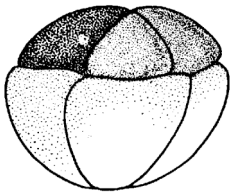

MINUTES OF THE XENOPUS RESOURCE DEVELOPMENT MEETING - JUNE 26TH 2009, MARINE BIOLOGICAL LABORATORY, WOODS HOLE MA

Present:
Mustafa Khokha (Yale)
John Wallingford (University of Texas, Austin)
Robert Grainger (University of Virginia)
Richard Harland (UC Berkeley)
Edward Marcotte (University of Texas, Austin)
Jon Henry (University of Illinois / MBL Woods Hole)
Aaron Zorn (Cincinnati Children's)
Frank Conlon (University of North Carolina)
Kris Kroll (Washington University)
Karen Liu (King's College London)
Todd Stukenberg (University of Virginia)

1. Xenopus Community Resources and need to organize Committees on Xenopus Resource Development

The Xenopus Community has greatly benefitted from a number of resources that have been developed for Xenopus including genomic sequence, full length cDNA sequencing, BAC libraries, etc. In order to develop additional resources to further benefit the Community coordinating with all of the different fields within the Xenopus Community (including developmental biology, genomics, cell biology and molecular biology) is essential. To this end, we propose a series of meetings.

1. Committees on Xenopus Resource Development Meeting Fall 2009
   a. Funding available through the BBSRC grant to Karen Liu to develop international collaborations
   b. To be held at MBL, Woods Hole MA
2. NIH Meeting Spring 2010
   a. Present research programs to NIH officials
   b. Describe resources needed – Coordinate with James Coulombe
3. International Xenopus Meeting Sept. 2010, Calgary Canada
   a. Integrate sessions between cell, molecular, and developmental biologists
   b. Discuss further resources needed across the international community

Action Items:

1. Set date and time for Fall 2009 meeting
   a. Wallingford to coordinate date/time with cell biologists – Bement, Stukenberg, Heald, Walter
   b. Karen Liu to coordinate with European group – Zimmerman, Stemple, Pollet, Amaya, Papalopulu, Gilchrist, Guille etc.
2. Create a Resources Document outlining the resources currently available for Xenopus researchers – post to Xenbase/Xine – Khokha.

2. Xenopus tropicalis genome paper

Uffe Hellsten in Dan Rokhsar's group and Richard Harland are coordinating the Xenopus tropicalis genome paper. The Xenopus genome is particularly interesting due to its degree of synteny with other vertebrates especially chicken and mammals especially human.

Multiple assemblies now available – Assembly 4 was generated at JGI using JAZZ while assembly 5 was generated using ARACHME by Jeremy Schultz. The version 5 assembly "lost" some genes that were not well assembled in version 4. Version 6 genome has included these version 4 missing genes into the version 5 assembly (which statistically is a better assembly than v4). The Genome has 95% of the genes expected from a vertebrate set, but needs to improve the long-range continuity. WashU recently did significant coverage of the genome using 454 sequencing However, not a lot of additional sequence was found indicating that sequence is all there and eliminating the concerns that cloning may have led to bias.

Richard Harland and Dan Rokhsar have funding to improve the X. tropicalis genome including long-range contiguity using HAPPY mapping and meiotic mapping of SNPs. It is anticipated that once this is completed the X. tropicalis genome will have 10 scaffolds with a chromosome scale assembly with concordance between genetic and physical maps. Current assemblies will be distributed to the usual databases- Ensemble and UCSC.

One problem is that the X. tropicalis genome has not been propagated through various databases such as NCBI and proteome databases like Mascot and Tornado.

Action item:

1. Conlon and Zorn to assemble a small group of people who will advocate to have Xenopus sequences deposited into searchable repositories/ pipelines/ databases including NCBI.
2. Khokha and Wallingford will contact James Coulombe, NIH and Steve Klein, NSF to see if they can also advocate for this and/or provide additional contacts that could propagate the Xenopus genome.
3. Drafts of the genome paper are underway

3. Stock Center

A P40 application after resubmission to the NIH/NCRR to establish a Xenopus Resource Center at the Marine Biological Laboratory, Woods Hole MA received a very good score (17 under the new NIH scoring system). Jon Henry has been identified as the Director, and Rob Grainger is the PI on the grant.
Meetings in AM with MBL on 6/26/09 appear conducive for a vibrant XRC with a number of neighboring resources such as microscopy and extensive teaching spaces during the non-summer months. Loeb basement will hold the new facility and is to be completely remodeled. Anticipate center to open in summer of 2010.
Missions of center:

1. house lines of disease-free wild type animals, transgenic and genetic lines of X. laevis and tropicalis
2. Tilling/reverse genetics resource: animals to be generated and housed/stocks frozen. Have discussed possibility to generate an in house sequencing component, since both Sanger and 454 capacity strong there.
3. Training component: Xenopus mini-courses, transgenics, imaging, cell biological, extract production.
4. Serve as a venue for the Xenopus community to assemble
5. Transgenic line generation resource- stock center will create transgenic lines for the community, which is especially critical for X. laevis where the long generation time often restricts users from creating these highly valuable lines. A method to prioritize lines is in place. Cost recovery will include nominal fee-for-services, expect around 10-15% cost recovery by end of 5 years. Mini-courses will also provide some cost-recovery.

