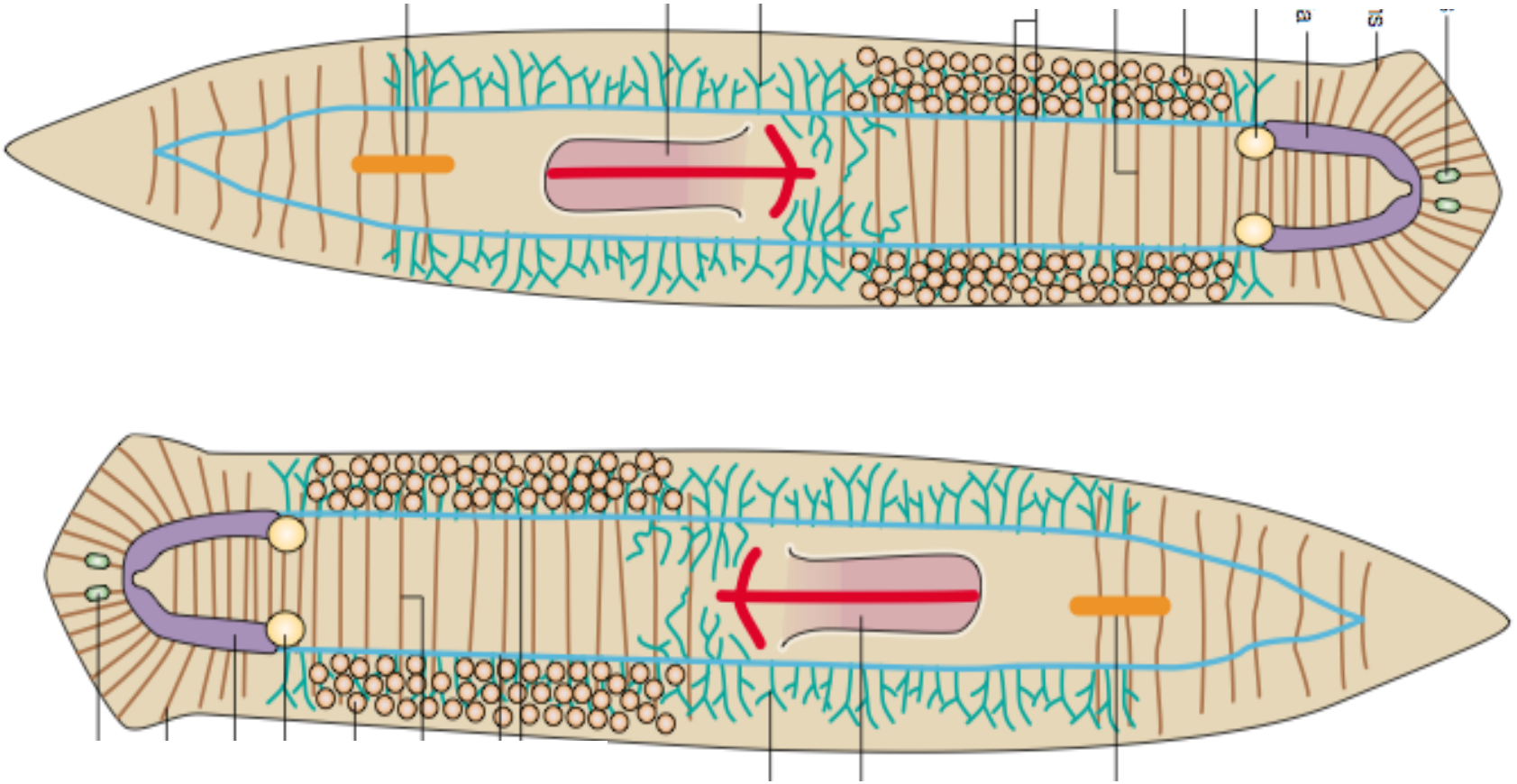


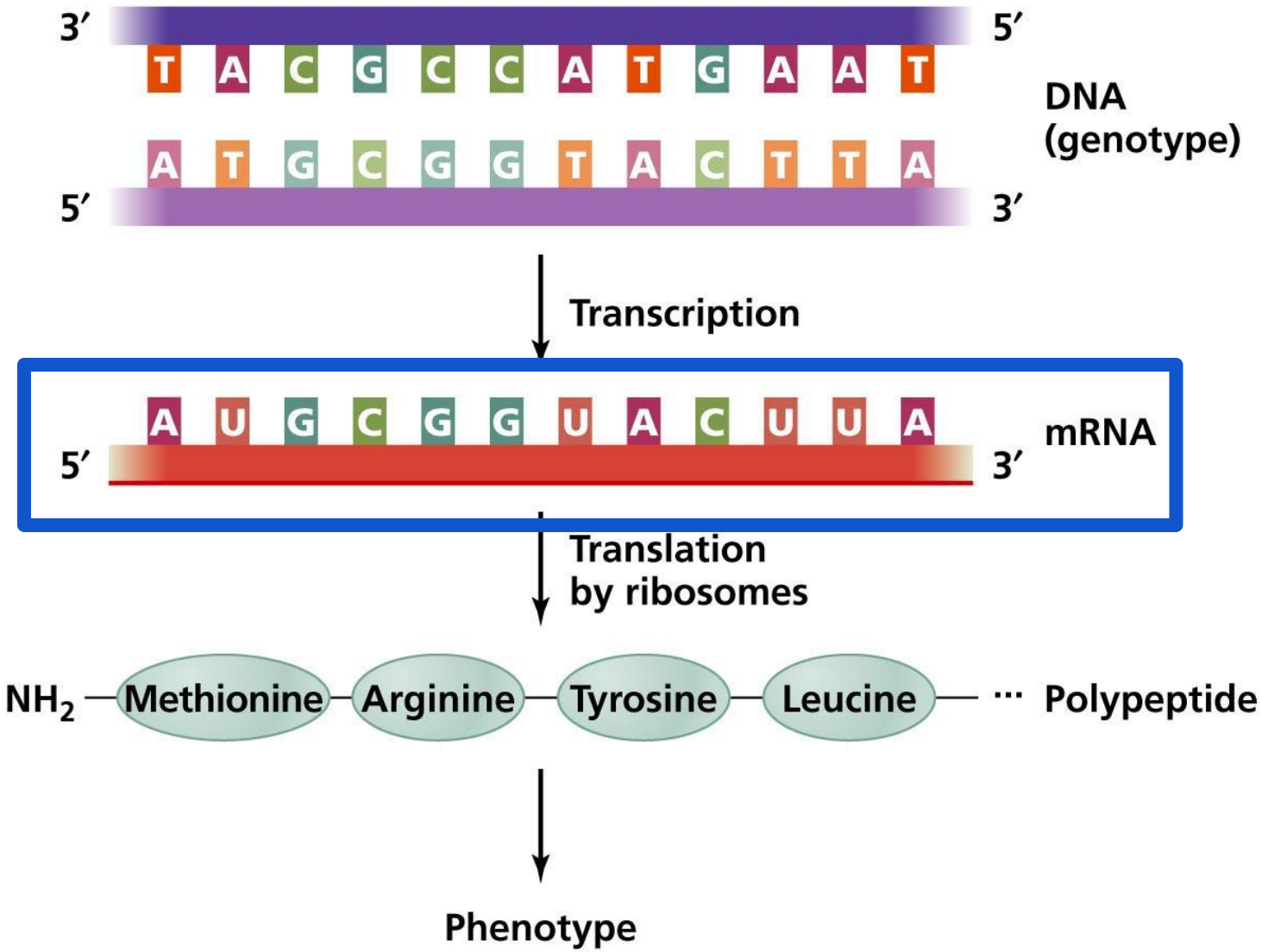
Kerry Zimdars & Ellen Siefkes

RNA-Seq: a revolutionary tool for transcriptomics

Zhong Wang, Mark Gerstein, and Michael Snyder

What is the Central Dogma?

What is the Central Dogma?



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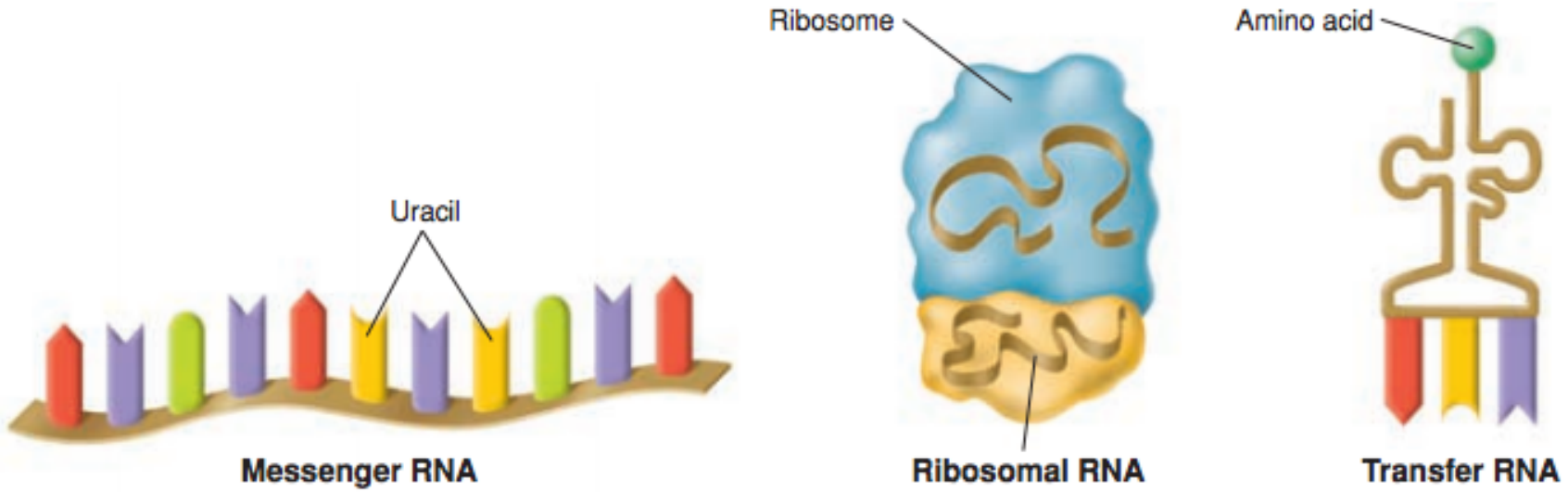
http://academic.pgcc.edu/~kroberts/Lecture/Chapter%207/07-07_CentralDogma_L.jpg

What is a Transcriptome?

What is a Transcriptome?

