Bioline, your partner for plant research

If you are working in plant research, you face daily challenges in plant molecular biology. It is well documented that plant material contains many compounds that are potent inhibitors of PCR reactions.

Whether your samples are leaf, root, shoot, seed or grain, the robust performance and tolerance to PCR inhibitors demonstrated by Bioline products will allow you to enhance your experiments.

If your research is centered on projects as diverse as transgenic recovery, transgenic screening, genotyping, amplification and cloning of difficult targets, let Bioline be your skilled partner for plant genetics research.

rice · wheat · barley · cotton · corn · sugar · legumes · soy

For further information on how Bioline can help you with your plant research, speak to your local distributor or go to www.bioline.com

BIOLINE

A Meridian Life Science® Company

Key Bioline solutions for plant research

MyTaq™ HS DNA Polymerase
High yield and high quality amplification from difficult samples, such as repetitive regions or in the presence of inhibitors

SensiFAST™ real-time reagents
Unparalleled speed and sensitivity for your plant gene expression studies. Detect expression levels down to single copies

ISOLATE II Plant DNA Kit
Rapid extraction of extremely pure and high yield genomic DNA from a wide variety of plant species

ISOLATE II RNA Plant Kit
Developed for extraction of highest quality and maximal yields of RNA from different plant species

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).

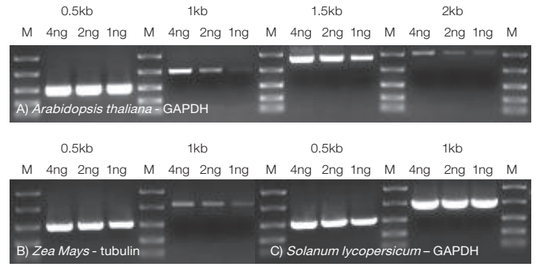

Fig 1. PCR of Genomic DNA extracted from plant leaves. Genomic DNA was extracted from A) *Arabidopsis thaliana*, B) *Zea Mays* (corn) and C) *Solanum lycopersicum* (tomato) leaves using an ISOLATE II Plant DNA Kit (BIO-52070). Decreasing amounts of DNA were amplified using MyTaq HS (BIO-25048). The GAPDH 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).

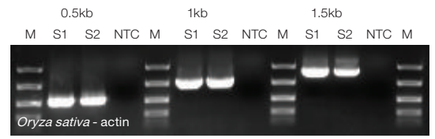

Fig 2. PCR of Genomic DNA extracted from one grain of rice. Genomic DNA was extracted from *Oryza sativa* (rice) using an ISOLATE II Plant DNA Kit. Two extractions (S1 and S2) were performed and amplified 4ng DNA with 0.5kb, 1kb, and 1.5kb fragments of the *Oryza sativa* actin gene using MyTaq HS. M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

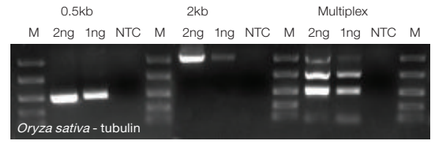

Fig 3. PCR of Genomic DNA extracted from one grain of rice. Genomic DNA was extracted from *Oryza sativa* (rice) using an ISOLATE II Plant DNA Kit. Decreasing amounts of DNA were amplified using MyTaq HS. Two fragments of the tubulin gene of *Oryza sativa* were amplified in singleplex (0.5kb and 2kb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

MyTaq™ HS DNA Polymerase

FEATURES AND BENEFITS

- New generation of antibody-based hot-start polymerase
- Highest specificity and superior performance
- Novel buffer system, including ultra-pure dNTPs and $MgCl_2$
- Red dye for direct gel loading
- Convenient all-in-one mastermix

APPLICATIONS

- Fast and reproducible genotyping of plant and crop samples
- Studies of genetic diversity and cultivar identification
- Multiplex assays using short tandem repeats (STRs)/ microsatellite markers for genetic characterization
- Specific amplification of difficult templates (GC rich)
- Screening of recombinant clones with plant DNA inserts by Colony PCR

The most common uses of PCR technology in plant research and agricultural product development are in gene discovery and cloning, vector construction, transformant identification, genetic screening and characterization and seed quality control. MyTaq HS is a new generation of very high performance, antibody-mediated hot-start PCR enzyme, ideal for fast and highly successful amplification from a variety of plant and crop DNA templates, in a wide range of downstream applications.

