

The Investigation of the Structure and Function of hCRY2 in the Circadian Rhythm



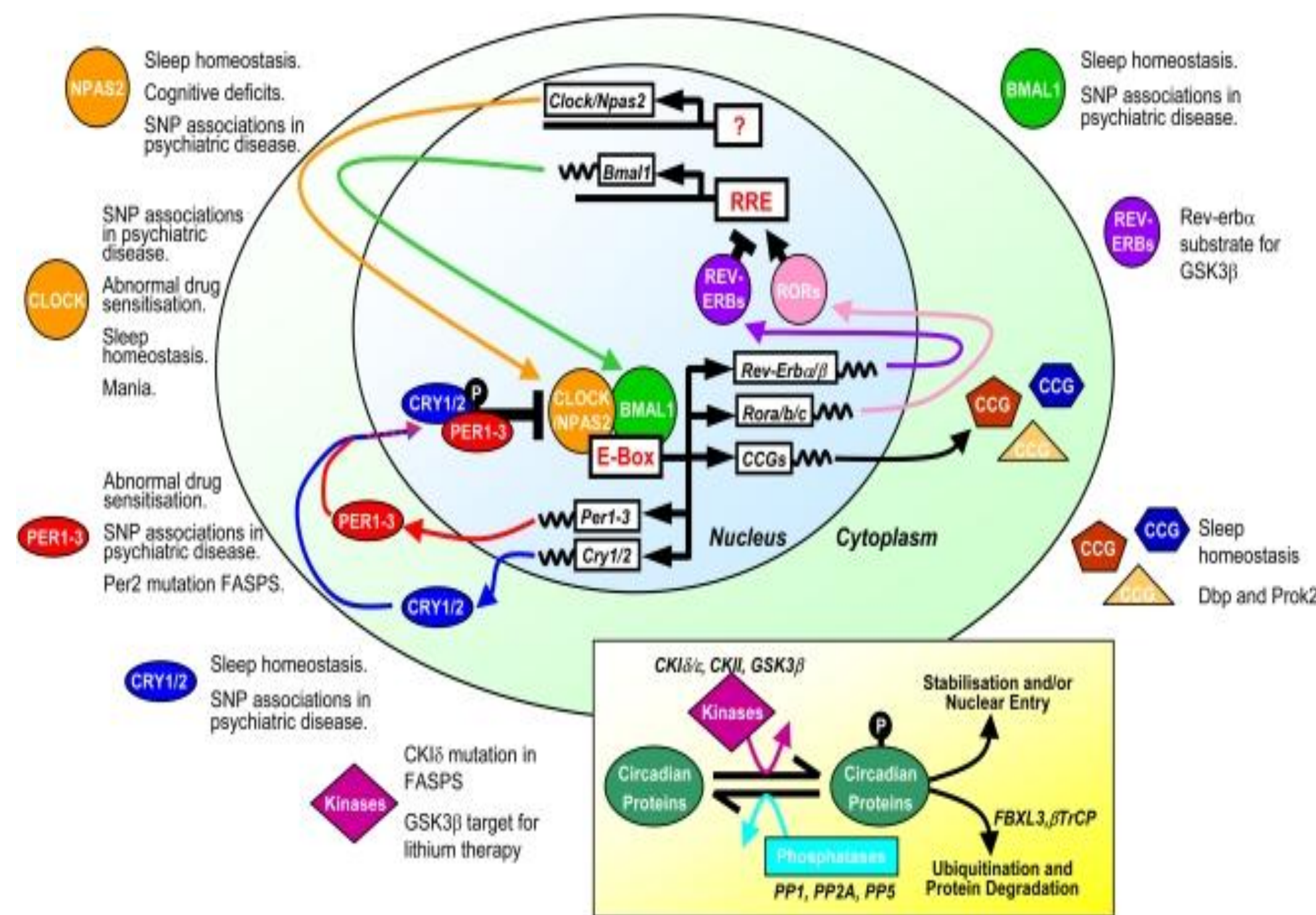
Arturo Mendoza¹, David Bojorquez¹, Isai Retana¹, Martin Chacon², Chuan Xiao²,

