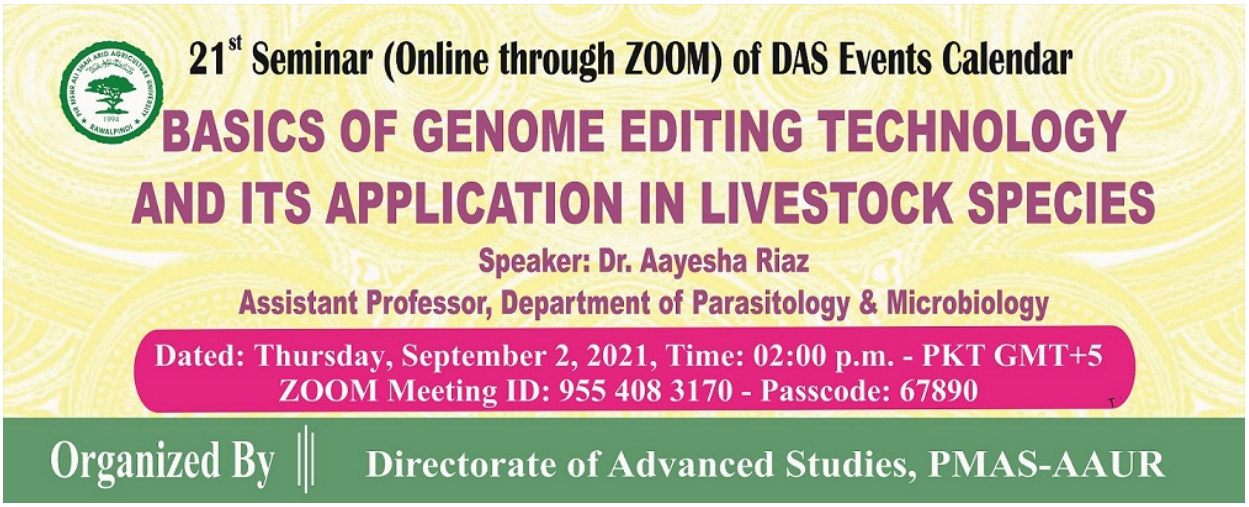
DIRECTORATE OF ADVANCED STUDIES
EVENT CATALOGUE
2021

21ST SEMINAR OF DAS EVENTS CALENDAR – 2021

BASICS OF GENOME EDITING TECHNOLOGY AND ITS APPLICATION IN LIVESTOCK SPECIES



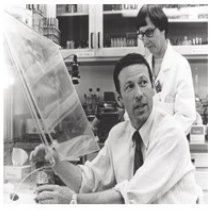
ACTIVITIES

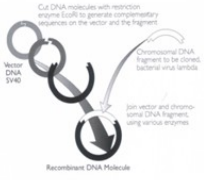
Contents

- Introduction
- What is gene Editing
- Gene Editing Tools
- CRISPR-Cas 9 Technique
- How gene editing works in Livestock
- Applications of this gene editing in Livestock
- Policy Changes
- Future

The Birth of Biotechnology – 1970s

Wally Gilbert and Fred Sanger, 1970s DNA sequencers



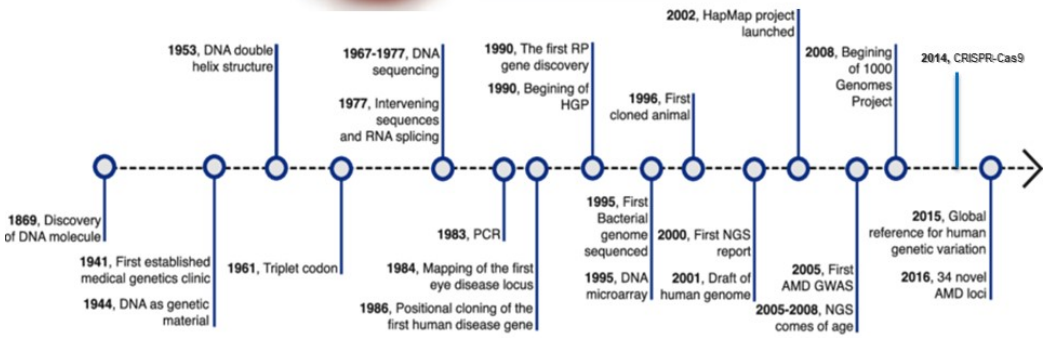


Paul Berg, Stanford, 1971 gene-splicing experiment where he inserted DNA from the lambda virus into the SV40 virus making recombinant DNA.

Biotech Today – What's Changed?

