Supplementary Information

CRISPR/Cas9-Mediated Generation of Guangxi Bama Minipigs Harboring Three Mutations in α-Synuclein Causing Parkinson's Disease

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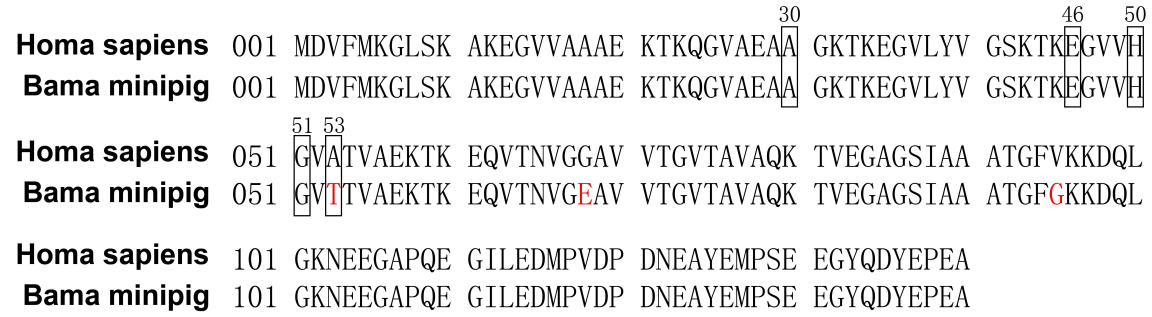
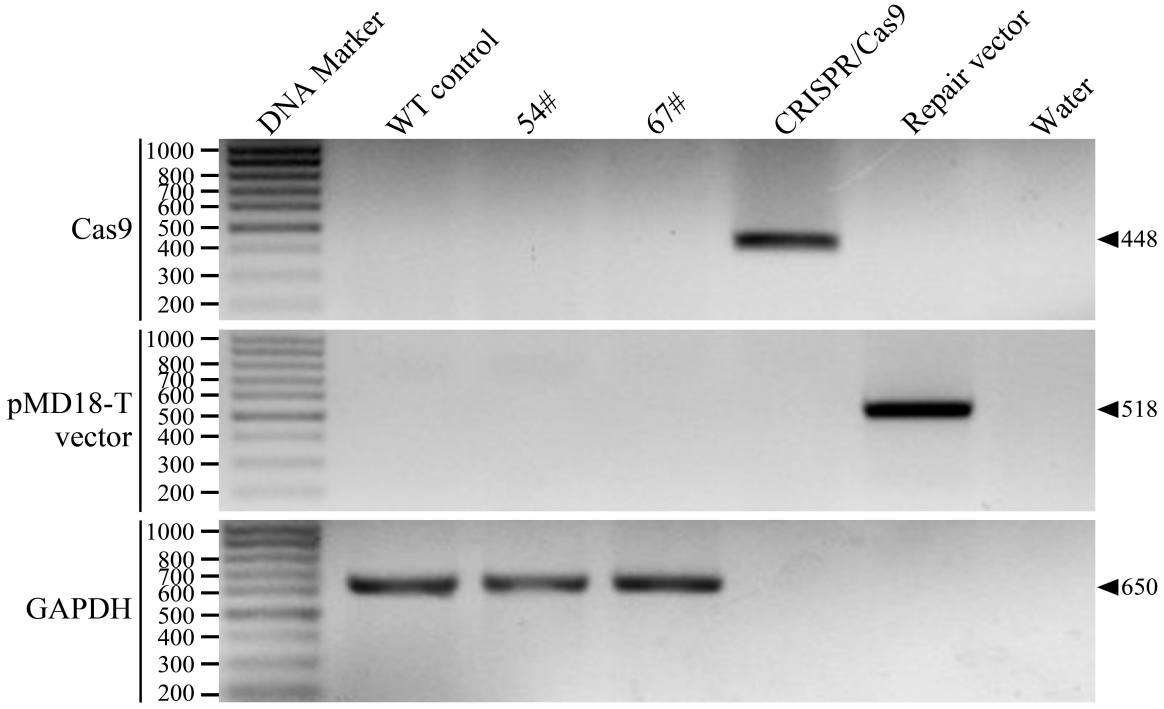
Supplementary Table S1 Off-target sites used for analysis of off-target mutations in gene-edited cell colonies

