Plant Seed Direct PCR Kit

Quick preparation of template DNA from desiccative Plant Seed for PCR without DNA Isolation

Kit Contents

Cat. No.	TQ2900-01	TQ2900-02	TQ2900-03
Preps	20 preps	100 preps	500 preps
PS1 Buffer	2 ml	12 ml	60 ml
Proteinase K	3mg	11mg	5 x 11mg
PS2 Buffer	1 ml	1 ml	5 x 11ml
PS3 Buffer	2 ml	12 ml	60 ml
2 x PCR Master Mix	1 ml	2 x 1 ml	8 x 1 ml
Distilled water	2 ml	10 ml	50 ml

Shipping and Storage

The Plant DNA Direct Kit is shipped at 2-8℃. 2 x PCR Master Mix should be stored at -20℃.

Product Description

The Plant Seed Direct PCR Kit contains all of the reagents required to rapidly extract and amplify genomic DNA from desiccative plant seeds. Briefly, the DNA is extracted from whole or ground seed by incubation in the Extraction Solution at 56°C for 10 minutes, then put it in 95°C for 5 minutes. After an equal volume of the Dilution Solution is added to the extract to neutralize inhibitory substances, the extract is ready for PCR. An aliquot of the diluted extract is then combined with the 2 x PCR MasterMix and user provided PCR primers to amplify target DNA. 2 x PCR MasterMix is a 2 x Reaction Mix containing buffer, salts, dNTPs, and Taq DNA Polymerase. It is optimized specifically for use with the extraction reagents.

Protocol for Plant Seed Extraction

Prepare Proteinase K Solution: Add PS2 Buffer to dissolve Proteinase K and Store at -20°C.

TQ2900-01: Add 120 µl PS2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ2900-02: Add 550 µl PS2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ2900-03: Add 550 µl PS2 Buffer to each tube of Proteinase K, gently mix to dissolve Proteinase K.

A: Protocol for grinding plant seeds.

Pretreatment：Grinding seeds

Note: with arabidopsis or similar sized seeds, approximately 50 seeds should be placed in a single well.

1. **grind using a bead mill**
   a. place one seed into each well of a 2 ml square well block.
   b. Pipette nuclease-free water into the well according to the following volumes:

      800 ul for soybean or similar sized seeds

      600 ul for cotton or similar sized seeds

      200 ul for canola, sorghum, wheat, or similar sized seeds

100ul for arabidopsis or similar sized seeds

c. Place a 4 mm stainless steel grinding ball in each well of the 2 ml 96 square well block and cover with sealing mat.

Place block in the bead mill and shake at 1,500 rpm for 10 min.

d. Continue to Extract seeds DNA.

2. grind individually using a plastic pestle or mortar

a. place one seed into a 1.5 ml microcentrifuge tube.

b. Pipette nuclease-free water into the well according to the above step b.

c. **I**ncubate the seed with water for 1 hour at 55℃.

d. Grind hydrated seeds in tube using a plastic pestle or mortar.

e. Continue to Extract seeds DNA

3. grind individually using liquid nitrogen

a. grind seed into a fine powder in liquid nitrogen using a mortar and pestle.

b. Transfer between 5 and 100 mg of ground seed material into a pre-weighed 1.5 ml microcentrifuge tube. Record the mass of the transferred seed material.

c. Pipette 4 ul of nuclease-free water for every mg of transferred ground seed material into the sample tube and vortex to mix.

d. Continue to Extract seeds DNA

Extract of ground seeds DNA

1. **Add 45 µl PS1 Buffer and 5 µl PS2 Buffer to the collection tube.** Close the tube and vortex briefly. Make sure the ground seeds is covered by the Extraction Solution.
2. Incubate at 56°C for 10-20 minutes.
3. Incubate at 95°C for 5 minutes.
4. **Add 50 µl PS3 buffer and vortex to mix.**
5. Store the extraction at 2-8°C.

B: Protocol for whole plant seeds.

1. Add 45 ul – 95 ul **PS1 Buffer and 5 µl PS2 Buffer to the collection tube.** Close the tube and vortex briefly. Make sure the ground seeds is covered by the Extraction Solution.
2. Incubate at 56°C for 1 hours.
3. Incubate at 95°C for 5 minutes.
4. **Add 50 -100 µl PS3 Buffer and vortex to mix.**
5. Store the extraction at 2-8°C.

PCR Protocol

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions, such as incubation times, temperatures, and amount of template DNA, may vary and must be individually determined.

1. **Thaw primer solutions.** Keep on ice after complete thawing, and mix well before use.
2. **Mix the PCR Master Mix by vortexing briefly.** It is important to mix the PCR Master Mix before use to avoid localized differences in salt concentration.
3. Prepare one of the following reaction mixes on ice: (For a 25 µl reaction volume)

Component	Volume	Final Concentration
2X PCR Master Mix	12.5 µl	1X
Upstream Primer, 10 µM	0.5 µl	0.1–1.0 µM
Downstream Primer, 10 µM	0.5 µl	0.1–1.0 µM
DNA Template	4 µl	<500 ng
Nuclease-Free Water to		25 µl

4. Gently mix the reaction and spin down in microcentrifuge.
5. **Set up program for a routine PCR reactions:**

Initial Denaturation	94-95°C for 1-5 min
25-40 cycles	94-95°C for 30 sec
	45-70°C for 10-30 sec
	72°C for X min(1min/kb)
Final extension	72°C for 5 min
Final soak	4-10°C

6. For a simplified hot start, proceed as described in step 7. Otherwise, place the PCR tubes in the thermal cycler and start the cycling program.
7. **Simplified hot start:** Start the PCR program. Once the thermal cycler has reached 94°C, place the PCR tubes in the thermal cycler. In many cases, this simplified hot start improves the specificity of the PCR.