Biotechnology → Genetic Engineering → Gene Editing

Biotech – the early years



Gene Editing

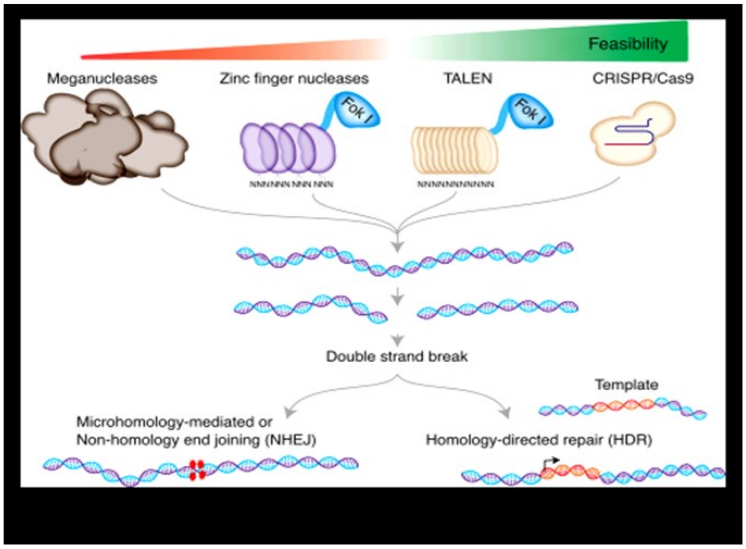
- Removal, modification, or addition of a functional element into the genome of a living cell or organism
  - Genes, regulatory regions
- **Random changes**
  - Insertion of plasmids or viruses at any of many accessible targets
- **Directed changes**
  - Homologous recombination
  - Sequence-specific DNA binding macromolecules
    - Protein domains: Zn fingers, TALENs: CRISPR

Gene editing tools

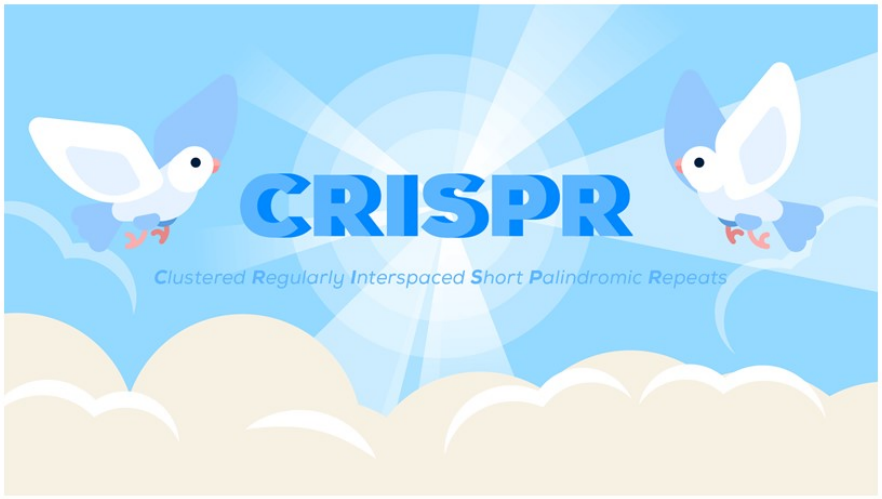
- Genome editing was pioneered in the 1990s.
- **Genetic Scissors:**

  As of 2015 four major classes of engineered nucleases were used:
- Meganucleases
- Zinc finger nucleases (ZFNs),
- Transcription activator-like effector based nucleases (TALENs) and
- The CRISPR-Cas9 (the clustered regularly interspaced short palindromic repeats) system.
- These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome.

Gene editing tools



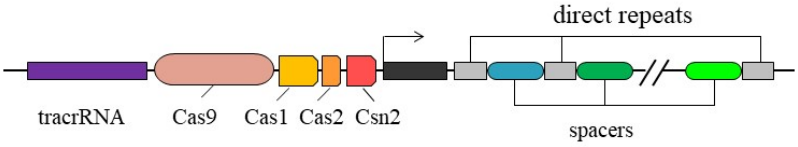
Gene editing tools



CRISPR – Bacterial Immunity

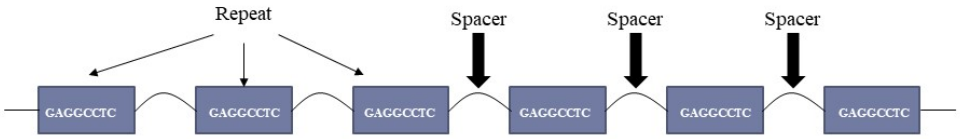
- **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
- Acquired adaptive immunity in bacteria against viruses
- First described in 1987

