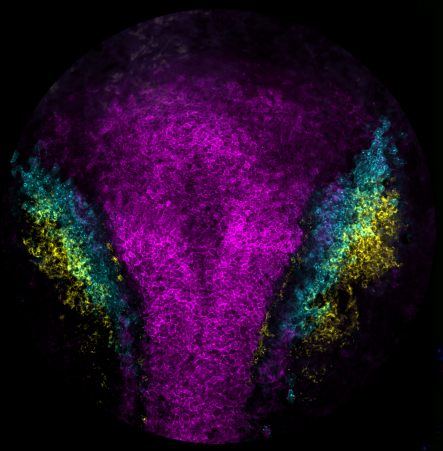
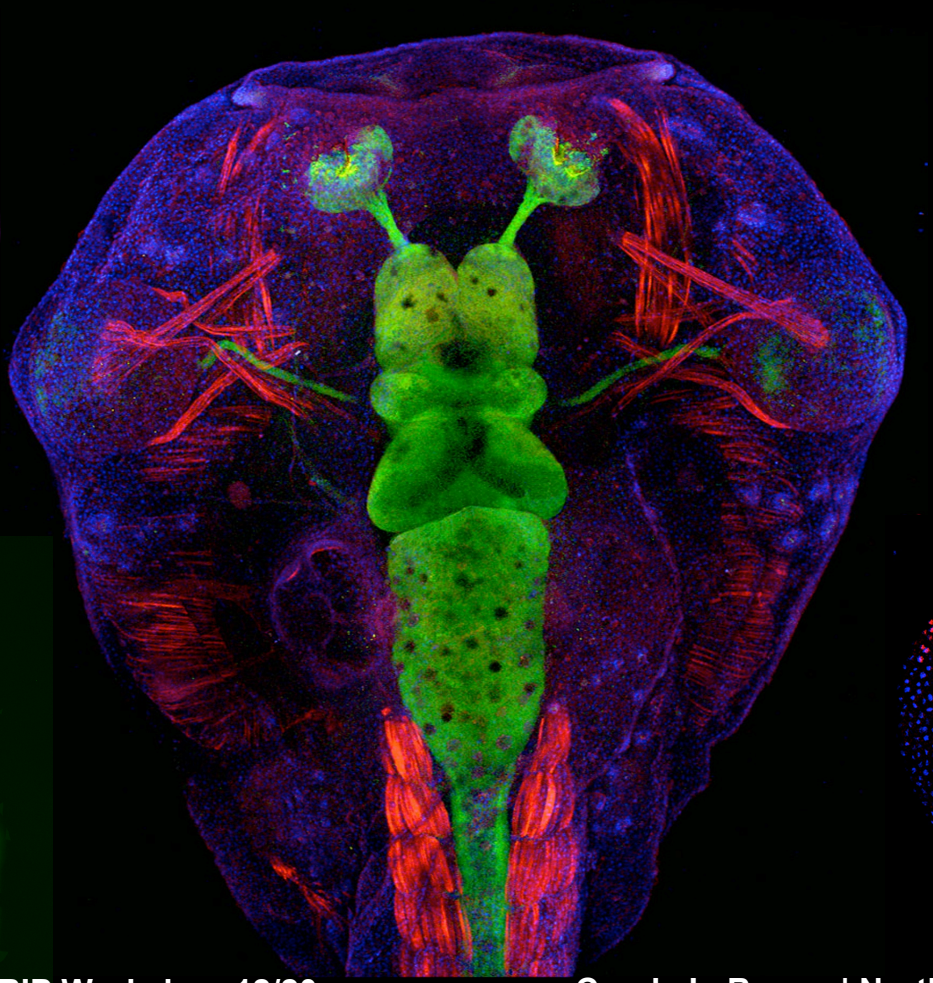
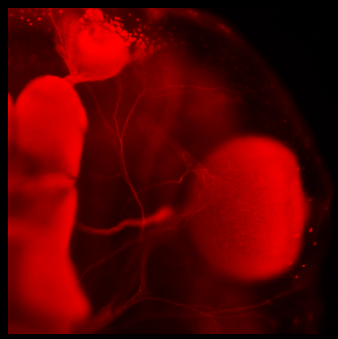
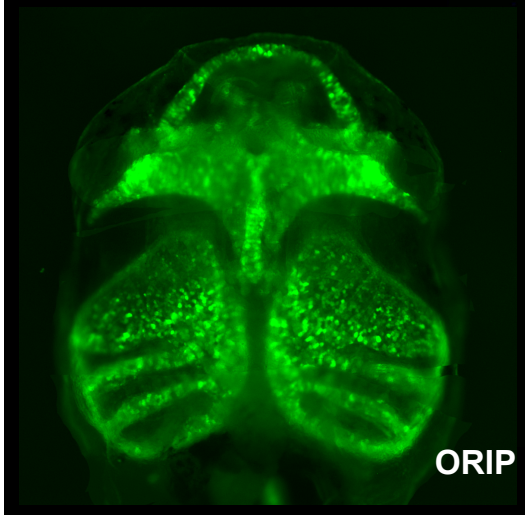
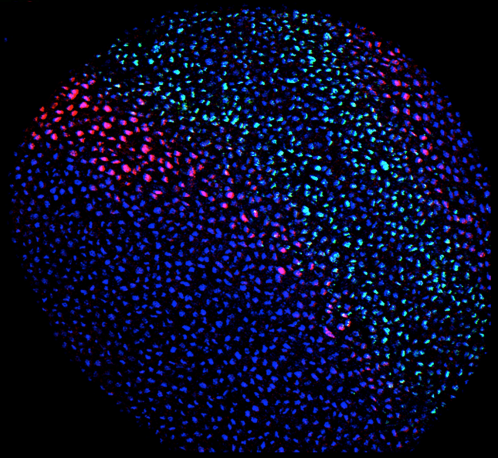
Xenopus: A Powerful System for Modeling Human Disease and Congenital Defects







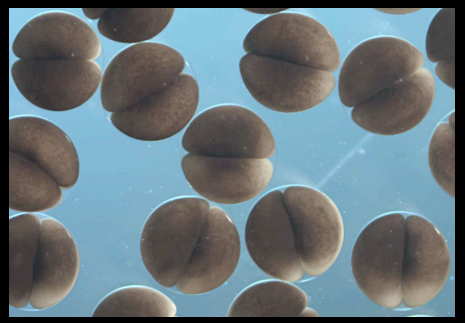




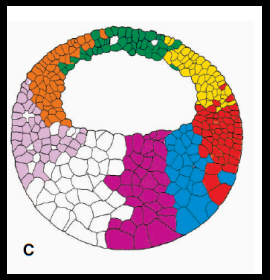
Carole LaBonne | Northwestern University

Some Advantages of the *Xenopus* System

Large Numbers of Synchronously Developing Embryos



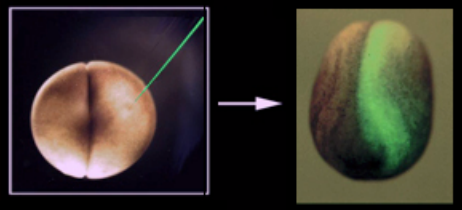
Validated Fate Maps and Powerful Lineage Tracing



