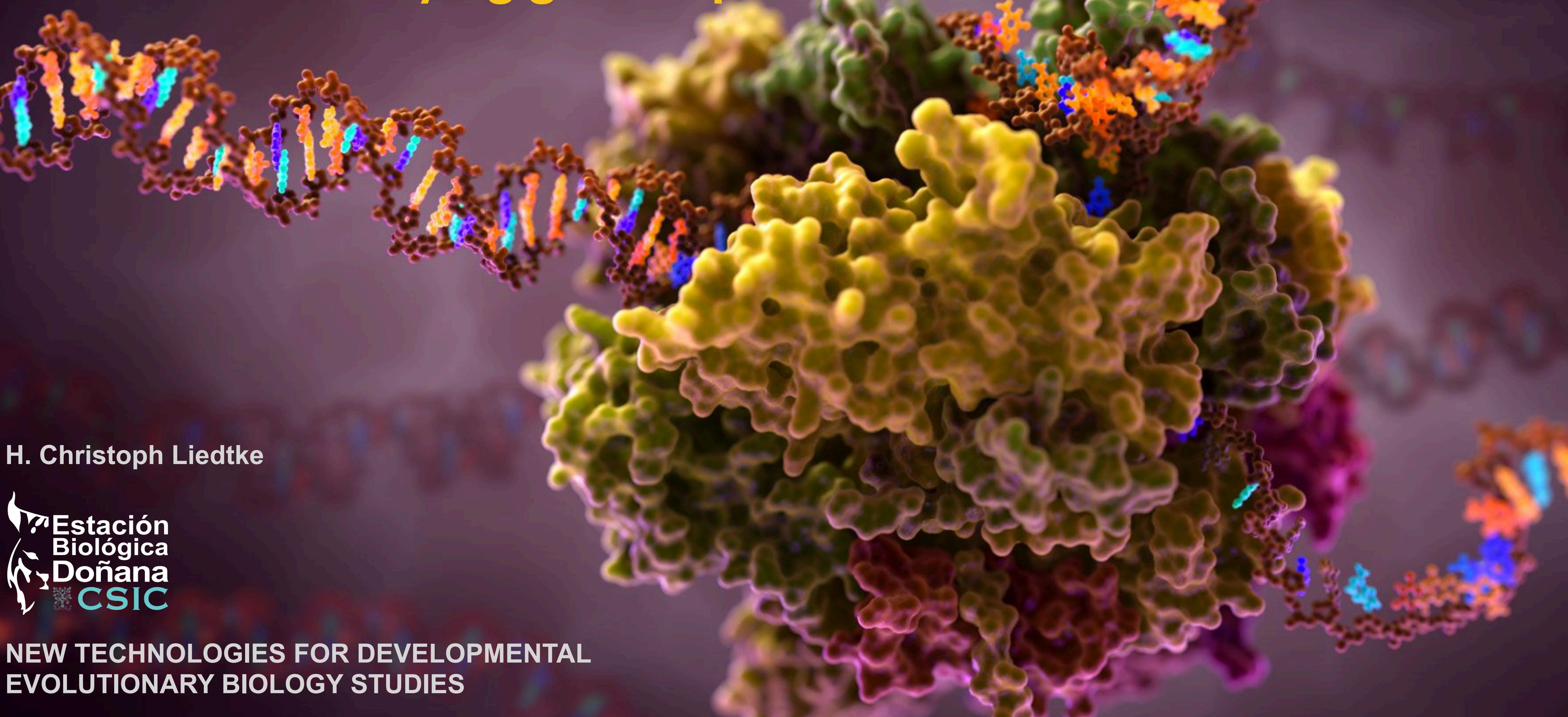


Plastic Phenotypes

And their underlying gene expression



H. Christoph Liedtke



NEW TECHNOLOGIES FOR DEVELOPMENTAL
EVOLUTIONARY BIOLOGY STUDIES

What generates phenotypic diversity?



Development

Evolution

Phenotype

Ecology

Development

Evolution

Phenotype

Ecology

Outline

Hands-off

- The definition of evolution according to the modern synthesis
- Eco-evo-devo thinking, and plastic phenotypes in evolutionary biology
- Transcribing genotypes to phenotypes
- RNAseq as a “new” toolkit for studying plastic phenotypes

Hands-on

- Introduction to the Tidyverse for programming in R [phenotypic plasticity practical]
- Exploring gene expression data with the Tidyverse and DESeq2 [RNAseq practical]

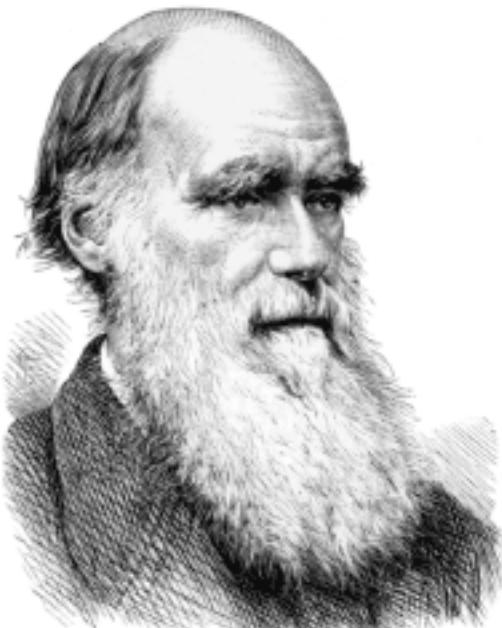
What is the definition of evolution?

Evolution consists of changes in the heritable traits of a population of organisms as successive generations replace one another

National Academy of Sciences, USA



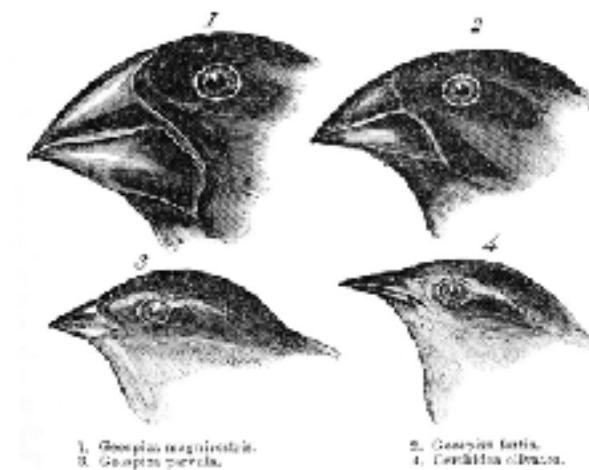
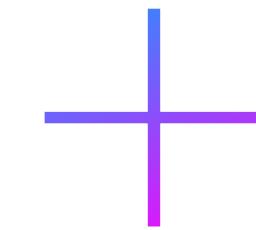
The Modern Synthesis



CHARLES DARWIN



ALFRED RUSSEL WALLACE



Huxley



Stebbins



Simpson



Mayr



Haldane



Fisher



Wright



Dobzhansky

The Modern Synthesis

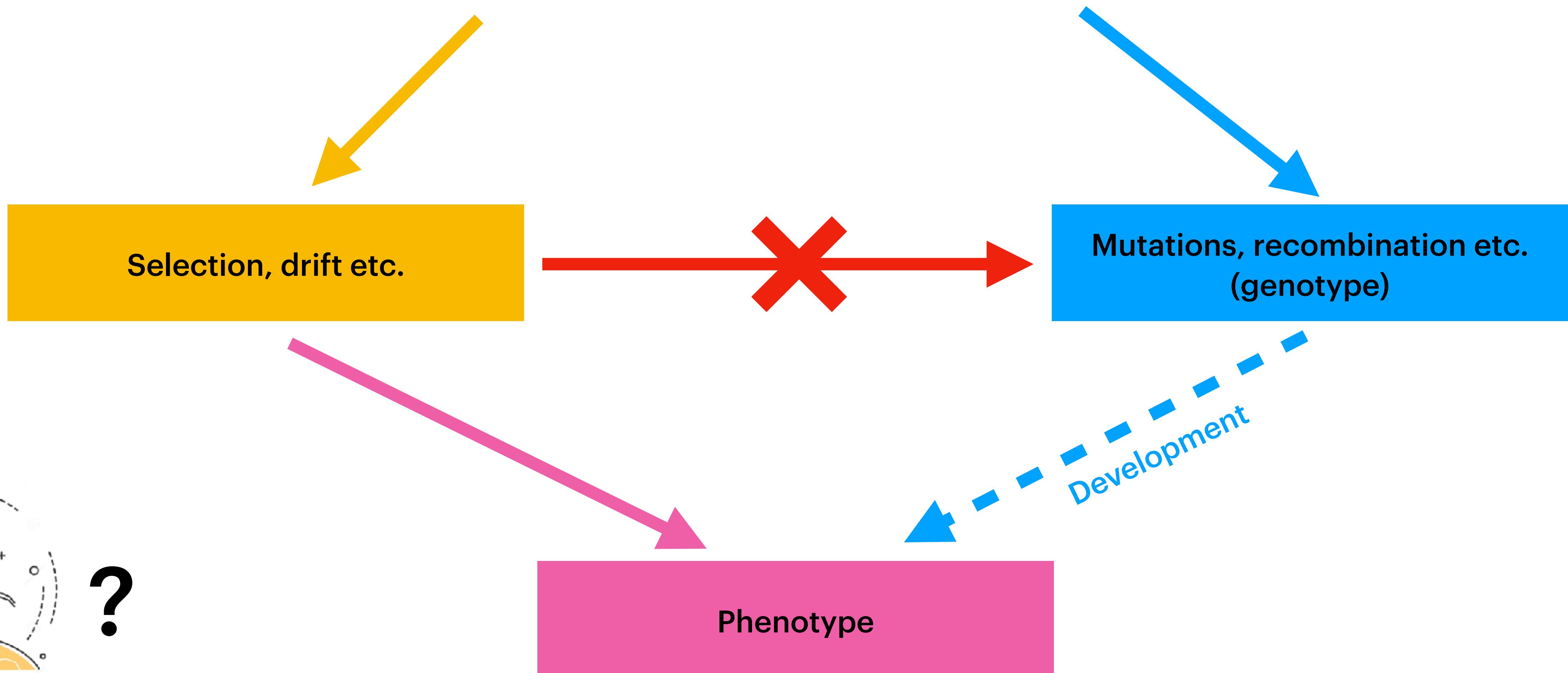
- Populations harbour genetic variation that emerges randomly through mutation and recombination.
- Populations evolve through changes in allele frequencies due to drift, gene flow, and especially, natural selection.
- The majority of genetic variants have a small phenotypic effect, so that the resulting phenotypic changes are gradual.
- Diversification occurs through speciation, which is mostly the result of reproductive isolation between allopatric populations.
- These processes, operating over long enough timescales, give rise to cumulative changes of such magnitude that the resulting species are grouped at higher taxonomic levels.

The Modern Synthesis

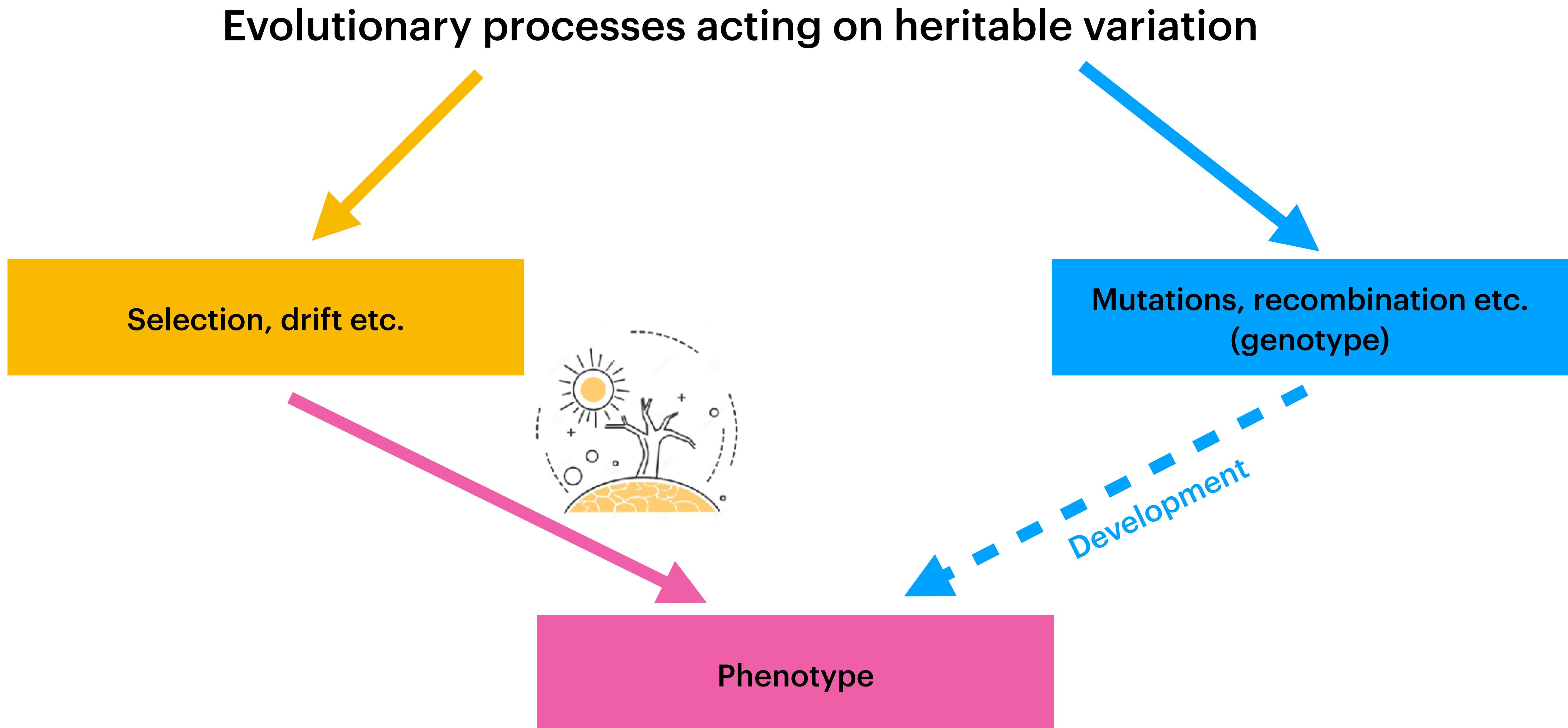
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How do phenotypes evolve?

Evolutionary processes acting on heritable variation



How do phenotypes evolve?



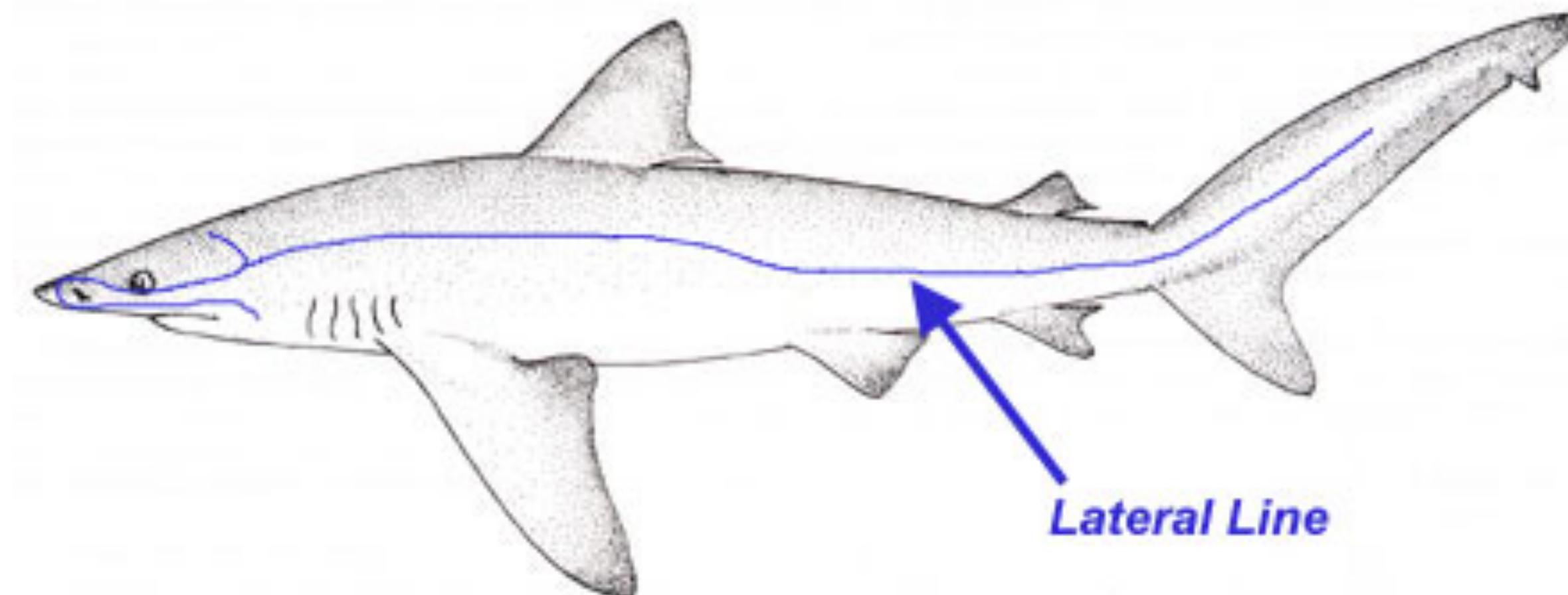
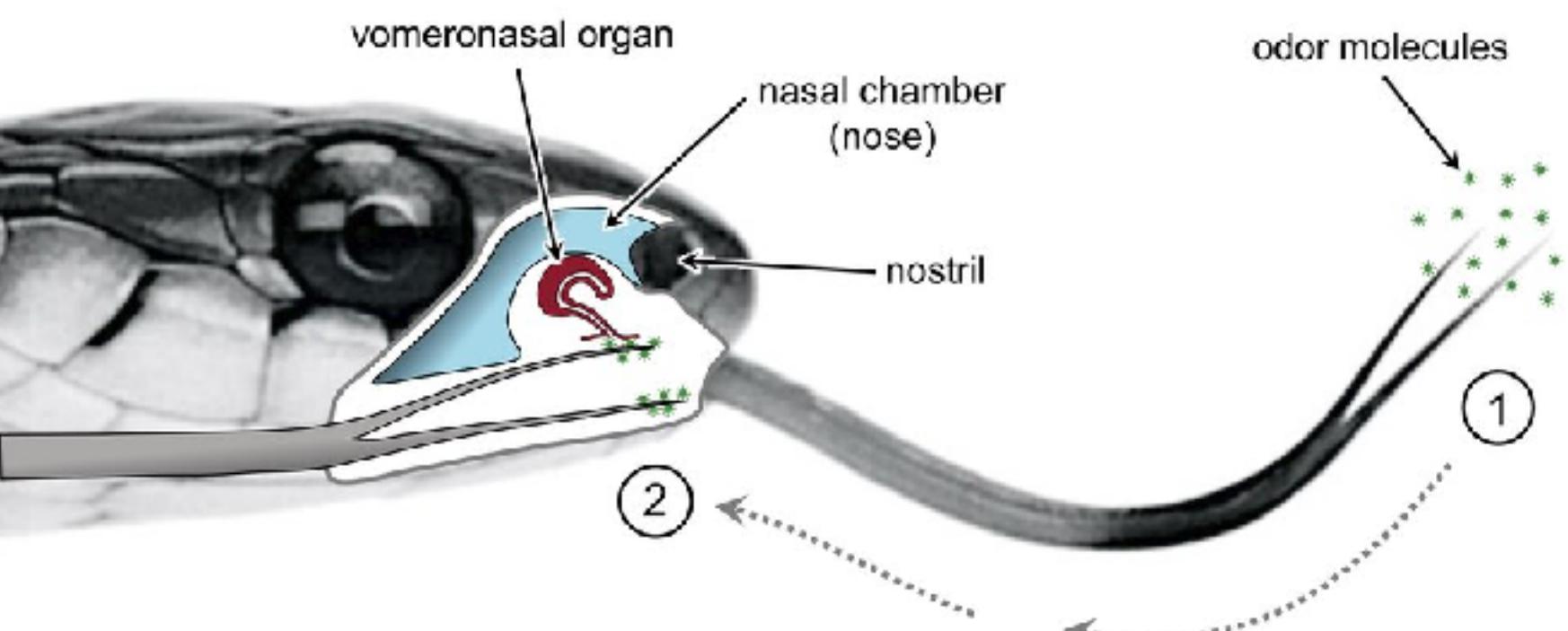
The environment as a passive filter?



