# PoE: Evolutionary Ecology

## Transcriptomics of Plastic Phenotypes

H. Christoph Liedtke

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

https://hcliedtke.github.io/PoE

# Crypsis through Background Matching

Photo credits: Doug Waylett, Michael Kidwell, Robert Jensen, Anna Veselova, Kris Wiktor, Mircea C, Davem Hunt

# Crypsis through Background Matching

Pigmentation plasticity




# Pigmentation Plasticity in Amphibians



Non-breeding male



Breeding male



Night



Day

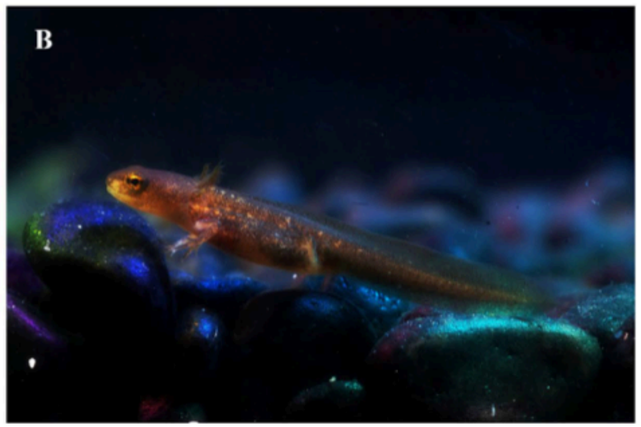


No predators



Predators


Light background

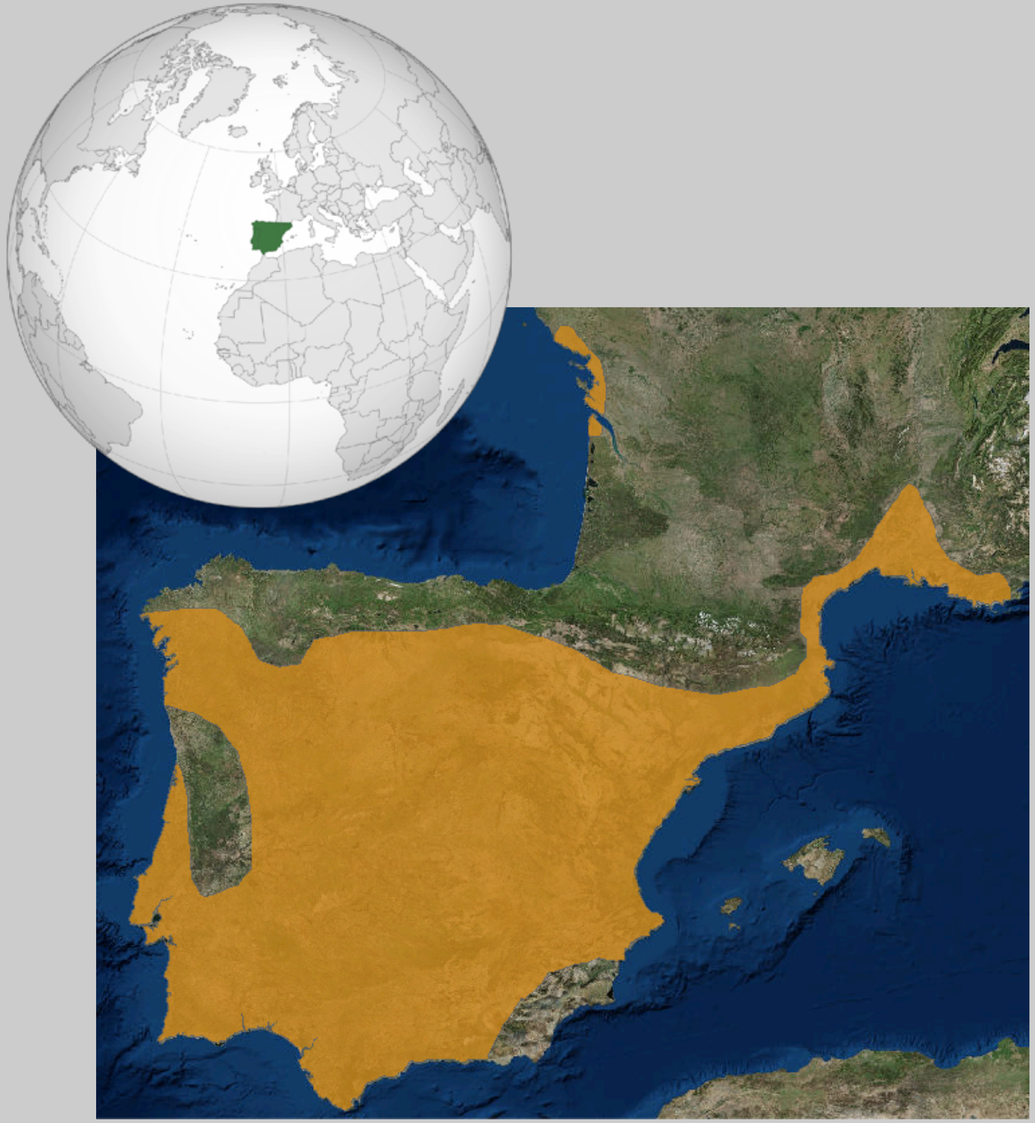



Dark background

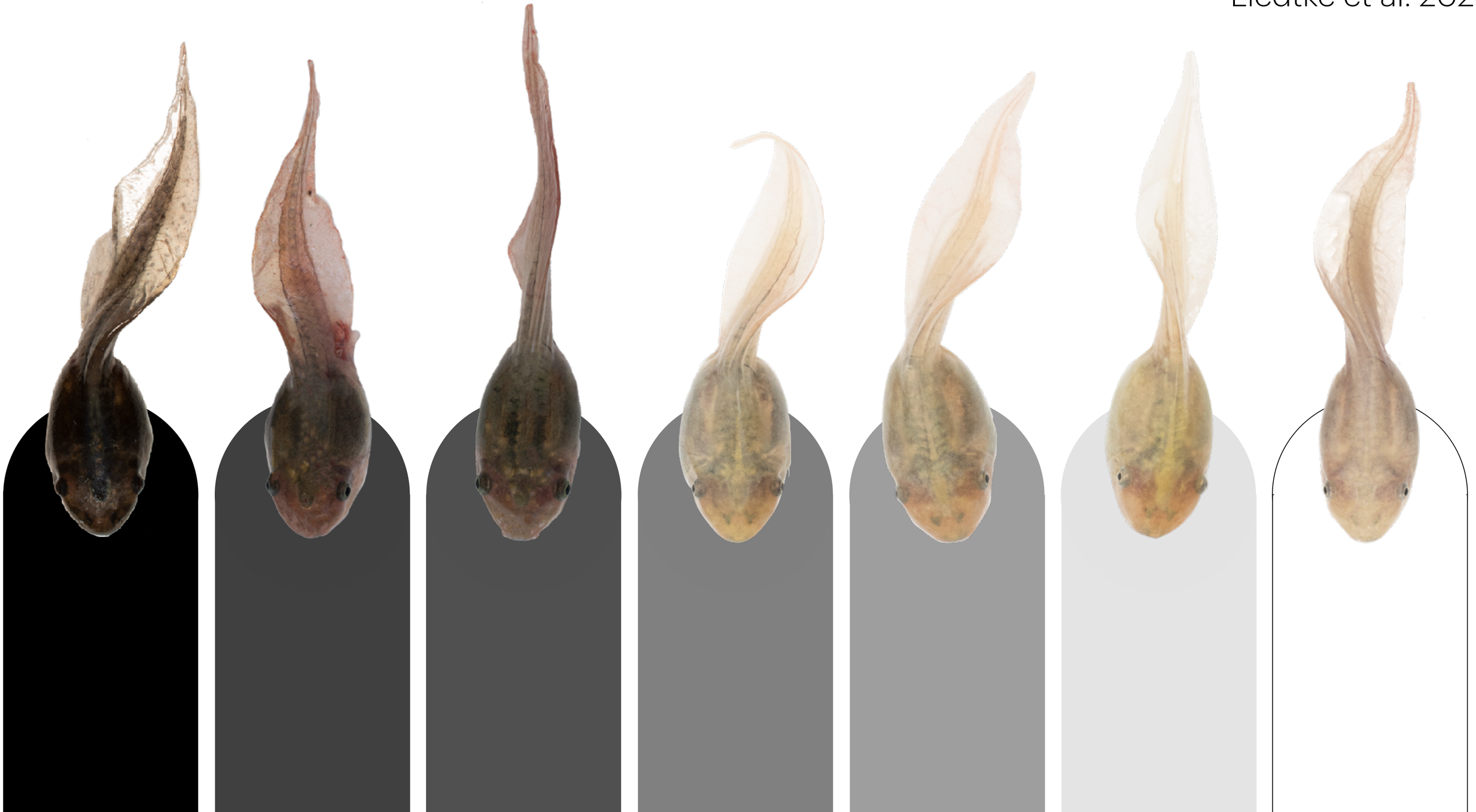
Photo credit: Nuria Polo, Michael F. Benard, Gabriela Bittencourt

