Standard Operating Procedures (SOPs)
Laboratorio de Genómica Viral y Humana
Facultad de Medicina UASLP

RT-PCR amplification of mammalian 18S rRNA

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This protocol describes the procedure for 18S rRNA amplification for a wide array of molecular applications when working with eukaryotes. 18 Svedberg ribosomal RNA (18S rRNA) is the only rRNA molecule making up the small eukaryotic ribosomal subunit. 18S rRNA is a component of the small eukaryotic ribosomal subunit (40S) and a homologue of prokaryotes and mitochondrial 16S rRNA. The 18S rRNA genes are widely used in molecular analysis to reconstruct phylogeny but also made their way into molecular methods as internal control templates for both PCR and RT-PCR. This protocol has been specifically optimized for evaluating the PCR functionality of extracted RNA following other quality evaluations such as spectrophotometric and electrophoretic integrity assays. This protocol can be easily translated for work with genomic DNA for internal PCR controls and the like.

Oligonucleotide primers

<table>
<tr><th>Name</th><th>Sequence*</th><th>bp</th><th>%GC</th><th>Tm</th><th>Hair</th><th>HmD</th><th>HtD</th><th>Amplicon</th><th>Ref</th></tr>
<tr><td>18S-F</td><td>5'-CgA-CgA-CCC-ATT-CgA-ACg-TCT-3'</td><td>21</td><td>57.1</td><td>59.3</td><td>-2.61</td><td>-10.65</td><td rowspan="2">-5.02</td><td rowspan="2">312 bp</td><td rowspan="2">1</td></tr>
<tr><td>18S-R</td><td>5'-gCT-ATT-ggA-gCT-ggA-ATT-ACC-g-3'</td><td>22</td><td>50</td><td>55.7</td><td>-1.35</td><td>-6.34</td></tr>
</table>

* 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.

Oligonucleotide specificity

```
Rattus_norvegicus            CGACGACCCATTCGAACGTCTGCCCTATCAACTTTCGATGGTAGTCGCCGTGCCTACCATGGT
Mus_musculus                 ---------------------------------------------------------------
Homo_sapiens                 ---------------------------------------------------------------
Drosophila_melanogaster      ----AGAT-T---A--T------------------T---------AT-TAG-A----------
Aedes_aegypti                ----AGAT-T---A--TA---------------A-T---------ATAGAG-A----------
Aedes_albopictus             ----AGAT-T---A--TA---------------A-T---------ATAGAG-A----------
Culex_pipiens                ----AGAT-T---A--TA---------------A-T---------ATAGAG-A----------
Anopheles_pseudopunctipennis ----AGAT-TCG-A--T----------------A-T---------AT-GAG-A----------
Musa_coccinea                -----CAT-----A--TT-----------------------GATAG-G------------

Rattus_norvegicus            GACCACGGGTGACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCAC
Mus_musculus                 --------------------------------------------------------------
Homo_sapiens                 --------------------------------------------------------------
Drosophila_melanogaster      TG-A------A---------------------------------------------------
Aedes_aegypti                TG-A------A-----------------------------------------T---------
Aedes_albopictus             TG-A------A-----------------------------------------T---------
Culex_pipiens                TG-A------A-----------------------------------------T---------
Anopheles_pseudopunctipennis T--A------A-----------------------------------------T---------
Musa_coccinea                -GTG-----------A----T-----------------------------------------
```

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```
Rattus_norvegicus            ATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGACCCGGGGAGGTAGTGACGAAAA
Mus_musculus                 ---------------------------------------------------------------
Homo_sapiens                 ---------------------------------------------------------------
Drosophila_melanogaster      ---T-------------------T---------------AG-T--------------------
Aedes_aegypti                -----------------------T----------A-----G-A-----------------G--
Aedes_albopictus             -----------------------T----------A-----G-A-----------------G--
Culex_pipiens                -----------------------T----------A-----G-A-----------------G--
Anopheles_pseudopunctipennis -----------------------T----------A-----G-A-----------------G--
Musa_coccinea                ----------------------------------A---T---A---------------A-T--

Rattus_norvegicus            ATAACAATACAGGACTCTTT••CGAGGCCCTGTAATTGGAATGAGTCCACTTTAAATCCTTTA
Mus_musculus                 ---------------------------------------------------------------
Homo_sapiens                 ---------------------------------------------------------------
Drosophila_melanogaster      -----------------A-ATC------------------------A----------------
Aedes_aegypti                ---------T-A--------TAT--C-T-T-A--------------TG-GCA---------C-
Aedes_albopictus             ---------T-A--------TAT--C-T-T-A--------------TG-GCA---------C-
Culex_pipiens                ---------T-A--------TAT--T-TTT-A------------AC-G-GCA---------CG
Anopheles_pseudopunctipennis ---------TGAA-------AAT--T-TTTCA--------------TG-GCA-----------
Musa_coccinea                ----------C--G-----C--••GA-T-TG---------------A--A-C-------C---

