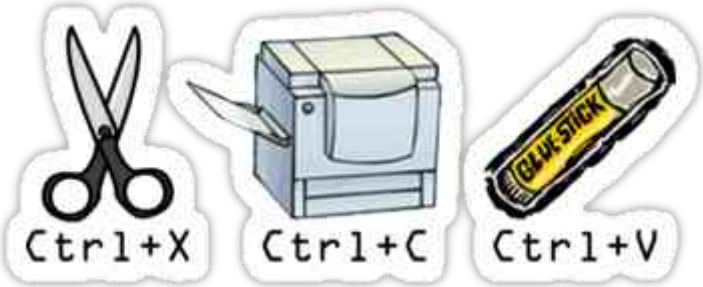
Le forçage génétique ou "gene drive" : état des lieux et enjeux associés à cette nouvelle biotechnologie

Virginie Courtier-Orgogozo



CRISPR

Marion Montagne

Agraulis vanillae

dorsal *ventral*

Wild-type **mutant *optics* CRISPR** **Wild-type** **mutant *optics* CRISPR**

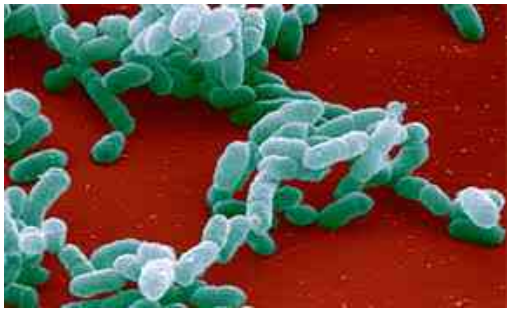
Reed et al. 2017

Fundamental research is important

bacteria *Thermus aquaticus*

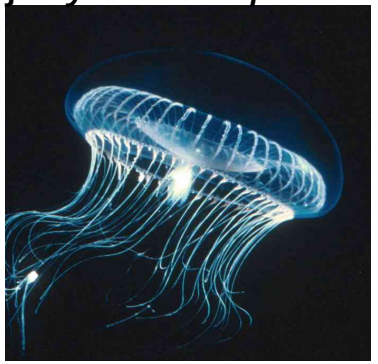
1969 → Taq-polymerase to amplify DNA

bacteria *Haemophilus influenzae*



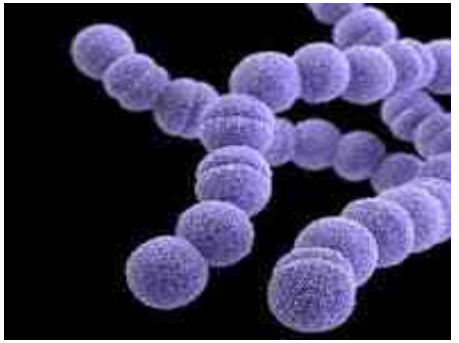
1970 → Restriction enzymes To cut DNA

jellyfish *Aequorea*



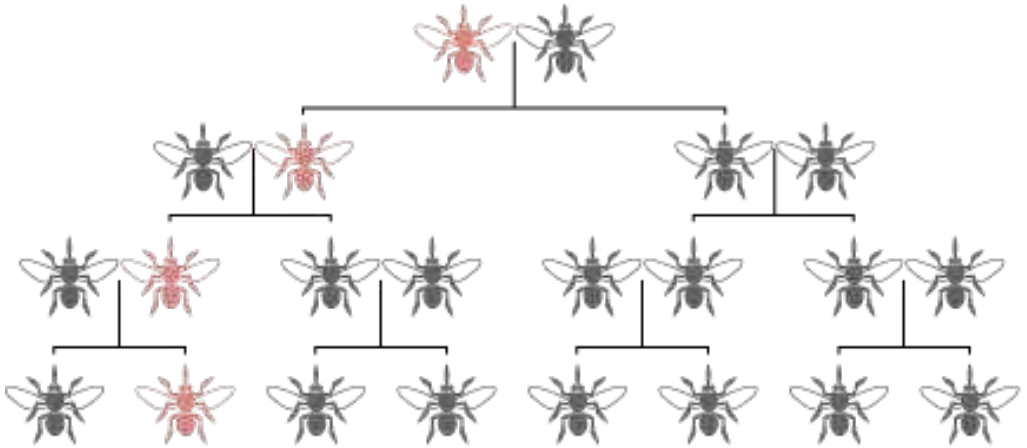
1992 → Fluorescent proteins

bacteria *Streptococcus pyogenes*

