

NEW TECHNOLOGIES FOR DEVELOPMENTAL EVOLUTIONARY
BIOLOGY STUDIES - 2024

Plastic Phenotypes

And their underlying gene expression

H. Christoph Liedtke



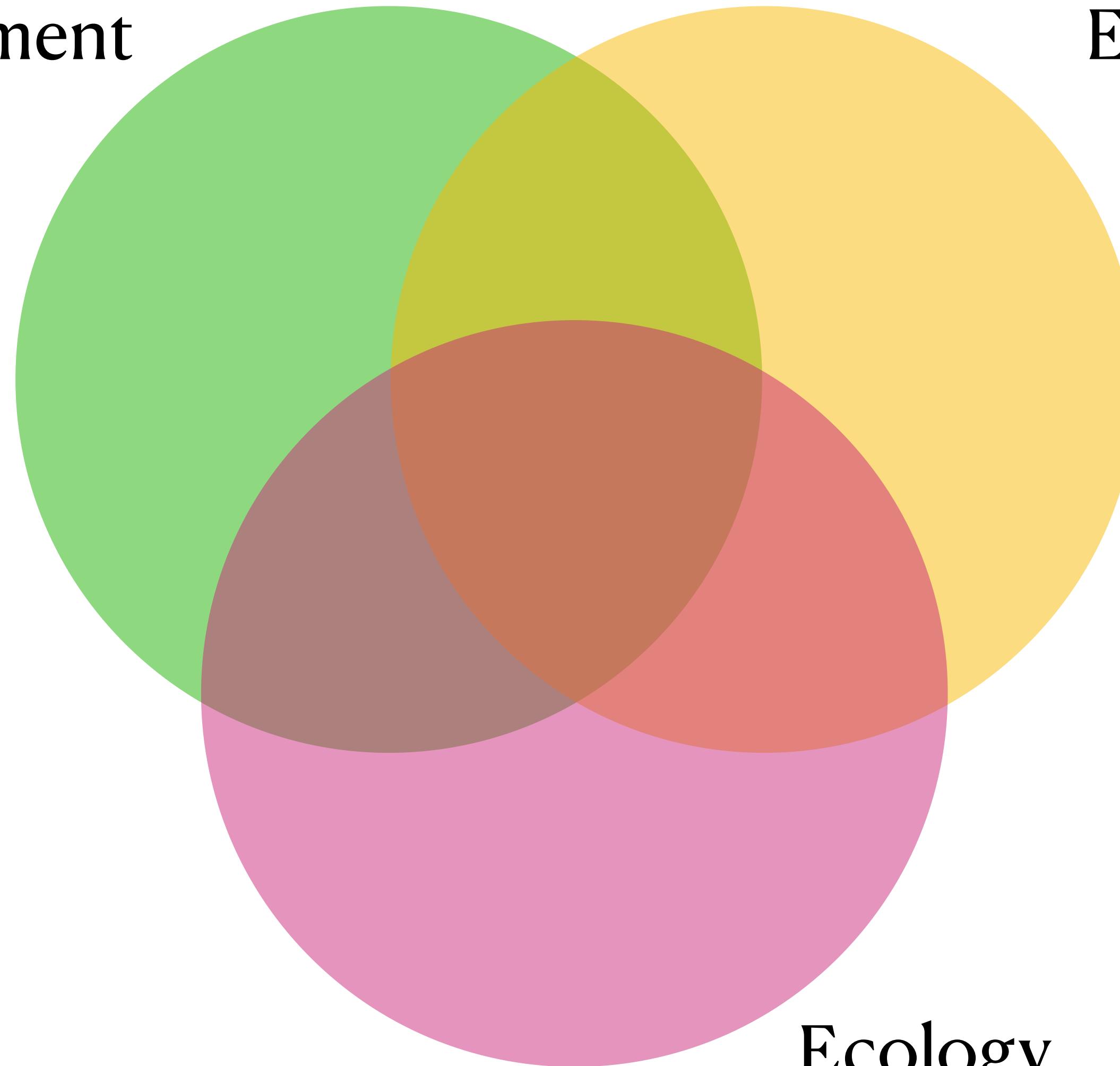
What generates phenotypic diversity?



Development

Evolution

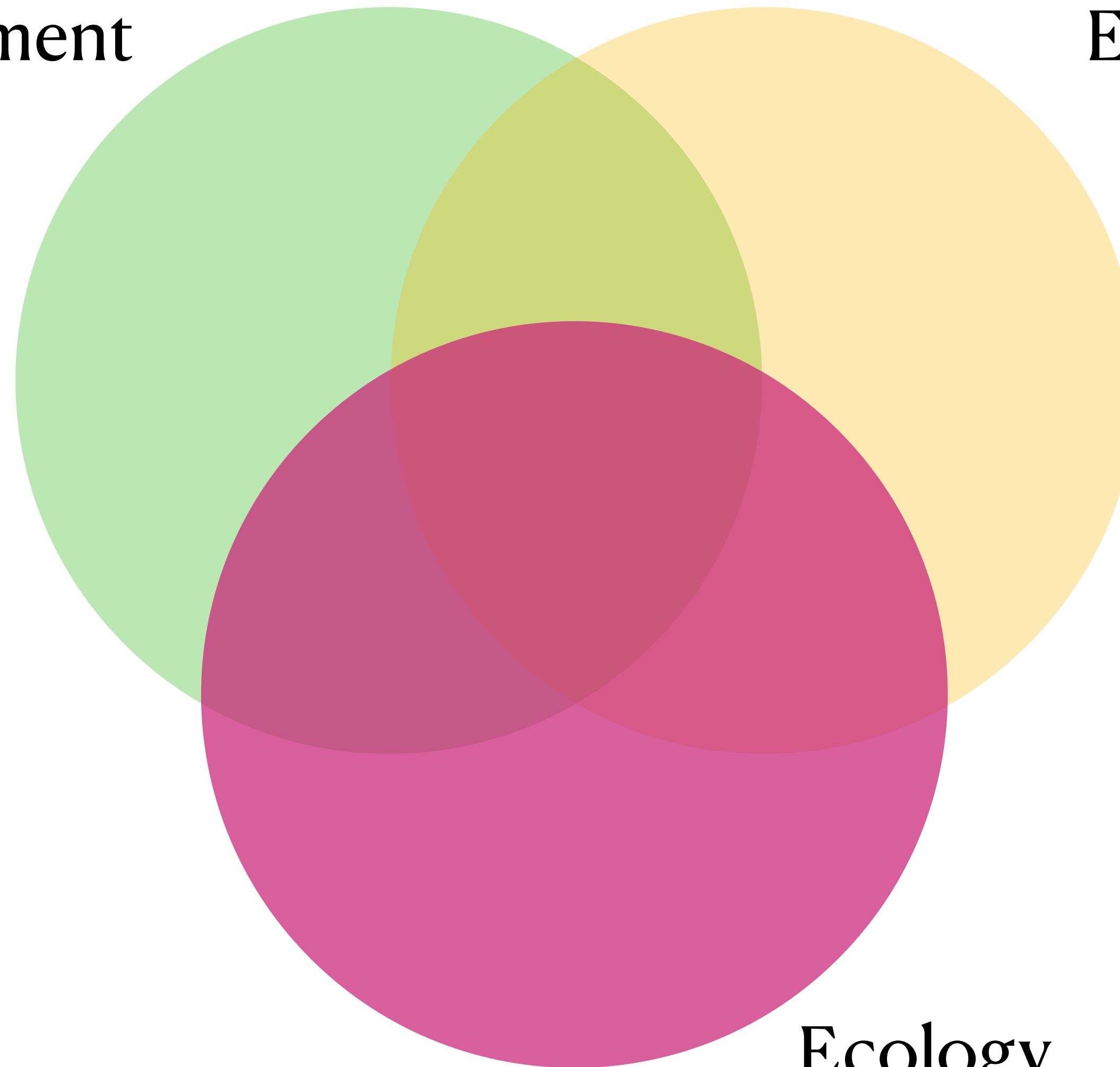
Ecology



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]

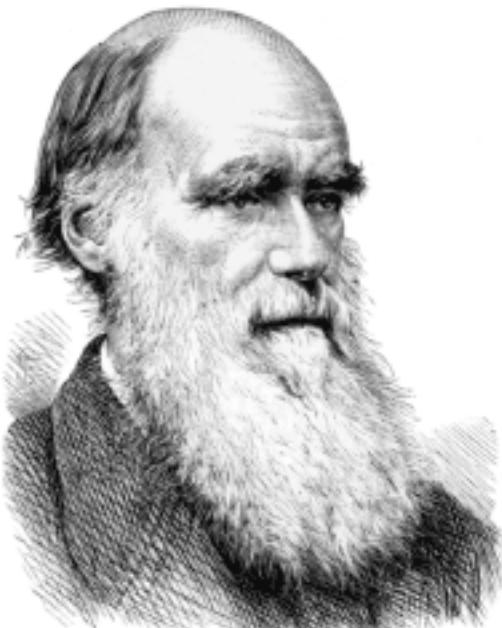
What is the definition of evolution?

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

National Academy of Sciences, USA



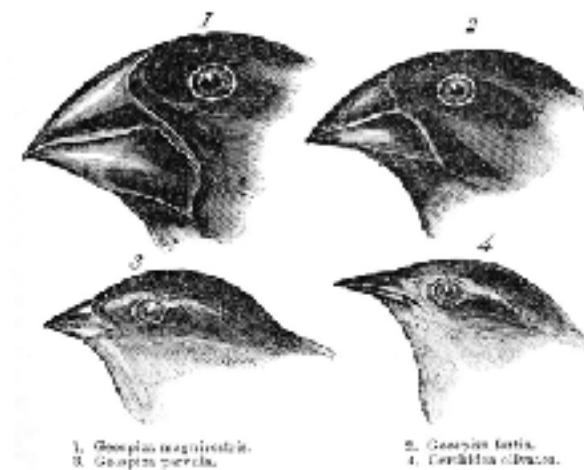
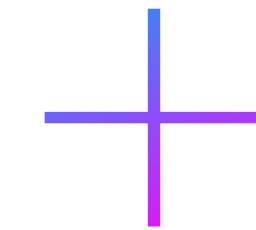
The Modern Synthesis



CHARLES DARWIN



ALFRED RUSSEL WALLACE



Huxley



Stebbins



Simpson



Mayr



Haldane



Fisher



Wright



Dobzhansky

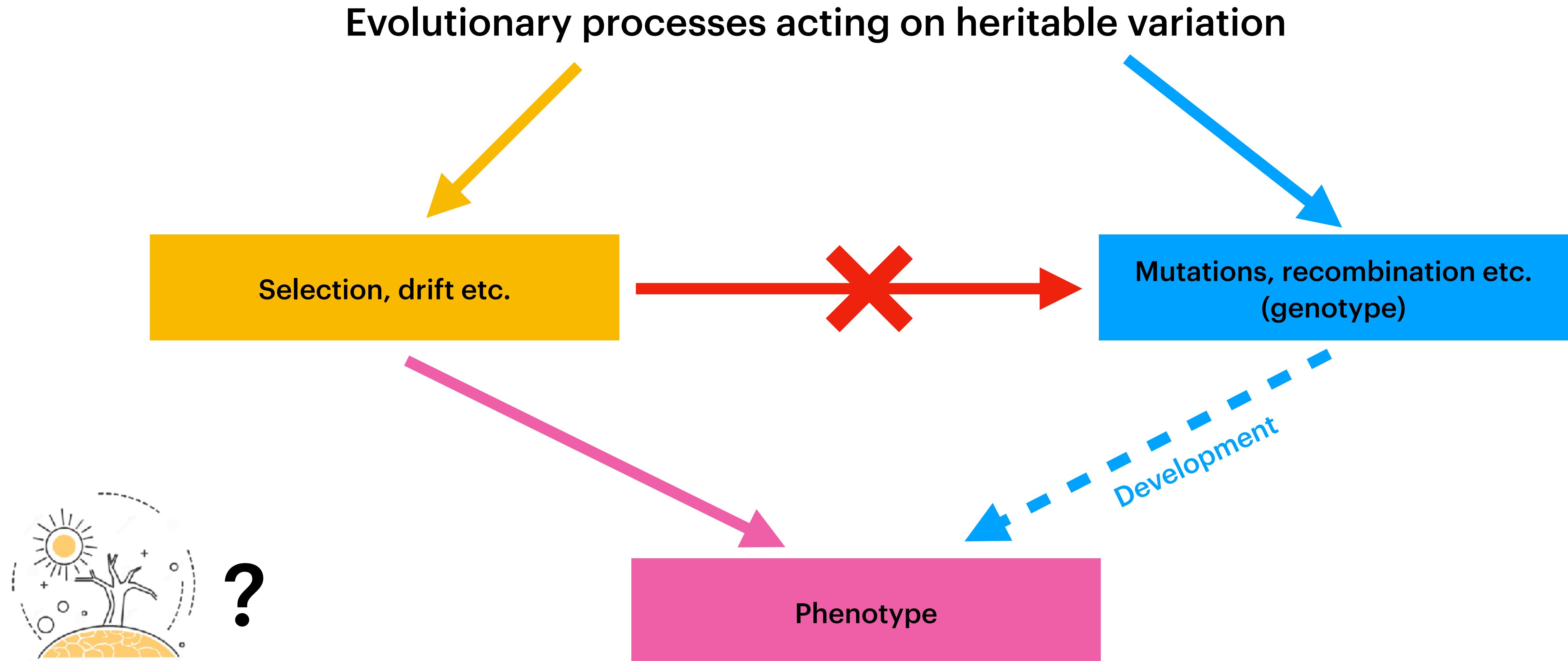
The Modern Synthesis

- 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.

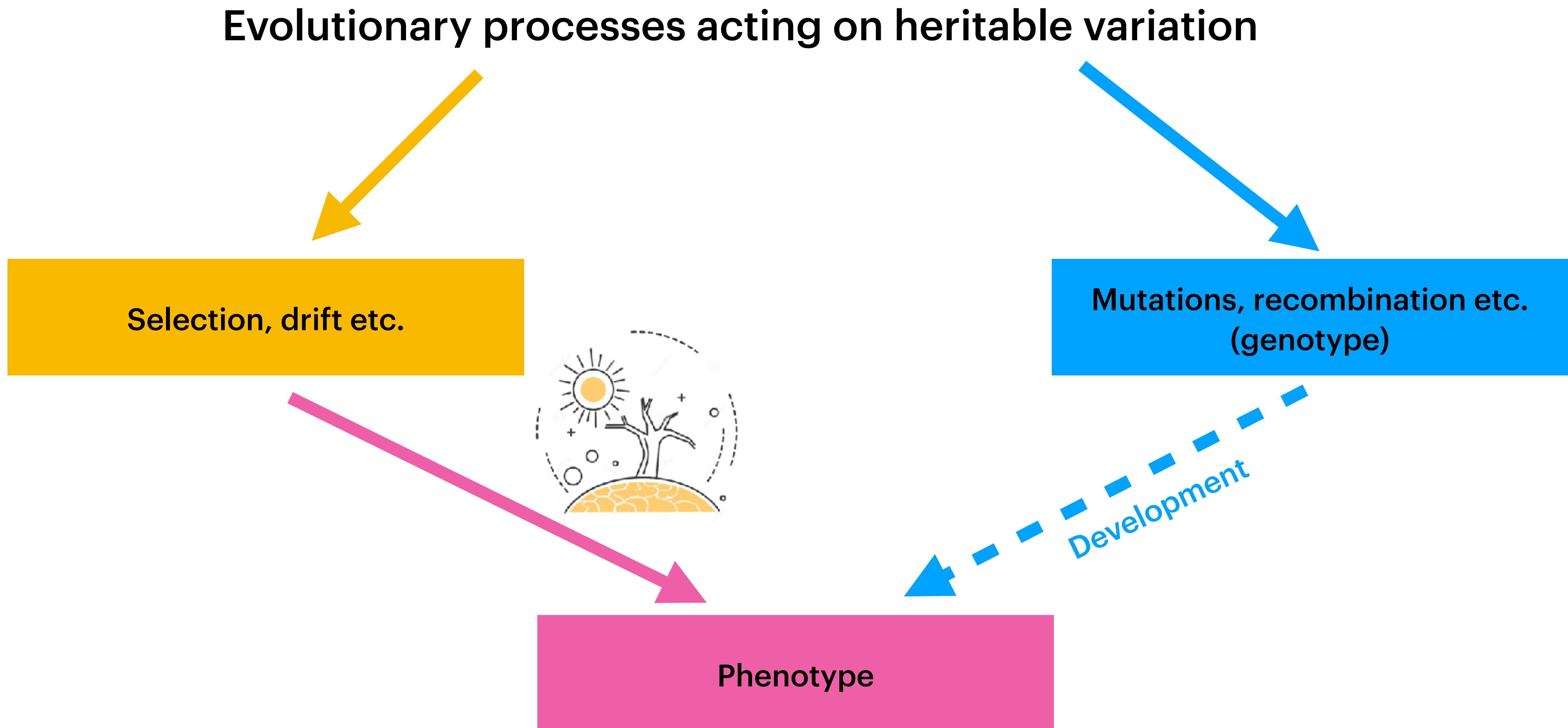
The Modern Synthesis

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What generates phenotypic diversity?



What generates phenotypic diversity?



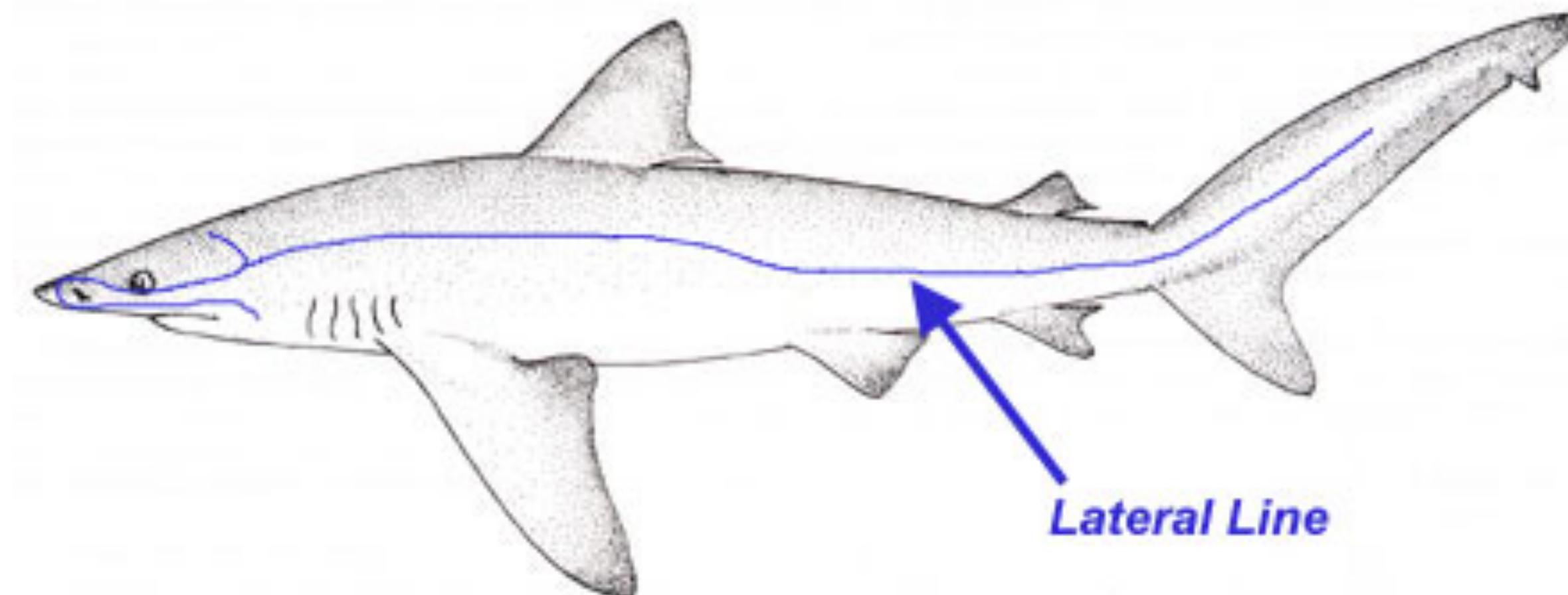
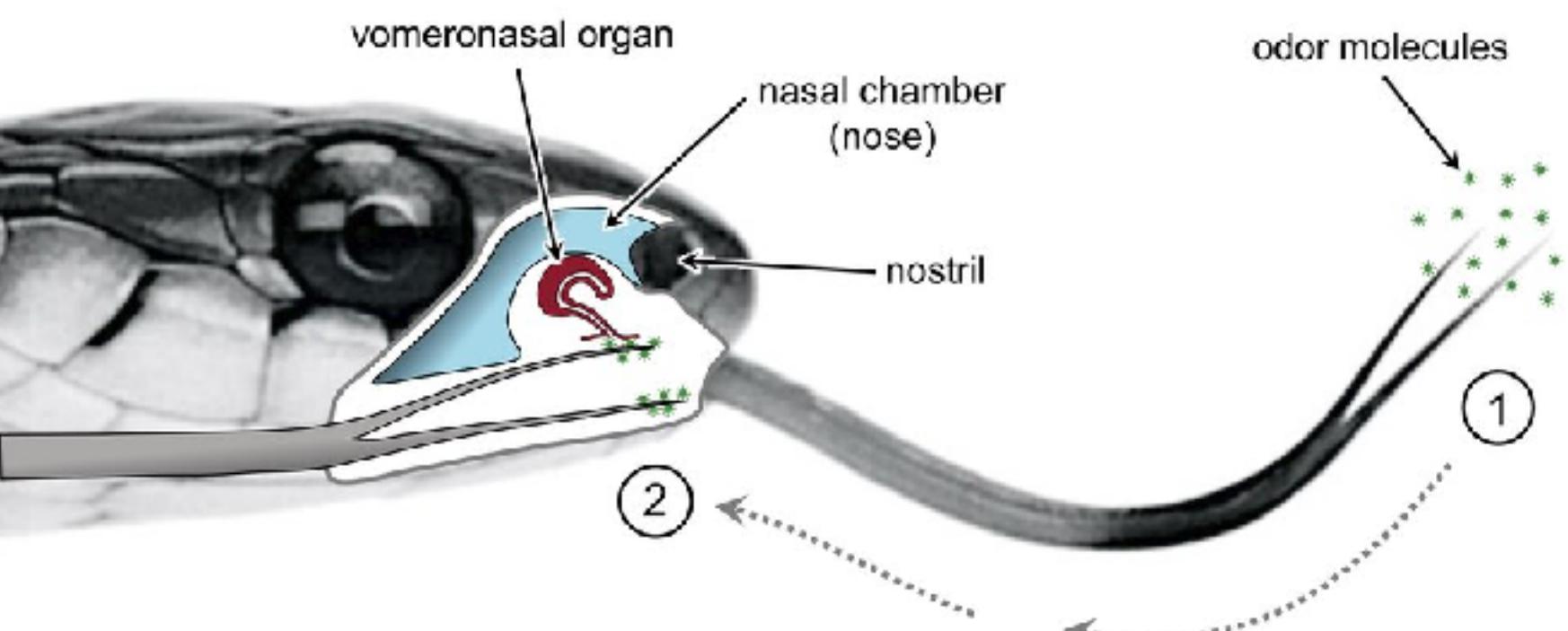
The environment as a passive filter?



