SUPPLEMENTARY MATERIALS AND METHODS

CONSTRUCTION OF THE *pKT3TS-gT* VECTOR

A linker sequence containing *Cla*I and *Spe*I restriction enzyme sites was cloned into the *Pvu*II site of the *pK* plasmid backbone[1] with 5′ phosphorylated oligonucleotides 5′-ATCGATACTAGT GCGGCCGCCCGC-3′ and 5′-CCGCGGGCGGCCG CACTAGTATC-3′ to produce vector *pK-Cla*I*Spe*I. The *TAL-lac*Z-*Fok*I sequence was amplified from the *pT3TS-gT* vector with KOD (Novagen) using primers F1-*Cla*I 5′-GCTTATCGATAGAGCGCCC AATACGCAA-3′ and R2-*Spe*I 5′-ATCCACTAGTA ACGACGGCCAGTGAATT-3′ and cloned into the pCRII-TOPO blunt vector (Invitrogen). The *Cla*I-*Spe*I *TAL-lac*Z-*Fok*I fragment was subcloned into the *pK-Cla*I*Spe*I backbone to make *pKT3TS-gT*. Positive clones were sequenced with the following primers: F1-*pK* 5′-ATGGCTCATAACACCCCTTG-3′ and R1-*pK* 5′-CGCCTTTGAGTGAGCTGATA-3′. A sequence verified clone was submitted to Addgene for distribution.

COMPARISON OF TARGETING EFFICIENCY BY TALENS ASSEMBLED IN *pT3TS-gT* AND *pKT3TS-gT*

TALENS targeting a *Bcl*I site in the second exon of the zebrafish *rb1* gene were assembled in the *pT3TS-gT* and *pKT3TS-gT* vectors. *rb1* left TALEN sequence (+ strand) 5′-CCAGTCCACTAACTCC AT-3′; *rb1* right TALEN sequence (– strand) 5′-TCTCCCTCTCCCATATTCT-3′. TALENs previously designed to target the *Hinc*II restriction site in exon 3 of the zebrafish *cdh5* gene[2] were subcloned into the *pKT3TS-gT* vector. *pKT3TS-gT rb1* and *cdh5* TALEN plasmids were grown under kanamycin selection. The plasmids were linearized using *Spe*I for *in vitro* mRNA synthesis with the T3 mMessage Machine Kit (Ambion). mRNA was purified using the Qiagen RNA Easy Kit. An amount of 50 pg of mRNA was injected into the 1-cell-stage wild-type WIK zebrafish embryos (WIK from the Zebrafish International Stock Center). DNA was extracted 2 days postfertilization as described.[2] TALEN mutagenesis efficiency was analyzed by PCR amplification on single embryos across the target sites in *rb*1 and *cdh*5 with GoTaq (Promega) using the following primers: *rb1*-F 5′-TTTCCA GACACAAGGACAAGG-3′; *rb1*-R-5′-GCGGTAAA GCAGATATCAGAAGA-3′; *cdh5*-L 5′-TTGTTGTC CTTGCAAAGCTG-3′; *cdh5*-R 5′-TCTAGAGGATT CGCTGAT-3′. PCR products were digested with *Bcl*I for *rb1* and *Hinc*II for *cdh5*, and analyzed on a 10% polyacrylamide gel.

REFERENCES

1. Hyland KA, Olson ER, Clark KJ, et al. Sleeping Beauty-mediated correction of Fanconi anemia type C. J Gene Med 2011;13:462–469.

2. Hyatt TM, Ekker SC. Vectors and techniques for ectopic gene expression in zebrafish. Methods Cell Biol 1999;59:117–126.