Application Note

Automated MIRU-VNTR genotyping of *Mycobacterium tuberculosis* strains using the QIAxcel® Advanced Systems

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Introduction

MIRU-VNTR (mycobacterial interspersed repetitive units–variable number of tandem repeats) genotyping is commonly applied in studies of *Mycobacterium tuberculosis* strains. The protocol based on mini-satellite allele numbering developed by Supply et al. (1) is widely used.

The conventional method for MIRU-VNTR genotyping requires PCR amplification of each targeted locus, followed by detection using high-resolution agarose gel electrophoresis. The allele calling is done manually based on the gel analysis data. Poor gel quality or human error can lead to significant rates of false interpretation. An alternative method is needed to simplify the procedure and increase the reliability of the data assessment to help with the implementation of *M. tuberculosis* genotyping in high-burden countries.

The QIAxcel Advanced System is a high-throughput capillary electrophoresis system with automated data interpretation features. The aim of this study was to assess the instrument's performance in terms of ease of *M. tuberculosis* genotyping and reliability of MIRU-VNTR pattern generation. Sizing accuracy, reproducibility, repeatability, and automated allele calling analysis were studied using a MIRU-24 genotyping panel of the most prevalent strains.

Materials and Methods

Amplification

All of the samples were amplified via PCR using the QIAGEN HotStarTaq® Master Mix Kit, as previously described (2). Two controls were included in each run: one known sample (H37Rv or H37Ra) and one PCR blank (molecular-grade pure water).

Conventional method for electrophoresis and allele calling

Electrophoresis was run on NuSieve® 3:1 3% Agarose 100 mL Gel for 5 h at 120 V in the presence of the intercalating agent and using several size markers per gel. Allele calling was manually calculated using theoretical tables (2) and reported before submission to a free online genotyping database.

QIAxcel-based method for electrophoresis and allele calling

PCR products were analyzed using a QIAxcel DNA High Resolution Kit on the QIAxcel Advanced System (protocol OM1700). The QIAxcel Alignment Marker 15 bp/3 kb was run simultaneously with all of the samples to frame or delineate DNA fragments with two clearly visible peaks. The QIAxcel DNA Size Marker 100 bp – 2.5 bp ▷

was used to estimate the size of the PCR amplicons. Sizing was done automatically using the QIAxcel ScreenGel® software.

Allele calling was performed automatically using a peak calling table that defines the size of the alleles according to theoretical tables (2) and a tolerance in % for each locus to exclude any overlapping data. Then the results were merged into an Excel® spreadsheet and submitted to the free online genotyping database.

Reference sizing method

Sanger sequencing was used as a reference method to determine the amplicon sizes.

Table 1. Summary of MIRU-VNTR analyzed in this study

	Alias/Locus	Minimum size (bp)	Maximum size (bp)	Allele Distribution												
				0	1	2	3–3s	4	5	6	7	8	9	10	15	Mixture
15-locus MIRU-VNTR panel (n = 82)	MIRU 04/580	250	757		2	63	3	7	2	3	2					
	MIRU 10/960	590	1262			8	48	17	2	3	2		1			1
	MIRU 16/1644	565	821	1	4	19	57									1
	MIRU 26/2996	335	649		4	8	10	6	40	2	11					1
	MIRU 31/3192	492	816		1	10	24	3	40	3						1
	MIRU 40/802	398	692		8	9	42	10	12							1
	Mtub 04/424	602	920		1	25	12	38	6							
	ETR C/577	270	445			3	14	63	2							
	Mtub 21/1955	164	1053		1	16	14	8	42						1	
	QUB-11b/2163b	140	626		6	12	6	11	2	38	5	1				1
	ETR A/2165	274	732		1	6	17	50		2	5					1
	Mtub 30/2401	248	533	1	7	22		52								
	Mtub 39/3690	387	783			7	53	8	9	2	1	1	1			
	QUB-26/4052	385	1317			1	6	4	7	17	11	29	4	1		2
	QUB-4156/4156	560	849	4	8	56	8	6								
24-locus MIRU-VNTR (n = 66)	MIRU 02/154	456	513		3	62										1
	MIRU 20/2059	508	605		2	64										
	MIRU 23/2531	306	472				1		59	5						1
	MIRU 24/2687	439	505		59	7										
	MIRU 27/3007	644	665				65									1
	MIRU 39/4348	584	700		1	17	47									1
	Mtub 29/2347	451	579			1	7	58								
	ETR B/2461	403	647		1	57	1	6	1							
	Mtub 35/3171	386	492		1		64									1

Results and Discussion

MIRU-VNTR loci

The 15-locus panel for epidemiological studies and the 24-locus panel for phylogenetic resolution were used as the standard sets for identification of the main mycobacterial genotypes (Table 1). Other locus panels can be used when addressing specific lineages, such as Beijing strains.

