Bacterial Transformations (of chemically competent cells)

*****NOT for commercial Top10 *E. coli* cells*****

1. Add ≤ 5µl (≤ 1/10 of the cell aliquot amount) of the DNA sample to a chemically competent cell aliquot. Mix by pipetting gently, then incubate on ice for 30 to 45 minutes. (30)
2. Heat shock at 42°C for 60-75s
3. Incubate on ice for 5 minutes
4. Add 250µl of plain LB or SOC media aseptically, then incubate for 30 to 90 minutes at 37°C, shaking (45)
    - If resistance is Kan, must incubate for *at least* 1 hour
    - SOC media= more nutrient rich media that may help growth
5. Plate 100µl (if big plates) or 50µl (if small or half plates) aseptically
6. Incubate plate at 37°C overnight or until growth is observed
    - NOTE: Usually colonies that appear after two days are false positives

*If transformation fails:

- Spin down transformed cells for 5 minutes to pellet
- Resuspend pellet in 100µl of media
- Plate and incubate

Troubleshooting Tips:

1. Failure to see colonies:
    a. Cells are not competent (enough):
        i. Competence of cells can decrease the longer they're stored in the -80 freezer
        ii. Homemade chemically competent cells can be much less competent than commercially available cells and not very good at taking up low quality or low concentration ligation products
        iii. Try a different procedure to make chemically competent cells
    b. Low quality DNA product
        i. Try miniprepping again and adding more DNA
        ii. Doing a reaction clean-up of ligation products
        iii. Add more ligation product
    c. Too much DNA added
    d. Too little DNA added (you can usually some colonies even with small amounts of DNA, but your cells should be very competent and your DNA should be of good quality)
        i. Add more ligation product
        ii. Ethanol precipitation to concentrate your DNA
    e. Try plating more sample, incubating for longer, or using SOC media

f. Try pelleting cells, resuspending in a new LB media and plating (concentrate the cells)
g. Try using electroporation instead if possible (electroporation is more full-proof)

2. Lawn growth
    a. IF AMP PLATES: B-lactamase is secreted by cells during incubation, so by the time you plate the sample the antibiotic in the plate is degraded very easily
        i. Incubate for 30-40min instead of 1hr
        ii. Plate less sample
        iii. Dilute the sample before plating
    b. Antibiotics in plates are not correct/at a high enough concentration/expired
        i. If using organisms other than *E. coli* they will have different basal levels of resistance to antibiotics, so make sure your plates have the right amount of antibiotic according to the species
        ii. Increase amount of antibiotics in the plate
        iii. Make new antibiotic stocks
    c. Transformation was extremely successful
        i. Try diluting the sample before plating, or plating less
        ii. Try incubating sample and growing plate overnight at a lower temperature (if this doesn't interfere with anything → some plasmids have temperature sensitive origin of replications)
        iii. Incubate sample for less time before plating

3. Appearance of satellite colonies= Small colonies surrounding large colonies (these are FALSE POSITIVES)
    a. Happens frequently with Amp plates (Carbenicillin is a good substitute for ampicillin)
        i. The cells in the large colony are able to degrade the antibiotic in the surrounding area allowing satellite colonies to grow
    b. Pick the large colonies for cPCR