Axolotl

Newsletter of the Ambystoma Genetic Stock Center
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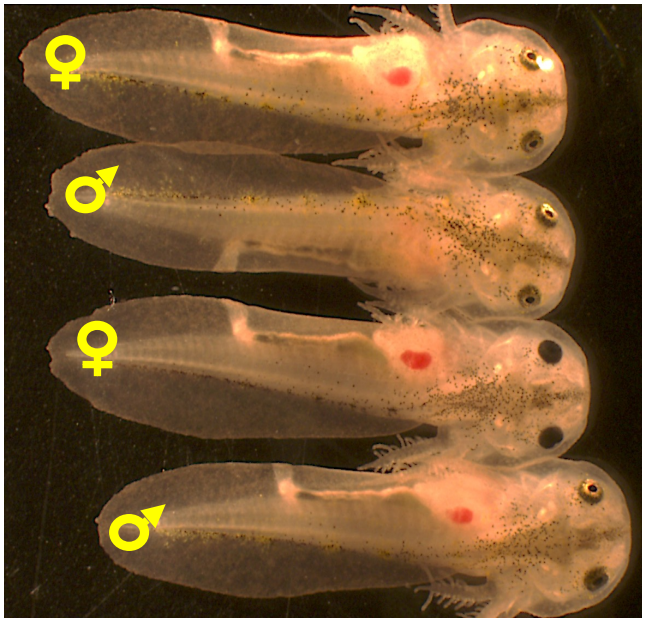
2017 Issue 2



Director's Note – Randal Voss

This issue of the *Axolotl Newsletter* was meant to be distributed at the end of 2017, I am sorry for the delay. However, we wanted to wait a few months to document and share some of the exciting and ongoing infrastructure improvements to the AGSC. Last July, my lab and the AGSC moved to the University of Kentucky, College of Medicine (UKCOM). We still occupy the same physical space, but because of the generosity of UKCOM, we were able to replace the recirculating systems that Chris Muzinic pioneered for axolotl husbandry some 20 years ago. The new systems are state-of-the-art with many automated features that will make axolotl husbandry more efficient. Most importantly, our axolotls give them two gill-flicks up!

In this issue, we are excited to share a short article that describes how the sex of an axolotl can be determined by performing a simple PCR. This is a timely article given recent concerns and interest in studying sex as a biological variable, and from a practical perspective, it can make axolotl husbandry more efficient. The lead author on this work is Melissa Keinath who completed her dissertation last year in Jeramiah Smith's lab at UK. Melissa is currently a post-doc in Joe Gall's lab at the Carnegie Institute of Science. The parent article for the methods paper, *Miniscule Differences Between Sex Chromosomes in a Giant Vertebrate (Salamander) Genome*, was recently submitted for peer review and publication. Keep your eyes open for this paper and learn how Melissa and colleagues identified a needle-in-a-haystack, the axolotl sex-specific region of the genome.

Rigor and reproducibility continue to be issues of concern in biological research. Not only is it important to carefully detail how experiments are performed in the Methods section of our papers, it is equally essential that we document the resources that we use. The Resource Identification Initiative is a database-driven initiative that seeks to "barcode" all of the critical reagents and tools that are used in the course of scientific research with Research Resource Identifiers (RRIDs). An RRID is useful because it allows rapid and ready access of information about the availability and use of specific resources in scientific research. Such transparency is essential for ensuring reproducibility in science. A growing number of journals now require RRIDs in submitted manuscripts, and *Neuron* has a very nice description of how one goes about obtaining RRIDs in support of their research. The AGSC, Sal-Site, and the axolotls that we distribute have RRIDs that can be obtained from SciCrunch.org. For example:

Resource	RRID
Sal-Site	**RRID:SCR_002850**
AGSC	**RRID:SCR_006372**
White hatchling axolotl	**RRID:AGSC_101H**

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.

AGSC & Community News

Progress in axolotl genomics and transcriptomics

This past year has seen considerable progress made in developing essential axolotl transcriptomic and genomic resources. The first axolotl assembly was made public by the Salamander Genome Project (Sal-Site) in July 2017, and then earlier this year, a more contiguous assembly was made available by the Tanaka, Myers, Hiller, and Schloissnig labs in Europe (Nowoshilow et al., 2018). This assembly and transcripts that were generated during this project are available from Axolotl-Omics.org. An extensive collection of tissue-specific transcripts were generated by the Whited lab in collaboration with the Broad Institute (Bryant et al., 2017), and these data can be accessed here. Finally, tissue-specific coding and non-coding RNAs were sequenced by Caballero-Pérez et al. (2018) for axolotls collected directly from Xochimilco. These exciting new resources will accelerate studies using the axolotl and further improvements of the axolotl genome assembly will be coming soon.

