MyTaq™ DNA Polymerase | Colony PCR

DNA amplification plays a critical role in many molecular biology procedures. Molecular analysis of thousands of genes and DNA templates is now routine, due to the advent of the PCR and the subsequent development of microarraying and high-throughput sequencing technology. For sequencing projects, the recombinant DNA template is normally purified from the host cell and then amplified by conventional PCR using a thermostable DNA polymerase. Alternatively, colony PCR can be performed by adding a single recombinant colony into a DNA polymerase PCR master mix, omitting the step of template purification. The use of this method however remains limited due to the inherent limitations of *Taq* DNA polymerase in crude sample PCR applications. *Taq* is easily inhibited by debris from bacterial cells and components of culture media, giving inconsistent results and only short fragments of cloned inserts can be interrogated.

MyTaq™ is a new generation of very high performance PCR products, designed to deliver outstanding results on all templates, including complex genomic DNA templates. MyTaq 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. The enzyme is supplied with an industry-leading novel buffer system, specifically formulated and validated for the unique properties of MyTaq.

In order to assess the suitability of MyTaq for colony PCR, the MyTaq Mix was compared with other similar polymerase mixes (see data below).

Competent Cells were chemically transformed with an 844 bp PCR fragment and a 2.6 kb PCR fragment cloned into M13 vectors and were then referred to as R844 and R2600 cell strains respectively.

PCR SETUP

Bacterial cells debris inhibition - In order to test the effect of cells debris on the PCR reaction, a serial dilution of R844 and R2600 was used. 10 mL of overnight culture (OD_{600}=362.5) was centrifuged and resuspended in 40 µL LB media, a five-fold serial dilution was then made in LB and 1 µL of each dilution used in a 25 µL PCR reaction (12.5 µL MyTaq Mix, 20 µM forward and reverse primer, up to 25 µL water).

LB inhibition - In order to test the effect of LB inhibition 1 µL of overnight culture (OD_{600}=1.45 (R844 and R2600)) was used and 1 µL increments of LB added to a 25 µL (see above) PCR reaction.

Agar inhibition - In order to test the effect of agar inhibition 1 µL of overnight culture (OD_{600}=1.6 (R844) and 1.45 (R2600)) was used and 0.5 µL increments of LB agar to a 25 µL (see above) PCR reaction.

Colony picking - To show the reproducibility in picking colonies from LB agar plates, <1 mm colonies grown overnight in petri dishes with solid LB agar with ampicillin were picked using sterile pipette tips or wooden tooth picks and inoculated directly into individual 25 µL (see above) PCR reactions.

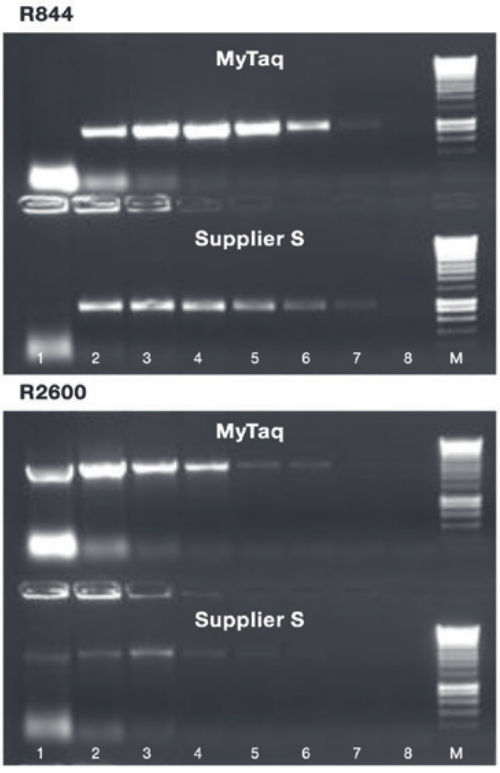

Fig. 1 Bacterial cells debris inhibition.
1 µL of a 1/5 serial dilution of a concentrated overnight culture of R844 and R2600, lanes 1-8 respectively, used in a 25 µL PCR reaction. 5 µL was run on a 1% agarose gel. Marker is HyperLadder™ 1kb (M).

