ZFIN NEWS

The Zebrafish Information Network

http://zfin.org

Volume 2, Number 2
Summer 2005

In this issue:

- *Gene Expression Surfs the Literature (**pg. 1**)*
- *Dissecting the Zebrafish with the Anatomical Ontology (**pg. 3**)*
- *Morpholino Curation at ZFIN (**pg. 4**)*

Gene Expression Surfs the Literature

Have you ever noticed a particularly informative figure in a journal article, but couldn't find it later? Or maybe you wished for a fast way to see gene expression in your favorite mutant? If you answered "yes," read on.

Now you can use ZFIN's familiar gene expression search page to retrieve figures from journal articles.

ZFIN already holds a rapidly growing collection of gene expression images that have been submitted directly to us (Table 1). The scientific literature is a vast additional source of gene expression figures. By incorporating published figures, ZFIN combines the power of a model organism database with the wealth of published data.

Bringing published figures to ZFIN presents two major hurdles – acquisition and annotation.

Be sure to attend the Genome Resources Workshop in Dresden!

Table 1: Growth in gene expression

	Jan 2005	Jun 2005
Annotated figures from publications	--	1,223
Submitted expression patterns	3,796	5,376

Acquisition: Open-access journals make it easy for ZFIN to display their figures. Traditional journals are more of a challenge because of copyright restrictions. Several major commercial journals have already granted figure permissions.

We anticipate that including figures in ZFIN will benefit the journals as well as the research community, because our new figure search tools will enable researchers to cite articles that they would not have found otherwise.

Annotation: Searching requires annotation – the association of figures with objects in the ZFIN database. ZFIN curators associate each figure with appropriate genes, fish, stages and anatomical terms. A typical figure with ZFIN annotation is shown on the next page (Figure 1).

When publisher permissions allow, curators enter figure images and captions along with gene expression annotation. When publisher permissions are not available, curators enter only the gene expression annotation. That way, even if we can't show a figure, we can show all the associated gene expression data. If a journal decides to grant figure permissions in the future, it's easy to update the previously annotated records with images and captions.

The Anatomical Ontology (AO) plays a central role in our annotation of gene expression. In turn, detailed curation of the literature for gene expression helps to improve and expand the AO with new structures and relationships.

Curation of gene expression now includes morpholinos.

(continued on page 2)

Gene Expression

(continued from pg. 1)

Searching for gene expression in a "morphant" background is no more difficult than searching in a mutant background.

Progress: ZFIN's gene expression resources have been completely redesigned to include published figures along with directly-submitted images.

Figures from about 200 articles have been added in fully annotated and searchable form. First priority in annotation is given to current articles. Because curating figure data entails a significant investment of curator time, systematic back-curation of older papers for gene expression is not yet feasible. We are able to do a limited amount of back-curation on an *ad hoc* basis.

Author annotation? To facilitate figure annotation, especially for older papers, we are considering making a web form to allow authors to annotate figures from their own publications. With sufficient community participation, this approach could become very valuable.

Figure 1: A published figure (A) and corresponding gene expression annotation (B) as they appear in ZFIN. Clicking the thumbnail opens up a full-size image.

A.

