Miniprep Plasmid Isolation

Plasmid big scale isolation

1. Overnight culture is putted into falcon 50 ml (2 falcon)
2. Centrifuge 3500 rpm 10 menit
3. Discard the supernatant, excess 1 ml (Pelet contain cell + plasmid)
4. Resuspention pelet with excess supernatant and move it into 1,7 mL tube
5. Centrifuge 12000 rpm, 1 minute then discard the supernatant
6. + P1 250 (125) µL, up down
7. + P2 250 (125) µL, invert 4 s.d. 6 x
8. + solution N 3 350 µL, invert 4-6 x
9. Centrifuge 12000 rpm, 10 min then take the supernatant
10. 850 µL next, move the supernatan to the spin column based nuclelic acid purification
11. centrifuge 12000 rpm, 1 minute
12. Discard lysate to the column (repeat it twice then centrifuge)
13. + PB 500 (200) µL then centrifuge 12000 rpm, 1minute then make the lysate
14. + PE + Etoh 700 (500) µL, (centrifuge 12000 rpm for 1minutes then discard the lysate) repeat twice
15. Move column into new microtube 1,7 mL
16. + 50 µL 1/3 EB, let it 1 minute
17. centrifuge 12000 rpm, 1 minute
18. + 20 µL 1/3 EB, let it 1 minute
19. centrifuge 12000 rpm, 1 minute
20. (Plasmid in the new microtube 1,7 mL)

- PE + Etoh with ration 1 Vol PE : 4 Vol Etoh
- 1/3 EB, 1 Vol EB in total 3 vol $\sum H_2O$
  Ex : 30 µL EB + 60 µL $\sum H_2O$