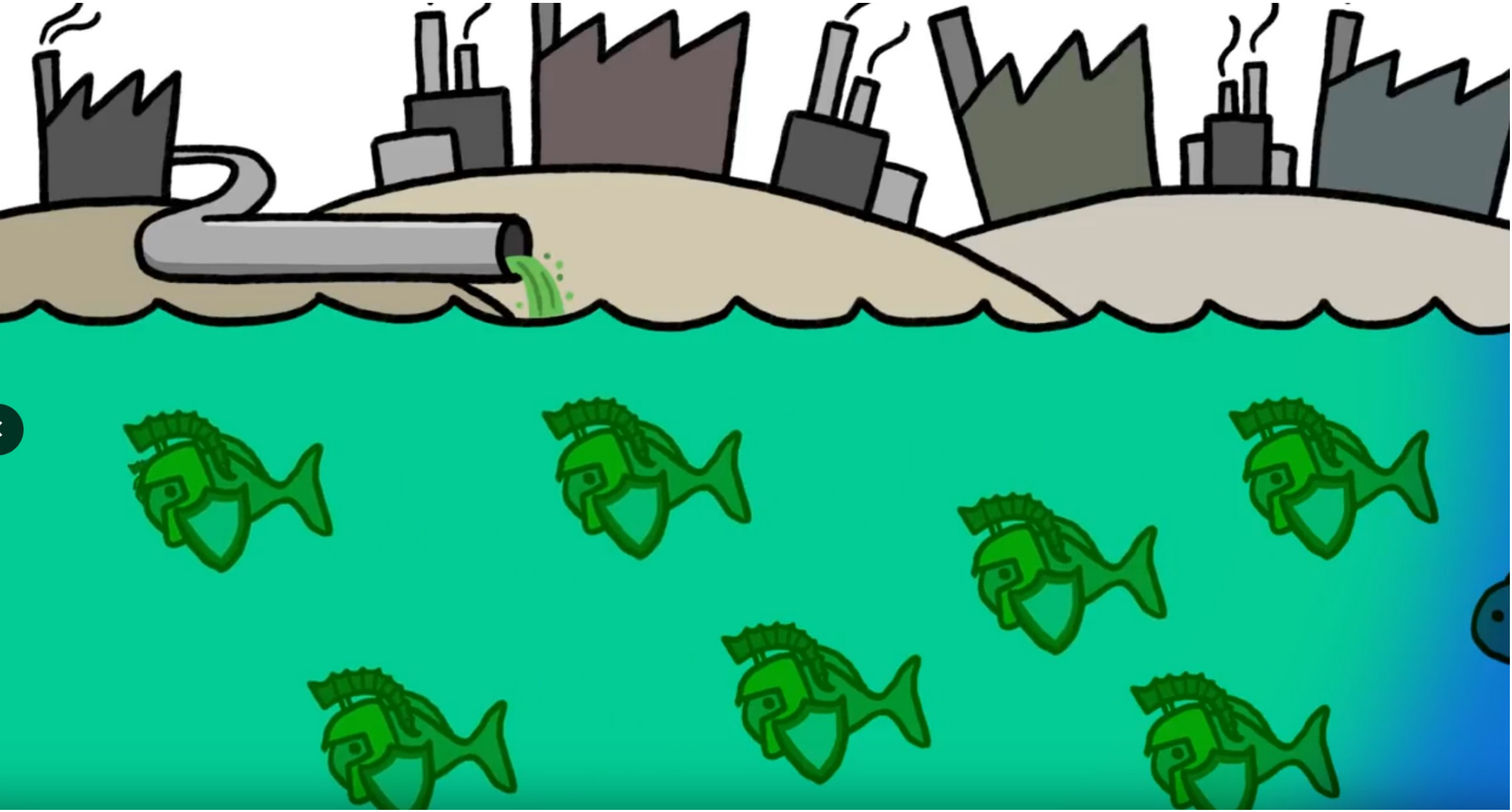
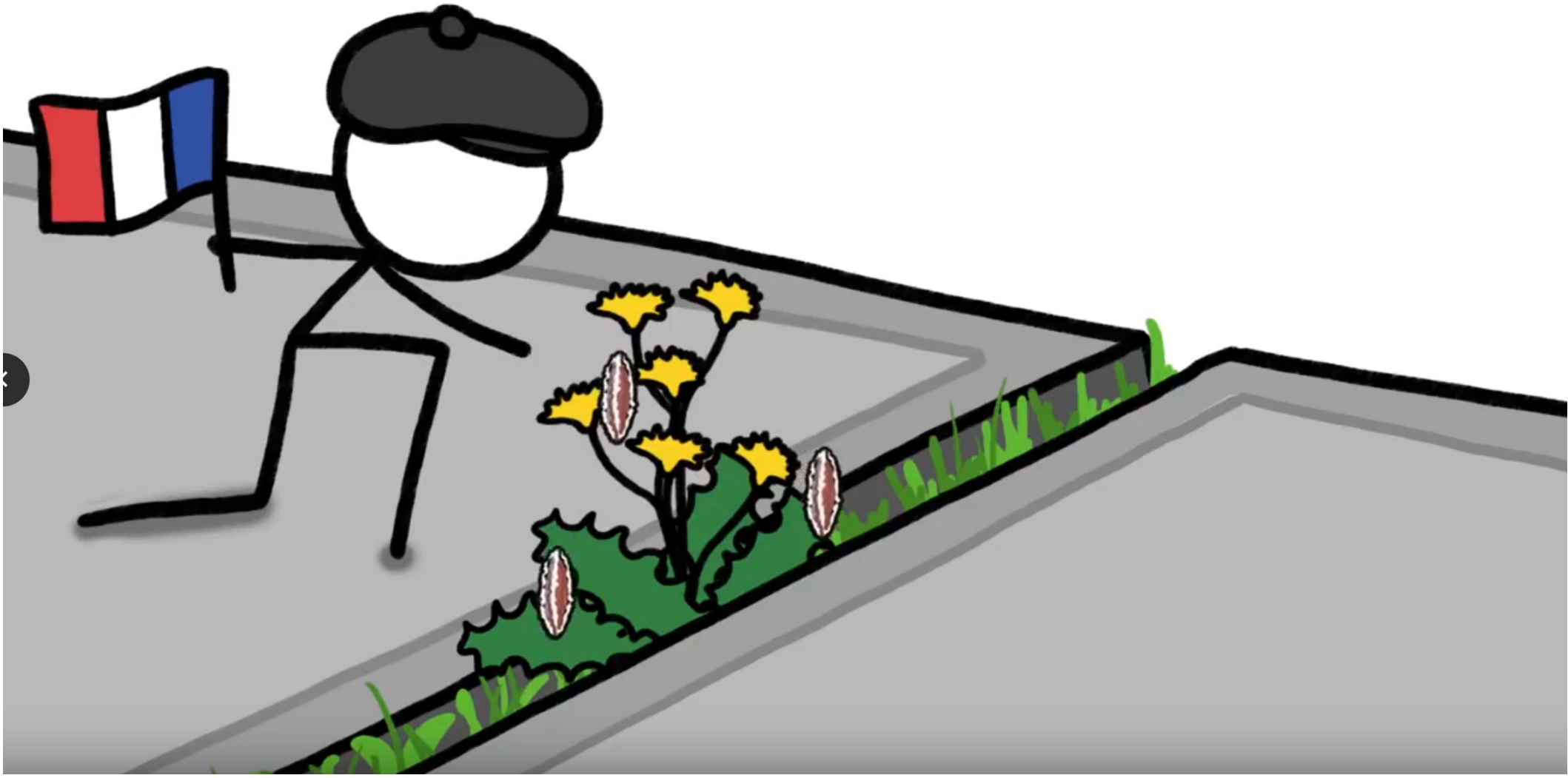
Evolution of PCB resistance in tomcods

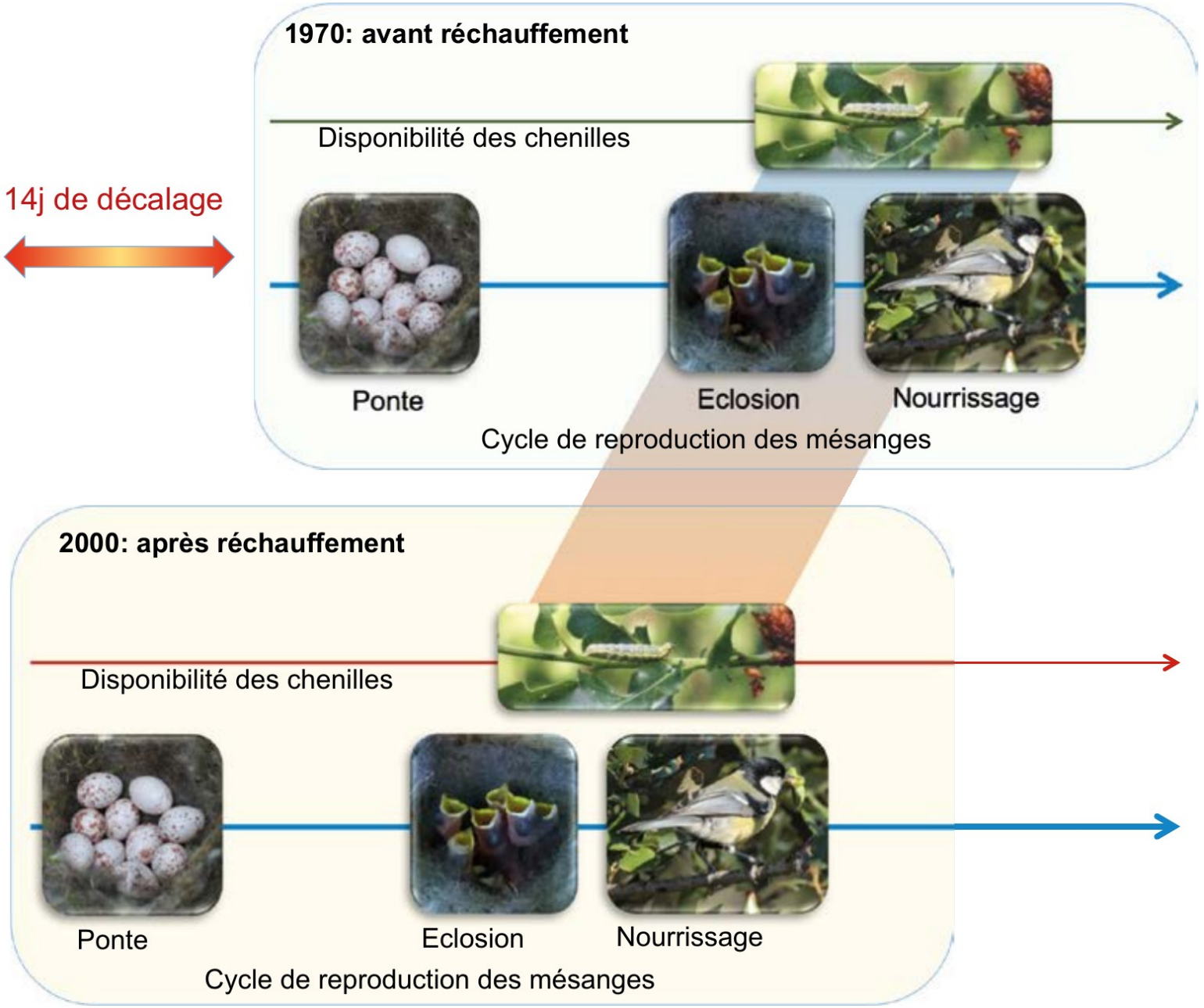


Evolution of *Crepis sancta*

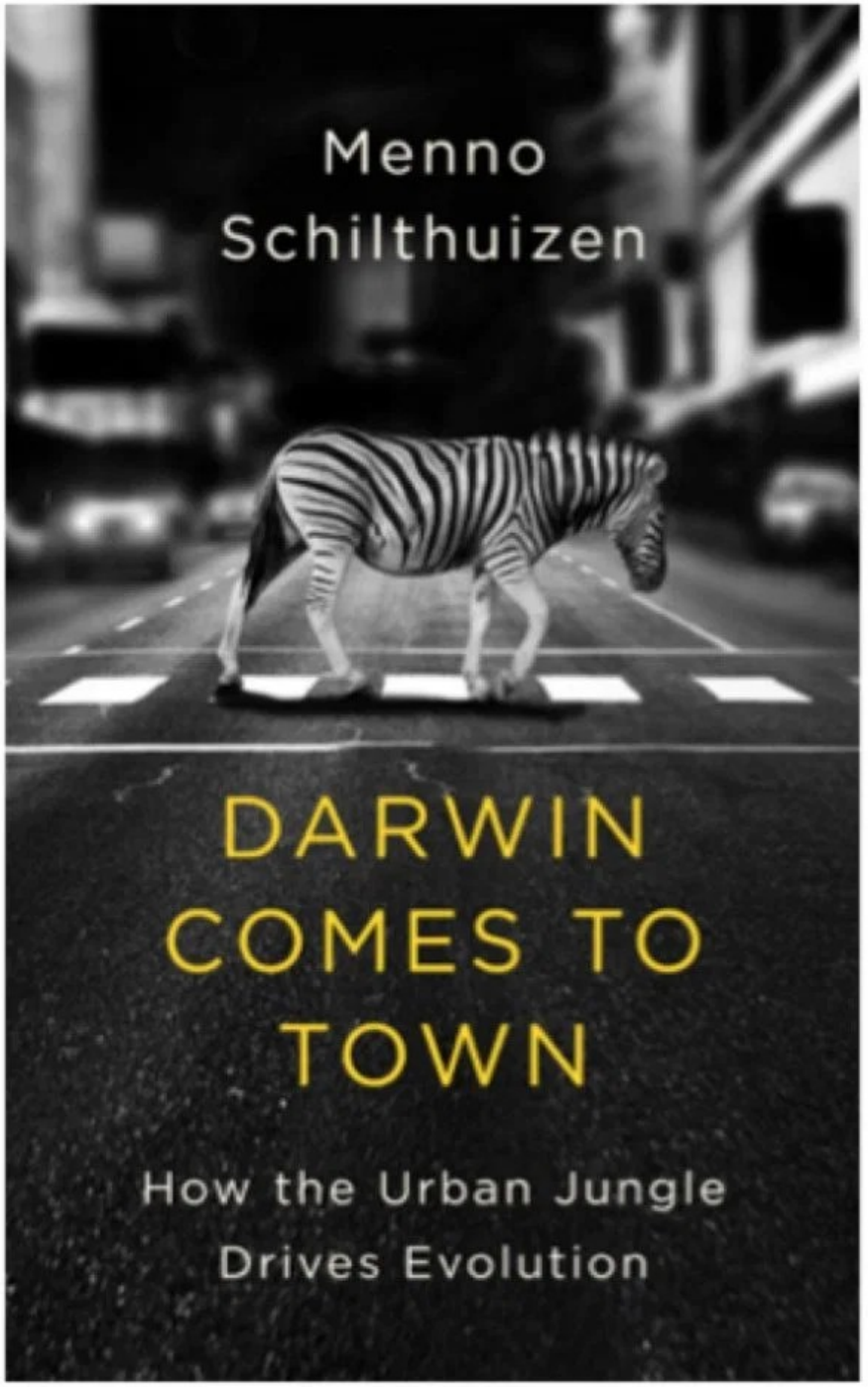


Coyotes become more nocturnal

Rapid evolution of great tits

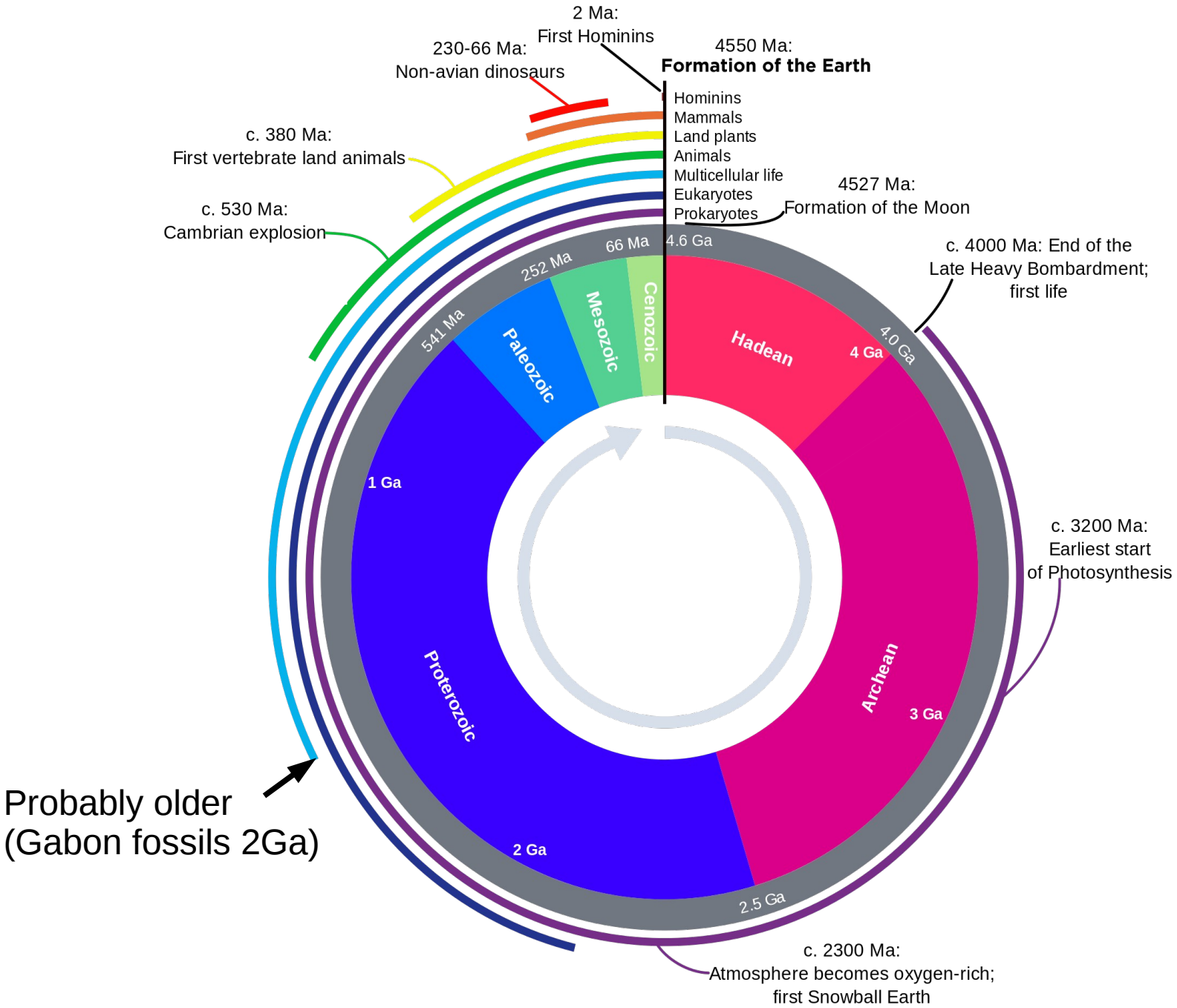


Etonnant vivant : découvertes et promesses du XXIe siècle (2017)