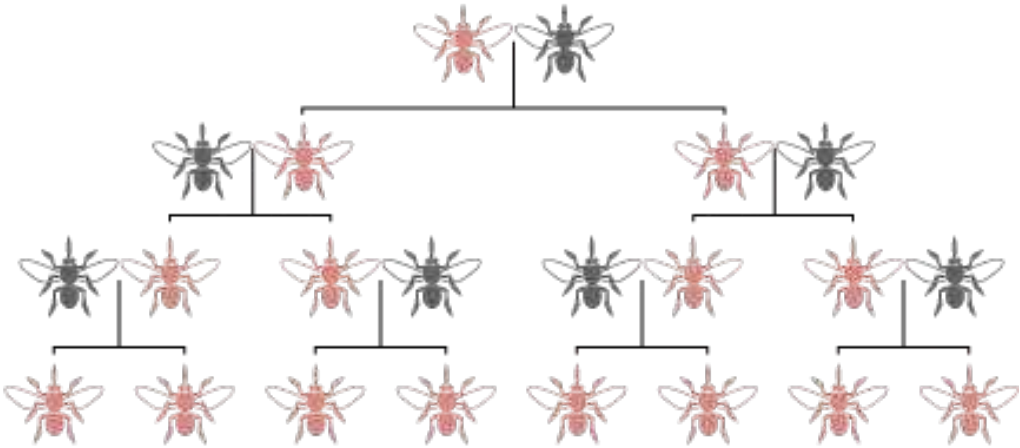


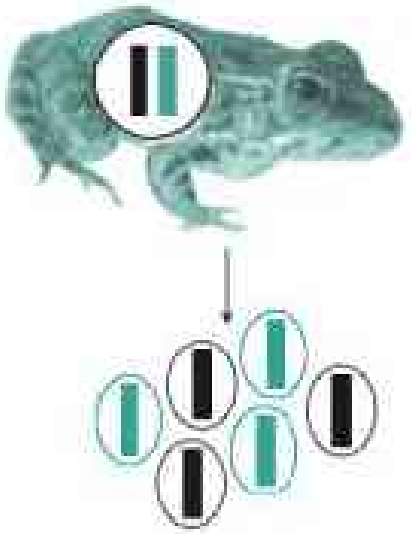
2012 → CRISPR

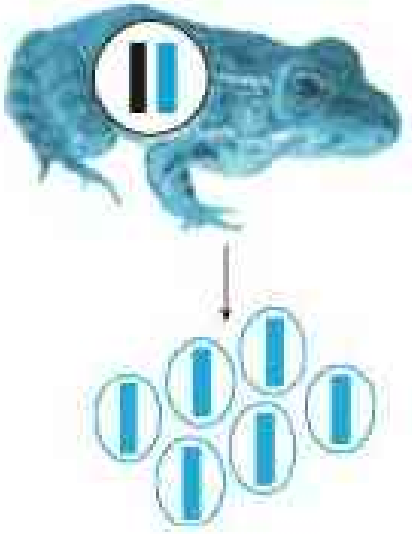
Normal reproduction



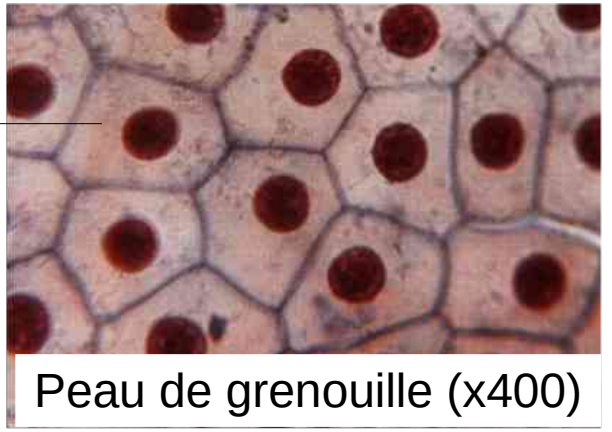
Reproduction with gene drive

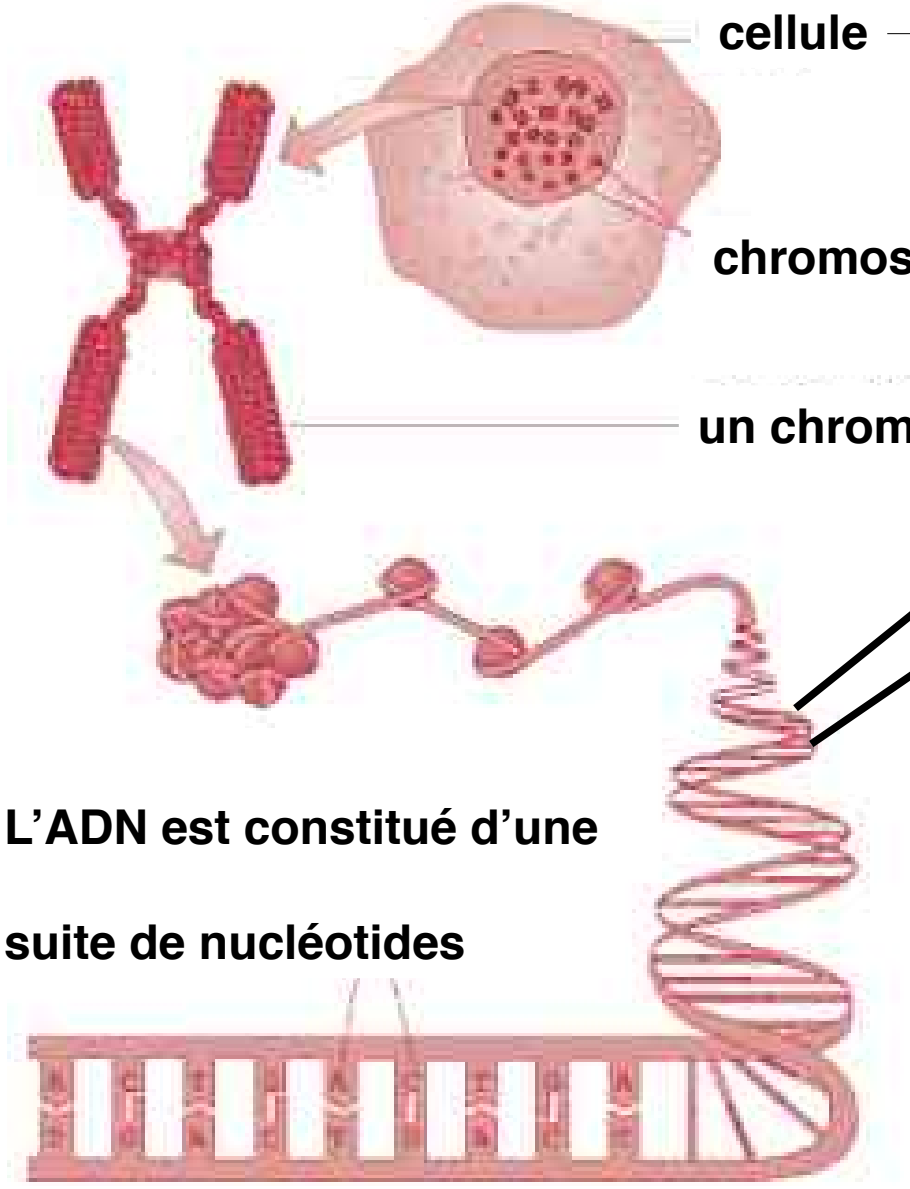




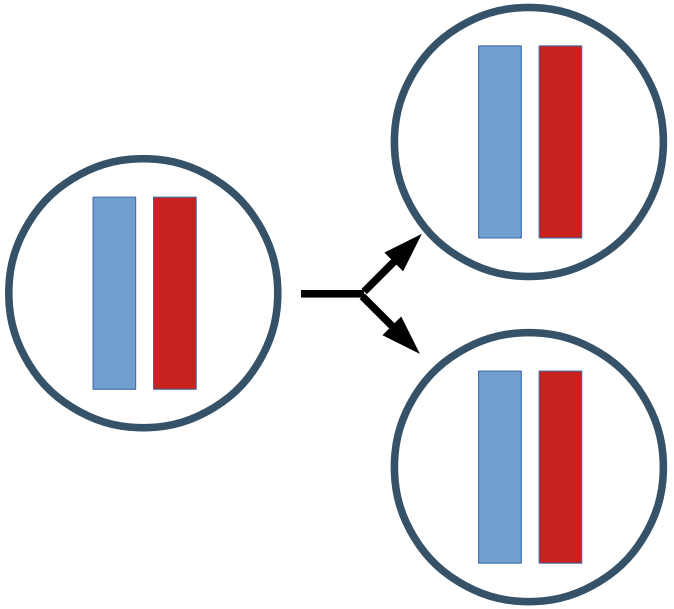


Rode et al. 2019

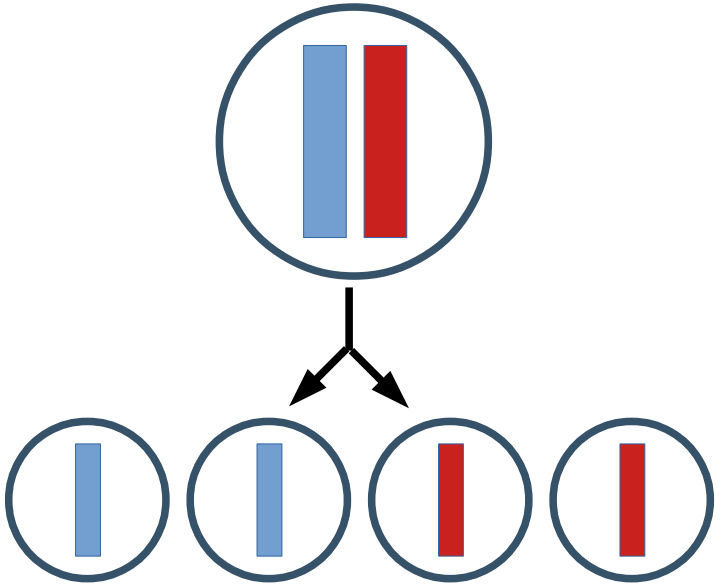

Peau de grenouille (x400)


cellule
chromosomes
un chromosome
ADN non codant
ADN codant
protéines
L'ADN est constitué d'une
suite de nucléotides
ADN

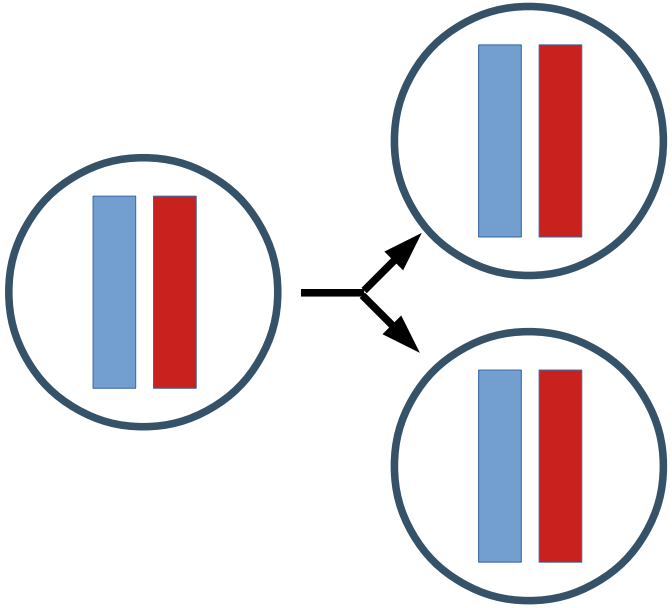
Division cellulaire (mitose)



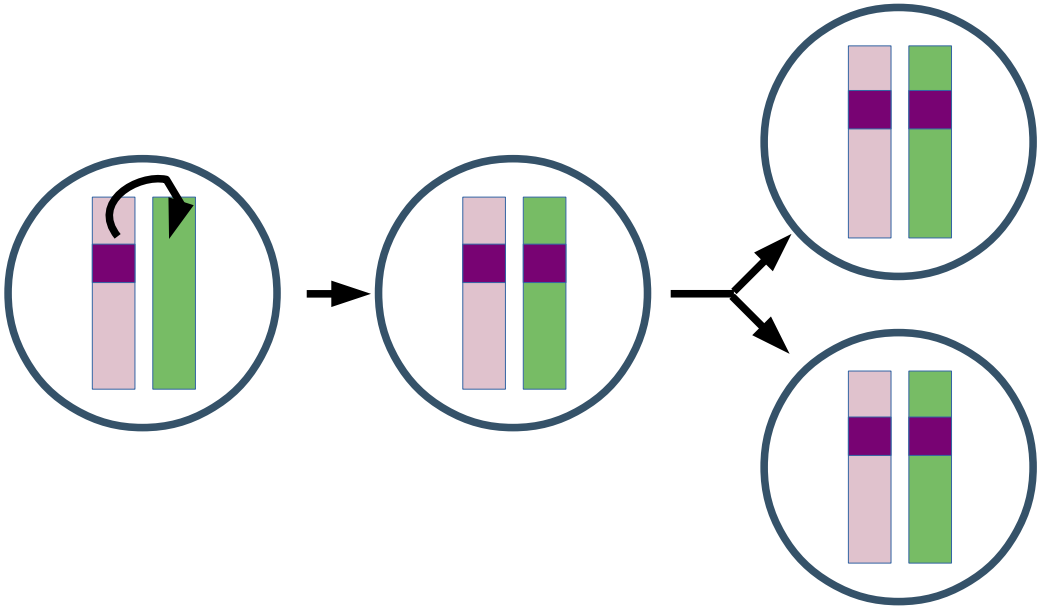
Reproduction normale



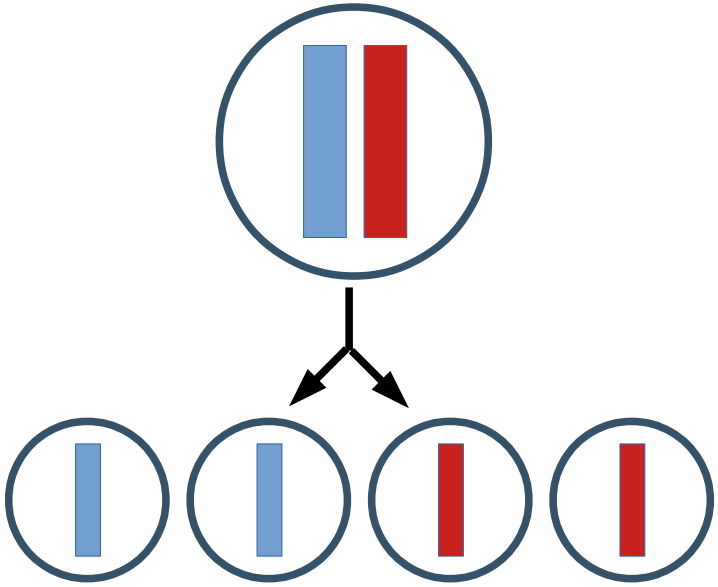
Division cellulaire (mitose)



