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Operon *Campylobacter coli* Genome Array Ready Oligo Set™ (Version 1.0)

The *Campylobacter coli* RM2228 Genome Array Ready Oligo Set™ (AROS, version 1.0) is currently available to the user community for microarray printing. It was created by the Operon scientists using the proprietary oligonucleotide probe design platform. The design was realized through a uniformed framework for multi-species/strain AROS, which adopts the design principles and selection criteria of *C. jejuni* subsp. *jejuni* AROS (version 1.0) as the base for the related species and strains. The AROS is composed of a main probe set (2,074 probes) and a control probe set (136 probes). The details of the main set are illustrated in the accompanied genelist. The control probes include positive controls, negative controls, stringency controls, randomly-generated negative controls, alien spike controls and tracking oligo.

Release date: February, 2006

I. The sequence sources

The following assembled contigs, genomes and plasmids from Genbank were the primary sources to obtain the gene sequences and annotations for probe design. The sequences and their annotations are accessible at the National Center for Biotechnology Information (www.ncbi.nih.gov).

NZ_AAFL01000001 (GI:57167558)
NZ_AAFL01000002 (GI:57167936)
NZ_AAFL01000003 (GI:57168306)
NZ_AAFL01000004 (GI:57168498)
NZ_AAFL01000005 (GI:57168607)
NZ_AAFL01000006 (GI:57168720)
NZ_AAFL01000007 (GI:57168839)
NZ_AAFL01000008 (GI:57168929)
NZ_AAFL01000009 (GI:57169007)
NZ_AAFL01000010 (GI:57169050)
NZ_AAFL01000011 (GI:57504571)
NZ_AAFL01000012 (GI:57504644)
NZ_AAFL01000013 (GI:57504720)

NZ_AAFL01000014 (GI:57504776)
NZ_AAFL01000015 (GI:57504869)
NZ_AAFL01000016 (GI:57504930)
NZ_AAFL01000017 (GI:57504961)
NZ_AAFL01000018 (GI:57504992)
NZ_AAFL01000019 (GI:57505023)
NZ_AAFL01000020 (GI:57505041)
NZ_AAFL01000021 (GI:57505056)
NZ_AAFL01000022 (GI:57505082)
NZ_AAFL01000023 (GI:57505098)
NZ_AAFL01000024 (GI:57505119)
NZ_AAFL01000025 (GI:57505129)
NZ_AAFL01000026 (GI:57505136)
NZ_AAFL01000027 (GI:57505144)
NZ_AAFL01000028 (GI:57505154)
NZ_AAFL01000029 (GI:57505160)
NZ_AAFL01000030 (GI:57505168)
NZ_AAFL01000031 (GI:57505174)
NZ_AAFL01000032 (GI:57505177)
NZ_AAFL01000033 (GI:57505180)
NZ_AAFL01000034 (GI:57505184)
NZ_AAFL01000035 (GI:57505188)
NZ_AAFL01000036 (GI:57505191)
NZ_AAFL01000037 (GI:57505193)
NZ_AAFL01000038 (GI:57505196)
NC_006134 (GI:51209432)
NC_007142 (GI:68164390)
NC_007143 (GI:68235689)
X82079 (GI:557228)
NC_000913 (GI:49175990)
NC_003197 (GI:16763390)

II. The design and selection criteria:

Once a gene or ORF sequence was included in the dataset for probe design, a pool of 70-mer candidates were generated. From which a final probe was selected with an optimal set of selection parameters described below.

- 1) The probe length was set at 70 bases.
- 2) Probes were restricted within the range of $71 \pm 5^{\circ}\text{C}$ for melting temperature (T_m). T_m was calculated with the formula: $T_m = 81.5 + 16.6 * \log[\text{Na}^+] + 41 * (\#G + \#C)/\text{length} - 500/\text{length}$, where $[\text{Na}^+] = 0.1 \text{ M}$ and $\text{length} = \#A + \#C + \#G + \#T$.

- 3) Probes were filtered for a preset distances from both 5'- and 3'-ends of ORFs for random priming.
- 4) Contiguous single nucleotide base repeat or poly (N) tract within probe was limited to 8 bases or less.
- 5) Potential hairpin stem length was constrained at 9 bases or shorter.
- 6) Probes were screened against a preset normalized score of simple repeats.
- 7) Cross-hybridization to other non-self transcripts was less than 70% identity.
- 8) Contiguous match to non-self transcripts was no greater than 20 bases.

