Genomic DNA purification

Introduction

This procedure will help you through how to purify genomic DNA quickly and efficiently and follows the ThermoFisher "Genomic DNA Purification Kit". Product Number: The protocol can be used for Blood, Sera, cell cultures, epithelium cells, tissues and bacterial cultures. This protocol is specific to working with bacterial cultures.

Materials

- Lysis Solution
- Precipitation Solution (10X)
- NaCl solution (1.2M NaCl)
- TE-buffer
- 70% Ethanol (cold)
- Chloroform
- 1.5ml microcentrifuge tubes

Procedure

Bacterial Cultures - Sample Preparation

1. Centrifuge the bacterial culture for 10min at 5000g
2. Place 10-20mg of bacterial cells in 1.5ml microcentrifuge tubes
3. Resuspend in 200µl of TE buffer
4. Note that thawed cells are not recommended.

Bacterial Cultures

1. Mix 200µl of sample with 400µl of lysis solution
2. Incubate at 65°C for 5 min.
3. If a frozen sample is used, lysis solution should be added before the thawing. Then the sample is incubated at 65°C for 10 min with occasional inverting of the tube.
4. Immediately add 600µl of chloroform, gently emulsify by inversion (3-5 times)
    - ➔ invert up to 5 min (that's the step where proteins get denatured so you want to get sure that the chloroform reaches all proteins that are in the solution)
5. Centrifuge the sample at 10,000 rpm for 2 min

- ➔ if you cannot see a clear line between the denatured proteins and the aqueous phase add more chloroform then centrifuge again, also increase rpm

6. Prepare precipitation solution by mixing 720µl of sterile dH20 with 80µl of 10X concentrated precipitation solution
7. Transfer the upper aqueous phase containing DNA to a new tube and add 800µl of freshly prepared precipitation solution
8. Mix gently by several inversions at RT for 1-2 min
    - ➔ here you should see DNA precipitating. If you cannot see any fine white DNA strands add more precipitation buffer (I added 15 more µl, but it depends on how much cells you put in the beginning and how much DNA you expect. As an alternative to the precipitation buffer isopropanol can be used to precipitate DNA.
9. Centrifuge at 10,000 rpm for 2 min
10. Remove supernatant completely (do not dry) and dissolve DNA pellet in 100µl of NaCl solution by gently vortexing (pipette up and down)
11. Make sure that the pellet is completely dissolved.
12. Add 300µl of cold ethanol, let the DNA precipitate at -20°C for 10min
13. Centrifuge at 10,000 rpm for 3-4 min
14. Remove the ethanol and wash with ethanol once (add about 200 µl) then remove all ethanol that is left in the tube and let pelett dry in air (open tube). Dissolve the DNA in 100ul /25 µl of sterile deionized H20 by gentle vortexing.
15. Measure the DNA concentration spectrophotometrically or visually after electrophoresis in agarose gel
16. Check the absence of RNA and estimate the average length of obtained DNA
17. Use 1-5µl of DNA solution for 50µl of PCR.