

Pisum sativum Genome Array-Ready Oligo Set™ (Version 1.0)

The Operon *Pisum sativum* AROS™ Version 1.0. The set contains 5220 longmer oligonucleotide probes representing 5220 genes of *Pisum sativum* genome. Each probe contains an amino linker at its 5' end.

Sequence source and gene selection

Gene sequences used for probe design were obtained from University Bielefeld, Germany.

Probe design and selection rules

The longmer probes are selected with an optimal set of parameters as described below.

1. The melting temperatures (T_m) of the probes are restricted within the range of 74 ± 5 °C. T_m is calculated using the following formula: $T_m = 81.5 + 16.6 * \log[Na+] + 41 * (\#G + \#C)/length - 500/length$ where $[Na+] = 0.1$ M and $length = \#A + \#C + \#G + \#T$
2. The contiguous single nucleotide repeat or poly (N) tract within each probe is limited to 7 bases or shorter.
3. The hairpin stem length of each probe is controlled at 8 bases or shorter.
4. The cross hybridization score for the probe against other non-representing (non-self) genes in the genome is set 70% or less of BLAST percent identity score.
5. The contiguous base match to other non-self genes is constrained at 20 bases or less.
6. The selection distances of the oligos are within 1,000 bases from the 3' end of each gene sequence.

The probes with the highest specificity (or the least cross-hybridization scores) are selected from a pool of candidates satisfying all the rules as described above.

The exceptions (relaxation of one or more selection rules) are made for the probe candidates of 570 genes (10.9%), which don't meet the rules as indicated above. 52 probes of them are matched to multiple genes at the identity score of 100% due to the highly conserved sequences of genes in *Pisum sativum* genome.

SUMMARY

Selection rules	Threshold	Probe number
Probe length (bases)	70	4650
Melting temperature (°C)	74 ± 5	
Poly (N) tract length (bases)	< 8	
Hairpin stem length (bases)	< 9	
Distal distance from 3' end	< 1000	
Cross-hybridization score (identity %)	<= 70	
Contiguous base match to non-self genes (bases)	<= 20	
Exceptions		570
Total		5220

The following illustrations show the distribution of all 5220 probes for oligo length, melting temperature, GC content, distance from 3' end, hairpin stem length, and cross-hybridization identity.

