

Array-Ready Oligo Sets™ for the Anthrax Genome Version 1.0 and the Anthrax Genome Extension Version 1.0

We are pleased to announce Version 1.0 of our Anthrax Genome Oligo Set and our Anthrax Genome Oligo Extension Set. The Anthrax Genome Oligo Set contains 5309 arrayable 70mers representing 5311 open reading frames (ORFs) from *Bacillus anthracis* strain Ames, 4879 ORFs from *Bacillus anthracis* strain A2012, and 3645 ORFs from *Bacillus cereus* ATCC 14579. The Anthrax Genome Oligo Extension Set Version 1.0 contains 2478 arrayable 70mers representing 1587 ORFs from *Bacillus cereus* ATCC 14579, 663 ORFs from *Bacillus anthracis* strain A2012, 21 ORFs from *Bacillus cereus* ATCC 14579 plasmid *pBclin15*, 203 ORFs from *Bacillus anthracis* A2012 plasmid *pXO1*, 102 ORFs from *Bacillus anthracis* A2012 plasmid *pXO2*, 142 ORFs from *Bacillus anthracis* virulence plasmid *pXO1*, and 82 ORFs from *Bacillus anthracis* virulence plasmid *pXO2*. 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

Anthrax Array-Ready Oligo Set™

Primary source

The 5309 probes for the Anthrax Array-Ready Oligo Set (AROS) are designed from ORFs from the NCBI *refseq* NC_003997 for *Bacillus anthracis* strain Ames updated April 2003 (Source: <http://www.ncbi.nih.gov>).

Secondary source

A secondary set of ORFs was obtained from NCBI *refseq* NC_004722 for *Bacillus cereus* ATCC 14579 updated in June 2003, and NC_003995 for *Bacillus anthracis* strain A2012 updated in June 2003 (Source: <http://www.ncbi.nih.gov>).

ORF sequence selection

All oligos are directly designed from the primary source, *B. anthracis* Ames ORFs, but the probes are selected such that a large number of *B. cereus* ATCC 14579 and *B. anthracis* A2012 ORFs are also represented. A *B. cereus* or *B. anthracis* A2012 ORF is said to be

represented by an oligo if the oligo has greater than 93% identity over the length of the oligo to the *B. cereus* or *B. anthracis A2012* ORF.

The final set of 5309 oligos in this set also represents 3645 *B. cereus ATCC 14579* ORFs and 4879 *B. anthracis A2012* ORFs. The gene list contains information for oligos and their corresponding *B. anthracis Ames*, *B. anthracis A2012*, and *B. cereus* ORFs.

Table 1: Anthrax AROS

	<i>NCBI RefSeq</i>	Number of ORFs represented at > 93%	Number of ORFs represented at 100%
<i>Bacillus anthracis Ames</i>	<i>NC_003997</i>	5311	5311
<i>Bacillus anthracis A2012</i>	<i>NC_003995</i>	4879	4763
<i>Bacillus cereus ATCC 14579</i>	<i>NC_004722</i>	3645	233

Anthrax Extension AROS

The 2478 probes for the Anthrax Extension AROS are designed from ORFs from the sources in Table 2. ORFs that are not represented by an oligo at >93% identity over the oligo length in the Anthrax AROS are included in the extension. Most oligos are directly designed from one ORF. A small number of *A2012* ORFs are represented by *B. cereus* oligos that are >93% over the length of the oligo to the *A2012* ORF.

Table 2: Anthrax Extension AROS

Description	<i>NCBI RefSeq</i>	Number of ORFs represented > 93%	Number of ORFs represented at 100%
<i>Bacillus anthracis strain A2012</i>	<i>NC_003995</i>	663	529
<i>Bacillus cereus ATCC 14579</i>	<i>NC_004722</i>	1587	1587
<i>Bacillus cereus ATCC 14579 plasmid pBClin15</i>	NC_004271	21	21
<i>Bacillus anthracis strain A2012 plasmid pXO1</i>	NC_003980	203	203
<i>Bacillus anthracis strain A2012 plasmid pXO2</i>	NC_003981	102	102
<i>Bacillus anthracis virulence plasmid pXO1</i>	NC_001496	142	141
<i>Bacillus anthracis virulence plasmid pXO2</i>	NC_002146	82	80

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 Anthrax and Anthrax Extension AROS.

