

## Original Article

**Molecular epidemiology and drug resistance of *Mycobacterium tuberculosis* in a tertiary care hospital in northeastern Mexico**

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**Abstract**

**Introduction:** Tuberculosis (TB) is a re-emerging disease considered a public health concern. In the present study, we analyzed the epidemiology and drug resistance of *Mycobacterium tuberculosis* strains isolated from patients with pulmonary TB.

**Methodology:** *Mycobacterium tuberculosis* isolates ( $n = 190$ ) were obtained from patients with pulmonary TB admitted to Dr. José Eleuterio González University Hospital (UH). Each *M. tuberculosis* isolate was analyzed by spoligotyping (spacer oligonucleotide typing) and MIRU-VNTR (Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeat). Drug resistance was evaluated using the Anyplex™ II MTB/MDR/XDR assay.

**Results:** The predominant spoligotypes observed were X1 (SIT 119,  $n = 46$ ), T1 (SIT 53,  $n = 40$ ), H3 (SIT 50,  $n = 13$ ), Beijing (SIT 1,  $n = 11$ ), and EAI2-Manila (SIT 19,  $n = 8$ ). MIRU-VNTR analysis showed that the locus QUB-26 had the highest allelic variability. The observed drug resistance included monoresistance to rifampicin (2.6%;  $n = 5$ ), isoniazid (3.2%;  $n = 6$ ), and fluoroquinolones (1.6%;  $n = 3$ ) as well as multidrug resistance (5.3%;  $n = 10$ ). All of the Beijing strains were susceptible. Regarding comorbidities, 13.7% (26/190) of the patients were co-infected with TB and HIV (TB<sup>+</sup>HIV<sup>+</sup>), and 31.6% (55/190) had TB along with diabetes (TB + diabetes).

**Conclusions:** The most prevalent lineages were X1 (SIT 119; 24.3%) and T1 (SIT 53; 21%). An alarming proportion (12.6%) of *M. tuberculosis* isolates presented drug resistance. To effectively manage TB, continuous surveillance of regional strain dissemination, drug resistance profiles, and TB-associated comorbidities is crucial.

**Key words:** *Mycobacterium tuberculosis*; spoligotyping; MIRU-VNTR; drug resistance.

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**Introduction**

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* [1,2]. TB is the second leading cause of death by a single infectious disease after COVID-19 [2,3]. In 2021, the World Health Organization (WHO) estimated that there were 10.6 million new cases of TB and 1.6 million TB-related deaths worldwide [2]. Approximately 2 billion individuals experience latent TB infections; 5%–10% of these individuals will develop active TB during their lifetime [4,5]. In 2019, of the 22,285 new cases of TB reported in Mexico, 80% were pulmonary TB. The national incidence of pulmonary TB was 14.1 new cases per 100,000 inhabitants [6].

The risk factors favoring infection and progression to active TB disease [2,7,8] include a compromised immune system (such as that in patients with HIV/AIDS) and the incidence of multidrug-resistant *M. tuberculosis* strains, as well as malnutrition, diabetes mellitus, tobacco smoking, poverty, undocumented migration [7,8], and social disparities. Undiagnosed cases and non-adherence to treatment have perpetuated TB as a significant public health challenge.

There are two major genotyping techniques used to identify and track *M. tuberculosis* strains. One of these is spoligotyping (spacer oligonucleotide typing); a rapid genotyping method for *M. tuberculosis* that uses the principle of reverse hybridization. It targets the highly

polymorphic direct repeat (DR) locus [9,10]. The other technique is mycobacterial interspersed repetitive units–variable number tandem repeats (MIRU-VNTR); this method determines the number and size of repeats at each locus by PCR amplification of the interspersed repetitive units [10]. The timely detection of patients with TB and the identification of relationships between *M. tuberculosis* strains will help to control the spread of TB infection in the population. In the present study, we analyzed the molecular epidemiology of *M. tuberculosis* strains using spoligotyping and MIRU-VNTR techniques and determined the drug-resistance patterns of isolates collected from a tertiary health care hospital in Northeast Mexico.

## Methodology

### *Ethics statement*

The local ethics committee of the Dr. Jos  Eleuterio Gonz lez University Hospital (UH) in Monterrey, Mexico, approved this study (approval no. PC18-00001).

### *Study population and data collection*

Each patient read and signed an informed consent form. The study comprised 190 samples reported as culture-positive and acid-fast bacillus positive from male and female patients with pulmonary TB attending the Clinical Pathology Department of Dr. Jos  Eleuterio Gonz lez University Hospital (UH), from August 2017 to June 2021. Only samples from cases of pulmonary TB were included in this study; all extrapulmonary TB samples were excluded. Medical records collected at the time of sampling for each TB+ patient included age, HIV status, and blood glucose level. Based on these data, patients were classified as HIV-positive or as having diabetes in accordance with the American Diabetes Association's recommendation, which defines diabetes as a fasting blood glucose level of 126 mg/dL or higher. No additional testing or follow-up was conducted. The age range of the patients spanned from 4 months to 97 years.

### *Sample collection and microbiological detection*

Samples were collected in a sterile and leak-proof container and processed using the Petroff-Hausser counting chamber method. Acid-fast bacilli were identified by Ziehl–Neelsen staining and cultivated on L wenstein–Jensen medium [11].

### *DNA extraction*

Bacterial colonies were resuspended in molecular-grade water and inactivated in a dry bath at 100 C for

90 min. DNA was extracted using the Isolate II Genomic DNA Kit (Bioline, Memphis, TN, USA) in accordance with the manufacturer's instructions. DNA was quantified using a NanoDrop spectrophotometer (8000, Thermo Fisher Scientific, Waltham, MA, USA), adjusted to a concentration of 20 ng/ L with nuclease-free water, and stored at –20  C.

