

# AXOLOTL NEWS

## Salamander Genome Project

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During this era of high-throughput technology and informatics, a genome initiative for the Mexican Axolotl would seem unlikely given its enormous genome size. Surprise! We have recently received NSF funding to initiate genetic and genomic analyses of the Mexican axolotl (*Ambystoma mexicanum*) and the Eastern tiger salamander (*A. tigrinum tigrinum*). Our primary goal is to develop a comprehensive genome map by locating the positions of several hundred expressed sequence tags (ESTs) among the chromosomes of *Ambystoma*. Once completed, the genome map will provide an amphibian perspective to vertebrate genome evolution, and will facilitate identification of gene correspondence between salamanders and human. Our work over the next five years will generate several resources that will be of use to urodele researchers, and thus extend the Mexican axolotl as a research organism in both biomedical and natural history research. Here we describe our project and anticipated resources, and encourage participation of urodele researchers in this initiative.

From an evolutionary perspective, the extension of genome analysis to additional vertebrate species is needed to identify homologous chromosomal features among all the major vertebrate groups, and thus facilitate comparative studies of genome evolution. As such, our project is partially motivated by the need to provide an amphibian perspective in the reconstruction of vertebrate genome evolution. However, the development of a genome map for ambystomatid salamanders will also have practical importance for biomedical research. For example, if a homologous mammalian chromosomal segment can be identified for a salamander chromosomal segment that is known to contain a salamander mutant phenotype, then knowledge of the genes on the mammalian chromosomal segment can be used to exclude or implicate candidate genes for the salamander phenotype. Given that complete knowledge of genome structure will be obtained for several vertebrates, candidate gene approaches are likely to play an important role in the development of emerging model organisms because they make use of existing genome resources and thus do not require full-scale genome initiatives. Candidate gene approaches have clearly played an important role in the development of zebrafish as a model organism, by allowing the identification of genes underlying numerous mutant phenotypes (Talbot and Hopkins, 2000).

Our recent data indicate that chromosomal homologies can be identified among *Ambystoma*, amniotes,

and fishes, and *Ambystoma* is a very efficient organism for genetic linkage analysis because of the high level of polymorphism that segregates in interspecific crosses (Voss et al. 2001). At this point, we are developing several hundred molecular markers, derived from ESTs, to comprehensively map the ambystomatid genome. These loci are being sampled from regenerating limb (axolotl) and retina (tiger salamander) to bias the collection of sequences towards genes of special interest to urodele researchers. Thus, a by-product of our work to build a genome map for *Ambystoma* will be the generation of the first EST databases for axolotl and tiger salamander. EST databases for other model organisms have served as valuable resources for microarray analysis, mutant identification, and probe generation for developmental, evolutionary, quantitative genetic, and population genetic research. We expect salamander ESTs to be of similar use to the community of urodele researchers. ESTs are being deposited in the dbEST database of GenBank for public access, and summaries of the on-going effort, as well as mapping and characterization data can be found at our website (<http://lamar.colostate.edu/~srvoss/SGP>).

Previously cloned urodele genes that have already been deposited in GenBank represent another important source of marker loci that we are using for the genome map. To date, we have mapped approximately 25 such loci. We will also make every effort to map genes upon request and to establish the orthology of genes by genome cross-referencing, thus we encourage researchers to send us axolotl or tiger salamander sequences in which they are particularly interested. During the first few years of the project, we expect the map will serve as a resource for establishing gene orthologies between salamanders and other models. Over the long term, when the map becomes saturated with protein-coding loci that are easily typed by PCR, the map will serve as a resource for mutant and gene identification, as well as quantitative trait locus mapping.

This is a very exciting time for the axolotl. With the development of fundamental genome resources and the likelihood of transgenic approaches in the near future, axolotl will continue to be a unique and powerful vertebrate model.

### ACKNOWLEDGEMENTS

The National Science Foundation (IBN-0080112, SRV) is supporting our work. We thank David Gardiner, Sue Bryant, and Susan Amara for resources, support and advice.

### REFERENCES

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Voss, S.R., J.J. Smith, D.M. Gardiner, and D.M. Parichy. 2001. Conserved vertebrate chromosomal segments in the large salamander genome. *Genetics* **158**:735-746.

On 10/01/01, 887 ESTs from a limb regeneration library were deposited into Genbank and dbEST. This list includes approximately 460 non-redundant protein coding sequences with presumptive human orthologues.