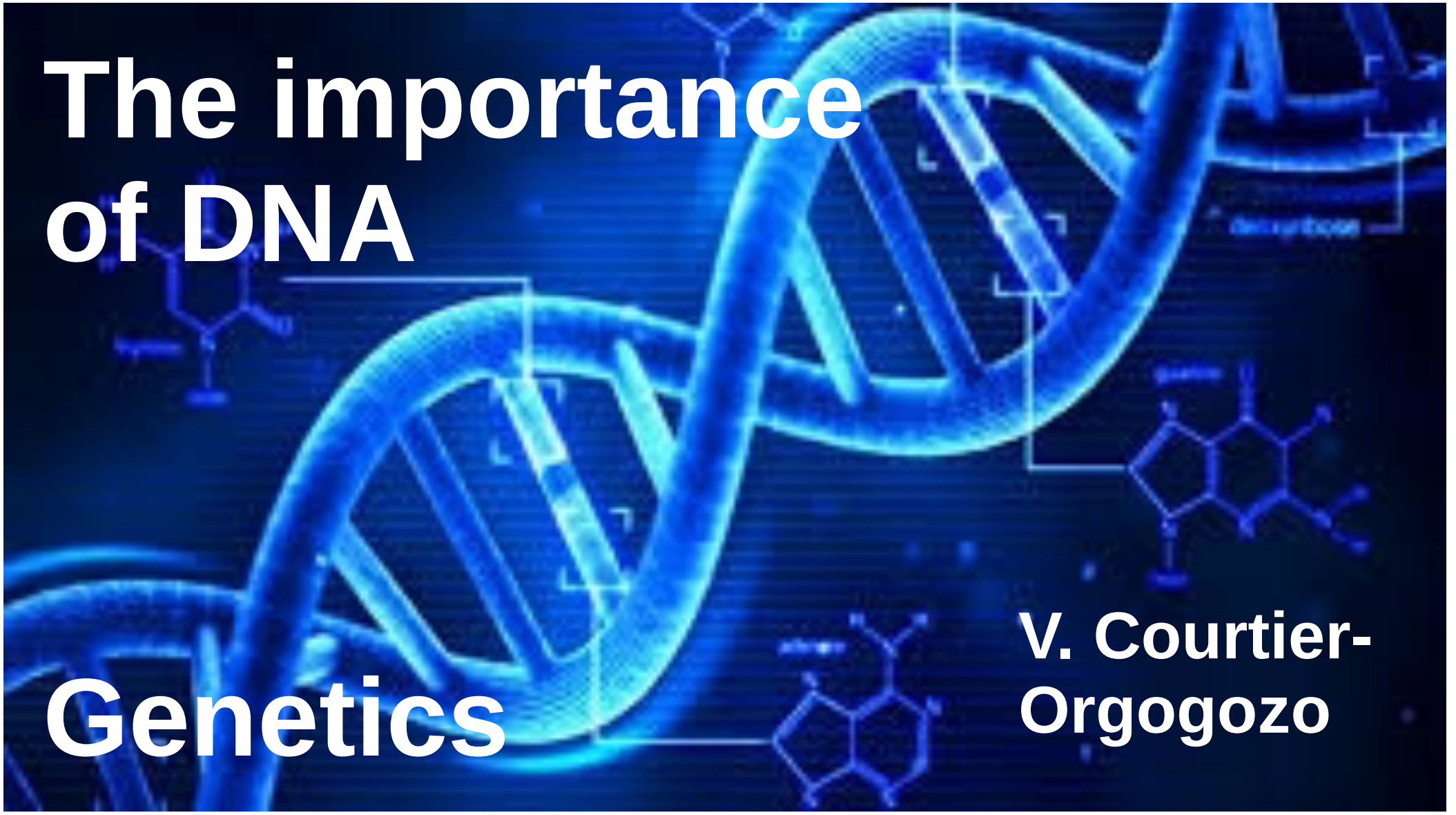
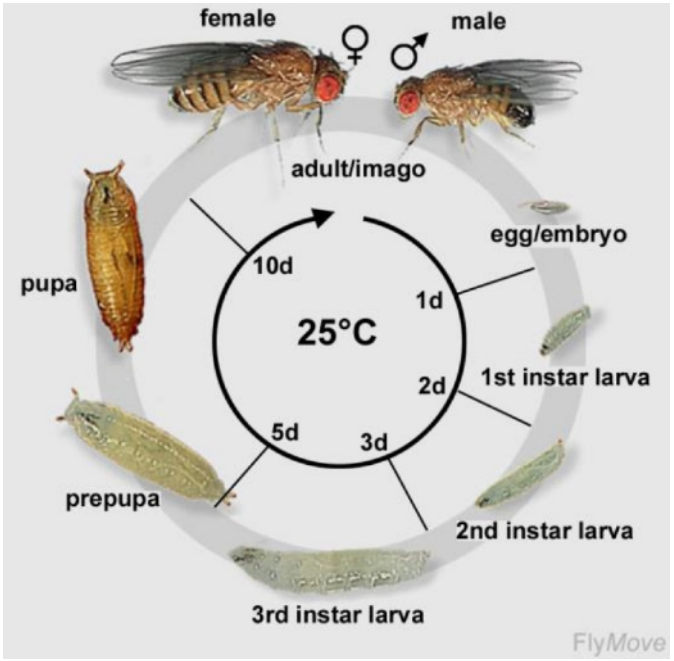
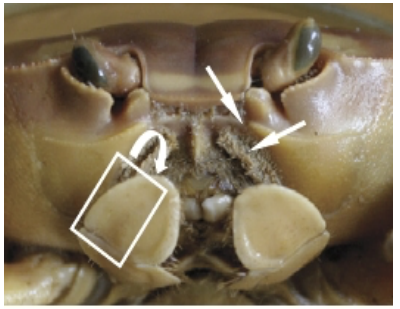

The importance of DNA
Genetics
V. Courtier-Orgogozo

My research: Drosophila glue



crab



soil

D. grimshawi

rotten strawberry

D. suzukii

grape

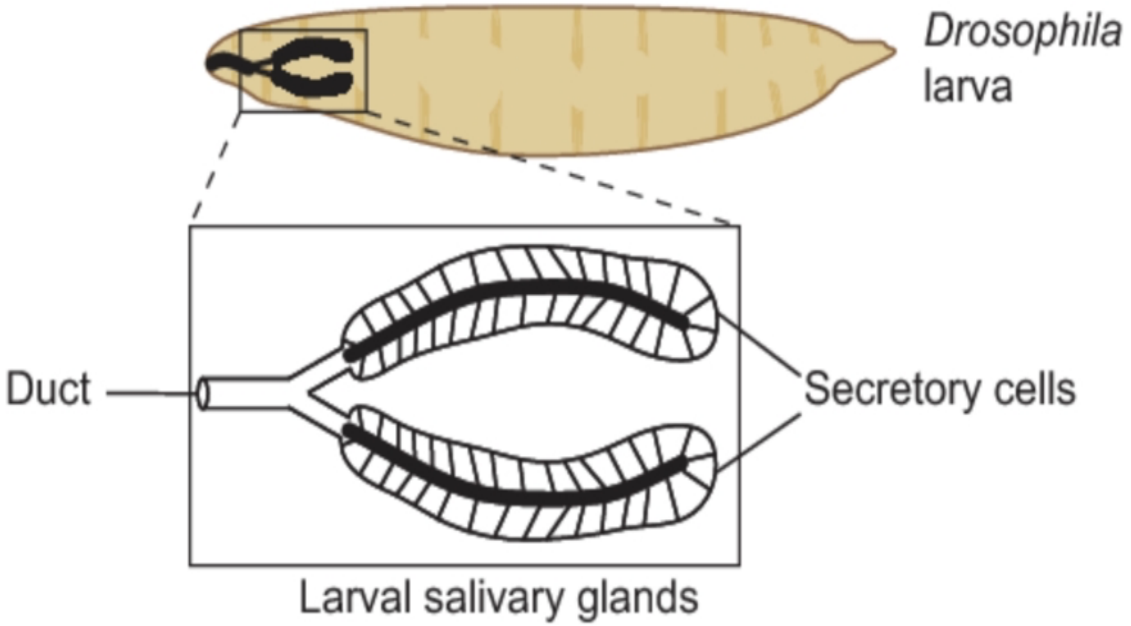
D. melanogaster

potato

D. repleta

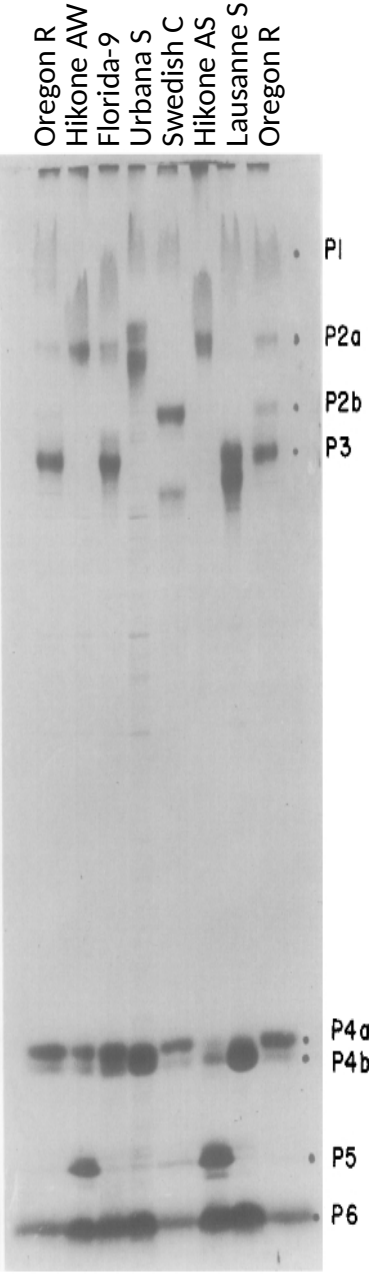
D. carcinophila

Glue proteins vary greatly between *D. melanogaster* strains



Tran & Hagen 2017

Extraction of salivary content
acrylamide gel
protein staining



Beckendorf & Kafatos 1976

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)

A model & to Develop New Bioadhesives

to Understand Adaptation

Genes mediating interactions with the environment

Immunity
Stress response
Reproduction

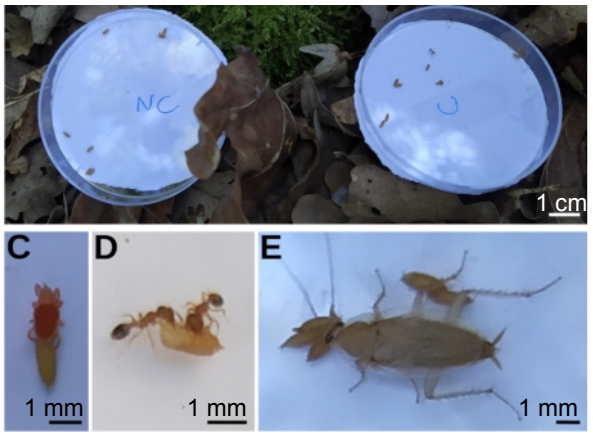
Rapidly evolving

Most studied bioadhesive
Mussel glue (water)

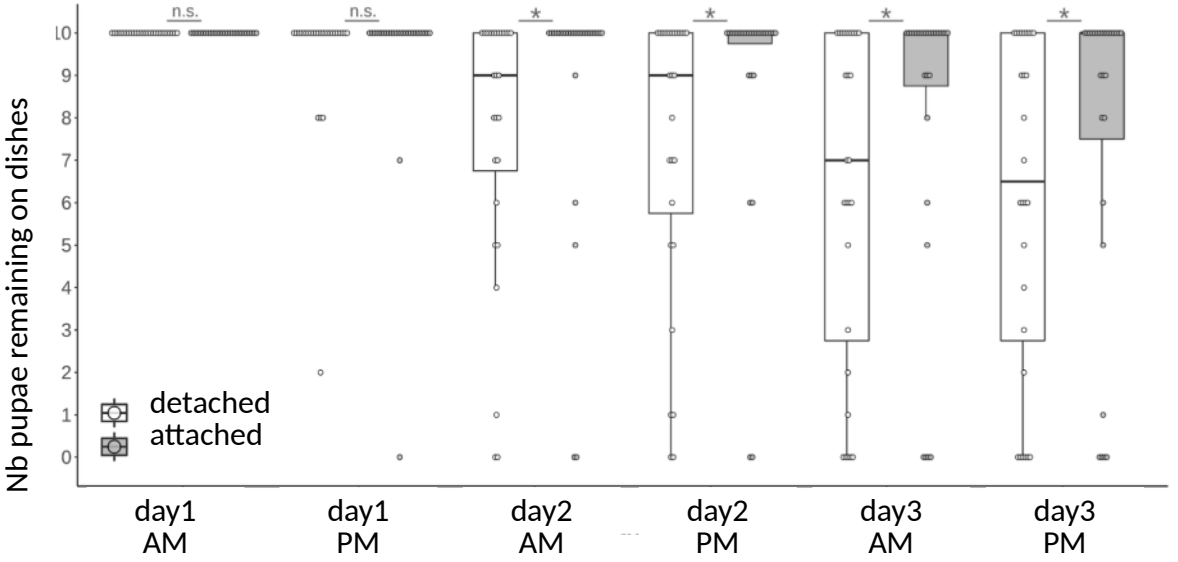
Function of the glue?
How adhesive is the glue?
What makes it adhesive?

In forest: detached pupae are gone more rapidly

detached attached



In Bois de Vincennes (Paris)
In each bucket (n=28):
one Petri dish w/ 10 detached pupae
one Petri dish w/ 10 attached pupae
Checked twice a day

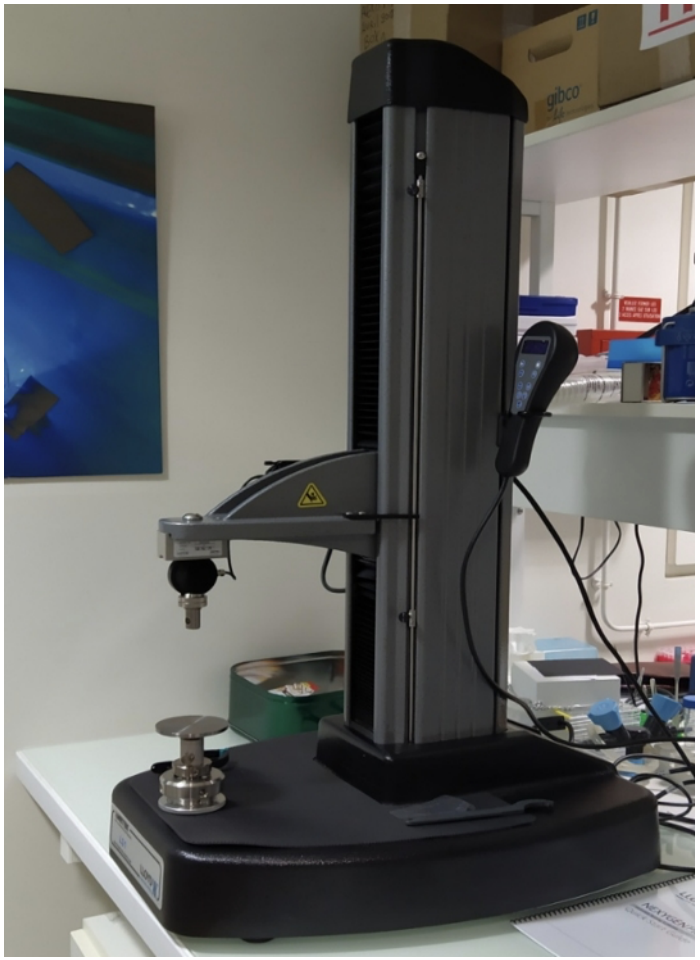


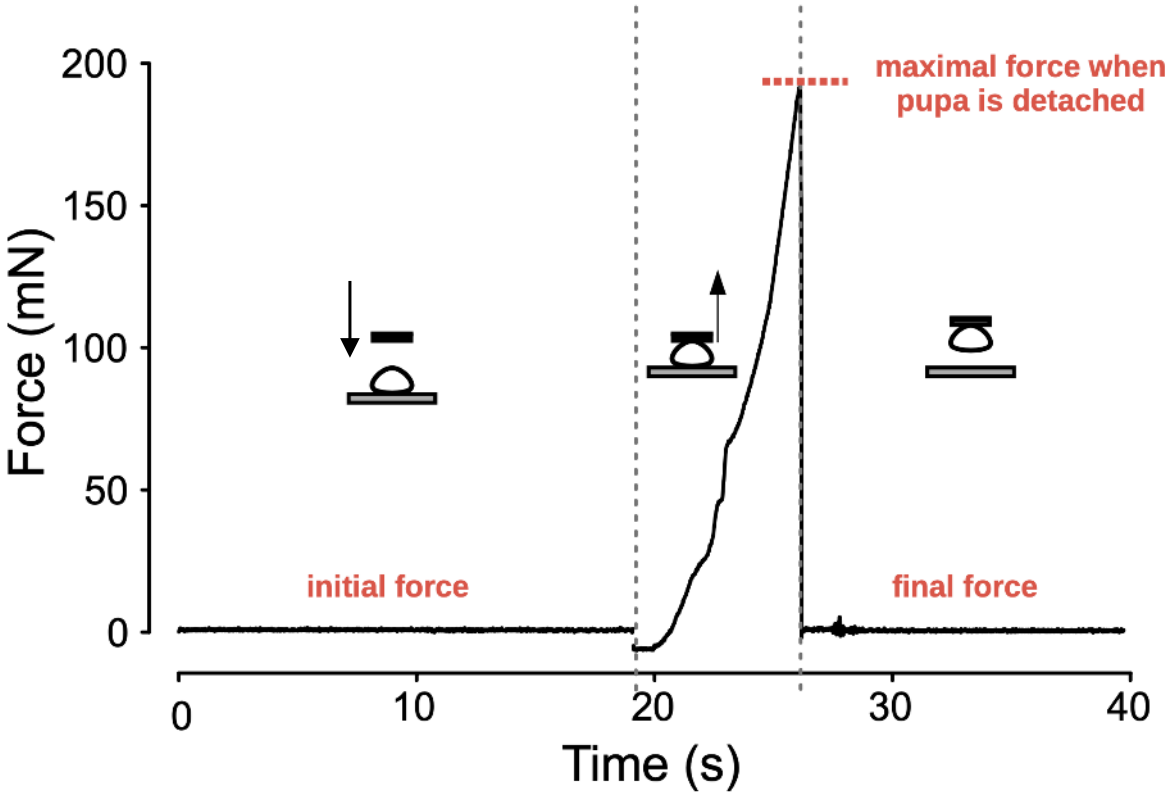
In the lab:

attached pupa

detached pupa

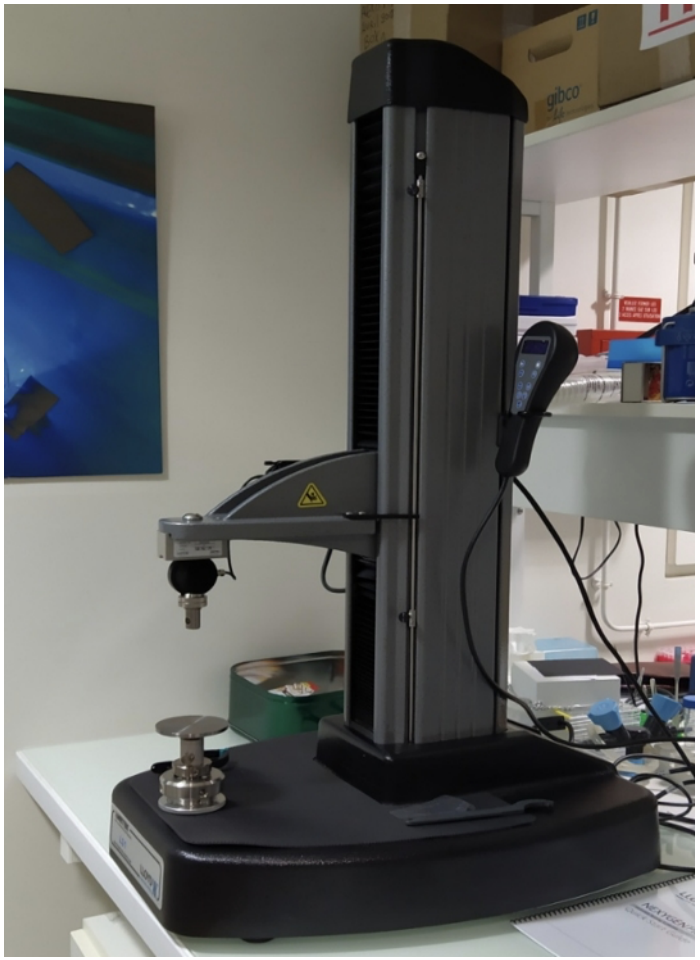
A new method to measure Drosophila glue adhesion





