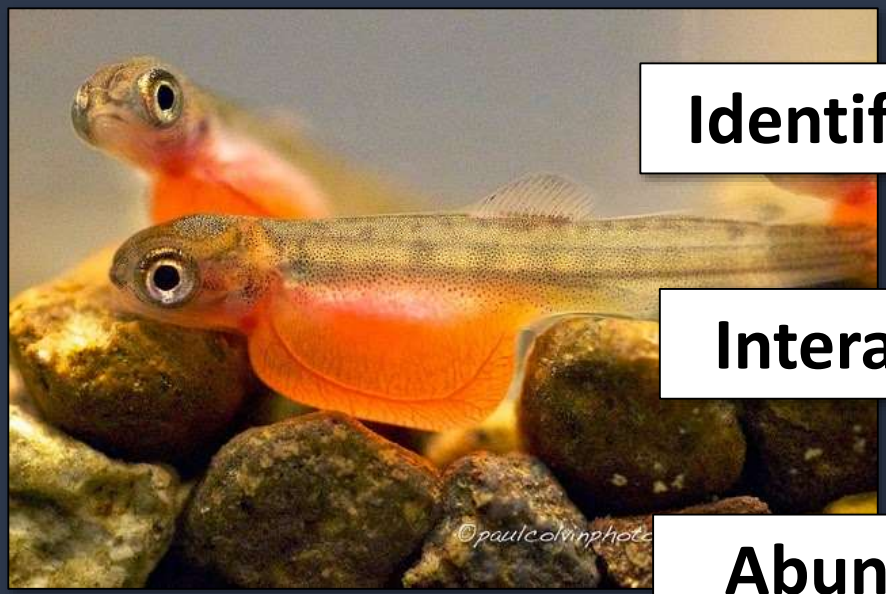
Understanding Aquatic Ecosystems through Genome Editing

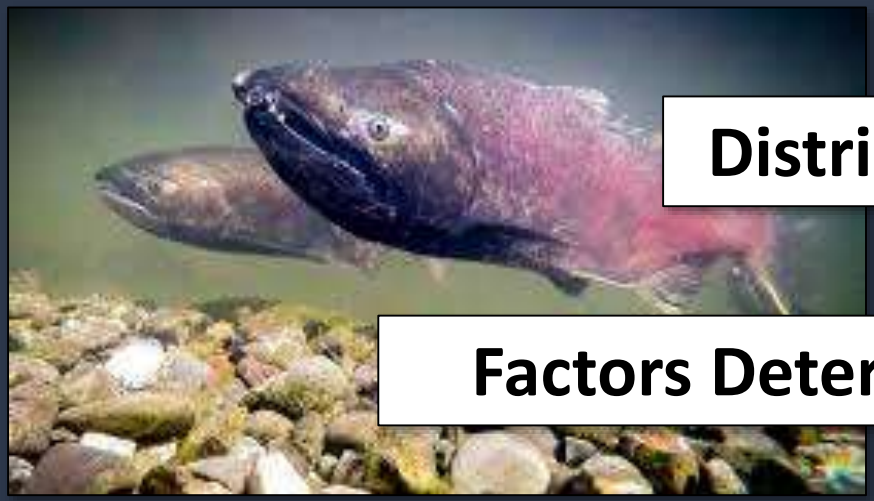
Michael P. Phelps



Identification

Interactions

Abundance



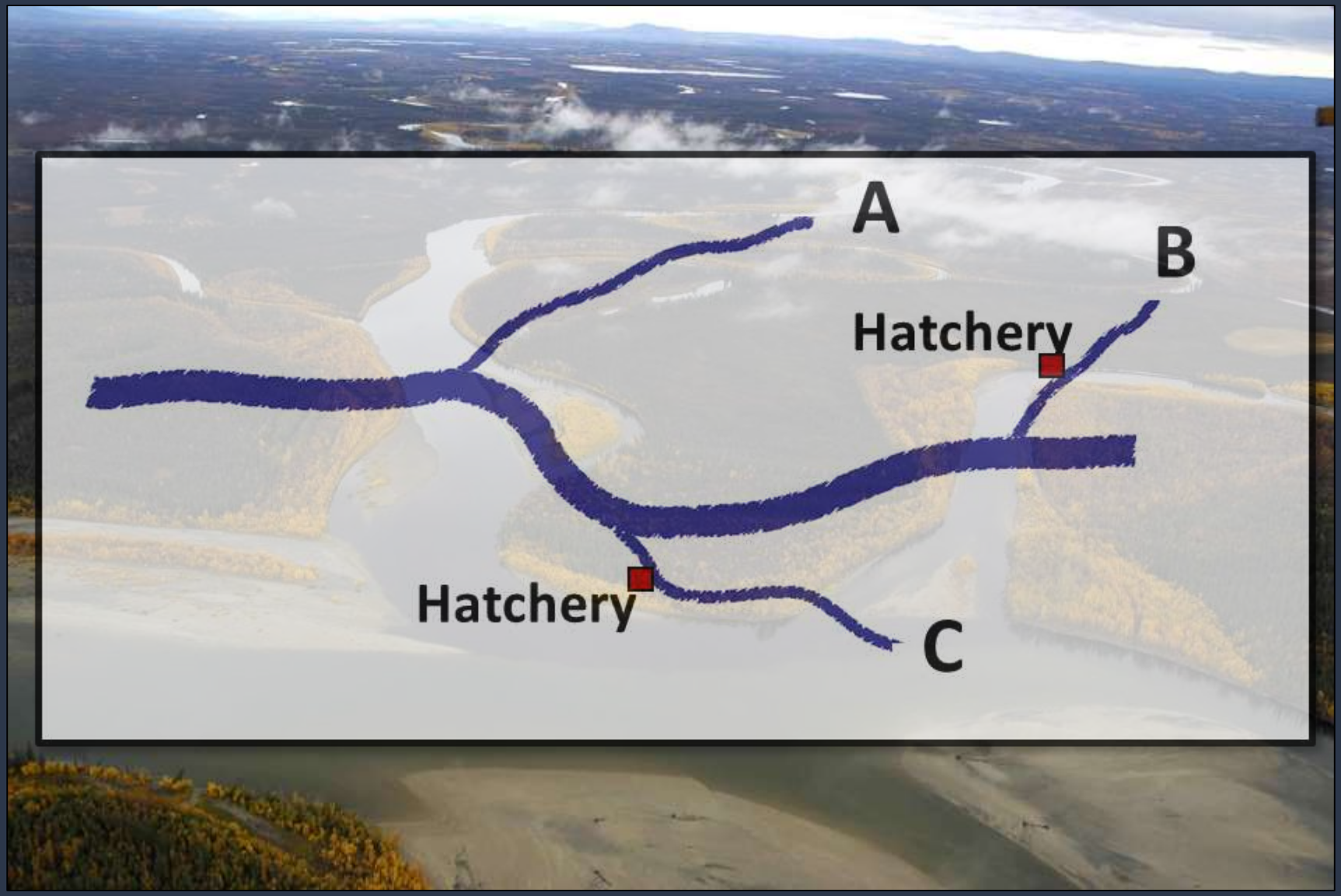
Distribution

Factors Determining Success

CRISPR Genome Editing

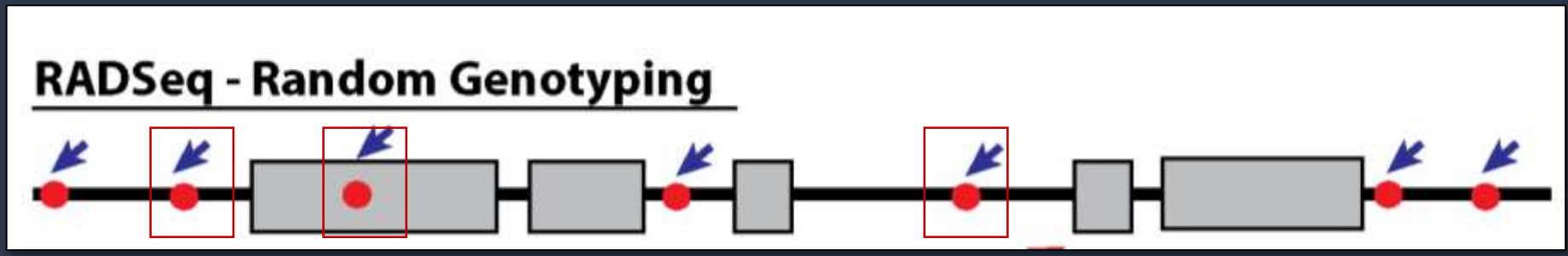
How Will Genome Editing Technology Impact Fisheries Research?

