MPOB TAQ-POLYMERASE (MTaq)

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MPOB INFORMATION SERIES • ISSN 1511-7871 • JUNE 2007 MPOB TS No. 19

The polymerase chain reaction (PCR) has become the *sine qua non* of many molecular biology laboratories. It basically uses enzymes to mass replicate a portion of a deoxyribonucleic acid (DNA) strand for easier analysis, such as in searching for genes of interest. Like the nuclear chain reaction, PCR is an exponential process that continues as long as the raw materials for sustaining the reaction are available. In contrast to DNA replication in the natural world, PCR can only replicate fairly small pieces of DNA, with an upper ceiling of about 2-3 kilo base pairs (kb). A modern PCR requires six basic components to work: the DNA segment to be copied, primers to delimit the segment, Taq polymerase to do the copying, DNA nucleotides as feedstock, a chemical buffer environment, and a machine called the thermal cycler.

Taq polymerase is the quintessential enzyme for performing PCR. It was first extracted from a deep-sea, thermal vent-dwelling bacterium, *Thermus aquaticus*, and has since existed mostly in the recombinant form. It works well, but not quite perfectly, for copying with commercially available Taq DNA polymerase having an error rate of one in 10 000 nucleotides and typically producing 16% mutated 1 kb PCR products in a reaction. It can amplify a 1 kb strand of DNA in roughly 30 s at 72°C. Taq DNA polymerase also yields fragments with A (Adenine) overhangs. This is particularly useful in 'TA Cloning,' in which a cloning vector (such as a plasmid) is used with T (Thymine) overhangs complementing the A overhangs of the PCR product to increase the ligation efficiency.

ECONOMIC ANALYSIS

The most expensive component in PCR is the enzyme, largely due to the fact that Taq polymerase is patented. With expiration of the patent in 2005, it is now possible to make Taq polymerase available to the masses without infringing on patent rights. Although, the patent has expired for more than two years, users are still awaiting to witness a drastic reduction in the price of commercial Taq polymerase. Not until the cost issue is settled, production of in-house Taq polymerase still remains the viable alternative. In the preparation of probes for the oil palm high density chip, high throughput amplification was done on our vast collection of ESTs to generate thousands of amplicons. As a result, much expenditure went into purchasing commercial Taq polymerase. To reduce the cost of generating the probes, our lab decided to produce our own Taq polymerase. According to our estimates, a saving of nearly 90% is possible (based on commercial Taq polymerase priced at RM 160 for 500 U), making it an economical solution for large scale PCR applications.

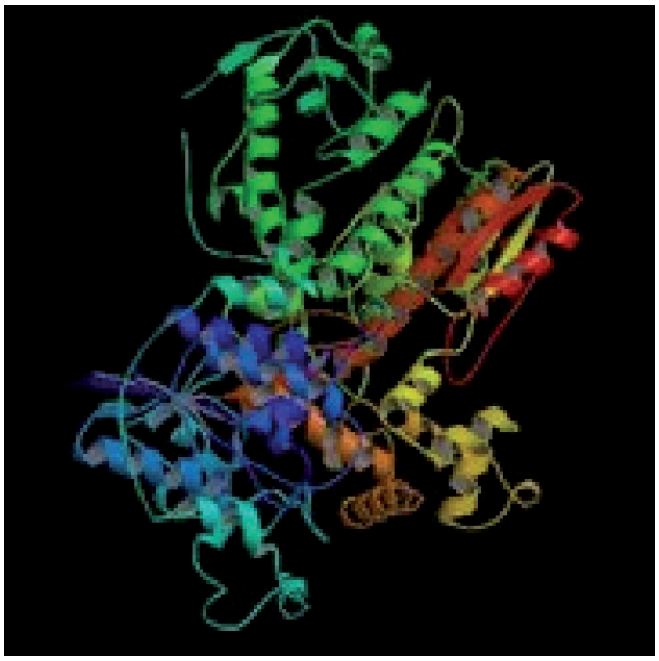

Figure 1. Molecular model of Taq polymerase (image from the Protein Data Bank).

