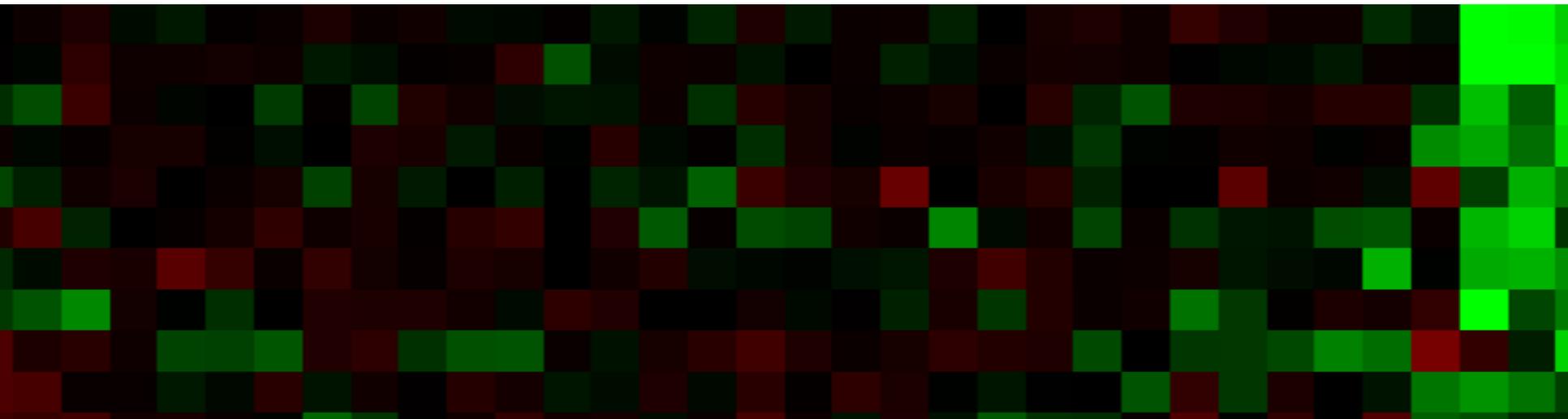
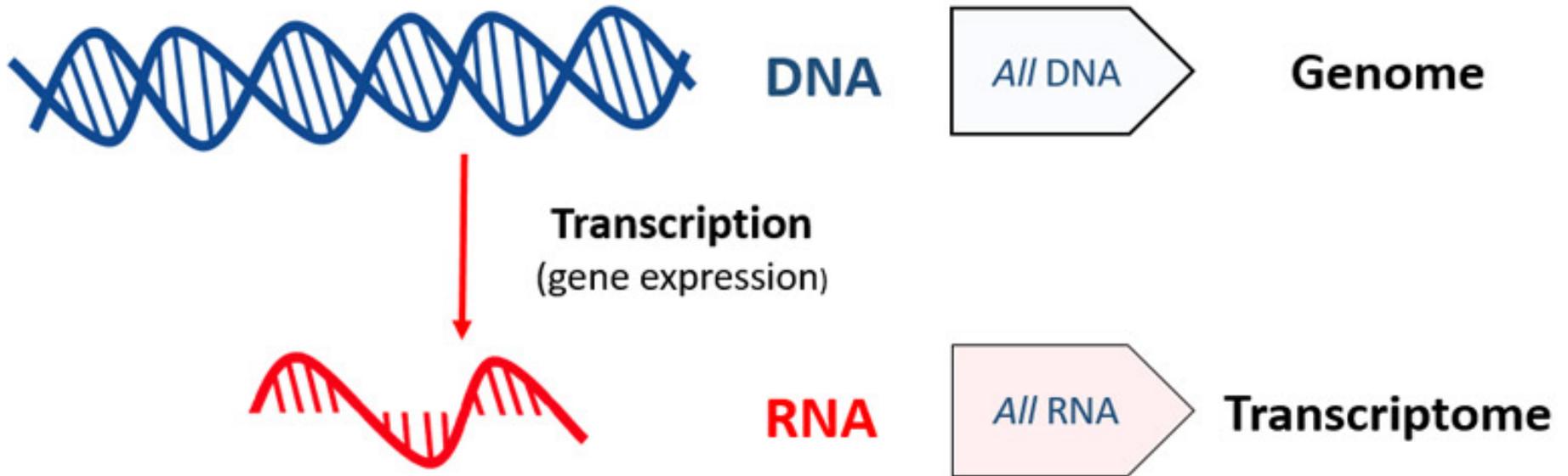


# Transcriptomics

Julie Fischer & Eddie Ruiz



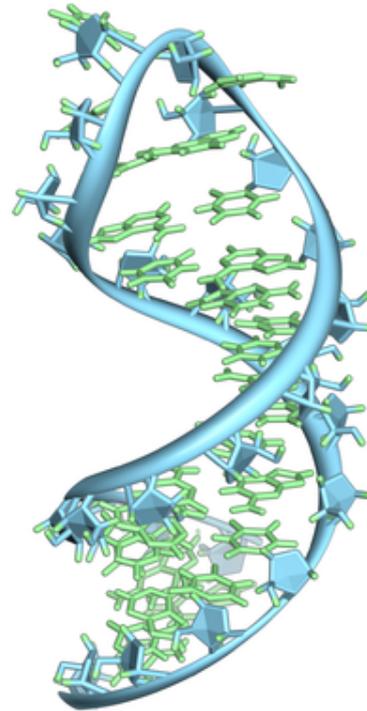
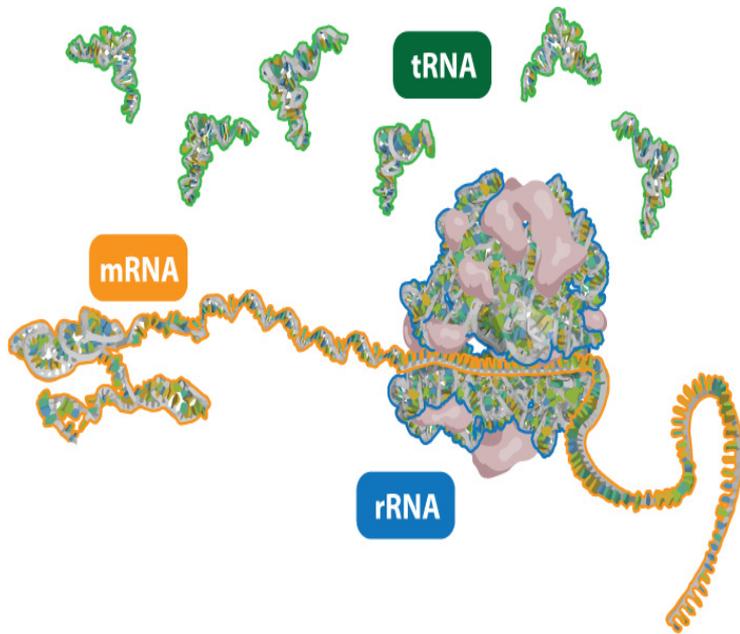
# What is the **transcriptome**?



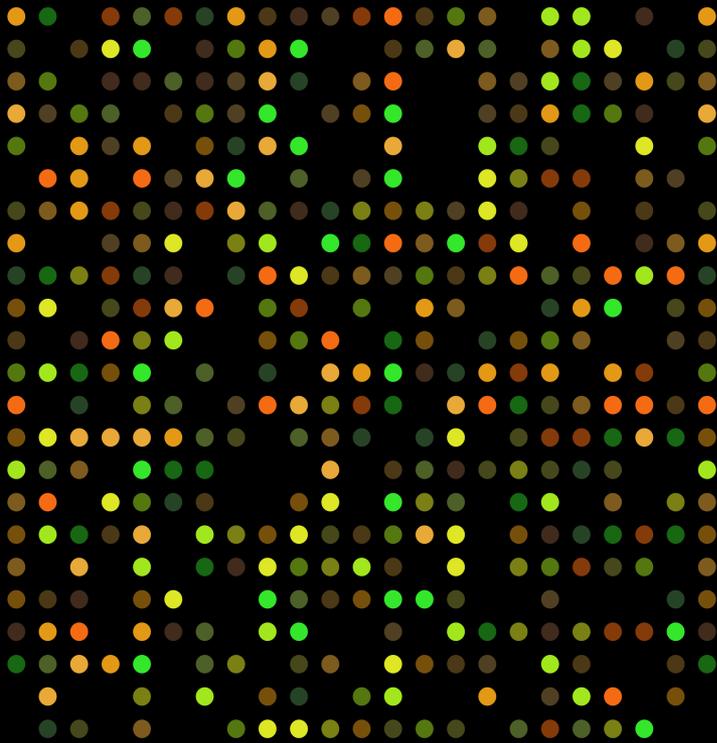
**“The complete set of transcripts in a cell, and their quantity, for a specific developmental stage or physiological condition.”**

-Wang et al. *Nature* 2009

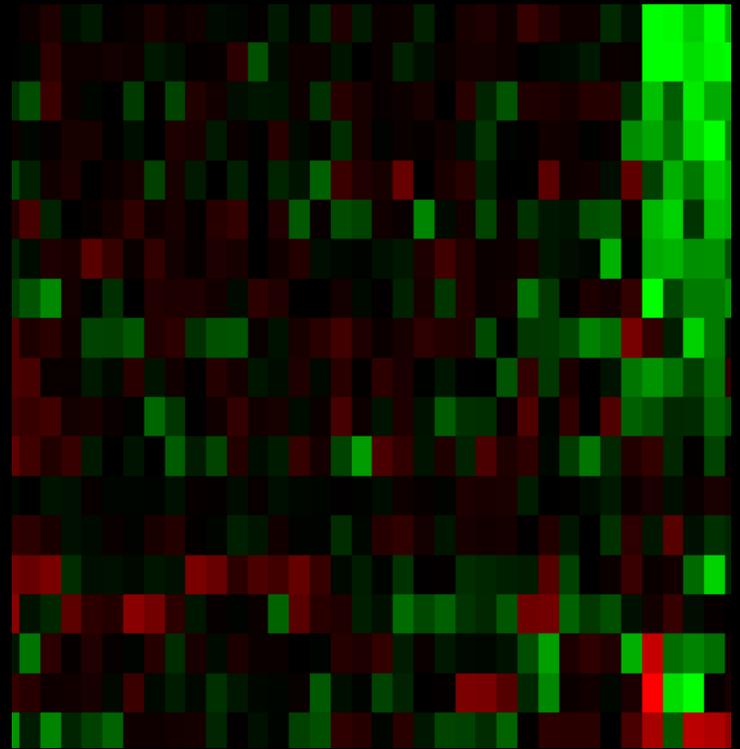
# What are the 3 key aims of transcriptomics?



# How can we study the transcriptome?

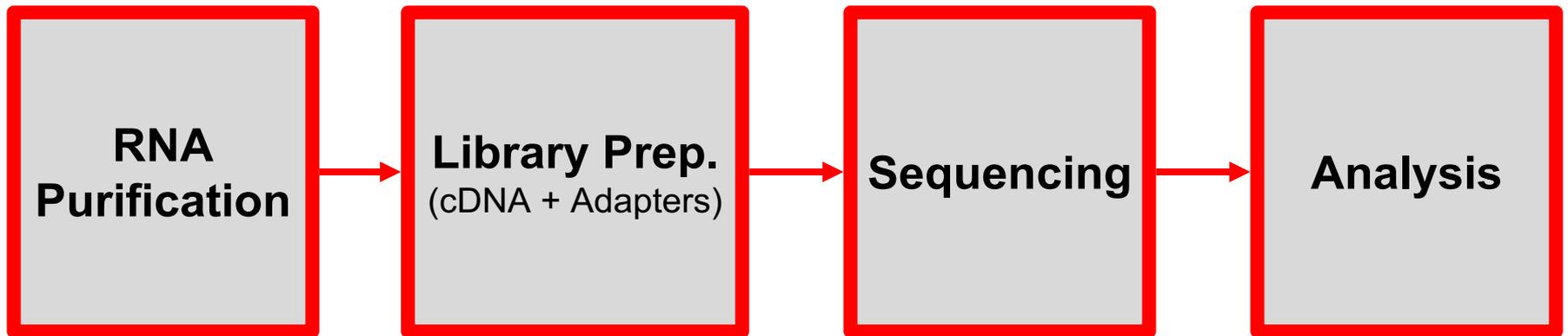


**Hybridization**

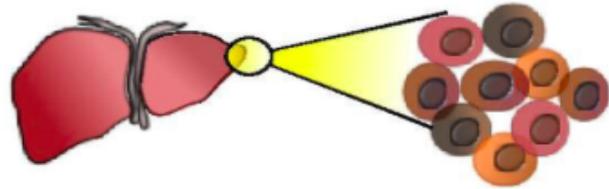


**Sequencing**

# How does RNA-seq work?



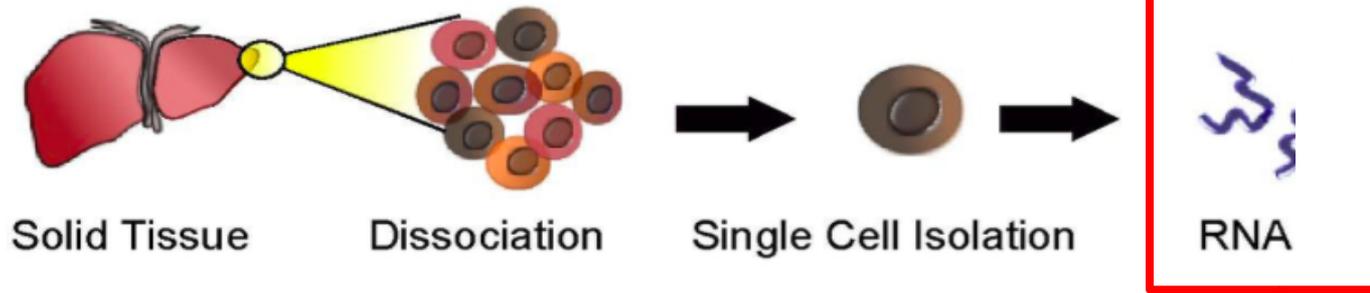
# How does RNA-seq work?