The complete set of transcripts (RNA molecules) in a cell, and their quantity, for a specific developmental stage or physiological condition.



Types of RNA The three main types of RNA are messenger RNA, ribosomal RNA, and transfer RNA. Ribosomal RNA is combined with proteins to form ribosomes.

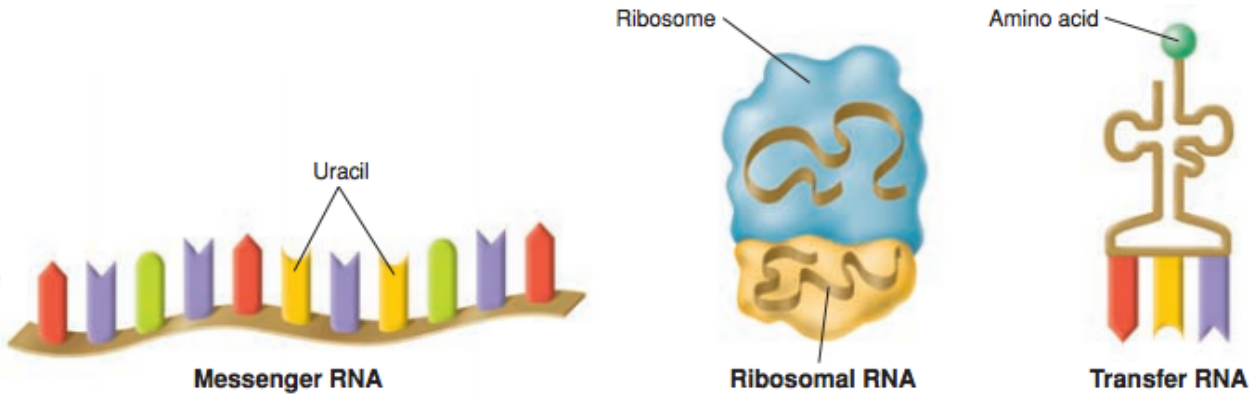
RNA-Seq: a revolutionary tool for transcriptomics
http://www.magrinscience.com/wp-content/uploads/2013/02/types-of-RNA.png

Why is understanding the transcriptome important?

1. interpret functional elements of genome

2. ?

3. ?



Types of RNA The three main types of RNA are messenger RNA, ribosomal RNA, and transfer RNA. Ribosomal RNA is combined with proteins to form ribosomes.

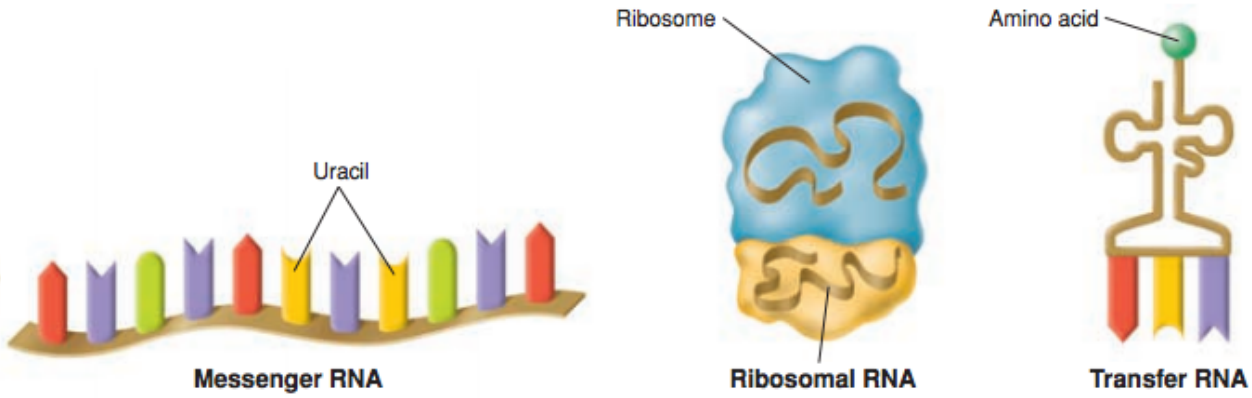
http://www.magrinscience.com/wp-content/uploads/2013/02/type

Why is understanding the transcriptome important?

1. interpret functional elements of genome

2. reveal molecular constituents of cells and tissues

3. understand development and disease



Types of RNA The three main types of RNA are messenger RNA, ribosomal RNA, and transfer RNA. Ribosomal RNA is combined with proteins to form ribosomes.

http://www.magrinscience.com/wp-content/uploads/2013/02/type

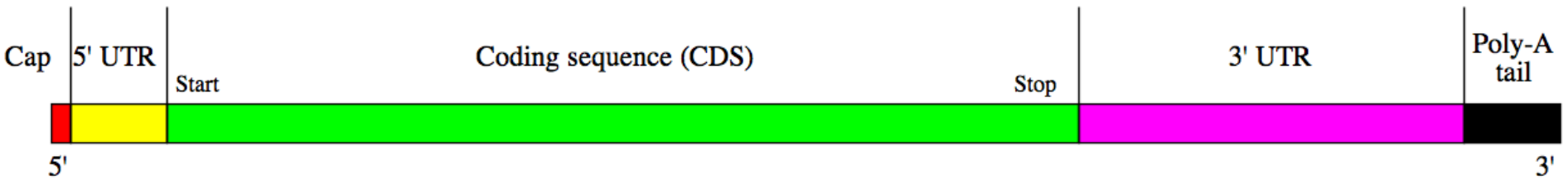
What are the key aims of transcriptomics?

What are the key aims of transcriptomics?

1. To catalogue **all** transcripts

2. To determine the transcriptional **structure** of genes*

3. To quantify the **changing** expression levels of each transcript



http://upload.wikimedia.org/wikipedia/commons/b/ba/MRNA_structure.svg

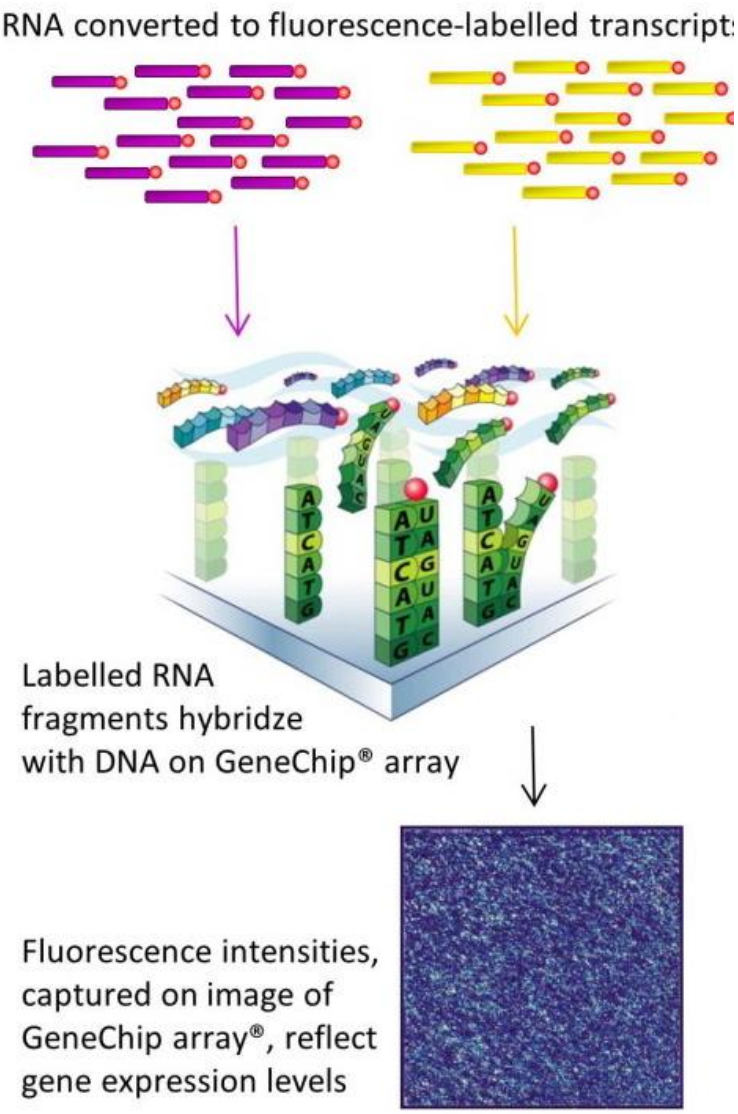
How do we study the transcriptome?

(Hint: 2 approaches)

How do we study the transcriptome?

1. Hybridization-based approaches

-Genomic tiling microarrays



http://www.intechopen.com/source/html/38870/media/image1.jpeg

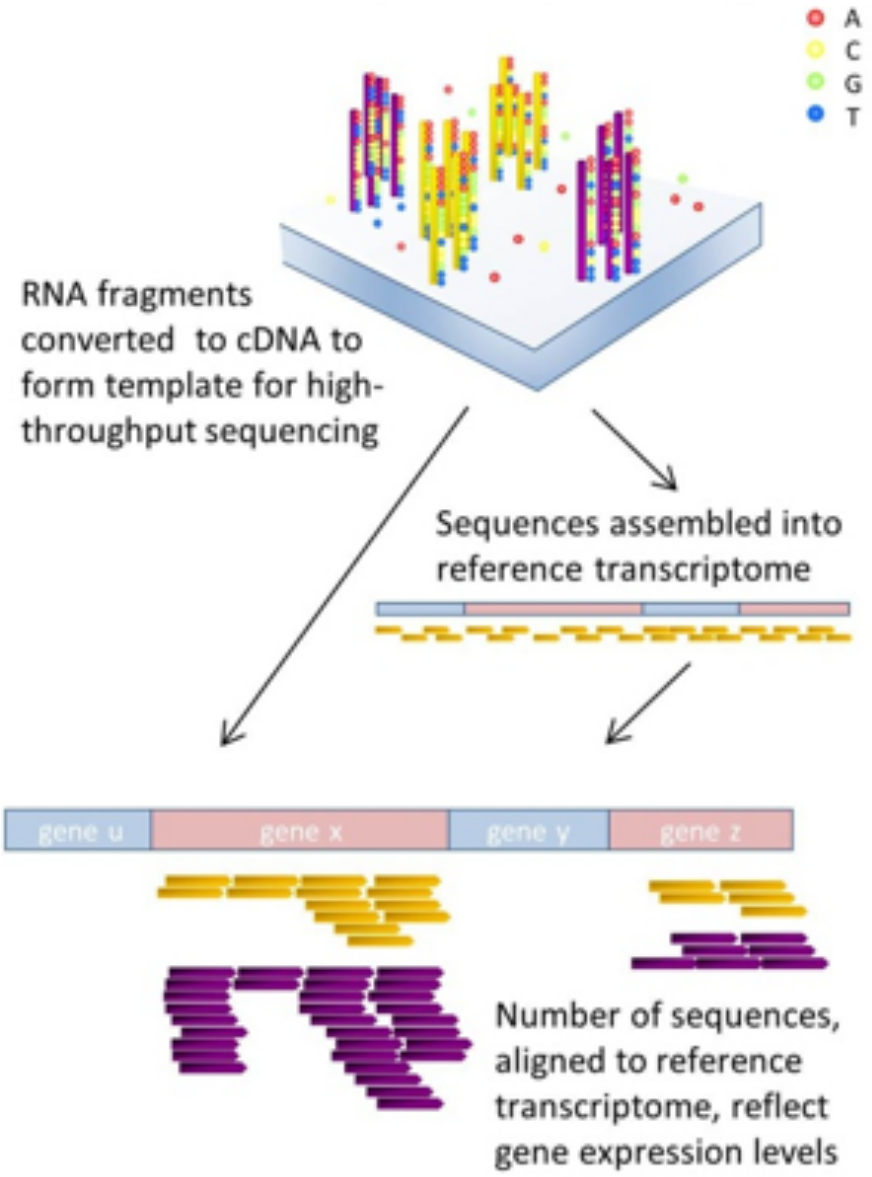
How do we study the transcriptome?