Axolotl PI meeting in Austria

In the spirit of building a more connected and united community of researchers that work with the axolotl and other salamander species, a salamander PI meeting will be held at the Research Institute for Molecular Pathology in Vienna, Austria on 12-13 July, 2018. During this meeting, a core group of researchers will share their data, knowledge, insights, and perspectives on how an international community of researchers can work together to elevate each other's work and salamander models in the eyes of science. One of the goals of this initial meeting will be to establish a regular schedule for hosting a meeting that will be open to everyone that works with salamanders. The organizers of the meeting (Elly Tanaka, Jessica Whited, Karen Echeverri, and Randal Voss) will generate and distribute a summary report, stay tuned for further information about this important initiative to further research efforts using salamander models.

Rear your own juvenile animals

The AGSC is seeing an increase in orders for juvenile animals and users are requesting more frequently that animals have intact limbs. To ensure that juveniles have intact limbs, AGSC staff rear individuals in separate containers. The amount of time that AGSC staff have to rear individuals singly is limited and we are encouraging users with high demands for juvenile animals to purchase small larvae and rear these in-house to the size and stage needed for experiments. If you would like advice on how best to do this, do not hesitate to contact AGSC staff.

AGSC getting a facelift

The AGSC is undergoing a major renovation of recirculating systems that house the adult axolotl population. The new custom Aquatic Enterprises/Aquarius Fish Systems will replace systems that have been in place since 2005. The renovation was made possible by the College of Medicine at the University of Kentucky

Techie Details

Each system is constructed of powder coated steel or aluminum racks with molded polycarbonate boxes. The life support system includes an 80-gallon sump per rack (8 per system) with screen filers, self-cleaning rotating drum filter, high efficiency pumps, 100-W UV sterilizer, carbon filter, with an automatic water change system, drawing water from a 100 gal reservoir tank. Each system is automated using a NEMA protected ProFilux touchscreen monitoring system that includes sensors for pH, conductivity, temperature, and water level. Dosing tanks maintain pH at 7.5 and conductivity at 4.2 μS (based on our current 40% Holtfretter's concentration).

The first set of systems was installed in March 2018 and the next set is slated for installation later this summer. Housing/cage space for each animal will be significantly upgraded. In our old systems, each male was housed in a converted 4L mouse cage. In the new systems, each male will be housed in an 8L baffled cage. Our females, currently pair housed in 11L converted rat cages, will now be housed in groups of three in 23L boxes. This will allow more females to be maintained in recirculating systems, decreasing the number that are statically housed. Larvae and juvenile animals will continue to be statically housed to meet user needs in a timely manner.

New male systems in room 122

A PCR Based Assay to Efficiently Determine the Sex of Axolotls

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An individual's sex can have profound effects on its physiology, gene expression, and even genome content (for species with differentiated sex chromosomes). As such, the ability to discern the sex of experimental animals can provide new research opportunities and better insure the generation of reproducible results among studies. Indeed, NIH now requires investigators to consider sex as a biological variable in their proposed studies (Clayton 2017). The development of diagnostic markers for sex in axolotls would allow investigators to directly account for sex-specific effects, improve husbandry practices and enable studies of sex-determination (and sex reversal) in this important amphibian model.

Sex is controlled by a Mendelian factor in the axolotl and it is determined at the time of fertilization when the Z or W-bearing oocyte meets the Z-bearing sperm (Humphrey 1945; Humphrey 1948; Smith and Voss 2009). However, gonadogenesis doesn't take place until 30-70 days post hatching and sexes are essentially indistinguishable prior to gonadal differentiation. Even after differentiation, dissection and examination of gonadal tissue is often necessary for proper identification. Alternatively, axolotls can be raised to maturity, but care and space for these animals can be costly and time consuming (~1-1.5 years), particularly in cases where many animals are needed for an experiment. Moreover, some experimental treatments may alter gonadal differentiation or maturation, preventing assessment of sexual genotype on the basis of gonadal morphology. Importantly, identifying sex prior to differentiation will be useful for studies of sex determination in the axolotl, an area which remains largely unexplored.

