B6-CAG-cas9-tdTomato

Strain Name： B6/JGpt-*Rosa26*$^{em1Cin(CAG\text{-}cas9\text{-}tdTomato)}$/Gpt
Strain Type： Knock-in
Strain Number： T004285
Background： C57BL/6JGpt

Description

Genetically modified mice (knockout, knock-in, mutation, heterologous gene expression, etc) have become important models for gene function and disease research. With the advent of new technologies such as ZFN, TALEN and CRISPR/Cas9, the technical difficulty of gene editing has been reduced. so the cost and time of making genetically modified mice models. especially the emergence of the CRISPR/Cas9 system, makes it easy to modify multiple sites at the same time[1-3].

However, it is still difficult to modify genes in tissue- and cell-specific regions using the CRISPR/Cas9 system. Because it is difficult to find a promoter expressing Cas9 specifically, so the expression region and timing are difficult to control. It is commonly used viral vectors such as AAV(adeno-associated virus) and LV (lentivirus) to expression CRISPR/Cas9 system in the tissues or cells of living mice, but the loading capacity of AAV and LV was limited[4-6]. It is difficult to simultaneously express sgRNA and Cas9 protein. High-pressure fluid tail vein injection can overcome the size limitation of vectors, but it is only effective in a few organs such as the liver and has low efficiency.

GemPharmatech based on the T002249 B6-CAG-LSL-cas9-tdTomato mouse model, mated with the systemic expression of Cre recombinase to remove the STOP element and open the expression of cas9 and tdTomato, remove the expression of Cre by backcrossing, established of B6-CAG-cas9-tdTomato strain. It has been verified that the liver, spleen, lung, kidney, brain, pancreas, stomach, bladder, and intestine tissues expressed red fluorescence and Cas9 protein in different intensity, but it was hardly detected Cas9 protein in heart and muscle. When using this strain to editing the tissue or cell in living mice, it is no longer to use AAV and LV which simultaneously express specific promoter, sgRNA and Cre recombinase for infection, and only need to provide a vector expressing sgRNA. It not only saves the trouble of searching for a specific promoter, but also greatly saves the virus loading space and can improve the efficiency of virus transfection and gene editing.

Risk warning: According to the internal test results, this model cannot be used as fluorescent reporter mice alone, especially for labeling of

cardiomyocytes, skeletal muscles and CD45+ immune cells. Notably, the Cas9 protein is barely expressed in cardiac muscle, skeletal muscle and CD45+ immune cells.

Strategy

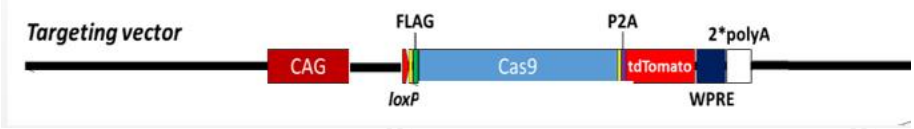

Fig1. B6-CAG-cas9-tdTomato model strategy

Application

1.CRISPR/Cas9 gene editing
2.Study single or multiple gene knock-out/knock-in in vivo
3.Conditional gene editing
4.Cancer related research

Data Support

1. Systemic expression of tdTomato assay

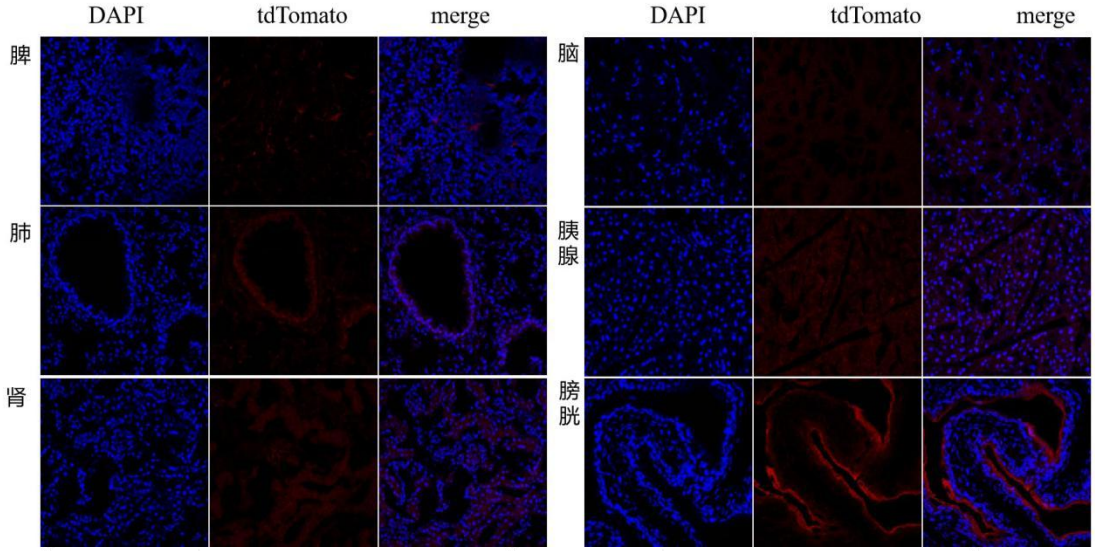

Fig2. Detection of red fluorescent protein expression in B6-CAG-cas9-tdTomato.

