Genetic and Genomic Needs for *Xenopus* Research

Report of the Workshop on March 2-3, 2000

Sponsored by the National Institute of Child Health and Human Development and the
Trans-NIH Non-Mammalian Model Coordinating Committee

CONTENTS

History of the Process Leading to this Report — Page 1

Value of *Xenopus* and Need for the Recommended tools, projects and resources — Page 2

RECOMMENDATIONS:

Tools, Projects and Resources — Page 5

Uses and Potential of Xenopus — Page 6

Details of the Recommendations — Page 10

- Genomic Tools for *Xenopus*
  - Expressed Sequence Tags — Page 10
  - Full Length cDNA Libraries — Page 12
  - MicroArrays — Page 13
- *Xenopus tropicalis*
  - Genetics Studies — Page 14
  - Gene Mapping — Page 16
- Resource Centers
  - Internet Resources — Page 19
  - Stock Centers — Page 21
  - Training Centers — Page 22

APPENDICES

Appendix I
Workshop Agenda and Participant List — Page 23

Appendix II
Recommendations and Participants from *Xenopus* Portion of NIH Non-Mammalian Model Meeting — Page 30

Appendix III
Uses of *Xenopus* for Research Supported by NIH Institutes — Page 33

Appendix IV
Table of federal funding for *Xenopus* work — Page 34

Appendix V
Procedure for ESTs submissions — Page 35

I. HISTORY of REPORT

This report is the product of the Workshop 'Identifying the Genetic and Genomic Needs for *Xenopus* Research', which was convened at the National Institutes of Health on March 2 and 3, 2000. The workshop was sponsored by the National Institute of Child Health and Human Development and by the Trans-NIH Non-Mammalian Models committee. At the workshop, members of the *Xenopus* research community recommended the genetic and genomic tools and resources to improve Xenopus' formidable ability to elucidate the cellular, molecular and genetic mechanisms that control cellular processes, including embryonic development. The participants recommended tools and resources that will benefit the broader biomedical research community in addition to those using Xenopus. Researchers who work on other models participated in the workshop. (See Workshop Agenda and Participant List, Appendix I)

This Workshop was designed to enable a broad representation of the *Xenopus* community to alter, revise and/or define the parameters of the recommendations of a previous meeting, the *Xenopus* portion of the 'NIH Non-Mammalian Models Meeting', February 1999. At that meeting, a small group of *Xenopus* researchers devised a prioritized list of needs for *Xenopus* research. Researchers who use other animal models participated in those discussions (see Recommendations and Participants, Appendix II). That list was based on input from many members of the *Xenopus* community that was solicited during the preceding six months.

The Non-Mammalian Models Meeting was, in turn, based on a previously convened meeting, the 'National Cancer Institute's Working Group on Non-Mammalian Models of Human Cancers', June 1997. That meeting recommended that the National Cancer Institute concentrate its efforts on five non-mammalian models for cancer research. One of these animals was Xenopus. When these recommendations were presented to the Director of the NIH, he noted that these models had much to offer all aspects of biomedical research, and that focusing on only cancer research was too narrow. He recommended that the NIH hold a meeting to determine what tools and resources these animals needed to benefit the broader biomedical research endeavor.

Thus, this report is the culmination of over three years of discussions amongst the members of the *Xenopus* research community and of at least three meetings convened by the National Institutes of Health. Of the five non-mammalian models that were advocated for cancer research in 1997, and were advocated for research into human disease and development in 1999, *Xenopus* still has the smallest amount of community-wide genetic and genomic resources. The resources and tools recommended in this report will produce enormous improvements in our ability to elucidate the cellular, molecular and genetic mechanisms that govern many critical aspects of embryonic development and cell biological processes.

II. VALUE OF *XENOPUS* AND NEED FOR RECOMMENDATIONS

Xenopus laevis is a unique resource for two critical vertebrate biological areas: early embryonic development and cell biology. In the former, ***Xenopus laevis*** has led the way in identifying the mechanisms of early fate decisions, patterning of the basic body plan, and organogenesis. Contributions in cell biology and biochemistry include seminal work on chromosome replication, chromatin and nuclear assembly, cell cycle components, cytoskeletal elements, and signaling pathways. In fact, *Xenopus* has become a major vertebrate model for the cellular and developmental biology research that is supported by most of the Institutes of the NIH (Appendix III). Information amassed from these studies provides a strong underpinning for future work, and, although ***Xenopus laevis*** is superb for characterizing the activities of particular genes, only a tiny fraction have as yet been assayed. A major goal now is to examine the expressed genome in the context of the biological phenomena mentioned above using genomic technology, such as ESTs and full-length cDNA libraries. This information will provide an extraordinary resource for a clearer evaluation of the cellular and developmental processes for which *Xenopus* has been so useful, for example by permitting the production of microarrays to be used for systematically evaluating gene expression.

Xenopus' unique abilities to elucidate the cellular and molecular aspects of important cell and molecular processes render it a valuable model for genomic and genetic analyses. Thus, the community's highest priority is to produce the resources required to initiate genomics in Xenopus. Specifically, this report recommends the production of *Xenopus* Expressed Tag Sequences (ESTs), full-length *Xenopus* cDNA libraries, and *Xenopus* microarrays. These resources will enable examination of the expressed genome in the context of the biological phenomena mentioned above. The availability of ***Xenopus laevis*** ESTs, cDNAs, and microarrays will also benefit research in ***Xenopus tropicalis***, the newly available genetic *Xenopus* species. The sequence data generated by these projects will be available via the internet and will help the broader research community to determine the identity of genes discovered in their systems.

In recent years, *Xenopus tropicalis* has emerged as a complementary system in which to combine genetic approaches with the established strengths of the ***Xenopus laevis*** system. New strategies will be feasible when genetic variants are examined in an embryological context, e.g. by making genetic chimeras, and generation of stable transgenic reporter lines in the short-generation ***Xenopus tropicalis*** will increase the feasibility of many embryological assays. Since the degree of sequence similarity and functional interchangeability is high, ***Xenopus tropicalis*** studies will also benefit from the ***Xenopus laevis*** ESTs and cDNAs. This new system will permit evaluation of gene interactions and hierarchies that are essential for understanding the complex developmental decisions and the processes like morphogenesis.

Xenopus' unique combination of features has enabled it to become one of the most important models for the study of early embryonic development and cell biology, despite the absence of those critical community-wide resources that have maximized the potential of other model systems. The *Xenopus* research community now recommends that the

NIH support the production of critical community-wide resources so that *Xenopus* can realize its enormous potential as a model for embryonic development, organogenesis and cell biological processes. A lower vertebrate model that combines embryological, cell-biological, genetic and genomic approaches will create an arsenal of methodologies in one biological system that will permit exciting progress on many previously intractable complex biological questions.

III. TABLE OF RECOMMENDATIONS

Here is presented the recommendations of the *Xenopus* community in a tabulated format, the detailed version of these recommendations are discussed in section IV.

In order to utilize the advantages of both *Xenopus laevis* and *tropicalis*, we the *Xenopus* community have set up three categories of recommendations. The first category is the need for **Genomic Tools** (1 - 3), the second is the **Development of the *Xenopus tropicalis* System** (I - V), and third the need for **Resource Centers** (A- C).