Menno
Schilthuizen
DARWIN
COMES TO
TOWN
How the Urban Jungle
Drives Evolution


DARWIN COMES TO TOWN
HOW THE URBAN JUNGLE DRIVES EVOLUTION
PICADOR
MENNO SCHILTHUIZEN

Geologic time scales



Wikipedia

Geologic time scales

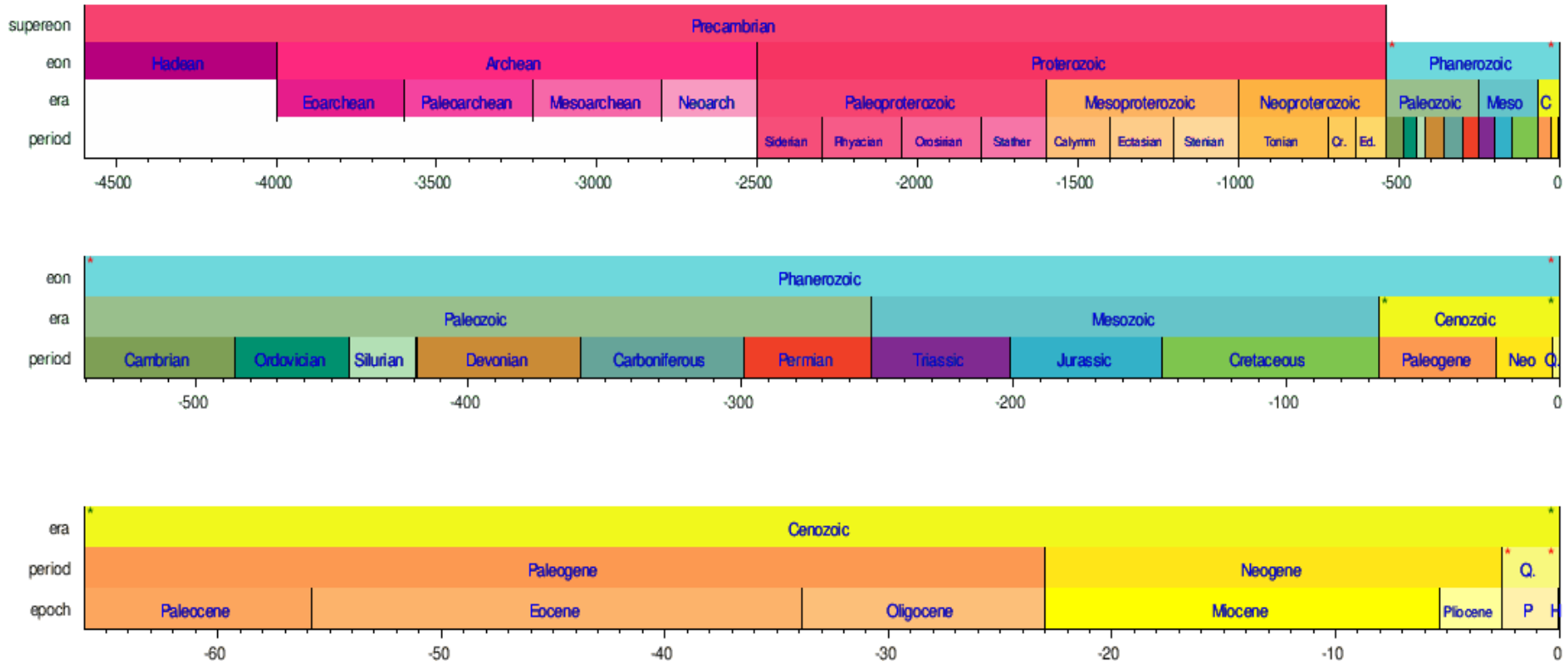
Eons:
Hadean, Archean, Proterozoic, Phanerozoic

Precambrian

Era:
Phanerozoic (-541Ma to present)= 3 eras: Paleozoic + Mesozoic + Cenozoic

Period:
Paleozoic (-541Ma to -252Ma)= 6 periods: Cambrian + Ordovician + Silurian + Devonian + Carboniferous + Permian
Cenozoic (-66Ma to present)= 3 periods: Paleogene + Neogene + Quaternary (Tertiary= obsolete= Paleogene + Neogene)

Epoch:
Quaternary=
Pleistocene (-2.5Ma to -11000) + Holocene (-11000 to present) (+ Anthropocene?)


supereon
eon
era
period
Precambrian
Phanerozoic
Hadean
Archean
Proterozoic
Eoarchean
Paleoarchean
Mesoarchean
Neoarch
Paleoproterozoic
Mesoproterozoic
Neoproterozoic
Paleozoic
Meso
C
Siderian
Rhyacian
Orosirian
Statherian
Calymm
Ectasian
Stenian
Tonian
Cr.
Ed.
-4500
-4000
-3500
-3000
-2500
-2000
-1500
-1000
-500
0
eon
era
period
Phanerozoic
Paleozoic
Mesozoic
Cenozoic
Cambrian
Ordovician
Silurian
Devonian
Carboniferous
Permian
Triassic
Jurassic
Cretaceous
Paleogene
Neo
Q.
-500
-400
-300
-200
-100
0
era
period
epoch
Cenozoic
Paleogene
Neogene
Q.
Paleocene
Eocene
Oligocene
Miocene
Pliocene
P
H
-60
-50
-40
-30
-20
-10
0

Wikipedia


DNA
V. Courtier-Orgogozo

Why is DNA an important molecule in biology?

Slide from S. Gilbert

SAME DNA. SMALLER CHROMOSOMES.

THE ALL-NEW MIDSIZE H3. LIVING UP TO THE OFF-ROAD REPUTATION HUMMER MADE FAMOUS.
COMING SOON. STARTING AT $29,500. VEHICLE SHOWN $30,195.*

HUMMER
LIKE NOTHING ELSE.™

*MSRP. TAX, TITLE, LICENSE, DEALER FEES AND OPTIONAL EQUIPMENT ARE EXTRA. 1.800.REAL.4WD

DNA still sells cars in the USA

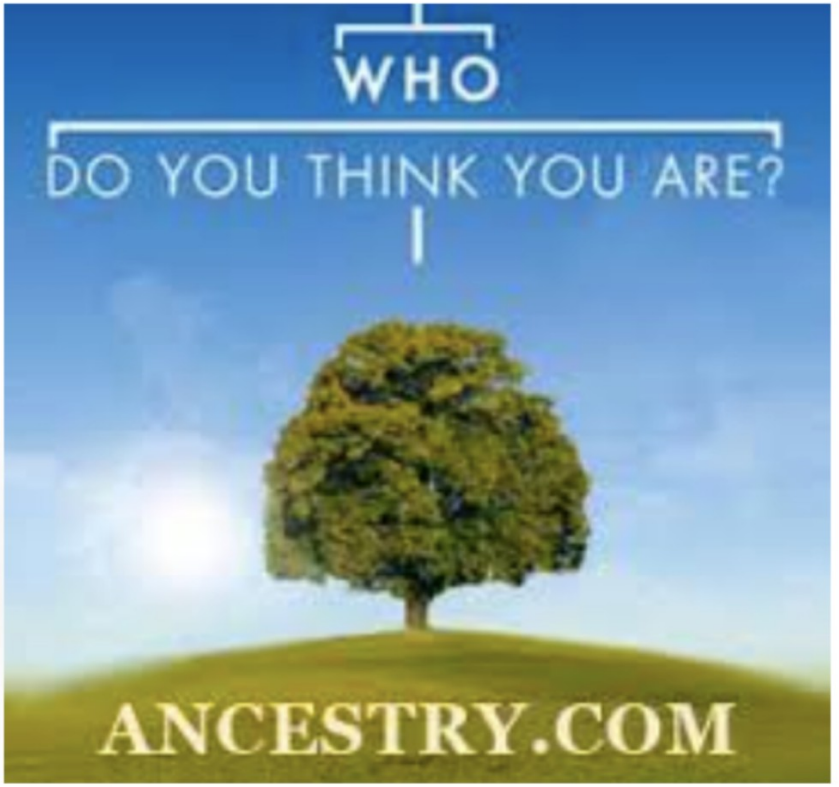
Subaru: “Genetic superstar”
Toyota: “Has a great set of genes

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

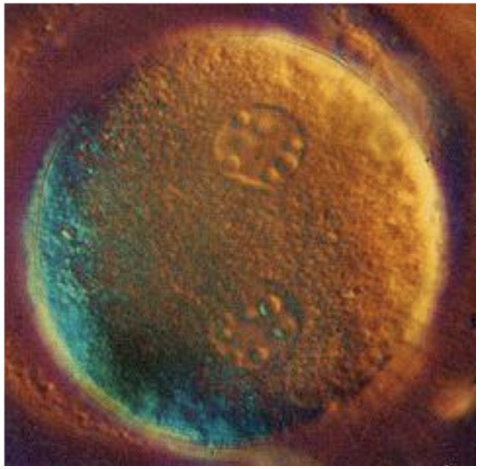
Genes determine who you are, and they act the same in each person.

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 ?

Extract, purify

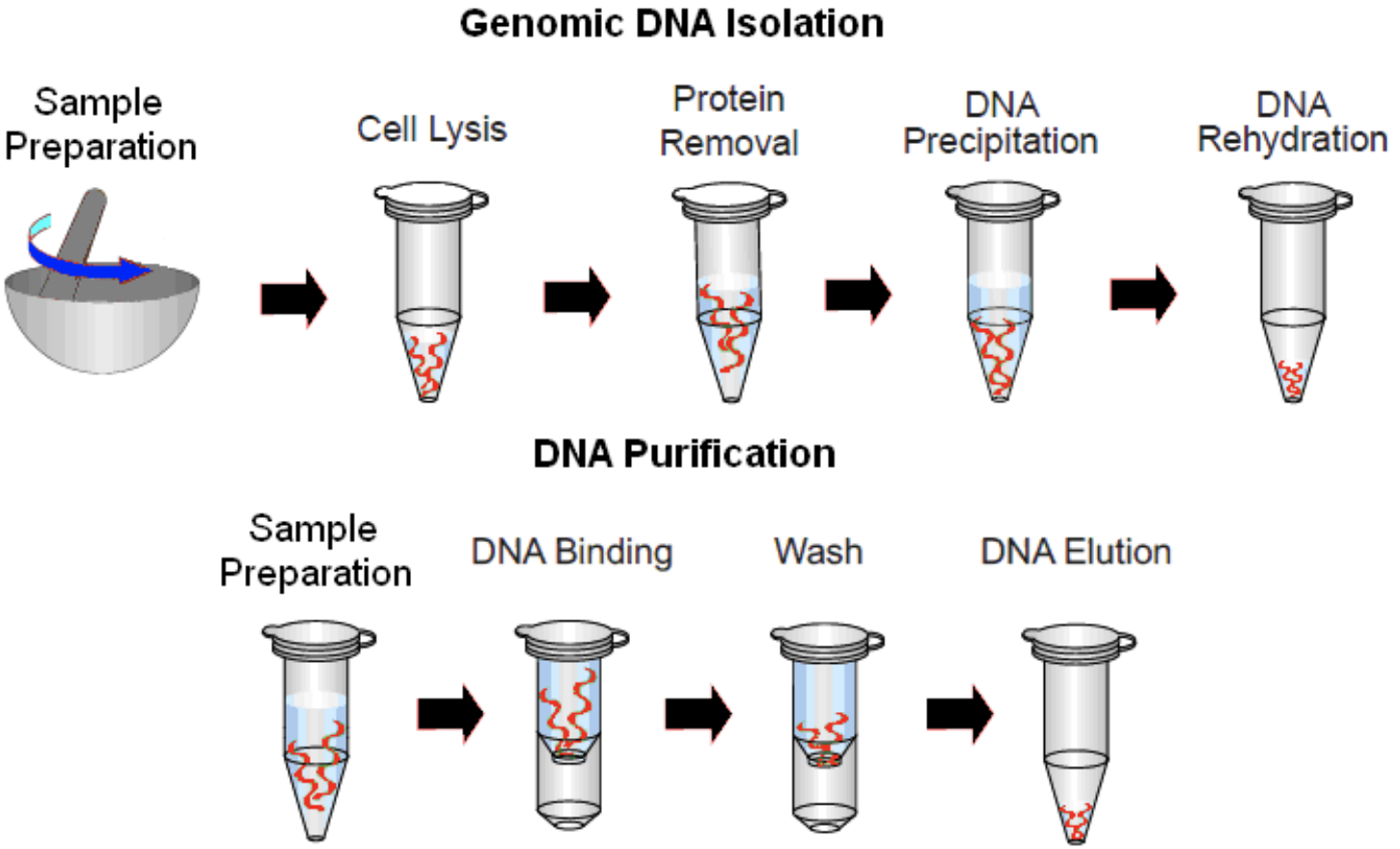
Make more
Amplify
Clone
Synthetize

Examine
Quantify
Examine length
Stain, probe
Sequence

Modify
Cut
Ligate
Recombine fragments
Introduce foreign DNA
Mutate

Extract DNA

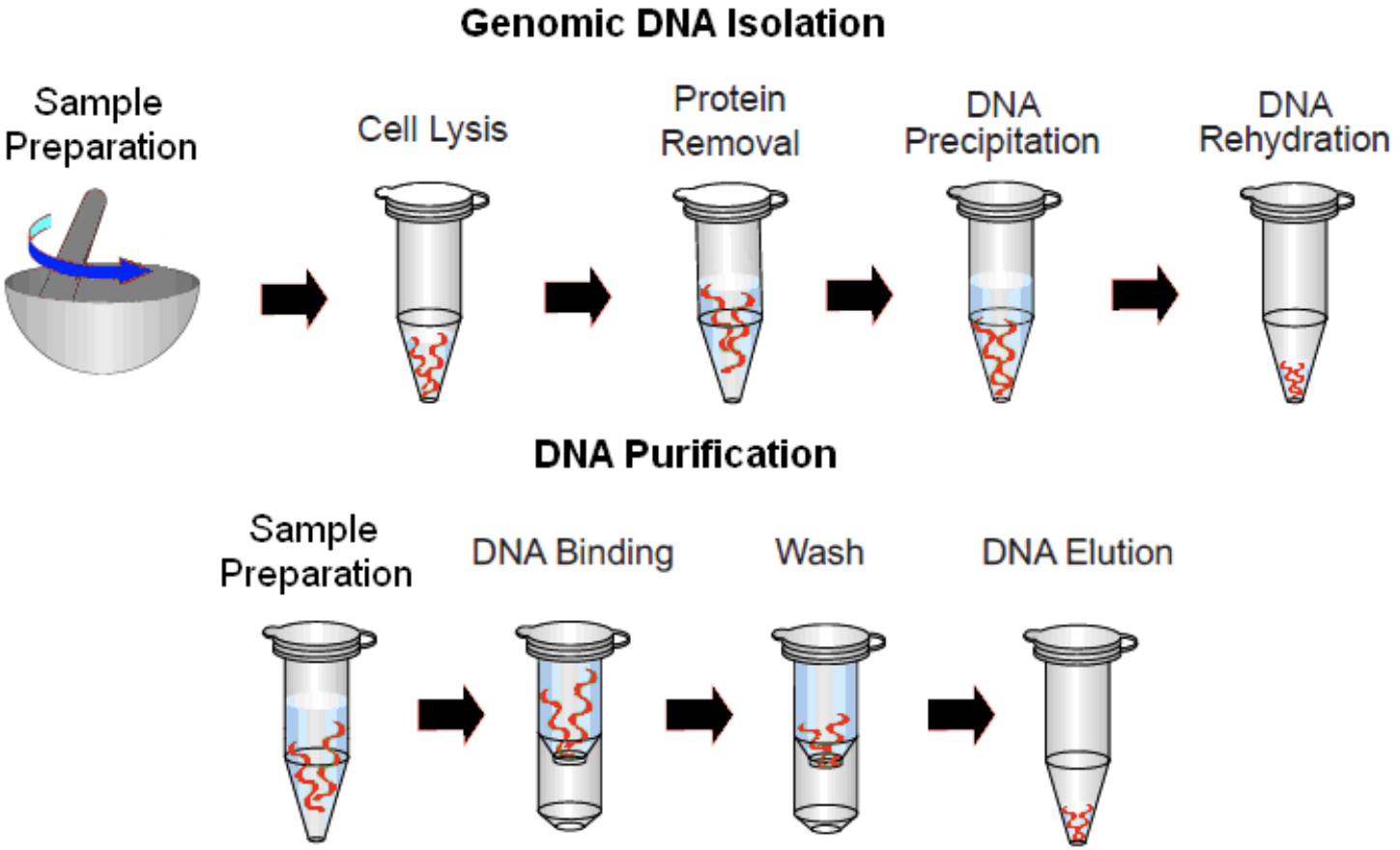
Break cells, remove lipids and proteins,
precipitate DNA, remove liquid, resuspend in aquaeous solution



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 ($MgCl_2$)

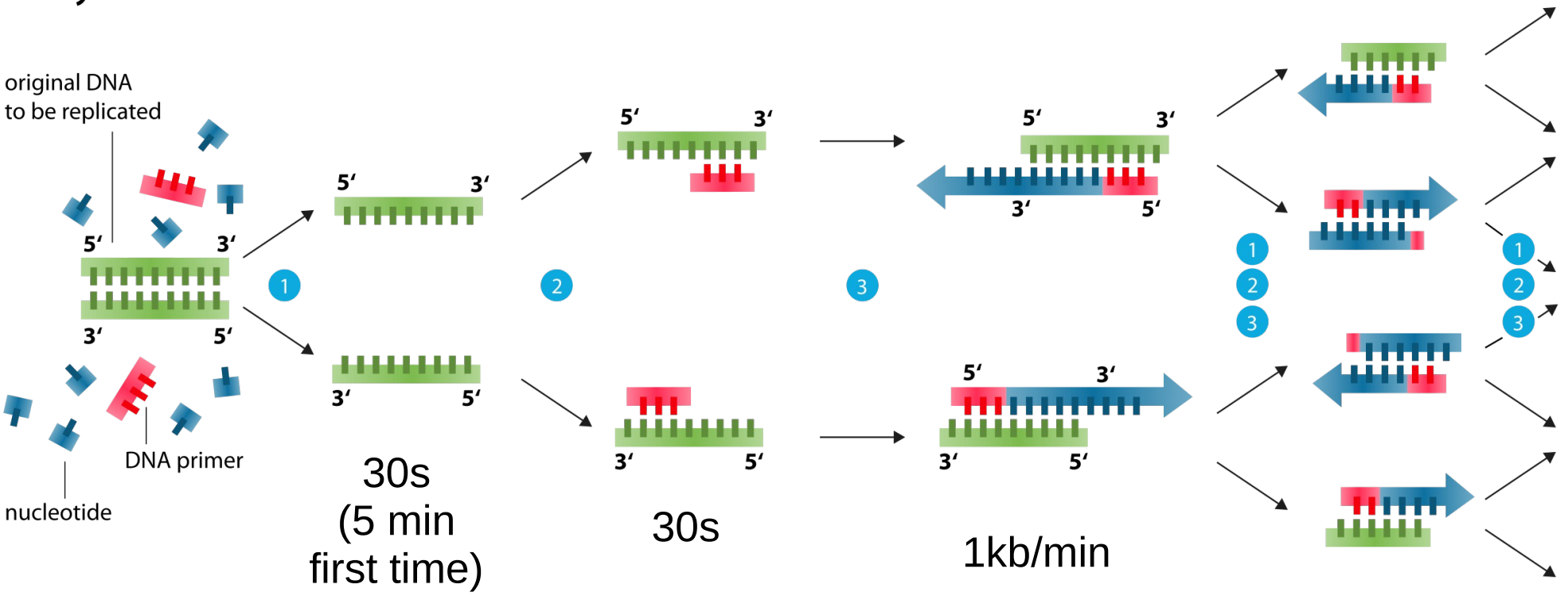
Cycles of Denaturation, Annealing, Elongation

