

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	-5.02		

* 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

SURe v1.0 Sequence Unanimity Reformatting of CLUSTAL 0(124) multiple sequence alignment

	18S Forward
	CGACGACCCATTCGAACGTCT
Sus_scrofa	CGG <mark>CGACGACCCATTCGAACGTCT</mark> GCCCTATCAACTTTCGATGGTAGTCGCCGTGCCTACCATGGTGACCACGGGTGACGGGGAATCAGGGTTCGATTCCG
Homo_sapiens	
Gorilla gorilla	<mark></mark>
Bos taurus	T
Balaenoptera	
Canis lupus	
Felis catus	
Rattus norvegicus	
Desmodus rotundus	
Pteropus vampyrus	
Dipodomys merriami	
Gallus gallus	
Bufo bufo	<mark></mark> T <mark>G</mark> T <mark></mark> G-TC-TT-T-CC-TT-T-C
Podarcis raffonei	<mark></mark>
Danio rerio	<mark></mark> GTT <mark></mark> T



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Sus_scrofa Homo_sapiens Gorilla_gorilla Bos_taurus Balaenoptera Canis_lupus Felis_catus Rattus_norvegicus Desmodus_rotundus Pteropus_vampyrus Dipodomys_merriami Gallus_gallus Bufo_bufo Podarcis_raffonei Danio_rerio	GAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGG
Sus_scrofa Homo_sapiens Gorilla_gorilla Bos_taurus Balaenoptera Canis_lupus Felis_catus Rattus_norvegicus Desmodus_rotundus Pteropus_vampyrus Dipodomys_merriami Gallus_gallus Bufo_bufo Podarcis_raffonei Danio_rerio	183 Reverse AGGACTCTTTCGAGGCCCTGTAATTGGAATGAGTCCACTTTAAATCCTTCCGCGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC
Sus_scrofa Homo_sapiens Gorilla_gorilla	188 Forward AGCTCCAATAGC AGCTCCAATAGC AGCTCCAATAGC AGCTCCAATAGCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCTTGGGAGCG•GG•CGGGCGGCCGCCGCGGGGCGAGCCACCGCCGT

Admo_Saplens Gorilla_gorilla Bos_taurus Balaenoptera Canis_lupus Felis_catus Rattus_norvegicus Desmodus_rotundus Pteropus_vampyrus Dipodomys_merriam Gallus_gallus Bufo_bufo Podarcis_raffonei Danio rerio

	A <mark>GETECAATAGE</mark> A <mark>GETECAATAGE</mark> GTATATTAAAGTTGETGEAGTTAAAAAGETEGTAGTTGGATETTGGGAGEG•GG•EGGGEGEGEGEGEGEGEGAGECGAGECGA
a	G_••G
cus	
dus rus	
iami	C
nei	

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 ₂ O	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		Total tir	ne: 6 min	
		$\mathbf{\Psi}$		95 °C	2 min	1 avala
	Run 2	RT-1 program	→	4 °C	2 min	1 cycle
		$\mathbf{+}$				
RT Buffer	1x cf	2 μL				
RT Enzyme 250 IU/µl	5 IU/μL	0.4 µL				
		vf: 20 µl		Total tir	ne: 1:12 hrs	
		38 °C	60 min			
Run RT-2 program				95 ℃	5 min	1 cycle
				4 °C	5 min	
		$\mathbf{\Lambda}$				
Polymerase Chain Reacti	on (PCR)	1x				
dH ₂ O	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		Тс	Total time: 2:10 hrs	
100 ng/µL cDNA	10 ng	4 µL		94 °C	3 min	
		vf: 15 µl		94 °C	30 sec	
		\bullet		58 °C	30 sec	35 cycles
	→	72 °C	45 sec			
				72 °C	5 min	

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



Electrophoresis conditions

Add 5 μ L of orange loading buffer to the PCR reaction, mix thoroughly by pipetting, and then load 15 μ L 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.

3000 bp		NC _	BALB/c-01	BALB/c-02	BALB/c-03	BALB/c-04	
1200 bp	-			· · ·			
900 bp	=						
600 bp	=						
400 bp ——							
200 bp u aso	_						
100 bp 60 80 10	100 bp MWM						

First lane corresponds to 100 bp molecular weight marker (MWM), NC corresponds to a dH₂O 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.



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References

1. Hadziavdic K, Lekang K, Lanzen A, Jonassen I, Thompson EM, Troedsson C. <u>Characterization of the 18S rRNA gene for designing universal eukaryote specific primers</u>. 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.