PCR & RT-PCR Amplification of Mammalian 18S rRNA gene

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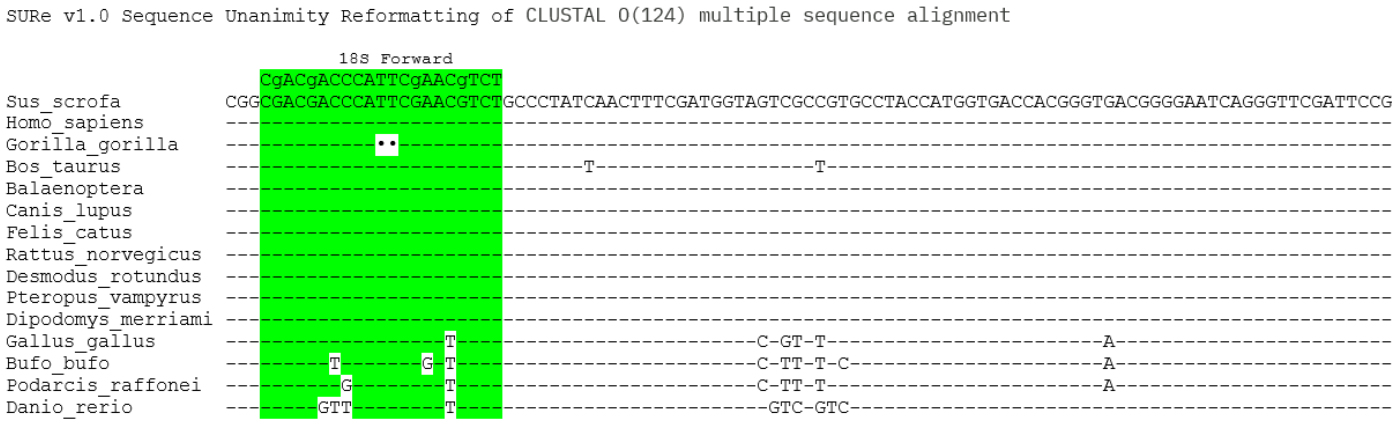
The PCR and RT-PCR Amplification of Mammalian 18S rRNA protocol outlines a standardized method for amplifying the 18S ribosomal RNA (rRNA) gene in mammalian samples. The 18S rRNA gene serves as a highly conserved molecular marker, commonly used for assessing DNA or RNA integrity, detecting inhibitors in nucleic acid extractions, assessing DNA extraction yield, PCR applicability of extracted DNA and for normalizing gene expression in quantitative assays. This protocol describes the steps for both PCR-based DNA amplification and RT-PCR of RNA templates, ensuring reliable and reproducible results in molecular biology applications.

