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Development of *Xenopus* Resource Centers: the National Xenopus Resource and the European Xenopus Resource Center

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Abstract

Xenopus is an essential vertebrate model system for biomedical research that has contributed to important discoveries in many disciplines, including cell biology, molecular biology, physiology, developmental biology and neurobiology. However, unlike other model systems no central repository/stock center for *Xenopus* had been established until recently. Similar to mouse, zebrafish and fly communities, which have established stock centers, *Xenopus* researchers need to maintain and distribute rapidly growing numbers of inbred, mutant and transgenic frog strains, along with DNA and protein resources, and individual laboratories struggle to accomplish this efficiently. In the last five years two resource centers were founded to address this need: the European *Xenopus* Resource Center (EXRC) at the University of Portsmouth in England, and the National *Xenopus* Resource (NXR) at the Marine Biological Laboratory (MBL) in Woods Hole, MA, USA. These two centers work together to provide resources and support to the *Xenopus* research community. The EXRC and NXR serve as stock centers and acquire, produce, maintain and distribute mutant, inbred and transgenic *X. laevis* and *X. tropicalis* lines. Independently, the EXRC is a repository for *Xenopus* cDNAs, fosmids and antibodies; it also provides oocytes and wild type frogs within the UK. The NXR will complement these services by providing research training and promoting intellectual interchange through hosting minicourses and workshops and offering space for researchers to perform short-term projects at the MBL. Together the EXRC and NXR will enable researchers to improve productivity by providing resources and expertise to all levels, from graduate students to experienced PIs. These two centers will also enable investigators that use other animal systems to take advantage of *Xenopus*' unique experimental features to complement their studies.

Keywords

stock center; minicourse; transgenesis; tropicalis; laevis; husbandry; oocyte; ORFeome

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Xenopus: An essential resource for scientific discovery

Animal models are crucial for biomedical research advances, and basic research in vertebrate model systems accelerates our understanding of human health and diseases. However, with increased model system use, centralized resource repositories become essential, and research in mouse, zebrafish, and fly has been greatly enhanced by The Jackson Laboratory, ZFIN, and the Bloomington *Drosophila* Stock Center at Indiana University, respectively. Similarly, the broad use of *Xenopus* frogs in biomedical research, accompanied by the recent creation of a large number of mutant and transgenic lines, now necessitates the creation of stock centers to house several hundred animal lines and other community resources.

A primary rationale for creating these Centers derives from the creation of these frog lines as well as several important innovations and research needs within the *Xenopus* research community. By way of introduction, there are now primarily two species used by the community, *Xenopus laevis* and *Xenopus tropicalis*, serving complementary functions for researchers (Harland and Grainger, 2011). *X. laevis* has been the primary species used by researchers, providing large eggs and amounts of embryonic material ideal for both embryological and biochemical work. While its long life cycle and allotetraploid genome provide some challenges for genomic and genetic studies, it remains a mainstay for many research problems. Because of its long generation time however, it has been difficult for researchers to generate the critical transgenic lines needed for a multitude of purposes; for example, carrying reporter constructs to identify various tissues, organs and cellular structures. But this is exactly the sort of resource that stock centers can readily provide for researchers. On the other hand, *X. tropicalis* is a diploid species that grows more rapidly and adds a new and powerful genetic dimension. But this ease of generating lines (inbred, mutant and transgenic) means that in just the short time that this model has been in use, there is already a pressing need to house these many lines that would otherwise be virtually impossible to distribute via the many labs that have generated them. Thus the new stock centers have become a critical clearinghouse for the entire *Xenopus* community.

Xenopus has had and continues to make considerable impact in scientific research due to its accessible embryos, large experimental tool kit, close evolutionary relationship with mammals and extensive synteny to the human genome (Harland and Grainger, 2011; Hellsten *et al.*, 2010). In the genomic era, its simple high-throughput gain- and loss-of-gene-function assays, rapid transgenesis methods, and the ability to generate mutations in *X. tropicalis*, means that its usefulness to the biomedical research community will continue to grow, and is already increasingly providing key insights into human disease (Harland and Grainger, 2011).