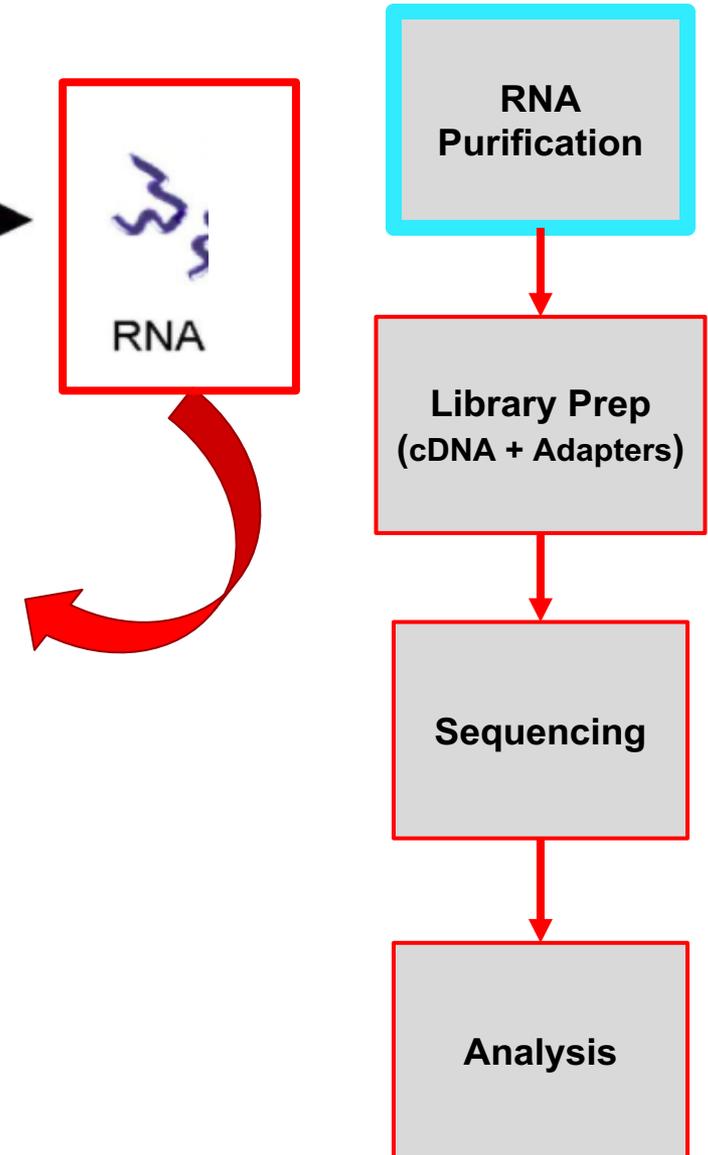
Solid Tissue

Dissociation

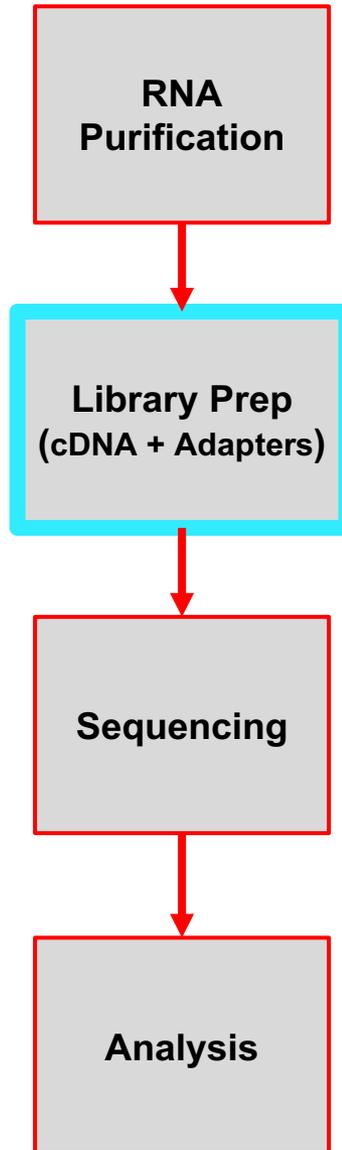
# RNA-Seq: RNA Purification



Large RNA	Small RNA
<b>mRNA</b>	<b>miRNA</b>
<b>lncRNA</b>	<b>siRNA</b>
<b>rRNA</b>	<b>piRNA</b>
	<b>tRNA</b>



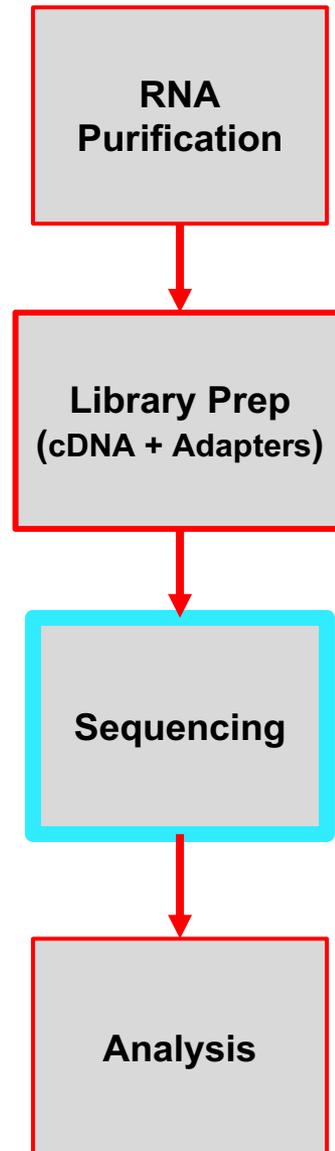
# RNA-Seq: Library Preparation



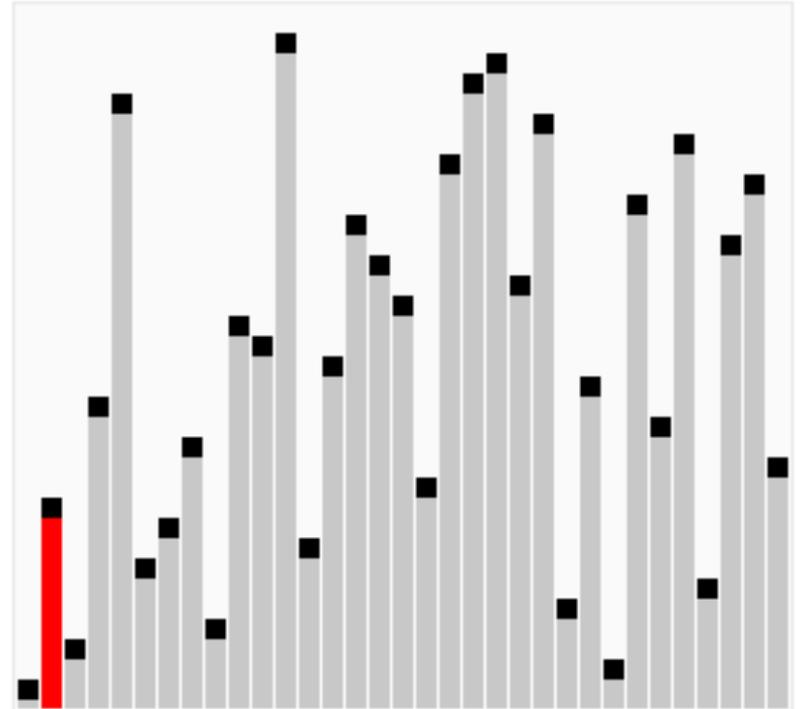
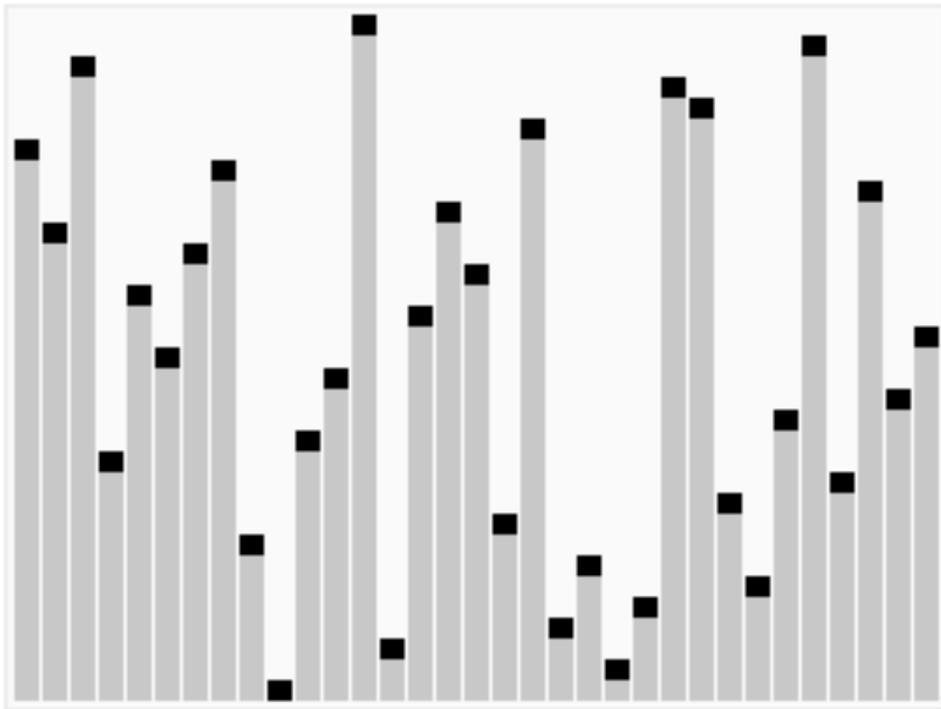
# RNA-Sequencing

```
ATCACAGTGGGACTCCATAAATTTTCT  
CGAAGGACCAGCAGAAACGAGAGAAAA  
GGACAGAGTCCCAGCGGGCTGAAGGGG  
ATGAAACATTAAAGTCAAACAATATGAA  
.....
```

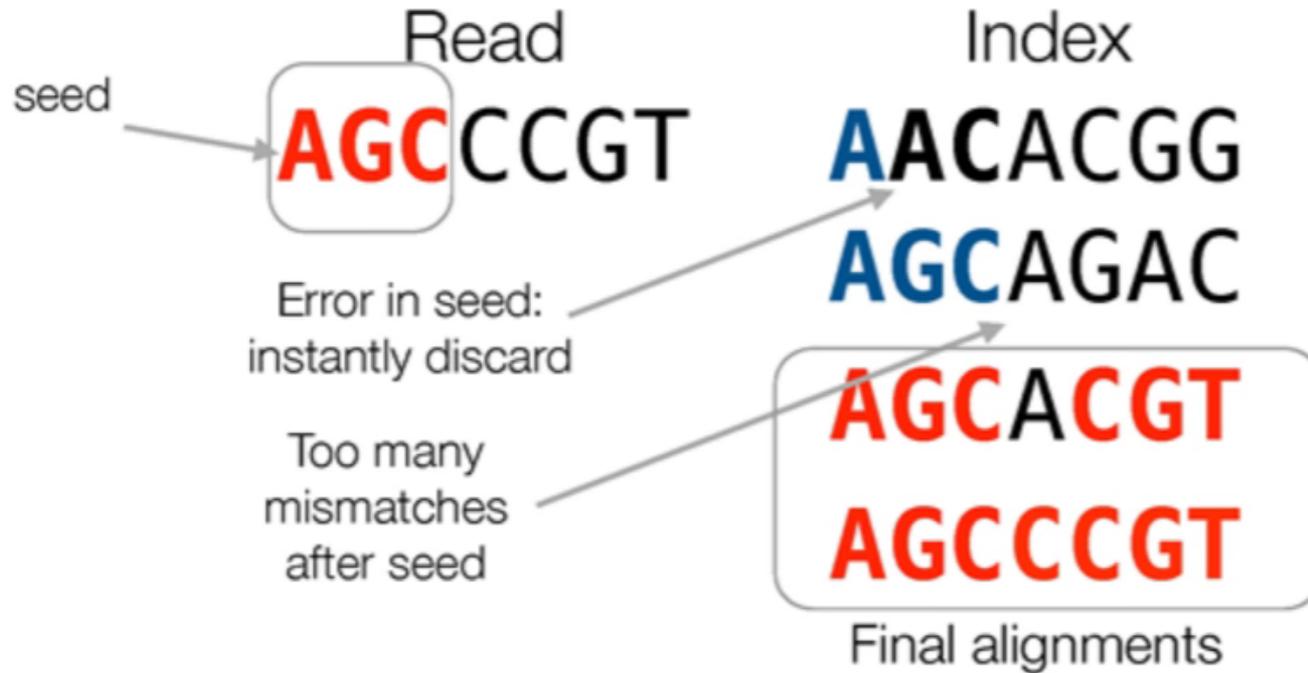
Short sequence reads



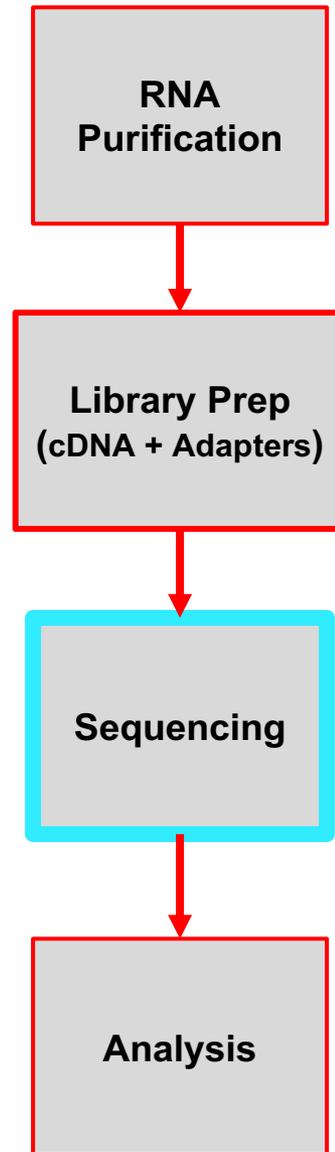
# RNA-Sequencing: Algorithmic alignment



# RNA-Sequencing: Transcriptome



## Bowtie algorithm



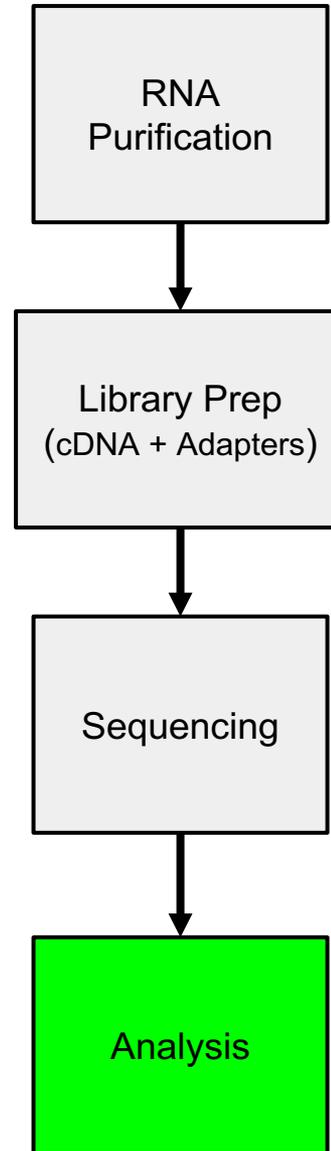
# RNA-Seq: Analysis of read per kilobase mm.

