Gene Editing in Animals

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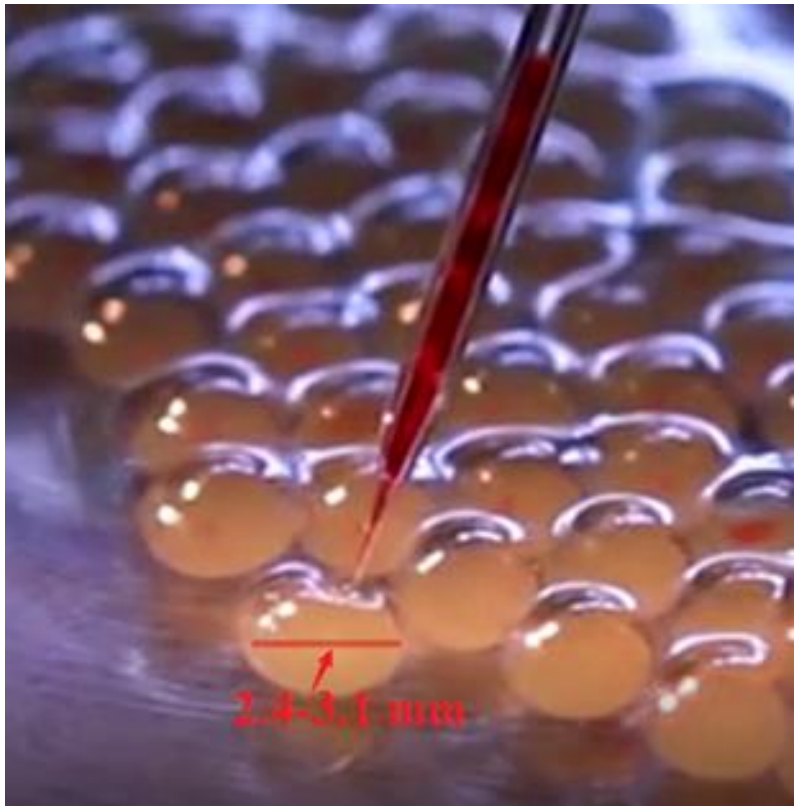
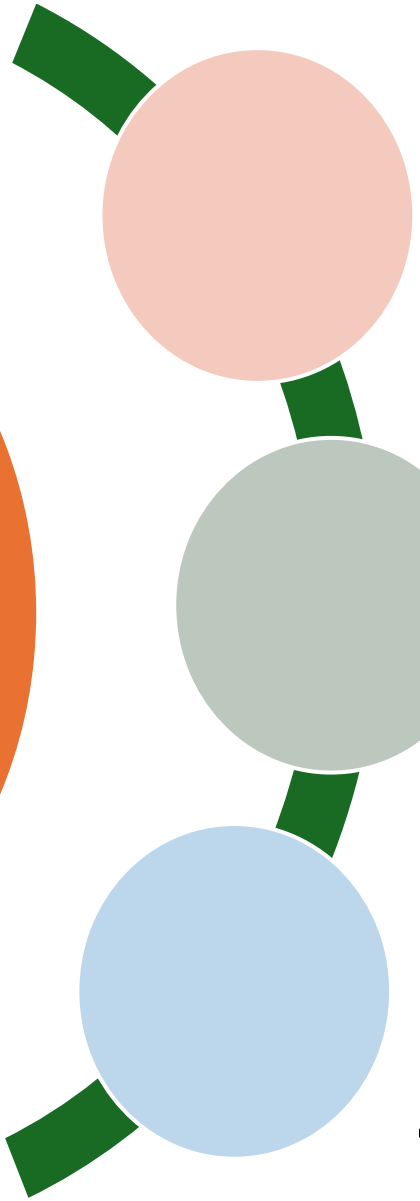
University of the Philippines Visayas

ISAAA Webinar
August 22, 2024

CRISPR/Cas9 microinjection in catfish eggs



Lecture Outline



Overview of gene editing techniques in animals

Recent developments in gene edited animals/fish

Overview of the status of gene editing regulations in the Philippines

What is gene-editing?

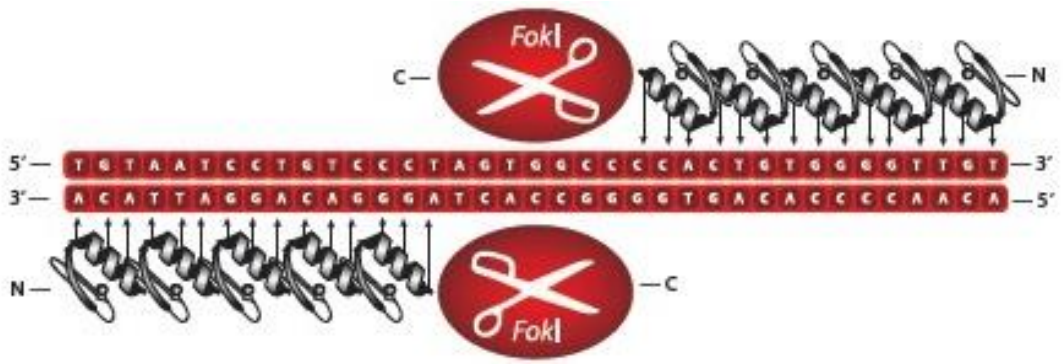
the ability to make highly specific changes in the DNA sequence of a living organism

- **Altering the sequence of DNA “in situ”**
- **Precisely and efficiently modify DNA within the cell**
- **Targeted mutagenesis**
  - Knock-outs
  - Point mutations
  - Gene insertions or “trait landing pads”
- **Ideally leaving no transgene footprint** (this is true for gene knock-outs)

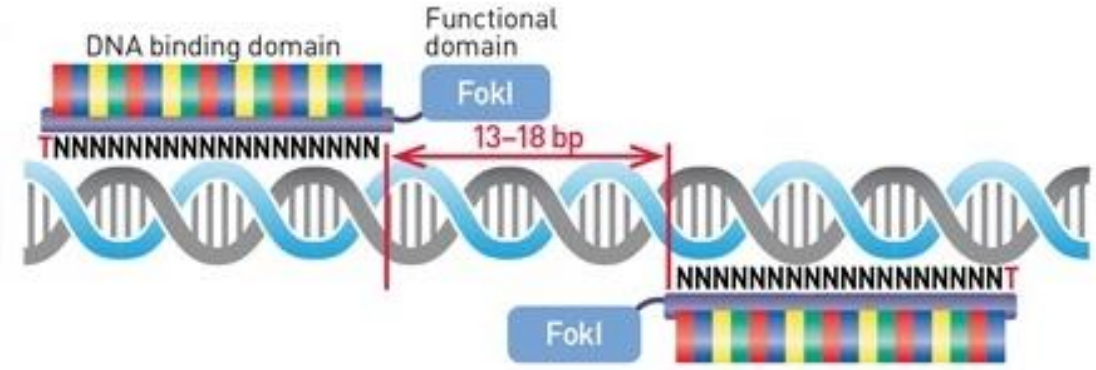
Gene-editing technologies

Common Feature: engineered nucleases (enzymes that make cuts at specific DNA sequences)