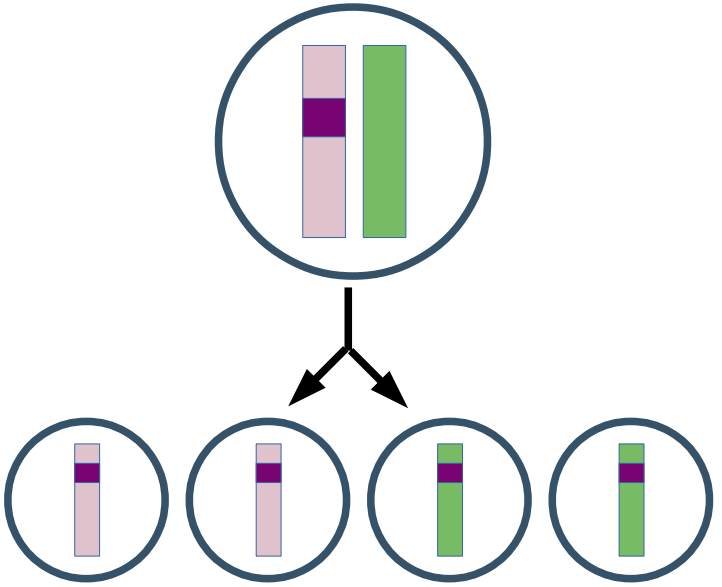
Gene drive

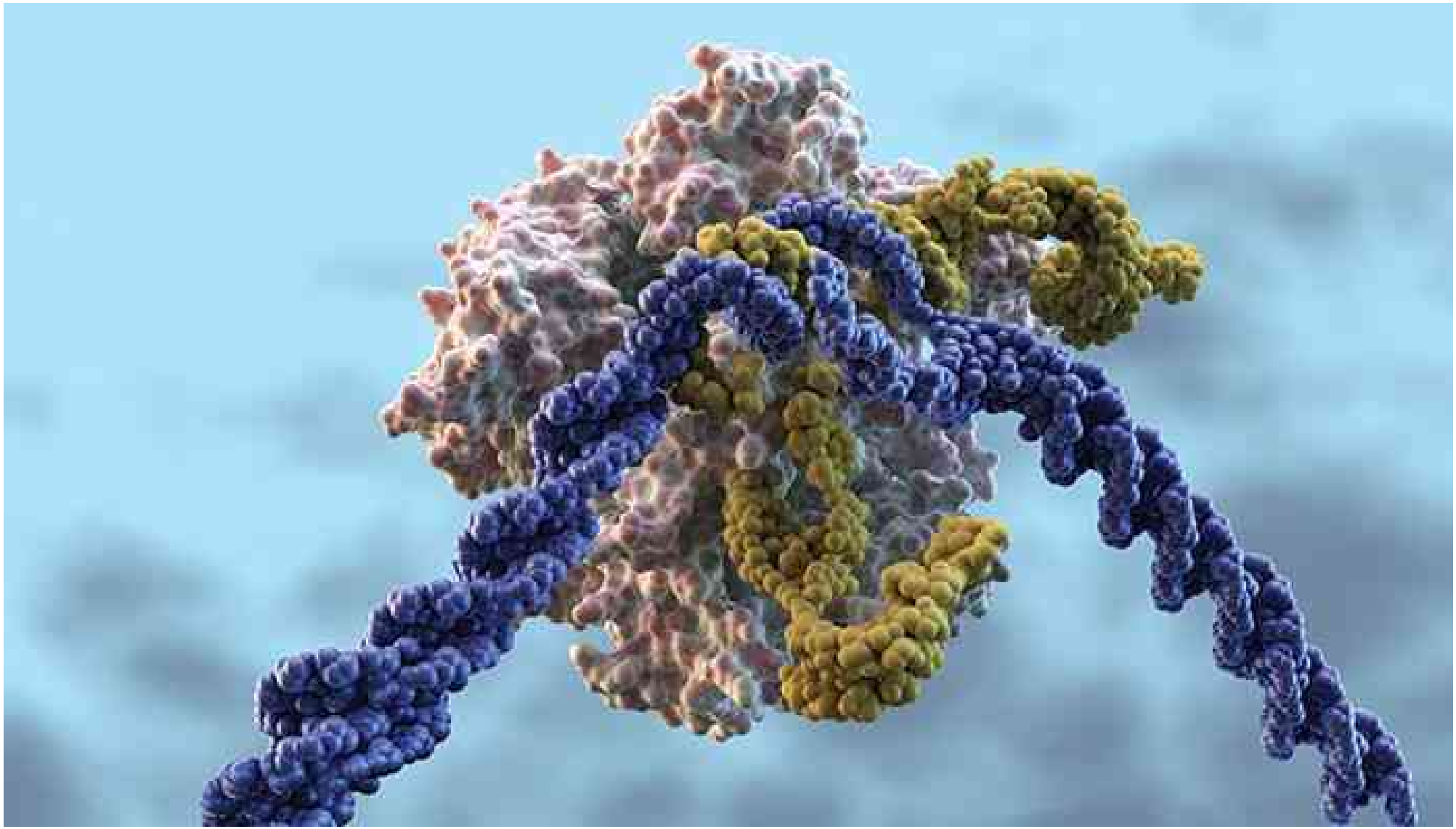


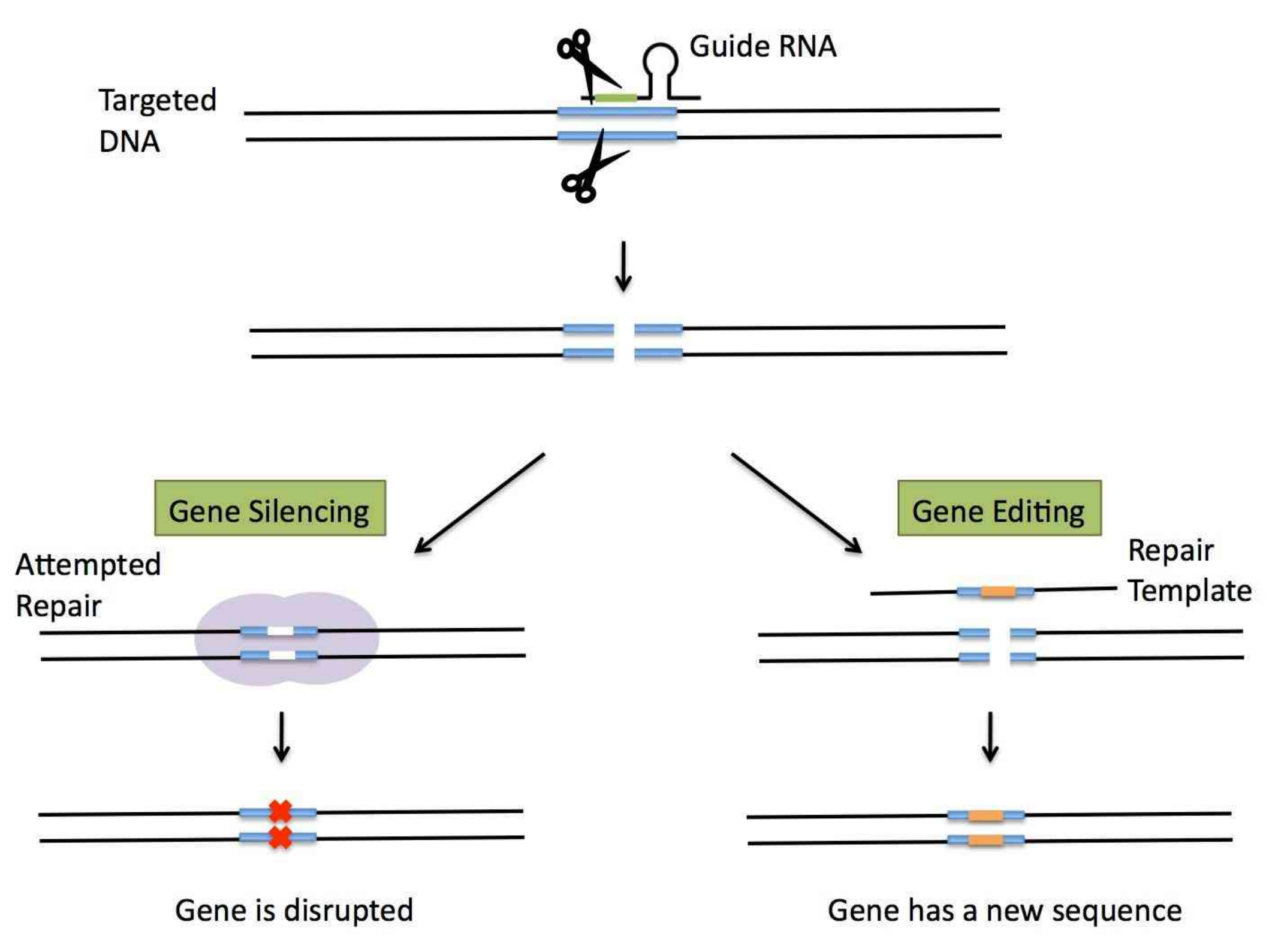
Reproduction normale



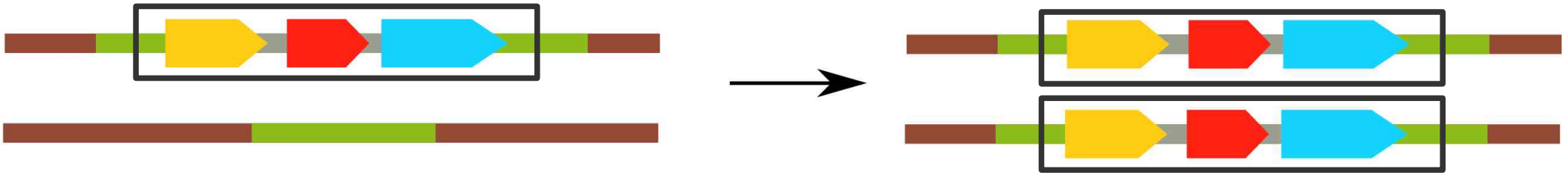
Gene drive

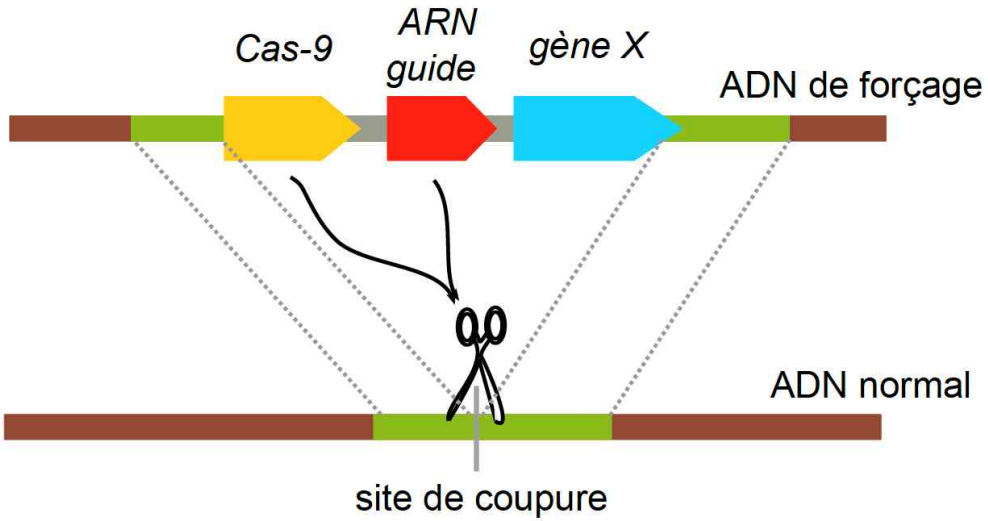


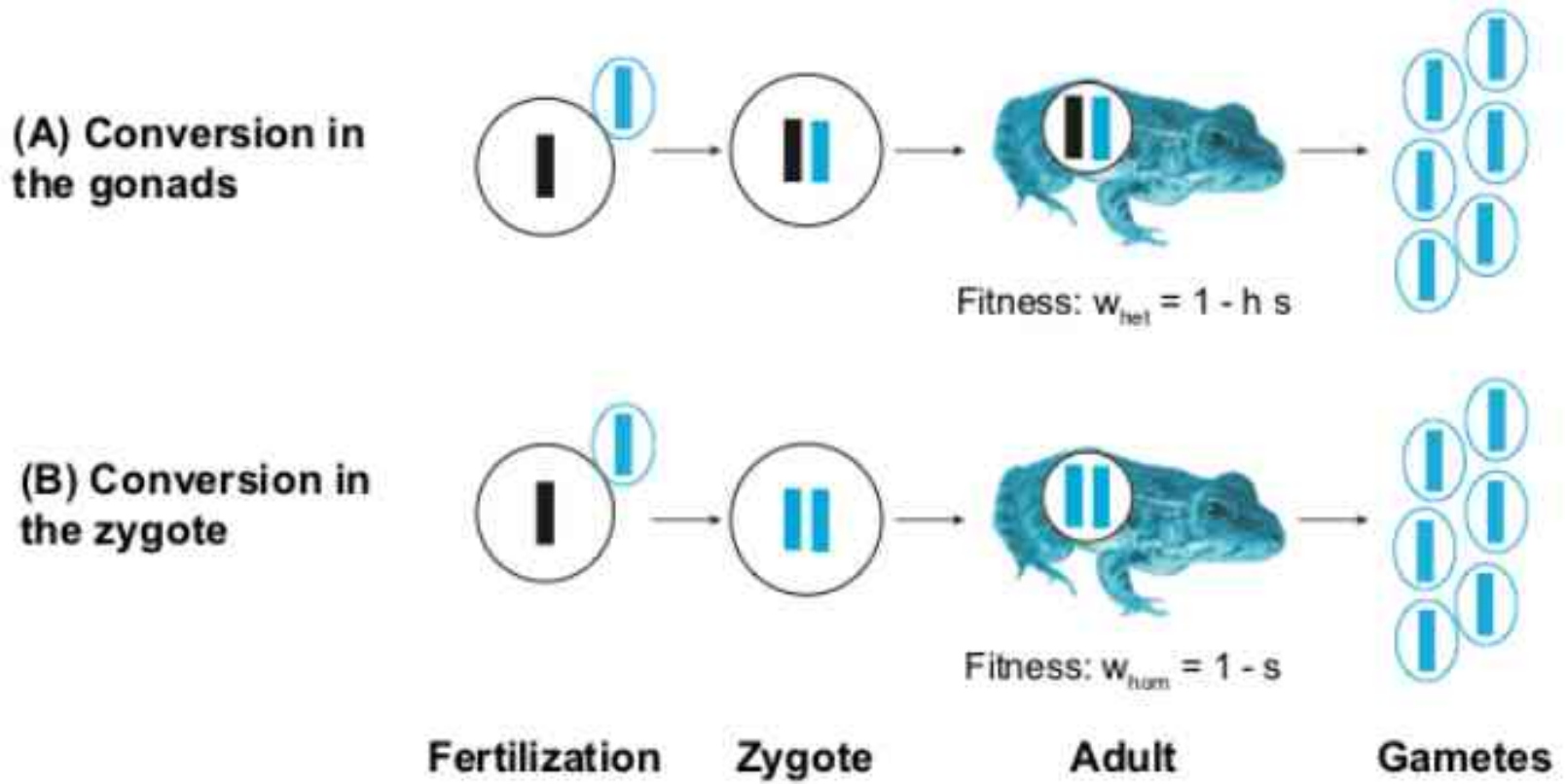



Guide RNA
Targeted
DNA
Gene Silencing
Gene Editing
Attempted
Repair
Repair
Template
Gene is disrupted
Gene has a new sequence

Comment une cassette gene drive se recopie







Rode et al. 2019

Potential applications of Gene Drive

(A) ERADICATION DRIVES

spreading strongly deleterious mutations in invasive populations

Eradicating invasive pest species

Eradicating invasive black rats that threaten the kereru (New Zealand pigeon) and other endemic species in New Zealand (NASEM 2016)
Image: David Mudge; Ngā Manu Nature Images

Eradicating invasive disease vectors

Eradicating invasive mosquitos, vector of avian malaria in Hawaiian honeycreeper birds (NASEM 2016)
Image: Sean McCann; Flickr

(B) SUPPRESSION DRIVES

spreading mildly deleterious mutations in invasive populations

Reducing the height of invasive common ragweed to decrease its competitive pressure on native plants (Neve 2018)
Image: Ashley Bradford; inaturalist.org

(C) RESCUE DRIVES