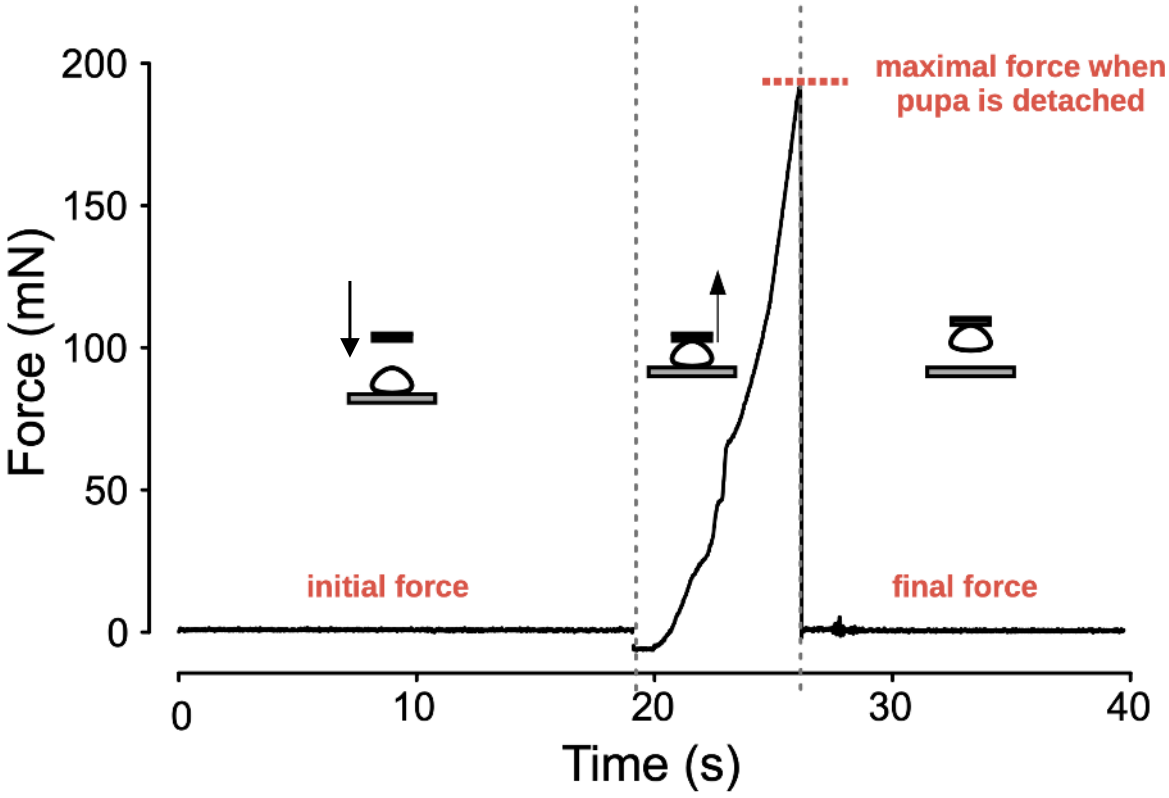
Borne et al 2020 JEB

D. melanogaster glue sticks as strongly as our most adhesive tapes



100-300kPa

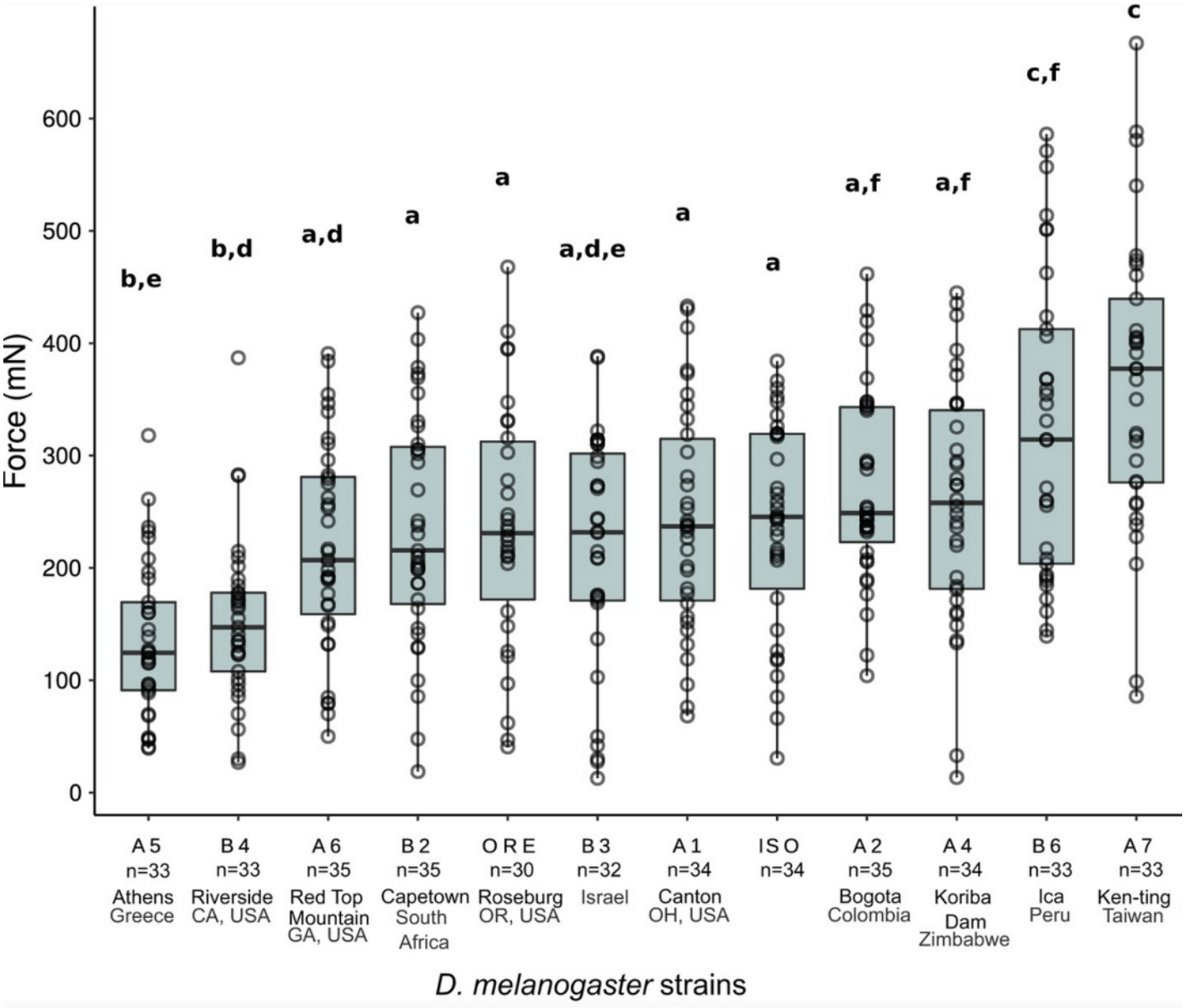
10MPa



Borne et al 2020 JEB

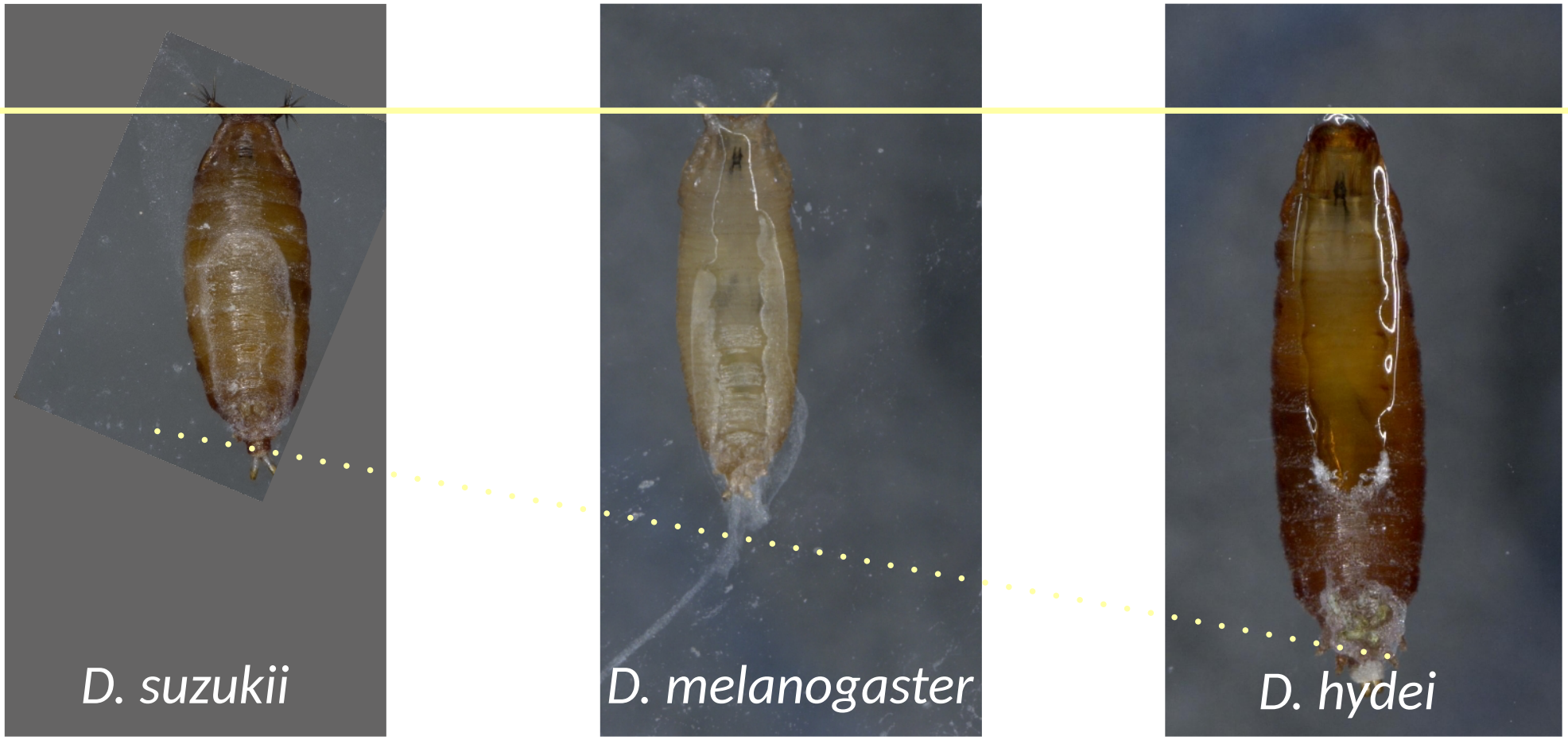
Adhesion force varies within species

D. melanogaster lines
DSPR



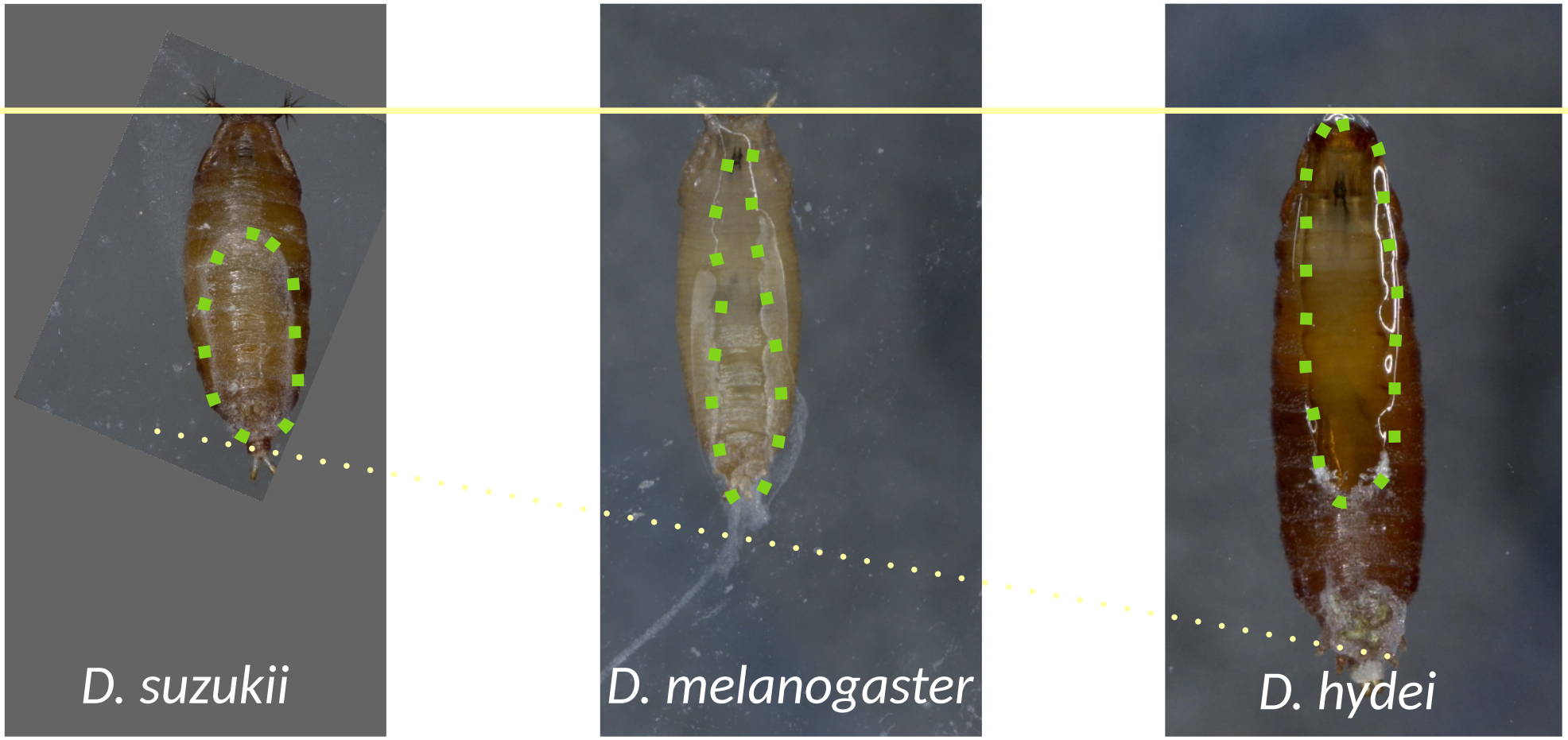
Borne et al. 2021 GBE

Pupa size and glue area vary between species



Monier et al. in revision

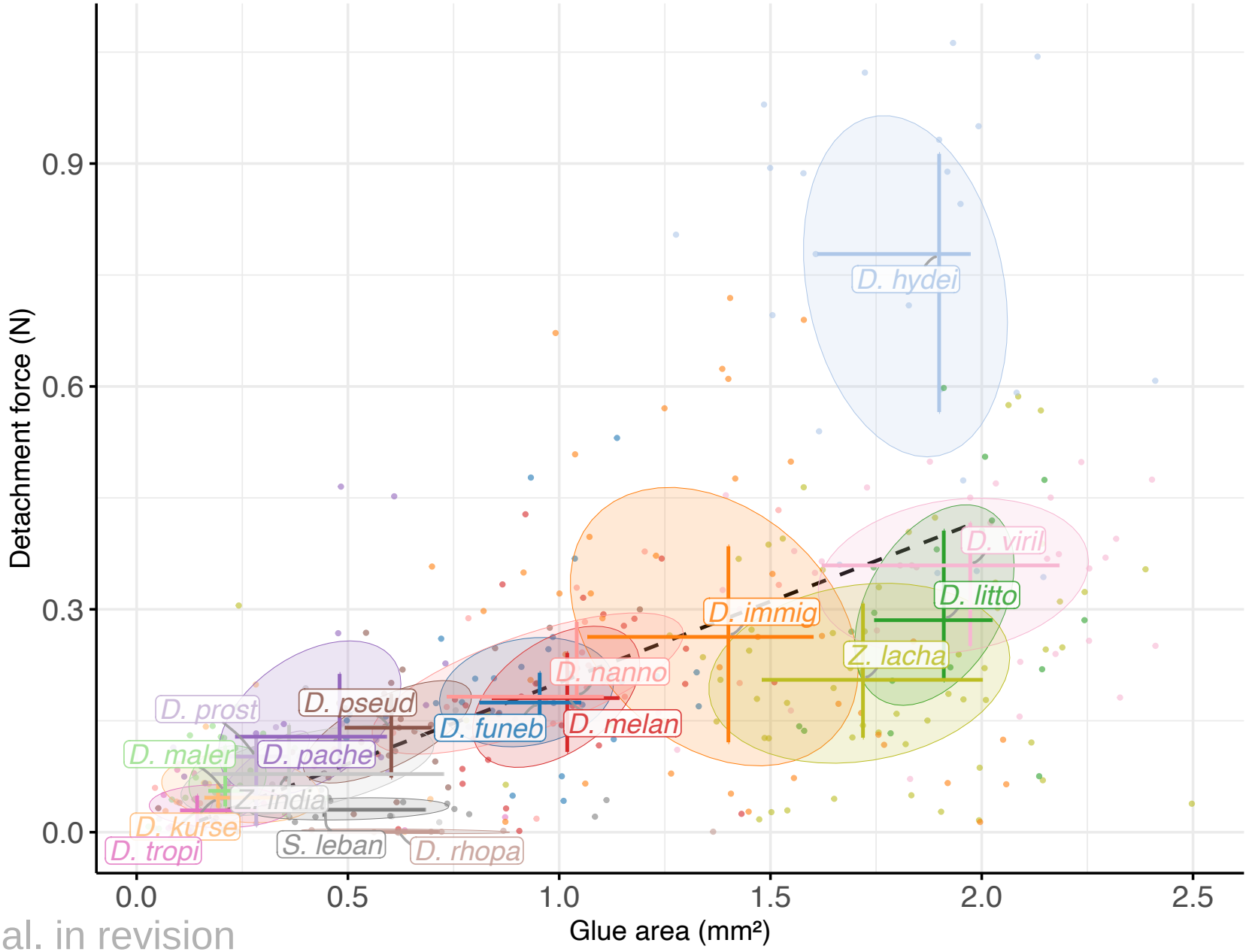
Pupa size and glue area vary between species