The environment is dynamic



Organisms sense their environment



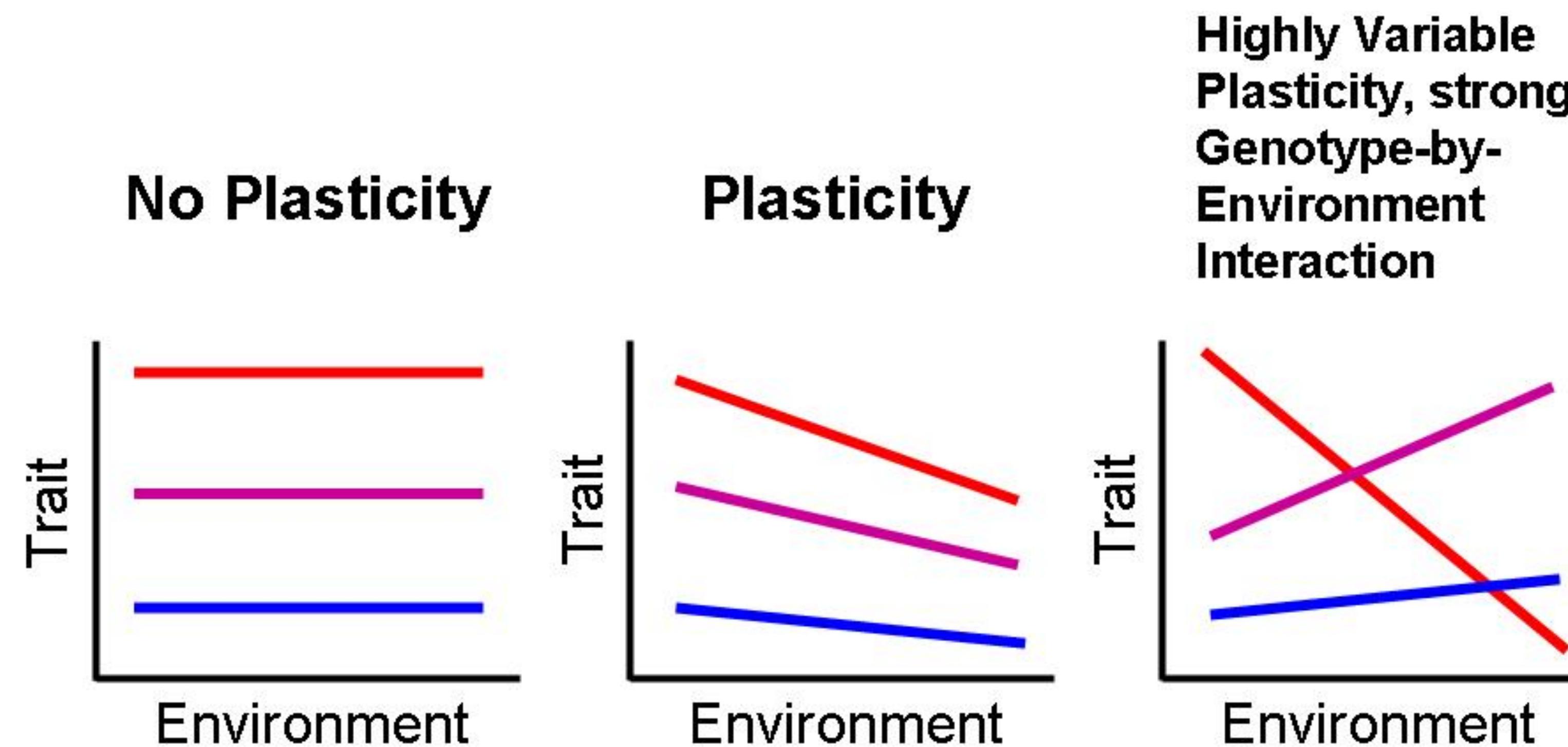
Phenotypes are environment-dependent



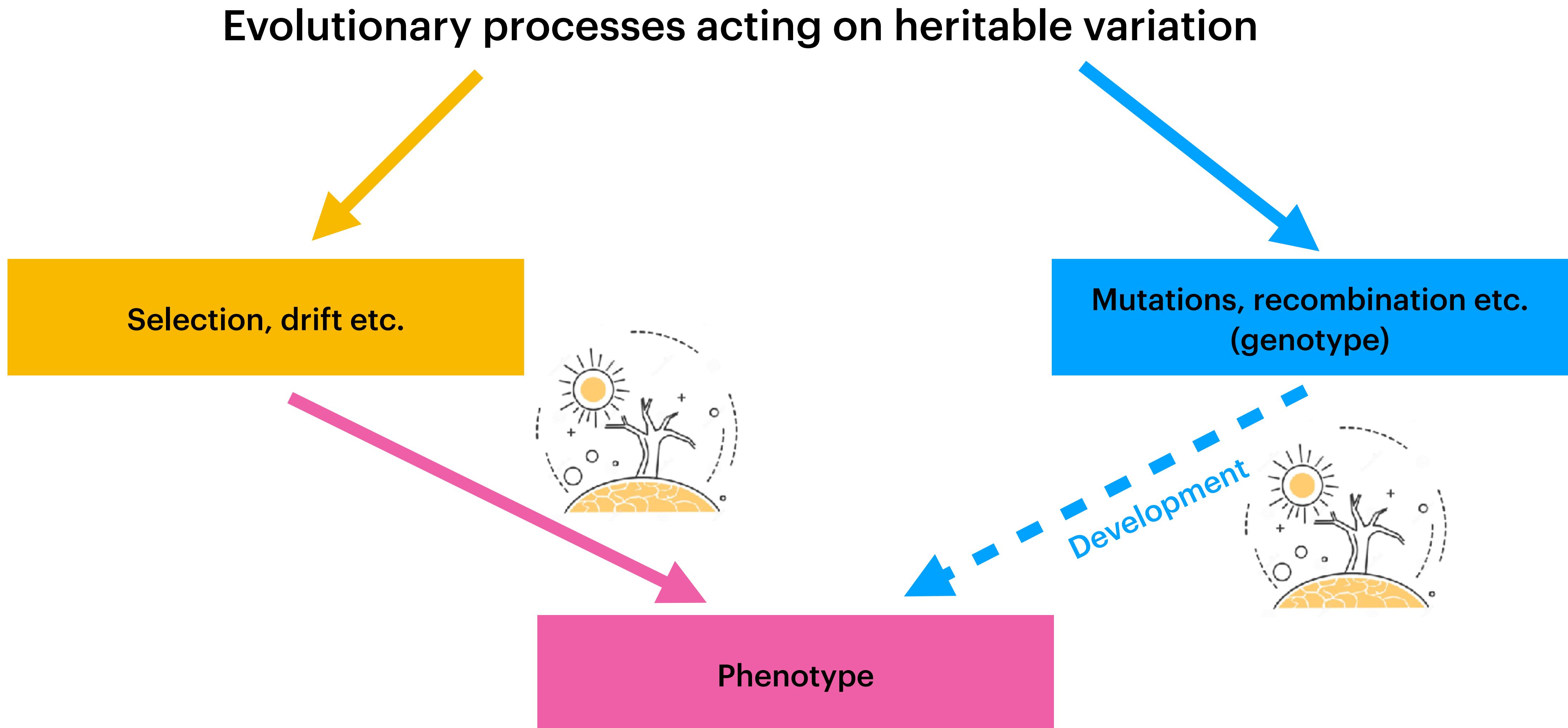
Nemoria arizonaria, Greene 1989 Nature

Phenotypic plasticity

Changes in an organism's behaviour, morphology and physiology (i.e. its phenotype) in response to a unique environment



How do phenotypes evolve?