ZFN- Zinc finger nucleases

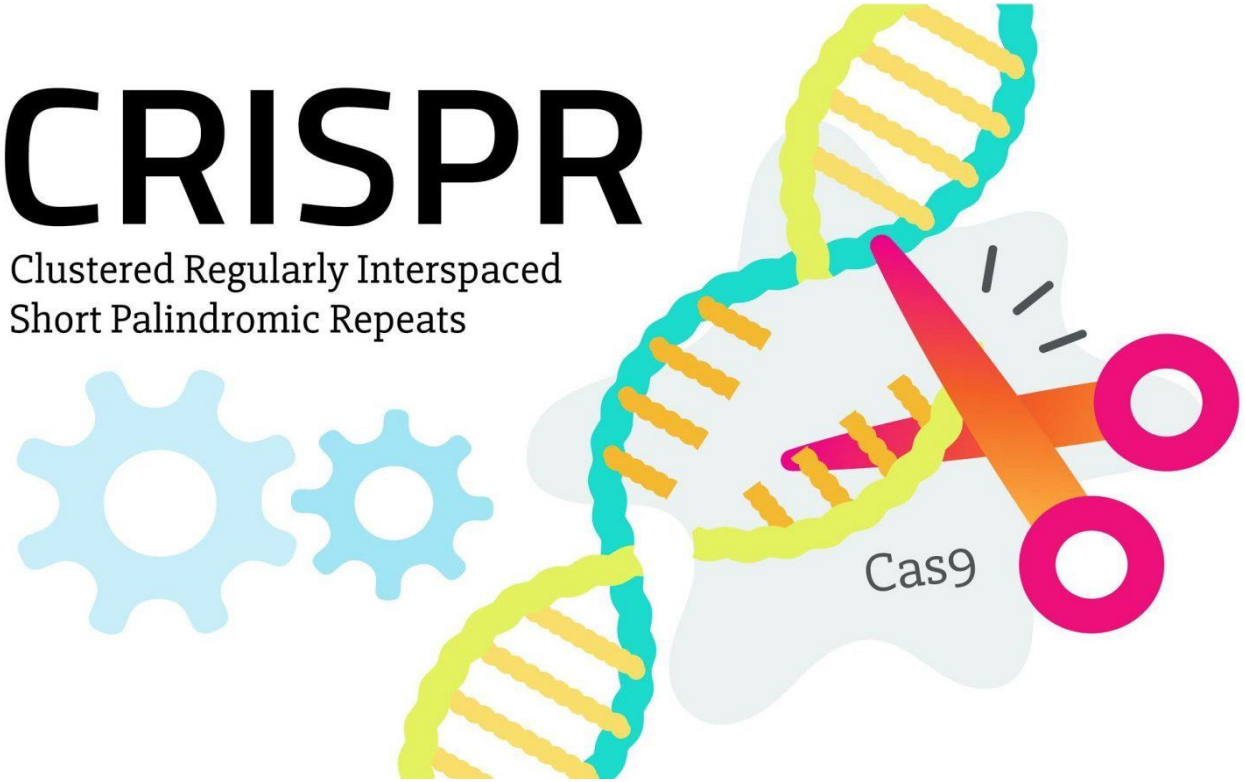


TALEN -transcription activator-like effector nucleases



CRISPR

Clustered Regularly Interspaced Short Palindromic Repeats

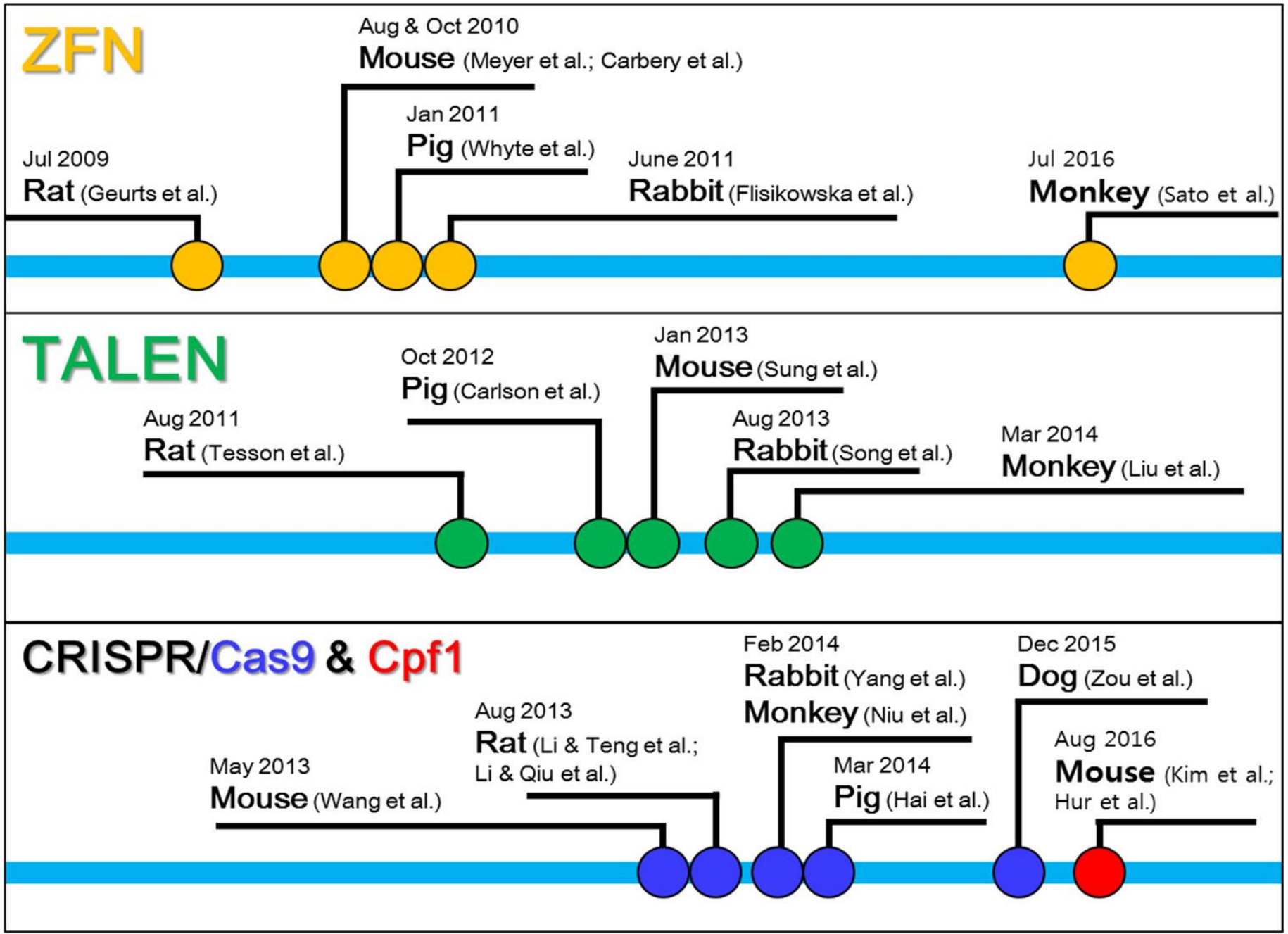


- CRISPR is cheap, easy to use and more efficient than TALEN and ZFN
- Based on bacterial immune system

Comparison of the different gene-editing technologies

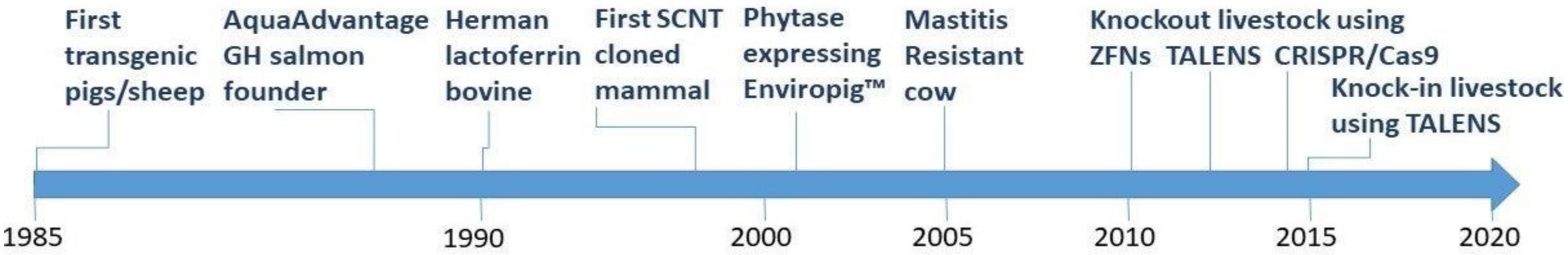
Factors	ZFN	TALEN	CRISPR/Cas9
DNA recognition	Multimeric protein-DNA interaction	Protein-DNA interaction	RNA-DNA Watson-Crick base pairing
DNA cleavage	Coupling to non-specific nuclease Fork1	Coupling to non-specific nuclease Fork1	Innate to Cas9
