Summer, Winter, Night, and Day: Cloning of Environmental Genes for Proteorhodopsin into Artificial Chimeric Construct

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Introduction

Proteorhodopsin (PR) is a transmembrane ion pump typically found in microbes that operates in the presence of light. Therefore, it is responsible for numerous changes inside their systems, such as pH variance and cation concentrations. It uses retinal as a chromophore, analogous to the one responsible for human sight. Due to these attributes, this protein is one of the most exciting proteins in optogenetics, the field applying light to activate single neurons in live organisms.

However, isolating functional PRs in an environmental sample is difficult, due to the vast array of unculturable organisms in such samples. Therefore, in order to gain a greater understanding of their characteristics, their DNA must be extracted, amplified using Polymerase Chain Reaction (PCR) and then cloned and expressed by substitution into a chimeric vector with retinal.

The goal of the investigation is to locate PRs in the samples and display them, in the hopes of increasing the number of known proteorhodopsins. As our samples were taken from the Red Sea (Figure 3) over the course of two days, six months apart, and were densely populated with microbial organisms, we also hope to see the difference in activity as a function of light exposure.

Methods and Procedures

Selecting Samples: Chose six marine samples from each day, taken four hours apart

PCR: Amplifying the PR DNA Sample x 68 billion

Gel Electrophoresis: Isolation and analysis of DNA using electrical current (Figure 2)

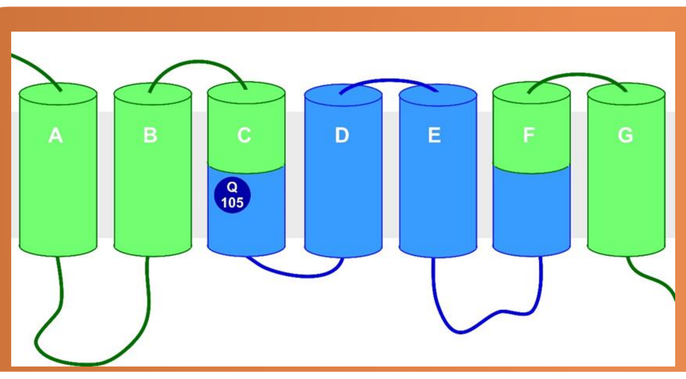
Molecular Cloning: Insertion into a chimeric vector made of GPR (Figure 1)

Electroporation: Apply voltage to the chimera and *E. coli* mixture; the chimera enters some bacterium as plasmid DNA

Growth: The largest bacteria colonies are mixed with AMP and glycerol and incubated in 96-well plates

Expression: IPTG and retinal is used to induce expression

Spectral Analysis: PRs are exposed to the full visible spectrum to determine their phenotype



DNA cut from marine samples, inserted into a chimeric vector to allow full length expression

Green Proteorhodopsin (GPR) complements the sample, necessary to make the insert viable for analysis

Figure 1: Diagram of the chimeric vector.

