used instead of the Vernier light probe. The IR detector was connected to an impedance amplifier (UFI, model 2991, Morro Bay, CA, USA) in which the output is then passed through an AD board (MacLab 4s interface, ADInstruments, INC; Colorado Springs, CO, USA) before being collected on a computer with the MacLab Chart (version 5) software. As with the earlier method, Styrofoam is used to cut out holders for aligning the infrared light path and glass alley way tube. The emitter and detector were aligned by use of a larger plastic pipette that had a cut away for placing the glass fly tube (Figure 1).

With either approach, Vernier or impedance detector, the IR emitter (model 276-142, Radio Shack) can be powered by a 9V battery for about 24 hrs. I found the battery strength runs down quickly. Alternatively, use of a DC power source from a 120V AC to a 9V DC transformer is suggested to be used. Each emitter requires a 66 Ohm resistance on one lead. With the transformer as a power source a number of emitters can be used. I used 8 in parallel with 1 resistor in series from the transformer the 8 emitters in parallel. The rate of acquisition, not to over collect but to catch quick movements, is best about 25,000 samples/hour for the Vernier software. Collecting data for 4 hours or less in each set keeps files small enough as to readily open and analyze. The impedance amplifier with the MacLab 4s interface one can collect for 8 hours at 1 KHz to sufficiently detect movements and keep files small enough to manage.

The responses from either method are then plotted as beam breaks over time for determining the activity of individual flies. These methods allow for various experimentations to be utilized, such as readily altering food sources, effects of compounds mixed with food and changes in environmental lighting (Sheward *et al*., 2007; Yoshii *et al*., 2007). Light conditions can be readily altered to total darkness or with visible light while still monitoring the adult locomotor activity if the IR emitter is used as a light source for the detector. Background level of absolute intensity on the Vernier detector will vary when using the IR emitter while the white light is turned on or off as the detectors pick up some of the white light signal.

I found the Vernier LabPro connected directly to a computer to be the easiest approach to set up and monitor activity. The impedance amplifier can saturate and requires monitoring often. Also the LabPro allows 4 probes to be connected simultaneously while the impedance amplifier monitors a signal detector. The net cost is also cheaper with the Vernier hardware as 4 detectors and one Vernier LabPro costs about $500 (USD) while each impedance amplifier costs about $350 (USD).

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References: Klarsfeld, A., J.C. Leloup, and F. Rouyer 2003, Behavioural Processes 64: 161-175; Pittendrigh, C.S., and S. Daan 1976, J. Comp. Physiol. [A] 106: 223–252; Sheward, W.J., E.S. Maywood, K.L. French, J.M. Horn, M.H. Hastings, J.R. Seckl, M.C. Holmes, and A.J. Harmar 2007, J. Neurosci. 27(16): 4351-4358; Yoshii, T., K. Fujii, and K. Tomioka 2007, J. Biol. Rhythms 22(2): 103-114.

Two robust multiplex PCR reactions for high-throughput microsatellite genotyping in *Drosophila melanogaster*.

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Microsatellites are currently among the most commonly used genetic markers for population genetic studies, because they allow the inference of several important population parameters, such as

population genetic structure, effective population size, and dispersal rate. Microsatellites are also commonly used for a range of other applications including paternity assignment, population of origin assignment, detection of population bottlenecks, and inbreeding assessment. Single amplification of microsatellite loci is time-consuming, expensive and can require large quantities of template DNA. An alternative to single amplification is multiplex PCR, where multiple microsatellite loci are amplified simultaneously in a single reaction. Despite all of the advantages of multiplex PCR and the common use of *D. melanogaster* in population genetics, there are very few published examples of successfully multiplexed microsatellite loci (England *et al.*, 1996; Schlötterer *et al.*, 1997). We have designed two robust PCR reactions that successfully amplified ten microsatellite loci, and we extensively tested these reactions in 176 *Drosophila melanogaster* populations. We present the basic population genetic data and PCR failure rates obtained from the 4,224 genotyped individuals.