1. Irvin High School, El Paso, Tx, USA & 2. Department of Chemistry, The University of Texas at El Paso, TX, USA



Introduction

The circadian rhythm is a roughly 24 hour cycle in the physiological processes of living beings, including plants, animals, fungi, and cyanobacteria. Long-term disruption of the circadian rhythm can cause health problems such as sleeping disorders, diabetes, obesity, and increased risk of cancer. The human cryptochrome 2 (hCRY2) protein is one of the major components in the circadian rhythm. The hCRY2 plays a vital role as a light-independent inhibitor of the circadian clock. hCRY2 more than likely regulates transcriptional cycling by activating both the activator and the feedback inhibitor. In order to study hCRY2, functional analysis must be completed. The initial steps began with a transformation of hCRY2 into BL21 cells, followed by plasmid purification to verify that the hCRY2 plasmid successfully went into the BL21 cells. A digest screening was performed to confirm that the plasmid contained the gene of hCRY2. Finally, a small scale protein expression was utilized to check if the hCRY2 protein expressed properly at a high concentration. The future work and goals for this project are to optimize protein expression in order to purify hCRY2 protein using different chromatography techniques. hCRY2 will then be crystallized after reaching a high homogenous state. Finally, the structure of hCRY2 will be studied using the cryo-EM. Determination of the structure of hCRY2 will facilitate a better understanding of the mechanism of the circadian rhythm. The findings of hCRY2 structure can help find ways to treat certain diseases.



Background

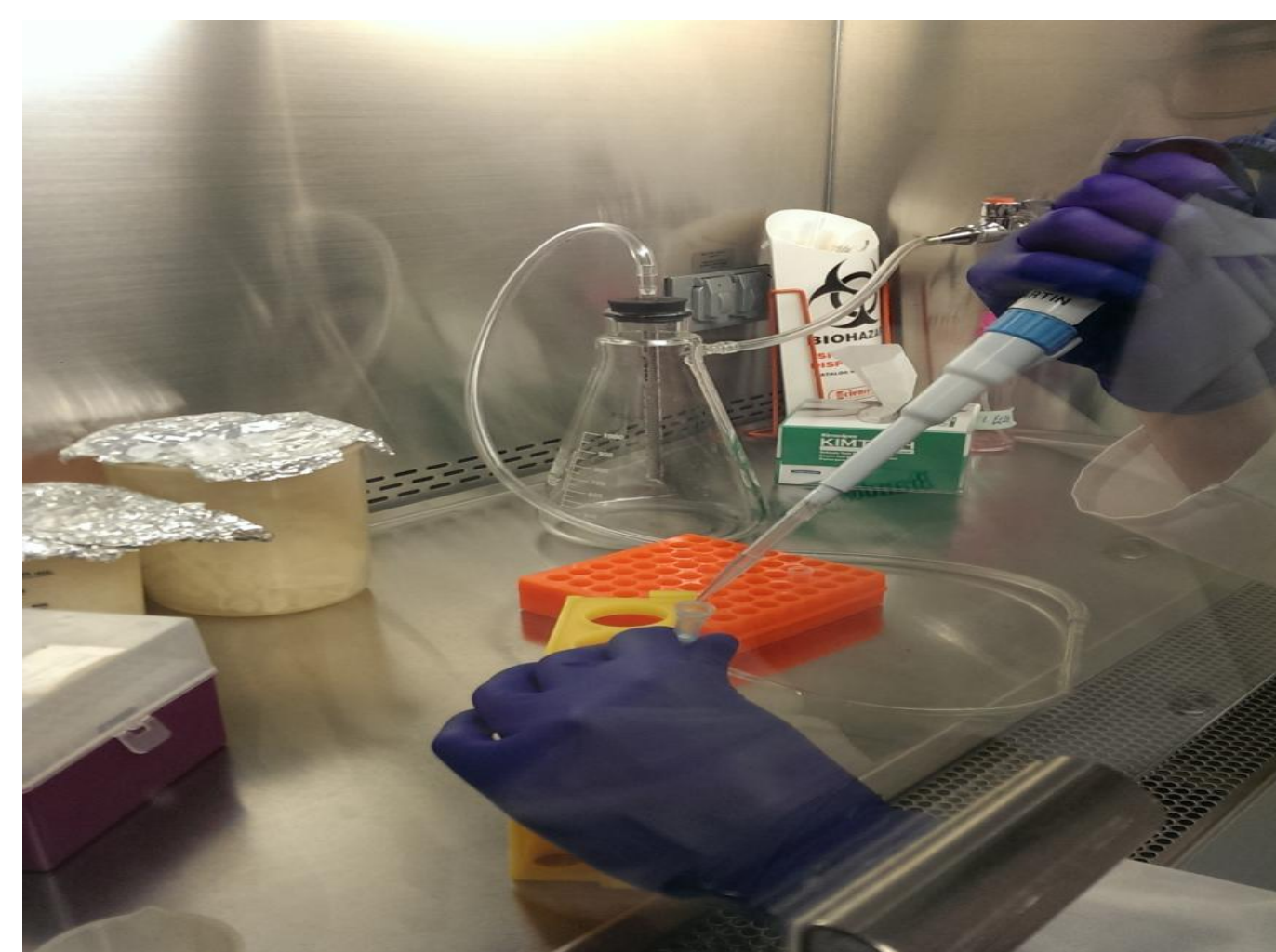
- The circadian clock plays a crucial role in the regulation of virtually every physiological process within an organism.
- Cryptochrome is found in plants and animals.
- The protein hCRY2 serves as a positive element that works together with other chemical compounds that make the circadian clock work appropriately.

Methods

Transformation



Plasmid Purification



Digest Screening



Protein Expression



Results

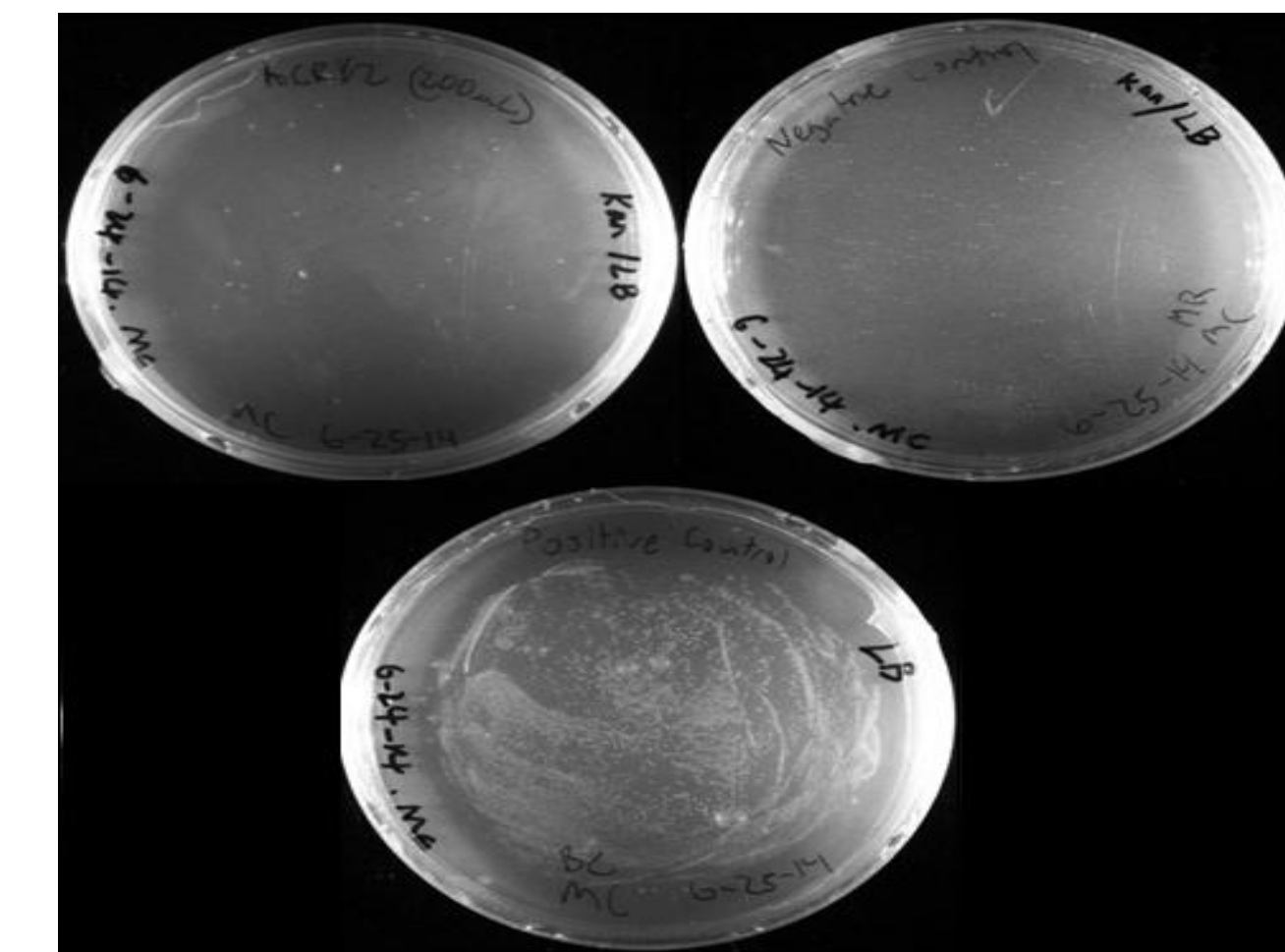


Fig 1. Transformation of hCRY2 plasmid, positive and negative control.

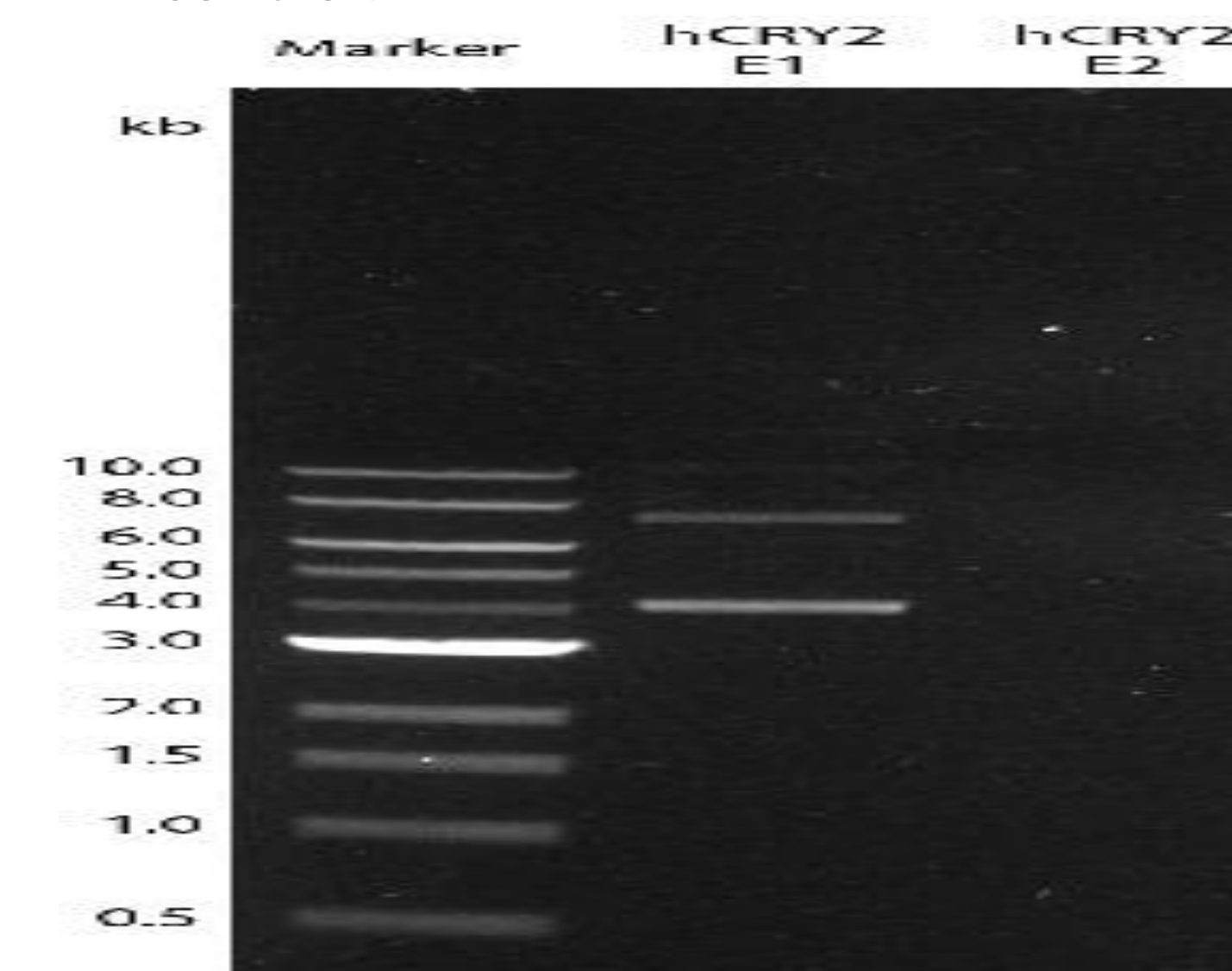


Fig 2. Agarose gel electrophoresis of hCRY2 plasmid after purification.

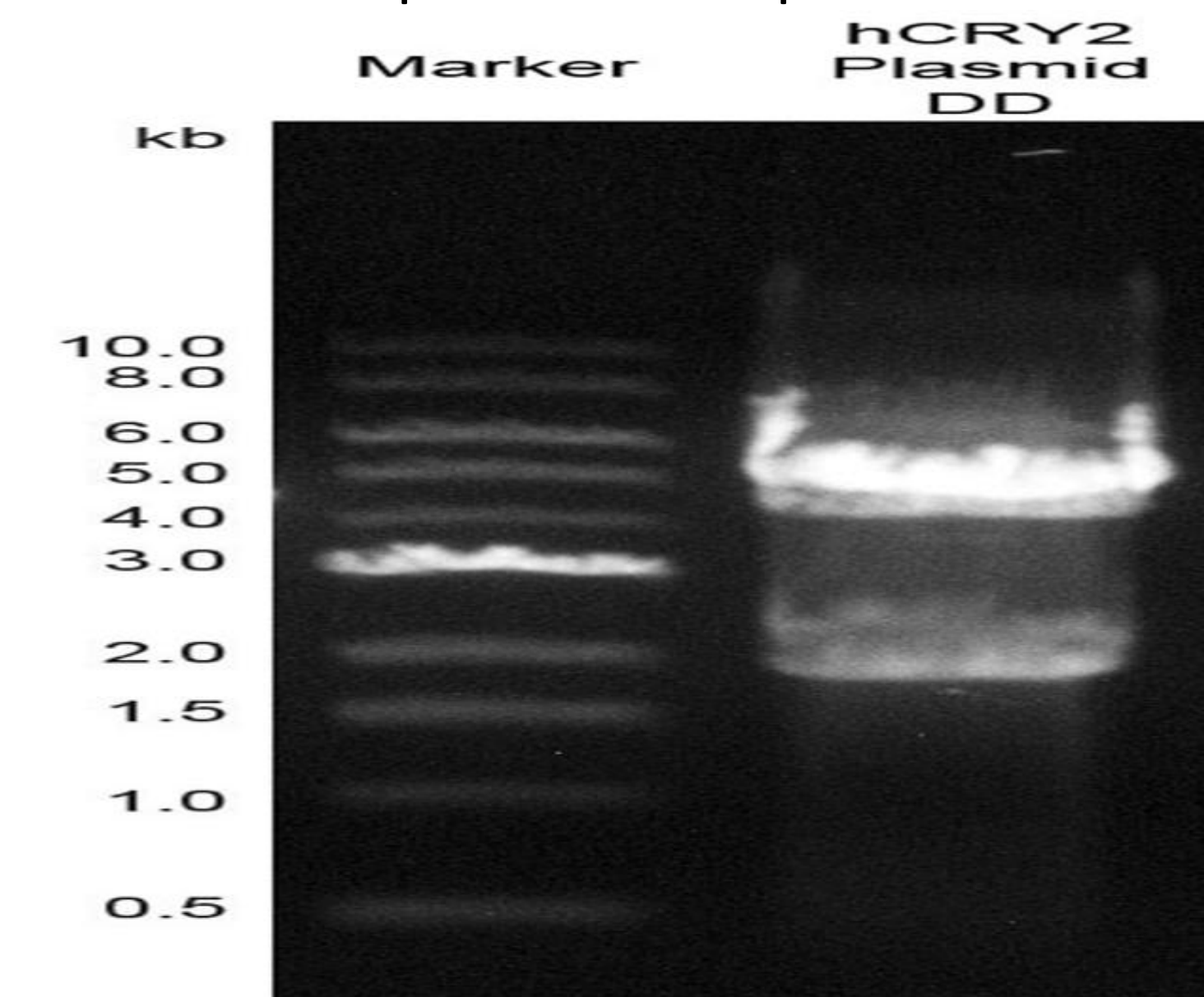


Fig 3. Digest screening of the hCRY2 gene (1.8kb).

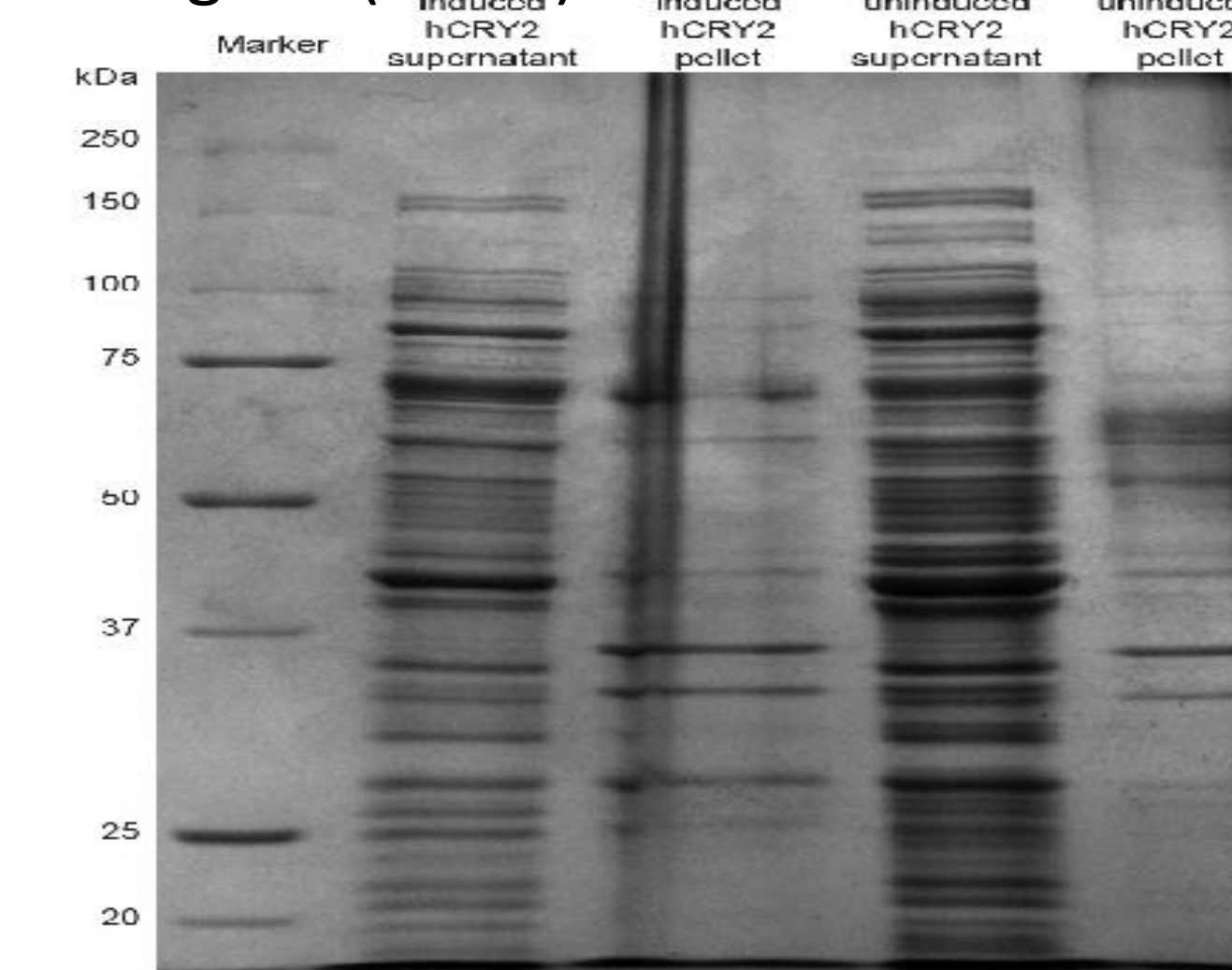


Fig 4. SDS-PAGE of hCRY2 in a small scale protein expression.

Discussion

- The hCRY2 gene has been successfully cloned and verified by restriction enzyme digestion showing a 1.8 kb insertion
- The small scale protein expression showed unspecific expression
- 37°C expression might be the cause of over expression other intrinsic proteins.

Future Work

- Optimize protein expression.
- Purify protein using different chromatography techniques.
- After reaching a high homogenous state, begin crystallization trials.
- After crystallization, study structure using X-ray crystallography.

Acknowledgements

- We would like to thank The University of Texas at El Paso for providing me with a chance to participate in the Work With a Scientist program.(NSF Grant DRL-1322600)
- The authors would like to express special thanks to the staff of the Genomic Analysis Core Facility of The University of Texas at El Paso. This core is supported by Grant G12MD007592 from the National Institutes on Minority Health and Health Disparities (NIMHD), a component of the National Institutes of Health.(NIH)
- Group members and friends: Mason Argobast, Cameron Wilson, Francisco Arriaga, Yuejiao Xian, Yating Yang

References

- J. van der Horst, G. T., J. Hoeijmakers, J. H., & Yasui, A. Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *nature*, 627-630. Retrieved April 23, 2014.
- Sack, R., Auckley, D., & Auger, R. Sleep. *Circadian Rhythm Sleep Disorders: Part I, Basic Principles, Shift Work and Jet Lag Disorders*, 30, 1460-83. Retrieved April 23, 2014
- Anand, S. N., Maywood, E. S., & Chesham, J. E. Distinct and Separable Roles for Endogenous CRY1 and CRY2 within the Circadian Molecular Clockwork of the Suprachiasmatic Nucleus, as Revealed by the Fbxl3Afh Mutation. *The Journal of Neuroscience*, 33, 7145-7153. Retrieved April 23, 2014