LAMDA BIOTECH

PRODUCT INFORMATION

Catalog No.: D303-100 & D303-1000

Product Name: **Seed-Direct™ PCR Kit**

Description: **Seed-Direct™ PCR Kit** contains all the reagents needed for quick preparation of genomic DNA from plant seeds and PCR amplification with the prepared seed DNA samples. Any plant seeds can be used, such as seeds from Arabidopsis, carrot, corn, cucumber, pepper, soybean, turnip, wheat or any other seeds as long as PCR primers are correctly designed. The PCR product can be directly loaded onto agarose gel without addition of loading dye for visualization of the experimental results.

Kit Contents:	D303-100	D303-1000
Size:	100 rxns	1000 rxns
DNA Prep Solution:	10ml	2x50ml
2X Direct-PCR Mix:	1ml	10x 1ml

Storage: The whole kit can be stored at 4°C for up to three months or at -20°C for long-term.

General Protocol

I. Seed Sample Preparation:

Seed amount needed: To use this kit, 1-2mg of seed is sufficient for each sample. It would be difficult and tedious to weigh each sample, especially when a lot of different samples are involved. Rule of thumb, in many cases it can be estimated that $1mm^3$ seed sample weighs about 1mg, a sesame seed is about 2mg, in other words only a small portion of a seed will be needed for this kit.

Seed Sample Preparation: Traditionally, there are many ways to prepare seed samples, such as: to use a mortar and pestle to grind seeds, liquid nitrogen, a mill, metal beads, or soften the seeds in water and grind using a disposable plastic pestle. You can still use all these methods if you prefer, however, this kit can eliminate all of the above complicated and sometimes tedious procedures by using the following technique to prepare the seed samples:

1. Wrap one or a few seeds (if the seeds are too tiny) with a piece of plastic film, such as Saran Wrap.
2. Use a regular pliers, any kind, to break the seeds.
3. Transfer one or a few pieces (about total 1 - 2mg, or the size of a sesame seed) of the broken seeds to a PCR tube, and then proceed to the following step for DNA Sample Preparation.

II. DNA Sample Preparation:

1. Place the sample into a PCR tube.
2. Add 100µl of the **DNA Prep Solution** into the tube containing the sample.
3. Heat the sample for 10 minutes at 95°C in a PCR machine.
4. Take out the sample and mix a few times. The sample is now ready for PCR. **Optional:** the sample can be centrifuged briefly and use the supernatant for PCR. Avoid any undigested tissue or debris.

II. PCR Amplification:

1. To set up the PCR reaction, add the following reagents to a PCR tube or PCR plate, as an example:

2X Direct-PCR Mix:	10µl
Primers:	1µl
Sample:	1µl
Water:	8µl
Total volume:	**20 µl**

Note:

A. Scale up your PCR according to your specific case, such as using 50µl as PCR reaction volume.

B. When large numbers of samples are processed, the 2X Direct-PCR Mix, water and primers can be premixed and aliquoted.

2. Mix the PCR reaction gently, avoid creating foam or bubbles. A brief centrifugation may be needed to collect the reaction mix to the bottom of the PCR tube or plate.

3. Perform the PCR thermal cycling. The following table is a typical example of a PCR.

Step	Temperature	Time	Cycles
Initial Denature	95°C	3 min	1
Denature	95°C	0.5-1 min	30-35
Annealing	50-65°C	0.5-1 min	
Extension	72°C	1 min/kb	
Final Extension	72°C	7 min	1
Hold	4°C	∞	

4. After PCR, the amplified products can be directly loaded onto an agarose gel for checking the results.

Troubleshooting: Problems and Solutions

Q1. Samples are not completely digested or dissolved.

A1. Samples are not expected to be digested or dissolved completely. Do not worry. Sufficient DNA will be released for PCR without complete digestion of the samples.

Q2. Little or no PCR product is detected.

A2. General Solution:

a) Make sure that there are no PCR components missed.
b) More PCR cycles may be needed.
c) Primers may not be designed optimally.
d) Try different annealing temperature and extension time or use a touchdown PCR program.
e) Too much sample may have been used, in that case the samples can be easily diluted 10 times with H_2O or 10mM Tris-HCl buffer, pH 8.5.

f) If you have tried all above and it is still not working, read the Specific Solution below.

A2. Specific Solution: Some templates and primers are difficult for PCR. If you have tried changing all the possible PCR parameters and your PCR is still not working well, we recommend that you try our **Conquest™ Genotyping PCR Optimizing Kit** (**Cat. No. D911**). This kit has successfully performed PCR on many tough PCR templates and primers. The Master Mixes within the **Conquest™ Genotyping PCR Optimizing Kit** are formulated with multiple thermal DNA polymerases and pre-optimized PCR buffers and enhancers. It is in four different ready-to-use 2X format combinations. Using this **Conquest™ Genotyping PCR Optimizing Kit** simplifies the process to determine optimal conditions for each unique combination of template and primers. You will easily find a specific Master Mix that works well with your templates and primers.

Q3. High background or multiple PCR products.

A3. Adjust your annealing temperature or use touchdown PCR program.

Q4. Negative control shows PCR product or false positive result.

A4. Reagents or your samples may be contaminated.

END