DETECTION AND PHYLOGENETIC ANALYSIS OF MYCOBACTERIUM LEPRAE IN PREHISTORIC SKULL BONE FROM LEWOLEBA, FLORES ISLAND-LEMBATA INDONESIA BASED ON TTC REGIONS

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ABSTRACT

Introduction: Identification of the genetic material of pathogenic organisms in the prehistoric networks provide important information for the study of certain infectious diseases in prehistoric populations. In addition, the identification of bacterial DNA provides direct evidence and the frequency of occurrence of infectious diseases in prehistoric populations and may provide information about the evolution of microorganisms and related diseases. Several recent reports have succeeded in isolating several *mycobacterium* by using PCR technique, because the PCR technique, although very small amount of DNA prehistoric biomaterials such as bone or soft tissue but can be identified. **Objectives:** To perform detection and phylogenetic analysis of *M.leprae* in prehistoric skull bone from Lewoleba Flores island-Lembata Indonesia based on TTC regions. **Methods and Material:** Lewoleba site of origin of the skull bone, Lembata island-Flores, Indonesia (code LL 1/5) which has been determined based on the C14 shows antikuitas age 2990 +/-160 BP. DNA extraction using Qiagen kit (and Proteinase-K for lysis). PCR run using primers LpF-R and Lp1,2 and Lp1,2-Lp3,4 (nested PCR). Purification and sequencing performed on 129 basepairs (bp) of PCR products, phylogenetic analysis based on TTC regions. **Results:** Swab the outside of the skull bones obtained one sample with 19 repetitions TTC and one sample with 14 repetitions TTC, whereas the inner bone obtained by 13 repetitions TTC. **Conclusion:** Isolates of *M. leprae* has been identified from Lewoleba Flores island-Lembata Indonesia (code LL 1/5) with a PCR-based region of TTC. Generally seen that the same pattern obtained with TTC motifs derived from *M. leprae* isolates in Southeast Asia.

Keywords: Skull-Bone, TTC regions, *M. leprae*, Prehistoric, PCR

INTRODUCTION

Identification of the genetic material of pathogenic organisms in the prehistoric networks provide important information for the study of certain infectious diseases in prehistoric populations [1]. In addition, the successful identification of bacterial DNA from tissue samples provide direct evidence of prehistoric and frequency of occurrence of infectious diseases in prehistoric populations and may provide information about the evolution of microorganisms and related diseases [1]. Over the last two decades, DNA identification techniques derived from prehistoric populations were PCR and hybridization, although the PCR technique is considered much more sensitive than hybridization techniques.

This is related to a very limited amount of DNA from prehistoric tissue due to degradation, however the high level of sensitivity of PCR is extremely vulnerable to contamination by DNA that contaminated the environment at this time [2,3]. Several recent reports have succeeded in isolating several *Mycobacterium* by using PCR technique, because the PCR technique, although very small amount of DNA in prehistoric biomaterials such as bone or soft tissues but have been identified [4]. This new approach not only broaden the horizon of knowledge related to the evolution of different strains of *mycobacteria*, but may also provide correlative data on the influence of environment on the development of *mycobacteria* and diversity. Nevertheless among anthropologists and museum curators still have concerns and objections to the possibility of damage to the bones of prehistoric high value due to the sampling of DNA from the bones of prehistoric[5].

Site Lewoleba, Lembata island-Flores is a grave site is located along the Gulf Lewoleba, Lebaktukan, Flores East which stretches from east to west along approximately 10 km [5]. Site Lewoleba, antiquities 1500 - 4000 years, from the late Neolithic period to the beginning of the metal age [5]. Polarization between racial elements become more light on this period, where the west and north of the archipelago, are stronger or as the only element is the Mongoloid element, while the east and south of the archipelago is Australomelanesid element. This situation still continues to this day and process more and more towards the east Monggolidisasi [5]. Archaeological artifacts have been found on the site is in the form of pottery Lewoleba either plain or geometric, and stone flakes parent to shale [5]. Time of animals that exist around the human frame includes pigs, dogs, some small herbivores and broken shells and snails [5]. Time frame of grave human primary acquired five individuals in

the supine position with head to the south and the rest of the human frame from a secondary grave in a state in situ, the entire contents of these jars with the matrix is already petrified [5]. However until now has never reported any *Micobacterium* especially Mycobacterium leprae was found in a prehistoric skull from Indonesia, so too has not been widely reported throughout the world of *M. leprae* found in the bones of prehistoric ages 2990 + / -160 BP.