Monier et al. in revision

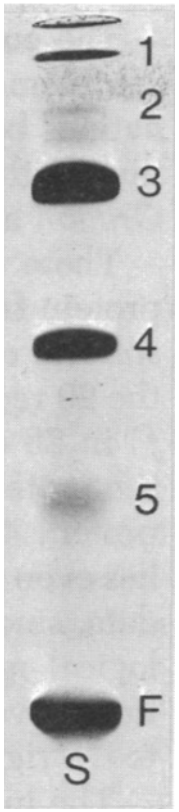
D. hydei has the highest adhesion strength

Adhesion strength = detachment force / glue area



Monier et al. in revision

D. melanogaster glue is composed of 8 proteins



Korge 1975

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

Long, repeats rich in Ser, Thr, Pro

Disordered

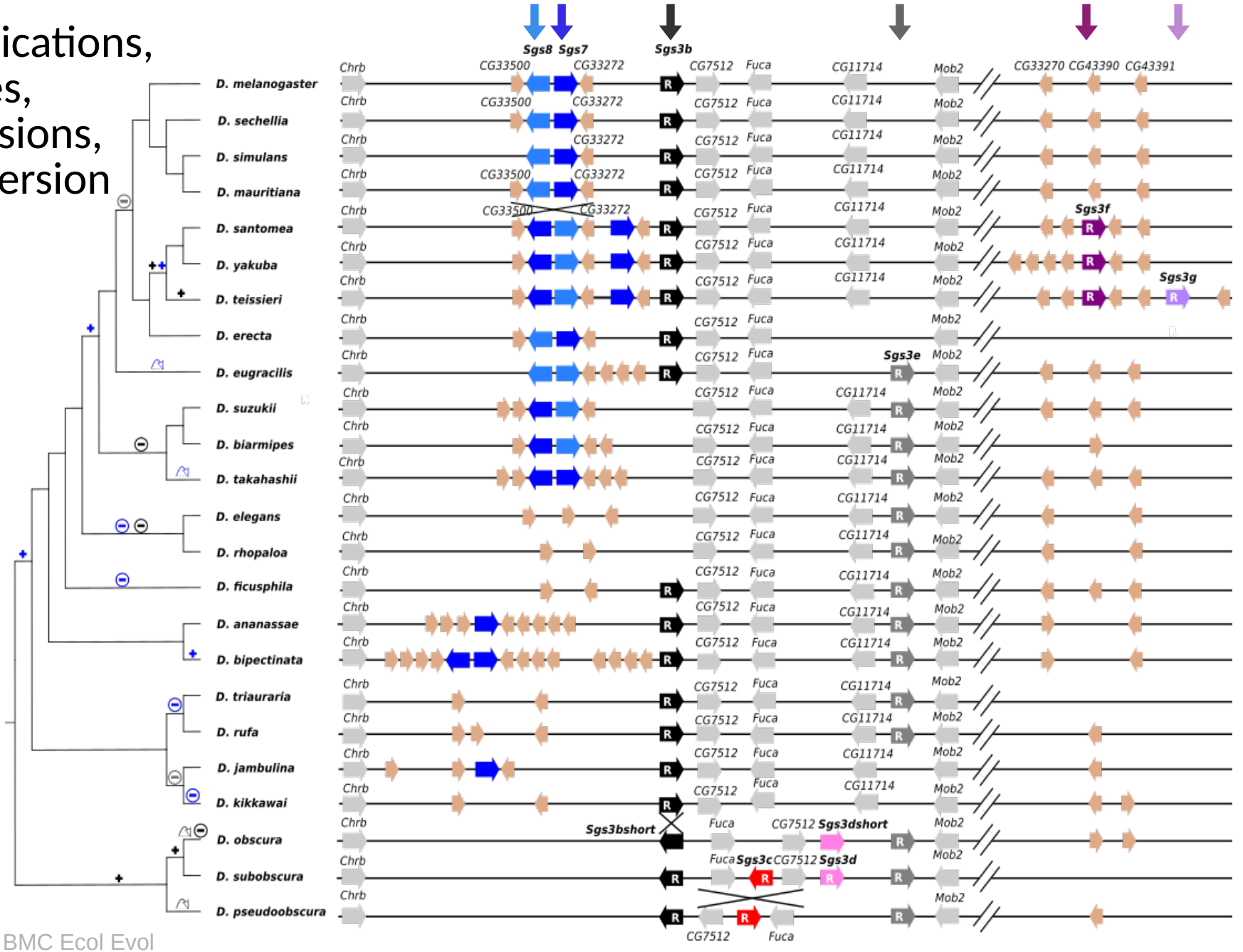
O-glycosylated

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

Short, rich in Cys

Rapid evolution of glue genes between species

Gene duplications,
gene losses,
local inversions,
gene conversion



Monier et al. 2024 BMC Ecol Evol

Conclusion

Drosophila glue as a model to understand adaptation

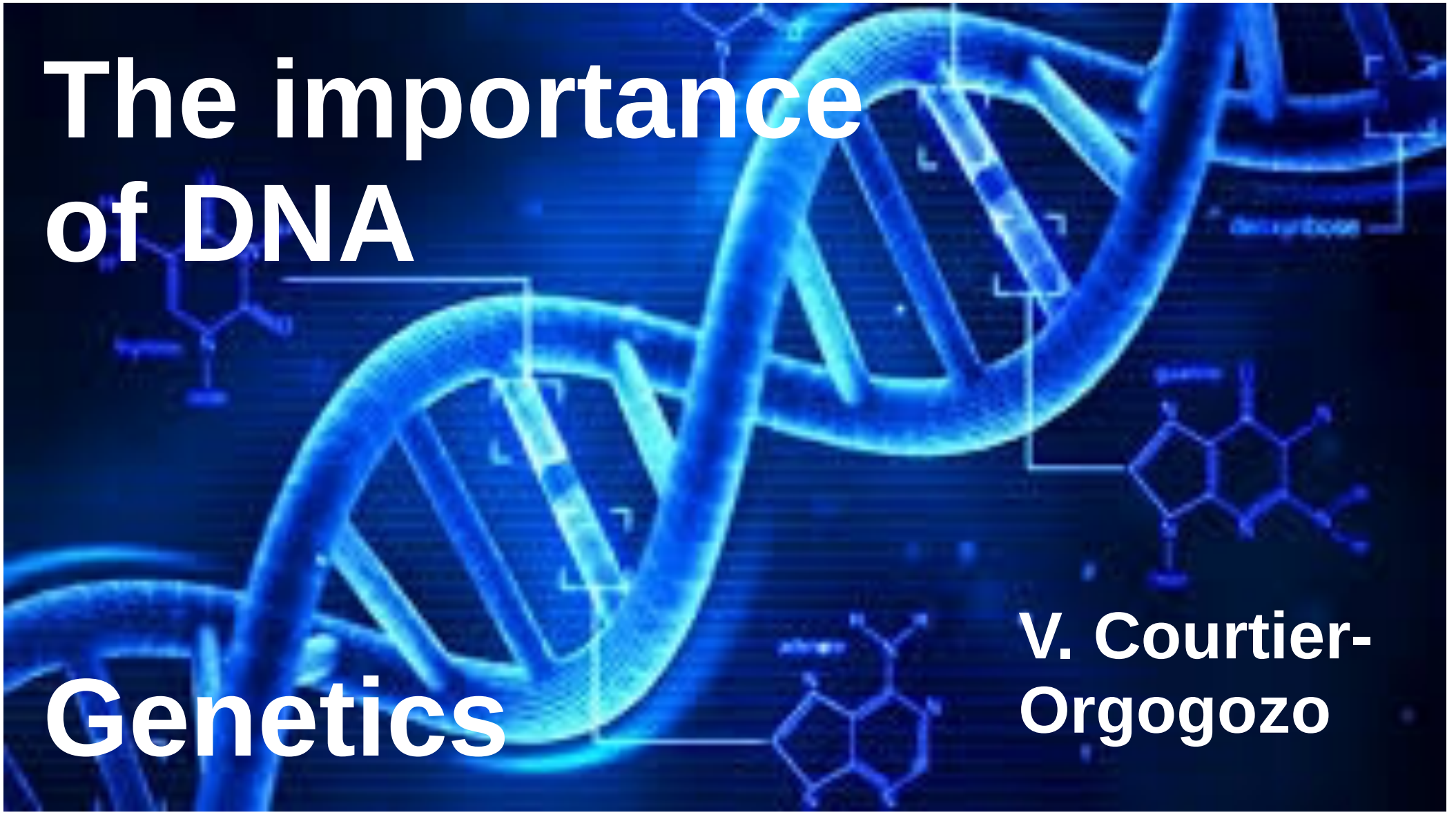
- protects from ant predation
- intra- and inter-species variation

to develop new bioadhesives

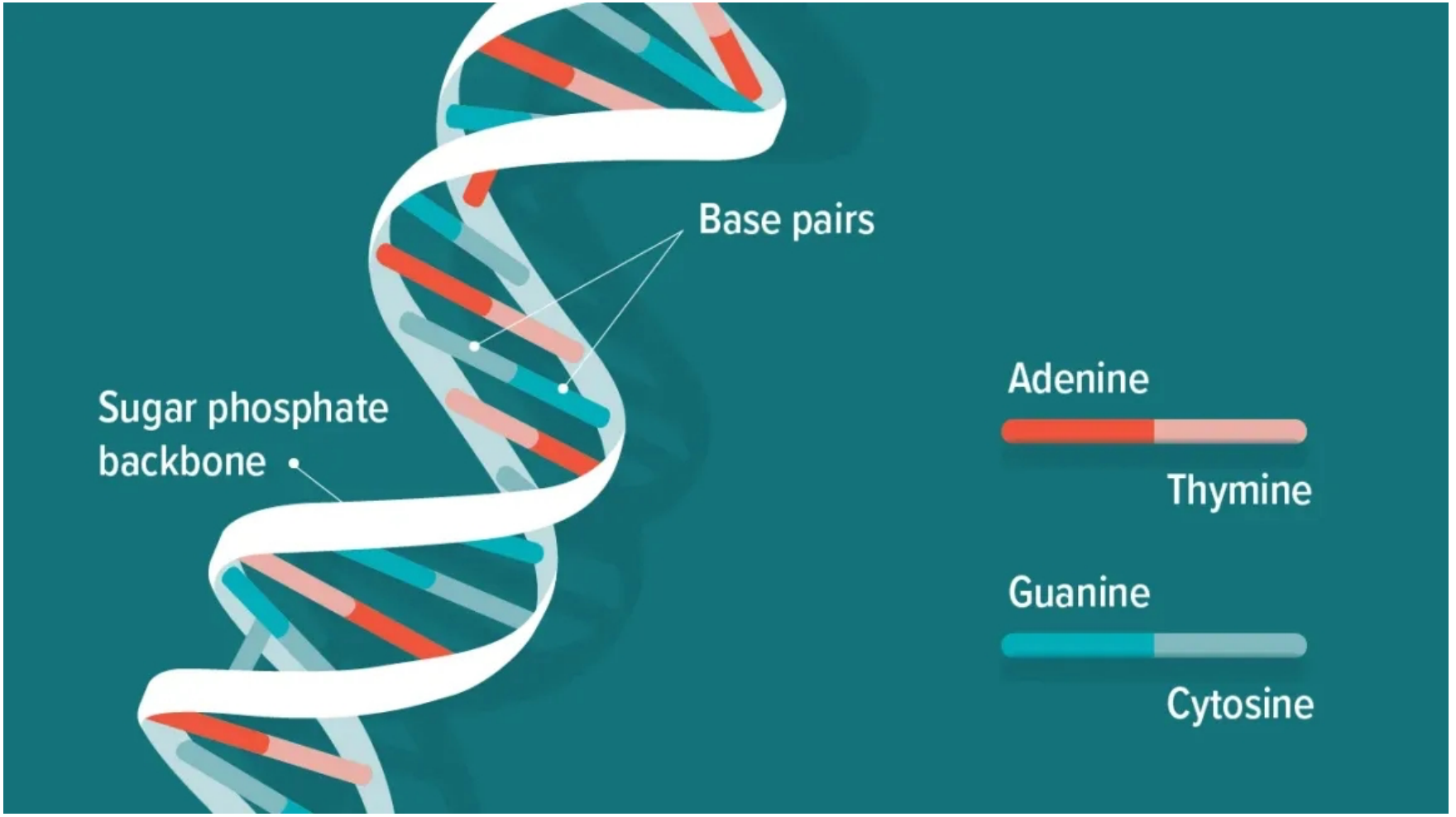
- as adhesive as most adhesive tapes
- *D. hydei* is the most adhesive
- Sgs3, Sgs7, Sgs8 are good candidates

Perspectives

Genetics Genomics	Characterize the function of each glue gene (CRISPR)
	Identify other potential glue genes (RNAseq)
Physics	Improve the adhesion assay, Test natural substrates
Physiology	Test antimicrobial properties of the glue
Biochemistry	Develop new bioadhesives


The importance of DNA
Genetics
V. Courtier-Orgogozo

Why is DNA an essential molecule in biology?



How can a single-letter flip in DNA lead to dramatic changes?

Should we be worried about GMOs?

Can genetics help us improve the future?

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

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 = 2 million years, forensic)

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

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

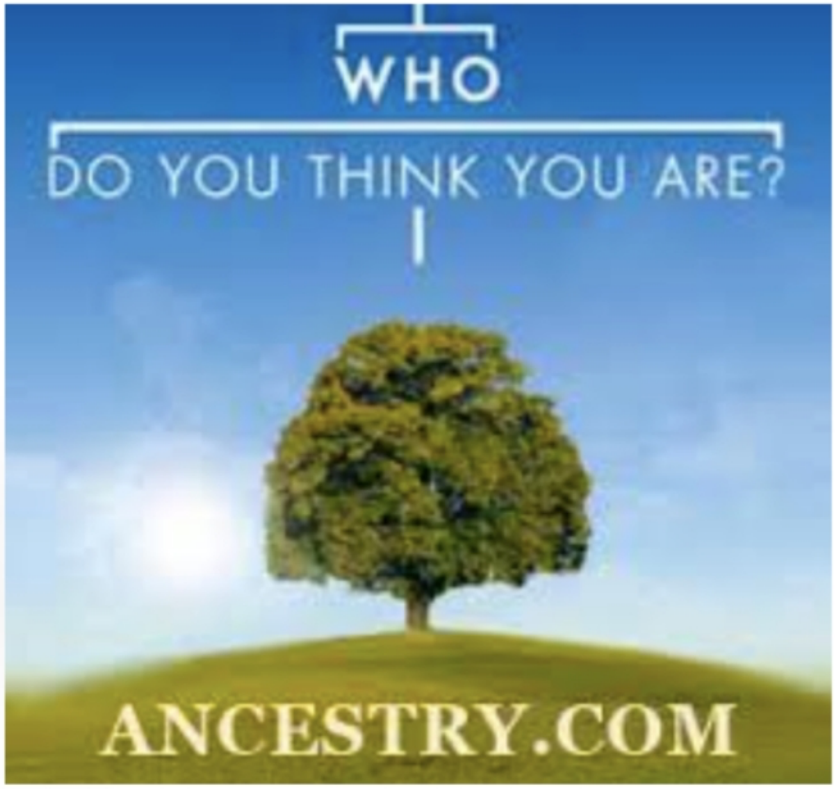
Several genes associated with autism, depression, etc. were “lost” in larger studies

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

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

Manipulating DNA

What can we do with DNA ?

What can we do with DNA ?

