# Fruit fly diagnostics

# DIAGNOSTIC METHODS USED TO IDENTIFY FRUIT FLIES

| Scientific name | PCR-RFLP TEST 1 | PCR-RFLP TEST 2 | DNA barcoding | qPCR[1] |
|---|---|---|---|---|
| *Anastrepha curvicauda* | | | 6/6 | |
| *Anastrepha distincta* | | | 0/59[e]* | |
| *Anastrepha fraterculus* | | ✓ | 49/167[e]* | |
| *Anastrepha grandis* | | ✓ | 3/39 | |
| *Anastrepha ludens* | ✓ | ✓[b] | 68/176[e]* | |
| *Anastrepha obliqua* | ✓ | ✓ | 52/134[e]* | |
| *Anastrepha serpentina* | ✓ | ✓ | 8/58* | |
| *Anastrepha striata* | | ✓ | 18/68 | |
| *Anastrepha suspensa* | | ✓[b] | 5/28[e]* | |
| *Bactrocera albistrigata* | ✓ | | 24/24 | |
| *Bactrocera aquilonis* | ✓[a] | ✓[c] | 24/38[f] | ✓ |
| *Bactrocera bryoniae* | ✓ | | 7/11 | |
| *Bactrocera carambolae* | ✓ | ✓ | 108/117 | |
| *Bactrocera caryeae* | | | 2/2[g] | |
| *Bactrocera correcta* | | | 54/228 | |
| *Bactrocera curvipennis* | ✓ | ✓ | 2/2 | |
| *Bactrocera dorsalis s.s.* [2] | ✓ | ✓ | 1075/804[g] | |
| *Bactrocera facialis* | ✓ | ✓ | 1/1 | |
| *Bactrocera frauenfeldi* | ✓ | ✓[d] | 8/16 | |
| *Bactrocera jarvisi* | ✓ | ✓ | 6/7 | |
| *Bactrocera kandiensis* | | | 18/17[g] | |

[1] Real-time PCR

[2] Includes *B. papayae, philippinensis* and *invadens* since their synonymisation with *dorsalis* sensu stricto as per Schutze et al. (2015). This is a taxonomic update of the previous list as opposed to an omission of these species.

| Scientific name | PCR-RFLP TEST 1 | PCR-RFLP TEST 2 | DNA barcoding | qPCR[1] |
|---|---|---|---|---|
| *Bactrocera kirki*[3] | ✓ | ✓[d,3] | 5/5 | |
| *Bactrocera kraussi* | | | 3/32 | |
| *Bactrocera latifrons* | ✓ | ✓ | 83/97 | |
| *Bactrocera melanotus* | | ✓ | 3/3 | |
| *Bactrocera minax* | | | 57/60 | |
| *Bactrocera musae*[4] | ✓ | ✓ | 22/25[5] | |
| *Bactrocera neohumeralis* | ✓[a] | ✓[c] | 4/4[f] | ✓ |
| *Bactrocera obliqua* | | | 0/0 | |
| *Bactrocera occipitalis* | | | 31/30[g] | |
| *Bactrocera oleae* | | ✓ | 108/114 | |
| *Bactrocera passiflorae* | ✓ | ✓ | 0/1 | |
| *Bactrocera psidii* | ✓ | ✓ | 2/2 | |
| *Bactrocera pyrifoliae* | | | 0/0 | |
| *Bactrocera trilineola* | | ✓[d] | 2/2 | |
| *Bactrocera trivialis* | | ✓ | 3/4 | |
| *Bactrocera tryoni* | ✓[a] | ✓[c] | 54/54[f] | ✓ |
| *Bactrocera tsuneonis* | | | 21/21 | |
| *Bactrocera tuberculata* | | | 5/13 | |
| *Bactrocera umbrosa* | ✓ | ✓ | 39/40 | |
| *Bactrocera xanthodes* | ✓ | ✓ | 6/50 | |
| *Bactrocera zonata* | ✓ | ✓ | 38/66 | |
| *Ceratitis capitata* | ✓ | ✓ | 206/244 | |
| *Ceratitis quilicii* | | | 0/5 | |
| *Ceratitis rosa* | ✓ | ✓ | 46/50* | |
| *Dacus longicornis* | | | 2/4 | |
| *Dacus solomonensis* | | ✓ | 0/0 | |
| *Dirioxa pornia* | ✓ | ✓ | 3/3 | |

