Plasmid DNA Lab Protocol

Day 1: Transformation of competent E.coli with plasmid DNA

OBS! The person pipetting should hold the tubes they are pipetting from and to
Always add the solutions to the bottom of the tube
Ask help from your lab assistant anytime you aren´t sure how to do a step

1. Add 5 µl of plasmid (P) to the tube with competent E.coli cells (C) and mix gently by flicking the tube.
2. Incubate the mixture on ice for 10 minutes
3. Heat shock the mixture on the heating block (37°C) for 3 minutes, then place back on ice for 1 minute
4. Mark an LB agar plate (plate contains ampicillin, IPTG and x-gal) with your name and group, add the 10ul transformation mixture to the plate and use a sterile spreader to distribute it homogeneously on the surface of the plate
5. Place back the lid and leave the plate upside down on your lab bench, your lab assistant will take it and place it in the incubator at 37°C incubator overnight
6. Discard all used reagent tubes and clean your benchtop, keep the ice.

Day 2: Picking of bacterial colonies and cultivation of bacteria

1. Label each 50ml falcon tube lid and on the side with your name and group, label one of them with “white” and one with “blue”
2. Using 2 different pipette tips, pick one white and one blue colony from your agar plate and drop the pipette tips in each 50ml falcon tube (the tip will stay in the tube), screw the caps properly and leave on your bench, your lab assistant will take them and place it in the shaking incubator at 37°C, overnight

Day 3: A. Plasmid preparation, B. Restriction enzyme digestion and C. Electrophoresis

A. Plasmid preparation

OBS! Label all tubes carefully on the lid and sides and change pipette tips between reagents and samples

1. Pick up your overnight culture (check successful growth)
2. Label 2 eppendorf tubes and pipette 1.4 ml (700µl twice) from respective 50ml falcon tube with the overnight cultures into the respective eppendorf tube, centrifuge at 12000g for 2 minutes NOTE: Balance in the centrifuge!
3. Use one of your used 50ml falcon tubes as a liquid waste container. Remove the supernatant from your centrifuged eppendorf tubes with a pipette (700µl twice). Do not disturb the cell pellet at the bottom, while taking away as much supernatant as possible.
4. Add 250µl of buffer A1 and resuspend pellets by pipetting up-down several times
5. Add 250µl of buffer A2 to lyse the cells and invert the tubes 4-6 times
6. Add 350µl of buffer A3 to neutralize the samples and invert the tubes 4-6 times
7. Centrifuge at full speed for 10 minutes

8. Label 2 spin columns (label on collection tube and **NOT** the actual spin column) and transfer **supernatants** (where your plasmid DNA will be dissolved in) from previous step. Centrifuge for 1 minute
9. Discard the flow-through (liquid in the lower collection tube) – the plasmid DNA should be bound to the membrane inside the spin column
10. Add 500µl of buffer A4 to the columns for washing, centrifuge for 1 minute and discard the flow-through again
11. Centrifuge the columns again for 1 minute to dry them out
12. Label 2 new eppendorf tubes, discard the plastic collection tube and transfer the spin column into the new eppendorf tubes
13. Add 75µl of distilled H_2O directly to the column silicon matrix, but avoid touching the matrix with the tip, centrifuge for 2 minutes to elute plasmid DNA

Now your 2 **plasmid preparations** are done!

B. Restriction enzyme digestion

1. Prepare a **master solution** of the restriction enzyme by mixing 24 µl of H_2O with 3 µl of "10X BamHI buffer" in an Eppendorf tube, then add 3 µl of BamHI enzyme and mix the master solution by pipetting up and down gently Note: The enzyme must be kept cold at all times, so keep master solution on ice after it´s prepared
2. Label two new Eppendorf tubes with “white-cut”; “blue-cut” and transfer 10 µl of each plasmid preparation to the newly labeled eppendorf tubes
3. Add 10 µl of BamHI master solution to each tube and incubate the tubes at 37°C for 1h
4. Add 4 µl of 6x loading dye to the digested samples and mix by pipetting up and down
5. For the undigested controls, label two new eppendorf tubes “white-uncut”; “blue-uncut” and transfer 10 µl of the white and blue plasmid preparation into the newly marked eppendorf tubes, add 2 µl of "6X loading dye" and mix by pipetting up and down

C. Agarose gel electrophoresis (gels: made by one group but used for 3/4 groups)

OBS! Ask for lab assistants’ help when you are about to start making gels

1. Weigh 1 g of agarose and pour it into a 200 ml glass flask
2. Add 100 ml of 1xTAE buffer
3. Melt the agarose in the microwave, until there are no visible agarose particles left ***(Note: wear protection gloves when holding the flask, and keep the flask away from your face and other students when you gently mix it!!!)***
4. Allow it to cool down slightly then add 4µl SYBR Safe and mix
5. Pour your gel mix into the gel-casting cassette (avoid air bubbles) and place the combs inside (thick side down), let the gel solidify for about 20 minutes
6. Fill up the gel-running tank with 1xTAE running buffer up to almost half-centimeter above the gel surface and remove the combs straight up slowly and carefully not to damage the wells
7. **Load each sample according to the order: 5 µl size marker, 10 µl white uncut, 10 µl white cut, 10 µl blue uncut, 10 µl blue cut**
8. Set the voltage to 120V and let the electrophoresis run for 60 minutes
9. Photograph the gel under UV light