TB Typing Kit VNTR-9

Project number: T665540
Storage: 2-8℃ for 1 month, -20℃ for 1 year

Product content

appliance	Cat. No.	T665540-50T
Identification of strains as human Mycobacterium tuberculosis	Mtb Identification	1ml
DNA template quality characterization	16S rRNA	1ml
Identification of strains as Beijing genotypes	RD105	1ml
9-locus VNTR genotyping assay	QUB-11b	1ml
	QUB-18	1ml
	QUB-26	1ml
	MIRU26	1ml
	MIRU31	1ml
	MIRU40	1ml
	Mtub21	1ml
	Mtub04	1ml
	VNTR2372	1ml
DNA Molecular Weight Standard I	Marker I	1ml
DNA Molecular Weight Standard II	Marker II	400μl

Product Introduction

This kit is a genotyping product for human Mycobacterium tuberculosis based on the latest advances in molecular epidemiology1) and optimized by process. It utilizes variable-number tandem repeats (VNTR) polymorphisms in the Mycobacterium tuberculosis genome for genotyping to differentiate clinical strains, and is a powerful tool for studying the molecular epidemiology of Mycobacterium tuberculosis and for monitoring the status of tuberculosis transmission. Compared with other existing Mycobacterium tuberculosis VNTR typing systems based on the VNTR principle, this typing system has a stronger ability to discriminate between strains prevalent in China1,2,3), and is therefore particularly suited to the needs of Chinese users.

By carefully optimizing the primer sequences of each PCR reaction and the composition of the premixed reaction solution, this product has a strong anti-interference power. Compared with the user's own reagents, this product significantly improves the signal intensity of specific bands and reduces the appearance of non-specific bands when using crude templates (boiling bacterial solution), which makes the experimental operation easier and quicker, and at the same time, improves the success rate of the test. The premixed reaction solution is chemically stable and can effectively withstand repeated freezing and thawing (10 times) and a longer period of time (one week) at room temperature, which is better adapted to the user's need for flexibility in the detection work.

This product integrates the identification of human-type Mycobacterium tuberculosis (MTBC), the identification of non-Mycobacterium tuberculosis (MOTT) and template mass, the identification of Mycobacterium tuberculosis Beijing

family bacteria, and the subsequent identification of 9-locus VNTR typing in a single kit, so that the user only needs a single kit and 12 PCR reactions to obtain the complete information necessary for the genotypic identification of the strain to be tested. The resolution index (Hunter-Gaston index (HGI) of the assay can reach 0.9891). Moreover, the genotype identification results can be digitally recorded, which makes it easy to compare and pool the experimental data between different batches of samples, different regions, different times, and different researchers, and greatly improves the efficiency of data use. For samples identified as clustered (all 9 loci with the same number of repeats) of the VNTR-9 genotype, further typing is required to determine whether there is a recent transmission relationship between strains using the companion TB Genotyping Kit HV-3. (The combination of the two products, VNTR-9 and HV-3, can be used to increase the HGI of the assay to 0.9931). For more information about the TB Genotyping Kit HV-3, please refer to its datasheet.

References

1）Luo T et al. Development of a hierarchical variable-number tandem repeat typing scheme for Mycobacterium tuberculosis in China. PLoS One. 2014 Feb 25;9(2)
2）Sun G et al. Discriminatory potential of a novel set of Variable Number of Tandem Repeats for genotyping
3）Mycobacterium marinum. Vet Microbiol. 2011 Aug 26;152(1-2)
4）Zhang L et al. Highly polymorphic variable-number tandem repeats loci for differentiating Beijing genotype strains of Mycobacterium tuberculosis in Shanghai, China. FEMS Microbiol Lett. 2008 May;282(1):22-31.

