FastAmp® Plant Tissue/ Seed Genotyping Kit

Intact Genomics®

Catalog #	4617	4619
Package Size	250 reactions	1,000 reactions
Concentration	2x	

Description

FastAmp® Plant Tissue/Seed Genotyping Kit is suitable for amplification of DNA directly from plant samples without purifying DNA. This kit is based on specially engineered *Taq* DNA polymerase, proprietary buffer system, dNTP, $MgCl_2$, PCR facilitators and dye mix which makes it extremely robust and tolerant of plant PCR inhibitors such as complex polysaccharides, polyphenols and others. This PCR master mix has been tested with leaves and seeds from a wide variety of plant species. This kit includes a complete set of optimized reagents and detailed protocols making it an ideal choice for high-throughput genotyping from various Plant tissue/seeds without DNA purification steps .

Highlights

- Direct PCR- no need to purify DNA
- Specially engineered *Taq* DNA polymerase with highest sensitivity and specificity
- Extremely short PCR protocol times
- Master mix format with premixed gel loading dye to reduce cross-contamination and sample handling errors
- Optimized for both low and high GC templates

Applications

- Genotyping
- Transgene detection
- Knockout analysis
- Sequencing

Product Includes

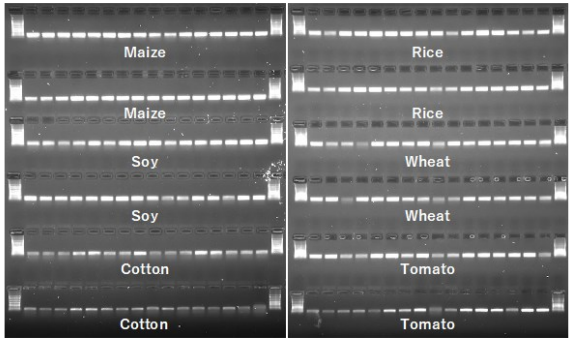
- FastAmp® Plant Direct PCR Master Mix (2x)
- FastAmp® Plant Direct PCR/Genotyping Solution

Storage Temperature: -20ºC

Quality Control Assays

FastAmp® Plant Tissue/Seed Genotyping Kit has been tested with tissue from a wide variety of plant species, some of the results are included here:

96 plate base Genotyping PCR



Genotyping PCR analysis from Plant leaf tissue in 96 plate using FastAmp® Plant Tissue/Seed Genotyping PCR kit.
Control Primer F - AGTTCGAGCCTGATTATCCC
Control Primer R - GCATGCCGCCAGCGTTCATC

Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.
Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.

Reminder

Please completely follow the protocol to perform your experiments.

General Guidelines Before Starting

A. Sample handling

5mm x 5mm cut leaf tissue or 2mg seed powder should be placed in 20µl of FastAmp® Plant Direct PCR/ Genotyping Solution. Then the sample should be mixed thoroughly and incubated at room temperature for 5min. No need to heat the sample to lyse the tissue.

B. PCR conditions

B-1. Denaturation

An initial denaturation of 8 minutes at 95°C is sufficient for most amplicons. Longer denaturation times can be used (up to 10 minutes) for difficult templates.
During thermocycling, the denaturation step should be kept to a minimum. Typically, a 20–30 second denaturation at 95°C is recommended for most templates.

B-2. Annealing

Optimize the annealing temperatures for the target gene specific amplification by keeping annealing temperature at least 5 °C below Tm values. Typically, use a 10–30 second annealing step. A temperature gradient can also be used to optimize the annealing temperature for each primer pair.
During thermocycling, the denaturation step should be kept to a minimum. Typically, a 20–30 second denaturation at 95°C is recommended for most templates.

B-3. Extension

The recommended extension temperature is 72°C. Extension times are generally 1 minute per kb for complex genomic samples but this can be reduced to 30 seconds per kb for simple templates.

FastAmp® Plant Tissue/ Seed Genotyping Kit

Intact Genomics®

General Guidelines Before Starting, *cont.*

When amplifying products >2 kb, it is often helpful to increase the extension time. A final extension of 5 minutes at 72°C is recommended.

B-4. Cycle number

Generally, 35–40 cycles yield sufficient product.

B-5. Primers

Forward and reverse primers are generally used at the final concentration of 0.1-0.6 µM each. If the primer concentration is too high, the specificity of priming may be reduced, resulting in non-specific products.

B-6. PCR product

The PCR products generated using Taq DNA Polymerase have dA ends. If cloning is the next step, then T/A-cloning is preferred.

Protocol

The reaction mix typically contains all the components needed for PCR except DNA template (leaf punch/other sources).

1. Thaw 2x master mix, Plant Direct PCR/Genotyping Solution, primers and mix thoroughly and spin down before use.
2. Cut 5mm x 5mm leaf tissue in 1.5ml tube or 96 well plate and add 20µl FastAmp® Direct PCR/Genotyping Solution and mix thoroughly
3. Prepare a reaction mix according to the following table:

PCR Reaction Set Up:	
Cut leat tissue/FastAmp® Plant Direct PCR/Genotyping Solution	1.0 µL
FastAmp® Plant Direct PCR master mix (2x)	10.0 µl
Forward primer (3.2 µM)	1.0 µl
Reverse primer (3.2 µM)	1.0 µl
FastAmp® Plant Direct PCR/ Genotyping Solution	7.0 µl

4. Mix the reaction mixture thoroughly.
5. Program the thermal cycler according to the manufacturer's instructions. A typical PCR cycling program is outlined in the following table:
6. Place the PCR tubes in the thermal cycler and start the cycling program.

PCR Cycling Conditions			
Steps	**Temp.**	**Time**	**Cycles**
Initial denaturation	95 ºC	8 min	1
Denaturation	95 ºC	20-30 sec	35-40
Annealing	Tm-5 ºC	20-30 sec	
Extension	72 ºC	1 min / kb	
Final extension	72 ºC	5 min	1
Hold	4-12 ºC	∞	

7. Run 10.0 µl of PCR products in 1% agarose gel (120 volts for 45 min).

Troubleshooting

No product at all or low yield
If the positive control with purified DNA is not working: • Optimize annealing temperature • Make sure the cycling protocol was performed as recommended • Increase the number of cycles up to 40

Non-specific products
• Increase annealing temperature • Shorten extension time • Decrease primer concentration • Check the purity and concentration of primers • Re-design new primers and test several pairs of primer

Related Products

- ig® SYBR Green qPCR 2x Master Mix (Cat.# 3354)
- FastAmp® Plant Direct PCR Kit (Cat.# 4612)
- FastAmp® Plant Direct PCR & Genotyping Solution (Cat.# 4611)
- GV3101 Chemically Competent Agrobacterium (Cat.# 1082-06)
- GV3101 Electrocompetent Agrobacterium (Cat.# 1282-12)
- AGL1 Chemically Competent Agrobacterium (Cat.# 1083-06
- AGL1 Electrocompetent Agrobacterium (Cat.# 1283-12)