MyTaq™ HS 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 development of microarraying and high-throughput sequencing technology. For genome sequencing projects, the recombinant DNA template is normally purified from the host cell and then amplified by conventional PCR using a thermostable DNA polymerase. Alternately, 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.

The MyTaq™ is a new generation of very high performance PCR products developed by Bioline, 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, 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, this should make it the perfect choice for colony PCR.

In order to assess the suitability of MyTaq for colony PCR, the MyTaq HS Mix was compared with other similar polymerase mixes (the results of supplier S are shown).

One-Shot® TOP10 cells were chemically transformed with an 844bp PCR fragment and a 2.6Kb PCR fragment cloned into M13 vectors and were then referred to as R844 and R2600 cell strains respectively.

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. 10ml of overnight culture (OD600=362.5) was centrifuged and resuspended in 40µl LB media, a five-fold serial dilution was then made in LB and 1µl of each dilution used in a 25µl PCR reaction (12.5µl MyTaq HS Mix, 20µM forward and reverse primer, up to 25µl water).

LB inhibition - In order to test the effect of LB inhibition 1µl of overnight culture (OD600=1.45 (R844 and R2600)) was used and 1µl increments of LB added to a 25µl (see above) PCR reaction.

Agar inhibition - In order to test the effect of agar inhibition 1µl of overnight culture (OD600=1.6 (R844) and 1.45 (R2600)) was used and 0.5µl increments of LB agar to a 25µl (see above) PCR reaction.

Colony picking - To show the reproducibility in picking colonies from LB agar plates, <1mm 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 25µl (see above) PCR reactions.

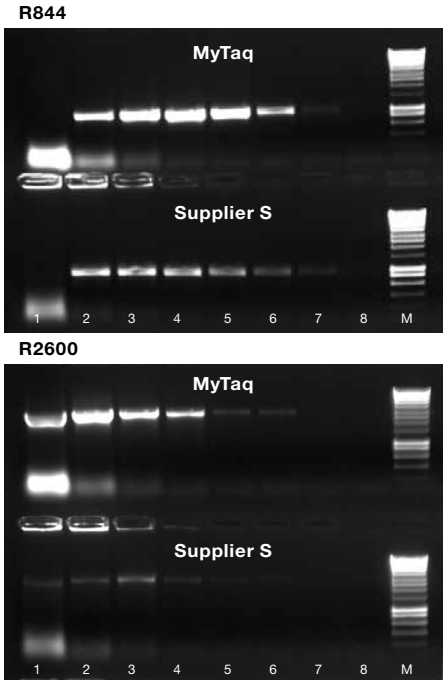

Fig. 1 Bacterial cells debris inhibition.
1µl of a 1/5 serial dilution of a concentrated overnight culture of R844 and R2600, lanes 1-8 respectively, used in a 25µl PCR reaction. Marker is HyperLadder™ I (M).

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 5µl of the final product run on a 1% agarose gel and stained with ethidium bromide.

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 HS 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 HS 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), offering the opportunity to interrogate full-length inserts with generic vector-specific primers, and facilitates the identification of clones carrying large deletions or insertions, or looking at the quality of a library.

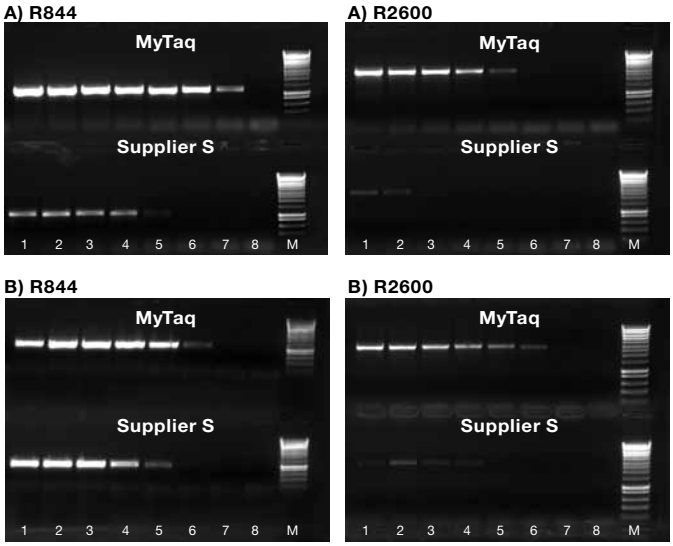

Fig. 2 A) LB inhibition.
0,1,2,3,4,5,6,7,8µl of LB, lanes 1-8 respectively, used in a 25µl PCR reaction. B) Agar inhibition 0.5, 1, 1.5, 2, 2.5, 3 ,3.5µl of LB agar, lanes 1-8 respectively, used in a 25µl PCR reaction. Marker is HyperLadder™ I (M).

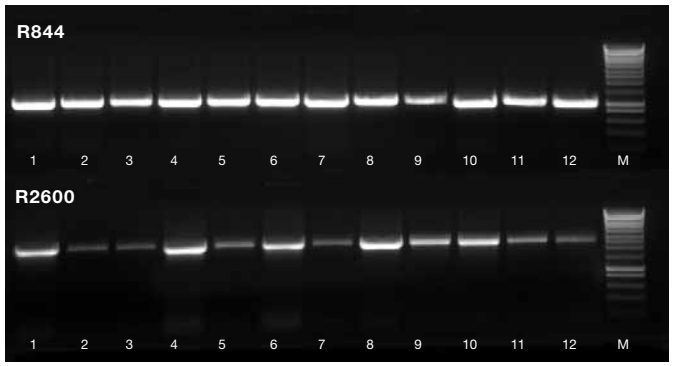

Fig.3 Colony picking.
12 colonies were picked with sterile pipette tips and washed directly into 25µl MyTaq HS Mix.

SUMMARY

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

Please visit www.bioline.com/mytaq to request a sample of MyTaq.

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