WOAH FOOD SAFETY WEBINAR
Understanding allergy of livestock products

Lecture 3: **Approaches to control allergenicity through animal biotechnology**

Shigeru KAKUTA, PhD, DVM.
Associate Professor
Department of Veterinary Medical Science / Research Center for Food Safety
Graduate School of Agricultural and Life Sciences,
The University of Tokyo

The New York Times

In a First, Man Receives a Heart From a Genetically Altered Pig

The breakthrough may lead one day to new supplies of animal organs for transplant into human patients.

Jan. 10, 2022

A 57-year-old man with life-threatening heart disease has received a heart from a genetically modified pig, a groundbreaking procedure that offers hope to hundreds of thousands of patients with failing organs.

It is the first successful transplant of a pig's heart into a human being. The eight-hour operation took place in Baltimore on Friday, and the patient, David Bennett Sr. of Maryland, was doing well on Monday, according to surgeons at the University of Maryland Medical Center.

"It creates the pulse, it creates the pressure, it is his heart," said Dr. Bartley Griffith, the director of the cardiac transplant program at the medical center, who performed the operation.

"It's working and it looks normal. We are thrilled, but we don't know what tomorrow will bring us. This has never been done before."

Dr. Bartley Griffith, left, performed the operation on David Bennett Sr. to receive a new heart from a genetically modified pig. University of Maryland School of Medicine

The New York Times

Genetically Modified Pig's Heart Is Transplanted Into a Second Patient

The first patient to receive such an organ died after two months. "At least now I have hope," the second recipient said before the surgery.

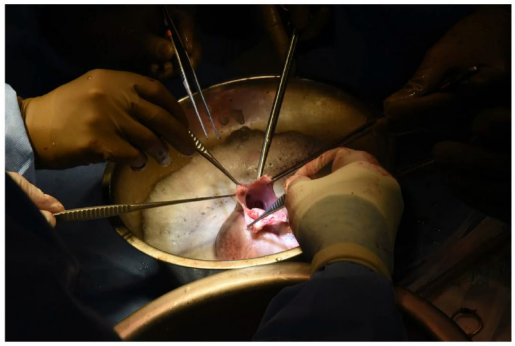
Sept. 22, 2023

Surgeons in Baltimore have transplanted the heart of a genetically altered pig into a man with terminal heart disease who had no other hope for treatment, the University of Maryland Medical Center announced on Friday.

It is the second such procedure performed by the surgeons. The first patient, David Bennett, 57, died two months after his transplant, but the pig heart functioned well and there were no signs of acute organ rejection, a major risk in such procedures.

The second patient, Lawrence Faucette, 58, a Navy veteran and married father of two in Frederick, Md., underwent the transplant surgery on Wednesday and is "recovering well and communicating with his loved ones," the medical center said in a statement.

Mr. Faucette, who had terminal heart disease and other complicated medical conditions, was so sick that he had been rejected from all transplant programs that use human donor organs.



Surgeons examine a pig heart during Lawrence Faucette's transplant surgery. Mark Teske/University of Maryland School of Medicine, via Associated Press

Mr. Faucette, a 20-year Navy veteran with heart failure from Frederick, Md., and his wife, Ann Faucette, before the surgery. Mr. Faucette, 58, received a genetically altered pig heart at the University of Maryland Medical Center. University of Maryland Medical Center

Table S1: Genetic Modifications of Source Animal for Cardiac Xenotransplantation

Genetic Modification	Mechanisms	Properties
Xenogeneic Carbohydrate Knockout		
Galactose-α-1,3-galactose KO (GTKO)	Deletion of immunogenic Galactose-α-1,3-galactose (Gal) glycan through knockout of the synthetic enzyme alpha1,3-galactosyltransferase (GT)	Anti-Immunogenic
β1,4-N-acetylgalactosyltransferase KO (B4GalKO)	Deletion of immunogenic blood group SDa antigen through knockout of the synthetic enzyme (B4GalNT2)	
CMP-N-acetylneuraminic acid hydroxylase KO (CMAHKO)	Deletion of immunogenic glycan N-glycolylneuraminic acid (Neu5Gc) through knockout of the synthetic enzyme CMP-N-acetylneuraminic acid hydroxylase (CMAH)	
Growth Hormone Receptor Knockout (GHRKO)	Reduction of downstream insulin growth factor-1 (IGF-1) signaling	Reduce intrinsic graft growth
Human Transgene Expression		
CD46	Suppress human complement activity by mediating cleavage of C3b and C4b complement deposition	Complement Regulation
Decay Accelerating Factor (DAF)	Inhibits C3 and C5 convertase enzymes and downstream complement activation	
Endothelial Cell Protein C Receptor (EPCR)	Activates Protein C	Anti-Coagulation
Thrombomodulin (TBM)	Binds human thrombin, and activates Protein C via activated thrombin	
Hemeoxygenase-1 (HO-1)	Decreases oxidative products	Anti-Inflammatory
CD47	Interacts with macrophage signal regulatory protein (SIRP)α to prevent opsonization and phagocytosis of xenogeneic tissue	