### *Spoligotyping*

Spoligotyping assays were performed using the method described by Kamerbeek *et al.* [12]. Briefly, the PCR reaction was performed using GoTaq<sup> </sup> Green Master Mix (Promega, Madison, WI, USA), molecular-grade water (Corning, New York, NY, USA), and oligonucleotides for spoligotyping provided by Mapmygenome (Hyderabad, India) (DRa 5'-GGTTTTGGGTCTGACGAC-3' and DRb 5'-CCGAGAGGGGACGGAAAC-3'). Positive and negative control samples were included in each run. The thermocycling conditions were as follows: 3 min at 96  C; 30 cycles of 1 minute at 96  C, 1 minute at 55  C, and 30 seconds at 72  C; and a final extension step of 5 minutes at 72  C. PCR products were hybridized using a commercial membrane (MapMyGenome) containing a set of 43 spacer probes in accordance with the manufacturer's instructions. The hybridization profile was revealed via chemiluminescence using an enhanced chemiluminescence detection system and X-ray film (Hyperfilm ECL, Amersham Biosciences, Little Chalfont, UK). The obtained profiles were compared against the international spoligotype database SITVIT2 of the Pasteur Institute of Guadeloupe (<http://www.pasteur-guadeloupe.fr:8081/SITVIT2/>).

### *MIRU-VNTR*

The MIRU-VNTR genotyping technique was performed using the protocol provided on the MIRU-VNTRplus platform (<https://www.miru-vntrplus.org/MIRU/in-dex.faces>). The PCR amplification of the different regions was carried out with specific primers for each of the following 15 loci: 424, 577, 580, 802, 960, 1644, 1955, 2163b, 2165, 2401, 2996, 3192, 3690, 4052, and 4156. Primer sequences are shown in Supplementary Table 1.

PCR was performed using GoTaq<sup> </sup> Green Master Mix (5  L), specific primers for each region (0.5  L of primer A and 0.5  L of primer B), molecular-grade water (8  L), and DNA at a concentration of 20 ng/ L (5  L) in a final reaction volume of 19  L.



The volume of primers for locus 4156 was increased to 1  $\mu$ L, maintaining a final volume of 20  $\mu$ L. As a positive control, we used DNA from the *M. tuberculosis* H37Rv strain, and as a negative control, we used molecular-grade water. Amplification cycles included initial denaturation at 96  $^{\circ}$ C for 15 minutes; 40 cycles of denaturation at 96  $^{\circ}$ C for 1 minute, annealing at 55  $^{\circ}$ C for 1 minute, and extension at 72  $^{\circ}$ C for 1 minute; and a final extension step at 72  $^{\circ}$ C for 1 minute.

Gel images were analyzed using the VisionWorks software (*LTF Labortechnik GmbH & Co. KG, Wasserburg, Bodensee, Germany*). The number of tandem repeats per allele was calculated after the molecular weight was determined [13], and the patterns of the 15 MIRU-VNTR loci were obtained.

#### Genetic drug-resistance testing

To determine genetic mutations conferring drug resistance, we used the multiplex real-time PCR assay Anyplex<sup>TM</sup> II MTB/MDR/XDR Detection (Seegene Inc., Seoul, South Korea). Resistance to isoniazid, rifampicin, fluoroquinolones, amikacin, kanamycin, and capreomycin was determined.

Each reaction tube contained PCR mix (5  $\mu$ L), MTB/MDR or MTB/XDR primer mix (5  $\mu$ L), molecular-grade water (5  $\mu$ L), and DNA (5  $\mu$ L), resulting in a final volume of 20  $\mu$ L. MTB/MDR primer mix was used to detect multidrug-resistant TB isolates, and the MTB/XDR primer mix was used to detect extensively drug-resistant TB isolates in two independent tubes. We used the wild-type and negative controls included in the kit.

Real-time PCR was run in the CFX96<sup>TM</sup> real-time thermal cycler (Bio-Rad, Hercules, CA) with the following program: initial denaturation at 95  $^{\circ}$ C for 15 minutes; 50 cycles of denaturation at 95  $^{\circ}$ C for 30 seconds, annealing at 60  $^{\circ}$ C for 1 minute, and extension at 72  $^{\circ}$ C for 30 seconds; and a final extension at 55  $^{\circ}$ C for 1 minute. The melting curve analysis was performed

over a temperature range of 55  $^{\circ}$ C to 85  $^{\circ}$ C, with a ramp rate of 0.5  $^{\circ}$ C per 5 seconds. The obtained data were analyzed using the Seegene Viewer software.

## Results

### Data collection

The age distribution of the patient sample cohort is as follows: 14 patients aged 0–20 years; 32 patients aged 21–30 years; 50 patients aged 31–40 years; 30 patients aged 41–50 years; 29 patients aged 51–60 years; and 23 patients aged over 61 years. The average age was 41.7 years. Age data was missing for 12 patients. In the cohort of patients with TB, the gender distribution was 30% female ( $n = 57$ ) and 70% male ( $n = 133$ ) (Table 1).

The distribution of the comorbidities was as follows: 26 patients (13.7%) were co-infected with pulmonary TB and HIV, while the remaining 164 patients (86.3%) were HIV-negative. Regarding the comorbidity of TB and diabetes, 55 patients (28.9%) were diagnosed with both TB and diabetes, while 135 patients (71.1%) had TB without concurrent diabetes. Only 5 patients with TB (2.6%) had both HIV and diabetes as comorbidities (Table 1).

### Spoligotyping

Table 2 displays the 42 spoligotype patterns identified among the 190 *M. tuberculosis* strains analyzed in this study, along with the corresponding clade assignments for each pattern, as per the SITVIT2 database. The three predominant clades were X1 (SIT 119) with 46 isolates (24.3%), T1 (SIT 53) with 40 isolates (21.0%), and H3 (SIT 50) with 13 isolates (6.8%). The fourth most prevalent clade was Beijing (SIT 1), comprising 11 isolates (5.8%), while the fifth was EAI2-Manila (SIT 19) with 8 isolates (4.2%).

### MIRU-VNTR analysis

The MIRU-VNTR analysis indicated that the QUB26 locus exhibited the highest allelic diversity, conferring it with the highest discriminatory capability. By contrast, the MIRU04 locus exhibited lower discriminatory power. The genotypes identified using the MIRU-VNTR analysis were the X ( $n = 46$ ; 24.2%), Ghana ( $n = 16$ ; 8.4%), Beijing ( $n = 11$ ; 5.8%), EAI ( $n = 9$ ; 4.7%), S ( $n = 7$ ; 3.7%) Haarlem ( $n = 6$ ; 3.2%), Uganda ( $n = 5$ ; 2.6%), LAM ( $n = 4$ ; 2.1%).