spreading beneficial mutations in endangered populations

Protecting lowland leopard frogs from highly pathogenic fungus (Esvelt et al 2014)
Image: Brian Gratwicke; Flickr

Rode et al. 2019

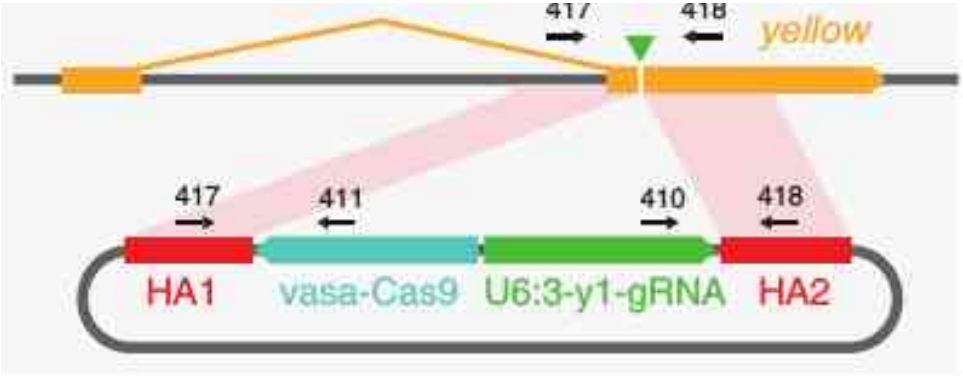
First gene drive organisms

GENOME EDITING

Science, April 2015

The mutagenic chain reaction: A method for converting heterozygous to homozygous mutations

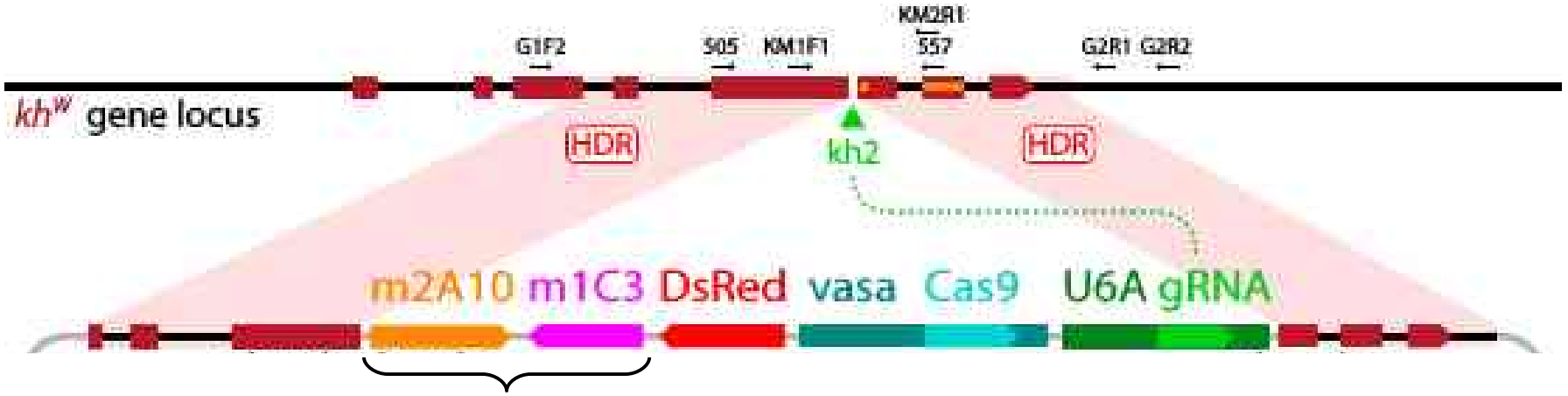
Valentino M. Gantz* and Ethan Bier*



E

	y^- ♂	y^- ♀	mosaic ♀	y^+ ♂	y^+ ♀	total
y^{MCR} ♂ x y^+ ♀	0	40	0	50	1	91
y^{MCR} ♀ x y^+ ♂	214	203	11	2	6	436

Mosquitoes without parasites



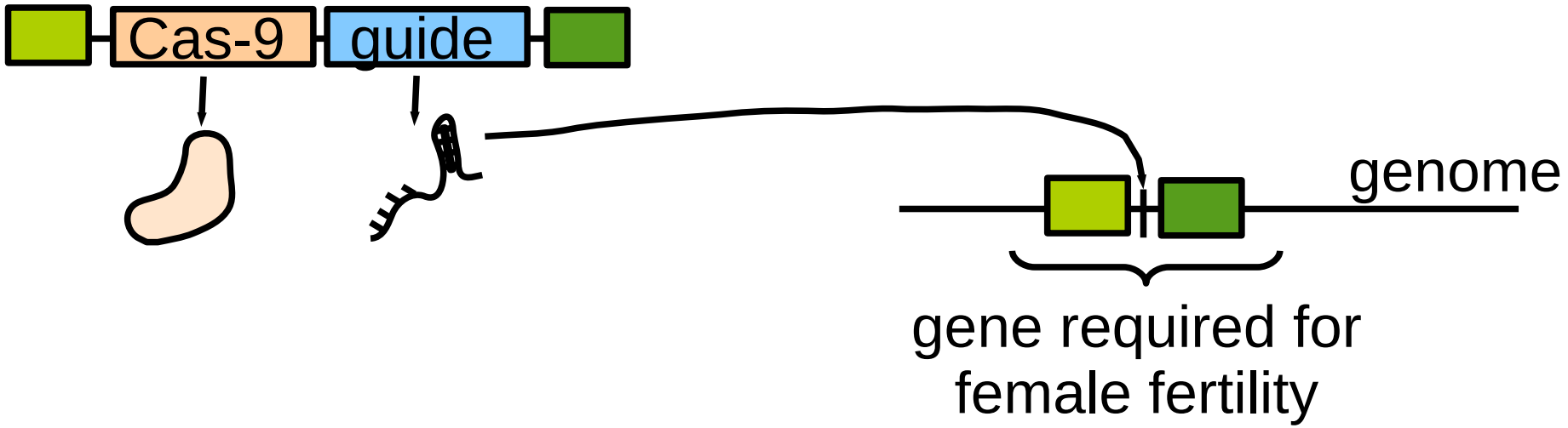
antibodies
anti-parasites

PNAS, November 2015

Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*

Valentino M. Gantz[a,1], Nijole Jasinskiene[b,1], Olga Tatarenkova[b], Aniko Fazekas[b], Vanessa M. Macias[b], Ethan Bier[a,2], and Anthony A. James[b,c,2]

Sterile mosquitoes



Nature Biotechnology, décembre 2015

A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*

Andrew Hammond[1], Roberto Galizi[1], Kyros Kyrou[1], Alekos Simoni[1], Carla Siniscalchi[2], Dimitris Katsanos[1], Matthew Gribble[1], Dean Baker[3], Eric Marois[4], Steven Russell[3], Austin Burt[1], Nikolai Windbichler[1], Andrea Crisanti[1] & Tony Nolan[1]

