Benchling

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Introduction

Use the Miniprep kit to extract DNA from liquid overnight cultures.

Materials

- MINIPREP KIT
  - and the RNASe stored in 4°C fridge
- Additional materials
  - 1.5mL tubes, all pipettes, MQ, gloves

Procedure

Miniprep (omega biotek kit - quick guide)

https://www.omegabiotek.com/wp-content/uploads/2017/08/D6945-Quick-Guide-011217.pdf

1. grow 1-5mL culture overnight in a 10-20mL culture tube (shoul be be happy shaking in the shaker incubator)
2. Centrifuge at 2,000 x g for 10 minutes at room temperature 22°C (Big cetrifuge). Wear gloves: Decant the liquid in the liquid Biowaste (needs to get autoclaved) -> we need white sandy bacteriastuff t the bottom
3. Add 250 µL Solution I mixed with RNase A (stored in fridge 3 at 4°C). Vortex to mix thoroughly.
4. Transfer suspension into a new 1.5mL microcentrifuge tube.
5. Add 250pL Solution II. G*ently i*nvert the tube by hand several times to obtain a clear lysate, (gently so dont disrupt the DNA, avoid vigorous mixing. Do this for 2-3 min. DO NOT exceed 5 min!
6. Add 350pL Solution III. Immediately invert several times until a flocculent white precipitate forms. Centrifuge at max speed (13,000xg) for 10 min. A compact white pellet will form.
7. Insert a HiBincl. DNA Mini Column into a 2mL Collection Tube. Pay attention to not touch anything with your hands where there will be DNA afterwards, so dont contaminate stuff.
8. Transfer the cleared supernatant liquid from Step 5 by CAREFULLY pipetting it into the HiBind• DNA Mini Column+ 2mL tube (should be aroung 850µL). Pay attention to not touch or suck in the white pellet (this is just proteinwaste-> dicard the tube in Biowaste autoclaved) Centrifuge at ma speed (13'000) for 1 minute. Discard the liquid filtrate in the liqiuid Biowaste and r euse the collection tube. The plasmid is in the column filtermembrane.
9. Add 500 µL HBC Buffer (yellow bottle) diluted with 100% isopropanol Centrifuge at max speed for 1 min. Discard the filtrate and reuse the collection tube. NOTE: if there is a cross on the bottelcap it is already mixed with iso and ready to use
10. Add 700 µL DNA Wash Buffer (green bottle) diluted with 100% ethanol. Centrifuge at max speed for 1 min. Discard the filtrate and reuse the collection tube.NOTE: if there is a cross on the bottelcap it is already mixed with et and ready to use)
11. Centrifuge the empty HiBind® DNA Mini Column II at max speed for 2 min to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.
12. Transfer the HiBind® DNA Mini Column II into a nuclease-free 1.5mL microcentrifuge tube. (our normal tubes)
13. Add 30-100 (kyle: 50) µL Elution Buffer or sterile deionized water. Let sit at room temperature for 60 seconds. Centrifuge at max speed for 1 min. ATTENTION: the centrifuge goes anticlockwise so the caps of the tubes need to be in the correct direction otherwise they get destroyed by the force.

14. Store eluted DNA at -20°C in fridge