- 1) All oligos are within $75^{\circ}\text{C} \pm 5^{\circ}\text{C}$ using the following formula:
$$T_m = 81.5 + 16.6 \times \log[\text{Na}^+] + 41 \times (\#G + \#C)/\text{length} - 500/\text{length}$$
where $[\text{Na}^+] = 0.1 \text{ M}$ and $\text{length} = \#A + \#C + \#G + \#T$
- 2) An oligo cannot have a contiguous single nucleotide base repeat or poly (N) tract longer than 8 bases.
- 3) An oligo cannot have a potential hairpin structure with a stem length longer than 9 bases.
- 4) An oligo has less than or equal to 70% cross-hybridization identity to all other non-self ORFs. Non-self ORFs are all ORFs other than the ORF(s) that represents the oligo. For all oligos in the Anthrax AROS, using BLAST, each oligo is aligned against all 5311 *Ames* strain ORFs. Using the alignment with the candidate oligo versus the highest scoring non-self ORF, a cross-hybridization identity score is computed. The highest scoring non-self ORF is defined as the sequence that yields the most matched bases in an alignment.

In the Anthrax Extension AROS, oligos directly designed from *A2012* are aligned using BLAST against all *A2012* ORFs including plasmids to compute cross-hybridization identity. Oligos designed directly from *B. cereus* ORFs are aligned using BLAST against all *B. cereus* ORFs including plasmids to compute cross-hybridization identity. Oligos designed directly from the virulence plasmids ORFs are aligned using BLAST against all virulence plasmid ORFs plus all ORFs from *B. anthracis Ames* to compute cross-hybridization identity.

- 5) An oligo cannot have greater than 20 contiguous bases common to any other non-self ORF. Oligos are aligned using BLAST using a similar method as mentioned above for cross-hybridization identity.

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

Note: For 99 of the sequences in the Anthrax AROS and 96 of the sequences in the Anthrax Extension AROS that did not yield oligos satisfying all the above criteria, certain rules were relaxed.

SUMMARY

Oligo selection criteria	Criteria values	Number of oligos in the Anthrax Genome Oligo Set	Number of oligos in the Anthrax Genome Extension Set
Length Melting temperature Poly(N)tract length Stem length in potential hairpin Cross-hybridization identity to all other sequences [§] Contiguous base match to any other sequence [§]	70mer 75°C ±5°C <=8 <=9 <=70% <=20	5210	2382
Total number of oligos not satisfying one or more of the above criteria		99	96
Length	35 < X <=50	2*	6**
Melting temperature	66 < X < 70°C	29*	25**
Stem length in potential hairpin	9 < X <=11	5*	0**
Contiguous base match to any other sequence [§]	>20	24*	48**
Cross-hybridization to all other sequences [§]	>70%	63*	66**
Total		5309	2478

* Out of 99 probes.

** Out of 96 probes.

[§]Cross-hybridization identity and contiguous base match for oligos designed directly from *A2012* are aligned using BLAST against all *A2102* ORFs including plasmids. Oligos designed directly from *B. cereus* ORFs are aligned using BLAST against all *B. cereus* ORFs including plasmids. Oligos designed directly from the virulence plasmids ORFs are aligned using BLAST against all virulence plasmid ORFs plus all ORFs from *B. anthracis* Ames.

The following illustrations show the distribution of all 5309 oligos in the Anthrax AROS and 2478 oligos in the Anthrax Extension AROS for melting temperature, GC content, location from 3' end, longest stem length, and cross-hybridization identity.