Two advanced gene drives

Drosophila suzukii

Invasive pest species

Scott et al. 2018

Anopheles mosquitoes

Vector of malaria

TARGET MALARIA

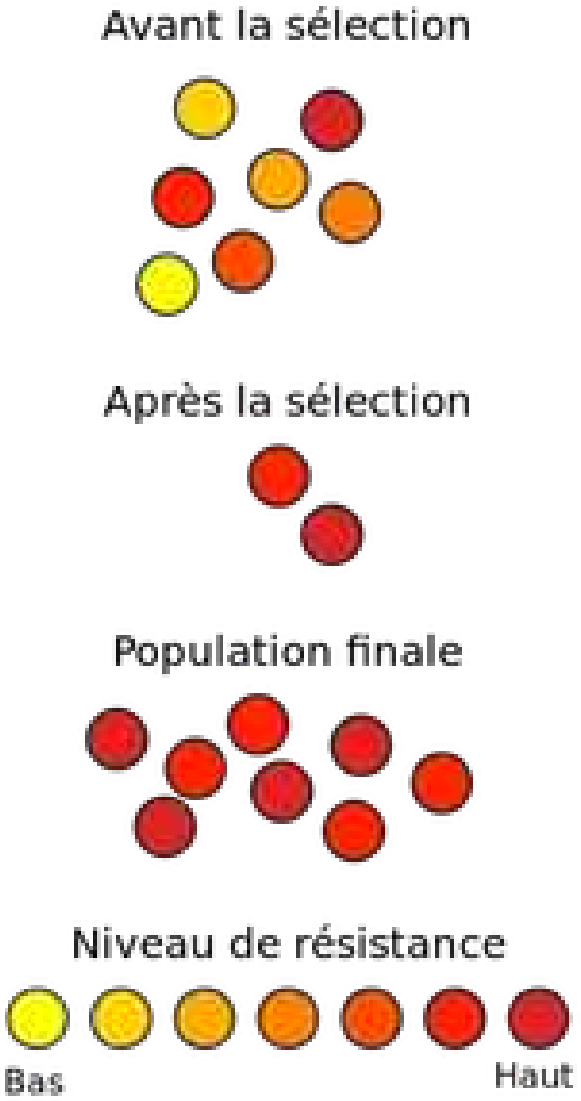
https://targetmalaria.org

What is novel about gene drive?

several DNA pieces assembled together
Eukaryote cis-regulatory regions with bacteria coding regions

Manipulates 2 pillars of evolution
- mutation
- transmission
(selection)

Résistance aux antibiotiques



Classical Darwinian Evolution

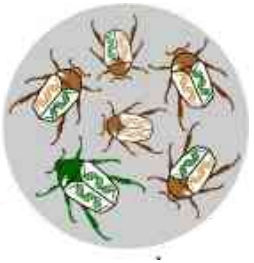
1

Variation

Mutations in DNA

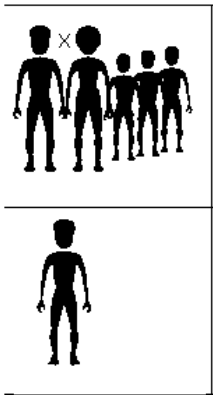
2

Transmission to the next generation



3

Reproduction Variability between individuals



Selection

Genetic Drift

http://evolution.berkeley.edu

What is novel about gene drive?

several DNA pieces assembled together
Eukaryote cis-regulatory regions with bacteria coding regions

Manipulates 2 pillars of evolution
- mutation
- transmission
(selection)

Potentially more effective than other biotechnologies
- ease of use
- speed of change
- unprepared regulatory environment

What are the risks?

Molecular off-targets

Propagation to non-target populations

Propagation to non-target species

Consequences for ecosystems

Failure of counter-measures

What are the risks?

Molecular off-targets

Propagation to non-target populations

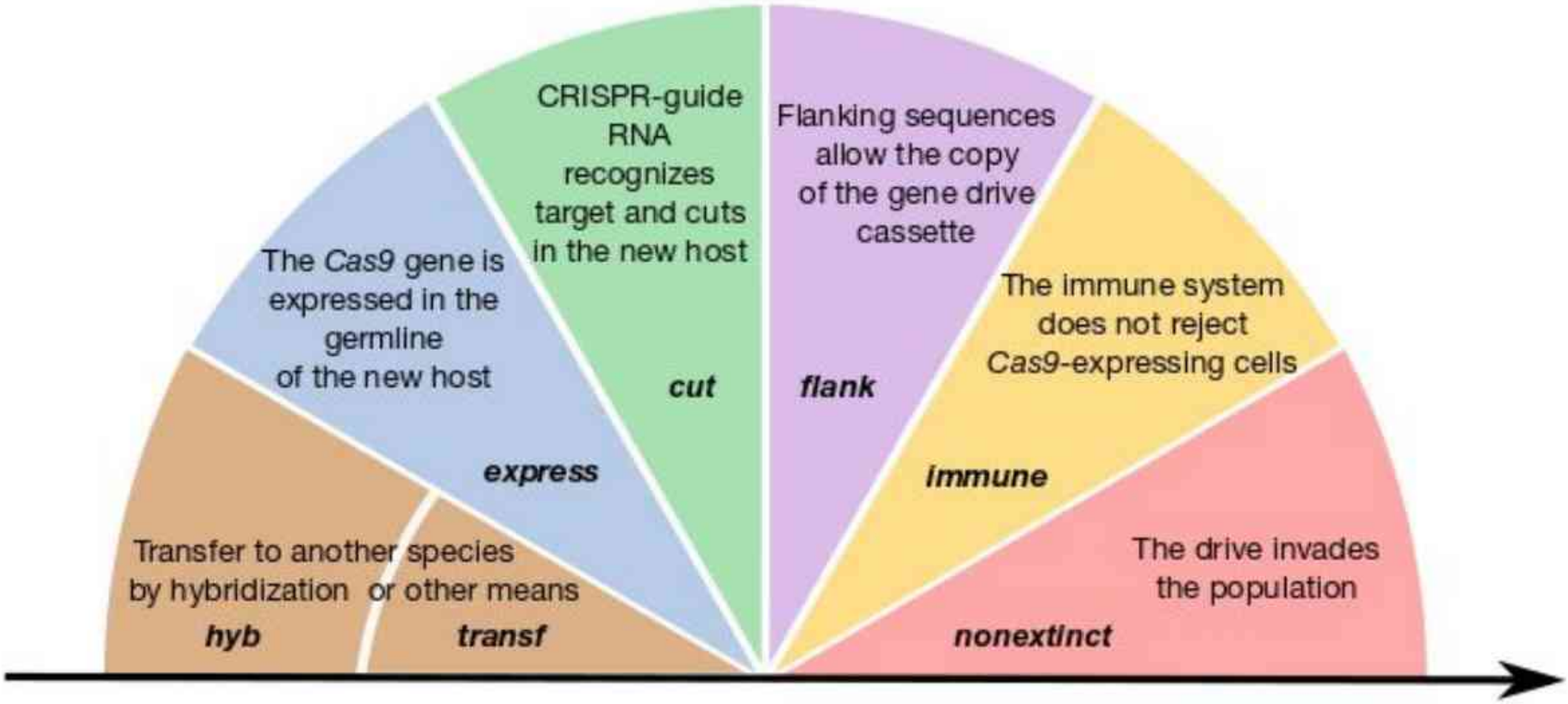
Propagation to non-target species

Consequences for ecosystems

Failure of counter-measures

The risk of gene drive contaminating a non-target species

Courtier-Orgogozo, Danchin, Gouyon and Boete - BioRxiv


CRISPR-guide RNA recognizes target and cuts in the new host
cut
Flanking sequences allow the copy of the gene drive cassette
flank
The immune system does not reject Cas9-expressing cells
immune
The drive invades the population
nonextinct
The Cas9 gene is expressed in the germline of the new host
express
Transfer to another species by hybridization or other means
transf
hyb

Risk of hybridization

Drosophila suzukii
Invasive pest species

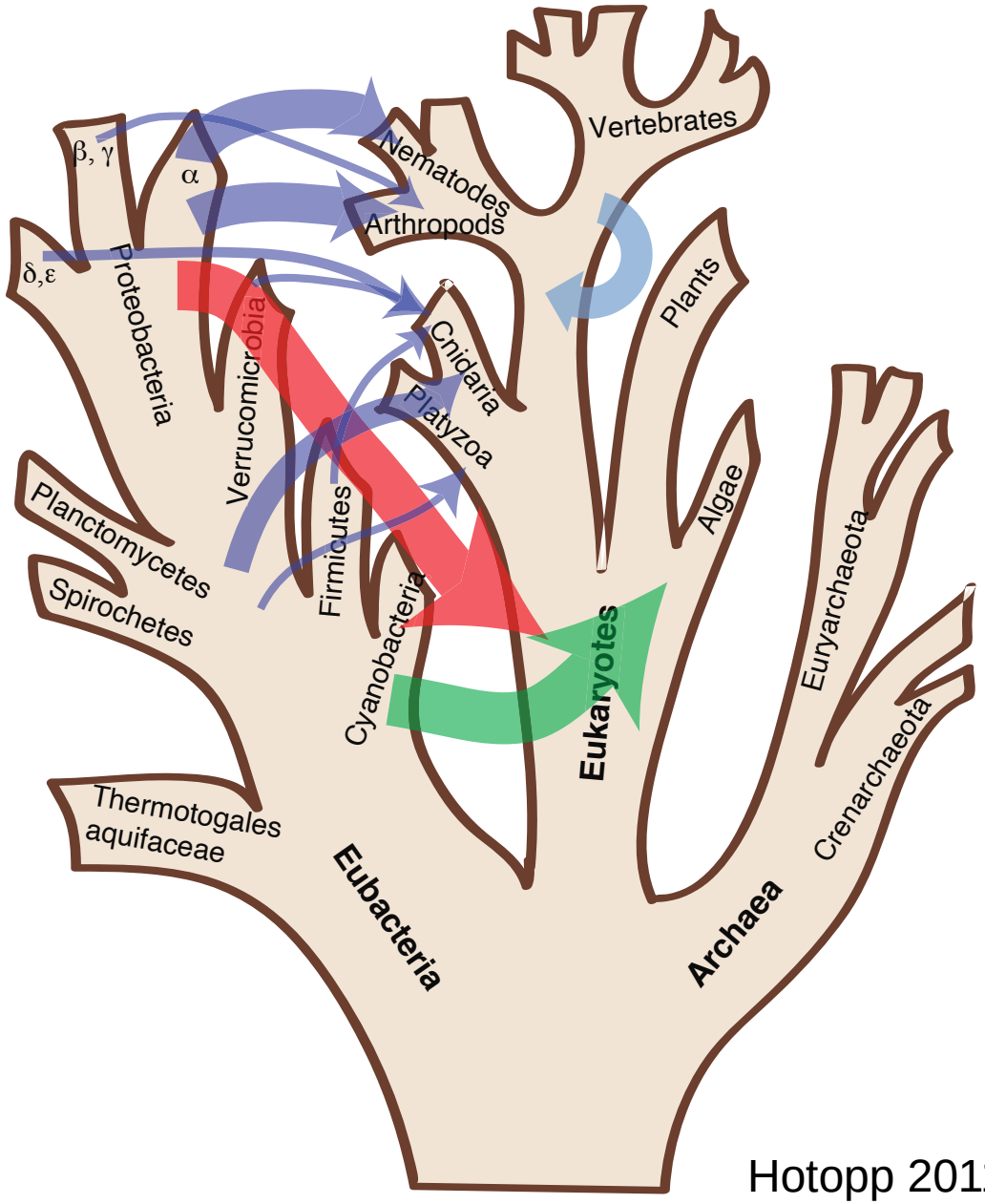
D. subpulchrella India, South East Asia, China, Japan
D. pulchrella India, South East Asia, southern China

temperate
tropical

Anopheles mosquitoes
Vector of malaria

An. gambiae s.s.
An. arabiensis
An. coluzzii
An. amharicus
An. melas
An. merus
An. bwambae
An. quadriannulatus