Extract, purify **Store**

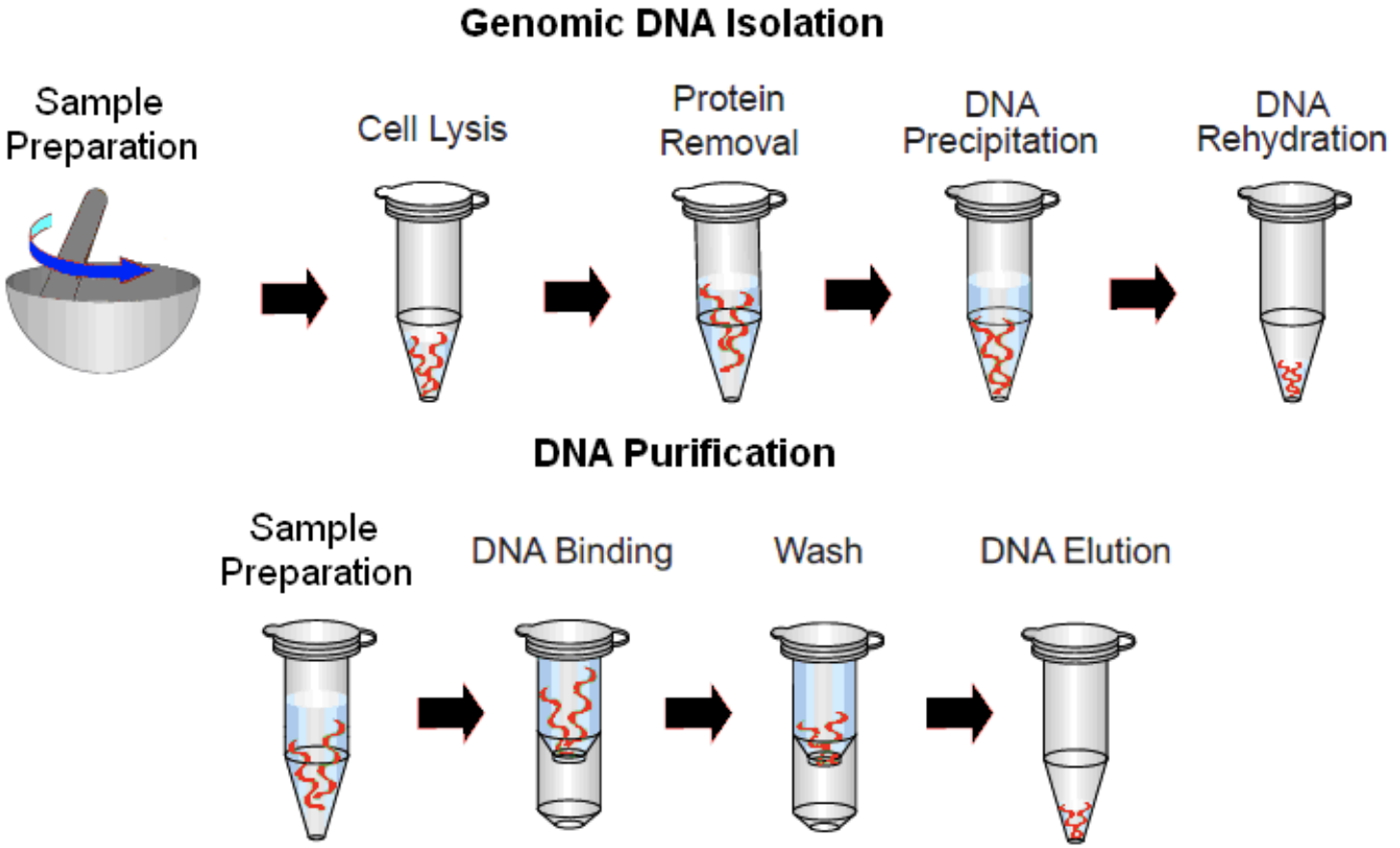
Make more
Amplify
Clone
Synthetize

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

Modify
Cut
Ligate
Recombine fragments
Introduce foreign DNA
Mutate

Extract DNA

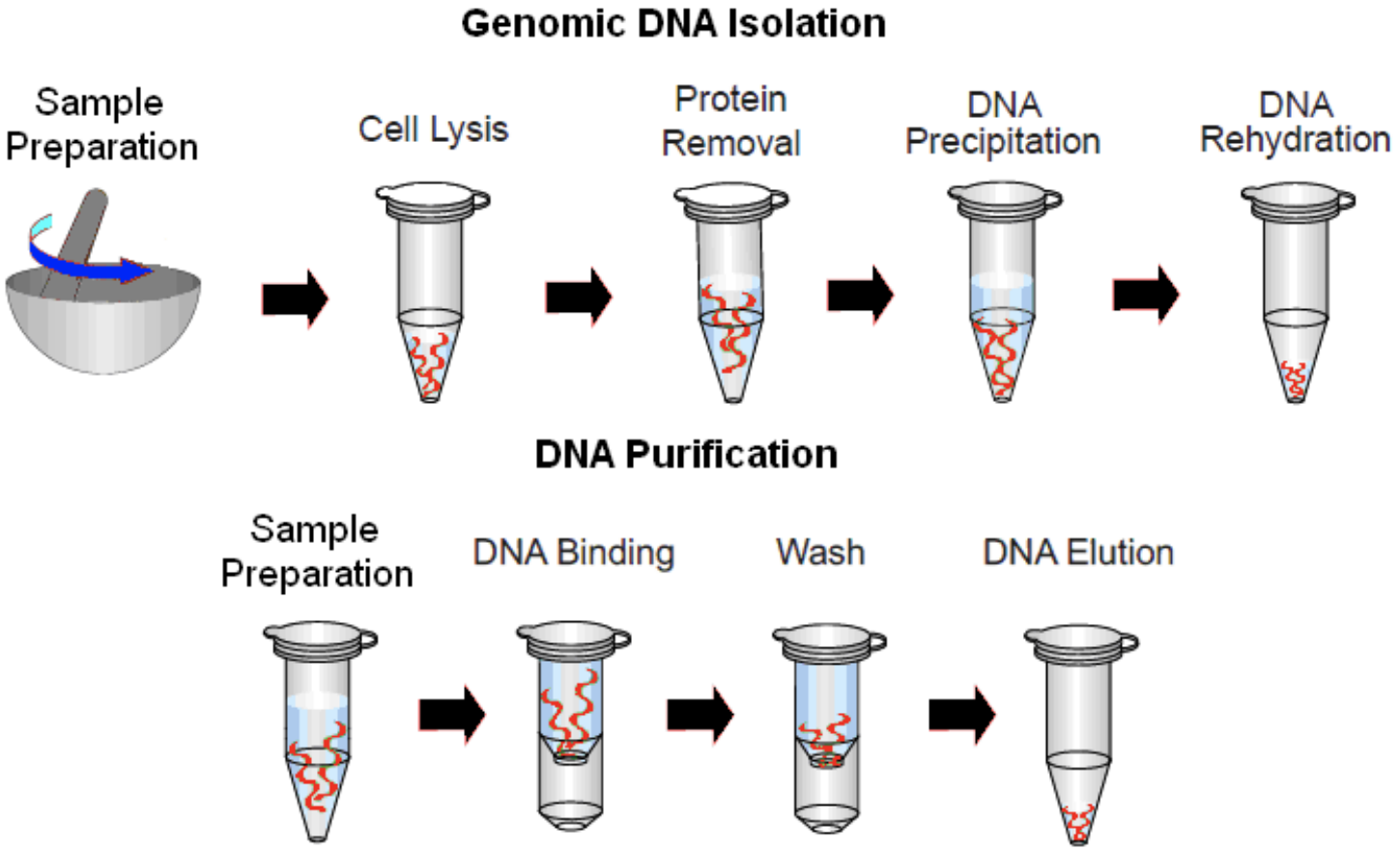
Break cells, remove lipids and proteins,
precipitate DNA, remove liquid, resuspend in aquaeous solution



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

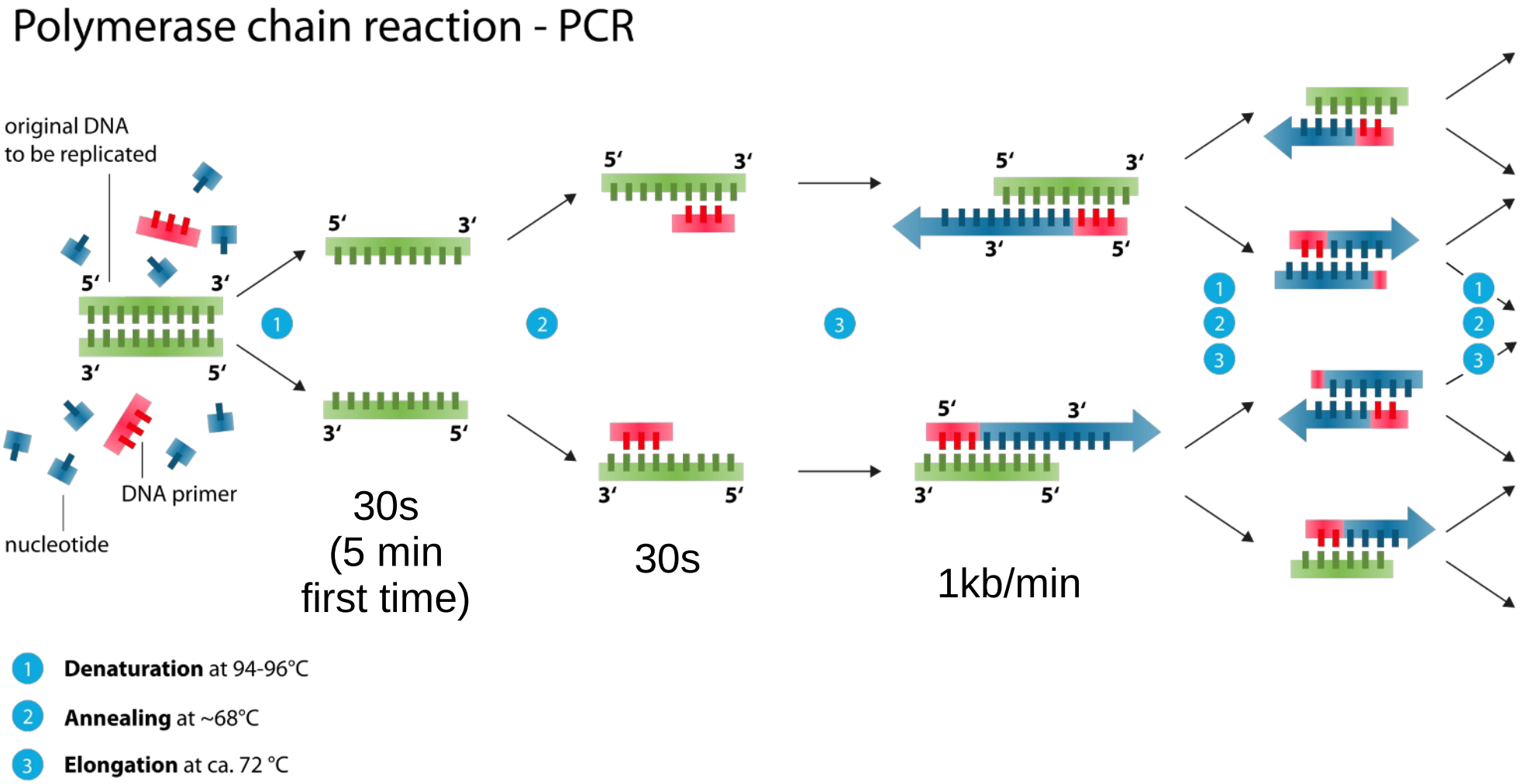
Mix:
Genomic DNA
Probes (oligonucleotides)
Nucleotides
Taq polymerase
Ions ($MgCl_2$)

Cycles of Denaturation, Annealing, Elongation

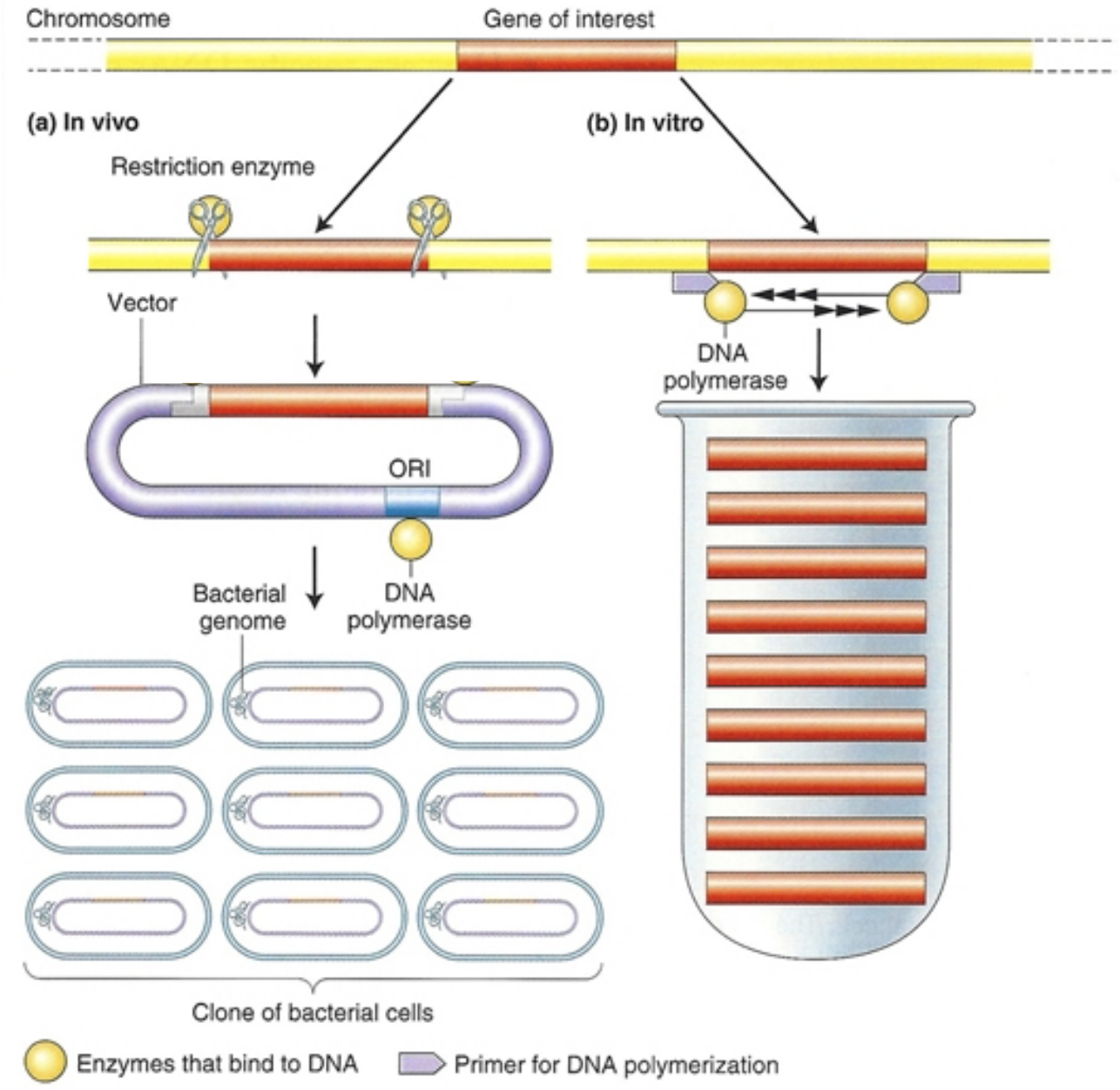
PCR: Polymerase Chain Reaction

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

Amplify DNA


Polymerase chain reaction - PCR
original DNA
to be replicated
5′
3′
3′
5′
DNA primer
nucleotide
1
5′
3′
3′
5′
30s
(5 min
first time)
2
5′
3′
3′
5′
30s
3
5′
3′
3′
5′
5′
3′
3′
5′
1kb/min
1
2
3
1
2
3
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)

Commande d'ADN sur internet

GenScript
Make Research Easy

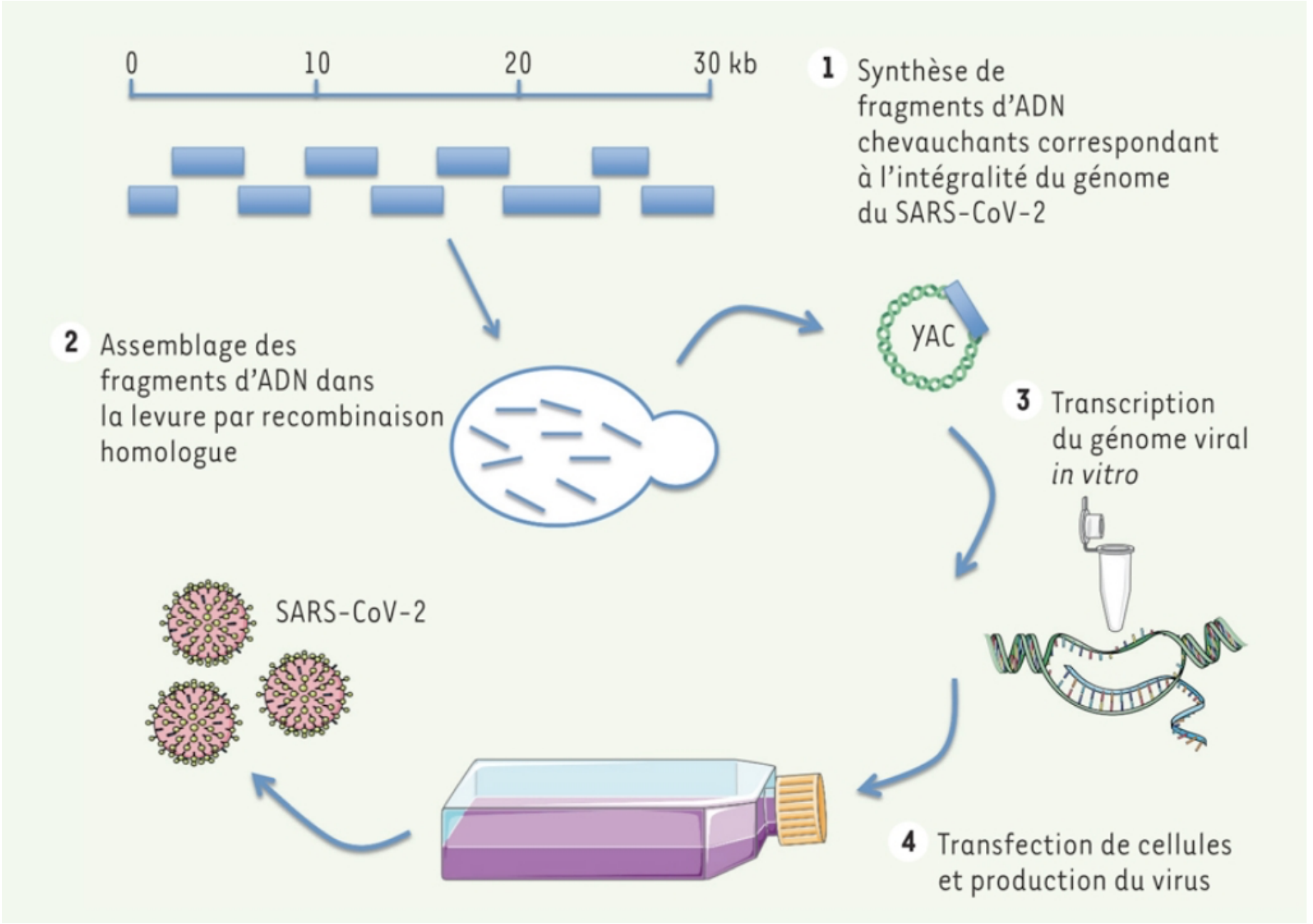
	Fast	Standard	Economy	GenBrick
Length	≤ 5 kb	≤ 8 kb	≤ 8 kb	> 8 kb
Turnaround time (starting from)*	5 business days (bd)	8 business days (bd)	10 business days (bd)	23 business days (bd)
Complex genes	✖	✔	✖	✔
Overview	Custom industry-leading genes, 100% sequence accuracy guaranteed			

