Wei-Shou Hu Laboratory
Department of Chemical Engineering
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University of Minnesota

Protocols for PCR Clean Up

Materials

- 3 M NaOAc pH 5.2
- IPA
- 70% Ethanol (made from 95% Ethanol)
- Spotting buffer (3 X SSC + 1.5 M Betaine)
- Costar 96-well v-bottom polypropylene plates
- Greiner 384-well v-bottom polypropylene plates

Equipments

- Centrifuge with plate rotor of maximum speed > 4500 g

Precipitation Method

1. After PCR reaction, add 10 µl 3 M NaOAc pH 5.2 to each well
2. Transfer reactions to a Costar 96-well V-bottom Polypropylene Plate. To save on pipette tips, first add 100 µl room temperature IPA to Costar plates and then transfer PCR + NaOAc pH 5.2 to IPA. Mix well with pipette
3. Transfer PCR reaction mixture (to which NaOAc has been added)
4. Spin plates at 4700 rpm for at least 2 hrs (at most 6 plates a time)
5. Quickly discard the supernatant right after the spinnin
6. Add 100 µl of ice cold 70% EtOH
7. Centrifuge the plates for half an hour at 4700 rpm
8. Discard the supernatant
9. Let plates air dry or dry in the speed vacuum with the plate rotor.
10. Resuspend the DNA pellets in spotting buffer for at least 10 hrs and save in -20 °C. Make sure the plates are sealed well. The PCR products can be directly transferred to Greiner 384 Well Polypropylene Plates at any time.

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Filtration Method Using Millipore Filter Plates

Materials

- Montage PCRμ96 Filter Plate (Millipore)
- TE buffer
- Spotting buffer (3 X SSC + 1.5 M Betaine)
- Greiner 384-well v-bottom polypropylene plates

Equipments

- MultiScreen® Resist Vacuum Manifold (Millipore)
- Vacuum pump
- Shaker

1. Add 5-μL TE buffer to each well of the 96-well plate containing the PCR product to be purified.
2. Transfer the PCR product and buffer to a Millipore Montage $PCR_{\mu 96}$ filter plate.
3. Place the filter plate on the vacuum holder. Turn on vacuum pump and adjust pressure to 20-inHg.
4. Allow the PCR product to filter until the filter is dry and shiny looking. (Approximately 12-18 minutes).
5. Place an additional 50-μL TE buffer to each well of the filter plate, and allow the vacuum to run until the filter is again dry and shiny looking.
6. Remove filter from vacuum system, and blot the bottom of the filter dry with a paper towel.
7. Add spotting buffer to each well of the filter plate, and place the filter on a shaker for 20-30 minutes.
8. Transfer the purified PCR product directly to Greiner 384 Well Polypropylene Plates. Seal the plate well store at -20 °C.

Note: Filter Plates are good for PCR product more than 100 bp long.