MangoTaq™

MangoTaq™ DNA Polymerase and MangoMix™: Robust performance in PCR amplification

MangoTaq™ DNA Polymerase is a formulation of *Taq* DNA Polymerase supplied with reaction buffer, which separates into two colors (red & orange) during electrophoresis. It also provides the added benefit of direct loading of your PCR samples onto an agarose gel. This application note demonstrates the use of Mango*Taq* in a variety of PCR applications.

Taq DNA polymerase is the most popular thermostable DNA Polymerase used for amplifying DNA by PCR. Many applications now require the handling of large numbers of samples, so time and convenience are key requirements for most assays. Meridian has developed Mango*Taq* DNA Polymerase, which enables direct loading of PCR reactions onto agarose gels. A pre-mixed, pre-optimized 2x formulation is also available, which requires the addition of just template, primers and water, therefore increasing reproducibility and reducing the time required for reaction set-up.

Mango*Taq*™ DNA POLYMERASE REACTION BUFFER

The 10x reaction buffer supplied with Mango*Taq*™ DNA Polymerase contains two inert, non-toxic dyes (red and orange) to assist with the direct gel loading of PCR samples onto agarose gels. The red dye migrates at the same rate as a 250 bp fragment on a 2% agarose gel, whereas the orange dye migrates at that of a 30 bp fragment on a 2% agarose gel.

COLORED DNA LOADING BUFFER DYE MIGRATION (APPROX.)

AGAROSE	RED	ORANGE
0.7%	1500 bp	60 bp
1.0%	750 bp	50 bp
1.5%	500 bp	40 bp
2.0%	250 bp	30 bp
3.0%	75 bp	20 bp

This property enables the user to monitor the migration of the samples and to ensure that the gel is run adequately and no loss of sample occurs (fig. 1)

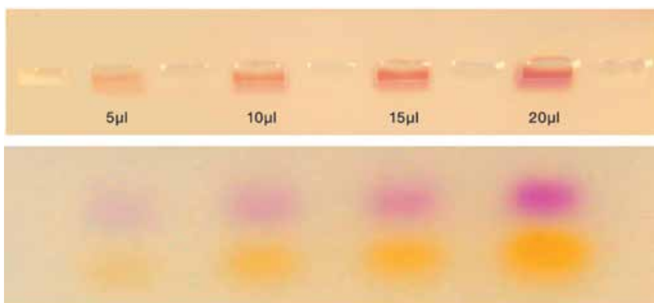

Figure 1. Separation of the red and orange dyes contained in the Mango*Taq* Reaction Buffer during electrophoresis. 5, 10, 15 and 20 µL of the amplification reactions were loaded onto a 1% agarose gel with TAE buffer and were subjected to electrophoresis.

Mango*Taq* DNA POLYMERASE PERFORMS OVER A WIDE RANGE OF $MgCL_2$ CONCENTRATIONS

A PCR may require different concentrations of $MgCl_2$ depending on the specific application. An experiment was performed to assess the ability of Mango*Taq* DNA Polymerase to amplify DNA in a range of $MgCl_2$ concentrations. A 626 bp fragment of the Rhodopsin gene was amplified from human genomic DNA using 1 Unit of Mango*Taq* DNA Polymerase in a 50 µL reaction. Mango*Taq* DNA Polymerase was able to amplify the fragment in concentrations as low as 2 mM and up to 10 mM $MgCl_2$ (fig. 2). The highest yield of the PCR product was observed in reactions containing 3.5-7 mM $MgCl_2$.

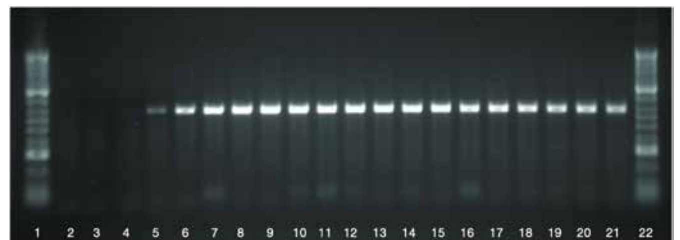

Figure 2. Amplification of a fragment of the Rhodopsin gene from human genomic DNA using Mango*Taq* DNA Polymerase in a range of $MgCl_2$ concentrations. A 626 bp fragment of the Rhodopsin gene was amplified from 100 ng human genomic DNA.
Lane 1. & 22. HyperLadder II
Lane 2. to 21. 0.5 mM to 10 mM $MgCl_2$ increasing in 0.5 mM increments

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ROBUST PCR PERFORMANCE

It is often necessary to amplify fragments from low quantities of starting material. An experiment was performed to determine the minimum starting concentration of DNA for successful PCR amplification. A 1 kb fragment was amplified from a 10-fold serial dilution of 0.5 ng Lambda DNA and from a 5-fold serial dilution of 25 ng of pGEM3zf(+), using 1 Unit of Mango*Taq* DNA Polymerase in a 50 µL reaction. The data demonstrate that Mango*Taq* DNA Polymerase can amplify the fragment from as little as 0.5 pg of Lambda DNA (fig. 3) or 40 pg of plasmid DNA (fig. 4), in the assay conditions tested.

