

**Using microfluidic chip and allele-specific PCR to rapidly identify drug
resistance-associated mutations of *Mycobacterium tuberculosis***

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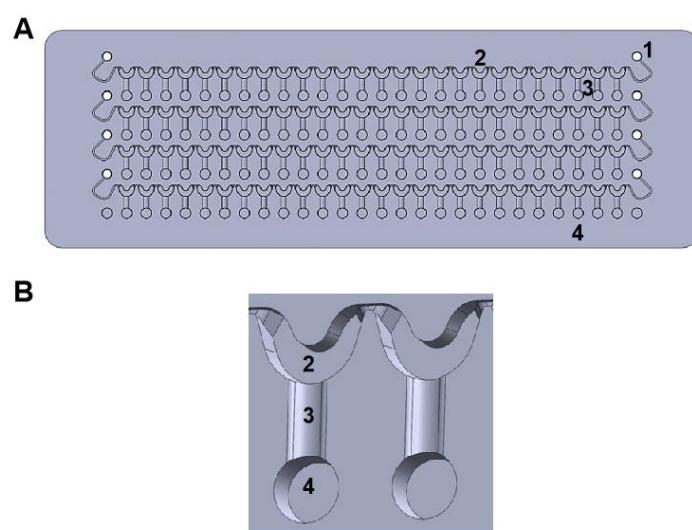


Figure S1: (A) The structure of the chip. The structures from 1 to 4 are as follows: inlet/outlet, infusing channel, linking channel and reaction chamber. (B) 3D illustration of single PCR reaction chamber.

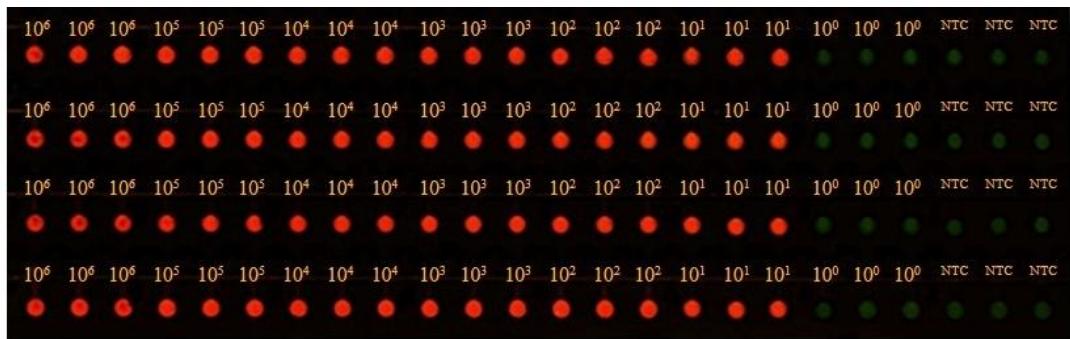


Figure S2: Fluorescence intensities of reaction chambers for sensitivity tests. The concentration of four different samples was diluted to 10^6 - 10^0 copies/ μl , respectively. The light green of reaction chambers indicate the failure amplifications that not reach the criteria for positive results.

Table S1 KASP primer sequences

Primers	Type	Allele-specific forward primers*		Reverse primer
		Tail 1	Tail 2	
<i>rpoB511Pro1</i>	C/T	TGTTCTGGTCCATGAATTGGCTCA	GTCGCCGCGATCAAGGAGTTCTT	
		Tail 2- GTTCTGGTCCATGAATTGGCTCG		
<i>rpoB513Leu</i>	A/T	Tail 1- GCACCAGCCAGCTGAGCCA	AGCGGGTTGTTCTGGTCCATGAAT	
		Tail 2- GCACCAGCCAGCTGAGCCT		
<i>rpoB513Pro2</i>	A/C	Tail 1- GCACCAGCCAGCTGAGCCA	AGCGGGTTGTTCTGGTCCATGAAT	
		Tail 2- CACCAGCCAGCTGAGCCC		
<i>rpoB516Val</i>	G/T	Tail 1- AGCCAGCTGAGCCAATTCATGG	TCAACCCCGACAGCGGGTTGTT	
		Tail 2- CAGCCAGCTGAGCCAATTCATGT		
<i>rpoB516Leu</i>	A/T	Tail 1- GCCAGCTGAGCCAATTCATGGA	TCAACCCCGACAGCGGGTTGTT	

		Tail 2- GCCAGCTGAGCCAATTGATGGT	
<i>rpoB</i> 526Arg	A/G	Tail 1- GCCGACAGTCGGCGCTTGT	AGAACAAACCCGCTGTCGGGGTT
		Tail 2- CCGACAGTCGGCGCTTGC	
<i>rpoB</i> 526Tyr	C/T	Tail 1- CCGACAGTCGGCGCTTGTG	AGAACAAACCCGCTGTCGGGGTT
		Tail 2- GCCGACAGTCGGCGCTTGTGTA	
<i>rpoB</i> 526Leu	A/T	Tail 1- GCCGACAGTCGGCGCTTGT	AGAACAAACCCGCTGTCGGGGTT
		Tail 2- GCCGACAGTCGGCGCTTGA	
<i>rpoB</i> 531Leu	C/T	Tail 1- CCCACAAGCGCCGACTGTC	ACAGACCGCCGGGGCCCCA
		Tail 2- GACCCACAAGCGCCGACTGTT	
<i>rpoB</i> 533Pro	T/C	Tail 1- CAGACCGCCGGGCCCCA	TTGACCCACAAGCGCCGACTGT
		Tail 2- AGACCGCCGGGCCCCG	
<i>rpsL</i> 43Arg	A/G	Tail 1- CGTGTACACCACCACTCCGAG	CAACCTTCCGAAGCGCCGAGTT
		Tail 2- CGTGTACACCACCACTCCGAA	
<i>rpsL</i> 43Thr	A/C	Tail 1- CGTGTACACCACCACTCCGAC	CAACCTTCCGAAGCGCCGAGTT
		Tail 2- CGTGTACACCACCACTCCGAA	
<i>rpsL</i> 88Arg	A/G	Tail 1- GCACACCAGGCAGGTCCC	CACAAACCTGCAGGAGCACTCGAT
		Tail 2- CGCACACCAGGCAGGTCCCT	
<i>rpsL</i> 88Thr	A/C	Tail 1- GCACACCAGGCAGGTCCG	CACAAACCTGCAGGAGCACTCGAT
		Tail 2- CGCACACCAGGCAGGTCCCT	
<i>rrs</i> 1401G	A/C	Tail 1- GGTGTTACCGACTTCATGACGC	GGTGAATACGTTCCCGGGCCTT
		Tail 2- GGTGTTACCGACTTCATGACGT	
<i>rrs</i> 514C	A/C	Tail 1- GGCCAACTACGTGCCAGCC	GACAACGCTCGCACCTACGTA

