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

Muhammad Mumtaz Khan, Sajid Ali and Mustafa Kamal\*

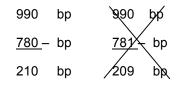
Department of Biotechnology, University of Karachi, Karachi-75270, Pakistan

**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).

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

| Target | Primer | Sequence                             | Product size | Product size |
|--------|--------|--------------------------------------|--------------|--------------|
| katG   | TB86   | 5'-GAAACAGCGGCGCTG <b>G</b> 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

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

<sup>\*</sup>Address corresponding to this author at the Department of Biotechnology, University of Karachi, Karachi-75270, Pakistan; Tel: 0333-3613589; E-mail: mustafacamal@hotmail.com

| Khan | et | al. |
|------|----|-----|
|------|----|-----|

| *(990)330 329 328 327 326 325 324 323 322 321 320 319 318 317  |        |  |  |  |  |  |  |
|--|--------|--|--|--|--|--|--|
| 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42         |        |  |  |  |  |  |  |
| <u>GTTGTCCCA</u> TTT <u>CGT</u> CGG <u>GGT</u> GTT <u>CGT</u> CCA <u>TAC</u> GAC <u>CTC</u> GAT                              | 4<br>- |  |  |  |  |  |  |
| N D W K T P T N T W V V E I  |        |  |  |  |  |  |  |
|  |        |  |  |  |  |  |  |
| 316 315 314 313 312 311 310 309 308 307 306 305 304 303  |        |  |  |  |  |  |  |
| 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 8 | 4      |  |  |  |  |  |  |
| <u>GCCGCTGGT GATCGCGTCCTTACCGGTTCCGGTGCC ATA</u> CGA   | L      |  |  |  |  |  |  |
| GSTIADKGTGTGYS   |        |  |  |  |  |  |  |
|  |        |  |  |  |  |  |  |
| 302 301 300 299 298 297 296 295 294 293  |        |  |  |  |  |  |  |
| 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 11-                     | 4      |  |  |  |  |  |  |
| <u>GCT CTT CCA GCC CAA</u> GCC <u>CAT</u> CTG <u>CTC</u> CAG   |        |  |  |  |  |  |  |
| SKWGLGMQEL   |        |  |  |  |  |  |  |
|  |        |  |  |  |  |  |  |
| 292 291 290 289 288 287 286 285 284 283  |        |  |  |  |  |  |  |
| 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 14       |        |  |  |  |  |  |  |
| <u>COOACCACCTC COOCTTC COOCCCCACCAC</u>  | ř.     |  |  |  |  |  |  |
| PAAEPEPGVL   |        |  |  |  |  |  |  |
| 282 281 280 279 278 277 276 275 274 273  |        |  |  |  |  |  |  |
| 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174      |        |  |  |  |  |  |  |
| <u>A T C G G C C G G G C C G G C C A T G</u> G G T <u>C T T</u> A C C  |        |  |  |  |  |  |  |
| D A P G A G H T K G  |        |  |  |  |  |  |  |
|  |        |  |  |  |  |  |  |
| 272 271 270 269 268 267 266 265 264 263  |        |  |  |  |  |  |  |
| 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204      |        |  |  |  |  |  |  |
| <u>G A A A G T G T G A C C G C C G A C G A T C A G C G C C G C</u>   |        |  |  |  |  |  |  |
| F T H G G V I L A A  |        |  |  |  |  |  |  |
|  |        |  |  |  |  |  |  |
| 262 261  |        |  |  |  |  |  |  |
| 205 206 207 208 209 210  |        |  |  |  |  |  |  |
| T G T T C  |        |  |  |  |  |  |  |
| T E (781)*   |        |  |  |  |  |  |  |
| * No of base pair katG gene in reference strand of H37Rv < http://ncbi.nlm.nih.gov>  |        |  |  |  |  |  |  |

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.

## REFERENCES

- Bostanabad SZ, Bahrmand AR, Poorazar S, *et al.* Mutations in codon 315 of the katG gene associated with high-level resistance to isoniazid. Tanaffos 2007; 6(3): 11-9.
- [2] Bostanabad SZ, Titov LP, Slizen W, Taghikhani M, Bahrmand A. katG mutations in isoniazid-resistant strains of Mycobacterium tuberculosis isolates from Belarusian patients. Tuberkuloz ve Toraks 2007; 55(3): 231-7.
- [3] Bostanabad SZ, Titov LP, Bahrmand A, Nojoumi SA, Detection of mutation in isoniazid-resistant Mycobacterium tuberculosis isolates from tuberculosis patients in Belarus. Indian J Med Microbiol 2008; 26(2): 143-7. http://dx.doi.org/10.4103/0255-0857.40528
- [4] Bostanabad SZ, Titov LP, Karimi A, et al. Molecular characterization and tree evolution of rifampicine and isoniazid-resistance in multi drug resistance strains isolated from primary and secondary tuberculosis diseases in

southern endemic border of Iran. Turk Resp J 2008; 9(1): 24-33.

- [5] Helal ZH, Gomaa FAM, Shehata MMK. Effect of low dose of gamma radiation on multidrug resistant Mycobacterium tuberculosis. J Am Sci 2010; 6(10): 774-80.
- [6] Molina-Torres CA, Moreno-Torres E, Ocampo-Candiani J, et al. Mycobacterium tuberculosis spoligotypes in Monterrey, Mexico. J Clin Microbiol 2010; 48(2): 448-55. http://dx.doi.org/10.1128/JCM.01894-09
- [7] Saeed ZB, Nojoumi SA, Krimi MK, et al. Characterization of molecular evolution in multi-drug resistant Mycobacterium tuberculosis in patients with active pulmonary tuberculosis of different regions in Belarus. Biol Med 2009; 1(4): 39-49.
- [8] Telenti A, Honoré N, Bernasconi C, et al. Genotypic assessment of isoniazid and rifampin resistance in Mycobacterium tuberculosis: a blind study at reference laboratory level. J Clin Microbiol 1997; 35(3): 719-23.
- [9] Zakerbostanabad S, Molla KV, Rahimi MK, et al. Multiplemutations in the katG gene of Mycobacterium tuberculosis isolates correlate with high-level of resistance to isoniazid in patients with active pulmonary tuberculosis from Belarus. Iran. J Microbiol 2009; 1(1): 13-21.

Received on 23-01-2012

Accepted on 18-02-2011

Published on 28-02-2012

DOI: http://dx.doi.org/10.6000/1927-5129.2012.08.01.28 © 2012 Khan *et al.*; Licensee Lifescience Global.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<u>http://creativecommons.org/licenses/by-nc/3.0/</u>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.