The environment is dynamic



Organisms sense their environment



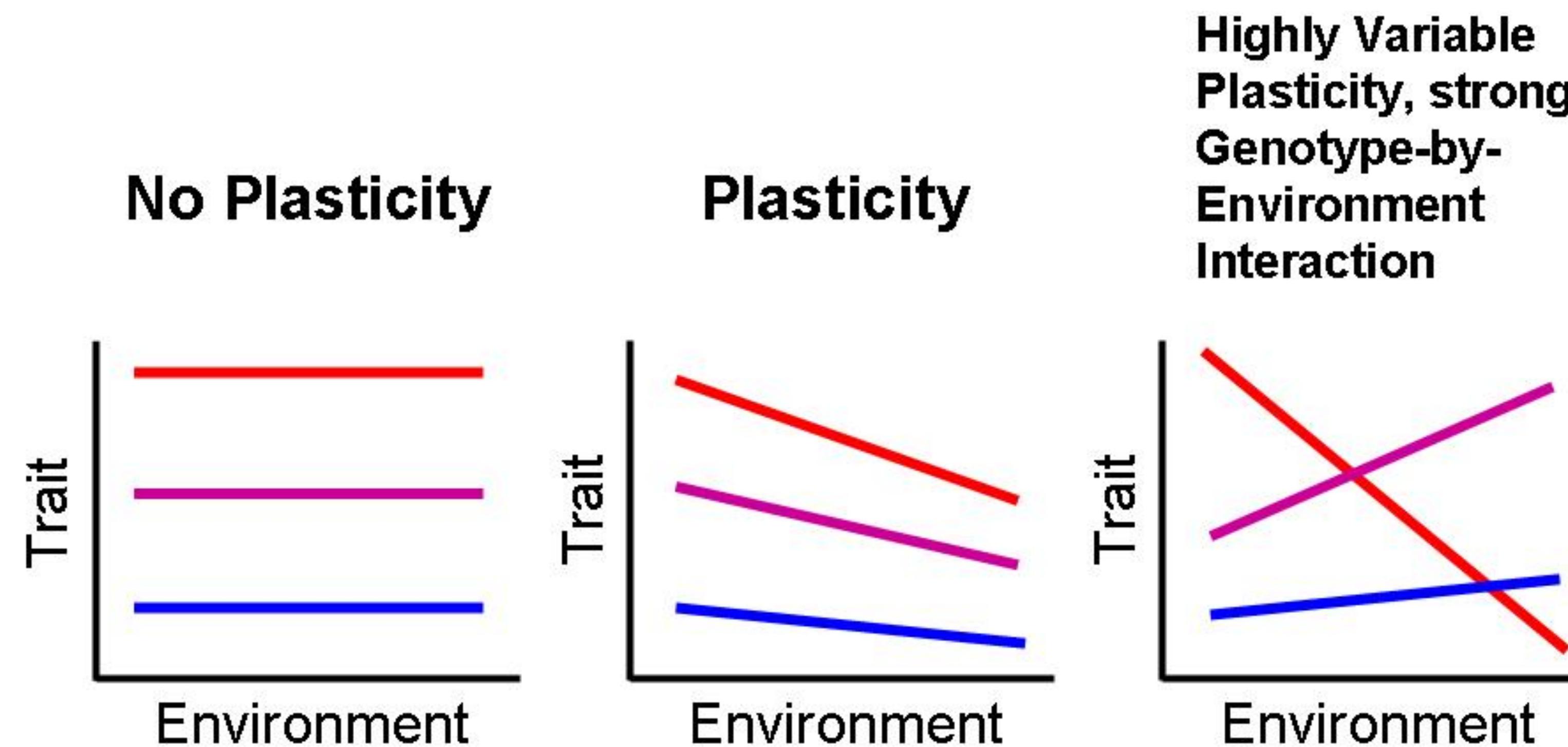
Phenotypes are environment-dependent



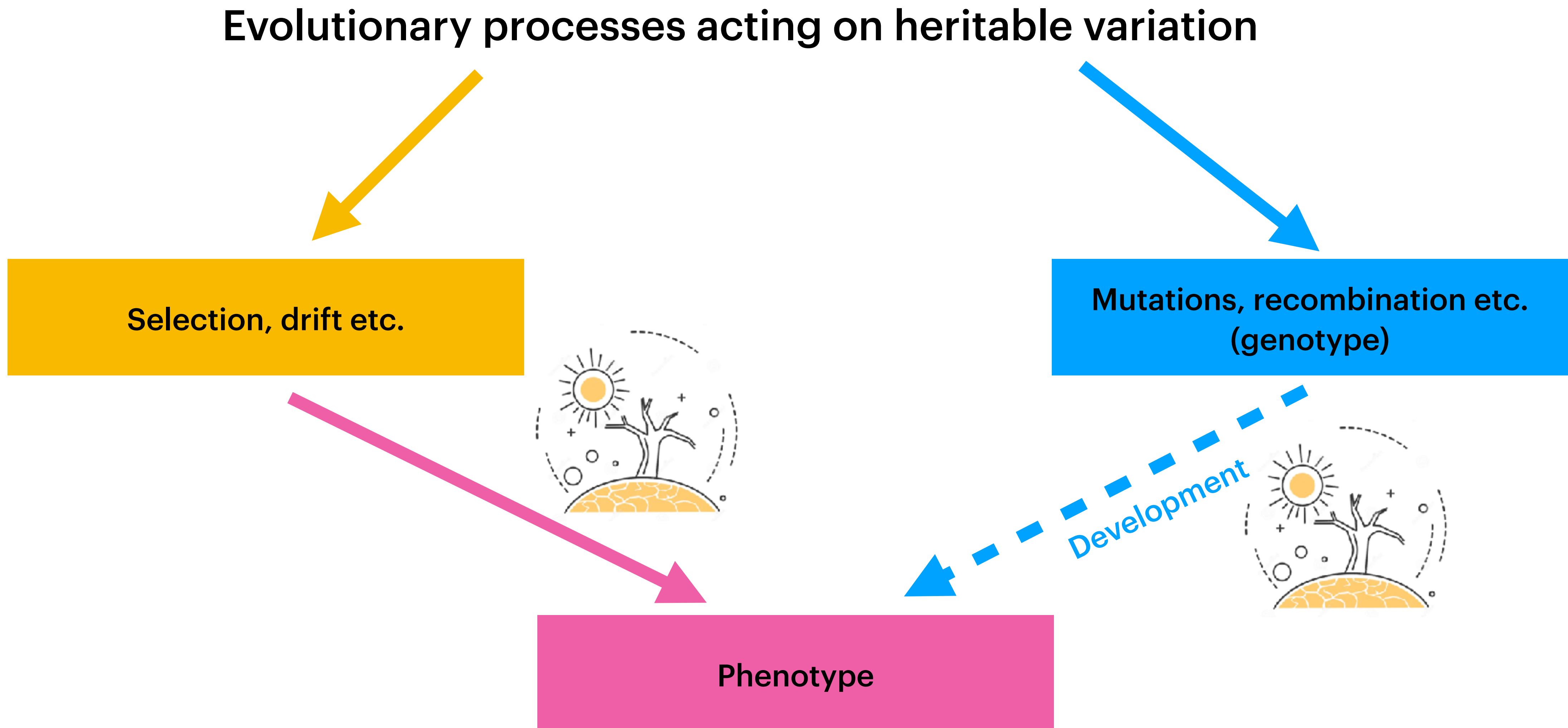
Nemoria arizonaria, Greene 1989 Nature

Phenotypic plasticity

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



What generates phenotypic diversity?

