

PCR Oligos design for absolute quantification of *S. salivarius* and *S. intermedius*

The PCR target region was identified by multiple sequence alignment programs (Clustal omega) by means of the complete 16S rRNA gene sequences, available in the DNA data Bank (GenBank). The selected sequence regions (Figure 1), showed in 3' region a differential nucleotide motif between *S. intermedius* and *S. salivarius* K12, these were able to discriminate the two microbial species. In these conditions the PCR real time reaction amplified a 600 base pair amplicon and an annealing temperature of 50°C for both strains (Table I). The theoretical melting temperatures of the primers (T_m), the formation of oligonucleotide dimers and self-complementarity were evaluated using Oligo Program version 7 (MedProbe, Oslo, Norway) and by DINA melt Web server for DNA Hybridisation prediction, homodimer simulation with salt condition to 2 mM $[Mg^{2+}]$ and 50 mM $[Na^+]$. Real time PCR reactions were performed by using a Light Cycler instruments ver. 2.2 and SYBR Premix Ex Taq Kit (TaKara-Clontech®) according to manufacturer's instructions. The real time PCR standard curve was made on DNA extracts, obtained from different *S. salivarius* and *S. intermedius* suspensions with a concentration range from 10^7 to 10^2 DNA genomes/2µl. We have calculated that the two bacterial genomes, approximately, were inside these parameters : (i) genome length = 2.19 Mb for *S. salivarius* and 1.94 Mb for *S. intermedius* (NCBI-genome, median values), (ii) we have used for mass conversion this subsequent computational formula, Dolzel *et al.*, 2003,

$$[bp = M * 0.978 * 10^6] \rightarrow [M = bp * 0.978 * 10^{-6}] \text{ were:}$$

M = DNA mass [fg]

bp = base pair of DNA fragment

Thus 1.7 in and 1.5 femtograms/genome respectively for *S. salivarius* and *S. intermedius*

The amount of bacterial genomes was calculated by an interpolated threshold cycle with a standard curve performed for each strain, as example figure 3 *S. salivarius* amplification curve, figure 4 standard curves for both bacterium.

Fig. 1. 16S rRNA, nucleotide sequence and position of the PCR oligos.

<i>S. intermedius</i>	TTTTCGGATC	GTAAAGCTCTGTTGTTAAGG	AAGAACGAGTGTGAGAATGGAAAGTTCATA	480
<i>S. salivarius</i>	TTTTCGGATC	GTAAAGCTCTGTTGTTAAGTC	AAGAACGAGTGTGAGAGTGGAAAGTTCACA	428
***** * *****				
<i>S. intermedius</i>	CTGTGACGGTACTTAAC	CAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC		540
<i>S. salivarius</i>	CTGTGACGGTAGCTTACC	CAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC		488
***** * *****				
<i>S. intermedius</i>	GTAGGTCCCGAGCGTTGTCCGGATTATTGGGCGTAAAGCGAGCGCAGGCGGTTAGATAA			600
<i>S. salivarius</i>	GTAGGTCCCGAGCGTTGTCCGGATTATTGGGCGTAAAGCGAGCGCAGGCGGTTTGTATAA			548
***** *****				
<i>S. intermedius</i>	GTCTGAAGTTAAAGGCAGTGGCTCAACCATTGTAGGCTTTGGAAACTGTTTAACTTGAGT			660
<i>S. salivarius</i>	GTCTGAAGTTAAAGGCAGTGGCTCAACCATTGTAGGCTTTGGAAACTGTTTAACTTGAGT			608
***** *****				
<i>S. intermedius</i>	GCAGAAGGGGAGAGTGGAAATCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAAC			720
<i>S. salivarius</i>	GCAGAAGGGGAGAGTGGAAATCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAAC			668

<i>S. intermedius</i>	ACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGA			780
<i>S. salivarius</i>	ACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGA			728

<i>S. intermedius</i>	GCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGG			840
<i>S. salivarius</i>	GCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTGGA			788
***** *				
<i>S. intermedius</i>	TCCTTTCCGGGACTTAGTGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGAC			900
<i>S. salivarius</i>	TCCTTTCCGGGATTAGTGCCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGAC			848
***** * *****				
<i>S. intermedius</i>	CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCGCACAAGCGGTGGAGCATGTGGTT			960
<i>S. salivarius</i>	CGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCGCACAAGCGGTGGAGCATGTGGTT			908

<i>S. intermedius</i>	TAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCGATGC	CCGCTCTAGAG		1020
<i>S. salivarius</i>	TAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCGATGC	TATTTCTAGAG		968
***** *****				
<i>S. intermedius</i>	ATAGAGCTTTACTTCGGTACATCGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC			1080
<i>S. salivarius</i>	ATAGAAAGTTACTTCGGTACATCGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC			1028
***** *****				

Accession numbers:

GenBank: FJ154804.1

GenBank: AF104671.1

Tab. I. Nucleotide sequence of the PCR oligos used in this work for absolute quantitation.