Requirements	Two large protein constructs	Two large protein constructs	Simple 20 nt change to construct
Targeting	Poor	Good	Good
Feasibility	Difficult	Difficult	Easy
Cost	High	High	Low

First applications of engineered nucleases in animals



Lee et al. 2018

Major Milestones in GE Livestock



Microinjection of DNA into early stage zygotes

Precomplexing DNA with sperm (SMGT)

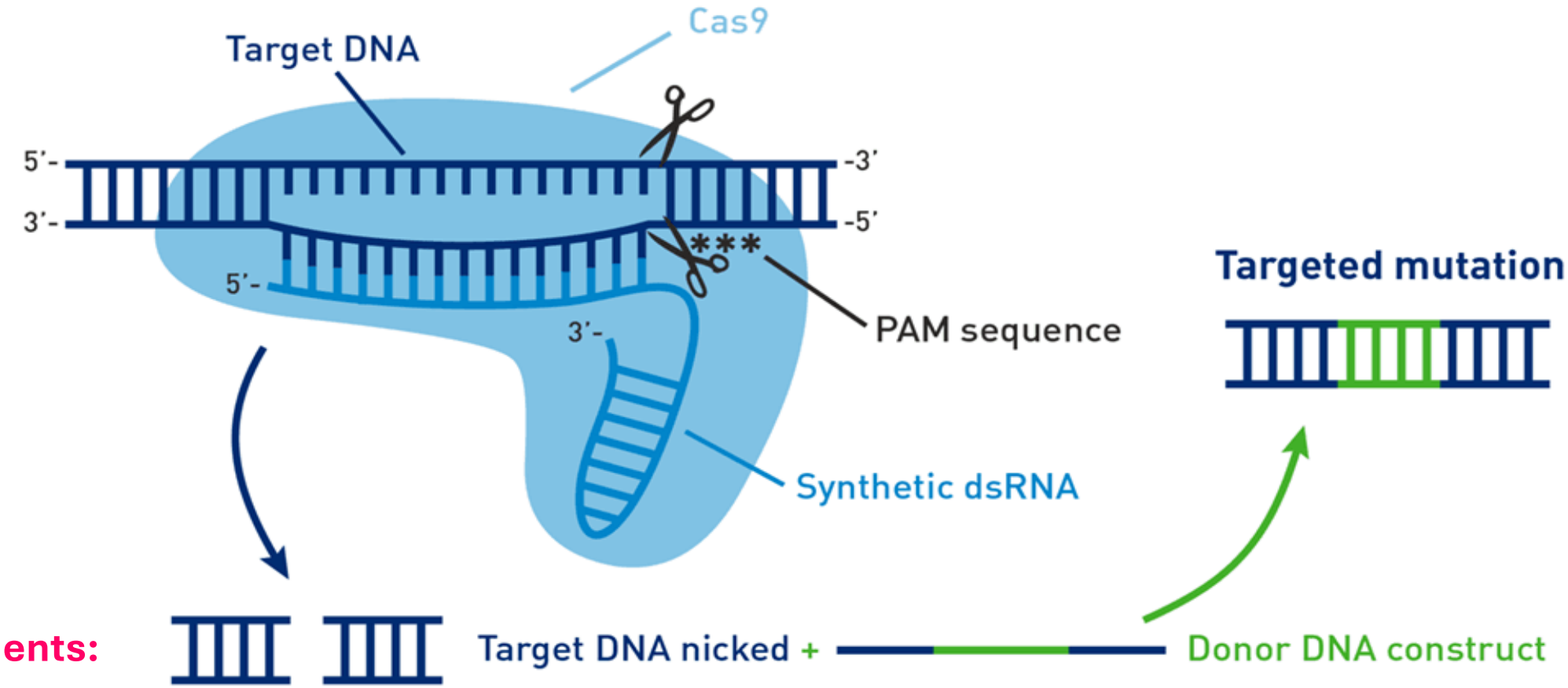
Retroviral delivery into zygotes

SCNT of genetically engineered cells

Transposon-mediated integration

Genome editing-based genomic alterations (ZFNs, TALENs, CRISPR/Cas9)