PCR: Polymerase Chain Reaction

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

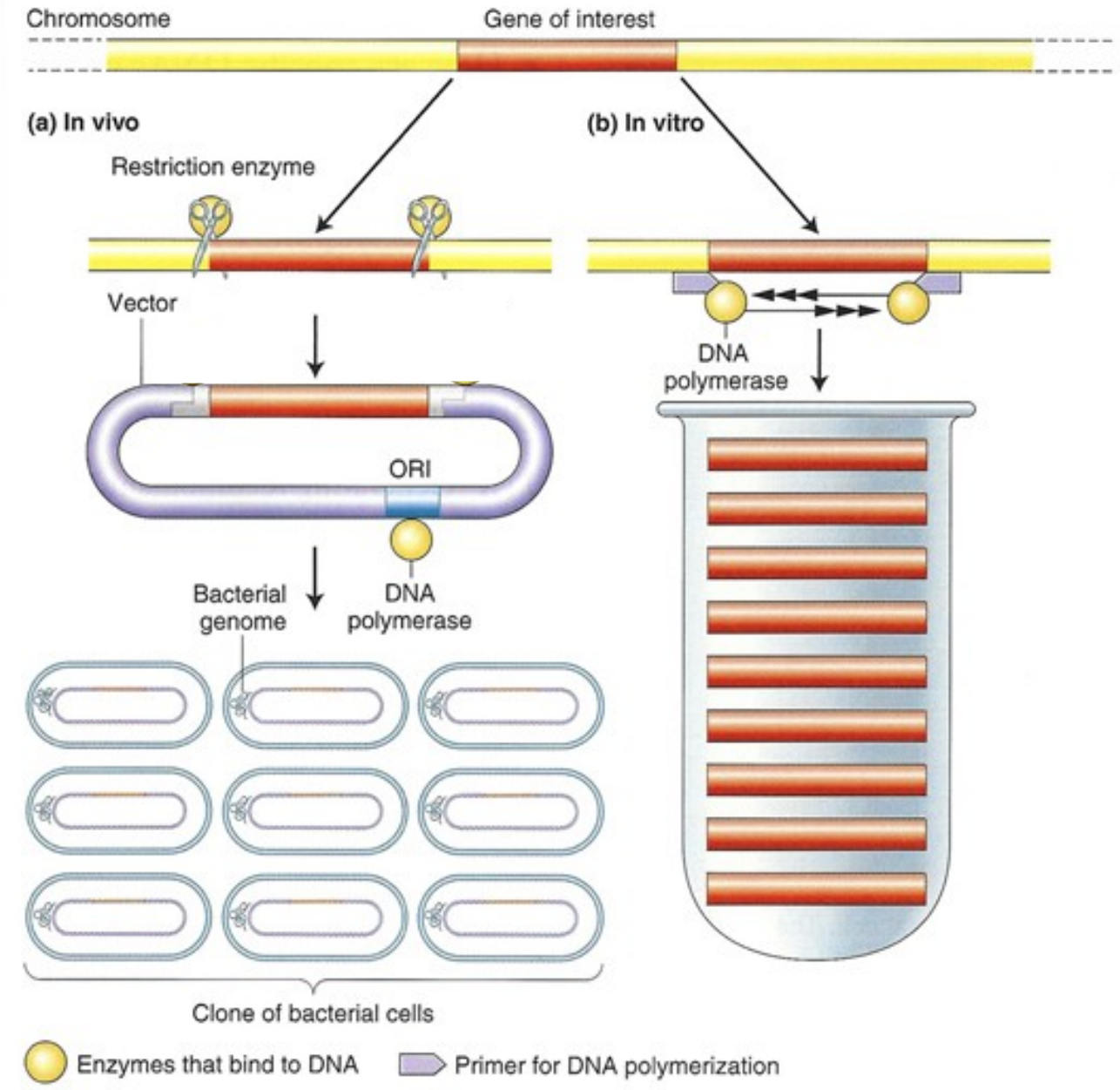
Amplify DNA

Polymerase chain reaction - PCR



1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **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)

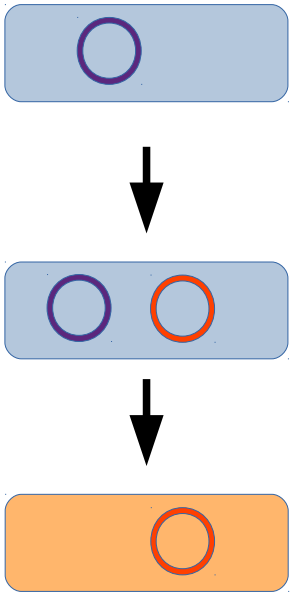
First “synthetic” cell developed by scientists

