QIAquick PCR Purification and Gel Extraction

Centrifugation Protocol

All centrifugation steps are carried out at 13,000 rpm (~*17,900 x g*) in a conventional table-top microcentrifuge.

The following procedure should be performed at room temperature (15-30°C).

- Gel extraction:
    1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.
    2. Weigh the gel slice in a colorless tube. Add 3 volumes of buffer QG to 1 volume of gel (100 mg, or approximately 100 µl)
    3. Incubate at 50°C for 10 minutes until the gel slice has completely dissolved. To help dissolve gel, mix by vortexing the tube every 2 – 3 minutes. (Increase incubation time for agarose gel with higher percentages)
    4. After the gel slice has dissolved completely, check for the yellow color of the mixture.
    5. Add 1 gel volume of isopropanol to the sample and mix.
    6. Place a QIAquick spin column in a provided 2 ml tube.
    7. To bind DNA, apply the sample to the QIAquick column, centrifuge for 1 min.
    8. Discard flow-through and place the spin column back into the same collection tube.
    9. Recommended: add 0.5 ml of buffer QG to QIAquick column and centrifuge for 1 min.
    10. To wash, add 0.75 ml of buffer PE into the QIAquick column and centrifuge for 1 min.
    11. Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at *7,900 x g* (13,000 rpm).
    12. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.
    13. To elute DNA, add 50 µl of buffer EB (10mM TrisCl, pH 8.5) or water (pH 7.0 – 8.5) to the center of QIAquick column and centrifuge for 1 min. to increase the DNA yield, the elution volume could be decreased to 30 µl and incubate up to 4 min at room temperature.

- PCR purification:
    1. Add 5 volumes of buffer PB to 1 volume of PCR sample and mix.
    2. If pH indicator I has been added to buffer PB, check for yellow color of the mixture.
    3. Place a QIAquick spin column in a provided 2 ml tube.
    4. To bind DNA, apply the sample to the QIAquick column, centrifuge for 30 – 60 s.
    5. Discard flow-through and place the spin column back into the same collection tube.
    6. To wash, add 0.75 ml of buffer PE into the QIAquick column and centrifuge for 30 – 60 s.
    7. Discard the flor-through and place the QIAquick column into the same tube. Centrifuge the column for an additional 1 min.
    8. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.
    9. To elute DNA, add 50 µl of buffer EB (10mM TrisCl, pH 8.5) or water (pH 7.0 – 8.5) to the center of QIAquick column and centrifuge for 1 min. to increase the DNA yield, the elution volume could be decreased to 30 µl and incubate for 1 min at room temperature.
    10. For analyzation of the purified DNA in gel, add 1 volume Loading Dye to 5 volumes of purified DNA. Mix solution by pipetting up and down before loading the sample to the gel.

From: iGEM Bielefeld-CeBiTec