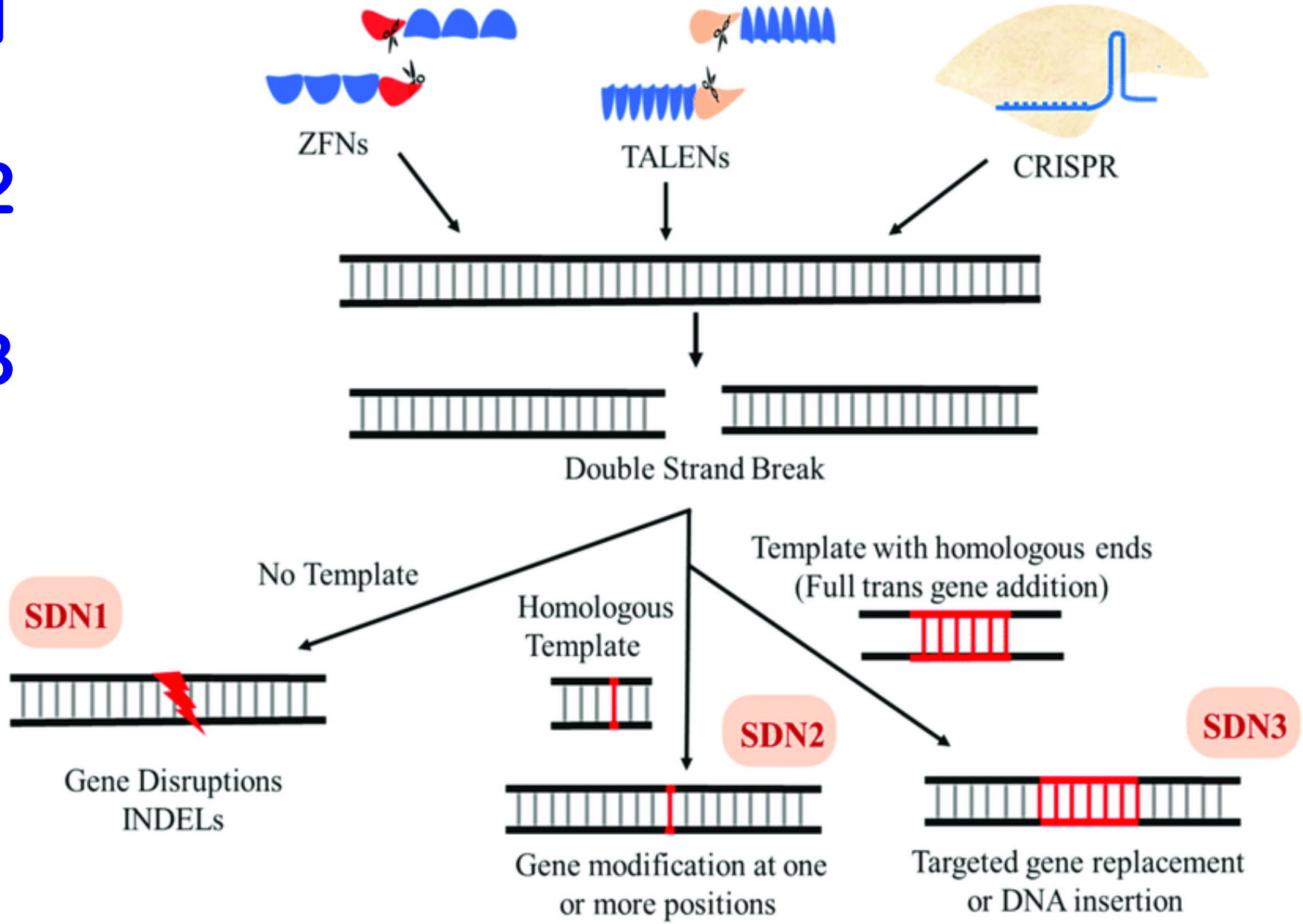
Basic DNA editing using CRISPR/Cas systems



Two components:

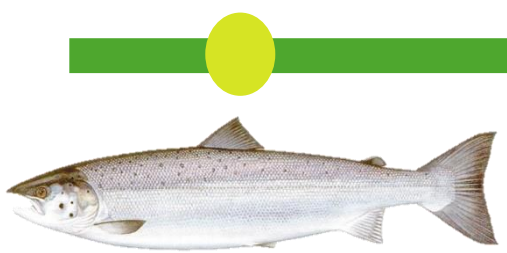
- **Guide RNA-** specific RNA sequence that recognizes the target DNA region of interest and directs the Cas9 nuclease
- **Associated protein Cas9** (nuclease/RNA-guided cutter)

SDN1 vs SDN2 vs SDN3



Ahmad et al. 2021

CRISPR/Cas9 Applications in Non-Model Fish



Atlantic Salmon

- knock-out of *dnd*
- knock-out of tyrosinase and slc45 responsible for pigmentation

(Edvardsen *et al*. 2014; Wargelius *et al*. 2016)

Nile Tilapia

- genes like *nanos2*, *nanos3*, *dmrt1*, and *foxl2* have been edited and found germ cell-deficient gonads and masculinization

(Li *et al*. 2014)

Common Carp

- disrupt muscle-specific *mstnba* gene displaying significant muscular growth

(Zhong *et al*. 2016)

Channel Catfish

- knock-out of myostatin gene resulted to more muscle cells and increase body weight
- increased mutation rates for RBL and TICAM1 genes

(Khalil et al. 2017; Elaswad et al. 2018; Kishimoto et al., 2018)

