MyTaq™ HS Mix

Increasing Complexity with Multiplex PCR

Multiplex PCR is a powerful technique that allows amplification of two or more products in parallel in a single reaction tube. This technology was first described in 1988 as a method to detect deletions in the dystrophin gene[1] and has since been applied in diverse research areas, including analyses of gene expression[2], forensic studies[3], linkage analysis[4], SNPs[5], mutations[6], microsatellites[7], polymorphisms[8] and pathogen identification[9].

Multiplex PCR ensures standardization because identical reaction conditions and template amounts are used, pipetting and cycling condition variations are eliminated and reliable comparison of results from different fragments is achieved. Multiplex PCR also saves time and reagents, hence its wide usage in various genotyping applications.

The key to successful multiplex PCR is the ability to define a set of common reaction parameters to all of the primer sets in the reaction, that ensure highly specific annealing to their target sequences and comparable extension efficiencies, even though the amplicons are different sizes (different enough to form distinct bands when visualized by gel electrophoresis). MyTaq™ HS Mix is a new generation of very high-performance PCR products developed by Meridian, designed to deliver outstanding results on all templates, including complex genomic DNA templates. MyTaq HS Mix is based on the latest technology in PCR enzyme preparation, engineered to increase affinity for DNA, so resulting in significant improvements to yield, sensitivity and speed. Together with a uniquely formulated buffer, this makes MyTaq HS Mix perfect for multiplex PCR.

This application note demonstrates the ability of MyTaq HS Mix to amplify from human genomic DNA, up to 16 PCR fragments simultaneously, without the need for specialized kits or large amounts of optimization.

TWO-STEP PROTOCOLS

Since MyTaq HS Mix is a ready to use solution containing all the reagents at their optimal concentration, lengthy optimization procedures, such as adjusting the amounts of DNA polymerase, Mg^{2+} and other reagents are virtually eliminated. MyTaq HS Mix contains a unique combination of salts and additives to ensure comparable efficiencies for annealing and extension of all primers in the reaction. Initially the recommended two-step amplification protocol, with a 4-plex reaction of larger amplicons was used (Fig. 1).

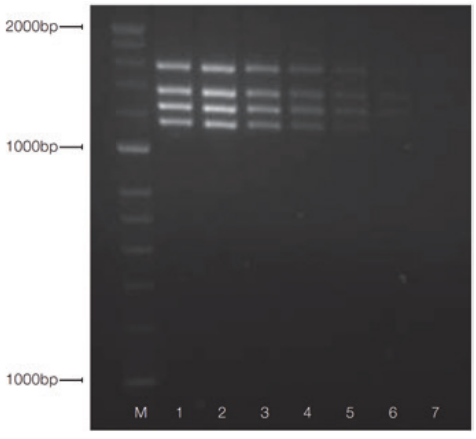

Fig. 1 Efficient 4-plex PCR from decreasing amounts of template using the two-step amplification protocol.
Decreasing amounts of human genomic DNA were used as a template (500 ng, 166 ng, 55 ng, 18 ng, 6 ng, 2 ng and 0.7 ng; lanes 1-7 respectively) in a 25 µL PCR reaction, with primers to produce amplicons of 1145 bp, 1247 bp, 1360 bp and 1563 bp. The cycling was performed under the following conditions: 95°C for 2 min, followed by 25 cycles at 95°C for 30s, 65°C for 4 min. Marker is HyperLadder 50 bp (Cat No. BIO-33039).

Once it was established that MyTaq HS Mix worked very efficiently without any further optimization, the complexity was increased successively to 7-plex, 11-plex (Fig. 2) and finally 16-plex (Fig. 3).

The results demonstrate the ability of MyTaq HS Mix to perform 7-plex (Fig. 2A) and 11-plex PCR (Fig. 2B) just by using the recommended two-step cycling protocol. With the 11-plex the smearing observed with the largest amplicons (1684 bp, 1989 bp, 2194 bp and 2608 bp) with the highest amount of template (500 ng) is eliminated with lowering the amount of template in the reaction.

MyTaq HS Mix is ideal whether performing singleplexing (Fig. 3A) or multiplexing (up to 16-plex) (Fig. 3B).

