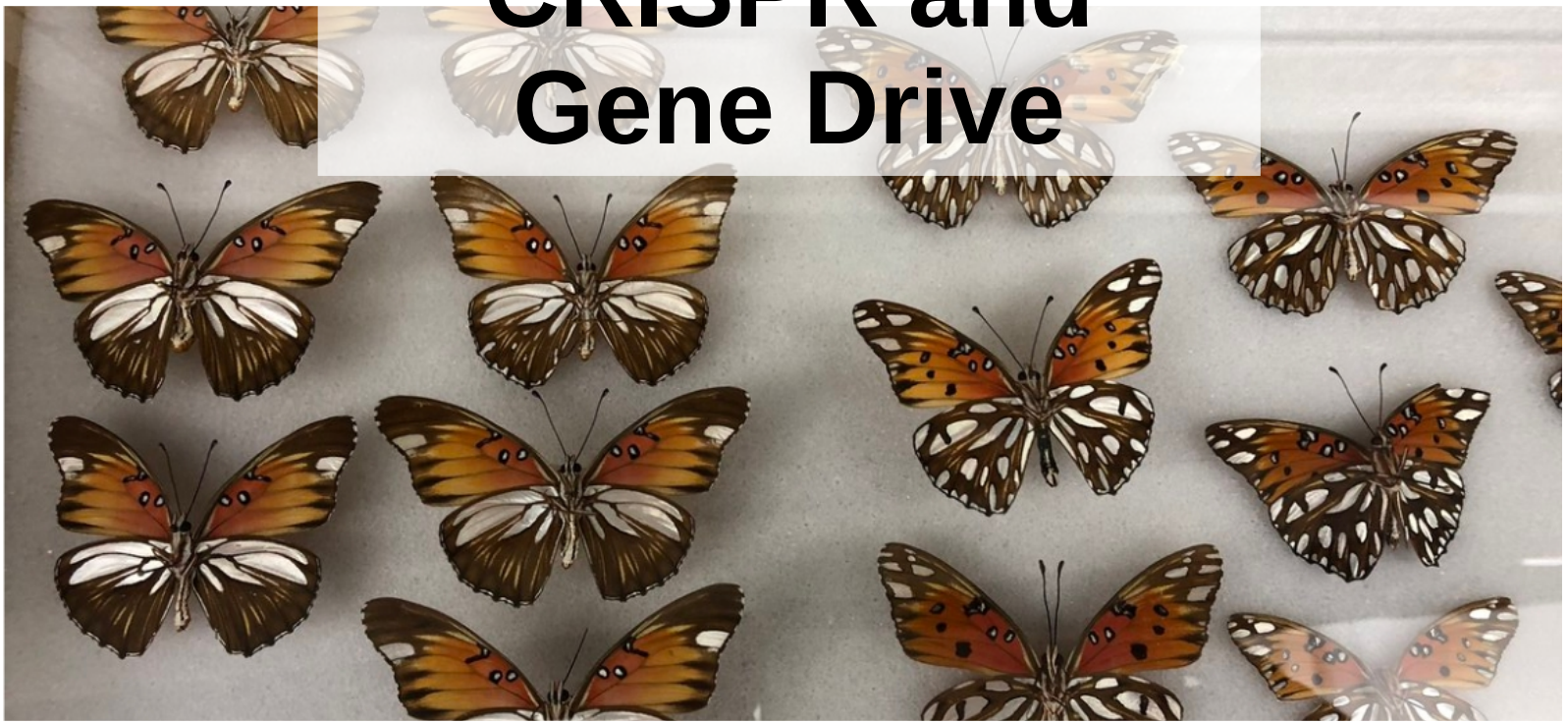
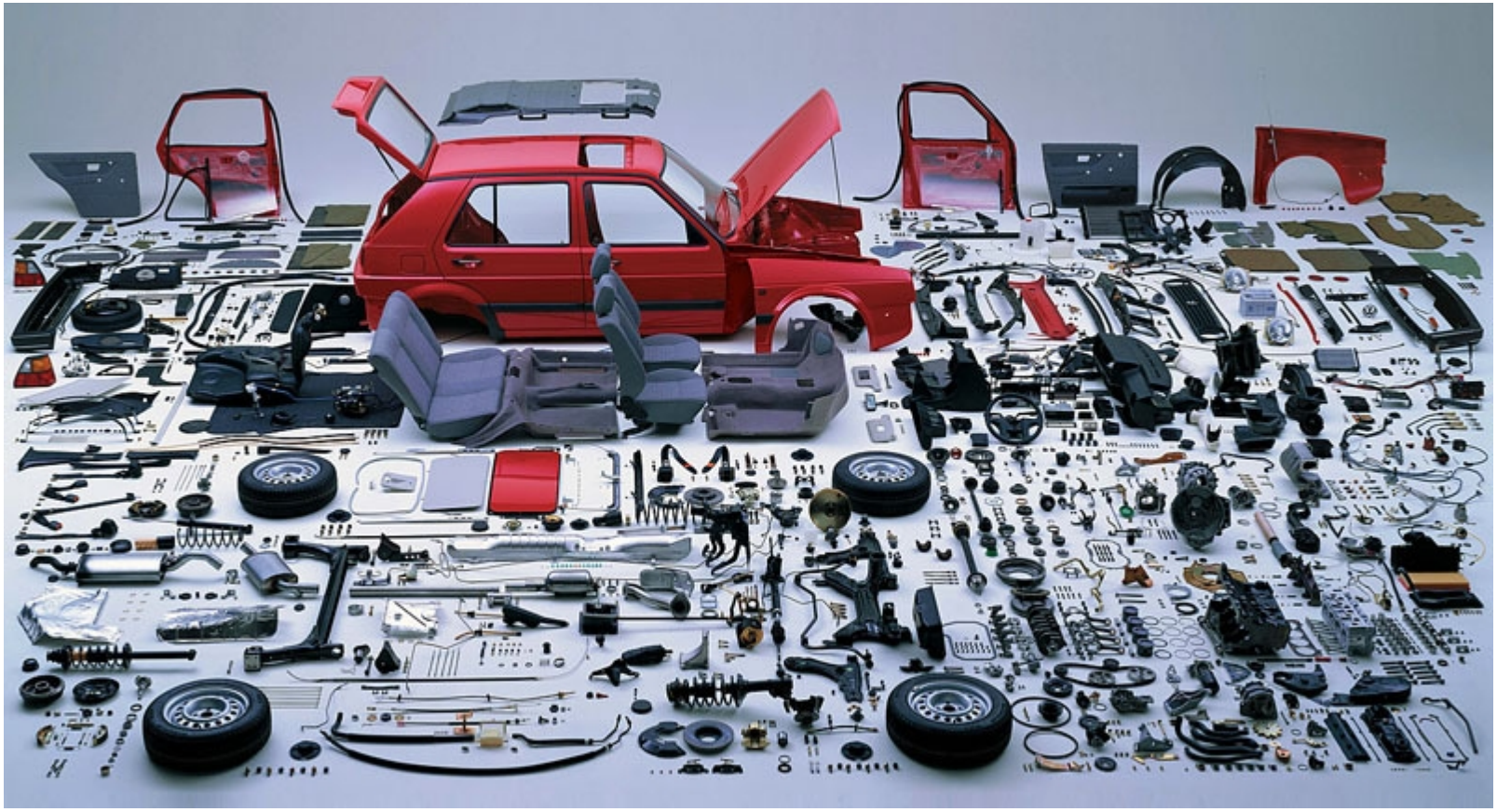
Genetic screens, CRISPR and Gene Drive



Virginie Courtier-Orgogozo
Institut Jacques Monod, Paris

Biochemistry versus Genetics



Genetic screens

unbiased approach:
Biochemical screen (kinase assay, methylation assay, etc…)
Genetic screen (for genes)

A genetic screen

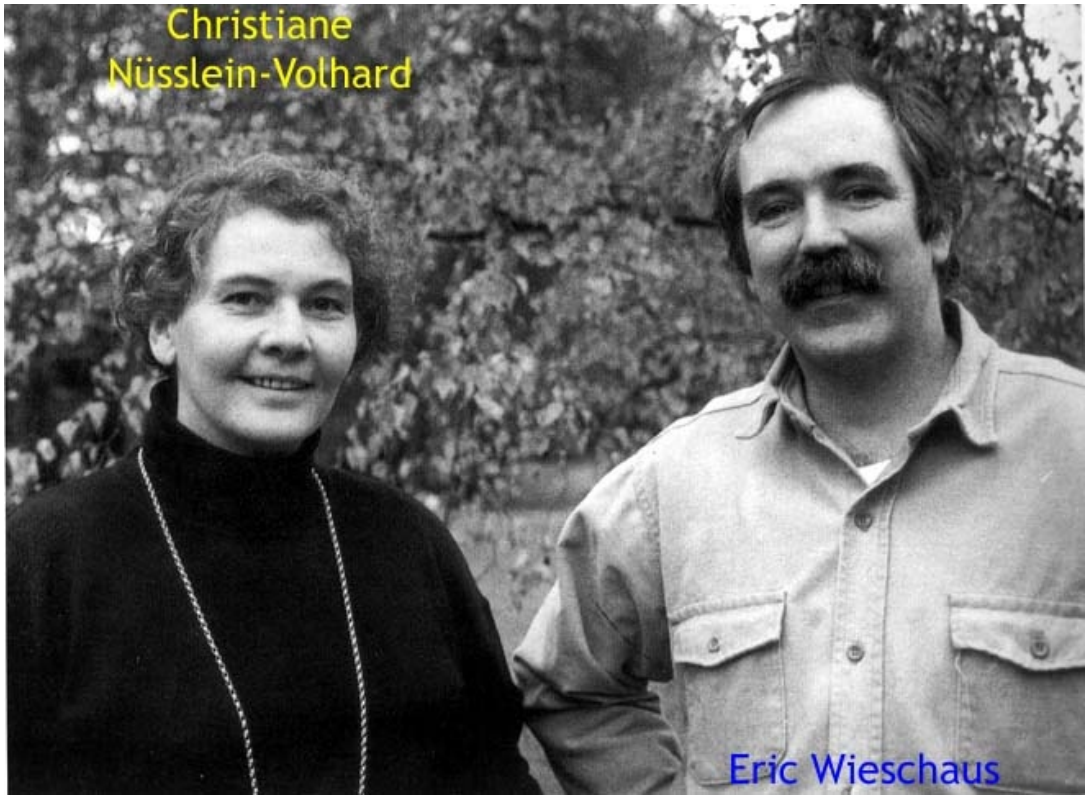
is a method used to find genes involved in a given phenotype
3 steps :
1. production of mutations
2. selection of individuals with the phenotype of interest
3. identification of the underlying genes

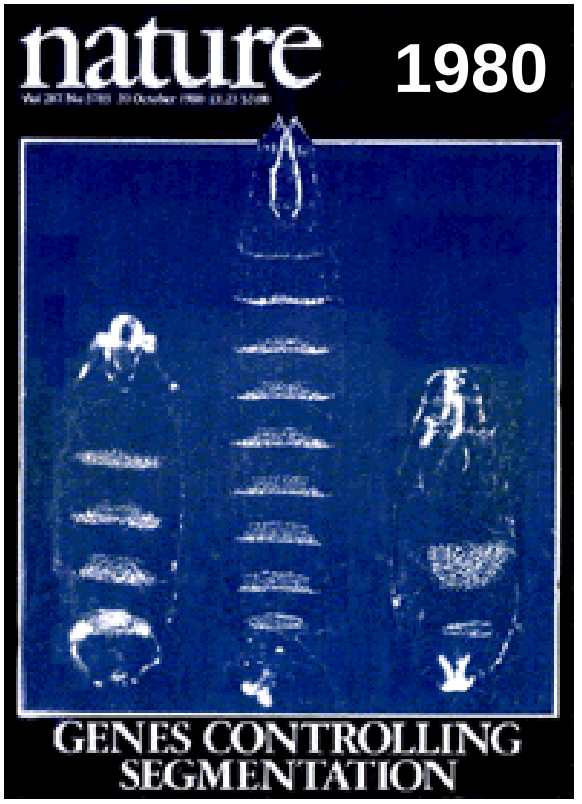
Historical screen
by C. Nüsslein-Wolhard et E. Wieschaus

General principles

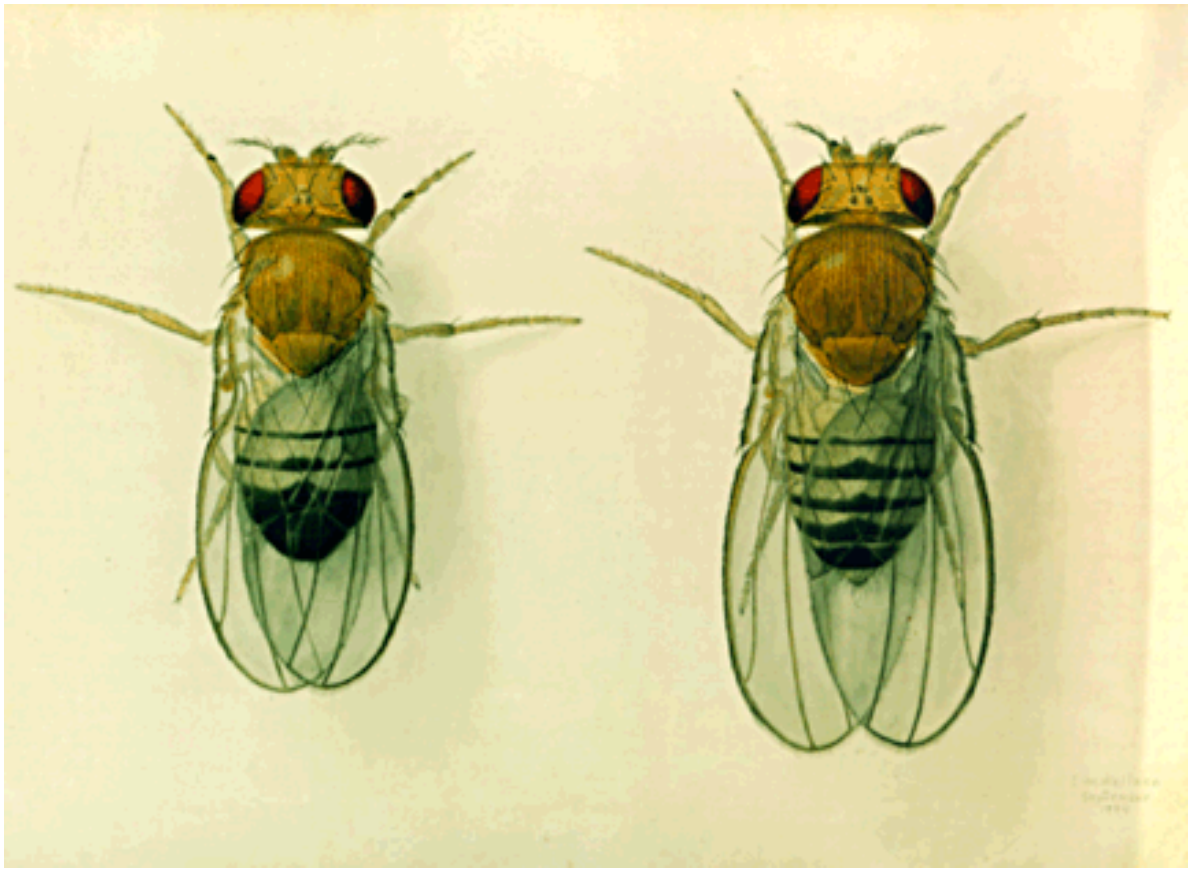
Other types of screen

Historical screen
by C. Nüsslein-Wolhard et E. Wieschaus





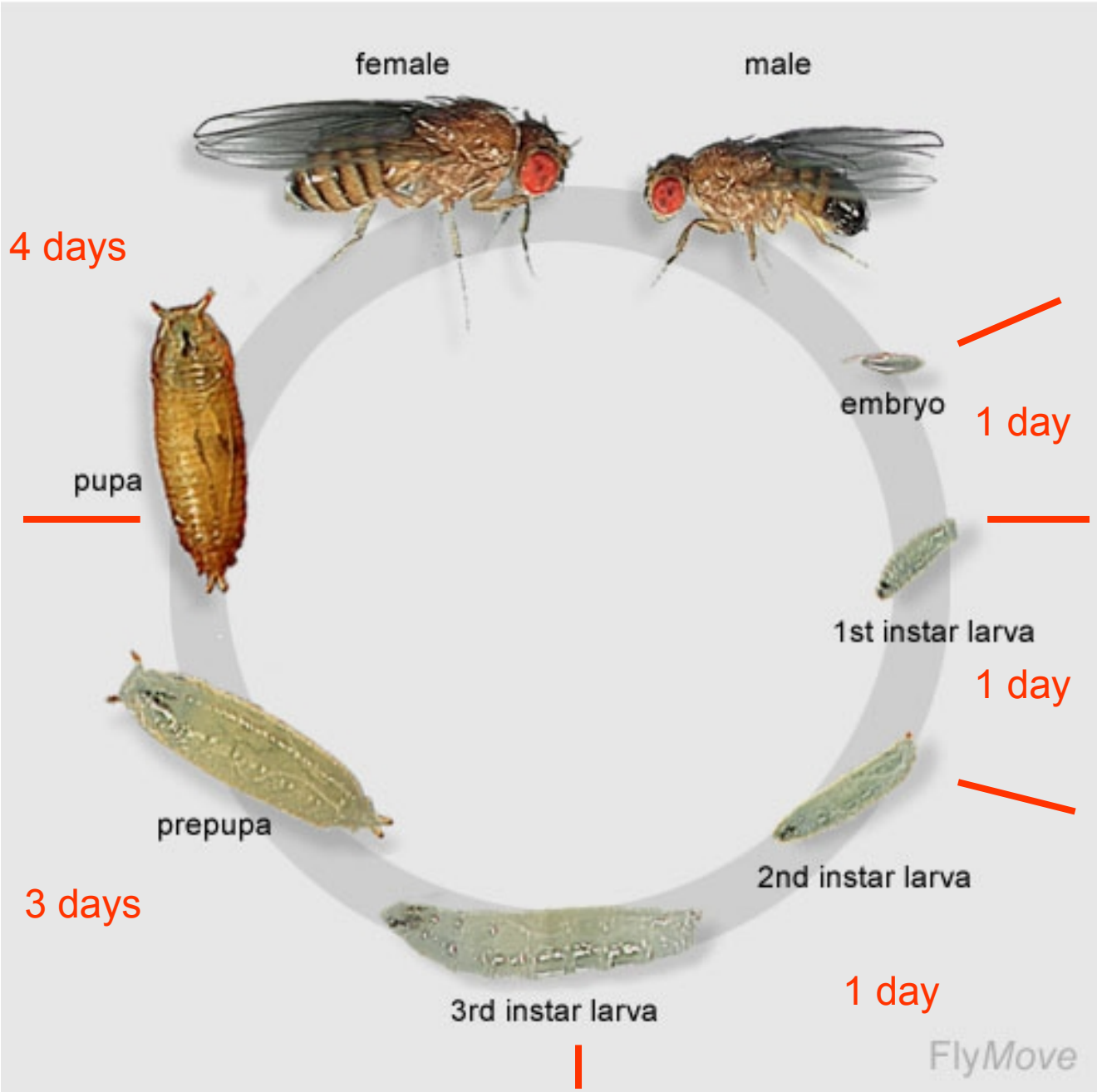
Nobel Price in Physiology/Medecine in 1995
with E. Lewis



♂ ♀


1521U
UAS GFP
813
FlyMove

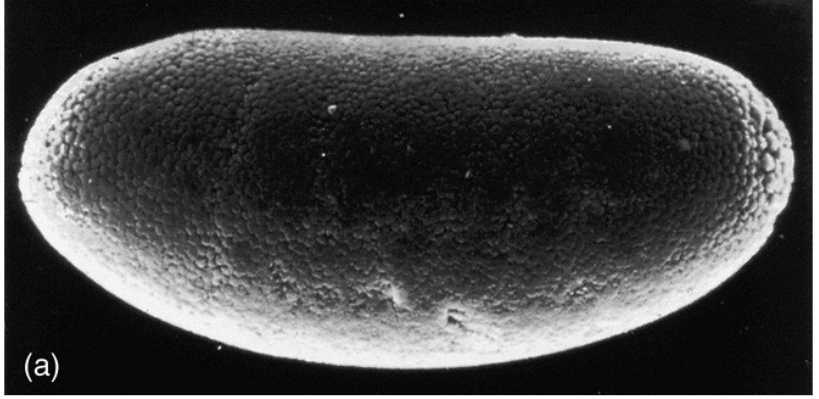
The life cycle of *Drosophila melanogaster*