Figure 3. Amplification of a 1 kb fragment from Lambda DNA using Mango*Taq*™ DNA Polymerase. A 10-fold serial dilution was performed on 0.5 ng Lambda DNA as follows:
Lane 2. 0.5 ng Lambda DNA
Lane 3.0.05 ng Lambda DNA
Lane 4. 5 pg Lambda DNA
Lane 5. 0.5 pg Lambda DNA
Lane 6. 0.05 pg Lambda DNA
Lane 7. 5 fg Lambda DNA
Lane 8. 0.5 fg Lambda DNA
Lane 9. 0.05 fg Lambda DNA
Lane 1 & 10. HyperLadder I

Figure 4. Amplification of a 1 kb fragment from pGEM3zf(+) using Mango*Taq*™ DNA Polymerase. A 5-fold serial dilution was performed on 25 ng pGEM3zf(+) DNA as follows:
Lane 2. 25 ng pGEM3zf(+)
Lane 3. 5 ng pGEM3zf(+)
Lane 4. 1 ng pGEM3zf(+)
Lane 5. 0.2 ng pGEM3zf(+)
Lane 6. 40 pg pGEM3zf(+)
Lane 7. 8 pg pGEM3zf(+)
Lane 8. 1.6 pg pGEM3zf(+)
Lane 9. 0.32 pg pGEM3zf(+)
Lane 1 & 10. HyperLadder I

HIGH YIELD PCR AMPLIFICATION PRODUCTS

Robust PCR performance is desirable in most PCR applications. We compared the yield of PCR products amplified using Mango*Taq* DNA Polymerase (accompanied by its colored buffer) or by using the equivalent Taq Polymerases from suppliers P and Q. PCR was performed with 1 Unit of each of the enzymes and 2.5 mM $MgCl_2$ in 50 µL reaction. The data illustrate that Mango*Taq* DNA Polymerase produces similar or higher yields of the PCR products tested in comparison to suppliers P and Q (fig. 5 & 6).

Figure 5. Amplification of a fragment of the ß-actin gene from human genomic DNA using Mango*Taq*™ DNA Polymerase and supplier P's and Q's Taq DNA Polymerases. A 5-fold serial dilution was performed on human genomic DNA as follows:
Lane 1. 200 ng human genomic DNA
Lane 2. 40 ng human genomic DNA
Lane 3. 8 ng human genomic DNA
Lane 4. 1.6 ng human genomic DNA
Markers (M) are HyperLadder I

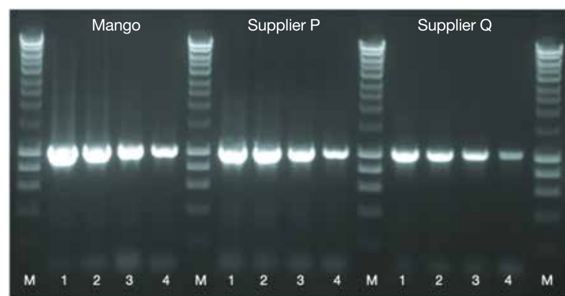

Figure 6. Amplification of a 1 kb fragment from Lambda DNA using Mango*Taq*™ DNA Polymerase and supplier P's and Q's Taq DNA Polymerases. A 5-fold serial dilution was performed on Lambda DNA as follows:
Lane 1. 0.5 ng Lambda DNA
Lane 2. 0.1 ng Lambda DNA
Lane 3. 0.02 ng Lambda DNA
Lane 4. 4pg Lambda DNA
Markers (M) are HyperLadder I

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Mango*Taq* DNA POLYMERASE IN HIGH SPECIFICITY ASSAYS

Often, depending on the template source and primer design some fragments are more difficult to amplify than others. The difficulty depends largely on GC content and the presence of secondary structure. The ability of Mango*Taq* DNA Polymerase to amplify fragments with a high GC content was assessed. A range of fragments from different human genes were amplified using Mango*Taq* DNA Polymerase and Supplier P's Taq DNA Polymerase and colored buffer. Figure 7 indicates that Mango*Taq* DNA Polymerase is able to amplify a range of fragments with varying GC content more efficiently than Supplier P's Taq DNA Polymerase.