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

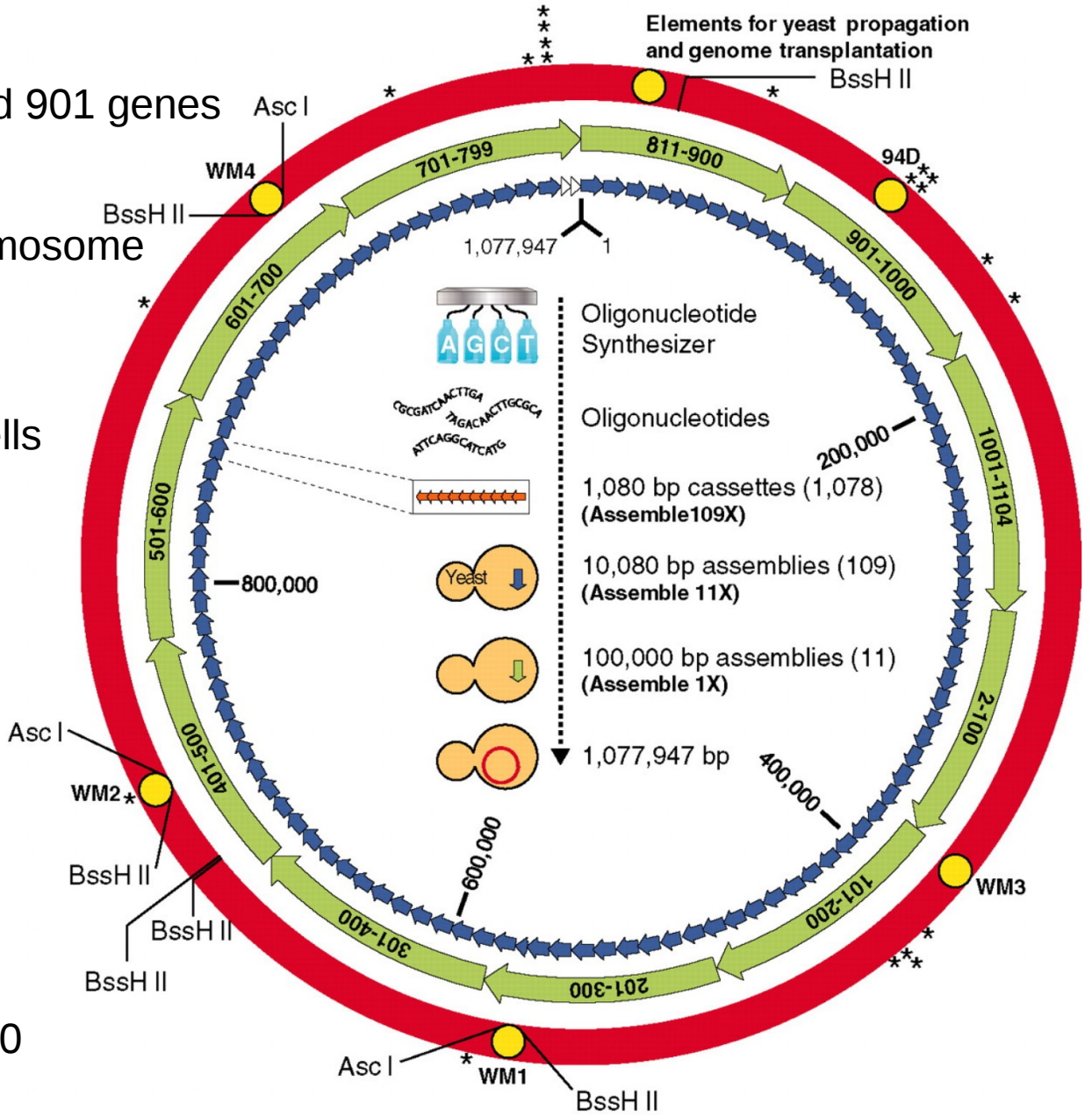
single yeast artificial chromosome

+

M. capricolum recipient cells

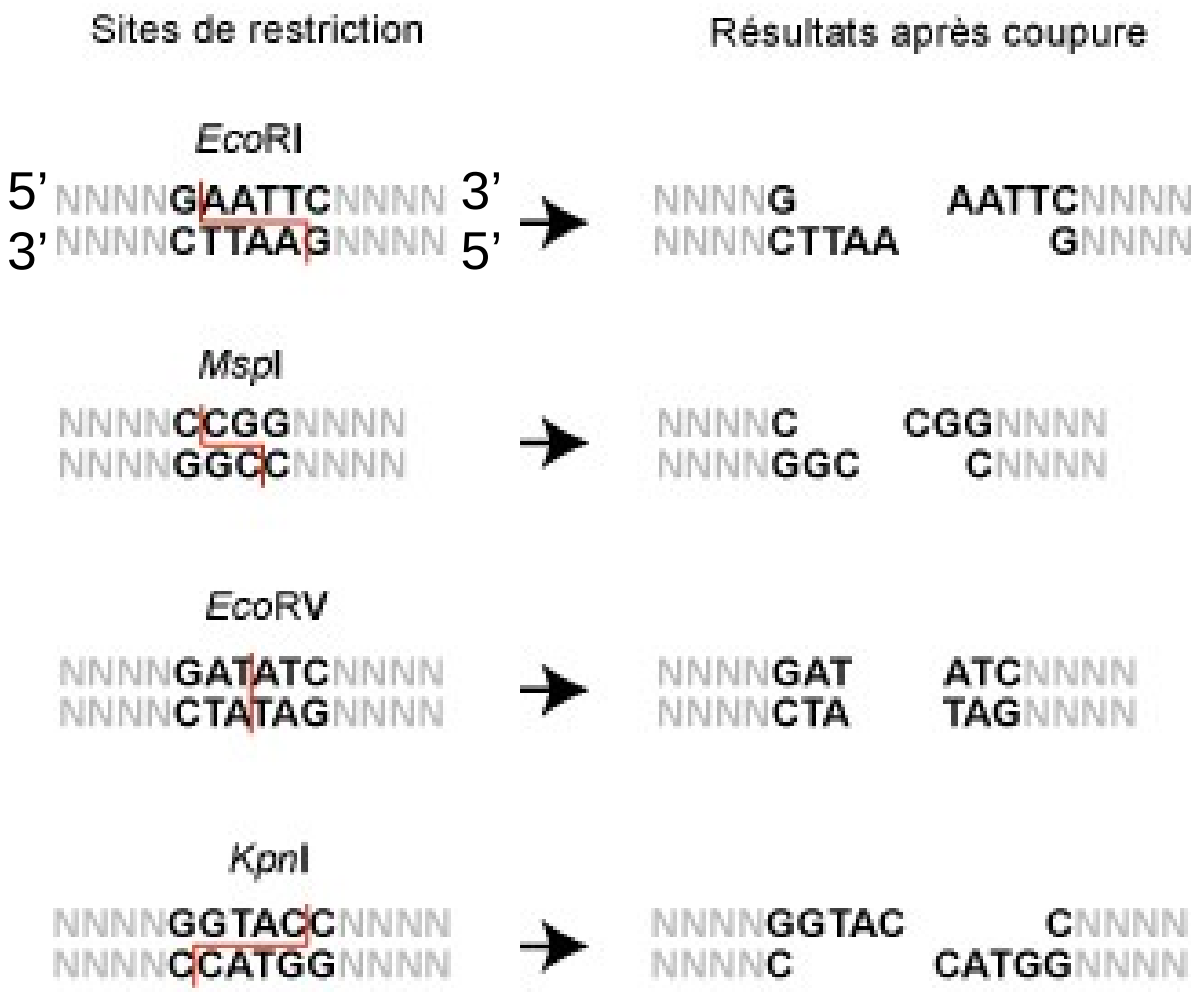


M. mycoides JCVI-syn1.0



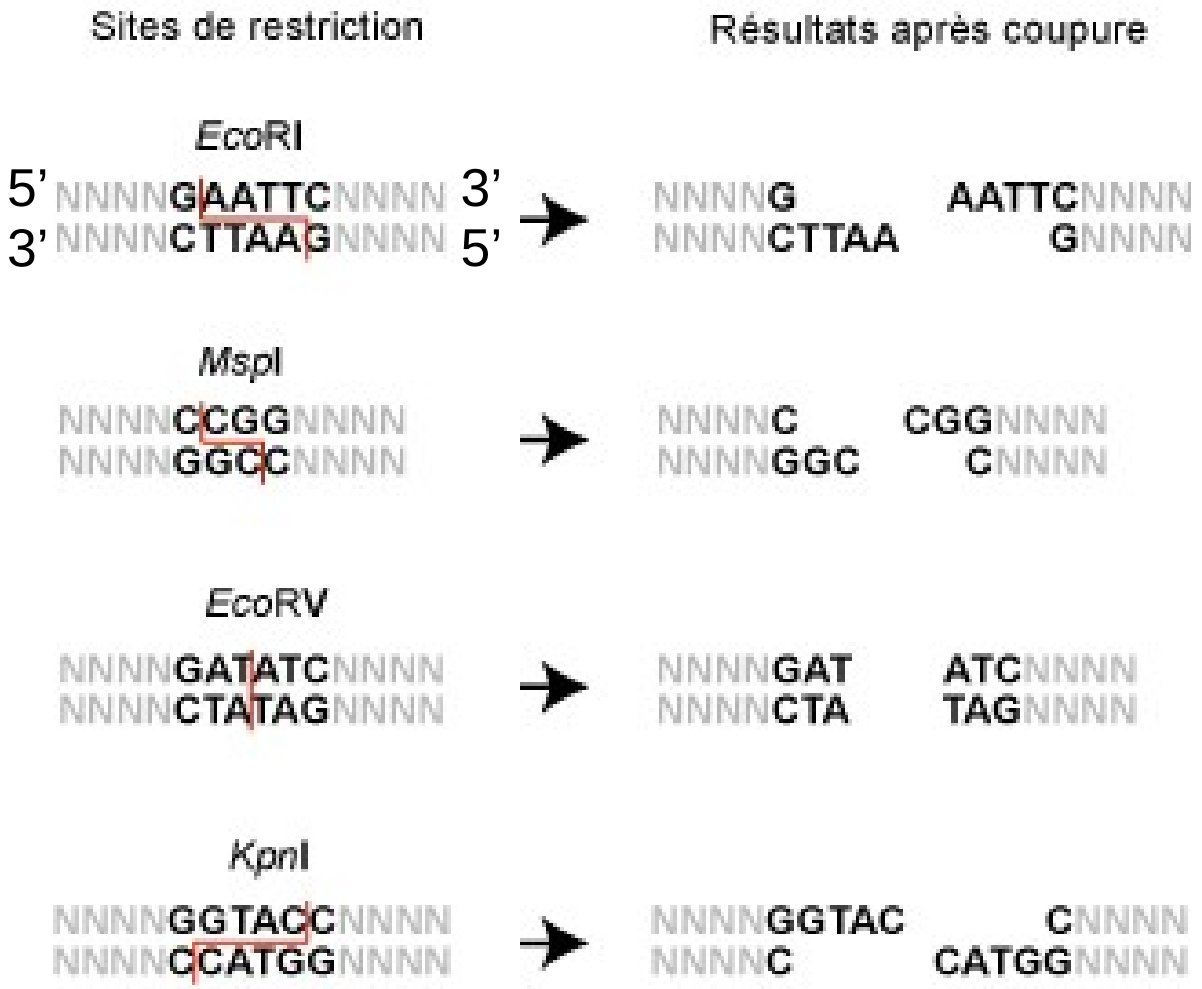
Gibson et al. 2016

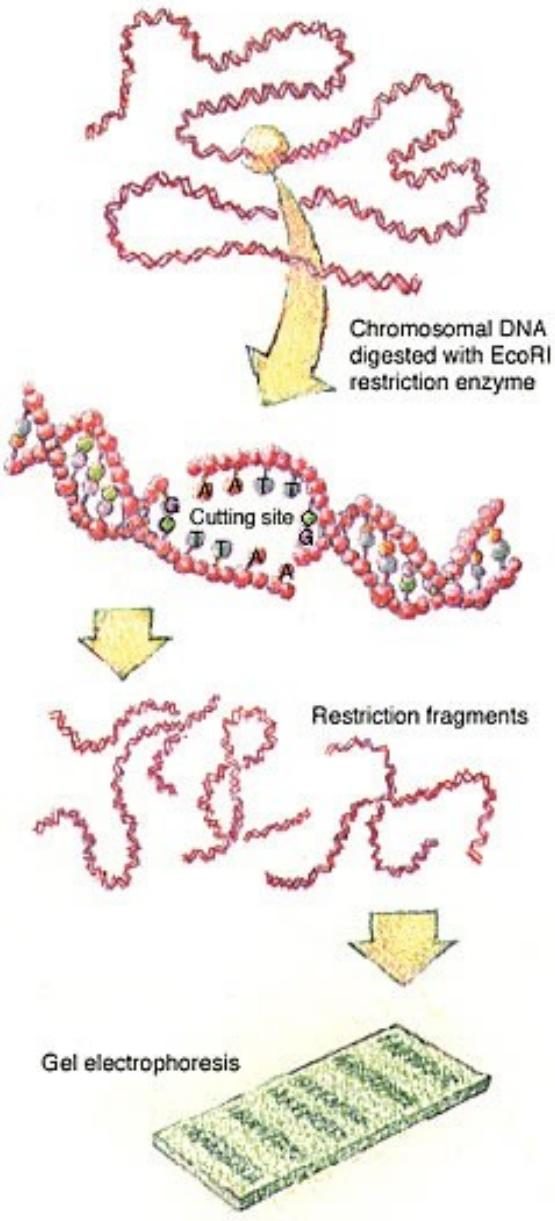
Cut DNA with restriction enzymes



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

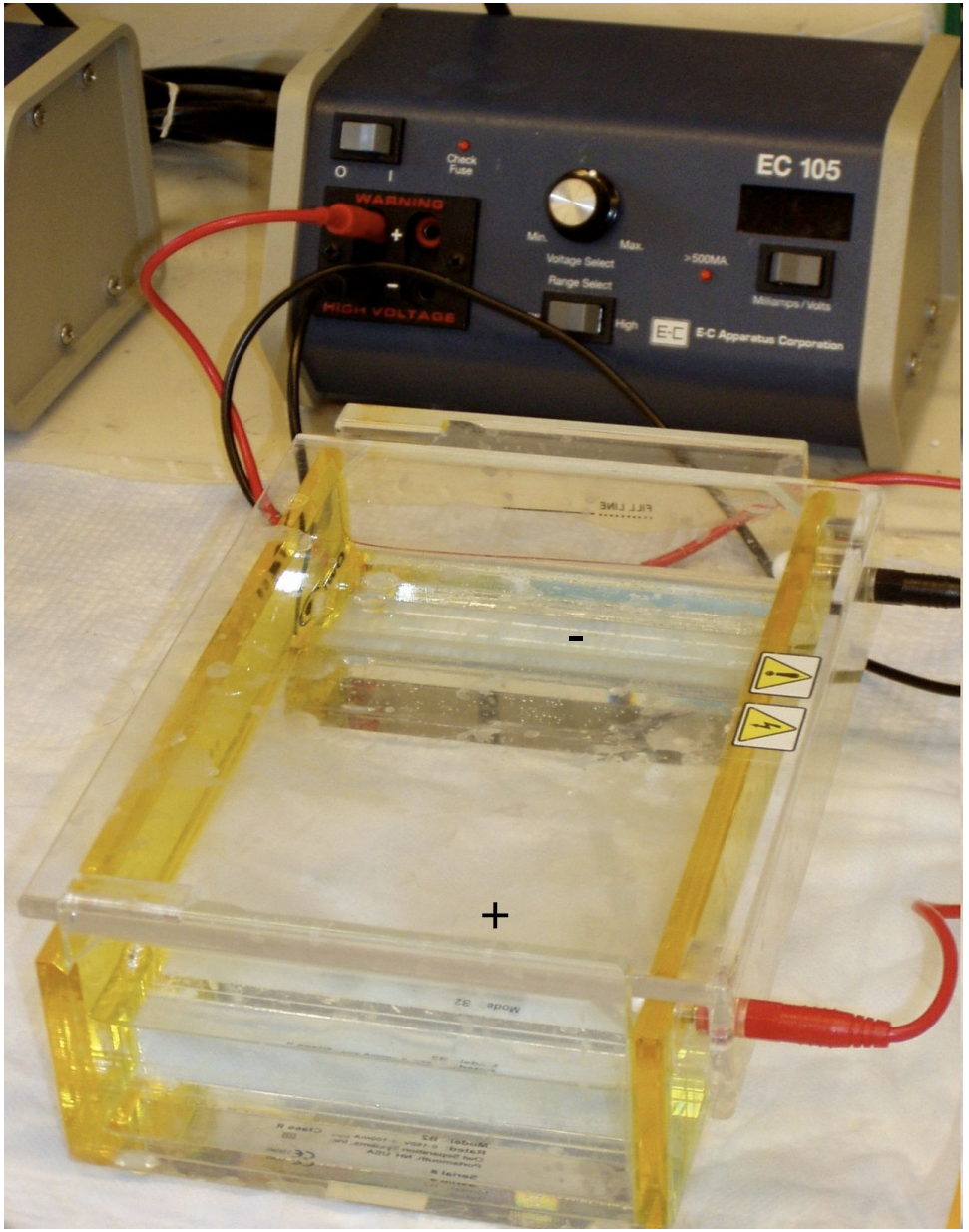
Cut DNA with restriction enzymes



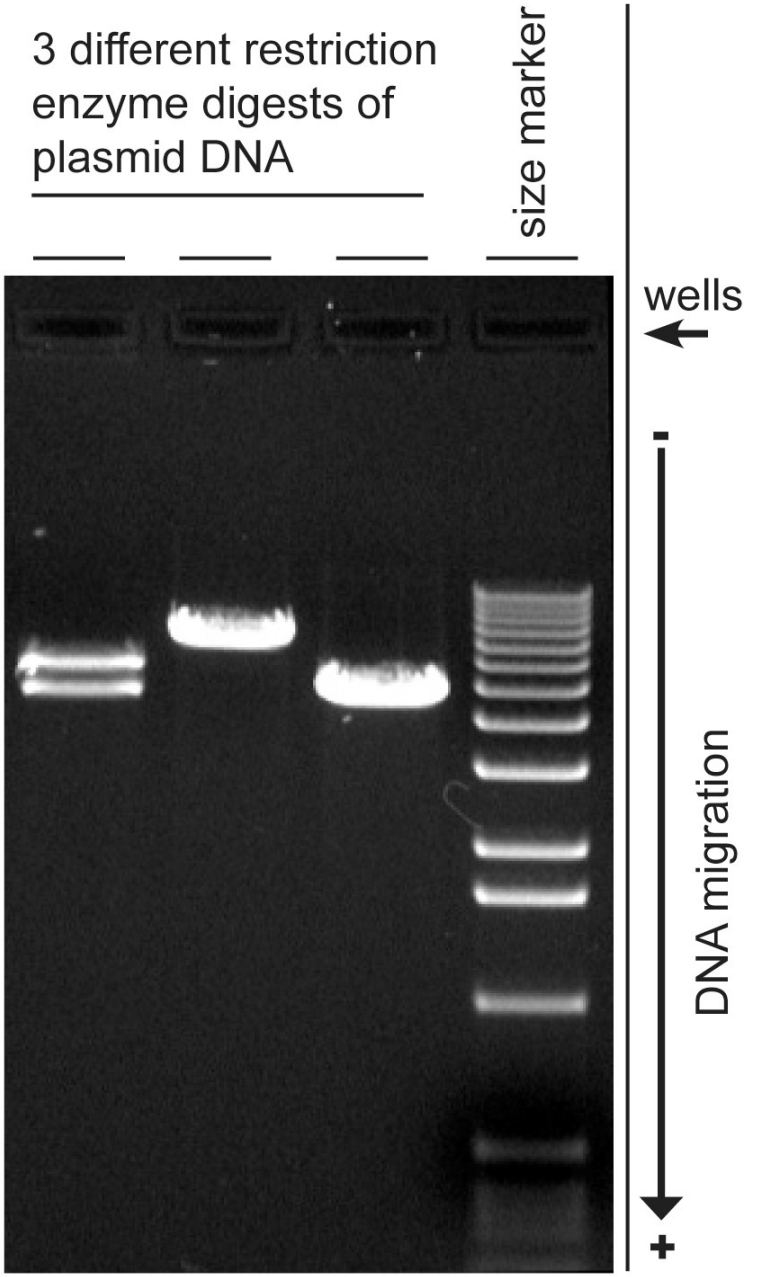


Blunt ends, 3' protruding ends, 5' protruding ends

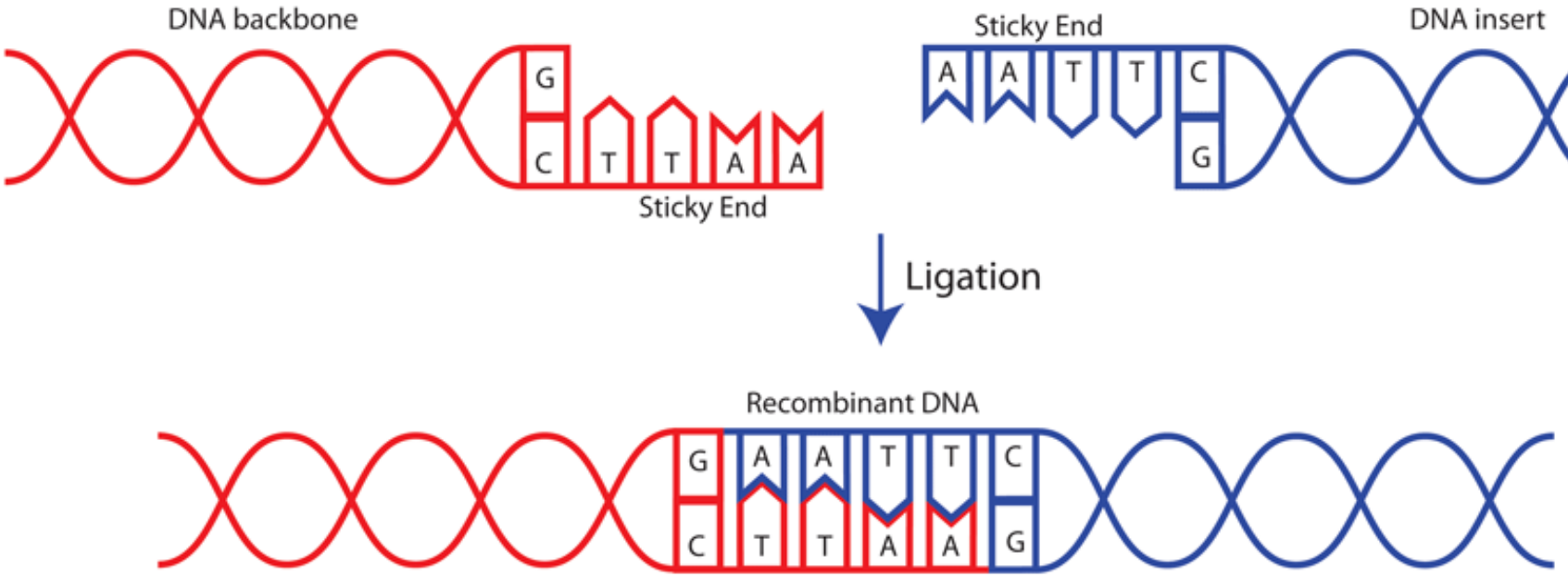
Examine length of DNA

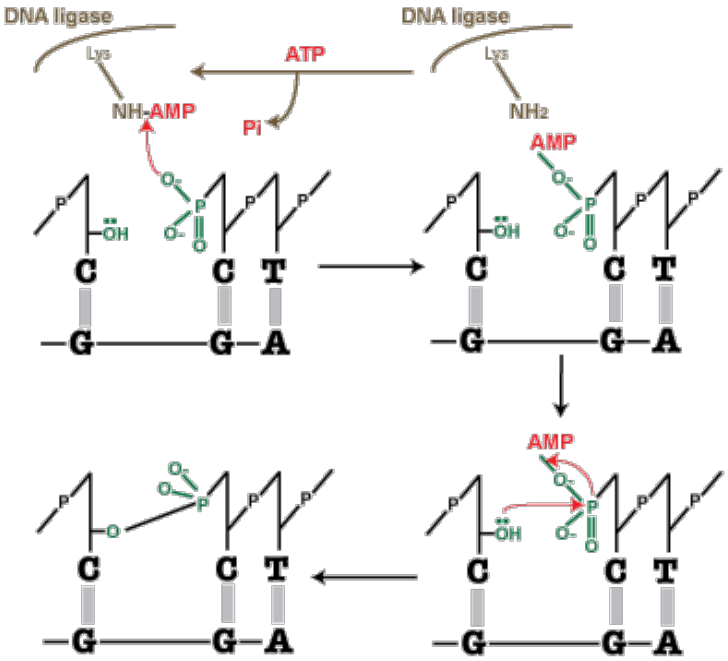


TAE (Tris-acetate-EDTA) buffer



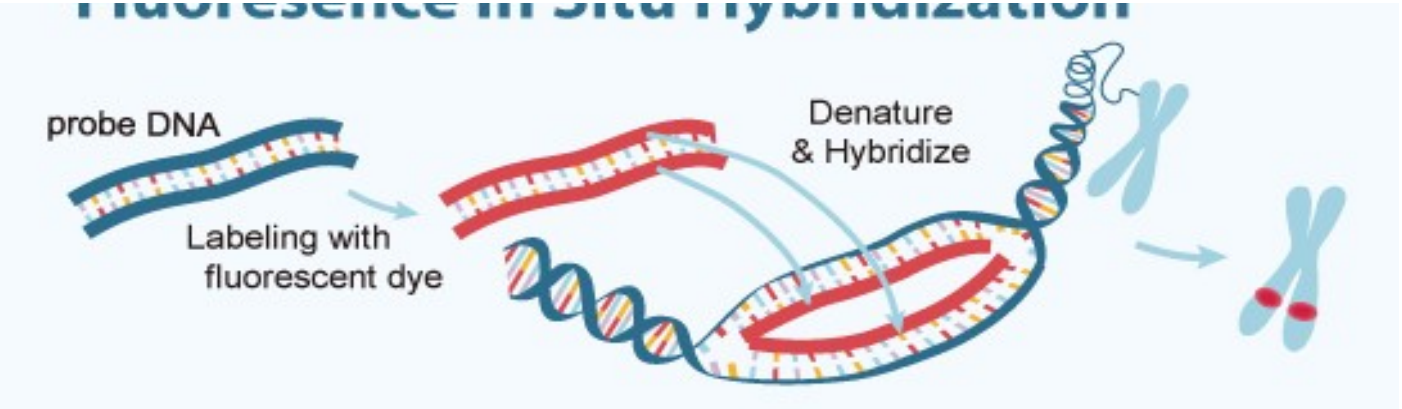
Ligate DNA

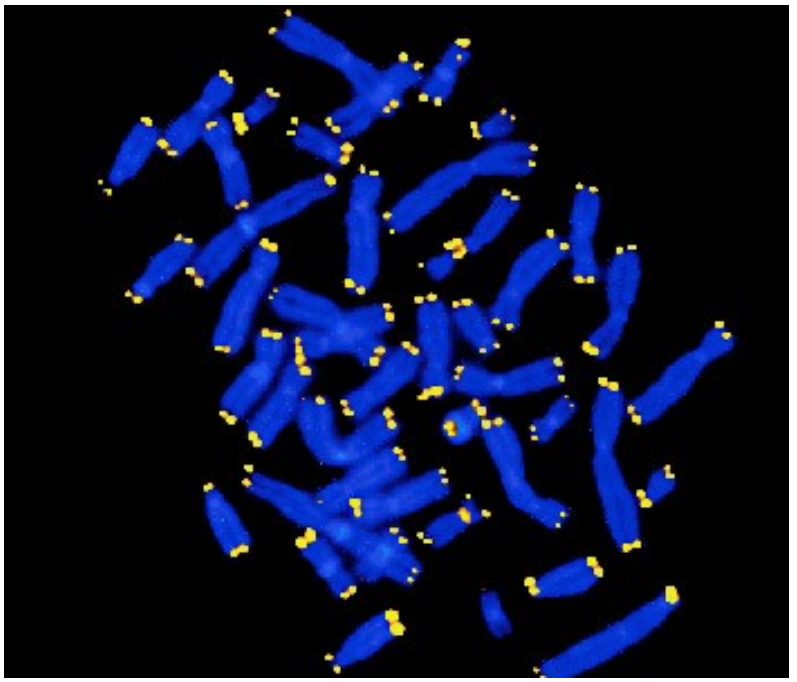




Fragments have to be phosphorylated but only on one strand
Dephosphorylate the vector to inhibit self-circularization

