

Co-infection with two closely related *Treponema pallidum* subsp. *pertenue* strains in a yaws patient from Namatanai, Papua New Guinea

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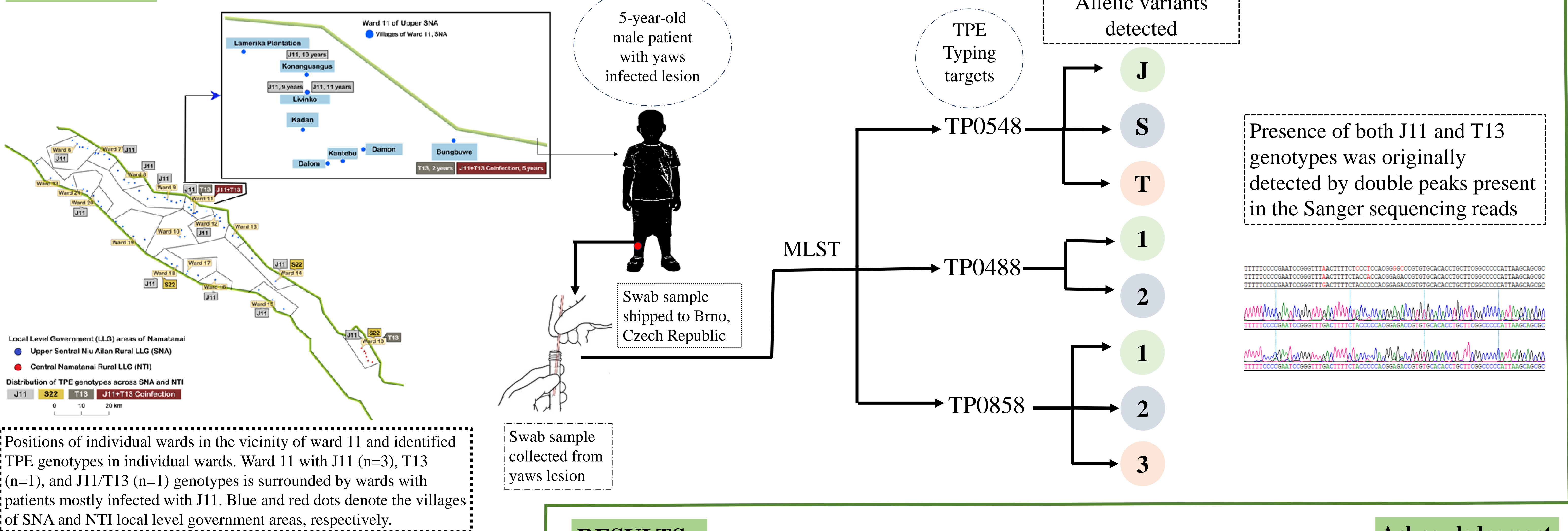
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INTRODUCTION

