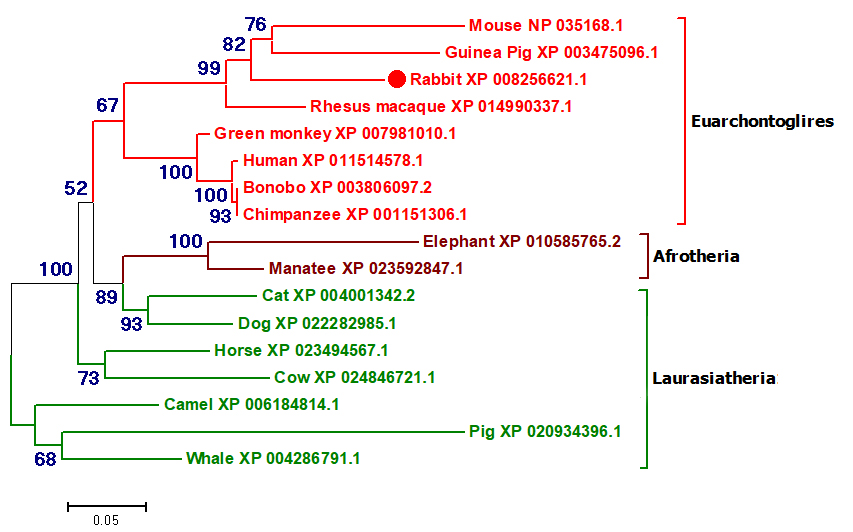
**Supplemental figures**

**Figure S1.**



**Figure S1. Evolutionary relationships of *PAX4* gene in Eutheria.**

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). &#32 The optimal tree &#32with the sum of branch length = 1.64719055 &#32is shown. &#32 The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). &#32 The tree is drawn to scale, with branch lengths &#32in the same units as those of the evolutionary distances used to infer the phylogenetic tree. &#32 The evolutionary distances were computed using the JTT matrix-based method (Jones *et al*. 1992) and are in the units of the number of amino acid substitutions per site. &#32 The rate variation among sites was modeled with a gamma distribution (shape parameter = 6). The analysis involved 17 amino acid sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 329 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al*. 2016).

Felsenstein, J., 1985 Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Jones, D. T., W. R. Taylor, and J. M. Thornton, 1992 The rapid generation of mutation data matrices from protein sequences. Comput. Appl. Biosci.8: 275–282.

Kumar, S., G. Stecher, and K. Tamura, 2016 MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870–1874.

Saitou, N., and M. Nei, 1987 The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.

**Figure S2.**

Figure S2

**Figure S2. The gene expression of *PAX4* was determined by qRT-PCR.**

WT, wild type control; PAX4+/-, heterozygous *PAX4* gene knockout rabbits; PAX4-/-, homozygous *PAX4* gene knockout rabbits. All experiments were repeated for three times for each gene. Data are presented as mean ± SEM, and analyzed by t-tests using Graphpad Prism software 6.0. \* *p* < 0.05; \*\*\* *p* < 0.001.

**Figure S3.**

**Figure S1**

**Figure S3. Mutation detection of 8 F0 rabbits obtained by T-cloning and Sanger sequencing.**

The sequences of sgRNAs are shown in green, the PAM sites are highlighted in red; deletions (-); WT, WT control. 2-1 - 2-8 represent the 8 pups used in this study.

**Supplemental tables:**

**Table S1.** The primers of potential off-target sites (POTS) used in this study

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Potential Off Target Site** | **Position** | **PCR Primer** |
| s1 | CGAGGGCATCACTACCGCTCTGG | chr4:-82175741 | F: CCGTCTATAATGCAGGGTTGT  R: TACAGCTCATGGTGTTGTCC |
| CCAGGCCCTTACTACCCCACTGG | chrUN0:+6791 | F: CTGCCAGCTTCCTACTTCAG  R: TCTACCCATACTCACCCTTCTC |
| CTAGGGCATCACGACCTCACAGG | chr18:+10523100 | F: CTGCAAGAAAGCTACAGCAAAG  R: TAGATCAGAGAGTGAGGCAAGA |
| GGAGGGCGTTGCTACTGCACAGG | chr7:-90526418 | F: GAGGAGTGATGTGCTGAGACTA  R: AAAGGAGCCGAACAGGTAGA |
| CCAGGGCTTTGCTACGGCACTGG | chrUN0:+3584352 | F: CCCTTCAGCCCAATGACTT  R:GTCTCCTTTCCTATCCACACTTC |
| s2 | TCTGGAGTGGTCACCGGAATGGG | chr12:+75520987 | F:GGTGTAGTGAGGATGTCAGTATC  R: CTGCCTTTCCTGGACAAATG |
| AAGCCACTGGTCCCCGGAATGGG | chr3:+41677265 | F:AGCCTTGAAACACAAGTCAGATA  R: GGTCGTGGAAGAAAGAGAAAGG |
| CAGAGACAGCTCACTGGAATAGG | chr17:+28450946 | F: TATTCCCAGCTGTGGCTTAAC  R:CCAAGACTGAACCATGGAGATAC |
| CCGGGACTGAGCACCGGAAGGGG | chrUN0:+67599 | F: TCCTGTGAGGGCGAGAG  R: TAGGGAGGTGGAGCTGTG |
| CAGGCGCTGTTCACCGAAATGGG | chr4:+75062479 | F: TTCCGTCGGTGATGTTTCTC  R: TAGCTGTCCCTCCCTCATC |

**Table S2:** Primers used for quantitative real-time RT-PCR

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Primers | Sequence(5’-3’) | Produce size (bp) |
| PAX4 | PAX4 - F  PAX4 - R | GGGCTCTTTGTGAATGGCCG  TACCTTAAGGCTCCGGGAGAT | 108 |
| GAPDH | GAPDH - F  GAPDH - R | TTCCACGGCAGGTCAAGGC  GGGCACCAGCATCACCCCAC | 99 |

**Table S3:** Results of urinalysis

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Rabbits** | **BLO** | **NIT** | **pH** | **URO** | **BIL** | **PRO (mg/dl)** | **GLU (mg/dl)** | **KET (mg/dl)** |
| WT | Negative | Negative | 9.0 | Normal | Negative | Negative | Negative | Negative |
| PAX4+/- | Negative | Negative | 9.0 | Normal | Negative | Negative | Negative | 2.5±3.5 |
| PAX4-/- | Negative | Negative | 5.0 | Positive | Negative | 100 | 2000 | 160 |