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Application note

PlexTaq® 5x qPCR Multiplex Master Mix

for *Mycobacterium tuberculosis* detection by microarray

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Introduction

The spread of multi-drug resistant clinical tuberculosis (TB) and its co-infection scenarios have seriously limited the prevention and treatment of TB. The causative agent of TB, *Mycobacterium tuberculosis* (MTB), has become resistant to the early first-line anti-TB drugs such as rifampicin (RIF) and isoniazid (INH). Tuberculosis is then defined as rifampicin-resistant (RR-TB) only or multidrug-resistant (MDR-TB) when resistant to both anti-bacterial drugs. The WHO Global tuberculosis report from 2022 estimated that of 3.6% newly diagnosed and 18% of previously treated TB cases had MDR-TB. Considering the present methods for detecting MTB being slow and time-consuming, there is an urgent need for sensitive and robust molecular diagnostic techniques for MTB detection.

Rifampicin notably plays a critical role in the treatment of tuberculosis due to its bactericidal effects. MTB resistance to RIF is primarily associated with mutations present in the *rpoB* gene encoding the RNA polymerase β subunit. Specifically, 66 of the 70 mutations associated with drug resistance in the *rpoB* gene are concentrated in an 81 bp RIF-resistance determining region (RRDR).[1,2] Thus, there is great value in utilising such a gene region for an early clinical diagnosis of resistance, establishing a rapid detection method of *rpoB* gene mutations in this region and the relative likelihood of RIF resistance.

In this study, PlexTaq® 5x qPCR Multiplex Master Mix (myPOLS Biotech GmbH, part of Medix Biochemica) and an alternative commercially well-known 5x multiplex master mix were compared in detecting two human gDNA targets (*β-actin* and *KRAS genes*) extracted from blood spiked with synthetic *Mycobacterium tuberculosis* RRDR of the *rpoB* gene on a multiplex microarray. The method is based on a solid-phase hybridization of fluorescently-labeled amplicons upon PCR, which combines the physical-optical properties of the silicon substrate with the surface chemistry used to coat the substrate to obtain better sensitivity.[3]

Methods

PlexTaq® 5x qPCR Multiplex Master Mix (myPOLS Biotech GmbH, part of Medix Biochemica) was compared to a competitor multiplex master mix. Human genomic DNA extracted from 300 ul of human whole blood by use of Maxwell's RSC Whole Blood DNA Kit (PROMEGA, Madison, Wisconsin, United States) according to the manufacturer's instructions, was spiked with ~10^8 copies of synthetic RRDR gene. The thermal cycling conditions for both mixes are presented in Tables 1 and 2, respectively, and the 3-target (β-actin, KRAS, RRDR) multiplex reaction setup is described in Table 3.

After PCR, dual domain reporters were introduced, and a temperature gradient was applied followed by the microarray detection of the Cy3-labeled amplicons (Figure 1). Dual-domain reporters are specific oligonucleotide reporters whose sequence consists of two domains. The 5' domain (discriminating domain) is complementary to the genome sequence comprising the mutation, whereas the 3' domain is a "barcode" sequence (barcode domain) that recognizes complementary oligonucleotide probes (capture probes) spotted at specific locations on the silicon chip. [4]

Table 1. Thermal cycling conditions for PlexTaq® 5x qPCR Multiplex Master Mix (run time 47min).

Step	Temperature	Time	Cycles
1.	95°C	2 min	1x
2.	95°C	15 sec	25x
3.	60°C	1 min	

Table 2. Thermal cycling conditions for the alternative multiplex master mix (run time 1h 07min).