Figure 1. Anthrax AROS: Melting Temperature

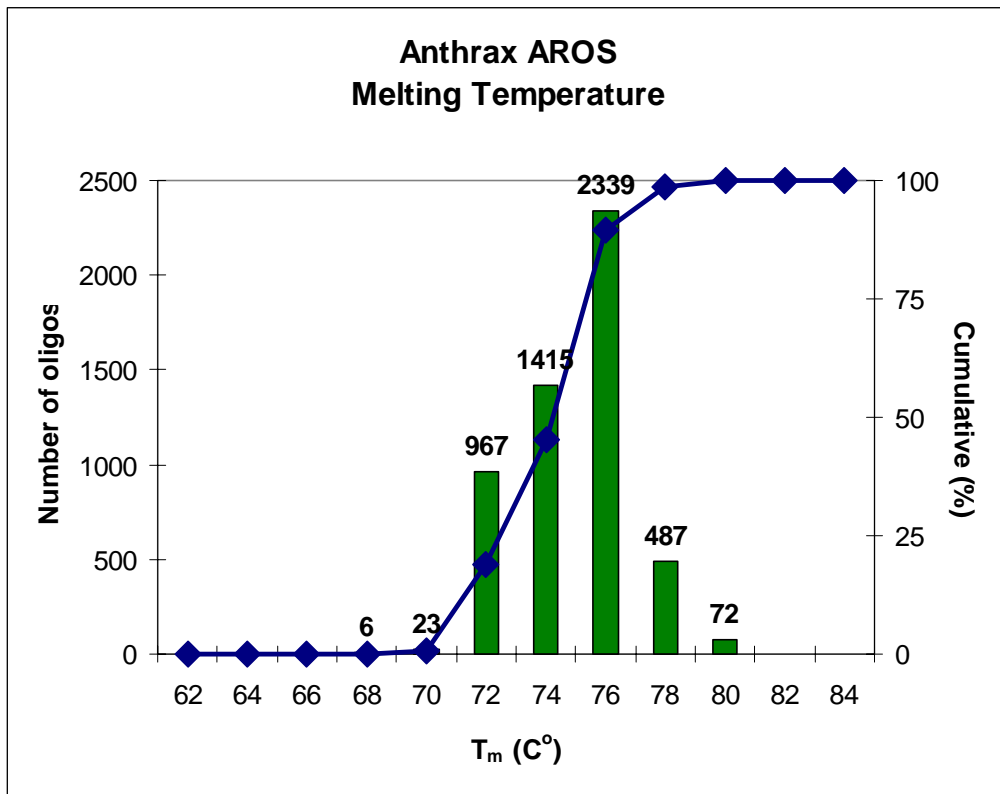


Figure 2. Anthrax AROS: GC Content

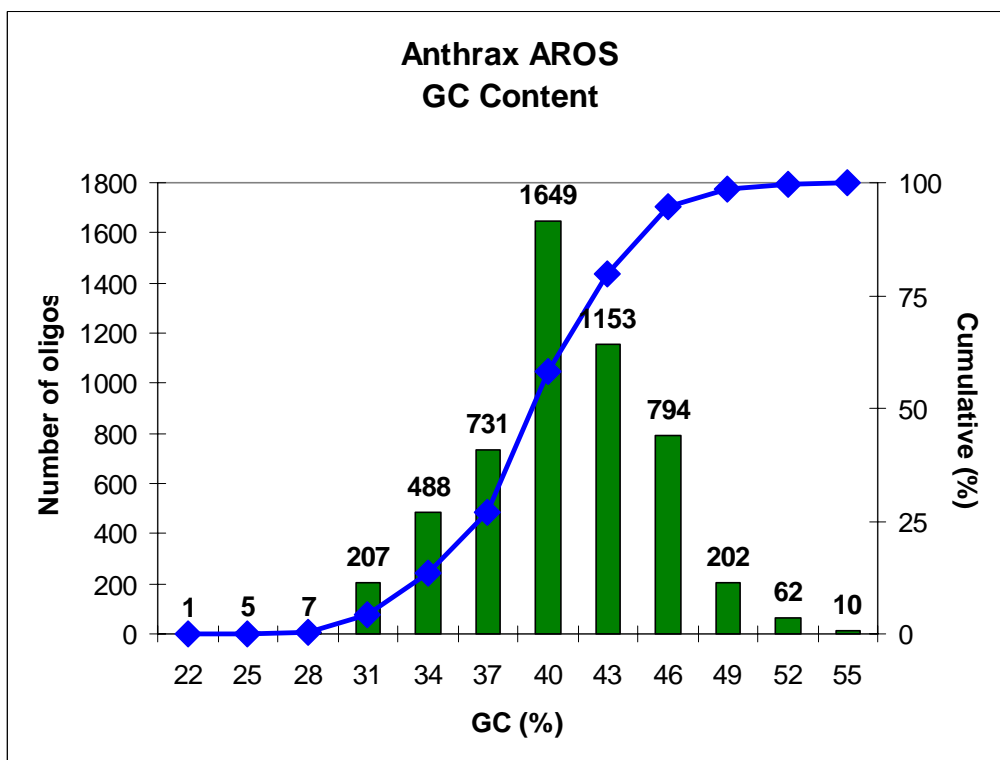


Figure 3. Anthrax AROS: Location from 3' End

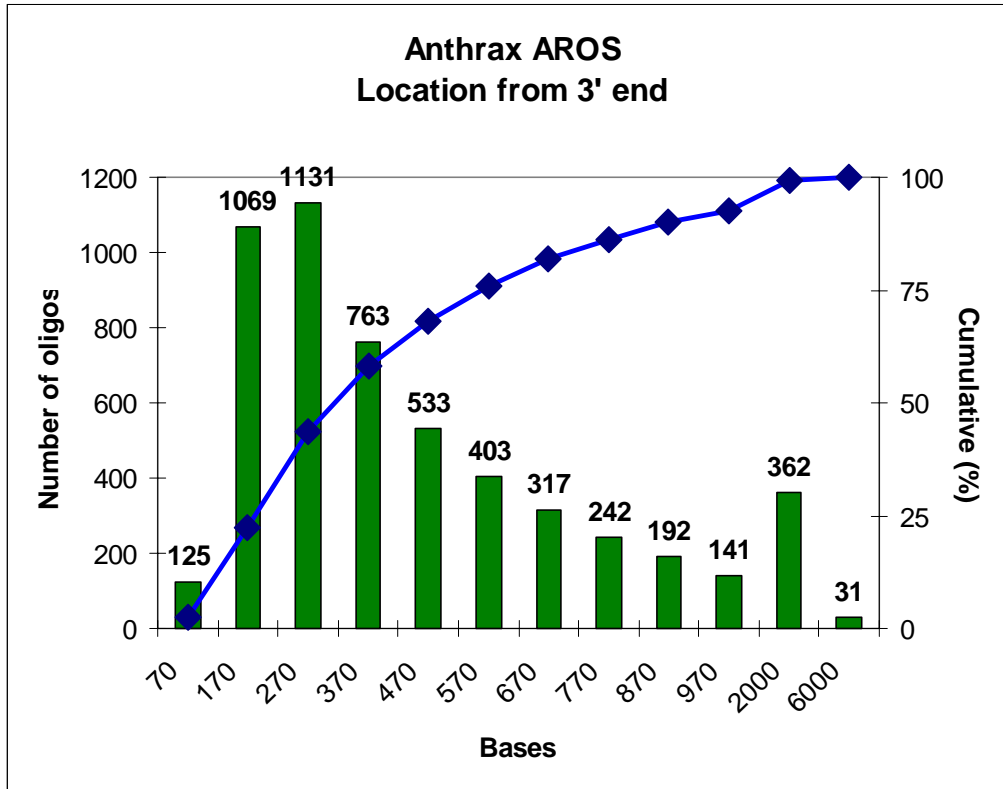