The first recommendation in each category represents the highest priority for funding. The community, because of immediate need and timing, unanimously agrees that the recommendations in these three categories be pursued at the same time. By prioritizing the EST projects, for example, we will be able to come up with a uniset *Xenopus laevis* and *tropicalis* DNA micro-array very quickly, which can then be used by the entire community. Unlike the situation with Drosophila, mouse and human, there is no commercial source to obtain these micro-arrays and these types of support are the only resources currently available. The genetic studies of *Xenopus tropicalis* need to begin now so that gene mapping can be initiated soon. Centralized facilities as well as shared databases will increase the coherence of the *Xenopus* community and will allow global communication among researchers. This will lead to a coordinated effort and also a better sense of a community as present in other focus research groups such as the zebrafish community or the mouse community.

There is a sense of urgency in the community for these powerful genetic tools to be available and there are several reasons for this. First, as highlighted above, the addition of genomic tools to the *Xenopus* will endow this model system with the best attributes of both experimental as well as genetic systems. Second, centralizing these efforts will, in the long run, allow synergy among different investigators and will eliminate funding for overlapping efforts in different laboratories. **A**s such, it will increase the productivity of both NIH and the *Xenopus* community. Third, recent breakthroughs in the *Xenopus* system, namely the development of a very efficient transgenesis system and development of the genetically-tractable *Xenopus tropicalis*, make this an especially opportune time to develop these new tools. Finally, organizing the *Xenopus* community by accommodating these needs will inevitably benefit researchers in other fields by giving them access to databases and other information that will be pertinent to their work.

Genomic tools for *Xenopus* laevis	Development of the *X. tropicalis* System	Resource Centers
1) Produce ESTs From existing cDNA libraries and from new libraries prepared specifically for this purpose. Use existing libraries as much a possible and make new ones to fill in gaps. stages of *laevis* development: Ovary and egg, Stages 10-11/12, 15-20, 28-36, 45 XTC cell line Stages of *tropicalis* development egg, 10-12, 15-20. Adult laevis organs Heart, male liver, brain, pancreas, kidney, fat body. Timing: 6 to 8 years Cost: $11.34/clone and 100,000 attempts/year	**I. *X. tropicalis* Genetics** Provide direct evidence for the diploidy and initiate genetic studies, in order of priority: a) Insertional Mutagenesis b) Gynogenesis c) Chemical Mutagenesis d) Preparation of Deletions Recommend 6 R01-sized projects.	**A. Database** • Gene expression • Fate maps • cDNA Libraries • *X. tropicalis* Genetics • News and technical innovations • Links to Microarray Sites • Links to EST database Cost to set up: $1 million for the first two years. Cost to Maintain: $0.5 million per year.
2) Produce and sequence full length cDNA Libraries a) *Xenopus laevis* ovary, egg and embryonic stages; b) *Xenopus laevis* organs; c) *Xenopus laevis* late stages; d) *Xenopus laevis* early stages Cost and time table: $15 million over three years.	**II.** ***Xenopus tropicalis*** **Gene Mapping** a) Large insert genomic DNA library,10X coverage b) Evaluation of genome organization c) Radiation hybrid panels d) Preliminary mapping with microsatellite or other polymorphic markers $200K for PAC/BAC libraries, and $200K per year for screening libraries. Recommend $100K for two years for genome studies and $250K for three years for RH panels and mapping.	**B. Animal Stock Center** Maintain and distribute about 200 transgenic and mutant lines of *Xenopus laevis* & *Xenopus tropicalis*. Include a training component. $250K for first year and $500K per year for subsequent years
1) Microarrays Recommended establishing 3 *Xenopus* Microarray facilities These facilities should also distribute clones Cost: about$500k/year each (not including clone storage and distribution). Provide supplements to existing grants for Array Readers ($50-70K). Duration: until competitive continuation	**III. Transition Funding** To enable *Xenopus laevis* labs to begin to use *X. tropicalis* via supplements to existing *Xenopus laevis* labs– about $20K each **IV. Sequencing the Genome of *X. tropicalis*** Preliminary discussion and planning for a genome-wide sequencing project is recommended.	**C. Training Center** 1) Continue and enlarge existing CSH lab *Xenopus* Course 2) Establish new training facilities that emphasize techniques such as Transgenesis

IV. USES AND POTENTIAL OF *XENOPUS*

The frog *Xenopus laevis* is a unique animal model system which has strongly contributed, and continues to contribute to many different disciplines in basic biological research. Among the strongest influences are the critical roles that this model system has played in both early vertebrate embryonic development and in cell biology.

Despite tremendous resources put into the zebrafish and the mouse systems, *Xenopus* continues to be the leading resource for understanding the early phases of vertebrate development. In fact most of the knowledge currently accumulated in vertebrate embryology is derived from studies of amphibian embryos with *Xenopus* as the leading model. Large numbers of embryos (up to a few thousand) can easily be obtained in the laboratory on a daily basis using two or three females, so the source of biological material is seldom limiting. The large size of embryos coupled to external development make the embryo accessible to study development from the time of fertilization. The size of the embryos makes them easily amenable to micro-surgery and micro-injections. In *Xenopus* embryos yolk is partitioned during development so that all cells have an autonomous supply of nutrients; this property makes the embryos ideal for experimental embryological approaches. For example, explants can be derived from normal or manipulated embryos and can be cultured in simple media without extrinsic influences.

In the 1960s and 1970s, it was demonstrated that experiments done on other amphibians are reproducible in the frog *Xenopus* and with the emergence of the first fate maps, *Xenopus* embryology was poised for the application of new molecular methods. Molecular approaches have been strongly influenced by the property that explants of embryos can be made with predictable fates and potencies. For example the “animal cap” assay exploits the reproducible restriction of animal cap tissue to ectodermal fates, but allows responses to extrinsic signals to be measured and interpreted in light of the fate map.

The fate map has also influenced experiments in the whole embryo, where specific grafts or injected molecules can be placed in predictable locations, and the effects on development monitored by any one of a variety of lineage tracing methods. In the case of lineage tracing, the embryo does not undergo net growth, so the tracers are not diluted extensively over time. Availability of good clearing methods provides a detailed whole mount image of fixed specimens. Development of whole-mount in situ and immunohistochemistry has complemented, and largely superseded, classical histology. There are now excellent gene expression maps that can be correlated with fate and specification maps. The development of RT-PCR, and molecular markers as diagnostic tools for cell types, has endowed the *Xenopus* system with further powerful molecular approaches. The more recent application of the DNA micro-array prototype promises an even higher level of resolution in the understanding of early embryological events. *Xenopus* is an ideal organism for micro-array analysis because of the ease of dissection of identified regions with known potencies. The combination of these approaches has provided the backdrop for successful analysis of the molecular basis of embryonic induction, axis formation, cell fate determination, competence, migration, morphogenesis,

thus establishing many of the currently accepted principles and paradigms for early vertebrate development.

The *Xenopus* embryo has also been extensively used to dissect the molecular components of signaling pathways. The molecular resolution of the main signaling pathways such as those of the TGFβs, Wnts, hedgehogs, and receptor tyrosine kinases have all used the *Xenopus* embryo to validate the biochemical findings mostly performed in mammalian cell culture systems. The power of reverse genetics using gain of function experiments with candidate genes has been instrumental in the resolution of these pathways. In addition, new components have been identified using expression cloning approaches. For example more than any other organism *Xenopus* has been instrumental in elucidating the TGFβ pathway. The specificity of the TGFβ ligands, receptors, Smads and transcriptional effectors of these pathways were most clearly resolved using Xenopus.

