

NEW TECHNOLOGIES FOR DEVELOPMENTAL EVOLUTIONARY  
BIOLOGY STUDIES - 2024

# Plastic Phenotypes

## And their underlying gene expression

H. Christoph Liedtke



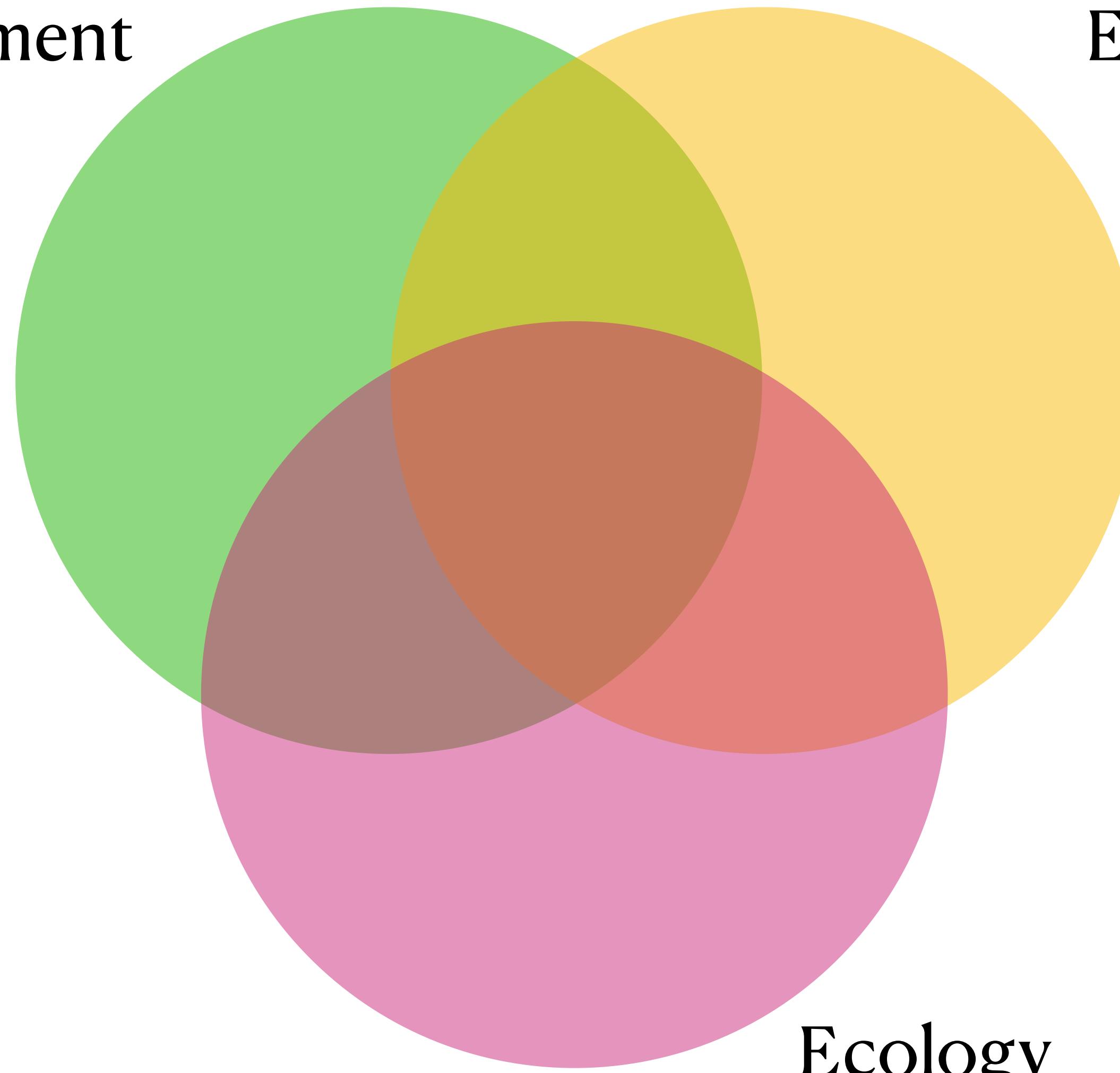
# What generates phenotypic diversity?



Development

Evolution

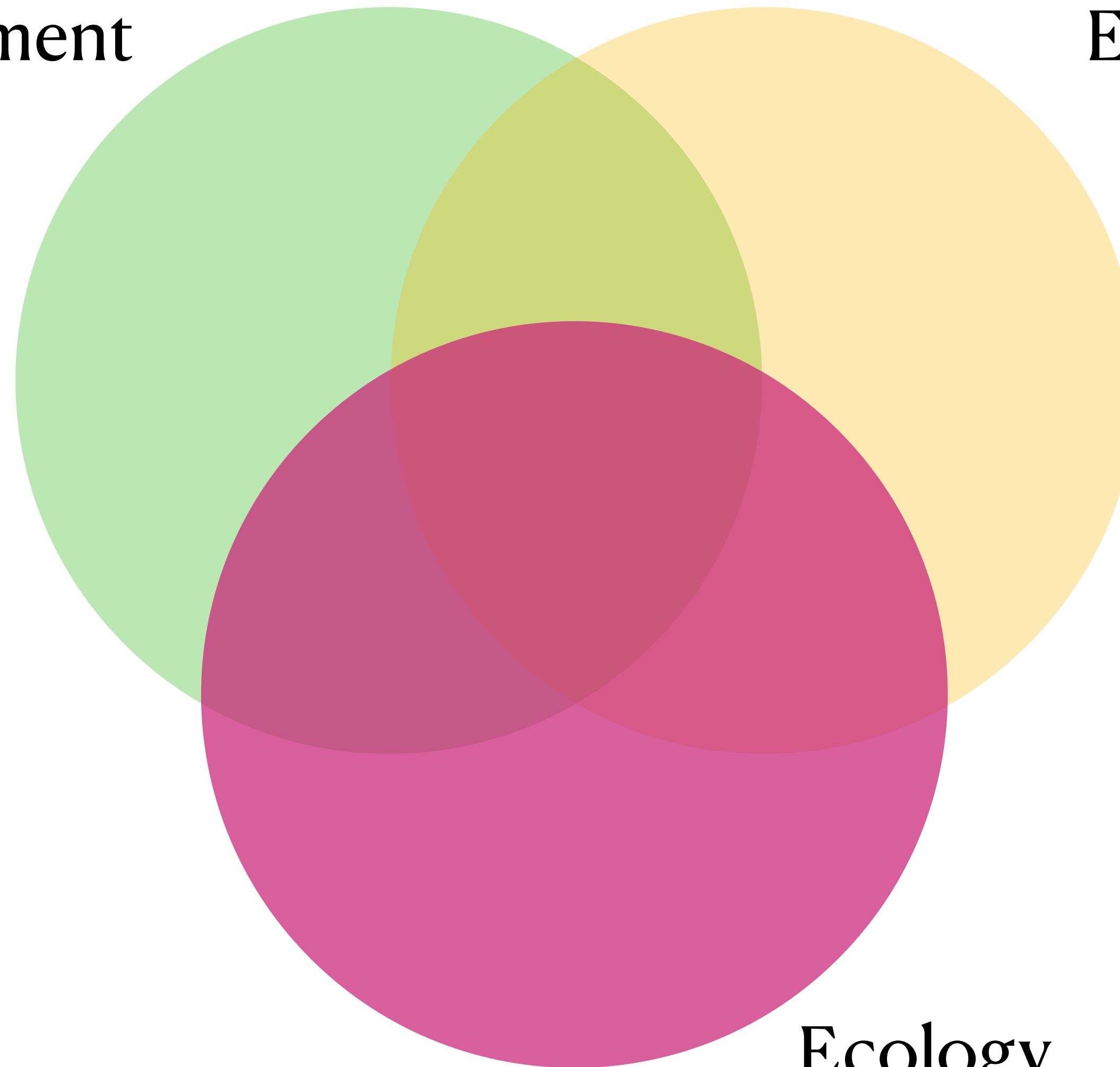
Ecology



Development

Evolution

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]

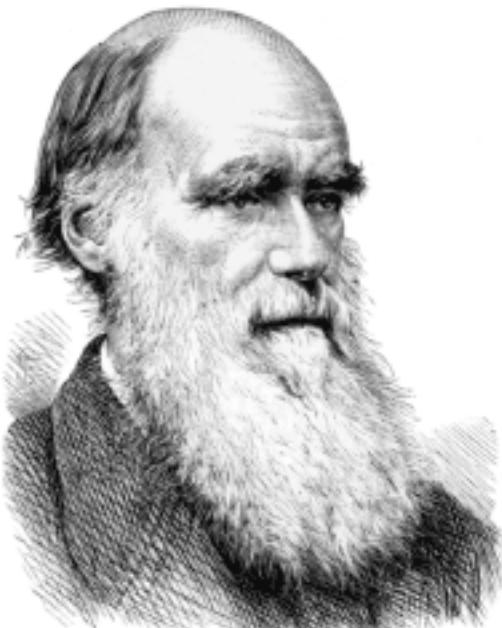
# What is the definition of evolution?

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

*National Academy of Sciences, USA*



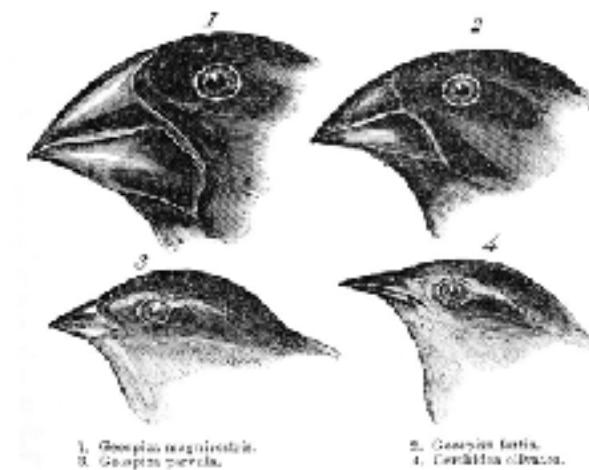
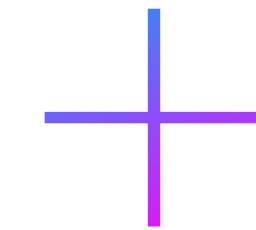
# The Modern Synthesis



CHARLES DARWIN



ALFRED RUSSEL WALLACE



Huxley



Stebbins



Simpson



Mayr



Haldane



Fisher



Wright



Dobzhansky

# The Modern Synthesis

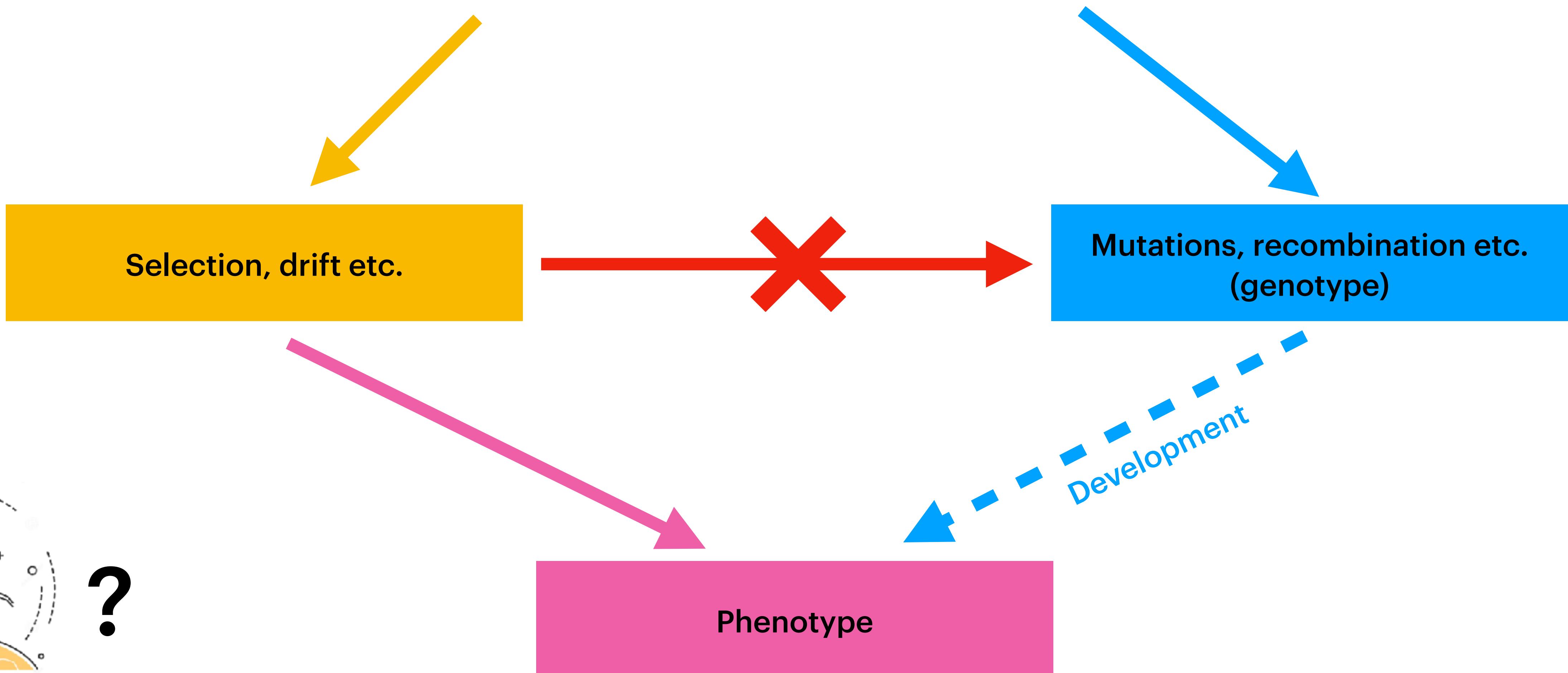
- Populations harbour genetic variation that emerges randomly through mutation and recombination.
- Populations evolve through changes in allele frequencies due to drift, gene flow, and especially, natural selection.
- The majority of genetic variants have a small phenotypic effect, so that the resulting 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

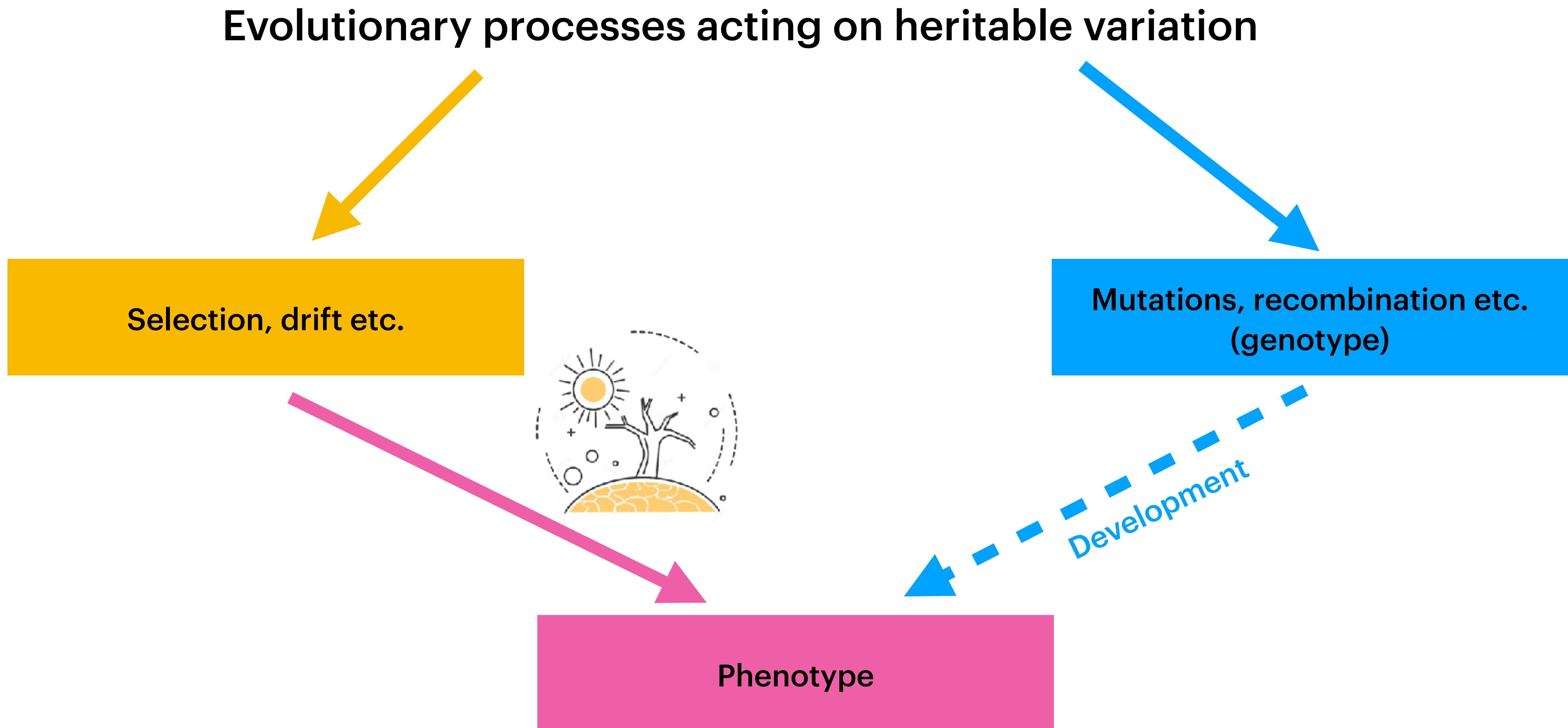
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# How do phenotypes evolve?

Evolutionary processes acting on heritable variation



# How do phenotypes evolve?



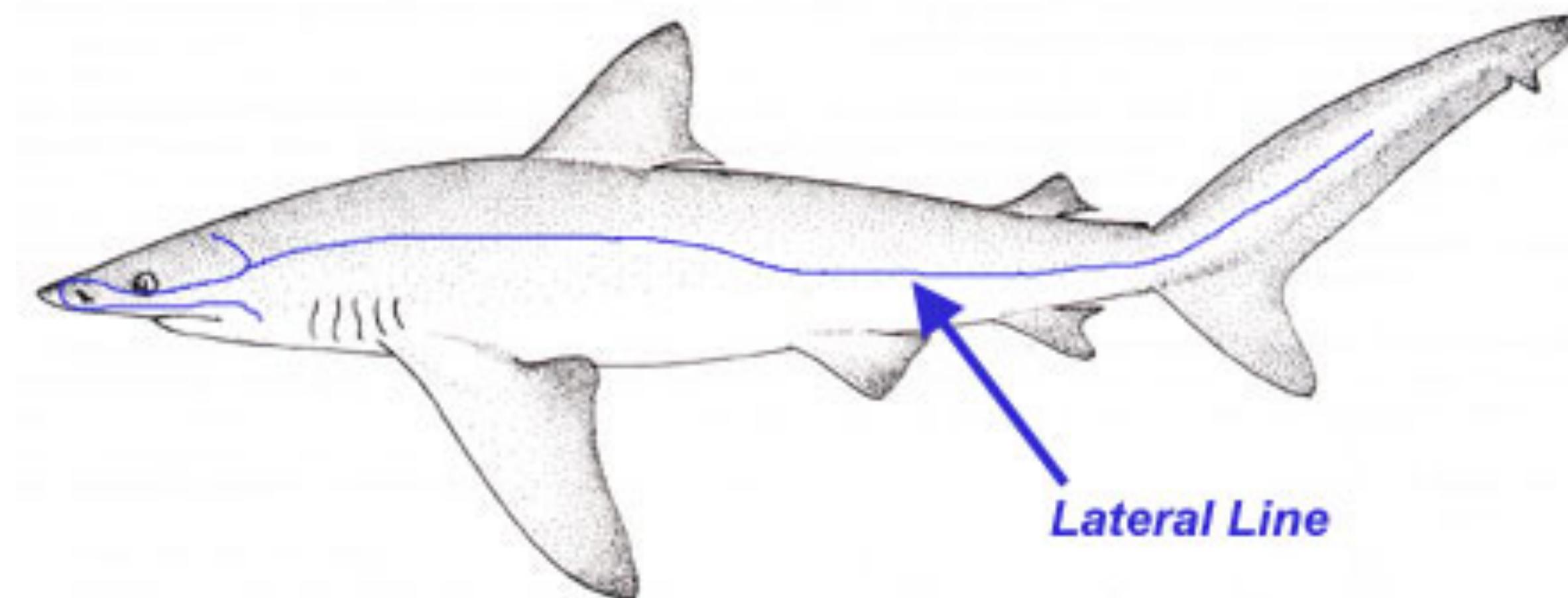
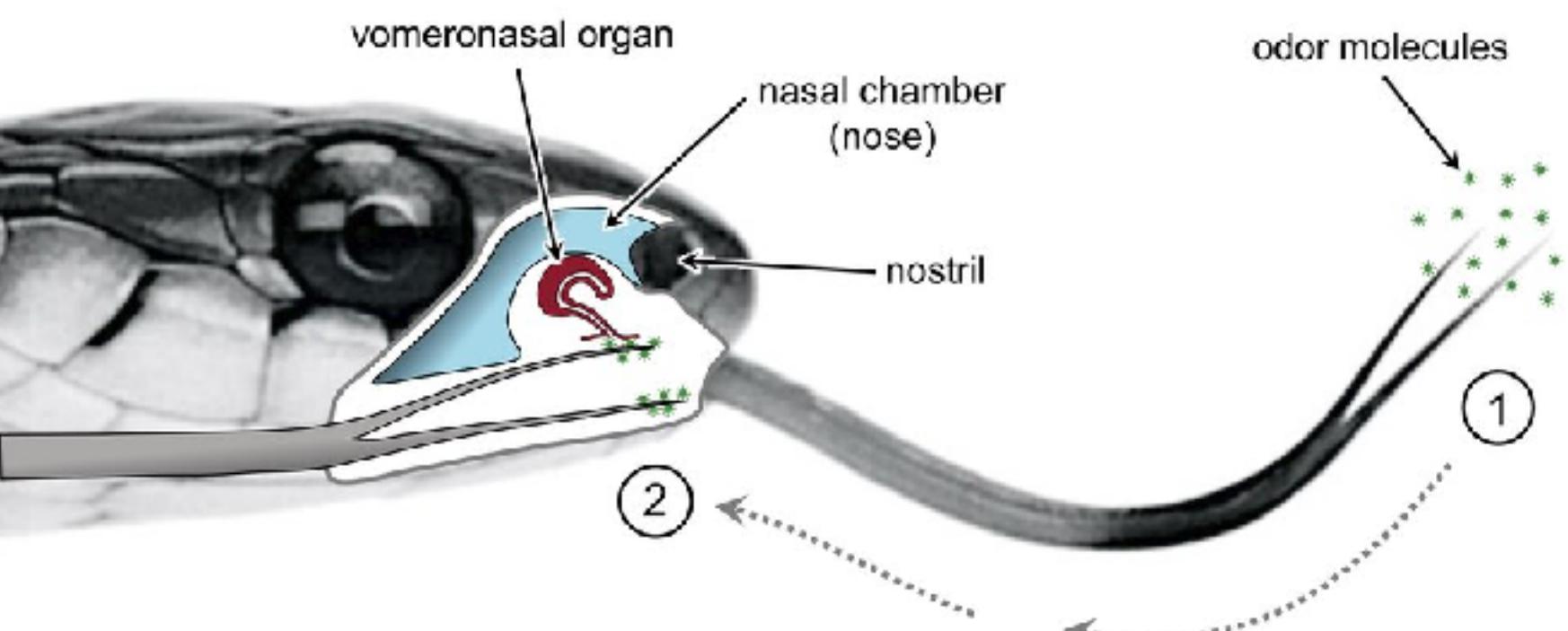
# The environment as a passive filter?



