Production of gene-edited pigs harboring orthologous human mutations via double cuttings by CRISPR/Cas9 with long single-stranded DNAs as homology-directed repair templates by zygote injection

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Abstract

Precise gene edition is required for modeling human diseases in model organisms. In this study, by using in vitro transcribed CRISPR RNA reagents and double cuttings by CRISPR/Cas9 at two sites flanking pig GJB2 (pGJB2) CDS with long single-stranded DNAs (lssDNA) as homology-directed repair (HDR) templates, we generated two gene-edited pigs of which GJB2 CDSs were replaced with a human GJB2 (hGJB2) CDS containing c.235delC mutation and a pGJB2 CDS containing p.V37I mutation both commonly observed in hearing loss patients, respectively. Genotyping showed that the HDR-derived mutation efficiencies in founders were as high as 80% (4/5) and 50% (2/4), respectively, while no mutation was observed in the group of single cutting with one sgRNA covering the 235th nucleotide C in pGJB2 CDS using a short single-stranded oligo DNA (ssODN) containing c.235delC mutation as HDR template. Besides, the HDR-derived mutations were extensively integrated into various tissues in founder and capable of germline transmission. This study indicated that the "two cuttings with lssDNA templates" method, which expands sgRNA selection scope and avoids direct cutting of gene CDS, can precisely introduce human mutations into mammalian genomic sites, especial those resistant to gene editing, with CRISPR RNAs instead of rebonucleoproteins used in previous reports.

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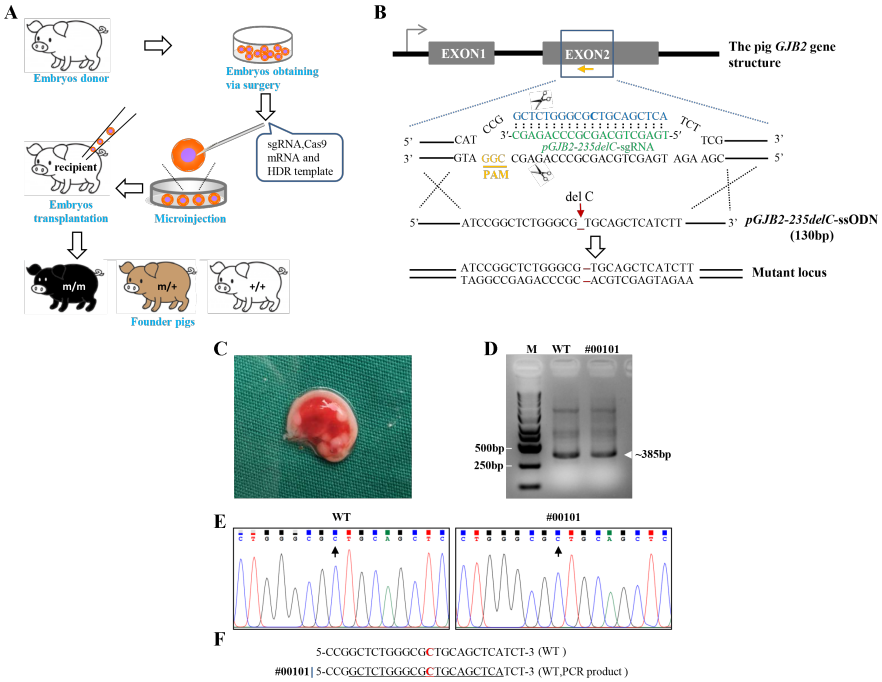
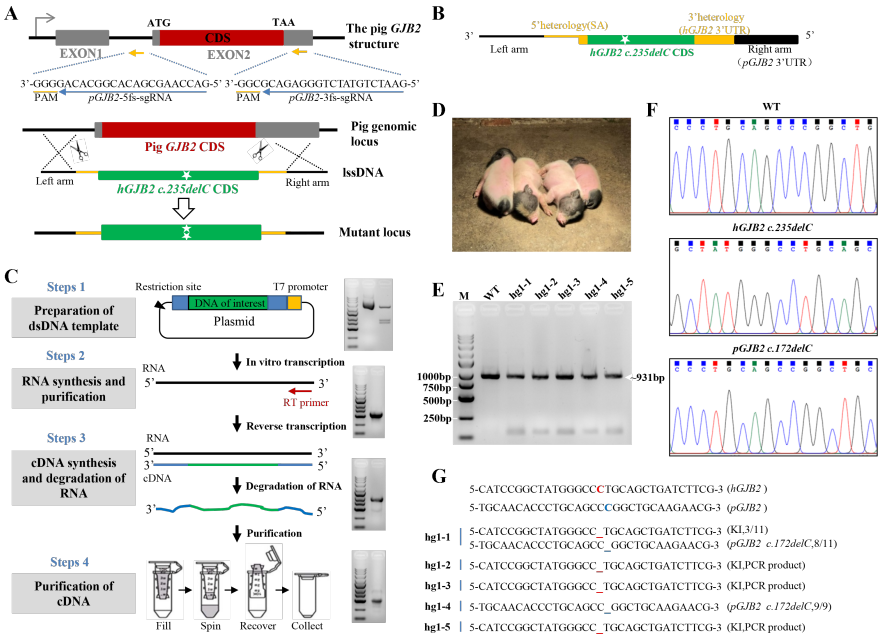
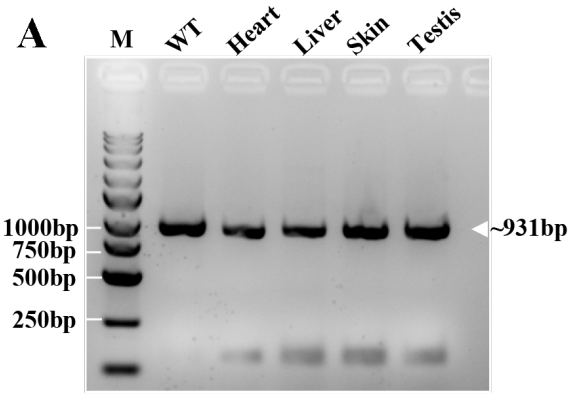
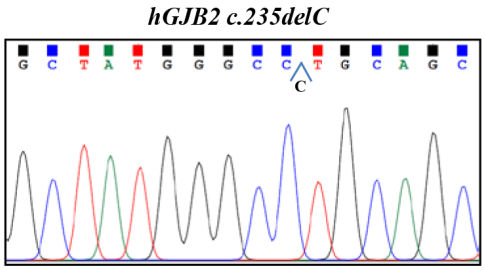
`Manuscript for human mutation.doc` available at https://authorea.com/users/298828/articles/428159-production-of-gene-edited-pigs-harboring-orthologous-human-mutations-via-double-cuttings-by-crispr-cas9-with-long-single-stranded-dnas-as-homology-directed-repair-templates-by-zygote-injection









C

5-CACATCCGGCTATGGGCC**C**TGCAGCTGATCTTCG-3(*hGJB2*)

Heart | 5-CACATCCGGCTATGGGCC_TGCAGCTGATCTTCG-3 (KI,PCR product)

Liver | 5-CACATCCGGCTATGGGCC_TGCAGCTGATCTTCG-3 (KI,PCR product)

Skin | 5-CACATCCGGCTATGGGCC_TGCAGCTGATCTTCG-3 (KI,PCR product)

Testis | 5-CACATCCGGCTATGGGCC_TGCAGCTGATCTTCG-3 (KI,PCR product)