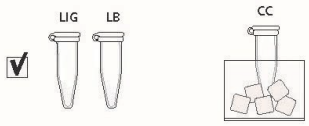
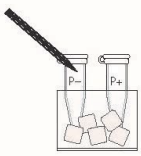
6 – Inserting recombinant plasmids into bacteria

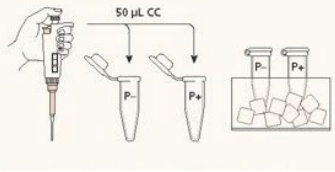
Lab 6: bacterial transformation using ligation mix



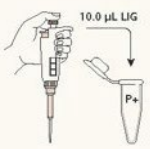
Check reagents (LIG, LB) and locate Competent Cells (CC), on ice



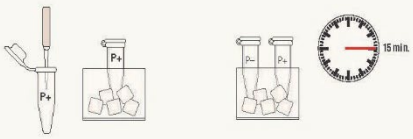
Label 2 empty tubes, P- and P+, put on ice



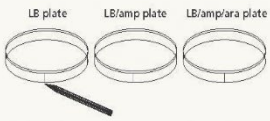
Resuspend CC before adding 50 µl to both P- and P+



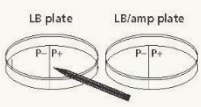
Add 10 µl LIG to P+ tube
Add 10 µl H_2O to P- tube



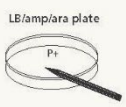
Mix using tip, keep tubes on ice for 15 min

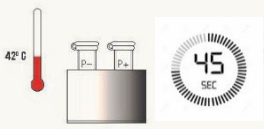


In this time, collect 3 different agar plates and label

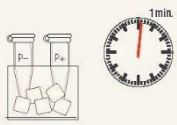


Divide the LB and LB/amp plates in two. Label one half 'P-' the other 'P+'. Label the third LB/amp/ara plate 'P+'

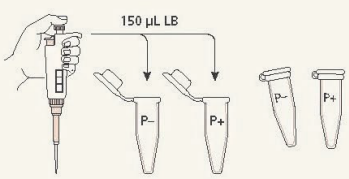




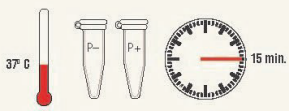
Check water bath is at 42 C. Place the P- and P+ tubes in water-bath for **exactly** 45 sec



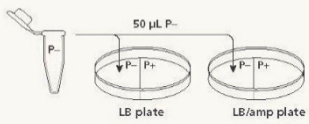
Immediately return tubes to ice, leave for 1 min.



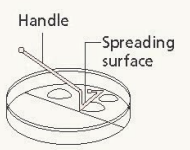
Add 150 µl LB into the P- and P+ tubes. Mix gently.



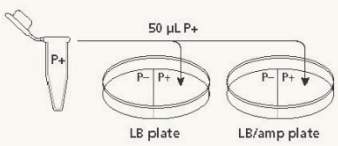
Incubate at room temperature for 15 minutes



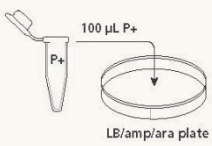
Pipette 50 µl of P- onto the P- half of the LB and LB/amp plates.



Use a sterile cell spreader to spread cells across the surface of the agar.



Using clean pipette tip, add 50 µl of P+ onto the P+ half of these same plates



Pipette 100 µl of P+ onto the LB/amp/ara plate then spread across the surface.

Let plates sit, right side up, for 5 min before taping all 3 plates together and labelling

Plates will be left in a warm environment for 24-36 hours to allow bacterial growth.