2. Sequence-based approaches

-Sanger sequencing

-Tag-based methods (SAGE, CAGE, MPSS)

-RNA-Seq



http://www.intechopen.com/source/html/38870/media/image1.jpeg

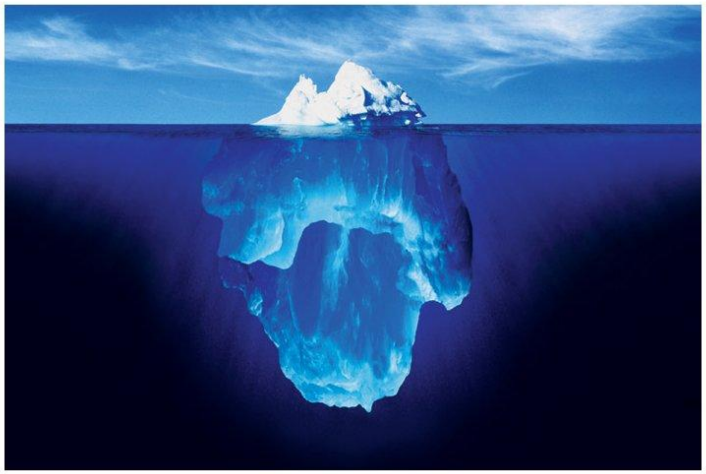
Hybridization-based Approaches

Positives:

-high throughput
-inexpensive*

Negatives:

-relies on current knowledge
-high background levels*
-limited dynamic range
-hard to compare across experiments



http://www.benbruno.com/wp/assets/piggybank.jpg
http://blog.gustavkaser.com.au/wp-content/uploads/2013/09/iceberg-poster.jpg

Sequence-based Approaches

Positives:

-high throughput

-precise gene expression levels

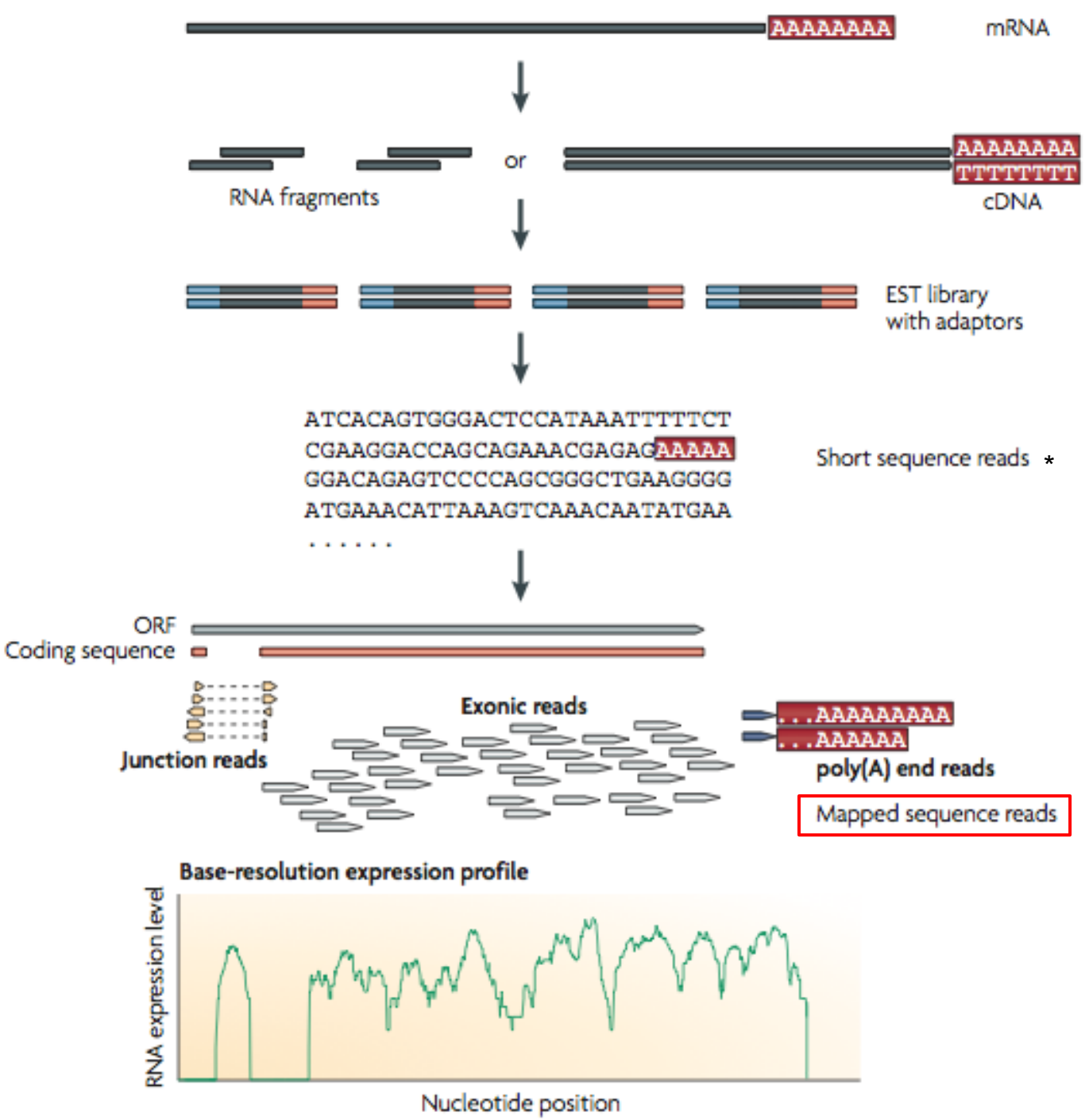
Negatives:

-expensive
-short tags are hard to map*
-only partial analysis

http://www.mhhe.com/physsci/chemistry/chang7/esp/folder_structure/ch/m2/s2/assets/images/chm2

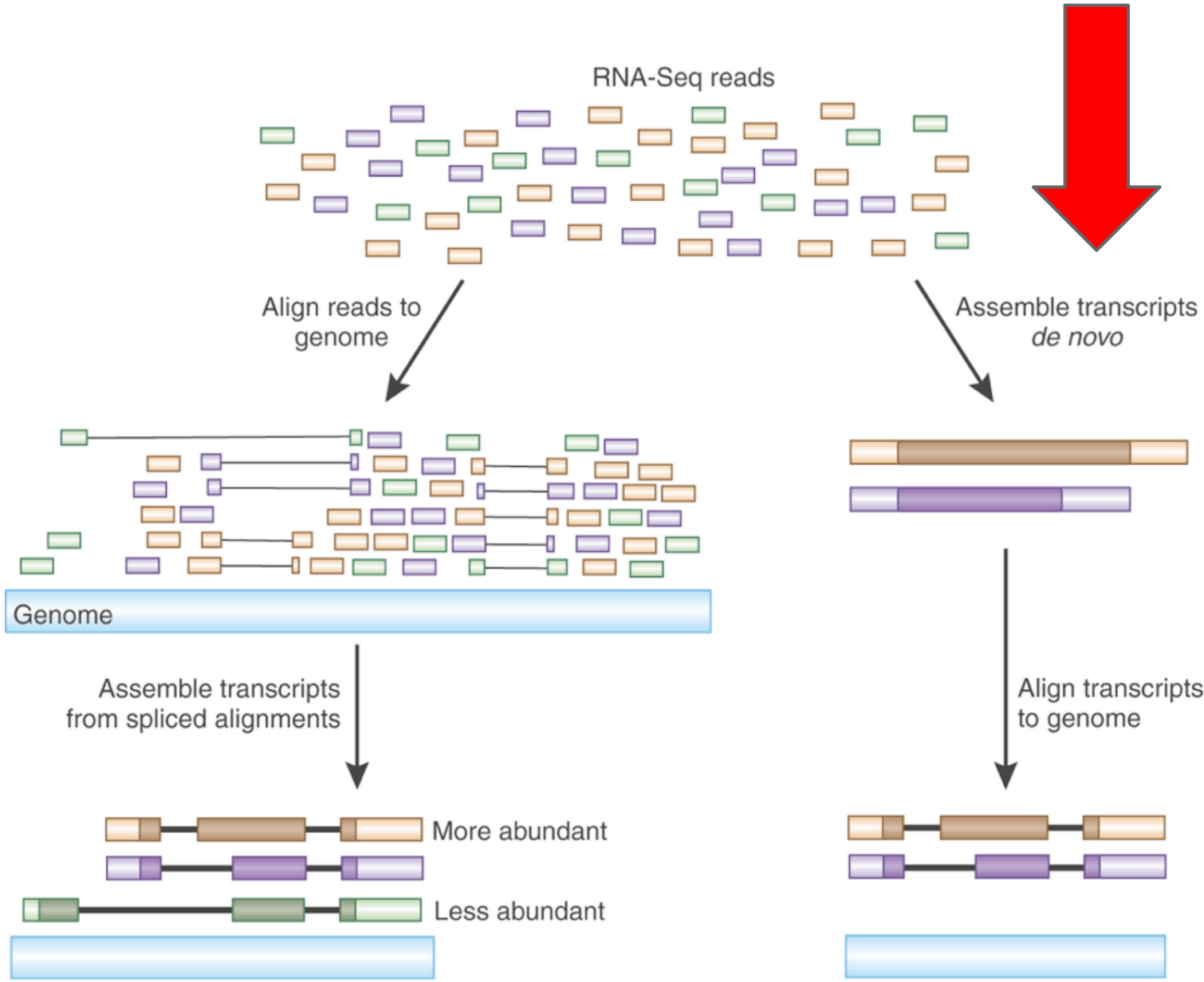
http://2.bp.blogspot.com/-UNt6fJkYVzQ/Tbz93JnjdMI/AAAAAAAABjI/i5QY-1YbK9o/s1600/dollar-sign.jpg

RNA-Seq



RAN-Seq: a revolutionary tool for transcriptomics

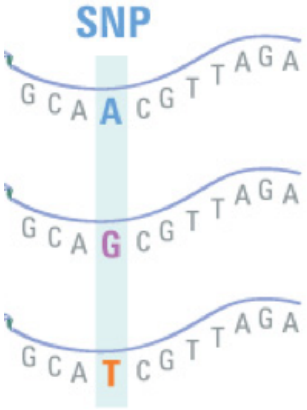
RNA-Seq



http://2.bp.blogspot.com/-J0eXeoo5Soc/TdV9n6rhvLI/AAAAAAAAuEk/hNfo7zq7oT4/s1600/Assembly.png

What are the key advantages of RNA-Seq?

