The Base "G" in TB86 Primer Frequently Used in the Amplification of *katG* Isoniazid Resistance Gene of *Mycobacterium tuberculosis* is an Insertion

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Abstract: Tuberculosis is a re-emerging disease caused by *Mycobacterium tuberculosis* and isoniazid is the major drug used in chemotherapy to treat this infection. The mutations detected in *katG* gene are usually responsible for resistance in *Mycobacterium tuberculosis*. In our work, we have found that the primer TB86, usually used to amplify the *katG* gene, has an insertion when compared to *katG* gene of H37Rv.

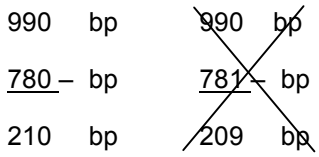
Keywords: *Mycobacterium tuberculosis*, primer TB86 of *katG,* reference strain H37Rv.

Tuberculosis (TB) is an alarming disease well spread in the World and it is responsible for millions of deaths every year. Samples with resistance to at least two major anti-TB drugs, rifampin (RIF) and isoniazid (INH) are characterized as multidrug-resistant TB (MDR-TB) strains and the emergence of new resistant strains has increased the fatality rate to 20% [5]. Patients diagnosed with MDR-TB strains do not have a good prognostic and are considered to be a potential threat. Strains resistant to isoniazid are commonly found around the world, especially in developing countries and this phenotype is associated to a *katG* gene. In our study we used the primer TB86, frequently used by several research groups [1-9]. Primers TB86 and TB87 (Table **1**) are used to amplify *katG* gene from codon 330 to codon 261 (Figure **1**).

Table 1. Primers Used for Amplification and Sequencing of *katG* Gene

Target	Primer	Sequence	Product size	Product size
katG	TB86	5′-GAAACAGCGGCGCTG**G**ATCGT-3′	(209 bp) x	210 bp
	TB87	5′-GTTGTCCCATTTCGTCGGGG-3′		

Primer TB86 consists of a fragment of 21 bp and the 16th base is the nucleotide "**G**", which is an insertion in the sequence. In the present study, twenty-five *Mycobacterium tuberculosis* clinical isolates were amplified using TB86 and it was observed in our sequencing results that insertion of "**G**" is present in all samples. When our results were compared with the H37Rv reference strain the same mismatch was not found (http://blast.ncbi.nlm.nih.gov) (Figure **1**). The sequencing was performed by Macrogen, Korea. Other research groups also reported the product size of 209 bp by using those primers [6, 8]. If we do not consider the additional base pair "**G**" in the primer the product size would be of 210 bp. The complete sequence product per H37Rv has been presented (Figure **1**) we found that the product starts from amino acid Asparagine (N) and ends at amino Glutamic Acid (E).



We believe that different groups may have calculated the size of the product by subtracting 781 of 990. We would like to emphasize that at 990 bp of the codon (330) base "G" is in 990 position, while codon (261) base "C" is in position 781. The subtraction of 781 from 990 shows that the calculation is wrong. If we do it manually, the product size will be 210bp, as shown in Figure (**1**). While the primer TB86 shows an inappropriate insertion and can lead to erroneous description of primer. Here we described two major objection, mathematically wrong calculation and poor design of primer. An appropriate primer can be designed, a new one, to make the amplification. However, we would like to show the problem to avoid

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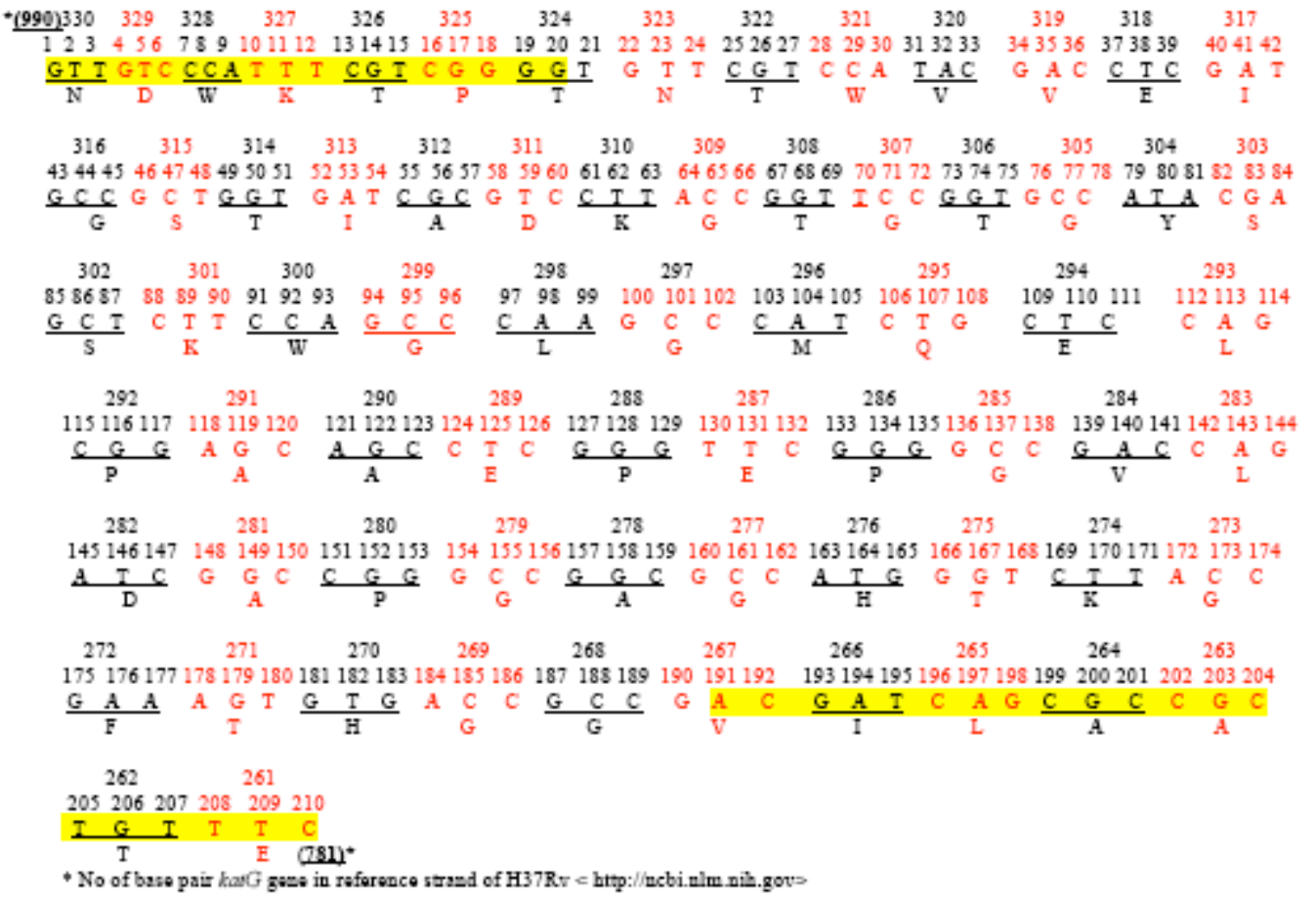

Figure 1: Sequence of *katG* gene showing region of 781bp to 990bp of *Mycobacterium tuberculosis* wild type strain H37Rv.

misunderstanding for other researchers. Actually, many groups are using this primer in their experiments since 1997 to 2010.

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