Genetic Pest Management Goals:

1. **Reduce Plant or Animal or Human Disease**
2. **Combat Invasive Species**

Genetic Pest Management Techniques:

1. **Sterile Insect Technique**
2. **Wolbachia**
3. **Genetic engineering**

From World Health Organization *(https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases)*

"... Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700 000 deaths annually. They can be caused by either parasites, bacteria or viruses. ...

Malaria is a parasitic infection transmitted by Anopheline mosquitoes. It causes an estimated 219 million cases globally, and results in more than 400,000 deaths every year. Most of the deaths occur in children under the age of 5 years. ...

Dengue is the most prevalent viral infection transmitted by Aedes mosquitoes. More than 3.9 billion people in over 129 countries are at risk of contracting dengue, with an estimated 96 million symptomatic cases and an estimated 40,000 deaths every year. ..."

Other viral diseases transmitted by [mosquitos] include chikungunya fever, Zika virus fever, yellow fever, West Nile fever, Japanese encephalitis ..."

Genetic Pest Management

Fred Gould – NCSU Entomology Professor / Genetic Pest Management Expert
(kindly helped with much of today's material)

Genetic Engineering and Society (GES) Center at NCSU

(see https://research.ncsu.edu/ges/)

GES CENTER

Integrating scientific knowledge and diverse public values in shaping the futures of biotechnology

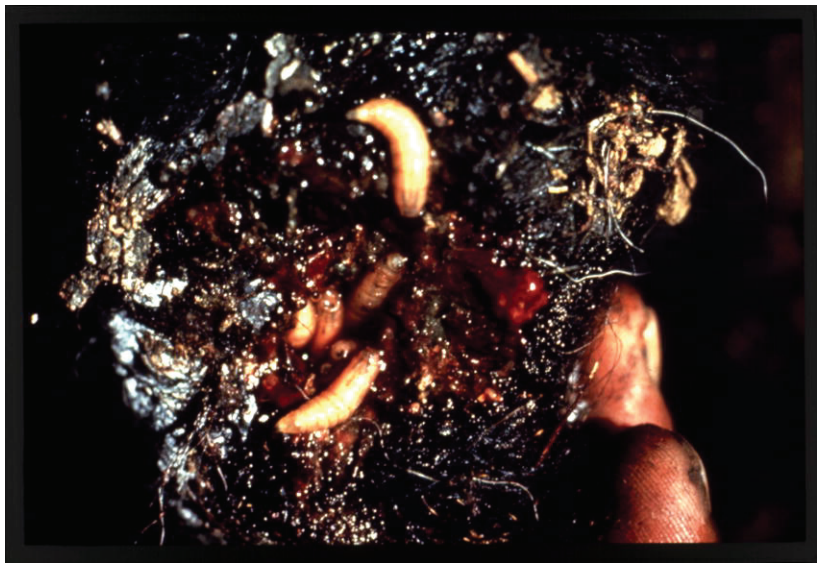
from http://www.nal.usda.gov/speccoll/collect/screwworm/chapters/01/001.htm

A cow infested with screwworm ...

New World Screwworm (Cochliomyia hominivorax)

- fly native to the Americas that lays eggs near wounds of mammals
- females mate only once

"In 1935, screwworms resulted in 180,000 livestock deaths in under half the counties in Texas, in spite of the man-power and constant effort invested in keeping the insects at bay. Endemic to the Southwest, screwworms spread to the Southeast when producers unknowingly transported infested livestock there in 1933. ..."



"USDA estimates that the U.S. livestock industry benefits by more than $900 million a year as a result of the eradication of the screwworm."

"Mexican producers and consumers saved about $2 billion from the beginning of eradication to 1991"

--photos and quotes from http://www.nal.usda.gov/speccoll/collect/screwworm/

Screwworm Eradication -

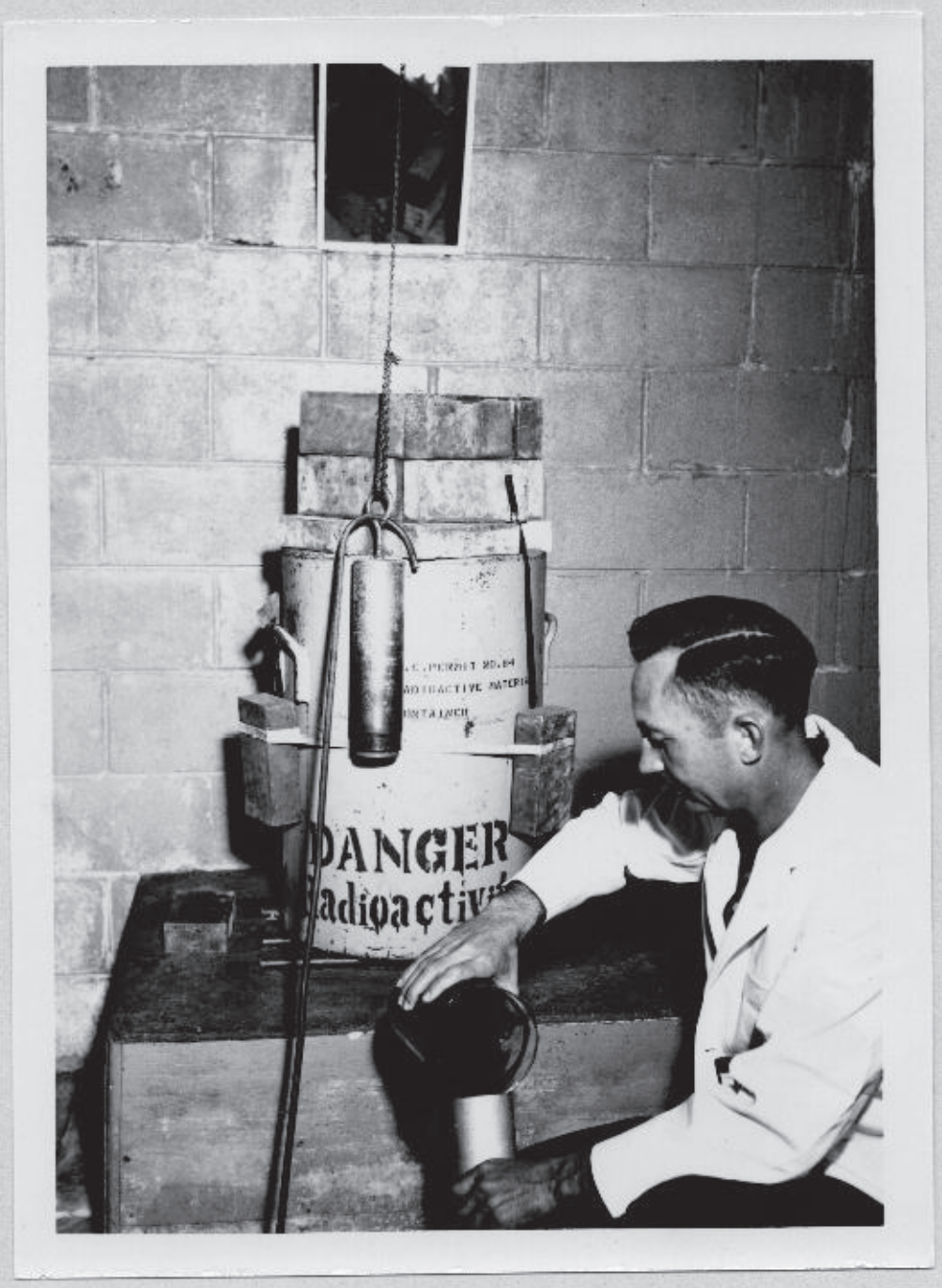
(1) Raise Large Number of males

(2) Irradiate Males

(3) Release MANY Irradiated males and attract females with "bait" (wounded animal?)

