Construction and Evaluation of *Desulfovibrio vulgaris* Whole-Genome Oligonucleotide Microarrays

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**Abstract**

*Desulfovibrio vulgaris* Hildenborough has been the focus of biochemical and physiological studies in the laboratory, and the metabolic versatility of this organism has been largely recognized, particularly the reduction of sulfite, fumarate, iron, uranium, and chromium. In addition, a *Desulfovibrio* sp. has been shown to utilize arabinose as the sole electron acceptor. *D. vulgaris* is a free-living bacteria with a genome size of 5.6 Mb and 1584 ORFs. The whole genome microarray of 95,179 ORFs has been constructed using *Desulfovibrio vulgaris* Hildenborough. All ORFs in the genome were represented with 3471 (97.1%) unique probes and 103 (2.9%) non-specific probes that may have cross-hybridization with other ORFs. In preparation for use of the experimental conditions, artificial probes and targets were designed to assess specificity and identify optimal hybridizations. The results indicated that for 50mer and 70mer oligonucleotide arrays, hybridization at 45ºC to 50ºC, washing at 37ºC and a wash time of 2.5 to 5 minutes obtained specific and strong hybridization signals. It is to evaluate the performance of the microarrays in experiments, growth conditions were selected that were expected to give significant hybridization differences for different sets of genes. The initial evaluations were performed using *D. vulgaris* cells grown in log phase and stationary phase. Transcriptional analysis of 95,179 *D. vulgaris* cells sampled during log phase growth were indicated that 23% of annotated ORFs were upregulated and 7% of annotated ORFs were downregulated compared to stationary phase cells. The upregulated genes included ORFs predicted to be involved with acyl chain biosynthesis, amino acid ABC transporter, translational initiation factors, and ribosomal proteins. In the stationary phase growth cells, the few up or down-regulated ORFs (370-540) were determined as a carbon/nitrogen starvation and/or a 23C-stabilized D-serine-2.5A-cycloallophosphatase (MECDP) enzyme. Polyamines are polyamines that are typically abundant in rapidly dividing cells and are essential growth factors in *D. vulgaris* growth. Polyamines are thought to stabilize DNA by the association of the amino groups with the phosphate residues of DNA and can also enhance DNA and ribosome stability. The MECDP synthase enzyme is essential in the de novo-synthetic pathway of polyamines; it catalyzes the formation of a polyamine from a diaminopimelic acid. polyamines and/or oxyphilic polyamines are responses of *D. vulgaris* during stationary phase. The MECDP synthase results indicate that the recently predicted polyamine biosynthesis pathway is functional. We are currently synthesizing growth conditions in log phase *D. vulgaris* cells to monitor whole-genome expression levels.

**Comparisons of Hybridization Signal Intensity to Probes of Different Length (50mer, 70mer, 100mer, 150mer or PCR product) with DNA or cDNA**

**Average Signal Intensity or SNR of 96 Probes of Different Length (50mer, 70mer, 100mer, 150mer, PCR-probe)**

**Relationship between Probe Target Similarity and Signal Intensity for *D. vulgaris* ORF Probes**

**Conclusions**

- *D. vulgaris* oligonucleotide probes perform in similar fashion to PCR-based microarrays under the tested conditions.
- The results indicated that for 50-mer and 70-mer oligonucleotide arrays, hybridization at 45ºC to 50ºC, washing at 37ºC and a wash time of 2.5 to 5 minutes gave specific and strong hybridization signals.
- The arrays worked well when whole-genome expression patterns were compared for log phase and stationary phase cells.
- Future work includes RT-PCR to confirm expression levels of selected ORFs.
- Work is currently underway to determine the expression profile of *D. vulgaris* cells when grown in the presence of sulfite, chromium, and uranium as electron acceptors.
- The newly identified specific responses of *D. vulgaris* that respond to increasing levels of stress and nutrient stresses.

**Comparisons of Designed Probe Information for *Desulfovibrio vulgaris* using different programs (probe length = 70-mer)**

<table>
<thead>
<tr>
<th>Array/OligoSelector</th>
<th>OligoArray1</th>
<th>OligoArray2</th>
<th>OligoPicker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Designed Probes</td>
<td>3574 (99.7%)</td>
<td>1628 (45.4%)</td>
<td>3441 (96.0%)</td>
</tr>
<tr>
<td>Specific probes</td>
<td>3471 (97.1%)</td>
<td>1590 (44.4%)</td>
<td>3412 (95.2%)</td>
</tr>
<tr>
<td>Non-specific probes</td>
<td>103 (2.9%)</td>
<td>38 (1.1%)</td>
<td>29 (0.8%)</td>
</tr>
<tr>
<td>Total ORFs</td>
<td>3584</td>
<td>3584</td>
<td>3584</td>
</tr>
<tr>
<td>Run Time</td>
<td>-5 hrs</td>
<td>-6.5 hrs</td>
<td>-5 min</td>
</tr>
</tbody>
</table>

**Graphs**

- **Graph A**: Comparison of Hybridization Signal Intensity to Probes of Different Length (50mer, 70mer, 100mer, 150mer or PCR product) with DNA or cDNA.
- **Graph B**: Average Signal Intensity or SNR of 96 Probes of Different Length (50mer, 70mer, 100mer, 150mer, PCR-probe).
- **Graph C**: Relationship between Probe Target Similarity and Signal Intensity for *D. vulgaris* ORF Probes.