

***E. coli* Transformation (Heat Shock)**

https://www.addgene.org/protocols/bacterial-transformation/?gclid=CjwKCAiAt4rfBRBKEiwAC678KfyAJUFANM02v7k-lp10eajjR1oCPbJmf5mqPpdBeQa7PpPmwq8kJxoCSHAQAvD_BwE

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.

1. Take competent cells out of -80°C and thaw on ice for **20-30 min**
2. Remove agar plates (containing the appropriate antibiotic) from storage at 4°C and let warm up to RT (or put **inside the incubator at 37°C**)
3. Mix 5 ul of DNA (usually 10 pg – 100 ng) into 100 ul of competent cells in a 1.5 ml tube. GENTLY mix by flicking the bottom of the tube with your finger a few times
For large plasmid, e.g., pCambia1300 (21 kb), could transform 100 ng.
4. Incubate the competent cell/DNA mixture on ice for 20 min

5. Heat shock each transformation tube by placing the tube into a **42°C** water bath for **90 secs**

Heat shock transformation uses a calcium rich environment provided by calcium chloride to counteract the electrostatic repulsion between the plasmid DNA and bacterial cellular membrane. A sudden increase in temperature creates pores in the plasma membrane of the bacteria and allows for plasmid DNA to enter the bacterial cell.

6. Put the tubes back on ice for 2 min

7. Add 1 ml LB or SOB media (without antibiotics) to the bacteria and grow in 37°C shaking incubator (200-300 rpm) for 1 hour

This outgrowth step allows the bacteria time to generate the antibiotic resistance proteins encoded in the plasmid backbone so that they will be able to grow once plated on the antibiotic containing agar plate

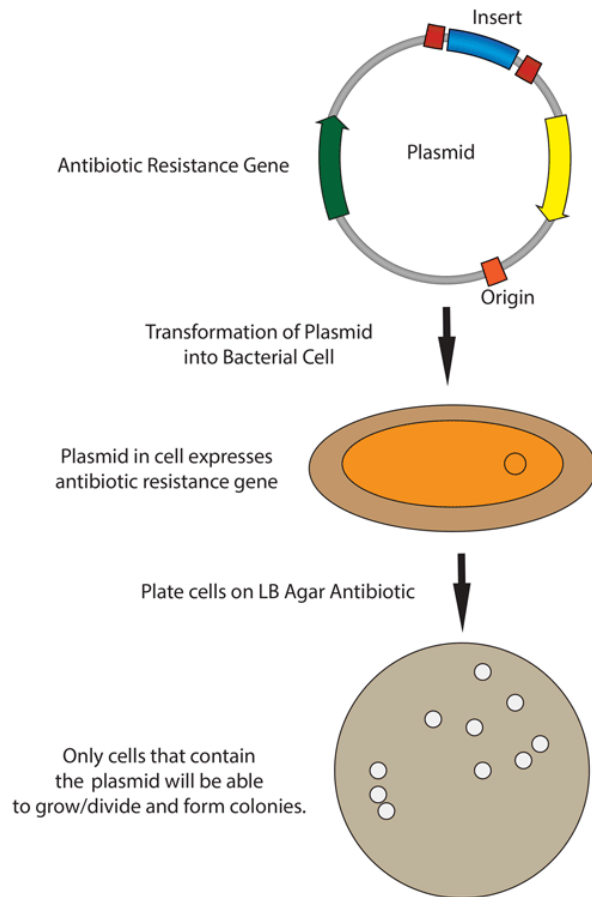
8. Plate some or all of the transformation onto a LB agar plate containing the appropriate antibiotic

We recommend that you plate 50-100 µL on one plate and the rest on a second plate. This gives the best chance of getting single colonies, while allowing you to recover all transformants.

For plasmid confirmation (such as plasmid extracted from Agro), use 50-100 µl unconcentrated solution to spread the plate

9. Incubate plates at 37°C overnight

If the culture volume is too big, gently collect the cells by centrifugation and resuspend in a smaller volume of LB so that there isn't too much liquid media on the agar plates. If the agar plate doesn't dry adequately before the cells begin dividing, the bacteria diffuse through the liquid and won't grow in colonies.



Timeline:

- Day 1: afternoon ~4 pm start transformation and spread the plate
- Day 2: afternoon ~5 pm pick colonies and shake in LB liquid medium
- Day 3: morning ~9 am (after 15 hours) start plasmid extraction