DNA

V. Courtier-Orgogozo

Why is DNA an important molecule in biology?

Newsweek, May 23, 2005

Slide from S. Gilbert

DNA still sells cars in the USA

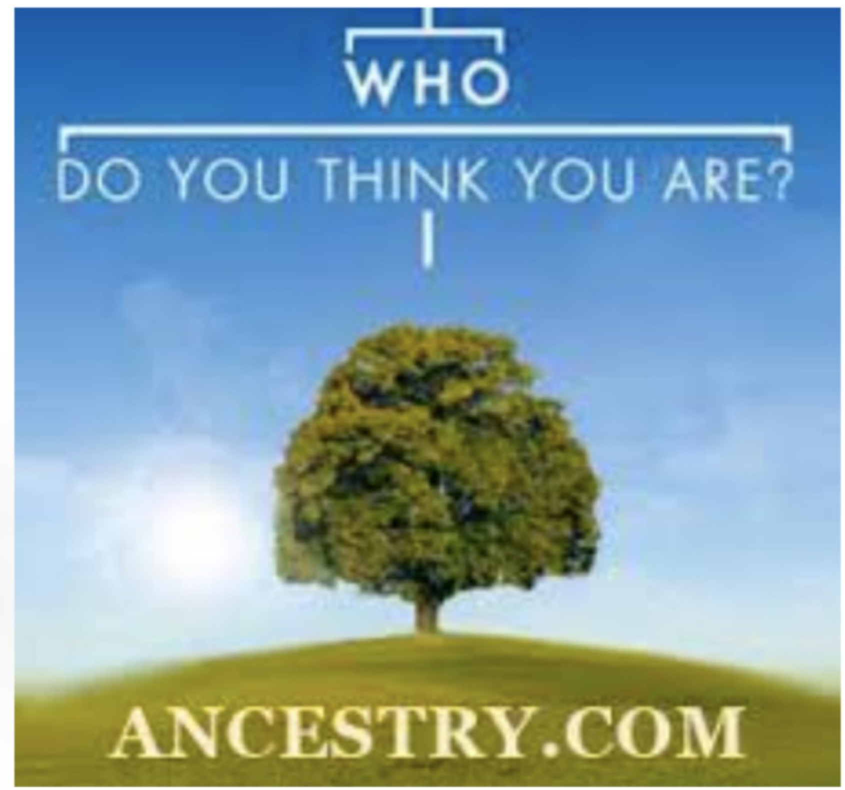
Subaru: “Genetic superstar”
Toyota: “Has a great set of genes

Slide from S. Gilbert

GENETIC INDIVIDUALITY:

Slide from S. Gilbert

Each of us is a genetically unique individual, and the genes determine who we are.



"…revealing what it is that makes you, you."

-American television ad for ancestry.com 2015

The importance of DNA in biology

Major basis of heritable variation (genotype-phenotype)

Transmitted (can help reconstruct history)

Present in all living entities (DNA/RNA)

Stable molecule (ancient DNA – oldest = horse in permafrost = 500 000 years, forensic)

String of letters, can be easily analyzed with computers (compared to anatomical traits for taxonomy)

Genetic Individuality

Slide from S. Gilbert

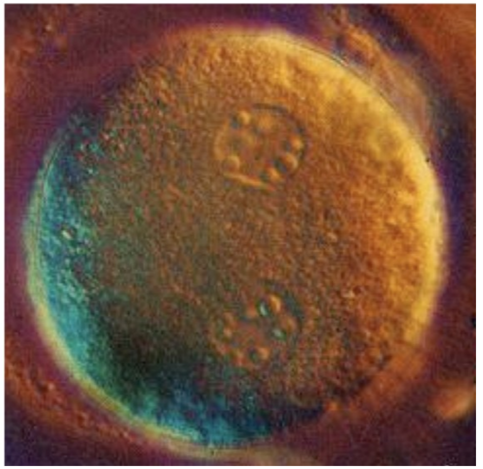
Genes determine who you are, and they act the same in each person.

LIFE Magazine, *First Days of Creation,* 1990:

"The result of fertilization is a single nucleus that contains an entire biological blueprint for a new individual, genetic information governing everything from the length of the nose to the diseases that will be inherited."

Standupgirl.com (anti-Choice website):

"And even more amazingly, intelligence and personality—the way you look and feel—were already in place in your genetic code. At the moment of conception you were essentially and uniquely you."



Disclaimer:
DNA is not the cause of everything

Monozygotic twins are not identical

Cardiovascular disease associates better with lifestyle than with DNA sequence (Mozaffarian 2008)

Lung cancer associated with smoking habits

Drug metabolism is mostly due to the microbiome

Several genes associated with autism, depression, etc. were “lost” in larger studies

Distilbene: anti-miscarriage drug, increases cancer risks in daughters and malformations in grand-daughters

What the HGP Taught us with the first genome sequenced: Genes act differently and non-additively in different people

Cockayne syndrome: **Mutation in the DNA repair enzyme ERCC6 at position 5q12.1.** Homozygous recessive persons are characterized by growth failure, impaired neural development, premature aging, sensitivity to sunlight.

Usher Syndrome: **Mutation in the retinal and cochlear basement membrane myosin MYO7A at 11q13.** Homozygous recessive persons are characterized by congenital deafness and gradual loss of vision.

James Watson, presently 90 years old; not deaf, blind, nor stunted

Slide from S. Gilbert

Manipulating DNA

What can we do with DNA ?

What can we do with DNA ?

Extract, purify

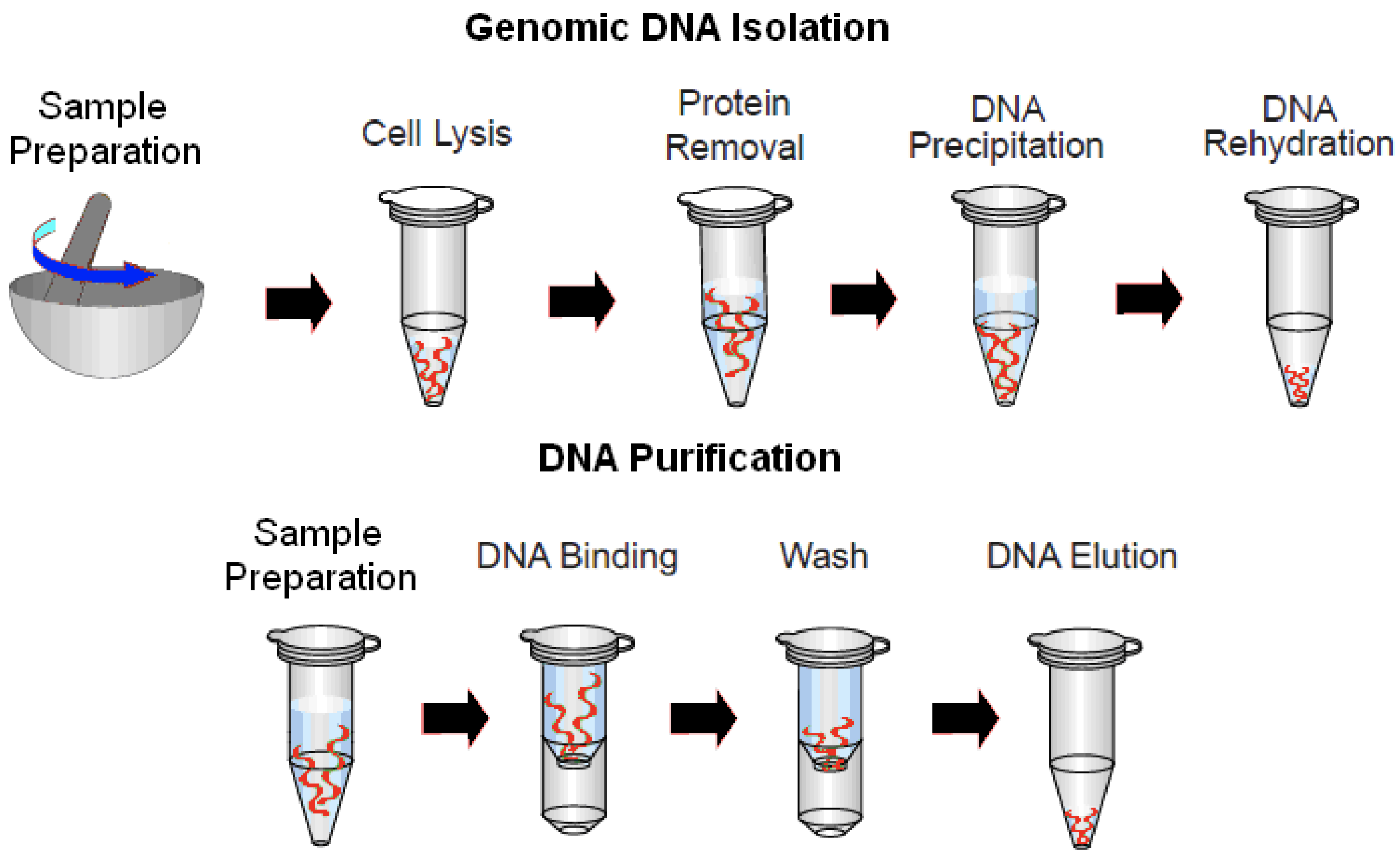
Make more
Amplify
Clone
Synthetize

Examine
Quantify
Examine length
Stain, probe
Sequence
Examine 3D structure
Measure tension of DNA molecules

Modify
Cut
Ligate
Recombine fragments
Introduce foreign DNA
Mutate

Extract DNA

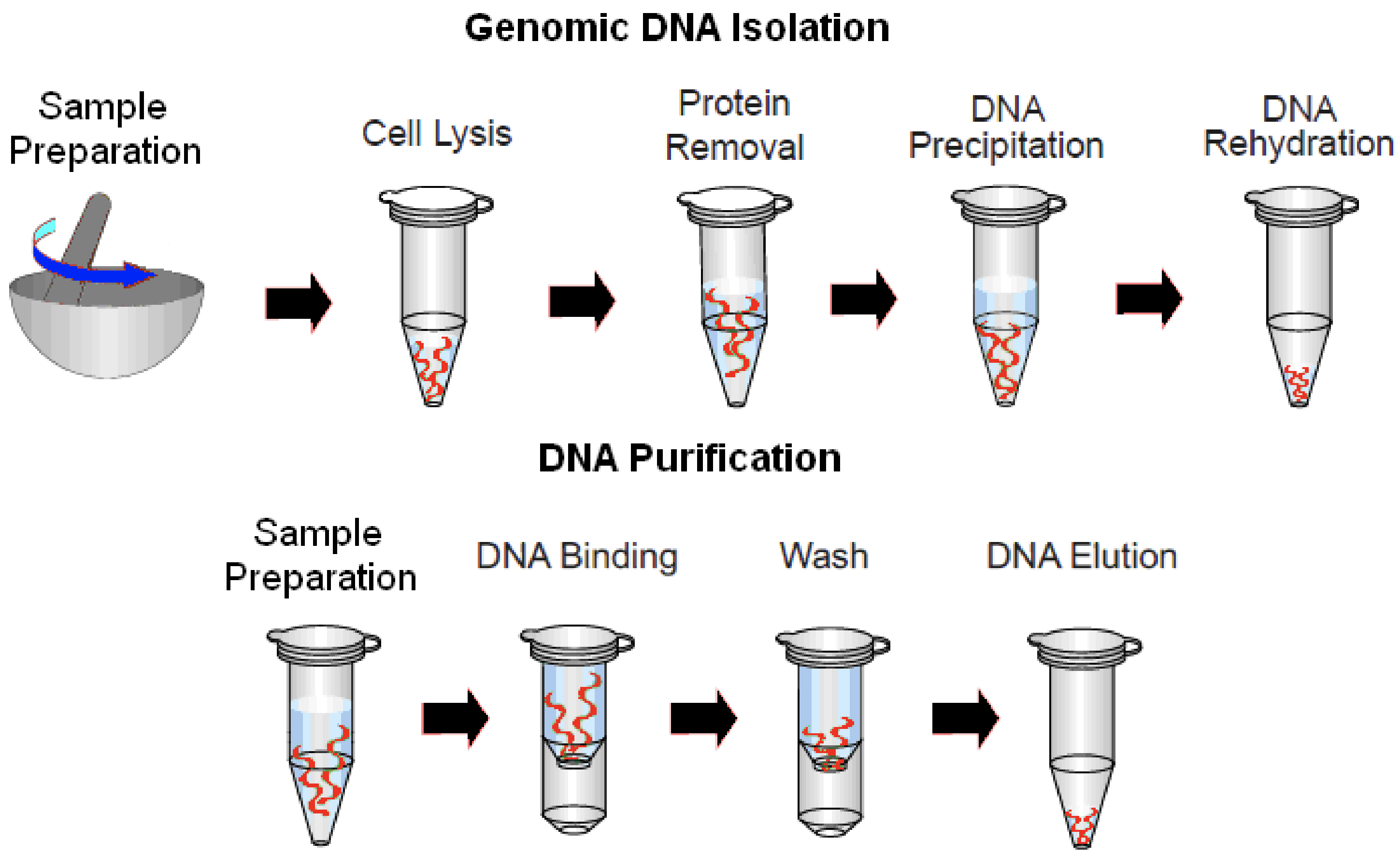
Break cells, remove lipids and proteins,
precipitate DNA, remove liquid, resuspend in aquaeous solution



Be aware of contaminants!

Extract DNA

Break cells, remove lipids and proteins,
precipitate DNA, remove liquid, resuspend in aquaeous solution



Be aware of contaminants!
(DNA from mitochondria, viruses, bacteria, researcher, symbionts...)

Amplify DNA

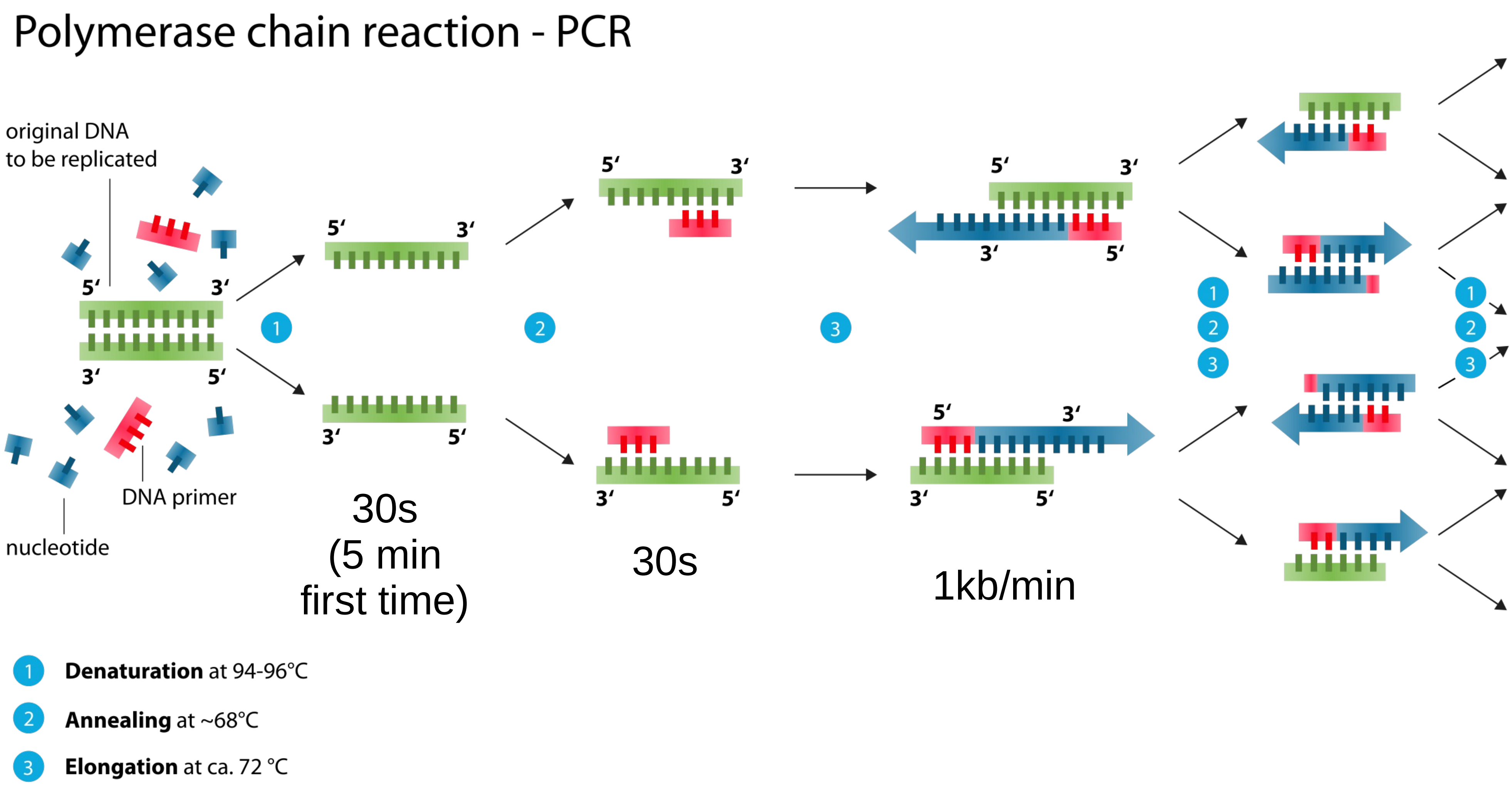
Mix:
Genomic DNA
Probes (oligonucleotides)
Nucleotides
Taq polymerase
Ions (MgCl2)

Cycles of Denaturation, Annealing, Elongation

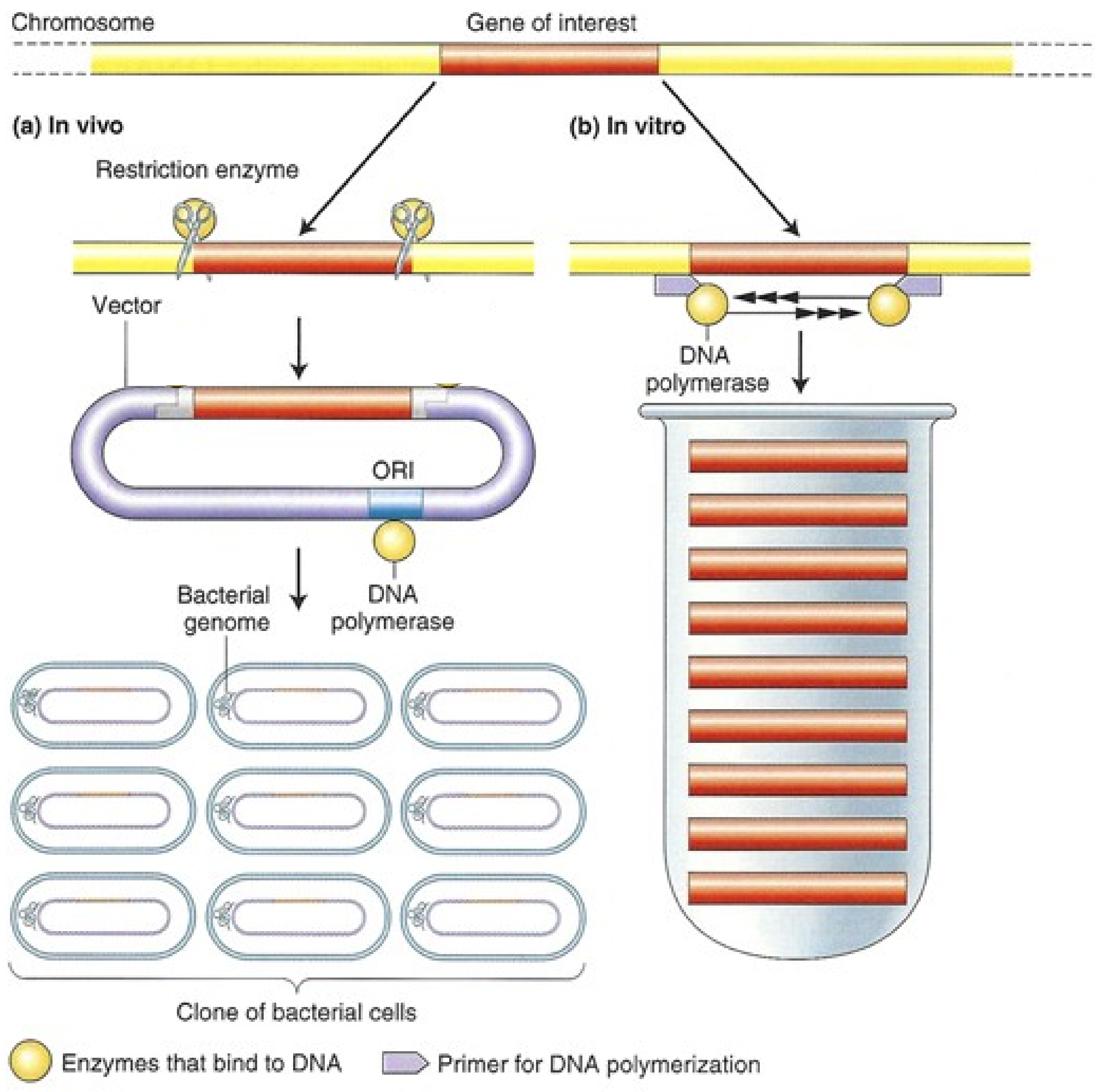
PCR: Polymerase Chain Reaction

Amplifies DNA fragments of between 0.1 and 10 kb (up to 40 kb)

Amplify DNA


Polymerase chain reaction - PCR
original DNA
to be replicated
5'
3'
3'
5'
DNA primer
nucleotide
1
2
3
30s
(5 min
first time)
30s
1kb/min
Denaturation at 94-96°C
Annealing at ~68°C
Elongation at ca. 72 °C

Cloning vs. PCR



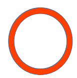
Amplify DNA

DNA fragments
5 kb-15 kb: plasmids in bacteria
~10 kb: lambda phage-based vectors
Up to 40 kb: fosmids in bacteria
~100-300 kb: bacterial artificial chromosomes (BAC)

