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Purification

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[1]iGEM_NTHU_Taiwan

iGEM NTHU

igemnthuth

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Protocol status: Working
We use this protocol and it's working

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Abstract

PCR product purification using the QIAGEN DNA Purification Kit.

Materials

- QIAquick PCR Purification Kit
- PCR reaction sample (20 µL)
- Benchtop centrifuge (capable of ≥13,000 rpm)
- Microcentrifuge tubes (1.5 mL)
- Pipettes and tips

Purification

1 **Mix PCR product with binding buffer**
Add 20 µL of PCR reaction mixture into a clean 1.5 mL microcentrifuge tube. Then add 100 µL of Buffer PB (5× the PCR volume). Mix thoroughly with a pipette. Do not centrifuge.

2 **Load the mixture onto the purification column**
Carefully transfer the entire mixture onto the center of a QIAquick spin column placed inside a 2 mL collection tube. Ensure all liquid is applied directly to the center of the membrane.

3 **First centrifugation and discard flow-through**
Centrifuge at 13,000 rpm for 1 minute. Discard the flow-through collected in the tube, but keep the spin column.

4 **Wash the silica membrane**
Add 750 µL of Buffer PE (pre-diluted with 96–100% ethanol according to the manual) to the spin column. Centrifuge at 13,000 rpm for 1 minute.

5 **Remove residual ethanol**
Discard the flow-through and place the column back into the empty collection tube. Centrifuge again at 13,000 rpm for 1 minute to completely remove residual ethanol, which could interfere with downstream applications.

6 **Elute DNA**
Transfer the spin column to a clean 1.5 mL microcentrifuge tube. Add 30 µL of Buffer EB directly to the center of the membrane (avoid touching the column walls). Let stand at room temperature for 1 minute to allow DNA release from the silica membrane.

7 **Final centrifugation and collection**
Centrifuge at 13,000 rpm for 1 minute to collect the eluate. The flow-through contains the purified PCR product.