

Diptera-Specific Polymerase Chain Reaction Amplification Primers of Use in Molecular Phylogenetic Research

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ABSTRACT DNA sequence data from a variety of mitochondrial and nuclear gene regions are significant components of phylogenetic research in entomology. Polymerase chain reaction (PCR) amplification primers for many gene regions have been developed that are specific to a range of dipteran groups. Here, we review the existing Diptera-specific PCR amplification primers that have been published for 11 mitochondrial and nuclear gene regions: 12S small ribosomal subunit, cytochrome *b*, cytochrome oxidase *c* subunit I, 28S ribosomal RNA, alanyl-tRNA synthetase, the carbamoyl phosphate synthase region of CAD, elongation factor-1 α , 6-phosphogluconate dehydrogenase, triose phosphate isomerase, *white*, and *wingless*. We also have designed in total 94 new PCR amplification primers for use in these same gene regions. Our new primers have been developed and tested using our DNA sequence database of >1,600 specimens representing 40 families of Diptera. All of the past and newly developed primer sequences are presented in tables, and their locations are shown on gene maps. This combined data will facilitate future molecular phylogenetic research within Diptera.

KEY WORDS gene maps, phylogenetics, mitochondrial DNA, nuclear DNA, ribosomal DNA

The most recent review of published, DNA sequence-based, phylogenetic studies of insects (Caterino et al. 2000) lists a large number of studies using a broad range of target taxa and gene regions. In the time since the publication of this review, many more papers have been published that use many different gene regions to study relationships between a variety of taxa. Although some of these studies include the development of new, taxon-specific polymerase chain reaction (PCR) amplification primers, many rely on existing, published primers. These existing primers, however, may not be appropriate for the taxa being investigated and may lead to inefficiency or sequencing failure.

Generally, the primers used to generate DNA sequence data were developed for use in groups of insects other than those that are the focus of the new study. For these primers to be of use in sequencing taxa that have never before been sequenced, universal primers are a necessity. Universality takes the form of oligonucleotide degeneracy or an acceptable level of oligonucleotide mismatch. Both situations can make the amplification and sequencing of target gene regions extremely difficult, if not impossible. Degenerate primers can produce nonspecific amplification,

multiplex PCR product, and the necessity of isolating the desired PCR product before sequencing. Although new techniques exist (Ma and DiFazio 2008, Gibson et al. 2010a) to facilitate the isolation of desired PCR products from multiplex PCR products, these methods require additional time and money. Also, when degeneracy is included in a primer sequence, each different possible version of the primer sequence is produced and included in the manufactured product. This can lead to an exponential increase in the number of primer sequences present in the PCR reaction. For example, a manufactured primer oligonucleotide sequence containing four N's and three Y's would actually consist of 2,048 different nucleotide sequences, only one of which would match the genomic template DNA sequence. This leads not only to a greatly reduced concentration of the correctly matching primer but also a disruption in reaction kinetics as genomic template primer locations are blocked by poorly matching versions of the primer. Primers with less degeneracy, but developed for distantly related groups, often lead to sufficient nucleotide mismatch to result in amplification failure.

Another consideration is the condition of the specimen from which DNA is being extracted. In molecular phylogenetic research, the specimens being used may not have been prepared or stored under optimal conditions. These conditions may have led to degradation and fragmentation of genomic DNA. In these instances, a number of alternate primer pairs may be necessary to amplify and sequence the target gene region in smaller segments.

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Table 1. Number of specimens of each Diptera family included in data set used to create new PCR amplification primers for each gene region

Family	Gene region										
	12S	Cytb	COI	28S	AATS	CAD	EF-1 α	PGD	TPI	<i>white</i>	<i>wingless</i>
Atelestidae	1	0	1	1	0	1	0	0	0	0	0
Bombyliidae	1	0	1	1	0	1	0	0	0	0	0
Brachyostomatidae	1	0	1	1	1	1	1	1	1	1	1
Conopidae	71	65	69	71	66	11	6	8	8	9	10
Curtonotidae	1	0	1	1	0	1	0	0	0	0	0
Cypselosomatidae	1	0	1	1	0	1	0	0	0	0	0
Diopsidae	4	0	3	4	3	4	4	3	4	2	4
Dolichopodidae	1	0	1	1	0	1	0	0	0	0	0
Drosophilidae	1	1	1	1	1	1	1	1	1	1	1
Empididae	1	0	1	1	0	1	0	0	0	0	0
Heleomyzidae	1	1	1	1	1	0	0	0	0	0	0
Hybotidae	1	0	1	1	0	1	0	0	0	0	0
Ironomyiidae	1	0	1	1	0	1	0	0	0	0	0
Lauxaniidae	2	2	3	2	2	2	2	1	1	2	2
Lonchopteridae	2	1	2	2	1	2	1	1	1	1	1
Marginidae	1	0	1	1	0	1	0	0	0	0	0
Megamerinidae	1	0	1	1	1	1	1	1	1	1	1
Micropezidae	46	1	38	13	5	42	11	5	5	4	35
Muscidae	2	1	2	2	1	2	0	0	1	1	1
Neriidae	1	0	1	1	1	1	1	1	1	1	1
Opetiidae	1	0	1	1	0	1	0	0	0	0	0
Palloppteridae	1	1	1	1	1	1	1	1	0	1	1
Phoridae	3	1	3	3	1	3	1	1	1	0	1
Pipunculidae	88	42	80	2	44	78	1	1	1	1	1
Platypezidae	2	1	2	2	1	2	1	1	1	0	1
Platystomatidae	1	1	1	1	1	1	1	1	1	1	1
Psilidae	2	0	2	2	2	2	2	1	1	2	2
Pyrgotidae	1	1	1	1	0	1	1	1	0	1	1
Richardiidae	1	0	1	1	0	1	0	0	0	0	0
Sciadoceridae	1	0	1	1	0	1	0	0	0	0	0
Sciomyzidae	1	0	1	1	0	1	0	0	0	0	0
Somatiidae	1	0	1	1	1	1	1	1	1	1	1
Sphaeroceridae	26	22	24	23	18	2	1	0	1	1	1
Strongylophthalmyiidae	2	1	2	2	2	2	2	1	2	2	2
Syringogastridae	1	0	0	1	1	1	1	1	1	1	1
Syrphidae	44	39	44	40	32	39	1	1	1	1	1
Tachinidae	1	0	1	1	0	1	0	0	0	0	0
Tanypezidae	1	0	1	1	1	1	1	1	1	1	1
Tephritidae	1	1	1	1	1	1	1	1	0	0	0
Therevidae	1	0	1	1	0	1	0	0	0	0	0
Total no. specimens included	319	182	298	193	189	215	44	35	36	36	72
Total no. families included	40	17	39	40	24	39	23	22	21	21	23

Due to the potential problems introduced by overly degenerate or mismatched primers and the necessity for primers located throughout the length of target gene regions, a variety of taxon-specific primers are essential. In the case of Diptera, many primers are used time and time again despite the fact that they were designed originally for use in nondipteran taxa. These primers are often not adequate to amplify and sequence target gene regions in dipteran taxa, leading to considerable frustration and waste. Diptera-specific primers for a variety of gene regions are needed to reduce time and effort spent and to increase sequencing success in molecular phylogenetic research.

Our purpose is to review unique PCR amplification primers that have been developed and published as part of research on Diptera phylogenetics. We have chosen eleven gene regions that include the most commonly sequenced gene regions as well as some that have only recently been developed. These gene regions are small ribosomal subunit (12S), cytochrome *b* (Cytb), cytochrome oxidase *c* subunit I

(COI), 28S ribosomal RNA (28S), alanyl-tRNA synthetase (AATS), the carbamoyl phosphate synthase region of CAD (CAD), elongation factor-1 α (EF-1 α), 6-phosphogluconate dehydrogenase (PGD), triose phosphate isomerase (TPI), *white*, and *wingless*. To the list of existing primers, we seek to add our own newly designed primers that will provide further options to future researchers that wish to amplify a given gene region for their own dipteran target taxa. We intend to provide sufficient primer alternatives, both old and new, such that any of these eleven gene regions could be amplified and sequenced in any future molecular phylogenetic research involving Diptera.

Materials and Methods

We surveyed the scientific literature to identify unique PCR amplification primers developed as a part of phylogenetic research involving Diptera. Primers that were different from past primers by at least one nucleotide were included in both primer tables and

Table 2. PCR amplification primers developed to amplify the 12S gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
F14029	ATTTAATAAACSCGTGATACAC	Oliveira et al. (2005)	13897	F (J)	21	<i>Drosophila</i> (Drosophilidae)
S12A	CATTCTAGATACACTTTCCAGT	Han and Ro (2002)	14176	F (J)	22	Tephritoidea
DRMT2279N	GTCATTCTAGATACACTTTCCAGTAC	Jenkins et al. (1996)	14178	F (J)	26	<i>Drosophila</i> (Drosophilidae)
SR-J14197	TACATCTACTATGTTACGACTT	Simon et al. (2006) ^a	14197	F (J)	22	Universal
SR-J-14199	TACATCTACTATGTTACGACTTAT	Kambhampati and Smith (1995)	14199	F (J)	24	Universal
SR-N14220	ATATGTACACATCGCCCGTC	Simon et al. (2006) ^a	14220	R (N)	20	Universal
12Sbi	AAGAGCGACGGCGGATGTGT	Simon et al. (1994) ^a	14233	F (J)	20	Universal
12Sc	AAGGTGGATTTGGTAGTAAA	Simon et al. (1994) ^a	14294	R (N)	20	Universal
S12F	CTACACCTTGATCTGATATA	Han and Ro (2002)	14382	F (J)	20	Tephritoidea
DRAT3S	GTAATCGATAATCCACGATGGACC	Jenkins et al. (1996)	14492	R (N)	24	<i>Drosophila</i> (Drosophilidae)
12Sj	TACAAAACAGGTCCCTCTG	Simon et al. (1994) ^a	14508	F (J)	18	Universal
12S-Dipt-14525R	CGGTATTTTAKTCTIDTYCAGAGG	New	14525	R (N)	23	Brachycera
12Se	ACTTAAAAAATTGGCCGCT	Simon et al. (1994) ^a	14540	R (N)	19	Universal
DRAT2S	CTAGGATTAGATACCCCTATTA	Jenkins et al. (1996)	14609	R (N)	20	<i>Drosophila</i> (Drosophilidae)
SR-J14610	ATAATAGGGTATCTAATCCTAGT	Simon et al. (2006) ^a	14610	F (J)	23	Universal
12Sai	AAACTAGGATTAGATACCCCTATTAT	Simon et al. (1994) ^a	14612	R (N)	25	Universal
12Sair	AGGCTTCTAATCCTAGTTT	Simon et al. (1994) ^a	14612	F (J)	20	Universal
A12C	CTAGGATTAGATACCCCTATTAT	Han and Ro (2002)	14612	R (N)	22	Tephritoidea
SR-N-14594	AAACTAGGATTAGATACCC	Kambhampati and Smith (1995)	14612	R (N)	19	Universal
R14735	AWAAACTAGGATTAGATACCC	Oliveira et al. (2005)	14614	R (N)	21	<i>Drosophila</i> (Drosophilidae)
DRAT1S	AAAAGAAAATTGAATTTATTAGTG	Jenkins et al. (1996)	14696	R (N)	25	<i>Drosophila</i> (Drosophilidae)
SR-N14745	GTGCCAGCAGTCGGGTTATAC	Simon et al. (2006) ^a	14745	R (N)	22	Universal
DRMT1653S	GGTGCCAGCAGTCGGGTTA	Jenkins et al. (1996)	14768	R (N)	20	<i>Drosophila</i> (Drosophilidae)
12S-Dipt-14771R	GCTGCCAGCAGTYGCCG	New	14771	R (N)	17	Brachycera
12Sh	GACCAAAATGGTGCCACAGT	Simon et al. (1994) ^a	14776	R (N)	21	Universal
12Sz	AGTATTGGTAAAATTTGTGCCAGC	Moulton (2000)	14779	R (N)	24	Simuliidae
A12DD	TTTATATGTAATTTTTGTGTG	Han and Ro (2002)	14880	R (N)	22	Tephritoidea
12Sgi	AAGTTTTATTTTGGCTTA	Simon et al. (1994) ^a	14939	R (N)	18	Universal
A12X	TAAAGTTTTATTTTGGCTT	Han and Ro (2002)	14942	R (N)	20	Tephritoidea

The 3' location is based on published *D. yakuba* sequence (Clary and Wolstenholme 1985). Direction F, forward; R, reverse (J, majority; N, minority as per Simon et al. 1994). Sequences in bold are newly developed for this study.

^a Sequences from Simon et al. (1994, 2006) are those matching *Drosophila* without any degeneracy.

gene maps for each gene region. We noted primers that were developed to be species-specific sequencing primers but did not include them in primer tables or maps. In a very few cases, we included primers that were developed as part of non-Diptera research, but that have been used extensively in Diptera molecular phylogenetics.

We also analyzed 1619 DNA sequences obtained for eleven gene regions (Table 1). These sequences were obtained as a part of ongoing phylogenetic research on a number of families of Diptera. The sequence data set included representatives of 40 families of Diptera from across Brachycera (Table 1). Although several other gene regions (e.g., 16S ribosomal DNA, 18S ribosomal DNA, cytochrome oxidase *c* subunit II, and the internal transcribed spacers I and II) have been used in phylogenetic research in the past, they are not included in the current study. We did not have sufficient numbers of sequences from these gene regions in our database to generate alignments and new primer sequences.

All DNA sequences from all fly families available (Table 1) for a given gene region were compiled into a single alignment. Using these alignments, we located small nucleotide sequences that were conserved across the diversity of the sequences included. These candidate primer locations are exact matches, when degenerate sites are included, for all of the taxa in-

cluded in the alignment. We sought to develop primers that are as Brachycera-specific as possible with a minimum of degeneracy. Overall, we also sought to locate potential primers that would, in combination with other new or existing primers, allow amplification and sequencing of each gene region in 500–1,000-bp segments.