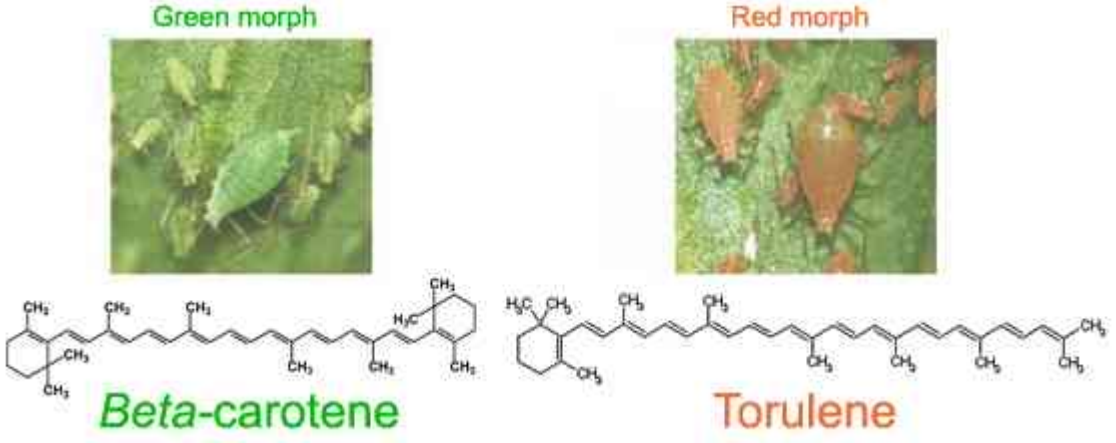
Risk of horizontal transfer

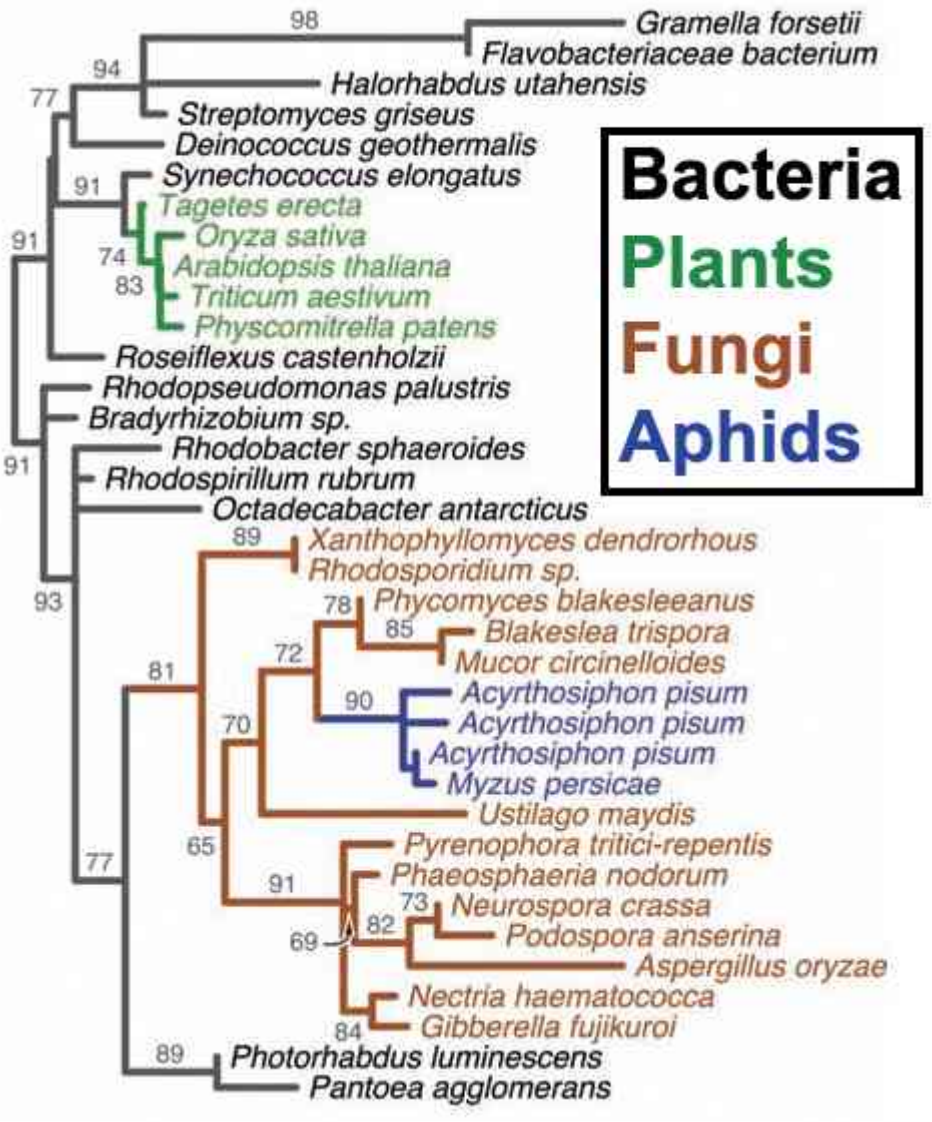


Size of transferred DNA
several kb
up to 150 kb in plants (Dunning et al. 2019) and animals (Inoue et al. 2017)

Hotopp 2011
TRENDS in Genetics

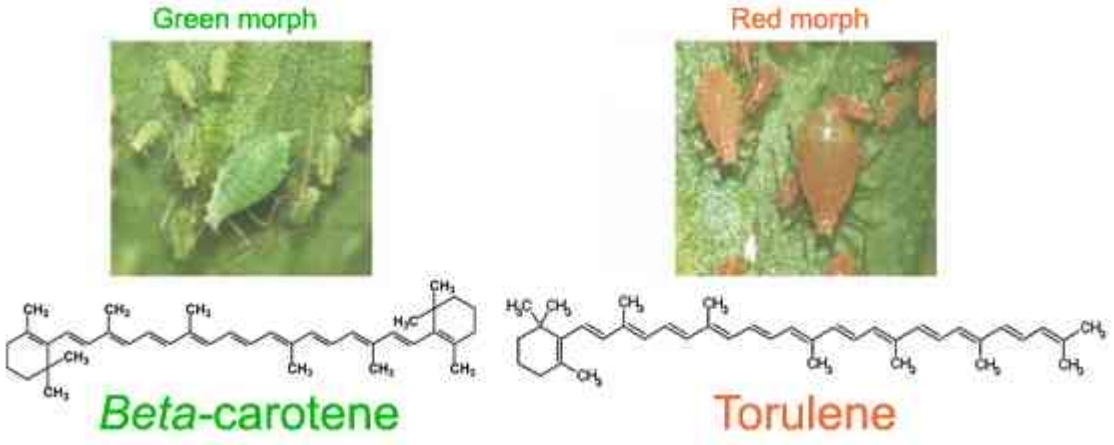
Genetic plagiarism of body color in aphids



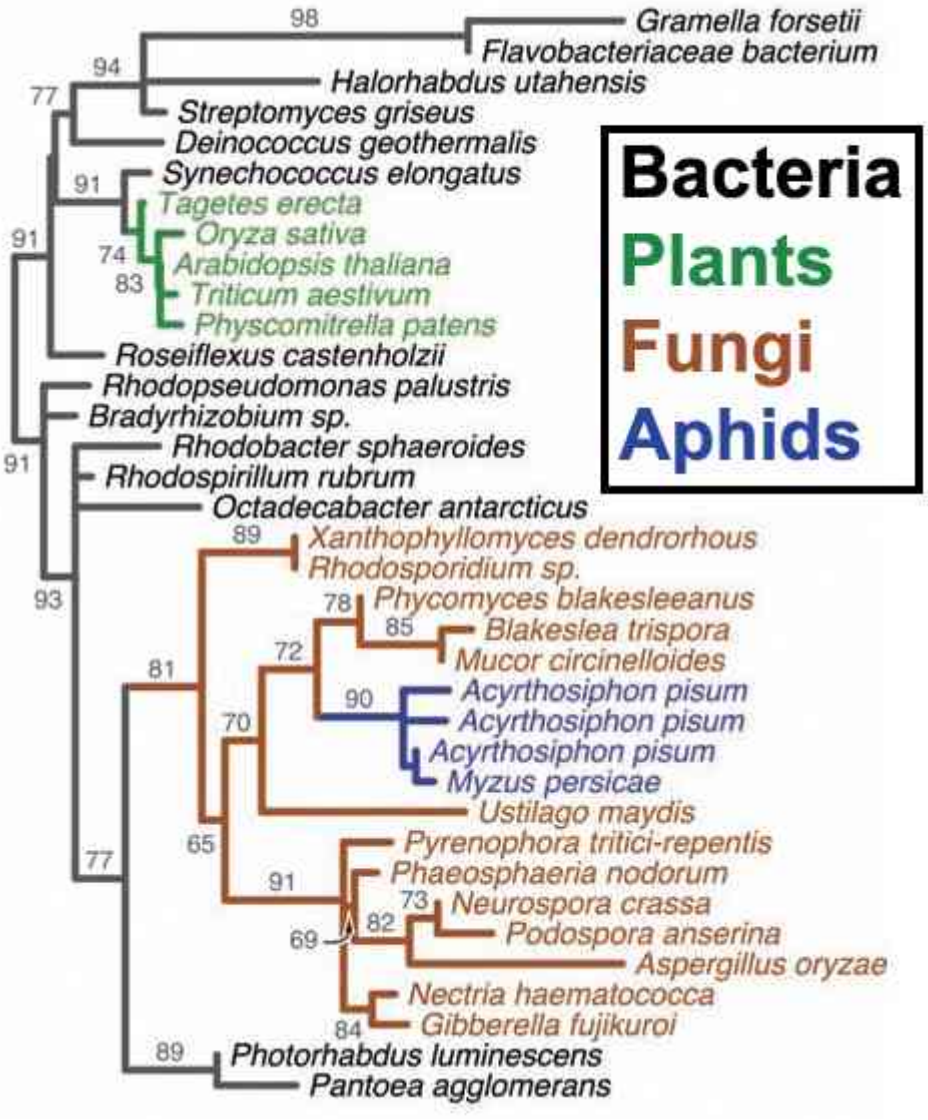


Moran and Jarvik 2010 Science

Genetic plagiarism of body color in aphids

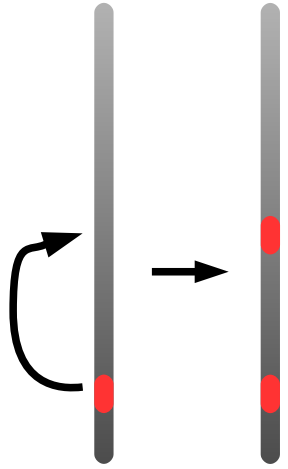


Carotenoid biosynthesis enzymes transferred also from fungi to the two-spotted spider mite (Altincicek 2012)