Long DNA of *M. leprae* has long 3,268,203 bp [5,6]. From the mapping results are known of the region with the coordinates of 2,785,435 bp nucleotide sequence in which the repetition occurs TTC. Regio TTC is now widely studied to distinguish strains of germs *M. leprae*. Repetition of nucleotide TTC was first introduced by Shin *et al* that aims to differentiate strains of *M. leprae*[7]. TTC repetition of this phenomenon is only found in *M. leprae* and not found *M. tbc, M. avium, M. marinum, mycobacterium sp* and others, until recently has found a variety of strains of *M. leprae* which showed differences in the number of repetitions of nucleotide TTC[7,8].

OBJECTIVES

The purpose of this study is to perform detection and phylogenetic analysis *M.leprae* on prehistoric skull Lewoleba site of origin, the island of Flores Lembata-based PCR products from the region of the TTC.

METHODS AND MATERIAL

Lewoleba site of origin of the skull bone, Lembata Island-Flores, Indonesia (code LL 1/5) which has been determined based on the C14 shows antiquities age 2990 +/-160 BP. DNA extraction using Qiagen kit (and Proteinase-K for lysis). PCR run using primers LpF-R and Lp1,2 and Lp1,2-Lp3,4 (nested PCR). Purification and sequencing performed on 129 basepairs (bp) of PCR products, phylogenetic analysis based on TTC regions.

RESULTS

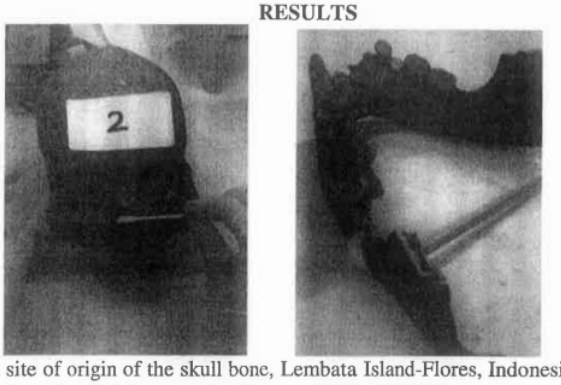


Figure 1. Lewoleba site of origin of the skull bone, Lembata Island-Flores, Indonesia (code LL 1/5) which has been determined based on the C14 shows antiquities age 2990 +/-160 BP

