Resources

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TALEN and CRISPR/Cas9 technologies

TALEN plasmid vectors: The vectors are constructed in a configuration similar to the widely used TALEN plasmid (Nat. Biotech. 29:143, 2011), but using our own proprietary TALEN sequences. The FokI nuclease domain is made of obligate heterodimers (Nature Method 8:74, 2011) and with sharkey mutations (J. Mol. Biol. 400:96, 2010). There are a total of three sets of vectors either with different promoters (CMV or CAGGS) or with the presence or absence of a different fluorescent tag (EGFP or mCherry) between the two obligate heterodimers. The fluorescent tags facilitate live cell imaging or cell sorting for isolating cells expressing both TALEN obligate heterodimers simultaneously. These vectors are compatible with the TALE tetramer library (see below) for assembly of TALEN in a single step (with 17 repeats) or two steps (with 18-21 repeats) of Golden Gate reaction. TALENs assembled with these vectors have successfully been used for mutating endogenous genes in cultured cells and in creating mutant rodents (both mice and rats) by pronuclear injection.

a) **pCMV-TALEN** (KKR-NG, KKR-NI, KKR-HD, KKR-NN, ELD-NG, ELD-NI, ELD-HD, ELD-NN)
b) **pCAGGS-TALEN** (NG, HD)
c) **pCMV-TALEN-2A-tag** (EGFP: KKR-NG, KKR-NI, KKR-HD, KKR-NN; mCherry: ELD-NG, ELD-NI, ELD-HD, ELD-NN)

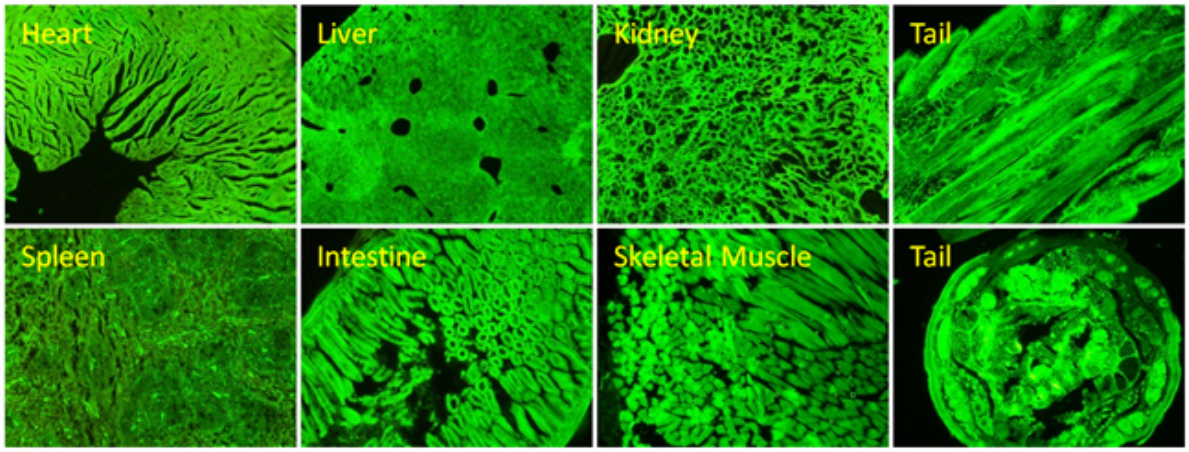
TALE Tetramer Library: The library contains a total of 1024 tetramer clones. With the TALEN vectors described above the tetramer clones allow for extremely high fidelity of assembly of TALEN with 17-21 repeats by a single or two steps of Golden Gate reaction within 3 working days. Importantly, the library supports low cost, high-throughput TALEN assembly in any laboratory with basic molecular biology setup. One person is able to generate 48 pairs of TALEN in a 96-well plate format in 3-4 working days.

Episomal reporters for TALEN activity assay: a simple double fluorescence episomal vector allows for cell-based (*e.g.* HEK293) TALEN activity testing. A derivative with drug resistance genes can be used as an episomal selection marker for TALEN-mediated endogenous gene mutagenesis in cultured cells.

Episomal reporters for gRNA assay: a simple fluorescence episomal vector allows for cell-based (*e.g.* HEK293) quick screening of a panel of candidate gRNAs.

Examples of Animal Models Currently Available

"Green mice": with a single copy of the EGFP gene under a CAGGS promoter inserted at the *Rosa26* locus. Heterozygotes (and homozygotes) exhibit a high level of EGFP expression in virtually all embryonic and adult tissues examined. These mice can be used as a source of donor tissues or cells for transplantation assays, allowing easy visualization and tracing of the transplanted material.



EGFP/WT, 6 month old

LRRK2 G2019S mice: with a glycine to serine mutation in the endogenous mouse LRRK2 gene at the position equivalent to human LRRK2 amino acid 2019, mimicking the human G2019S allele that results in predisposition to Parkinson's disease. The model was created by TALEN-mediated single nucleotide mutagenesis and has two unique features over the commonly used LRRK2 models: 1) homozygotes only produce G2019S mutant but not wild type LRRK2; and 2) the mutant LRRK2 is expressed at the physiological level under control of the endogenous regulatory mechanisms. Thus, this model is a valuable tool for studying LRRK2 G2019S biology and pathology *in vivo*.