One Generation
=
10 days

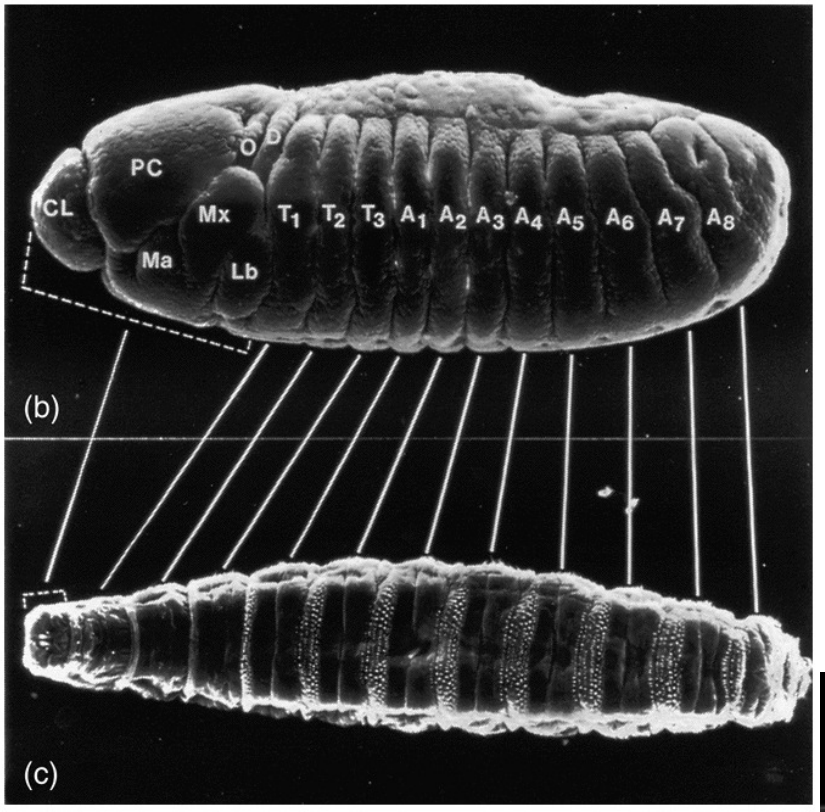
Cellular bastoderm stage

3 h



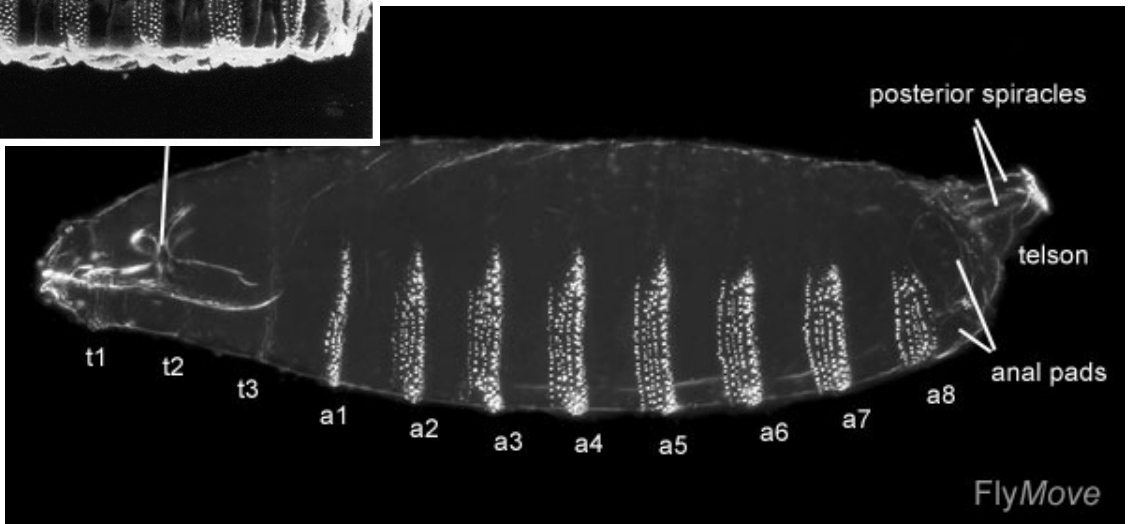
Segmented embryo

10 h



Larva

Wild-type larva



General Strategy

Random mutagenesis of the *Drosophila* genome and screen for mutant phenotypes

↓

Identification of the mutated gene

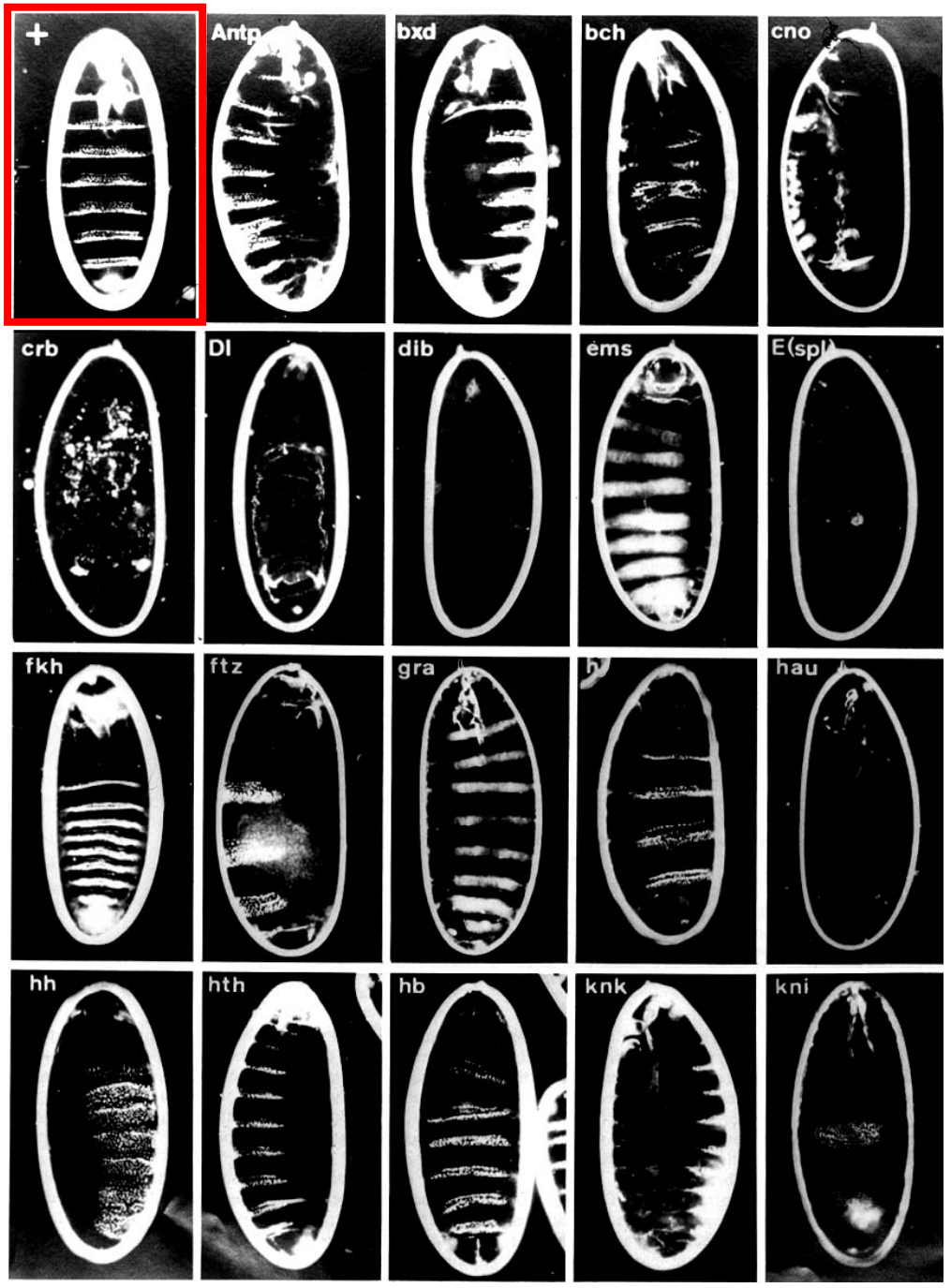
↓

Molecular analysis of the protein function

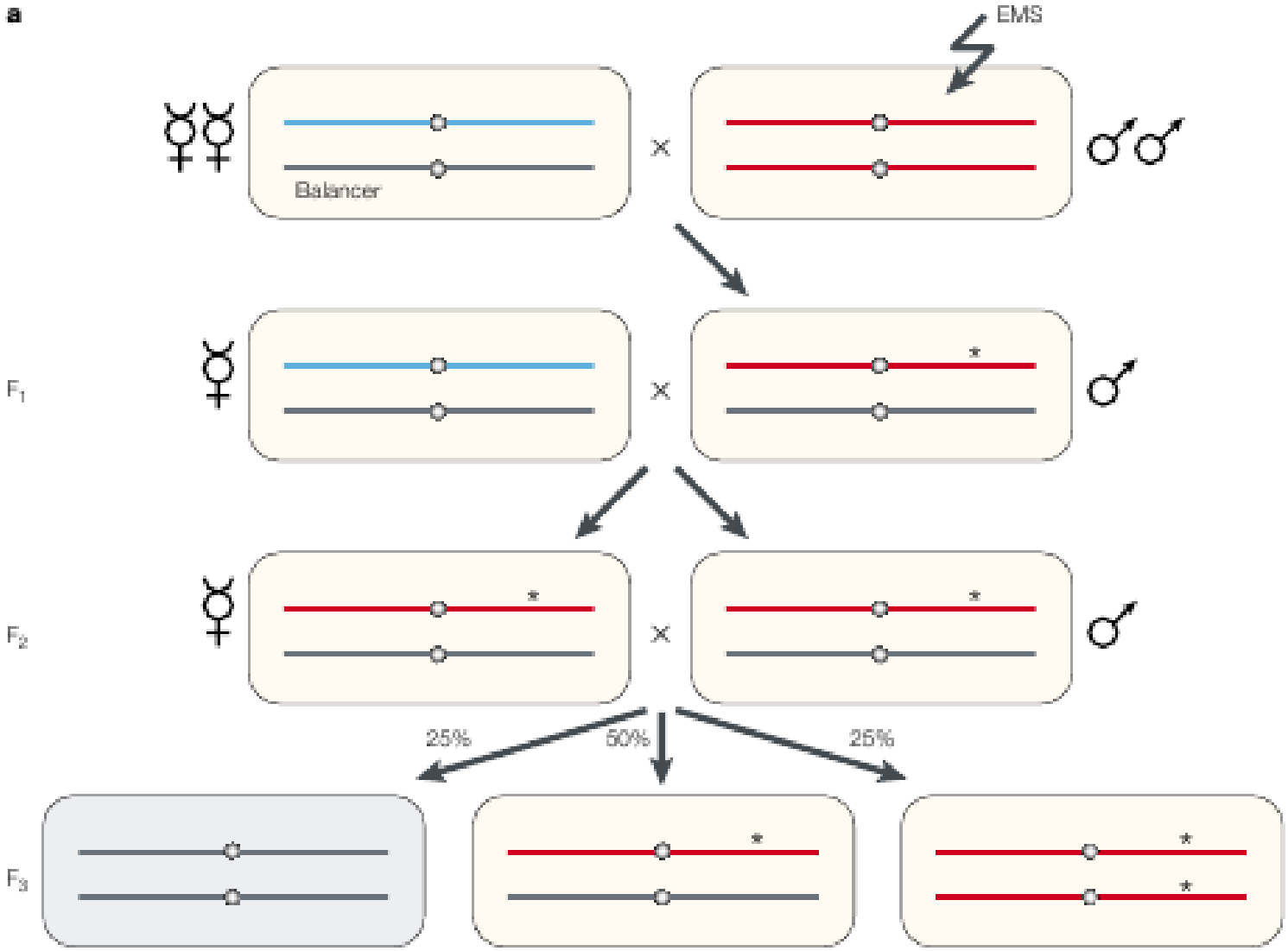
Wild-type

Others= mutants

(aberrant position /shape of trichomes)



Using balancers to screen recessive lethals



St Johnston, NRG 2002

Screen of chromosome 2

Production of 5.764 lines
    including 4.217 homozygote lethal lines

Identification of 7.600 lethal mutations
    including 2.843 mutations causing embryonic lethality
        and 272 mutations embryonic phenotypes

Complementation test for mutations with the same phenotype:
48 **complementation groups** containing on average 5.4 alleles
13 alleles are complémented by all other mutants

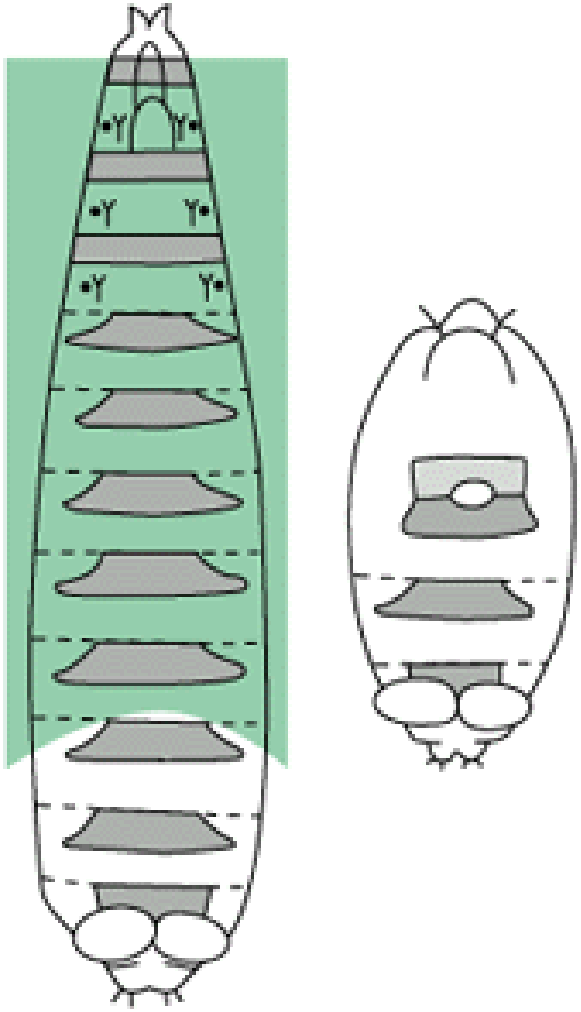
= 61 genes in total

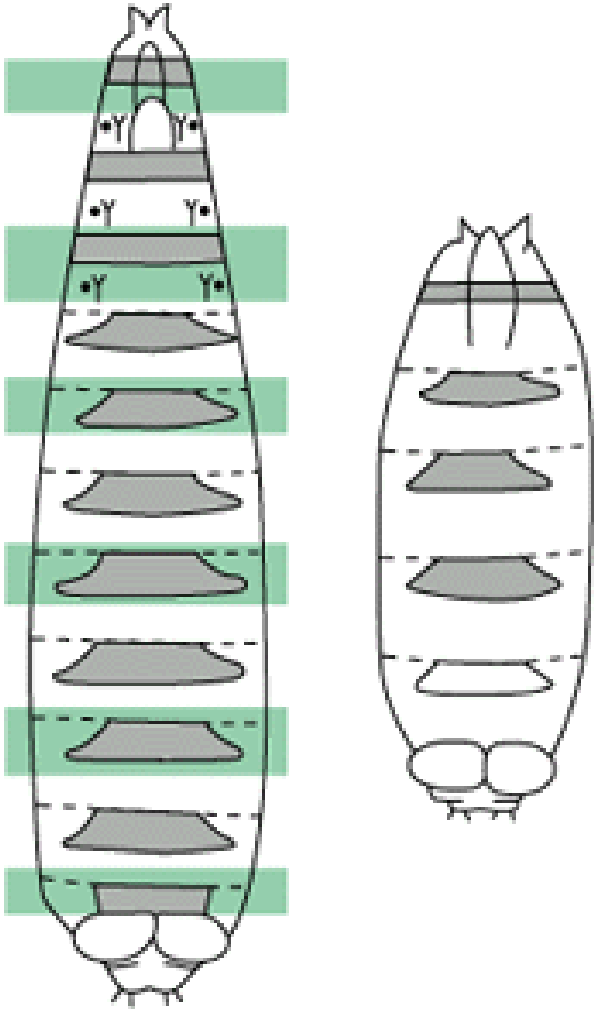
Nusslein-Volhard, Wieschaus (1984) Roux's Arch Dev 193: 267

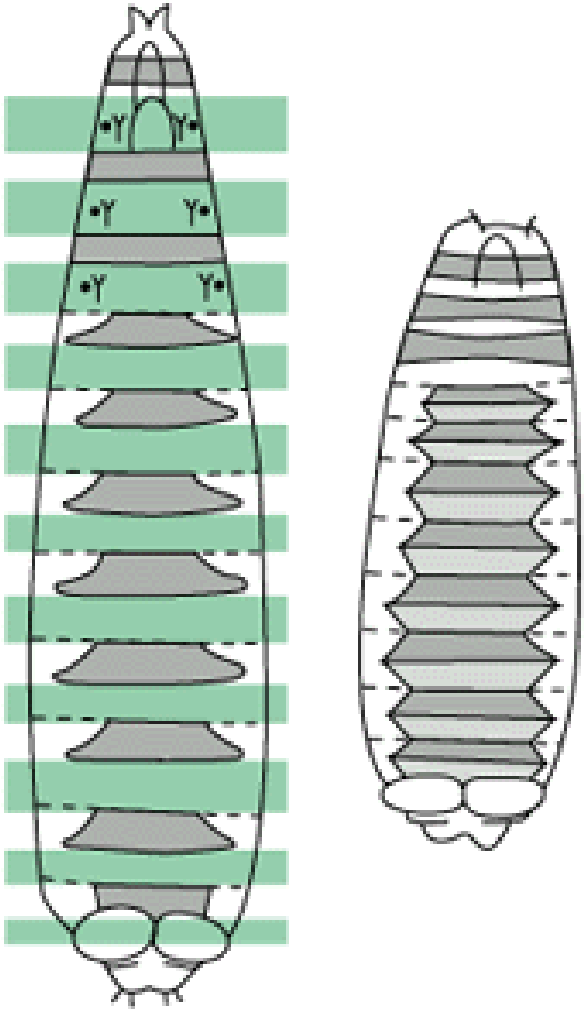
GAP GENE *(Krppel)*

PAIR-RULE GENE
(even-skipped)