Probe DNA:
Fluorescent In Situ Hybridization

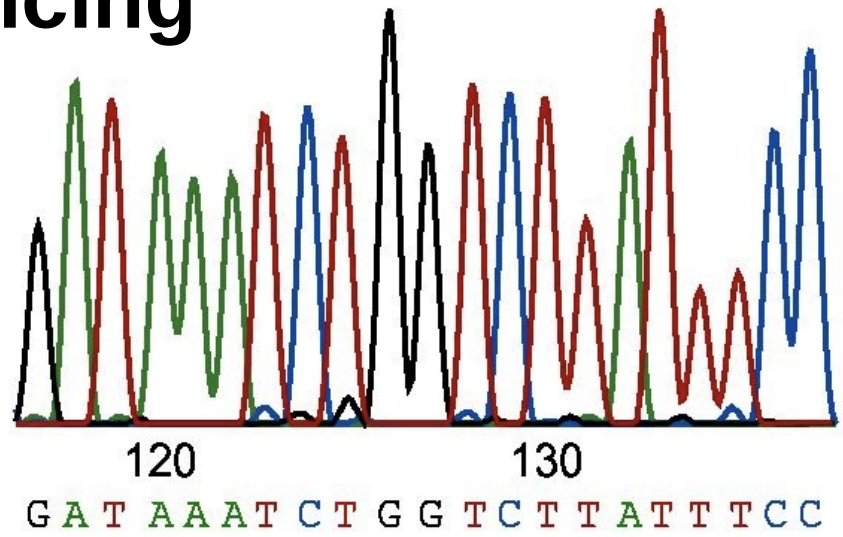




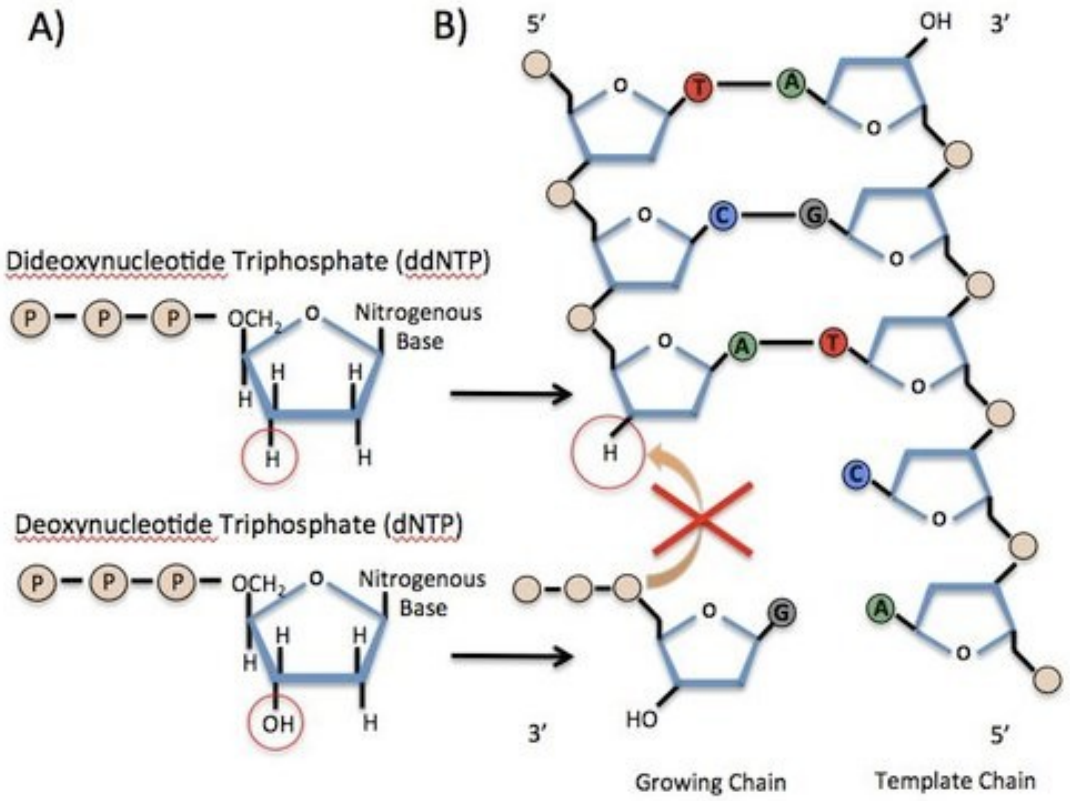
Probes for telomere sequences

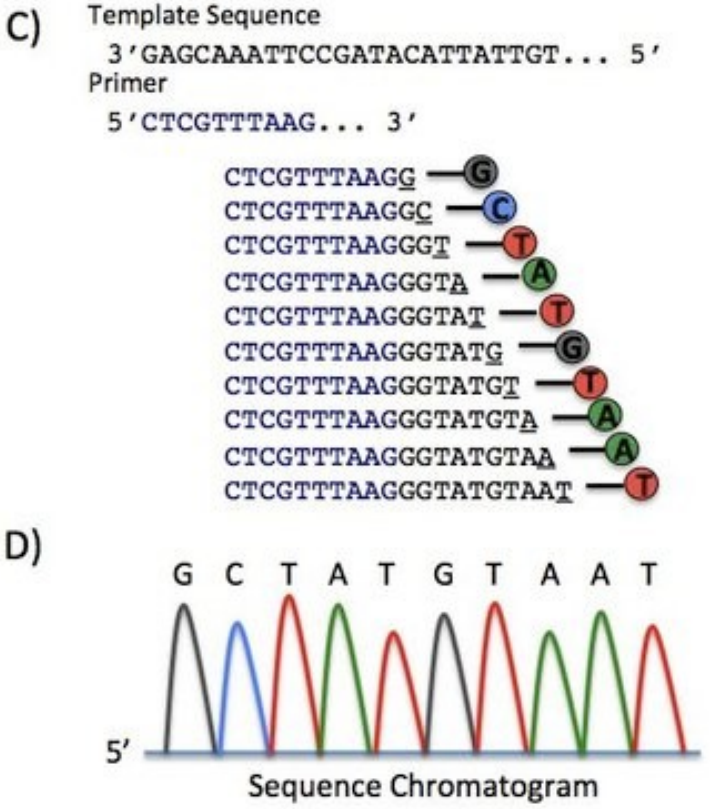
Sanger sequencing

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



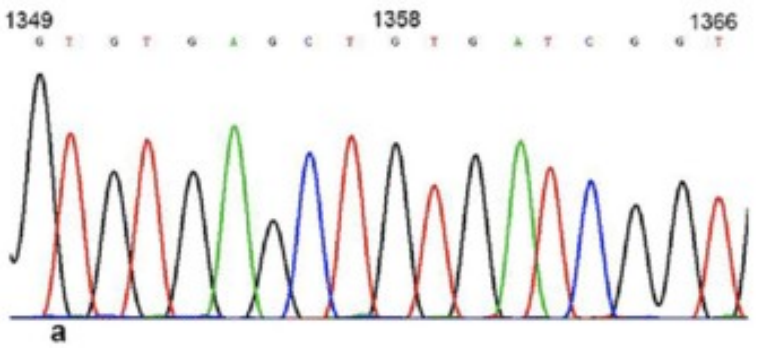
Dye terminator sequencing

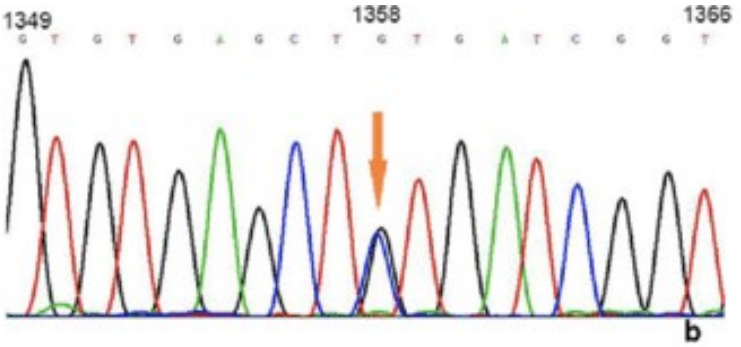


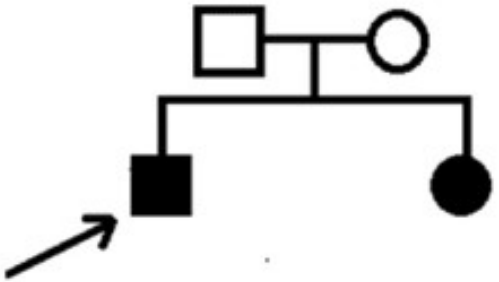


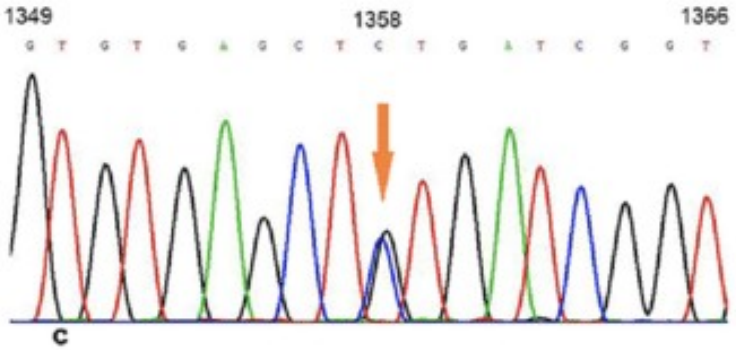
McGovern 2015

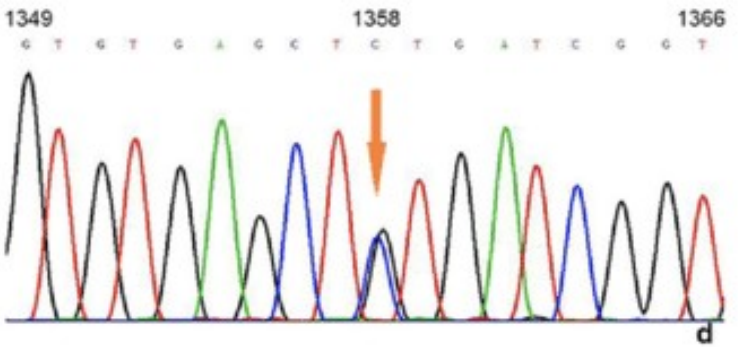
GTGTGAGCTGTGATCGGT



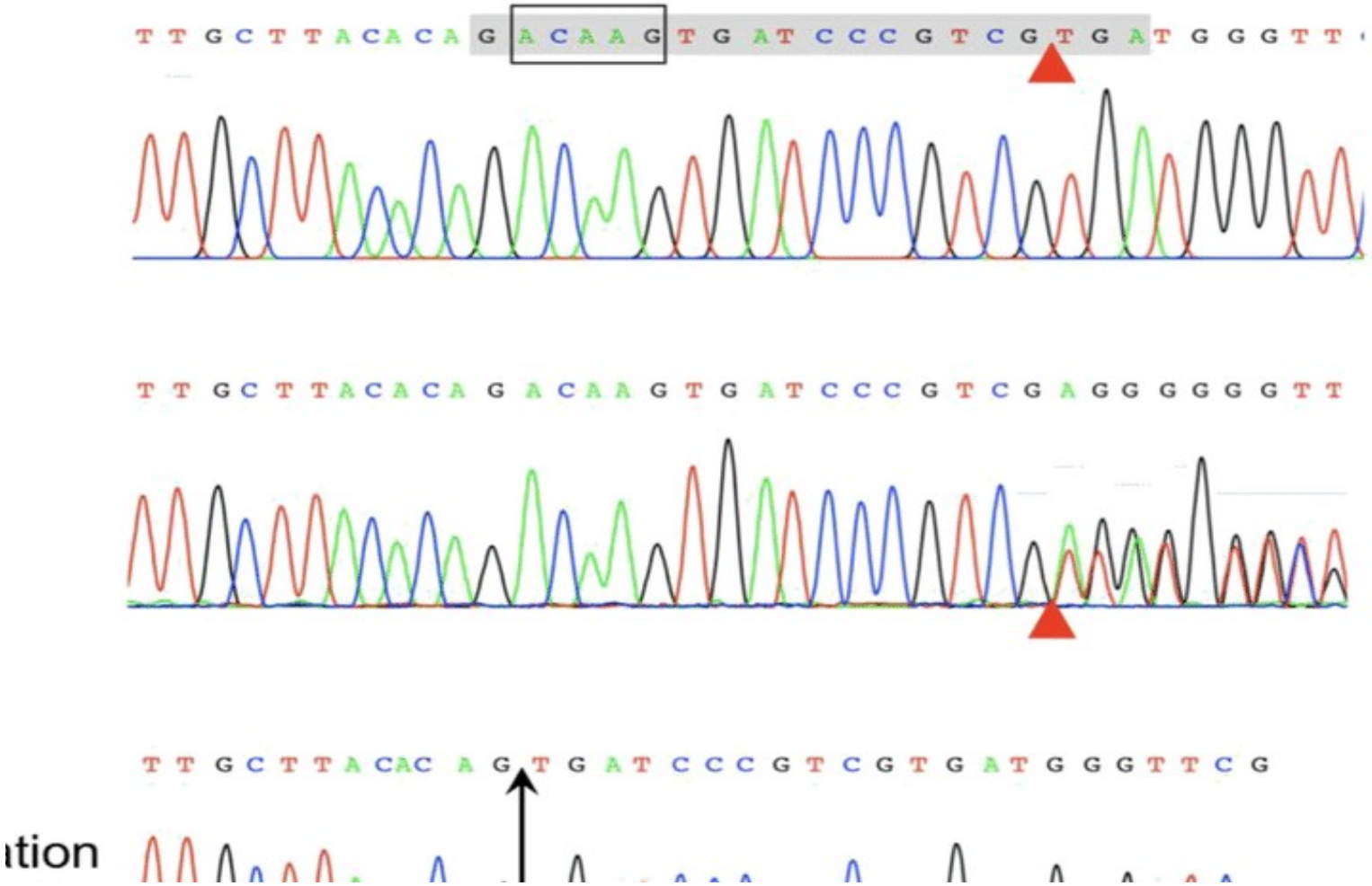






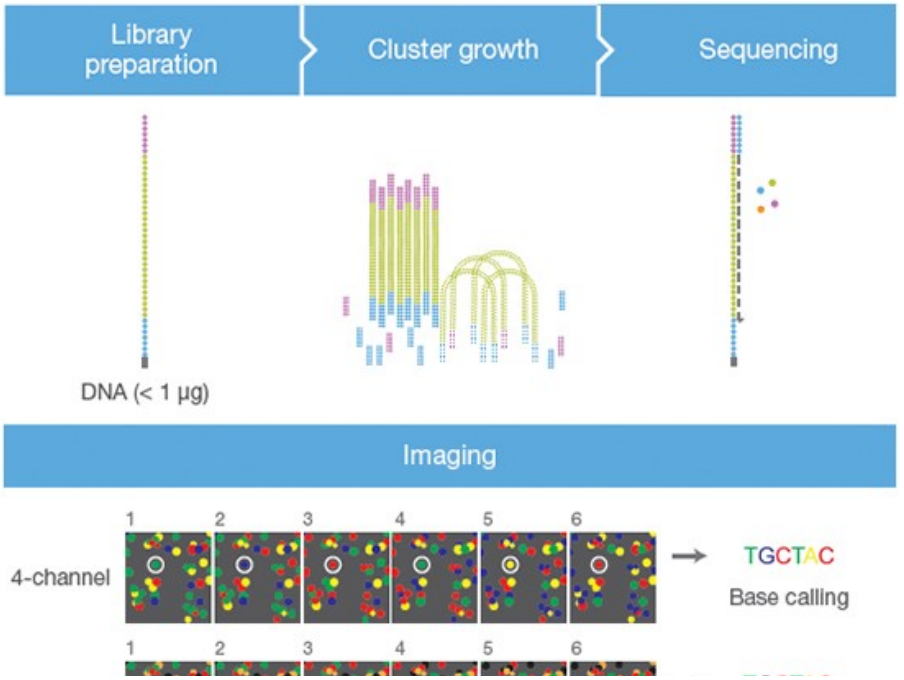


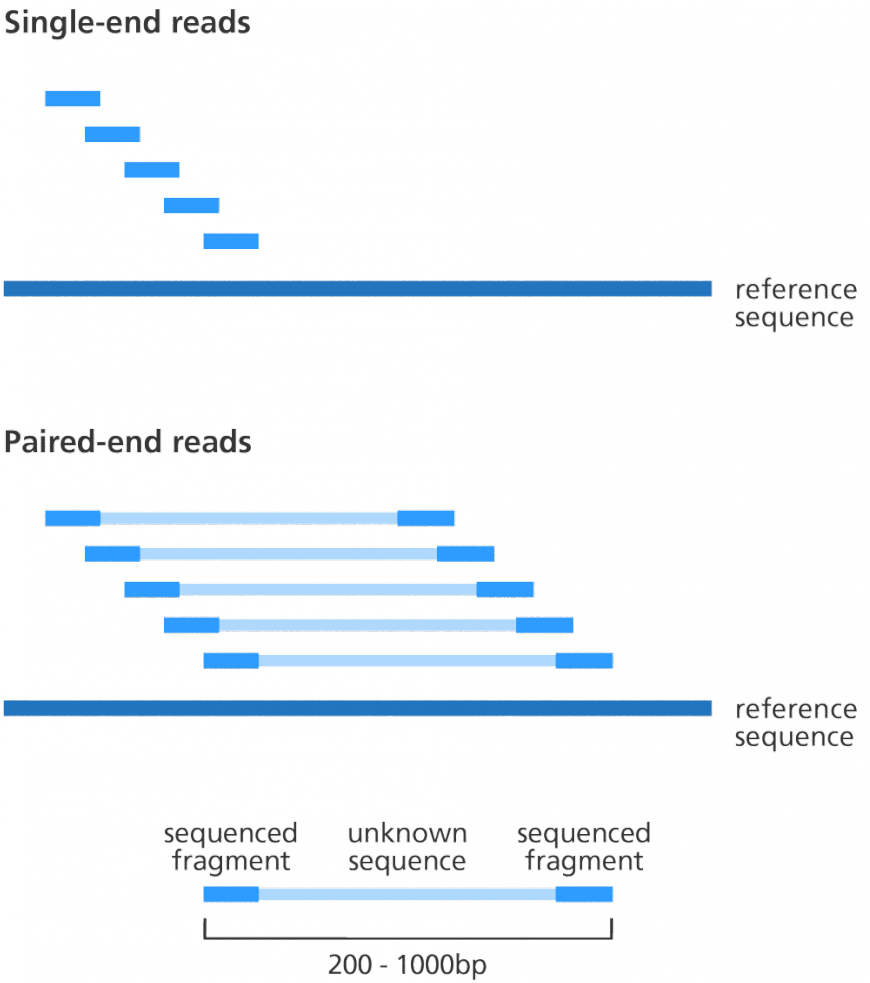
Ding et al 2015


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

Illumina sequencing

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

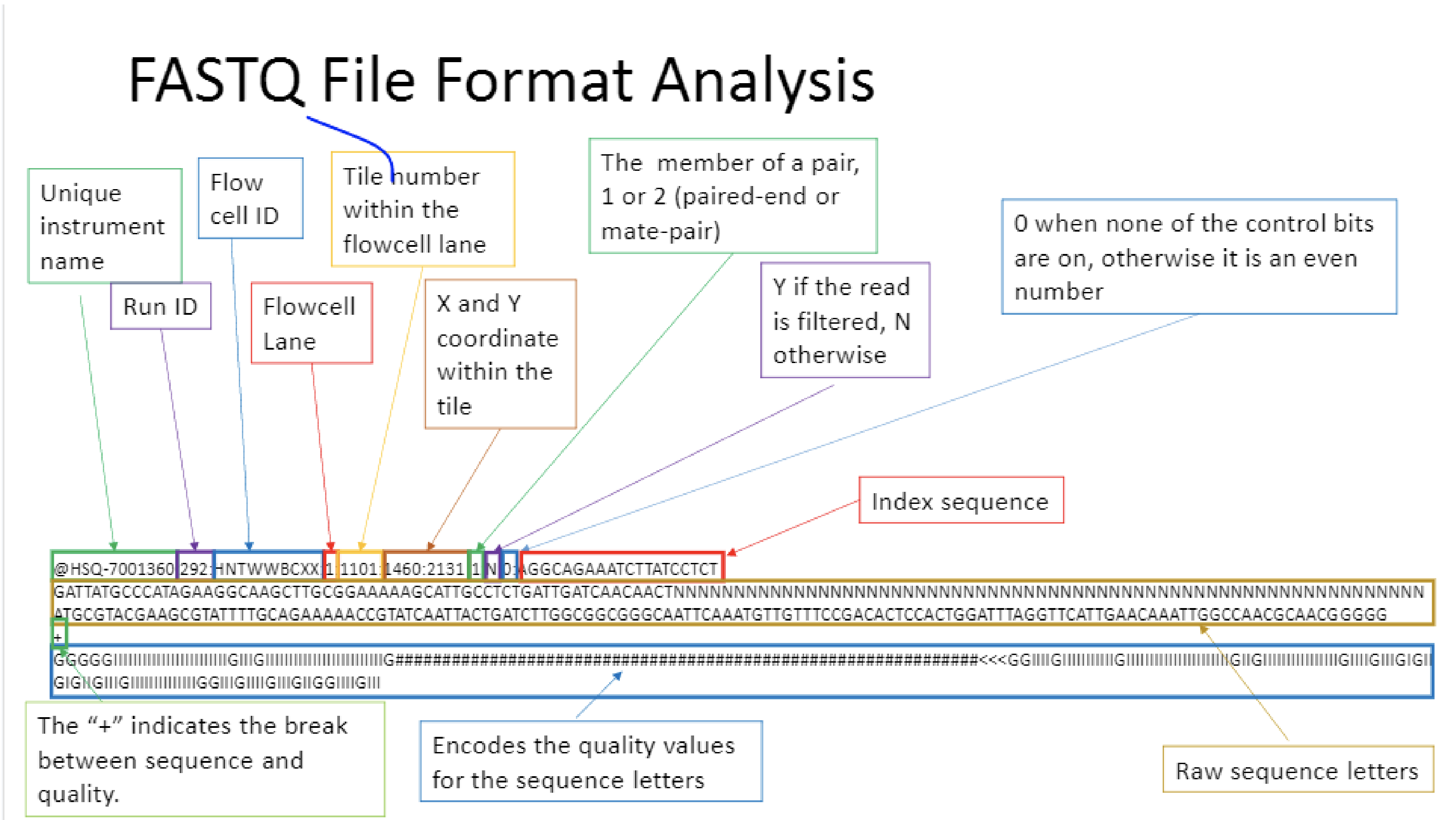




For transcriptome: 2x 75 bp
For whole genome: 2x 150 bp

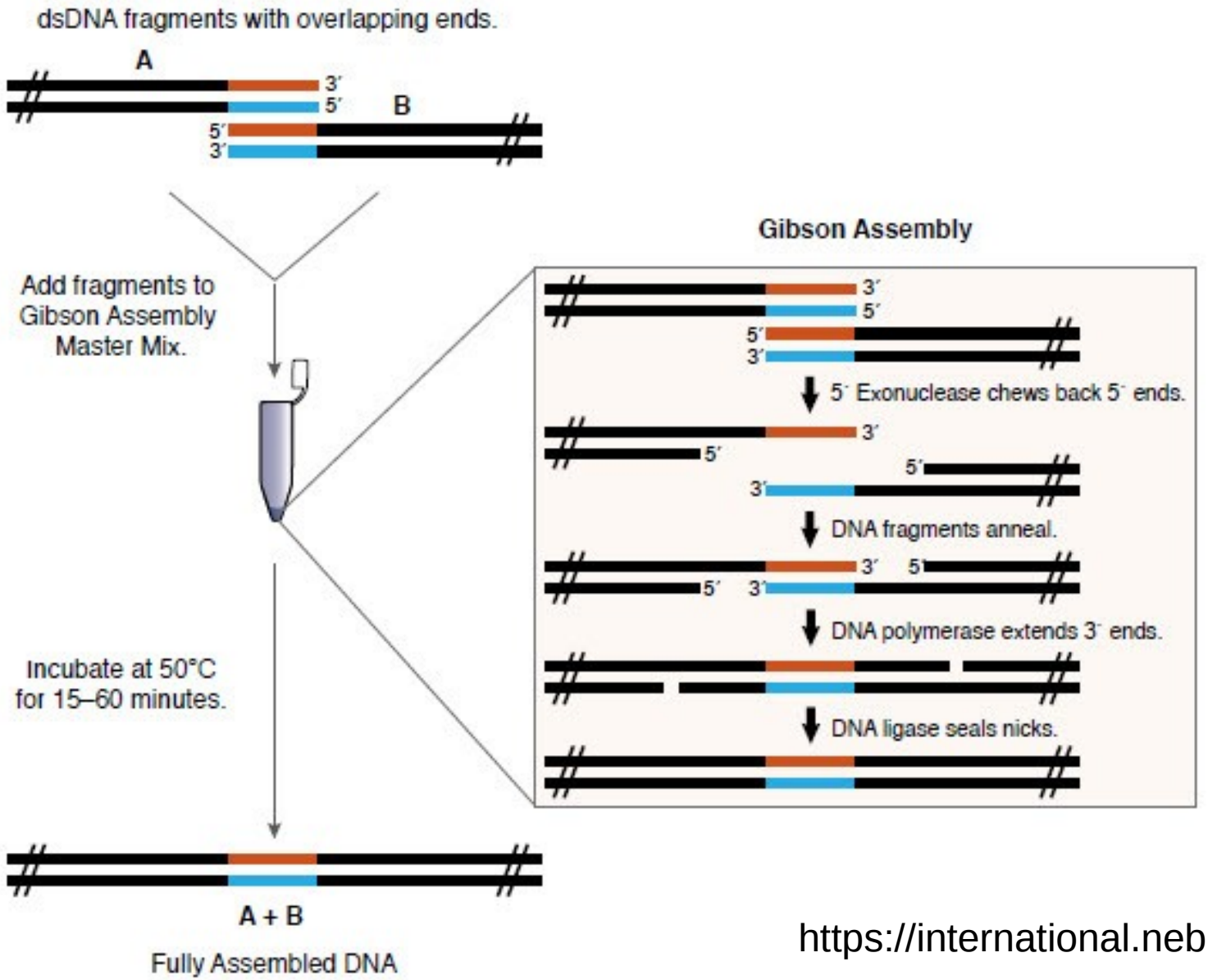
https://www.illumina.com/science/technology/next-generation-sequencing/sequencing-technology/2-channel-sbs.html

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

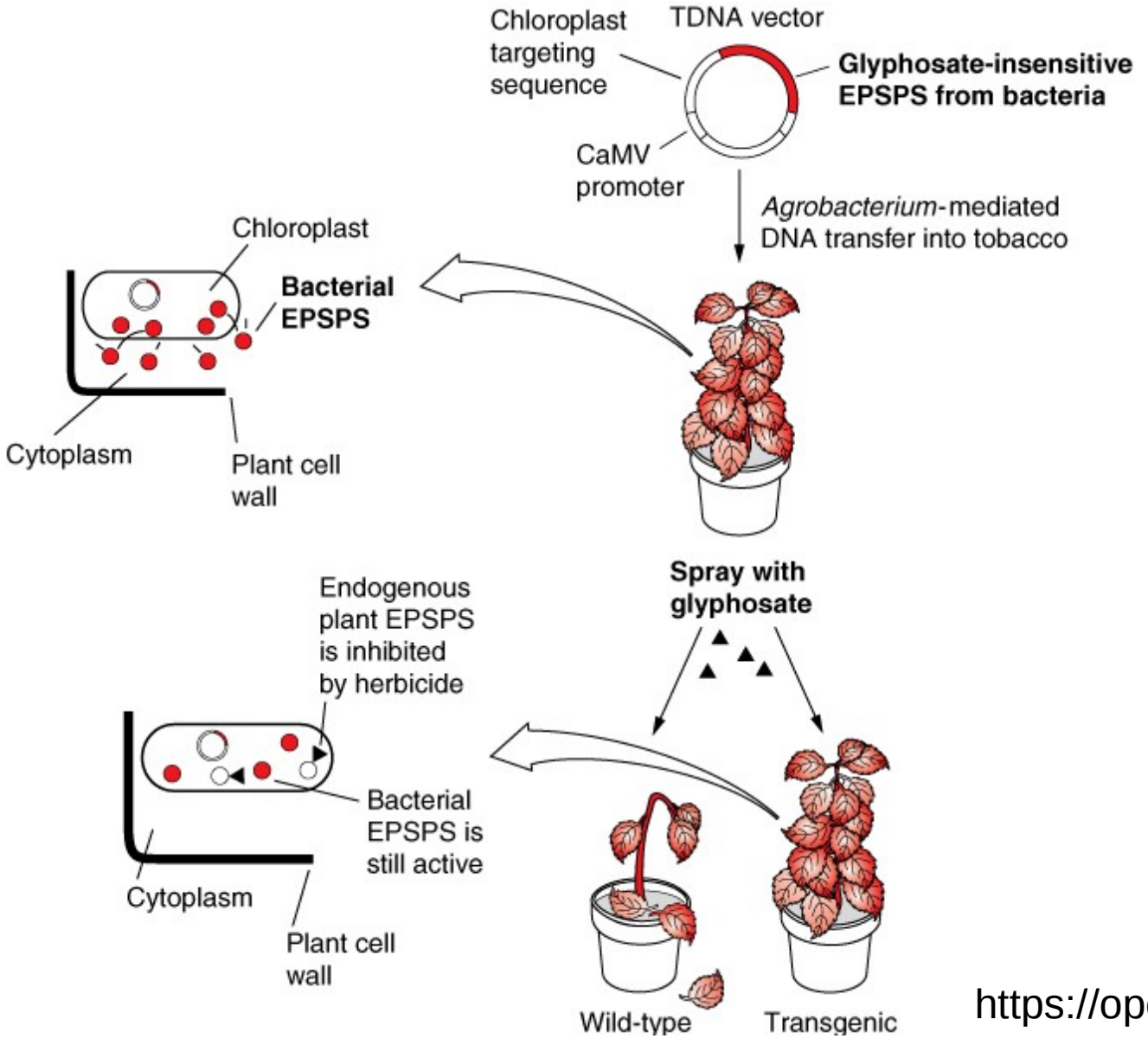
Gene Synthesis Service Options

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

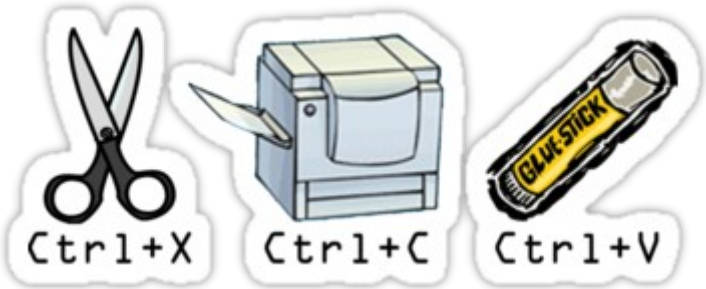
Introduce foreign DNA

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

In tobacco:



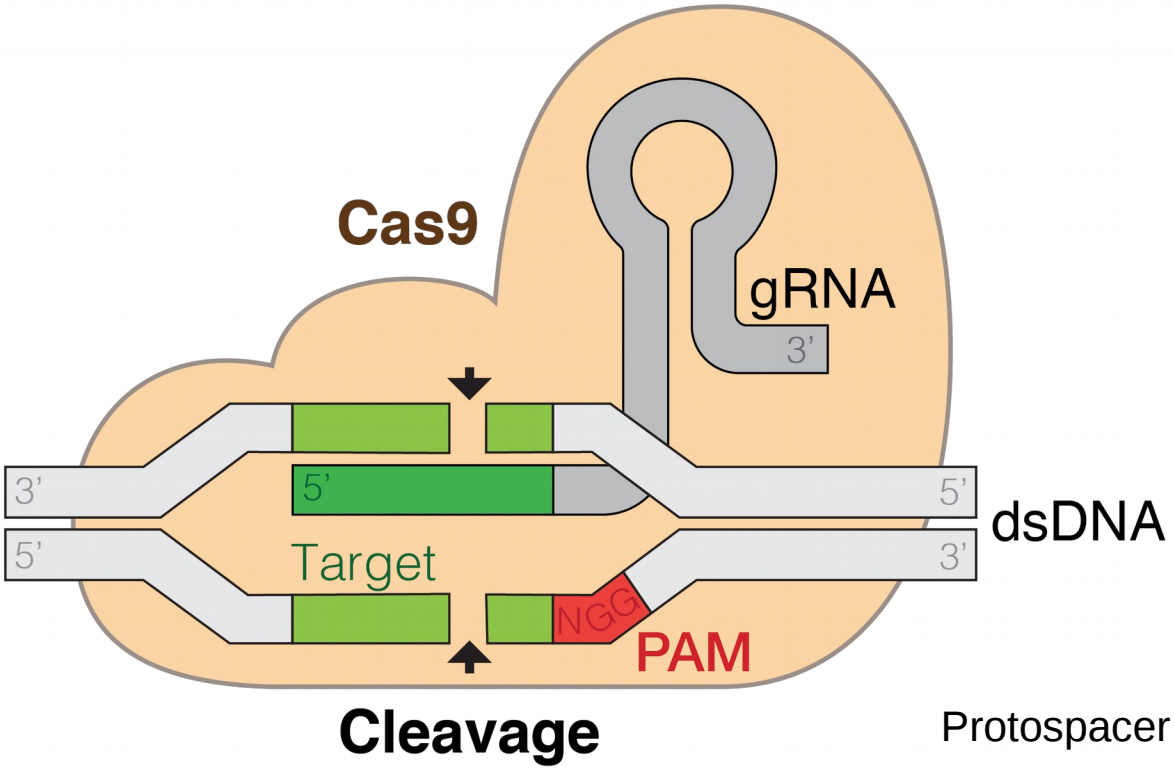
https://openwetware.org



CRISPR

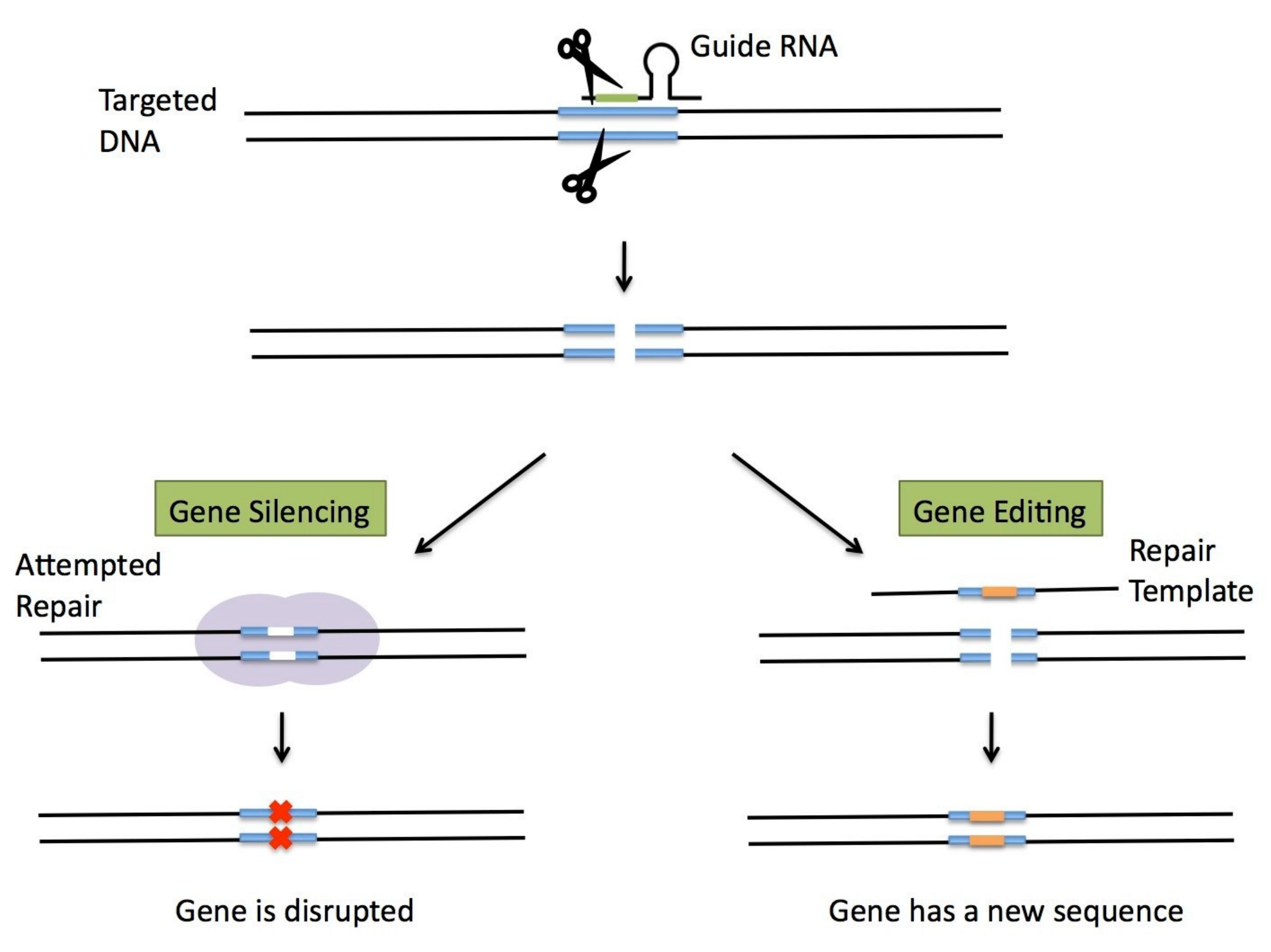
Marion Montagne

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

dorsal *ventral*

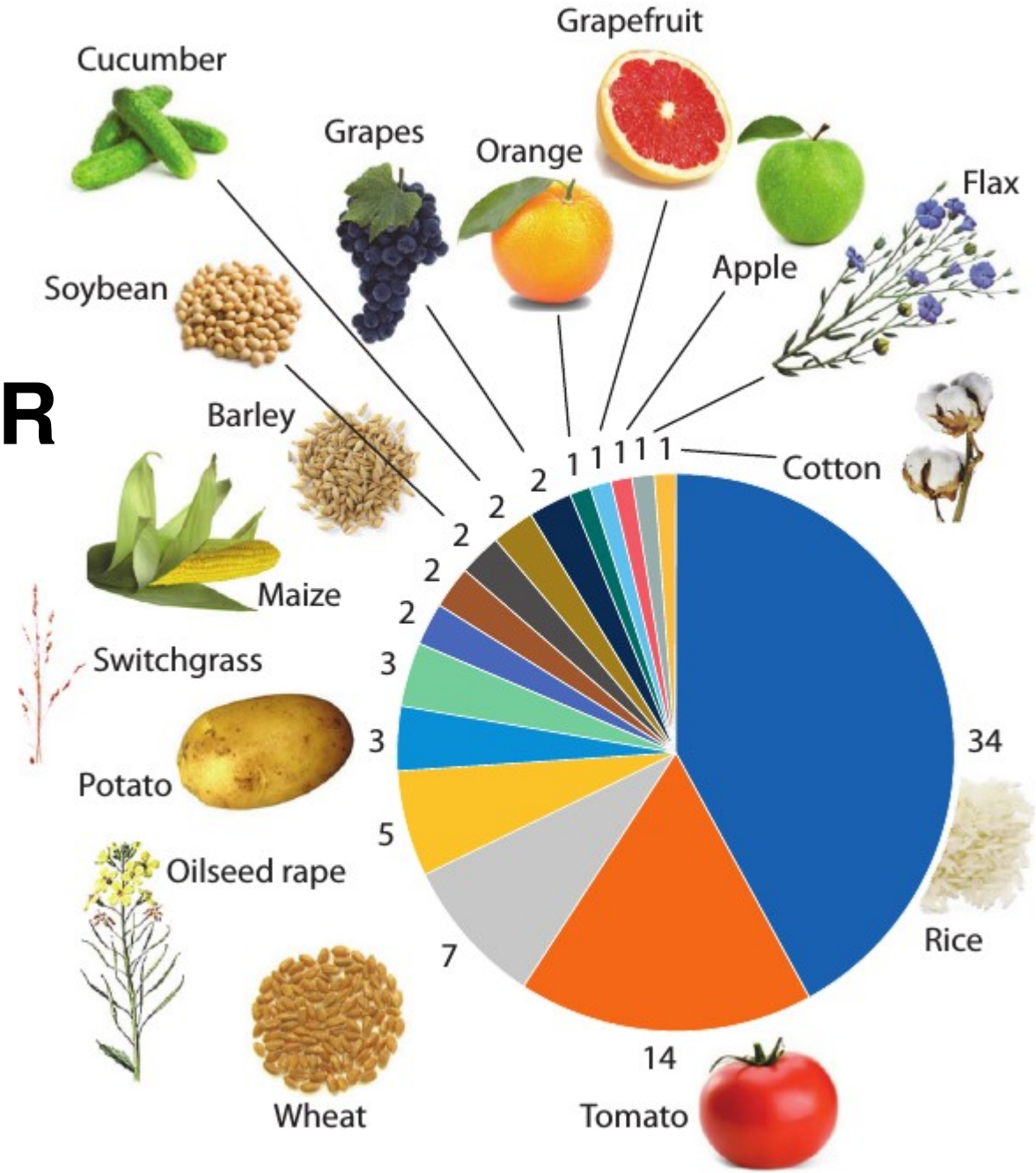
Wild-type **mutant *optics* CRISPR** **Wild-type** **mutant *optics* CRISPR**

Reed et al. 2017

normal

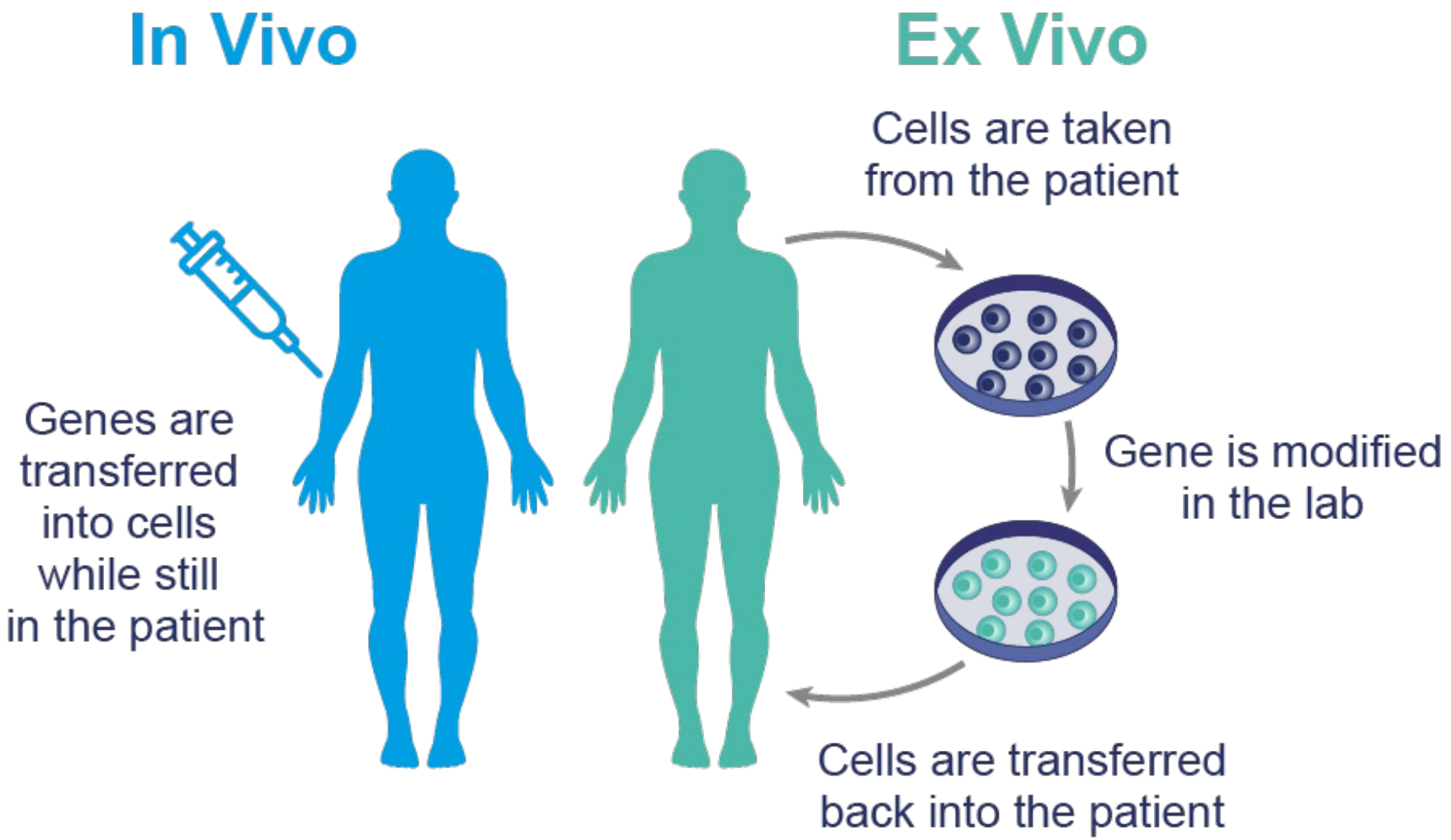
mutant
***WntA* CRISPR**

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

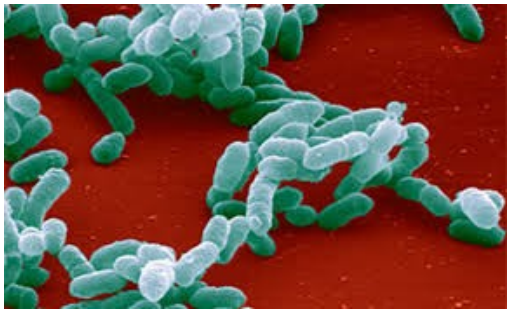
Modify
Cut
Ligate
Recombine fragments
Introduce foreign DNA
Mutate

Fundamental research is important

bacteria *Thermus aquaticus*

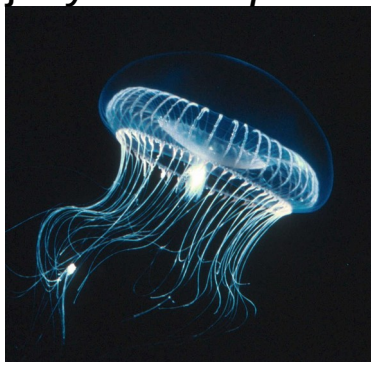
1969 → Taq-polymerase to amplify DNA

bacteria *Haemophilus influenzae*



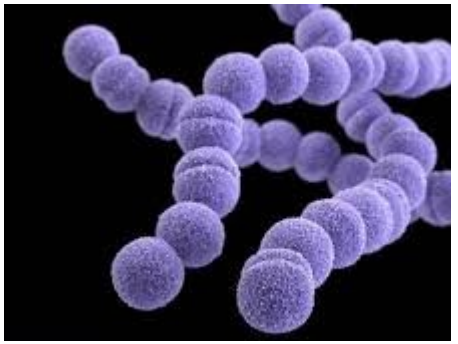
1970 → Restriction enzymes To cut DNA

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 ?

