5th International Workshop on Regulatory Approaches for Agricultural Applications of Animal Biotechnologies

Application of genome editing technology in marine fish aquaculture in Japan

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Genome-edited food products for which notification has been completed in Japan				
No.	Name	Date of Notification	Notifiers	Market launch date
1	Tomatoes with partially modified glutamic acid decarboxylase gene to increase GABA content	December 11, 2020	Sanatech Seed Co.,Ltd.	September 1, 2021
2	Increased edible portion red sea bream	September 17, 2021	Regional Fish Institute, Ltd.	October 1, 2021
	Additin of strains	December 5, 2022	Regional Fish Institute, Ltd.	January 1, 2023
3	High-growth tiger puffer	October 29, 2021	Regional Fish Institute, Ltd.	November 1, 2021
	Additin of strains	December 5, 2022	Regional Fish Institute, Ltd.	January 1, 2023
4	PH1V69 CRISPR-Cas9 waxy corn	March 20, 2023	Corteva Japan Ltd.	undecided
5	Tomatoes with partially modified glutamic acid decarboxylase gene to increase GABA content	July 27, 2023	Sanatech Seed Co.,Ltd.	undecided
6	High-growth Japanese flounder	October 24, 2023	Regional Fish Institute, Ltd.	undecided

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Disruption of *mstn* in Red sea bream

Target Fish :

Red sea bream
（*Pagrus major*）

Target gene :

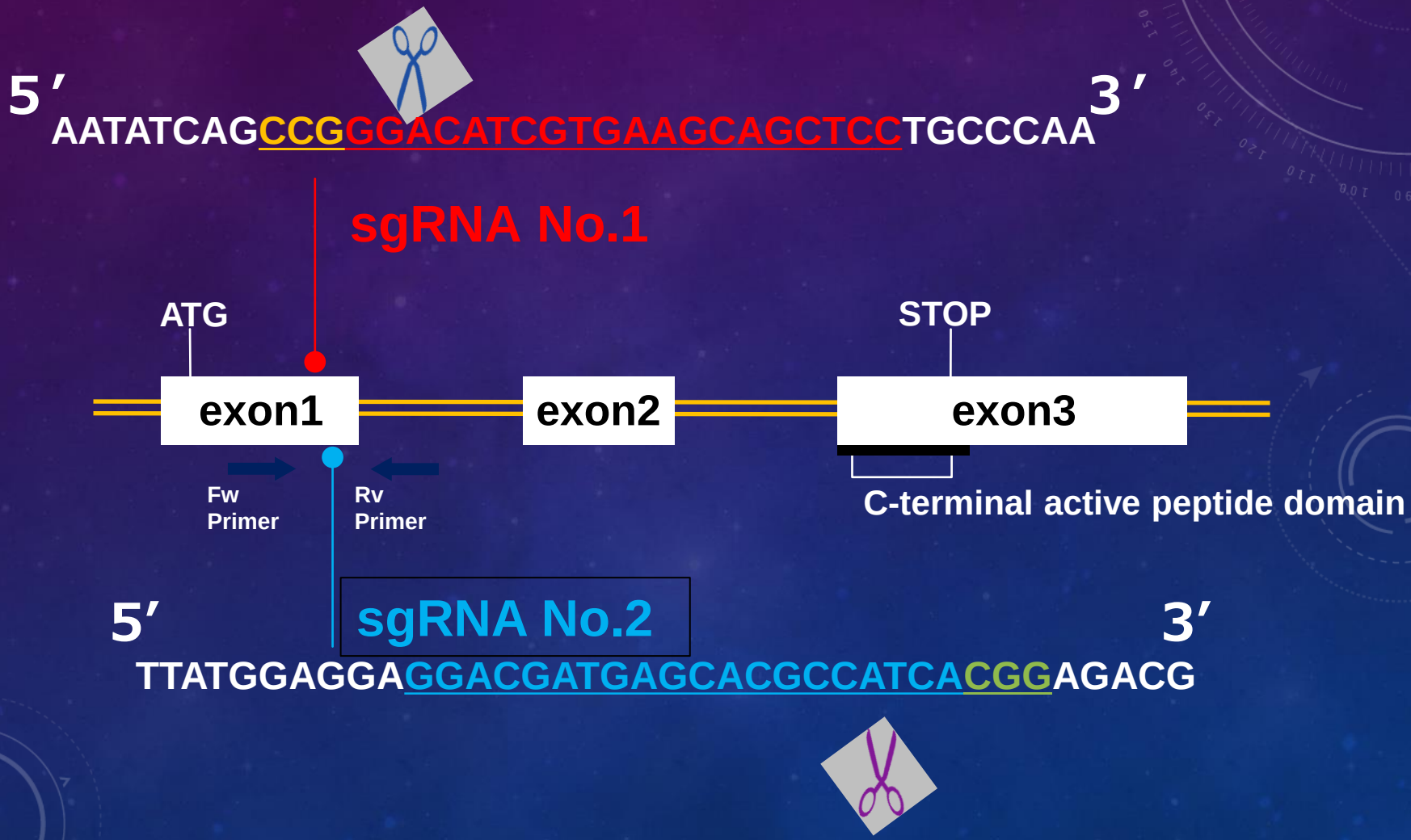
myostatin gene
(*mstn*)

Genome editing tool :

