Procedure for M. leprae molecular viability by qPCR

Leonardo Ribeiro,María Cristina Vidal Pessolani,Chyntia Díaz

Abstract

Goal:

This SOP has the objective to instruct the users and establish a standard protocol for determining *M. leprae* molecular viability using qPCR method.

General considerations:

This protocol is based on the method developed by Martinez *et al* (2009) (1) with few alterations. qPCR reaction is performed using hydrolysis probes from taqman detection system, therefore needing taqman reagents. In this protocol, only the rRNA 16S gene is used as a target for both cDNA and DNA samples.

Experimental proceedings:

1. Clean PCR booth with 70% ethanol and turn on U.V. light for 10 minutes.
2. Prepare PCR mix adding the reagents according to table 1 so as to obtain 10 µL for each reaction. Primers and probes sequences are presented in table 2

Table 1 – PCR mix components

Reagents	Volume
Taqman Universal PCR Master Mix (2X)	5,0 µL
Primers 10 µM (forward + reverse)	0,2 µL
Taqman probe 10 µM – gene *16S*	0,2 µL
H_2O	2,6 µL

Table 2 – Primers and probe sequences used in qPCR reaction.

Gene/probe	Sequence
ML 16S – Forward primer	5′- GCATGTCTTGTGGTGGAAAGC -3′
ML 16S – Reverse primer	5′- CACCCCACCAACAAGCTGAT -3′
ML 16S – probe	5’ FAM – CATCCTGCACCGCA- MGBNFQ 3’

3. Pipet 8 µL from PCR mix in each well of a plate or strip fitted for the thermocycler that will be used, considering additional space for the negative control of the reaction, in which water will be added, instead of a sample. Both cDNA and DNA samples must be previously diluted to the concentration of 5 ng/µL.
4. Outside the PCR booth, add 10ng of cDNA or DNA, 2 µL, in each well, as well as add 2 µL of water in the wells corresponding to the reaction negative control.
5. After sealing the plate, it must be submitted to a short centrifugation until complete removal of all bubbles.
6. Run PCR reaction at the thermocycler, choosing the adequate detection system (Taqman) and for relative determination of gene expression. The reaction must initiate with a step at 50 °C for 2 minutes, followed by heating at 95 °C for 10 minutes, proceeding to 40 amplification cycles of 95°C for 15 seconds and 60 °C for 1 minute
7. Result analysis should go through an initial step to determine the reaction efficiency using LinRegPCR software (2), followed by the analysis using comparative CT method, considering efficiency corrections as described in (3). For the calculation of viability percentage, cDNA samples were normalized against its corresponding DNA samples and viability was determined as a percentage, considering non treated samples as control, 100%, and comparing the viability of treated samples to non treated controls, as the equation demonstrated below:

% Viability = (÷) x 100

E = Efficiency

CT = Cycle threshold

OBS 1: Since PCR reaction uses the same probe for cDNA and DNA detection, each sample must be added individually, in which one well is destined to the cDNA sample and another to DNA sample.

OBS 2: The amount of cDNA or DNA added to the reaction can be optimized adjusting the volume of water in the mix. However, it is important to maintain the alterations for all following batches of experiments.

OBS 3: The plate design should consider a technical replicate for each sample, thus, each sample should be added at least in duplicates on the PCR plate.

References:

1. Martinez AN, Lahiri R, Pittman TL, Scollard D, Truman R, Moraes MO, et al. Molecular determination of Mycobacterium leprae viability by use of real-time PCR. J Clin Microbiol. 2009;47(7):2124–30.
2. Ramakers C, Ruijter JM, Lekanne Deprez RH, Moorman AFM. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Lett. 2003;339(1):62–6.
3. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res [Internet]. 2001;29(9):2002–7. Available from: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/29.9.e45

Citation: Leonardo Ribeiro,María Cristina Vidal Pessolani,Chyntia Díaz Procedure for M. leprae molecular viability by qPCR. **protocols.io**
dx.doi.org/10.17504/protocols.io.pmwdk7e
Published: 22 Apr 2018

Protocol