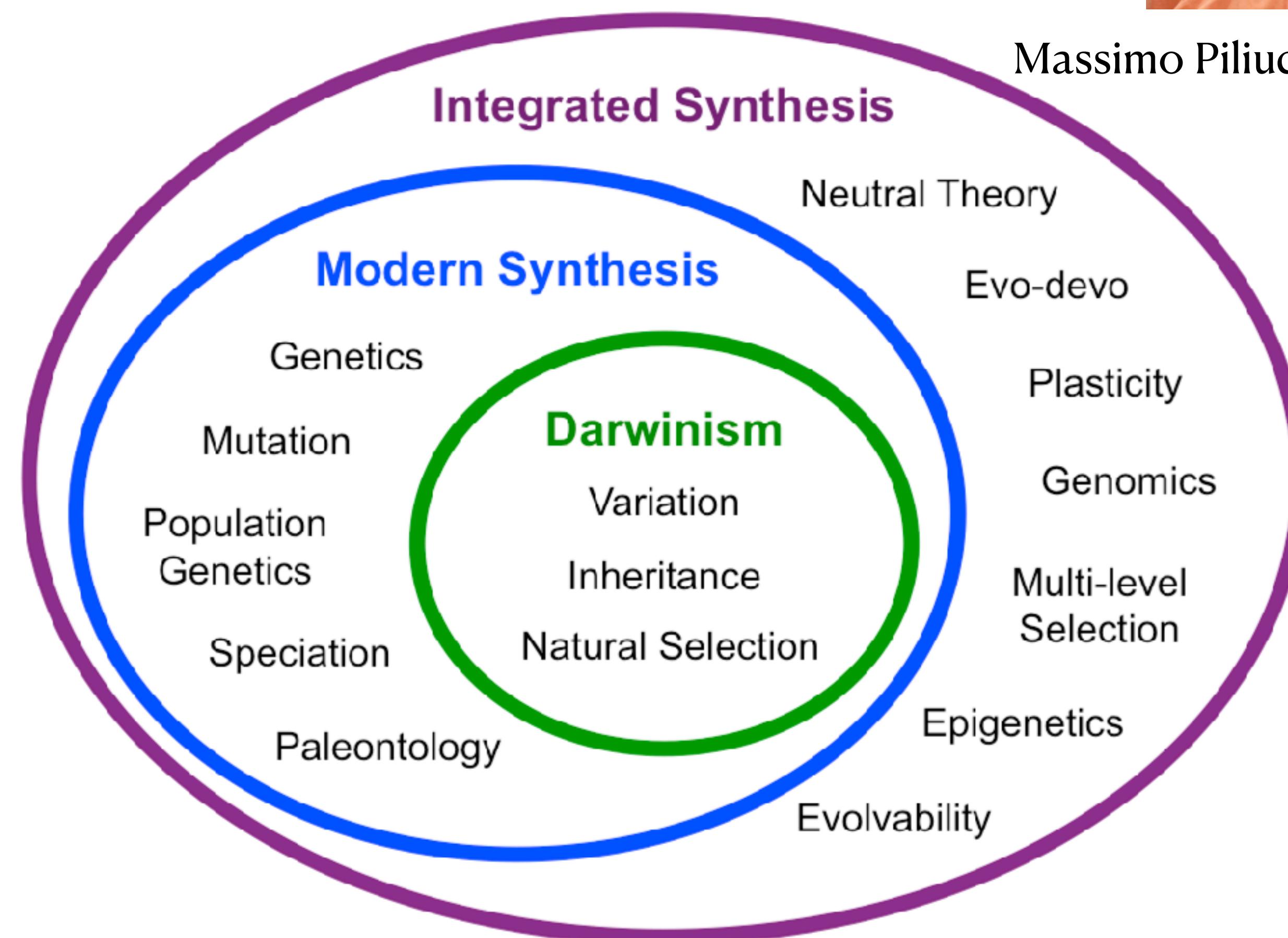




Extended Evolutionary Synthesis



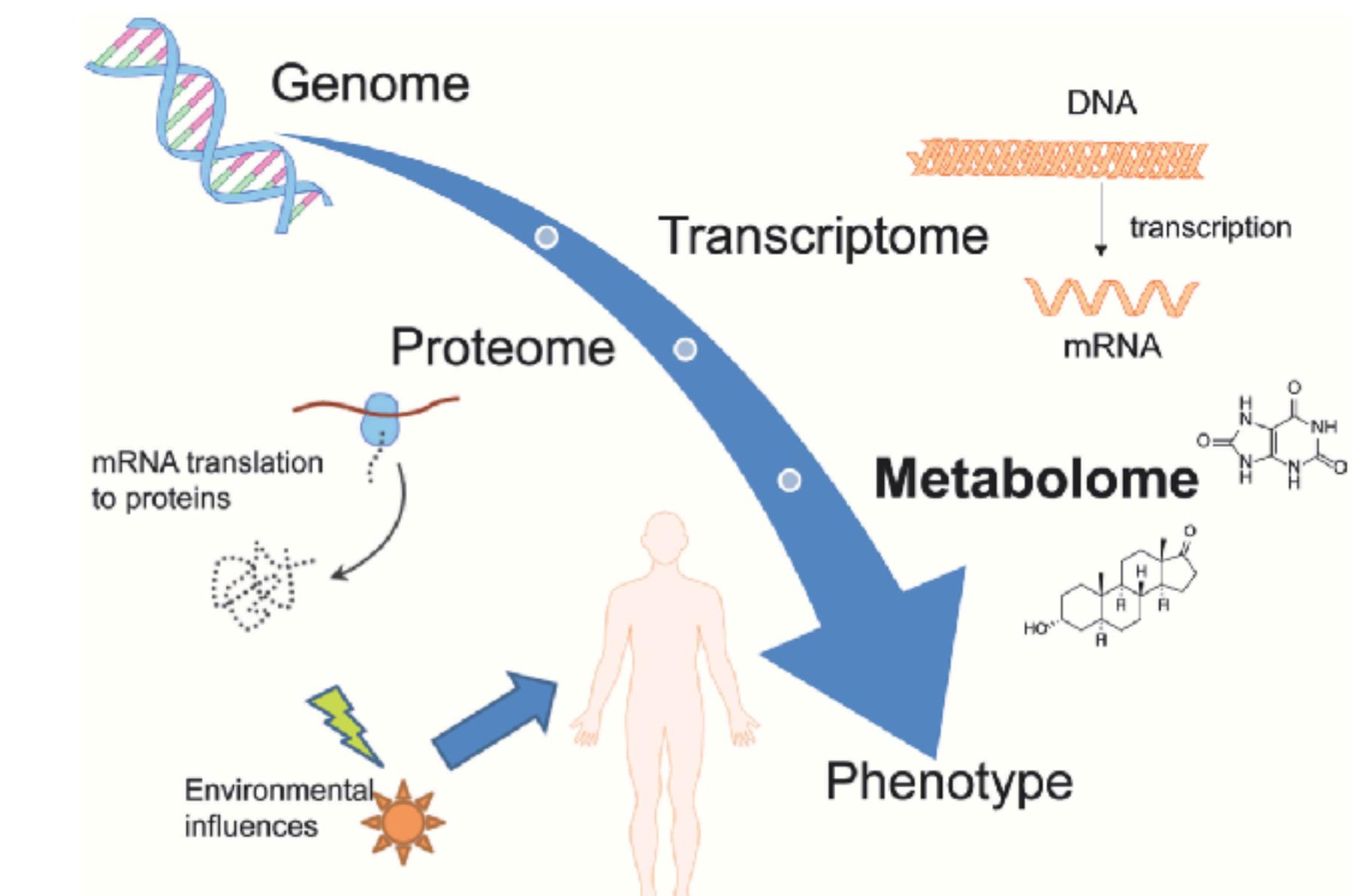
Massimo Piliucci



Data wrangling and visualisation with the Tidyverse



From genotypes to phenotypes with transcriptomics



Colour plasticity for background matching in an amphibian tadpole



H. Christoph Liedtke

Eco-Evo-Devo Group
Estación Biológica de Doñana - CSIC



Crypsis through Background Matching



Crypsis through Background Matching

Pigmentation plasticity



Pigmentation Plasticity in Amphibians



Non-breeding male



Breeding male



No predators



Predators



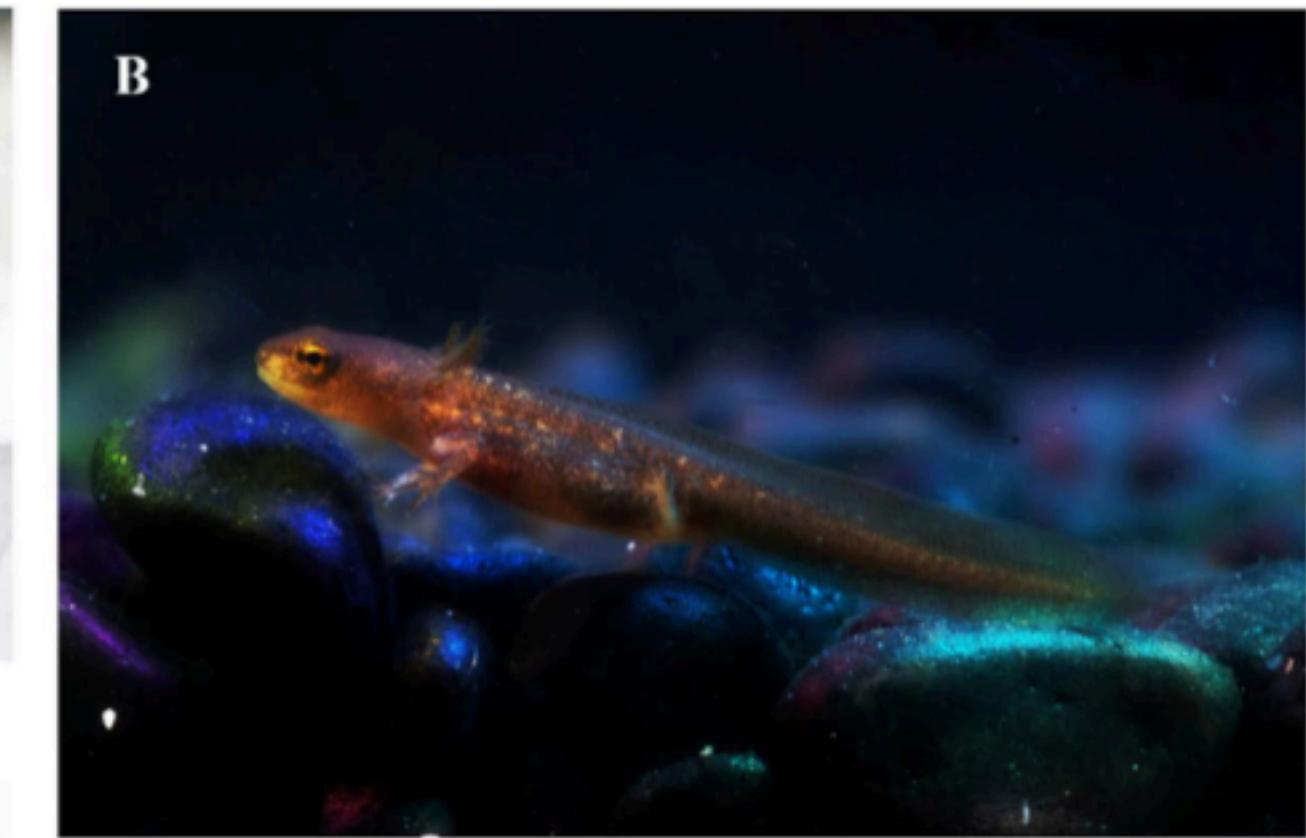
Night



Day



Light background



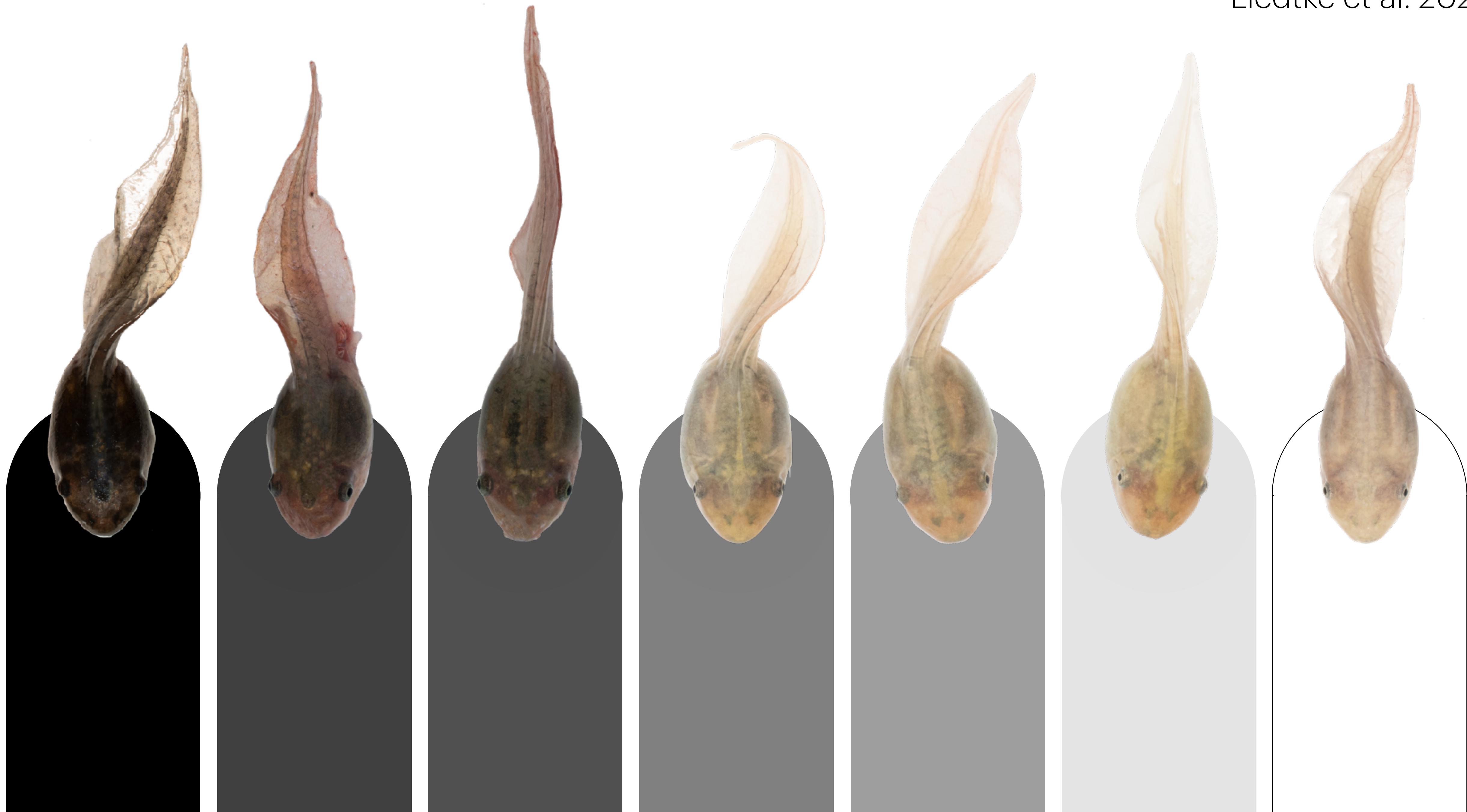
Dark background

Pelobates cultripes

Western Spadefoot toad





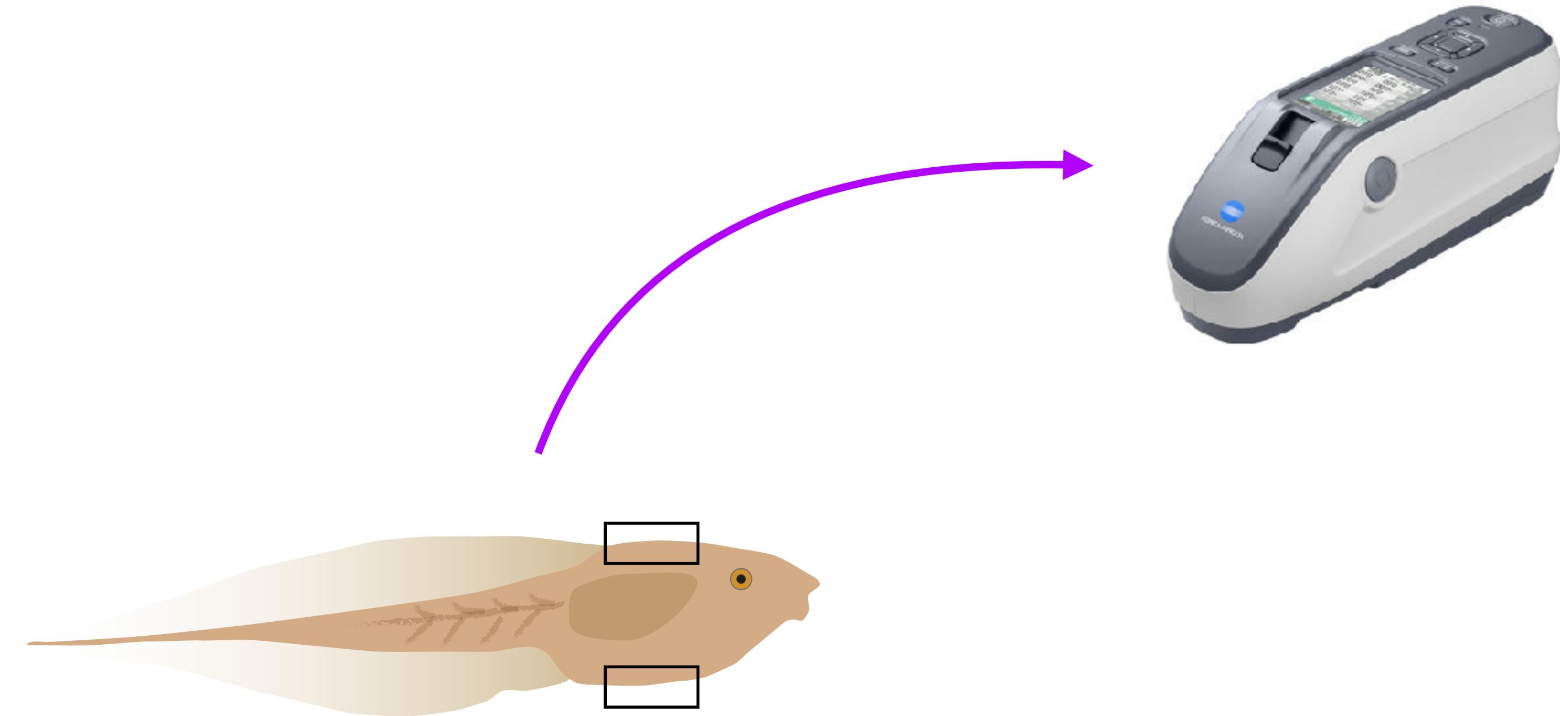


Colour change is reversible



Background matching experiment

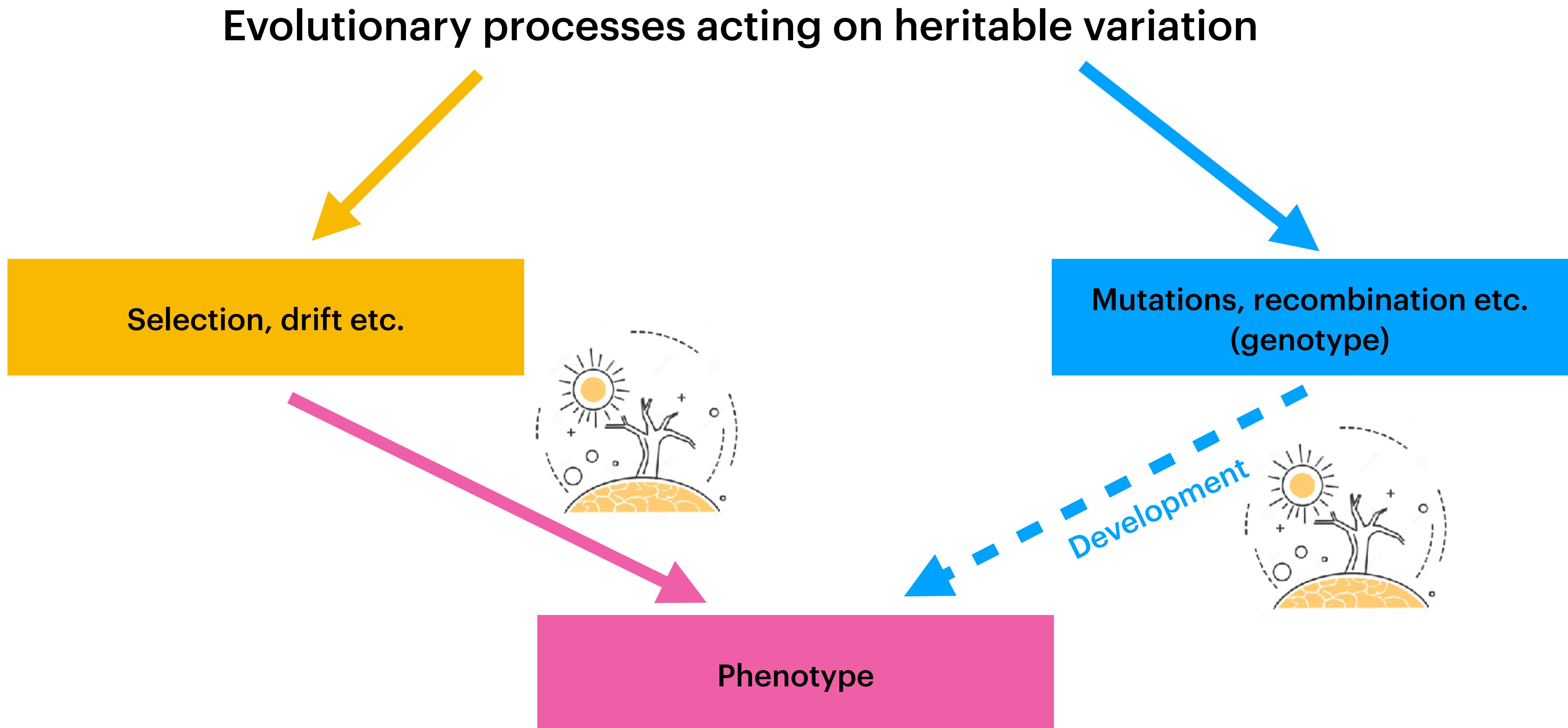
Spectrophotometry



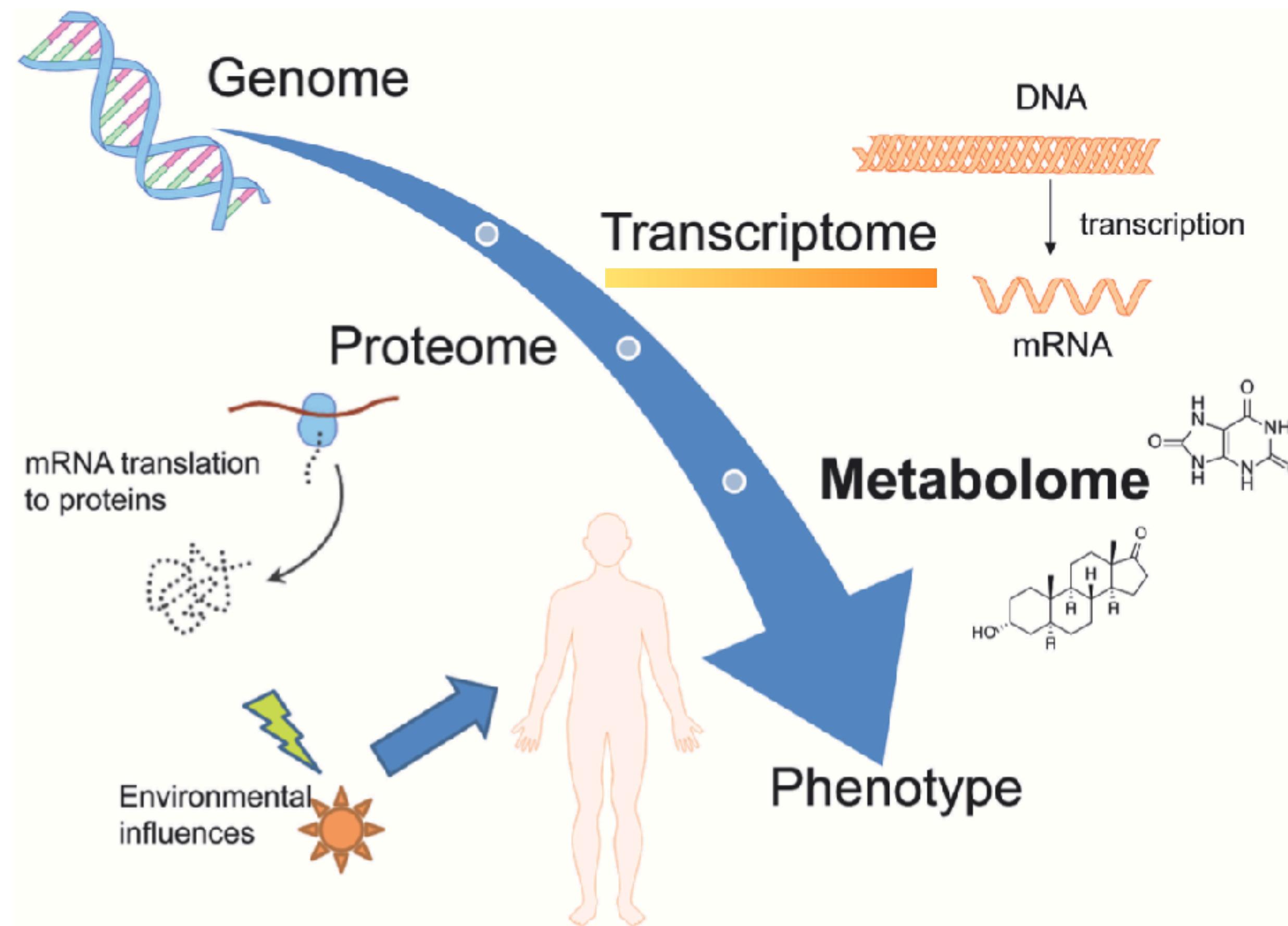
Data wrangling and visualisation with the Tidyverse

https://hcliedtke.github.io/UAM_NewTech

How do phenotypes evolve?

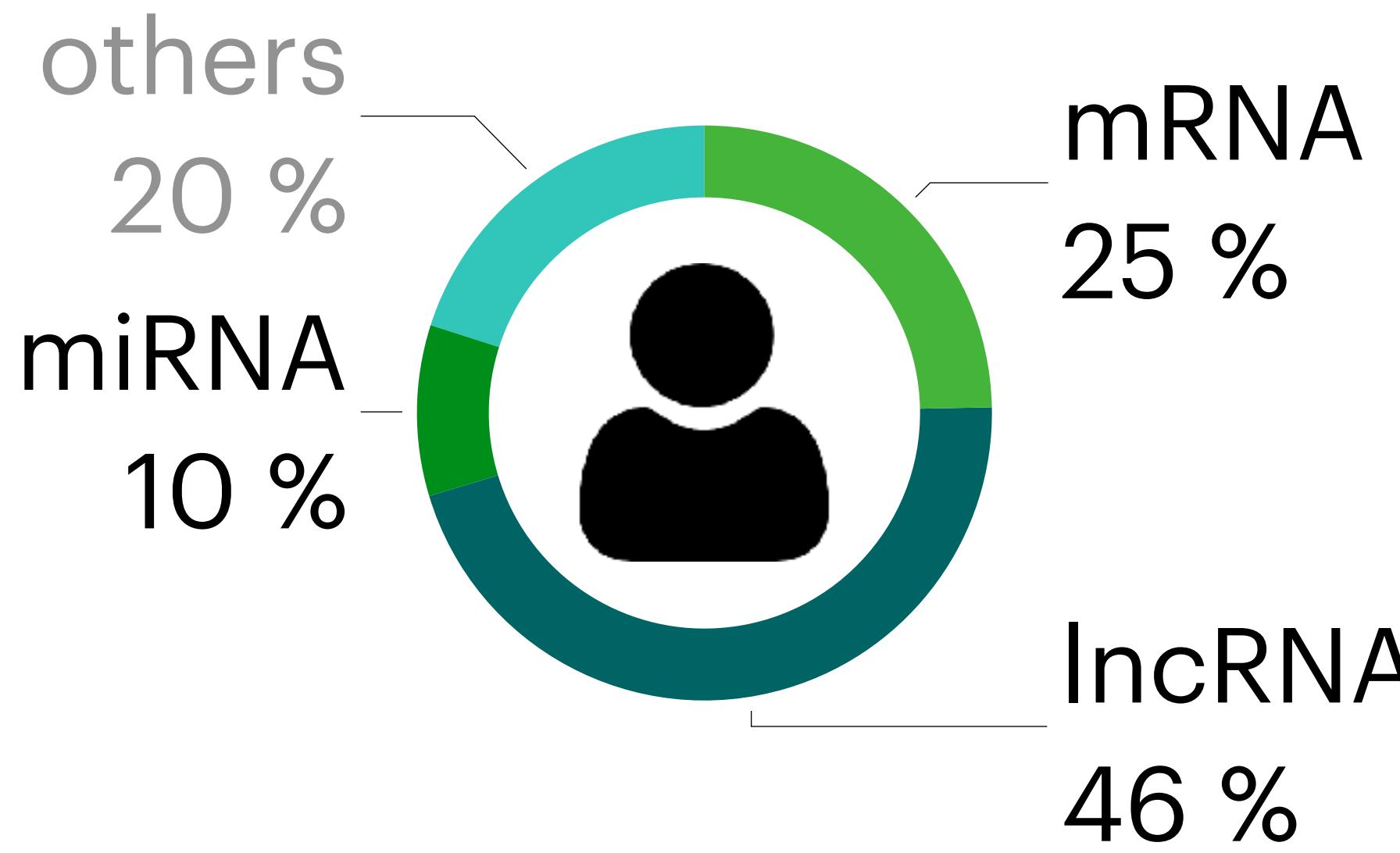


From genotype to phenotype



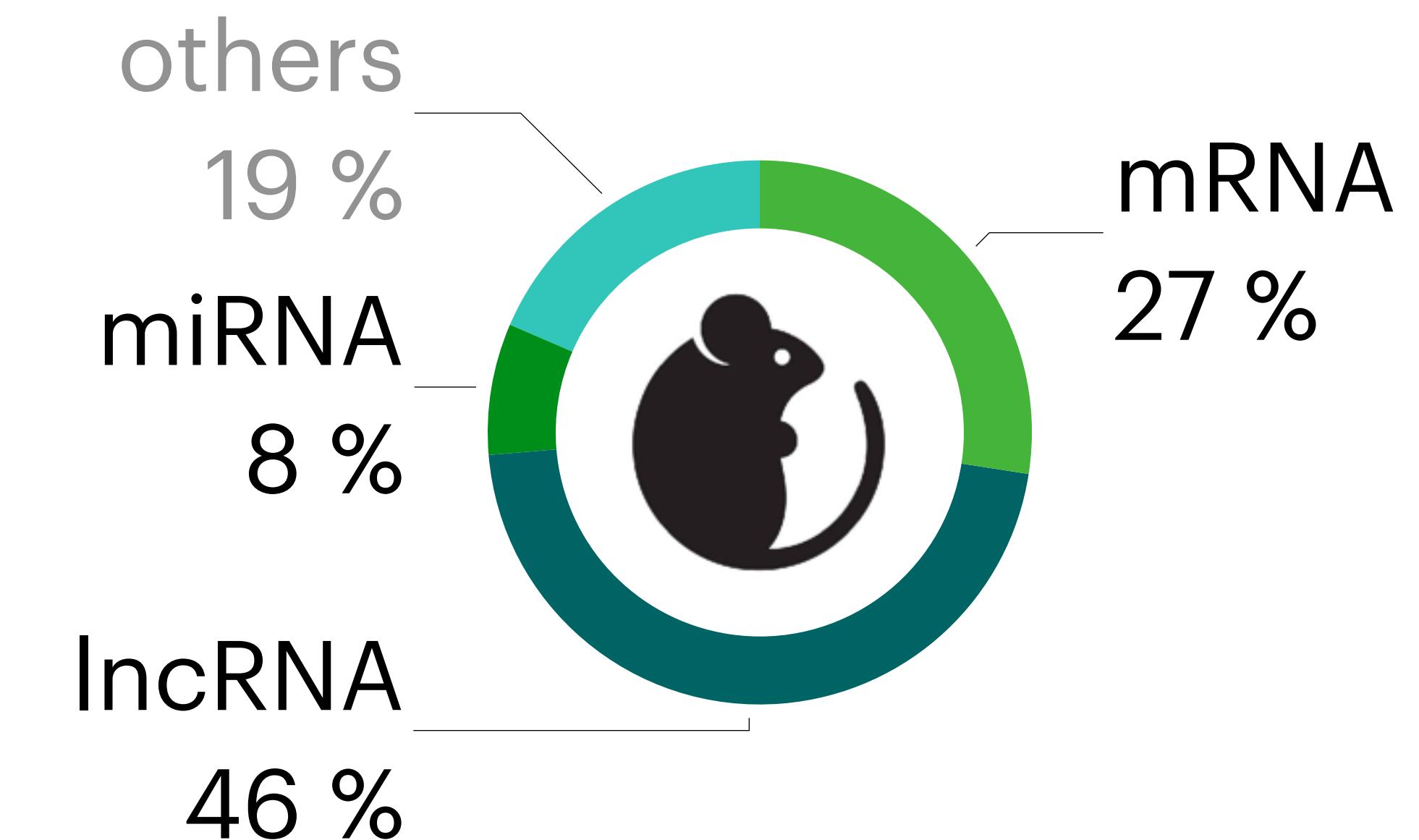
What is the transcriptome?

the set of all RNA transcripts (coding and non-coding) in an individual or a population of cells.