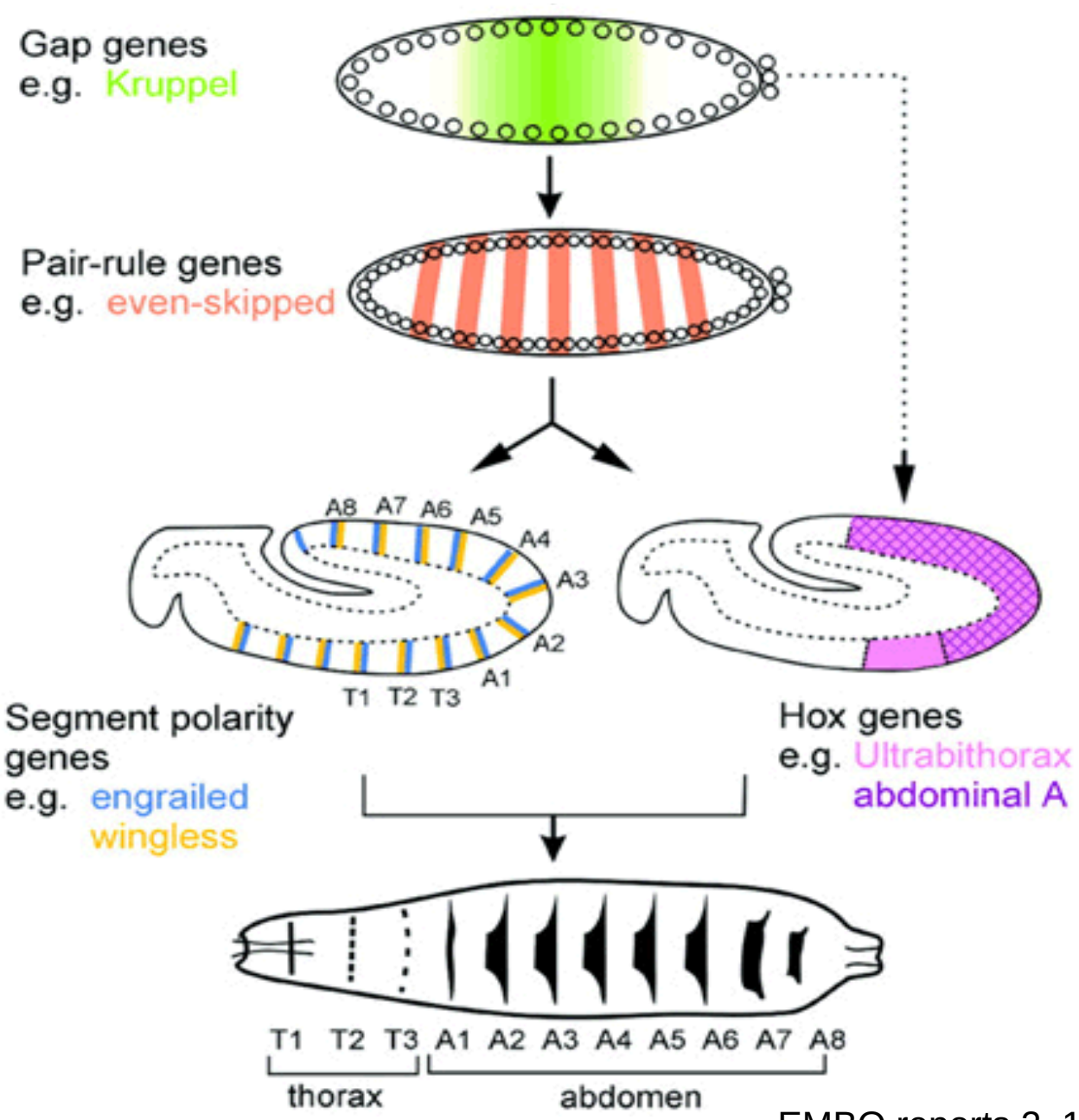
SEGMENT-POLARITY
GENE *(gooseberry)*





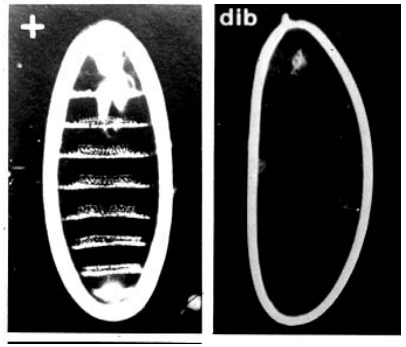


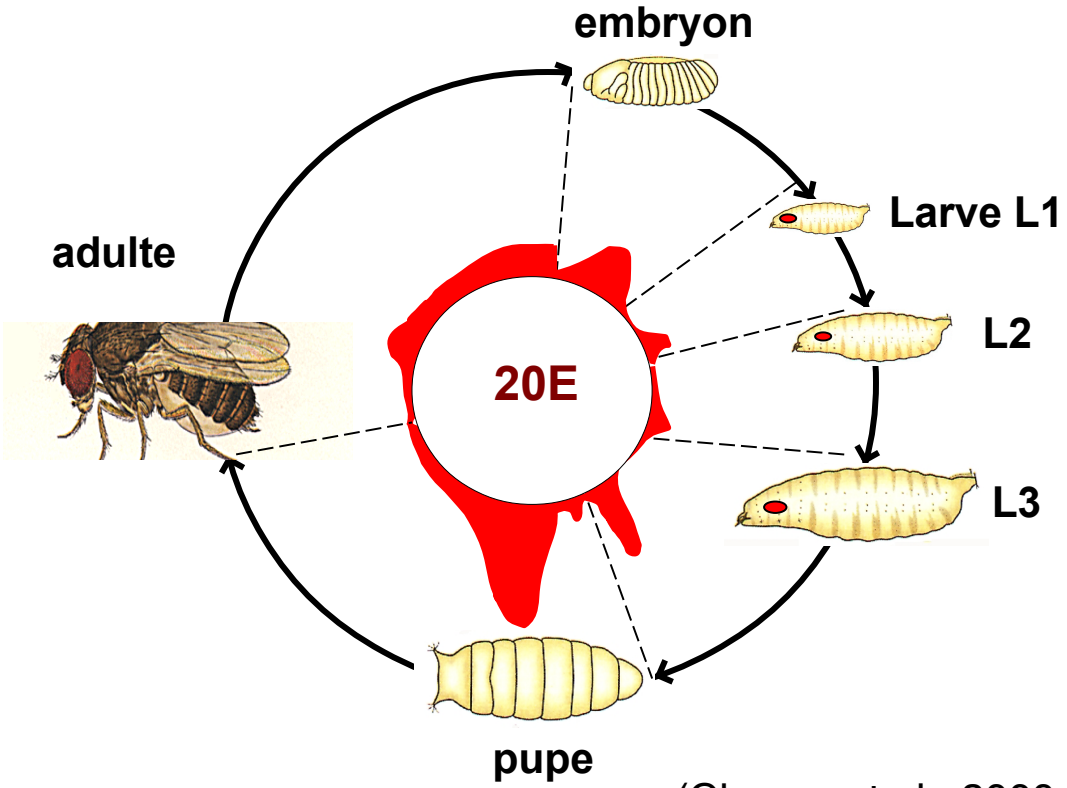
Embryonic development of Drosophila



EMBO reports 2, 12, 1083–1088 (2001)

Halloween mutants: steroid hormone biosynthesis





cholesterol

↓ *nvd*

7-dehydro-cholesterol

↓ *spo, sro*

ketodiol (2,22,25dE)

↓ *phm*

↓ *dib*

↓ *sad*

ecdysone (E)

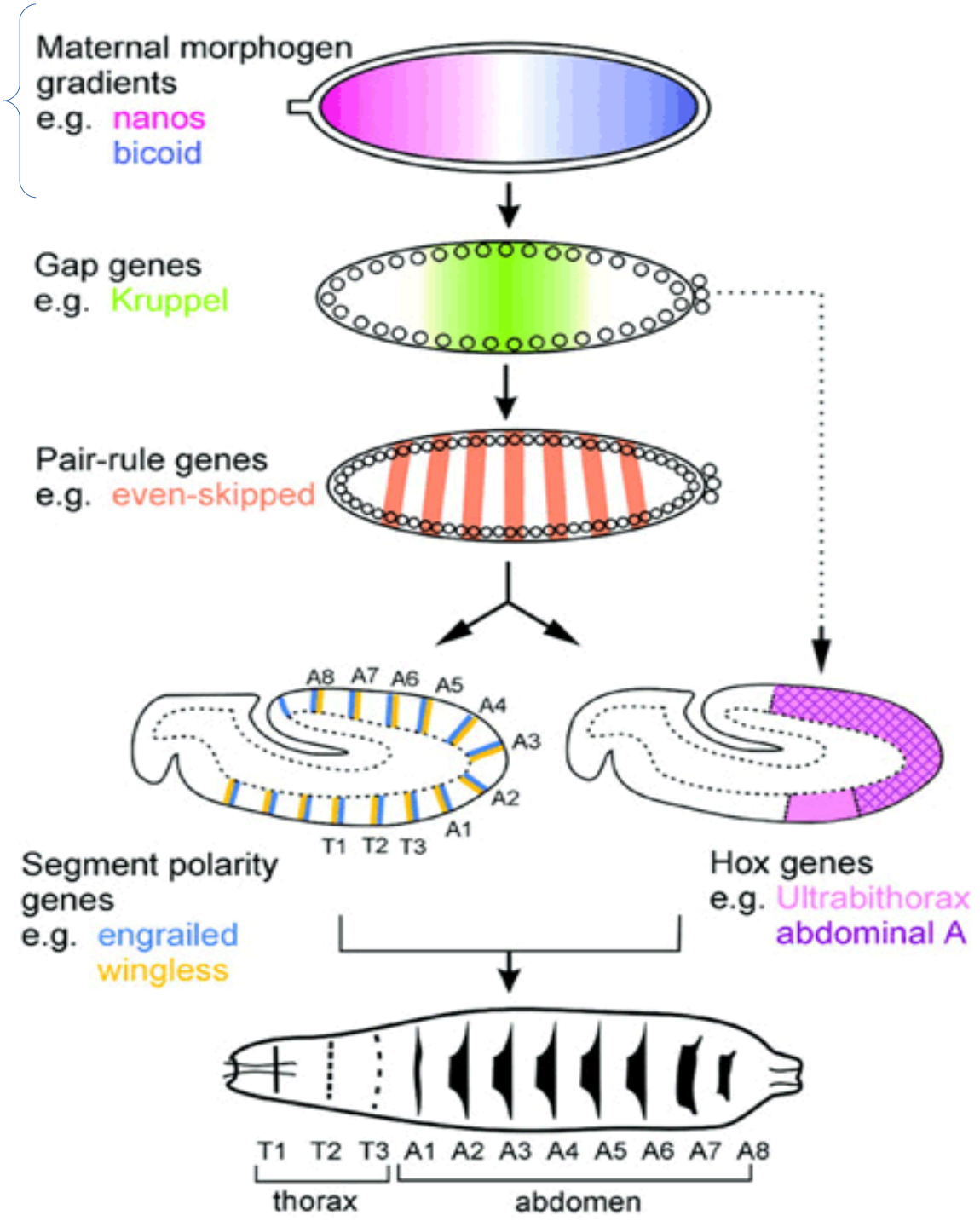
↓ *shd*

20-hydroxy-ecdysone (20E)

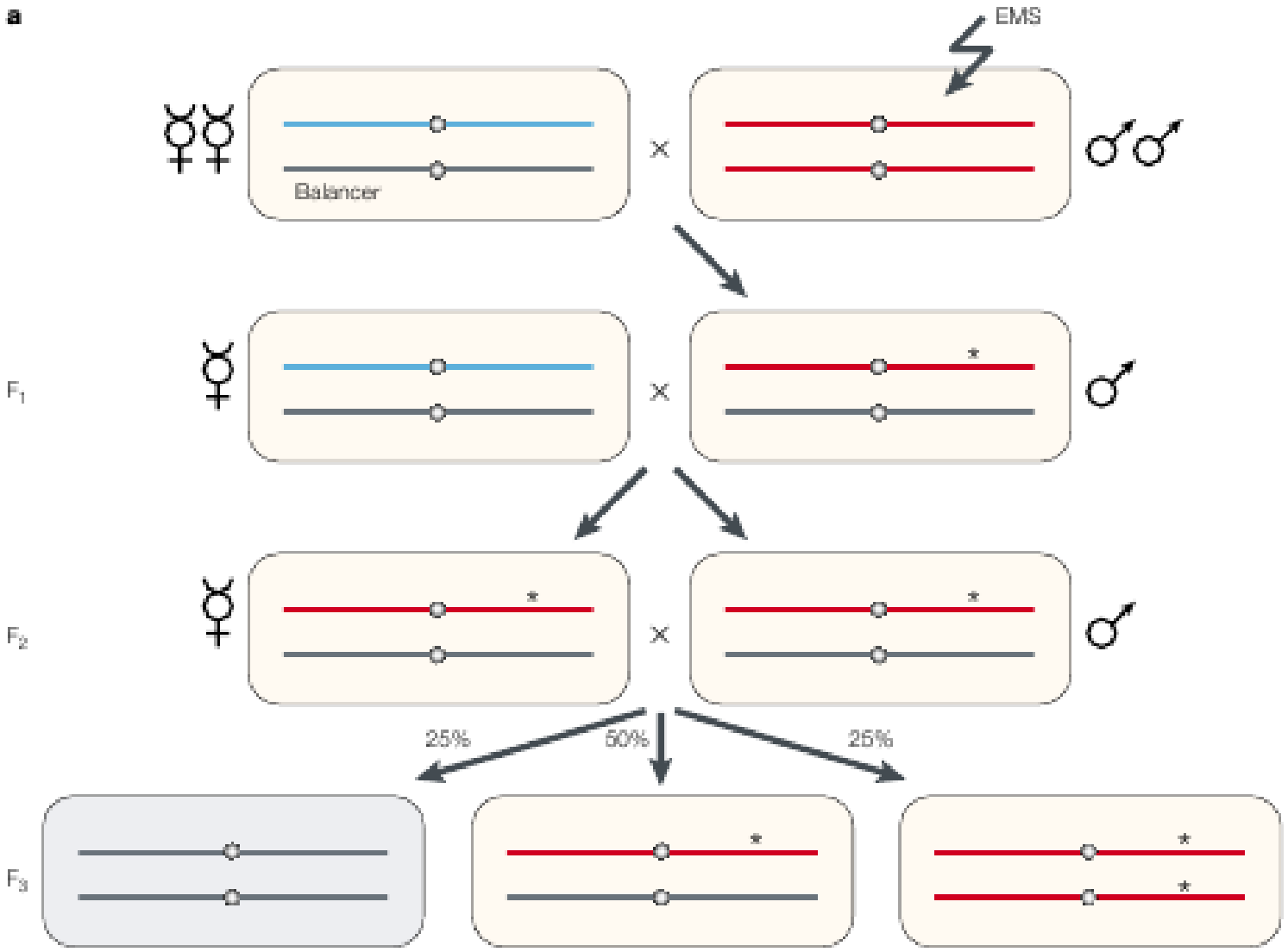
(Chavez et al., 2000; Warren et al., 2002; Petryk et al., 2003; Niwa et al., 2004; Warren et al., 2004; Namiki et al., 2005; Yoshiyama et al., 2006)

Maternal genes

ARNm deposited in the egg before fecundation



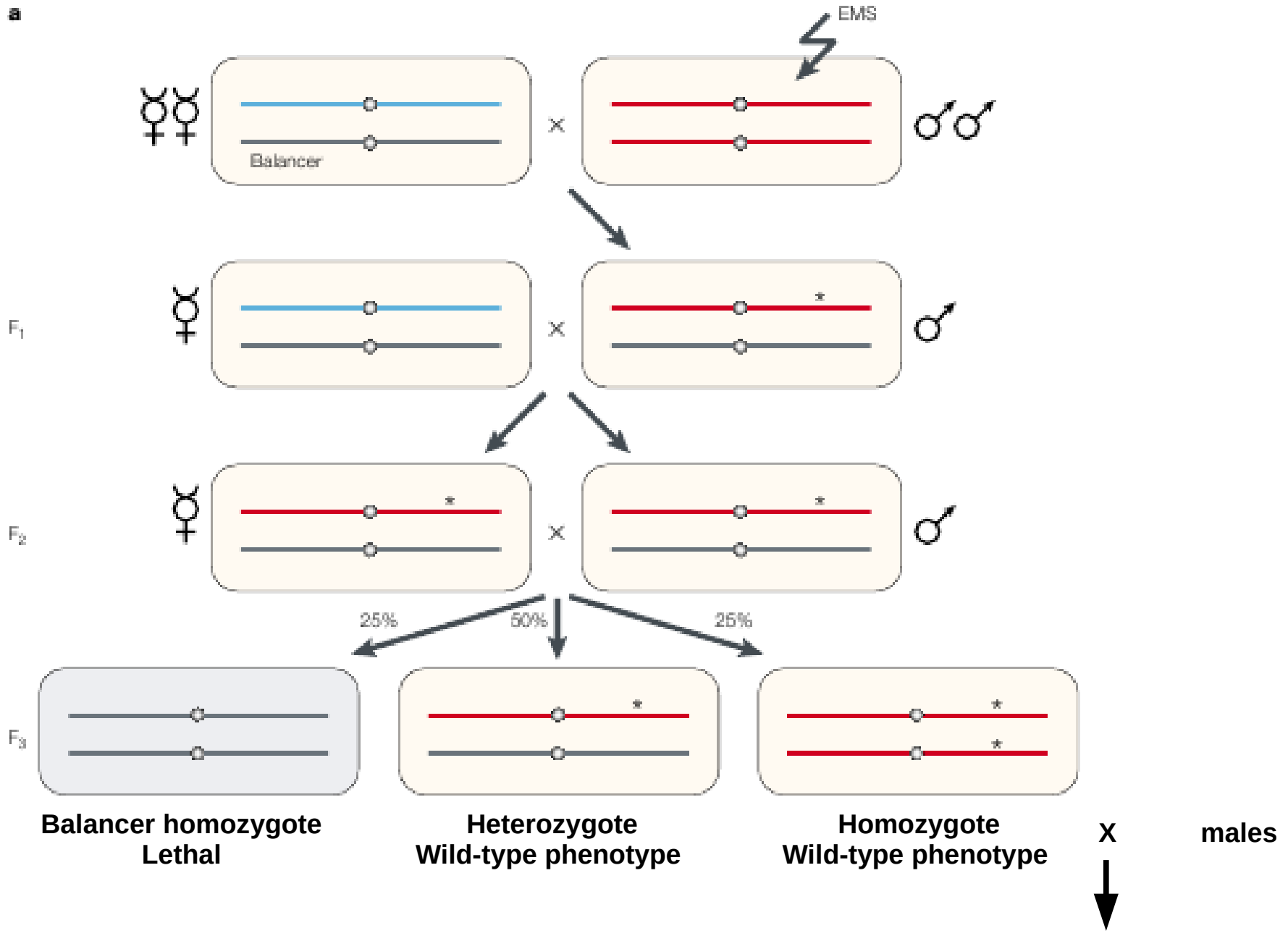
Screen for maternal effect genes



X males

Examination of progeny

Screen for maternal effect genes



Homozygous lethals cannot be identified
(ex : *Fz, Dsh, Apc, Nvd*)

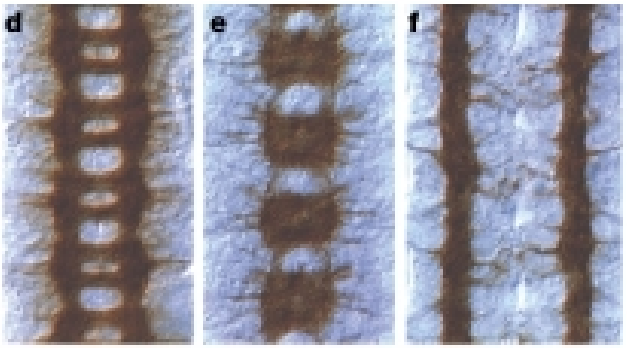
Certain genes involved in embryonic development were not identified with this screen

Maternal effect genes whose mutation is recessive lethal

Genes involved in the development of internal structures (brain, gut, etc.)