GGTA1 gene

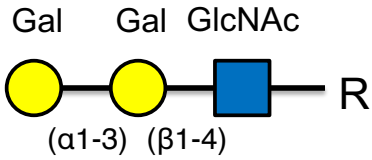
N Engl J Med 387:35-44 (2022)

Galactose-α-1,3-galactose (α-Gal) is

present in organs and muscles from most mammals (but not humans, apes, and old world monkeys) as a glycan conjugated to both proteins and lipids.

biosynthesized by α1,3-galactosyltransferase encoded in *GGTA1* gene, which has inactive mutation in humans, apes, and old world monkeys.

recognized as target epitopes of immunoglobulin (Ig)E, that commonly contributed to clinical symptoms in α-Gal syndrome.



news

Check for updates

First GM pigs for allergies. Could xenotransplants be next?

The FDA greenlights α-Gal allergy-safe meat, but its makers have organs for transplants in their sights.

Nat Biotechnol 39: 393-400 (2021)

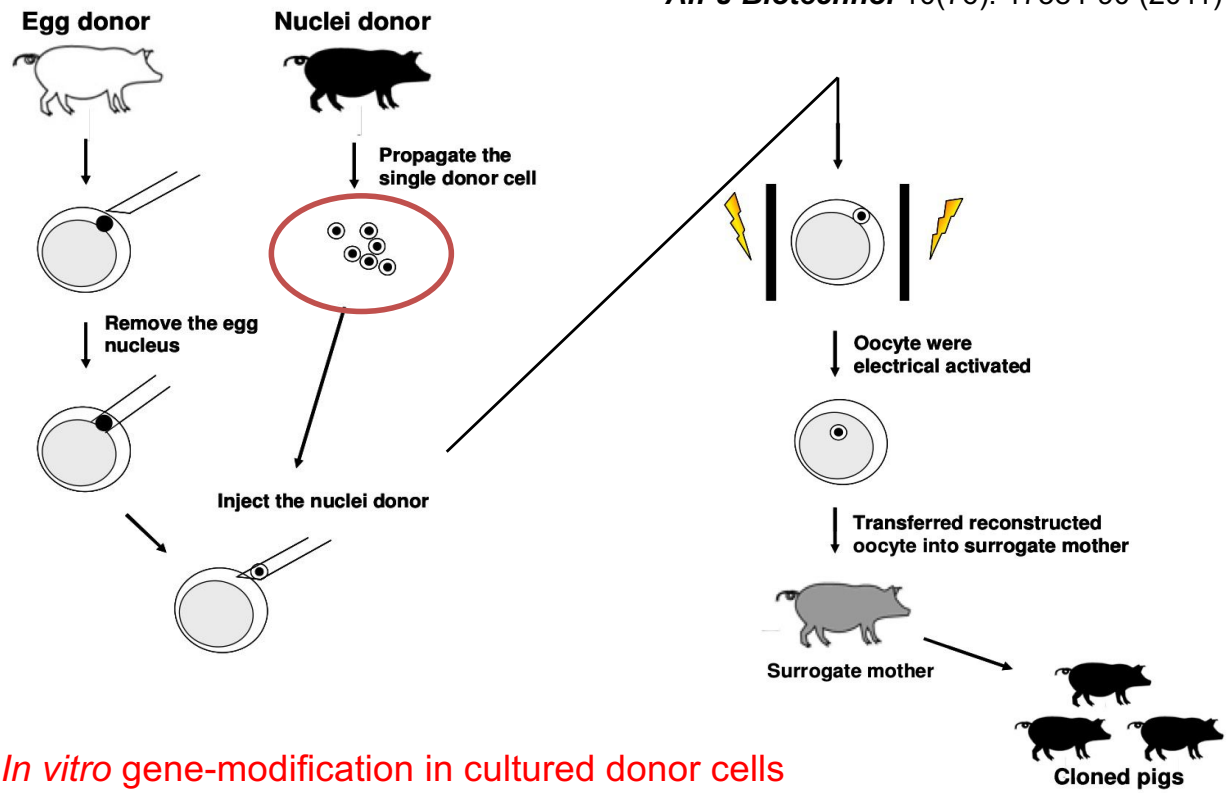
GalSafe pigs

could now provide a source of meat for people who develop tick bite-induced allergic reactions to the sugar, a condition known as **α-Gal syndrome**, including red meat allergy.