Off-target No.	Chr.	Strand	Position	Sequence*	Score	Gene	Primers for PCR and sequencing	Amplicon (bp)
sgRNA	Chr. 8	1	138648226	ACCAAGGAAGGAGTGGTTCATGG	100	NCBI Gene ID: 641350	-	-
Off-target 1#	Chr. 1	-1	84002721	AACAGGGAAGGAGTGGTTCAGGG	5.722891566	None	PF: GATGGATAGATCTTGTCTGG PR: CGATTCCAGGACTCTTAGAG	504
Off-target 2#	Chr. 18	-1	20227588	GAGATGGAAGGAGTGGTTCATGG	1.30580855	None	PF: GGATACACGTACATGCAGTC PR: TCACCAAGGATCTCACCATG	451
Off-target 3#	Chr. 6	1	27376728	ACCAAGGTAGGAGTCAGTCAGAG	0.025390982	NCBI Gene ID: 397478	PF: TACAGTGCAGCTCAGAGAGG PR: ACCTAGCATCCAGCTCAAAC	531

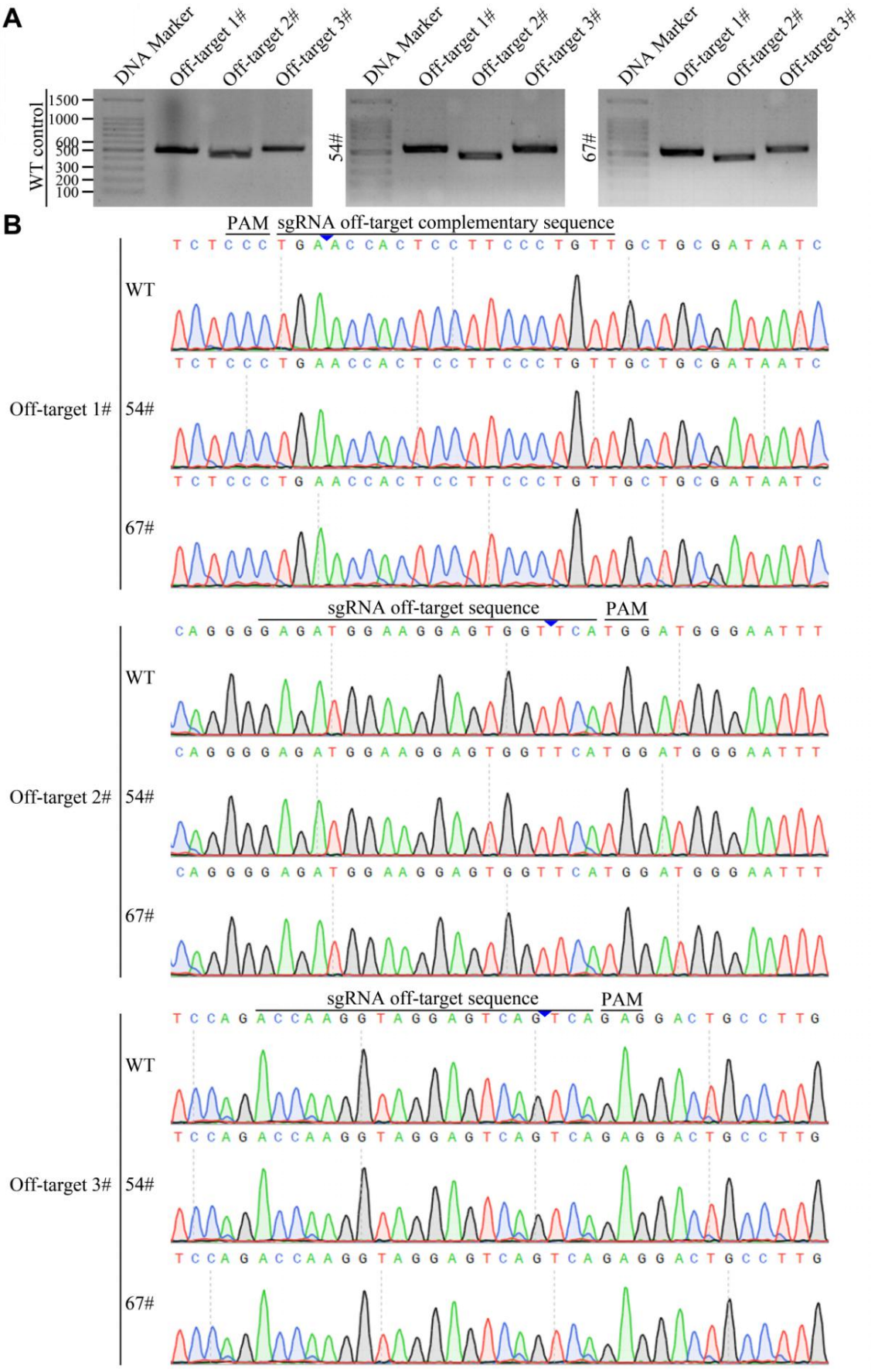
*Red letters are showing the unmatched nucleotides in off-target sequences aligned to sgRNA. PAM sequences are labeled with underline.