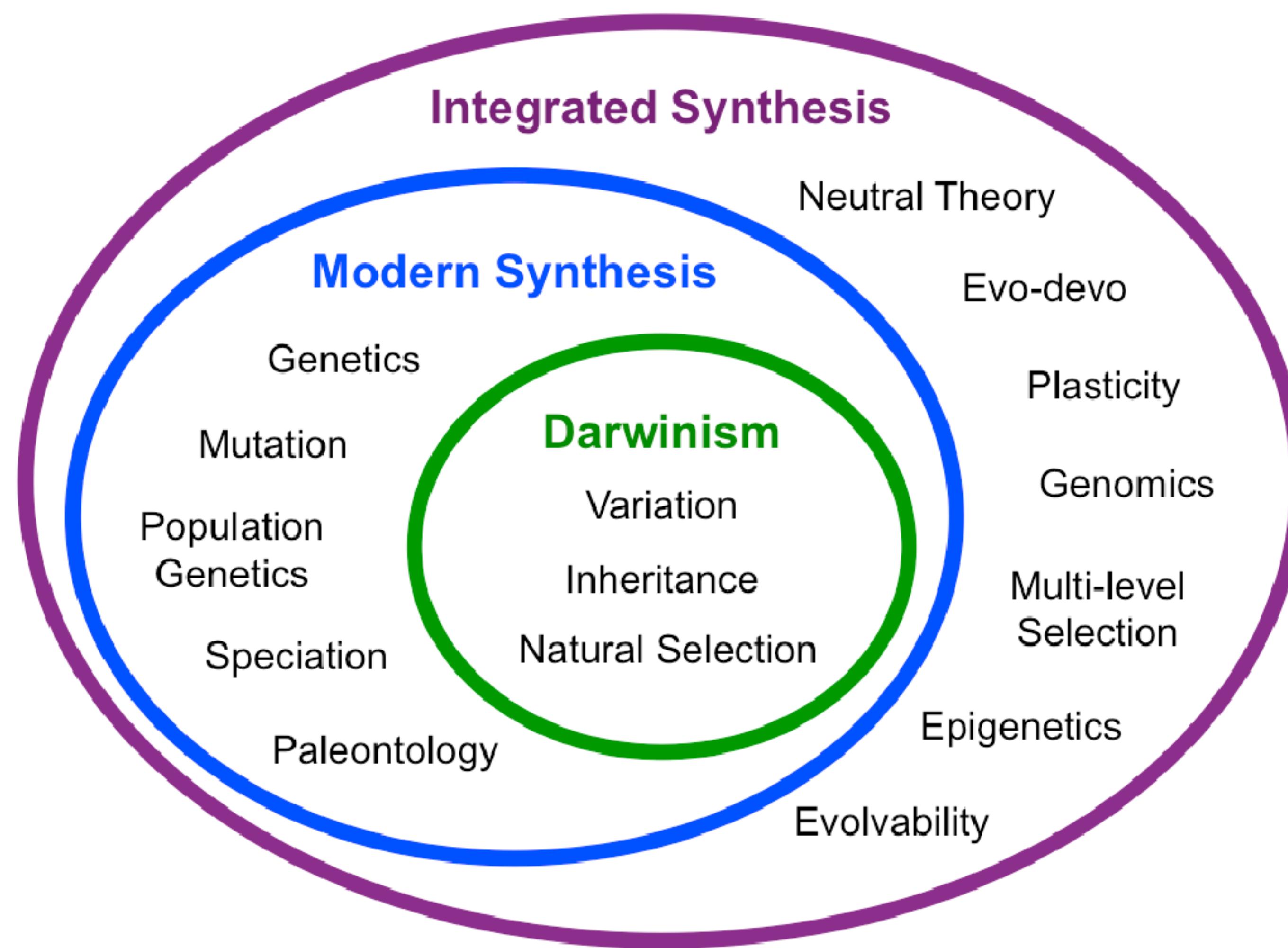




Extended Evolutionary Synthesis



Massimo Piliucci



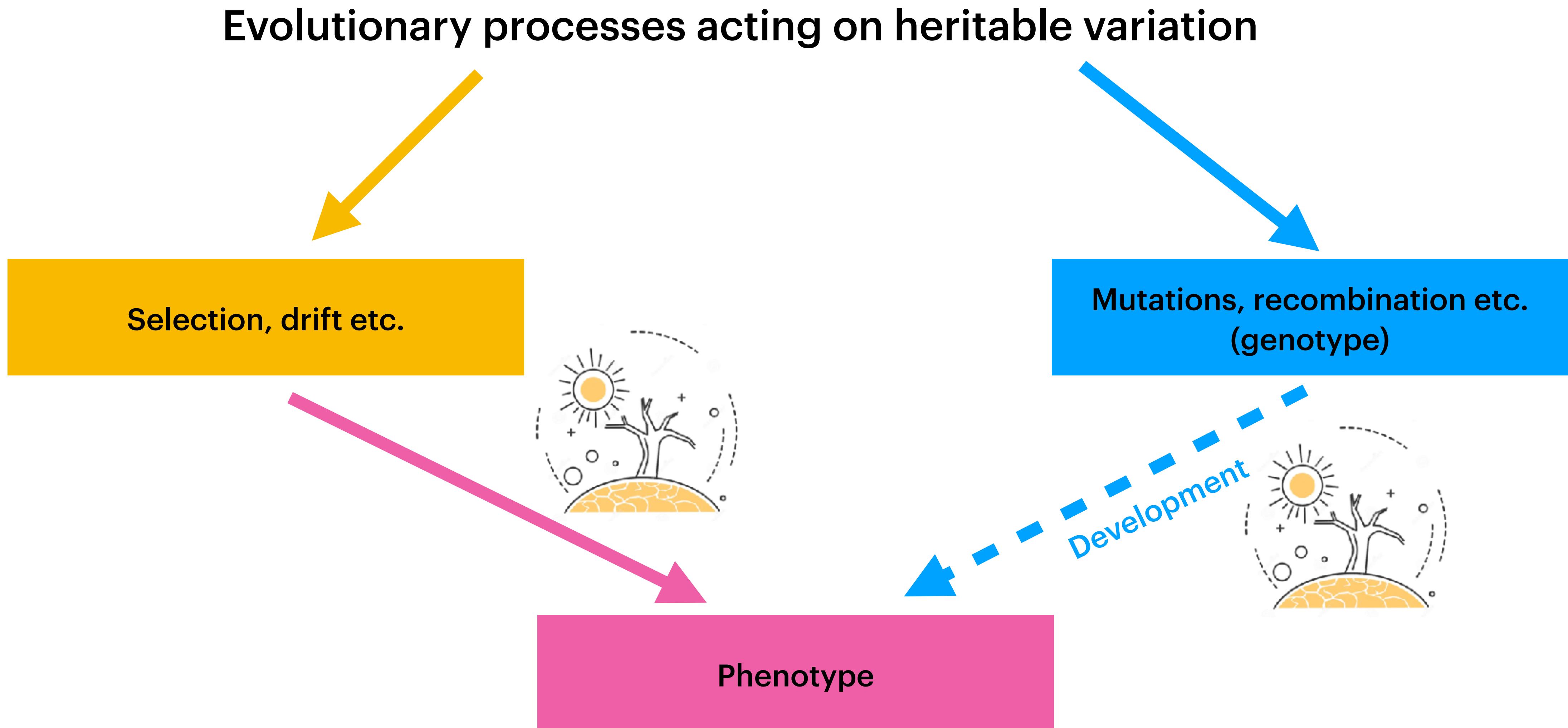
DEVELOPMENTAL
PLASTICITY
AND EVOLUTION



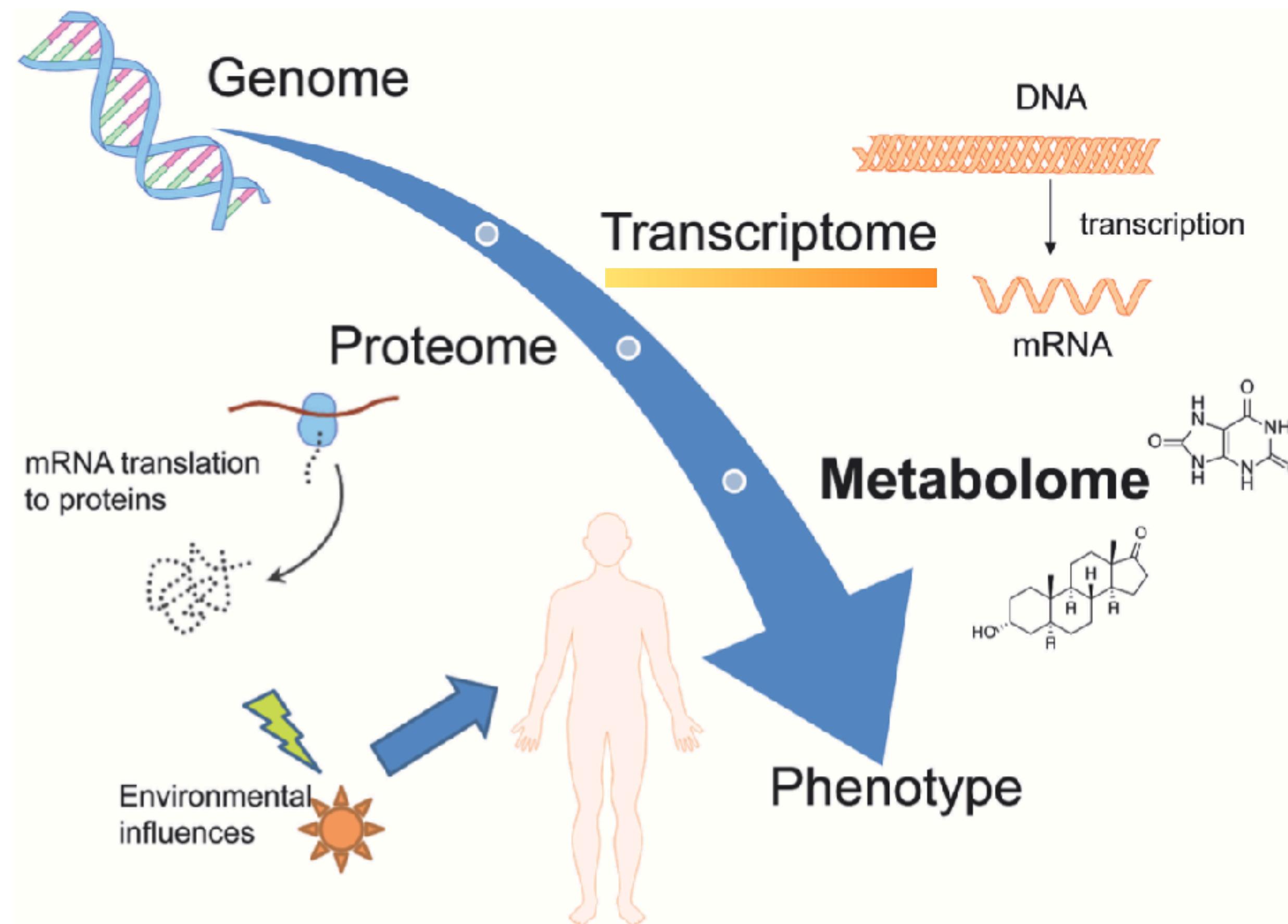
MARY JANE WEST-EBERHARD

Mary Jane West-Eberhard

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

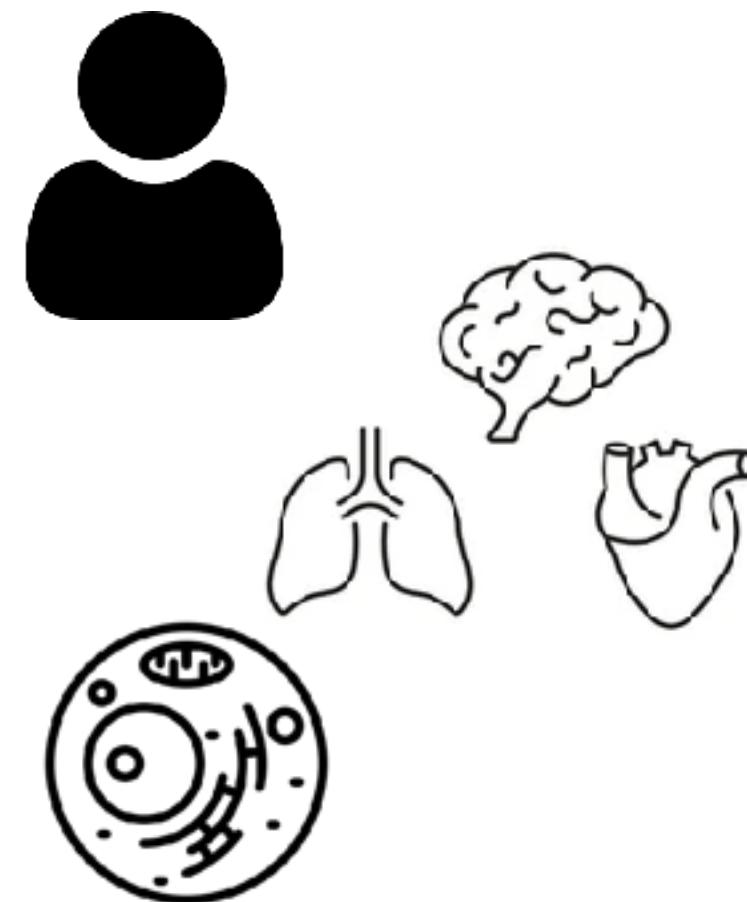
78724 genes



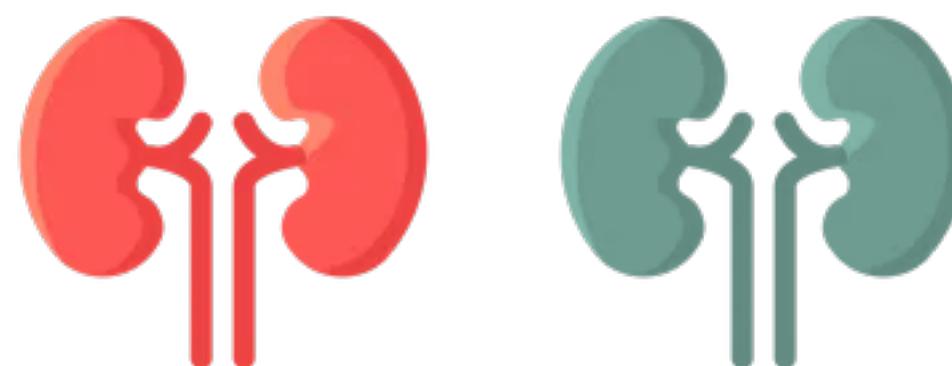
2.7bp genome

78239 genes

What is the transcriptome?



- Organism > organ > tissue > cell



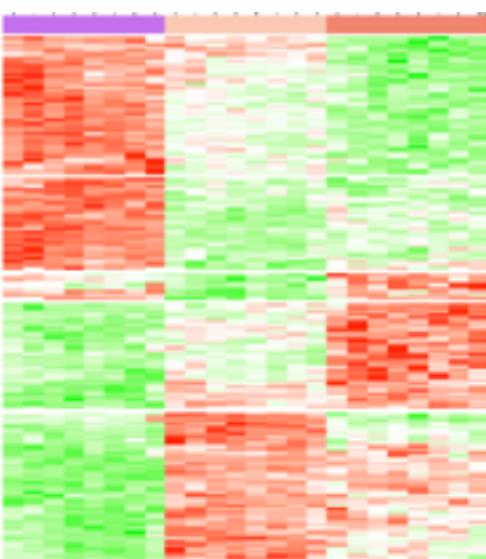
- Variable and state-dependent

How do we study the transcriptome?



Qualitatively

- Identify expressed genes
- Gene discovery



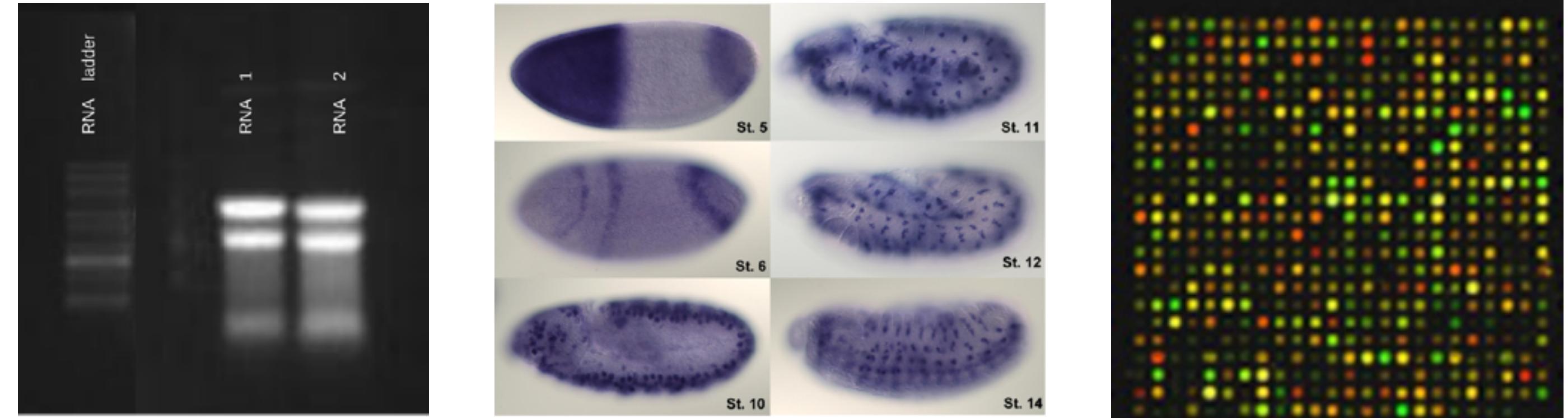
Quantitatively

- Compare expression levels of genes or transcripts

History of studying transcripts

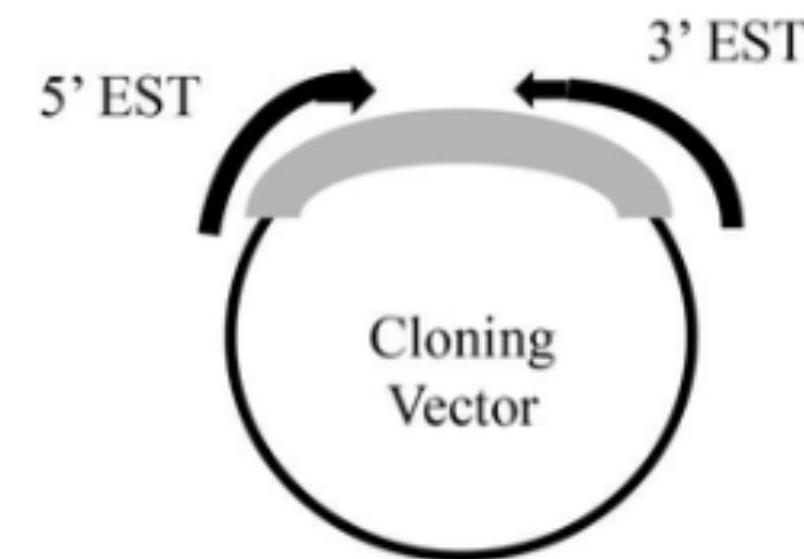
Quantitative

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

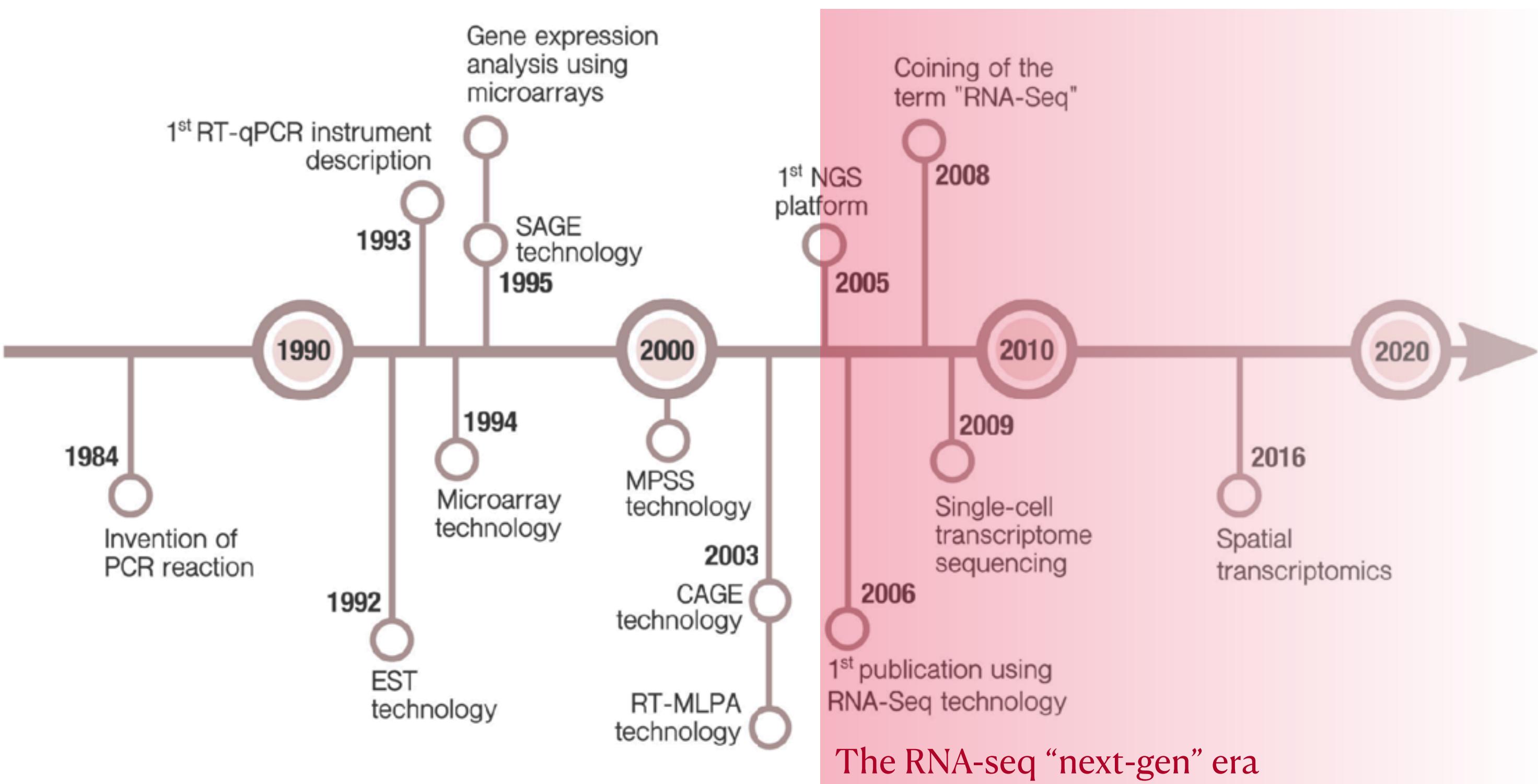


Qualitative

- Expressed sequence tags 1990s



History of studying transcripts

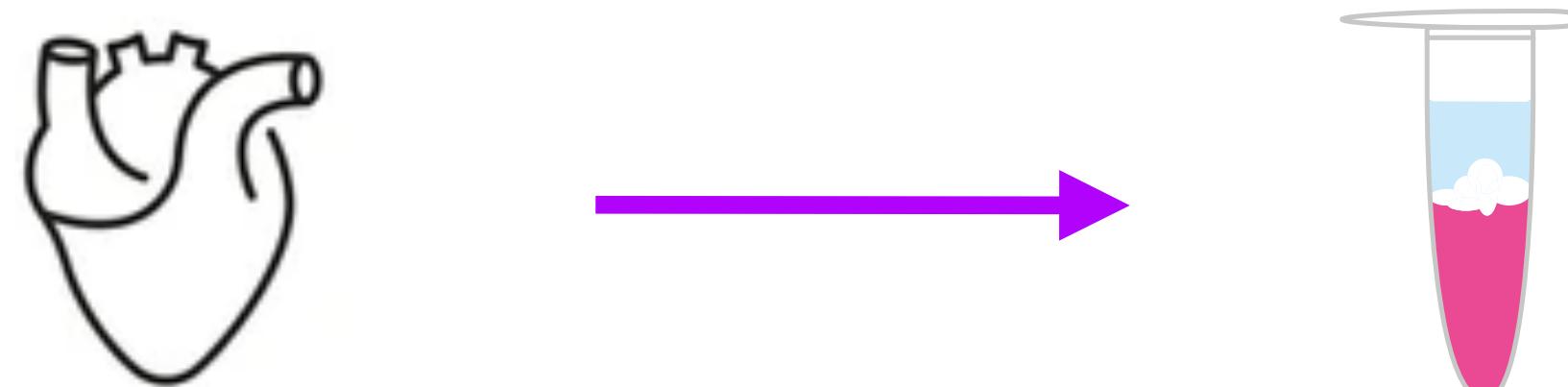


RNA-seq

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

RNA-Sequencing (lab)

1. RNA extraction



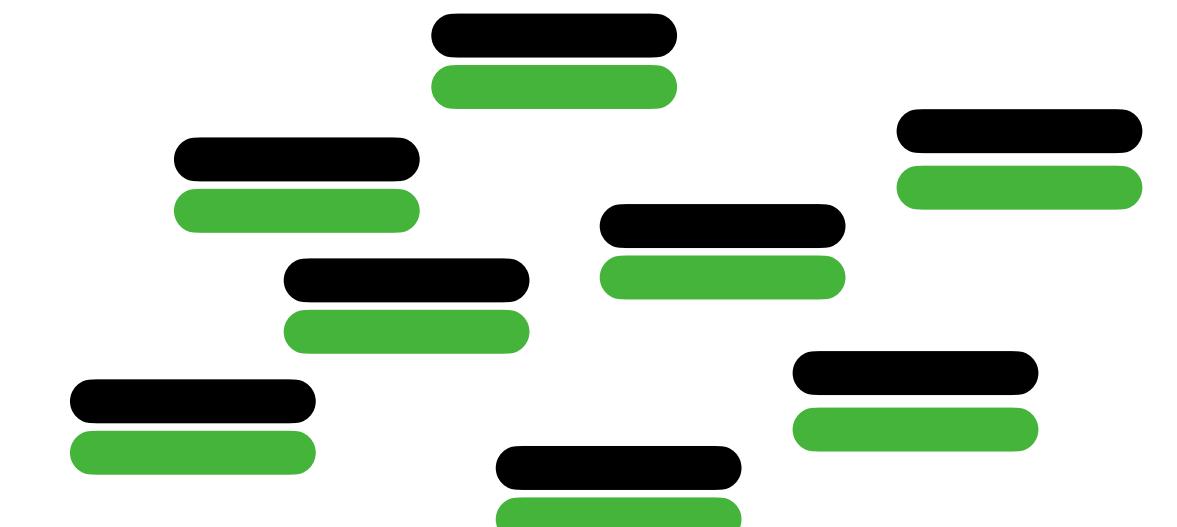
2. Isolation/purification (e.g. poly-A enrichment)