Gene-edited pigs could solve the human organ transplant shortage. Credit: Pulsar Imagens / Alamy Stock Photo

Somatic cell nuclear transfer (SCNT) in pig

Afr J Biotechnol 10(76): 17384-90 (2011)



In vitro gene-modification in cultured donor cells

1st generation of *GGTA1* gene deficient pigs

Production of α-1,3-Galactosyltransferase Knockout Pigs by Nuclear Transfer Cloning

Liangxue Lai,[1] Donna Kolber-Simonds,[3] Kwang-Wook Park,[1] Hee-Tae Cheong,[1,4] Julia L. Greenstein,[3] Gi-Sun Im,[1,5] Melissa Samuel,[1] Aaron Bonk,[1] August Rieke,[1] Billy N. Day,[1] Clifton N. Murphy,[1] David B. Carter,[1,2] Robert J. Hawley,[3] Randall S. Prather[1]*

The presence of galactose α-1,3-galactose residues on the surface of pig cells is a major obstacle to successful xenotransplantation. Here, we report the production of four live pigs in which one allele of the α-1,3-galactosyltransferase locus has been knocked out. These pigs were produced by nuclear transfer technology; clonal fetal fibroblast cell lines were used as nuclear donors for embryos reconstructed with enucleated pig oocytes.

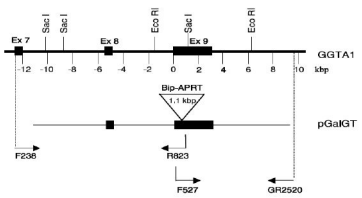


Fig. 1. pGalGT targeting vector and genomic PCR assays for targeting (35).

Table 1. Pregnancies carried to term after transfer of embryos reconstructed with GGTA1 knockout cell lines.

Surrogate (estrus day)	Donor line	NT embryos	Outcome
O212 (0)	F7-H6	116	Mated surrogate Seven born 9/21/01 One NT-derived female piglet
O226 (1)	F3-C5	92	Four NT-derived female piglets born 10/19/01
O230 (1)	F7-H6 cultured	130	Two NT-derived female piglets born 10/15/01

Science 295:1089-92 (2002)

Targeted disruption of the α1,3-galactosyltransferase gene in cloned pigs

Yifan Dai[1*], Todd D. Vaught[1], Jeremy Boone[1], Shu-Hung Chen[1], Carol J. Phelps[1], Suyapa Ball[1], Jeff A. Monahan[1], Peter M. Jobst[1], Kenneth J. McCreath[2], Ashley E. Lamborn[1], Jamie L. Cowell-Lucero[1], Kevin D. Wells[1], Alan Colman[2], Irina A. Polejaeva[1], and David L. Ayares[1]

Galactose-α1,3-galactose (α1,3Gal) is the major xenoantigen causing hyperacute rejection in pig-to-human xenotransplantation. Disruption of the gene encoding pig α1,3-galactosyltransferase (α1,3GT) by homologous recombination is a means to completely remove the α1,3Gal epitopes from xenografts. Here we report the disruption of one allele of the pig α1,3GT gene in both male and female porcine primary fetal fibroblasts. Targeting was confirmed in 17 colonies by Southern blot analysis, and 7 of them were used for nuclear transfer. Using cells from one colony, we produced six cloned female piglets, of which five were of normal weight and apparently healthy. Southern blot analysis confirmed that these five piglets contain one disrupted pig α1,3GT allele.

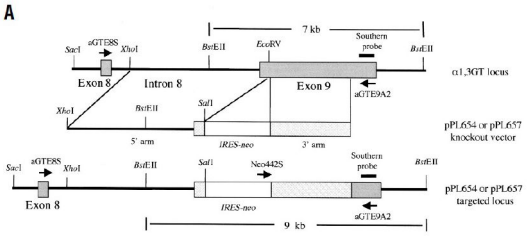


Figure 3. Five α1,3GT gene knockout piglets at 2 weeks of age.

