Axolotl

Newsletter of the
Ambystoma Genetic Stock Center
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Director's Note 1

AGSC News 2

AGSC to pilot a microinjection service
Progress towards an axolotl genome sequence
SGP awarded grant to perform chemical genetic screening
AGSC offering new axolotl aquaria kits
AGSC advisory board members selected
Axolotl husbandry – Don't hesitate to contact AGSC staff

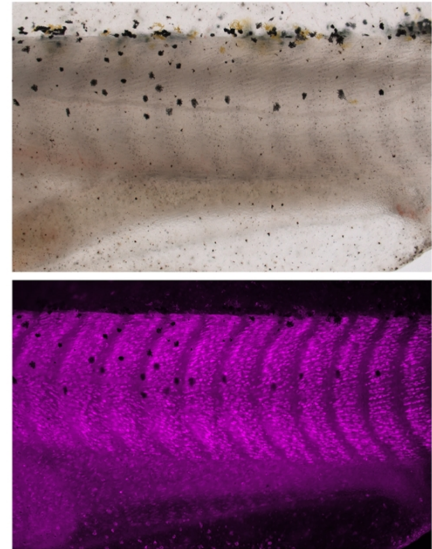
A Genotyping Assay to Identify Carriers of *albino* 5

Cover photo: Matthew Niemiller

Director's Note – Randal Voss

I am happy to report that the Ambystoma Genetic Stock Center (AGSC) will circulate *Axolotl*, a newsletter in support of axolotl research. From 1976 – 2003, Indiana University published the *Axolotl Newsletter* to provide useful information about axolotl biology, including methods for husbandry, experimental protocols, non-peer reviewed research reports and reviews, and community news. We hope that an electronic version of *Axolotl* distributed once or twice a year will facilitate research and educational efforts, and build community spirit. This inaugural issue is thin; it would be great to receive content from community members for upcoming issues. If you'd like to contribute a protocol or short paper to *Axololt*, please do not hesitate to contact me. *Axolotl* will be emailed to AGSC users and also made available for download on Sal-Site.

Much has happened since the axolotl collection at Indiana University moved to the AGSC in 2005. More than 4,000 shipments have been sent to hundreds of research and educational institutions, supplying over 200,000 embryos, larvae, juveniles, and adults. In addition to supplying wildtype axolotls and several historical mutants (*albino, white, melanoid, axanthic, cardiac, shortoes, eyeless)*, the AGSC also now ships GFP and RFP expressing axolotls. Almost certainly we will see an increase in requests for older axolotls and production of more transgenic and mutant axolotls. To facilitate community efforts, we are increasing the size of the axolotl collection and will initiate a microinjection service to assist users in the development of mutants/transgenics (see story below).



Example of an available AGSC transgenic stock - RFP (nucCherryRed+ CMV:Chicken β-actin) expression in the somites of a developing embryo. Image provided by David Parichy, U. Washington.

We are excited to be a NIH P40 National Animal Resource Center. Through NIH support, we will sustain and distribute stocks, and perform applied research projects to improve AGSC operations, products, and services. To ensure long-term support, it will be vital for us to document research and protocol advances within the community to show the axolotl's significance across multiple NIH biomedical areas of interests. I encourage each of you to share these advances with me so that I can bring them to the attention of NIH and members of our community. And please, if you use axolotl stocks from the AGSC, acknowledge this in your publications and reference our funding mechanism (P40-OD019794). We appreciate all of your support and look forward to serving your needs and interests into the future. Please do not hesitate to ask for our help.

AGSC & Community News

AGSC to pilot embryo microinjection service

It is exciting that methods are now available to create transgenic and mutant axolotls. However, approaches are currently limited to labs that have a breeding axolotl colony to generate embryos for microinjection, and animal care and molecular infrastructures that are not available to many investigators. We see a need for a service that makes transgene knock in and gene knock out approaches more widely available to investigators in the community. The AGSC has one of the largest breeding populations of axolotls in the world and is the primary source of axolotl stocks for investigators. It is a logical place to establish a microinjection service for the axolotl.

Beginning Fall 2016, we will pilot a microinjection service for a few NIH-funded researchers. We envision a service where the AGSC works closely with users to achieve high rates of transgene incorporation and embryo survival. Users will send ready to inject mRNA, DNA, or protein for microinjection. After molecules are injected, embryos will be shipped back to users via overnight courier. In anticipation of initiating this service, we have generated germline Tol2 transgenics, CRISPR/CAS9 and TALEN mutants, and are currently working to identify best practices in serving the needs of investigators. If you currently have NIH funding and are interested in performing a pilot experiment this fall, please contact us.

Progress towards an axolotl genome sequence

An objective of the Salamander Genome Project (SGP) is to develop an axolotl genome sequence, a resource that would greatly enhance community research efforts. It will take a bit more time to generate a genome sequence because the axolotl's genome exceeds 30 Gigabases and assembly of the genome poses an extreme challenge to existing assembly pipelines. However, considerable progress has been made in developing approaches and methods for chromosome and genome scale sequencing and assembly. This past year Keinath et al (2015) presented some early results of their genome sequencing approach, which uses laser capture microscopy to develop DNA sequencing libraries from single chromosomes. This approach effectively samples DNA for independent partitions of the genome, enriching for sequence reads that have a higher probability of forming contiguous sequence assembly than if the sequence reads were generated randomly from the overall genome. This approach reduces the computational burden associated with assembling large genomes. The SGP is currently pursuing several approaches to increase contiguity of the assembly, including working with Dovetail Genomics. Go to Sal-Site for SGP genome sequencing updates as they become available.

