BRIEF REPORT

GoldyTALEN Vectors with Improved Efficiency for Golden Gate TALEN Assembly

Jordan M. Welker,[1] Wesley A. Wierson,[1] Ying Wang,[1] Tanya L. Poshusta,[2] Melissa S. McNulty,[2] Ellen E. Tisdale,[1] Staci L. Solin,[1] Stephen C. Ekker,[2] Karl J. Clark,[2] Maura McGrail,[1] and Jeffery J. Essner[1,*]

[1]*Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, Iowa;* [2]*Department of Biochemistry and Molecular Biology, Mayo Clinic Cancer Center, Rochester, Minnesota.*

TALEN-MEDIATED GENOME EDITING has widespread utility in many systems for reverse genetic approaches. *GoldyTALEN,*[1] a Golden Gate–compatible platform for TALEN assembly,[2] was previously optimized by Bedell et al.[3] We found that the *pT3Ts-goldyTALEN* (*pT3TsgT*) Golden Gate vector is prone to recombination, leading to an increased number of false-positive colonies during the final TALEN assembly step. Here we report an updated vector, called *pKT3Ts-goldyTALEN* (*pKT3TsgT*), which was modified to prevent recombination and allow for selection with the more stable antibiotic kanamycin. The *pKT3TsgT* vector is compatible with the Golden Gate TALEN assembly protocol and produces TALENs with the same assembly efficiency and *in vivo* activity as the previous *pT3TsgT* vector. These modifications in pKT3TS-gT allowing for efficient assembly during automated high throughput TALEN production.

The GoldyTALEN scaffold was previously cloned into the mRNA expression vector *pT3TS*[4] to create *pT3TS-gT*.[3] We found that initial TALEN assemblies with *pT3TS-gT* were nearly 100% efficient, with 10/10 white colonies representing complete TALEN clones. However, with newer preparations of *pT3TS-gT* propagated in bacteria, the number of false-positive clones (white colonies) lacking TAL repeats routinely reached 90–95%, lowering the efficiency to 5–10%. Examination of the *pT3TS-gT* vector sequence revealed two *lac*Z sequences on opposing strands flanking the TAL RVD cloning site that could recombine and remove the RVD restriction sites and the *Fok*I sequence necessary for TALEN assembly (Fig. 1A). We observed both blue and white colonies after *pT3TS-gT* transformation, indicating that the plasmid stock contained a population of vectors lacking the *lacZ* selection gene (Fig. 1C). We re-engineered the vector to remove the secondary *lac*Z sequence (Fig. 1B and Supplementary Materials and Methods; Supplementary Data are available online at www.liebertpub.com/hum) and prevent recombination (Fig. 1D).

To verify that the new backbone did not alter TALEN mutagenesis activity, we compared TALEN mRNAs targeting the zebrafish *rb1* and *cdh5* genes produced with the *pT3TS-gT* and *pKT3TS-gT* vectors. For both *rb1* and *cdh5* genes, TALEN injection resulted in PCR amplicons that contained a restriction site in the targeted region and were almost completely resistant to restriction digestion (Fig. 1E), suggesting that insertion or deletion alleles were induced at frequencies that approach 100%. In contrast, control PCR amplification products from uninjected embryos were fully digested. These data indicate that TALENs generated from either vector will target mutations with equal efficacy *in vivo*. In summary, the modified *pKT3TS-gT* vector avoids recombination of the parental plasmid in bacteria, allowing for more efficient and reliable generation of TALENs for genetic studies in any TALEN-compatible system and aiding small laboratories in adopting this

**Correspondence: Dr. Jeffery J. Essner, Department of Genetics, Development, and Cell Biology, 1210 Molecular Biology Building, Iowa State University, Ames, IA 50011. E-mail: jessner@iastate.edu*