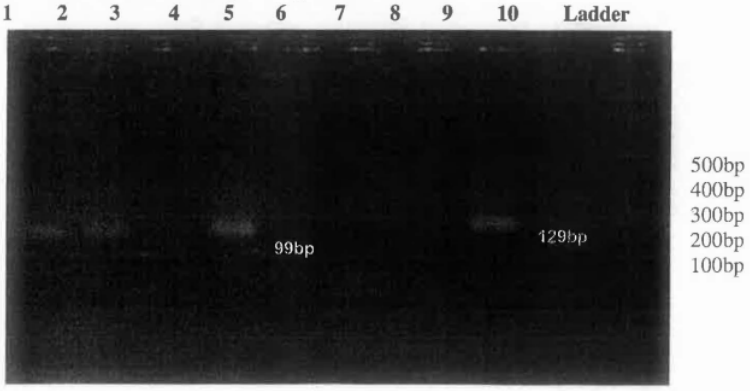
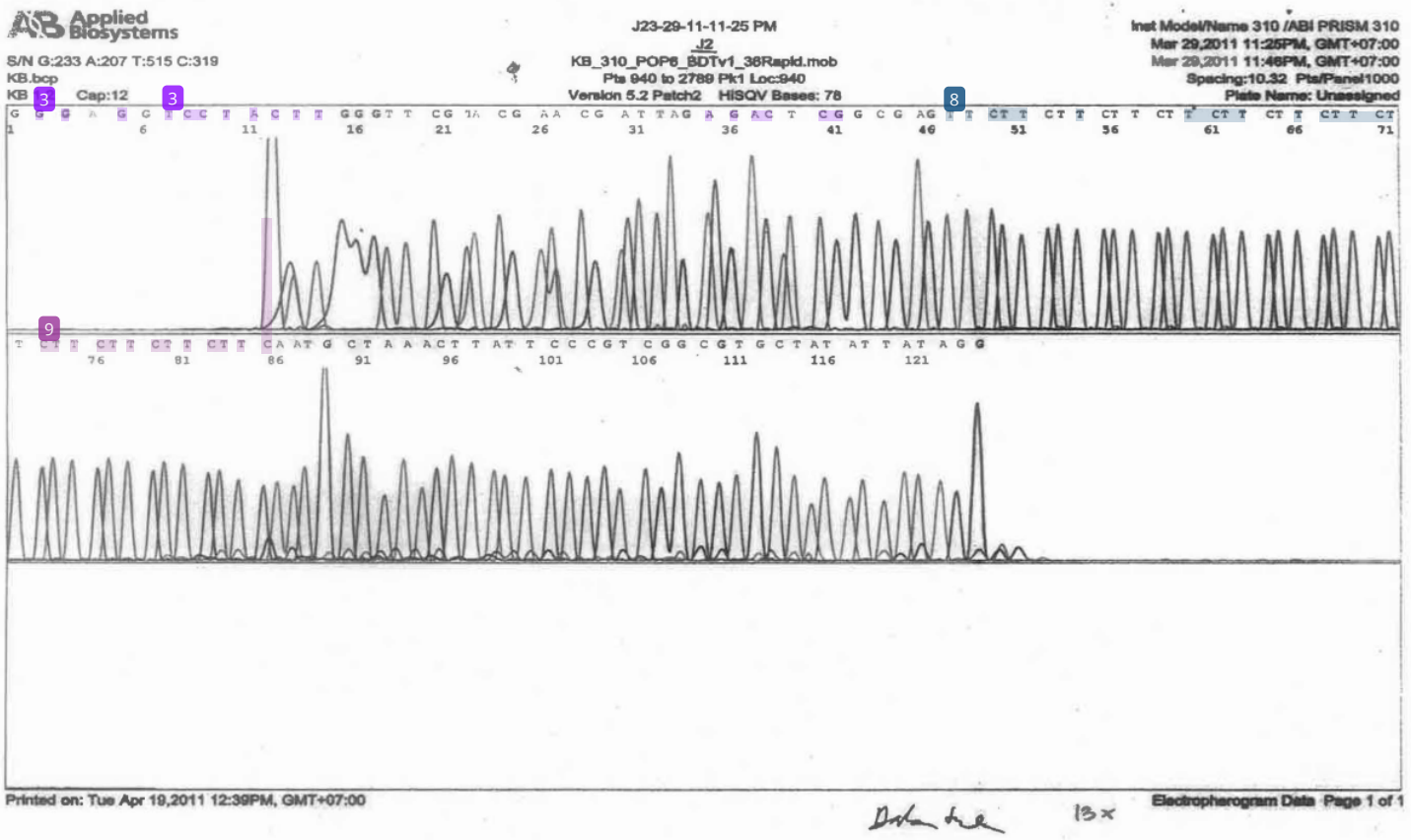


Figure 2. PCR products of DNA *M.leprae*. Lane 1-4 : PCR products (99 bp) using primers Lp1-Lp2 (*outer*) and Lp3-Lp4 (*inner*). *Lane* 1 : PCR products using Kit Qiagen methods, *Lane* 2 : PCR products using conventional methods, *Lane* 3 : Negative controle, *Lane* 4 : Positive controle (DNA *M.leprae Thai*53), **Lane 5-8** : PCR products (129 bp) using primers LpF-LpR (*outer*) and Lp1-Lp2 (*inner*). *Lane* 5 : PCR products using Kit Qiagen methods, *Lane* 6 : PCR products using conventional methods, *Lane* 7 : Negative controle, *Lane* 8 : Positive controle (DNA *M.leprae Thai*53). *Lane* 10 : 100bp DNA ladder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13x

Figure 3.