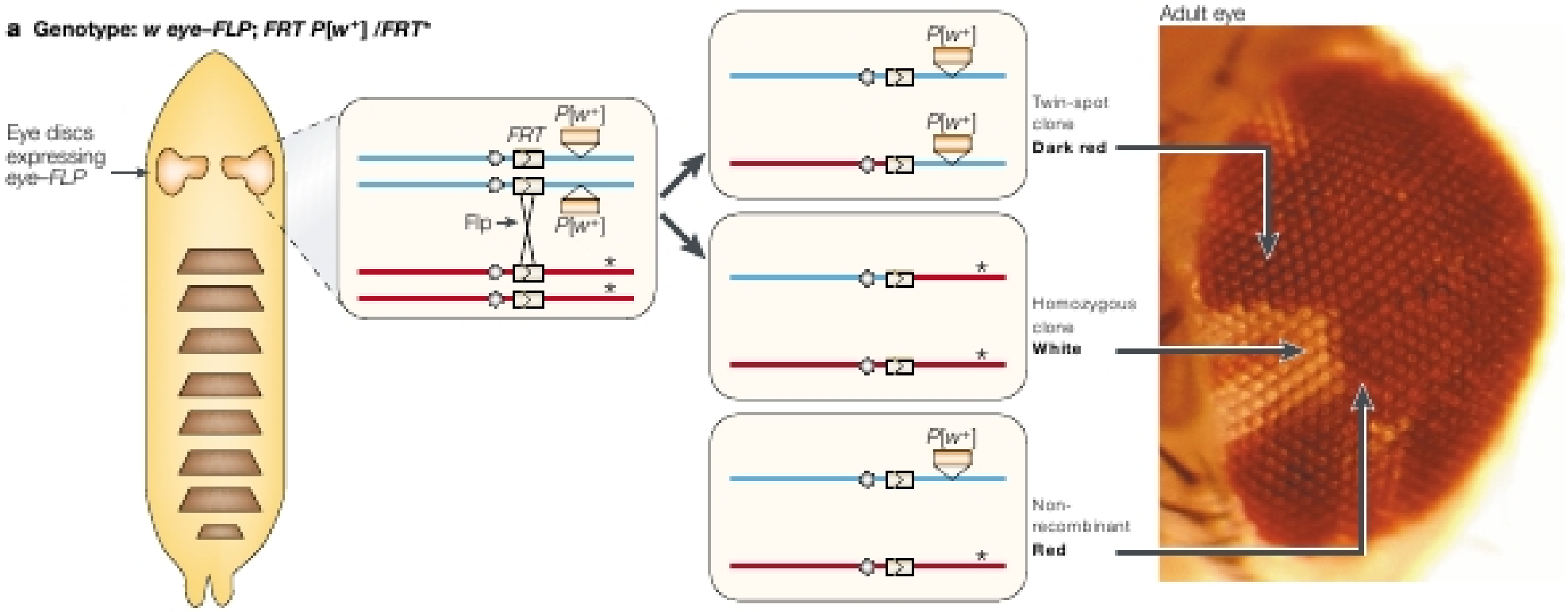
Redundant genes

WT *roundabout commissureless*



With such screens, only the first essential function of a gene can be identified.

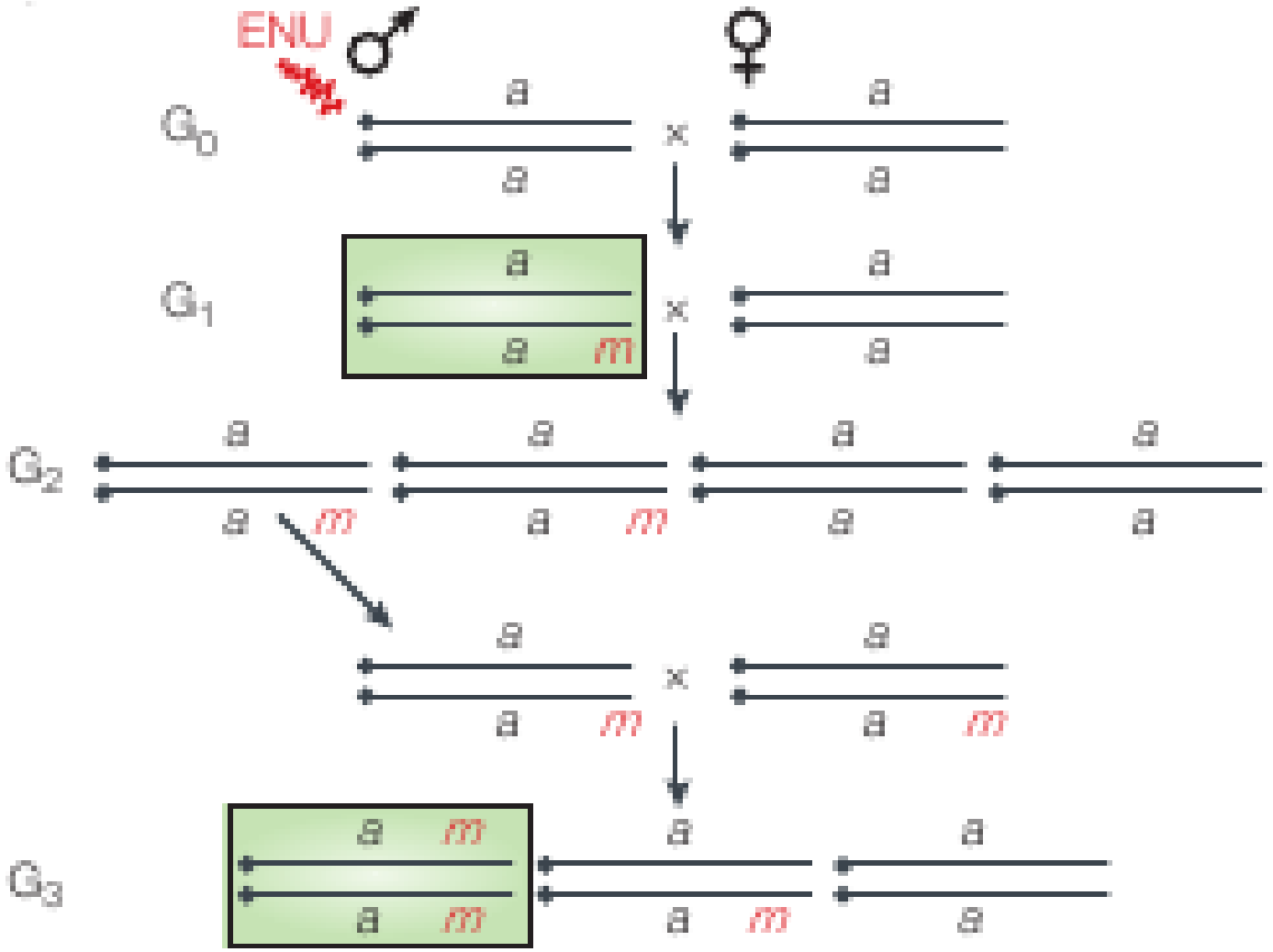
Mitotic clones



Flp = Flippase
FRT = Flippase Recombination Target

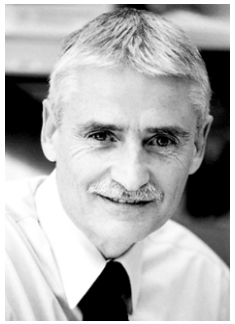
St Johnston NRG 2002

Screen for suppressors or enhancers



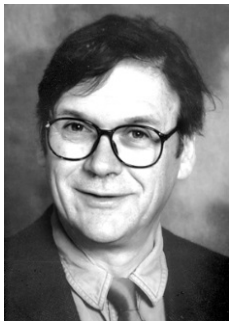
a/a individuals are viable and fertile.
Screen for enhancers or suppressors of the phenotype.

Three Nobel prizes associated with genetic screens



Leland Hartwell

Paul Nurse



Tim Hunt

Eukaryotic cell division
(Hartwell et al. 1974; Nurse et al. 1976)

Seymour Benzer

Circadian rhythms
(Konopka and Benzer 1971)

The Nobel Prize in Physiology or Medicine 2017

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Jeffrey C. Hall

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Michael Rosbash

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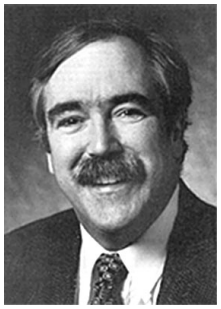
Michael W. Young

C. Nusslein-Volhard



Eric Wieschaus

Development
(Lewis 1978; Nusslein-Volhard and Wieschaus 1980)

General principles

Mutagens

Crosses

Types of phenotype

Identification and validation of causal mutations

Mutagens (1)

X rays : breaks in double-stranded DNA, resulting in large deletions of pieces of chromosome or chromosomal re-arrangements. → good to map by cytological examination of chromosomes, but often not limited to single genes

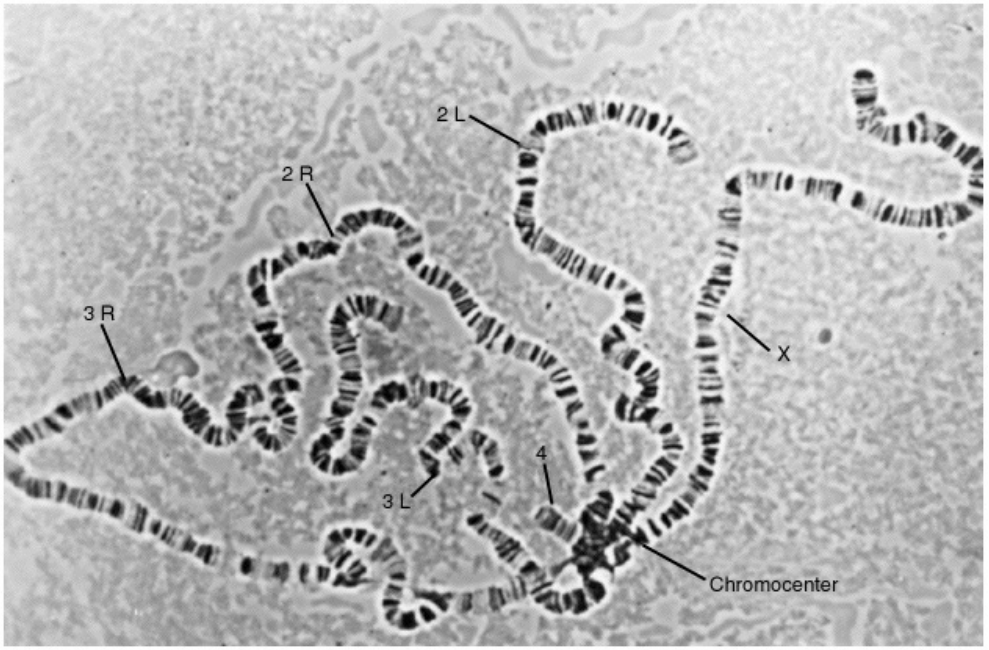
Chemical:
Ethylmethane sulfonate (EMS) : very efficient, alkylation agent (GC to AT), point mutations

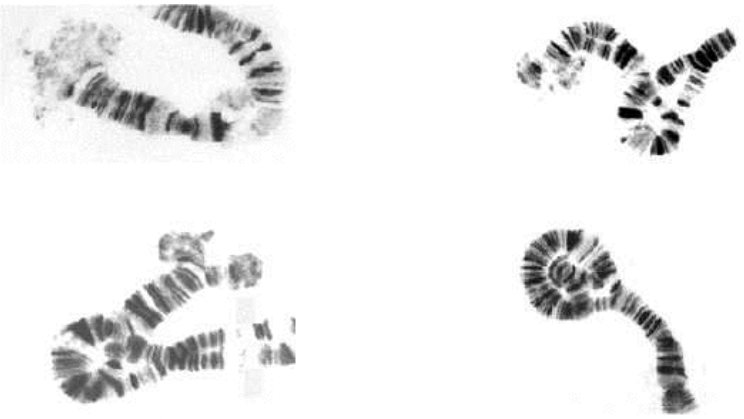
In Drosophila, EMS can produce ~10^{-3} mutations per gene

→ how many mutated genes on average on one chromosome containing 5000 genes?

→ if 6000 such EMS-treated chromosomes are generated, how many alleles per gene can be expected from the screen?

X rays





Photomicrographs of polytene chromosomes; name the mutation, if any

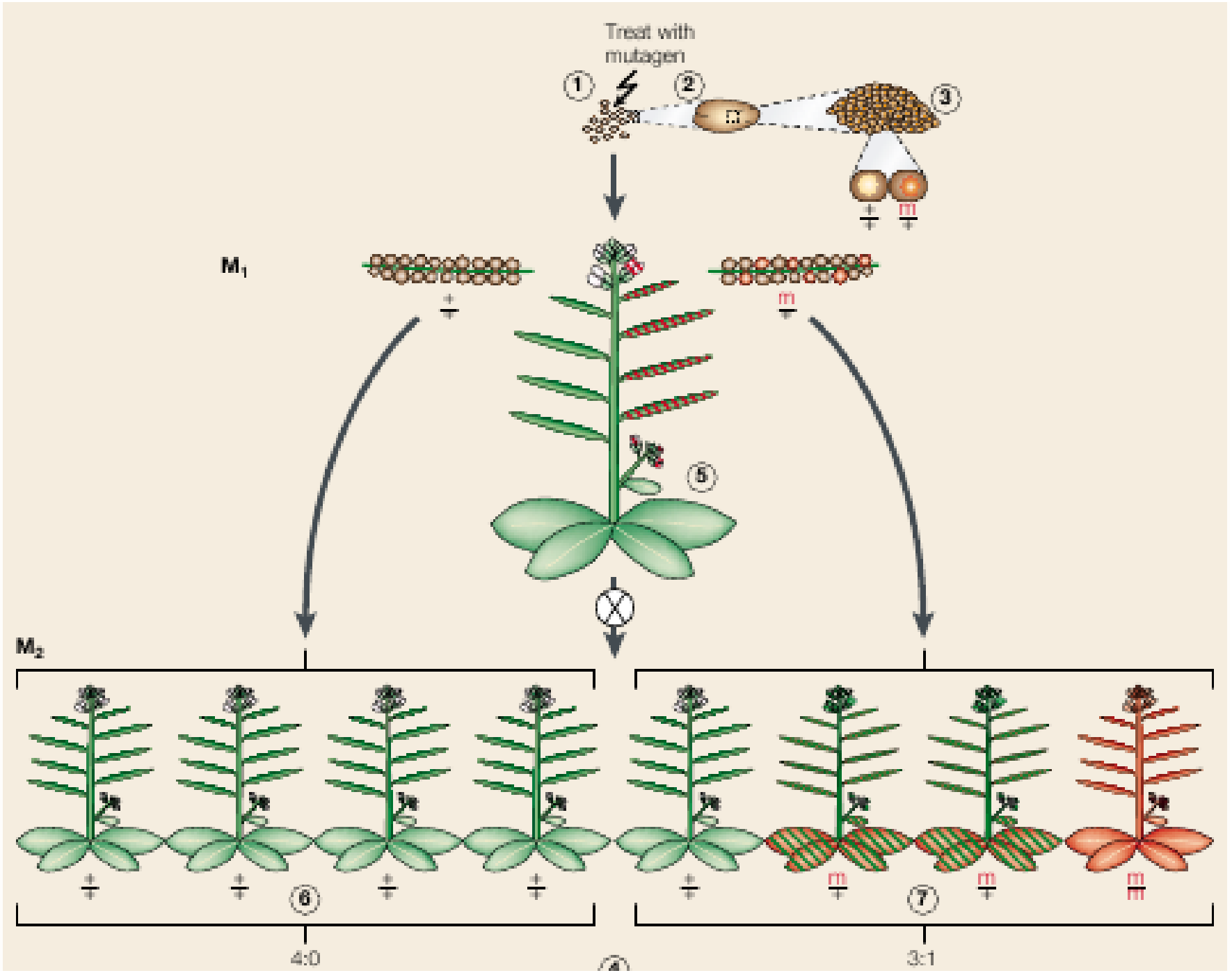
Mutagens (2)

<u>Chemical</u>:
Methylmethane sulfonate (MMS) : agent alkylant, moins efficace que l'EMS pour la drosophile, induit un peu plus de délétions que l'EMS.

N-nitroso-N-ethylurea (ENU) : ethyle oxygen atoms (O2 and O4 of T, AT to GC, O6 of G, GC to AT), fewer aberrations than EMS

Triethylmelanine (TEM) : deletions
Formaldehyde : deletions

Chemical mutagens create mosaic individuals



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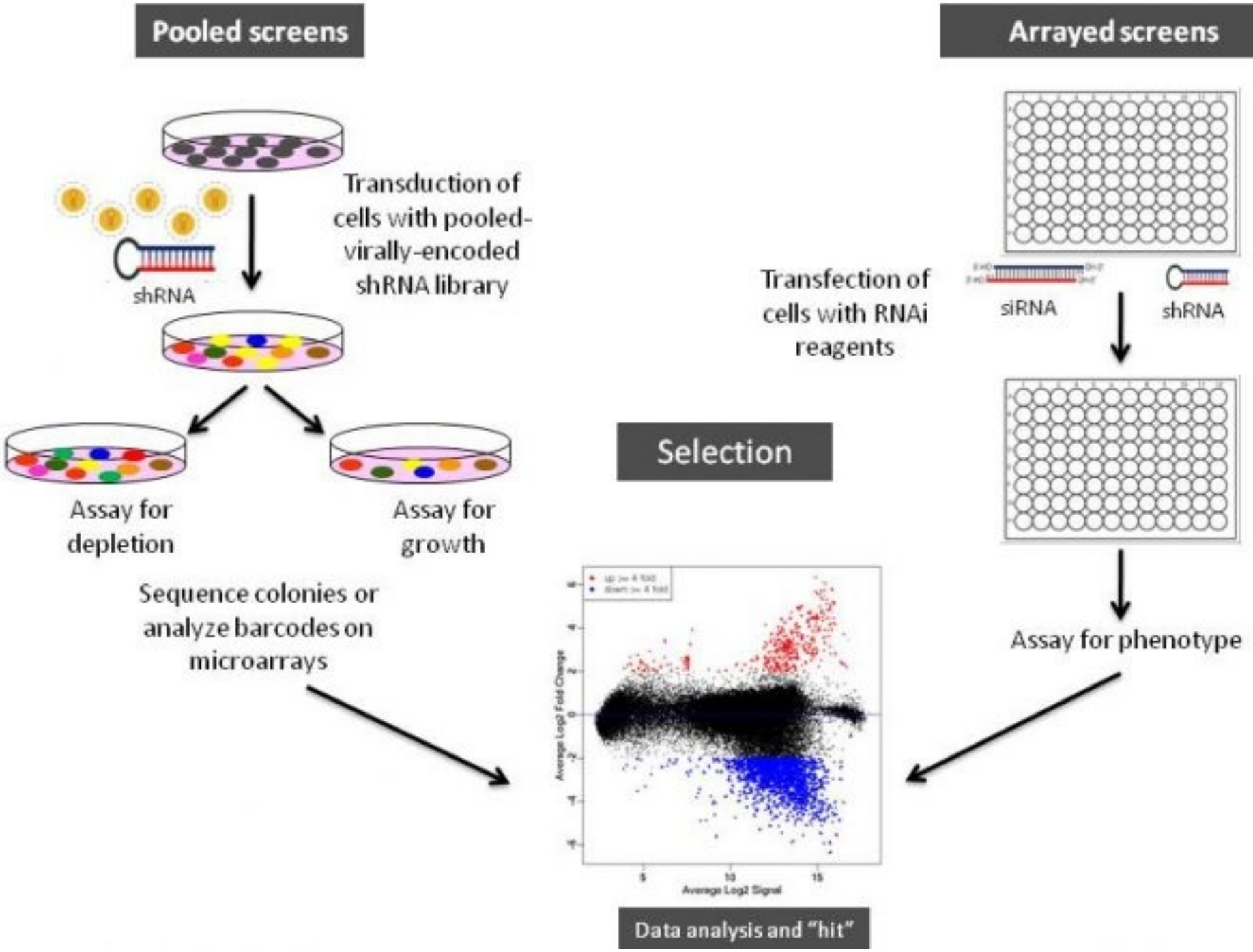
Triethylmelanine (TEM) : deletions
Formaldehyde : deletions