All of these advantages and experiments has led to a situation where much of the hypothesis driven work on induction in higher vertebrates such as the mouse relies on work done in frogs. The application of dominant negative approaches for loss of function studies during embryogenesis were first performed, optimized and given validity using *Xenopus* embryos. In addition to dominant negative approaches, maternal mRNA ablation approaches developed in *Xenopus* provide another powerful approach to address the requirement for specific molecules or pathways in development. Robust antisense approaches exploiting new chemistries of oligonucleotide synthesis (such as the morpholinos) will add to the repertoire of knock out experiments. Finally, *Xenopus* has also strongly contributed to promoter analysis and the study of gene regulation. The first eukaryotic genes to be isolated were from *Xenopus* (40S pre-rRNA and 5S RNA), which permitted the first eukaryotic transcription factor to be purified and molecularly cloned. TFIIIA was isolated using *Xenopus* oocytes and its functioning analyzed in vitro in extracts of oocytes.

The *Xenopus* oocyte has also served as a paradigm for the analysis of localized RNAs, and in fact was one of the first cell types in which subcellularly localized RNA were identified. Among the first localized mRNA was Vg1 a member of the TGFb superfamily of growth factors. Since that discovery over twenty localized RNAs have been identified in the oocyte (examples include: VegT, an1, an2, and Xdazl) and the mechanisms of subcellular RNA localization has progressed very rapidly. Similar research has been virtually impossible to pursue in the significantly smaller oocytes of other vertebrates.

With the recent success in transgenesis, characterization of regulatory elements in vivo in a fast developing system such as the frog promises to complement, and perhaps replace, these kinds of approaches in the mouse because it is so easy, inexpensive and efficient. In addition transgenesis will allow later embryological events, such as organogenesis to be amenable to molecular analysis in the frog and molecular manipulation by targeted gene expression will be available as more promoters are characterized. The empirical observation of lack of position effects suggest that analysis of the P1 generation will be effective without the necessity to breed transgenic lines. It is also important to remember that the first cloned animal was the frog Xenopus. Following

on Briggs work, Gurdon using nuclear transplantation techniques successfully reported the first cloned animal and set the stage for the cloning of mammals.

In addition to its leading role in vertebrate embryological studies, *Xenopus laevis* has contributed significantly to research areas in cell biology and biochemistry. *Xenopus* oocyte and egg extracts have been the starting material of choice for study of many complex cellular processes. The oocytes and eggs, also termed “the spherical test tube" by Brenner, provided a coupled transcription translation system for cloned animal sequences in plasmid DNAs. Immediately following the pioneering work of Lingrel et al using the reticulocyte lysates for in vitro translation of mRNAs, the first in vivo translation of exogenous animal mRNA was performed in *Xenopus* oocytes by injection of globin RNA. Also the first expression cloning (of interferon) used *Xenopus* oocytes to express mRNAs. Proteins with signal sequences are secreted into the medium where they can be assayed. The size of the oocyte means that useful biochemical amounts of protein can be made. Radiolabelling is facile with low background since injection can subvert most of the translational machinery. The ability to analyze the physiology of the oocyte, for example by electrophysiological recording, has allowed expression cloning and analysis of ion channels. Similarly oocyte injection has been used for the isolation and study of signaling molecules. The *Xenopus* oocyte remains currently an ideal system, and often the first step for expression of recombinant proteins. Seminal work on transcription, translation, chromosome replication, chromatin and nuclear assembly, cell cycle components, cytoskeletal elements, nuclear import and export, and signaling pathways have been performed using the *Xenopus* eggs and oocytes and cell-free systems derived from them. For example the first assays for animal promoters were performed in *Xenopus* oocytes and this was done even before 5S genes were molecularly cloned. The first faithful in vitro systems for transcription were developed exploiting the enormous size of oocyte nuclei. Much of the biochemical work on the cell cycle emerged from studies on oocyte maturation and the ability to reconstitute the entire mitotic cell cycle in vitro. *Xenopus* has been the source for much of the biochemistry of chromosomal DNA replication, chromosome and chromatin structure, microtubule and actin function in the cellular environment, nuclear transport, nuclear assembly, and RNA splicing.

Oocyte and egg extracts lend themselves to depletion with antibodies. Thus loss of function approaches with power similar to genetic loss of function, can be carried out in extracts. Such extracts can be biochemically complemented by addition of recombinant protein to establish and further analyze the contribution of proteins to a variety of processes.

While oocyte and egg extracts are an ideal source for purification of proteins, progress is hampered by the difficulty of going from fragments of protein sequence to cloned DNA. This alone justifies a large scale EST project. The benefit of the EST project to embryological studies will be an important and crucial bonus.

The information amassed from these studies provides a strong underpinning for future work, and, although *Xenopus laevis* is superb for characterizing the activities of particular genes, only a tiny fraction have as yet been assayed. A major goal now is to examine the

expressed genome in the context of the biological phenomena mentioned above using genomic technology, such as ESTs and full-length cDNA libraries.

In very recent years, *Xenopus tropicalis* is emerging as a complementary system in which to combine genetic approaches with the established strengths of the *Xenopus laevis* system. New strategies will be feasible when genetic variants are examined in an embryological context, e.g. by making genetic chimeras, and generation of stable transgenic reporter lines in the short-generation *Xenopus tropicalis* will increase the feasibility of many embryological assays. Since the degree of sequence similarity and functional interchangeability is high, *Xenopus tropicalis* studies will also benefit from the *Xenopus laevis* ESTs and cDNAs.

It is also interesting to note that studies in *Xenopus* will benefit the mission of each of the NIH Institutes. For example the study of the cell cycle is relevant to the goals of NIGMS; study of signaling pathways is directly in line with the goals of NCI; studies on the formation of the nervous system and brain relevant to NINDS NEI and NIMH and studies of mesoderm fates leading to the formation of heart and blood are relevant to NHLBI.

Finally, several good books and reviews are available which highlight the advantages of the *Xenopus* system in biological and biochemical studies. A few are listed below:

Sive, H.L. Grainger, R.M. and Harland, R.M. (1999) Early development of *Xenopus* laevis: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

Methods in Cell Biology, 36. *Xenopus* laevis: Practical uses in Cell and Molecular Studies. Edited by B. Kay and H. P. Peng. 1991.

The Biology of Xenopus. Edited by R.C. Tinsley and H.R. Kobel. 1992.

Normal table of *Xenopus laevis* (Daudin). A systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis. Nieuwkoop, P.D. and Faber, J. Amsterdam, North-Holland Pub. Co., 1956 reprinted by Garland Press, 1994.

Xenopus' unique combination of features has enabled it to become one of the most important models for the study of early embryonic development and cell biology, despite the absence of those critical community-wide resources that have maximized the potential of other model systems. The *Xenopus* research community now recommends that the NIH support the production of critical community-wide resources so that *Xenopus* can realize its enormous potential as a model for embryonic development, organogenesis, toxicology, neurophysiology, evolutionary biology, and cell biological processes.

V. DETAILS OF RECOMMENDATIONS

This section provides a more detailed version of the community's recommendations.

