***XENOPUS COMMUNITY WHITE PAPER 2016***

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 I. Executive Summary

***A. Xenopus* is an essential vertebrate model system for biomedical research**

Model animals are crucial to biomedical research. Among the commonly used model animals, the amphibian, *Xenopus,* has tremendous impact because of its unique experimental advantages, cost effectiveness, and close evolutionary relationship with mammals. Over the past 50 years the use of *Xenopus* has made possible many fundamental contributions to biomedicine, and it is a cornerstone of research in neurobiology, physiology, molecular biology, cell biology, and developmental biology. Looking forward, *Xenopus* continues to be a powerful system to study fundamental biological and disease mechanisms. Moreover, recent advances in high throughput DNA sequencing, genome editing, proteomics, and pharmacological screening are particularly well suited to *Xenopus,* enabling rapid functional genomics and human disease modeling at a systems level.

**This White Paper highlights recent contributions of *Xenopus* research to the NIH’s mission and outlines recommendations for how continued, focused NIH investment can maximize the impact of biomedical research using the *Xenopus* system**.

**B. Past NIH investment in research using *Xenopus* has significantly advanced biomedical research**

The NIH’s stated mission is “*to seek fundamental knowledge about the nature and behavior of living systems and to apply that knowledge to enhance health, lengthen life, and reduce illness and disability*”. As one means to achieve these goals, the NIH has made a large and sustained investment in biomedical researchusing *Xenopus*.

* In FY FY2015-2016, the NIH (17 different Institutes plus the Office of the Director) supported **316** active “*Xenopus*” grants with over **$117 million**.
* In the last four years (2012-2015) the NIH provided a total investment of more than **$501 million**.
* These grants have supporting numerous research programs and training opportunities. They have also supported over 20 projects that have developed critical resources, including the *Xenopus* *tropicalis* and *Xenopus laevis* genome sequences, mutational resources, the *Xenopus* ORFeome, as well as extensive epigenetic, transcriptomic, and proteomic datasets.
* The outstanding return on this investment is evident from the large number of published research contributions that demonstrate how *Xenopus*: 1) provides fundamental knowledge about biological processes; 2) plays a crucial role in elucidating the basic cellular, molecular and biochemical mechanisms underlying a wide spectrum of human pathologies; 3) is a powerful system for elucidating the function of human disease genes; and 4) is a superb system for modeling human diseases.

**C. Community recommendations for continued NIH investment in *Xenopus* research**

The discoveries already made using *Xenopus* attest to the **productivity and impact** the *Xenopus* system can have on the NIH’s goals to discover fundamental new knowledge and to translate that knowledge to issues impacting human health. *Xenopus* is a particularly compelling model system in the current fiscal climate because it provides outstanding value for money, allowing the study of conserved biomedical mechanisms at a fraction of the cost of mammalian models and at a much faster rate. To maximize the NIH’s investment and realize the full potential of *Xenopus* research*,* we need to:

1. continue to invest in research utilizing this model; and
2. maintain the established community resources that have already led to major advancements.

To this end, members of the *Xenopus* community, representing broad and diverse fields of inquiry, met in September 2015 at the Marine Biological Laboratory to establish priorities for continued investment that would have the largest impact on the mission of the NIH. There was broad community-wide consensus that six resources are currently needed to support the existing research community and to make this powerful model system more accessible to biomedical researchers from other fields. These were prioritized into two categories: **Immediate Needs** and **Essential Resources**.

**Immediate needs of the *Xenopus* research community (highest priority):**

**1.** Development of protein resources (ORFeome; Antibodies; Proteome)

**2.** Development and maintenance of “Big Data” repositories (Xenbase, XenMine)

**Essential resources needed by the *Xenopus* research community:**

In addition, the community identified four additional Essential Resources that should be developed as soon as possible so that *Xenopus* research can more effectively fulfill the missions of the NIH. The *Xenopus* community considers all of these additional resources to be essential, but understands that priorities must be set, and therefore ranks these as indicated below:

1. Enhancement of efficient gene editing approaches and mutant lines
2. Enhancement of transgenic resources
3. Improvement and expansion of amphibian genomic resources
4. Support for *Xenopus* training and meetings

To accomplish these goals, the *Xenopus* community strongly advocates:

* Issuing “Resource” PARs to develop protein resources
* Issuing “Resource” PARs to enable renewed funding of Xenbase, XenMine and the *Xenopus laevis* Research Resource for Immunobiology (XLRRI) ([Section IV-2](#Repositories)), in support of “Big Data” repositories, enhancement of gene editing approaches, and providing mutant and transgenic lines.
* Continuing to support “Resource” study sections and R24 “Resource” grants in consideration that “Resource” projects are essential to the NIH mission and have a broad and long-lasting impact on the entire biomedical community.
* Ensure that new RFAs/PARs addressing models of human disorders or functional genomics specifically include *Xenopus* in the language. This would allow investigators responding to new RFAs/PARs in these areas to leverage the established advantages of *Xenopus* with functional genomics ([Section IV-2](#Repositories)), genome editing ([Section IV-3](#GeneEditing)), or improved transgenesis ([Section IV-4](#Transgenic)). Such studies would facilitate systems-level analyses of disease *in vivo* and have a significant impact on translation of “Big Data to Knowledge” that would align well with the NIH [BD2K](http://bd2k.nih.gov/#sthash.zjpNAOQm.dpbs) initiative.
* Increase support for training in cutting edge techniques, such as those provided at the Cold Spring Harbor Laboratory and the National *Xenopus* Resource (NXR), and support for incorporating *Xenopus* into laboratories using other animal models. Increase support to attend international and national meetings for junior researchers.

**D. Long term goal of using *Xenopus* to understand human disease:**

The major long-term goal of the *Xenopus* research community is to accelerate the use of this animal as a model for understanding human diseases. All of the essential resources in this White Paper **will help achieve the overarching objective of optimizing the utility of *Xenopus* to study human disease and improve human health**. Coupling the experimental advantages of *Xenopus* with recent advances in genome editing and high throughput analysis will establish a cost-effective platform for the rapid identification, validation, and characterization of genes involved in human disease. It will also provide mechanistic insight into potential therapeutic design. From John Gurdon’s initial identification of nuclear reprogramming, through Tim Hunt’s identification of cyclins, and many more recent findings, *Xenopus* research has produced many seminal discoveries that have helped elucidate the cellular mechanisms underlying human diseases. By revealing fundamental biological processes, *Xenopus* research informs our understanding of how gene dysregulation can lead to disease and has provided critical insight into how these pathways might be manipulated for regeneration, repair and aging. **II. Introduction**

Model animals are essential for biomedical research. Among the most commonly used laboratory-based model animals, the frog, *Xenopus,* has had tremendous impact because of its **unique experimental advantages**, **cost effectiveness**, and **close evolutionary relationship with mammals**. The ability to directly observe all stages of the *Xenopus* life cycle free of maternal influences, from fertilized egg to adult, is optimal for the study of a multitude of developmental and physiological processes, pathologies and regenerative responses. The rapid developmental time scale and the uniquely large and accessible eggs and embryos further enhance this capacity. *Xenopus* provides a remarkably broad experimental platform in neurobiology, physiology, molecular biology, cell biology, and developmental biology. As an amphibian tetrapod, *Xenopus* also bridges the gap between costly mammalian models and the evolutionarily more distant zebrafish model. Over the past 50 years, studies using *Xenopus* have provided many fundamental contributions to biomedicine, revealing critical information about the basic biological mechanisms that underlie normal human health and disease. For example, in 2012 Sir John Gurdon was awarded the Nobel Prize for his discovery in *Xenopus* that adult cells can be reprogrammed to become pluripotent, revolutionizing our understanding of cell differentiation and paving the way for methods to induce pluripotent stem cells in humans; this finding has transformed regenerative biomedical research. Looking forward, *Xenopus* continues to be a powerful system to study fundamental biological and disease mechanisms. Moreover, recent technological advances in high-throughput DNA sequencing, genome editing, proteomics, and pharmacological screening are particularly well suited to *Xenopus,* enabling rapid functional genomics and human disease modeling at a systems level.

**Thus, *Xenopus* has proven to be an essential vertebrate model system for:**

1. understanding fundamental biological processes;
2. applying fundamental knowledge to pathological processes;
3. deciphering the function of human disease genes; and
4. modeling human diseases.

We provide a representative list of recent publications that utilized *Xenopus* to elucidate fundamental biological mechanisms and understand and model human disease processes.

**A. *Xenopus* provides fundamental knowledge** to elucidate the cellular, molecular and biochemical mechanisms underlying a wide spectrum of biological processes.

**Cell division:**

Petridou and Skourides. 2014. Nat. Commun. 5:5240. http://www.ncbi.nlm.nih.gov/pubmed/?term=PMID%3A+25341507

Jiang et al. 2015. Cell 163: 108-122. <http://www.ncbi.nlm.nih.gov/pubmed/26388440>

Sanuki et al. 2015.Cell Cycle 14: 1010-1023. <http://www.ncbi.nlm.nih.gov/pubmed/25602506>

Thuret et al. 2015. Biol. Open. 4: 1772-1781. <http://www.ncbi.nlm.nih.gov/pubmed/26621828>

Wilczynska et al. 2016. PLoS ONE 11: e0146792. <http://www.ncbi.nlm.nih.gov/pubmed/26829217>

Malhotra et al. 2016. Dev. Cell 36: 94-102. <http://www.ncbi.nlm.nih.gov/pubmed/26766445>

**DNA repair and replication:**

Kalb et al. 2014. Cell Rep. 8:999-1005. http://www.ncbi.nlm.nih.gov/pubmed/25131202

Long et al. 2014. Mol. Cell 56: 174-185. <http://www.ncbi.nlm.nih.gov/pubmed/25219499>

Räschle et al. 2015. Science 348:1253671. <http://www.ncbi.nlm.nih.gov/pubmed/25931565>

Shintomi et al., 2015. Nature Cell Biol. 17: 1014-1023. http://www.ncbi.nlm.nih.gov/pubmed/26075356

Zhang et al. 2015. Nat. Struct. Mol. Biol. 22: 242-247. http://www.ncbi.nlm.nih.gov/pubmed/25643322

**Genome organization, transcriptional regulation and epigenetics:**

Wang et al. 2014. Epigenetics Chromatin 7: 22. <http://www.ncbi.nlm.nih.gov/pubmed/25302076>

