FastAmp® Plant Direct PCR Kit

Intact Genomics®

Catalog #	4612	4615
Package Size	250 reactions	1,250 reactions
Concentration	2x	

Description

FastAmp® Plant Direct PCR 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 amplification of plant DNA without DNA purification. **For Seed Tissue, please refer to our FastAmp Plant Tissue-Seed Genotyping Kit.**

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

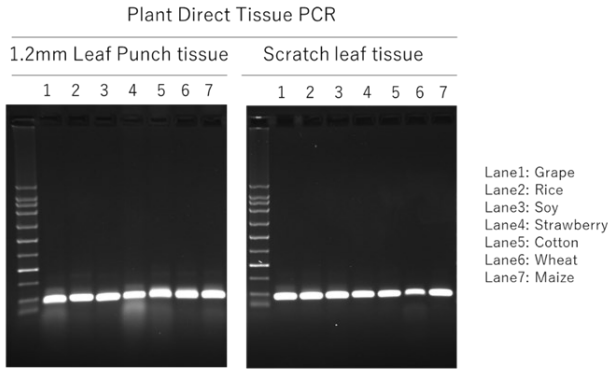
Product Includes

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

Storage Temperature: -20ºC

Quality Control Assays

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



Direct PCR analysis from various 1.2mm punched leaf/tissue and scratch tissue using FastAmp® Plant Direct PCR kit

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

1.2mm punch leaf tissue should be placed directly into 8µl FastAmp® Plant Direct PCR/Genotyping Solution in PCR tube.
A leaf punch can be obtained by placing the leaf puncher (i.e. Uni-Core punch by GE Healthcare, Product # WB100028, or any other commercial sources) in perpendicular position over the expanded leaf and rotating it. The tip of puncher needs to placed inside the PCR tube and expunge to drop the sample. It is critical that plant tissue materials/leaf punch is completely inside the PCR solution in tube. Occasionally, the leaf punch may stick to the side of PCR tube and therefore does not reach the bottom of tube. If this occurs, use a 10 µl pipet tip to manually drive the leaf punch to the bottom of PCR tube. This can be done right before capping the PCR tubes and starting the thermocycling program. For reusing the puncher, it is very important to clean the cutting edge properly with 70% ethanol to prevent cross-contamination between samples.

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 ºC 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.

General Guidelines Before Starting, *cont.*

B-3. Extension

The recommended extension temperature is 72°C. Extension times are generally 1 minute per kb for complex, genomic samples, but can be reduced to 30 seconds per kb for simple templates. 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. Place scratch leaf tissue or punch leaf tissue into 8µl FastAmp® Direct PCR/Genotyping Solution and mix thoroughly
3. Prepare a reaction mix according to the following table:

PCR Reaction Set Up:	
Leaf punch/scratch tissue/with FastAmp® Plant Direct PCR/	8.0 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

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:

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

6. Place the PCR tubes in the thermal cycler and start the cycling program.
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 Tissue & Seed Genotyping Kit (Cat.# 4617)
- 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)