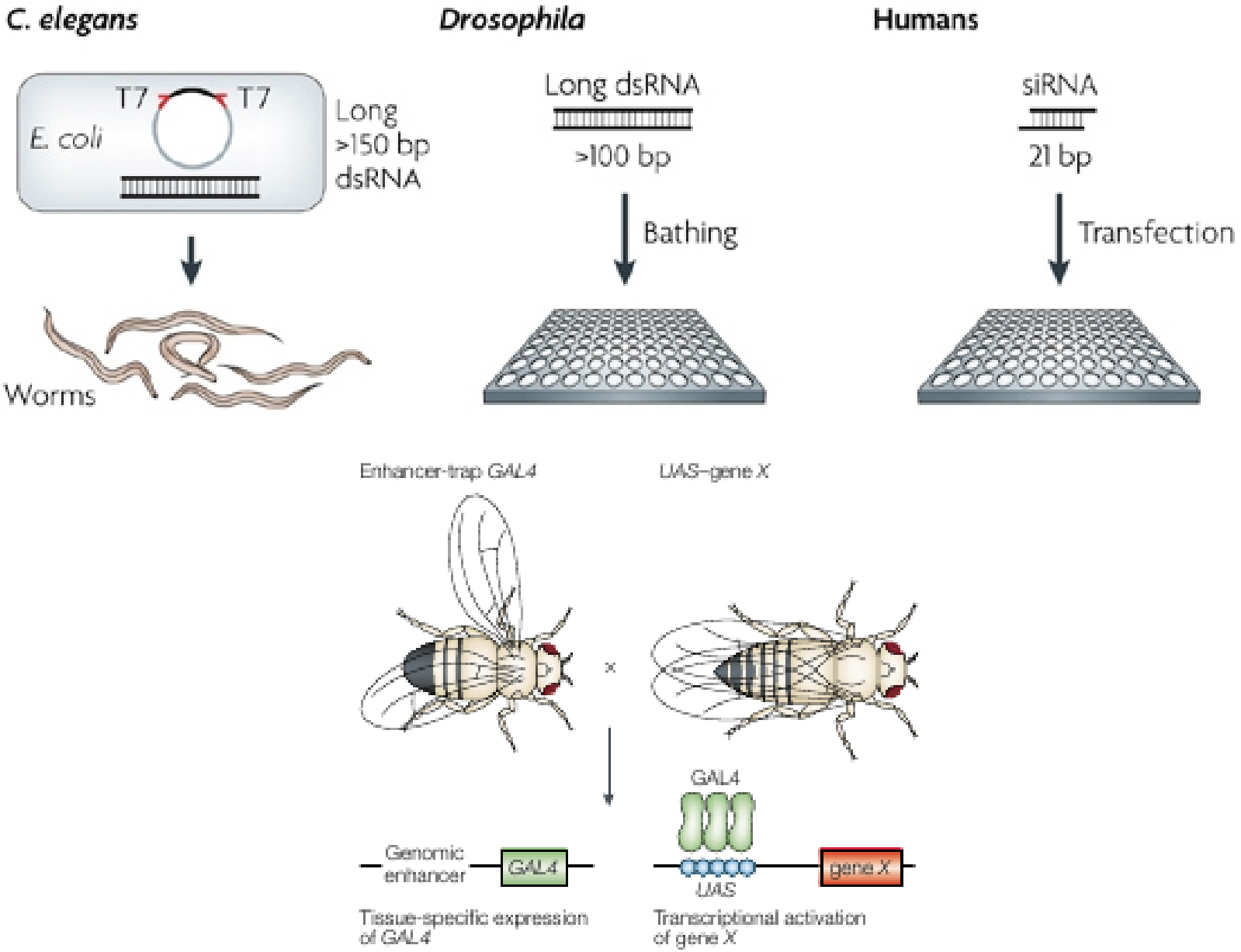
Insertational:
Transposable elements without transposase: integrate into the genome, facilitates identification of the mutation

RNAi

CRISPR-Cas-9


Pooled screens
Arrayed screens
Transduction of cells with pooled-virally-encoded shRNA library
shRNA
Transfection of cells with RNAi reagents
siRNA
shRNA
Assay for depletion
Assay for growth
Selection
Sequence colonies or analyze barcodes on microarrays
Assay for phenotype
Average Log2 Fold Change
Average Log2 Signal
Data analysis and "hit"

RNAi screens



Boutros et Ahringer (2008) Nat Rev Genetics 9, 554

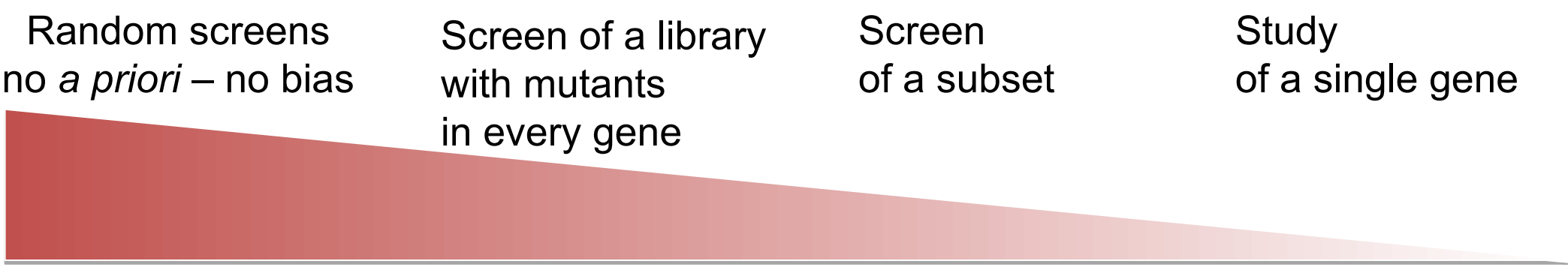
Forward genetics

Genetic screen for a
phenotype of interest,
identification of the mutated gene etc...

Reverse genetics

Start from a gene of interest
knockout, transgenesis, etc...

The distinction is fuzzy when one starts from a subset of a library of mutants



Crosses

Since chemical mutagens create mosaic individuals, the progeny must be screened

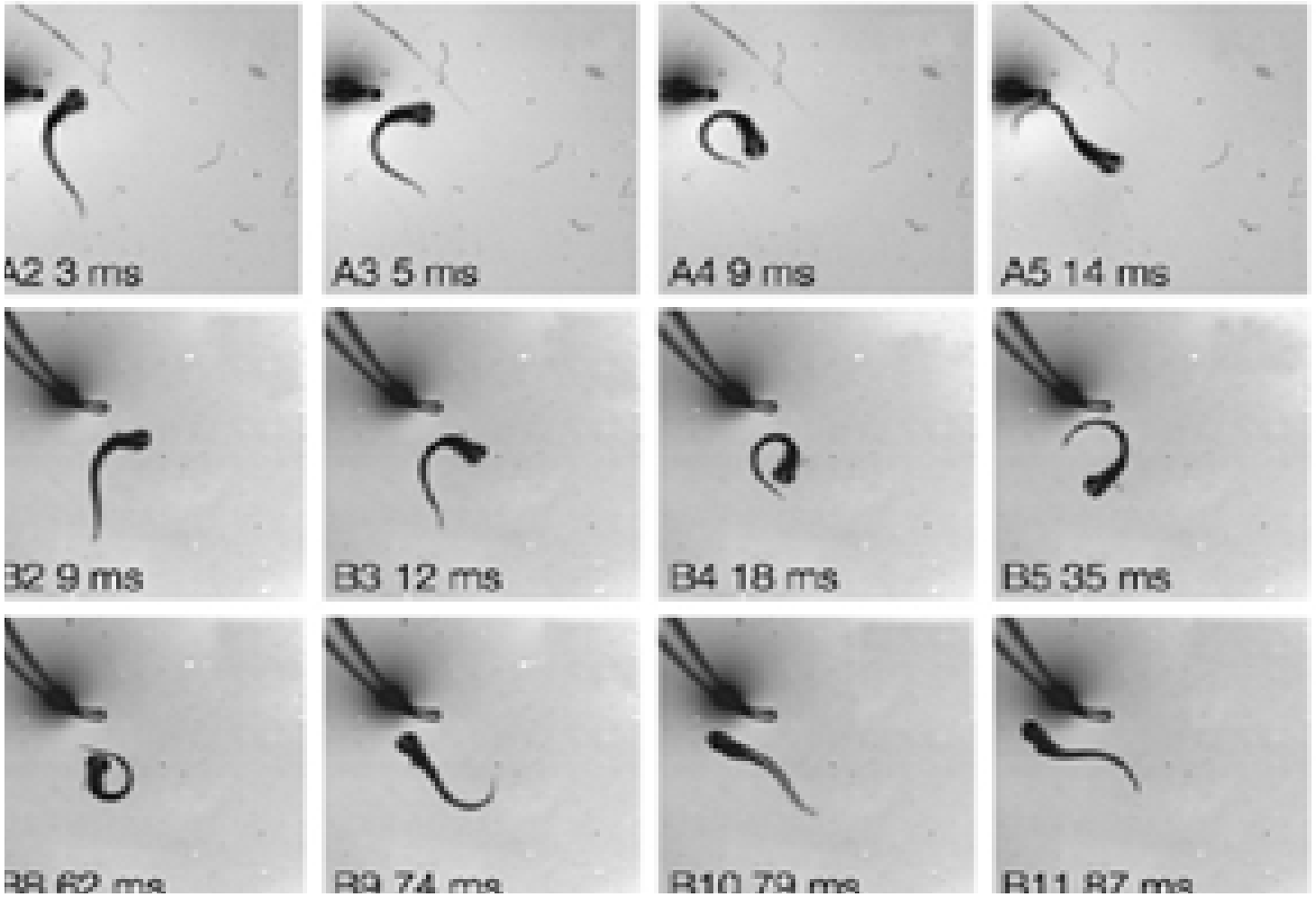
F1 screen: screen for suppressors and enhancers

F2 screen: screen for recessive mutations

F3 screen: screen for maternal effect genes

Phenotypes

Morphology, Physiology, Behavior



space cadet Zebrafish mutant

Phenotypes

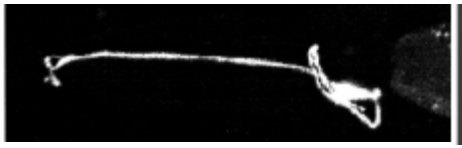
Direct observation

WT

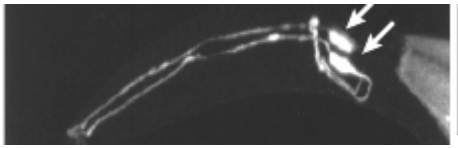
agamous-1

Staining (GFP, antibodies)

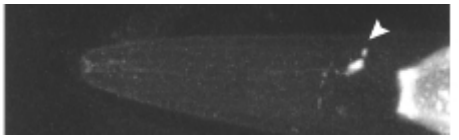
WT



mutant 1

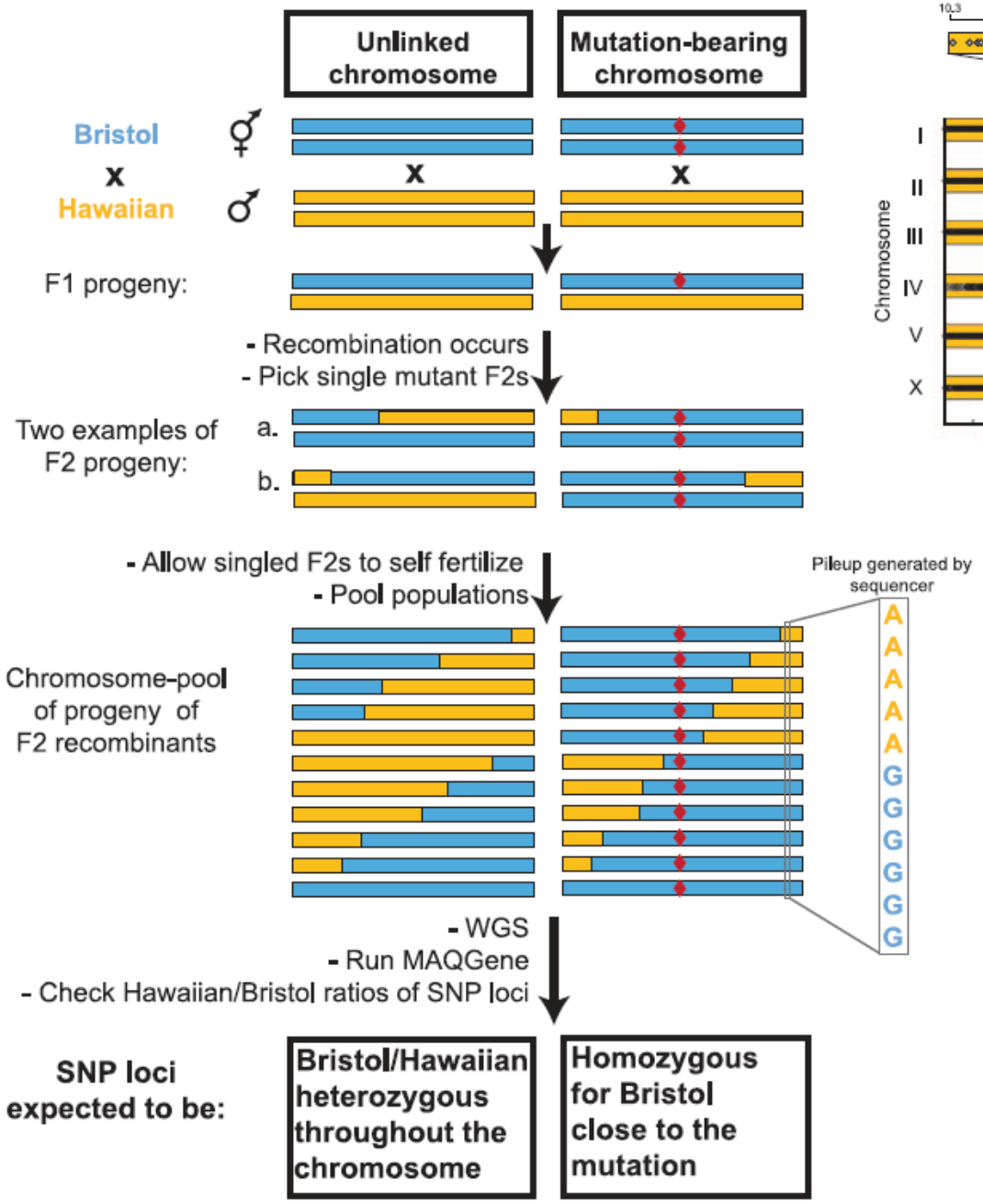


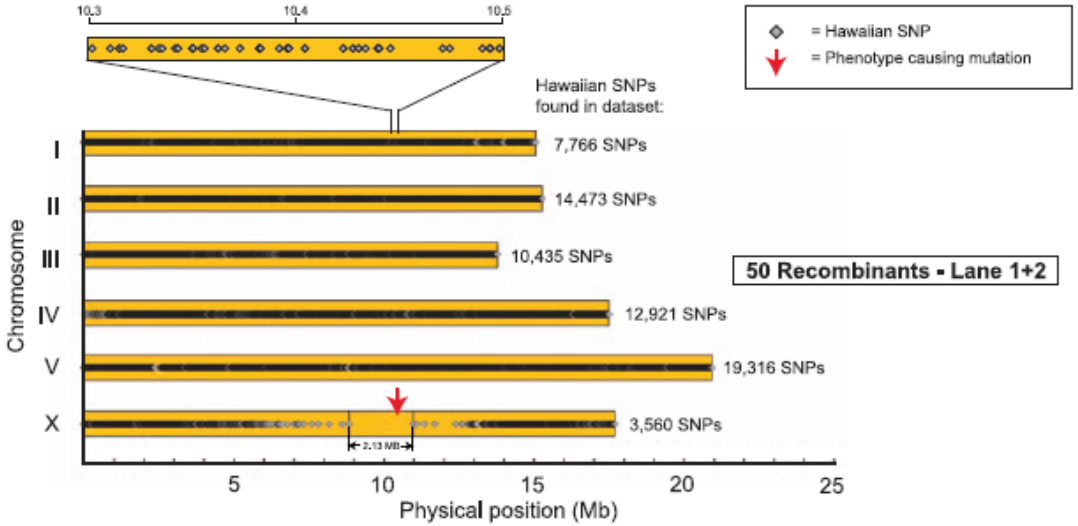
mutant 2



str2::GFP

Identification of the mutation





Requires nucleotide divergence and many recombinants

Oliver Hobert lab

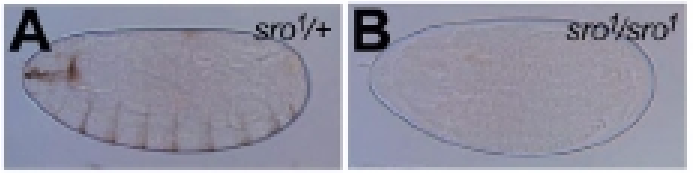
Identification of the mutation

Once a small region is identified

Complementation test with deletions/mutants already available

Analysis of candidate gene expression

Rescue of the mutant phenotype with transgenes



Niwa et al. (2010) Development 137, 1991

Table 1. *sro*^{1} lethality was rescued by *sro/nm-g* overexpression

Genotype	Number of adults
+/+; 2-286-GAL4 sro^{1}/+ sro^{1}	0 (308)
UAS-sro/+; sro^{1}/sro^{1}	0 (170)
UAS-nm-g/+; sro^{1}/sro^{1}	0 (138)
UAS-sro/+; 2-286-GAL4 sro^{1}/+ sro^{1}	128 (286)
UAS-nm-g/+; 2-286-GAL4 sro^{1}/+ sro^{1}	57 (270)

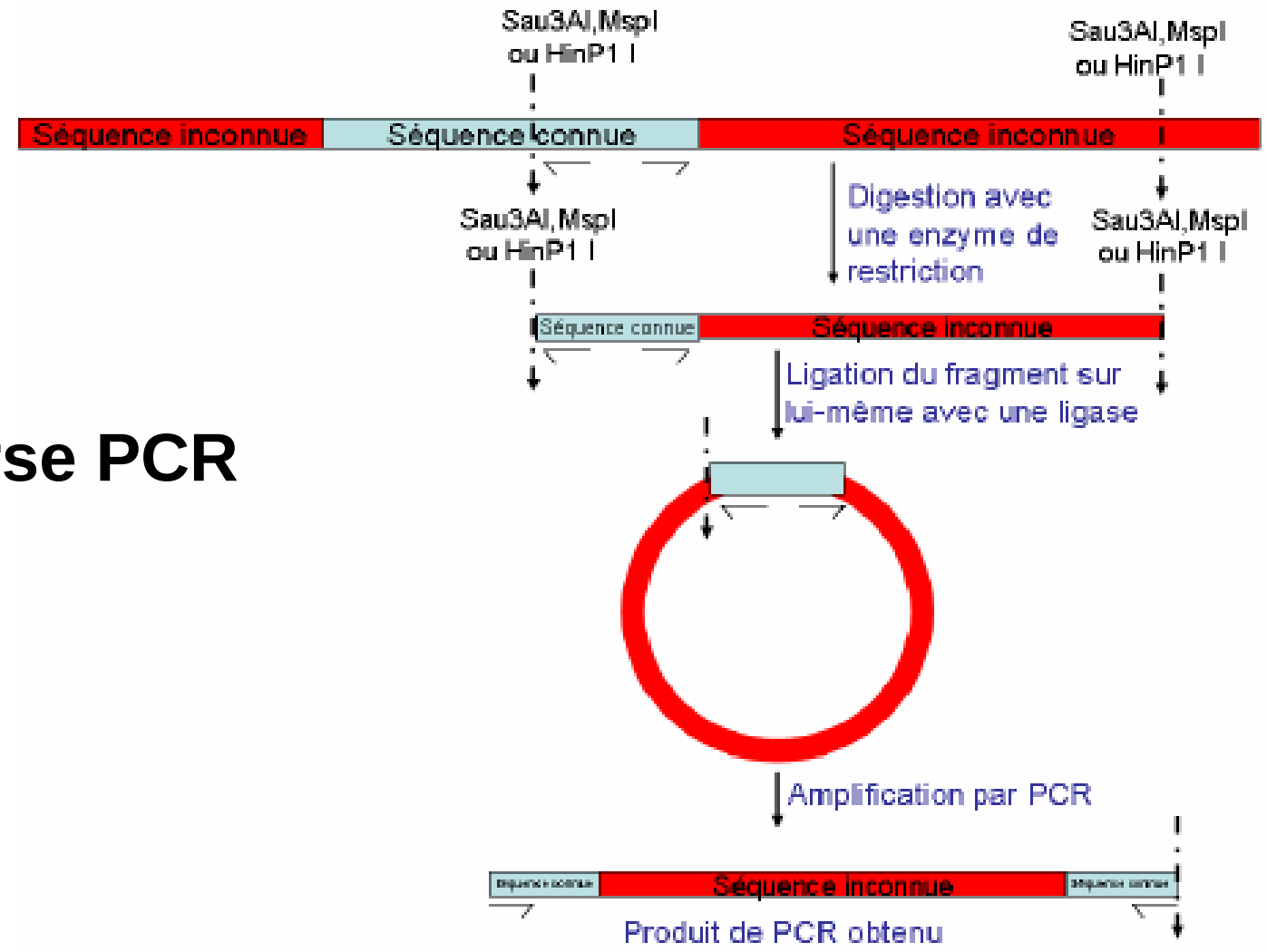
The numbers of viable adults were scored. Parentheses indicate the number of viable progeny with the presence of balancer markers from the parental strains.