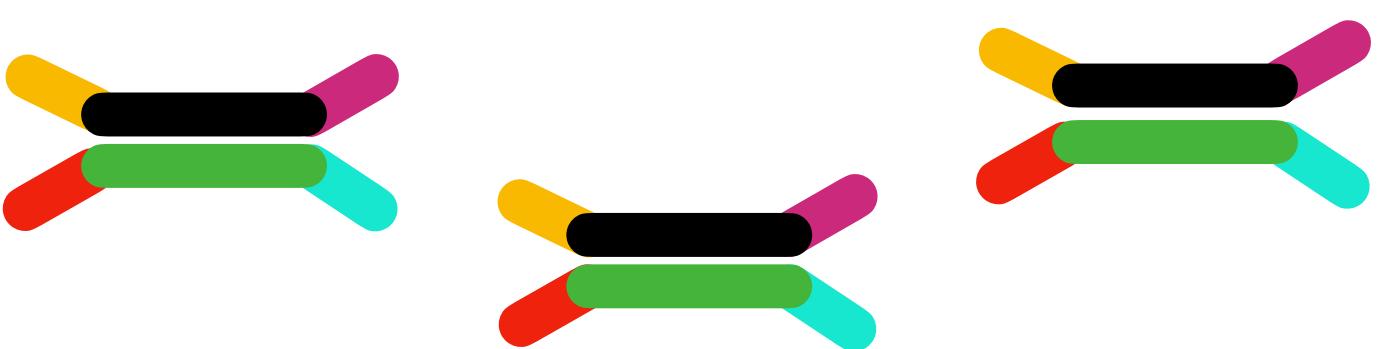
3. Fragmentation



4. cDNA synthesis



5. Adaptor ligation

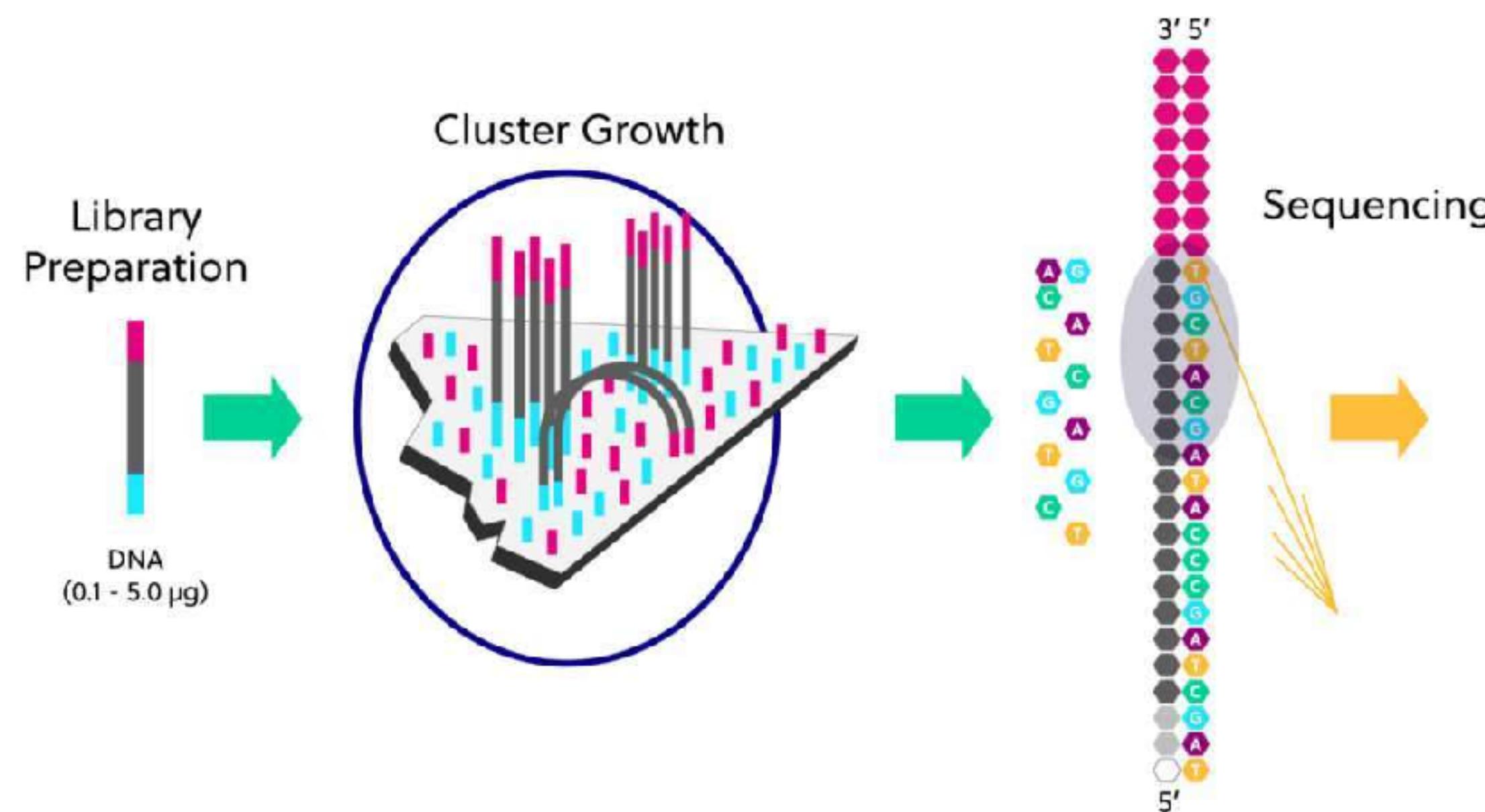


Parallel sequencing



Next-Gen Sequencing

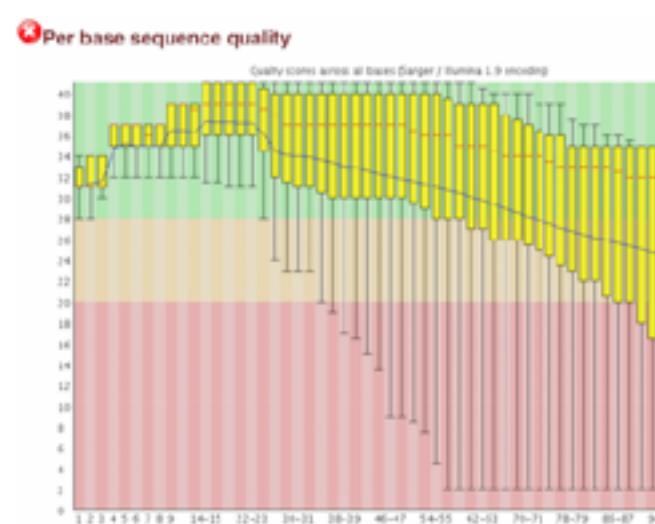
Illumina short reads technology



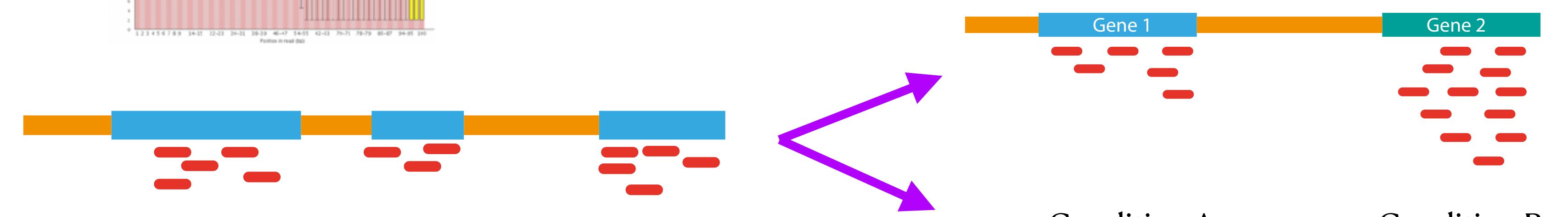
- Usual read length: 100bp
- Usual quantity of reads for RNAseq: 20-30 million reads per sample
- Single “lanes” allow running many samples at once (25 million - 20 billion reads depending on technology)

RNA-Sequencing (in silico)

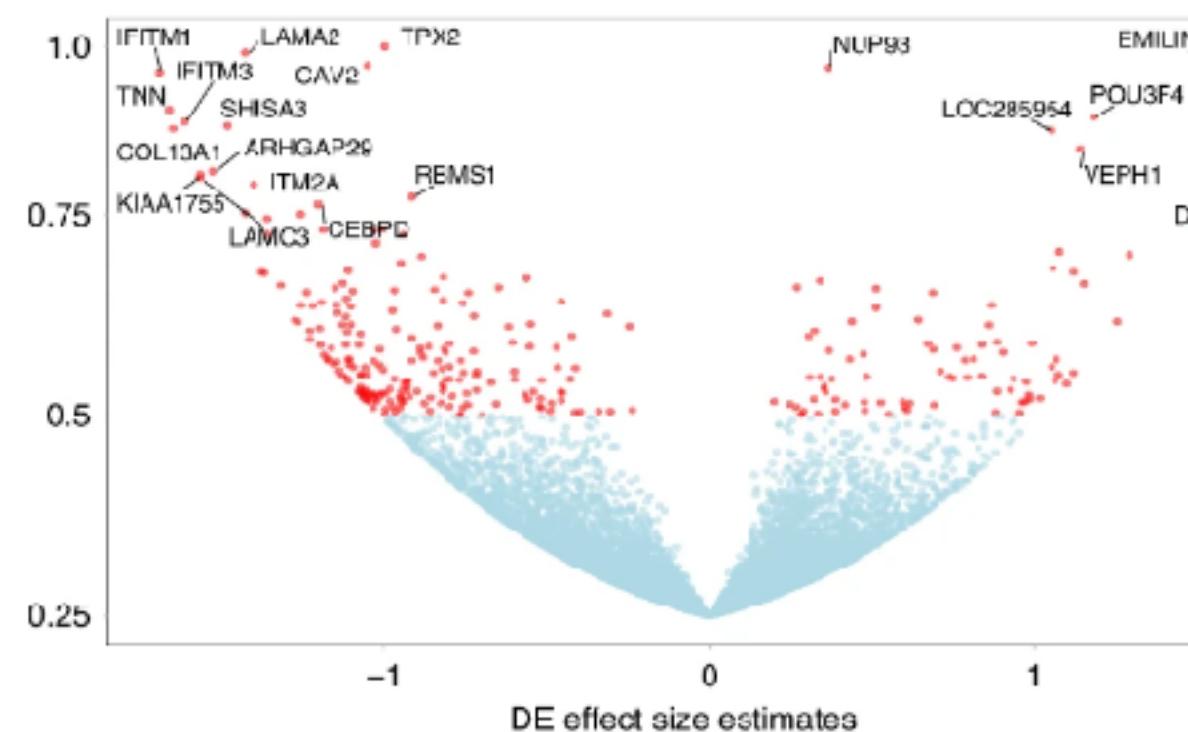
1. Sequence cleaning



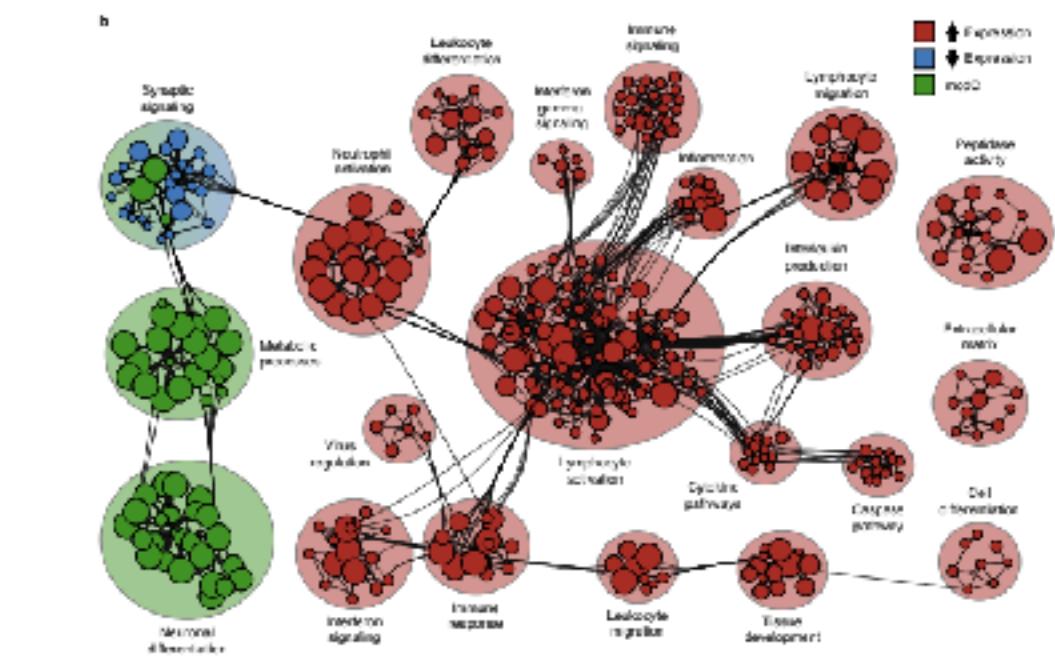
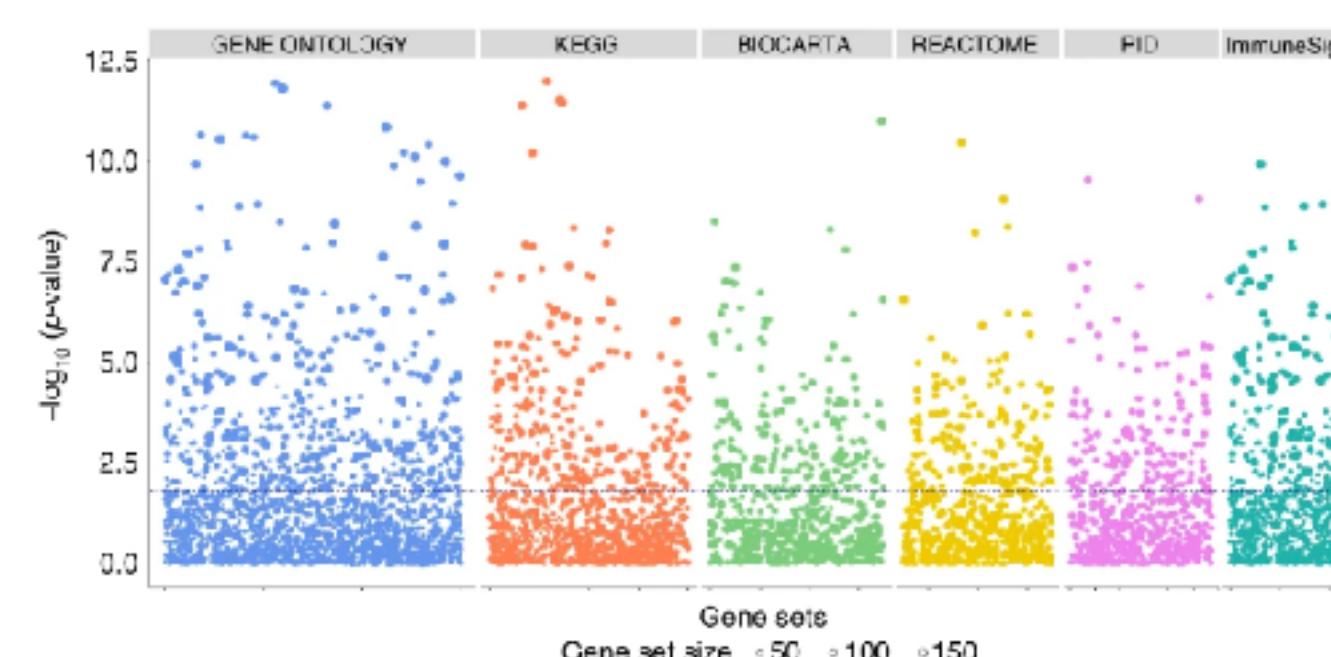
2. Mapping/quantification



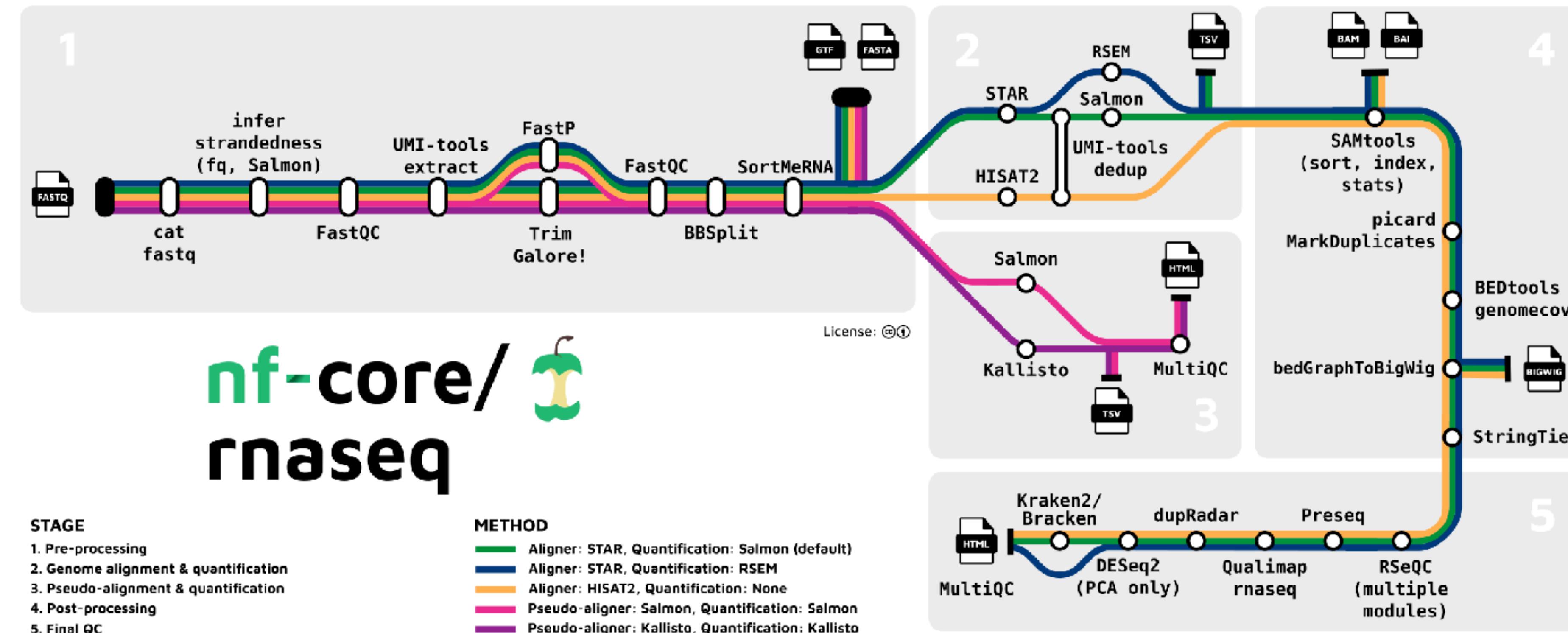
3. Differential gene expression



4. Functional insights



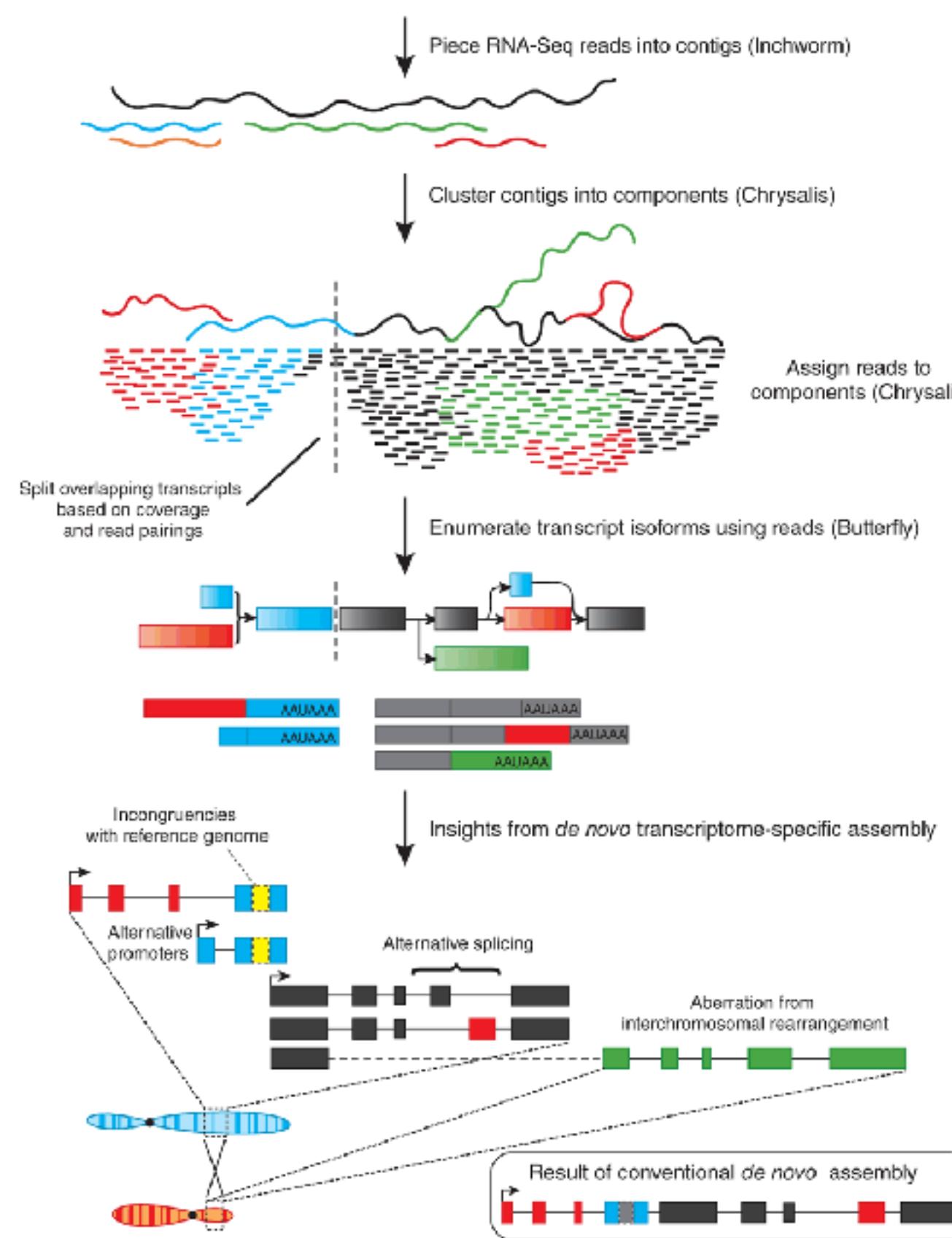
Pipelines and Work Flows



What if I don't have a reference genome?

No Genome? No problem!