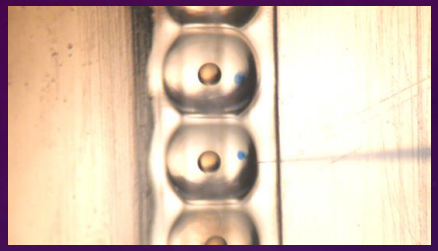
CRISPR-Cas9 system

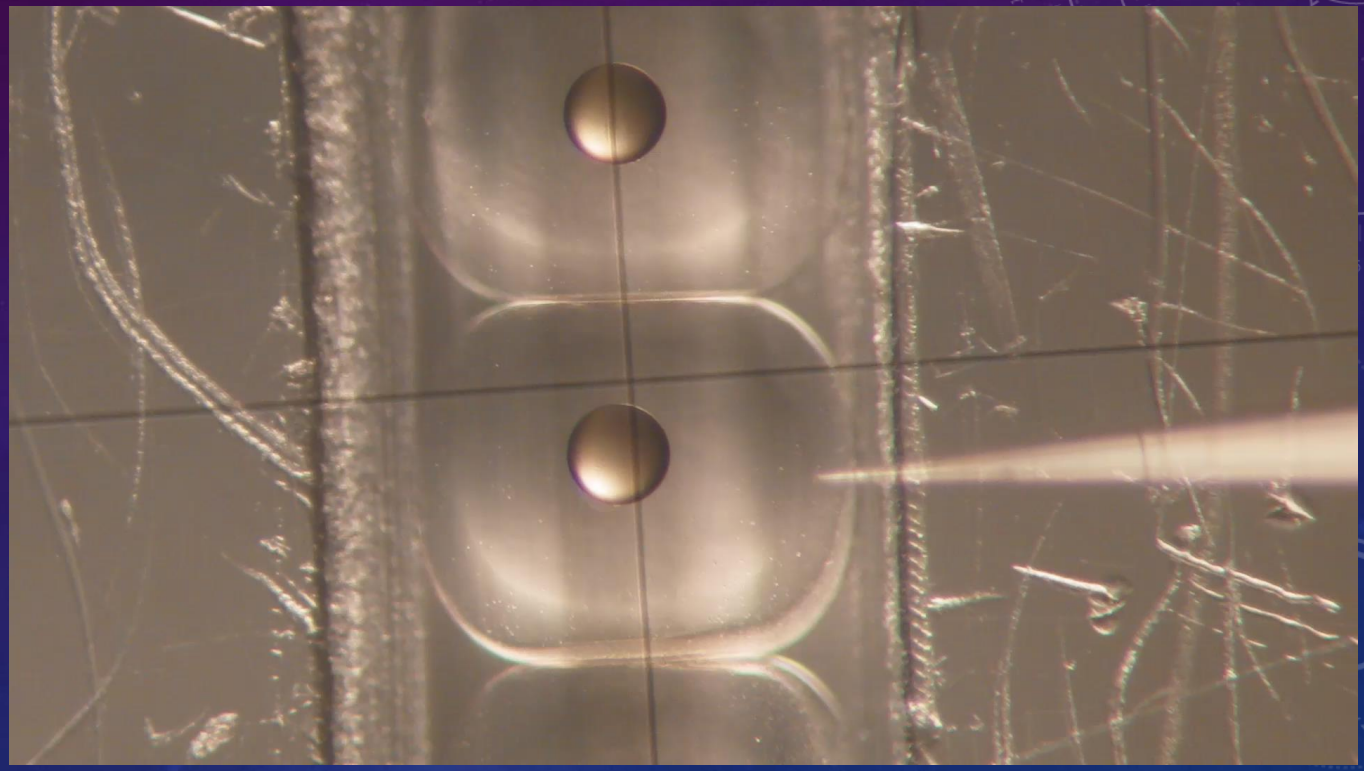
Design CRISPR-Cas9 (sgRNA)