# *Pelobates cultripes*

## Western Spadefoot toad



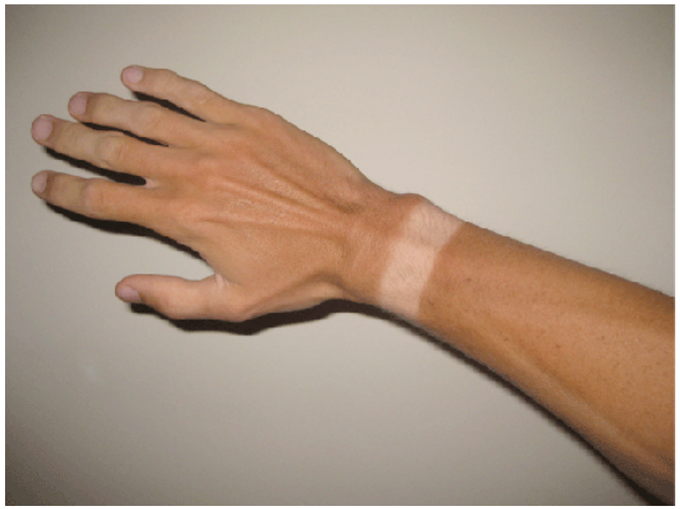
Liedtke et al. 2023

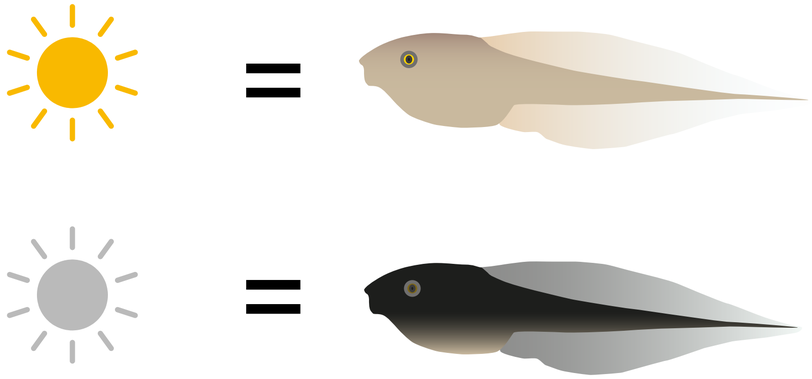


# Colour change is reversible

Liedtke et al. 2023

# Inverse photosensitivity of plastic melanisation

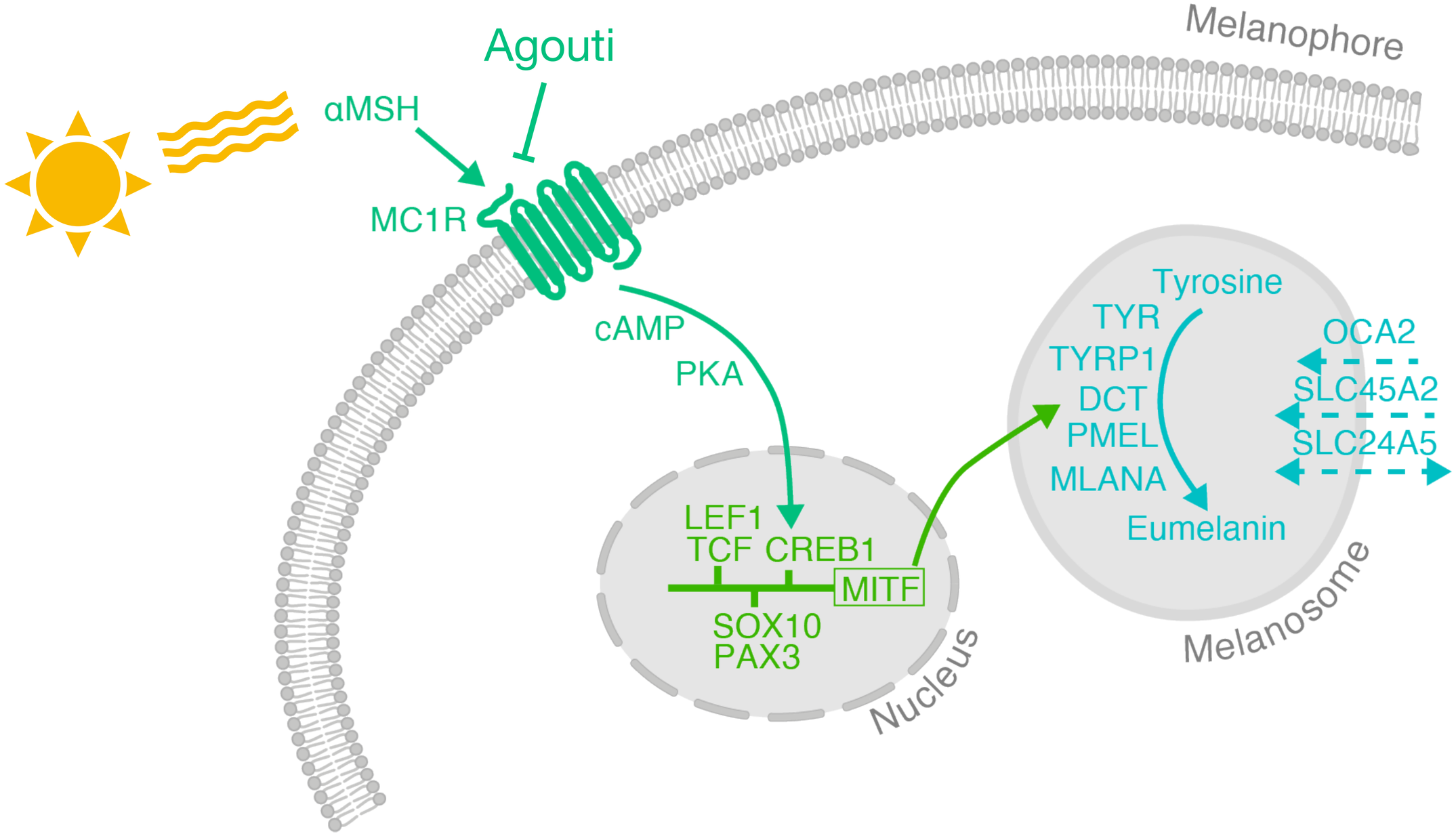


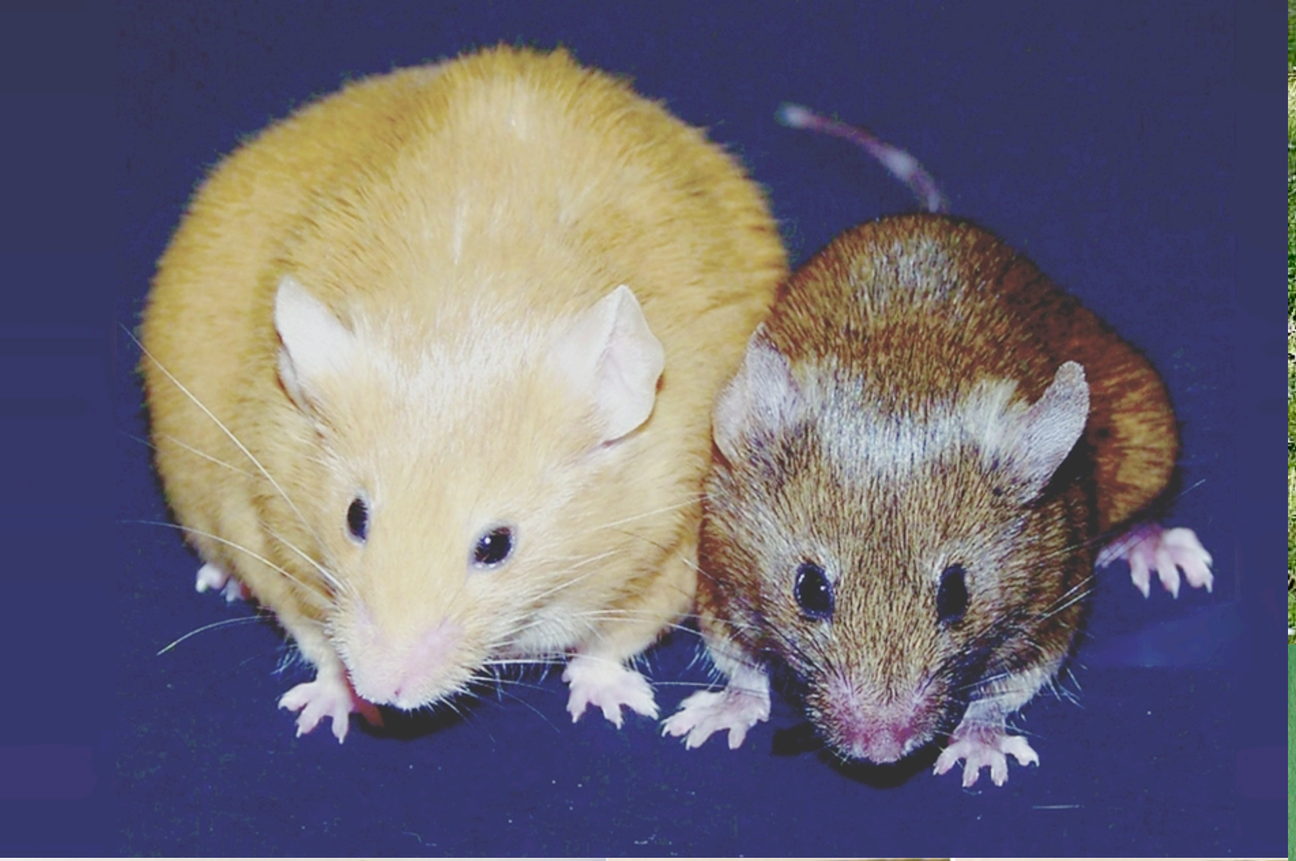


