Functional analysis of an E. coli DNA repair protein - BIO 5009A

MONDAY, 9/25/2023

Aim:

We want to understand the role of three genes in the mismatch repair (MMR) pathway. We will compare three mutant lines of *E.coli* each defective in a different gene that is supposed to be part of MMR and confirm that these produce a higher rate of spontaneous mutants compared to wild type.

Introduction

To be completed - include some context and relevant background reading

List of E.coli strains

	Bacterial Line	Mutation
1	JW2799	knockout - mutH
2	JW4128	knockout mutL
3	JW2702	knockout mutS
4	BW25113	wild type

Procedures

A. Assess the phenotypes of wt, JW2799, JW4128, JW2702 E.coli strains

- According to Protocol A, take 1µL of each cell type and inoculate into LB Broth (without Antibiotics).

I noted the following important changes in procedure

- This whole procedure was done under sterile conditions at a close but safe distance from a blue-flamed bunsen burner.
- Exactly 1µL of each strain of *E.coli* was separately inoculated into 10mL of LB Broth, then left to grow overnight.
- Sample labelled as follows - PL 27/9/23.

B. Functional test of pRB318

- According to Protocol B, transform pRB318 into the following four strains E. coli wild type, and the three mutants each containing a single gene knockout (mutS, JW2702; mutH, JW2799; mutL, JW4128) and plate onto LB-Amp (see Protocol B). Incubate O/N at 37oC.

I noted the following changes:

- The heat block used was a mini block heater, Greiner Bio-One
- One change was that it was important for the LB medium and E. coli to be in a shaking incubator so that the cells would mix and all be exposed to the LB medium.
- for step 5, a shaking incubator was used and set to 37°c
- steps 6 and 7 were completed in sterile conditions at a close but safe distance to a blue flamed bunsen burner

- My plate was labelled as PL, JW2702 + pRB318, 27/9/23
- The other three transformations carried out by my group:

Transformation Plates

	Group member	Labelled Sample
1	Steve Mcqueen	SM, JW2799+pRB318, 27/9/23
2	Joe Bloggs	JB, JW4128+pRB318, 27/9/23
3	Nicholas Cage	NC, BW25113+pRB318, 27/9/23

Plates put in a static incubator at 37 degrees overnight.

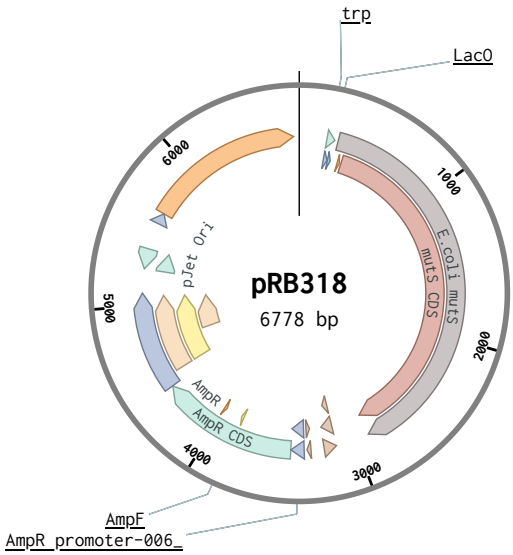
Next Time:

After the O/N growth for the Luria Delbruck assay, the technical team will plate these cultures onto Rifampicin antibiotic plates. I will need to establish the number of colonies which have grown on the Rifampicin Plates for each strain as part of Experiment A

To be completed - include some carefully thought out predictions about what you expect to see next time

For Experiment B, I will need to recover potential transformants, confirm this and repeat the Luria Delbruck assay for transformant cells that now carry plasmid *prB318*.

pRB318



Key References

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