

Four sgRNAs sequences designed to uniquely target ZBED6

sgRNA	sgRNA-1	sgRNA-2	sgRNA-3	sgRNA-4
sequence 5'→3'	agaagggtttgcgaattaag	gtaaggatttgggatctggg	agagcaaagacttccattgtg	gtataacctggc gggcaattt
gene knockout efficiency	0%	0%	7.50%	0%

Efficiency of ZBED6 cloning mutation

sgRNA	Number of cell colonies	Efficiency of wildtype	Efficiency of gene mutation	
			68% (39/57)	
sgRNA-3	57	32% (18/57)	Efficiency of biallelic mutation 49% (28/57)	Efficiency of allelic mutation 19% (11/57)

Summary of embryo transfer results from gene-targeted PEFs

Pig ID surrogate	Donor cells	Embryos transferred	Pregnancy in 28 days	Piglets	KO	Survival piglets
J-10	Z17(+1 bp/+1 bp)	187	Yes	5	Yes	0
J-12	Z23(-1 bp/-1 bp)	212	Yes	5	Yes	1(#182) 5
J-11	Z28(-1 bp/-1 bp)	182	Yes	6	Yes	(#175,#176,#177,#178, #180)
Ttotal	3	581	3	16	16	6