Belikov et al. 2015. Nucleic Acids Res. pii: gkv1350. <http://www.ncbi.nlm.nih.gov/pubmed/26657626>

Buisine et al. 2015. PLoS ONE 10: e0137526. <http://www.ncbi.nlm.nih.gov/pubmed/26348928>

Hontelez et al. 2015. Nat. Commun. 6: 10148. <http://www.ncbi.nlm.nih.gov/pubmed/26679111>

Wen et al. 2015. FASEB J. 29: 385-393. <http://www.ncbi.nlm.nih.gov/pubmed/25366346>

Bogdanovic et al. 2016. Nature Genetics 48: 417-426. <http://www.ncbi.nlm.nih.gov/pubmed/26928226>

Gazdag et al. 2016. Development. <http://www.ncbi.nlm.nih.gov/pubmed/26952988>

Gao et al. 2016. Development 143: 492-503. <http://www.ncbi.nlm.nih.gov/pubmed/26700681>

Owens et al. 2016. CellRep.14: 632-647. <http://www.ncbi.nlm.nih.gov/pubmed/26774488>

Tamaoki et al. 2016. Cell Biosci. 6:2. <http://www.ncbi.nlm.nih.gov/pubmed/26798452>

**Hematopoiesis and vascular development:**

Metikala et al. 2015. Angiogenesis. [Epub ahead of print]. <http://www.ncbi.nlm.nih.gov/pubmed/26678600>

Mimoto et al. 2015. Dev. Biol. 407: 1-11. <http://www.ncbi.nlm.nih.gov/pubmed/26365900>

Tanizaki et al. 2015. Exp. Hematol. 43: 125-136. <http://www.ncbi.nlm.nih.gov/pubmed/25448492>

Agricola et al. 2016. Dev. Dyn. 245: 47-55. <http://www.ncbi.nlm.nih.gov/pubmed/26264370>

**Morphogenesis and organogenesis:**

Bestman et al. 2015. Dev*.* Bio. 408: 269-291. <http://www.ncbi.nlm.nih.gov/pubmed/25818835>

Nie and Bronner. 2015. Cardiovasc. Res. 106: 67-75. <http://www.ncbi.nlm.nih.gov/pubmed/25691536>

Okada et al. 2015. Cell Biosci. 5: 74. <http://www.ncbi.nlm.nih.gov/pubmed/26719790>

Ossipova et al. 2015. Dev. Bio. 408: 316-327. <http://www.ncbi.nlm.nih.gov/pubmed/26079437>

**Nuclear reprogramming and stem cells:**

Ali et al. 2014. Development 141: 2216-2224. <http://www.ncbi.nlm.nih.gov/pubmed/24821983>

Jullien et al. 2014. Mol Cell 55: 524-536.<http://www.ncbi.nlm.nih.gov/pubmed/25066233>

Kole et al. 2014. Cell Reprogram.16: 18-28. <http://www.ncbi.nlm.nih.gov/pubmed/2440506>

Maza & Hanna 2014. Mol. Cell 55: 507-509. <http://www.ncbi.nlm.nih.gov/pubmed/25148360>

Buitrago-Delgado et al. 2015. Science 348: 1332-1335. <http://www.ncbi.nlm.nih.gov/pubmed/25931449>

Kurmann et al. 2015. Cell Stem Cell 17: 527-542.<http://www.ncbi.nlm.nih.gov/pubmed/26593959>

Paranjpe & Veenstra 2015. BiochBiophysActa1849:626-36. <http://www.ncbi.nlm.nih.gov/pubmed/25857441>

**Signal transduction pathways:**

Lee et al. 2015. Elife. 4: e08142. <http://www.ncbi.nlm.nih.gov/pubmed/26297804>

Zhang et al., 2015. Dev. Cell 32: 719-730. http://www.ncbi.nlm.nih.gov/pubmed/25771893

Lee et al. 2016. Dev. Biol. pii: S0012-1606(16)30027-6. <http://www.ncbi.nlm.nih.gov/pubmed/26806705>

Park et al. 2016. Development [Epub ahead of print]. <http://www.ncbi.nlm.nih.gov/pubmed/26811381>

Zhang et al. 2016. J. Biol. Chem. 291: 2435-2443. <http://www.ncbi.nlm.nih.gov/pubmed/26631728>

Zhao et al. 2016. Dev. Biol. pii: S0012-1606(15)30212-8. <http://www.ncbi.nlm.nih.gov/pubmed/26845534>

**B. *Xenopus* is used to apply this fundamental knowledge to understanding a variety of pathological processes.**

**DNA damage response and apoptosis:**

McCoy et al. 2013. J. Biol. Chem. 288: 8838-8848. <http://www.ncbi.nlm.nih.gov/pubmed/23400775>

Kermi et al., 2015. Dev. Cell 34: 364-372. <http://www.ncbi.nlm.nih.gov/pubmed/?term=26212134>

Olivera Harris et al. 2015. DNA Repair 28: 1-7. <http://www.ncbi.nlm.nih.gov/pubmed/25697728>

Shi et al. 2015. FASEB J. 29: 4914-4924. <http://www.ncbi.nlm.nih.gov/pubmed/26268927>

Tammaro et al. 2016. Nucleic Acids Res. 44: 221-231. [http://www.ncbi.nlm.nih.gov/pubmed/26420828](http://www.ncbi.nlm.nih.gov/pubmed/26420828%20)

**Immune and inflammatory responses:**

Grayfer & Robert. 2014. J. Leukoc. Biol. 96:1143-1153. <http://www.ncbi.nlm.nih.gov/pubmed/25190077>

Edholm et al. 2015. J. Immunol. 2015. 195:576-586. <http://www.ncbi.nlm.nih.gov/pubmed/26062996>

Haynes-Gimore et al. 2015. Dev. Biol. 408: 205-212. <http://www.ncbi.nlm.nih.gov/pubmed/?term=25601449>

Paredes et al. 2015. Dev. Biol. 408:213-228. <http://www.sciencedirect.com/science/article/pii/S0012160615001219>

Wangkanont et al. 2016. J. Biol. Chem. M115.709212. <http://www.jbc.org/content/early/2016/01/11/jbc.M115.709212>

**Regenerative plasticity and wound healing:**

Hyashi et al. 2015. Dev. Biol. 406:271-282. <http://www.ncbi.nlm.nih.gov/pubmed/26282893>

Munoz et al. 2015. Dev. Biol. 408: 229-243. <http://www.ncbi.nlm.nih.gov/pubmed/25797152>

Wang et al. 2015. Mech. Dev.138 Pt 3:256-267. <http://www.ncbi.nlm.nih.gov/pubmed/26862980>

Wang and Beck. 2015. Regeneration 2: 19-25. <http://www.ncbi.nlm.nih.gov/pubmed/24823862>

Franchini & Bertolotti. 2016. Acta Histoch. 116:1141-1147. <http://www.ncbi.nlm.nih.gov/pubmed/24998030>

**Response to environmental toxicity:**

Chen et al. 2015. Env. Toxic. Chem. 34:1770–1777. <http://onlinelibrary.wiley.com/doi/10.1002/etc.2980/full>

Hellyer et al. 2015. J. Neurochem. 135:479-91. [http://www.ncbi.nlm.nih.gov/pubmed/?term=26173951](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hellyer++xenopus)

Sai et al. 2015. Env. Contam. Toxicol. 95:157-163. <http://www.ncbi.nlm.nih.gov/pubmed/25634327>

**Therapeutics development:**

Rodrigues et al. 2015 Neuropharmacology105:241-257. <http://www.ncbi.nlm.nih.gov/pubmed/26801076>

Quadri et al. 2016. Bioorg. Med. Chem. 24:286-93.

[http://www.unboundmedicine.com/medline/citation/26707847/Dissection\_of\_N,N-diethyl-N](http://www.unboundmedicine.com/medline/citation/26707847/Dissection_of_N%2CN-diethyl-N)

Volkman et al 2016 PloS One 11:e0148129. <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0148129>

***C. Xenopus* is a powerful system for deciphering the function of human disease genes.** As new genes that cause human disease are identified, understanding their normal and disease functions is critical to designing therapeutic approaches. The use of *Xenopus* eggs and embryos for *in vivo* analyses of disease gene expression and function has exploded in recent years.

**Alzheimer’s, Depression, Autism and other Neurodevelopmental Disorders:**

James et al. 2015. J. Neurosci. 35: 3218-3229. <http://www.ncbi.nlm.nih.gov/pubmed/25698756>

Park et al. 2015. BMC Anesthesiology 15:116 <http://bmcanesthesiol.biomedcentral.com/articles/10.1186/s12871-015-0098-5>

Ramirez-Vizcarrando et al. 2015. FASEB J. 21 <http://www.fasebj.org/content/29/1_Supplement/657.1>

Ullah et al. 2015. PLoS ONE 10: e0137357. <http://www.ncbi.nlm.nih.gov/pubmed/26348728>

**Cancers:**

Haynes-Gimore et al. 2015. Carcinogenesis 35:1808 http://carcin.oxfordjournals.org/content/35/8/1807.long

Van Nieuwenhuysen et al. 2015. Oncoscience 19: 555-566. <http://www.ncbi.nlm.nih.gov/pubmed/26097888>

Green et al. 2016. Gene Expression Patterns 20: 55-62. <http://www.ncbi.nlm.nih.gov/pubmed/26631802>

Wei et al. 2016. Cancer Sci. [Epub ahead of print]. <http://www.ncbi.nlm.nih.gov/pubmed/26749017>

**Congenital Heart Defects:**

Endicott et al. 2015. Development 142: 4068-4079. <http://www.ncbi.nlm.nih.gov/pubmed/26493400>

Torres-Prioris et al. 2015. Lab. Anim. 49 (3S): 60. <http://riuma.uma.es/xmlui/handle/10630/10732>

Silva et al. 2016. Mol. Biol. Cell 27:48-63. <http://www.ncbi.nlm.nih.gov/pubmed/26538025>

**Craniofacial and Auditory Malformations:**

Griffin et al. 2015. PLoS Genet. 11: e1005018. <http://www.ncbi.nlm.nih.gov/pubmed/25756904>

Moody et al. 2015. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 178:16-24.
<http://www.ncbi.nlm.nih.gov/pubmed/26117063>