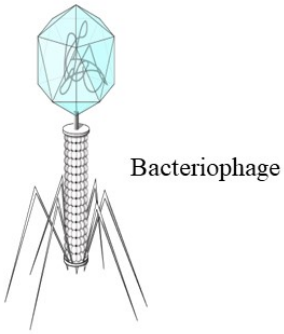
Streptococcus pyogenes CRISPR array



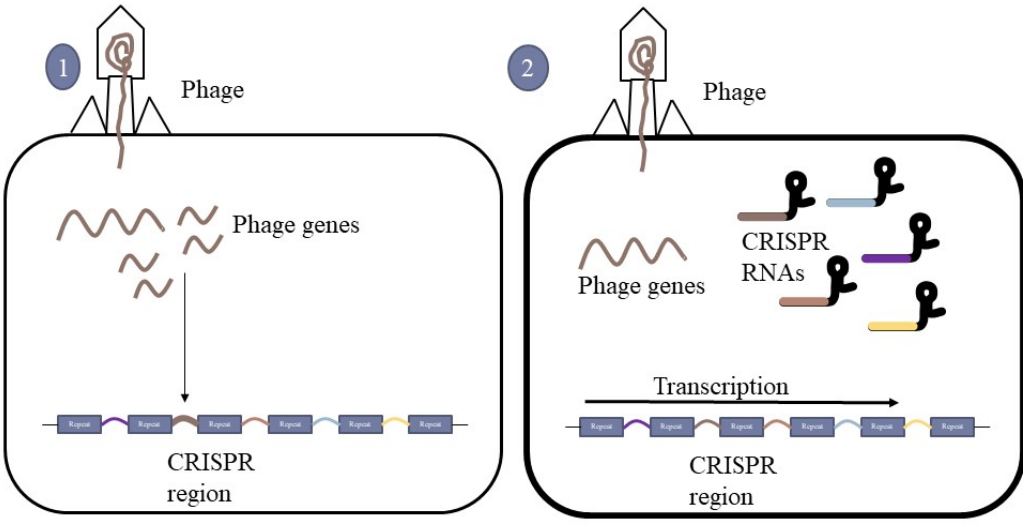
Yoshizumi Ishino

Clustered Regularly Interspaced Short Palindromic Repeats





Clustered Regularly Interspaced Short Palindromic Repeats



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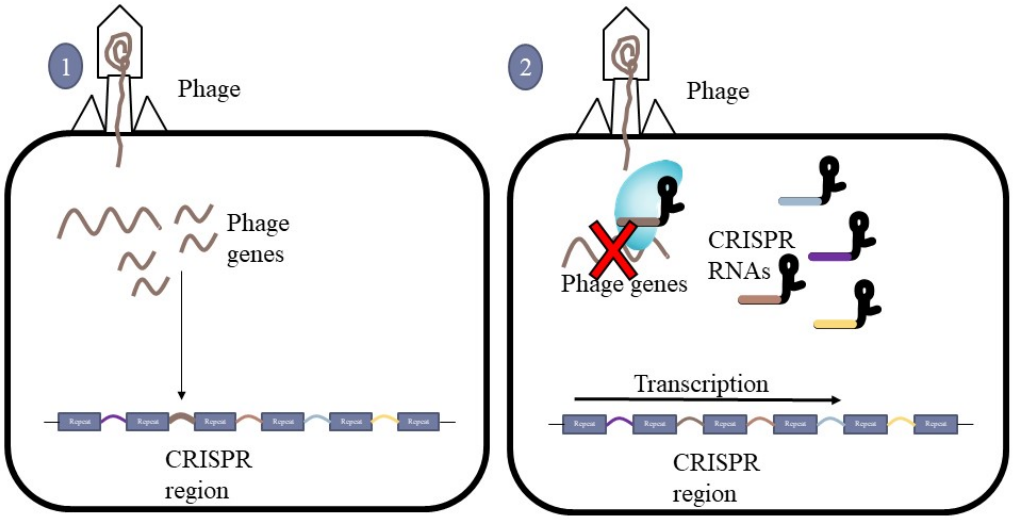
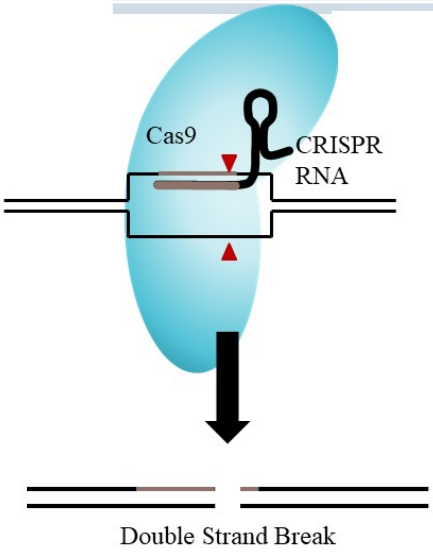
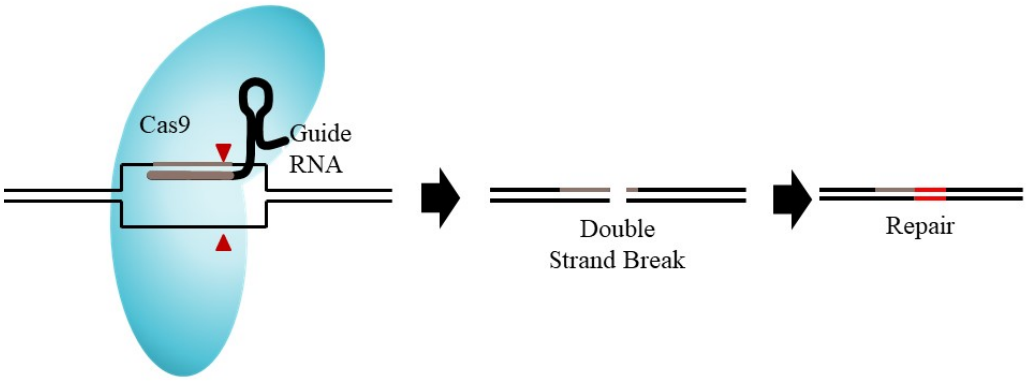


