PCR purification (QIAquick PCR purification kit)

Materials

Buffer PE
Buffer PB
Buffer EB
3M sodium acetate
QIAquick column
0.8 % agarose gel
Loading dye

Steps

Add ethanol (96-100%) to buffer PE, and add 1:250 volume pH indicator I to buffer PB. The yellow color of buffer PB after addition of pH indicator I indicates a pH of ≤7.5.

1. Add 5 volumes Buffer PB to 1 volume of the PCR reaction and mix. If the colour of the mixture turns orange or violet, add 10 µl of 3M sodium acetate, pH 5 and the colour of the mixture will turn yellow.
2. Place a QIAquick column in a provided 2 ml collection tube.
3. Transfer the sample to the QIAquick column and centrifuge at 13,000 rpm for 1 min to bind DNA. Discard flow-through.
4. For washing, add 750 µl buffer PE to the column and centrifuge at 13,000 rpm for 1 min then discard the flow-through.
5. Centrifuge again at 13,000 rpm for 1 min to remove any residual PE buffer.
6. Place the QIAquick column in a clean 1.5 ml eppendorf tube.
7. For elution, add 20-30 µl buffer EB to the center of the QIAquick membrane without touching it. Let stand at room temperature for 5 mins then centrifuge at 13,000 rpm for 1 min. Do not throw the flow-through as it contains the purified PCR product.
8. Use the nanodrop to determine concentrations of the PCR purification product.
9. To verify the nanodrop concentrations, run 2-5 µl of the samples on 0.8% agarose gel.

Adapted from Qiagen