CRISPR Technology and Eco-Restoration

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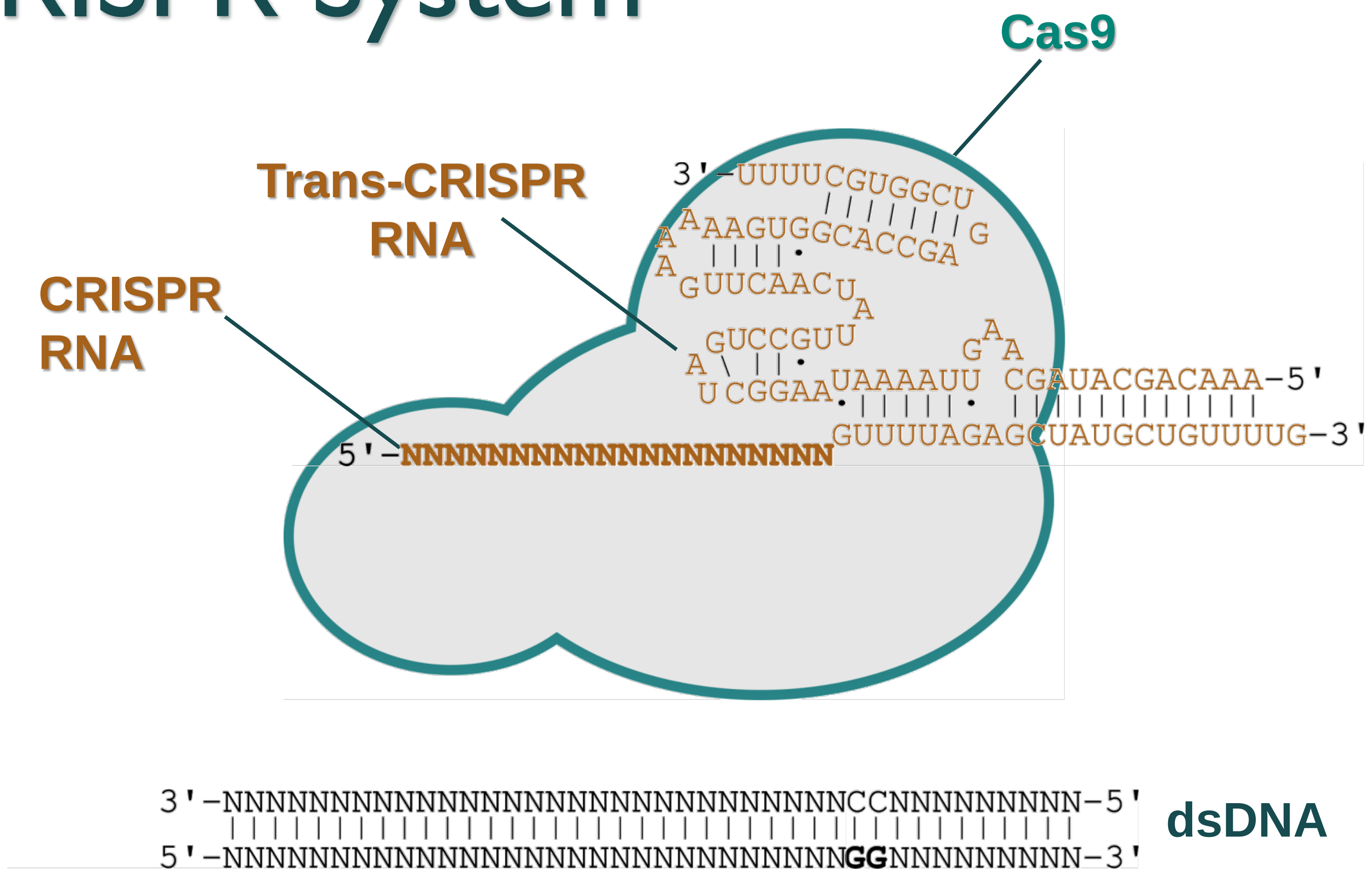
10/7/2015

WYSS INSTITUTE

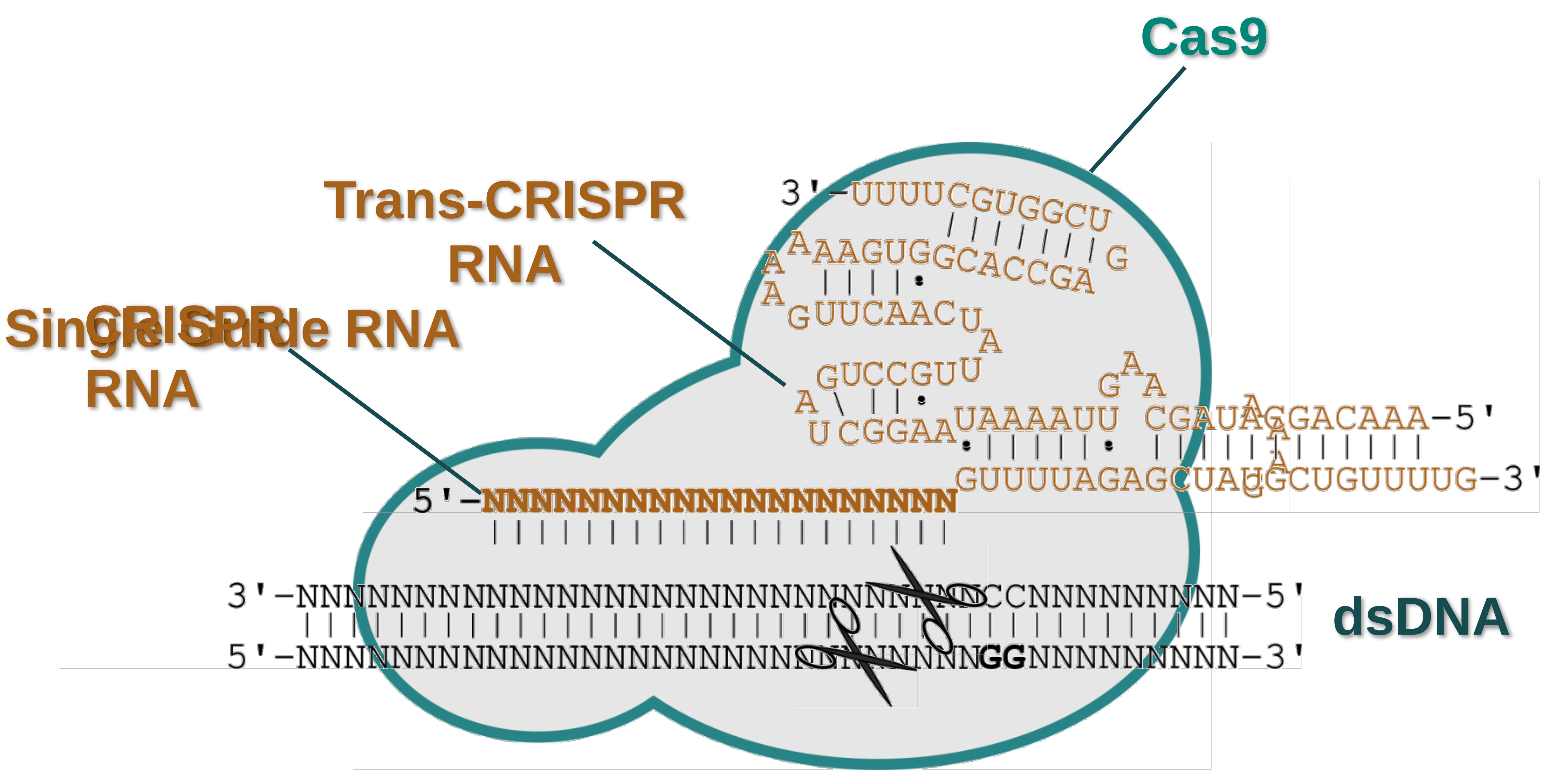
Outline

- What is CRISPR
- Current environmental engineering projects involving CRISPR
- CRISPR and AIS

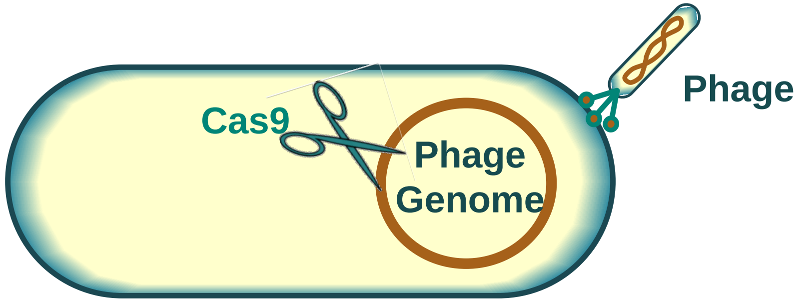
The CRISPR System



Deltcheva E*, Chylinski K*, Sharma CM*, Gonzales K, Chao Y, Pirzada ZA, Eckert MR, Vogel J, Charpentier E (2011) *Nature*



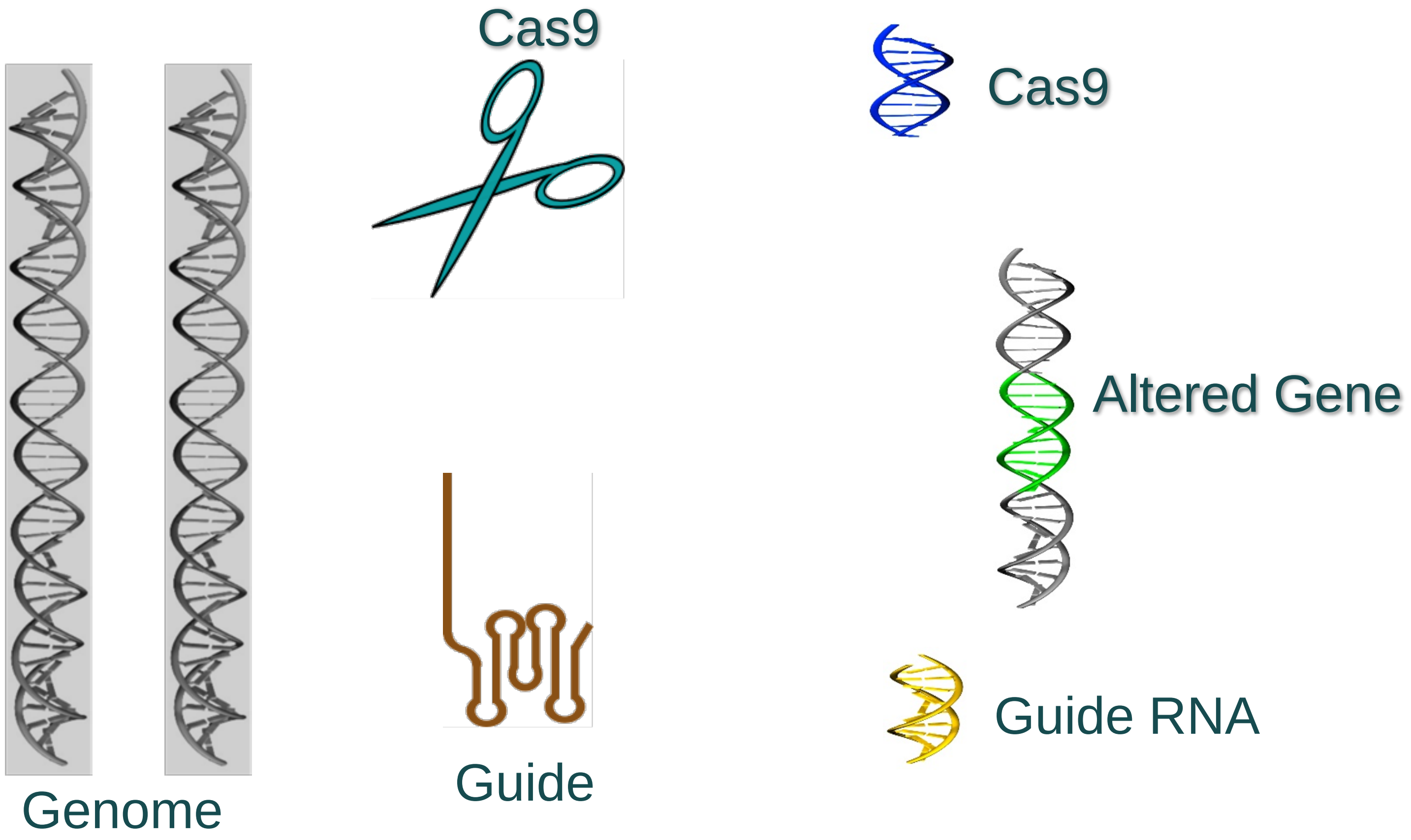
Bacterium with Cas9, CRISPR RNA vs phage genome



Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) *Science*

Deltcheva E*, Chylinski K*, Sharma CM*, Gonzales K, Chao Y, Pirzada ZA, Eckert MR, Vogel J, Charpentier E (2011) *Nature*

Using CRISPR/Cas9 to edit genes



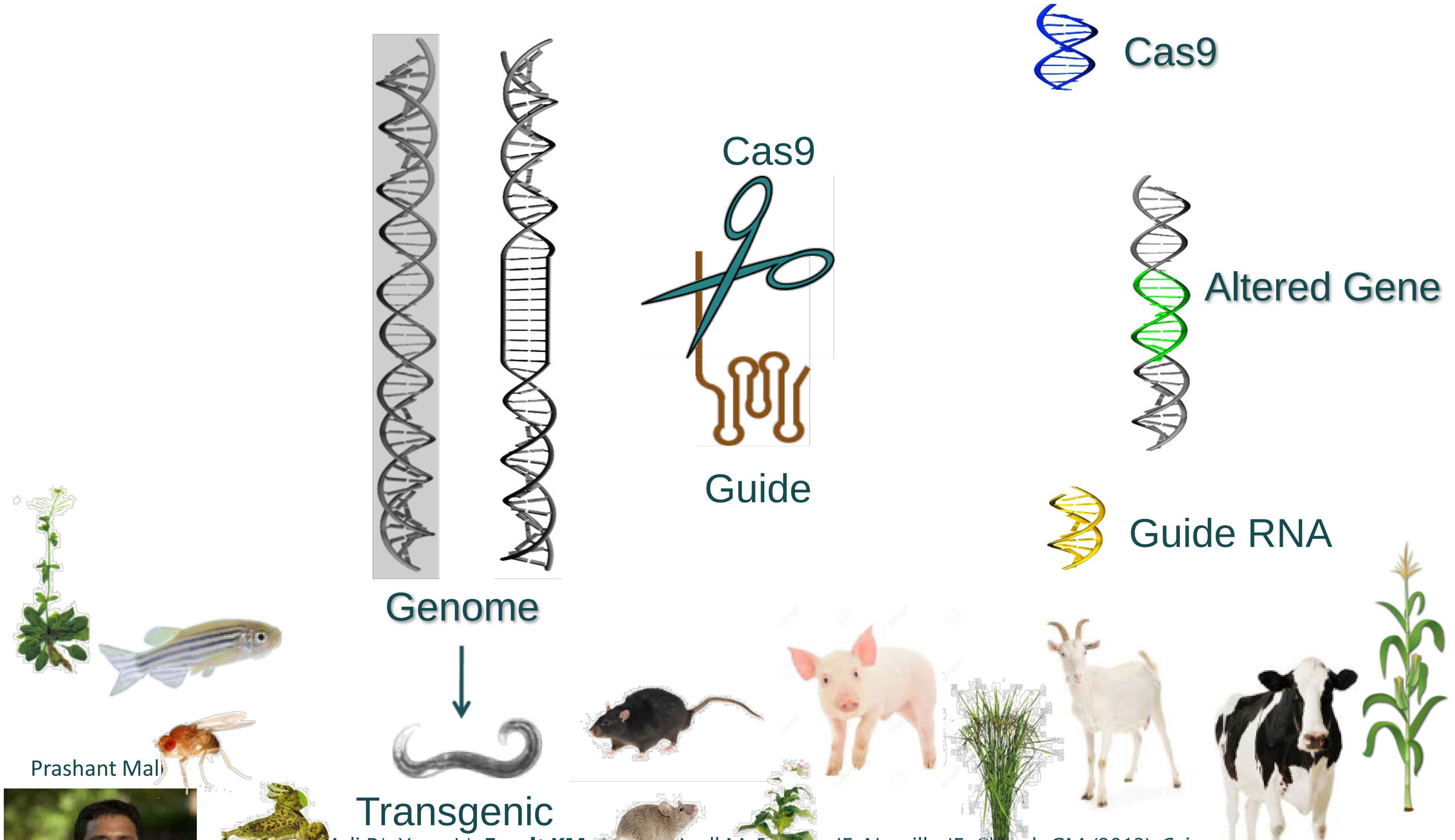
Prashant Mali

Mali P*, Yang L*, **Esvelt KM**, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM (2013) *Science*

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini L, Zhang F (2013) *Science*

Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J (2013) *eLife*

Using CRISPR/Cas9 to edit genes



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Mali P*, Yang L*, **Esvelt KM**, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM (2013) *Science*