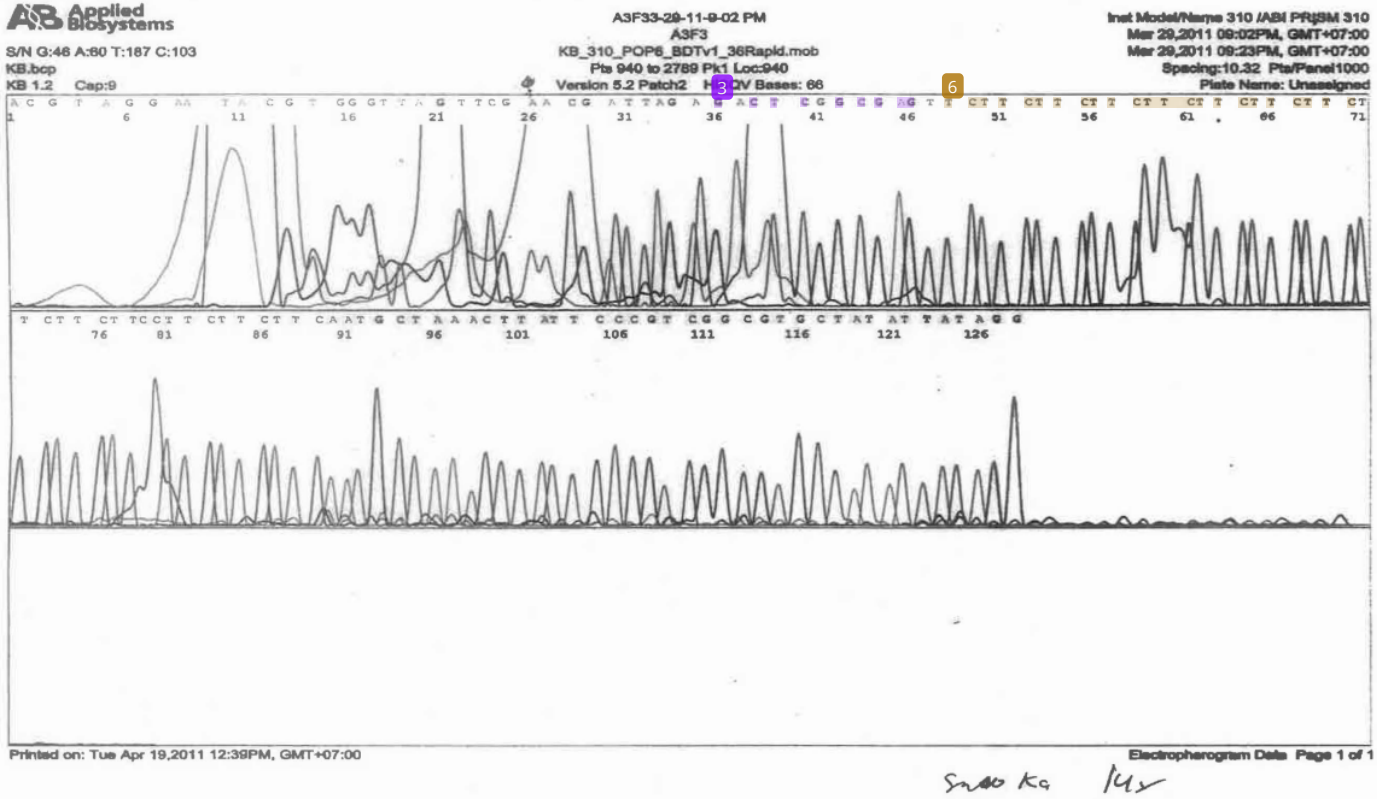
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Figure 4.