Summary Table of Design and Selection Criteria

Probe selection criteria	Value
Probe length (bases)	70
Melting temperature (°C)	71 ± 5
Distal distance from 3' end (bases)	>110
Poly(N) tract length (bases)	≤8
Stem length in potential hairpin (bases)	≤9
Contiguous match to non-self transcripts (bases)	≤20
Cross-hybridization identity to all other genes	≤70%

III. The specifications and characteristics of *Campylobacter coli* AROS (version 1.0)

The two major categories of the *C. coli* AROS V1.0 are described below.

- 1) Main oligos: 2,074 oligos covers the full ORF set, RNA genes and pseudogenes within the *C. coli* RM2228 genome.
- 2) Control oligos:
 - Negative/cross-hybridization control oligos: 14 of them were selected from the K12 strain of Operon *Escherichia E. coli* AROS V2.0. 15 from LT2 strain of Operon *Salmonella S. typhimurium* AROS 1.0. The detailed gene annotations are tabulated in the accompanied genelist. In addition, they were analyzed against the transcript sequences of *C. coli* RM2228 genome for non cross-hybridization property.

- Positive control oligos: 96 oligos from this *C. coli* AROS set were selected as the positive controls. Most of them represent the genes for various biochemical pathways and so should be stably expressed. The gene information of the selected oligos is described in the genelist.
- Hybridization stringency control oligos: They were generated based on Stratagene alien oligos and the 10 selected oligos from the main AROS set. The selected oligos are opCcV0100000243, opCcV0100000560, opCcV0100001111, opCcV0100001200, opCcV0100001264, opCcV0100001292, opCcV0100001415, opCcV0100001679, opCcV0100001806, opCcV0100002092, representing the genes for rpoA (DNA-directed RNA polymerase, alpha subunit), ftsA (cell division protein FtsA), gap (glyceraldehyde-3-phosphate dehydrogenase), accA (acetyl-CoA carboxylase), flaA (flagellin), topA (DNA topoisomerase I), 50S ribosomal protein L6, infB (translation initiation factor IF-2), dnaK (heat shock protein) and dnaA (chromosomal replication initiator protein) respectively. The oligo sequences were mutated randomly to generate the mis-matching stringency at the identity scores of 100%, 90%, 80%, 70%, 60%, 50%, anti-sense and complement only. They're intended to serve as the standards to calibrate the microarray hybridization conditions.
- 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 has one for quality assurance.
- Stratagene alien spike control oligos: They are licensed from Stratagene (www.stratagene.com). In coupling 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.

Caution: The T_m of Stratagene alien spike control oligos range from 78 to 81, which are different from those of the main AROS set (71 +/- 5).

The following figures illustrate characteristics of T_m, GC content, distances from 3' end, hairpin stem length, and cross-hybridization score of 2,074 probes in the *C. coli* AROS (version 1.0).