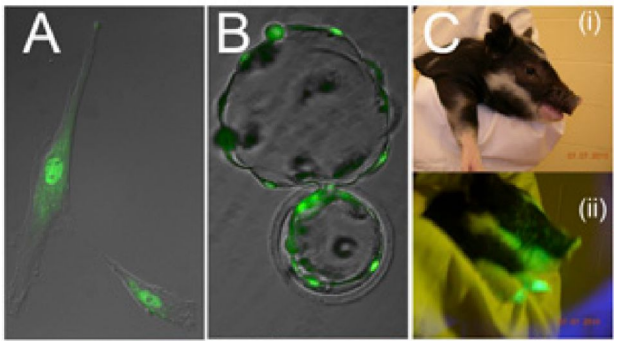
Nat Biotechnol 20:251-55 (2002)

For Food Safety,

1, transgene insertion, including drug selection marker gene, vector sequence, etc.

2, unexpected (off-target) mutation

in genetically modified animals are not appropriate.



Transgenic pig carrying
green fluorescent protein (GFP)
derived from jellyfish
PNAS 110(16):6334-39 (2013)

How generate target gene-modified animals "without" exogenous gene integration?

Genetic scissors: a tool for rewriting the code of life

The Nobel Prize in Chemistry 2020

Emmanuelle Charpentier
Jennifer A. Doudna

Share this

The Nobel Prize in Chemistry 2020

© Nobel Media. Ill. Niklas Elmehed.

Emmanuelle Charpentier

Prize share: 1/2

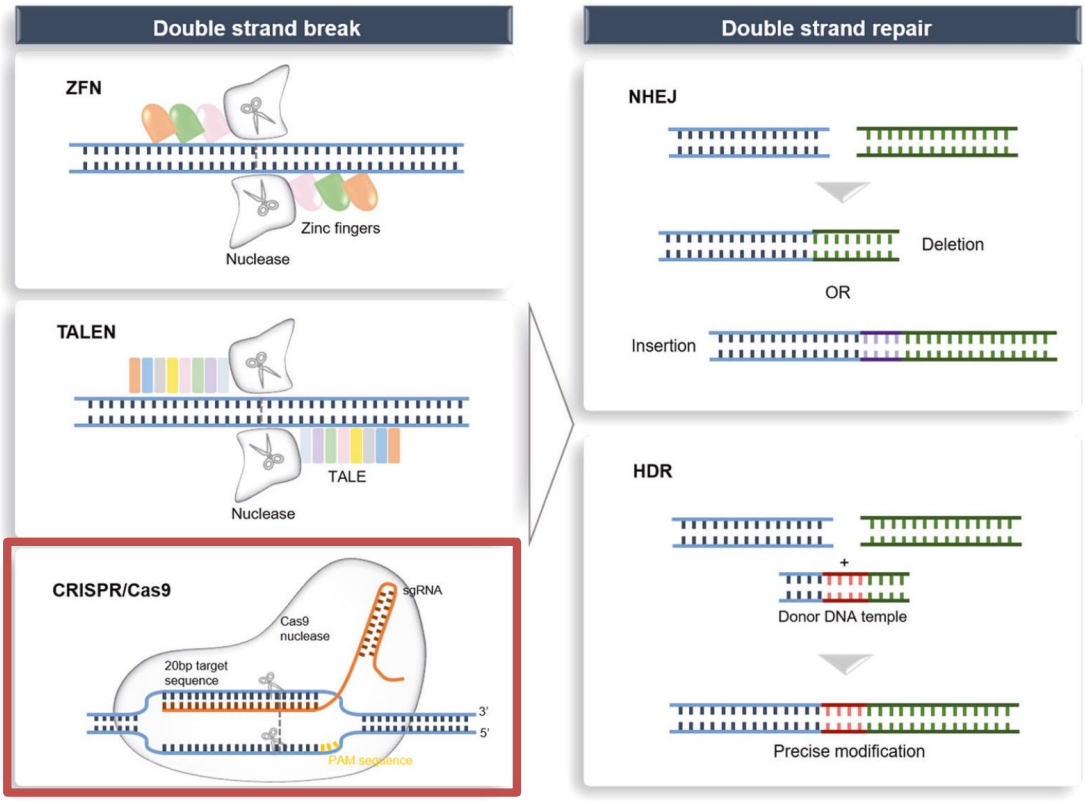
© Nobel Media. Ill. Niklas Elmehed.

