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

Expt 1. 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).

Protocol A - Functional assay of MMR in E.coli

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.

Expt 2. 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 37°C.

Protocol B - Transformation of E.coli with plasmid DNA

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

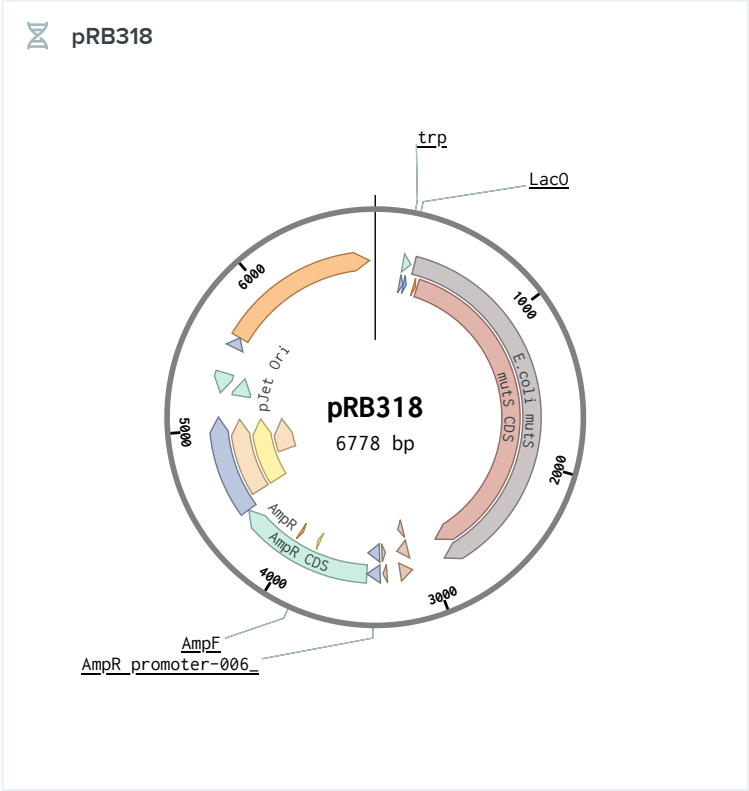
Plates put in a static incubator at 37 degrees overnight.

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 1

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

For Experiment 1, I will need to recover potential transformants, confirm this and repeat the Luria Delbruck assay for transformant cells that now carry plasmid *prB318*.



Plasmid map pRB318

Key References

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Kunkel, TA and Erie, DA (2005) DNA Mismatch Repair. *Annu. Rev. Biochem.* **74** 681-710.

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Obmolova, G, Ban, C, Hsieh, P and Yang, W (2000) Crystal structures of mismatch repair protein MutS and its complex with a DNA substrate. *Nature* **407** 703-710.

WEDNESDAY, 9/27/2023

Aim:

Continued experimental work from 25th September.

Today I aim to review the LB-Amp transformation plates for Experiment 2. I will count the number of colonies on each plate - these should contain the pRB318 plasmid because this provides Ampicillin resistance through the *AmpR* gene. I will confirm this transformation by carrying out PCR confirmation of the plasmid DNA

Procedures

Expt 2. Functional test of pRB318.
According to Protocol D, pick single colonies for PCR and transfer to LB Amp Broth

Protocol D - Colony PCR

This protocol followed exactly - including thermocycler conditions with the primer set as follows:

Colony PCR primers

	Primer Name	Sequence
1	AmpF	GCGGCCAACT TACTTCTGAC
2	AmpR	CAACGTTGTT GCCATTGCT

According to the pRB318 plasmid map this will produce a 171bp fragment.

Observations

On my LB Amp plate I found 230 colonies, indicating a good rate of transformation - from this plate I picked a single colony to put through colony PCR

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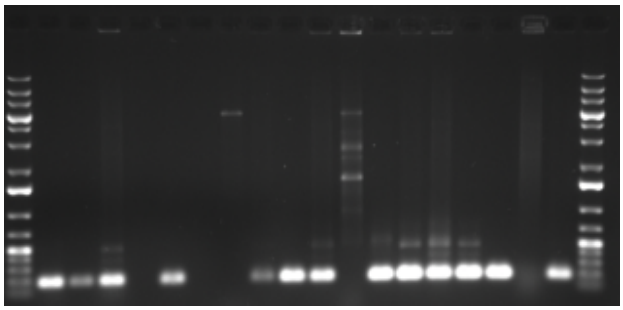

A 1% Agarose gel stained with EtBR - multiple experiments run here. Original image can be found on Lab UV illuminator system
Lane 1 = gene ruler 1kb plus ladder from Fisher
Lane 2 = my sample, PL, JW2702 + pRB318

According to the DNA ladder - this PCR produce appears to be the expected size.

Reflections

A few PCR reactions did not work well - indicated by the empty wells on the gel, we noticed that some people did not have enough PCR mastermix when preparing their reactions. After discussion with our MO we concluded the most likely reason was inaccurate pipetting and using more of the reagents than necessary, leaving too little for those prepping their PCRs last. We should practice our pipetting using water, weigh boats and microbalances next time so that we can practice our accuracy.

Next Time:

This confirmed transformation has been transferred to LB-Amp Broth in order to grow through the non-selective Luria Delbruck Assay - a repeat of Protocol A.
Insert a prediction about the group's results for next time!