# The environment is dynamic



# Organisms sense their environment



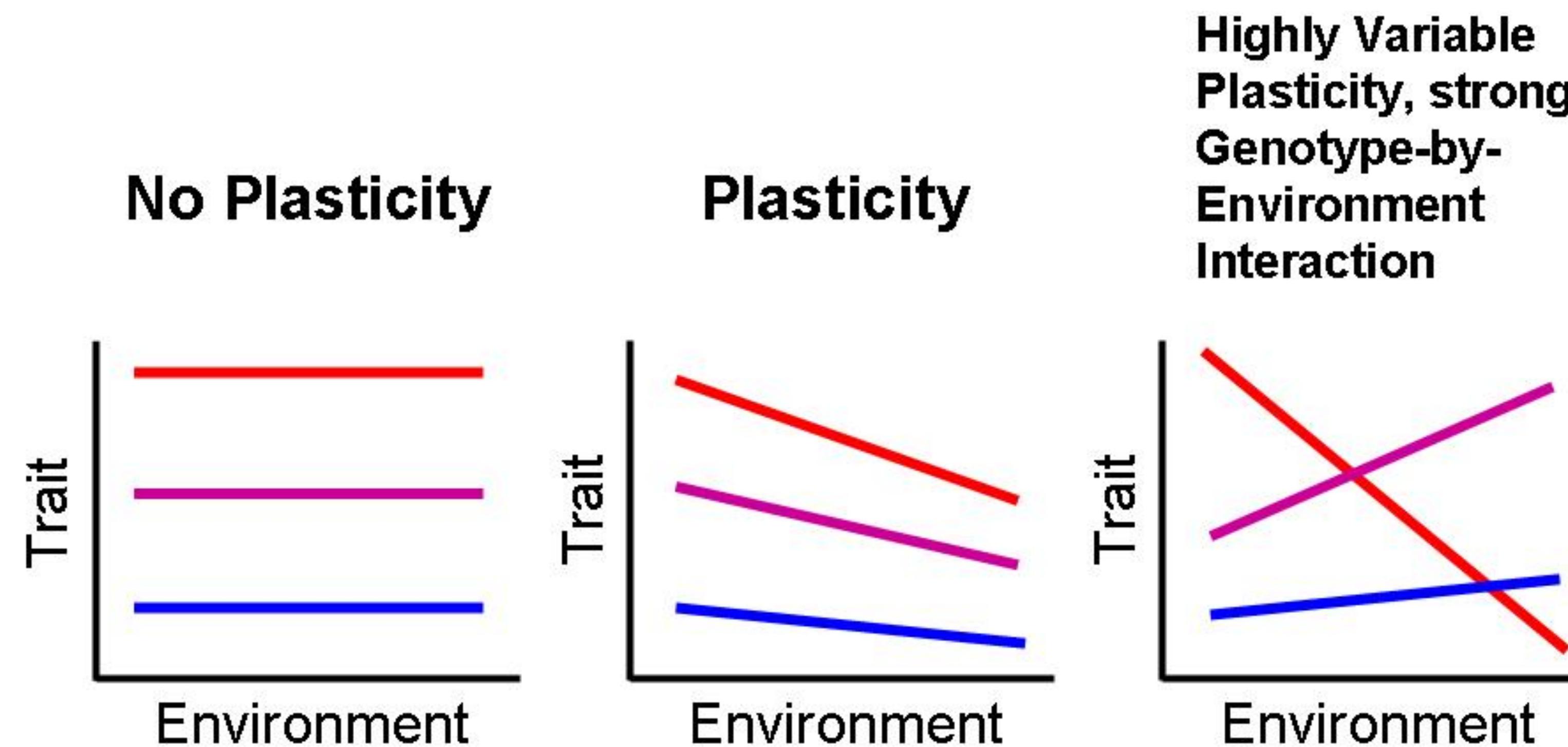
# Phenotypes are environment-dependent



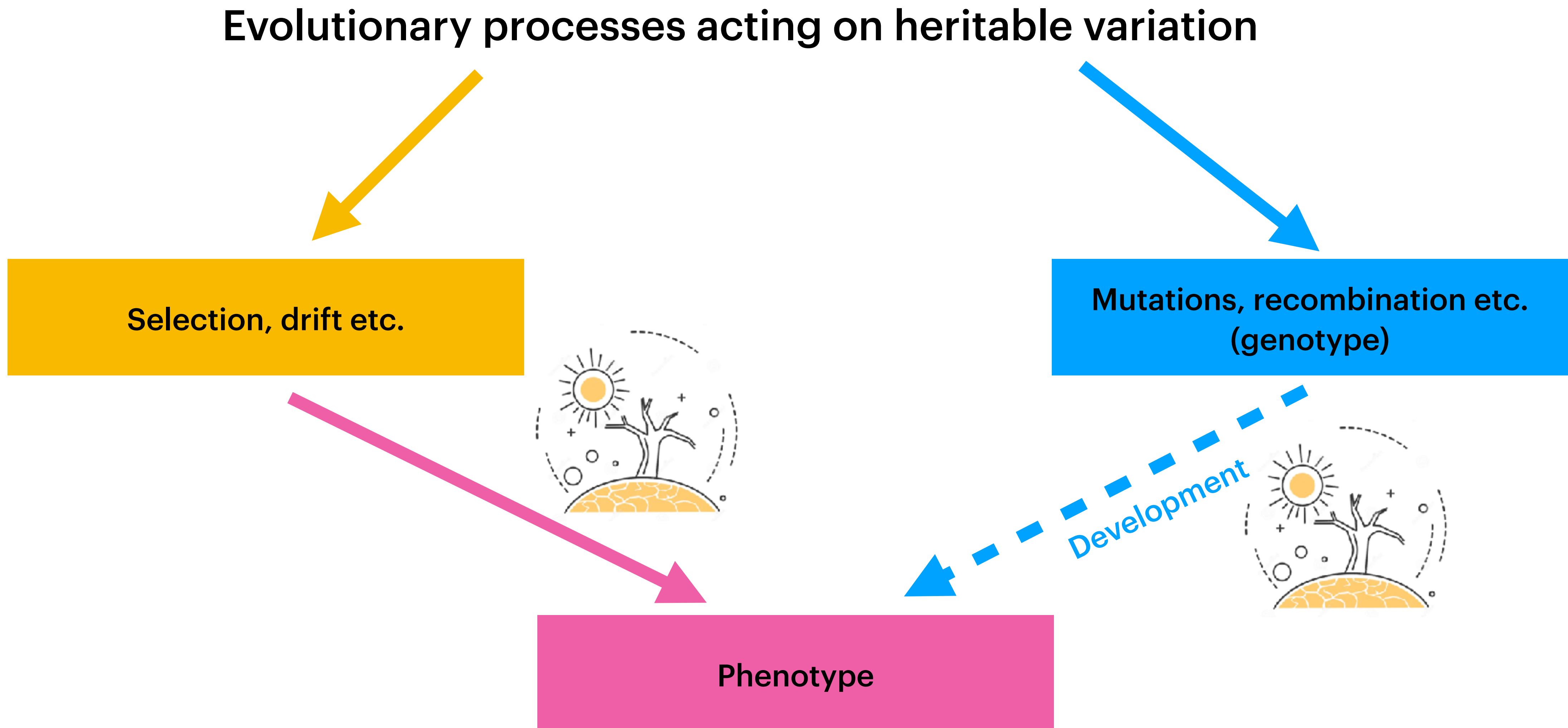
Nemoria arizonaria, Greene 1989 Nature

# 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?

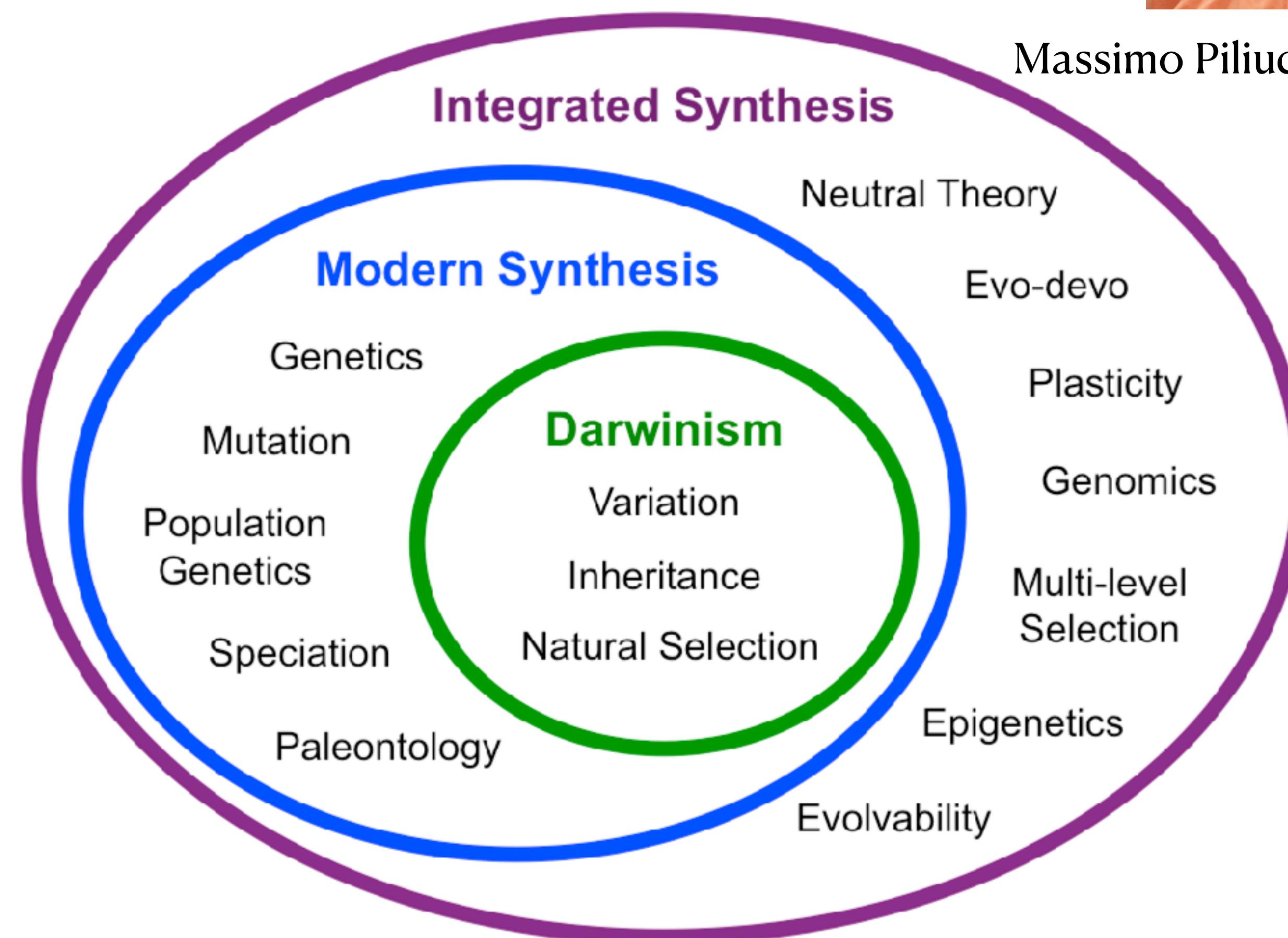




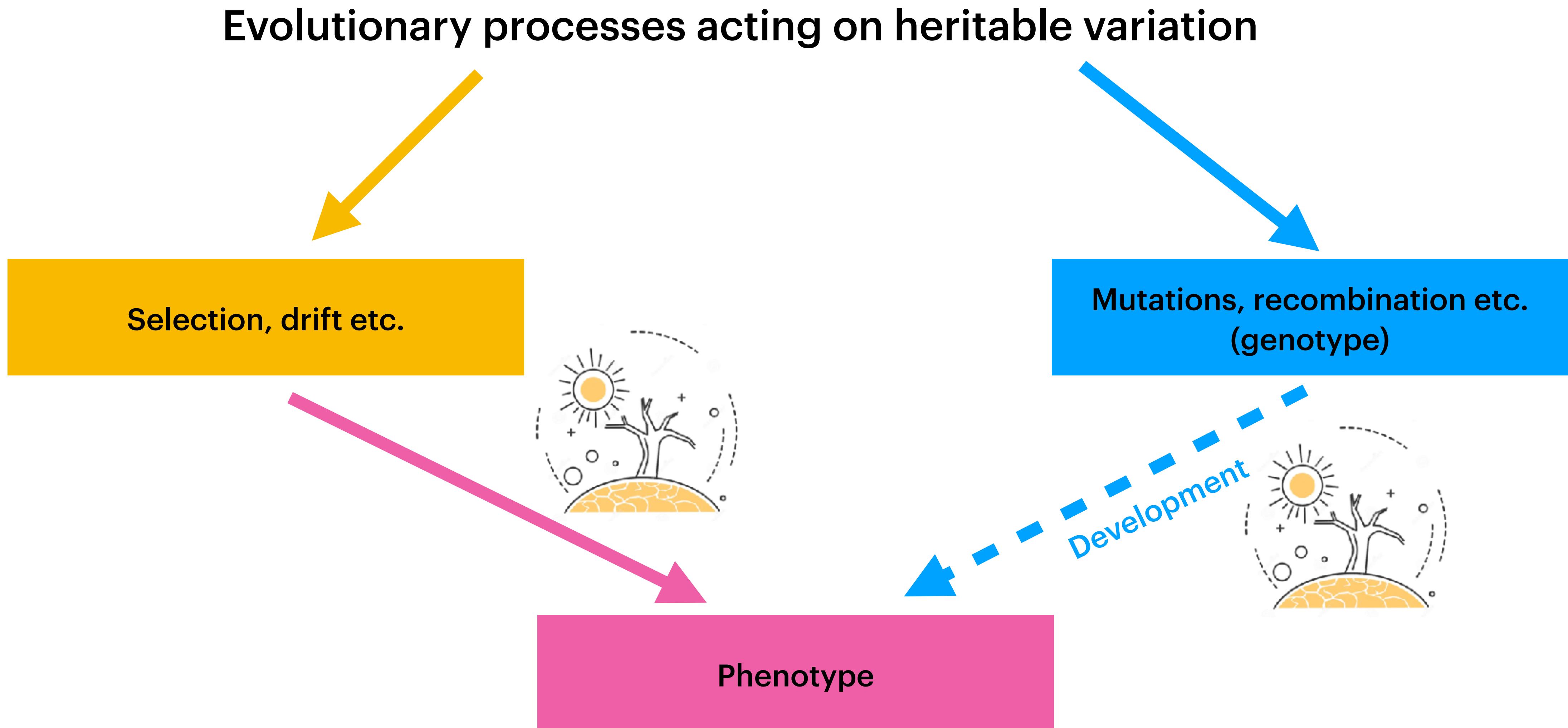
# Extended Evolutionary Synthesis



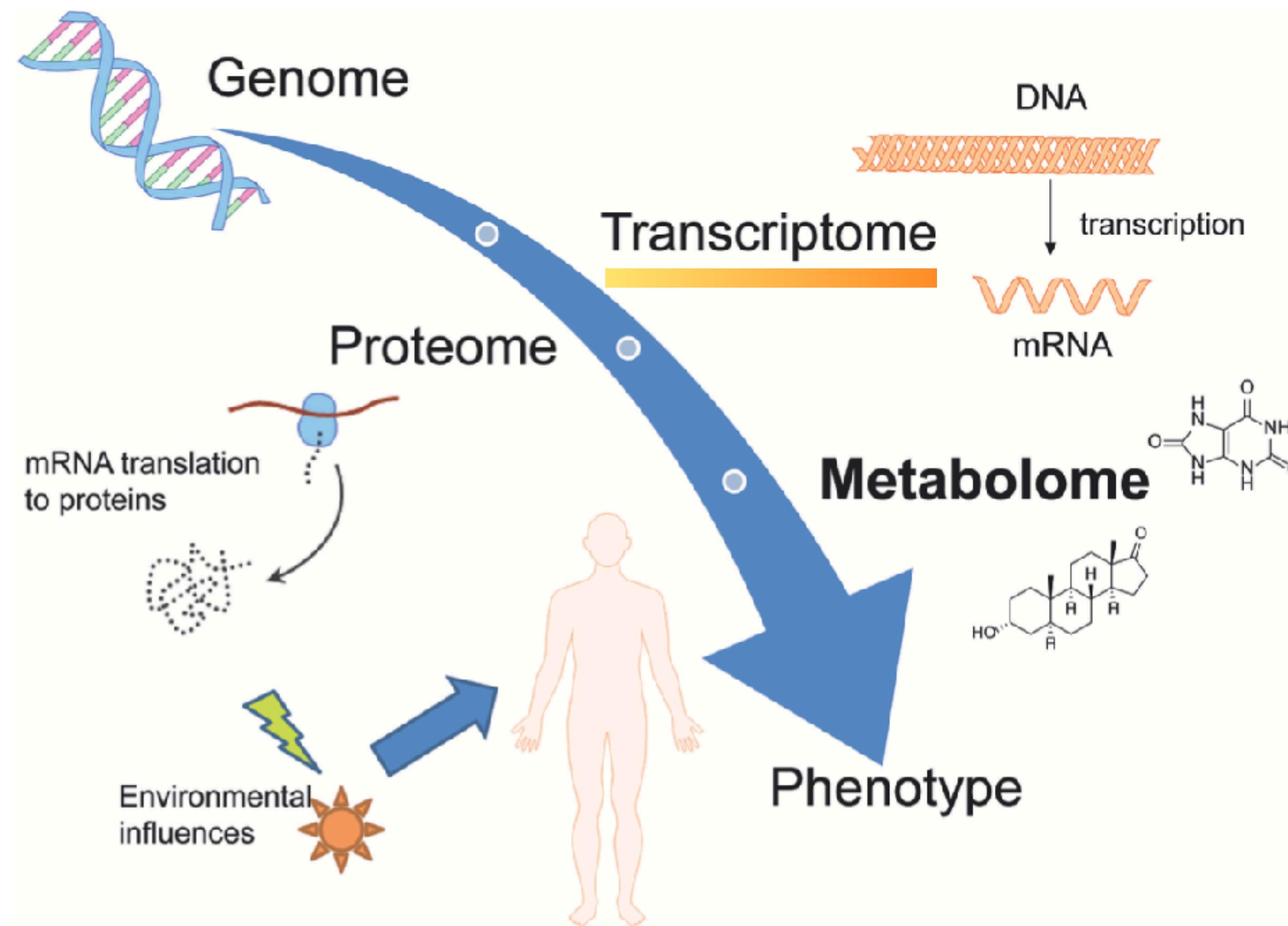
Massimo Piliucci



# 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

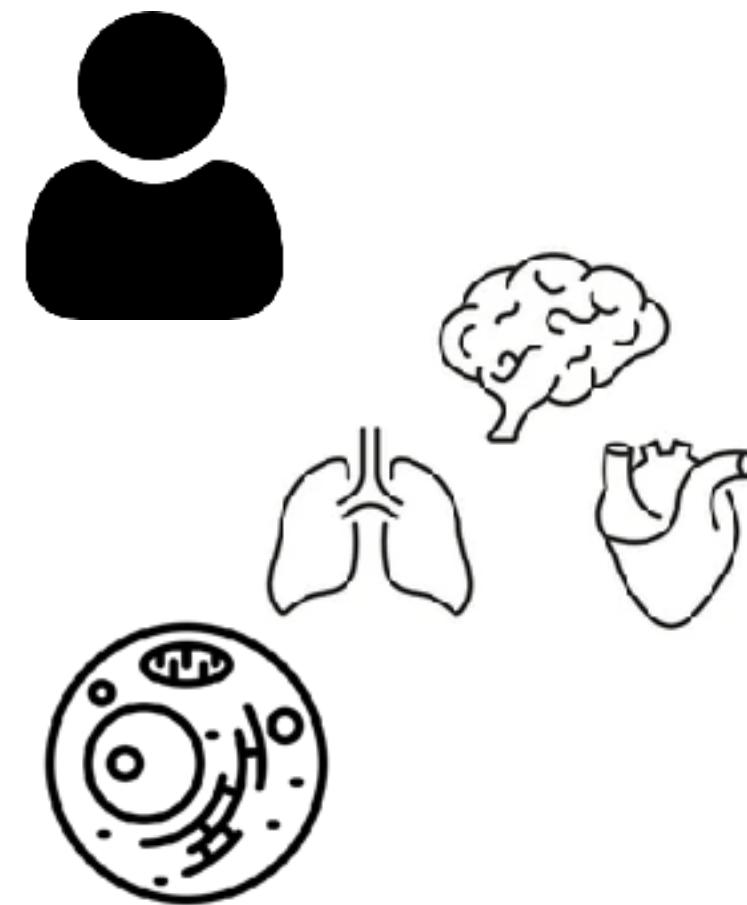
78724 genes



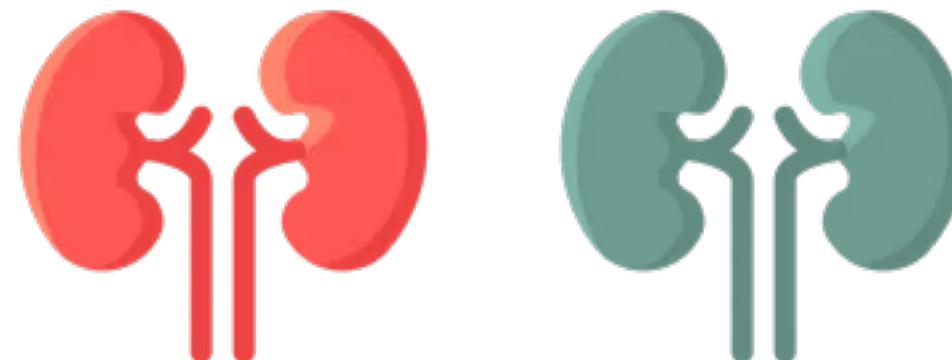
2.7bp genome

78239 genes

# What is the transcriptome?



- Organism > organ > tissue > cell



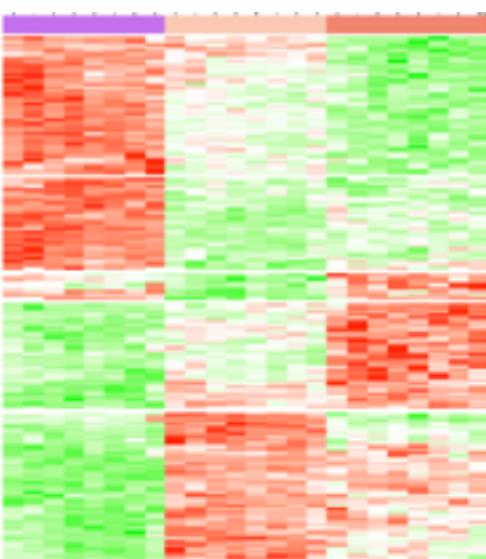