Trinity *de novo* transcriptome assembly



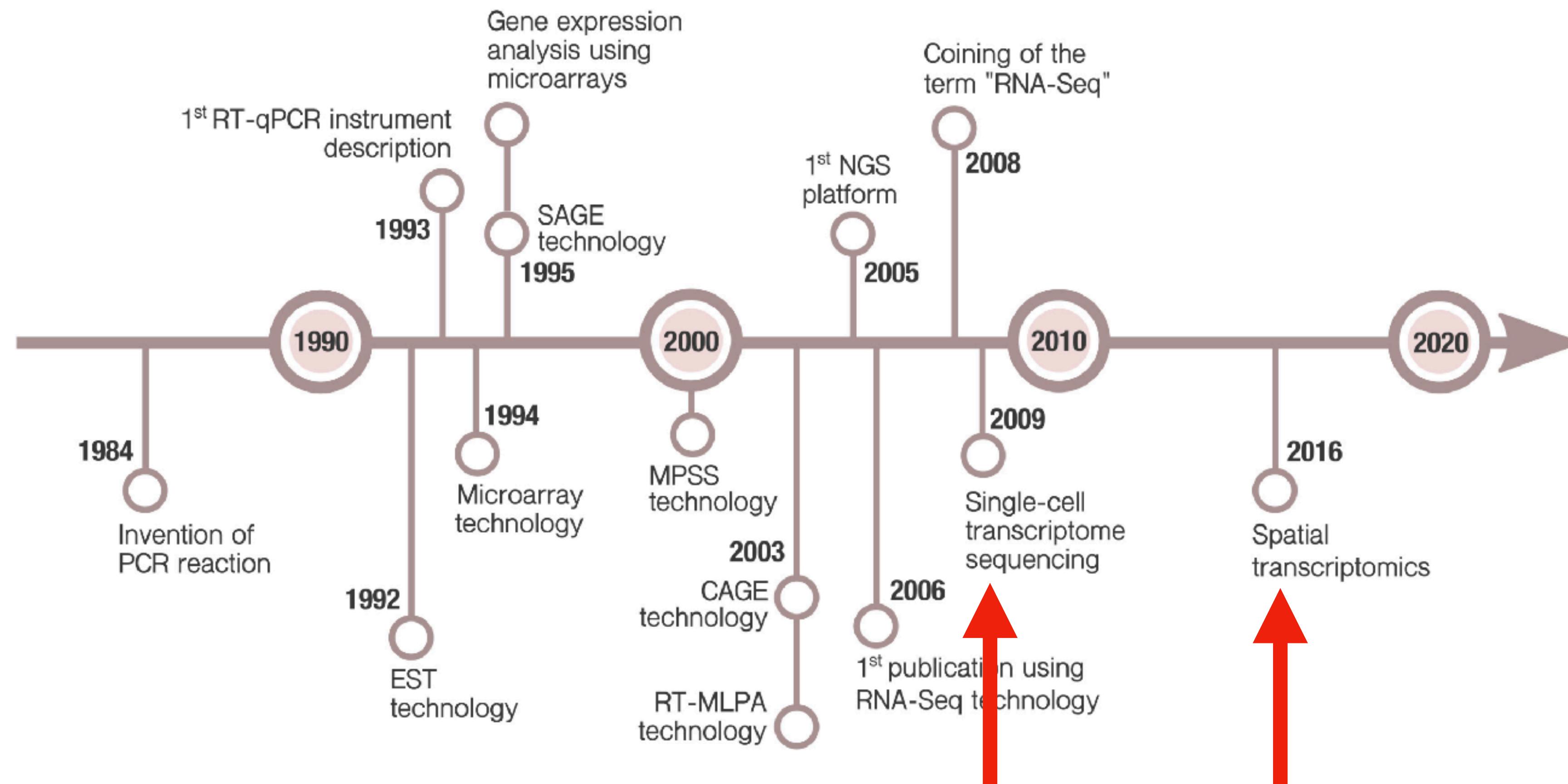
Genomes:

- Higher accuracy
- Better annotations (sometimes)

De novo Transcriptomes:

- Better than nothing
- Avoids mapping bias

“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

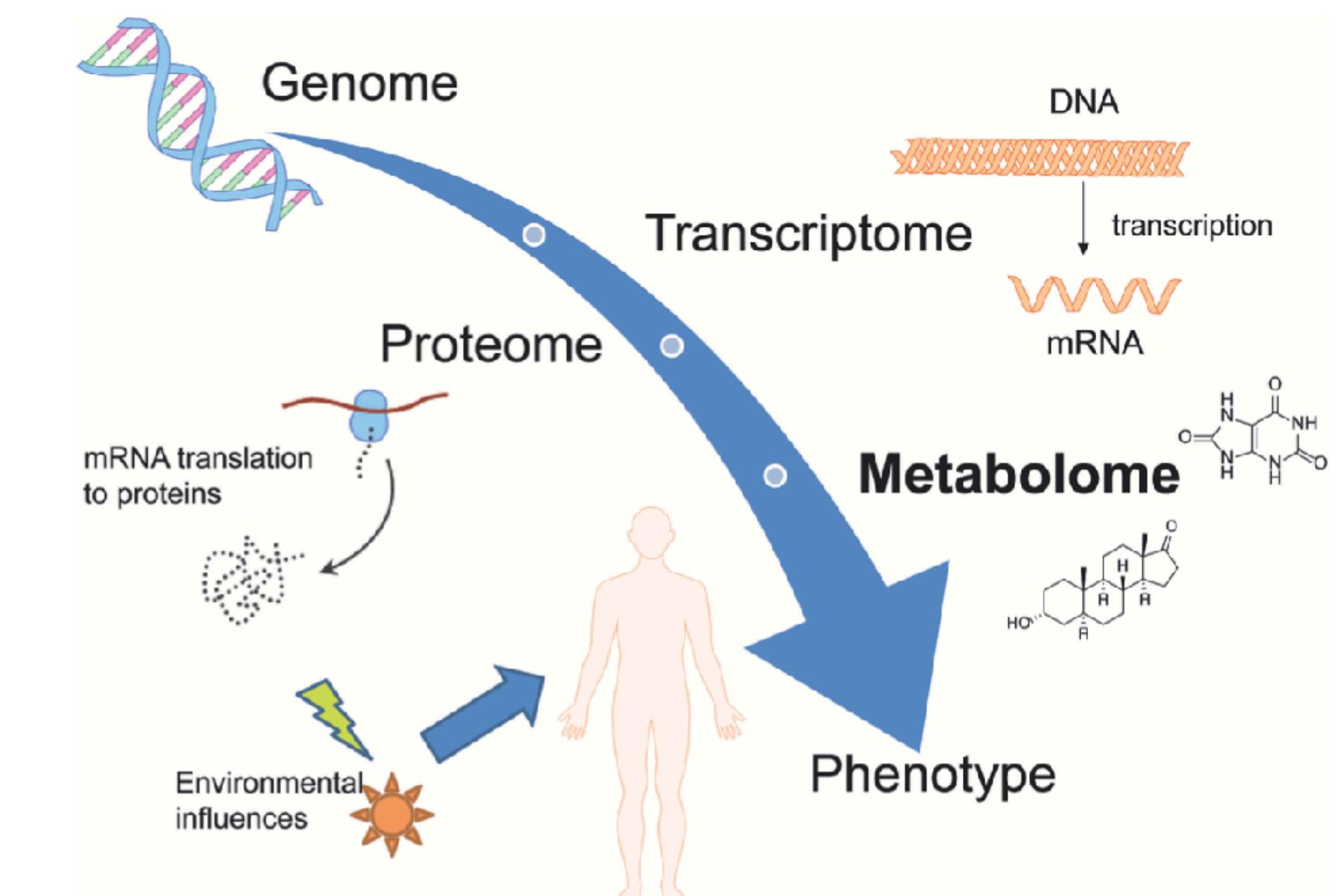
Main uses of bulk RNA-Seq:

- 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

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



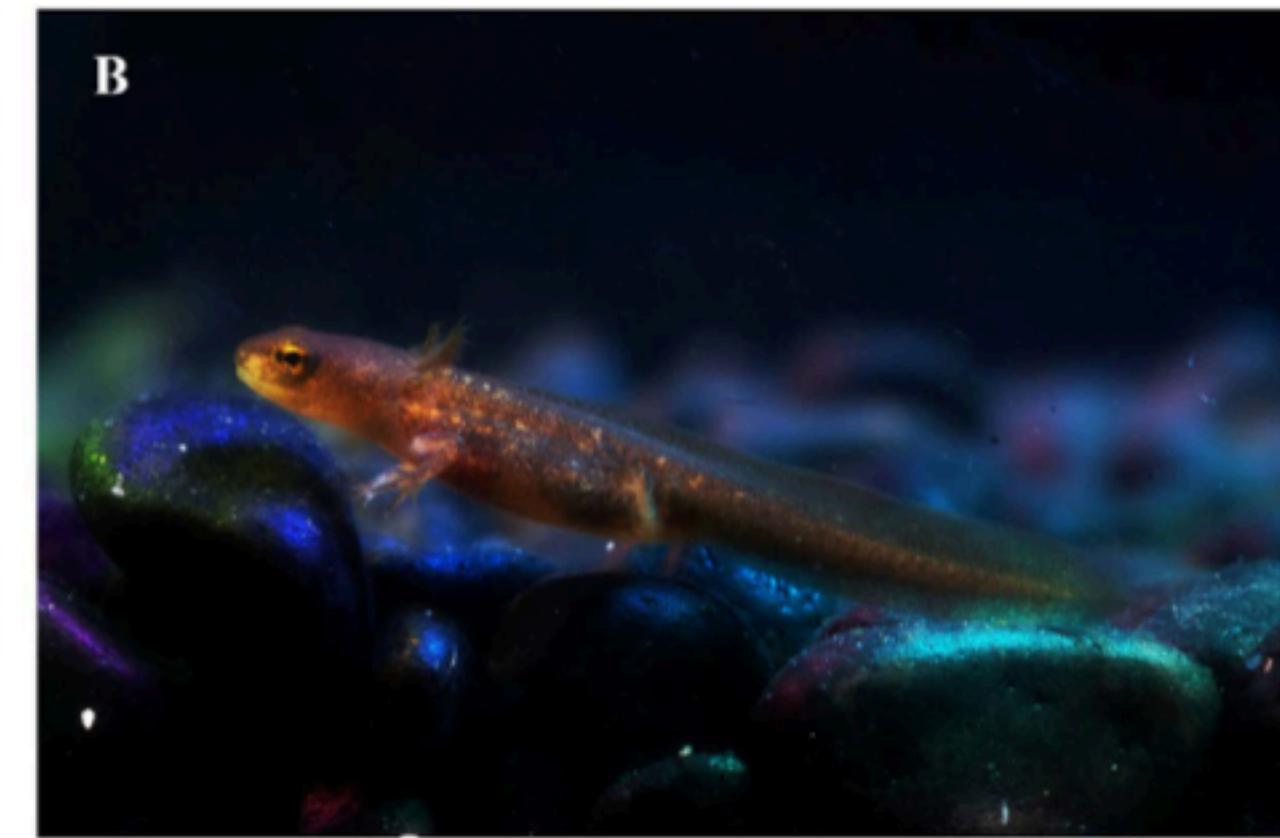
Night



Day



Light background



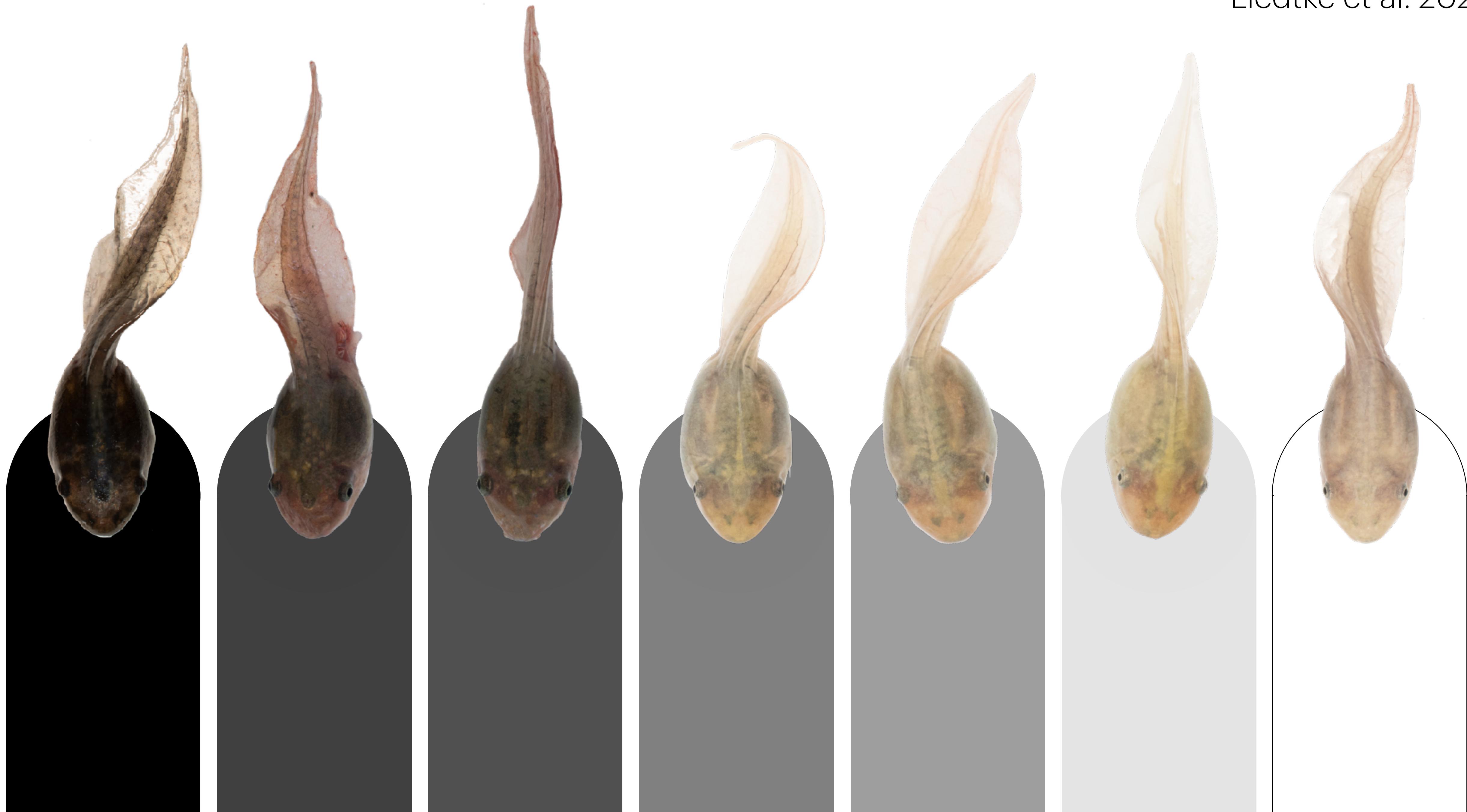
Dark background

Pelobates cultripes

Western Spadefoot toad



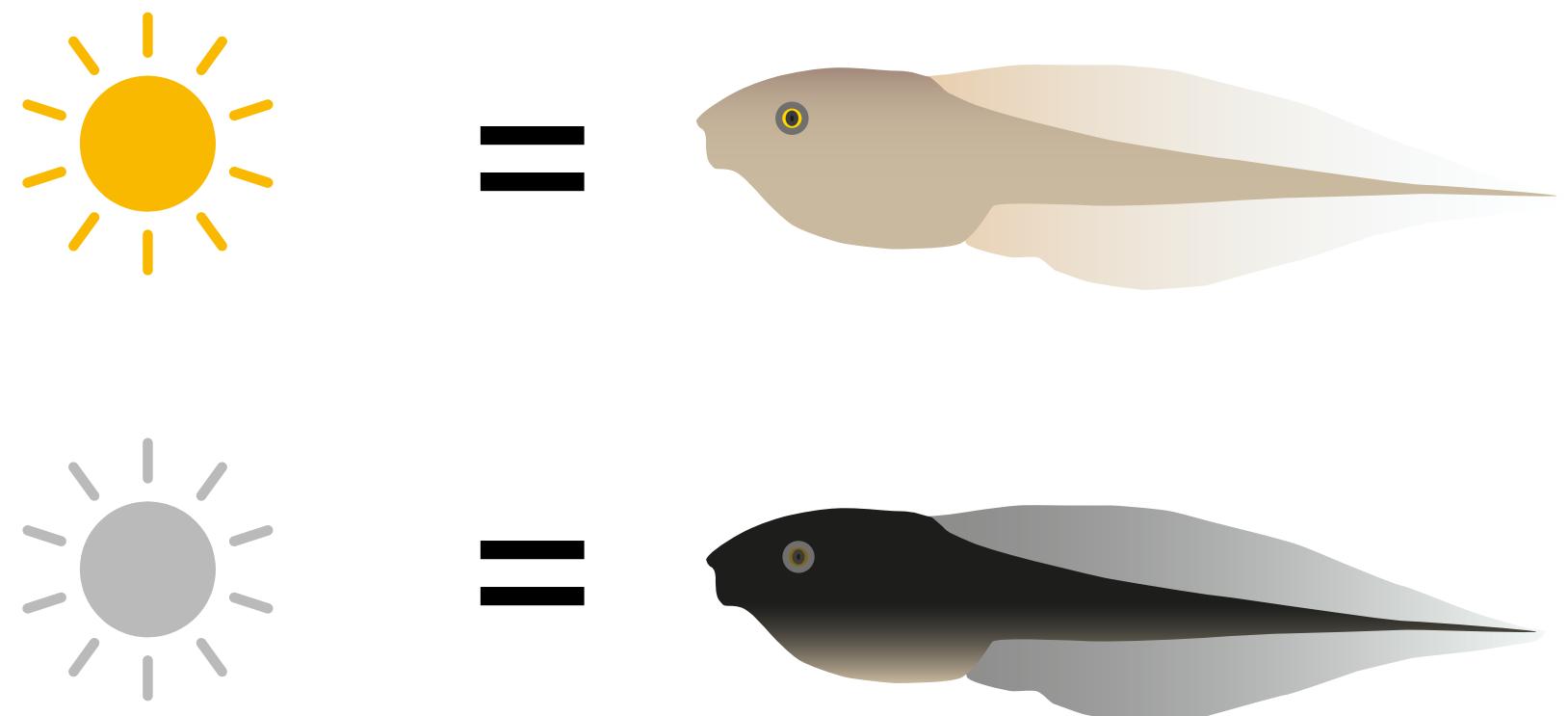




Colour change is reversible



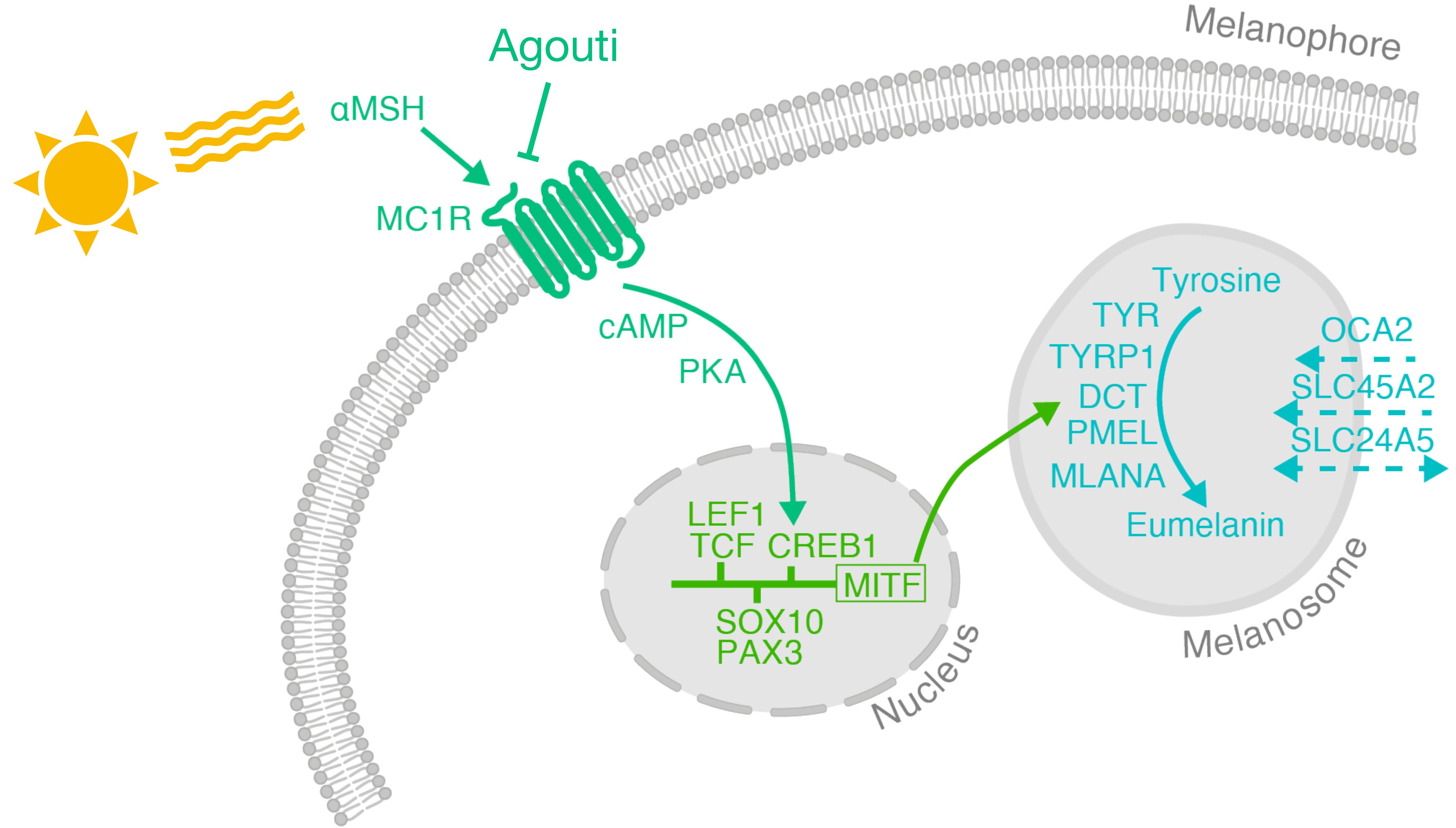
Inverse photosensitivity of plastic melanisation

