

DATABASE

Open Access

zTrap: zebrafish gene trap and enhancer trap database

Koichi Kawakami^{1,2*}, Gembu Abe¹, Tokuko Asada¹, Kazuhide Asakawa^{1,2}, Ryuichi Fukuda¹, Aki Ito¹, Pradeep Lal^{1,2}, Naoko Mouri¹, Akira Muto¹, Maximilliano L Suster^{1,5}, Hitomi Takakubo¹, Akihiro Urasaki¹, Hironori Wada³, Mikio Yoshida⁴

Abstract

Background: We have developed genetic methods in zebrafish by using the *Tol2* transposable element; namely, transgenesis, gene trapping, enhancer trapping and the Gal4FF-UAS system. Gene trap constructs contain a splice acceptor and the GFP or Gal4FF (a modified version of the yeast Gal4 transcription activator) gene, and enhancer trap constructs contain the zebrafish *hsp70l* promoter and the GFP or Gal4FF gene. By performing genetic screens using these constructs, we have generated transgenic zebrafish that express GFP and Gal4FF in specific cells, tissues and organs. Gal4FF expression is visualized by creating double transgenic fish carrying a Gal4FF transgene and the GFP reporter gene placed downstream of the Gal4-recognition sequence (UAS). Further, the Gal4FF-expressing cells can be manipulated by mating with UAS effector fish. For instance, when fish expressing Gal4FF in specific neurons are crossed with the UAS:TeTxLC fish carrying the tetanus neurotoxin gene downstream of UAS, the neuronal activities are inhibited in the double transgenic fish. Thus, these transgenic fish are useful to study developmental biology and neurobiology.

Description: To increase the usefulness of the transgenic fish resource, we developed a web-based database named zTrap <http://kawakami.lab.nig.ac.jp/ztrap/>. The zTrap database contains images of GFP and Gal4FF expression patterns, and genomic DNA sequences surrounding the integration sites of the gene trap and enhancer trap constructs. The integration sites are mapped onto the *Ensembl* zebrafish genome by in-house Blat analysis and can be viewed on the zTrap and *Ensembl* genome browsers. Furthermore, zTrap is equipped with the functionality to search these data for expression patterns and genomic loci of interest. zTrap contains the information about transgenic fish including UAS reporter and effector fish.

Conclusion: zTrap is a useful resource to find gene trap and enhancer trap fish lines that express GFP and Gal4FF in desired patterns, and to find insertions of the gene trap and enhancer trap constructs that are located within or near genes of interest. These transgenic fish can be utilized to observe specific cell types during embryogenesis, to manipulate their functions, and to discover novel genes and *cis*-regulatory elements. Therefore, zTrap should facilitate studies on genomics, developmental biology and neurobiology utilizing the transgenic zebrafish resource.

Background

Zebrafish has been used as a model vertebrate because of high fecundity, rapid embryonic development, transparency during embryonic stages and inexpensive and easy breeding. We have developed a transposon technology by using the medaka fish *Tol2* transposable element in this model vertebrate [1-5]. With the *Tol2* transposon

technology, it is now possible for researchers to perform highly efficient transgenesis in zebrafish and powerful genetic approaches such as gene trapping, enhancer trapping and targeted gene expression by the Gal4-UAS system [4,6-10]. These have greatly advanced genetic studies in zebrafish and increased the usefulness of zebrafish as a vertebrate model.

We have constructed different types of *Tol2*-based gene trap and enhancer trap constructs. T2KSAG and T2KSAGFF (referred to as T2KSAGFF(LF) hereafter) are gene trap constructs that contain the GFP and Gal4FF

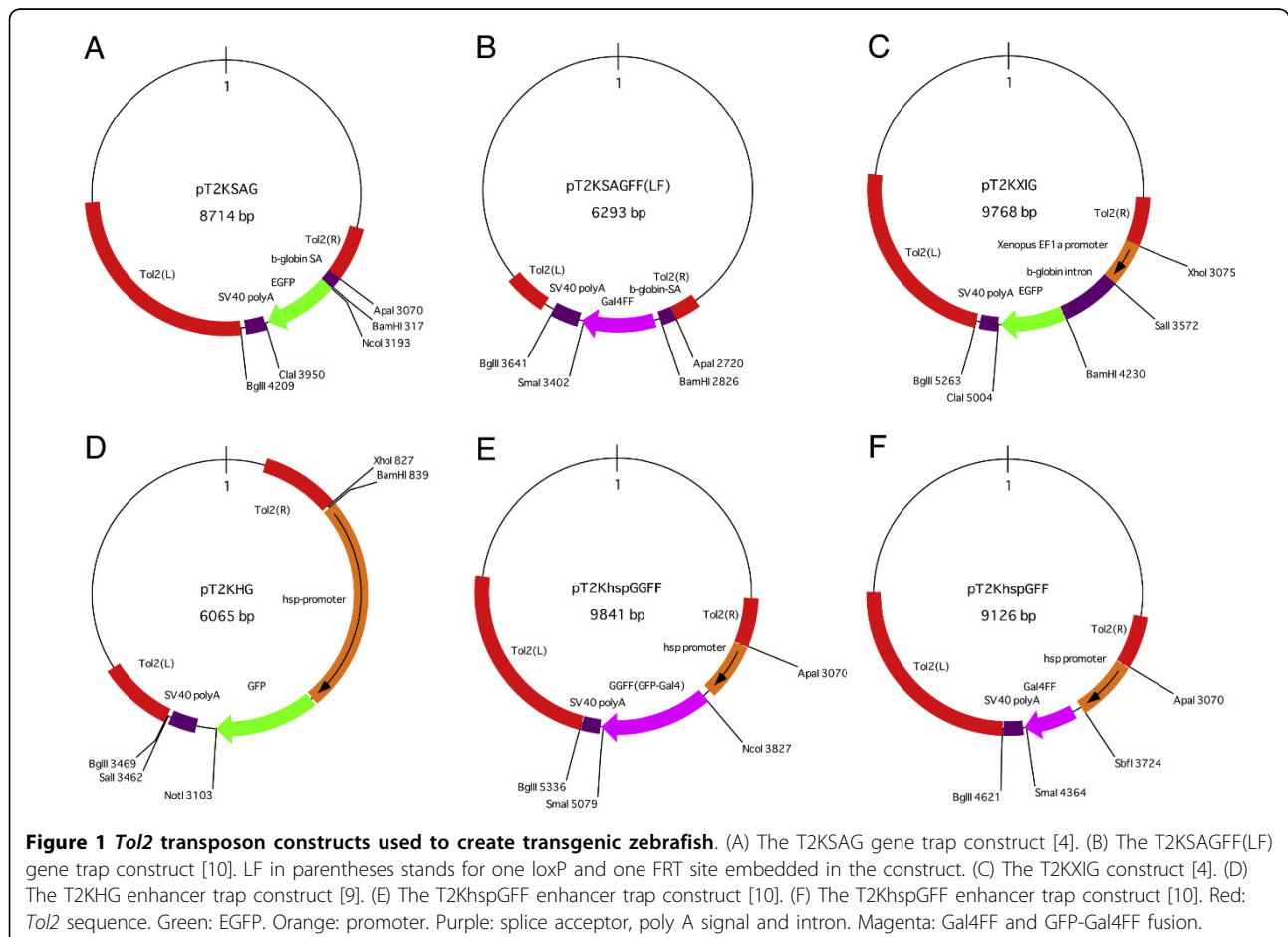
* Correspondence: kokawaka@lab.nig.ac.jp