- Variable and state-dependent

# How do we study the transcriptome?



## Qualitatively

- Identify expressed genes
- Gene discovery



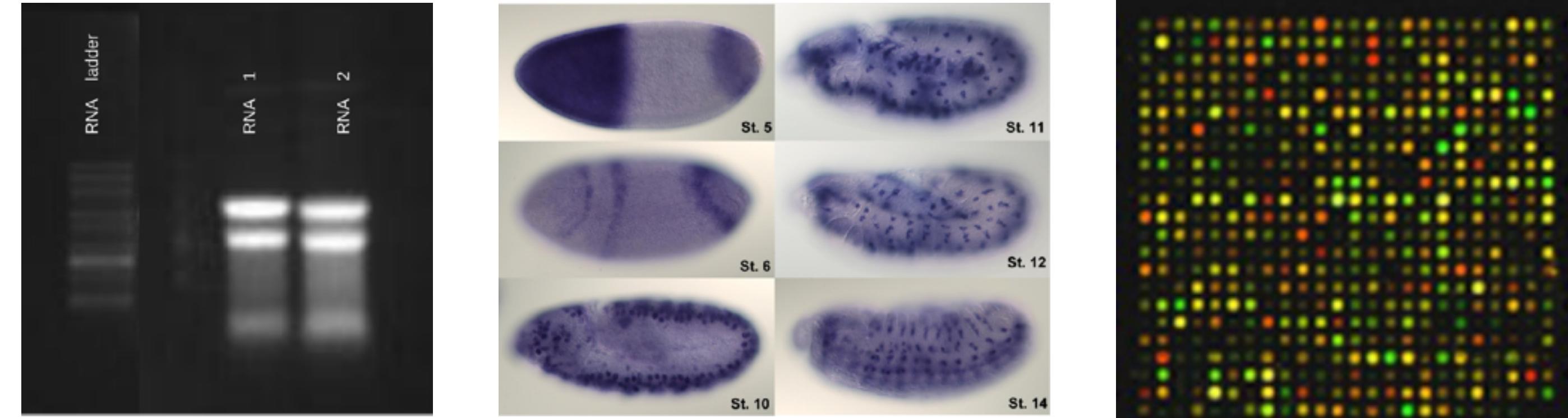
## Quantitatively

- Compare expression levels of genes or transcripts

# History of studying transcripts

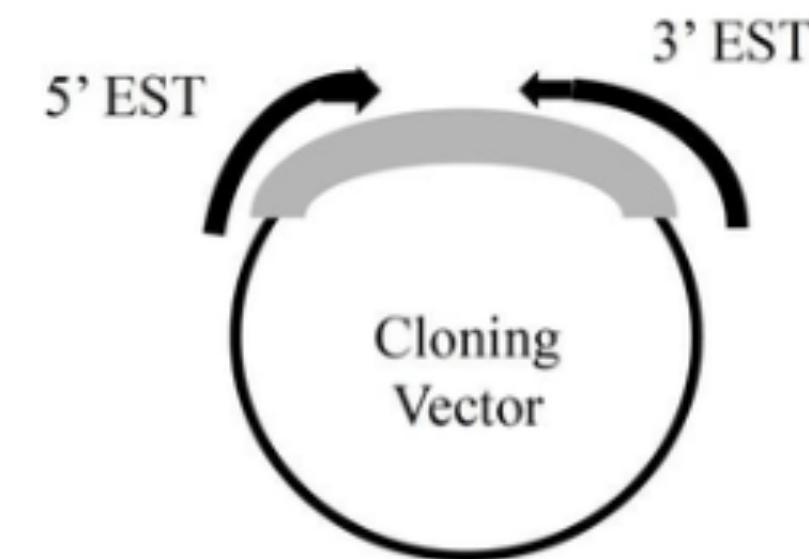
## Quantitative

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

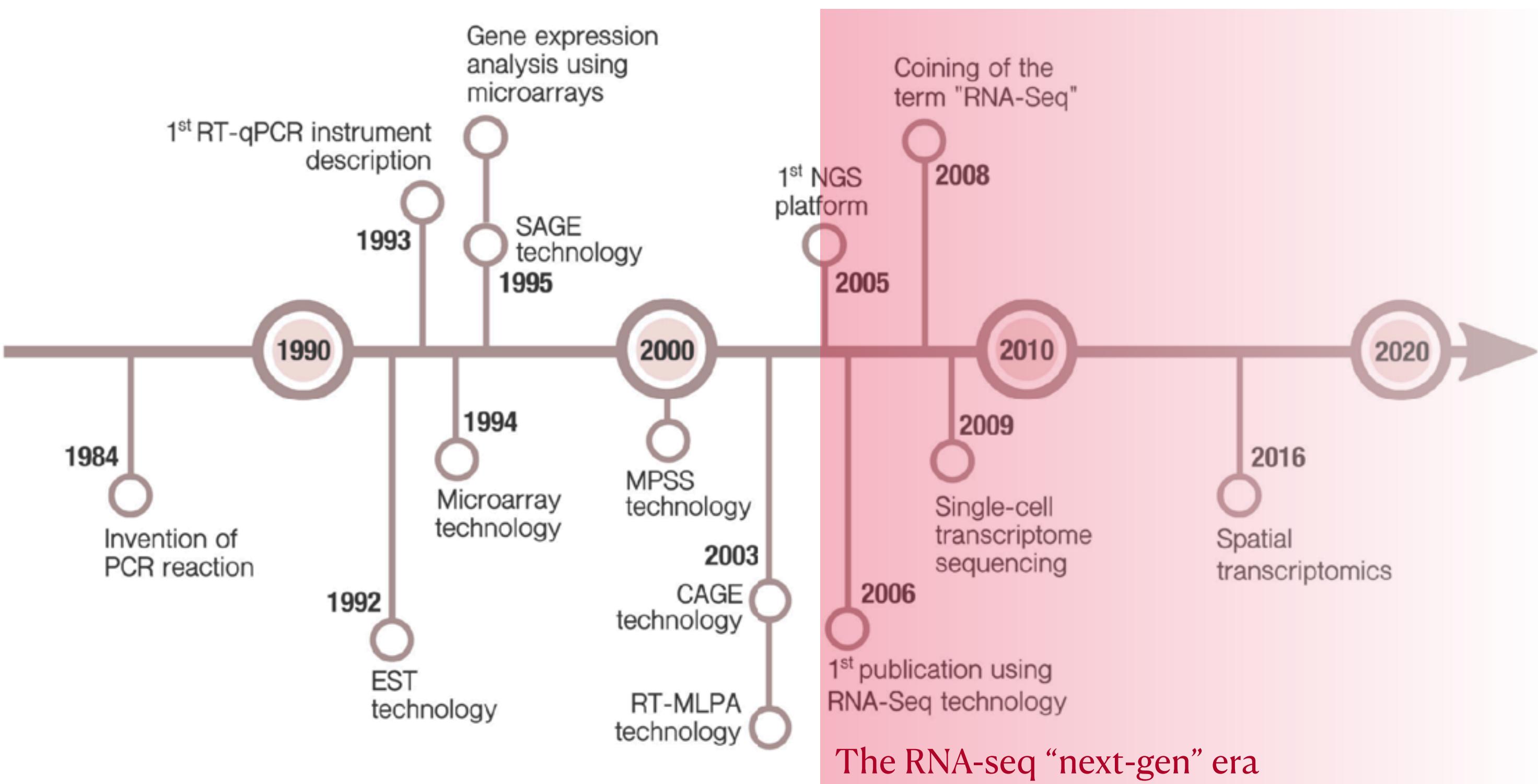


## Qualitative

- Expressed sequence tags 1990s



# History of studying transcripts

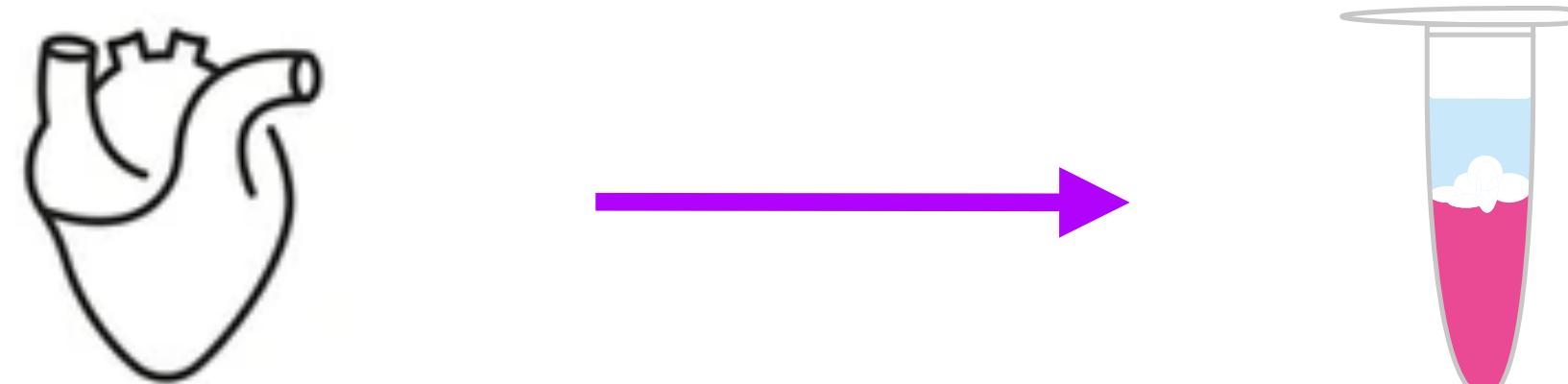


## RNA-seq

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

# RNA-Sequencing (lab)

1. RNA extraction



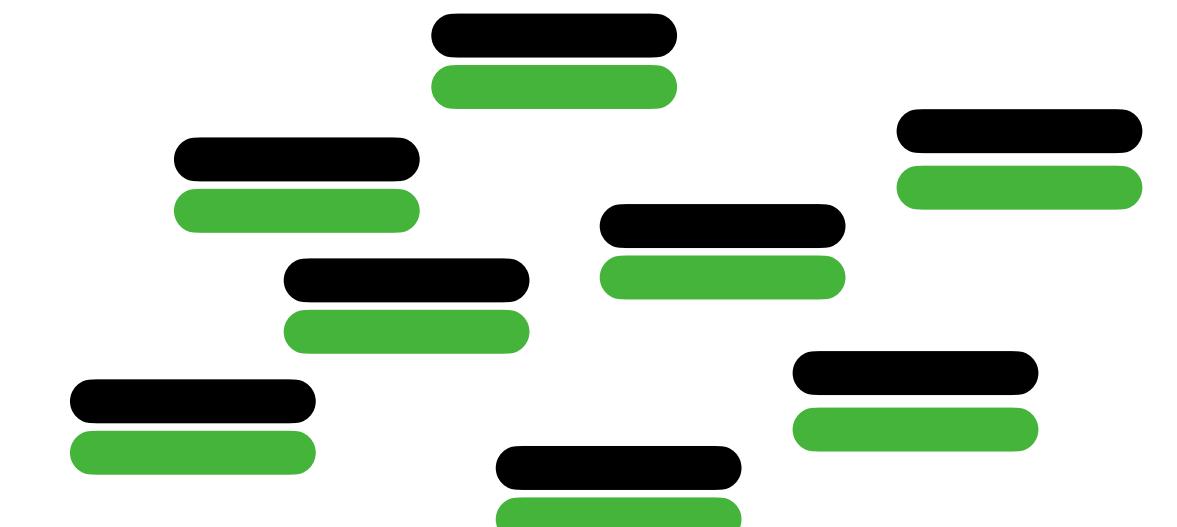
2. Isolation/purification (e.g. poly-A enrichment)