Genes

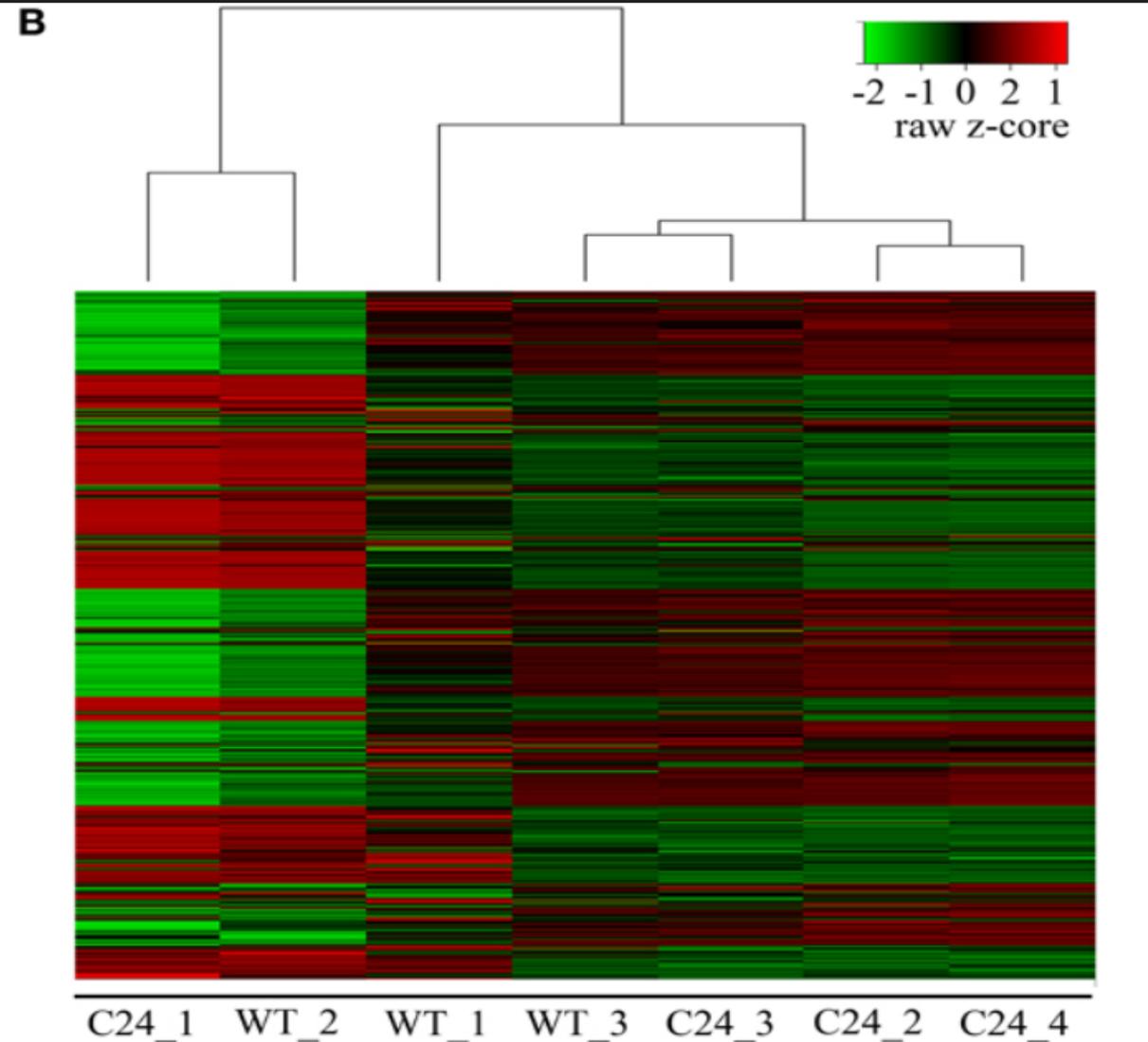
Gene Name	Rep1 Counts	Rep2 Counts	Rep3 Counts
A (2kb)	10	12	30
B (4kb)	20	25	60
C (1kb)	5	8	15
D (10kb)	0	0	1

Replicates (Samples)

Gene Name	Rep1 RPKM	Rep2 RPKM	Rep3 RPKM
A (2kb)	1.43	1.33	1.42
B (4kb)	1.43	1.39	1.42
C (1kb)	1.43	1.78	1.42
D (10kb)	0	0	0.009



# RNA-Seq: Analysis



RNA Purification

Library Prep  
(cDNA + Adapters)

Sequencing

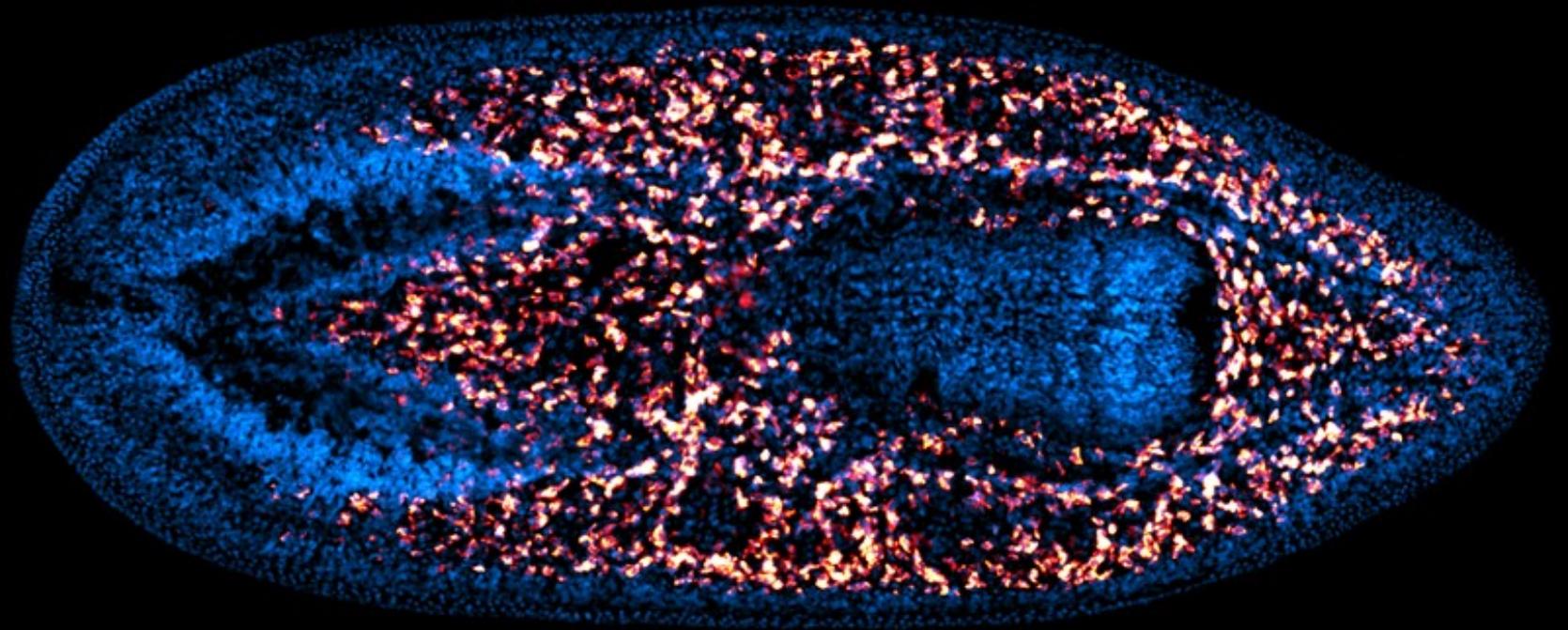
Analysis

# So why is **RNA-seq** preferred?

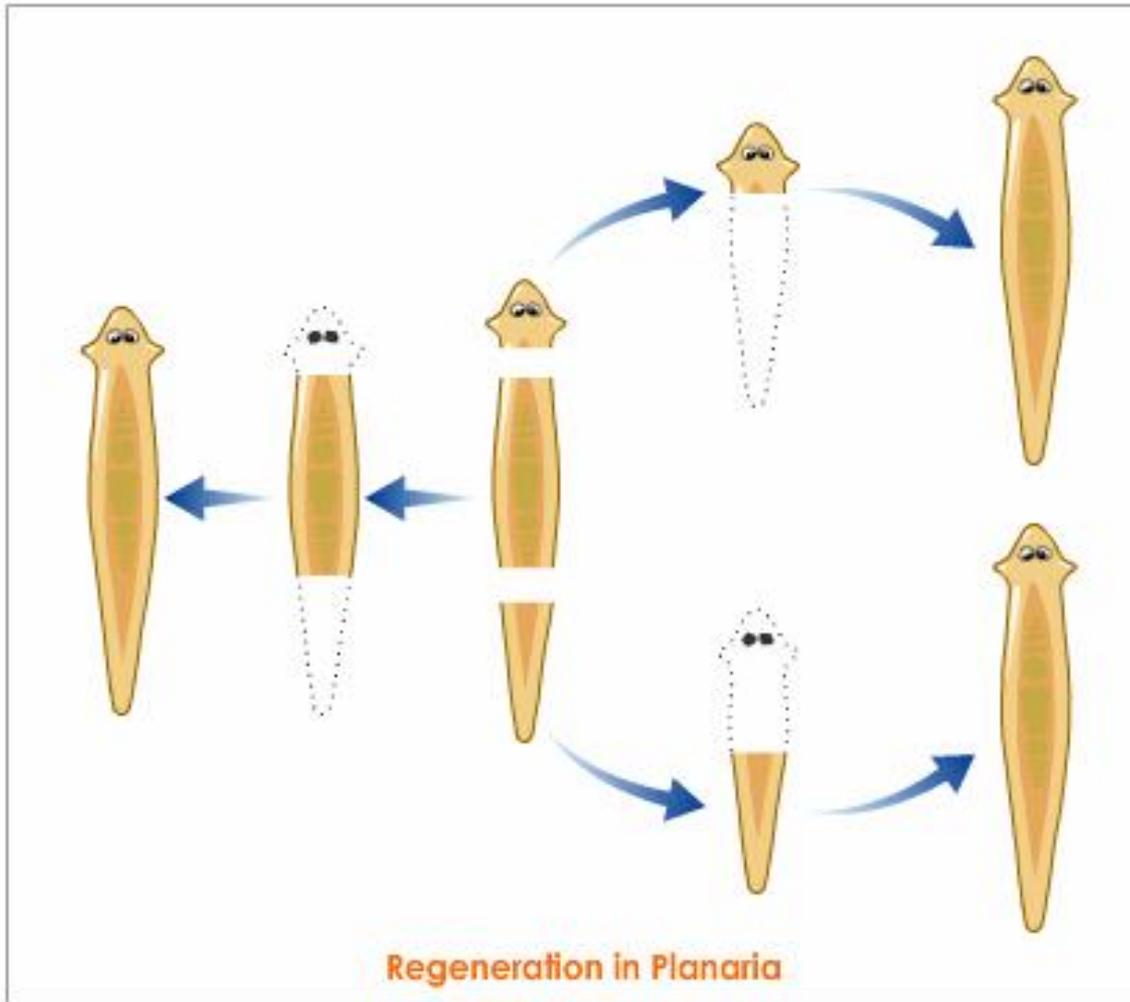
Technology	Tiling microarray	cDNA or EST sequencing	RNA-seq
<i>Technology specifications</i>			
Principle	Hybridization	Sanger sequencing	High-throughput sequencing
Resolution	From several to 100 bp	Single base	Single base
Throughput	High	Low	High
Reliance on genomic sequence	Yes	No	In some cases
Background noise	High	Low	Low
<i>Application</i>			
Simultaneously map transcribed regions and gene expression	Yes	Limited for gene expression	Yes
Dynamic range to quantify gene expression level	Up to a few-hundredfold	Not practical	>8,000-fold
Ability to distinguish different isoforms	Limited	Yes	Yes
Ability to distinguish allelic expression	Limited	Yes	Yes
<i>Practical issues</i>			
Required amount of RNA	High	High	Low
cost for mapping transcriptomes of large genomes	High	High	Relatively low

**How can you use RNA-seq in your project?**

# What are Planaria?



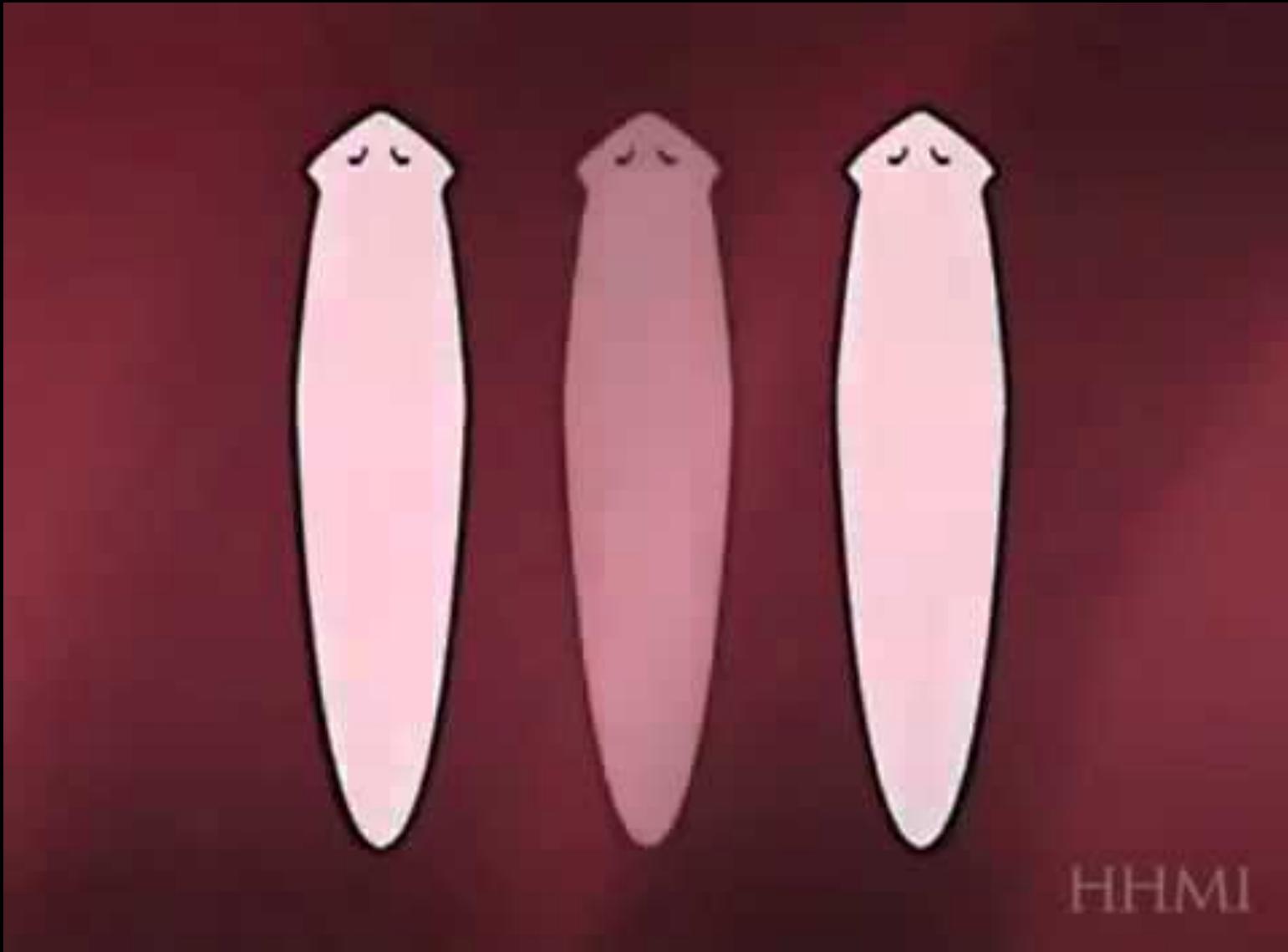
# Why use Planaria as a model system?