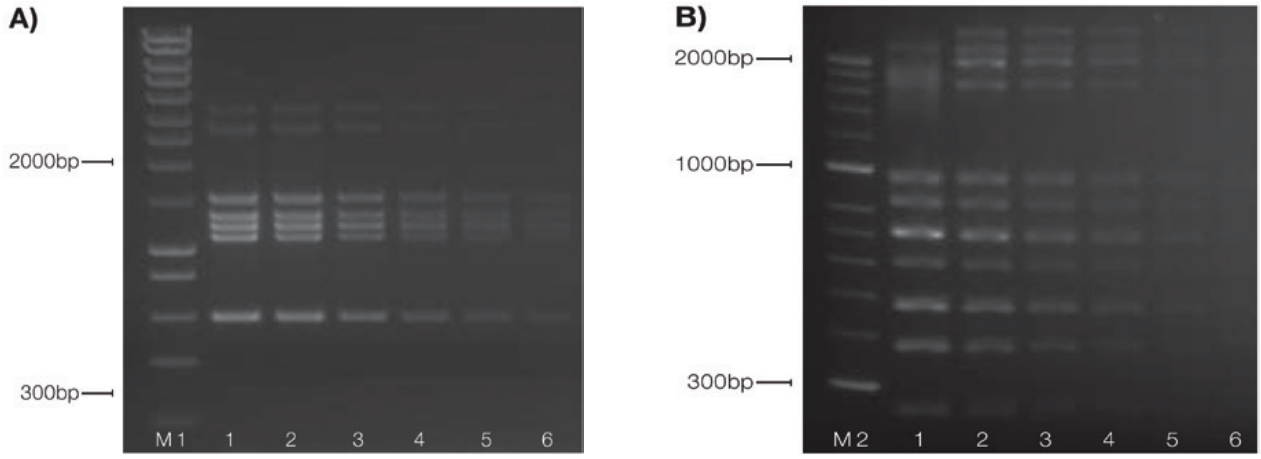

Fig. 2 Efficient 7- and 11- plex PCR from decreasing amounts of template.
Decreasing amounts of human genomic DNA were used as a template (500 ng, 166 ng, 55 ng, 18 ng, 6 ng and 2 ng; lanes 1-6 respectively) in a 25 µL PCR reaction, with primers to produce amplicons of A) 614 bp, 1145 bp, 1247 bp, 1360 bp, 1563 bp, 2818 bp and 3533 bp (7-plex) B) 256 bp, 383 bp, 484 bp, 612 bp, 726 bp, 857 bp, 972 bp, 1684 bp, 1989 bp, 2194 bp and 2608 bp (11-plex). The cycling was performed under the following conditions: 95°C for 2 min, followed by 25 cycles at 95°C for 30s, 65°C for 4 min. Marker is HyperLadder 1 kb (M1) (Cat No. BIO-33053). Marker is HyperLadder 50 bp (M2) (Cat No. BIO-33039).