Step	Temperature	Time	Cycles
1.	95°C	12 min	1x
2.	95°C	20 sec	25x
3.	60°C	30 sec	
4.	72°C	30 sec	
5.	72°C	7min	1x

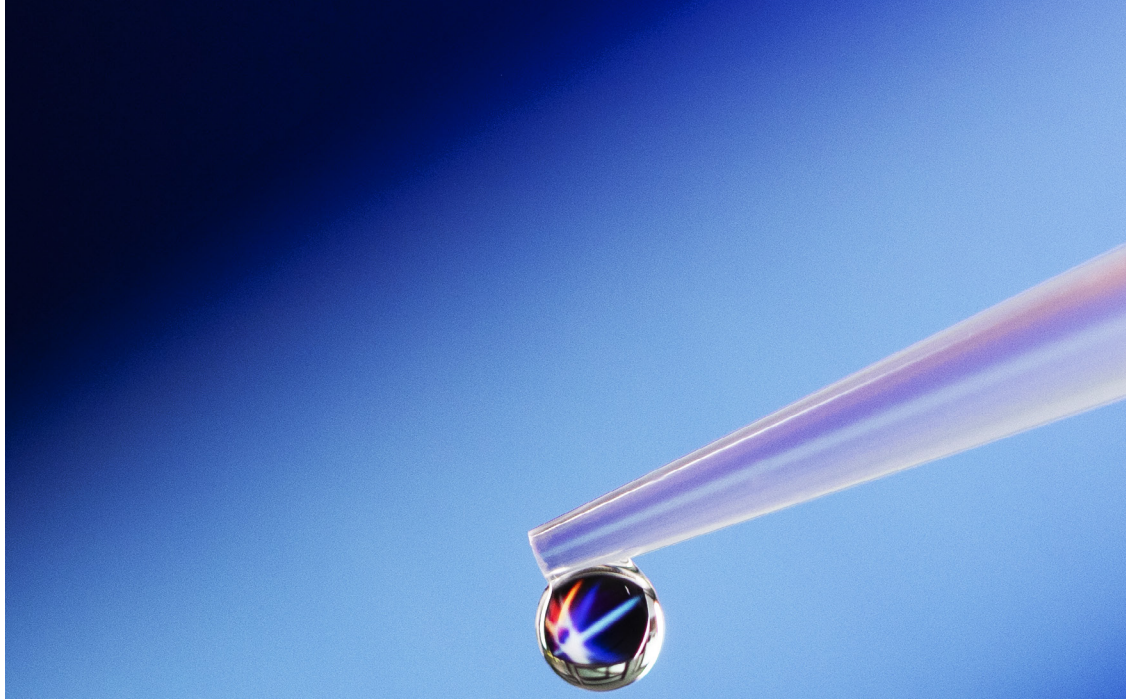


Table 3. Reaction setup.

Component	Final conc.	Vol (µL)
5x PCR Master Mix	1x	5
Forward human β-actin primer (10µM)	0,5µM	1,25
Reverse human β-actin primer (10µM)	0,5µM	1,25
Forward human KRAS primer (10µM)	0,5µM	1,25
Reverse human KRAS primer (10µM)	0,5µM	1,25
Forward bacterial RRDR primer (10µM)	0,5µM	1,25
Reverse bacterial RRDR primer (10µM)	0,5µM	1,25
Human genomic DNA (2000 copies/µL)	100 copies/µL	1,25
Synthetic bacterial DNA (10^8 copies/µL)	$5*10^6$ copies/µL	1,25
H2O		10
Total volume		**25**

Figure 1. Multiplex microarray workflow.