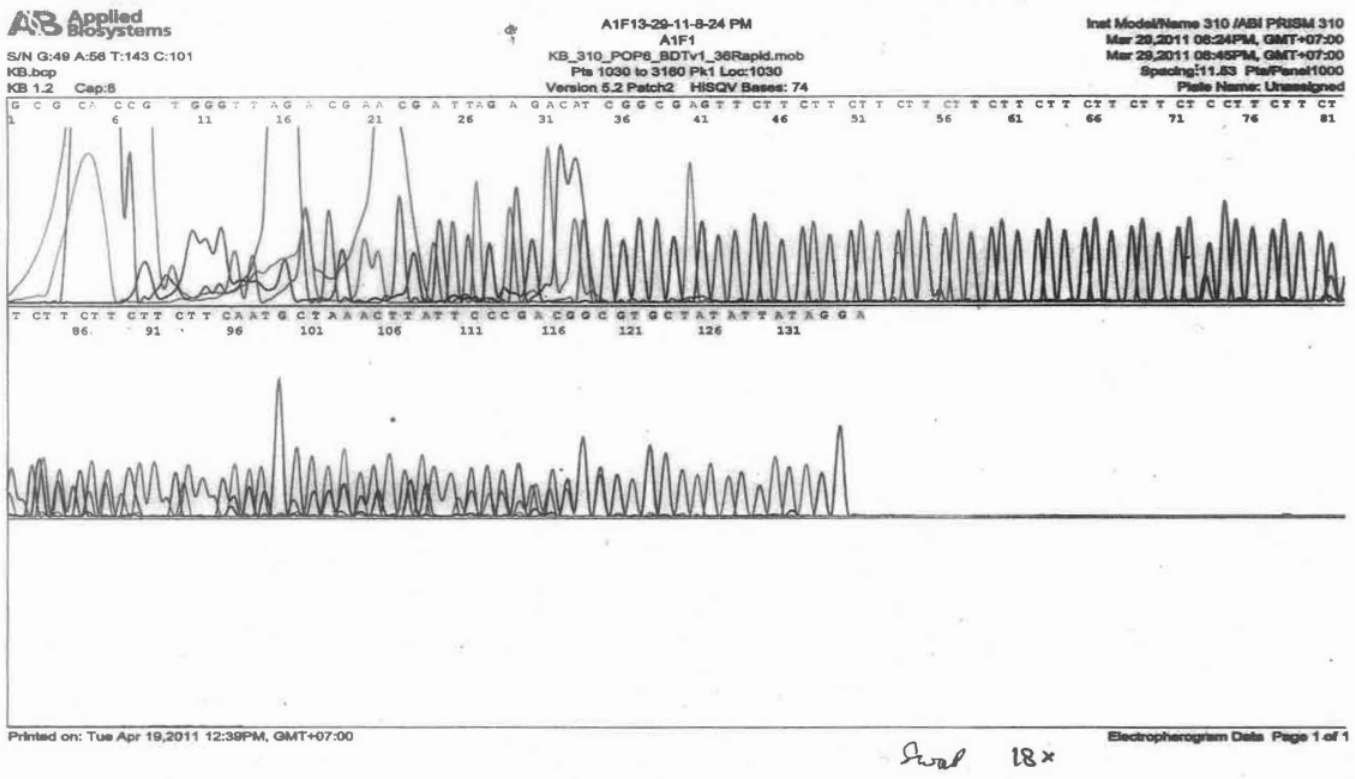
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Figure 5.