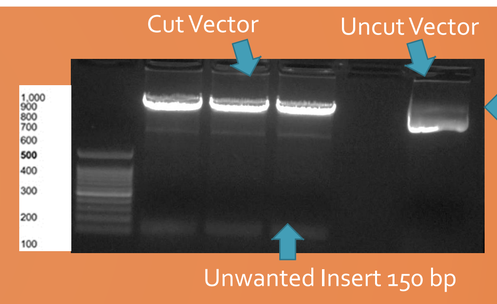
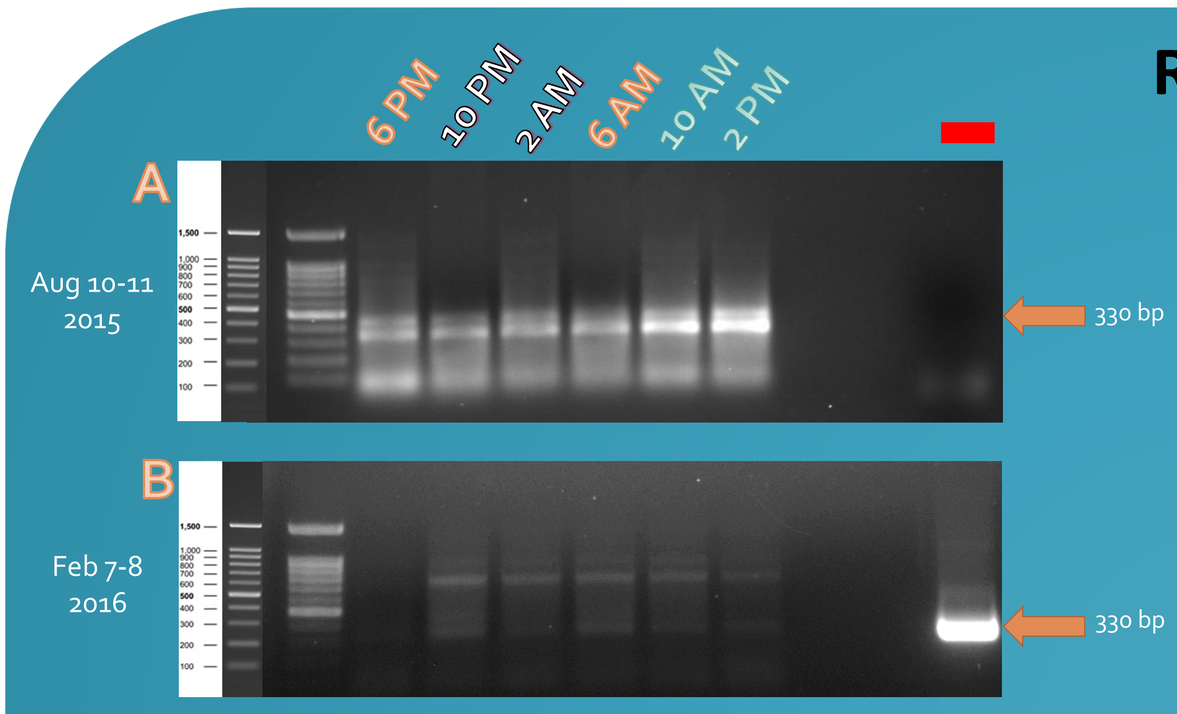


Figure 2: Gel image of the cut DNA vector to be used for ligation.

Figure 3: Map of Israel with Eilat highlighted on the coast of Red Sea.

Results



Figures 4A and 4B: Gel images of the original PCR. Brightness in the results indicate the presence of PRs in the samples. The orange arrows indicate the desired DNA concentration.

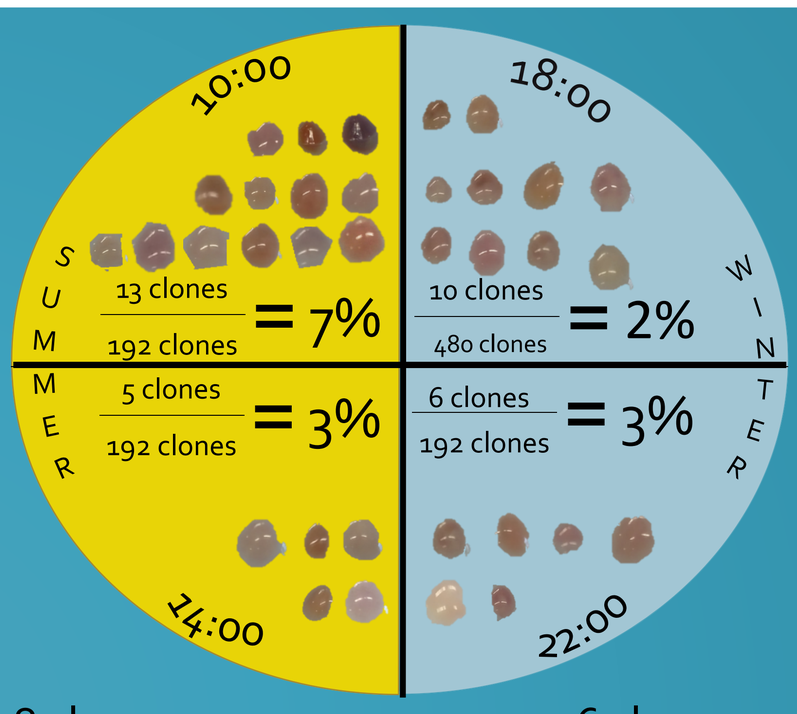


Figure 5: Color scheme of the different Rhodopsins identified during our project.