Cas nucleases

- Nuclease – a protein that cuts nucleic acids
- Cas9 makes a double strand break in target DNA
- Directed to a sequence by an RNA – in bacteria the RNA produced from the CRISPR loci.
- Nearly ANY sequence



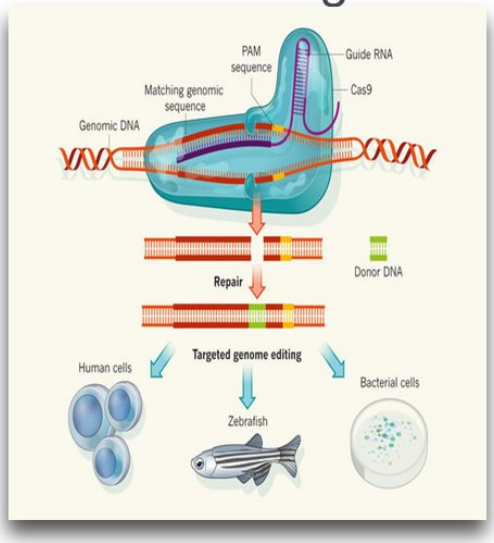
CRISPR/Cas9 as a gene editing tool



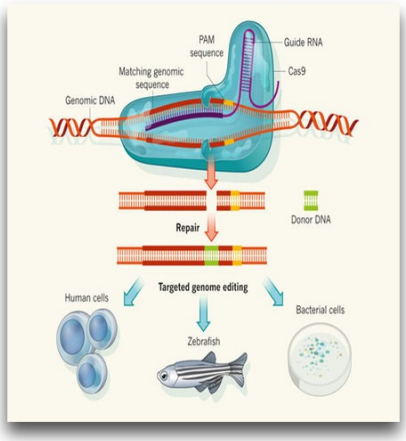
Emmanuelle Charpentier and Jennifer Doudna won the Nobel Prize in Chemistry 2020 for their groundbreaking work on CRISPR technology, for the development of the gene editing tool.

By using this technique they can cut any DNA molecule at a predetermined site.

CRISPR-Cas9 – a highly specific genome editor



CRISPR-Cas9 – a highly specific genome editor



Knock genes out – disable them

Precisely replace genes or insert new ones

Increase or decrease expression of genes

CRISPR/Cas9 – two kinds of editing

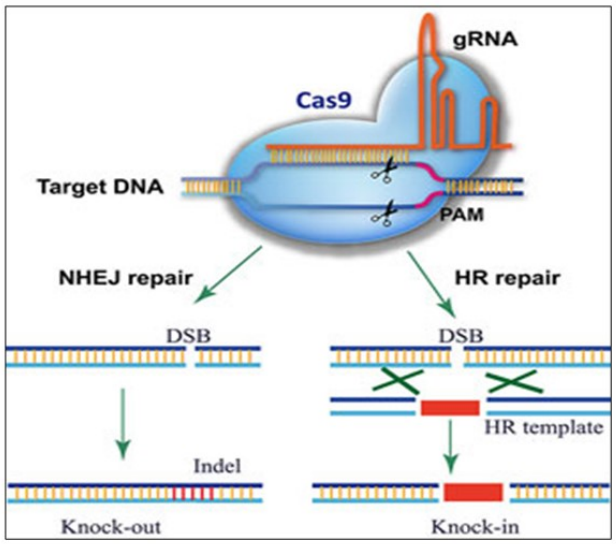
1. Error prone DNA repair (NHEJ)
2. DNA repair using a template (HR)

Faster, cheaper, more accurate, more efficient.

CRISPR genome editing

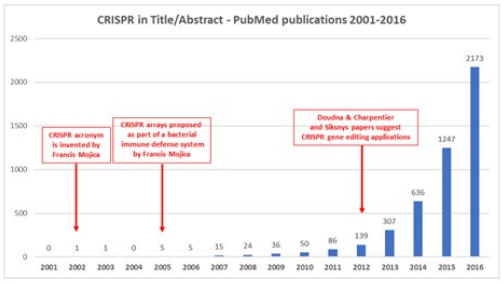
CRISPR/Cas9 – two kinds of editing

1. Error prone DNA repair also called as non homologous end joining (NHEJ)
2. DNA repair using a template, also called as homology-directed repair (HR).