Naming of all of the primers we have developed follows a common convention: an abbreviation of the name of the gene region amplified, followed by "Dipt" for Diptera-specific, followed by a location number corresponding to the 3'-most base of the primer compared with a published DNA sequence, followed by F or R for forward or reverse primers, respectively (e.g., 28S-Dipt-3385 F). Although we have not adopted the J and N naming system suggested by Simon et al. (1994), we do include J and N designations in our tables for primers of mitochondrial gene regions. The DNA sequence used to determine the location number within each name varies with the gene region in question.

Results and Discussion

Existing and newly developed primers for each gene region are summarized in Tables 2–12. In each table, the name as provided in the original reference, sequence, length, direction, and original reference is

Table 3. PCR amplification primers developed to amplify the *Cytb* gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
CB-J-10612	CAAATTAATATTTCAAGATGATGAAA	Simon et al. (1994) ^a	10612	F (J)	26	Universal
CB-J-10612	AAAAAGCTTCCATCCAAATCTCAGCATGATGAAA	Gasparich et al. (1995)	10612	F (J)	35	<i>Ceratitis</i> (Tephritidae)
CB-J-10621	TCAAGATGATGAAAATTTTGGATC	Simon et al. (2006) ^a	10621	F (J)	24	Universal
CB-N10608	CAAAGTAATGATCCAAAATTTCA	Simon et al. (2006) ^a	10630	R (N)	23	Universal
CB-N-10671	AGACGACACATTACATATCG	Gasparich et al. (1995)	10690	R (N)	20	<i>Ceratitis</i> (Tephritidae)
CYT BF2	TACCATGAGGWCAAATATCATWTTGAG	Lyman et al. (1999)	10916	F (J)	27	Universal
CYT BF1	GGTCAAATATCATTTTGGAGKAGCWACWG	Lyman et al. (1999)	10923	F (J)	28	Universal
CYT BF	GGACAAATATCATTTTGGAGGACCAACAG	Lyman et al. (1999)	10923	F (J)	28	Universal
CB-J-10933	TATGTTTTACCTTGAGGACAAATATC	Simon et al. (1994) ^a	10933	F (J)	26	Universal
CB-J10933	GTTTTACCTTGAGGACAAATATC	Simon et al. (2006) ^a	10933	F (J)	23	Universal
CB-N-10920	TCCTCAAAATGATATTTGCTCTCA	Simon et al. (1994) ^a	10943	R (N)	24	Universal
CB-N-10920	AACTCGAGCCCCTCAGAATGATATTTGCTCTCA	Gasparich et al. (1995)	10943	R (N)	34	<i>Ceratitis</i> (Tephritidae)
CB-N11010	TATCTACAGCAAATCTCTCTCA	Simon et al. (2006) ^a	11010	R (N)	22	Universal
Cytb-9F	ATGAATTTGAGGGGATTTG	Rao et al. (2006)	11022	F (J)	20	Tipulidae
CytB-Dipt-11035F	GGNTTYKCNCTNGAYAAAYGC	New	11035	F (J)	20	Brachycera
CBsunA	AATGTTACAAGAATTC	Dusfour et al. (2004)	11047	F (J)	17	<i>Anopheles</i> (Culicidae)
CytB-Dipt-11074F	CGATTTTYACHITTYCAITTYATYHTNC	New	11074	F (J)	29	Brachycera
SunSS	TATCATTCTGAGGAGCC	Dusfour et al. (2007)	11143	R (N)	17	<i>Anopheles</i> (Culicidae)
CytB-Dipt-11146F	GGNTCHAAAYAYCNAATNGG	New	11146	F (J)	20	Brachycera
SunE	ATGATTTTTACCAATTTTC	Dusfour et al. (2007)	11230	R (N)	19	<i>Anopheles</i> (Culicidae)
CBsunB	TTAGCTATACATTTATC	Dusfour et al. (2004)	11296	R (N)	17	<i>Anopheles</i> (Culicidae)
Cytb-308R	AGAGCGTTAGCTGGGATAAA	Rao et al. (2006)	11302	R (N)	20	Tipulidae
Forward 11226	GAATGATATTTTTTATTTGC	Hodgkinson et al. (2002)	11328	F (J)	20	Psychodidae, Culicidae
CB-N-11328	AGCAAATAAAAAATATCATTC	Simon et al. (1994) ^a	11328	R (N)	21	Universal
CB3-PDR	CAYATTC AACCCWGAATGATA	Ready et al. (1997)	11335	F (J)	20	Psychodidae
CB-J11335	CACATTC AACCCAGAATGATA	Simon et al. (2006) ^a	11335	F (J)	20	Universal
CB-J11335	CACATTC AACCCAGAATGATATTT	Simon et al. (1994) ^a	11338	F (J)	23	Universal
CB-N-11367	ATAACTCTCTCTAATTTATTAGGAAT	Simon et al. (1994) ^a	11367	R (N)	26	Universal
PDR-WF01	CTTCGTTCTATTCCTAAT	Hall et al. (2001)	11375	F (J)	18	<i>Chrysomya</i> (Calliphoridae)
CYT BR1	ATTTATTAGCAATWGATCGTAAAATWG	Lyman et al. (1999)	11379	R (N)	27	Universal
CytB-Dipt-11389R	ACTCCYCCARITTRITDGG	New	11389	R (N)	20	Brachycera
CYT BR	ATTAATCTCCTAGCTTATTTAGGAATTC	Lyman et al. (1999)	11392	R (N)	28	Universal
CB3-R3A	GCTATTAATCCYCTAACTTRIT	Essegheir et al. (2000)	11395	R (N)	23	Psychodidae
CYT BR2	ATTTGATATAACTAAWGCAATWACTCCTCC	Lyman et al. (1999)	11411	R (N)	30	Universal
CB-N11526	TTCAACTGGTCCGACTCCAATTC	Simon et al. (2006) ^a	11526	R (N)	24	Universal
CB-J11545	ACATGAATGGAGCTCGCAACTG	Simon et al. (1994) ^a	11545	F (J)	23	Universal
CytB-Dipt-11545R	ACDGGDCDGGCYCCRATTC	New	11545	R (N)	20	Brachycera
PDR-WF03	GCACGACCTGTAGAAGA	Hall et al. (2001)	11551	F (J)	17	<i>Chrysomya</i> (Calliphoridae)
PDR-WR02	GGCTCTTCAACAGTCCG	Hall et al. (2001)	11554	R (N)	17	<i>Chrysomya</i> (Calliphoridae)
CytB-Dipt-11554R	GGRTBTTCADCDGGNCC	New	11554	R (N)	17	Brachycera
PDR-WR04	ATTTCCAGCTACTTAACT	Hall et al. (2001)	11675	R (N)	18	<i>Chrysomya</i> (Calliphoridae)
TS1-N-11683	AAATTCTATCTTATGTTTTTCAAAC	Simon et al. (1994) ^a	11683	R (N)	25	Universal
Reverse 11587	CITATGTTTTTCAAGACATATGC	Hodgkinson et al. (2002)	11699	R (N)	22	Psychodidae, Culicidae

The 3' location based on published *D. yakuba* sequence (Clary and Wolstenholme 1985). Direction F, forward; r, reverse (J, majority; N, minority as per Simon et al. 1994). Sequences in bold are newly developed for this study.

^a Sequences from Simon et al. (1994, 2006) are those matching *Drosophila* without any degeneracy.

included for each existing primer. We have also included our newly developed primers, named according to our naming system. We have calculated the location of each primer based on a previously published gene region map. For each primer, existing or newly developed, we have given an approximation of the breadth of taxa for which the primer was developed. In existing primers, this is based on the information given in the original reference and ranges from genus-specific to universal (i.e., primers developed for use in Diptera plus other Insecta). For new primers, the breadth of taxa is determined by the diversity of specimens included in the sequence database used to develop the primers (Table 1). All newly developed primers, except two developed for *wingless*, are developed to be useful across all Brachycera.

12S. In their compendium of primers useful in amplifying animal mitochondrial DNA, Simon et al. (1994) included eight unique primers that they had matched with the 12S region of the published *Drosophila yakuba* Burla sequence (Clary and Wolsten-

holme 1985). Four more primers were added in their later animal mitochondrial compendium (Simon et al. 2006). Two modified primers were designed for use across insects and ticks (Kambhampati and Smith 1995). Five unique 12S primers were designed for use in a population dynamics study of *Drosophila* (Drosophilidae) (Jenkins et al. 1996). One additional primer was developed as part of a study of the phylogenetics of Simuliidae (Moulton 2000). Five unique 12S primers were developed in a study on the phylogeny of Tephritoidea (Han and Ro 2005). Oliveira et al. (2005) designed two more primers for use in a study of a *Drosophila* species complex.

We have developed two new 12S primers. The naming of our new primers is based on the published mitochondrial genome of *D. yakuba* (Clary and Wolstenholme 1985). In total, 29 primers are listed and mapped (Fig. 1; Table 2). The entire length of the 12S gene region (≈ 800 bp) can be sequenced using existing primers. The actual length of segments amplified

Table 4. PCR amplification primers developed to amplify the COI gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
UEA1	GAATAATTCOCATAAATAGATTTACA	Lunt et al. (1996)	1438	F (J)	26	Universal
TY-N-1438d	GAAWAATTCOCYATAAWTARATTTACA	Zhang and Hewitt (1996)	1438	F (J)	26	Universal
CCOIF	GTCTATTGCCTAAACTTCAGCC	Chen et al. (2004)	1460	F (J)	22	Calliphoridae
TY-J-1460B	TATCGCCTAAACTTCAGCC	Lonsdale et al. (2010)	1460	F (J)	19	Clusiidae
primer 1	TACAATTTATGCGCTAAACTTCAGCC	Sperling et al. (1994)	1460	F (J)	26	Calliphoridae
TY-J-1460	TACAATCTATGCGCTAAACTTCAGCC	Bernasconi et al. (2000b)	1460	F (J)	26	Muscoidea
TY-J-1461	TTTACARITTTACGGCCTATTRTCAGCCA	Winkler et al. (2009)	1461	F (J)	28	Agromyzidae
COI-1	ATCGCCTAAACTTCAGCCAC	Wang et al. (2006)	1462	F (J)	20	<i>Drosophila</i> (Drosophilidae)
L1 CDHIM	TGCGCTAAACTTCAGCCATT	Virgilio et al. (2009)	1463	F (J)	20	<i>Dacus</i> (Tephritidae)
COI-F1	CGCCTAAACTTCAGCCACTT	He et al. (2009)	1464	F (J)	20	Drosophilidae
LC01490-L	GGTCWACWAATCATAAAGATATTGG	Nelson et al. (2007)	1514	F (J)	25	Universal
LC01490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)	1514	F (J)	25	Universal
911	TTTCTACAAATCATAAAGATATTGG	Guryev et al. (2001)	1514	F (J)	25	Chironomidae
COX1F	TCAACWAATCATAAAGATATTGG	Sayar et al. (2009)	1514	F (J)	23	Tephritidae
L1440d	TYTCAACWAATCATAAARGATATTGG	Van Houdt et al. (2010)	1514	F (J)	25	Tephritidae
Uni Minibar F	TCCACTAATCACAARGATATTGGTAC	Meusnier et al. (2008)	1517	F (J)	26	Universal
Cl-J-1535	ATTGGAACTTTATATTTTATATTGG	Scheffer and Wiegmann (2000)	1535	F (J)	26	Agromyzidae
Cl-N-1560	TCTTCTACTATTCGGCTCA	Simon et al. (1994) ^a	1540	R (N)	21	Universal
COI1532	TYTGGAGCTTGATCGGNGATA	Ekrem (2006)	1551	F (J)	21	Tanytarsini (Chironomidae)
Cl-J-1632	TGATCAAATTTATAAT	Kambhampati and Smith (1995)	1632	F (J)	16	Universal
Uni Minibar R	GAAAATCATAATGAAGGCATGAGC	Meusnier et al. (2008)	1668	R (N)	24	Universal
COI-Dipt-1682F	ATTTYTYTATRTGNATRCC	New	1682	F (J)	20	Brachycera
COI-Dipt-1703F	CCHRHTATRTATYGGWGNNTYGG	New	1703	F (J)	23	Brachycera
Cl-N-1687	CAATTTCCAAATCTCCAATTAT	Wells and Sperling (1999)	1709	R (N)	23	<i>Chrysomya</i> (Calliphoridae)
Cl-J1709	AATTGGGGGGTTTGGAAATTG	Simon et al. (2006) ^a	1709	F (J)	21	Universal
Cl-J-1709	ATAATTGGAGGATTTGGAAATTG	Wells and Sperling (1999)	1709	F (J)	23	<i>Chrysomya</i> (Calliphoridae)
Cl-J-1718mod	GGAGGATTTGCAAAATTCATTACT	Dallas et al. (2003)	1715	F (J)	23	Universal
Cl-J-1718	GGGGGGTTTGGAAATTCATTACTGCGC	Simon et al. (1994) ^a	1718	F (J)	26	Universal
forward	GGATTTGCAAAATTCATTACTCCTT	Pradeep Kumar et al. (2007)	1720	F (J)	25	Culicidae
Cl-N1738	TTTATTCGTGGGAATGCTATGTC	Simon et al. (2006) ^a	1738	R (N)	23	Universal
COI-Dipt-1751R	GGRAADGCVATRACWGGDCMHCC	New	1751	R (N)	23	Brachycera
Cl-J-1751 (alias Ron)	GGAGCTCCTGCATCAGATTCC	Simon et al. (1994) ^a	1751	F (J)	23	Universal
Cl-J-1751b	GGATCCCCTGATATAGCYTTTCC	Wells and Sperling (1999)	1751	F (J)	23	<i>Chrysomya</i> (Calliphoridae)
UEA3	TATAGCATTCGCCAGATAAATAA	Lunt et al. (1996)	1763	F (J)	24	Universal
COI-Dipt-1769F	GCHTYCCNCGNATRAAAYAYATRAG	New	1769	F (J)	26	Brachycera
af281	CGAATAAAATATAAGATTGTA	Song et al. (2008)	1776	F (J)	24	Sarcophagidae
L280d	CGAATAAAATATAAGATTGTTGAYT	Van Houdt et al. (2010)	1778	F (J)	26	Tephritidae
L280	CGAATAAAATATAAGATTGTTGATTA	Van Houdt et al. (2010)	1779	F (J)	27	Tephritidae
Lc/HL-S395F	GTTTACCTCTGCGATTAACITTA	Chen et al. (2004)	1800	F (J)	23	Calliphoridae
H343	CCAGCTCCGTTTCTACTAT	Van Houdt et al. (2010)	1816	F (J)	20	Tephritidae
Cl-N-1843d	GMWARWGWGGRATAWACWGTTCA	Zhang and Hewitt (1996)	1843	R (N)	23	Universal
UEA2	TCAAGATAAAGGAGATAAACAGTTTC	Lunt et al. (1996)	1844	R (N)	26	Universal
K699R	GGGGCTAAACTTCGATCC	Wahlberg (2010)	1858	R (N)	19	Nymphalidae
COI-Dipt-1858R	GGRTANACNGTYCANC	New	1858	R (N)	17	Brachycera
Cl-J-1859 (alias RonII)	GGTACAGCTTGAACCTGTTTACCCTCC	Simon et al. (1994) ^a	1859	F (J)	26	Universal
Cl-N-1958	CGTATATTAATAATTTGTTGAATAA	Scheffer and Wiegmann (2000)	1958	R (N)	25	Agromyzidae
L499	ATTAATATACGATCAACAGGAAT	Van Houdt et al. (2010)	1994	F (J)	23	Tephritidae
H526	ACAAATAAAGGTATTCGGTCAAA	Van Houdt et al. (2010)	1999	R (N)	23	Tephritidae
Cl-J-2050	GATCGAATACCTTTATTTGTTTC	Lonsdale et al. (2010)	2024	F (J)	23	Clusiidae
UEA4	AATTTCCGTCAGTTAATAATATAG	Lunt et al. (1996)	2057	R (N)	24	Universal
Cl-J-2090	TGTTTTAGCTGGAGCTATTTACTAT	Bernasconi et al. (2000a)	2090	F (J)	24	Scathophagidae
UEA5	AGTTTTAGCAGGAGCAATTTACTAT	Lunt et al. (1996)	2090	F (J)	24	Universal
COIFg	AGTATTAGCAGGAGCAATTTACTAT	Sallum et al. (2002)	2090	F (J)	24	Culicidae
Cl-J-2090A	AGTTTTAGCAGGAGCAATTTACTAT	Bernasconi et al. (2007)	2090	F (J)	24	Dolichopodidae
Cl-N-2096d	GANGTATTWARRTTTCGRTCWGTTA	Zhang and Hewitt (1996)	2096	R (N)	25	Universal
Cl-J-2101	GGAGCAATTACAATACTATTAACAG	Scheffer and Wiegmann (2000)	2101	F (J)	25	Agromyzidae
COI2121	CCTCCTCCAGCAGGRTCAAAAAAAG	Ekrem (2006)	2121	R (N)	25	Tanytarsini (Chironomidae)
COIF-5'	CCAGCTGGAGGAGGAGATCC	Palumbi (1996)	2150	F (J)	20	<i>Drosophila</i> (Drosophilidae)
R3 688	CCAAAGAATCAAAATAAATGTTTC	Park et al. (2009)	2161	R (N)	23	Calliphoridae
COX1R	CCAAARAATCAAAATAAATGTTTC	Sayar et al. (2009)	2161	R (N)	23	Tephritidae
HCO2198	TAAACTTCAGGCTGACCAAAAAATCA	Folmer et al. (1994)	2173	R (N)	26	Universal
HCO2198-L	TAAACTTCWGGRTGWCCAAAAATCA	Nelson et al. (2007)	2173	R (N)	26	Universal
H2123d	TAWACTTCWGGRTGWCCAAAAATCA	Van Houdt et al. (2010)	2173	R (N)	26	Tephritidae
COI-Dipt-2183F	CABCAYTATTTGATTTTTCGG	New	2183	F (J)	23	Brachycera
F3 710	CAACATTTATTTGATTTTCGG	Park et al. (2009)	2183	F (J)	23	Calliphoridae
Cl-J-2183 (alias Jerry)	CAACATTTATTTGATTTTTCGG	Simon et al. (1994) ^a	2183	F (J)	23	Universal
Cl-J-2183C	CAACATTTATTTGATTTTTCGG	Bernasconi et al. (2007)	2183	F (J)	23	Dolichopodidae
Cl-N-2191mod	CAGGTAAAAATTAATAAATAAATCTCTGG	Dallas et al. (2003)	2188	R (N)	28	Universal
Cl-N-2191 (alias Nancy)	CGCGGTAAAAATTAATAAATAAATCTCTGG	Simon et al. (1994) ^a	2191	R (N)	26	Universal
Cl-N-2191	CCTGAAAAATTAATAAATAAATCTCTGG	Kambhampati and Smith (1995)	2191	R (N)	23	Universal
COI-M-2	CCTGATTCCTGACTAATAATATG	Wang et al. (2006)	2191	R (N)	23	<i>Drosophila</i> (Drosophilidae)