Supplementary Fig. S1 Alignment of α-synuclein protein sequences of human (Homa sapiens; NCBI Gene No. 6622) and Bama minipig. Caged residues are showing SNCA mutations (A30P, E46K, H50Q, G51D and A53T) causing human Parkinson's disease (PD). Notice that the porcine α-synuclein normally contains a threonine (T) at position 53, indicating A53T which cause PD in human will not be functional in pigs. Residues not evolutionary conserved are marked in red.

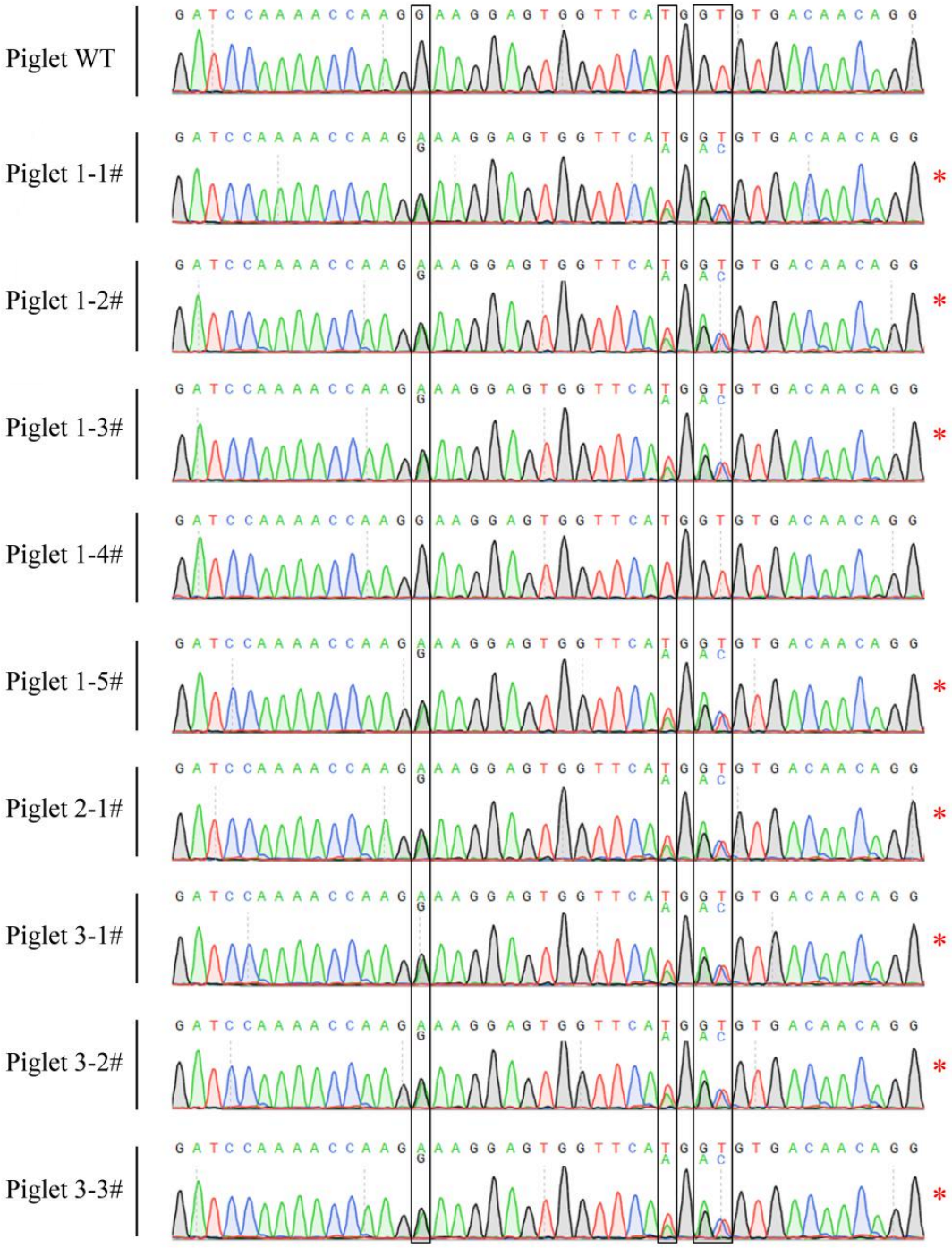


