PLANT DNA ISOLATION AND AMPLIFICATION

Plant material contains an abundance of potent PCR inhibitors such as polyphenols, tannins and complex polysaccharides, which makes amplification of plant-derived DNA a challenging task.

MyTaq HS and ISOLATE II Plant DNA are new generations of very high performance products from Bioline, developed to deliver outstanding results on all templates including the complex genomic DNA templates found in plants. MyTaq is based on the latest technology in PCR enzyme preparation and engineered to increase affinity for DNA, which results in significant improvements to yield, sensitivity and speed. ISOLATE II Plant DNA Kit is designed for the rapid purification of genomic DNA from a variety of wet or dry plant material, including leaves, bark, roots, fruits, etc. In order to show the suitability of MyTaq HS for the PCR of plant DNA, samples were initially prepared from a number of different plant types with the Bioline ISOLATE II Plant DNA Kit.

The leaves from plants such as *Arabidopsis thaliana*, corn and tomato are commonly used for agricultural research and are a convenient source of DNA without causing too much damage to the main plant. The combination of ISOLATE II and MyTaq means that high quality DNA can be extracted rapidly from many leaves and used in subsequent PCR (fig. 1).

Rice is a staple food for over half of the world's population, as it is the only crop that can be grown continuously without the need for rotation and can produce up to three harvests a year. However, as nutrients are lost during milling and polishing research is being carried out to improve the nutritional properties of this important crop. PCR from DNA isolated from milled rice samples has been difficult to perform for a number of reasons:

- The low level of DNA is difficult to isolate.
- PCR inhibition through high levels of polysaccharides (>90% starch normally found in the bran layer and embryo alone).
- DNA shearing and/or degradation that may have occurred during the processes of desiccation, storage and milling of mature rice grains.

Here we used a combination of the Bioline ISOLATE II Plant DNA Kit and MyTaq HS Red Mix to show how easy it can be to perform PCR (fig. 2) and to demonstrate multiplex PCR from such materials (fig. 3).

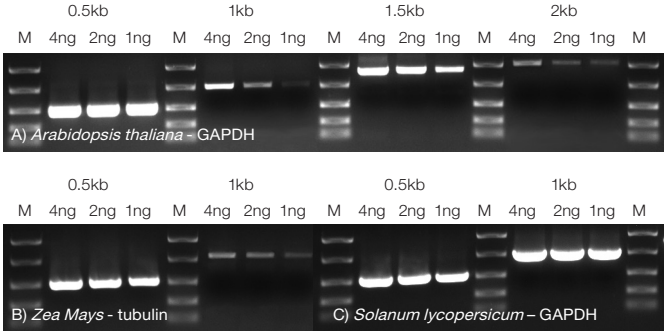

Fig 1. PCR of Genomic DNA extracted from plant leaves. Genomic DNA was extracted from A) Arabidopsis thaliana, B) Zea Mays (corn) and C) Solanum lycopersicum (tomato) leaves using an ISOLATE II Plant DNA Kit (BIO-52070). Decreasing amounts of DNA were amplified using MyTaq HS (BIO-25048). The GAPDH gene was amplified in Arabidopsis thaliana (0.5kb, 1kb, 1.5kb, and 2kb) and Solanum lycopersicum, two fragments (0.5kb, 1kb). Two fragments (0.5kb, 1kb) of the tubulin gene were amplified in Zea Mays. M - EasyLadder I (BIO-33046).

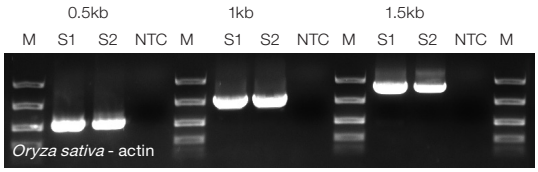

Fig 2. PCR of Genomic DNA extracted from one grain of rice. Genomic DNA was extracted from Oryza sativa (rice) using an ISOLATE II Plant DNA Kit. Two extractions (S1 and S2) were performed and amplified 4ng DNA with 0.5kb, 1kb, and 1.5kb fragments of the Oryza sativa actin gene using MyTaq HS. M - EasyLadder I.

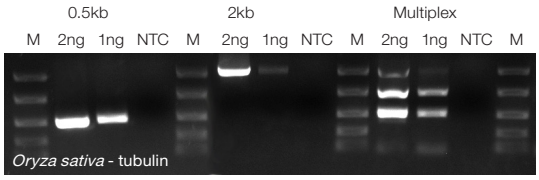

Fig 3. PCR of Genomic DNA extracted from one grain of rice. Genomic DNA was extracted from Oryza sativa (rice) using an ISOLATE II Plant DNA Kit. Decreasing amounts of DNA were amplified using MyTaq HS. Two fragments of the tubulin gene of Oryza sativa were amplified in singleplex (0.5kb and 2kb fragments) and as a multiplex (0.5kb, 1kb, 2kb fragments) using MyTaq HS. M - EasyLadder I.

SUMMARY

MyTaq HS is demonstrated to be a highly robust and versatile polymerase. The combination of MyTaq HS and ISOLATE II offers superior tolerance to a wide range of common PCR inhibitors, which results in unsurpassed performance in PCR from plant materials.

Reference

Murray, S.A., *et al. Appl. Envir. Microbiol.* **77,** 7050-7057(2011)