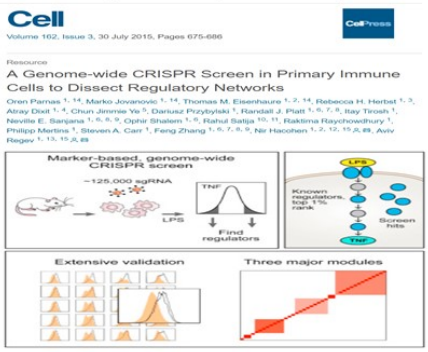
CRISPR-Cas9 explosion

- Fantastic research tool - knock out individual genes at high efficiency



*Over 3000 mentions in 2017

http://www.cnb.csic.es/~montoliu/CRISPR/

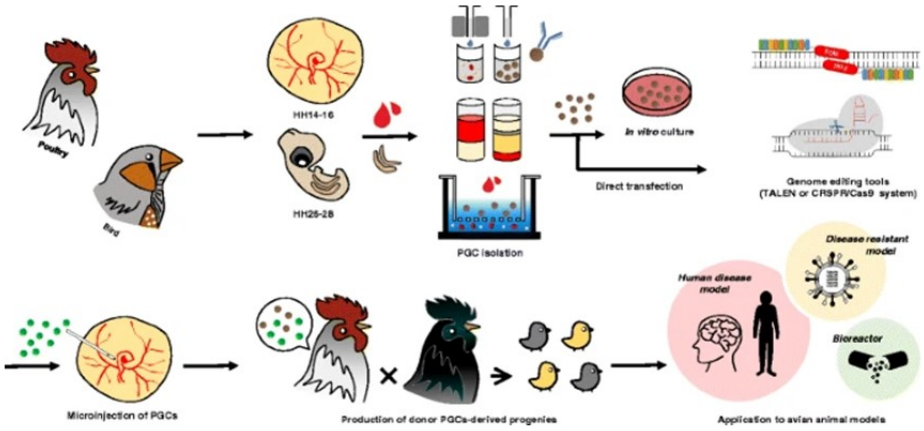


CRISPR/CAS9 in Livestock

- Since conventional genetically modified organism (GMO) has foreign gene or uncontrolled random mutation, there has been public concern about the safety issue of food derived from GMO due to unknown allergen reaction or use of antibiotic resistance genes.

- On the other hand, genome-edited chickens and other livestock can be produced by controlled precise genome editing technology similar to mutations in intrinsic genomic sequences, like natural mutations, rather than foreign gene insertion as in conventional GMO.