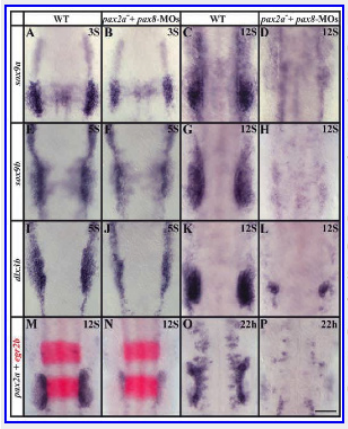


Fig. 3 Pax2a and Pax8 are required for maintenance of otic cell fates. Pax2a and Pax8 are required together for preotic expression of sox9a (A-D), sox9b (E-H) and pax2a (M-P) but not of dlx3b (I-L). Cells of the presumptive otic placode express sox9a in wild-type embryos at the three-somite stage (A) at higher levels than in pax2a– mutants after pax8-MOs injection (B). At the 12-somite stage, sox9a is expressed throughout the otic placode in wild-type embryos (C) but no otic sox9a expression can be detected in pax2a– mutants depleted of Pax8 (D). (E-H) The sox9a duplicate, sox9b, shows similar behavior. In wild type at the five-somite stage, sox9b is expressed in the preotic region and neural crest (E). The neural crest expression is unaffected in pax2a– mutants injected with pax8-MOs, but expression in the preotic domain is reduced (F). At the 12-somite stage, sox9b is expressed strongly in the otic placode in wild-type embryos (G) but is absent in pax2a– mutants injected with pax8-MOs (H). dlx3b expression is strong in cells of the future otic placode in wild-type embryos (I) at the five-somite stage and this domain is smaller but still recognizable in pax2a– mutants after pax8-MOs injection (J). At the 12-somite stage, dlx3b is expressed throughout the otic placode in wild-type embryos (K) but in pax2a– mutants with a knockdown of Pax8, only a few residual cells express dlx3b (L). pax2a expression is strong in the otic placode of wild-type embryos at the 12-somite stage (M) but expression is severely reduced in pax2a– mutants after pax8-MOs injection (N). At 22 h, when the otic vesicle has formed in wild-type embryos, pax2a expression is restricted to the ventromedial region (O) but is completely absent in pax2a– mutants depleted of Pax8 (P). Expression of egr2b (red) in rhombomeres 3 and 5 is unchanged in pax2a– mutants after pax8- MOs injection (N) in comparison with uninjected wild-type embryos (M). Dorsal views, anterior towards the top. Scale bar: 120 µm.

B.

Gene	Fish	Stage	Qualifier	Anatomy	Assay
dlx3b	WT	5-9 somites		ectoderm	ISH
		10-13 somites		otic placode	ISH
	noi^{tu29a}, MO:pax8	5-9 somites		ectoderm	ISH
		10-13 somites		otic placode	ISH
egr2b	WT	10-13 somites		presumptive hindbrain	ISH
	noi^{tu29a}, MO:pax8	10-13 somites		presumptive hindbrain	ISH
pax2a	WT	10-13 somites		otic placode	ISH
		26+ somites		otic vesicle	ISH
	noi^{tu29a}, MO:pax8	10-13 somites		otic placode	ISH
		26+ somites	*Not detected*	otic vesicle	ISH
sox9a	WT	1-4 somites		ectoderm	ISH
		10-13 somites		otic placode	ISH
	noi^{tu29a}, MO:pax8	1-4 somites		ectoderm	ISH
		10-13 somites	*Not detected*	otic placode	ISH
sox9b	WT	5-9 somites		ectoderm	ISH
		10-13 somites		otic placode	ISH
	noi^{tu29a}, MO:pax8	5-9 somites		ectoderm	ISH
		10-13 somites	*Not detected*	otic placode	ISH

Acknowledgments:
ZFIN wishes to thank the journal Development for permission to reproduce figures from this article. Please note that this material may be protected by copyright.

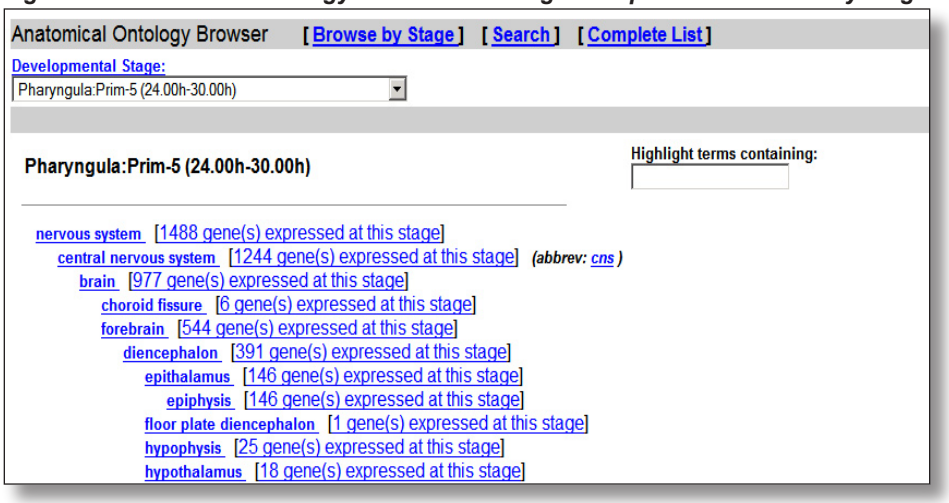
Dissecting the Zebrafish with the Anatomical Ontology