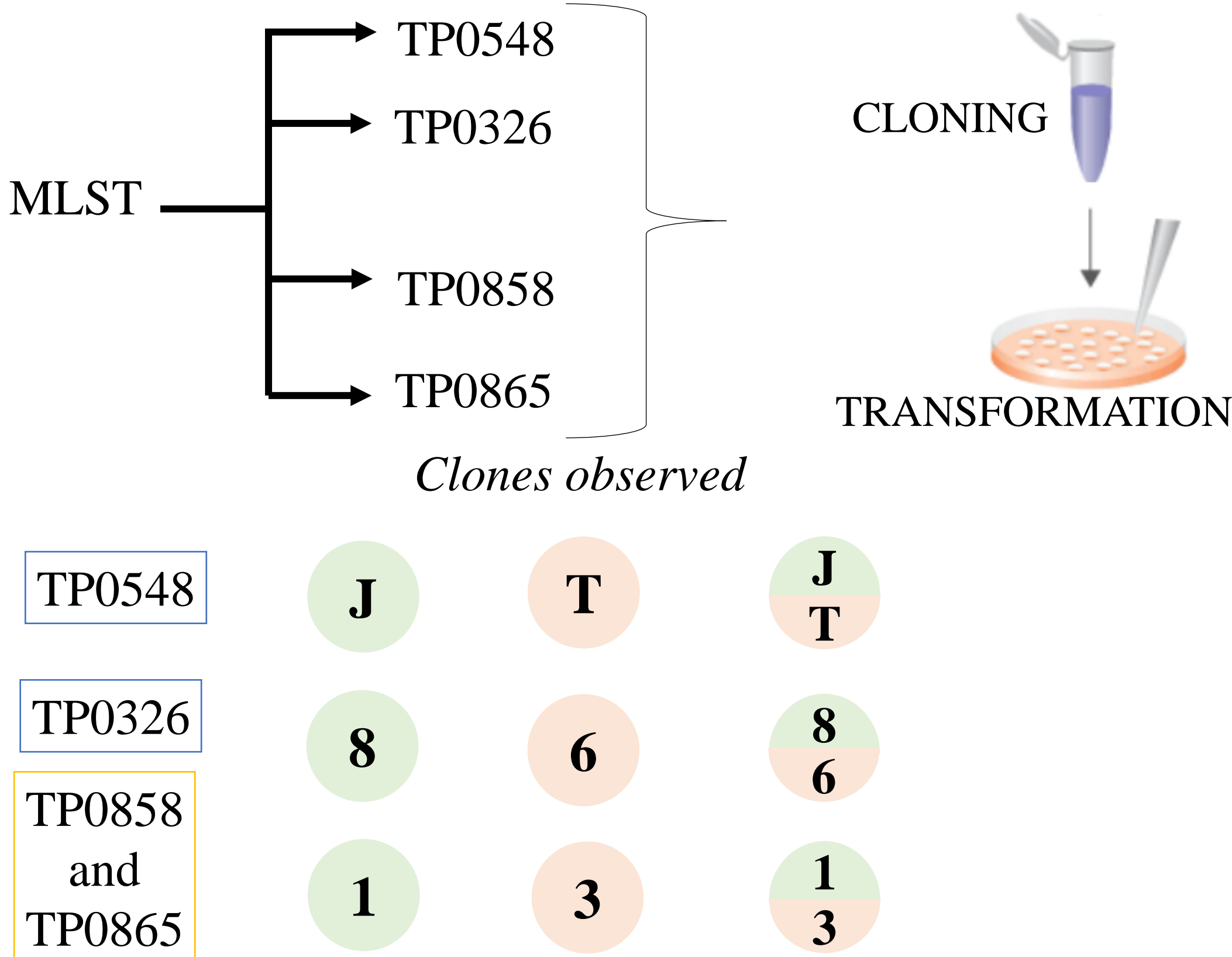
Yaws, a prevalent tropical disease primarily affecting children, is characterized by the presence of erythematous lesions predominantly found in the lower limb. Through a comprehensive multi-locus analysis of 255 fully typed clinical isolates, it was discovered that three allelic profiles (APs) namely J11, S22 and T13 are present. In this study, we present a unique case of a five-year-old male patient from Papua New Guinea (PNG) who was found to be coinfecting with two TPE strains, exhibiting allelic profiles J11 and T13. While the presence of both J11 and T13 genotypes was originally detected by double peaks present in the Sanger sequencing reads, cloning of PCR products and the subsequent sequencing of individual clones proved that there is a combination of two different genotypes in the original sample. Interestingly, about 21% of analyzed clones showed chimeric character, combined sequences of both J11 and T13 genotypes in a single sequence.

