

Supplemental Material

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1. Attached movie. The movie begins with the 7 piglets as viewed through the correct filters and with the correct lighting (GFsP-5, Biological Laboratory Equipment Maintenance and Service, LTD. H-1165 Budapest, Zselyi Aladar u.31. Hungary, <http://www.bls-ltd.com>). Then the lights are turned on to reveal the rest of the cloned piglets. Note that it takes a few seconds for the camera to adjust the lighting.

2. The results from the first 3 SCNT/ETs.

Date of Transfer	Surrogate	Donor Cell ID	# transferred to the surrogate	Day of cycle	Result
7/15/2010	200543	ZFN JZ	135	1	6 piglets delivered 11/8/2010 - 5 KO.
7/16/2010	O910	ZFN JZ	180	0	1 piglet delivered 11/8/2010 - 1 KO.
7/22/2010	O925	ZFN JZ	181	1	Euthanized 8/5/2010; 9 fetuses - 7 KO.

3. Supplemental Table 1. ZFN-mediated mutations in eGFP sequence from cultured adult ear fibroblasts and SCNT piglets derived from ear fibroblast donor cells. Sanger sequencing data are listed with tissue sample type. Double-stranded wild-type eGFP DNA sequence is shown with ZFN binding sites highlighted in gray and the nuclease cleavage site underlined. Sample mutations as determined by sequence analysis are indicated by dashes (-) for deletions and bold nucleotides for insertions.

Sample ID; Phenotype	Mutation	Mutation Location
WT eGFP; green*	none	ACA <u>ACTACAACAGCCACAACGTCTAT</u> <u>ATCATGGCCGACAAGCA</u> TG <u>TTGATGTTGTCGGTGTTC</u> <u>CAGATATAGTACCGGCTGTTTCGT</u>
Fibroblasts #5 & 18; KO	333 bp deletion	CATCTTCTT-----//-----CAACGAAAG
Fibroblast #10; KO	6 bp deletion	ACA <u>ACTACAACAGCCACAAC</u> -----ATCATGGCCGACAAGCA
Fibroblast #13; KO	222 bp deletion, 113 bp insertion	CGCGCCGAGG-----//-----ACCCCCAT
d12 fetus 1; KO	2 bp insertion	ACA <u>ACTACAACAGCCACAACGTCTA</u> <u>GAT</u> ATCATGGCCGACAAGCA
d12 fetus 5; green*	none	ACA <u>ACTACAACAGCCACAACGTCTAT</u> ATCATGGCCGACAAGCA
d12 fetus 7 KO	76 bp deletion	AACAGCCACAAC <u>GTCT</u> -----//-----TGCAGCTCGCCGACC
d12 fetus 2, 3, 4, 6, and 8; KO	---	no sequence data
d12 fetus 9; green*	none	ACA <u>ACTACAACAGCCACAACGTCTAT</u> ATCATGGCCGACAAGCA
Piglet 165-1; KO	3 bp insertion	ACA <u>ACTACAACAGCCACAACGTCTA</u> <u>GCT</u> ATCATGGCCGACAAGCA
Piglet 165-2; KO	1 bp deletion 4 bp insertion	CAACTACAACAGCCACAAC <u>GATA</u> - <u>TCTATATCATGGCCGACAAGCA</u>

Sample ID; Phenotype	Mutation	Mutation Location
Piglet 165-3; KO	7 bp deletion	ACAACACTACAACAGCCACAA-----ATCATGGCCGACAAGCA
Piglet 165-4; KO	9 bp insertion	ACTACAACAGCCACAAC <u>CGTC</u> GTTGATATC <u>TATAT</u> CATGGCCGACA
Piglet 165-5; KO	2 bp insertion	ACAACACTACAACAGCCACAAC <u>GTCTA</u> GA <u>TAT</u> CATGGCCGACAAGCA
Piglet 165-6; green*	none	ACAACACTACAACAGCCACAAC <u>GTCTATAT</u> CATGGCCGACAAGCA
Piglet 166-1; KO	2 bp insertion	ACAACACTACAACAGCCACAAC <u>GTCTA</u> GA <u>TAT</u> CATGGCCGACAAGCA

* Sample has normal eGFP sequence and expresses fluorescent eGFP protein.

Note added in proof:

Supplemental Table 2: The results from the fourth SCNT/ET.

Date of Transfer	Surrogate	Donor Cell ID	# Transferred to the surrogate	Day of cycle	Result
7/23/2010	O909	ZFN JZ	202	1	6 piglets delivered 11/15/2010 - 6 KO

Supplemental Table 3: ZFN-mediated mutations in eGFP sequence from **Litter 170** SCNT piglets derived from ear fibroblast donor cells. Sanger sequencing data are listed with tissue sample type. Double-stranded wild-type eGFP DNA sequence is shown with ZFN binding sites highlighted in gray and the nuclease cleavage site underlined. Sample mutations as determined by sequence analysis are indicated by dashes (-) for deletions.

Sample ID; Phenotype	Mutation	Mutation Location
WT eGFP; green*	none	ACA AACTACAACAGCCACAACGTCTAT <u>AT</u> CATGGCCGACAAGCAGAAGAACGGC TGTGATGTTGTCGGTGTTCAGATATAGTACCGGCTGTTTCGTCTTCTTGCCG
Pig 170-1	2 bp deletion	ACA AACTACAACAGCCACAACGT-- <u>AT</u> CATGGCCGACAAGCAGAAGAACGGC TGTGATGTTGTCGGTGTTC <u>CA</u> -- <u>T</u> ATAGTACCGGCTGTTTCGTCTTCTTGCCG
Pig 170-2	21 bp deletion	ACA AACTACAACAGCCACAA-----GCAGAAGAACGGC TGTGATGTTGTCGGTGTTC-----CGTCTTCTTGCCG
Pig 170-3	700 bp deletion	ACA AACTACAACAGCCACAAC-----/ /-----GTCAAAGGGCGAAAAACC TGTGATGTTGTCGGTGTTC-----/ /-----GGTTTTTCGCCCTTTGAC
Pig 170-4	21 bp deletion	ACA AACTACAACAGCCACAA-----GCAGAAGAACGGC TGTGATGTTGTCGGTGTTC-----CGTCTTCTTGCCG
Pig 170-5	21 bp deletion	ACA AACTACAACAGCCACAA-----GCAGAAGAACGGC TGTGATGTTGTCGGTGTTC-----CGTCTTCTTGCCG
Pig 170-6	21 bp deletion	ACA AACTACAACAGCCACAA-----GCAGAAGAACGGC TGTGATGTTGTCGGTGTTC-----CGTCTTCTTGCCG

* Sample has normal eGFP sequence and expresses fluorescent eGFP protein.