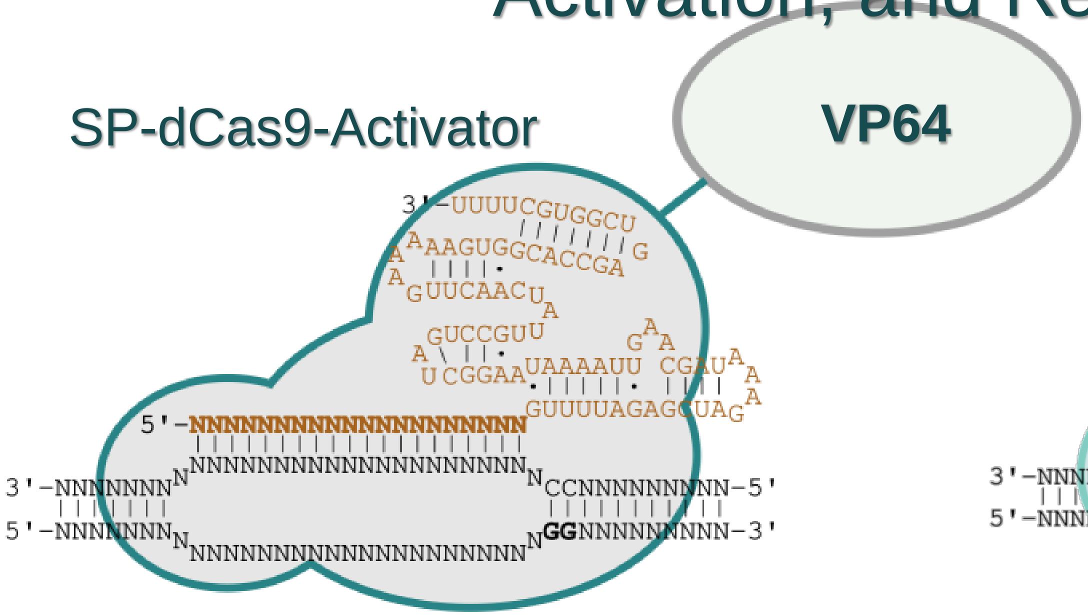
Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini L, Zhang F (2013) *Science*

Friedland A, Tzur YB, **Esvelt KM**, Colaiacovo MP, Church GM, Calarco JA (2013) *Nature Methods*

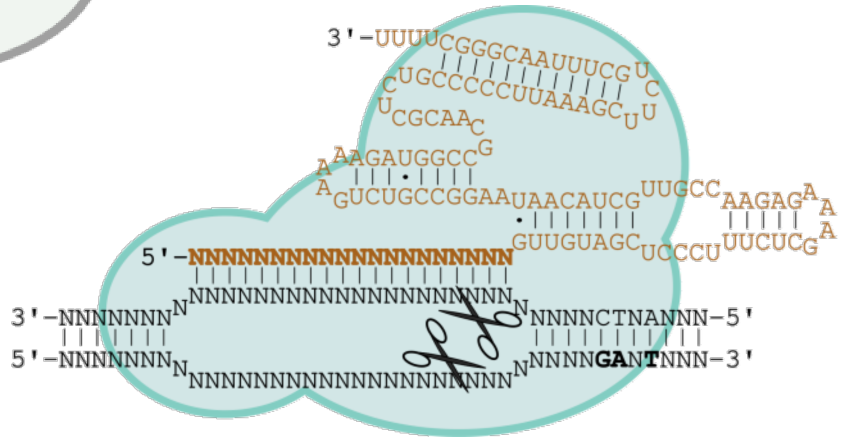
Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J (2013) *eLife*

Orthogonal CRISPR Proteins Permit Concurrent Editing, Activation, and Repression

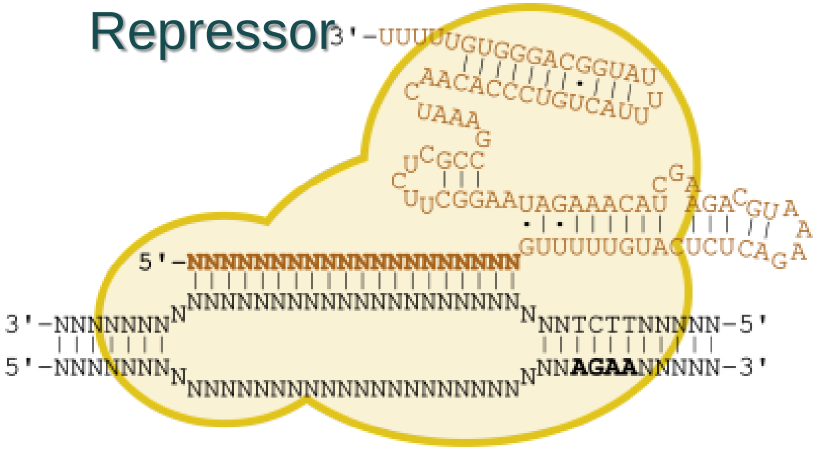
SP-dCas9-Activator



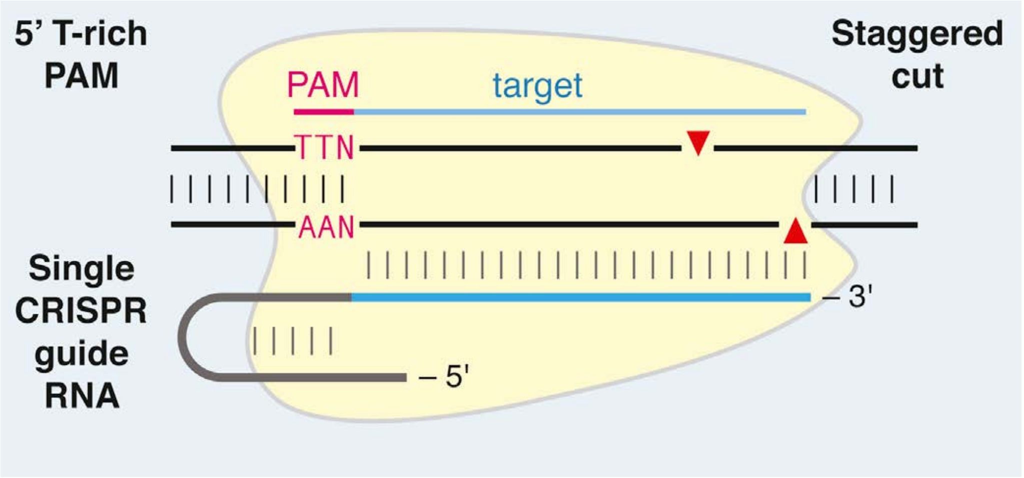
NM-Cas9 Editor



ST1-dCas9-Repressor



Cpf1 (Cas9 Alternative



Esvelt KM*, Mali P*, Braff J, Moosburner M, Yaung SJ, (2013) *Nature Methods*
Bernd Zetsche, Jonathan S. Gootenberg, Omar O. Abudayyeh, ..., Aviv Regev, Eugene V. Koonin, Feng Zhang, (2015) *Cell*

Outline

- What is CRISPR
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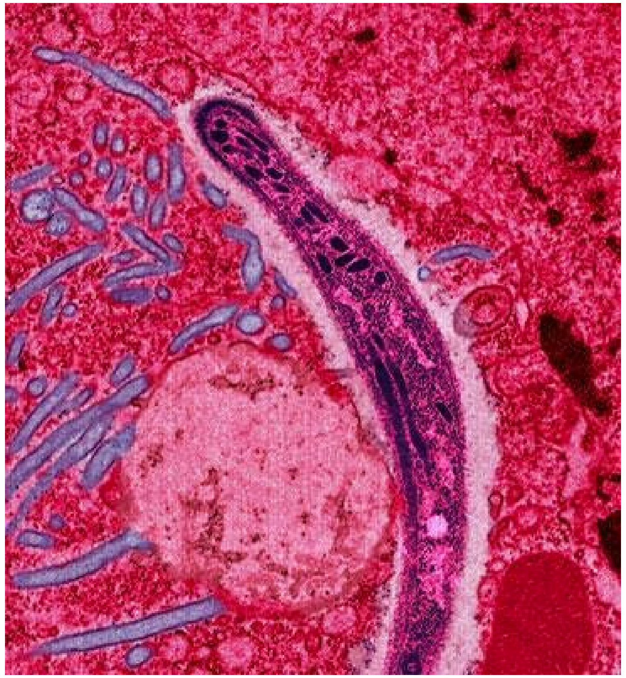
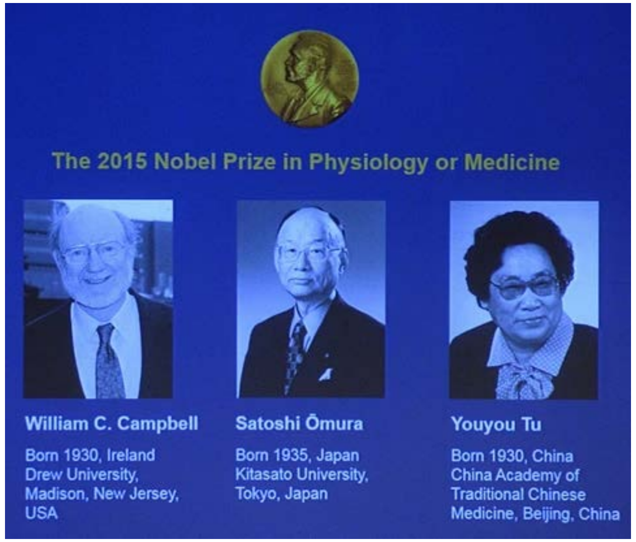
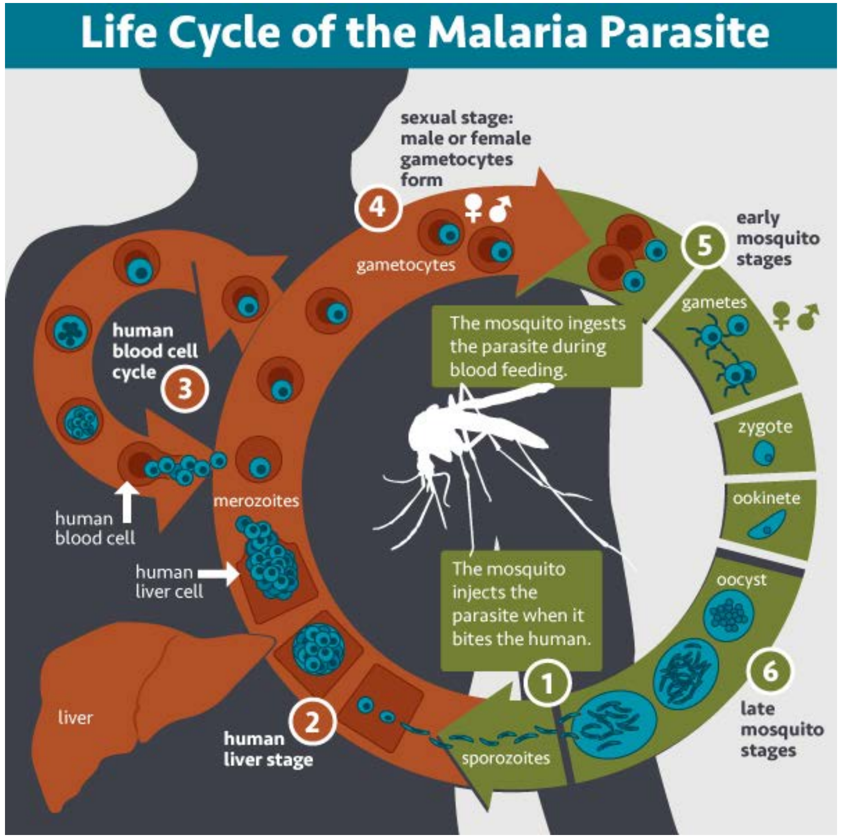
The malaria epidemic

- *Plasmodium,* a parasitic single-celled organism
- Carried by mosquitoes, infects humans and other animals
- Affects liver and blood cells
- 2010: >200 Million cases worldwide with >655,000 deaths (90% in Africa)
- State of the art drug: Artemisinin



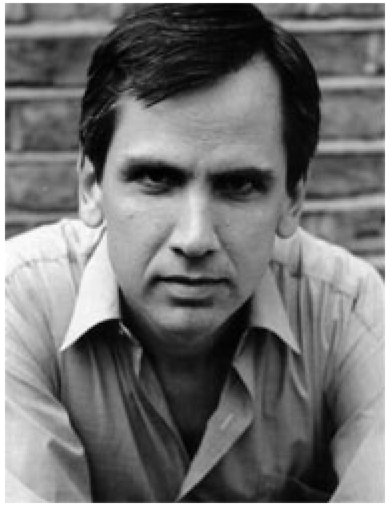


Case Study: Malaria



"Life Cycle of the Malaria Parasite" by NIH and HIAID / CC BY

- *Anopheles* Mosquitos are its primary host
- Engineered SM1 peptide in the mosquito gut appear to halt Malaria development
- If we can replace wild-type mosquitos with GM ones, we can eradicate Malaria



Austin Burt first proposed to harness natural endonuclease gene drives to alter wild populations in 2003

Problem #1: Retargeting natural homing endonuclease enzymes is very difficult

Problem #2: Even if you could, the resulting gene drive would not be evolutionarily stable