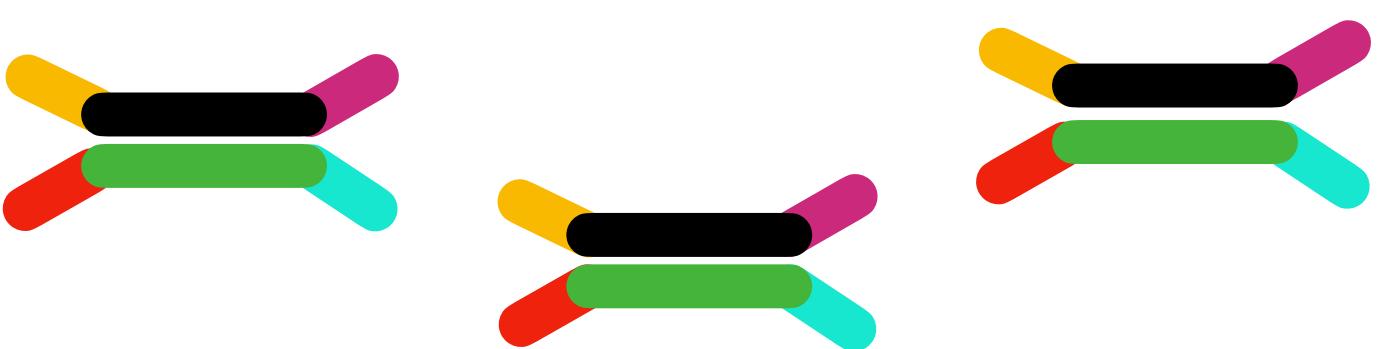
3. Fragmentation



4. cDNA synthesis



5. Adaptor ligation

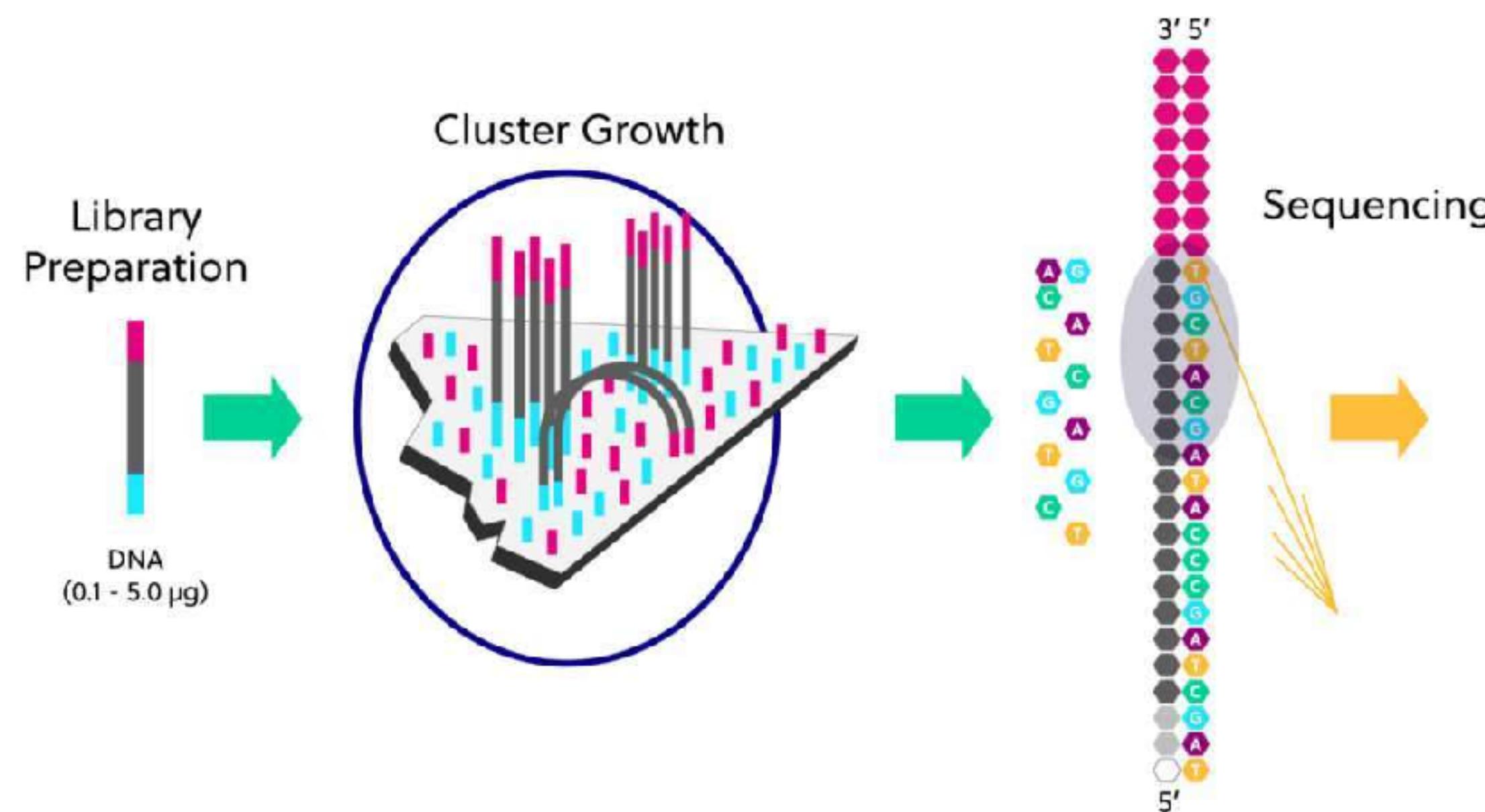


Parallel sequencing



# Next-Gen Sequencing

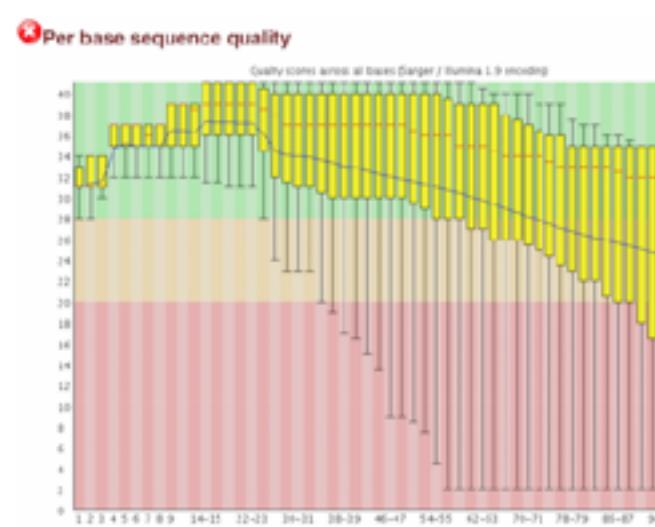
## Illumina short reads technology



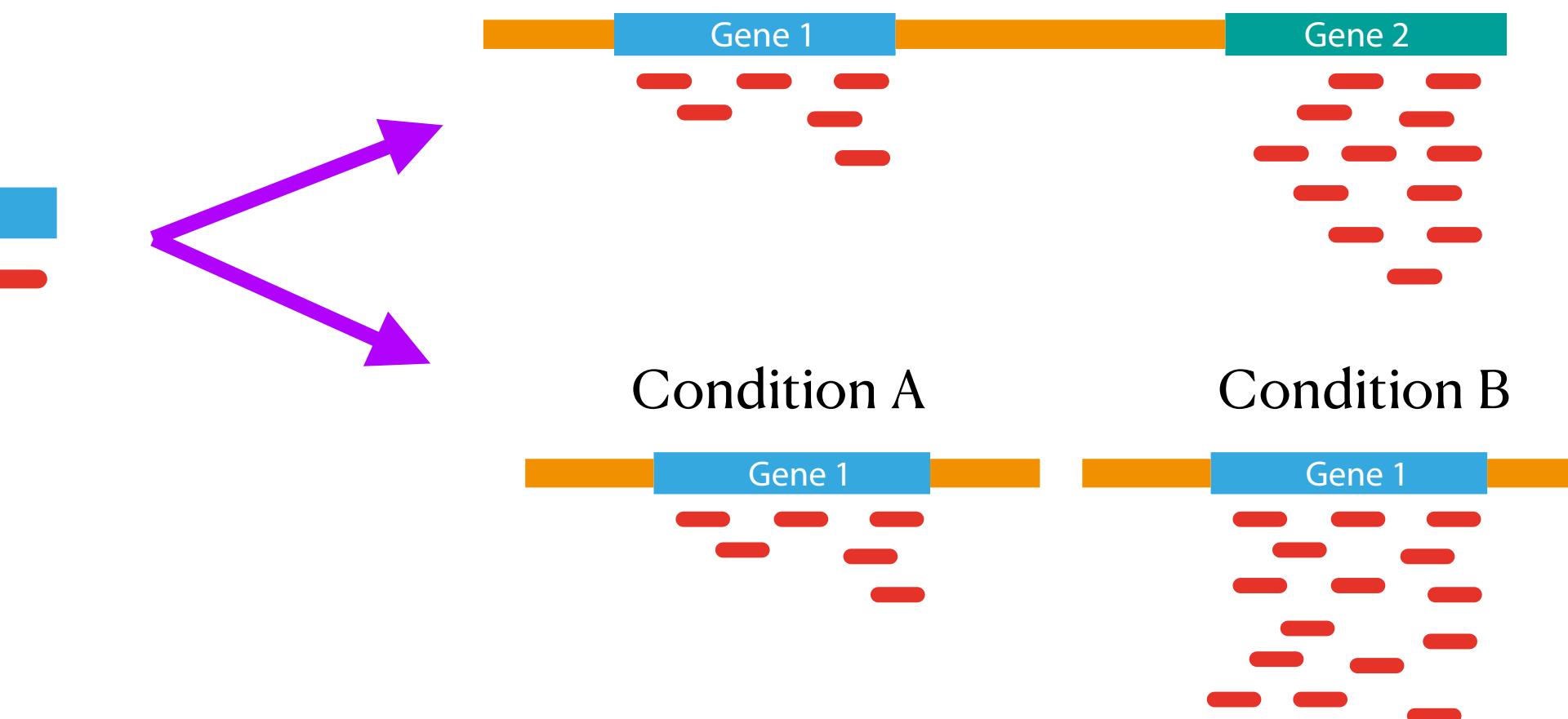
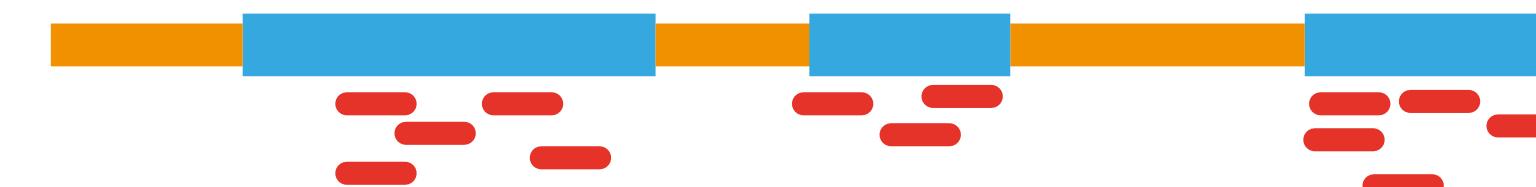
- Usual read length: 100bp
- Usual quantity of reads for RNAseq: 20-30 million reads per sample
- Single “lanes” allow running many samples at once (25 million - 20 billion reads depending on technology)

# RNA-Sequencing (in silico)

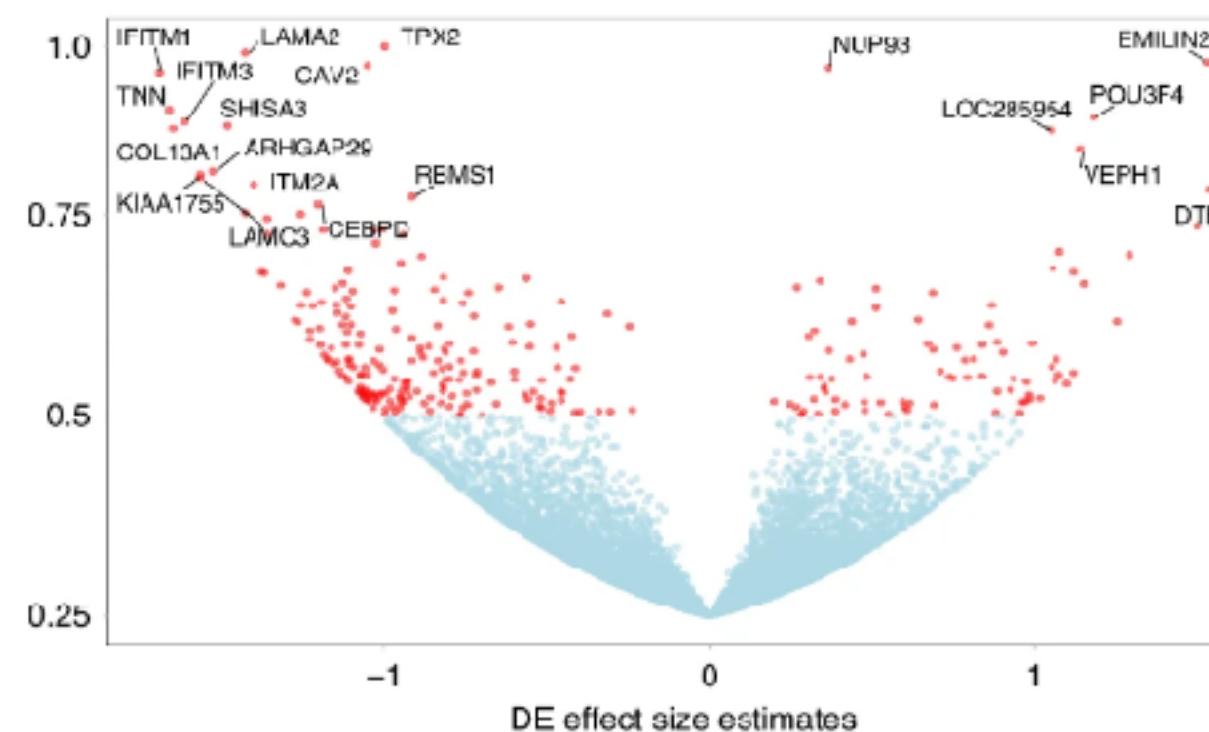
## 1. Sequence cleaning



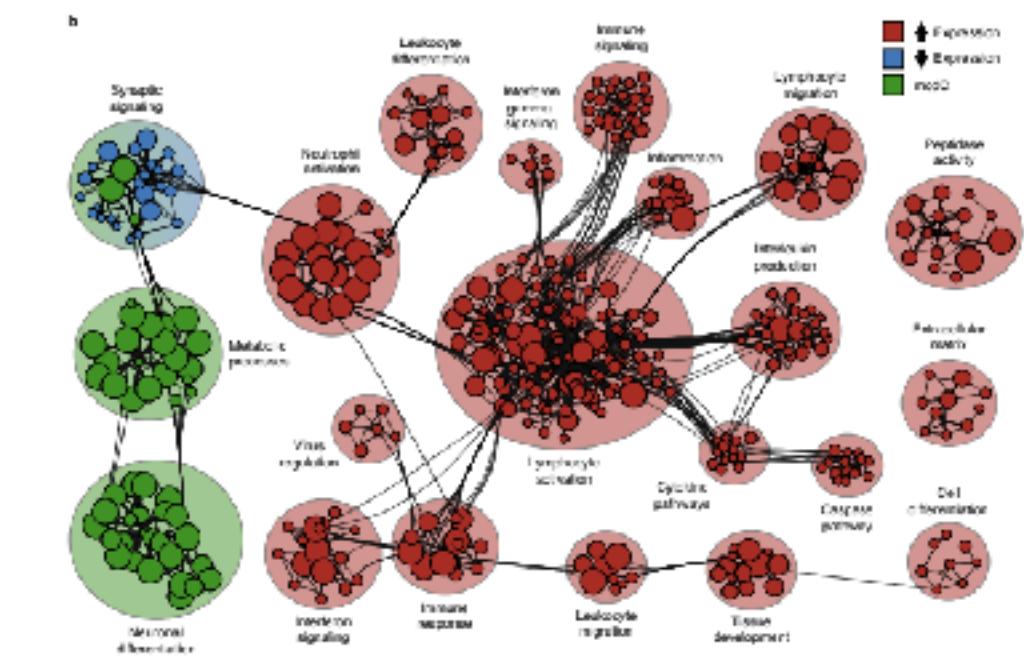
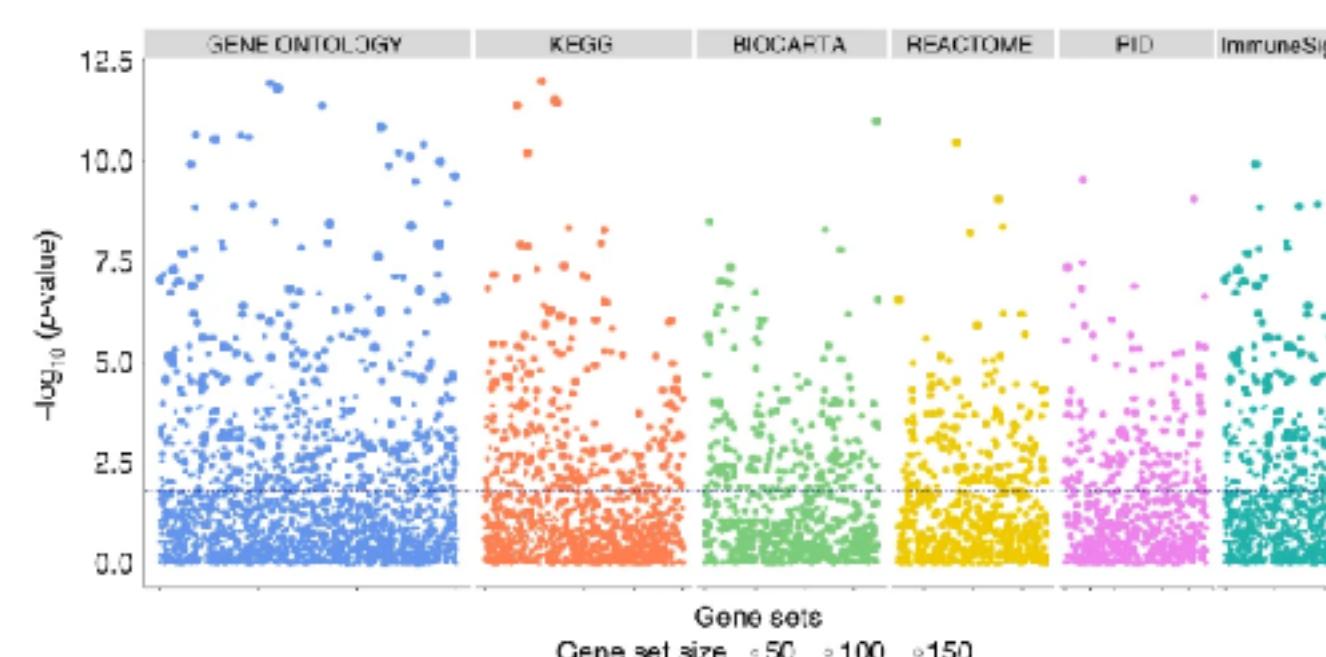
## 2. Mapping/quantification



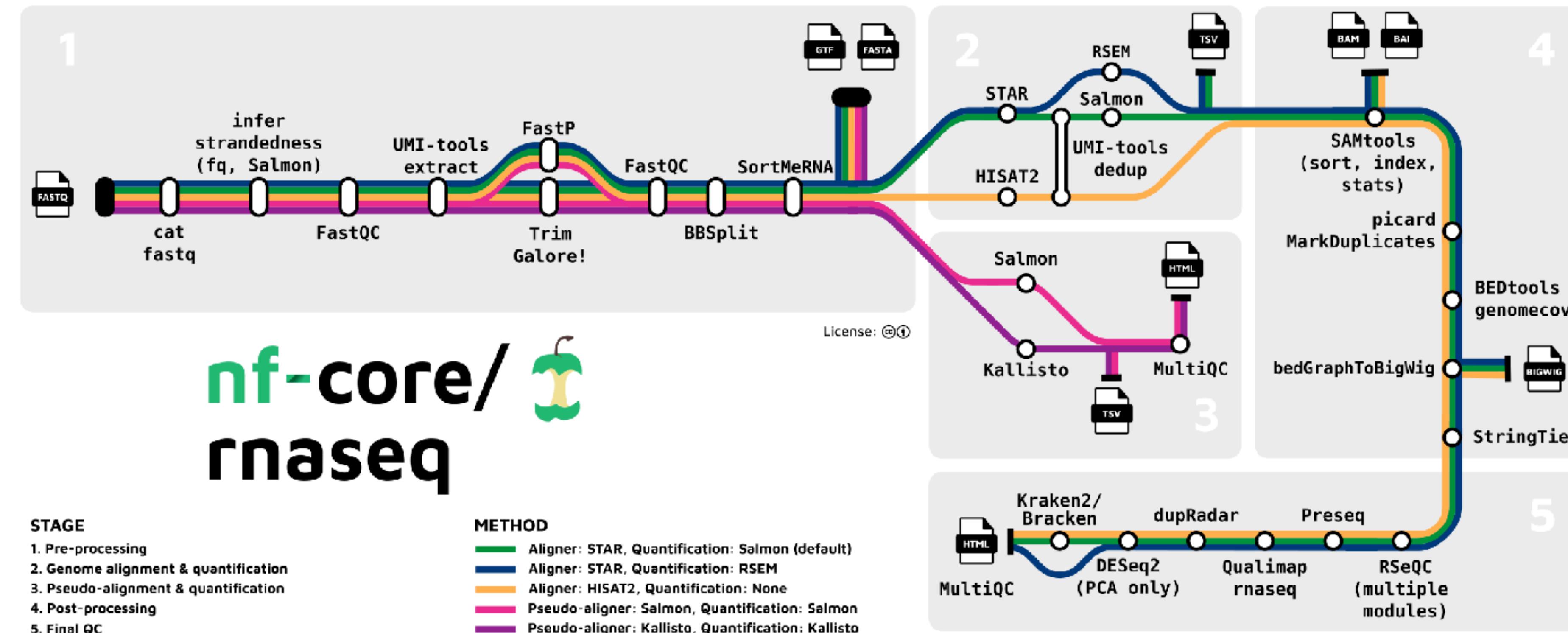
## 3. Differential gene expression



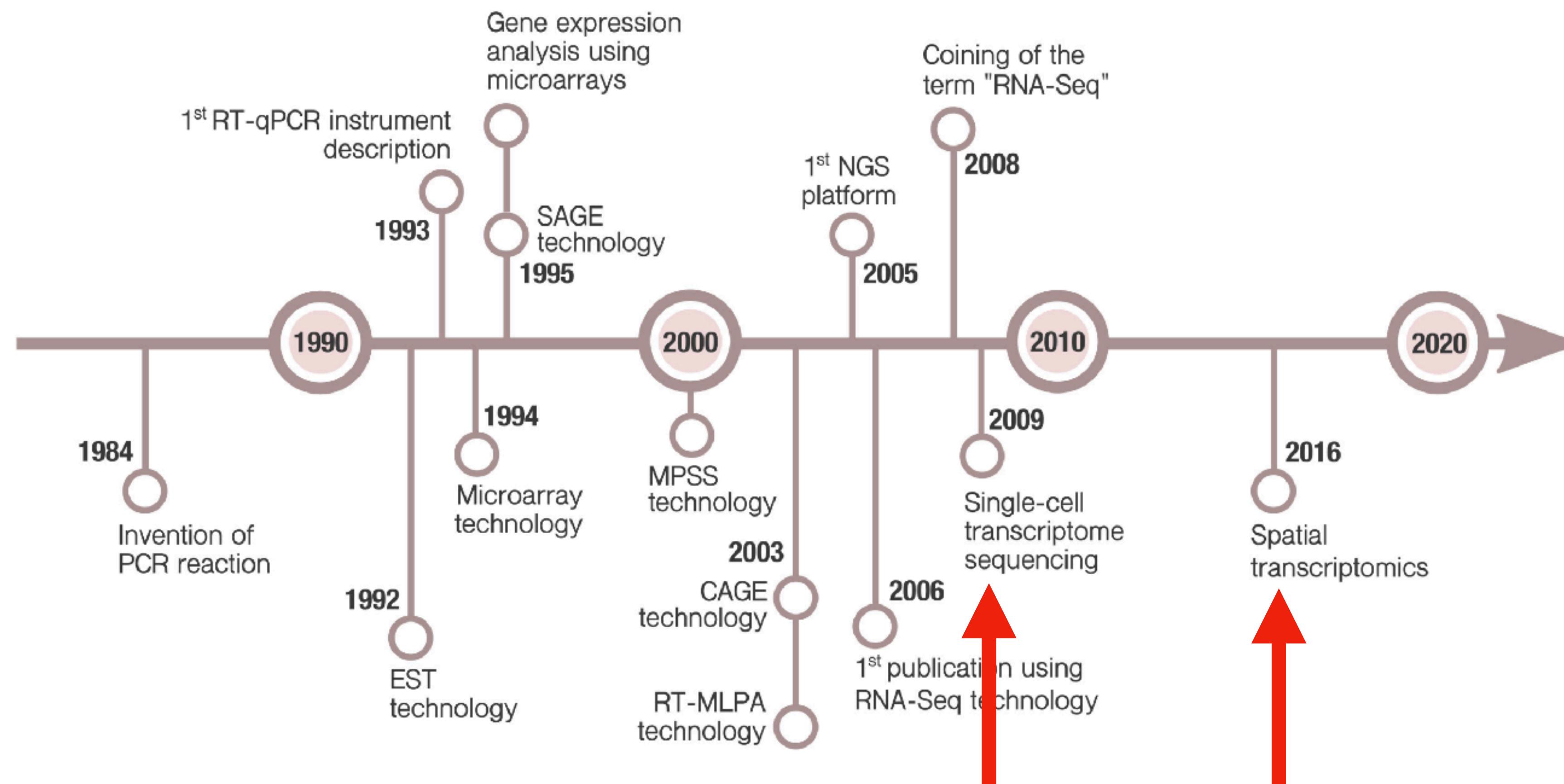
## 4. Functional insights



# Pipelines and Work Flows



# “BULK” RNA-seq vs. Other RNA-Seq



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

## Disadvantage of bulk RNA-Seq:

- Averaging effects (loss of cellular heterogeneity)