Supplementary Fig. S2 PCR amplification of Cas9 and repair vector confirmed no integration of exogenous genes in gene-edited cell colonies. Plasmids were represented as positive control, and wild-type (WT) cells were represented as negative control. GAPDH was used to confirm the DNA quality of all the samples.

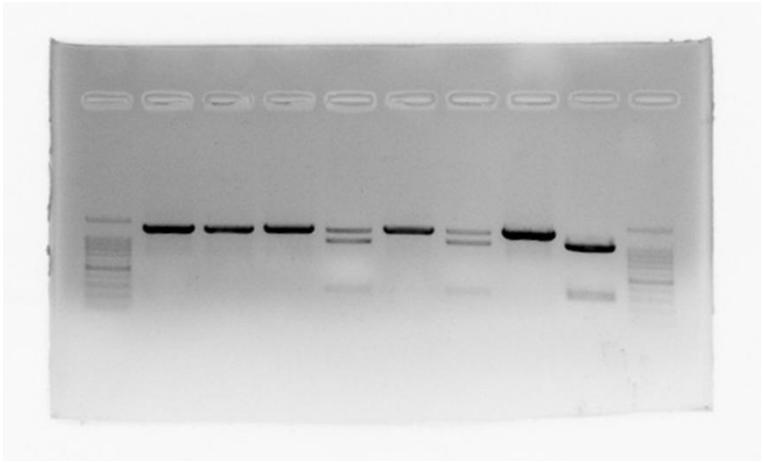


Supplementary Fig. S3 Analysis of the off-target effects by DNA sequencing. (A) PCR amplifications were