VNTR allele-calling accuracy

The overall concordance on allele calling observed between the two methods is 1803/1824 (98.8%). For the size range of 140–900 bp, it is 99.9%. We observed 21 discrepancies: 19 on locus VNTR4052 (alleles 8 to 10), 1 on locus VNTR3690 (allele 8), and 1 on locus VNTR1955 (allele 15). Sanger sequencing confirmed all the allele sizes obtained by conventional gel electrophoresis. The QIAxcel data showed overestimated sizes for alleles above 900 bp. Optimization of the sizing algorithm for OM1700 is in progress. An in-house allelic ladder can act as an alternative internal size marker for accurate allele calling.

Influence of DNA concentration on sizing

It has been proved that conventional gel electrophoresis is negatively affected by high DNA concentrations. Therefore, we investigated the influence of DNA concentration on the resolution and size estimation for the two methods, and compared the results (Figure 1). Dilution series of the same PCR products were used to estimate the impact of DNA concentration on sizing accuracy.

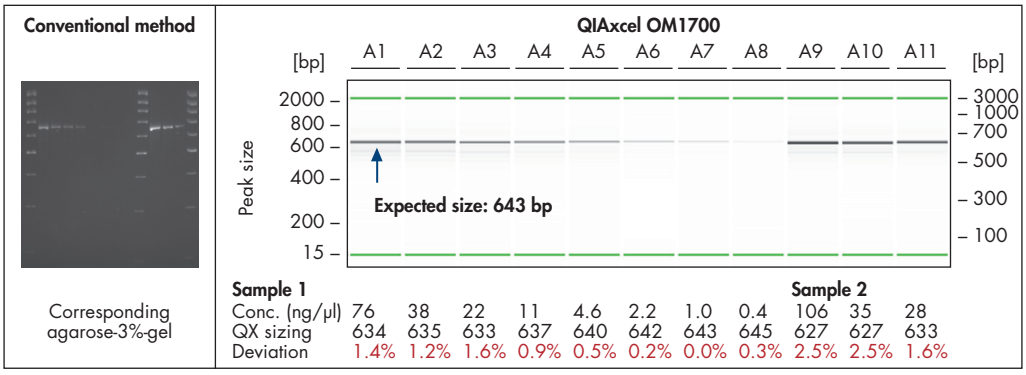

Figure 1. QIAxcel Advanced performs accurate DNA fragment sizing. Gel electrophoresis and fragment sizing of DNA fragments at various concentrations were performed using conventional gel electrophoresis and the QIAxcel Advanced instrument. Locus MIRU10 has repeats of 55 bp. The 643-bp fragment represents 3 alleles.

The results show no difference in allele calling at concentrations between 0.4 and 106 ng/µl. The QIAxcel Advanced System enables detection of DNA at concentrations as low as 0.4 ng/µl. The limit of detection using the conventional method is 2.2 ng/µl. Thus, PCR DNA quantification and/or sample dilution are not required before the QIAxcel run under these conditions.

Reproducibility and repeatability

A number of variables (operator, different alignment marker and cartridge batches, and cartridges of different ages) were tested using a ladder of previously sequenced amplicons of known sizes in order to cover the broad size range (100, 206, 382, 348, 562, 639, 681, 1065 bp). Tests were done with two cartridges. The maximum deviation for the estimated sizes ranged between 1.9 bp (100-bp amplicon) and 6.5 bp (1065-bp amplicon). The results indicate that the automated analysis with the QIAxcel protocol is repeatable (0.4 and 0.8% at the beginning and end of kit shelf life, respectively) and reproducible (0.4 and 1.2%) for MIRU-VNTR genotyping.

Conclusions

- The precision and accuracy of sample size estimation of Mycobacterium tuberculosis DNA with the QIAxcel Advanced method is compatible with that of conventional methods, and the repeatability and reproducibility are better.
- The QIAxcel Advanced method is significantly faster and less expensive.
- Implementing QIAxcel Advanced-based MIRU-VNTR genotyping of M. tuberculosis in the field would benefit epidemiological studies on tuberculosis worldwide.

References

1. Supply, P. et al. (2006) Proposal for Standardization of Optimized MIRU-VNTR Typing of Mycobacterium Tuberculosis. J. Clin. Micr. **44(12)** 4498.
2. http://www.miru-vntrplus.org/MIRU/files/MIRU-VNTRtypingmanualv6.pdf
3. Gauthier, M. et al. (2014) High-Throughput MIRU-VNTR Genotyping for Mycobacterium Tuberculosis Epidemiological Studies. J. Clin Micr. Accepted for publication November 2014.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel Alignment Marker 15 bp/3 kb (1.5 ml)	Alignment marker with 15 bp and 3 kb fragments	929522
QIAxcel DNA Size Marker 100 bp – 2.5 bp (50 µl)	DNA size marker with fragments of varying size from 100 to 2500 bp; concentration 100 ng/µl	929559

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

See the benefits of how automated gel electrophoresis at **www.qiagen.com/QIAxcel**!