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Genomic deletion induced by *Tol2* transposon excision in zebrafish

Peng Huang[1], Linjie Xu[1], Wei Liang[1], Chi Ian Tam[1], Yutian Zhang[1], Fei Qi[1], Zuoyan Zhu[1], Shuo Lin[2,*] and Bo Zhang[1,*]

[1]Key Laboratory of Cell Proliferation and Differentiation of the Ministry of Education, College of Life Sciences, Peking University, Beijing 100871, P. R. China and [2]Department of Molecular, Cell & Developmental Biology, University of California Los Angeles, Los Angeles, CA, USA

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ABSTRACT

Genomic deletions induced by imprecise excision of transposons have been used to disrupt gene functions in *Drosophila*. To determine the excision properties of *Tol2*, a popular transposon in zebrafish, we took advantage of two transgenic zebrafish lines *Et(gata2a:EGFP)pku684* and *Et(gata2a:EGFP)pku760*, and mobilized the transposon by injecting transposase mRNA into homozygous transgenic embryos. Footprint analysis showed that the *Tol2* transposons were excised in either a precise or an imprecise manner. Furthermore, we identified 1093-bp and 1253-bp genomic deletions in *Et(gata2a:EGFP)pku684* founder embryos flanking the 5′ end of the original *Tol2* insertion site, and a 1340-bp deletion in the *Et(gata2a:EGFP)pku760* founder embryos flanking the 3′ end of the insertion site. The mosaic *Et(gata2a:EGFP)pku684* embryos were raised to adulthood and screened for germline transmission of *Tol2* excision in their F_1 progeny. On average, ~42% of the F_1 embryos displayed loss or altered EGFP patterns, demonstrating that this transposon could be efficiently excised from the zebrafish genome in the germline. Furthermore, from 59 founders, we identified one that transmitted the 1093-bp genomic deletion to its offspring. These results suggest that imprecise *Tol2* transposon excision can be used as an alternative strategy to achieve gene targeting in zebrafish.

INTRODUCTION

In *Drosophila*, genomic deletions induced by transposon excision of *P* or *Minos* element are one of the most commonly used methods to disrupt genes of interest (1,2). However, no similar approach has been documented in other organisms. In zebrafish (*Danio rerio*), several transposons, including the *Tol1*, *Tol2*, *Sleeping Beauty* and *Ac/Ds* elements, have been used for a variety of purposes (3–6). As the *Tol2* transposon is highly efficient, it is widely used in zebrafish, and many transgenic fish lines have been generated through several large-scale genetic screens based on it (7–16).

The *Tol2* transposon is a member of the *hAT* transposon family, whereas *P* element belongs to the *P* family. Although they are not closely related, the *Tol2* transposon and *P* element are similar in certain properties, such as transposition through the mode of cut-and-paste, generation of 8-bp DNA duplications at the original insertion sites and leaving footprints after excision (17). Footprints are generated by the error-prone non-homologous end-joining repair of DNA double-strand breaks, which are induced during transposition (18). The excision of the *Tol2* transposon is reported to be either precise or imprecise in medaka (*Oryzias latipes*) and zebrafish (19,20). Small nucleotide deletions and insertions (indels) have been detected after imprecise *Tol2* excision; however, relatively large genomic deletions (>1 kb) similar to that induced by *P* element have not been reported (19–22).

Here, we investigated the excision efficiency and the footprints of the *Tol2* transposon using two transgenic fish lines, *Et(gata2a:EGFP)pku684* and *Et(gata2a:EGFP)pku760*, in which the *Tol2* transposon containing

*To whom correspondence should be addressed. Tel: +86 10 6275 9072; Fax: +86 10 6275 9072; Email: bzhang@pku.edu.cn
Correspondence may also be addressed to Shuo Lin. Tel: +1 310 267 4970; Fax: +1 310 267 4971; Email: shuolin@ucla.edu

The authors wish it to be known that, in their opinion, the first three authors should be regarded as joint First Authors.

an enhancer trap cassette with an *EGFP* reporter gene was inserted at 140-bp upstream of the *lmo2* (*LIM domain only 2*) gene and in the first intron of the *nav3* (*neuron navigator 3*) gene, respectively. We identified 1093-bp and 1253-bp genomic deletions in the *Et(gata2a:EGFP)pku684* founder embryos and a 1340-bp deletion in the *Et(gata2a:EGFP)pku760* founder embryos adjacent to the *Tol2* insertion sites. Furthermore, we identified the 1093-bp genomic deletion in the progeny from one out of 59 *Et(gata2a:EGFP)pku684* founder fish, indicating that genomic deletions induced by *Tol2* excision is heritable through germline transmission. Our results showed that *Tol2* transposon excision may be a feasible and efficient new approach for mutagenesis in zebrafish.

