Standard Operating Procedure for iSPEED point-of-use real-time PCR

SOP published in:

In Situ Processing and Efficient Environmental Detection (iSPEED) of tree pests and pathogens using point-of-use real-time PCR

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Published: April 2, 2020 • https://doi.org/10.1371/journal.pone.0226863

Material:

- Portable thermocycler Franklin (Biomeme)
- 100 µL PCR tube strips and caps (Applied biosystems, ref 4358293 and ref 432032)
- 20 µL Pastettes (Alpha laboratories, ref LW4730-500)
- 5 mL screw cap tubes (Axygen, SCT-5ML-S)
- Tools to prepare the samples (tweezers, scalpel, punch)
- Tris base (Fisher Scientific, ref BP152-1)
- Ethylenediamine Tetraacetic Acid, Disodium Salt Dihydrate (EDTA) (Fisher Scientific, ref BP120-1)
- NaCL (Fisher Scientific, ref BP358-1)
- Sodium Dodecyl Sulfate (SDS) (Bioshop, ref SDS001-500)
- PolyVinylPolyPirrolidone (PVPP) (Sigma-Aldrich, ref 77627-100G)
- D-(+)-Trehalose dihydrate (Sigma-Aldrich, ref T9531-5G)

- Water (Sigma-Aldrich, ref W4502)
- Mineral Oil (Ward's Science, ref 470108-800)
- QuantiTect Multiplex PCR, noROX kit (Qiagen, ref 204743)
- Suitable TaqMan assays (various manufacturers)

Solutions to prepare:

- Edwards: Tris 200 mM pH 8.0, EDTA 25 mM, NaCl 250 mM, SDS 0.5% (w/v) (+ PVPP 1% (w/v) for plant samples)
- Trehalose 30% (w/v)
- Water aliquots for DNA dilution in 5 mL screw-caps tube (980 µL or 1980 µL)

Method:

1 Prepare lyophilized reactions

- Mix 10 uL Quantitect mastermix with primers and probe (200-500 nM each)
- Add trehalose 30% to reach 5% final
- Freeze to -20°C
- Lyophilize in the dark for 60-90 min

2 DNA extraction/real-time PCR setup

- Depending on sample, place 2.5-20mg of material in an empty 100 µL PCR tube
- Add 40 µL of Edwards buffer to the tube with a clean Pastette, cap and incubate for 10 min at 95°C in the portable thermocycler
- Dilute 20 µL of the DNA extract in pre-aliquoted water in the 5 mL screw-cap tube (980 µL for 1:50, 1980 µL for 1:100) with a clean Pastette

- Mix and distribute 20 µL of the diluted extract to a freeze-dried reaction with a clean Pastette
- Add 40 µL of mineral oil and close the tube with a clean Pastette

3 real-time PCR

- run the reactions with the following conditions:

  95°C for 15 min

  40 cycles of:

  95°C for 15 sec

  60°C for 1min30