Sequencing and search for mutations (nonsense, deletions, etc.)

Identification of the mutation

For transposable elements

Inverse PCR



Other types of screens

Gene expression screens

RNAseq

In situ hybridization of all genes

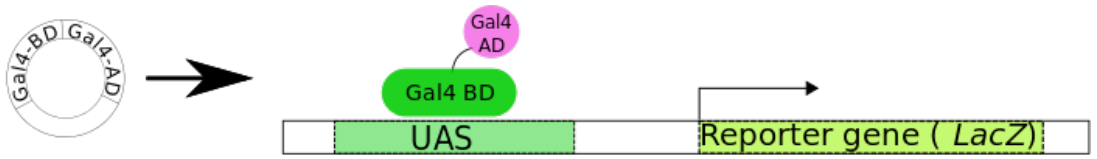
Screen of DNA sequences

Library with all the genes coding for transcription factors

Two-hybrid screen

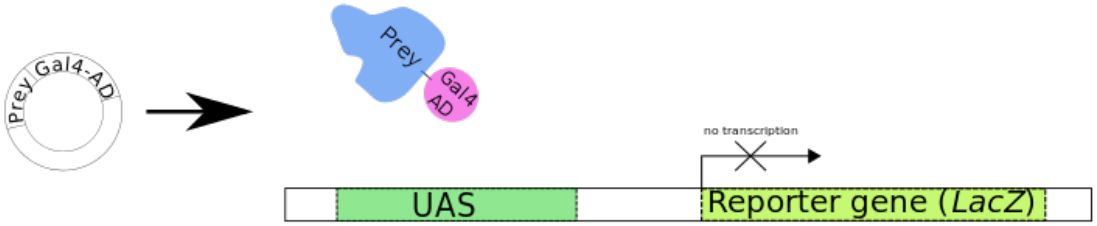
etc.

Yeast two-hybrid screen

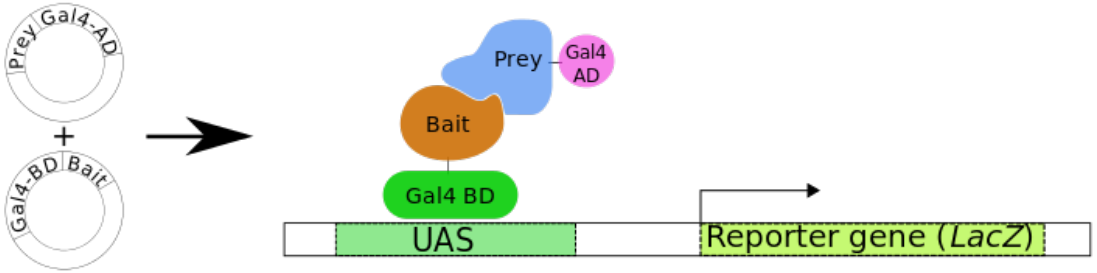


A. Regular transcription of the reporter gene

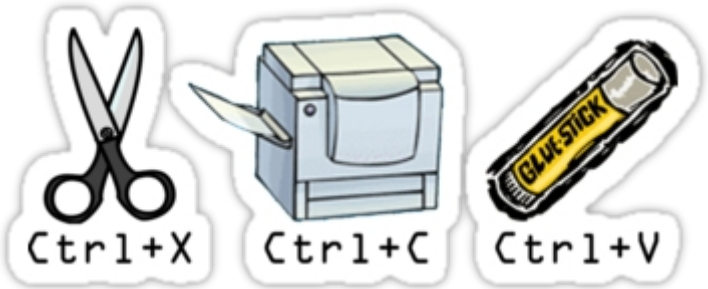
B. One fusion protein only (Gal4-BD + Bait) - no transcription



C. One fusion protein only (Gal4-AD + Prey) - no transcription



D. Two fusion proteins with interacting Bait and Prey

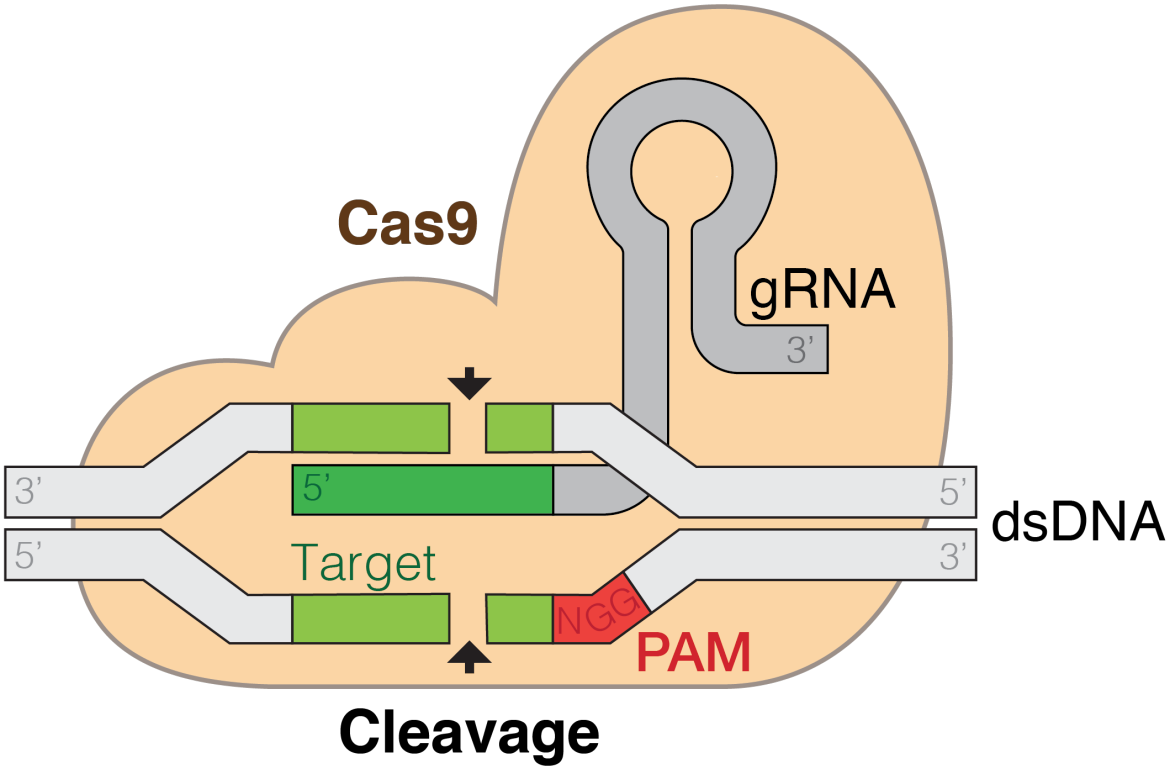

Ctrl+X
Ctrl+C
GLUE STICK
Ctrl+V

CRISPR

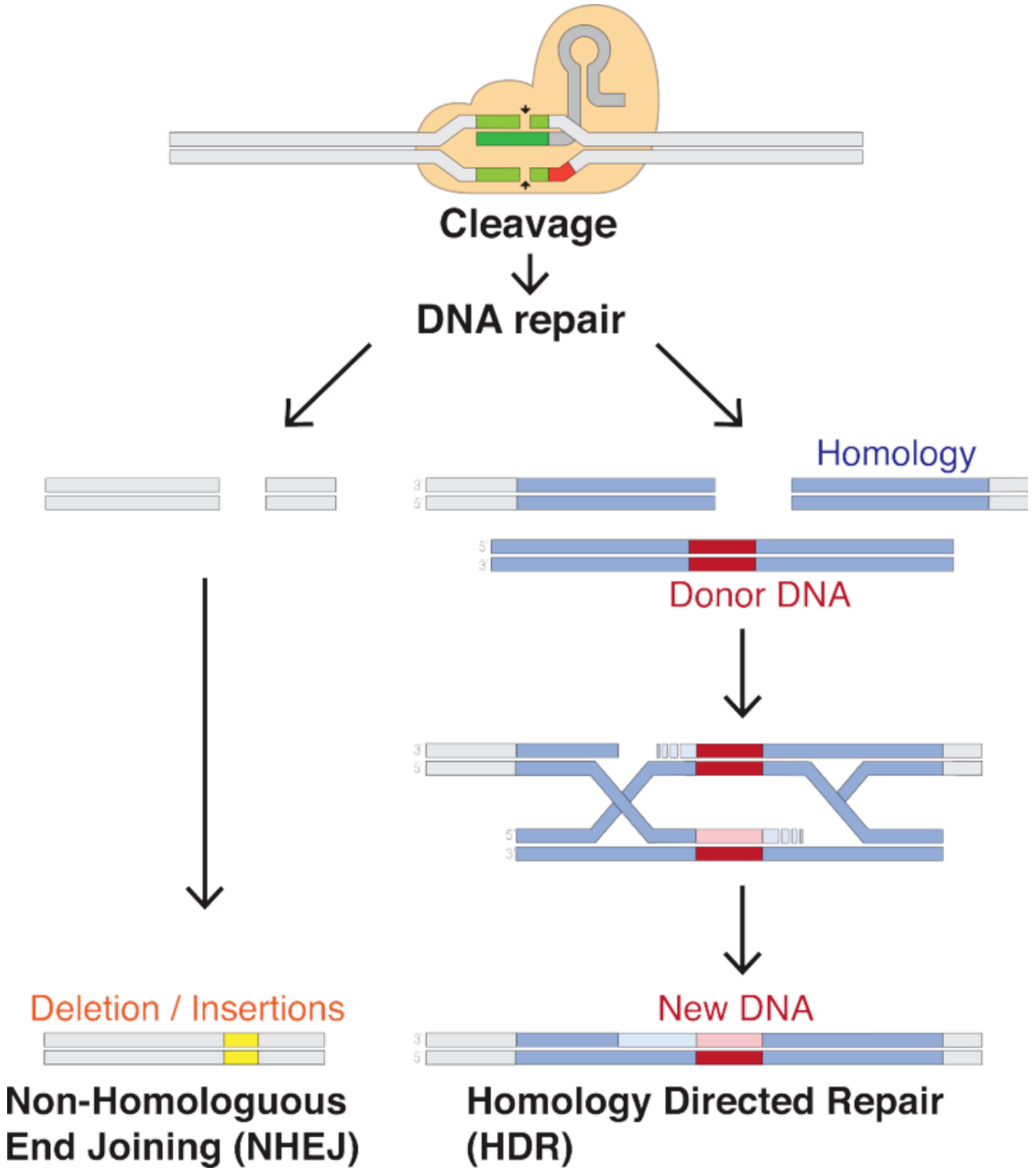

YAAAAAA!
TARGET


TAA!
TATATATA
TATATATA!
TA!
TA!

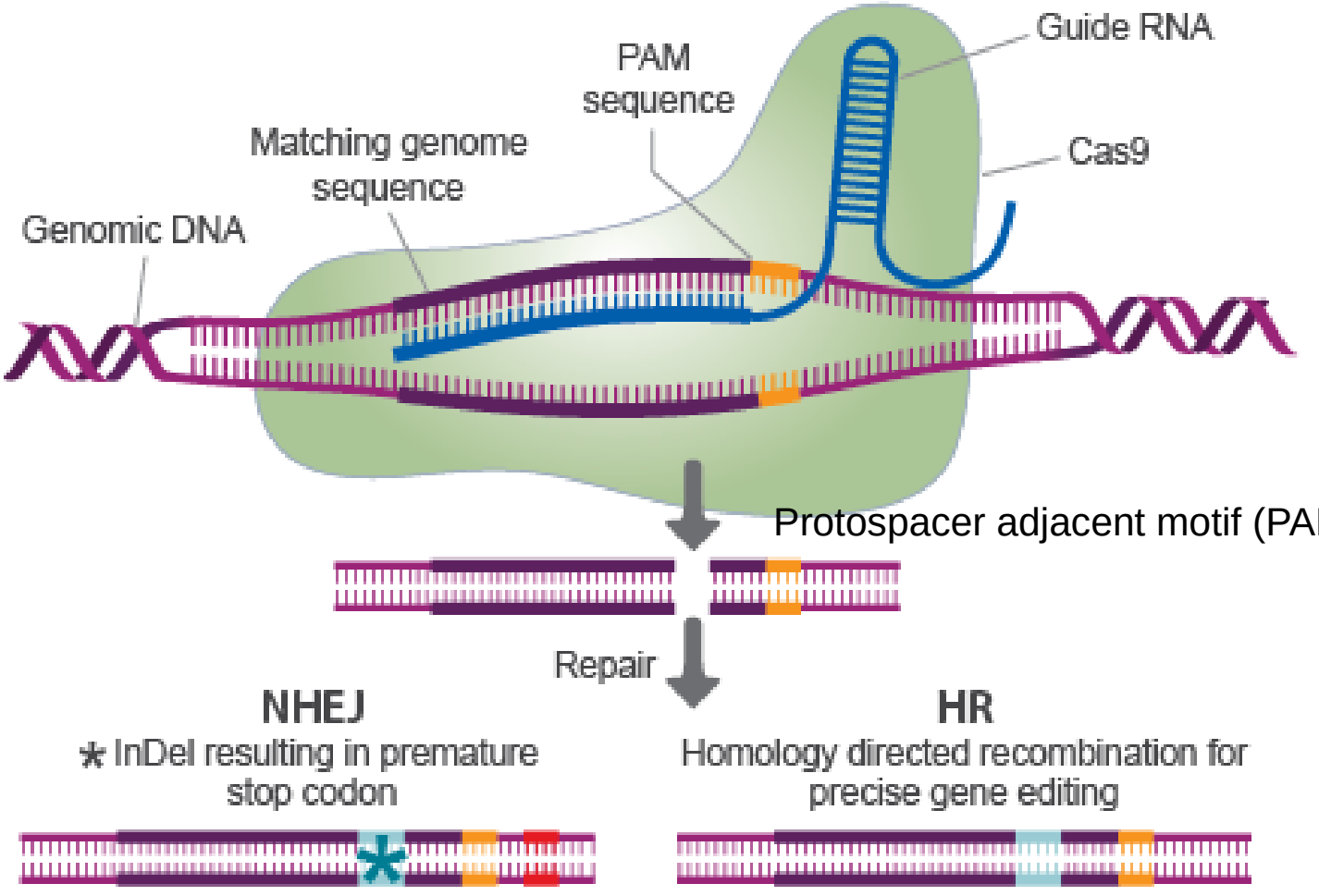
CRISPR = clustered regularly interspaced short palindromic repeats
= family of DNA sequences present in bacteria and used to detect and destroy virus DNA



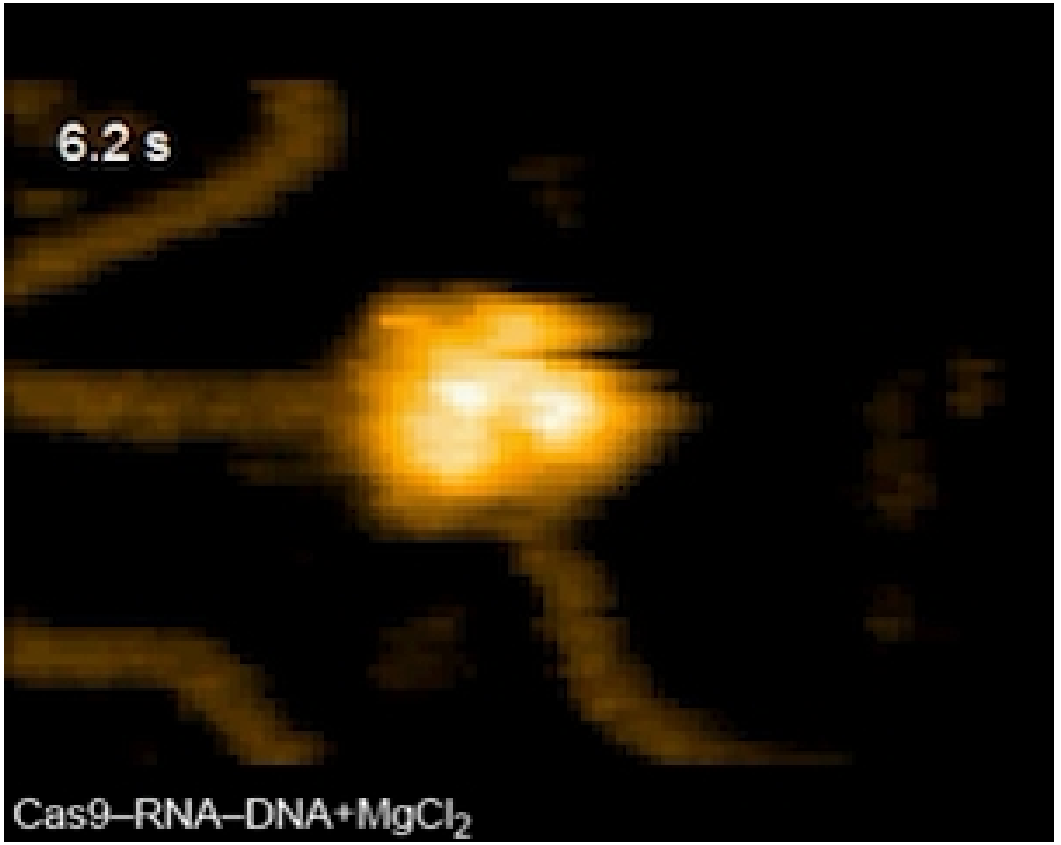
Can recognize and cut a specific DNA sequence (recognized by guide RNA)
More versatile than restriction enzymes, Zn finger nucleases and transcription activator-like effector nucleases (TALENs).


Cleavage
DNA repair
Homology
Donor DNA
Deletion / Insertions
New DNA
Non-Homologuous
End Joining (NHEJ)
Homology Directed Repair
(HDR)

Creating mutants with CRISPR/Cas9



GAGTTCTACAGCGTGAACCACATCAACCAGACGTACGAGTTTGTGCAGCGGATGCG Wild type
GAGTTCTACAGCGTGAACCACATCAACCAGACGTACGAGTTTG--CAGCGGATGCG
GAGTTCTACAGCGTGAACCACATCAACCAGACGTACGAGT------AGCGGATGCG
GAGTTCTACAGCGTGAACCACATCAACCAGACGTACG--------CAGCGGATGCG Deletion
GAGTTCTACAGCGTGAACCACATCAACCAGACGTA----------CAGCGGATGCG
GAGTTCTACAGCGTGAACCACAT----------GCGGATGCG
AGTTCTACAGCGTGAACCACATCAACCAGACGTACGAGTTTGTGACAGCGGATGCG
TACAGCGTGAACCACATCAACCAGACGTACGAGTTTGTGGCTTTAAAGCGGATGCG
CAGCGTGAACCACATCAACCAGACGTACGAGTTTGTGCAAGGAAACTGCGGATGCG Insertion



High-speed atomic force microscopy (HS-AFM) movies of three representative pre-assembled Cas9–RNA–DNA molecules on the AP-mica surface, showing that the HNH domain undergoes fluctuations and then adopts the docked conformation, followed by the release of the cleaved DNA. The cleavage reaction was initiated by the addition of MgCl2 during the HS-AFM observations. Fluctuations of the HNH domain are indicated by magenta arrows. The cleavage products released from Cas9–RNA are indicated by blue arrows.

Shibata 2017

Heliconius melpomene

Heliconius erato

Same genes in co-mimics?