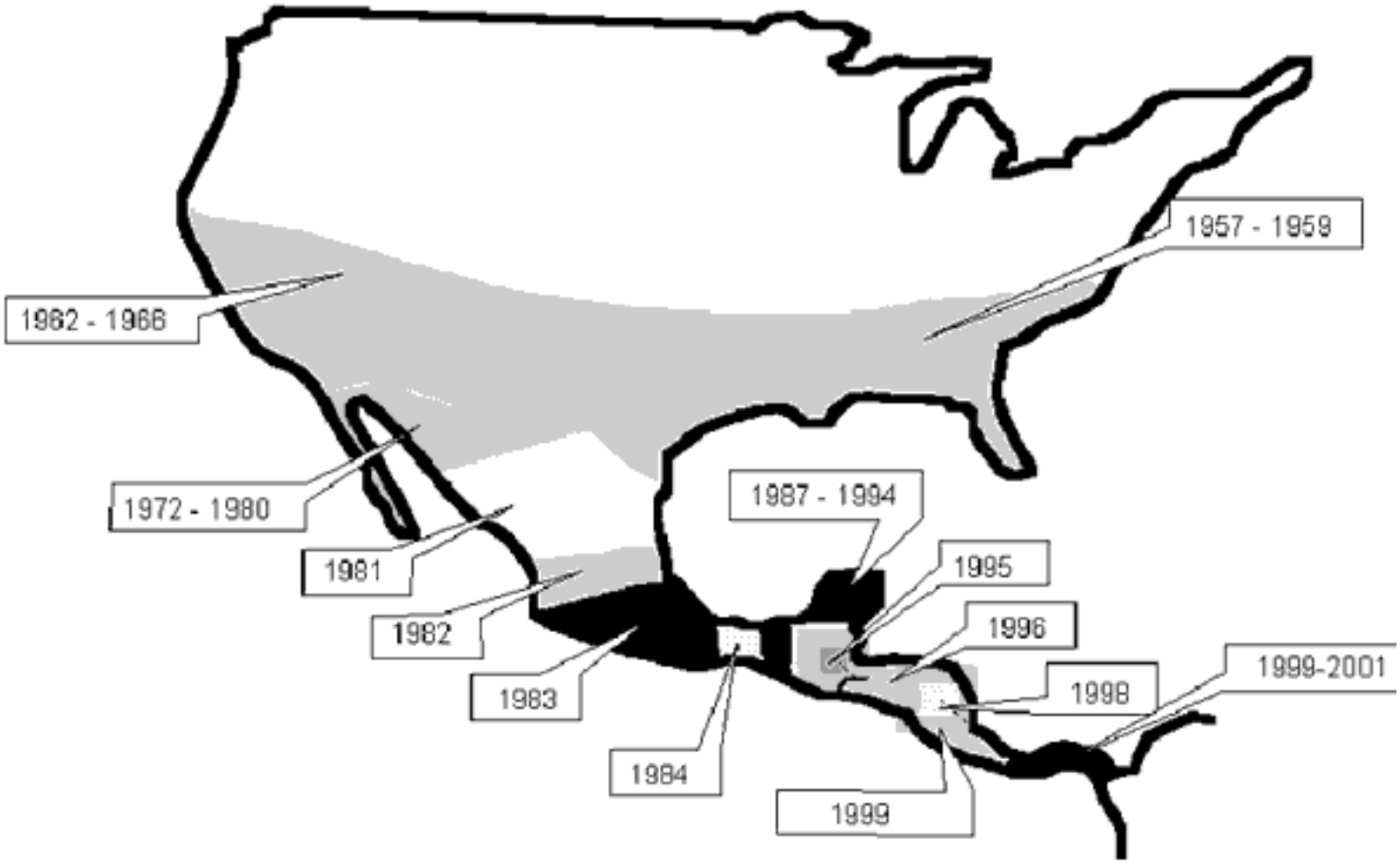
(4) Irradiated males much more numerous than wild males ... Females mate mainly with irradiated males. Offspring of Irradiated males do not survive due to chromosomal damage (double-strand breaks).

Pop'n density decreases



Photograph No. 10. Loading an irradiation chamber with screw-worm pupae. (The sealed 70-curie cobalt-60 experimental source is in the background. The chamber is screwed on the steel plug and lowered by chain and pulley into the source. Note open port visible in the mirror above the source.)

Screwworm distribution over time ...



from http://www.fao.org/docrep/004/y2022e/y2022e02.htm

Sterile Insect Technique Success stories: Screwworm fly (Eradicated from U.S., Mexico, ...), Medfly (successful control in Israel, California, Central America etc.)

Current Targets: Anopheles mosquito - Malaria vector; Tsetse fly - sleeping sickness vector; Painted Apple Moth (Lep: Lymantriidae) in Auckland, New Zealand; Aedes mosquitoes - vectors for filariasis, Dengue and yellow fever.

Drawbacks:

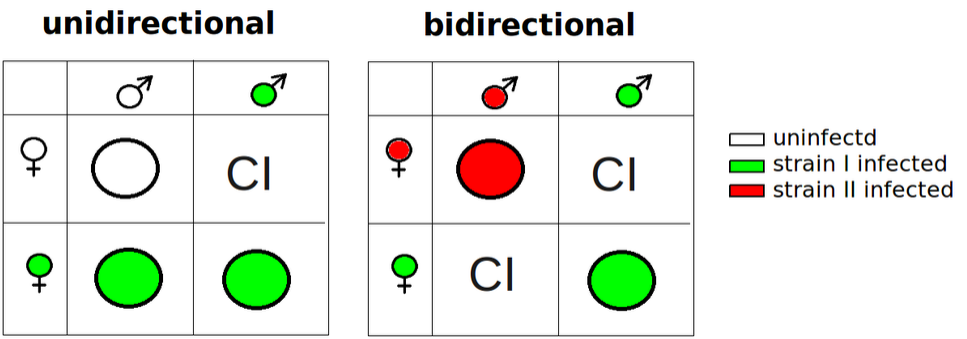
1. Repeated treatment is required to exterminate the population.
2. Sex separation is difficult for some species
3. Radiation treatment in some cases affects the health of males, so sterile males at disadvantage when competing for females.
4. Technique is species specific (e.g., 22 species of Tsetse fly in Africa) and the technique must be implemented separately for each.
5. Many fertile pest insects must be grown before sterilization and housed securely to prevent their escape or release [in Feb. 2003, irradiation machinery at a plant in Mexico failed and 4 million fertile screwworms released before the problem was spotted.
6. Migration of insects from outside the control area will repopulate.
7. Cost can be prohibitive

