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Array-Ready Oligo Set™ for the *Campylobacter jejuni* Genome
Version 1.0

We are pleased to announce the release of our Array-Ready Oligo Set™ (AROS) for the *Campylobacter jejuni* genome containing 1601 70mer probes representing 1546 Open Reading Frames (ORFs) from *C. jejuni* subsp. *jejuni* NCTC 11168 (GenBank sequence AL111168); 51 ORFs from *C. jejuni* 81-176 virulence plasmid pVir (GenBank sequence AF226280); and 4 ORFs from *C. jejuni* plasmid pCJ01 (GenBank sequence AF301164). All 1601 probes are designed within predicted ORFs. For probe design we use state-of-the-art methodology and proprietary software. An amino linker is attached to the 5' end of each oligo.

ORF sequence source and selection

A total of 1601 probes in the *C. jejuni* Genome Oligo Set were designed from one strain and two plasmids. Each set of ORF sequences was obtained from the corresponding GenBank entries: AL111168, AF226280, and AF301164 . Complete *C. jejuni* genome GenBank entries were downloaded from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

Probe design and selection rules

Once an ORF has been selected to be included in the set, a probe is selected with an optimal set of parameters. Sufficient numbers of 70mer candidate probes for each ORF are selected using the following criteria for the *C. jejuni* Genome Oligo Set.

1) All oligos are within 71°C ± 5°C using the following formula:
T_m = 81.5 + 16.6 x log[Na+] + 41 x (#G + #C)/length – 500/length where [Na+] = 0.1 M and length = #A + #C + #G + #T

2) Each oligo is selected anywhere within the ORF but does not occur within 70 bases from the 3' end of the ORF. This design allows the researcher to use either random priming or ORF-specific priming. Location from the 3' end is defined as the coordinate of the first base on the 5' end of the oligo from the 3' end of the ORF. All coordinates start from 1.

3) An oligo cannot have a contiguous single nucleotide base repeat or poly (N) tract longer than 8 bases.

4) An oligo cannot have a potential hairpin structure with a stem length longer than 8 bases.

5) A normalized score is assigned to each oligo based on the number of repeats. Oligos with more repeats having a normalized score greater than a certain threshold are filtered out.

6) Using the Basic Local Alignment Search Tool (BLAST), each oligo was aligned against all ORFs represented *C. jejuni* genome and two plasmids. This BLAST percent identity is also referred to as cross-hybridization identity of the non-self gene. Each oligo selected has less than or equal to 70% identity to all other ORFs within the genome or plasmid. BLAST alignment results were used for final selection of unique oligos within the genome or plasmid. The highest scoring non-self gene is defined as the sequence that yields the most matched bases in an alignment. This cross-hybridization percent identity score is dependent on the size of the sequence database used to BLAST against, oligo sequence, and use of no-gap alignment method.

7) Each oligo cannot have greater than 20 contiguous bases common to any nonself ORF.

Once oligo candidates have been selected satisfying all the selection rules mentioned above, each oligo is ranked based on BLAST percent identity as computed in Step 6. One final oligo for each gene is selected with the minimum cross-hybridization identity.

Oligo Selection Criteria	Value	Number of oligos in genome set satisfying these criteria (out of 1601)
Length	70mer	1598
Melting temperature	71°C ± 5°C	
Location from 3' end	› 70	
Poly(N) tract length	≤ 8	
Stem length in potential hairpin	≤ 8	
Contiguous bases common to any non-self ORF	≤ 20	
Cross-hybridization identity to all other genes	≤ 70%	

By request, cross-hybridization identity score and other score values of each oligo will be provided to the customer.

The following illustrations show distribution of melting temperature (T_m), GC content, location from 3' end of the ORF, and length of longest stem for all 1601 oligos.

