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### **Author**

Mukhopadhyay, Aindrila

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## ESPP2: Study of Two component signal transduction systems in *Desulfovibrio vulgaris* Hildenborough.

Aindrila Mukhopadhyay<sup>1,3</sup>, Eric Luning<sup>1,3</sup>, Jayashree Ray<sup>1,3</sup>, Adam Deutschbauer<sup>1,3</sup>, Kimberly Keller<sup>2,3</sup>, Jarrod Robertson<sup>2,3</sup>, Grant Zane<sup>2,3</sup>, Morgan Price<sup>1,3</sup>, Swapnil Chhabra<sup>1,3</sup>, Judy Wall<sup>2,3</sup>, Adam Arkin<sup>1,3</sup>, Terry Hazen<sup>1,3</sup>, Jay Keasling<sup>1,3</sup>

<sup>1</sup>Lawrence Berkeley National Laboratory, <sup>2</sup>University of Missouri, <sup>3</sup>Virtual Institute of Stress and survival (vimss.lbl.gov).

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Two component systems, comprised of Histidine Kinase and Response regulator proteins, represent the primary and ubiquitous mechanism in bacteria for initiating cellular response towards a wide variety of environmental conditions. In *D. vulgaris* Hildenborough, more than 60 such systems have been predicted, but remain mostly uncharacterized. The ability of *D. vulgaris* to survive in its environment is no doubt linked with the activity of genes modulated by these two component signal transduction systems. Recent methods developed for in vitro confirmation of specific phosphotransfer from histidine kinases to their cognate response regulators provides a valuable method for high throughput mapping of two component systems. This is aided by *in silico* methods to predict candidate partners for histidine kinases or response regulators that are either ORFans or have no proximal genes that may be supposed to be their cognate partners. Such methods have been developed by several groups and can be checked for additional corroboration from microarray data in our VIMSS database. Use of these computation methods are being done in collaboration with the Computational Core of ESPP. However, adopting such a workflow requires highly pure, active proteins for the phosphotransfer assay. Using Gateway® Cloning system, expression vectors for all response regulator candidates and the soluble portions of histidine kinases candidates were created. His-tagged proteins were purified under native conditions. The purified histidine kinases with the corresponding cognate response regulators have been used successfully in *in vitro* phosphotransfer mapping for selected interesting two component systems in *D. vulgaris*. The immediate applications are for two component systems discovered to be involved in specific stress studies. Availability of a library of response regulators and histidine kinase also allows us test a variety of broader questions experimentally. For example, do the many highly homologous histidine kinases in *D. vulgaris*, still maintain specific interactions with one or few response regulators? Beyond testing such hypothesis, this library of purified proteins can also be used to map the entire two component signal transduction cascade. In addition, availability of a library of histidine kinase knockout mutants, constructed over the last several years of this project, also allows us to rigorously follow-up conclusions from the in vitro phosphotransfer tests. Technology that will aid a high throughput phenotypic analysis of knockout mutants under defined stress/ signal inducing conditions have been developed for *D. vulgaris* by the Applied and environment core of ESPP.