For research use only Version Number：1.0

Plant Seed Direct PCR Kit I/II

For performing PCR directly from plant seed (containing low polysaccharide and polyphenol components) without prior DNA purification

I	Kit composition (20μl system)	TP-0311T	TP-03111	TP-03112	TP-03113
		50T	200T	500T	2000T
Part I	Buffer SP1	10 ml	40 ml	100 ml	400 ml
	Buffer SP2	3 ml	10 ml	25 ml	100 ml
	6× DNA Loading Buffer	1.5 ml	1.5 ml	1.5 ml	1.5 ml × 4
Part II	2× Seed PCR Easy™ Mix	500 μl	1 ml × 2	1.7 ml × 3	1.7 ml × 12
Instruction		1	1	1	1
II	**Kit composition (20μl system)**	**TP-0313T**	**TP-03131**	**TP-03132**	**TP-03133**
		50T	200T	500T	2000T
Part I	Buffer SP1	30 ml	120 ml	100 ml × 3	400 ml × 3
	Buffer SP2	3 ml	10 ml	25 ml	100 ml
	6× DNA Loading Buffer	1.5 ml	1.5 ml	1.5 ml	1.5 ml × 4
Part II	2× Seed PCR Easy™ Mix	500 μl	1 ml × 2	1.7 ml × 3	1.7 ml × 12
Instruction		1	1	1	1

Product Introduction

This product uses a unique lysis reaction system, which can quickly release genomic DNA from plant seed samples with low polysaccharide and polyphenol content (such as: rice, wheat, tobacco, corn, soybeans, etc.) for use in PCR reactions, so it is particularly suitable Large-scale genetic testing. In order to meet the needs of different detection tests, this kit can use multiple types of samples (such as whole seeds, small tissue cuts, or ground samples of multiple seeds) for experiments. Among them, for seed samples that still need to be germinated, you can choose to cut out micro-tissue pieces (1-5 mg) other than seed embryos for experiment; for experiments that need to be sampled in a large number of samples, you can choose to mix multiple samples and grind them into particles with a diameter of about 0.5mm before proceeding with the experiment (at least 0.1% of the target sample can be detected).

The process of releasing genomic DNA from the lysis buffer can be completed within 5-10 minutes. No other processes to remove protein, RNA or secondary metabolites are required, and the released trace DNA can be used as a template for PCR reactions.

Transport and storage conditions

1. Transportation conditions: The whole process is transported in a low-temperature ice box to ensure that the Part II kit is at <4℃.

2. Storage conditions: Part I of this kit is stored at room temperature or 2-8℃; Part II is stored at -20℃.

Features

1. Time-consuming and expensive DNA purification are not required.
2. The sample demand is small, and the experiment can be carried out only by taking a single seed.
3. No special treatments such as grinding and crushing are required, and the operation is simple.
4. Optimized PCR system makes PCR have higher specificity and stronger tolerance to PCR reaction inhibitors.

Precautions:

- This kit is only suitable for seed samples with low polysaccharide and polyphenol content. For samples with high polysaccharide and polyphenol content, please choose the Plant Seed Direct PCR Plus Kit I/II.
- Pay attention to the cleaning of the experimental equipment and the operating methods of the experiment to avoid cross-contamination between samples.
- Please try to use seeds within 1 year for experimentation. If the seeds are stored for more than 1 year or the seeds are particularly dry, please pick the seed coat or use the crushed seeds when lysing.
- If Buffer SP1 has precipitated out, it can be placed at 37°C until the precipitate disappeared, and the solution should be shaken before use.
- 2× Seed PCR Easy™ Mix should avoid repeated freezing and thawing, otherwise it will affect the PCR efficiency.
- If the ambient temperature is too high, the 2× Seed PCR Easy™ Mix may become turbid. It can be placed on ice for 1-2 minutes. When the solution is clear, mix it upside down and mix 3-5 times before use.
- During electrophoresis detection, do not use Loading Buffer containing SDS, otherwise a large group of tailing bright bands will appear in the lane during electrophoresis, which will affect the experimental results.

Operation guide

A: Sample release

1. Take an appropriate amount of seed sample and add it to a 200 μl or 2 ml centrifuge tube.

Note: If the material is a particularly dry seed that has been stored for more than one year, the seed coat or the seed must be broken up before the lysis reaction can proceed.

2. Add an appropriate amount of Buffer SP1 according to the size of the seed or tissue block (see Table 1 below for reagent dosage).

Note: Add up to 200 μl Buffer SP1 per lysis for small and medium seeds, and up to 600 μl Buffer SP1 per lysis for large seeds.