First “synthetic” cell developed by scientists

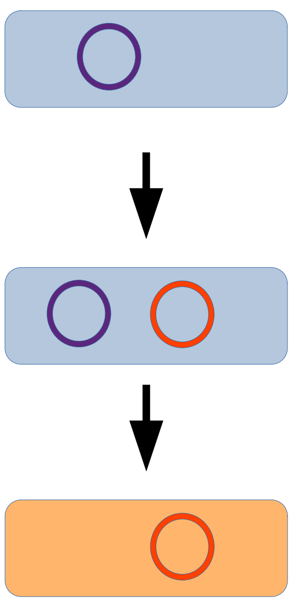
M. mycoides JCVI-syn1.0
1.08 million base pairs and 901 genes

single yeast artificial chromosome

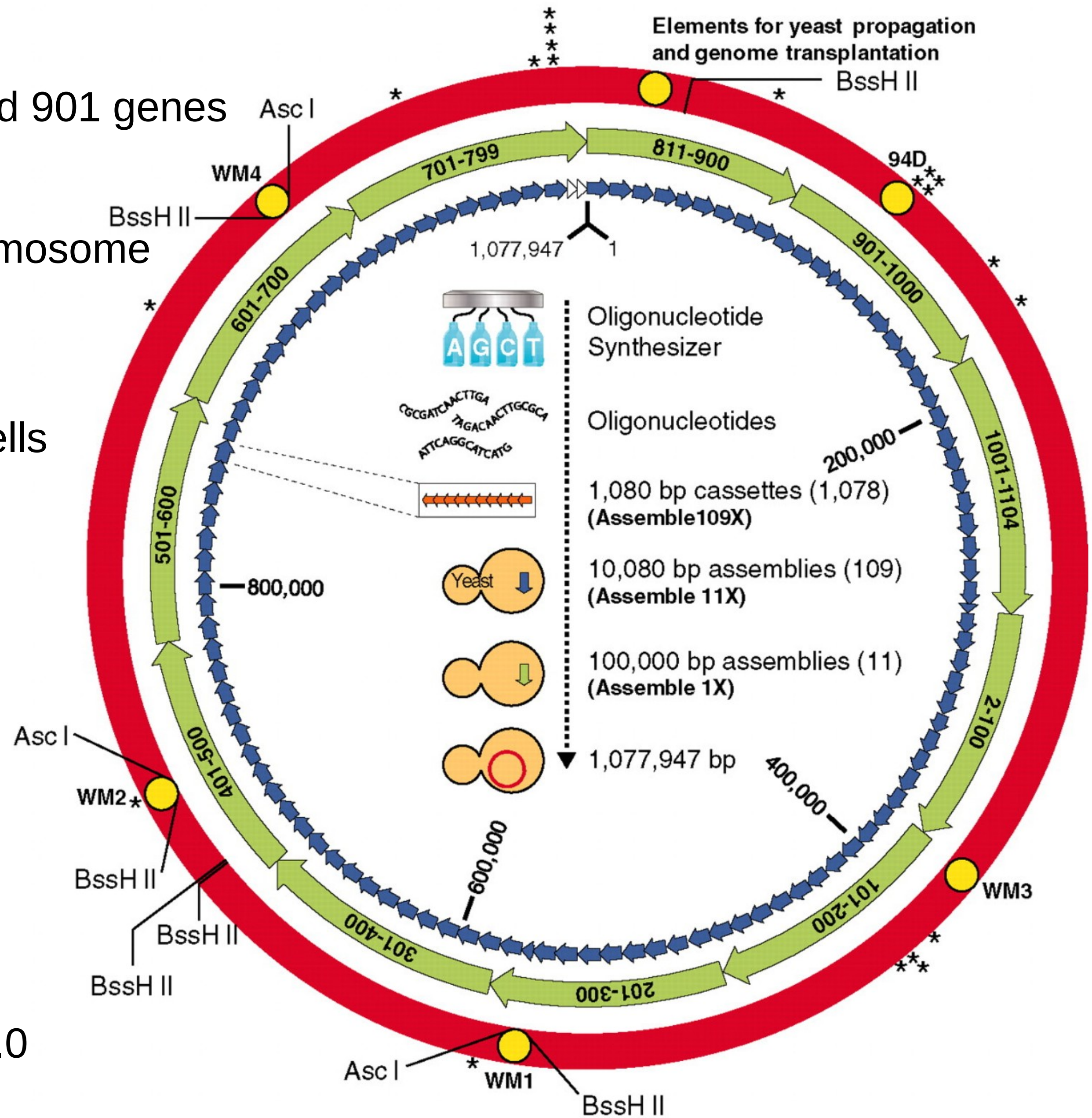


+

M. capricolum recipient cells



M. mycoides JCVI-syn1.0

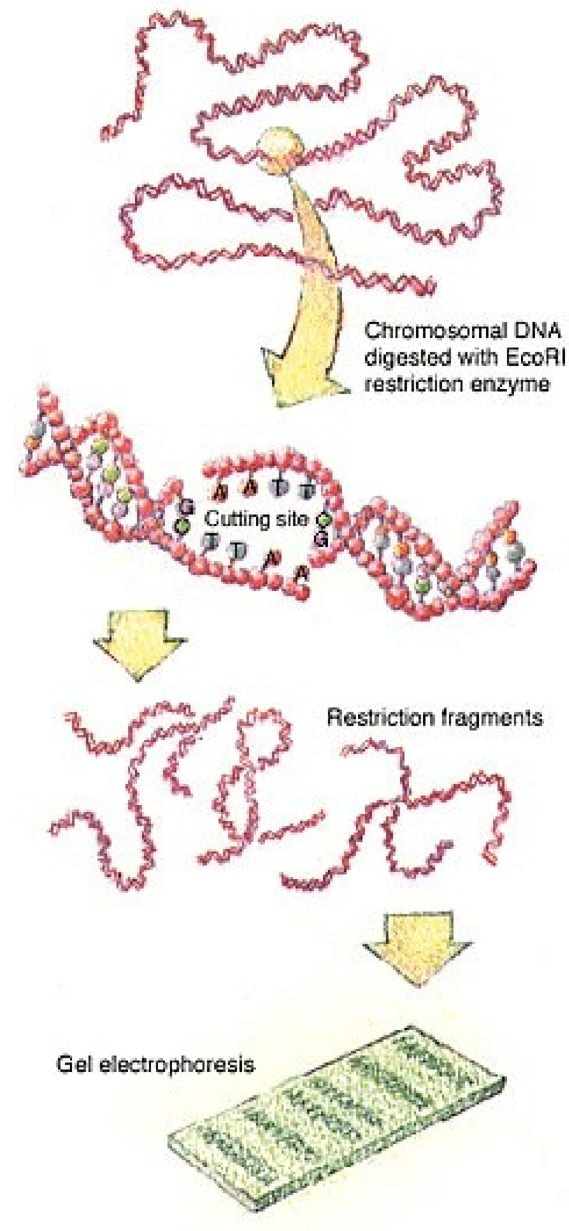


Gibson et al. 2016

Cut DNA with restriction enzymes

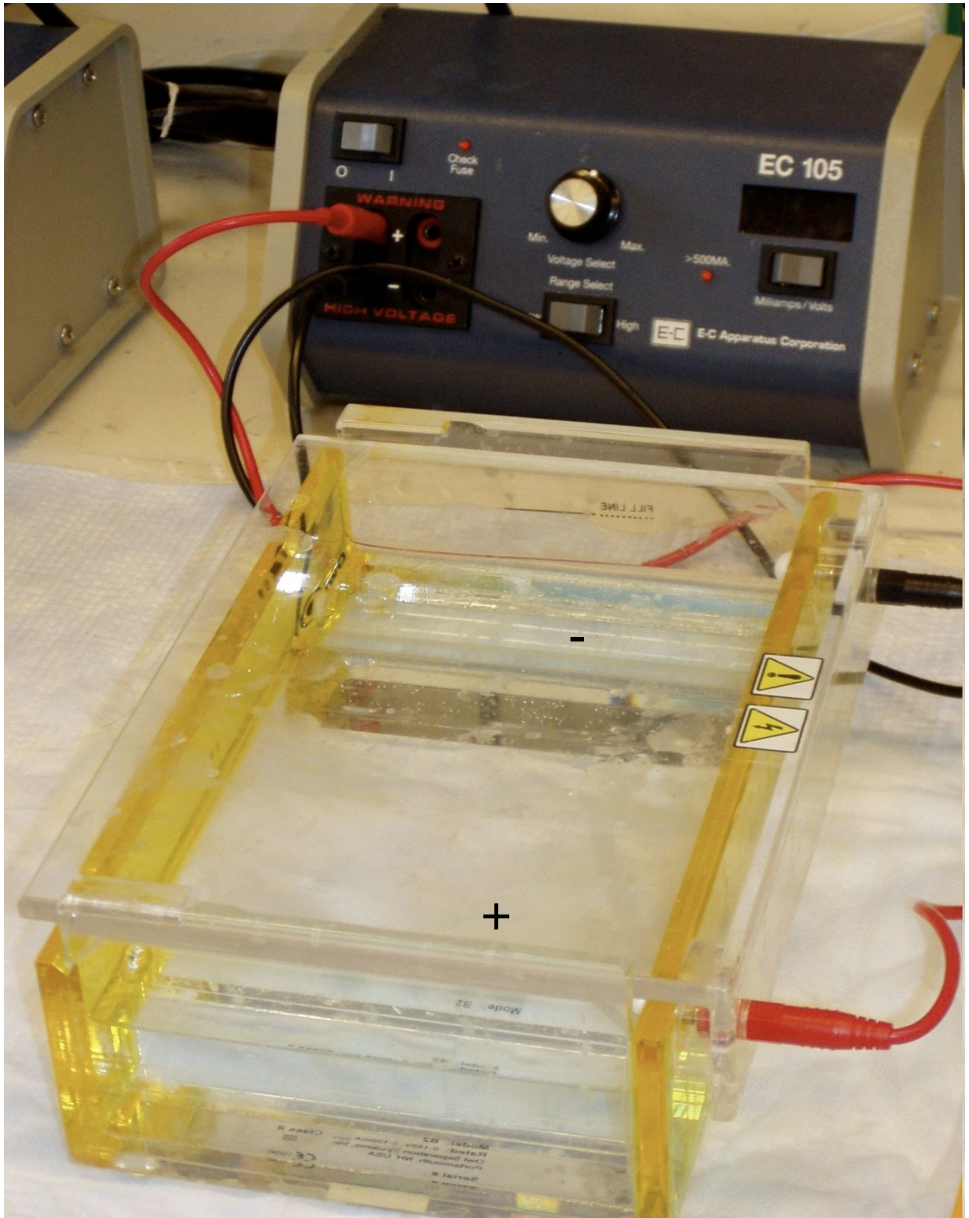
Blunt ends, 3’ protruding ends, 5’ protruding ends

Cut DNA with restriction enzymes

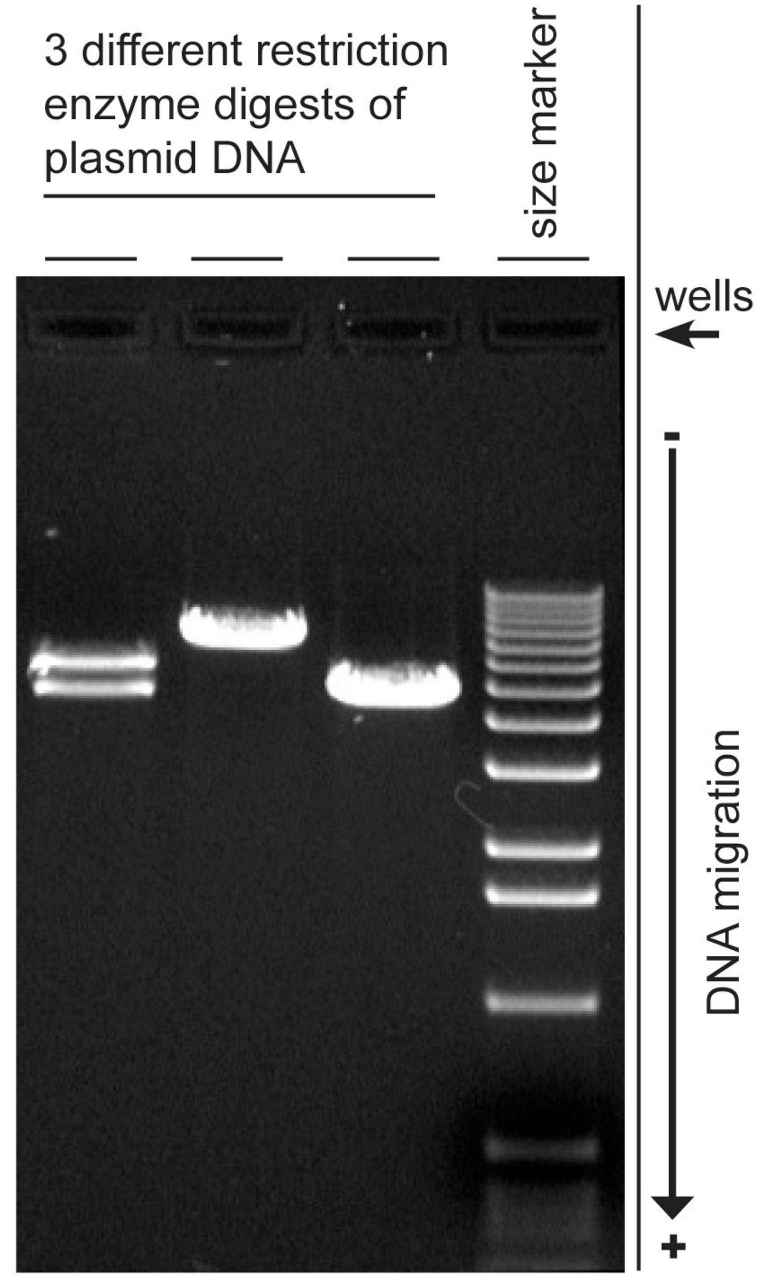


Blunt ends, 3' protruding ends, 5' protruding ends

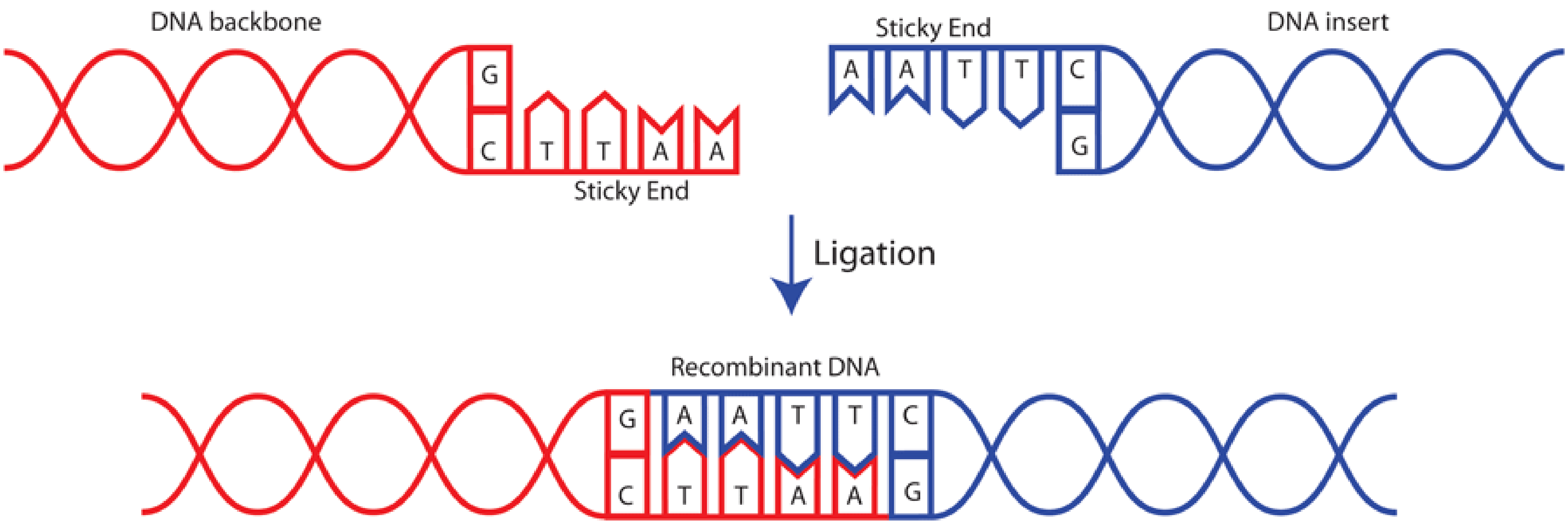
Examine length of DNA

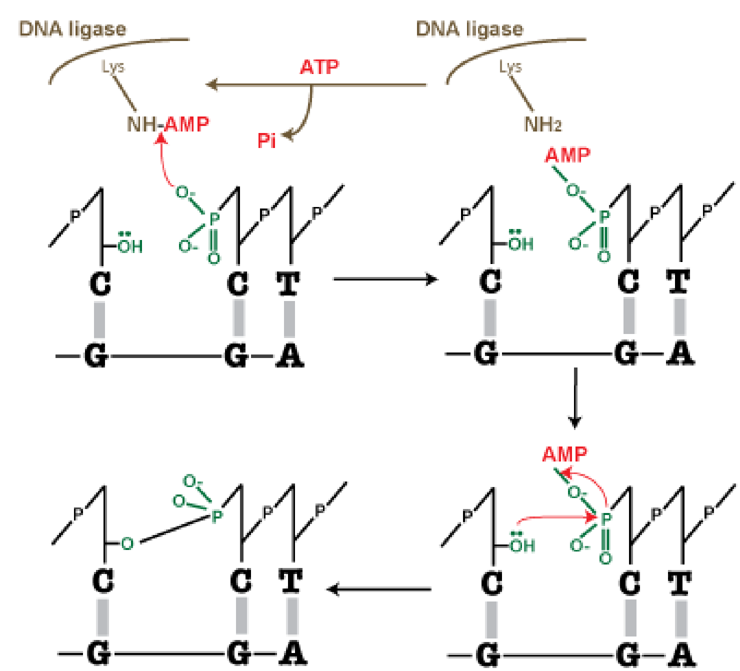


TAE (Tris-acetate-EDTA) buffer



Ligate DNA

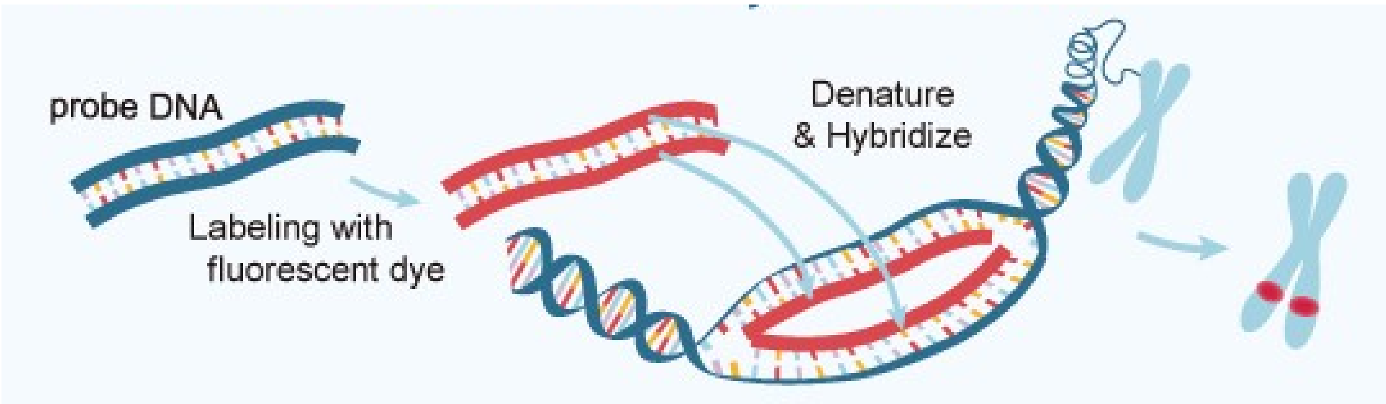


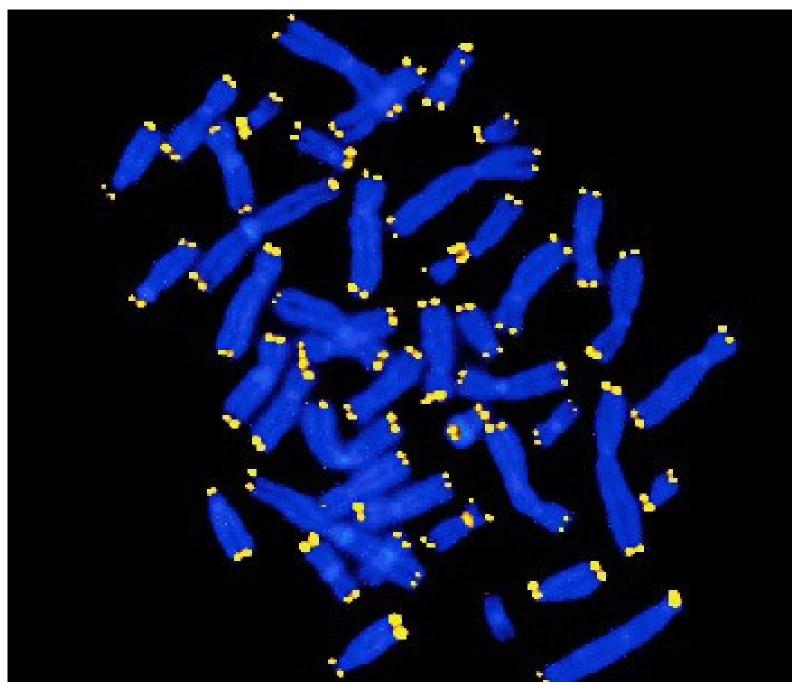


Fragments have to be phosphorylated but only on one strand

Dephosphorylate the vector to inhibit self-circularization

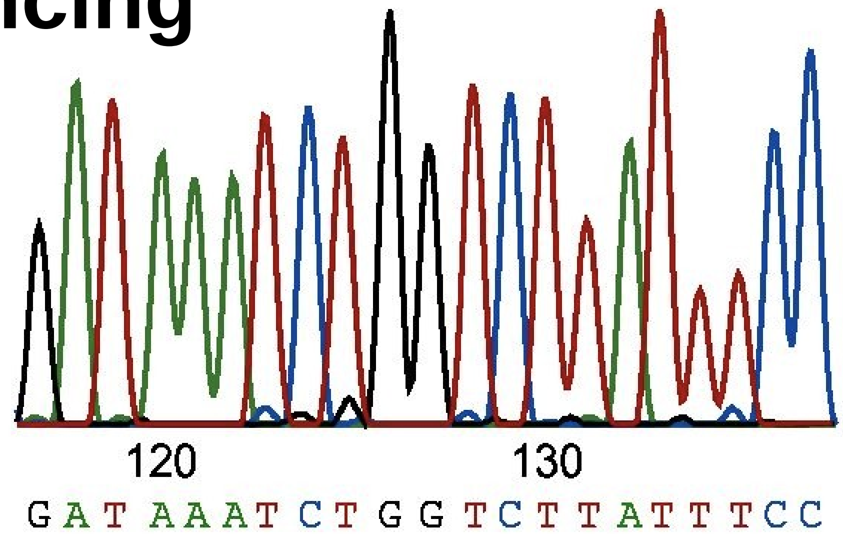
Probe DNA:
Fluorescent In Situ Hybridization





