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|  | 6. Plasmid preparation and Digestion test  Kit: Use QIAprep miniprep buffers  Note: works well with XL1-Blue(Enda1-strains)  Does not work with Enda1+strains(HB101 etc)  Preparations:  Make buffer P2  P1, store at 4°C (RNAse Added)  P2, store at  P3, store at RT  100% EtOH, Store at -20  EB  P1 (50 mM Tris-HCl, 10 mM EDTA, 100 ug/mL RNase A, pH 8.0)  P2 (1% SDS, 0.2 M NaOH )  P3 (3.0 M Potassium Acetate, pH 5.5)  0. Stransfer overnight culture 2mL into a 2mL Eppendorf tube (bigger one that can holds all 2mL),  1. Spin at 16000g 3min  2. Remove supernatant (by vacuum or hand).  Resuspend pellet with buffer P1(Tris-HCl, EDTA, RNase A)  3. Add 100µL of ice-cold buffer P1.  4. Vortex for 5-10 seconds (enough to completely resuspend the pellet)  5. Let sit on bech for 5min.  Lysis of cell membrane, release plasmid  6. Add 200µL P2 (SDS, NaOH)  7. Mix by inverting several times. Do not vortex.  8. Incubate on ice for 5min.  Precipitate proteins and genomic DNA  9. Add 150µL ice-cold P3 (Potassium Acetate). Vortex 3~5sec to mix.  10. Incubate on ice for 5 min  11. While waiting, prepare and label Eppendorf tubes.  Remove precipitate  12. Spin samples from step 10 at 16000g 1min.  13. Transfer 400µL of supernatant (containing plasmids) to labeled Eppendorf tubes, then add 800µL ice-code 100% EtOH. Discard original tubes with precipitates.  Precipitate Plasmid DNA  14. Mix samples gently by inverting 4 times.  15. Let sit on ice/-20°C for 1min.  16. Spin samples at 16000g for 5min. A pellet should be seen after spin.  Washes and dry  17. Remove EtOH.  18. Wash pellet with 800µL 70%EtOH  19. Spin samples at 16000g for 3min.  20. Carefully remove all supernatant without touching the pellet.  21. Air dry for 5~10min  (Use 1.5mL tips aspirate the supernatant, invert the tube after removed the last drop of supernatant, put the tube on a 30 degree leaned surface with caps open. Open caps is let the EtOH evaporate, angle the tubes is to let the H2O stay away from the pellet. )  Elute  22. Add 50µL (or 30µL) of EB/H2O to elute DNA.  Expected  400ng/µL 50µL elution  900ng/µL 30µL elution |