A. *Xenopus* EST project

The sequencing of expressed sequence tags (ESTs) from *Xenopus laevis* has lagged behind efforts on many other common experimental organisms and man, partly because of the pseudotetraploid nature of the *Xenopus* genome. Nonetheless, large collections of *Xenopus* ESTs would be useful in gene discovery, oligonucleotide-based knockout studies, gene chip analyses of normal and perturbed development, mapping studies in the related diploid frog X. *tropicalis*, and for other reasons. Several libraries are currently being sequenced to generate *Xenopus* ESTs. In the largest such effort to date, the NIEHS intramural group successfully sequenced 13,879 ESTs out of 16,607 attempts (83.6% success rate) from a normalized library of cDNAs from unfertilized *Xenopus* eggs. These mostly 5' ESTs, averaging 508 bp in length, have been deposited in GenBank. Using a fragment assembly program, these ESTs were assembled into 8,985 "contigs" comprised of up to 11 ESTs each. When these contigs were used to search publicly available databases, 46.2 % bore no relationship to protein or DNA sequences in the database at the significance level of 1e-06. Examination of a sample of 100 of the assembled contigs revealed that most (~ 87 %) were comprised of two apparent allelic variants. Expression profiles of 16 of the most prominent contigs showed that 12 exhibited some degree of zygotic expression. These findings have implications for sequence-specific applications for *Xenopus* ESTs, particularly the use of allele-specific oligonucleotides for knockout studies, differential hybridization techniques such as gene chip analysis, and the establishment of accurate nomenclature and databases for this species.

Other EST collections are being established from other developmental stages, and sequences are being generated by the Washington University center as well as European centers and are being deposited in GenBank. High quality, normalized libraries from various developmental stages are urgently needed for this effort.

Although the group assigned parallel studies in X. tropical a lower priority for this effort, there was strong agreement that this project should be supported for both *Xenopus laevis* and *tropicalis*. ESTs from both species may be useful in genetic mapping as well as cross-species hybridization-based assays.

The community strongly recommends that the sequences and other information generated by the EST project, as well as the clones themselves, be freely available. They also strongly supported the concept of linking the EST information with other available information, e.g., on BLAST searches, through the development of centralized databases with links to the various relevant sites.

The sub-priorities for the EST projects are as follows:

1- First priority: Developmental stages and cell line

1-1- EST projects from different stages of *laevis* development:
- Ovary and egg (maternal genes)
- Stages 10-11/12 (early zygotic and gastrula genes)
- Stages 15-20 (early to late neurula)
- Stages 28-36 (tailbud and hatching genes)
- Stages 45 (organogenesis and neuronal ionic channels)
- XTC cell line (cell cycle components)

1-2- EST project for *tropicalis*
- Stages 28-36 (tailbud to hatching)

2- Second priority: *tropicalis* developmental stages:
- egg, (maternal genes)
- 10-12, (early zygotic and gastrula)
- 15-20, (neurula)

3- Third priority: Adult organs from *laevis*:
- Heart, male liver, brain, pancreas, kidney, fat body.

Mechanism:

The community agreed that the EST sequencing projects should be done as part of the ongoing library project at Washington University in St. Louis (see appendix V for protocols for submissions), with arraying at Lawrence Livermore National Labs. Other efforts using other sequencing facilities, e.g. the NIH intramural sequencing center, will continue in parallel. A cDNA subcommittee (under the leadership of Marc Kirschner, see below) will make sure that different laboratories send their libraries into pilot project for quality validation and, if indicated, sequencing. Generation of tissue-specific libraries in collaboration with CGAP will be organized by Igor Dawid. Normalization of libraries will be performed on new libraries, and, if possible, subtraction will be performed on high quality libraries to remove sequenced clones.

Contacts

The EST contacts will be Perry Blackshear and Richard Harland, and the full length library contact will be Marc Kirschner. Current cDNA libraries should be sent to Steve Johnson and Sandra Clifton for sequencing; they will coordinate the generation of arrays by LLNL for sequencing. Sandra will prepare instructions on how to do this. The ESTs will be then sent to LLNL for the preparation of nylon filters (contact to be named) and the Brivanlou lab for glass micro-array. Both centers will make the arrays immediately available to the community without any intellectual propriety restrictions. Other arrays may be generated for specialized purposes, e.g., the NIEHS developmental toxicology arrays and filters.

B. *Xenopus* full length libraries

The power of expression cloning in *Xenopus* has provided the rationale to engage in a collective effort to generate full length cDNA libraries from eight developmental stages, as well as libraries derived from different regions of the *Xenopus laevis* embryo. As mentioned above, one of the strengths of the *Xenopus* system is the availability of large numbers of embryos which are synchronized in their development, from the first cell cycle of life, by whole batch in vitro fertilization. The size of the early embryos (1.2 mm in diameter from the fertilized egg until mid neurulation) also allows large scale microsurgery to isolate (or explant) different regions, germ layers, and organs to generate region specific expression libraries. Once made these libraries will be available to all and will be fully sequenced. It was agreed that for *tropicalis*, there is currently no need for full length clones, however, this will be reevaluated in the future as the needs of the *tropicalis* community becomes better defined.

Sub-priorities for the full length cDNA libraries for *Xenopus laevis* are as follows:

1- Expression libraries from developmental stages
Maternal: Oocyte and eggs
Gastrula: Three libraries representing the onset (stage 10), mid-gastrula (stage 11), and a late gastrula (stage 12).
Neural plate (stage 14)
Neural groove (stage 18)
Neural tube (stage 20)
Late neurula (stage 25)
Tailbud (stage 28)
Swimming tadpole (stage 45)

1- Region specific expression libraries:
Gastrula (stage 10.25): Ventral marginal zone (VMZ) and Dorsal marginal zone (DMZ)
Early neurula (stage14): Neural plate specific libraries
Tailbuds: Head libraries, endoderm libraries, tail-bud libraries

Mechanism:
Marc Kirschner, has taken responsibility for this. He will follow the CGAP efforts and will adopt measures they have used with human and mouse, since it is part of an existing system. Marc has also formed a subcommittee to assist him with both this effort and independent EST projects he will set up to complement the effort of Washington University.

Contact
Marc Kirschner (Harvard Medical School) and the Kirschner subcommittee.

C. *Xenopus* Micro-arrays

The community suggested the formation of three centers to print and distribute *Xenopus* DNA micro-arrays. One center will be on the East coast, one in the mid-west and one in the west coast. The rationale behind several centers instead of one is several fold. First, since this technology requires biologists, bio-informaticians and engineers collectively working toward the molecular biology, programming/clustering and that having three centers is advantageous because it will have different combinations of the groups working with different strengths. Second, in terms of security is best not to centralize this effort in a single place. In case of accidents (fire, flood or other nightmares) other centers will provide a additional security net. A prototype *Xenopus* chip is currently available from the Brivanlou lab, free of charge for distribution to the community. Data analysis software and availability on the web is already established and functional (see http://arrays.rockefeller.edu/xenopus/.

The input for the chips will be provided by the EST projects following the priorities described above. This will complement the Brivanlou lab effort currently printing a 12,000 genes *Xenopus laevis* DNA array. Dr. Perry Blackshear from NIEHS has also agreed to provide about 4000 maternal *Xenopus* clones currently sequenced and available in his lab to the chip center ASAP. The ultimate goal is to have all *Xenopus laevis* cDNAs represented in one "master chip" as well as DNA arrays from specific developmental stages, adult organs and cell culture (same priority as above). Pilot experiments will be performed between the Amaya, Grainger and Brivanlou lab to assess the feasibility of using *laevis* chips in *tropicalis* experiments. This is very likely to be the outcome, but if this turns out not to be the case then we will print *tropicalis* chips again following the priorities described for the ESTs.

The community also recommends that the array facilities be used as a centralized stock center for distribution of *Xenopus* clones to the community.

Mechanism:
The microarray facilities will take input of genes from four different sources listed below
1- Sandra Clifton will send ESTs
2- NIEHS will contribute additional clones
3- Brivanlou lab efforts with Robotics at The Rockefeller
4- Individual laboratories will contribute sets of genes
It was suggested that several RFAs be established as soon as possible to accommodate the financial need of the Centers and Ali Hemmati Brivanlou has accepted responsibility for the *Xenopus laevis* micro-arrays and their distribution to the community with minimum cost (presently 1 cent/spot) with no legal strings attached. Because of the urgency to have this technology available ASAP it was suggested that funds from the NIH be available to support this ongoing effort until the RFA is advertised. Funds will be required also to establish within each center: cataloging, quality control and distribution of *Xenopus* clones to the entire community.
Contacts
Ali Brivanlou at The Rockefeller University.