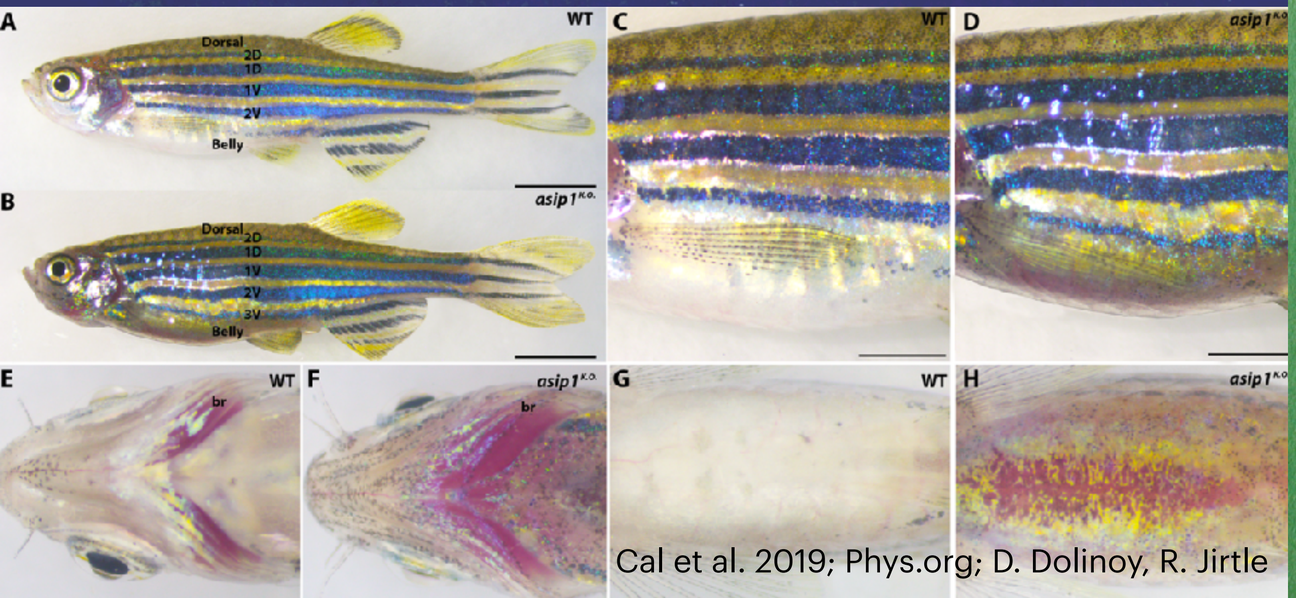
# Non-plastic countershading

Photo credits: Nicolas Rohner

# Photosenstive melanin biosynthesis

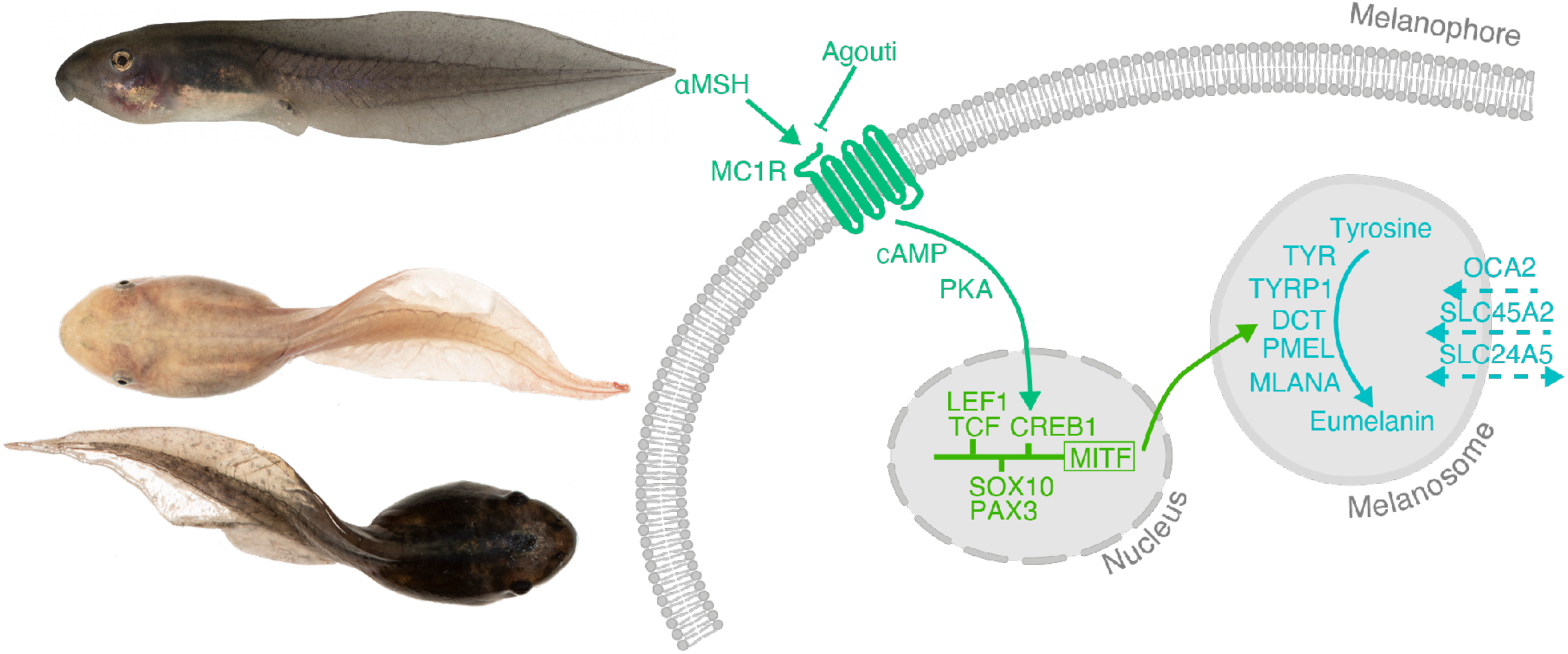







Cal et al. 2019; Phys.org; D. Dolinoy, R. Jirtle

# Photosenstive melanin biosynthesis



# Background matching experiment

40-day exposure

