N O T E

Inexpensive Molecular Weight Markers[1]

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Two of the most expensive components of PCR and subsequent analysis of the amplified fragments using agarose or polyacrylamide gels are *Taq* DNA polymerase and molecular weight markers or standards. Each gel on which PCR amplification products are to be electrophoresed and visualized usually requires the use of one or more lanes of molecular weight markers to estimate the size of the PCR product. The cost of commercially available molecular weight markers varies. In general, a cost of about US$1-2 per lane is routine. For example, a 100 bp ladder (100-1500 bp; Cat. No. G2101; Promega Corp., Madison, Wi) is currently listed at US$99 (excluding shipping charges) for 50 lanes, yielding a cost of about US$2 per lane. The cumulative cost of assaying a large number gels (e.g., in population genetic studies) can be prohibitive. Therefore, we developed a set of inexpensive molecular weight markers that can be produced quickly and inexpensively in a laboratory using routine PCR. In addition, the method can be used to produce customized molecular weight markers to suit specific applications.

We developed two sets of markers. The first set (MW12S400) is more suited for DNA fragments of small (<400 bp) expected molecular weight and consists of 100 bp, 200 bp, 300 bp, and 400 bp fragments. The second set of markers (MW18S1000) is a 100 bp ladder with fragments ranging in size from 100-1000 bp in 100 bp increments. The MW12S400 marker set was derived from the sequence of a portion of the mitochondrial 12S rRNA gene of the wood-feeding cockroach, *Cryptocercus darwini* Burnside et al. (Aldrich et al. 2004, Biochem. Gen. 42: 149-163; Burnside et al. 1999, J. Kansas Entomol. Soc. 72: 361-378). We chose *C. darwini* because we have a large number of specimens of this species on hand due to our ongoing studies on the evolutionary genetics of *Cryptocercus* spp. The MW18S1000 marker set was derived from the 18S rRNA gene sequence (Tautz et al. 1987, J. Mol. Biol. 195: 525-542; GenBank accession no. M21017) of *Drosophila melanogaster* Meigen. We chose the

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18S rRNA gene because of its size (~1800 bp) and *D. melanogaster* because specimens of this fly are easily available worldwide. However, the molecular weight markers can be derived from any organism.

From the DNA sequence of each gene fragment, we manually designed primers to amplify the fragment of the desired size (Table 1). A common forward primer was coupled with a reverse primer to generate a specific fragment. Thus, for the 12S rRNA gene fragment of *C. darwini*, the primers were designed to amplify 100 bp, 200 bp, 300 bp, and 400 bp fragments. For the 18S rRNA gene fragment of *D. melanogaster,* the primers were designed to amplify fragments ranging from 100 bp to 1000 bp at 100 bp intervals.

DNA was extracted from individual *C. darwini* or from 10 individuals of *D. melanogaster* (wild type strain w1118) using the Qiagen DNAEasy kit (Qiagen, Inc., Va-

Table 1. Primer sequences for the amplification of 18S rRNA and 12S rRNA gene fragments. The 18SFOR primer was used in all amplifications of the 18S rRNA fragments in combination with one of the other 18S rRNA "R" primers. For example, PCR with 18SFOR and 18S100R results in the amplification of a 100 bp fragment from *D. melanogaster.* Similarly, PCR with 12SFOR and 12S100R results in the amplification of a 100 bp fragment from *C. darwini.*

Primer name	Primer sequence (5′ – 3′)
18S rRNA gene	
18SFOR	CTG GTT GAT CCT GCC AGT AG
18S100R	ATA ATG AGC CTT TTG CGG TT
18S200R	CAC ACG TCC CAT AAG GTT CA
18S300R	GAA AGA TCT GTC GTC GGT AC
18S400R	CTC AGG CTC CCT CTC CGG AA
18S500R	CGG ATA TGA GTC CTG TAT TG
18S600R	CTA TTG GAG CTG GAA TTA CC
18S700R	TAC ATA AAG GTA TAG TAC TA
18S800R	TCG TTT AAG AGC ACT AAT GC
18S900R	GTC TTA TTT CAT TAT CCC ATG
18S1000R	GTC CAA GAA TTT CAC CTC TC
12S rRNA gene	
12SFOR	TAC TAT GTT ACG ACT TAT
12S100R	GTA ATT ATT ATT TTA GAT AG
12S200R	TTC AGG TCA TGG TGC AAT
12S300R	CGT TGG ACC TTA CTT GAT T
12S400R	ATT GTT TAA CTT GAG TAG

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lencia, Ca) following manufacturer's instructions. PCR was set up in 50 µL volumes as described (Kambhampati et al. 1992, J. Med. Entomol. 29: 939-945). The amplification conditions for the MW12S400 fragments were: 10 cycles of 95°C for 30 sec, 45°C for 45 sec, 72°C for 30 sec followed by 25 cycles of 95°C for 30 sec, 50°C for 45 sec, 72°C for 30 sec. The MW18S1000 fragments were amplified at 95°C for 30 sec, 60°C for 45 sec, 72°C for 30 sec for 35 cycles. An initial denaturing step of 94°C for 3 min and a final extension step of 72°C for 10 min were added in both cases.

