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***Mycoplasma pneumoniae* Genome Array Ready Oligo Set™ (Version 1.0)**

Mycoplasma pneumoniae Genome AROS (version 1.0) was designed based on *M. pneumoniae* strain M129 (ATCC 29342) which consists of 816394 bp DNA on a circular genome. *M. pneumoniae* AROS, consisting of 741 oligonucleotide probes, represent all gene sequences. *M. pneumoniae* AROS is supplemented with a set of positive controls, randomly-generated negative controls, stringency controls, SpotReport™ Alien™ spike controls and tracking control for experimental validation and quality control.

Release: December, 2006

I. Sequence source

M. pneumoniae strain M129 genome sequence (Accession = U00089 gi:26117688) 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 *M. pneumoniae* strain M129.

- 1) Gene-specific oligos: 741 oligos cover all gene-specific sequences.
- 2) Control oligos: 119 total.
 - 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 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 oligos were generated based on the Stratagene SpotReport Alien 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: It 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 in the 384-well and 96-well plates so that each 384-well plate has four, and each 96-well plate and provides a unique hybridization signature for each 384-well plate of the AROS for identification purposes.

Figure 1. Melting Temperature

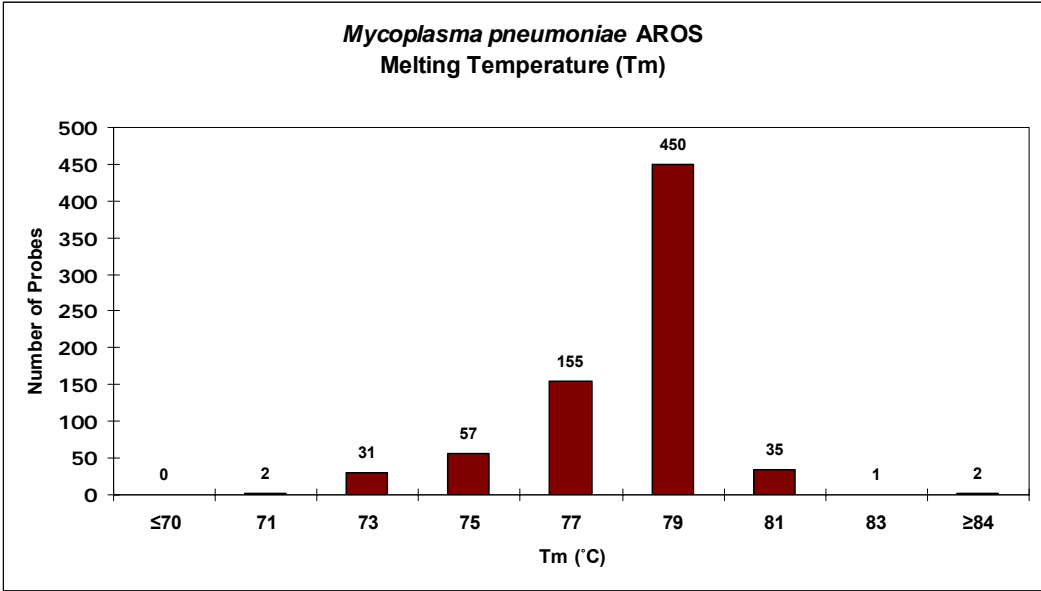


Figure 2. GC content

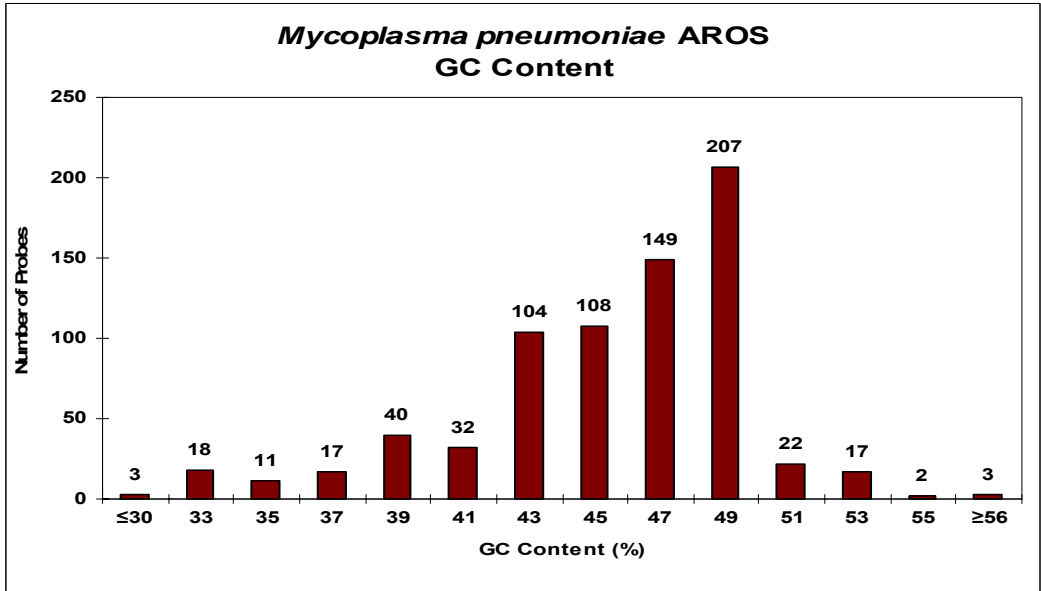


Figure 3. Distribution of potential hairpin stem lengths

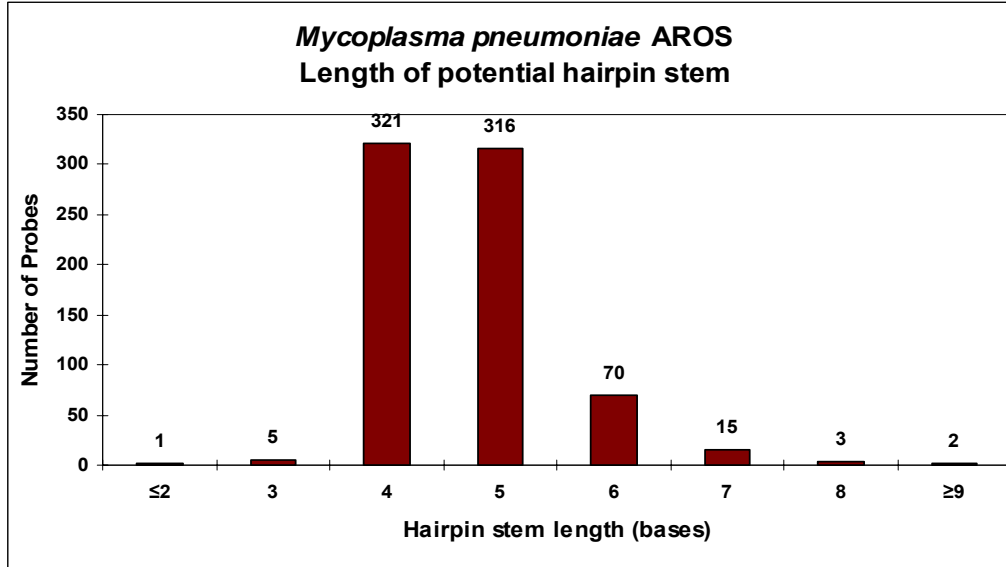
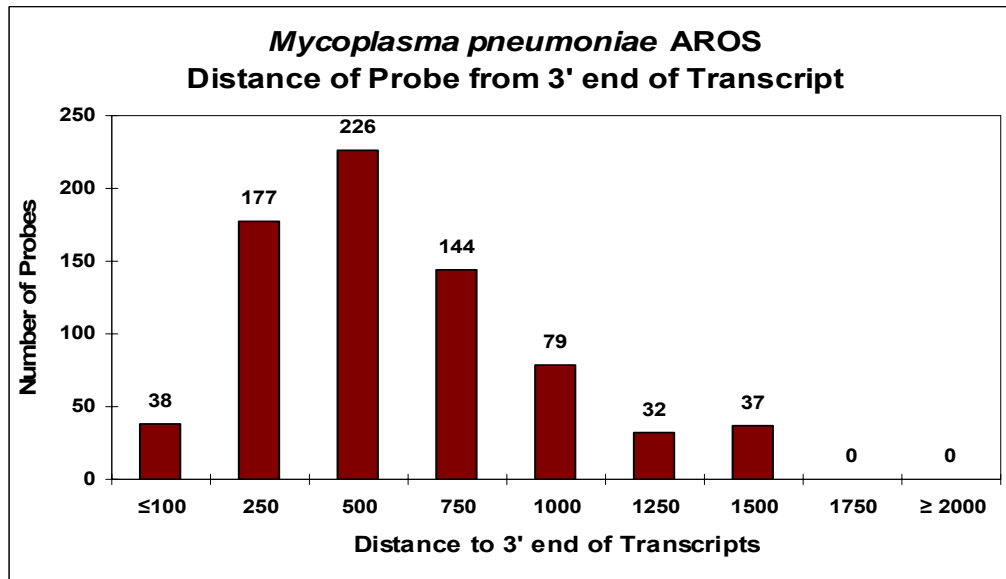


Figure 4. Distribution of probe distance from 3' end of transcript



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 $77 \pm 5^\circ\text{C}$ 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 pre-selected to avoid both 5'- and 3'-end of transcripts as well as a maximal distance of less than 1200 bases from the 3'-end.
- 4) The oligo candidates were screened against the low complexity of sequences
 - a. Contiguous single nucleotide base repeat or poly (N) tract (≤ 8 bases);
 - b. Pre-set normalized simple repeat score.
- 5) Oligo probe candidates were selected against the potential hairpin structure with stem length of over 9 bases.
- 6) Oligo probe candidates were screened against 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.
- 7) Exceptions were made if no qualified oligos were available to meet all selection criteria.