For research use only Version Number：1.0

Plant Leaf Direct PCR Kit

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

Kit composition (20μl system)		TP-0211T	TP-02111	TP-02112	TP-02113
		50T	200T	500T	2000T
Part I	Buffer P1	3 ml	10 ml	25 ml	100 ml
	Buffer P2	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× Leaf PCR Easy™ Mix	500 μl	1 ml × 2	1.7 ml × 3	1.7 ml × 12
Instruction		1	1	1	1

Product Introduction

1. This product uses a unique lysis buffer system, which can quickly release genomic DNA from leaf samples of plants with low polysaccharide and polyphenol content (such as rice, wheat, tobacco, corn, soybeans, rape, etc.) for use in PCR reactions. When the leaves are treated with lysis buffer, the leaves do not need to be ground or shredded, so they are particularly suitable for large-scale genetic testing.
2. 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 in a state of <4℃.

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

Features

- Time-consuming and expensive DNA purification are not required.
- The sample demand is small, and the experiment can be carried out with a blade with a diameter of 2 mm (1 mg).
- No special treatments such as grinding and crushing are required, and the operation is simple.
- Optimized PCR system makes PCR have higher specificity and stronger tolerance to PCR reaction inhibitors.

Precautions:

- This kit is only suitable for plant samples with low polysaccharide and polyphenol content. For samples with high polysaccharide and polyphenol content, please choose the Plant Leaf Direct PCR Plus Kit.
- 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 fresh young leaves of plants for experiments. If you choose mature leaves, please avoid using the tissues of the main veins of the leaves.
- If there is precipitation in Buffer P1, it can be placed at 37°C until the precipitation disappears, and the solution should be shaken before use.
- 2× Leaf PCR Easy™ Mix should avoid repeated freezing and thawing, otherwise it will affect the PCR efficiency.
- If the ambient temperature is too high, 2× Leaf PCR Easy™ Mix may become turbid, it can be placed on ice for 1-2 minutes, until the solution is clear, inverted and mixed 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

Direct method (only for plant leaf samples with low polysaccharide & polyphenol content)

1. Add 2× Leaf PCR Easy™ Mix to a 200 μl PCR tube, then add the corresponding primers, and dilute to 1× with ddH2O (see Table 1 for PCR system preparation).

2. Cut 1-2 mg leaf fragments (diameter 2-3 mm) and add them to the PCR system prepared above.

Note: Ensure that the leaf fragments are completely immersed in the PCR reaction solution, and do not add excessive leaf tissue.

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.

Lysis method

A: Sample release

1. Cut 3-5 mg leaf tissue (diameter 5-7 mm) into a 200 μl or 1.5ml centrifuge tube.

Note: Do not add excessive leaf tissue.

2. Add 50 μl Buffer P1 to ensure that the lysate can completely immerse the leaf tissue.

3. Cover the centrifuge tube lid, place it in a PCR machine or a metal bath, and lyse at 95℃ for 10 min.

Note: After heating, if there is a lot of liquid on the tube wall, the liquid can be collected to the bottom of the centrifuge tube by instant centrifugation.

4. Add 50 μl Buffer P2, pipette or vortex to mix.

5. The resulting lysis mixture can be stored at 4℃ (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℃.

B: PCR reaction identification

1. Add the corresponding 2× Leaf 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, but not more than 6 μl).

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× Leaf 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*]
Leaf tissue or lysis mixture[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

Steps	Temperature	Time	cycles	Content
1	94℃	3 min	1	Predenaturation
2	94℃	10 sec	30-40	denaturation
3	55-65℃	20 sec		Primer annealing
4	72℃	x min (2kb/min)[2*]		extend
5	72℃	5min	1	Final extension

1*: 2× Leaf PCR Easy™ Mix has a good amplification ability for templates with high GC content. When performing PCR, we recommend that the annealing temperature of all primers is 2℃ 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.

Please be sure to take samples as shown in the figure below, and the sample size of the blade should not exceed that shown in the figure below:

Direct method (scale = 1: 1)

Pyrolysis method (scale bar = 1: 1)