Red sea bream *myostatin* gene



Microinjection



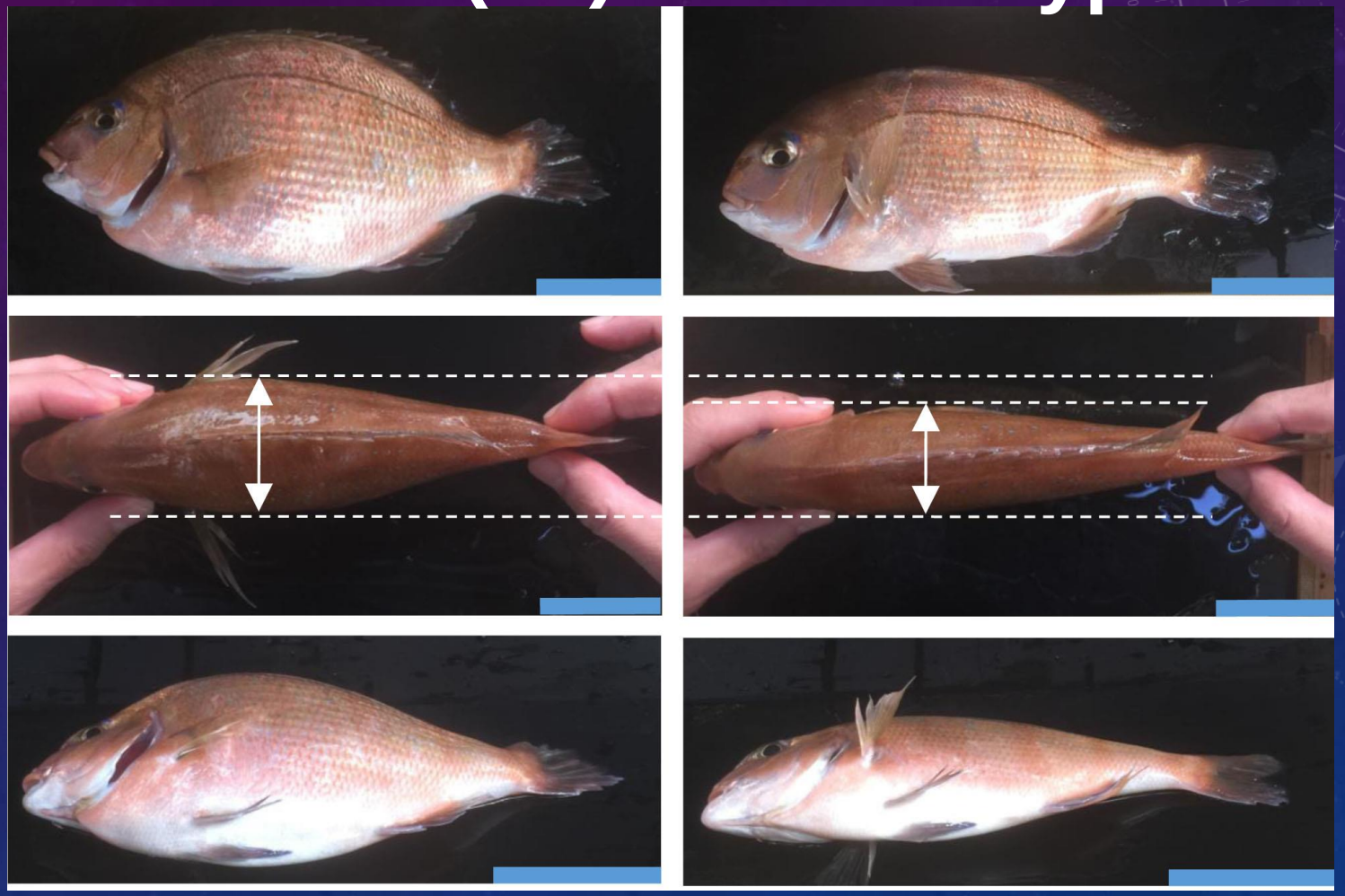


sgRNA : 25ng/ul
Cas9 RNA: 100ng/ul

1,000-3,000 eggs / person / day

Increased muscle mass in 2nd generation

mstn KO (F2) Wildtype



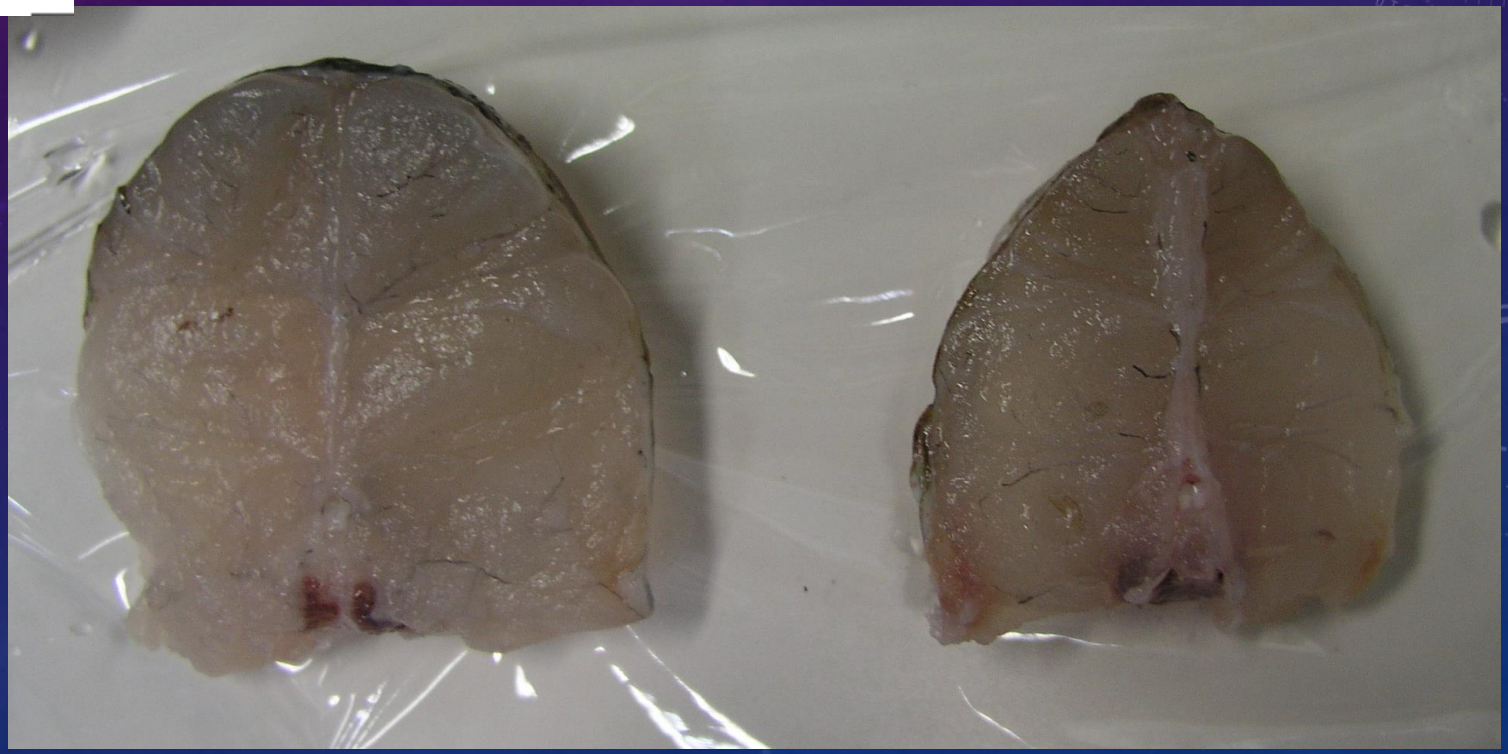
329-days old

Kishimoto et al. (2018) Aquaculture 495, 415–427

Increased muscle mass in 2nd generation

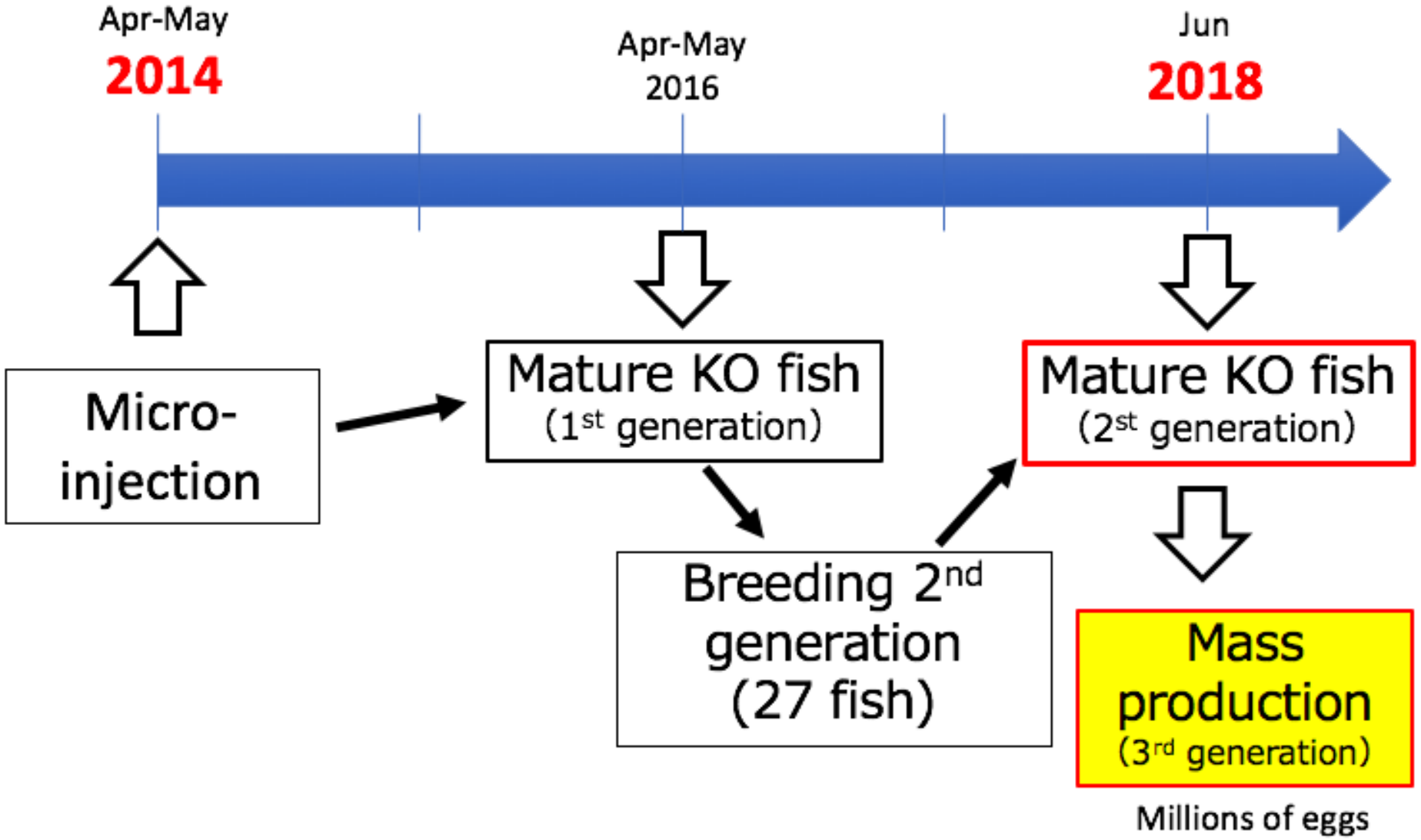
1 year old

mstn KO (F2) Wildtype



20% increase in edible portion !