Integrating Genome Editing Technology into Fisheries

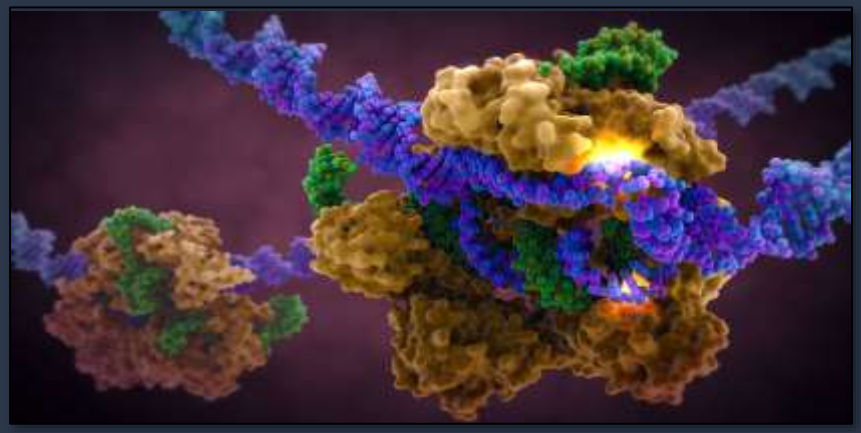


Determining Genetic Differences Between Populations

Site-Specific Endonuclease (RADSeq)



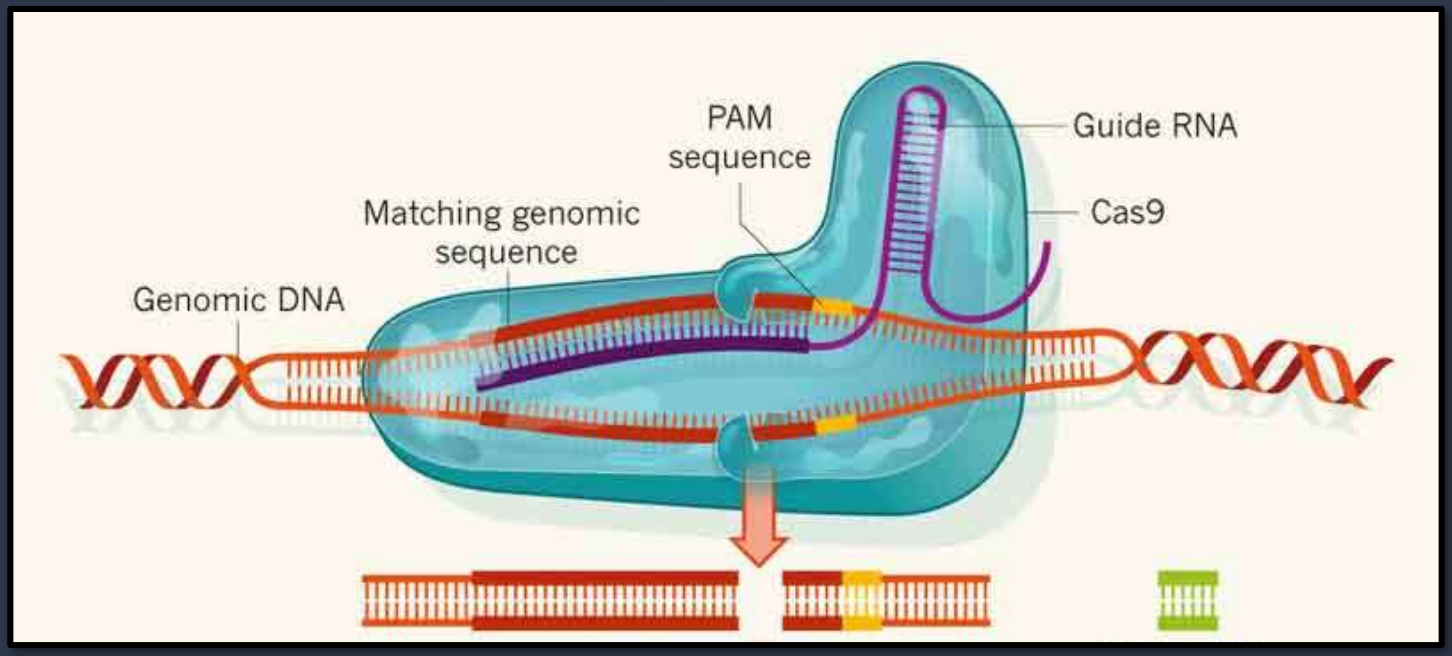
CRISPR Genome Editing



Two Component Gene Editing System

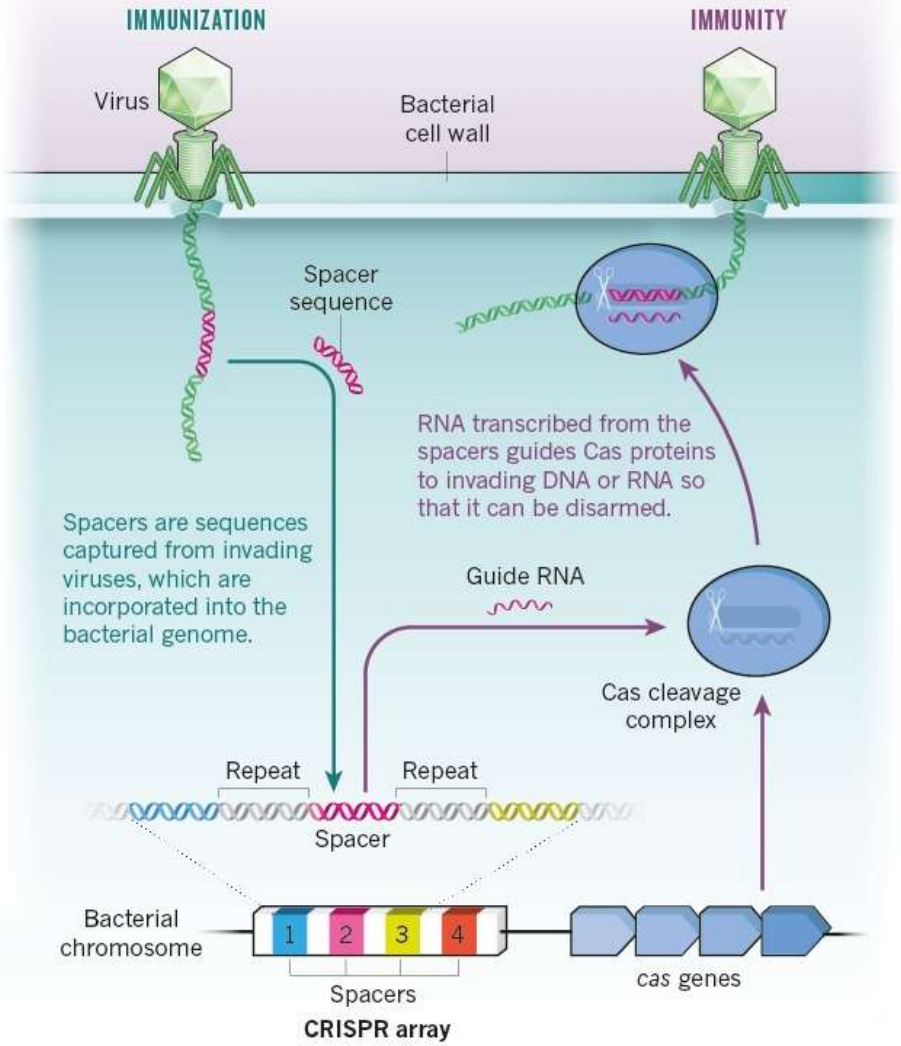
1) Programmable Nuclease

2) Small "Guide" RNA (gRNA)

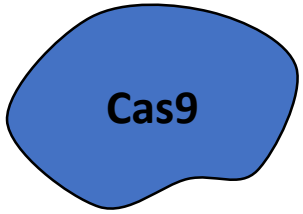


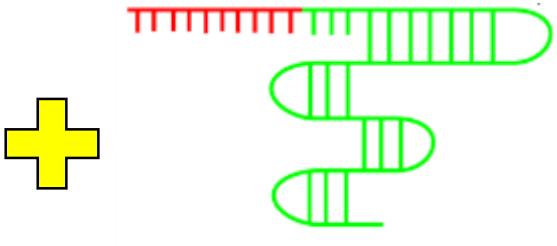
LASTING PROTECTION

About 90% of known archaea and one-third of bacteria have some form of CRISPR–Cas immunity. This is controlled by a cluster of short DNA repeats separated by 'spacer' sequences and a series of nearby genes that encode CRISPR-associated (Cas) proteins.



