SAVING • CORALS WITH **CRISPR**

Jeanne Ye and Sheena Caldetera



Purpose/Problem: To understand the potential of genetically engineering coral reefs to be more resistant toward effects of climate change.



Here is what we plan to look into:

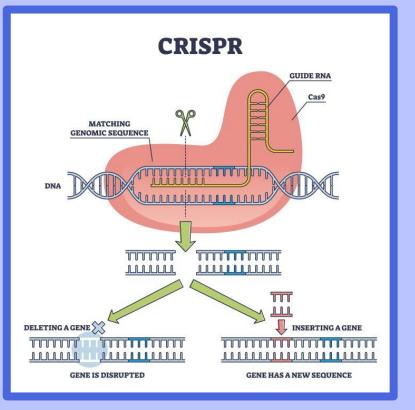
- The different methods of genetic engineering, such as CRISPR Cas9 through vectors, and understanding the limits of genetic technology in cnidarians.
- Possible methods to implement CRISPR Cas9 genetic engineering in corals to increase heat tolerance.
- The potential consequences of genetically altering groups of marine organisms and the impact they could have on their ecosystem.
- Interviewing some climate change and genetics experts
- Possible ways to respond to coral reef population declination.

What is Coral Bleaching?



What is CRISPR?

Clustered Regularly Interspaced Short Palindromic Repeats

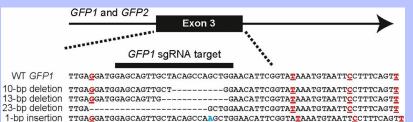


Study 1: Reduced Thermal Tolerance by Phillip Cleaves

Table 1.

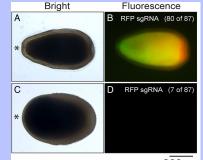
Numbers of injected and surviving A. millepora zygotes

	Night 1			Night 2	
Target gene(s) <u>*</u>	FGF1a	GFP	RFP	FGF1a	None (Cas9- only)
No. of individuals injected successfully_	146	123	147	246	227
No. of individuals surviving until 12 h postfertilization <u>‡</u>	74	88	79	116	176
Percent of individuals surviving until 12 h postfertilization, % <u>†</u>	51	72	54	47	78



GFP2 sgRNA target

WT GFP2	TTGA <mark>A</mark> GATGGAGCAGTTGCTACAGCCAGCTGGAACATTCGGTA <mark>A</mark> AAATGTAATT <mark>T</mark> CTTTCAGT <mark>A</mark>
Complex	TTGA <mark>A</mark> GATGGAGCAGTTGCT <mark>GG</mark> AACATTCGGTA <mark>A</mark> AAATGTAATT <u>T</u> CTTTCAGT <u>A</u>
13-bp deletion	TTGAAGATGGAGCAGTTGCTACATTCGGTAAAATGTAATTTCTTCAGTA
1-bp insertion	TTGAAGATGGAGCAGTTGCTACAGCCAAGCTGGAACATTCGGTAAAATGTAATTTCTTTC
1-bp insertion	TTGA <mark>A</mark> GATGGAGCAGTTGCTACAGCC T AGCTGGAACATTCGGTA <mark>A</mark> AAATGTAATT <mark>T</mark> CTTTCAGT <mark>A</mark>

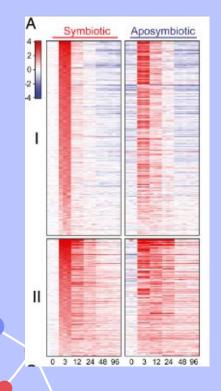


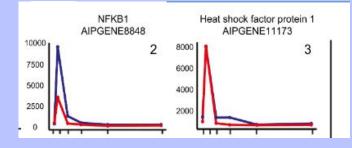


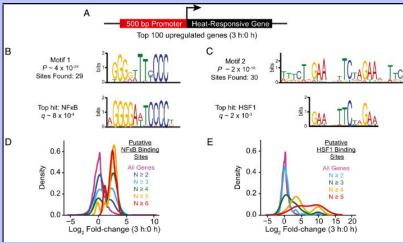




Study 2: Aiptasia and the HSF1 Gene







Study 3: Philip Cleaves' Coral **CRISPR Experiment**

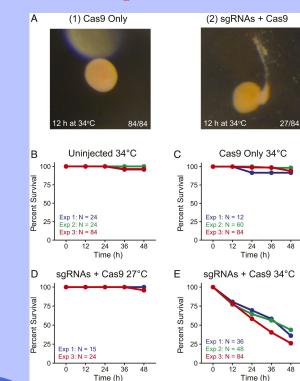


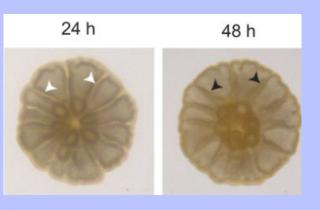
Table 1.