¹Division of Molecular and Developmental Biology, National Institute of Genetics, 1111 Yata, Mishima, Shizuoka 411-8540, Japan
Full list of author information is available at the end of the article

gene, a modified version of the yeast Gal4 transcription activator, downstream of the rabbit β -globin splice acceptor (SA), respectively. T2KHG, T2KhspGGFF and T2KhspGFF are enhancer trap constructs that contain the GFP, Gal4FF-GFP fusion, and Gal4FF gene downstream of the zebrafish *hsp70l* promoter, respectively [4,9,10] (Figure 1). To visualize Gal4FF expression, we constructed transgenic fish carrying the GFP or RFP reporter gene downstream of the Gal4 recognition sequence (UAS:GFP and UAS:RFP). By using these constructs and transgenic fish, we have performed genetic screens, and generated a large number of transgenic fish that express GFP and Gal4FF in specific tissues, cells and organs.

These transgenic fish have been useful for various studies in developmental biology, genomics and neurobiology. For example, a novel *hoxC* transcript was revealed by the SAGp22A line [4], new functions for the *tcf7* gene and the *synembryn-like* gene were uncovered using HG21C and HGn8H [9], mitochondrion-rich cells on the skin were visualized using HG9B [11], dilatation of the cardiac ventricle was analyzed using SAG4A [12], the *misty somites* gene encoding a maternal factor

involved in somitogenesis was discovered in SAG20A [13,14], innervation of hair cells by afferent neurons was visualized in HGn39D [15], and nasal-temporal patterning of the retina by Fgf signaling was demonstrated using HGn42A [16]. More recent examples include studies of cell-fate determination in the notochord using SAGFF214A [17], characterization of the somatotopic projections of the lateral line afferent neurons in the CNS using hspGFF53A [18], sex-reversal in the *fancl* mutant HG10A [19], patterning of the lymphatic system (SAGFF27C;[20]), and lens fiber differentiation (SAGFF168A;[21]). Furthermore, the Gal4FF-expressing transgenic fish can be used for targeted expression of a desired gene in a desired place. For instance, we created transgenic fish carrying the tetanus neurotoxin gene downstream of the Gal4 recognition sequence (UAS:TeTxLC and UAS:TeTxLC:CFP). When these UAS effector fish were crossed with transgenic fish that expressed Gal4FF in subsets of the spinal neurons or olfactory neurons (SAGFF31B, SAGFF36B, and SAGFF27A), double transgenic fish exhibited specific behavioral abnormalities, demonstrating successful



inhibition of the specific neural circuits by targeted expression of the tetanus toxin [10,22].

Previously, GFP enhancer trap screens using *Tol2* [6] or *Sleeping Beauty* [23], an enhancer trap screen by using a retroviral vector [24,25], gene trap and enhancer trap screens to generate transgenic fish that express Gal4 in specific patterns [7,8] were carried out also in other laboratories. Although a database containing the data from 27 GFP-expressing fish was created previously [26]<http://plover.imcb.a-star.edu.sg/~zetrp/ZETRAP.htm>, it is at present difficult to search most of these transgenic fish because of the absence of searchable databases. To make the transgenic fish generated in our laboratory more useful, we aimed to develop a database equipped with search functions that contains the expression pattern data and the integration site data from our transgenic fish. Here we report a web-based database named zTrap (zebrafish gene trap and enhancer trap database).