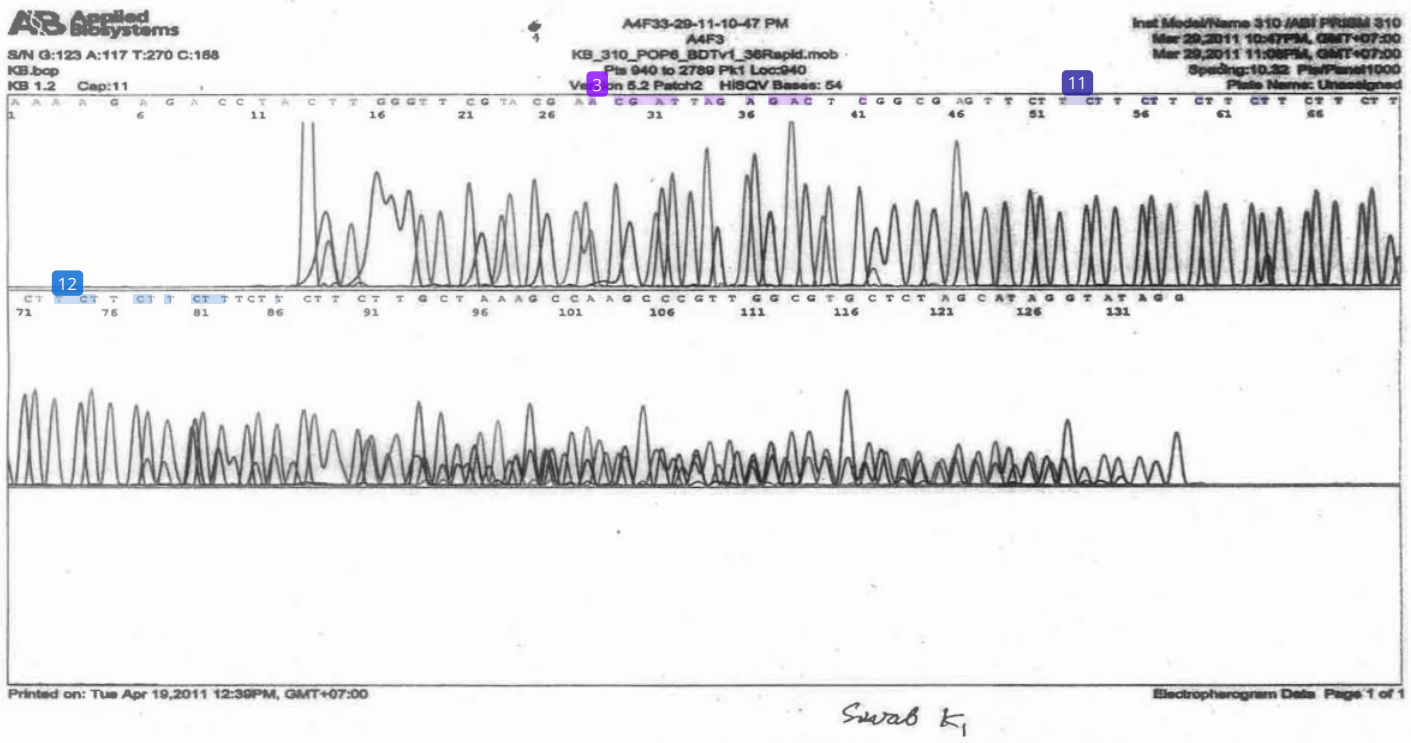
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Figure 6.

DISCUSSION

Research on the analysis of DNA from pathogens during the last 15 years, concentrated on efforts to uncover the disease of unknown cause and associated with pathogens that live and are found on prehistoric bones. The pathogen that is currently studied are: malaria; E. Coli; tuberculosis[9,10,11]. The variation in the number of repetitions on the examination of nucleotide TTC genotypes of M. leprae open the possibility to investigate the variations in strains of germs M. leprae [6].

This study used a sample of the skull bones of prehistoric origin Lewoleba site, Lembata island-Flores (code LL 1 / 5) both of bone and swab the inside of the outer bone (Fig. 1.). For the analysis of *M. leprae* using a nested PCR with primers LPF-R and Lp1, 2 (122 bp) and Lp1,2-LP3, 4 (99 bp) (Figure 2.)

The number of repetitions of nucleotid : TTC is found to vary, namely: 13 copies (in bone, figure 3); swab left temple (14 copies, figure 6); swab right temple (14 copies, figure 4); and forehead swab (18 copies, figure 5). It seems that isolate fro Lewoleba site of origin of the skull bone, Lembata Island-Flores, Indonesia (code LL 1/5), and mostly from South East Asian region such as the *M.leprae* strain *Thai*-53 from Thailand (TTC-14 copy) and from Philippines (mostly TTC-14, followed by TTC-24 and TTC-25 copies) that has been reported by [7]. It is diffe nt than the isolates that found in the Africa and India which are have longer repeated (*M.leprae* strain Tamil Nadu India has TTC-21 copy; *M.leprae* strain Ethiopia has TTC-29 copy). Based on ese molecular typing, it could be related with the origin of leprosy that came from Indian subcontinent and from India, leprosy is thought to have spread to China, Japan reaching Pacific Islands until America as described below by Monot [12].

CONCLUSION

Isolates of *M. Leprae* has been identified from Lewoleba Flores Island-Lembata Indonesia (code LL 1/5) with a PCR-based region of TTC. Generally seen that the same pattern obtained with TTC motifs derived from *M. Leprae* isolates in Southeast Asia.

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