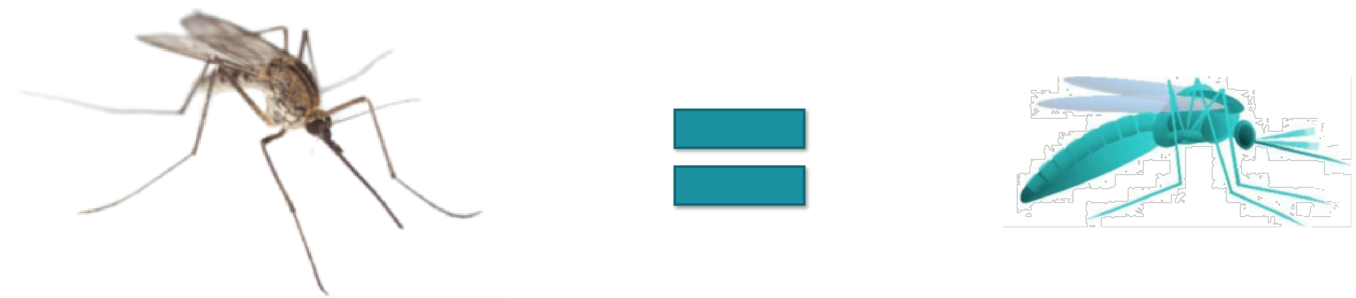
Burt A (2003) *Proc. R. Soc. Lond. B*

Windbichler N et al. (2011) *Nature*

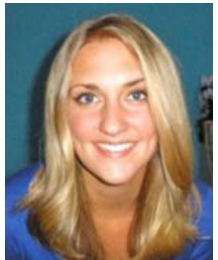
Chan YS et al. (2011) *Genetics*

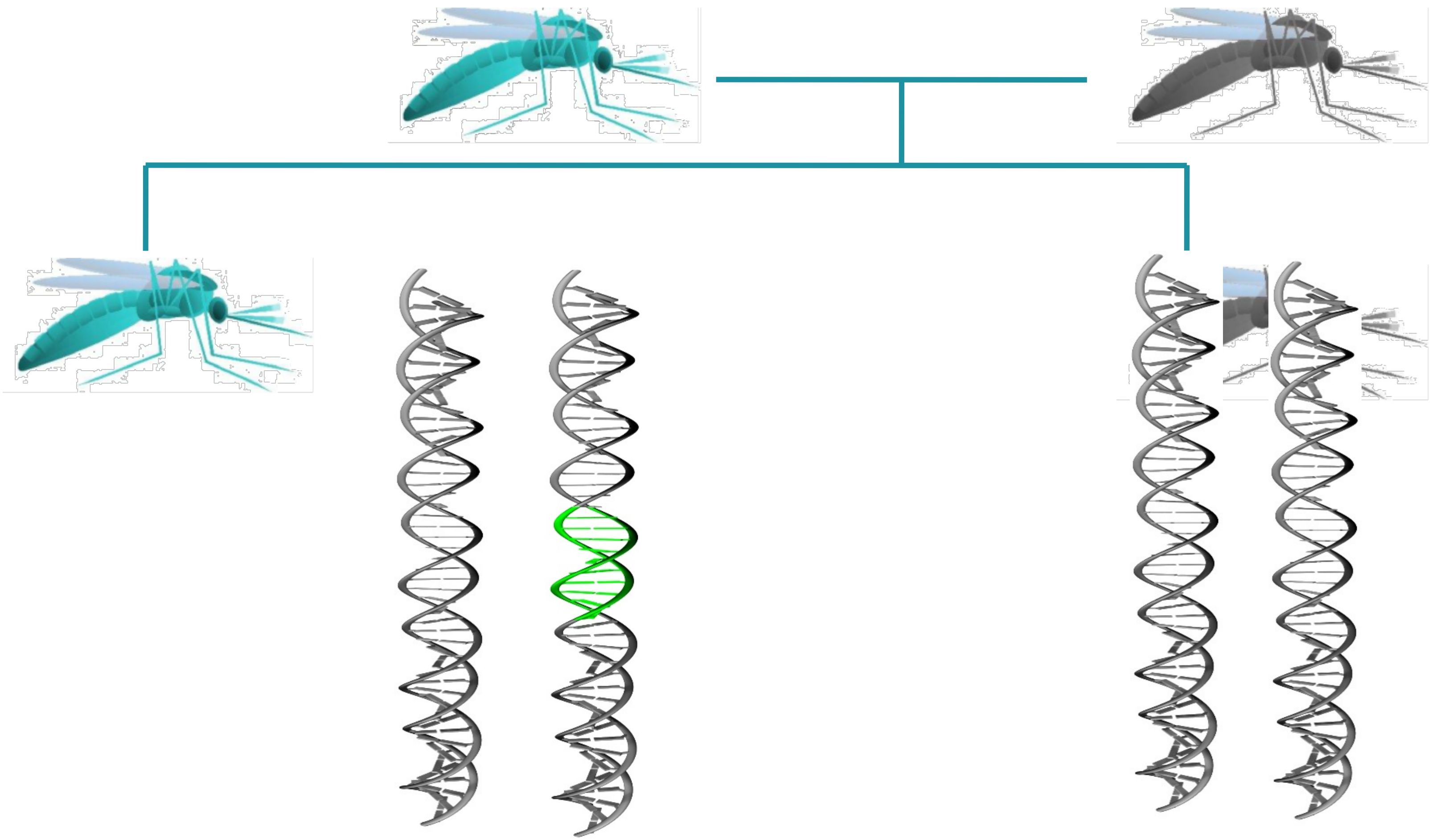
Chan YS et al. (2013) *PLoS One*

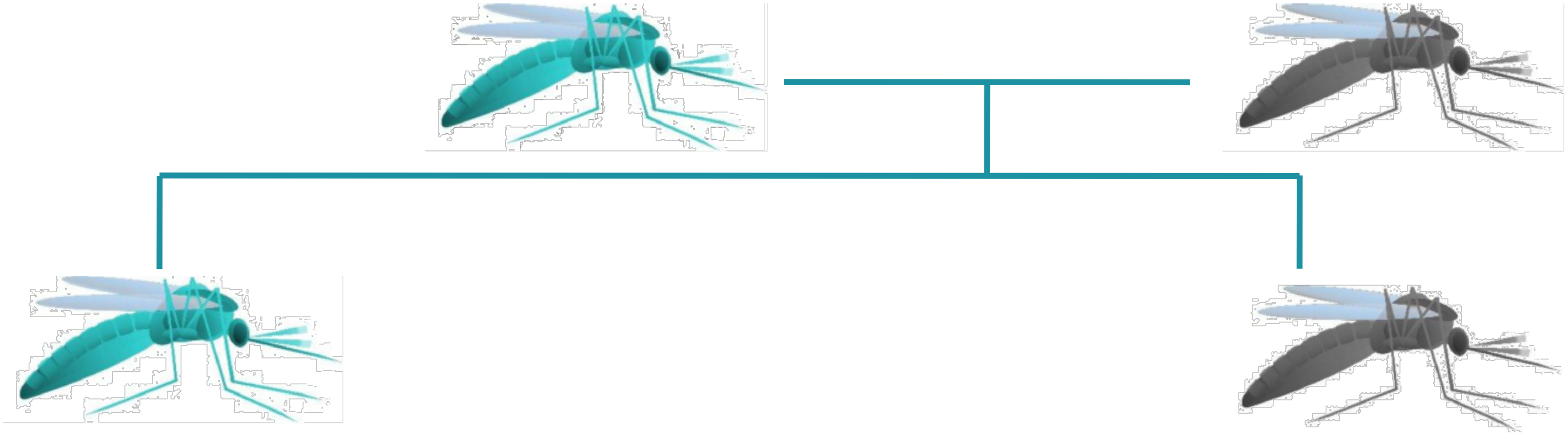
Gene-Drives for Malaria Eradication

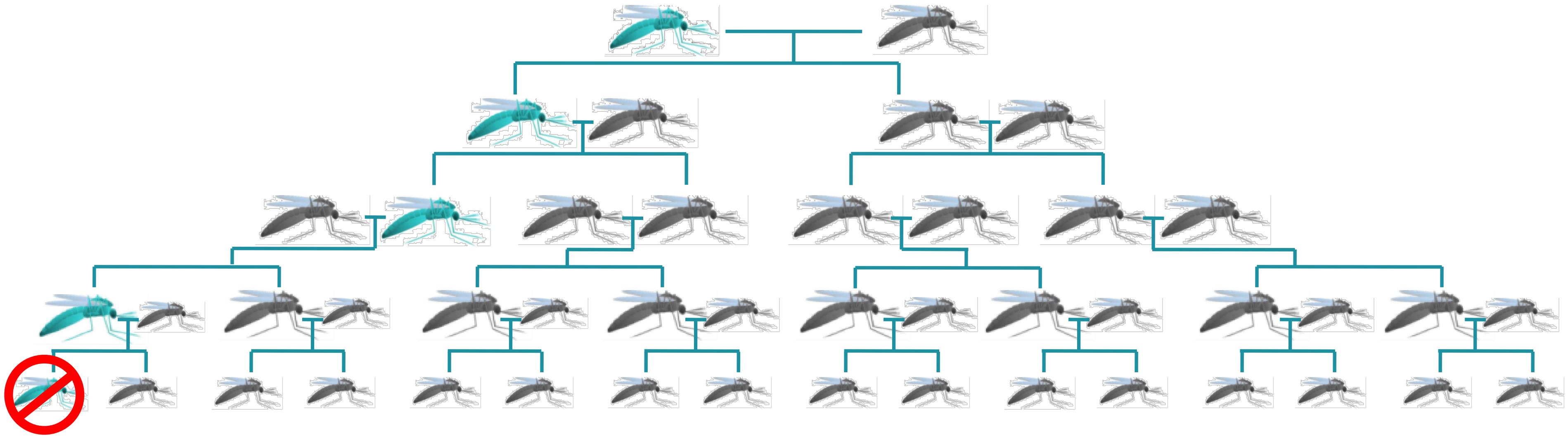


Andie Smidler





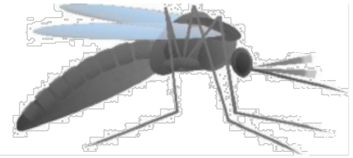


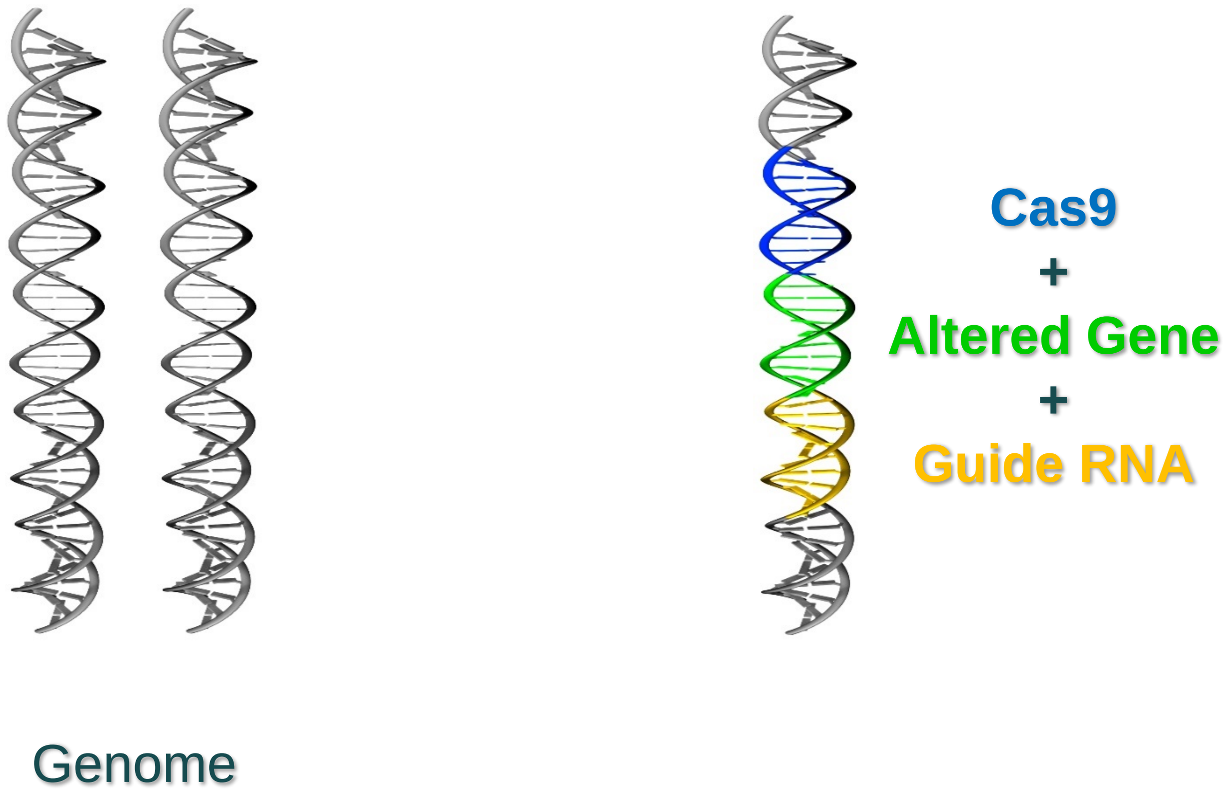


Altering an organism almost always reduces its ability to survive and reproduce in the wild

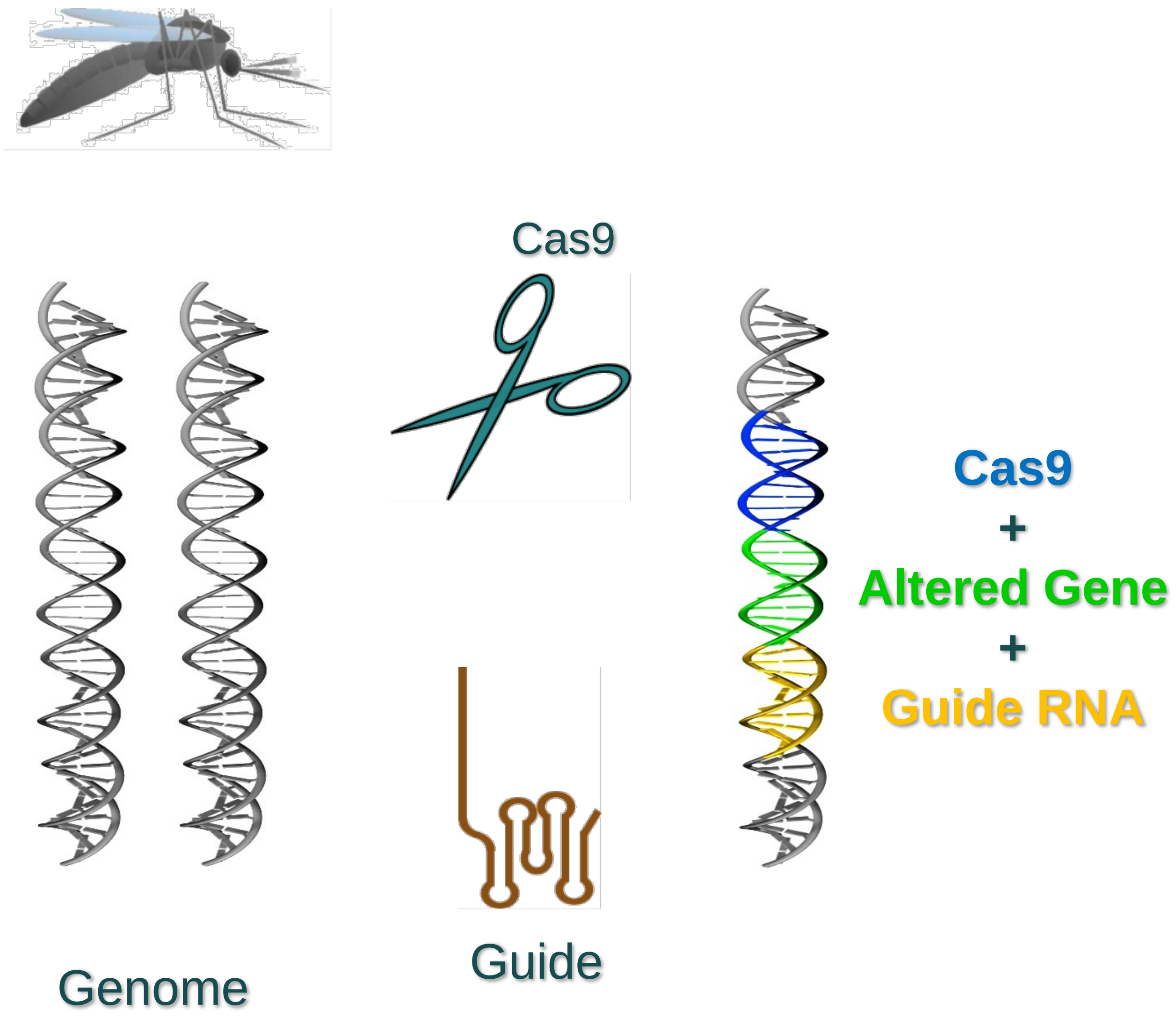
"Natural Selection... is a power incessantly ready for action, and is as immeasurably superior to Man's feeble efforts as the works of Nature are to those of Art."
- Charles Darwin, On the Origin of Species

Is there a general way to evade natural selection?





Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*



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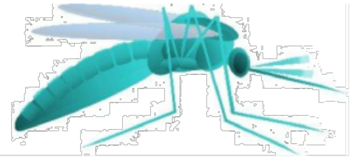

