Technische Universiteit
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Biomedical Engineering

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PCR Purification

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1 PCR Purification

Estimated bench time: 30 minutes
Estimated total time: 30 minutes
Purpose: Purifying the product obtained from a PCR reaction.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

Important before starting are the following points (retrieved from QIAGEN):

- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Add 1:250 volume pH indicator I to Buffer PB (i.e., add 120 µl pH indicator I to 30 ml Buffer PB or add 600 µl pH indicator I to 150 ml Buffer PB). The yellow color of Buffer PB with pH indicator I indicates a pH of #7.5.
- Add pH indicator I to entire buffer contents. Do not add pH indicator I to buffer aliquots.
- If the purified PCR product is to be used in sensitive microarray applications, it may be beneficial to use Buffer PB without the addition of pH indicator I.

1.1 Materials

- Autoclaved Eppendorf tubes
- Autoclaved H_2O
- MiniSpin Centrifuge
- PCR tubes with PCR product
- Pipettes and tips
- QIAquick PCR Purification Kit

1.2 Setup & Protocol

- Mix 1 volume of the PCR sample with 5 volumes of buffer PB.
- Load the sample on a QIAquick spin column which is inserted in a collection tube (with a maximum of 800 µl per run).
- Centrifuge the sample for 1 minute at 13,400 rpm. Weight-balance the sample well.
- Discard the flow-through.
- Wash the sample with 750 µl of PE buffer and centrifuge for 1 minute at 13,400 rpm.
- Discard the flow-through.
- Dry spin the sample for 1 minute at 13,400 rpm.
- Transfer the spin column to a new autoclaved Eppendorf tube.
- Load 42 µl of autoclaved H_2O on the column (pipette drops in the middle of the membrane, do not touch the membrane). Incubate for 1 minute and centrifuge for 1 minute at 13,400 rpm.
- The resulting elution product will contain purified PCR product.