Ramirez-Gordillo. 2015. BMC Res. Notes 8: 691. <http://www.ncbi.nlm.nih.gov/pubmed/26582541>

Dickinson. 2016. Semin. Cell Dev. Biol. S1084-9521. <http://www.ncbi.nlm.nih.gov/pubmed/26778163>

**Diabetes:**

Pearl et al., 2011. Dev Biol. 351: 135-145. http://www.ncbi.nlm.nih.gov/pubmed/21215266

Salanga and Horb. 2015. Curr Pathobiol Rep. 3:137-145. <http://www.ncbi.nlm.nih.gov/pubmed/26236566>

Kofent & Spagnoli. 2016. Semin Cell Dev Biol S1084-9521. <http://www.ncbi.nlm.nih.gov/pubmed/26806634>

**Kidney Disease:**

Desgrange et al. 2015. Cells. 4:483-499. <http://www.ncbi.nlm.nih.gov/pubmed/26378582>

Stiburkova et al. 2015. Am. J. Med. Sci. 350: 268-271. <http://www.ncbi.nlm.nih.gov/pubmed/26418379>

Leinkamp 2016. Semin. Dev. Cell Biol. S1084-9521. <http://www.ncbi.nlm.nih.gov/pubmed/26851624>

**Lennox-Gastaut Syndrome:**

Hammer et al. 2015. PLoS ONE 10: e0120239. <http://www.ncbi.nlm.nih.gov/pubmed/25798598>

**Zimmermann-Laband syndrome:**

Kortüm et al. 2015. Nature Genet. 47: 661-667. <http://www.ncbi.nlm.nih.gov/pubmed/25915598>

***D. Xenopus is a superb system for modeling human diseases.*** The ability to make humanized mutations quickly in founder lines and expression vectors, and the similarity in anatomy and function of most organs across the tetrapods (amphibian, reptiles, avians and mammals), make *Xenopus* an ideal system in which to model human diseases.

 **Aniridia:**

Nakayama et al. 2015. Dev. Biol. 408: 328-344. <http://europepmc.org/abstract/MED/25724657>

**Blood brain barrier dysfunction**:

De Jesus Andino et al. 2016. Scientific Reports 6: 22508. http://www.nature.com/articles/srep22508

**Congenital heart disease:**

Battacharya et al. 2016. Dev. Biol. 408: 196-204. <http://www.ncbi.nlm.nih.gov/pubmed/26546975>

**Demyelination diseases:**

Sekizar et al. 2015. Dev. Neurosci. 37: 232-242. <https://www.karger.com/Article/FullText/380817>

**Holoprosencephaly:**

Nakayama et al. 2015. Genesis 51:835-843. <http://www.ncbi.nlm.nih.gov/pubmed/24123613>

**Huntington’s Disease:**

Haremaki et al. 2015. Dev. Biol. 408: 305-315. <http://www.ncbi.nlm.nih.gov/pubmed/26192473>

**Myasthenia gravis:**

Yeo et al. 2015. Dev. Biol. 408: 244-251.
<http://www.sciencedirect.com/science/article/pii/S0012160615000871>

**Pneumonia:**

Walentak et al. 2015. Dev. Biol. 408: 292-304. <http://www.ncbi.nlm.nih.gov/pubmed/25848696>

**Tumor progression:**

Hardwick & Philpott. 2015. Dev. Biol. 408: 180-187. <http://www.ncbi.nlm.nih.gov/pubmed/25704511>

**III.** **Past NIH Investment in Research Using *Xenopus* has Significantly Advanced Biomedical Research**

The NIH’s stated mission is “*to seek fundamental knowledge about the nature and behavior of living systems and to apply that knowledge to enhance health, lengthen life, and reduce illness and disability*”. As one means to achieve these goals, the NIH has made a large and sustained investment in biomedical researchusing *Xenopus*. A February 2016 search of the NIH RePORT database using the term “*Xenopus*” returned **316 active grants** of over **$117,000,000 in FY2015-2016**, funded by **17 different Institutes plus the Office of the Director (OD)**. This includes ~$12 million awarded through a multi-institute “Genetic and Genomic Analysis of *Xenopus*” PAR-12-250/1 that supported over 20 projects leading to many critical resources including the *Xenopus* *tropicalis* and *Xenopus laevis* genome sequences, mutational resources, the *Xenopus* ORFeome and extensive epigenetic, transcriptomic and proteomic datasets. Included in this figure is a >$5 million investment by NICHD and the OD to establish and maintain the National *Xenopus* Resource (NXR) in Woods Hole, MA and Xenbase, the *Xenopus* online bioinformatics database, and a >$3.3 million investment by the NIAID to establish and maintain the *Xenopus laevis* Research Resource for Immunobiology (XlRRI) at the University of Rochester, NY. These enabling infrastructure, resources and technologies continue to have a major impact on most R01-funded *Xenopus* research.

The outstanding return on this investment (>$501 million between 2012-2015) is evident from the large number of published research contributions listed in **Section II** that demonstrate how *Xenopus*: 1) provides fundamental knowledge about biological processes; 2) plays a crucial role in elucidating the basic cellular, molecular and biochemical mechanisms underlying a wide spectrum of human pathologies; 3) is a powerful system for understanding the function of human disease genes; and (4) is a superb system for modeling human diseases. The *Xenopus* research community strongly believes that the full potential of this model could be realized if current resources are maintained, additional resources were made available, and the model were made more easily accessible to a broader field of biomedical researchers. **The goal of this 2016 White Paper is to outline these resources, justify their need and importance to the broad biomedical community, and provide recommendations on how to obtain them.**

**IV.** **Recommendations for Continued NIH Investment in *Xenopus* Research**

To identify resources needed by the *Xenopus* community, 67 *Xenopus* researchers met in September 2015 at the Marine Biological Laboratory (MBL) in Woods Hole, MA. This group discussed the progress made since the 2014 *Xenopus* White Paper, identified emerging opportunities and agreed upon a prioritized list of resources that would maximize the NIH’s investment and have the biggest impact on research using *Xenopus* to understand the fundamental process of human health and disease. Recognizing the need to balance the exciting new opportunities in the *Xenopus* system with the current fiscal climate, the *Xenopus* community identified **two** **immediate needs** and **four** **essential resources** for which new and continued NIH support is needed for optimal leverage of previous investments, accelerate current research and training efforts using *Xenopus*, and promote and facilitate the use of *Xenopus* for modeling human diseases.

**Immediate needs of the *Xenopus* research community:**

**1.** Development of protein resources (ORFeome; Antibodies; Proteome)

**2.** Development of “Big Data” repositories (XenMine, Xenbase)

**Essential resources needed by the *Xenopus* research community:**

In addition to these most-pressing needs, the community has identified four other Essential Resources that should be developed as soon as possible so that *Xenopus* research can more effectively fulfill the missions of the NIH. Although each of these resources is essential, the *Xenopus* community understands that priorities must be set, and therefore ranks these as indicated below:

1. Enhancement of efficient gene editing approaches and mutant lines
2. Enhancement of transgenic resources
3. Improvement and expansion of amphibian genomic resources
4. Support for *Xenopus* training and meetings

These priorities represent the consensus view of the *Xenopus* research community. The priorities were established during the 2015 Xenopus PI/Researcher meeting at the MBL and a draft document was then prepared by several members of the *Xenopus* community, covering expertise in cell, molecular, computational, and developmental biology, genetics, neurobiology, endocrinology, and immunology (Appendix 1). The draft was posted on Xenbase for a period of 4 weeks, and announcements were sent by email from Xine, the online *Xenopus* newsletter, in order to solicit comments and input from the entire *Xenopus* community. Feedback was incorporated, and the resulting 2016 White Paper represents a broad consensus of the community.

**1. Development of Protein Resources**

***1A. Impact of developing protein resources for Xenopus***

Current research in many areas of biology and biomedicine integrates systems level analyses performed in the context of the whole genome and proteome. Despite the advance in *Xenopus* genomic and genetic resources, the availability of proteomic tools specific to *Xenopus* proteins remains limited. There are three key resources that are essential: a complete ORFeome, a wide range of antibodies, and detailed proteomic data for different cell types and biological processes. We anticipate that acting upon this priority will result in the generation of a number of invaluable research resources for both the *Xenopus* and broader biomedical research communities. In particular:

* **Complete *Xenopus* ORFeome:** The generation of the complete coding sequence of the *Xenopus* genome in easily manipulated expression vectors will transform our ability to carry out large-scale analyses of gene function, especially investigations that integrate mechanistic and functional studies. This resource will accelerate advances in many fields, such as cell signaling, gene regulation, organogenesis, and neuroscience. It also will facilitate the construction of humanized genes for the study of syndromic and non-syndromic diseases.
* **Antibody Resources:** The generation of a large number of antibodies will be invaluable for a wide range of applications. We envision that both conventional and recombinant antibodies will be widely utilized for the analysis of the subcellular localization of proteins, protein-protein interactions and gene regulatory networks (via chromatin immunoprecipitation), in structural biology, for functional interference and for signal transduction studies.
* **Proteomics**: The expansion of current proteomic datasets and the development of new methods for quantifying individual proteins will provide a powerful toolkit for the Xenopus cell biology community, while opening new opportunities for *in vivo* systems-level analyses of developmental and disease processes.

***1B. Key protein resources that are needed***

**Complete *Xenopus* ORFeome:** The *Xenopus* “ORFeome” refers to a fully sequenced, validated set of *Xenopus* full-length open reading frames (ORFs) encoded in the genome. The ORFs are cloned into Gateway vectors, which facilitate transfer to various cassettes for modification (e.g., tags, hormone-inducible sequences, Engrailed repressor or VP16 activator sequences, etc.). The establishment of a complete ORFeome would be a major advance for a broad range of studies and would promote the use of the system across the entire biomedical research community. ORFeomes facilitate rapid functional characterization of proteins. New methodologies for high throughput screens and proteomics have greatly increased our ability to identify new proteins that are involved in a given process. Because of the ease with which proteins can be expressed *in vivo* or *in vitro*, *Xenopus* provides a simple, rapid, flexible, and very cost-effective system for protein expression studies. Importantly, protein expression in *Xenopus* encompasses over- or mis-expression, as well as expression of epitope- or fluorescent protein-tagged constructs, thereby allowing the integration of imaging-based protein localization studies and biochemical protein-protein interaction analyses. Moreover, expression of a tagged protein at large scale allows researchers to generate massive amounts of biological material, which positions *Xenopus* as a premier system for high-throughput proteomics.

