Methylation Status of Three Genes Over Time in *Escherichia coli*

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**Introduction**

- GATC sequences can have a methyl group in the adenine in the *E. coli* genome.
- Methylation can either allow or block gene expression and it can also block restriction enzymes from cutting up DNA sequences.
- Methylation also stops viruses from damaging the DNA.
- There is a gene called *dam* (DNA adenine methyltransferase) and it methylates GATC sequences and blocks restriction enzymes from cutting them.
- The genes we investigated are *marR*, *mtlA*, and *hupB* because studies have shown that there is a difference in gene expression when *dam* is not present.

**Motivation**

- We can learn the role that methylation plays in the ability of pathogens to invade a host if we know more about methylation.
- Also, because methylation occurs across the entire genome, it could have a big effect on gene expression.

**Purpose**

- To observe how much methylation occurs on a specific gene at a specific time point.

**Methods**

1. I added *MboI*, a restriction enzyme that cuts unmethylated GATC sites, to DNA that was extracted at different time points.
2. I digested DNA from a *dam* mutant, which served as a control.
3. I then used primers specific for *marR*, *hupB*, and *mtlA* on a polymerase chain reaction (PCR).
   a. There were 7 samples of *E. coli* DNA from different time points (4hrs, 8hrs, 16hrs, 24hrs, 72hrs, 96hrs, 120hrs), 1 sample of DNA that did not include the *dam* gene, and 1 sample that included no DNA (control).
4. I ran a gel electrophoresis on the PCRs for 30 minutes.
5. I placed the gel in an Ethidium Bromide (EtBr) mix for 20 minutes.
6. I then placed the gel under a UV light and observed the different bands.
7. Finally, I quantified the pixel intensity of each band using ImageJ software.

**Conclusion**

- In *mtlA* GATC and *hupB* GATC there was a spike in methylation at 96hrs, however *mtlA* also had a spike at 120hrs.
- In *marR* GATC there was a consistent level of methylation.
- When there is a gene that contains a GATC sequence within or upstream of it, if the GATC is not methylated the sample is not able to produce a PCR product.
- No *dam* gene = GATC sequence will not be methylated, thus allowing restriction enzymes to cut up the DNA and no PCR product will form.
- If the GATC is methylated it won’t be cut by *MboI* and a PCR product will be made.

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