QIAquick column and centrifuge for 30–60 s.

7. Discard flow-through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1 min.
IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation. EtOH may interfere with subsequent experiments.

8. Place QIAquick column in a clean 1.5 ml microcentrifuge tube.

9. To elute DNA, add 30-50 µl of H_2O to the center of the QIAquick membrane and let the column stand for 1 min, and then centrifuge.
IMPORTANT: Ensure that the H_2O is dispensed directly onto the QIAquick membrane for complete elution of bound DNA (but don't touch the membrane with your tip). The

average eluate volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 µl elution buffer. It may be desirable to keep the DNA concentrated if it requires manipulation later on (e.g gel extraction, another enzyme digest), as additional procedures may dilute it. In such cases, elute in 30 µl of H_2O.
Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent.