The Cloning and Expression of Human Neuronal PAS Domain Protein 2 for Structural and Functional Studies

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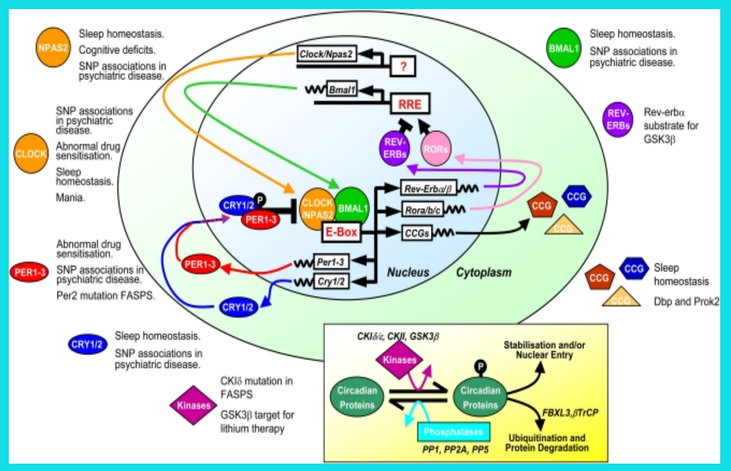
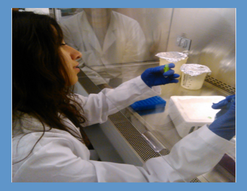
Background

The circadian rhythm maintains the daily biological cycle that controls the sleep-wake patterns in most mammals. Long-term disruption of the circadian rhythm can cause health problems such as sleeping disorders, diabetes, obesity, and risk of cancer. The human neuronal PAS domain (hNPAS2) protein works together with the aryl hydrocarbon receptor nuclear translocator-like (ARNTL) protein as a heterodimeric transcription factor that controls the gene expression in a 24-hour period. The mammalian NPAS2 gene encodes a functional analog of the circadian locomotor output cycles kaput (CLOCK) protein, but is expressed in a distinguished pattern of tissues including the prefrontal cortex of the brain. The project goal is to study the structure and the function of hNPAS2. The initial steps began by executing a transformation of an expression vector with hNPAS2 gene into BL21 cells, followed by plasmid purification to verify that the plasmid properly entered the BL21 cells. Restriction enzyme digestion was utilized to confirm the hNPAS2 gene was properly cloned in the plasmid. A small scale protein expression was performed to check the expression level of hNPAS2. Future goals include optimizing the protein expression of hNPAS2 followed by different chromatography techniques to purify hNPAS2 to high homogenous state. After crystallizing the highly purified protein. The structure of hNPAS2 will be studied using X-ray crystallography. Discovering the structure of hNPAS2 will give a better understanding of how it functions in the circadian rhythm. New treatments to the diseases associated with the disruption of the circadian rhythm will be developed based on the mechanism revealed from the structure of hNPAS2.



Methods

Transformation



Plasmid Purification

Digest Screening

Protein Expression

Results

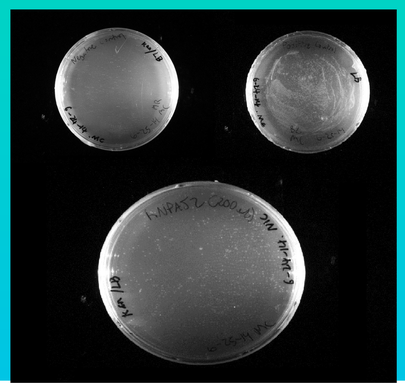


Figure 1: Transformation products of hNPAS2 also includes positive and negative controls.

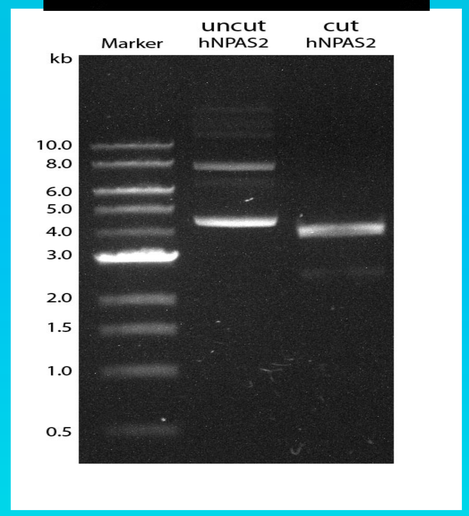


Figure 2: Agarose gel showing plasmid purification and digest screening.

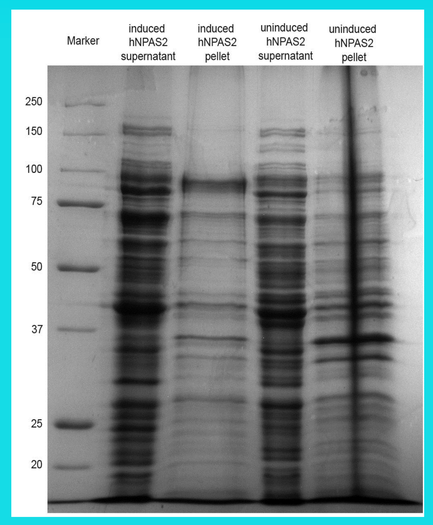


Figure 3: Small scale protein expression of hNPAS2.

Discussions

- Cloning of hNPAS2 was successful.
- The small scale protein expression trial showed unspecific expressions.
- Reclone hNPAS2 into pCold-TF vector and use a cold expression system (16°C) to express the hNPAS2 protein.

Future Work

- Improve protein expression of hNPAS2.
- Purify hNPAS2 using various chromotography techniques.
- After reaching a high homogenous state, start crystallization trials.
- After crystallizing hNPAS2, study structure using a cryo-EM.

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References

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