The Anatomical Ontology (AO) is a hierarchical vocabulary of zebrafish anatomical terms, including definitions and synonyms. The AO must contain a high level of detail to allow a rich description of gene expression patterns and mutant phenotypes. It also needs to be flexible enough to facilitate cross-species comparisons. Ongoing changes to the AO help achieve these objectives.

New hierarchy: The AO is now based on a stage-independent fish combining terms from the one-cell embryo to the adult. Within this fish, terms are arranged by functional system. This arrangement makes it much easier to search for anatomical structures.

New terms: Many new terms and definitions have been added, and stage ranges of existing terms updated. For relatively simple cases, we derive term definitions from the literature. For more complex cases, we consult a consortium of researchers who serve as experts for particular sets of anatomical structures. Members of the consortium can be found at: http://zfin.org/zf_info/anatomy/dict/mem.html. In addition to the consortium, we sometimes consult other researchers who specialize in particular anatomical areas. We also welcome user suggestions for new terms or changes to existing terms, and plan to have a "proposed anatomy term" form available to the public later this year.

Figure 2: Anatomical Ontology browser showing a sample search return by stage.



New unknown stage: Each anatomical structure in the AO exists within a defined stage range. When the stage range of a structure is not yet well-defined, we indicate that the stage is "unknown." If you see a term with an unknown stage and you know the stage, please let us know!

New relationship types: We have implemented new relationship types between terms, making the AO more robust than a simple hierarchical list of structures. A given term may have multiple relationship types to multiple terms. We have added types "is_a," "part_of," and "develops_from." For example, the lateral line "is_a" sensory system and "part_of" the peripheral nervous system; the neural keel "develops_from" the neural plate.

Anatomy search and display: The ZFIN anatomy browser allows searching by name and shows the hierarchical structure at each stage (Figure 2). Next to each anatomical structure, the number of annotated genes expressed in that structure or any of its sub-structures (children) is listed. Clicking this number will take you to a list of these genes. The anatomy term display shows the full term name, abbreviation, synonyms and stage range. It has links to genes that have been annotated using this term, as well as links to parents and children of this term. Publications with this term in the abstract are also displayed.

Anatomy templates for researchers: Laboratories who wish to annotate gene expression or phenotype data using a core subset of current AO terms can download a FileMaker template at the following location: http://www.cs.uoregon.edu/~eriks/Documents/ZFIN_templates_home.html. The full AO can be downloaded at: http://zfin.org/zf_info/downloads.html#ad. We welcome expression data submitted by users. Community use of the AO will help standardize the description of anatomical structures in the zebrafish literature. The more the AO is used, the better it will be!

Morpholino Curation at ZFIN

Morpholinos (MOs) have become a cornerstone technique for evaluating gene function in zebrafish. ZFIN curators are now recording MOs from the published literature, making it easy to locate MOs that have been effectively used by others to target a specific gene, and to locate papers that report using a specific MO. Efforts are also under way to include all the MOs from the Stephen Ekker lab in the future.

The Morpholino Page: Each curated MO has a unique name, sequence and data page in ZFIN (Figure 3). Sequences are shown as the MO sequence in the 5'-3' orientation, regardless of how they are reported. A 'BLAST IT' button has been included to facilitate independent sequence analysis against the most current sequence databases. Clicking the 'BLAST IT' button takes you to an NCBI BLASTN page where the MO sequence is populated in the sequence box, the species is limited to *Danio rerio*, and the expect and word size parameters are optimized to locate short exact matches (1000 and 7 respectively).

