MangoTaq™

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

Mango*Taq*™ DNA Polymerase is a formulation of *Taq* DNA Polymerase supplied with a red 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. Bioline 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.

MangoTaq™ 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 250bp fragment on a 2% agarose gel, whereas the orange dye migrates at that of a 30bp fragment on a 2% agarose gel.

COLORED DNA LOADING BUFFER DYE MIGRATION (APPROX.)		
AGAROSE	RED	ORANGE
0.7%	1500bp	60bp
1.0%	750bp	50bp
1.5%	500bp	40bp
2.0%	250bp	30bp
3.0%	75bp	20bp

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).

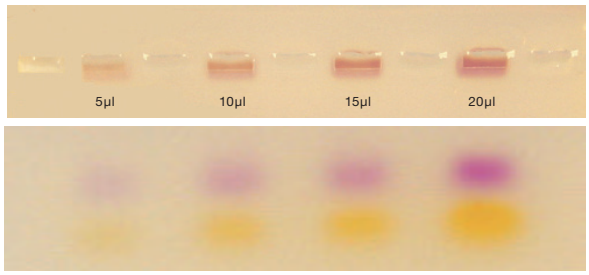

Figure 1. Separation of the red and orange dyes contained in the Mango*Taq* Reaction Buffer during electrophoresis. 5, 10, 15 and 20µl of the amplification reactions were loaded onto a 1% agarose gel with TAE buffer and were subjected to electrophoresis.

MangoTaq 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 626bp fragment of the Rhodopsin gene was amplified from human genomic DNA using 1 Unit of Mango*Taq* DNA Polymerase in a 50µl reaction. Mango*Taq* DNA Polymerase was able to amplify the fragment in concentrations as low as 2mM and up to 10mM $MgCl_2$ (fig. 2). The highest yield of the PCR product was observed in reactions containing 3.5-7mM $MgCl_2$.

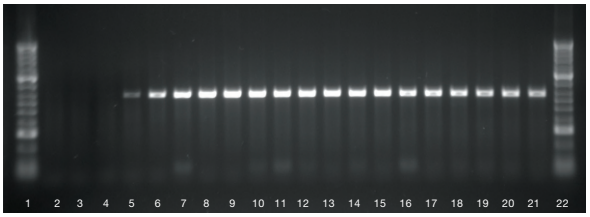

Figure 2. Amplification of a fragment of the Rhodopsin gene from human genomic DNA using Mango*Taq* DNA Polymerase in a range of $MgCl_2$ concentrations. A 626bp fragment of the Rhodopsin gene was amplified from 100ng human genomic DNA.
Lane 1. & 22. HyperLadder II
Lane 2. to 21. 0.5mM to 10mM $MgCl_2$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

BIOLINE

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 1Kb fragment was amplified from a 10-fold serial dilution of 0.5ng Lambda DNA and from a 5-fold serial dilution of 25ng of pGEM3zf(+), using 1 Unit of Mango*Taq* DNA Polymerase in a 50µl reaction. The data demonstrate that Mango*Taq* DNA Polymerase can amplify the fragment from as little as 0.5pg of Lambda DNA (fig. 3) or 40pg of plasmid DNA (fig. 4), in the assay conditions tested.

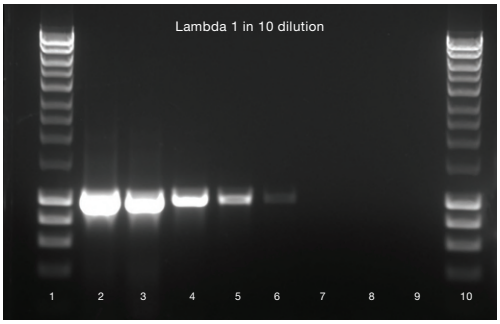

Figure 3. Amplification of a 1Kb fragment from Lambda DNA using Mango*Taq*™ DNA Polymerase. A 10-fold serial dilution was performed on 0.5ng Lambda DNA as follows:
Lane 2. 0.5ng Lambda DNA
Lane 3. 0.05ng Lambda DNA
Lane 4. 5pg Lambda DNA
Lane 5. 0.5pg Lambda DNA
Lane 6. 0.05pg Lambda DNA
Lane 7. 5fg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