CRISPR Genome Editing





CRISPR Nuclease **Guide RNA (gRNA)**

Programmable DNA Digestion

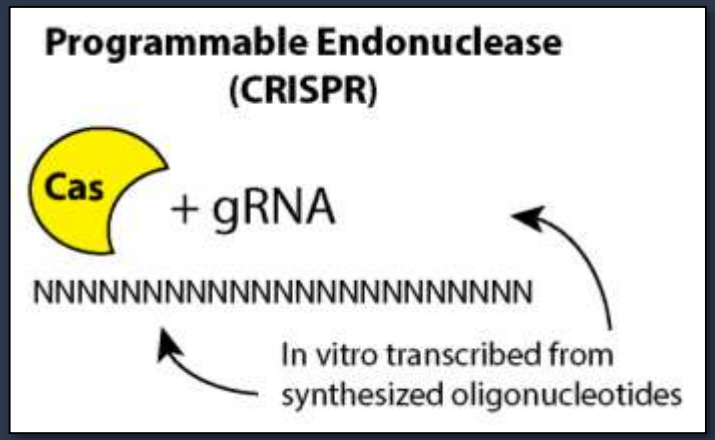
TCTGTCCCGATACTAGCTTGGAAGA
TCTGTCCCGATACTTGCTTGGAAGA
TCTGTCCCGATACTA-TCTGGAAGA
TCTGTCCCGATAC--GCTTGGAAGA
TCTGTCCCGATAC---CTTGGAAGA

Targeted DNA Mutations

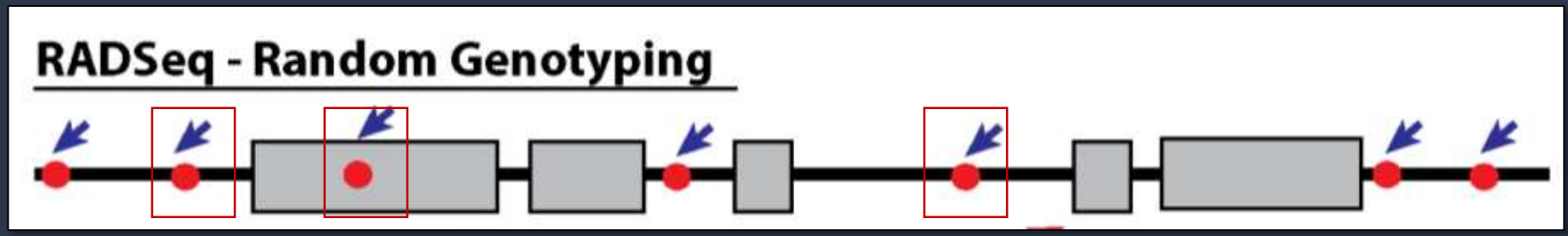
CAGAGGGAAATGGCCCGT
GTCTCCCTTTACCGGGCA

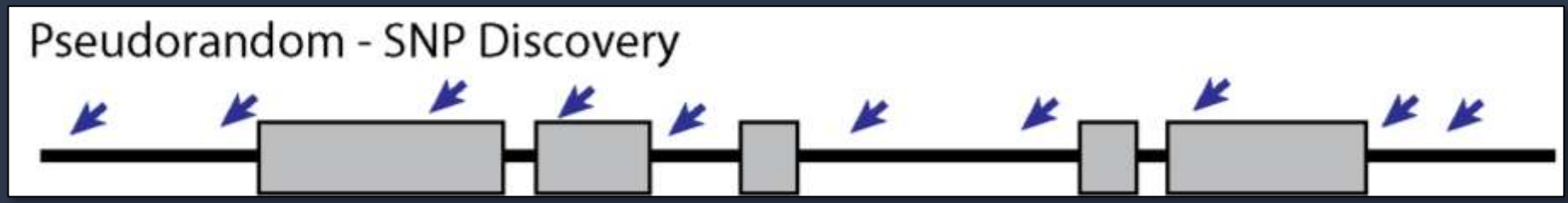
Precise Genetic Modifications

Determining Genetic Differences Between Populations



CRISPR Sequencing Locations are Not Restricted to Specific Sites

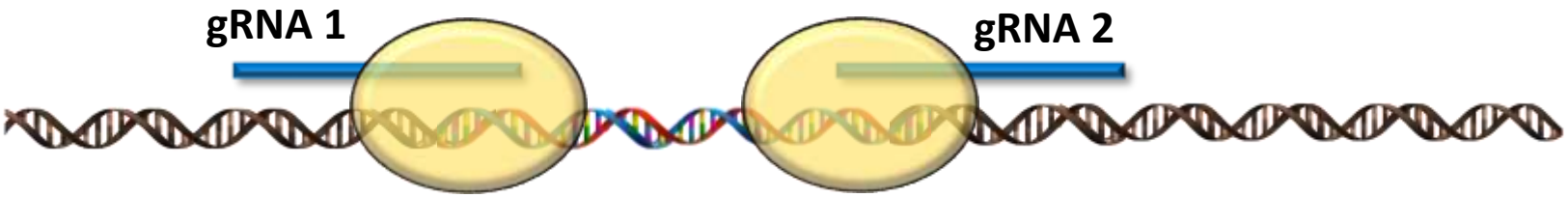




CRISPR Targeted Sequencing

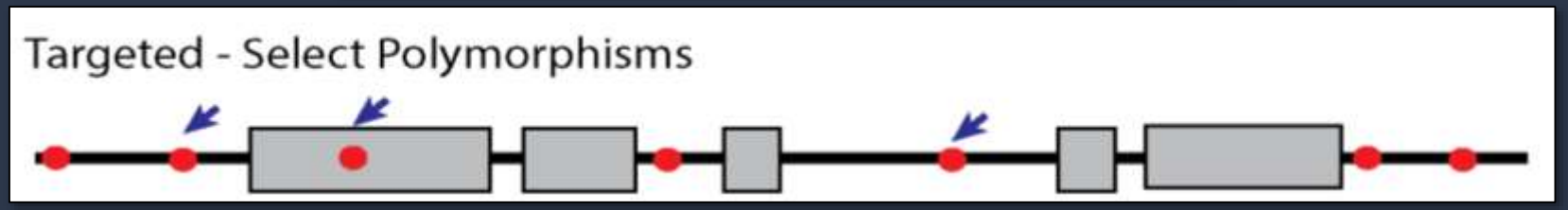
CRISPR

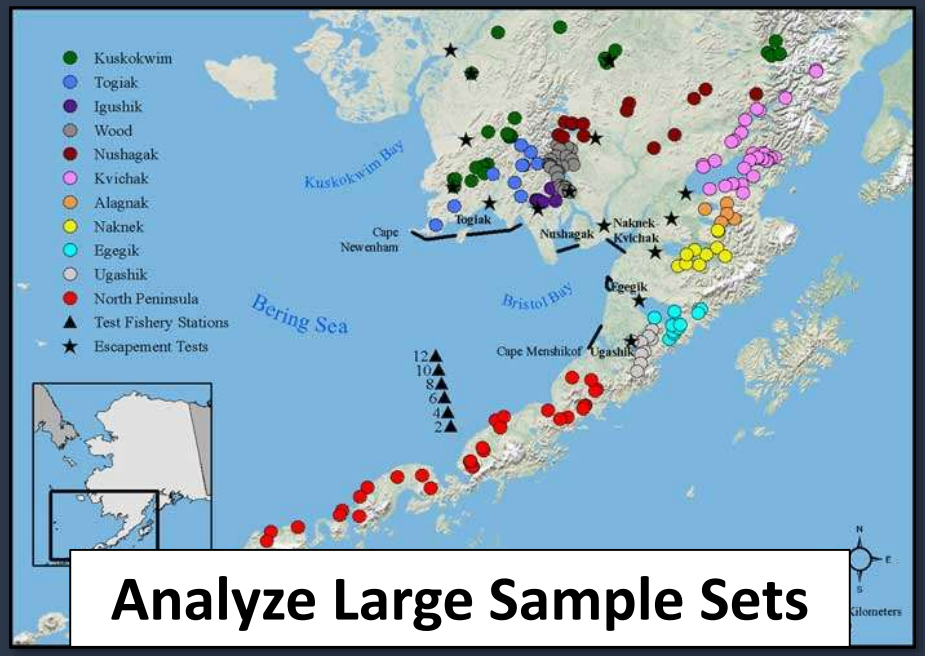
Fragmentation-Based Targeted Sequencing



Determining Genetic Differences Between Populations

CRISPR Rapidly Sequence Important SNPs or Microsatellites





Analyze Large Sample Sets

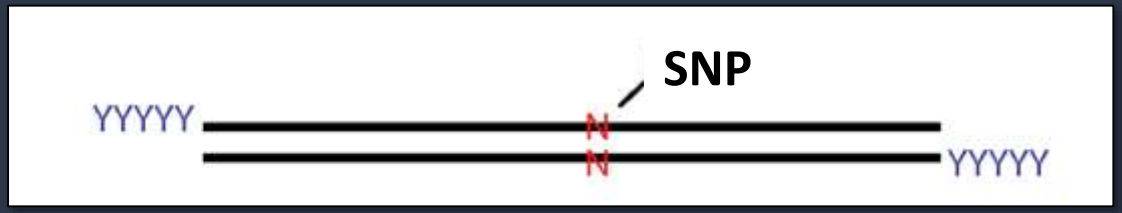
Parentage Based Tagging (PBT)