Moran and Jarvik 2010 Science

Transposable Element

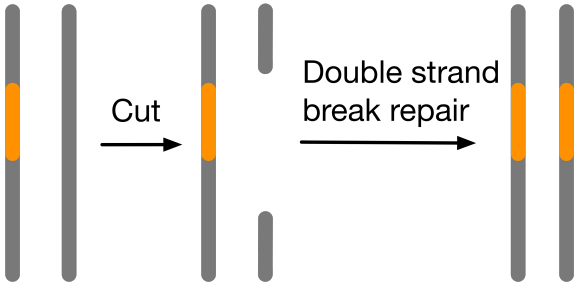


Many per genome

Cuts and inserts itself at multiple sites

Have mechanisms to integrate into DNA

Homing endonuclease



One per genome

Cuts and inserts itself at a target site

Gene drive

One per genome

Cuts and inserts itself at a target site

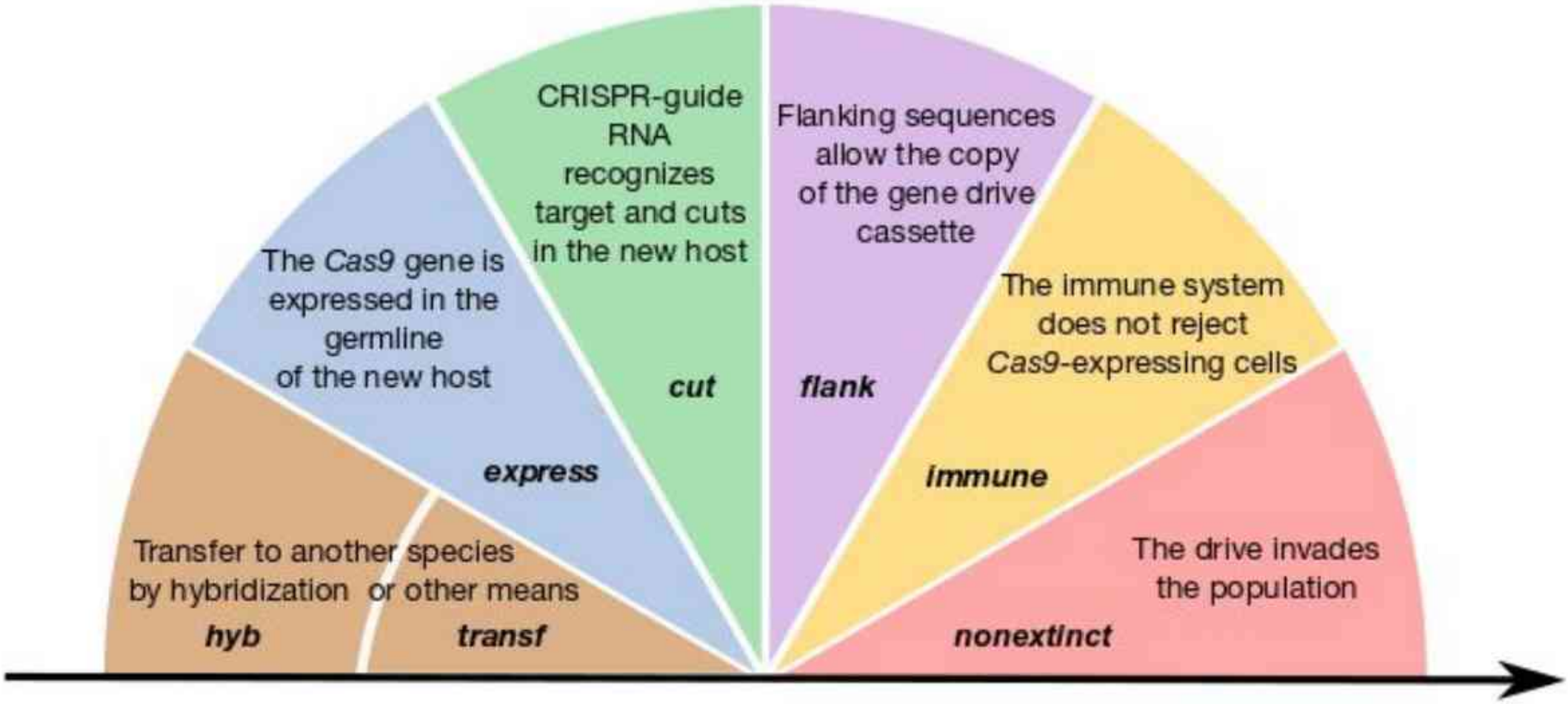
Rate of Horizontal Transfer

0.035 events per TE family per million years between 3 Drosophila species (Bartolomé et al. 2009)

Unknown

cox1: 70 events in 165 plant species (Sanchez-Puerta et al. 2008)

Unknown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express
Transfer to another species by hybridization or other means
transf
hyb

Drosophila, Mosquitoes → Insects/Diptera
Mice → Mammals

Target Species	Cis-regulatory sequences for Cas9 expression	Cis-regulatory sequences for guide RNA expression	Reference
Drosophila melanogaster	*D. melanogaster vasa*	*D. melanogaster U6:3*	(Gantz and Bier 2015)
Anopheles stephensi	*A. stephensi vasa*	*A. stephensi U6A*	(Gantz *et al.* 2015)
Anopheles gambiae	*A. gambiae vasa2*	*A. gambiae U6*	(Hammond *et al.* 2016)
Drosophila melanogaster	*D. melanogaster vasa* *D. melanogaster nanos*	*D. melanogaster U6:3*	(Champer *et al.* 2017)
Drosophila melanogaster	*D. melanogaster Sry-alpha* *D. melanogaster DNApol-α180* *D. melanogaster Rcd-1r*	*D. melanogaster U6:3*	(KaramiNejadRanjbar *et al.* 2018)
Anopheles gambiae	*A. gambiae zpg*	*A. gambiae U6*	(Kyrou *et al.* 2018)
Mus musculus	*M. musculus vasa* *M. musculus Stra8*	*H. sapiens U6*	(Grunwald *et al.* 2019)

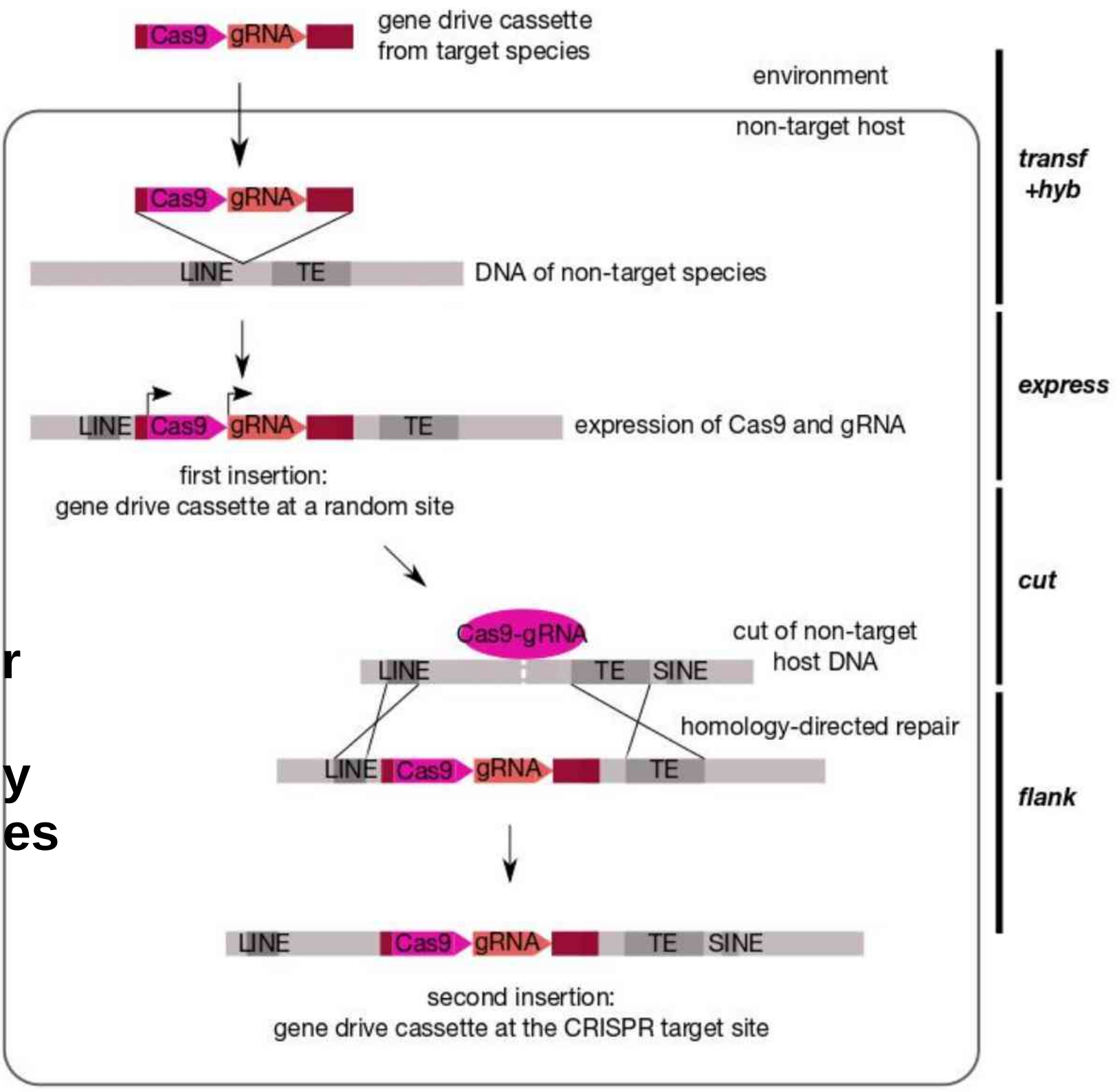
CRISPR-guide RNA can cut DNA in other species