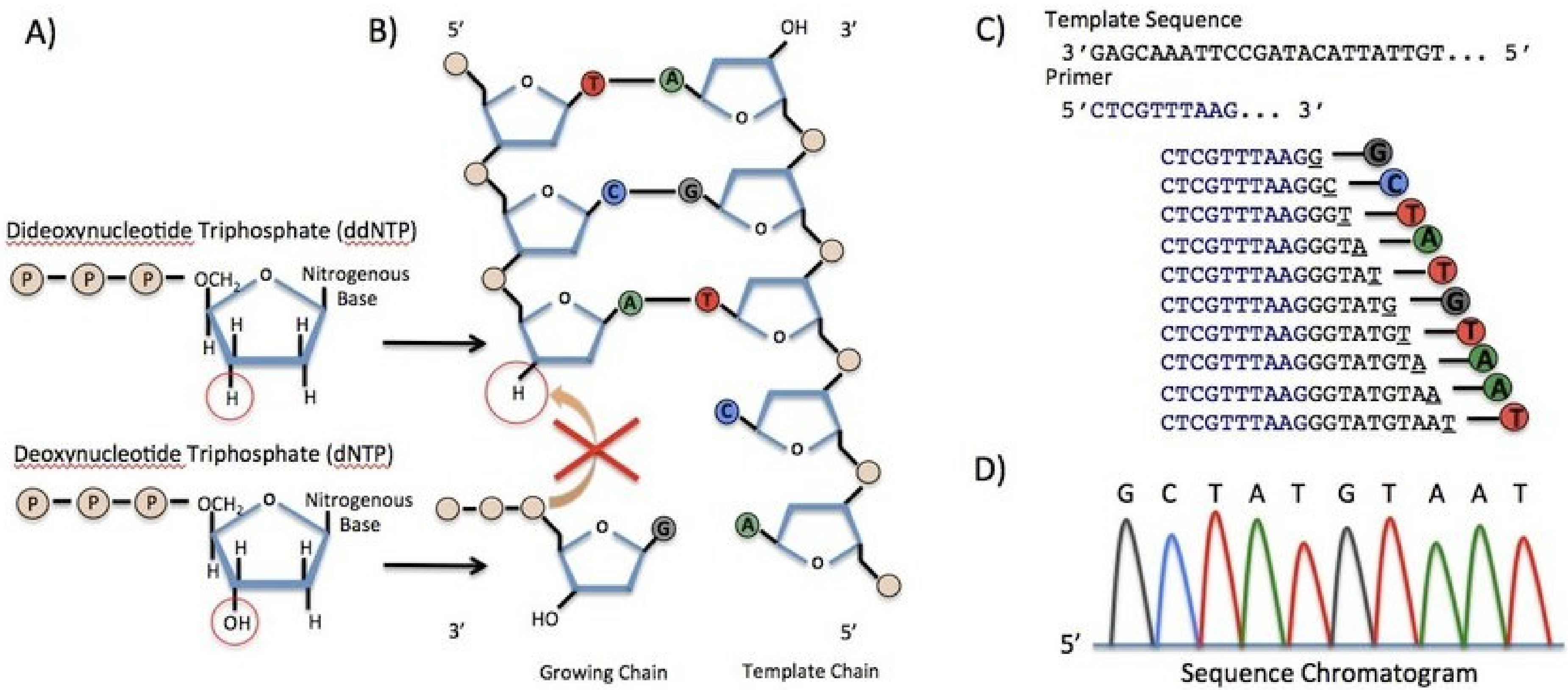
Probes for telomere sequences

Sanger sequencing



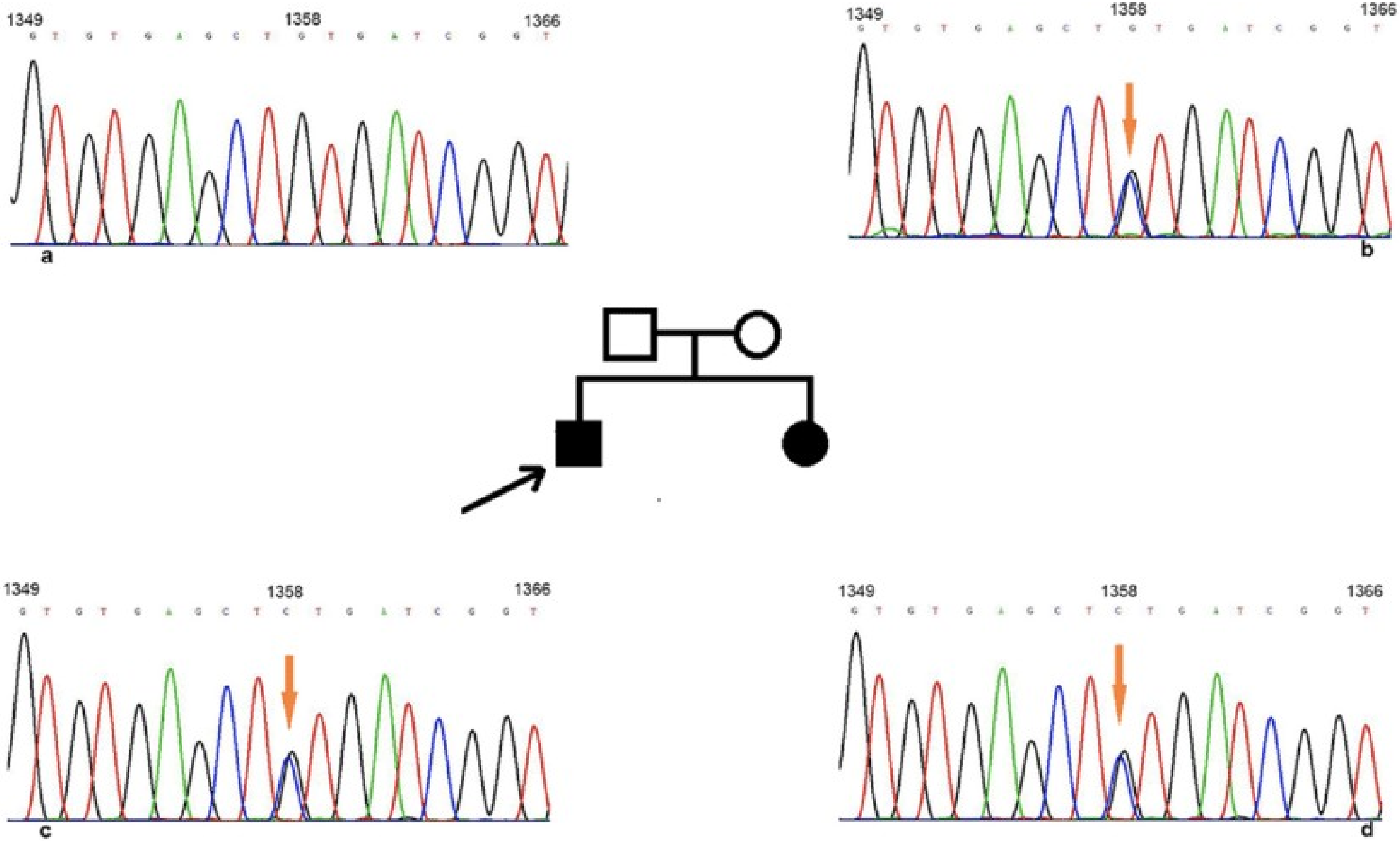
800 bp long
Starts based on oligonucleotide (primer)
~4 euros per reaction

Dye terminator sequencing

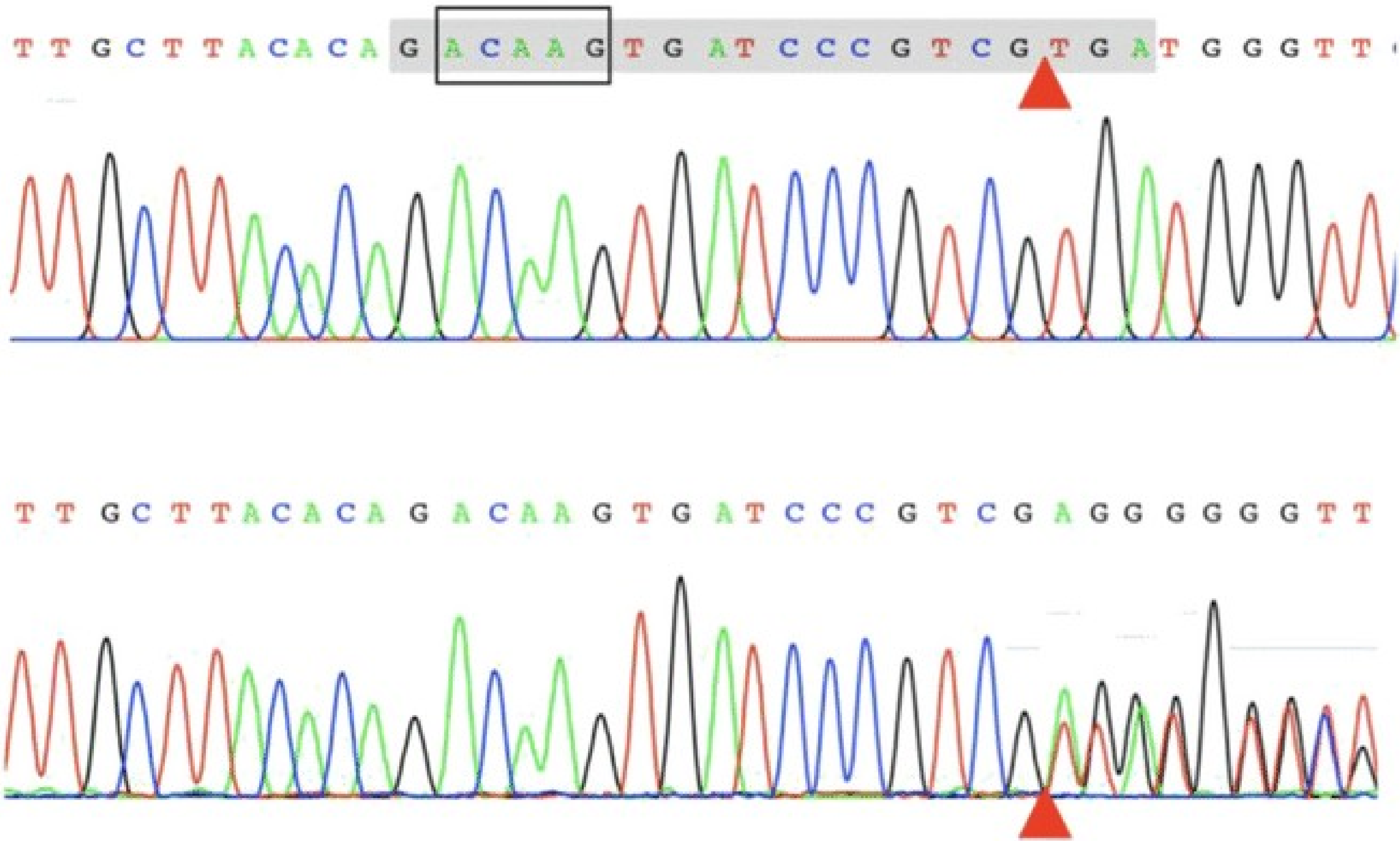


McGovern 2015

GTGTGAGCTGTGATCGGT

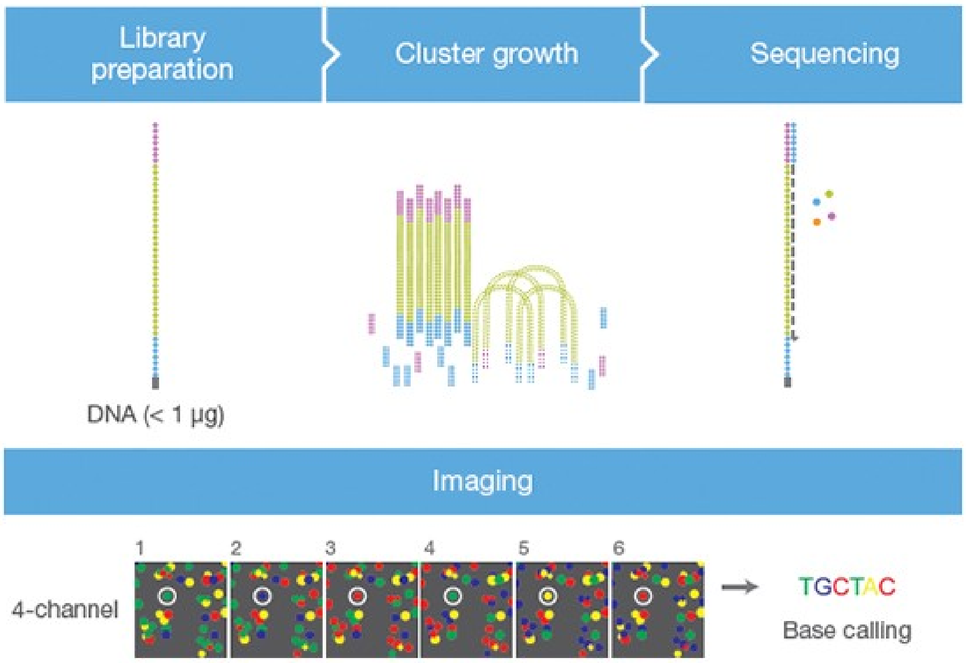


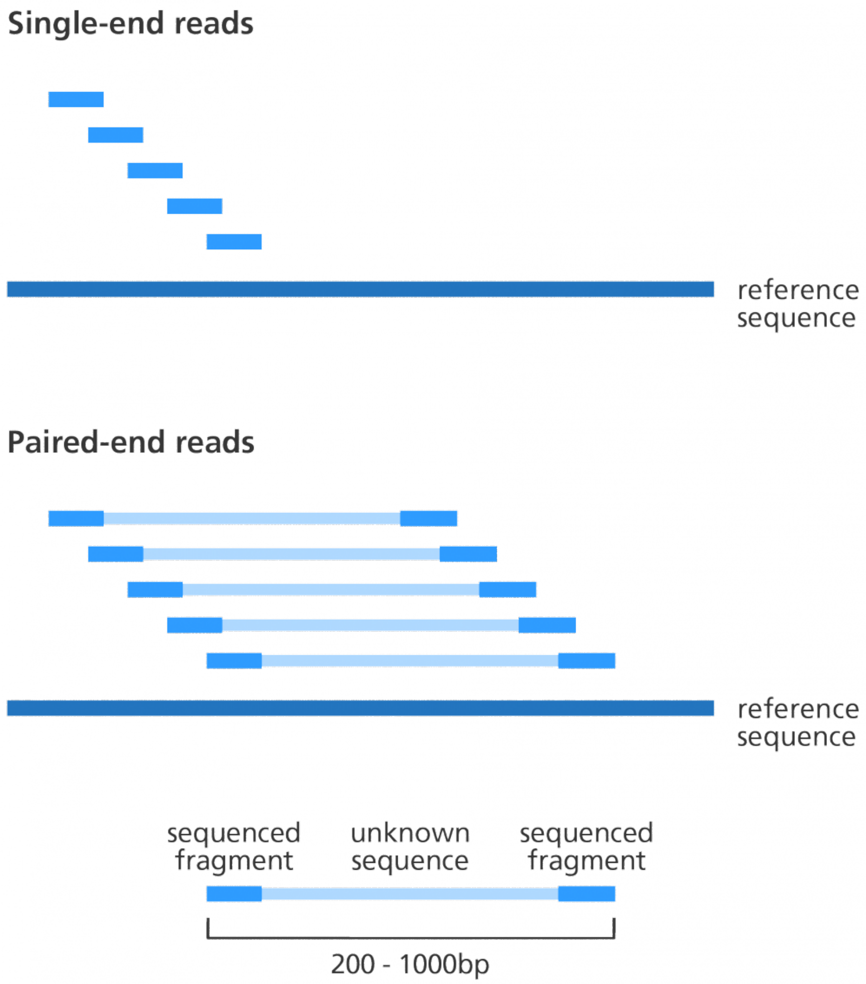
Ding et al 2015


T T G C T T A C A C A G A C A A G T G A T C C C G T C G T G A T G G G T T
T T G C T T A C A C A G A C A A G T G A T C C C G T C G A G G G G G G T T

Illumina sequencing

Millions of reads, each ~100 bp long
Starts at all possible positions
~500 euros per run