Lateralized Injections and CRISPR Mutagenesis – particularly powerful for studying bilateral tissues and organs

High Conservation of Genome to Human

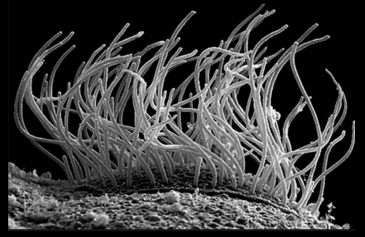
Organs Most Aquatic Models Lack - including septated heart ventricles, mucociliary epidermis, limbs, lungs



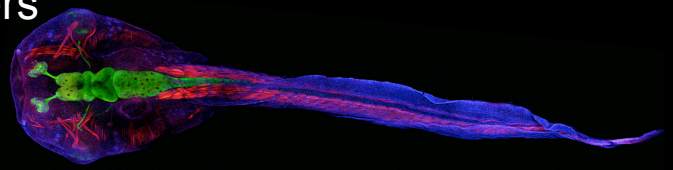
Ease of Tissue Explants / Transplants and Organ Culture

Excellent System for Studying Regeneration

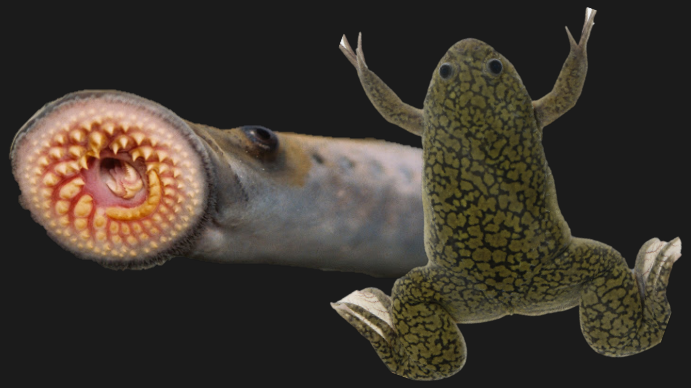
Embryonic Stem Cell Explants that Lineage Restrict in Culture On the Same Time Scale as In vivo (~7hrs)



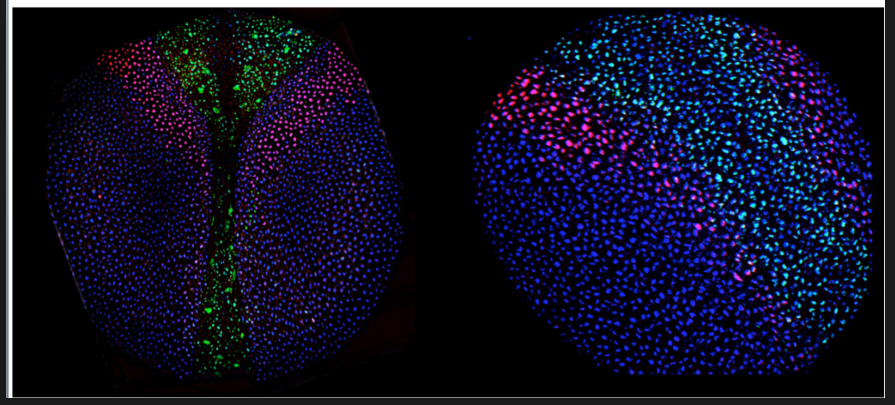
Rapid Inexpensive Validation of Mutations implicated in Human Diseases and Disorders



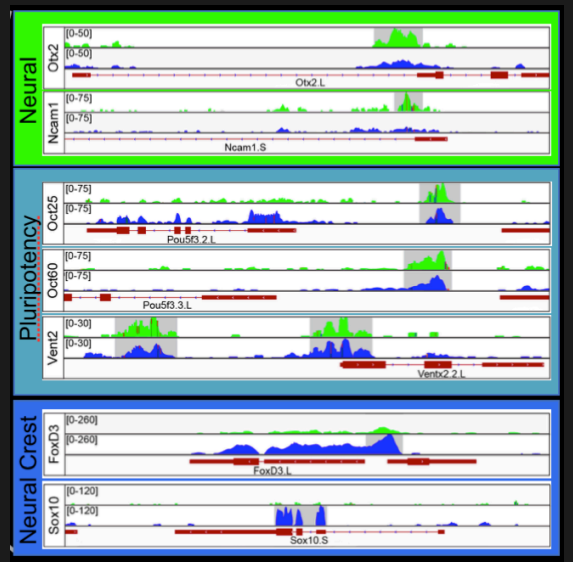
Development and Evolution of the Vertebrate Neural crest

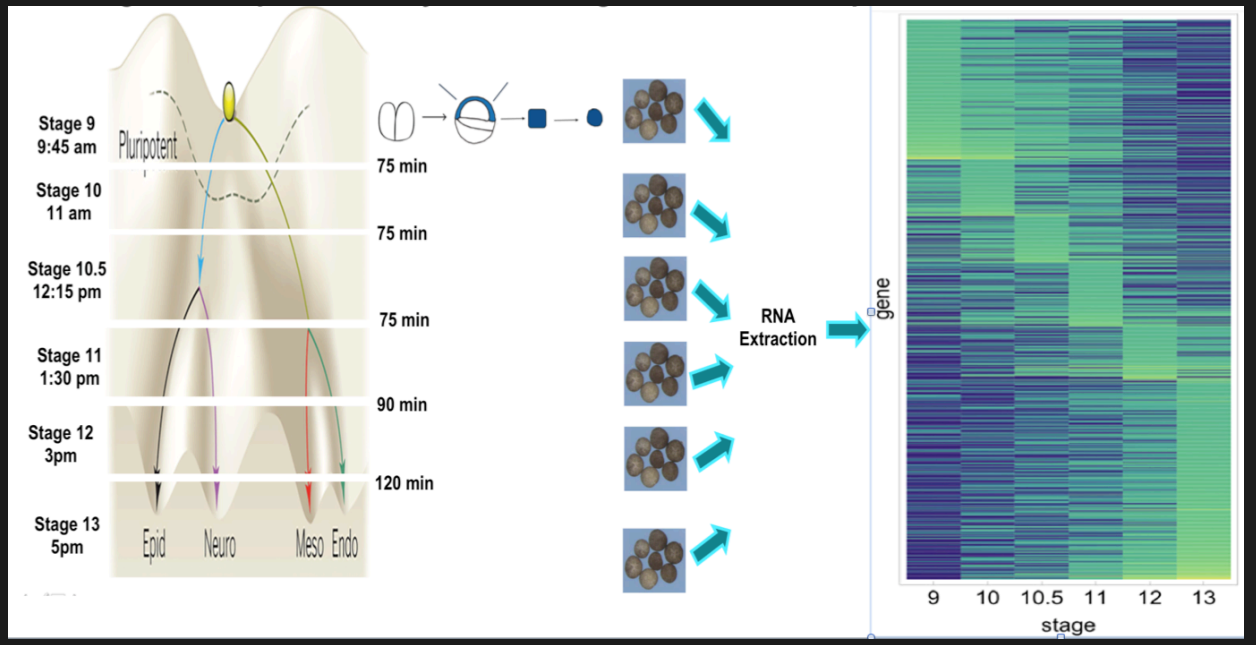


Building quantitative imaging tools to validate gene expression dynamics during cell state transitions and lend spatial insights