METHODS



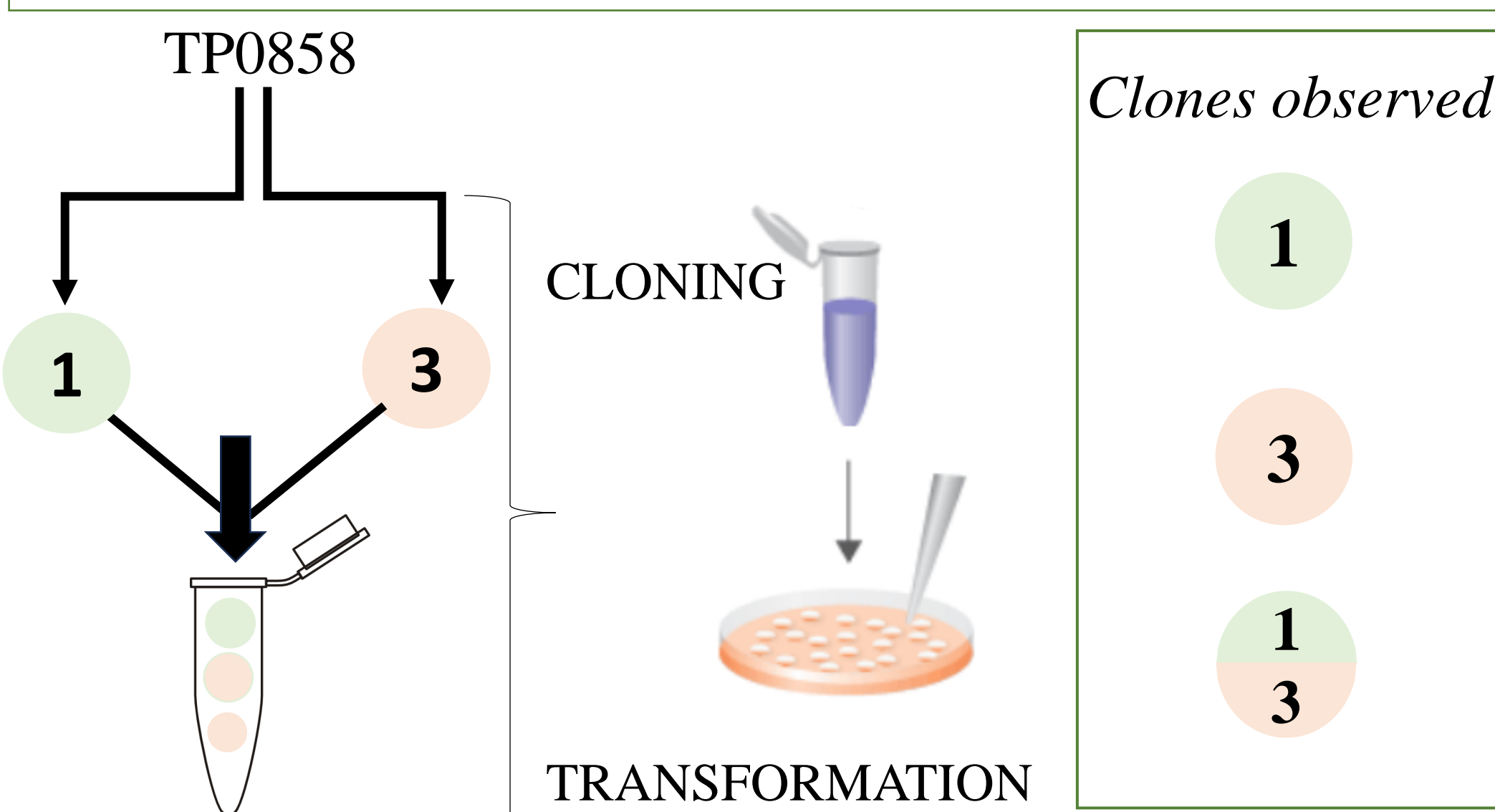
Detection of Coinfection

Coinfection was detected by cloning of PCR products of four genomic loci namely TP0548, TP0326, TP0858 and TP0865. Clones containing amplified PCR products of TP0548 (n=8), TP0326 (n=23), TP0858 (n=96) and TP0865 (n=33) were analysed for coinfection



Detection of PCR chimera

To assess whether the cloned PCR product chimeras are due to chimeric template or due to chimeras emerging during PCR amplification, amplification of TP0858 locus was performed with mixture of pure target DNA of both J11 and T13 in the same ratio to mimic the same conditions as in the original coinfecting sample. Under these conditions, 96 clones of TP0858 were analysed



RESULTS

Determination and analysis of sample from coinfecting patient

The coinfection was confirmed by detection of two distinct genotypes (allelic profiles) using cloning of PCR products of four genomic loci including TP0548, TP0326, TP0858, and TP0865. Both TPE allelic profiles J11 and T13 differed in TP0548, TP0326, TP0858, and TP0865, in 10, 7, 32 and 14 nucleotide positions, respectively.

