Fruit Fly ID Australia

Fruit fly molecular diagnostics

AMPLIFICATION OF DIAGNOSTIC LOCI: COI, POP4, EIF3L, RPA2 AND DDOSTS2

MITOCHONDRIAL CYTOCHROME C OXIDASE SUBUNIT I (COI)

The DNA barcode region of the COI gene is PCR amplified using a fruit fly-specific priming system, producing a ~550 bp amplicon for sequencing.

Notes

- Easy to amplify in most specimens/species – the 'gold standard' of DNA barcoding.
- In fruit flies, COI barcoding using universal primers (Folmer et al. 1994) is known to suffer from problems with nuclear encoded mitochondrial pseudogenes (in particular members of the B. tryoni complex), and poor amplification of sub-optimally preserved specimens.
- New dacine-specific COI primers have been designed to overcome these issues (Krosch et al. unpublished). The universal primers of Folmer et al. (1994) were modified to be more dacine-specific: these primers will amplify the full length COI barcode. Additionally, two internal primers have been designed to assist in the amplification of the barcode region for empty pupal cases and sub-optimally preserved samples. The internal primers are used in two separate reactions with the respective modified universal primers.
- Distinguishes most species consistently under phylogenetic analysis, but resolution within some species complexes is poor (e.g. *B. tryoni*, *B. frauenfeldi* complexes).
- Can be used as the sole molecular diagnostic locus, or in combination with other loci.

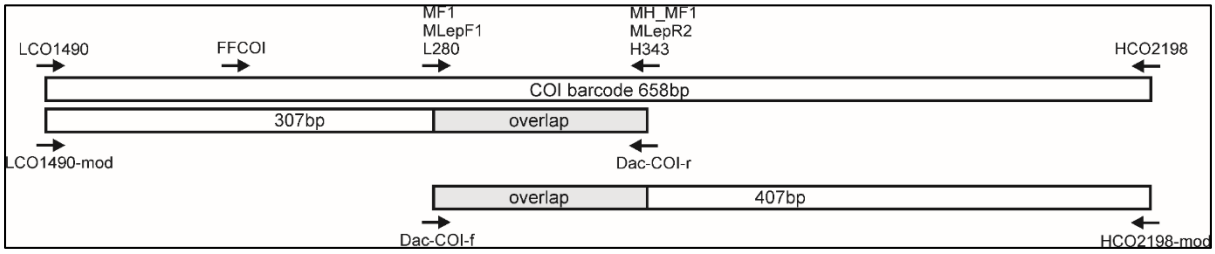

Figure 1. Conceptual diagram of alternative primer designs for the mitochondrial COI barcode, including the 'universal' primers of Folmer et al. (1994) (LCO1490/HCO2198), the modified forward primer of Blacket et al. (2012) (FFCOI), and the novel primer sets presented here (LCO1490-mod, HCO2198-mod, MLepF1-mod, MLepR2-mod).

Download validated COI alignment

PRIMER INFORMATION

Gene	Primer	Sequence	Reference
Dacine specific COI	Forward Primer: LCO1490-modF	5' TYTCAACAAATCATAAAGATATTGG 3'	Krosch et al 2018*
	Reverse Primer: HCO2198-modR	5' TAAACTTCAGGGTGWCCAAARAATCA 3'	Krosch et al 2018*
Internal 1	Forward Primer: LCO1490-modF	5' TYTCAACAAATCATAAAGATATTGG 3'	Krosch et al 2018*
	Reverse Primer: Dac-COI-r	5' GTTCAACCTGTACCVGCYCCGTTTTC 3'	Krosch et al 2018*
Internal 2	Forward Primer: Dac-COI-f	5' GCHTTCCCHCGAATAAATAATA 3'	Krosch et al 2018*
	Reverse Primer: HCO2198-modR	5' TAAACTTCAGGGTGWCCAAARAATCA 3'	Krosch et al 2018*

PCR RECIPE (25 µL REACTION)