We initially chose twelve microsatellite loci that provided whole genome coverage and minimised physical linkage of loci (Table 1). These twelve loci were organised into two multiplex reactions (MP1 and MP2) (Table 1). Loci with overlapping allele size ranges were labelled using different 5'-fluorescent labels on the forward primer (6-Fam, VIC, PET, NED) (Table 1). All unlabelled reverse primers were tailed with a commercially available seven base sequence (tail) that promotes +A DNA polymerase activity and thus reduces stutter-bands that are associated with poly-A tailing (Applied Biosystems, Foster City, California). Primers in a single multiplex reaction were checked for complementarity and hairpin structures using the programs MULTIPLEX MANAGER (Holleley and Geerts, in prep.) and AUTODIMER (Vallone and Butler, 2004).

Table 1.

	Reaction Conditions			General Microsatellite Information					Primer Sequences	
Short Name	Mplex	Fluro Dye	Primer Conc. Final Reaction	Published Name	Repeat	Chrom.	Cytological Location	Genetic Location (cM)	Forward/ Reverse (5' - 3')	References
Msat 1	MP1	6-FAM	0.2 µM	*DMZW3K25*	$(AT)_{14}$	X	3A	1	ATTGTCATTTTATTGCTGCCG	a, b
									TAACGAAGAGAGTTGCGGAGA	
Msat 2	MP1	6-FAM	0.2 µM	*su.var*	$(TG)_{12}$	II	29A5-B4	31	GGTTGCTGGGAGAAAGAC	c, d
									GCCACACATTCGCATCTC	
Msat 3	MP1	VIC	0.2 µM	*Cad-GA*	$(GA)_{11}$	II	38D4-E1	54	AGGCACTCTCTGGGCGAAAC	d, e
									CGTCACTAGGTCGGGGTATC	
Msat 4	MP1	NED	0.2 µM	*DROMHC*	$(CA)_{13}$	II	60 E9	107	AAACCCACACAACAACTGCA	a, b
									GACATTACCGATATTGGATGCA	
Msat 5	MP1	NED	0.2 µM	*DROGPAD*	$(GT)_{9}$	II	47A	62	GAAATAGGAATCATTTTGAATGGC	a, f
									AATTAAAAACAAAAAACCTGAGCG	
Msat 6	MP1	PET	0.2 µM	*3L8939767ct*	$(CT)_{10}$	III	66F	28	CCGTCCCGCTCTGGTTTGG	b, g
									GTTGCTGCTCCTCCGCTGA	
Msat 7	MP2	6-FAM	0.1 µM	*DRO17DC2Z*	$(CT)_{9}$	III	84	48	TTCGTGCAAAGGTGTTTTCC	a
									ATGCAGATACCAGAAACCGC	
Msat 8	MP2	6-FAM	0.4 µM	*DMU1951*	$(TA)_{16}$	III	93C	71	GGGTCTTTCTGCTTCAGTTACC	a, b
									GGAATACACGAATCCCCCTT	
Msat 9	MP2	VIC	0.1 µM	*Adh-TC*	$(TC)_{11}$	II	35B2-B4	50	CAGCACCAGCATCCAAGTAC	d, e
									AGTCTCTGTGGCAGTGTGAG	
Msat 10	MP2	NED	0.1 µM	*DMTENA*	$(TA)_4CC(AT)_{14}$	X	11A6	38	CTCTTAGTGCGCAGGGATTC	a, b
									GAGTCGCTCAATGGCAGG	
Msat 11	MP2	NED	0.1 µM	*G410*	$(TC)_{11}(TG)_4$	II	33E9-E10	46	TTCGGCTCTTTGTTTGCTTG	d, h
									AAGCTTAAACCGATCGAAAAC	
Msat 12	MP2	PET	0.1 µM	*DMTROPONI*	$(CA)_{11}$	X	16F3-6	59	CAAGAGATCCCGAGAGAGAGA	a, b
									ACGTGTGCGTGTTGTTTCTC	