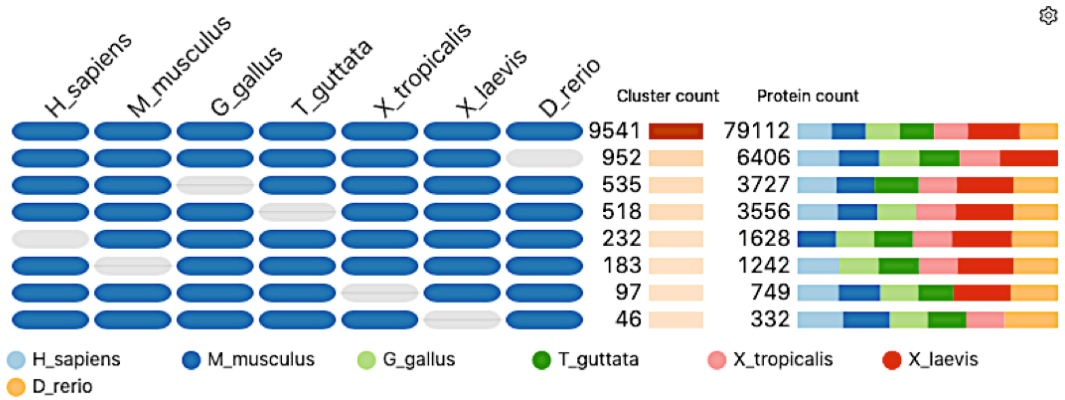
NC Syndromes associated with mutations in SoxE genes





LaBonne Lab | Northwestern University **@LaBonneLab**

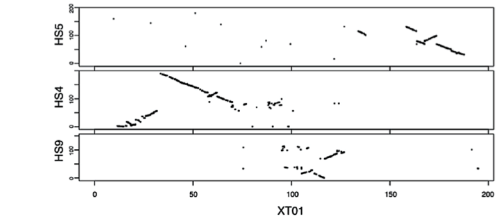
Xenopus genomes includes more orthologs of mammalian genes than chicken or zebrafish



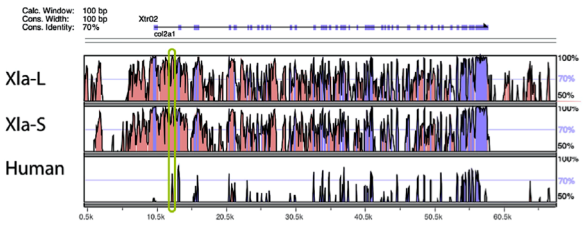
952 vertebrate orthologs missing in zebrafish
535 vertebrate orthologs missing in chicken
…
…
97 vertebrate orthologs missing in xenopus

Bredeson et al, in preparation

Relationships between human and Xenopus genomes



Long runs of collinear synteny between human and Xenopus tropicalis (free of genome duplication as in zebrafish) facilitate genetic comparisons



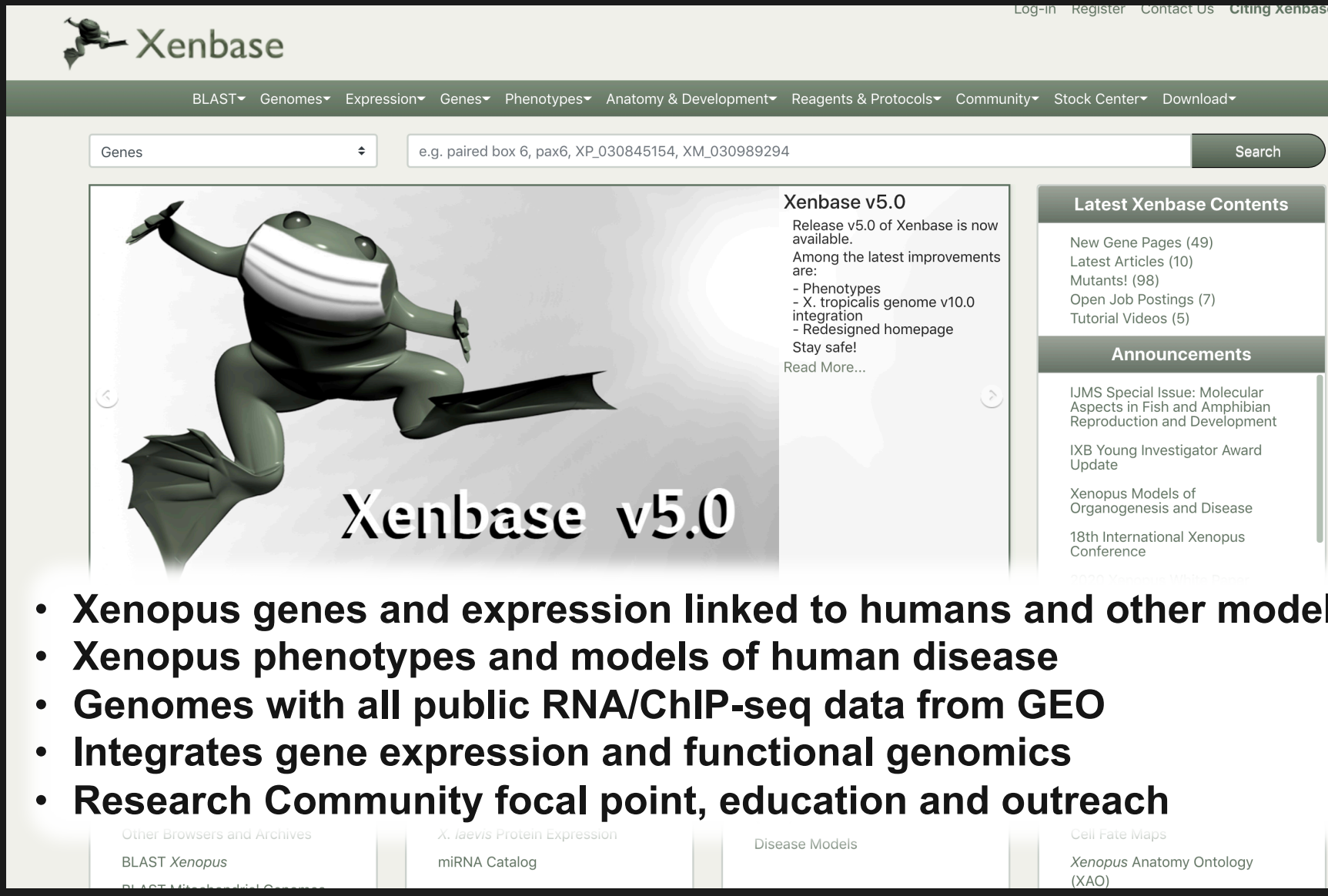
Phylogenetic "sweet spot": human and Xenopus tropicalis conserve most exons and some regulatory elements; X. laevis (and 25 other frog genomes) provides finer resolution.