## Advantages of bulk RNA-Seq:

- Relatively cheap
- Flexible starting material quantities
- Very straightforward protocols
- No specialised equipment
- Well established bioinformatic pipelines

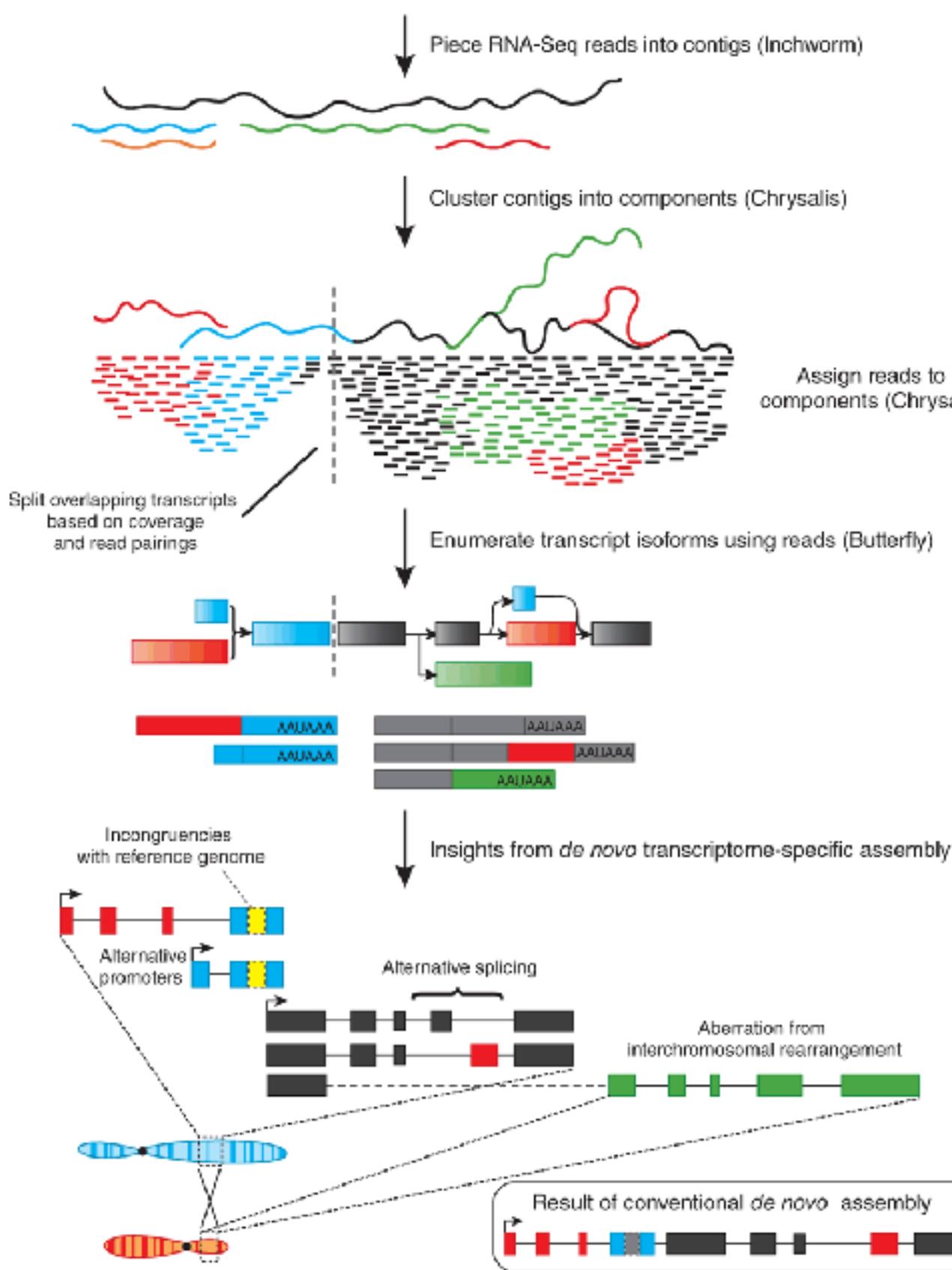
## Main uses of bulk RNA-Seq:

- Gene Expression Profiling:
  - Across tissues
  - Across developmental stages
  - Across conditions
- Genome annotation
- 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

# What if I don't have a reference genome?

## No Genome? No problem!

Trinity *de novo* transcriptome assembly



### Genomes:

- Higher accuracy
- Better annotations (sometimes)

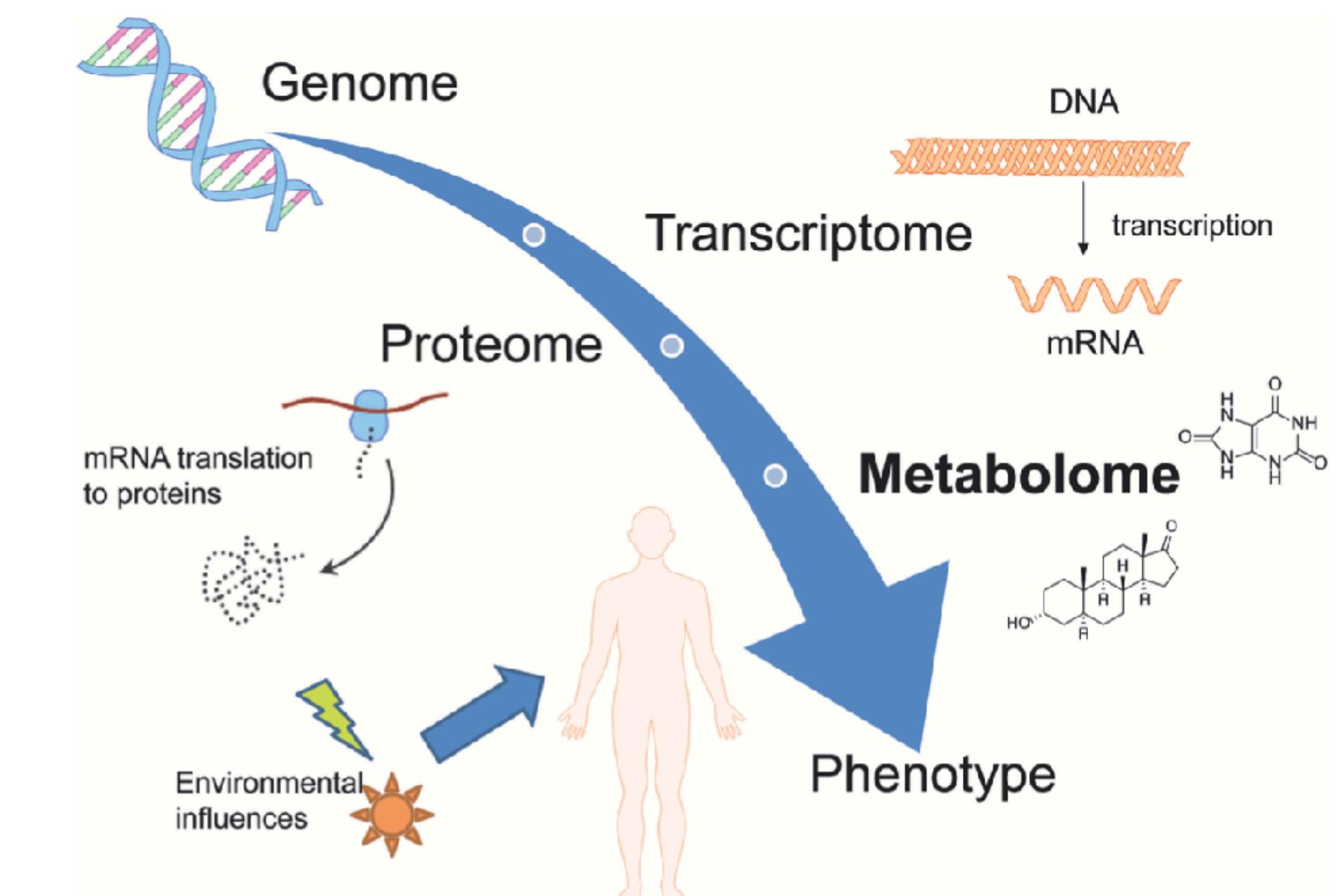
### *De novo* Transcriptomes:

- Better than nothing
- Avoids mapping bias

# 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



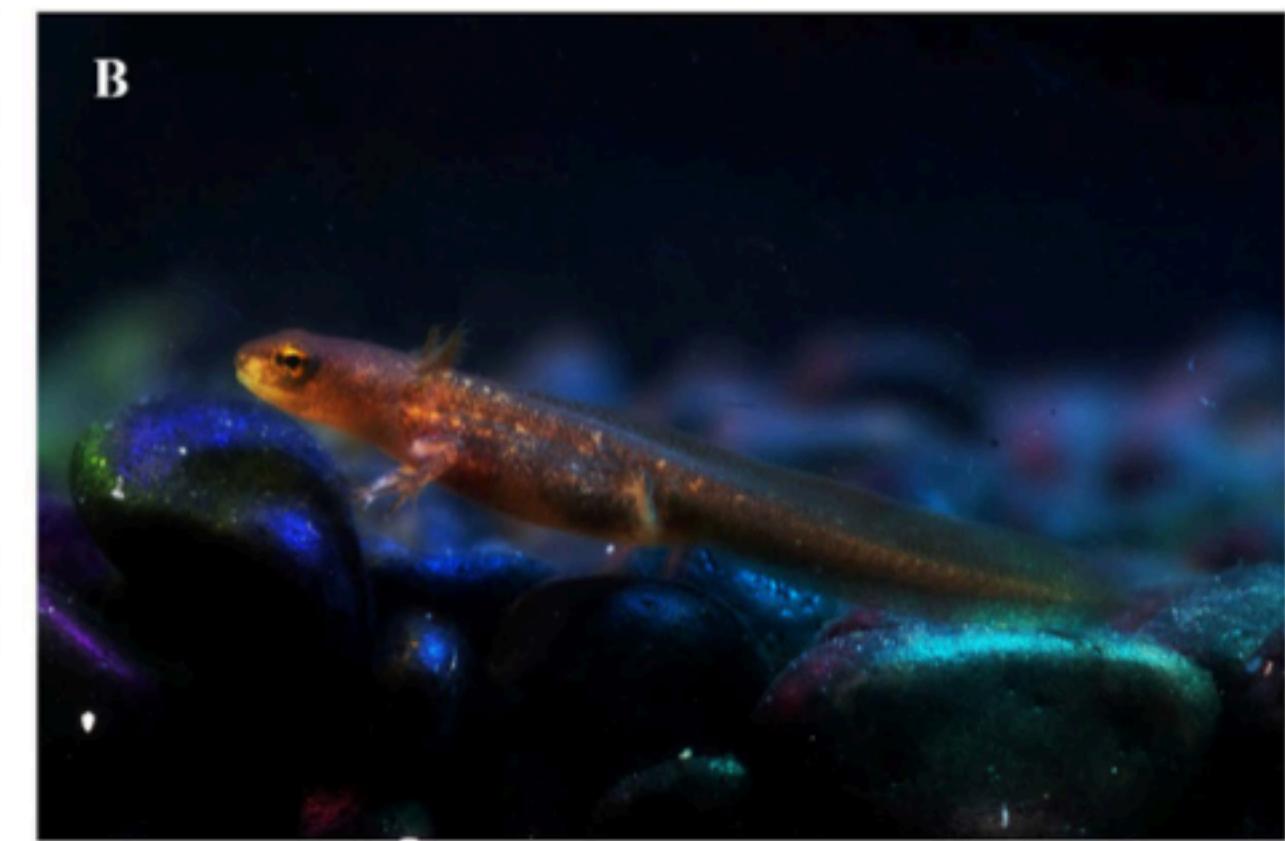
Night



Day



Light background



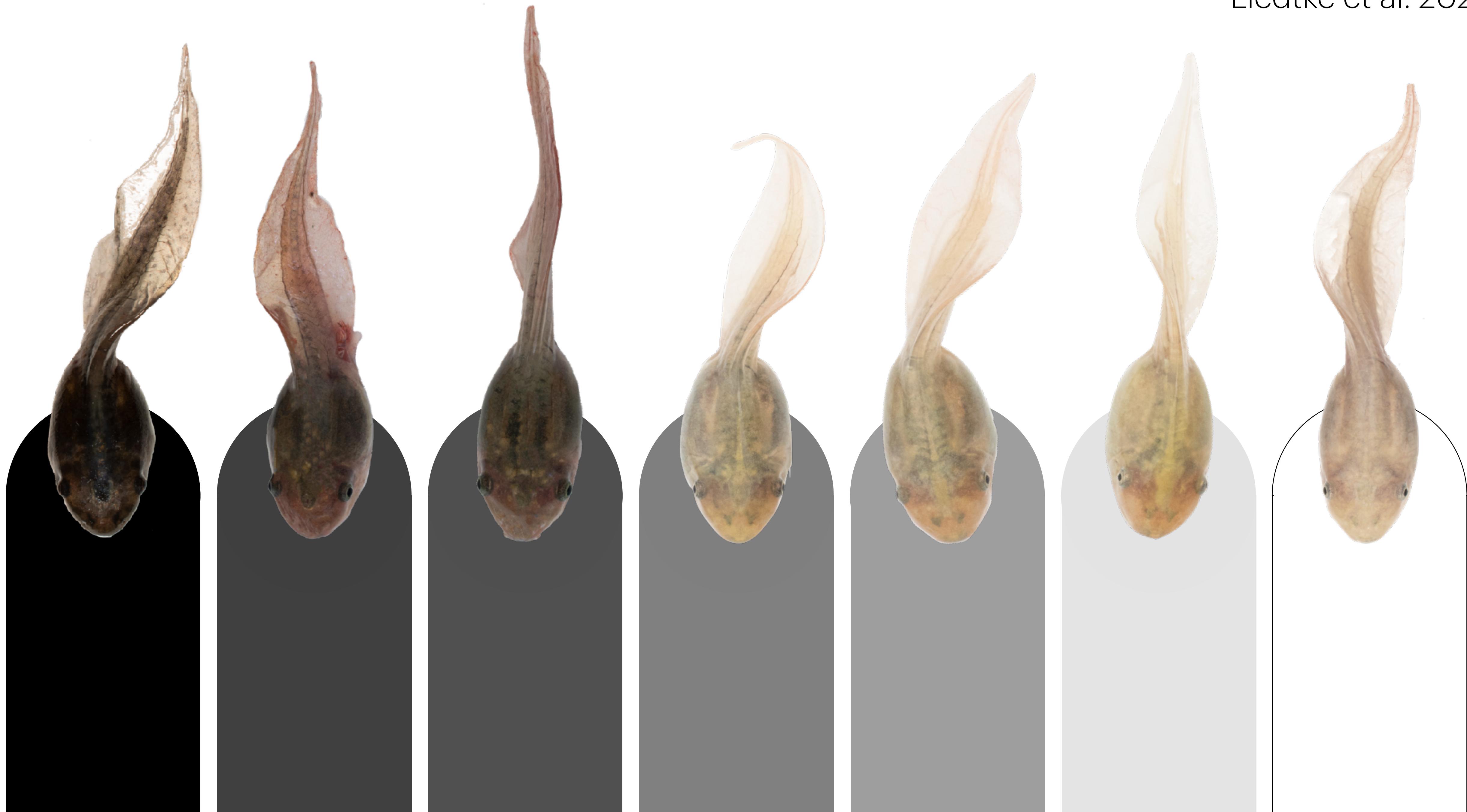
Dark background

# *Pelobates cultripes*

Western Spadefoot toad



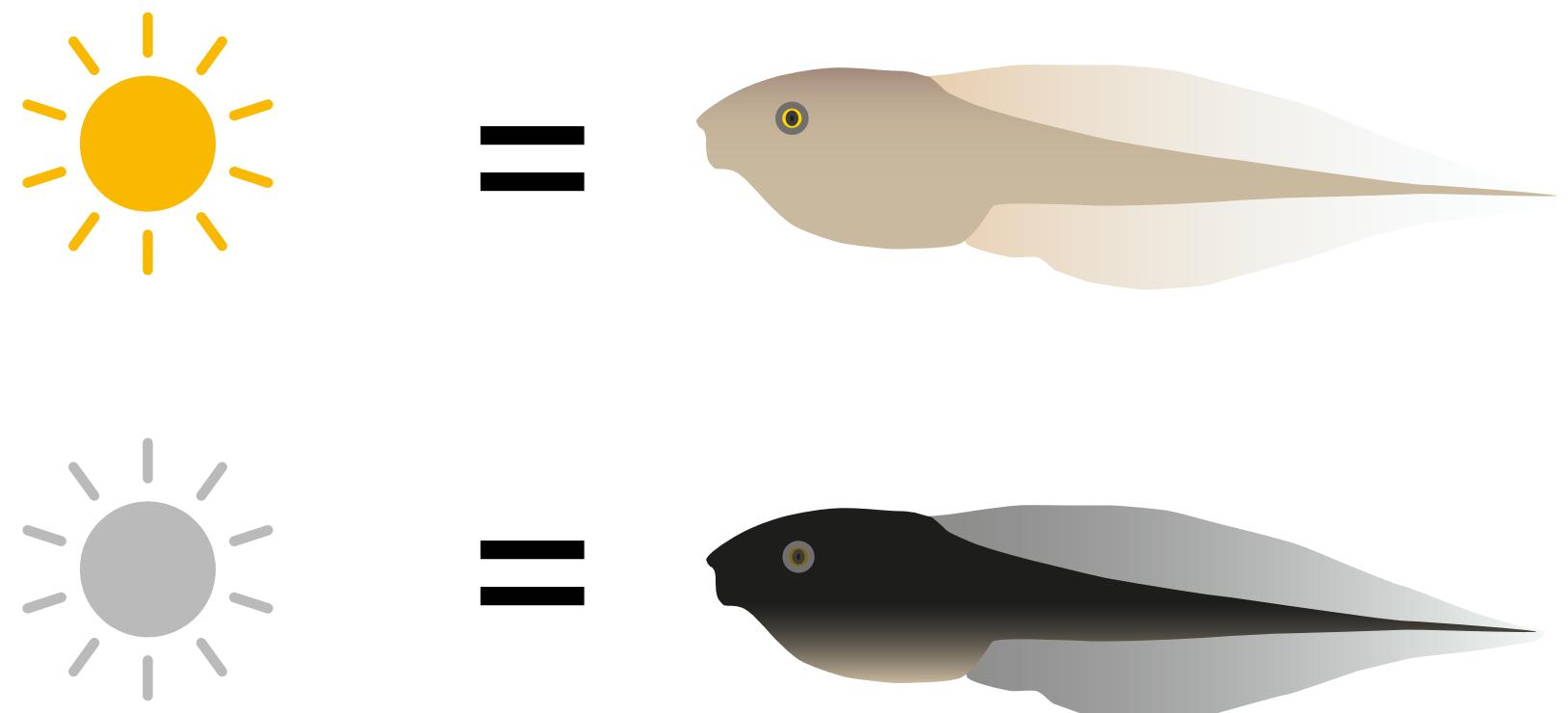




# Colour change is reversible



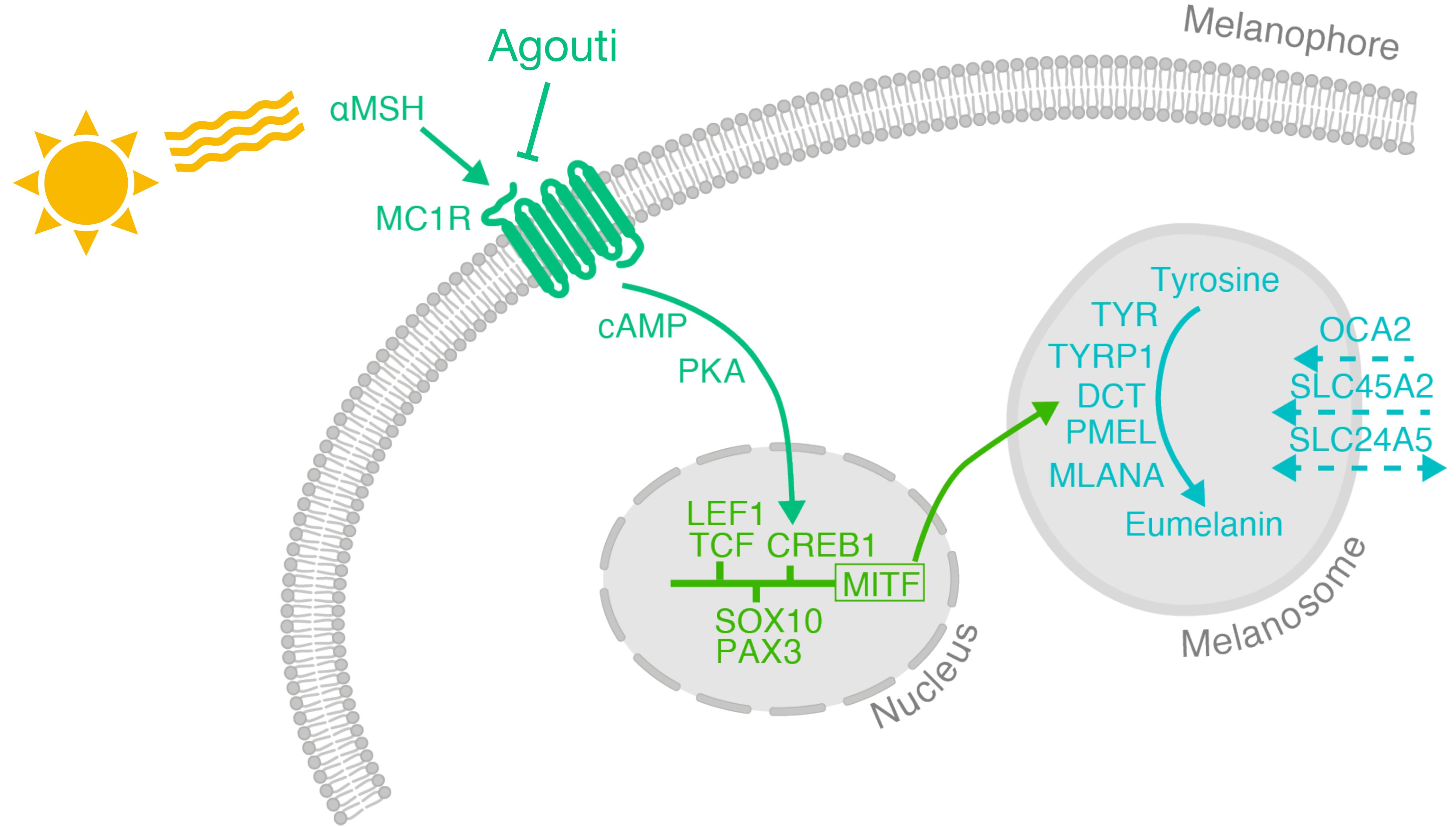
# Inverse photosensitivity of plastic melanisation

