QIAGEN Supplementary Protocol:

Purification of DNA fragments from dye-labeled reactions using the QIAquick® PCR Purification Kit

This protocol is designed to purify single- or double-stranded DNA fragments from dye-labeled reactions. Fragments ranging from 100 bp to 10 kb are purified from dye-labeled nucleotides (e.g. Cy3-dNTP or Cy5-dNTP), primers, polymerases, and salts using QIAquick® Spin Columns in a microcentrifuge.

Please be sure to read the *QIAquick Spin Handbook* carefully before beginning this procedure.

Important notes before starting

- Recoveries for fragments less than 200 nucleotides may be reduced.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifuge steps are at ≥10,000 x *g* (~13,000 rpm) in a conventional tabletop microcentrifuge.

Procedure

1. **Add 5 volumes of Buffer PB to 1 volume of the sample and mix. It is not necessary to remove mineral oil or kerosene.**
   For example, add 500 µl of Buffer PB to 100 µl sample (not including oil).
2. **Place a QIAquick Spin Column in a provided 2 ml collection tube.**
3. **To bind DNA, apply the sample to the QIAquick Spin Column and centrifuge for 30–60 s.**
4. **Discard flow-through. Place the QIAquick Column back into the same tube.**
   Collection tubes are re-used to reduce plastic waste.
5. **To wash, add 0.75 ml of a 35% guanidine hydrochloride aqueous solution (35 g in 100 ml) and centrifuge for 30-60 s.**
6. **Discard flow-through and place the QIAquick Column back into the same tube.**
7. **For the second wash step, add 0.75 ml Buffer PE to the QIAquick Column and centrifuge for 30–60 s.**
8. **Discard flow-through and place the QIAquick Column 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 unless the flow-through is discarded before this additional centrifugation.
9. **Place QIAquick Column in a clean 1.5 ml microcentrifuge tube.**

10. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAquick membrane and centrifuge the column for 1 min. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.

IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 µl elution buffer.

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. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected handbooks can be downloaded from **www.qiagen.com/literature/handbooks/default.asp**.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp**.