Xenopus tropicalis genome

Essential *Xenopus* Resources

Xenbase is critical to maximizing NIH's investment in *Xenopus* research. Without Xenbase most *Xenopus* research data would be lost in the literature, not accessible by computer searches and not linked to humans or other species/models

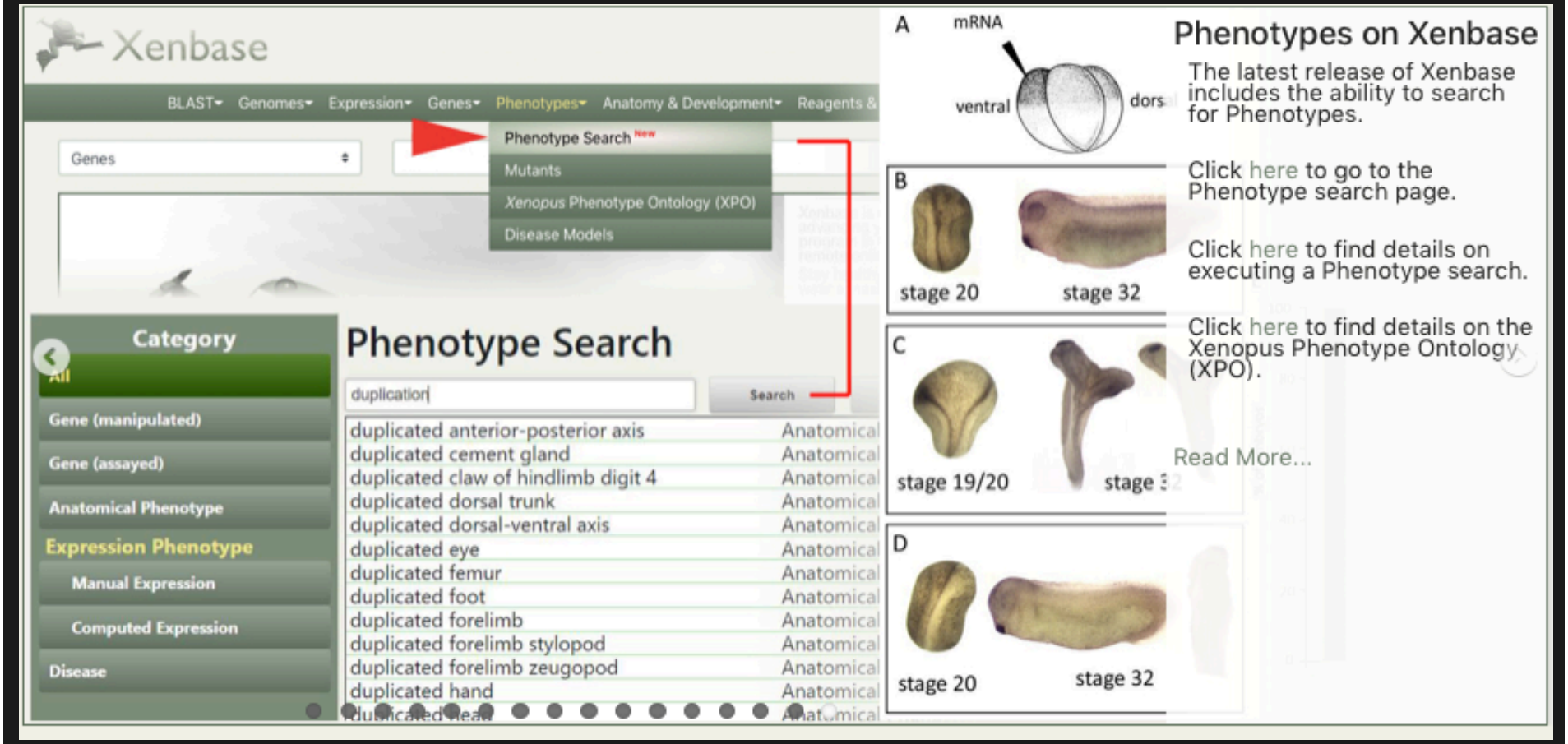
Essential *Xenopus* Resources



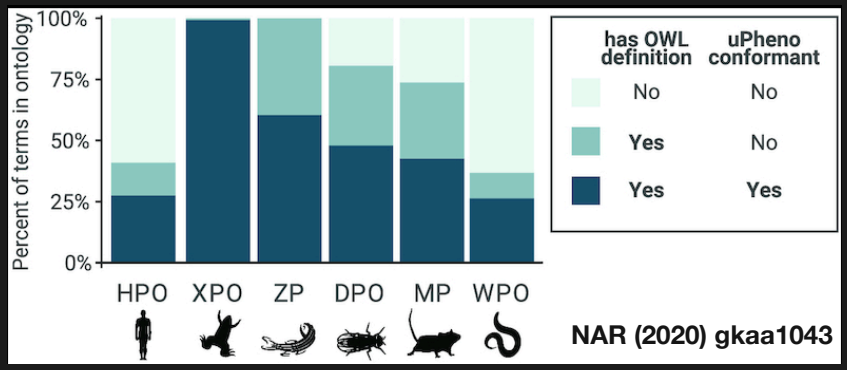
- **Xenopus genes and expression linked to humans and other models**
- **Xenopus phenotypes and models of human disease**
- **Genomes with all public RNA/ChIP-seq data from GEO**
- **Integrates gene expression and functional genomics**
- **Research Community focal point, education and outreach**

Xenbase has one of the most advanced systems for genomics support - reprocessing all of the public data in GEO with a standardized pipeline with sophisticated visualization tools. MGI and ZFIN are interested in adapting Xenbase's system

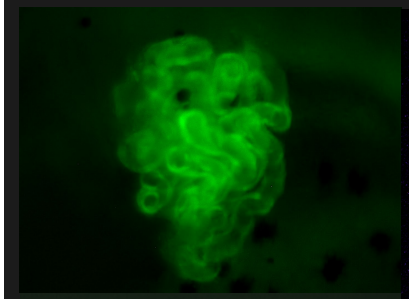
Essential *Xenopus* Resources

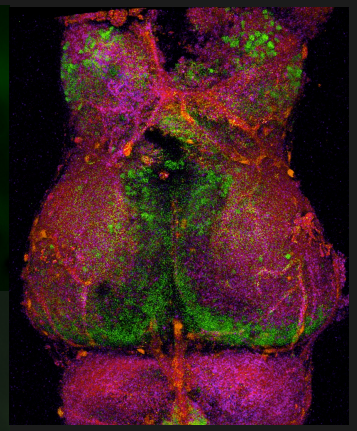


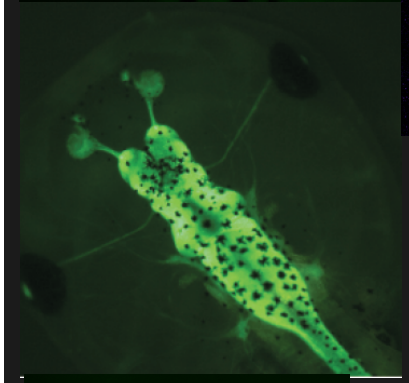
Xenbase has the most advanced phenotype ontology (Xenopus Phenotype Ontology: XPO) that allows Xenopus phenotypes to directly link to human diseases and to other species.