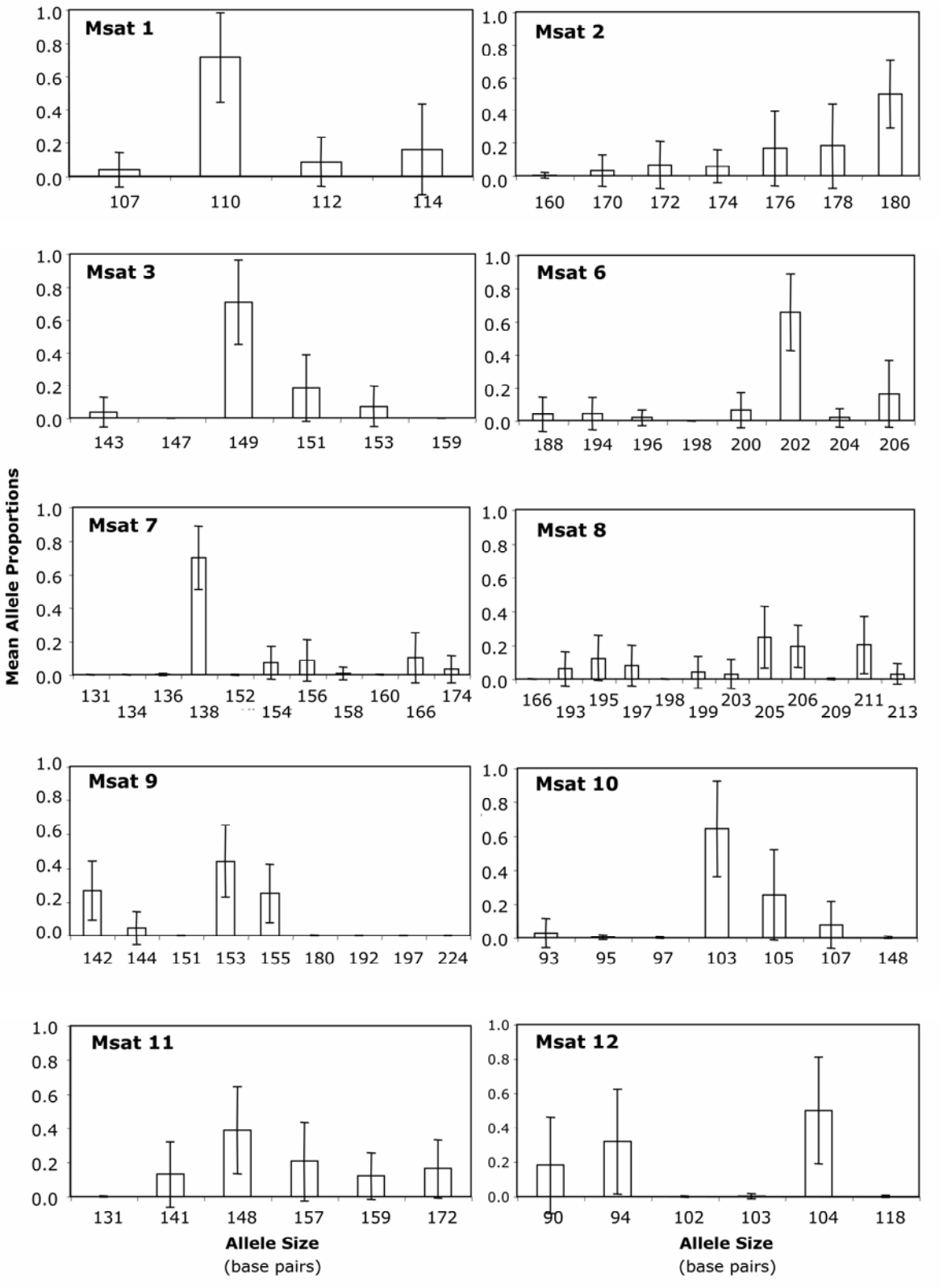

Figure 1. Mean allele proportions of the ten microsatellites amplified in multiplex reactions MP1 and MP2 (N = 176 populations). Bars indicate the standard deviation of the mean.