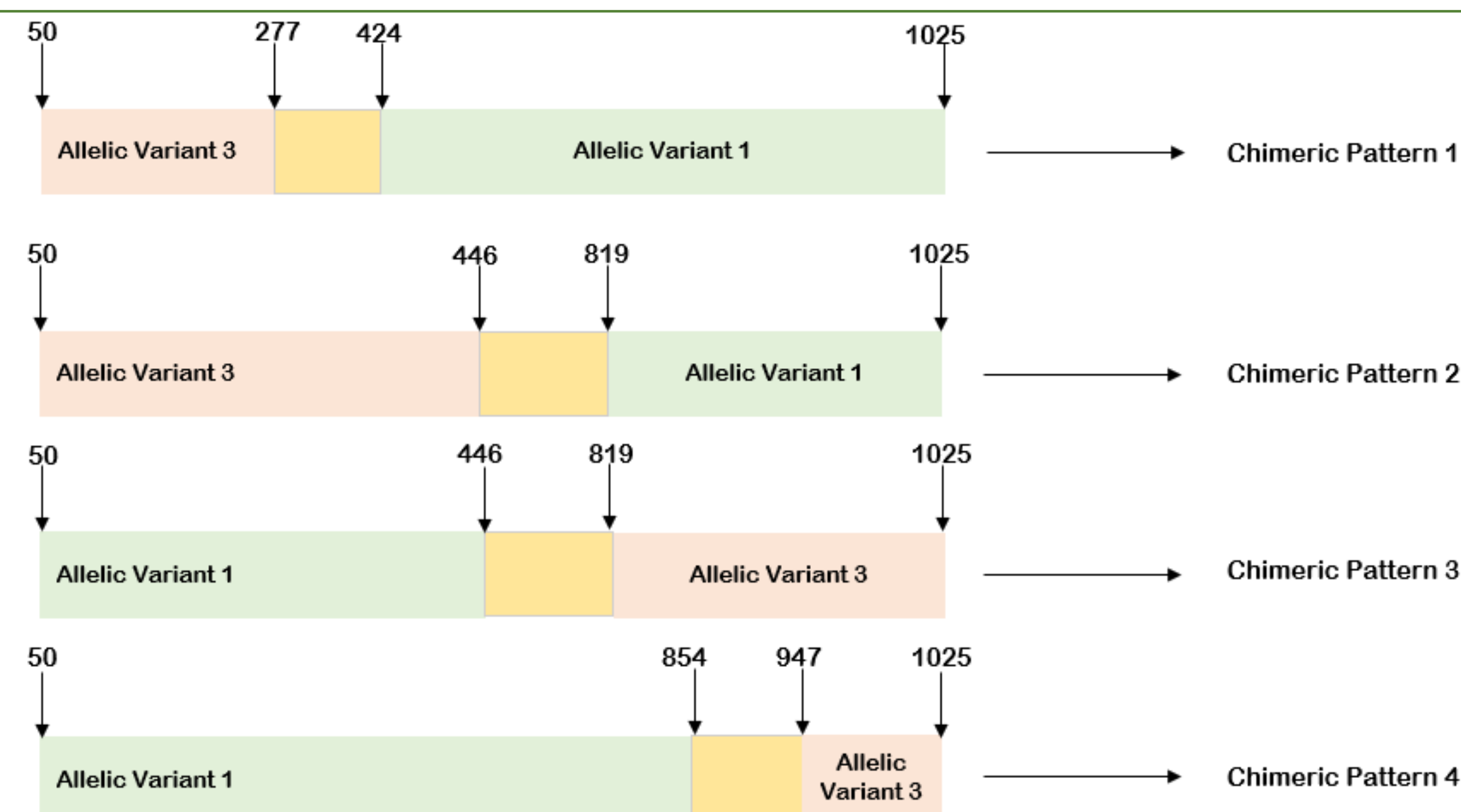
Locus	J	T	J/T
TP0548	n=2	n=3	n=3
TP0326	n=1	n=16	n=6
TP0858	n=17	n=63	n=16
TP0865	n=2	n=22	n=9

Clones containing amplified PCR products of TP0548 (n=8), TP0326 (n=23), TP0858 (n=96) and TP0865 (n=33), consistently revealed co-infection with predominance of AP T13 in comparison to J11 profile. Interestingly, several cloned PCR products resembled molecular chimeras

Allelic variants are determined for each tested locus. A total of 160 clones were screened.

Analysis of PCR product chimeras

A total of 11 chimeric patterns were found of which the predominant four chimeric patterns are depicted below. A total of 96 clones were screened



Yellow blocks represent gene sequences where the transition from one sequence type to the other sequence type has occurred. Chimeric pattern 1 was observed only in PCR products from the clinical sample whereas chimeric patterns 2, 3, and 4 were observed in PCR products prepared from both target DNA preparations and clinical sample.

Acknowledgement

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References

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- [2] Pětrošová H, Zobaniková M, Čejková D, Mikalová L, Pospíšilová P, Strouhal M, et al. Whole Genome Sequence of *Treponema pallidum* ssp. *pallidum*, Strain Mexico A, Suggests Recombination between Yaws and Syphilis Strains. *PLoS Neglected Tropical Diseases*. 2012 Sep 20;6(9): e1832.

Abbreviations

- Papua New Guinea (PNG)
- Allelic profiles (APs)
- *Treponema pallidum* subsp. *pallidum* (TPA)
- *Treponema pallidum* subsp. *pertenue* (TPE)
- *Treponema pallidum* subsp. *endemicum* (TEN)
- *Treponema pallidum* (TP)

ISGlobal Barcelona Institute for Global Health



National Institute of Virology and Bacteriology



DISCUSSION

- The occurrence of coinfection in a single human with two genetically distant strains of TPA, TPE, or TEN is an extremely rare phenomenon.
- Although intrastrain heterogeneity can sometimes mimic coinfection with different strains, it is typically limited to a few nucleotide positions and specific genetic loci.
- While the coinfection of a single individual with two genetically distinct TP strains may have limited epidemiological and clinical implications, its evolutionary potential is of significant importance. Previous research has suggested that coinfection with different TP species and subspecies is necessary for observed recombination events between TP subspecies