We recently characterized the sex chromosomes of the axolotl and our work is currently under-review for publication. From this work, we describe a PCR based assay that investigators can use to determine the sex genotype of any axolotl at any stage of development. PCR requires a small amount of tissue for DNA, which can be acquired through minimally invasive procedures, such as a tail clip or external body scrape. Because PCR amplification products can be rapidly separated by gel electrophoresis, the assay can be performed within a few hours. This assay will be a valuable resource for colony management and experimental studies that consider sex to be an important covariate.

METHODS

A sex-specific PCR primer pair was designed for the sex candidate genomic region using Primer3 (Untergasser et al. 2012). These primers (Forward Primer AACCCAATACAAAAGGTAAACATGTAG and Reverse Primer

AGAAAGAGAAATTGGGCTTACTTTAAC) amplify a 219bp DNA fragment that is diagnostic for female axolotls but not males. As a control for PCR amplification in males, a second primer pair is included in the PCR reaction to amplify a 486bp DNA fragment (E16E2) in both sexes. This primer pair was previously reported by Smith et al. (2005): (Forward Primer GGTTTTAATTGTGATCAGTGGTACAG and Reverse Primer AAAGAAATAAATAGGGCAAACAACAC).

The isolated axolotl DNA in Figure 1 came from tail tip amputations; however, PCRs have been validated in a variety of tissue types, including liver, blood and a simple scrape with a pipette tip along the external body. All tail tip amputations and dissections were performed under Institutional Animal Care and Use protocol #01087L2006 at the University of Kentucky. The axolotls were anesthetized with 0.02% benzocaine in 40% modified Holtfretter's Solution (Armstrong et al. 1989). DNA was isolated using standard phenol/chloroform extraction (Sambrook and Russell 2001). PCR was performed using the following PCR conditions (150ng DNA, 57.5ng E16E2 primer, 42.5ng female-specific primer, 200 mM each dATP, dCTP, dGTP, dTTP; thermal cycling at 94°C for 4 minutes; 34 cycles of 94°C for 45 seconds, 55°C for 45 seconds, 72°C for 30 seconds; and 72°C for 7 minutes). Gel electrophoresis was performed using a 1% agarose gel, gelred for staining and a benchtop UVP Transilluminator for visualization. Only those samples that showed a band for the non-sex-specific fragment (E16E2) were considered, and presence/absence was recorded for the smaller female-specific fragment.

RESULTS & DISCUSSION

In our paper under review, we identified female-specific (W-specific) DNA from more than 40 female-specific scaffolds in the current version of the *Ambystoma* genome. From a single marker, we developed a novel PCR based assay using a primer pair that amplifies a fragment in the female-specific genome, presumably a W-specific fragment. As an internal control, we use a previously published primer pair for a larger non-sex-specific fragment (E16E2) (Smith et al. 2005).

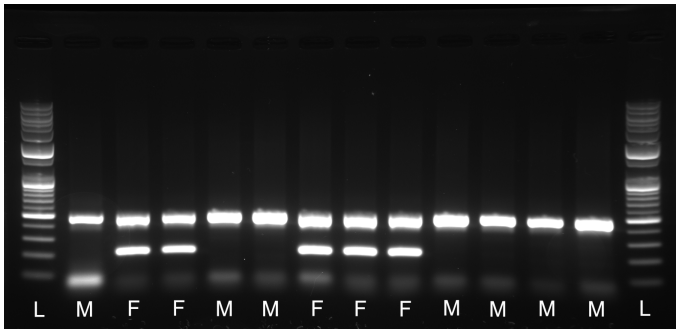

Figure 1. Determination of the sex of axolotls using E16E2/female-specific PCR. Genomic DNA from male and female juvenile axolotls was isolated from tail clips and used as template for PCRs. The control fragment (486bp) from E16E2 is shown in every lane, in both males and females. The sex-specific fragment is only present in female axolotls (219bp). L denotes the 100-bp ladder, M and F denote male and female, respectively.

The primers for the PCR based assay have been tested for accuracy in many different axolotls at multiple stages of development. Initially, DNA was extracted from known sex animals (adults that contributed to a successful spawn) and validated across 6 males and 6 females. Juvenile tail tip DNA from 10 unrelated animals was extracted and tested with the primers, and dissections confirmed the PCR findings. Lastly, the tail tips of 12 hatchlings were collected for DNA

isolation and PCR, and animals were housed separately and dissected after gonadogenesis to verify the PCR findings.

In summary, we show that biplex PCR using primers designed to amplify a female-specific fragment and a non-sex-specific fragment is a reliable and efficient method for determining genetic sex in the axolotl. This PCR assay will allow rapid sexing of all stages of development without dissection and is the only sexing option for axolotls that have undifferentiated gonads.

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