*Schmidtea  
Mediterranea*

- Regeneration
- Ease of culture
- High turnover
- Diploid (*Schmid.*)
- Plasticity

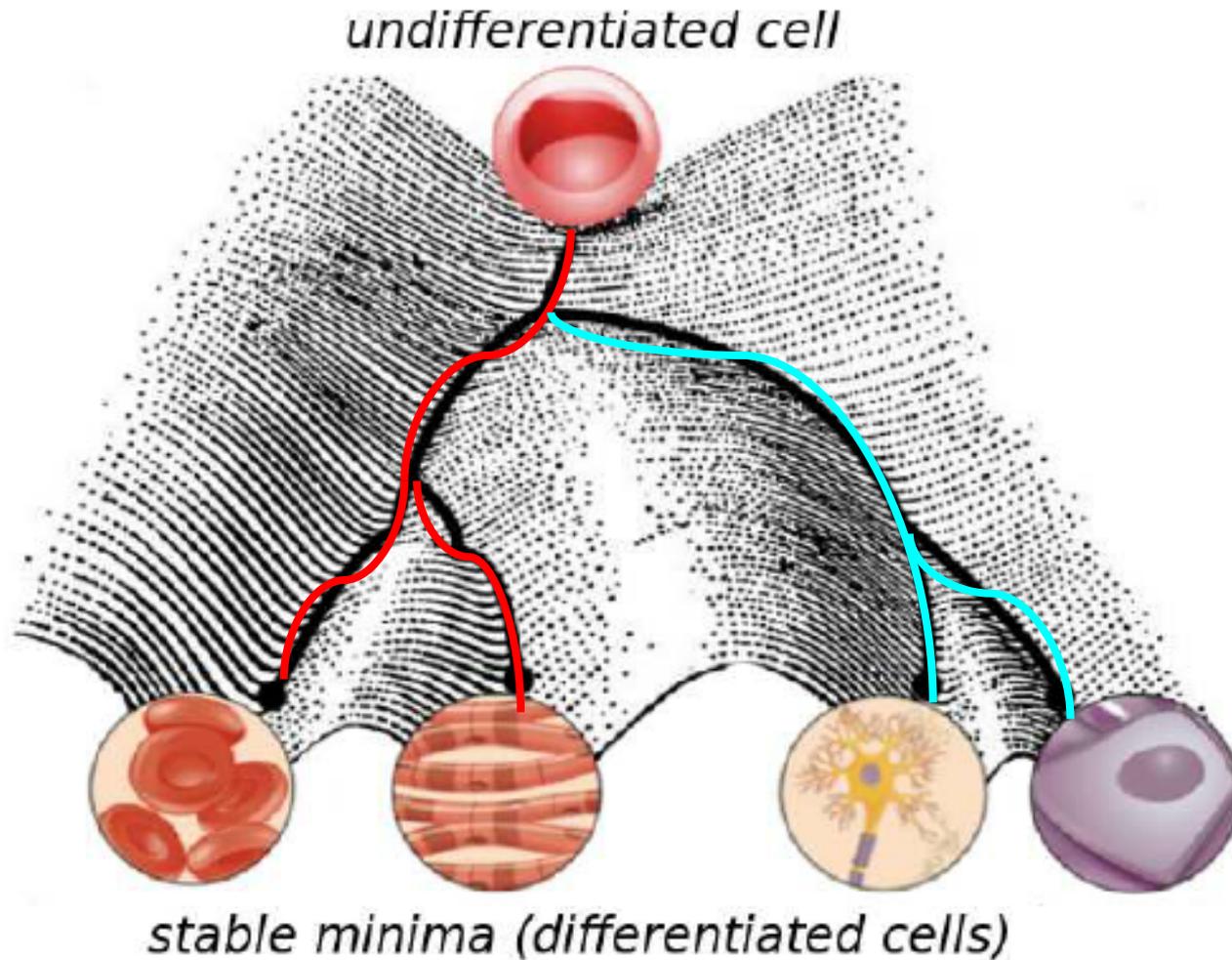
# How do Planaria relate to regeneration?



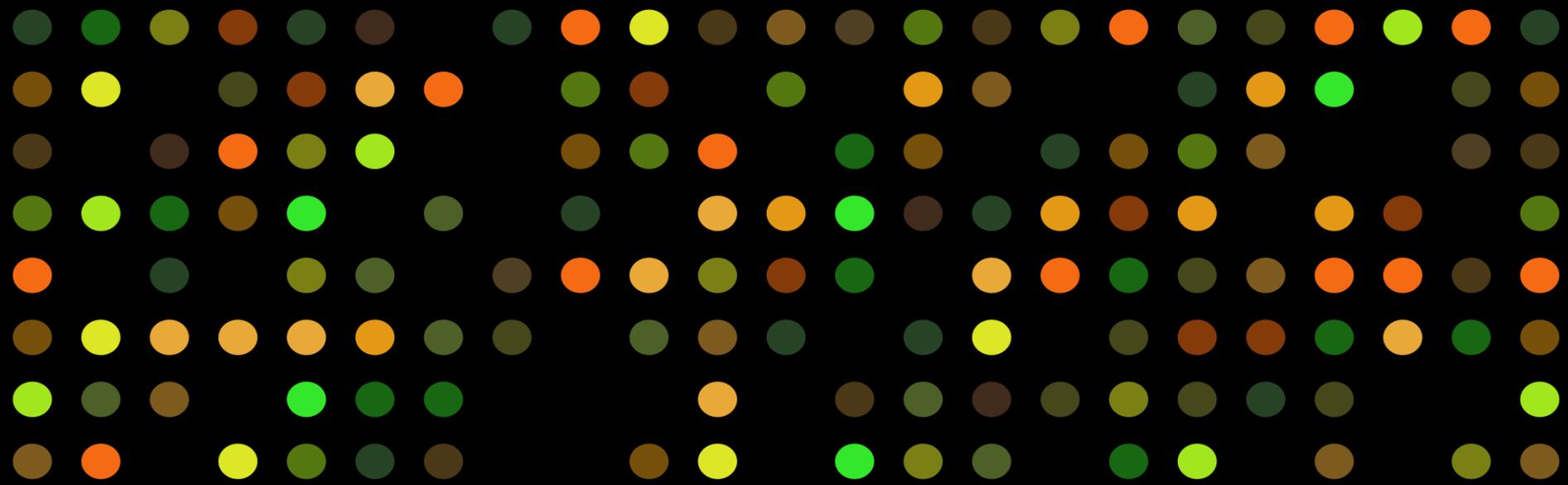
... “incredible extremes”



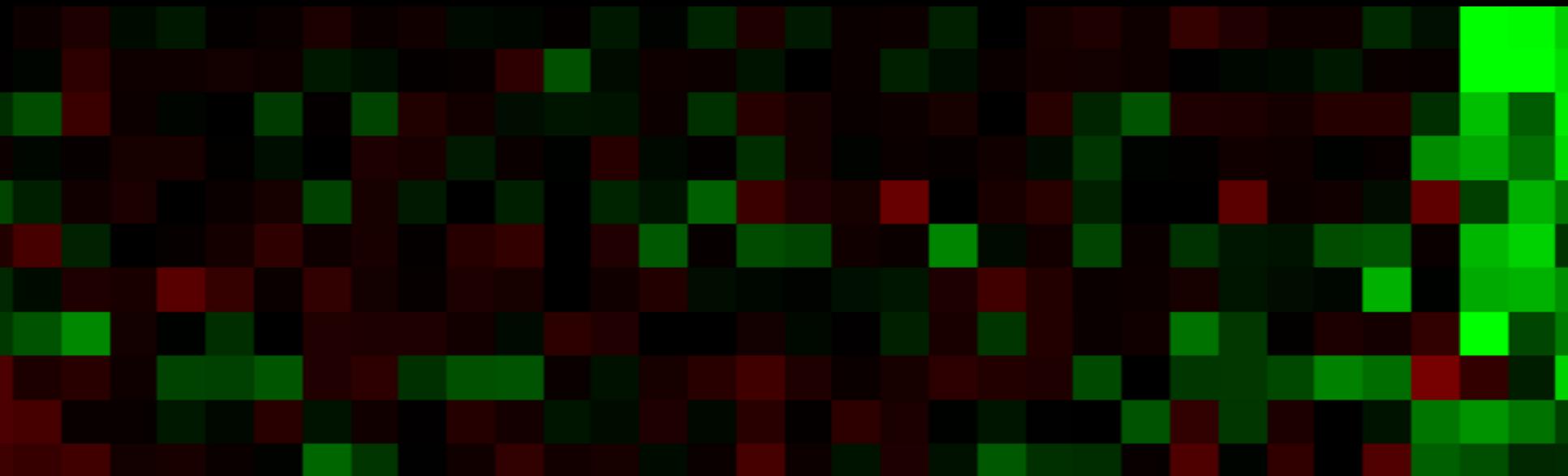
# What are stem cells?

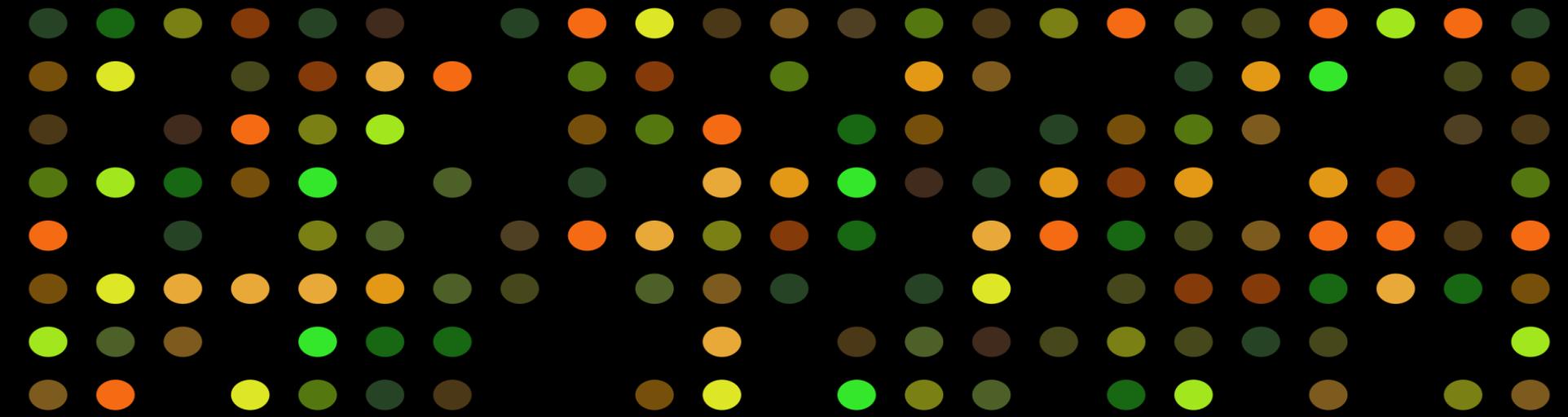


**Waddington's Epigenetic Landscape**

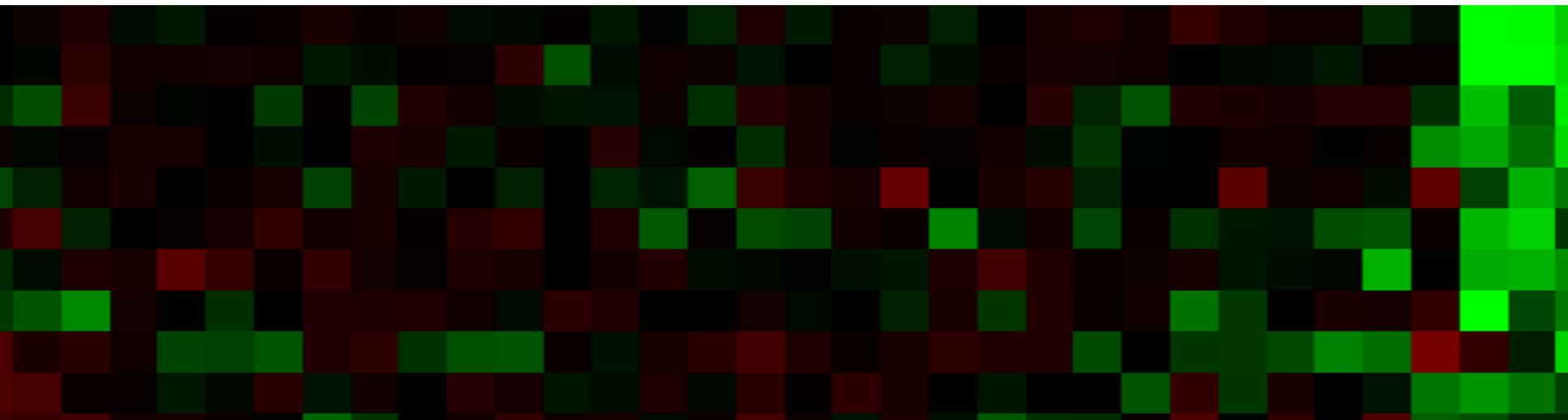


Questions?

