Published in final edited form as:

Dev Dyn. 2008 September ; 237(9): 2460–2465. doi:10.1002/dvdy.21673.

G2R Cre Reporter Transgenic Zebrafish

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Abstract

The Cre/loxP site-specific recombination system has been widely used to manipulate DNA in vivo and to study gene function in the mouse by inducible transgenic and conditional gene targeting. To fully use this powerful genetic tool in a relatively new animal model, zebrafish, we generated reporter transgenic lines for easy detection of Cre recombinase activity in vivo. The transgenic fish lines, designated G2R, express two fluorescent protein genes, GFP and RFP, under the control of the ubiquitous promoter of the *Xenopus EF1 alpha* gene. The G2R animals change their color from green to red (G2R) after Cre-mediated recombination and are useful for development of cell type specific Cre transgenic lines and for cell lineage and fate mapping studies in zebrafish.

Keywords

Cre/loxP; transgenic zebrafish; Cre-reporter fish; conditional gene expression

Introduction

Site-specific recombination systems, including Cre/loxP, Flp/FRT, and PhiC31 int/att, have provided powerful genetic tools to manipulate DNA not only in vitro at the plasmid level, but also in vivo at the chromosome level (van der Weyden et al., 2002; Branda and Dymecki, 2004). Cre recombinase functions in organisms ranging from bacteria to mammals and has revolutionized mouse genetics for efficient inducible transgenic studies and conditional gene targeting (Lakso et al., 1992; Orban et al., 1992; Gu et al., 1994). Cre-transgenic mice are important components of the Cre/loxP system for gene functional analysis and require a sensitive Cre-reporter to monitor their recombinase activity. Several such Cre-reporter lines have been established in mice (reviewed in Branda and Dymecki, 2004). Rosa26R is one of the most commonly used reporter mouse lines (Soriano, 1999). In this mouse, the *lacZ* gene is integrated into the ubiquitously expressed *Rosa26* locus with a floxed neo cassette (the neo gene is flanked with two loxP sites) upstream of it so that expression of the *lacZ* gene is prevented before Cre recombination. After excision of the neo cassette, *lacZ* starts to be expressed and can be detected by X-gal staining. Z/AP is another commonly used reporter mouse line (Lobe et al., 1999). The transgene of the Z/AP mouse encodes two enzymes, alkaline phosphatase (AP) and beta-galactosidase (LacZ) and is designed to switch the expression of AP to LacZ by Cre-mediated recombination for positive-negative detection. These Cre reporter mice have greatly facilitated the generation of tissue-specific Cre transgenic mice for spatially and temporally controlled gene activation and disruption.

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The zebrafish (*Danio rerio*) has recently emerged as a model animal to study vertebrate development, genetics, and human diseases (Lieschke and Currie, 2007; Kari et al., 2007). Zebrafish allows for powerful genetic analysis to be performed and combines the advantages of both invertebrates such as *Drosophila* and *C. elegans* (which can be subjected to large-scale mutagenesis and chemical screening) and vertebrates such as the mouse (which shows more similarity to the human than invertebrate models). The Cre/loxP system should be useful for zebrafish genome manipulation because it is functional in zebrafish (Langenau et al., 2005; Thummel et al., 2005; Pan et al., 2005). Two recent studies generated Cre-reporter transgenic zebrafish with the same strategies used in the Rosa26R and Z/AP mouse lines (Thummel et al., 2005; Pan et al., 2005). Fluorescence color switch from green (GFP) to red (RFP) was designed for detection of the Cre-mediated recombination in these studies. Although GFP signals in the transgenic fish disappeared after activation of Cre recombinase, the RFP signal was not well detected by fluorescence microscopy. Several possible reasons for the low level of RFP were noted, such as a coding frame shift by the ATG in the loxP site, slow maturation of the prototype DsRed (Baird et al., 2000; Bevis and Glick, 2002), and poor or mosaic expression by a mammalian viral CMV promoter in zebrafish cells (Thummel et al., 2005; Pan et al., 2005). In this report, we show the establishment of stable transgenic zebrafish lines that exhibit a color conversion from green to red by Cre-mediated recombination. This was accomplished with the use of the Tol2 system along with the following changes: (1) the transgenic expression of fluorescent proteins by elimination of extra translation start signals in the expression vector, (2) using the fast maturing variant DsRed.T1 (Bevis and Glick, 2002), and (3) using the *Xenopus elongation factor 1 alpha (EF1a)* promoter (Johnson and Krieg, 1994), which has been reported to show high and ubiquitous expression in zebrafish (Kawakami et al., 2004).