Jennifer A. Doudna

Prize share: 1/2

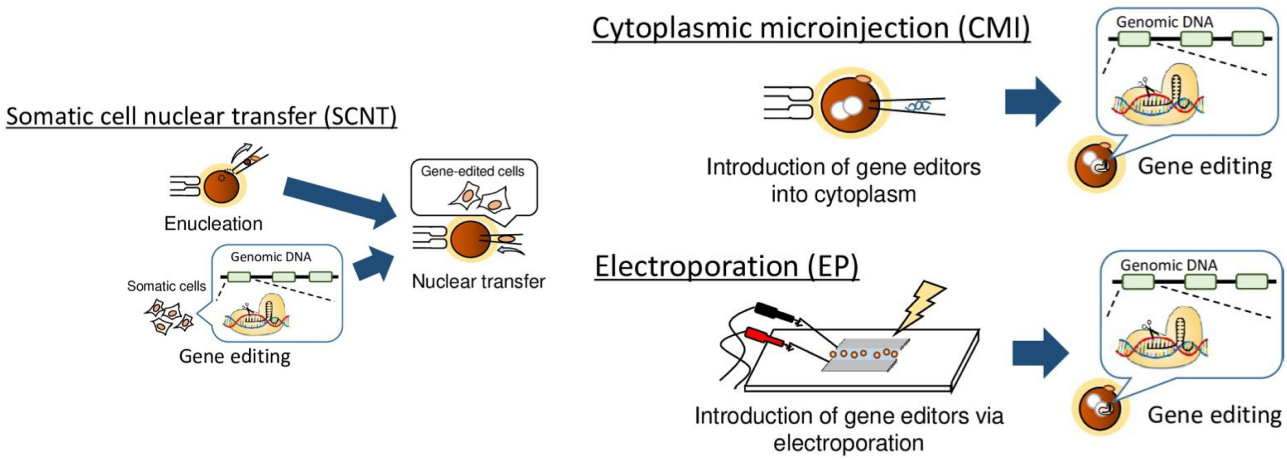
"for the development of a method for genome editing"

Genome editing platforms and mechanisms for DSB repair with endogenous DNA.



Signal Transduction and Targeted Therapy 5:1 (2020)

Major methods for generating genetically modified pigs using gene editors



J Reprod Dev 67: 177–187 (2021)

Tanihara *et al. BMC Biotechnology* (2020) 20:40
https://doi.org/10.1186/s12896-020-00638-7

BMC Biotechnology

RESEARCH ARTICLE Open Access

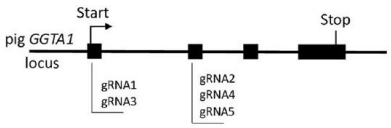
Efficient generation of *GGTA1*-deficient pigs by electroporation of the CRISPR/Cas9 system into in vitro-fertilized zygotes

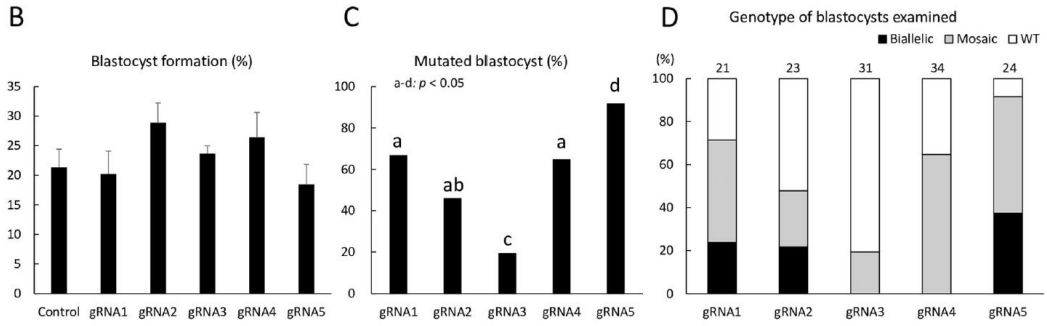
Fuminori Tanihara[1], Maki Hirata[1*], Nhien Thi Nguyen[1], Osamu Sawamoto[2], Takeshi Kikuchi[2], Masako Doi[2] and Takeshige Otoi[1]

Tokushima University, Japan

A

gRNA	Target Sequence	PAM	Strand
gRNA1	TGTTTTGGGAATACATCAAC	AGG	Sense
gRNA2	GGATTAAACCAGTCCACTAG	CGG	Antisense
gRNA3	AACTGTAATGGTTGTGTTTT	GGG	Sense
gRNA4	GAGAGCTTCCGCTAGTGGAC	TGG	Sense
gRNA5	AGACGCTATAGGCAACGAAA	AGG	Sense