The initial version of the *Xenopus* ORFeome represents approximately 1/3 of the total number of genes in *X. laevis* and *X. tropicalis*, cloned in a format in which any ORF sequence can be easily transferred into a diverse array of expression vectors. This set includes >90% of the currently cloned ORFs from both species andis available as an entire set or as single clones from four companies and the European *Xenopus* Resource Centre (EXRC; <https://xenopusresource.org>). In the second phase of the ORFeome project, the remaining ORFs, for which there are no available clones, will be cloned into Gateway vectors. We will focus solely on *X. tropicalis* ORFs, simplifying issues around the homeologous paired genes in the allotetraploid *X. laevis*. The expansion of the *Xenopus* ORFeome leverages the rapid and powerful functional genomics that is a key asset of this model system. Large-scale, genome-wide screens of gene function have been carried out in *Xenopus* for nearly 20 years, leading to a wealth of important discoveries. Improvements to the *Xenopus* ORFeome will expand the potential of these large-scale functional genomic screens by allowing new flexibility and greater genomic coverage. Finally, the *Xenopus* ORFeome will provide clones for all *Xenopus* genes for *in vivo* gene expression analysis by *in situ* hybridization, a major benefit to the entire biomedical research community.

**Antibody Resources**: Antibodies are widely recognized as one of the most useful tools for protein studies because of their high-affinity interaction with the antigen and a large number of developed immunoassays. They are used for tissue, cellular and subcellular localization, protein detection and quantitation, protein-protein interactions and protein-nucleic acid interactions. Although *Xenopus* is an outstanding system for the *in vivo* analysis of biochemical mechanisms underlying cellular regulatory processes, the broader impact of the *Xenopus* model system for *in vivo* cell, biological and biochemical approaches, as well as FACS sorting of purified cell populations, has been compromised by the lack of high affinity, highly specific antibodies. The most useful antibody tools would be those that can be easily maintained, are self-sustaining in availability, and are readily accessible to the community, such as monoclonal antibodies raised against defined peptide sequences or recombinant antibody libraries (nanobodies). Outside of the fields of cell cycle control, DNA repair and cytoskeletal regulation, relatively few *Xenopus*-specific antibodies have been generated. Furthermore, with a few notable exceptions, there is relatively low cross-reactivity with existing or commercial antibodies. Therefore, there is a widespread and acutely perceived need to have an open access database of antibodies tested by the *Xenopus* community and to develop new antibodies for use in the *Xenopus* system. In the first case, many *Xenopus* PIs have collaborated to donate existing antibodies to the EXRC, and to compile lists of commercially available antibodies that work well in *Xenopus*. Xenbase has also developed and is actively curating a [*Xenopus* antibody database](http://www.xenbase.org/reagents/searchAntibody.do?searchIn=1&searchValue=*&resultsPerPage=10&method=search) containing information on all of the community-generated, commercial and published antibodies that have been validated in *Xenopus*.

Nonetheless, **further development of new antibody resources will be of enormous benefit to the entire biomedical research community and will allow an expansion into new research questions and assays.** Two approaches have generated encouraging preliminary data for producing self-sustaining *Xenopus*-specific antibodies that would be of high value in the *Xenopus* community. One approach is to develop recombinant antibody libraries, including those generated from single domain antibodies (nanobodies); one such project has recently received NIH funding. Another approach is to produce fully characterized monoclonal antibodies to transcription factors and cell surface proteins, including signaling receptors and ligands as well as tissue specific markers that have roles in developmental and disease processes and for which there are no cross-reactive commercial antibodies. Immunogens would include both recombinant proteins expressed in mammalian cells and complex protein extracts isolated from *Xenopus* embryos. These immunizations would yield more than 10,000 clonal IgG-expressing hybridomas that would be screened by immunofluorescence, whole mount immunostaining, immunoprecipitation and mass spectrometry. These monoclonal antibodies, together with the characterization data, would be freely distributed to the community via the Developmental Studies Hybridoma Bank. The generation of a wide range of antibodies would facilitate **all** aspects of research in *Xenopus*, including high-throughput “omics” approaches, neurobiology, immunology, developmental, cellular, and molecular biology.

**Proteomics**: *Xenopus* research has long relied on mRNA expression (*in situ* hybridization, RT-PCR, qPCR, RNA-Seq) to analyze experimental perturbations. It is recognized in many systems, however, that during developmental and disease processes, not all mRNAs are translated. Proteomic analyses provide two benefits to the community. First, they offer fundamental information regarding the molecular complexity of early embryonic development, cell fate decisions during organogenesis, and tissue stem cell behaviour. This in turn allows researchers to evaluate hypotheses regarding the roles of translational control mechanisms. Second, they provide reference datasets for investigators who incorporate proteomic studies in other investigations. With the development of highly sensitive mass spectrometry techniques, it is becoming possible to identify the protein composition of embryos, tissues and even single cells at different stages of differentiation. Quantification of proteins offers a broad range of challenges, depending on the abundance and type of protein, as well as the cell or tissue type. *Xenopus* eggs and embryos provide an easy source to obtain large amounts of protein from highly interesting biological states, making *Xenopus* a superb model for mass spectrometry-based proteomics. Recent developments in proteomics enable the reliable and quantitative measurement of abundance for thousands of proteins across multiple conditions. Despite these advances, many key molecules are present at levels below the level of detection for this approach (e.g., we can measure ~20,000 different mRNA transcripts with RNA-Seq but only ~10,000 proteins with proteomics). While this is a general problem for proteomics, it is a particular challenge when evaluating signaling pathway components or gene regulatory networks, in which many essential proteins are present at levels below the current limits of detection.

The *Xenopus* community will benefit from methodological advances that can reliably measure these low-abundance proteins. To this end, it is desirable to develop shotgun proteomics methods that allow the quantification of low-abundance proteins. In addition, development of antibodies for FACS and the generation of knock-in transgenic lines so that purified cell types could be obtained would greatly benefit the detection of low abundance tissue-specific factors. Of particular interest for the community is the development of targeted proteomics methods that will allow one to predefine a set of ~100 potentially very low abundance proteins of interest, e.g. transcription factors, which can be targeted for quantification. Once feasible, the data generated by these various approaches would be published in an accessible format on Xenbase for maximum benefit to the broader biomedical research community.

***1C. Community recommendations***

The *Xenopus* community voiced as an immediate need the development of three key protein resources:

* **The expansion of the *Xenopus* ORFeome**: The initial ORFeome was based on the *Xenopus* Gene Collection (XGC: <http://xgc.nci.nih.gov/>); over 90% of these clones, representing ~1/3 of protein-coding genes, are now available in Gateway-compatible vectors, and destination vectors have been generated to allow rapid transfer of clones carrying in-frame tags, in constructs compatible with *in vitro* transcription or transgenesis. In order to realize the full potential of this resource, the ORFeome must be expanded to a ***complete collection*** of full-length Open Reading Frame (ORF) clones; these should be made available in standardized, multifunctional Gateway vectors. The first step will be to generate a complete *X. tropicalis* ORFeome, which will facilitate experiments in both species. The focus on *X. tropicalis* will simplify both the generation and use of this resource. These cloneswill be made available through the EXRC and commercial suppliers for use by the broader biomedical research community.
* **The continued curation by Xenbase of a database of verified antibodies:** This database should include both antibodies generated by community members as well as commercially available antibodies raised against other species that have been tested for cross-reactivity to *Xenopus*. Annual surveys of *Xenopus* PIs will help keep this database up-to-date. Moreover, the antibody database will be cross-referenced with Xenbase gene-specific pages to seamlessly indicate antibody resources available to proteins of interest. Additional input will be solicited at the community meetings (International *Xenopus* Conferences and MBL PI/Researcher meetings).
* **The generation of specific high-affinity antibodies against *Xenopus* proteins**: Funding opportunities should be issued for the generation of these critically needed antibodies. This collection of antibodies will need to be maintained and made widely available to the biomedical research community. For example, conventional monoclonal antibodies should be deposited in the Developmental Studies Hybridoma Bank.
* **The development of proteomic analyses of oocytes, early embryos, and embryonic tissues, as well as efforts to advance methodologies for proteomic analysis and quantification of low-abundance proteins in embryonic tissues**. The availability of comprehensive proteomic datasets in early development will facilitate advances across many areas within molecular and cell biology, developmental biology, physiology, and neuroscience. Moreover, methodological advances in the quantitative analysis of low-abundance proteins will benefit ongoing investigations throughout the biomedical research community. All data sets should be deposited in Xenbase to promote the broadest community access.
* “Resource” RFAs/PARs of R01/R21/R24 level projects should be issued to achieve these goals.
* “Resource” study sections to review R24 applications (http://grants.nih.gov/grants/guide/pa-files/PAR-13-253.html) should continue to be supported because these projects have a broad and long-lasting impact on the entire biomedical community.

**2. Development of “Big Data”** **Repositories**

***2A. Impact of providing “Big Data” repositories for Xenopus***

[Xenbase](http://xenbase.org/common/), the *Xenopus* model organism bioinformatic database, is an essential web-accessible resource that integrates the diverse genomic, expression and functional data available from *Xenopus* research. Comparative functional genomics between humans and model organisms has led to a wealth of discoveries, and databases such as Xenbase are essential to translate this vast body of data into a meaningful biological synthesis. Xenbase enhances the value of *Xenopus* data through high quality curation, data integration, providing bioinformatics tools optimized for *Xenopus* experiments, and linking *Xenopus* data to human genes. Xenbase also plays an indispensible role in making *Xenopus* data accessible to the broader biomedical community through data sharing with NCBI, UniProtK and Ensembl. Xenbase content, tools and usage have grown tremendously in recent years. These include a new genome browser, new BLAST server, powerful gene expression and text mining tools, support for an antibody database, and ongoing annotation of the scientific literature. Xenbase now has over 1000 unique visitors a day. It hosts over 15,500 gene pages, supports both the *Xenopus* *tropicalis* and *Xenopus* *laevis* genomes, contains >44,000 *Xenopus* publications, and offers >50,000 gene expression images. It also provides a number of unique large-scale datasets not available at other databases. Xenbase plays a critical role in making data from NIH’s >$100 million annual investment in *Xenopus* research accessible in a meaningful way.