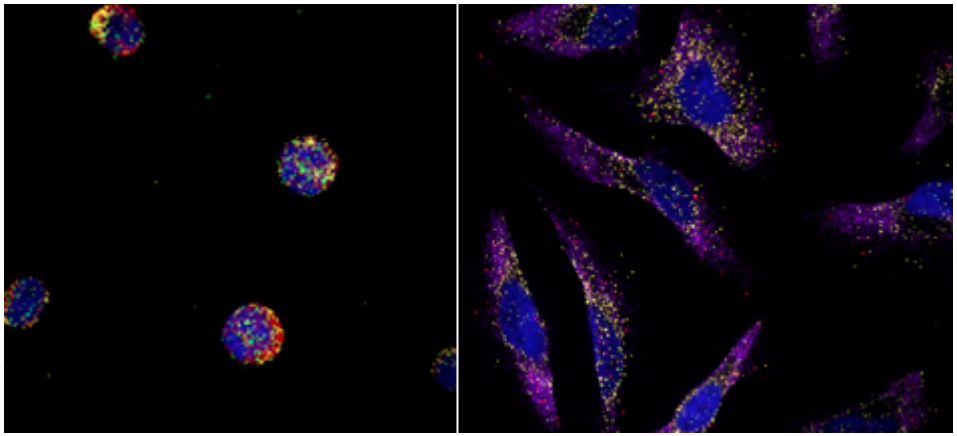
1. Not limited to existing genomic sequences

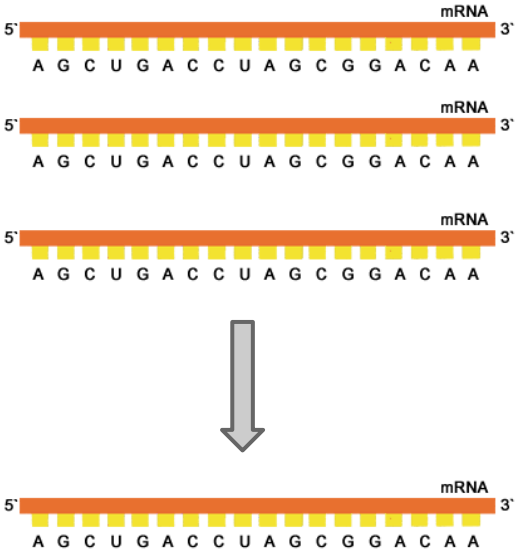


ButterflyL http://butterflywebsite.com/gallery/maier20.htm
GG: http://themedicalbiochemistrypage.org/images/spliceconsensus.jpg
SNP: http://www.broadinstitute.org/files/news/stories/full/SNP_260x260.jpg

Key Advantages

2. Low/No background signal



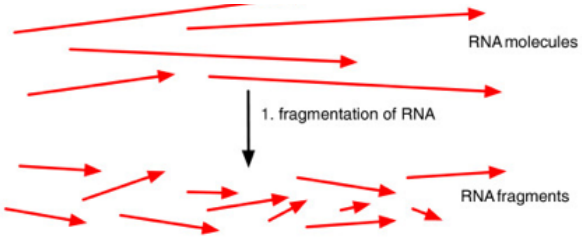


rna FISH: http://upload.wikimedia.org/wikipedia/commons/a/a1/Multiplex_ViewRNA_FISH_Assay_in_Jurkat_and_HeLa_cells.jpg
mrna strand: http://library.thinkquest.org/C004535/media/transcription.gif

What are challenges of RNA-Seq?

What are challenges of RNA-Seq?

1. Library construction



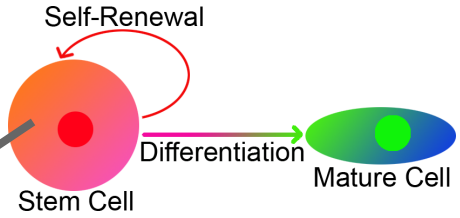
2. Bioinformatic challenges

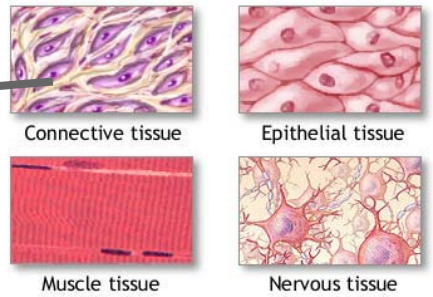
3. Coverage vs. Cost

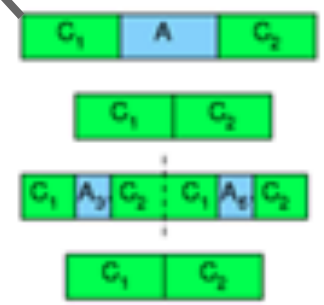
library: http://genomebiology.com/content/figures/gb-2011-12-3-r22-1.jpg
bioinfo: http://www.uptimeus.com/pictures/Services/ist2_4610923-binary-code.jpg
money: http://www.onwravens.net/ravendaily/files/2013/10/money-03.jpg

So why bother with it?

It provides an unprecedented global view of the transcriptome!



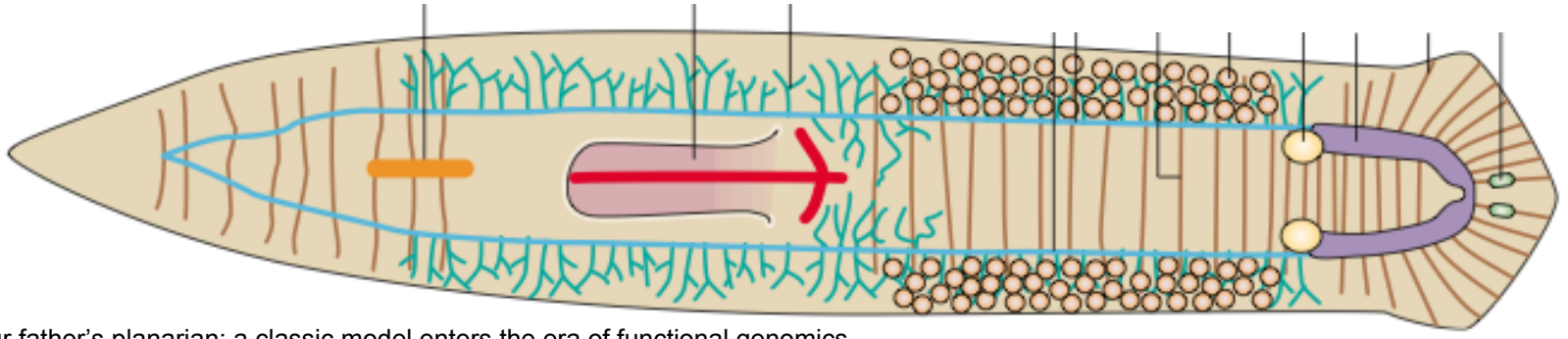




Ah-Ha: http://www.rippleeffect.com/wp-content/uploads/2010/12/learn.jpg, revise: https://ccconline.org/media/library/proofread.jpg
splice: http://www.psi.toronto.edu/index.php?q=splicing%20code ; sc: http://www.allthingsstemcell.com/wp-content/uploads/2009/07/StemCell.png
tissues: http://www.bio.davidson.edu/people/kabernd/berndcv/lab/epithelialinfoweb/index_clip_image001.jpg

A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals

Labbe, Irimia, Currie Lin, Zhu, Brown, Ross, Voisin, Bader, Blencowe & Pearson

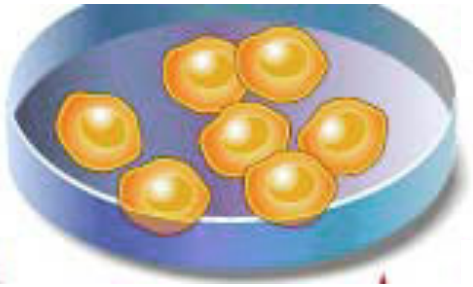


Not your father's planarian: a classic model enters the era of functional genomics

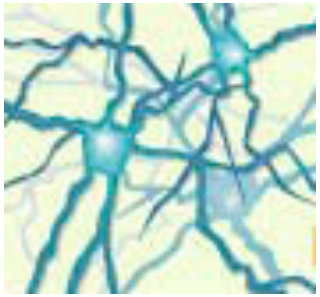
What is a stem cell?