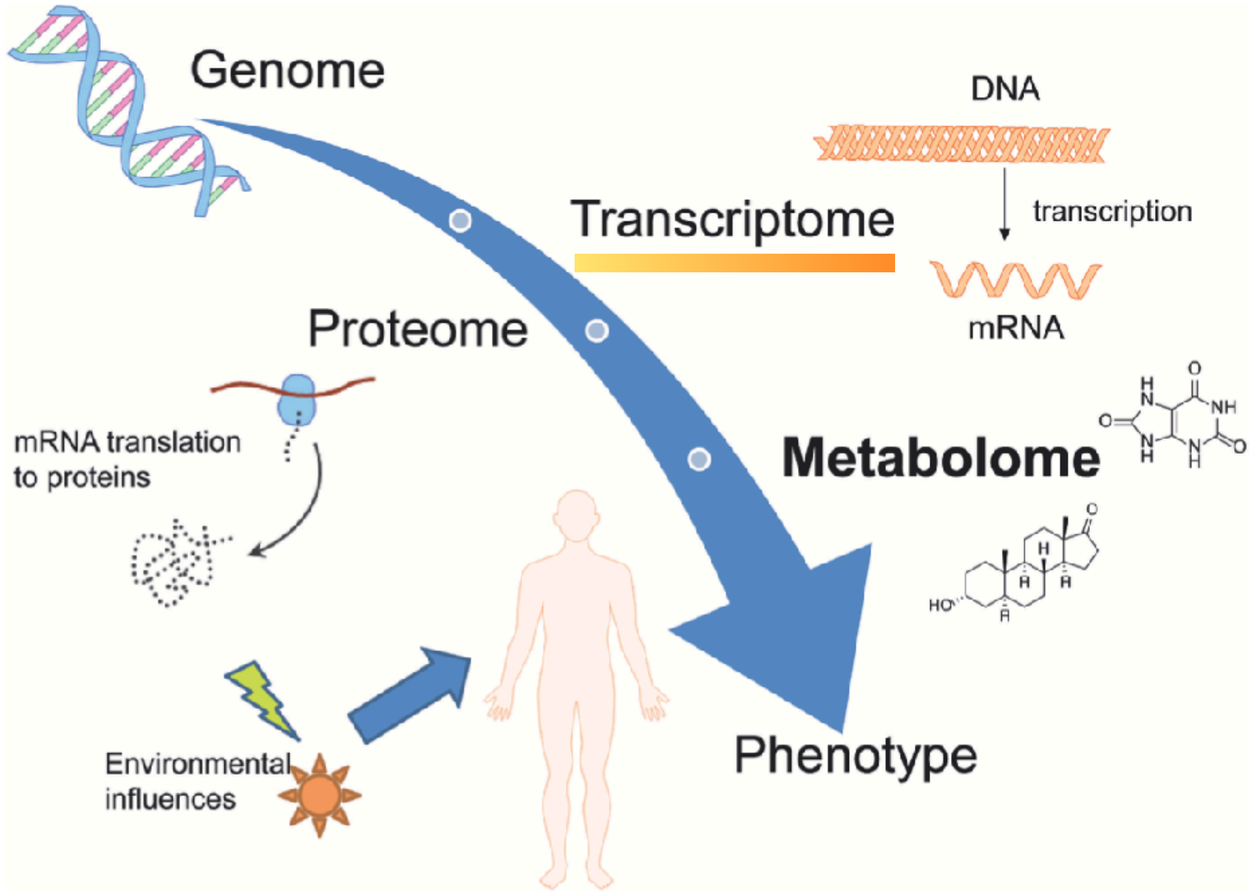
Spectrophotometry


RNAseq

# Spectrophotometry practical

**https://hcliedtke.github.io/PoE**

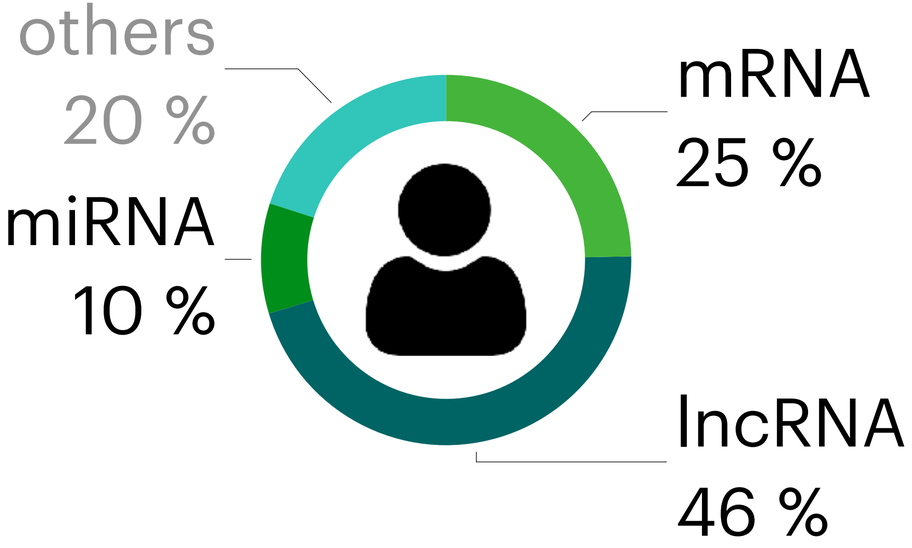
# From genome to phenotype




Steuer et al. 2019, Frontiers in Chemistry

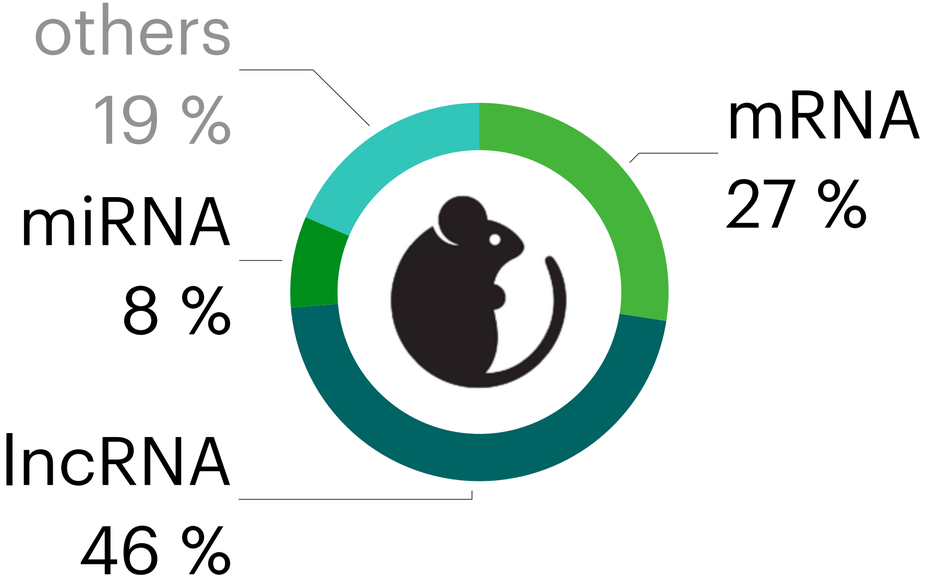
# What is the transcriptome?