- disease resistance gene knock-in (Simora et al., 2020

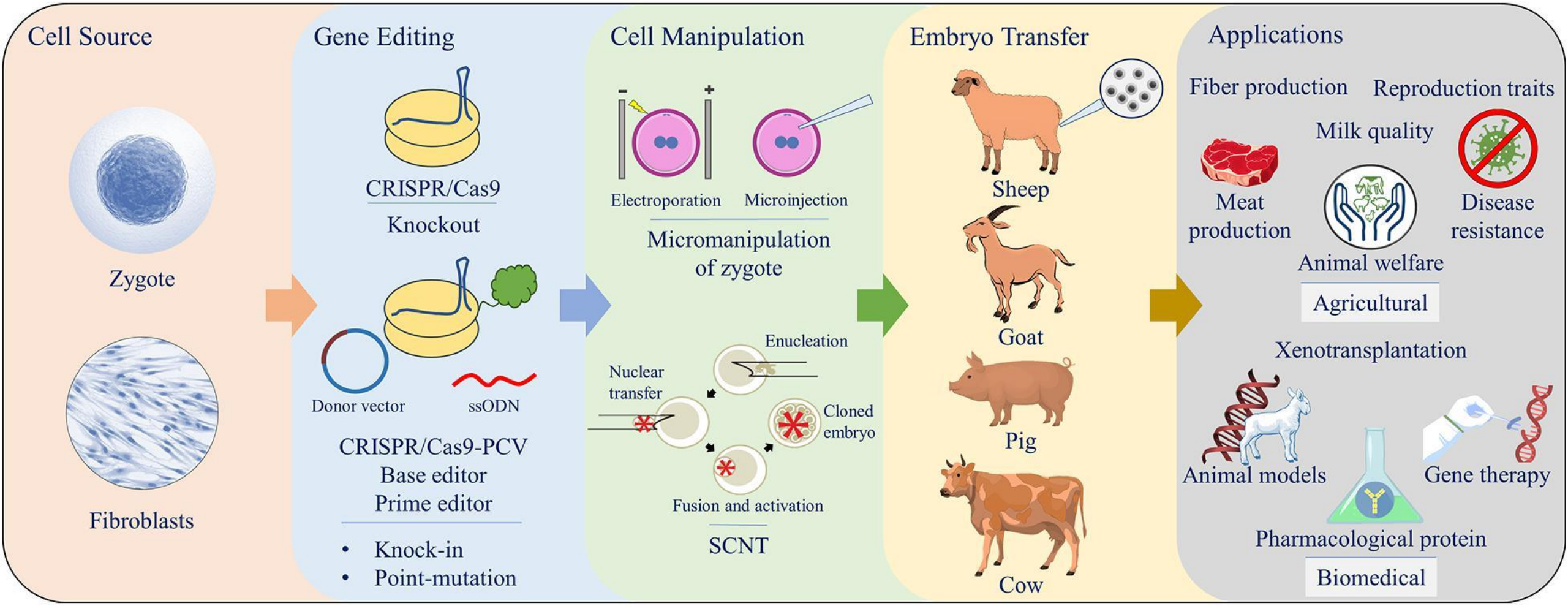
Sea Bream

Rainbow trout

- disrupt expression of duplicated insulin-like growth factor binding protein-2b gene

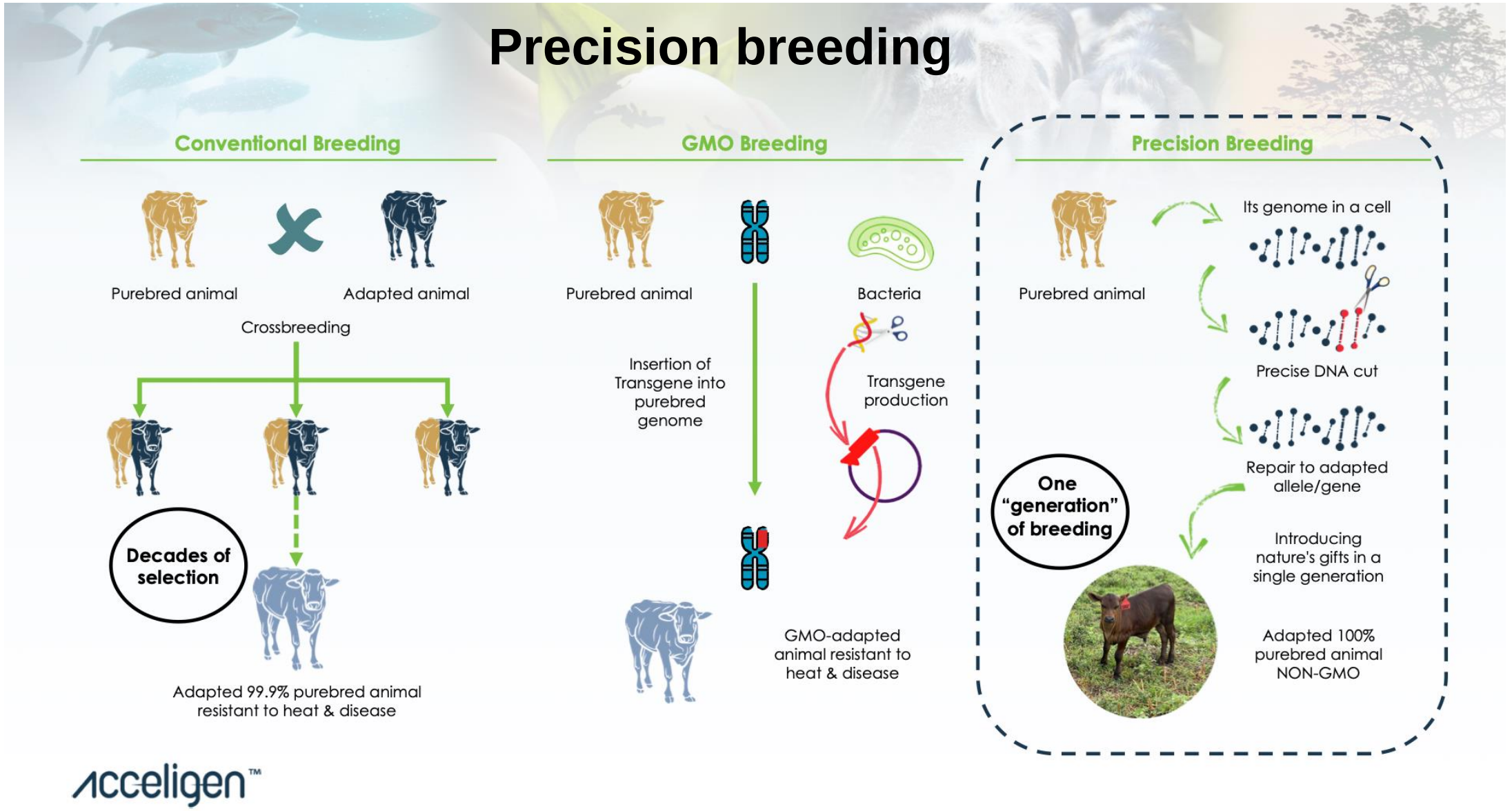
(Cleveland et al. 2018)

Summary of CRISPR/Cas9 gene editing using either zygote micromanipulation or somatic cell nuclear transfer (SCNT) for generation of livestock animals for various applications