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Table 4. Continued

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
cox1R	TTTTTTGGTCATCCAGAAGT	Castro et al. (2002)	2195	F (J)	20	Universal
Cl-J-2195 mod	TGATTYTTTGGTCATCCNGAAGT	Lehr et al. (2005)	2195	F (J)	23	Culicidae
Cl-J-2195	TTGATTTTGGTCATCCAGAAGT	Roehrdanz (1993)	2195	F (J)	24	Universal
Cl-J2195	TGATTTTTGGTCACCCCTGAAGT	Simon et al. (2006) ^a	2195	F (J)	23	Universal
Cl-N-2229	GATTCCTGACTAATAATATGAGAAAT	Lonsdale et al. (2010)	2227	R (N)	26	Clusiidae
Cl J2231	TACCTGGATTYGGRAITRATTC	Lewis et al. (2005)	2231	F (J)	22	<i>Drosophila</i> (Drosophilidae)
Ra DCHIM	TTCCTTTTCCCGGATTCIT	Virgilio et al. (2009)	2233	R (N)	20	<i>Dacus</i> (Tephritidae)
Cl-J-2228	ATTTCTCATATTAATGCAAGAATC	Lonsdale et al. (2010)	2252	F (J)	26	Clusiidae
Cl-N-2293a	ACTAAACCAATTGCTAGTATAGC	Wells and Sperling (1999)	2293	R (N)	23	<i>Chrysomya</i> (Calliphoridae)
Cl-N-2293b	ATGGCATAAATTATTCCTAAAGC	Wells and Sperling (1999)	2293	R (N)	23	<i>Chrysomya</i> (Calliphoridae)
Cl-J-2319	TAGCTATTGGAYTATTAGG	Wells and Sperling (2001)	2318	F (J)	19	Calliphoridae
Fly5IP	GGATTATTAGGATTTATTGT	Sallum et al. (2002)	2327	F (J)	20	Culicidae
Cl-N-2329 (alias K525)	ACTGTAAATATATGATGAGCTCA	Simon et al. (1994) ^a	2329	R (N)	23	Universal
COIF2 g	CGATTATTGTTTGGAGCTCA	Sallum et al. (2002)	2336	F (J)	20	Culicidae
sf856	ACTTGTAAATATATGATGATCTCA	Song et al. (2008)	2351	F (J)	24	Sarcophagidae
Cl N2353	GCTCGTGTATCAACGCTCTATWCC	Lewis et al. (2005)	2353	R (N)	23	<i>Drosophila</i> (Drosophilidae)
Cl-N2353	GCTCGTGTATCAACGCTCTATWCC	Simon et al. (2006) ^a	2353	R (N)	23	Universal
UEA7	TACAGTTGGAATACAGCTTGATAC	Lunt et al. (1996)	2369	F (J)	24	Universal
COIR2AS	GAAATAAAATGAGCTCG	Sallum et al. (2002)	2371	R (N)	17	Culicidae
Pl	CGTGCCTATTTCACTTCAGC	Shi et al. (2005)	2390	F (J)	20	<i>Bactrocera</i> (Tephritidae)
Cl-N-2393	CCTGTAGGAACAGCAATAATTATTG	Scheffer and Wiegmann (2000)	2393	R (N)	25	Agromyzidae
UEA-6 mod	TTAATTCCTGTAGGNACAGCAATAATTAT	Lehr et al. (2005)	2395	R (N)	29	Culicidae
UEA6 reverse	TTAATWCCWGTWCCGNACNGCAATRATTAT	Lunt et al. (1996)	2395	R (N)	29	Universal
	AAAAATTTTAAATTCAGCTTGGAAACGC	Pradeep Kumar et al. (2007)	2404	R (N)	27	Culicidae
COI-Dipt-241F	GCHACWATAATTATTGCHGTNCC	New	2411	F (J)	23	Brachycera
Cl-N-2413	TCARCTRAAAATTTTAAATTCCTGT	Winkler et al. (2009)	2413	R (N)	24	Agromyzidae
COI-Dipt-241R	GCHADTCAADCTRAAAATTTTRATNCC	New	2411	R (N)	26	Brachycera
Cl-J-2441 mod	CCTACAGGAATTAATAATTTTATGTTGATTAGC	Scheffer et al. (2004)	2441	F (J)	32	Agromyzidae
Cl-J-2441 (alias Dick) primer 2	CCTACAGGAATTAATAATTTTATGATGATTAGC	Simon et al. (1994) ^a	2441	F (J)	32	Universal
Brian F	CAGCTACTTATAGCCTTTAGG	Sperling et al. (1994)	2495	F (J)	22	Calliphoridae
Cl-N-2508	CTTCTATATTATGAAGATTAGG	Wahlberg (2010)	2495	F (J)	22	Nymphalidae
Cl-N-2514	CTCCAGTAAATCCTCCAAGTGTAAAT	Scheffer et al. (2004)	2495	R (N)	26	Agromyzidae
	AACTCCAGTAAATCCTCCTAC	Wells and Sperling (2001)	2515	R (N)	21	Calliphoridae
COIF2AS	GCTCATTTCATTATGT	Sallum et al. (2002)	2606	F (J)	17	Culicidae
cox1F	ATTGCAAAATACCTGCACCTAT	Castro et al. (2002)	2614	R (N)	20	Universal
Cl-N-2629	AAATCCTGCTATAATAGCAAATAC	Lonsdale et al. (2010)	2623	R (N)	24	Clusiidae
Cl-J-2630	TTTATCAATAGGAGCAGTATTTCG	Bernasconi et al. (2007)	2630	F (J)	24	Dolichopodidae
Cl J2636	ATAGGRGCGTATTTTGCYATTAT	Lewis et al. (2005)	2636	F (J)	23	<i>Drosophila</i> (Drosophilidae)
F2640	GCWGTMTTTCGCTATATAGCAGC	Oliveira et al. (2005)	2642	F (J)	23	Universal
Cl-J-2628	GTATTTGCTATTATAGCAGGATTT	Lonsdale et al. (2010)	2646	F (J)	24	Clusiidae
2672r	CCAGTAAATATGGGTATCAGTG	Gleason et al. (1997)	2650	R (N)	23	<i>Drosophila</i> (Drosophilidae)
Cl-N-2659 (alias Milal)	GTCAAATCCAGTAAATAATGG	Simon et al. (1994) ^a	2659	R (N)	20	Universal
UEA5	AAAAATGTGAGGCAAAAATGTTA	Lunt et al. (1996)	2735	R (N)	24	Universal
GaRev	AAAAATGCTGGGGCAAGAAATGTTA	Otranto et al. (2003)	2735	R (N)	24	Oestridae
UEA 9 mod	GTAATTTTAAACATTTTTTCCYCAACA	Bernasconi et al. (2000)a	2753	F (J)	26	Muscoidea
UEA9	GTAACCTAAATCTTTTCCCTCAACA	Lunt et al. (1996)	2753	F (J)	26	Universal
GaFor	GTAACAATAACATTTCTCCCCAGCA	Otranto et al. (2003)	2753	F (J)	26	Oestridae
Cl-J2756	ACATTTTTCCCCCAACATTT	Simon et al. (2006) ^a	2756	F (J)	20	Universal
UEA9.2	CTAAGATTTTTTCTCAACATTTTTTAGG	Sallum et al. (2007)	2762	F (J)	29	Culicidae
COIR2 g	CGTGCAGGTAATTCGGGCTAA	Sallum et al. (2002)	2764	R (N)	20	Culicidae
Cl N2776	TAATCTGAATAACGCTCCNGG	Lewis et al. (2005)	2776	R (N)	20	<i>Drosophila</i> (Drosophilidae)
Cl-N2776	GGTAATCTGAATAACGCTCCAGG	Simon et al. (2006) ^a	2776	R (N)	22	Universal
Cl-J-2792b	ATACCTCGGCATATCTCTGA	Wells and Sperling (1999)	2792	F (J)	20	<i>Chrysomya</i> (Calliphoridae)
Cl-J-2797	CCTCGACGTTATTACGATTACC	Simon et al. (1994) ^a	2797	F (J)	22	Universal
R2 1327	CAAGTTGTGTAAGCATC	Park et al. (2009)	2800	R (N)	17	Calliphoridae
primer 3	CATTTCAAGYCTGTAAAGCATC	Sperling et al. (1994)	2800	R (N)	22	Calliphoridae
CO1a-3'	AGCTAAGCATCAGGGTAATC	Palumbi (1996)	2809	R (N)	21	Universal
Fly10IP	GCAAATAATGAAATGTTCT	Sallum et al. (2002)	2839	R (N)	20	Culicidae
P2	CAGCTGGAGGGGTATTTTGA	Shi et al. (2005)	2952	R (N)	20	<i>Bactrocera</i> (Tephritidae)
UEA10.2	TTATATGTTAATAAYGGTARTTCTG	Sallum et al. (2007)	2984	R (N)	25	Culicidae
TL2-N-3013	TCCATTACATATAATCTGCCATATTAG	Wells and Sperling (1999)	3013	R (N)	27	<i>Chrysomya</i> (Calliphoridae)
R3037	TYCATTGCACCTAATCTGCCATATTAG	Oliveira et al. (2005)	3013	R (N)	26	Universal
TL2-N-3014 mod	AATGCACCTAATCTGCCATATTAG	Lehr et al. (2005)	3013	R (N)	23	Culicidae
TL2-N-3014 (Pat)	TCCATTGCACCTAATCTGCCATATTAG	Simon et al. (1994) ^a	3014	R (N)	25	Universal
UEA10	TCCAATGCACCTAATCTGCCATATTAG	Lunt et al. (1996)	3014	R (N)	25	Universal
TL2-N-3015	ATTGCACTAATCTGCCATATTAG	Lonsdale et al. (2010)	3012	R (N)	24	Clusiidae
TL-N-3017	CTTAAATCCATTCGCAATATCTGCCATA	Scheffer et al. (2004)	3017	R (N)	28	Agromyzidae
CCOIR	CCATTCGACTAATCTGCCA	Chen et al. (2004)	3019	R (N)	19	Calliphoridae
COI-2	TCCATTGCACCTAATCTGCCA	Wang et al. (2006)	3019	R (N)	20	<i>Drosophila</i> (Drosophilidae)
CULR	TGAAGCTTAAATTCATTCGACTAATC	Dyer et al. (2008)	3024	R (N)	26	Glossinidae

The 3' location based on published *D. yakuba* sequence (Clary and Wolstenholme 1985). Direction F, forward; R, reverse (J, majority; N, minority as per Simon et al. 1994). Sequences in bold are newly developed for this study.