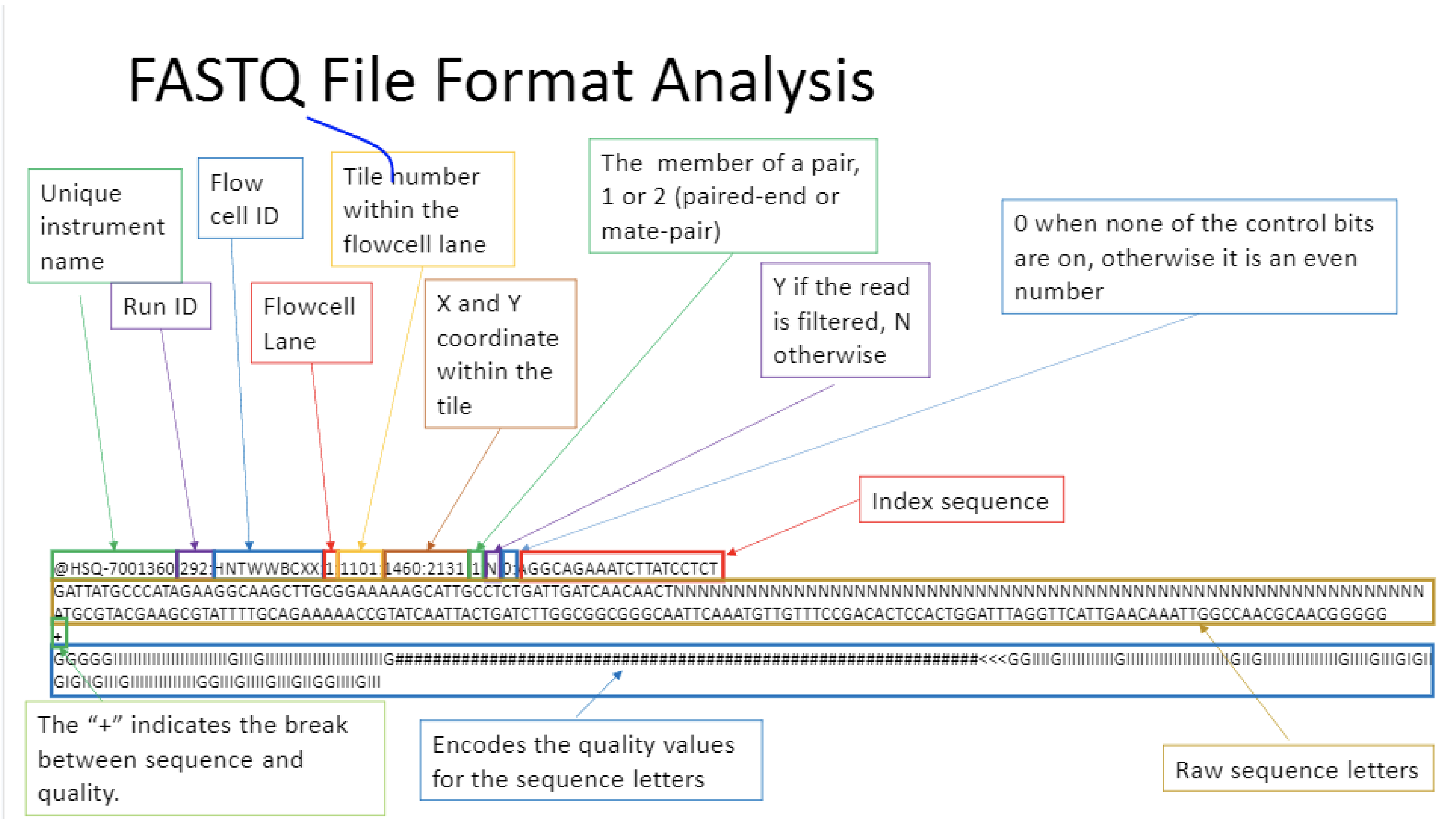




For transcriptome: 2x 75 bp
For whole genome: 2x 150 bp

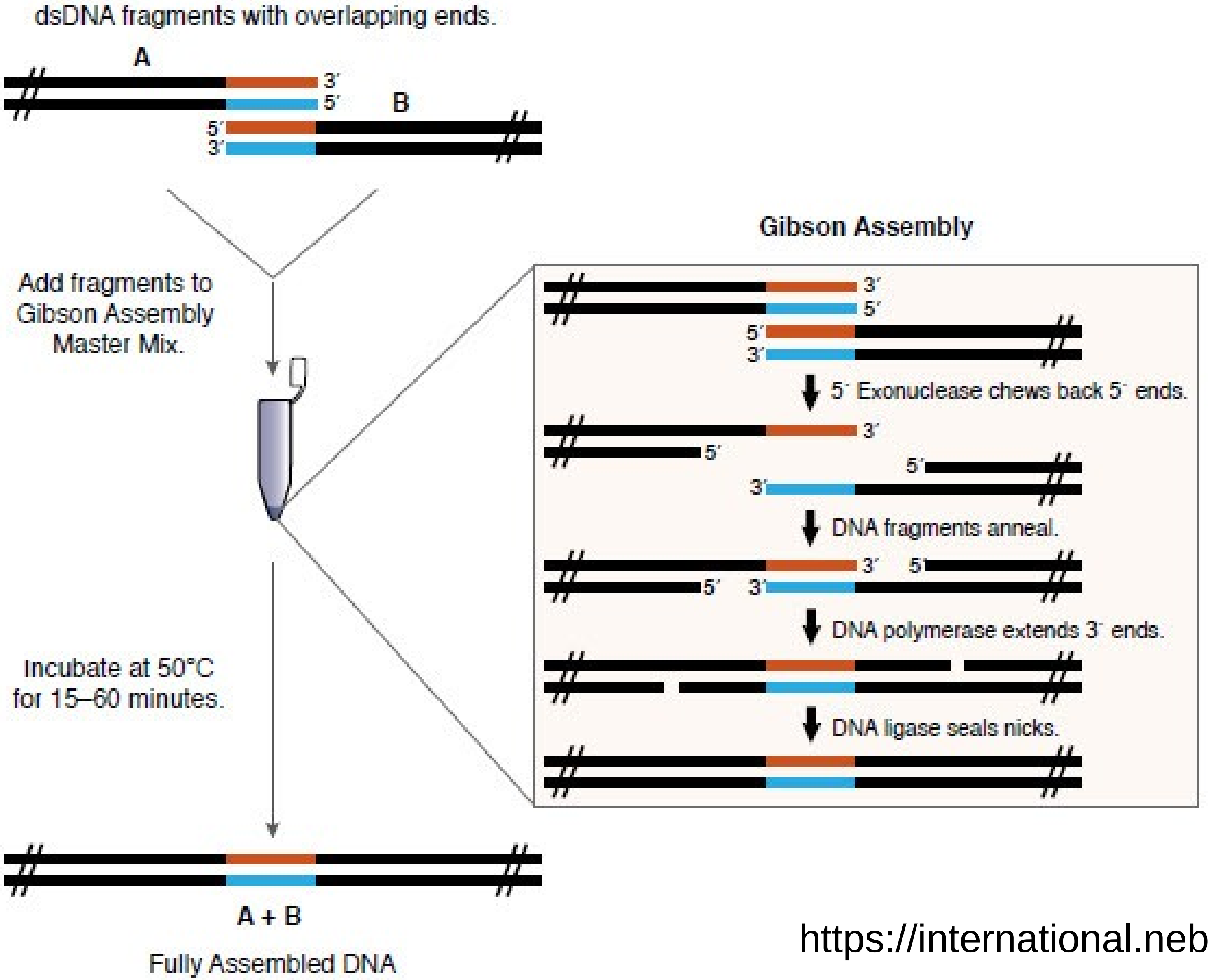
https://www.illumina.com/science/technology/next-generation-sequencing/sequencing-technology/2-channel-sbs.html

Output of Illumina sequencing



Recombine DNA: Gibson cloning

Prepare fragments using PCR and special primers



https://international.neb.com/products

Synthetize DNA

GenScript
Make Research Easy

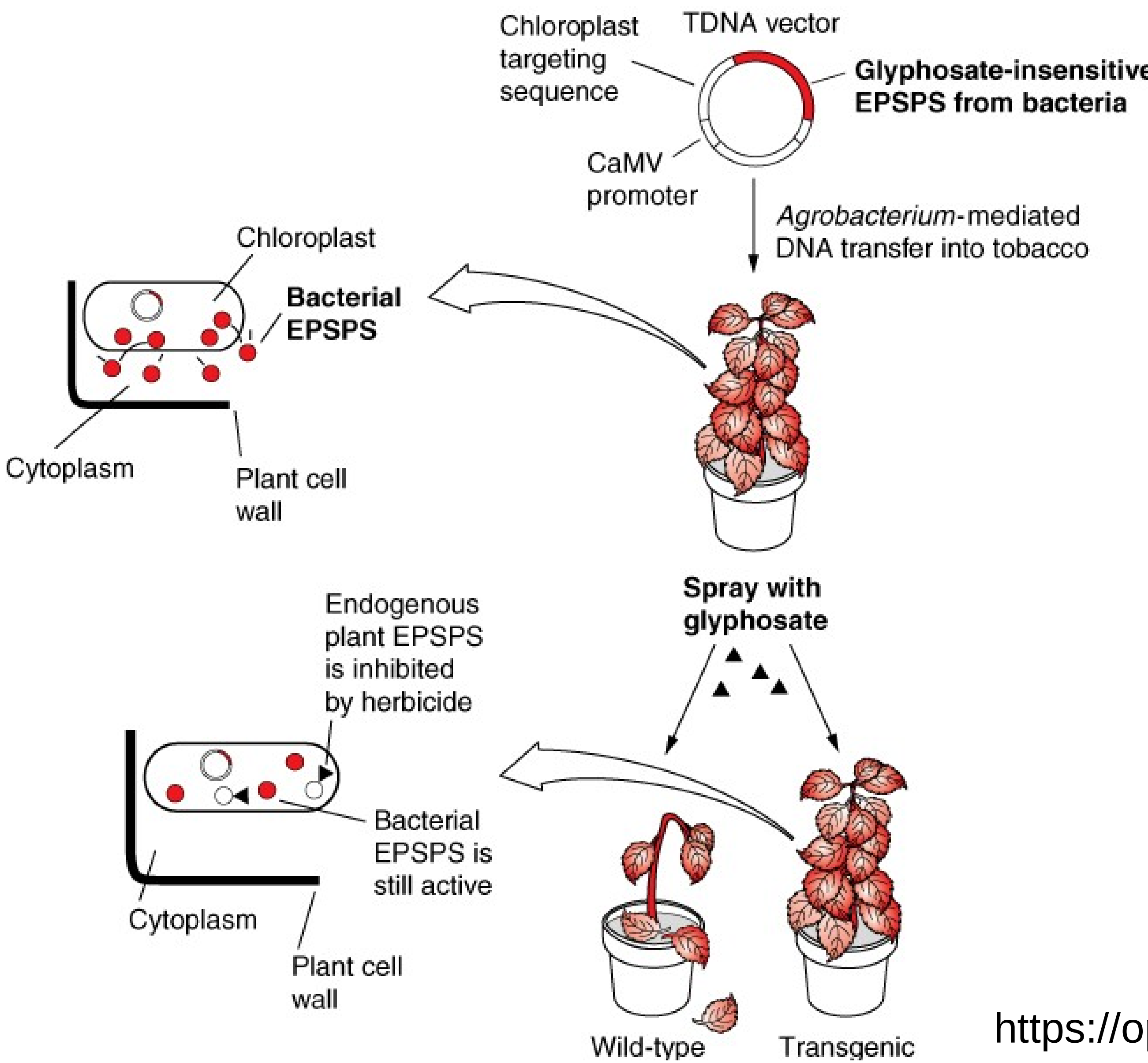
Gene Synthesis Service Options

Types	Gene Length	Price (No hidden charge promise) †	Starting Turnaround Time (Business Days) *	Starting Turnaround Time with Plasmid Prep Service (Business Days)
Standard Gene Synthesis *Guaranteed*	≤ 8 kb	View your discounted price online in as short as 1 minute	8	10
Fast Gene Synthesis *Guaranteed*	≤ 5 kb		7	9
Rush Gene Synthesis *Guaranteed*	≤ 4 kb		4 *US Manufacture*	6 *US Manufacture*
GenPlus HT Gene Synthesis	≤ 3 kb		18	20
GenPlus Economy Gene Synthesis	≤ 8 kb		15	17
GenBrick® Gene Synthesis	> 8 kb		23	25

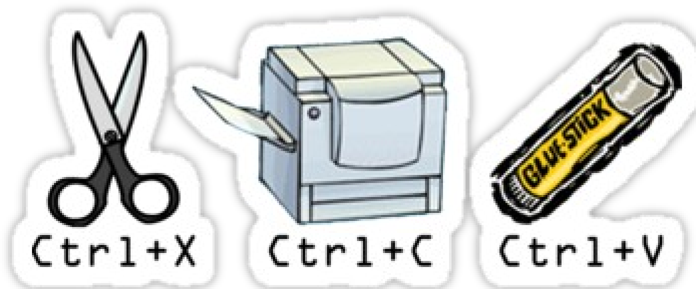
Introduce foreign DNA

most widespread transgenic crop in 2005-2015 = soybean resistant to glyphosate

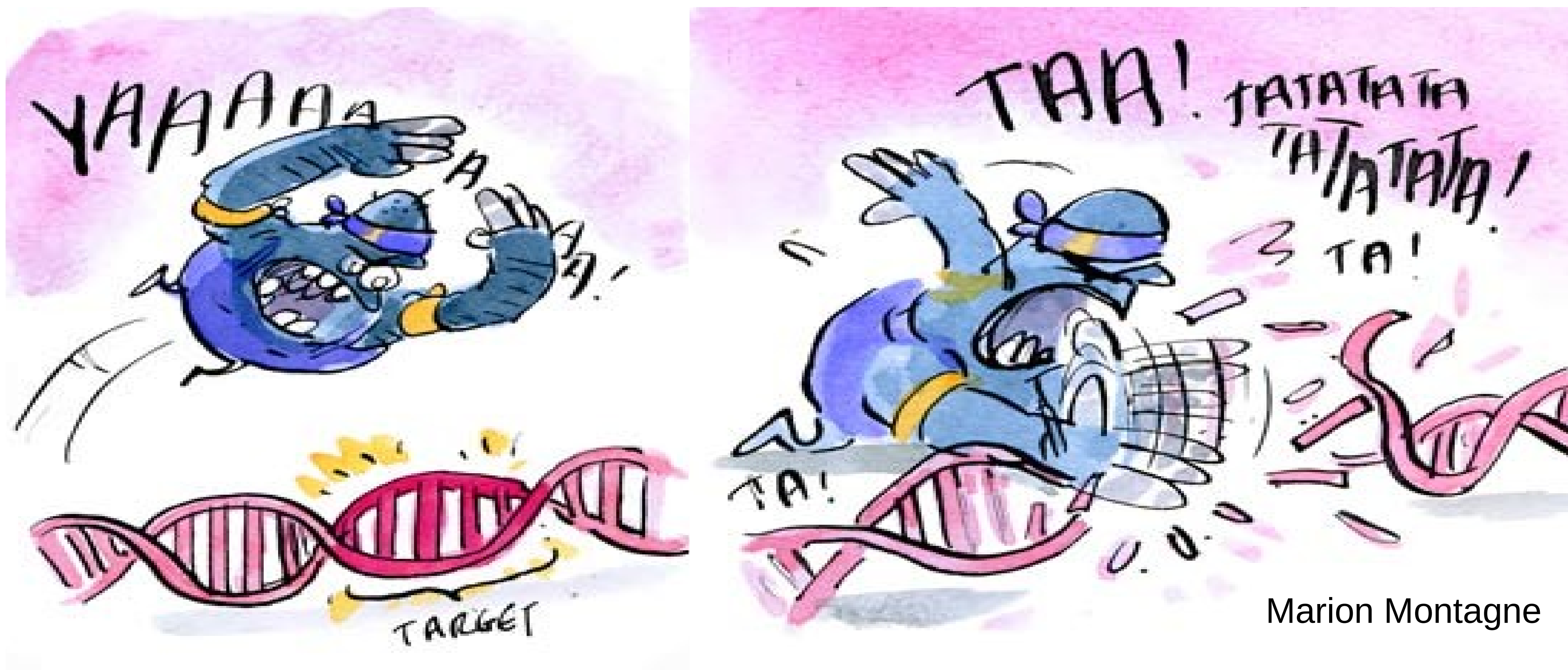
In tobacco:



https://openwetware.org

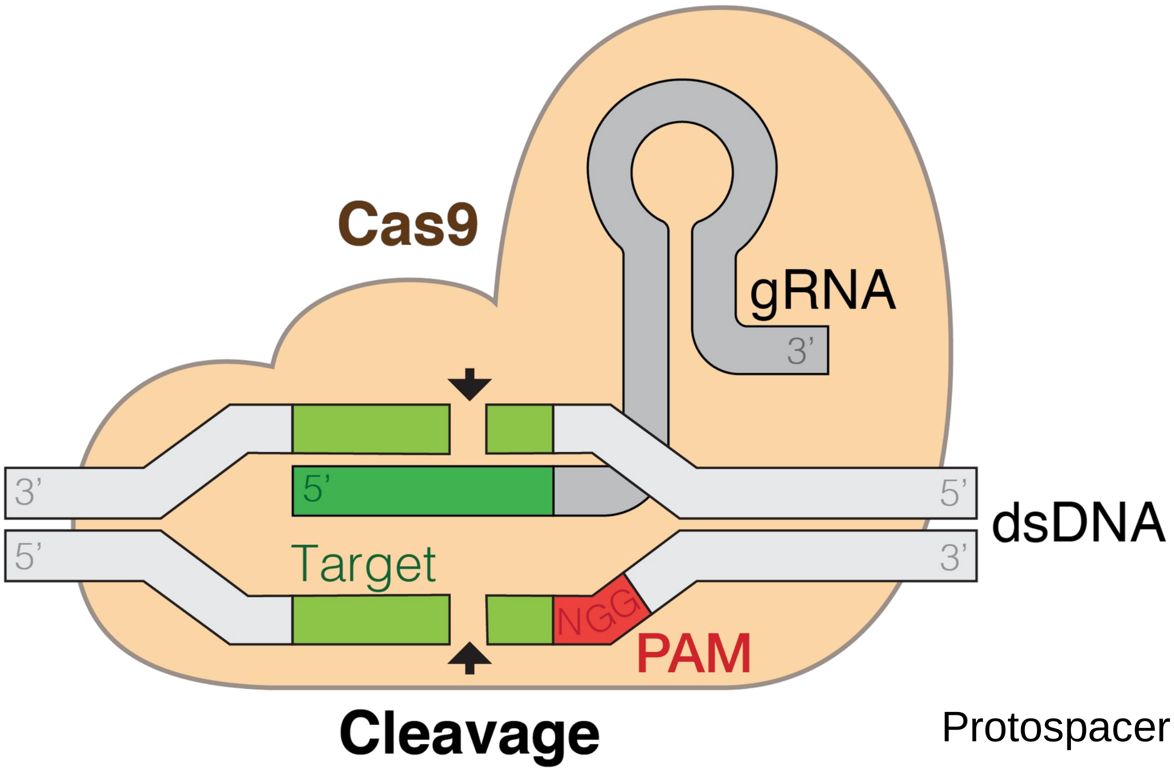


CRISPR



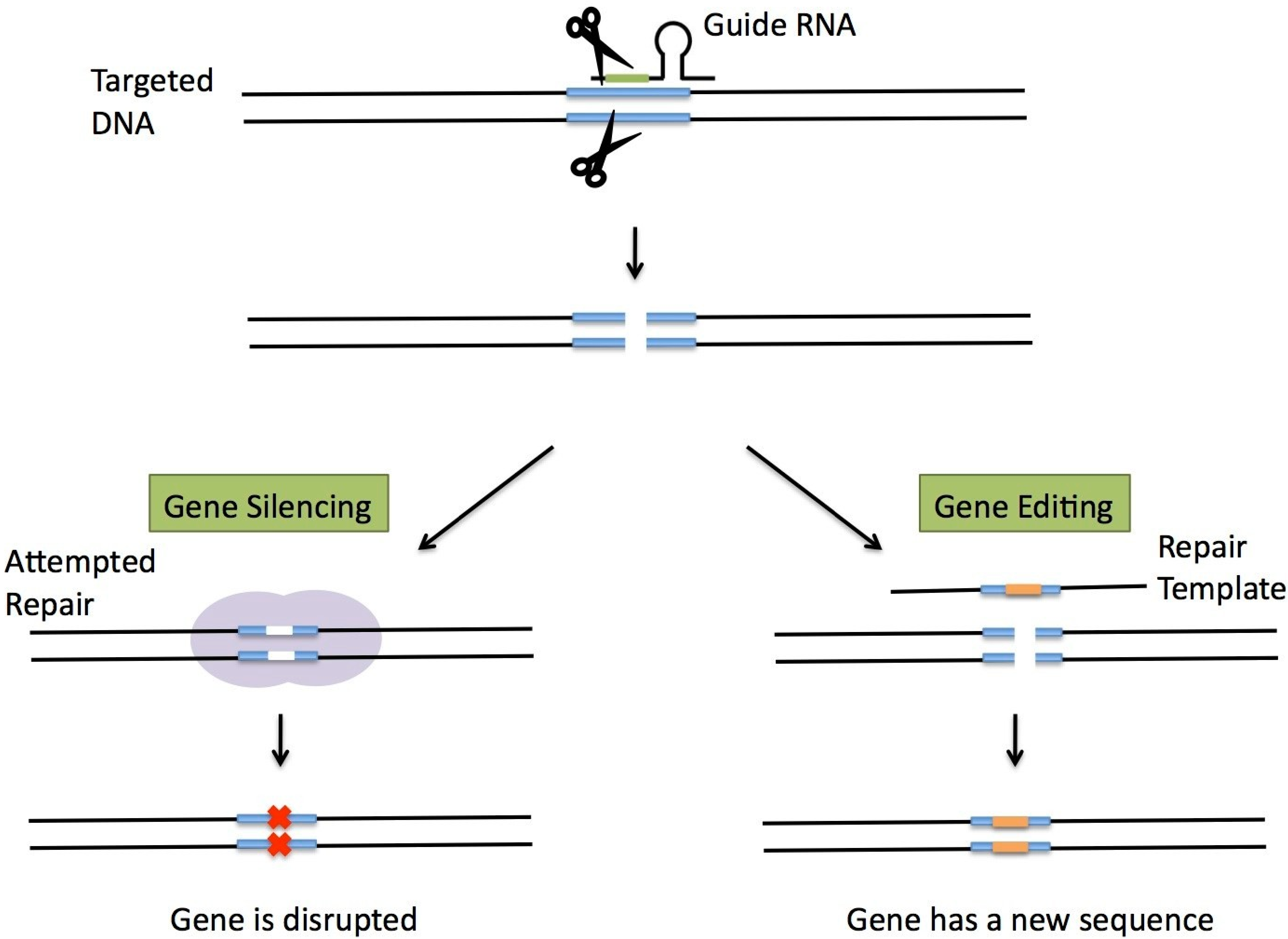
Marion Montagne

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

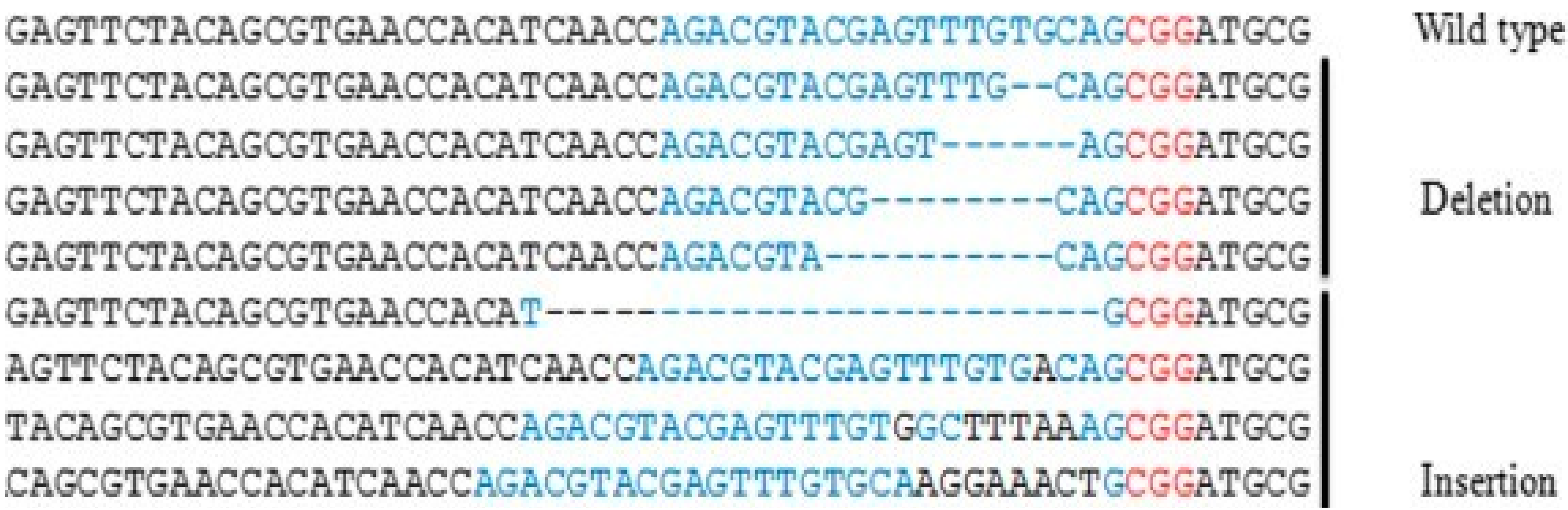


Protospacer adjacent motif (PAM)

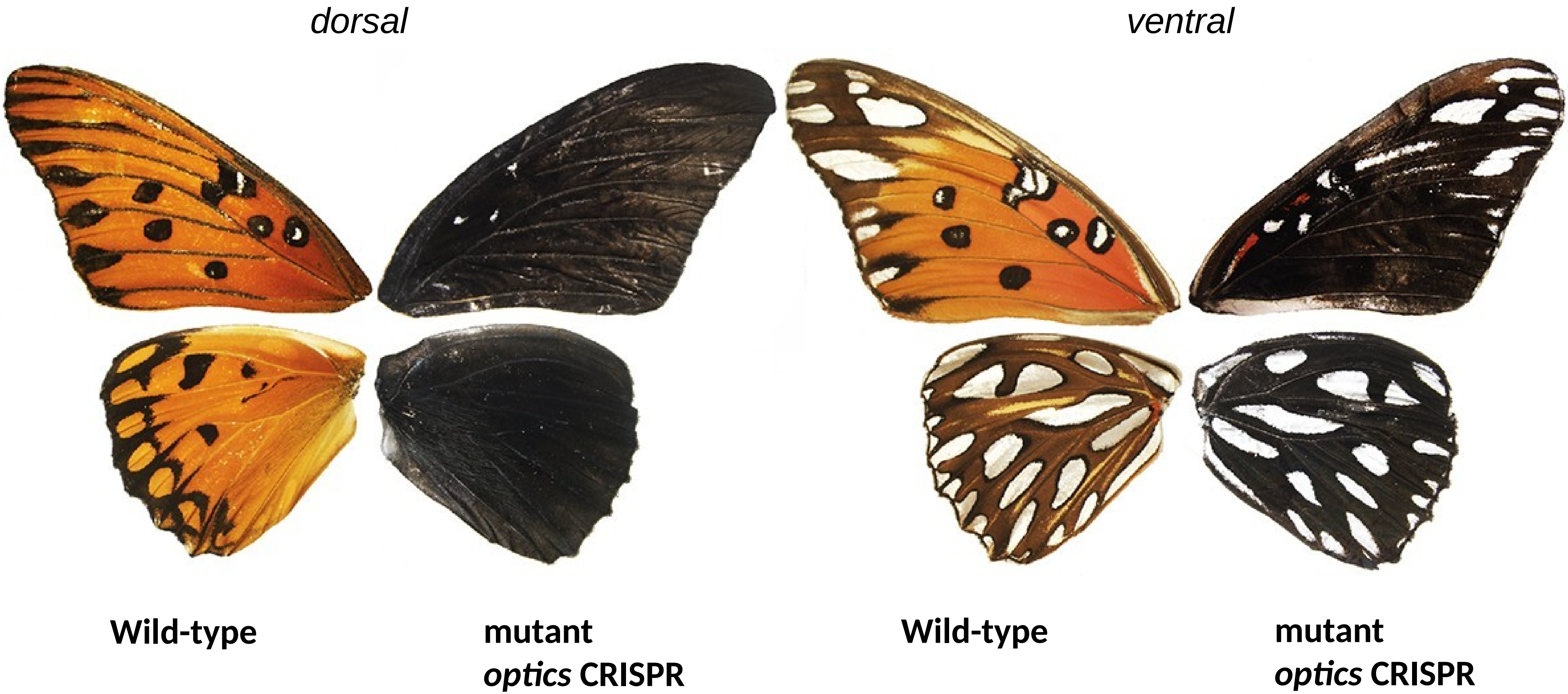
Can recognize and cut a specific DNA sequence (recognized by guide RNA)
More versatile than restriction enzymes, Zn finger nucleases and transcription activator-like effector nucleases (TALENs).