ADULT STEM CELLS

STEM CELL PROGENY

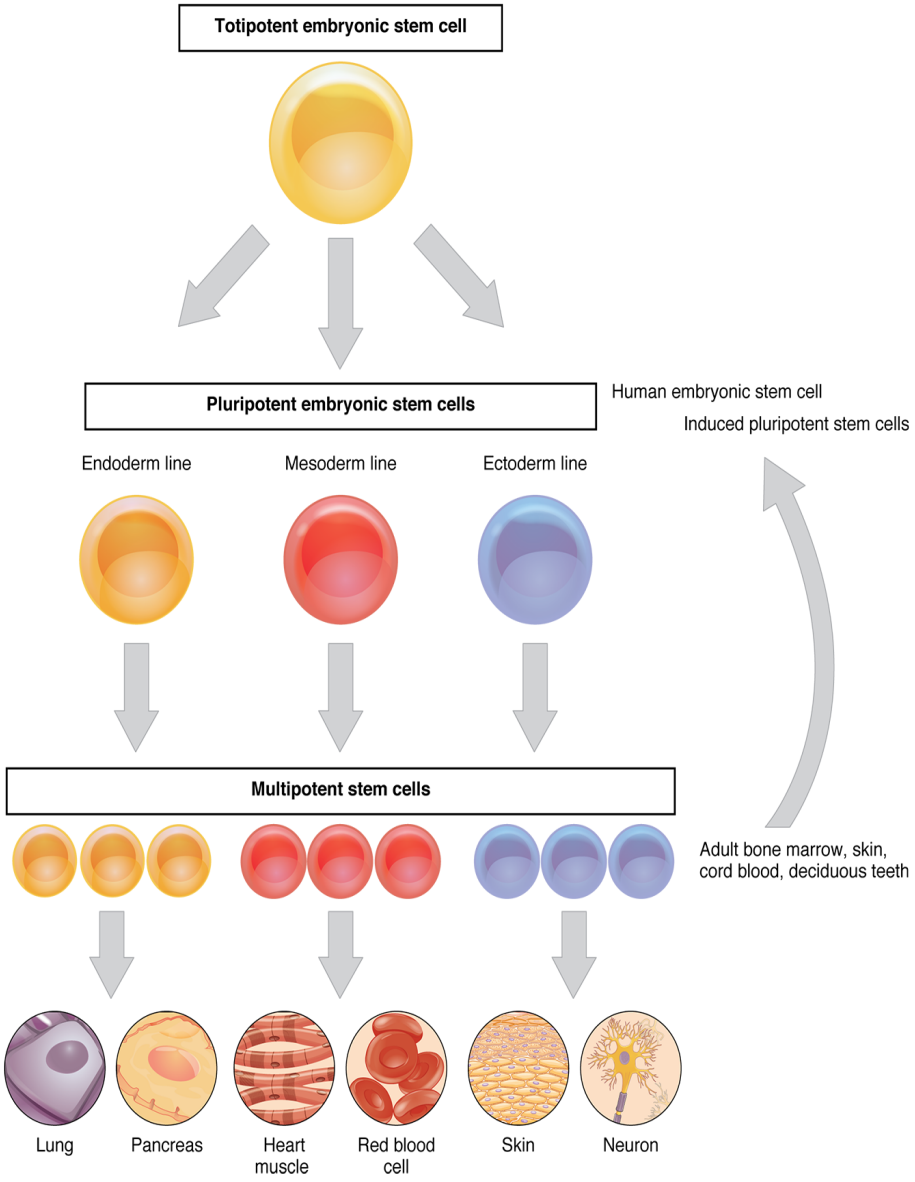


DIFFERENTIATED CELLS



http://universe-review.ca/I10-17-stemcells.jpg

Totipotency and Pluripotency

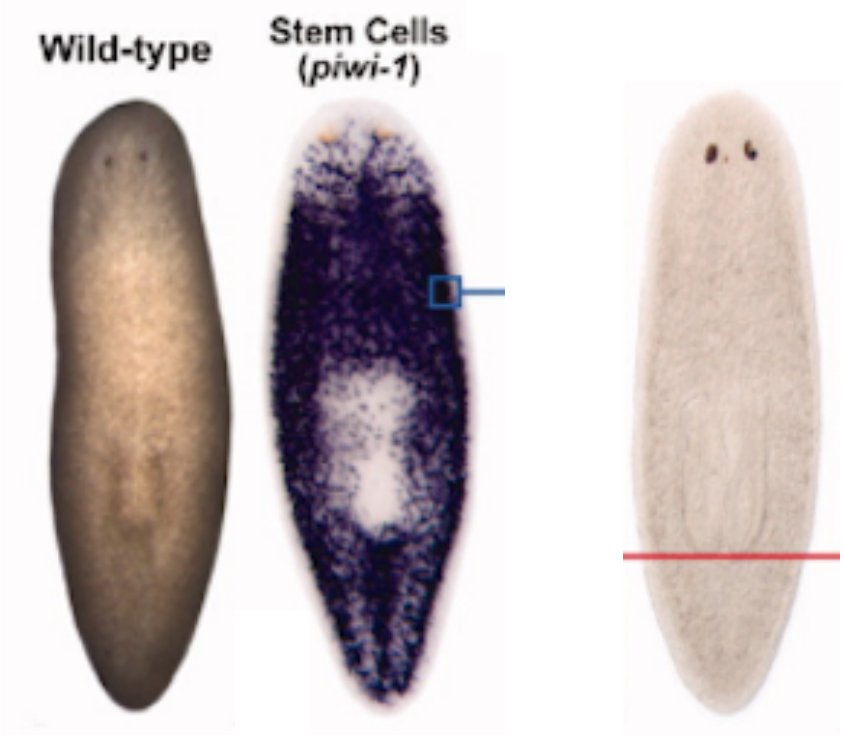


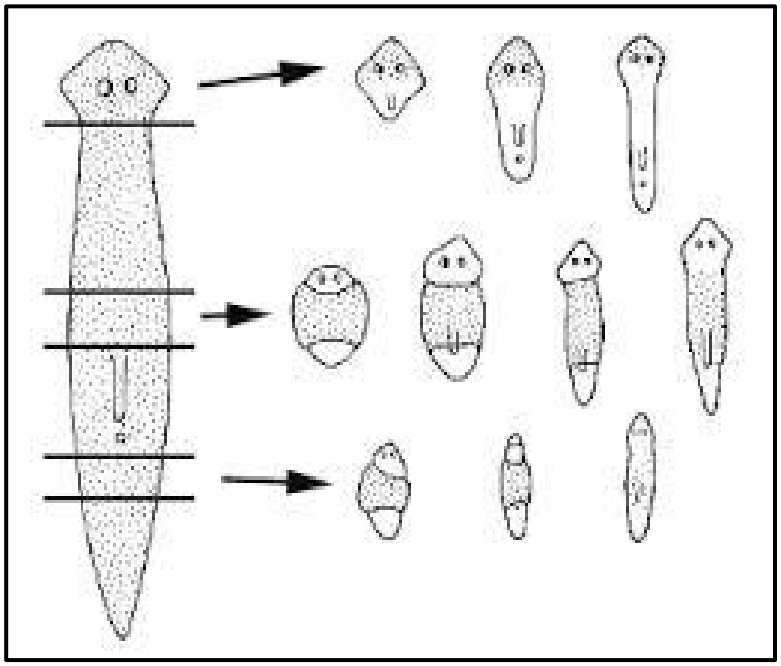
Totipotency: cells that can form all cell types in an organism, including extraembryonic

Pluripotency: cells that give rise to all cell types that make up a body

http://cnx.org/content/m46036/latest/?collection=col11540/latest

Planaria have stem cells & regeneration abilities





Schmedtea mediterranea- grow and shrink

A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals

Planaria as a model organism

http://www.hhmi.org/biointeractive/planarian-regeneration-and-stem-cells

How do we sort stem cells from planaria?

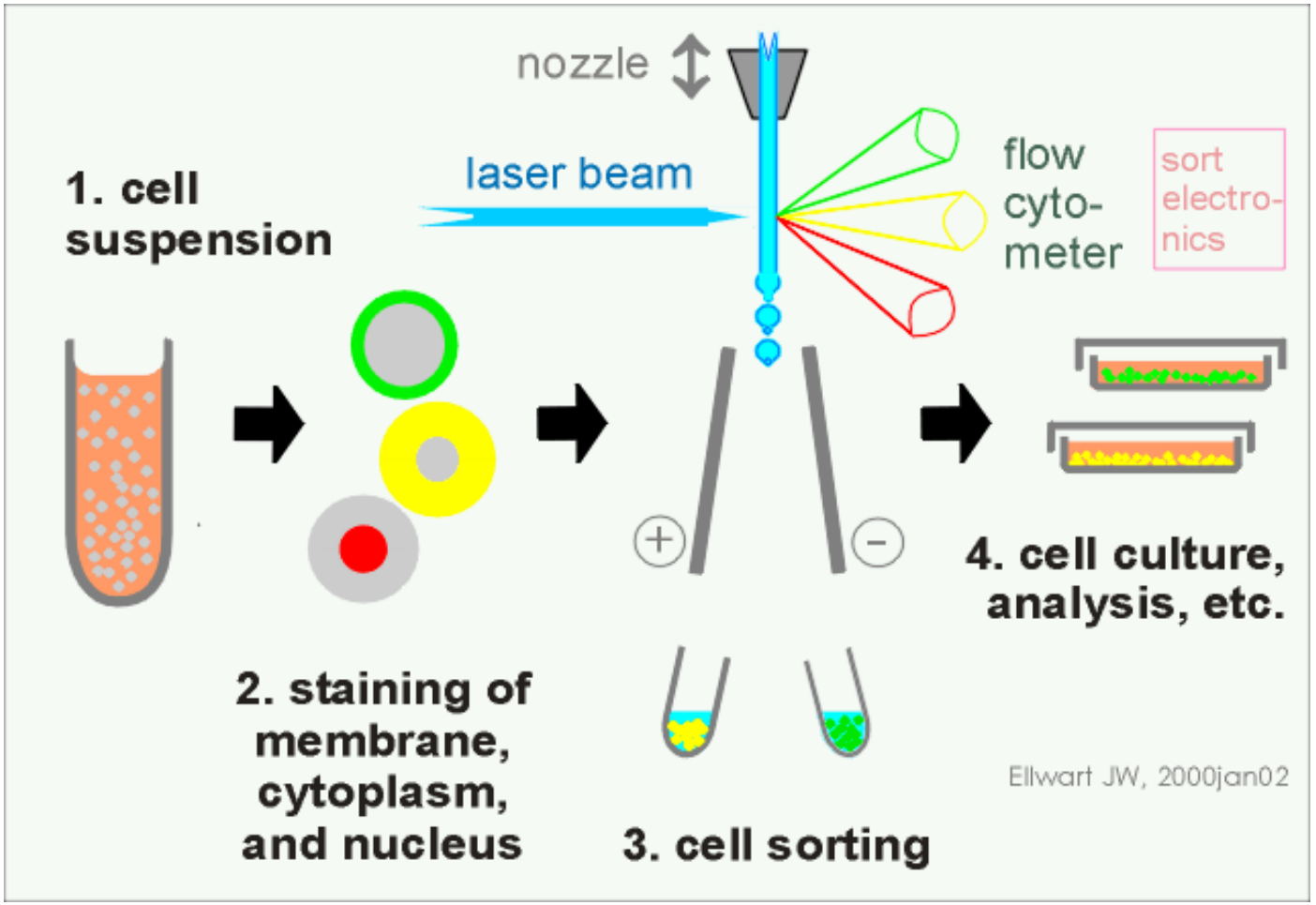
How do we sort stem cells from planaria?