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




3.2bp genome

78724 genes

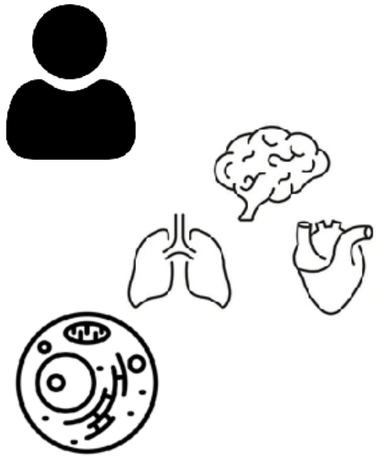



2.7bp genome

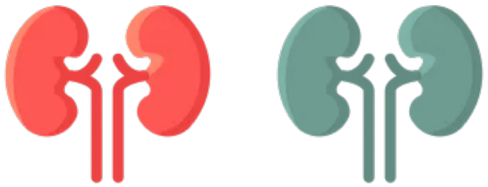
78239 genes

https://www.gencodegenes.org/

# What is the transcriptome?



- Organism > organ > tissue > cell

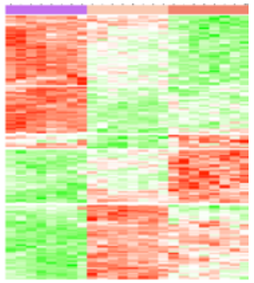


- Variable and state-dependent

# How do we study the transcriptome?

**Qualitatively**

- Identify expressed genes
- Gene discovery



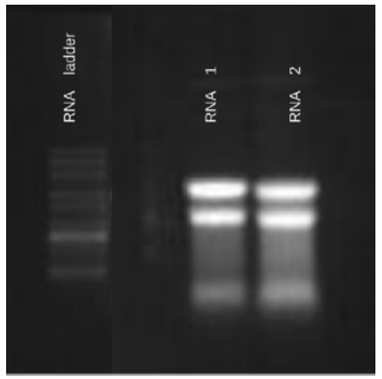
**Quantitatively**

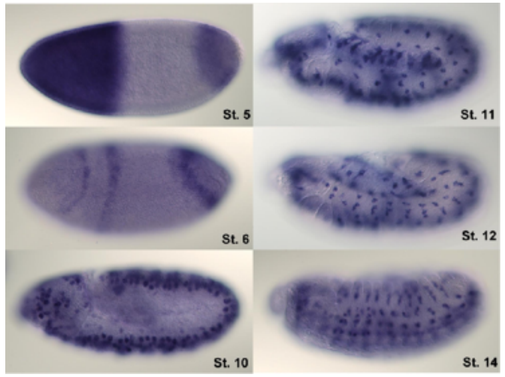
- Compare expression levels of genes or transcripts