D. *Xenopus tropicalis*

Establishing the *Xenopus tropicalis* system

The prospect of using *Xenopus tropicalis* to complement studies of *Xenopus laevis* offers extraordinary possibilities for the *Xenopus* system as a whole. Indeed development of genetics will allow a quantum leap forward in the potential of the system. As a potential genetic system *X. tropicalis* presents very important advantages. When compared to *Xenopus laevis* it develops to sexual maturity in 1/3 the time, has 1/2 the genome size (and is believed to be diploid), and requires 1/5 the housing space. It nonetheless shares the embryological advantages of *Xenopus laevis* and gene sequences are sufficiently similar that probes cross-react between the two species. The facility with which one can make haploid and gynogenetic diploid embryos greatly reduces the time (and space) needed to make inbred lines and to perform genetic screens when compared, for example, to the resources needed for mouse genetics. As a vertebrate genetic model, this system offers significant advantages over the mouse system, which costs far more to house and maintain, and has raised more controversy as a model because it is a mammal. The evolutionary conservation of developmental processes revealed in recent years among all vertebrates gives us confidence that information revealed from studies of lower vertebrates will, however, at least in large part, apply to mammalian systems.

1. The utility of a genetic approach in frogs over mice is highlighted in other ways as well. Simply having the large numbers of eggs in a typical *Xenopus* spawning is also of great value. Of course ability to manipulate the frog embryo is significantly better, an advantage that is underscored when one considers the possibility of making genetic chimeras. In frogs it should be possible to make chimeras of mutant and wildtype tissue to be able to very readily study the effects of gene manipulation on particular developmental events by obviating early, more general lethal effects that have diminished the ability to interpret many mouse knockouts.

The urgent need for developing a genetic component to *Xenopus* research now emerges when one considers the implications of recent breakthroughs arising from this system. Very novel findings, for example, regarding signal transduction (e.g. concerning the Wnt and BMP pathways) have resulted from analysis in *X. laevis*. However, as we try to integrate the many components of these pathways the ability of reagents traditionally used in this system (e.g. antisense and dominant negatives) are very limited when compared to the prospect of having mutations in these genes for epistasis studies. The same principle applies to tissue and organ formation, where, for example, in the eye, brain and heart, many regulatory genes have been identified but the subtleties of gene interaction remain poorly understood because there is no formal genetics. To take full advantage of the *Xenopus* system the genetic dimension must be developed.

Some may ask whether the development of a new lower vertebrate genetics system, in light of recent successes with zebrafish, is warranted. There are a number of points that provide strong support for the new venture. First, the *Xenopus* system has an enormous wealth of information about a multitude of developmental processes and gene activities

that act as an unparalleled resource to complement genetic studies. Second, amphibian embryos are generally much more amenable to embryological manipulations than are fish embryos, particularly at gastrula and neurula stages, an issue which becomes important for example, in making genetic chimeras. Third, at least at present, transgenic technology is more advanced in the frog system. Finally, the zebrafish genome is now believed to have undergone a duplication event, so that the genome is tetraploid relative to mammals, diminishing at least to some extent its potential as a genetic system. There is overwhelming evidence that *X. tropicalis* is a diploid species and efforts in progress in many laboratories will establish this fact unequivocally.

The community recommends as its highest priority for *X. tropicalis* the examination of its potential for genetic manipulation by supporting 6 R01 projects that test the feasibility and utility of the system for isolating and characterizing mutations, and identifying the gene products affected by these mutations. The strategies and priorities listed below are the ones that are clear at the time of writing this proposal, but others may emerge in the near future that should be considered. For example, a strategy to perform targeted gene inactivation has not been developed for *Xenopus*, but if the feasibility of such a strategy were suggested, it would become a very high priority.

Within the genetics category, the community believes that the highest priority at present should be given to insertional mutagenesis strategies because these procedures, though probably less efficient than classical chemical mutagenesis, allow immediate identification of the mutated gene. The feasibility of an efficient gene trap procedure suggests that this will lead to the identification of many mutations in genes that can be readily identified. Preliminary work using transposable elements suggests that this may also be a useful strategy.

The second priority for mutagenesis is to use the method known as gynogenesis. This procedure allows one to reveal recessive mutations in offspring of animals carrying mutant alleles without having to perform the traditional crosses, and taking the time, associated with conventional brother-sister matings of offspring from carriers. The technique entails making haploid embryos by fertilizing eggs with U.V. irradiated, and thereby inactivated, sperm. Shortly after fertilization these embryos are then subjected to several thousand pounds of pressure for a few minutes, a procedure which leads to the fusion of the second polar body with the embryo. The reintroduced polar body nucleus results in an embryo that is "diploidized" but in effect largely haploid because in many cases large segments of the genome from the second polar body and the egg nucleus are identical. The frequency of haploidy at a given locus in a population of pressure-treated embryos depends on the degree of meiotic crossing over that has occurred during oogenesis. However there are always a significant fraction of embryos (between 10% and 50%) that are haploid at a particular locus and so recessive phenotypes are readily scorable. This method has been used in both *X. laevis* and *X. tropicalis* to identify a number of mutant phenotypes. The procedure has a relatively high priority because it simplifies the identification of mutant phenotypes and can be successfully performed immediately on wild caught animals, which bear a large number of developmental mutations.

Chemical mutagenesis was given a somewhat lower priority than insertional mutagenesis because mutated genes are not likely to be readily cloned. But, because this may also be a very rapid way to identify mutants, it remains an important strategy. The highly successful utilization of ENU mutants by the zebrafish community is a strong testament to the value of this approach for developmental biologists. Recently generated transgenic lines bearing reporter genes that express fluorescent proteins in tissue-specific patterns provides far more subtle, and focused, ways of screening for such mutants than has been possible in the past. The gynogenesis procedure can be used in conjunction with chemical mutagenesis to uncover potential mutations in a rapid way.

While finding mutations in wild-caught animals by gynogenesis, and generating mutants by chemical mutagenesis will result in many mutations in which target genes cannot be readily identified, expression cloning procedures that work well in the *Xenopus* system may allow subsequent cloning of genes affected in these mutations. For example, these methods have been used to identify novel gene activities, such as noggin, that can rescue early axial defects caused by U.V. treatment.

Generation of deletion mutants, while not a high priority initially, will become more important as mutants are generated by other techniques. Large deletions are very useful in helping to map genes and experience with the zebrafish system suggests that they should be fairly easy to generate.

2. The second, and complementary, priority for *X. tropicalis* is to develop strategies for genomic mapping that will become extremely important as mutations are identified. The first goal among this set of recommendations is to prepare large insert DNA libraries (PAC and BAC libraries). The high efficiency transgenesis procedure recently developed for *Xenopus* creates the need for large insert libraries for cloning and analysis of genomic sequences, specifically for promoter analysis of the *X. tropicalis*, diploid genome. These libraries will also be essential for clarifying the organization of groups of genes (e.g. the *Hox* clusters) and will be extremely important to use in conjunction with mapping (and cloning) of mutations.