MATERIALS AND METHODS

Zebrafish lines

All the zebrafish used in this study were maintained at 28.5°C in the fish facility of Peking University. The *Tol2* transposon insertion sites of the transgenic fish lines *Et(gata2a:EGFP)pku684* and *Et(gata2a:EGFP)pku760* were mapped using linker-mediated polymerase chain reaction (PCR) as previously described and confirmed by PCR genotyping (23).

Whole-mount *in situ* hybridization

A 1358-bp fragment of the *lmo2* gene was amplified from cDNAs of 24 hours post fertilization (hpf) embryos by PCR (5′-ATAGGACTGAATGCGTGGTGACA-3′ and 5′-AAGATGGGATTGAAGACTGCTGAA-3′). The PCR product was ligated into the pBluescript vector. Antisense RNA probe was prepared by *in vitro* transcription using T7 RNA polymerase (Promega) and labeled with digoxigenin-UTP RNA labeling mix (Roche). The whole-mount *in situ* hybridization procedure was carried out as described previously (24,25).

Image acquisition and processing

The *in situ* hybridization results were captured using a Zeiss Stemi 2000-C dissecting microscope equipped with a color digital CCD camera (AxioCam MRc5, Zeiss). Fluorescent images of *Et(gata2a:EGFP)pku684* were taken under a Zeiss Axioimager Z1 fluorescence microscope equipped with a monochrome CCD camera (AxioCam MRm, Zeiss) and Zeiss filter set 10. Pseudo-color was added using the supplied AxioVision software (Zeiss). Fluorescent images of *Et(gata2a:EGFP)pku760* were taken under a Zeiss Axioimager A1 fluorescence microscope equipped with the color digital CCD camera.

Injection of mRNA encoding *Tol2* transposase, footprint analysis and screening for large chromosomal deletions

The mRNA encoding *Tol2* transposase was synthesized using pCS-TP plasmid by *in vitro* transcription using an SP6 mMESSAGE mMACHINE kit (Ambion) (7). One-cell stage homozygous *Et(gata2a:EGFP)pku684* or *Et(gata2a:EGFP)pku760* embryos were injected with 50-pg transposase mRNA to induce *Tol2* transposition. The *Et(gata2a:EGFP)pku684* founders were raised to adulthood and outcrossed with homozygous transgenic fish for footprint analysis and with wild-type fish for screening of chromosomal deletions. To examine the footprints of *Et(gata2a:EGFP)pku684*, the original *Tol2* insertion site in founder embryos or individual F_1 embryos was amplified by PCR (5′-TTATGTCATTTACTTTTATTGTTG-3′ and 5′-GTTTCTGCTCTTTTCCGACTT-3′) from genomic DNA and analysed by sequencing. To evaluate large deletions of *Et(gata2a:EGFP)pku684* in founder embryos, genomic DNA was extracted from groups of three to five 3 days post fertilization (dpf) embryos, and potential deletions were determined by sequencing after PCR amplification and electrophoresis (5′-TCAGGCAGAGATGAGCATCAG-3′ and 5′-ACGAGCTCAAACACGGAGTC-3′ for 5′ detection; 5′-TTATGTCATTTACTTTTATTGTTG-3′ and 5′-GCCCCATTCTCAGATTATTAC-3′ for 3′ detection). To screen for heritable genomic deletions in *Et(gata2a:EGFP)pku684*, F_1 embryos negative for EGFP or expressing EGFP in the non-intermediate cell mass (ICM) region were picked out under a microscope. Twenty selected F_1 embryos from the same founder were pooled, and genomic DNA was extracted at 5 dpf. Genomic deletions in these embryos were isolated by electrophoresis after PCR and determined by sequencing (5′-GTTTCTGCTCTTTTCCGACTT-3′ and 5′-ACGATGGAAGTGAATGGTT-3′ for 5′ detection; 5′-TTATGTCATTTACTTTTATTGTTG-3′ and 5′-TTCAGAAAGAAGCGGTCTC-3′ for 3′ detection). Footprints and large deletions in *Et(gata2a:EGFP)pku760* founders were examined similarly as for *Et(gata2a:EGFP)pku684* (5′-TTCTCAAGAGCCCTTGCTTG-3′ and 5′-AAGGACGCAGCAGGGAAG-3′ for footprint detection, 5′-TTCTCAAGAGCCCTTGCTTG-3′ and 5′-TGTGCTTTTGAGGGCAGTAG-3′ for 5′ deletion detection, 5′-GCGTGTTGTTTGGAGCCT-3′ and 5′-CCCGCATGATGTTTGTATG-3′ for 3′ deletion detection).