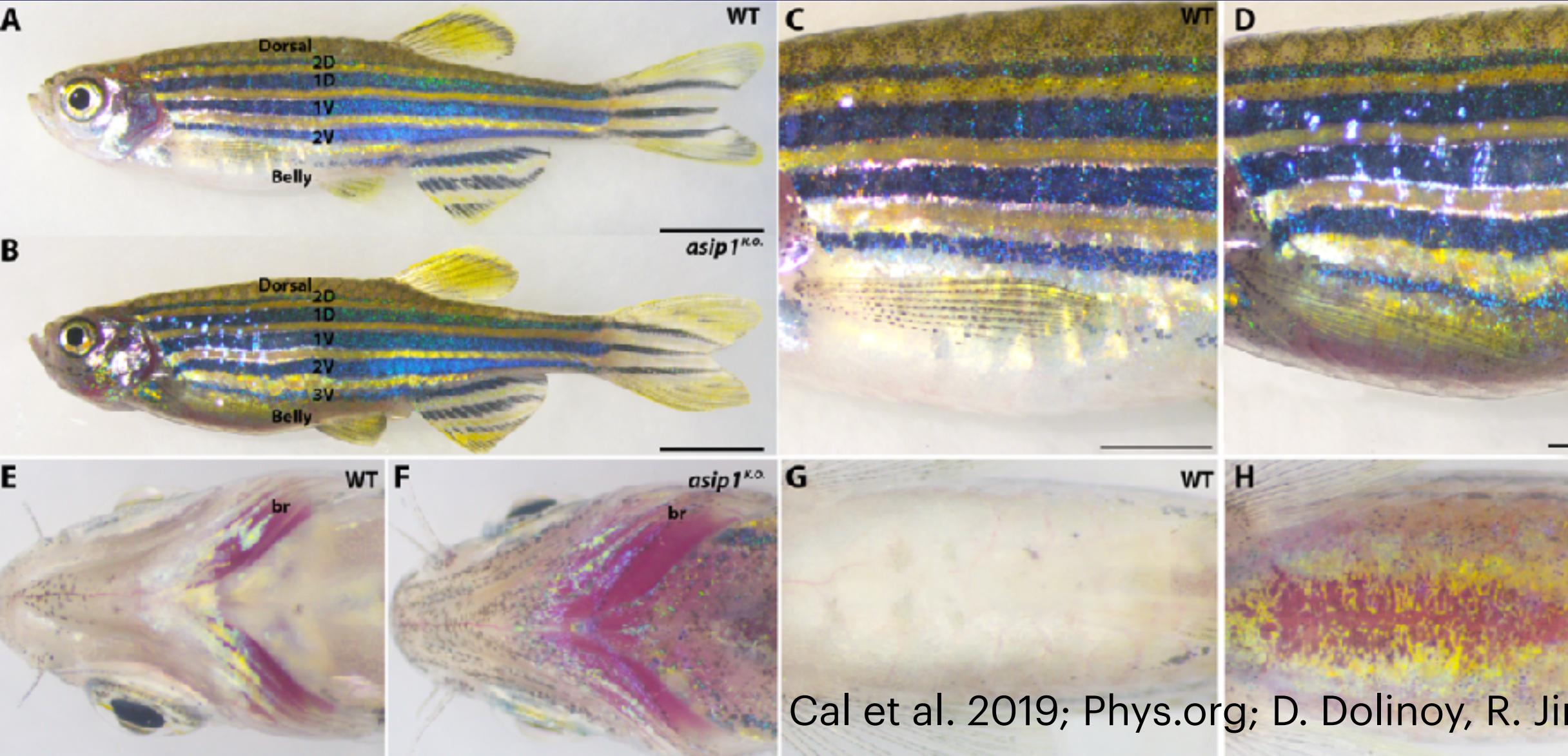
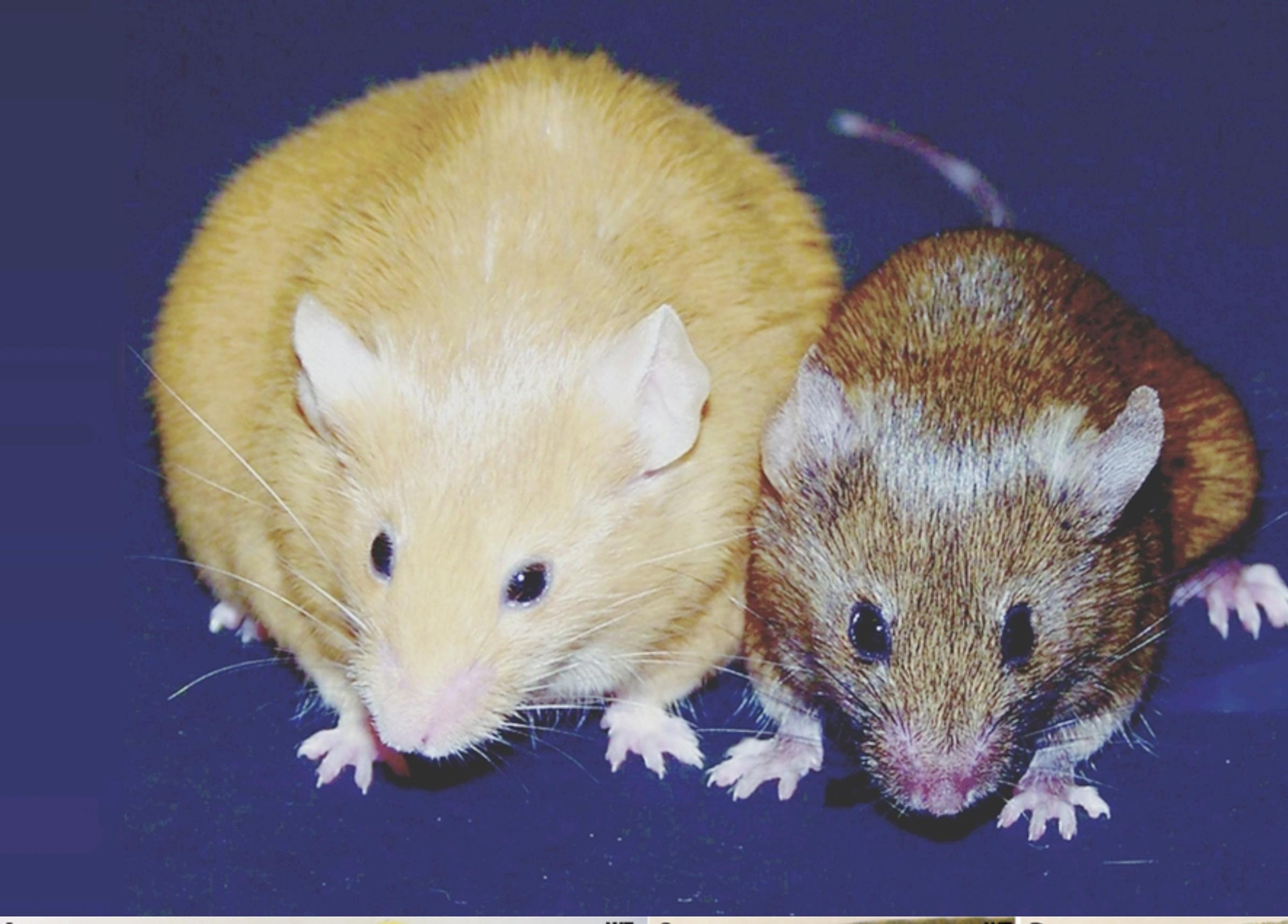


