

Proposal for constructing a BAC library from *Monodelphis domestica* (Paul Samollow, Chris Amemiya)

Why target a marsupial for BAC construction?--Marsupials are phylogenetically distinct from existing mammalian biomedical models, all of which are eutherian (placental) species. However, marsupials and eutherians are more closely related to one another than to any other vertebrate model species (i.e., birds or amphibians). Thus, marsupials represent a unique midpoint between existing mammalian and non-mammalian vertebrate models. As a legacy of their common ancestry, marsupials and eutherians share basic genetic mechanisms and molecular processes that represent fundamental (ancient) mammalian characteristics. Nevertheless, since their divergence from a common ancestor approximately 140-165 million years ago, eutherian and marsupial mammals have evolved many distinctive morphologic, physiologic, and genetic variations on these elemental mammalian designs. These phylogenetically restricted differences can be used as comparative tools for examining the underlying molecular and genetic processes that are common to all mammalian species, and thereby help to reveal how variations in these mechanisms lead to differences in gene regulation, expression, and function. Moreover, as the closest sister group to eutherian mammals, marsupials are the most appropriate "outgroup" for comparative analyses among eutherian species.

Monodelphis domestica as a research model:--*Monodelphis domestica* (a.k.a. the laboratory opossum) is a small South American marsupial which has been established as a model organism for research on a broad range of topics that are relevant to human development, physiology, and disease susceptibility. It is the most widely used laboratory-bred marsupial for basic biologic and biomedical research worldwide (Samollow and Graves 1998; unpublished results from literature searches). Recent research utilizing *M. domestica* as an experimental model (revealed by a search of the PubMed database) includes: normal and abnormal physiology; fetal ontogeny (skeletal, endocrine, integumentary, neural); normal and regenerative neuronal growth in central and peripheral nervous systems; structure and function of the major histocompatibility and immunoglobulin systems; dental biomineralization and cranial development; reproductive behavior; and numerous genetic topics (e.g., gene structure; photobiology and cancer (melanoma) genetics; gene regulation and expression; DNA repair; gene and genome evolution; immunogenetics and antigen receptor evolution; reproductive biology and gametogenesis; sex determination and differentiation; lipoprotein metabolism and response to dietary fat and cholesterol; X-linked gene expression; X-chromosome inactivation). Topics for which *M. domestica* appears to have outstanding research potential include its use as a model for ultraviolet radiation-induced melanoma (e.g.; Robinson and Dooley 1995; Robinson et al 1994; 1998; Chan et al 2001; Wang et al 2001), structural and functional evolution of the mammalian immune system (e.g., Belov et al 1999; Miller et al 1999; Miska and Miller 1999; Stone et al. 1999; Baker et al 2001), genomic imprinting of autosomal and X-linked genes (e.g., Keohane et al 1998; O'Neill et al 2000; Killian et al 2001), and genetic mechanisms that influence neuronal growth and axon targeting during central and peripheral nervous system regeneration (e.g.; Lepre et al 1998; Saunders et al 1998; Nicholls et al 1999).

Size of the research community / Potential uses of *M. domestica* BACs: There is a rapidly growing community of researchers who have expressed strong enthusiasm for *M. domestica* as a model system in basic biological and biomedical research. An indication of the size of this community can be inferred from the large number (28) of letters that were provided by investigators from Australia, Canada, Italy, the United Kingdom, and the United States in support of Dr. Samollow's recent linkage mapping grant application (described beyond). With regard to BAC resources, Dr. Samollow has received inquiries from several investigators who wish to use large-insert genomic DNA (especially BAC) libraries to conduct various structural and functional investigations of specific genomic regions. Examples include: genomic imprinting (Dr. John Greally, Albert Einstein College of Medicine; Dr. Robert Nicholls, University of Pennsylvania; Dr. Paul Vrana, University of California, Irvine), the organization and expression of immunoglobulin and major histocompatibility loci (Dr. Robert Miller, University of New Mexico), the regulation of X-linked gene expression during gametogenesis and