Xenopus is used extensively in cell biology, molecular biology, developmental biology and neurobiology for a number of reasons. *Xenopus* have large, abundant, externally developing embryos that are hardy and provide large quantities of material for biochemical and molecular biological analyses. In addition, they can be injected with gene products or constructs at early cleavage stages (e.g., for tissue specific overexpression or knockdown, to observe expression of various gene constructs, or to create transgenic animals) or surgically manipulated (e.g. grafting to study tissue interactions) (Bajpai *et al.*, 2010; Fakhro *et al.*, 2011; Jarikji *et al.*, 2009; Nie *et al.*, 2009). *Xenopus* oocytes have long been used as a powerful system for monitoring gene activities, such as identifying or assaying ion channel activities (Gantress *et al.*, 2003; Tochinai and Katagiri, 1975). *Xenopus* egg extracts, on the other hand, have been exceptionally useful for *in vitro* experiments, such as cell cycle regulation and DNA damage (Ben-Yehoyada *et al.*, 2009; Raschle *et al.*, 2008). The hardiness and large size of the embryos has also made them a preferred system in which to

study neurophysiological development (Lambert *et al.*, 2009; Roberts *et al.*, 2010, 2011; Straka and Simmers, 2011). In recent years, the ability to combine genetic approaches with well-established *Xenopus* embryology and cell biology techniques is providing unique research opportunities and insights into developmental regulation mechanisms at the subcellular level. The cost of generating lines and maintaining animals in a *Xenopus* colony is a fraction of that of a mouse colony, and much of the protein and gene function data obtained from *Xenopus* are highly translatable to understanding human development and disease (Harland and Grainger, 2011). The importance of *Xenopus* in a multitude of research areas is highlighted by the amount of resources awarded by the US government with 678 National Institutes of Health (NIH) funded grants utilizing *Xenopus* for the funding year 2009-2010 (from a search of the NIH grant database rePORT).

Further resources are required to fully utilize the advantages of Xenopus

Over the last 15 years there has been a substantial increase in the number of inbred, mutant and transgenic *Xenopus* lines necessitating a centralized repository to ensure maintenance and distribution of an estimated 200 lines in total, similar to resources established for other animals such as *Drosophila*, zebrafish and mouse (Beck *et al.*, 2009; Beck and Slack, 1999; Day and Beck, 2011; Harland and Grainger, 2011; Hirsch *et al.*, 2002; Horb *et al.*, 2003; Rankin *et al.*, 2009; Rankin *et al.*, 2011).

For *Xenopus laevis* researchers, a pressing need is the generation of reporter lines alluded to above. Such lines will greatly facilitate assays of tissue and organ formation, which are so readily and effectively studied in *Xenopus*, as will reporter lines demarcating subcellular structures, permitting advanced analysis of cell biological issues in a whole animal context or in cell extracts, both strengths of the *Xenopus* system. While the transgenic technology in *Xenopus* is extremely efficient, the husbandry associated with generating these lines is not commonly undertaken by most researchers using *Xenopus laevis*, but will be a straightforward initiative for a stock center. In addition the stock center will house other high priority lines, for example, the inbred MHC homozygous J strain that is being used for sequencing the *X. laevis* genome (Gantress *et al.*, 2003; Tochinai and Katagiri, 1975). These animals will provide a more uniform genetic background that will enhance research in a number of ways, for example, in helping to accurately design antisense reagents without concern for the sequence polymorphisms seen in outbred animals.

For researchers using *X. tropicalis*, the stock centers will be a repository for inbred, mutant, and transgenic lines. It is estimated that the stock centers will hold living stocks for approximately 200 such lines, with less frequently used lines stored as frozen sperm (Sargent and Mohun, 2005). Inbred lines have been generated for both Nigerian-derived and Ivory Coast-derived animals, which show considerable polymorphisms to each other and are therefore useful for genetic mapping experiments (Wells *et al.*, 2011). Both lineages have been highly inbred and used for preparing animals for the *X. tropicalis* genome project, BAC libraries, and in genetic screens. Other wild caught populations will be housed as well to be a source of genetic diversity for future studies. Mutant lines will be raised where they are likely to be useful by many investigators. For example, lines that have already been identified as having reduced pigmentation will be extremely valuable for *in vivo* imaging for many researchers. Lines affecting axis formation (e.g. left-right axis mutations) and organ/tissue formation (e.g. eye, heart, limb and neural crest formation) are also of great interest to many investigators. Lastly, there are already a multitude of transgenic lines that have been produced in this species because multigenerational studies are simpler than in *X. laevis*. Here many dozens of existing tissue-specific reporter lines will be housed, in addition to newly produced lines, and other types of transgenic lines, for example, those which allow conditional gene expression by the Gal4/UAS system (Chae *et al.*, 2002).

