

DNA replication uses DNA polymerase to copy the cell genome, however, DNA polymerase has an error rate of 1 in 10^5 - 10^7 for every nucleotide copied¹. Errors that do occur must be corrected as these mutations can affect the rest of the genome by producing mutated proteins and potentially causing genetic disorders, such as cancer, neurological disorders, and diabetes². To address these mistakes, the cell utilizes mismatch repair (MMR) to replace erroneous bases and prevent downstream effects. Previous research into MMR focuses on *Escherichia Coli* (*E. coli*) UvrD, a helicase which plays a role in prokaryotic MMR, as our current understanding of eukaryotic MMR is limited. We are investigating the characteristics and functions of *Thermus aquaticus* (*Taq*) UvrD, which can then be compared to those of *E. coli* to better understand eukaryotic MMR. We will transform *E. coli* cells with a plasmid containing the *Taq* UvrD gene for expression. The protein will then be expressed using autoinduction and purified through ammonium sulfate precipitation and column chromatography. We will analyze the *Taq* UvrD function using FRET, mobility shift assays, helicase assays, FRET helicase assays, and ATPase assays. Through these techniques, we will potentially be able to identify the direction of translocation, the unwinding velocity, and the preferred binding site of the *Taq* helicase. We expect these characteristics to be similar to those of *E. coli* UvrD, due to the similarities in the proteins' structure and function. By gaining a deeper understanding of the function of *Taq* UvrD, we will elucidate further information regarding the role of helicases within eukaryotic MMR, which can then be used in therapeutic development for various genetic disorders.

References

1. Lee DF, Lu J, Chang S, Loparo JJ, Xie XS. Mapping DNA polymerase errors by single-molecule sequencing. *Nucleic Acids Res.* 2016 Jul 27;44(13):e118. doi: 10.1093/nar/gkw436. Epub 2016 May 16. PMID: 27185891; PMCID: PMC5291262.
2. Lee TI, Young RA. Transcriptional regulation and its misregulation in disease. *Cell.* 2013 Mar 14;152(6):1237-51. doi: 10.1016/j.cell.2013.02.014. PMID: 23498934; PMCID: PMC3640494.