4 years to establish new breed



4 years to establish new breed

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Jun

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Toward distribution of genome-edited fish

Estimated Food Safety Risks for Genome-Edited Red Sea Bream

1. Residual genome editing tool (Cas9, sgRNA)

2. Effects of off-target mutations

3. Biosynthesis of new proteins due to partial deletion of target genes

4. Changes in metabolite composition due to loss of function of the target gene

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Detection of Cas9 and sgRNA, and the DNA sequences used in their synthesis

NextSeq 550

16.25-120Gb Output range

Genome size of red sea bream: about 800Mb

Analysis of about 30 Gb of the genome of genome-edited fish (About 40 times as many bases as the genome of the sea bream)

↓

No Cas9, sgRNA, etc. detected

↓

No genome editing tools remain

Toward distribution of genome-edited fish

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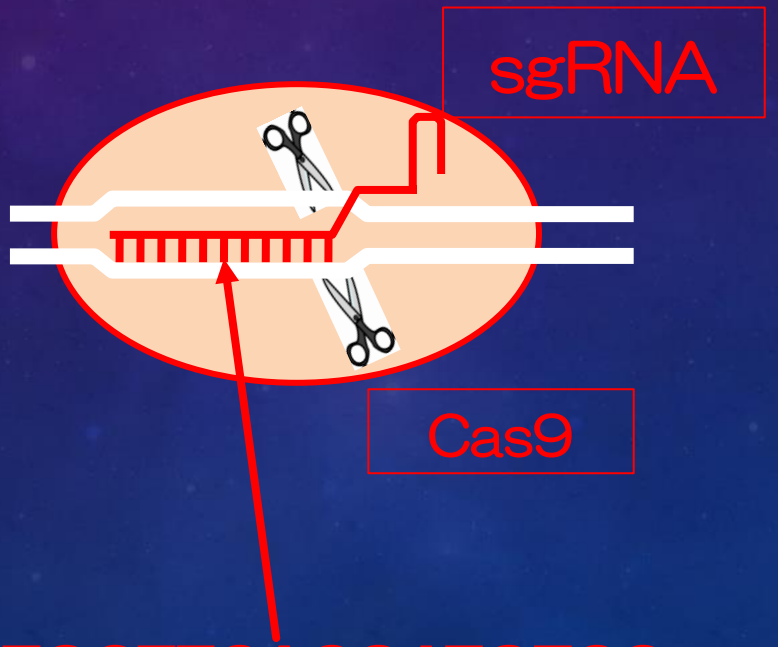
2. Effects of off-target mutations

In the case of the CRISPR-Cas9 system, it recognizes and cleaves one part (18 bases) of the target gene, but it is possible that it may incorrectly cleave a similar sequence.



Example of off-target analysis

Targeted DNA sequence	Number of mismatched bases from the original sequence	Changes due to genome editing
AGCTGCTTCACGATGTCC	Original sequence	-
AGGTGCTTCACGCTGTCC	2	Not detected
AGCTGCTTCAGGATGTCA	2	Not detected
AACTGGTTCACGATGTCC	2	Not detected
AGCTGCTTCATGATGACC	2	Not detected
ATCTGCCTCACGATGTCC	2	Not detected
AGC−GCTTCACGGTGTCC	2	Not detected
AGCTGC−TCAAGATGTCC	2	Not detected
ATCTGCTTCACG−TGTCC	2	Not detected
AGCAGCTTCACGATGTC−	2	Not detected
AGCTGCTTCA−G−TGTCC	2	Not detected
AGC−−CTTCACGATGTCC	2	Not detected
AGCTGCTTCAC−A−GTCC	2	Not detected
AGCTGCTTTCAC−GATGTCC	2	Not detected
AGCTGCTGTCACGA−GTCC	2	Not detected

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Allergen analysis of genome-edited fish
14 base deleted individuals (-14)

50

WT: MHPSQIVLYL SLLIVLGPVV LSDQETQQQQ QQQQQQQPSA TSPEDTEQCA
-14: MHPSQIVLYL SLLIVLGPVV LSDQETQQQQ QQQQQQQPSA TSPEDTEQCA

WT: TCEVRQQIKT MRLNAIKSQI LSKLRMKEAP NISRDIVKQL LPKAPPLQQL
-14: TCEVRQQIKT MRLNAIKSQI LSKLRMKEAP NISRDIVKQL LPKAPPLQQL

100

WT: LDQYDVLGDD NRDVVMEEDD EHAITETIMM MATEPESVVQ VDGEPRCCFF
-14: LDQYDVLGDD NRDVVMEEDD EHDYDDGH

128

150

WT: SFTQKIQANR IVRAQLWVHL RASDEATTVF LQISRLMPVT DGNGHIHIRS
LKIDVNAGVG SWQSIDVKQV LSVWLRQPET NWGIQINAFD SRGNDLAVTS
AEPGEDGLQP FMEVKISEGP KRVRRDSGLD CDENSPESRC CRYPLTVDFE
117
DFGWDWIIAP KRYKANYCSG ECEYMHLQKY PHTHLVNKAN PRGSAGPCCT
PTKMSPINML YFNRKEQIIY GKIPSMVVDR CGCS

388

Allergen Prediction Results for Genome-Edited red sea bream

Table 1. Full-length sequence comparison (wild type retrieved up to 150 amino acids)

	-14　(1-128 a.a.)	WT (1-150 a.a.)
FAO/WHO (>35% in 80 a.a)	VL(1)GPVVLS(1)D QE(1)TQQQQQQQ QQQQP （gliadin）	VL(1)GPVVLS(1)D QE(1)TQQQQQQQ QQQQP （gliadin）

Table 2.　Comparison of the new amino acid sequence and its upstream 10 amino acids as a query

	-14 (10＋new a.a.)	WT
FAO/WHO (6 a.a exact match)	No	-
Motif-based(ADFS)	No	-
Epitope search (ADFS)	No	-

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Transcriptome and metabolome analysis

