quickAmp™ Seed Genomic DNA PCR Kit

Storage
The kit should be stored at −20°C.

Procedure
All steps are performed at room temperature unless otherwise noted.

A. Seeds Grinding

1. Grind using a bead mill.

    a. Place 1 seed into each well of a 2 ml square well block.

    b. Pipette the following volumes of water into the well:

    800 µl for soybean or similar sized seeds.
    600 µl for cotton or similar sized seeds.
    200 µl for canola, sorghum, wheat, or similar sized seeds.
    100 µl 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 minutes. Continue with Section B, Step 1.

2. Grind using a plastic pestle

    a. Place 1 seed into a 1.5 ml microcentrifuge tube.

    b. Pipette the following volumes of water into the tube:

    800 µl for soybean or similar sized seeds.
    600 µl for cotton or similar sized seeds.
    200 µl for canola, sorghum, wheat, or similar sized seeds.
    100 µl for *Arabidopsis* or similar sized seeds.

    c. Incubate the seed for 1 hour at 55°C.

    d. Grind hydrated seeds in tube using a plastic pestle. Continue with Section B, Step 1.

3. Grind using liquid nitrogen.

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

b. Transfer between 5–100 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 µL of water for every mg of transferred ground seed material into the tube and vortex to mix well. Continue with Section B, Step 1.

B. Seeds DNA Extraction

1. Pipette 45 µl of Extraction Solution into a microcentrifuge tube or a multiwell plate well. Add 5 µl of Preparation Solution to the tube or well and pipette up and down to mix.

Note: If several extractions will be performed, sufficient volumes of Extraction Solution and Preparation Solution may be mixed in a ratio of 9:1 up to 2 hours before use.

2. Pipette 5 µl of the ground seed suspension from Section A into the tube or well and vortex to mix.

3. Incubate samples at 55°C for 10 minutes.

4. Incubate samples at 95°C for 3 minutes.

5. Add 50 µl of Neutralization Solution to each sample and mix by vortexing.

6. Store the neutralized seed extract at 4°C or use immediately in PCR. Continue with Section C, Step 1.

C. PCR Amplification

ACTGene *Taq* mastermix, ACTGene Hot-Start *Taq* mastermix, ACTGene Blue/Red *Taq* mastermix, or ACTGene Blue/Red Hot-Start *Taq* mastermix can be used for the following PCR amplification steps. Typical final primer concentrations are approximately 0.4 µM each. Primer concentrations and thermal cycling parameters should be optimized depending on the system.

1. Add 10 µl of PCR mastermix (2x), 4 µl of seed extract, and proper amount of primers to a thin-walled PCR microcentrifuge tube or plate. Bring the total volume to 20 µl with PCR grade water. Gently mix well.

Note: If less than 4 µl of tissue extract is added to the PCR reaction volume, use a 50:50 mixture of Extraction Solution : Neutralization Solution to compensate the volume of seed extract to 4 µl.

2. Perform thermal cycling using optimized amplification parameters.

3. Load the amplified DNA onto an agarose gel after the PCR is completed.