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

Creating mutants with CRISPR/Cas9



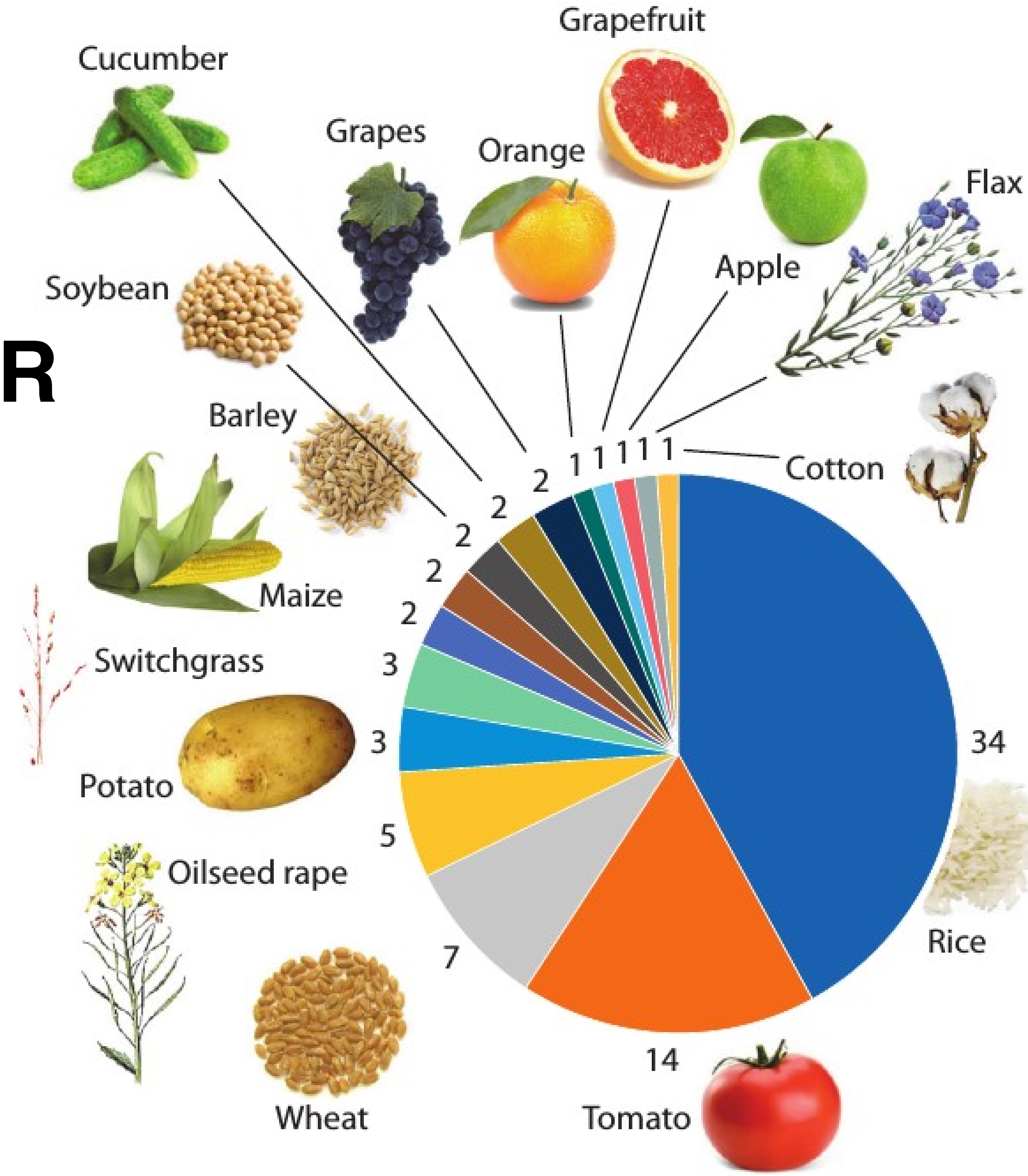
Agraulis vanillae



Reed et al. 2017

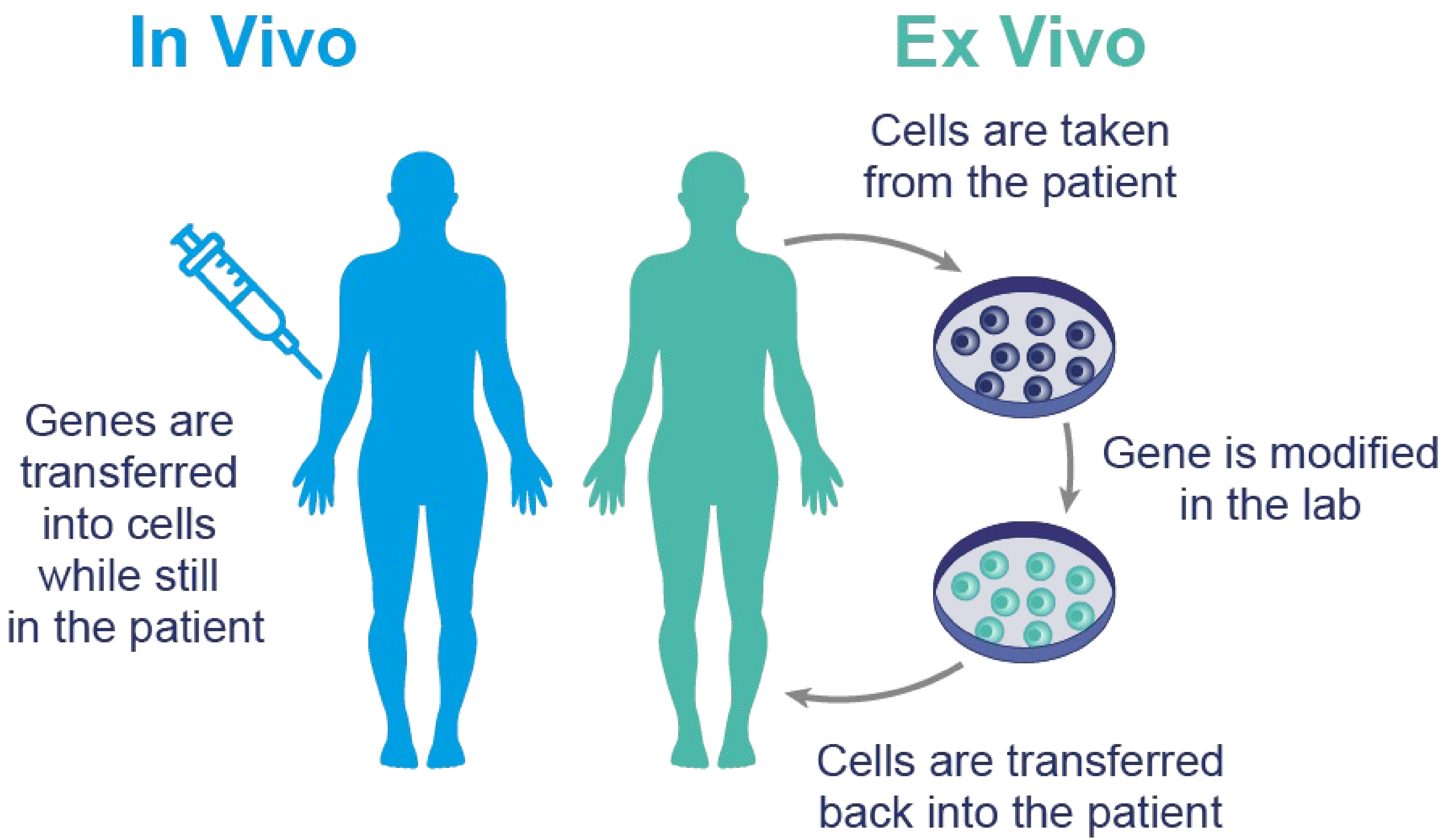

normal
mutant
WntA CRISPR

Numerous genes modified via CRISPR



Korotkova et al. 2019

Ongoing clinical trials using CRISPR



Retina disease

Beta-thalassemia
Sickle cell disease

What can we do with DNA ?

Extract, purify

Make more
Amplify
Clone
Synthetize

Examine
Quantify
Examine length
Stain, probe
Sequence

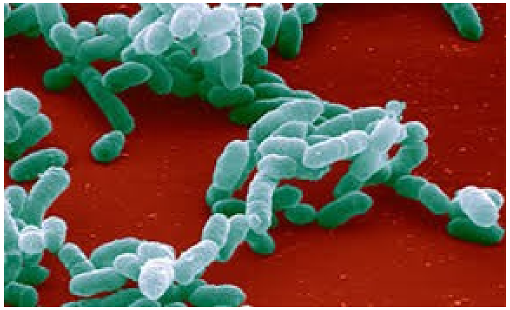
Modify
Cut
Ligate
Recombine fragments
Introduce foreign DNA
Mutate

Fundamental research is important

bacteria *Thermus aquaticus*

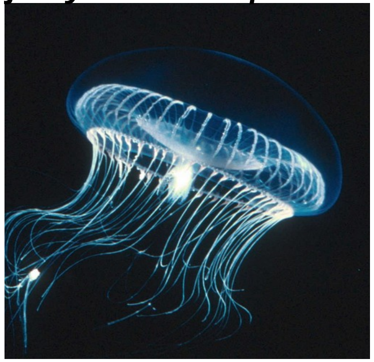
1969 → Taq-polymerase to amplify DNA

bacteria *Haemophilus influenzae*



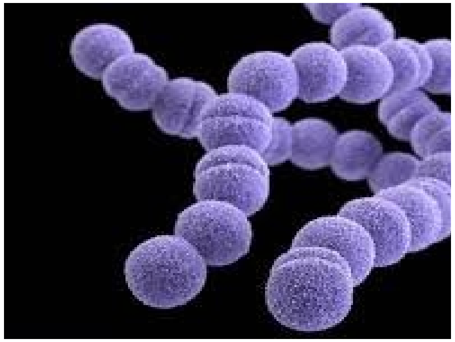
1970 → Restriction enzymes To cut DNA

jellyfish *Aequorea*



1992 → Fluorescent proteins

bacteria *Streptococcus pyogenes*



2012 → CRISPR

DNA and its observable effects

The distinction between genotype and phenotype is the basis of genetics

“The view of natural inheritance as realized by an act of transmission, viz., the transmission of the parent's (or ancestor's) personal qualities to the progeny, is the most naive and oldest conception of heredity.”

“All "types" of organisms, distinguishable by direct inspection or only by finer methods of measuring or description, may be characterized as "**phenotypes**.”

“ A "**genotype**" is the sum of all the "genes" in a gamete or in a zygote.”

Johansen 1911

Phenotype = observable attributes of an individual

Genotype = inheritable genetic material
= DNA or RNA

How do genotypes map onto phenotypes ?

Aberration Types

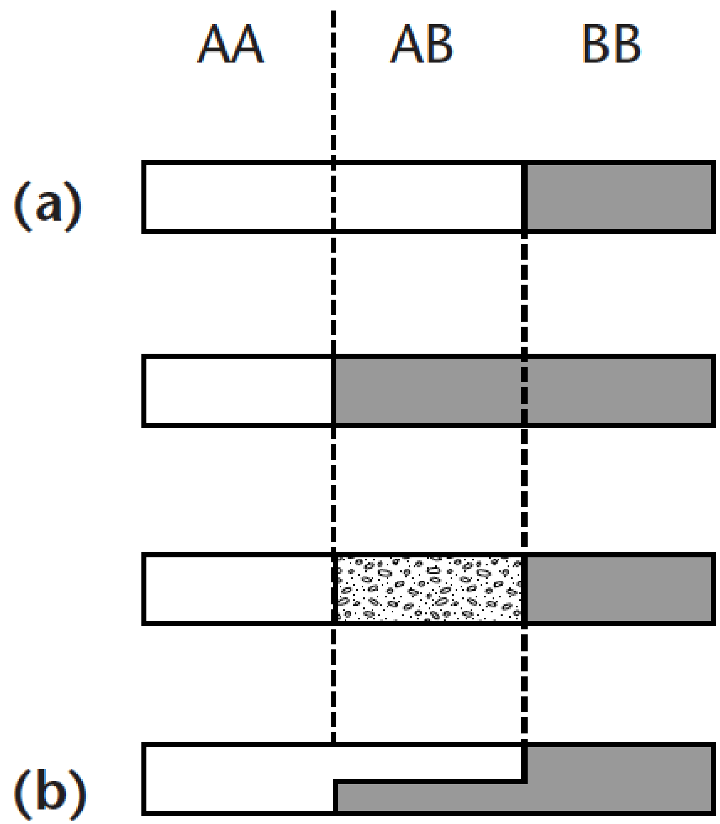
SNP
Insertion (CNV)
Deletion
Indel
Inversion
Translocation
Complex change
(Epigenetic change)

Estimation of mutation rates
Mutation accumulation lines, sequencing family trio, across a phylogeny

Coding versus cis-regulatory

Coding
Cis-regulatory
Gene loss
Gene amplification
(Gene rearrangement)

Levels of dominance



A is dominant to B

A is recessive to B

A and B are semidominant

Ex: pink flowers

A and B are codominant

Ex: AB blood groups, red and white flowers

Can be quantified as deviation from midpoint between parents

Dominance is not an intrinsic to an allele

- It is relative to another allele, not to *all* other alleles

- It is a property of their effect on a given phenotypic trait

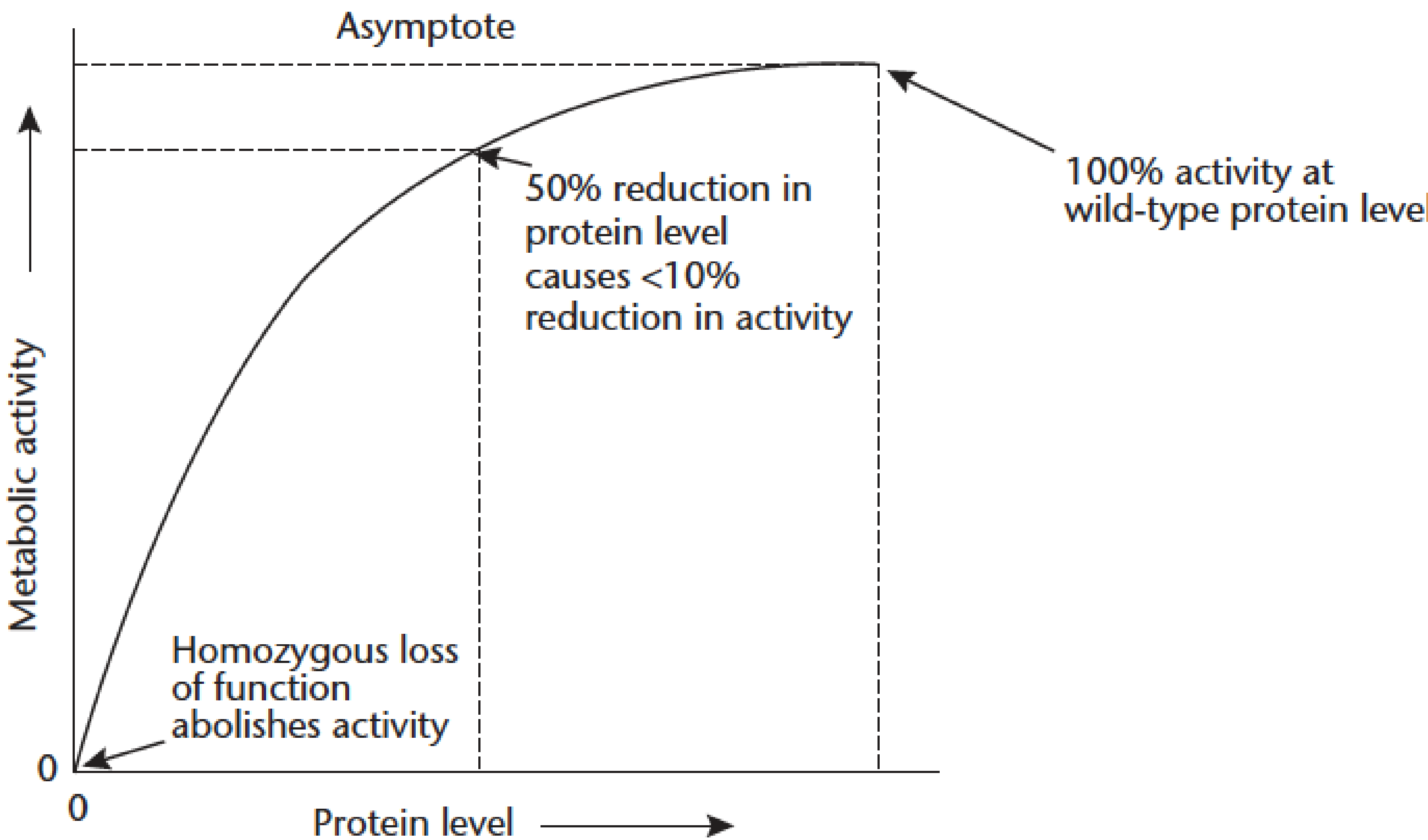
as in "dominance of *a1* over *a2* for a particular trait"

Ex:

yellow allele is **dominant** over the + allele for coat color
yellow allele is **recessive** over the + allele for lethality

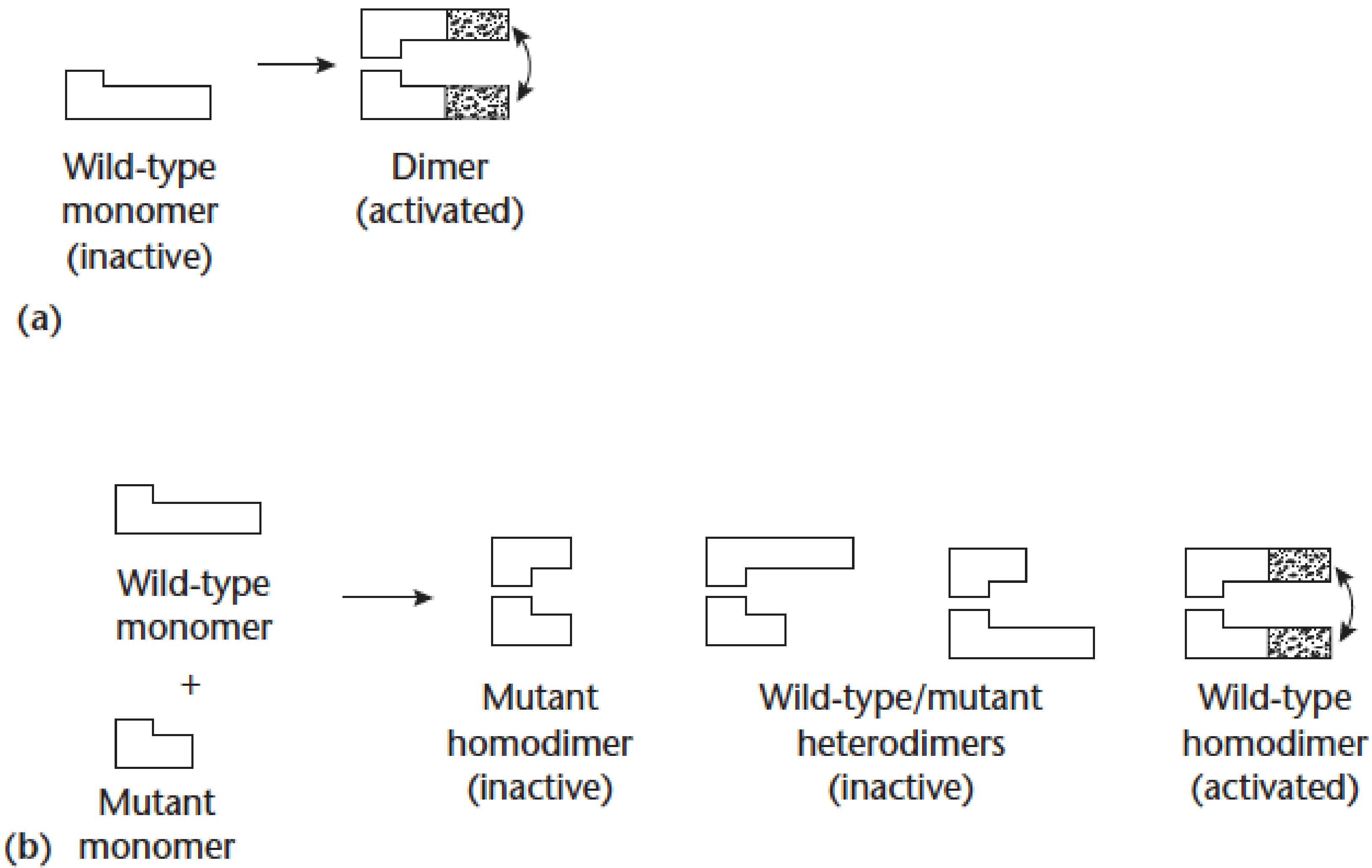
Most wild alleles are dominant

Robustness to half-dose:



Wilkie 2005

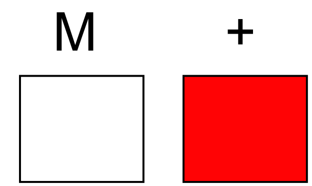
One type of dominant-negative mutation: sequestration of wild-type in dimer



Wilkie 2005

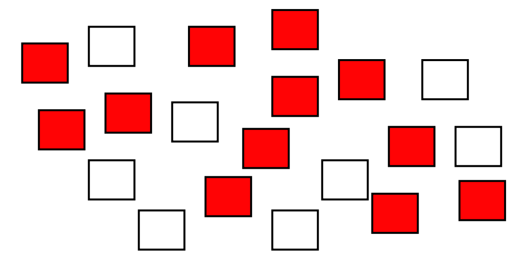
Penetrance

Discrete binary phenotype



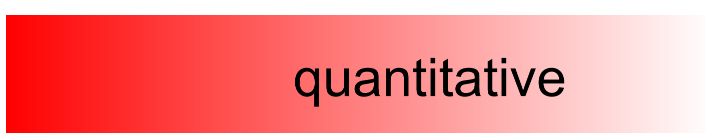
% of individuals showing the phenotype

ex: 40% of individuals have a white color
Partial penetrance

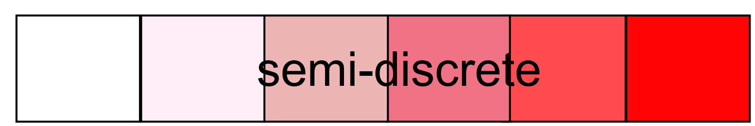


Expressivity

Phenotype with different degrees of severity



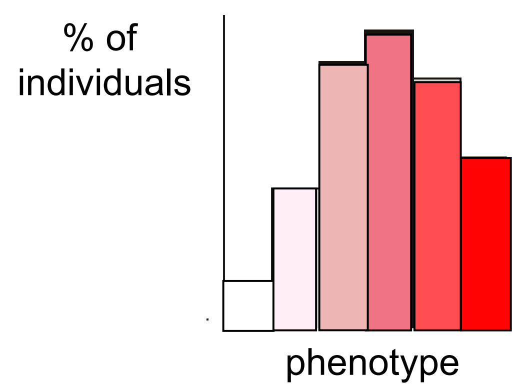
or



Degree of severity of the phenotype

ex: - number of affected ommatidia
- light pink color

often shown as a distribution of
phenotypic values of individuals:



Maternal (or paternal) effect

The genotype of the parent matters,
not that of the individual itself.

Frequent for mutations affecting early embryonic development

m/m ♀ x *+/+* ♂
phenotype ? phenotype ?

F1 *m/+* x *+/+* ♂ or *m/m* or *m/+*
phenotype **M**

F2 *m/+* or *m/m* or *+/+*
phenotype **+**

+/+ ♀ x *m/m* ♂
phenotype ? phenotype ?

F1 *m/+*
phenotype **+**

Heredity with sex-linked transmission

Example: mutation on the X chromosome
in a species reproducing with XX ♀ x XY ♂

m/m ♀ x *+/Y* ♂
phenotype M phenotype +

↓

F1 *m/+* ♀ + *m/Y* ♂
females of phenotype + males of phenotype M

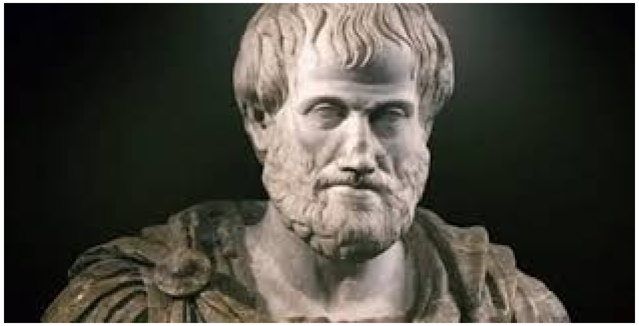
Heredity with sex-linked expression

Example: mutation that affects the phenotype only in females

Different kinds of phenotypes

Morphology

- Color
- Size and shape
- Presence/absence
- Position



Aristote, Historia animalium, book I, 2, 300BC

Physiology

Behavior

Genotype & Phenotype

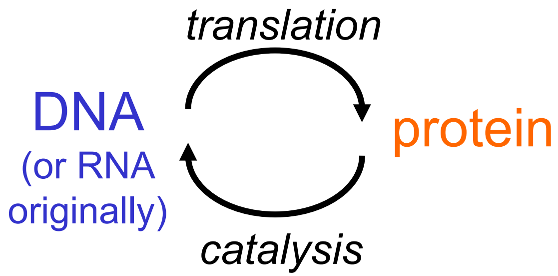
= what engenders = what is apparent

DNA/RNA

- **Regulation of gene expression**
- **Biochemical reactions**
- **Subcellular architecture**
- **Assembly of cells**
- **Organism morphology and behavior**

etc.

distinction appeared at the origin of life:



Francis Crick Central Dogma
A reductionist view of the GP relationship

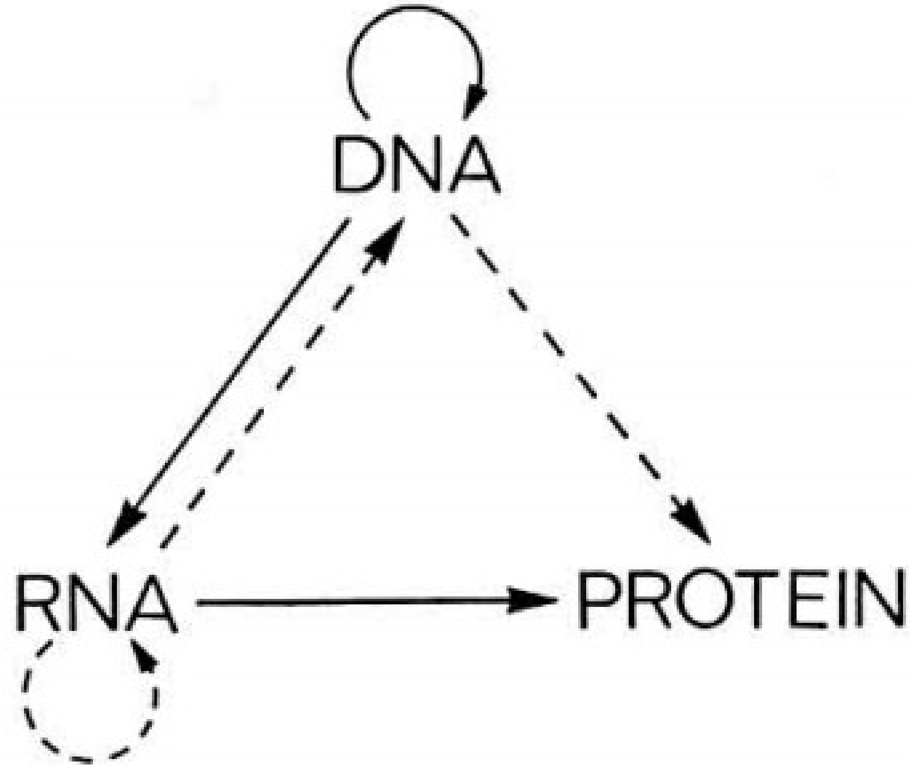
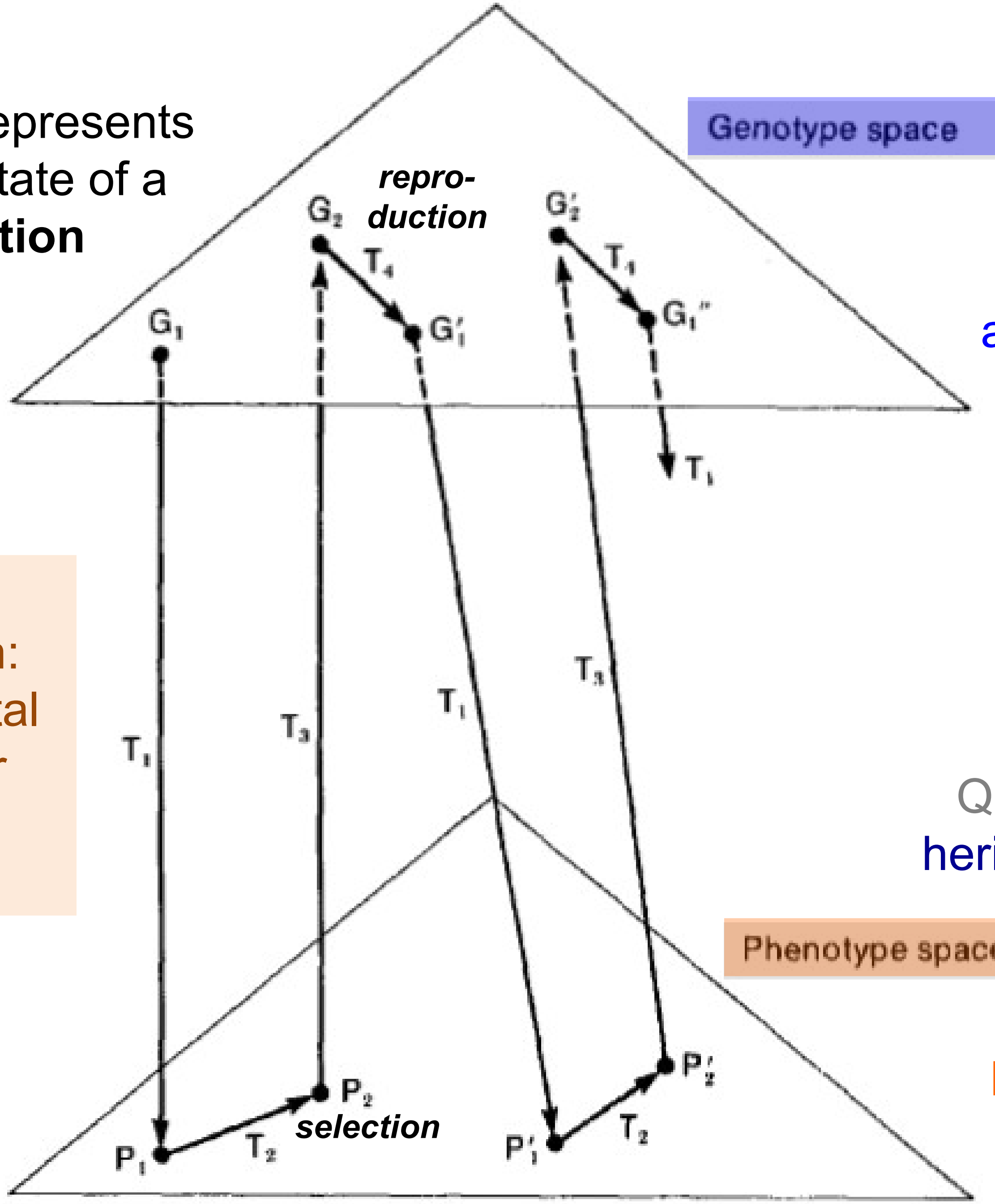


Fig. 3. A tentative classification for the present day. Solid arrows show general transfers; dotted arrows show special transfers. Again, the absent arrows are the undetected transfers specified by the central dogma.