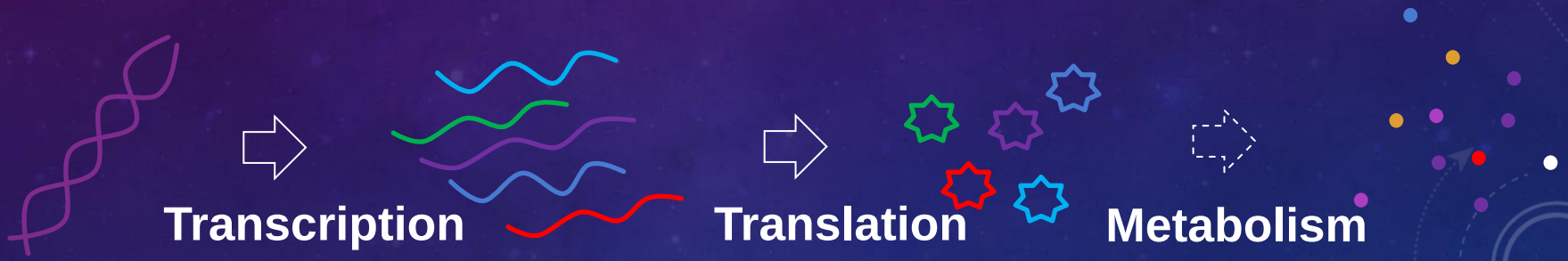
①Transcriptome analysis of skeletal muscle

(3 mutant fish, 3 WT fish)

②Metabolome analysis of skeletal muscle

(6 mutant fish, 3 WT fish)

Proteins
Nucleic acids
Sugars
Lipids, etc.



Genome

mRNA
Transcriptome

Proteins
Proteome

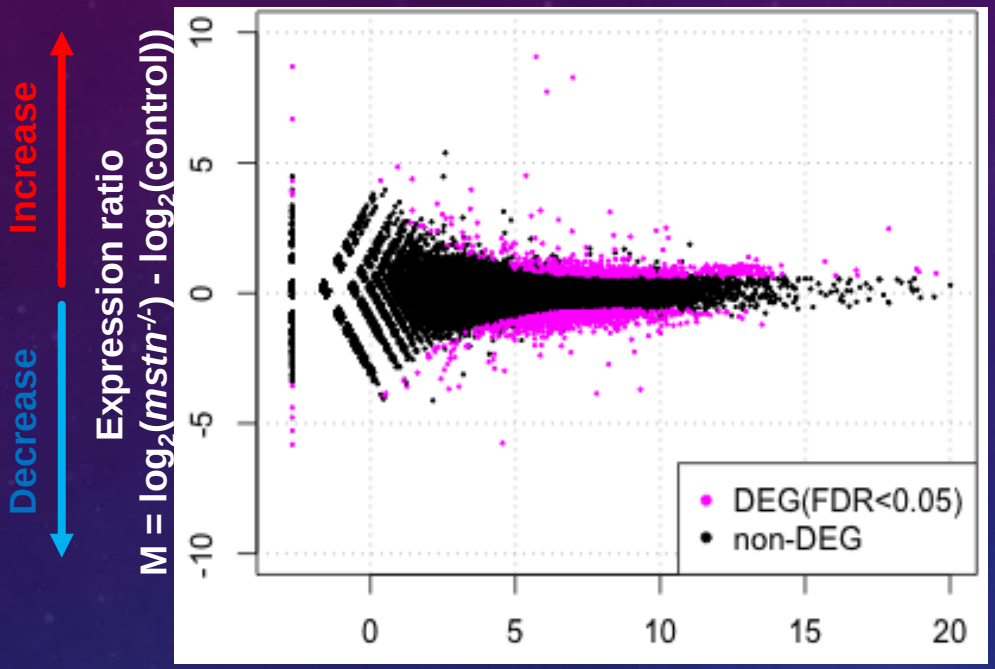
Metabolites
Metabolome

Sample

Left side dorsal muscle: Transcriptome 50mg, Metabolome 1 g

Transcriptome analysis
—Comparison between WT and Mutant fish—

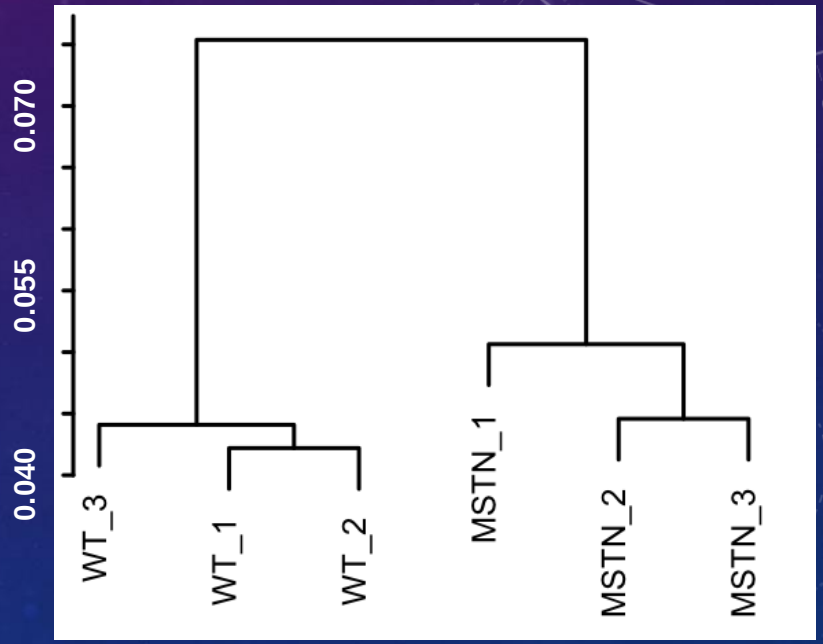
MA plot



※ DEG : Differentially Expressed Gene
FDR < 0.05 ; 1159
FDR < 0.1 ; 1718
total genes ; 22878