MyTaq HS has been engineered to increase affinity for DNA, resulting in significant improvements in yield, sensitivity and speed. The enzyme is supplied with an industry-leading novel buffer system, consisting of a proprietary formulation containing dNTPs, $MgCl_2$ and enhancers at optimal concentrations, removing the need for optimization while delivering superior amplification.

MyTaq HS Mix is a ready-to-use 2x mix for fast, highly-specific, hot-start PCR. The advanced formulation of MyTaq HS Mix allows fast cycling conditions to be used, greatly reducing the reaction time without compromising PCR specificity and yield (fig. 1).

MyTaq HS is also available with an inert red dye, to facilitate direct agarose gel loading of samples following PCR. MyTaq HS is the ideal choice for the most demanding of needs in plant molecular biology, including high throughput applications.

PRODUCT	PACK SIZE	CAT NO.
MyTaq HS DNA Polymerase	250 Units	BIO-21111
	1000 Units	BIO-21112
	2500 Units	BIO-21113
MyTaq HS Red DNA Polymerase	250 Units	BIO-21114
	1000 Units	BIO-21115
	2500 Units	BIO-21116
MyTaq HS Mix, 2x	200 Reactions	BIO-25045
	1000 Reactions	BIO-25046
MyTaq HS Red Mix, 2x	200 Reactions	BIO-25047
	1000 Reactions	BIO-25048

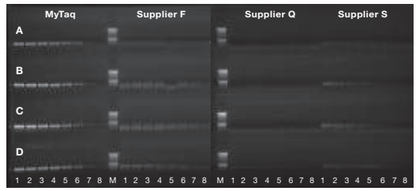

Fig. 1 Fast amplification (26.3 minutes) was carried out on a range of genes from genomic DNA
A) A 340bp and B) a 450bp fragment of the myc gene, C) a 525bp fragment of the EGFR gene and D) a 530bp fragment of the AGRI1 gene were amplified with MyTaq HS and the results were compared with amplifications performed with hot-start DNA polymerases from other suppliers. The process used a serial dilution of genomic DNA (100ng, 33ng, 10ng, 4ng, 1ng, 33pg, 10pg and 3pg genomic DNA, lanes 1-8 respectively), incubated for 3 mins at 95ºC followed by 35 cycles of 15s at 95ºC, 55ºC and 72ºC. Marker is HyperLadder 1kb (M). MyTaq HS performed well across all four genes. MyTaq HS clearly performed better than any of the alternative polymerases.

Selected Product Citations

MyTaq HS

Gilding, E.K. *et al.*, **Allelic variation at a single gene increases food value in a drought-tolerant staple cereal** *Nat. Commun.* 4:1483 (2013).

Mieog, J.C. *et al.*, **Fast-tracking development of homozygous transgenic cereal lines using a simple and highly flexible real-time PCR assay** *BMC Plant Biology* 13:71 (2013).

MyTaq

Schrumpfová, P.P. *et al.*, **Telomere Repeat Binding proteins are functional components of Arabidopsis telomeres and interact with telomerase.** *Plant J.* doi: 10.1111/tpj.12428 (2014).

Jaške, K. *et al.*, **A telomerase-independent component of telomere loss in chromatin assembly factor 1 mutants of Arabidopsis thaliana** *Chromosoma* 122(4): 285-293 (2013).

Robb, S.M. *et al.*, **The use of RelocaTE and unassembled short reads to produce high-resolution snapshots of transposable element generated diversity in rice** *G3* 3(6):949-957 (2013).

Obinna-Echem, P.C. *et al.*, **Evaluation of the microbial community, acidity and proximate composition of aka-mu, a fermented maize food** *J. Sci. Food Agric. doi:* 10.1002/jsfa.6264 (2013).

Valdivia, E.R. *et al.*, **Regulation of secondary wall synthesis and cell death by NAC transcription factors in the monocot *Brachypodium distachyon*** *J. Exp. Bot.* 64(5):1333-1343 (2013).

Runo, S. *et al.*, **Striga parasitizes transgenic hairy roots of Zea mays and provides a tool for studying plant-plant interactions** *Plant Methods* 8(1):20 (2012).