(this slide is only tiny modification from part of wikipedia entry on Sterile Insect Technique)

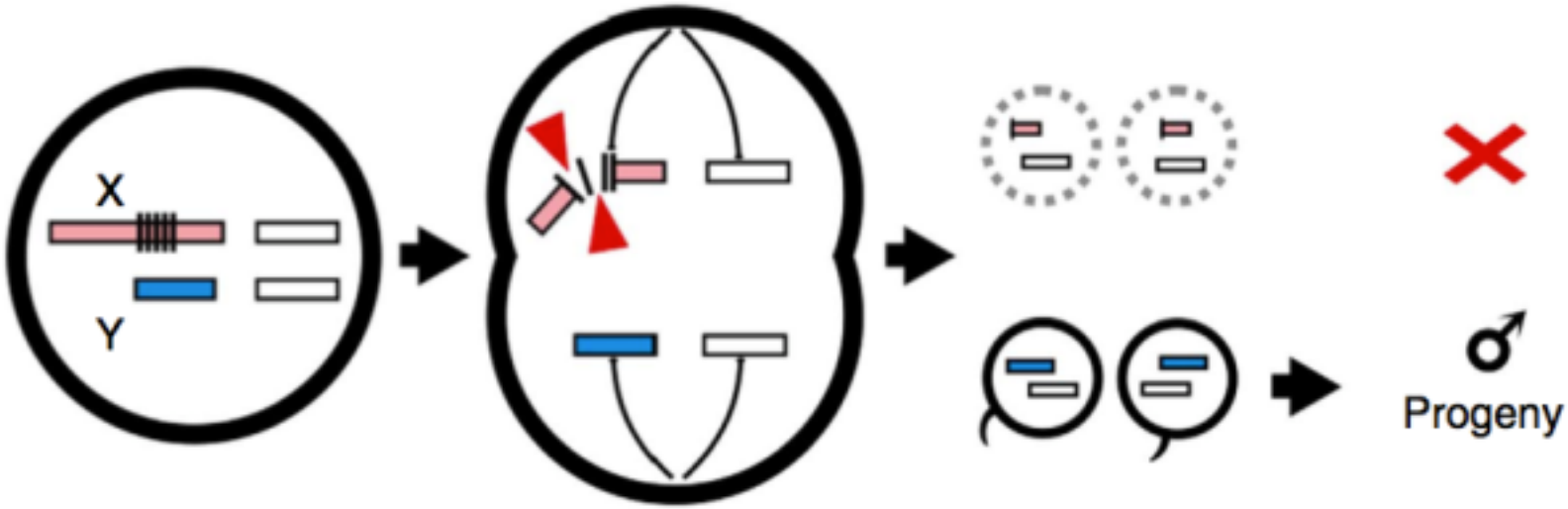
Wolbachia is a kind of intracellular bacterium that can infect mosquitos, fruit flies, and other insects.

1. *Wolbachia* can cause "cytoplasmic incompatibility" (CI) that facilitates its spread throughout insect population **because** it is transmitted from insect to insect via insect eggs and **because** infected **females** have reproductive advantage over uninfected females.

2. *Wolbachia* also lessens or prevents transmission of some (but not all) virusses as well as transmission of *Plasmodium falciparum* organism that causes malaria.



from: https://en.wikipedia.org/wiki/Cytoplasmic_incompatibility



Galizi et al. (Nature Communications 5, 2014. Article No. 3977) introduce an engineered homing endonuclease (I-Ppol) into *Anopheles* mosquito autosomes. Engineered protein expressed in males during spermatogenesis. Causes X-chromosomes to be cut ("shredded") so that only sperm with Y-chromosomes can lead to viable offspring.

This "X-shredder" could lead to eventual extinction of a species in which it is introduced ...

figure taken from http://www.psmag.com/nature-and-technology/mosquitos-83134

CRISPR consists of (interrupted) repeats in prokaryotes

Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A (December 1987). Journal of Bacteriology. 169 (12): 5429–33. PMC 213968. PMID 3316184.

Rodolphe Barrangou
(now at NCSU)

CRISPR/Cas System gives acquired immunity to prokaryotes that have it

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., et al. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. Science 315, 1709–1712. doi: 10.1126/science.1138140

CRISPR/Cas System can be used for targetted genome editing

numerous papers on this topic ...