A distinct MIRU-VNTR profile was identified in 49 (25.8%) of the isolates, while 37 samples (19.4%) failed to amplify (Table 3 and Supplementary Table 2).

**Table 3.** Lineages of *M. tuberculosis* obtained by MIRU-VNTR.

| Lineage | <i>n</i> | %    |
|---------|----------|------|
| X       | 46       | 24.2 |
| Ghana   | 16       | 8.4  |
| Beijing | 11       | 5.8  |
| EAI     | 9        | 4.7  |
| S       | 7        | 3.7  |
| Haarlem | 6        | 3.2  |
| Uganda  | 5        | 2.6  |
| LAM     | 4        | 2.1  |
| Unique  | 49       | 25.8 |
| NA      | 37       | 19.5 |
| Total   | 190      | 100  |

NA: no amplification.

**Drug resistance**

Anyplex™ assays indicated that 12.6% (*n* = 24) of the samples exhibited drug resistance. Table 2 presents the specific drug-resistance profiles of the isolates. Monoresistance was observed in 7.4% (*n* = 14) of the strains, dual resistance in 4.2% (*n* = 8), and triple resistance in 1% (*n* = 2). Monoresistance to rifampicin (*rpoB*; 2.6%; *n* = 5), isoniazid (*katG*; 1.6%; *n* = 3), isoniazid (*inhA*; 1.6%; *n* = 3), fluoroquinolone (*gyrA*; 1.6%; *n* = 3), and isoniazid (*katG, inhA*; 0.5%; *n* = 1) was observed. Multidrug resistance to rifampicin (*rpoB*) and isoniazid (*katG*; 3.7%; *n* = 7) and to rifampicin (*rpoB*), isoniazid (*katG*), and fluoroquinolones (*gyrA*; 1; *n* = 2) was noted (Table 4). Table 5 illustrates the correlation between spoligotyping clades and resistance profiles.

**Discussion**

The genetic variability of *M. tuberculosis* is associated with its geographic distribution, epidemiology, virulence, and drug resistance patterns [2]. The primary aim of the present study was to analyze the diversity of *M. tuberculosis* strains collected from patients with pulmonary TB in a tertiary care hospital using spoligotyping and MIRU-VNTR. The Dr. Jos  Eleuterio Gonz lez University Hospital (UH) is a regional hospital providing care to individuals from the northeastern states of Mexico, including Tamaulipas, Nuevo Le n, Coahuila, and San Luis Potos . Our research identified that the lineages X1 and T1 are the most prevalent. In 12.6% of all *M. tuberculosis* isolates,

**Table 4.** Drug-resistance pattern of *M. tuberculosis* isolates.

| Type of resistance  | n (%)     |
|---|-----------|
| <b>Monoresistance</b>   |           |
| RIF ( <i>rpoB</i> )   | 5 (2.6)   |
| INH-R1 ( <i>katG</i> )  | 3 (1.6)   |
| INH-R2 ( <i>inhA</i> )  | 3 (1.6)   |
| FQ ( <i>gyrA</i> )  | 3 (1.6)   |
| INH-R1 ( <i>katG</i> ), INH-R2 ( <i>inhA</i> )                  | 7 (3.7)   |
| <b>MDR</b>  |           |
| RIF ( <i>rpoB</i> ), INH-R1 ( <i>katG</i> )                     | 1 (0.5)   |
| RIF ( <i>rpoB</i> ), INH-R1 ( <i>katG</i> ), FQ ( <i>gyrA</i> ) | 2 (1)     |
| Subtotal  | 24 (12.6) |

RIF: rifampicin; INH: isoniazid; FQ: fluoroquinolones.

drug resistance was detected to at least one or multiple antitubercular drugs. Additionally, diabetes emerged as a significant comorbidity among TB patients within our study population.

In this study, spoligotyping revealed that the most prevalent families in the population were SIT 119 (*n* = 46; 24.2%), SIT 53 (*n* = 45; 23.7%), SIT 50 (*n* = 13; 6.8%), and SIT 1 (Beijing *n* = 11; 5.8%). In a prior study, Molina-Torres *et al.* observed that within the same region, the two predominant spoligotypes were SIT 53 (23.8%) and SIT 119 (15.5%) [14]. Vera *et al.* found that spoligotype SIT 53 was the most prevalent in Nuevo Le n, while SIT 119 was the dominant spoligotype in central Mexico [15]. Flores-Trevi o *et al.* [16] reported that in western Mexico (Guadalajara, Jalisco), the Haarlem and LAM lineages were predominant in *M. tuberculosis clinical isolates*.

In a recent study by Molina-Torres *et al.* [17] conducted in a Mexican–Amerindian population from

**Table 5.** *M. tuberculosis* clades and drug resistance.

| Clade        | n  | Strain resistant | %    |
|--------------|----|------------------|------|
| X1           | 46 | 11               | 23.9 |
| T1           | 45 | 3                | 6.7  |
| T2           | 6  | 3                | 50   |
| H3           | 13 | 2                | 15.4 |
| T3 T2        | 5  | 2                | 40   |
| LAM          | 5  | 1                | 20   |
| X3           | 7  | 1                | 14.3 |
| X            | 4  | 1                | 25   |
| Beijing      | 11 | 0                | 0    |
| Orphan       | 11 | 0                | 0    |
| S            | 7  | 0                | 0    |
| EAI2 Manilla | 6  | 0                | 0    |
| X2           | 5  | 0                | 0    |
| LAM 9        | 6  | 0                | 0    |
| LAM 2        | 3  | 0                | 0    |
| H3           | 2  | 0                | 0    |
| LAM 3        | 2  | 0                | 0    |
| LAM 5        | 2  | 0                | 0    |
| T5 Madrid    | 1  | 0                | 0    |
| LAM 8        | 1  | 0                | 0    |
| LAM 1        | 1  | 0                | 0    |
| MANU 2       | 1  | 0                | 0    |

Los Altos, Chiapas, Mexico, SIT 53 was identified as the most predominant strain, while SIT 119 ranked third in prevalence. Our data showed similar trends in strain distribution; however, in contrast to the findings of Molina-Torres *et al.*, SIT 119 emerged as the primary spoligotype in our study. As mentioned, patients receiving care at the UH come from multiple states, leading to a more diverse demographic than would be seen in a small, localized town.

