

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

We are pleased to announce Version 1.0 of our *Neisseria* Genome Oligo Set which contains 2872 arrayable 70mers representing 2098 Open Reading Frames (ORFs) from *Neisseria meningitidis* serogroup B strain MC58, 2119 ORFs from *Neisseria meningitidis* serogroup A strain Z2491, 2131 ORFs from *Neisseria meningitidis* serogroup C strain FAM18, and 2067 ORFs from *Neisseria meningitidis* ALPHA14. For probe design we use state-of-the-art methodology and proprietary software.

ORF Sequence Source and Selection

ORF sequences for the four organisms are as follows: *Neisseria meningitidis* serogroup B strain MC58 (NCBI RefSeq: NC_003112: <http://www.ncbi.nlm.nih.gov>), *Neisseria meningitidis* serogroup A strain Z2491 (NCBI RefSeq: NC_003116), *Neisseria meningitidis* serogroup C strain FAM1. (http://www.sanger.ac.uk/Projects/N_meningitidis/seroC/NMC.embl), and *Neisseria meningitidis* ALPHA14 (Frosch M, University of Wuerzburg, Germany).

2078 oligos are directly designed from the primary source, *Neisseria meningitidis* serogroup B strain MC58 ORFs, but the probes are selected such that a large number of *Neisseria meningitidis* serogroup A strain Z2491, *Neisseria meningitidis* serogroup C strain FAM18 ORFs, and *Neisseria meningitidis* ALPHA14 ORFs are also represented. An 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 ORF.

296 oligos are directly designed from *Neisseria meningitidis* serogroup A strain Z2491 ORFs. 102 oligos are directly designed from *Neisseria meningitidis* serogroup C strain FAM18 ORFs. 396 oligos are directly designed from *Neisseria meningitidis* ALPHA14 ORFs. A number of these oligos also represent ORFs from another organism other than from the organism they were designed from.

Table 1: *Neisseria* AROS

	<i>NCBI RefSeq</i>	Number of ORFs represented at > 93%	Number of ORFs represented at 100%
<i>Neisseria meningitidis</i> serogroup B strain MC58 (BMC58)	NC_003112	2098	2087
<i>Neisseria meningitidis</i> serogroup A strain Z2491 (AZ2491)	NC_003116	2119	1275
<i>Neisseria meningitidis</i> serogroup C strain FAM18 (CFAM18)		2131	1235
<i>Neisseria meningitidis</i> ALPHA14 (ALPHA14)		2067	1313

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 Neisseria AROS.

1) All oligos are within $78^{\circ}\text{C} \pm 5^{\circ}\text{C}$ using the following formula:

$$T_m = 81.5 + 16.6 \times \log[\text{Na}^+] + 41 \times (\#G + \#C)/\text{length} - 500/\text{length} \text{ where } [\text{Na}^+] = 0.1 \text{ M and length} = \#A + \#C + \#G + \#T$$

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 designed from *BMC58*, using BLAST, each oligo is aligned against all *BMC58* ORFs. For all oligos designed from *AZ2491*, using BLAST, each oligo is aligned against all *AZ2491* ORFs. For all oligos designed from *CFAM18*, using BLAST, each oligo is aligned against all *CFAM18* ORFs. For all oligos designed from *ALPHA14*, using BLAST, each oligo is aligned against all *ALPHA14* 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.

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.

For 284 of the sequences in the Neisseria AROS that did not yield oligos satisfying all the above criteria, certain rules were relaxed.

SUMMARY

Oligo selection criteria	Criteria Value	Number of oligos in the Neisseria AROS
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	2588
Total number of oligos not satisfying one or more of the above criteria		284
Length	35 < x ≤ 50	12*
Melting temperature	66 < x < 70°C 80< x < 81°C	9*
Stem length in potential hairpin	9 < X ≤ 14	5*
Contiguous base match to any other sequence§	> 20	251*
Cross-hybridization to all other sequences§	> 70%	236*
Total		2872

* Out of 284 probes

The following illustrations show the distribution of all 2872 oligos in the Neisseria AROS for melting temperature, GC content, location from 3' end, longest stem length, and cross-hybridization identity.

