Bacterial Transformation

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Transformation is the process by which foreign DNA is taken up by bacteria. Bacteria in a state called “competent” are primed to take up such DNA. This protocol describes how to transform chemically-competent bacteria (i.e. made competent by chemical means) with plasmids of interest.

1. Take a single 50 µL aliquot of competent *E. coli* (strain of interest depending on application) from the -80ºC freezer and place directly on ice.
2. Take agar plates from 4ºC storage (with appropriate resistance antibiotic) and place them on the lab bench to reach room temperature.
3. Check to see that the competent cell solution from Step 1 is thawed. Be gentle; do not flick or swirl.
4. **Under sterile conditions**, gently add 1-5 µL of the plasmid DNA to the thawed competent cells aliquot. Be gentle and do not mix! Incubate on ice for 30 min.
    a. We work with a flame on the benchtop.
    b. The amount of DNA to be added depends on the competency of the cells. For cloning applications downstream of recombination or ligation reactions, generally 4 µL of plasmid DNA are used. Otherwise 10 ng of plasmid DNA is typically sufficient.
5. Heat-shock cells by incubating in a 42°C water bath for exactly 45 seconds without shaking.
    a. It is advisable to double-check the water bath temperature with a calibrated thermometer, as temperature of this heat shock is a critical covariate.
6. Immediately place tubes back on ice for 2 minutes.
7. Add 250 µL sterile SOC media (Invitrogen 15544-034) to each vial under **sterile conditions**.
8. Shake horizontally for 1h at 37°C in an orbital shaker.

    a. This step allows the selection markers to be expressed by transformed bacteria. It is absolutely essential for kanamycin, but not as essential for ampicillin.
9. Under sterile conditions, carefully transfer and spread DNA/cell mix onto agar plate with appropriate resistance.
    a. For spreading, we use a glass Pasteur pipette that has been sterilized by the open Bunsen flame creating a sterile environment. The heat is also used to bend the pipette to a ~90^{o} angle, and then it is briefly cooled by rapid shaking in the air before spreading. Make sure to only use one pipette per tube.
    b. Otherwise you can spend a lot of money on fancy spreaders.
10. Incubate at 37°C overnight to grow cultures (16-18 hours ideally).
    a. Some plasmids (such as lentiviral plasmids or empty gateway vectors) are incubated at 30^{o}C.
11. Next day, examine for colonies.
    a. Colonies can be selected from the plates for minipreps, maxipreps, glycerol stocks, etc. (see other protocols).
12. Place plates at 4°C wrapped in parafilm (along edges) until further use. Plates are good for at least several weeks, if not months.