The PCR products were electrophoresed on a 1% agarose gel in TAE buffer. A 100 bp ladder (Bioline USA, Inc., Randolph, Ma.) was used to estimate the size of the amplified products. The gel was stained with ethidium bromide and the fragments visualized and photographed under UV light.

The results of the amplification of the MW12S400 and MW18S1000 sets are shown in Fig. 1. Each primer set resulted in a fragment of the predicted size as determined in relation to the commercial molecular weight marker. The combination of 18SFOR and 18S700R primers for the 700 bp fragment resulted in visible, but weak, amplification. To prepare the molecular weight markers for routine use, we combined equal quantities of each PCR product to yield a given set of molecular weight markers (Fig. 1). However, specific fragments can be made to appear brighter on the gel under UV light by adding an excess amount of those PCR products. A single set of amplifications of 50 µL each results in about 500 µL of molecular weight marker cocktail for the MW18S1000, sufficient for about 100 lanes. Furthermore, the

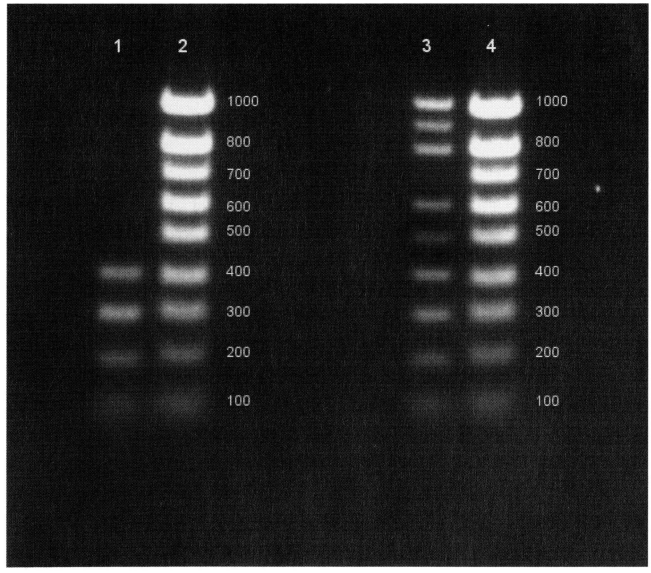

Fig. 1. Gel with molecular weight markers. Lane 1. MW12S400. Lanes 2 and 4. Commercial marker (Bioline USA, Inc.), Lane 3. MW18S1000. Sizes of fragments are indicated in base pairs. The commercial marker does not contain a 900 bp fragment. The 700 bp fragment in lane 3 is not visible because of weak amplification.

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molecular weight marker can be obtained in about 24 h, including the time required for DNA isolation, PCR amplification, and gel electrophoresis.

The cost of generating either molecular weight marker is extremely low. The cost of primer synthesis was US$0.35 per base, with the average length of each primer being 20 nucleotides, resulting in a total cost of about US$7 per primer. However, the amount of each primer synthesized is sufficient for undertaking over 1000 PCRs. We estimate the total cost of all PCRs for the MW18S1000 to be about US$2 resulting in a per lane cost of US$0.02, relative to US$1-2 per lane for commercial molecular weight markers. The cost of generating the MW12S400 marker set is even lower because of the fewer number of primers and PCRs involved.

Another advantage of the method we described here is the ability to customize the marker set for a specific application, for example, by making a cocktail of only a subset of the fragments. Primers can also be designed to amplify a fragment of any size.

A BLAST (Altschul et al. 1990, J. Molec. Biol. 215: 403-410) search indicated that most of the primers we developed match the respective genes of other insects as well. However, we caution against the use of these primers to amplify target fragments from species other than those used here because of the potential for size polymorphism. In the unlikely event that *D. melanogaster* specimens are not easily available, it is advisable to design a new set of primers for the 18S rRNA (or other) gene for the most easily available species.

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