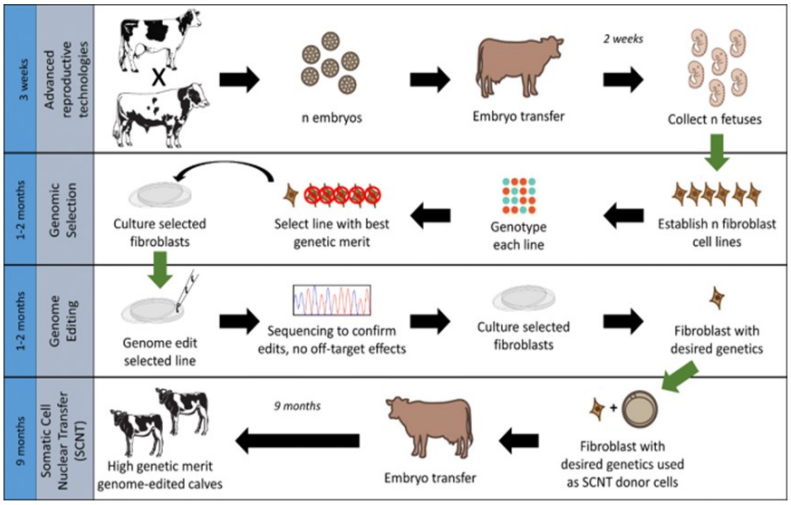
The Process



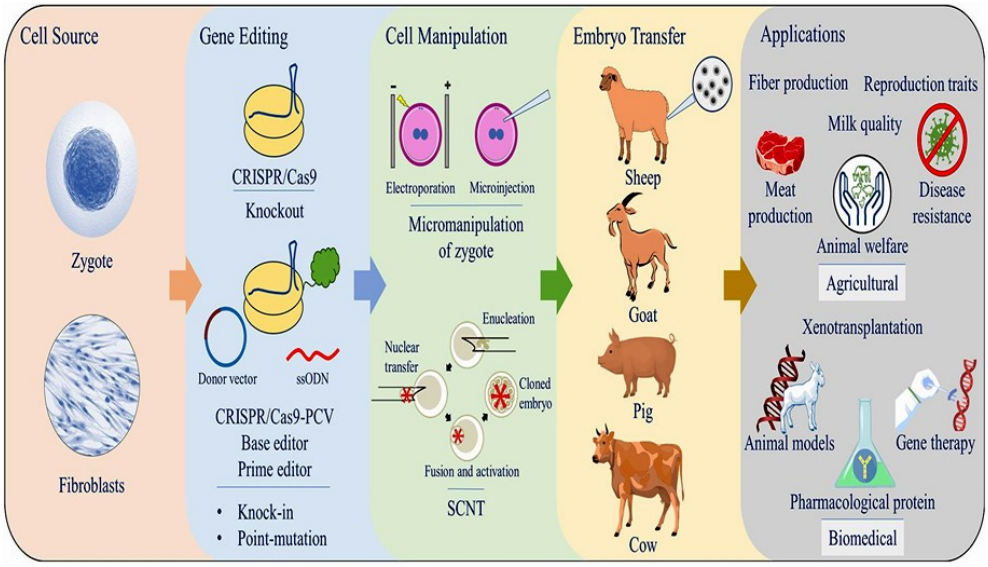
Strategies for the production of genome-edited birds. Avian PGCs can be isolated from embryonic blood (HH stages 14–16) and embryonic gonads (HH stage 26–28) by cell-surface antibody-mediated methods, density gradient centrifugation, and size-dependent isolation methods. Genome-edited birds can be produced by transplanting directly isolated or in vitro cultured PGCs into the blood vessels of recipient embryos after the introduction of genome editing tools. Avian genome editing systems can be applied to produce various avian models, such as avian disease resistance models, bioreactor models, and human disease models.

Han and Park, 2018

The Process



The Process



Applications

- Disease resistance (e.g. tuberculosis, mastitis, Avian Influenza)
- Production (e.g. myostatin knockout)
- Elimination of allergens (e.g. beta-lactoglobulin knockout)
- Heat Tolerance (poultry)
- Therapeutic Use
- Welfare (e.g. hornlessness)

Examples of successful gene edited agricultural applications in food animal species.

Species	Target	Publication	Trait/Goal
Cattle	Intraspecies *POLLED* allele substitution	Tan *et al.* [22]; Carlson *et al.* [13••]	No horns
	Myostatin KO	Proudfoot *et al.* [23] Luo *et al.* [24]	Increased muscle yield
	Beta-lactoglobulin KO	Yu *et al.* [25]	Elimination of milk allergen
	Lysostaphin transgene	Liu *et al.* [15]	Disease resistance
	Lysozyme transgene	Liu *et al.* [16]	Disease resistance
	SP110 transgene	Wu *et al.* [17]	Resistance to tuberculosis
Chicken	Ovalbumin KO	Park *et al.* [26]	Elimination of ovalbumin in egg
	Immunoglobulin heavy chain locus	Dimitrov *et al.* [27]	Germline gene editing
Goat	Myostatin	Ni *et al.* [28]	Increased muscle growth
	Prion protein KO		Elimination of prion protein
	Beta-lactoglobulin KO		Elimination of milk allergen
Pig	CD163 KO	Whitworth *et al.* [29•]	PRRS Virus Resistance
	RELA interspecies substitution	Lillico *et al.* [14••]	African Swine Fever Resistance
	Myostatin KO	Wang *et al.* [30] Qian *et al.* [31]	Increased muscle yield
Sheep	Myostatin KO	Proudfoot *et al.* [23] Crispo *et al.* [32] Han *et al.* [33]	Increased muscle yield

KO: knock out or inactivation of gene function.

Disease Resistance

Single Cas9 nickase induced generation of *NRAMP1* knockin cattle with reduced off-target effects

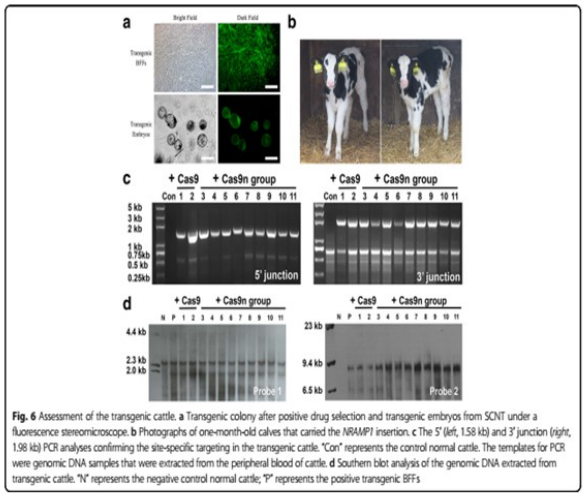


Fig. 6 Assessment of the transgenic cattle. **a** Transgenic colony after positive drug selection and transgenic embryos from SCNT under a fluorescence stereomicroscope. **b** Photographs of one-month-old calves that carried the *NRAMP1* insertion. **c** The 5′ (*left*, 1.58 kb) and 3′ junction (*right*, 1.98 kb) PCR analyses confirming the site-specific targeting in the transgenic cattle. "Con" represents the control normal cattle. The templates for PCR were genomic DNA samples that were extracted from the peripheral blood of cattle. **d** Southern blot analysis of the genomic DNA extracted from transgenic cattle. "N" represents the negative control normal cattle; "P" represents the positive transgenic BFFs