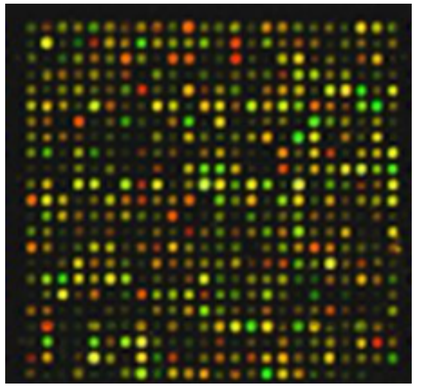
# History of studying transcripts

## Quantitative

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

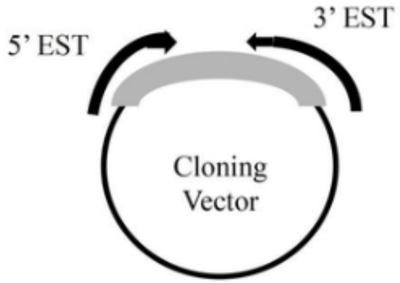


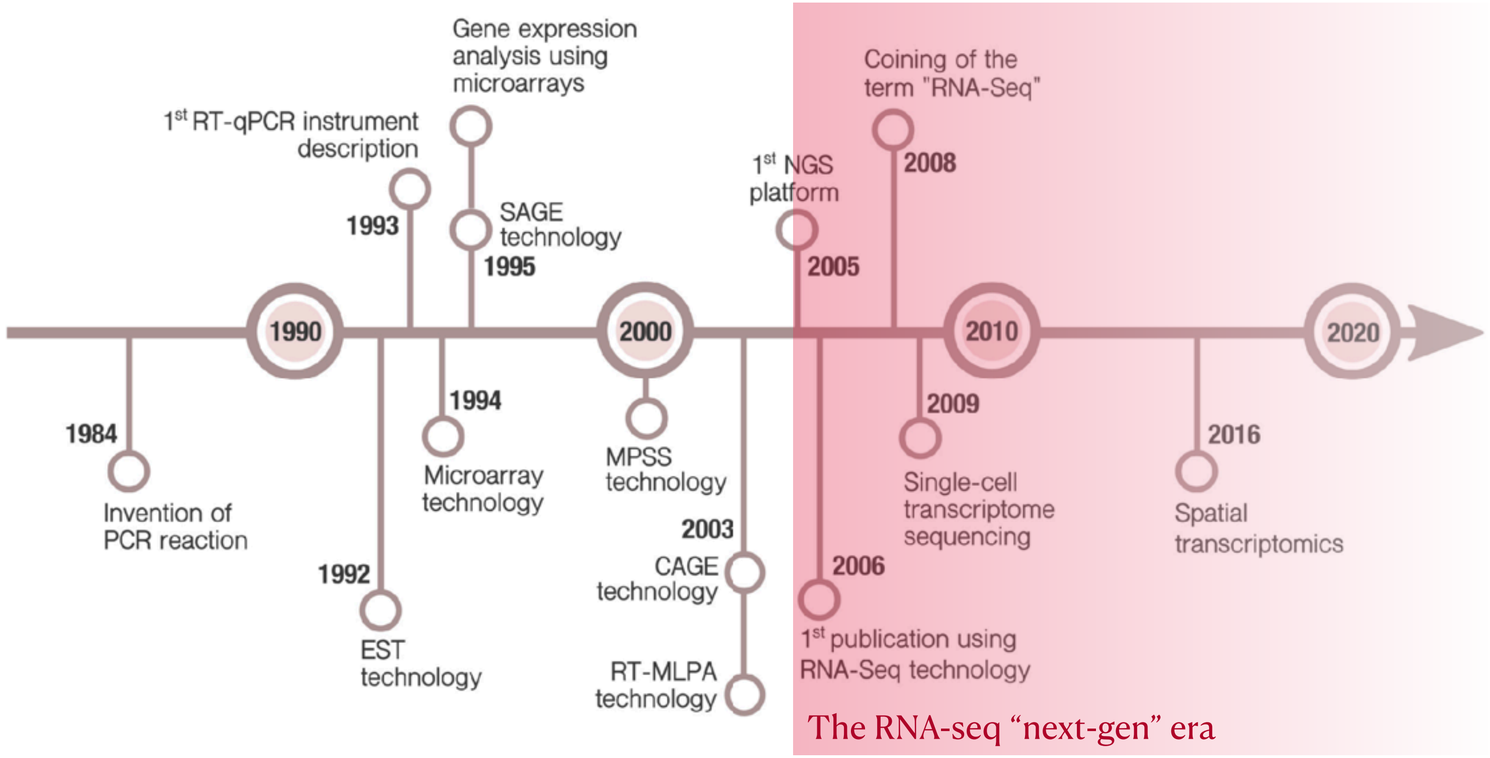



## Qualitative

- Expressed sequence tags 1990s



# History of studying transcripts



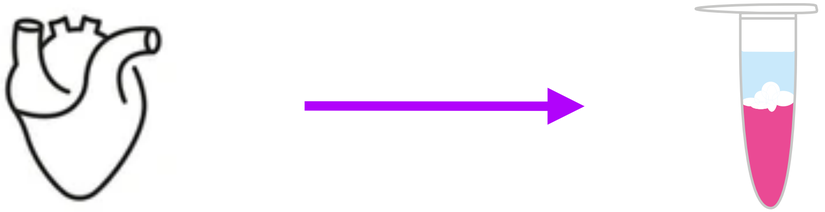

## RNA-seq

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

Martín-Perez et al. 2022

# RNA-Sequencing (lab)

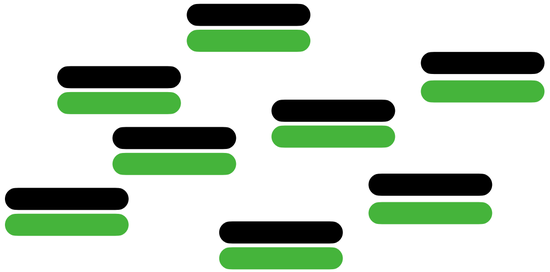
1. RNA extraction



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


3. Fragmentation

4. cDNA synthesis

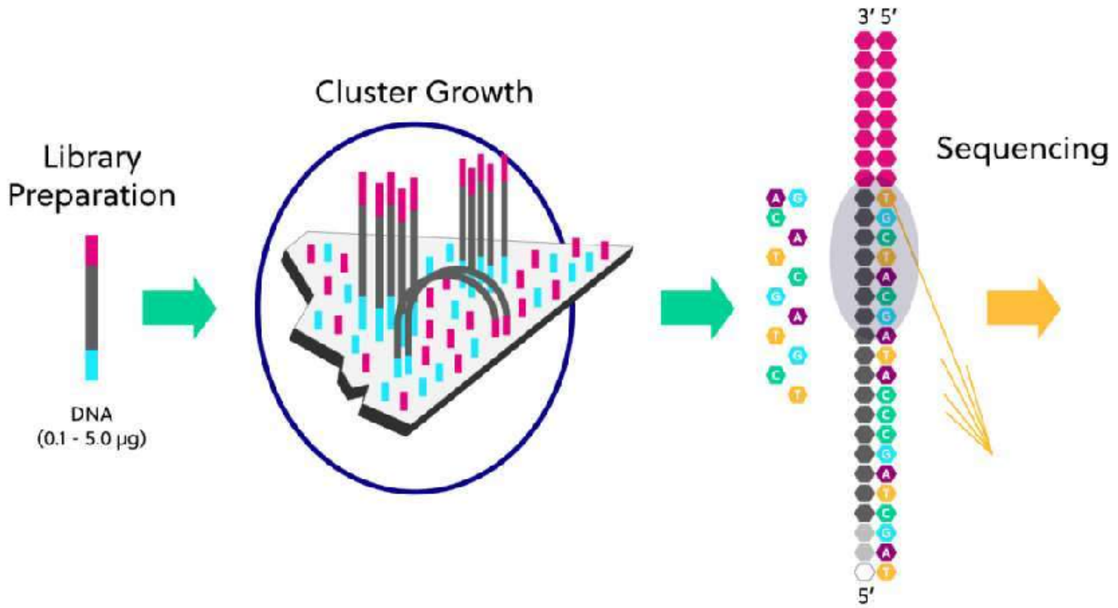


5. Adaptor ligation

Parallel sequencing

# Next-Gen Sequencing

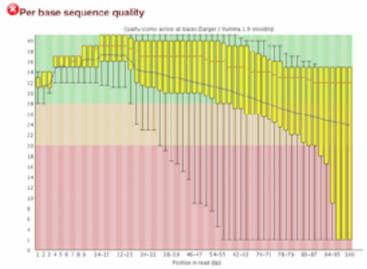
## Illumina short reads technology



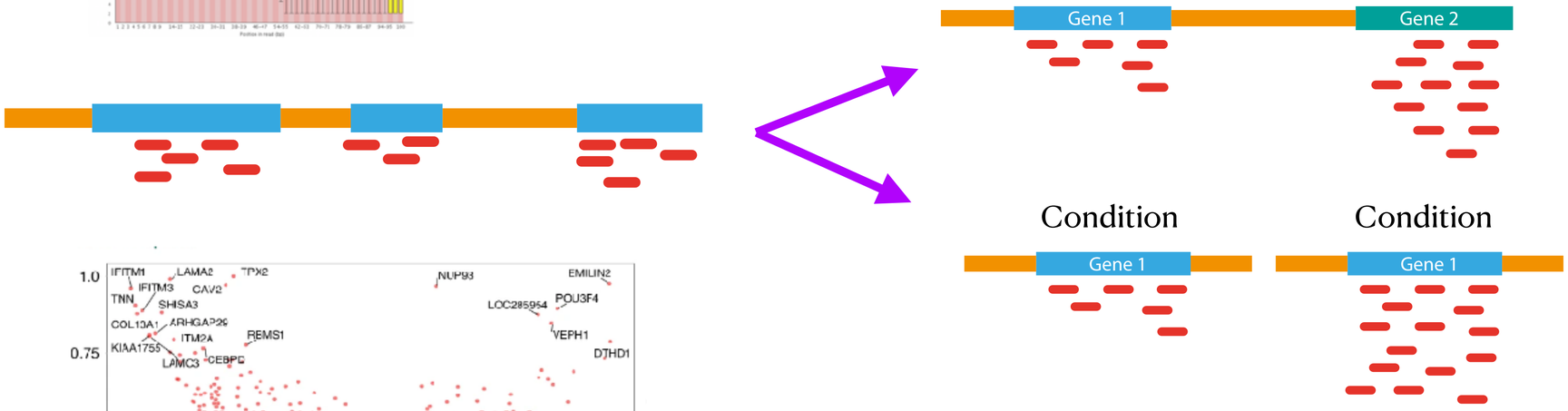

- Usual read length: 100bp
- Usual quantity of reads for RNAseq: 20-30 million reads per sample
- Single “lanes” allow running many samples at once (25 million - 20 billion reads depending on technology)

