



Operon Biotechnologies, Inc.
2211 Seminole Drive
Huntsville, AL 35805
www.operon.com

***Helicobacter pylori* Genome Array Ready Oligo Set™ (Version 1.0)**

Helicobacter pylori Genome AROS (version 1.0) was designed based on *H. pylori* 26695, a 1,667,867 bp circular DNA genome. Other synonyms for this helicobacter strain are *Helicobacter pylori* ATCC700392, *Helicobacter pylori* KE26695, and *Helicobacter pylori* (strain 26695).

H. pylori AROS V1.0 consists of 1706 non-redundant oligonucleotide probes representing the gene sequences annotated for this genome. The *H. pylori* AROS is supplemented with a set of positive controls, randomly-generated negative controls, stringency controls, SpotReport™ Alien™ spike controls and a tracking control for experimental validation and quality control. The total number of oligos included in the set is 1903.

Release: December, 2006

I. Sequence source

Helicobacter pylori 26695 genome sequence (Accession = AE000511.1 GI:6626253) and its associated annotations available through NCBI (<http://www.ncbi.nlm.nih.gov>)

II. Specifications and characteristics of *M. pneumoniae* AROS (version 1.0)

This AROS encompasses the annotated gene sequences of *H. pylori* 26695.

- 1) Gene-specific oligos: 1706 oligos cover all gene-specific sequences. Fifty *H. pylori* AROS oligos chosen at random are repeated within the set to increase experimental verification.
- 2) Control oligos: 203 total.
 - Positive control oligos: Oligo probe sequences expected to be expressed at a variety of levels were selected for inclusion within the Control oligos portion of the AROS (plate Hepy5). Fifty *H. pylori* AROS oligos chosen at random are repeated on plate Hepy5 of this AROS set for use in experimental verification.
 - Negative control oligos: Randomly-generated oligo sequences, and were selected after filtering against the transcripts and intergenic sequences for non cross-hybridization property.
 - Stratagene SpotReport™ Alien™ spike control oligos which are licensed by Operon from Stratagene (www.stratagene.com). By using the associated 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.

- Hybridization stringency control oligo sequences were generated based on the Positive Control Oligos and the Stratagene SpotReport Alien spike control oligo sequences with sequence homology of 50%, 60%, 70%, 80%, 90% identity to the original oligo sequences. An additional antisense control is also included.
- Production tracking oligo (opHsV04NC000001) is a randomly-generated oligo sequence with a length of 30 bases. It was filtered against the cross-hybridizations with the gene sequences used in the AROS design. The tracking oligo is randomly positioned every 96 wells in the 384-well plate so that each 384-well plate has four and provides a unique hybridization signature for each 384-well plate of the AROS for identification purposes.

