18th June

First lab preparation session. Practiced pipetting different volumes into eppendorfs using micropipettes.

7th July

Starting the real lab work! We were briefed about lab safety and learnt about sterile technique. We then carried out transformation of T7 210-THS luciferase plasmids into E. Coli.

8th July

No cultures grew after overnight incubation and we came up with possible mistakes:

1. Wrong calculations
2. Poor streaking
3. Problem with competent cell storage/ transportation

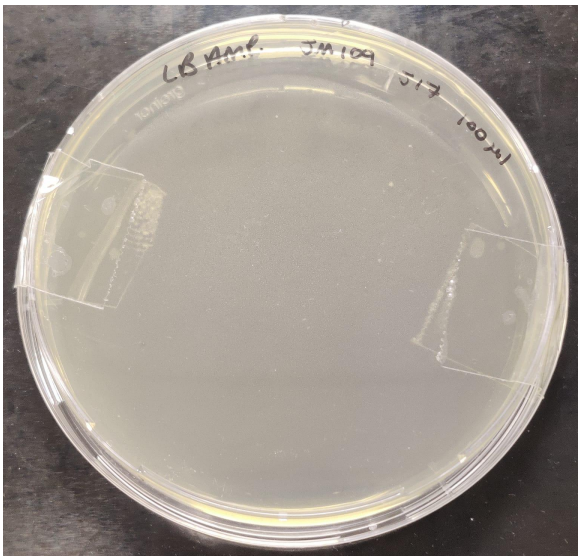
We bought competent cells from KCL to retry transformation using plasmid controls and normal agar.

12th July

Second attempt at transformation with JM109 and DH5α, we were very careful with each step and hope to have learned from our mistakes.

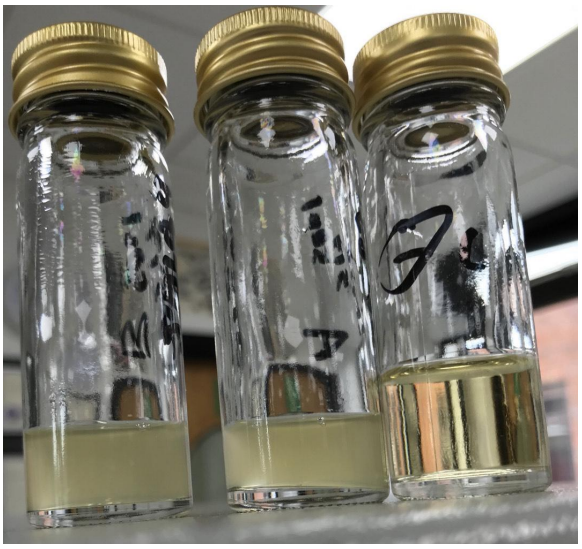
13th July

Air bubbles were observed in most plates rather than colonies. Luckily, one colony was found in our JM109 miR-517-5p plate (as shown below) and we transferred it to LB.



14th July

LB turned cloudy overnight which indicates that bacteria is present and our second transformation was successful!