SGP awarded grant to perform chemical genetic screening

The SGP in collaboration with the University of Kentucky Center for Pharmaceutical Research and Innovation was awarded an NIH R24 grant to use an axolotl embryo

assay to screen 10,000 small molecules for affect on tail regeneration. This project will also generate transgenic and knockout axolotl lines. The data from this project will be made available through Sal-Site and the lines will be distributed by the AGSC.

AGSC offering new axolotl aquaria kits

Axolotls are one of the best animals to bring into classrooms, learning centers, and museums. Their unusual traits and charming behavior capture the attention of students of all ages, making them perfect for teaching science. The AGSC is now offering durable Axolotl Aquaria that include everything needed to rapidly set-up and integrate an axolotl into learning environments, including the axolotl! Several options are available to tailor Axolotl Aquaria for different learning experiences. Please help us spread the word.

AGSC advisory board members selected

The first meeting of the AGSC Advisory Board was held in Lexington, KY on May 19, 2016. The Advisory Board members are Dr. Jeramiah Smith (University of Kentucky), Dr. Heather Eisthen (Michigan State University), Dr. David Parichy (University of Washington), and Dr. James Hanken (Harvard University). The AGSC is excited to work with Drs. Smith, Eisthen, Parichy, and Hanken as they bring a wealth of outside scientific expertise to advise on AGSC center operations, functions, and goals.

Axolotl husbandry – Don't hesitate to contact the AGSC

As a reminder, AGSC staff are available to provide advice about axolotl husbandry should questions arise. The axolotls that the AGSC distributes have been maintained under standard conditions for many decades. In other words, AGSC axolotls have been artificially selected to thrive under conditions that were originally established by the Indiana University Axolotl Colony. To ensure reproducibility across studies, we recommend that the community follow AGSC husbandry methods.

The AGSC would like your feedback concerning interest in an annual course that introduces axolotl biology, care and use. The course would provide conceptual and hands-on training in working with axolotl embryos, larvae, and adults. Additionally, the course would cover axolotl anesthesia, surgery, and euthanasia. Please let us know if you see need for such a course.

A Genotyping Assay to Identify Carriers of *albino*

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Test crossing is performed in captive populations to identify individuals that carry recessive alleles for single locus phenotypes. Typically, an individual with a dominant wild type phenotype is crossed to an individual that presents a recessive phenotype. If approximately 50% recessive phenotype progeny arise from the cross, the wild type individual is identified as a heterozygous carrier of the recessive allele. Test crossing provides a reliable way to track recessive alleles in a captive population but it is inefficient from an animal management perspective. More potential carriers of recessive alleles are reared to maturity for test crossing than are needed to sustain mutant lines. This burdens animal care infrastructure, especially when the animal model has a lengthy time to first maturity.

DNA typing provides a more efficient method for managing recessive alleles in a captive population. If a single-nucleotide polymorphism (SNP) or insertion-deletion polymorphism is identified near a recessive allele, the polymorphism can be typed to identify likely carriers of the recessive allele. DNA typing only requires a small amount of tissue, which can be collected from individuals using minimally invasive methods (e.g. tail clip) at early stages of development. After DNA is isolated, PCR and a robust genotyping method can be used to rapidly determine an individual's genotype in just a few hours. Thus, DNA typing allows prospective management of a captive population. Individuals are prioritized for rearing according to their genotypes at an early stage of development, thus negating the need to rear individuals to maturity for test crossing.

Several recessive alleles are segregating in the AGSC axolotl collection, including *albino, white, melanoid, axanthic, eyeless, cardiac,* and *short-toes*. *albino* continues to be a highly requested AGSC mutant because absence of pigmentation is ideal for histology, immunohistochemistry, and fluorescence applications. To better manage this valuable mutant in captivity, we describe a method for identifying wild type individuals that carry the *albino* recessive allele.

METHODS

All animal work was performed under an approved Institutional Animal Care and Use protocol at the University of Kentucky. Axolotls were anesthetized in pH buffered 0.02% benzocaine and 1-3 mm of the distal tail tip was amputated for DNA isolation. DNA was isolated using a traditional phenol/chloroform approach (Voss 1993). PCR was performed using standard protocols and DNA sequencing was performed by the Advanced Technology Genetic Center at University of Kentucky. PCR primers (Forward Primer CAGCGTACTTATGTTTTATTTCAATGTAAGC, Reverse Primer GTACAGCACACACAATAGTCGACTT) were designed to amplify a 144 bp DNA

fragment containing a SNP that distinguishes tyr^{alb} and tyr^{wt} alleles. TaqMan® probes for tyr^{alb} (FAM-TGGACACCGTTTTACT) and tyr^{wt} (VIC-CTGGACACCATTTTACT) alleles were designed by ABI and the assay was performed with TaqMan® GTXpress™ Master on an ABI StepOnePlus™ Real-Time PCR System using the following conditions: 95° C for 20 sec followed by 40 cycles of 95° C for 3 sec, 60° C for 30 sec.