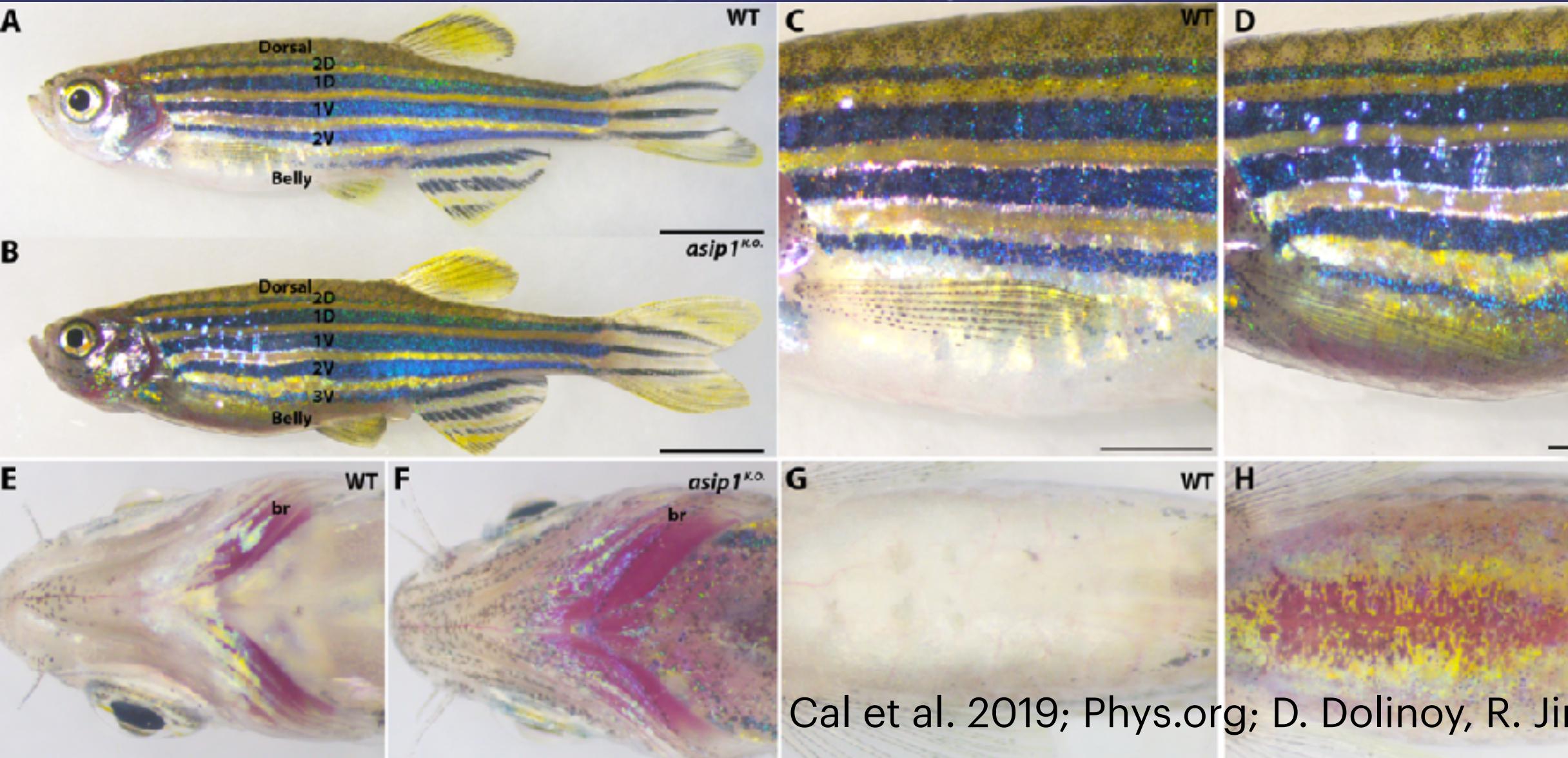
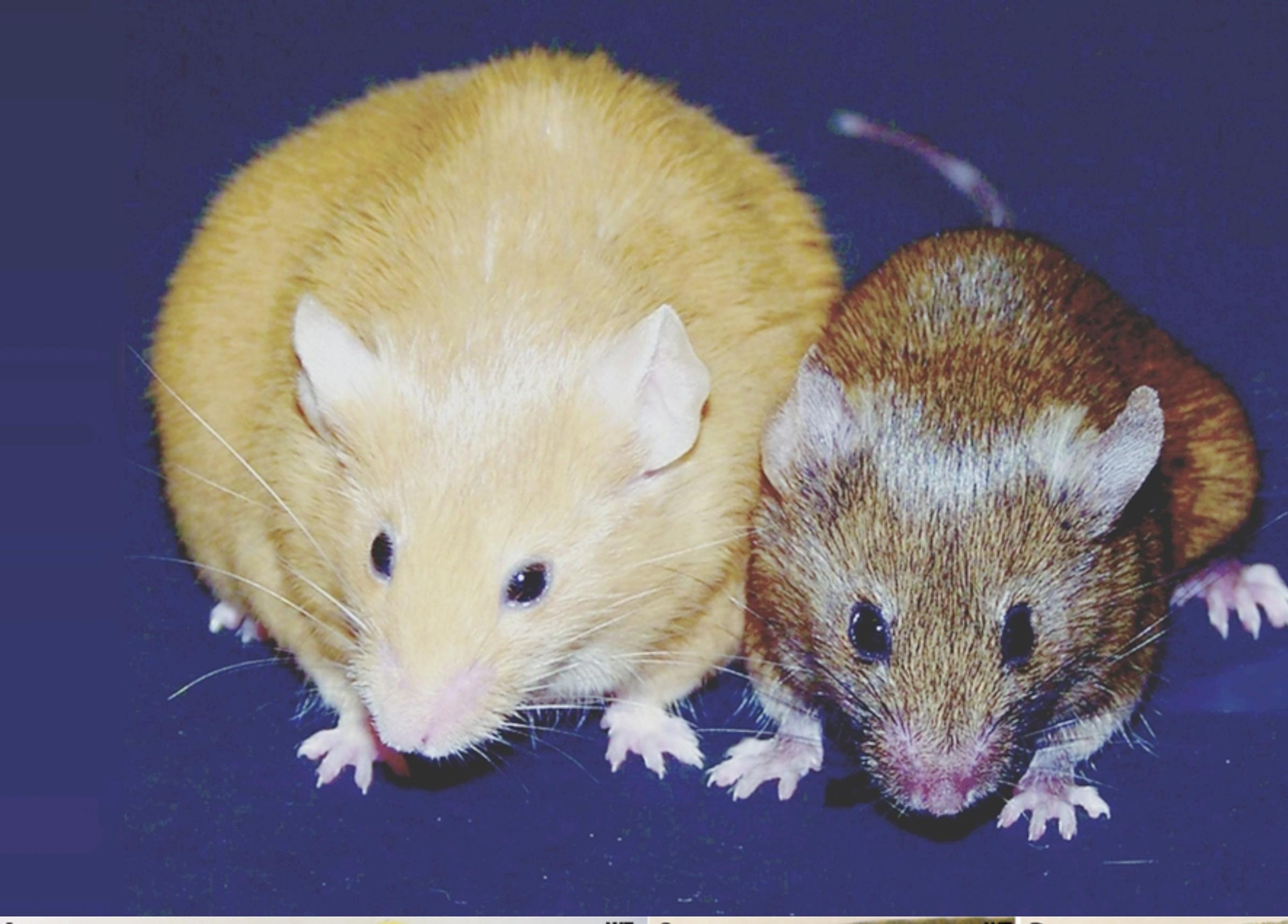