Xenbase is **the single most important clearinghouse for *Xenopus* data**. It provides high quality annotation, tools specific for *Xenopus* research, and integrates diverse data types in a way not available at any other single database. For example, Xenbase inter-relates *Xenopus* genomic, epigenetic, mRNA and protein sequence data, with gene expression and gene function, as well as physical reagents such as antibodies, morpholinos and transgenic lines. Xenbase links *Xenopus* data to other model organisms and human disease databases, such as OMIM. By administering the Human Genome Nomenclature Committee-approved *Xenopus* gene nomenclature, Xenbase associates *Xenopus* gene centric data to the correct (orthologous) human genes and then through automated weekly data sharing Xenbase provides this curated *Xenopus* data to many external resources including; NCBI, Entrez Gene, UniProtK and Ensembl. Xenbase also integrates critical data from sharing infrastructure for the *Xenopus* Stock centers (e.g., NXR, EXRC) and many other NIH funded initiatives including, pre-release *Xenopus* genomes (Rokhsar & Harland; HD065705); epigenomics data (Veenstra; HD069344, Baker HD076839); the ORFeome (Stukenberg et al; HD069352); and large-scale expression screens (Amaya, Ciau-Uitz, Kirschner, Pollet, Perone, Ueno, Wheeler, & Zorn labs). The community needs Xenbase to develop new infrastructure, data pipelines, bioinformatics tools and annotation teams to support the exponential growth in NextGen sequence data and the increasing use of *Xenopus* phenotypes as a tool to model human diseases. Immediate plans include support for the *Xenopus* transcriptome (Khokha et al.; GM099149), *Xenopus* proteome (Kirschner; GM103785) and XenMine bioinformatics tools (Baker; GM095346). Without Xenbase the data from these important projects would be much less accessible.

**Xenbase enables both discovery and the integration of findings from disparate datasets, allowing investigators to make novel connections between molecular pathways in *Xenopus* and models of human disease. These connections are critical to the NIH’s new “Big Data to Knowledge” (**[**BD2K**](http://bd2k.nih.gov/#sthash.CvUxmJb6.dpbs)**) initiative, which seeks to enable biomedical scientists to capitalize more fully on the diverse and expanding range of genomic data generated by contemporary molecular analyses.**

***2B. Why it is essential to develop XenMine and integrate it with Xenbase***

XenMine was recently established to meet a critical need within the *Xenopus* community to access and compare all emerging genomic datasets. In essence, XenMine ([www.xenmine.org](http://www.xenmine.org)) is a central space to view, search and analyze data, providing essential information on gene expression changes and regulatory elements present in the genome. XenMine currently houses published genomic datasets from *X. tropicalis and X. laevis*. To compare these sequences, XenMine created a standard analysis pipeline where all published datasets are uniformly processed with the latest genome releases. Information from these datasets can be extracted and compared using an array of pre-built or custom templates. With these search tools, users can easily extract sequences for all putative regulatory domains surrounding a gene of interest, identify the expression values of a gene of interest at different developmental timepoints, and analyze lists of genes for gene ontology terms and publications. ***Additionally, XenMine hosts an in-house genome browser that allows users to visualize all available ChIP-Seq data, extract specifically marked sequences, and aid in identifying important regulatory elements within the genome.*** Altogether, XenMine is an excellent tool for visualizing, accessing and querying analyzed datasets rapidly and efficiently.

Moving forward, XenMine will need to be continually maintained, upgraded and improved to meet the needs of the growing *Xenopus* genomic research now in progress. Current work is focused on re-analyzing all datasets currently housed on XenMine against the latest stable release of both the *X. laevis* and *X. tropicalis* genomes (version 9.1). This updated analysis, with a genome containing far better assembly and annotations, will allow XenMine to link stably to other MODMines to access information on gene orthologues, phenotypes, interactants, and other critical information from a number of different species. ***XenMine plans to establish links to other databases, including OMIM, which allows users to associate genes with specific human diseases, a central goal toward advancing Xenopus as a model for human pathologies***. The newest genome assemblies are also connected to Biogrid, where *Xenopus* researchers can extract direct interactions among proteins of interest.

Expansion of XenMine is necessary to keep pace with the onslaught of genomic and proteomic data. The data housed within XenMine will be expanded to include recently published datasets from both species, particularly in *X. laevis* where the rise in genomic profiling will greatly complement the wealth of embryonic characterizations. Working together with InterMine software developers, XenMine will also offer an extensive battery of tools including:

* The expansion of the analysis pipeline to allow users to compare differentially expressed transcripts that are significantly changing amongst multiple datasets.
* The creation of a central repository in which mass spectrometry proteomic and metabolomic data can be deposited, analyzed, and accessed by the community.
* The expansion of visual tools, such as interactive heat maps to display mass spectrometry, ChIP-Seq and RNA-Seq data.
* ***The inclusion of other sequencing data types, including CAGE-Seq, ATAC-Seq, and chromatin capture techniques,*** ***allowing the entire Xenopus community easy access to an enormous array of datasets for research.***

As next-generation sequencing has become more accessible, the collection of genome-wide datasets has rapidly increased. However, the ability to browse and manipulate such information remains underdeveloped. XenMine provides a platform to query all publicly available *Xenopus* genomic data, providing user-friendly tools for further hypothesis generation. XenMine also provides a quick and easy way to recover genomic sequence for given features in the genome, allows querying and visualization of multiple ChIP-Seq experiments, offers a platform for simple list analysis, and allows users to manipulate template queries for their own specific needs. As genomic assays continue to evolve, XenMine will serve as a web resource to investigate the most efficient and powerful means to obtain biologically relevant genomic information to serve the *Xenopus* community. ***Given that such important genomic resources for Xenopus research are currently lacking, it is critical that XenMine be expanded and maintained in order to facilitate ongoing Xenopus research and its connections to human disease mechanisms.*** The role of XenMine is well aligned with the NIH’s new BD2K initiative, which seeks to enable the integration and translation of large, complex and diverse data sets into meaningful knowledge that can improve human health.

***2C. Community recommendations***

The *Xenopus* community voiced as an immediate need the maintenance and further development of the existing databases as “Big Data” repositories.

* Xenbase must be maintained and expanded to continue ongoing curation of *Xenopus* research data. It is an essential web-accessible resource that integrates the diverse data available from *Xenopus* research, and makes it freely available to the entire biomedical research community.
* Xenbase support should be enhanced for large-scale NextGen sequencing data and mass spectrometry metabolomics and proteomic data.
* XenMine should be expanded and integrated with Xenbase to facilitate the connection of *Xenopus* research with human disease mechanisms.
* RFAs/PARs should be issued to achieve these goals. Study sections/review panels that are charged with recognizing that database projects have a broad and long-lasting impact on the entire biomedical research community should review these grants.

# 3. Enhancement of Efficient Gene Editing Approaches and Mutant Lines

***3A. Impact of further developing efficient gene editing approaches and mutant lines***

*Xenopus* has been a powerful system for rapid functional genomics using both mRNA-mediated over-expression, ectopic expression and morpholino knockdown. Recent genetic modification techniques (TALENs and CRISPR/Cas9) have been shown to work exceptionally well in *Xenopus,* making *Xenopus* amenable to gene knockout studies that will help expand the repertoire of experimental possibilities. Enhancement of these genome-editing techniques, enabling the mutation and genetic modification of any sequence in the genome, will be transformative, especially when coupled to the established experimental advantages of *Xenopus*. To facilitate the use and impact of *Xenopus* as a **model for human health and disease,** these new loss-of-function technologies must continue to be optimized, standardized, and disseminated. Greater flexibility for knockout and knockdown technology, as well as as gene editing using modified Cas9, will allow *Xenopus* investigators to develop and exploit loss-of-function strategies in *Xenopus* oocytes, cell-free extracts, developing embryos, and adults. **These approaches are essential for fully exploiting the *Xenopus* system to model human diseases.**

These technologies are easily adapted to the *Xenopus* system, as they only require microinjection into early embryos and can be used in either *X. laevis* or *X.* *tropicalis*. Gene function can be initially assessed with bi-allelic gene targeting in F0 embryos and tadpoles. Because of the mosaic nature of the mutations, however, heterozygous animals must be bred and maintained to create true null homozygous mutant offspring. To facilitate the use of this technology, the National Xenopus Resource ([NXR](http://www.mbl.edu/xenopus/)) now offers the generation of mutant lines on a fee-for-service basis. Although *X. laevis* is allotetraploid, complicating its use for genetic knockout studies, the gene editing features of TALENs/CRISPRs, such as the introduction of epitope tags into protein coding genes, should be extremely powerful in combination with the excellent cell biological and biochemical approaches that are standard in *X.* *laevis*. The knock-in of tags also will facilitate FAC sorting of cells and ChIP assays for which there are not adequate antibodies. In addition, mutant cells or tissues generated using inbred *Xenopus* lines could be transferred to normal recipients for further characterization of the process of interest; these transplantation-based characterizations will be even more feasible in athymic *X. tropicalis*, which show minimal allograft rejection (Nakai et al., 2016, Genes Cells, doi: 10.1111/gtc.12337). Rapid dissemination of new technologies to the community will be greatly facilitated by continued support of and coordination with two critical centers: the NXR and XLRRI.

***3B. What is needed to enhance genome-editing technologies in Xenopus***

Rapid and straightforward genome-editing technologies will transform the use of *Xenopus* in the biomedical research community by providing means to knock out genes of interest and to generate targeted mutations in genes/genomic regions to model human diseases. While the use of TALENs/CRISPRs are now routine techniques for the rapid generation of mutant *Xenopus* lines, the methodologies need to be optimized and standardized for both *X. tropicalis* and *X. laevis*. This will increase the use of mutant lines in both fundamental biological and disease-related research. Approaches to be developed and/or refined include:

* Generating advanced tools for establishing knock-in strategies in *Xenopus*, including gene replacement, gene editing, targeted insertions, tagging proteins and creation of dominant active or repressive genes.
* Standardizing and enhancing the use of different TALEN and CRISPR systems, such as “high Fidelity” versions of Cas9 (Slaymaker et al., 2016, Science 351: 84; Kleinstiver et al., 2016, Nature 529: 490). These efforts should include further development and testing of strategies designed to reduce off-target effects, (e.g., nickase, Fok1-dCas9 fusions; Guilinger et al., 2014, Nat. Biotechnol. 32: 577).
* Optimizing a strategy to target expression of Cas9 or TALENs to germ cells only, thus bypassing somatic mutations in the F0 generation.
* Developing CRISPR/TALEN-based strategies for tissue-specific gene knockouts. These efforts are ongoing, and preliminary findings suggest that tissue-specific expression of Cas9 may not be sufficient to generate a tissue-specific knockout. Alternative strategies should be developed.

