Liu et al. (8) assessed the usefulness of five mycobacterial interspersed repetitive-unit (MIRU) loci and RD105 deletion-targeted multiplex PCR (DTM-PCR) (1) to predict M. tuberculosis Beijing strains. They concluded that negative amplification of Mtb02 had sensitivity and specificity comparable to that of DTM-PCR. Mtb02 is a 9-bp repeated sequence that is located 21 bp downstream of the ATG codon of Rv0071 and 25 bp before the RD105 deletion (Fig. 1). This locus repeats five times in H37Rv, whereas variability has been observed in Beijing strains, where the number of repeats has been seen to range from three to nine (13). The primers Liu et al. used for Mtb02 were designed according to the genome of H37Rv (6), and the 3′ end of the reverse primer (TTCGTTCCAGGAACCTCCAAGG) was located in the 48 bp downstream of the RD105 deletion, which explains the negative amplifications in Beijing strains. Thus, the prediction of Beijing strains by Mtb02 is not based on allele variations but on the detection of the RD105 deletion, which is the same result as that determined by RD105 DTM-PCR. Further study indicated that RD105 defines the East Asia lineage of M. tuberculosis and that the Beijing family is a sublineage of the East Asia lineage (4). However, as the RD105-deleted non-Beijing strains have been found very rarely (1, 12, 15), RD105 deletion is still a reliable marker of Beijing strains.

Except in Mtb02 studies, Liu et al. and several previous researchers recommend using single or several MIRU loci to predict Beijing strains (2, 8, 11). However, all these studies represented only a limited diversity of strains of M. tuberculosis. By reviewing previous publications (5, 10, 14) and our unpublished data (MIRU genotyping of 203 Beijing strains collected from Shanghai), we calculated the prediction sensitivities and found large variations among regions (Table 1). The prediction sensitivities of MIRU 26, MIRU 31, and ETR-A are relatively low in all settings, and Mtb30 shows high prediction sensitivities in Chinese and Russian studies but relatively low sensitivity in Japanese studies. The specificities of these methods are affected by the prevalence of non-Beijing strains in the studied populations. According to a previous study (7), the non-Beijing strains in Sichuan province (near Chongqing) mainly belong the European-American lineage, which is genetically distant from the Beijing family and shows MIRU profiles distinct from those of the Beijing family (4). Accordingly, the specificity of using MIRU loci to predict Beijing strains was found to be very high in the study by Liu et al. However, to apply their methods in settings such as Iran and Afghanistan would be problematic, because most of the prevalent strains of Beijing family and Rim of Indian Ocean lineage share the same alleles in MIRU 26, MIRU 31, and ETR-A (3, 9). The convergent evolutions at Mtb30 seen with Beijing family and West Africa lineage strains would also lead to low specificity in applying these methods in settings in Africa (4).

In summary, our analysis does not support using single or several MIRU loci for the prediction and identification of Beijing strains. The most reliable and cost-effective approach would be based on the detection of phylogenetically robust markers such as RD105 and Beijing family-specific single nucleotide polymorphisms (SNPs).

### REFERENCES


### TABLE 1. The sensitivity of four MIRU loci for prediction of Beijing strains in different settings

<table>
<thead>
<tr>
<th>Locus</th>
<th>Prediction sensitivity by region (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chongqing (n = 130)</td>
</tr>
<tr>
<td>MIRU 26</td>
<td>84.6</td>
</tr>
<tr>
<td>MIRU 31</td>
<td>83.1</td>
</tr>
<tr>
<td>ETR-A</td>
<td>87.7</td>
</tr>
<tr>
<td>Mtb30</td>
<td>97.7</td>
</tr>
</tbody>
</table>

* The values representing prediction sensitivity in Chongqing are according to the study by Liu et al. (8). For Shanghai, sensitivities were calculated according to our unpublished data. For Beijing, Japan, and Russia, sensitivities were calculated according to data from references 5, 14, and 10, respectively.