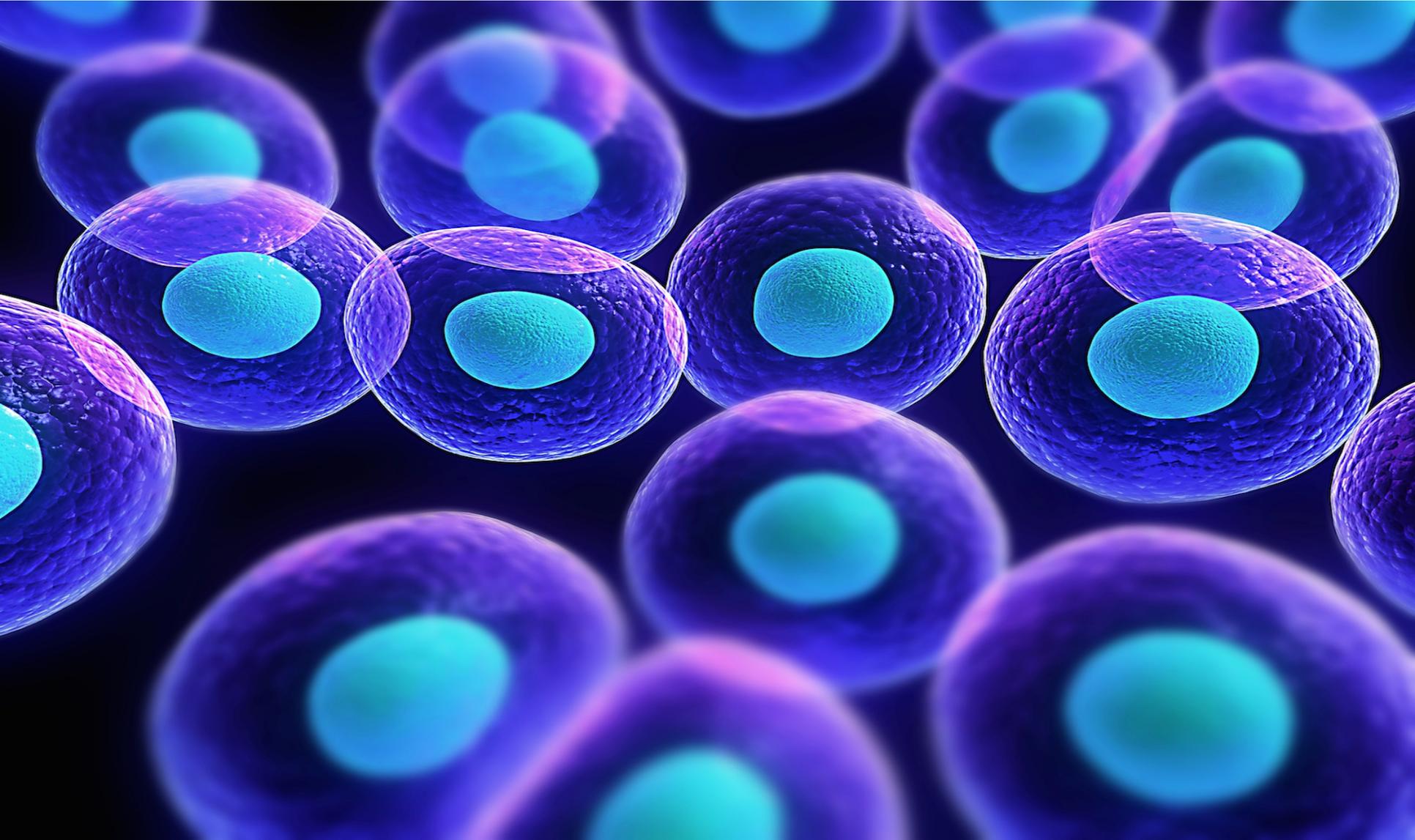




**A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals.**  
-- Labbe et al. *Stem Cells*. 2012



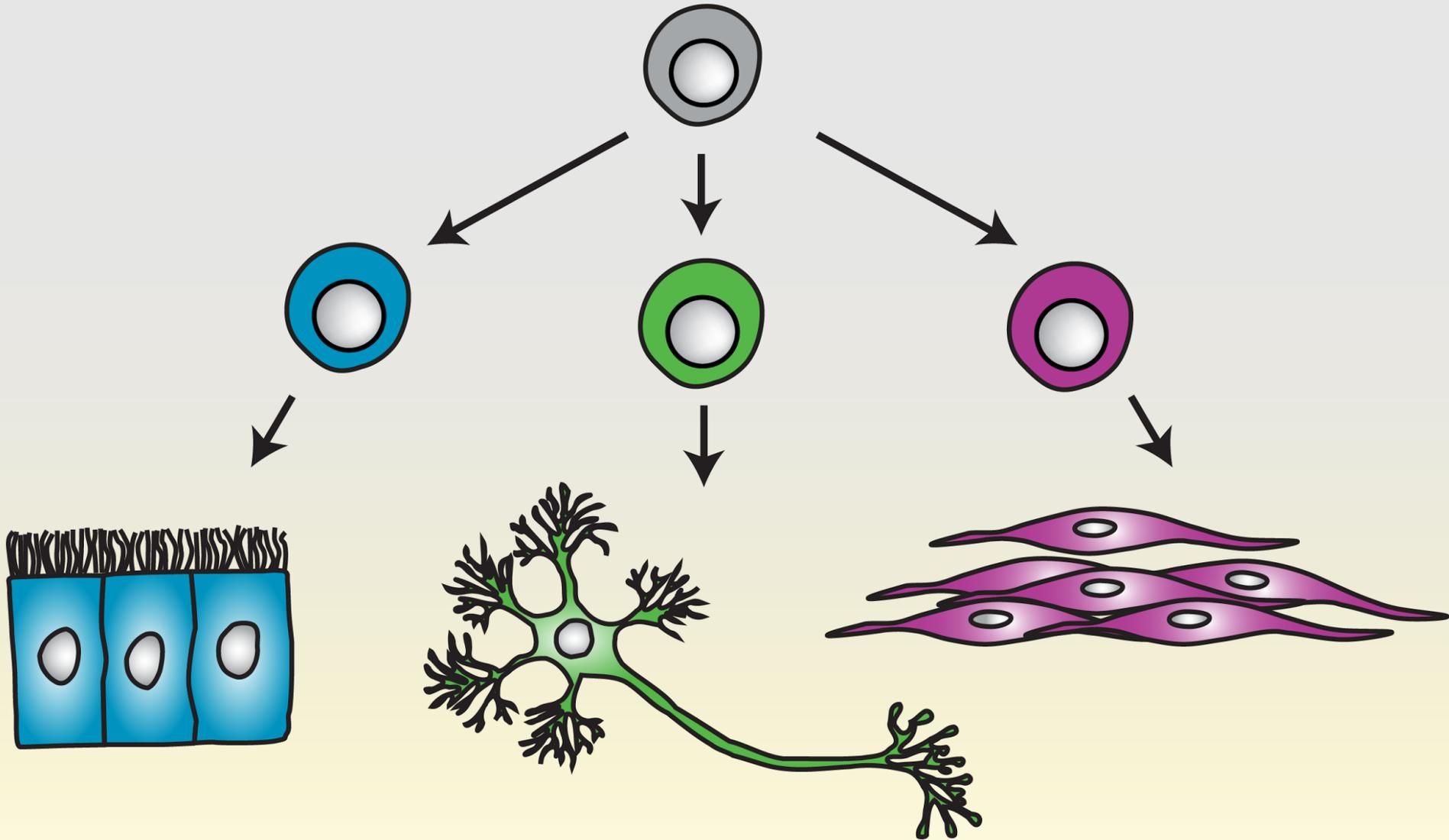
**What was the purpose of the study?**



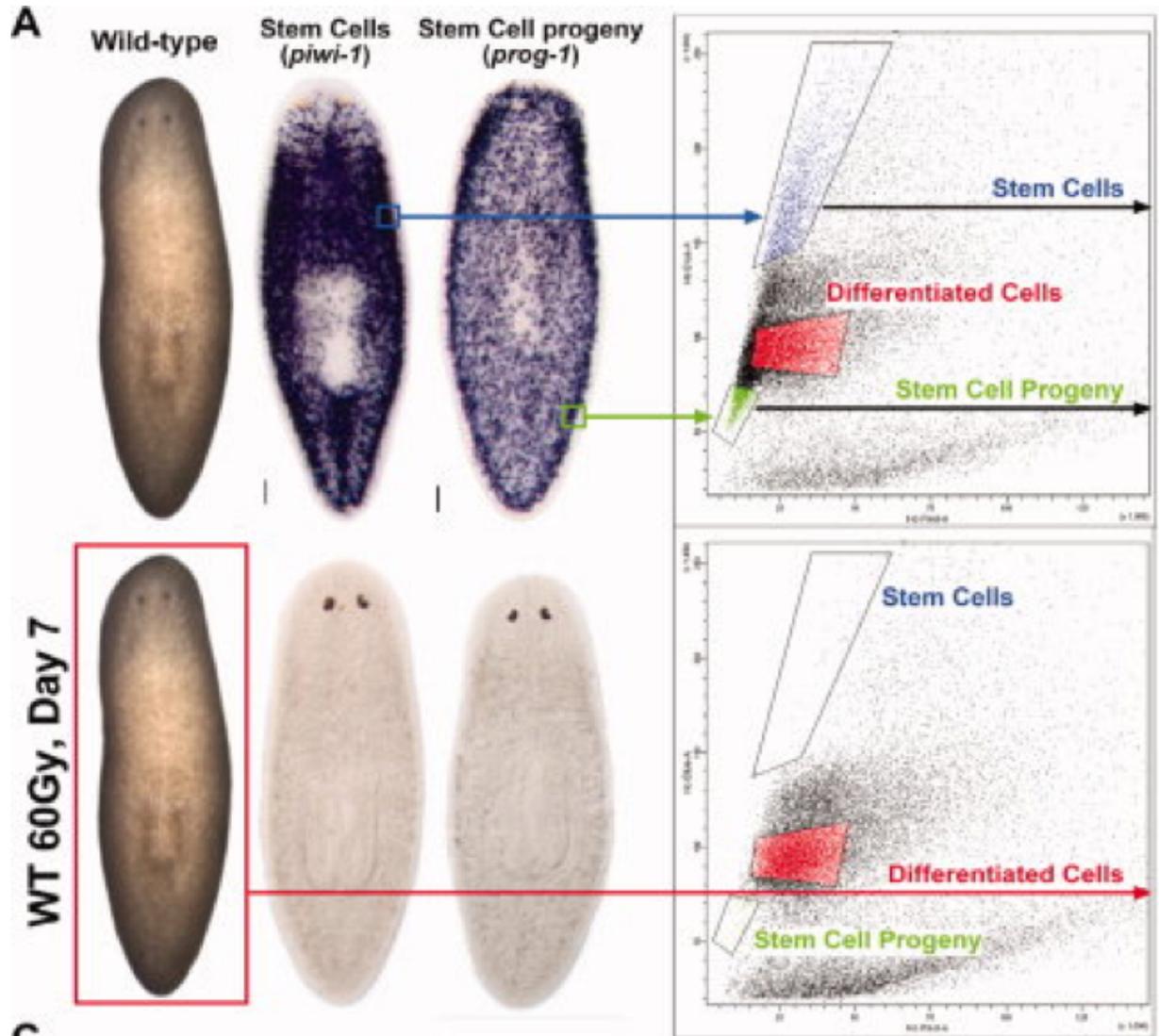
**What organisms were compared?**



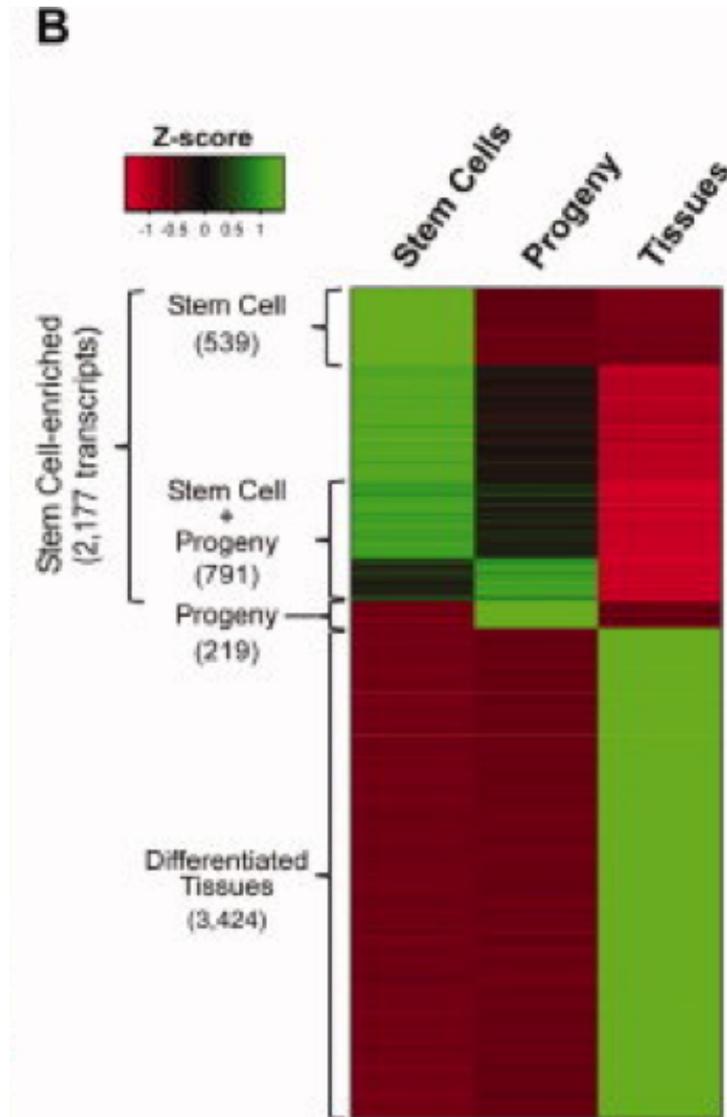
# What cell types were investigated?