matters needing attention

1. To avoid contamination, it is recommended that sample preparation and PCR Mix preparation be performed within separate locations and with separate pipettes.
2. Care should be taken to label the samples at all stages of DNA collection, extraction and amplification, and to prevent cross-contamination between different samples.
3. Commonly used reagents and consumables need to be autoclaved before experimentation.
4. Each tube of PCR Mix contains different primers and cannot be mixed. It can be dispensed into different quantities at once according to the experimental needs to avoid repeated freezing and thawing.
5. To avoid splashing the reaction solution when opening the reaction tube, centrifuge briefly before opening the cap and collect the liquid at the bottom of the tube. In case of accidental splashing on gloves or table, change gloves immediately and wipe the table with 75% alcohol or dilute acid.
6. Be careful not to cross-contaminate the PCR Mix when aspirating, and it is recommended to wipe the pipette head with 75% alcohol 2 times before taking Mix each time.
7. Pre-experiment preparation: 1×TE buffer (PH=8.0), 0.5×TBE buffer, agarose, ethidium bromide (EB), common PCR instrument, DNA electrophoresis equipment and gel imager, 0.2 ml of PCR reaction tubes, octreotide rows or 96-well PCR tubes, and pipettes of different sizes: 0.5-10 μl and 20-200 μl.

Operation steps

1. DNA template preparation:

1.1 Scrape a small amount (1-2 inoculation loops) of sample from the solid medium, resuspend in 100 μl of TE, and inactivate at 80° C for 30 minutes.

1.2 After inactivation, take the strain out of the P3 laboratory and do the following: boil at 100° C for 10 minutes (the cap of the EP tube may burst open during boiling, try to avoid this, fasten the EP tube tightly and do not allow water to enter the tube), place it on ice immediately for 2 minutes, centrifuge it at 12,000 rpm (~13,400 x g) for 10 minutes, then take the supernatant and put it into another sterile EP tube, mark it and - 20° C.

2. Testing procedures:

2.1 Remove the TB Genotyping Kit VNTR-9, allow the liquid to equilibrate to room temperature, mix by shaking slightly 3-4 times, and centrifuge at 12,000 rpm for 5 seconds to allow the liquid on the cap to collect in the tube.

2.2 Mycobacterium tuberculosis genotype analysis:

2.2.1 Identify the sample as human Mycobacterium tuberculosis (Important! This step should not be omitted!) :

A. PCR amplification: the reaction system was 20 μl.

Add 19 μl Mtb Identification PCR Mix, 1 μl DNA template to each PCR tube separately and mix well.

B. Response procedures:

Steps	Temperature	time	
Pre denaturation	95° C	10min	
denaturation	94° C	30s	30 cycles
annealing	60° C	30s	
Extend	72° C	30s	
Final extension	72° C	7min	

C. Gluing, Electrophoresis

PCR amplification products were electrophoresed using a 1% agarose gel.

To prepare 1% agarose gel, 12×6 cm gel tray was used to make the gel, each gel was 40 ml.

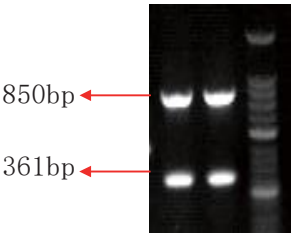
(1) Weigh 0.4g agarose, add 40ml 0.5×TBE, weigh it on the balance and put it into the microwave oven, heat it on high for 2-3 minutes to make the agarose dissolve completely, shake it well, and observe it as a homogeneous and transparent solution without particles, then weigh it again on the balance and replenish it with an appropriate amount of double-distilled water to keep the concentration of the gelatinous gel unaffected.

2) When the melted gel was cooled to about 55° C, 2 μl of ethidium bromide (10ug/ml) was added and gently rotated to mix well. Use an 18-tooth comb to make the gel and pour the warm gel into the gel tray.

3) When the gel is completely solidified (left at room temperature for 30 minutes), carefully pull out the comb, remove the tray and put it into the electrophoresis tank. Add 0.5×TBE buffer to the electrophoresis tank, not exceeding the gel surface by 1-2mm.

4) PCR product up-sampling: 3μl PCR product was added to each well, one well was left to add 5μl MarkerⅠ to each piece of gel, and one H37Rv was added to each piece of gel as quality control. The voltage was 150V and the electrophoresis time was 45 minutes.

The results show:



D. Analysis of results:

(1) If two bands of 850 and 361bp appear, it means that the template DNA is human-type Mycobacterium tuberculosis, and you can proceed to step F (see later) for the analysis and identification of Mycobacterium tuberculosis Beijing genotype, and further use this kit for the identification of 9-locus VNTR genotype analysis of this strain.

(2) If only a band of 850 bp is amplified, it means that the strain belongs to Mycobacterium tuberculosis complex group, but is not human-type Mycobacterium tuberculosis, and this product is not suitable for genotypic analysis and identification.