		Tail 2- CGGCCAACTACGTGCCAGCA	
<i>rrs517T</i>	C/T	Tail 1- CGCACCCCTACGTATTACCGCA	GGAGAAGAAGCACC GGCCA ACTA
		Tail 2- CGCACCCCTACGTATTACCGCG	
<i>rrs513T</i>	C/T	Tail 1- ACCCTACGTATTACCGCGGCTA	GGAGAAGAAGCACC GGCCA ACTA
		Tail 2- CCCTACGTATTACCGCGGCTG	
<i>rrs516T</i>	C/T	Tail 1- GCCCAACTACGTGCCAGCAGT	GACAACGCTCGCACCC TACGTA
		Tail 2- GCCAACTACGTGCCAGCAGC	

Abbreviations: * Universal sequence of allele specific primers connected at the 5', tail

1=GAAGGTGACCAAGTTCATGCT, tail 2= GAAGGTCGGAGTCAACGGATT.

Table S1 KASP primer sequences (Continue)

Primers	Type	Allele-specific forward	
			Reverse primer
<i>embB306Val</i>	A/G	Tail 1- GACGGCTACATCCTGGCG	CGAACCGCGAAATAGTTGGACAT
		Tail 2- CGACGGCTACATCCTGGCGA	
<i>embB306Ile1</i>	G/C	Tail 1- GGTCGGCGACTCGGGCG	ACGACGGCTACATCCTGGGCAT
		Tail 2- GGTCGGCGACTCGGGCC	
<i>embB306Ile2</i>	G/T	Tail 1- GGTCGGCGACTCGGGCA	ACGACGGCTACATCCTGGGCAT
		Tail 2- GGTCGGCGACTCGGGCC	
<i>embB306Ile3</i>	G/A	Tail 1- GGTCGGCGACTCGGGCT	ACGACGGCTACATCCTGGGCAT
		Tail 2- GGTCGGCGACTCGGGCC	
<i>embB306Leu1</i>	A/C	Tail 1- GACGGCTACATCCTGGCC	CGAACCGCGAAATAGTTGGACAT
		Tail 2- CGACGGCTACATCCTGGCA	

		Tail 1- CGACGGCTACATCCTGGCT	CGAACCGCGAAATAGTTGGACAT
<i>embB306Leu2</i>	A/T	Tail 2- CGACGGCTACATCCTGGCA	
		Tail 1- GAGCCGAGCGCGATGATGG	CCGCGTGGATGCCGTTCAACAA
<i>embB406Ala</i>	G/C	Tail 2- GAGCCGAGCGCGATGATGC	
		Tail 1- CCATACGACCTCGATGCCGC	CGTATGGCACCGGAACCGGTAA
<i>katG315Thr</i>	G/C	Tail 2- CCATACGACCTCGATGCCGG	
		Tail 1- CCATACGACCTCGATGCCGC	CGTATGGCACCGGAACCGGTAA
<i>katG315Asn</i>	G/A	Tail 2- CCATACGACCTCGATGCCGT	
		Tail 1- CCGTAAGGACGCGATCACCA	GGTGTTCGTCCATACGACCTCGAT
<i>katG315Arg1</i>	A/C	Tail 2- CGGTAAGGACGCGATCACCC	
		Tail 1- CCATACGACCTCGATGCCGC	CGTATGGCACCGGAACCGGTAA
<i>katG315Ile</i>	G/T	Tail 2- CCATACGACCTCGATGCCGA	
		Tail 1- CCGTAAGGACGCGATCACCA	GGTGTTCGTCCATACGACCTCGAT
<i>katG315Gly</i>	A/G	Tail 2- CGGTAAGGACGCGATCACCG	
		Tail 1- GGTAAGGACGCGATACCAGC	GGTGTTCGTCCATACGACCTCGAT
<i>katG315Arg2</i>	C/G	Tail 2- GGTAAGGACGCGATACCAGG	
		Tail 1- ATCTCGAGGAAACTGTTGTCCC	GTATGGACGAACACCCCGACGAA
<i>katG315Arg3</i>	T/C	Tail 2- CTCGAGGAAACTGTTGTCCCG	
		Tail 1- ACGAACACCCCGACGAAATGG	CGTACAGGATCTCGAGGAAACTGTT
<i>katG315Cys</i>	G/T	Tail 2- GACGAACACCCCGACGAAATGT	

Abbreviations: * Universal sequence of allele specific primers connected at the 5', tail

1=GAAGGTGACCAAGTTCATGCT, tail 2= GAAGGGTCGGAGTCAACGGATT.