Figure 1. Tm

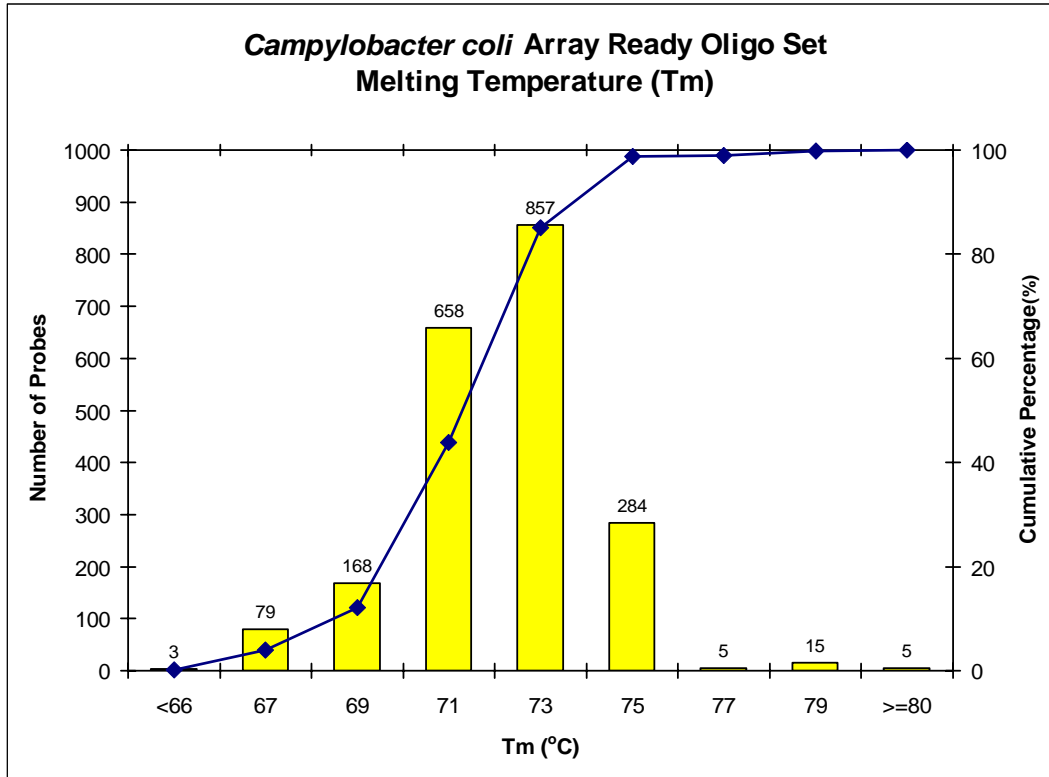


Figure 2. GC content

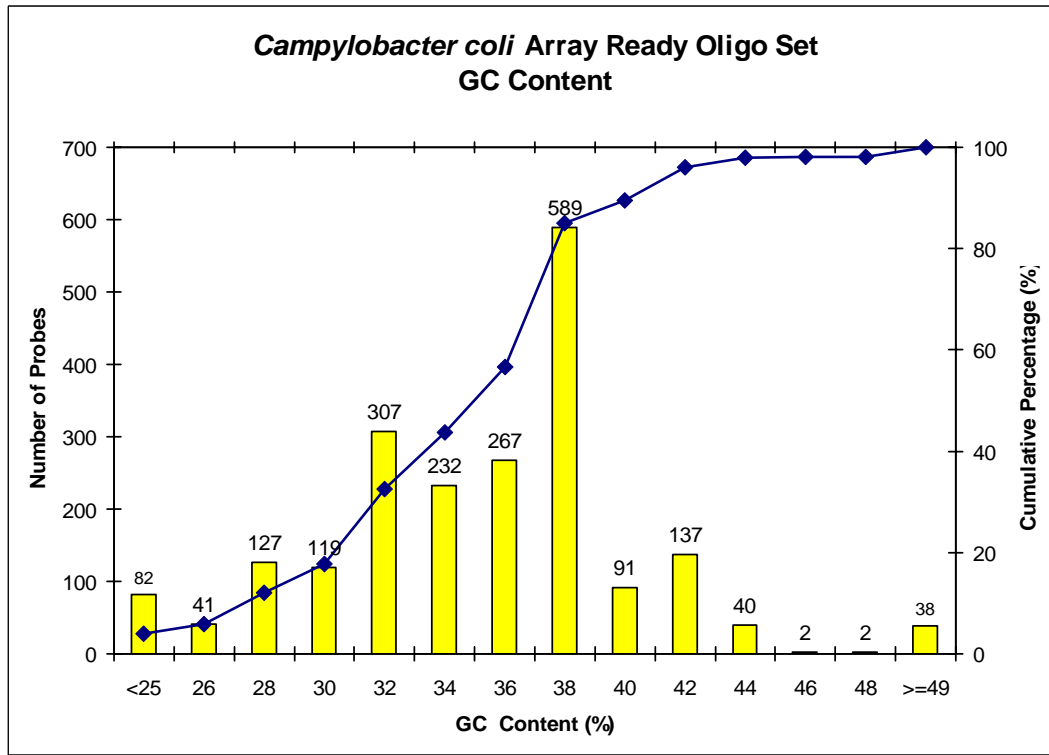


Figure 3. Distance to the 3'-end of transcripts