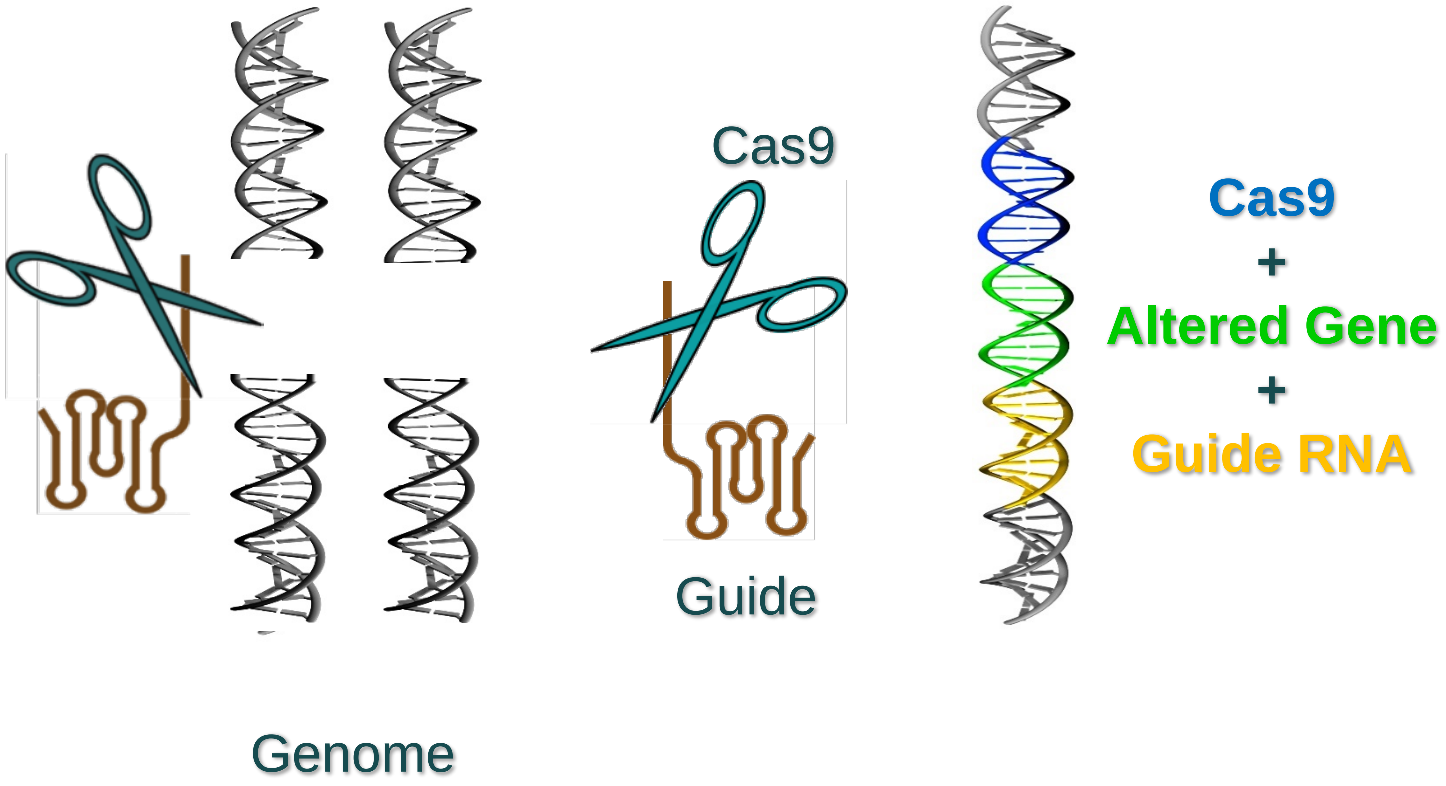

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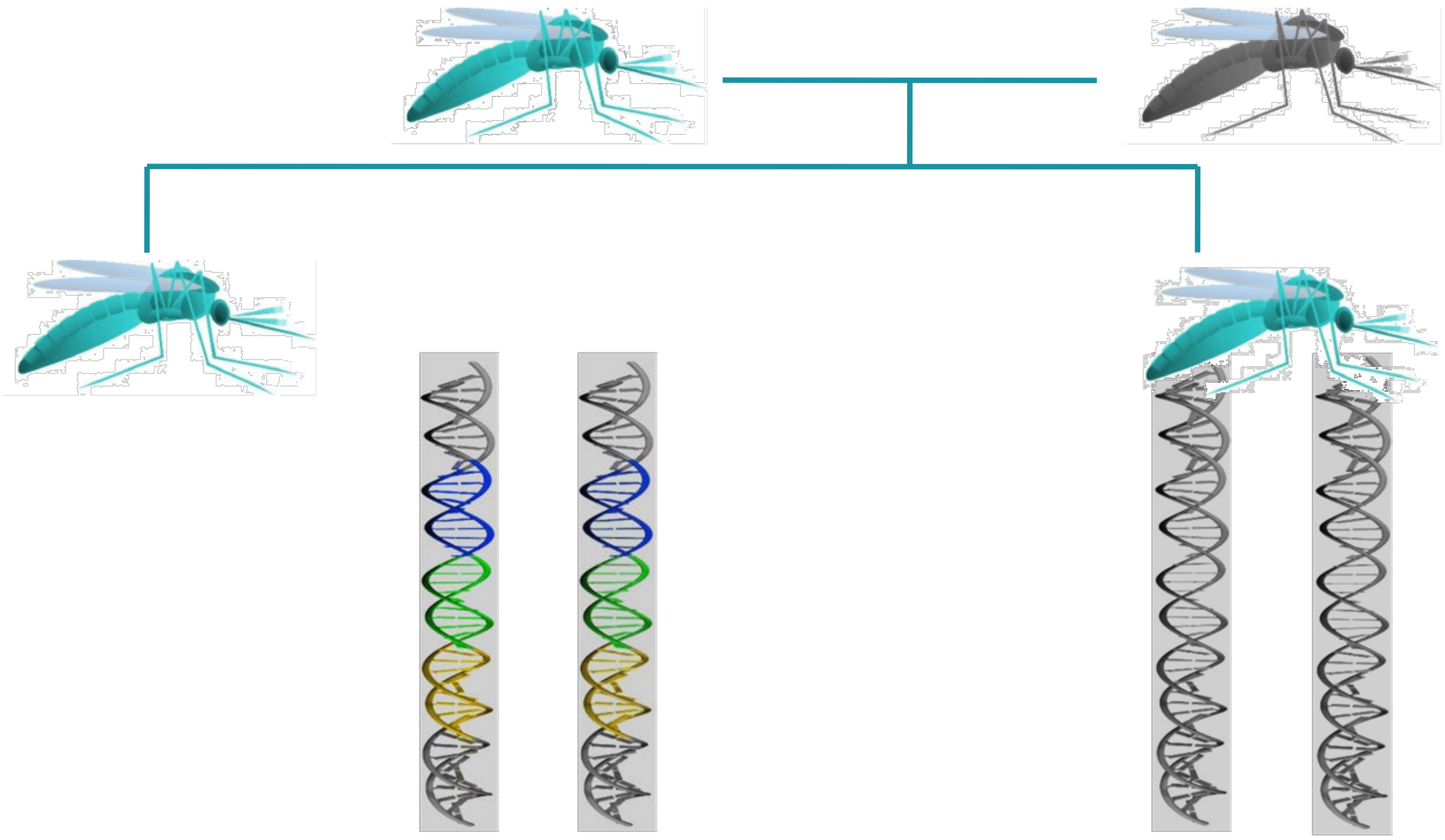

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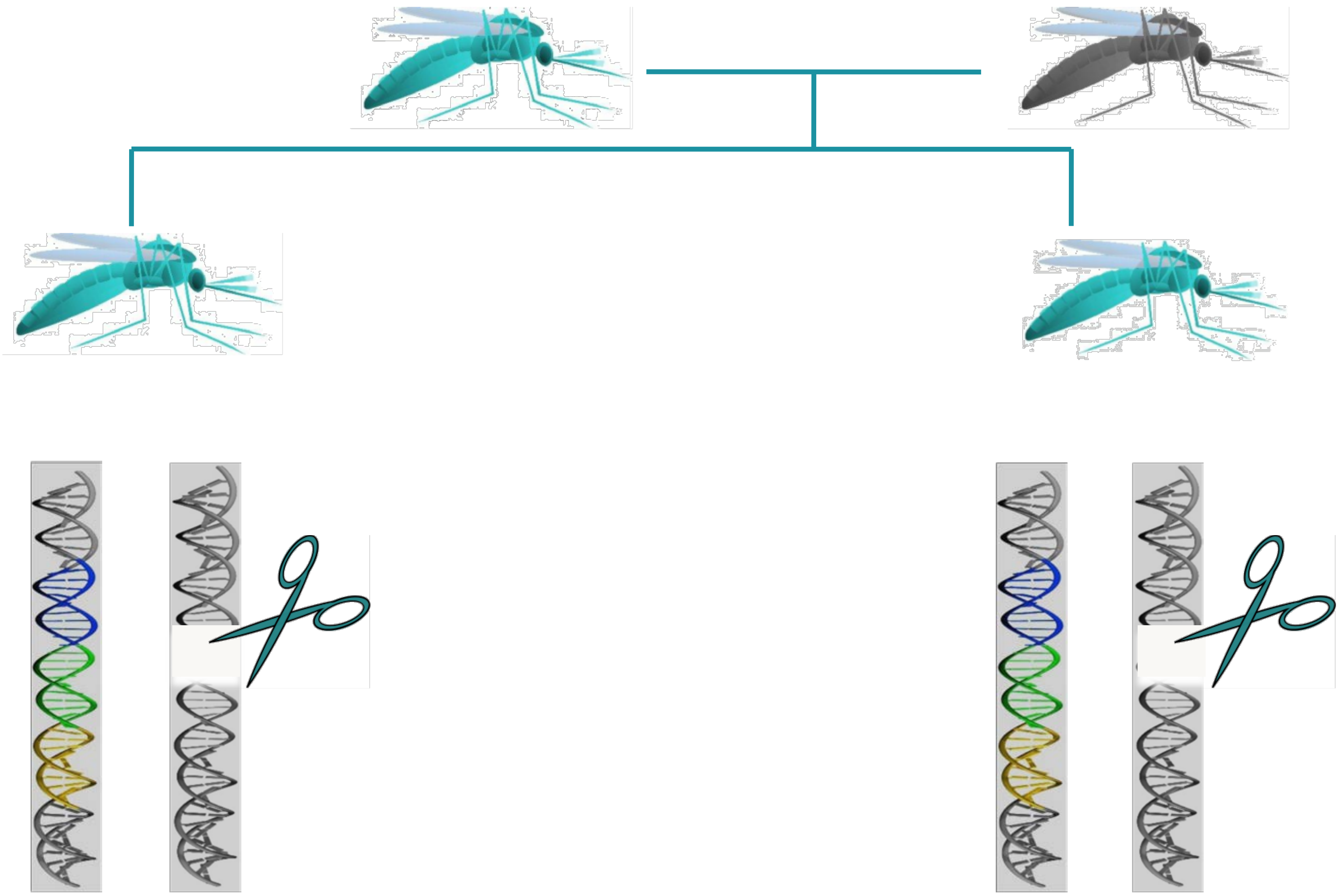

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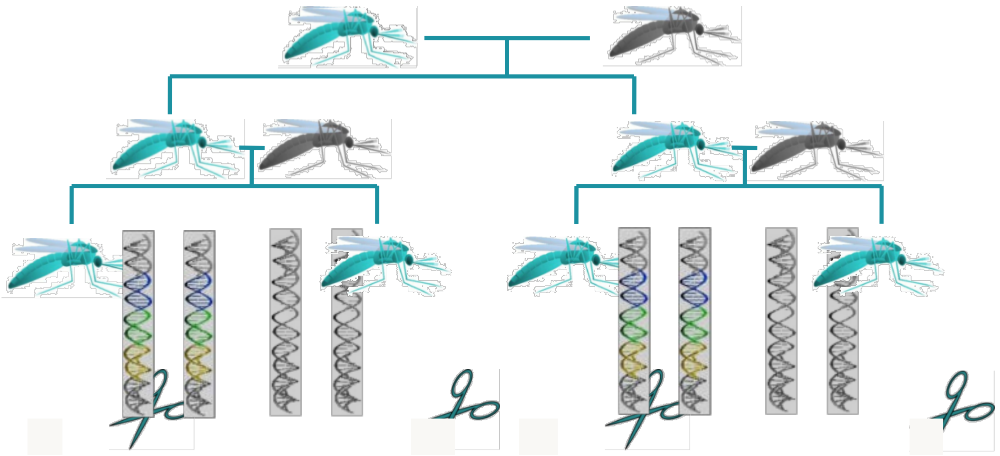

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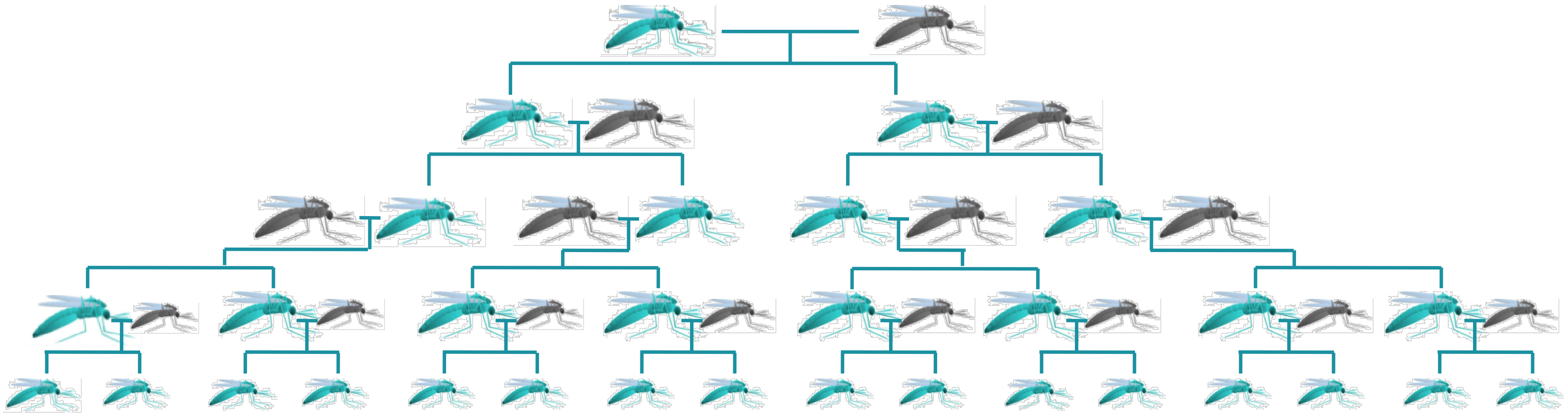


Cas9 + guide RNAs "drive" the altered gene through the population

Natural gene drives are present in the genomes of just about all organisms

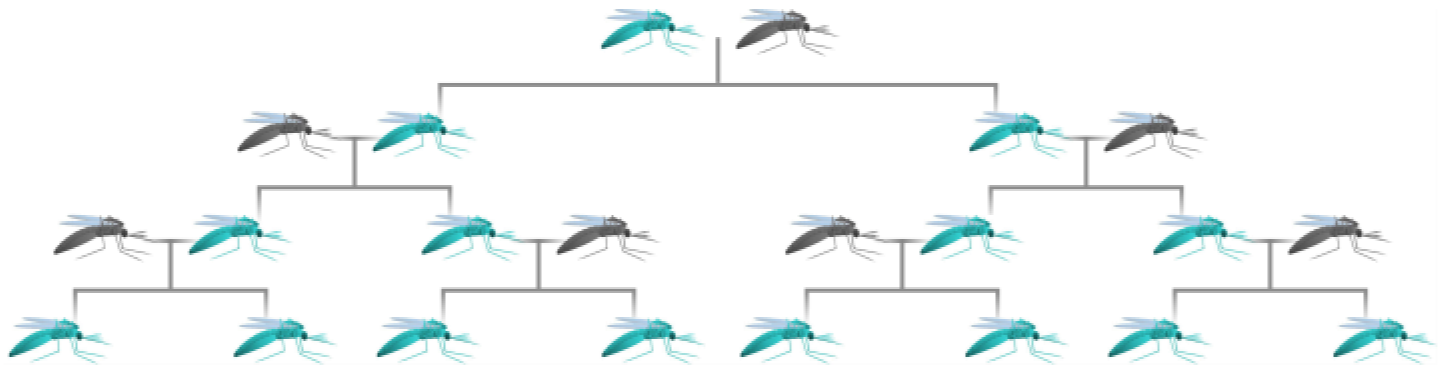
- many employ this exact DNA-cutting mechanism (e.g. Homing Nucleases)

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

Caveats

1) Bacteria, viruses, and other asexual organisms can't be affected

2) Fast-reproducing organisms only (Great for most invasives!)



Releasing 1 drive organism per 10,000 wild-type = 16 generations minimum

3) The difficulty of building gene drives will vary between species

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*
Oye K*, **Esvelt KM***, Appleton E, Catteruccia F, Church GM, Kuiken T, Lightfoot S, McNamara J, Smidler A, Collins JP (2014) *Science*

Outline

- What is CRISPR
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Can Gene Drives Work on Invasive Species?

Rhinella marina

(Australia)

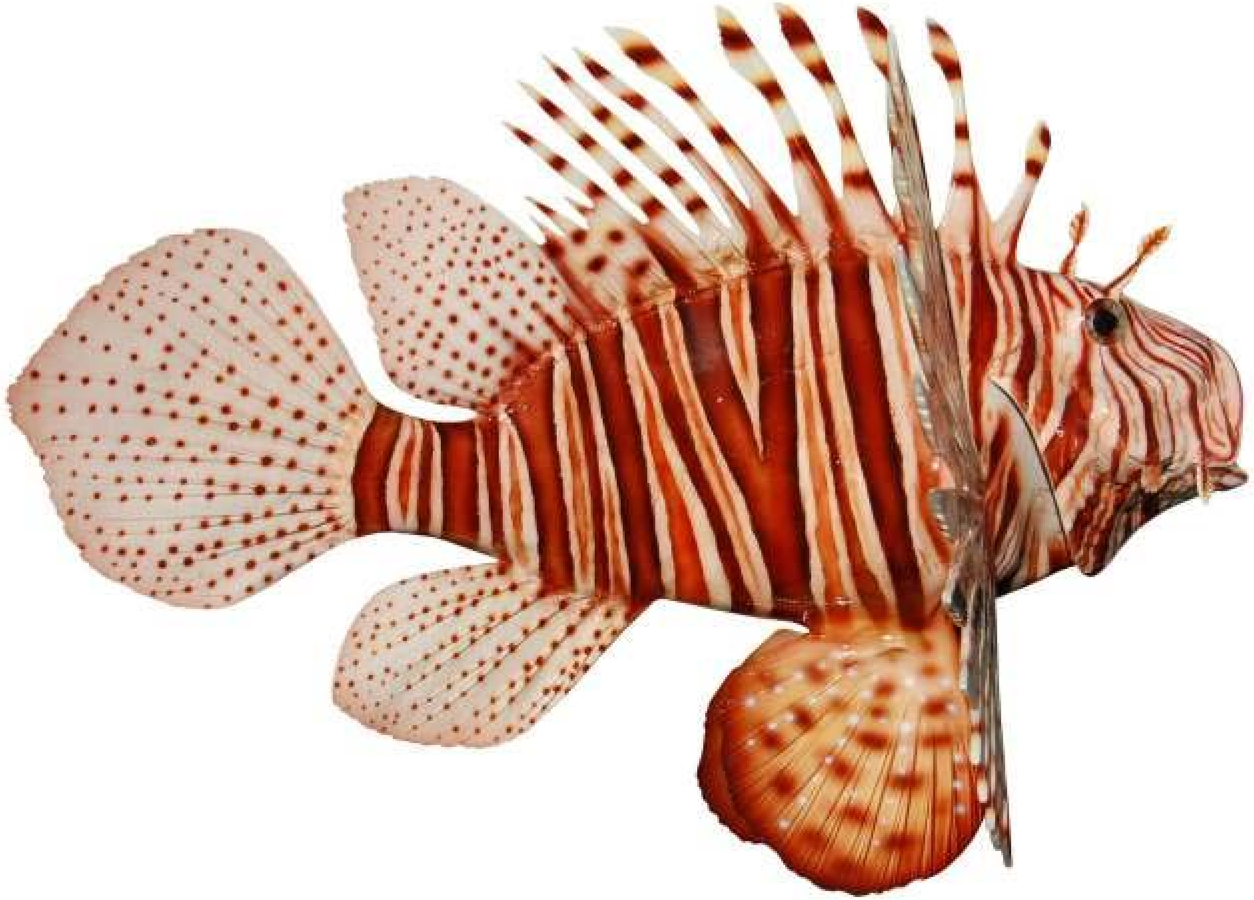
Rattus norvegicus

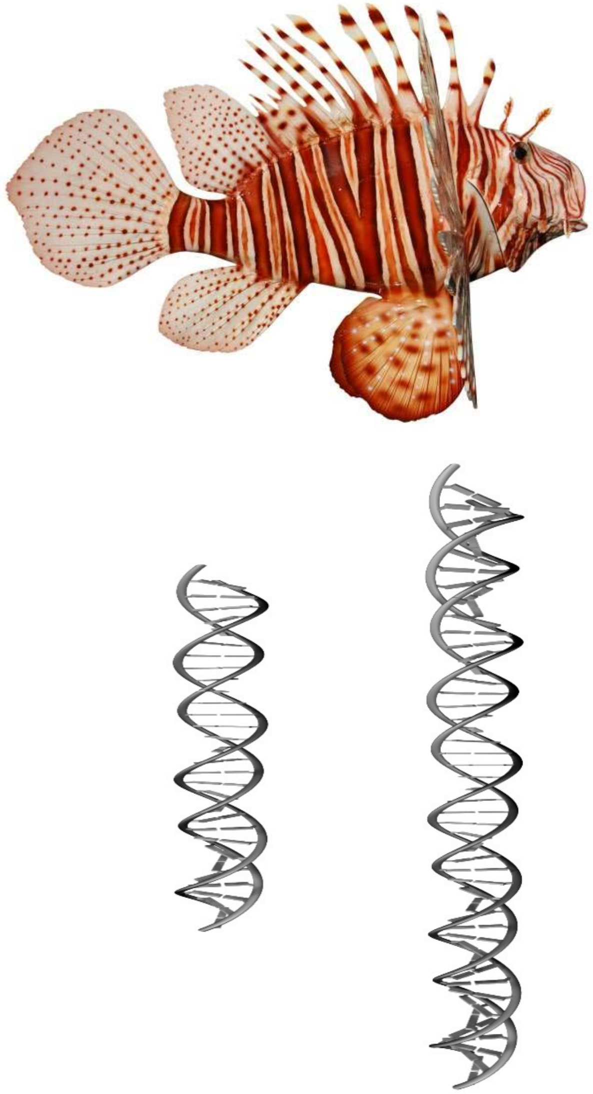
(Galapagos)