^a Sequences from Simon et al. (1994, 2006) are those matching *Drosophila* without any degeneracy.

Table 5. PCR amplification primers developed to amplify the 28S gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
rc28AB	ACTACCCCTGAATTTAAGCA	Bertone et al. (2008)	3334	F	21	Diptera
D1F	CCCSCCTAAAYTTAAGCATAT	Friedrich and Tautz (1997)	3337	F	20	Diptera
rc28A	AGCCGAGGAAAAGAAAC **	Bertone et al. (2008)	3359	F	17	Diptera
D1 SP	GGGAGGAAAAGAACTAAC	Moulton (2000)	3363	F	19	Simuliidae
28y	CAAGGATTCCCTTAGTAGCG	Stireman (2002)	3382	F	20	Tachinidae
28S-Dipt-3385F	GGATTTTCTTAGTAGCGGG	New	3385	F	20	Diptera
28y	CTAACAAGGATTTTCTTAGTAGCGGGGAGT	Hillis and Dixon (1991)	3388	F	30	Universal
28yy	CTAACAAGGATTTTCTTAGTAGCGGGGAGC	Tachi and Shima (2010)	3388	F	30	Tachinidae
28S-Dipt-3394F	GTAGCGGGCAGGAAAAG	New	3394	F	18	Diptera
CP12	GTGGATCCAGTCGTGTGCTTATAGTGCAG	Porter and Collins (1996)	3570	F	31	<i>Anopheles</i> (Culicidae)
28kk	ACTAGGATTAACATAAGTACCG	Hillis and Dixon (1991)	3638	F	21	Universal
28kk-1	ACCGATAGTAAACAAGTACCG	Tachi and Shima (2010)	3638	F	21	Tachinidae
28S-Dipt-3661F	GGGAAAGTTCAAAAAGAACT	New	3661	F	20	Diptera
D1R	ACTCTCTATTACARAGTCTTTT	Friedrich and Tautz (1997)	3673	R	22	Diptera
28Sforward	AGAGAGAGTTCAAGAGTACGCT	Castro et al. (2002)	3686	F	22	Universal
D2a	ACGTGAAACTGCTTAGAGGTT	Ruiz Linares et al. (1991)	3702	F	21	<i>Drosophila</i> (Drosophilidae)
rc28B	CCCGTCTTGAACACGGACC ^a	Bertone et al. (2008)	4067	F	20	Diptera
28Sreverse	TGGTCCGTTTTCACAGCCGG	Castro et al. (2002)	4069	R	22	Universal
28z	AGACTCCTTGGTCCCGTGTTCACAGC	Hillis and Dixon (1991)	4076	F	26	Universal
D3 PCR	TGAAACACGGACCAAGGAGTCTA	Moulton (2000)	4077	F	24	Simuliidae
CP15	GTGAATTTCTGGTCCGTGTTCACAGCCGG	Porter and Collins (1996)	4077	R	30	<i>Anopheles</i> (Culicidae)
D2b	ATGTTAGACTCCTTGGTCCGT	Ruiz Linares et al. (1991)	4081	R	21	<i>Drosophila</i> (Drosophilidae)
D3 SP	GCATAGTTCCACATCTTTC	Moulton (2000)	4285	R	19	Simuliidae
10i	GTGCAATTCGATTTGTCAGA	Pawlowski et al. (1996)	4358	F	19	Culicomorpha
28b	TCCGAAAGAACCCAGCTACTA	Stireman (2002)	4413	R	20	Tachinidae
0ic	GAAGTTTCYCTCAGGATAGC	Pawlowski et al. (1996)	4431	F	20	Culicomorpha
rc28C	CCGAAGTTTCCCTCAGGATAGC ^a	Wiegmann et al. (2000)	4431	F	22	Diptera
28S-Dipt-4534F	CCTATTTCTCAAACCTTAAATGGG	New	4534	F	23	Diptera
28S-Dipt-4610F	GGGCCACTTTTGGTAAGCAG	New	4610	F	20	Diptera
12r	CCAGTTCTGCTTACCAA	Pawlowski et al. (1996)	4616	R	17	Culicomorpha
12i	GTAAGCAGAACTGCTGCT	Pawlowski et al. (1996)	4620	F	18	Culicomorpha
28S-Dipt-4632R	GGTTTCATCCACAGCCGC	New	4632	R	18	Diptera
S28E	AGCAGGACGGTGGACATGGA	Han et al. (2002)	4721	F	20	Tephritoidea
11	GTTACRCACTCCTTARCRG ^a	Pawlowski et al. (1996)	4749	R	19	Culicomorpha
D6 SP	CGCTAAGGACTGTGTAAAC	Moulton (2000)	4749	F	18	Simuliidae
28ee	ACTCGCTAAGGAGTGTCTAAACAATCACC	Hillis and Dixon (1991)	4757	F	29	Universal
A28D	ACTTAAGCCCATCCATTTT	Han et al. (2002)	4797	R	20	Tephritoidea
rc28P	TGCTATGCGTGAAGTGTTCGCC	Wiegmann et al. (2000)	4906	F	23	Diptera
28P	GGCTACGCCAACACTTCTAGCC	Wiegmann et al. (2000)	4913	R	24	Diptera
28S-Dipt-4964F	GGTGTAGTAGCAAATAATCC	New	4964	F	21	Diptera
14i	CGATGRCTGAAGTCCA	Pawlowski et al. (1996)	4992	F	16	Culicomorpha
28S-Dipt-4997F	CGAGGACTGAAGTGGAGAAGG	New	4997	F	21	Diptera
D7F	CTGAACTGGAGAAGGGT	Friedrich and Tautz (1997)	4999	F	17	Diptera
S28G	GAAGTGGAGAAGGGTTTCCGT	Han et al. (2002)	5004	F	20	Tephritoidea
D7int1	AGGGTTTCCGTGTGAACAG	Friedrich and Tautz (1997)	5012	F	18	Diptera
A28F	TGCAACCCGATTTCCCTTTCC	Han et al. (2002)	5150	R	20	Tephritoidea
28S-Dipt-5161F	CGGTTCCAAITTCGGTAAAC	New	5161	F	19	Diptera
D7int2	TTCCAAACCMTTACTCT	Friedrich and Tautz (1997)	5181	R	16	Diptera
D7int3	CGATTTTCAAGGTCC	Friedrich and Tautz (1997)	5378	R	15	Diptera
rc28D	CGGCAGCTGGTCTCCAAG	Wiegmann et al. (2000)	5438	F	18	Diptera
15i	TCTATCGACTAGAGACTC	Pawlowski et al. (1996)	5461	R	18	Culicomorpha
A28HL	CTTACCTACATTTCTATCGACT	Han et al. (2002)	5475	R	24	Tephritoidea
D7R	GACTTCCCTTACTACAT	Friedrich and Tautz (1997)	5482	R	18	Diptera
28S-Dipt-5497F	GGAAGTCCGCAAATTAGATCCG	New	5497	F	22	Diptera
28E	CCTTATCCCGAAGTTACG	Wiegmann et al. (2000)	5513	R	18	Diptera
28II	GATCCGTAACCTCCGGATAAGGATTGGCTC	Hillis and Dixon (1991)	5521	F	30	Universal
28S-Dipt-5532R	CTCAATCTTACAGGCCAATCC	New	5532	R	21	Diptera
D8 SP	GCACTGGGCAGAAATCA	Moulton (2000)	5842	R	17	Simuliidae
28F	CAGAGCACTGGCAGAAATCAC	Lonsdale et al. (2010)	5846	R	22	Diptera
28v	AAGGTAGCCAAAGTCCCTGATC	Hillis and Dixon (1991)	5930	F	21	Universal
D9 SP	AGCCAAATGCCCTCGTATC	Moulton (2000)	5933	F	18	Simuliidae
rc28H	CTACTATCCAGCCGAAACC ^a	Wiegmann et al. (2000)	6000	F	18	Diptera
28S-Dipt-6018R	GGCCGTCCCTTGGCTCTGG	New	6018	R	20	Diptera
28I	GGTCTTCTTTCCCGCT	Lonsdale et al. (2010)	6047	R	18	Diptera
21	CTCAARCTCAAMAGGGTC	Pawlowski et al. (1996)	6060	R	18	Culicomorpha
D8 PCR	TTAGAGTCAAGCTCAAAGGGTCTTCT	Moulton (2000)	6065	R	27	Simuliidae
28w	CCTTTGACCTTGACTCTAATCTG	Hillis and Dixon (1991)	6097	F	24	Universal
D10c	TGAAATACCCTACTCT	Ruiz Linares et al. (1991)	6154	F	17	<i>Drosophila</i> (Drosophilidae)
rc28Q	GGACATTGCCAGGTAGGGAGTT	Wiegmann et al. (2000)	6406	F	22	Diptera
28Q	AACTCCCTACTGGCAAT	Yang et al. (2000)	6406	R	18	Diptera
D10d	CGGCCAGTCAAACCTCCC	Ruiz Linares et al. (1991)	6418	R	19	<i>Drosophila</i> (Drosophilidae)
D10 SP	TACCGCCCGACTCAAAC	Moulton (2000)	6420	R	17	Simuliidae
D10 PCR	TGAGAGATGTACCCGCCAGTCAA	Moulton (2000)	6429	R	24	Simuliidae
28S-Dipt-6462F	GGTGTCCCAAGGCCAGCTCAG	New	6462	F	21	Diptera
28S-Dipt-6565F	CGGCCTATCGATCCTTTTGG	New	6565	F	20	Diptera

Continued on following page

Table 5. Continued

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
28J	CCCTGTGGTAACTTTTCT	Lonsdale et al. (2010)	6610	R	18	Diptera
28S-Dipt-6635F	CGCTTGTGGCGGCCAAGCG	New	6635	F	19	Diptera
28S-Dipt-6647R	CGTCGCTATGAACGTTGGCC	New	6647	R	21	Diptera
28K	GAAGAGCCGACATCGAAC ^a	Wiegmann et al. (2000)	6678	R	18	Diptera
28x	GTGAATTTTGGCTTCATCAATGCTAGGAAGAGCC	Hillis and Dixon (1991)	6702	R	32	Universal
28S-Dipt-6723F	CCAAGCGTTGGATTGTTACCC	New	6723	F	22	Diptera
28M	AACCCAGCTCACGTTCCC	Lonsdale et al. (2010)	6747	R	18	Diptera
28jj	AGTAGGGTAAACTAACCT	Hillis and Dixon (1991)	6782	R	19	Universal
28S-Dipt-6834F	CGCTAGTACGAGAGCAACCG	New	6834	F	20	Diptera
28S-Dipt-6916R	GAGGCGTTACGGCATAATCC	New	6916	R	20	Diptera
rc28X	CGCCTCTAAGTCCGTATCCG ^a	Wiegmann et al. (2000)	6930	F	20	Diptera
28S-Dipt-7176R	CCACTTACAACACCTTGCC	New	7176	R	19	Diptera
28Zc	TGGATCGCAGTATGGCAGCT	Bertone et al. (2008)	7202	R	20	Diptera
28Z	CCAAAGGATAAGCTTCAGTGG	Wiegmann et al. (2000)	7220	R	21	Diptera

The 3' location based on published *D. melanogaster* sequence (Tautz et al. 1988). Direction F, forward; R, reverse. Sequences in bold are newly developed for this study.

^a Reverse-complement versions of these primers have been published with the same location and opposite direction.

and sequenced will vary across taxa due to expansion segments within the gene region.

Cytb. Simon et al. (1994) presented eight primers that could be used to amplify the *Cytb* gene region across Animalia. Their later update (Simon et al. 2006) added six more primers. Gasparich et al. (1995) designed three unique *Cytb* primers as part of a project to sequence the mitochondrial DNA of *Ceratitidis capitata* (Wiedemann) (Tephritidae). A phylogeographic study of Psychodidae (Ready et al. 1997) included one unique *Cytb* primer. Although working on triatomine bugs (Hemiptera: Reduviidae), Lyman et al. (1999) designed six primers for the *Cytb* region based on GenBank sequences of three Diptera species plus the sequences of a bee, a locust, and a crustacean. In another molecular phylogenetic study of Psychodidae, Essegir et al. (2000) presented a new primer. While developing molecular markers for Old World populations of *Chrysomya* (Calliphoridae), Hall et al. (2001) developed four unique primers. Two more *Cytb* primers were developed as part of population genetics studies of Psychodidae and Culicidae (Hodgkinson et al. 2002). Analyses of the population genetics of *Anopheles* (*Cellia*) included four unique primers

specific to Culicidae as well as several primers specific to individual *Cytb* haplotypes (Dusfour et al. 2004, Dusfour et al. 2007). A study of invasive species of Tipulidae included two unique *Cytb* primers (Rao et al. 2006).