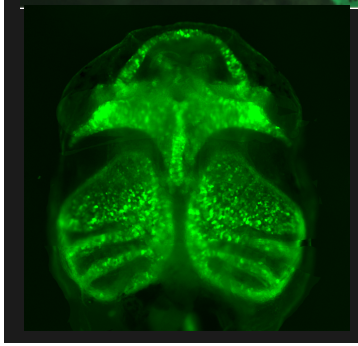


Essential *Xenopus* Resources









National Xenopus Resource

P40 OD010997 National *Xenopus* Resource Center (Marko Horb)

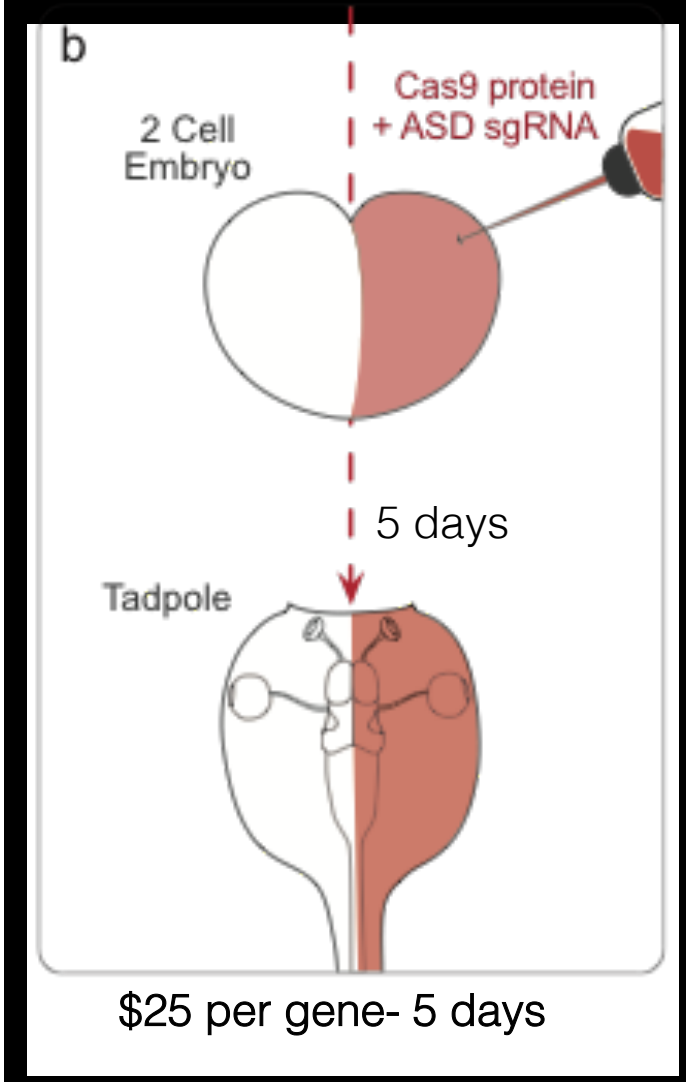
R21 OD023810 Enhancing CRISPR-CAS for Disease Modeling (Marko Horb)

R24 OD030008 *Xenopus* Mutant Resouce (Marko Horb)


Marko Horb

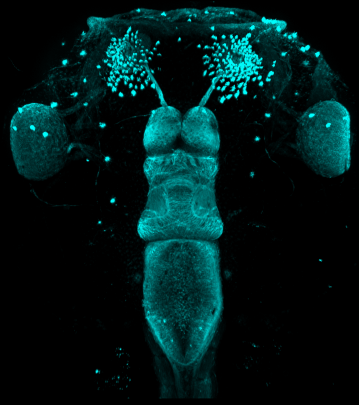
- Maintains and distributes transgenic/mutant/inbred frogs
  -over 250 different lines – including as frozen sperm
- Holds Advanced training workshops
  -genome editing, advanced imaging and bioinformatics
- Hosts visiting researchers -short term projects taking advantage of lines available at the NXR
- Custom transgenic and knockout frogs -currently working on generating 200 germline CRISPR mutants

