# Plant Direct PCR Kit

PD105

Version 9.1

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## Introduction

Plant Direct PCR Kit is designed to perform PCR directly from plant leaves, seeds, etc., and can be used for high-throughput screening of non-polysaccharide and polyphenolic plant samples. The directly engineered DNA polymerase, which maintains high resistance to PCR inhibitors in plants and extremely high amplification performance, is suitable for amplification of DNA fragments length within 5 kb. The unique Lysis Buffer A in kit can be used to lyse fresh or frozen plant tissue with easy handling, and the lysate yield can be used as template with no need of purification. The protective agent contained in the lysate allows the crude product to be efficiently amplified after multiple freeze-thaw cycles. Amplification can be directly performed as primers and templates are added to pre-prepared 2 × Plant Direct Master Mix, which reduce pipetting operation and improve the throughput of detection and reproducibility of results. The amplification product is blunt end and is applicable to the ClonExpress Rapid Cloning Kit (C112/C113/C114).

## Package Information

| Components | PD105-01 50 rxn (50 μl/rxn) | PD105-02 200 rxn (50 μl/rxn) |
|---|---|---|
| 2 × Plant Direct Master Mix | 1.25 ml | 4 × 1.25 ml |
| Plant Direct Lysis Buffer A | 5 ml | 20 ml |
| Plant Direct Lysis Buffer B* | 5 ml | 20 ml |

* Plant Direct Lysis Buffer B is an optional reagent for neutralizing Plant Direct Lysis Buffer A to extend sample storage time and can be used according to actual conditions.

## Storage

2 × Plant Direct Master Mix should be stored at -20˚C and avoid repeated freezing and thawing; Plant Direct Lysis Buffer can be stored at -20˚C or 2˚C - 8˚C.

## Protocol

### Sample Preparation

**For Plant leaf**

**Direct Protocol:** It is recommended to use young leaves. In order to obtain a small and uniform sample, it is recommended to use a punch with a fixed diameter of 0.5 - 3 mm to punch sample1, and add the sample directly into the PCR reaction system (50 μl system is recommended). Be careful to ensure that the sample is in the PCR solution, not on the tube wall. If the direct PCR method is used to amplify longer fragments and complex samples, taking a smaller diameter (0.5 - 1 mm) sample as a template will help to obtain better results.

**Grinding Protocol:** It is recommended to use young leaves. Take a small piece of leaf (about 1 - 3 mm in diameter), place it in 20 μl Plant Direct Lysis Buffer A2, and grind it as much as possible (you can use a 100 μl pipette tip to squeeze the leaves to smash the sample). If the leaf tissue is used in a large amount (do not exceed 7 mm), increase the volume of the dilution buffer to 50 μl. After the leaves are ground, the solution should appear green. After brief centrifugation, 1 μl of the supernatant was added to the PCR reaction system as template3.

**Heat Protocol:** It is recommended to use young leaves. Take a small piece of leaf (about 1 - 3mm in diameter) and place it in 20 μl Plant Direct Lysis Buffer A, 95˚C for 5 - 10 min, and prolong the lysis time (not more than 20 min) for the leaves hard to lyse. If the leaf tissue is used in a large amount (do not exceed 7 mm), increase the volume of the lysis buffer to 50 μl. After heating, the mixture was briefly centrifuged and adds 1 μl of the supernatant to the PCR reaction system as template.

**Plant Seed**

**Grinding Protocol:** Cut the seed with a diameter of about 5 mm using a scalpel, added it to a 100 μl Plant Direct Lysis Buffer A, and the sample was ground with a pipette tip or the like. After brief vortexing, it was allowed to stand at room temperature for 3 - 5 min. Make sure the seed sample is immersed in the dilution buffer. After a brief centrifugation, 1 μl of supernatant was added to the PCR reaction system as reaction template3.

**Heat Protocol:** Cut the seed with a diameter of about 5 mm using a scalpel, add it to 100 µl of Plant Direct Lysis Buffer A, heat at 95˚C for 5 - 10 min, and prolong the lysis time (not more than 30 min) for the leaves hard to lyse. After the end of the centrifugation, 1 µl of supernatant was added to the PCR reaction system as template.

1. Scissors or other tools can also be used to cut samples; if the punch or scissors are used repeatedly, they should be cleaned with 2% sodium hypochlorite solution before use to prevent cross-contamination.
2. Before use, ensure that the Plant Direct Lysis Buffer is fully melted, if it is viscous or precipitated; please heat it at 37˚C to completely melt before use.
3. The template volume in the reaction system can be appropriately adjusted according to the difference plant material and the difference volume of the diluted solution.

