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Reproduction, Fertility and Development

Supplementary Material

Production of light-coloured, low heat-absorbing Holstein Friesian cattle by precise embryo-mediated genome editing

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Supplementary Information

Supplementary Table S1. Sequences for primers, probes, and repair template.

ID	Function	Sequence
1249	PCR primer, fwd	TGCTTTAAGATGAGACTGACC
1250	PCR primer, rev	ATGTCCTCACTAAATCAAATGG
1283	PCR primer, fwd	TTGCTGGAAGGAAGAACAGG
1284	PCR primer, rev	GGAGACACCTGAAGCACTAC
1285	HDR FAM probe	TGATGGGTGTTCTGGCTGTAGGGACCACA
1321	Ref HEX probe	TGCACACCTGCTCTGGTTTTTCTCTCCCT
1289	Dark probe	GGCTCTGATGGGTGTTCTTCTGGCTGTAGGGACCACAG
1282	+ve control, gBlock®	GAGTCTTTGGTTGCTGGAAGGAAGAAGAACAGGATGGATCTGGTGCTGAGAAA ATACCTTCTCCATGTGGCTCTGATGGGTGTTCTGGCTGTAGGGACCACAGAA GGTGAGTGTTGGATGTTGGACATGAACAAGTGTGAATTCGGGGTTGCACAC CTGCTCTGGTTTTTCTCTCCCTAAAATGGAAGATATCAGTAGTGCTTCAGGTG TCTCCACCCATTTG
1288	ssODN template	GAAAATACCTTCTCCATGTGGCTCTGATGGGTGTTCTGGCTGTAGGGACCAC AGAAGGTGAGTGTTGGATGTTGGACATGAACAAGTGTGAATTTGGGGTTGC ACACCTGCTCTGGTTTTTCTCTCC
1607	PCR primer, plasmid	GACCTGAGAACCCTGGGCTA
1608	PCR primer, plasmid	CTTGCGCATCTTCAGCAGCT
211	PCR primer, <i>LALBA</i>	TGCCCCAGAGAAGAGAAGG
212	PCR primer, <i>LALBA</i>	ATTGCTAACGGGAGTGAAGTAAGT



Supplementary Fig. S1. Coat color phenotype of PMEL-edited and non-edited control calves at 5 - 7 1/2 months of age.

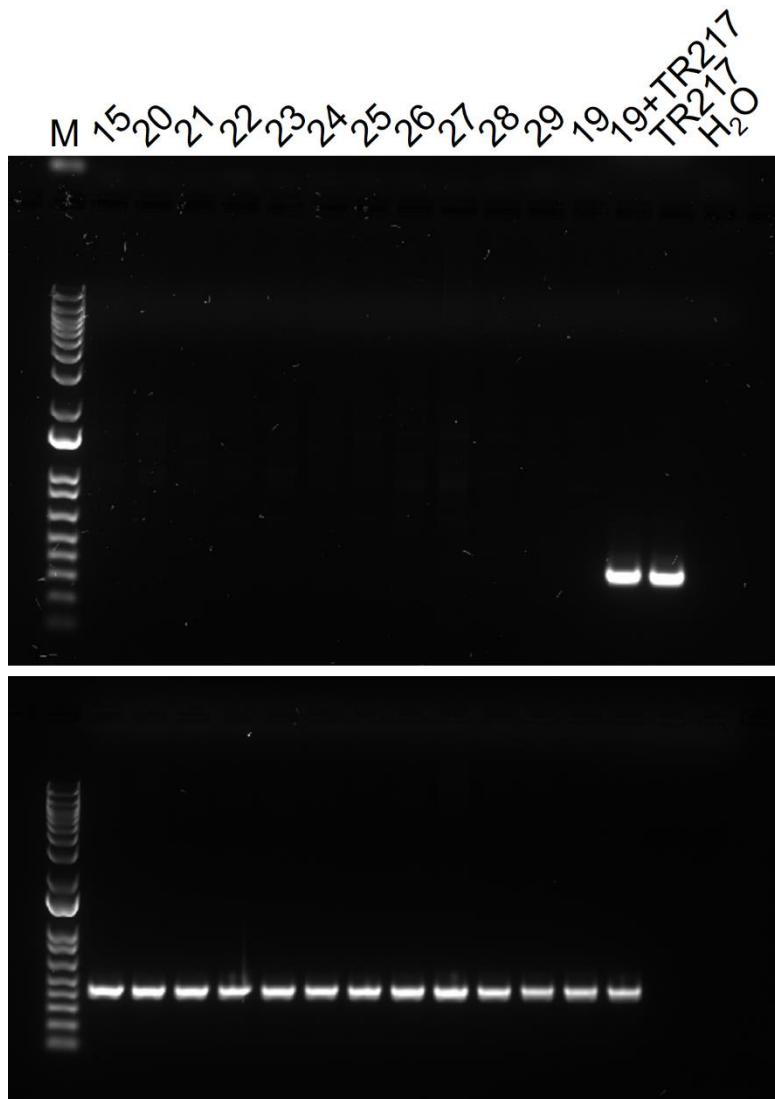


Fig. S2. Absence of a plasmid-specific fragment in genomic DNA from edited calves. (A) Amplification of PMEL-TALEN vector sequence from genomic DNA (50 ng) of *PMEL*-edited calves (15, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29). Non-edited calf 19, water (H₂O), TALEN TR217 plasmid spiked into calf 19 genomic DNA and TALEN TR217 plasmid (both 2 pg), served as negative and positive controls, respectively. M: DNA size marker. (B) The same samples analyzed for the amplification of a genomic fragment specific for the endogenous *LALBA* gene, encoding alpha-lactalbumin.

OPU PMEL
(100 % edited)

PMEL #22		Visible Spectrum	Infrared Spectrum
Original Image			
	Scored Image		
		Animal Absorbance Score (visible)	46
	Animal Absorbance Score (infrared)	58	
	Animal Absorbance Score (total)	104	

PMEL #24		Visible Spectrum	Infrared Spectrum
Original Image			
	Scored Image		
		Animal Absorbance Score (visible)	50
	Animal Absorbance Score (infrared)	60	
	Animal Absorbance Score (total)	110	

OPU controls

Control #14		Visible Spectrum	Infrared Spectrum
Original Image			
	Scored Image		
		Animal Absorbance Score (visible)	96
	Animal Absorbance Score (infrared)	98	
	Animal Absorbance Score (total)	194	

Control #16		Visible Spectrum	Infrared Spectrum
Original Image			
	Scored Image		
		Animal Absorbance Score (visible)	97
	Animal Absorbance Score (infrared)	98	
	Animal Absorbance Score (total)	195	

White patch
(OPU controls)

Control #14 ("White Patch" only)		Visible Spectrum	Infrared Spectrum
Original Image			
	Scored Image		
		Animal Absorbance Score (visible)	61
	Animal Absorbance Score (infrared)	74	
	Animal Absorbance Score (total)	135	

Control #16 ("White Patch" only)		Visible Spectrum	Infrared Spectrum
Original Image			
	Scored Image		
		Animal Absorbance Score (visible)	66
	Animal Absorbance Score (infrared)	74	
	Animal Absorbance Score (total)	140	

Supplementary Fig. S3. Images of scanned body regions used to deduce overall absorbance scores. Displayed are the original images and the scored images where sections not belonging to the animal were edited and excluded (blackened regions). Absorbance scores for visible and NIR are given below the scored images.