SensiFAST™ Real-Time PCR Kits

FEATURES AND BENEFITS

- Fast - Optimized for rapid cycling conditions (under 30 minutes), ideal for high-throughput
- High specificity - Leading to better efficiency and more confidence in your results
- Ultra-sensitive - Perfect for low copy number samples
- Universal - Compatible with all standard and fast cycling instruments

APPLICATIONS

- Determination of transgene copy number and zygosity in plants
- SNP genotyping of agricultural breeding lines
- Quantitative detection of genetically modified (GM) plants and crops
- Fast molecular detection of viral, fungal and bacterial pathogens in plants
- Specific amplification of difficult templates (GC-rich)

Real-time PCR is one of the most powerful and sensitive gene analysis techniques available and is widely used in plant and agricultural research laboratories. The new SensiFAST Kits are ideal for real-time PCR based studies with a wide range of plant and crop samples, from developmental studies of plant cellular transcription, foreign DNA identification, rapid plant pathogen detection, to transgenic copy number and zygosity analysis and genotyping of SNPs in crop breeding lines.

SensiFAST has been specifically designed to meet higher throughput needs, by combining a proprietary antibody mediated hot-start enzyme with an industry leading, unique buffer chemistry. As a result, SensiFAST formulations provide the fastest cycling times while maintaining the high performance, reliability and high reproducibility of conventional real-time PCR reagents. SensiFAST Real-Time PCR Kits deliver outstanding sensitivity and reproducibility with different plant species, whether using SYBR® Green (fig. 1) or Probe based assays (fig. 2).

Exceed the limit with SensiFAST Real-Time PCR kits, available in configurations matched to all your plant applications with both DNA and RNA templates.

PRODUCT	PACK SIZE	CAT NO.
SensiFAST SYBR® Hi-ROX Kit	500 Reactions	BIO-92005
SensiFAST SYBR® Lo-ROX Kit	500 Reactions	BIO-94005
SensiFAST SYBR® No-ROX Kit	500 Reactions	BIO-98005
SensiFAST SYBR® & Fluorescein Kit	500 Reactions	BIO-76001
SensiFAST Probe Hi-ROX Kit	500 Reactions	BIO-82005
SensiFAST Probe Lo-ROX Kit	500 Reactions	BIO-84005
SensiFAST Probe No-ROX Kit	500 Reactions	BIO-86005
SensiFAST Genotyping Kit	500 Reactions	BIO-36005

For a full list of products see www.bioline.com

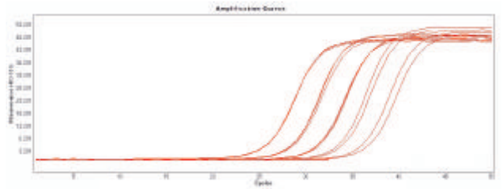

Fig. 1 SensiFAST SYBR® No-ROX
Genomic DNA was extracted from *Solanum Lycopersicum* (tomato) using an ISOLATE II Plant DNA Kit. A fragment of actin gene was amplified using fast cycling conditions with SensiFAST SYBR® No-ROX kit, from a five-fold serial dilution of tomato genomic DNA.

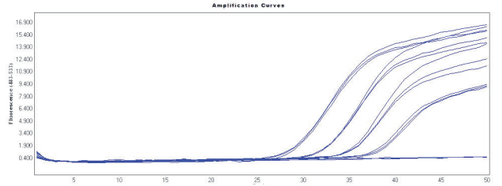

Fig. 2 SensiFAST Probe No-ROX
Genomic DNA was extracted from *Oryza Sativa* (rice) using an ISOLATE II Plant DNA Kit. Plant universal primers and TaqMan® probe Nes-2, was used to amplify from a 10-fold serial dilution of rice genomic DNA was amplified using fast cycling conditions with SensiFAST Probe No-ROX kit, using fast cycling conditions.

Selected Product Citations

Yates, S. *et al.*, **The temporal foliar transcriptome of the perennial C3 desert plant Rhazya stricta in its natural environment.** *BMC Plant Biol.* 14(2), (2014).

Romero-Campero, F.J. *et al.*, **A contribution to the study of plant development evolution based on gene co-expression networks** *Front. Plant Sci.* 4(291), (2013).

Gruner, K. *et al.*, **Reprogramming of plants during systemic acquired resistance** *Front. Plant Sci.* 4(96): (2013).

Gatica-Arias, A. *et al.*, **Over-expression of the transcription factor HIMYB3 in transgenic hop (Humulus lupulus L. cv. Tettnanger) modulates the expression of genes involved in the biosynthesis of flavonoids and phloroglucinols** *Plant Cell, Tissue, Organ Culture* 113(2):279-289 (2013).

Deveson, I. *et al.*, **Expression of human ARGONAUTE 2 inhibits endogenous microRNA activity in Arabidopsis** *Plant Mol. Biol.* 82(1-2): 85-96 (2013).