Genotype Every Broodstock Individual Across a Panel of SNPs

Compare Offspring to Parental SNP Variation to Identify Hatchery of Origin

CRISPR Parentage Based Tagging

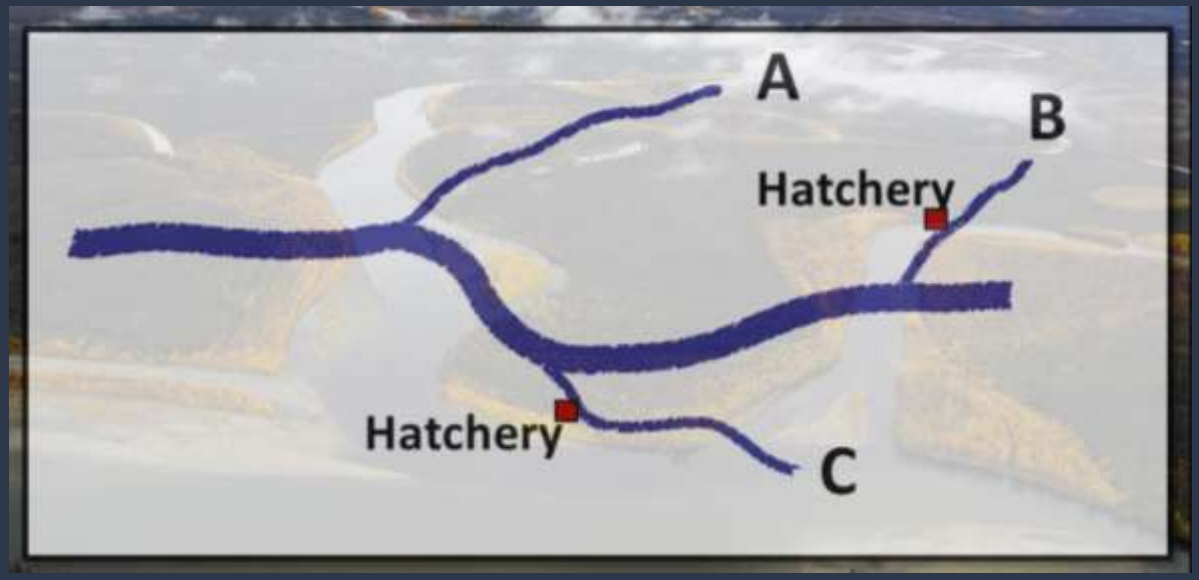
CRISPR Targeted Sequencing



Understand the Level of Hatchery:

Straying

Introgression



Any number of SNPs or microsatellites could be analyzed

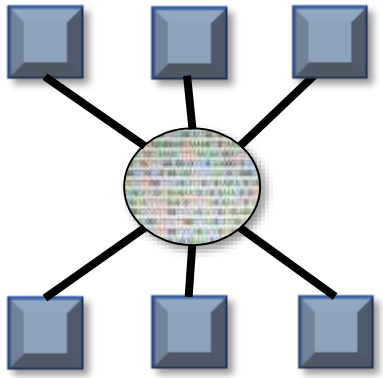
CRISPR and Nanopore: The Perfect Match

Nanopore DNA Sequencing

Real-Time, Field Compatible Sequencing

Sharing CRISPR Targeted Sequencing Tools

Open Source Sharing of CRISPR Targeted Sequencing Libraries



Challenges With Modern Fish Tagging

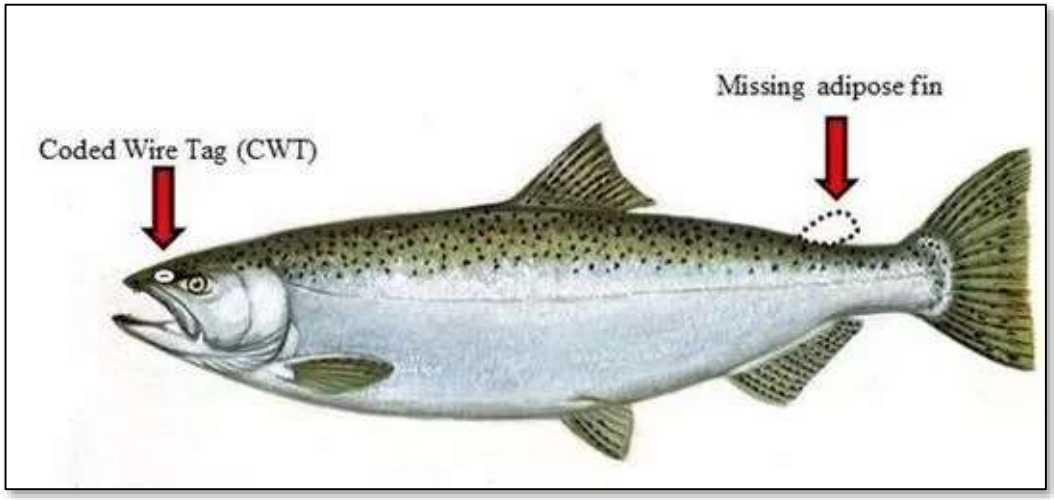
PBT, CWT, Pit tags

Need to Repeat Annually

Labor Intensive

Difficult for Closely Related Populations

Replacing PBT or Coded Wire Tags with CRISPR?



Barcode Site: ATGGCATAGGACCGATTACCCAAGACCGG

Barcode Location

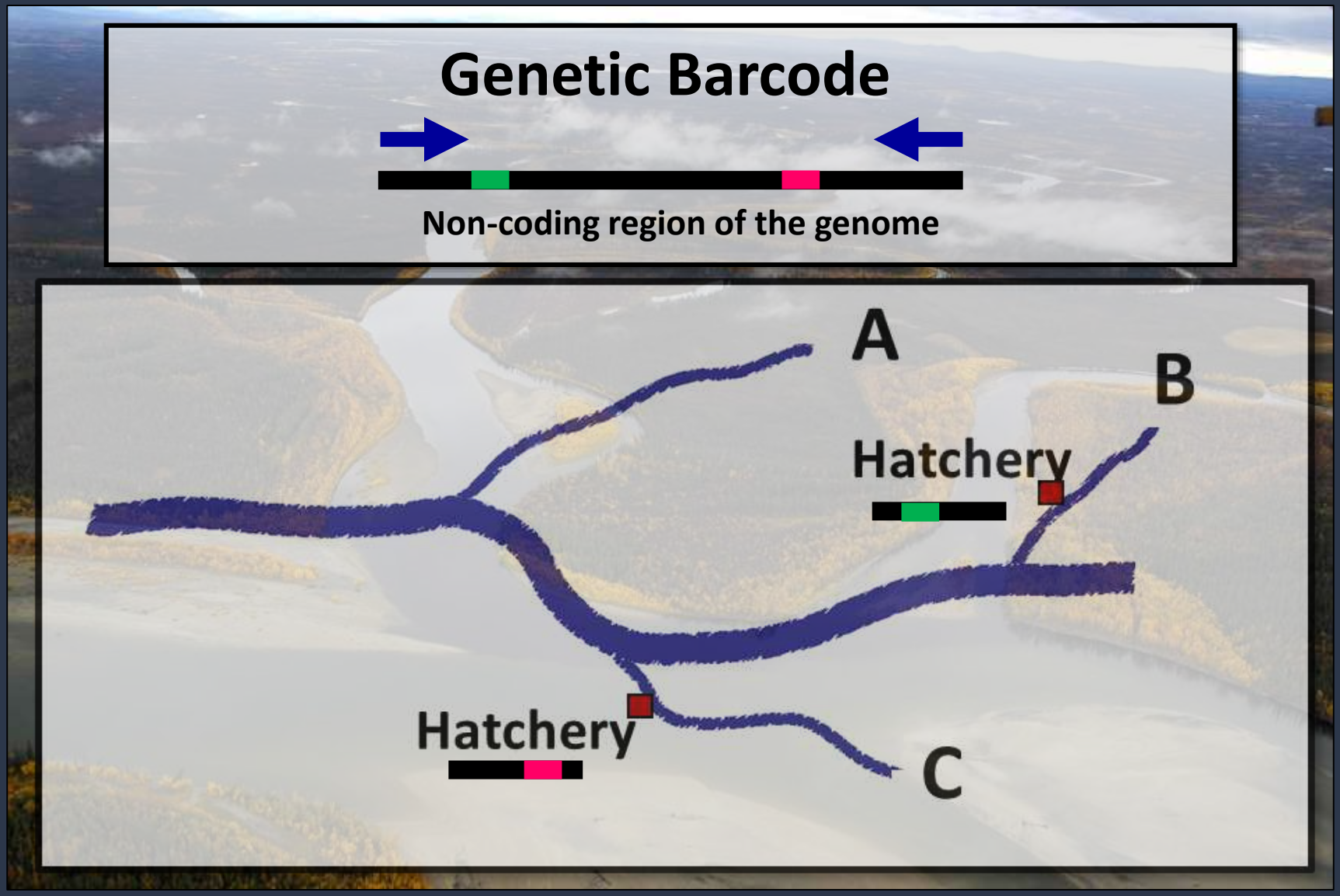
- Isolated away from genes in stable region of the genome
- Common among most or all target individuals