ⓘ The standard delivery includes:

- 4 µg of lyophilized plasmid containing your gene insert (1 µg for low-copy plasmid) *
- Sequence chromatograms or NGS read depth plot covering your gene (electronic)
- Construct map for the plasmid (electronic)
- Quality assurance certificate

https://www.genscript.com/gene_synthesis.html?src=home

Synthèse de SARS-CoV-2 en un mois

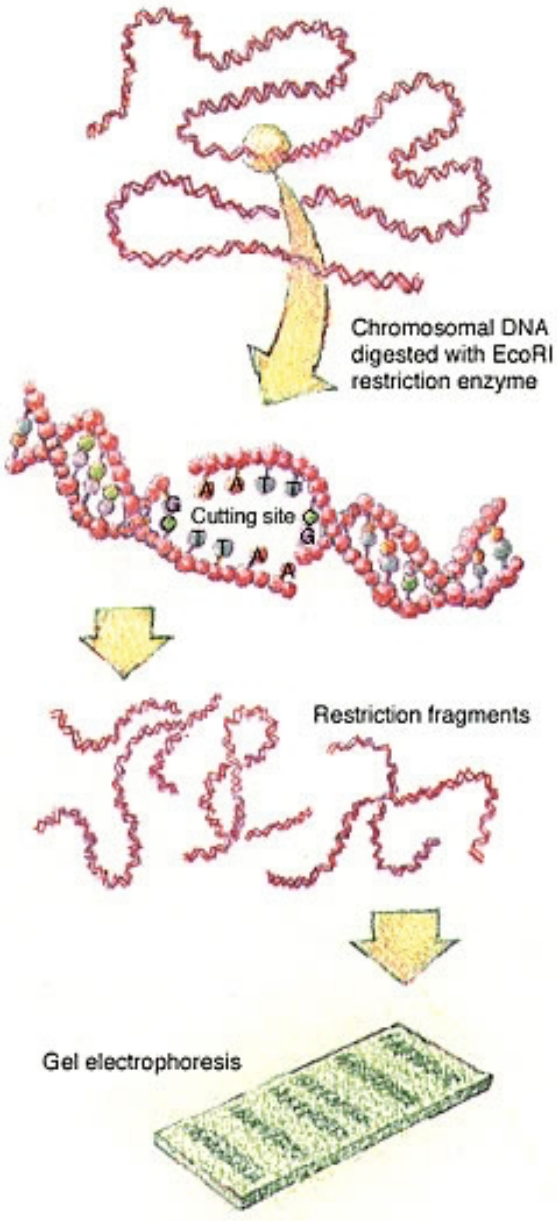


Iseni, Frédéric, and Jean-Nicolas Tournier. 2020. 'Une Course Contre La Montre-Création Du SARS-CoV-2 En Laboratoire, Un Mois Après Son Émergence !' Médecine/Sciences 36 (8–9): 797–802

Cut DNA with restriction enzymes

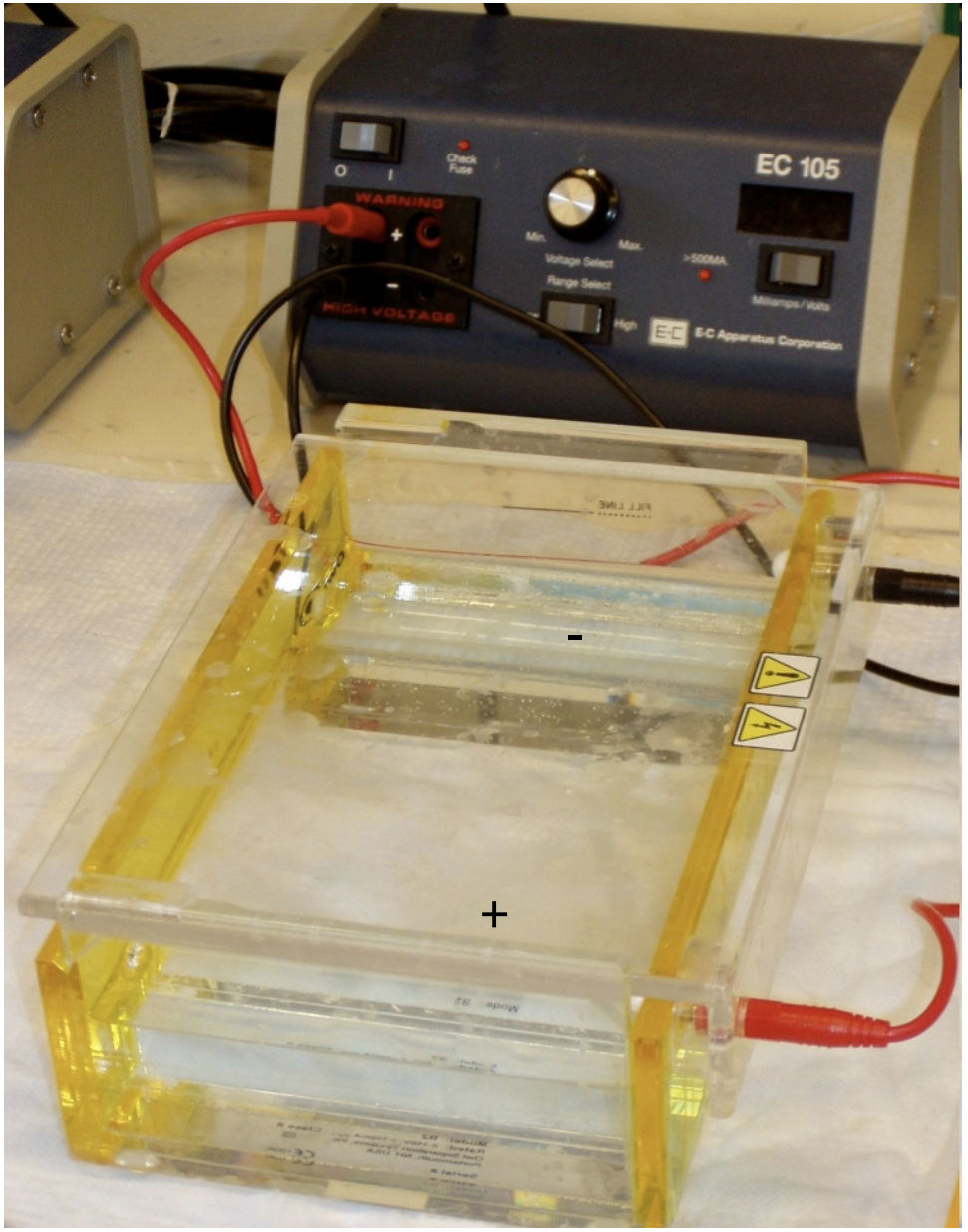
Blunt ends, 3' protruding ends, 5' protruding ends

Cut DNA with restriction enzymes

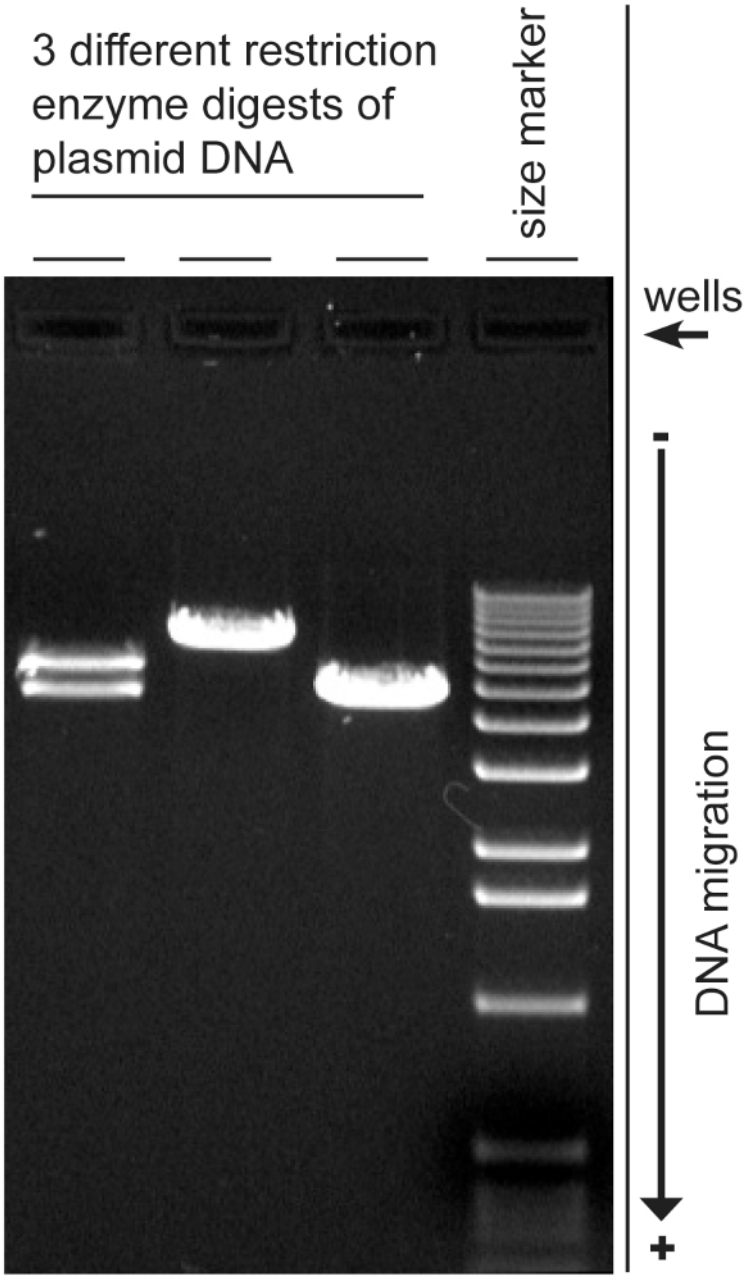


Blunt ends, 3' protruding ends, 5' protruding ends

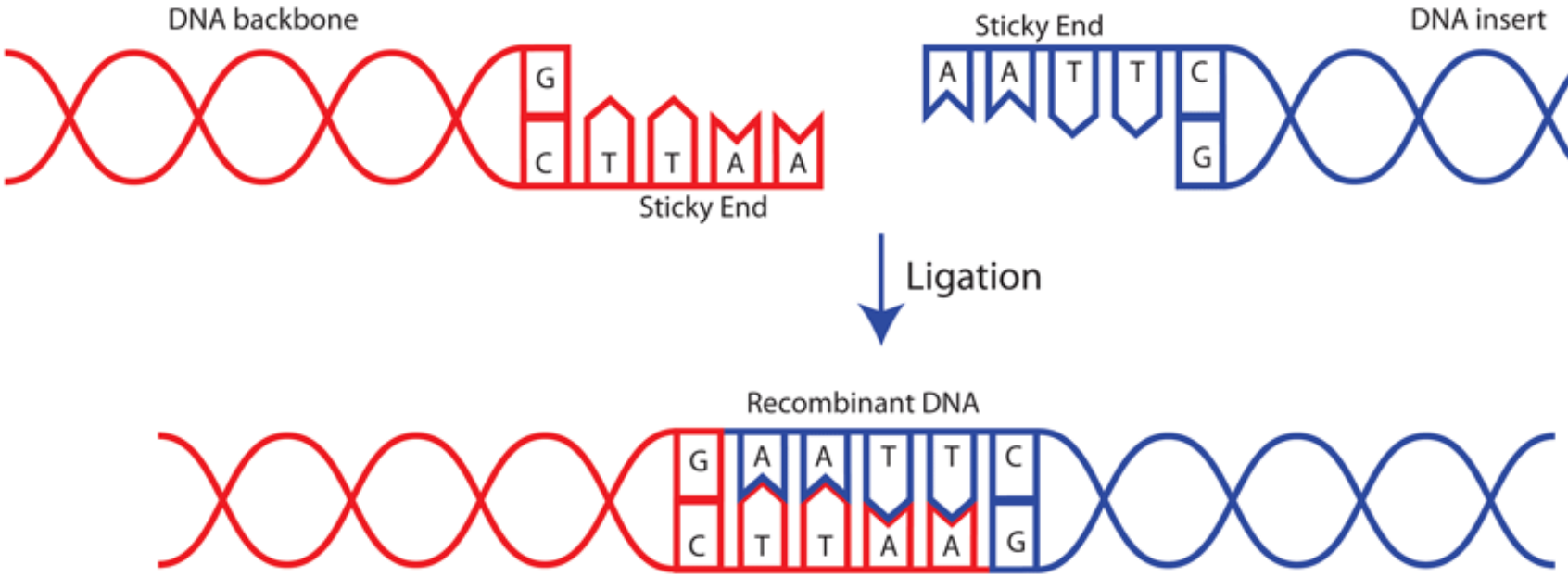
Examine length of DNA

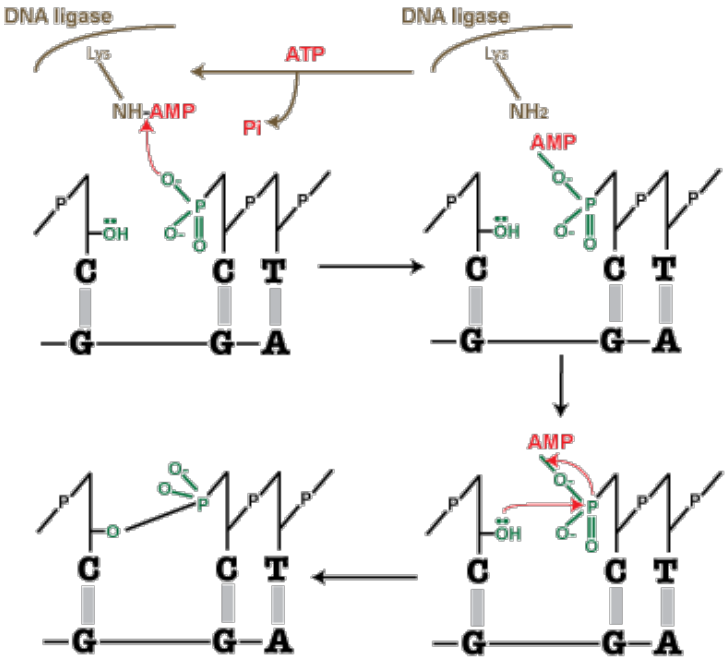


TAE (Tris-acetate-EDTA) buffer



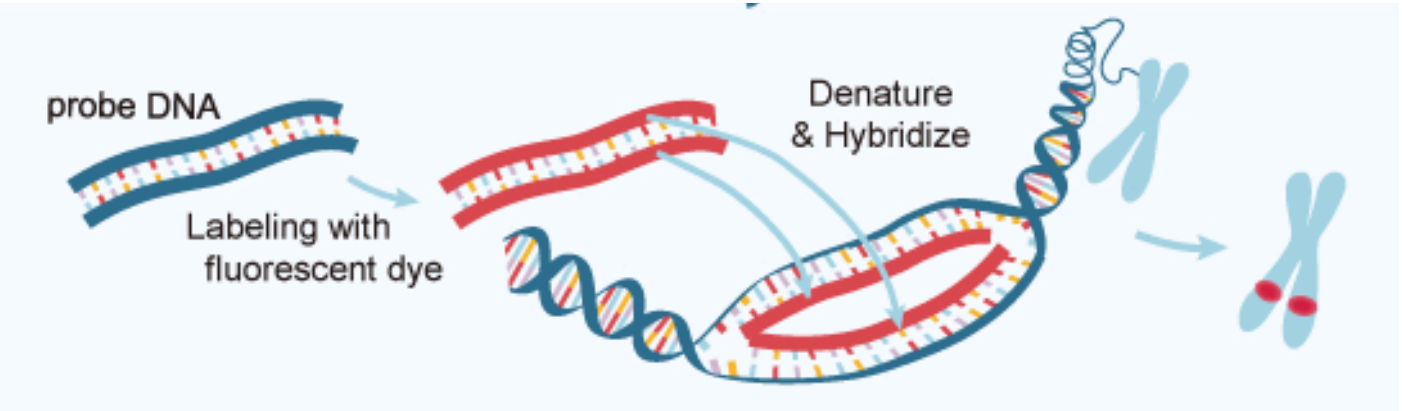
Ligate DNA

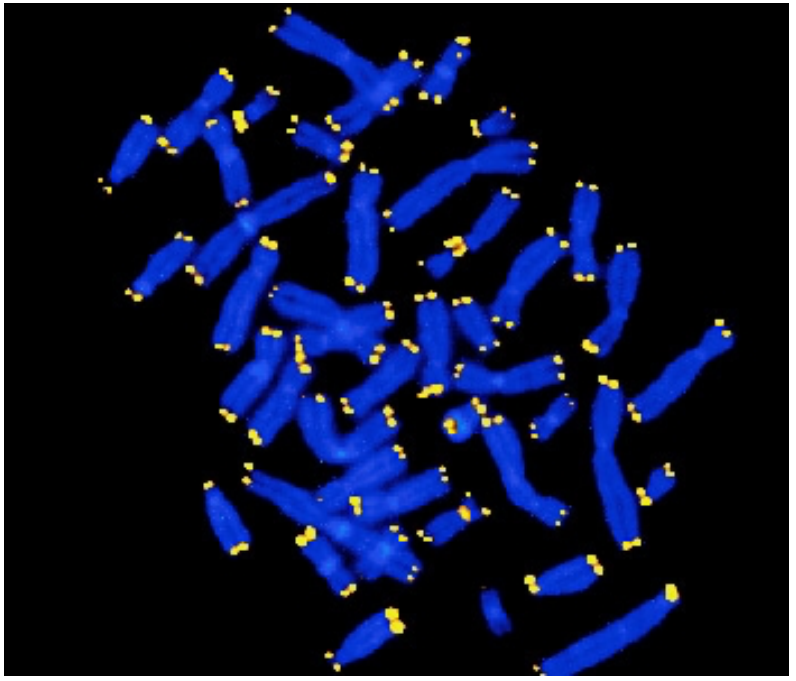




Fragments have to be phosphorylated but only on one strand
Dephosphorylate the vector to inhibit self-circularization