You, W. *et al.*, **Interplay among RNA polymerases II, IV and V in RNA-directed DNA methylation at a low copy transgene locus in Arabidopsis thaliana** *Eur. J. Plant Pathol.* 137(2): 403-413 (2013).

Herbel, V. *et al.*, **Lifetimes of Arabidopsis cryptochrome signaling states in vivo** *Plant J.* 74(4): 583-592 (2013).

Berkowitz, O. *et al.*, **Acclimation responses of Arabidopsis thaliana to sustained phosphite treatments.** *J. Exp. Bot.* 64(6): 1731-1743 (2013).

Mokrini, F. *et al.*, **Quantitative detection of the root-lesion nematode, Pratylenchus penetrans, using qPCR** *Plant J.* 74(4):583-592 (2013).

ISOLATE II Plant DNA Kit

FEATURES AND BENEFITS

- **Plant genomic DNA isolated in 30 minutes saving time over traditional methods**
- **High-purity DNA: typical A_{260}/A_{280} ratio 1.6 - 1.9 requires no further processing**
- **Choice of two lysis buffers for optimizing extraction protocol to sample type**
- **Extra filters for clarification of lysate included**
- **RNase A included to remove potential RNA contamination**

APPLICATIONS

- **Fresh, frozen or lyophilized plant tissue**
- **Dung, animal fecal, soil and compost samples**
- **Herbarium specimens**
- **Fungi**

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. Up to 100mg wet plant material and up to 20mg dry plant material can be processed per spin column. The protocol does not require the use of Proteinase K, which means that all components can be conveniently stored at room temperature. The plant samples are first homogenized by mechanical treatment. DNA is then extracted with chaotropic salts, denaturing agents and detergents. Crude lysates are cleared in order to remove polysaccharides, contaminants and residual cellular debris. The DNA is bound to a silica membrane, washed and the pure genomic DNA is eluted.

The kit contains two optimized lysis buffers based on the established CTAB and SDS methods and RNase A is included to remove RNA.

ISOLATE II Plant DNA Kit shows excellent recovery of plant DNA with a variety of homogenization techniques (fig. 1). High yields are obtained with every miniprep (fig. 2).

The online product manual has protocols for purifying plant genomic DNA, fungal genomic DNA, dung, animal fecal, soil and compost samples. The isolated DNA is ready for use in downstream applications such as PCR, real-time PCR, genotyping, restriction analysis and sequencing.

Isolated DNA is ready for all downstream applications such as PCR, real-time PCR and genotyping with no further purification required.

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Plant DNA Kit	10 Preps	BIO-52068
	50 Preps	BIO-52069
	250 Preps	BIO-52070

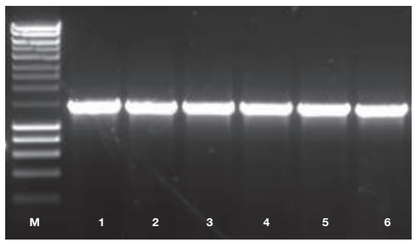

Fig. 1 Excellent recovery of plant DNA using different homogenization techniques
Freeze-dried budding leaves of Arabidopsis thaliana were homogenized in a mortar and pestle in the presence of liquid nitrogen (lanes 1-3 respectively) and with a rotor stator homogenizer (lanes 4-6 respectively). Genomic DNA was isolated using ISOLATE II Plant DNA Kit. A 1.4Kb fragment of AOS gene was amplified from the isolated DNA using MangoMix (BIO-25033). HyperLadder 1kb (M). The results illustrating that the ISOLATE II Plant DNA Kit gives consistent results with either extraction technique.

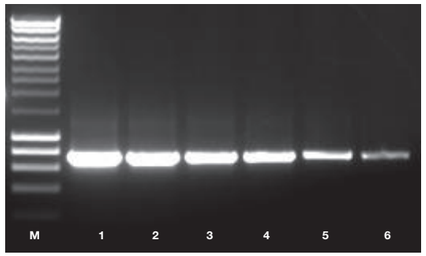

Fig. 2 High yields of plant genomic DNA
Genomic DNA was isolated from 20mg freeze-dried budding leaves of Arabidopsis thaliana using ISOLATE II Plant DNA Kit. Using a 2-fold dilution of the miniprep (200ng, 100ng, 50ng, 25ng, 12,5ng and 6.25ng, lanes 1-6 respectively), a 1.4kb fragment of AOS gene was amplified from the isolated DNA using MangoMix (BIO-25033). HyperLadder 1kb (M).