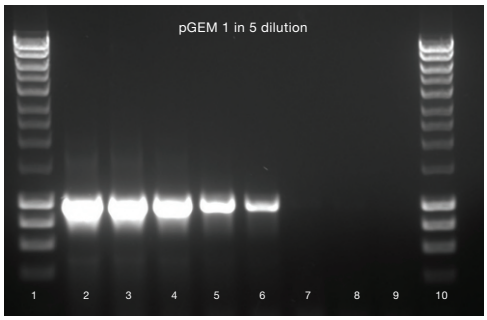

Figure 4. Amplification of a 1Kb fragment from pGEM3zf(+) using Mango*Taq*™ DNA Polymerase. A 5-fold serial dilution was performed on 25ng pGEM3zf(+) DNA as follows:
Lane 2. 25ng pGEM3zf(+)
Lane 3. 5ng pGEM3zf(+)
Lane 4. 1ng pGEM3zf(+)
Lane 5. 0.2ng pGEM3zf(+)
Lane 6. 40pg pGEM3zf(+)
Lane 7. 8pg pGEM3zf(+)
Lane 8. 1.6pg pGEM3zf(+)
Lane 9. 0.32pg 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 Competitors P and Q. PCR was performed with 1 Unit of each of the enzymes and 2.5mM $MgCl_2$ in 50µl reaction. The data illustrate that Mango*Taq* DNA Polymerase produces similar or higher yields of the PCR products tested in comparison to Competitors P and Q (fig. 5 & 6).

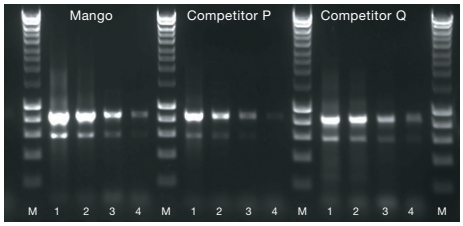

Figure 5. Amplification of a fragment of the β-actin gene from human genomic DNA using Mango*Taq*™ DNA Polymerase and Competitor P's and Q's *Taq* DNA Polymerases. A 5-fold serial dilution was performed on human genomic DNA as follows:
Lane 1. 200ng human genomic DNA
Lane 2. 40ng human genomic DNA
Lane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

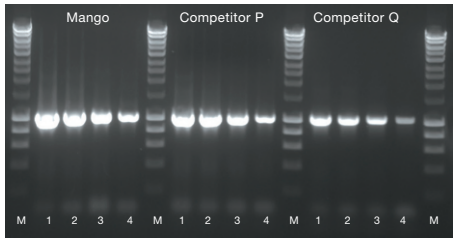

Figure 6. Amplification of a 1Kb fragment from Lambda DNA using Mango*Taq*™ DNA Polymerase and Competitor P's and Q's *Taq* DNA Polymerases. A 5-fold serial dilution was performed on Lambda DNA as follows:
Lane 1. 0.5ng Lambda DNA
Lane 2. 0.1ng Lambda DNA
Lane 3. 0.02ng Lambda DNA
Lane 4. 4pg Lambda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

MangoTaq 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 Competitors 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 Competitors P's *Taq* DNA Polymerase.

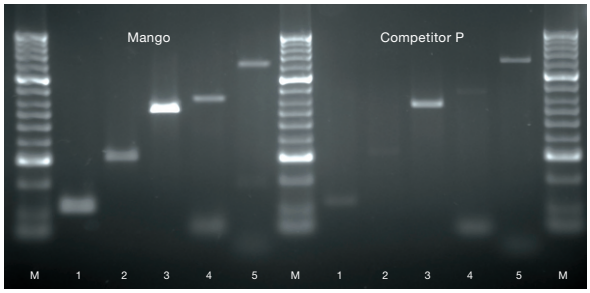

Figure 7. A range of fragments from different human genes were amplified using Mango*Taq*™ DNA Polymerase and Competitor P's *Taq* DNA Polymerase. The amplification products are as follows:
Lane 1. 119bp and 43% GC product amplified from the human glucocerebrosidase gene
Lane 2. 321bp and 37% GC product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

MangoTaq 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 5Kb 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 3mM $MgCl_2$ were used in 50µl reactions. The result demonstrates the suitability of Mango*Taq* DNA Polymerase to amplify fragments of up to 5Kb from Lambda DNA (fig. 8).