Hatchery 1 ATGGCAATAGGACCGATTACCCAAGACCGG

Hatchery 2 ATGGCATAGGACCGAT_ACCCAAGACCGG

Use tiny CRISPR induced mutations to genetically mark hatchery fish

CRISPR Genetic Barcoding



CRISPR Mutations

DNA Double Strand Break

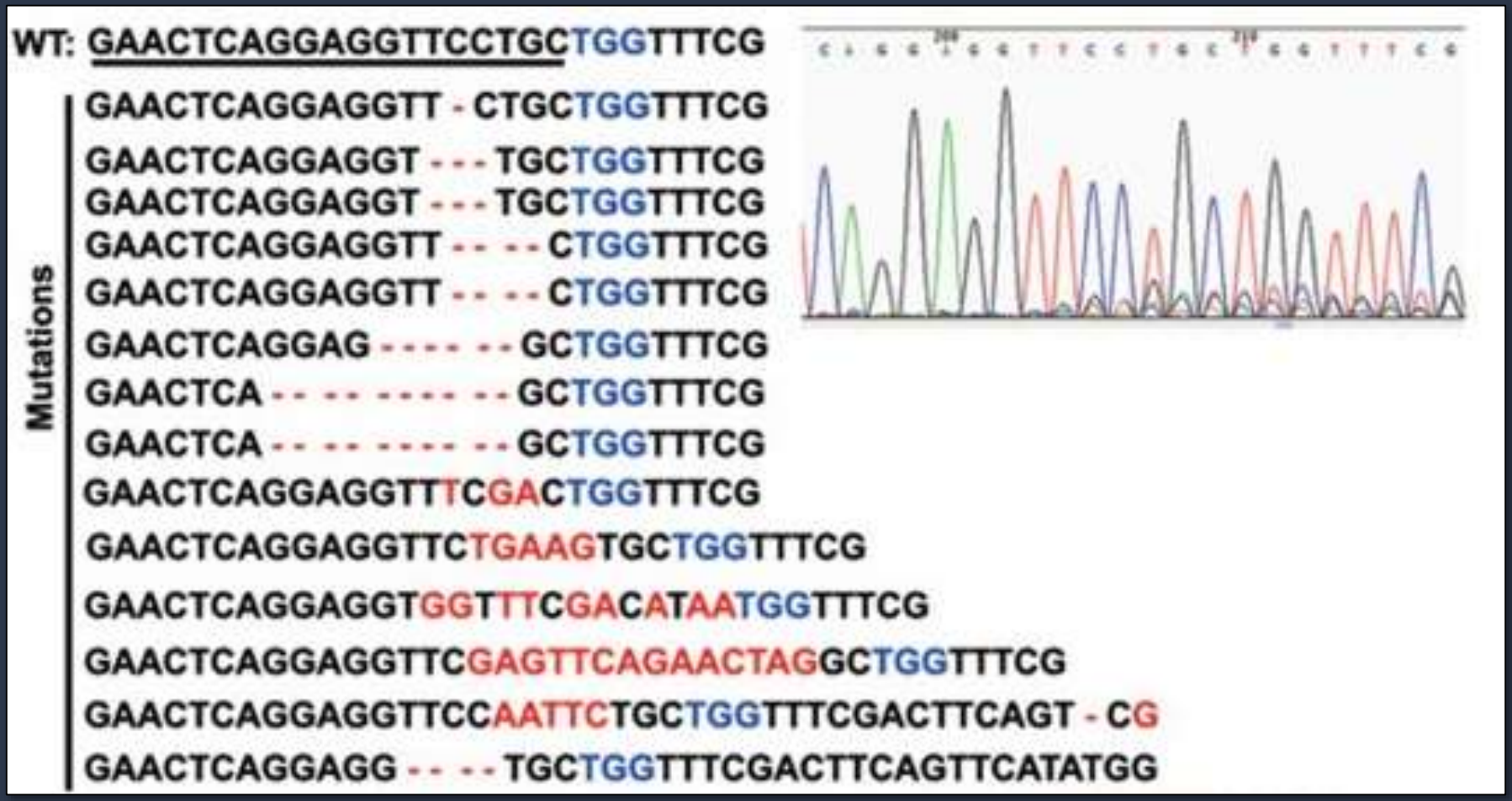
ATGGC ATAGGACCGATTACCCAAGACCGG
TACCG TATCCTGGCTAATGGGTTCTGGCC

Non-Homologous End Joining (NHEJ) "Error Prone"

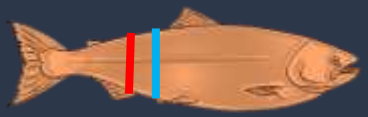
ATGGCAATAGGACCGATTACCCAAGACCGG
TACCGTTATCCTGGCTAATGGGTTCTGGCC

Insertion or Deletion

CRISPR Mutations



Identifying Individuals – Genetic Barcoding

	Single Locus	Multi Locus (Marking Year Class)
Year Class 1	 $1^{A/B}$	 $1^{A/B}$
Year Class 2	 $1^{A/B}$	 $1^{A/B}$ $2^{C/D}$
Year Class 3	 $1^{A/B}$	 $1^{A/B}$ $2^{C/D}$ $3^{E/F}$