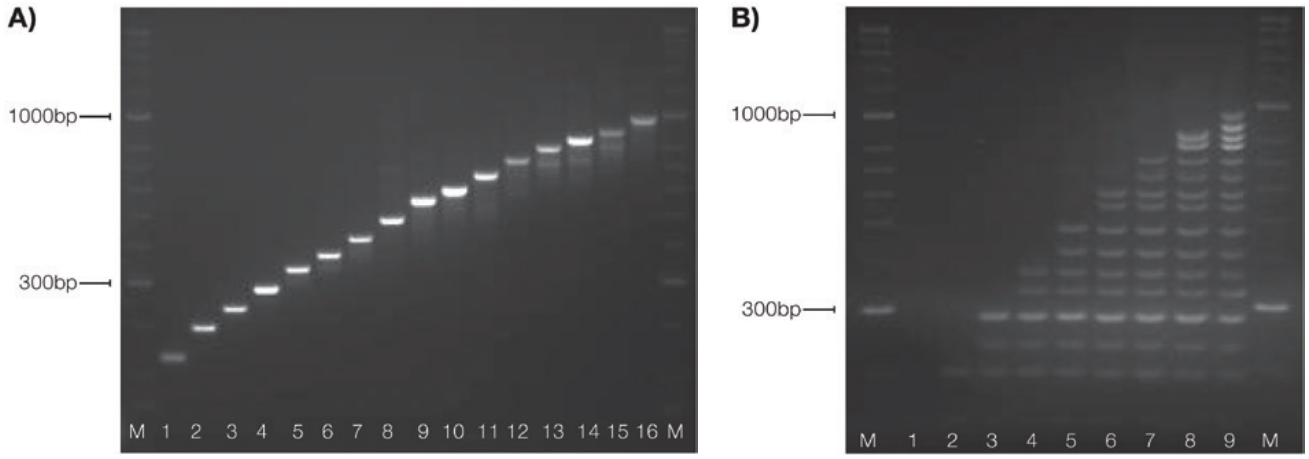

Fig. 3 Successful 16-plexing using MyTaq HS Mix.
50 ng of human genomic DNA was used as a template in a 25 µL PCR reaction, with primers to produce A) individual amplicons of 135 bp, 196 bp, 236 bp, 285 bp, 332 bp, 372 bp, 418 bp, 477 bp, 548 bp, 588 bp, 649 bp, 720 bp, 793 bp, 843 bp, 892 bp or 961 bp (lanes 1-16 respectively). B) 1-, 2-, 4-, 6-, 8-, 10-, 12-, 14-, and 16-plex reaction (lanes 1-9 respectively) using the same primers as above. The cycling was performed under the recommended multiplex conditions: 95°C for 2 min, followed by 25 cycles at 95°C for 30s, 65°C for 4 min. Marker is HyperLadder 50 bp (M) (Cat No. BIO-33039).

THREE-STEP PROTOCOLS

Although a two-step protocol is easier to adjust, as three-step protocols are commonly used, the multiplexing above was therefore repeated using a standard three-step protocol. 4-plex and 11-plex PCR reactions (from Figures 1 and 2) were repeated using a standard three-step amplification protocol recommended by other suppliers (in this case supplier Q).

The results demonstrate that MyTaq Mix gives high yields (Fig. 4A) and works even in a complex reaction without optimization (Fig. 4B) when using a three-step reaction.

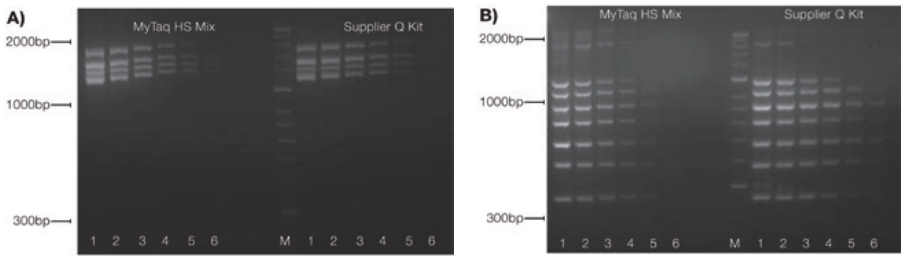

Fig. 4 4-plex, 7-plex and 11-plex PCR using three-step amplification.
Decreasing amounts of human genomic DNA were used as a template (500 ng, 166 ng, 55 ng, 18 ng, 6 ng and 2 ng; lanes 1-6 respectively) in a 25 µL PCR reaction, with primers to produce amplicons of A) 1145 bp, 1247 bp, 1360 bp and 1563 bp (4-plex), B) 256 bp, 383 bp, 484 bp, 612 bp, 726 bp, 857 bp, 972 bp, 1684 bp, 1989 bp, 2194 bp and 2608 bp (11-plex). The PCRs were performed under Supplier Q's recommended conditions: 94°C for 15 min, followed by 30 cycles at 94°C for 30s, 60°C for 90s and 72°C for 90s. Marker is HyperLadder 50bp (M) (Cat No. BIO-33039).

FAST MULTIPLEXING

MyTaq HS Mix is based on the latest technology in PCR enzyme preparation, resulting in significant improvements to yield, sensitivity and speed. This allows MyTaq HS Mix to be used with much shorter extension times than most of the Taq based kits available on the market, reducing the overall PCR cycling time, without the risk of compromising reaction performance, or having to invest in specialized PCR consumables.

The three-step reaction in Figure 3 takes approximately 143 minutes; this is reduced to 114 minutes using the two-step protocol in Figures 1 and 2. Using a two-step reaction and reduce the annealing/extension time from 4 minutes to 1 minute, the overall reaction time is further reduced to approximately 40 minutes (Fig. 5) without losing the ability to multiplex (Fig. 6).