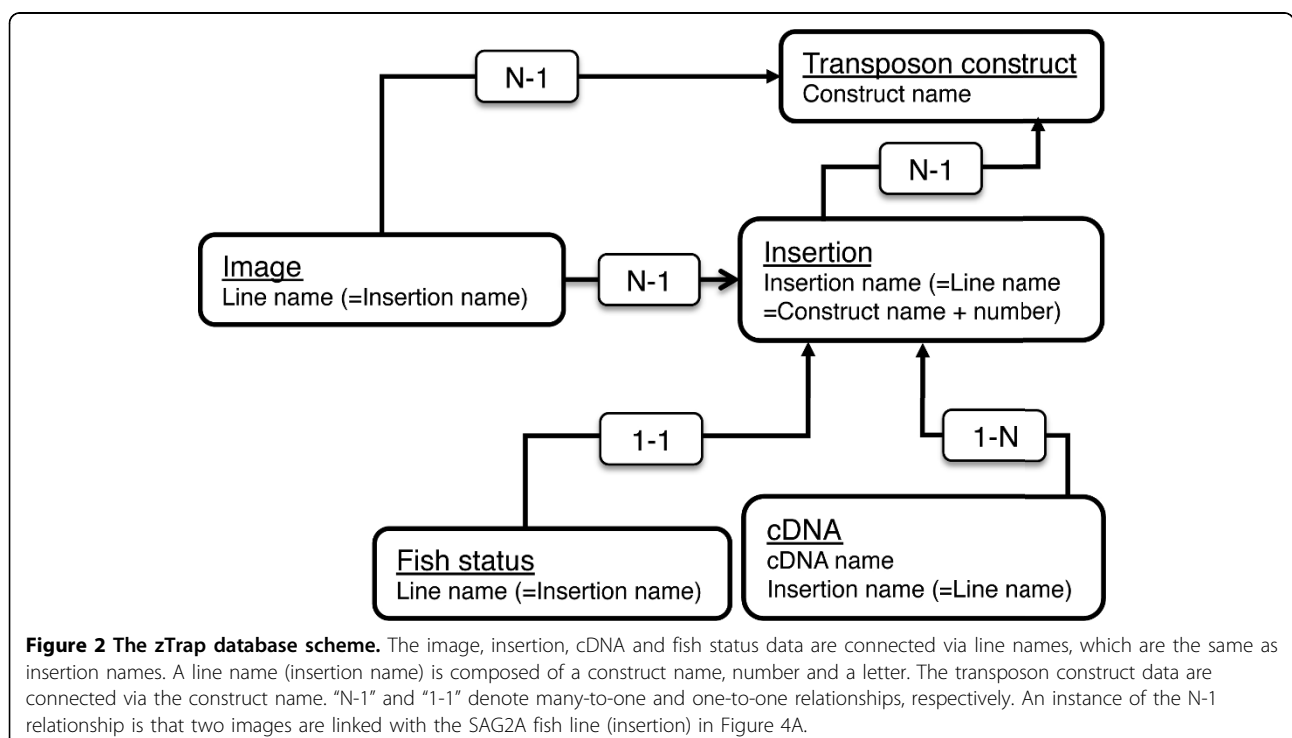
Construction and content

The zTrap database was constructed by using the following applications: OS, Red Hat Enterprise Linux; the database management system, MySQL 5.0; the application server, Tomcat 5.5; the user interface, Java Server Face 1. 1; the web server, Apache 2. The zTrap database scheme is depicted in Figure 2. The database contains image, insertion, cDNA, transposon construct, and fish status data. The image data have a line name, which

corresponds to the name of the insertion carried by the transgenic line, and thus the image and insertion data are connected. Also, the line name is used to connect these data to the fish status and cDNA data. A line name consists of transposon construct name, number and letter, and thereby the image and insertion data are connected to the transposon construct data.

(1) Image data: we have performed gene trap and enhancer trap screens and created transgenic fish that expressed GFP and Gal4FF in spatially and temporally restricted patterns. Image data were generated from these transgenic fish lines. The image data consist of image; line name (construct plus number); image type (selected from expression pattern, in situ or movie); effector (UAS lines used to visualize Gal4FF expression); line type (for now, transgenic only); stage (when the image was taken); region (where the expression was observed); and information (links to insertion and transposon construct data) (Figure 3A).

Images are acquired by photographing transgenic embryos with a CCD camera under a fluorescent stereo microscope. When we obtain an image of the offspring from fish injected with a *Tol2* construct, we first assign a line name to the image data. The line name is composed of an abbreviation of the *Tol2* construct used (namely, SAG for T2KSAG, HG for T2KHG, hspGGFF for T2KhspGGFF, hspGFF for T2KhspGFF, and SAGFF (LF) for T2KSAGFF(LF)), a number given to individual founder fish in the order of successful mating, and a



letter that discriminates different expression patterns observed in offspring from the same founder. To describe the GFP expression patterns, we selected 32 terms from anatomical ontology in ZFIN <http://zfin.org/action/anatomy/search>. By using these terms, expression annotation is carried out in our weekly screen meetings. New anatomical terms may be added to the data sheet when new expression patterns are observed. Currently, the image data were created for previously published transgenic lines; namely, 37 SAG lines [4], 72 HG lines [9], 28 hspGGFF lines [10], 1 hspGFF line [18], and 9 SAGFF(LF) lines [10,17,20-22], and more data for new transgenic fish are being added daily.

zTrap contains image data for UAS reporter and effector lines and other transgenic lines as well; i.e., the UASGFP, UASRFP, UASTeTxLC, UASTeTxLCCFP, and XIG lines. These transgenic fish were created in our laboratory also by *Tol2*-mediated transgenesis. Image data of UAS lines are acquired by crossing these lines with appropriate Gal4 drivers. The information of the Gal4 driver is also attached to the image data of the UAS lines (Figure 4B). The XIG fish carries the GFP gene under the control of the EF1 α promoter [4]. For UASTeTxLCCFP, movie files were uploaded to show behavioral defects in double transgenic fish more clearly.