Plasmid-based excision assay

Transient excision of the *Tol2* transposable element from plasmid DNA pTol2-GT2MP after injection into zebrafish embryos was examined as described previously (20,23). Fifty picograms of the circular plasmid and 50-pg of capped transposase mRNA were co-injected into fertilized eggs at the one-cell stage. Each individual embryo was lysed at the bud stage followed by DNA extraction. Fragments of 560 bp were amplified from the DNA preparation by PCR (5′-CATCAGCCTCCCCGGTCCAT-3′ and 5′-GGCACGACAGGTTTCCCGAC-3′). The PCR products were gel purified and cloned into pMD18-T simple vector (Takara) for sequencing.

Southern blot

A 546-bp DNA fragment was amplified by PCR (5′-ACGATGGAAGTGAATGGTT-3′ and 5′-GATCTGTAAGTGCATAGAAGTC-3′) followed by ligation to pBluescript vector and used as the template for probe synthesis. The digoxigenin-labeled probe was synthesized using a PCR DIG Probe Synthesis Kit (Roche). Genomic DNA was extracted from adult fin clips, and 20 μg was digested

with PstI and SacI overnight. The digested DNA was separated on 0.8% agarose gel and transferred to nylon membrane (Amersham Hybond-N+). Hybridization was carried out following the manufacturer's instructions (Roche). The signal was detected using NBT and BCIP (Promega).

RESULTS

Identification of the *Et(gata2a:EGFP)pku684* and *Et(gata2a:EGFP)pku760* fish lines

Using a *Tol2* transposon cassette containing an *EGFP* reporter gene driven by a zebrafish *gata2a* minimal promoter, we performed a large-scale enhancer trap screen (unpublished data). From this screen, we identified mp684, a fish line also called *Et(gata2a:EGFP)pku684*, in which the EGFP signal was first detected in the two stripes of lateral mesoderm at 12 hpf. At 24 hpf, the EGFP expression was restricted to the ICM region and the sprouting intersegmental vessels (Supplementary Figure S1a). This indicated that both hematopoietic and endothelial cells were labeled in this line. To identify the trapped gene, we cloned the sequence flanking the *Tol2* transposon insertion site by linker-mediated PCR (Supplementary Figure S1c and Supplementary Table S1). Sequencing and Basic Local Alignment Search Tool (BALST) searching results showed that the *Tol2* enhancer trap cassette was inserted at 140-bp upstream of the transcription start of *lmo2*, a gene essential for hematopoiesis and angiogenesis (Supplementary Figure S1d). We further confirmed the insertion site by PCR with two primers across the transposon and one primer located within the transposon (Supplementary Figure S1e). Whole-mount *in situ* hybridization showed that the EGFP pattern of *Et(gata2a:EGFP)pku684* recapitulated endogenous *lmo2* expression (Supplementary Figure S1b). In addition, *Et(gata2a:EGFP)pku684* showed a fluorescence pattern similar to two previously reported *lmo2* transgenic fish lines, which were generated using *dsRed* and *EGFP* as reporters under the control of a 2.5-kb *lmo2* promoter (26). All these results suggested that the *Tol2* transposon trapped the enhancer of the *lmo2* gene in *Et(gata2a:EGFP)pku684* fish line.

Similarly, we identified another line *Et(gata2:EGFP)pku760*, also called MP760GFP or mp760a, in which the *Tol2* enhancer trap cassette was inserted in the first intron of gene *nav3* (Supplementary Figure S2e and Supplementary Table S1) (27). In this line, the EGFP signal was detected in endoderm, central nervous system and spinal cord, which was consistent with the *nav3* gene expression pattern (Supplementary Figure S2) (28,29).

Analyses of the efficiency and footprints of *Tol2* transposon excision

The *Tol2* transposon is mobilized from the genome in the presence of its transposase. We first evaluated the properties of *Tol2* transposon excision in *Et(gata2:EGFP)pku684* fish line. After injection of *Tol2* transposase mRNA into homozygous *Et(gata2:EGFP)pku684* embryos, DNA fragments flanking the *Tol2* insertion site were amplified by PCR and analysed by sequencing (Figure 1). Among 35 clones that were sequenced, 10 (28.6%) showed exactly the same sequence as the wild-type, indicating precise excision of the *Tol2* transposon (i.e. a complete reversal to the wild-type genomic sequence after *Tol2* excision). Footprints with various indels were detected in the remaining 25 (71.4%) clones (Figure 2a), suggesting that imprecise excision is dominant during *Tol2* transposition. We also examined the *Tol2* excision in *Et(gata2:EGFP)pku760* fish line using similar method. Among 41 clones that were sequenced, nine (22.0%) showed precise excision, and the remaining 32 (78.0%) clones displayed footprints with different indels (Figure 2b), which is comparable with the situation in *Et(gata2:EGFP)pku684*.