Our analysis revealed that 11 (5.8%) of the 190 isolates were classified as part of the Beijing lineage. These isolates were all devoid of the RD207 region, which encompasses spacers 1–34, and exhibited the distinctive hybridization pattern of spacers 35–43, thus presenting the unique spoligotype signature associated with Beijing strains. The Beijing lineage, with a global distribution, is associated with increased transmissibility, enhanced virulence, and elevated levels of drug resistance [18,19]. Vera-Cabrera *et al.* [15] reported 7 (1.7%) Beijing strains in 414 collected samples from 12 Mexican states, and Flores-Trevi o *et al.* [16] reported a 1.5% (1/68) prevalence of Beijing strains in the city of Guadalajara, located in western Mexico. A report on Beijing strains in Latin America showed that most countries had a low presence of this strain ( $\leq 5\%$ ), except for Cuba and Peru, where the prevalence was higher. Continued surveillance of the genotypes of *M. tuberculosis* strains in the region is critical. Furthermore, epidemiological research on this lineage in Latin America is necessary to track the dissemination of Beijing strains and enhance TB control programs in alignment with the WHO's 2035 targets [2].

TB affects individuals of all ages. The gender distribution of patients with TB in this study aligns with the global distribution reported by the WHO, with 37% female and 63% male patients [2]. In Mexico, the prevalence of TB infection is 31% among women and 69% among men [6]. Bello *et al.* analyzed surveillance data from 2007 to 2017 in Mexico and reported a higher number of cases in men, which also increased during that period [20]. This difference is frequently attributed to biological and epidemiological factors, as well as socioeconomic and cultural barriers that impede access to healthcare.

The incidence and prevalence of TB are indicative of extreme poverty and gender inequality. As stated in the technical report by The Global Fund [21], males represent 64% of global TB cases. The observed rise in TB incidence among men has been partly ascribed to occupational hazards, as they are frequently employed in high-risk sectors such as construction and mining.

Moreover, the risk is compounded for those who smoke or consume alcohol or drugs. [22]

TB comorbidities pose a challenge to the control of the disease. Patients with HIV often present with TB co-infection. As these patients have a suppressed immune system, their risk of developing TB is increased. In 2021, the WHO [2] estimated that 1.6 million deaths were attributable to TB, of which 187,000 occurred in patients co-infected with HIV. We found that 13.7% ( $n = 26$ ) of the isolates originated from HIV-positive patients, exceeding the prevalence reported by the WHO in 2021 [2].

TB-diabetes comorbidity is significant because diabetes mellitus increases the risk of developing active TB by 2–3 times and is also associated with poorer TB treatment outcomes [23].

The Health and Nutrition National Survey [24] reported that 10.3% of individuals with TB in Mexico also had diabetes, while the WHO indicated that globally, 3.5% of TB patients were affected by diabetes. In our study, 28.9% of patients were co-infected with TB and diabetes (TB+DM+), which is three times higher than the prevalence reported in Mexico and almost nine times higher than the global prevalence reported by the WHO [2].

The Anyplex<sup>TM</sup> test identified a 12.6% drug resistance rate ( $n = 24$ ) in the samples from patients treated at the UH. Zenteno-Cuevas *et al.* [24] reported on drug-resistant *M. tuberculosis* strains isolated in Veracruz City. They observed resistance to at least one first-line drug in 60 isolates (30%): streptomycin in 36 isolates (18%), isoniazid in 52 (26%), rifampicin in 41 (20.5%), ethambutol in 19 (9.5%), and pyrazinamide in 25 (12.5%). Differences in the percentages of drug-resistant strains may be attributed to the varying prevalence of these strains in the two different regions of the country. Veracruz City, a port on the Gulf of Mexico, reports the highest incidence of drug-resistant TB and multidrug-resistant TB in both the state and the country [24]. Anyplex<sup>TM</sup> is a molecular method that simultaneously detects 25 mutations associated with multidrug-resistant tuberculosis (MDR-TB) and 13 mutations associated with extensively drug-resistant tuberculosis (XDR-TB). However, phenotypic detection of drug resistance using the mycobacterial growth indicator tube (MGIT) system, or the agar proportion method, will be necessary to corroborate these molecular results.

We analyzed samples from 190 patients using MIRU-VNTR genotyping. Of the samples tested, 153 exhibited amplification, while 37 did not. QUB 26 was the locus exhibiting the highest allelic diversity

according to this analysis, making it the most discriminatory locus. Moreover, QUB 26 was reported as the most discriminatory locus in both Veracruz City [24] and Colombia [10]. Although these reports from various regions in Latin America concur that QUB 26 is a discriminatory locus for *M. tuberculosis*, further studies are necessary to substantiate this finding.

Using MIRU-VNTR analysis, we determined that 49 (25.8%) of the isolates exhibited unique MIRU-VNTR patterns. The predominant *M. tuberculosis* lineages reported in Veracruz City differed from those we observed [24]: H (32%), T (23%), LAM (18%), and X (12%). The predominant *M. tuberculosis* lineages in Colombia [10] were LAM (54.8%) and Haarlem (25.8%). These differences confirm that the regional distribution of *M. tuberculosis* lineages is influenced by factors that remain incompletely understood.

Genotyping using spoligotyping and MIRU-VNTR techniques has enabled the determination of the predominant *M. tuberculosis* lineages in our region and the association of specific lineages with particular clinical conditions. Further studies involving whole-genome sequencing (WGS) of each *M. tuberculosis* strain would yield comprehensive data to analyze genomic variations and track the distribution of these isolates within the population. The sample size in this study is limited and not representative of the entire *M. tuberculosis* population in the region or country. However, it does contribute to the epidemiological surveillance of *M. tuberculosis* lineages in our area.