One of the difficulties faced by the *Xenopus* community in using mutant lines is the increased housing costs and housing space required to raise and breed the lines. This difficulty is currently being addressed by encouraging labs to interact with the NXR and XLRRI to create specific mutants. The NXR also is helping to coordinate the efforts to generate mutations in several hundred key genes that are studied by many investigators. The long-term goal would be to mutate all genes in the genome; mutant sperm could easily be stored frozen and distributed to the community. This would reduce duplication of effort and generate a resource similar to JAX for mice and ZIRC for fish, which have proven to be very successful. With this goal in mind, the NXR is soliciting a list of genes from the *Xenopus* community to prioritize for mutation. Together the proposed generation, distribution and data collection would be a modest, but highly productive, investment relative to the already substantial NIH investment. Another difficulty faced is expanding from *X. laevis* to *X.* *tropicalis*, since these animals require different housing and handling. This problem could be addressed via administrative supplements to existing *X. laevis* grants to cover the cost of new *X. tropicalis* housing systems.

Increased use of genome-editing technologies also will require additional support for other resources, such as:

* A more complete annotation of both *X. laevis* and *X. tropicalis* genomes, as outlined below ([Section IV-5](#genome)) to facilitate genome editing and creation of knockouts.
* Bioinformatic support to identify CRISPR/TALEN sites in the *Xenopus* genomes, and collation of data on the resulting mutant animals and phenotypes ([Section IV-2](#Repositories)).

***3C. Community recommendations***

The *Xenopus* community identified as an essential resource the further development of genome-editing technologies in order to allow the rapid generation of mutant lines requested by the community, such as key disease-related genes, and to enhance the development of *Xenopus* as a non-mammalian model system for human disease research.

* Generate new mutant lines. The NXR has integrated TALEN and CRISPR/Cas9 mutagenesis into its portfolio as a fee-based service. Community-requested mutant lines can be generated based on demand, ideally as a supplement to the NXR grant. The NXR is standardizing these techniques and will continue to generate new mutant lines for the community.
* “Resource” RFAs/PARs of R01/R21/R24 level projects should be issued to generate advanced genome editing tools, as outlined above.
* “Resource” study sections to review R24 applications (http://grants.nih.gov/grants/guide/pa-files/PAR-13-253.html) should continue to be supported, because these projects have a broad and long-lasting impact on the entire biomedical community.
* Supplements to existing grants should be available to allow individual labortories to utilize mutant lines in their research projects and/or to establish *X. tropicalis* colonies.
* Support should be provided for advanced genome annotation and bioinformatics support to facilitate genome-editing efforts.

# 4. Enhancement of Transgenic Resources

***4A.******Impact of enhancing transgenic resources***

There is a strong and increasing need for transgenic *Xenopus* resources. Significant results in cell and developmental biology have come from using transgenic systems in frogs, but the transgenic *Xenopus* lines currently available are insufficient. More extensive characterization of existing transgenic lines and production of new lines are critical for advances in basic cell and developmental biology, development of pluripotent stem cells, and the developmental origins of adult disease. Transformative advances will emerge from combining transgenic resources with the established advantages of the *Xenopus* model. Although there are several highly efficient methods to generate transgenic *Xenopus*, individual researchers often lack the space, time, and technical expertise required to make and characterize them. *Xenopus* stock centers, such as the NXR, can generate or facilitate production of transgenic lines, as well as accept lines for distribution. For example, the NXR has acquired over 100 transgenic lines from individual labs and has bred them for distribution to the community. The demand for these transgenic lines has grown each year, as they are useful for gene reporter assays and for purifying specific cell populations for high-throughput assays such as RNA-Seq, ChIP-Seq and ATAC-Seq. However, there are still many lines in the community that need to be acquired and established at the NXR.

Transgenic resources in *Xenopus* have revealed significant biological insights and illustrate paths for expanded development of these resources.

* Reporter lines: These lines allow visualization of a subcellular component, tissue, or whole body via expression of a fluorescent protein. Such lines are employed for a wide variety of studies, including live imaging, lineage tracing, developmental signaling pathways, enhancer trap screens, analysis of promoter/enhancer activity of non-coding sequences, FAC sorting of specific cell populations, and small molecule screens. These uses are particularly advantageous in *Xenopus* because of the large number of offspring, transparent larvae, and ease of embryonic gene expression and tissue manipulation.
* Inducible lines: These lines allow ubiquitous inducible expression of transgenes, utilizing heat shock or small molecule ligands as inducing agents. Numerous early and late developmental events have been studied using inducible lines, which can be used to eliminate undesired early embryonic effects of overexpression via other means.

The XLRRI has generated several immune-system relevant lines; the NXR has been successful in generating a number of new transgenic lines, both through individual requests and community-initiated requests. Demand for new lines continues to increase each year. It is clear that transgenic lines are needed both for both *X. laevis* and *X. tropicalis*. Although *X.* *laevis* has a longer generation time, its large size makes it more amenable to cell biology and biochemistry. The shorter generation time and diploid nature of *X.* *tropicalis* make it ideally suited for binary transgenic systems and genetic analyses. Expansion of transgenic resources would bolster ongoing studies in both systems; it would also invigorate the potential for new investigations by providing a new experimental model for interrogation of later developmental processes.

Promising opportunities exist to merge transgenic resources with the advantages of the *Xenopus* model and with ongoing efforts to develop resources (genome sequences, ORFeome, XvivoENCODE). For example, the systematic generation of transgenic lines for binary expression systems could provide a platform for high-throughput *in vivo* analyses of gene function in specific tissues and developmental time points, which are not feasible in other vertebrate model systems. Moreover, appropriate transgenic lines could establish the basis for large-scale pharmacological or small molecule screens *in vivo.*

***4B.******What is needed to enhance transgenic resources***

A few tissue-specific reporter lines are currently available, including some cellular components, rod cells, heart myocardium, exocrine pancreas and immune system. However, this set must be expanded to other tissues, such as neural crest, specific brain regions, liver, lung, and kidney, in order to realize the full potential of *Xenopus* as a model for developmental and disease processes.

Inducible lines can be used to eliminate undesired early embryonic effects of over-expression but they lack the flexibility of binary expression systems. Binary expression systems enable precise temporal and spatial control of transgene expression and are comprised of two parts: 1) a promoter line controlling tissue-specific expression of a ligand-activated transgenic transcription factor and 2) a transgene line activated upon ligand expression in a specific tissue determined by the promoter line. Systems shown to work in frogs include the GAL4/UAS, Tet-On, and Cre/lox systems. These transgenic systems have been used to investigate mechanisms of pancreas development, and the molecular regulation of development and regeneration of muscle and liver. A significant advantage of binary expression systems comes from the combinatorial possibilities of having separate promoter and transgene lines, which multiplies the combinations of tissue specificity and transgenes of interest readily available to researchers. Despite this great potential, few characterized transgenic lines suitable for study of disease processes are currently available.

***4C. Community recommendations***

The *Xenopus* community identified as an essential resource enhancement of transgenic lines. These will invigorate the potential for new investigations by providing a new experimental model for interrogation of later developmental processes and facilitate the development of *Xenopus* as a non-mammalian model system for human disease research.

* Consolidation of existing transgenic constructs from the research community. While a number of excellent transgenic constructs have already been generated, such as the modular gateway-compatible pTrangenesis plasmid collection and cell-component GFP-fusion reporters, transgenic animals carrying these constructs are not available in many cases. The first step will be to consolidate and prioritize these constructs for the preparation of new transgenic lines. This is already underway, as the European and Japanese *Xenopus* stock centers distribute these plasmids to the community, and the pTransgenesis system is used at the NXR in transgenic line production. The NXR and EXRC (and in some instances with help from the Japanese stock center) have already established a network to exchange lines.
* Identify tissue-specific promoter/enhancers. Promoters from other species are often effective in frogs. Novel candidate promoters will be identified from the published literature, as well as from ENCODE-type prediction of cis-regulatory modules in the *Xenopus* genome. In both cases, promoters must be validated in frogs for appropriate expression characteristics for use as a reporter or for tissue-specific transgene expression. Additional transgenic vectors will also need to be generated with the emphasis on Gateway-compatible modules that can be reused for many purposes.
* Production and characterization of lines. After promoters and constructs have been validated with transient assays, transgenic founders need to be made, reared, and tested for germ-line transmission as well as continued appropriate transgene expression. They further need to be characterized as to how many transgene inserts are in the animal and at what loci. Transgenic lines will be produced in the inbred J strain *X. laevis*, which will permit adoptive cell, tissues or organ transfers.
* Development of knock-in strategies. This will be particularly useful to tag proteins for numerous assays such as FAC sorting to obtain purified populations of cells, proteomic approaches, ChIP-Seq, and ATAC-seq.
* A larger initial investment is recommended to jump-start the widespread use of binary expression systems. A strategic effort with community input to produce a priori a critical mass of 10 to 15 promoter and transgene lines that could be used combinatorially would enable a large number of projects "off the shelf". Production of such a select set of transgenic lines could be done at the NXR with input from the community. With promoter and transgene lines in hand, it would then be cost-effective for individual investigators to use these lines or custom-make a single promoter or transgene line.

# 5. Improvement and Expansion of Amphibian Genomic Resources

***5A. Impact of improving and expanding the Xenopus*** ***genome***

The continual improvement of the *Xenopus* genome sequence is a top priority of the *Xenopus* research community. Advances in quantitative DNA sequencing technology and a revolution in genome editing have enabled an unprecedented analysis of cell function, development, homeostasis, and disease at both a genome-wide level and high-resolution mechanistic depth. The experimental advantages of *Xenopus* make it ideally suited for this type of systems-level analysis. Well-annotated *Xenopus* genome assemblies are required in order to realize this potential.

