High Throughput Identification and Structural Characterization of Multi-Protein Complexes During Stress Response in Desulfovibrio vulgaris: Microbiology Subproject

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High Throughput Identification and Structural Characterization of Multi-Protein Complexes During Stress Response in Desulfovibrio vulgaris: Microbiology Subproject

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**Summary**

The Microbiology Subproject is part of the Virtual Institute for Microbial Stress and Survival (VIMSS) supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomes Program. The project focuses on the identification and characterization of multi-protein complexes that are involved in stress response pathways in Desulfovibrio vulgaris, a model anaerobic sulfur-reducing bacterial strain. The project aims to understand the role of these complexes in stress response and metal and radionuclide reduction, and to develop tools for protein complex characterization and imaging by the other groups.

**Construction of inframe tags**

The construction of a combined STF-SNAP is being constructed in order to increase throughput by using one tag for all constructs. The Gateway® Cloning procedure, verifies the recombinant approach to be adapted from the VIMSS to date: O

**High Throughput Phenotyping of Tagged Strains**

- Produce Engineered Strains with Tagged Proteins
- HTP Tagged Constructs Current Capacity

**TOPO-GATEWAY High Throughput Strategies for Tagged-Strain Generation**

Details in the TOPO-GATEWAY strategy and the Recombineering approach are in the adjoining paper.

**Phenotyping of Tagged Strains**

- Synthesis, consistent self-production for transcription, proteomics, metabolomics, and lipomics
- To date: >300 biomass productions, >1700 L

**Large-Scale Biomass Production and Harvesting**

1. 4 x 5 L and 2 x 3 L non-metallic fermenters, for anaerobic conditions
2. Potential production capacity: batch culture up to 3 L in stepwise or stress & control, and continuous
3. Currently running continuous flow productions 100 L scale

**Construction of inframe tags**

- For tagged proteins that do not have an associated antibiotic marker, the gene of interest and a portion of the downstream from the gene is amplified with the tag in the center, and that construct put into an entry vector that could then be put into the Gateway® plasmid containing Speciﬁcally mutant resistance gene and the kan promoter/ups gene.

**Environmental Stress**

The environment is the context in which genomes evolved, function, and continue to evolve. It is the only context in which they can be fully understood.

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