Figure 1. Oligo length

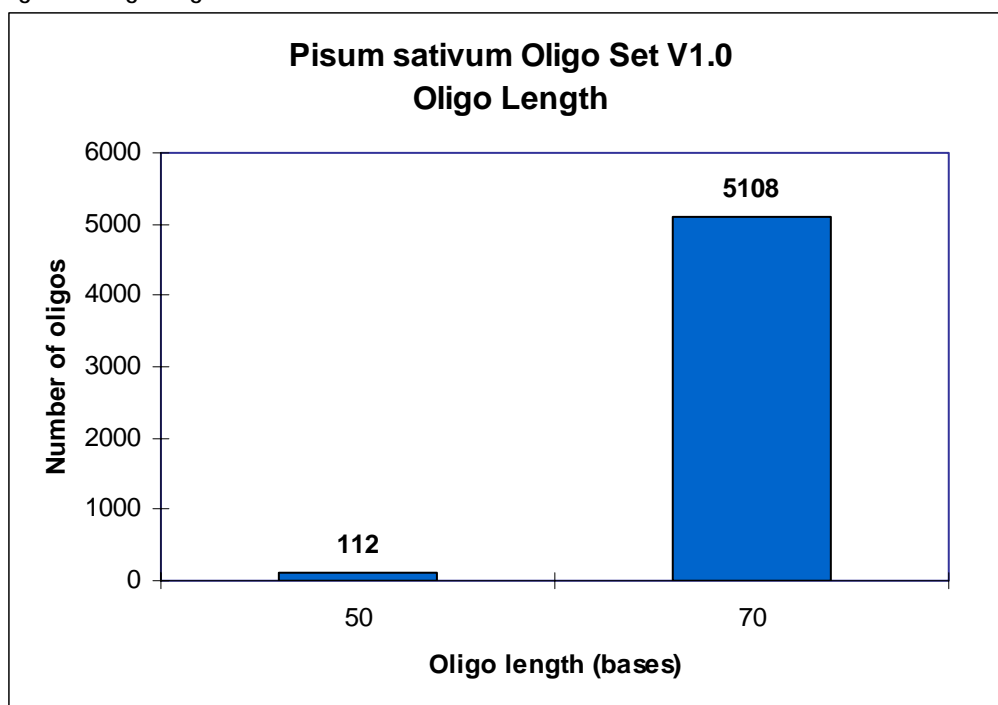


Figure 2. Melting temperature

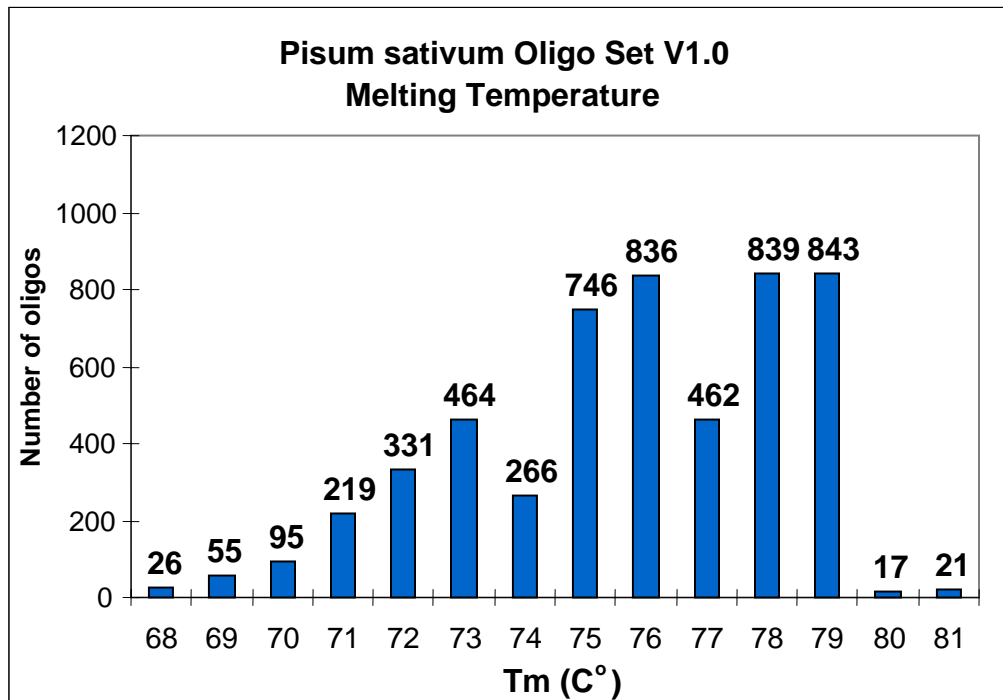


Figure 3. GC Content

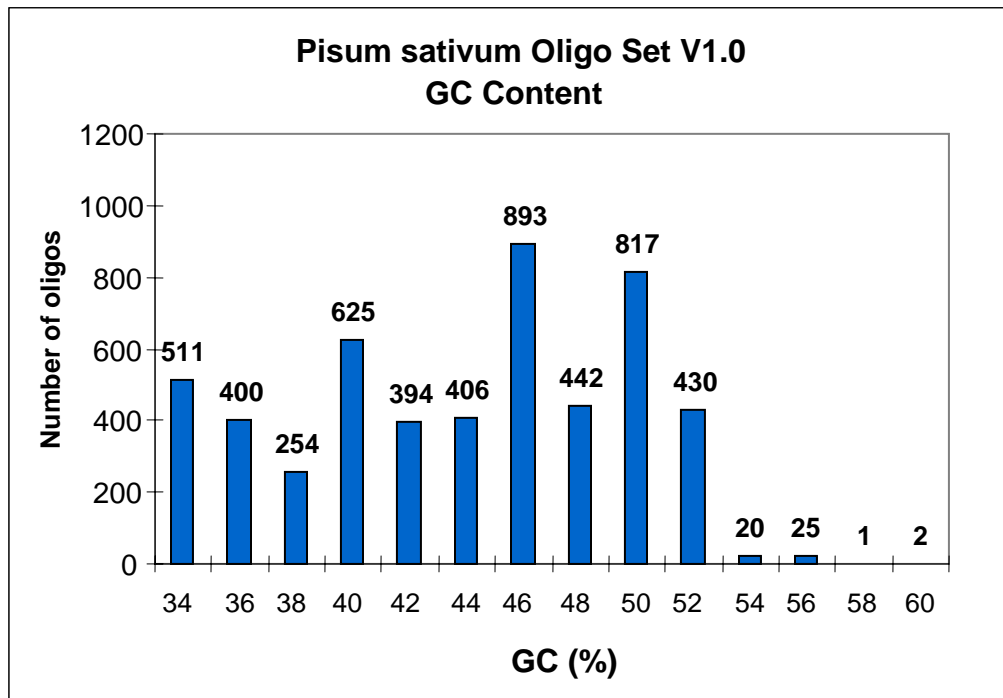


