SOP: SP073.1
Modified: 12/16/2022 by RAS

PCR on *Mycobacterium leprae* genomic DNA

Materials and Reagents:

1. *M. leprae* genomic DNA
2. TaKaRa Ex Taq HS Polymerase
3. dNTP mixture 2.5 mM each
4. 10X PCR Buffer
5. PCR primers (Note 1)
6. Sterile DPEC treated water
7. Ice bucket and ice
8. 0.2 mL PCR reaction tubes
9. 10 and 20 µl plugged micropipette and tips
10. Bio-Rad T100 Thermocycler

Protocol:

1. _____Collect reagents 1-6 and place on ice. Any frozen reagents should be thawed before placing on ice.

2. _____Collect four PCR tubes; three for template reactions, plus one to use as a non-template negative control.

3. _____ Calculate all reagent volumes prior to starting reaction. (Note 1)

4. _____ Create "master mix". (Note 2)

5. _____ Add calculated volume of DPEC water to each reaction tube so that final volume will be 50 µl.

6. _____ Add 9 µl of "master mix" to each reaction tube.

7. _____Add 1 µl of 5 µmol stock solution of both M.lep.0277 forward, and reverse primer to **one** properly labeled tube.

8. _____Repeat step 7 for M.lep.0333 and M.lep.0393 in their respective tubes, making sure that each reaction thus far contains only one set of reverse and forward primers, and is properly labeled to reflect which primer set it contains.

9. _____To the fourth tube (non-template control), add 1 µl of 5 µmol stock solution of **every** primer set to generate a multiplex reaction.

10. _____ Add 10-100 ng of sample template gDNA to each PCR reaction tube **except** for the non-template control tube.

11. _____ Add 0.25 µl of TaKaRa Ex Taq HS to each reaction tube.

12. _____Run an appropriate PCR protocol in the thermocycler (Note 3)

13. _____PCR product can be stored for 2-3 weeks at 4 ºC, and long-term storage is achieved at -20 ºC

14. _____Run PCR product from each tube on a 1.2% agarose gel to observe amplicons (Note 4)

Notes:

1. Each reaction tube should have the following:

Tube #	**1**	**2**	**3**	**5**
	0.0277	0.0233	0.0393	Non-template Control with All Primers
10x PCR Buffer	5µl	5µl	5µl	5µl
dNTP	4µl	4µl	4µl	4µl
Template	*	*	*	none
F/R Primers	1µl/1µl	1µl/1µl	1µl/1µl	3µl/3µl
taq	.25µl	.25µl	.25µl	.25µl
DPEC	#	#	#	34.75µl
Total =	50µl	50µl	50µl	50µl

*Calculate volume based on 50-100 ng/µl

#Calculate so total volume is 50 µl

2. Add 5 µl 10X PCR buffer and 4 µl dNTP mixture, for **each** reaction to create "master mix". Vortex briefly. Keep all reactions on ice until PCR is performed.

3. Primers designed in house as: M.lep.0277 (forward: TCAAGTCCAGAATTATTCCGGC reverse: CCATGCTGCGTTTGTTGATAAGGG), M.lep.0333 (reverse: GAATGGCCTGTATCGACCTTAACGC forward: GAATCCACCAGCTGTATTGCCGC) and M.lep.0393 (reverse: GTAAAGTGAGGTCCACCGTGCTGGG forward: CGTACGTGATTGCCTCCAGATAGCG)

4. Initial denaturation 30 seconds at 94 ºC, followed by 30 cycles of [98 ºC for 10 seconds; 55 ºC for 30 seconds; 72 ºC for 30 seconds], followed by annealing state at 72 for 5 minutes. Final hold @ 12 ºC.

5. Reference Dobos SOP: SP018. Use 1 µl of ladder due to low gDNA yield.

Gene From Genome Accession Number AL450380.1*	Total Amplicon Size (bp)	Product	Name
ML0277	313	PPE-family protein (pseudogene)	PPE
ML0333	415	Conserved hypothetical protein	ML0333c
ML0393	411	Probable hydrolase	ML0393c

*M. leprae TN - sequenced on 2015