The mandate for these stock centers was generated by an NIH *Xenopus* initiative was developed at the non-mammalian workshop in 1999 (http://www.nih.gov/science/models/xenopus/index.html). This workshop outlined recommended tools, projects and resources for the *Xenopus* community, with the aim of providing support to enable critical scientific breakthroughs that utilize the experimental advantages of the *Xenopus* system. This was followed up by several White Papers from the *Xenopus* community, the most recent being the 2011 *Xenopus* White Paper (http://www.xenbase.org/community/xenopuswhitepaper.do) (see Khokha, 2012 this issue). These reports recommended establishing Resource Centers as crucial focal points that would provide shared resources to accelerate and enhance biomedical research.

To implement these recommendations, the European *Xenopus* Resource Center (EXRC) was established in 2006 at the University of Portsmouth, England, and the National *Xenopus* Resource (NXR) began operations in 2010 at the Marine Biological Laboratory (MBL) in Woods Hole, USA. Together these two centers aim to provide resources, courses and support not only for the *Xenopus* community, but also for researchers outside the community who wish to utilize the experimental advantages of *Xenopus* in their own research programs To provide the widest possible dissemination of information about available resources, the EXRC and NXR will work in close collaboration with Xenbase, the *Xenopus* database (http://www.xenbase.org) (Woodland and Zorn, 2008). Below is a brief description of the centers, including the future resources they expect to offer to *Xenopus* researchers.

Housing and distribution of Xenopus

Both the EXRC and the NXR acquire, produce, maintain, and distribute wild type, inbred, transgenic and mutant lines of *Xenopus laevis* and *Xenopus tropicalis*. Each resource center will also generate novel transgenic strains, initially producing a range of tissue- and subcellular-structure-specific fluorescent protein reporter lines. Strains will be duplicated at the EXRC and NXR to insure against potential disease outbreaks at either facility. The EXRC website (http://www.port.ac.uk/research/exrc) includes links to the lines currently housed at the EXRC, and Xenbase also links to the EXRC website (http://www.xenbase.org/stockCenter/index.do). The EXRC currently distributes animals to research laboratories worldwide (with shipping paid by the receiving laboratory), and has recently begun distribution of testes and frozen sperm where shipping of live animals is impractical. The NXR will begin distribution of animals in the near future, when the colonies bred from donated lines are established; detailed information of available lines will be posted on its website (http://www.mbl.edu/xenopus/index.html) and on Xenbase (http://www.xenbase.org). The NXR also aims to offer made-to-order transgenic lines and frozen sperm from transgenic animals.

Scale of operations

Both stock centers are designed to hold thousands of *Xenopus laevis* and *Xenopus tropicalis* animals. The EXRC currently houses 3400 *X. laevis*, 1100 of which are transgenic, and 1600 *X. tropicalis*, 800 of which are transgenic. The NXR will gradually increase its capacity. Currently the NXR houses 1200 *X. laevis*, 400 of which are transgenic, and 1200 *X. tropicalis*, 500 of which are either transgenic or mutant lines, with the other 700 being from various inbred wild type lines. The capacity of the mature NXR facility will take several years to reach, but is expected to be 4000 *X. laevis* and 8000 *X. tropicalis*. The proportions of wild type, transgenic and mutant lines will vary to respond to the needs of the community as the resource centers grow. As noted earlier, it is expected that for *X. laevis*, inbred lines and transgenic reporter lines will comprise the preponderance of animals. For *X.*

tropicalis, these centers will house the multitude of inbred and outbred lines that have already been produced by other laboratories, as well as a large number of mutant and transgenic lines, as described above. In addition, it is anticipated that populations of mutagenized animals will be housed in these centers as a resource for possible forward genetic screens and for reverse genetic studies identifying mutations by "TILLING," as discussed below.