Crick 1958
Crick 1970

The first genotype-phenotype map

Here a dot represents the mean state of a **population**



Population genetics: stochastic processes and selection coefficient

Phenotype construction: developmental and cellular biology physiology

Quantitative genetics: heritability of phenotypes

Evolutionary biology of phenotypes, evolutionary ecology

Lewontin 1974

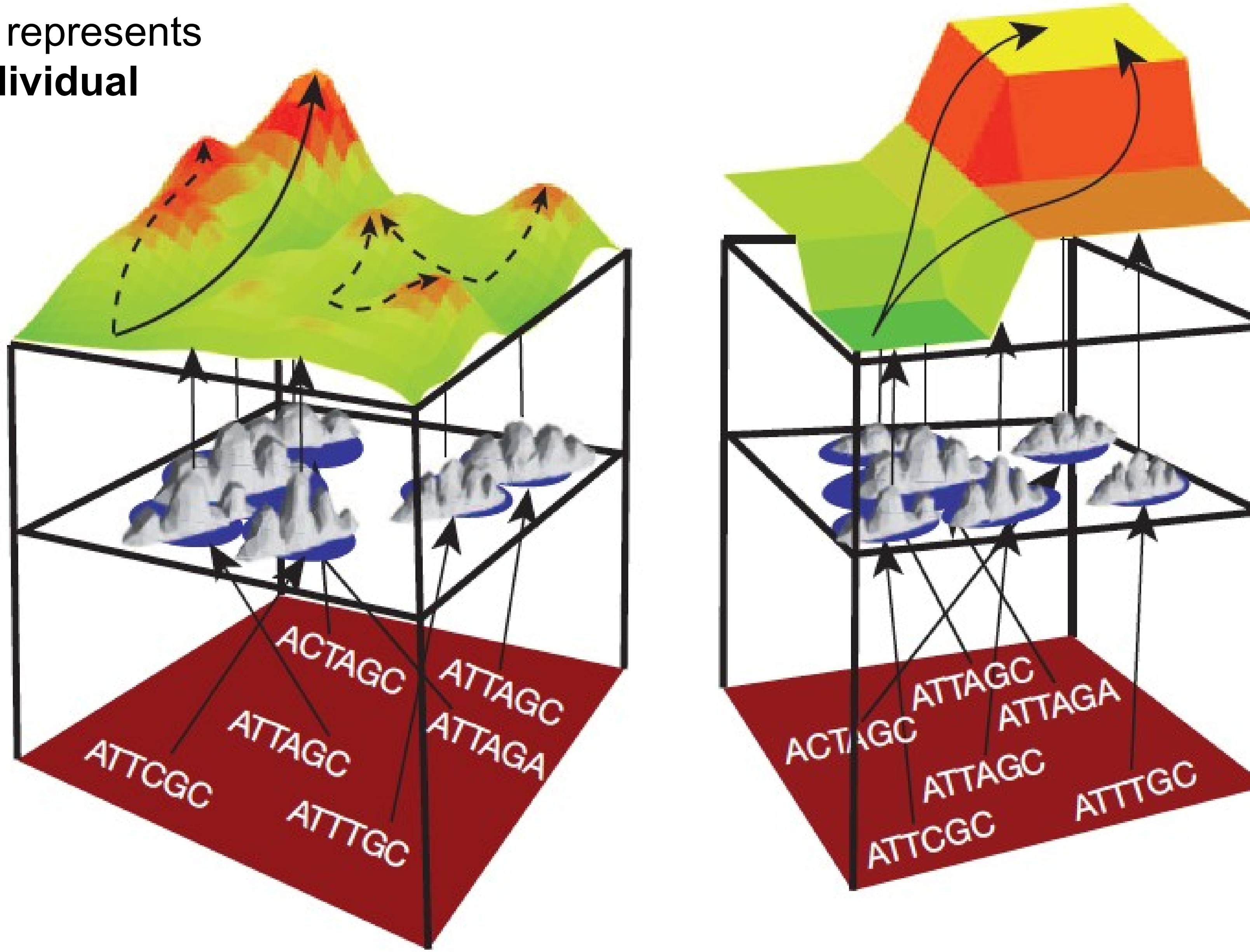
The genotype-phenotype-fitness map

Here a dot represents one **individual**

Fitness

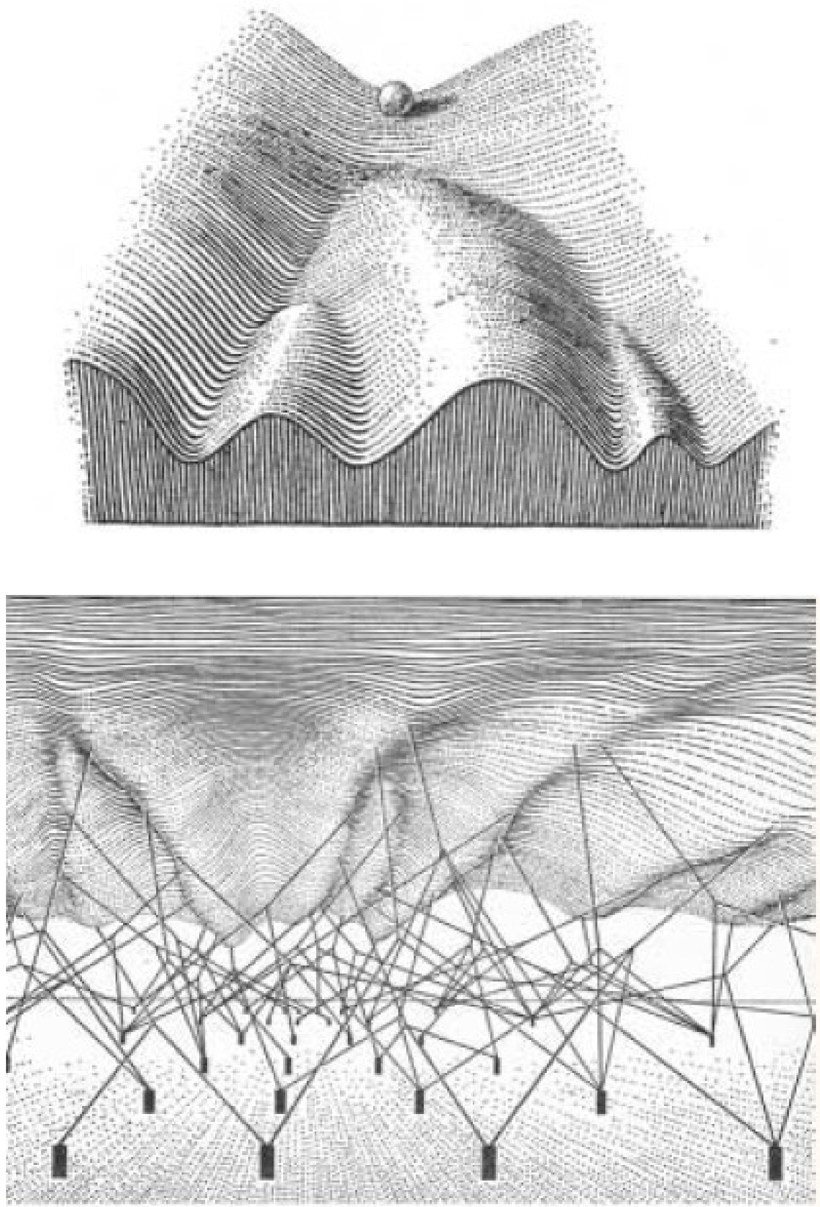
Phenotype
Tooth morphology

Genotype



Salazar-Cuidad & Martin-Riera 2013

The Epigenetic Landscape
A metaphor for the G-P relationship

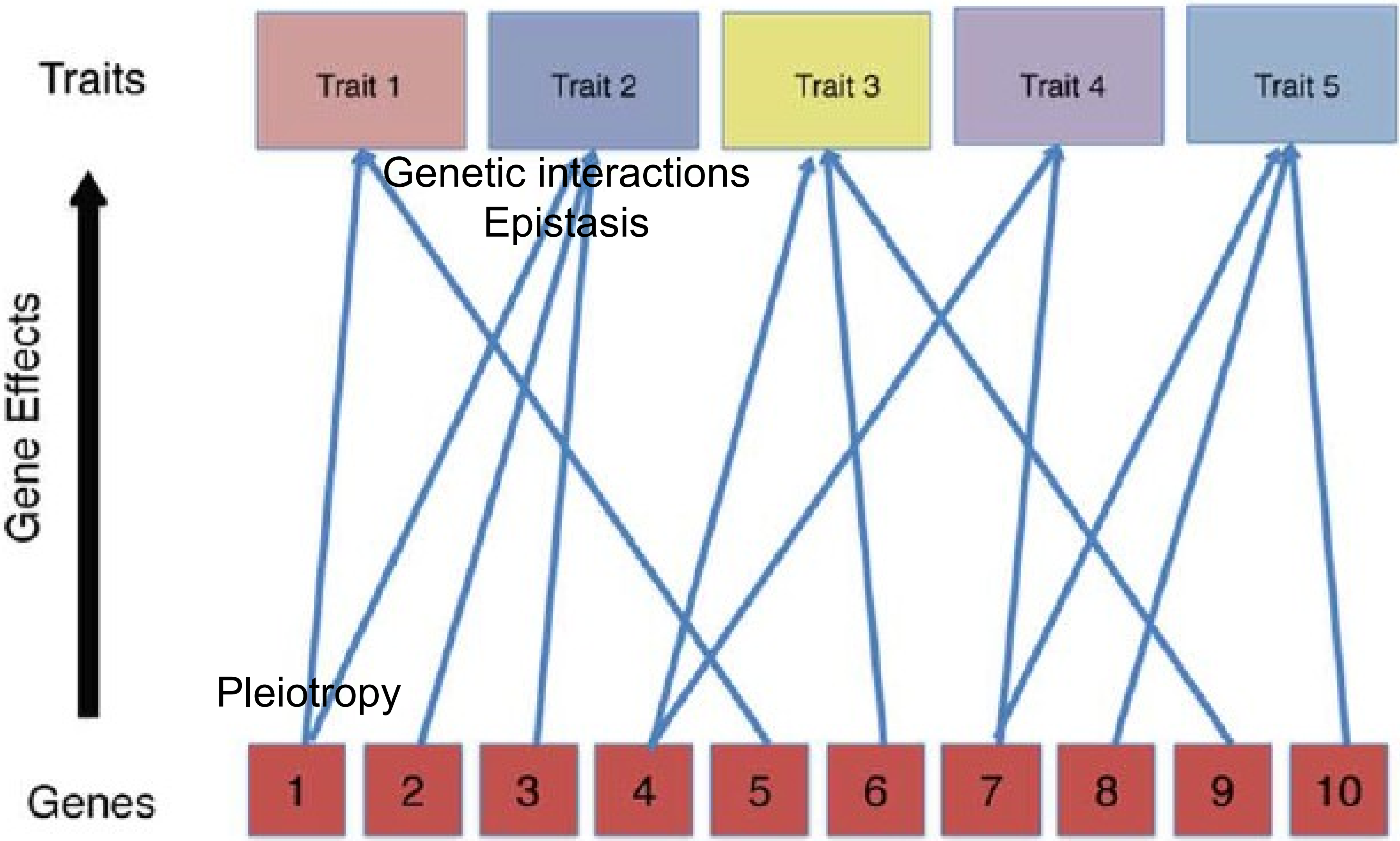


Development

Canalization

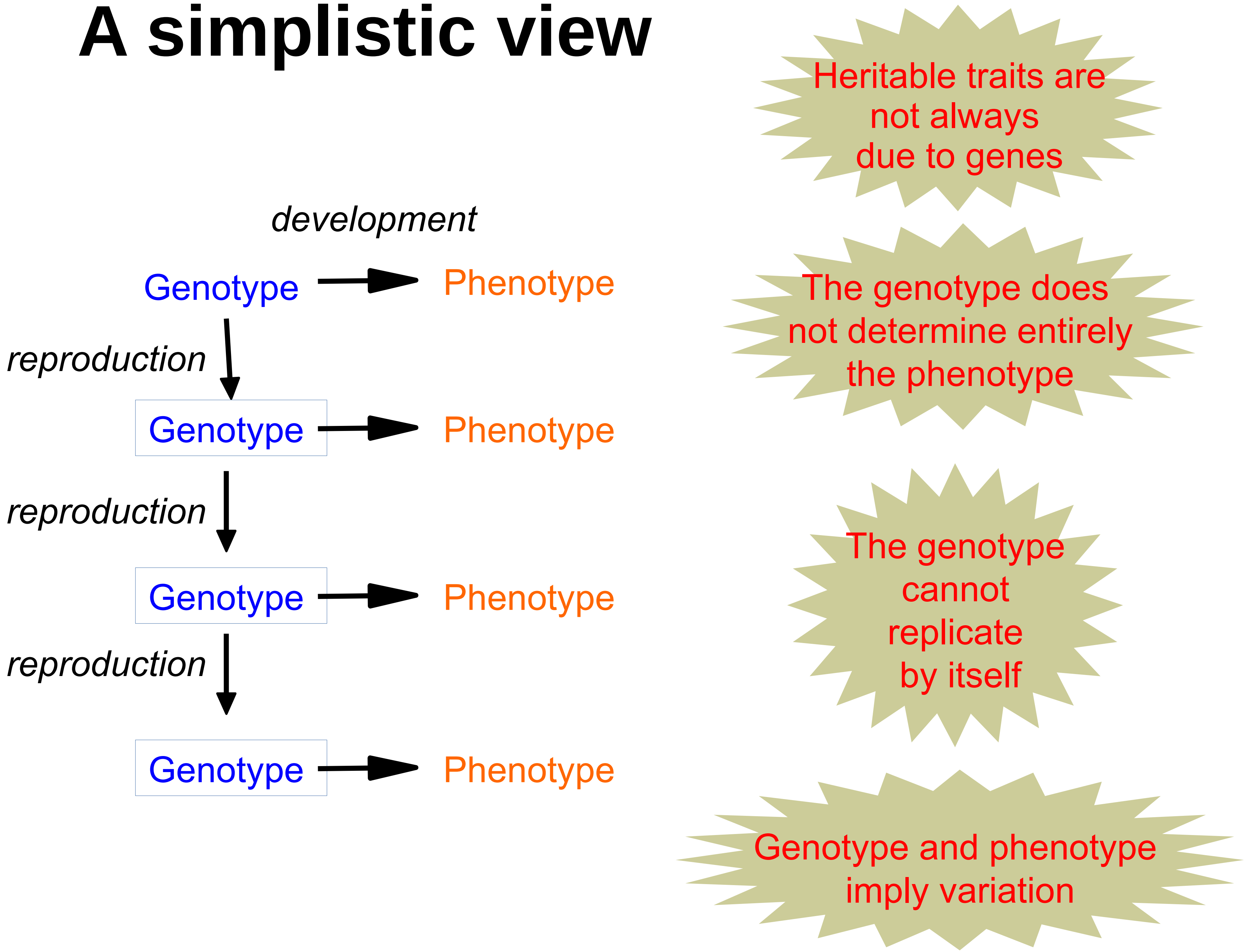
Genes underlying
the landscape

Waddington 1957



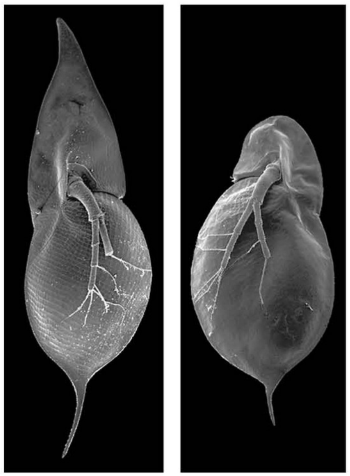
Hallgrimsson et al. 2014

A simplistic view



Plasticity: one genotype → several phenotypes

Daphnia



with helmet | without helmet

Nemoria arizonaria caterillars

spring: caterpillars feed on catkins

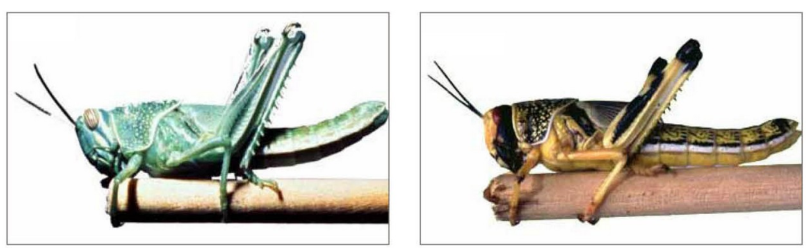
summer: caterpillars feed on leaves

Water crowfoot plant

leaves growing above water

leaves growing below water

Desert locusts



solitary

gregarious

Commodore butterfly

winter

summer

Commodore butterly: Michael Wild, CC-BY-SA-3.0 (winter), Svdmolen, CC-BY-SA-3.0 (summer)

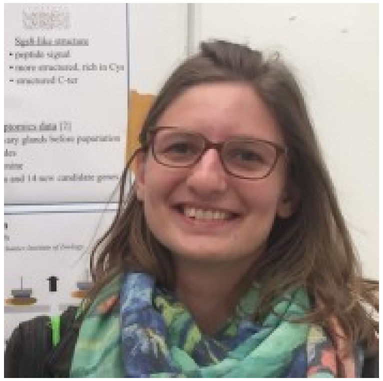
Daphnia: Agrawal *et al* (1999)

Nemoria arizonaria caterillars: Sadava *et al* (2014)

Water crowfoot plant: J R Crellin, CC BY-NC-ND 3.0

My lab
Fly Glue Evolution

A Lalouette (genitalia)
JN Lorenzi (SARS-CoV-2)
R Vijendravarma (organ size)



Flora Borne
PhD (3 years)

Manon Monier
M2+PhD (3 years)

Isabelle Nuez
Technician

Co-supervision: F. Graner & I

Genetics ↔ Chemistry Molecular biology ↔ Physics ↔ Ecology Behavior

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Genetics ↔ Chemistry / Molecular biology ↔ Physics ↔ Ecology / Behavior

Genetics
JL Da Lage
R Kulathinal (Philadelphia)

Chemistry / Molecular biology
JM Camadro
Y Guerardel
K Hagen (USA)

Physics
S Gorb (Kiel)
A Kovalev (Kiel)
L Corté
F Graner

Ecology / Behavior
M Molet

Bioadhesives

Natural polymer that can act as an adhesive: binds two items together and resists their separation

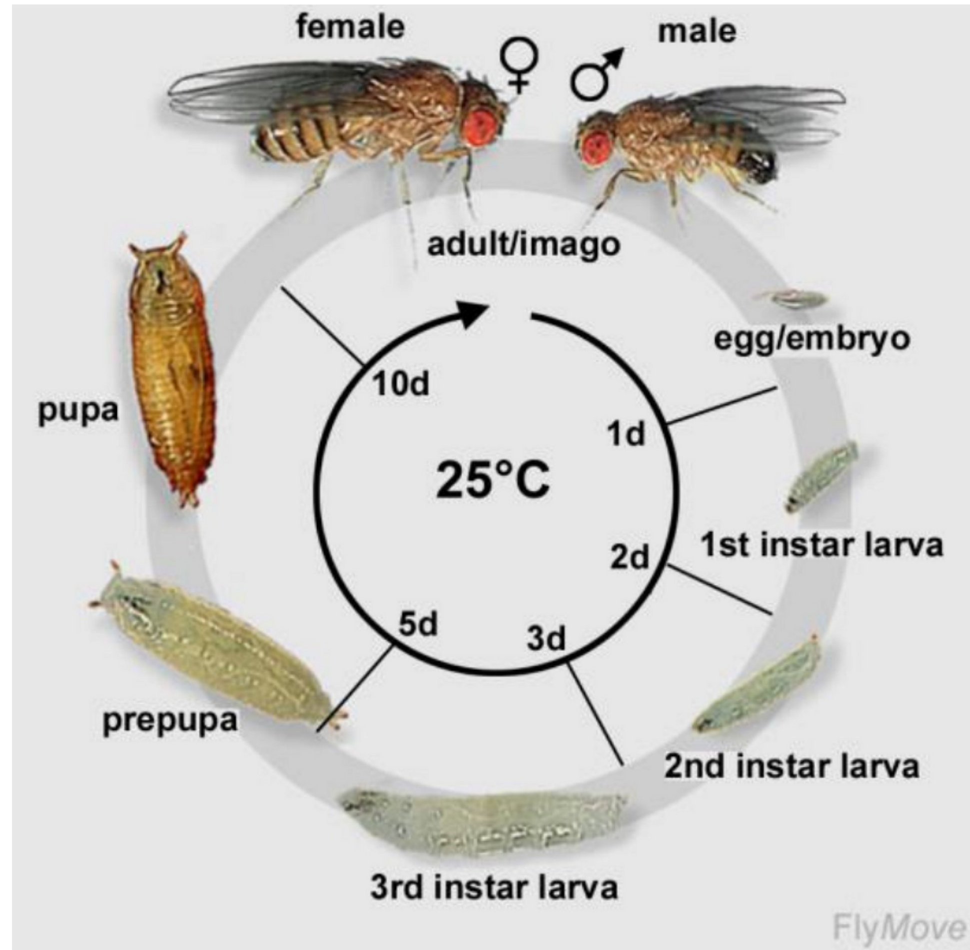
Mussel glue

water resistant

25 proteins

3,4-dihydroxyphenylalanine (DOPA)

Fly Glue



female
male
adult/imago
egg/embryo
10d
pupa
1d
25°C
1st instar larva
2d
5d
3d
prepupa
2nd instar larva
3rd instar larva
FlyMove

Stick to a wide variety of substrates

soil

D. grimshawi

rotten strawberry

D. suzukii

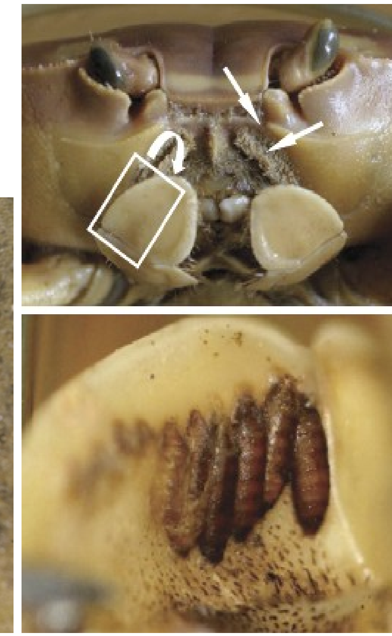
grape

D. melanogaster

potato

D. repleta

crab

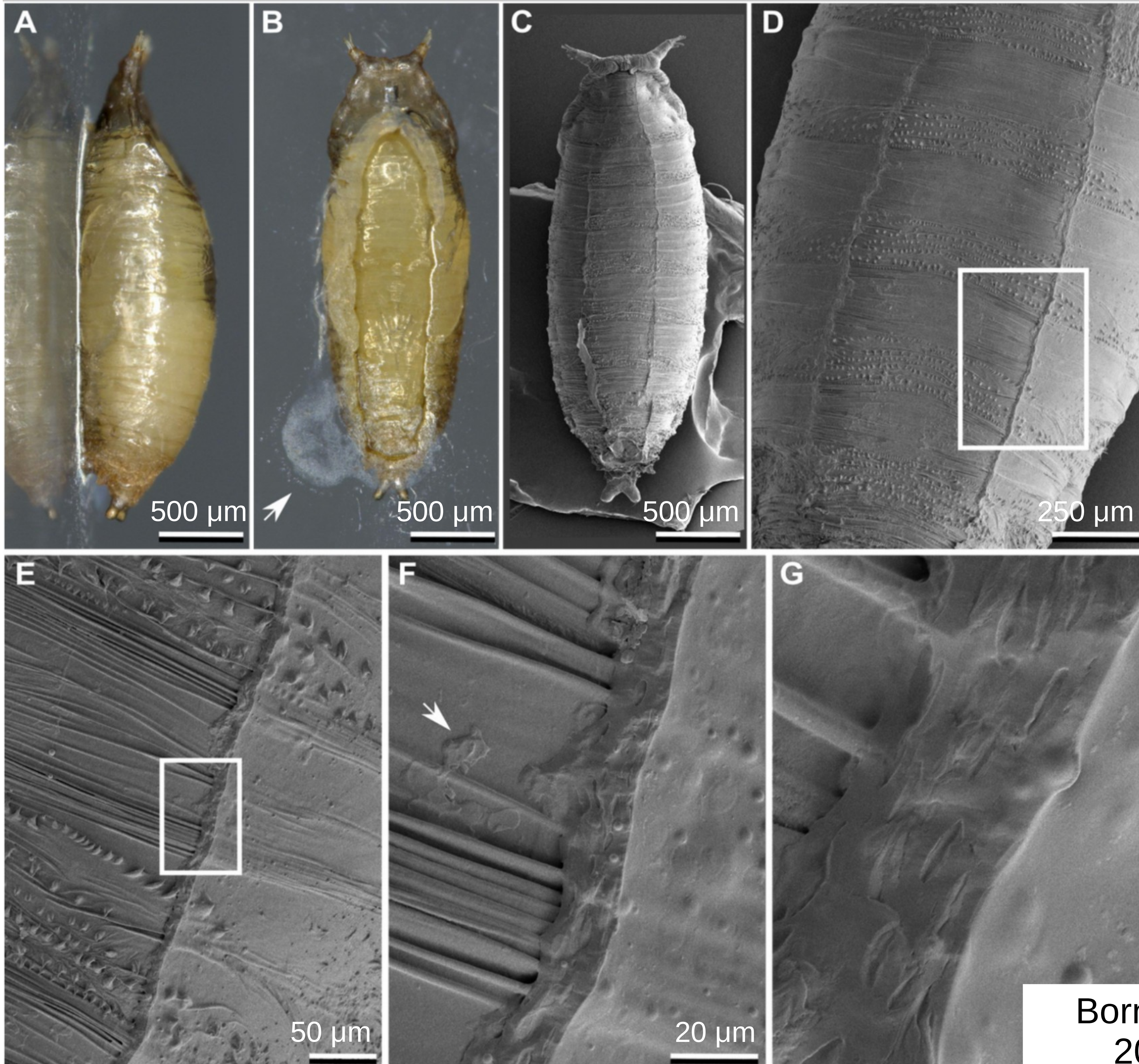


D. carcinophila

Glue genes ↔ Chemistry Molecular biology ↔ Adhesion ↔ Ecology

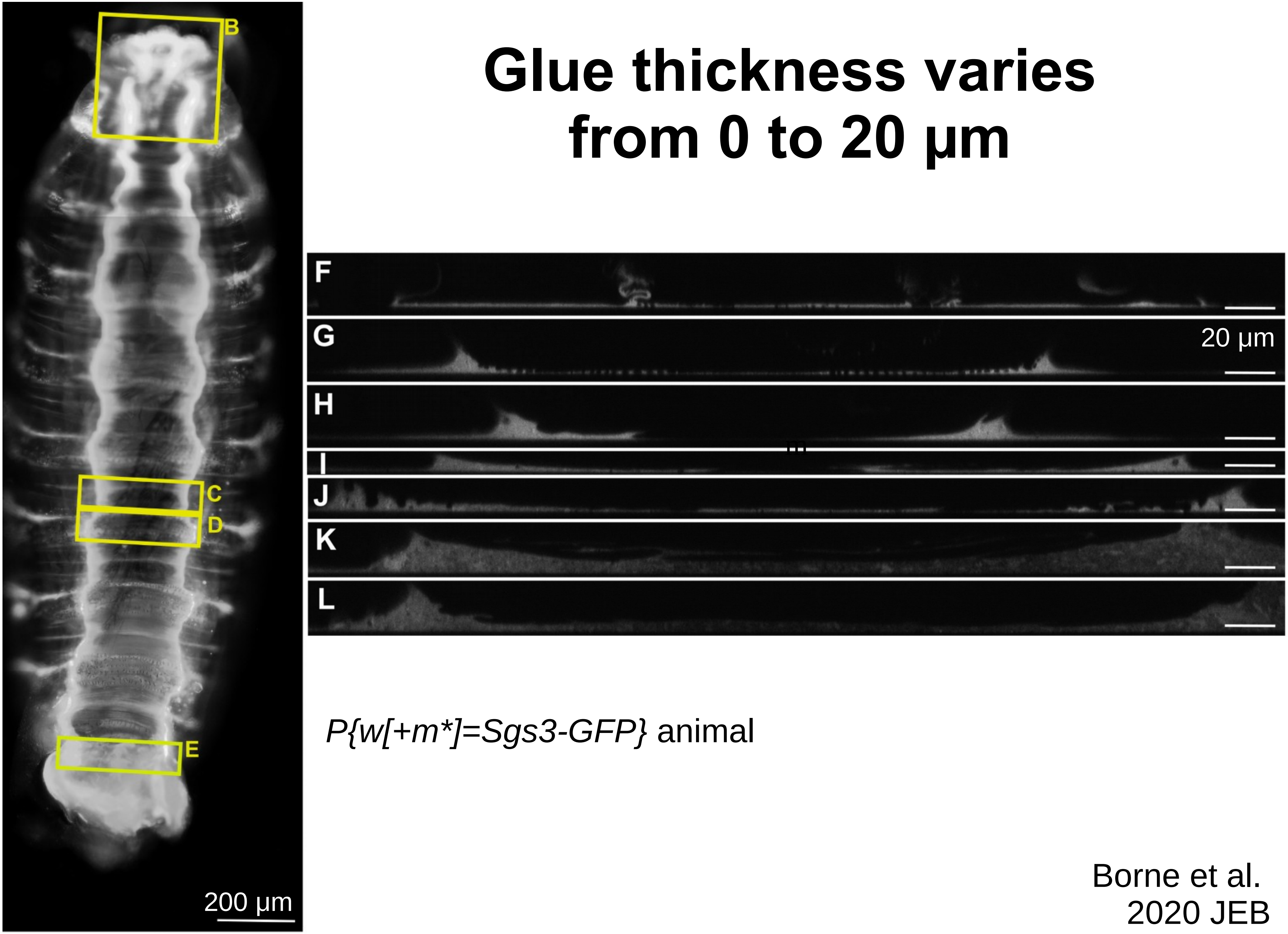
How adhesive is the glue?

What makes it adhesive?