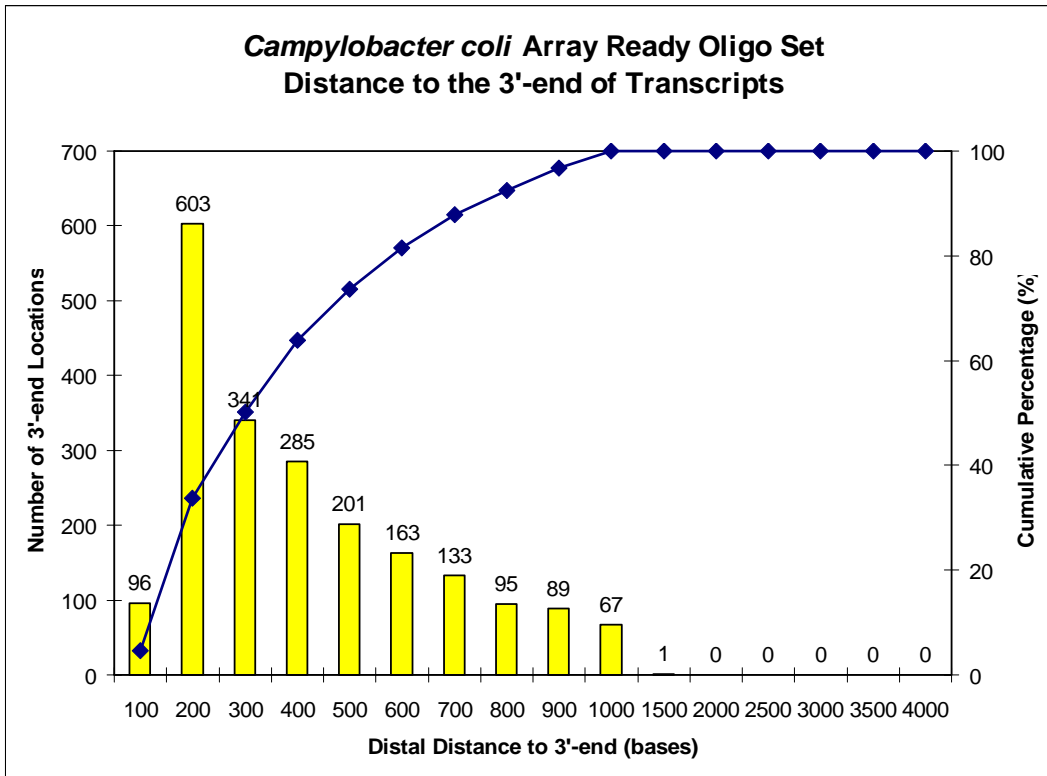


Figure 4. Hairpin stem length

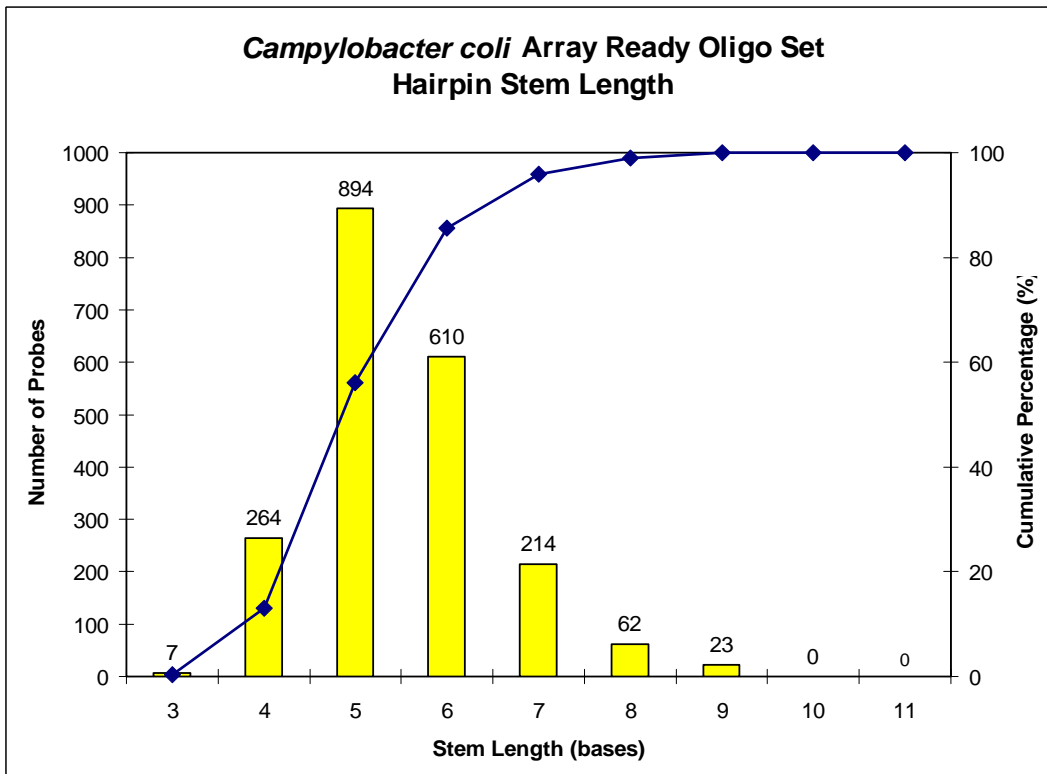


Figure 5. Cross-hybridization