early development (Dr. John McCarrey, University of Texas at San Antonio); the identification of QTLs influencing craniofacial development (Dr. Kathleen Smith, Duke University); the order and timing of gene expression in dental/craniofacial development (Dr. Ken Weiss, Penn State University); the structure and expression of tooth matrix genes (amelogenin and enamelin) in early development (Dr. James Simmer, University of Texas Health Science Center at San Antonio); comparative mapping of marsupial and eutherian genomes (Dr. Jennifer Graves, Australian National University), and others, including Dr. Chris Amemiya (Virginia Mason Research Center and member of the BLRN), who is interested in comparative genomics of the *Dlx* gene clusters. In addition, Dr. Samollow plans to utilize *M. domestica* BAC clones for fine structure mapping of regions harboring recently detected QTLs that influence variation in diet-induced hypercholesterolemic responsiveness (Rainwater et al 2000). Eight letters of support from these and other investigators interested in using *M. domestica* BAC resources are appended to this request. Finally, it may be important to point out that many Australian (and other) researchers who work with other marsupials feel that *M. domestica* is the most appropriate marsupial species for continued development as a genetic research model.

Availability of animal and DNA resources:--*Monodelphis domestica* is a small (100-150g), highly prolific (mean litter size ~8; up to three litters per year) species that is housed and bred under conditions that compare favorably with those used for laboratory rodents (VandeBerg and Robinson 1997). Initiated from a small founding stock in 1979, the research colony at the Southwest Foundation for Biomedical Research (SFBR) has been repeatedly infused with new genetic material from wild caught populations and exhibits very high genetic diversity (VandeBerg and Robinson 1997; P.B. Samollow unpublished data). More than 70,000 fully pedigreed animals have been produced from the SFBR colony, and the distribution of experimental animals and breeding stock has resulted in the establishment of several additional research colonies worldwide (Australia, Italy, Switzerland, UK, and several in the US). The SFBR colony maintains a steady state of approximately 2,400 animals including outbred and partially inbred strains derived from founders originating from several regions in Brazil and Bolivia (Vandeberg and Robinson 1997). The colony is fully pedigreed and all pedigree records are maintained in an extensive pedigree management database. DNA is readily available from the living colony animals.

Choice of *M. domestica* strain for DNA source:--There is no obvious rationale for choosing any particular outbred or partially inbred strain as the source of DNA for BAC library construction. We will utilize DNA from a descendent of the original founder strain, Population 1 (VandeBerg and Robinson 1997), for BAC construction simply because it is the most widely distributed of all *M. domestica* strains on a worldwide basis. The source animal will be unambiguously identified (by its permanent ID number), and its unused tissues and DNA will be archived.

Genome characteristics:--Physical genome size has not been determined for *M. domestica*, but has been estimated for other marsupials including several species in the family Didelphidae, of which *M. domestica* is a member. Didelphid species, including the closely related *Monodelphis dimidiata*, have DNA contents in the 3.9-4.3 pg/genome range (Gregory 2001) and thus have genomes roughly equivalent in size to other mammals in general. Moreover, the genomes of marsupials are highly conservative, both in karyotype and DNA content (Hayman 1990), and there is no reason to believe that *M. domestica* is exceptional in this regard. The nine pairs of *M. domestica* chromosomes (8AA; X,Y) are large and easily discriminated on the basis of size, centromere position, and banding patterns revealed by standard G-banding methodologies (Pathak et al 1993). As mentioned previously, the research colony at SFBR exhibits high genetic diversity.

Other genomic resources for this species:--An NIH-funded research program (RR 14214: Genomic Resource Development in the Laboratory Opossum; P.B. Samollow, P.I.) is underway to complete a 2.5-5.0 cM (centiMorgan) linkage map of the *M. domestica* genome, comprised of both anonymous markers and functional gene loci. The primary objective is to establish a resource that will enable the

localization of quantitative trait loci (QTLs) that contribute to normal and abnormal physiologic and developmental variation, and will simultaneously provide comparative information regarding the evolution of gene synteny and linkage relationships among distantly related mammalian species. The current linkage map subsumes more than 80 loci distributed among 8 autosomal linkage groups and the X-chromosome. Additional resources include normal and tumor-derived cell lines available from individual investigators and commercial sources.

Time frame for library availability:--Several research programs are already awaiting the availability of *M. domestica* BAC clones (see appended letters).

Availability of other *M. domestica* BAC libraries:--None known to exist.

Other proposals and support for *M. domestica* BAC library construction:--None.

Specifications for the library: A 10X coverage BAC library will be made using *EcoRI* partial digests with average insert size of 150 kb. This library should suffice for isolating most genes that will be screened for and will be useful for generating templates and STCs for whole genome sequence, should it be attempted.

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