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New nuclear and mitochondrial primers for systematics and comparative genomics in Drosophilidae.

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Introduction

Several reviews of both mitochondrial (Simon *et al*., 1994) and nuclear (Brower and DeSalle, 1994) primers useful for molecular systematics and molecular evolution have recently been published. Our laboratory has been developing a battery of primers capable of amplifying a wide range of Drosophilid species. Here we report on a number of primer pairs useful for examining a wide range of divergences (from the population to genus level). Primer design and amplification protocols for high throughput applications can be found in Zilversmit *et al*. (2002). These primers should prove useful to a researchers studying population genetics, molecular evolution and phylogenetic systematics in the family Drosophilidae.

Mitochondrial Primers

We have developed a series of primers that will amplify an entire *Drosophila* mitochondrion. Below are a number of primer pairs that work well in a large range of species and constitute about 1/4 of the mitochondrial sequence.

N2-J-1006	TAGGTGGACTACCTCCATTTTYAGG
C1-N-1560	TGTTCCTACTATTCCGGCTCA
C1-J-1718	GGAGGATTTGGAAATTGATTAGTTCC
C1-N-2191	CCCGGTAAAATTAAAATATAAACTTC
C1-J-2183	CAACATTTATTTTGATTTTTTGG
C1-N-2659	GCTAATCCAGTGAATAATGG
C2-J-3696	GAAATTTGYGGRGCWAATCATAG
A8-N-4102	AARTTTGTTATCATTTTC
C2-J-3696	GAAATTTGYGGRGCWAATCATAG
A8-N-4478	GTTGTGTATGATTAATTCAACC
C3-J-5014	TTATTTATTKTWTCWGAAGT
C3-N-5460	TCAACAAAGTGTCAGTATCA
C3-J-5041	TTATTTATTKTWTCWGAAGT

C3-N-5460	as above
C3-J-5778	TGAATGYGGRTTTGAYCC
N5-N-6708	GGTTCWATATGATTTATACC

Nuclear Primers

Nuclear primers have recently become used in an effort to examine a variety of phylogenetic questions. The complete genome sequence of *Drosophila melanogaster* (Adams *et al*., 2000) has made design of nuclear primers much more tractable. Below we list several that we have developed in our laboratory and are useful at a variety of levels.

Several primer pairs flank non-coding or highly variable regions in the species we have surveyed. CG3869, an unnamed gene of unknown function, has a large intron of up to 400 base pairs in some taxa. The bride of sevenless (*boss*) gene also contains an intron in some species. Short non-coding regions can also be found in sans fille (*snf*) and lethal (2) neighbor of *tid* (tumorous imaginal discs). The glass gene also has some interesting variation in some groups. Two other genes we have examined, seven in absentia (*sia*) and forkhead (*fkh*), show little variation, but amplify in a wide range of taxa, including vertebrates.

A number of other nuclear primers are also being explored in our laboratory. These include *wee*, extra sex combs (*esc*), and wingless (*wg*). Other primers have been designed to genes discovered by the *Drosophila melanogaster* genome project, but not associated with any phenotype or function. This latter class of primers is assigned only a "CG" number below. Finally, many of our primers have been engineered to contain the T7 and T3 universal priming sites. This facilitates rapid sequencing by high throughput methodology (Zilversmit *et al*., 2002). Some sequences we have had positive results with include *fkh*, glass, amylase (*amy*), *esc*, mago nashi (*mago*), *ntid*, *boss*, *snf*, and *sia*. All primers are listed 5'— 3'.

CG3869F	CCCAACATCTTCATCCTGAACAAYMGNTGGGA
CG3869R	GCGGACTGGGAGATGCAYTCYTCRAA
BossF1	ACCAGATGCCCTGGGGNGARAA
BossR1	TGGACAGGGAGCCGCKNARCCARTT
T3/BossF1	ATTAACCCTCACTAAAGACCAGATGCCCTGGGGNGARAA
T7/BossR1	AATACGACTCACTATAGTGGACAGGGAGCCGCKNARCCARTT
snfL	GAAGATGCGGGGCCARGCNTTYGT
snfR	GAACAGCATGGACAGCATCATYTCRTT
T3/snfL	ATTAACCCTCACTAAAGGAAGATGCGGGGCCARGCNTTYGT
T7/snfR	AATACGACTCACTATAGGAACAGCATGGACAGCATCATYTCRTT
ntidF1	GGGCCGCATCTTCGARCAYAARTGG
ntidR1	TGGAGGGGTAGGTGTTCCARCARTA
T3/ntidF1	ATTAACCCTCACTAAAGGGGCCGCATCTTCGARCAYAARTGG
T7/ntidR1	AATACGACTCACTATAGTGGAGGGGTAGGTGTTCCARCARTA
glass1	TTTCGATTGCGGCGGNTGYTTYGA

glass2	GCCGTGGTGCATGGTCATRTTCAT
T3/glass1	ATTAACCCTCACTAAAGTTTCGATTGCGGCGGNTGYTTYGA
T7/glass2	AATACGACTCACTATAGGCCGTGGTGCATGGTCATRTTCAT
sia1	TCGAGTGCCCCGTGTGYTTYGAYTA
sia2	GAAGTGGAAGCCGAAGCAGSWYTGCATCAT
T3/sia1	ATTAACCCTCACTAAAGTCGAGTGCCCCGTGTGYTTYGAYTA
T7/sia2	AATACGACTCACTATAGGAAGTGGAAGCCGAAGCAGSWYTGCATCAT
T3/fkhL	ATTAACCCTCACTAAAGTCCCTACTCCTACATCTCCCTGATHACNATG T7/fkhR AATACGACTCACTATAGCGCAGGTAGCAGCCGTTYTCRAACATRT
weeL	GCCTGGGCCGAGGAYGAYCAYATG
weeR	TCACGTGGCCCAGGTCNCCDATYTT
escL	GGCCATCAACGAGCTGAARTTYCAYCC
escR	TTCCAGCACACGATGGCRTTYTCRCA
T3/escL	ATTAACCCTCACTAAAGGGCCATCAACGACGTGAARTTYCAYCC
T7/escR	AATACGACTCACTATAGCGAACCACTGCACGCAGTCNACRTARTT
wgL	GCAGTTCCGGAACCGGMGNTGGAAYTG
wgR	GGACATGCCGTGGCACTTRCAYTCYTG
T3/amyF1	ATTAACCCTCACTAAAGCGCCCCTGGTGGGARMGNTA
T7/amyR1	AATACGACTCACTATAGCGCGCAGGCCCACNARYTCRCA
T3/magoL	ATTAACCCTCACTAAAGCCACAAGGGCAAGTTCGGNCAYGARTT
T7/magoR	AATACGACTCACTATAGCACTTCAGGTCCTGCACCARRTARTARAA

References: Adams, M.D., *et al.,* 2000, The genome sequence of *D. melanogaster*. Science 287: 2185-2215; Brower, A., and R. DeSalle 1994, Practical and theoretical considerations for choice of DNA sequence region in insect molecular systematics, with a short review of published studies using nuclear gene regions. Ann. Rev. Entomol. 87: 702-716; Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook 1994, Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Rev. Entomol. 87: 651-701; Zilversmit *et al.,* 2002, High Throughput Sequencing Protocols for a Survey of Genomic Characters in the Family Drosophilidae. Dros. Inf. Serv. 84: (this issue).