## Non-plastic countershading

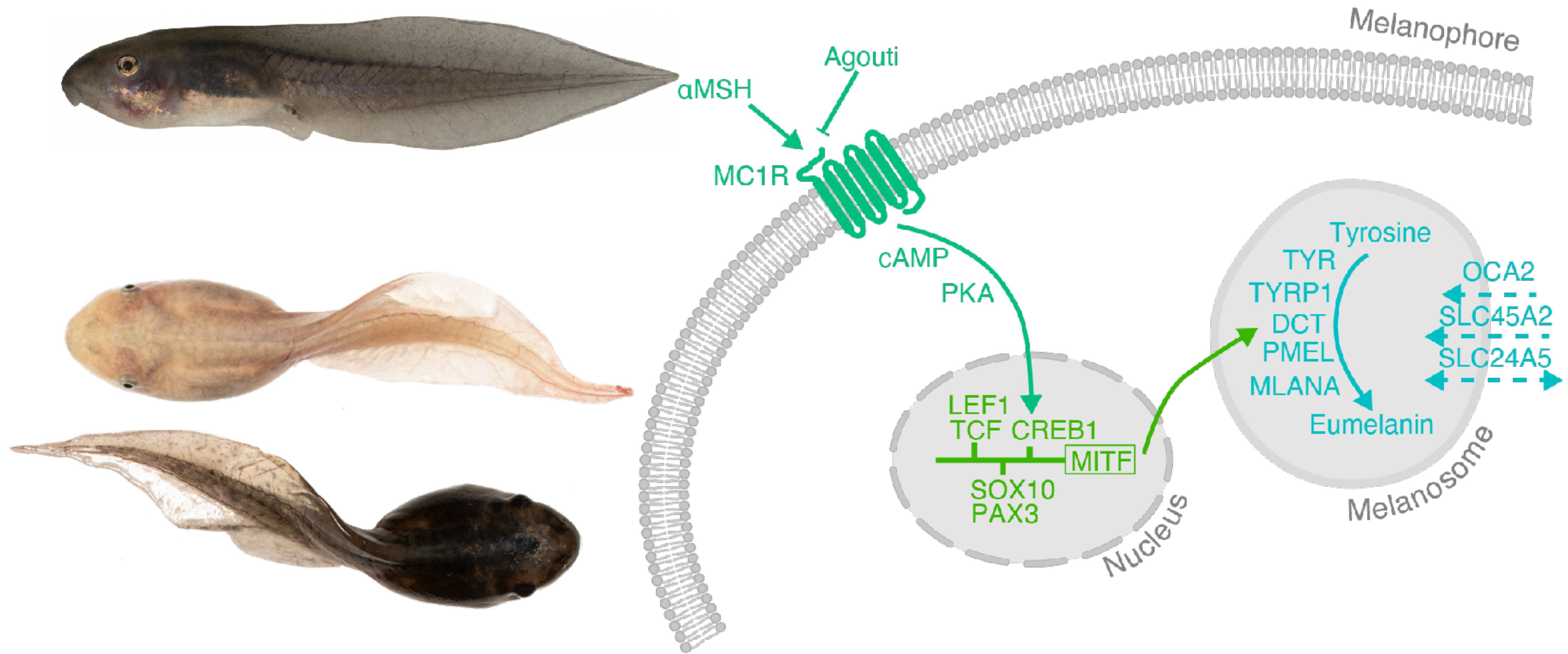


# Photosensitive melanin biosynthesis





# Photosensitive melanin biosynthesis

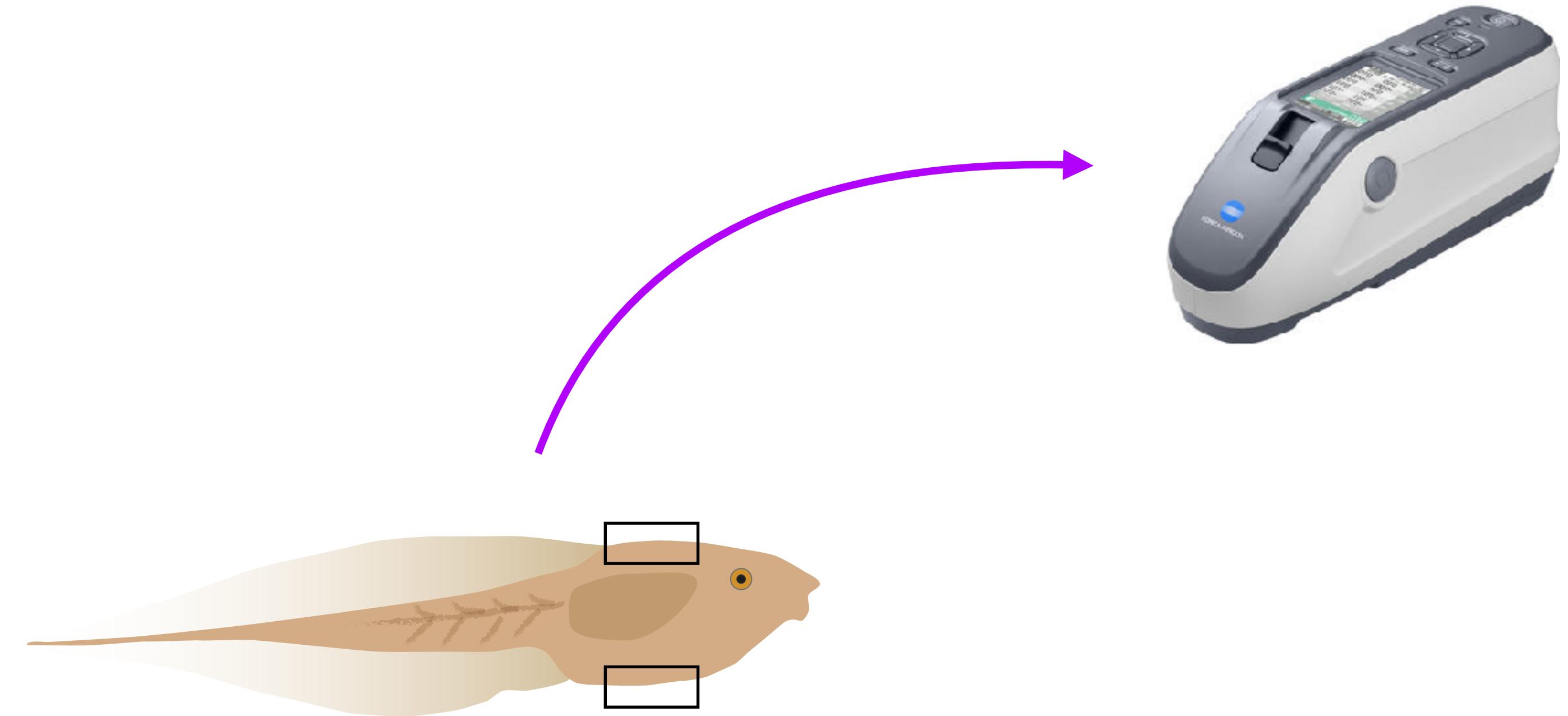


# Background matching experiment

Spectrophotometry



40-day exposure

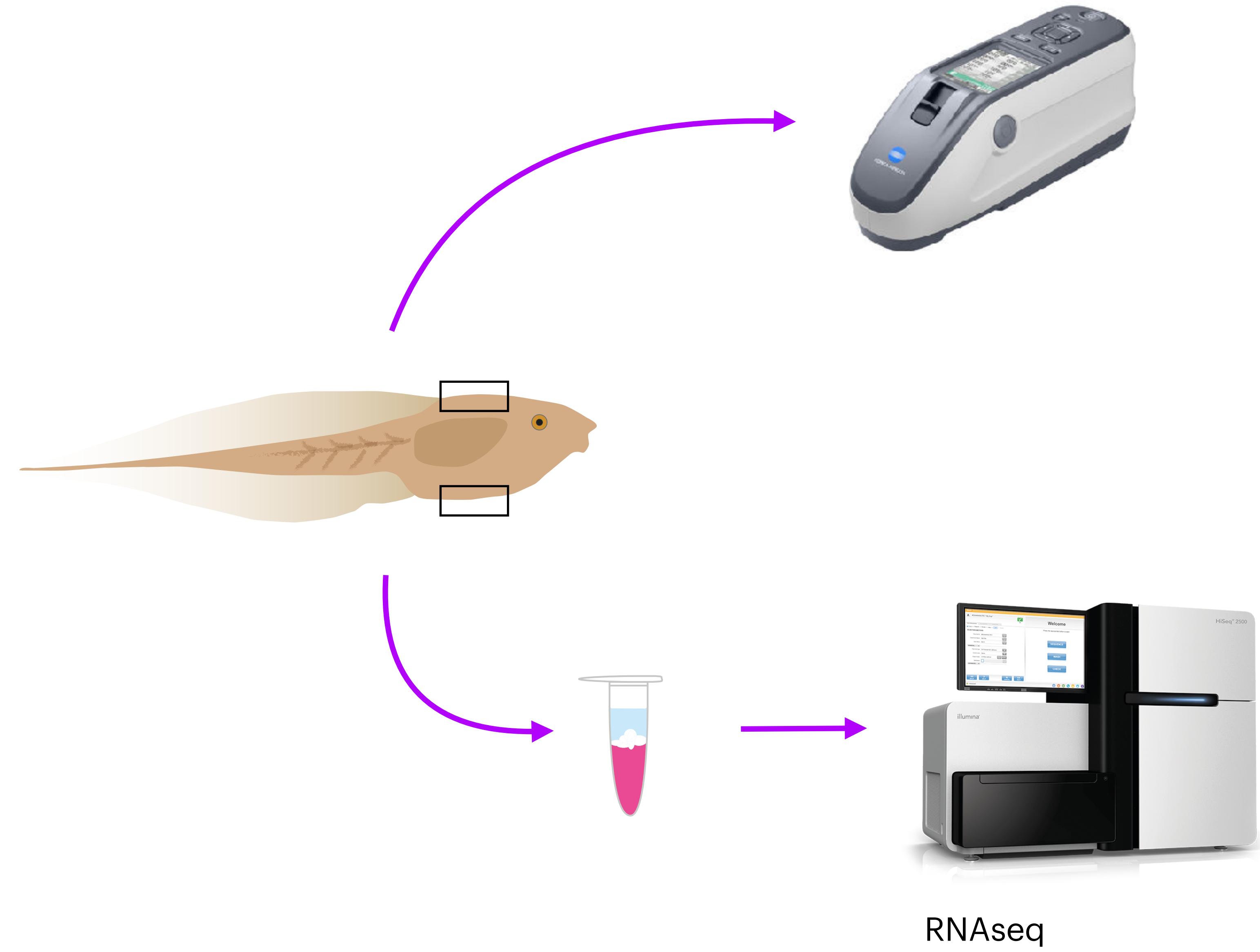


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Spectrophotometry



40-day exposure



# Data wrangling and visualisation with the Tidyverse

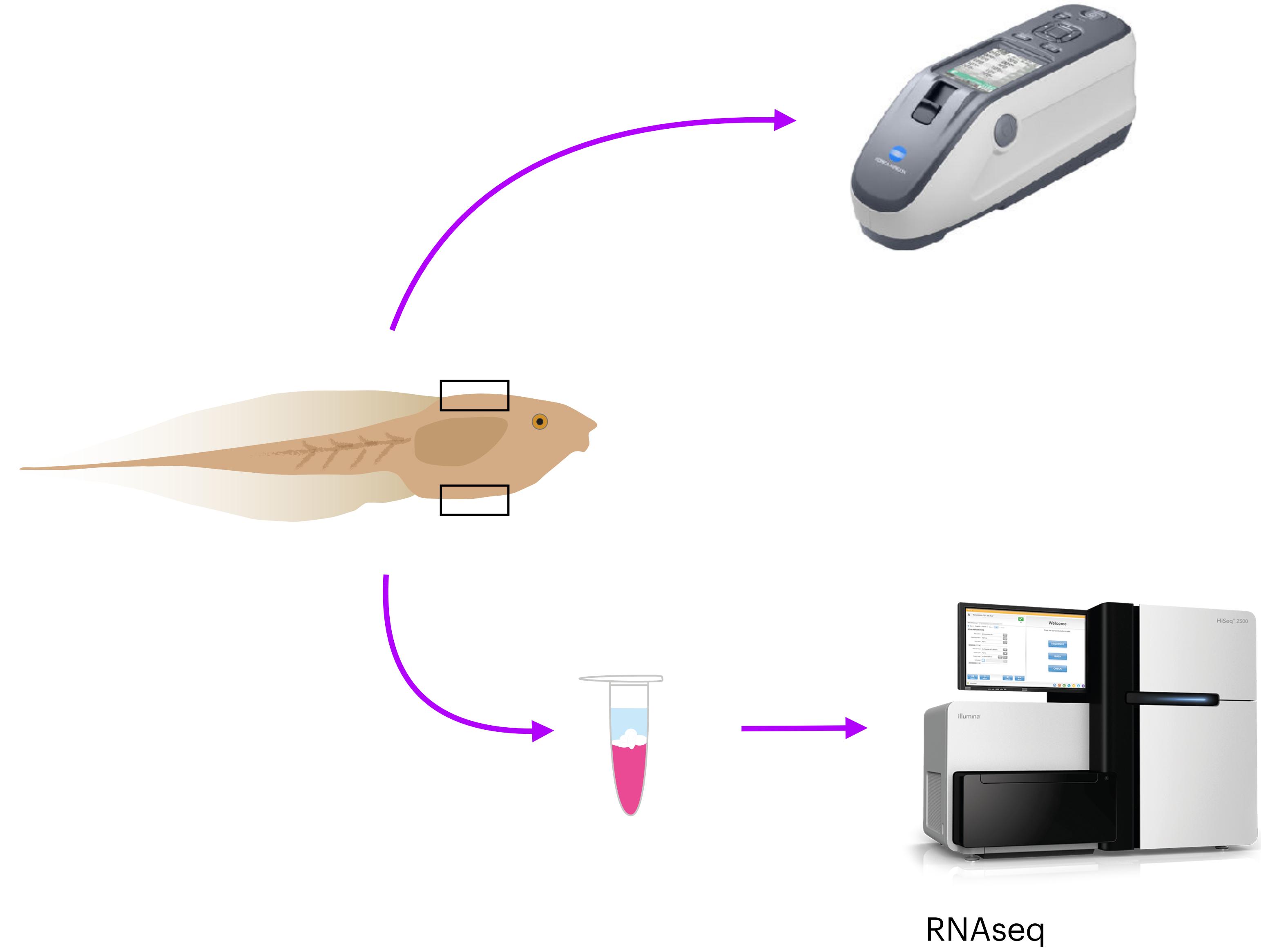
**[https://hcliedtke.github.io/UAM\\_NewTech](https://hcliedtke.github.io/UAM_NewTech)**

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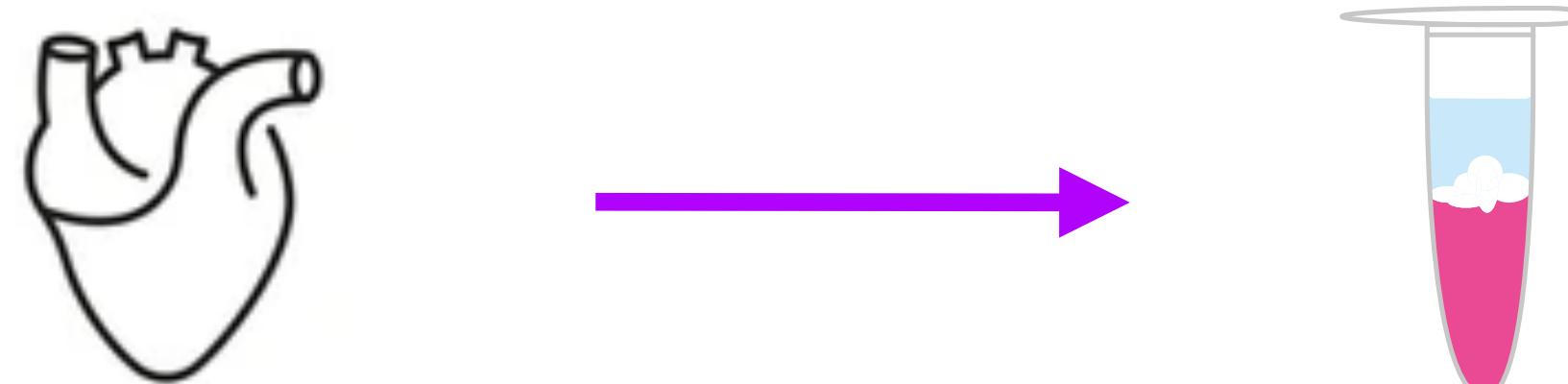


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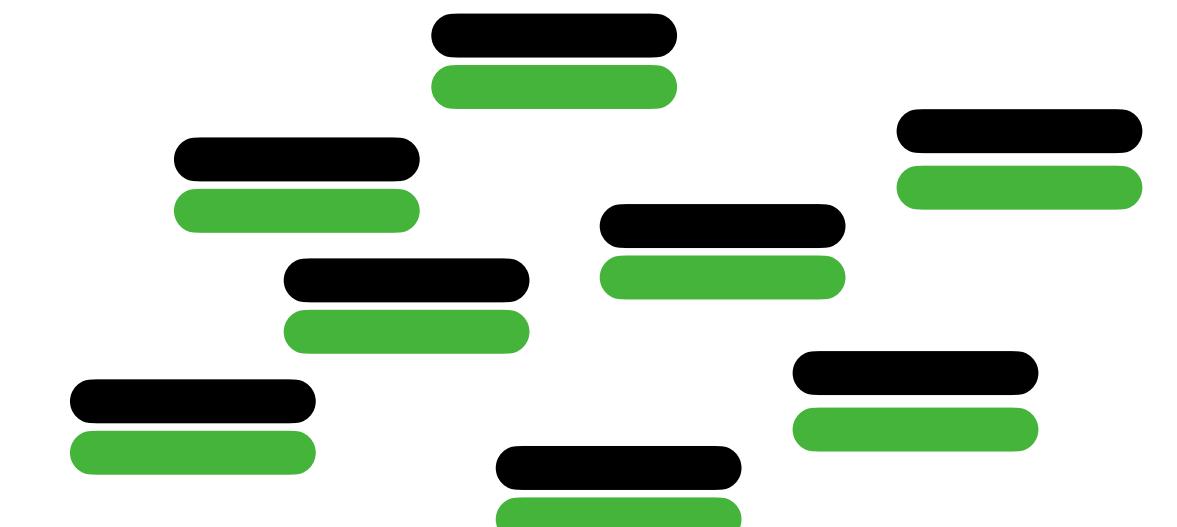
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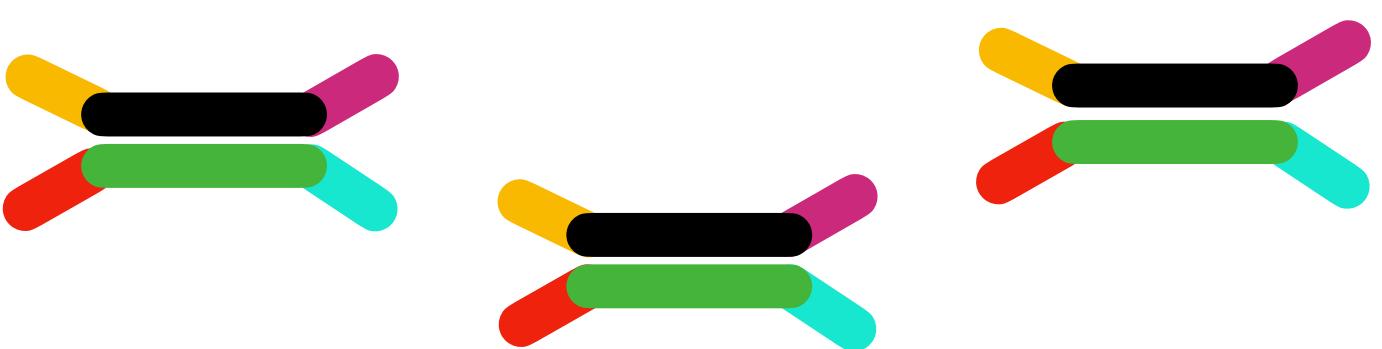
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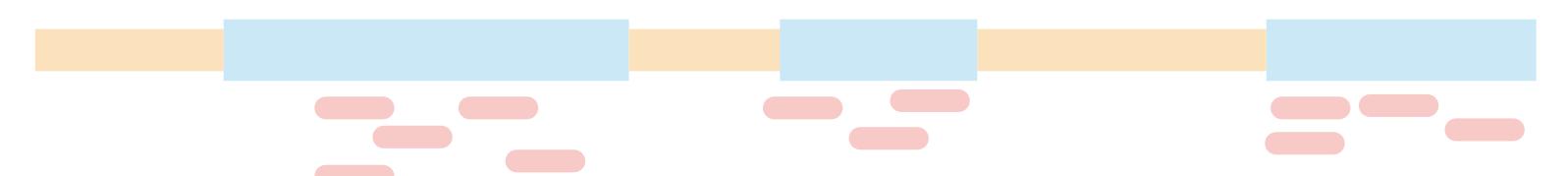
Parallel sequencing



# RNA-Sequencing (in silica)

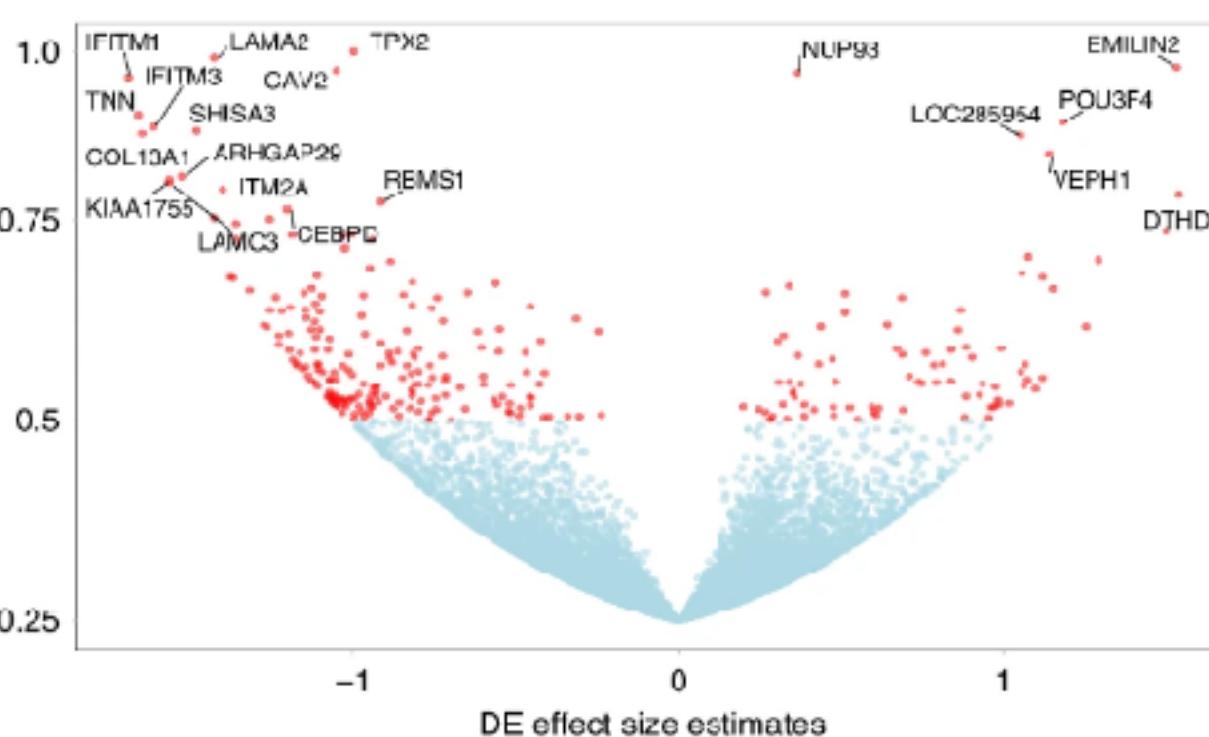
## 1.Sequence cleaning

## 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) at positions 150, 200, 250, and 300. Gene 2 has nine transcripts at positions 350, 400, 450, 500, 550, 600, 650, 700, and 750. A large purple arrow points from the left towards Gene 1.

