Genomic DNA isolation (Easy-DNA™ Kit, cat.no. K1800-01, from Invitrogen,)

Experimental overview: Cells are lysed by the addition of Solution A and subsequent incubation at 65°C. Proteins and lipids are precipitated and extracted by the addition of solution B and chloroform. The solution is then centrifuged to separate the solution into two phases with a solid interface separating the two phases. The DNA is in the upper, clear aqueous phase, the proteins and lipids are in the solid interface, and the chloroform forms the lower phase. The DNA is then removed, precipitated with ethanol, and resuspended in TE buffer.

Day 1: G-: Culture the strains on blood agar plates.
G+: Prepare the liquid culture of the strains by transferring 1 loop of bacteria to 10 ml of BHI. Incubate overnight at 37°C, with gentle shaking (75 rpm).

Day 2:

Before starting:

- Chill 96% and 80% ethanol in a -20°C freezer.
- Thaw RNase and keep it on ice.
- Equilibrate heat block at 65°C, and an incubator (or a heat block) at 37°C.
- Prepare two sets of tubes[1], "**low bind"** Epp. Tubes, one for the isolation of DNA, one for the DNA precipitation.

Preparation G+:

- Thaw Lysozyme (10 mg/ml) or Lysostaphin (10 mg/ml) and Proteinase K (20 mg/ml)
- Centrifuge the culture: 3.000 rpm; 4ºC; 5 min
- Discard the supernatant and turn the tube upside down on a Kleenex.
- Resuspend the pellet gently in 200 µl PBS (cat. no. 10010-23)
- Transfer the suspension to an Epp Tube.
- Add 30µl Lysozyme or 15 µl Lysostaphin and incubate at 37ºC for 20 min. (possibly shaking with 550 ppm)
- Add 30µl 10% SDS. Mix by turning the tube up and down.
- Add 15 µl Proteinase K and incubate at 37ºC for 20 min.

Preparation G-:

- Harvest from BA: ¾ of a (Blue) 10 µl loop into 1 ml PBS (cat. no. 10010-23)
- Centrifuge at 20.000 G for 5 min - decanted the buffer. Resuspend cell pellet in 200 µl 1 × PBS

Isolation of DNA:

lysis
1. Add 350 µl Solution A to cell suspension and vortex in 1 second intervals until evenly dispersed.
2. Incubate at 65°C for 10 min.
3. Let the tubes cool, at the table or in the refrigerator

Precipitation of proteins and lipids
4. Add 150 µl solution B and vortex vigorously until the precipitate moves freely in the tube, and the sample is uniformly viscous. If the precipitate is hard and fixed to the tube, loosen it by knocking at the tube with a pair of scissors.

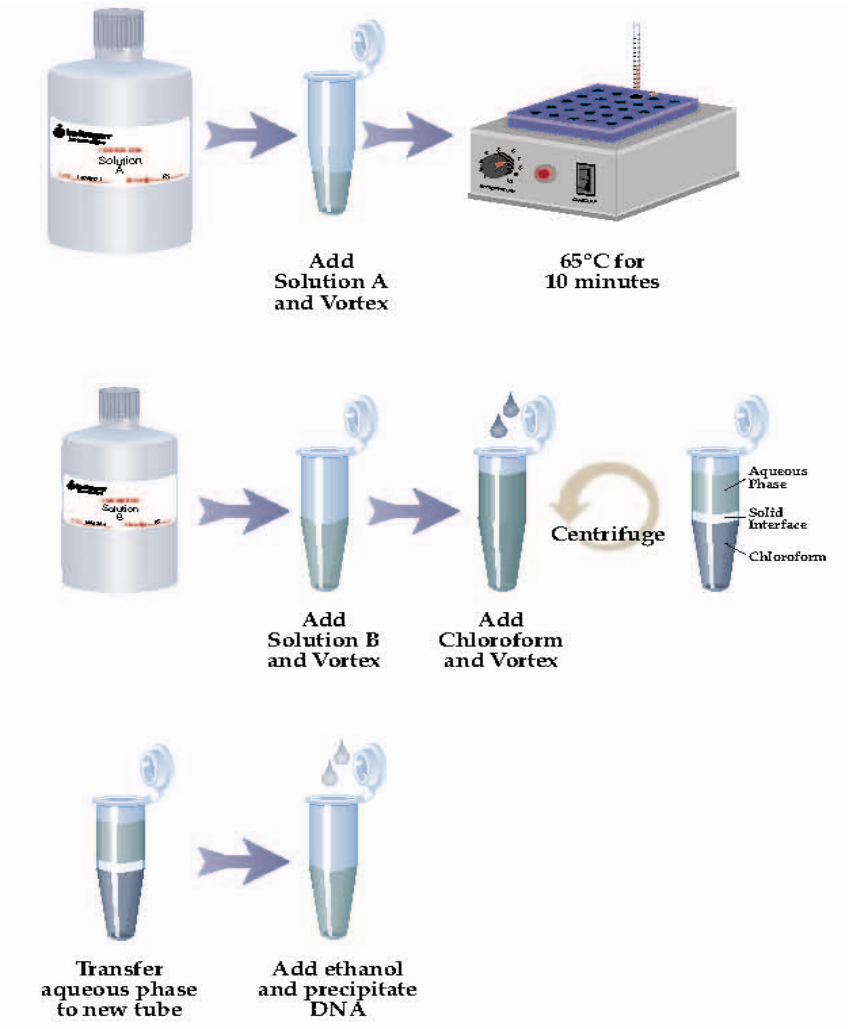
[1] If both sets are in low bind tubes, it will give a higher yield. If you don't need the high yield it's ok to use normal eppendorf tubes as the first set with the PBS.

5. Add 500 µl chloroform (work in a fume cupboard, find chloroform:isoamylalcohol – SigmaC-0549-1PT) and vortex until viscosity decreases and the mixture is homogeneous (10 sec -1 min).
6. Centrifuge at 20.000 g for 10 min. at 4°C to separate phases.
7. In the meantime add 1 ml of 96% ethanol (-20°C) into the second tube (**a low bind eppendorf tube**), keep at 5°C.
8. After centrifugation, transfer 500 µl of the upper phase into the 1 ml of ethanol. Mix by turning the tube up and down. Look for the DNA.
9. Incubate tube on ice for 30 min. (If you can see the DNA this step can by omitted).

You can keep the tubes at -20°C and continue with the precipitation another day.
Or continue right away:

DNA precipitation:

10. Centrifuge at 20.000 g for 10-15 min at 4°C. Decant the ethanol.
11. Add 500 µl of 80% ethanol (-20°C) and loosen the pellet using a pipette.
12. Centrifuge at maximum speed for 3-5 min at 4°C. Decant the ethanol.
13. Centrifuge shortly at maximum speed for 3-5 min at 4°C. Remove the residual ethanol with a pipette. Let air dry 5 min, until no ethanol can be smelled.
14. Prepare TE-RNase: 100 µl TE + 2 µl 2 mg/ml RNase pr. tube (prepare for one extra tube)
15. Resuspend pellet in 100 µl TE-RNase
16. Incubate at 37°C 1-2 hours.
17. Leave ON at 4°C
18. Measure the DNA concentration on Quibit with 2 µl of your DNA sample.


Solution A
Add Solution A and Vortex
65°C for 10 minutes
Solution B
Add Solution B and Vortex
Add Chloroform and Vortex
Centrifuge
Aqueous Phase
Solid Interface
Chloroform
Transfer aqueous phase to new tube
Add ethanol and precipitate DNA