(2) Insertion data: all of transgenic fish created in our laboratory have been analyzed by Southern blot hybridization to identify fish with single *Tol2* insertions. When fish turn out to carry multiple insertions, they are mated with non-transgenic fish to generate fish with single insertions in the next generation [27]. This process is important to exclude ambiguity concerning the relationship between an expression pattern and a causative insertion, and also makes it easy to amplify genomic DNA surrounding the *Tol2* insertions by inverse PCR and adaptor-ligation PCR. Insertion data are created based on these analyses.

The insertion data consist of the insertion name (which corresponds to a line name); a transposon construct used to create the insertion; gene (when *Tol2* is integrated within a gene); keywords (given manually); checkboxes to show whether it is located within exon or intron; its chromosomal position; genomic DNA sequence at the integration site retrieved from inverse PCR or adaptor-ligation PCR; and the result of in-house Blat analysis using the DNA sequence. The position of the 8-bp target site duplication created upon integration is highlighted in blue in the DNA sequence (Figure 3B).

The genomic DNA sequence surrounding the integration site is analyzed by in-house Blat search against the *Danio rerio* genomic data that is downloaded from the *Ensembl* FTP server ftp://ftp.ensembl.org/pub/current_fasta/danio_rerio/dna/. Currently *Zv8* is used, and a newer version of the genome data will be downloaded

when it becomes available. When the integration site is successfully mapped on the *Ensembl* genome, the chromosome number and the start and end positions are incorporated into the insertion data. The mapping process is in part carried out manually when the in-house Blat analysis showed no hit or multiple hits. If the integration site is mapped within an annotated transcript, the gene name and specific location within an exon or intron are added manually later. Keywords are added also manually. If transcripts surrounding the insertion are analyzed by cDNA cloning, the cDNA data are linked to the corresponding insertion data. The insertion data have been created for all of 152 transgenic lines described above, and more data are being added daily.

(3) cDNA data: when we analyzed transcripts trapped by gene trap insertions or located near integration loci by RT-PCR, 5'RACE, 3'RACE or other methods, cDNA data are created. The cDNA data contain the cDNA name, the corresponding insertion name, a gene type (known, unknown or predicted), a type of experiment (RT-PCR, 5'RACE or 3'RACE), sequence itself and the result of in-house Blat analysis using the sequence (Figure 3C). This analysis is optional and is not carried out for all transgenic lines. Of the 147 insertions of the gene trap and enhancer trap constructs described above, 20 were analyzed for cDNA data.

(4) Transposon construct data: these data contain the restriction map and sequence information of the transposon constructs used to create the transgenic fish (Figure 3D). Currently, these include the gene trap and enhancer trap constructs (T2KSAG, T2KSAGFF(LF), T2KHG, T2KhspGGFF, and T2KhspGFF), the T2KXIG construct (Figure 1), and the UAS reporter and effector constructs (UAS:GFP, UAS:RFP, and UAS:TeTxLC:CFP).

(5) Fish status data: these data indicate the status of transgenic fish in our laboratory; i.e., whether they are kept alive in the fish room (a "fish tank" icon) stored as frozen sperms (a "tube" icon), or whether they have been terminated or lost (a "fish skeleton" icon)(Figure 3E).

Utility and Discussion

Find image

The "Find Image" page is the main page of the zTrap database (Figure 4A and additional file 1). From this page, users can search the database by clicking a term in the "by region" column, a construct name in the "by construct" column and a number in the "by number" column, and then thumbnail images of corresponding transgenic lines will appear on the right. Thus, users can find transgenic fish lines that show expression patterns of interest or that are created by using a construct of interest. In these columns, "ALL" can also be selected. The "advanced search" allows users to select regions and stages with more flexibility. When the thumbnail image

A National Institute of Genetics KAWAKAMI LAB ZTRAP Zebrafish Gene Trap and Enhancer Trap Database

Kawakami Lab Home | Find Image | Find UAS | Find Insertion | Gene to Insertion | Login

Find Image advanced search

by region: ALL (brain/head, (rostrum), (midbrain), (eye), (forebrain), (floor plate), (proliferative neuroblast), pineal gland, nose, eye vesicle, skin, (of arches/jaw), gill, rhombus, notochord, somite/muscle, heart, blood, blood vessel, fin, (of), (medial), (lateral), (ventral), (posterior), mouth, hatching gland, yolk, post-vent region (tail), whole organism)

by construct: ALL (XG, SB, HG, hspGGFF, SAGFFL)

by number: ALL (2A, 4A, 4B, 10A, 11A, 14A, 16A, 20A, 36A, 38A, 50A, m11A, m11C, m11E, m11K, m17A, m17B, m17C, m18A, m18C, m1A, p11A, p22A, p22D, p27A, p48A, p48B, p48C, p53A, p53B, p57A, p57A, p84A)

1 - 5 of 37 linenames Show Image List Show Line List

Previous 1 2 3 4 5 6 7 8 Next

SAG2A

day 1 day 1

SAG4A

day 1 day 1 day 1

SAG4B

day 1 day 1 day 1

SAG10A

day 1 day 1 day 1

SAG11A

day 1 day 1 day 1

B National Institute of Genetics KAWAKAMI LAB ZTRAP Zebrafish Gene Trap and Enhancer Trap Database

Kawakami Lab Home | Find Image | Find UAS | Find Insertion | Gene to Insertion | Login

Find UAS advanced search

by construct: ALL (UASGFP, UASRFP, UASfTetL, UASfTetL, UASfTetL, UASfTetL)

by number: ALL (1A)

1 - 2 of 2 linenames Show Image List Show Line List

Previous Next

UASGFP1A

hspGGFF1B

day 1 day 1

UASRFP1A

hspGGFF1B

day 1 day 1

C Find Insertion

Search Clear Show All

Insertion name: HG

Transposon construct: T2KHG

Insertion within: Exon Intron

Keywords:

Chromosome:

Search Clear Show All

Search Results

Refresh

Insertion (1 - 6 of 6)

Insertion	Transposon construct	Gene	Exon(s)	Intron(s)	Chr.	Start	End	Integration site	Length	
HG10A	T2KHG	foxp4	<input checked="" type="checkbox"/>	<input type="checkbox"/>	13	2753870	2753921	CAGATCAG	502	z! e! View
HG10C	T2KHG	ENSDART0000010341	<input checked="" type="checkbox"/>	<input type="checkbox"/>	16	27574069	27574486	TGGTATC	391	z! e! View
HG21C	T2KHG	tdcf	<input checked="" type="checkbox"/>	<input type="checkbox"/>	21	46139050	46138850	GAGGTGG	504	z! e! View
HG5B	T2KHG	s:cn21-6/rt6.9	<input checked="" type="checkbox"/>	<input type="checkbox"/>	9	13666805	13666731	TTCAATGCC	75	z! e! View
HG13B	T2KHG	AHA1, activator of heat shock protein ATPase homolog 1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	20	26609115	26608877	CTCATCAG	246	z! e! View
HG43A	T2KHG	Pleckstrin homology domain-containing family D member 1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	19	41648848	41649059	CTCTTAGG	812	z! e! View

D Gene-to-Insertion Search

Search Clear

fox

Search Results for 'fox'

Refresh

Ensembl (1 - 3 of 3)

Name	Chr.	Strand	Start	End	Ensembl	References	Insertion	Map
foxr4	5	-	17324214	17338069	ENSDART0000006094	ZFIN ZDB-GENE-990415-277 ZFIN ZDB-GENE-990415-277 forkhead box N4 [Source RefSeq peptide Acc# NP_571174]	UASfTetL:3A chr:5 strand:1 position:17356506 - 17360191	z!
foxd11	5	+	33369363	33370146	ENSDART0000040341	ZFIN ZDB-GENE-04020-2094 ZFIN ZDB-GENE-04020-2094 forkhead box D1 like [Source RefSeq peptide Acc# NP_990976]	HG43A chr:8 strand:1 position:33420652 - 33421417	z!
foxd1.1	7	+	29728199	29729097	ENSDART0000007921	ZFIN ZDB-GENE-990415-77 ZFIN ZDB-GENE-990415-77 forkhead box B1.1 [Strand: 1], mRNA [Source RefSeq peptide Acc# NP_531283]	SAG4B chr:7 strand:1 position:29728270 - 29728803	z!

Figure 4 The functionality to search fish and insertions. (A) "Find image" is a main page to find gene trap and enhancer trap fish with desired patterns. Thumbnails of images of fish lines appear when an expression pattern and/or construct name is selected in the "by region", "by construct" and "by number" columns on the left. Here, ALL in "by region", SAG in "by construct" and ALL in "by number" are chosen, and all "SAG" transgenic fish are seen on the right. These can be seen also as a list style by clicking "Show Image List" or "Show Line List". Icons next to the line name link to the fish status data, the insertion data and the *z!* and *e!* genome browsers. (B) "Find UAS" allows users to find transgenic fish carrying a reporter or an effector gene downstream of the Gal4 recognition sequence. Here, double transgenic fish carrying the hspGGFF1B insertion and UASGFP or UASRFP are shown. Icons next to the line names link to the fish status data, the insertion data and the *z!* and *e!* genome browsers. (C) "Find insertion" allows users to search the insertion data by filling in the blanks. Here, "HG" is typed in "insertion name" and the "exon" checkbox is checked, and all insertions that are created by using the T2KHG construct and located within an exon are seen. (D) "Gene-to-Insertion Search" allows users to find insertions located near a gene of interest. Here, "fox" is typed, and all insertions located within a distance of 100-kb from the fox genes are picked up.

is clicked, the image data will open. To view many images at the same time, the image data can be shown as a list by clicking “Show Image List” or “Show Line List” (Figure 4A).

There are four types of icons next to the line name. A “fish tank” icon opens the fish status data. These data are useful when users request transgenic fish from us. A “transposon icon” (depicted in a shape of a inverted triangle) opens the corresponding insertion data (Figure 4A). “z!” and “e!” icons link with the zTrap and *Ensembl* genome browsers, respectively, and appear next to the line name when the genomic DNA sequence at the integration site was successfully mapped onto the *Ensembl* genome by in-house Blat analysis.

Find UAS

The UAS-reporter and UAS-effector fish that carry a reporter or effector gene downstream of the Gal4 recognition sequence are useful to visualize and manipulate Gal4FF-expressing cells. Such UAS transgenic fish can be explored by clicking “Find UAS” on the menu bar (Figure 4B). The “Find UAS” page has a similar function to that of the “Find Image” page but lacks the “by region” column since it is not applicable to the UAS fish. UAS reporter or effector fish of interest are easily found by clicking a construct in the “by construct” column. Thumbnail images (in some cases, movies) of the UAS fish, the line names, icons, and the information on Gal4FF drivers will appear on the right. We also analyzed the integration sites of the UAS constructs by inverse PCR and adaptor-ligation PCR. Thus, insertion data are created for all of the UAS fish lines and linked to the UAS image data.

Find Insertion

“Find Insertion” on the menu bar opens dialog boxes to search insertion data by an insertion name, a transposon construct name, checkboxes (“Exon” and “Intron”), keywords, and a chromosome number (Figure 4C). The corresponding insertion data will be shown as a list. From the list, it is possible to view the insertion site and jump to the insertion data and the genome browsers. The “Exon” and “Intron” checkboxes are useful to find possible insertional mutations caused by integration of a *Tol2* construct. These two checkboxes can be checked simultaneously. Search by the chromosome number is useful to identify insertions located near a locus of interest. Such insertions may be used for genetic mapping or as dominant markers on a balancer chromosome to maintain a lethal mutation.