Figure 1. Melting Temperature

Average T_m = 73.3 °C

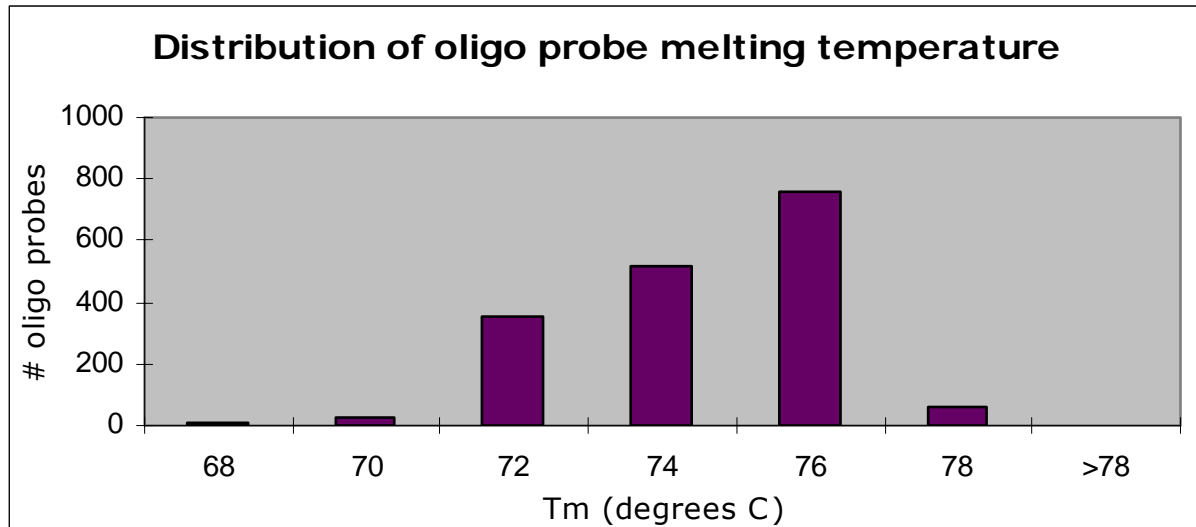


Figure 2. Distribution of GC content within *H. pylori* AROS V1.0

Average G/C content = 39.2%

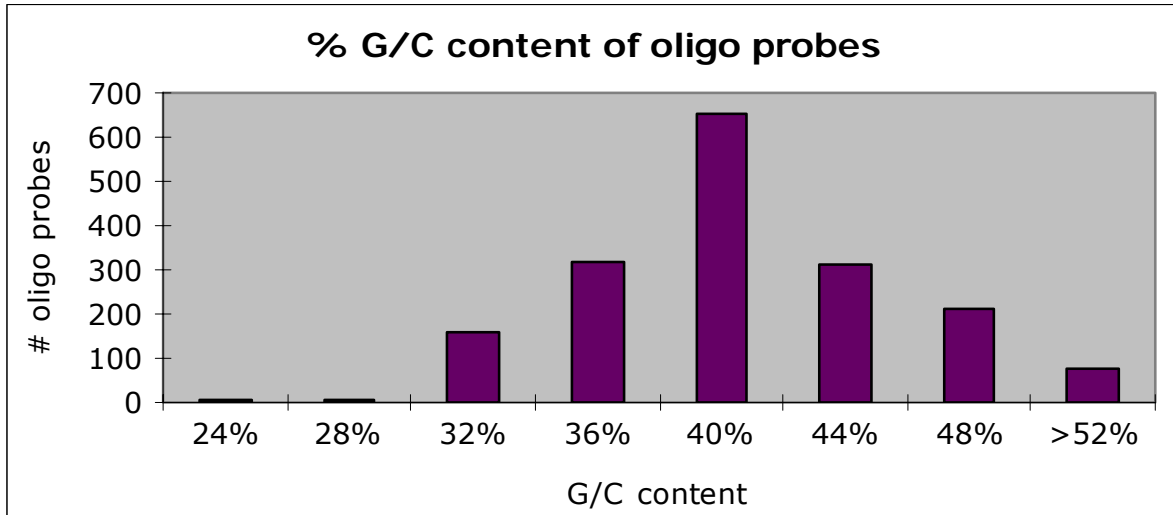


Figure 3. Distribution of potential hairpin stem lengths

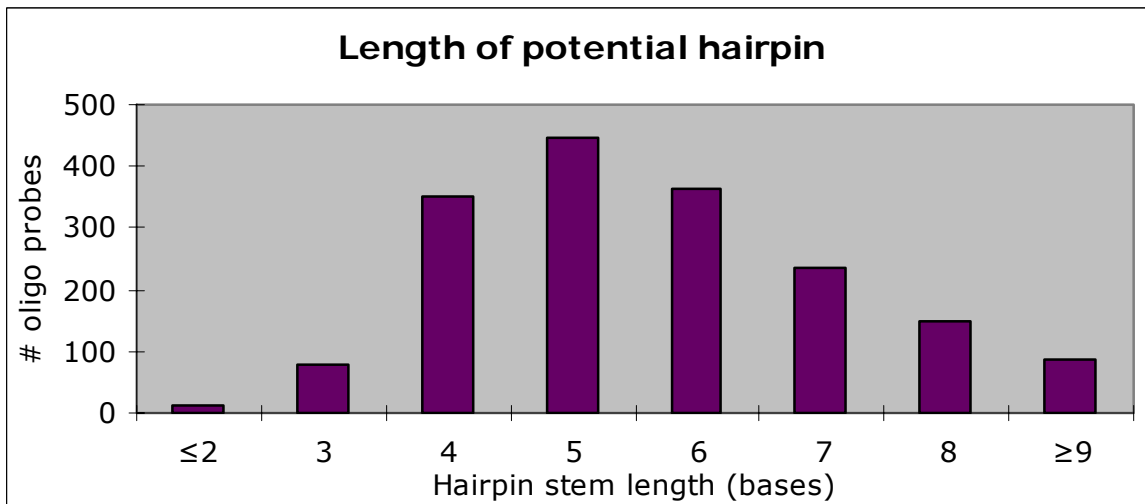
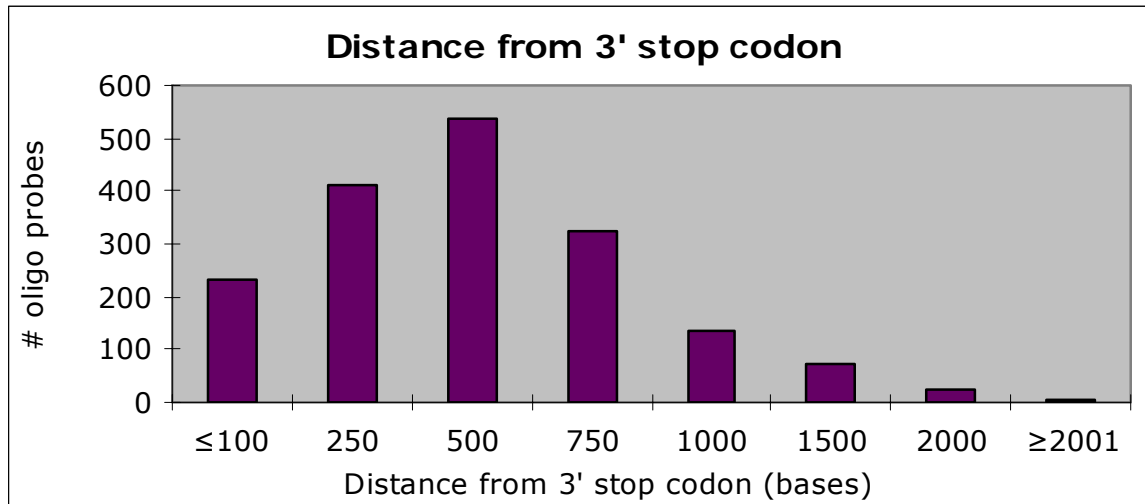


Figure 4. Distribution of probe distance from 3' end of transcript

Average = 424 bases



III. Design criteria and selection rules:

- 1) Target length of oligo probe sequences was set at 70 bases with the exceptions for genes without qualified oligo candidates.
- 2) The melting temperatures (T_m) of $73 \pm 5^\circ\text{C}$ was used for oligo screening. T_m was calculated according to the nearest neighbor program developed by Le Novère (*Bioinformatics*, **17**: 1226-7.)
- 3) The oligo candidates were screened for low complexity of sequences
 - a. Contiguous single nucleotide base repeat or poly (N) tract (≤ 8 bases);
 - b. Pre-set normalized simple repeat score.
- 4) Oligo probe candidates were selected for potential hairpin structure with stem length less than 9 bases wherever possible.
- 5) Oligo probe candidates were screened for the cross-hybridization of the non-self transcripts. Non-self transcript is any transcript not represented by this specific oligo.
 - a. Cross-hybridization identity score $\leq 70\%$
 - b. Contiguous match length to any non-self transcript ≤ 20 bases.
- 6) Exceptions were made if no qualified oligos were available to meet all selection criteria.