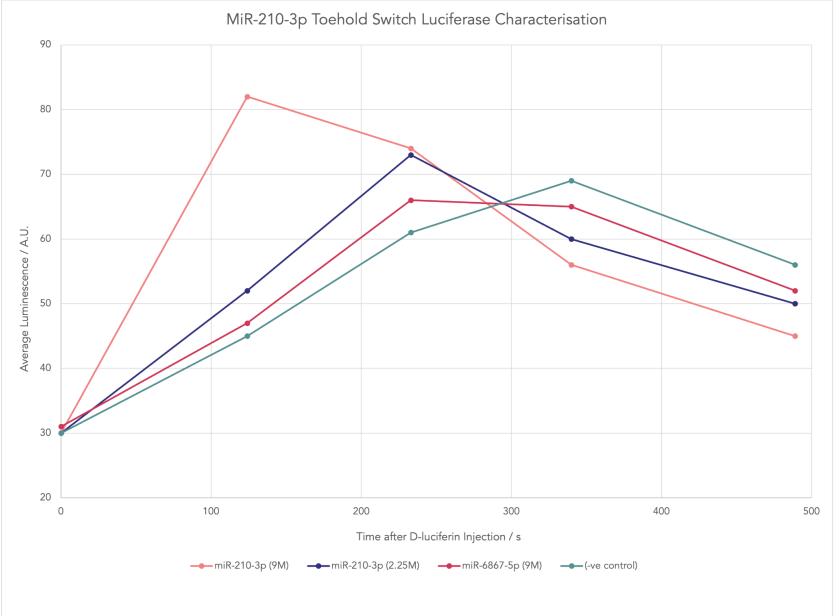


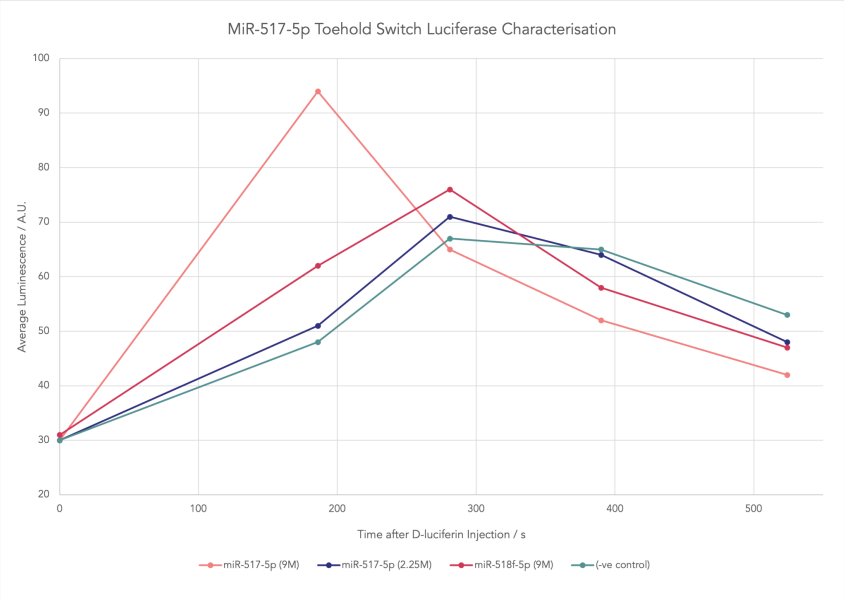
We carried out mini-prep.

25th September

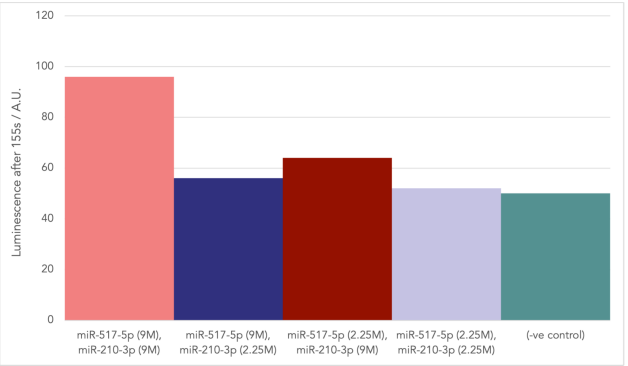
We characterised our gen2 (miR-210-3p & miR-517-5p) and gen3 (AND-Gate) toehold switches.

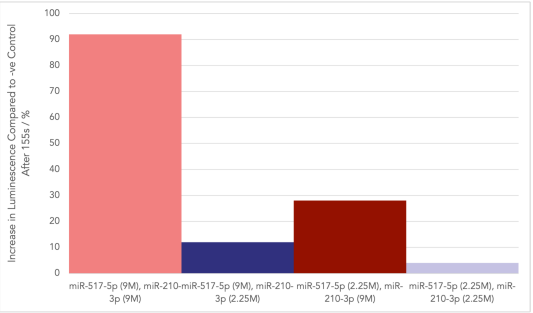
The gen2 toehold switches cause a noticeable increase in translation of luciferin with increased miRNA concentration.





The gen3 toehold switches also cause a noticeable increase in translation when both miRNAs are at a high concentration (9M), compared to when one of both of them is at a low concentration (2.25M), which gives strong evidence that our AND-gate design works.





7th October

We amplified miR-517-5p miRNA with miRPA. However, we ran into some problems due to inexperience which we aim to improve in our next session.

11th October

We amplified miR-210-3p and miR-517-5p miRNAs with miRPA, performed PCR to check that ligation has worked and carried out gel electrophoresis to see if we had amplified a sufficient amount.

14th October

Gel electrophoresis results:

We did not have access to gel ladders which could discriminate between <100bp DNA strands, but our gel showed that the target miRNAs miR-210-3p and miR-517-5p were amplified more than the homologs.

miRPA Product with Target miRNAs and Homologs