© 2016 by Mary Ann Liebert, Inc.
DOI: 10.1089/hum.2016.012

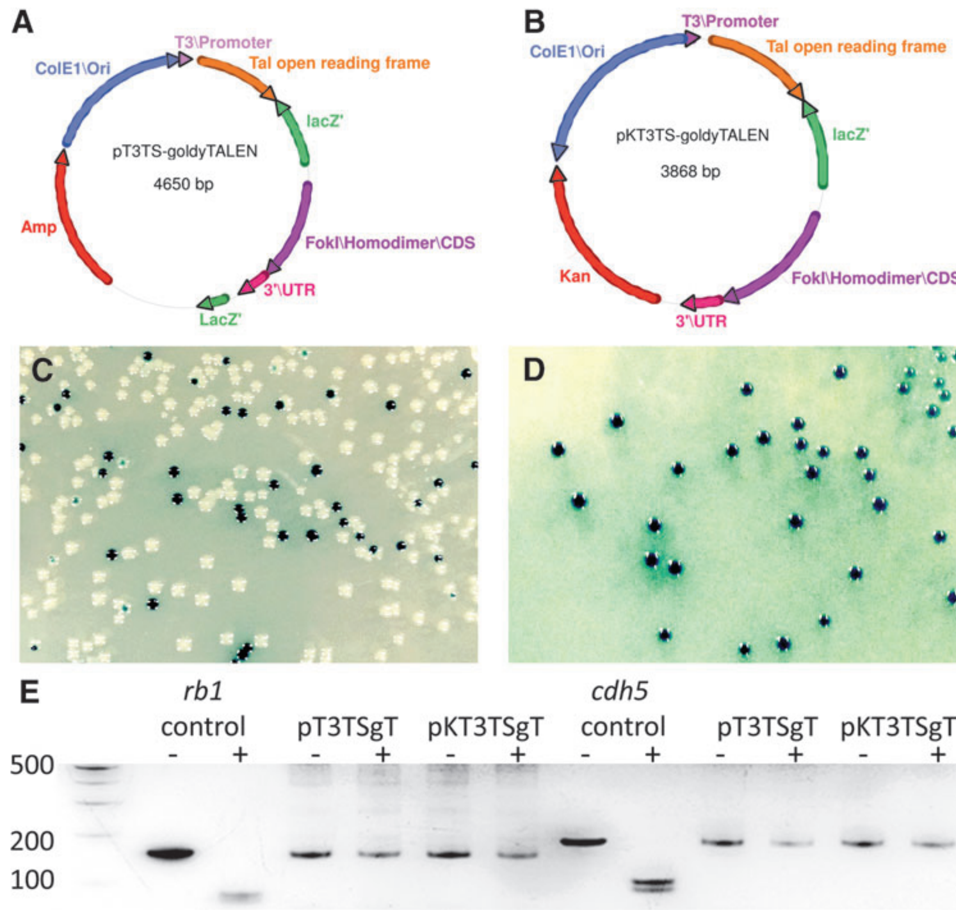

Figure 1. Comparison of recombination between the original pT3Ts-goldyTALEN (pT3TsgT) and the modified pKT3Ts-goldyTALEN (pKT3TsgT) vectors. **(A)** The original pT3TsgT vector has two *lacZ* sequences on opposite strands, allowing for recombination and excision of the RVD cut sites and the *Fok*I gene. **(B)** The modified pKT3TsgT vector lacks the second *lacZ* site and has a backbone that contains the kanamycin resistance gene. **(C)** Transformation of pT3TsgT and plating on LB-Xgal plates with the appropriate antibiotic produces 5 times as many white colonies as blue. **(D)** Plating pkT3TsgT on LB-Xgal plates results in only blue colonies. **(E)** Comparison of targeting efficiency between TALENs assembled in the pT3TsgT and pKT3TsgT vectors. Each TALEN pair targeting either *rb1* or *cdh5* resulted in 100% biallelic inactivation after injection into individual embryos. +, digested; –, undigested PCR amplicons.

technology. We anticipate that the increased vector stability of *pKT3TS-gT* will also facilitate efficient one-step TALEN assembly.[5]

ACKNOWLEDGMENTS

This work was funded by National Institutes of Health Grants GM088424 (J.J.E.), GM63904 and P30DK84567 (S.C.E., K.J.C.), and P30DK090728 (S.C.E.); the State of Minnesota Grant MNP #13.11 (S.C.E.); and the Roy J. Carter Charitable Trust Grant #14-4418 (M.M.).

AUTHOR DISCLOSURE

J.J.E. and K.J.C. own equity in Recombinetics, Inc. Other authors do not declare competing interests.

REFERENCES

1. Carlson DF, Tan W, Lillico SG, et al. Efficient TALEN-mediated gene knockout in livestock. Proc Natl Acad Sci U S A 2012;109:17382–17387.

2. Cermak T, Doyle EL, Christian M, et al. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res 2011;39:e82.

3. Bedell VM, Wang Y, Campbell JM, et al. *In vivo* genome editing using a high-efficiency TALEN system. Nature 2012;491:114–118.

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

5. Ma AC, McNulty MS, Poshusta TL, et al. FusX: A rapid one-step Transcription Activator-Like Effector Assembly System for Genome Science. Hum Gene Ther 2016 [Epub ahead of print].

Received for publication January 29, 2016;
accepted after revision April 16, 2016.
Published online: April 19, 2016.