Morpholino Nomenclature: Each MO is given a unique name in the format MO#-targeted_gene_symbol, which has been approved by the Zebrafish Nomenclature Committee. The # in the name is a sequential number indicating the order in which MOs are entered into ZFIN. For example, the MO page shown in the figure is the second MO targeting *wnt11* that has been entered into ZFIN. Published MO names that deviate from this format are retained as previous names and are linked to the appropriate publications.

Morpholino Sequence Evaluation: Prior to creating MO records in ZFIN, BLAST analysis is used to verify the sequence and to determine if it is already in ZFIN. The authors of the publication will typically be contacted for clarification if this analysis results in any uncertainty about the published MO sequence. A new record is then created in ZFIN, or the publication reporting use of the MO is added to the publication list of an existing MO record. A notes field on each MO page is used to describe changes to the sequence of the MO if the published sequence was incorrect in some way.

Locating Morpholinos in ZFIN: MOs can be located in ZFIN in two ways. From our Genes/Markers/Clones search page, you can search specifically for MOs by selecting "Morpholino" from the "Types" pull down menu. To search for MOs that target a specific gene, select "Morpholino" from the Types pull down menu and enter all or part of the gene symbol in the search box. Morpholino information can also be found at the top of each gene page, in the "Mutants and Targeted Knockdowns" section.

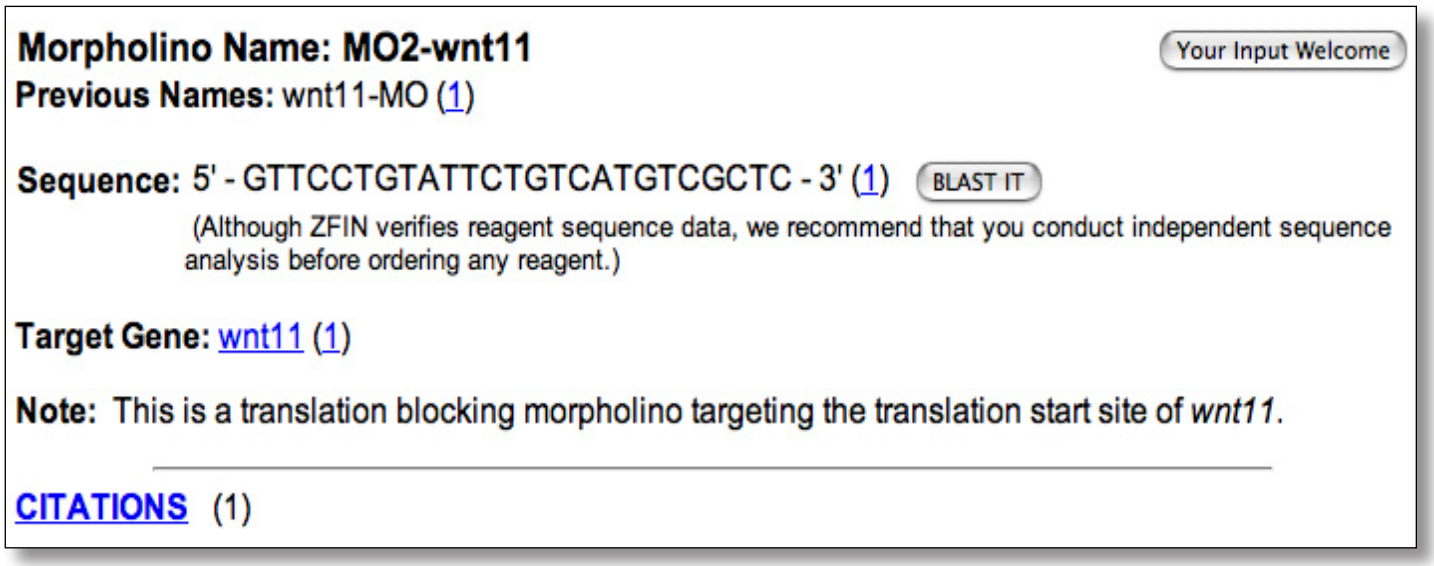



Figure 3: Each morpholino in ZFIN has a dedicated page including the name, previous names, sequence, link to the targeted gene, notes, and a citation link where all papers that used that MO can be found.