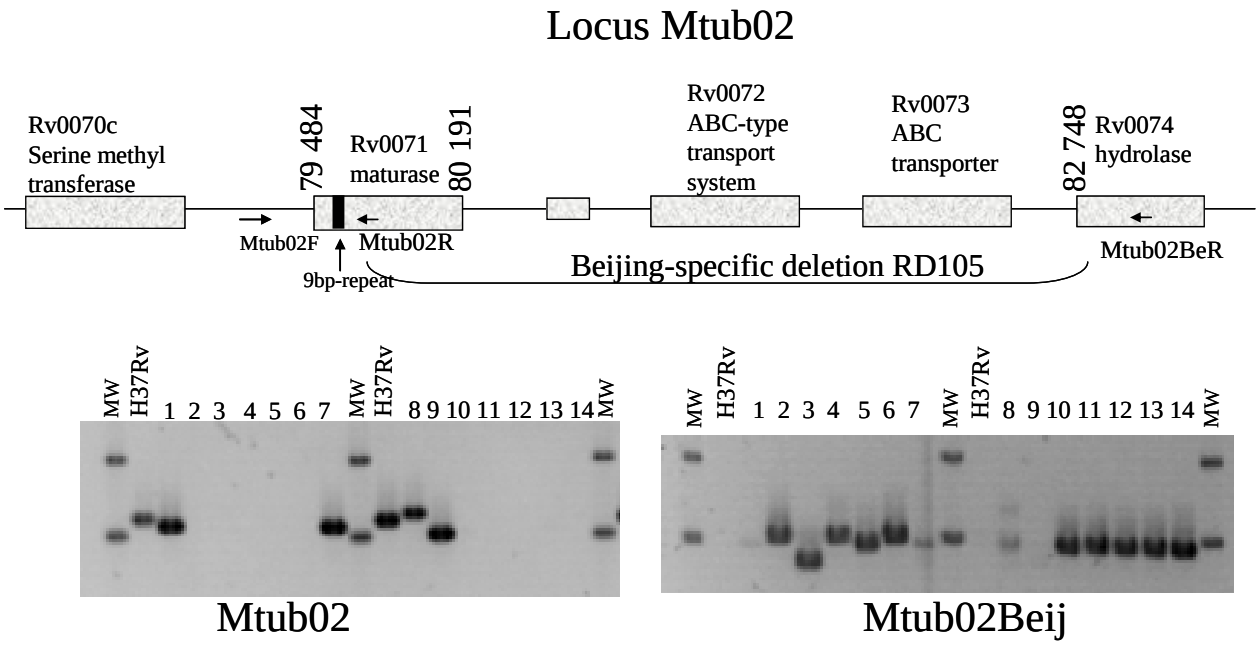
Mtub02/RD105 Analysis----Beijing Family Identification

Principle

TB strains belonging to the Beijing family possess a specific deletion called RD105 by Tsolaki *et al*.(J. Clin. Microbiol. 2005. Vol. 43, p3185-3191) and that we independently observed using primers tagging the Mtub02 VNTR.




```
79441 tttgtcggcg gctggcgggg cgtggcggcg atagcattcg aatatgagtt cgatcacggt
79501 gtcggtggac ccggtggacc cggtggaccc ggtggacccg gtggacccgg tggacgccgt
79561 ggtcgcc                                                  gtgggac
83041 gggcgcgacg gcacgccgcg gccgcgttgc gctccgggat caccacgatt cgcgatctcg
83101 gcgaccgtga ctatgcggcc ttggcgctgc gggaggagta tcggcagaaa acgacggtgg
83161 ggccggaact ggtggtttct gggccaccat tgactcgcag cggcgggcat tgctggttcc
```

By combining two PCRs with primers either flanking the deletion Mtub02Beij (Mub02F + Mtub02BeR) or primers inside the deletion Mtub02 (Mtub02F + Mtub02R) it is possible to quickly separate isolates into Beijing-type and non-Beijing type.

On the figure above the samples 1, 7, 8, and 9 and the control strain H37Rv are non-Beijing type strains. The samples 2, 3, 4, 5, 6, 10, 11, 12, 13, and 14 are Beijing-type strains.

1. PCR amplification
1) Sample DNA
Use 2μl of DNA extract (5ng/μl) per amplification reaction.
2) Primers
(1) Mtub02
L): 5'-CGTGCACAGTTGGGTGTTTA-3'
R): 5'-TTCGTTCAGGAACTCCAAGG-3'
(2) Mtub02Be

Mtub02RevBeijing CGTCGTTTTCTGCCGATACT

3) Preparation of working master mix.

(1) Preparation for the working master mix for 100 reactions

Reagent	Volume(μl)	*100Rns(μl)
10XBuffer(Including $MgCl_2$)	1.5	150
dNTP	1.5	150
5MBetain	3.0	300
Primers(Left and Right mixture)	1.5	100
Taq E (5U/μl)	0.05	5
Distilled Water	5.5	600
Total	13.0	1300

(2) Dispense 13μl of working master mix to each PCR reaction tube.

(3) Add 2μl of DNA samples(5ng/μl) to each reaction vial as given below.

4) Thermal cycling protocol

The cycling protocol is as follows:

94℃ 5min

94℃ 30sec

62℃ 30sec 35cycles

72℃ 45sec

72℃ 10min

20℃ until the gel is loaded for electrophoresis

2. Detection and Analysis of PCR Products by Agarose Gel Electrophoresis(AGE).