High-throughput processing of VNTR analyses using the QIAxcel® system

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Fragment sizes automatically calculated using the QIAxcel system were used to determine the number of repeats in variable number tandem repeat (VNTR) loci. Results obtained using the QIAxcel system enabled discrimination of *M. tuberculosis* strains.

Introduction

The number of tandem repeats in JATA loci vary in different strains of *M. tuberculosis* (1). As the size of the amplified fragment is directly correlated to the number of tandem repeats in the sequence, a reliable size-determination method would enable rapid, high-throughput discrimination of these strains.

Materials and methods

Strains, DNA purification, and PCR amplification

Genomic DNA was purified from 192 isolates of *M. tuberculosis* as well as the H37Rv laboratory standard strain using the ISOPLANT Kit (NIPPON GENE). Amplification reactions were prepared with purified DNA (1 µl), primers (25 pmol each), dNTPs (200 µM each), GC buffer I (Takara Bio, Inc.), Takara® EX *Taq* DNA polymerase (0.5 U, Takara Bio, Inc.), and distilled water (6.3 µl) using primers for 15 JATA loci. The amplification reaction was subjected to the following cycling conditions: 5 minutes at 94°C for initial denaturation, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 63°C, and 3 minutes at 72°C.

Fragment-size analysis

Unless otherwise stated, amplification reactions were diluted with 5 volumes of QX DNA Dilution Buffer (QIAGEN). Diluted PCR products were analyzed on the QIAxcel system using the QIAxcel DNA High Resolution Kit. Separation was performed with the OM2100 method using 10 seconds injection time and 2100 seconds separation time. The size and concentration of DNA fragments were automatically calculated using BioCalculator Software.

As a control, the 100 bp DNA Ladder (Toyobo) was analyzed using the QIAxcel system to determine the accuracy and reproducibility of size calculation.

Estimating number of tandem repeats from fragment size

The size of PCR products amplified from JATA loci can be calculated from the size of a fragment exhibiting no repeats, the length of the repeated sequence, and the number of repeats in the locus (Table 1). Using this data, the observed size of an amplified fragment can be used to estimate the number of repeats at the locus.

Table 1. Calculated fragment sizes of JATA amplification products

			Size (bp)																
JATA no.	Name	Locus	Repeated unit	Amplified fragment (according to number of tandem repeats) 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Mtub 04	0424	51	535	586	637*	688	739	790	841	892	943	994	1045	1096	1147	1198	1249	1300
2	MIRU 10	0960	53	483	536	589	642*	695	748	801	854	907	960	1013	1066	1119	1172	1225	1278
3	Mtub 21	1955	57	208	265*	322	379	436	493	550	607	664	721	778	835	892	949	1006	1063
4	Mtub 24	2074	56	14	70	126	182	238*	294	350	406	462	518	574	630	686	742	798	854
5	QUB 11b	2163b	69	200	269	338	407	476	545*	614	683	752	821	890	959	1028	1097	1166	1235
6	VNTR 2372	2372	57	176	233	290*	347	404	461	518	575	632	689	746	803	860	917	974	1031
7	MIRU 26	2996	51	285	336	387	438*	489	540	591	642	693	744	795	846	897	948	999	1050
8	QUB 15	3155	54	71	125	179	233	287*	341	395	449	503	557	611	665	719	773	827	881
9	MIRU 31	3192	53	491	544	597	650*	703	756	809	862	915	968	1021	1074	1127	1180	1233	1286
10	QUB 3336	3336	59	98	157	216	275	334	393	452	511	570*	629	688	747	806	865	924	983
11	QUB 26	4052	111	168	279	390	501	612	723*	834	945	1056	1167	1278	1389	1500	1611	1722	1833
12	QUB 4156	4156	59	510	569	628	687*	746	805	864	923	982	1041	1100	1159	1218	1277	1336	1395
13	ETR A	2165	75	184	259	334	409*	484	559	634	709	784	859	934	1009	1084	1159	1234	1309
14	QUB 11a	2163a	69	170	239	308*	377	446	515	584	653	722	791	860	929	998	1067	1136	1205
15	QUB 18	1982	78	231	309	387	465	543	621*	699	777	855	933	1011	1089	1167	1245	1323	1401

Table reproduced from the cited reference.

* Length of fragment amplified from genomic DNA of the H37Rv strain of *M. tuberculosis*.

Results

Fragments amplified from 15 JATA loci were analyzed for 192 *M. tuberculosis* strains (total of 2880 analyses) using the QIAxcel system (Figure 1) and visual examination of agarose gels (data not shown). The observed fragment size was used to estimate the number of tandem repeats in the amplified products according to the data in Table 1.