Action items:

1. Rob Grainger to develop a G20 application to offset the aquarium/mod costs and/or James may be able to offset costs through NICHD. This would likely make the P40 highly fundable through NCRR
2. Jon and Rob to also explore NSF for support. John Wallingford mentions interest by evolutionary biologists at UT Austin who might be good contacts to push for advocacy/education component. Getting X. laevis and tropicalis BAC sequence comparisons would facilitate these efforts.

4. Status of X. tropicalis genome

Currently, version 6 of the X. tropicalis gneome is available via FTP and a password protected Metazome site for X. trop genome. Contact Richard Harland for access. The genome is still fragmented and lacks long-range contiguity. To improve contiguity two parallel efforts are underway – HAPPY mapping, and meiotic mapping of SNPs. Harland anticipates that these efforts will produce 10 super scaffolds, but not clear whether these will contain all of the individual scaffolds. Plan for assembly:

1. Jiang collaborator at Wash State U to do non-cloning based Happy-mapping, Illumina sequencing to get physical linkage.
2. 50 individual SNP map, Derek Stemple advocates for meiotic mapping to generate linkage map—Richard planning to deep sequencing with Illumina to genotype these individuals.
3. For genetics, improving the meiotic map is essential. For proteomics applications, obtaining the missing 5% of genes is a goal. Where are the missing genes? Are they in the trace? Not clear—haven't done a mapping between EST clusters in Gilchrist database and genome.
4. Would be great to get additional BAC-end sequences. Nicolas Pollet may be planning some BAC end sequencing.

Action items:

1. Identify missing genes - trace analysis of the genome and of the 454 sequences (now accessible). Richard can send access to these. The goal here is to identify which genes are missing which will be important to improve in the genome as well as for full length libraries as listed below.
2. BAC end sequencing Mustafa will ask Pollet about status of this. Jeremy Schmutz also has the Lucigen sheared library, if Pollet efforts appear productive, might advocate for more end sequencing of this.

5. Tilling/reverse genetics

Success to date: First mutations identified through the Sanger sequencing efforts including a hit to noggin2 and Rx. 1:250-350,000 hit rate from Rob's libraries, and Richard's rate was similar (ENU). Frank and Mustafa also have an effort ongoing for ENU and ICR191 –requires some optimization and a calculation of hit rate.

1. Frank mentions that there are technical challenges to TILLING by sequencing. Both Rob and Frank suspect from a set of detected base pair changes 20-50-fold are PCR errors rather than true mutations. While this is high, rescreening hits should eliminate this problem.
2. Detecting polymorphic rate – since certain TILLING strategies like CelI can be complicated by polymorphic rates Frank has been calculating the SNP rates in various lines. Ball-park estimate is 0.03% per bp (3 changes per 10,000; varies depending on coding vs non-coding). This is the data for the NASCO Nigerian F6 being sold to the community.
3. There does seem to be significant inbreeding issues. F6 animals appear fertile but then due to homozygosity of "lethal" background alleles fertility often drops in further generations although Khokha and Harland have noticed a significant improvement in fertility of F12 generation Nigerains. Also, finding allelic dropout/non-Mendelian inheritance of some alleles, which may affect use of the meiotic map.
4. Frank plans to use CelI and agarose gel analysis with silver stain. This may work well with LICOR Bioanalyzers because unlike dHPLC you aren't subject to amplicon size.

Action items: Rob to discuss the numbers and progress with the other fish tilling groups and see whether there is capacity to include us there.

6. Xenbase

Currently Xenbase has been significantly improved by a R01 grant from the NIH. A number of members were highly impressed with the latest version of Xenbase and stated that it is now the first website that they go to for gene related content. The R01 grant is now ending and a P41 grant was submitted to maintain and expand the focus of Xenbase with Aaron Zorn and Peter Vize as PIs. Aims of the application are to:

1. Maintain Xenbase and curate Xenopus research data.
2. Enhance Xenbase functionality by introducing morpholino and mutant phenotypes
3. Support new content on Xenbase gene pages
4. Continue and expand our collaborative research and service efforts

Expanding the number of users, have a record of who is listed on the white pages and, in general, how many hits the site gets from different institutions.

Action items:

Aaron to discuss a few things with Peter:

1. Xenbase Discussion board – would be helpful to have a discussion board and Wiki for issues from policy to technical methods.
2. Connections with Xenmark- blast'able database of in situ images, and/or inclusion of a similar blast search on Xenbase.