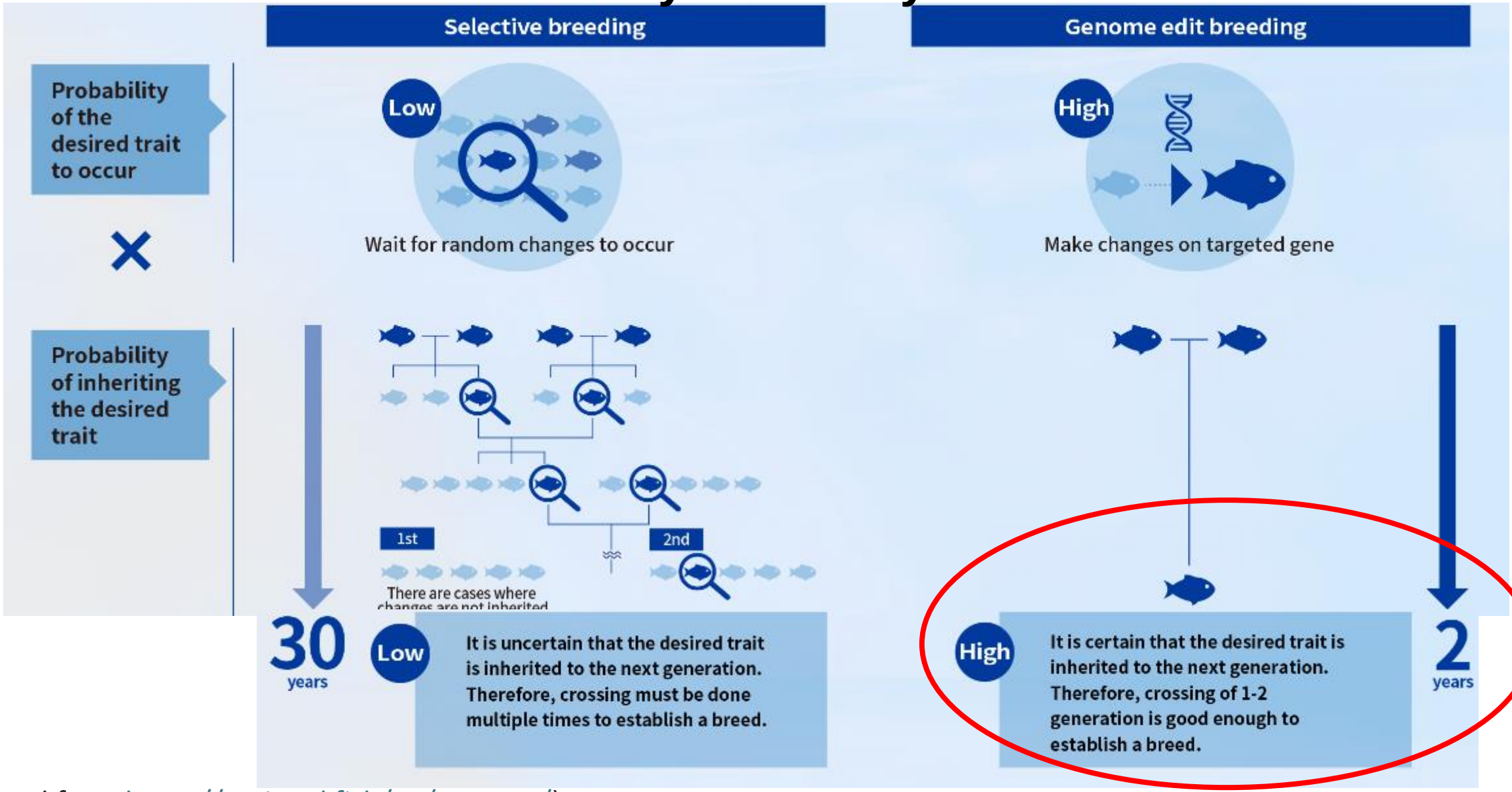
Perisse et al. 2021

Recent developments in gene edited animals/fish



acceligen™

Gene editing can forward the breeding process in fish from 30 years to 2 years!



(Adapted from https://regional.fish/en/genome/)

Fishes are excellent systems for genome editing

- Fishes have high fecundity.
- Protocols for artificial induction of spawning exist for many species.
- The eggs are relatively large, fertilization is external and easily conducted *in vitro*.
- Embryonic and larval development occur outside the mother; egg incubation and larval rearing methods are well established.
- Generation times range from one (tilapias) to several (carps, salmonids, catfishes) years.

(Adapted from Dr. Hallerman's lecture)

Did you know?

As of 2024, three genetically-engineered food animal products are commercially available in the United States.

- 2015, the FDA granted its first approval for a genetically engineered (GMO) food animal, fast growing line of Atlantic salmon modified by AquAdvantage
- 2020, Galsafe pig which can be eaten by people who are allergic to a molecule called alpha-gal in unmodified pigs
- 2022, FDA approved a gene-edited short-haired, heat-tolerant beef cattle for human consumption

Gene-edited lines of fish are advancing along the pathway towards commercial production

Species	Country	Company	Year	Modification
FLT-01 Nile Tilapia (*Oreochromis niloticus*)	Argentina	AquaBounty	2018	CRISPR/Cas9 knock out of myostatin gene to increase muscle mass (16% improved growth rate)
Red sea bream (*Pagrus major*)	Japan	Regional Fish Institute, Ltd. (Kyoto and Kindai Universities)	2019	CRISPR/Cas9 knock out of myostatin gene to increase muscle mass (20% more meat)
Tiger pufferfish (*Takifugu rupripes*)	Japan	Regional Fish Institute, Ltd. (Kyoto and Kindai Universities)	2021	CRISPR/Cas9 knock out of genes controlling appetite (1.9 times heavier than wild type)

Introducing… the gene-edited olive flounder

December 2023

RFI has completed the official procedures to commercialize "fast growth olive flounder" developed using genome editing technology.

Source: RFI website

How about a catfish that has more muscles?

Catfish with increased muscle mass

Our research at Auburn University Fish Genetics Lab

Naturally occurring disruption of myostatin locus

Knock-out of Myostatin Gene

Double-muscled cattle

SCIENTIFIC REPORTS

OPEN

Generation of *Myostatin* Gene-Edited Channel Catfish (*Ictalurus punctatus*) via Zygote Injection of CRISPR/Cas9 System

Received: 11 April 2017
Accepted: 26 June 2017
Published online: 4 August 2017

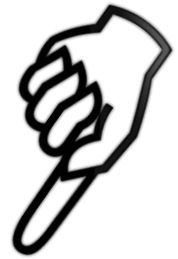
Karim Khalil[1,2], Medhat Elayat[2], Elsayed Khalifa[2], Samer Daghash[2], Ahmed Elaswad[1,4], Michael Miller[3], Hisham Abdelrahman[1,5], Zhi Ye[1], Ramjie Odin[1], David Drescher[1], Khoi Vo[1], Kamal Gosh[1], William Bugg[1], Dalton Robinson[1] & Rex Dunham[1]

Or adding an alligator gene to reduce infections in catfish?