Figure 4. Anthrax AROS: Longest Hairpin Stem length

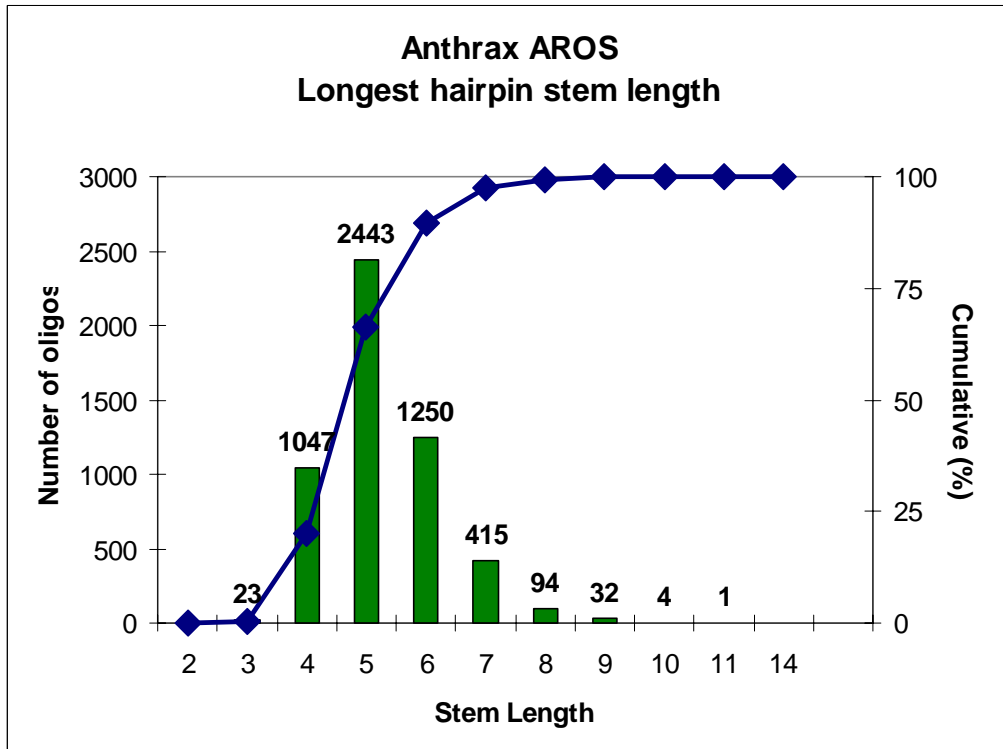


Figure 5. Anthrax AROS: Cross-Hybridization Identity

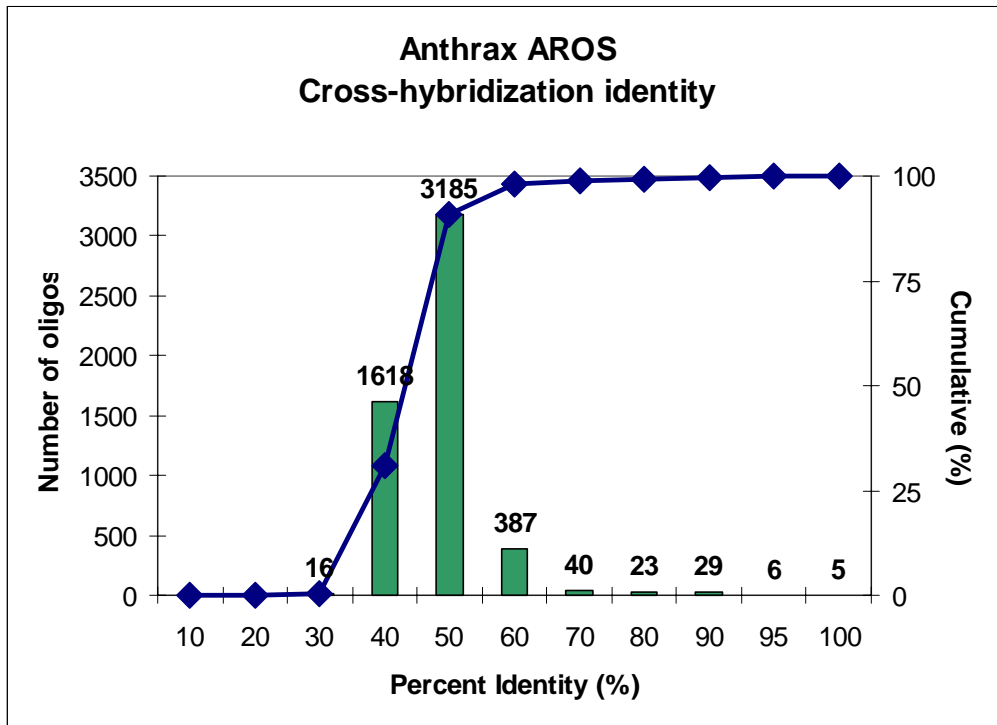


Figure 6. Anthrax Extension AROS: T_m