# Figure 1a: How were **cells** sorted?



# Figure 1b: What genes were enriched?



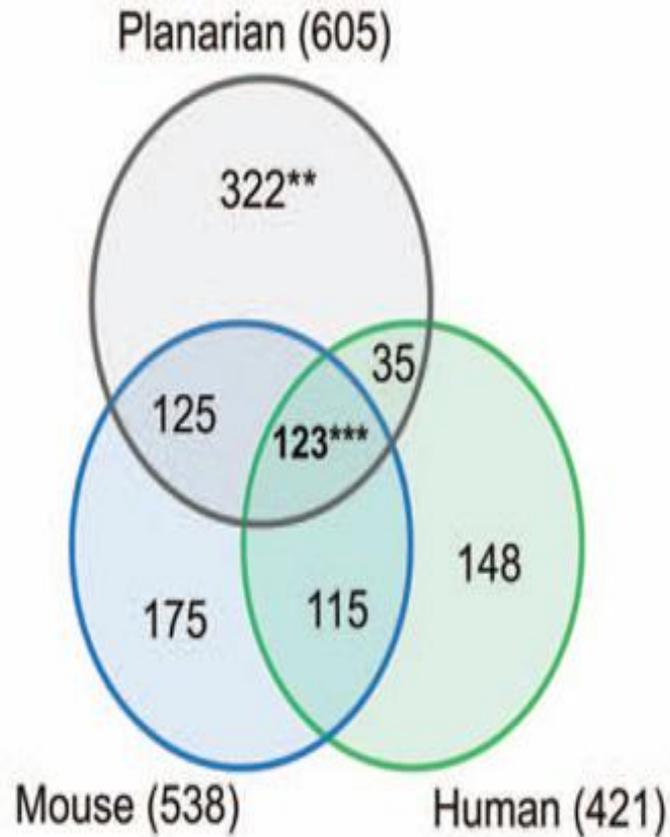
# How were 1:1:1 orthologs determined?

**blastx**

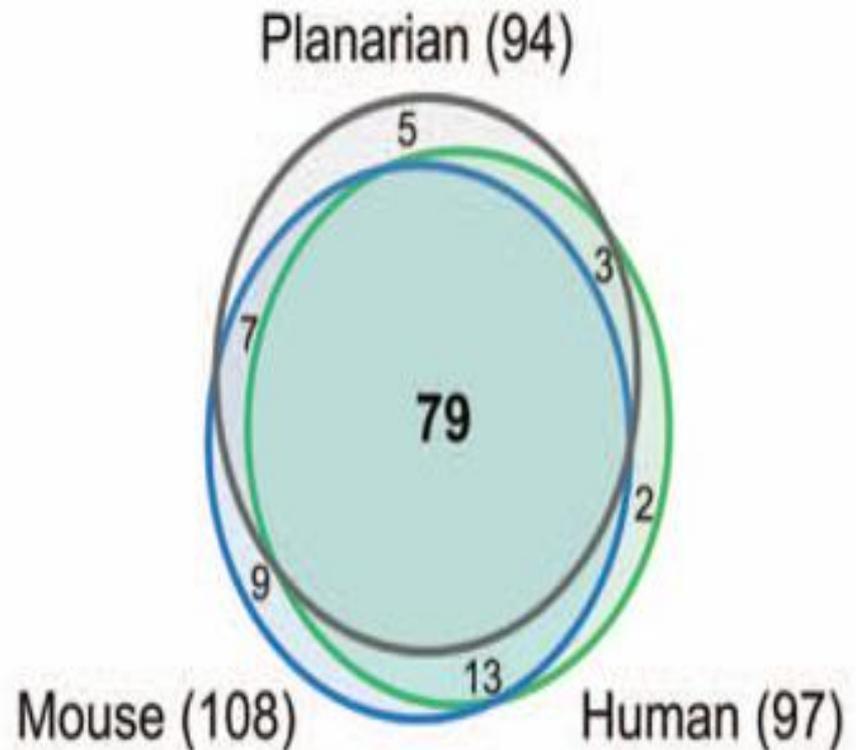
translated nucleotide ► protein

# Figure 3: What were the BLASTx results?

1:1:1 orthologs (4,432)  
≥ 5-fold DE in all stem cells

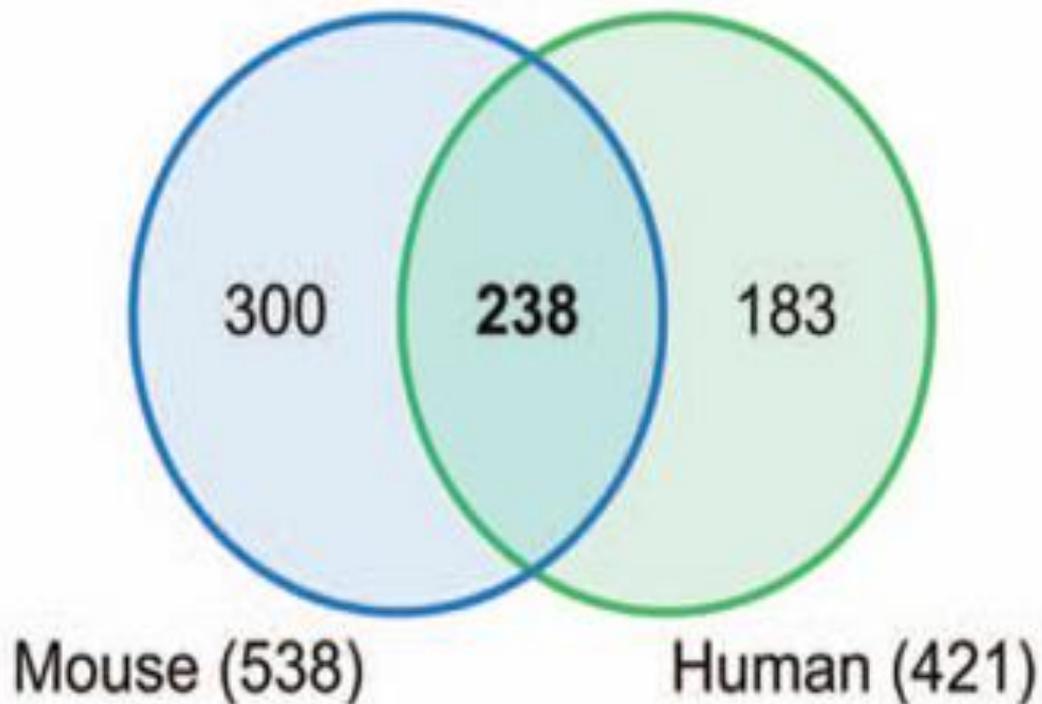


PluriNet genes (127 total out of 4,432 orthologs) ≥ 5-fold DE in stem cells



# Fig 3c: How similar are **mouse** and **human** stem cells?

**1:1:1 orthologs (4,432)**  
**≥ 5-fold DE in ESCs**



# Fig 3b: How do planarian stem cells **compare** to **mammalian** stem cells?

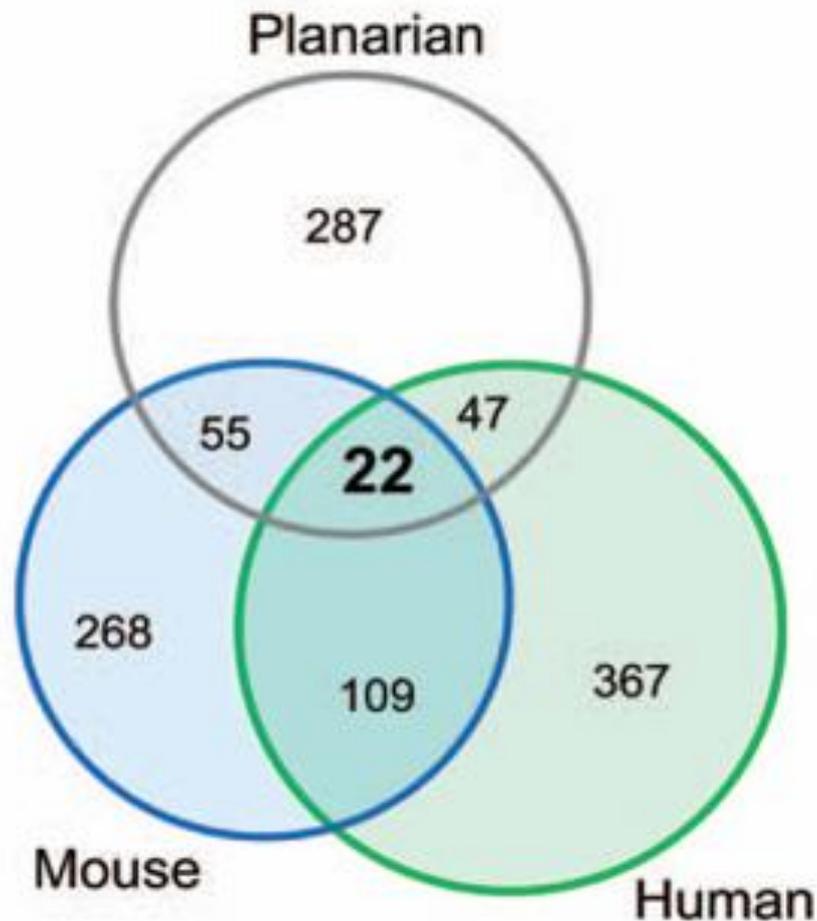
Total planarian genes  
≥ 5-fold DE in stem cells (2,177)



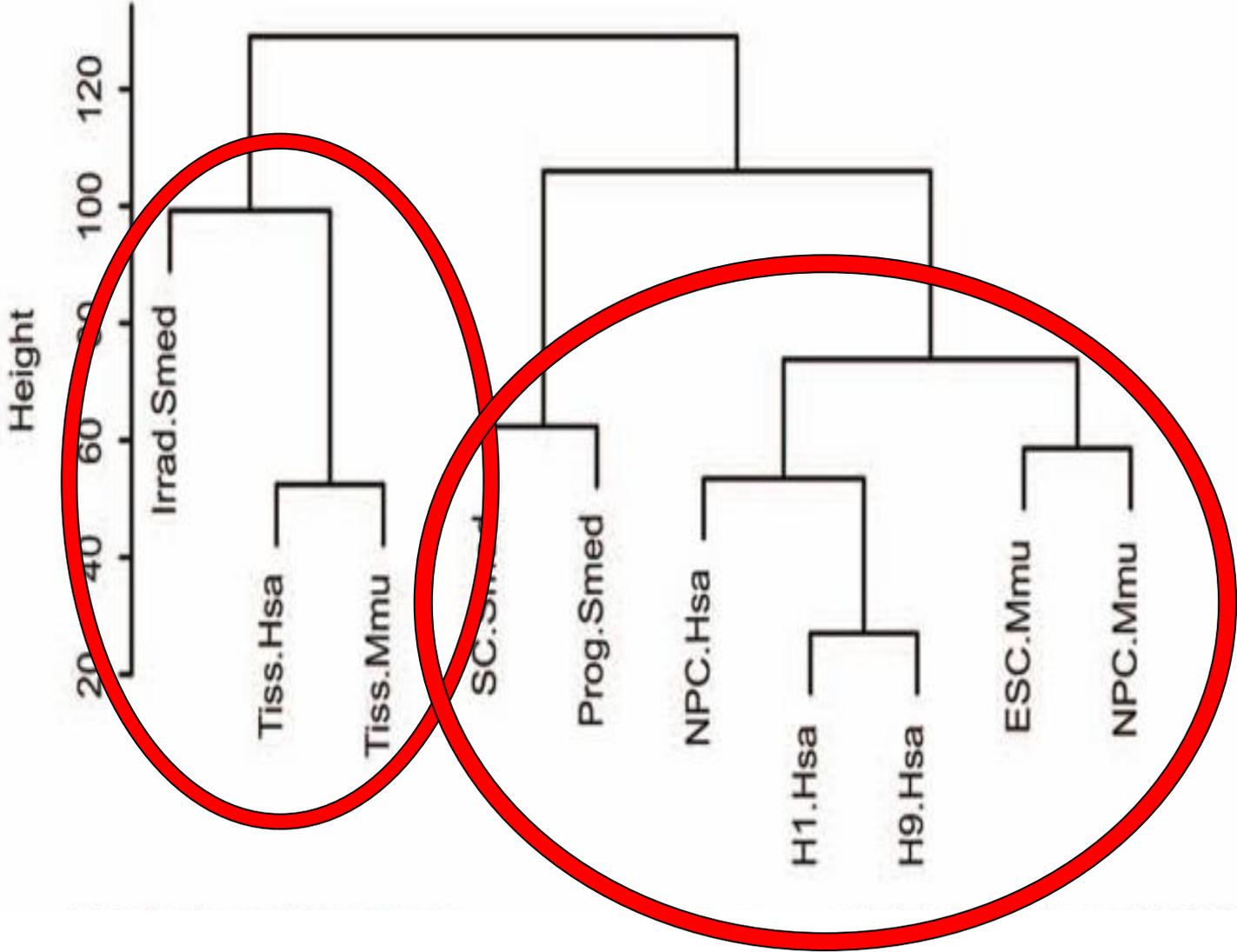
1:1:1 orthologs (4,432)