Tracking Individuals with Genetic Barcodes

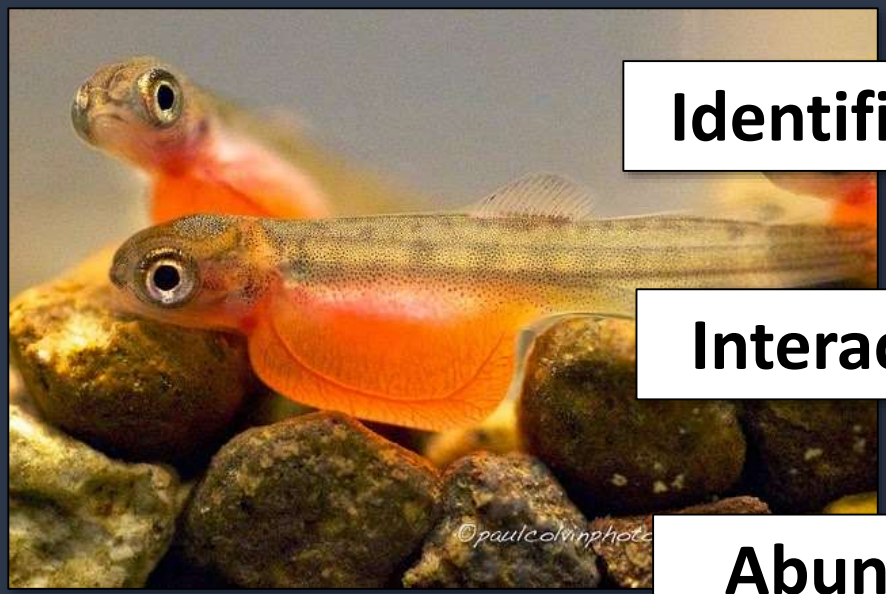
Real Time Genetic Monitoring

DNA Fish Passage Sampling

Smart DNA sampling trawls

Environmental DNA Sampling

Direct Sampling



Identification

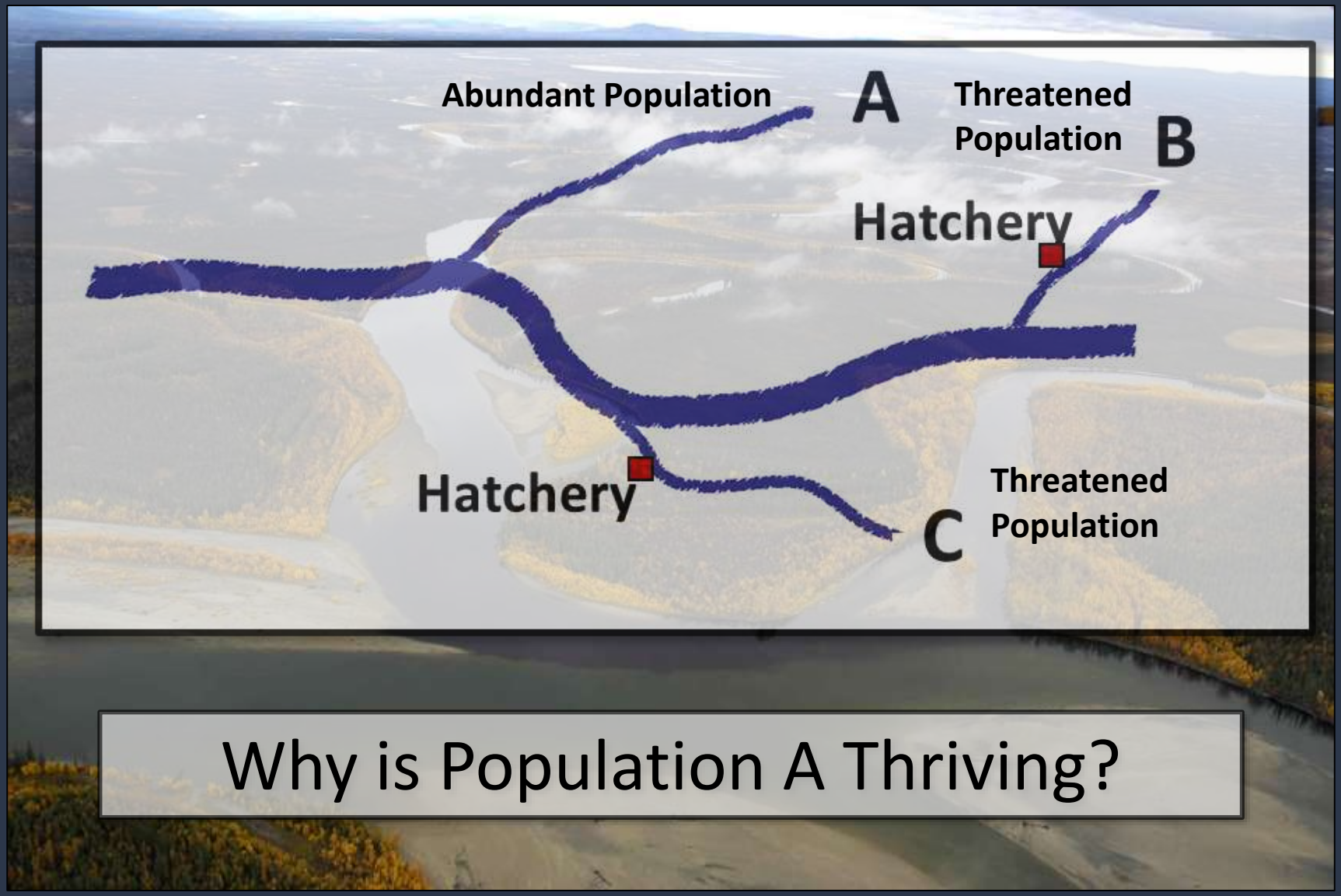
Interactions

Abundance

Distribution

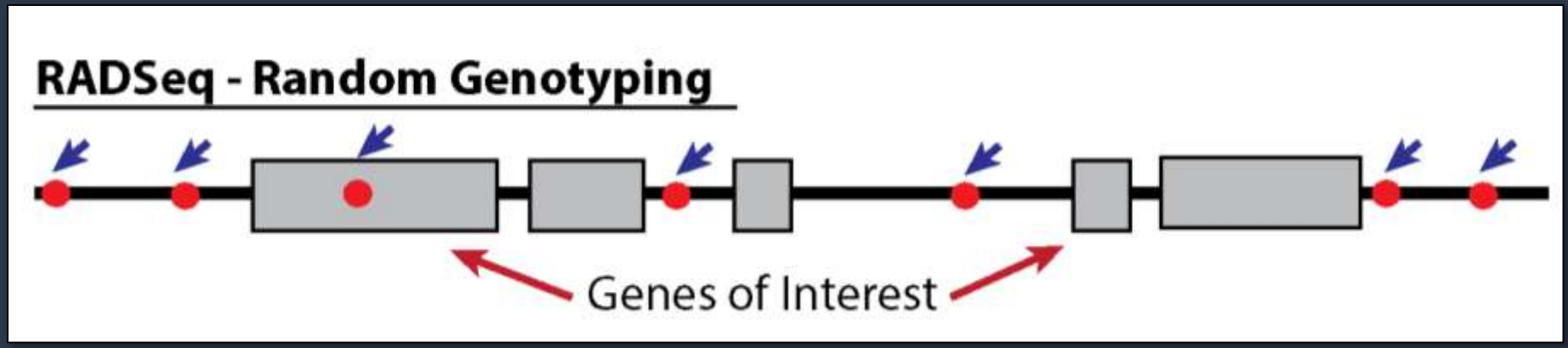
Factors Determining Success

Identifying Adaptive Traits

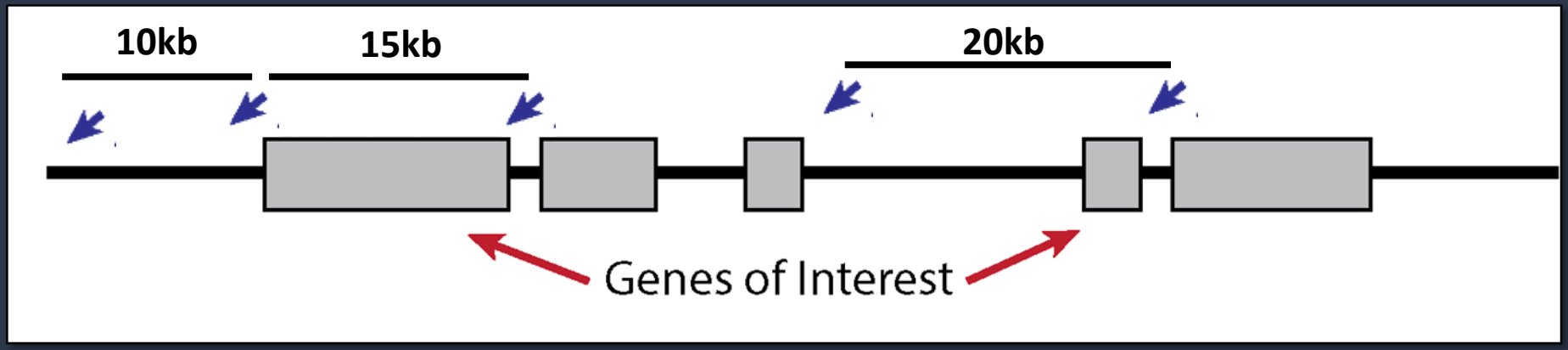


Why is Population A Thriving?

