Optimization of 64 microsatellite loci primer pair annealing temperatures of *Drosophila mediopunctata*.

Cavasini, Renato, Marcos R.D. Batista, Klélia A. Carvalho, and Louis B. Klaczko.
Departamento de Genética, Evolução e Bioagentes, Instituto de Biologia, Universidade Estadual de Campinas – UNICAMP, Cx. Postal 6109, Campinas, 13083-970 SP, Brazil. E-mail: LBK@unicamp.br

Drosophila mediopunctata is a model organism that our research group has been studying for almost 30 years (Klaczko, 2006). Laborda *et al.* (2009) developed and characterized 134 microsatellite *loci* for this species. They performed PCR in a gradient temperature (*touchdown PCR*), and, although the temperature range was established, for many *loci* the exact annealing temperature (T_a) was not determined.

Preliminary tests using homokaryotypic strains from the laboratory and samples from natural populations of *D. mediopunctata* revealed unspecific alleles for several *loci*. In an attempt to make our genotyping more reliable, eliminating unspecific alleles from our analysis, we tried to set accurately the annealing temperature (T_a) for several *loci* primer pairs.

Genomic DNA was isolated from four individuals, two from each of a pair of standard homokaryotypic strains (*ITC 229ET* and *ITA 24P*). Polymerase chain reactions (*PCRs*) were performed in 25 µl reactions with: 11.5 µl of Milli-Q water, 1 µl of DMSO, 2.5 µl of buffer 10×, 2.5 µl of dNTP Mix (2 mM), 2 µl of $MgCl_2$ (25 mM), 1 µl of *Taq* DNA polymerase, 1.25 µl of each primer (10 mM), and 2 µl of genomic DNA (approximately 10 ng/µl). Thermocycler parameters were: 95°C for 5 min, 40 cycles of 94°C for 1 min, T_a for 1 min and 15 s, 72°C for 1 min and 30 s, 72°C for 30 min.

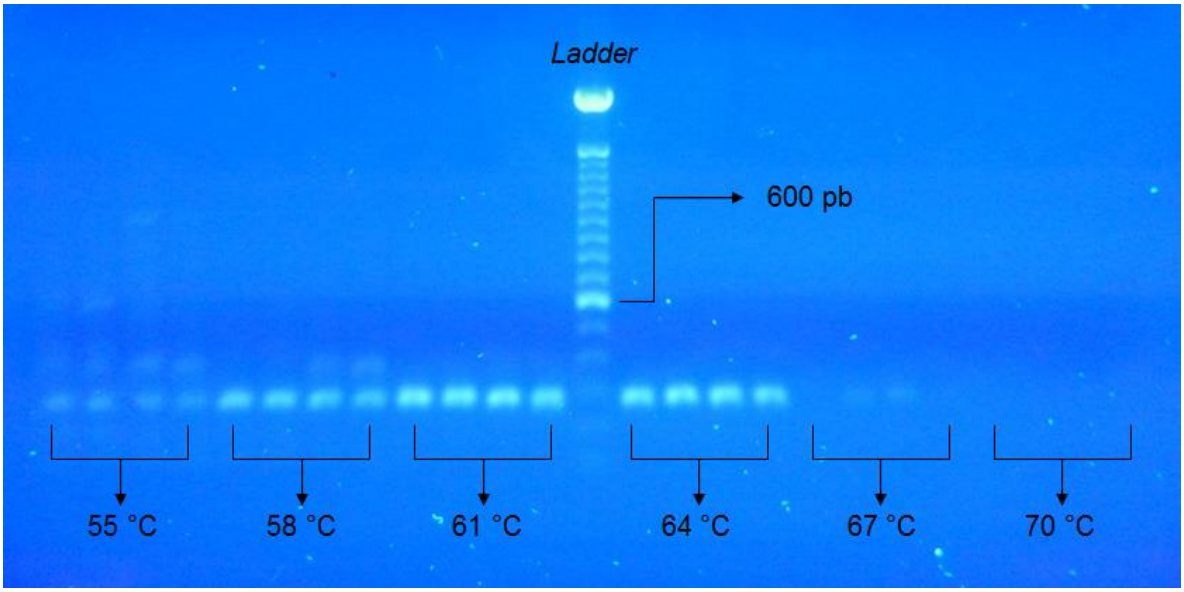

Figure 1. Locus *Dmed*048 amplified products visualized in 1% agarose gel (TBE 1×), stained with *SYBR-safe*. Annealing temperature (T_a) is 64ºC.

We tested the amplification of 64 *loci* in six specific annealing temperatures (T_a = 55ºC; 58ºC; 61ºC; 64ºC; 67ºC; and 70°C). The highest temperature that showed positive amplification with minimum unspecific alleles was assumed to be the optimal annealing temperature for each *locus* primer pair. PCR products were visualized in 1% agarose gel (TBE 1×) stained with *SYBR-safe* (Figure 1). Table 1 shows the specific annealing temperature settled for each locus primer pair.

Table 1. Annealing temperatures (T_a) of 64 primer pair loci of *Drosophila mediopunctata*.

Locus	T_a (ºC)	*Locus*	T_a (ºC)	*Locus*	T_a (ºC)	*Locus*	T_a (ºC)
*Dmed*003	58	*Dmed*040	58	*Dmed*071	61	*Dmed*100	58
*Dmed*006	64	*Dmed*044	61	*Dmed*072	58	*Dmed*102	58
*Dmed*011	58	*Dmed*045	55	*Dmed*074	58	*Dmed*103	64
*Dmed*012	55	*Dmed*046	61	*Dmed*076	61	*Dmed*106	61
*Dmed*014	61	*Dmed*048	64	*Dmed*078	61	*Dmed*107	67
*Dmed*015	61	*Dmed*049	64	*Dmed*080	61	*Dmed*109	61
*Dmed*017	61	*Dmed*051	61	*Dmed*084	58	*Dmed*112	58
*Dmed*018	61	*Dmed*053	58	*Dmed*085	58	*Dmed*113	61
*Dmed*020	61	*Dmed*054	58	*Dmed*086	61	*Dmed*114	55
*Dmed*021	58	*Dmed*058	64	*Dmed*087	61	*Dmed*115	61
*Dmed*025	58	*Dmed*060	61	*Dmed*091	61	*Dmed*117	58
*Dmed*027	61	*Dmed*061	61	*Dmed*094	58	*Dmed*118	61
*Dmed*028	61	*Dmed*064	61	*Dmed*095	61	*Dmed*120	55
*Dmed*030	58	*Dmed*067	64	*Dmed*096	61	*Dmed*123	61
*Dmed*035	61	*Dmed*069	61	*Dmed*097	67	*Dmed*130	61
*Dmed*037	61	*Dmed*070	58	*Dmed*098	67	*Dmed*131	61

Loci names are abbreviated, such as *Dmed*058 for Dmed^{UNICAMP}_ssr058.

Acknowledgments: We thank Iuri M. Ventura, Mitsue T. Brianti, and Maurício P. de Arruda for cogent suggestions; and Claudete do Couto for technical help. Funds were provided by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES); Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP); Fundo de Apoio ao Ensino e à Pesquisa (FAEPEX-UNICAMP).

References: Klaczko, L.B., 2006, Genetica 126: 43-55; Laborda, P.R., G.M. Mori, and A.P. Souza 2009, Conserv. Genet. Resour. 1: 297-307.