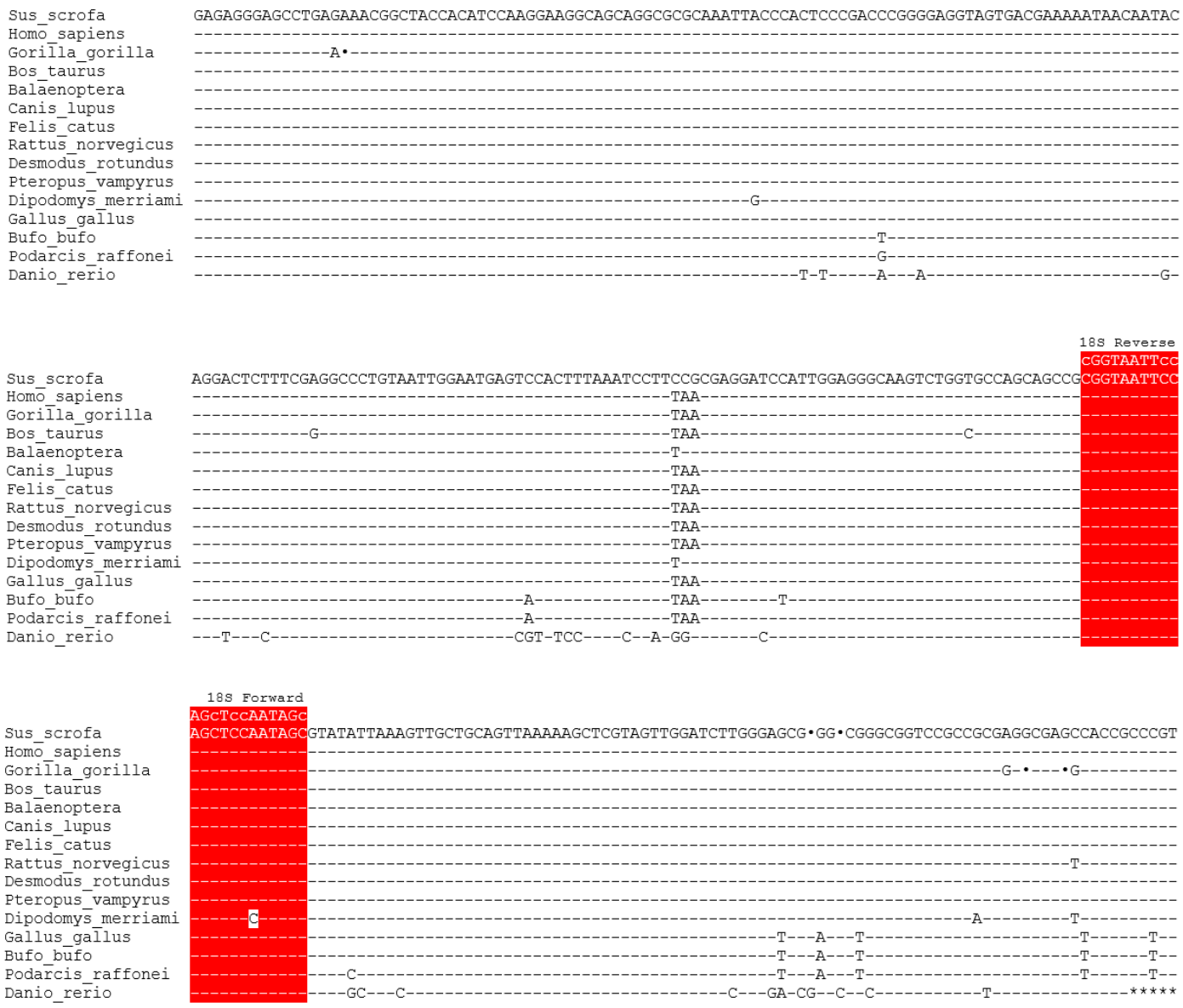
Oligonucleotide primers

Name	Sequence*	bp	%GC	Tm	Hair	HmD	HtD	Amplicon	Ref
18S-F	5'-CgA-CgA-CCC-ATT-CgA-ACg-TCT-3'	21	57.1	59.3	-2.61	-10.65	-5.02	312 bp	1
18S-R	5'-gCT-ATT-ggA-gCT-ggA-ATT-ACC-g-3'	22	50	55.7	-1.35	-6.34			

***** Reverse oligonucleotide primer sequences given in this table are the reverse-complement of the sequence present in the alignments (see below) and as they should be ordered for synthesis. Hairpin (Hair), homodimer (Hm) and heterodimer (Ht) ΔQ's are given in kcal/mol.

Sequence alignments and primer map





NOTE: Be advised, oligos are known to NOT bind to arthropods, crustaceans and lower order animals.

DNA amplification components (PCR) using ABM Taq DNA polymerase

(Applied Biological Materials Inc, British Columbia Canada Cat# G009)

		1x
dH_2O	cf	2.6 µL
5 µM Forward oligo	1.125 µM	2.25 µL
5 µM Reverse oligo	1.125 µM	2.25 µL
10 mM dNTPs	250 µM	0.5 µL
RNA	-	10 µL
		vf: 17.6 µl

↓

Run RT-1 program →

Total time: 6 min		
95 ºC	2 min	1 cycle
4 ºC	2 min	

↓

RT Buffer	1x cf	2 µL
RT Enzyme 250 IU/µl	5 IU/µL	0.4 µL
		vf: 20 µl

↓

Run RT-2 program →

Total time: 1:12 hrs		
38 ºC	60 min	1 cycle
95 ºC	5 min	
4 ºC	5 min	

↓

Polymerase Chain Reaction (PCR)