Agraulis vanillae

dorsal *ventral*

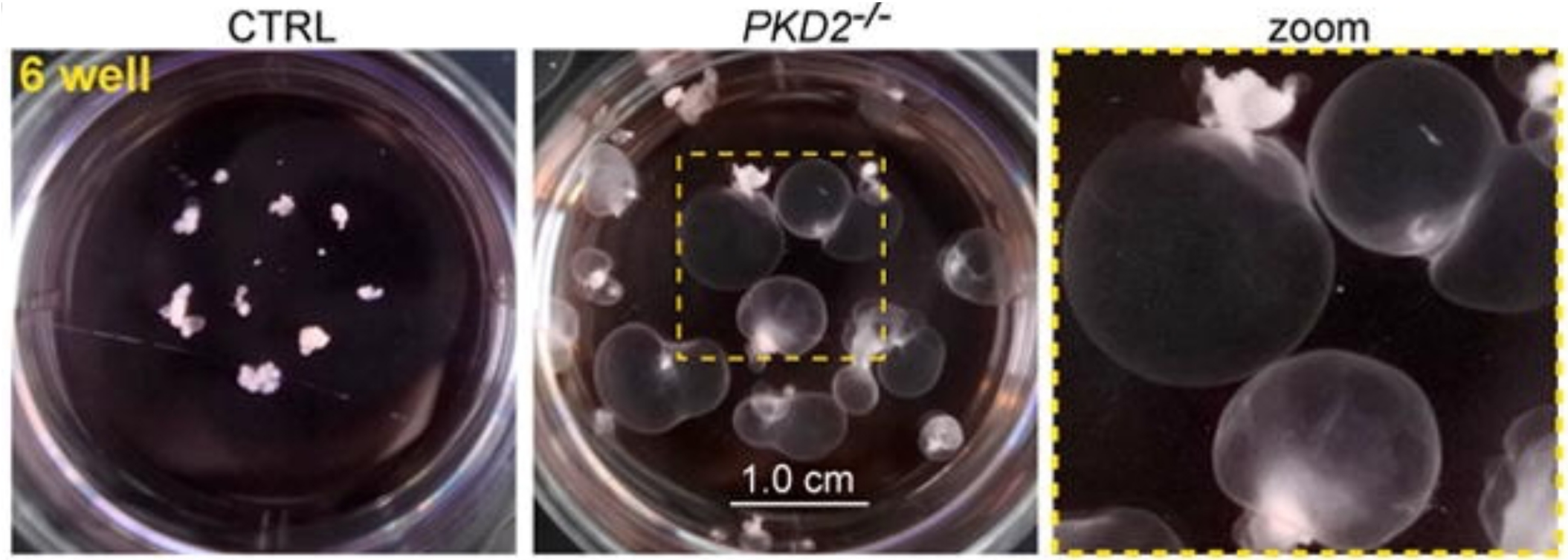
Wild-type **mutant *optics* CRISPR** **Wild-type** **mutant *optics* CRISPR**

Reed et al. 2017

normal

mutant
***WntA* CRISPR**

CRISPR and organoids

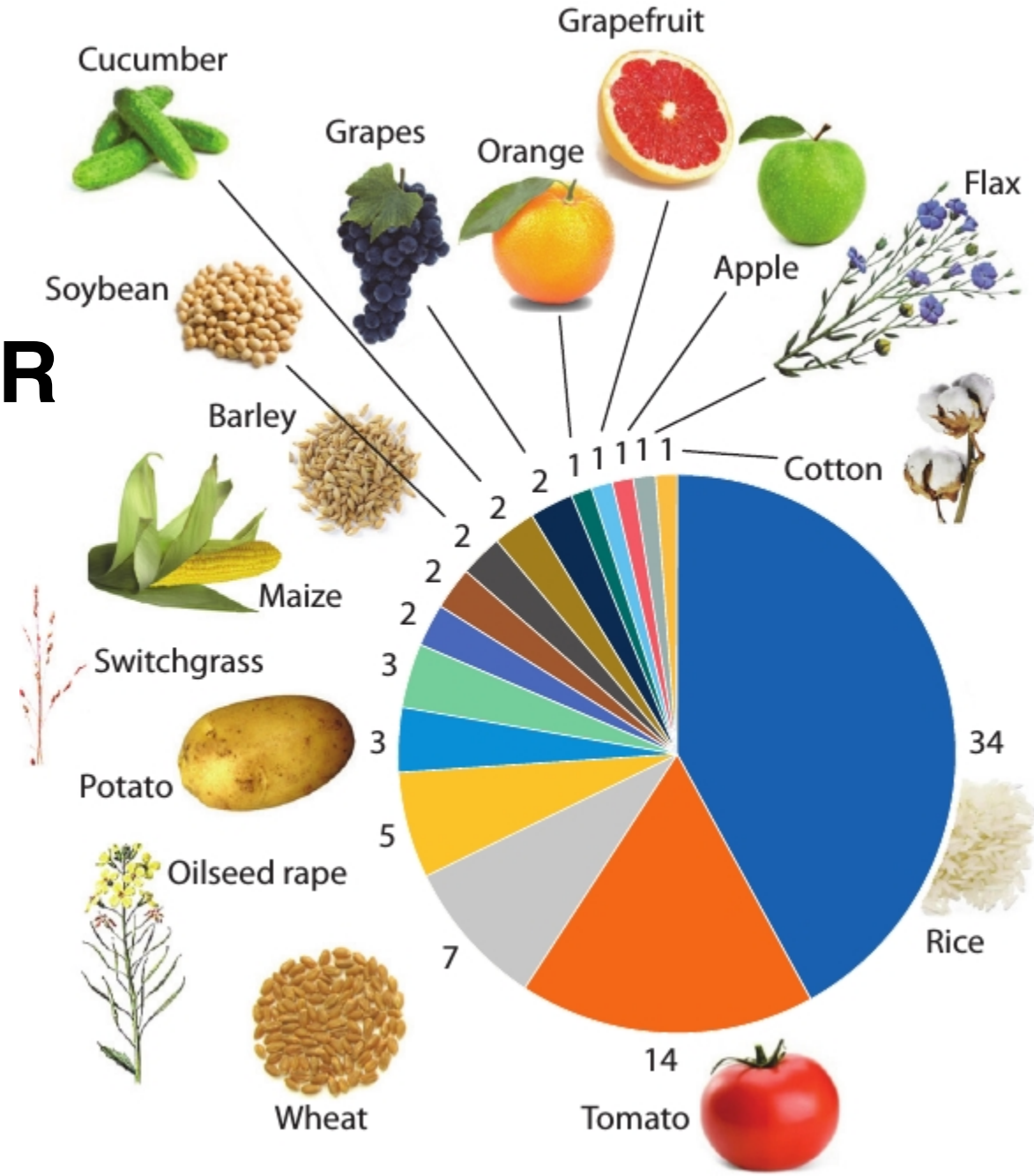


6-well (3.5 cm) dishes containing PKD or control organoids
after 9 months of culture

Human pluripotent stem cells → CRISPR → kidney organoids

Cruz et al. Nature Materials 2017

Numerous genes modified via CRISPR



Korotkova et al. 2019

The first CRISPR food

Mutation of several polyphenol oxidase genes

"Animal and Plant Health Inspection Service (APHIS) has concluded that your CRISPR/Cas9-edited white button mushrooms as described in your letter do not contain any introduced genetic material. APHIS has no reason to believe that CRISPR/Cas9-edited white button mushrooms are plant pests"

FDA does not consider CRISPR-edited food as GMO

Europe position on CRISPR-edited organisms

July 2018: the European Court of Justice (Case C-528/16) ruled that organisms obtained by directed mutagenesis techniques are to be regarded as genetically modified organisms (GMOs)

exemption of mutagenesis in Annex 1B of the Directive applies only to organisms obtained through techniques of mutagenesis which have long been used in conventional breeding and were deemed by the Directive to have a long safety record

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Large deletions induced by Cas9 cleavage

ARISING FROM H. Ma et al. *Nature* **548**, 413–419 (2017); https://doi.org/10.1038/nature23305

Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements

2017

Michael Kosicki, Kärt Tomberg & Allan Bradley

Allele-specific genome editing using CRISPR-Cas9 causes off-target mutations in diploid yeast

2018

Arthur R. Gorter de Vries, Lucas G. F. Couwenberg, Marcel van den Broek, Pilar de la Torre Cortés, Jolanda ter Horst, Jack T. Pronk and Jean-Marc G. Daran*

Cas9-mediated introduction of a DSB resulted in large scale loss of heterozygosity affecting DNA regions up to 360 kb that resulted in introduction of nearly 1700 off-target mutations, due to replacement of sequences on the targeted chromosome by corresponding sequences from its non-targeted homolog.

Dr Soledad Miranda @NeuroLatina · 25 juil.

En réponse à @carlzimmer

Sad, how ignorance harms the development of new technology that have the potential to bring food to the ones who need it most. We scientist need to go out more and explain how GMO can help.

Traduire le Tweet

The first CRISPR-edited foods are not “to save the world”

Herbicide-tolerant oilseed rape

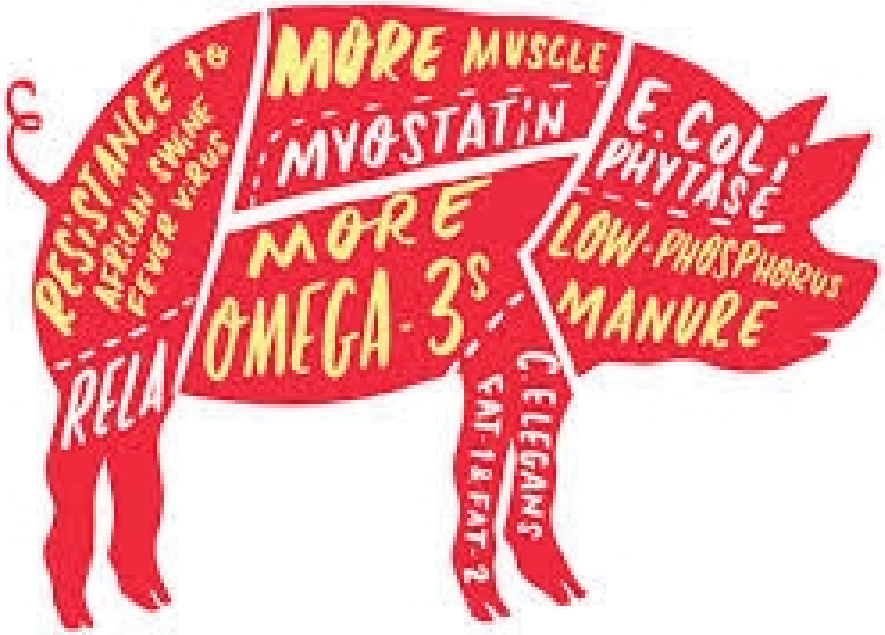
(in 2015, the German authorities authorized the release of oilseed rape developed by the US company CIBUS without being regulated as genetically engineered.)

Soybean with modified fatty acid composition
Potato with improved storage capacity at cool temperatures
“Waxy” maize with a modified starch composition
Herbicide-tolerant flax
Sweeter-tasting strawberries
Seedless tomatoes

Ueta et al. 2017

Making plants that are resistant to drought or salt requires exogenous genes

CRISPR-Cas9 can knock-out many genes at once

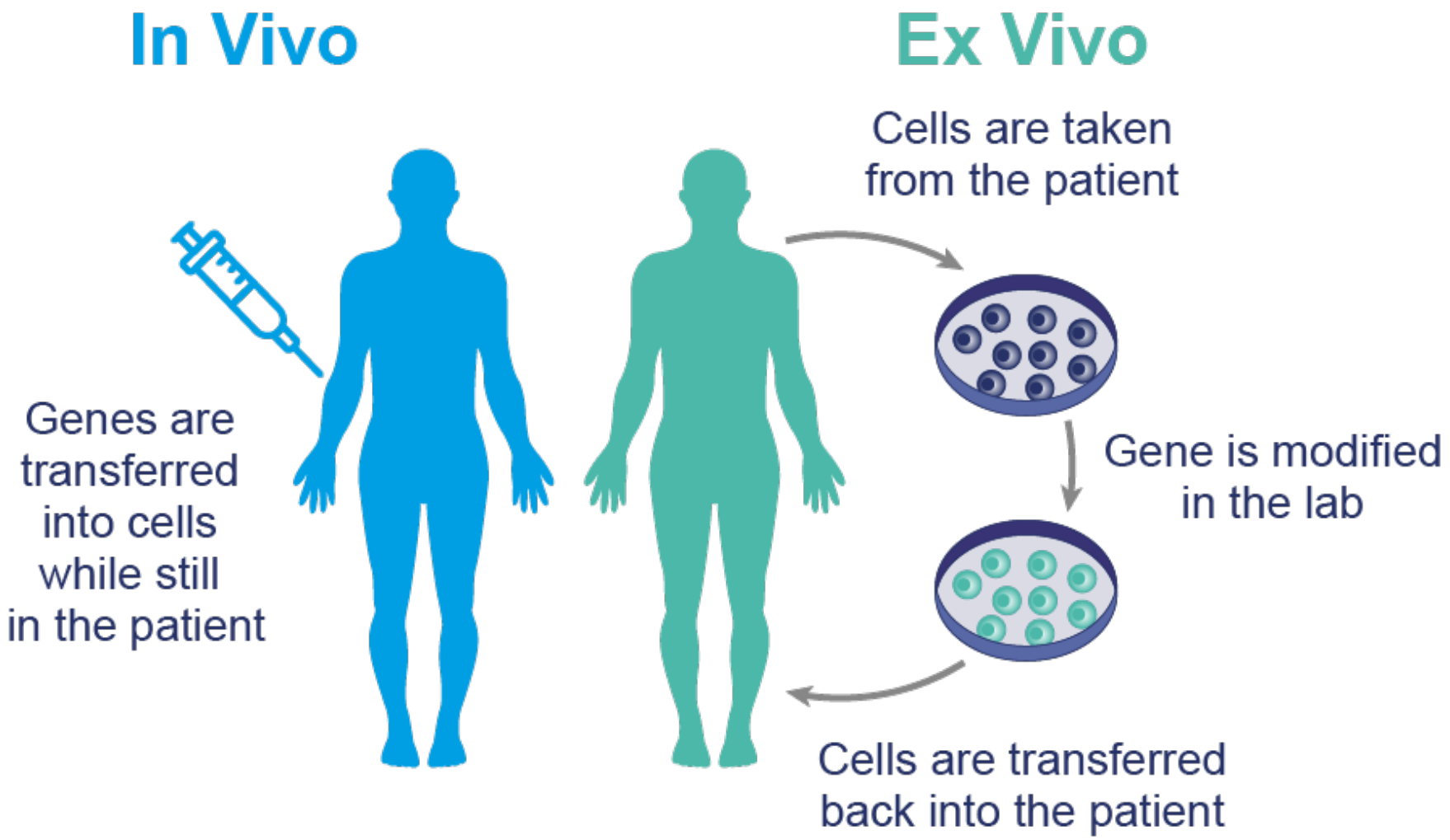


CRISPR-Cas9 was used to inactivate all the 62 porcine endogenous retrovirus sequences in a porcine primary cell line.

Several PERV-inactivated pigs were then produced via somatic cell nuclear transfer.