(3) If there is no specific band amplification, it means that the strain is a non-Mycobacterium tuberculosis or the quality of template DNA is not good enough for this product to be used for genotyping and identification, so you can either abandon the sample or go to step E (see later) and test the quality of the DNA template.

E. DNA template quality characterization:

If no specific bands are amplified in steps A-D above, the template DNA is of poor quality or the strain is not Mycobacterium tuberculosis, in which case proceed as follows.

1) PCR amplification: the reaction system was 20 μl.

Add 19 μl 16S rRNA PCR Mix, 1 μl DNA template to each PCR tube separately and mix well.

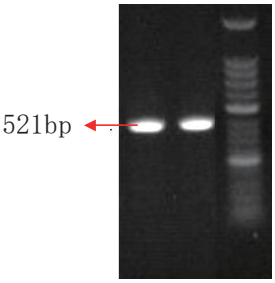
2) Reaction Procedures:

Steps	Temperature	time	
Pre denaturation	95° C	10min	
denaturation	94° C	30s	30 cycles
annealing	55° C	30s	
Extend	72° C	30s	
Final extension	72° C	7min	

3) Electrophoresis

1% agarose gel electrophoresis at 150 V for 45 min;

4) The results show:



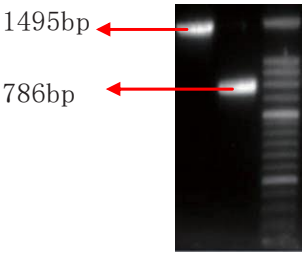
5) Analysis of results:
16S rRNA sequences are present in all bacteria, and if no amplification product is present in the sample, the amount or quality of template DNA is not sufficient for VNTR genotyping. If an amplification product band is present, the strain is a non-Mycobacterium tuberculosis and should not be genotyped using this kit.

F. Methods for identification of genotyped strains in Beijing:
After the strain was identified as Mycobacterium tuberculosis, it was further distinguished whether it was a Beijing genotype strain.
1) PCR amplification: the reaction system was 20 μl.
Add 19 μl RD105 PCR Mix, 1 μl DNA template to each PCR tube separately and mix well.
2) Reaction Procedures:

Steps	Temperature	time	
Pre denaturation	95° C	10min	
denaturation	94° C	30s	30 cycles
annealing	68° C	30s	
Extend	72° C	3min	
Final extension	72° C	7min	

3) Electrophoresis
1% agarose gel electrophoresis at 150 V for 45 min.

4) Results display:



5) Analysis of results:
If the amplification product is 1495 bp, the strain is a non-Beijing genotype strain;
If the amplification product is 786 bp, the strain is a Beijing genotype strain.

2.2.2 Mycobacterium tuberculosis 9-locus VNTR genotyping method: genotyping of clinical strains of Mycobacterium tuberculosis and preliminary identification of cluster-forming strains.A. PCR amplification: the reaction system was 20 μl. Take 9 PCR reaction tubes, add 19 μl of PCR Mix of QUB-11b, QUB-18, QUB-26, MIRU26, MIRU31, MIRU40, Mtub21, Mtub04, VNTR2372 to each PCR tube, add 1 μl of DNA template and mix well.

B. Response Procedures:

Steps	Temperature	time	
Pre denaturation	95° C	10min	
denaturation	94° C	30s	30 cycles
annealing	58° C	30s	
Extend	72° C	30s	
Final extension	72° C	7min	

C. Gluing and electrophoresis:

C-1: Notes:

Important! Positive (H37Rv strain DNA) and negative controls (deionized water) need to be set up for each experiment.

Key! This experiment is based on agarose gel electrophoresis to interpret the genotype of VNTR locus, therefore, in order to make the results of different laboratories accurately comparable with each other, it is necessary to follow a unified standard operation in this step of electrophoresis, and the following points should be noted:

1) The comb used for glue making is 18 holes.

(2) The two holes on the left and right sides of the gel are easily deformed during electrophoresis, which affects the interpretation of the results, so they are discarded or a negative control is placed in one of the holes, and the remaining 16 holes are divided into 12 samples, 3 DNA markers and 1 positive control. The order of spotting was "1, 2, M, 3, 4, 5, 6, M, 7, 8, 9, 10, M, 11, 12, H37Rv", the numbers represent samples, and M represents DNA Marker.