Non-plastic countershading

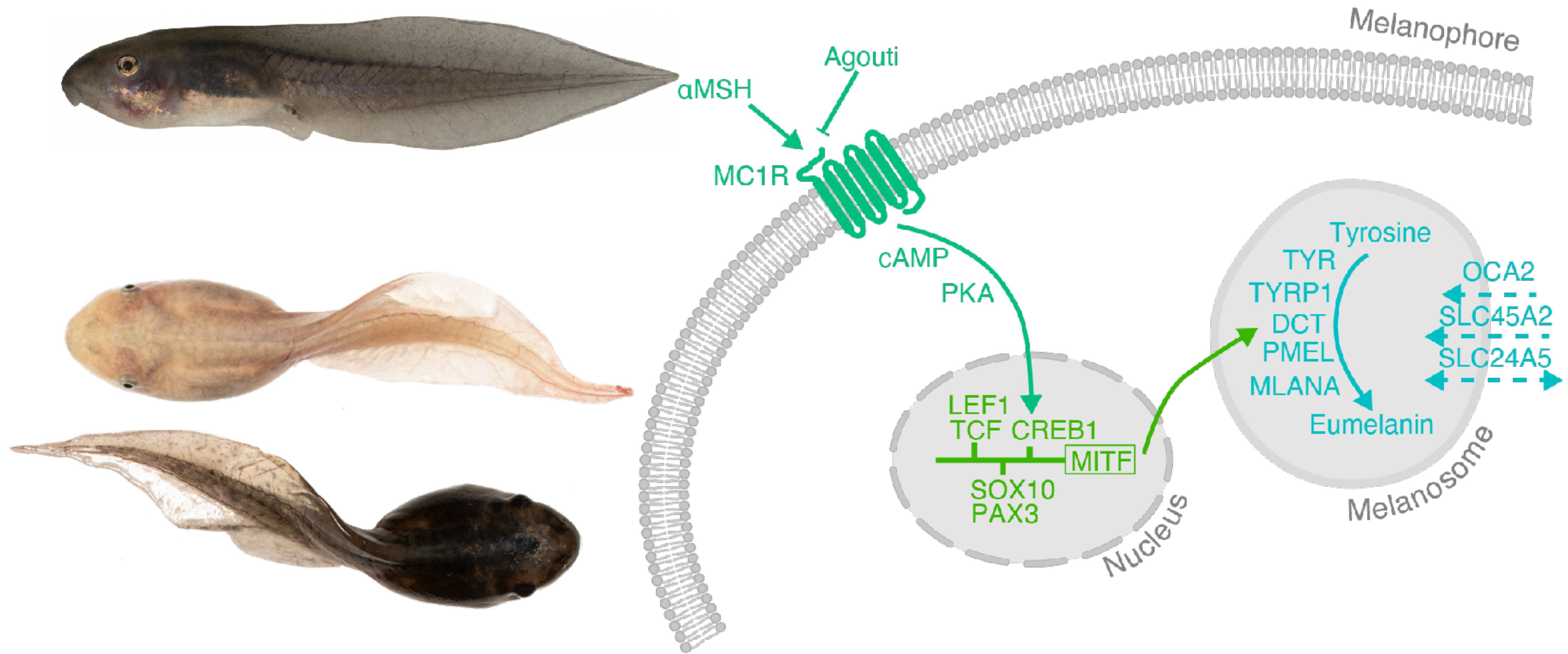


Photosensitive melanin biosynthesis



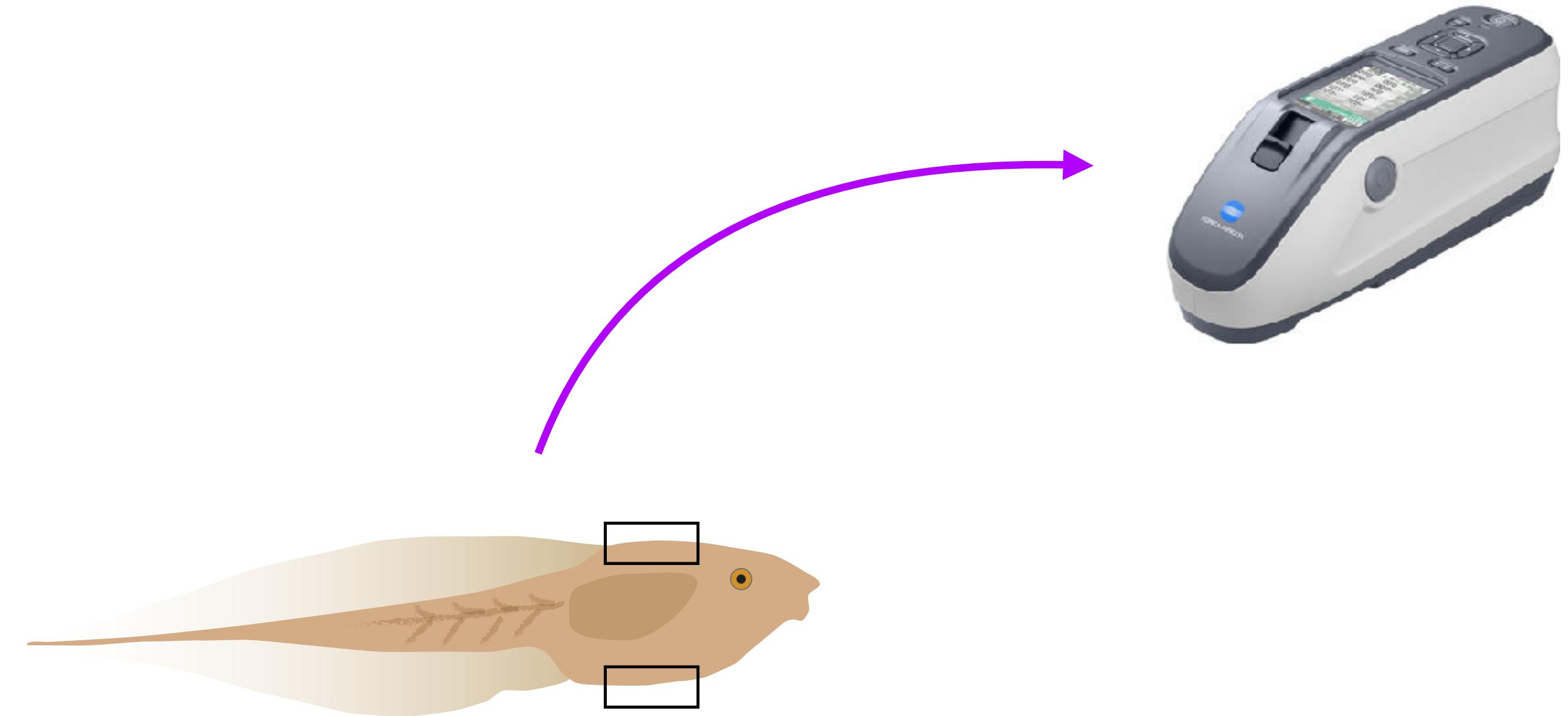


Photosensitive melanin biosynthesis



Background matching experiment

Spectrophotometry

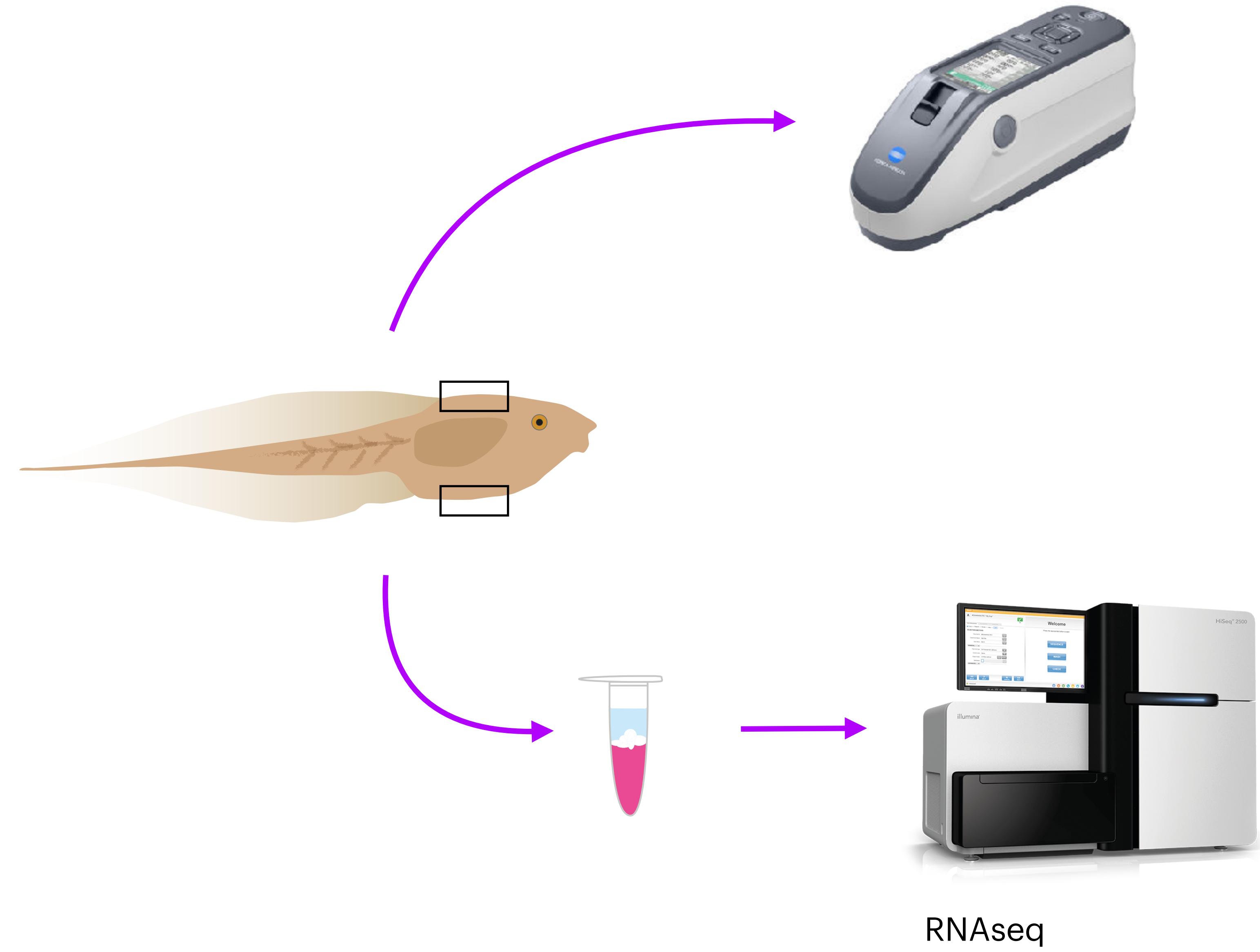


Background matching experiment

Spectrophotometry



40-day exposure



Data wrangling and visualisation with the Tidyverse

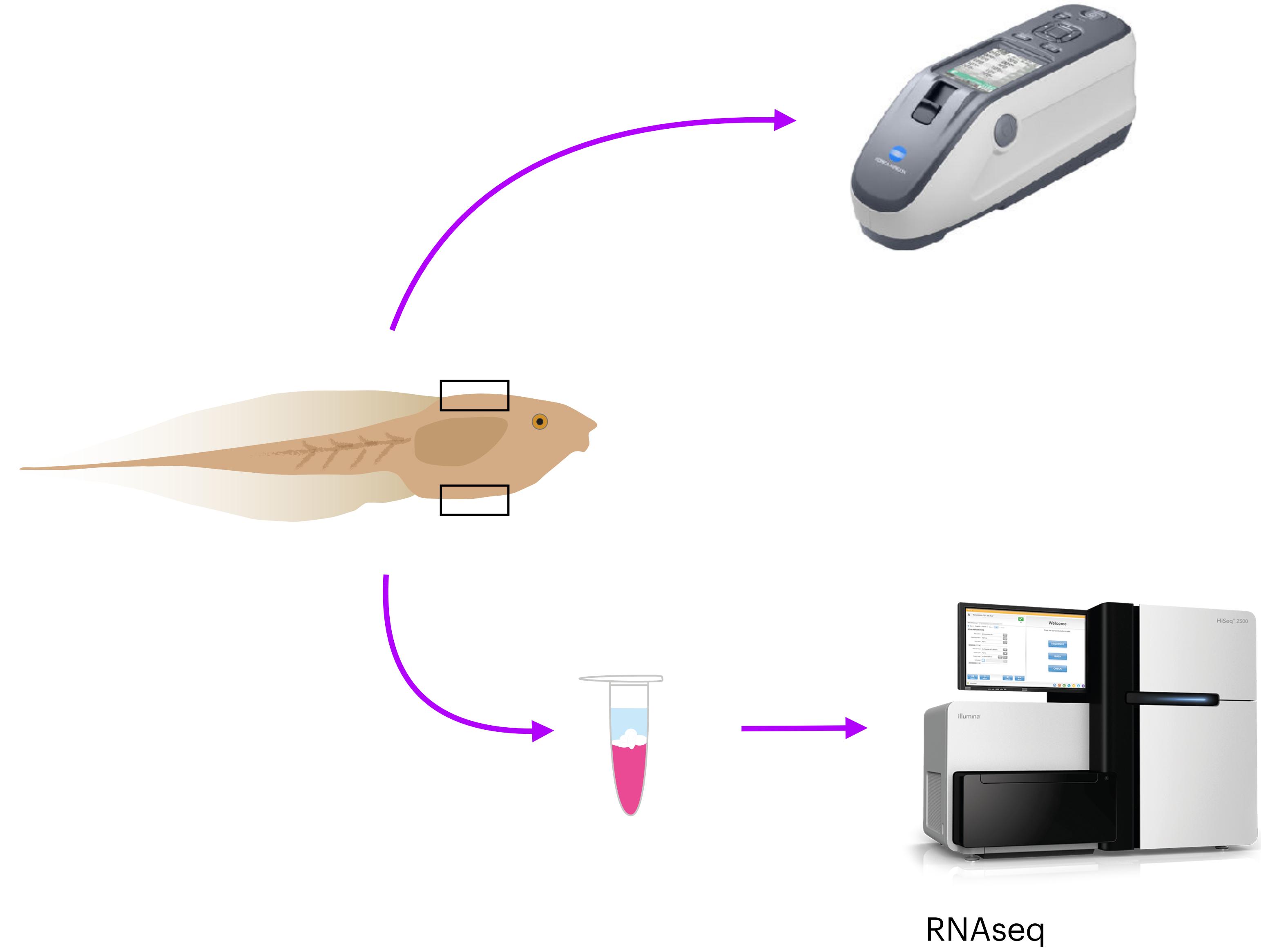
https://hcliedtke.github.io/UAM_NewTech

Background matching experiment

Spectrophotometry

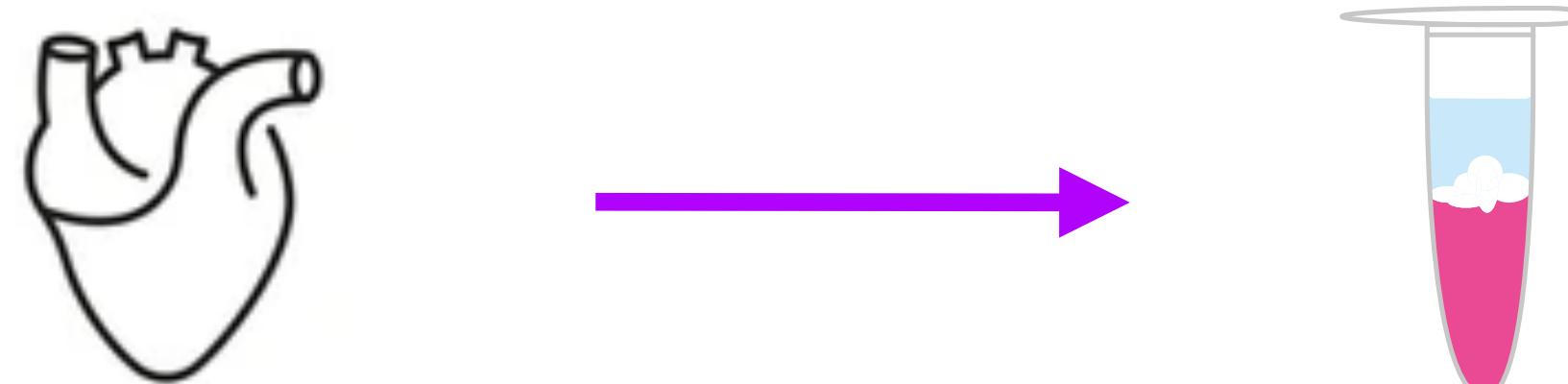


40-day exposure



RNA-Sequencing (lab)

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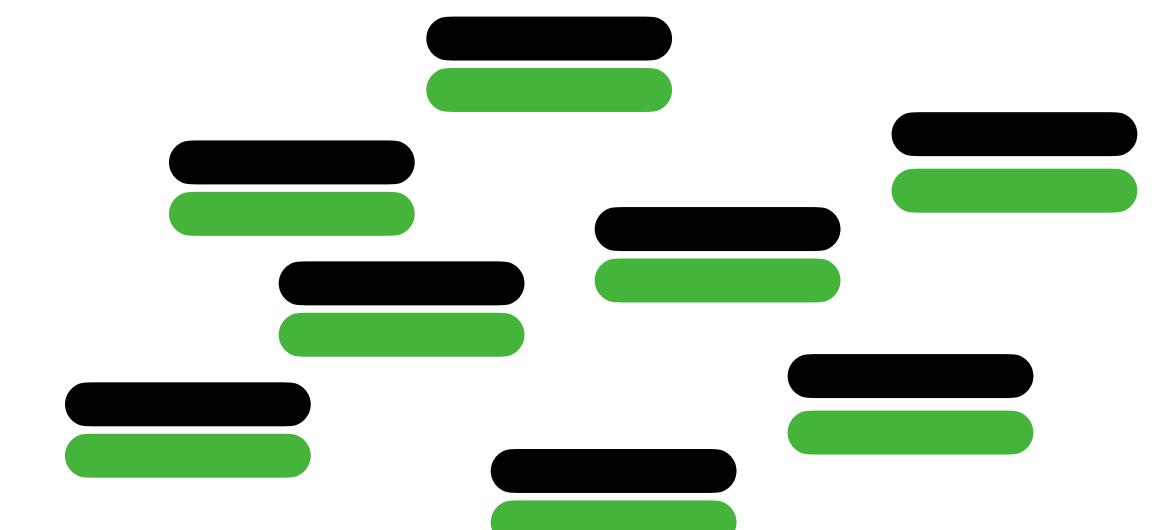
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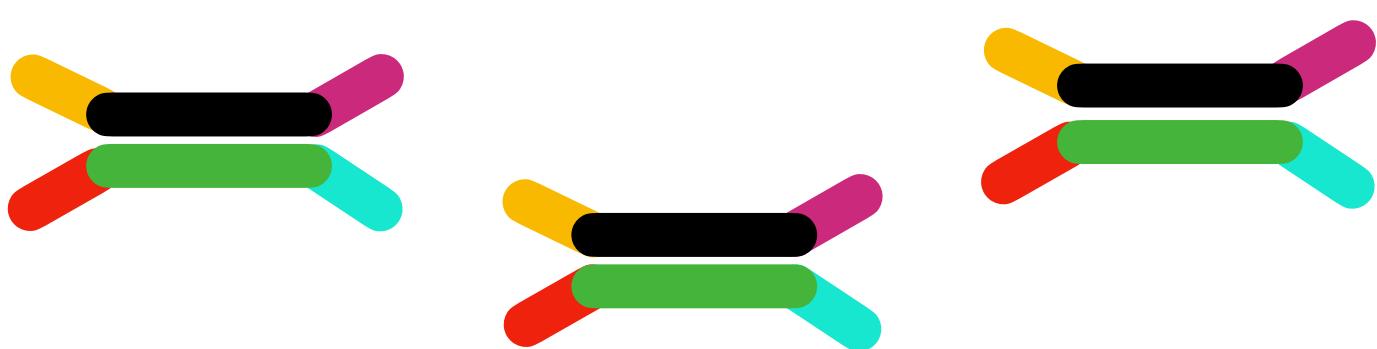
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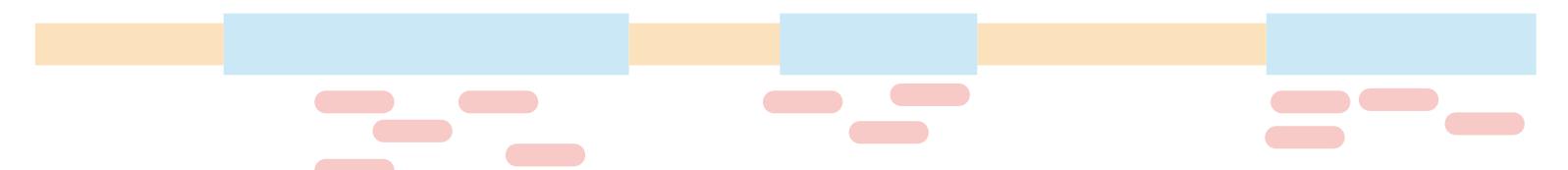
Parallel sequencing



RNA-Sequencing (in silica)

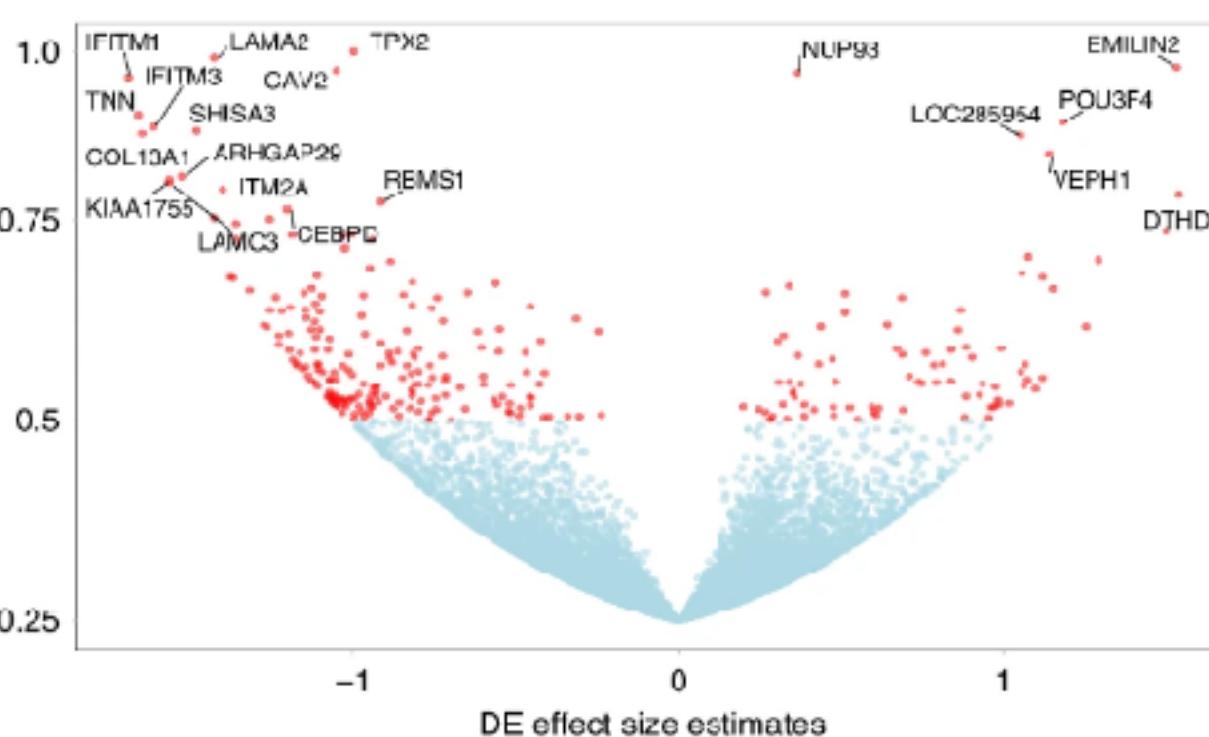
1.Sequence cleaning

2. Mapping/quantification



The diagram illustrates gene expression levels across two conditions. At the top, two genes are shown: Gene 1 (blue box) and Gene 2 (teal box). Below each gene, several pink horizontal bars represent RNA molecules. In the first condition (left), Gene 1 has 6 RNA molecules and Gene 2 has 7 RNA molecules. In the second condition (right), Gene 1 has 7 RNA molecules and Gene 2 has 6 RNA molecules. A large purple arrow at the bottom points from left to right, indicating a transition or comparison between the two conditions.

3. Differential gene expression



4. Functional insights

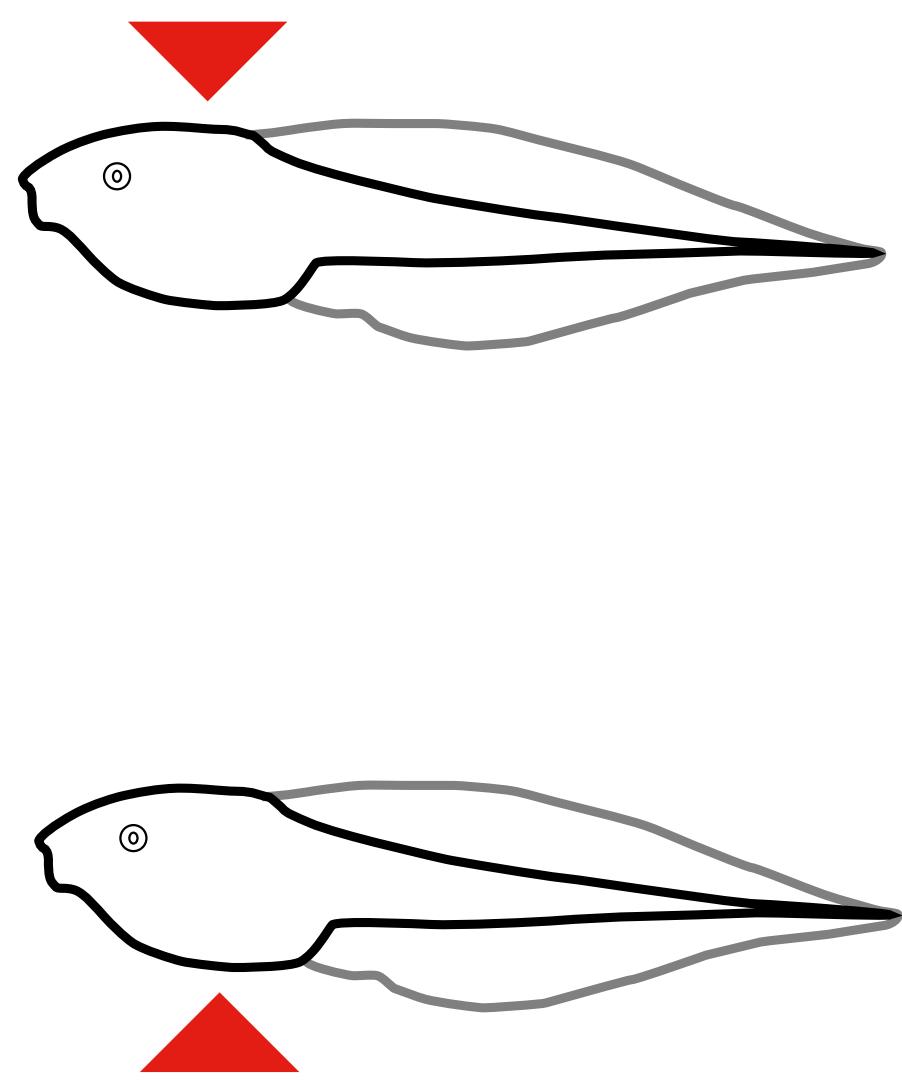
RNA-seq counts matrix

	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

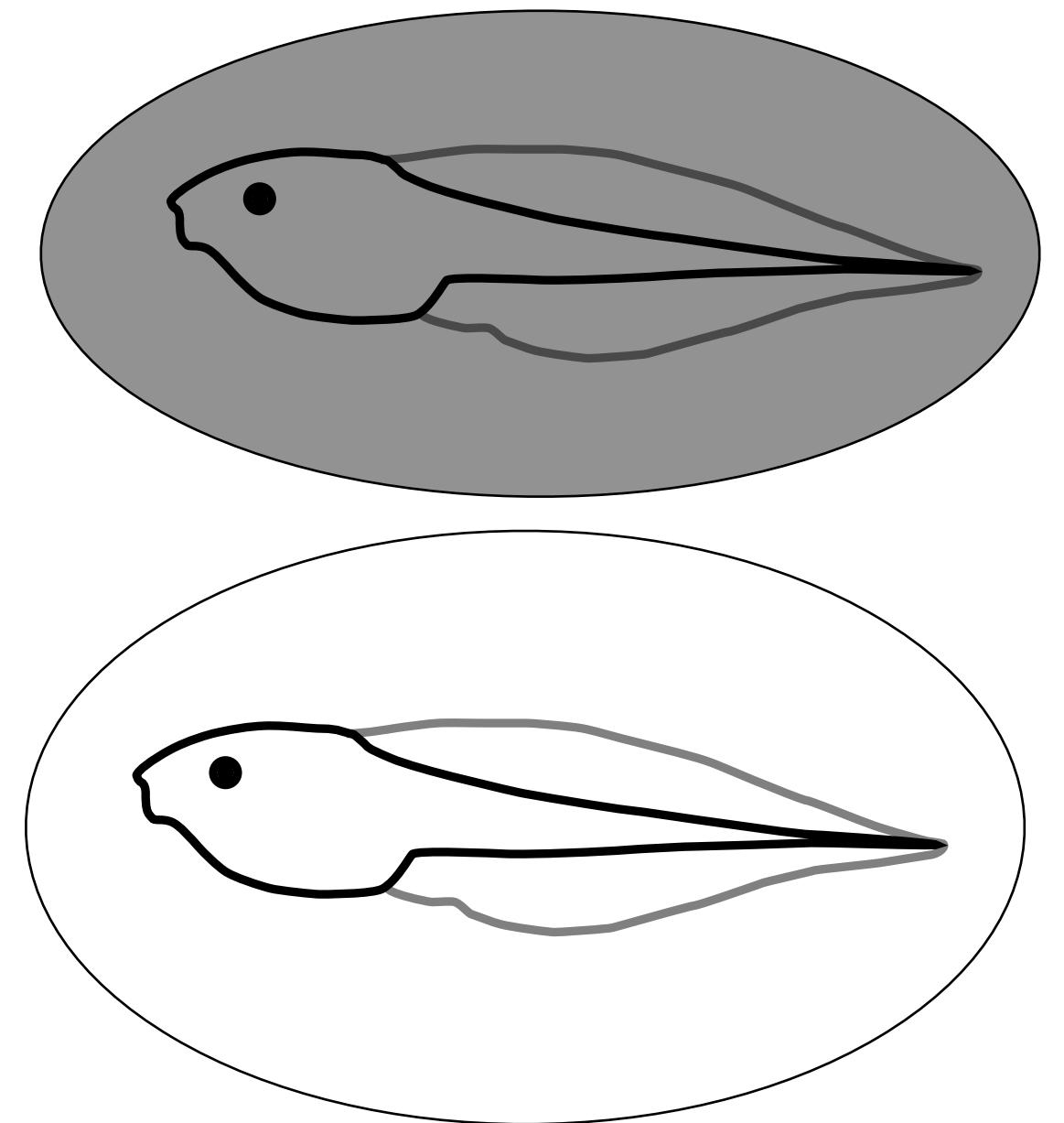


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Anatomical effect

+

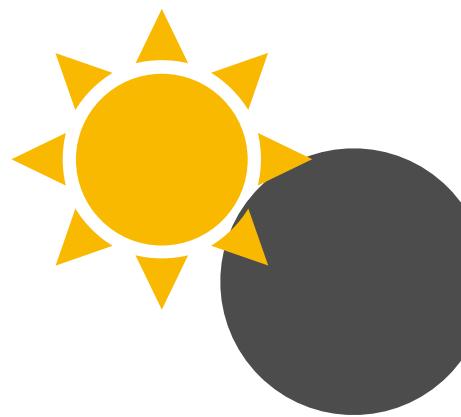


Background effect

Differential Gene Expression

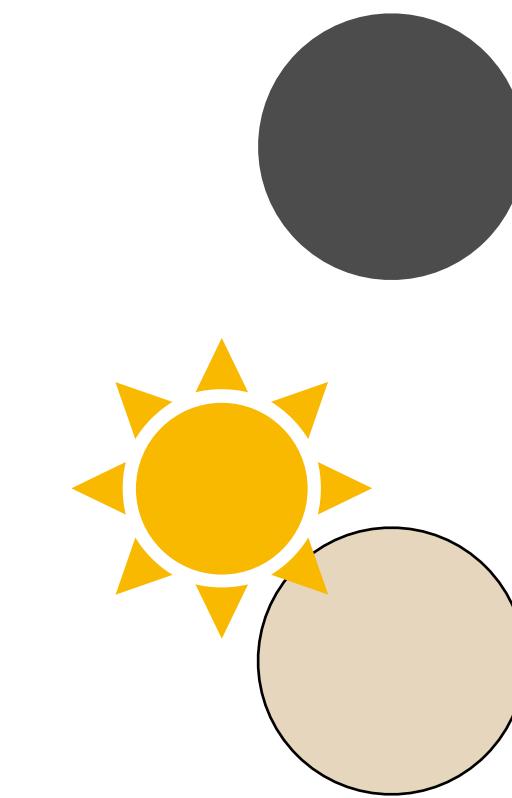


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Fixed pigmentation

+



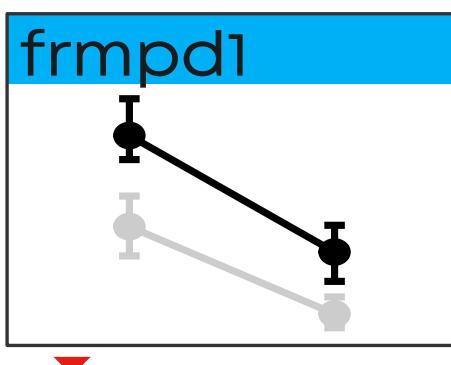
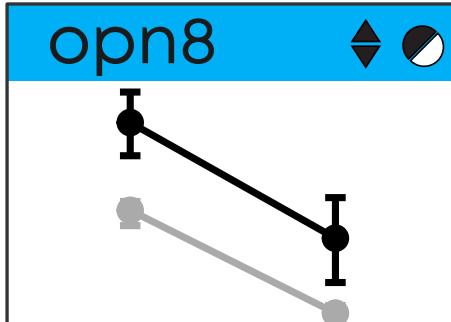
Plastic pigmentation