Tuberculosis resistant cows developed for the first time using CRISPR technology.

--By using CRISPR/Cas9n a tuberculosis resistance gene, called NRAMP1, was successfully inserted into the cow genome.

--Scientists were then able to successfully develop live cows carrying increased resistance to tuberculosis.

--Importantly, their method produced no off target effects on the cow genetics meaning that the CRISPR technology employed may be better suited to producing transgenic livestock with purposefully manipulated genetics."

Gao et al., 2017

Disease Resistance

Species specific differences in use of ANP32 proteins by influenza A virus

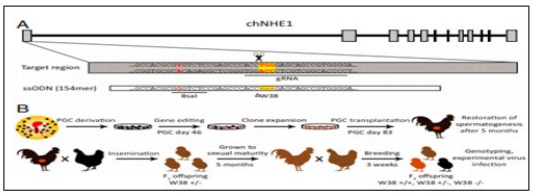
Lab grown Gene-edited chicken cells resist bird flu virus.

Viral replication is abrogated in chicken cells lacking ANP32A

The data above suggest that chANP32B cannot substitute for chANP32A in support of IAV polymerase in chicken cells. Since chicken cells that completely lack expression of chANP32A show no polymerase activity in the minigenome assay, they might be refractory to IAV infection. Multi-cycle

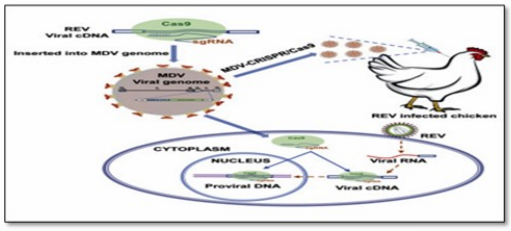
Long et al., 2019

Precise CRISPR/Cas9 editing of the NHE1 gene renders chickens resistant to the J subgroup of avian leukosis virus



Koslova et al., 2020

Prevention of Avian Retrovirus Infection in Chickens Using CRISPR-Cas9 Delivered by Marek's Disease Virus

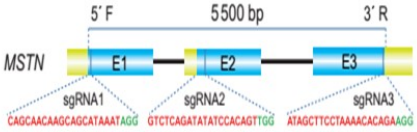


Li et al., 2020

Muscle Productivity

The CRISPR/Cas9 induces large genomic fragment deletions of *MSTN* and phenotypic changes in sheep

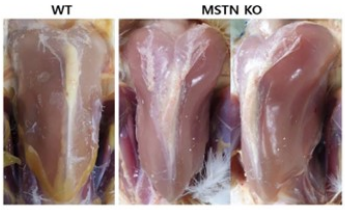
The average daily gain of *MSTN*-disrupted animals was higher (164 g d–1>142.2 g d–1) than that of wildtype lambs.



Yi et al., 2017

Generation of myostatin-knockout chickens mediated by D10A-Cas9 nickase

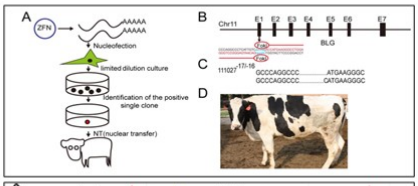
•In this study it has been seen that MSTN KO chickens exhibited significantly larger skeletal muscles.
•The abdominal fat deposition was dramatically lower in MSTN KO chickens than in wild-type chickens.



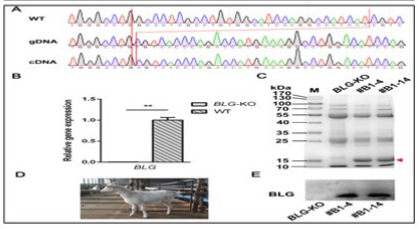
Kim et al., 2020

Allergen free food

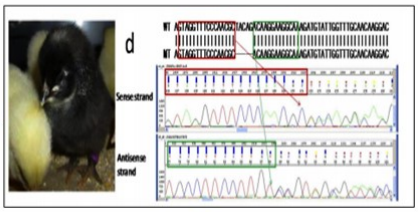
Production of hypoallergenic milk from DNA-free beta-lactoglobulin (BLG) gene knockout cow using zinc-finger nucleases mRNA. (Sun et al., 2018). For the frst time DNA-free BLG bi-allelic knockout cow by zinc-fnger nuclease (ZFNs) mRNA was produced and produced BLG-free milk.