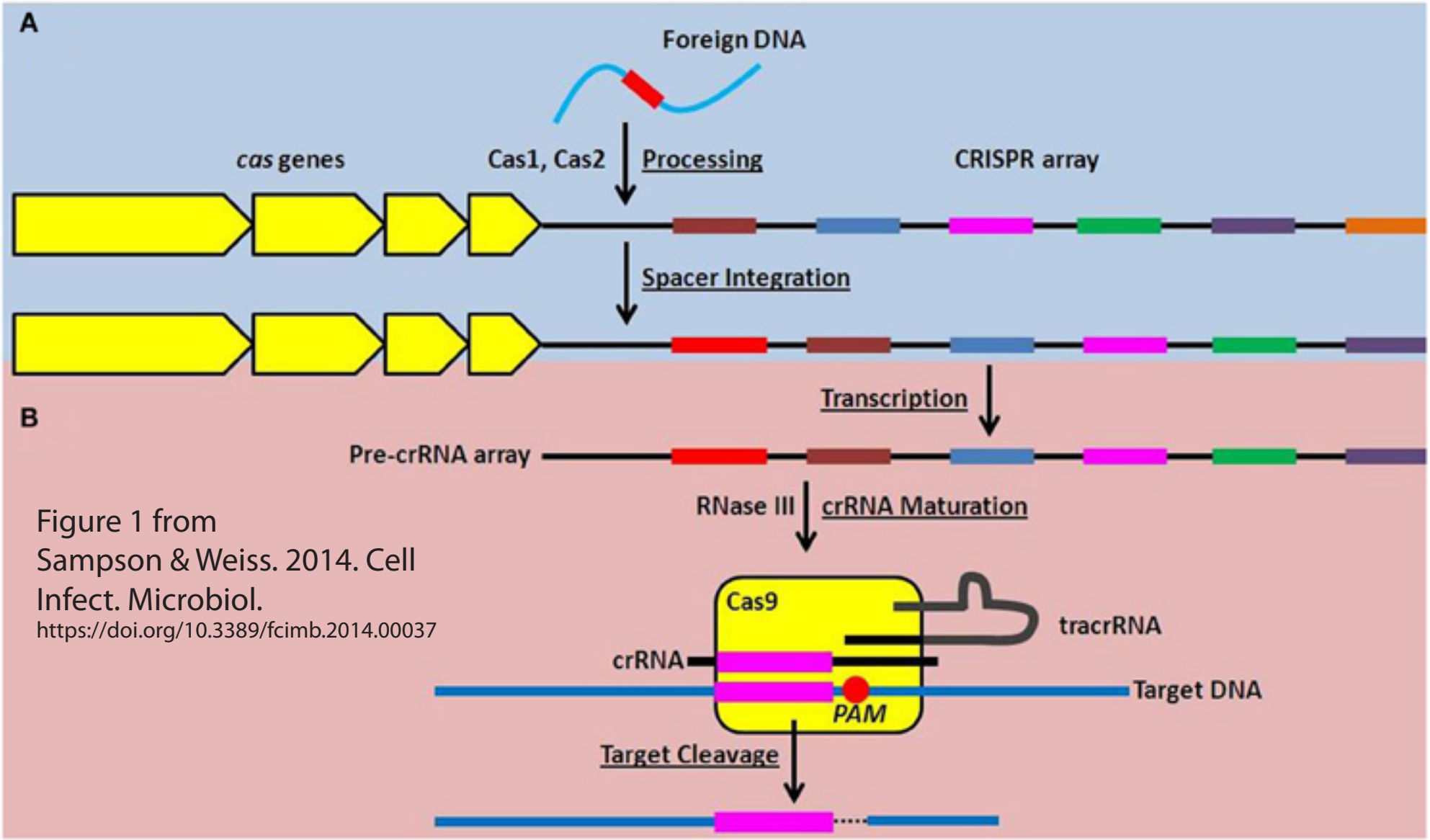
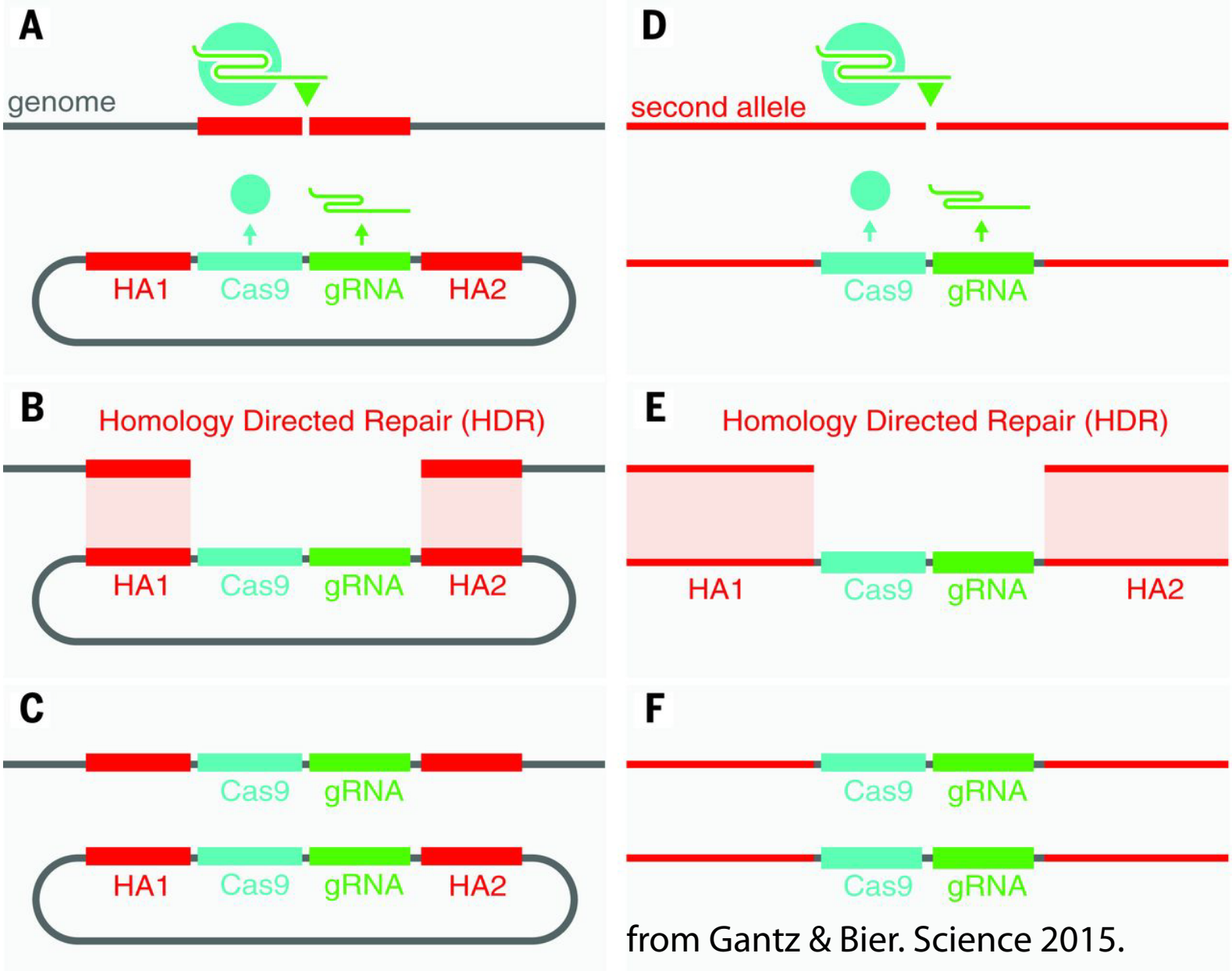


Figure 1 from
Sampson & Weiss. 2014. Cell Infect. Microbiol.
https://doi.org/10.3389/fcimb.2014.00037

Figure 1. Function of the Type II CRISPR-Cas system in adaptive nucleic acid restriction. (A) Foreign DNA is recognized by Cas1 and Cas2 and is processed into a new spacer sequence (red) within the CRISPR array (Adaptation phase, blue). (B) To restrict foreign DNA, the CRISPR array is transcribed as a single transcript (pre-crRNA array) and matured into small targeting crRNAs in a process requiring RNase III and tracrRNA. The dsRNA complex of crRNA and tracrRNA is associated with Cas9 and the spacer sequence within the crRNA can hybridize to complementary DNA sequences. Cas9 then mediates cleavage of the targeted DNA downstream of the proto-spacer adjacent motif, or PAM, highlighted by the red circle (Effector phase, pink).