We have developed five new primers for the *Cytb* gene region. The naming is based on the published mitochondrial genome of *D. yakuba* (Clary and Wolstenholme 1985). In total, 42 primers are listed and mapped (Fig. 2; Table 3). The entire length of the gene region (≈1035 bp) can be sequenced using the listed primers. There are no known introns within the *Cytb* gene region.

COI. A COI primer designed using Diptera sequence data were first developed by Roehrdanz (1993). Folmer et al. (1994) designed what are now considered the “barcoding region primers,” perhaps the two most commonly used COI primers for use across Metazoa. They were developed using *Drosophila* (Drosophilidae) and *Anopheles* (Culicidae) sequences among others. Research on forensically important species of Calliphoridae (Sperling et al. 1994; Wells and Sperling 1999, 2001; Chen et al. 2004; Nelson et al. 2007; Park et al. 2009) has introduced nineteen

Table 6. PCR amplification primers developed to amplify the AATS gene region using Diptera exemplars

Primer Name	Sequence	First reference	3' location	Direction	bp	Developed for
AATS-Dipt-463F	ATCAAHCARTTYAARCC	New	463	F	17	Brachycera
IF40	GNATGAAAYCARTTYAARCCNAT	Feng-Yi Su et al. (2008)	466	F	22	Universal
unnamed	CGATCCCAACAGCGARATGTCCAA	Feng-Yi Su et al. (2008)	505	F	24	Universal
AATS-Dipt-547F	CARAARTGYATHCGNGCHGG	New	547	F	20	Brachycera
AATS-Dipt-559F	CGNCGHGGHCGHAARCAAYA	New	559	F	20	Brachycera
AATS-Dipt-598F	GGNAARGAYGNTAYCAYCAYAC	New	598	F	23	Brachycera
2F	TAYCAYCAYACNTTYTYTGARATG	Regier (2008)	611	F	24	Universal
AATS-Dipt-631F	ATGYTNGGHAMTYGGTCNTTYGG	New	631	F	23	Brachycera
AATS-Dipt-828R	CCCATYTCCCARAARTTRTC	New	828	R	20	Brachycera
AATS-Dipt-840R	GGNCCNVYTCNCCCATYTCCC	New	840	R	22	Brachycera
4F	ATGAARGAYAAAYTTYTGGGARATGGG	Regier (2008)	847	F	26	Universal
unnamed	ATGAACACCAGATTCCAGATYTCCA	Feng-Yi Su et al. (2008)	946	R	25	Universal
AATS-Dipt-962R	CGATTTRWAYTGWTRAAANACHARRTTCC	New	962	R	28	Brachycera
IR244	CATNCCRCARTCNARTGYTT	Feng-Yi Su et al. (2008)	1017	R	21	Universal
5R	GGRAANCCRTANGTRTCRTA	Regier (2008)	1677	R	20	Universal

The 3' location based on published *D. melanogaster* sequence (Adams et al. 2000). Direction F, forward; R, reverse. Sequences in bold are newly developed for this study.

Table 7. PCR amplification primers developed to amplify the CAD gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
CAD-60F	GARGTNGTNTTYCARACNGGNAT	Lonsdale et al. (2010)	142	F	23	Clusiidae
CAD-Dipt-144F	TGNTTYCARACNGGNATGG	New	144	F	20	Brachycera
54F	GTNGTNTTYCARACNGGNATGGT	Moulton and Wiegmann (2004)	145	F	23	Eremoneura
68F	GGATCGTTTCATTCGTCGACA	Barr and Wiegmann (2009)	187	F	21	Ceratitis (Tephritidae)
CAD-Dipt-757F	AGYAAAYGCNCNGGHGAYCC	New	757	F	20	Brachycera
320F	ATHTTYGGNATYTYGYTGGNCAYCA	Moulton and Wiegmann (2004)	844	F	26	Eremoneura
CAD-Dipt-844R	TCRTGDCCYARRCADATNCC	New	844	R	20	Brachycera
287nR	TTRTGNCNCCKRTRCCRTA	Regier (2008)	844	R	20	Universal
338F	ATGAARTAYGGYATCGTGGHCAYAA	Moulton and Wiegmann (2004)	907	F	26	Eremoneura
CAD-Dipt-964F	ATGACNTCNARAAYCAYGG	New	964	F	20	Brachycera
CAD359R	CCATGGTTTTGWCANGTCAT	Barr and Wiegmann (2009)	964	R	20	Ceratitis (Tephritidae)
AG-360AR	CCATGATYTTGTGARGTCAT	Scheffer et al. (2007)	964	R	20	Agromyzidae
AG-360BR	CCRTGRTTYTGTGAYGTCA	Scheffer et al. (2007)	964	R	20	Agromyzidae
364R	TCNACNCGRAANCRTGRTTYG	Moulton and Wiegmann (2004)	976	R	23	Eremoneura
CAD-Dipt-1085F	CCNTAYTTYCDGTNCARTTYCATCC	New	1085	F	26	Brachycera
356F	CARTTYCAYCNGARCA	Regier (2008)	1094	F	17	Universal
350R	RTGYTCNCGRTGRAAYTG	Regier (2008)	1095	R	18	Universal
405R	CNGTTRTYTCNCGRTGRAAYTG	Moulton and Wiegmann (2004)	1100	R	23	Eremoneura
CAD-Dipt-1100R	GMHGTRTYTCNCGRTGRAAYTG	New	1100	R	23	Brachycera
CAD-410R	GGNCCNCGNTRTYTCNCGRTG	Lonsdale et al. (2010)	1106	R	23	Clusiidae
CAD-Dipt-1294F	GGNARGCNGNGGARTTYGA	New	1294	F	20	Brachycera
267fin2F	CGNCGNARTTYGAYTA	Regier (2008)	1297	F	17	Universal
CAD-Dipt-1326F	GGNTCNARGCNATHAARGC	New	1326	F	20	Brachycera
581F2	CGWGGWCAAAACWGCWYTTMAAYTYGG	Moulton and Wiegmann (2004)	1507	F	26	Eremoneura
496F	CARACNCGNNTYAAAYTYGG	Regier (2008)	1507	F	20	Universal
581F	GANACTGARGAYMGRAAAATMTTYGC	Moulton and Wiegmann (2004)	1610	F	26	Eremoneura
576R	TCNTCYTCRTRTTNGCRAA	Regier (2008)	1646	R	20	Universal
CAD-Dipt-1756R	GCRAANCCNAGNCCNARNCCNCC	New	1756	R	23	Brachycera
613F	TGCAARGARTNGARTAYGARGT	Regier (2008)	1864	F	23	Universal
606nR	ACNACYTCRTAYTCNACYTCYTTCCA	Regier (2008)	1867	R	26	Universal
680R	AANGCRTCNCGNACMACYTCRTAYTC	Moulton and Wiegmann (2004)	1879	R	26	Eremoneura
CAD-Dipt-1911F	TGYATHACNGTNTGYAAYATGG	New	1911	F	22	Brachycera
267fin3R	TTYTCCATRTTRCANAC	Regier (2008)	1915	R	17	Universal
681F	GARTGYAAYRTNCARTAYGC	Regier (2008)	2065	F	20	Universal
CAD-Dipt-2065F	GGNGARTGYAAYATHCARTAYGC	New	2065	F	23	Brachycera
787F	GGDGTNACNCGNTYTYTGARCC	Moulton and Wiegmann (2004)	2248	F	26	Eremoneura
806F	CTNGTNAARATGCCNMGNTGGGA	Moulton and Wiegmann (2004)	2287	F	23	Eremoneura
850F	RAAYATHGGHAGTTCBATGA	Winkler et al. (2009)	2334	F	20	Agromyzidae
CAD-Dipt-2341F	TGGHAGYTCNATGAARAGYGT	New	2344	F	21	Brachycera
843R	GCYTTYTGRAANGCYTCYCRAA	Moulton and Wiegmann (2004)	2393	R	23	Eremoneura
CAD-Dipt-2393R	GCYTTYTGNAANGCYTCYTC	New	2393	R	20	Brachycera
843R2	TCNACCATWCKNARWGCYTTYTGRAA	Moulton and Wiegmann (2004)	2408	R	26	Eremoneura
CAD-Dipt-2803F	GTNGCNGGARTGCGCCNCC	New	2803	F	20	Brachycera
CAD-Dipt-2927F	TCNTCNGTNGARTTYGAYTGG	New	2927	F	21	Brachycera
970F	GARTTYGAYTCGTYGC	Regier (2008)	2932	F	17	Universal
970R	TRTCRTARTCNGTGGAHACRGTTCNCG	Winkler et al. (2009)	3018	R	28	Agromyzidae
1057F	GTNTCNACNGAYTAYGAYATGTC	Moulton and Wiegmann (2004)	3020	F	23	Eremoneura
CAD-Dipt-3127F	TCNATGGGGHGCNARYTRCC	New	3127	F	20	Brachycera
1098R	TTNCGNAGYTGCCNCCCAT	Moulton and Wiegmann (2004)	3130	R	20	Eremoneura
CAD-Dipt-3130R	TTNCGNARYTGCCNCCCAT	New	3130	R	20	Brachycera
1025R	TRRTTNGGNARYTGCCNCCCAT	Regier (2008)	3133	R	23	Universal
CAD-Dipt-3144F	CCNAAAYAYATHCGNATGG	New	3144	F	19	Brachycera
CAD-Dipt-3202F	CCNGARTCNATHGAYAGYGC	New	3202	F	20	Brachycera
1124R	CATNCCNGARAAAYTTAARGATTYTC	Moulton and Wiegmann (2004)	3227	R	27	Eremoneura
CAD-Dipt-3370F	GGNCGNGHATGAAYGTNGC	New	3370	F	20	Brachycera
1201F	GARGCNAARGARATYGAYTNGAYGC	Moulton and Wiegmann (2004)	3504	F	26	Eremoneura
CAD1201Fc	GAAGCNAARGAAATYATGTGGATGC	Lonsdale et al. (2010)	3504	F	26	Clusiidae
CAD-Dipt-3504F	GCNAARGARATYGAYTNGAYGC	New	3504	F	23	Brachycera
CAD-Dipt-3682F	CCNTTYAAYATGCARYTNATYGC	New	3682	F	23	Brachycera
1278R	TCRTTNTTYTTWGCRTYAAAYTCAT	Moulton and Wiegmann (2004)	3694	R	26	Eremoneura
CAD-Dipt-3925F	GGHGTNGARATGGCNCNACHGG	New	3925	F	23	Brachycera
CAD-Dipt-3943F	GGNGARGTNGCNTGYTYGG	New	3943	F	20	Brachycera
CAD-Dipt-4000R	GGDATYTGRAADCCNCGTNGAYATC	New	4000	R	24	Brachycera
1436R	CCRTGYTCNCGRTARAARTC	Moulton and Wiegmann (2004)	4228	R	20	Eremoneura

The 3' location based on published *D. melanogaster* sequence (Freund and Jarry 1987). Direction F, forward; R, reverse. Sequences in bold are newly developed for this study.

unique COI primers. Simon et al. (1994) included eleven COI primers in their compendium of mitochondrial insect primers; their later compendium (Simon et al. 2006) added six more unique primers. Two modified primers were designed for use across insects and ticks (Kambhampati and Smith 1995). Palumbi

(1996) added two COI primers, modified to suit *Drosophila* (Drosophilidae) sequences, in his book chapter on PCR. Ten unique COI primers were tested across Insecta, including six Diptera species, by Lunt et al. (1996). Three COI primers were deemed to be useful across invertebrates, including *Drosophila*

