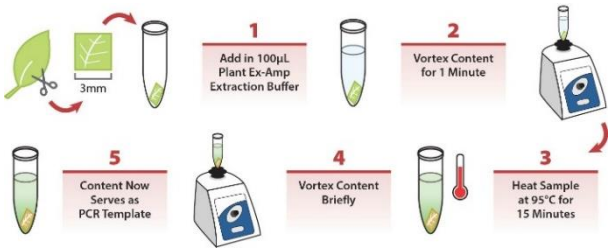
Plant Advance™ PCR Kit

(Cat# M1144-25; 25 preps, 200 x 50 µl Rxn; Store at -20°C)

I. Introduction:

Plant Advance™ PCR Kit provides all ingredients necessary for PCR in a premixed and optimized format that simplifies the Plant PCR workflow. Plant Advance™ PCR Kit offers the ultimate convenience for plant PCR. The hasslefree gDNA extraction process eliminates the conventional freezing of plant tissues with liquid nitrogen, mechanical disruption, organic extraction, column DNA purification, or alcohol precipitation. It only takes 15 easy min to "vortex-boil-vortex" a small piece of plant sample in BioVision's proprietary extraction buffer to obtain PCR-ready template. The kit comes with BioVision's sophisticated Advance™ 2X PCR Master Mix with dye that allows an unbeatable robustness and extreme fidelity in a streamlined and efficient PCR setup. BioVision's Plant Advance™ PCR Kit is also very effective against some commonly known difficult samples such as pine-tree-like samples. BioVision's Advance™ 2X PCR Master Mix with dye contains a green dye blend which resolves during gel electrophoresis into a turquoise band at ~4000 bp and a yellow band at the ~50 bp region. The resolving bands of dye allow easy visualization of the real time progress of your gel electrophoresis, where the yellow band indicates the migrating front on the gel.



II. Application:

- Plant PCR
- Gene/transgene detection
- Plant sample genotyping

III. Key Features:

- No time-consuming DNA purification steps
- Only small amount of sample needed
- Simple universal protocol for various plant samples
- Advance™ 2X PCR Master Mix with Dye for Robust PCR and gel-loading-ready PCR products.

IV. Kit Contents:

Product Name	Quantity	Part No.
Plant Ex-Amp Extraction Buffer	5.0 ml (200 Rxns)	M1144-XX-1
10X Plant PCR Enhancer	1 ml	M1144-XX-2
Advance™ 2X PCR Master Mix with dye	5 X 1 ml	M1144-XX-3

V. User Supplied Reagents and Equipment:

- PCR Tubes
- PCR Instrument
- Pipettes
- Water, Nuclease-free
- Primers (forward and reverse)
- Template DNA

VI. Shipment and Storage:

Keep at -20°C for long term storage; Master Mix may not freeze at -20ºC. A small amount of salt precipitation may occur after thawing but can be re-dissolved into the Master Mix by mixing well. Advance™ 2X PCR Master Mix with dye is stable at 4°C for one month or at least fifteen freeze-thaw cycles (-80ºC). For daily use, we recommend keeping an aliquot at 4°C.

VII. PCR Protocol:

PCR reactions should be assembled in a nuclease-free environment. DNA sample preparation, reaction mixture assemblage and the PCR process, in addition to the subsequent reaction analysis, should be performed in separate areas. The use of "clean", automatic pipettors designated for PCR and aerosol resistant barrier tips are recommended. Always keep the control DNA and other templates to be amplified isolated from the other components.

A control reaction, omitting template DNA, should always be performed to confirm the absence of contamination.

DNA Extraction:

E1. Place a single piece (3 mm X 3 mm) of leaf sample into a microcentrifuge tube.
E2. Add 100 µl of room temperature Plant Ex-Amp Extraction Buffer.
E3. Vortex contents for 1 minute.
E4. Incubate sample mixture/tube at 95°C for 15 min.
E5. Briefly vortex contents. The solution containing the gDNA can be directly used as template in PCR. Additional freeze-thaw/vortex cycles can be used for difficult samples.

PCR Amplification:

A1. Add the following components to a sterile 0.2 ml PCR tube sitting on ice.

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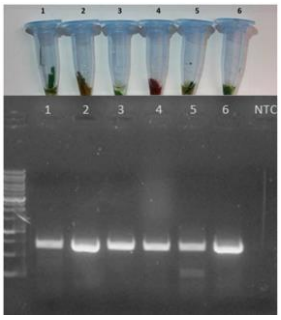
Components	Volume	Final concentration
Advance™ Master Mix with Dye	25 µl	1X
10 µM Forward Primer	1-2.5 µl	500 nM

10 µM Reverse Primer	1-2.5 µl	500 nM
Template from previous step (E5)	0.1-2 µl	Varies
10X Plant PCR Enhancer (Optional)	0.5 µl	1x
Water, Nuclease-free	Up to 50 µl	-

We recommend preparing a pre-mix for multiple reactions to minimize reagent loss and enable accurate pipetting.
The addition of 10X Plant PCR Enhancer is optional; only use it for weak and/or difficult amplifications.
A2. Mix contents of tube and centrifuge briefly.
A3. Incubate reactions in a thermal cycler at 94°C for 3 mins to completely denature the template.
A4. Perform 30 - 40 cycles of PCR amplification as follows:
Denature: 94°C for 30 secs; Anneal: 45°C - 72°C for 30 secs; Extend: 72°C for 1 min/1 kb template
A5. Incubate for an additional 5 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
A6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or other DNA staining. Use appropriate molecular weight standards.

VIII. Sensitivity:



A 700 bp of plant 28S was amplified from various leaves samples.

IX. Related Products:

BV Product Name	BV Cat. No.
M1127-200	Ready™ PCR Mix
M1127-1000	Ready™ PCR Mix
M1128-200	Ready™ PCR Mix-Dye
M1128-1000	Ready™ PCR Mix-Dye
M1129-200	Image Ready™ PCR Mix
M1130-200	Robust Ready™ PCR Mix
M1130-1000	Robust Ready™ PCR Mix
M1131-200	Robust Ready™ PCR Mix-Dye
M1131-1000	Robust Ready™ PCR Mix-Dye
M1132-200	Rigor™ PCR Mix
M1133-200	Rigor™ PCR Mix-Dye
M1134-200	Breeze™ PCR Mix
M1135-200	Breeze™ PCR Mix-Dye
M1136-200	Distant™ PCR Mix
M1137-200	Distant™ PCR Mix-Dye
M1138-200	Image Distant™ PCR Mix
M1139-200	Advance™ PCR Mix
M1140-200	Advance™ PCR Mix-Dye
M1141-200	Fire Start™ PCR Mix
M1142-200	Fire Start™ PCR Mix-Dye
M1143-200	Whole Blood PCR Mix
M1144-25	Plant Advance™ PCR Kit
M1145-100	Tissue Advance™ PCR Kit
M1146- M1153	DNA Polymerases

FOR RESEARCH USE ONLY! Not to be used on humans.