Generation of beta-lactoglobulin knock-out goats using CRISPR/Cas9. (Zhou et al., 2017)
BLG protein had been abolished in the milk of the BLG knock-out goat.



Targeted Mutagenesis in Chicken using CRISPR/Cas 9 System (Oishi et al., 2015)
Two egg white genes, ovalbumin (OVA) and ovomucoid (OVM) were efficiently (>90%) mutagenized in cultured chicken primordial germ cells (PGCs) by transfection of circular plasmids encoding Cas9, a single guide RNA.

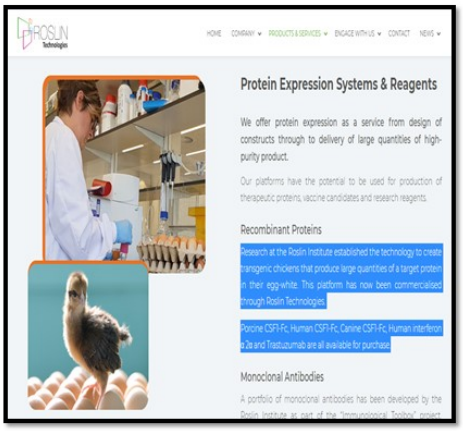


Therapeutic Use

Gene modified chickens 'lay medicines'

•Researchers at the University of Edinburgh's Roslin Institute have genetically modified chickens to produce human proteins in their eggs.
•Just three eggs contain a clinically significant dose.

•It's the egg white that contains the treasure: large quantities of medically important proteins.

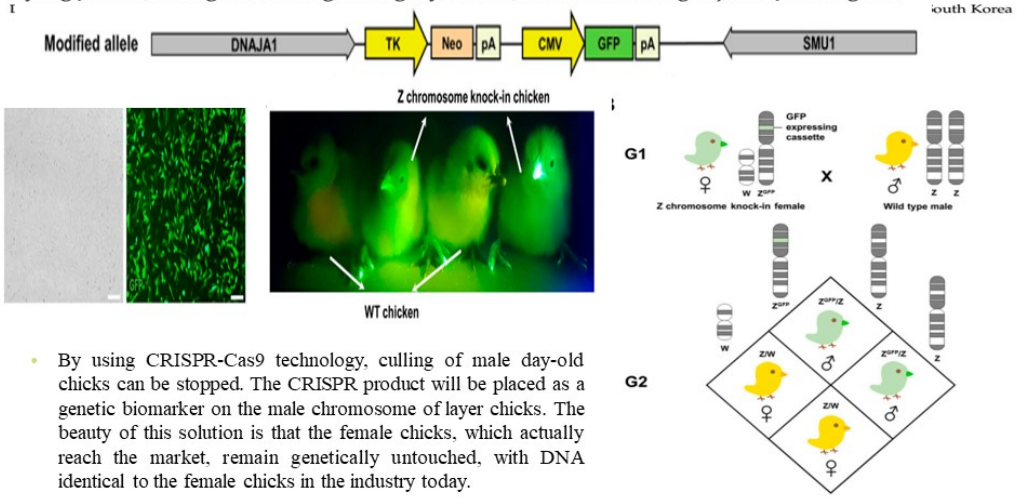


•Human CSF1-Fc, Canine CSF1-Fc and Human interferon α 2α are all available for purchase.
•The chickens themselves are unaffected by the presence of human proteins in their systems.

BioMarker

Targeted gene insertion into Z chromosome of chicken primordial germ cells for avian sexing model development

Hong Jo Lee, Jong Won Yoon, Kyung Min Jung, Young Min Kim, Jin Se Park, Kyung Youn Lee, Kyung Je Park, Young Sun Hwang, Young Hyun Park, Deivendran Rengaraj, and Jae Yong Han[1]
[1] ...outh Korea



- By using CRISPR-Cas9 technology, culling of male day-old chicks can be stopped. The CRISPR product will be placed as a genetic biomarker on the male chromosome of layer chicks. The beauty of this solution is that the female chicks, which actually reach the market, remain genetically untouched, with DNA identical to the female chicks in the industry today.

Policy Challenges

- Enormous potential
- Regulatory challenges
- Biohacking – positives and negatives
- How to stay on top of fast-moving science
- Old policies won't fit new technologies in health, food, environment
- Balance between innovation and regulation
- Education and public engagement on complicated science

The future is here

- Genetic diseases
  - More than 10,000 diseases are due to mutations to a single gene
- Viral diseases
- Cancer
- Forestry, agriculture, environment
  - Climate change mitigation and adaptation
  - Increased yields
  - Reduced pesticides and antibiotics
- Enhanced opportunities for synthetic biology