Cite this as: Xinyi LI, Bing SUN, Hongrun QIAN, Jinrong MA, Magdalena PAOLINO, Zhiying ZHANG. A high-efficiency and versatile CRISPR/Cas9-mediated HDR-based biallelic editing system[J]. Journal of Zhejiang University Science B, 2022, 23(2): 141-152.
https://doi.org/10.1631/jzus.B2100196

A high-efficiency and versatile CRISPR/Cas9-mediated HDR-based biallelic editing system

Keywords: biallelic editing, CRISPR/Cas9, Homology-directed repair (HDR), homozygote

Research Summary

- **Puro-TK-GFP/Zeo-TK-RFP**

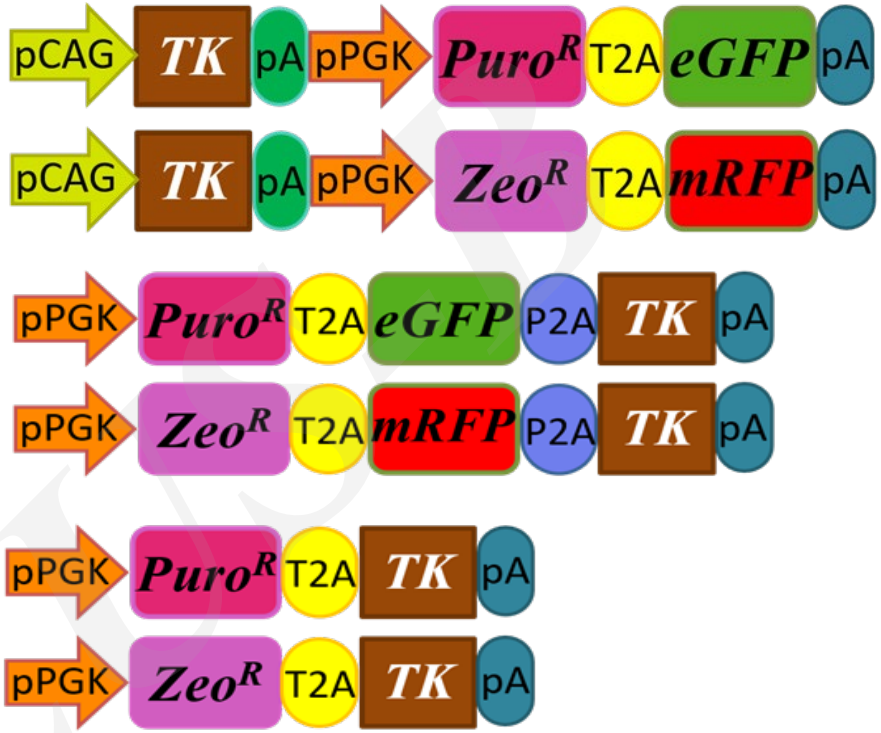
  TPG/TZR 5.1kb/4.9kb

- **Puro-GFP-TK/Zeo-RFP-TK**

  PGT/ZRT 3.2kb/2.9kb

- **Puro-TK/Zeo-TK**

  PT/ZT 2.4kb/2.2kb



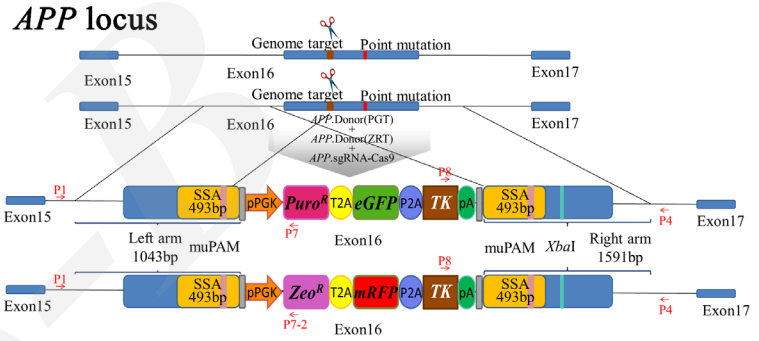
We generated **three pairs** of selection cassettes to achieve high efficiency of biallelic editing at different loci, allowing the insertion of 2kb-5kb fragments into the genome flanked with 0.8-1.6kb homology arms for homology-directed repair. *APP* and *PSEN1* loci were chosen to be tested in 293T cells, and reached up to 82% positive efficiency.

Innovation points

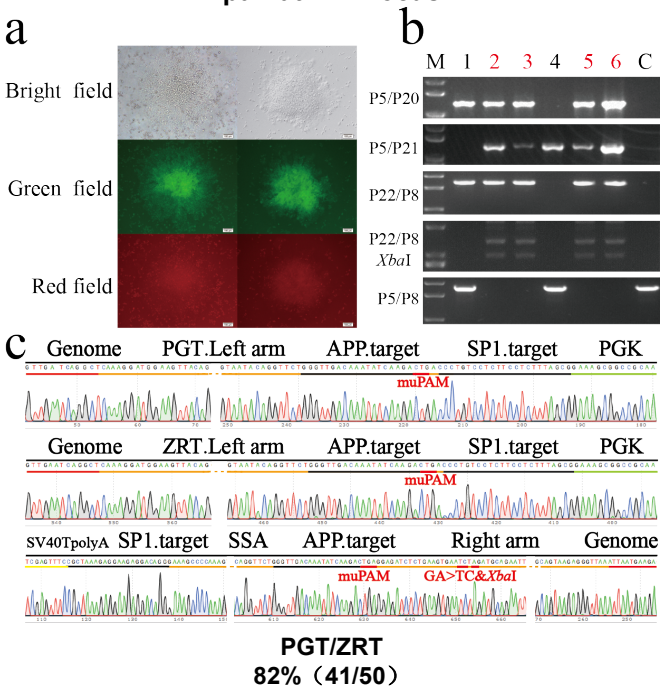
- We used **two donor vectors**, each containing a different fluorescent protein as well as a drug-resistant gene, could lead to higher biallelic editing efficiency.

- All **three pairs** of donors for targeted allele integration were proved to be efficient and versatile, allowing a highly efficient selection of biallelic targeted clones.

PGT/ZRT-dependent biallelic genome editing at the *APP* locus.



We got highest efficiency using PGT/ZRT pair at *APP* locus



PGT/ZRT
82%（41/50）