3. Place it in a PCR machine or a metal bath, and lyse at 95°C for 10-30 minutes.

Note: Most samples can be lysed for 10 minutes. For samples that are difficult to lyse or have been

stored for too long, the lysis time can be extended to 30 minutes.

4. Centrifuge at 13400 × g for 2 min at room temperature, take 50 μl of the supernatant of the lysate into a new centrifuge tube, add 50 μl of Buffer SP2, and mix by pipetting. Micro-tissue cuts in the range of 1-5 mg can be directly added to the reaction tube by adding 50 μl Buffer SP2 for neutralization after the lysis is complete.

5. The resulting lysis mixture can be stored at 4°C (within 5 days) or directly used as a template for PCR reaction. If long-term storage is required, the lysis mixture can be stored at -20°C.

Table 1: Lysis related parameters of samples with low polysaccharide and polyphenol content

Sample type	Sample size (mg)	Buffer SP1 (μl)	Neutralization reaction (μl)	
			lysis product	Buffer SP2
Rapeseed or similar seeds[1*]	~6	100	50	50
Single seed (rice similar size)	~30	200	50	50
Single seed (corn similar size)	~250	600	50	50
Tissue dicing	1-5	50	- [2*]	50
	5-30	200	50	50
Ground sample[3*]	100	600	50	50

1*: When experimenting with tobacco or similar-sized seeds, 10-30 seeds (about 1-6 mg) need to be used for lysis.

2*: For micro-tissue lysis within the range of 1-5 mg, you can directly add 50 μl Buffer SP2 to the reaction tube for neutralization after the lysis is completed.

3*: In order to ensure the strength and uniformity of pyrolysis, it is advisable to control the particle diameter of the ground sample within 0.5 mm.

B: PCR reaction identification

1. Add the corresponding 2× Seed PCR Easy™ Mix and specific primers to the PCR tube for later use.

2. Take an appropriate amount of the lysis mixture processed in step A and add it to the PCR system prepared above (see Table 1 for system preparation).

Note: The amount of template should be between 10-20% of the PCR system, and should not exceed 30% (for example, in a 20 μl PCR system, add 2-4 μl of lysis buffer).

3. Carry out the PCR reaction according to the optimized PCR conditions (annealing temperature, etc.) (see the following table 2 for the reaction conditions).

4. Agarose gel electrophoresis test results.

Table 1: PCR reaction system preparation

PCR system additions	Consumption		Final concentration
2× Seed PCR Easy™ Mix	10 μl	25 μl	1×
Forward Primer (10 μM)	0.5 μl	1 μl	0.2-0.25 μM [1*]
Reverse Primer (10 μM)	0.5 μl	1 μl	0.2-0.25 μM [1*]
Lysis Mix (DNA template)[2*]	X μl	X μl	
ddH_2O (Sterilized distilled water)	(9-X) μl	(23-X) μl	
Total Volume	20 μl	50 μl	

1*: Usually the final primer concentration is 0.2-0.25 μM to get better results. When the reaction performance is poor, the primer concentration can be adjusted within the range of 0.1-0.5 μM.

2*: The lysate is used as a PCR template, and the amount of addition is between 1-10% of the PCR system. In actual operation, you can explore the conditions of template addition to find the best template amount.

Note: The preparation of this system is for reference only. The laboratory can adjust the size of the PCR system as needed by adding an appropriate ratio of lysis mixture. Prepare the PCR reaction system, place it on a vortexer, vortex and mix, and centrifuge briefly to collect the reaction solution at the bottom of the tube.

Table 2: Examples of reaction conditions

Step	temperature	time	Cycles	content
1	94℃	3 min	1	Predenaturation
2	94℃	10 sec	30-40	transsexual
3	55-65℃	20 sec		Primer annealing
4	72℃	x min (2kb/min) [2*]		extend
5	72℃	5min	1	Final extension

1*: 2× Seed PCR Easy™ Mix has good amplification ability for templates with high GC content. When performing PCR, we recommend that the annealing temperature of all primers is 2°C higher than the TM value.

2*: For DNA fragments within 1kb, the recommended extension time is 30 sec.

Note: The PCR conditions in this table are for reference only. The PCR reaction conditions vary depending on the structural conditions of the template, primers, etc. In the specific operation, it is necessary to design the optimal reaction conditions, including annealing temperature, extension time, etc. according to the specific conditions of the template type, the size of the target fragment, the base sequence of the amplified fragment, and the GC content and length of the primer.