Cold Spring Harbor Laboratory Course on:
Cell & Developmental Biology of *Xenopus*
March 30- April 11, 2022

INSTRUCTORS:
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COURSE TEACHING ASSISTANTS:
Cameron Exner, University of California San Francisco, San Francisco, CA
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In vivo animal models are an important tool for the understanding of human development and disease. Studies using the frog *Xenopus* have made remarkable contributions to our understanding of fundamental processes such as cell cycle regulation, transcription, translation and many other topics. *Xenopus* is remarkable for studying development and disease, including birth defects, cancer, and stem cell biology. Because *Xenopus* are easy to raise, producing many thousands of eggs per day, these frogs have emerged as a premiere model for understanding of human biology from the fundamental building blocks to the whole organism.

The recent development of CRISPR/Cas9 technology has made it easy to target genes of interest using *Xenopus.* This course has been designed with that in mind. Our goal was for each student to design a set of experiments focusing on their gene or biological interest. Prior to starting the course, students were expected to choose gene(s) of interest, and the instructors generated sgRNAs targeting these genes. These were either the students' own genes, or chosen from a bank provided by the instructors. The gene targeting experiments will be combined with other manipulations, such as tissue explants and transplants and live imaging to analyze the function of the genes.

Xenopus is increasingly being used as imaging test-bed to investigate the roles of cytoskeleton and intracellular trafficking in cell biological and morphogenetic contexts. The course maintains stock mRNAs for targeting fluorescent proteins to specific structures for studying cell shape and cytoskeletal dynamics but students are encouraged to bring or suggest additional tools, including fluorescent biosensors, tension-sensors, etc. The power of Xenopus can be leveraged when live-cell fluorescence imaging is combined with microsurgery, grafting, and dissociated cell culture.

During the course, the students analyzed phenotypes generated from CRISPR/Cas9based gene depletion and learned the diverse array of techniques available in *Xenopus*. In previous courses, we have guided students in the ablation of a wide variety of genes and helped them design suitable assays for their biological interests. Most recently, students have targeted autism genes, thyroid genes and immune modulators, several of which have already led to publications. Approaches covered included microinjection and molecular manipulations such as CRISPR/Cas9 knockouts, antisense morpholino-based depletions, transgenics, and mRNA overexpression. In addition, students combined these techniques with explant and transplant methods to simplify or test tissue level interactions. Additional methods included mRNA in situ hybridization and protein immunohistochemistry as well as basic bioinformatic techniques for gene comparison and functional analysis. Biochemical approaches such as proteomics and mass spectrometry and biomechanical concepts were discussed. Finally, to visualize subcellular and intercellular

activities, we introduced a variety of sample preparation and imaging methods including time-lapse, fluorescent imaging, optical coherence tomography and confocal microscopy. These were facilitated by state-of-the-art equipment from Nikon, Leica, Thorlabs, and Bruker.

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PARTICIPANTS:

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16 Participants (11 Female, 5 Male, 4 URM)

* NIH Scholarship support

SEMINARS:

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Organizer cell signaling

Lance Davidson, University of Pittsburgh, Pittsburgh, PA
Leveraging Xenopus mechanics and morphogenesis

Hironori, Funabiki, The Rockefeller University, New York, NY
Using Xenopus cell extract for chromatin studies

Douglas Houston, University of Iowa, Iowa City, IA
Maternal control of development

Mustafa Khokha, Yale University, New Haven, CT
Patient Driven gene discovery - Oxygen, Mitochondria, and Xenopus Power

Carole LaBonne, Northwestern University, Evanston, IL
Neural crest development

Karen Liu, King's College London, London, United Kingdom
Using Xenopus model to study human diseases

Roberto Mayor, University College London, United Kingdom
Neural crest development

Rachel Miller, UTHealth Houston, McGovern Medical School, Houston, TX
Kidney morphogenesis

Brian Mitchell, Northwestern University, Chicago, IL
Cytoskeleton and ciliogenesis

Gert Jan Veenstra, Radboud University, Nijmegen, Nijmegen, Netherlands
Genomic and epigenomic regulation of cell fates

Sarah Woolner, University of Manchester, Manchester, United Kingdom
Using Xenopus to investigate how mechanical force regulates cell division

Martin Wuhr, Princeton University, Princeton, NJ

Proteomic Approaches to Xenopus Biology

Helen Willsey, **University of California San Francisco, San Francisco, CA**
Neurodegenerative disease models

Jan Witkowski, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Ethics, Rigor and Reproducibility lecture

