Transformation of Plasmid DNA into bacterial cells

Aim: Transformation of pUC18 Plasmid into *E.coli* DH5α Competent Cells

Introduction:

Transformation is the process by which foreign DNA is introduced into a cell. Transformation of bacteria with plasmids is important not only for studies in bacteria but also because bacteria are used as the means for both storing and replicating plasmids. Because of this, nearly all plasmids, even those designed for use in mammalian cells; carry both a bacterial origin of replication and an antibiotic resistance gene for use as a selectable marker in bacteria.

Protocol:

1. Take competent cells out of -80°C and thaw on ice (approximately 20-30min).

2. Take agar plates (containing the appropriate antibiotic, here Ampicillin 100 µg/ml) out of 4°C to warm up to room temperature or place in 37°C incubator.

3. Mix 1 to 5µl of DNA (usually 1ng to 100ng) into 50 - 100µL of competent cells in a micro-centrifuge (1.5 ml). GENTLY mix by flicking the bottom of the tube with your finger a few times (6- 8 times).

4. Place the competent cell/DNA mixture on ice for 40 min without any disturbances.

5. Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 90 seconds.

6. Put the tubes back on ice for 2 min.

7. Add 700µl pre-warmed LB (without antibiotic) and grow in 37°C shaking incubator for 45min.

(**Note:** This outgrowth step allows the bacteria time to generate the antibiotic resistance proteins encoded on the plasmid backbone so that they will be able to grow once plated on the antibiotic

containing agar plate. This step is not critical for Ampicillin resistance but is much more important for other antibiotic resistances.)

8. Harvest cells by centrifugation at 3000 g for 10 min at 4 °C and resuspend in 100 µl of LB media.

8. Plate some or all of the transformation onto a LB agar plate containing the appropriate antibiotic.

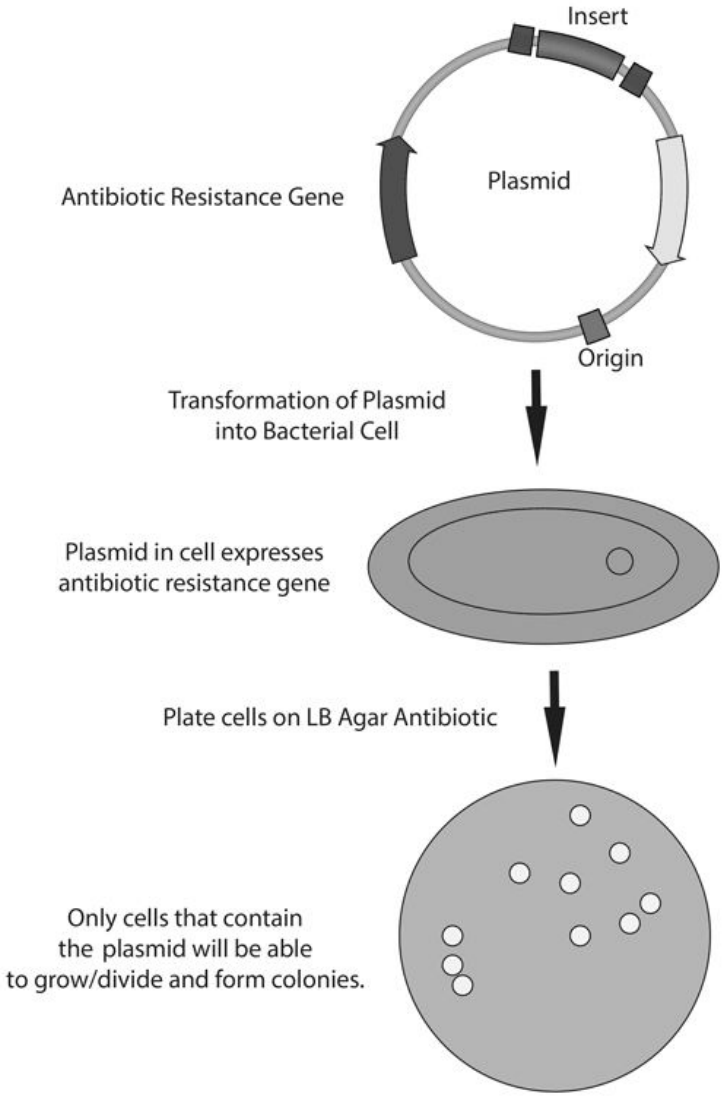
9. Incubate plates at 37°C overnight.

Note:

1. Thaw cells in ice. DO NOT thaw by warming them with your hands

2. To maintain competency, bacteria should be kept on ice at all times. Once they have warmed up, they are no longer competent and will not take up DNA.

3. Add LB or SOC using sterile procedures (ie in the hood)


Insert
Antibiotic Resistance Gene
Plasmid
Origin
Transformation of Plasmid into Bacterial Cell
Plasmid in cell expresses antibiotic resistance gene
Plate cells on LB Agar Antibiotic
Only cells that contain the plasmid will be able to grow/divide and form colonies.