48

Numbers of injected and surviving A. millepora zygotes

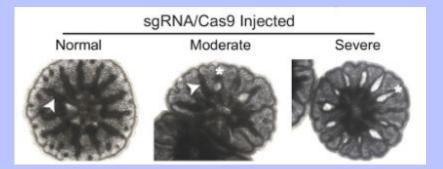
Injected material <u>*</u>	Experiment 1		Exp	eriment 2	Experiment 3	
	Cas9 alone	sgRNA/Cas9	Cas9 alone	sgRNA/Cas9	Cas9 alone	sgRNA/Cas9
Nos. of individuals for which injection was attempted	100	200	200	200	300	300
Nos. of individuals surviving until 12 h postfertilization <u>†</u>	68	180	147	148	239	205
Nos. of survivors that had been injected successfully <u>‡</u>	52	125	117	133	190	154

SLC4y: The bicarbonate transporter

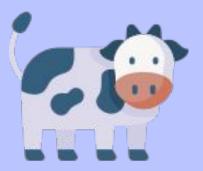




arrows indicate where septa should form at different times (indicated by arrows)



Arrows indicate where the septa formed properly. Asterisks indicate where gaps where septa should have formed.



Different promoter types were used to drive GFP vectors... found that ROSA26 was supportive of all to an extent

ROSA26: The Safe Harbour Locus

- Safe harbour loci allow for ubiquitous & stable expression with different promoters
- Yet to find safe harbour locus in anthozoans, but can utilize genomic sequence homology analyses to help us find one

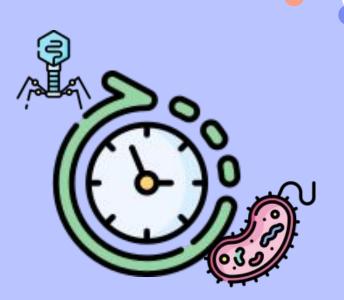


Rosa26 has only been around since 1991, but it has been used for many different knock-in experiments since.



CRISPR: A Brief History

- Found in 1987 in Escherichia coli during an analysis of phosphate metabolism
- Early 2010s, found its role in the immune system
- Currently being used in many different studies, including one to treat mosquitoes to prevent spread of malaria!



Ethics and Public Concerns



Ecosystem

"How can we guarantee that the modifications won't affect the food chain?"



"Naturalness"

"Humans have no right to change what was created as perfect"



Use

"Frankencoral" "...Technology could be used in terrible ways."



Evidence

"There a lack of scientific evidence that proves this an effective solution."



An Ideal Experiment

Electroporation and Growth

Deliver Cas9 through electroporation

Application

Using the Aiptasia as a model for future engineering 2

4

3

Preparation

Preparation of sgRNA HSF1 Cloning zygotes

Observing and testing growth

Testing the Aiptasia in an ecosystem environment and in a heat shock environment

Present Solutions to Slow Down and/or prevent Dying Coral Reefs in Response to Climate Change



Approaches to Coral Reef Conservation. (2018, September 10). Coral Reef Alliance. Retrieved March 9, 2024, from https://coral.org/en/blog/restoration/



Mitra, S. (n.d.). reduce Your Carbon Footprint Now! 10 Shockingly Simple Ways to Save the Planet. Let's Talk Geography. Retrieved March 9, 2024, from https://letstalkgeography.com/reduce-your-carbon -footprint/

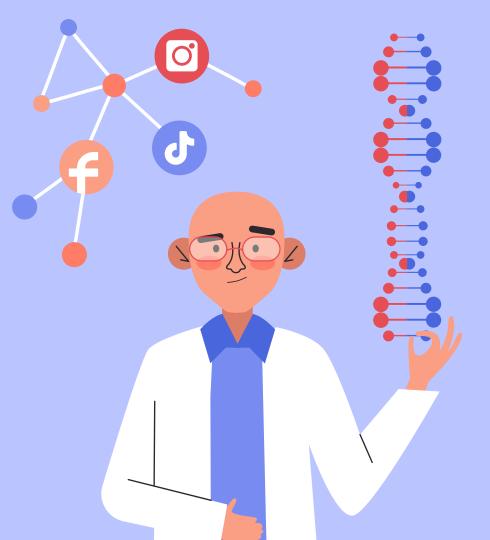




CONCLUSIONS

Currently, coral restoration has been a common practice to restore coral reefs. The genome of a coral still needs to be studied. Genetic engineering provides a promising method to relieve dying reefs. Nevertheless, it is our responsibility to reduce our carbon footprint to slow down climate change.





THANKS!

CREDITS: This presentation template was created by **Slidesgo**, and includes icons by **Flaticon** and infographics & images by **Freepik**