Gene-to-Insertion Search

We developed the “Gene-to-Insertion” program to further facilitate searching the insertion data. “Gene-to-Insertion” on the menu bar opens a dialog box (Figure 4D). Users

can type a gene name (*hox*, *sox*, etc.), gene ID starting from *zgc* (*ZFIN* gene name) or ENSDARG (*Ensembl* genes), or any words linked to the *Ensembl* transcript database. The Gene-to-Insertion program first identifies genes that have the typed word in their descriptions, and then finds insertions that are located within a distance of 100-kb from the identified genes. Thus, users may find transgenic fish lines that express GFP or Gal4FF in a pattern that corresponds to that of a gene of interest or may find an insertional mutation of a gene of interest.

The zTrap genome browser

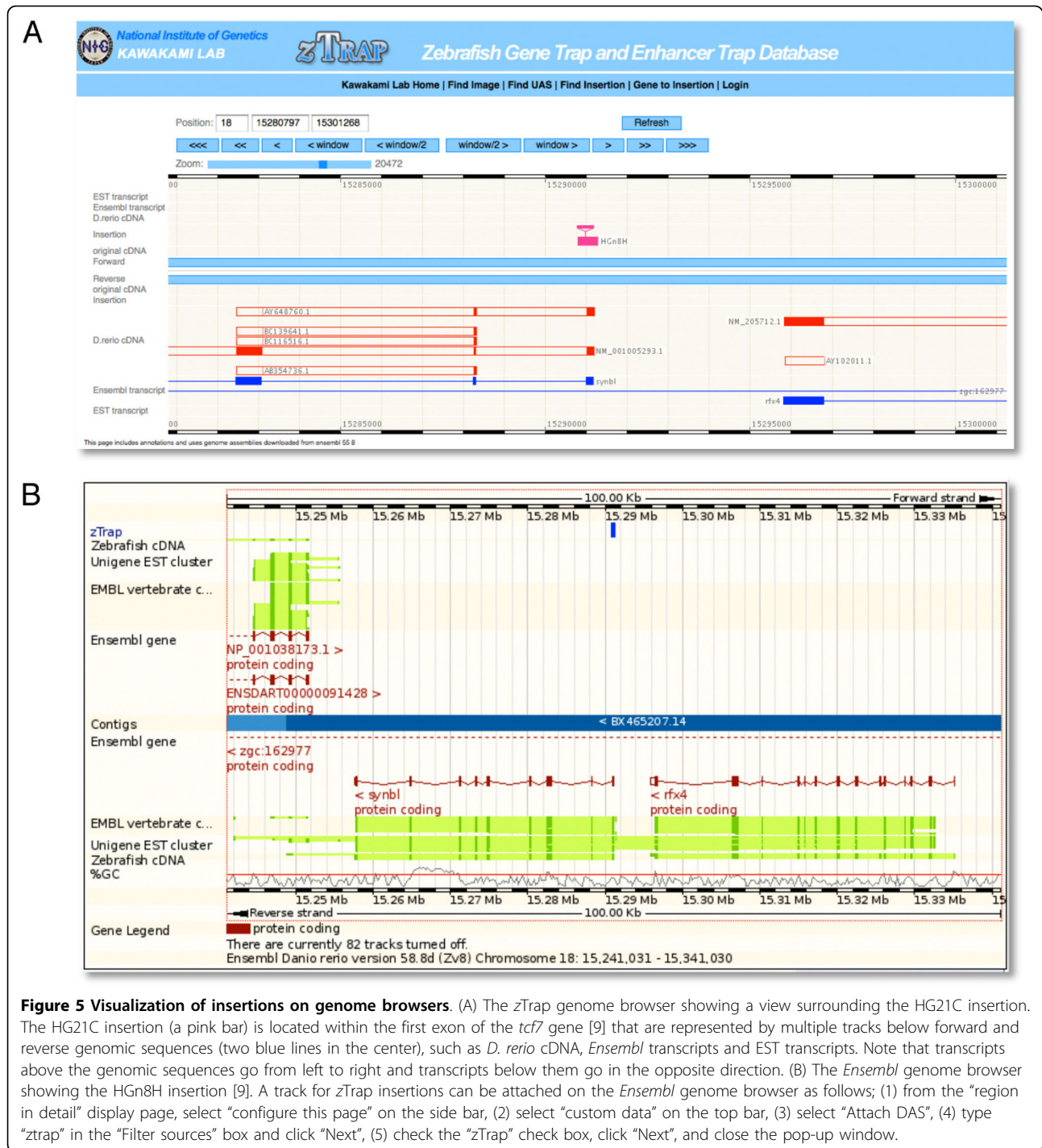
In order to see the genomic landscape surrounding the integration site quickly, we developed the zTrap genome browser. The “z!” icon found in many places is used to open the browser (Figure 5A). On the zTrap genome browser, 100-kb of the genomic sequence surrounding a *Tol2* insertion (indicated by a pink bar with an inverted triangle) can be viewed with zoom-in and zoom-out functions. The *Tol2* construct can be inserted in two different orientations. We previously defined the left (L) and right (R) ends of *Tol2* with respect to the direction of the transposase gene [28]. When the insertion is placed above the genomic sequence (two blue lines in the center), the L end of *Tol2* is located on the left, and when the insertion is placed below the genomic sequence, the L end is located on the right. The z! browser essentially contains the same data as the *Ensembl* browser. The *D. rerio* cDNA, *Ensembl* transcript and EST transcript data are downloaded from *Ensembl* and can be seen on multiple tracks of the z! browser. Descriptions about the insertion, *D. rerio* cDNA, *Ensembl* transcript and EST transcript appear as pop-ups when they are clicked. In addition, other insertions and original cDNA located within a distance of 100-kb can be seen simultaneously.