# RNA-Sequencing (in silica)

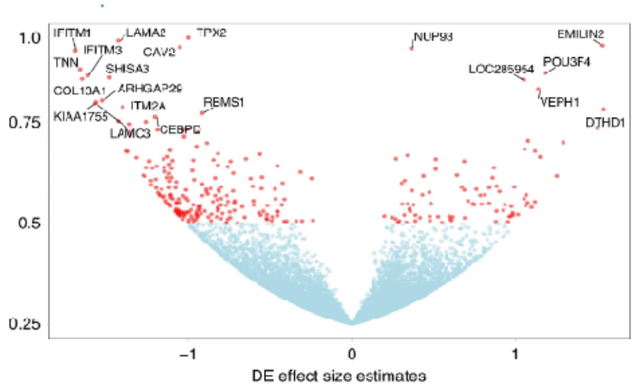
1.Sequence cleaning



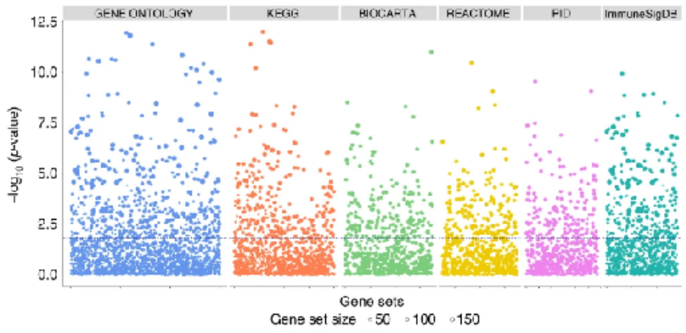

2. Mapping/quantification

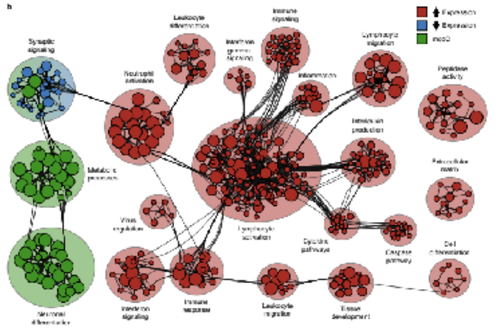



3. Differential gene expression




4. Functional insights

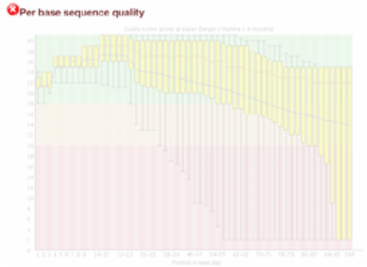


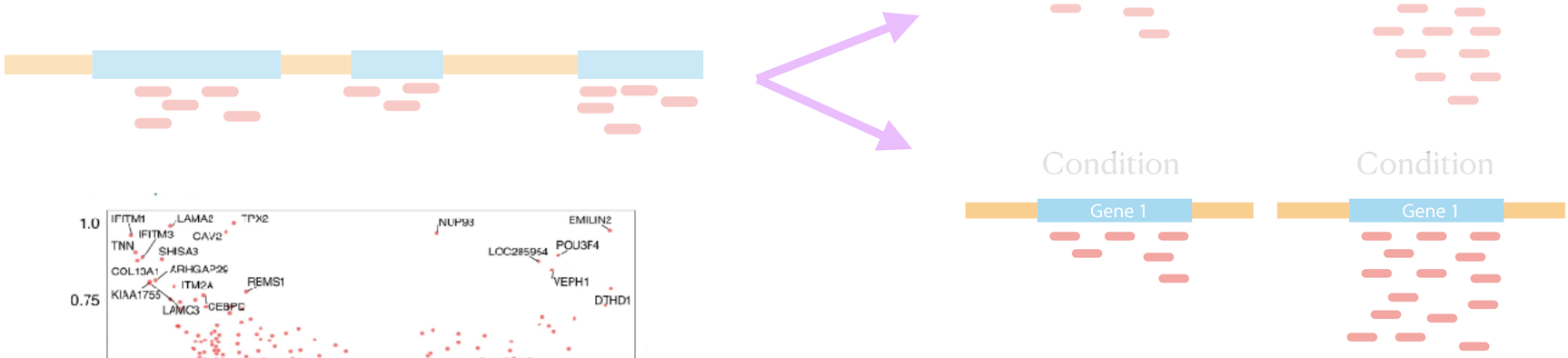
Marshall et al. 2020

# RNA-Sequencing (in silica)

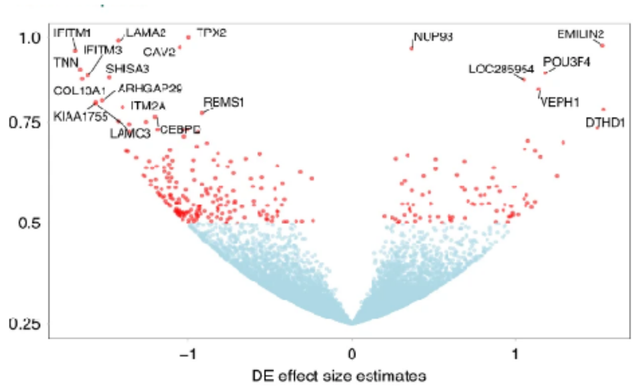
1.Sequence cleaning



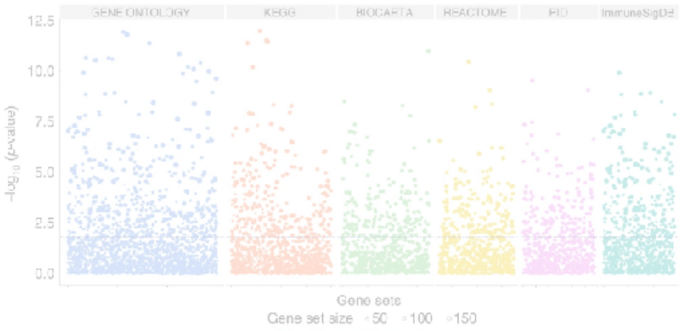

2. Mapping/quantification




3. Differential gene expression




4. Functional insights



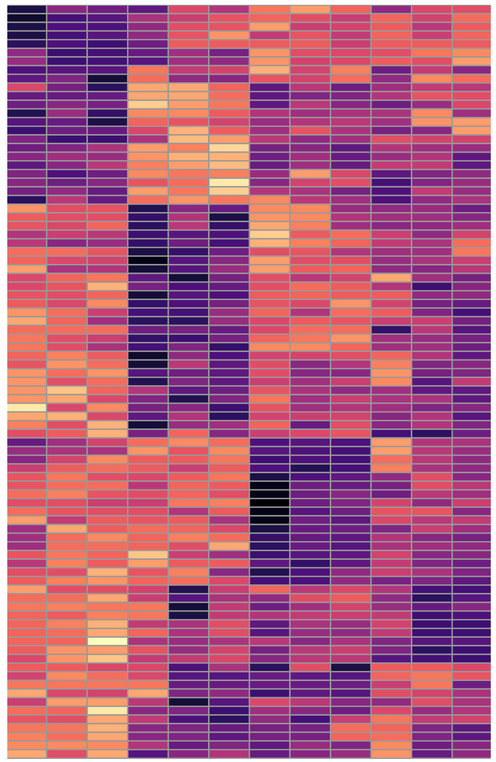



Marshall et al. 2020

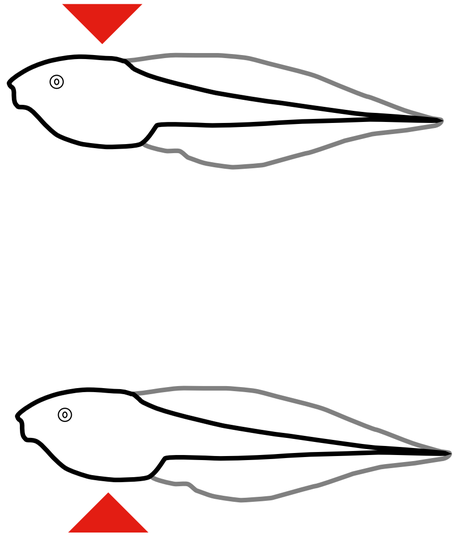
# RNA-seq counts matrix

| | 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

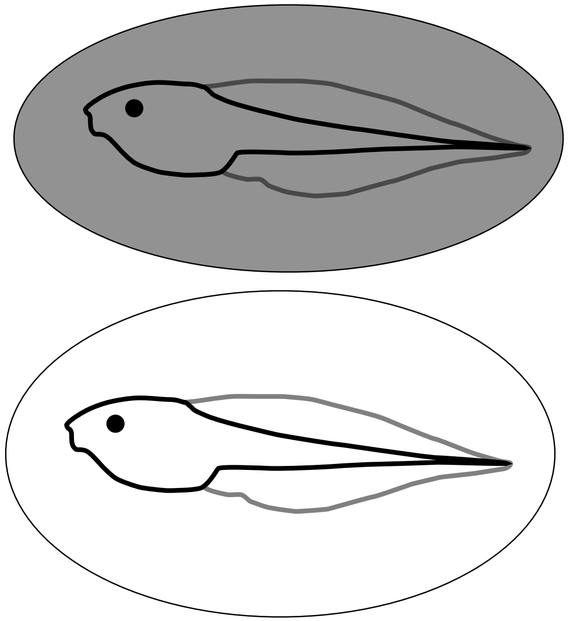


~



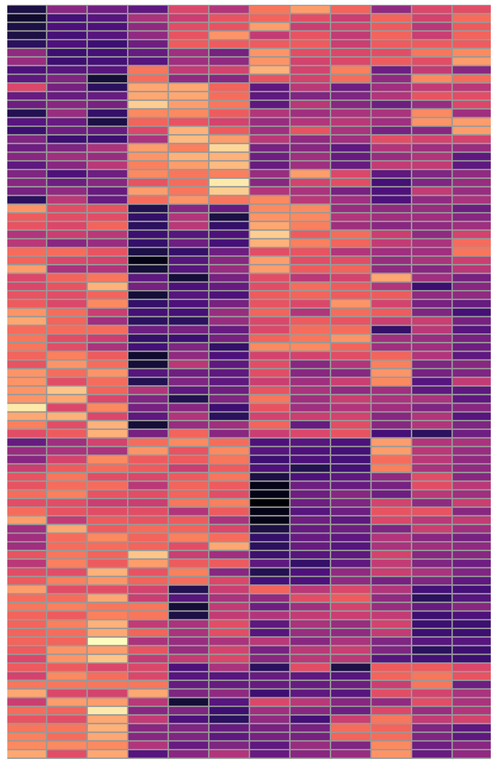
Anatomical effect

+

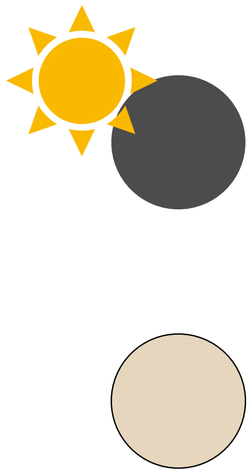


Background effect

# Differential Gene Expression

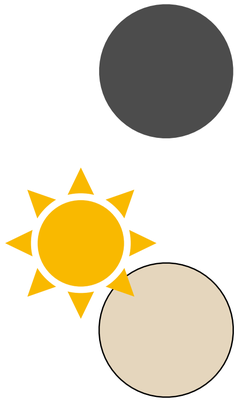


~



Fixed pigmentation

+



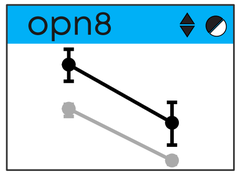
Plastic pigmentation

# RNA-seq practical

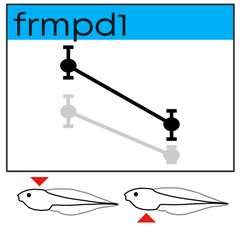
https://hcliedtke.github.io/PoE

# Differential Gene Expression

## Photosensitive signalling

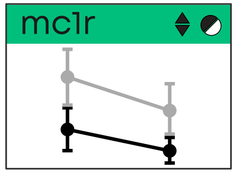