Aedes aegypti

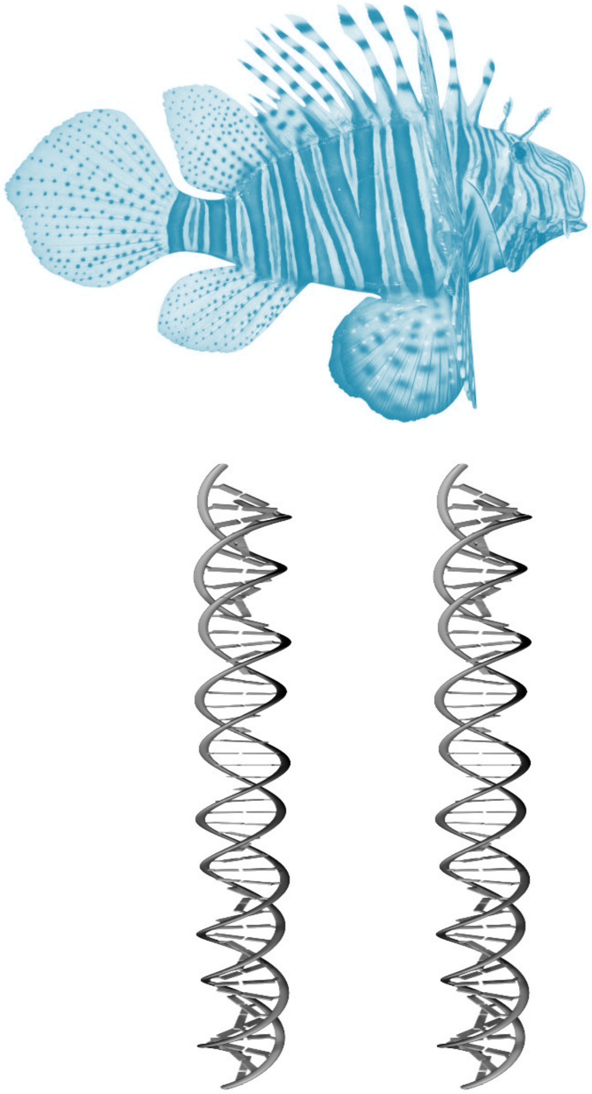
(Hawaii)

Gender-biasing Gene-Drive for *AIS*





Z / W Female

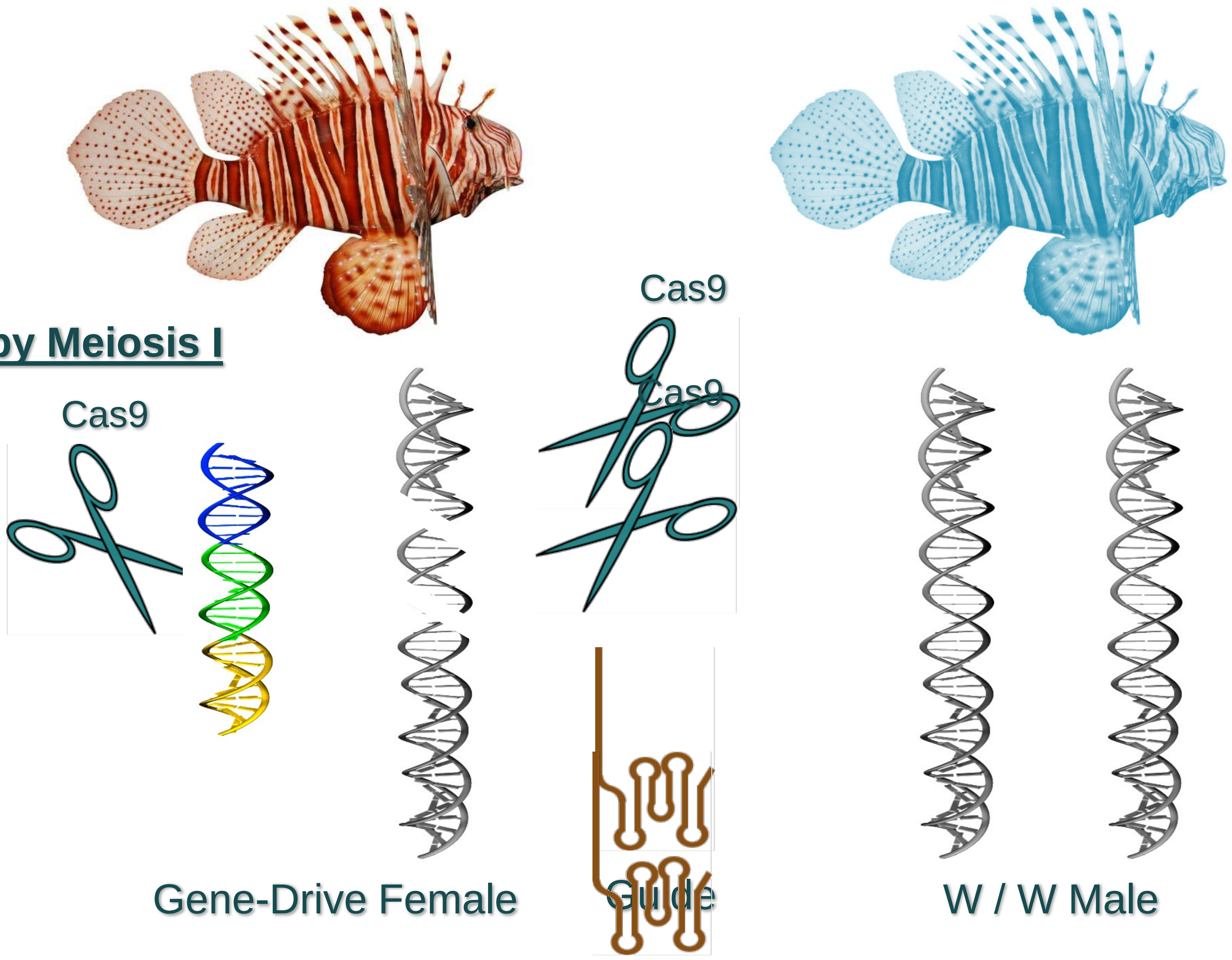


W / W Male

Burt, 2003; Schliekelman et al., 2005; Huang et al., 2007; Deredec et al., 2008; Marshall, 2009; Yahara et al., 2009; Deredec et al., 2011; Alphey and Bonsall, 2014

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

Activated by Meiosis I



Burt, 2003; Schliekelman et al., 2005; Huang et al., 2007; Deredec et al., 2008; Marshall, 2009; Yahara et al., 2009; Deredec et al., 2011; Alphey and Bonsall, 2014

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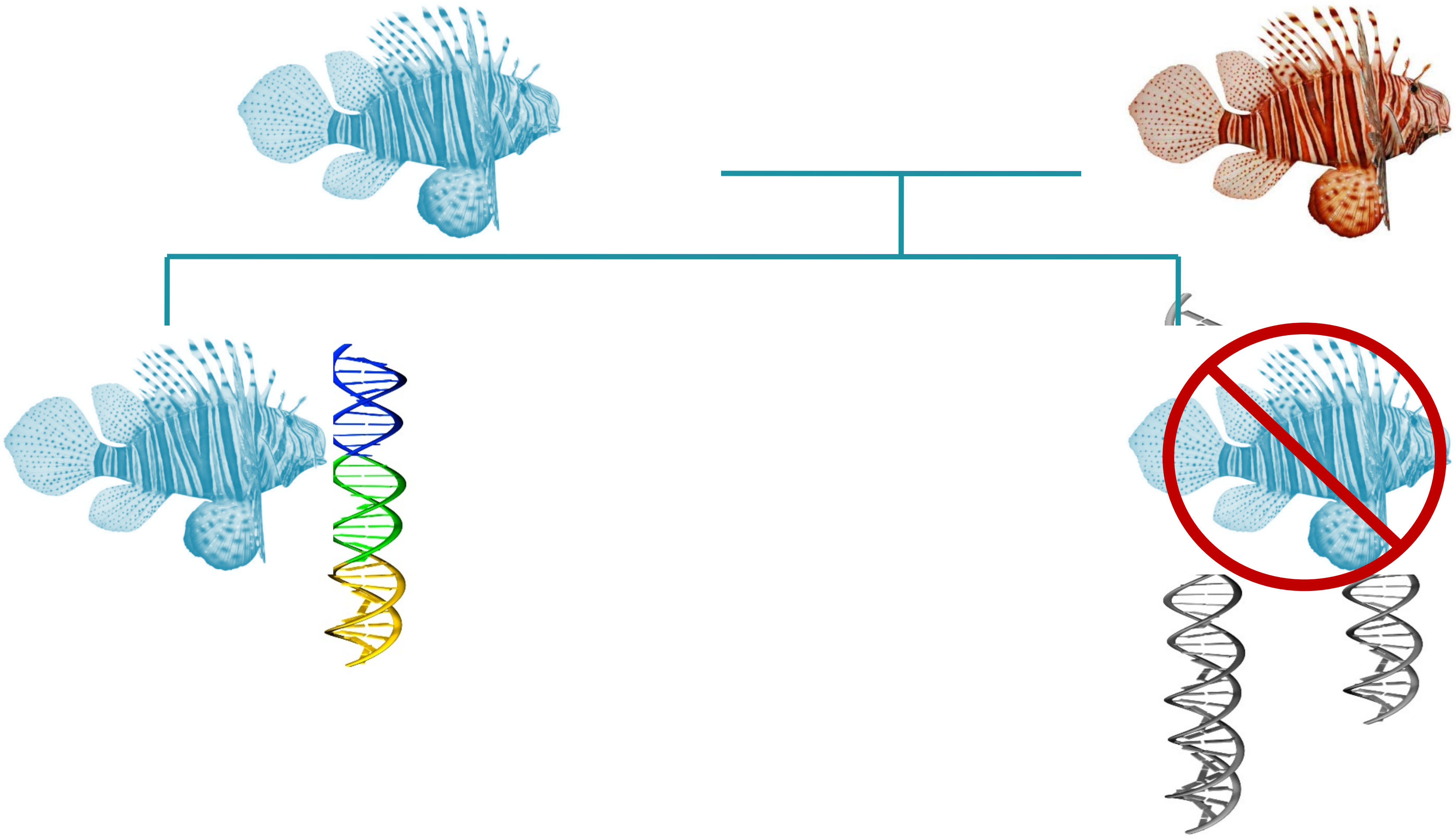


All CRISPR editing is only activated during male meiosis

Burt, 2003; Schliekelman et al., 2005; Huang et al., 2007; Deredec et al., 2008; Marshall, 2009; Yahara et al., 2009; Deredec et al., 2011; Alphey and Bonsall, 2014

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

Safety & Containment

- Whether a standard gene drive will **<u>spread</u>** through a target population depends on molecular factors such as homing efficiency, fitness cost, and evolutionary stability (Marshall&Hay *J. Theoretical Biology 2012*)

- **<u>Immunity</u>** is expected due to genetic diversity. Immunization can also be "engineered" by releasing organisms with mutations the guide-RNA target

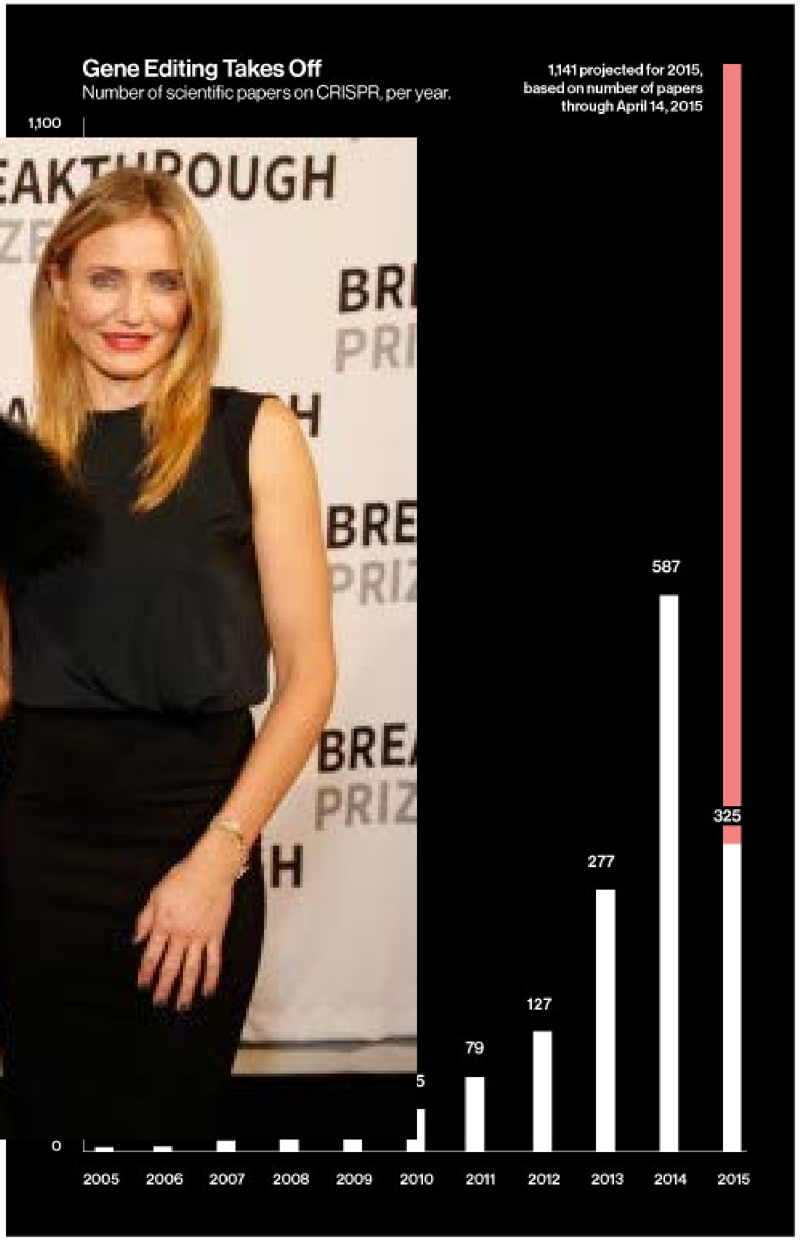
- CRISPR/Cas9 have demonstrated remarkable **<u>specificity</u>** to the extent that several companies are already investing $100M+ into human therapeutic applications

- **<u>Reversal Drives</u>** can be designed using similar principles, and should spread faster than the effector Gene Drive due to it having minimal fitness impact

- Longterm / **<u>multigenerational</u>** (50+ generations) effects and possibility for **<u>species cross-over</u>** are currently being investigated in nematode models (*Caenorhabditis*). I expect results within 1 year

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*
Oye K*, **Esvelt KM***, Appleton E, Catteruccia F, Church GM, Kuiken T, Lightfoot S, McNamara J, Smidler A, Collins JP (2014) *Science*

Cost: (cheaper every day)



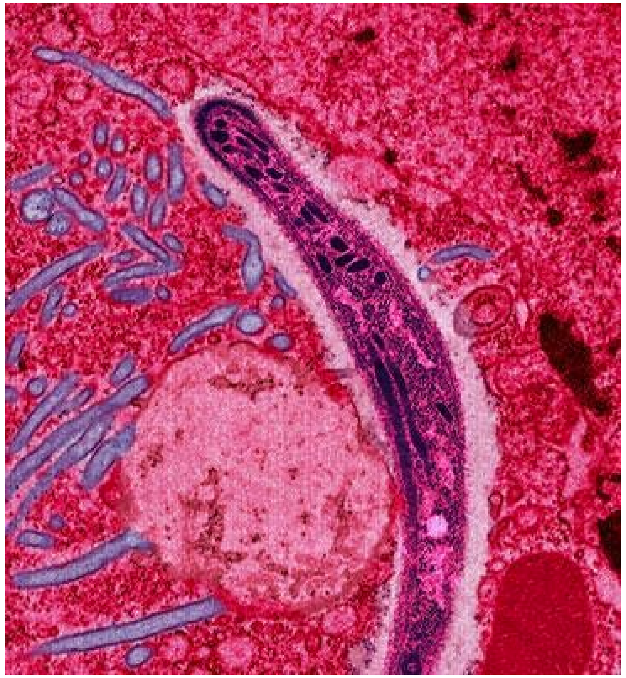
Source: National Library of Medicine

MIT Technology Review

NIT / MIT tech review

Public Image

Drug Production



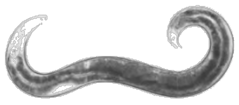
Infectious Diseases

Nutrition Supplement

Pest Control?