To analyse the excision efficiency and properties of the *Tol2* transposon in the zebrafish germ line, we raised the mosaic *Et(gata2a:EGFP)pku684* founder embryos to adulthood and outcrossed the founders with wild-type fish. The excision events were determined according to the EGFP patterns in F_1 embryos at 24 hpf (Figure 1). Negative or altered EGFP patterns indicated that the *Tol2* transposon had been excised from the original site in these F_1 embryos. We analysed 1882 F_1 embryos from 18 founders, and the excision efficiency of the *Tol2* transposon was 42.0% on average, ranging from 1.4% to 100% (Table 1). To determine the footprints left by *Tol2* excision, we crossed 13 mosaic founders from the above with homozygous *Et(gata2a:EGFP)pku684* fish. The flanking sequences at the original insertion site of individual F_1 embryos were PCR amplified and analysed by sequencing. On average, ~36.5% of the offspring, ranging from null to 100%, carried exactly the same sequence as the wild-type, resulting from the precise excision of the *Tol2* transposon, and 63.5% showed indels compared with the wild-type sequence, indicating that imprecise excision had occurred (Table 2 and Figure 3a). These results indicated that *Tol2* transposon could be efficiently excised from the zebrafish genome, and imprecise excision is also dominant in heritable germline cells after remobilization of *Tol2* transposon.

The *Tol2* transposon usually generates an 8-bp duplication of the flanking genomic target sequence after its integration, as seen in both of the two transgenic fish lines; however, we did not detect any footprints with a remaining duplication of exactly 8-bp after *Tol2* remobilization in any of the two lines. Instead, a full reversal to the wild-type genomic sequence (i.e. precise elimination of one copy of the 8-bp duplication) is readily detectable in a relatively high frequency in both fish lines and also in the heritable germline cells of *Et(gata2a:EGFP)pku684* fish. These observations are consistent with the previous reports showing that *Tol2* excision could be either precise or imprecise (19,20).

To further analyse the properties of *Tol2* transposon excision, we performed a plasmid-based excision assay in zebrafish embryos (Supplementary Figure S3a). The pTol2-GT2MP plasmid contains the *Tol2* transposon flanked by an 8-bp duplication in the plasmid backbone

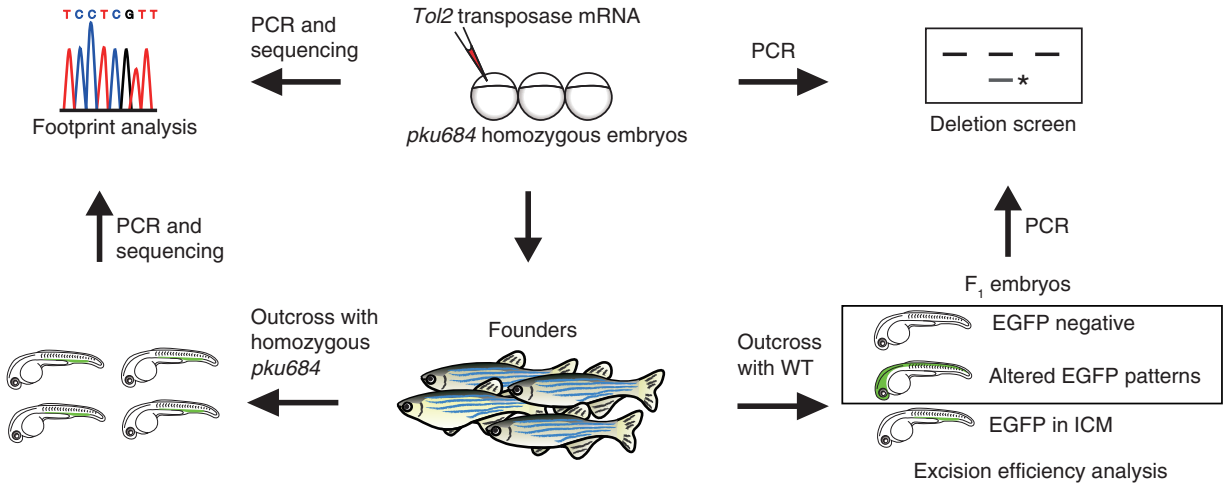

Figure 1. Schematic diagram showing the strategy for *Tol2* excision analysis and large genomic deletion screening using *Et(gata2a:EGFP)pku684* as an example. The *Tol2* transposon was considered to be excised from the original insertion site in embryos with negative or altered EGFP patterns. Asterik, PCR band containing relatively large genomic deletions; WT, wild-type. *Et(gata2a:EGFP)pku684* is simplified to *pku684*.