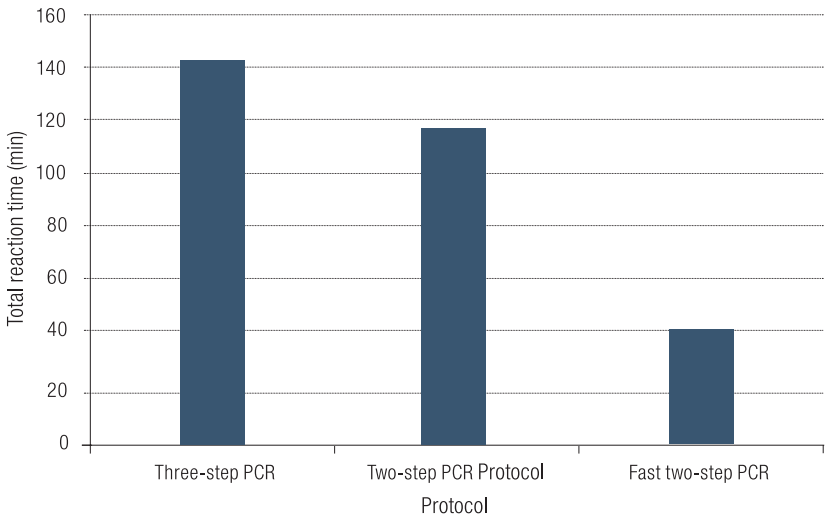

Fig. 5 Comparison of the speed of different multiplex protocols.
MyTaq HS Mix is designed for fast multiplex PCR, offering faster cycling times and improved reaction efficiency, resulting in time savings of up to 75% in comparison to other suppliers.

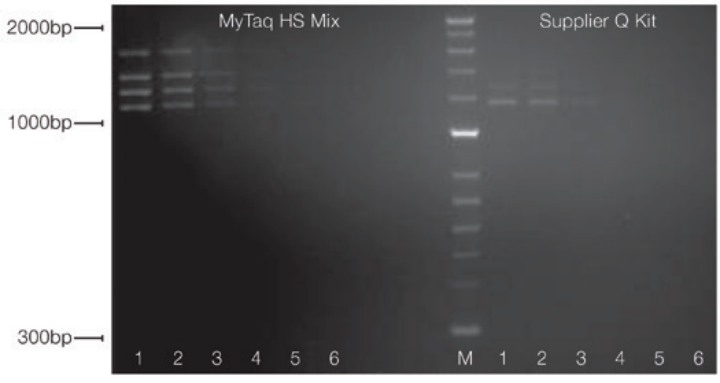

Fig. 6 4-plex PCR from decreasing amounts of template using a fast two-step amplification protocol.
Decreasing amounts of human genomic DNA were used as a template (500 ng, 166 ng, 55 ng, 18 ng, 6 ng, and 2 ng; lanes 1-6 respectively) in a 25 µL PCR reaction, with primers to produce amplicons of 1145 bp, 1247 bp, 1360 bp and 1563 bp. The cycling was performed under the following conditions: 95°C for 2 min, followed by 25 cycles at 95°C for 30s, 65°C for 1 min. Marker is HyperLadder 50bp (M) (Cat No. BIO-33039).

CONCLUSION

This application note demonstrates that MyTaq HS enables success in multiplex PCR at the first attempt, whether you are using a two-step or a three-step reaction. Furthermore, MyTaq HS can even be used for fast multiplex PCR, without the need of resorting to specialist kits, buffers or optimization, making multiplex PCR with MyTaq HS Mix easy for all your research needs.

REFERENCES

1. Chamberlain J. S., *et al. NAR* **16** (23): 11141–11156 (1988).
2. Avent N. D., *et al. Blood* **89** (7): 2568-2577 (1997).
3. Paracchini S., *et al. NAR* **30** (6): e27 (2002).
4. Barton A., *et al. Hum. Mol. Genet.* **10** (18): 1901-1906 (2001).
5. Hayden M. J., *et al. BMC Genomics* **9**: 80 (2008).
6. Garcia-Yoldi D., *et al. Clin. Chem.* **52** (4): 779-781 (2005).
7. Lepais O. L. & Bacles, C. F. J. *Hered.* **102** (5): 627-632 (2011).
8. Singh B. K., *et al. App. Environ. Micro.* **72** (11): 7278-7285 (2006).
9. Gafan G. P., *et al. J. Clin. Microbiol.* **42** (9): 4141-4146 (2004).