PCR CONDITIONS

A standard 3-step cycling profile was used, 95°C for 1 min, followed by 35 cycles of 95°C for 15s, 60°C for 15s and 72°C for 10s. These were set-up at room temperature and subsequently 5 µL of the final product was run on a 1% agarose gel and stained with ethidium bromide.

RESULTS AND DISCUSSION

At very high bacterial concentrations, PCR is inhibited, but at lower concentrations (under normal working concentrations) the cell debris is not very inhibitory to either MyTaq Mix or Supplier S mix (Fig. 1), suggesting that cell debris is not normally a major contributing factor to poor colony PCR.

Both LB (Fig. 2A) and agar (Fig. 2B) are inhibitory at high concentrations, particularly with larger PCR fragments; however MyTaq was able to tolerate much higher concentrations than Supplier S.

MyTaq Mix can be used to successfully screen recombinant clones starting with crude colony preparations or suspensions, in a high-throughput method. The mix is robust enough to amplify a wide range of fragment sizes using different primer pairs, for amplicons of up to about 3 kb (Fig. 3).

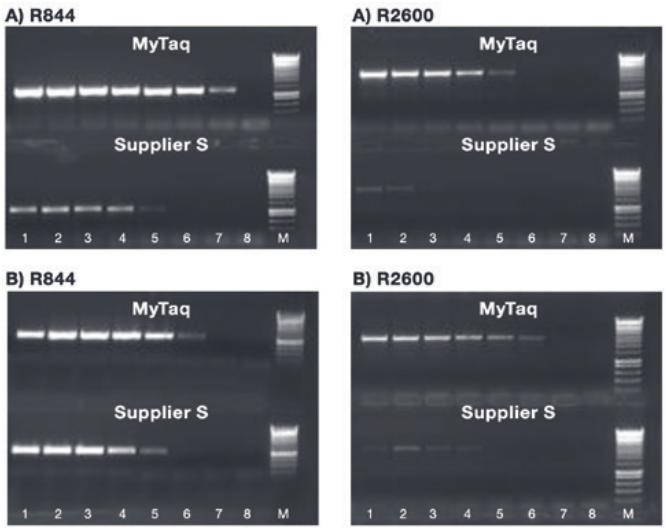

Fig. 2 LB and Agar inhibition.
A) LB inhibition 0,1,2,3,4,5,6,7,8 µL of LB, lanes 1-8 respectively, used in a 25 µL PCR reaction. B) Agar inhibition 0.5, 1, 1.5, 2, 2.5, 3, 3.5 µL of LB agar, lanes 1-8 respectively, used in a 25 µL PCR reaction. 5 µL was run on a 1% agarose gel. Marker is HyperLadder™ 1kb (M).

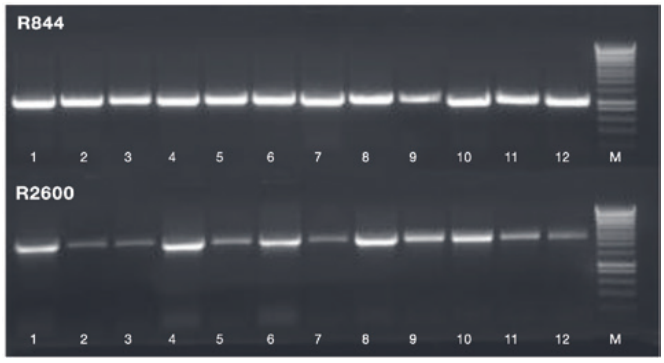

Fig.3 Colony picking.
12 colonies were picked with sterile pipette tips and washed directly into 25 µL MyTaq Mix. For both R844 and R2600 5 µL of a 50 µL reaction was run on a 1% agarose gel. Marker is HyperLadder™ 1 kb (M).

SUMMARY

MyTaq is evidently a highly robust and versatile polymerase and together with a novel buffer, delivers high performance in chemically complex reaction conditions. The result is superior tolerance to a wide range of common PCR inhibitors, which results in unsurpassed performance in colony PCR. An added advantage is that MyTaq can also be used to directly screen overnight LB cultures, so minimizing the cost of plasmid preparation of uninteresting clones.

The use of MyTaq HS, a hot-start polymerase, also allows convenience of leaving the PCR reaction at room temperature before processing, which can be advantagous when working with a large number of colonies.