Link to Ensembl

When the “e!” icon found in many places is clicked, the positional information of the integration site is transferred to the *Ensembl* genome browser, and the “region in detail” display of the corresponding genomic locus will open. Furthermore, we developed a DAS (Distributed Annotation System) server. The zTrap track appears on the *Ensembl* genome browser by executing the following procedure (Figure 5B); (1) from the “region in detail” display page, select “configure this page”, (2) select “custom data” on the top bar, (3) select “Attach DAS”, (4) type “ztrap” in the “Filter sources” box and click “Next”, (5) check the “zTrap” check box and click “Next”, and close the pop-up window.

Conclusions

The zTrap database developed in this study allows users to rapidly search a large number of transgenic fish lines



on two major criteria: i.e., expression patterns of interest and genes of interest. Using these search functions, researchers may find transgenic fish that express GFP or Gal4FF in desired patterns which can be used in several ways. First, transgenic fish may serve as live markers for studies of cell proliferation, differentiation and migration during embryogenesis and organogenesis. Second,

transgenic fish expressing Gal4FF may be used to manipulate specific cell types in combination with appropriate UAS-effector fish. Third, by analyzing the genomic locus surrounding the integration site and associated transcripts, a novel gene, transcript and/or *cis*-regulatory elements responsible for the expression pattern may be discovered. Finally, it is possible that the

transposon insertion could disrupt the function of a gene of interest or a previously uncharacterized gene. Altogether, the zTrap database and our transgenic zebrafish resources should facilitate studies on genomics, developmental biology and neurobiology.

Availability and requirements

The zTrap database is publicly accessible at <http://kawakami.lab.nig.ac.jp/ztrap/>. Browsers recommended are: Firefox 2 or later; Internet Explorer 6 or later; Safari 2 or later. The copy and use of the content (text, graphics, images and other materials) are permitted with prior agreement from the corresponding author.

Additional material

Additional file 1: zTrap database navigation. A figure that shows how to jump to the contents from "Find image" page.

Acknowledgements

We thank T. Kotani, K. Mizusawa, S. Nagayoshi, and H. Kikuta for creating initial data, N. Kimura for sperm cryopreservation, Y. Kanebako, M. Mizushima, and M. Suzuki for fish maintenance, and M. Masame and K. Onozawa for administrative supports on this project. This work was supported by a grant from Transdisciplinary Research Integration Center of Research Organization of Information and Systems, the National BioResource Project, and the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Author details

¹Division of Molecular and Developmental Biology, National Institute of Genetics, 1111 Yata, Mishima, Shizuoka 411-8540, Japan. ²Department of Genetics, Graduate University for Advanced Studies (SOKENDAI), 1111 Yata, Mishima, Shizuoka 411-8540, Japan. ³PRESTO, Japan Science and Technology Agency (JST), Honcho 4-1-8, Kawaguchi, Saitama 322-0012, Japan. ⁴Intec Systems Institute Inc., 1-3-3 Shinsuna, Koto-ku, Tokyo 136-0075, Japan. ⁵Current Address: Sars International Centre for Marine Molecular Biology, Thormøhlensgate 55, N-5008 Bergen, Norway.

Authors' contributions

KK designed the database and wrote the manuscript. GA, TA, KA, RF, AI, PL, NM, AM, MLS, HT, AU and HW created the data. MY designed and constructed the database. All authors read and approved the manuscript.

