Module 2 Remapping the Body of the World

Evidence worksheet\_04 “Bacterial Rhodopsin Gene Expression”

**Learning objectives:**

* Discuss the relationship between microbial community structure and metabolic diversity
* Evaluate common methods for studying the diversity of microbial communities
* Recognize basic design elements in metagenomic workflows

**General Questions:**

• *What were the main questions being asked?*

The main goal was to more fully describe proteorhodopsin (PR) photosystem genetics and biochemistry. PRs are retinal-containing proteins that catalyze light-activated proton efflux across the cell membrane and are found globally in the ocean’s photic zone and in a diverse array of Bacteria and Archaea.

What are the minimal heterologous genetic level transfers required for the transfer of the phenotype?

• *What were the primary methodological approaches used?*

High-density colony macroarrays

* Screening for PR expression

Fosmid library screen

* Screened for PR-containing clones on retinal-containing LB agar plating medium
* Used a copy-control system that allowed a controlled transition from one copy per cell to multiple (up to 100) vector copies upon addition of the inducer L-arabinose

HPLC Analysis

* For separation of carotenoids

Proton-Pumping Experiments

* Change in pH of a water bath as a result of a clone’s proton pump was the detection method

ATP Production Assays

* ATP measured with a luciferase-based assay

• *Summarize the main results or findings.*

Took DNA from the environment, created a library out of it, and performed a screen in E. coli for a phenotype with genes it does not normally have.

* A powerful way of mining the uncultivated diversity found around the world and using it to engineer microbes to discover the minimal number of genes required to generate a specific phenotype

Discovered that it only required 7 genes to create the PR photosystem phenotype, which is a small enough number of genes that they could easily fit on a single F1 plasmid, enabling extensive horizontal gene transfer

* Importantly, it is apparent that the PR photosystem is ubiquitous among diverse microbial taxa

• *Do new questions arise from the results?*

• *Were there any specific challenges or advantages in understanding the paper (e.g. did the authors provide sufficient background information to understand experimental logic, were methods explained adequately, were any specific assumptions made, were conclusions justified based on the evidence, were the figures or tables useful and easy to understand)?*