The Cloning and Expression of the Circadian Rhythm Protein, Human Period 2 for Functional and Structural Investigations

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Introduction

The protein human period 2 (hPER2) is accountable for controlling the circadian rhythm, an intrinsic 24-hour biological clock that synchronizes body activities throughout the day and night. Not only could humans be affected by this rhythm but so can plants, animals, and any living organism. Interruption of this rhythm results in irregular sleeping patterns, eating disorders, lack of energy, and can even lead to cancer. To fully understand the role of the hPER2 protein and the mechanism of the circadian rhythm, hPER2 will be investigated for its structure and function. The expression vector with the synthesized hPER2 gene (3.8kb) was first transformed into the bacterial BL21 cells. Plasmid purification and restriction enzyme digestion then confirmed that the expression vector with hPER2 gene properly entered the bacterial cells. Small scale protein expression was also performed to test the expression of hPER2. Future plans include the optimization of hPER2 expression and purification of hPER2 using different chromatography techniques. After reaching a high homogenous state, crystallization of hPer2 will be tried to obtain crystals of hPer2 for X-ray crystallographic studies. Since hPER2 has an enormous role on the circadian rhythm by helping synchronize this biological clock, finding hPER2s' structures and functions will facilitate finding cures to circadian related diseases, contributing with positive long term effects on humanity

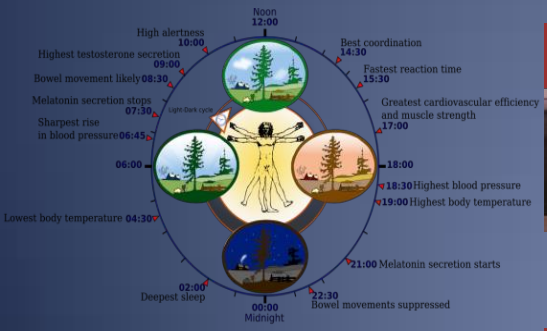


Figure 1: Circadian diagram describing energy levels

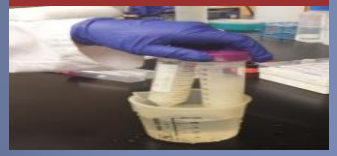
Methods

Transformation

Plasmid purification

Digest screening

Small scale protein expansion



Results

Transformation

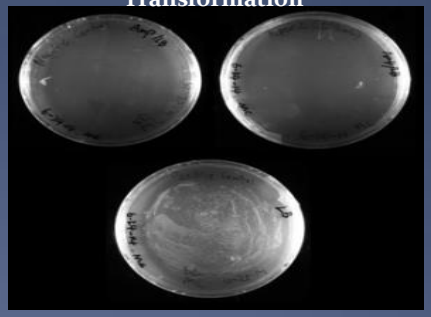


Figure 2: Transformation of hPER2 to negative and positive control very little colonies

Plasmid Purification

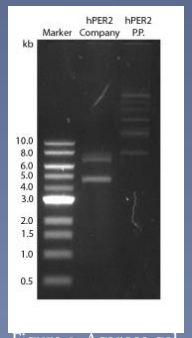


Figure 3: Agarose gel electrophoresis of hPER2

Digest Screening

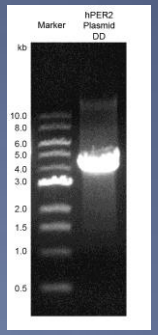


Figure 4:Agarose gel showing digest screening of hPER2

Protein Expression

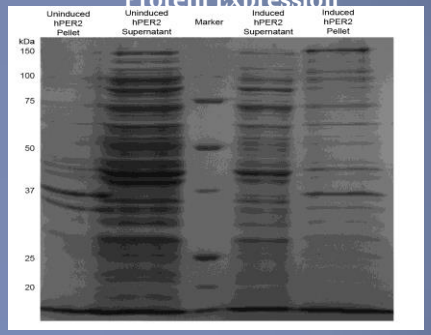


Figure 5: SDS-PAGE of hPER2 in small scale expression

Discussions

- Cloning was not successful as seen in results
- Digest screening showed an absence of the insert (3.8kb)
- The small scale protein expression trial showed unspecific expression
- Send plasmid into sequencing to see if hPER2 gene is present and if any mutations occurred.
- Retransformation of hPER2 may be necessary due to the fact the size of the plasmid (8.3kb) is too large.

Future work

- Optimize protein expression making sure hPER2 is expressing at a high concentration.
- Purify hPER2 using chromatography techniques.
- begin crystallization trials to study protein structures using x-ray crystallography

Acknowledgements

- This project is funded by the National Science Foundation Grant DRL-1322600.
- We thank BB(Grant G12MD007592)
- We would also like to thank our friends and supporters Cameron Wilson, Francisco Arriaga, Yuejiao Xian, Yating Yang

References

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