

Cloning and Transformation of PCR products

1. Obtain your PCR products from the professor / TA. If your PCR did not work we will provide you with one with a good product.
2. Add the following reagents to the 0.5 ml microfuge tube labeled "LIG". This tube contains 1 μ l of ligase from bacteriophage T4. Ligase is temperature-sensitive so keep the ligase on ice.

Add in order: 1 μ l of linearized TA overhang vector ("VEC")
5 μ l of 2x ligation buffer ("BUF")
3 μ l of PCR product
10 μ l final volume

3. This tube is your "ligation reaction." Briefly centrifuge the tube (30 seconds), then mix it **GENTLY** by flicking the tube, centrifuge again (30 seconds).
4. Incubate the ligation reaction at room temperature for 20 minutes. While you are doing this, make sure that the electroporation cuvette is cooling on ice.
5. Add 1 μ l of the ligation reaction into the tube of electroporation competent *E. coli* cells keeping the cells on ice (do not take the tube out of the ice). Mix by stirring the cells with the pipette tip in the tube. This tube is your transformation reaction. Keep the rest of the ligation – we will let the reaction go overnight then try again if you don't have sufficient ligation in the 20 minute incubation.
6. Add the mixture of ligation reaction and competent cells to the ice-cold electroporation cuvette. Make sure that the liquid is all between the electrodes, and that the liquid forms a contact between both electrodes so that your ligation and cells will complete the electrical circuit.
7. Electroporate the cells at 1.8 kV for 5 ms. Check the time constant after electroporation. If you see a spark, or the time constant is less than 3.5 ms, you may want to retry the electroporation reaction. This indicates salt contamination and that the conductivity of the mix is too high, which generally results in the cells being overheated and killed.
8. IMMEDIATELY after electroporation, add 500 μ l of LB broth to the electroporation cuvette and mix.
9. Transfer the mixture of broth and competent cells from the electroporation cuvette to a clean, sterile 1.5ml tube.
10. Cap the tube tightly and place at 37°C for 30 minutes.
11. While the cells are incubating, spread 100 μ l of 100mM IPTG and 40 μ l 20mg/ml X-GAL solution on an LB/Ampicillin plate. The instructors will demonstrate how to spread onto the agar plate using sterile glass beads. Let the plate dry for 5-10 minutes with the lid on and the glass beads left on the surface.

12. Centrifuge the cells for one minute at top speed. Remove the clear supernatant leaving the cell pellet.

13. Resuspend the cell pellet in 50 μ l of LB medium by pipetting, and add all 50 μ l onto the LB/Ampicillin plate.

14. Spread the cells using the glass beads remaining on the plate surface, wait for the liquid to soak into the agar, then shake again. Carefully remove the beads by pouring them out of the plate back into the tube they came in, once they are dry.

15. Label the plates ON THE BOTTOM, with your name and group number. Place the plates upside down in the 37°C incubator. The instructor/ TA's will remove the plates tomorrow morning and put at 4°C for storage until next week.

Bacterial Growth Media

LB (Luria-Bertani) Medium

1.0% Tryptone

0.5% Yeast Extract

1.0% NaCl

pH 7.0

For Agar plates add 15g/liter agar before autoclaving the media.

For antibiotic plates cool the media to 55 C, then add ampicillin to 100 ug/ml final concentration.