MyTaq™ Blood PCR Kit

LONG-AMPLICON, MULTIPLEX HUMAN GENOTYPING PCR

As personalized medicine grows, there is a demand for efficient and cost effective PCR-based workflows using patient samples that are easily collected. Often, the preferred sample collection technique is non-invasive such as saliva or buccal cell collection. However, saliva and buccal swab specimens are rife with PCR inhibitors that prevent long-amplicon PCR. Traditionally, removal of these inhibitors involves a silica membrane or magnetic bead-based DNA purification step. Such DNA extraction protocols are labor intensive and expensive when processing samples in a high throughput workflow. To overcome the disadvantages of these classic DNA purification protocols, a new workflow is presented that circumvents the DNA purification step and instead uses a buccal cell lysate. The unpurified DNA, together with the MyTaq Blood PCR kit, is used for detection of single nucleotide polymorphisms (SNPs) and insertion/deletion events within a gene of clinical relevance.

The MyTaq Blood PCR Kit is renowned for performing optimally in the presence of even very stringent inhibitors such as proteinases, immunoglobins and other substances from the bacterial flora. In this application, the MyTaq Blood PCR Kit succeeds in overcoming buccal specimen inhibitors during multiplex PCR, followed by analyte-specific primer extension. The kit was able to; (1) eliminate nonspecific amplification during room temperature set up because of the hot-start polymerase; (2) achieve the specificity and robustness required for selectively amplifying the CYP450 2D6 gene that is homologous to the CYP450 2D7 gene; (3) amplify 1.5 kb and 3 kb amplicons simultaneously during the 1st step of gene amplification followed by the 2nd step of analyte-specific primer extension of 29 different primers without additional MyTaq enzyme; (4) amplify detectable quantities of all amplicons despite the variability in number of buccal cells collected from patient to patient and (5) outperform all tested alternative products designed to work on samples without DNA purification or “direct PCR” scenarios.

This robust and high yield PCR mix exceeds the results of competitor products and is able to significantly streamline sample isolation and subsequent downstream amplification without compromise to amplicon yield even when the amplicons are >1kb.

MATERIALS AND METHODS

Human buccal cells were harvested with a flocked swab then stored in a DNA preserving agent until lysis. Commercially available human genomic DNA was used as a positive control, while a sample of the DNA preserving agent without cells was used as a no template control. The lysate was treated with a proteinase for 15 minutes. After heat inactivation of the protease, an aliquot (3 µL) of this crude lysate was added to MyTaq Blood PCR mix (12 µL) containing primer mix A. Primer mix A will detect for CYP450 2D6 gene duplication and other SNPs. Another aliquot (3 µL) of cells is added to a second PCR mix (12 µL) containing primer mix B. Primer mix B will detect for CYP450 2D6 gene deletion and other SNPs. To increase primer specificity, a touchdown PCR protocol was used for the first 6 cycles of amplification (95 °C for 2 min initial hot-start step; 6x 95 °C for 15 s, 67 °C for 30 s (-1.0 °C/cycle), 68 °C for 3:00 min), then 27 additional standard PCR cycles (94 °C for 15 s, 61 °C for 15 s, 71 °C for 2.30 min) with a final extension step of 71 °C for 1 min.

After PCR was complete, 10 µL of Amp Mix A was combined with 10 µL of Amp Mix B and a PCR cleanup was performed using phosphatase and exonuclease to remove excess primers and dNTPs. Analyte-specific primers were then added to the purified PCR reaction. These primers were extended, by the MyTaq carried over from PCR, in the presence of fluorescent dCTP. Finally, these labeled DNA strands were then loaded onto the DNA microarray, or BioFilm chip from AutoGenomics followed by hybridization, washing, scanning and results analysis using instrumentation and software as part of the INFINITI® system from AutoGenomics.

RESULTS

The MyTaq Blood PCR kit delivers efficient and robust multiplex CYP450 2D6 gene amplification (Fig. 1). When the sample has a CYP450 2D6 gene deletion, a 3.2 kb amplicon is produced in Amp Mix B as well as a second 3.1 kb amplicon (lane 7). When the sample has a CYP450 2D6 gene duplication, a 3 kb amplicon is produced in Amp Mix A, as well as a second 1.5 kb amplicon (lanes 9, 12, and 16).