(3) When PCR amplification products are subjected to the first electrophoresis and Marker I is used, the gel concentration is 1%, and an electrophoresis tank with a distance between two electrodes of not less than 30 centimeters should be used, with a voltage of 150 V and a time of 100-120 minutes.

4) If the fragment of the amplification product is too large (>1000bp), and it is necessary to electrophoresis again and use Marker II, the concentration of the gel is 0.8%, and an electrophoresis tank with a distance of not less than 30 cm between the two electrodes should be used with a voltage of 150V and a time of 150 minutes.

C-2: Gel making as well as electrophoresis process:

PCR amplification products were electrophoresed using a 1% agarose gel.

To prepare 1% agarose gel, 12×12 cm gel tray was used to make the gel, each gel was 80 ml.

1) Weigh 0.8g of agarose, add 80ml of 0.5×TBE, weigh it on the balance and put it into the microwave oven, heat it on high for 2-3 minutes to make the agarose dissolve completely, shake it well, and observe it as a homogeneous and transparent solution without particles, then weigh it again on the balance and replenish it with an appropriate amount of double-distilled water to keep the concentration of the gum unaffected.

2) Add 4 μl of ethidium bromide (10ug/ml) when the melted gel is cooled to about 55° C, and gently rotate to mix well. The gel was made with an 18-tooth comb and the warm gel was poured into a 12×12 cm gel tray.

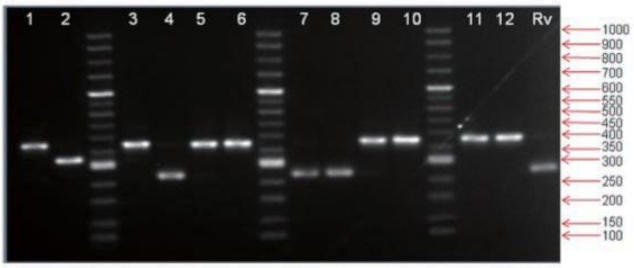
3) When the gel is completely solidified (40 minutes at room temperature), carefully pull out the comb, remove the tray and put it into the electrophoresis tank. Add 0.5×TBE buffer to the electrophoresis tank, not

exceeding the gel surface by 1-2mm.

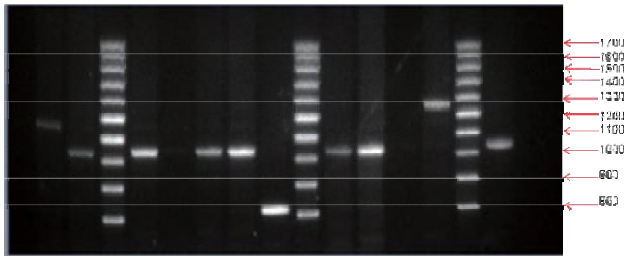
4) Sampling electrophoresis: add 12 samples to each piece of gel (the topmost wells are not sampled), add 3-5 μl PCR products to each well, meanwhile add three 5 μl DNA Marker Ⅰ to each piece of gel, and add one H37Rv as a quality control (the distribution of spotting wells is shown in the following figure). The voltage is 150V and the electrophoresis time is 100-120 minutes. This step is the key to the accuracy of the final readout of each spot and needs to be followed uniformly.

5) Some of the loci (QUB-18 and QUB-26) had amplification products greater than 1000bp in clinical strains, and these amplification products were then electrophoresed using 0.8% agarose gel, while DNA Marker Ⅱ was added as a control for the size of the bands, with a voltage of 150V and an electrophoresis time of 150 minutes.

Marker Ⅰ



Marker Ⅱ



D. Analysis of results:

According to the relative position of the amplified bands and Marker of each clinical strain, the size of each band was read out using gel analysis software. The number of repeat units of each strain at the VNTR site was calculated using the table of repeat unit readings for each VNTR site and the rules for reading VNTR sites, and the table of repeat unit readings for each VNTR site and the rules for reading VNTR sites are attached.