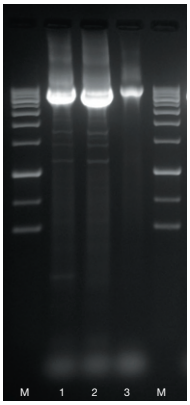

Figure 8. Amplification of a 5Kb fragment from Lambda DNA using Mango*Taq*™ DNA Polymerase and Competitor P's and X's *Taq* Polymerases.
Lane 1. Competitor P DNA Polymerase and colored buffer
Lane 2. Mango*Taq*™ DNA Polymerase
Lane 3. Competitor X DNA Polymerase
Markers (M) are HyperLadder III

MangoMix™

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

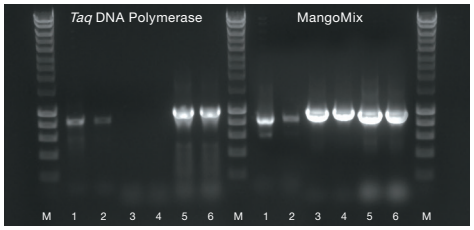

Figure 9. Amplification of fragments from three different templates using *Taq* DNA Polymerase and MangoMix™.
Lanes 1-2. 800bp fragment of the β-actin gene amplified from genomic DNA
Lanes 3-4. 1Kb fragment amplified from Lambda DNA
Lanes 5-6. 1Kb fragment amplified from pGEM3zf(+)
Markers (M) are HyperLadder 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 β-actin gene was amplified with MangoMix and with 3 competitors' mixes. The data shown demonstrate the robust performance of MangoMix (fig. 10).

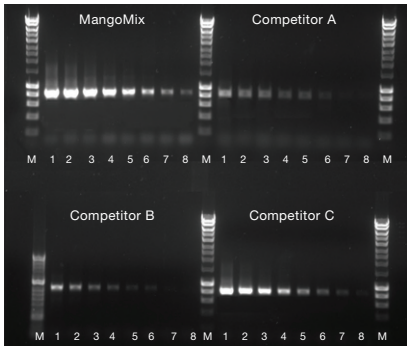

Figure 10. Amplification of a fragment of the β-actin gene from human genomic DNA using MangoMix™ and ready-to-go mixes from Competitors A, B and C. A 2-fold serial dilution was performed on human genomic DNA as follows:
Lane 1. 200ng human genomic DNA
Lane 2. 100ng human genomic DNA
Lane 3. 50ng human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

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 1Kb fragment was amplified from Lambda DNA, and half of the reaction was cleaned up using SureClean (Cat No. BIO-37042) according to the manufacturer's instructions. 1µl of the PCR product, either un-purified or purified using SureClean, was used in a TA cloning reaction with the vector PCR4-TOPO (Invitrogen). 3µl of the TA cloning reaction were transformed into α-Select Gold Efficiency Chemically Competent Cells (Cat No. BIO-85027), and plated on LB plates containing X-GAL, IPTG (Cat No. BIO-37086) and Ampicillin (Cat No. BIO-87025). 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. 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).

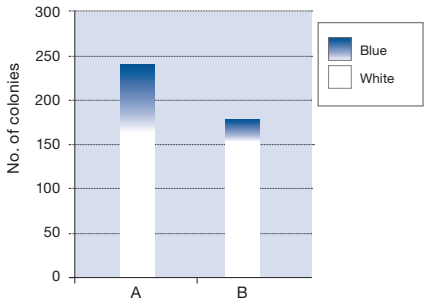

Figure 11. TA cloning using Mango*Taq*™ DNA Polymerase amplified PCR products. A 1Kb fragment was amplified from Lambda DNA using Mango*Taq*™ DNA Polymerase. Half of the PCR reaction was purified using SureClean (B) and the other half was left unpurified (A). The purified (B) and unpurified (A) PCR fragments 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 119bp and 5Kb 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 0.5pg Lambda DNA or 40pg 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 (Cat. No BIO-37042).

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

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