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Standard Operating Procedure for Real-Time PCR Reaction (qPCR) V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

Goal:
This SOP aims to guide users and establish a standardization for the real-time PCR protocol.

General considerations:
- This standard operating protocol was based on the protocols provided by the manufacturer of the Step One plus and ViiA 7 (Applied Biosystems) PCR machines available in the leprosy laboratory, Oswaldo Cruz Foundation, Brazil.
- Considering that the PCR principle is based on the amplification of DNA or cDNA molecules, it is essential that the material to be used (tubes, tips, water, etc.) is free of nucleases.

Procedures:

TaqMan® system (used for molecular feasibility testing)

1. Turn on the molecular biology cabinet and leave UV light on for 10 minutes;
2. Inside the cabinet, dilute the stock of primers and probe in nucleases free water (eg RF) or TE buffer (see information from this solution below) at 10 µM concentration in RNAse/DNAse-free PCR microtubes (eppendorf or similar). Typically, the stock of primers at an initial 100 µM concentration needs to be diluted 1:10.
3. Prepare the Mix containing the primers by adding:

	0.2 µL Primer Reverse (10 µM)
	0.2 µL Primer Sense (10 µM)
	0.2 µL Probe (10 µM)
	5.0 µL master mix
	2.4 µL Water RF

1. Add 8µL of the reaction MIX per well to the PCR plate (96-well or 384-well plate)
2. Outside of the cabinet, add 2 µL of cDNA or DNA (cDNA or DNA concentration 5 ng / µL) per reaction. In this way the reaction will have a final volume of 10 µL (The final volume indicated for each reaction above can be varied / increased according to need, in that case the volume of all the reagents must be adjusted to respect the recommended final concentration).
3. Take the plate to the thermocycler and start the reaction.

SYBR® Green System
1. Connect the molecular biology cabinet and leave in UV light for 10 minutes;
2. Inside the cabinet, dilute the primers at a concentration of 10 µM in a microtube (stock usually at 200 µM);
3. Prepare the Mix containing the primers, adding:

	0.2 µL Pair of primers (10 µM)
	5.0 µL master mix
	2.8 µL Water RF

1. Add 8 µL of reaction MIX per well to the PCR plate (96- or 384-well plate);
2. Outside the cabinet, add 2 µL containing 10 ng of cDNA per reaction to a final volume of 10 µL. (The final volume of the reaction may vary as needed, so all reagent concentrations should be adjusted.)

Programming software

1. Open the program on the ViiA 7 RUO software icon;
2. Click the "Experiment setup" option;
3. In the side menu of the window, in the item "experiments properties" it is possible to: name the experiment, select type of plate (96 or 384), type of experiment, type of reagent used and finally if the run will be "standard" (normal time) or "fast" (fast tempo), according to the type of master mix used;
4. In the "define" option, name the target genes in the tab called "targets", number of samples in the "samples" tab and, if necessary, define the biological replicates in the "Biological replicates group" tab;
5. In the "assign" option, select the names and genes and targets in the "targets" and "samples" tab, and construct the design of the PCR plate in the "Plate Layout" tab, selecting with the mouse the area on the board and defining both the sample and the selected gene;
6. In the "run methods" option, in the Graphical view tab, indicate the number of reaction cycles and reaction volume desired (usually following the manufacturer's recommendations);
7. Finally, in the "Run" option, start the run with the green button indicating "start run".

Materials:

1. Eppendorf tubes (free from RNAse / DNAse)
2. Micropipettes
3. Plate Centrifuge
4. Optical plate sealer
5. PCR plate 96 or 384 wells
6. Master Mix Syber Green or Master Mix Taqman
7. Rnase/Dnase free tips with filter
8. RF Water (RNase free water)
9. TE buffer (5 mM TrisHCl and 0.1 mM EDTA pH 8.0)

References:

https://tools.thermofisher.com/content/sfs/manuals/cms_095288.pdf

https://tools.thermofisher.com/content/sfs/manuals/cms_046736.pdf