Figure 7. A range of fragments from different human genes were amplified using Mango*Taq*™ DNA Polymerase and Supplier P's Taq DNA Polymerase. The amplification products are as follows:
Lane 1. 119 bp and 43% GC product amplified from the human glucocerebrosidase gene
Lane 2. 321 bp and 37% GC product amplified from the Angiotensin Receptor II gene
Lane 3. 626 bp and 56% GC product amplified from the Rhodopsin gene
Lane 4. 762 bp and 33% GC product amplified from the ß-globin gene
Lane 5. 1200 bp and 54% GC product amplified from the α-1-antitrypsin gene
Markers (M) are HyperLadder II

Mango*Taq* DNA POLYMERASE IN AMPLIFICATION OF LARGER FRAGMENTS

An experiment was performed to assess the ability of Mango*Taq* DNA Polymerase to amplify larger fragments. A 5 kb fragment was amplified from Lambda DNA using Mango*Taq* DNA Polymerase and Competitor P's and X's equivalent *Taq* DNA Polymerases. One unit of enzyme and 3 mM $MgCl_2$ were used in 50 µL reactions. The result demonstrates the suitability of Mango*Taq* DNA Polymerase to amplify fragments of up to 5 kb from Lambda DNA (fig. 8).

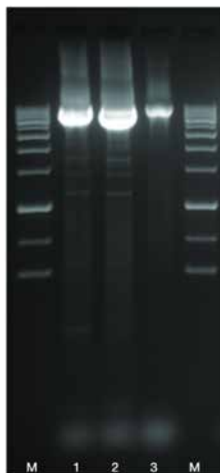

Figure 8. Amplification of a 5 Kb fragment from Lambda DNA using Mango*Taq*™ DNA Polymerase and Supplier P's and X's Taq Polymerases.
Lane 1. Supplier P DNA Polymerase and colored buffer
Lane 2. Mango*Taq*™ DNA Polymerase
Lane 3. Supplier X DNA Polymerase
Markers (M) are HyperLadder III

MangoMix™

Mango*Taq* DNA Polymerase is also available as a convenient pre-mixed, pre-optimized 2x master mix, Mango*Taq*™. Performance of MangoMix was similar to or better than that of *Taq* DNA Polymerase in the PCR assays performed (fig. 9).

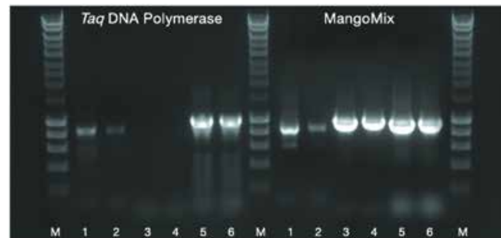

Figure 9. Amplification of fragments from three different templates using *Taq* DNA Polymerase and Mango*Taq*™.
Lanes 1-2. 800 bp fragment of the ß-actin gene amplified from genomic DNA
Lanes 3-4. 1 Kb fragment amplified from Lambda DNA
Lanes 5-6. 1 Kb fragment amplified from pGEM3zf(+)
Markers (M) are HyperLadder I

In addition, the performance of MangoMix was compared to that of similar ready-to-go mixes from a number of competitors. A fragment of the ß-actin gene was amplified with MangoMix and with 3 supplier's mixes. The data shown demonstrate the robust performance of MangoMix (fig. 10).

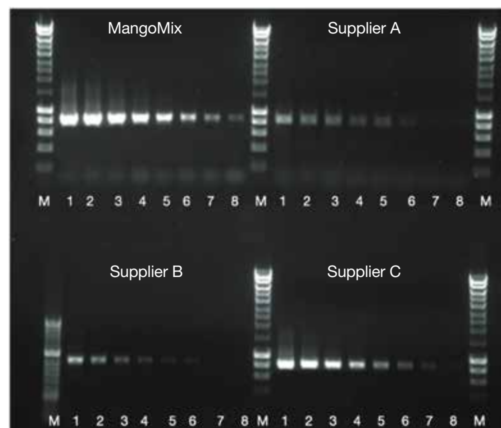

Figure 10. Amplification of a fragment of the ß-actin gene from human genomic DNA using Mango*Taq*™ and ready-to-go mixes from Suppliers A, B and C. A 2-fold serial dilution was performed on human genomic DNA as follows:
Lane 1. 200 ng human genomic DNA
Lane 2. 100 ng human genomic DNA
Lane 3. 50 ng human genomic DNA
Lane 4. 25 ng human genomic DNA
Lane 5. 12.5 ng human genomic DNA
Lane 6. 6.25 ng human genomic DNA
Lane 7. 3.375 ng human genomic DNA
Lane 8. 1.69 ng human genomic DNA
Markers (M) are HyperLadder I

