Bacterial Transformation

Cloning is copying (biotech definition) and relates specifically to making many copies of DNA molecules using plasmids and bacteria.

Competent cells- CaCl2: Helps cells to take up DNA
Aseptically streaked to attain single colonies.

Grow bacteria: Single colony in 10 mls of Luria broth. Place solution into vial of at least 2X the volume, and agitate vigorously at 37 ^{o}C. Inoculate a larger solution with 10 mls from previous days inoculum (serial inoculation.)

Centrifuge/wash: Ensure that the samples are treated in a sterile manner. Pellet is resuspended in isotonic saline and pelleted again. Add glycerol and CaCl2

Store bacteria frozen (optional): As a result of above, cells can be frozen and are considered COMPETENT.

If frozen over the long run, aliquot into microfuge tubes and freeze quickly (be immersing into liquid nitrogen.) Note that freezing of bacteria is done quickly, as opposed to eukaryotic cells that are frozen very slowly.

Heat shock transformation

Add competent cells: Many competent cells are commercially available. Thaw 100 ul of cells (but keep on ice.) Add plasmid DNA (maximum usually added is 5 ul or 10 ng of plasmid.) Chilled on ice then…

Heat shock: For 45 seconds at 42 ^{o}C. Then replace into ice bath. Heat helps bacteria to take up plasmid (by expansion and uptake) and immersion in ice helps to keep inside (with condensing of cellular contents.)

Cultivate bacteria: Media is added (non-selective, i.e. no antibiotic is added.) Samples loaded onto plates with antibiotics (for plasmid uptake selection.) If samples are suspected of being too dense, serial dilutions may be conducted.

B-galactosidase (AKA Lac-Z) gene. Will take B-galactosidase and turn it blue. If a bacteria has taken up this plasmid, will appear as a blue colony. If a gene has been inserted, it will insert in the middle of the B-galactosidase gene and deactivate it, so colony will appear white. So… in summary, blue colonies have taken up plasmid, and white have taken up novel plasmid with new insert.

Electroporation – Transformation/Electrical pulse.

Load bacteria and DNA into chamber: All chilled reagents are used, and 20 µl of bacteria and DNA are added to chamber. Exposed to 2.5 Kv burst. Small pores appear on surface of bacteria and DNA is taken in. The apparatus allows for variable voltage and or capacitance, as a function of different experimental parameters.