EXRC: an evolving resource for the Xenopus community

One of the primary goals of the EXRC is to support the "3Rs" in animal research: reduction, refinement and replacement. By housing many mutant and transgenic frog lines the EXRC enables laboratories to purchase animals when needed rather than housing a critical mass of each line they use; this service reduces the overall number of animals held globally. The EXRC has successfully established an oocyte delivery service, also reducing the number of frogs used; oocytes from a single female frog can be used by several research labs. This service reduces the cost burden on the receiving lab by eliminating animal husbandry costs, and makes better use of available resources by allowing fewer animals to be used overall. The EXRC is piloting the extension of this delivery service to include cell-free egg extracts for cell biology experiments. Replacement of animals in experiments involving *in vivo* work is not possible, but the introduction of improved experimental techniques and strategies, and utilization and teaching of good practice animal husbandry is refining animal experimentation, to acquire the most data from the animals used.

Xenopus cDNA collections

To limit unnecessary duplication of cloning of genes among the members of the *Xenopus* research community, the EXRC houses a collection of cDNA plasmids for *in situ* hybridization and over-expression experiments. All of the plasmids sent to the EXRC are confirmed by sequencing, with the files being directly available to users. In situ hybridization probes are also tested with the images from these tests available on Xenbase. The collection currently holds over 700 clones and is continuing to grow as researchers donate plasmids.

In addition to the cDNA collection the EXRC will house and distribute two *Xenopus* ORFeomes, one for *X. tropicalis* and one for *X. laevis*. The ORFeomes will represent complete sets of *Xenopus* sequence-validated protein-coding open reading frames (ORFs), including alternate splice variants, in vectors based on the Gateway system (Invitrogen/Life Technologies, California, USA), which enables bulk transfer of ORFs to a range of different expression vectors to facilitate rapid functional genomic screening. Expression screening experiments in *Xenopus* have been a highly successful strategy for identifiying novel components controlling key developmental processes in because *Xenopus* embryos are so well suited for injecting gene constructs (Smith and Harland, 1992). The creation of the ORFeome will permit researchers to capitalize on this historical strength, providing a much more complete, and manipulable, set of gene constructs for this kind of analysis. The ORFeomes will be created by Dr Todd Stukenberg of the University Virginia (USA) in collaboration with the Dana-Farber Cancer Institute. This resource will allow faster elucidation of the function of *Xenopus* genes, as researchers can simply purchase the gene-of-interest and potential interaction partners or downstream targets, and use the Gateway system to transfer them into the vector best suited to their purpose. Having a central repository for the ORFeome reduces unnecessary duplication of work in individual research laboratories, thus enhancing progress in research. Laboratories requiring the complete ORFeomes will be able to purchase them through commercial companies such as Open Biosystems and Source Biosciences.

The cDNA and ORFeome collections are useful to *Xenopus* researchers, but are also particularly valuable resources for comparative studies by non-*Xenopus* researchers. Workers using other animal models can order and use *Xenopus* cDNAs and ORFeomes to compare the activity of homologous gene products from different species and thereby define which regions of proteins are important for different functions. The Bloomington Drosophila Stock Center at Indiana University, from which anyone can order fly cDNA clones, is a good example for this kind of pan-model system utility. Cross-species comparisons help identify regions in a protein or protein family critical for a specific function (Tokmakov, 2011). This has been particularly valuable in comparing the function of specific frog and fly Bruno-like proteins (Horb and Horb, 2010), and in dissecting functional differences between species, as we recently showed for Ngn3 across mouse, human and frog (Oropeza and Horb, 2011).