It's not just the teeth that are formidable

- Cathelicidin from alligator was shown to have strong activity against multiple Gram-negative bacteria and is not cytotoxic towards mammalian cells (Barksdale *et al*. 2016)

Our team at AU published several papers related to CRISPR/Cas9 in catfish



scientific reports

Check for updates

OPEN

CRISPR/Cas9-mediated knock-in of alligator cathelicidin gene in a non-coding region of channel catfish genome

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

ELSEVIER

CRISPR/Cas9 microinjection of transgenic embryos enhances the dual-gene integration efficiency of antimicrobial peptide genes for bacterial resistance in channel catfish, *Ictalurus punctatus*

Jinhai Wang [a], Baofeng Su [a,*], Timothy J. Bruce [a], Allison L. Wise [a], Peng Zeng [b], Guanqun Cao [b], Rhoda Mae C. Simora [a], Logan Bern [a], Mei Shang [a], Shangjia Li [a], De Xing [a], Wenwen Wang [a], Andrew Johnson [a], Michael Coogan [a], Darshika U. Hettiarachchi [a], Jacob Al-Armanazi [a], Renata S. Farias [a], Rex A. Dunham [a]

[a] *School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL 36849, USA*
[b] *Department of Mathematics and Statistics, Auburn University, Auburn, AL 36849, USA*

Full siblings of channel catfish produced at Auburn University

Wild type

Gene edited

Healthier fish using CRISPR/Cas9

Knock-in to Improve Omega-3 Fatty Acid Content (*elovl2* gene) and Knock-out of LH gene

2022 Published Paper

Marine Biotechnology
https://doi.org/10.1007/s10126-022-10110-6

ORIGINAL ARTICLE

Check for updates

CRISPR/Cas9-Mediated Transgenesis of the Masu Salmon (*Oncorhynchus masou*) *elovl2* Gene Improves n-3 Fatty Acid Content in Channel Catfish (*Ictalurus punctatus*)

De Xing[1] · Baofeng Su[1] · Shangjia Li[1] · Max Bangs[1,2] · David Creamer[1] · Michael Coogan[1] · Jinhai Wang[1] · Rhoda Simora[1,3] · Xiaoli Ma[1] · Darshika Hettiarachchi[1] · Veronica Alston[1] · Wenwen Wang[1] · Andrew Johnson[1] · Cuiyu Lu[1] · Tasnuba Hasin[1] · Zhenkui Qin[1,4] · Rex Dunham[1]

Received: 17 October 2021 / Accepted: 2 March 2022

- Insertion of an *elovl2* transgene isolated from masu salmon to improve the n-3 fatty acids particularly the EPA and DHA contents of channel catfish.
- Simultaneous gene knock- in of *elovl2* gene and knockout of LH (luteinizing hormone) gene to achieve sterilization.

Successful applications of genome editing in some aquaculture species

TABLE 1 Successful applications of genome editing to date in some aquaculture species

Targeted traits	Species	Edited gene	New phenotypes	References
Growth	*Sparus aurata*	*myostatin*	Increased growth	89
	Ictalarus punctatus	*myostatin*	Increased growth	89
	Cyprinus carpio	*sp7a/sp7b/mstn(ba)*	Muscle development	76
	Oncorhynchus mykiss	*igfbp−2b1/2b2*	Increased growth	133
	Crassostrea gigas	*myostatin*	Increased growth	96
	Pagrus major	*myostatin*	Increased growth	87
	Pagrus major	*myostatin*	Increased growth	88
	Lethenteron morii	*kctd10/wee1/soxe2/wnt7b*	Growth related	134
	Megalobrama amblycephala	*myostatin*	Increased growth	97
	Paralichthys olivaceus	*myostatin*	Increased growth	27,94
Sex/reproduction	*Salmo salar*	*dnd, dmrt1/nanaos2-3/foxl2, gsdf*	Ablated germ cells, and sex	123,135
	Oreochromis niloticus	*sf-1, dmrt1, amhy, wnt1a/b, dmrt1 and foxl2*	Sex, gonad development, growth	23,24
	Ictalarus punctatus	*Lh*	sterilisation	43
	Acipenser ruthenus	*dnd1*	Surrogate production	93
	Silurus meridionalis	*cyp26a1*	Advanced meiotic initiation	136
	Paralichthys olivaceus	*gsdf*	Sex	27
Pigmentation	*Salmo salar*	*tyr/slc45a2*	Skin colour	135
	Cyprinus carpio	*asip 1 and asip 2*	Melanin aggregation	84
	Oreochromis niloticus	*pmel17, pomc*	Golden colour and sexual dimorphism	5,30
	Paramisgurnus dabryanus	*tyr*	Skin colour	137
	Lethenteron morii	*slc24a5*	Colour	134
	Betta spldens	*mitfa*	Skin colour	98
Fatty acid profiles	*Salmo salar*	*elov-2, δ5 and δ6 desaturases*	Fatty acid profiles	80,81
Diseases	*Ctenopharyngodon idella*	*gcjam-a*	Viral infection	92
	Ictalurus punctatus	*cathelicidin gene*	Disease resistance	91
	Paralichthys olivaceus	*maf1*	Viral infection	95

CRISPR/Cas9 gene editing has been applied to 18 species of fish.

Gratacap et al. 2019

Status of gene editing regulations

Selectively bred fish

- Can be adopted immediately in the aquaculture industry; no regulations
- Must be safe, regardless of the breeding method

Transgenic fish

- Regulation is a lengthy process.
- Will require approval by designated authorities and convincing benefits to the customers to alleviate antagonistic attitudes towards GM fish (more than 2 decades for GM salmon)

Gene-edited fish

- Separate regulation in some countries
- Regarding foreign DNA insertion, gene-edited fish fall into two categories:(i) gene-edited fish without insertion of foreign DNA (non-transgenic or transgene-free gene-edited fish) and (ii) gene-edited fish with foreign DNA insertion into the genome due to the use of gRNA/Cas9-expressing plasmid constructs (transgenic gene-edited fish).

Global Gene Editing Regulation Tracker

Click on a country (eg. Brazil, US) or region (eg. European Union) below to find which animal products and processes are approved or in development and their regulatory status.