## Conclusions

Despite concerted efforts to control tuberculosis (TB), the disease continues to pose a significant public health challenge. This study demonstrated that the predominant lineages were X1 (SIT 119; 24.3%) and T1 (SIT 53; 21%). Of the *M. tuberculosis* isolates studied, 12.6% exhibited drug resistance. In our population, diabetes is a common comorbidity among patients with TB. To effectively manage tuberculosis (TB), continuous surveillance of regional strain dissemination, drug resistance profiles, and TB-associated comorbidities is crucial.

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## Authors' contributions

All authors have read and approved the manuscript. Conceptualization: AF, LR. Formal analysis: AF, CV, RT. Supervision: AF, LR, JL, CR. Validation: AF, JZ, CV, NC. Writing: AF, CV. Original draft: AF, CV. Project administration: JZ, CV, JV. Investigation: JZ, CV, CR. Methodology: CV. Editing: CV. Writing, reviewing, and editing: CV, RT, JC. Funding acquisition: MO, JL. Resources: MO, JL. Visualization: CV, JC. Data curation: CV, NC. Software: CV.

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**Annex – Supplementary Items**

**Supplementary Table 1.** Primer sequences used in MIRU-VNTR genotyping.

| <i>Locus</i> | <b>Primer sequences</b>  |
|--------------|--|
| 424          | 5'CTTGCCGGCATCAAGCGCATTATT 3'<br>5'GGCAGCAGAGCCCGGATTCTTC 3'   |
| 577          | 5'CGAGAGTGGCAGTGGCGGTATCT 3'<br>5'AATGACTTGAACGCGCAAATTGTGA 3' |
| 580          | 5'GCGCGAGAGCCCGAACTGC 3'<br>5'GCGCAGCAGAAACGCCAGC 3'           |
| 802          | 5'GGTGTCTGGATGACAACGTGT 3'<br>5'GGGTGATCTCGGCGAAATCAGATA 3'    |
| 960          | 5'GTTCTTGACCAACTGCAGTCGTCC 3'<br>5'GCCACCTTGGTGATCAGCTACCT 3'  |
| 1644         | 5'TCGGTGATCGGGTCCAGTCCAAGTA 3'<br>5'CCCGTCGTGCAGCCCTGGTAC 3'   |
| 1955         | 5'AGATCCAGTTGTCGTCGTC 3'<br>5'CAACATCGCCTGGTCTGTGA 3'          |
| 2163b        | 5'CGTAAGGGGGATGCGGGAAATAGG 3'<br>5'CGAAGTGAATGGTGGCAT 3'       |
| 2165         | 5'AAATCGGTCCCATCACCTTCTTAT 3'<br>5'CGAAGCCTGGGGTGCCCGGATTT 3'  |
| 2401         | 5'CTGAAGCCCCGGTCTCATCTGT 3'<br>5'ACTTGAACCCCGAGCCATTAGTA 3'    |
| 2996         | 5'TAGGTCTACCGTCGAAATCTGTGAC 3'<br>5'CATAGGCGACCAGGCGAATAG 3'   |
| 3192         | 5'ACTGATTGGCTTCATACGGCTTTA 3'<br>5'GTGCCGACGTGGTCTTGAT 3'      |
| 3690         | 5'CGGTGGAGGCGATGAACGTCTTC 3'<br>5'TAGAGCGGCACGGGGAAAGCTTAG 3'  |
| 4052         | 5'AACGCTCAGCTGTCGGAT 3'<br>5'CGGCCGTGCCGCCAGGTCCTTCCCGAT 3'    |
| 4156         | 5'TGACCACGGATTGCTTAGT 3'<br>5'GCCGGCTCCATGTT 3'                |