RESULTS AND DISCUSSION

We recently mapped *albino* to *tyrosinase* (*tyr*), which encodes an enzyme that functions in melanin synthesis. Many different mutations of *tyr* have been identified among various animal models and humans. Using DNA sequencing, we found that *albino* has a 147 bp deletion of *tyr* protein coding sequence in exon 1 (tyr^{alb}), suggesting this is the causative mutation. We then sequenced and compared the 3' UTR of tyr^{alb} to homologous sequences obtained from wildtype axolotl (tyr^{wt}) to identify diagnostic polymorphisms (Figure 1). We initially validated these polymorphisms as diagnostic for pigmentation by sequencing alleles from AGSC axolotls of known wildtype and *albino* color. In all cases (N=32), *albino* axolotls only presented tyr^{alb} alleles while wildtype axolotls presented one (tyr^{wt}) or two (tyr^{alb}, tyr^{+}) different allele types. These results are expected if tyr^{alb} and tyr^{wt} are recessive and dominant alleles, respectively. Overall, these results suggested to us that DNA typing could be used to type *tyr* alleles that associate with *albino* and wildtype coloration.

DNA typing can be accomplished with great precision and efficiency using assays that are designed to specifically type alternate alleles at loci. We decided to develop a TaqMan assay because it allows gel-less resolution of alleles using any of several different real-time PCR platforms. We performed a cross (AGSC spawn #14067) between a known *albino* carrier (13655.2; female; +/*a*) and a wildtype non-carrier (13135.3; male; +/+). Embryos were reared to the time of hatching, anesthetized, and distal tail tip tissue was collected (2 mm) for DNA isolation. After tissue was collected for embryos, each was assigned a unique identifier and reared independently. Taqman analysis was performed using fluorescent probes (VIC, FAM) that were designed to bind and detect alternate tyr^{alb} and tyr^{wt} alleles (Figure 1). As expected, two genotypes were identified (Figure 2); tyr^{wt}/tyr^{alb} corresponding to heterozygotes (+/*a*) and tyr^{alb}/tyr^{alb} corresponding to homozygotes (+/+). These results show that early stage genotyping can be used to efficiently prioritize embryos to optimally manage an axolotl population.

We note one potential caveat in using the TaqMan assay described here - the 3' UTR SNP used to type tyr^{alb}, tyr^{wt} is a great distance from the presumptive causative mutation in exon 1, and thus the assay will not be informative if recombination has occurred and the tyr^{alb} 3' UTR SNP is no

CAGCGTACTTATGTTTTATTTCAATGTAAGCAGACGCTGGCAAAGTTAAGGGATCAT
GAA (C/**A**) GAATAT (C/T) CTTGCCGCAGTGAAAG**AGTAAAA (T/C) GGTGTCCAG**ACA
ACATGGGTTAAAATT**AAGTCGACTATTGTGTGTGCTGTAC**

Figure 1. A portion of axolotl *tyr* 3' UTR. The blue (tyr^{alb}) and red (tyr^{wt}) nucleotides indicate SNP sites. The boxed regions are forward and reverse PCR primer sequences and the underlined sequence is the target for TaqMan probes. Note the tyr^{wt} and tyr^{alb} TaqMan probes differ in length by one base.

longer in linkage disequilibrium with the causative mutation. While this is a possibility, we have yet to identify a recombinant in our screening of AGSC *albino* axolotls.

In summary, we show that the allele associated with *albino* can be efficiently and reliably genotyped using a Taqman assay. This assay will allow efficient, prospective management of captive axolotl populations. For example, prior to the development of this assay, standard operating procedure in the AGSC was to rear offspring of unknown genotype to maturity for the purpose of performing test crosses to identify axolotls that carry recessive alleles. This procedure is costly in terms of labor and space for an animal model that does not reach maturity until ~ 1-1.5 yrs of age. Looking forward, we have recently identified genes for several other axolotl mutants including *white, melanoid,* and *eyeless.* We will develop and make TaqMan assays available for these and other genes in the future.

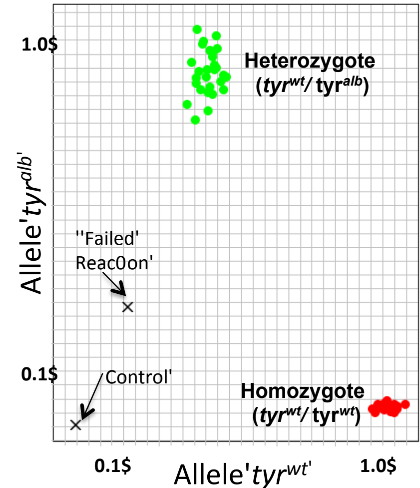

Fig. 2. Allele discrimination plot generated by the ABI StepOnePlus™ Real-Time PCR System. The control did not contain template.