Oligo name	Oligo sequence 5'---3'	Oligo name	Gene name GenBank accession	bp	
<i>S. salivarius</i>	GTAAAGCTCTGTTGTAAGTC	OG439	16S rRNA	600	Annealing temperature = 50° C
	AACTTTCTATCTCTAGAAATA	OG440	FJ154804		
<i>S. intermedius</i>	GTAAAGCTCTGTTGTTAAGG	OG437	16S rRNA	600	
	AAAGCTCTATCTCTAGAGCGG	OG438	AF104671		

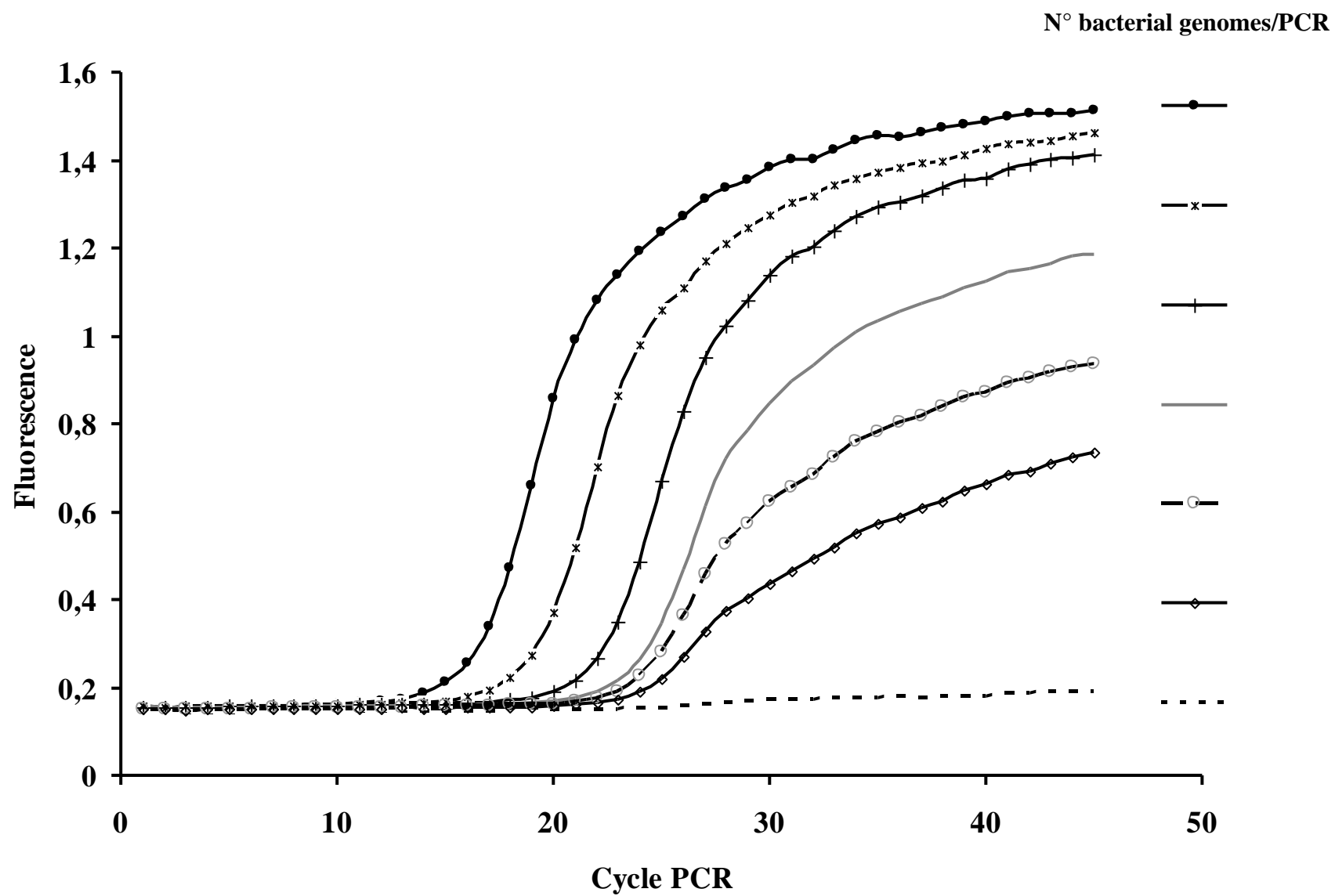


Fig. 3. *S. salivarius* K12 amplification curve, obtained with bacterial suspensions ranged from 10^2 to 10^7 genomes /2 μ l for each strain.