Results and Discussion

The G2R (green to red) plasmid was constructed based on a modified Tol2 transposon vector (Kawakami et al., 2004; Fig. 1). Two fluorescent protein genes, EGFP (GFP) and DsRed.T1 (RFP, Bevis and Glick, 2002), were separated by a triple, tandem repeat of SV40 polyadenylation signals (3xpA), which prevents RFP expression by any possible transcriptional read-through. The GFP cassette (GFP plus 3xpA) was flanked by two loxP sites and the fluorescent protein expression was driven by the ubiquitous promoter of the *Xenopus EF1a* gene (Johnson and Krieg, 1994; Kawakami et al., 2004). An intron cassette from rabbit *beta-globin* was added between the promoter and the 5' loxP site. G2R is designed to switch the color from green to red once the floxed GFP cassette is excised out by Cre-mediated recombination. After removal of the transcriptional termination signals, GFP expression is stopped and RFP expression is initiated.

Several improvements were incorporated into this G2R plasmid compared with the previous studies (Thummel et al., 2005; Pan et al., 2005). To make certain that the RFP is correctly and efficiently expressed after excision of the GFP cassette, no additional translation start codon (ATG) was introduced into the plasmid. The RFP variant (DsRed.T1; Bevis and Glick, 2002) used in G2R matures 10–15 times faster than the wild-type protein cloned from *Discosoma* coral (DsRed or drFP583; Matz et al., 1999) and produces enhanced fluorescence, providing a high detection sensitivity. The *Xenopus EF1a* promoter (Johnson and Krieg, 1994) in the G2R plasmid is one of the commonly used ubiquitous promoters in zebrafish (Kawakami et al., 2004). This promoter includes an intron and enhancer sequences and provides stable and maximal expression of the transgene (Burket et al., 2008). We used the Tol2 transposon system (Kawakami et al., 2004) for efficient germ line transmission to establish stable transgenic fish lines. This Tol2 transposon-based system has been widely used for zebrafish transgenesis (reviewed in Kawakami, 2005, 2007).

To test this color conversion system, transient transgenic analysis was performed. Embryos at the one-cell stage were injected with the G2R plasmid only or co-injected with the G2R plasmid and the in vitro synthesized Cre mRNA. Examination with a dissecting fluorescence microscope at 1 day postfertilization (dpf) revealed that 98% (122/124) of the co-injected embryos showed strong red fluorescent signals while 95% (84/88) of the control embryos injected with the plasmid only displayed green signals (Supplementary Figure S1, which can be viewed online). This result clearly indicates that Cre recombinase is functional in the zebrafish embryos as previously reported (Langenau et al., 2005; Thummel et al., 2005; Pan et al., 2005) and that our G2R construct permits detection of Cre activity in vivo by a color conversion as we expected.

Three transgenic lines (*Tg(EF1a:loxP-GFP-loxP-RFP)* 6211, *Tg(EF1a:loxP-GFP-loxP-RFP)* 622, and *Tg(EF1a:loxP-GFP-loxP-RFP)* 627) were established and used for further studies of the color conversion system. In the stable transgenic embryos, GFP expression was detected at 10–12 hours postfertilization (hpf). To evaluate the Cre-mediated recombination in the G2R fish, 1-cell stage embryos of the G2R fish were injected with Cre mRNA and their fluorescence color patterns were observed at 1 dpf. Injected transgenic embryos from all three transgenic lines showed uniform and strong RFP (red) signals and no GFP (green) expression, indicating that the Cre-mediated DNA recombination is highly efficient in stable transgenic fish. In contrast, the uninjected transgenic embryos displayed only GFP (green) signals (Supplementary Table S1). These experiments further confirmed our results from transient transgenic analysis.