**Supplementary Table 2.** MIRUVNTR CODE octal database.

| <b>ID</b> | <b>424</b> | <b>577</b> | <b>580</b> | <b>802</b> | <b>960</b> | <b>1644</b> | <b>1955</b> | <b>2163b</b> | <b>2165</b> | <b>2401</b> | <b>2996</b> | <b>3192</b> | <b>3690</b> | <b>4052</b> | <b>4156</b> | <b>MIRUVNTR CODE</b> |
|-----------|------------|------------|------------|------------|------------|-------------|-------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|----------------------|
| 1         | 2          | 3          | 2          | 5          | 4          | 3           | 4           | 4            | 3           | 4           | 5           | 3           | 6           | 8           | 3           | 232543443453683      |
| 4         | 4          | 6          | 3          | 1          | 1          | 3           | 2           | 5            | 4           | 2           | 5           | 4           | 4           | 4           | 11          | 4631132542544112     |
| 6         | 3          | 4          | 3          | 4          | 7          | 3           | 2           | 3            | 3           | 2           | 5           | 4           | 2           | 9           | 3           | 343473233254293      |
| 7         | 3          | 3          | 2          | 5          | 3          | 3           | 4           | 3            | 3           | 4           | 4           | 3           | 3           | 6           | 1           | 332533433443361      |
| 14        | 2          | 5          | 2          | 3          | 0          | 3           | 2           | 5            | 3           | 4           | 4           | 3           | 4           | 7           | 2           | 252303253443472      |
| 15        | 2          | 3          | 2          | 4          | 1          | 3           | 1           | 3            | 3           | 4           | 4           | 3           | 4           | 3           | 2           | 232413133443432      |
| 17        | 2          | 3          | 2          | 5          | 4          | 3           | 4           | 4            | 3           | 4           | 5           | 3           | 6           | 8           | 3           | 232543443453683      |
| 18        | 2          | 5          | 2          | 3          | 1          | 2           | 1           | 6            | 3           | 4           | 3           | 2           | 1           | 5           | 3           | 252312163432153      |
| 19        | 2          | 5          | 2          | 3          | 8          | 10          | 2           | 4            | 2           | 2           | 2           | 5           | 3           | 5           | 3           | 2523810242225353     |
| 21        | 2          | 6          | 3          | 3          | 4          | 6           | 2           | 5            | 4           | 2           | 2           | 5           | 4           | 2           | 2           | 263346254225422      |
| 22        | 1          | 4          | 5          | 2          | 4          | 3           | 10          | 9            | 4           | 2           | 2           | 4           | 2           | 7           | 1           | 1452431094224271     |
| 23        | 2          | 3          | 2          | 5          | 4          | 3           | 4           | 4            | 3           | 4           | 5           | 3           | 6           | 8           | 3           | 232543443453683      |
| 24        | 5          | 3          | 2          | 4          | 6          | 6           | 3           | 3            | 3           | 5           | 6           | 3           | 3           | 1           | 3           | 532466333563313      |
| 25        | 4          | 4          | 2          | 3          | 4          | 7           | 3           | 4            | 2           | 3           | 4           | 4           | 3           | 2           | 2           | 442347342344322      |
| 26        | 1          | 4          | 5          | 2          | 4          | 3           | 10          | 9            | 4           | 2           | 2           | 4           | 2           | 7           | 1           | 1452431094224271     |
| 27        | 2          | 3          | 2          | 5          | 4          | 3           | 4           | 4            | 3           | 4           | 5           | 3           | 6           | 8           | 3           | 232543443453683      |
| 28        | 3          | 3          | 2          | 5          | 3          | 3           | 4           | 3            | 3           | 4           | 4           | 3           | 3           | 6           | 1           | 332533433443361      |
| 29        | 2          | 3          | 2          | 5          | 4          | 3           | 4           | 4            | 3           | 4           | 5           | 3           | 6           | 8           | 3           | 232543443453683      |
| 30        | 2          | 3          | 2          | 5          | 4          | 3           | 4           | 4            | 3           | 4           | 5           | 3           | 6           | 8           | 3           | 232543443453683      |
| 31        | 3          | 4          | 4          | 7          | 4          | 5           | 3           | 6            | 4           | 2           | 8           | 5           | 2           | 3           | 2           | 344745364285232      |
| 32        | 3          | 6          | 3          | 7          | 3          | 3           | 3           | 2            | 5           | 3           | 8           | 4           | 3           | 0           | 2           | 363733325384302      |
| 34        | 3          | 4          | 3          | 5          | 3          | 3           | 1           | 4            | 3           | 2           | 5           | 3           | 4           | 4           | 0           | 343533143253440      |
| 35        | 2          | 3          | 2          | 3          | 2          | 3           | 3           | 6            | 3           | 4           | 5           | 3           | 3           | 6           | 3           | 232323363453363      |
| 36        | 2          | 3          | 2          | 5          | 4          | 3           | 2           | 3            | 3           | 4           | 6           | 3           | 2           | 1           | 2           | 232543233463212      |
| 37        | 2          | 3          | 2          | 5          | 4          | 3           | 4           | 4            | 3           | 4           | 5           | 3           | 6           | 8           | 3           | 232543443453683      |
| 38        | 2          | 3          | 2          | 5          | 4          | 3           | 4           | 4            | 3           | 4           | 5           | 3           | 6           | 8           | 3           | 232543443453683      |
| 39        | 2          | 4          | 2          | 4          | 1          | 3           | 2           | 5            | 3           | 4           | 5           | 4           | 1           | 2           | 2           | 242413253454122      |
| 40        | 2          | 3          | 2          | 5          | 4          | 3           | 4           | 4            | 3           | 4           | 5           | 3           | 6           | 8           | 3           | 232543443453683      |
| 41        | 2          | 3          | 2          | 3          | 2          | 3           | 3           | 6            | 3           | 4           | 5           | 3           | 3           | 6           | 3           | 232323363453363      |
| 42        | 1          | 4          | 5          | 2          | 4          | 3           | 10          | 9            | 4           | 2           | 2           | 4           | 2           | 7           | 1           | 1452431094224271     |
| 43        | 3          | 4          | 3          | 5          | 3          | 3           | 1           | 4            | 3           | 2           | 5           | 3           | 4           | 4           | 0           | 343533143253440      |