CRISPR Gene Drives

Improving our understanding



Candidate applications

<u>Wide-spread implementation</u>

Acknowledgments


Kevin Esvelt


George Church

Gene Drive Safeguards and Regulation

Kenneth Oye
James P. Collins
Todd Kuiken
Andrea Smidler
Shlomiya Lightfoot
Simon Bulllock
Kevin Cook
Fillip Port

CRISPR/Cas9 for Genome Editing and Regulation

Prashant Mali
Stephanie Yaung
Jonathan Braff
John Aach
John Calarco
Ari Friedland
Monica Colaiacovo

Gene Drive Experiments in Mosquitoes

Andrea Smidler
Flaminia Catteruccia

Gene Drive Experiments in Yeast

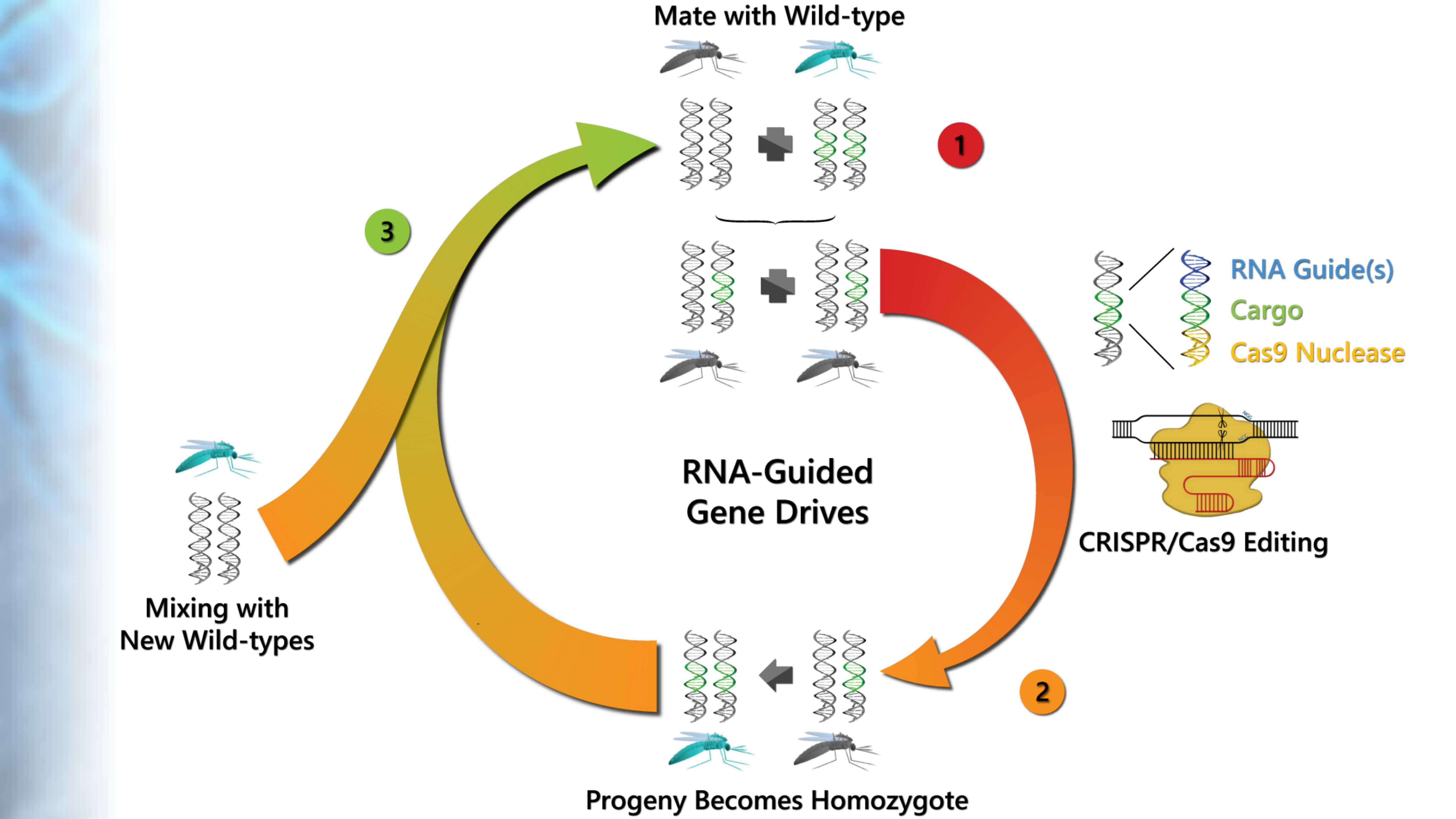
James DiCarlo
Alejandro Chavez
Sven Dietz

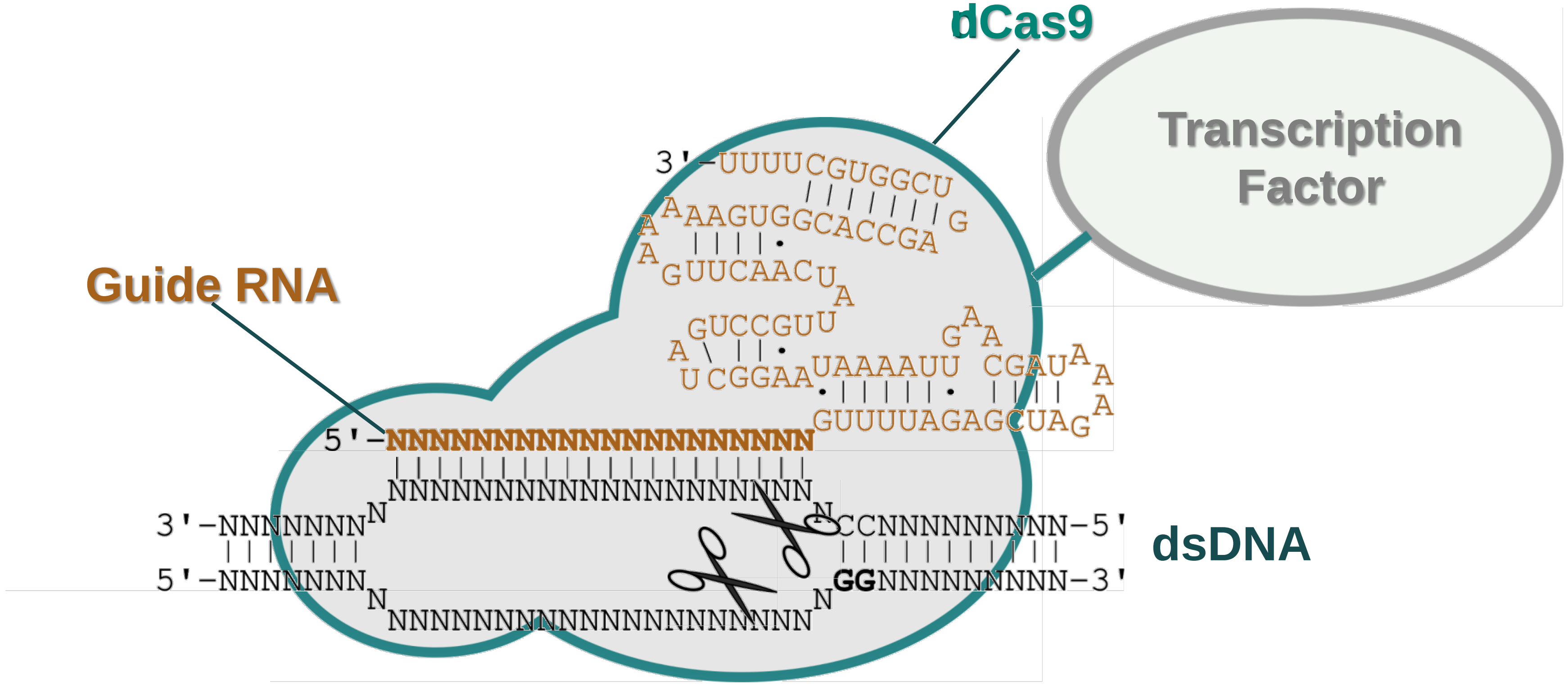
Gene Drive Modeling

Charleston Noble
Fred Gould
Martin Nowak

VE RI TAS
the Hertz Foundation freedom to innovate
NSF Graduate Research Fellowship Program
HHMI
National Institutes of Health
K99DK102669
Wyss Institute
Harvard Medical School

Developing Collective Technologies


Mate with Wild-type
1
RNA Guide(s)
Cargo
Cas9 Nuclease
3
RNA-Guided
Gene Drives
CRISPR/Cas9 Editing
Mixing with
New Wild-types
2
Progeny Becomes Homozygote



Knock out 1 nuclease domain = nickase for more precise genome editing

Knock out 2 nuclease domains = DNA-binding protein for gene regulation

Mali P*, Aach J*, Stranges PB, **Esvelt KM**, Moosburner M, Kosuri S, Yang L, Church GM (2013) *Nature Biotechnology*
Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA (2013) *Cell*
Mali P*, Aach J*, Stranges PB, Esvelt KM, Moosburner M, Kosuri S, Yang L, Church GM (2013) *Nature Biotechnology*

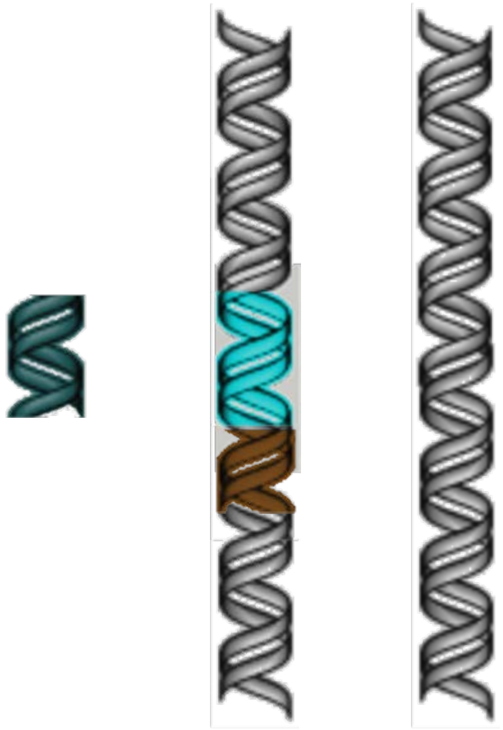
Safeguards for Self-Propagating Biologics

Gene drives must *never* accidentally escape the laboratory!

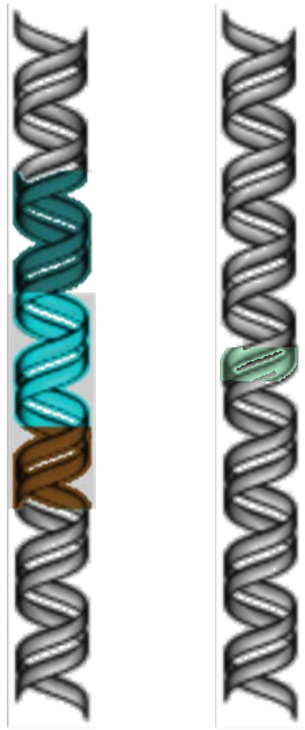
Confinement measures must be *proof against human error*

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*
Oye K*, **Esvelt KM***, Appleton E, Catteruccia F, Church GM, Kuiken T, Lightfoot S, McNamara J, Smidler A, Collins JP. (2014) *Science*

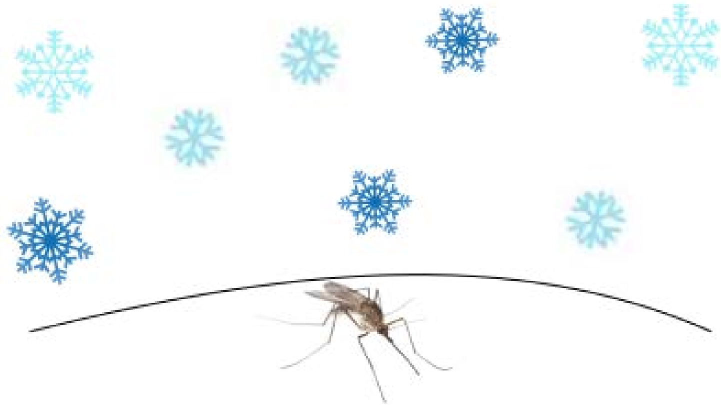
Separate *cas9* from guide RNAs



Target a synthetic site

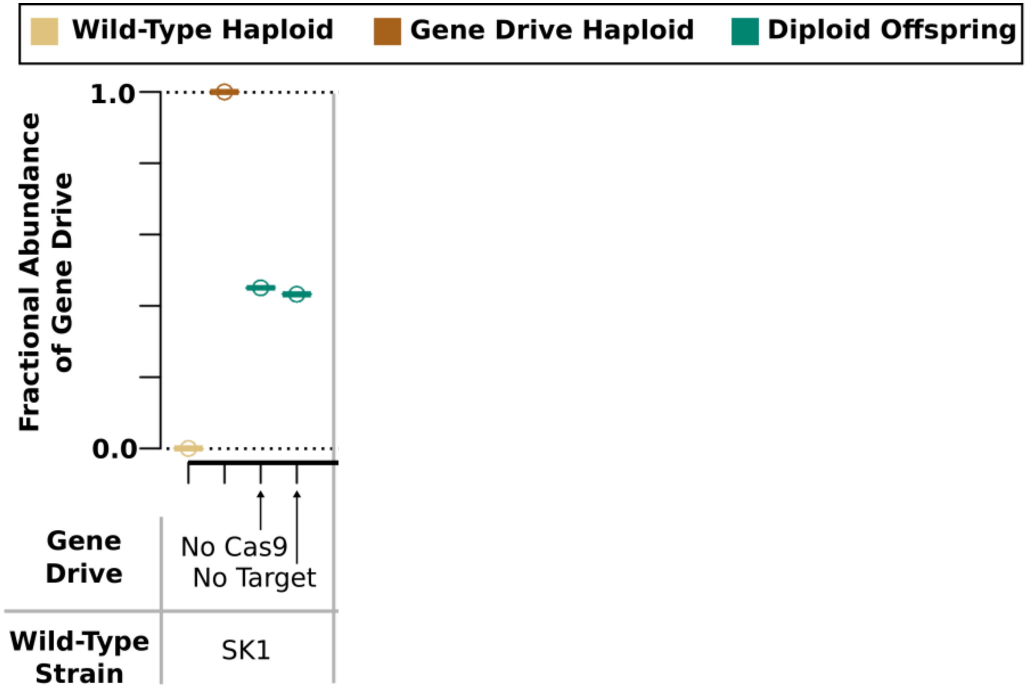


Build gene drives in regions where the organisms can't survive



Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

Testing RNA-Guided Gene Drive and Confinement in Yeast



⟵ Diverse yeast strains from around the world ⟶

Efficiency of copying is **>99.5%** for all drives over successive generations

That is *at least as good* as natural homing endonuclease gene drives (e.g. I-SceI)

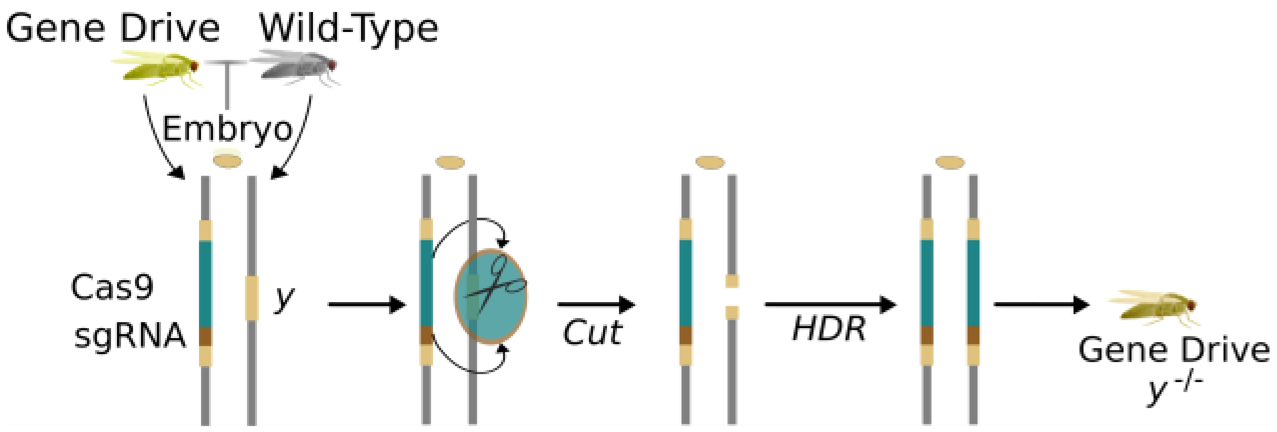
James DiCarlo

Alex Chavez

Sven Dietz

DiCarlo J*, Chavez A*, Dietz S, **Esvelt KM°**, Church GM° (2015) *bioRxiv* preprint, *in revision*

RNA-Guided Gene Drive in Fruit Flies (Bier lab)



Efficiency of copying: 97%

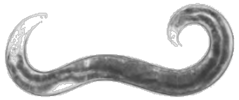
Gantz VM, Bier E (2015) *Science* aaa5945