Approximately 50% of embryos from heterozygous fish of lines *Tg(EF1a:loxP-GFP-loxP-RFP)* 6211 and *Tg(EF1a:loxP-GFP-loxP-RFP)* 622 were fluorescence-positive (Supplementary Table S1), suggesting these two transgenic lines carried a single copy of the transgene. *Tg(EF1a:loxP-GFP-loxP-RFP)* 622 was then used for generation of double transgenic animals (dTg) to investigate whether our G2R Cre-reporter transgenic fish is useful for detection of Cre activity in an inducible system. Expression of Cre in transgenic line HSP70-EGFP-cre (HS-Cre) is inducible by a brief heat shock (Thummel et al., 2005). In this HS-Cre fish, the fusion protein of GFP and Cre is controlled by the zebrafish *heat shock protein 70* (*hsp70*) promoter. To further characterize induction of Cre in this transgenic line, embryos at 1 dpf were incubated at 38°C for 1 hr. Expression of the GFP and Cre fusion protein was detected 3–4 hr after the heat shock. This heat-induced transgene expression was transient. The green signal in the heated HS-Cre embryos was greatly reduced in 24 hr and almost completely disappeared by 48 hr (Supplementary Figure S2). Heterozygous G2R fish were crossed to homozygous HS-Cre fish to generate dTg embryos. Green embryos (dTg) at 1 dpf were incubated at 38°C for 1 hr and observed from 5 to 48 hr postheat shock. RFP expression started at 5 hr in a mosaic pattern and became strong and ubiquitous by 24 hr (Supplementary Table S2). At 3 dpf (48 hr postheat shock), the green signal in the heated dTg embryos was significantly reduced compared with the unheated dTg and G2R single transgenic (sTg) controls (Fig. 2A). These residual weak GFP signals in the heated dTg embryos were not likely derived from the GFP-Cre fusion protein as these signals were nearly absent at this stage (Supplementary Fig. S2). Rather, they might result from the G2R transgene due to an imperfect heat induction and/or the prolonged stability of GFP mRNA and protein. All heated dTg embryos ($n = 109$, Supplementary Table S2) displayed strong and ubiquitous RFP expression while G2R sTg embryos showed only the autofluorescence in the yolk. A strong red signal in the ocular lens of the unheated dTg embryos (Fig. 2A, arrowheads) was an indication of the GFP cassette excision by Cre activity, which may be a result of the spontaneous transcriptional activity of the zebrafish *hsp70* promoter in the lens under non-stressed conditions (Blechinger et al., 2002). No detectable green signals in the lens of HS-Cre sTg embryos (Supplementary Figure S2) indicated that the GFP-Cre fusion protein was expressed at a relatively low level, which was sufficient to catalyze the excision of the GFP cassette as evidenced by very strong

to moderate red signals in the lens of the heated and unheated dTg embryos (Fig. 2A), respectively. These results suggest a high sensitivity of G2R to monitor the recombination mediated by Cre. Low but detectable red signals in the trunk region of the unheated dTg embryos were also observed (Fig. 2A). This nonlens expression of RFP was likely a result of the Cre-mediated recombination rather than a read-through from GFP to RFP, because such red signals were not observed in G2R sTg (Fig. 2A). The HS-Cre line used in this experiment might be leaky for the transgene expression at a low level. It is also possible that residual Cre expression might result from minor stress to the embryos under our embryonic culture conditions.

The Cre-mediated recombination was further confirmed by genomic polymerase chain reaction (PCR). At 48 hr after heat shock, embryos of three groups, heated dTg (red), unheated dTg (red-eyed), and heated G2R sTg (green), were collected. Genomic DNA was then extracted and used for PCR analysis. Four primers (p1 to p4) were designed (Fig. 1; Supplementary Table S3) to amplify recombinant and nonrecombinant forms of the G2R transgene. All three groups contained the G2R transgene as demonstrated by the presence of the PCR product amplified by the p3+p4 primers (300 bp) at a similar intensity (Fig. 2B). The quality of genomic DNA was assessed with successful PCR amplification of cytoplasmic actin (*bactin1*). The heated dTg embryos showed a great reduction in the non-recombinant-specific bands (p1+p2, 741 bp and p1+p4 upper, 2,041 bp) and a strong recombinant-specific band (p1+p4 lower, 554 bp). This result was consistent with the microscopic observation of the fluorescent color conversion. The unheated red-eyed embryos showed a partial recombination as they had both non-recombinant- and recombinant-specific bands (Fig. 2B). The p1+p4 lower band (554 bp) in the unheated dTg embryos may be derived predominantly from tissue-specific recombination in the lens. It should be noted that the relative intensity of the upper and lower bands amplified by p1 and p4 primers was not a direct reflection of the molar ratio of nonrecombinant and recombinant, because preferential amplification of smaller fragments of DNA by PCR likely occurred. As expected, no Cre- and recombinant-specific PCR products were detected in G2R sTg embryos.

