

Figure S2. Map of *pX330-pRosa26*. A plasmid expressing Cas9 nuclease and sgRNA targeting the *pRosa26* locus (5'-GCTCCTTCTCGATTATGGGC-3').

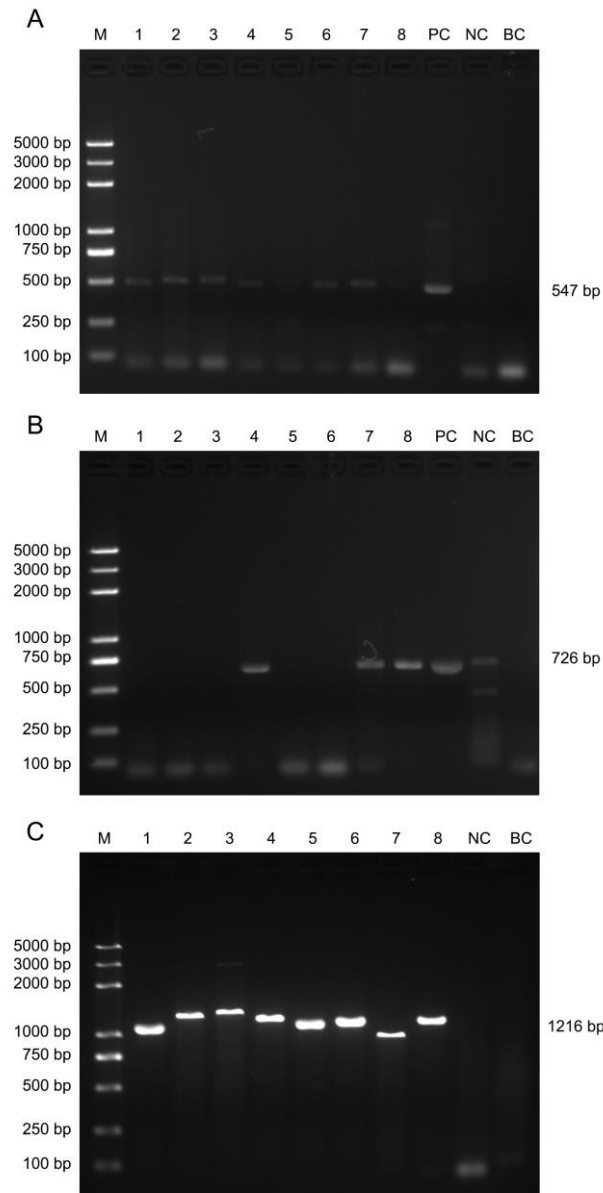


Figure S3. PCR analysis for the identification of site-specific *pbd-2* knock-in porcine fetal fibroblasts (PFFs). (A) Amplification for the dual *pbd-2* gene; (B) Amplification for the selectable marker gene *neoR*; (C) Amplification for the fragment which represents on-target insertion of the transgene. M: Molecular mass marker; Lane 1-8: Numbers for different cell colonies; PC: Positive control; NC: Negative control; BC: Blank control.

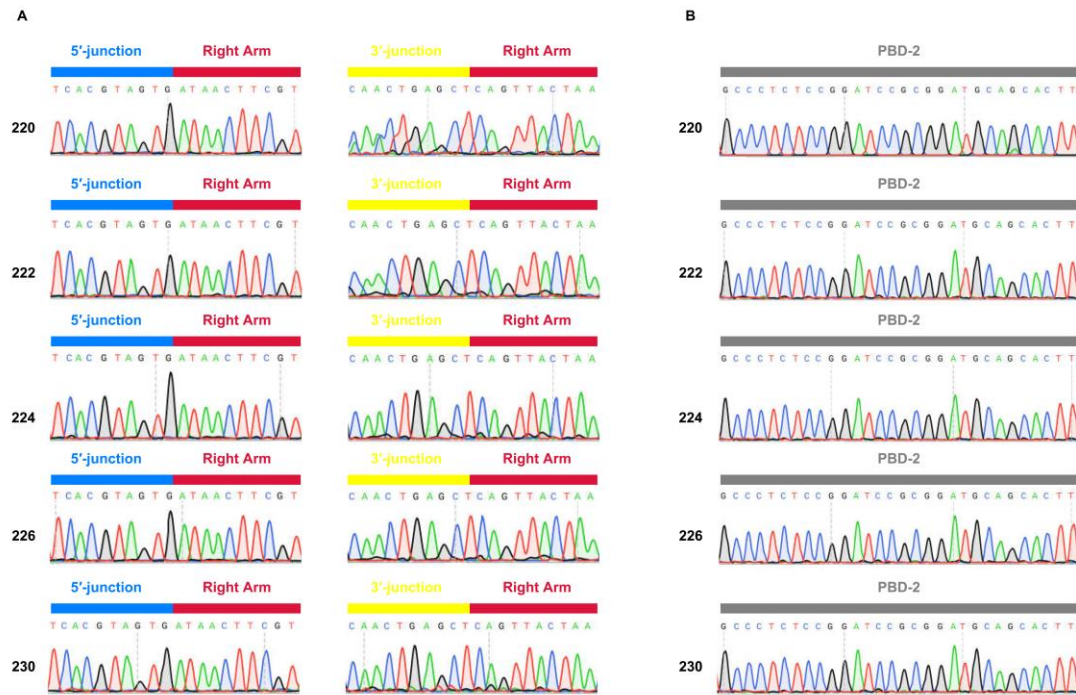


Figure S4. Sanger sequencing analyses to confirm on-target integration of *pbd-2* in cloned pigs. Numbers represent different transgenic pigs. **(A)** Sequencing results of the On-target fragment amplified with primers OT-F and OT-R. Red line indicates the sequence in the right homology arm; blue line indicates the sequence upstream of the right homology arm; yellow line indicates the sequence downstream of the right homology arm. **(B)** Sequencing results of the PBD-2 fragment amplified with primers vTG-F and vTG-R. Gray line indicates the sequence in the *pbd-2* gene.

Table S1. Information of primer pairs for identification of transgenic PFFs.

Fragment	Primer	Sequence (5'-3')	Length (bp)
PBD-2	vTG-F	GCTGGTTGTTGTGCTGTCTCATCA	547
	vTG-R	CCCTCTAGACTCGAGTCAGGGTCAGC	
Marker	marker-F	GATGGATTGCACGCAGGTTCTCC	726
	marker-R	GATACCGTAAAGCACGAGGAAGCG	
Locus	f-locus	CCTTCTATCGCCTTCTTGACGAGTT	1216
	r-locus	AACTGTCCCAGCAACACCTAAGATT	

Table S2. Information of primer pairs for amplification of predicted off-target loci.

Fragment	Primer	Sequence (5'-3')	Length (bp)
OT-1	OT1-F	ATAAAACAATGGGGGAATGAGAC	429
	OT1-R	AGCCAGAGGCAAAGTGATAGATA	
OT-2	OT2-F	CACAGAAGGAATCCCTGAGAGA	389
	OT2-R	CATGCTTCTCTGCACACACTA	
OT-3	OT3-F	TCGGTGTCTTGCCAAGTCG	417
	OT3-R	CACCTTCCTGGCATTAAATCCCT	
OT-4	OT4-F	AGTTAATGATTCCTAGGACATGCTT	367
	OT4-R	TCAAAGTGGCGGGTAAGGGAA	
OT-5	OT5-F	GGGTCTGTGTTGTTGCATGAGAA	350
	OT5-R	CACAGTGGGTGAGGTAAGAAATT	