
### 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 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**: TGTTCCCTACTATTTCCCGCTCA
- **C1-J-1718**: GGAGGATTTGGAAATGTAGTTCC
- **C1-N-2191**: CCCGGTAAATTAAAAATAAAACTTC
- **C1-J-2183**: CAACATTTATTTTGATTTTTGG
- **C1-N-2659**: GCTAATCCAGTGAAATGG
- **C2-J-3696**: GAAATTTGYGGRGCWAATCATAG
- **A8-N-4102**: AARTTTGTTATCATTTC
- **C3-J-5014**: TTATTTATTGTKTWTCCGAAGT
- **C3-N-5460**: TCAACAAGTGTCAGTATCC
- **C3-J-5041**: TTATTTATTGTKTWTCCGAAGT
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 CCAACATCTCTCACTCTGAACAAAYMGNTGGGA  
CG3869R GCAGACTGGGAGATGCAYTCYTCRAA

BossF1 ACCAGATGCCCTGCGGNGARAA  
BossR1 TGGACAGGGAGCGCKNARCCARTT

T3/BossF1 ATTAACCCTCACAAAGACCAGATGCCCTGCGGNGARAA  
T7/BossR1 AATACGACTCACTATAGTGGACAGGGAGCGCKNARCCARTT

snfL GAAGATGCCGCGGCGCCARGCNYTTYG  
T3/snfL ATTAACCCTCAGAAAGGAAGATGCNGCCARGCNYTTYG

T7/snfR AATACGACTCAGGACGGAGCGCCARGCNYTTYG

ntidF1 GGGCCGCATCGCTCGARCAAYARTG  
ntidR1 TGGAGGGTAGGTGTCCARCARTA

T3/ntidF1 ATTAACCCTCAGAAAGGACCGCATCTCGARCAAYARTG  
T7/ntidR1 AATACGACCTCAGGACGGAGGGTAGGTGTCCARCARTA

glass1 TTTCGATTGCCGGGNTGYTTYG