<u>FACS</u>

Fast

Objective

Quantitative



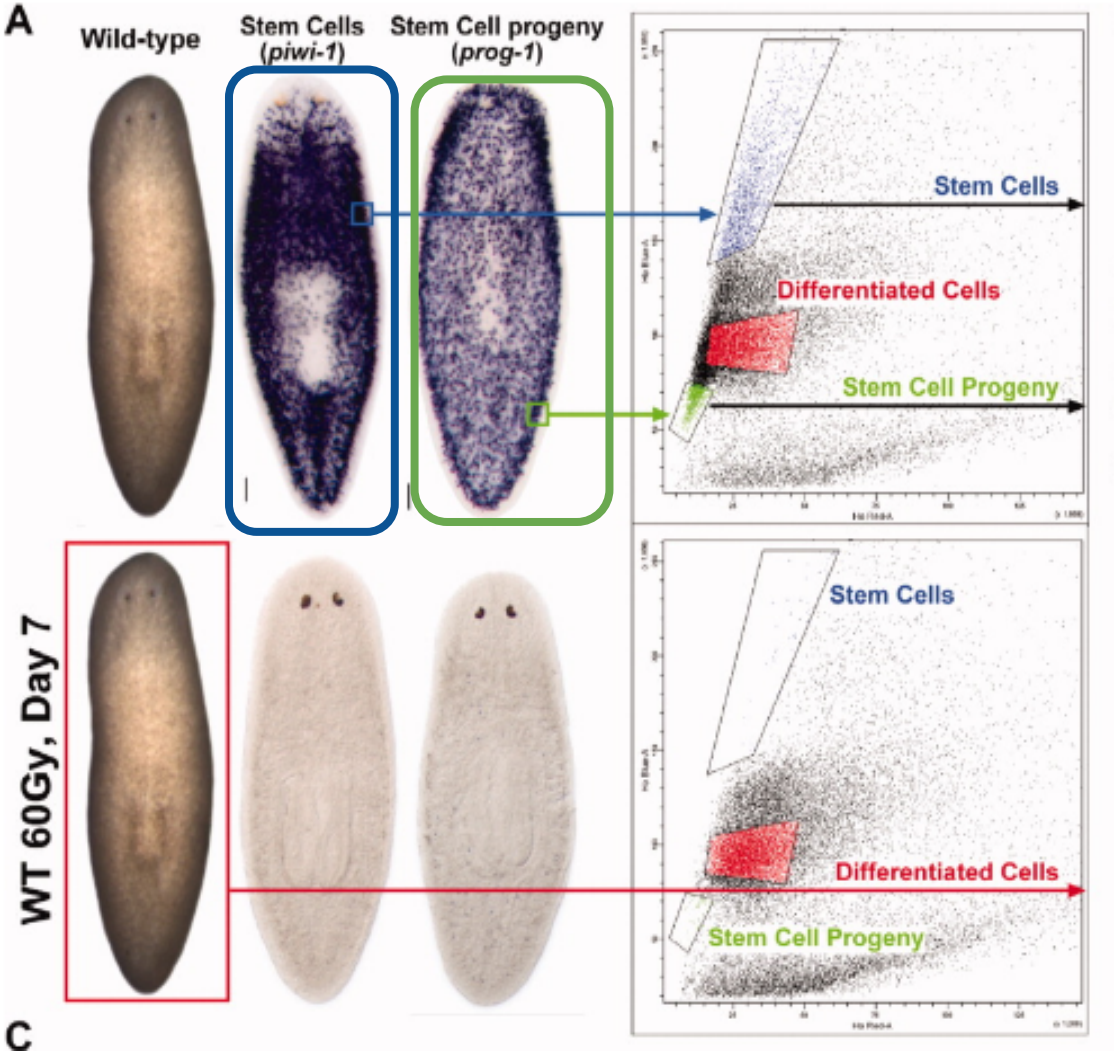
Fluorescence-activated cell sorter

http://flowlab-childrens-harvard.com/new_user

Using FACS in Planaria

piwi-1 stem cell marker

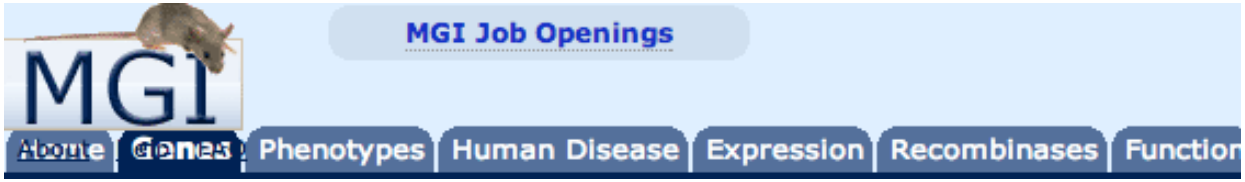
prog-1 stem cell progeny marker



A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals

Why was ontology used in this paper?

