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## ***Vibrio salmonicida* Genome Array Ready Oligo Set™ (Version 1.0)**

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*Vibrio salmonicida* V1.0.1 AROS is designed using state-of-the-art methodology and proprietary software for detection of transcripts from *Vibrio salmonicida* strain LFI1238 in collaboration with Prof. Nils-Peder Willassen of the Protein Research Group Department of Molecular Biotechnology, Institute of Medical Biology University of Tromsø. *Vibrio salmonicida* is a gram negative marine bacterium that is a cause of cold-water vibriosis in Atlantic salmon; it is related to the human pathogens *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. The chromosome is ~4.2 Mb with a G+C content of ~45%, and there are two large plasmids of ~ 450 bp and ~250 kb, along with several smaller plasmids.

As with all probe sets for bacterial genomes, each oligo probe is fully contained within the transcript it detects which makes this probe set also applicable for CGH (comparative genome hybridization) experiments. The *V. salmonicida* AROS V1.0 contains 4,231 probes including 240 Positive Stringency control oligos. Additionally, 45 Production control oligos used by Operon for internal checks and also useful to researchers for plate layout checks are included free of charge. These AROS control probes supplement with the set to facilitate experimental validation and quality control.

Release date: June, 2007

### **I. The sequence sources**

*Vibrio salmonicida* genome sequence and the associated information are taken from the *S. salmonicida* sequencing collaboration between Dr. Willassen and the Sanger Institute. Additional information on annotation can be found through ([http://www.sanger.ac.uk/Projects/V\\_salmonicida/](http://www.sanger.ac.uk/Projects/V_salmonicida/)).

### **II. Design criteria and selection rules:**

The following selection rules were executed in the design.

- 1) Target length of oligo probe sequences was 70 bases. Exceptions for genes and regions without qualified oligo candidates at this length are routinely made in AROS

probe sets in order to maintain tight control of hybridization characteristics to allow for highly stringent experimental conditions. These exceptions are based on the inherent characteristics of the *C. familiaris* genome and not a limitation of the design algorithm.

- 2) The melting temperatures ( $T_m$ ) of  $66 \pm 5^\circ\text{C}$  was used for first round probe candidate screening.  $T_m$  was calculated using the nearest neighbor algorithm.
- 3) The oligo candidates were screened against the low complexity of sequences: (a) The contiguous single nucleotide base repeat or poly (N) tract ( $\leq 8$  bases); (b) the pre-set normalized simple repeat score.
- 4) The oligo candidates were selected against the potential hairpin structure with stem length of over 9 bases.
- 5) The oligo candidates were screened against the cross-hybridization of the non-self transcripts. The non-self transcript is the transcript not represented by a specific oligo. The selection criteria were used in the selection: (a) the cross-hybridization identity score  $\leq 70\%$ ; (b) contiguous match length to any non-self transcript  $\leq 20$  bases.
- 6) Limited exceptions were made if no qualified oligos were available from the selection. Information covering these exceptions is detailed in the *V. salmonicida* AROS V1.0 genelist.

### **III. Annotation notes:**

Oligos were identified by using NCBI BLAST and the Sanger BLAST utility for *Vibrio salmonicida* to map each oligo to the sequences in the databases described above. Additional information was provided by Dr. Willassen. Identifiers are retained, added, or deleted according to the BLAST score (score  $>97\%$  identity).

The oligos are also mapped with BLAST to the genome represented by the reference chromosome sequences to determine their potential for comparative genome hybridization (CGH) experiments. An oligo that is unique is indicated as 'Predicted\_CGH\_Oligo' and an oligo whose maximum cross-hybridization match is less than 85% identical is indicated by 'Predicted\_CGH\_Oligo\_Potential'. The maximum cross-hybridization score is provided.

### **IV. Characteristics of *Vibrio salmonicida* AROS V1.0 Control Probes**

- 1) Control Oligos:
  - Negative Control Oligo Probes: Randomly-generated oligo sequences which were selected after filtering against the transcripts. These oligos are also used in the *E. coli* V2.0 control plate.
  - Positive Control Oligo Probes: oligo probes were selected from this AROS as the positive controls including both known and predicted transcripts. They and the corresponding Hybridization Stringency Control Oligos (see below) are randomly scattered throughout the AROS set to be used to check a variety of potential issues including wash stringency and variation of hybridization stringency in different areas of the array.

- Hybridization Stringency Control Oligos: Generated based on several positive control oligo sequences with sequence homology of 50% 60% 70%, 80%, 90% and antisense identity to the original oligo probe sequences. They're intended as the standards to calibrate the microarray hybridization conditions and stringency.
- Stratagene SpotReport™ Alien™ Spike Control Oligos: Licensed from Stratagene ([www.stratagene.com](http://www.stratagene.com)). Coupled with the Alien mRNA spikes in Stratagene SpotReport Alien Oligo Array Validation System, they are intended as the internal controls for the normalization and standardization of dye incorporation, microarray hybridization and data analyses.
- Production Tracking Oligo opHsV04NC000001: It is a randomly-generated oligo sequence with a length of 30 bases. The tracking oligo is randomly positioned in the 384-well and 96-well plates so that each 384-well plate has four, and each 96-well plate has one for the purpose of quality control in production. The CyDye labelled antisense probe for this oligo can be used in a QC assay to ensure the correct placement/order of the probe printing plates on the spotting robot.