3.2bp genome

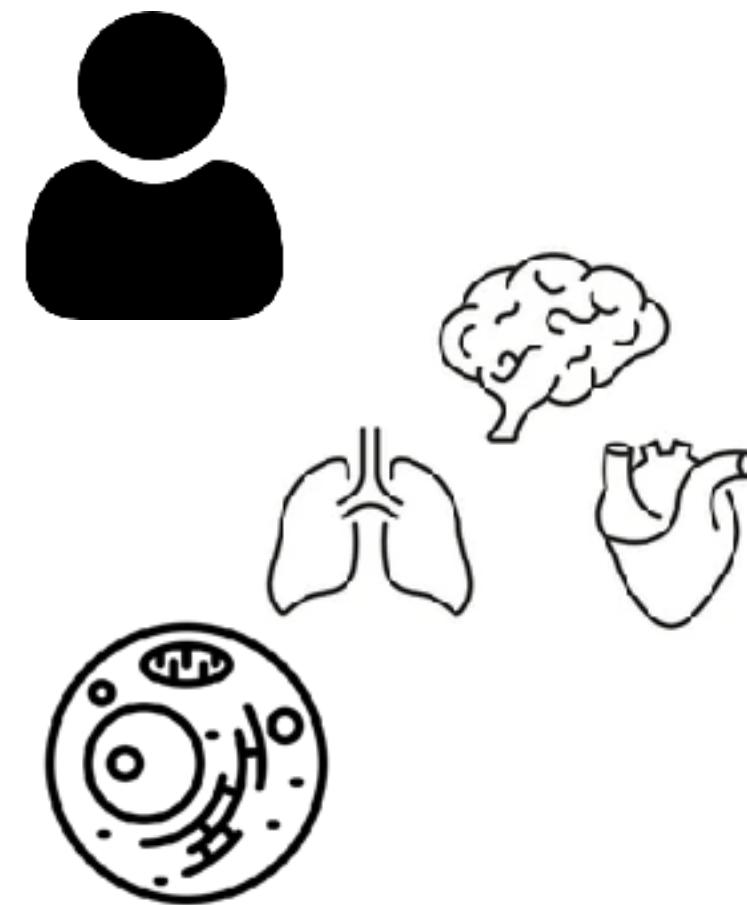
78724 genes



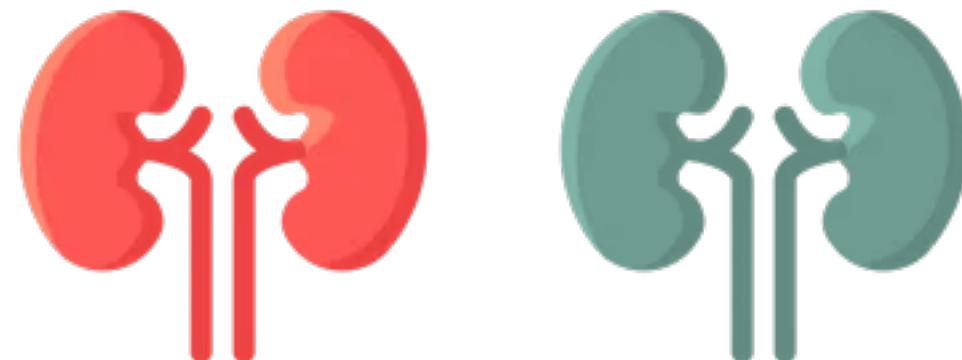
2.7bp genome

78239 genes

What is the transcriptome?



- Organism > organ > tissue > cell



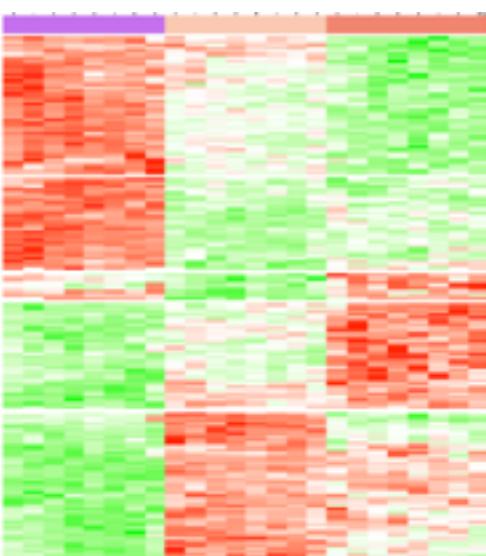
- Variable and state-dependent

How do we study the transcriptome?



Qualitatively

- Identify expressed genes
- Gene discovery



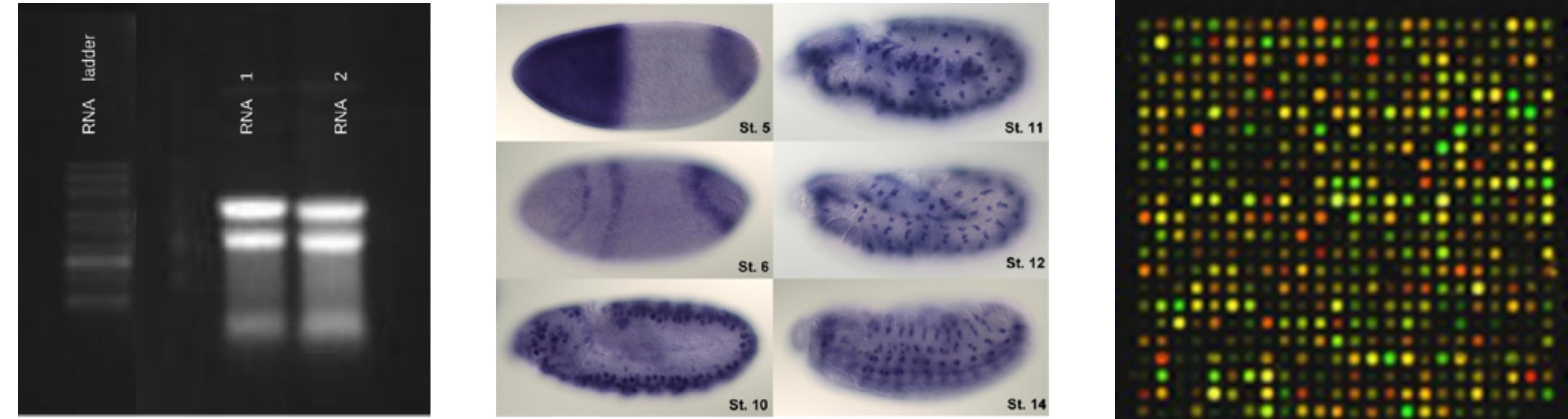
Quantitatively

- Compare expression levels of genes or transcripts

History of studying transcripts

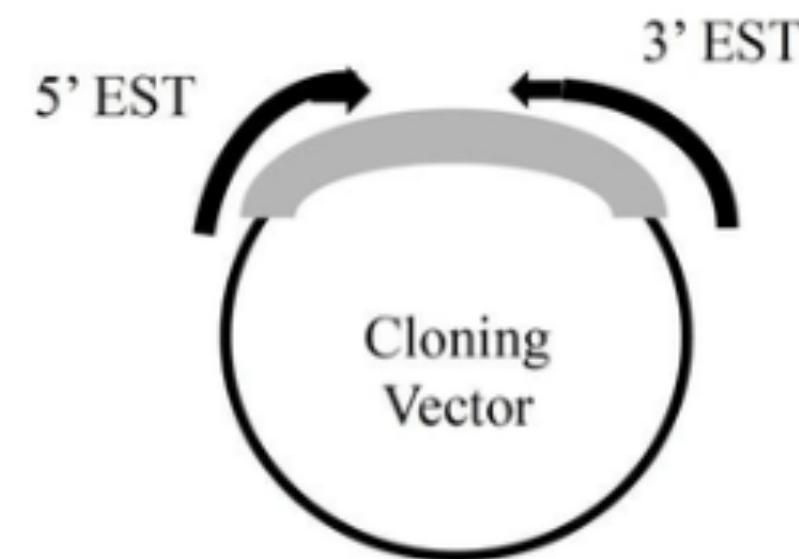
Quantitative

- Northern Blotting: 1970s
- *In situ* hybridisation: 1980s
- qPCR: 1990s
- Microarray: 1990s

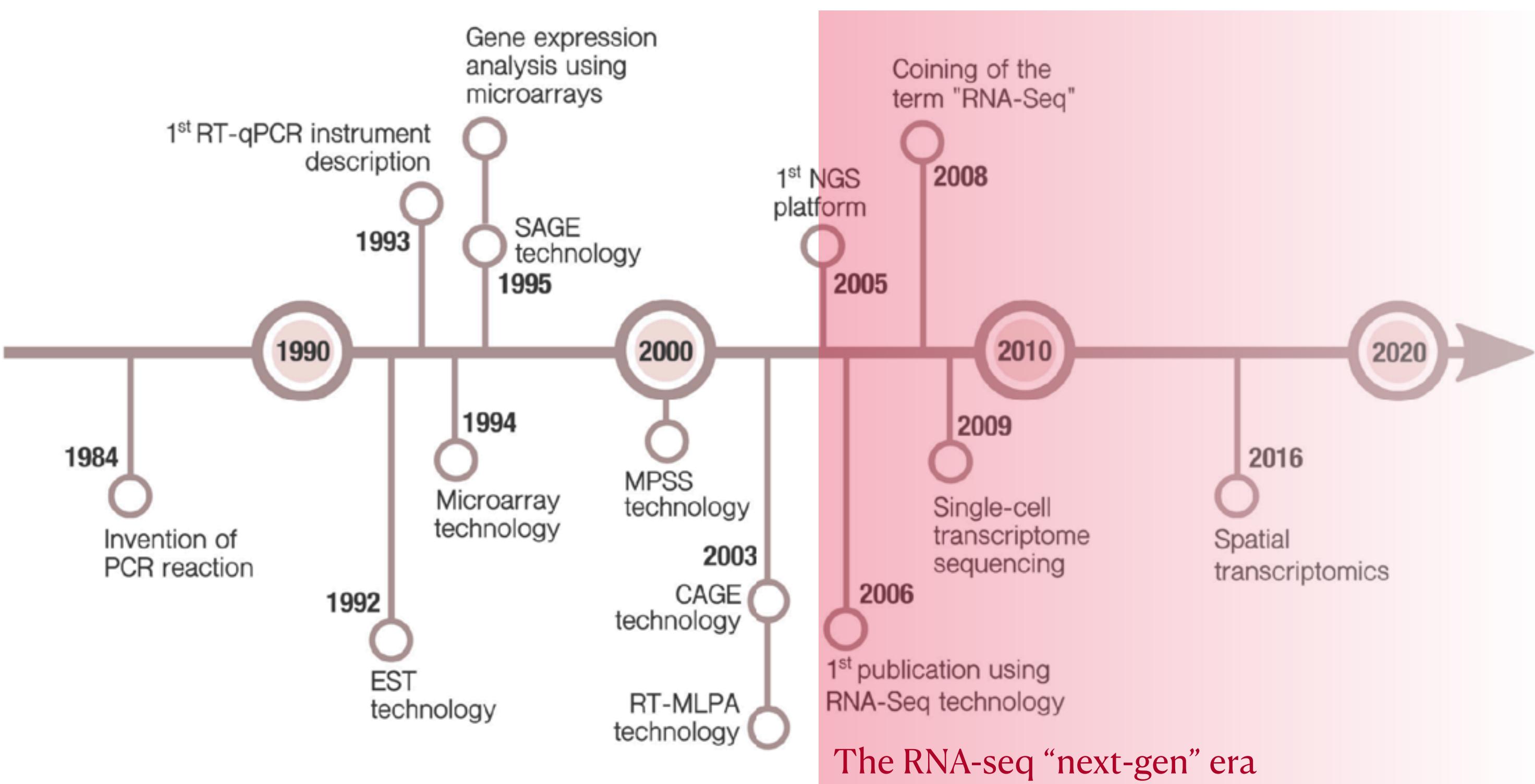


Qualitative

- Expressed sequence tags 1990s



History of studying transcripts

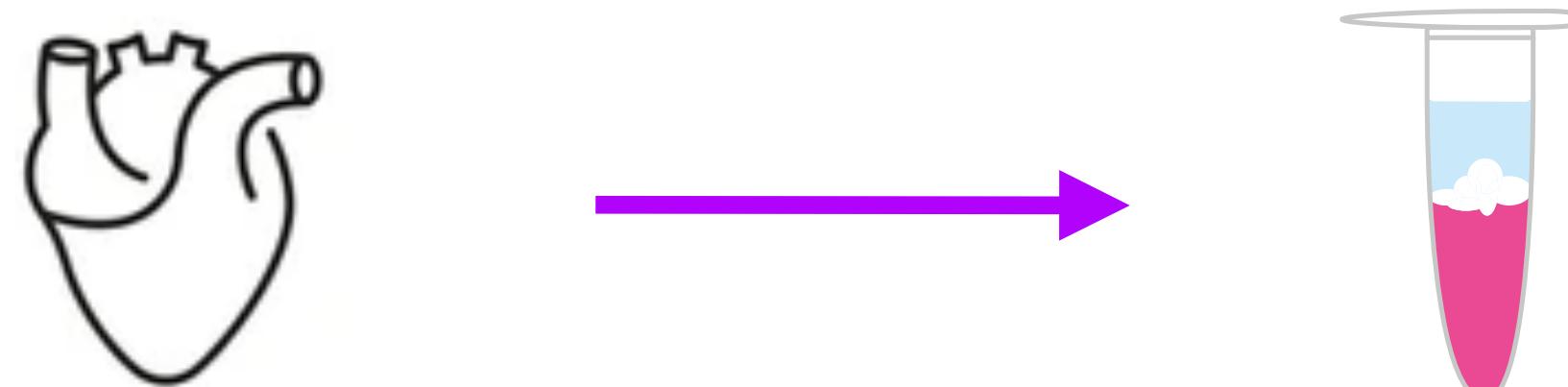


RNA-seq

- whole transcriptome at once
- no prior knowledge of gene sequences
- quantitative and qualitative

RNA-Sequencing (lab)

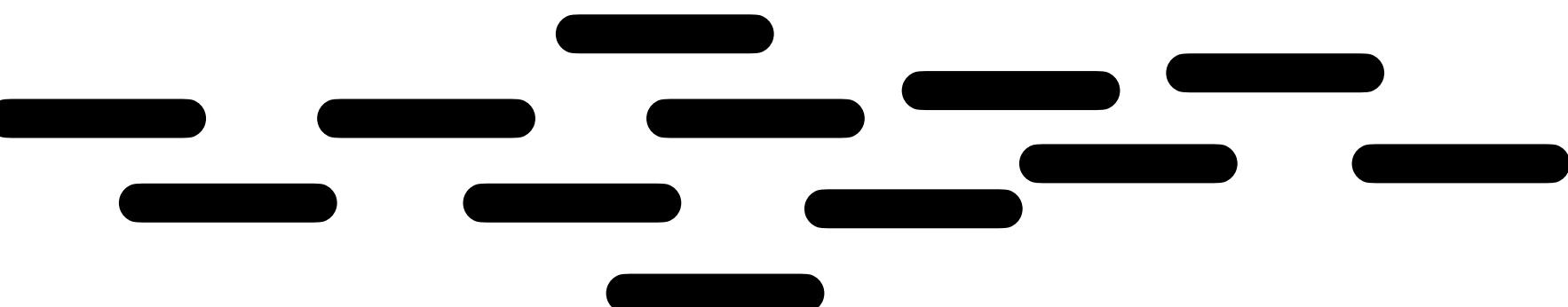
1. RNA extraction



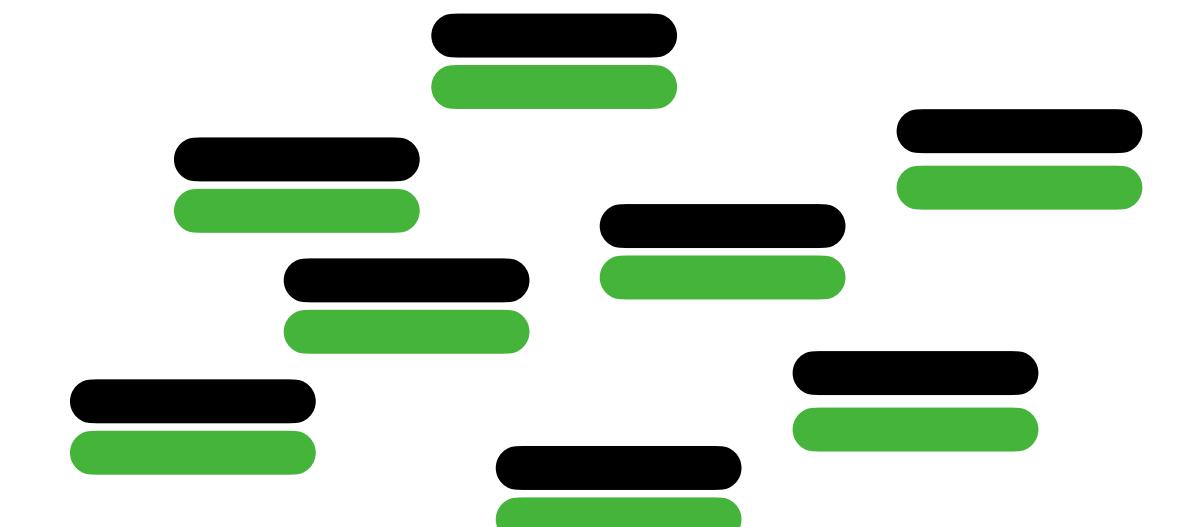
2. Isolation/purification (e.g. poly-A enrichment)