Table 8. PCR amplification primers developed to amplify the EF-1a gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
M3	CACATYAACATGTGCGTSATYGG	Cho et al. (1995)	2103	F	23	Universal
30F	CAYATYAAYATHGTSNTATHGG	Regier (2008)	2103	F	23	Universal
rc40	GTCGTSATYGGWCACGTMGATTCTYGG	Yang et al. (2000)	2118	F	26	Therevidae
40.6F	ATYGARAARTTYGARAARGARGC	Regier (2008)	2199	F	23	Universal
EFs175	GGAAATGGGAAAAGGCTCCTCAAGTAYGCTYGGG	Stireman (2002)	2237	F	35	Tachinidae
40.71F	TCNNTTYAARTAYGCNTGGGT	Regier (2008)	2238	F	20	Universal
EF-SE	TGAGCGTCAGCGTGCTATC	Esseghir et al. (2000)	2275	F	19	Psychodidae
M44-1	GCTGACGGYGARCGTGGTATCAC	Cho et al. (1995)	2277	F	23	Universal
rcEF4	GARGGCTGATYACMATTGA	Yang et al. (2000)	2283	F	20	Therevidae
2284-2302(S)	TATYGGCTTTRTGAAATTTCG	Baker et al. (2001)	2303	F	20	Diopsidae
rcM44.9	CTTGATGAAATCYCTGTGTCC	Cho et al. (1995)	2362	R	21	Universal
42.8R	ATCATRTTYTTDATRAARTC	Regier (2008)	2370	R	20	Universal
Sam	YGATTGTCGCCCGGCTACTGCTGAAT	Moulton (2000)	2429	F	27	Simuliidae
2477-2495(S)	CTTGCTTTCACHTTGGGCTG	Baker et al. (2001)	2495	F	19	Diopsidae
45.71F	GTNGSNGTNAAYAARATGGA	Regier (2008)	2529	F	20	Universal
EF1a-Dipt-2544F	ATGCAYTNTCYGARCCACC	New	2544	F	20	Brachycera
M46-1	GAGGAAATYAARAAGGAAG	Cho et al. (1995)	2582	F	19	Universal
46	TGAGGAAATCAARAAGGAAG	Yang et al. (2000)	2582	F	20	Therevidae
EF46M	GAGGAAATYAARAARGAAGT	Collins and Wiegmann (2002)	2583	F	20	Empidoidea
EF1a-Dipt-2583F	GARGAAATHAARAARGAAGT	New	2583	F	20	Brachycera
EF1a-Dipt-2655F	CCHATYTCYGGHTGCAAYGG	New	2655	F	20	Brachycera
51	CATGTTGTCRTGCCATCC	Yang et al. (2000)	2662	R	18	Therevidae
EF0-5'	TCCGGATGGCACGGCGAGAACATG	Palumbi (1996)	2665	F	24	Universal
rcM51-1	CATRTTGTCKCCGTCGCCAKCC	Cho et al. (1995)	2665	R	21	Universal
Joe-2	CCGTGGTWCAGGGATGG	Moulton (2000)	2704	F	18	Simuliidae
Joe	CHTGGTWCAGGGATGGAA	Moulton (2000)	2706	F	19	Simuliidae
EF1a-Dipt-2724F	GGYTTYAACGTAARAACG	New	2724	F	19	Brachycera
M51.9	CARGACGTATACAAAATCGG	Cho et al. (1995)	2832	F	20	Universal
52R	CCDATYTTTANACRTCYTG	Regier (2008)	2832	R	20	Universal
EF-1A 5' SP	TGTTTACAAAATTTGGCGGTAT	Moulton (2000)	2838	F	21	Simuliidae
Shemp	TCCRAATACCNAARATTTTGTAT	Moulton (2000)	2842	R	21	Simuliidae
EF-1A 3' SP	TTCCAAATACCGCAATTTTG	Moulton (2000)	2843	R	20	Simuliidae
Curly	GTAAGTGTCCGATACCGCC	Moulton (2000)	2849	R	19	Simuliidae
rc47	CGAACAGTACCYGTGGGTCC	Yang et al. (2000)	2859	F	20	Therevidae
EF1a-Dipt-2859F	GGHACAGTACHGTNGGTCC	New	2859	F	20	Brachycera
2869-2889(S)	CGTGTDTTCAAACCGAGTTG	Baker et al. (2001)	2889	F	20	Diopsidae
52.4F	TCNGTNGATGCAYCAYG	Regier (2008)	2951	F	19	Universal
52.5R	TCRTGRTGCATYTCNAC	Regier (2008)	2952	R	17	Universal
2934-2954(A)	CTTCGTGATGCATTTCAACCG	Baker et al. (2001)	2954	R	21	Diopsidae
EF1a-Dipt-2954R	CTTCGTGATGCATTTCAACRG	New	2954	R	21	Brachycera
rcM52.6	CCYTCGTGCTGCATYTCAC	Cho et al. (1995)	2955	R	20	Universal
EF1-5'	GACAACCGTTGGCTTCAACGTAAGAACC	Palumbi (1996)	3005	F	28	Universal
EF1a-Dipt-3005R	CGTTTYTNACGTTGAARCC	New	3005	R	19	Brachycera
M52.7	GTCAAGGARYGTGGTCTGG	Cho et al. (1995)	3030	F	20	Universal
EF-SE2	CGGGTGGTTACGTACGATGA	Esseghir et al. (2000)	3112	R	20	Psychodidae
EF1a-Dipt-3162R	TGRGCDGTGTRCAATC	New	3162	R	17	Brachycera
EF2-3'	ATGTGACGGGTGGCAATCCAA	Palumbi (1996)	3165	R	23	Universal
EF2	ATGTGACGAGTGTGGCAATCAA	Stireman (2002)	3165	R	22	Tachinidae
53.5R	ATRTGVMNGTRTGRCAATC	Regier (2008)	3165	R	20	Universal
rcM53-2	GCAATGTGRGCGTCTGGCA	Cho et al. (1995)	3168	R	20	Universal
EF5	CTCATATCACGTACAGCRAARGG	Yang et al. (2000)	3351	R	23	Therevidae
41.21R	TGYCTCATRTCDGVCACRGCRAA	Regier (2008)	3354	R	23	Universal
rcM4	ACAGCVACKGTYTGYCTCATRTC	Cho et al. (1995)	3366	R	23	Universal

The 3' location based on published *D. melanogaster* sequence (Hovemann et al. 1988). Direction F, forward; R, reverse. Sequences in bold are newly developed for this study.

melanogaster Meigen, by Zhang and Hewitt (1997). One unique primer was developed for use in *Drosophila* species phylogenies (Gleason et al. 1997). Nine additional COI primers were developed in studies of the phylogenetics of Agromyzidae and Fergusoniniidae (Scheffer and Wiegmann 2000, Scheffer et al. 2004, Winkler et al. 2009). In a pair of papers inves-

tigating the Muscoidea, Bernasconi et al. (2000a,b) introduced unique primers. A study of the systematics of Chironomidae included one COI primer (Guryev et al. 2001). As part of a comparison of molecular evolution between parasitic Diptera and parasitic Hymenoptera, including representatives of 14 dipteran families, Castro et al. (2002) developed two COI primers.

Table 9. PCR amplification primers developed to amplify the PGD gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
pgdfor	GGAGCCGACTCGCTNGARGAYATG	Brisson et al. (2004)	1468	F	24	<i>Drosophila</i> (Drosophilidae)
pgdrev	CGCGGCCTCGTGCCNCCNGGCAT	Brisson et al. (2004)	3151	R	24	<i>Drosophila</i> (Drosophilidae)
PGDF	AARATGGTNCAYAAAYGGNAT	Scott et al. (1993)	3270	F	20	<i>Ceratitis</i> (Tephritidae)
2F	ATHGARTAYGGNGAYATGCA	Regier (2008)	3288	F	20	Universal
2.5AF	ATGAARACCCTYGGCATGTC	Winkler et al. (2009)	3333	F	20	Agromyzidae
PGD-Dipt-3495F	GGNACNGGNAARTGGAC	New	3495	F	17	Brachycera
2.5R	ATRC AACNCCRCGCCACAT	Winkler et al. (2009)	3795	R	20	Agromyzidae
PGD-Dipt-3805F	GGNTGYATHATHMGNAGG	New	3805	F	18	Brachycera
PGDR	CTRTGNGCNCCRAARTARTC	Scott et al. (1993)	4056	R	20	<i>Ceratitis</i> (Tephritidae)
4R	CCNGTCCARTTNGTRTC	Regier (2008)	4107	R	17	Universal

The 3' location based on published *D. melanogaster* sequence (Scott and Lucchesi 1991). Direction F, forward; R, reverse. Sequences in bold are newly developed for this study.

Three analyses of the phylogenetics of Culicidae included eleven unique primers (Sallum et al. 2002, 2007; Pradeep Kumar et al. 2007). Otranto et al. (2003) developed two COI primers in their study of Oestridae. Dallas et al. (2003) developed improved versions of two COI primers as part of their study of Ceratopogonidae. Lehr et al. (2005) included three unique primers in their study of cryptic species of *Anopheles* (Culicidae). Analyses of species of Drosophilidae (Lewis et al. 2005, Oliveira et al. 2005, Wang et al. 2006, He et al. 2009) have included ten unique primers. Two primers were designed based on published gene sequences of several species of *Bactrocera* (Tephritidae) (Shi et al. 2005). Ekrem (2006) designed two primers using sequences of Chironomidae. A phylogenetic study of Dolichopodidae (Bernasconi et al. 2007) included three COI primers. A study of tsetse flies (Glossinidae) yielded one unique COI primer (Dyer et al. 2008). As part of study on universal DNA mini-barcode, Meusnier et al. (2008) developed two new primers. Two more primers were developed as part of research on the systematics of Sarcophagidae (Song et al. 2008). Virgilio et al. (2009) developed two COI primers designed to be specific to the genus *Dacus*

(Tephritidae). Another study of Tephritidae (Sayar et al. 2009) included two unique primers. Also working with Tephritidae, Van Houdt et al. (2010) developed seven unique primers to facilitate DNA barcoding of museum specimens. A phylogenetic study of Clusiidae (Lonsdale et al. 2010) included eight additional COI primers. Wahlberg (2010) and the members of the Nymphalidae Systematics Group published two unique primers for Nymphalidae online that have been successfully used for Diptera.

In addition, many studies have developed species-specific COI primers for use in population genetics and identification studies. We list them here but do not include them in our primer table or map. Species-specific COI primers exist for species in: *Chrysomya*, *Lucilia*, and *Hemipyrellia* (Calliphoridae) (Chen et al. 2004, Saigusa et al. 2005); *Culicoides* (Ceratopogonidae) (Pagès and Sarto i Monteys 2005, Nolan et al. 2007, Matsumoto et al. 2009, Pagès et al. 2009, Schwenkenbecher et al. 2009); *Aedes*, *Anopheles*, and *Culex* (Culicidae) (Morlais and Severson 2002, Van Bortel et al. 2002, Hemmerter et al. 2007, Pedro and Sallum 2009); *Drosophila* (Drosophilidae) (Spicer 1995, Goto et al. 1999, de Brito et al. 2002, Dyer and

Table 10. PCR amplification primers developed to amplify the TPI gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
tabmothF	CTNGCNGGNACYTGAA	Tyshenko and Walker (1997)	2267	F	17	Tabanidae
TPI-Dipt-2276F	GGAACCTGGAAGATGAACGG	New	2276	F	19	Brachycera
1F	AAATGGAARATGAAYGG	Regier (2008)	2276	F	17	Universal
TPI 111Fb	CGNAAATGGAARATGAAYGG	Bertone et al. (2008)	2276	F	20	Diptera
TPI-2	TGGAAGATGAAAYGGNGAYATGC	Tittiger et al. (1993)	2285	F	23	<i>Culex</i> (Culicidae)
TPI-Dipt-2459F	GGHGCNTTYACNGGNGA	New	2459	F	17	Brachycera
TPI-Dipt-2525F	GGNCAYTCNGARCGHCG	New	2525	F	17	Brachycera
TPI-Dipt-2735R	GCCASCASGGYTCTASGC	New	2735	R	20	Brachycera
275R	GCCCANACNGGYTCRTANGC	Bertone et al. (2008)	2735	R	20	Diptera
tabR	GCCCANACNGGYTCUTA	Tyshenko and Walker (1997)	2735	R	17	Tabanidae
2R	GCCCANACNGGYTCRTA	Regier (2008)	2735	R	17	Universal
277R	CCDATNGCCCANACNGGYTC	Bertone et al. (2008)	2740	R	20	Diptera
TPI-Dipt-2740R	CCDATYGGCCAVMMBGGYTC	New	2740	R	20	Brachycera
mosR	GTCTGGCGTTGACAACT	Tyshenko and Walker (1997)	3024	R	18	Culicidae
TPIREV	GTCTGGCGTTGACAACTG	Tittiger et al. (1993)	3026	R	20	<i>Culex</i> (Culicidae)

The 3' location based on published *D. melanogaster* sequence (Shaw-Lee et al. 1991). Direction F, forward; R, reverse. Sequences in bold are newly developed for this study.

Table 11. PCR amplification primers developed to amplify the *white* gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
wh-S1	TYGCNTATGTNCARCARGA	Tachi and Shima (2010)	11275	F	20	Tachinidae
11404S	TYGCNTATGTNCARCARGAYGA	Baker et al. (2001)	11278	F	23	Diopsidae
white-Dipt-11297F	GAYTNTTYATWGGNTC	New	11297	F	17	Brachycera
white-Dipt-11315F	GGNTCNHTNACNGCMGNGARCA	New	11315	F	23	Brachycera
white-Dipt-11563R	GGNYTNGAYTCNTTYAYGGC	New	11563	R	20	Brachycera
white-Dipt-11739R	GCNGYNGAYTYYTYDCNTACT	New	11739	R	22	Brachycera
wh-R	ATGTARTRTRTRGGNCANTGNGCRCC	Tachi and Shima (2010)	11828	R	26	Tachinidae
WZ2E	AAYTAYAAAYCCNGCNGAYTYYTA	Besansky and Fahey (1997)	11844	F	23	Culicidae
wh-A1	ACYTGNACRTAAAARTCNCG	Tachi and Shima (2010)	11849	R	20	Tachinidae
11975R	ACYTGNACRTAAAARTCNCGNGG	Baker et al. (2001)	11852	R	23	Diopsidae
WZ11X	TTNARRAARAANCCNCCRAA	Besansky and Fahey (1997)	12866	R	20	Culicidae

The 3' location based on published *Drosophila melanogaster* sequence (O'Hare et al. 1984). Direction F, forward; R, reverse. Sequences in bold are newly developed for this study.

Jaenike 2004, Nunes et al. 2008); *Gasterophilus* (Oestridae) (Pawlas-Opiera et al. 2010); *Simulium* and *Prosimulium* (Simuliidae) (Finn et al. 2006, Gaudreau et al. 2010); *Lydella* and *Pseudoperichaeta* (Tachinidae) (Agusti et al. 2005); and *Bactrocera* (Tephritidae) (Yu et al. 2004).

We have developed eight new primers for the COI gene region. The naming is based on the published mitochondrial genome of *D. yakuba* (Clary and Wolstenholme 1985). In total, 141 primers are listed and mapped (Fig. 3; Table 4). The entire length of the gene region (≈ 1540 bp) can be sequenced using listed primers. There are no known introns within the COI gene region.

28S. The first published 28S primers specific to Diptera (Hillis and Dixon 1991) were those based on the

published genome of *D. melanogaster* (Tautz et al. 1988). Nine primers were included with little difference in primer sequence across Eukaryote taxa. These primers were also published with reverse-complement primers given separate names. They are not included separately on our map or table. Four primers, designed especially to amplify the D2 and D10 expansion segments of 28S were designed for species of *Drosophila* (Drosophilidae) (Ruiz Linares et al. 1991). Two primers were designed to amplify the D2 expansion segment of species of *Anopheles* (Culicidae) (Porter and Collins 1996). Eight unique primers were designed to amplify the D4–D7 region of 28S for Culicomorpha (Pawłowski et al. 1996). Seven primers were designed to amplify the D1 and D7 expansion segments across Diptera (Friedrich and Tautz 1997).