Borne et al.
2020 JEB

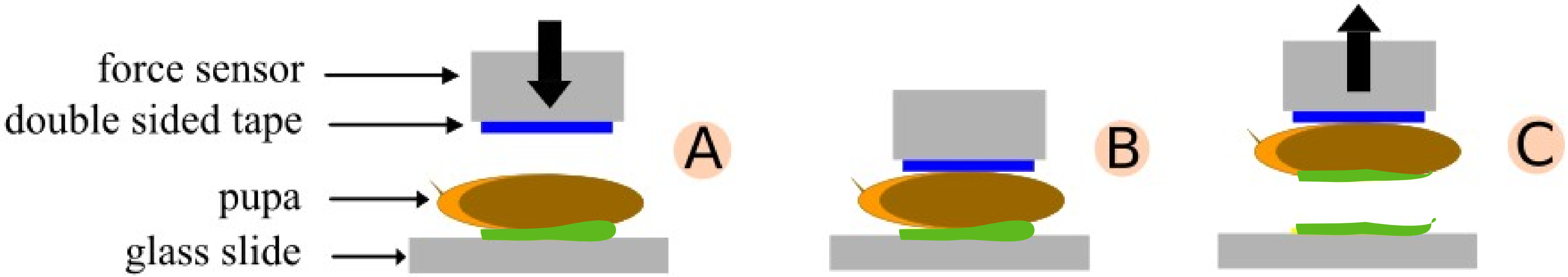
Glue thickness varies from 0 to 20 µm

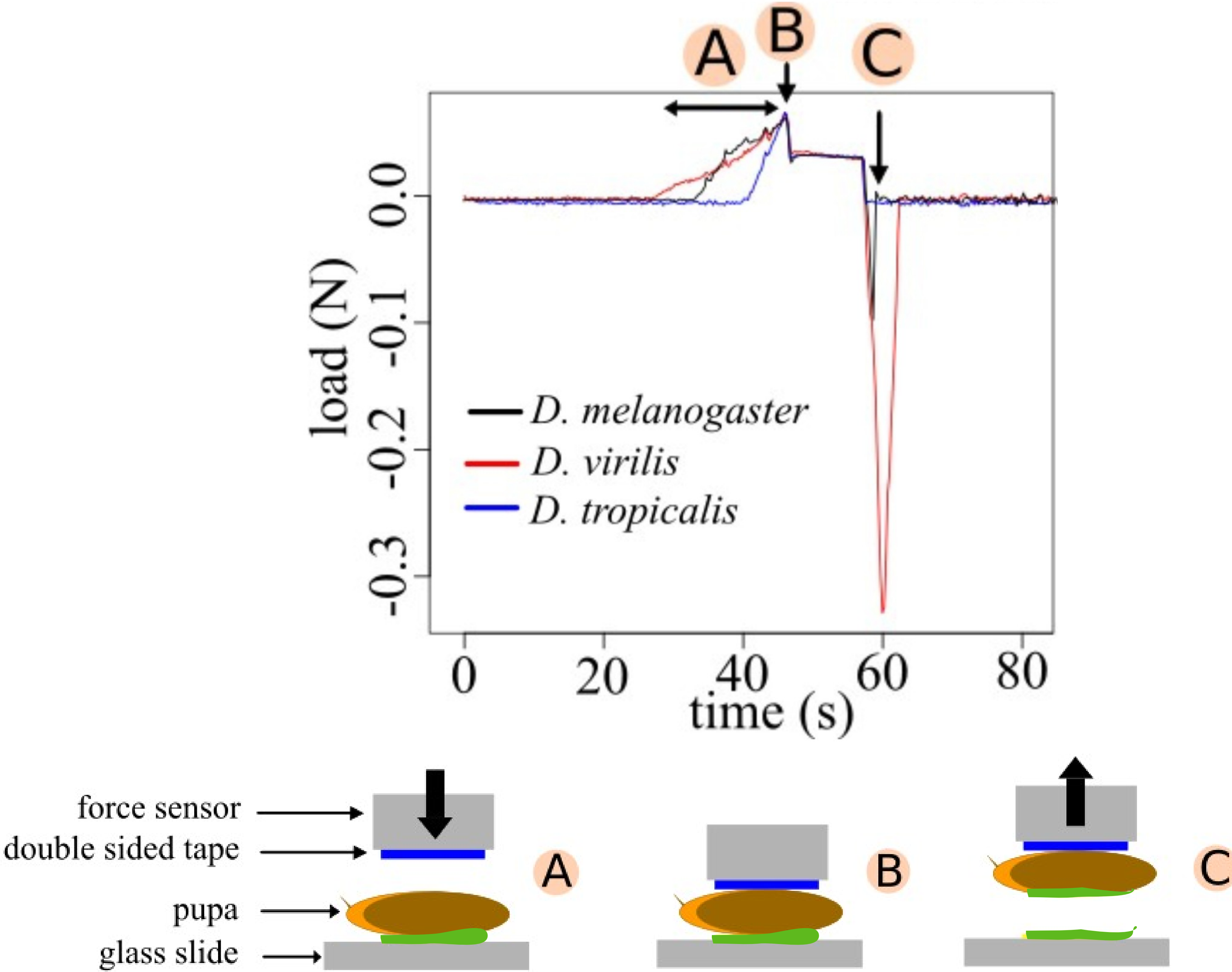


P{w[+m]=Sgs3-GFP}* animal

Borne et al. 2020 JEB

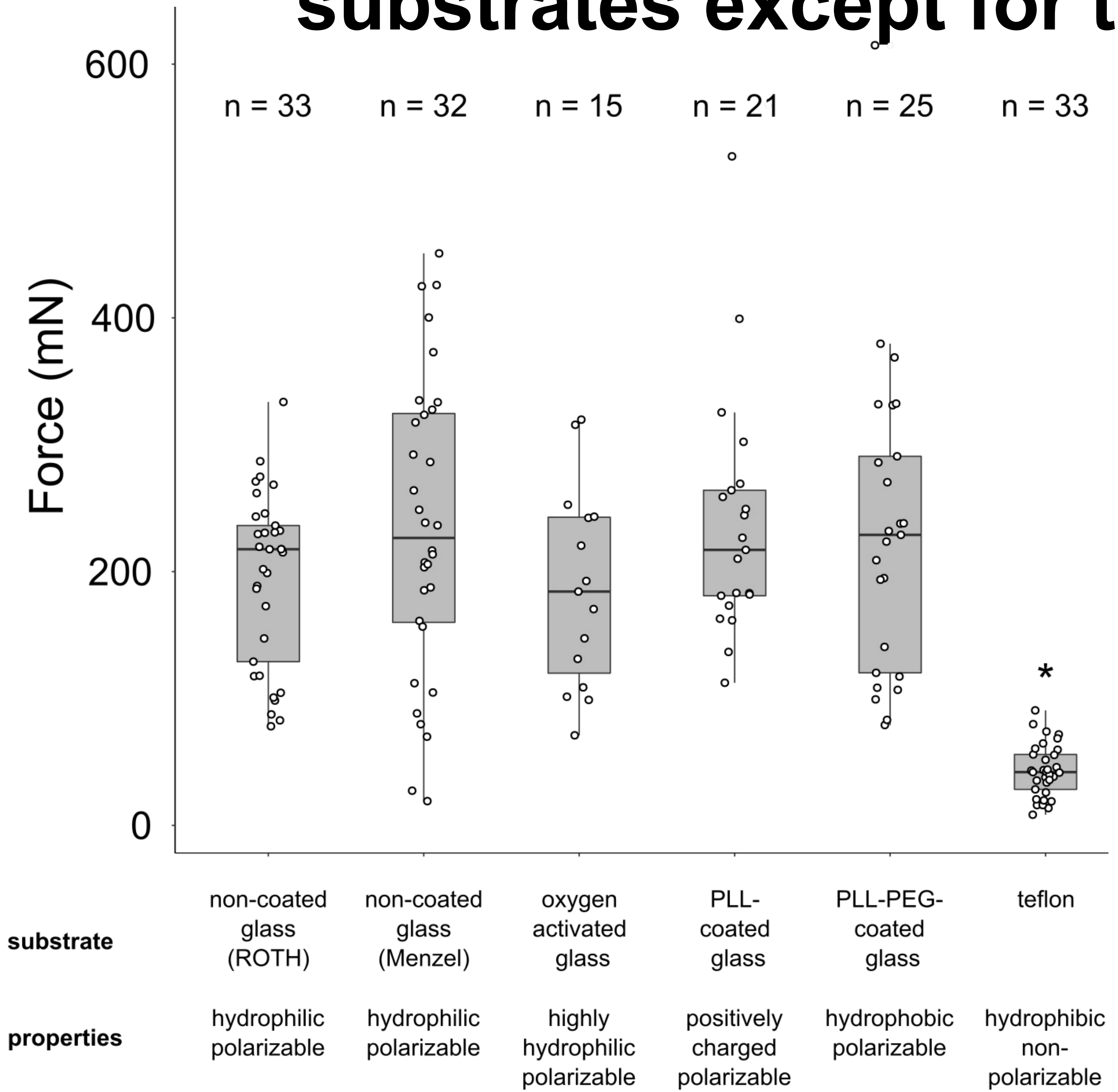
Experimental set up





A
B
C
load (N)
0.0
-0.1
-0.2
-0.3
D. melanogaster
D. virilis
D. tropicalis
0
20
40
60
80
time (s)
force sensor
double sided tape
pupa
glass slide
A
B
C

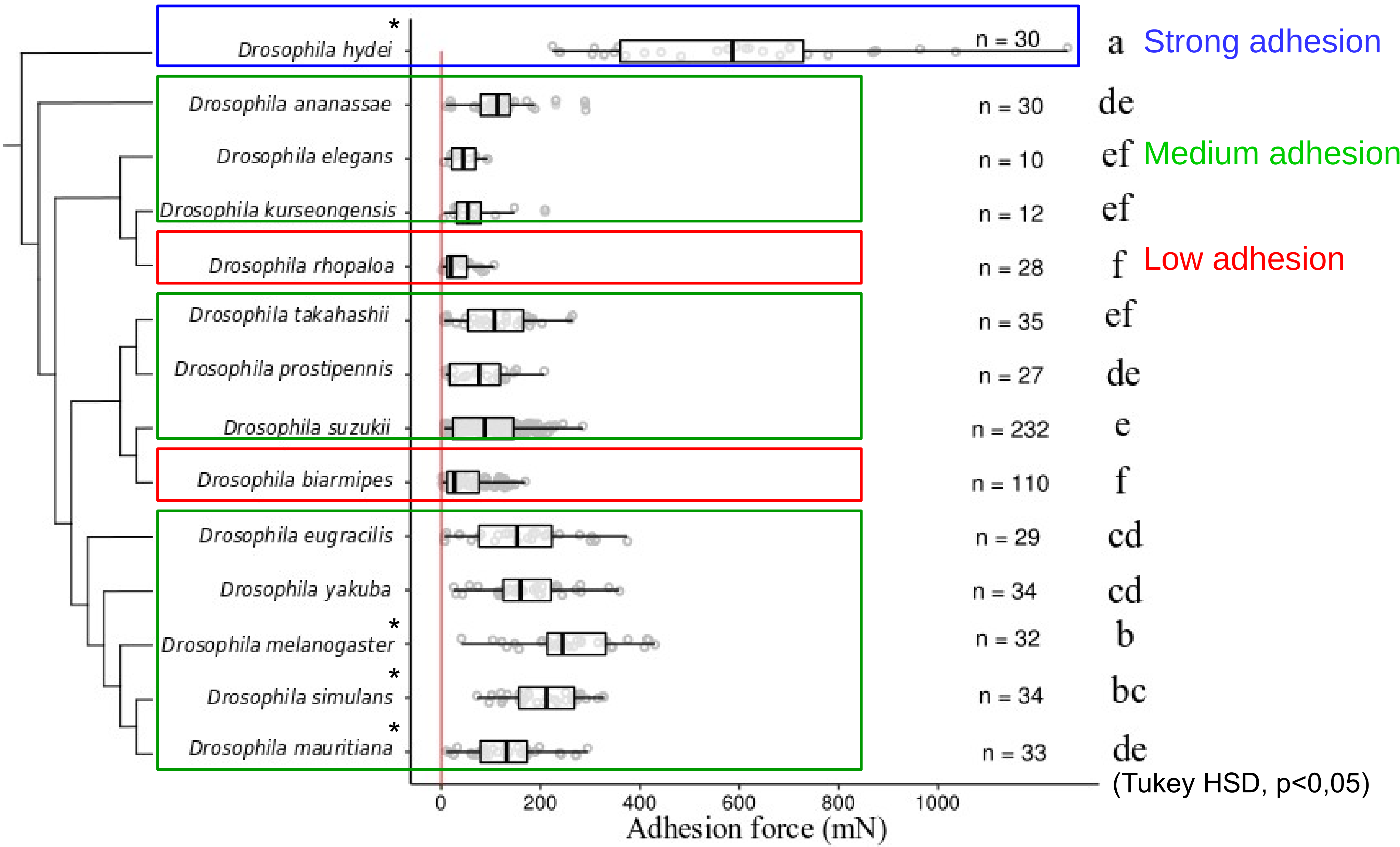
Adhesion force does not vary between substrates except for teflon



* teflon different from every other substrates (ANOVA followed by Tukey tests, $p < 0.05$)

Borne et al. 2020 JEB

Low, Medium and Strong Adhesion Species



Monier, unpublished

How adhesive is the glue?

D. melanogaster glue is universal.

moth eggs < Drosophila glue < mussel, barnacle < superglue
10-100 kPa 100-300 kPa 300-1000 kPa 10 MPa

~15 000 times the pupa weight
Like very strong adhesive tapes

Borne et al. 2020 JEB

What makes it adhesive?

The glue is composed of 8 proteins

Sgs1, Sgs3, Sgs4, Eig71Ee (1286, 307, 287, 445 aa)

Long, repeats rich in Ser, Thr, Pro
Disordered
O-glycosylated
Interact with water and polarizable substrates?

Sgs5, Sgs5bis, Sgs7, Sgs8 (163, 142, 74, 75 aa)

Short, rich in Cys
Prevent aggregation and allow secretion?

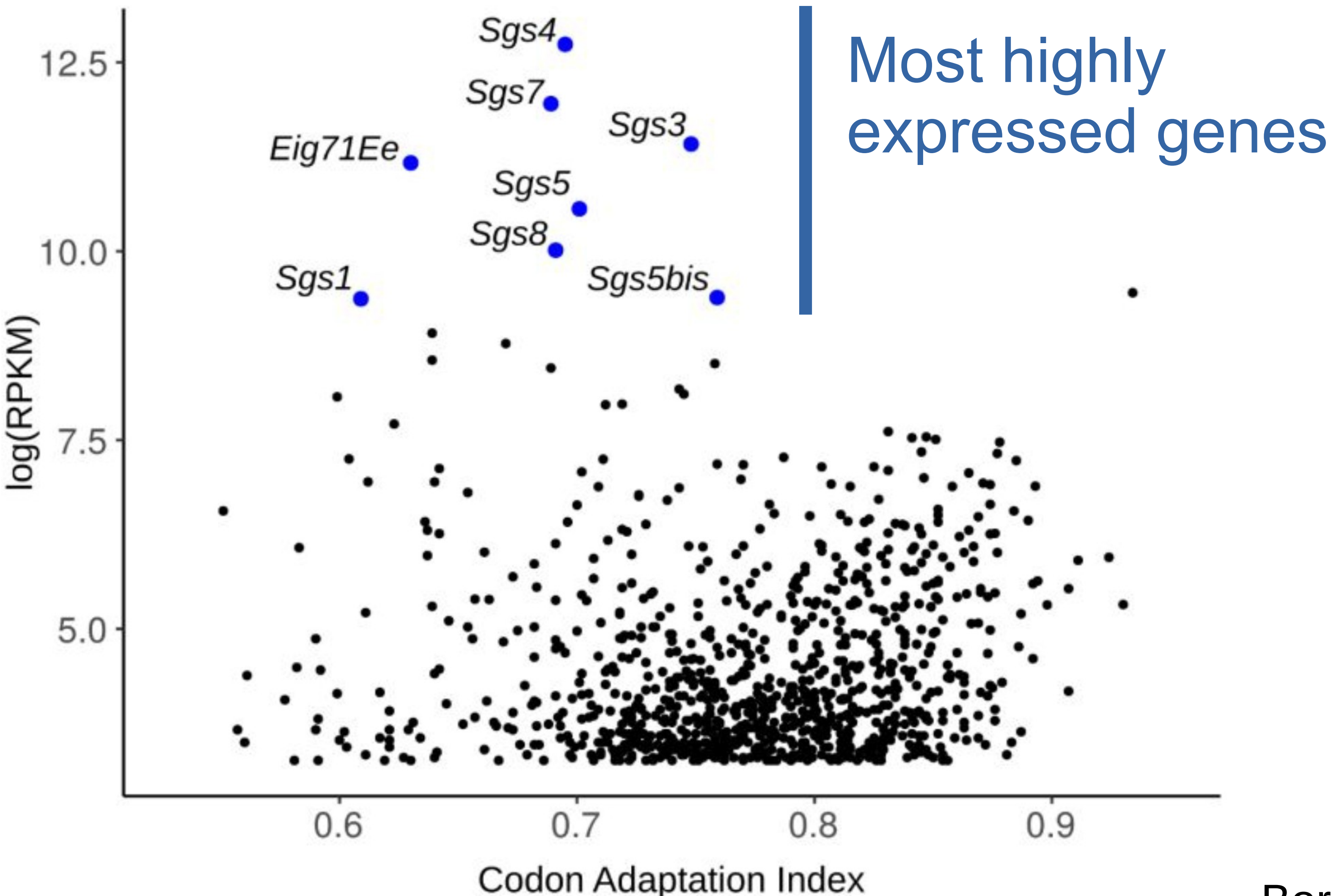
Monier and Courtier-Orgogozo 2022
Insects

Protein	Species	Repeat motif	Number of repeats
Sgs1	*D. melanogaster*	PTTTTPR/STTTTSTSR	85
	D. mauritiana	CAPTTTTPR	13
	D. simulans	CAPTTTTPR	40
Sgs3	*D. melanogaster*	KPTT	24-31
	D. mauritiana	CAPPTRPPCTSPTTTTTTT	5
	D. simulans	T-rich stretches	
Sgs4	*D. melanogaster*	CRTEPPT	18-26
	D. mauritiana	CNTEPPT	25-35
	D. simulans	CDTEPPT	8
Eig71Ee	*D. melanogaster*	CTCTESTTRTNPT	7-9
	D. mauritiana	CTCTDSTTRTNPT	2-4
	D. simulans	CTDSTTKTTNPPCT	8

Da Lage et al. 2019 BMC Evol Biol

Transcriptomics

Salivary glands from wandering 3rd instar larvae
(modENCODE Graveley 2011)



Borne et al 2021 GBE

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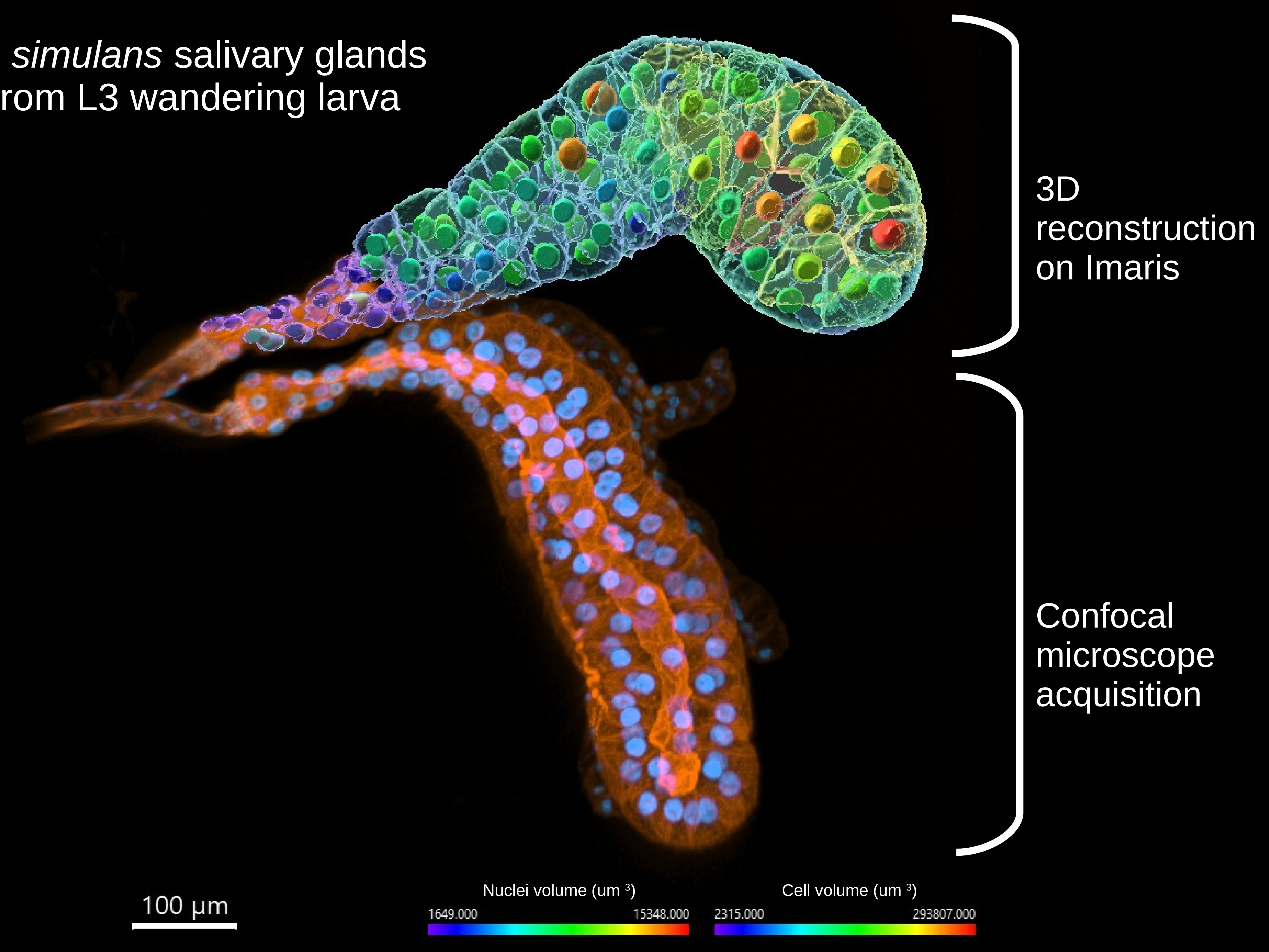
Borne et al. 2020 JEB

What makes it adhesive?

8 major proteins
Ongoing RNAi and CRISPR

unpublished



simulans salivary glands
rom L3 wandering larva
3D reconstruction on Imaris
Confocal microscope acquisition
100 µm
Nuclei volume (um ³)
1649.000
15348.000
Cell volume (um ³)
2315.000
293807.000

Next time: bring your laptop!