Antibodies and fosmids

Another goal of the EXRC is to increase *Xenopus* community access to antibodies and fosmids. At present, *Xenopus*-specific antibodies are not broadly available. Commercially, Novus Biologicals (Colorado, USA) and the Developmental Studies Hybridoma Bank (http://dshb.biology.uiowa.edu/)) sell antibodies reactive to a small number of *Xenopus* antigens. On the whole, access to *Xenopus* antibodies is limited by the small number of laboratories that have created them and those laboratories' ability to distribute the antibodies they produced for their own research. In contrast, antibodies for other model organisms are readily available commercially. To improve this situation, the EXRC is becoming a central repository for *Xenopus* antibodies, collecting and distributing monoclonal and polyclonal antibodies gathered from various researcher collections. In addition, the EXRC is collaborating with Xenbase to create a Wiki page (a multi-user-editable webpage) outlining how commercial antibodies created against non-*Xenopus* antigens perform in *Xenopus*.

A heavily used molecular resource available from EXRC is the *X. tropicalis* fosmid collection, which contains much of the genome in approximately 40kb fragments. These fosmids are extremely useful as templates for preparing fragments of the genome by PCR, which in turn are useful for analyzing gene regulation or for driving transgene expression. The position of each fosmid is mapped onto the *X. tropicalis* genome when viewed from Xenbase. This makes it simple to identify fosmids containing the desired target sequences, facilitating generation of transgene and other genomic constructs.

NXR: a new resource for the Xenopus community

The newly established National *Xenopus* Resource (NXR) at the MBL will complement resources available at the EXRC. As mentioned above, both centers will house and distribute mutant, inbred and transgenic lines. While the EXRC is a repository for reagents, the NXR will offer workshops, minicourses and hands-on experience for visiting researchers. The NXR will benefit tremendously from the MBL's established expertise in hosting workshops and courses, its extensive laboratory space, teaching facilities and student housing that allow it to host various sized research groups. Therefore, in addition to serving as a stock center, the NXR will run training courses and workshops tailored to those planning to utilize *Xenopus* in their research. The initial goal is to offer one or two courses per year, but the number of courses offered will grow with the NXR to meet community demand. Some of the future course topics the NXR expects to cover include bioinformatics, husbandry, transgenesis, cell-free extract preparation, imaging and genetic screening. Due to the previous lack of community resources for the *Xenopus* model, it has been difficult for scientists wishing to take advantage of its versatility to initiate rapidly changing new technologies in their laboratories. These courses, together with the availability of the above

mentioned resources from the centers, will make it much easier to incorporate *Xenopus* experiments into a group's research repertoire.

Bioinformatics

The advent of high throughput sequencing has increased the importance of computational methods in molecular biology, and intensified the pressure on laboratory scientists to find ways to access these new paradigms. Conventional web-based bioinformatics approaches now need to be augmented with core computational skills to develop data analysis competencies without depending on overworked bioinformatics experts. It makes sense to develop these skills within a model organism community. Each model system has its own distinctive profile of data resources and repertoire of experimental techniques. A course tailored to these, taught by experts from within the community, will have much greater impact than a more generally based course. For the *Xenopus* community, the gaps in the genome assembly for *X. tropicalis* and the emerging genomic information only now beginning to be available for the allotetraploid species *X. laevis* provide unique challenges for many researchers who require further bioinformatics training to adequately utilize these resources. RNA-seq and other similar technologies, so well suited to the availability of tissues for detailed expression analysis in *Xenopus* embryos, provide another example where bioinformatics training is urgently needed to augment the training of many researchers. The NXR will take advantage of the excellent teaching facilities of the MBL to facilitate the integration of these important analyses into this already powerful experimental system. The first workshop the NXR will offer will therefore focus on bioinformatics.

Husbandry

One of the main issues for any *Xenopus* researcher is the practical maintenance of a healthy colony of adult frogs to generate oocytes and fertilized eggs for experimentation. In contrast to the range of resources offered to individuals working with mice, *Xenopus* researchers often must rely on anecdotal knowledge when establishing and maintaining their colony. Many researchers were never offered courses on *Xenopus* maintenance because the labs in which they trained already had fully functional colonies supervised by technical staff. For those who have worked exclusively on *X. laevis* there has in the past been little impetus to raise animals, since experiments have relied on the purchase of outbred adult animals for generating oocytes, eggs and embryos. An animal husbandry workshop will provide best practices for *Xenopus* care, including housing and breeding. There will be a *Xenopus* health component to the course, focusing on diagnosing and treating disease in both laboratory *Xenopus* species. This workshop will be especially useful to new faculty who are initially establishing their *Xenopus* colonies, as well as researchers wishing to add *Xenopus* to established research programs.