Study Example:
Whole brain genetic analysis of ASD risk genes in *Xenopus*

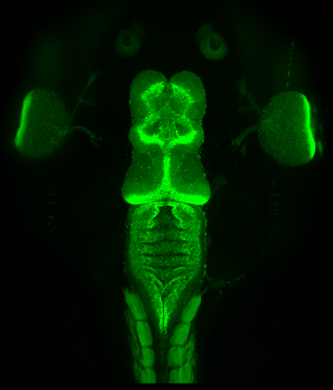


$25 per gene- 5 days

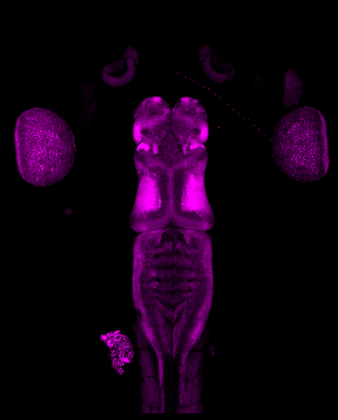
All Neurons



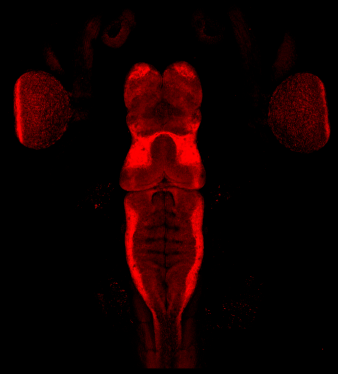
Progenitors



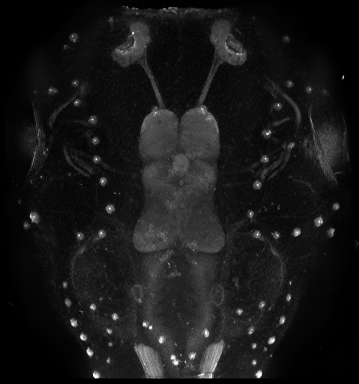
Excitatory Neurons



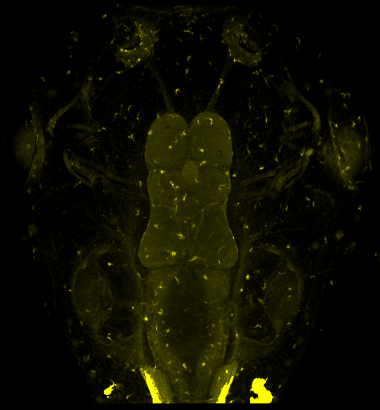
Inhibitory Neurons



Astrocytes

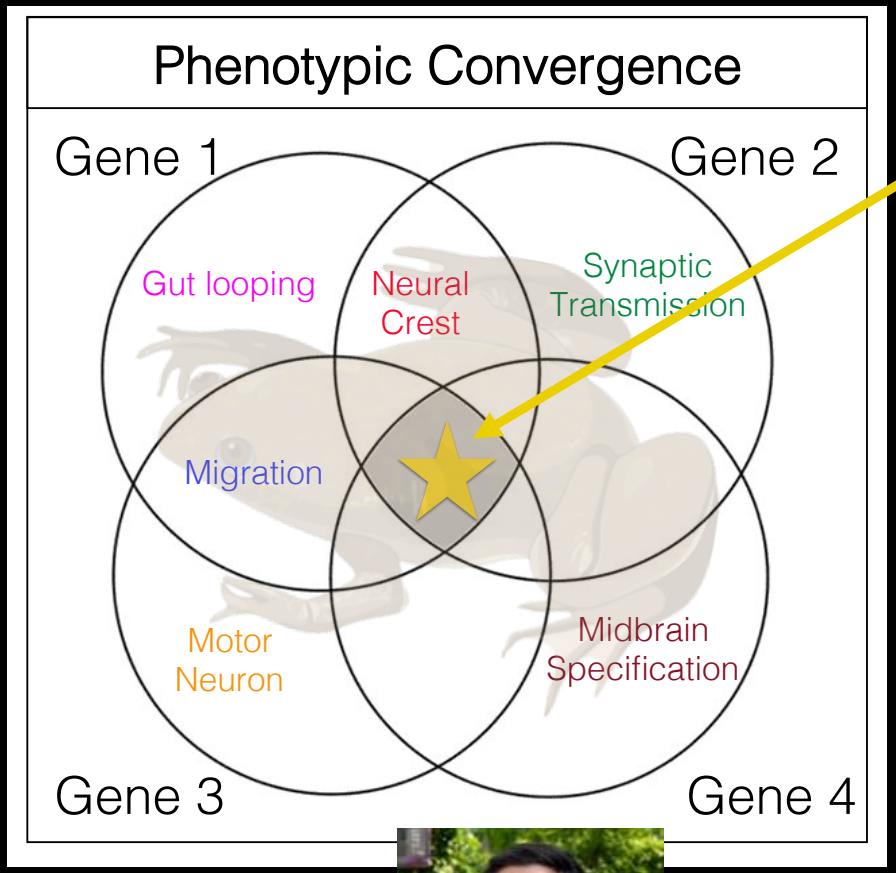


Microglia

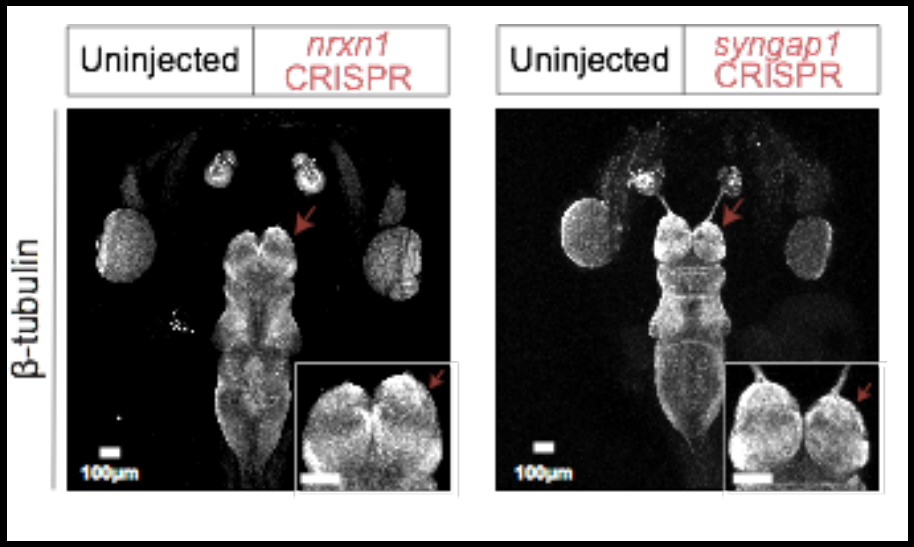


Helen Willsey | UCSF

ASD risk genes and estrogen converge during neurogenesis



Changes in Forebrain Growth (Neurogenesis)



Drug Screening: Estrogen Rescues

Helen Willsey | UCSF

Willsey, et al., *Dev Bio* 2018
Willsey & Xu, et al., *Development* 2020
Willsey, et al., *resubmitted*
Rosenthal & Willsey, et al., *under review*
Exner & Willsey, *genesis, accepted*

Other examples of modeling of Human Disease Genes

> J Med Genet. 2020 Jul 6;jmedgenet-2019-106805. doi: 10.1136/jmedgenet-2019-106805. Online ahead of print.

Journal of medical genetics

***DLG5* variants are associated with multiple congenital anomalies including ciliopathy phenotypes**

> Am J Hum Genet. 2020 Oct 1;107(4):727-742. doi: 10.1016/j.ajhg.2020.08.013. Epub 2020 Sep 4.

Mutations of the Transcriptional Corepressor ZMYM2 Cause Syndromic Urinary Tract Malformations

> J Hum Genet. 2020 Oct;65(10):911-915. doi: 10.1038/s10038-020-0776-0. Epub 2020 May 21.

Novel compound heterozygous variants in NHLRC2 in a patient with FINCA syndrome

> Hum Mol Genet. 2020 Jul 21;29(11):1900-1921. doi: 10.1093/hmg/ddaa050.

Novel truncating mutations in CTNND1 cause a dominant craniofacial and cardiac syndrome

Case Reports > BMC Nephrol. 2019 Jul 17;20(1):271. doi: 10.1186/s12882-019-1458-z.

Identification of novel mutations and phenotype in the steroid resistant nephrotic syndrome gene NUP93: a case report

> Development. 2018 Oct 18;145(20):dev166181. doi: 10.1242/dev.166181.

RPSA*, a candidate gene for isolated congenital asplenia, is required for pre-rRNA processing and spleen formation in *Xenopus

Review > Cold Spring Harb Perspect Biol. 2020 Jun 1;12(6):a037200. doi: 10.1101/cshperspect.a037200.

***Xenopus*: Experimental Access to Cardiovascular Development, Regeneration Discovery, and Cardiovascular Heart-Defect Modeling**

Comparative Study > PLoS Biol. 2019 Sep 6;17(9):e3000437. doi: 10.1371/journal.pbio.3000437. eCollection 2019 Sep.

Conservation and divergence of protein pathways in the vertebrate heart

> Front Physiol. 2020 Feb 18;11:75. doi: 10.3389/fphys.2020.00075. eCollection 2020.

Modeling Bainbridge-Ropers Syndrome in *Xenopus laevis* Embryos

Case Reports > Hum Genet. 2020 Nov;139(11):1363-1379. doi: 10.1007/s00439-020-02175-x. Epub 2020 May 18.

De novo mutations in FBRSL1 cause a novel recognizable malformation and intellectual disability syndrome

> Nat Genet. 2012 May 13;44(6):709-13. doi: 10.1038/ng.2259.