These results strongly suggest that Cas9 can drive <u>most alterations</u> through wild populations both <u>reliably and *reversibly*</u>

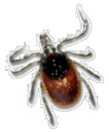
Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*
Oye K*, **Esvelt KM***, Appleton E, Catteruccia F, Church GM, Kuiken T, Lightfoot S, McNamara J, Smidler A, Collins JP. (2014) *Science*

Gene Drives

Improving our understanding



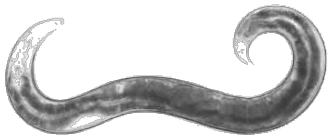
Candidate applications



Wide-spread implementation

Model Organisms

Safely study gene drives in large populations over many generations



Caenorhabditis

- fitness
- stability
- evolvability
- spread into related species

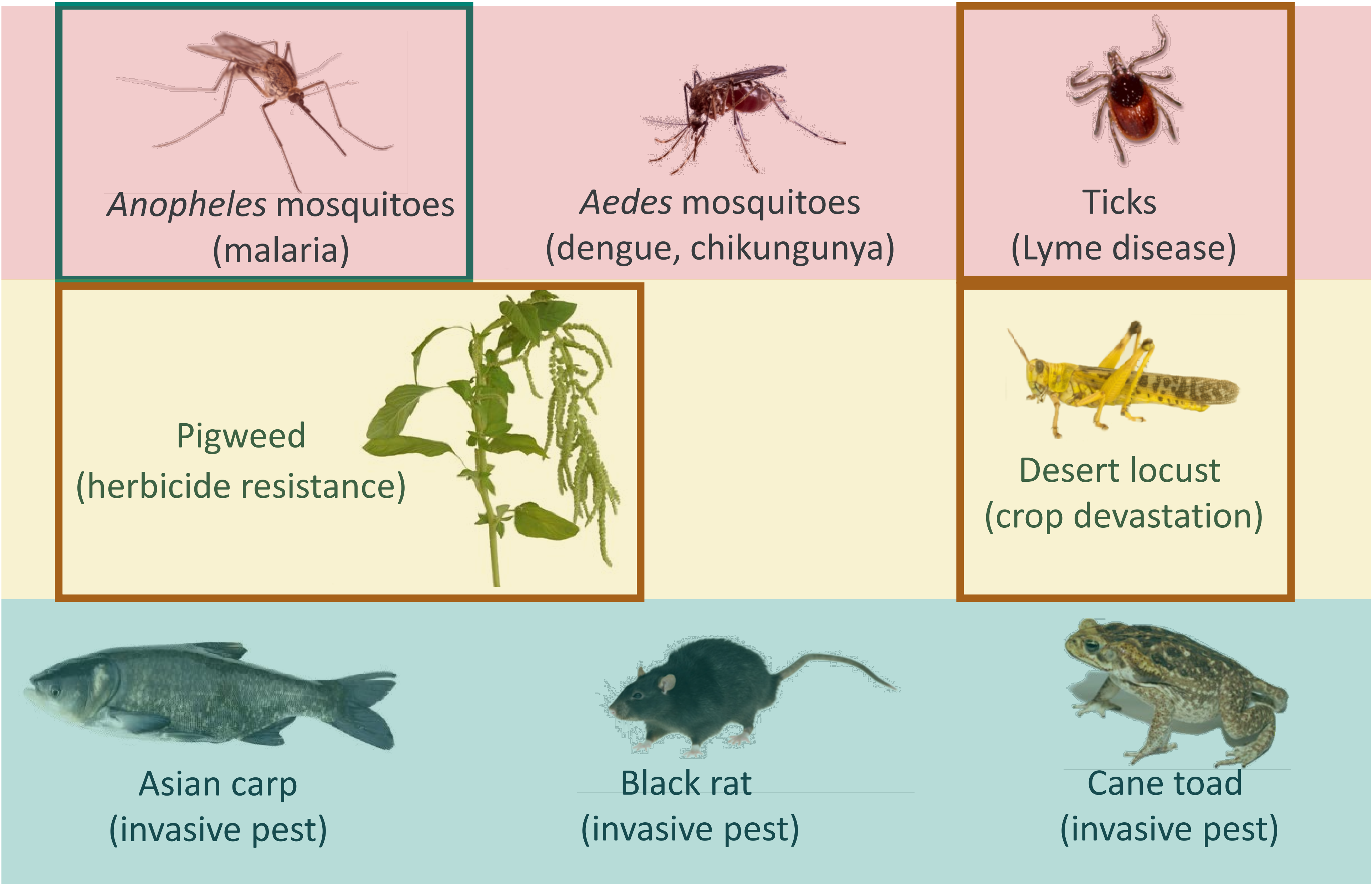
Develop drive architectures:

immunizing reversal	Undo button: overwrites earlier change
genetic load / sex biasing	Suppress or eliminate target population
sensitizing	Make populations vulnerable to an otherwise harmless small molecule -> now a "pesticide"
constrained	Control extent of gene drive spread

Burt A (2003) *Proc. R. Soc. Lond. B*

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

Candidate Applications in Target Organisms



Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

Oye K*, **Esvelt KM***, Appleton E, Catteruccia F, Church GM, Kuiken T, Lightfoot S, McNamara J, Smidler A, Collins JP. (2014) *Science*

Developing Collective Technologies

Safeguards

Transparency

Community

Responsiveness

Goats in the Galapagos

- We have introduced rats, pigs, and even goats to the Galapagos

- The goats were destroying local habitats and wild life on the Galapagos

What we did about it

“Marines make explosive impact in Afghanistan” by Marines / CC BY

- Goat population peaked at ~80,000
- The problem was solved in 2006 by ex-military exterminators
- There are still rats on the Galapagos

Mosquitos around the world

- We brought mosquitos to Hawaii

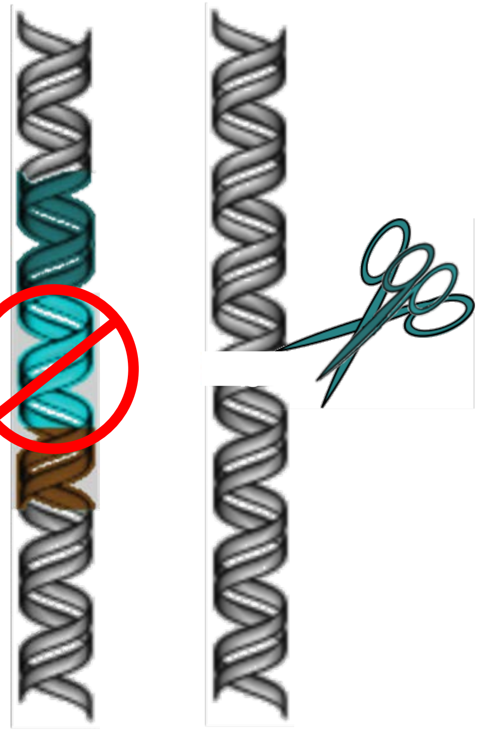
- Associated diseases have driven over 1/3 of of Hawaiian honeycreeper to extinction

We tried to fix the mosquito problem

- "Sterile insect release" technique, pioneered by Bushland and Knipling in 1950s

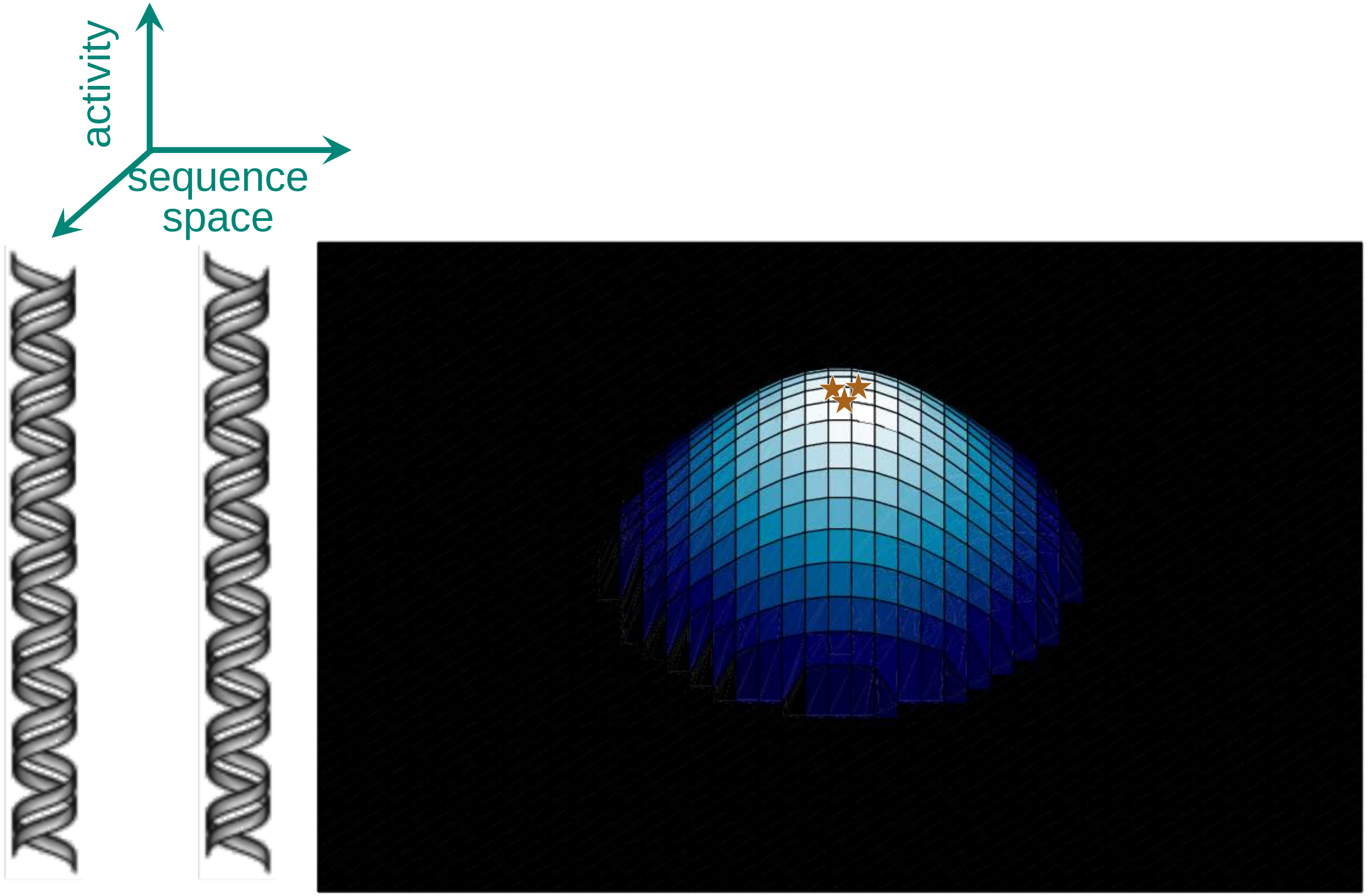
- Limited successes, require repeated large-scale release on islands

Endonuclease gene drives will sometimes create “drive-resistant” mutations

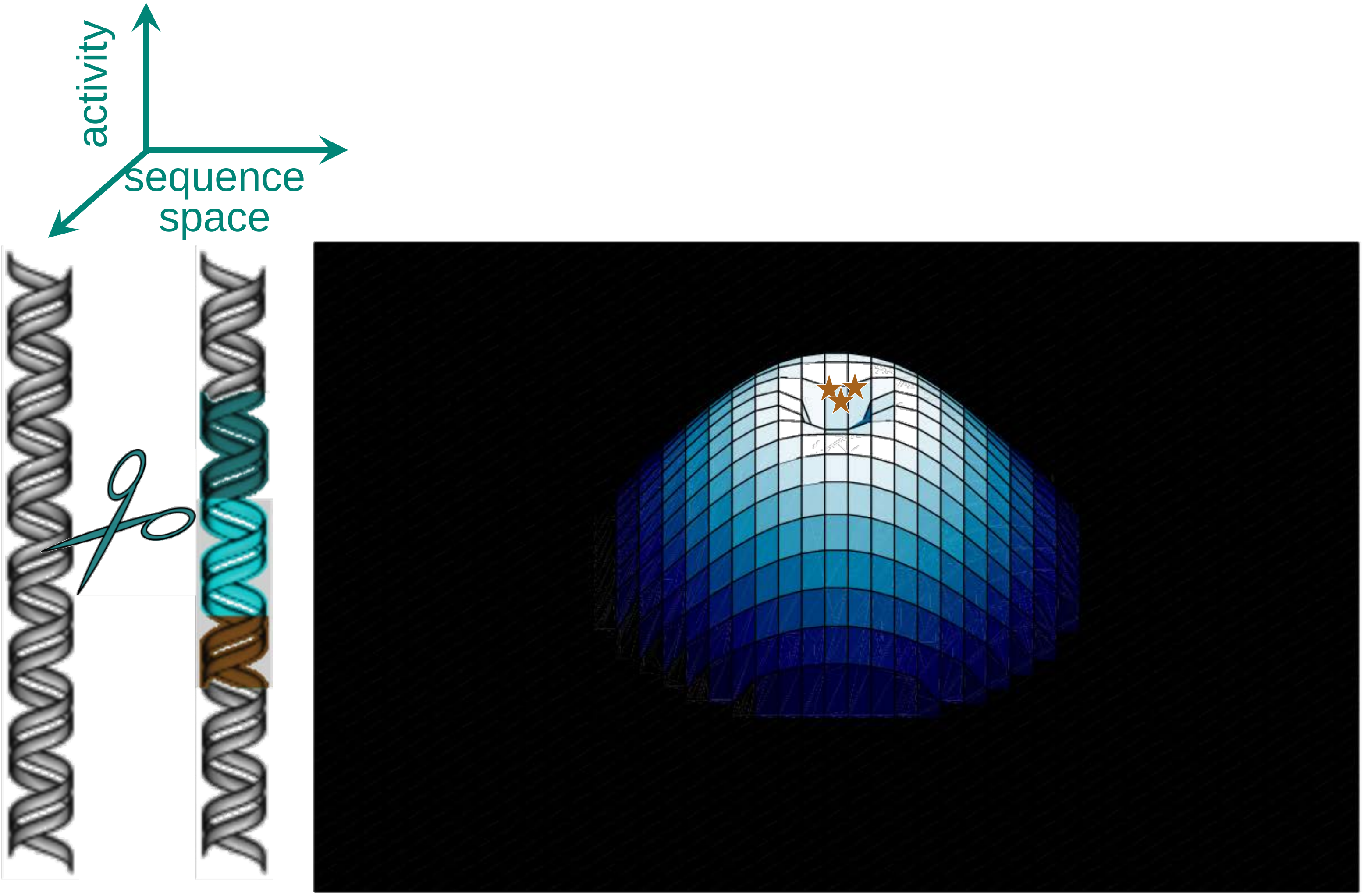


How can we build evolutionarily stable gene drives?