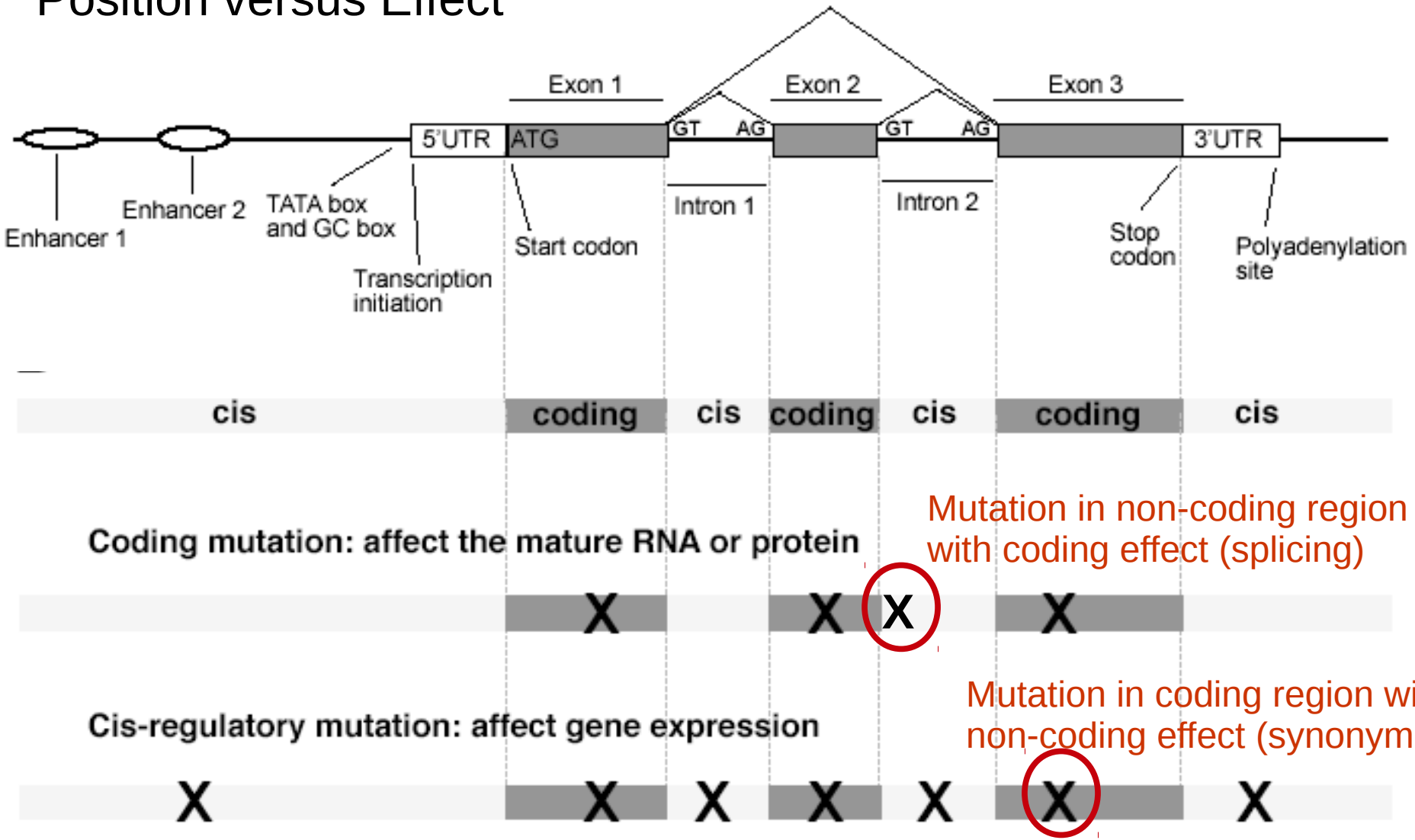
Aberration Types

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

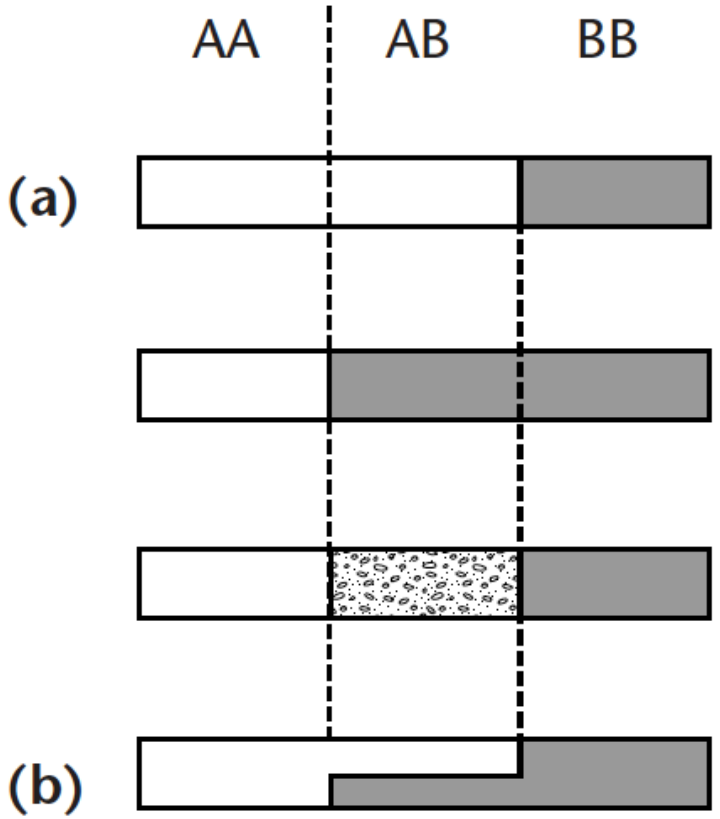
Position versus Effect



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

Levels of activity of the various alleles

- **Null**: no activity, equivalent to gene deletion
- **Hypomorph ou loss-of-function**: reduced activity
- **Silent**: no influence on the phenotype
- **Hypermorph or gain-of-function**: increased activity
- **Neomorph**: new activity, can be ectopic expression

Relationship with dominance

- Dominant (or semi-dominant) null allele:
  haplo-insufficiency – dose effect
  - Dominant-negative via a poison effect
  - Dominant gain-of-function/neomorph: common when gain-of-function, also with neomorph

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

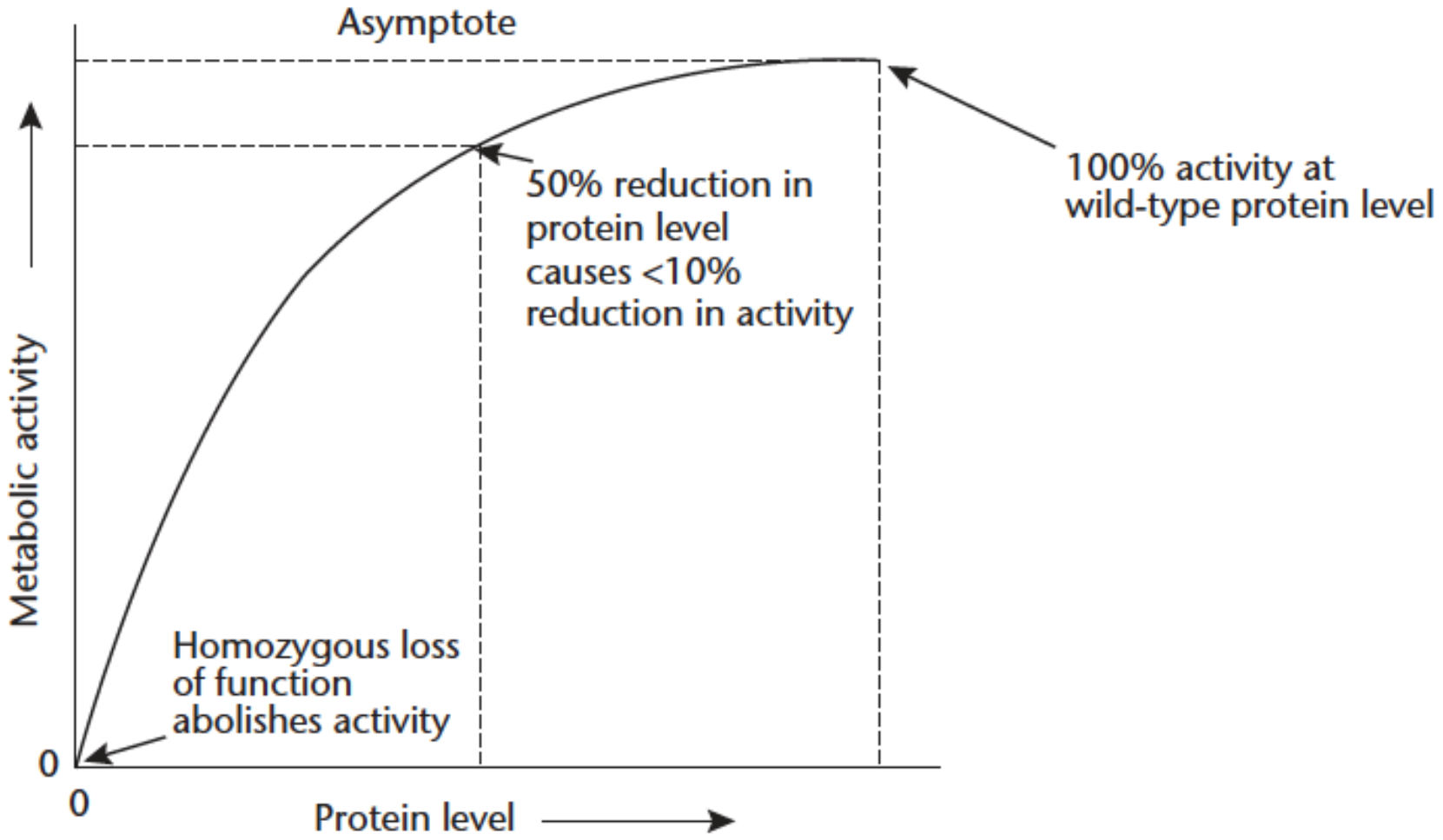
as in "dominance of *a1* over *a2* for a particular trait"

Ex:

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

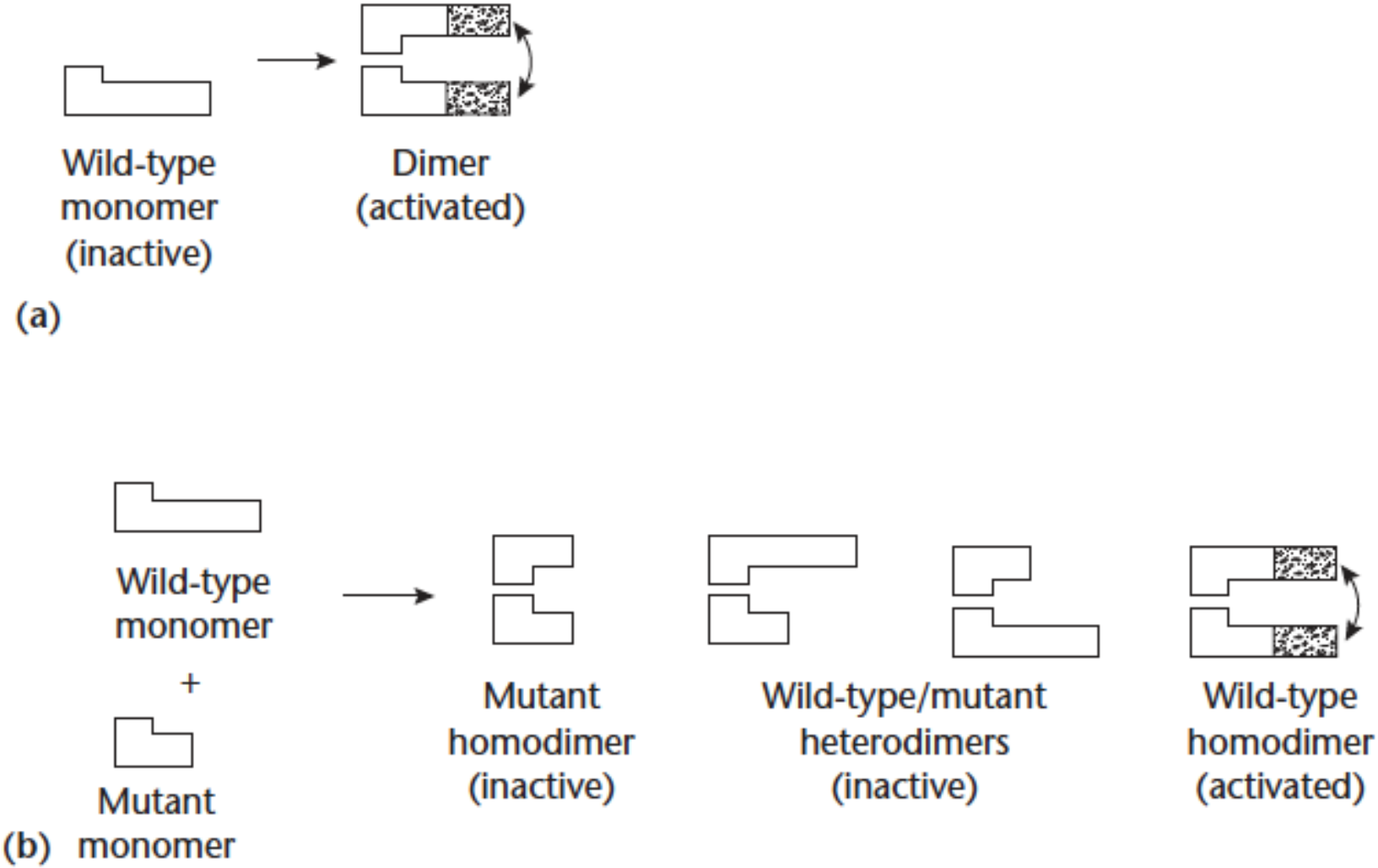
Most wild alleles are dominant

Robustness to half-dose:



Wilkie 2005

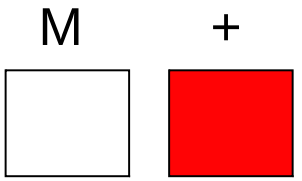
One type of dominant-negative mutation: sequestration of wild-type in dimer



Wilkie 2005

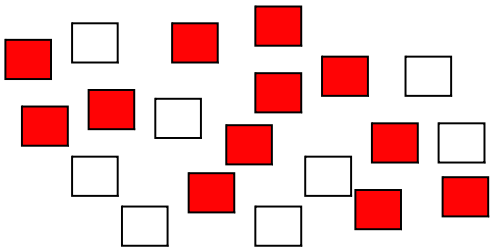
Penetrance

Discrete binary phenotype



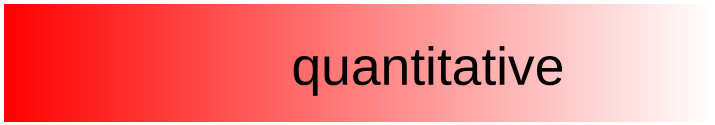
% of individuals showing the phenotype

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



Expressivity

Phenotype with different degrees of severity

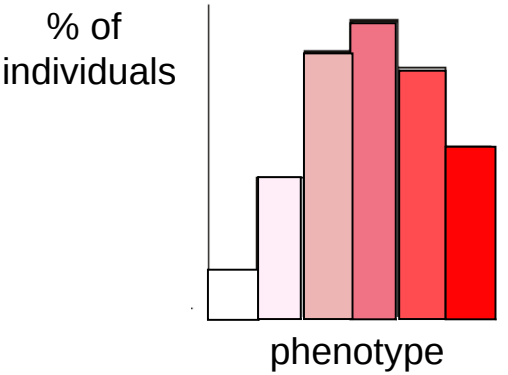


or

Degree of severity of the phenotype

ex: - number of affected ommatidia
- light pink color

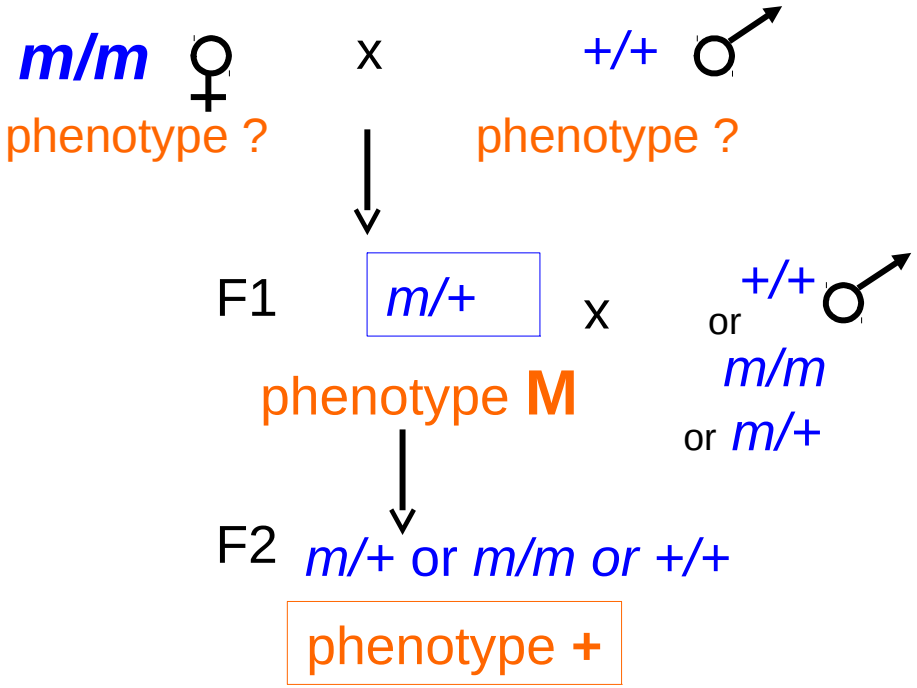
often shown as a distribution of phenotypic values of individuals:

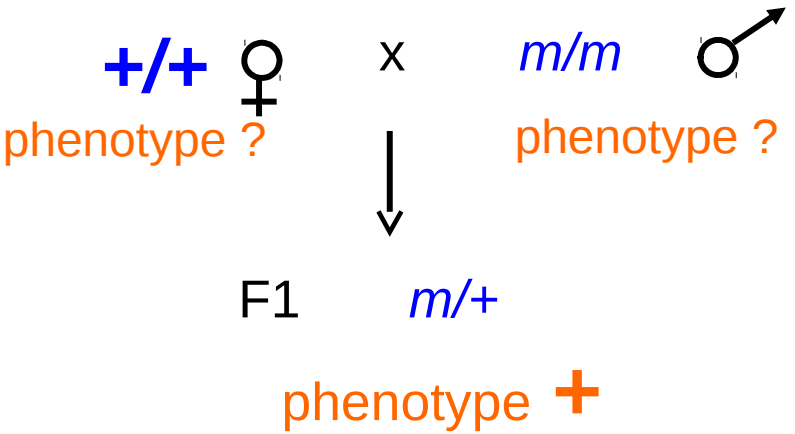


Maternal (or paternal) effect

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

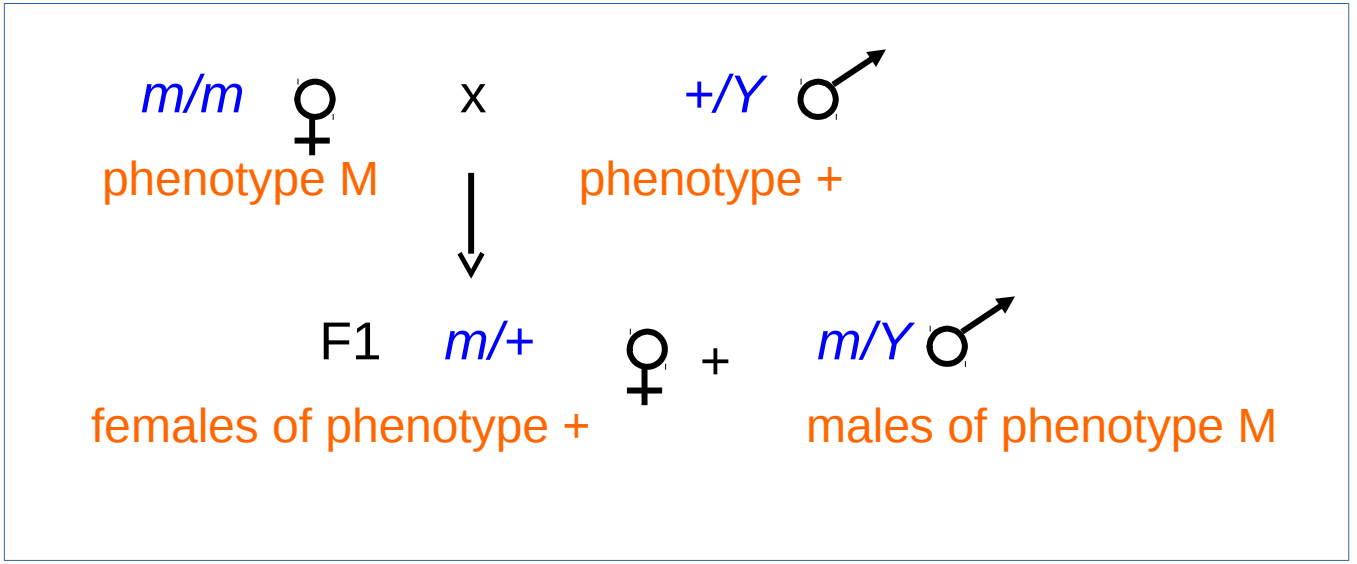
Frequent for mutations affecting early embryonic development