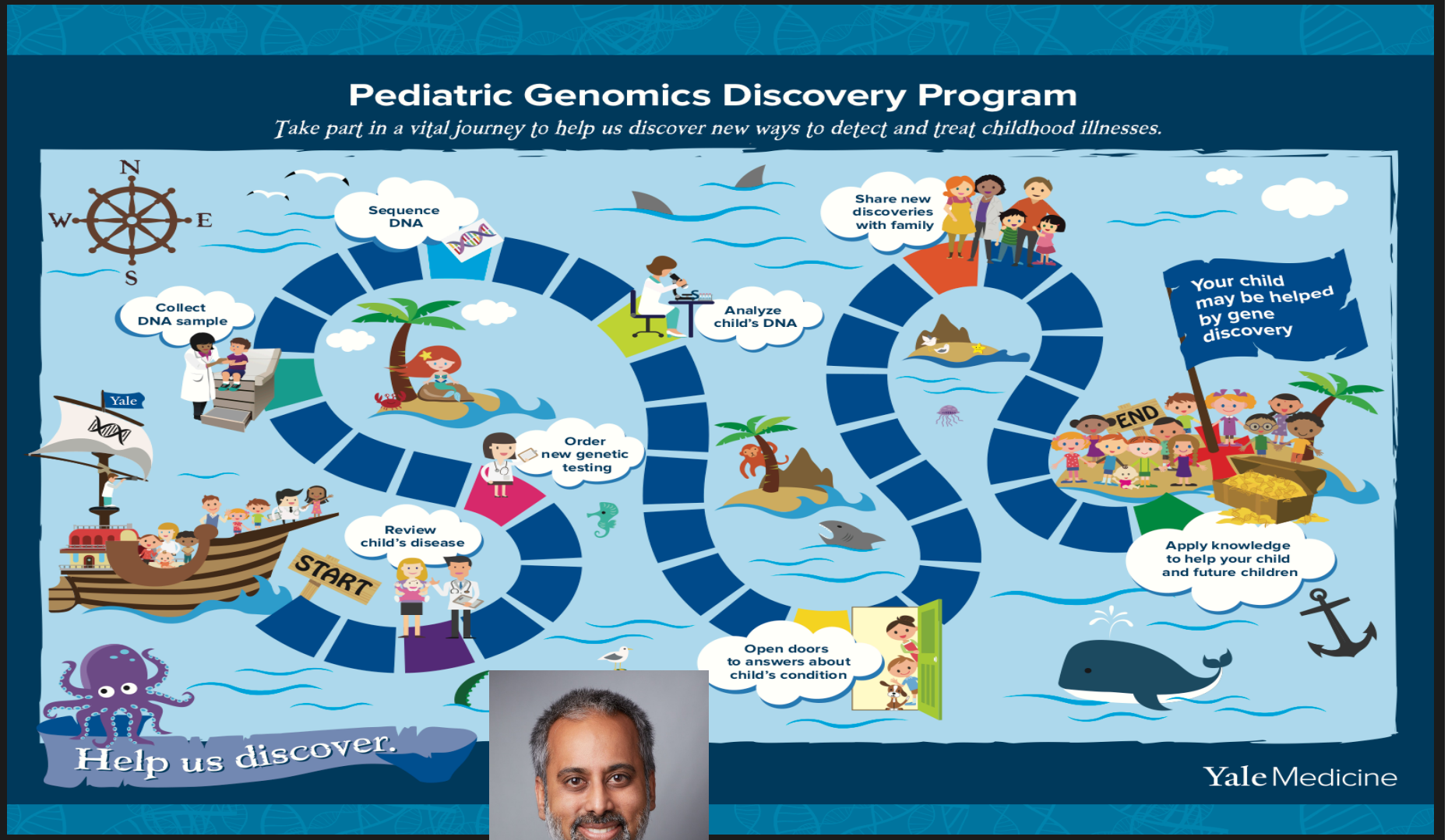
Mutations in IRX5 impair craniofacial development and germ cell migration via SDF1

> J Biol Chem. 2018 Jun 22;293(25):9841-9853. doi: 10.1074/jbc.RA118.003104. Epub 2018 May 10.

FSHD2- and BAMS-associated mutations confer opposing effects on SMCHD1 function

Face Validity – Construct Validity – Predictive Validity

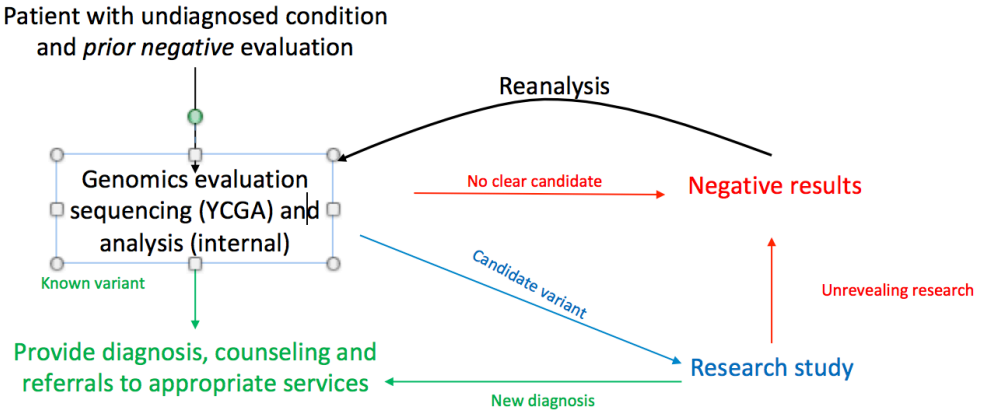
Examples of Established Vertical Integration

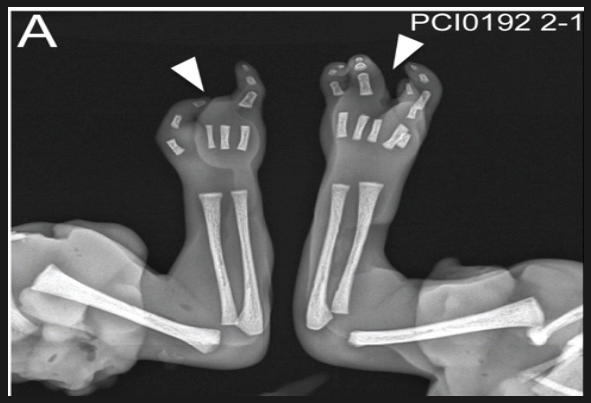


Mustafa Khokha

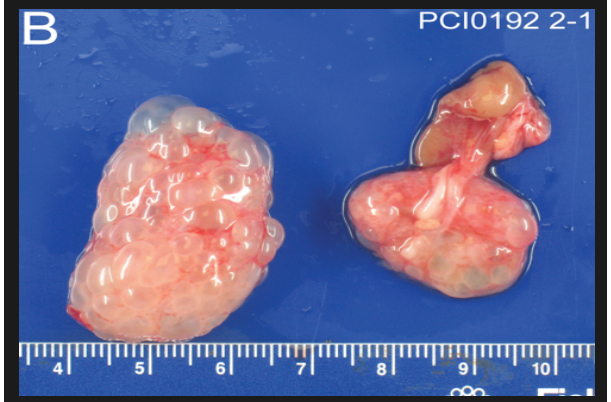
Examples of Established Vertical Integration

