# Preparation of plasmid DNA using the alkaline lysis protocol and inoculation of an E. coli culture

This preactical consists of three separate tasks.

A. Prepare plasmid DNA from the Taq or Pfu production strain. B. Preparation of LB liquid medium C. Innoculation of a small E. coli culture

## A. Preparation of plasmid DNA using the alkaline lysis protocol

1. Spin 1-3 mL cell culture in an Eppendorf tube in a microcentrifuge at top speed for 30s. The tube can only hold 1.5-2mL at a time depeding on the tube.
2. Resuspend pelleted bacterial cells in 200 µl Buffer P1 (kept at 4 °C) and transfer to an Eppendorf tube. Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.
3. Add 200 l Buffer P2 and gently invert the tube 4–6 times to mix. Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.
4. Add 200 l Buffer P3 and invert the tube immediately but gently 4–6 times. To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition. The solution should become cloudy.
5. Centrifuge for 10 min at full speed (at least 13,000 rpm ~17,900 x g) in a table-top microcentrifuge. A compact white pellet will form. Continue with step while tubes are spinning 5.
6. Prepare one Eppendorf tube with 1 mL 96% or 99% ethanol for each sample.
7. Pour supernatant from step 4 into the tube with ethanol, avoiding the precipitate. Mix by inversion 4-5 times. Centrifuge for 10 min at full speed.
8. Pour off supernatant by invertin.
9. Add 500 µl of 70% ethanol.
10. Spin 1-2 min at full speed.
11. Remove ethanol with a pipett. Take as much as you can. Spin again if necessary (if there is visible liquid).

12. Air dry pellet.
13. Add 50 µl TE buffer to the dried DNA.
14. Mark your Eppendorf tube with group, and date.

## B. Preparation of 50 mL LB liquid medium for *E. coli*

1. Weigh the appropriate amounts of each media component into a piece of paper.
2. Transfer the media components to a 250 mL Erlenmeyer flask.
3. Add 50 mL distilled water.
4. Plug the flask with a cotton stopper.
5. Wrap some aluminium foil around the neck of the bottle.
6. Put a small piece of autoclave tape on the bottle.
7. Mark with group and date.

## C. Inoculation of an E. coli culture

Clean the bench with 70% ethanol and dry the surface with tissue paper.

Light an alcohol lamp and perform the following steps always in the vicinity of the flame:

1. Pour ~5 mL sterile LB medium into a sterile Erlenmeyer flask. Be careful not to contaminate the medium. Use a 15mL Falcon to measure the volume.
2. Take a small amount of cells from the plate containing the *E. coli* strain using a sterile wooden skewer or a toothpick.
3. Add the cells to the medium in the test tube or Erlenmeyer flask.
4. Add amicillin stock solution to the medium.
5. Put the test tube or Erlenmeyer flask in the 37°C incubator with shaking at 150 - 200 rpms.

## Recepies & Material

### LB medium recepie

10 g/L Tryptone or Peptone 5 g/L yeast extract 10 g/L NaCl

**Material needed for each group:**

Sterile Eppendorf tubes Tube rack for Eppendorf tubes Tube rack for 50mL Falcon tubes Alcohol lamp 250 mL Erlenmeyer flask (not sterile) Cotton plug for Erlenmeyer flask Tissue paper P200 P1000 Yellow tips Blue tips Sterile test tube with cap or small (~50mL) Erlenmeyer flask

**Material needed for all groups:**

Microcentrifuge Lighter Liquid LB medium (25 mL) Aluminium foil 1L distilled water (not sterile, not ultrapure!) Peptone Tryptone NaCl Spoons for chemicals balance buffer P1 50 mM Tris-HCl, pH8, 10 mM EDTA, 100 µg/mL RNaseA buffer P2 200 mM NaOH, 1% SDS buffer P3 3.0M potassium acetate, pH 5.5 autoclave tape permanent markers sterile skewers