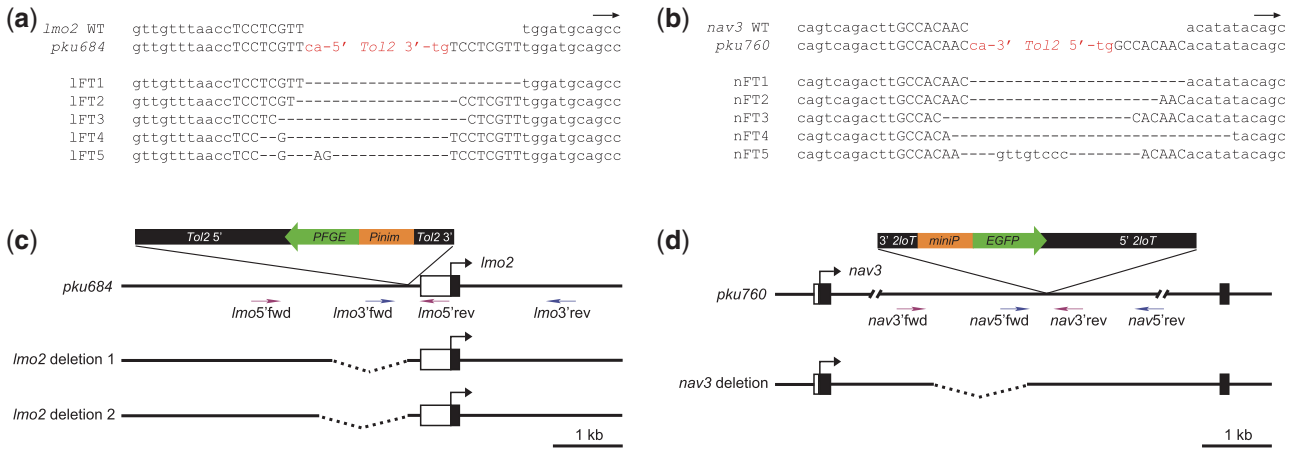

Figure 2. Analysis of footprints and genomic deletions induced by *Tol2* excision in zebrafish founder embryos. (**a**) Representative footprints detected at the *lmo2* locus after *Tol2* transposon excision. lFT1: precise excision; lFT2-5: imprecise excision. The arrow indicates the direction of transcription of *lmo2* gene. (**b**) Representative footprints detected at the *nav3* locus after *Tol2* transposon excision. nFT1: precise excision; nFT2-5: imprecise excision. The arrow indicates the direction of transcription of *nav3* gene. (**c**) Schematic diagram showing the 1093-bp and 1253-bp genomic deletions at the *lmo2* locus induced by *Tol2* excision. Inverted letters indicate that these elements (*miniP* and *EGFP*) were inserted reversely into the endogenous gene. Dashed lines indicate deletions. (**d**) Schematic diagram showing the 1340-bp genomic deletion at the *nav3* locus induced by *Tol2* excision. Inverted letters indicate that these elements (*Tol2* 5′ and *Tol2* 3′) were inserted reversely into the endogenous gene. Dashed lines indicate deletions. The 8-bp genomic duplication is shown in uppercase. WT, wild-type. *Et(gata2a:EGFP)pku684* is simplified to *pku684*; *Et(gata2a:EGFP)pku760* is simplified to *pku760*.

to mimic the situation after genomic insertion. DNA fragments with expected size (560 bp) were only amplified in the plasmid and transposase co-injected wild-type embryos, suggesting successful excision of the *Tol2* element and repair of the plasmid in the presence of the transposase (Supplementary Figure S3b). Among 24 clones that were sequenced, seven (29.2%) were exactly the same as the empty vector, indicating precise excision of the *Tol2* transposon. Footprints with various indels were detected in 15 (62.5%) clones. Interestingly, two clones were found to give rise to more than 100-bp deletions flanking the insertion site after *Tol2* excision, one being 173 bp at the 5′ end and the other 160 bp at the 3′ end (Supplementary Figure S3c). These results showed that precise excision leading to exact elimination of one copy of the 8-bp duplication flanking the *Tol2* integration site can also occur in the plasmids outside the zebrafish genome, and the ratio of the precise excision was also comparable with that of *Et(gata2a:EGFP)pku684* and *Et(gata2:EGFP)pku760*.

Taken together, all the aforementioned results demonstrated that the *Tol2* transposon can be efficiently excised from the original insertion site in both precise and imprecise manners with the imprecise excision being dominant. More importantly, both excision types could be efficiently inherited through germline cells.