Compared to competitor products, MyTaq Blood PCR mix delivered higher yield and improved specificity. The same volume of crude DNA solution was used in all PCR assays and the manufacturers' recommended PCR conditions were used for all 3 experiments. Only the MyTaq Blood Kit generated a high yield of the expected PCR products (Fig. 2).
After hybridization and analysis of the INFINITI® microarray chip (Fig. 3), detection of 29 SNPs was easily achieved. No PCR inhibition from salivary factors or epithelial cells was noted.

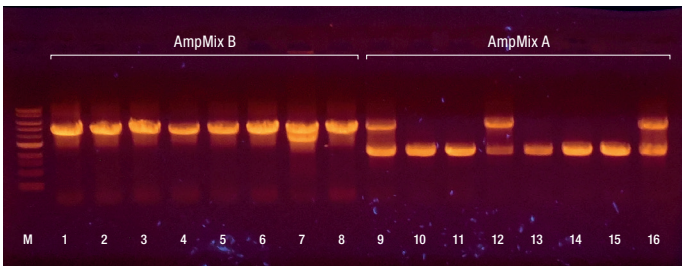

Fig 1. Results from 8 different CYP450 2D6 amplifications using MyTaq Blood PCR mix revealing a gene deletion event in lane 7 from Amp Mix B and duplication events in lanes 9, 12 and 16 from Amp Mix A. A 1kb DNA ladder was loaded for size reference.

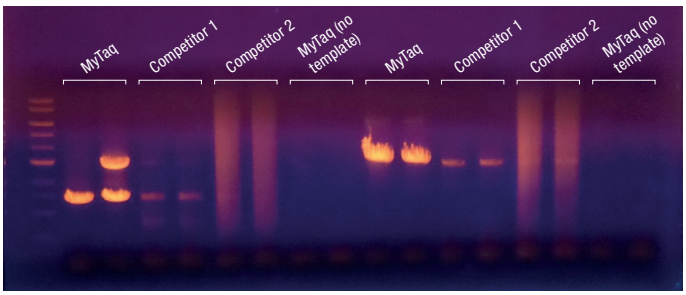

Fig 2. Gel of MyTaq Blood Kit vs competitor products over Amp Mix A and B
Robust amplification is visible for both Amp Mix A and B. A 1kb DNA ladder was loaded for size reference.

SUMMARY

The MyTaq Blood PCR Kit allows for rapid, robust and long (>1kb) amplicon multiplex amplification of human genomic DNA from buccal cells. Because a DNA purification step with silica membranes or magnetic beads is unnecessary, there is an approximate 10-fold cost savings and about a 4-fold time saving prior to the PCR step. The sample preparation and amplification presented here is conducive to a high throughput, automated workflow. In addition, the MyTaq Blood PCR Kit efficiently neutralized potential PCR inhibitors present in a buccal cell lysate. After protease treatment, buccal DNA was used directly in the PCR MyTaq amplification mix containing PCR primers. Compared to other direct PCR reagents, only the MyTaq Blood PCR kit reliably multiplexed long amplicons from the human CYP450 2D6 gene. The PCR products were of sufficient yield and specificity to drive efficient CYP450 2D6 genotyping when using the CYP450 2D6 assay as part of the INFINITI® system from AutoGenomics.

Buccal DNA	Analysis	Buccal DNA	Analysis
2850C	M [CYP2D6*2]	2615_7delAAG	
2850T		100C	W
2549A	W	100T	
2549delA		124G	W
1846G	W	124A	
1846A		1023C	W
1707T	W	1023T	
1707delT		1659G	W
2935A	W	1659A	
2935C		-1584C	M [CYP2D6 -1584G]
1758T		-1584G	
1758G	W	2988G	W
1758A		2988A	
2615_7AAG	W	ConXN	P [CYP2D6 *XN]
M = Mutant	W = Wildtype	H = Heterozygous	P = Positive

Fig 3. INFINITI® CYP450 2D6 genotype results from a patient that has a gene duplication.
Under "Buccal DNA" are all the clinically relevant SNPs. SNP location within the 2D6 gene is indicated by the number in front of the SNP, and genotype for each SNP is shown under "Analysis"

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