Fruit fly molecular diagnostics

REAL-TIME PCR TAQMAN DETECTION OF THE ISLAND FLY, DIRIOXA PORNIA

INTRODUCTION

Tephritid fruit fly species, the Island fly, *Dirioxa pornia* has a wide range of hosts and attack ripe, damaged or rotting and fallen fruits. *D. pornia* belongs to a small group of flies that are morphologically as well as geographically distinct from each other. *D. pornia* is mainly distributed in Australasian-Oceanian (Carroll et al., 2006) whereas the sibling species, *D. fuscipennis*, is restricted to some Polynesian islands, such as Vanuatu (Hancock and Drew, 2003).

D. pornia has been often encountered at New Zealand's borders in citrus imports from Australia as eggs or larvae and can lead to delays in species identification if only using morphological features, thus the real-time PCR protocol targeting the COI gene for rapid identification was developed and validated, which provide rapid detection of those interceptions. The COI gene of *D. pornia* is distinct and easily separated from commonly intercepted fruit flies. The real-time PCR assay for *D. pornia* assay is species-specific, no cross-reaction observed with other fruit fly species tested, such as the related interceptions, *B. tryoni* complex, *B. jarvisi* (Dhami et al. 2016). The routine application of this assay at the New Zealand borders is already easing the quarantine decision making, leading to faster handling and more reliable decision making.

AIM

This assay aims to provide a rapid method for the identification of the *Dirioxa pornia*, from any life stage, using the real-time TaqMan technique.

TARGET

Dirioxa pornia

PROCEDURE

DNA extraction protocols

See DNA extraction page on Fruit Fly Identification website.

Real-time PCR assay for *Dirioxa pornia*

All the real-time PCR setup and analysis are the same as for *B. tryoni* complex (see Real time PCR page on the Fruit Fly Identification Australia website) except the following reagents and steps listed below.

Equipment and material

- Primers and Probe (Dhami *et al.,* 2016)
  - Forward primer: Dpor2F, 5'- CACCAGATATAGCCTTCC -3'
  - Reverse primer: Dpor2R, 5'- TGAGCAATTACAGAGGATAA -3'
  - Probe: Dpor2P, FAM*- ATATAAGTTTCTGACTTCTTCCACCTT-BBQ1
- Positive control to monitor the performance of the real-time PCR
    - DNA samples of *Dirioxa pornia*
    - Plasmid DNA of COI insert of *Dirioxa pornia* (available from MPI PHEL, NZ on request)

Method

- The real-time PCR compositions

Table: TaqMan real-time PCR for D. pornia assay using SsoAdvanced Universal Probe Supermix (BioRad)

Reagents	1 x reaction (µl)[a]	10x reactions (µl) [b]
Sterile distilled H_2O	4.8	48
2 x Probe Supermix	10.0	100
5 µM Dpor2F (200 nM)	0.8	8
5 µM Dpor2R (200 nM)	0.8	8
5 µM Dpor2P (200 nM)	0.8	8
BSA (10mg/ml)	0.8	16
DNA template	2.0	

[a] The compositions for 20 µl are listed in this table, halve the volumes for each reagent if using in 10 µl volume.

[b] Using 10x reaction as an example, calculate the volumes of each reagents using the number of reactions you are going to test when conducting the assay; note, one or two extra reaction volumes should be included to account for loss due to retention of liquid in pipette tips.

Real-time PCR cycling parameters

1 x cycle	95 °C, 2 min
40 x cycles	95 °C, 15 sec
	60 °C, 45 sec
	Plate read after each cycle

ANALYSIS OF REAL-TIME PCR RESULTS

- Interpretation of the results – *D. pornia* assay (use the PCR competent DNA samples only):
    a. For *Cq* values ≤35 cycles, the sample is considered as positive for *D. pornia*,
    b. The negative threshold for the assay is *Cq* values >35, at which point the samples should be considered as negative for *D. pornia*, and identification by another method is necessary.

References: see References page on the Fruit Fly Identification Australia website