Table 12. PCR amplification primers developed to amplify the *wingless* gene region using Diptera exemplars

Primer name	Sequence	First reference	3' location	Direction	bp	Developed for
1099-1118S	GAAATGCCNCARGARTGYAA	Baker et al. (2001)	1118	F	20	Diopsidae
LepWG1	GARTGYAARTGYCAYGGYATGTCTCG	Brower and DeSalle (1998)	1136	F	26	Nymphalidae
Wing-Dipt-1145F	GGYATGTCGTGNTCDIGYAC	New	1145	F	20	Brachycera
Földvári wg1	GTTAGAACATGTTGGATGCG	Földvári et al. (2007)	1166	F	20	Diopsidae
1147-1166S	GTTAGAACWTGYTGGATGCG	Baker et al. (2001)	1166	F	20	Diopsidae
Wing-Dipt-1181F	GGATGCCNYTVGCNAAYTTYCG	New	1181	F	22	Brachycera
Wing-Dipt-1226F	CGHTTYGAYGGNGCNTCNCG	New	1226	F	20	Brachycera
B&D wg3	GGATTCGATGGCCACACCGCTCCA	Baker and DeSalle (1997)	1232	F	26	Drosophilidae
Wing-Dipt-1393R	YGATGSCGATCGTATG	New	1393	R	16	Micropezidae
Wg290F	GCWTRACTCACAGYATCGC	Pilgrim et al. (2008)	1459	F	20	Vespoidea
1448-1469A	GAATNCGTGATACACTRTTCG	Baker et al. (2001)	1470	R	22	Diopsidae
1451-1471S	AYAGTGTATCACGNAATTCGG	Baker et al. (2001)	1471	F	21	Diopsidae
Wing-Dipt-1505F	GGACGNGGACGTCARGG	New	1505	F	17	Micropezidae
B&D wg1	CCCCTCGRTACTGAAACGA	Baker and DeSalle (1997)	1539	F	20	Drosophilidae
B&D wg2	GGAGTCAACAAGACTGTCTTTGA	Baker and DeSalle (1997)	1575	R	24	Drosophilidae
1597-1617A	ATYTTTTTCRCAAAARCTTGG	Baker et al. (2001)	1617	R	21	Diopsidae
Wing-Dipt-1703R	CCRCARCACATYARRTCRCA	New	1703	R	20	Brachycera
1723-1742A	CGYTCNACNACAATRACCTC	Baker et al. (2001)	1751	R	20	Diopsidae
Földvári wg2	CGTTCAACGCAARTGACCTC	Földvári et al. (2007)	1751	R	20	Diopsidae
Wing-Dipt-1771R	GCAAGCACCAGTGGAAATGTRC	New	1771	R	21	Brachycera
PompWg2 rev	ACTGCCGAGCACCAGTGGAAATGTRCA	Pilgrim et al. (2008)	1774	R	26	Vespoidea
LepWG2a	ACTICGCARCACCARTGGAATGTRCA	Brower and DeSalle (1998)	1774	R	26	Nymphalidae
LepWG2	ACTICGCARCACCARTGGAATGTRCA	Brower and DeSalle (1998)	1774	R	25	Nymphalidae
1756-1775A	ACYTCRCARCACCARTGRAA	Baker et al. (2001)	1775	R	20	Diopsidae

The 3' location based on published *D. melanogaster* sequence (Rijeswijk et al. 1987). Direction F, forward; R, reverse. Sequences in bold are newly developed for this study.

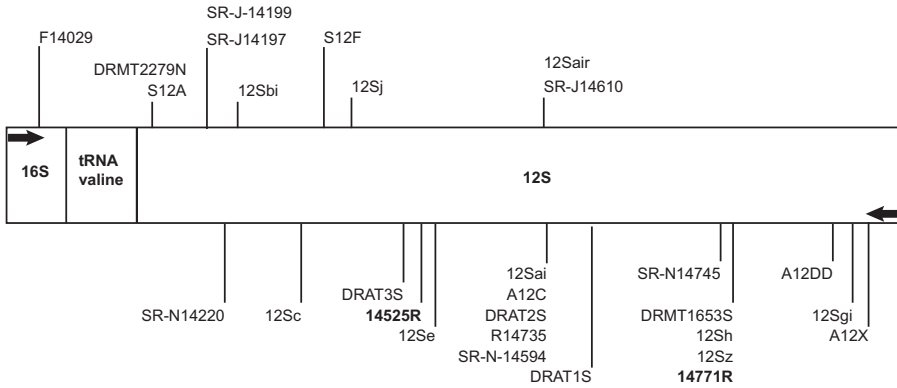


Fig. 1. Map of the 12S gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 2. Primers in bold are newly designed for this study and have had their name abbreviated from “12S-Dipt-xxxxR.” Map is not to scale.

A study of the phylogenetic relationships within Simuliidae yielded nine new 28S primers (Moulton 2000). Four new primers were designed to sequence representatives of Tachinidae (Stireman 2002, Tachi and Shima 2010). Five primers were designed to amplify the D4–D7b region for Tephritoidea (Han et al. 2002). In a comparison of rates of molecular evolution between parasitic Diptera and parasitic Hymenoptera, including representatives of 14 dipteran families, Castro et al. (2002) developed two 28S primers. Nineteen primers, designed for use in Diptera, were developed as a part of a series of studies of the relationships of Therevidae, Tabanomorpha, “lower Diptera,” and Clusiidae (Wiegmann et al. 2000, Yang et al. 2000, Bertone et al. 2008, Lonsdale et al. 2010). Both forward and reverse-complement versions were developed for most of these new primers, and only the unique primer sequences are included in our table and map.

We have developed a further 20 new primers for the 28S gene region. The numbers in the names are based on matching 3’ positions compared with the published ribosomal RNA sequence for *D. melanogaster* (Tautz et al. 1988). In total, 89 primers are listed and mapped

(Fig. 4; Table 5). Although 3’ location numbers of a pair of primers can provide a rough guide to the length of the segment being amplified, variation in expansion segment length, especially D2, D8, and D10, can lead to large fluctuations in amplified sequence length between taxa. These primers can be used in combination to sequence nearly the entire gene, including all expansion segments (≈ 3945 bp for *D. melanogaster*).

AATS. This gene region has only recently been developed for use in dipteran phylogenetics. The compilation of Regier (2008) of nuclear gene region primer sequences for use across Arthropoda includes three AATS primers. Four unique primers are included in a phylogenetic analysis of Sepsidae (Feng-Yi Su et al. 2008). Although none of the primers listed in this paper are attributed to a source, these primers were developed for use in the FLYTREE Assembling the Tree of Life project (Wiegmann et al. 2011). These primers also were used in studies of Asilidae (Dikov 2009) and Schizophora (Gibson et al. 2010b).

We have developed eight new primers for the AATS gene region. The naming is based on the published genome of *D. melanogaster* (Adams et al. 2000). In total, 15

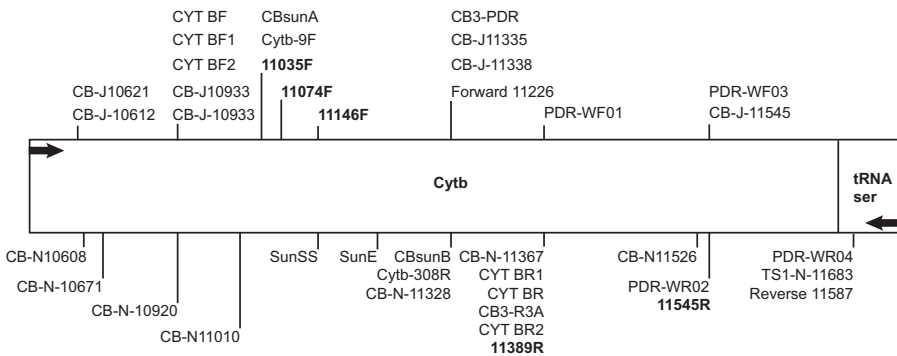


Fig. 2. Map of the Cytb gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 3. Primers in bold are newly designed for this study and have had their name abbreviated from “Cytb-Dipt-xxxxF/R.” Map is not to scale.

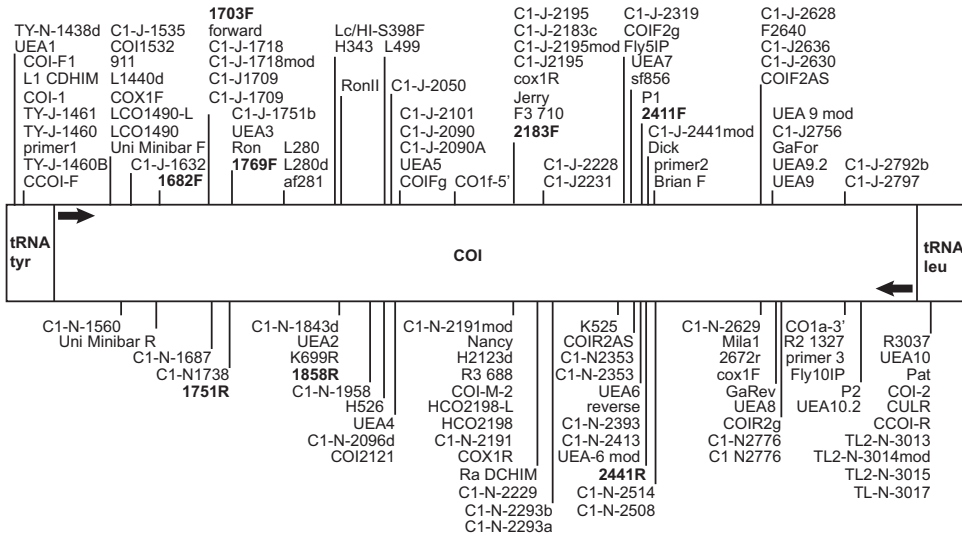


Fig. 3. Map of the COI gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 4. Primers in bold are newly designed for this study and have had their name abbreviated from “COI-Dipt-xxxxF/R.” Map is not to scale.

primers are listed and mapped (Fig. 5; Table 6). A large portion of the gene region (≈ 1200 bp) can be sequenced using listed primers. There are no known introns within the alanyl-tRNA synthetase gene region.

CAD. In their analysis of the phylogenetic relationships within Eremoneura, Moulton and Wiegmann (2004) developed eighteen CAD primers and used them to amplify and sequence representatives of 18 families of Diptera. Five more unique primers were added in a pair of studies on the phylogenetics of Agromyzidae (Scheffer et al. 2007, Winkler et al. 2009). Regier (2008) added 14 additional primers, designed to be useful across Arthropoda. Two more

CAD primers were developed as a part of a study involving species of *Ceratitis* (Tephritidae) (Barr and Wiegmann 2009). Four more CAD primers were developed as a part of research on the systematics of Clusiidae (Lonsdale et al. 2010).

We have developed 25 novel primers using our current data set. Naming of the new primers is based on the published CAD sequence for *D. melanogaster* (Freund and Jarry 1987). In total, 64 primers are listed and mapped (Fig. 6; Table 7). Using the given primers, the entire carbomoylphosphate synthase domain of CAD (≈ 4000 bp for *D. melanogaster*) can be sequenced including a number of taxon-specific introns.

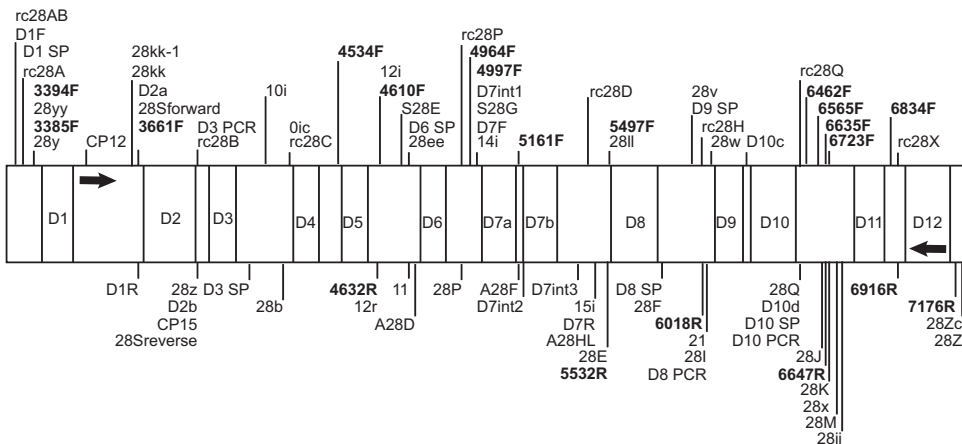


Fig. 4. Map of the 28S gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 5. Primers in bold are newly designed for this study and have had their name abbreviated from “28S-Dipt-xxxxF/R.” Symbols D1–D12 denote expansion segments as identified and labeled in Hancock et al. (1988). Map is not to scale.

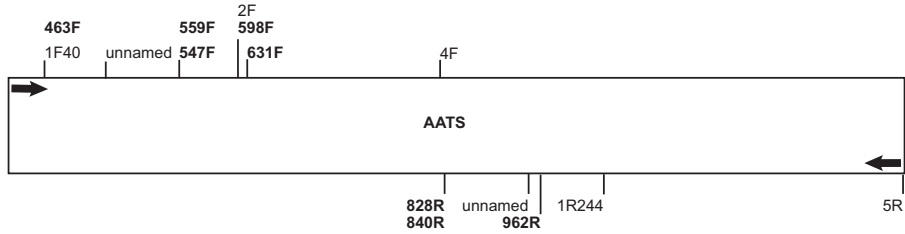


Fig. 5. Map of the AATS gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 6. Primers in bold are newly designed for this study and have had their name abbreviated from “AATS-Dipt-xxxxF/R.” Map is not to scale.

EF-1 α . Although developed as a part of a study on the phylogenetics of heliothine moths, Cho et al. (1995) designed ten EF-1 α primers using published sequences, including *Drosophila*. These primers have been used in many subsequent Diptera phylogenetic papers (Baker et al. 2001, Gibson et al. 2010b). Palumbi (1996) added three unique primers in his book chapter on PCR primers of use across Animalia. A study of the phylogenetic relationships within Simuliidae (Moulton 2000) included seven EF-1 α primers. Research into the systematics of Psychodidae (Essegir et al. 2000) included two unique primers. In their study of higher level phylogenetics of Therevidae, Yang et al. (2000) included six new EF-1 α primers. Four primers were developed as part of a study of the molecular systematics of Diopsidae (Baker et al. 2001). Stireman (2002) developed two unique primers as part of his study of Tachinidae. Collins and Wiegmann (2002) included a modification of an existing primer in their analysis of relationships within Empidoidea. Regier, in the online guide to primer development (Regier 2008), included 10 unique, and quite degenerate, EF-1 α primers, designed to be useful across Arthropoda.