| ID  | 424 | 577 | 580 | 802 | 960 | 1644 | 1955 | 2163b | 2165 | 2401 | 2996 | 3192 | 3690 | 4052 | 4156 | MIRUVNTR CODE       |
|-----|-----|-----|-----|-----|-----|------|------|-------|------|------|------|------|------|------|------|---------------------|
| 45  | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 46  | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 48  | 7   | 4   | 3   | 4   | 5   | 9    | 3    | 4     | 5    | 8    | 5    | 4    | 5    | 6    | 2    | 743459345854562     |
| 49  | 7   | 6   | 3   | 5   | 4   | 9    | 2    | 5     | 5    | 8    | 5    | 4    | 7    | 6    | 2    | 763549255854762     |
| 50  | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382     |
| 51  | 1   | 4   | 5   | 2   | 4   | 3    | 10   | 9     | 4    | 2    | 2    | 4    | 2    | 7    | 1    | 1452431094224271    |
| 52  | 8   | 4   | 2   | 5   | 4   | 9    | 2    | 3     | 4    | 6    | 5    | 4    | 5    | 5    | 3    | 842549234654553     |
| 53  | 2   | 4   | 2   | 1   | 3   | 8    | 2    | 5     | 5    | 7    | 6    | 4    | 3    | 5    | 2    | 242138255764352     |
| 54  | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 55  | 5   | 5   | 3   | 6   | 3   | 7    | 2    | 3     | 5    | 2    | 4    | 3    | 4    | 6    | 2    | 553637235243462     |
| 56  | 8   | 5   | 2   | 2   | 4   | 6    | 2    | 1     | 2    | 1    | 5    | 3    | 3    | 7    | 2    | 852246212153372     |
| 57  | 2   | 3   | 2   | 3   | 3   | 7    | 2    | 2     | 3    | 2    | 5    | 4    | 4    | 12   | 2    | 2323372232544122    |
| 58  | 2   | 2   | 1   | 6   | 4   | 6    | 2    | 3     | 3    | 2    | 5    | 5    | 4    | 5    | 2    | 221646233255452     |
| 59  | 1   | 5   | 2   | 3   | 3   | 2    | 3    | 3     | 4    | 2    | 5    | 4    | 2    | 4    | 2    | 152332334254242     |
| 60  | 6   | 3   | 2   | 3   | 4   | 9    | 2    | 15    | 6    | 3    | 2    | 8    | 3    | 6    | 3    | 6323492156328363    |
| 61  | 8   | 6   | 2   | 2   | 5   | 7    | 2    | 2     | 3    | 2    | 5    | 5    | 5    | 6    | 3    | 862257223255563     |
| 63  | 4   | 4   | 2   | 1   | 3   | 2    | 3    | 3     | 2    | 1    | 4    | 3    | 2    | 8    | 2    | 442132332143282     |
| 64  | 2   | 7   | 4   | 7   | 10  | 10   | 3    | 3     | 3    | 2    | 12   | 11   | 3    | 1    | 2    | 2747101033321211312 |
| 65  | 10  | 8   | 4   | 4   | 4   | 12   | 3    | 3     | 4    | 2    | 2    | 12   | 3    | 0    | 3    | 108444123342212303  |
| 66  | 4   | 6   | 4   | 4   | 10  | 12   | 3    | 3     | 6    | 9    | 2    | 11   | 5    | 0    | 2    | 464410123369211502  |
| 67  | 6   | 2   | 3   | 9   | 10  | 11   | 3    | 6     | 5    | 7    | 12   | 12   | 4    | 1    | 2    | 6239101136571212412 |
| 68  | 7   | 2   | 4   | 5   | 6   | 11   | 3    | 8     | 5    | 7    | 14   | 12   | 2    | 3    | 3    | 724561138571412233  |
| 69  | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361     |
| 70  | 7   | 7   | 3   | 7   | 8   | 14   | 2    | 4     | 3    | 3    | 5    | 12   | 4    | 0    | 3    | 77378142433512403   |
| 71  | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382     |
| 72  | 6   | 7   | 3   | 9   | 9   | 12   | 2    | 3     | 3    | 3    | 10   | 12   | 0    | 2    | 7    | 673991223331012027  |
| 73  | 8   | 7   | 2   | 3   | 10  | 10   | 2    | 2     | 3    | 2    | 5    | 10   | 2    | 0    | 2    | 872310102232510202  |
| 74  | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382     |
| 75  | 6   | 8   | 5   | 6   | 8   | 11   | 2    | 7     | 7    | 2    | 11   | 9    | 2    | 3    | 5    | 68568112772119235   |
| 76  | 4   | 6   | 3   | 3   | 7   | 11   | 2    | 5     | 3    | 3    | 11   | 6    | 2    | 2    | 2    | 46337112533116222   |
| 77  | 2   | 3   | 3   | 5   | 7   | 12   | 2    | 4     | 3    | 2    | 11   | 9    | 3    | 0    | 3    | 23357122432119303   |
| 78  | 4   | 2   | 3   | 6   | 8   | 10   | 2    | 3     | 4    | 5    | 10   | 8    | 3    | 1    | 2    | 42368102345108312   |
| 79  | 4   | 3   | 3   | 2   | 2   | 9    | 2    | 3     | 2    | 7    | 10   | 9    | 2    | 0    | 2    | 4332292327109202    |
| 80  | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 81  | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 82  | 5   | 5   | 8   | 1   | 10  | 7    | 3    | 5     | 2    | 2    | 11   | 9    | 3    | 2    | 3    | 55811073522119323   |
| 83  | 8   | 5   | 8   | 1   | 3   | 3    | 4    | 5     | 2    | 3    | 11   | 8    | 3    | 12   | 3    | 85813345231183123   |
| 84  | 6   | 5   | 10  | 1   | 9   | 8    | 4    | 5     | 5    | 2    | 2    | 4    | 3    | 5    | 3    | 6510198455224353    |
| 85  | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 86  | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 87  | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361     |
| 88  | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382     |
| 89  | 2   | 2   | 2   | 0   | 11  | 7    | 3    | 5     | 5    | 6    | 11   | 6    | 6    | 8    | 3    | 22201173556116683   |
| 90  | 3   | 5   | 2   | 1   | 7   | 7    | 3    | 4     | 4    | 4    | 11   | 7    | 2    | 8    | 2    | 3521773444117282    |
| 91  | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361     |
| 92  | 4   | 3   | 8   | 3   | 4   | 10   | 3    | 3     | 3    | 5    | 9    | 8    | 3    | 12   | 5    | 43834103335983125   |
| 95  | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361     |
| 97  | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 98  | 4   | 6   | 3   | 1   | 1   | 3    | 2    | 5     | 4    | 2    | 5    | 4    | 4    | 11   | 2    | 4631132542544112    |
| 99  | 1   | 4   | 5   | 2   | 4   | 3    | 10   | 9     | 4    | 2    | 2    | 4    | 2    | 7    | 1    | 1452431094224271    |
| 100 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 104 | 4   | 4   | 2   | 1   | 3   | 2    | 3    | 3     | 2    | 1    | 4    | 3    | 2    | 8    | 2    | 442132332143282     |
| 105 | 4   | 4   | 2   | 1   | 3   | 2    | 3    | 3     | 2    | 1    | 4    | 3    | 2    | 8    | 2    | 442132332143282     |
| 106 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 107 | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382     |
| 108 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 109 | 7   | 6   | 3   | 9   | 11  | 11   | 3    | 4     | 5    | 2    | 6    | 9    | 2    | 12   | 5    | 763911113452692125  |
| 110 | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361     |
| 111 | 6   | 7   | 3   | 7   | 9   | 10   | 2    | 4     | 5    | 4    | 6    | 10   | 1    | 9    | 5    | 67379102454610195   |
| 112 | 1   | 4   | 5   | 2   | 4   | 3    | 10   | 9     | 4    | 2    | 2    | 4    | 2    | 7    | 1    | 1452431094224271    |
| 113 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 114 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 115 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 116 | 5   | 6   | 2   | 8   | 5   | 9    | 2    | 3     | 4    | 4    | 6    | 4    | 4    | 5    | 2    | 562859234464452     |
| 117 | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361     |
| 118 | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361     |
| 119 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |
| 120 | 4   | 3   | 1   | 5   | 4   | 7    | 2    | 3     | 6    | 2    | 10   | 6    | 2    | 10   | 0    | 43154723621062100   |
| 121 | 4   | 3   | 1   | 7   | 5   | 8    | 3    | 3     | 3    | 2    | 12   | 4    | 2    | 11   | 1    | 43175833321242111   |
| 122 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683     |