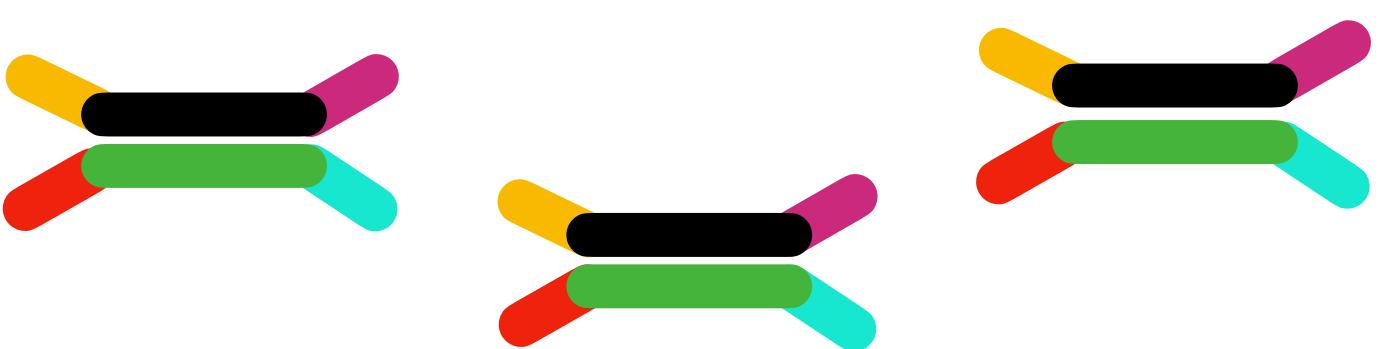
3. Fragmentation



4. cDNA synthesis



5. Adaptor ligation

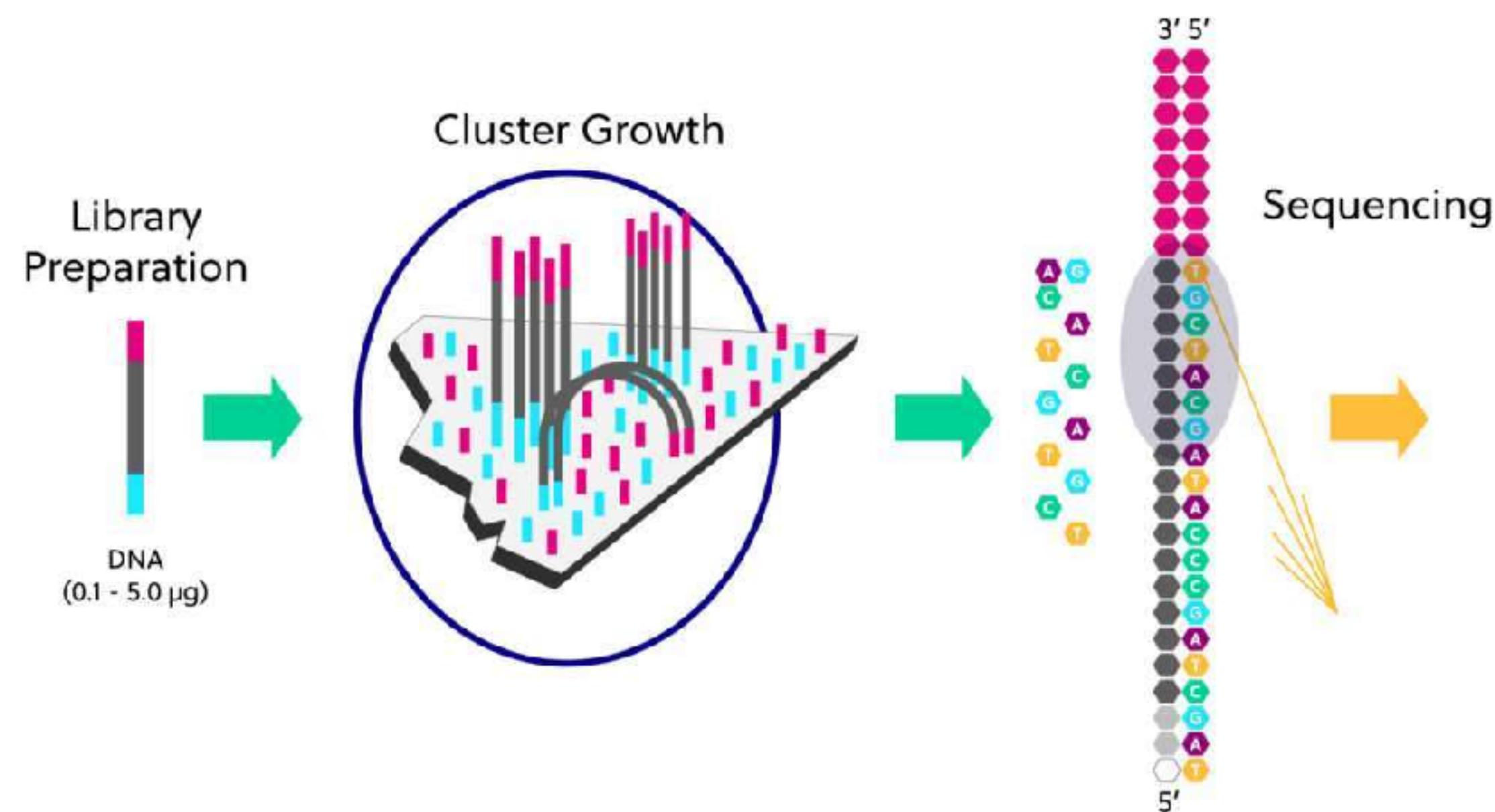


Parallel sequencing



Next-Gen Sequencing

Illumina short reads technology



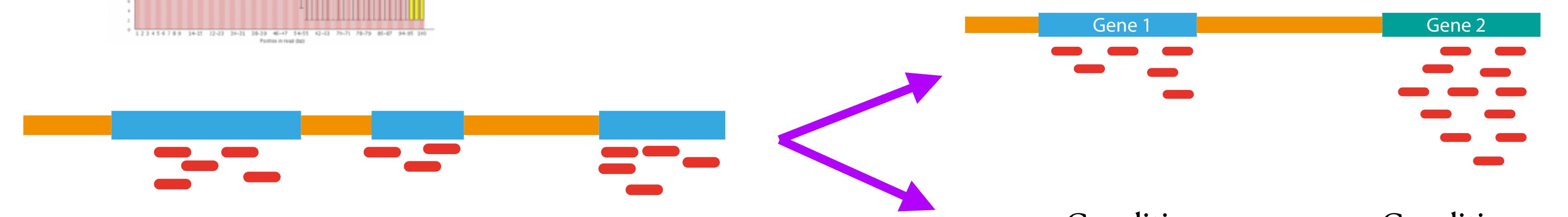
- Usual read length: 100bp
 - Usual quantity of reads for RNAseq:
20-30 million reads per sample
 - Single “lanes” allow running many samples at once (25 million - 20 billion reads depending on technology)

RNA-Sequencing (in silico)

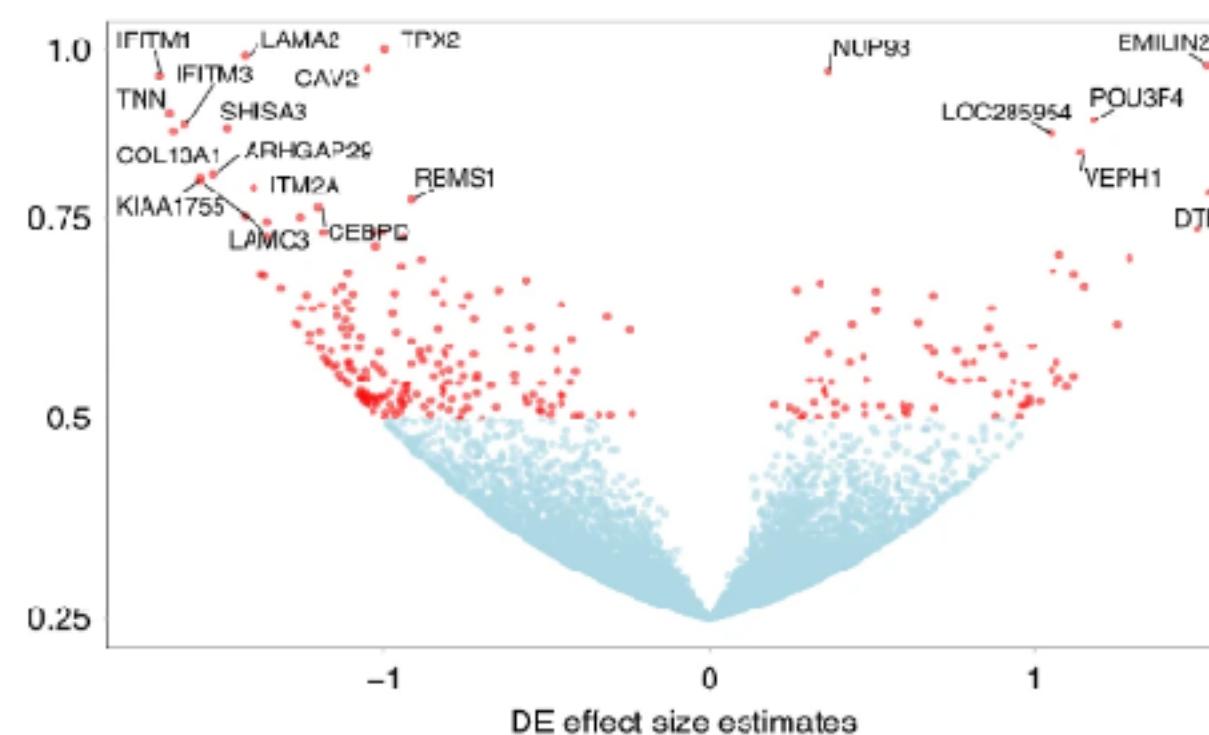
1. Sequence cleaning



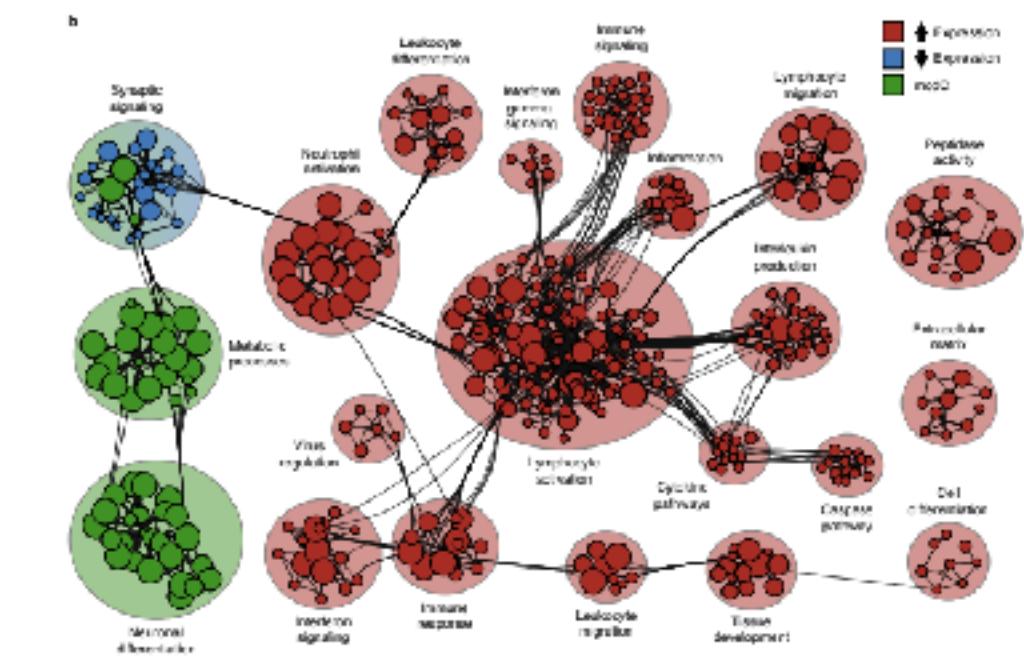
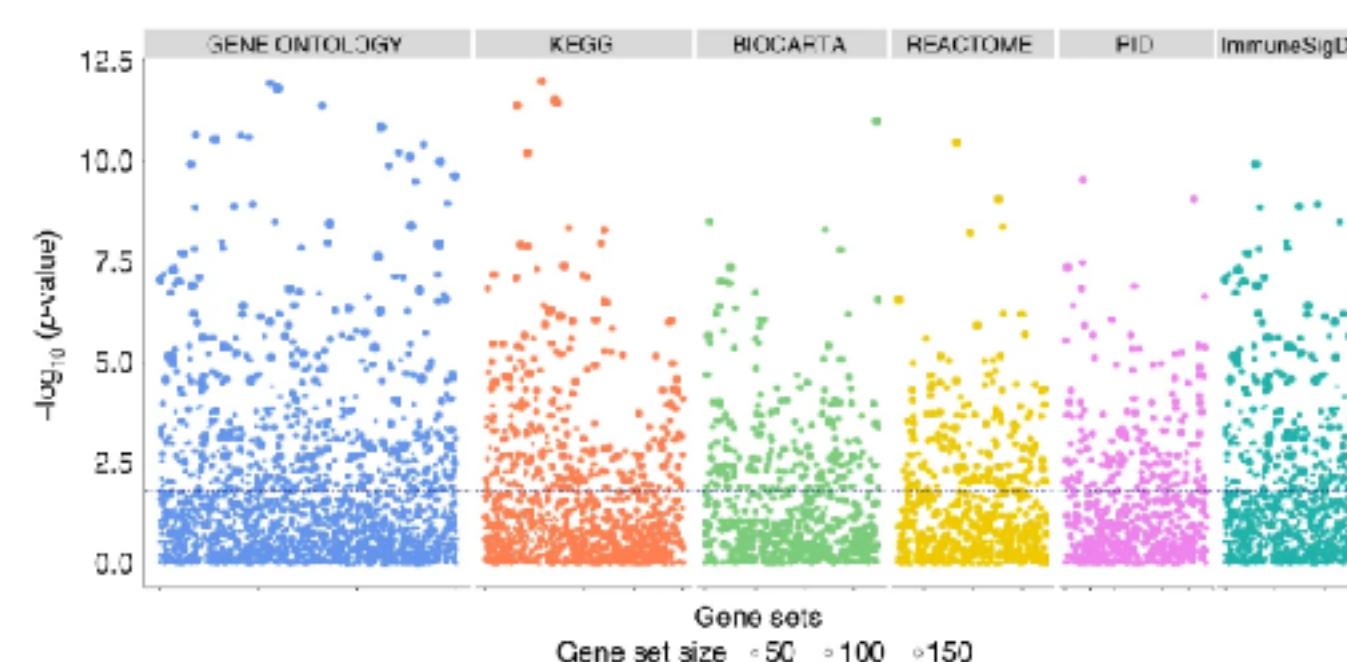
2. Mapping/quantification



3. Differential gene expression



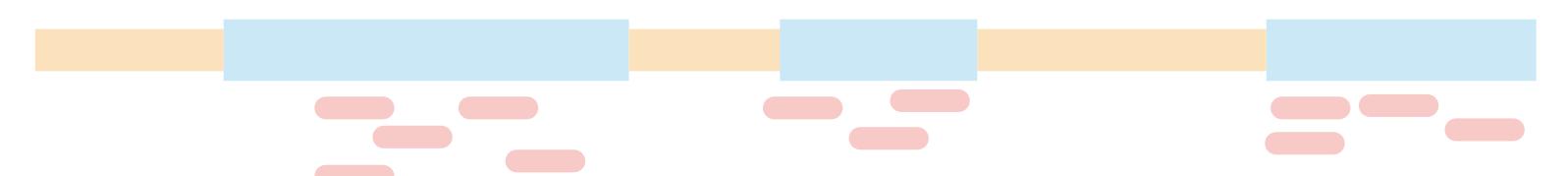
4. Functional insights



RNA-Sequencing (in silica)

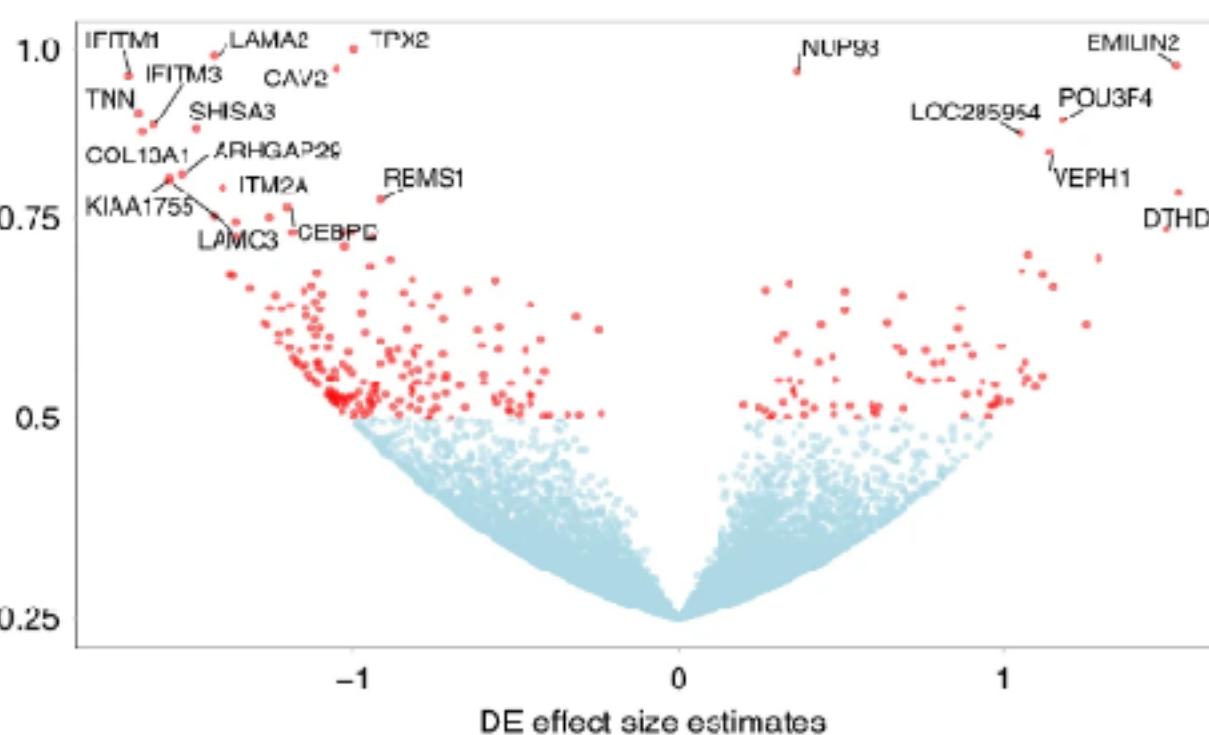
1.Sequence cleaning

2. Mapping/quantification



The diagram illustrates a genomic region with two genes, Gene 1 and Gene 2, represented by blue and green boxes respectively. Gene 1 is located between positions 100 and 300, and Gene 2 is between 300 and 500. Gene 1 has four transcripts (red ovals) spanning from approximately position 150 to 350. Gene 2 has ten transcripts spanning from approximately position 350 to 500. A large purple arrow on the left points towards Gene 1.