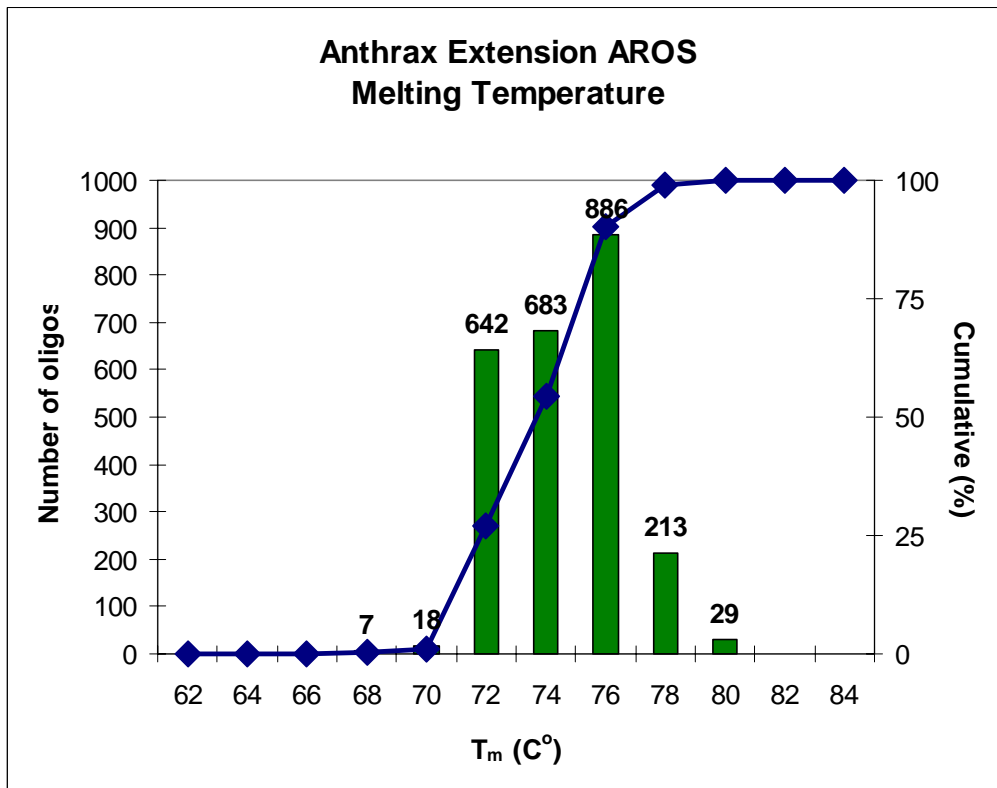


Figure 7. Anthrax Extension AROS: GC Content

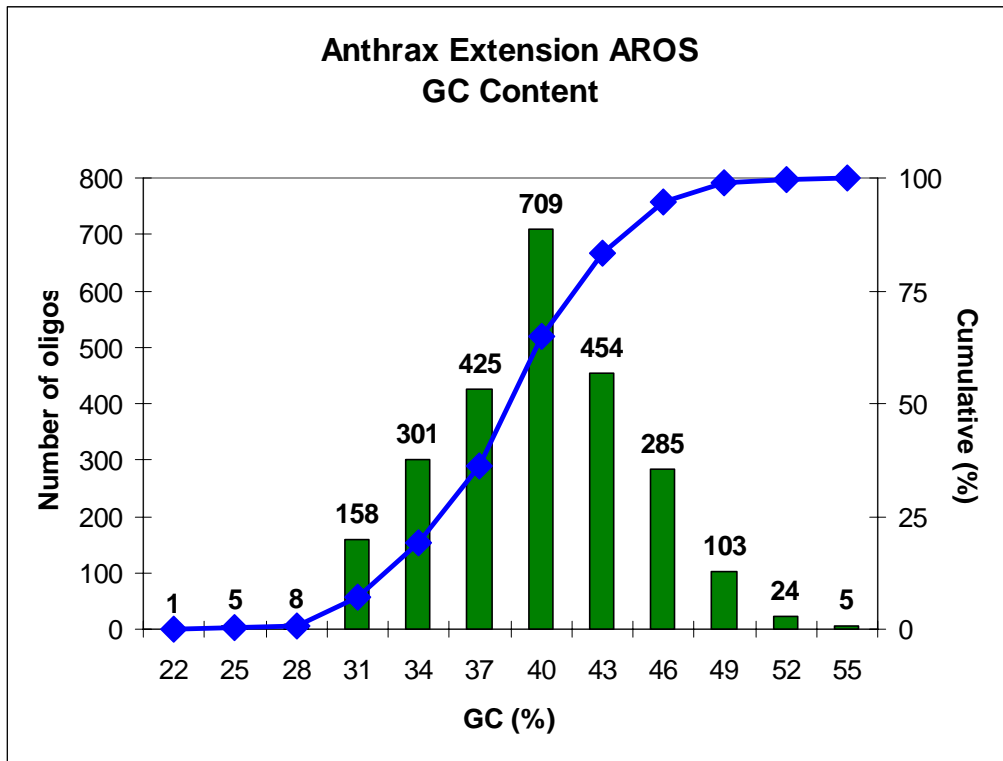


Figure 8. Anthrax Extension AROS: Location from 3' End

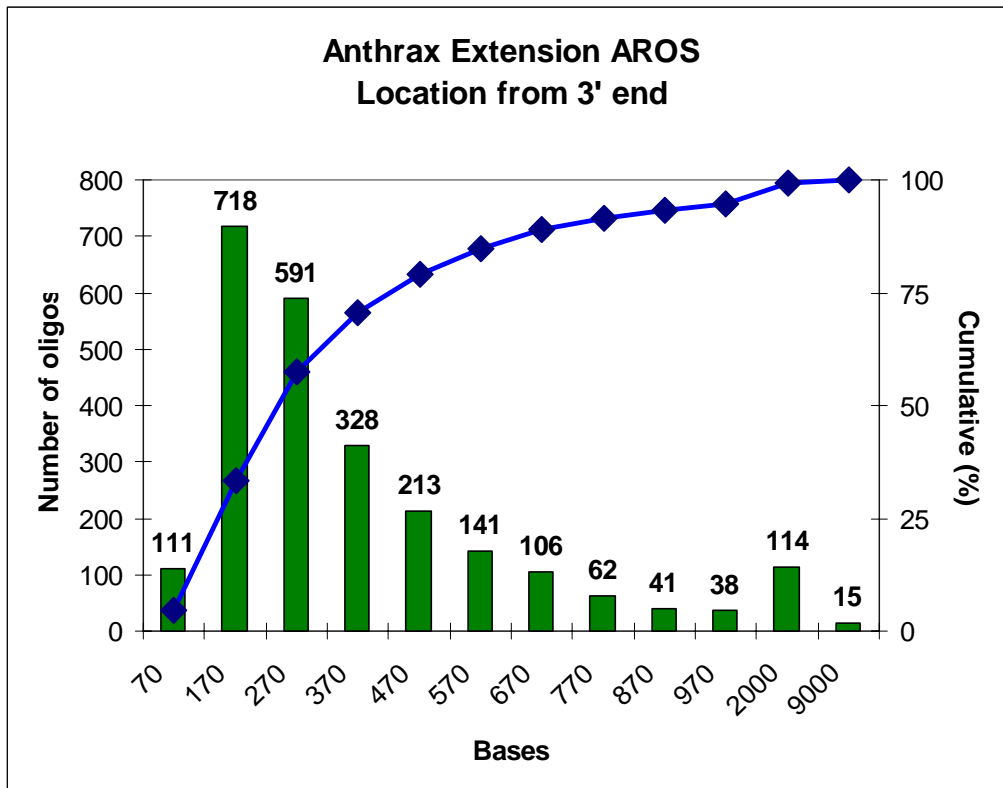