The PCR reactions consisted of QIAGEN® Multiplex Master Mix [1x], RNase-free water, approximately 20 ng of template DNA (Holleley, 2007) and an equimolar concentration of primers Msat 1 – Msat 6 [0.2μM] for multiplex one (MP1) (Table 1). In multiplex two (MP2), the concentration of Msat 8 was increased [0.4μM] relative to the equimolar concentration of the remaining primers Msat 7 – Msat 12 [0.1μM] to compensate for differential amplification efficiency among loci (Bercovich *et al.*, 1999) (Table 1). Both multiplex reactions were amplified using a step-down PCR protocol. The initial denaturing step of 15 minutes at 95˚C was followed by 10 cycles of 94˚C for 30 seconds, 64˚C for 90 seconds, 72˚C for 90 seconds. After this high stringency cycling, the annealing temperature was reduced in a step-wise fashion from the initial 64˚C to 60˚C in the next 10 cycles, then 56˚C in the next 10 cycles, and 50˚C in the final set of 10 cycles. After the 40 cycle step-down protocol was complete, there was a final extension period of 72˚C for 10 minutes. We found that MP1 amplified best if the above conditions were modified slightly, such that the extension temperature of the 10 × 53˚C annealing temperature cycles was 70˚C instead of 72˚C. This step-down PCR approach allowed co-amplification of primers with different annealing temperatures. Additionally the gradient of high stringency to relaxed stringency cycling increased specificity and yield (Henegariu *et al.*, 1997). This approach can result in preferential amplification of primers with higher annealing temperatures, thus we designed both multiplexes to minimise the range of annealing temperatures (MP1 55˚C – 57.3˚C; MP2 53˚C – 55˚C). PCR products were run on a 48-Capillary 3730 DNA Analyser (Applied Biosystems, Foster City, California), and fragment size analysis was conducted using GENEMAPPER® SOFTWARE 3.7 (Applied Biosystems 2004).

Table 2. Basic population genetic data for twelve microsatellite loci from 176 *Drosophila melanogaster* populations (*N* = 24 individuals per population). For each locus we present the size range of amplified fragments in base pairs, the total number of alleles observed over all populations (Total *Na*), the mean number of alleles per population (Mean *Na*), mean observed heterozygosity per population (H_o), mean expected heterozygosity (H_e), the percentage of populations that significantly deviated from Hardy-Weinberg equilibrium (HWE) expectations using Fisher's Exact test with Bonferroni correction and the percentage of PCR reactions that failed to amplify. Means are presented ± the standard deviation. Dash (–) indicates that information was not available.

Short Name	Published Name	Allele size range	Total *Na*	Mean *Na*	Mean H_o	Mean H_e	% HWE deviation	% Failure
Msat 1	*DMZW3K25*	106-114	4	2.1 ± 0.2	0.297 ± 0.2	0.279 ± 0.2	0	0.21
Msat 2	*su.var*	160-180	7	2.9 ± 0.1	0.503 ± 0.2	0.487 ± 0.1	1.1	0.14
Msat 3	*Cad-GA*	143-159	6	2.3 ± 0.2	0.350 ± 0.3	0.331 ± 0.2	1.1	0.05
Msat 4	*DROMHC*	-	-	-	-	-	-	-
Msat 5	*DROGPAD*	168-212	9	1.7 ± 0.2	0.071 ± 0.1	0.193 ± 0.2	29	1.35
Msat 6	*3L8939767ct*	188-206	8	2.8 ± 0.2	0.402 ± 0.2	0.403 ± 0.2	3.4	0.14
Msat 7	*DRO17DC2Z*	131-174	11	2.8 ± 0.2	0.392 ± 0.2	0.397 ± 0.2	2.3	0.36
Msat 8	*DMU1951*	166-213	12	4.9 ± 0.1	0.738 ± 0.1	0.695 ± 0.1	2.8	0.97
Msat 9	*Adh-TC*	142-224	9	3.2 ± 0.1	0.598 ± 0.2	0.558 ± 0.1	1.7	0.05
Msat 10	*DMTENA*	93-148	7	2.3 ± 0.2	0.359 ± 0.3	0.342 ± 0.2	0.6	0.71
Msat 11	*G410*	131-172	6	3.5 ± 0.2	0.584 ± 0.2	0.552 ± 0.2	3.4	0.21
Msat 12	*DMTROPONI*	90-118	6	2.2 ± 0.2	0.366 ± 0.2	0.351 ± 0.2	0	0.07