"Fig. 1. Scheme outlining the mutagenic chain reaction (MCR). (A to C) A plasmid consisting of a core cassette carrying a Cas9 transgene, a gRNA targeting a genomic sequence of interest, and flanking homology arms corresponding to genomic sequences abutting the target cleavage site (A) inserts the core Cas9-gRNA cassette into the targeted locus via HDR [(B) and (C)]. (D to F) In turn, the inserted cassette expresses both Cas9 and the gRNA, leading to cleavage (D) and HDR-mediated insertion of the cassette into the second allele, thereby rendering the mutation homozygous [(E) and (F)]. HA1 and HA2 denote the two homology arms that directly flank the gRNA-directed cut site."



from Gantz & Bier. Science 2015. 348:442-444

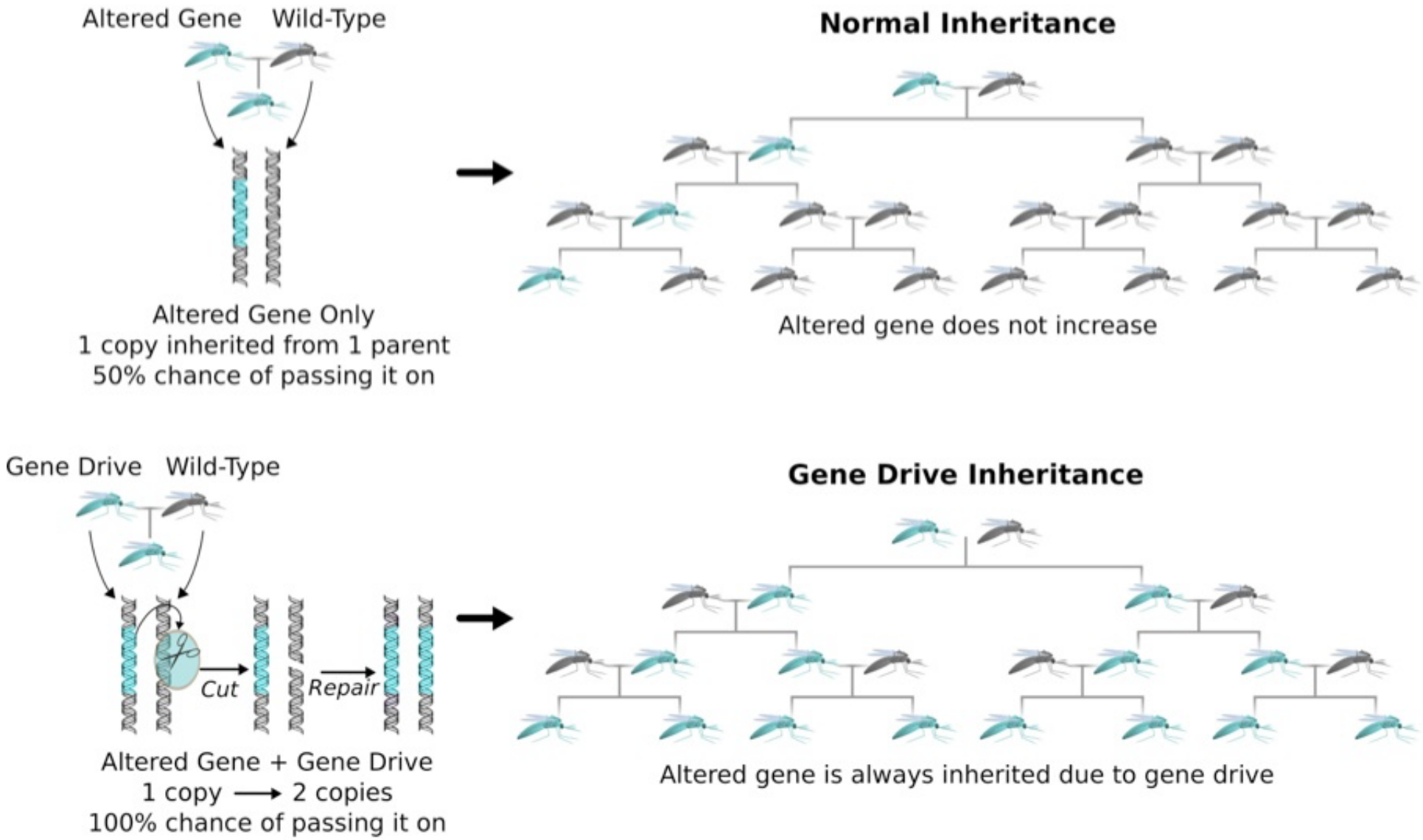


Figure taken from Scientific American guest blog by Esvelt et al.

see http://blogs.scientificamerican.com/guest-blog/gene-drives-and-crispr-could-revolutionize-ecosystem-management/

