Miniprep (QIAprep Spin Miniprep Kit)

Materials

Ethanol (96-100%)
Buffer P1
Buffer P2
Buffer N3
Buffer EB
QIAprep 2.0 spin column
Agarose gel
Loading dye

Steps

Add LyseBlue reagent and RNase A solution to Buffer P1, mix and store at 4°C. Add 99.8% ethanol to Buffer PE.

1. Centrifuge overnight cultures at 10,000 rpm for 10 mins at 4°C, and discard the supernatant.
2. Resuspend the pellet with a 250 µl of P1 buffer and transfer the sample into fresh 1.5 ml eppendorf tubes.
3. Add 250 µl of P2 buffer and mix by inverting 5 times not exceeding 5 mins or else plasmid will degrade . The solution will turn blue indicating cell lysis.
4. Add 350 µl of N3 buffer and mix by inverting till a white precipitate is observed.
5. Centrifuge at 13,000 rpm for 10 mins.
6. Transfer 800 µl of the supernatant into a QIAprep 2.0 spin column. Centrifuge at 13,000 rpm for 1 min.
7. Wash twice by adding 750 µl of PE buffer and centrifuge the spin columns at 13,000 rpm for 1 min. Discard wash-through and centrifuge at 13,000 rpm for 1 min.
8. Transfer the spin column to a fresh eppendorf . To elute, add 50 µl of EB buffer and let stand for 5 mins. Centrifuge at 13,000 rpm for 1 min. Do not discard the flow-through as it contains the plasmid. For concentrated plasmid, elute in 30 µl of EB buffer
9. Determine purified plasmid concentration using nanodrop.
10. For verification of the nanodrop result, run 2-5 µl of the purified plasmid on 0.8% agarose gel.

Adapted from Qiagen