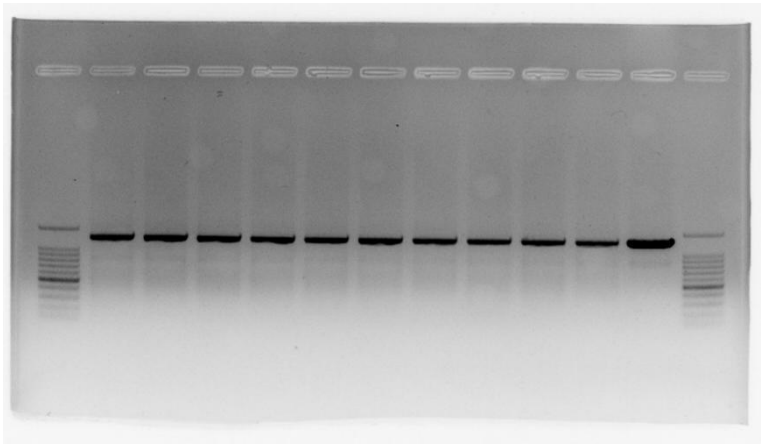
performed with two gene-edited cell colonies in three off-target loci (Off-target 1#~3#). (B) DNA sequencing suggested no mutations were occurred in all of the detected off-target loci in these two positive gene-edited cell colonies. The predicted cleavage sites in off-target sequences were marked with blue triangles.



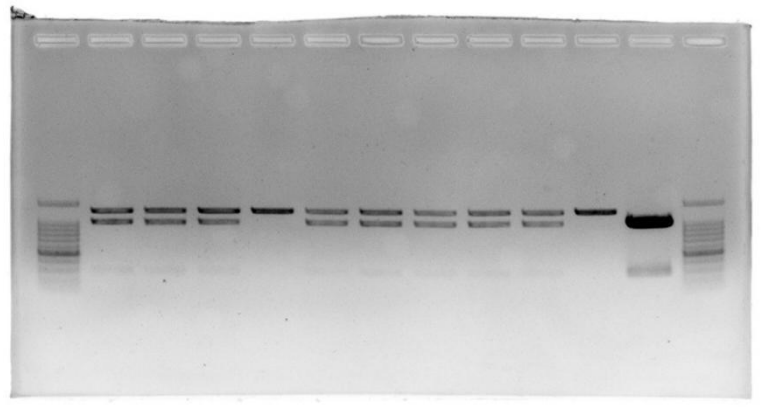
Supplementary Fig. S4 DNA sequencing confirmed that the cloned Guangxi Bama minipigs harboring designed mutations in *SCNA* locus. One wild-type (WT) pig was used as negative control, and 8 mutant minipigs were marked with red asterisks.



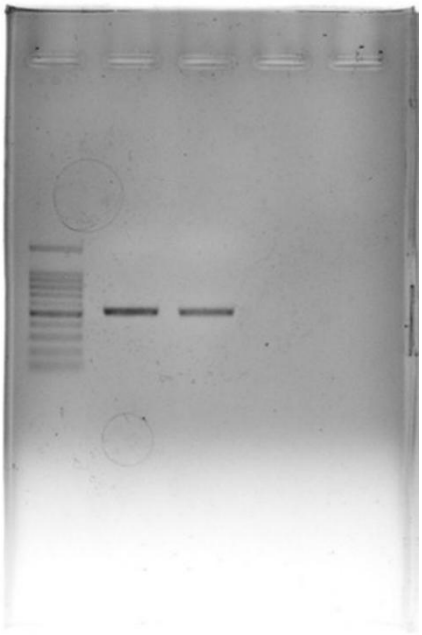
Full-length gel picture of Fig. 1B



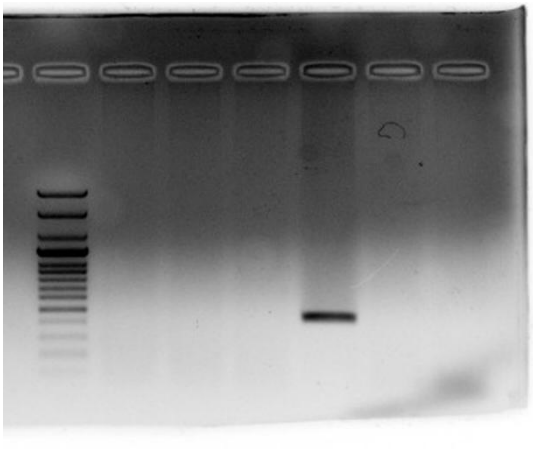
Full-length gel picture of Fig. 2B Upper gel