Table 1. Efficiency of *Tol2* transposon excision in *Et(gata2a:EGFP)pku684* germline cells

Founder	EGFP patterns of F_1 embryos[a]			Total F_1 embryos[a]	*Tol2* excision efficiency (%)[c]
	ICM[b]	Negative	Non-ICM		
Male 1	45	5	0	50	10.0
Male 2	127	23	3	153	17.0
Male 3	0	15	0	15	100.0
Male 4	91	27	0	118	22.9
Male 5	51	42	22	115	55.7
Male 6	79	15	10	104	24.0
Male 7	12	16	0	28	57.1
Male 8	18	60	5	83	78.3
Male 9	0	75	72	147	100.0
Male 10	155	27	10	192	19.3
Male 11	24	123	3	150	84.0
Male 12	5	38	3	46	89.1
Male 13	127	68	22	217	41.5
Male 14	69	1	0	70	1.4
Female 1	69	4	0	73	5.5
Female 2	141	8	3	152	7.2
Female 3	71	39	0	110	35.5
Female 4	54	5	0	59	8.5
Average	/	/	/	/	42.0

[a]The F_1 embryos were obtained from crosses of each corresponding founder with wild-type fish.
[b]EGFP expression in ICM was considered to indicate no excision.
[c]Efficiency was calculated as percentage of embryos showing either 'Negative' or 'Non-ICM' GFP patterns over total embryos.

Table 2. Footprints after *Tol2* transposition in *Et(gata2a:EGFP)pku684* germline cells

Founder	Sequence Reads[a]	Precise (WT)		Imprecise (Indels)	
		Reads	Frequency (%)	Reads	Frequency (%)
1	28	25	89.3	3	10.7
2	39	36	92.3	3	7.7
3	37	0	0.0	37	100.0
4	7	7	100.0	0	0.0
5	6	0	0.0	6	100.0
6	22	17	77.3	5	22.7
7	1	0	0.0	1	100.0
8	10	1	10.0	9	90.0
9	19	1	5.3	18	94.7
10	11	11	100.0	0	0.0
11	7	0	0.0	7	100.0
12	24	0	0.0	24	100.0
13	14	0	0.0	14	100.0
Average	/	/	36.5	/	63.5

[a]The sequence was obtained from individual F_1 embryos from the crosses of each corresponding founder with homozygous *Et(gata2a:EGFP)pku684* fish.

Identification of genomic deletions induced by *Tol2* transposon excision

To determine whether genomic deletions larger than the indel mutations (e.g. >1 kb) can be induced by *Tol2* excision, we first examined the flanking regions of the *Tol2* insertion sites in mosaic *Et(gata2:EGFP)pku684* and *Et(gata2:EGFP)pku760* founders by PCR. If large genomic deletions occurred, smaller PCR products should be detected by electrophoresis (Figure 1). In *Et(gata2:EGFP)pku684* founders, of 34 pools (107 founder embryos in total), we identified relatively large deletions in 10 pools (i.e. a much shorter PCR fragment than expected) 5′ to the original *Tol2* insertion site. Sequencing results revealed two kinds of slightly different deletions. A 1093-bp deletion was identified in nine pools, and a 1253-bp deletion was identified in one pool (Figure 2c and Supplementary Figure S4). In *Et(gata2:EGFP)pku760* founders, of 20 pools (100 founder embryos in total), we identified a 1340-bp deletion 3′ to the original *Tol2* insertion site in one pool (Figure 2d and Supplementary Figure S5). We did not find comparable large deletions either at 3′ of the *Tol2* transposon in *Et(gata2:EGFP)pku684* or at the 5′ of the *Tol2* transposon in *Et(gata2:EGFP)pku760* founders embryos.

Next, we went on to determine whether similar large genomic deletions can be induced in germline cells. We crossed the mosaic *Et(gata2a:EGFP)pku684* founders with wild-type zebrafish and picked out F_1 embryos with either negative or altered EGFP patterns, in which the *Tol2* transposon had been mobilized from the original insertion site. Of 59 founders, we identified one that transmitted a genomic deletion 5′ to the original *Tol2* insertion site to its EGFP-negative offspring (Figure 3b). Sequencing result showed that the corresponding allele (called $lmo2^{pku684ld}$) carried a 1093-bp deletion, the same as one of the two large deletions detected in the mosaic founder embryos (Figure 3c). However, we did not find any large deletion at 3′ to the *Tol2* insertion site from these founders. To further confirm this deletion, we performed Southern blot using a probe located 5′ upstream of the deletion region (Figure 4a). The fish heterozygous for $lmo2^{pku684ld}$ showed a 4.7-kb wild-type band and a 3.7-kb band owing to the deletion (Figure 4b), as expected. These results showed that *Tol2* transposon excision can induce relatively large genomic deletions after imprecise repair in both somatic and germline cells.