Total: 18 clones / 384 clones = 4.7%

Total: 16 clones / 652 clones = 2.5%

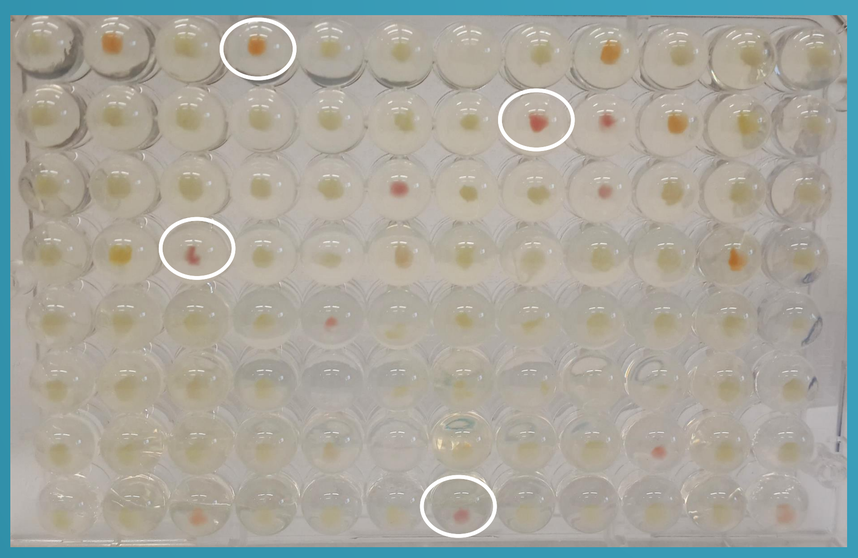


Figure 6: 96-well plate from August 11, 10 AM specimen after addition of retinal. Some of the *E. coli* colonies with expressed PR's of various colors are circled.

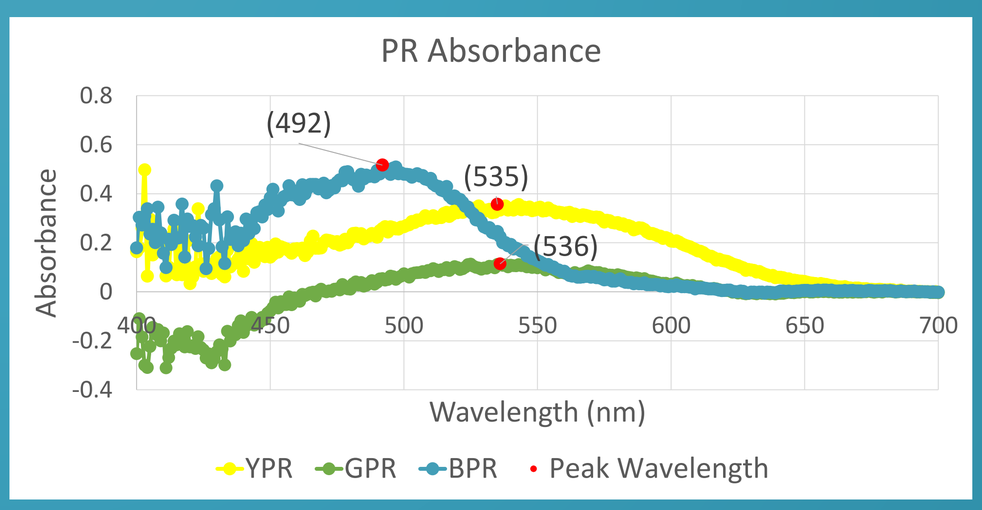


Figure 7: Graph of spectral analysis on some of the positive colonies, used to determine its phenotype (the color it absorbs).

Conclusions and Further Inquiries

- Possible discovery of 34 new PRs
- Notable difference in brightness between the summer (Figure 4A) and winter (Figure 4B) samples.
- Color diversity and concentration of PRs is a function of seasonal sunlight (Figures 5 and 6)
- Further Inquiry: Sequence the DNA to determine if the expressed PRs were previously undiscovered.
- Further Inquiry: Compare the phenotype (Figure 7) and genotype of the found PRs to see if there were any abnormalities and investigate

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References

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Map of Israel with Eilat circled. *Vicios de Viagem*, 12 Jan. 2017, viciosdeviagem.com/asia/israel/eilat/eilat-um-balneario-israelense-muito-cool/. Accessed 14 Aug. 2017.