The tissues of the liver, spleen, lung, kidney, brain, pancreas, stomach, bladder and intestine were all expressed red fluorescent protein with different intensities in B6-

CAG-cas9-tdTomatomice. The results indicate that B6-CAG-cas9-tdTomato mice can express tdTomato protein.

2. Cas9 protein expression assay

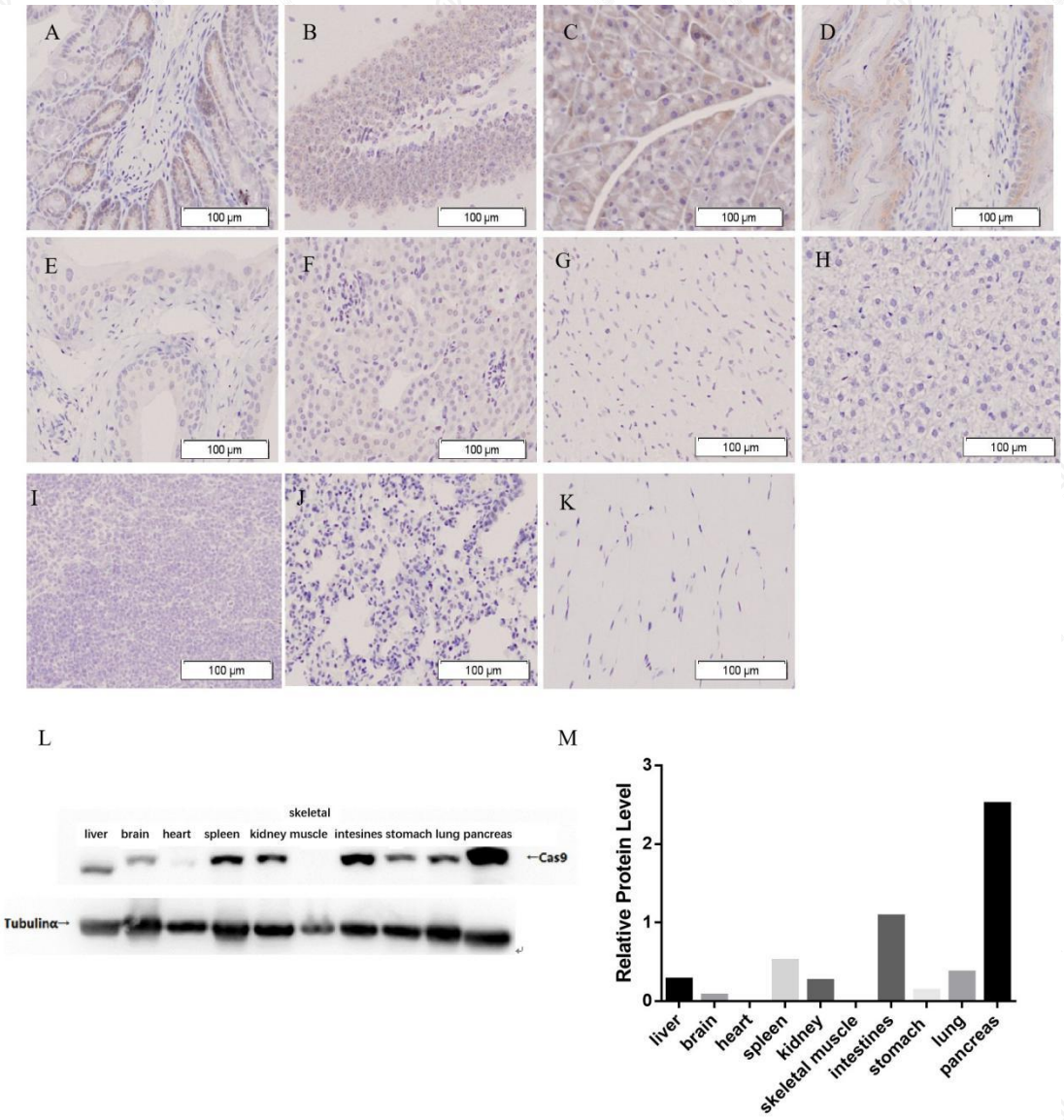

Fig3. Detection of Cas9 protein expression in B6-CAG-cas9-tdTomato.

To investigate the expression of Cas9 protein in different tissues of B6-CAG-cas9-tdTomato mice, we used immunohistochemical(A-K) and western(L-M) methods to detect the expression of Cas9 protein. The results showed that Cas9 protein was expressed in liver, spleen, lung, kidney, brain, pancreas and stomach. The main expression regions of brain are the hippocampus and the cortex, that it was hardly

expressed in the heart and muscle. The results indicated that Cas9 protein can be expressed in the main tissues of B6-CAG-cas9-tdTomato mice.

Reference

1. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. Science, 2013, 339(6121): 819-23.
2. Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science, 2012, 337(6096): 816-21.
3. Mali P, Yang L, Esvelt KM, Aach J, et al. RNA-guided human genome engineering via Cas9. Science. 2013, 339(6121): 823-6.
4. Flotte TR. Size does matter: overcoming the adeno-associated virus packaging limit. Respir Res, 2000, 1(1): 16-8.
5. Kumar M, Keller B, Makalou N, Sutton RE. Systematic determination of the packaging limit of lentiviral vectors. Hum Gene Ther, 2001, 12(15): 1893-905.
6. Wu Z, Yang H, Colosi P. Effect of genome size on AAV vector packaging. Mol Ther, 2010, 18(1): 80-6.