Dear Halcyon,

Thank you very much for having us and attending the Transformation Workshop. I wanted to share a few points following the workshop. The PowerPoints and protocol are in this drive folder:

https://drive.google.com/drive/folders/18-enm9EKrmOUxzk4JczLZnJgcKzNylDO?usp=sharing

First and foremost, could you please fill out this short survey to let us know your experience during the workshop? This would directly help us do better in the competition. Link to the form:

https://forms.cloud.microsoft/e/WGUamvEZHD



Secondly, a few technical points about the practical we did not have time to cover. Please read if you would like to fully wrap your head around the concept:

1. You plated -DNA on LB and LB-Amp (Antibiotic) plates, you repeated the process for +DNA. Cells without the DNA we added did not receive a gene, the instructions, to express (make) the ampicillin resistance enzyme, beta-lactamase. Therefore, you would expect NO growth for LB-Amp, -DNA.

   If transformation was successful, you will see colonies for +DNA on LB-Amp. This means that the bacteria successfully took up the DNA and made the relevant protein, thereby “saving” themselves from the damaging effects of the antibiotic.

LB plates without ampicillin are a positive control; since there is no selective pressure (i.e. nothing is killing the cells, LB is just the nutrient medium), you should see bacteria for both +DNA and -DNA.

Plate	Expected result	Interpretation
+DNA on LB-Amp	Growth	Bacteria are making the protein to degrade ampicillin, "saving themselves". They are reading the instructions we introduced to them.
−DNA on LB-Amp	No growth	Ampicillin kills cells; they do not have the relevant DNA, the instructions to grant them resistance.
+DNA on LB	Growth	No selective pressure, cells grow
−DNA on LB	Growth	No selective pressure, cells grow

2. The recovery step (step 11) which we skipped is typically needed to give cells enough time to make the antibiotic resistance-associated protein. If you plate cells on a medium with antibiotic, they will not be able to "defend" themselves and die.

3. This does NOT happen for ampicillin, because it disrupts bacterial cell wall integrity over time; bacteria only synthesise new cell walls after some time has passed since you plated them. They have enough time to make the resistance protein before they divide (which is when cell wall synthesis is required).
    a. For other antibiotics like kanamycin, the effects are much more drastic: crucial physiological functions are immediately inhibited and bacteria die; hence, you need a proper recovery phase (1 hour, 37˚C, in a shaking incubator).

4. The plasmid we used is a vector, it does not actually have a gene insert (for example, one coding for insulin). It is an empty information vehicle. Why does it carry the ampicillin resistance gene? We use it to tell if our transformation has worked. Only cells that have the DNA coding for the ampicillin resistance protein can survive over

time, when plated on LB-Amp. We can then select only the cells that we know have our plasmid vector.

5. Recall evolution: you need a selective pressure to drive genetic change. In the same way here, we are artificially introducing that selective pressure in the form of Ampicillin slowly killing cells. Therefore, it is more beneficial for cells to retain the DNA we transformed them with, rather than degrading it.
    a. In industrial applications, you want the cell to retain the DNA you introduced to ensure your protein of interest, such as insulin, is being made. Otherwise, there is no advantage for the bacterium to be making this protein – it won't use it and will just waste energy when making it. Antibiotic resistance is a fair tradeoff.

6. Transformations often fail. Some common reasons are inaccurate heat shock time (too long/short), cells not being on ice for enough time for DNA to bind and be stabilised, DNA not actually being introduced to the cells properly (it is a small volume that can get lost or even stay on the wall of the Eppendorf tube).
    a. Small tweaks, like using SOC media (more expensive) over LB in step 10, longer recovery (time given to express resistance protein) in a shaking incubator or using other methods, which we did not cover increase transformation efficiency by multiple-fold.

Should you have any questions, do not hesitate to reach out to igem2025@imperial.ac.uk.

Here are a few MCQs to test your understanding:

1. A human gene is put into *E. coli*, and the bacteria make the human protein. Why is this possible?
a) *E. coli* has human ribosomes
b) The plasmid converts the gene into bacterial DNA
c) All living things use the same genetic code
d) Human genes originally came from bacteria

2. A bacterium takes up DNA from a dead neighbour, and that DNA carries an antibiotic resistance gene. Which benefit of DNA uptake is this?
a) Genome diversification

b) Genome repair

c) Nutrition

d) Recovery

3. Why are the cells and DNA left on ice for 30 minutes before the heat shock?

a) To stop the cells dividing before the DNA is added

b) To kill any cells that won't take up DNA

c) To give the plasmid time to copy itself

d) To let the DNA bind onto the rigid cell surface

4. A student leaves their tubes in the 42°C water bath for 3 minutes instead of 45 seconds. What will they most likely see the next day after plating?

a) More colonies, because more DNA got in

b) Few/no colonies, because many cells died

c) Colonies growing on the −DNA LB-Amp plate

d) A full lawn of bacteria on every plate

5. Why do we add LB media and incubate at 37°C for an hour before plating on ampicillin?

a) The cells need time to make the resistance protein

b) The plasmid needs time to get inside the cells

c) It kills off cells that didn't take up the plasmid

d) The plasmid needs time to join the chromosome

6. The recovery step can usually be skipped with ampicillin, but not with some other antibiotics. Why?

a) Ampicillin is too weak to kill *E. coli* on its own

b) The resistance gene was already switched on before heat shock

c) Ampicillin only kills cells that are growing and dividing

d) Ampicillin breaks down within minutes of plating

7. Why does the plasmid carry the AmpR (Ampicillin Resistance) gene?

a) To make the protein we want more stable

b) To help the plasmid get through the membrane

c) To protect the cells from heat shock damage

d) So only cells that took up the plasmid can grow

8. A group finds a full lawn of bacteria on their −DNA LB-Amp plate. What is the most likely explanation?

a) The transformation worked extremely well

b) The ampicillin in the plate isn't working

c) Their heat shock went on for too long

d) Their competent cells were dead

9. What is the −DNA plate on plain LB (no ampicillin) there to check?

a) That the cells survived the protocol

b) That the plasmid contains AmpR

c) That the ampicillin is working

d) How many cells took up the plasmid

10. A company wants to make glucagon in *E. coli*. What is the correct order of steps?

a) Clone gene → Design construct → Transform → Purify → Express

b) Design construct → Transform → Clone gene → Express → Purify

c) Design construct → Clone gene → Transform → Express → Purify

d) Design construct → Clone gene → Express → Transform → Purify

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