**Fig 3d: How do stem cell progeny compare between species?**

**1:1:1 orthologs (4,432)  
≥ 1.5-fold DE in NPCs and progeny**



**Fig 3a: What does differential expression tell us?**



**Figure 2a: What was the difference between **stem cells** and stem cell progeny?**

**A: Stem cells vs. stem cell progeny**

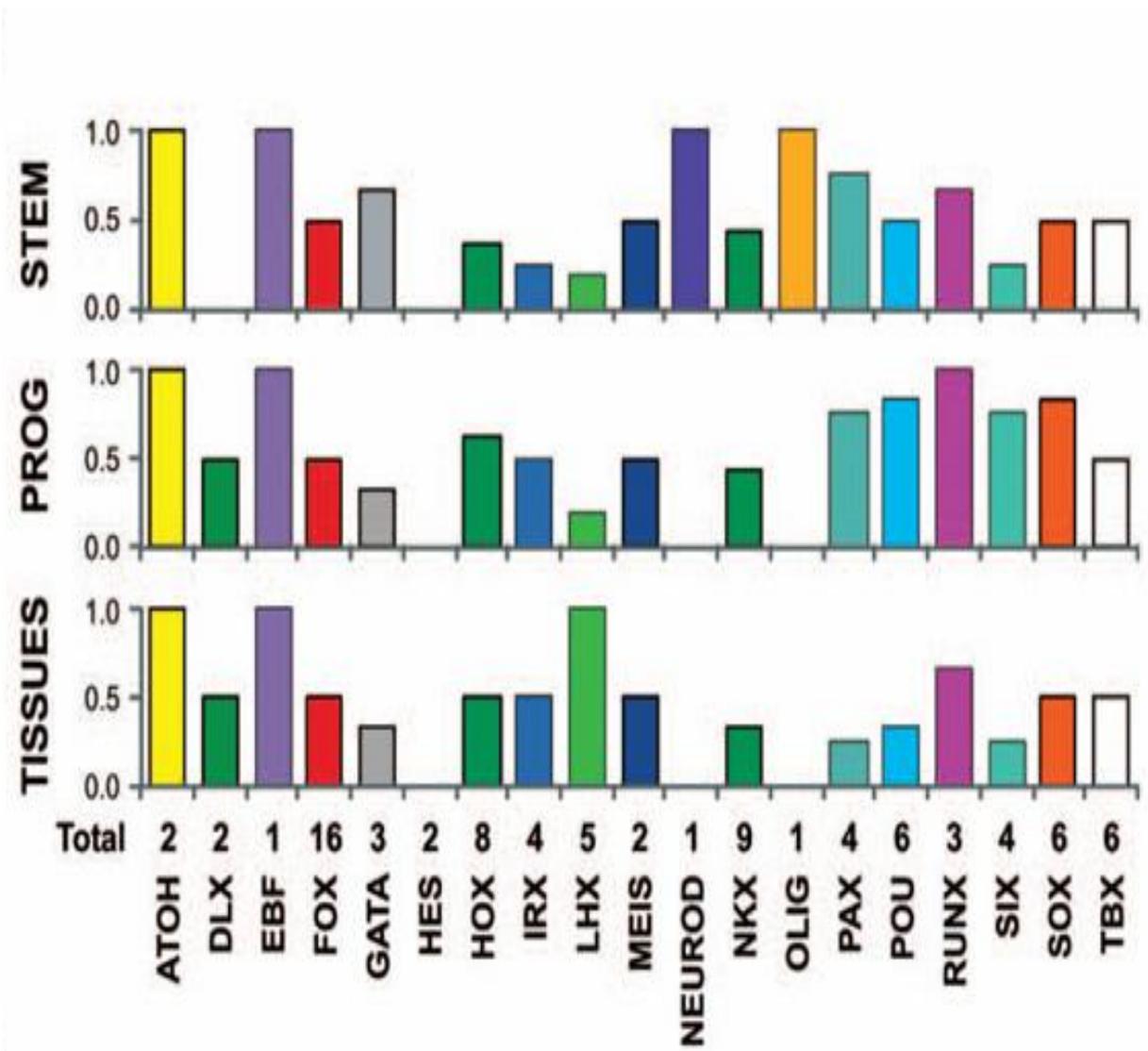
**Figure 2b: What was the difference between **stem cells** and **differentiated tissues**?**

**B: Stem cells vs. differentiated tissues**

**Figure 2c: What was the difference between stem cell progeny and **differentiated tissues**?**

**C: Stem cell progeny vs. differentiated tissues**

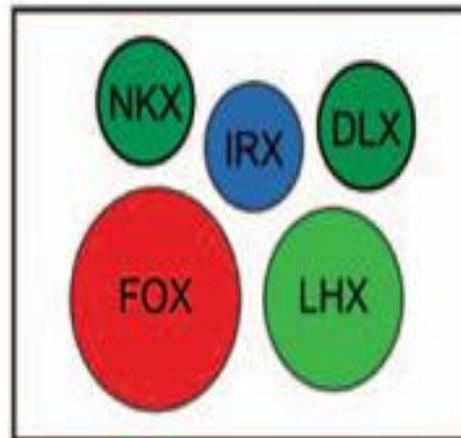
# What was enriched in planarian stem cell progeny?



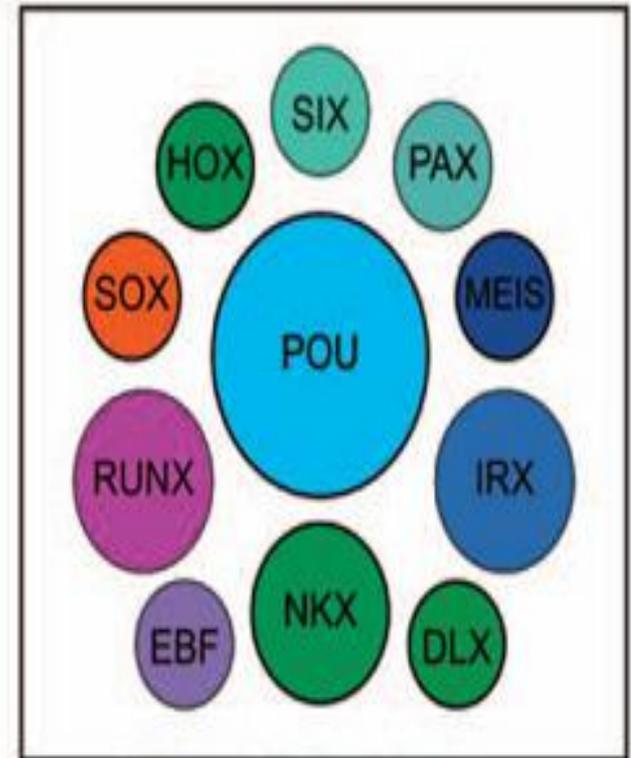
# What was enriched in planarian stem cell progeny?



**Stem Cells**



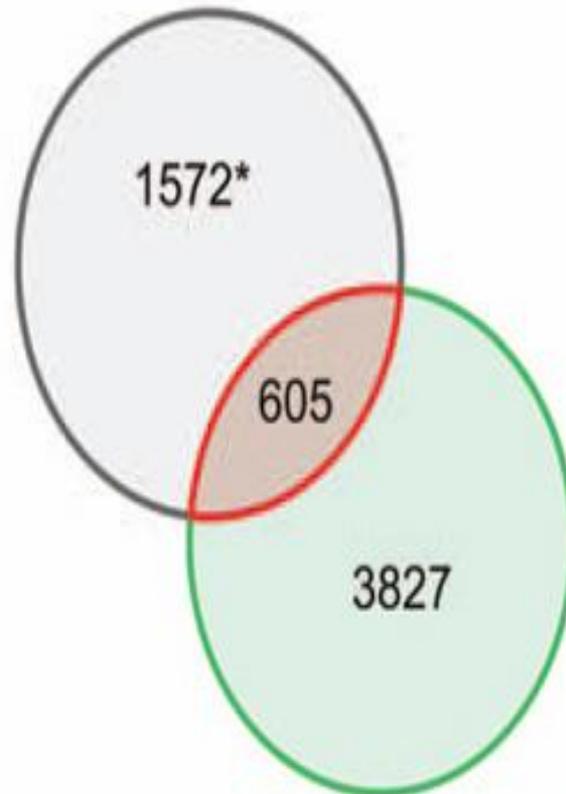
**Differentiated Tissues**



**Stem Cell Progeny**

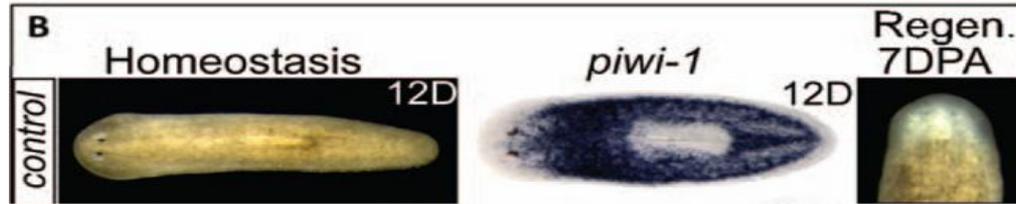
# What genes were enriched in only planarian stem cells?

Total planarian genes  
 $\geq$  5-fold DE in stem cells (2,177)



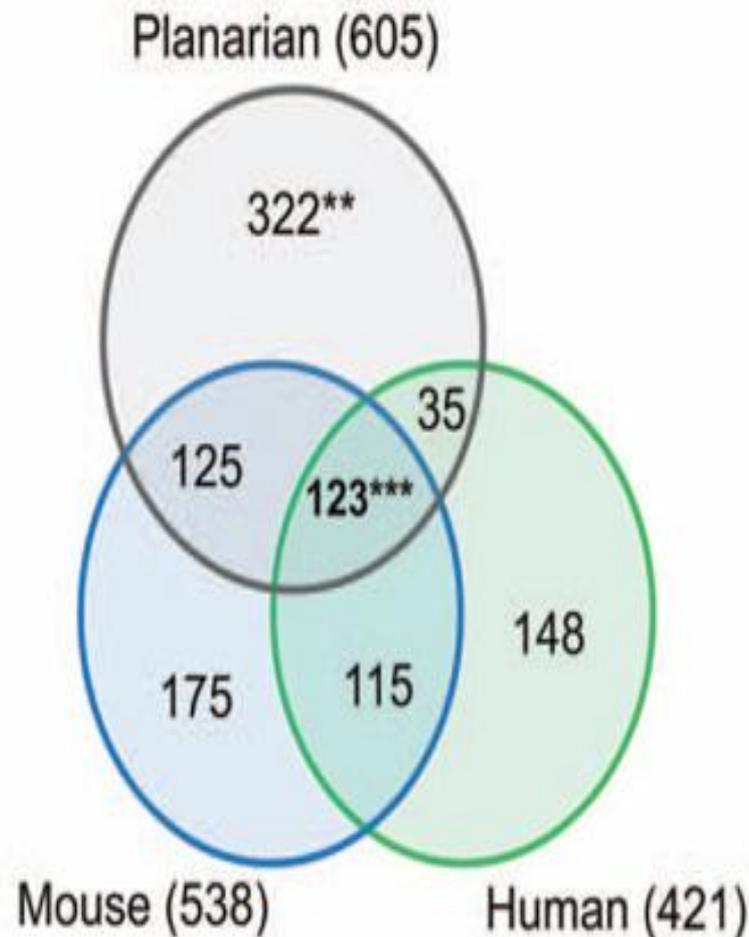
1:1:1 orthologs (4,432)