Probe DNA:
Fluorescent In Situ Hybridization

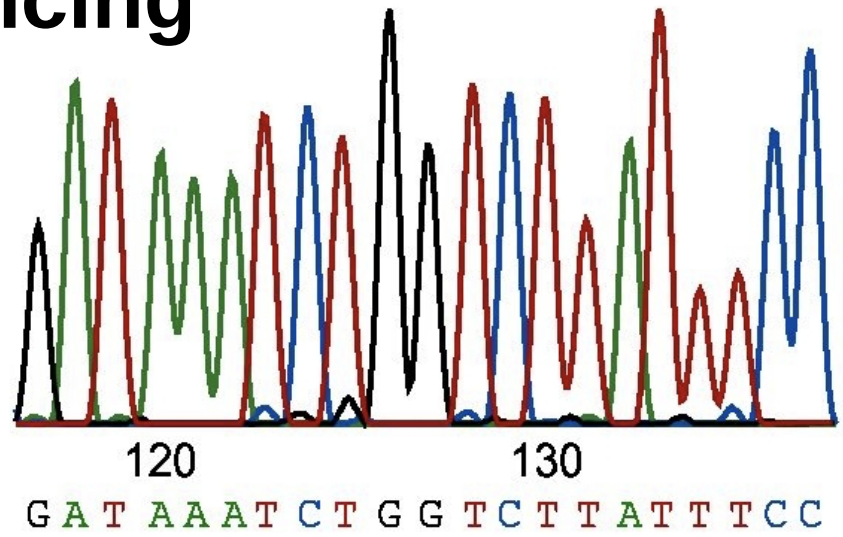




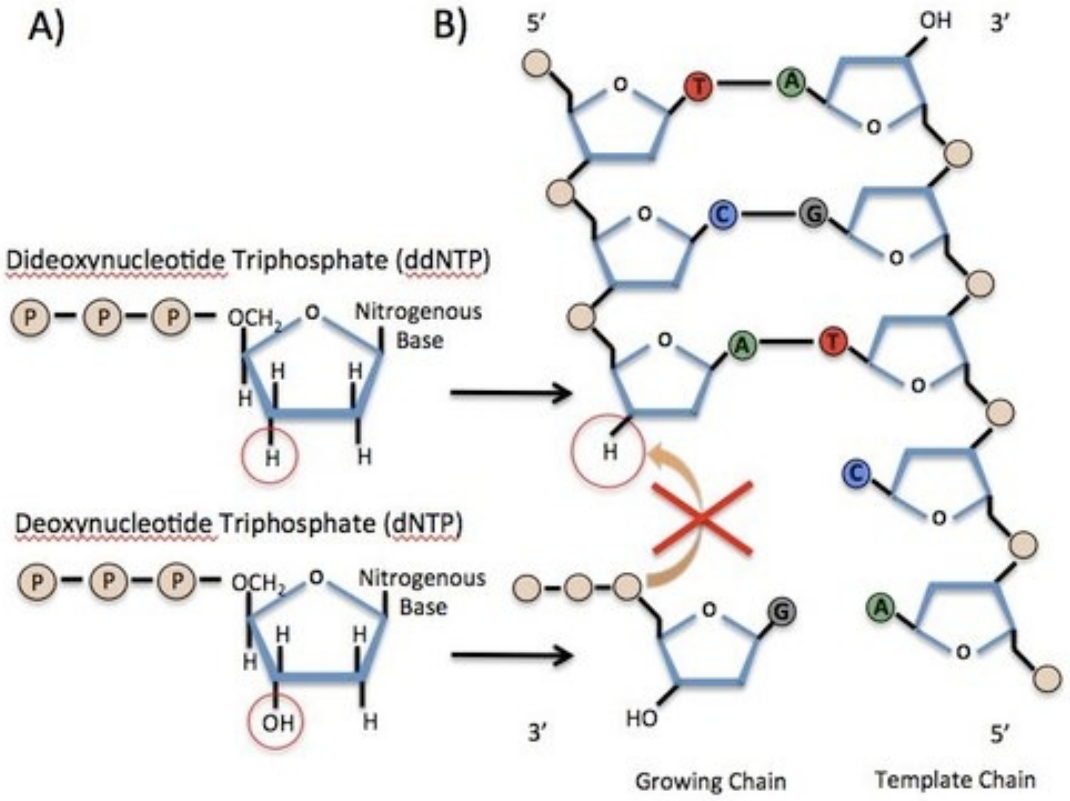
Probes for telomere sequences

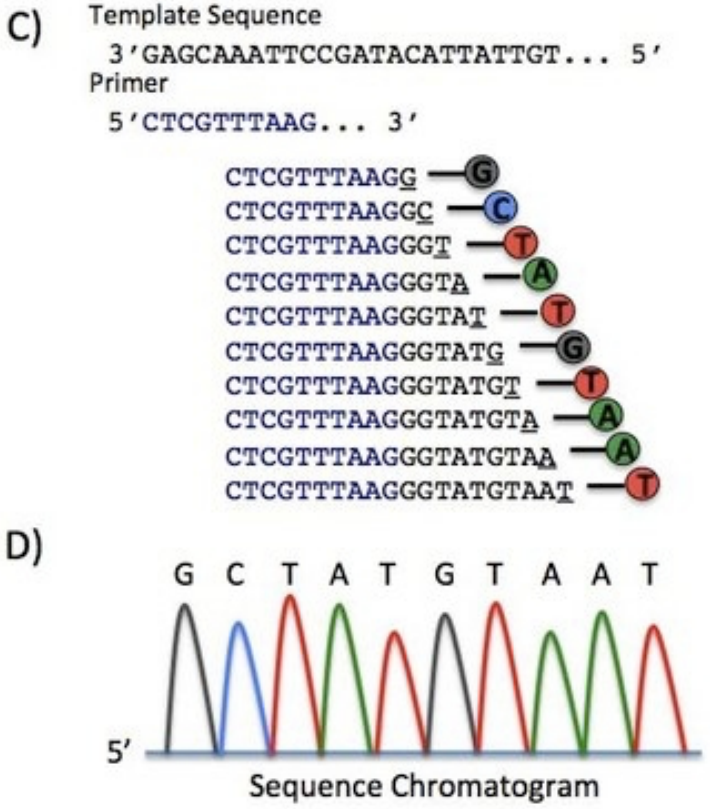
Sanger sequencing

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



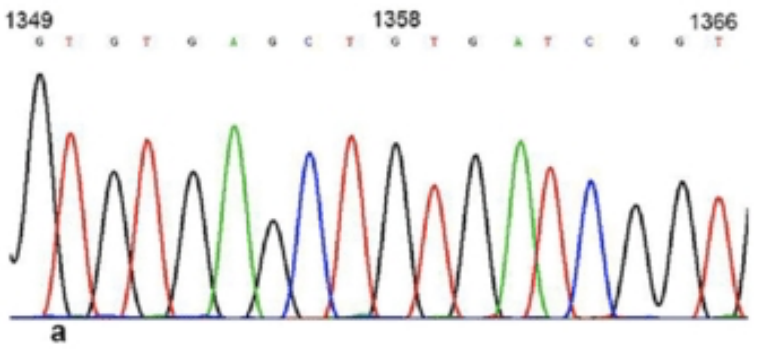
Dye terminator sequencing

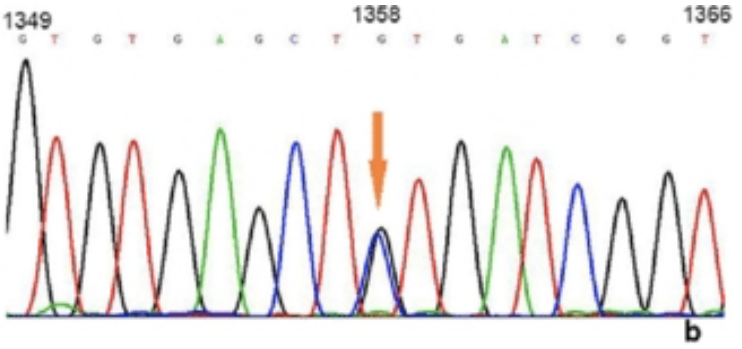


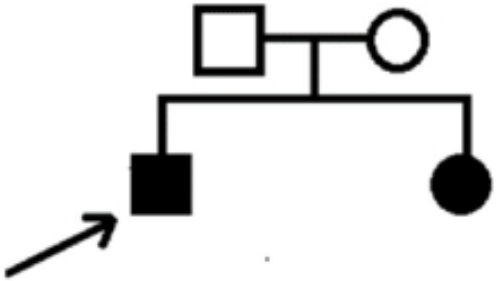


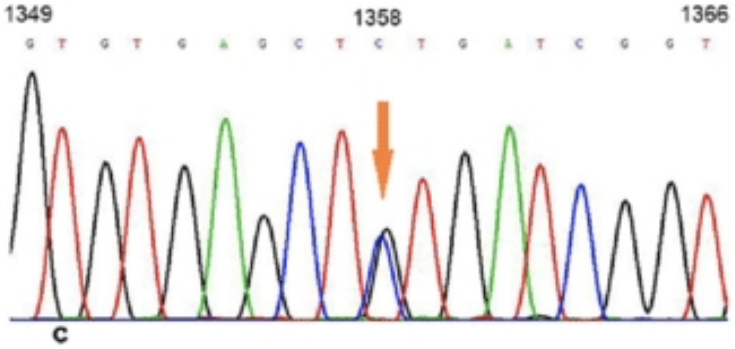
McGovern 2015

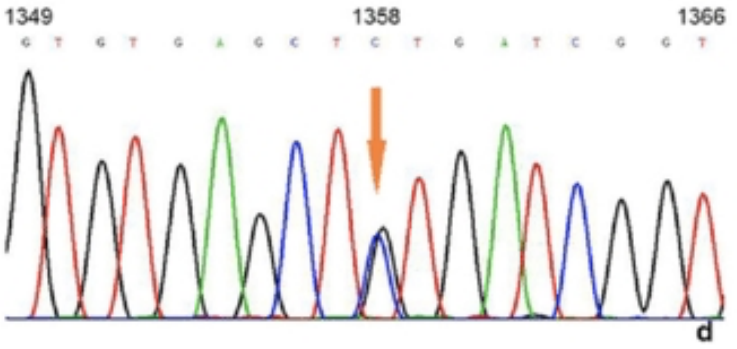
GTGTGAGCTGTGATCGGT



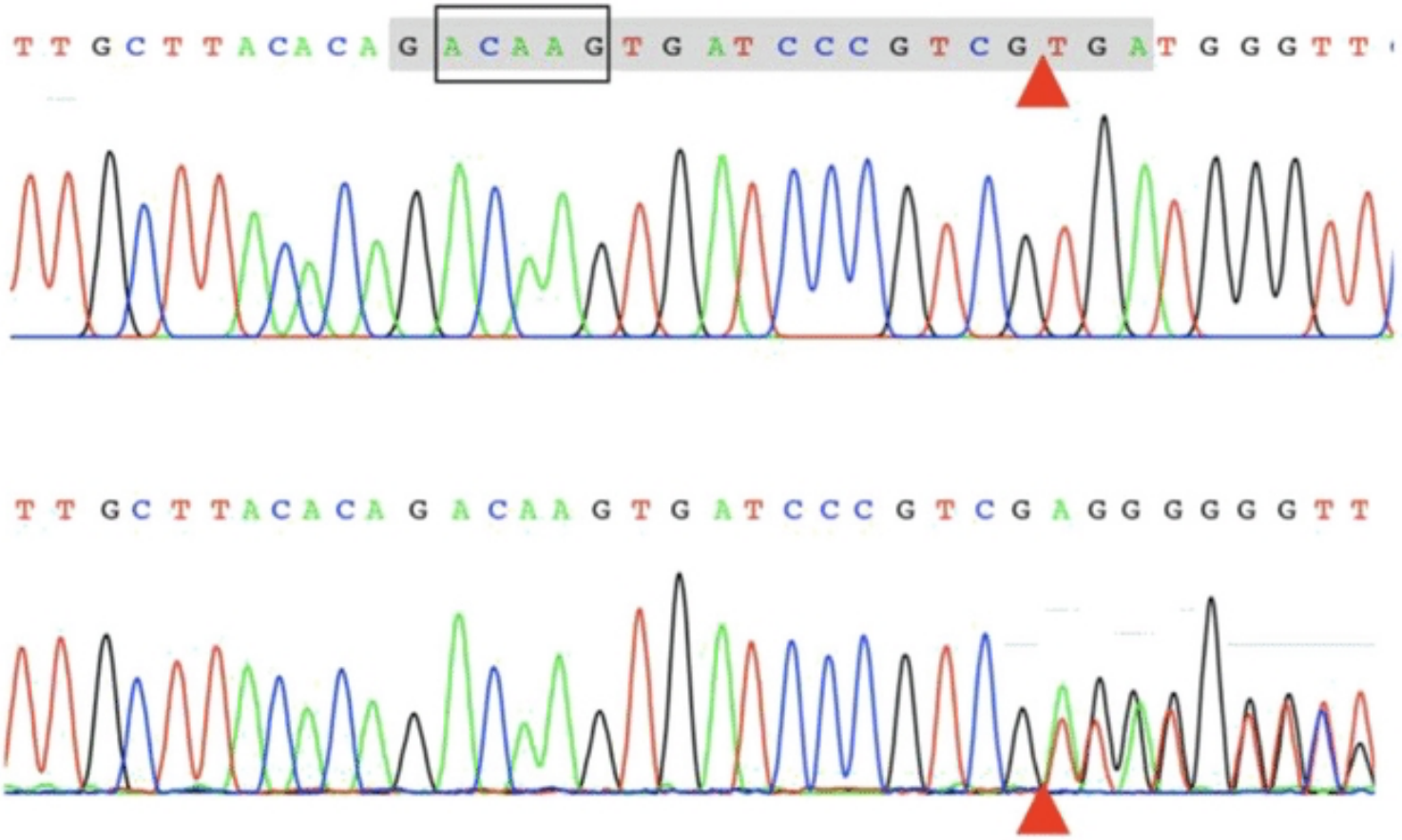






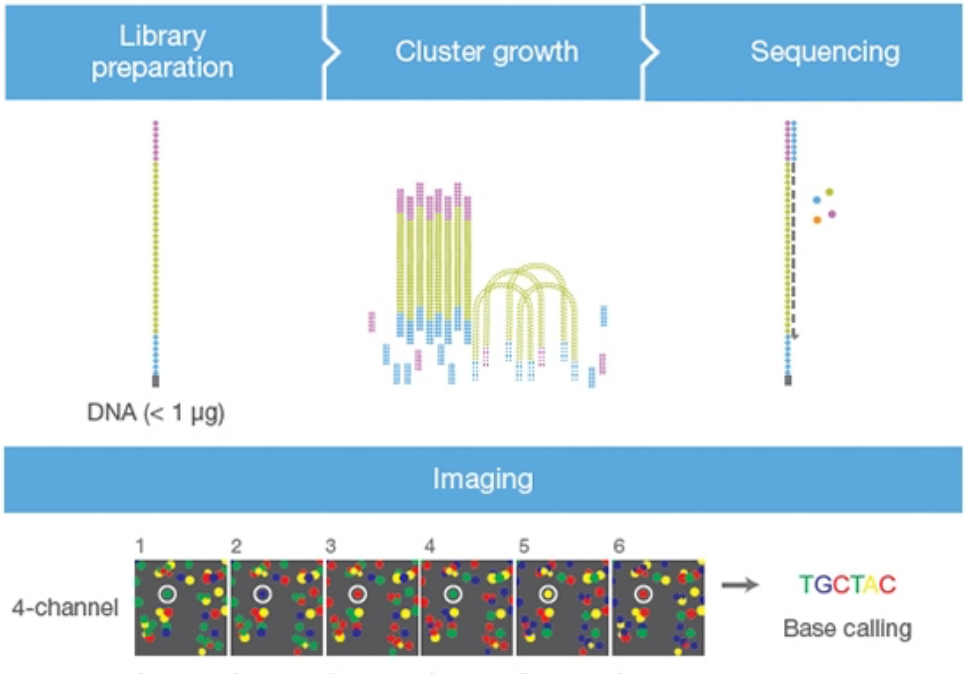


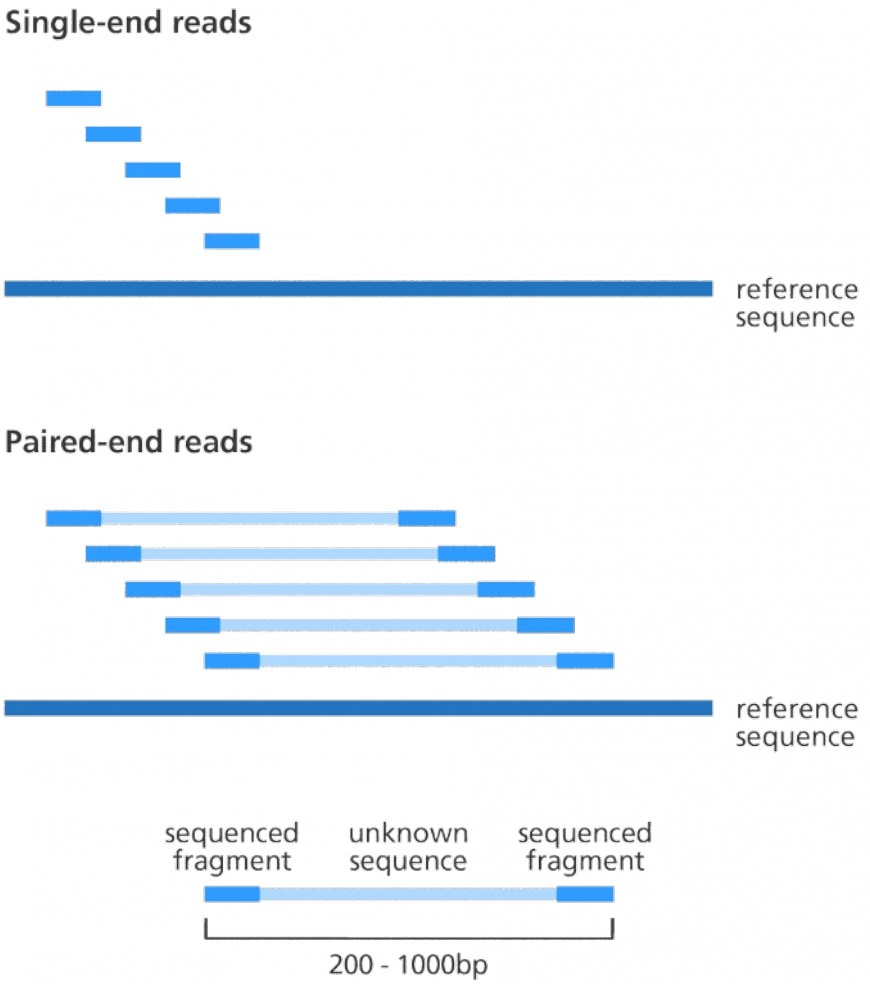
Ding et al 2015


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

Manipulating RNA

What can we do with RNA ?

What can we do with RNA ?