Reagents	x1 reaction		
	Dacine COI	Internal 1	Internal 2
dH_20	8 µL	8 µL	8 µL
One Taq	12.5 µL	12.5 µL	12.5 µL
Primers	0.5 µL	0.5 µL	0.5 µL
$MgCl_2$ (25mM)	1 µL	0.5 µL	0.5 µL
BSA (10%)	0.5 µL	1 µL	1 µL
gDNA	2 µL	2 µL	2 µL

PCR PROTOCOLS

Dacine COI		Internal 1		Internal 2	
2 min @ 94°C		2 min @ 94°C		2 min @ 94°C	
30 sec @ 94°C	X35*	30 sec @ 94°C	X 35*	30 sec @ 94°C	X 35*
30 sec @ 52°C		30 sec @ 53°C		30 sec @ 53°C	
45 sec @ 68°C		20 sec @ 68°C		20 sec @ 68°C	
2 min @ 68°C		2 min @ 68°C		2 min @ 68°C	

* It is recommended to increase the number of cycles from 35 to 40+ when working with degraded specimens

NUCLEAR EUKARYOTIC TRANSLATION INITIATION FACTOR 3 SUBUNIT L (EIF3L)

EIF3L is a component of the eukaryotic translation initiation factor 3 (eIF-3) complex, which is required for several steps in the initiation of protein synthesis.

Notes

- Easy to amplify using the protocol below.
- Distinguishes most species consistently under phylogenetic analysis, but resolution within some species complexes is poor (e.g. *B. tryoni*, *B. frauenfeldi* complexes).
- Can be used as the sole molecular diagnostic locus, or in combination with other loci.

Download validated EIF3L alignment

PRIMER INFORMATION

Primer	Sequence
Forward: EIF3L-f	5' CCCAAGGAAAYGATCCYCAA 3'
Reverse: EIF3L-r	5' GCTGACGCACTTCATCCATA 3'

PCR RECIPE (25 µL REACTION)

Reagent	x1 reaction
dH_20	7.75 µL
One Taq	12.5 µL
Primers	0.5 µL
$MgCl_2$ (25mM)	1 µL
BSA (10%)	0.75 µL
gDNA	2 µL

PCR PROTOCOL

PCR protocol	
2 min @ 94°C	
30 sec @ 94°C	
30 sec @ 52°C	X 34
30 sec @ 68°C	
5 min @ 68°C	

NUCLEAR RIBONUCLEASE P PROTEIN SUBUNIT P29 (POP4)

POP4 is a part of a protein complex that generates mature tRNA molecules by cleaving their 5′ ends.

Notes

- Distinguishes most species consistently under phylogenetic analysis, but resolution within some species complexes is poor (e.g. *B. tryoni*, *B. frauenfeldi* complexes).
- Substantial sequence differences recorded between *Zeugodacus*/ *Dacus* and *Bactrocera*. Genus-specific primers are used for targeted PCRs where a genus-level morphological identification has been made.
- When analysed as a single data set, genus-level differences can obscure relationships at the tips of the tree. Recommended to analyse each genus on its own with an appropriate outgroup.
- This locus is sometimes difficult to amplify and sequence cleanly in some taxa, especially *B. dorsalis* complex.
- Can be used as the sole molecular diagnostic locus, or in combination with other loci.

Download validated POP4 alignment

PRIMER INFORMATION

Gene	Primer	Sequence
POP4 *(Bactrocera)*	Forward: POP4-f	5′ ACATTACAATGTTGGAAGGGGG 3′
	Reverse: POP4-r-Bac	5′ TTGACGCTGCGCTCTGCGCT 3′
	*Reverse: POP4-r	5′ CTTYAYCTTYTTGACGCTGCG 3′
POP4 *(Zeugodacus/Dacus)*	Forward: POP4-f-Zeu	5′ GAACACATTACCATGTTGGA 3′
	Reverse: POP4-r-Zeu	5′ GACACTGCGCTCTGCGGGACG 3′

PCR RECIPE (25 µL REACTION)