Figure 4. Distance from 3'-end

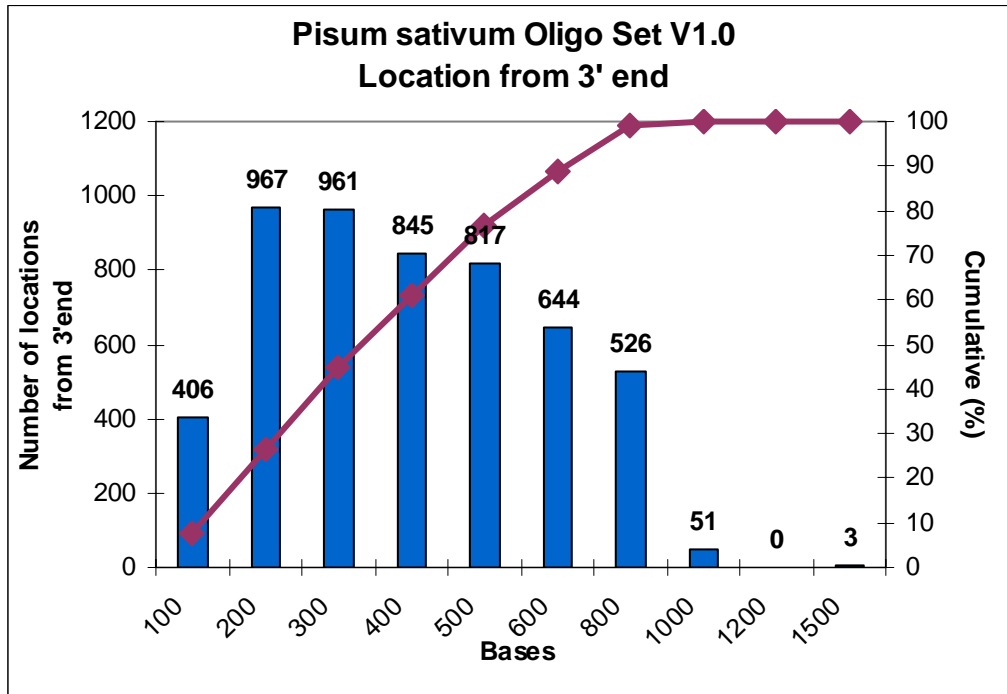


Figure 5. Hairpin stem length

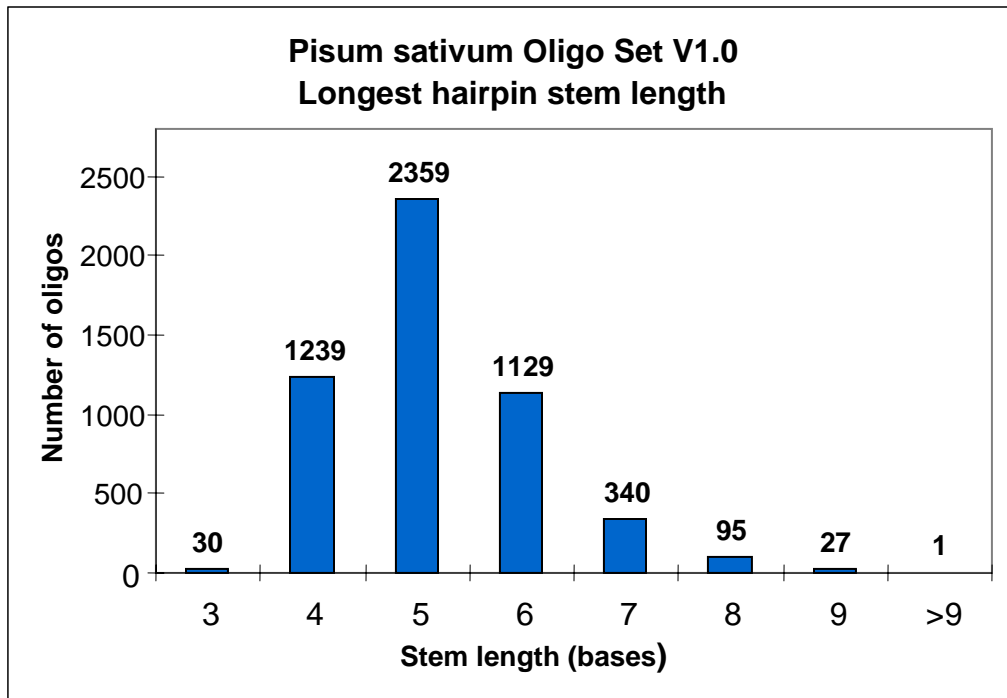


Figure 6. Cross-hybridization score