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ADDITIONAL APPLICATIONS
CLONING INTO TA VECTOR

Products amplified using Mango*Taq* DNA Polymerase possess A overhangs for cloning into T vectors. To demonstrate this and assess the effect of the red dye in TA-cloning, the following experiment was performed. A 1 kb fragment was amplified from Lambda DNA, and half of the reaction was cleaned up using SureClean Plus (Cat No. BIO-37047) according to the manufacturer's instructions. 1 µL of the PCR product, either un-purified or purified using SureClean, was used in a TA cloning reaction with the vector PCR4-TOPO (Invitrogen). 3 µL of the TA cloning reaction were transformed into Chemically Competent Cells, and plated on LB plates containing X-GAL, IPTG and Ampicillin. The percentage of recombinant colonies was calculated as follows:

(Number of white colonies ÷ total colonies) x 100, and was calculated as 68% when the PCR fragment had not been purified, and 91% after purification of the PCR fragment with SureClean Plus. Similar results were obtained when a colorless buffer was used in place of the Mango*Taq* reaction buffer (data not shown), indicating that the red dye does not affect the efficiency of cloning reactions. In addition, this experiment illustrates that PCR products amplified using Mango*Taq* DNA Polymerase and Mango*Taq* reaction buffer can be used directly in downstream applications (fig. 11).

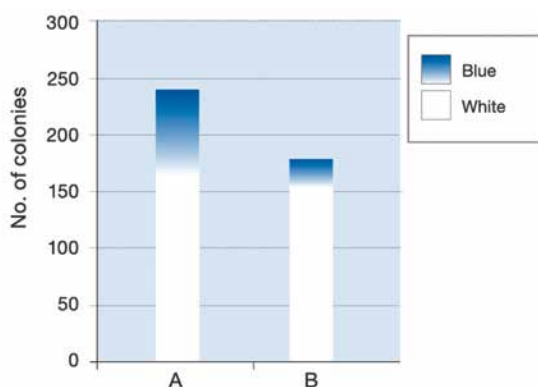

Figure 11. TA cloning using Mango*Taq*™ DNA Polymerase amplified PCR products. A 1 kb fragment was amplified from Lambda DNA using Mango*Taq*™ DNA Polymerase. Half of the PCR reaction was purified using SureClean Plus (B) and the other half was left unpurified (A). The purified (B) and unpurified (A) PCR fragments were cloned into a TA cloning vector and the percentage of positive transformants was estimated.

SUMMARY

Mango*Taq* DNA Polymerase and Mango*Taq* reaction buffer have been specifically designed for high-throughput applications, offering direct loading onto agarose gels, as well as the ability to monitor the migration of the PCR products in agarose gels during electrophoresis.

Using Mango*Taq* DNA Polymerase products between 119 bp and 5 kb were successfully amplified. Mango*Taq* DNA Polymerase was shown to give high yield amplification when compared to competitors equivalent Taq DNA Polymerases and colored buffers in the assays tested. Mango*Taq* DNA Polymerase was able to amplify PCR products from as little as 0.5 pg Lambda DNA or 40 pg of plasmid DNA. The ability of Mango*Taq* DNA Polymerase to perform a wide range of $MgCl_2$ concentrations and to amplify fragments with high GC content was also demonstrated.

The ready-to-go mix containing Mango*Taq* DNA Polymerase, MangoMix, was shown to require less optimisation than assays set-up with the stand alone components. MangoMix amplifies fragments with very high yield, requires little or no optimization and reduces the time required for reaction set-up, thereby increasing reproducibility and reducing the chances of contamination and pipetting errors.

Finally, it is illustrated that the dye present in Mango*Taq* Reaction buffer and MangoMix does not interfere in downstream processes such as TA-cloning. However, if the PCR product is to be analysed spectrophotometrically, or is to be used in sequencing reactions, we recommend initially cleaning up the product by a method such as the column-free nucleic acid purification using SureClean Plus (Cat No. BIO-37047).

Please visit **www.bioline.com/mangotaq** to request a sample of Mango*Taq*™ DNA Polymerase or MangoMix™.

Notes:

1. Mango*Taq* and MangoMix are trademarks of Bioline Reagents Ltd.
2. pGEM3zf(+) is a trademark of Promega Corporation.
3. PCR4-TOPO is a trademark of Invitrogen Corporation.

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