As part of the second recommendation in this section it will be very useful to characterize further the organization of the *X. tropicalis* genome with regard to possible duplication of genes. This will be especially important background information for studies using radiation hybrid panels for gene mapping. While all existing evidence defines *X. tropicalis* as a diploid organism, not a large number of genes have been examined to establish whether previously undetected chromosome duplications may have occurred in these amphibians. While there is no evidence for the kind of duplications that have occurred in the fish genome, examination of the inheritance patterns of genes from a larger number of gene families (20 or more) will establish whether they behave as diploid genes (the expected outcome) and thereby test more broadly the ploidy of the *X. tropicalis* genome. In *X. laevis* such tests reveal, as expected, that most genes behave as tetraploid sets. In the long term, but beyond the scope of this set of recommendations, the possibility of investigating more subtle rearrangements or duplications of genes in the genome will become possible as more genes are identified and mapped in *X. tropicalis*.

Generation of radiation hybrid panels is viewed as the most useful way to generate the first genetic maps for *X. tropicalis*. Well-established technology should make this a straightforward undertaking. In conjunction with this effort, mapping of polymorphic markers, probably from microsatellite sequences, and ESTs, generated as a result of the first set of recommendations in this report, should be initiated.

The third recommendation for *X. tropicalis* is to provide transitional funding for laboratories to enable them to rapidly initiate work on this system. The costs to set up animal facilities for *X. tropicalis* are modest by comparison to many other animal systems. However, there are differences from *X. laevis* (e.g. in water temperature) and expenses associated with acquiring animal stocks that will make it difficult for many investigators to start work on this system quickly without a modest infusion of funds for these purposes.

A final recommendation is that discussion and planning regarding a more extensive genomic sequencing project for *X. tropicalis* be initiated. At this early stage it is premature to request funds for a genome sequencing project, though when mapping of genes and mutations are underway, the utility of such a venture becomes quickly obvious, as in other organisms in which the genome has been sequenced. The value for gene identification, mapping of regulatory regions, elaboration of the organization of gene families, and studies of gene synteny, as a few examples, is extraordinary. The great utility of the *Xenopus* system for studies in cell biology and development, and now for its genetic potential, provide a strong underpinning for considering a project of this magnitude. From another perspective, *Xenopus* is an important organism to sequence because it is a tetrapod sufficiently divergent from mammals that its sequence and genome organization would provide key information about genome evolution. *Xenopus* is also likely to be similar enough to mammals that it would also be extremely useful as a genomic model.

Mechanism
It is recommended that the examination of the genetic potential of the *X. tropicalis* system would be best investigated by six R01 grants to laboratories interested in developing this new area of research. In the category of genomic resources, it is recommended that both large insert DNA libraries, each with 10X coverage, be made with two vectors (e.g. a BAC and PAC library) to insure that all genomic sequences are cloned. The estimated cost for construction of the two libraries is $200,000. While it is expected that these libraries might be housed in a number of laboratories, it is very costly to screen them to isolate a particular clone, and that investigators in general will not want to house whole libraries. Therefore, as is being done in the zebrafish community, we suggest that library pools be created that can be screened at other sites to target individual clones. It is estimated that this will cost approximately $200,000 a year. Evaluation of the ploidy status of 20 or more *X. tropicalis* genes can be investigated by following the inheritance patterns of these genes through crosses using highly polymorphic strains. It is estimated that this will require $100,000 a year for a two-year period. Creation of a radiation hybrid panel and subsequent mapping of polymorphic markers and EST's is estimated to cost $250,000 a year for three years. Transition funding for laboratories setting up the *X. tropicalis* system is recommended at a level of $20,000 per laboratory.

Regarding the issue of sequencing the *Xenopus* genome, a committee should be established to define key points regarding the importance of such a venture, and if the project is to proceed, to lay out a timetable and plans for obtaining funding for the project. Rob Grainger, Richard Harland and Ali Hemmati-Brivanlou, working together with Steve Klein, will take responsibility for organizing this committee.

Contacts
Rob Grainger and Lyle Zimmerman (University of Virginia) have taken responsibility to serve as a contact and coordinate the efforts for *X. tropicalis*.

E. Resource Centers

Proposal for the development of *Xenopus* web resources

There are two immediate goals. Firstly to expand the availability of information on *Xenopus* development available on the existing web sites, and secondly to initiate a new centralized database that will provide researchers with access to powerful relational database tools. In the first instance researchers will be provided with information, in the second they will be provided with tools to analyze this information.

An international group of *Xenopus* researchers has volunteered to help in the expansion and upgrading of the existing XMMR site. New managers include:

Enrique Amaya:	transgenics, genetics
Bruce Blumberg:	monthly magazine
Leon Browder:	gene traps, genetics
Mike Danilchik:	movies, imaging
Sally Moody:	fate maps, early development
Roger Patient:	gene expression patterns, microarrays
Derek Stemple/Amy Sater:	RH panels
Nato Ueno:	genomics
Lyle Zimmerman:	genetics, mutants

Short term improvements of speed will also be realized via the new linux server that Peter Vize is in the process of setting up. Issues of data ownership will be explored with journal publishers. A monthly magazine will be one of the new features.

In terms of a centralized database once again an international group of consultants is contributing to planning how this should be done. We are discussing design options with: NCBI, Flybase, Wormbase, ZFIN. Other consultants include: N. Pollet, F. Verbeek,

This new database will be an extremely powerful tool. A 3D atlas is under development (Verbeek/Destree), as are tools for 3D representation of gene expression patterns (Verbeek/Vize), and even developmental modeling (Phillips). Tools are already available that supply closest sequence matches between systems, and such tools, especially when linked to 3D visualization software, would be powerful tools for researchers in other systems to explore their genes function in frogs. We will create *Xenopus* versions of such tools linked to NCBI resources, and allow people to identify the nearest frog gene, view its expression pattern, explore developmental phenotypes, literature etc.

Compared to other systems our present database support is quite primitive. Once we embark upon genetic projects such support will be even more essential in providing up to date access to maps, mutants, strains etc. A database capable of complex analysis of more than one type of data, e.g. sequence, gene map, expression pattern, microarray data etc. would be ideal. Approaches to such combined analyses are being explored. If data is entered into databases in a format that allows such cross-examination, tools capable of

performing such analyses will be much easier to create, so we will be very careful regarding future compatibility.

A new database will take years to develop, and it is important that we start as soon as possible. It is also important that we upgrade our existing resources to help distribute information via existing sites while the new system is under development.

Contacts
Peter Vize (University of Texas) and Bruce Blumberg (UC Irvine).

F. Stock Centers

1. Stock Center for Maintenance and Distribution of *Xenopus* lines.

A consensus was reached on the need for Stock Centers for the maintenance and distribution of *Xenopus* transgenic and gene trap lines. It is estimated that there are currently 200 different *Xenopus* lines in existence (about equally shared between the UK and the US). At present, these comprise about equal numbers of *laevis* and *tropicalis* lines. The total number of lines is likely to increase greatly over the next few years, effectively exhausting the resources of existing research oriented laboratories to maintain the animals.

The Stock Center in the US will need to carry duplicates of lines maintained by any (hypothetical) British or European Stock Center. This is required, first, to guarantee redundancy in case of a disaster at one center and second, to avoid shipping and customs problems with moving live animals internationally.

The proposed *Xenopus* stock center would serve at least two additional roles.
a). Further research is needed into the practicality of freezing sperm (i.e. cryopreservation) as a long term strategy for maintaining a large number of *Xenopus* lines. Preliminary studies have reported successful freezing of *Xenopus* sperm, but this question needs to be studied in much more detail and the relevant parameters defined.

b). Further research is required on the efficient husbandry of *Xenopus tropicalis*. Some progress has been made in optimizing the conditions for maintaining *tropicalis* and also in reducing the generation time, but much more remains to be determined. This type of research is ideally carried out in a stock center.