### Plant Direct Lysis Buffer

The Plant Direct Lysis Buffer A included in this product is rigorously optimized to release the genome of most plant tissues and is applicable to short-term storage of crude plants at 4˚C. If the sample needs to be stored for a long period of time (eg 1 - 2 months), it is recommended to transfer the supernatant to a new EP tube and store at -20˚C. In order to store the sample more stably, an equal volume of Plant Direct Lysis Buffer B can be added to the transferred supernatant, mixed and stored at -20˚C. The storage time varies with plant samples and conditions.

### Reaction System

| | | |
|---|---|---|
| $ddH_2O$ | To 20.0 µl | To 50.0 µl |
| 2 × Plant Direct Master Mix [a] | 10.0 µl | 25.0 µl |
| Primer 1 (10 µM) [b] | 0.8 µl | 2.0 µl |
| Primer 2 (10 µM) [b] | 0.8 µl | 2.0 µl |
| Plant Leaf/Crude Sample [c] | Round piece of leaf with a diameter of 0.5 - 3 mm/x µl | Round piece of leaf with a diameter of 0.5 - 3 mm/x µl |

a. Containing a final concentration of 2 mM $Mg^{2+}$

b. It is recommended to use a final concentration of 0.4 µM per primer. Excessive amount of primers added will result in non-specific amplification.

c. The amount of sample used in single reaction can be adjusted between 2% and 20% of the total volume of the reaction. Excessive use may lead to an unsuccessful amplification.

### Reaction procedures

| Procedures | Temperature | Time | Cycle |
|---|---|---|---|
| Pre-denaturation [a] | 98˚C | 5 min | 1 |
| Denaturation | 95˚C | 10 sec | |
| Annealling [b] | 58 - 72˚C | 15 sec | 35 cycles |
| Extension [c] | 72˚C | 30 sec/kb | |
| Final Extension | 72˚C | 5 min | 1 |

a. Pre-denaturation (98˚C, 5 min) can promote plant tissue lysis, genomic DNA released can be used for PCR amplification. Do not shorten the time or lower the temperature.

b. Generally set equal to the primer Tm value or 2 - 4˚C higher than the Tm value. The Direct-PCR DNA polymerase used in this product is different from the ordinary Taq enzyme, and has special requirements for the reaction annealing temperature; high annealing temperature can effectively reduce non-specific amplification and improve the expansion efficiency. For complex templates, it is necessary to adjust the annealing temperature and extend the extension time to achieve efficient amplification.

c. If the length of the amplified product is ≤ 1 kb, the extension time is set at 30 sec/kb; if the length of the amplified product is > 1 kb, the extension time is set at 60 sec/kb.

d. For complex samples or samples with lower amplification yields, increase the number of cycles to 40 - 50 cycles.

## Application examples

Use this kit to directly amplify the five common plants in the laboratory. Tobacco leaves, Arabidopsis thaliana slices, wheat leaves, rice leaves and maize seeds were treated by grinding method, and the obtained crude product was used as a template, and the above five plant genomic DNAs extracted by CTAB method were used as controls. Use 2 × Plant Direct Master Mix to amplify seven different target fragments of 0.5 kb, 2.0 kb, 0.3 kb, 1.5 kb, 2.3 kb, 0.8 kb, and 0.3 kb respectively, and the primer Tm value was between 60 and 68˚C (calculated using Primer Premier 5). The reaction system and procedure are as follows:

### Reaction System

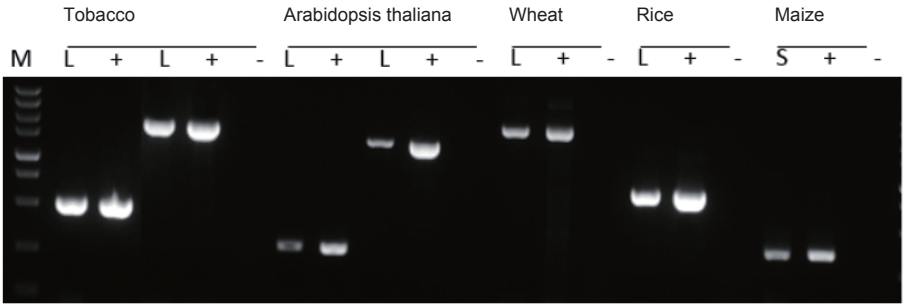
| | |
|---|---|
| $ddH_2O$ | To 20.0 µl |
| 2 × Plant Direct Master Mix | 10.0 µl |
| Primer 1 (10 µM) | 0.8 µl |
| Primer 2 (10 µM) | 0.8 µl |
| Plant crude sample | 1.0 µl |