Transgenesis

Since the inception of *Xenopus* transgenesis, one of the advantages is the ability to produce transgenic animals rapidly and in large numbers (Kroll and Amaya, 1996). Nonetheless, transgenic techniques have not become broadly used within the community. As has been shown in mice and zebrafish, transgenesis in *Xenopus* has been very useful for a variety of applications, such as functional overexpression, tissue-specific labeling and rapid assays for enhancer function (Fish *et al.*, 2011; Horb *et al.*, 2003; Jarikji *et al.*, 2007; Loeber *et al.*, 2009; Ogino *et al.*, 2008; Ogino *et al.*, 2006; Rankin *et al.*, 2011; Wheeler *et al.*, 2008); though in *Xenopus*, these methods are even more efficient and more likely to result in appropriate tissue-specific expression of gene constructs in injected (F0) embryos, allowing for potentially very rapid assays for biological effects of injected constructs. Many groups however, have struggled to establish the technique. A workshop focused on learning to perform and evaluate transgenesis in both species of *Xenopus* will help promote wider use

of this technology. The goal of this workshop will be twofold. First, it will teach the different techniques currently used to create transgenic animals, so that an individual researcher can choose the one most suitable for their particular experimental question and in the future create transgenic animals in their own laboratory. Second, for those researchers for whom creating transgenics in their own laboratories is not practical, the course will enable them to create transgenic animals at the NXR and take them back to their laboratories. Thus, this course will promote the more widespread use of transgenesis in *Xenopus*, which in turn will benefit to the entire research community by providing a wider range of transgenic animals.

Cell-Free Egg Extract

The *Xenopus* egg extract, which represents essentially undiluted cytoplasm from the egg, is a powerful system for cell-free reconstitution of cellular processes and characterization of the protein complexes that drive them (Chan and Forbes, 2006; Deming and Kornbluth, 2006; Kano *et al.*, 2006; Mirny and Needleman, 2010; Tutter and Walter, 2006). The use of Xenopus egg extracts has been highly valuable owing to the fact that one can isolate extract with precise competency for individual cell cycle states. This coupled with the ability to immuno deplete or add proteins has facilitated the detailed characterization of numerous cell biological processes. The Cell-free Egg Extract workshop will teach the preparation and use of egg extracts from both *X. laevis* and *X. tropicalis*, evaluation of extract quality, and design and implementation of typical extract experiments. The course will also cover essential elements of biochemistry and microscopy techniques used in extract experiments, including preparation and fluorescent labeling of antibodies and protein complexes, proteomics, practical aspects of different fluorescence microscopy methods, and image analysis. In addition to serving as a training resource, the workshop will serve as a focus for technique and reagent sharing not only for the world-wide *Xenopus* extract community but also for other researchers studying cell biological processes who wish to incorporate *Xenopus* cell free extracts into their experimental repertoire.

Imaging

As with bioinformatics, imaging in *Xenopus* presents specific challenges. Imaging at the cellular level on the surface of the embryo has been quite useful for basic cell biology studies (Woolner *et al.*, 2009; Yu and Bement, 2007). However, given that the embryo is pigmented, opaque until tadpole stages and many cells deep, conventional high resolution imaging of developmental processes is problematic. However, pioneering imaging approaches have been developed that combine classical embryology, the use of explant systems for studying tissue morphogenesis during early development, and state-of-the-art live cell imaging (Davidson *et al.*, 2008; Rolo *et al.*, 2009). Therefore, the imaging workshop will take advantage of the various highly sophisticated microscope facilities available at the MBL to demonstrate different techniques to prepare, mount and visualize both live and fixed samples.

Genetics

The introduction of the faster developing diploid species *Xenopus tropicalis* has now allowed genetic screens to be performed in a number of labs and several mutant genes to be positionally cloned (Geach and Zimmerman, 2011; Harland and Grainger, 2011). This approach involves generation of mutagenized animals, which is an unfamiliar technology for many labs. In addition, specialized methods are used for rapid screening, such as gynogenesis, which reveals recessive phenotypes quickly by using U.V.-treated (inactivated) sperm to fertilize mutant eggs that are subsequently diploidized to generate homozygous recessive mutant embryos for screening (Khokha *et al.*, 2009; Noramly *et al.*, 2005). The goal of this workshop is to introduce researchers to the various genetic resources currently

available, technologies required for genetic screening and analysis of already identified mutants. Mutagenized frogs and mutant lines at the NXR will provide material both for the workshop and for visiting researchers who want to perform customized screens.