Figure 1. Melting Temperature

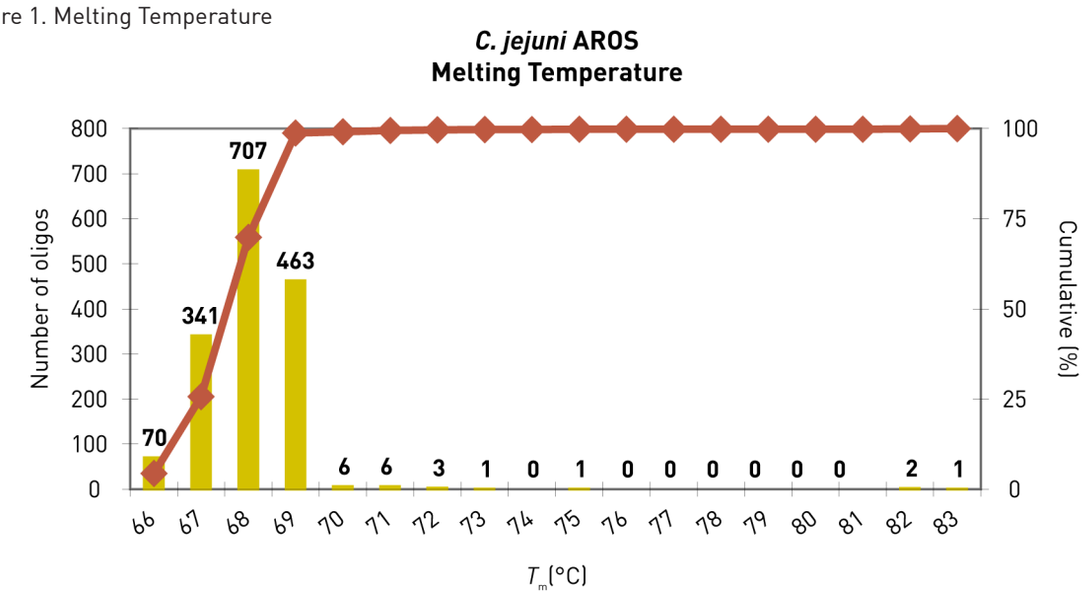


Figure 2. GC Content

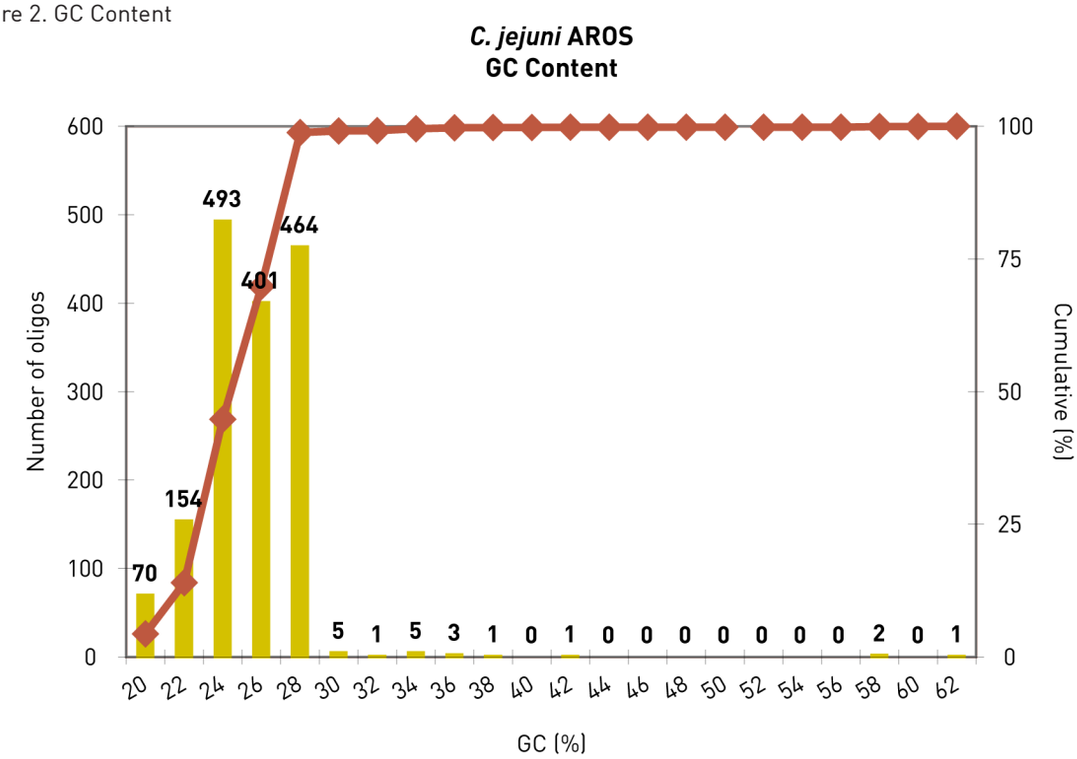


Figure 3. Location from 3' End

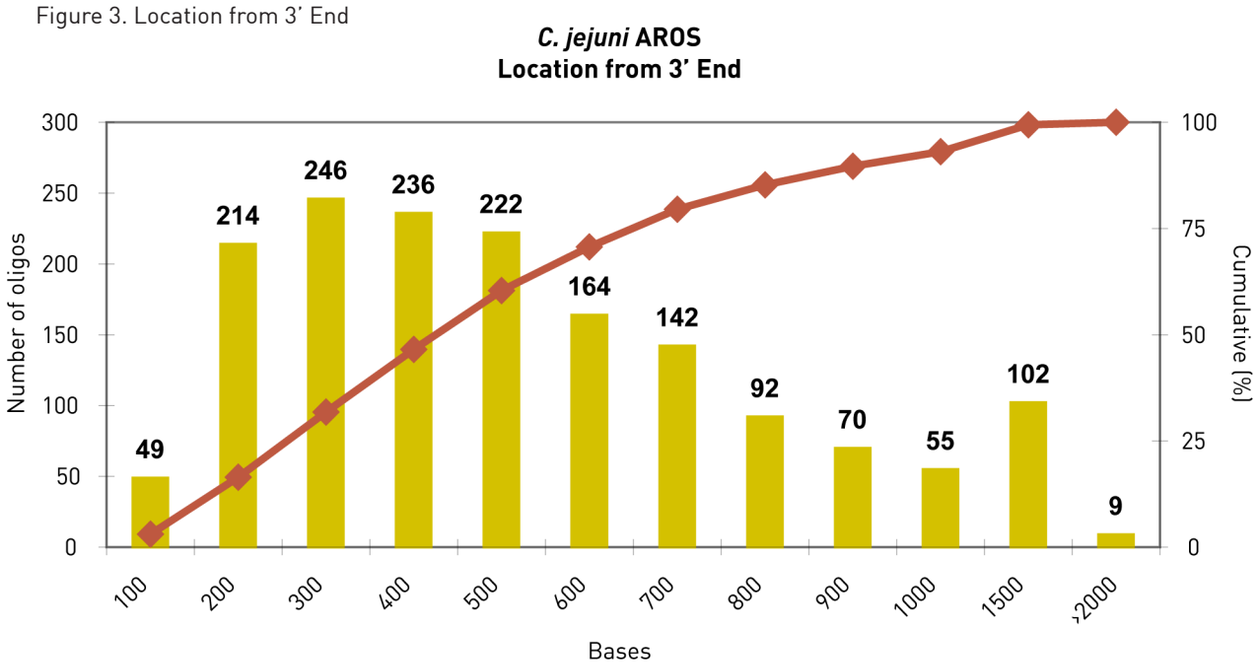


Figure 4. Length of the Longest Hairpin Stem

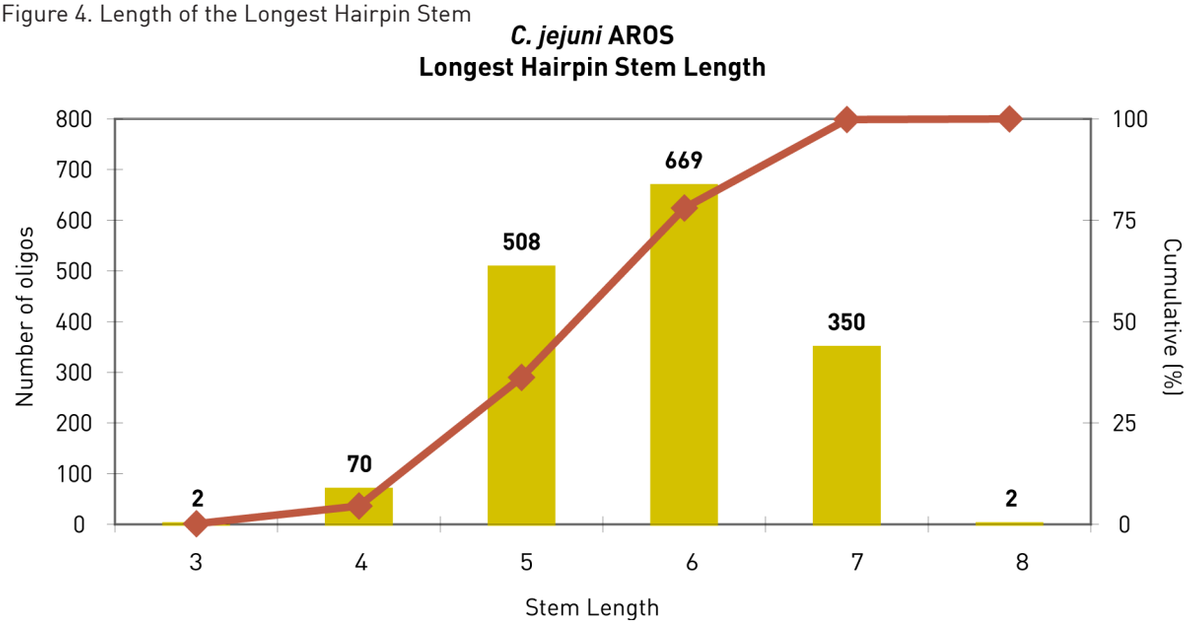
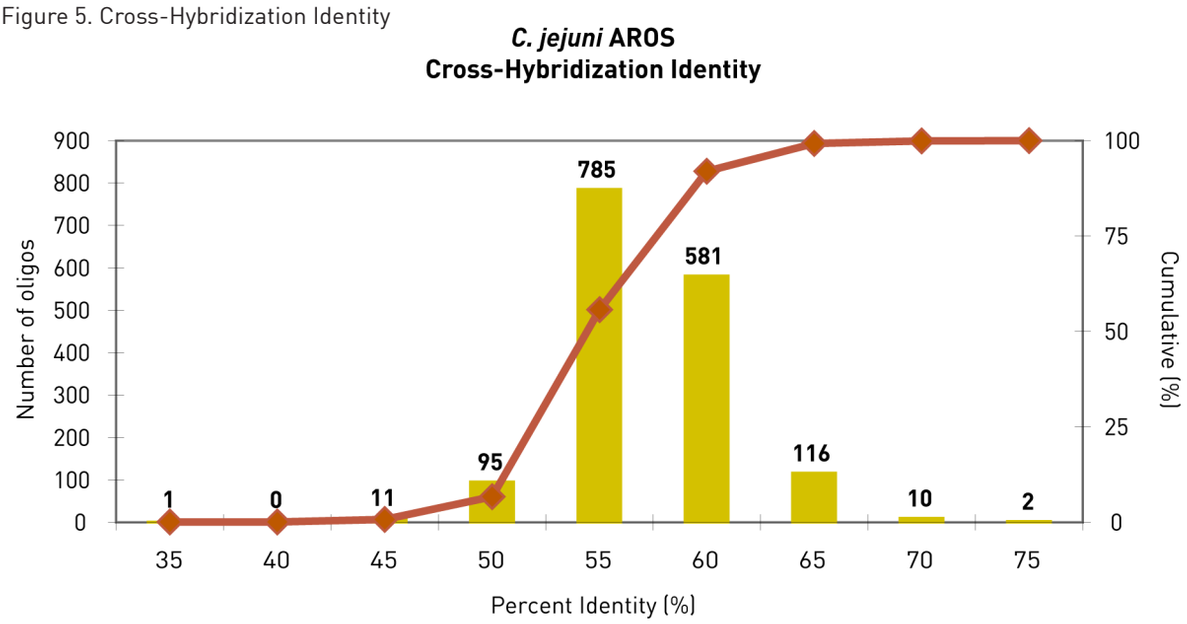


Figure 5. Cross-Hybridization Identity



Quality check of probe design specifications

Once the final oligo has been selected to represent a gene or ORF, each oligo undergoes design specifications quality control where we use an independent method to confirm that all oligos have met the specified design specifications. The table below summarizes data from our quality check for probe design specifications for all 1601 oligos in the set.

Probe design specification	Expected value	Verified range	Number of oligos
Melting temperature (°C)	71°C ± 5°C	66.0-76.0	1598
Poly(N) tract length	3-8	3-8	1601
Hairpin stem length (basepairs)	3-8	3-8	1601
Contiguous bases common to any non-self ORF	10-20	10-20	1601
Cross-hybridization identity (%)	34-70	34-70	1599