### Reaction Procedure

| Procedures | Temperature | Time | Cycle |
|---|---|---|---|
| Pre-denaturation | 98℃ | 5 min | 1 |
| Denaturation | 95℃ | 10 sec | |
| Annealling | 60℃ / 64℃ / 68℃* | 15 sec | 35 cycles |
| Extension | 72℃ | 30 sec - 2 min | |
| Final Extension | 72℃ | 5 min | 1 |

*When the 2.3 kb target fragment of wheat and the 0.3 kb target fragment of corn were expanded, the annealing temperatures were set at 68℃ and 64℃, respectively; the annealing temperature of the rest system primer was set to 60℃.

## Application examples




The figure shows that the five plants can be effectively amplified using this kit.
L, crude leaf; S, crude seed; +, genomic DNA; -, no template control; M, DL5,000 DNA Marker

## Precautions

1. For the amplification or direct amplification of crude plants, it is recommended to use purified genomic DNA as a positive control before the experiment to ensure the system, primers and operations are correct.
2. The direct- amplification DNA polymerase used in this kit has strong proofreading activity. Therefore, if the amplification product is used for TA cloning, DNA purification must be performed before adding A.
3. Primer design:

- The last base of the 3' end of the primer is G or C preferably;
- The last 8 bases at the 3' end of the primer should avoid a continuous mismatch;
- The hairpin structure should be avoided at the 3' end of the primer;
- It is better that the Tm value difference of the forward primer and the reverse primer are not more than 1℃, and the Tm value is preferably adjusted to 60℃-72℃ (the primer Tm value is recommended to be calculated using Primer Premier 5);
- The additional sequence of the primer, ie the unpaired sequence with the template, should not participate in the calculation of the primer Tm value;
- The GC content of the primer is controlled within 40% and 60%;
- The overall distribution of primers A, G, C, and T should be as uniform as possible, avoiding using the region with high GC content or with high AT content;
- Avoid complementary sequences of more than 5 bases in the primer or between the two primers, and control the complementary sequence less that 3 base at the 3' end of the two primers;
- After primer design, please use the NCBI BLAST function to search for primer specificity to avoid non-specific amplification.

## Troubleshooting and FAQ

### ◇Sample Processing

1. When using plant leaf to perform direct amplification, it is recommended to use a 50 μl reaction system and use young leaves with a diameter of 0.5 - 3 mm. The small reaction system and excessive leaf usage are likely to fail in amplification.
2. When using crude plant sample as a template for amplification, the superior amplification effect can be obtained by adjusting the crude treatment method and the amount of use. Try to mash the sample in Plant Direct Lysis Buffer A and incubate for 3 min at room temperature; adjust the template added among 0.5 μl to 5 μl. Or dilute the crude product in a ratio of 1:1 - 1:10, and take 1 μl as template. Note that there are inhibitory components of the PCR reaction in the plant sample and the crude product, and too much template adding can inhibit the reaction and cause the amplification failure. For samples that are difficult to amplify, try adding a final concentration of 2% beta-mercaptoethanol or 10 mM DTT to Plant Direct Lysis Buffer A.

### ◇Sample Processing

#### No product or less product

| | |
|---|---|
| Repeat Experiment | Confirm that the reaction system and the reaction procedure are set correctly |
| Primer | Optimize primer design, confirm primer concentration and purity |
| Annealing Temperature | Set the annealing temperature gradient to find the proper annealing temperature |
| Reaction System | Increase the concentration of $Mg^{2+}$ |
| Reaction Procedure | Increase the number of cycles, or extend the extension time |
| Template Usage | Try different template dosages, increase reaction volume or try to use BME/DTT |

#### Miscellaneous or diffuse band

| | |
|---|---|
| Primer | Optimize primer design and reduce primer concentration |
| Annealing Temperature | Try to increase the annealing temperature or set the annealing temperature gradient, reduce the annealing time |
| Reaction Procedure | Reduce the total number of cycles, the extension time does not exceed 60 sec/kb |
| Template Usage | Try different template dosages, increase reaction volume or try to use BME/DTT |

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