Emerging Technologies

Future workshops and minicourses will be created based on the demand from the community. As emerging technologies become available the NXR will promote specific applications to *Xenopus* through workshops similar to those described above. These workshops will provide researchers direct access to experts, including their experience in troubleshooting and optimizing the technique as it was being developed. Some examples of such emerging techniques include approaches for making mutant lines in genes of high interest to researchers, such as zinc finger nuclease and TILLING technologies, as well as new methods to produce loss-of-function phenotypes, such as siRNA (Harland and Grainger, 2011) .

In addition to these workshops and minicourses, the NXR is also perfectly situated to become a hub of *Xenopus* research, where scientists can come for days, weeks or months to interact with one another and utilize the resources of both the NXR and the MBL, thus fostering collaborations amongst research groups. Through hosting researchers for short-term projects, the NXR will enable researchers to perform experiments in collaboration with or guidance of colleagues. The NXR will provide bench space and access to resources such as microinjection apparatus and fluorescent and bright field microscopes, and limited animal housing. This arrangement is also ideally suited towards researchers for whom *Xenopus* is not their primary model organism, and who will benefit from utilizing the NXR frogs. The EXRC has successfully employed this “research hotel” concept to host researchers, and the NXR will expand upon this idea. This will allow a wider range of scientists to take advantage of the benefits of *Xenopus* for specific experiments, without committing to animal husbandry or requiring specialized equipment for their laboratory. For example, a clinical scientist studying diabetes-linked genes could perform morpholino knock-down experiments using embryos from the Elas-GFP transgenic frog line available at the NXR to establish if a specific morpholino alters the size, morphology or constituent cell types within the fluorescent pancreatic tissue. A *C. elegans* developmental biology researcher wanting to test functional conservation of their gene-of-interest could perform morpholino knock-down and overexpression experiments in *Xenopus* embryos, without investing in the needed *Xenopus* colony and equipment. Such use of *Xenopus* in “hybrid” model studies has been used in a multitude of high impact studies in recent years because of the unique advantages *Xenopus* offers for assessing so many complex biological problems *in vivo* (Cruciat *et al.*, 2010; Peng *et al.*, 2009; Yoon *et al.*, 2011)

Because the *Xenopus* Centers are likely to house stocks of mutagenized animals for genetic screening experiments they will also be very attractive sites for visitors interested in establishing new research programs focusing on the new genetics approaches available in *Xenopus*. For example, at present there are projects both in the U.S. (at the MBL) and in the UK (at the Sanger Centre) to identify mutations in gene of high interest to the research community using “TILLING” technology using high throughput sequencing to screen through populations of mutagenized animals (Comai and Henikoff, 2006; Moens *et al.*, 2008; Stemple, 2004). As they are generated, these mutagenized animals and mutant lines will become part of the resources of the stock centers and will be available to visiting scientists. The availability of mutagenized animals at these Centers will also potentially permit investigators to pursue forward genetic screening experiments as well.

Future growth of the Xenopus Resource Centers

The EXRC and the NXR are essential for expanding the use of *Xenopus* in biomedical research by providing the vast resources currently housed in individual laboratories to the entire research community, and by making the power of emerging technologies including transgenesis, next-generation sequencing, genomics and genetic resources widely accessible. By providing the *Xenopus* community with a central hub for disseminating resources (EXRC) and expertise (NXR) the advantages of *Xenopus* as a model organism will be augmented by rapid adoption of new technologies by the community. These benefits will also extend to researchers new to the *Xenopus* system by providing the resources and training that will allow them to rapidly acquire the ability to utilize this powerful experimental system. Future growth of the Resource Centers will require *Xenopus* community involvement, and continual consultation with the community will ensure that both the EXRC and the NXR develop new resources, support and workshops relevant to community needs.

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