Hierarchical clustering

Using R; Spearman's rank correlation coefficient and ward's method



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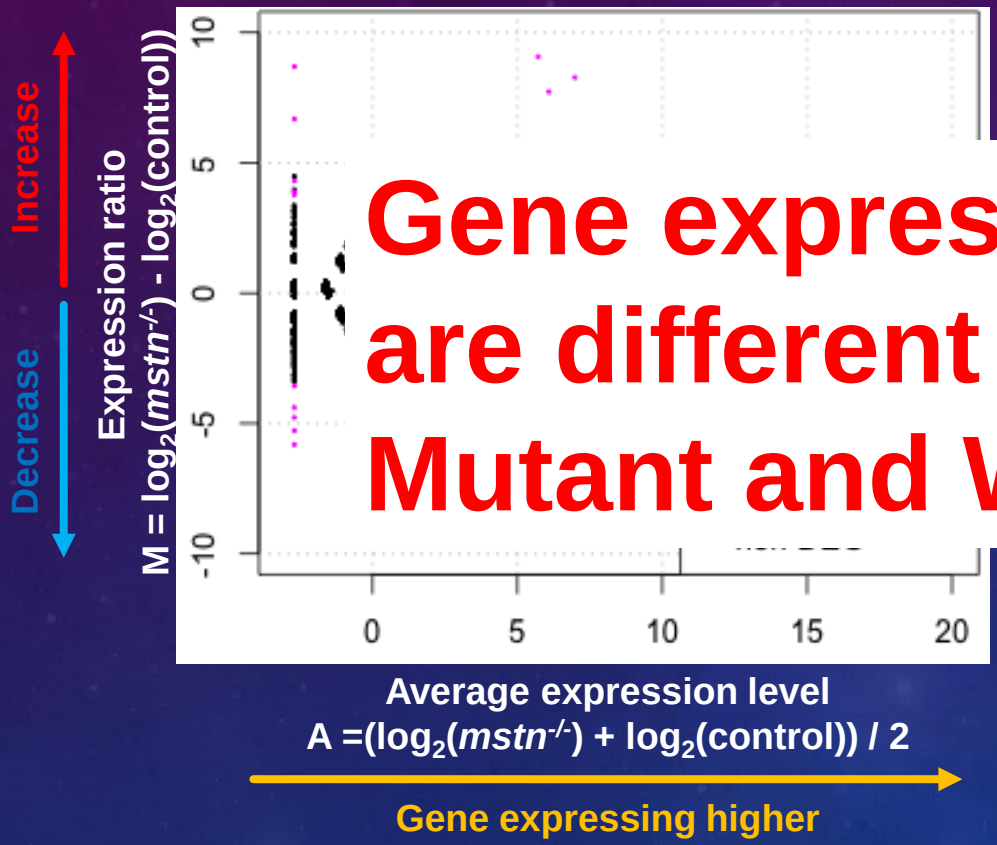

Hierarchical clustering

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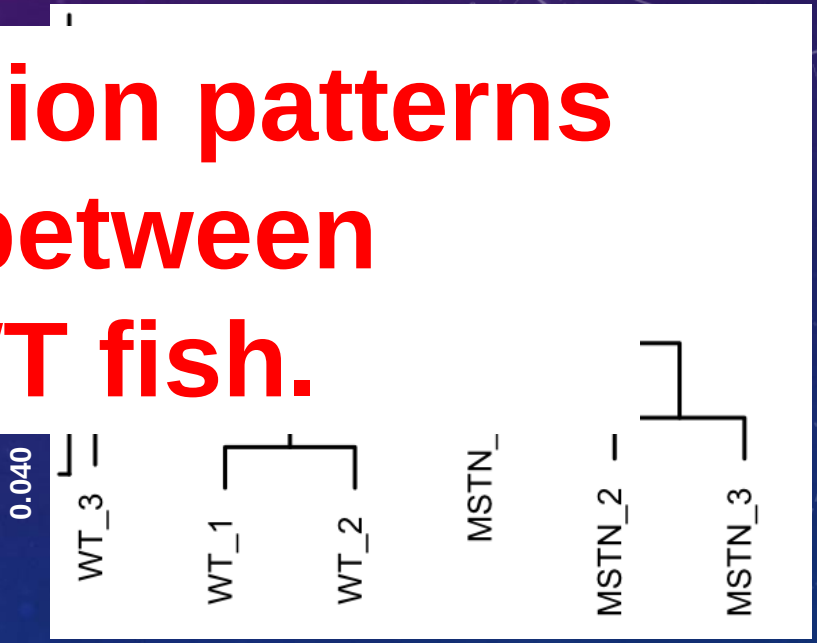

Gene expression patterns are different between Mutant and WT fish.

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Metabolome analysis
—Comparison between WT and Mutant fish—