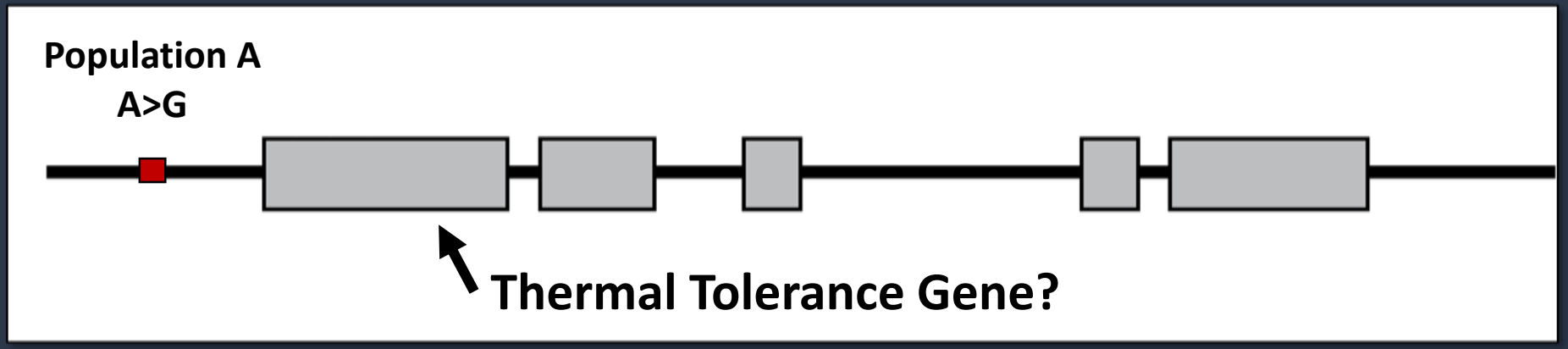
Identifying Adaptive Genes



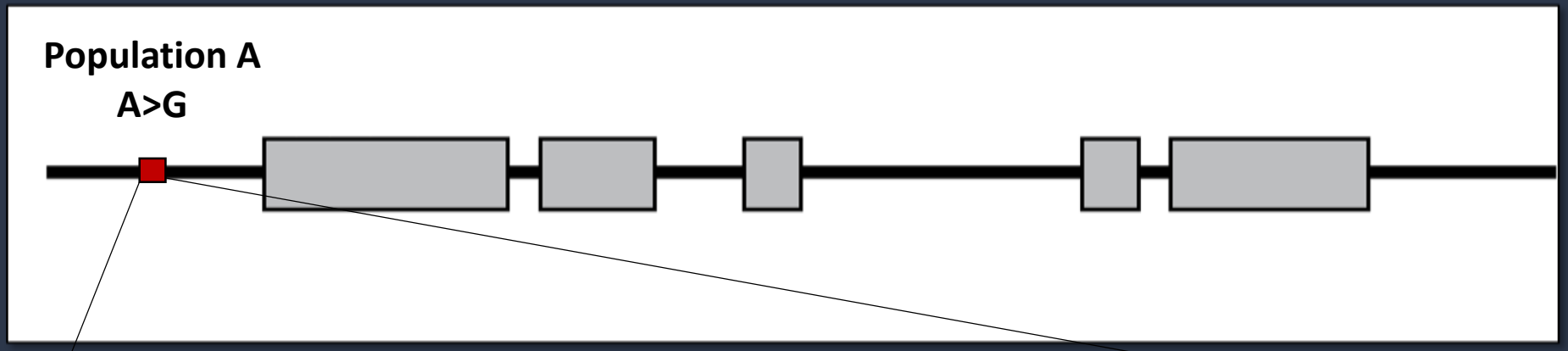
Thermal Tolerance Genes



Identifying Adaptive Genes



Investigating Genomic Correlations



CAGAGGGAAATGGCCCGTATAGACCATACATATCGGG
GTCTCCCTTTACCGGGCATATCTGGTATGTATAGCCC

Population A
Remove Adaptive Allele

Remove Thermal Tolerance

Population B
Add Adaptive Allele

Increase Thermal Tolerance

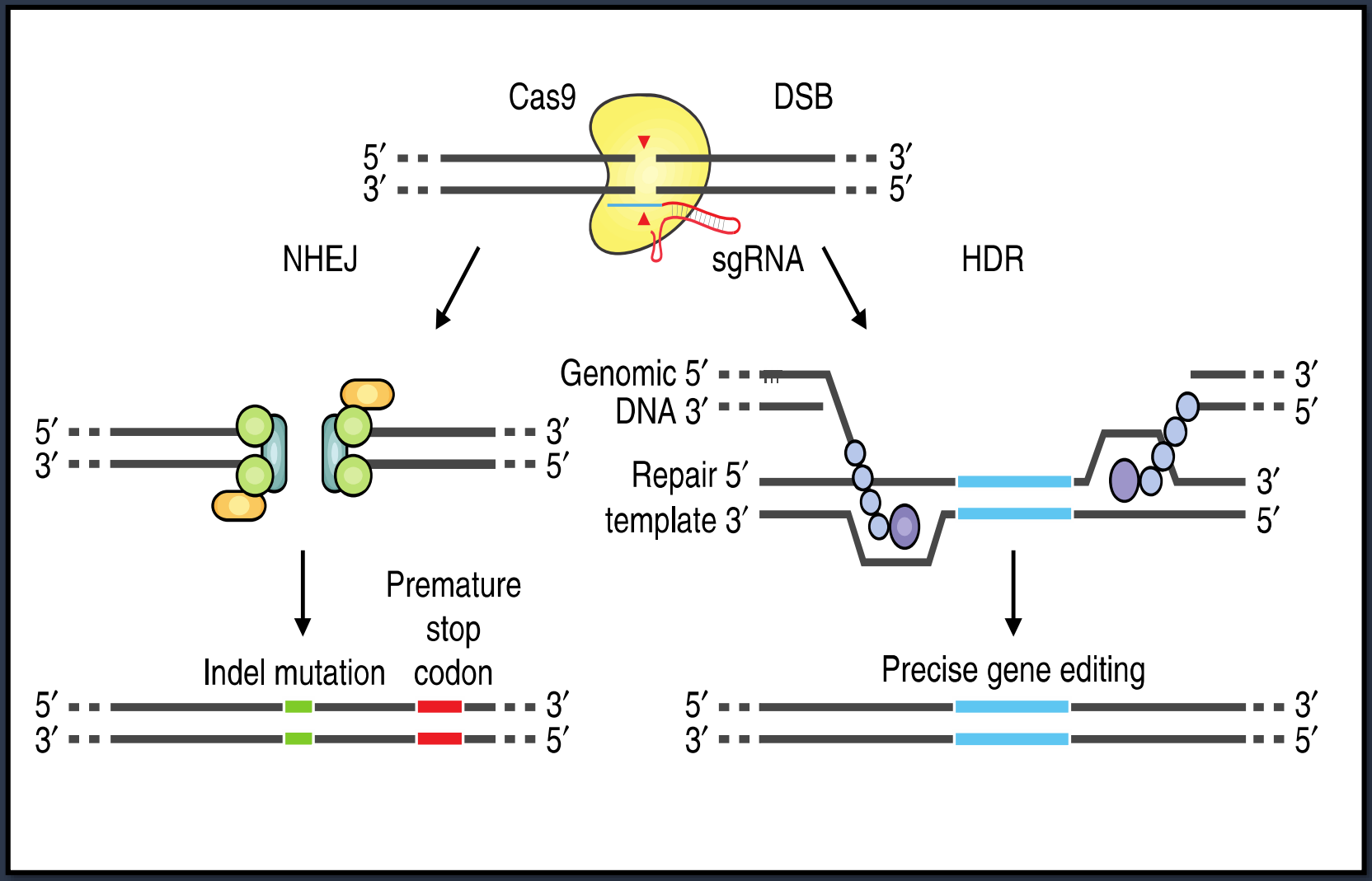
Investigating Genomic Correlations

Targeted Mutagenesis in Atlantic Salmon (*Salmo salar* L.) Using the CRISPR/Cas9 System Induces Complete Knockout Individuals in the F0 Generation

Rolf B. Edvardsen, Sven Leininger, Lene Kleppe, Kai Ove Skaftnesmo, Anna Wargelius