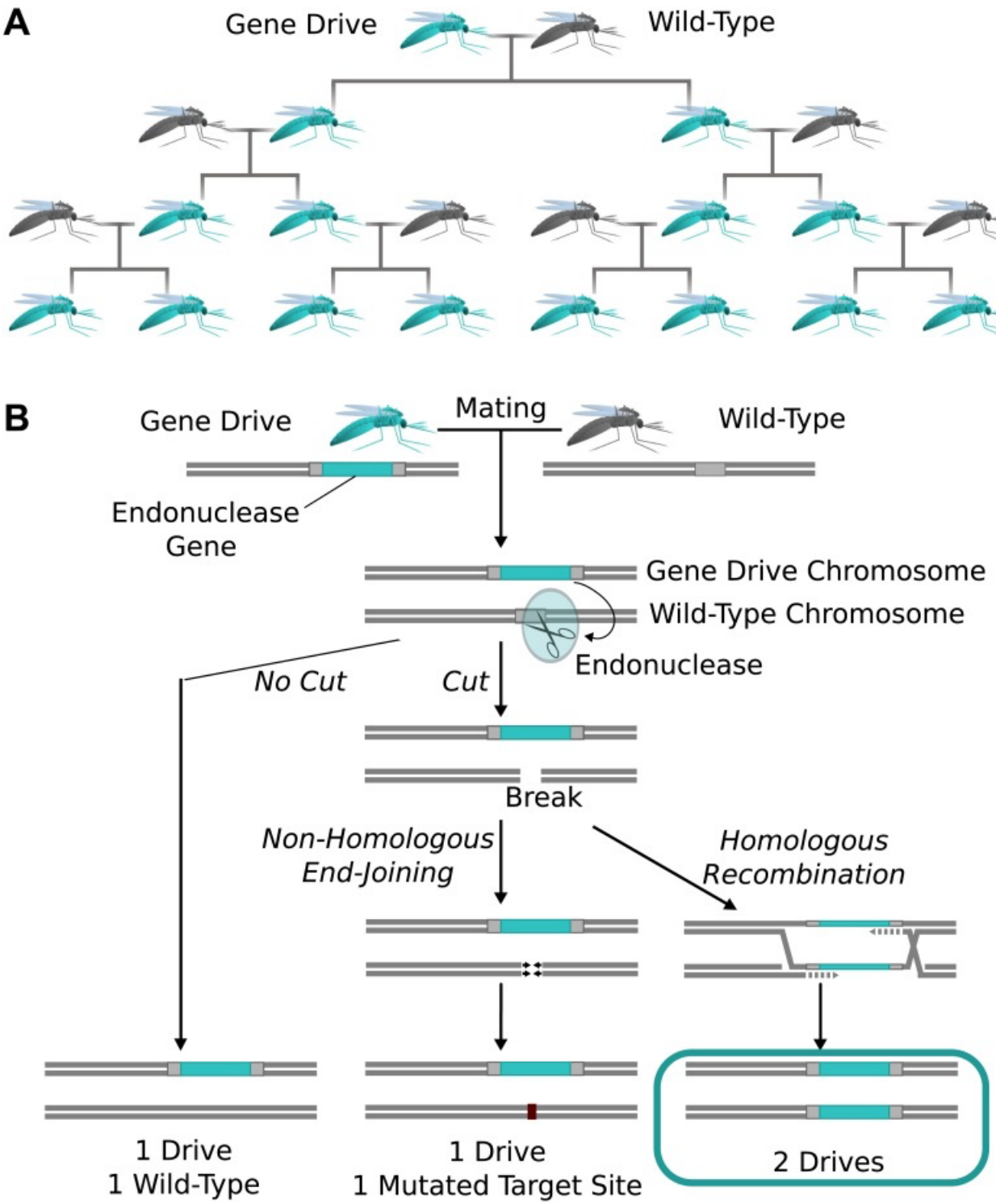


Figure 1 from Esvelt et al. 2014. Elife 3: e03401

"... (A) When an organism carrying an endonuclease gene drive (blue) mates with a wild-type organism (grey), the gene drive is preferentially inherited by all offspring. This can enable the drive to spread until it is present in all members of the population–even if it is mildly deleterious to the organism.

(B) Endonuclease gene drives are preferentially inherited because the endonuclease cuts the homologous wild-type chromosome. When the cell repairs the break using homologous recombination, it must use the gene drive chromosome as a repair template, thereby copying the drive onto the wild-type chromosome. If the endonuclease fails to cut or the cell uses the competing non-homologous end-joining repair pathway, the drive is not copied, so efficient gene drives must reliably cut when homology-directed repair is most likely. ..."

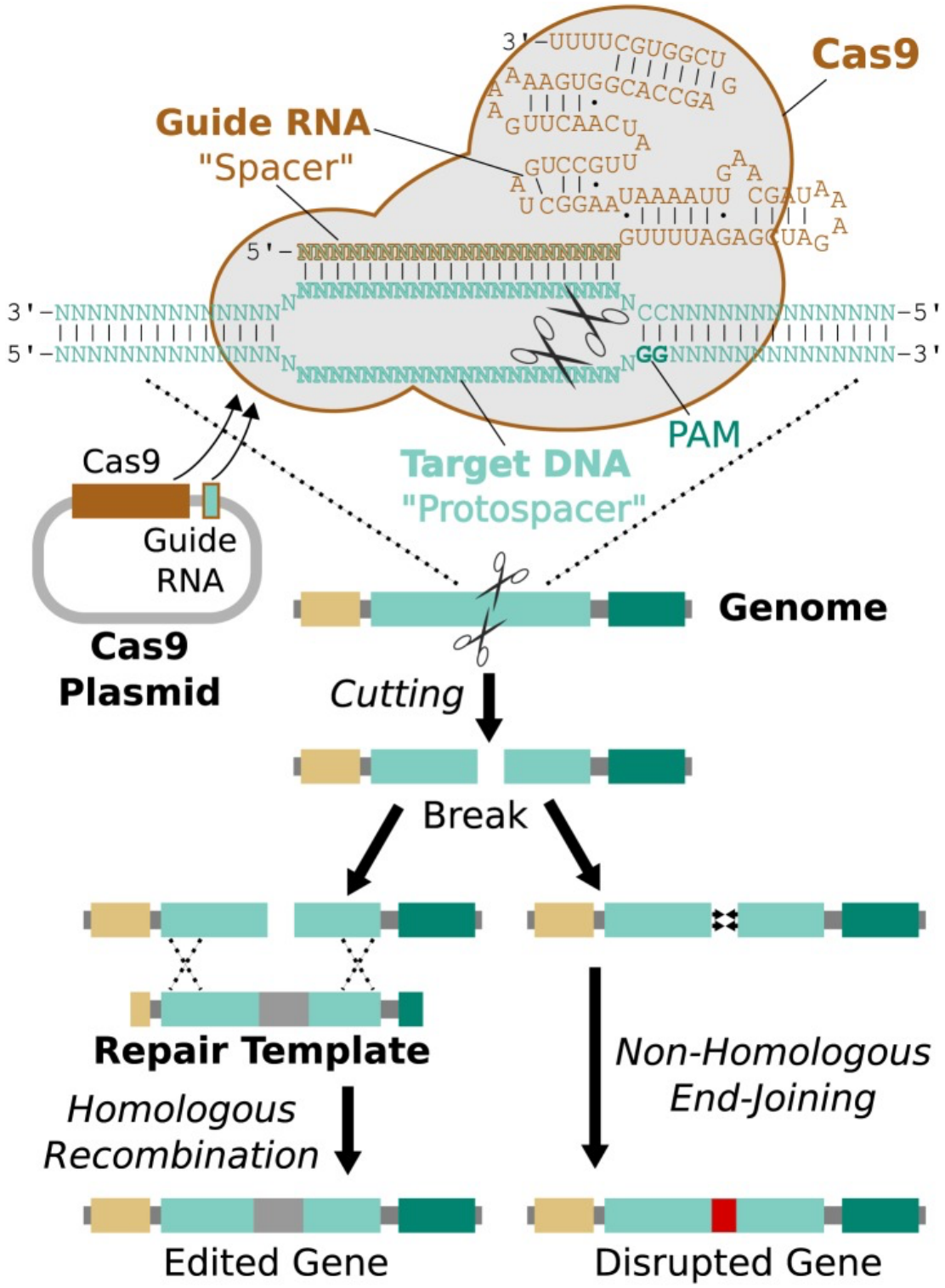


Figure 3 from Esvelt et al. 2014. Elife 3: e03401

"...The Cas9 nuclease protein and guide RNA must first be delivered into the target cell. This is often accomplished by transfecting DNA expression plasmids, but delivering RNA is also effective. The guide RNA directs Cas9 to bind target DNA 'protospacer' sequences that match the 'spacer' sequence within the guide RNA. Protospacers must be flanked by an appropriate protospacer-adjacent motif (PAM), which is NGG for the most commonly used Cas9 protein (Jinek et al., 2012). If the spacer & protospacer are identical or have only a few mismatches at the 5' end of the spacer, Cas9 will cut both strands of DNA, creating a blunt-ended double-strand break. If supplied with a repair template containing the desired changes and homology to the sequences on either side of the break, the cell may use homologous recombination to repair the break by incorporating the repair template into the chromosome. Otherwise, the break will be repaired by non-homologous end-joining, resulting in gene disruption. ... If the cell being edited is a germline cell that gives rise to eggs or sperm, the changes can be inherited by future generations. ..."