Hierarchical clustering

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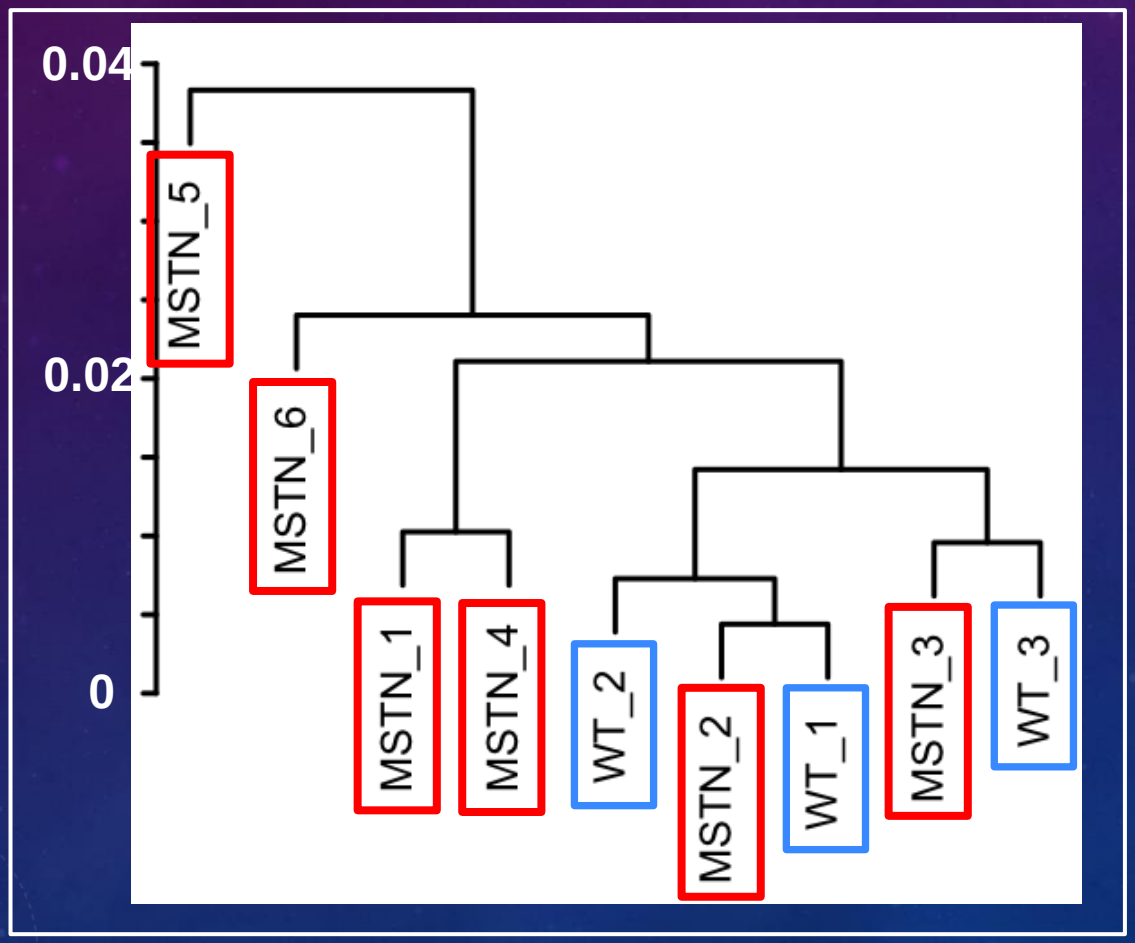
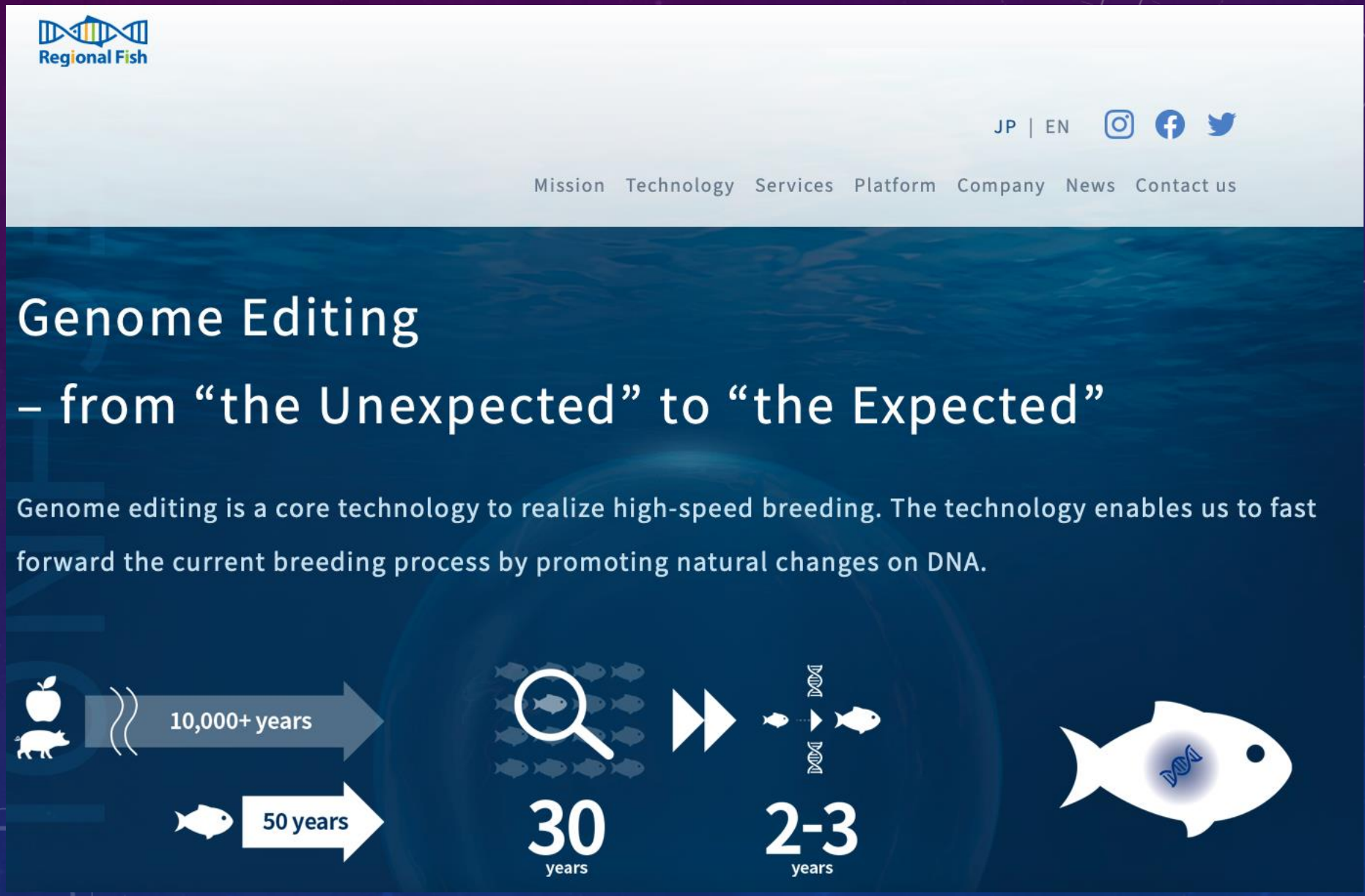


MSTN 1-3: -14/-14
MSTN 4-6: -14/-8

WT and mutant fish not separated

There is no clear difference between them.