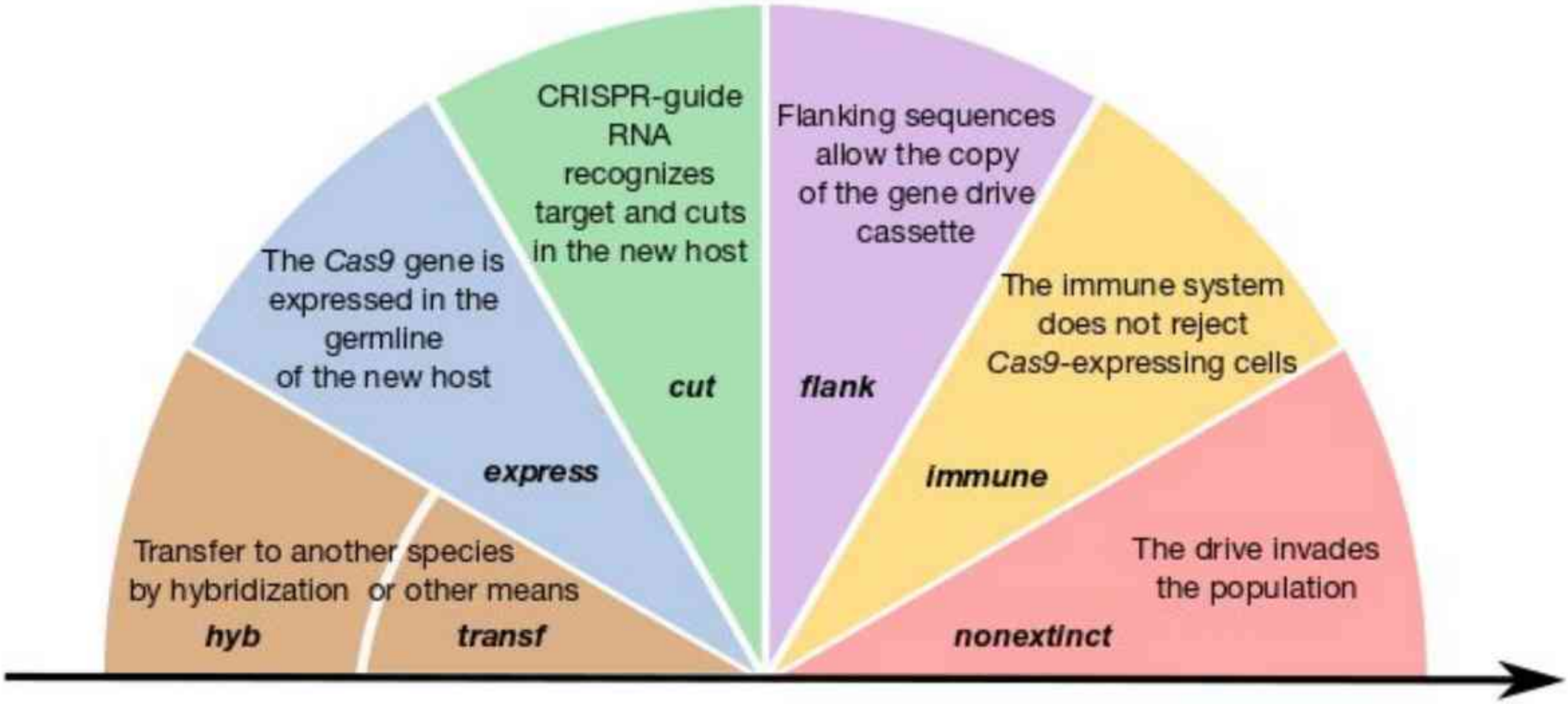
Sample of BLAST hits

Target: *tyrosinase* in *Mus musculus*			
ATGGCCGATAGGTGCAT**AGG**	ATGGCCGATAGGTGCAT**CGG**	ATGGCCGATAGGTGCAT**GGG**	ATGGCCGATAGGTGCAT**TGG**
Mammals	**Mammals**	**Mammals**	**Mammals**
		Pan troglodytes	*Cricetulus griseus*
			Grammomys surdaster
			Homo sapiens
	Amphibians	**Birds**	*Mus caroli*
	Rhinatrema bivittatum	*Streptopelia turtur*	*Mus musculus*
			Mus pahari
			Peromyscus leucopus
Bony fishes	**Bony fishes**	**Bony fishes**	
Gadus morhua	*Sparus aurata*	*Anabas testudineus*	
Betta splendens	*Sphaeramia orbicularis*	Cottoperca gobio	
		Gadus morhua	
		Parambassis ranga	
Insects	**Insects**	**Insects**	**Insects**
		Drosophila elegans	*Diabrotica virgifera*

(...)

Possible scenario for insertion in a distantly related species

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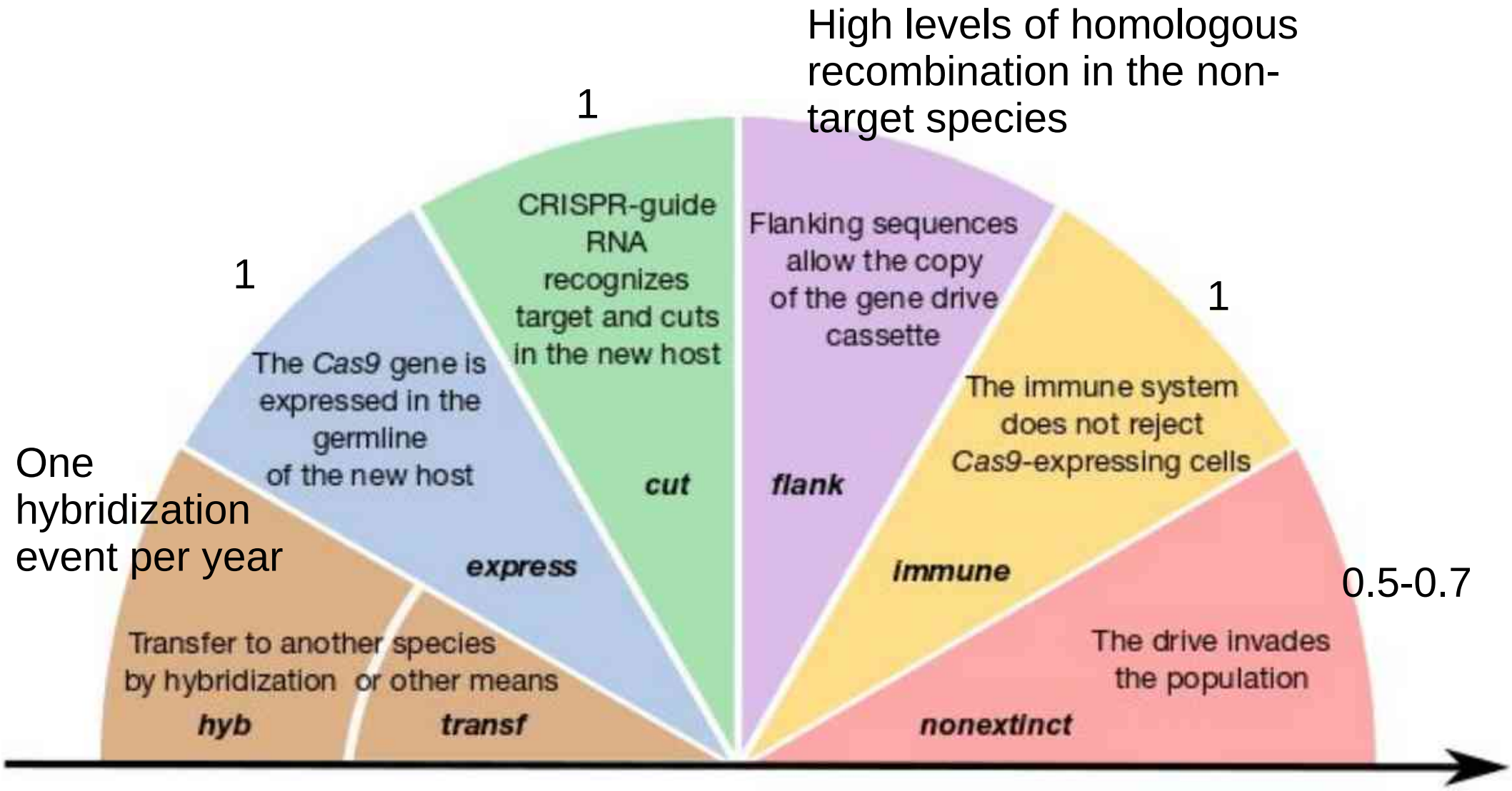
Transfer to another species by hybridization or other means
transf
hyb

In a worst-case scenario

High levels of homologous recombination in the non-target species



One hybridization event per year

Resulting risk of contamination: 0.5 per year

Gene drives: good or bad?

May eradicate diseases and pest species

Less expensive than other methods

Potentially faster than other methods

Potentially more powerful than other methods

Potentially less efficient than expected (resistance via mutations in the target site, cryptic species)

An uncontrolled system released in the wild

Impact on other species and ecosystems not quantified

DOI : 10.4267/2042/70673

LE FORÇAGE GÉNÉTIQUE (GENE DRIVE) ET SES APPLICATIONS

@Biol4Ever

GENE DRIVE AND ITS APPLICATIONS

Par Virginie COURTIER-ORGOGOZO[1]

Antoine Danchin
Pierre-Henri Gouyon
Christophe Boete

Florence Débarre
Clément Gilbert
Arnaud Martin
Nicolas Rode

bioRxiv
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Jean David
Scott Gilbert
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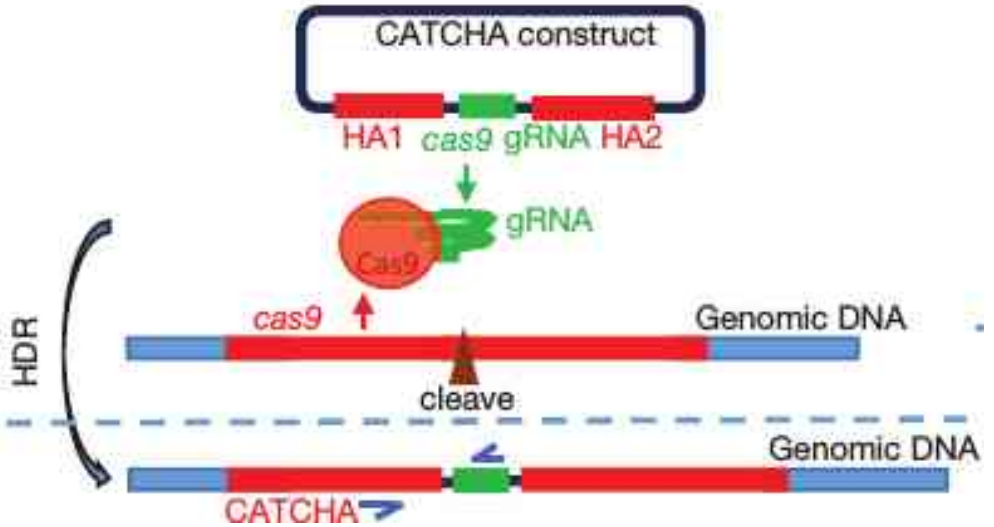
Bruno Lemaitre
Olivier Panaud
Thomas Pradeu
Benjamin Prud'homme
Michael Turelli
Mylène Weill

Need to stop a drive? Use another one!



CORRESPONDENCE

Nature Biotechnologies, février 2016

Bing Wu[1,2], Liqun Luo[1] & Xiaojing J Gao[1–3]

Cas9-triggered chain ablation of *cas9* as a gene drive brake