To examine whether the G2R color conversion could detect Cre activity in vivo at single cell resolution, a new expression vector (8xHSE-Cre) was constructed and used to induce Cre expression in the G2R embryos. In this vector, an artificial eight-time repeat of the heat shock-inducible element of the human *HSP70.1* gene (Cunniff and Morgan, 1993; Bajoghli et al., 2004) and a minimum promoter of the zebrafish *gsnl1* gene (Yoshikawa et al., 2007) is used to drive Cre expression. One-cell stage embryos from Tg(*EF1a:loxP-GFP-loxP-RFP*)^{XZ622} were injected with 8xHSE-Cre and heated at 24 hpf for short periods (2, 5, or 30 min) to obtain mosaic recombination. Twenty-four hours after the heat shock, mosaic color conversion in various ratios was observed (Fig. 3B,C). The recombination efficiency induced by transient expression of 8xHSE-Cre likely depended on mosaicism of the injected transgene. In less-efficiently recombined embryos a single or a small cluster of red cells were observed (Fig. 3E,F,H,I), indicating that the G2R color conversion is suitable for detection of Cre activity at the cellular level.

In conclusion, we established stable transgenic lines of zebrafish that change their fluorescent color from green to red by Cre-mediated DNA recombination. They are valuable reporter animals for detection of the Cre enzymatic activity in vivo. Because the fluorescent color conversion can be easily monitored in live animals, these transgenic fish lines allow for continuous observation of developing embryos and permit detection of the Cre activity regardless of tissue type or developmental stage. Using the G2R system, monitoring Cre activity in vivo is noninvasive, in contrast to the invasive requirements of LacZ and AP. The green and red fluorescent proteins in the G2R system have been commonly used as visual markers in the fields of cell and developmental biology and do not require special equipment

for their detection other than a fluorescence microscope. These features will allow numerous laboratories to use the G2R fish very easily. The G2R fish can greatly assist development of cell type-specific Cre transgenic zebrafish and could be useful for cell lineage and fate mapping studies when it is combined with random introduction of Cre by a viral vector or electroporation.

Experimental Procedures

Plasmid Construction

To create the G2R plasmid, three nonessential parts of pTol2000 (Kawakami and Noda, 2004) were removed by restriction enzyme digestion, filled-in with Klenow fragment, and re-ligated with T4 DNA ligase. The following enzymes were used: *KpnI* and *PshAI* for the medaka tyrosinase gene, *SacI* and *NotI* for the extra multiple cloning sites of the pBluescriptII backbone, and *SfiI* and *AscI* for the nonessential Tol2 transposable element. The *Xenopus EF1a* promoter plus the rabbit *beta-globin* intron cassette from pT2K-XIG (Kawakami et al., 2004), EGFP from pEGFP-1 (Clontech), and fast maturing variant DsRed.T1 (Bevis and Glick, 2002) plus *Xenopus globin* 3' untranslated region were sequentially sub-cloned in the opposite orientation to the Tol2 transcription. The triple tandem repeat of SV40 polyadenylation signals (3xpA) was generated by PCR with BsrG-SV40pA-F and SV40pA-Acc65-R primers (Supplementary Table S3). The PCR product was double digested with *BsrGI* and *Acc65I*, generating compatible 5' overhangs of GTAC. After purification, the fragment was ligated and double digested to obtain the tandemly repeated fragments. The fragment corresponding to three repeats was then isolated by agarose gel electrophoresis and cloned into the *BsrGI* site of EGFP. Finally double stranded oligo DNA fragments for the loxP site (Supplementary Table S3) were generated and inserted upstream of EGFP (*BamHI* site) and downstream of 3xpA (*NotI* site), respectively. To create the 8xHSE-Cre, the eight-time repeats of the heat shock-inducible element were isolated from pSGH2 (a kind gift from Thomas Czerny) by digestion with *SacI*, and then cloned upstream of the 353-bp 5' flanking region of zebrafish *gsnl1* containing a minimum promoter (Yoshikawa et al., 2007) to drive Cre recombinase expression.

Microinjection

To obtain stable transgenic fish, one-cell stage embryos were co-injected with Hanks' balanced salt solution containing 10 ng/μl G2R plasmid, 5 ng/μl capped Tol2 transposase mRNA and 0.1% phenol red as previously described (Yoshikawa et al., 2007). The injected volume was controlled by keeping the diameter ratio of the red injected solution to the spherical fertilized eggs in the range of 1:5 to 1:4 under the microscope, resulting in 0.8% to 1.6% of the total volume of the embryo. The pCS-TP plasmid (Kawakami et al., 2004) encoding the transposase was linearized with *NotI* and used as the template for in vitro transcription with mMessage mMachine (Ambion), according to the manufacturer's instruction. For transient Cre expression, 0.1 ng/μl mRNA was injected.