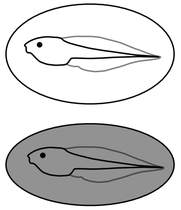

Opsin 8/ Neuropsin

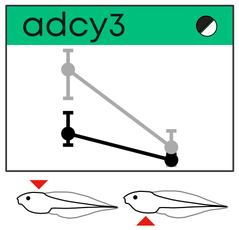


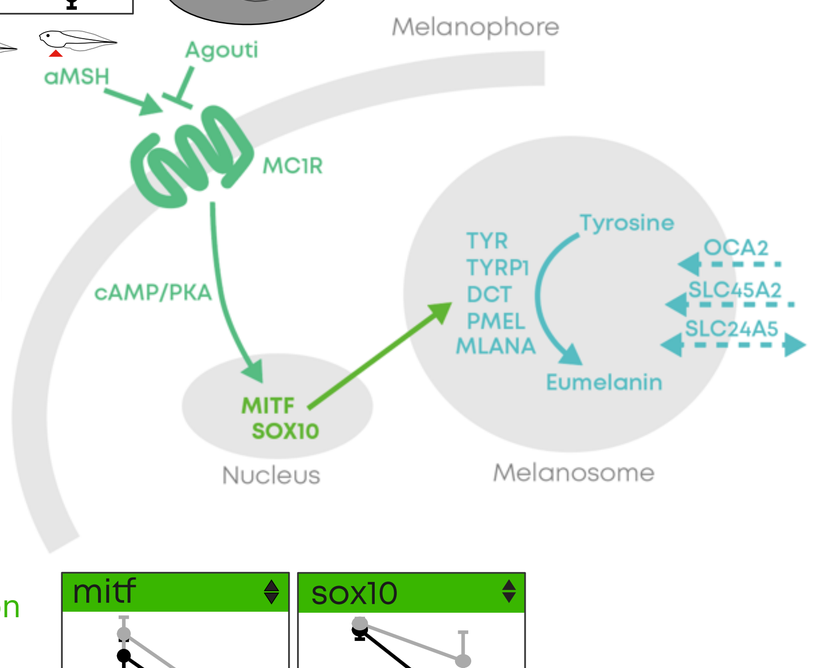

FERM And PDZ
Domain Containing 1

## Photosensitive signalling

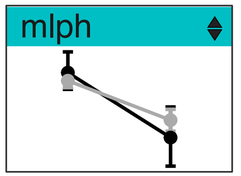


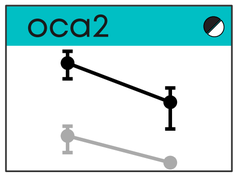



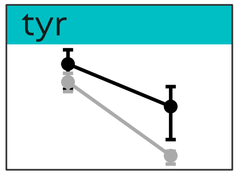


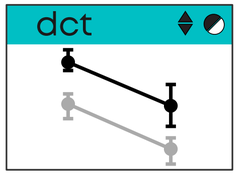



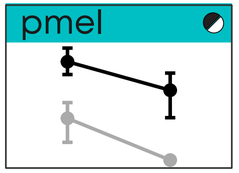

## Tyrosine metabolism

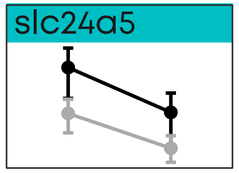


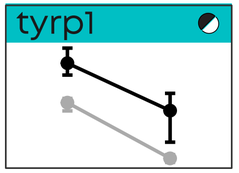



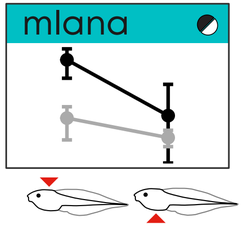



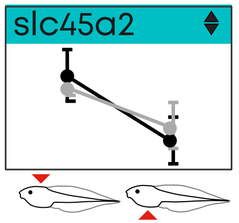



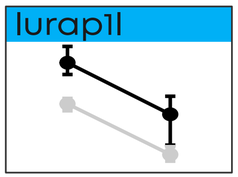



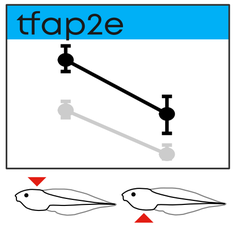




## Transcription regulators

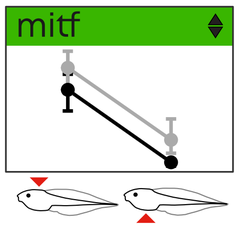


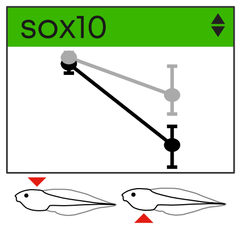

Leucine Rich Adaptor
Protein 1-Like



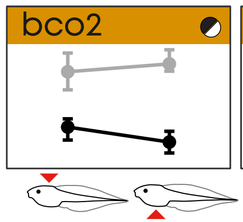

Transcription Factor
AP-2 Epsilon

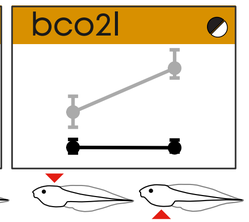
## Transcription regulators




## Carotenoid metabolism




Beta-Carotene
Oxygenase 2

# Summary

- Dorso-ventral gradients are controlled by highly conserved molecular mechanisms (αMSH-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) may play photoprotective roles.