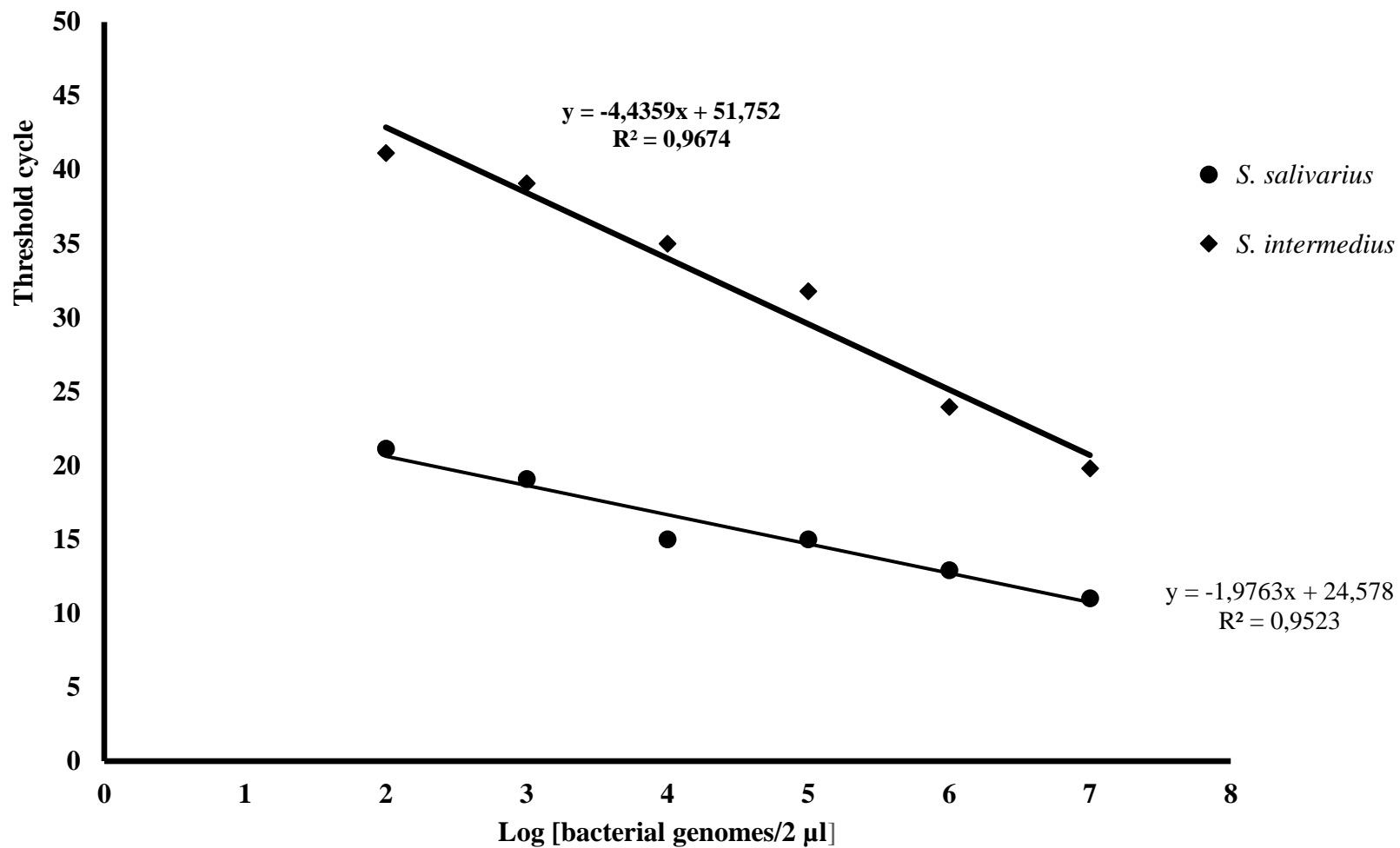


Fig.4. *S. intermedius* and *S. salivarius* standard curves obtained with bacterial suspensions ranged from 10^2 to 10^7 genomes /2 µl for each strain.

References

Dolezel J, Bartos J, Voglmayr H, Greilhuber J. (2003). Nuclear DNA content and genome size of trout and human. *Cytometry*, 51:127-8.

Markham, N. R. & Zuker, M. (2008) UNAFold: software for nucleic acid folding and hybridization. In Keith, J. M., editor, *Bioinformatics, Volume II. Structure, Function and Applications*, number 453 in *Methods in Molecular Biology*, chapter 1, pages 3–31. Humana Press, Totowa, NJ.

Arcadu B, Orrù M, Piga R, Orrù G. (2012). Designing of sequencing assay assisted by capillary electrophoresis based on DNA folding analysis: an application to the VCAM1 gene. *Electrophoresis*, 33:1215-9. doi: 10.1002/elps.201100590.

Footnotes

DNA calculator MW/length : <http://www.calctool.org/CALC/prof/bio/dna>

Clustal omega : <https://www.ebi.ac.uk/Tools/msa/clustalo/>

Genome-NCBI: (*S. intermedius*): <https://www.ncbi.nlm.nih.gov/genome/?term=streptococcus++intermedius>
(*S. salivarius*): <https://www.ncbi.nlm.nih.gov/genome/?term=streptococcus+salivarius>

The DINAMelt Web Server: <http://unafold.rna.albany.edu/?q=DINAMelt/Homodimer-simulations>

Reverse complement calculator : <http://reverse-complement.com/>

Oligo7: <https://www.oligo.net/downloads.html>