DISCUSSION

With the development of engineered endonuclease technologies, targeted disruption of genes of interest is now feasible in zebrafish (30–36). However, targeted deletion of relatively large DNA fragments in the zebrafish genome has not been reported, although it is also useful, and sometimes indispensable, for the dissection of gene functions and developmental mechanisms, as well as disease modeling. Here, we provide a strategy to achieve this type of genome modification by deleting the genomic region flanking the *Tol2* insertion site through imprecise excision of this transposon. We have detected >1-kb genomic deletions in two independent transgenic fish lines, with one deletion showing inheritable germline transmission. Several large-scale enhancer trap and gene trap screens based on the *Tol2* transposon have been carried out in our laboratory and by others (7–16,23). It has been shown that the *Tol2* transposon tends to insert

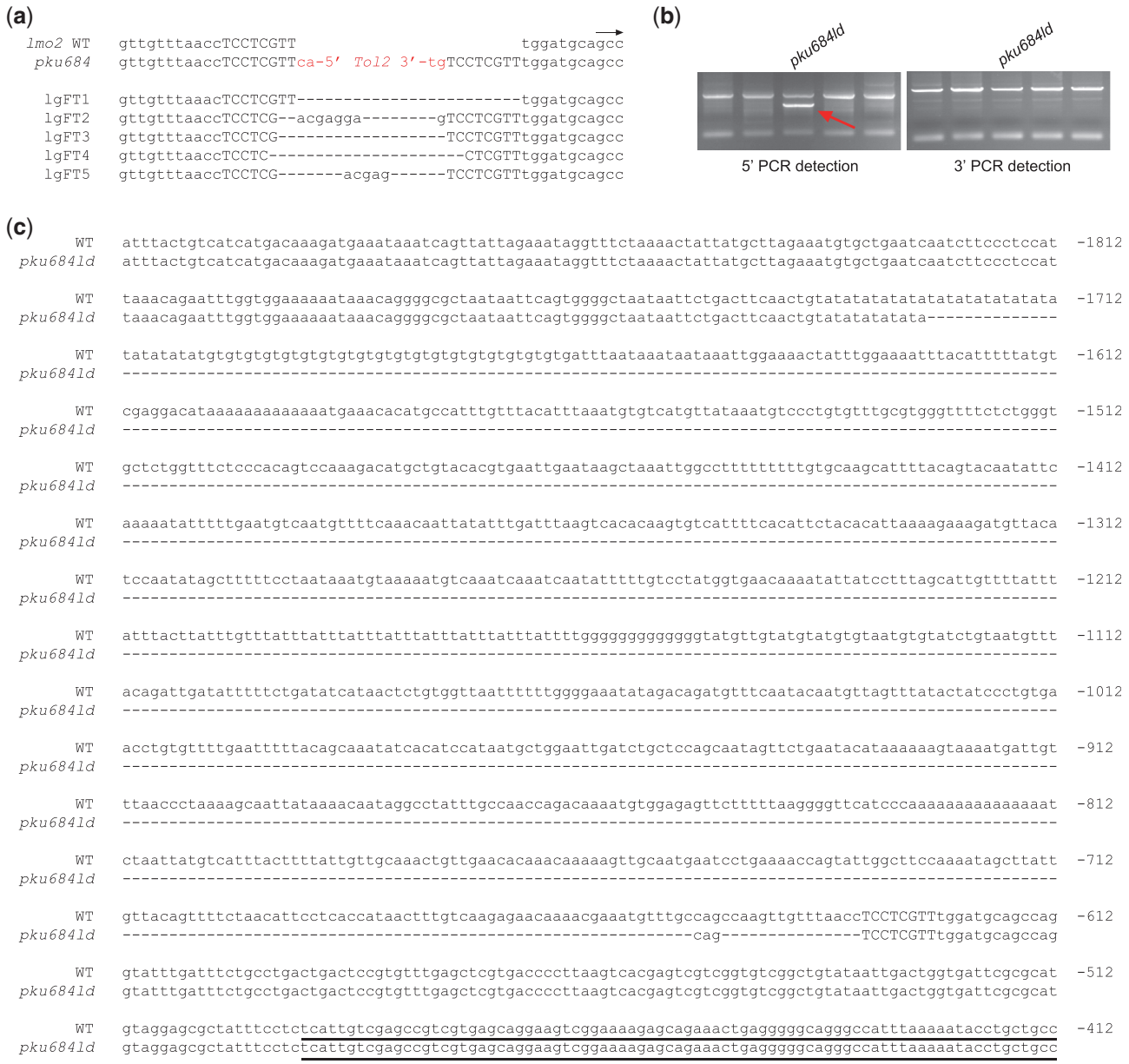

Figure 3. Analysis of footprints and genomic deletions induced by *Tol2* excision in the *Et(gata2a:EGFP)pku684* germline. (**a**) Representative footprints detected in F_1 embryos. lgFT1: precise excision; lgFT2-5: imprecise excision. The arrow indicates the direction of transcription of *lmo2* gene. (**b**) PCR results to detect large genomic deletion events in F_1 embryos. The red arrow denotes the PCR band containing relatively large genomic deletions. (**c**) Nucleotide sequence alignment showing the 1093-bp genomic deletion in $lmo2^{pku684ld}$. The transcribed region of the *lmo2* gene is underlined. The translation start site (ATG) is set as +1. The 8-bp genomic duplication is shown in uppercase. WT, wild-type. *Et(gata2a:EGFP)pku684* is simplified to *pku684*; $lmo2^{pku684ld}$ is simplified to *pku684ld*.

into or near genes, and many of its insertion sites have been identified (9,23). The collections of *Tol2* insertions within or near a variety of genes comprise unique resources to achieve gene disruption through transposon excision. In addition, here we reported the identification of two enhancer trap lines *Et(gata2:EGFP)pku684* and *Et(gata2:EGFP)pku760*, which faithfully recapitulates the expression pattern of the endogenous genes *lmo2* and *nav3*, respectively. To our knowledge, *Et(gata2:EGFP)pku760* is the first transgenic fish available to trace the live and dynamic expression of *nav3*, which is a valuable tool for studies of the function and regulation of this gene.