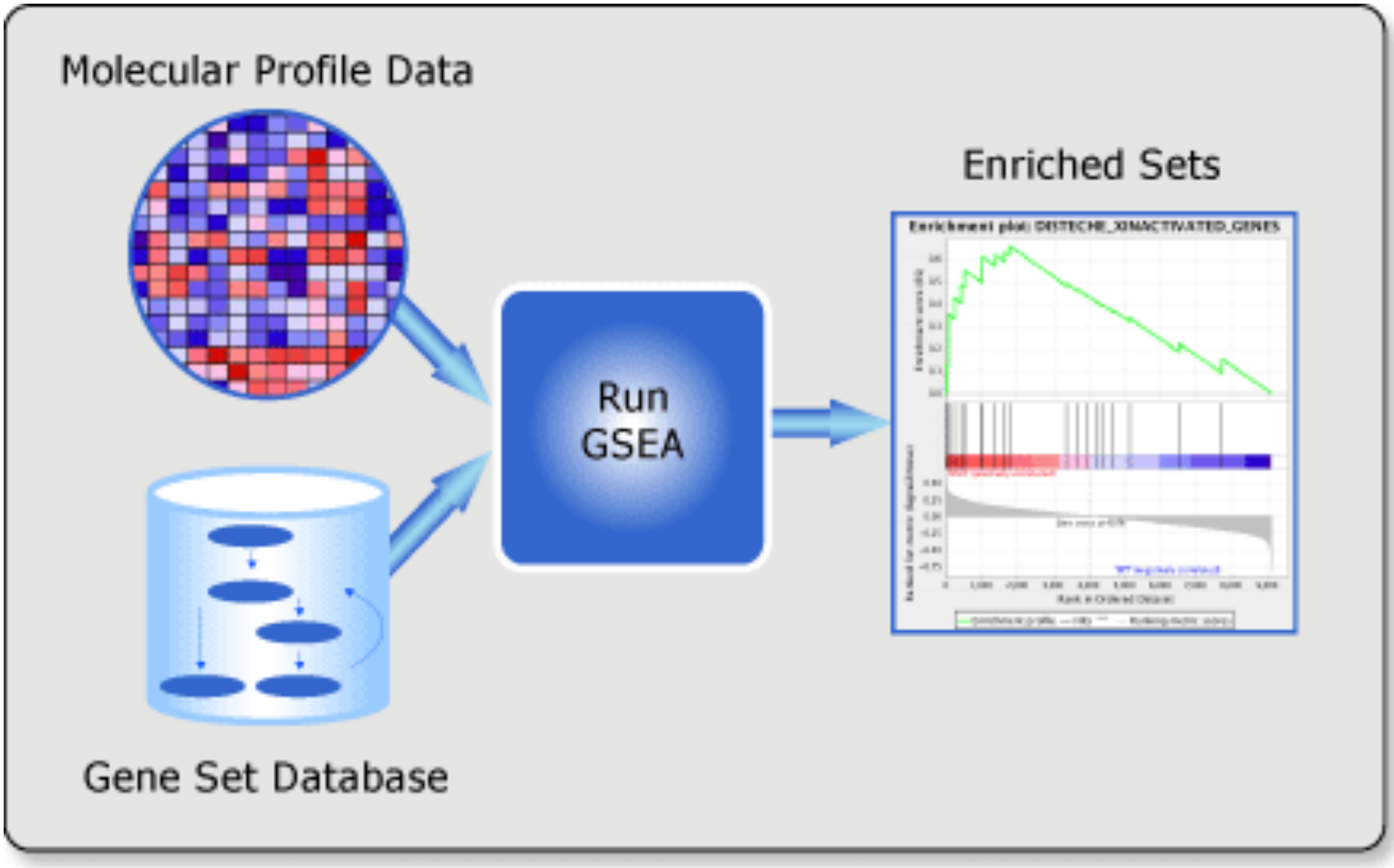
Planarian gene ontology



4,432 planarian transcripts were assigned as 1:1:1 orthologs

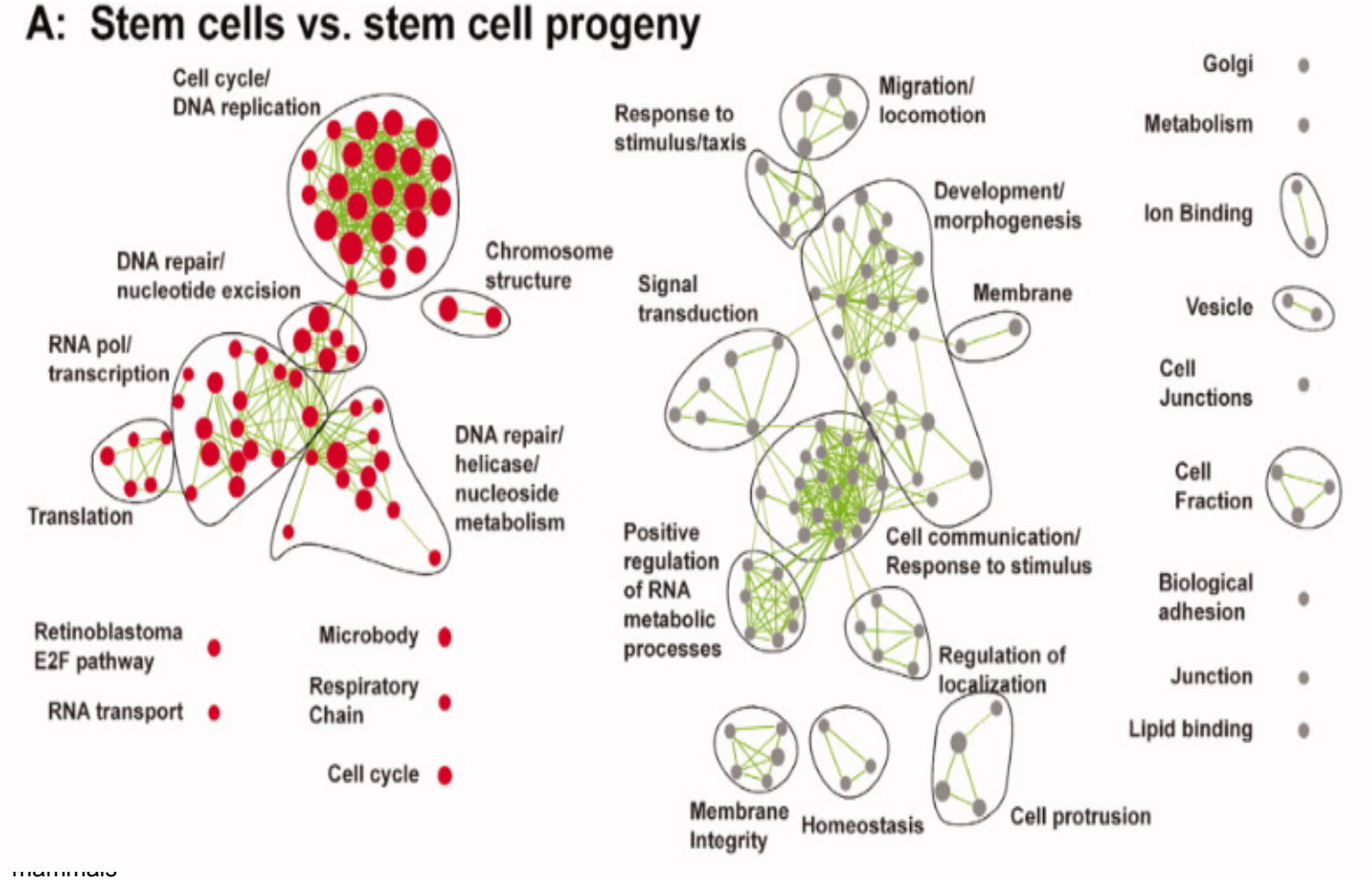
http://blast.ncbi.nlm.nih.gov/Blast.cgi http://www.informatics.jax.org/function.shtml http://www.geneontology.org/

Gene Set Enrichment Analysis (GSEA)



http://www.broadinstitute.org/gsea/index.jsp

Are cell pathways similar between stem cells & stem cell progeny in planaria?



Stem cell:

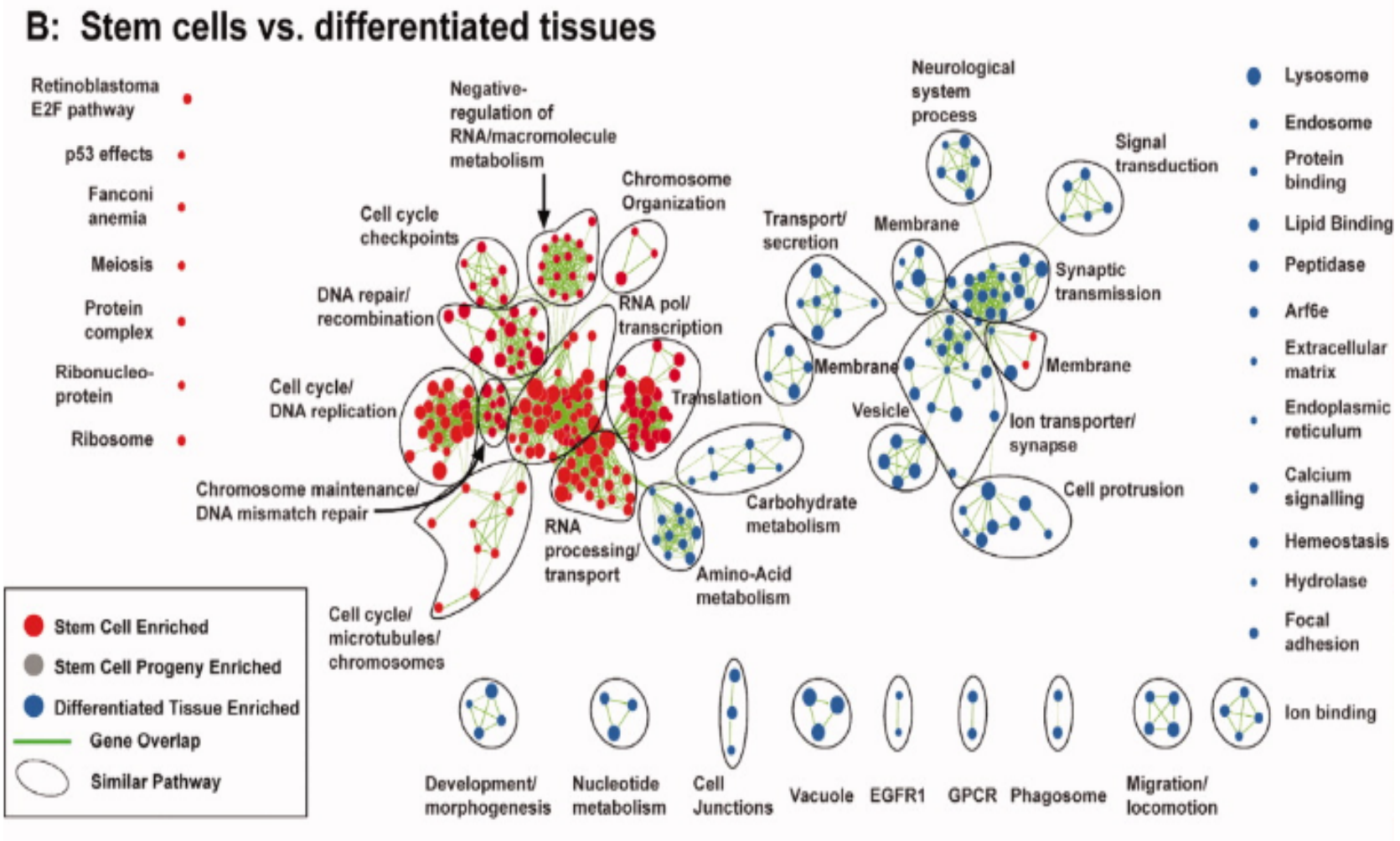
-Retinoblastoma pathway

Progeny cells:

-processes associated with differentiated cells

Are cell pathways similar between stem cells & differentiated cells in planaria?

A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and

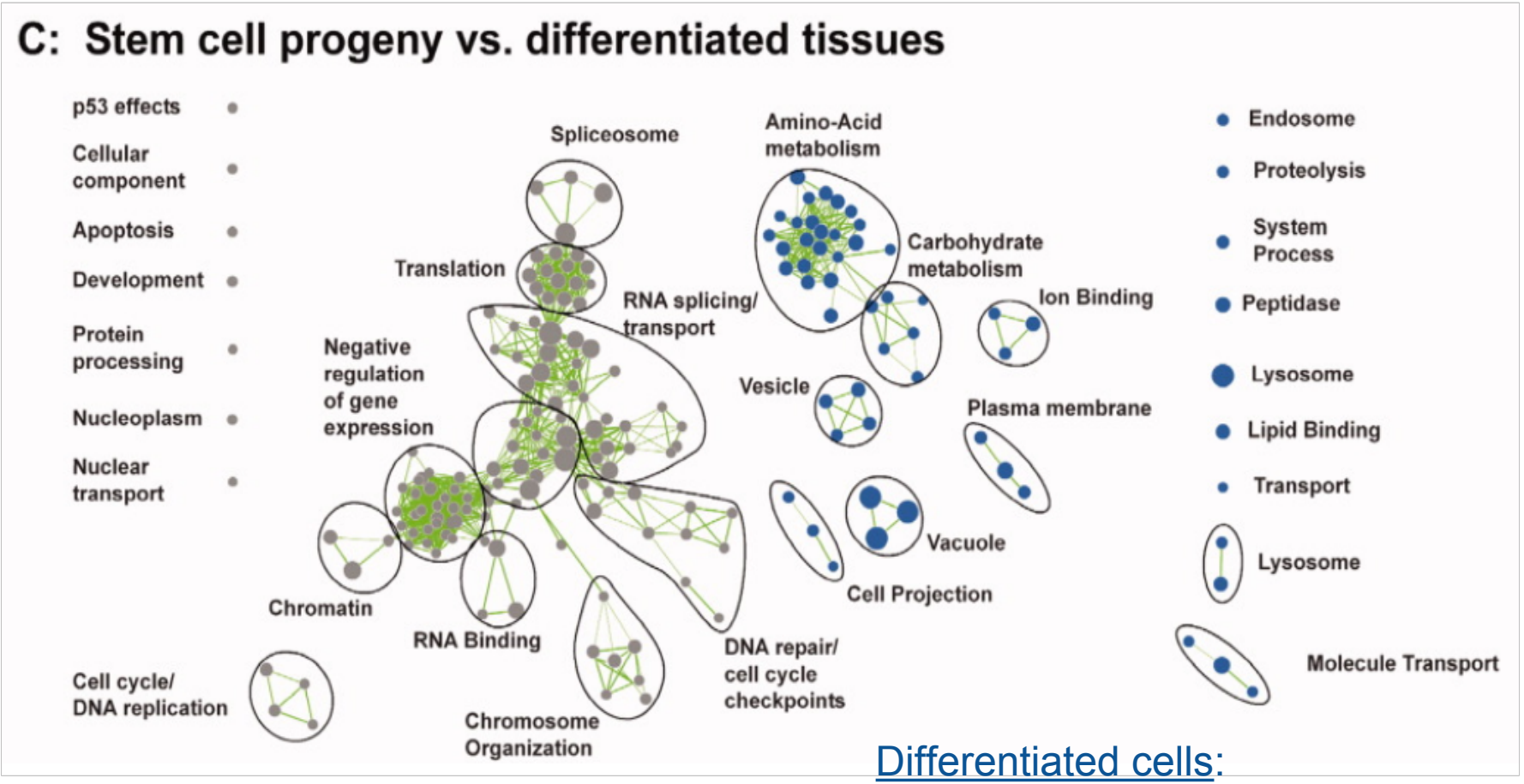


Stem cells:
-cell cycle pathways
-tumor suppressor pathways

Differentiated cells:
-nervous system function
-cell signaling
-metabolism

Are cell pathways similar between stem cell progeny & differentiated cells in planaria?

