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

Bonacum, J., R. DeSalle, P. O'Grady, D. Olivera, J. Wintermute, and M. Zilversmit. Division of Invertebrate Zoology, American Museum of Natural History, New York, NY 10024, USA.

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).