We add eight new EF-1 α primers based on our data set. Naming of the new primers is based on the published EF-1 α sequence for *D. melanogaster* (Hovemann et al. 1988). In total, 53 primers are listed and mapped (Fig. 7; Table 8). Using these primers, almost

the entire coding region of EF-1 α (\approx 1200 bp) can be amplified and sequenced.

PGD. The first PGD primers for Diptera were those developed by Scott et al. (1993) as part of an attempt to map the gene region within *C. capitata* (Tephritidae). Two primers were designed to amplify intron II of PGD in *Drosophila* (Drosophilidae) (Brisson et al. 2004). In his large compendium of primer sequences, Regier (2008) includes two unique primers useful for amplifying PGD across Arthropoda. Two additional primers were developed for a phylogenetic study of Agromyzidae (Winkler et al. 2009).

To these existing primers, we add two new primers. The names are assigned according to the nucleotide numbering system of the PGD gene region of *D. melanogaster* (Scott and Lucchesi 1991). In total, 10 primers are listed and mapped (Fig. 8; Table 9). These primers can be used to amplify most of exon II, all of intron II, and nearly all of exon III of the PGD gene region (\approx 2600 bp).

TPI. The first TPI primers developed for use in Diptera were two developed as part of a study of the gene region within species of *Culex* (Culicidae) (Tittiger et al. 1993). A study of intron development in TPI among representatives of Tabanidae, Culicidae, and *Heliothis* moths included three unique primers (Tyshenko and Walker 1997). A phylogenetic analysis of “lower Diptera” included three more primers (Bertone et al. 2008). The online

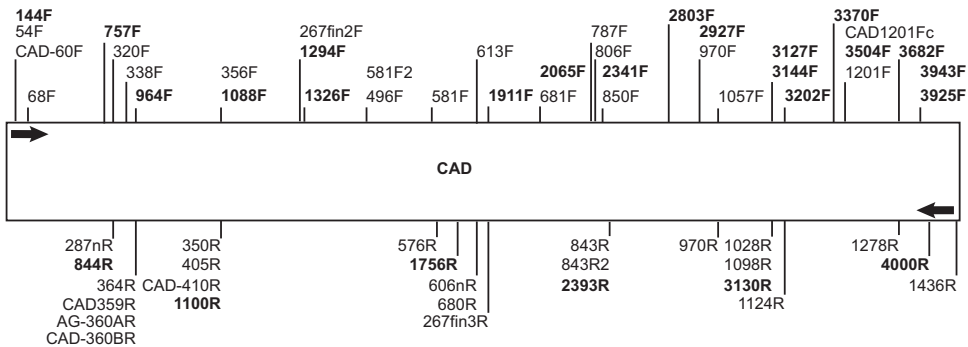


Fig. 6. Map of the CAD gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 7. Primers in bold are newly designed for this study and have had their name abbreviated from “CAD-Dipt-xxxxF/R.” Map is not to scale.

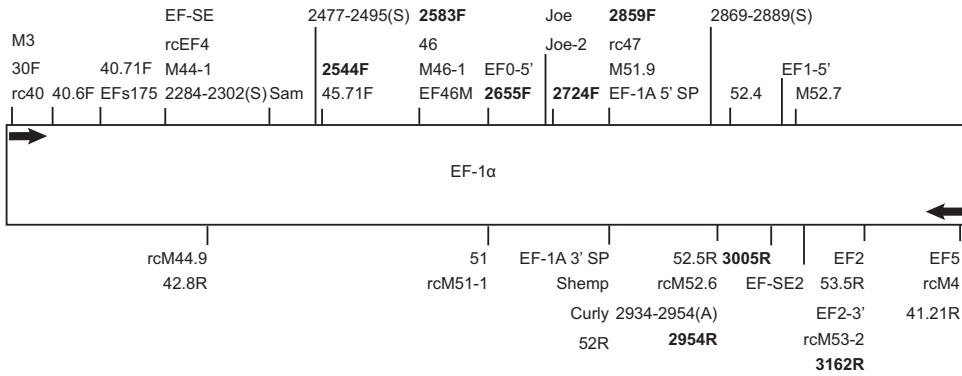


Fig. 7. Map of the EF-1 α gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 8. Primers in bold are newly designed for this study and have had their name abbreviated from “EF1 α -Dipt-xxxxF/R.” Map is not to scale.

compendium of primers for nuclear genes includes two TPI primers (Regier 2008). It should be noted that all of the existing primers represent variations on a single forward location and two reverse locations within the TPI gene region.

We have developed five new primers for the TPI gene region, including two new internal sites. The naming is based on the published TPI gene map of *D. melanogaster* (Shaw-Lee et al. 1991). In total, 15 primers are listed and mapped (Fig. 9; Table 10). Using the existing primers, the entire length of the gene region (≈ 759 bp) can be sequenced, including intron locations identified for *Drosophila* (Drosophilidae) (Shaw-Lee et al. 1991) and *Culex* (Culicidae) (Whyard et al. 1994, Tyshenko and Walker 1997).

White. A study of relationships among species of Culicidae included two unique *white* primers (Besansky and Fahey 1997). A multigene study of relationships among species of Diopsidae included two additional *white* primers (Baker et al. 2001). In their research on the molecular phylogeny of Tachinidae, Tachi and Shima (2010) added three more primers.

We have developed four new *white* primers. They are named using a modification of the *D. melanogaster* chromosome numbering system (O’Hare et al. 1984).

In the original reference, the gene is numbered from +11050 through 0 to -3050. For ease of use, these numbers have been converted to 1–14100. In total, 11 primers are listed and mapped (Fig. 10; Table 11). Primers exist to amplify nearly all of exon III, and all of intron III, exon IV, intron IV, exon V, and intron V (≈ 1500 bp for *D. melanogaster*). Care should be taken because the presence, size, and location of introns within the *white* gene region vary greatly across Diptera (Gomulski et al. 2001).

Wingless. Although the *wingless* gene region has been found to be highly conserved across Brachycera (Mellenthin et al. 2006), it belongs to the *Wnt* family of genes. As many as seven homologs of the *wingless* gene region have been discovered to date in *D. melanogaster* (Sidow 1992, Mellenthin et al. 2006). The homologs seem to be highly conserved such that multiple copies can be amplified using the same primer pairs, leading to an inability to establish homology between DNA sequences of different taxa (Gibson et al. 2010b).

The first *wingless* primers designed specifically for use in Diptera were those developed as part of a study of Hawaiian Drosophilidae (Baker and DeSalle 1997). Seven unique primers were developed specifically for

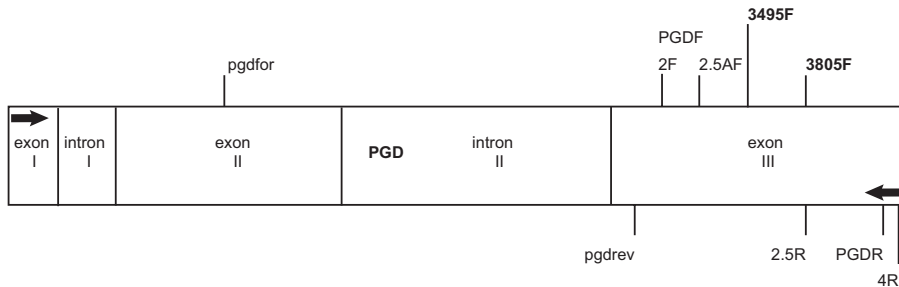


Fig. 8. Map of the PGD gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 9. Primers in bold are newly designed for this study and have had their name abbreviated from “PGD-Dipt-xxxxF/R.” Map is not to scale.

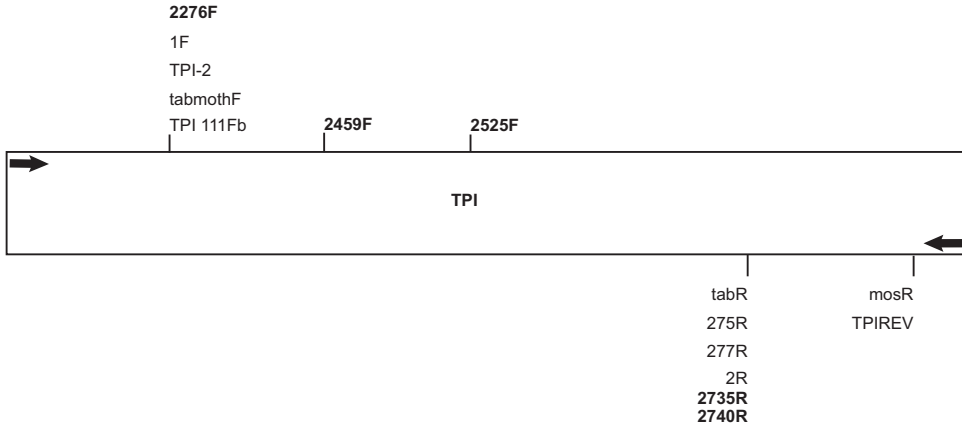


Fig. 9. Map of the TPI gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 10. Primers in bold are newly designed for this study and have had their name abbreviated from “TPI-Dipt-xxxxF/R.” Map is not to scale.

use in Diopsidae by Baker et al. (2001). Also working within Diopsidae, two additional *wingless* primers were developed by Földvári et al. (2007). Although not designed as a part of phylogenetic studies of Diptera, *wingless* primers designed for use in Nymphalidae (Lepidoptera) (Brower and DeSalle 1998) and Vespoidea (Hymenoptera) (Pilgrim et al. 2008) have been used extensively in dipteran phylogenetic studies (Kotrba and Balke 2006, Kronforst et al. 2007, Gibson et al. 2010b).

We have developed seven new primers for the *wingless* gene region. Two of these primers were designed to be most useful for specimens of Micropezidae. The naming is based on the published genome of *D. melanogaster* (Rijsewijk et al. 1987). In total, 24 primers are listed and mapped (Fig. 11; Table 12). A large portion of the gene region can be sequenced using these primers (≈650 bp for *D. melanogaster*).

In conclusion, in reviewing past literature, we noticed many instances in which the original references for the primers being used in a study were not easily determined. In some cases, this led to “novel” primers being proposed that had, in fact, been published previously. These instances lead to many problems, not least of which is confusion as to the name of a given

primer. We also noted that in some cases, a primer’s location within the gene region or even the direction of the primer was not included in a publication. These situations lead to difficulty in determining the potential usefulness of a primer in combination with other published primers.

We attempt to improve the present situation with this study. We record 399 previously published unique primers for 11 different gene regions. To this we add 94 primers newly designed as part of this study. We have designed our new primers to be an exact match across all brachyceran families included, yet still with a minimum of degeneracy. Generally, we also have targeted primer locations that will allow all gene regions to be sequenced in as small as 500-bp segments. It should be noted as well that a reverse-complement version of any of our primers could be used as a primer in the same location in the opposite direction. We feel that because our new primers have been designed to match exactly across Brachycera, they should be the first choice for anyone attempting to sequence these gene regions for any brachyceran specimen.

We have attempted to make all existing and new primers as user-friendly as possible through the use of detailed primer tables, gene maps, and a descriptive

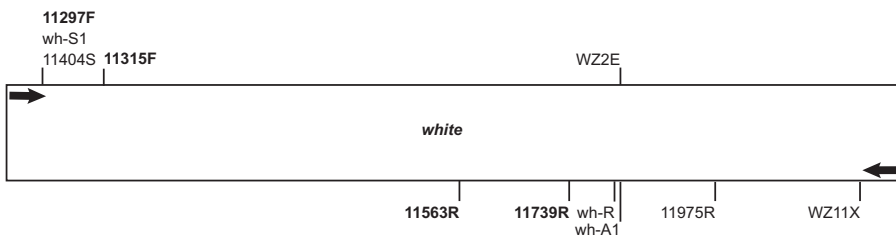


Fig. 10. Map of the *white* gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 11. Primers in bold are newly designed for this study and have had their name abbreviated from “white-Dipt-xxxxF/R.” Map is not to scale.

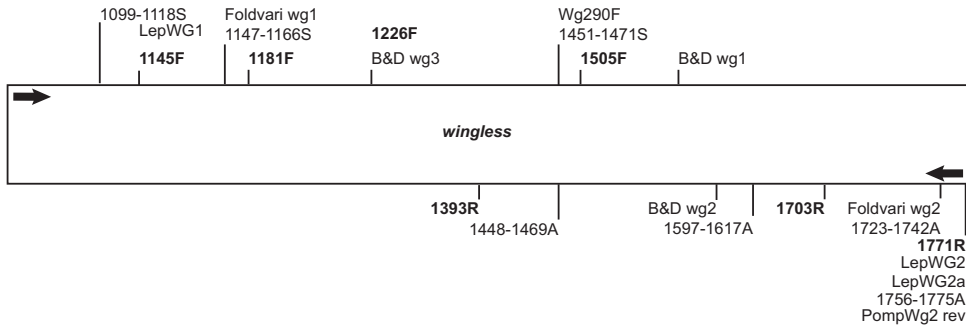


Fig. 11. Map of the *wingless* gene region locating all unique PCR amplification primers designed using Diptera exemplars. Primers above are in the forward direction; primers below are in the reverse direction. Original references and oligonucleotide sequences for each primer are given in Table 12. Primers in bold are newly designed for this study and have had their name abbreviated from “Wing-Dipt-xxxxF/R.” Map is not to scale.

naming system for new primers. With this information, anyone attempting to begin or continue a molecular phylogenetic project with dipteran specimens will have a large number of choices of both gene regions and PCR amplification primers. Although our research has been specifically focused on dipteran taxa, we believe that a similar approach could be taken in any other target group of organisms. The cataloguing, mapping, and proper naming of all existing and newly developed PCR amplification primers can only expedite the generation of DNA sequence data for use in phylogenetic study.

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