Niu et al 2017

Ongoing clinical trials using CRISPR



Retina disease

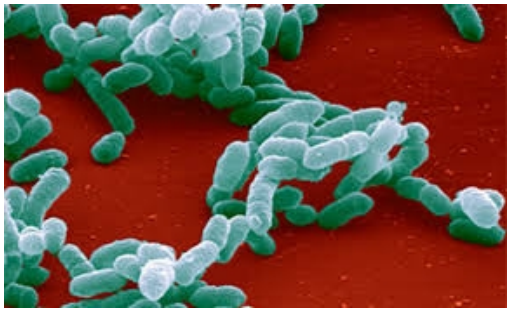
Beta-thalassemia
Sickle cell disease

Fundamental research is important

bacteria *Thermus aquaticus*

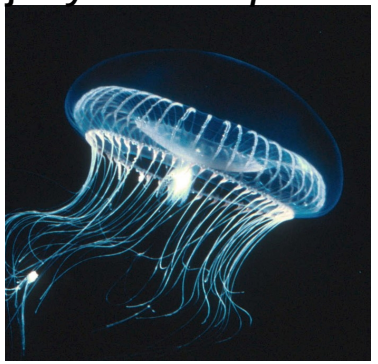
1969 → Taq-polymerase to amplify DNA

bacteria *Haemophilus influenzae*



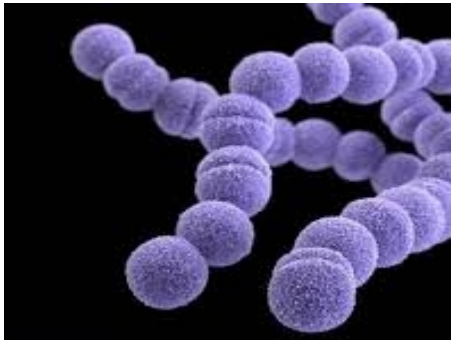
1970 → Restriction enzymes To cut DNA

jellyfish *Aequorea*



1992 → Fluorescent proteins

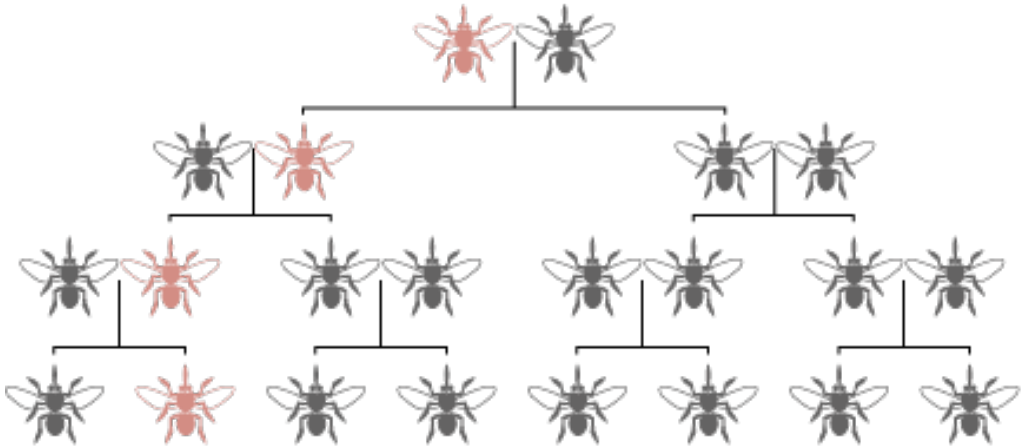
bacteria *Streptococcus pyogenes*



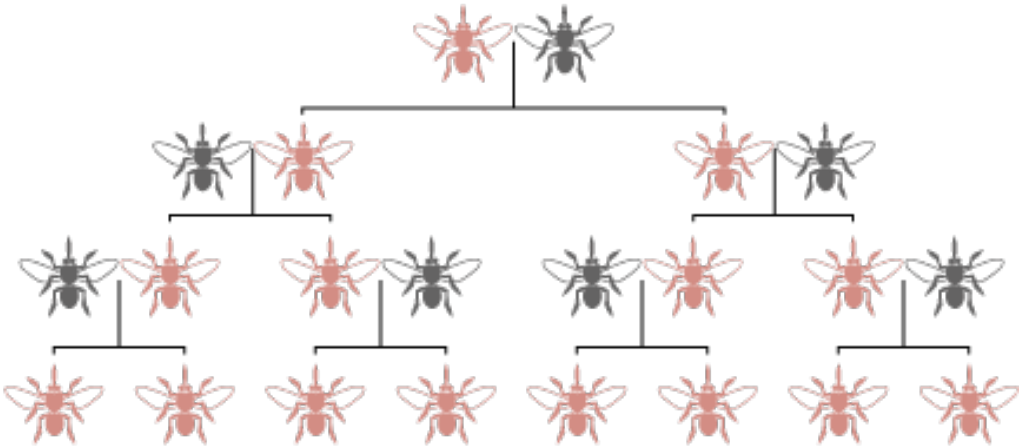
2012 → CRISPR

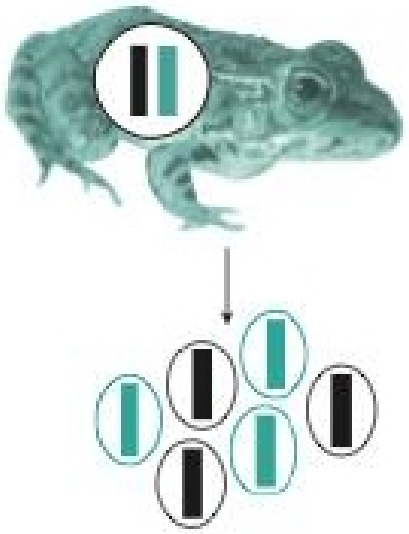
Gene drive

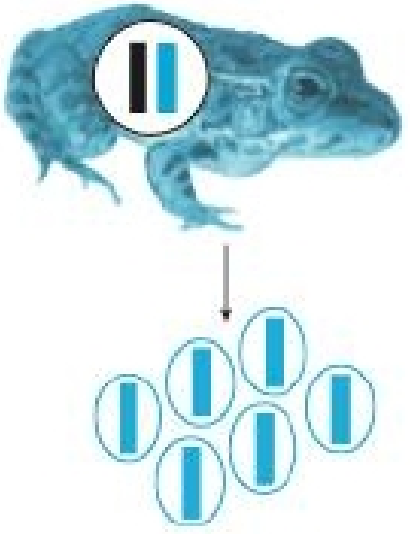
Normal reproduction



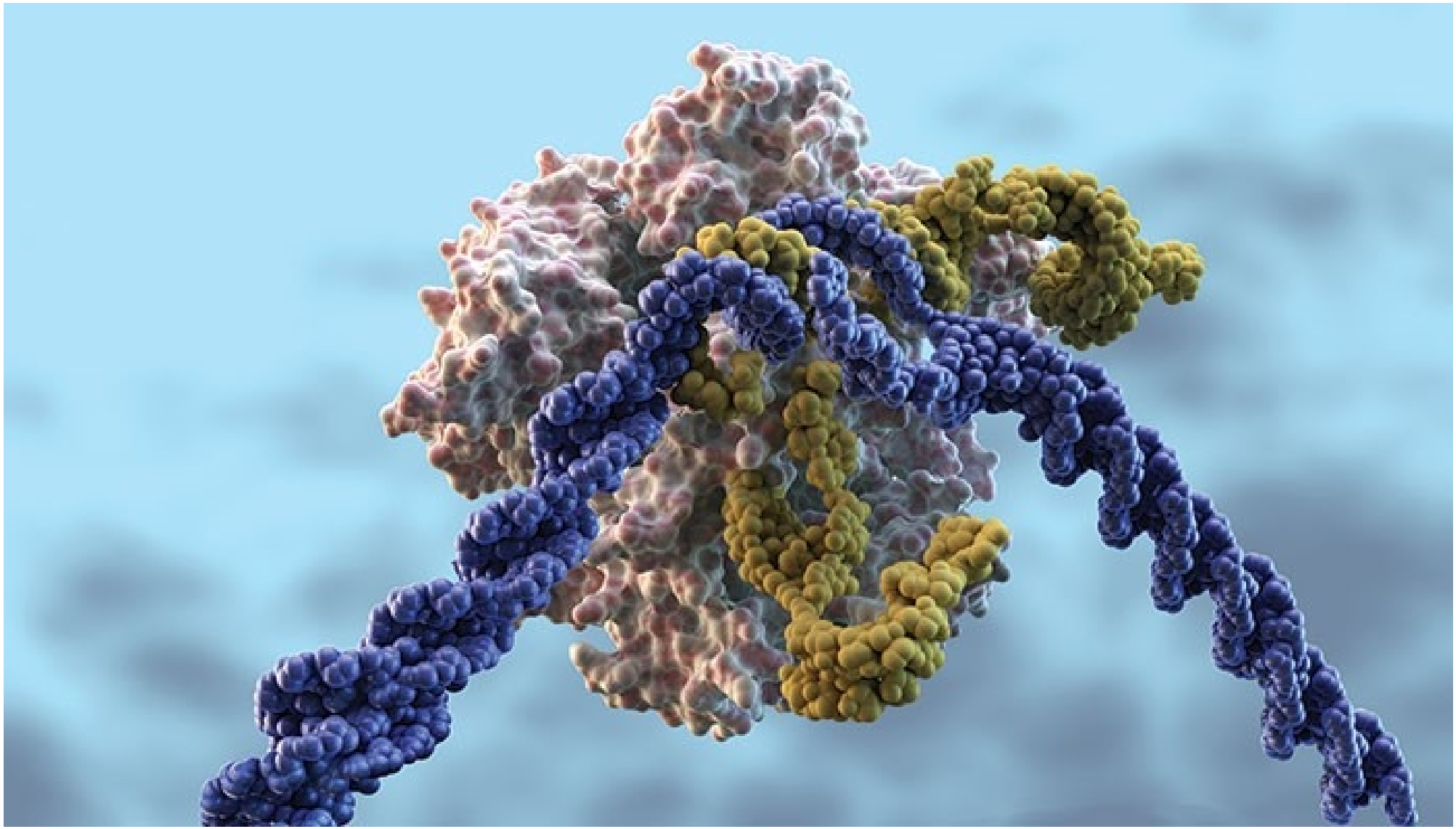
Reproduction with gene drive



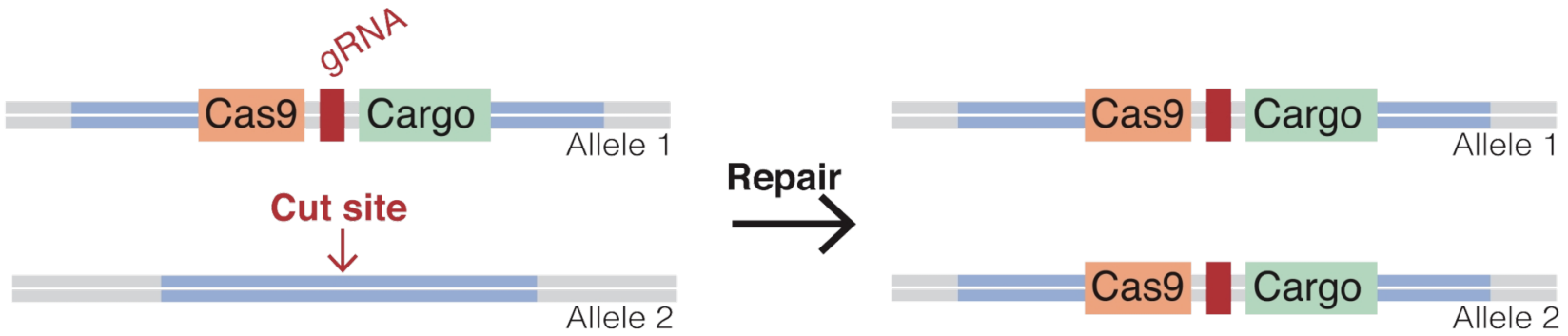




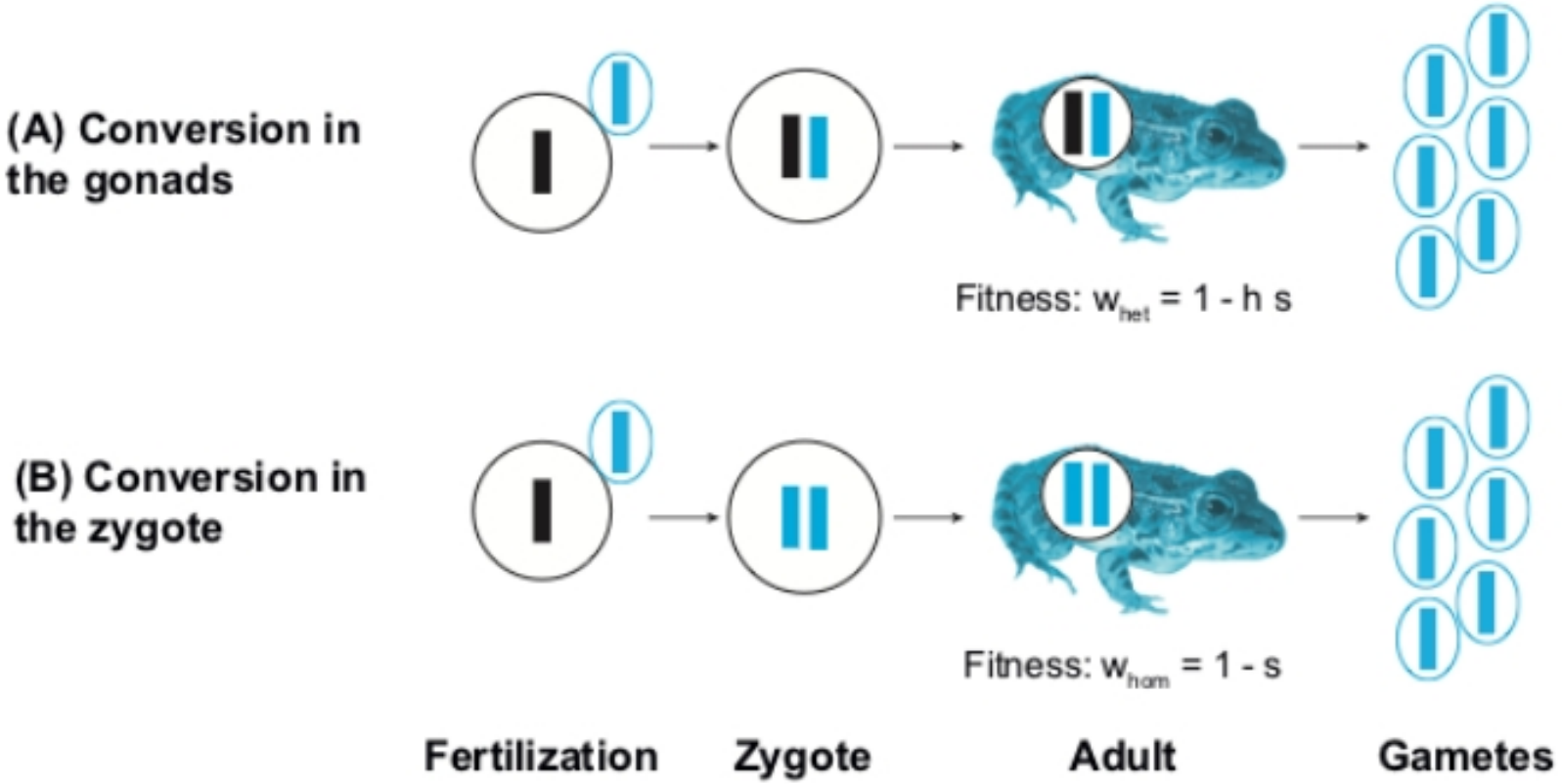
Rode et al. Submitted



How a gene drive cassette copies itself



Wikipedia



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

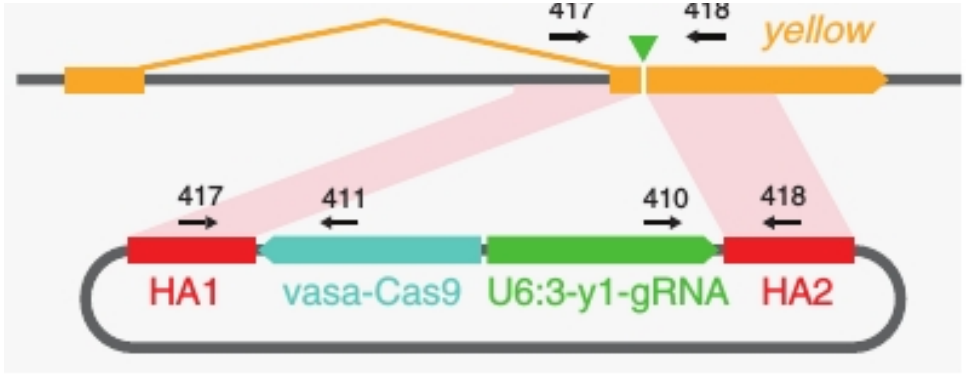
First gene drive organisms

GENOME EDITING

Science, April 2015

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

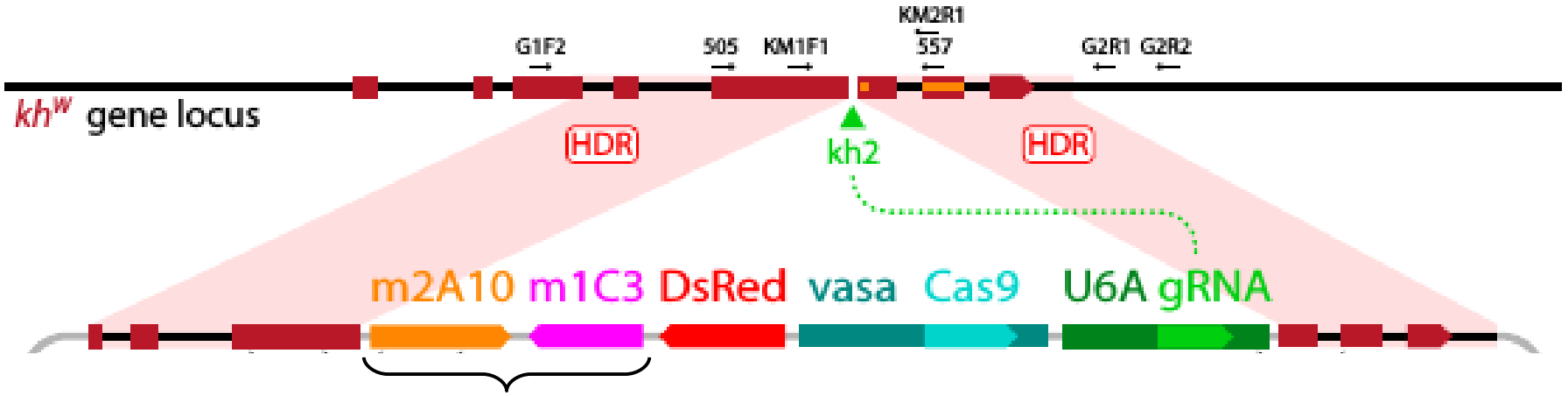
Valentino M. Gantz* and Ethan Bier*



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



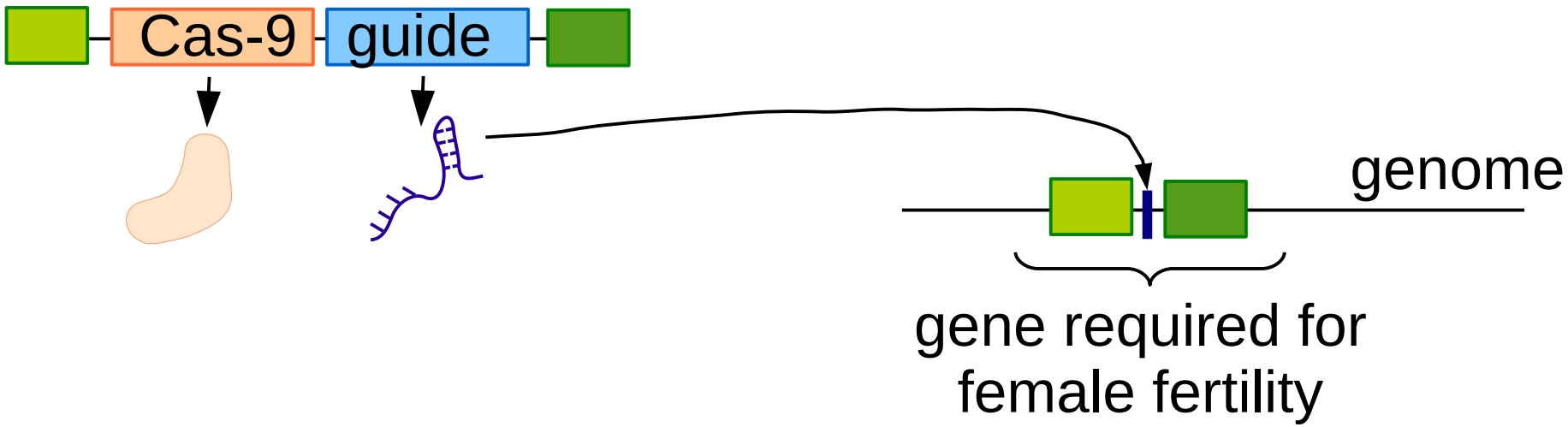
antibodies
anti-parasites

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*

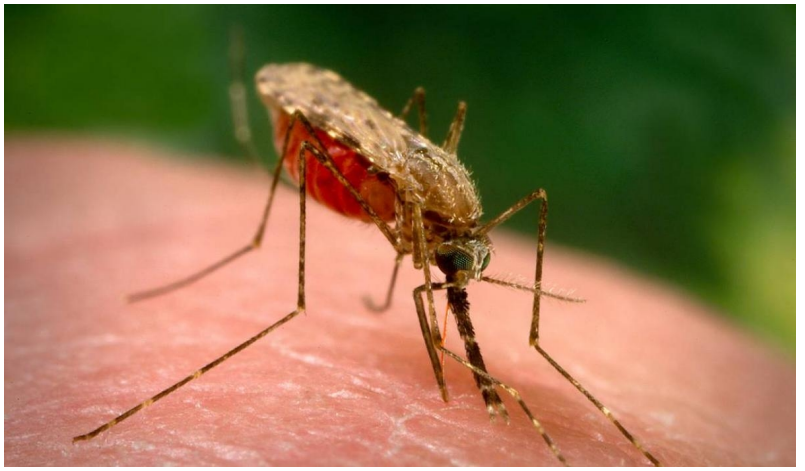
Andrew Hammond[1], Roberto Galizi[1], Kyros Kyrou[1], Alekos Simoni[1], Carla Siniscalchi[2], Dimitris Katsanos[1], Matthew Gribble[1], Dean Baker[3], Eric Marois[4], Steven Russell[3], Austin Burt[1], Nikolai Windbichler[1], Andrea Crisanti[1] & Tony Nolan[1]

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)

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

Classical Darwinian Evolution

1

Variation

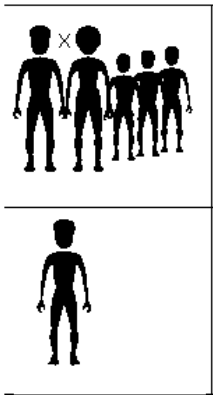
Mutations in DNA

2

Transmission to the next generation

3

Reproduction
Variability between individuals



Selection

Genetic Drift

http://evolution.berkeley.edu

What are the risks?

Molecular off-targets

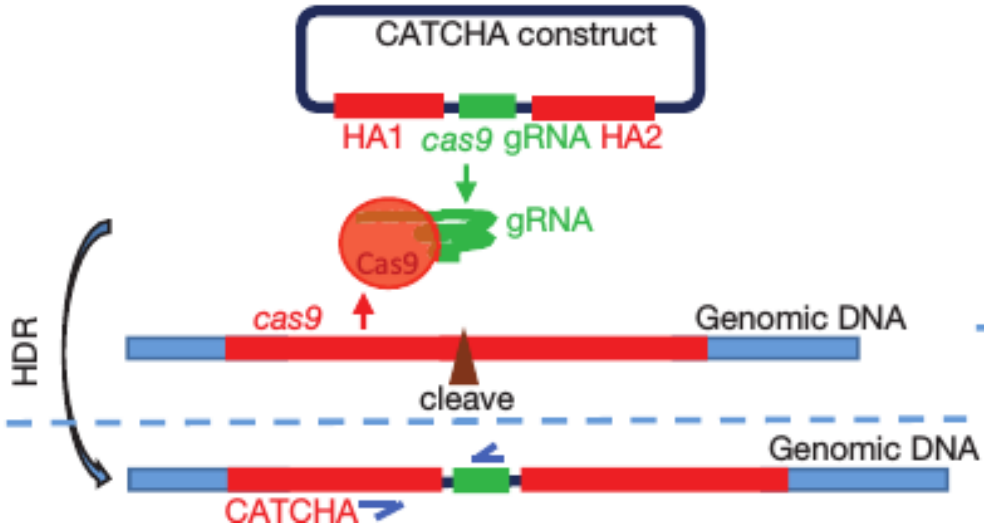
Propagation to non-target populations

Propagation to non-target species

Consequences for ecosystems

Failure of counter-measures

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

Good or bad?

May eradicate some diseases

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 ecosystems not quantified

What can we do with DNA ?

Extract, purify

Make more
Amplify
Clone
Synthetize

Examine
Quantify
Examine length
Stain, probe
Sequence

Modify
Cut
Ligate
Recombine fragments
Introduce foreign DNA
Mutate