Received: 11 May 2010 Accepted: 18 October 2010

Published: 18 October 2010

References

1. Kawakami K, Koga A, Hori H, Shima A: Excision of the *Tol2* transposable element of the medaka fish, *Oryzias latipes*, in zebrafish, *Danio rerio*. *Gene* 1998, **225**:17-22.
2. Kawakami K, Shima A: Identification of the *Tol2* transposase of the medaka fish *Oryzias latipes* that catalyzes excision of a nonautonomous *Tol2* element in zebrafish *Danio rerio*. *Gene* 1999, **240**:239-44.
3. Kawakami K, Shima A, Kawakami N: Identification of a functional transposase of the *Tol2* element, an *Ac*-like element from the Japanese medaka fish, and its transposition in the zebrafish germ lineage. *Proc Natl Acad Sci USA* 2000, **97**:11403-8.
4. Kawakami K, Takeda H, Kawakami N, Kobayashi M, Matsuda N, Mishina M: A transposon-mediated gene trap approach identifies developmentally regulated genes in zebrafish. *Dev Cell* 2004, **7**:133-44.
5. Kawakami K: Transposon tools and methods in zebrafish. *Dev Dyn* 2005, **234**:244-54.
6. Parinov S, Kondrichin I, Korzh V, Emelyanov A: *Tol2* transposon-mediated enhancer trap to identify developmentally regulated zebrafish genes *in vivo*. *Dev Dyn* 2004, **231**:449-59.
7. Davison JM, Akitake CM, Goll MG, Rhee JM, Gosse N, Baier H, Halpern ME, Leach SD, Parsons MJ: Transactivation from Gal4-VP16 transgenic insertions for tissue-specific cell labeling and ablation in zebrafish. *Dev Biol* 2007, **304**:811-24.
8. Scott EK, Mason L, Arrenberg AB, Ziv L, Gosse NJ, Xiao T, Chi NC, Asakawa K, Kawakami K, Baier H: Targeting neural circuitry in zebrafish using GAL4 enhancer trapping. *Nat Methods* 2007, **4**:323-6.
9. Nagayoshi S, Hayashi E, Abe G, Osato N, Asakawa K, Urasaki A, Horikawa K, Ikeo K, Takeda H, Kawakami K: Insertional mutagenesis by the *Tol2* transposon-mediated enhancer trap approach generated mutations in two developmental genes: *tcf7* and *synembryn-like*. *Development* 2008, **135**:159-69.
10. Asakawa K, Suster ML, Mizusawa K, Nagayoshi S, Kotani T, Urasaki A, Kishimoto Y, Hibi M, Kawakami K: Genetic dissection of neural circuits by *Tol2* transposon-mediated Gal4 gene and enhancer trapping in zebrafish. *Proc Natl Acad Sci USA* 2008, **105**:1255-60.
11. Nakada T, Hoshijima K, Esaki M, Nagayoshi S, Kawakami K, Hirose S: Localization of ammonia transporter Rhcg1 in mitochondrion-rich cells of yolk sac, gill, and kidney of zebrafish and its ionic strength-dependent expression. *Am J Physiol Regul Integr Comp Physiol* 2007.
12. Seguchi O, Takashima S, Yamazaki S, Asakura M, Asano Y, Shintani Y, Wakeno M, Minamino T, Kondo H, Furukawa H, et al: A cardiac myosin light chain kinase regulates sarcomere assembly in the vertebrate heart. *J Clin Invest* 2007, **117**:2812-24.
13. Kotani T, Kawakami K: *Misty somites*, a maternal effect gene identified by transposon-mediated insertional mutagenesis in zebrafish that is essential for the somite boundary maintenances. *Dev Biol* 2008, **316**:383-96.
14. Kotani T, Iemura S, Natsume T, Kawakami K, Yamashita M: *Mys* protein regulates protein kinase A activity by interacting with regulatory type alpha subunit during vertebrate development. *J Biol Chem* 2010, **285**:5106-16.
15. Faucherre A, Pujol-Marti J, Kawakami K, Lopez-Schier H: Afferent neurons of the zebrafish lateral line are strict selectors of hair-cell orientation. *PLoS One* 2009, **4**:e4477.
16. Picker A, Cavodeassi F, Machate A, Bernauer S, Hans S, Abe G, Kawakami K, Wilson SW, Brand M: Dynamic coupling of pattern formation and morphogenesis in the developing vertebrate retina. *PLoS Biol* 2009, **7**: e1000214.
17. Yamamoto M, Morita R, Mizoguchi T, Matsuo H, Isoda M, Ishitani T, Chitnis AB, Matsumoto K, Crump JG, Hozumi K, et al: *Mib-1-Notch* signalling regulates patterning and structural roles of the notochord by controlling cell-fate decisions. *Development* 2010, **137**:2527-37.
18. Pujol-Marti J, Baudoin JP, Faucherre A, Kawakami K, Lopez-Schier H: Progressive neurogenesis defines lateralis somatotopy. *Dev Dyn* 2010, **239**:1919-30.
19. Rodriguez-Mari A, Canestro C, Bremiller RA, Nguyen-Johnson A, Asakawa K, Kawakami K, Postlethwait JH: Sex reversal in zebrafish *fancl* mutants is caused by *Tp53*-mediated germ cell apoptosis. *PLoS Genet* 2010, **6**:e1001034.
20. Bussmann J, Bos FL, Urasaki A, Kawakami K, Duckers HJ, Schulte-Merker S: Arteries provide essential guidance cues for lymphatic endothelial cells in the zebrafish trunk. *Development* 2010, **137**:2653-7.
21. Imai F, Yoshizawa A, Fujimori-Tonou N, Kawakami K, Masai I: The ubiquitin proteasome system is required for cell proliferation of the lens epithelium and for differentiation of lens fiber cells in zebrafish. *Development* 2010, **137**:3257-3268.
22. Koide T, Miyasaka N, Morimoto K, Asakawa K, Urasaki A, Kawakami K, Yoshihara Y: Olfactory neural circuitry for attraction to amino acids revealed by transposon-mediated gene trap approach in zebrafish. *Proc Natl Acad Sci USA* 2009, **106**:9884-9.
23. Balciunas D, Davidson AE, Sivasubbu S, Hermanson SB, Welle Z, Ekker SC: Enhancer trapping in zebrafish using the Sleeping Beauty transposon. *BMC Genomics* 2004, **5**:62.
24. Ellingsen S, Laplante MA, Konig M, Kikuta H, Furmanek T, Hoivik EA, Becker TS: Large-scale enhancer detection in the zebrafish genome. *Development* 2005, **132**:3799-811.

25. Kikuta H, Laplante M, Navratilova P, Komisarczuk AZ, Engstrom PG, Fredman D, Akalin A, Caccamo M, Sealy I, Howe K, *et al*: **Genomic regulatory blocks encompass multiple neighboring genes and maintain conserved synteny in vertebrates.** *Genome Res* 2007, **17**:545-55.
26. Choo BG, Kondrichin I, Parinov S, Emelyanov A, Go W, Toh WC, Korzh V: **Zebrafish transgenic Enhancer TRAP line database (ZETRAP).** *BMC Dev Biol* 2006, **6**:5.
27. Asakawa K, Kawakami K: **The Tol2-mediated Gal4-UAS method for gene and enhancer trapping in zebrafish.** *Methods* 2009, **49**:275-81.
28. Urasaki A, Morvan G, Kawakami K: **Functional dissection of the Tol2 transposable element identified the minimal cis-sequence and a highly repetitive sequence in the subterminal region essential for transposition.** *Genetics* 2006, **174**:639-49.

doi:10.1186/1471-213X-10-105

Cite this article as: Kawakami *et al.*: zTrap: zebrafish gene trap and enhancer trap database. *BMC Developmental Biology* 2010 **10**:105.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