Received: 1 February 2019 | Revised: 27 March 2019 | Accepted: 18 April 2019
DOI: 10.1111/xen.12524

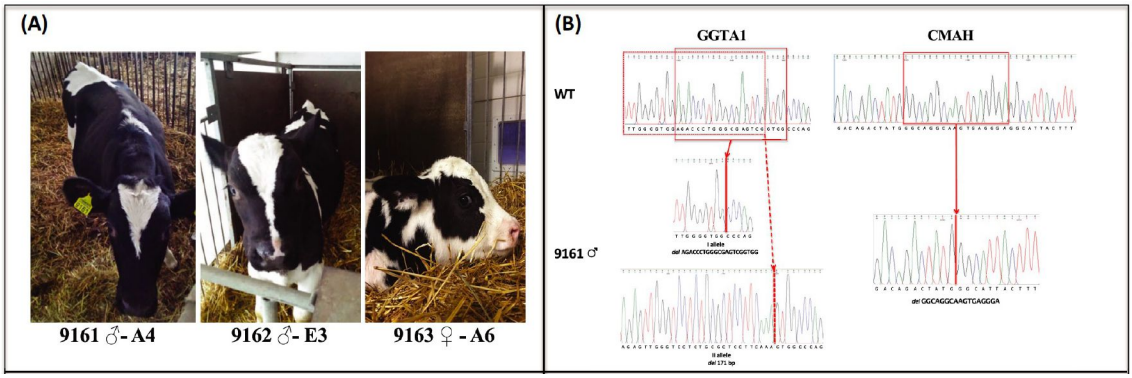
ORIGINAL ARTICLE

Xenotransplantation WILEY

Generation of cattle knockout for galactose-α1,3-galactose and N-glycolylneuraminic acid antigens

Andrea Perota[1] | Irina Lagutina[1] | Roberto Duchi[1] | Elisa Zanfrini[1] | Giovanna Lazzari[1,2] | Jean Paul Judor[3,4] | Sophie Conchon[3,4] | Jean Marie Bach[5] | Tomaso Bottio[6] | Gino Gerosa[6] | Cristina Costa[7] | Manuel Galiñanes[8] | Jean Christian Roussel[9] | Vered Padler-Karavani[10] | Emanuele Cozzi[11] | Jean Paul Soulillou[3,4] | Cesare Galli[1,2]

Laboratory of Reproductive Technologies, Cremona, Italy.

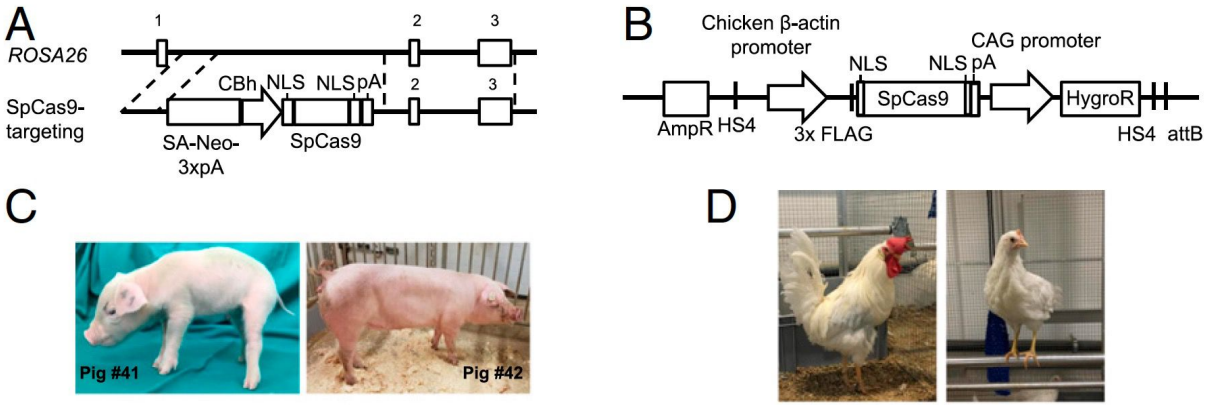


PNAS 118 (10): e2022562118 (2021)