Figure 1. Neisseria AROS: Melting Temperature

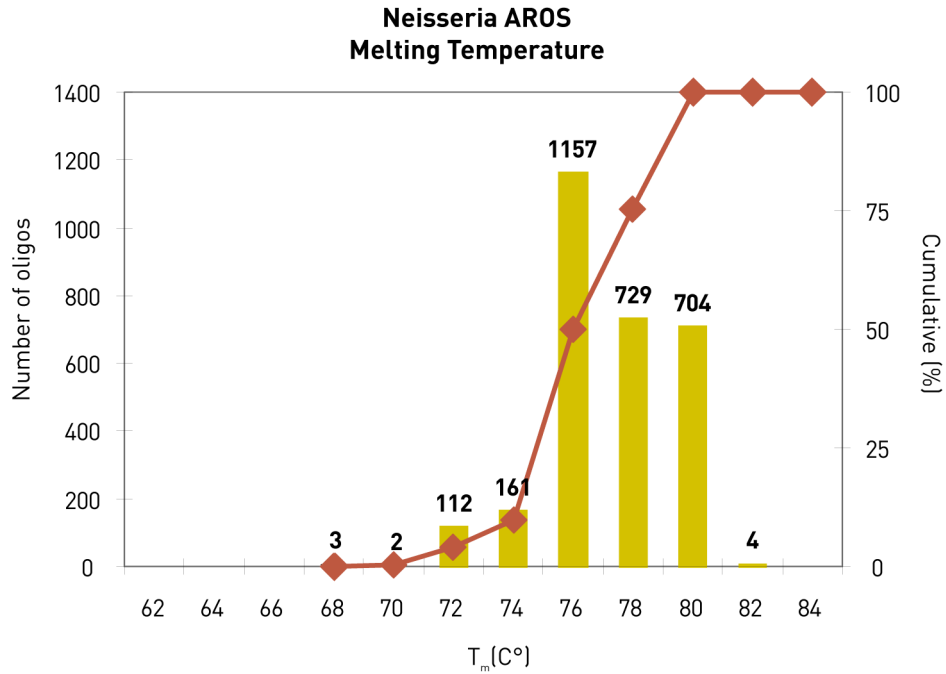


Figure 2. Neisseria AROS: GC Content

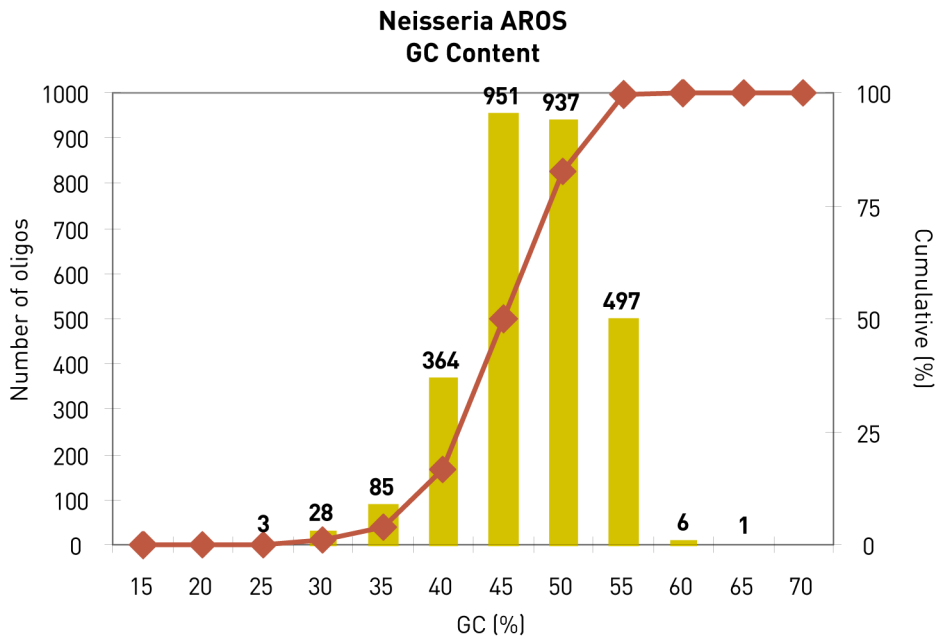


Figure 3. Neisseria AROS: Location from 3' End

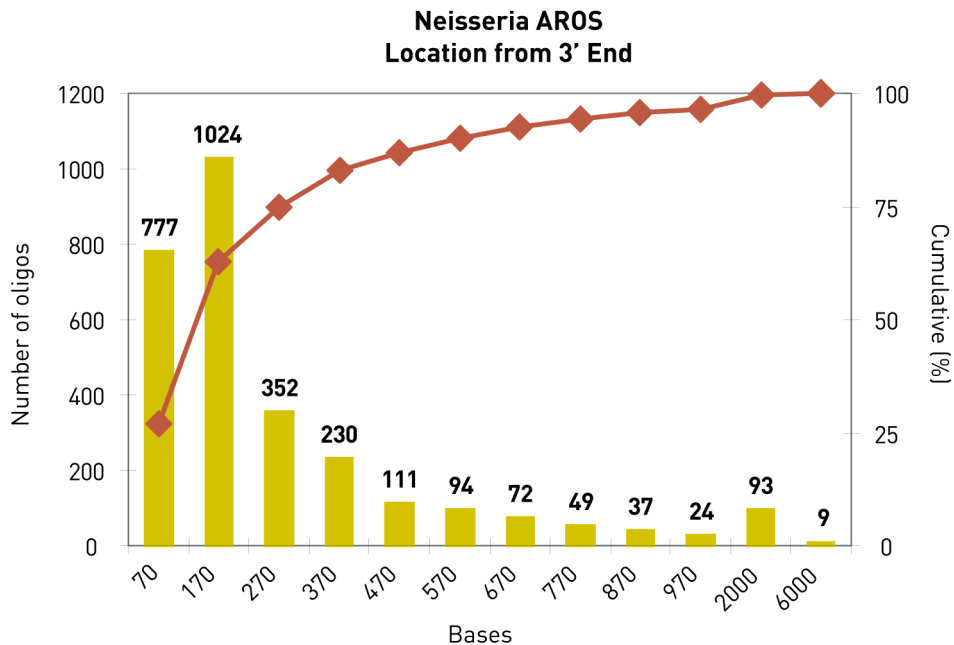


Figure 4. Neisseria AROS: Longest Hairpin Stem length

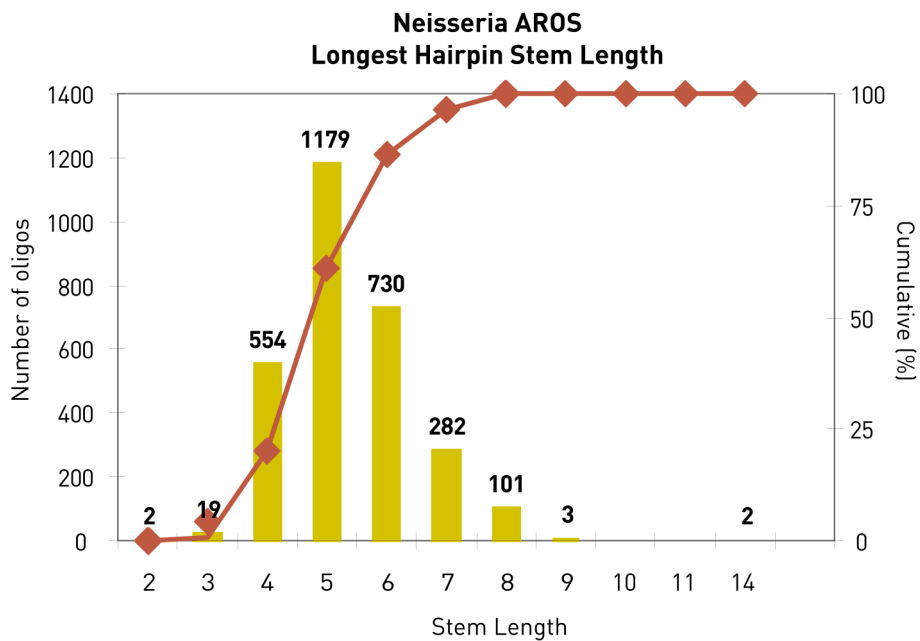
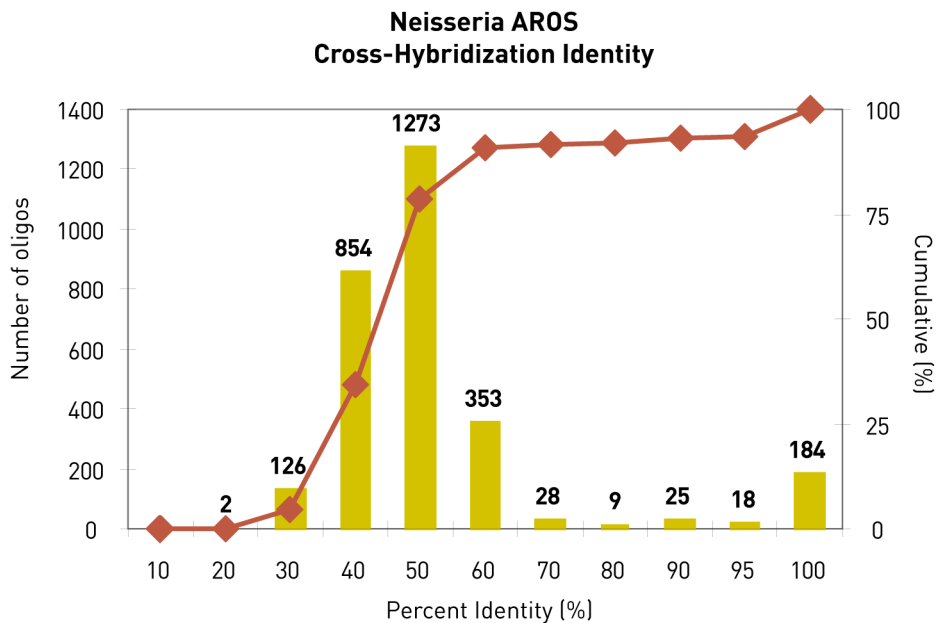


Figure 5. Neisseria AROS: Cross-hybridization identity



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.

Probe design specification	Expected value	Verified range	Number of oligos Neisseria AROS
Melting temperature [C°]	75°C ± 5°C	70.0–80.0	2863
Melting temperature [C°]	67.0–70.0	67.0–70.0	5
Melting temperature [C°]	80.0–81.0	80.0–81.0	4
Cross-hybridization identity (%)	≤ 70	17–70	2636
Cross-hybridization identity (%)	> 70	71–100	236