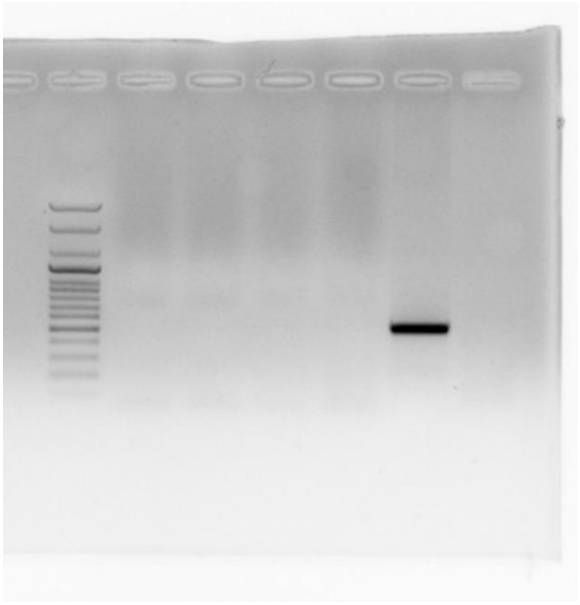
Full-length gel picture of Fig. 2B Lower gel



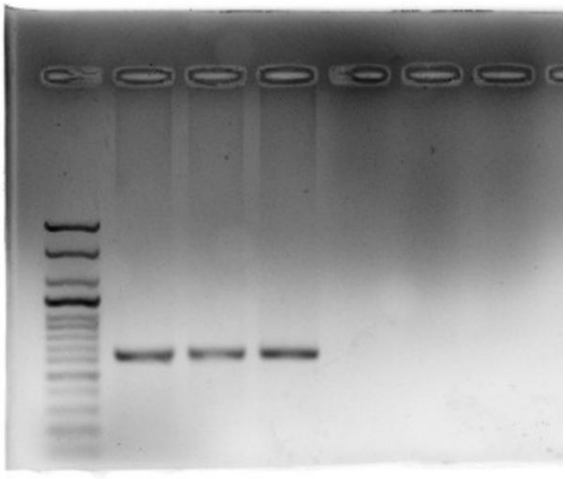
Full-length gel picture of Fig. 2D



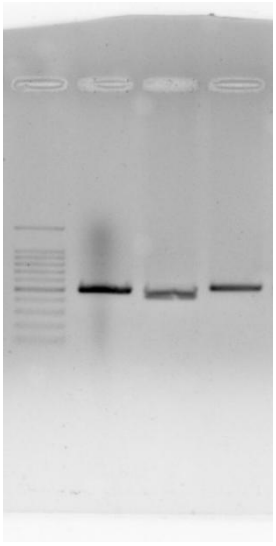
Full-length gel picture of Fig. S2 Upper gel



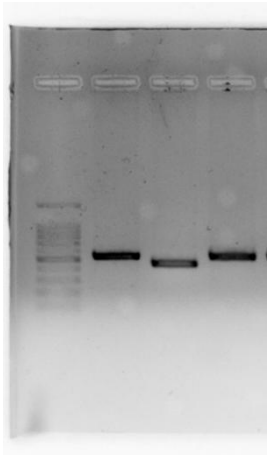
Full-length gel picture of Fig. S2 Middle gel



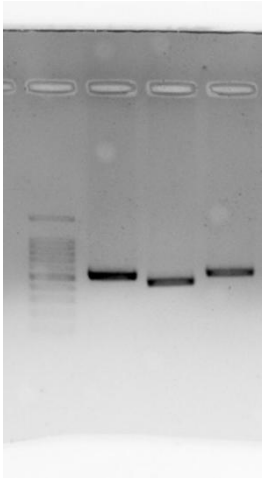
Full-length gel picture of Fig. S2 Lower gel



Full-length gel picture of Fig. S3A Left gel



Full-length gel picture of Fig. S3A Middle gel



Full-length gel picture of Fig. S3A Right gel