3. Differential gene expression



4. Functional insights

Colour plasticity for background matching in an amphibian tadpole

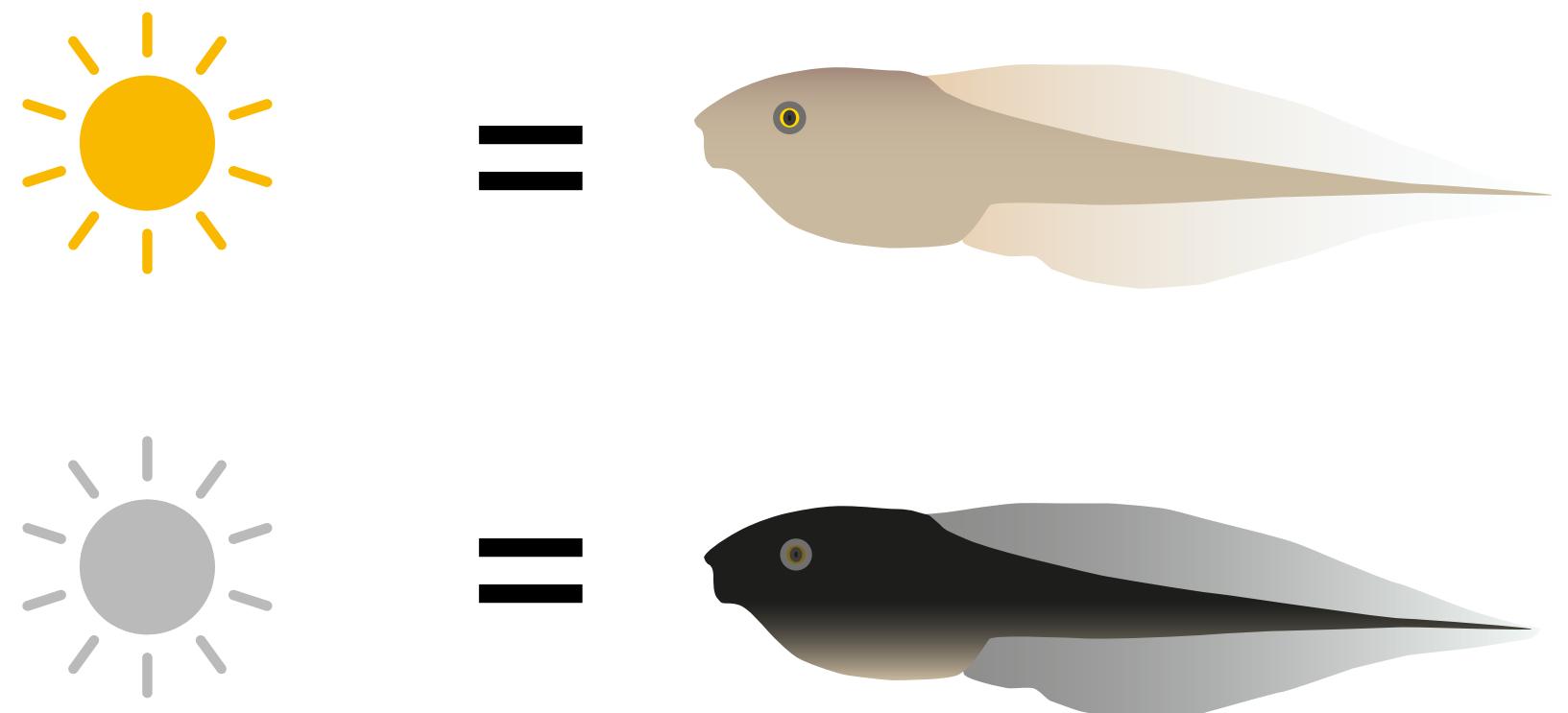


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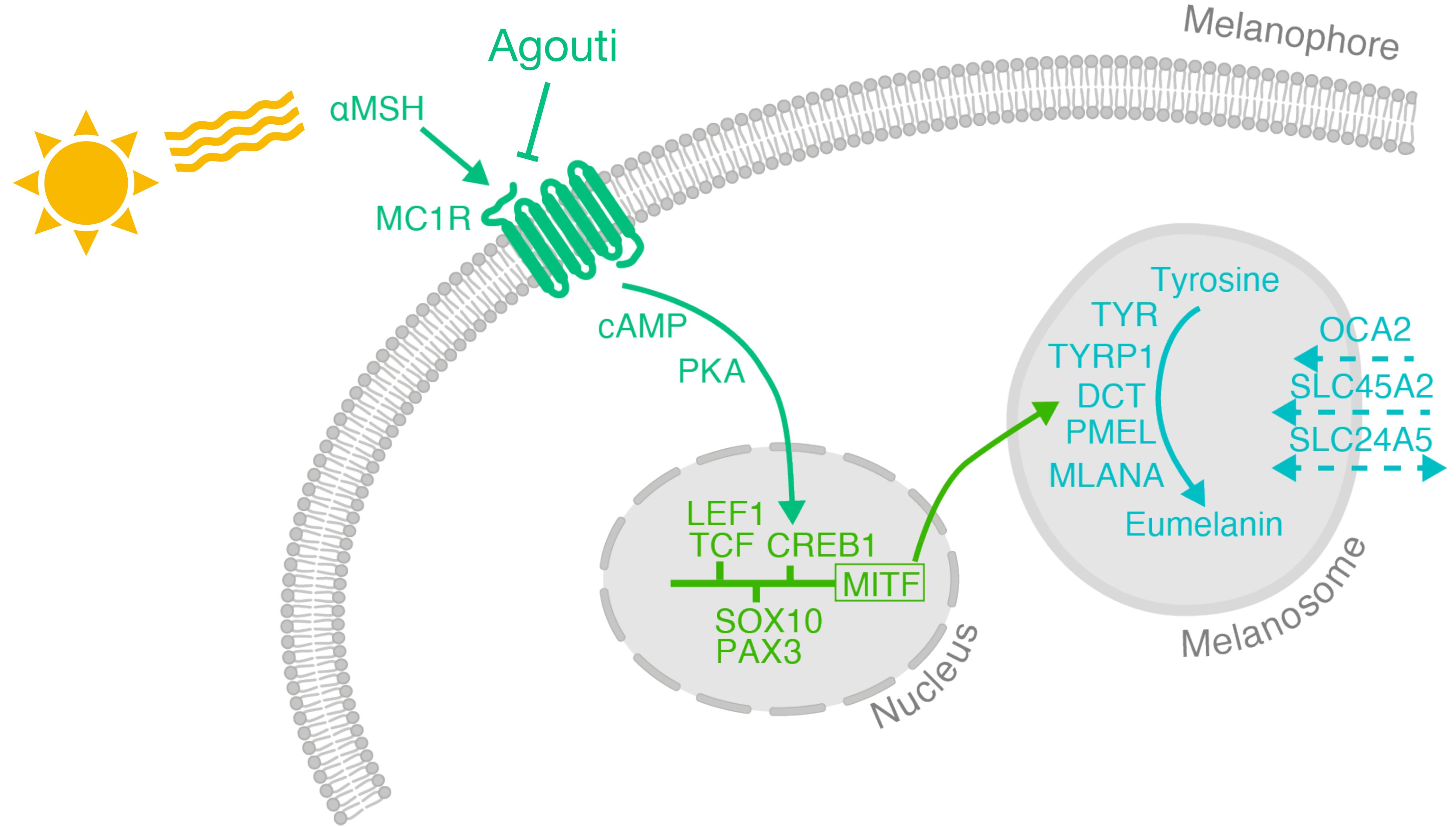
Inverse photosensitivity of plastic melanisation

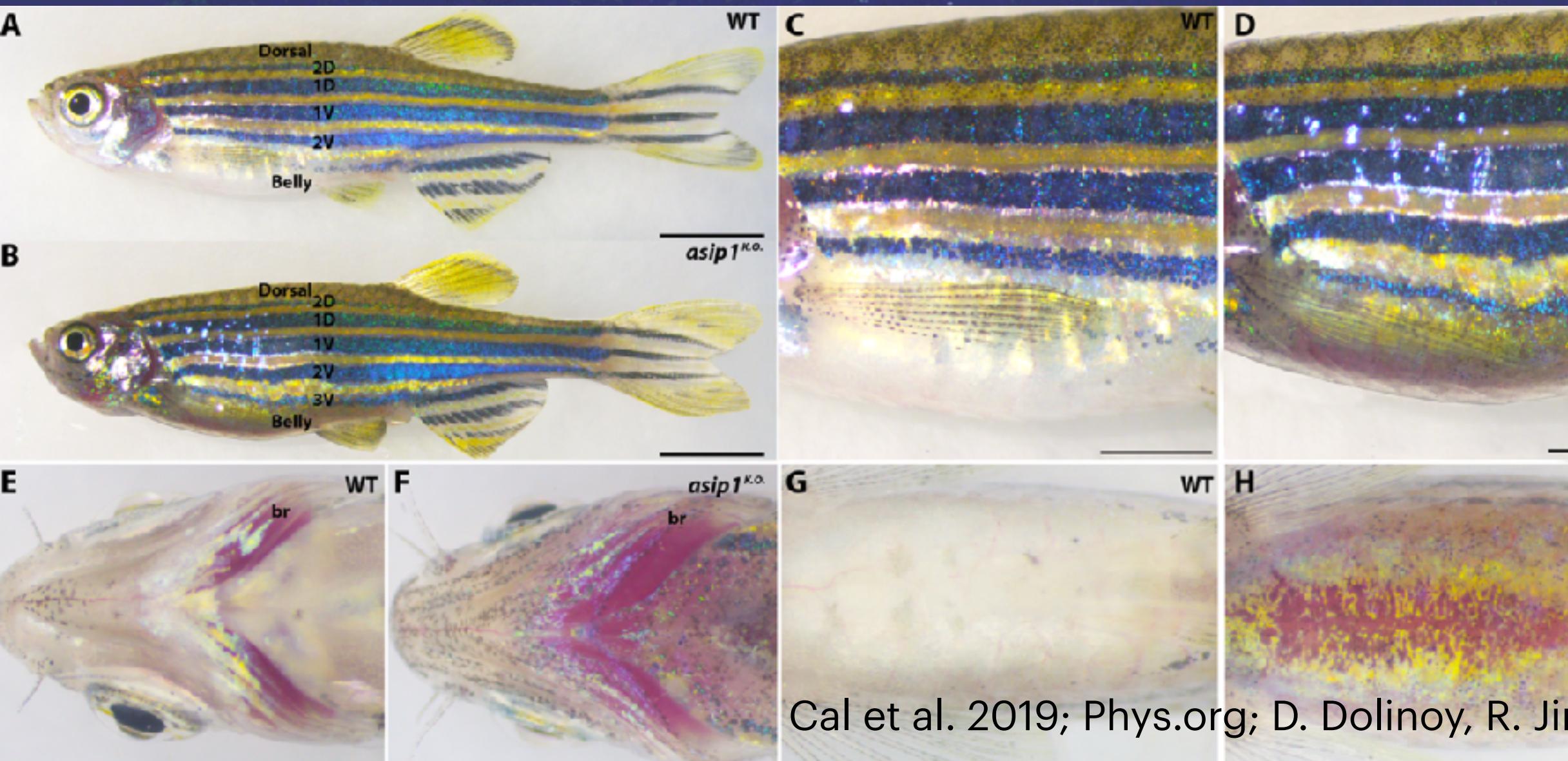
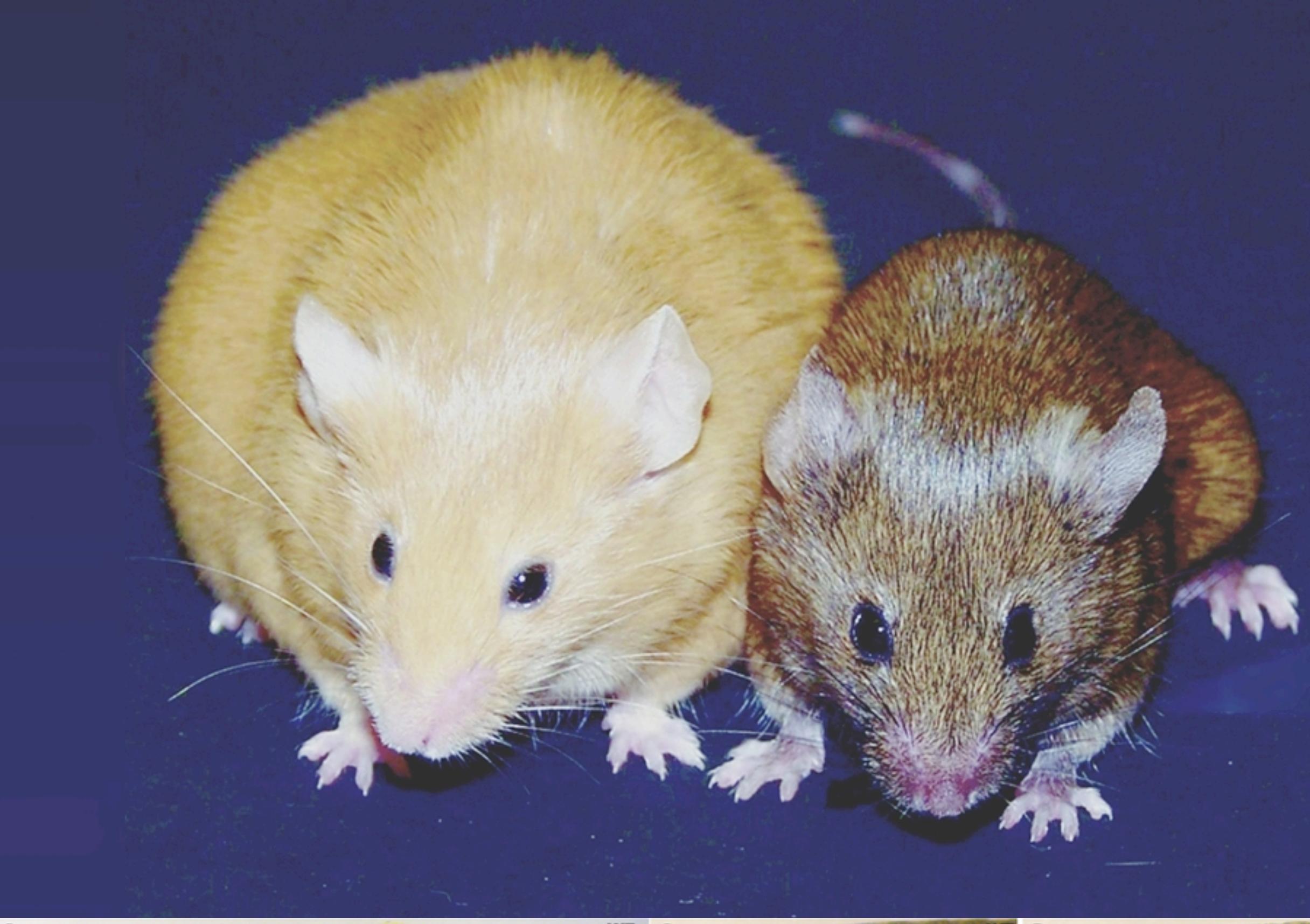


Non-plastic countershading

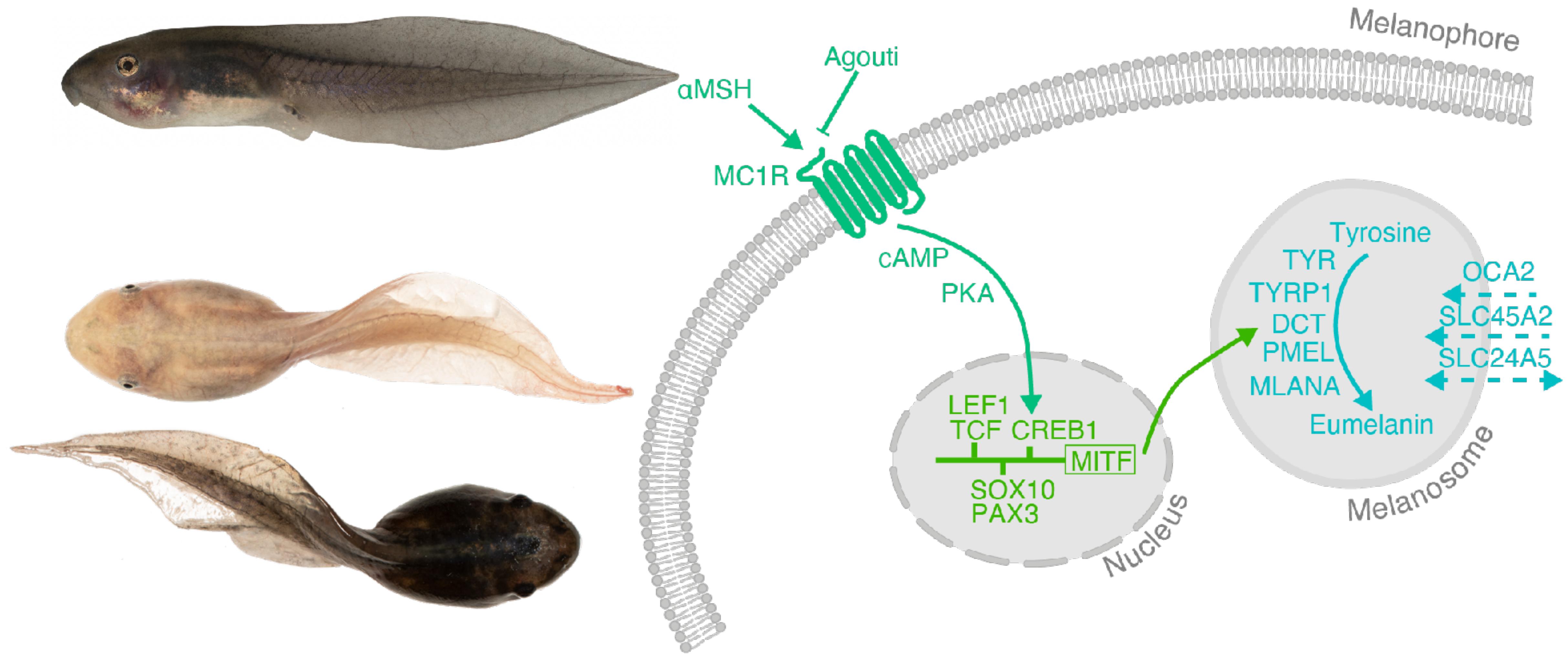


Photosensitive melanin biosynthesis





Photosensitive melanin biosynthesis

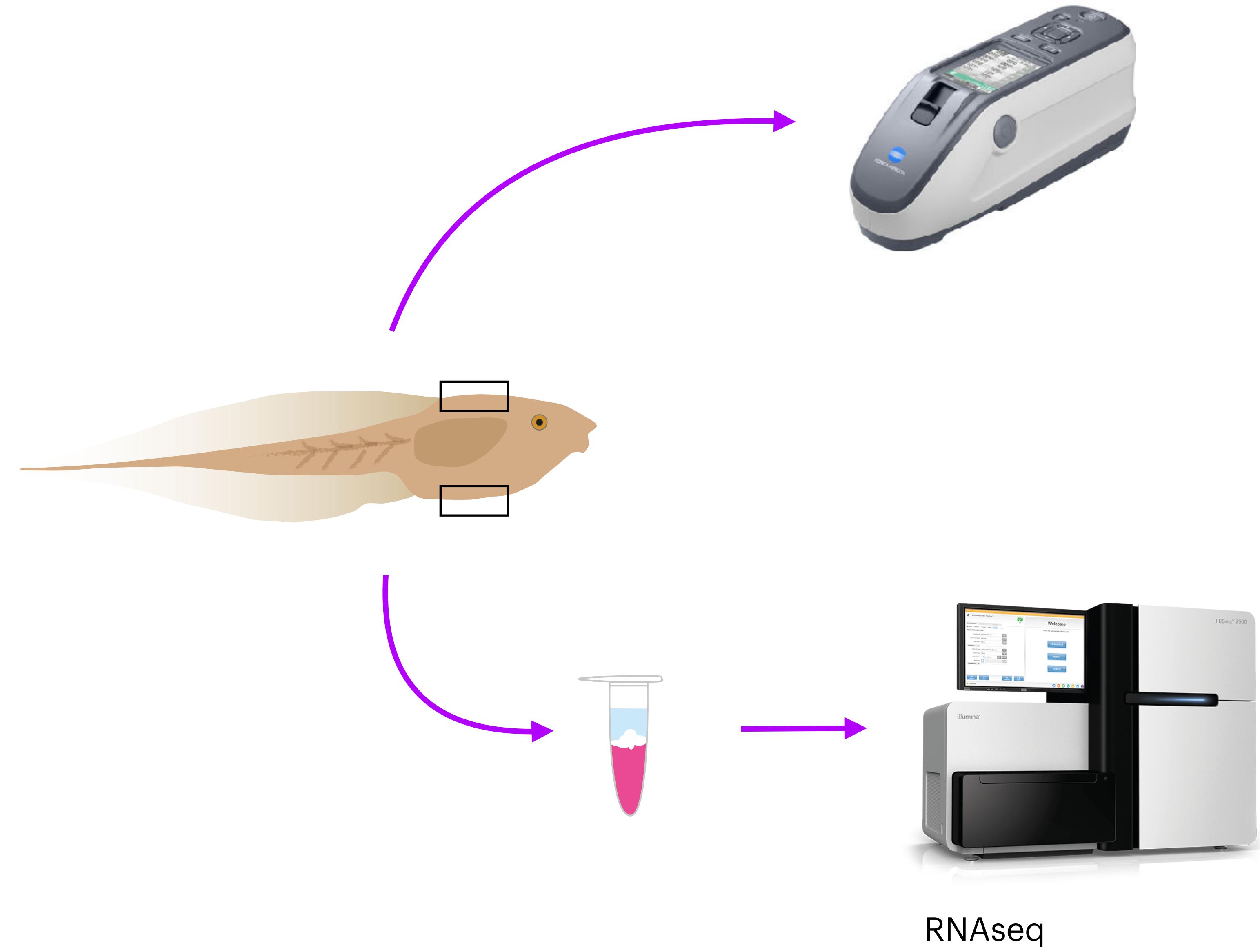


Background matching experiment

Spectrophotometry



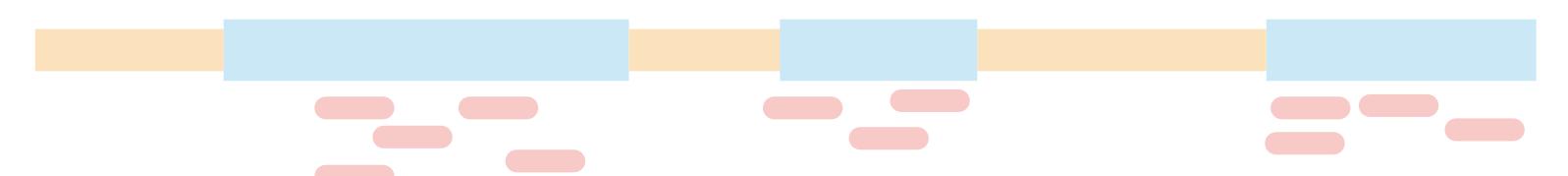
40-day exposure



RNA-Sequencing (in silica)