7. ORFeome/ complete gene set in gateway vectors

Given the power of expression cloning, overexpression, and expression/detection of tagged proteins in Xenopus, arrayed genome wide gene set would be a powerful resource that would be beneficial for all members of the Community. The NIH funded XGC project has generated ~9500 full length X. laevis clones and ~7300 X. tropicalis FL cDNAs that are available as individual clones or re-arrayed plates from Open Biosystems. The Sanger Centre / Gurdon Institute UK collaboration has independently generated a ~9000 X. tropicalis full length cDNA clone set that is available from MRC Geneservice UK. It is expected that the XGC and Sanger clone sets have considerable redundancy. Many (most?) of these clones are pCS-type expression vectors. The next goal would be to complete this gene set and place the coding regions into recombination-based gateway entry vectors and create a series of useful destination vectors, to add N- or C- terminal tags of various types (eg GFP, TAP, myc, etc).

Richard asks has this been done for mouse or human and will these work in Xenopus assays? Todd Stukenberg suggests that this will vary by gene and so there is validity to generating a Xenopus collection for this purpose. In addition, for things like in situ hybridization probes, Xenopus clones are essential.

Since we currently have X. tropicals genomic sequence, then generating a genome wide full length set of X. tropicalis clones is the first priority. This set would be supplemented with the 9500 X. laevis clones that are identified. As X. laevis genomic sequence is made available than X. laevis full length clones would also be desirable.

Action items:

1. Perform an analysis and determine all clones that are full length from the EST libraries or putative full length clones. Also determine which clones between trops and laevis are orthologous
2. Subclone the coding regions into a suitable entry clone
3. Using PCR, clone genes that are not found in the full length libraries

4. A deep sequencing effort would also provide important information regarding genes that are rare transcripts and splice information. In fact, deep sequencing of the egg may be very valuable since there seems to be a lot of spliced transcripts there.
5. The most efficient way to generate this resource is likely to hire a company to produce it – John/Aaron to explore options.

8. Xenopus laevis genome

Richard submitted a competitive supplement to generate X. laevis genomic sequence. He proposes to use illumina paired end reads to generate an assembly with alignments to X. tropicalis scaffolds. Efforts to initially get exon-sized sequences, hope to segregate A and B copies (may not be essential for proteomics). This is a major priority for the Community. Todd mentions that the X. tropicalis genome is not good enough for mass spec analysis. Frank and Todd both state that only one base change causes loss of a protease digestion site and makes the trop data inadequate for proteomics, morpholino design, non-coding regions.

Various efforts on laevis genome sequencing:

1. Austin, TX: John and Edward's group put together enough funding to do short read SOLID sequencing at 20X coverage and plan to use these to tile the exons and map to tropicalis. Will be moving forward with about 0.1% of genome to test the sequencing and assembly pipeline (trying to decide between reduced representation of genome versus doing BAC sequencing). $100K effort. If "desirable" BACs can be identified ASAP from the X. laevis BAC library, then this would be ideal as it would immediately be useful to the community.
2. Berkeley, CA: Richard's proposal for $300K effort.
3. Amy Sater/Houston (454-based, not clear what schedule is).
4. Takuya Nakayama/Japan committee – uncertain if there is an effort there.

Everyone will use the inbred J strain (generated by inbreeding rather than gynogenesis) from Jacques Robert (U. of Rochester)

Need to have a web site or posted link where one can deposit existing genomic sequences.

Action Items:

1. Todd to provide examples of mass spec analysis failing with the X. tropicalis genome. This will be important to justify X. laevis sequencing.
2. Coordination of efforts is essential since multiple "cottage industry" sequencing efforts may yield lots more information if different libraries are sequenced by different groups. Posting on Xenbase may help.
3. Wallingford to contact Ueno about the Japanese effort and Amy Sater about her effort.

9. PA—Xenopus genetics and genomics.

The previous PA was instrumental in advancing Xenopus genetics and genomics.

Action item: A new RFA seems unlikely in the current climate therefore lobby James Coulombe for extending the PA. At the PI meeting discuss strategies for developing community wide support for applications / projects that benefit Xenopus research. Todd says he'll try to facilitate including the cell biologists.

10. Antibody/Molecular resource—discuss with Jacques Roberts and Enrique Amaya status on this. Need to get the Portsmouth molecular resource web site up and running, they already have >400 clones and we will coordinate to add additional genes.

11. Morpholinos—negotiate for group discount for morpholinos. Can we get Gene Tools to hold the rest of the stock (or Portsmouth stock center could store and ship leftover MOs)? Xenbase will automatically collect these based on search terms. Eventually updating may be Wiki-based by users also.

2009 White Paper writing assignments:
Mustafa -X. tropicalis genome improvements
Frank – TILLING
Aaron – Xenbase
Richard – Xenopus laevis sequencing
Aaron/John – Arrayed full length libraries/Gateway expression system
John/Frank – proteomics
Rob/Jon – stock center

Mustafa, John and Aaron will compile into a White paper for distribution.

Xenopus researchers, if you wish to participate in meetings and write sections of the white paper or have comments/questions, please email:

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