Status of Xenopus laevis and Xenopus tropicalis microarrays

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Thank you very much. I am just going to give a very brief talk. I was asked to update the status of microarray availability for the amphibian systems *Xenopus laevis* and *Xenopus tropicalis*.

As you know, *Xenopus* is relatively widely used for toxicological studies with the assay FETAX being the most well known. But *Xenopus* is also a very important model system for cell biological and early embryonic development. The recent advent of transgenesis in *Xenopus*, opens the possibility to use it to study organogenesis and morphogenesis.

A benefit of using *Xenopus* is that there is a very large knowledgebase. We know a lot about early development during the times when the animals are sensitive to treatment with various chemicals. We also have the possibility to do all sorts of functional studies: embryonic manipulations, transgenesis, gene "knockouts" using morpholino antisense oligonucleotides. The early embryos are quite accessible. *Xenopus laevis,* of course, has a defect in that it is more or less tetraploid so that prevents genetics. It also has a long generation time of more than a year.

In recent years, people have become interested in using the related species, *Xenopus tropicalis*. *Tropicalis* is very closely related to *Xenopus laevis.* The probes cross hybridize for *in situ* hybridizations and for microarray analysis. Embryonic development is identical insofar as it has been studied.

Tropicalis has a number of advantages. It is a true diploid, which means you can do forward and reverse genetics, transgenesis, and insertional mutagenesis. The National Institutes of Health in the United States has recently funded five or six grants to do mutagenesis and mapping of the resulting mutants, so that will be a very big boost to the field.

Tropicalis has a short generation time, three to four months. Since they are much smaller they take up a lot less space. Also there is a *tropicalis* genome project being started. So soon we will have, yet another resource, the entire genomic sequence.

A few disadvantages. X. tropicalis is a little bit more difficult to keep in the lab, and there are not so many labs using it. The most recent EST database update shows that *Xenopus laevis* has climbed up very high. We are actually number eight on the list, and when Prof. Ueno releases his sequences, it is going to jump up to number six.

There are several *Xenopus* EST projects, one of the first ones was at NIESH (National Institute of Environmental Health Sciences), led by Perry Blackshear. They produced a relatively large number of ESTs from an egg library. In the past year and a half, the NIH has put a fair amount of money into EST sequencing, and now the total is 147,000, more or less, and they have at least 60,000 more to go because that is how many I recently sent to them.

Here in Japan at NIBB, Prof. Ueno has more than 60,000 EST sequences, and he reports that that is somewhere in the vicinity of 25,000 individual unique sequences.

Xenopus laevis is now a UniGene organism. What that means is that the National Center for Biotechnology Information in the U.S. is doing analysis of the sequences that have been generated. They have gotten through 58,000 so far, and of those 12,000 represent completely unique genes. So there are at least 12,000 genes, so far as we know.

Microarrays are coming along although not yet widely commercially available. There are two companies that produce small *Xenopus* microarrays. Much of the work right now has been done by academic labs, with the leading lab at NIBB — Prof. Ueno. They have distributing macroarrays and in

collaboration with Ken Cho's lab at UCI relatively large microarrays with 42,000 spots have been produced.

My laboratory is developing macroarrays from the ESTs, and we have 76,000 of those. Once those are sequenced they will be available on membranes. At Rockefeller University in Ali H. Brivanlou's lab, they have a chip that they are distributing for the cost of the chip that has 12,000 ESTs on it.

Xenopus tropicalis is just beginning an EST project. The NIH effort so far has got close to 30,000 and the Sanger Centre has a similar amount. My laboratory is making a large number of cDNA libraries that will be contributed to this EST project. *Tropicalis* arrays, unfortunately there are not any cDNA arrays yet available, but in my lab there will be macroarrays available as the EST sequences come, and as UniGene sets are available we will convert those into microarrays.

As I mentioned there is a *Xenopus tropicalis* genome project just starting up. One thing that you need for a genome project is BAC libraries. There are several being developed at NIBB; again, Prof. Ueno's lab has a reasonably good library. The Institute for Systems Biology is developing one, and there is now a grant proposal in to the National Human Genome Research Institute for two very large insert libraries that will be the basis of the genome sequencing.

There are at least two, and perhaps three, sequencing efforts underway. The Joint Genome Institute in Berkeley predicts that they will have three-fold coverage of the *tropicalis* genome by the end of 2002. There is a proposal for 7x coverage at the National Genome Research Institute. The Sanger Center also is involved and they are currently determining what their level of involvement will be.

That is all I have to say. I just want to show the laboratories that are contributing to this and I would be happy to answer any questions.