

PCR Purification - QIAquick Kit Protocol

This protocol is designed to purify single- or double-stranded DNA fragments from PCR. Fragments ranging from 100 bp to 10 kb are purified from primers, nucleotides, polymerases, and salts using QIAquick spin columns in a microcentrifuge.

Procedure

1. Add 5 volumes of Buffer PBI to 1 volume of the PCR sample and mix. For example, add 500 μ l of Buffer PBI to 100 μ l PCR sample.
2. Place a QIAquick spin column in a provided 2 ml collection tube.
3. To bind DNA, apply the sample to the QIAquick 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 750 μ l Buffer PE to the QIAquick column and centrifuge for 30–60 s.
6. Discard flow-through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1 min.
7. Place QIAquick column in a clean 1.5 ml microcentrifuge tube.
8. To elute DNA, add 30 μ l of water to the center of the QIAquick membrane, let the column stand for 1 min and centrifuge the column for 1 min.

Materials required

- Fresh PCR product
- QIAquick PCR purification kit
- 200 and 1000 μ l pipette tips
- Sterilized DI H₂O
- 1.5 ml microcentrifuge tubes

Equipment necessary

- 200 μ l micropipetter
- 1000 μ l micropipetter
- Benchtop centrifuge capable of 20,000 x g