Estimated costs: The initial US *Xenopus* stock center should be established with capacity to maintain a total of 200-300 lines of frogs (both *tropicalis* and *laevis*). The estimated cost for the tanks and water handling equipment is $250,000. Significant additional costs will be involved if a building needs to be renovated or constructed to house the facility. Personnel costs for a manager and two assistants to care for and distribute the animals is estimated at $200,000 annually. It is estimated that an additional $250,000 will be required after approximately 3 years to upgrade the facility to handle a total of 1000 frog lines.

2. Stock Center for maintenance and distribution of libraries, clones and other reagents.

A consensus was reached on the need for a Stock Center or Centers to store and distribute *Xenopus* genomics reagents. Particularly important in this respect, is a center to maintain and distribute gridded genomic and cDNA libraries. It was agreed that the most efficient approach will be to provide additional personnel support for existing laboratories that are already working with microarray libraries or that are already maintaining clone stocks.

Estimated costs: Funds sufficient for appointment of an assistant to an existing microarray laboratory, at approximately $50,000 per annum. In addition, funds for an extra assistant at the *Xenopus* animal stock center with the specific mission to maintain and distribute *Xenopus* clones and reagents, at approximately $50,000 per annum.

Mechanism: A stock center, supported by grants from the NIH, for the frog lines and the clones will be established in the mid-west (University of Cincinnati). The cost to establish such a center is estimated to be $250,000 for the first year and $0.5 M per year to maintain.

Contact: Chris Wylie, University of Cincinnati.

G. Training courses.

At present, research oriented laboratories are sharing the responsibility for training new investigators in the techniques required for production of transgenic lines. Due to the increasing interest in generation of transgenics and the exciting possibility of large scale insertional mutagenesis screens, individual laboratories can no longer meet the demands for transgenesis training. The community agrees that courses for training scientists and students in transgenic techniques are essential.

Estimated costs: A course dedicated to teaching *Xenopus* transgenic techniques should be established, perhaps based on the successful "Cell and Developmental Biology of *Xenopus*" Course held annually at Cold Spring Harbor Laboratories. Cost for 10 students is estimated at $50,000 per year.

Appendix I

Workshop Agenda and Participant List

AGENDA

Day 1

8:00	**Coffee/Tea/Pastries/etc.**	
8:30	**Welcome**	Steve Klein
8:45	**Introductory comments** **Products of the Meeting**	Meeting Chair, Ali Hemmati-Brivanlou

SESSION I Where We Are

9:00	**Uses and Advantages of Xenopus**	Richard Harland

State of the Field; Existing Tools and Resources

9:30	**XMMR Database**	Peter Vize
9:50	**mRNA Depletion**	Janet Heasman
10:10	**Transgenics**	Enrique Amaya
10:30	**Break**	
10:50	**X. *tropicalis***	Rob Grainger
11:10	**RH Panels**	Amy Sater
11:30	***Xenopus* Arrays**	Ali Hemmati-Brivanlou
11:50	**ESTs**	Steve Johnson
12:00	***Xenopus* ESTs**	Sandra Clifton
12:10	**cDNAs via CGAP**	Igor Dawid
12:15	**cDNA Committee**	Marc Kirschner

12:30- 1:30 LUNCH

SESSION II The Potential

1:30	**Cancer Genome Anatomy Project**	David Krizman
1:45	**Zebrafish Tools**	Tyl Hewitt
2:00	**FlyBase**	Bill Gelbart
2:15	**British *Xenopus* Group**	Hugh Woodland
2:30	**Wellcome Trust Support of** **Genetic & Genomics Resources**	Ted Bianco
2:45	**NCRR Support of Resources**	Jill Carrington

SESSION III The Process

3:00	**NIH Support of Genetic & Genomic Resources**	Steve Klein
3:15	**NIH Support of Zebrafish Resources**	Tyl Hewitt
3:25	**History & Potential of *Xenopus* Process**	Steve Klein
3:30	**BREAK**	

SESSION IV Identifying, Prioritizing & Defining the Needs for Xenopus

3:50 Goal of Discussions — Steve Klein

	Topics	Discussion Leader	
4:00	**1. ESTs**	Perry Blackshear	45 min
4:45	**2. cDNA Libraries**	Marc Kirschner	45 min
5:30	**3. Arrays**	Ali Hemmati-Brivanlou	30 min
6:00	**adjourn for the day**		

Day 2

8:00	**Coffee/Tea/Pastries/etc.**	
8:30	**NICHD's Interest in Animal Models**	Duane Alexander
8:40	**The Trans-NIH Non-Mammalian Models Committee**	Marvin Cassman

Discussions continue

8:45	**4. Database**	Peter Vize	45 min
9:30	**5. X. *tropicalis***	Rob Grainger	45 min
10:15	*BREAK*		
10:30	**6. *Xenopus* Center(s)**	Paul Krieg	30 min
11:00	**7. Other Topics/ Loose Ends**	Steve Klein	30 min

11:30 Summary of Topics

15 min each x 6; each lead by Discussion Leader

1:00 Conclusions – Next Steps — Ali Hemmati-Brivanlou

1:30 Adjourn

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Appendix III

Recommendations and Participants from *Xenopus* Portion of NIH Non-Mammalian Model Meeting

Xenopus laevis is a unique resource for two critical vertebrate biological areas: early embryonic development and cell biology. In the former, *X. laevis* has led the way in establishing the mechanisms of early fate decisions, patterning of the basic body plan, and organogenesis. Contributions in cell biology and biochemistry include seminal work on chromosome replication, chromatin and nuclear assembly, cell cycle components, cytoskeletal elements, and signaling pathways. Information amassed from these studies provides a strong underpinning for future work, and, although *X. laevis* is superb for characterizing the activities of particular genes, only a tiny fraction have as yet been assayed. A major goal now is to examine the expressed genome in the context of the biological phenomena mentioned above using genomic technology, specifically ESTs and full-length cDNA libraries.

In recent years, *Xenopus tropicalis* has emerged as a complementary system in which to combine genetic approaches with the established strengths of the *X. laevis* system. New strategies will be feasible when genetic variants are examined in an embryological context, e.g. by making genetic chimeras, and generation of stable transgenic reporter lines in the short-generation X. *tropicalis* will increase the feasibility of many embryological assays. Since the degree of sequence similarity and functional interchangeability is high, X. *tropicalis* studies will also benefit from the *X. laevis* EST and cDNA cloning experiments.

In preparation for this workshop, the opinions of the *Xenopus* community were canvassed and the proposals below are based on this information together with discussions at this workshop.

1. EST Database

The highest priority, one which is ready to be undertaken immediately, is the generation of an *X. laevis* EST database that should consist of 500,000 clones (estimated to be at least half of the expressed sequence complexity). Initially this should capitalize on existing cDNA libraries and should be complemented with normalized libraries from selected stages. A small EST database in X. *tropicalis* (50,000 clones) will provide a beginning for genomic research in this organism. Estimated cost: $5,500,000 over two years.

2. Full Length cDNA Sequences

Full length sequenced, unique cDNAs from *X. laevis* eggs and embryos are needed for functional studies (e.g. expression cloning strategies). Estimated cost for preparation, sequencing and arraying 50,000 full length cDNAs is $15,000,000 over three years.