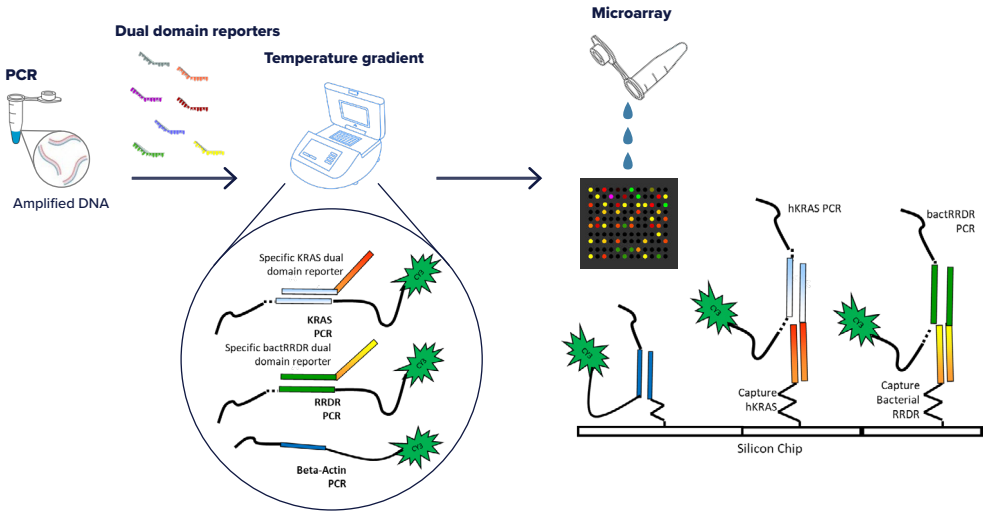


Figure 2. A) Spotting scheme of the human β-actin, KRAS and RRDR multiplex. B) Cy3 fluorescence images for PlexTaq® 5x qPCR Multiplex Master Mix and the alternative multiplex master mix.

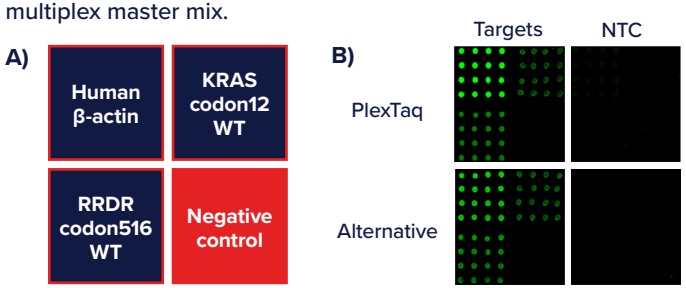
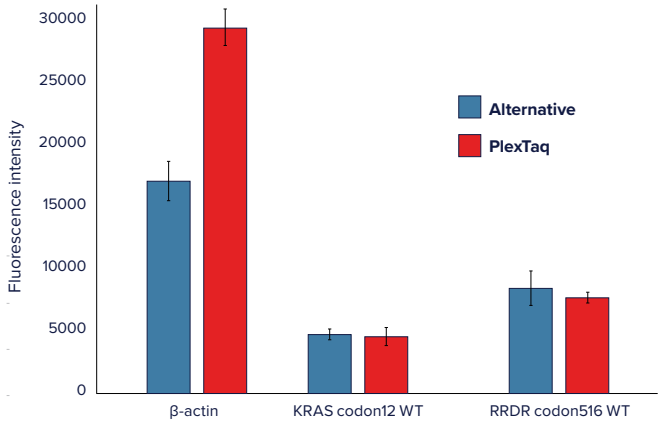


Figure 3. Fluorescence intensities for the three multiplex targets.



Results

The microarray detection spectrums for the three targets are presented in Figure 2 and their respective fluorescence intensities in Figure 3. The microarray fluorescence images are of similar intensity to the naked eye. The NTC were evidently without fluorescence. The fluorescence intensities were averaged from every four repeats for the two human targets and the MTB one. The fluorescence intensity for KRAS and RRDR were similar for both PlexTaq® 5x qPCR Multiplex Master Mix and the alternative mastermix. However, the fluorescence intensity for β-actin is visibly stronger in the case of PlexTaq® 5x qPCR Multiplex Master Mix compared to the alternative mastermix. This may suggest that the PlexTaq can accommodate multiple gene targets in a single reaction compared with the alternative mastermix.

Conclusions

This study demonstrated that both PlexTaq® 5x qPCR Multiplex Master Mix and the alternative multiplex master mix were able to amplify the three targets detected on a silicon microarray. However, PlexTaq® 5x qPCR Multiplex Master Mix outperformed the alternative mix with **better signal** for β-actin and 20 min **faster PCR run time.** This may suggest that the PlexTaq® 5x qPCR Multiplex Master Mix display superior multiplexing performance over the alternative mastermix.

PlexTaq® 5x qPCR Multiplex Master Mix is a ready-made mix that can be used for multiplex microarray and allows to detect simultaneously human and bacterial DNA.

References:

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4. Damin, F.; Galbiati, S.; Soriani, N.; Burgio, V.; Ronzoni, M.; Ferrari, M.; Chiari, M. Analysis of KRAS, NRAS and BRAF mu-tational profile by combination of in-tube hybridization and universal tag-microarray in tumor tissue and plasma of colorectal cancer patients. PLoS ONE (2018), 13, e0207876.

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