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Protocol for RPA-PCR Couple

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Reagents

TwistAmp Liquid Basic (TwistDx Inc., United Kingdom)
40 mM dNTPs mix (total) (VWR Life Science)
RPA primers
Routine PCR tubes strips with cap strips
Routine PCR Reagents and primers/ probes

Procedure

Design RPA Assay

1) Pick several forwards and reverse RPA primers of 30 to 35 nucleotides length from the region flanking your nucleic acid target.
2) Screen RPA primers to find optimal primer pair using TwistAmp Liquid Basic (TwistDx Inc.) and following related instructions from TwistDx manuals. Keep the nucleic acid template concentration high in the screening process.

Design PCR Assay

3) Design and optimize a PCR assay from your nucleic acid target.

Prepare RPA Reaction Mix (for slower reaction kinetics)

4) Prepare pre-master mix in routine 1.5 mL tube. The recipe for one reaction or 1X is following

2x Reaction Buffer = 25 µL
10x Basic E-mix = 2 µL
40 mM dNTPs (total) = 1 µL
Optimum forward RPA primer (10 µM) = 1.5 µL
Optimum reverse RPA primer (10 µM) = 1.5 µL

Water = 15.21 µL

5) Mix and spin the pre-master mix briefly.
6) Bring 20x Core Reaction Mix to room temperature and pipette mix it.
7) Add 20x Core Reaction Mix to the lid of pre-master mix vial and invert the vial 10 times to make the RPA master mix ready. Keep the volume of 20x Core Reaction Mix at only 1 µL per 1X recipe.

Prepare PCR Reaction Mix
8) Prepare PCR reactions as per manufacturer recommendations.

Crude Maceration
9) Takes plant tissue weighing from 25-100 mg in round bottom 2 ml Eppendorf tube and initially macerate in 100 µL water autoclaved distilled water with a plastic pestle.
10) Add more water to prepare suitable final dilution of macerate such as 1:10 or 1:20 (w/v) tissue weight by volume of water. Vortex and leave for particulate settlement for around one minute.

RPA-PCR couple
11) Pipette 47.21 µL of the RPA master mix to tube strips and add 1 µL macerate supernatant/template and 1.79 µL of 280mM MgOAc in lid strips.
12) Start RPA reaction by inverting tubes (six inverts at least) and incubate tubes at 39 ^{0}C for 20 min, with an agitation step after four minutes. Incubation can be accomplished in PTC-0200 DNA Engine Cycler with no lid heating.
13) After incubation, heat reaction at 85 ^{0}C for 1 min to denature enzymes and then cool at 4 ^{0}C.
14) Transfer 1 µL reaction directly to the PCR and store the rest of the RPA reaction at -20 ^{0}C for future use.
15) Run PCR in any PCR machine, keeping the number of cycles around 30.

Equipment
PTC-0200 DNA Engine Cycler or any other suitable small tubes incubator
Mx3000P QPCR System (Agilent, Germany) or any PCR machine
Routine centrifuges and vortexer

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