Both multiplexes had a low rate of PCR failure (0.07% – 1.4%) and a low mis-scoring error rate of 3.9% (Table 2). Microsatellite 4 (*DROMHC*) showed extreme allelic drop-out under these conditions and thus was excluded from analysis and not scored. Microsatellite 5 (*DROGPAD*) also exhibited evidence of allelic drop-out or null alleles as it had an excess of homozygotes (H_o = 0.071) and 29% of the populations significantly deviated from Hardy-Weinberg expectations (Table 2). Thus microsatellite 5 should be excluded from population genetic analyses. After excluding these two potentially compromised loci, MP1 and MP2 comprise ten microsatellite loci that amplify robustly and provide good but not complete genome coverage. The total number of alleles for the remaining ten loci ranges from 4 – 12 and the mean observed heterozygosity ranged from 0.3 – 0.7 (Table 2). The remaining ten loci adhered to Hardy-Weinberg expectations in 96.6 – 100% of populations, with the mean allele proportions displayed in Figure 1.

We have described a set of optimised PCR reaction conditions that, in conjunction with step-down PCR cycling conditions, result in robust, repeatable and non-preferential amplification of a suite of ten microsatellite markers. Thus this paper provides *Drosophila* researchers with an efficient and cost effective alternative to multiple single PCR reactions.

References: Bercovich, D., Z. Regrev, T. Ratz, A. Luder, Y. Plotsky, and Y. Gruenbaum 1999, BioTechniques 27: 762-770; Caracristi, G., and C. Schlötterer 2003, Molec. Biol. Evol. 20: 792-799; England, P.R., D.A. Briscoe, and R. Frankham 1996, Genet. Res. 67: 285-290; Goldstein, D.B., and A.G. Clark 1995, Nucleic Acids Res. 23: 3382-3386; Harr, B., 2000, Ph.D. Thesis, University of Konstanz Germany; Harr, B., and C. Schlötterer 2000, Genetics 155: 1213-1220; Henegariu, O., N.A. Heerema, S.R. Dlouhy, G.H. Vance, and P.H. Vogt 1997, BioTechniques 23: 504-511; Holleley, C.E., 2007, Dros. Inf. Serv. 90: 137-138; Schlötterer, C., C. Vogl, and D. Tautz 1997, Genetics 146: 309-320; Schug, M.D., T.F.C. Mackay, and C.F. Aquadro 1997, Nat. Genet. 15: 99-102; Schug, M.D., K.A. Wetterstrand, M.S. Gaudette, R.H. Lim, C.M. Hutter, and C.F. Aquadro 1998, Mol. Ecol. 7: 57-70; Swindell, W.R., and J.L. Bouzat 2006, Evolution 60: 1014-1022; Vallone, P.M., and J.M. Butler 2004, BioTechniques 37: 226-231.

An inexpensive and efficient method for obtaining *Drosophila* heads.

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During our investigation of calcium/calmodulin-dependent protein kinase II which is highly concentrated in the heads of *Drosophila melanogaster*, we have devised a method to quickly separate the heads of the flies from the bodies.

The flies are moved into a clear, empty vial with a foam cap. Then small chunks of dry ice are put into the vial. The carbon dioxide immobilizes flies within a few seconds, while the low temperature minimizes protein degradation. The vial is manually shaken for a few minutes until the wing tissue comes off, as evident by the clear specks on the sides of the vial. By this time most heads have snapped off. The contents of the vial (including remaining pieces of dry ice) are then examined under a dissecting microscope and the heads could be easily separated from the bodies using a fine brush or dissecting needle.