Our results and those of others have shown that *Tol2* transposon excision is either precise or imprecise (Table 2) (19,20). When *P* element mobilizes from the chromosomal DNA, the original insertion site can be repaired by using its wild-type homologous chromosome as the template, resulting in precise excision (1). As we injected *Tol2* transposase mRNA into homozygous transgenic embryos devoid of wild-type homologous chromosome, the wild-type sequence generated by *Tol2* mobilization could not come from its homologous chromosome. This indicates that mobilization of the *Tol2* transposon and elimination of the 8-bp duplication is an intrinsic activity/property

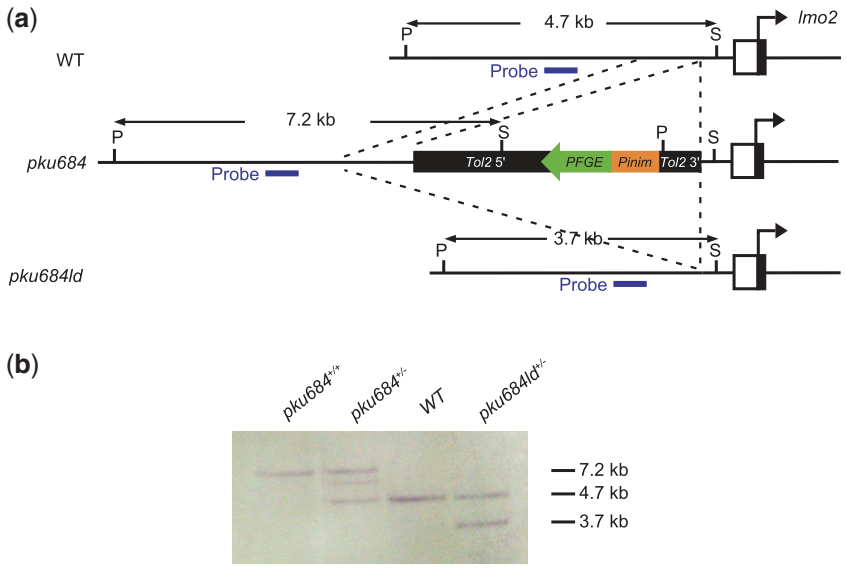

Figure 4. Southern blot analysis of the genomic deletion at the *lmo2* locus. (**a**) Schematic diagrams of the *lmo2* locus in wild-type, *Et(gata2a:EGFP)pku684* and $lmo2^{pku684ld}$ fish. Inverted letters indicate that these elements (*miniP* and *EGFP*) were inserted reversely into the endogenous gene. Blue bars indicate the position of the probe used for Southern blot. P and S indicate PstI and SacI, respectively. (**b**) Southern blot results of the *lmo2* locus. WT, wild-type. *Et(gata2a:EGFP)pku684* is simplified to *pku684*; $lmo2^{pku684ld}$ is simplified to *pku684ld*.

of *Tol2* transposase. We propose that *Tol2* transposase may create a complementary sticky end from each of the two 8-bp duplications during transposition at the original insertion site. In this way, the original insertion site could be restored exactly to the wild-type sequence after repair/ligation. The imprecise footprints left after *Tol2* excision may result from the modification of the DNA double-strand breaks through the non-homologous end-joining repair pathway. However, the precise mechanism of *Tol2* transposition still needs further investigation.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Table 1 and Supplementary Figures 1–5.

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