Results report: Excel file (the following table is an example of the results report output format)

Strain number	Region	Year	Mycobacterium tuberculosis	Beijing genotype	9-site VNTR results								
					QUB-11b	QUB-18	MIRU 26	QUB-26	Mtub 21	MIRU 31	Mtub 04	MIRU 40	VNTR2372
1	SH	2007	Y	N	0	1	2	1	1	1	0	3	2
2	SH	2007	Y	N	0	1	6	4	1	1	0	3	2
3	SH	2007	Y	Y	5	9	8	9	5	1	0	3	2

4	SH	2007	Y	Y	5	9	8	9	5	2	3	3	5
5	SH	2006	Y	Y	5	9	8	9	5	2	3	2	5
6	SH	2007	Y	Y	5	9	8	9	5	2	3	2	5
7	SH	2007	Y	Y	6	9	8	9	5	2	3	2	5

Compare the number of repeats of the 9 VNTR loci of different strains for cluster analysis: if two or more strains have the same 9-locus genotype, they are initially identified as clustered strains, and if necessary, the companion product, TB Genotyping Kit HV-3, can be used to do further typing identification in a more refined way. If the isolated strains have specific 9-locus genotypes, they will be identified as a single strain.

Appendix 1: VNTR site readout rules

VNTR site	Lateral sequence size (bp)	Repetitive unit size (bp)	Amplified fragment length (bp, Taking MTB H37Rv as an example) = Repeating unit length (bp) ×Number of duplicate units+Incomplete repeating sequence (bp) +Lateral sequence length (bp)
QUB-11b	67	69	422=69×5+10+67
QUB-18	182	78	621=78×5+49+182
QUB-26	129	111	708=111×5+24+129
MIRU26	243	48	387=48×3+0+243
MIRU31	108	52	264=52×3+0+108
MIRU40	354	54	408=54×14+0+354
Mtub21	92	57	206=57×2+0+92
Mtub04	137	51	269=51×2+30+137
VNTR2372	172	57	298=57×2+12+172

Appendix 2: VNTR Site Repeat Unit Readout Table

1		2		3		4	
QUB-11b		QUB-18		QUB-26		MIRU26	
repeats	Fragment size	repeats	Fragment size	repeats	Fragment size	repeats	Fragment size
1	146	0	231	1	264	1	291
2	215	1	309	2	375	2	339
3	284	2	387	3	486	3	387
4	353	3	465	4	597	4	435
5	422	4	543	5	708	5	483
6	491	5	621	6	819	6	531
7	560	6	699	7	930	7	579
8	629	7	777	8	1041	8	627
9	698	8	855	9	1152	9	675
10	767	9	933	10	1263	10	723
		10	1011	11	1374		
		11	1089	12	1485		
		12	1167				

Note: The repeated unit size of QUB-11b is 69bp。H37Rv: 67+69× 5+10=422bp	Note: The size of the QUB-18 repeat unit is 78bp, and most clinical strains have an incomplete repeat of 49bp in size.H37Rv: 182+78×5+49 =621bp	Note: The size of the QUB-26 repeat unit is 111bp, and some clinical strains have an incomplete repeat of 24bp in size.H37Rv: 129+111 ×5+24=708bp	Note: The size of the MIRU26 site repeat unit is 48bp。H37Rv: 243+48× 3=387bp

5		6		7		8		9	
MIRU31		MIRU40		Mtub21		Mtub04		VNTR2372	
repeats	Fragment size	repeats	Fragment size	repeats	Fragment size	repeats	Fragment size	repeats	Fragment size
1	160	1	408	1	149	0	167	1	241
2	212	2	462	2	206	1	218	2	298
3	264	3	516	3	263	2	269	3	355
4	316	4	570	4	320	3	320	4	412
5	368	5	624	5	377	4	371	5	469
6	420	6	678	6	434	5	422	6	526
7	472	7	732	7	491	6	473	7	583
8	524	8	786	8	548	7	524	8	640
9	576			9	605	8	575	9	697
10	628			10	662			10	754
Note: The size of the MIRU31 repeat unit is 52bp, and some clinical strains have an incomplete repeat of 24bp in size.H37Rv: 108+52× 3+24=264bp		Note: The size of MIRU40 repeating units is 54bp。H37Rv : 354+54 × 1=408bp		Note: The repeat unit size of Mtub21 is 57bp.H37Rv: 92+57× 2=206bp		Note: The repeat unit size of Mtub04 is 51bp, and some clinical strains have an incomplete repeat of 30bp in size.H37Rv: 137+51× 2+30=269bp		Note: VNTR2372 has a repeat unit size of 57bp, and some clinical strains have an incomplete repeat of 12bp in size.H37Rv: 172+57× 2+12=298bp	