2022 NEFU_China

PCR product purification

ABSTRACT

This protocol is used to purify PCR products (in case of single band detected by gel electrophoresis)

BEFORE STARTING

Prepare CP Buffer and Wash Buffer reagents

1.Add 4-5 times CP Buffer to the PCR product，Blow and mix well.

2.Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

3.Inhale the mixed liquid into the HiBind® DNA Mini Column

4.Centrifuge at 10000x gspeed for 1 minute.

10000 x g, Room temperature, 00:01:00

4. Discard the filtrate and reuse the collection tube.

5. Add 600 µL Wash Buffer

7.Centrifuge at maximum speed for 1 minute.

15000 x g, Room temperature, 00:01:00

8.Discard the filtrate and reuse collection tube.

9.Repeat step 6~8 once.

10.Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

11.Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

12.Add 30-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

13.Let sit at room temperature for 1 minute.

14.Centrifuge at maximum speed for 1 minute.

15000 x g, Room temperature, 00:01:00

This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

15.Suck out the solution from the tube and re-add it to the center of the column membrane to give a second centrifuge.

15000 x g, Room temperature, 00:01:00

16.Test the concentration and purity of DNA using NanoDrop.

17.Store DNA at -20°C.