QIAquick Gel Extraction Kit Protocol (using a microcentrifuge)

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.

Minimize the size of the gel slice by removing extra agarose.

2. Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 ul).

For example, add 300 ul of Buffer QG to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Buffer QG . 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 the gel, mix by vortexing the tube every 2-3 min during incubation.

IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.

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 ul 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 </= 7.5. Buffer QG ia a pH indicator which is yellow at pH </= 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 ul isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kn. Fo rDNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on the yield. DO not centrifuge the sample at this stage.

6. Place a QIAquick spun 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 ul. For sample volumes of more than 800 ul, simply load and spin again.

8. Discard the flow-through and place the QIAquick column back in the same collection tube.

Collection tubes are re-used to reduce plastic waste.

9. (Optional): Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min.

This step will remove all traces of agarose. It is only required when the DNA will subsequently be used for direct sequencing, in vitro transcription or microinjection.

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 senstitive 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 solumn for an additional 1 min at 13,000 rpm (~17,900 x g).

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 1.5 ml microcentrifuge tube.

13. To elute the DNA, add 50 ul of 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 ul elution buffer 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. The average eluate volume is 48 ul from 50 ul elution buffer volume, and 28 ul from 30 ul .

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 withing 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 (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.