Transgenic Screening

To establish stable transgenic lines of G2R, the injected embryos were raised to adulthood and the 3-month-old fish (F0) were crossed to wild-type fish. F1 embryos were examined under a fluorescence microscope for identification of germline-transmitted F0 founders. Positive F1 embryos were raised to adulthood and were then screened in the same way to estimate copy numbers of the transgene and to establish stable transgenic lines. Three independent lines (F2 and more advanced generations) were obtained, two of which contained a functional single copy of G2R.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Dr. Ryan Thummel for HSP-EGFP-cre fish, Dr. Shinichi Aota for DsRed.T1 and Dr. Thomas Czerny for pSGH2. X.C.Z. received funding from Hermann Eye Fund and National Eye Institute. The Department of Ophthalmology and Visual Science, University of Texas Health Science Center at Houston received funding from the National Eye Institute and Research to Prevent Blindness.

Grant sponsor: the Hermann Eye Fund; Grant sponsor: National Eye Institute; Grant number: R21EY018728; Grant number: P30EY10608; Grant sponsor: Research to Prevent Blindness.

References

- Baird GS, Zacharias DA, Tsien RY. Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proc Natl Acad Sci U S A* 2000;97:11984–11989. [PubMed: 11050229]
- Bajoghli B, Aghaallaei N, Heimbucher T, Czerny T. An artificial promoter construct for heat-inducible misexpression during fish embryogenesis. *Dev Biol* 2004;271:416–430. [PubMed: 15223344]
- Bevis BJ, Glick BS. Rapidly maturing variants of *Discosoma* red fluorescent protein (DsRed). *Nat Biotechnol* 2002;20:83–87. [PubMed: 11753367]
- Blechliger SR, Evans TG, Tang PT, Kuwada JY, Warren JT Jr, Krone PH. The heat-inducible zebrafish hsp70 gene is expressed during normal lens development under non-stress conditions. *Mech Dev* 2002;112:213–215. [PubMed: 11850198]
- Branda CS, Dymecki SM. Talking about a revolution: the impact of site-specific recombinases on genetic analysis in mice. *Dev Cell* 2004;6:7–28. [PubMed: 14723844]
- Burket CT, Montgomery CE, Thummel R, Kassen SC, LaFave MC, Langenau DM, Zon LI, Hyde DR. Generation and characterization of transgenic zebrafish lines using different ubiquitous promoters. *Transgenic Res* 2008;17:265–279. [PubMed: 17968670]
- Cunniff NF, Morgan WD. Analysis of heat shock element recognition by saturation mutagenesis of the human HSP70.1 gene promoter. *J Biol Chem* 1993;268:8317–8324. [PubMed: 8463341]
- Gu H, Marth JD, Orban PC, Mossmann H, Rajewsky K. Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting. *Science* 1994;265:103–106. [PubMed: 8016642]
- Johnson AD, Krieg PA. pXex, a vector for efficient expression of cloned sequences in *Xenopus* embryos. *Gene* 1994;147:223–226. [PubMed: 7926804]
- Kari G, Rodeck U, Dicker AP. Zebrafish: an emerging model system for human disease and drug discovery. *Clin Pharmacol Ther* 2007;82:70–80. [PubMed: 17495877]
- Kawakami K. Transposon tools and methods in zebrafish. *Dev Dyn* 2005;234:244–254. [PubMed: 16110506]
- Kawakami K. Tol2: a versatile gene transfer vector in vertebrates. *Genome Biol* 2007;8(suppl 1):S7. [PubMed: 18047699]
- Kawakami K, Noda T. Transposition of the Tol2 element, an Ac-like element from the Japanese medaka fish *Oryzias latipes*, in mouse embryonic stem cells. *Genetics* 2004;166:895–899. [PubMed: 15020474]
- 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;7:133–144. [PubMed: 15239961]
- Lakso M, Sauer B, Mosinger B, Lee EJ, Manning RW, Yu SH, Mulder KL, Westphal H. Targeted oncogene activation by site-specific recombination in transgenic mice. *Proc Natl Acad Sci U S A* 1992;89:6232–6236. [PubMed: 1631115]
- Langenau DM, Feng H, Berghmans S, Kanki JP, Kutok JL, Lood AT. Cre/lox-regulated transgenic zebrafish model with conditional myc-induced T cell acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A* 2005;102:6068–6073. [PubMed: 15827121]

- Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. *Nat Rev Genet* 2007;8:353–367. [PubMed: 17440532]
- Lobe CG, Koop KE, Kreppner W, Lomeli H, Gertsenstein M, Nagy A. Z/AP, a double reporter for Cre-mediated recombination. *Dev Biol* 1999;208:281–292. [PubMed: 10191045]
- Matz MV, Fradkov AF, Labas YA, Savitsky AP, Zaraisky AG, Markelov ML, Lukyanov SA. Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat Biotechnol* 1999;17:969–973. [PubMed: 10504696]
- Orban PC, Chui D, Marth J. Tissue- and site-specific DNA recombination in transgenic mice. *Proc Natl Acad Sci U S A* 1992;89:6861–6865. [PubMed: 1495975]
- Pan X, Wan H, Chia W, Tong Y, Gong Z. Demonstration of site-directed recombination in transgenic zebrafish using the Cre/loxP system. *Transgenic Res* 2005;14:217–223. [PubMed: 16022392]
- Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 1999;21:70–71. [PubMed: 9916792]
- Thummel R, Burket CT, Brewer JL, Sarras MP Jr, Li L, Perry M, McDermott JP, Sauer B, Hyde DR, Godwin AR. Cre-mediated site-specific recombination in zebrafish embryos. *Dev Dyn* 2005;233:1366–1377. [PubMed: 15977183]
- van der Weyden L, Adams DJ, Bradley A. Tools for targeted manipulation of the mouse genome. *Physiol Genomics* 2002;11:133–164. [PubMed: 12464689]
- Yoshikawa S, Norcom E, Nakamura H, Yee RW, Zhao XC. Transgenic analysis of the anterior eye-specific enhancers of eh zebrafish gelsolin-like 1 (gsnl1) gene. *Dev Dyn* 2007;236:1929–1938. [PubMed: 17576137]

Fig. 1.

Scheme of the transgenic vector G2R (Green to Red). Two fluorescent genes, GFP and RFP, are separated by the transcription termination signal (3xpA). Gene expression is controlled by the ubiquitous EF1a promoter. The GFP cassette (GFP plus 3xpA) is flanked by two loxP sites. The entire cassette is cloned into a Tol2 transposon-based vector. Before recombination, only GFP is expressed because the transcription stops at the 3xpA. However, after the Cre-mediated recombination, the GFP cassette is excised out and RFP starts to be expressed instead of GFP. Half arrows (p1 to p4) indicate the polymerase chain reaction primer positions.

Fig. 2.

Cre-mediated recombination of G2R in transgenic embryos is detected by fluorescent color change and polymerase chain reaction analysis. **A:** Fluorescent color conversion by heat induced Cre. Double transgenic embryos (dTg) of HS-Cre and G2R were observed under a dissecting fluorescence microscope 48 hr after the heat shock (left column). The expression of GFP was significantly reduced and RFP was induced, compared to the control embryos of the unheated dTg (center) and G2R single transgenic (sTg, right). The unheated dTg displayed red eyes (arrowheads) due to the spontaneous expression of Cre by the HS promoter in the lens under non-stressed conditions. The sTg retained the strong green signals and showed only the autofluorescent red signals in the yolk (brackets). **B:** Genomic PCR of recombined G2R. The positions of the primers are represented in Figure 1. Consistent with the color conversion, the non-recombinant-specific bands, p1+p2 and p1+p4 upper, were greatly reduced but the recombinant-specific band, p1+p4 lower, was intensive in heated dTg (left lane). The unheated dTg (center) showed a partial recombination while the sTg (right) showed only the non-recombinant-specific band.

Fig. 3.

Cre-mediated recombination at the single cell level is detected by color conversion from green to red in the 2 days postfertilization (dpf) G2R embryos injected with 8xHSE-Cre. **A,D,G:** Left column, GFP. **B,E,H:** Center, RFP. **C,F,I:** Right, merged images. **A,B,C:** The 8xHSE-Cre injected and heat-shocked embryo (top) showed mosaic expression of RFP induced by transient transgenic Cre expression, while injected and unheated (center) and uninjected and unheated (bottom) embryos displayed the background level of red signals only. **D,E,F:** High magnification of the color conversion in the head of an 8xHSE-injected and heat-shocked embryo shows four-cell and two-cell clusters of RFP-positive cells (indicated by arrowheads). **G,F,I:** High magnification of the color conversion in the trunk-tail region of an 8xHSE-injected, heat-shocked embryo indicates RFP-positive cells in clusters of various sizes. Scale bars = 200 μ m.