| ID  | 424 | 577 | 580 | 802 | 960 | 1644 | 1955 | 2163b | 2165 | 2401 | 2996 | 3192 | 3690 | 4052 | 4156 | MIRUVNTR CODE     |
|-----|-----|-----|-----|-----|-----|------|------|-------|------|------|------|------|------|------|------|-------------------|
| 123 | 1   | 3   | 1   | 4   | 4   | 8    | 3    | 3     | 5    | 5    | 5    | 7    | 3    | 10   | 0    | 1314483355573100  |
| 124 | 3   | 4   | 1   | 2   | 4   | 3    | 3    | 3     | 2    | 1    | 11   | 3    | 3    | 7    | 0    | 3412433321113370  |
| 125 | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361   |
| 126 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 127 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 128 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 129 | 2   | 3   | 1   | 4   | 5   | 9    | 2    | 3     | 4    | 5    | 10   | 4    | 3    | 7    | 4    | 2314592345104374  |
| 130 | 6   | 4   | 2   | 2   | 3   | 7    | 2    | 2     | 3    | 1    | 7    | 3    | 3    | 5    | 0    | 642237223173350   |
| 131 | 2   | 3   | 2   | 3   | 2   | 3    | 3    | 6     | 3    | 4    | 5    | 3    | 3    | 6    | 3    | 232323363453363   |
| 132 | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361   |
| 133 | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361   |
| 134 | 9   | 2   | 1   | 2   | 1   | 9    | 3    | 1     | 4    | 6    | 13   | 3    | 2    | 10   | 3    | 92121931461332103 |
| 135 | 7   | 2   | 1   | 1   | 4   | 9    | 3    | 1     | 4    | 2    | 11   | 3    | 3    | 10   | 3    | 72114931421133103 |
| 136 | 0   | 3   | 1   | 7   | 4   | 8    | 2    | 1     | 4    | 1    | 9    | 2    | 2    | 8    | 0    | 031748214192280   |
| 137 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 138 | 1   | 5   | 2   | 3   | 3   | 2    | 3    | 3     | 4    | 2    | 5    | 4    | 2    | 4    | 2    | 152332334254242   |
| 139 | 1   | 5   | 2   | 3   | 3   | 2    | 3    | 3     | 4    | 2    | 5    | 4    | 2    | 4    | 2    | 152332334254242   |
| 140 | 6   | 2   | 1   | 2   | 4   | 7    | 2    | 2     | 6    | 6    | 11   | 3    | 3    | 6    | 2    | 6212472266113362  |
| 141 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 142 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 143 | 9   | 2   | 0   | 3   | 1   | 8    | 3    | 2     | 7    | 5    | 11   | 4    | 3    | 4    | 2    | 9203183275114342  |
| 144 | 1   | 4   | 5   | 2   | 4   | 3    | 10   | 9     | 4    | 2    | 2    | 4    | 2    | 7    | 1    | 1452431094224271  |
| 147 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 148 | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361   |
| 149 | 1   | 8   | 2   | 3   | 4   | 6    | 3    | 7     | 8    | 2    | 10   | 4    | 3    | 6    | 1    | 1823463782104361  |
| 150 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 151 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 152 | 2   | 4   | 1   | 2   | 2   | 0    | 3    | 3     | 2    | 3    | 7    | 4    | 4    | 5    | 2    | 241220323274452   |
| 153 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 154 | 2   | 3   | 2   | 3   | 2   | 3    | 3    | 6     | 3    | 4    | 5    | 3    | 3    | 6    | 3    | 232323363453363   |
| 155 | 3   | 5   | 2   | 3   | 2   | 3    | 2    | 5     | 4    | 4    | 5    | 3    | 3    | 5    | 2    | 352323254453352   |
| 156 | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382   |
| 157 | 3   | 3   | 2   | 5   | 3   | 3    | 4    | 3     | 3    | 4    | 4    | 3    | 3    | 6    | 1    | 332533433443361   |
| 158 | 4   | 3   | 1   | 6   | 4   | 1    | 2    | 3     | 3    | 4    | 7    | 6    | 4    | 6    | 4    | 431641233476464   |
| 159 | 2   | 3   | 2   | 3   | 2   | 3    | 3    | 6     | 3    | 4    | 5    | 3    | 3    | 6    | 3    | 232323363453363   |
| 160 | 5   | 3   | 1   | 5   | 4   | 3    | 2    | 3     | 2    | 0    | 5    | 6    | 3    | 6    | 2    | 531543232056362   |
| 161 | 3   | 4   | 1   | 7   | 3   | 4    | 2    | 4     | 1    | 3    | 5    | 2    | 2    | 5    | 2    | 341734241352252   |
| 162 | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382   |
| 163 | 4   | 2   | 1   | 3   | 5   | 4    | 2    | 3     | 2    | 3    | 5    | 4    | 3    | 6    | 3    | 421354232354363   |
| 164 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 165 | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382   |
| 166 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 167 | 4   | 3   | 1   | 5   | 6   | 4    | 2    | 3     | 2    | 3    | 4    | 4    | 3    | 5    | 2    | 431564232344352   |
| 168 | 1   | 2   | 1   | 4   | 3   | 3    | 2    | 4     | 2    | 0    | 4    | 3    | 0    | 5    | 3    | 121433242043053   |
| 179 | 1   | 4   | 5   | 2   | 4   | 3    | 10   | 9     | 4    | 2    | 2    | 4    | 2    | 7    | 1    | 1452431094224271  |
| 181 | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382   |
| 184 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 186 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 187 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |
| 188 | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382   |
| 189 | 4   | 4   | 2   | 3   | 3   | 3    | 5    | 6     | 3    | 4    | 5    | 5    | 3    | 8    | 2    | 442333563455382   |
| 190 | 2   | 3   | 2   | 5   | 4   | 3    | 4    | 4     | 3    | 4    | 5    | 3    | 6    | 8    | 3    | 232543443453683   |