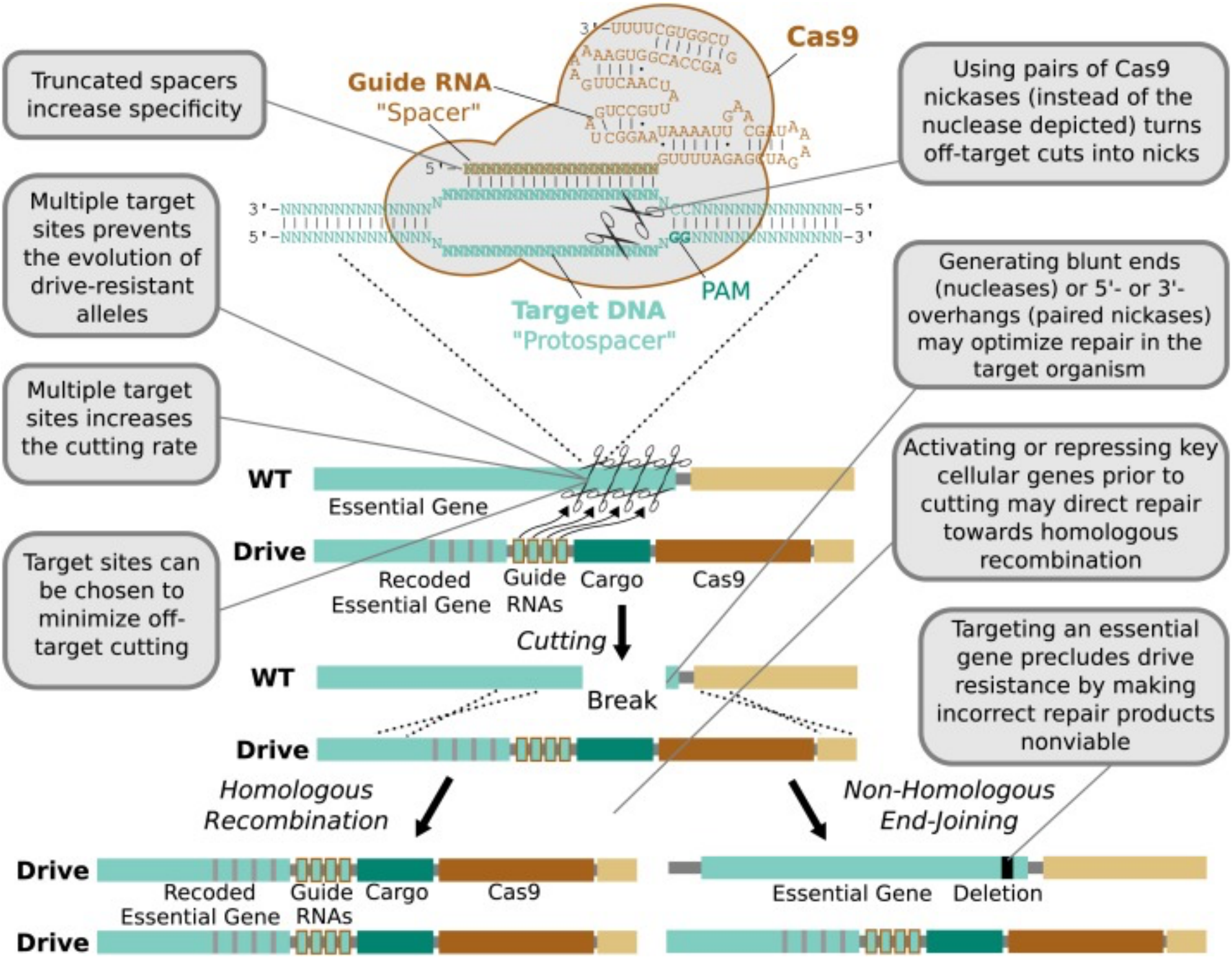


Figure 4 from Esvelt et al. 2014. Elife 3: e03401

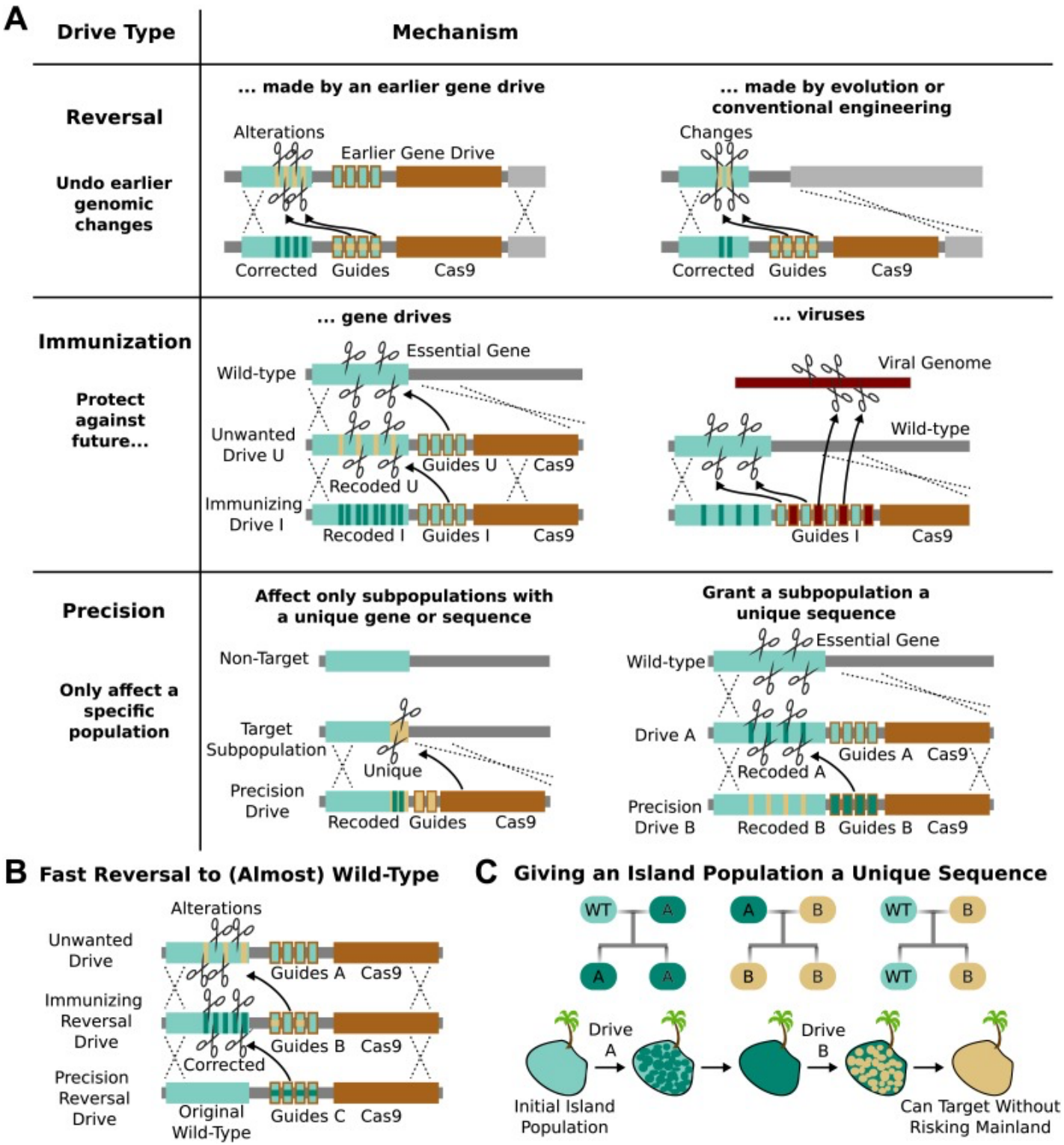


Figure 5 from Esvelt et al. 2014. Elife 3: e03401

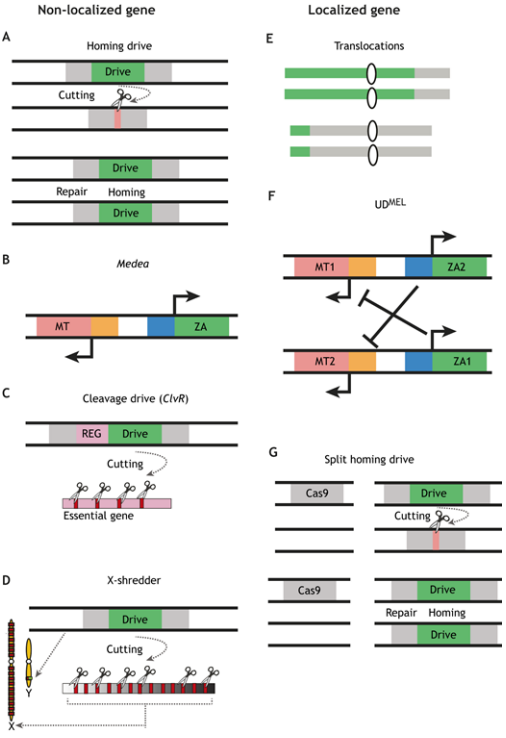
"...Gene drives can favorably bias the inheritance of a linked disease-refractory gene, which could possibly be exploited (i) to generate a vector population incapable of transmitting disease or (ii) to disrupt an essential gene for viability or fertility, which could eventually eliminate a population. ..."