Reagent	x1 reaction		
	POP4 *(Bactrocera)* POP4-r-Bac	*POP4 *(Bactrocera)* POP4-r	POP4 *(Zeugodacus/Dacus)*
dH_20	7.5 µL	6 µL	8 µL
One Taq	12.5 µL	12.5 µL	12.5 µL
Primers	0.5 µL	0.75 µL	0.5 µL
$MgCl_2$ (25mM)	1 µL	2 µL	0.5 µL
BSA (10%)	1 µL	1 µL	1 µL
gDNA	2 µL	2 µL	2 uL

PCR PROTOCOLS

POP4 *(Bactrocera)* POP4-r-Bac		*POP4 *(Bactrocera)* POP4-r		POP4 *(Zeugodacus)*	
2 min @ 94°c		2 min @ 94°c		2 min @ 94°c	
30 sec @ 94°C	X 38	30 sec @ 94°C	X 38	30 sec @ 94°C	X 35
30 sec @ 53°C		30 sec @ 50°C		30 sec @ 55°C	
30 sec @ 68°C		30 sec @ 68°C		30 sec @ 68°C	
5 min @ 68°C		5 min @ 68°C		5 min @ 68°C	

*Only use *Bactrocera* POP4-r primer to amplify problematic samples – primarily species within the dorsalis complex.

NUCLEAR REPLICATION PROTEIN A 32 KDA SUBUNIT (RPA2)

RPA2 is part of the heterotrimeric replication protein A complex (RPA/RP-A), which binds and stabilises single-stranded DNA intermediates that form during DNA replication or upon DNA stress.

Notes

- Easy to amplify using the protocol below.
- Distinguishes most species consistently under phylogenetic analysis, but resolution within some species complexes is poor (e.g. *B. tryoni*, *B. frauenfeldi* complexes).
- Not recommended for use as the only molecular diagnostic locus – accuracy is improved in concatenated analyses with other loci.

Download validated RPA2 alignment

PRIMER INFORMATION

Primer	Sequence
Forward primer: RPA2-f	5' ACAAATCCTATATTCGCBTGAGGG 3'
Reverse primer: RPA2-r	5' AATTTTTDTTGCAAYTCTTTGCGG 3'

PCR RECIPE (25 µL REACTION)

Reagent	x1 reaction
dH_20	7 µL
One Taq	12.5 µL
Primers	0.5 µL
$MgCl_2$ (25mM)	1.5 µL
BSA (10%)	1 µL
gDNA	2 µL

PCR PROTOCOL

PCR Protocol	
2 min @ 94°C	
30 sec @ 94°C	X 33
30 sec @ 53°C	
45 sec @ 68°C	
5 min @ 68°C	

NUCLEAR DOLICHYL-DIPHOSPHOOLIGOSACCHARIDE-PROTEIN GLYCOSYLTRANSFERASE SUBUNIT 2 ISOFORM X2 (DDOSTS2)

Essential subunit of the N-oligosaccharyl transferase (OST) complex which catalyses critical reactions between sugars, peptides and lipids in the endoplasmic reticulum.

Notes

- Easy to amplify using the protocol below.
- Distinguishes most species consistently under phylogenetic analysis, but resolution within some species complexes is poor (e.g. *B. tryoni*, *B. frauenfeldi* complexes).
- Not recommended for use as the only molecular diagnostic locus – accuracy is improved in concatenated analyses with other loci.

Download validated DDOSTs2 alignment

PRIMER INFORMATION

Primer	Sequence
Forward: DDOSTs2-f	5' GTGGCAGATCGTGTTGAAGA 3'
Reverse: DDOSTs2-r	5' GAGTATTATCGGCCTTTAAAGTTCC 3'

PCR RECIPE (25 µL REACTION)

Reagent	x1 reaction
dH_20	7 µL
One Taq	12.5 µL
Primers	0.5 µL
$MgCl_2$ (25mM)	1.5 µL
BSA (10%)	1 µL
gDNA	2 µL

PCR PROTOCOL

PCR protocol	
2 min @ 94°C	
30 sec @ 94°C	
30 sec @ 53°C	X 33
45 sec @ 68°C	
5 min @ 68°C	