### 3. Differential gene expression

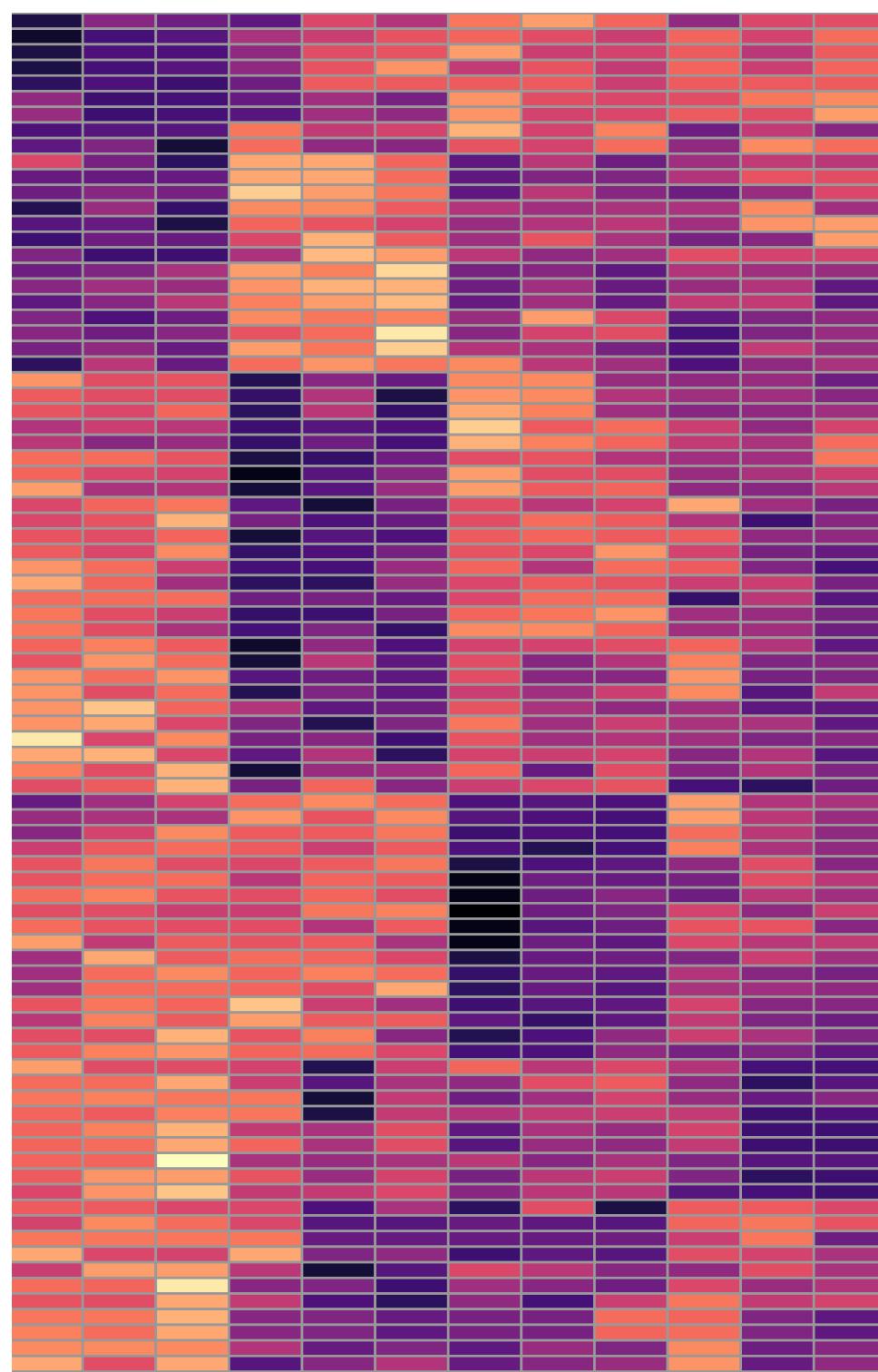


## 4. Functional insights

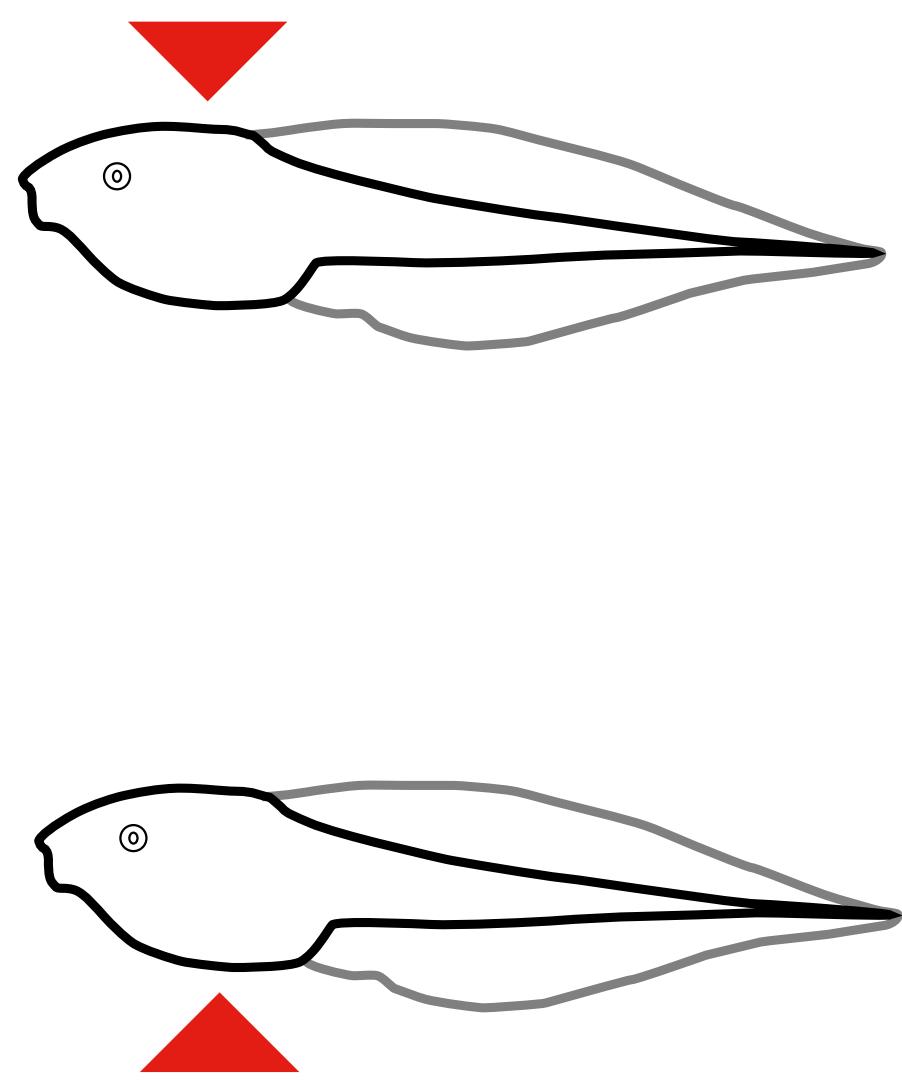
# RNA-seq counts matrix

	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

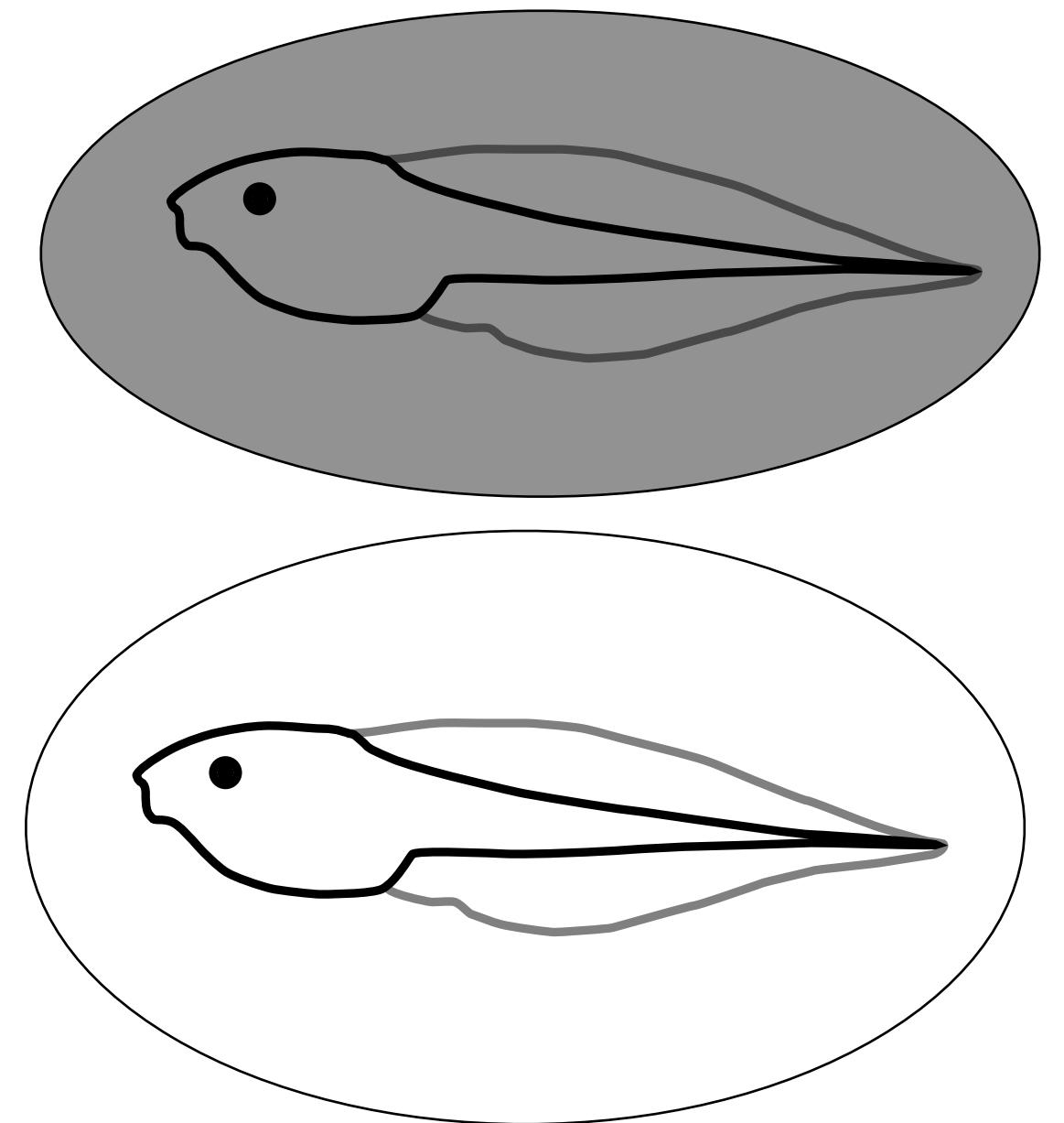


?



Anatomical effect

+

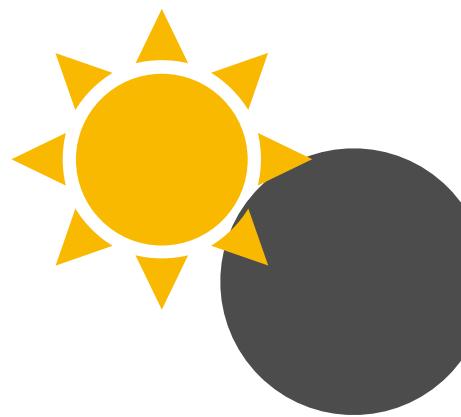


Background effect

# Differential Gene Expression

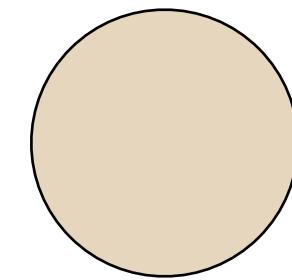


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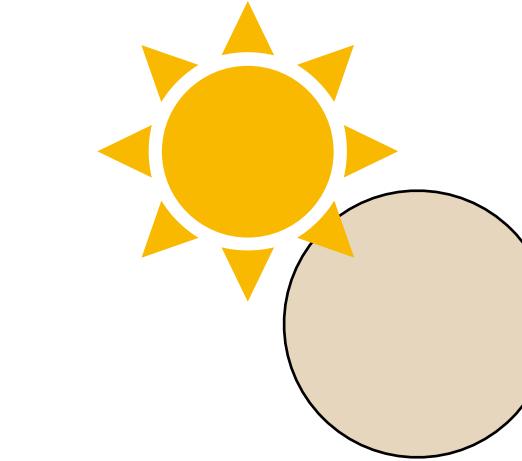
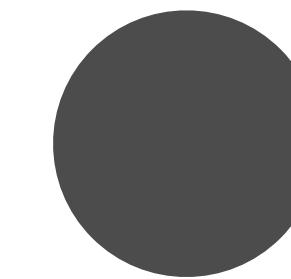


Fixed pigmentation

+



Plastic pigmentation

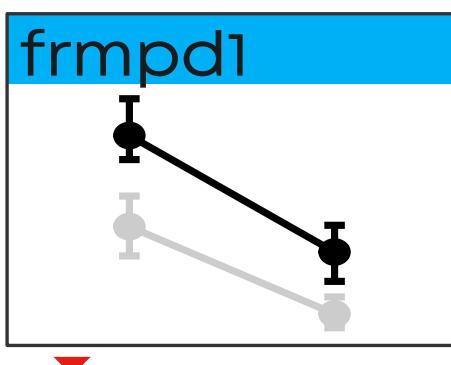
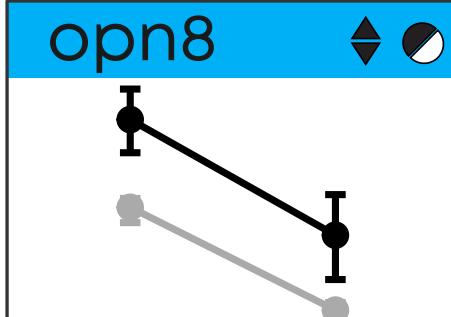


# RNA-seq practical

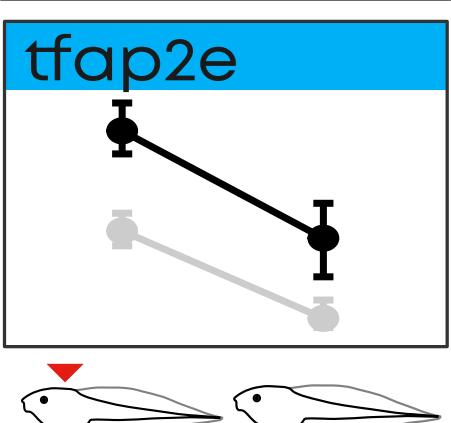
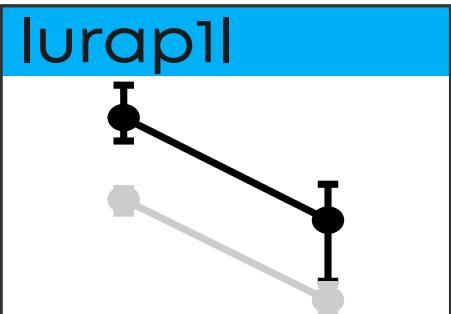
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# Differential Gene Expression

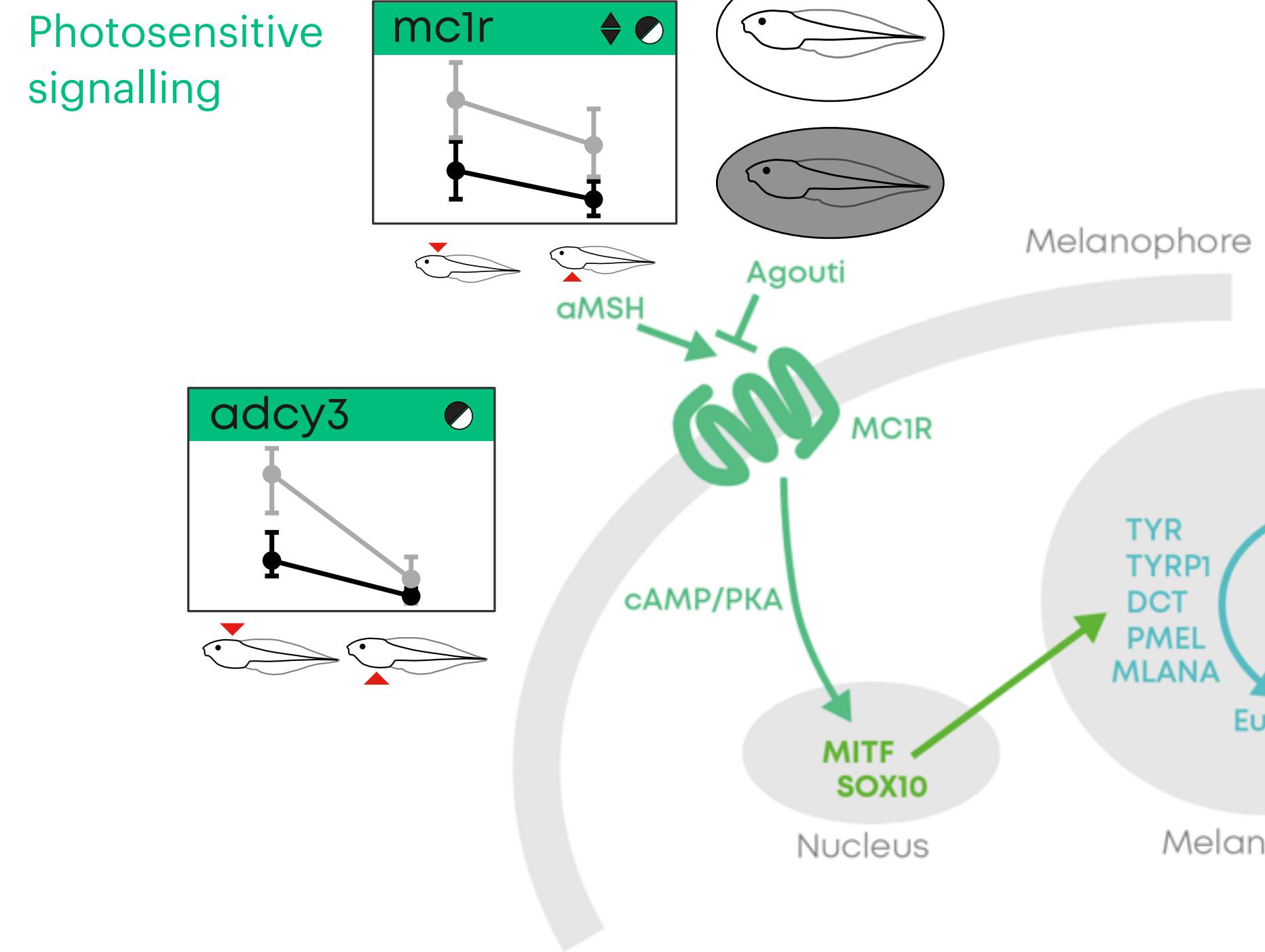
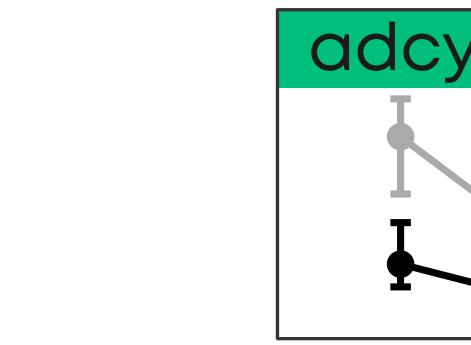
## Photosensitive signalling



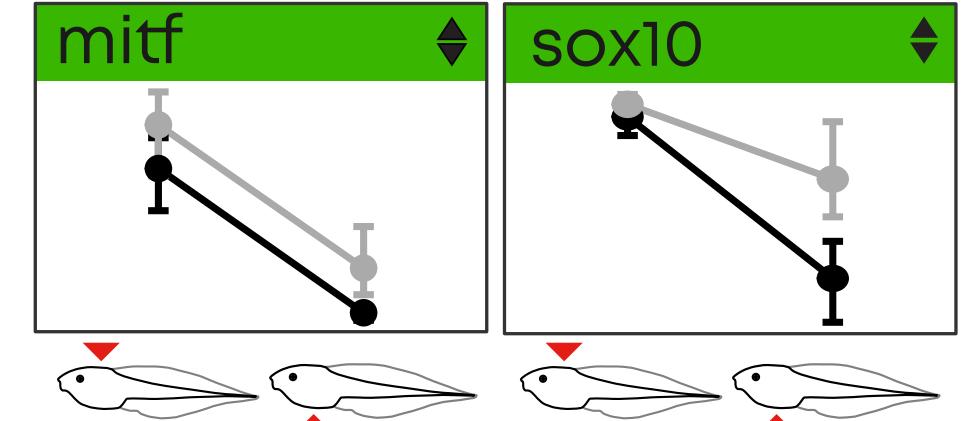
## Transcription regulators



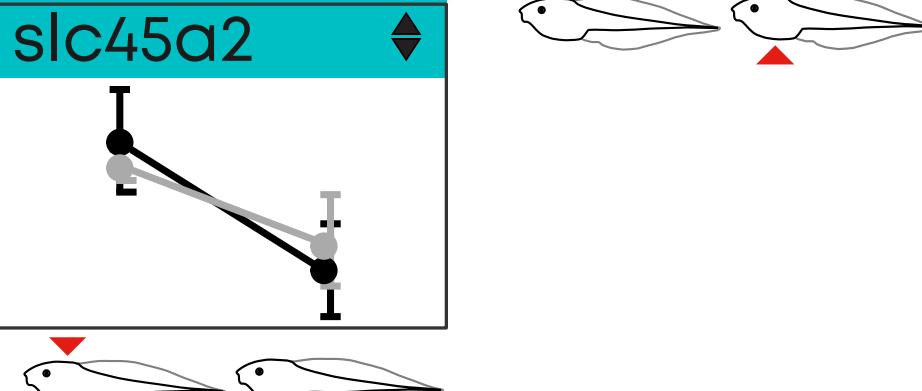
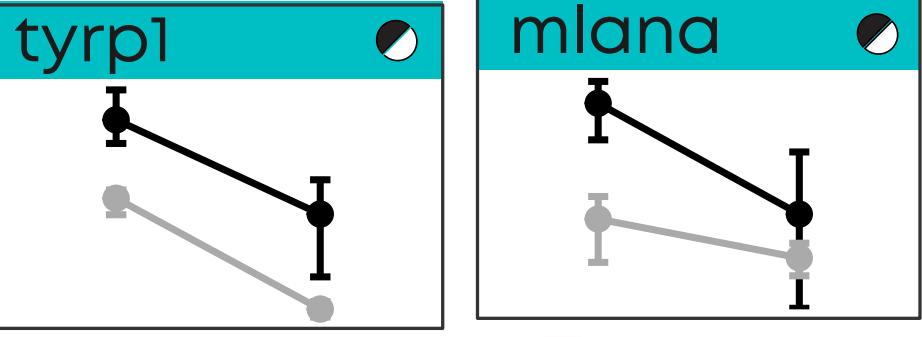
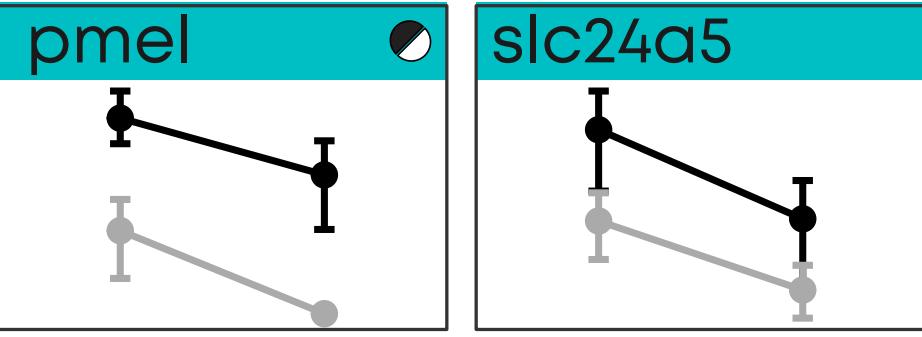
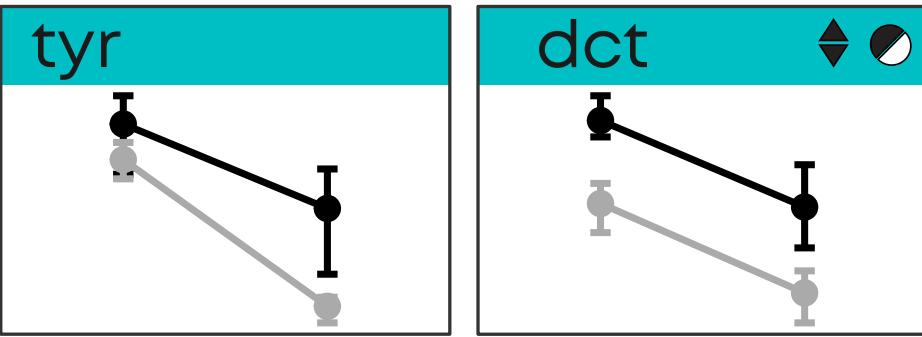
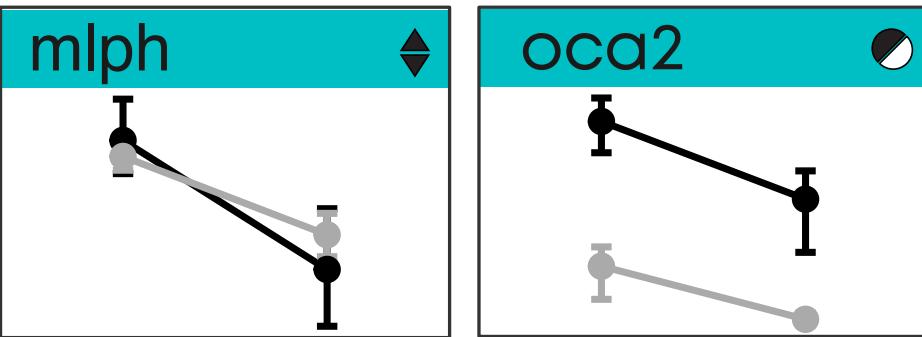
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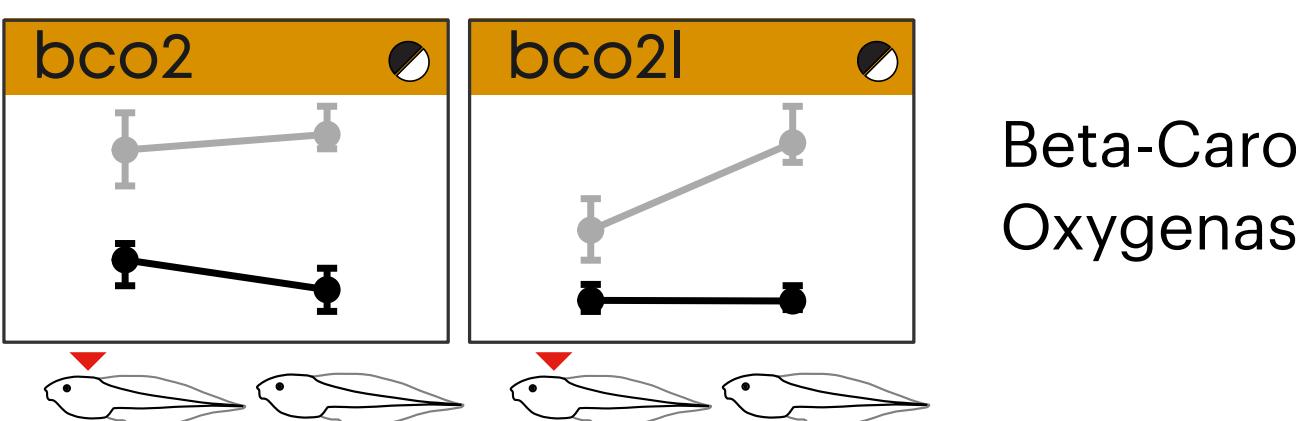
## Transcription regulators



## Tyrosine metabolism



## Carotenoid metabolism



## Beta-Carotene Oxygenase 2

# Summary

- Dorso-ventral gradients are controlled by highly conserved molecular mechanisms (aMSH-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