"... Non-localized drives are expected to spread beyond a release site and maintain themselves in the population for many generations. ...

[L]ocalized drives are expected to spread only into local populations and, in some cases, eliminate themselves from the population over time. ..."

from:
Raban et al. J Exp Biol (2020) 223 (Suppl_1): jeb208181.

Fig.1:



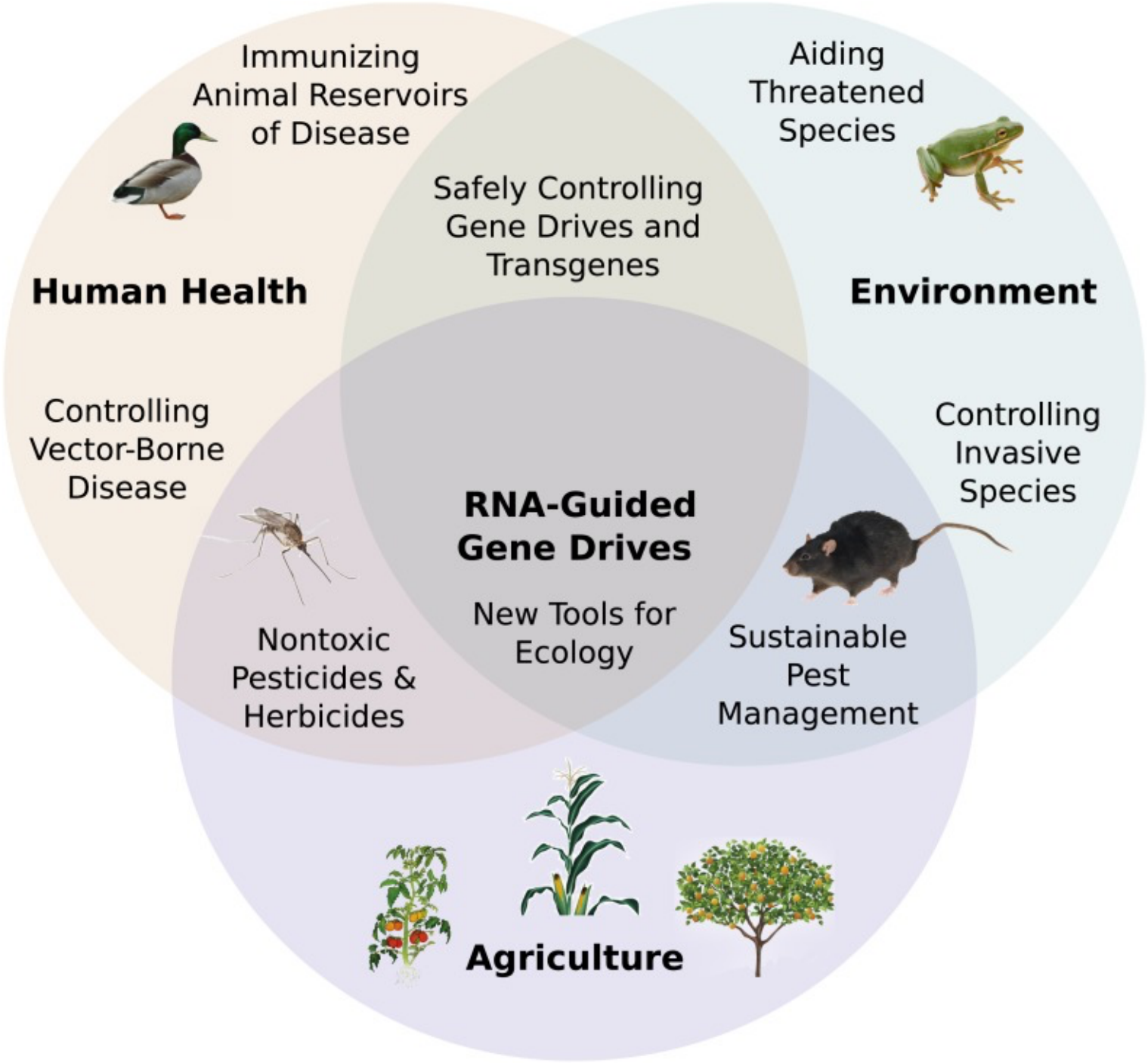


Figure 7 from Esvelt et al. 2014. Elife 3: e03401