Recognizing the importance of *Xenopus* the NIH and the Department of Energy (DoE/JGI) have made a substantial investment in sequencing two *Xenopus* genomes, resulting first in the X. *tropicalis* genome assembly, published in 2010. With strong community support and NIH funding (GM086321, HD065705), the Rokhsar and Harland groups have since generated an improved *X.* *tropicalis* genome version 9.0 assembly (Xt.v9.0). In addition, a *X.* *laevis* genome assembly (Xl.v9.1) at chromosome-scale is now available on Xenbase and will be published in 2016.

The current *Xenopus* genomes are outstanding resources that continue to have a tremendous impact on all areas of *Xenopus* research. However, consistent with experience from human, mouse, and zebrafish genomes, more work is needed to complete a fully annotated reference sequence. Continued investment in two key areas is required to realize the full potential of the *Xenopus* genomes:

* Continued improvement to the *Xenopus* genomes, including gap closure, curation, and annotation.
* Sequencing and assembly of additional amphibian genomes.

***5B. Why improve and expand amphibian genomic resources?***

A complete genome sequence with well-annotated gene models is essential for virtually all studies of gene expression and function. Identification of the transcription/translation start sites and intron-exon boundaries are critical for designing gene disruption experiments using morpholinos, shRNAs, TALENs and CRISPRs. Well-annotated genomes are critical for identifying cis-regulatory elements, needed for designing tissue-specific promoters used in transgenesis, and essential for systems-level functional genomics. Complete gene models are also essential for proteomics and thus are critical to the large and very productive *Xenopus* cell biology community that relies on mass spectrometry analyses. Both the *X.* *tropicalis* and *X. laevis* genomes need improvement. In addition to being essential for experiments in both species, comparative genomics between the two species has proven to be a powerful tool for characterizing conserved non-coding regulatory elements. Moreover, because *X.* *laevis* underwent an ancient whole-genome duplication, the two *Xenopus* genomes offer a unique model of vertebrate gene diversification and genomic evolution.

*Sequencing / Assembly of additional amphibian genomes:*

Comparative genomics has proved to be enormously useful in understanding molecular biology from both a mechanistic and an evolutionary perspective, deepening our understanding of genome organization and evolution, transcriptional control mechanisms, post-transcriptional regulation, and the genetic basis of disease and development. Genomic analysis of selected additional amphibian species will expand our understanding of amphibian genome organization and evolution, and provide a resource for comparative studies of transcriptional regulatory elements and possible roles of noncoding sequences. Equally important, each of the species the community has selected for genome sequencing and assembly reflect distinct opportunities for investigating the molecular genetics of development, behavior, speciation, and other life history traits. For example, the Tungara frog (*Engystomops pustulosus*), has provided spectacular insight into the role of sexual selection in behavioral evolution. A sequenced genome from this species will serve as a foundation from which to explore the genetic underpinnings of variation in amphibian communication as a basis for reproductive success.

In *Eleutherodactylus coqui*, the larval and metamorphic stages have been lost, and embryos develop directly into juvenile frogs within the egg capsule; this direct development is accomplished in part by a dramatic shift in the timing of limb development, which is initiated at a much earlier stage than in conventional amphibian embryos. This unusual life history is associated with profound differences in pulmonary and digestive development, the genetic basis of which is completely unknown. Similarly, the spadefoot toad, *Spea bombifrons*, displays divergent larval morphologies, as well as corresponding differences in life history characteristics, including drought tolerance and prolonged aestivation. *Xenopus borealis,* a close relative of *X. laevis*, shows striking differences in vocalization and corresponding modifications of laryngeal structures and neuroendocrinology, and also an entirely distinct genetic mechanism for triggering sex determination. From a phylogenetic perspective, these species sample a breadth of anuran phylogenetic diversity, including representative species from Neobatrachia (*Engystomops* and *Eleutherodactylus*) and Archaeobatrachia (*Spea* and *Xenopus*).

Genome sequencing and assembly for each of these species will unleash new advances in our understanding of the genetic control of development and behavior. In addition, it will establish a phylogenomic framework for further comparative studies of evolutionary genetics within amphibian lineages. These genomic analyses will also directly benefit the community of *Xenopus* researchers through the generation of reference genomes for more closely related species, which will aid in the identification and functional analysis of enhancers and other regulatory sequences. Comparative analysis of the complete genomes of the tetraploid species *X. borealis and X. laevis*, and the diploid *X. tropicalis*, the latter two species for which sequenced genomes are already competed, promises to further illuminate how duplicate genes evolve and diverge in function and patterns of expression.

***5C. Community recommendations***

A *Xenopus* Genome Steering Committee was established in 2009, which includes a diverse group of PIs and a panel of bioinformaticians. This committee, together with the broad *Xenopus* community prioritized two key areas of genome improvement; 1) closing gaps in the assembly and finishing the sequences; and 2) improving gene model annotations. *Xenopus* can leverage lessons learned from the genome projects of human and other species and incorporate many different data sources to improve the *Xenopus* genome sequence and annotation.

* Incorporate data from currently funded ongoing projects (e.g., proteomics, ORFeome, RNA-seq transcript profiling, ChIP-Seq epigenetic analysis) to improve genome assembly and annotation.
* Continued bioinformatics support is critical to maximize the value of *Xenopus* as a model for human disease. New bioinformatics tools and analytical approaches will need to be developed to integrate the diverse data types described above. We anticipate that a certain amount of manual curation will also be needed, as this has remained the gold standard from the human genome and multiple models including mouse, fly and worm. The community strongly recommends that simultaneous efforts to improving both *tropicalis* and *laevis* genomes be pursued. One compelling reason is that comparisons between the two species can help accelerate annotation, as gene models validated in one species can be used as template models in the other species. In addition to accelerating genome annotation, this comparative approach has proven very powerful for characterizing conserved regulatory elements (e.g., comparisons across mammals and across various *Drosophila* species). Analysis of the conserved synteny between *Xenopus*, human, and other vertebrate model systems, as well as between the two *Xenopus* species, allows precise assignment of orthology across species and will facilitate the use of *Xenopus* for human disease modeling. Xenbase will serve as a centralized repository for data integration and facilitate dissemination to public databases (NCBI, UCSC, and Ensembl). To this end, members of the *Xenopus* Genome steering committee have been in communication with NCBI leadership to improve data pipelines and increase the visibility of *Xenopus* data.
* Continued support and improved coordination of efforts to sequence genomes of selected amphibians. Strategies for integration and dissemination of amphibian genomic data, and tools to facilitate comparative genomic analyses, are also needed.

# 6. Support for *Xenopus* Training and Meetings

***6A. Impact of supporting Xenopus training and meetings***

Training the next generation of scientists is an important component of the NIH mission and a top priority of the *Xenopus* community. Training in *Xenopus* research is robust with the continued entry of new students, postdocs and junior faculty to the field and formal training through courses and meetings. These help enhance the NIH investment by providing scientists in the *Xenopus* field with access to the latest technology and opportunities to exchange research ideas. In addition, they serve to train researchers from other animal models to incorporate *Xenopus* in their establish programs. Historically, there has been one primary course held annually, the Cold Spring Harbor Laboratory (CSHL) course on “Cell and Developmental Biology of *Xenopus”*, and one International *Xenopus* Conference held biennially (even years). More recently, the NXR has developed advanced workshops, which provide more in-depth training in emerging technologies.

The CSHL course on “Cell and Developmental Biology of *Xenopus”* has been offered each year since 1992 ([Appendix 2](#Appendix2)), training over 300 students in basic practices, along with a range of advanced experimental approaches. Nearly 30 students who have taken the course are currently PIs working in the field. The course also attracts established PIs seeking training in emerging techniques or seeking to add *Xenopus* to their experimental repertoire.

The International *Xenopus* Conference was first conceived at a workshop in 1984, and a scientific meeting has been held every two years since 1986 ([Appendix 3](#Appendix3)). It is held on a rotating basis in Europe and North America to encourage broad participation. It was begun with a focus on developmental genetics, but has been expanded in recent years to include topics in cell biology, cell cycle control, immunology, organogenesis, neurobiology, birth defects and human disease, thus more deeply addressing the broader goals of biomedical research.

The National *Xenopus* Resource (NXR) is the nexus of the US *Xenopus* community. Located at the Marine Biological Laboratory in Woods Hole MA, the NXR serves several training functions essential for the global *Xenopus* effort. These include:

* Generation, maintenance, and distribution of transgenic and mutant lines (Sections IV-3, IV-4).
* Hosting of workshops and community meetings to provide advanced training, dissemination of information, and development of plans and priorities within the community.
* Hosting and collaboration with individual investigators to provide access to NXR resources.
* Coordination of activities across the international *Xenopus* community via regular interactions with European *Xenopus* Resource Center (EXRC), the National BioResource Project of Japan (NBRP) and XLRRI.
* Development of new technologies (e.g., Ratzan et al. 2016. Dev. Biol. March 15, doi: 10.1016/j.ydbio.2016.03.006

In particular, the NXR has hosted three different kinds of workshops for advanced training. These are small (20-25 students), week-long intensive workshops that are created and taught by experts in the field. The goal is to offer 2-3 workshops each year in important areas that impact research in *Xenopus*. To date these have included “Bioinformatics”, “Imaging”, and “Genome Editing”; there are plans for future workshops in “Egg extracts” and “Husbandry”.

***6B. Why improve training and meeting opportunities?***

Courses, meetings, workshops and training grants help develop, maintain, and renew human and scientific resources for *Xenopus* researchers and contribute to the training mission of the NIH. The CSHL course on “Cell and Developmental Biology of *Xenopus”* is critical for disseminating evolving techniques throughout the *Xenopus* community in a timely fashion, and it is taught by faculty who pioneered or refined the methodologies. The biennial International *Xenopus* Conference has been held since 1986. These are very well attended, and unlike other meetings, speakers are required to pay for themselves. This has become a burden for laboratories that wish to send postdoctoral fellows and graduate students to the meetings so they can become part of the international community, exchange ideas, and learn about novel approaches and reagents. It also is important to support the attendance of junior faculty as well as members of the biomedical research community that wish to incorporate *Xenopus* into their established research programs. The organizers of the past several meetings have made a special effort to include junior researchers as invited speakers. Continued support for these and similar training initiatives is essential to maintain and propagate the use of *Xenopus* in biomedical research.