COURSE ASSESSMENT

CSHL makes considerable efforts to measure the quality and effectiveness of each post-graduate course offered at the Laboratory, in terms of both the immediate and long-term impact on individual participants as well as on the field as a whole. To this end, the course program at CSHL employs two main evaluation instruments:

a) Student evaluations designed to measure the overall satisfaction of participants in a given course
b) Electronic surveys of past students, which are designed to assess the long-term impact of the course on participants' research projects, collaborations, publication records, and careers

a) Student Evaluations

Student evaluations are circulated on the final day of a course, and the students complete them before departing from CSHL so that all suggestions and criticisms are fresh in their minds. To encourage frankness in the students' comments, the evaluations are completely anonymous. Because of the timing and anonymity of these evaluations, response rates are always close to or at 100%. These surveys assess the immediate impact of course material on each trainee as well as the overall organization and logistical support of the course.

The student evaluations are reviewed independently by course instructors and CSHL staff within four weeks of a course's completion. This allows any significant criticisms to be dealt with immediately, and also allows constructive comments to be considered in the following year's course design. If a majority of numerical responses to a given question are less than 4, email or telephone conversations between CSHL staff and the course's instructors will occur to address the issue and rectify it for the following year. In extremely rare cases, student evaluations indicate a more serious problem and drastic steps must be taken by CSHL, up to and including the replacement of individual instructors. However, historic averages indicate that CHSL courses are consistently rated as exceptional, and evaluations tend to contain only minor suggestions for improvement that instructors incorporate easily in their planning for subsequent years.

Many CSHL courses have contributed significantly to the development of their respective fields through the connections and collaborations established within a given year's cohort of the course. It is clear from extensive informal feedback that beneficiaries of the course include not only the students but also the instructors, assistants, visiting faculty, and technical staff from companies who help support the course.

The table below is a summary of the average responses from participants of the supported course for the period 2011 – 2021. The scores are in the very-good-to-exceptional range for most questions, indicating a clear level of satisfaction amongst each student class upon completion of the course. The numerical responses range from 1 to 5, 5 equates roughly to "strongly agree/nothing should be changed" and 1 is "strongly disagree/changes are definitely needed." Copies of individual evaluation forms are available upon request.

CSHL Course: Cell & Developmental Biology of *Xenopus* (2011-2022)

Questionnaire / Response Average	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2022**
In general, did the course meet your needs/expectations?	4.7	4.1	4.6	4.6	4.9	4.7	4.4	4.7	4.3	4.9
Were the lecture topics well chosen?	4.3	4.2	4.1	4,2	4.8	4.5	4.9	4.7	4.1	4.7
Was the level of the lectures appropriate?	4.6	4.2	3.8	4.5	4.5	4.3	4.8	4.9	4.2	4.8
Were the presentations clear?	4.5	4.1	4.0	4.5	4.3	4.5	4.8	4.9	4.0	4.8
Were the instructors helpful?	4.8	4.5	4.7	4.9	5.0	4.9	4.8	4.9	4.8	5.0
Was the selection of lab exercises appropriate?	4.5	4.1	4.5	4.5	4.7	4.8	4.4	4.8	4.2	4.9
Was there sufficient/too much supervision of the lab?	4.6	3.9	4.4	4.5	4.8	4.5	4.8	4.4	4.4	4.3
Were the labs well enough equipped?	4.5	4.1	4.2	4.6	4.7	4.7	4.5	4.5	4.1	4.8
What was the utility and quality of the written experimental protocols?	4.5	4.1	4.5	4.6	4.6	4.6	4.1	4.5	4.1	4.9
How was the course work load?	4.4	4.0	4.0	4.3	4.7	4.3	4.5	4.7	4.0	4.6
AVERAGE	4.5	4.1	4.3	4.6	4.7	4.6	4.6	4.7	4.2	4.8

b) Long-Term Assessments

Long-term impact measures we collect and track include overall satisfaction, proportion of alumni still working in biomedical science, publication records, and publications attributed specifically to course participation. Because trainees may have switched institutional affiliations multiple times since taking a course, left science altogether, moved into industry, or changed names after marriage, it is frequently difficult to track down former students of CSHL courses. The CSHL staff currently uses a variety of search tools (Google and PubMed) and online profiles (LinkedIn and ResearchGate) in attempts to find former students. Students who can be found are solicited via email approximately every five years and directed to an online survey with questions designed to evaluate how a course contributed to their intellectual development, technical expertise, scientific collaborations, and publication records. Response rates to this kind of longitudinal survey currently run 25-50% depending on the course and years surveyed, and the majority of responses are overwhelmingly positive.