Cas9-expressing chickens and pigs as resources for genome editing in livestock

Beate Rieblinger[a,1], Hicham Sid[b,1], Denise Duda[b,1], Tarik Bozoglu[c,d,1], Romina Klinger[b], Antonina Schlickenrieder[b], Kamila Lengyel[b], Krzysztof Flisikowski[a], Tatiana Flisikowska[a], Nina Simm[a], Alessandro Grodziecki[a], Carolin Perleberg[a], Andrea Bähr[c,d], Lucie Carrier[e,f], Mayuko Kurome[g,h], Valeri Zakhartchenko[g,h], Barbara Kessler[g,h], Eckhard Wolf[g,h], Lutz Kettler[i], Harald Luksch[i], Ibrahim T. Hagag[j], Daniel Wise[k], Jim Kaufman[k,l], Benedikt B. Kaufer[j,2], Christian Kupatt[c,d,2], Angelika Schnieke[a,2], and Benjamin Schusser[b,2]

Technical University Munich



In vivo genome editing in SpCas9 transgenic pigs and chickens can be easily performed by transducing with an AAV virus encoding gRNAs

PNAS

BRIEF REPORT | AGRICULTURAL SCIENCES

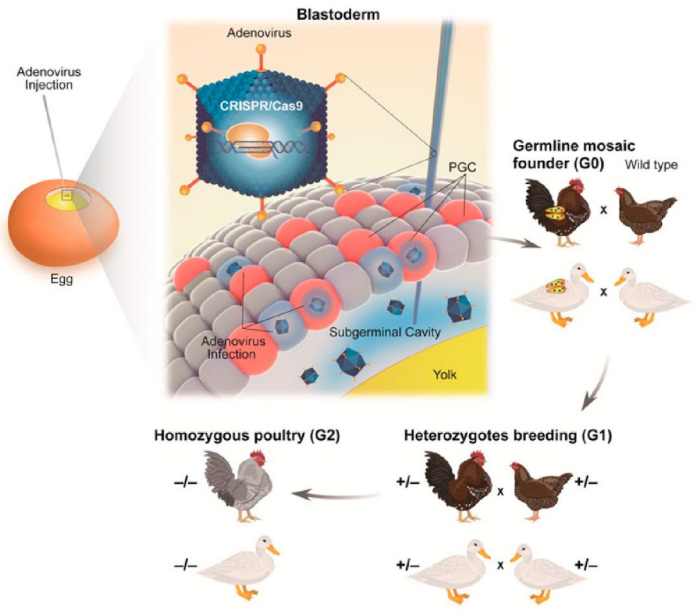
OPEN ACCESS

Generation of genome-edited chicken and duck lines by adenovirus-mediated in vivo genome editing

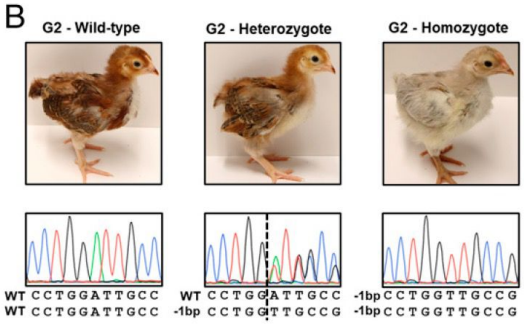
Joonbum Lee[a], Dong-Hwan Kim[a], Madeline C. Karolak[a], Sangsu Shin[a,b], and Kichoon Lee[a,1]

The Ohio State University

PNAS 119 (45):e2214344119 (2022)