# What happened when these genes were silenced?

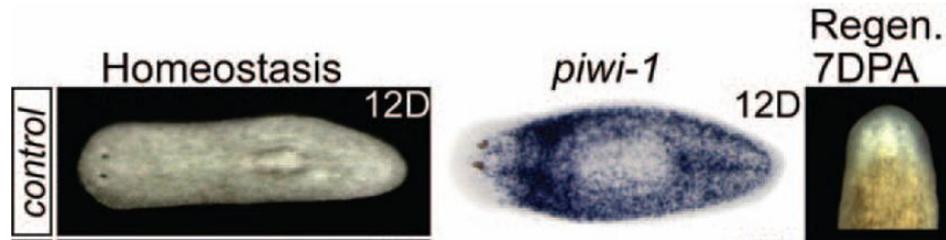


# What orthologs were only differentially expressed in planarian stem cells?

1:1:1 orthologs (4,432)  
≥ 5-fold DE in all stem cells

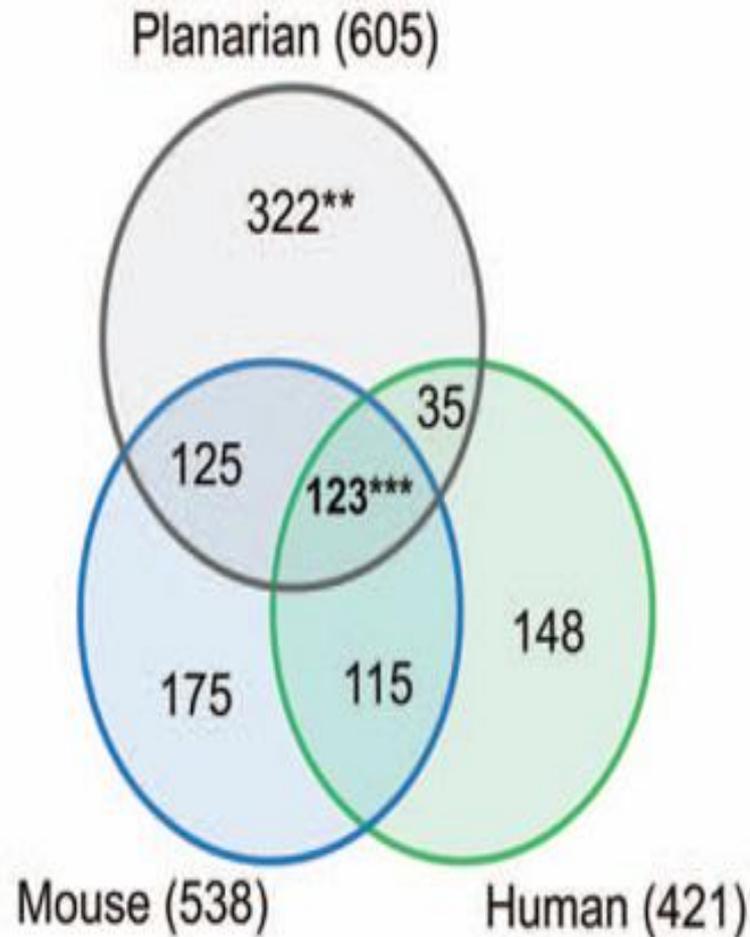


# What happened when these genes were silenced?

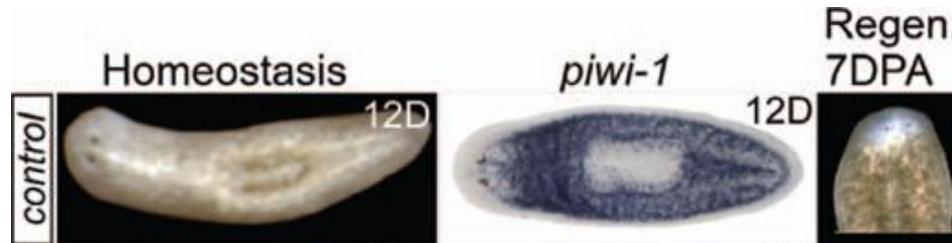


# What orthologs were differentially expressed in all stem cell types?

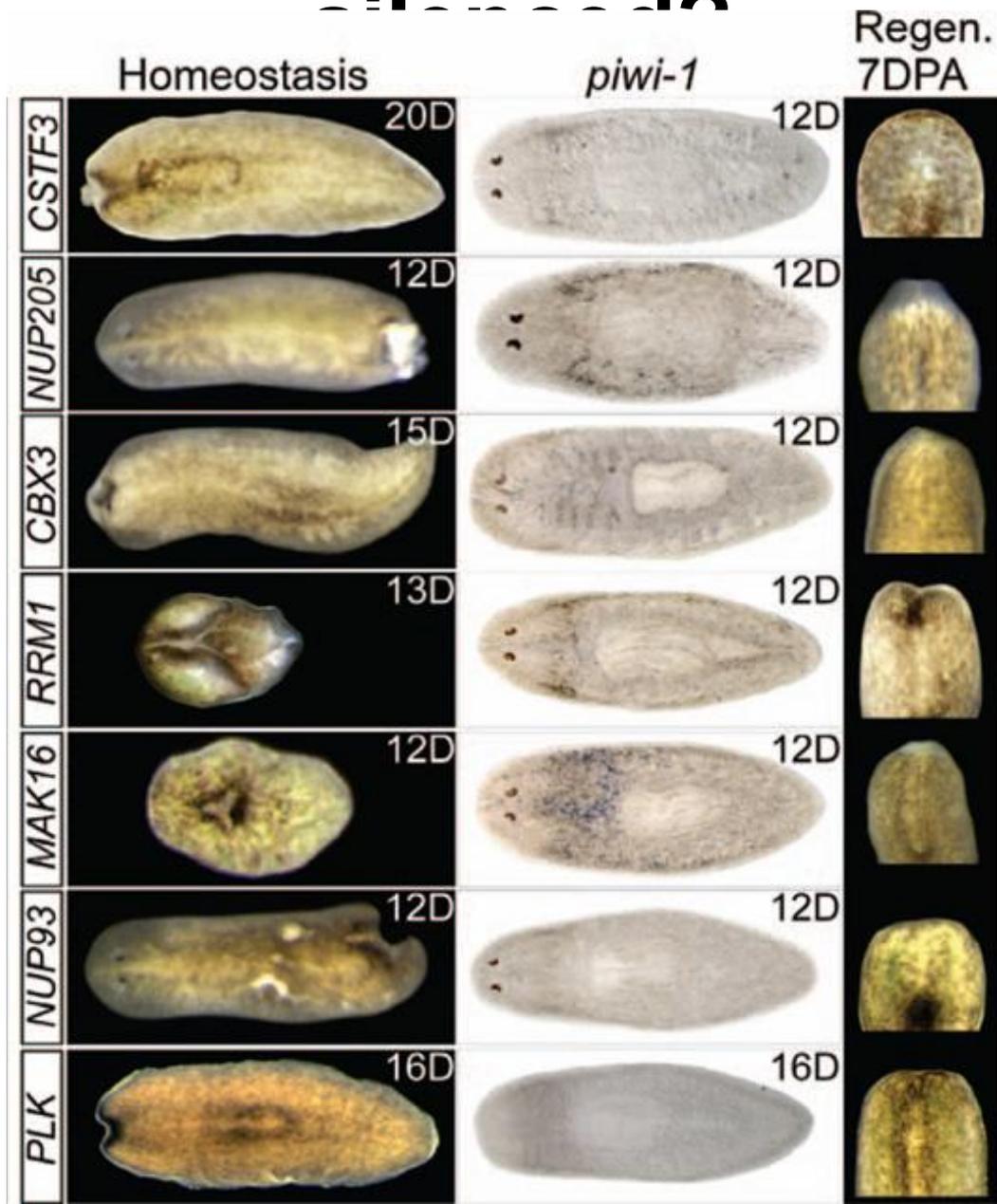
1:1:1 orthologs (4,432)  
≥ 5-fold DE in all stem cells



# What happened when these genes were silenced?



# What happened when these genes were



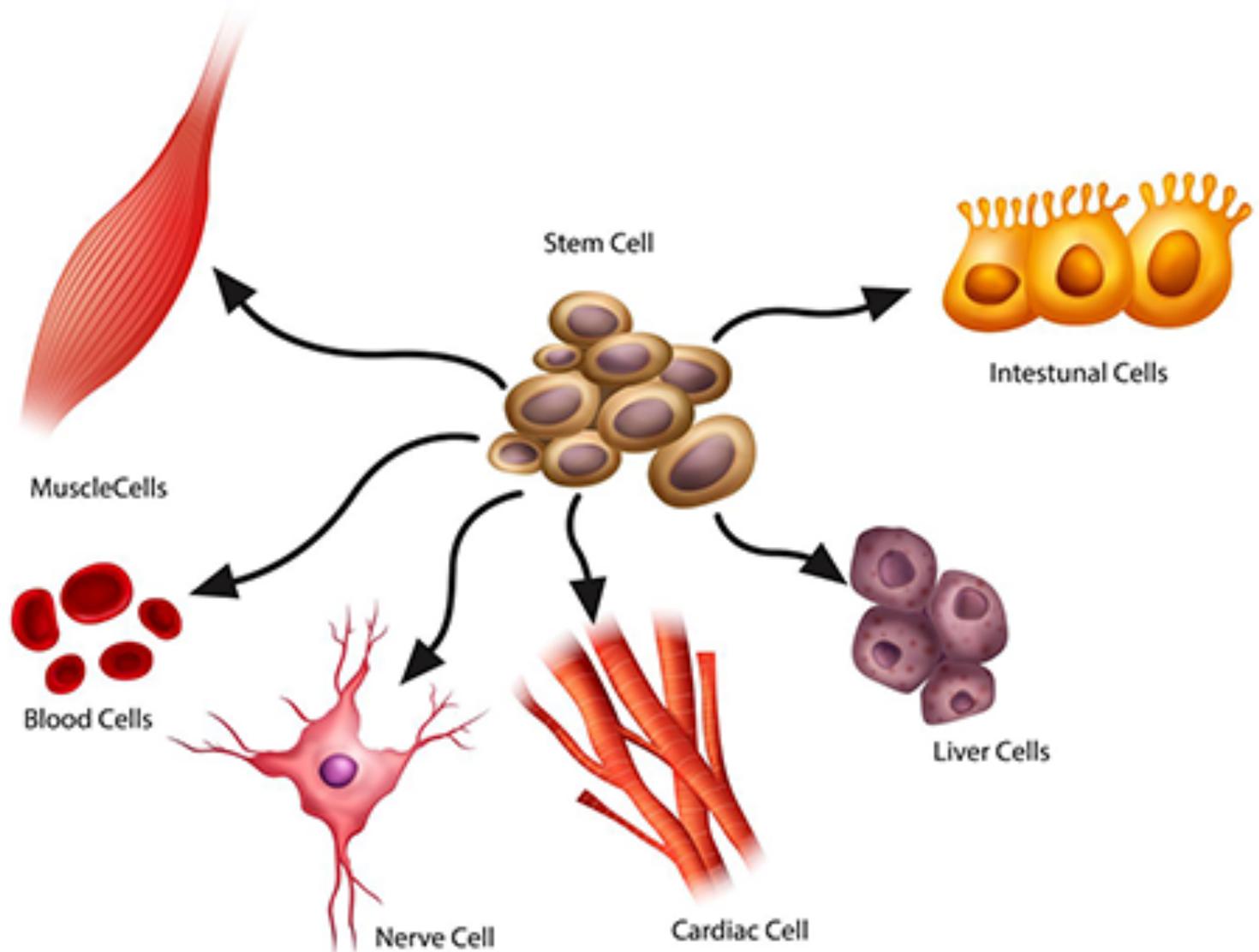
# **What were the main conclusions?**

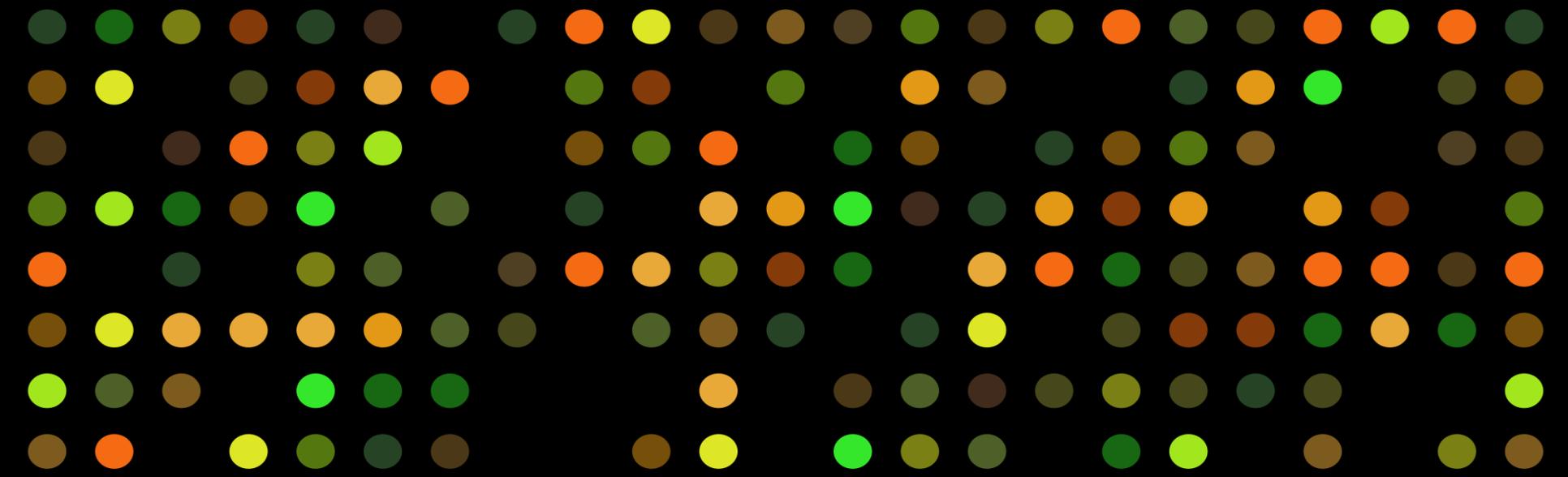
**Genes regulating planarian SC's are both unique and conserved**

**Novel planarian SC genes identified**

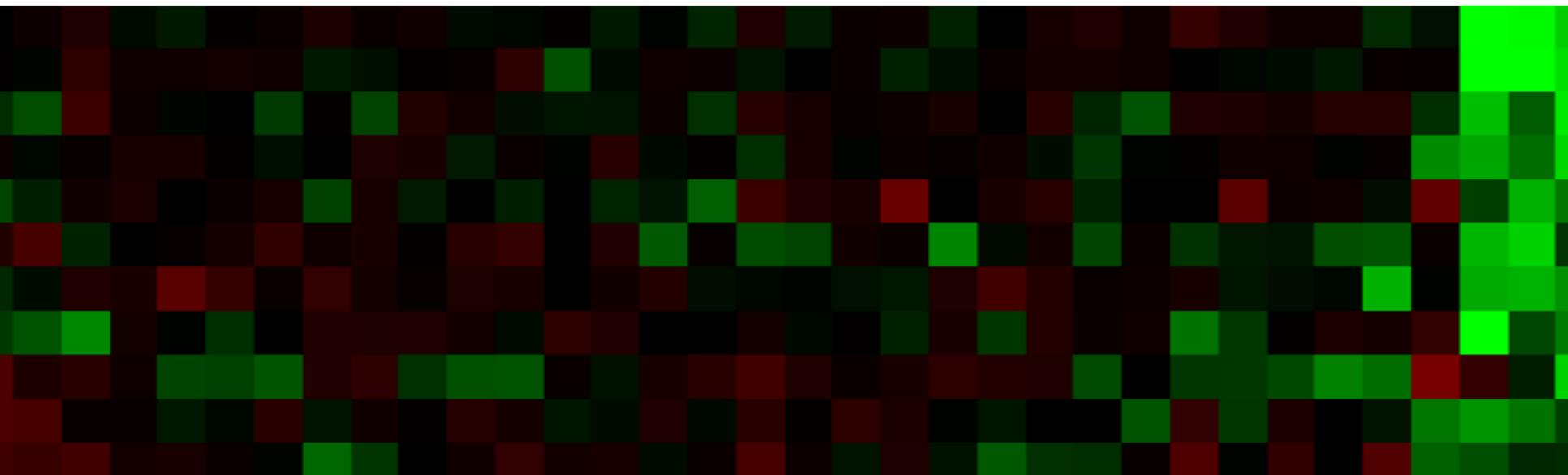
**Conserved SC genes identified**

# Why do we care?





**Questions?**



# References

1. [http://learn.genetics.utah.edu/content/basics/beyond/images/tmr\\_rna.jpg](http://learn.genetics.utah.edu/content/basics/beyond/images/tmr_rna.jpg)
2. <https://upload.wikimedia.org/wikipedia/commons/thumb/a/a4/Pre-mRNA-1ysv-tubes.png/220px-Pre-mRNA-1ysv-tubes.png>
3. [http://s7.computerhistory.org/is/image/CHM/xb93.80p-03-01?\\$re-medium\\$](http://s7.computerhistory.org/is/image/CHM/xb93.80p-03-01?$re-medium$)
4. <https://www.slideshare.net/ueb52/uebuat-bioinformatics-course-session-23-vhir-barcelona>
5. [https://upload.wikimedia.org/wikipedia/commons/thumb/a/aa/RNA-Seq\\_workflow-5.pdf/page1-1753px-RNA-Seq\\_workflow-5.pdf.jpg](https://upload.wikimedia.org/wikipedia/commons/thumb/a/aa/RNA-Seq_workflow-5.pdf/page1-1753px-RNA-Seq_workflow-5.pdf.jpg)
6. [http://www.frontiersin.org/files/Articles/77307/fgene-05-00043-HTML/image\\_m/fgene-05-00043-g001.jpg](http://www.frontiersin.org/files/Articles/77307/fgene-05-00043-HTML/image_m/fgene-05-00043-g001.jpg)
7. [http://study.com/cimages/multimages/16/dugesia\\_subtentaculata\\_1\\_resize.jpg](http://study.com/cimages/multimages/16/dugesia_subtentaculata_1_resize.jpg)
8. <https://www.youtube.com/watch?v=b4tVokh6Law>
9. <http://www.rna-seqblog.com/rpkm-fpkm-and-tpm-clearly-explained/>