The Yale PGDP model



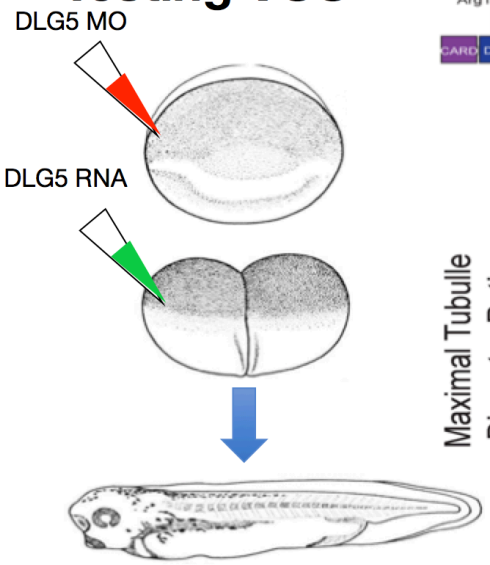


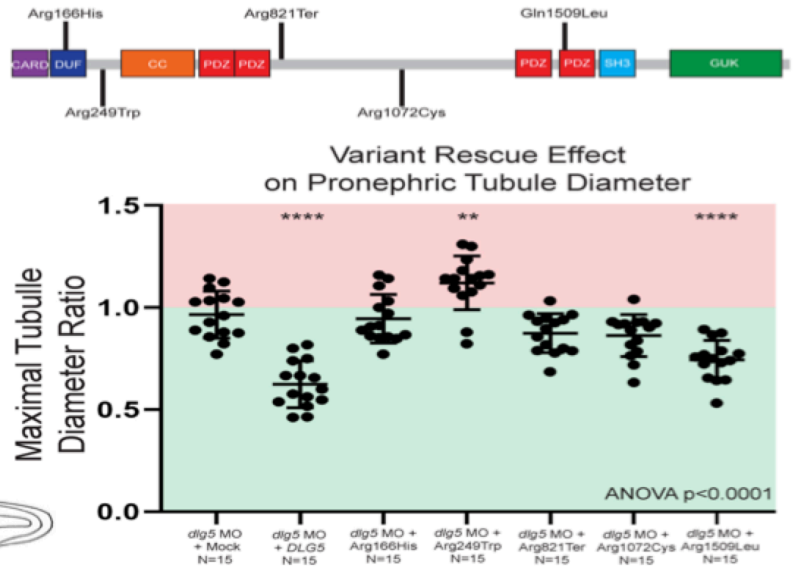
Ectrodactyly, club feet



Multicystic kidneys

Testing VUS



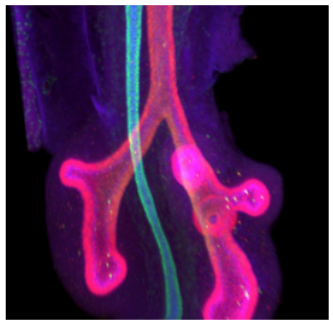


Examples of Established Vertical Integration

CLEAR Consortium Home About Researchers Families News

Lead investigator: Aaron Zorn (CCHMC)

Co-investigators: James Wells, Debora Sinner (CCHMC), Sang-Wook Cha (University of Central Missouri)



This project uses an innovative combination of frog (Xenopus) and mouse models to define the genetic and cellular mechanisms of TE development.

- Characterize the molecular and cellular mechanisms of trachea-esophageal development in Xenopus and mouse. Though several models have been proposed, the cellular processes controlling trachea-esophageal morphogenesis are unknown.
- To determine the cellular processes regulated by developmental cell signaling pathways. Disruption of key pathway genes can cause TEDs in humans, mouse, and Xenopus, but the cellular basis of these phenotypes are not known.
- To model trachea-esophageal defect-causing mutations from TED patients in Xenopus and mouse. Genes with unknown function in trachea-esophageal development and genetic variants from TED patients can be tested using animal models.

Aaron Zorn

P01 HD93363

Short Article

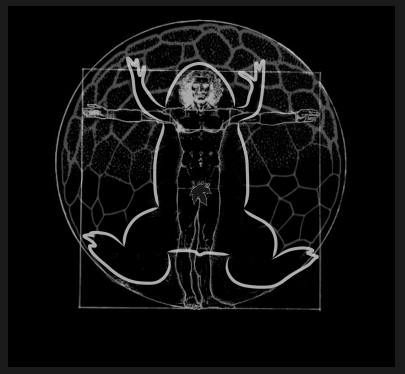
Developmental Cell

Endosome-Mediated Epithelial Remodeling Downstream of Hedgehog-Gli Is Required for Tracheoesophageal Separation

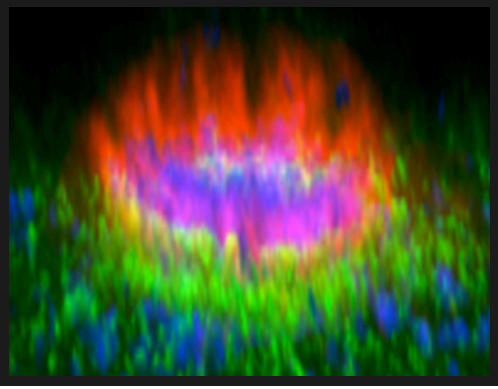
Demonstrated Face Validity for Trachea-Esophogeal Birth Defects - Used Xenopus to study de novo mutations from newborns to define the cell biology of these congenital defects – then confirmed in humans

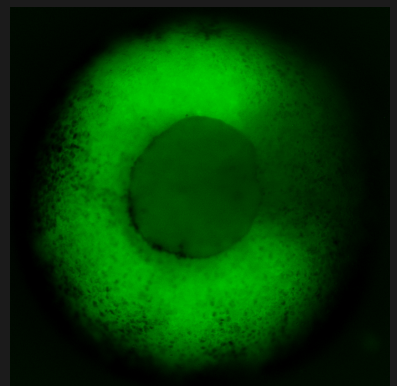
Technologies Needed to Refine Validation

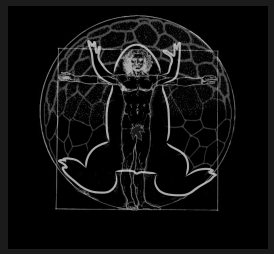
- Knock-in Technologies to Tag Proteins in Frame
- Improved CRISPR-based Generation of Disease Alleles (Humanized Frogs)
- Fuller set of Validated Antibodies
- Additional Transgenic and Mutant Lines
- Large Scale Protein Interaction Data



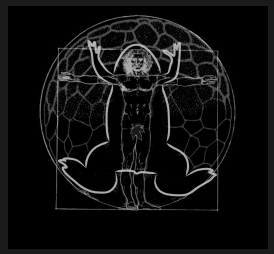
Continued Support of Xenbase and NXR is Essential to the *Xenopus* Community







Other Points:



Science needs a large and diverse set of research organisms – there is no perfect model

There is currently an over-reliance on a small number of organisms to the detriment of advances to human health

Study Sections need better education re the need for and advantages of different research organisms

Taking better advantage of organisms in which disease modeling is rapid and inexpensive can speed results to patients