RNA-seq practical

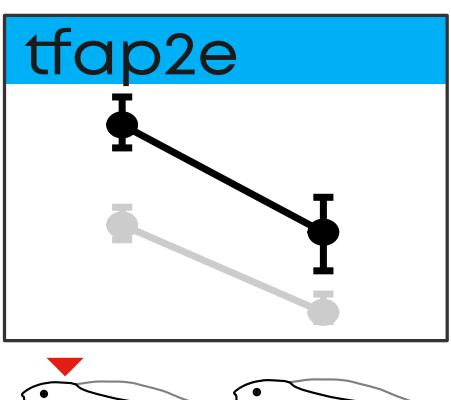
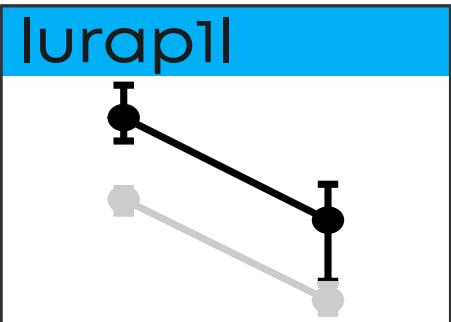
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Differential Gene Expression

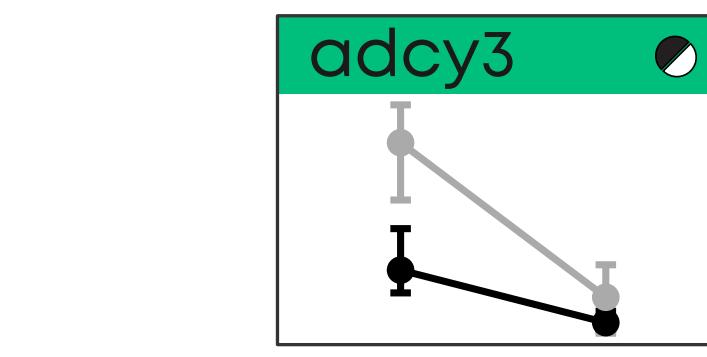
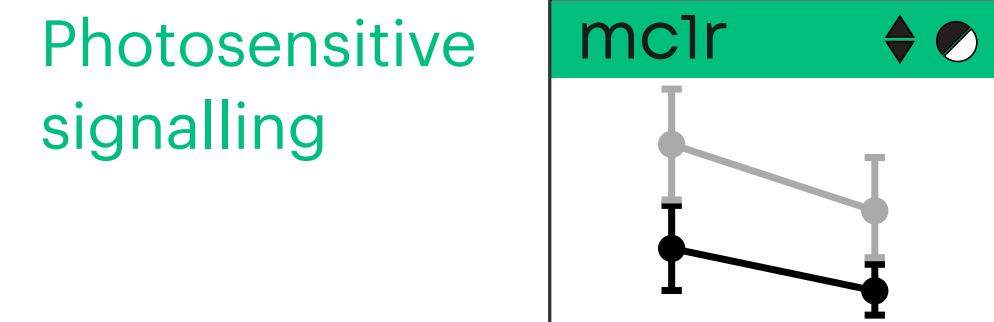
Photosensitive signalling



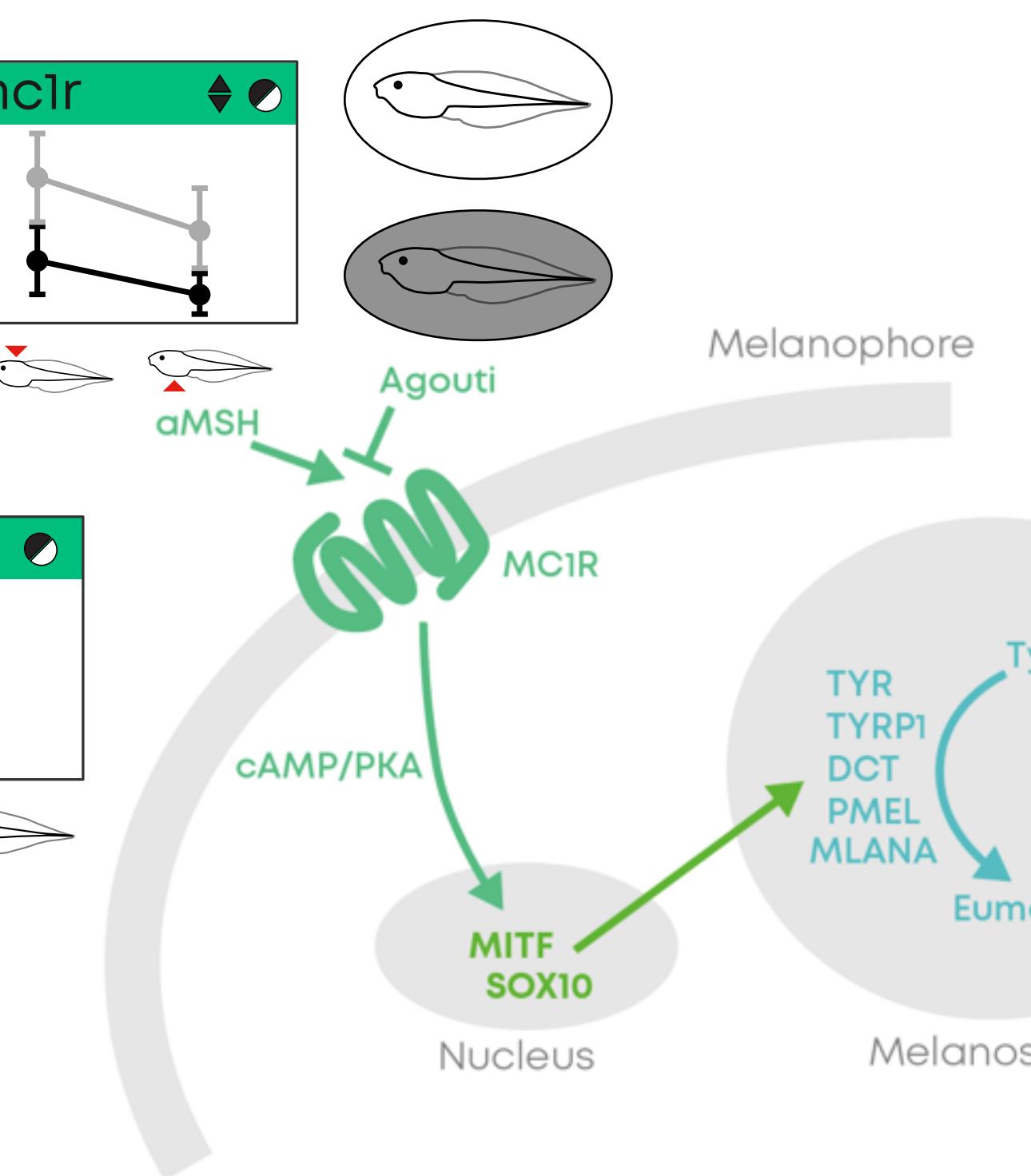
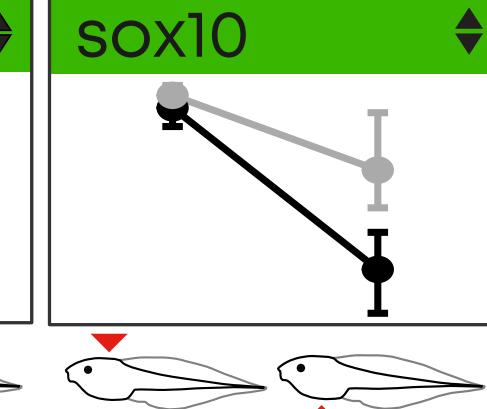
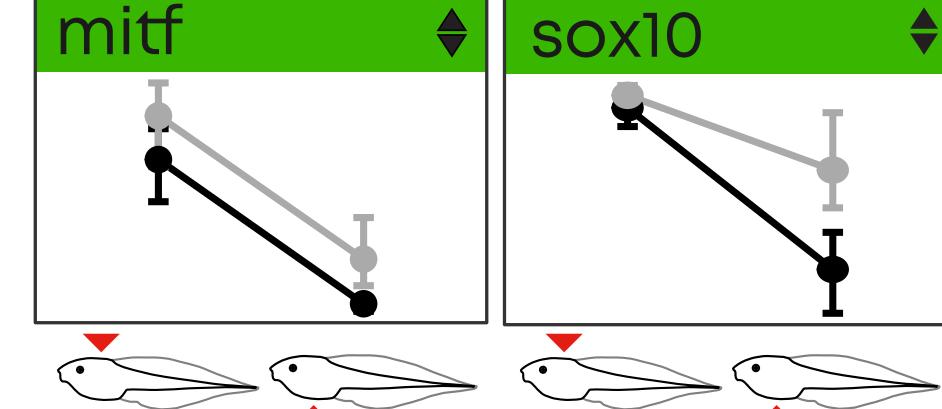
Transcription regulators



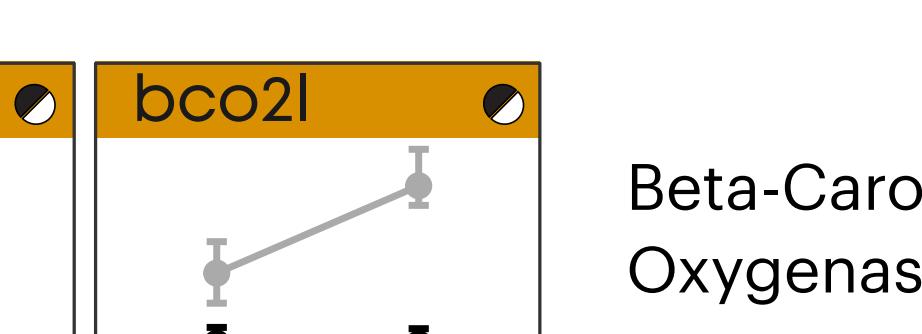
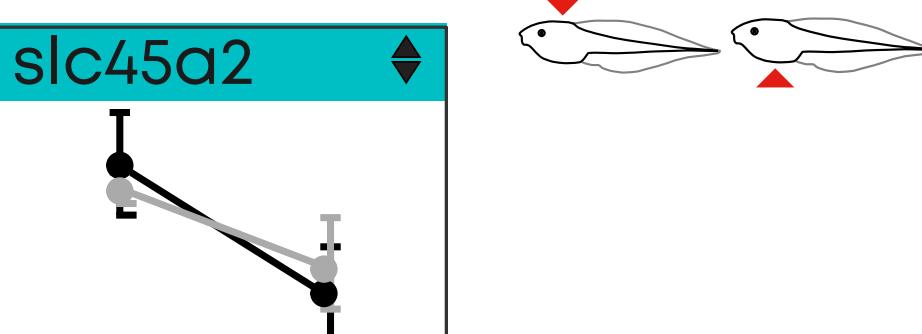
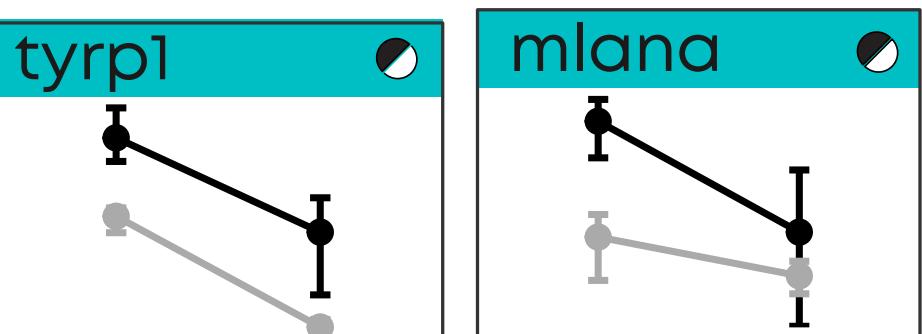
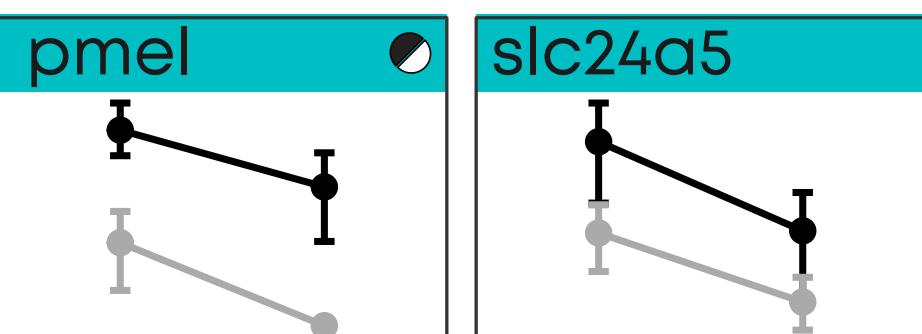
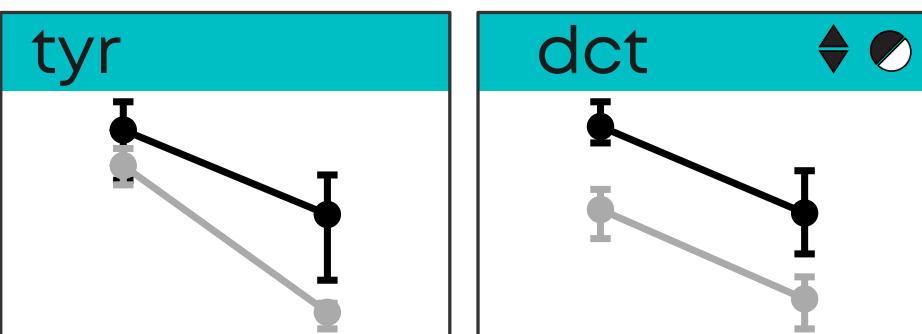
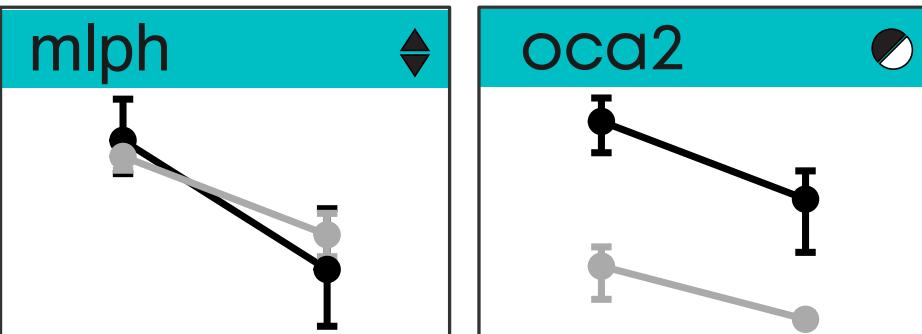
Photosensitive signalling



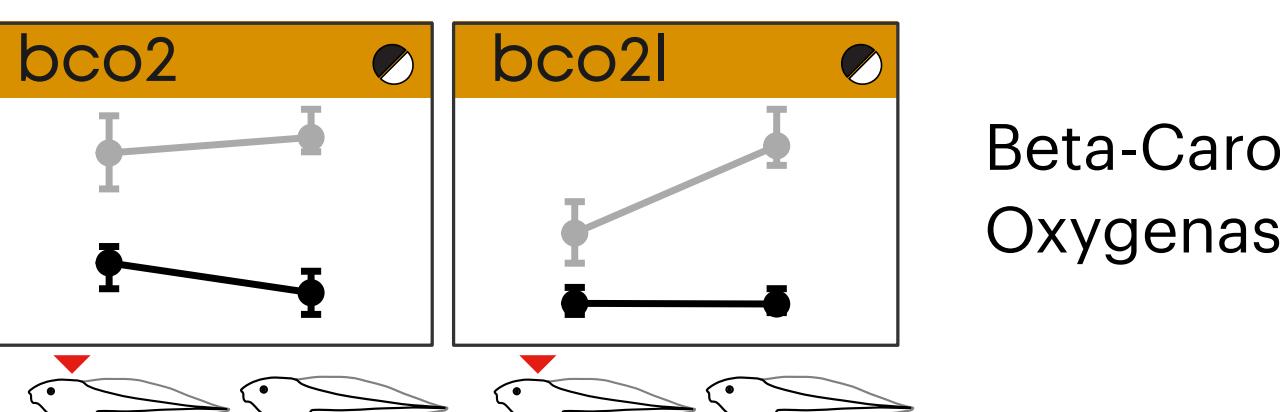
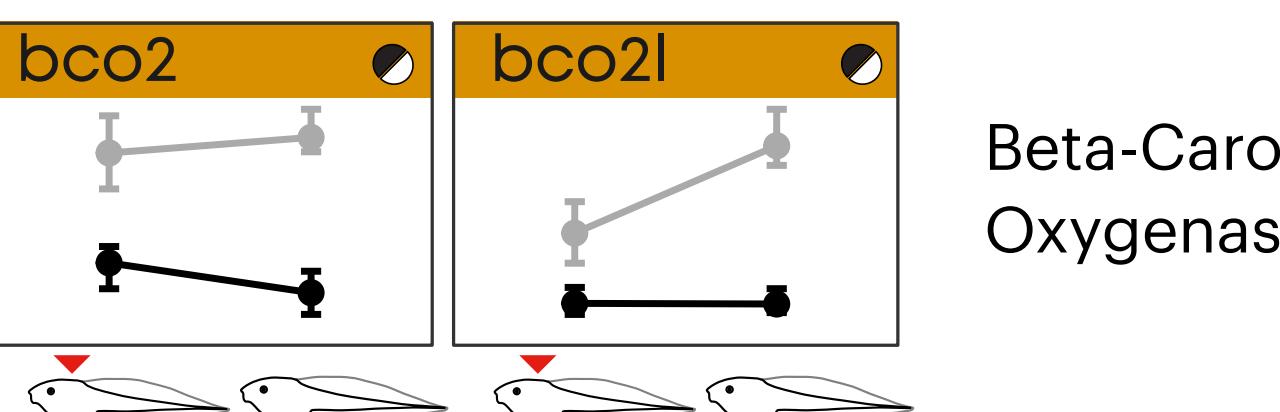
Transcription regulators



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