***6C. Community recommendations***

The *Xenopus* community identified as an essential resource the continued support and further development of opportunities for training in the use of *Xenopus* in all areas of biomedical research.

* The CSHL course on “Cell and Developmental Biology of *Xenopus* is currently supported by NIH funding that extends through 2019. This continued support for the CSHL *Xenopus* course is essential to maintain the vibrancy and usefulness of the *Xenopus* system.
* Continued and enhanced support for the biennial International *Xenopus* Conference is essential to ensure attendance of students, postdoctoral fellows, junior faculty and faculty who wish to begin to use *Xenopus*. Their participation would facilitate their long-term integration into this important community.
* Support for financial scholarships for the advanced training workshops at the NXR. There is a high level of enthusiasm for these workshops, but many applicants request financial aid, which currently is not available. Supplemental funding and support through the R25 mechanism to the NXR, allowing it to provide tuition assistance, will benefit the entire community.

**V.** **The Long Term Goal of Using *Xenopus* to Understand Human Disease**

***A. Impact of using Xenopus to understand human disease***

The major common objective of the *Xenopus* research community is to accelerate the use of this animal as a model for understanding human diseases. All of the essential resources in this White Paper **will help achieve the overarching goal of optimizing the utility of *Xenopus* to study human disease and improve human health**. Coupling the experimental advantages of *Xenopus* with recent advances in genome editing and high throughput analysis will establish a cost-effective platform for the rapid identification, validation, and characterization of genes involved in human disease. It will also provide mechanistic insight into potential therapeutic design. From John Gurdon’s initial discovery of nuclear reprogramming, through Tim Hunt’s identification of cyclins, and many more recent findings, *Xenopus* research has produced many seminal discoveries that have helped elucidate the cellular mechanisms underlying human diseases. By revealing fundamental biological processes, *Xenopus* research informs our understanding of how gene dysregulation can lead to disease and has provided critical insight into how these pathways might be manipulated for regeneration, repair and aging.

*Xenopus* has a number of features that make it ideally suited to model human disease. Sequencing and assembly of both the *Xenopus tropicalis* and *Xenopus laevis* genomes have revealed a high level of gene colinearity with the human genome. Organogenesis in *Xenopus* resembles human organogenesis both genetically and anatomically, which makes this model system particularly attractive for investigating the molecular mechanisms of human disease. Indeed, for some organ systems such as lungs or limbs, comparable studies are not possible in fish. Functional genomic analyses, including both gain- and loss-of-function, can be done efficiently in *Xenopus*, allowing the analysis of hundreds of candidate disease genes and characterization of their interacting pathways in just a few months at a fraction of a cost compared to mammals. Numerous studies have already demonstrated the utility of *Xenopus* in studying various human diseases, and as an excellent model for functional analysis of disease-related mutations. For example, *Xenopus* has proved useful in investigating congenital heart disease, heterotaxy, diabetes, ciliogenic-related disorders, kidney disease as well as brain disorders (see Section II). Moreover, *Xenopus* has a considerably longer life-span (~15 years) than do mice or zebrafish, and this may provide unforeseen advantages in studies of tissue repair and regeneration.

With the proliferation of Genome-wide association studies (GWAS), an increased understanding of gene-environment interactions, and the increasing use of patient-specific genome sequencing for precision medicine, the data on gene variants related to human disease are increasing exponentially. The major challenge will be deciphering, validating, and functionally characterizing specific gene variants/mutations and the impact they have on disease. In many cases, the necessary experimentation cannot be accomplished in tissue culture. For example, many of the variants identified in GWAS studies do not alter the protein-coding sequence, but rather change nucleotides in introns or intergenic regulatory regions that may only be active in the *in vivo* setting. With genome editing technologies and transgenics firmly in place, *Xenopus* is an excellent model system to interrogate candidate gene variants and to characterize pathways of human disease.

The ability to produce large numbers of synchronized *Xenopus* eggs and embryos, their easy culture without specialized media or temperature requirements, and their rapid development make them ideal for high-throughput screening for a number of medically relevant products, such as compounds that interfere with blood/lymphatic vessel growth, cancer-promoting regulatory pathways, or organogenesis. Pharmacological screens using *Xenopus* embryos have identified modulators of angiogenesis (Kalin et al., 2009, Blood 114: 1110), heterotaxy (Dush et al. 2011, Chem Biol. 18: 252), and aquaporin1 activity (Patil et al. 2015, Chem Biol. Drug Des. Dec 18. doi: 10.1111/cbdd.12713). In addition, high-throughput screens based on egg extracts have been used to identify modulators of the Fanconi Anemia DNA Damage Response pathway (Landais et al. 2009, Internatl J Cancer 124:783) and inhibitors of the Wnt pathway (Thorne et al., 2011, J. Biomol. Screen 16: 995).

***B. How can Xenopus be used for modeling and studying human disease?***

* Generate tools to induce and model human diseases in developing *Xenopus* embryos. Enhanced development of mutant alleles in *Xenopus* using TALENs and CRISPR/Cas should be encouraged.
* In addition to providing an effective system for *in vivo* screening of candidate GWAS genetic variants in human disease, unbiased studies of organ formation and function can reveal *Xenopus* phenotypes similar to human diseases and thus implicate novel disease candidates.
* Functional epistasis experiments in *Xenopus* can elucidate how different genes implicated in the same disease can interact in regulatory pathway, revealing alternative drug targets.
* New transgenic lines that mark specific lineages associated with human disease that can be used to target experimental and pharmacological manipulations.
* Identify and develop methods for rapid introduction of disease-specific alleles to study their altered functions in the developing embryo. For example, human mutations in the neonatal diabetes candidate gene, *RFX6*, were shown to produce nonfunctional proteins in developing *Xenopus* embryos.
* Many adult human diseases arise as a result of developmental defects. *Xenopus* is an excellent model to study the origins of such adult diseases.
* There is an increasing awareness that gene-environment interactions impact disease susceptibility at both the genetic and epigenetic level. *Xenopus* has long been used for teratogenic assays to test the exposure of tadpoles to toxins on the development of specific cell types.
* *Xenopus* is an excellent system for small molecule screens to assess the effects of newly described drugs. Screens can include *in vivo* analysis of organ development and function, as well as biochemical screens in cell free extract focused on specific drug targets.
* *Xenopus* is very useful to study regeneration and methods to improve regeneration in mammals. The tail, limb and eye regenerate in *Xenopus,* and studies investigating why regeneration occurs in early stages of *Xenopus* development but not later stages will be useful in identifying both the key components required to promote regeneration, and components that inhibit or impair regenerative processes.
* Robust cell biology, biochemistry and cell-free extracts as well as the compatibility with small molecule screens, enables structure-function analyses of potential therapeutic-target interactions.
* How do disease-causing mutations influence protein function and protein-protein interactions? Transgenic *Xenopus* can be created that carry specific mutations to study how these mutations affect protein function.

***C. Community recommendations***

Funding of the essential resources listed in this White Paper (Section IV) is the most immediate way to achieve the overarching goals of facilitating the use of *Xenopus* for understanding human disease. In addition, the community recommends:

* Focused initiatives with specific funding opportunities (FOAs) to encourage the use *Xenopus* as a tool to study human disease mechanisms would have significant impact. Indeed, many NIH Institutes have issued PARs calling for novel animal models for human disease modeling. We encourage the NIH to issue PARs specifically for the development of non-mammalian model systems in disease modeling.
* Establish a cooperative network in the *Xenopus* community to identify key target genes for mutagenesis. Such a network could develop pipelines to systemically screen human gene variants in *Xenopus*. This can be achieved in collaboration with the NXR.
* Develop a chemical screening center that can be used to test various compounds and their effects on the development of specific organ systems and for understanding the molecular basis of how various chemicals function.
* Enhance bioinformatics resources to correlate phenotypes from *Xenopus* gene function studies with human diseases and mouse models. This can be achieved in collaboration with Xenbase.
* Incorporate the *Xenopus* model as a component of larger multi-system consortia studying human disease. For example, screening in *Xenopus* should be combined with the current approaches of patient phenotyping, human genetics, and screening candidate variants *in silico* and in tissue culture.

VI. Appendices

Appendix 1 – Authors of the 2016 *Xenopus* Community White Paper

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**Appendix 2 –** **The Cold Spring Harbor Laboratory course on “Cell & Developmental Biology of *Xenopus*”**

[**http://meetings.cshl.edu/courses.aspx?course=C-XENO&year=16**](http://meetings.cshl.edu/courses.aspx?course=C-XENO&year=16)

1993-1996: Co-Directors: Hazel Sive (MIT), Rob Grainger (UVa), Richard Harland (UC Berkeley)

 1997-2000: Co-Directors: Paul Krieg (UT Austin), Sally Moody (GWU)

2001-2004: Co-Directors: Ken Cho (UC Irvine), Jan Christian (OHSU)

2005-2007: Co-Directors: Janet Heasman (CCHMC), Chris Wiley (CCHMC)

2008-2010: Co-Directors: Ray Keller (Univ. Virginia), Kristen Kroll (Washington Univ.)

2011-2014: Co-Directors: Amy Sater (Univ. Houston), Gerald Thomsen (Stony Brook Univ.)

2015-2017: Co-Directors: Karen Liu (King’s College London), Mustafa Khokha (Yale Univ.)

Supported by NIH grants and by the Howard Hughes Medical Institute

**Appendix 3 – The International *Xenopus* Conference**

1984: Organizational workshop at Airlie House, Warrenton, Virginia, USA

1986: University of East Anglia, UK

1988: Tulane University, New Orleans, LA, USA

1990: Les Diablerets Conference Center, Switzerland

1992: Asilomar Conference Center, California, USA

1994: Congress Center De Branding, The Netherlands

1996: YMCA of the Rockies, Estes Park, Colorado, USA

1998: Sardinia, Italy

2000: YMCA of the Rockies, Estes Park, Colorado, USA

2002: Cambridge University, Cambridge, UK

2004: Marine Biology Laboratory, Woods Hole, MA, USA

2006: Kazusa Akademia Park, Japan

2008: Leiwen, Germany

2010: Lake Louise, Canada

2012: Giens Peninsula, France

2014: Asilomar Conference Center, California, USA

2016: Orthodox Academy of Crete, Chania, Greece