Heredity with sex-linked transmission

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



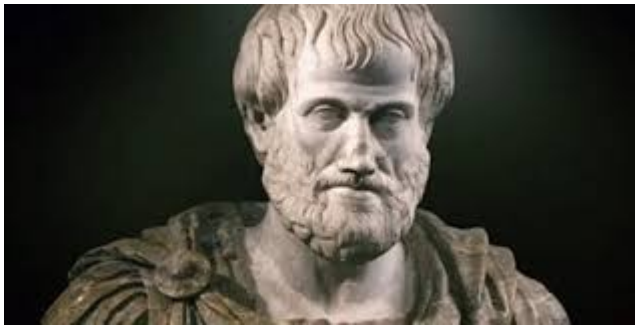
Heredity with sex-linked expression

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

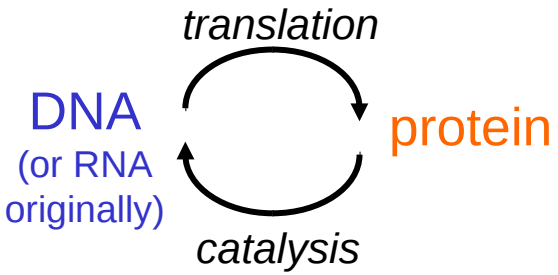
= what engenders = what is apparent

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

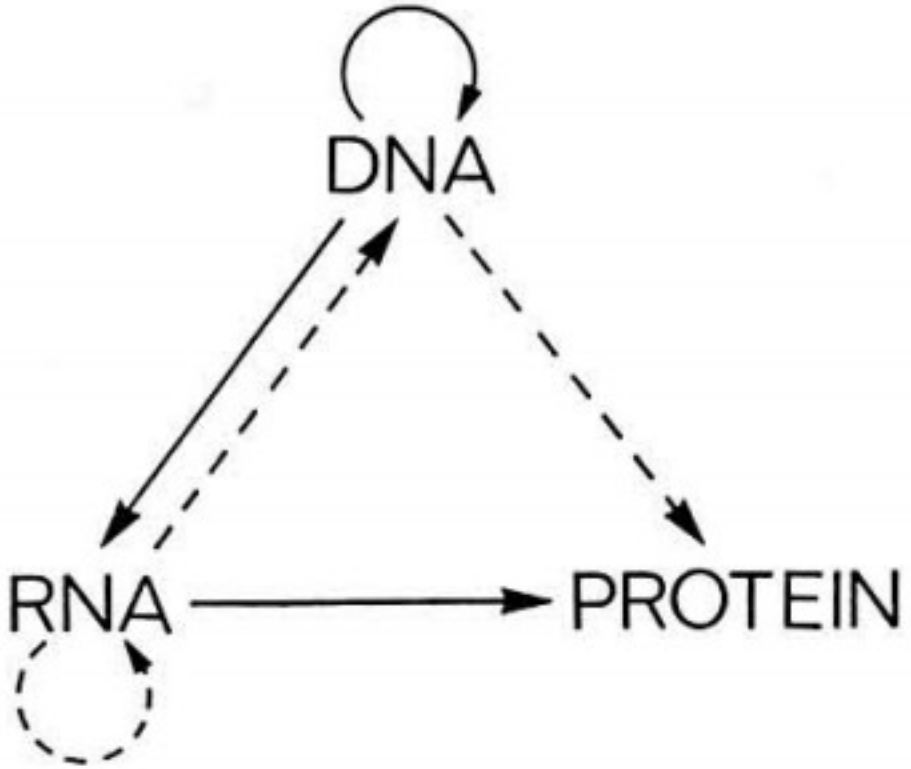
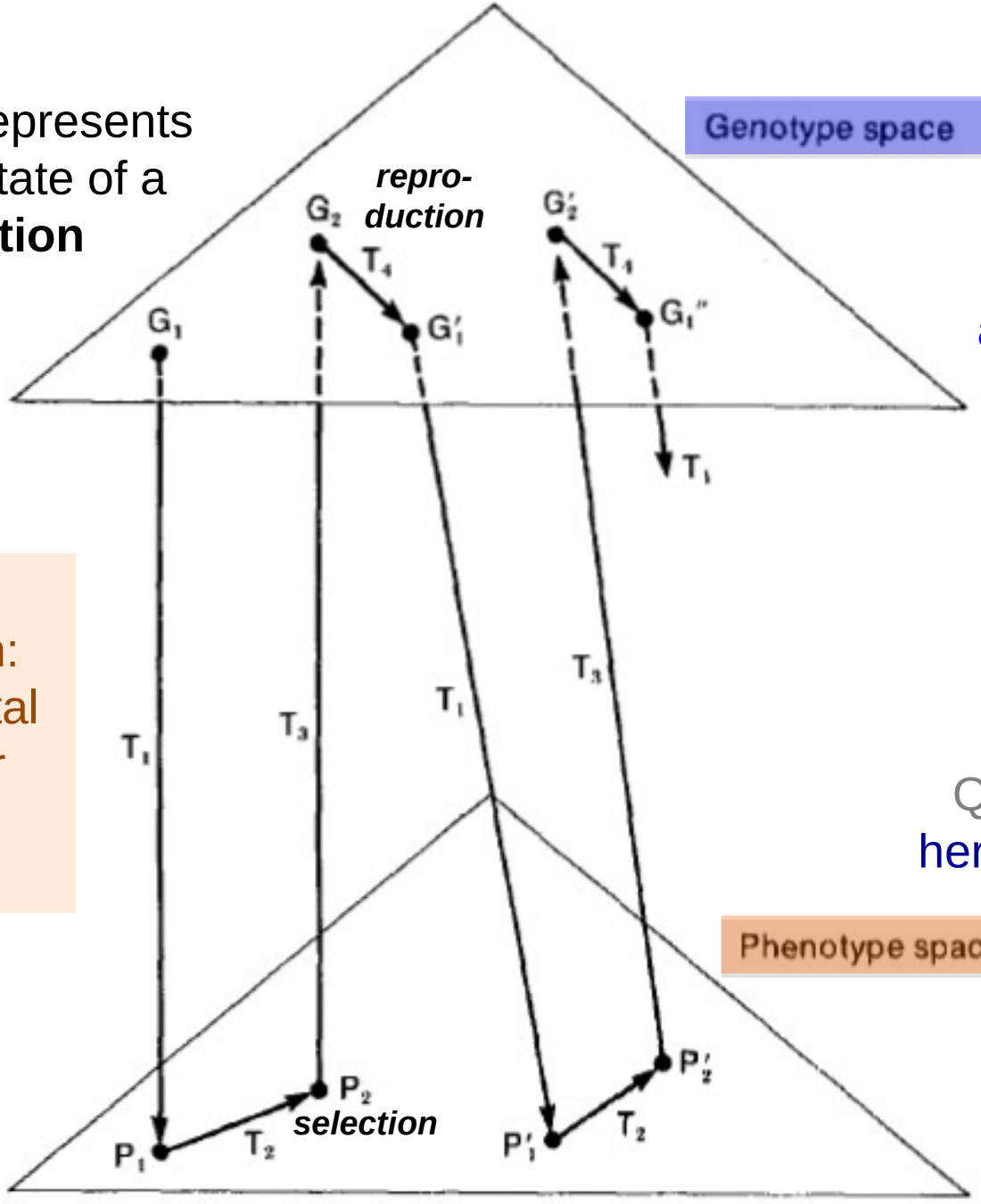


Fig. 3. A tentative classification for the present day. Solid arrows show general transfers; dotted arrows show special transfers. Again, the absent arrows are the undetected transfers specified by the central dogma.

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

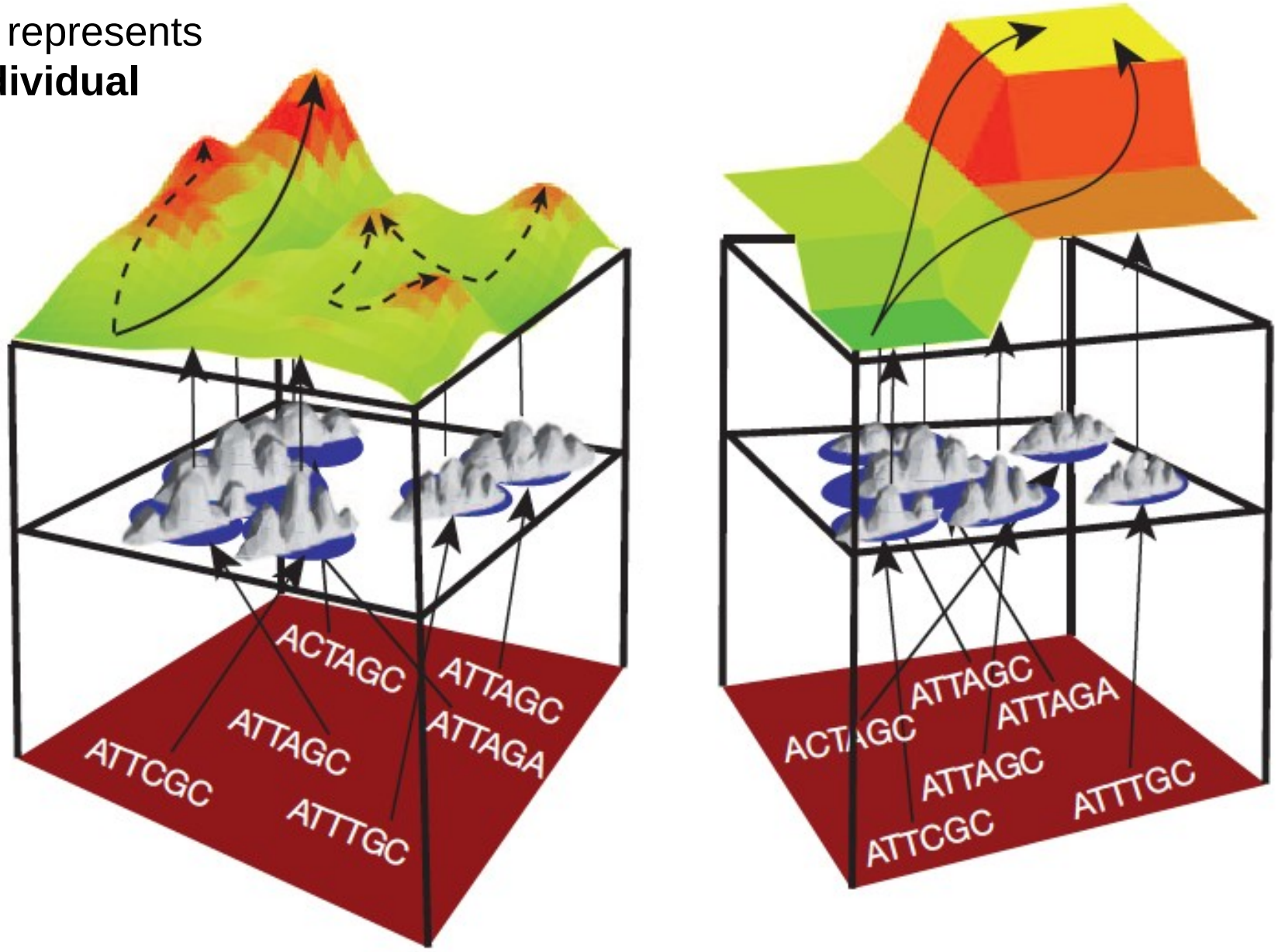
The genotype-phenotype-fitness map

Here a dot represents one **individual**

Fitness

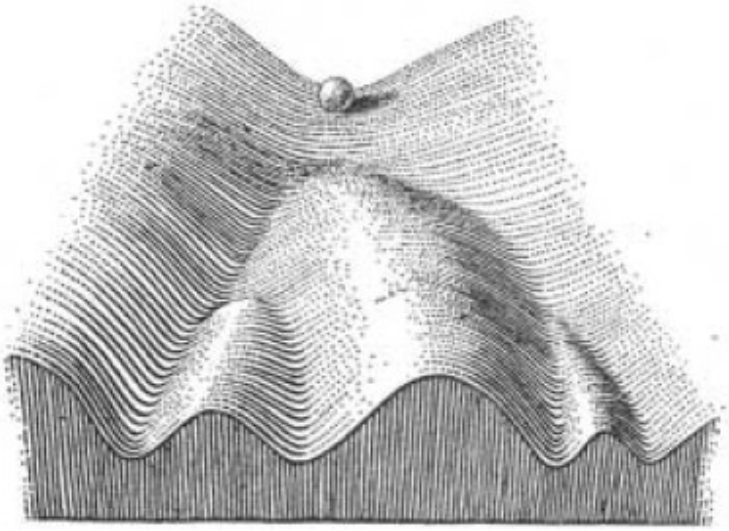
Phenotype
Tooth morphology

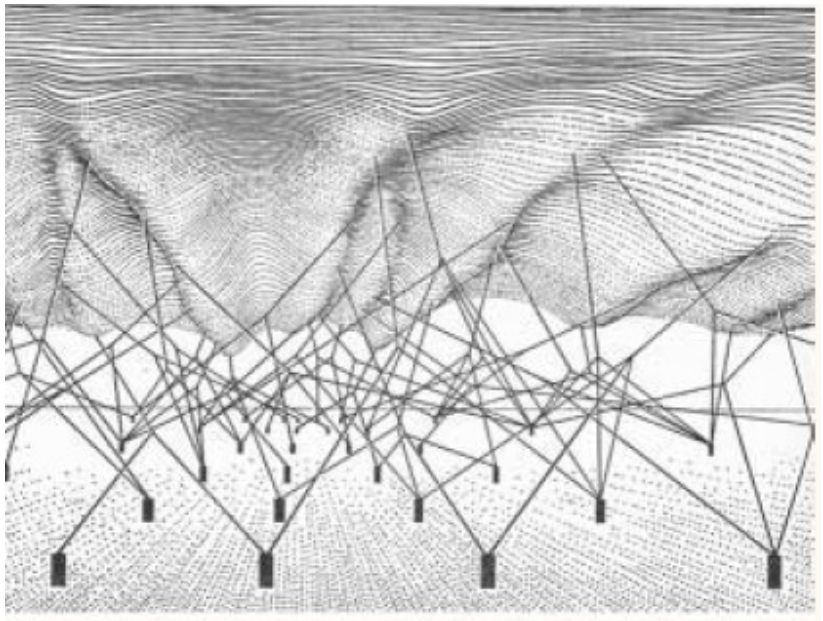
Genotype



Salazar-Cuidad & Martin-Riera 2013

The Epigenetic Landscape
A metaphor for the G-P relationship



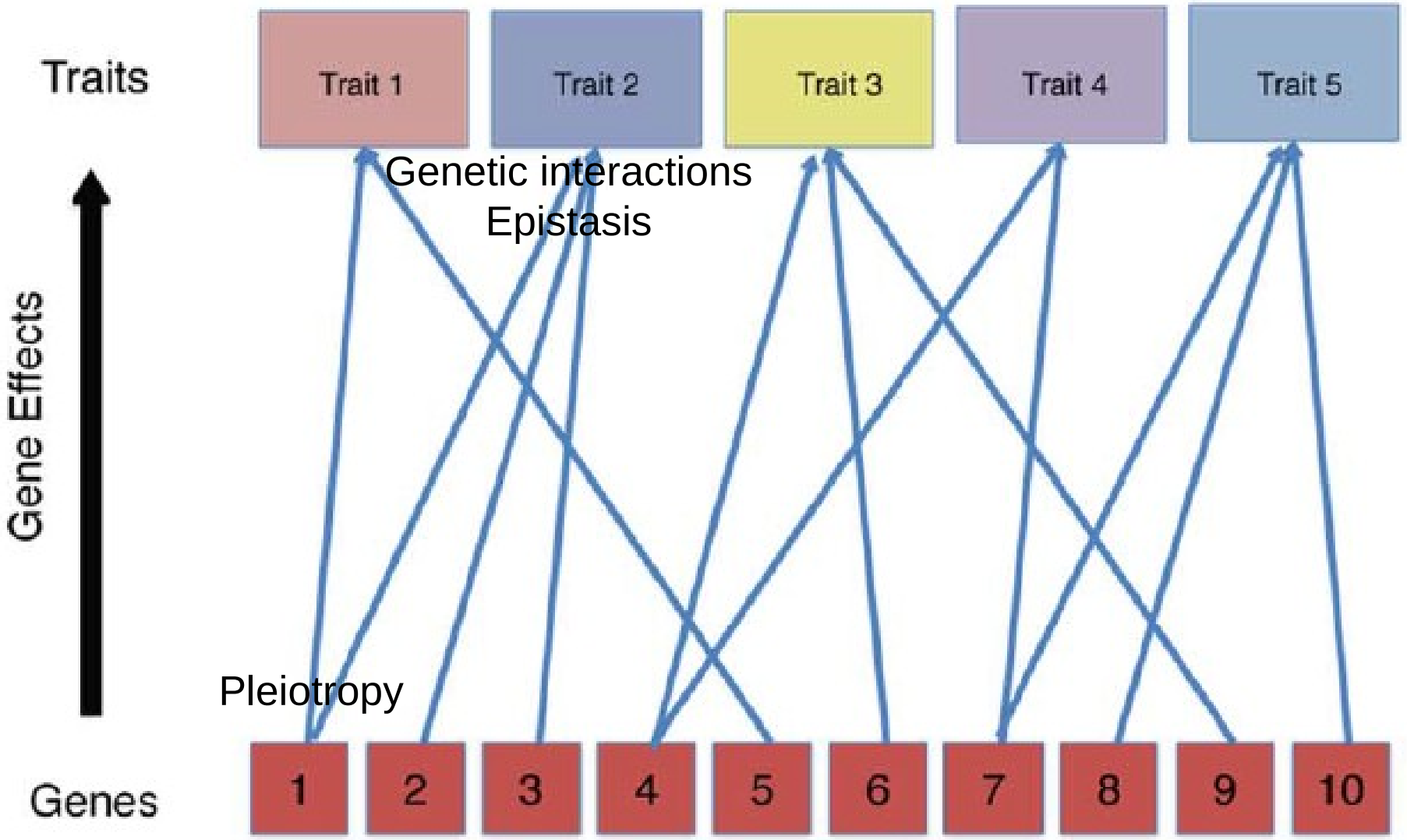


Development

Canalization

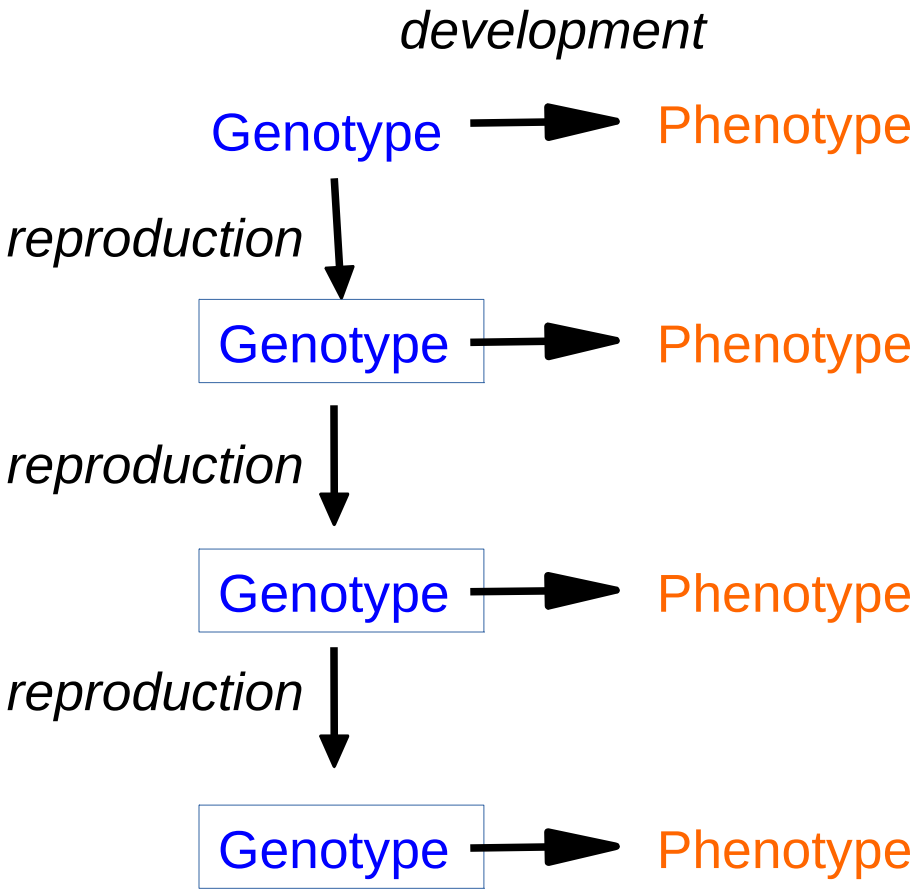
Genes underlying
the landscape

Waddington 1957



Hallgrimsson et al. 2014

A simplistic view



Heritable traits are not always due to genes

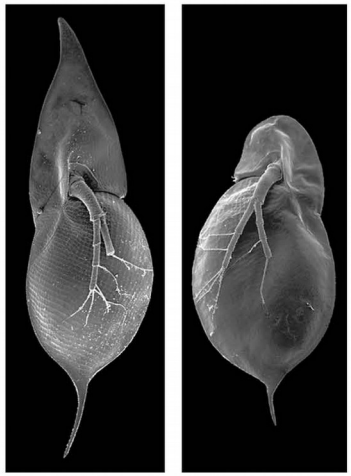
The genotype does not determine entirely the phenotype

The genotype cannot replicate by itself

Genotype and phenotype imply variation

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

Next time: bring your laptop!