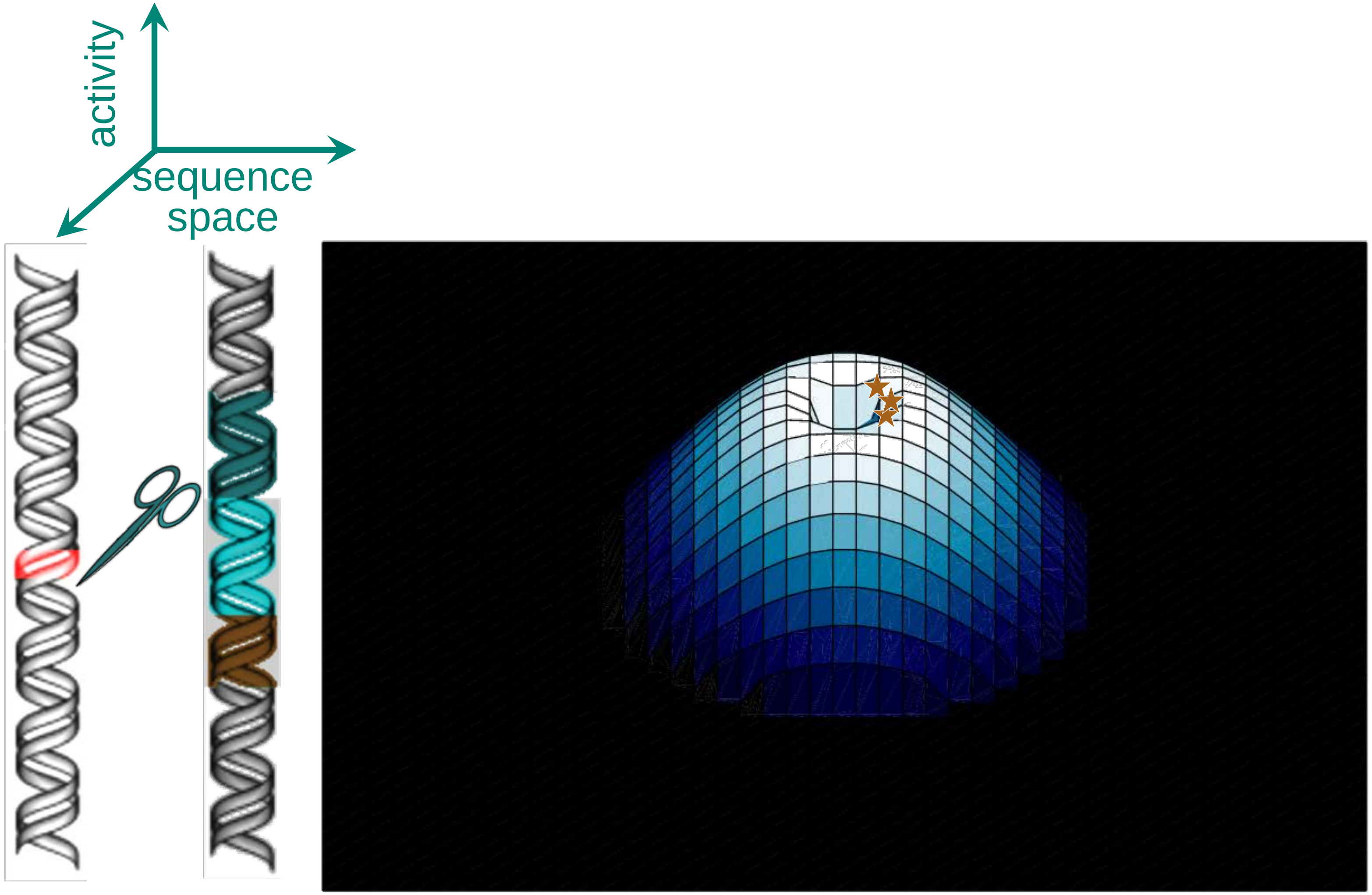
Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*



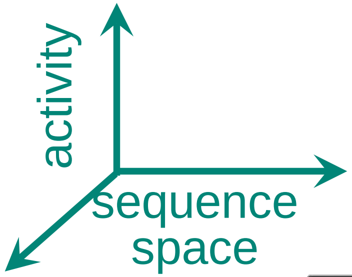
Fitness landscape of the target gene without the drive

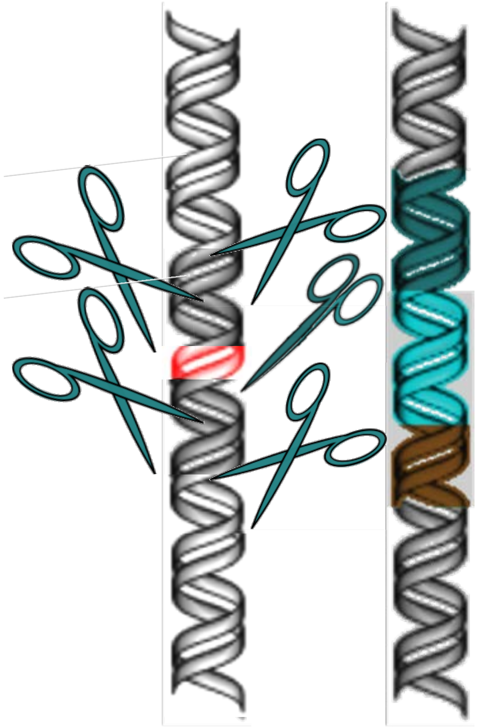


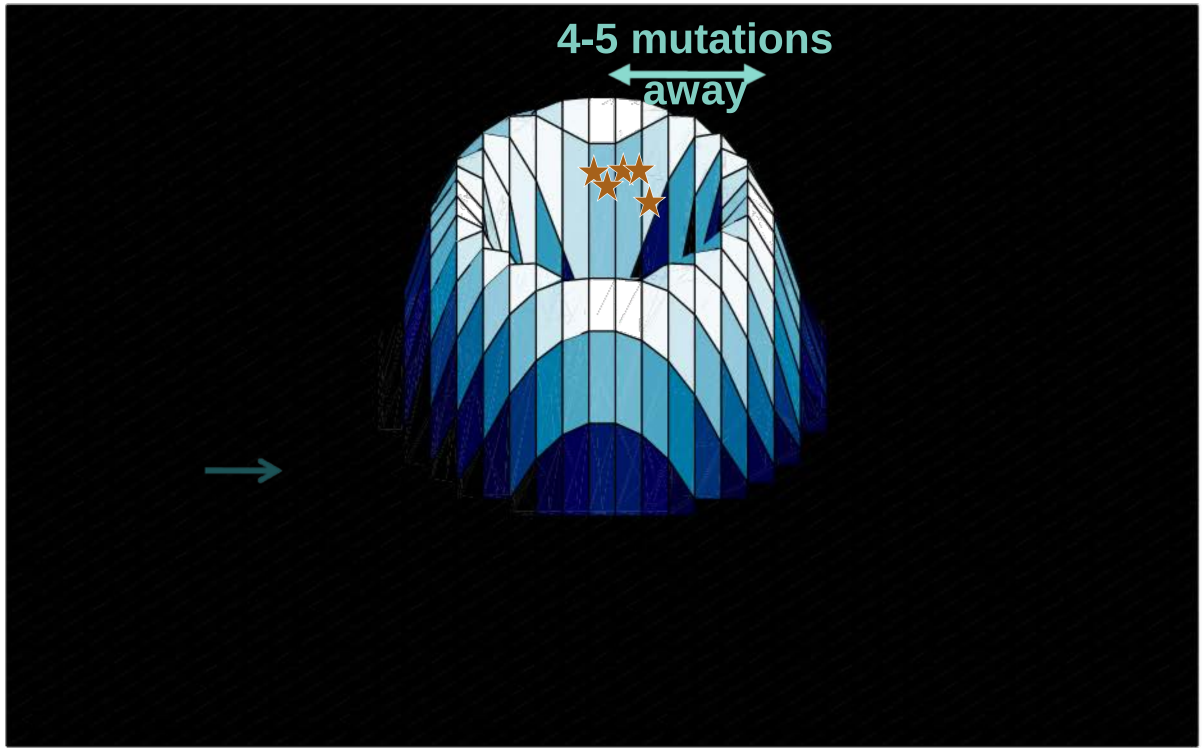
Fitness landscape of the target gene with the drive



Fitness landscape of the target gene with the drive



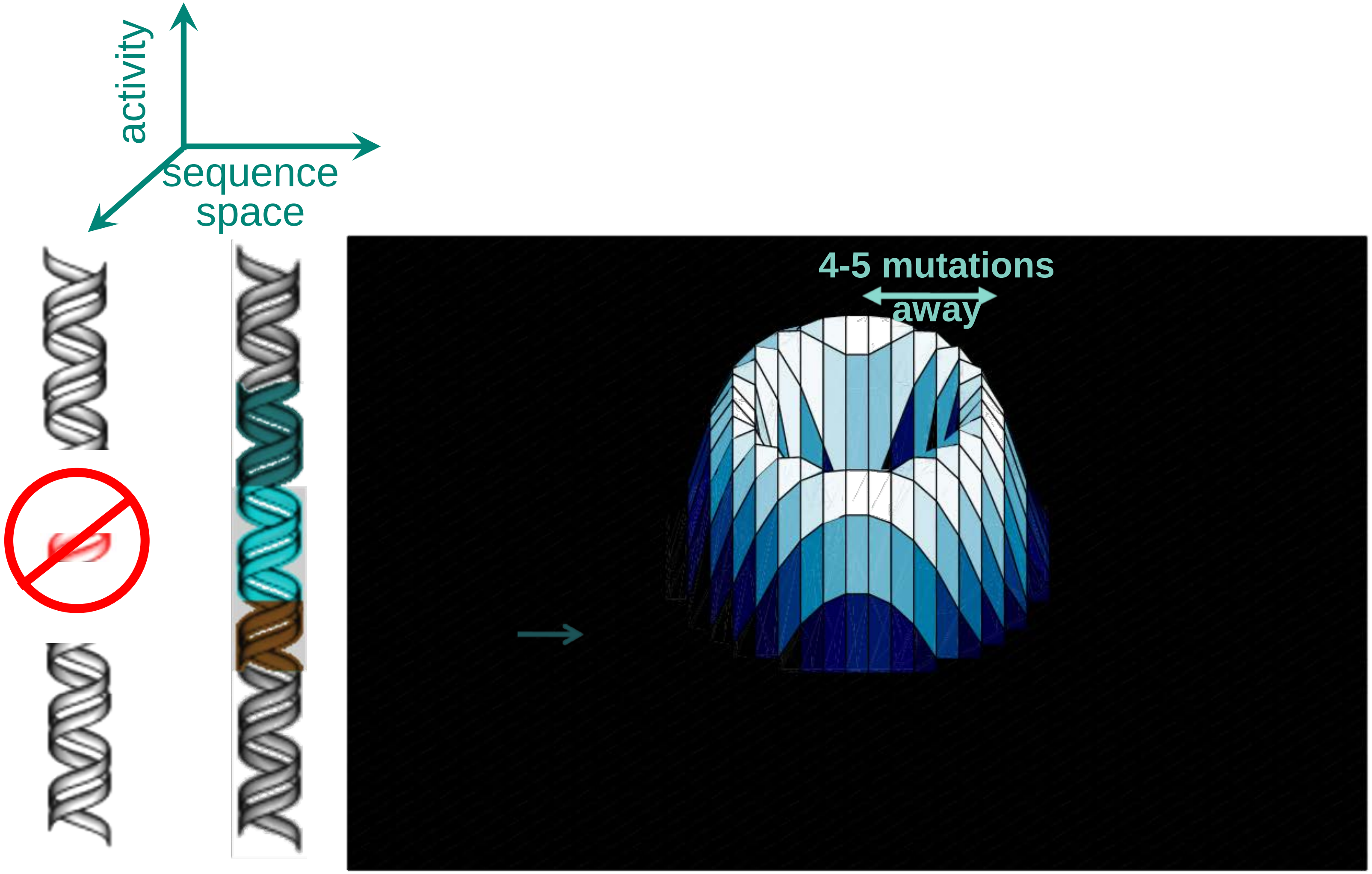




We can build evolutionarily stable gene drives by targeting *multiple sites…*

… within genes that are *important for fitness*

Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*



We can build evolutionarily stable gene drives by targeting *multiple sites…*

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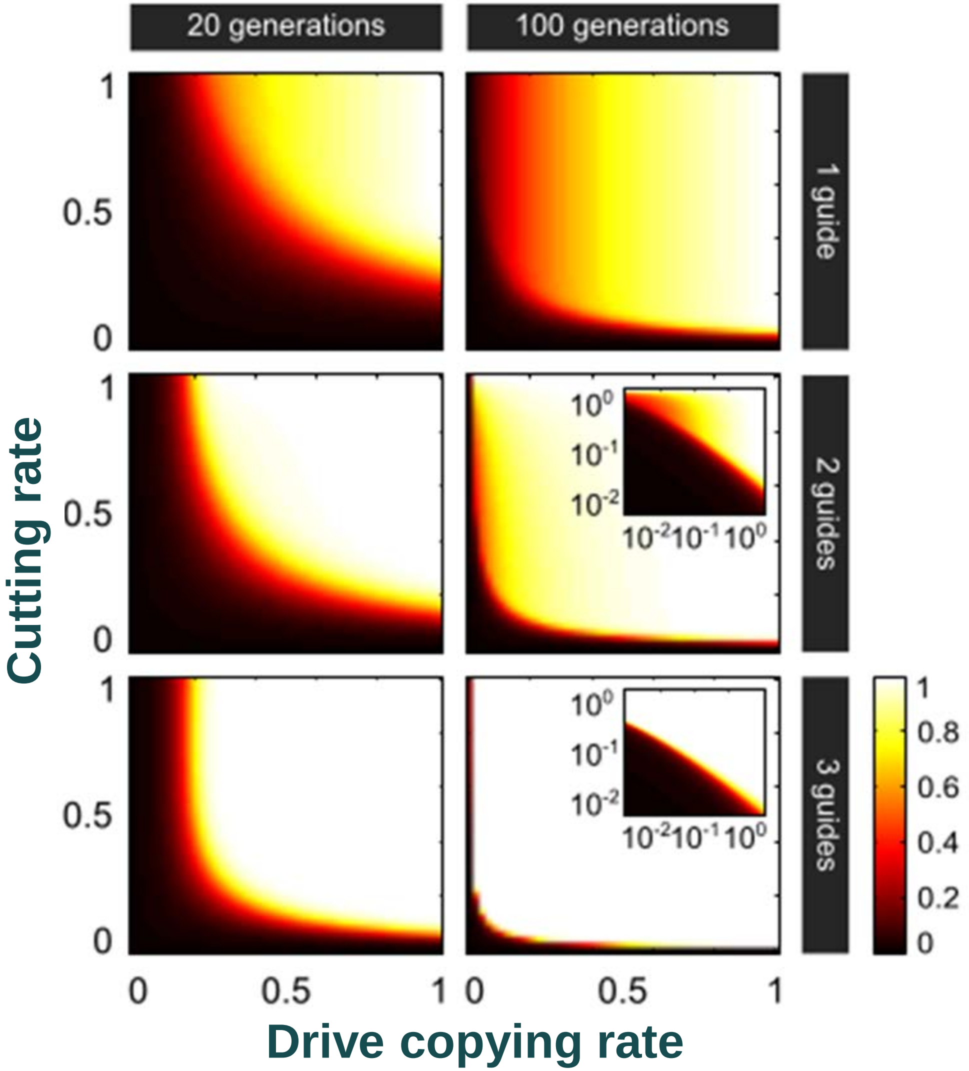
Esvelt KM, Smidler AL, Catteruccia F, Church GM (2014) *eLife*

Modeling Multiplex Cutting and Gene Drive Spread



Charleston Noble

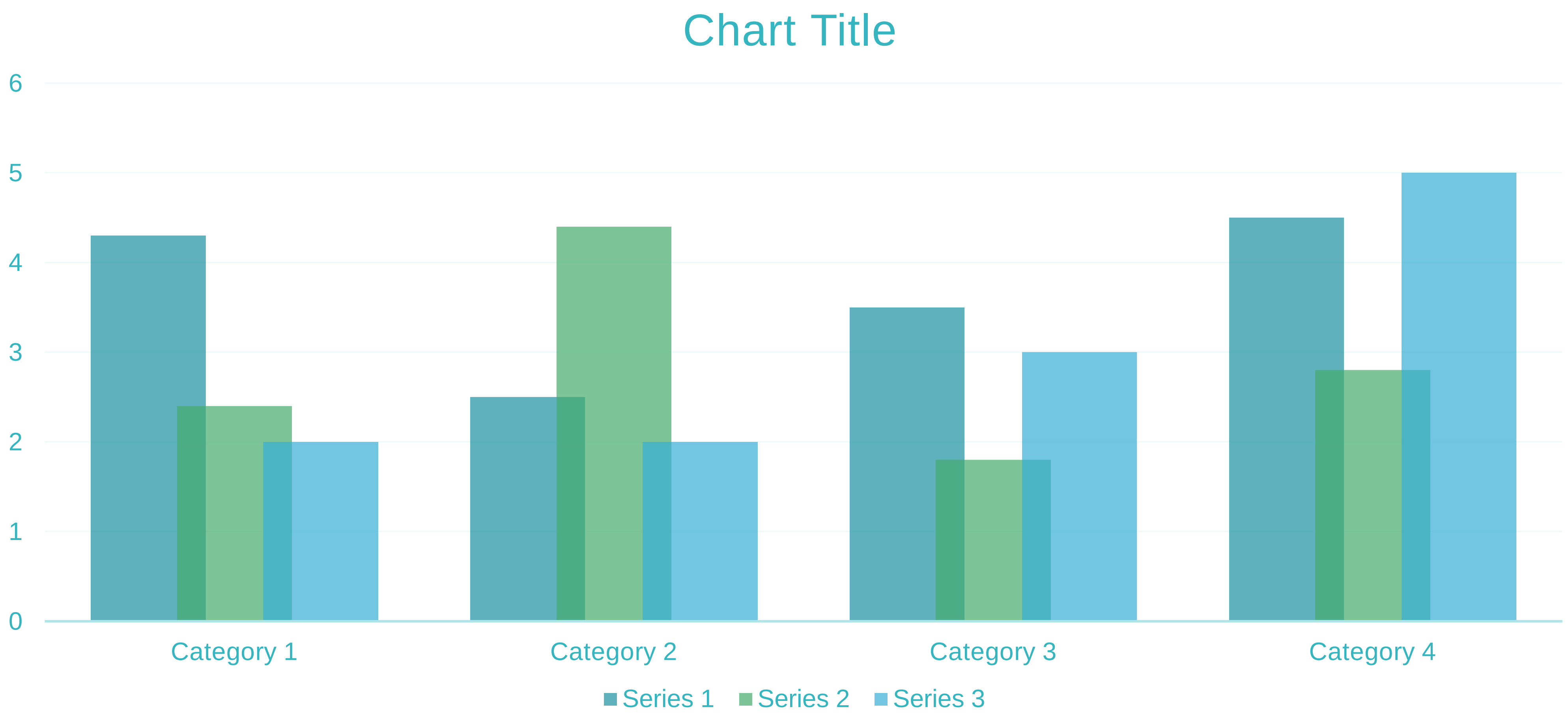
Targeting multiple sites could also enable gene drive even in species with low copying rates

Noble C, **Esvelt KM**, Nowak M, Church GM, *in preparation*

Title and Content Layout with List

- Add your first bullet point here
- Add your second bullet point here
- Add your third bullet point here

Title and Content Layout with Chart



Two Content Layout with Table

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- Second bullet point here
- Third bullet point here

	Group A	Group B
Class 1	82	95
Class 2	76	88
Class 3	84	90

Two Content Layout with Table

- First bullet point here
- Second bullet point here
- Third bullet point here

Group A	Group B
Task 1	Task 1
Task 2	Task 2
Task 3	Task 3
	Task 4