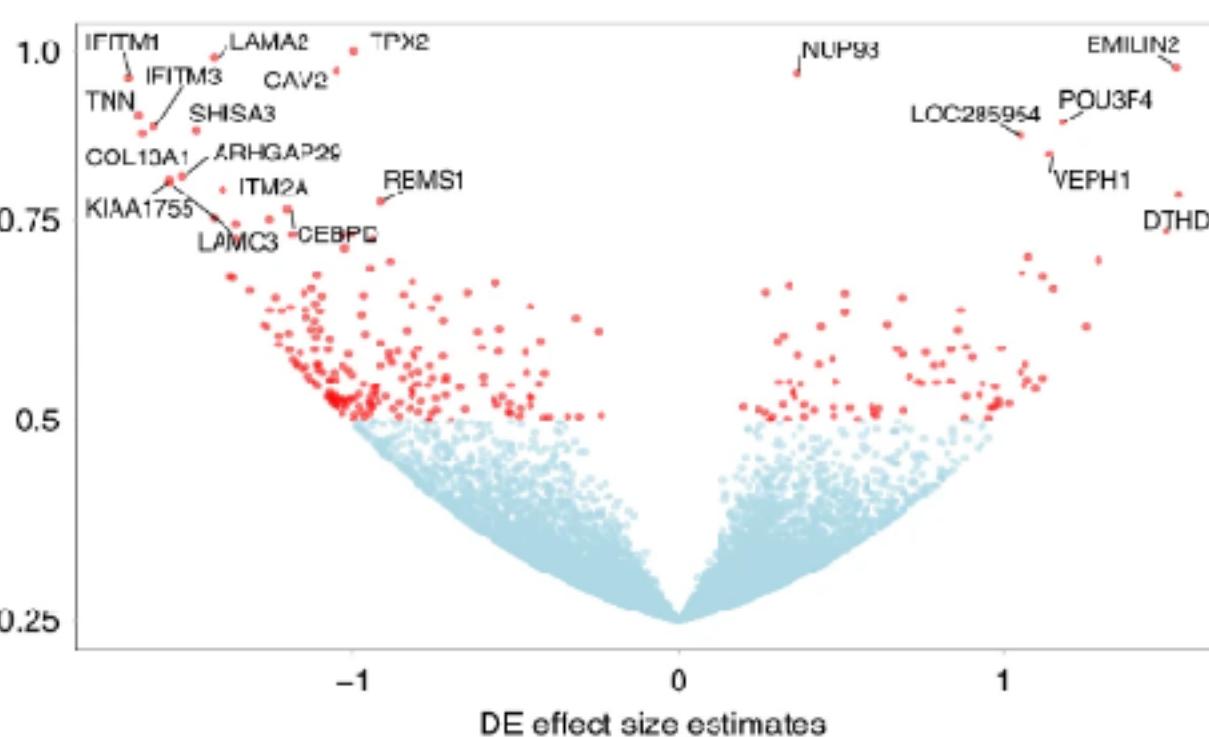
1.Sequence cleaning

2. Mapping/quantification



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3. Differential gene expression



4. Functional insights

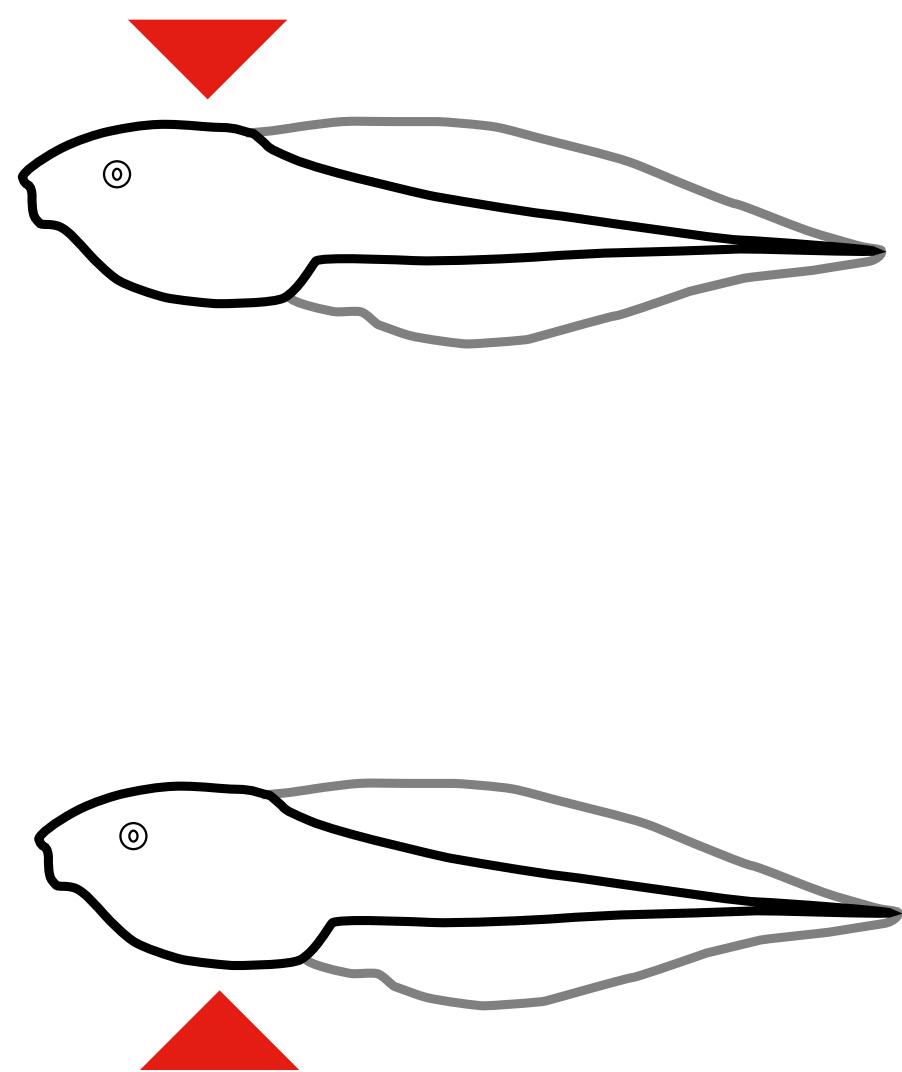
RNA-seq counts matrix

	Condition 1			Condition 2		
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
Gene1	72.7	75.7	66.3	55.0	51.2	35.9
Gene2	81.3	77.4	71.1	58.1	47.9	32.8
Gene3	80.2	75.3	70.0	57.1	51.9	43.3
Gene4	78.8	76.7	68.8	58.0	43.9	41.5
Gene5	79.8	74.6	67.9	60.2	45.3	38.5
	76.1	74.5	69.7	59.6	45.3	40.5
	78.8	79.0	74.5	58.0	52.8	50.8
	78.7	79.2	71.8	58.8	49.8	38.3
	76.8	74.0	70.5	64.1	46.6	33.4

Differential Gene Expression

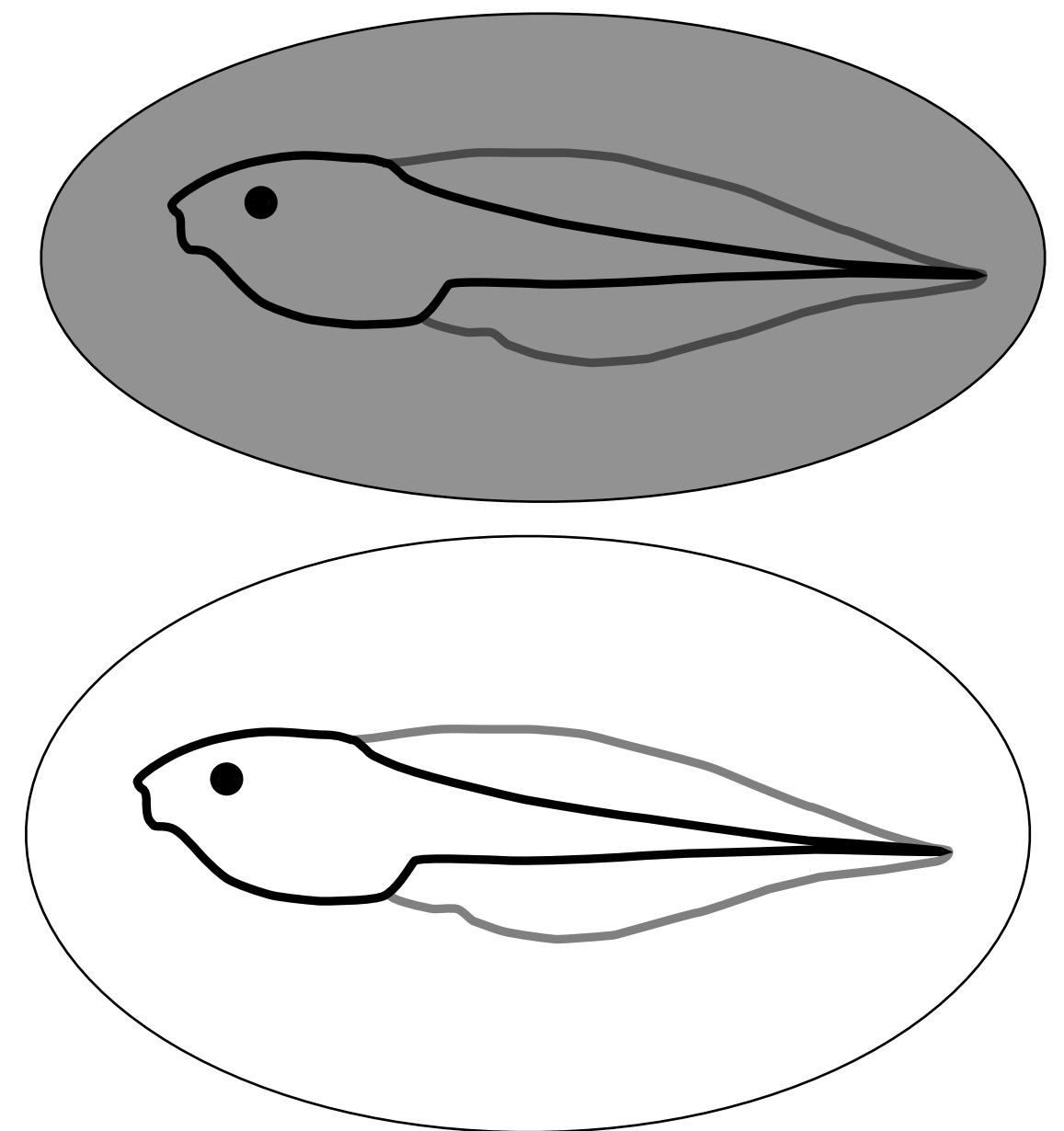


?



Anatomical effect

+

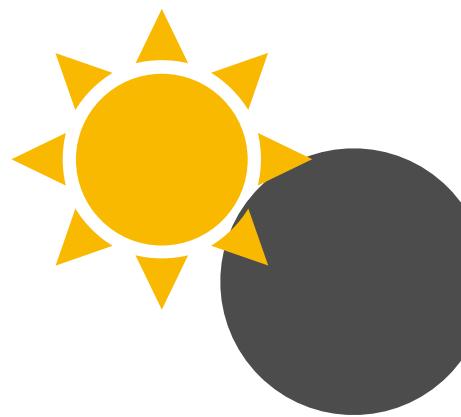


Background effect

Differential Gene Expression

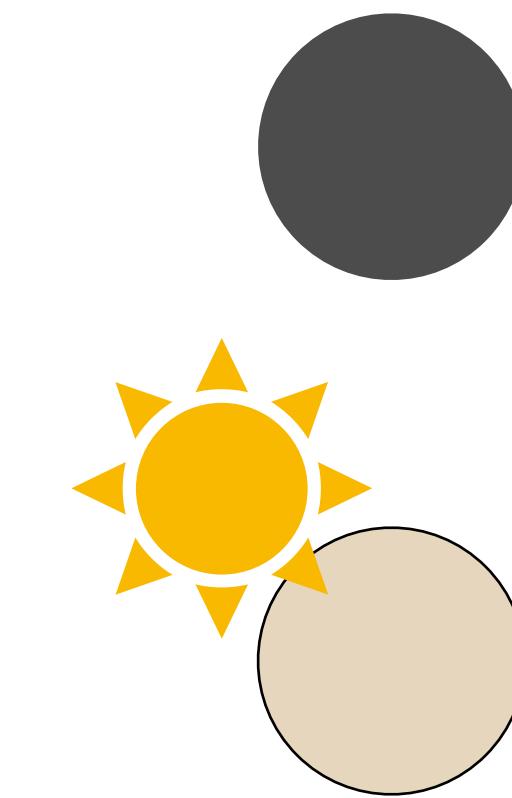


?



Fixed pigmentation

+



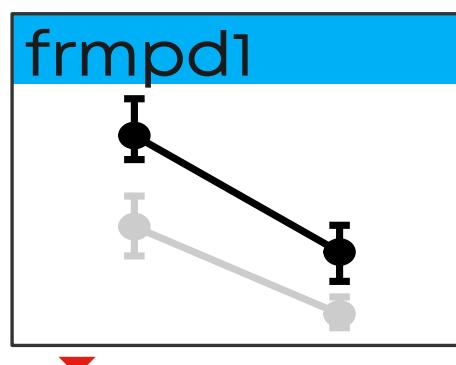
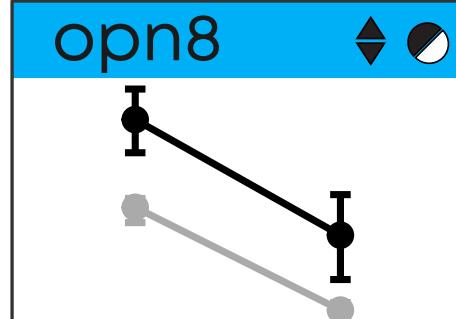
Plastic pigmentation

RNA-seq practical

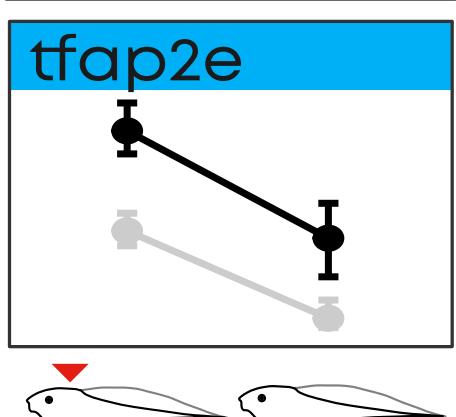
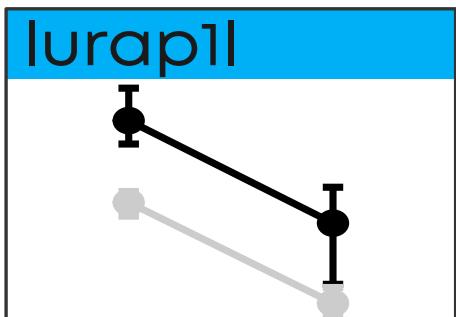
https://hcliedtke.github.io/UAM_NewTech