Figure 9. Anthrax Extension AROS: Longest Hairpin Stem length

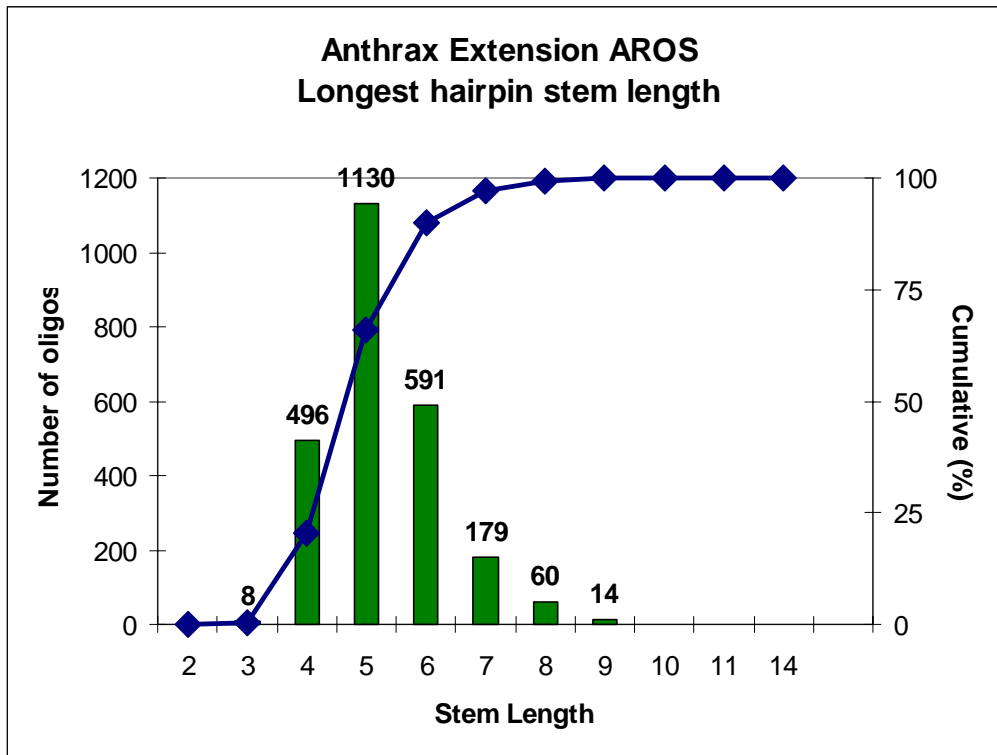
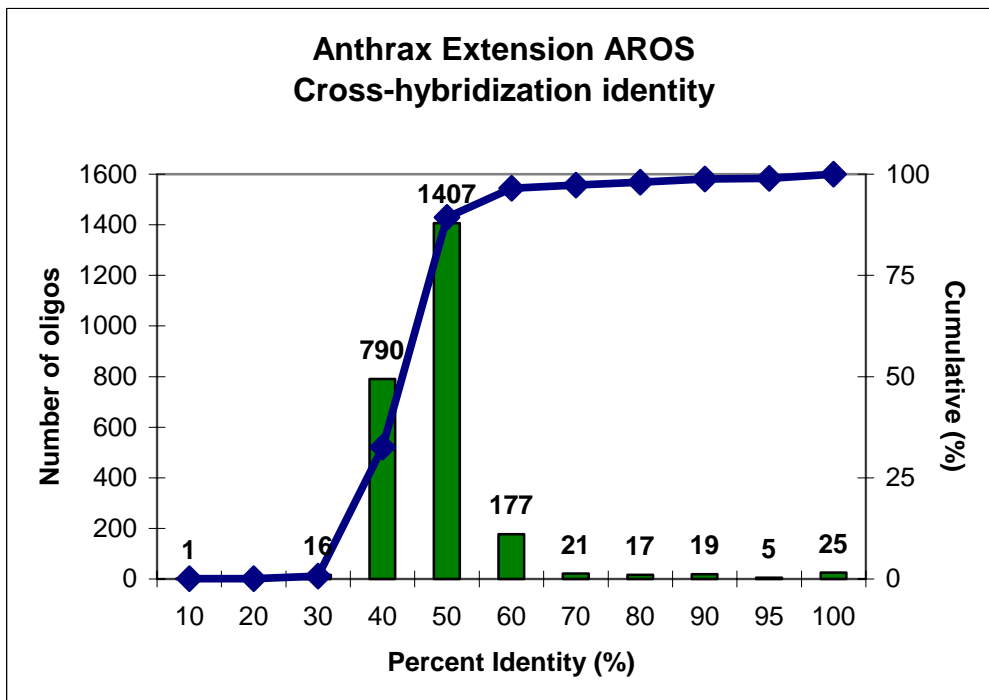


Figure 10. Anthrax Extension AROS: Cross-Hybridization Identity



Quality check of probe design specifications

Once the final oligo set has been selected to represent a gene, 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 probes.

Table 3: Quality check probe design specifications

Probe design specification	Expected value	Verified range	Number of oligos Anthrax AROS	Number of oligos Anthrax Extension AROS
Melting temperature (C°)	75°C±5°C	70.0–80.0	5280	2453
Melting temperature (C°)	66.0–70.0	66.0–70.0	29	25
Cross-hybridization identity (%)	< = 70	31–70	5246	2412
Cross-hybridization identity (%)	> 70	71–100	63	66