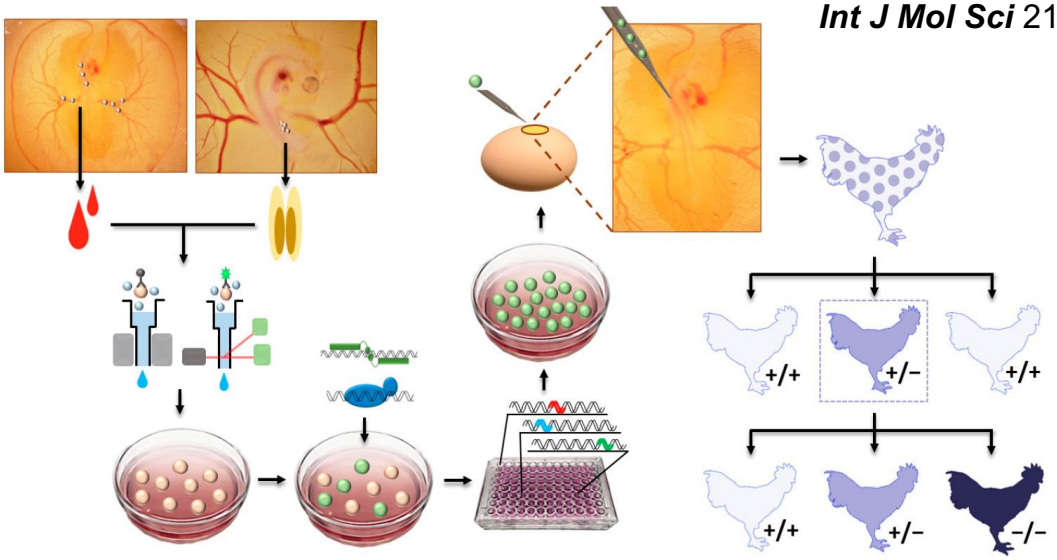
PGCs : primordial germ cells



Melanophilin (*MLPH*) gene deficient chickens

Ex vivo primordial germ cell (PGC)-mediated method for genome editing in the chicken

Int J Mol Sci 21: 3937 (2020)



1, Chicken PGCs are isolated from embryonic blood or gonad
2, *Ex vivo* genome editing in cultured PGCs
3, Selection of genome-edited PGCs
4, Injection into the dorsal aorta of recipient chicken embryos
5, Generation of potential germ-line chimeric chicken
6, Chimeras are mated with wild-type partners to produce heterozygous mutant (+/−) chickens

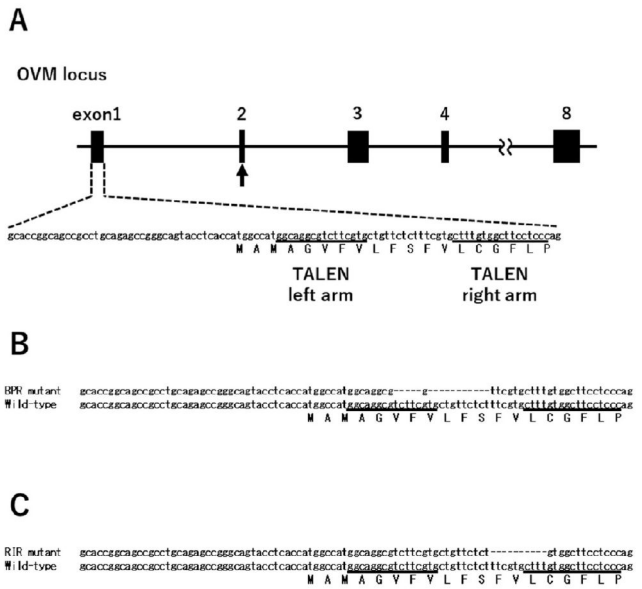
HIROSHIMA UNIVERSITY

Confirming the safety of genetically edited allergen-free eggs

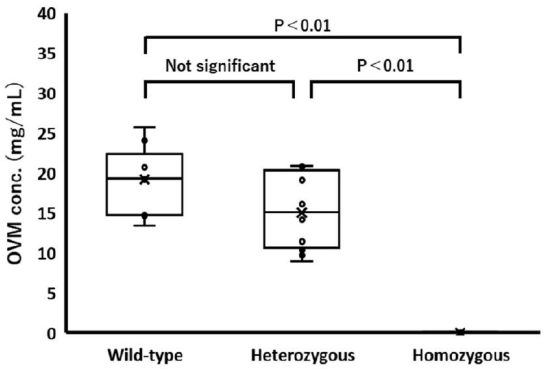
> Researchers have created a genetically edited allergen-free chicken egg that may be safe for those with egg white allergies.

Scientists from Hiroshima University produced OVM knocked out chickens using genome editing tools. Colored chickens are knockout chickens. (Ezaki et al. 2023, Food and Chemical Toxicology)

Ovomucoid (OVM) accounts for approximately 11% of all the protein in egg whites, and OVM is one of allergens for egg allergy.



OVM mutation in gene-targeted chickens



OVM protein was not detected in *OVM*-null egg.

Food and Chemical Toxicology 175:113703 (2023)

Summary

Current genetic engineering techniques enable to produce genetically modified livestock including pigs, cattle and chickens.

GGTA1 deficient pigs, which lack galactose-α-1,3-galactose (α-Gal) sugar molecule, for potentially allergy-free meat were established.

FDA approved *GGTA1* deficient pigs (facility in northern Iowa) for human food in 2021, but not yet commercially available.

Exogenous gene integration-free *GGTA1* deficient cattle, *OVM* deficient chickens were also generated.

These genetically modified animals are thought as new livestock for potentially allergy-free food, but it is still in the research stage.