Extract, purify

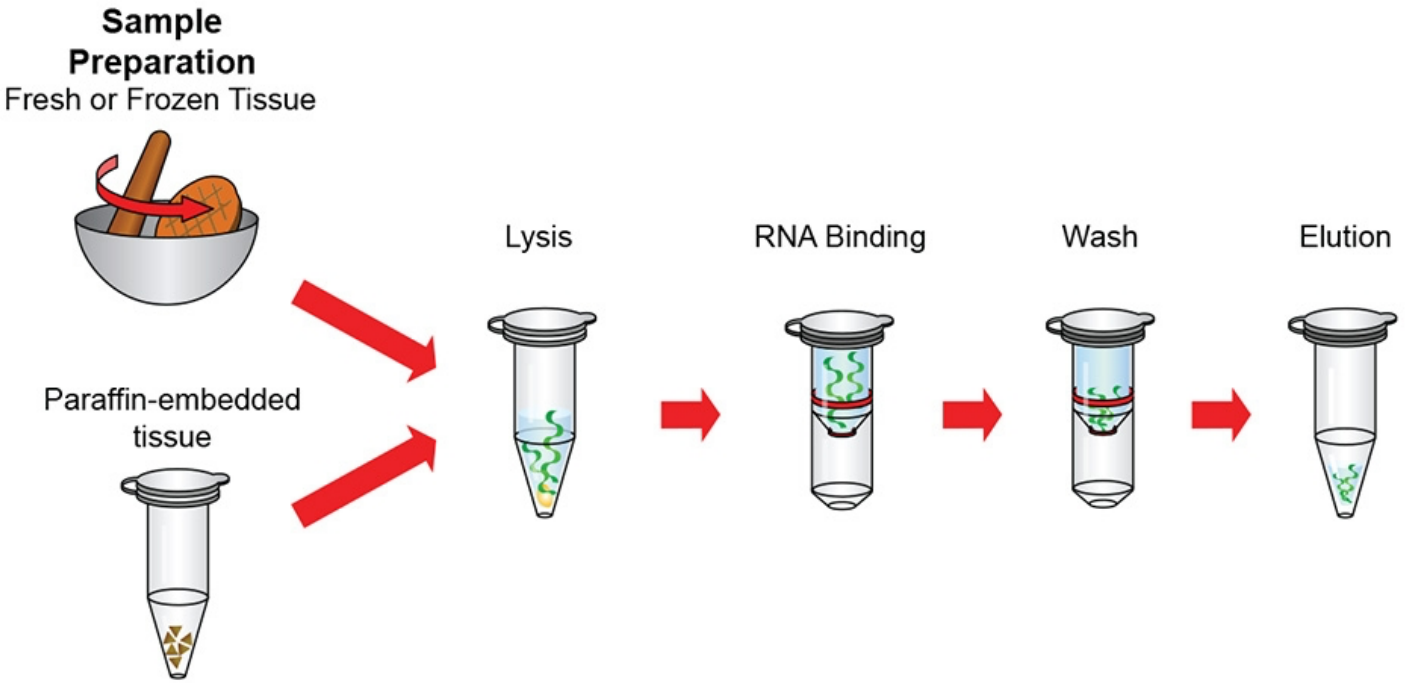
Make more
RNA → DNA → RNA (reverse transcription, transcription)

Examine
Quantify
Examine length
Stain, probe
Sequence
Examine 3D structure
Measure physical properties of RNA molecules

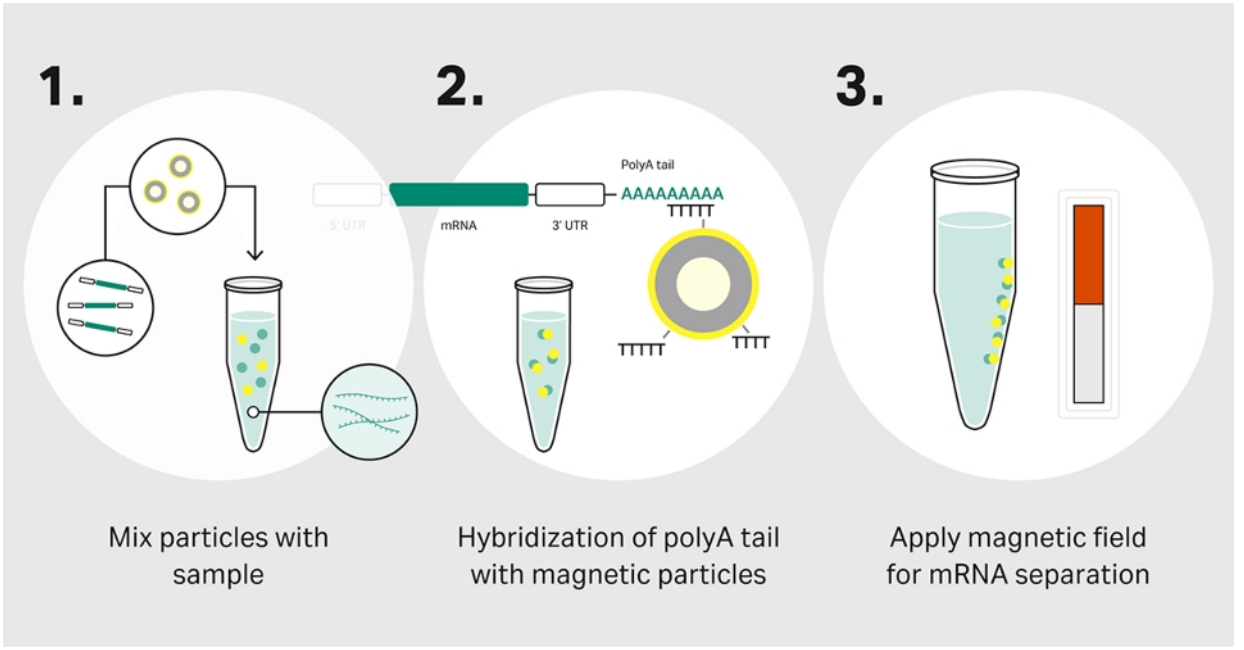
Modify
Mostly via DNA

Extract RNA

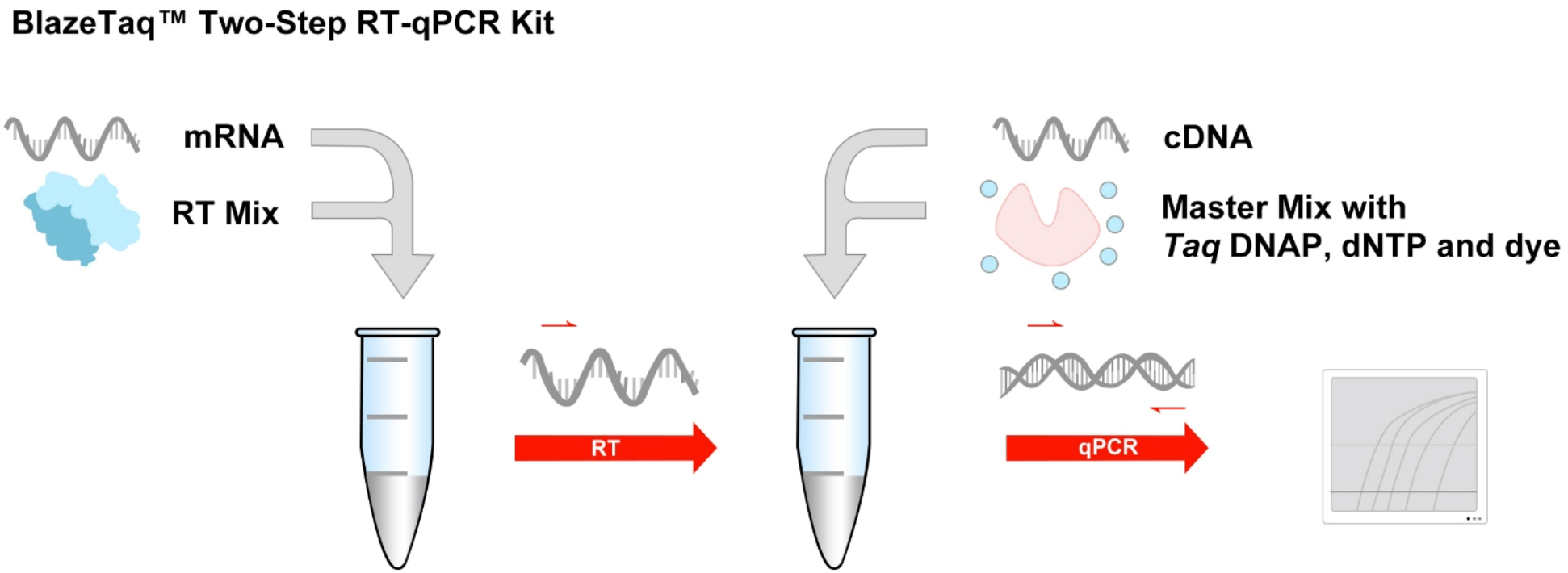
Total RNA



mRNA



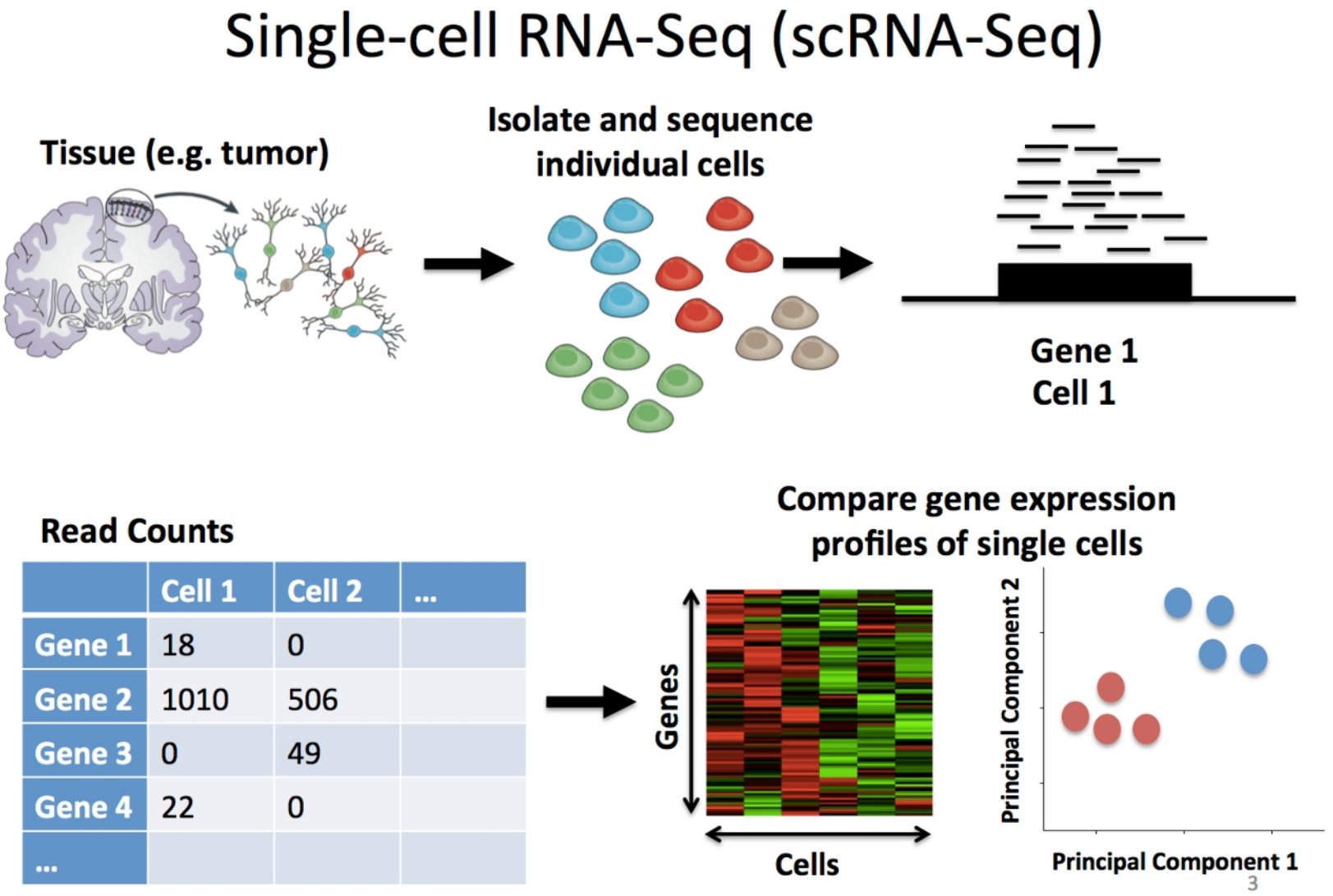
RT-qPCR



https://www.youtube.com/watch?v=iu4s3Hbc_bw

RNAseq

Starting with tissues/organs/single cells



	Cell 1	Cell 2	...
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
...			

DNA and its observable effects

The genotype-phenotype map

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

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

Aneuploidy = anomalous number of chromosomes

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

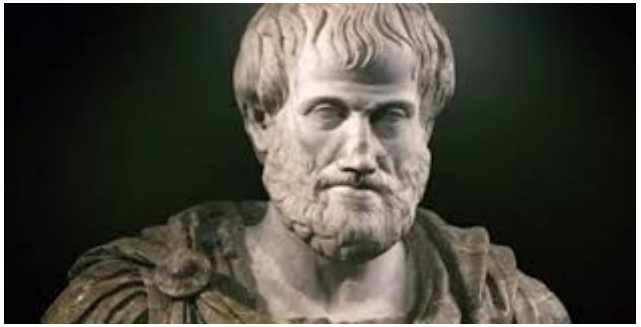
Coding versus cis-regulatory

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

Different kinds of phenotypes

Morphology

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



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

Physiology

Behavior

Genotype & Phenotype

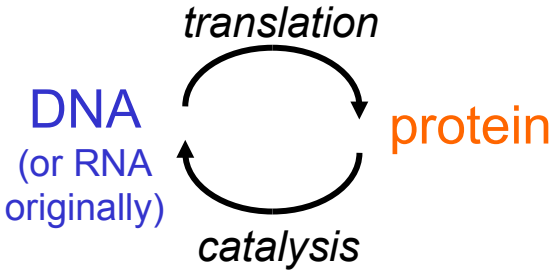
= what engenders = what is apparent

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

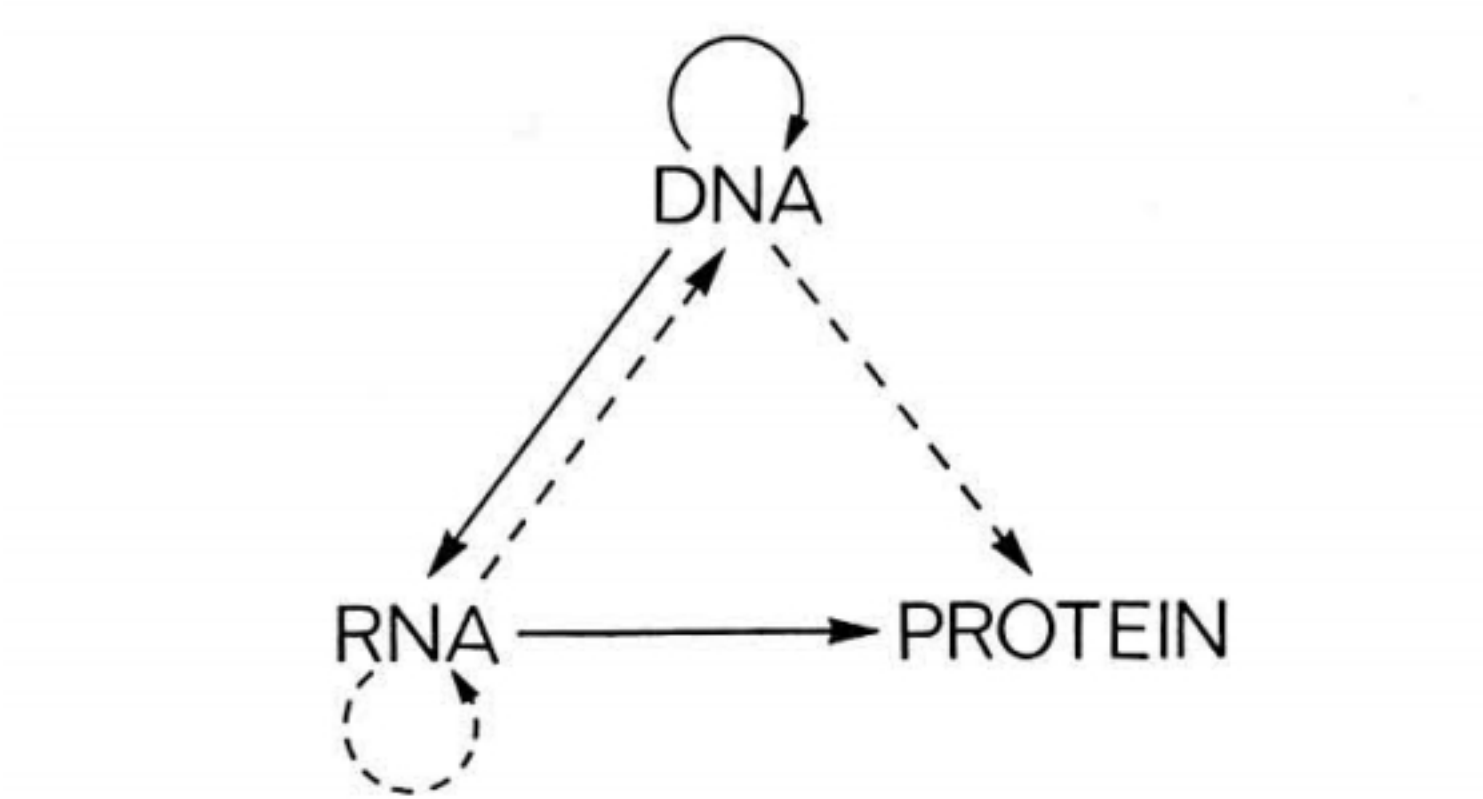


Fig. 3. A tentative classification for the present day. Solid arrows show general transfers; dotted arrows show special transfers. Again, the absent arrows are the undetected transfers specified by the central dogma.

Crick 1958
Crick 1970

How do genotypes map onto phenotypes ?

DEVELOPMENTAL BIOLOGY

EVOLUTIONARY GENETICS

Both are direct descendants of Morgan's school. Emphasis on genes.

How does an organism form from a single cell?

What makes one organism different from another one?