Ratings Guide

Regulation Status	Rating
Determined: No Unique Regulations*	10
Lightly Regulated	8
Proposed: No Unique Regulations†	6
Ongoing Research, Regulations In Development	5
Highly Regulated	4
Mostly Prohibited	2
Limited Research, No Clear Regulations	1
Prohibited	0

https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/

Status of gene editing regulations in the Philippines

Regulation of Modern Biotechnology in the Philippines

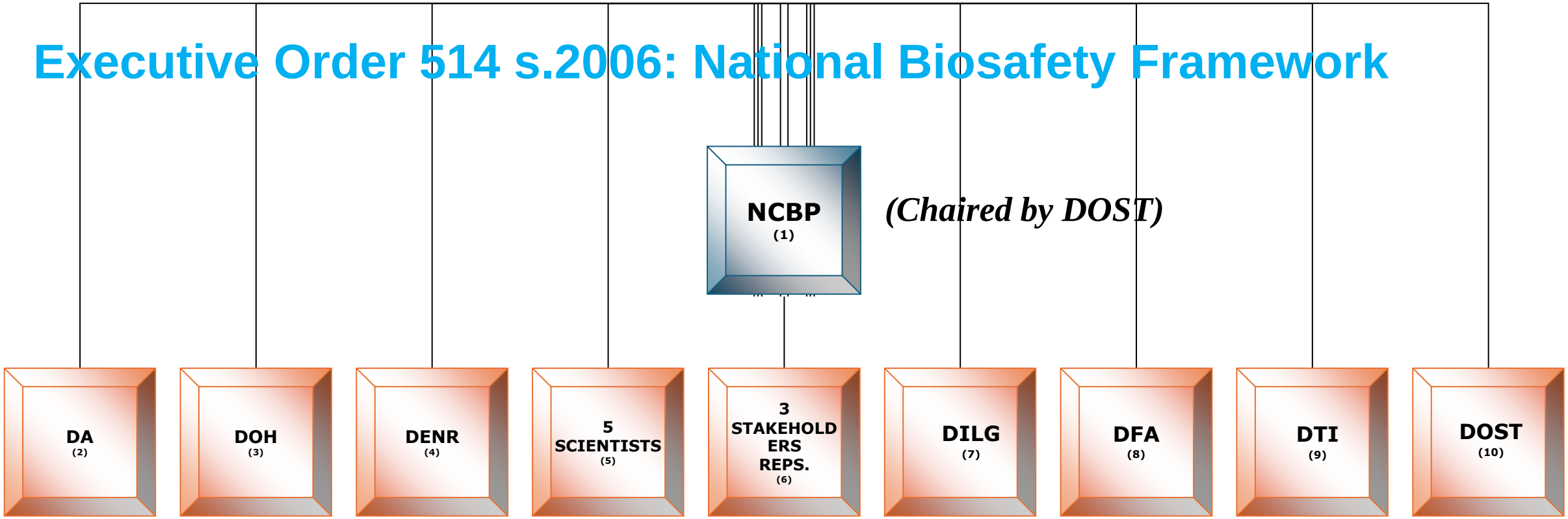
- National Biosafety Framework (2006)
- DOST-DA-DENR-DOH-DILG Joint Department Circular no. 1, series of 2021
- DA Memorandum Circular no. 8, series of 2022

•Draft Regulations for GM Animals and Animal Products 2022

•DOST Draft Guidance Document for the Contained Use of Genetically Modified (GM) and Gene edited (GEd) Fish - 2024

•DOST Draft Guidance Document for the Risk Assessment of Living Modified Fish for Contained Use - 2024

Executive Order 514 s.2006: National Biosafety Framework



MALACAÑANG
MANILA

BY THE PRESIDENT OF THE PHILIPPINES

EXECUTIVE ORDER NO. 514

ESTABLISHING THE NATIONAL BIOSAFETY FRAMEWORK, PRESCRIBING GUIDELINES FOR ITS IMPLEMENTATION, STRENGTHENING THE NATIONAL COMMITTEE ON BIOSAFETY OF THE PHILIPPINES, AND FOR OTHER PURPOSES

DONE, in the City of Manila, this 17th day of March in the year of our Lord two Thousand and Six.

Gloria Arroyo

By the President:

EDUARDO R. ERMITA
Executive Secretary

Agencies Involved in JDC1 (s2021) (The Competent National Authorities, CNAs)

DOST	DA	DENR	DOH	DILG
evaluates applications for “contained use” and “confined test”	**evaluates all applications for field trial, commercial propagation and direct use**	evaluates environmental impacts of GM application	evaluates environmental health impacts of GM application	supervise public consultation process for field trial

Draft Guidelines for the Regulation of GM Animals and Animal Products

DOST-DA-DENR-DOH-DILG
JOINT DEPARTMENT CIRCULAR (JDC)
NO. __ *Series of 2021*

Subject: **Rules and Regulations for the Research and Development, Handling and Use, Transboundary Movement, Release into the Environment, and Management of Genetically-Modified Animal and Animal Products Derived from the Use of Modern Biotechnology**

WHEREAS, Section 15, Article II of the 1987 Philippine Constitution declares that the State shall protect and promote the right to health of the people and instil health consciousness among them. Furthermore, Section 9, Article XVI provides that the State shall protect consumers from trade malpractices and from substandard or hazardous products. Toward these ends, the State shall maintain a farm to fork food safety

CRISPR/Cas9 gene-editing efforts in the Philippines

- Establishment of CRISPR/Cas9 gene-editing facility for fish at FBC and UPV
- Inauguration of FBC facility on November 13, 2023 and December 7, 2023 at UPV

FBC facility – microinjection, fish rearing, genotyping

UPV facility – gene construct design, guide RNA design, bioinformatics

Microinjection set-up at the FBC facility

Laboratory set-up at the UPV facility

Benefits of genetically enhanced organisms:

- ✓ Increase production, efficiency and profits
- ✓ Healthier, fitter animals-better animal welfare
- ✓ Genetically engineered animals are not "organic", but if they reduce or eliminate chemical and antibiotic use is that not as beneficial as organic?

Genetic improvement as a solution to animal diseases rather than antibiotics/chemicals

Thank you for your attention!

Auburn University
Fish Genetics Lab

UPV and FBC Fish CRISPR Team

WELS GRANT

World Expert Lecture Series
UP Office of International Linkages

Advances in CRISPR/Cas9 Gene-editing Technology in Fish

December 16 and 17, 2024
UP Visayas
(F2F and online)

Lecture 1: Applications of CRISPR/Cas9 gene-editing technology for commercially important fish species

Lecture 2: Transgenic and gene-edited fish: Is it safe to eat?

Dr. Rex Dunham

Auburn University

Designated as being in the Top 1.5% of Scientists Worldwide