Published: September 25, 2014 • https://doi.org/10.1371/journal.pone.0108622

Precision Genome Editing



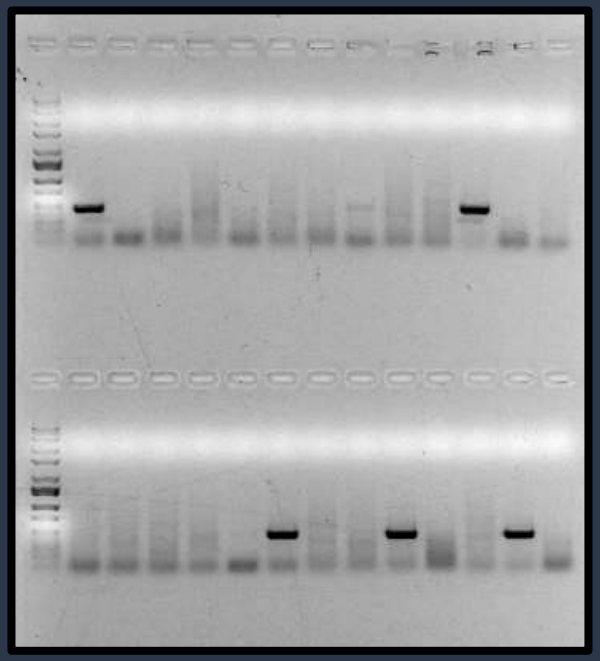
Investigating Genomic Correlations

Design gRNA Targeting Desired Location

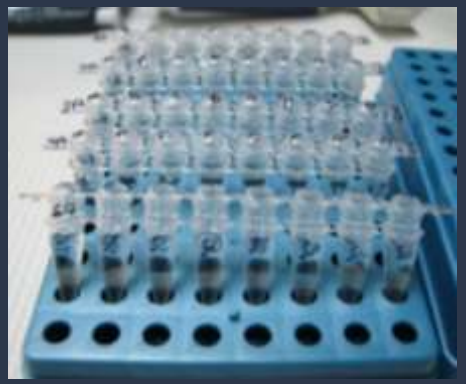
gRNA

TATCCTGGCTATGGGTTCTG
ATGGCATAGGACCGATACCCAAGACCGG

Microinject Eggs with gRNA and Cas Nuclease

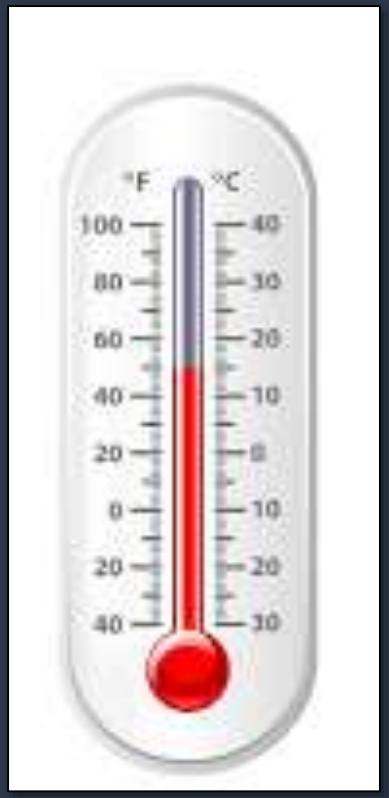


Identify Mutant Founders

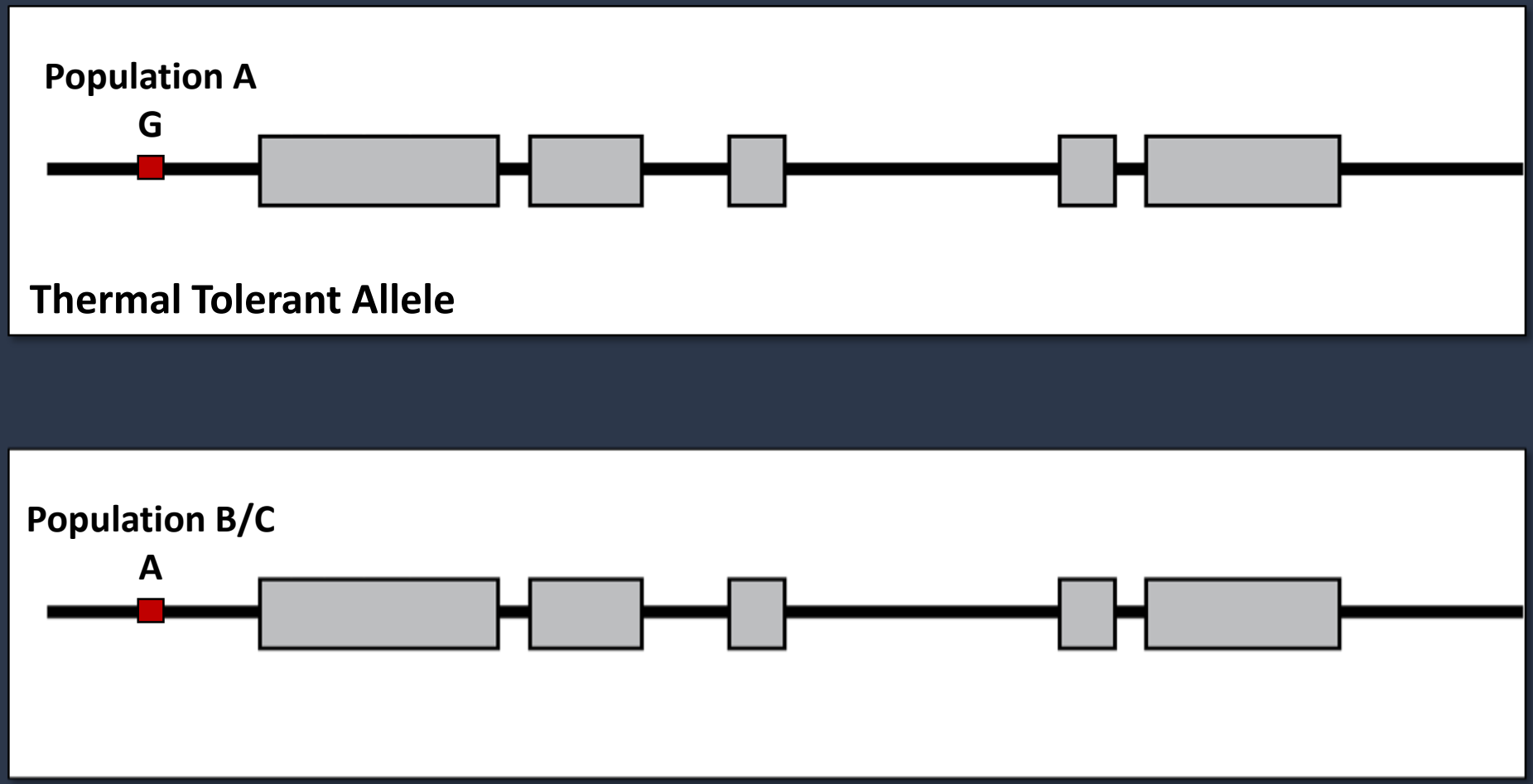


Investigating Genomic Correlations

Evaluate Phenotypic Change



Linking Genotype to Phenotype



Facilitating Adaptation

Selection vs. Assisted Gene Flow vs. Genome Editing

Assisted Gene Flow to Facilitate Local Adaptation to Climate Change

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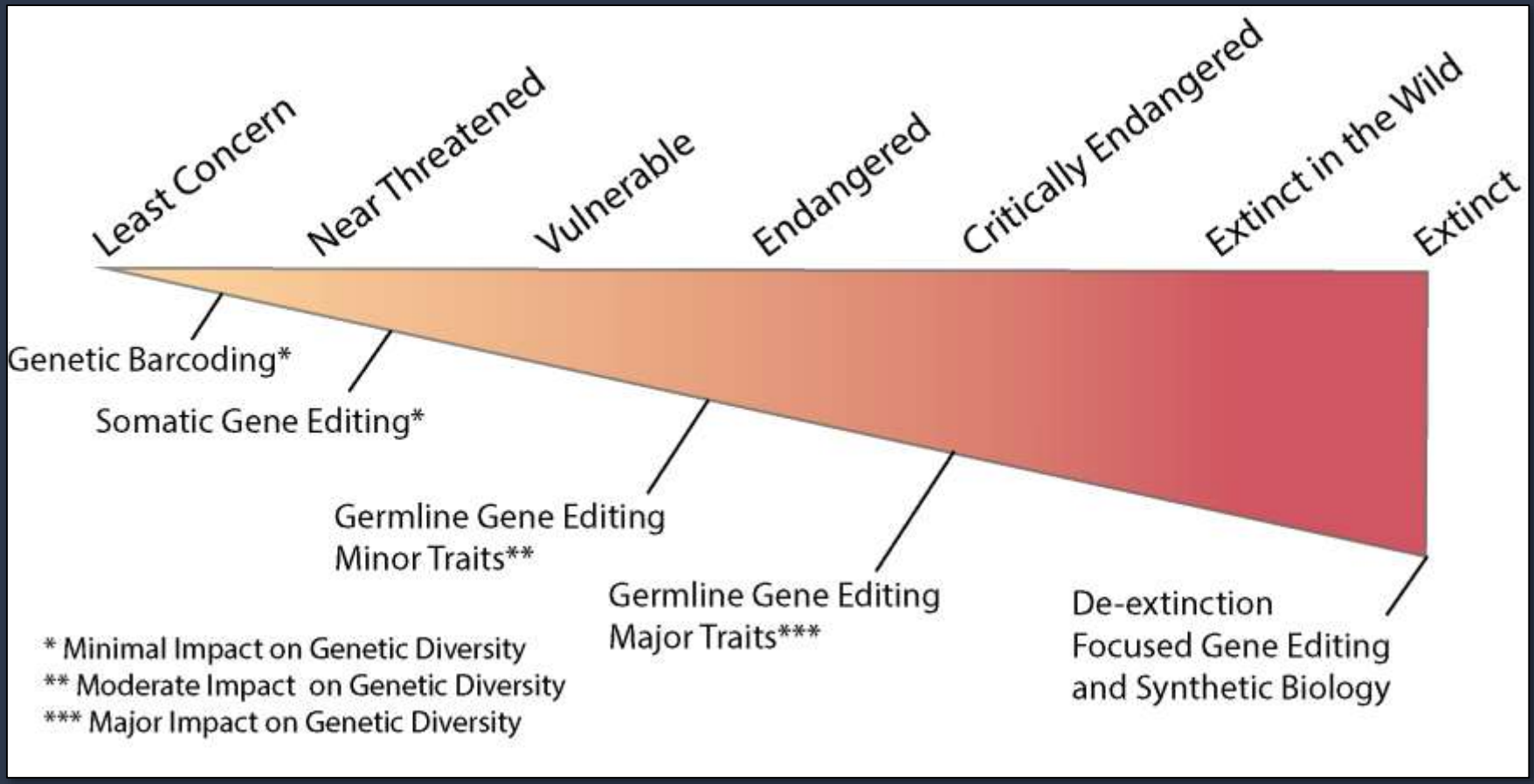
Genome Editing in Fisheries: Considerations Conservation Objective?

What Do We Want To Conserve?

Preserve Genetic Unit
vs.
Preserve Ecological Unit

Depends on the Conservation Status of Species/Populations

Ethics of Genome Editing in Fisheries



Phelps M, Seeb L, Seeb J. 2019; *Cons. Biol*.

Acknowledgements

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