		1x
dH_2O	cf	6.2 µL
5x Buffer	5 x	3 µL
5 µM Forward oligo	200 nM	0.6 µL
5 µM Reverse oligo	200 nM	0.6 µL
10 mM dNTPs	200 µM	0.3 µL
Taq DNA Pol		0.3 µL
100 ng/µL cDNA	10 ng	4 µL
		vf: 15 µl

↓

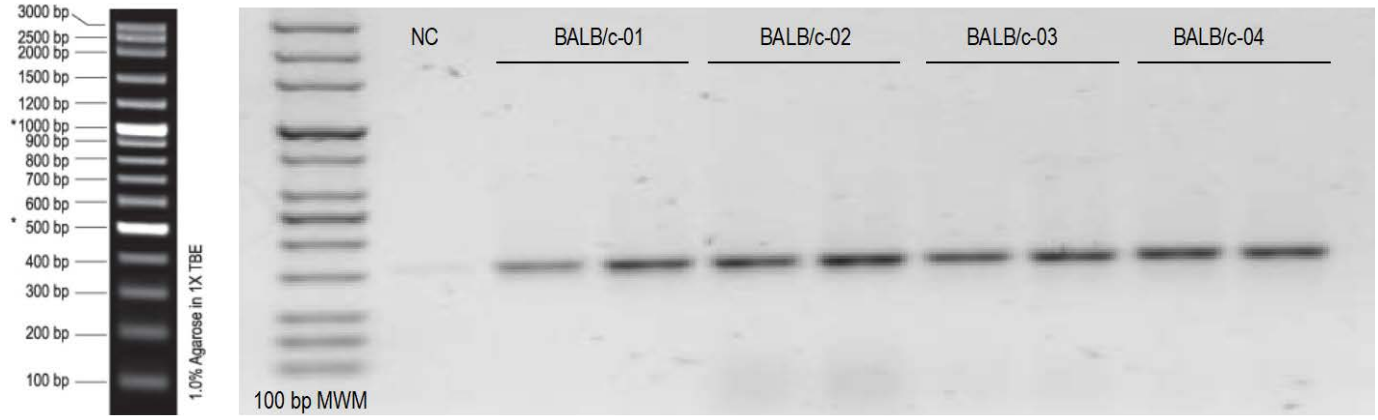
Run 18S program →

Total time: 2:10 hrs		
94 ºC	3 min	
94 ºC	30 sec	35 cycles
58 ºC	30 sec	
72 ºC	45 sec	
72 ºC	5 min	

NOTE: PCR programs validated for Axygen Maxygene and Applied Biosystems MiniAmp Plus cyclers.

Electrophoresis conditions

Add 5 µL of orange loading buffer to the PCR reaction, mix thoroughly by pipetting, and then load 15 µL of the mixture into each well of a 24-well rapid-load comb in a 2% agarose gel prepared in TAE buffer. Electrophorese the gel at 100 VDC for 60 minutes.



First lane corresponds to 100 bp molecular weight marker (MWM), NC corresponds to a dH_2O negative control, the following eight lanes correspond to duplicates of BALB/c intestinal tissue RNA extract. NOTE: Band present in NC corresponds to well 2 (BALB/c-01) overflow during gel loading.

Notes

1. RT-PCR / PCR reactions should be prepared within the Molecular Biology area Biological Ssafety Cabinet (BSC) with the blower off.
2. Clean BSC with 0.1% NaOCl and 70% Ethanol before and after preparing RT-PCR / PCR reactions.
3. Addition of PTC (positive template control, especially artificial genes or cloned fragments should only be performed in the workbench next to the real-time cycler (Molecular Biology area).
4. Prepare RT-PCR / PCR reactions on ice to prevent volume loss due to evaporation.
5. Vortex all reagents (except RNA, DNA and RT/Taq DNA polymerase) before preparing master mix.
6. Vortex master mix after adding all required reagents and before distribution to PCR tubes.
7. Vortex all PCR reactions after adding reagents and DNA. Spin briefly and load into cycler.

References

1. Hadziavdic K, Lekang K, Lanzen A, Jonassen I, Thompson EM, Troedsson C. Characterization of the 18S rRNA gene for designing universal eukaryote specific primers. PLoS One. 2014 Feb 7;9(2):e87624.

Revision history

1. Original document.
2. Protocol components corrected and optimized for ABM enzyme and Applied Biosystems MiniAmp Plus Cyclers, new sequence alignments included.