ISSN 1511-7871
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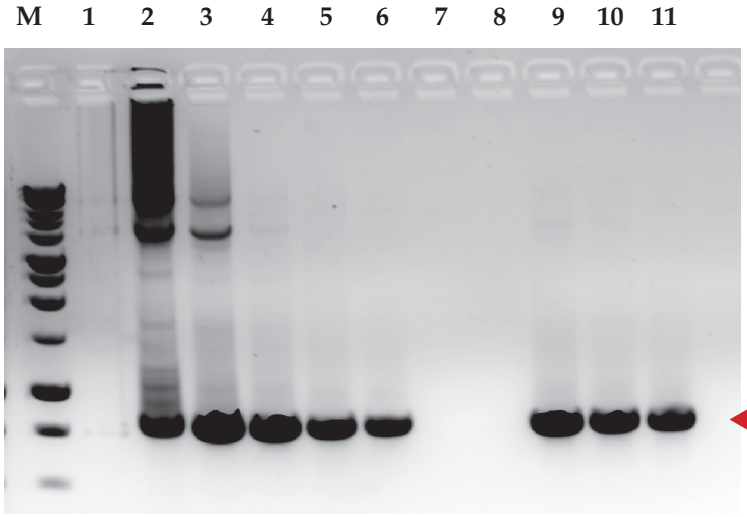

PCR Reaction: (25 µl)
10X PCR Buffer 2.5 µl
$MgCl_2$ (50 mM) 1.0 µl
dNTPS 0.2 µl
Forward primer (50 mM) 0.1 µl
Reverse Primer (50 mM) 0.1 µl
Template 0.05 µl

Marker 1 kb
Lane 1 Empty
Lane 2 Commercial Taq 5 U
Lane 3 Commercial Taq 2.5 U
Lane 4 Commercial Taq 1.25 U
Lane 5 Commercial Taq 0.625 U
Lane 6 Commercial Taq 0.3125 U
Lane 7 Commercial Taq 0.156 U
Lane 8 Empty
Lane 9 MTaq ~1.2 U
Lane 10 MTaq ~0.6 U
Lane 11 MTaq ~0.3 U

Note: As this Taq polymerase is in crude form, we do not recommend its use for reactions that require high fidelity Taq polymerases for now.

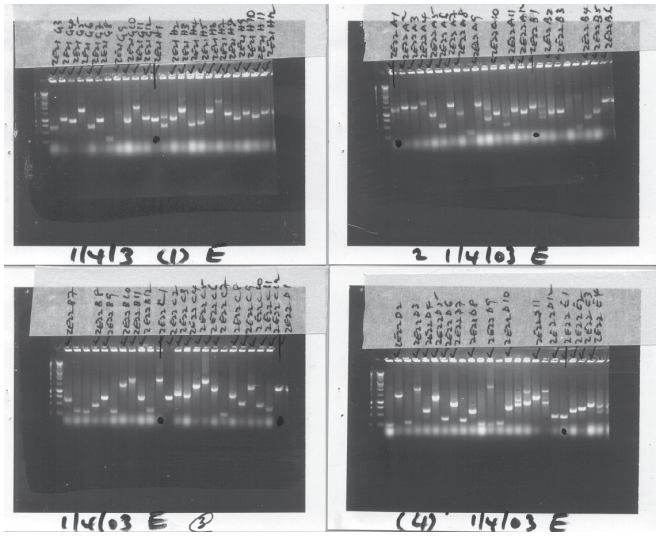

Figure 2. Example of high throughput amplification done on ESTs of zygotic embryo library.

COST

RM 50 per 500 units

If production services are required, the cost is based on unit of enzymes produced. Free sample (500 units) of MTaq (includes 10X Reaction Buffer) is available upon request. Limited to one free sample per customer.

For more information kindly contact:

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