Automatic size calculation using the QIAxcel system was 100% accurate for fragments less than 1000 bp (2658 fragments, 92.3%). However, discrepancies were observed with fragments of >1000 bp, amplified from the JATA 5 (VNTR2163b), JATA 10 (VNTR3336), JATA 11 (VNTR4052), JATA 14 (VNTR2163a), and JATA 15 (QUB18) loci which correspond to 0.5%, 2.1%, 54.7%, 9.4%, and 49.5% of analyzed strains, respectively.

To determine the effect of DNA concentration on automatically calculated fragment size, amplification products of known size were diluted to different concentrations before separation (Figure 2). Bands from high-concentration fragments appeared to be wider in the gel image than low-concentration fragments. As a result, calculated sizes for high-concentration fragments were smaller than the actual sizes (Table 2). This tendency was more pronounced for larger fragments.

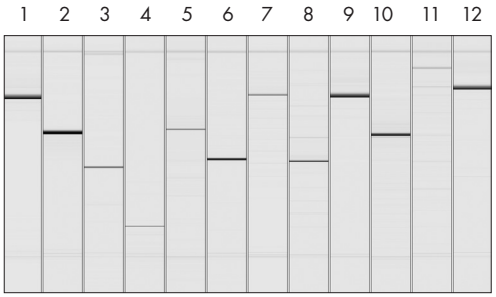

Figure 1. Separation of amplification products from a *M. tuberculosis* JATA locus. DNA from an *M. tuberculosis* isolate was amplified and analyzed using primers for the JATA 1–12 loci.

Table 2. Size determination of PCR dilutions

	Calculated fragment size (bp)				
	Dilution				
Actual fragment size (bp)	1:1	1:2	1:5	1:10	1:20
591	531.8	541.7	550.3	558.2	562.7
1028	846.1	877.9	937.1	961.0	981.1
1496	999.1	1069.7	1166.0	1273.3	1311.7

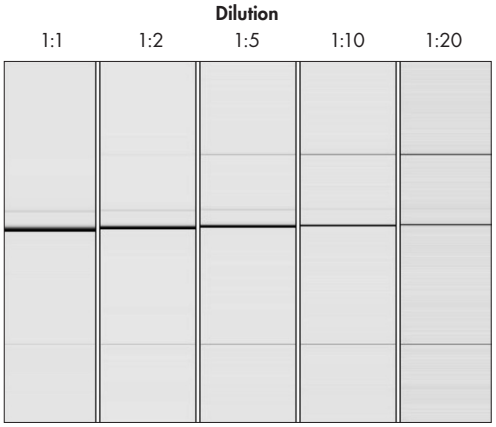

Figure 2. Concentration-dependent size difference. DNA from the H37Rv strain of *M. tuberculosis* was amplified, diluted as indicated, and analyzed using primers for the JATA 10 locus.

Table 3. Fragment size for the 100 bp marker

Actual (bp)	Calculated (bp)*	Standard deviation
1500	1557.9	19.3
1000	1061.8	2.7
900	922.3	1.4
800	809.7	1.3
700	699.1	1.1
600	598.4	1.2
500	494.6	1.1
400	399.0	1.1
300	301.5	0.9
200	203.8	0.7
100	100.4	0.8

* Average of 96 measurements.

Discussion

Fragment sizes automatically calculated using the QIAxcel system could be used to estimate the number of tandem repeats in JATA loci. Two factors affected the accuracy of the size determination: DNA concentration and fragment length.

Fragments sizes were calculated as smaller than actual for high-concentration DNA. This tendency was more pronounced with fragments of >1000 bp. Based on these results, we recommend analyzing dilutions for fragments of >1000 bp to ensure accurate fragment-size calculation. Furthermore, we also recommend that a standard strain of known fragment sizes is analyzed with unknown strains to aid correction of the size for long fragments.

The size of small fragments may be miscalculated if they are nearly the same size as the lower alignment marker. In these cases, the alignment marker peaks can be edited or deleted manually to obtain accurate size calculation. We recommend that the lower alignment marker is recognized correctly and that the size and concentration values of the amplified fragments are checked before exporting the data from the QIAxcel system for subsequent use.

Conclusions

- The QIAxcel system enables rapid, high-throughput VNTR analysis of *M. tuberculosis* strains.
- VNTR analysis using the QIAxcel system is less error prone and less labor intensive than manual analysis using visual inspection of conventional agarose gel electrophoresis (data not shown).
- Following recommendations improves the accuracy of size calculation for large and small fragments.

References

1. Maeda, S., Murase, Y., Mitari, S., Sugawara, I., and Kato, S. (2007) Rapid, simple genotyping method by the variable numbers of tandem repeats (VNTR) for Mycobacterium tuberculosis isolates in Japan — analytical procedure of JATA (12) VNTR. Kekkaku **83**, 673.

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