ISOLATE II RNA Plant Kit

FEATURES AND BENEFITS

- **Plant RNA isolated in under 30 minutes**
- **High-purity RNA (A_{260}/A_{280} ratio: 1.9 - 2.1)**
- **Excellent RNA recovery and integrity (RIN>9)**
- **Choice of two optimized lysis buffers for all plant types**
- **Filters to enhance sample homogenization included**
- **DNase I included to remove contaminating genomic DNA**

APPLICATIONS

Isolation of RNA from:

- **Fresh plant cells and tissue**
- **Frozen and lyophilized plant tissue**
- **Filamentous fungi**

ISOLATE II RNA Plant Kit is specially designed for the fast and efficient isolation of extremely pure total RNA from a wide variety of plant tissues, including leaves, bark, roots and fruits. Up to 100mg starting material can be processed per spin column.

The protocol is easy to follow on a step-by-step basis. Two highly optimized lysis buffers, containing guanidinium thiocyanate and guanidinium-HCl are provided to ensure lysis for all plant types. The lysis buffers also inactivate RNases, thus protecting the released RNA. The lysate is applied to a spin column to selectively remove contaminating genomic DNA, eliminating the need to perform a separate DNase I digestion step. The RNA is then bound to a silica membrane. Subsequent wash steps remove the remaining cell debris and pure RNA is eluted in the final step with RNase-free water.

The isolated RNA shows excellent performance in downstream applications such as real-time PCR (fig. 1), reverse transcription (fig. 2), Northern blot analysis, microarrays and RNA protection assays.

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II RNA Plant Kit	10 Preps	BIO-52076
	50 Preps	BIO-52077

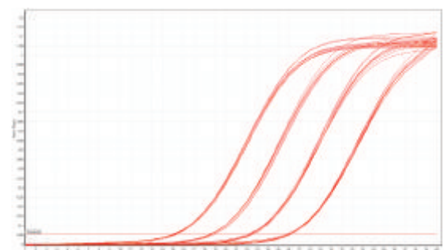

Fig. 1 Superior performance in real-time applications
RNA was isolated from 20mg freeze-dried budding leaves of *Arabidopsis thaliana* using ISOLATE II RNA Plant Kit. The RNA was split into twelve replicates and diluted in a 10-fold series. Real-time reactions were performed using primers for a fragment of UBQ10 gene and SensiFAST SYBR® No-ROX One-Step Kit (BIO-72005). The results illustrate the quality of the RNA extracted through the reproducibility of the amplification.

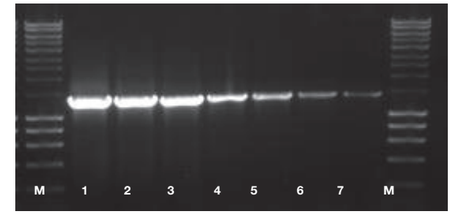

Fig. 2 High-quality RNA isolated from plant tissue
RNA was isolated from 20mg freeze-dried budding leaves of *Arabidopsis thaliana* using ISOLATE II RNA Plant Kit. The extracted RNA was diluted in a 2-fold serial dilution (1mg, 500ng, 250ng, 125ng, 60ng, 30ng and 15ng, lanes 1-7 respectively) and PCR was performed using MyTaq One-Step RT-PCR Kit (BIO-65048) to amplify a 1.4kb fragment of the allene oxide synthase gene. HyperLadder 1kb (M). Products were run on a 1.5% agarose gel. The results illustrate the quality of the RNA obtained, as it can be used for very sensitive cDNA synthesis and PCR without further purification.

Selected Product Citations

Kim S. *et al.*, **Arabidopsis chlorophyll biosynthesis: an essential balance between the methylerythritol phosphate and tetrapyrrole pathways.** *Plant Cell* doi: 10.1105/tpc.113.119172 (2013).

Here is what other plant researchers say about our products

"The speed of MyTaq is surprisingly fast, only 20 seconds for 1.8kb with great amplifying efficiency!"

- Plant Research Centre, Adelaide, Australia

"After getting nowhere with other kits we tried the SensiFAST SYBR® One-Step Kit and saw results first time, I cannot describe how wonderful this made us feel."

- Asia-Pacific Special Nutrients Sdn. Bhd., Selangor, Malaysia

"The MyTaq HS gave much better results in terms of multiplex amplification when genotyping milkweed microsatellites."

- Evolution, Ecology and Organismal Biology, Ohio, USA

"MyTaq HS Red DNA Polymerase reliably amplified alleles of varying amplicon size in the promoter region of common wheat *(Triticum aestivum)* membrane transporter."

- Department of Primary Industries, Horsham, Australia

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