[3] *B. kirki* can be distinguished from *B. frauenfeldi* and *B. trilineola* using an additional enzyme *Acc* I

[4] The 17 species within the *B. musae* complex (Drew *et al*. 2011) have not been tested by the RFLP method, but DNA barcodes will distinguish *B. musae* s.s. from at least the non-pest species *B. conterminal, B. prolixa, B. rufivitta,* and *B. tinomiscii*; adults of all species can be distinguished morphologically.

| Scientific name | PCR-RFLP TEST 1 | PCR-RFLP TEST 2 | DNA barcoding | qPCR[1] |
|---|---|---|---|---|
| *Drosophila suzukii* | | | 148/1233 | |
| *Rhagoletis cingulata* | | | 30/30 | |
| *Rhagoletis completa* | | ✓ | 21/19* | |
| *Rhagoletis fausta* | | | 7/2 | |
| *Rhagoletis indifferens* | | | 4/4 | |
| *Rhagoletis mendax* | | | 2/2[h] | |
| *Rhagoletis cerasi* | | | 24/41 | |
| *Rhagoletis pomonella* | | ✓ | 25/17[h] | |
| *Zeugodacus atrisetosus* | | | 0/0 | |
| *Zeugodacus cucumis* | ✓ | ✓ | 4/11 | |
| *Zeugodacus cucurbitae* | ✓ | ✓ | 244/502* | |
| *Zeugodacus decipiens* | | | 0/0 | |
| *Zeugodacus depressus* | | | 0/0 | |
| *Zeugodacus tau* | | | 85/136* | |

## EXTRA PCR-DNA BARCODING FOOTNOTES

Species with the same superscript letter cannot be distinguished from each other with DNA barcodes or can only be identified to the level of a species complex.

PCR-RFLP test 1 is best used when the unknown samples may be suspected as one for which the PCR-RFLP test 1 has not been developed; all RFLP patterns available in Armstrong and Cameron (1998) are listed in Restriction enzyme haplotype chart and Diagnostic restriction patterns, and includes other species not listed in this table, *A. albistrigata*, *A. sorocula*, *A. zenildae* and *B. quadriestosa*.

Numbers (x/y) refer to the x number of individuals of that species with publicly accessible DNA (COI) barcodes on the Barcode of Life (BOLD) data system v4 website and y number of total DNA barcodes (i.e. 'Species Level Barcode Records' with a COI minimum sequence length of 500b.p.) available if using the BOLD identification engine; the difference between x and y represents private barcodes unavailable for download as of 10 October 2017 (http://v4.boldsystems.org/).

Where there are no species-specific barcodes available (i.e. 6 x species above), other species in the genus could be used to at least obtain a genus-level identification; as of 10 October 2017, there are 197 *Bactrocera*, 58 *Ceratitis*, 84 *Dacus*, 27 *Rhagoletis*, and 3 *Toxotrypana* (synonymised with *Anastrepha*) species that have DNA barcodes available. The genus *Zeugodacus* is currently not recognised on the BOLD website, with the three *Zeugodacus* species with DNA barcodes above currently present as *Bactrocera* on BOLD.

Species with * are not well resolved amongst a number of other species in BOLD, although low divergence clades may be apparent. Used in conjunction with other information about the specimen this may still be sufficient to make a confident identification. Complimentary identification by ITS1 PCR-RFLP could also be considered.