Rattus_norvegicus            ACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
Mus_musculus                 --------------------------------------------------------------
Homo_sapiens                 --------------------------------------------------------------
Drosophila_melanogaster      --A----C-A----------------------------------------------------
Aedes_aegypti                --A------A-G---------------------------------------------C----
Aedes_albopictus             --A------A-G---------------------------------------------C----
Culex_pipiens                GTA------A-G---------------------------------------------C----
Anopheles_pseudopunctipennis G-A------A-G---------------------------------------------C----
Musa_coccinea                --------------------------------------------------------------
```

SURe v1.0 Sequence Unanimity Reformatting by UASLP's Viral & Human Genomics Laboratory (http://midasmap.uaslp.mx/suretool/) of Clustal Omega (1.2.4) multiple sequence alignment of GenBank retrieved accessions for Mus musculus (X00686.1), Rattus norvegicus (X01117.1), Homo sapiens (M10098.1), Drosophila melanogaster (M21017.1), Aedes aegypti (HE613439.1), Aedes albopictus (AB085210.1), Culex pipiens (HG001282.1), Anopheles pseudopunctipennis (U49735.1) and Musa coccinea (LC610755.1).

Dual (separate RT and PCR) components and conditions

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Dual (separate RT and PCR) uses Vivantis M-MULV (Cat. No.: ME2305) RT Enzyme and Vivantis Taq DNA Polymerase (Cat. No.: PL1202), Vivantis Technologies Sdn Bhd, Malaysia

First strand synthesis (RT)

		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 in Axygen TC-1 →

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 in Axygen TC-1 →

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	7.58 µL
2x Buffer	1 x	1 µL
10 µM Forward oligo	150 nM	0.15 µL
50 mM $MgCl_2$	1.5 mM	0.3 µL
10 mM dNTPs	200 µM	0.2 µL
5 µM oligo mix	200 nM	0.4 µL
5IU Taq DNA Pol	0.6 IU	0.12 µL
100 ng/µL cDNA	10 ng	0.4 µL
		vf: 10 µl

↓

Run generic program in Applied Biosystems 7500 →

Total time: 2:10 hrs		
94 ºC	2 min	
94 ºC	45 sec	35 cycles
58 ºC	30 sec	
72 ºC	90 sec	
72 ºC	10 min	

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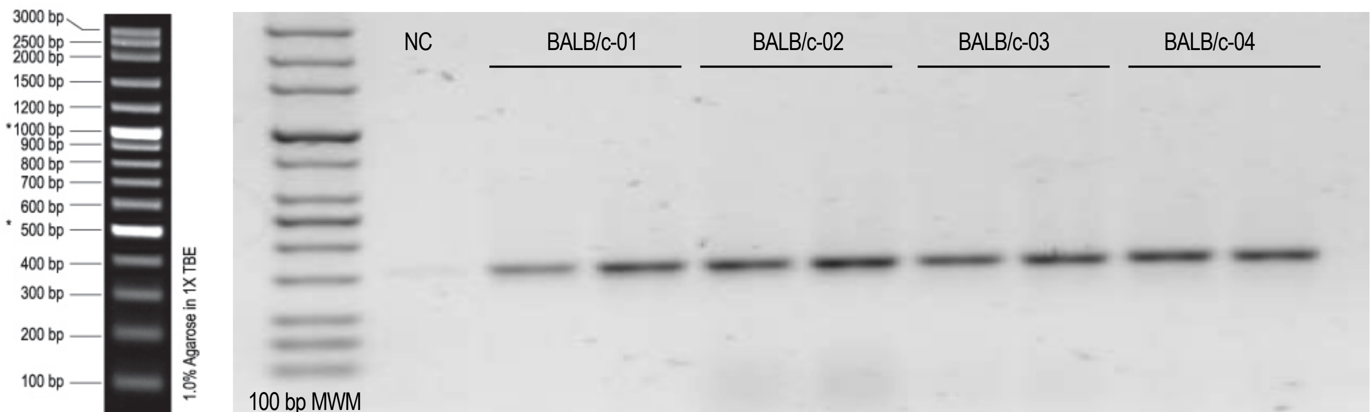
Electrophoresis conditions

Load 12 µL of 5 µL of orange loading buffer plus the complete PCR reaction volume (10 µL), mix by pipetting, use 2.5% agarose gel in TAE buffer and run at 80 VDC for 75 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. All RT-PCR reactions and mastermixes should be set up in the RT-PCR area ONLY!
2. Clean workbench with 0.1% NaOCl and 70% Ethanol before and after setting up the RT-PCR.
3. Preparation of all mastermixes should only be performed in the RT-PCR workbench.
4. Addition of sample RNA/DNA should only be performed in the RT-PCR workbench.
5. Prepare RT-PCR reactions on ice to prevent evaporation of small liquid volumes.
6. Vortex all reagents (except RNA, DNA and RT/Taq DNA polymerase) before dispensing into master mix.
7. Vortex master mix after adding all required reagents then distribute to each individual PCR tube.
8. Vortex all PCR reactions after adding reagents and DNA. Spin briefly and load into cycler.

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References

1. Zhou J. Microarrays for bacterial detection and microbial community analysis. Curr Opin Microbiol. 2003 Jun;6(3):288-94. Review. PMID: 12831906 https://doi.org/10.1016/S1369-5274(03)00052-3.

Revision history

1.0 Original document.

2.0 Expanded number of organisms included in alignments, changed title to indicate mammalian specificity of primers.

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