Regional Fish Institute, Ltd., established in April 2019



Toward distribution of genome-edited fish

Rules for the Use of Genome-Edited Foods and Relevant Ministries and Agencies

1. Whether or not the organism is genetically modified (the Ministry of the Environment)

2. Characteristics of the organism and how to cultivate it (the Ministry of Agriculture, Forestry and Fisheries)

3. Safety as food (the Ministry of Health, Labour and Welfare)

4. Labeling (the Consumer Affairs Agency)

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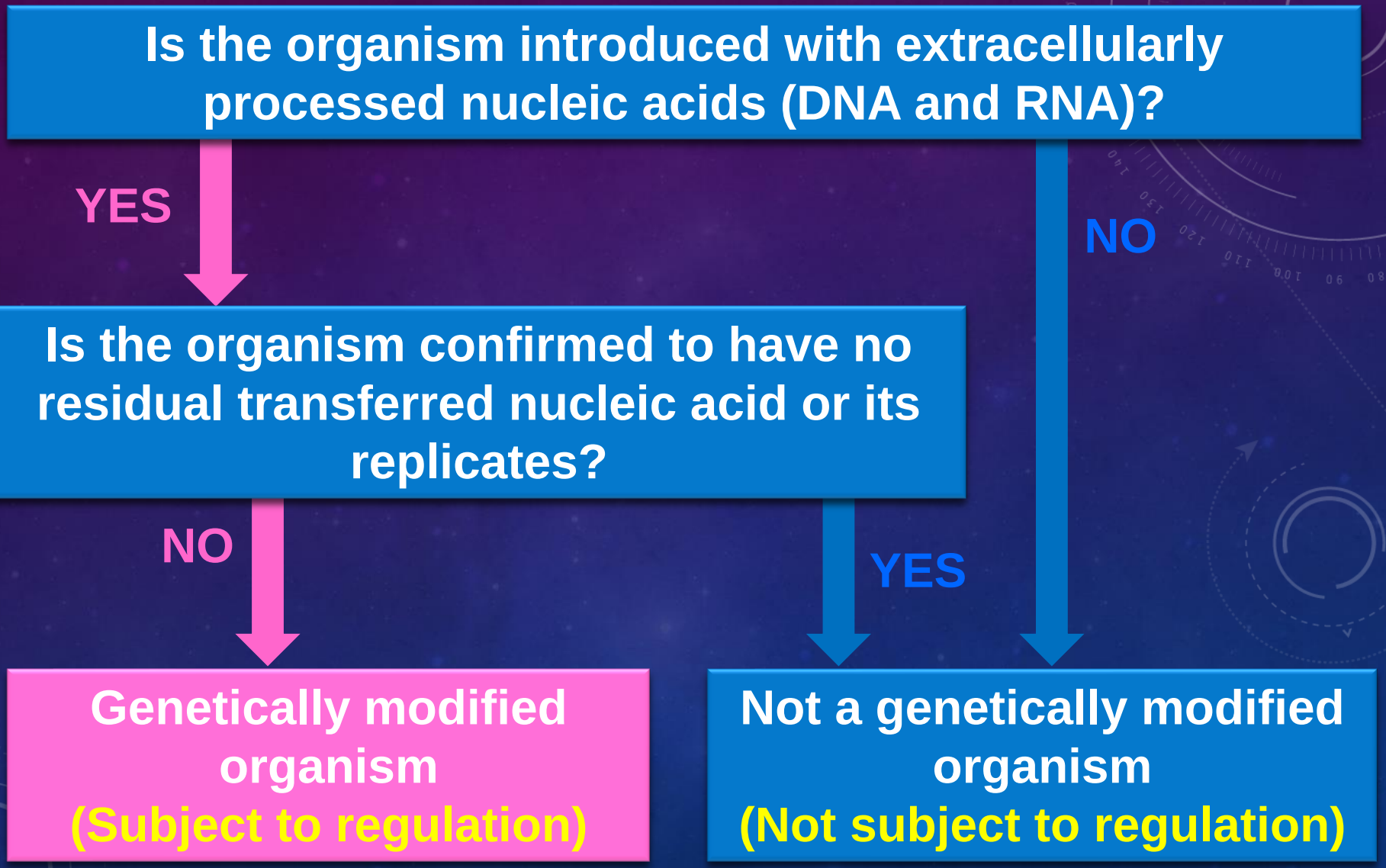
Whether or not the organism is genetically modified (Ministry of the Environment, 2019)



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Prior consultation and notification to the Ministry of Agriculture, Forestry and Fisheries (2021)

- Characteristics of the organism
- Genetically modified organisms or not
- Production methods and its process
- Rearing facilities and methods
- Use as a material for feed

↓

Hearing from academic experts

↓

Submit additional information if needed

↓

Verify additional information

↓

Determined to be applicable for notification

Submission of information form and report of when to start shipping the product (notification)

Toward distribution of genome-edited fish

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3. Safety as food (the Ministry of Health, Labour and Welfare)

4. Labeling (the Consumer Affairs Agency)

Prior consultation and notification to the Ministry of Health, Labour and Welfare (2019)

- Production methods and its process
- Genetically modified organisms or not
- Presence of off-target mutations
- Presence of allergens
- Effect on metabolism

↓

Expert Committee on Genetically Modified Foods, etc.

↓

If necessary, the Food Safety Commission discuss the matter

↓

Determined to be applicable for notification

Submission of information form and report of when to start shipping the product (notification)

Toward distribution of genome-edited fish

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3. Safety as food (the Ministry of Health, Labour and Welfare)

4. Labeling (the Consumer Affairs Agency)

Labeling (Consumer Affairs Agency, 2019)

The items notified to the Ministry of Health, Labour and Welfare are those for which the business operator provides information on labeling, etc. to the consumer.
(obligation to make effort)

Not subject to labeling under the Food Labeling Standard at this stage
（No obligation to label）

ゲノム編集で品種改良 身の量1.2倍のマダイ 販売開始へ 京都

2021年9月17日 18時41分

遺伝子を自在に操作できる「ゲノム編集」の技術を使って品種改良し、身の量を増やしたマダイについて、京都市のベンチャー企業が流通の際に求められる「ゲノム編集食品」としての届け出を厚生労働省に行いました。
会社では試験販売の受け付けを始めたということで「ゲノム編集食品」の販売は国内で2例目です。

届け出が行われたのは、ゲノム編集の技術を使って、身の量を通常よりおよそ1.2倍に増やしたマダイで、京都市のベンチャー企業「リージョナルフィッシュ」が京都大学や近畿大学と共同で開発しました。

- Press Conference on Completion of Notification (17 Sep 2021)

- This was the first case in the world where a government authorized the distribution of genome-edited fish.

Crowdfunding sales

CAMPFIRE

世界初！ゲノム編集技術を利用して開発された「22世紀鯛」を多くの人に届けたい！

リージョナルフィッシュ株式会社　フード・飲食店　京都府　コロナサポートプログラム対象

現在の支援総額

3,205,500円

320%

目標金額は1,000,000円

支援者数

231人

募集終了まで残り

終了

リージョナルフィッシュ株式会社は、ゲノム編集技術を利用した品種改良法「ナノジーン育種」によって、日本の水産業の再興・世界のタンパク質不足の解決に挑んでいます！この度私たちが開発した、日本の水産業を救う可能性を秘めた可食部増量マダイ「22世紀鯛」を、食卓で味わってみませんか？

シェア　ツイート　LINEで送る　URLコピー　QRコード　埋め込み

このプロジェクト
31人の支援により
9-30に募集を終了

Online store

Regional Fish Online

HOME　目指す未来　当社の技術　ブランドライン　ふるさと納税　お問い合わせ

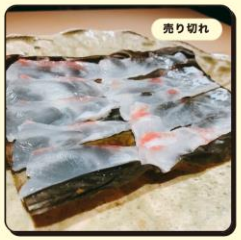
商品一覧

・送料は全国一律で1,500円です。
・配送日のご指定は、カートページにてご指定いただけます。
・領収書の発行をご希望の方は、お問い合わせフォームよりお問い合わせください。

〈新登場〉22世紀ふぐ×下鴨茶寮 贅沢セット

22世紀ふぐ　てっちりセット

¥3,290 JPYから



22世紀鯛　昆布締めセット【京都料亭コラボ】

Contributors

Kindai University : Yohei WASHIO

Kyoto University : Masato KINOSHITA

Regional fish institute: Mitsuki Ohama

Kenta KISHIMOTO

Tadanori UMEKAWA

National Institute of Genetics : Atsushi TOYODA

Japan Fisheries Research and Education Agency : Yasutoshi YOSHIURA

All stuffs and students involved in the selective breeding and genome editing of red sea bream in Kindai University


Thank you for your attention!