3. Microarrays

This rapidly evolving technology promises major advances. The utility of *Xenopus* for developmental studies, e.g. explantation, induction, and overexpression assays, make array technology especially valuable in this system. Array technology will become applicable as EST/cDNA sequences come on line. Therefore, funding to produce and make available *Xenopus* chips to the community should begin one year after EST/cDNA sequencing is initiated. Estimated cost: $1,000,000 over three years.

4. Model Organism Database

There is an immediate need for expansion of the present *Xenopus* database, the *Xenopus* Molecular Marker Resource (XMMR). The expanded database should encompass the existing data, more comprehensive information concerning gene expression patterns, additional fate maps and anatomical atlases of embryonic stages, and information generated from the EST database and cDNA libraries. Sequences should be organized along the lines of databases for other organisms so that data is easily retrievable. Initial requirements include computational facilities and a skilled data manager; as the sequence database matures, a bioinformatics professional will become essential. Estimated cost: $400,000 per year.

5. PAC and BAC Libraries

The high efficiency transgenesis procedure recently developed for *Xenopus* creates the need for large insert libraries for cloning and analysis of genomic sequences, specifically for promoter analysis of the X. *tropicalis*, diploid genome. Similar libraries in *X. laevis* will enable comparison of putative control elements. Estimated cost: $1,000,000.

6. X. *tropicalis* Genomic Resources

Creation and preliminary characterization of radiation hybrid panels are required in anticipation of genetic screens and transgenic insertions. Additional support is needed for pilot genetic studies, including chemical and insertional mutagenesis. Estimated cost: $150,000 for the RHP project and $300,000 a year for pilot genetic studies.

7. Training and Stock Centers

Resource centers are urgently needed for broader dissemination of new technology and for animal stocks. In the case of new technologies this should take the form of training centers in host labs experienced in transgenesis or antisense ablation. For animal stocks, including transgenic and genetically altered lines, one stock center is initially required with the expectation that this will expand as more permanent lines are established. Estimated cost: for training centers, $100,000 per year per host lab by supplementation of existing grants; for a stock center, $500,000-$1,000,000 for construction and $100,000 per year for operating costs.

Xenopus Breakout Group
From the Non–Mammalian Models Meeting

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Appendix III

Uses of *Xenopus* for Research Supported by NIH Institutes

Studies in *Xenopus* benefit the mission of most of the NIH Institutes. For example, studies of cellular function such as signaling pathways, and cell cycle are relevant to the goals of the National Institute of General Medical Sciences and the National Cancer Institute. Studies of fertilization and patterning during the early stages of embryogenesis are important to the National Institute of Child Health and Human Development. Studies on the formation of specific organs are important to the Institute that supports research on those organs. Studies on the formation of the nervous system are relevant to the National Institute of Neurological Disorders and Stroke and to the National Institute of Mental Health; studies on the formation of the heart and the circulatory system are relevant to National Heart Lung and blood Institute; studies on the formation and patterning of the digestive organs are important to the National Institute of Digestive Disorders and Kidney Disease. Studies on the development of the eye and the ear are important to the mission of the National Eye Institute and the National Institute on Deafness and Communication Disorders; studies on the formation of the head and face are important to the National Institute of Dental and Craniofacial Research. Studies that will enhance the use of Xenopus as a model system for biomedical research, or to develop research tools and resources for the Xenopus model, are important to the mission of the National Center for Research Resources.

Appendix IV
Table of federal funding for *Xenopus* work

TOTALS BY
AWARDING UNIT

Agency	DOLLARS AWARDED	NUMBER OF PROJECTS & SUBPROJECTS
GM	$23,610,094	101
NS	$13,400,066	64
DK	$10,790,737	49
HL	$9,505,939	41
HD	$7,544,598	35
EY	$6,787,402	32
AR	$2,830,744	12
AI	$2,629,293	11
CA	$2,150,584	15
AA	$1,550,233	9
DC	$1,432,463	8
DA	$1,291,538	8
AG	$1,146,244	5
MH	$822,007	7
RR	$434,498	2
DE	$211,771	1
ES	$151,500	2
NIH TOTAL	**$86,289,711**	**402**
NSF	$2,000,000	13

This table shows the amount of money (and the number of projects) spent last year (Fiscal 1999) by each NIH Institute/center and by the NSF on projects that include Xenopus. As shown, the NIH total is about $90 million dollars (total cost = direct & indirect) on about 400 grants. The Institutes are listed in order of the amount of money they spent on these projects. However, it's important to remember that this is undoubtedly an overestimate. Firstly, not all of these projects are actually interested in Xenopus, per se. Some of them are using *Xenopus* as an expression system. Secondly, the database shows the award's entire amount even if only part of the project uses Xenopus. This overestimation may be particularly large for *Xenopus* as compared to other animals. Nevertheless, similar budget figures from 1998 indicate that of the Non-mammalian models supported by the NIH, *Xenopus* ranked number 3 [after yeast (~ $150M) and Drosophila (~$110M); the forth place goes to chicken (~ $70M)].

Appendix V

Washington University Sequencing Center Procedure For Converting Phage to Plasmid Libraries for ESTs

For libraries in lambda ZapII, this procedure allows for inserts to be excised by use of the helper phage ExAssist (though excision of the pBluescript sequence in the lambda Zap).

1. The excision protocol should be carried out as suggested by Stratagene using their ExAssist Interference-Resistance Helper Phage kit. During the excision process you will notice that the initial step is to excise the pBluescript sequence using exassist in the host strain XL1Blue resulting in the secretion of single stranded bluescript phagemid particles into the medium. At this stage the solution is incubated in a heated waterbath.

2. We have found that extending the incubation for 10 min. longer than the Stragagene protocol suggests seems to ensure little or no bacterial contamination.

3. The next step is to get the single stranded phagemids into SOLR cells, as specified by the Stratagene protocol.

4. After infection of SOLR cells, you need to ensure that when selecting for transformants on amp plates you are getting single colonies and not lawns of cells.

5. Extract plasmid DNA from the SOLR cells.
Isolate the plasmid (Qiagen maxi-prep columns were used in this procedure)

This protocol was developed by Stavros Bashiardes, PhD. If you need any help whatsoever throughout the procedure, contact him at:

Genetics Department, RM320
Washington University School of Medicine
4559 Scott Ave.
St. Louis, MO 63110, USA
stavros@genetics.wustl.edu
Tel. (314) 7471059
Fax. (314) 7472489

6. Check for plasmid diversity.

7. Send acceptable plasmid preps to:

Stephen L. Johnson, Ph. D.
Asst. Professor
Dept. Genetics, Box 8232

4566 Scott Ave
Washington University Medical School
St. Louis, MO 63110
Tel. (314) 362-0362
fax (314) 362-7855
email sjohnson@genetics.wustl.edu

Steve will send your plasmid preps to Christa Prange at LLNL (He has a Material Transfer Agreement with the LLNL). She will do the transformation into DH10B, make the 384 well arrayed plates, and give them an LLNL number. One plate will be sent to Wash U Darwin sequencing group for EST construction, and copies will be sent to the distributors in the IMAGE consortium.

Christa prefers to have the DNA. However, if you wish to do the transformation yourself, and send Christa arrayed 384 well plates of frozen glycerol stock clones, please use the phage resistant DH10B cells, GeneHogs, available from Research Genetics. The following procedure has been successful in our hands.

Optional

8. Electroporate 2 micrograms of each plasmid library into GeneHog cells.

9. Prepare a glycerol stock and store at -80 degrees C.

10. Check for plasmid diversity: Plate out transformants and pick 10-20 colonies at random. These can be analyzed by colony PCR to ensure that you are getting a distribution of inserts that are of varying sizes.