Differential Gene Expression

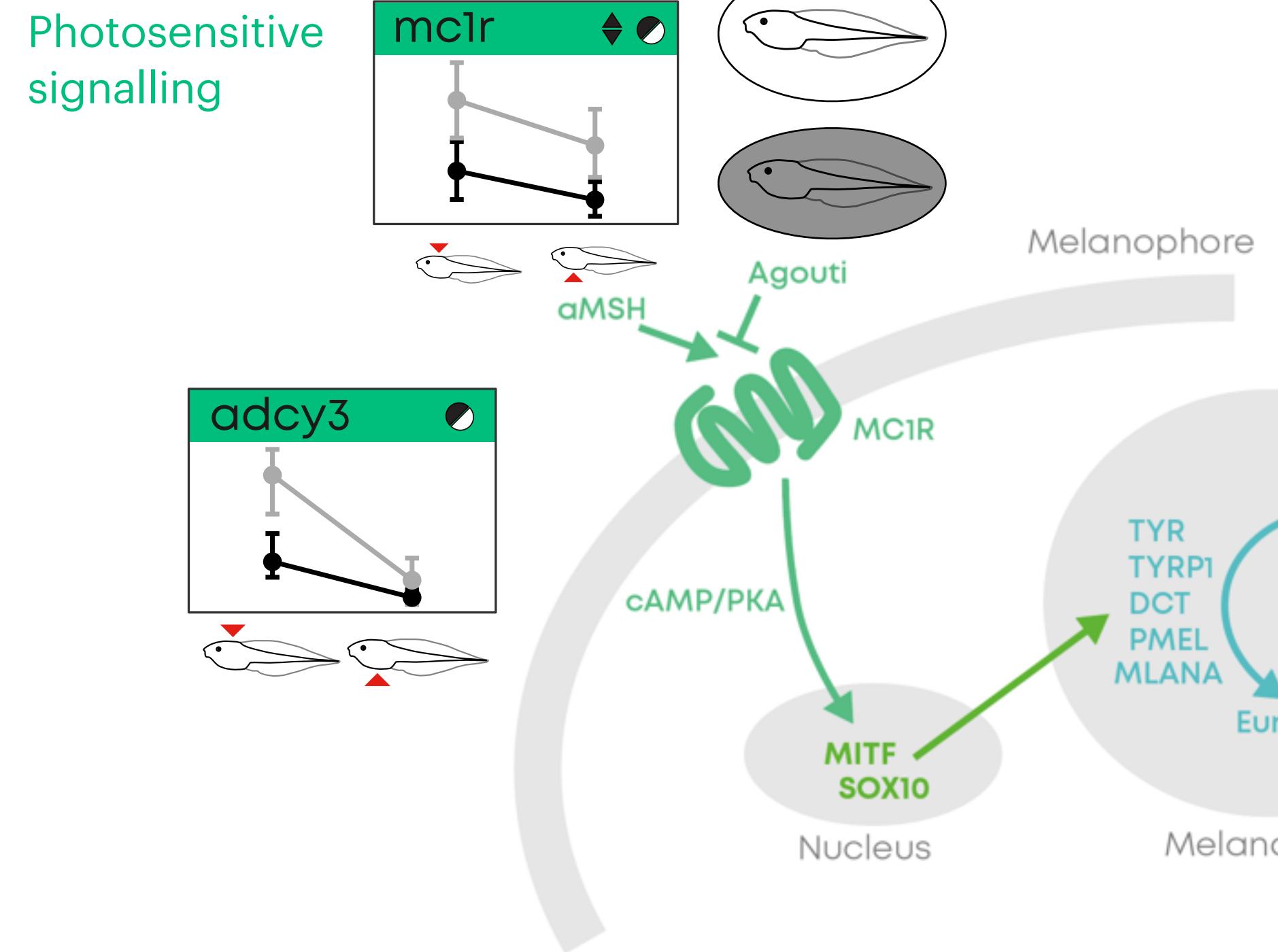
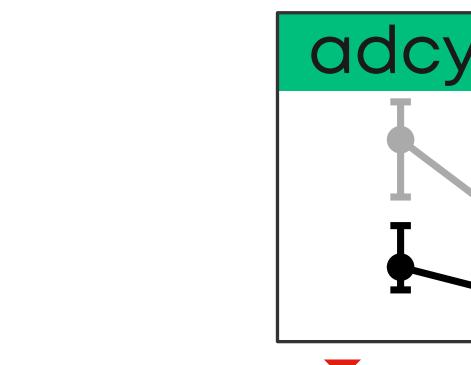
Photosensitive signalling



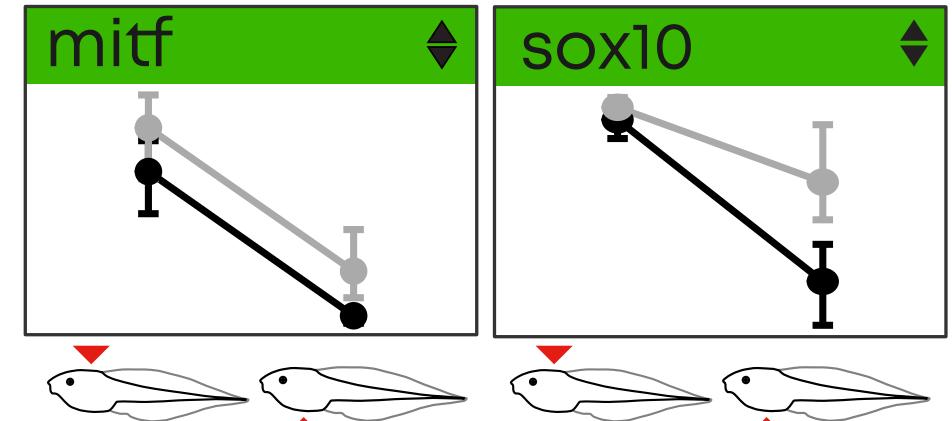
Transcription regulators



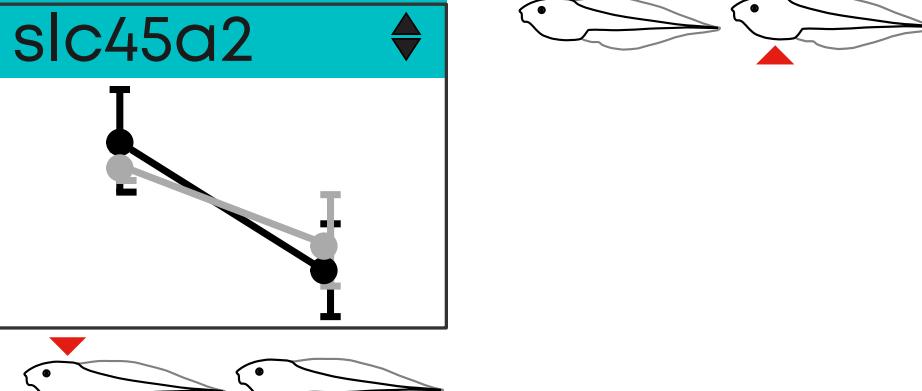
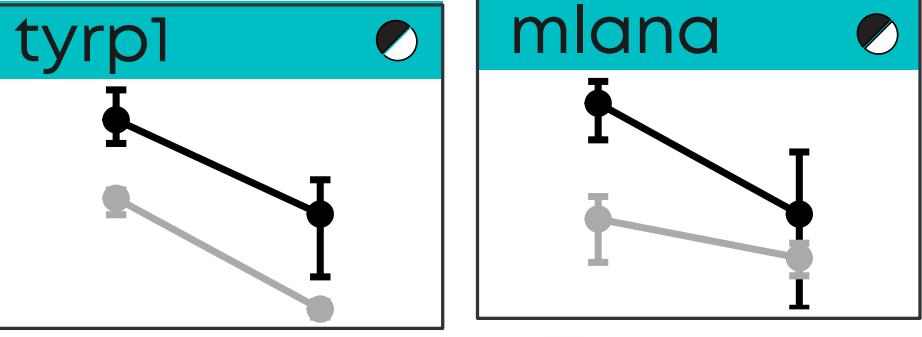
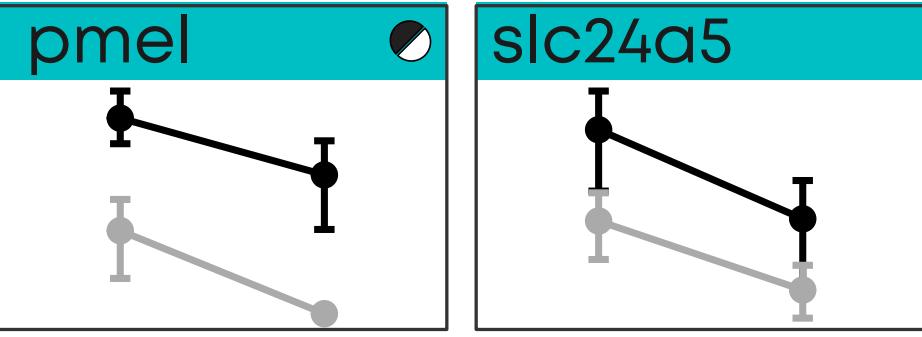
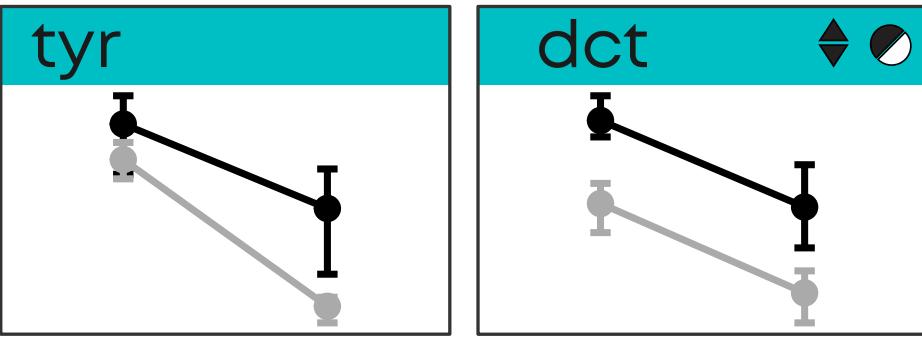
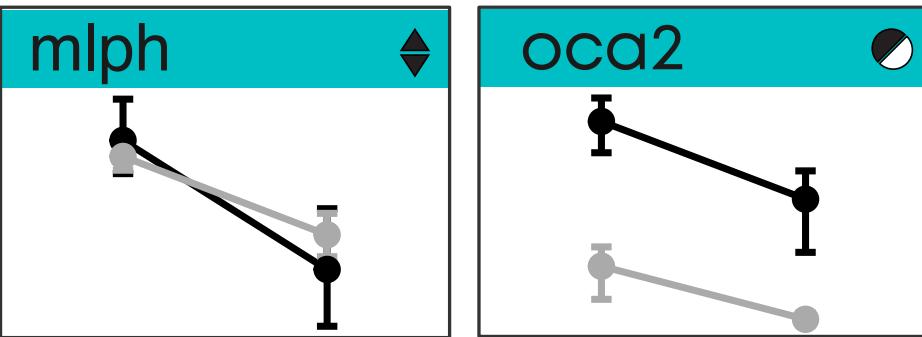
Photosensitive signalling



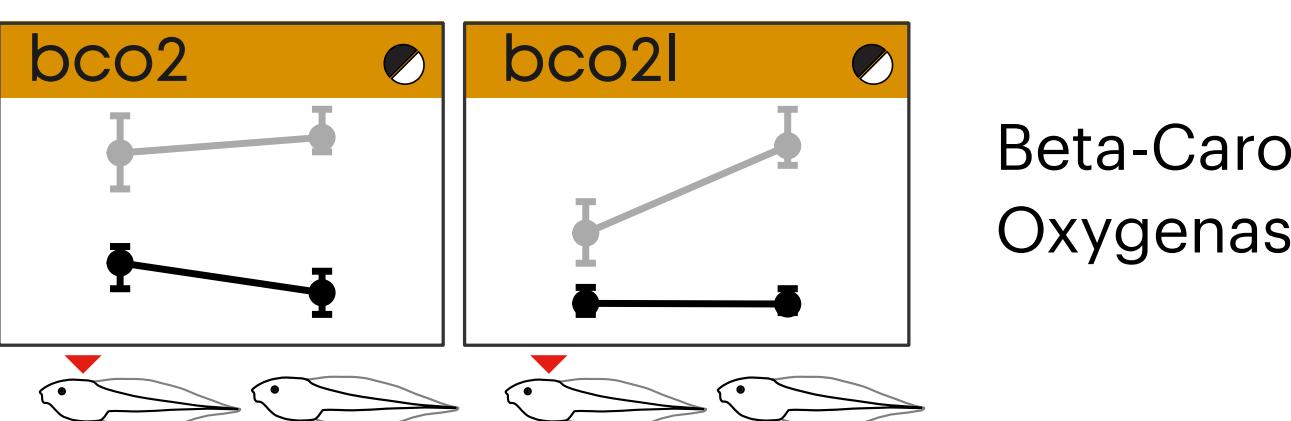
Transcription regulators



Tyrosine metabolism



Carotenoid metabolism



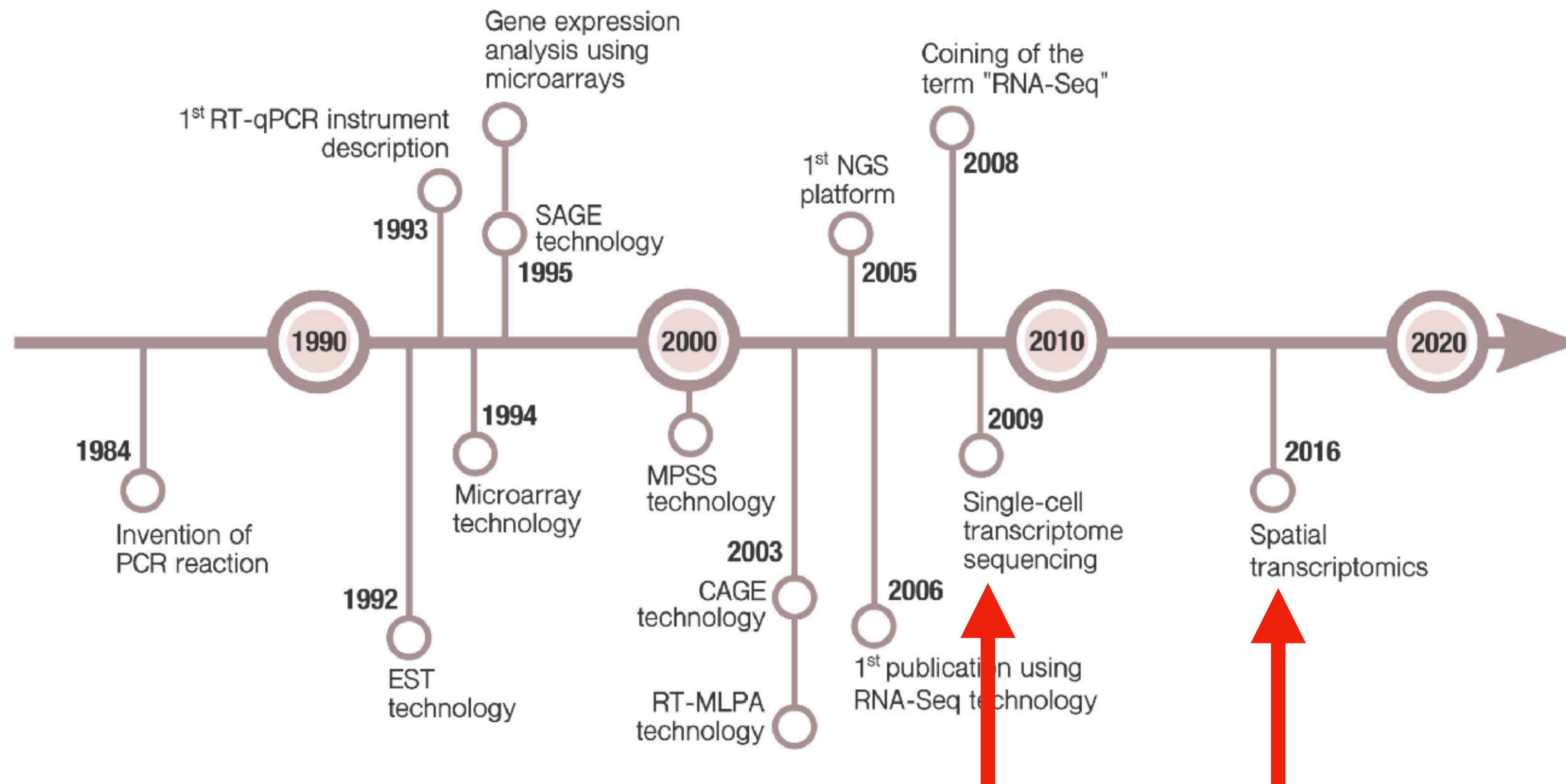
Beta-Carotene Oxygenase 2

Summary

- Dorso-ventral gradients are controlled by highly conserved molecular mechanisms (α MSH-agouti signalling)
- Tyrosine metabolism is decoupled from photosensitive MC1R to allow for background matching
- Other photosensitive pathways are regulating background matching
- Other pigments (e.g. carotenoids) are also being regulated



A closing remark on “BULK” RNA-seq



“Bulk” RNA-seq versus the new kids on the block

Main uses of bulk RNA-Seq:

- Gene Expression Profiling:
 - Across tissues
 - Across developmental stages
 - Across conditions
- Functional Genomics and Disease Research:
 - Identifying biomarkers
 - exploring disease mechanisms
 - drug response studies.
- Multi-Omics Integration:
 - Combining RNA-seq with other data types like proteomics and epigenomics
- Genome annotation

Disadvantage of bulk RNA-Seq:

- Averaging effects (loss of cellular heterogeneity)

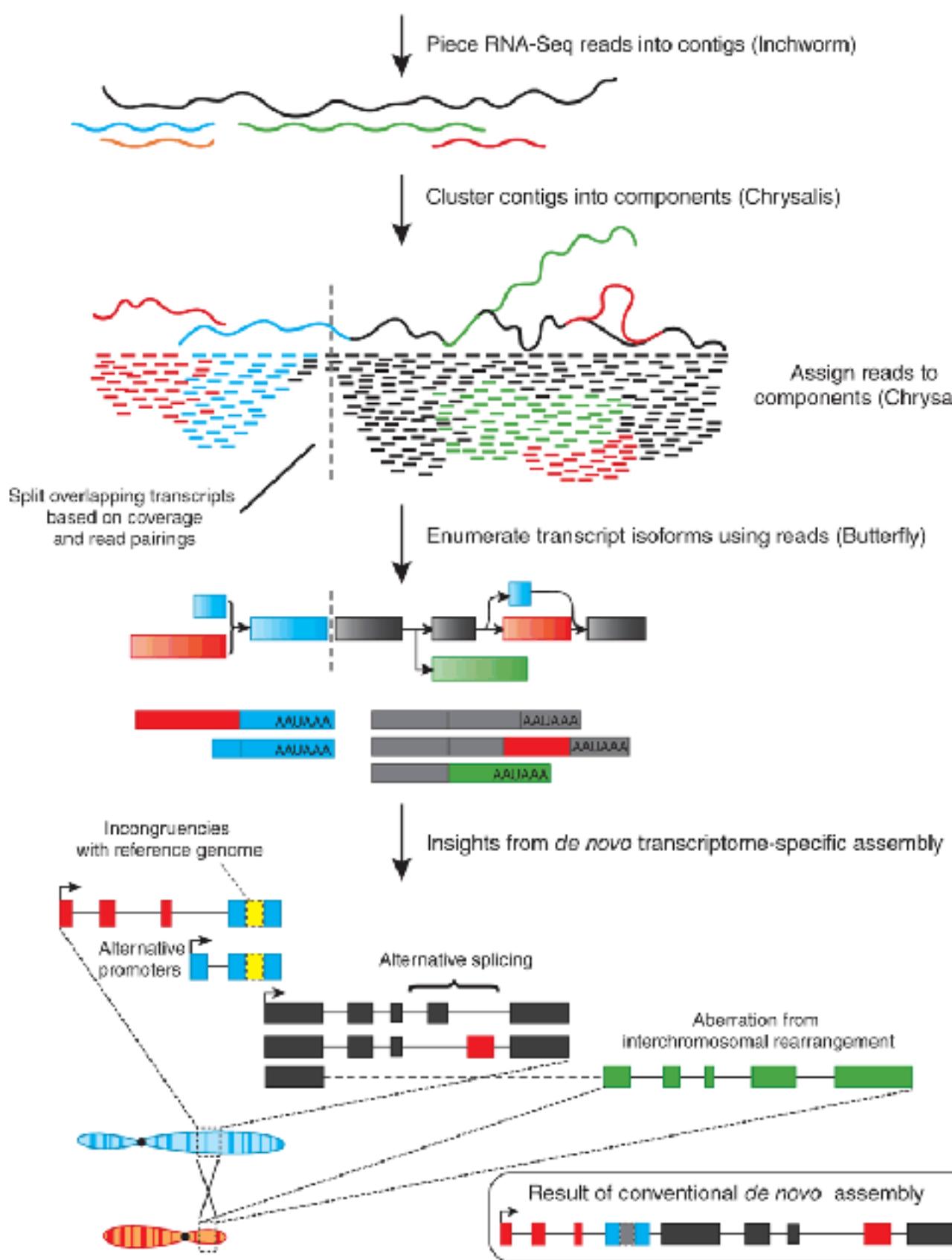
Advantages of bulk RNA-Seq:

- Relatively cheap
- Flexible starting material quantities
- Very straightforward (easy!)
- No specialised equipment
- Well established bioinformatic pipelines

What if I don't have a reference genome?

No Genome? No problem!

Trinity *de novo* transcriptome assembly



Genomes:

- Higher accuracy
- Better annotations (sometimes)

De novo Transcriptomes:

- Better than nothing
- Avoids mapping bias