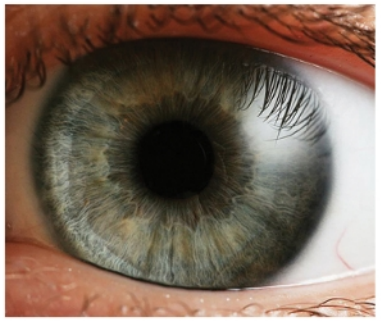
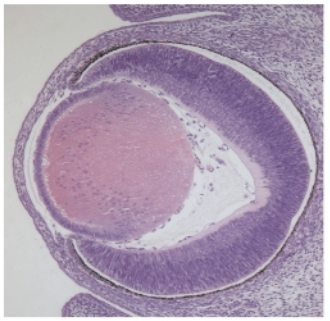
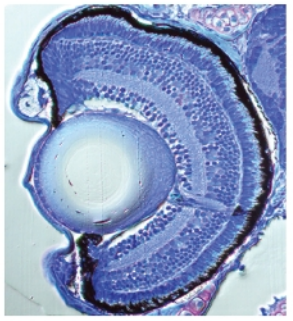
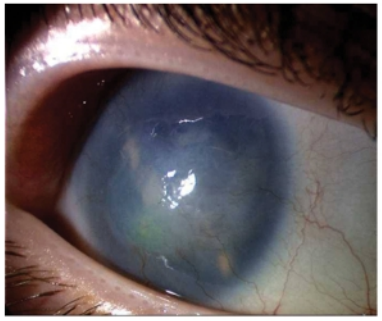
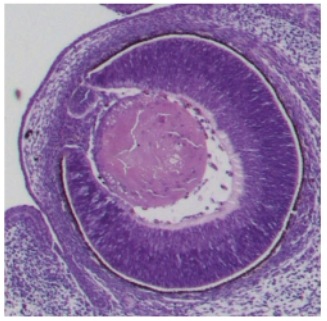
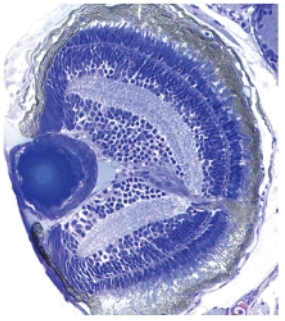
One of the central problems of biology is that of differentiation - how does an egg develop into a complex many-celled organism? That is, of course, the traditional major problem of embryology; but it also appears in genetics in the form of the question,
"How do genes produce their effects?

Sturtevant, 1932

How do genes produce observable traits?

Gene → Observable character

Pax6 : an eye gene ?

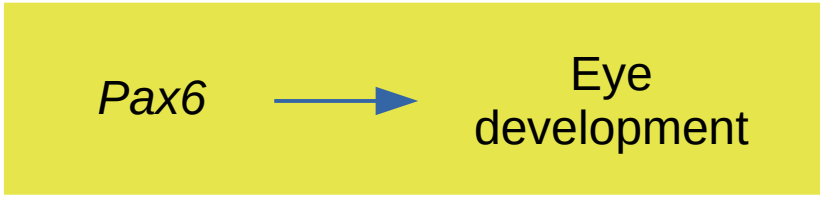
	Human	Mouse	Zebrafish	*Drosophila*
WT				
mut				
	***PAX6*$^{+/-}$**	***Pax6*$^{-/-}$**	***pax6b*$^{-/-}$**	***ey*$^{-/-}$**
EQs	cornea opaque iris absent retina degenerate lens opaque aqueous humor of eyeball increased pressure	eye decreased size lens fused_to cornea iris morphology anterior chamber absent	eye decreased size lens decreased size retina malformed	eye absent

Drosophila

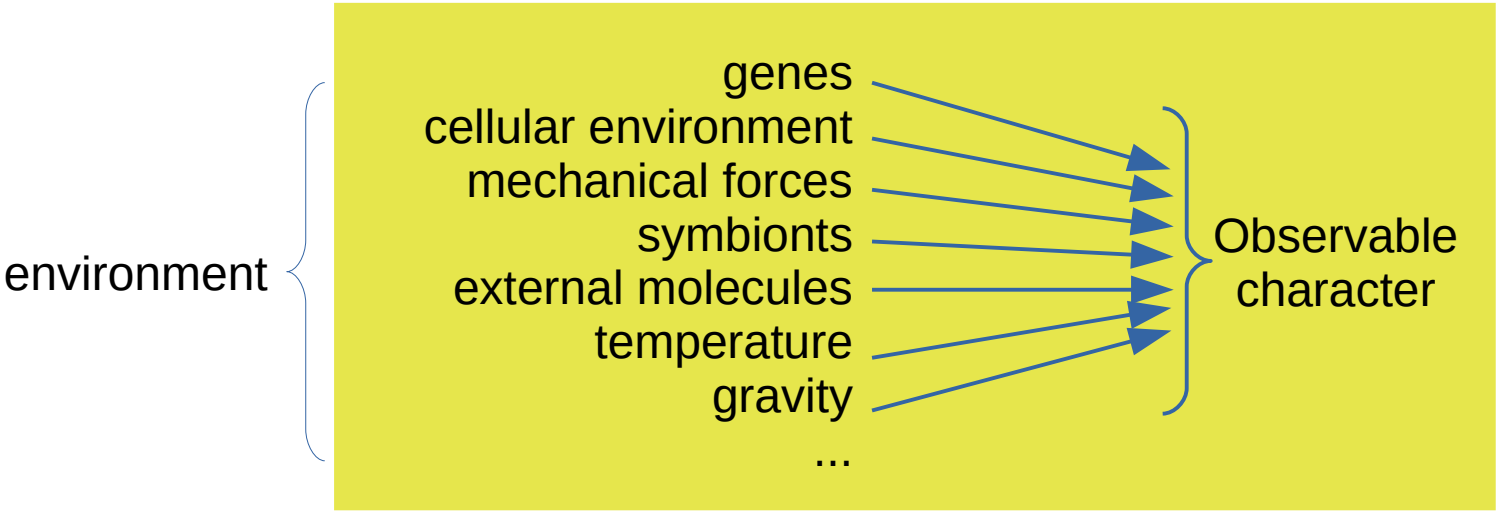
Ectopic expression of Pax6

Gene → Observable character

Pax6 → Eye development



Too simplistic

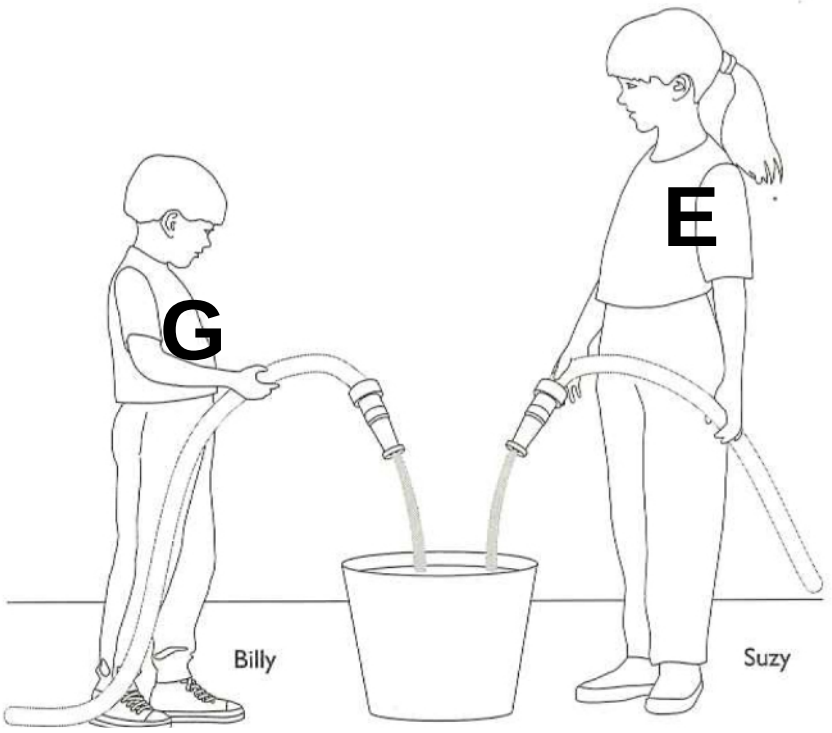


Better, but difficult to disentangle the effects

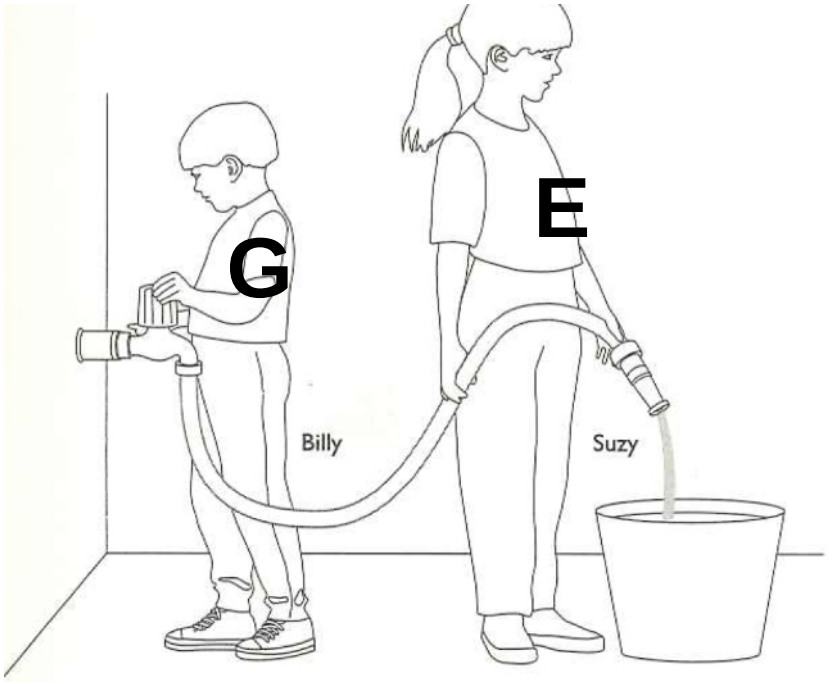
We all come from GxE (Genes x Environment)

Eero Mäntyranta, Finnish ski champion
7 Olympic medals in the 60s
Mutation enabling his blood to transport oxygen more efficiently
His family members were not Olympic champions

Impossible to quantify innate versus acquired influences



GxE

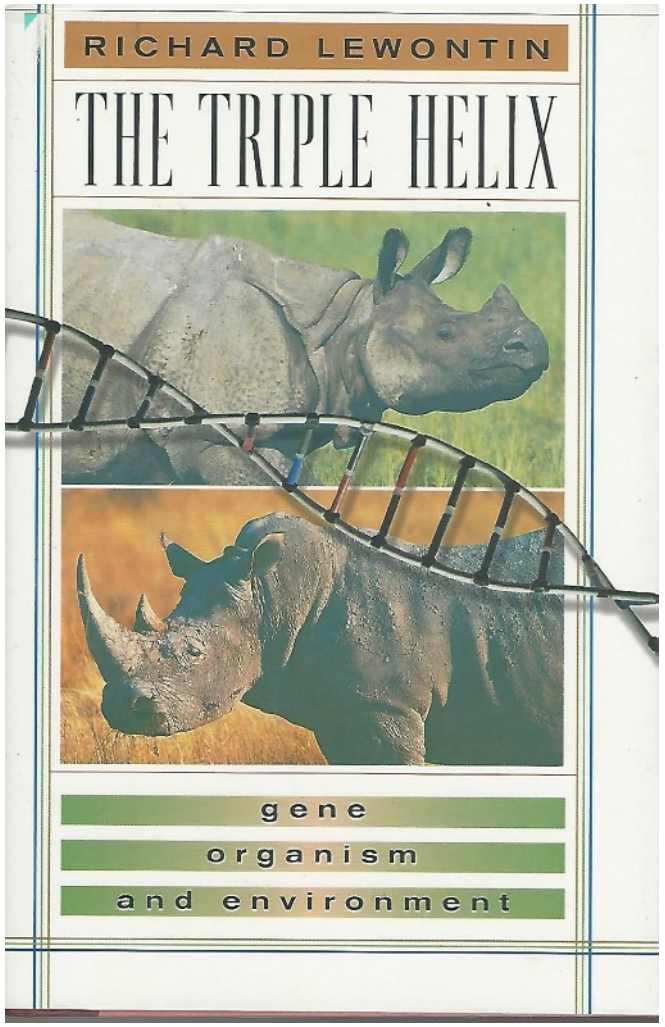


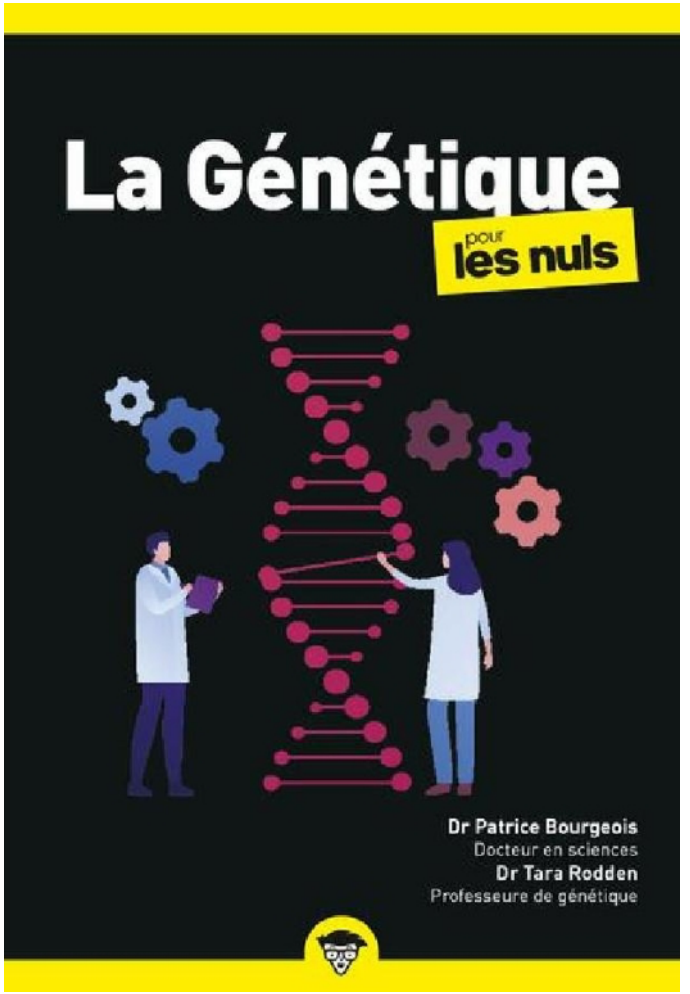
Fox Keller (2010)

The zombie fly

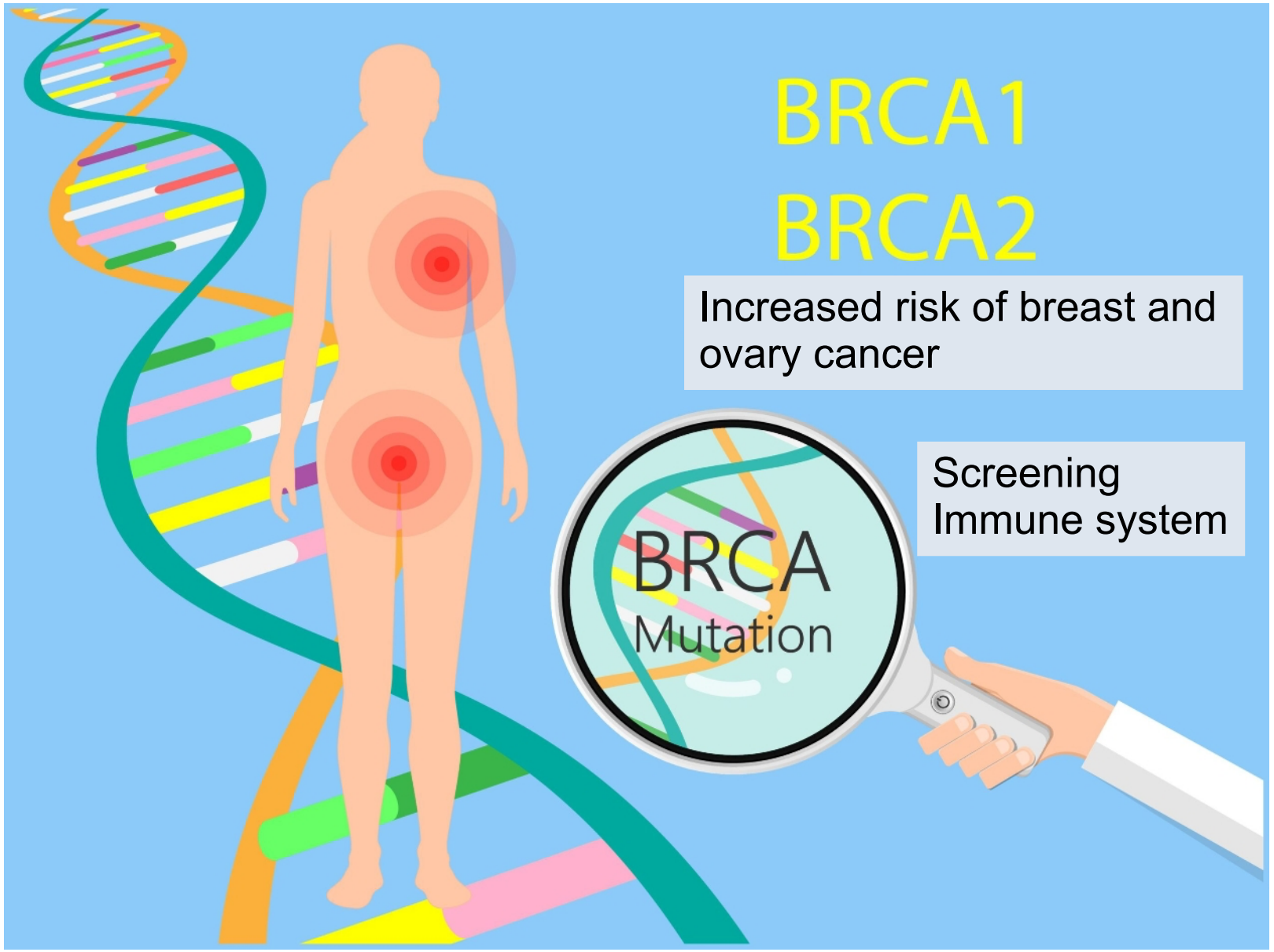
Entomophthora muscae
Parasite of
Musca domestica

https://www.biorxiv.org/content/10.1101/2021.10.21.465334v1


RICHARD LEWONTIN
THE TRIPLE HELIX
gene
organism
and environment


La Génétique
pour
les nuls
Dr Patrice Bourgeois
Docteur en sciences
Dr Tara Rodden
Professeure de génétique

Exercices!


BRCA1
BRCA2
Increased risk of breast and ovary cancer
Screening
Immune system
BRCA
Mutation

érable sycomore (Acer pseudoplatanus)