A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals



Progeny cells:

- *p53* pathway

Differentiated cells:

-nervous system function

-cell signaling

-metabolism

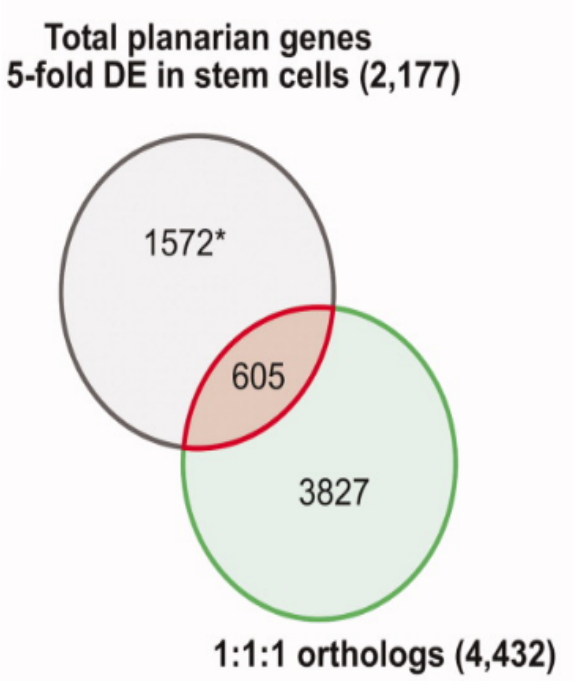
How many genes are conserved in mammals and planarians?

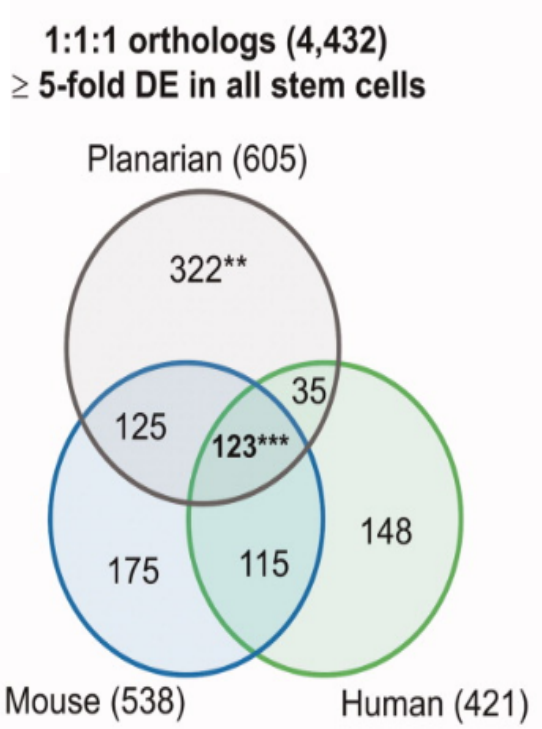
4,432 orthologs were assigned from planarian transcripts

-605 overlapped with genes in planarian stem cells

605 planarian genes are compared to mammalian genes

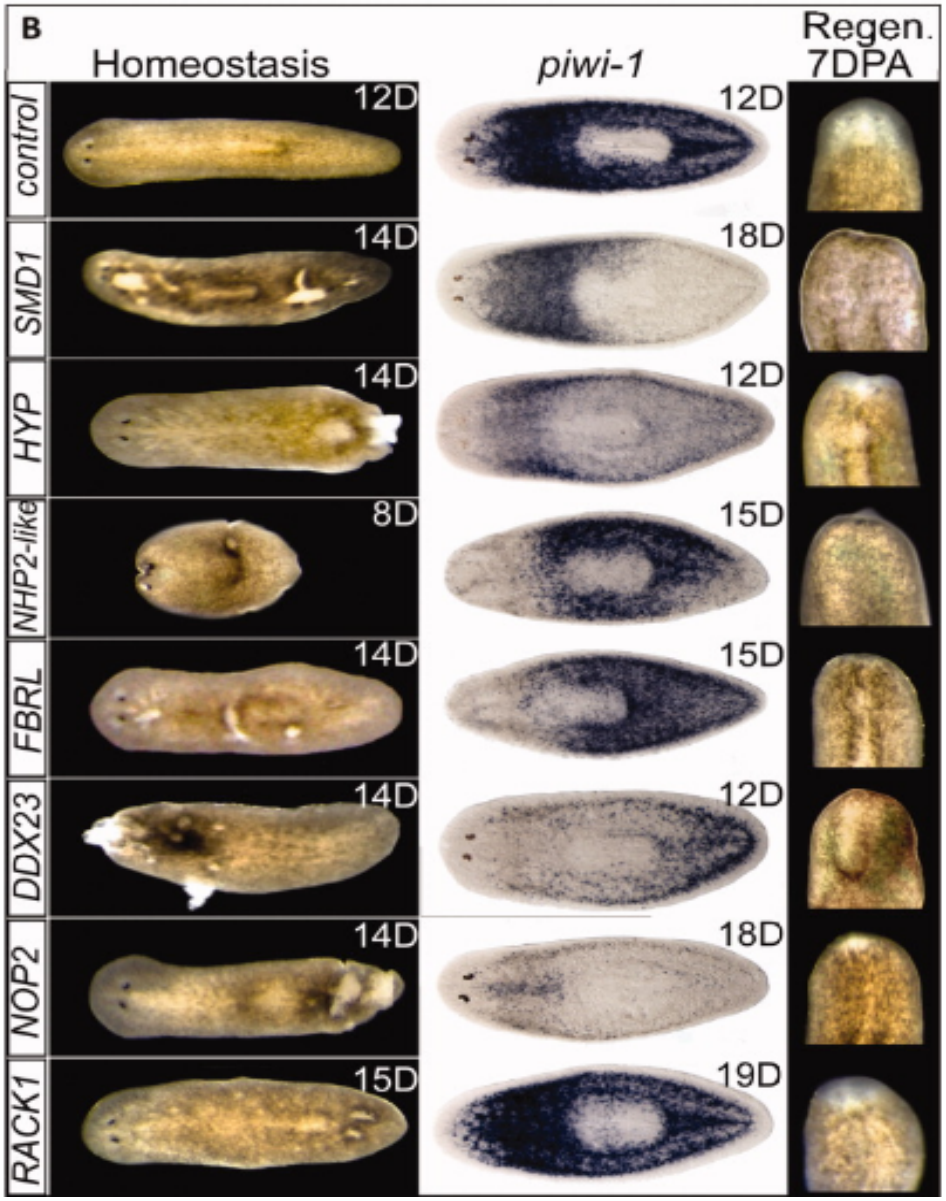
-123 common between all species





A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals

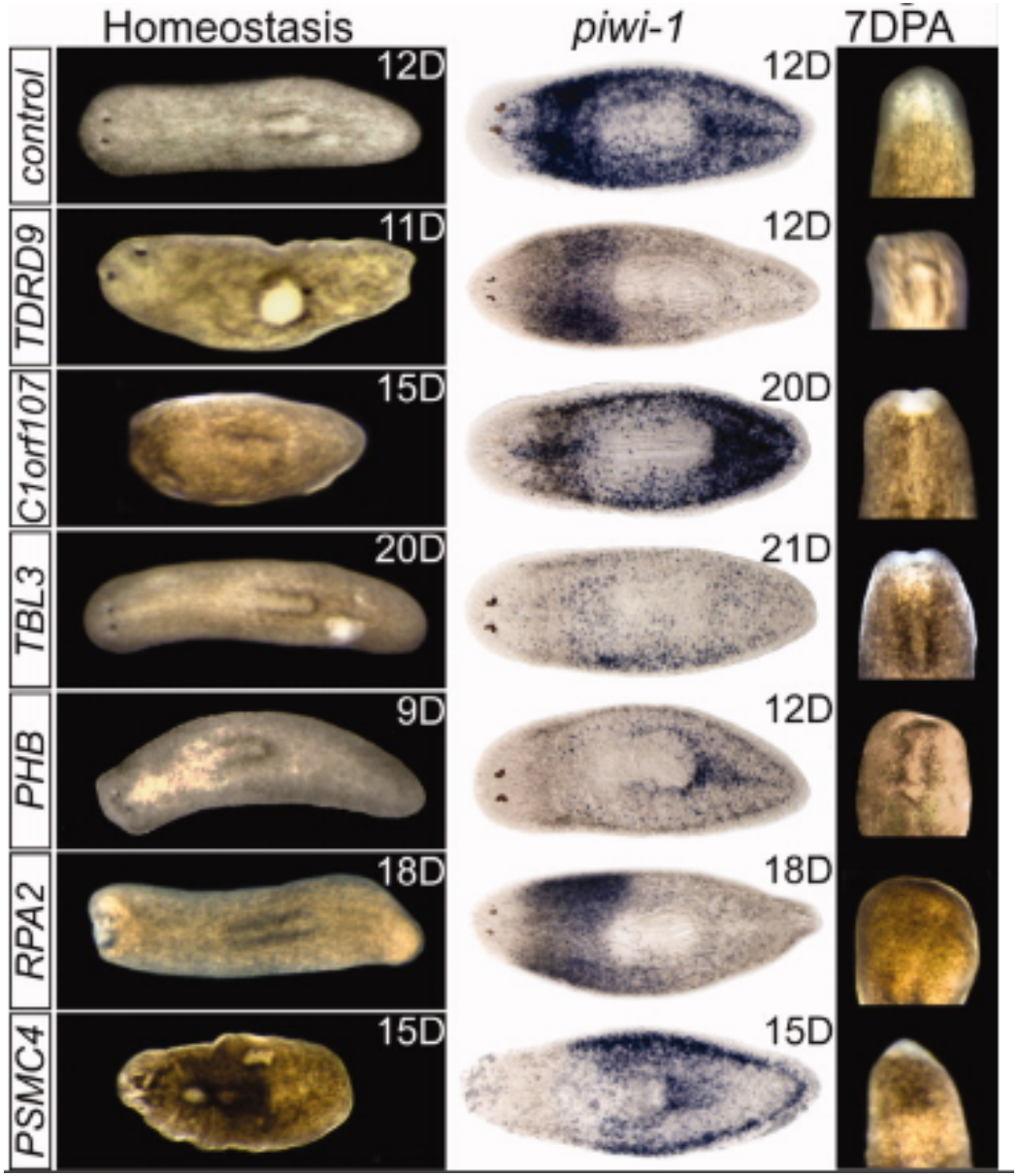
Do gene KOs affect stem cell expression and function?



RNAi induced KO of genes with no 1:1:1 ortholog but have 5 fold increase in differential gene expression in planaria models.

A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals

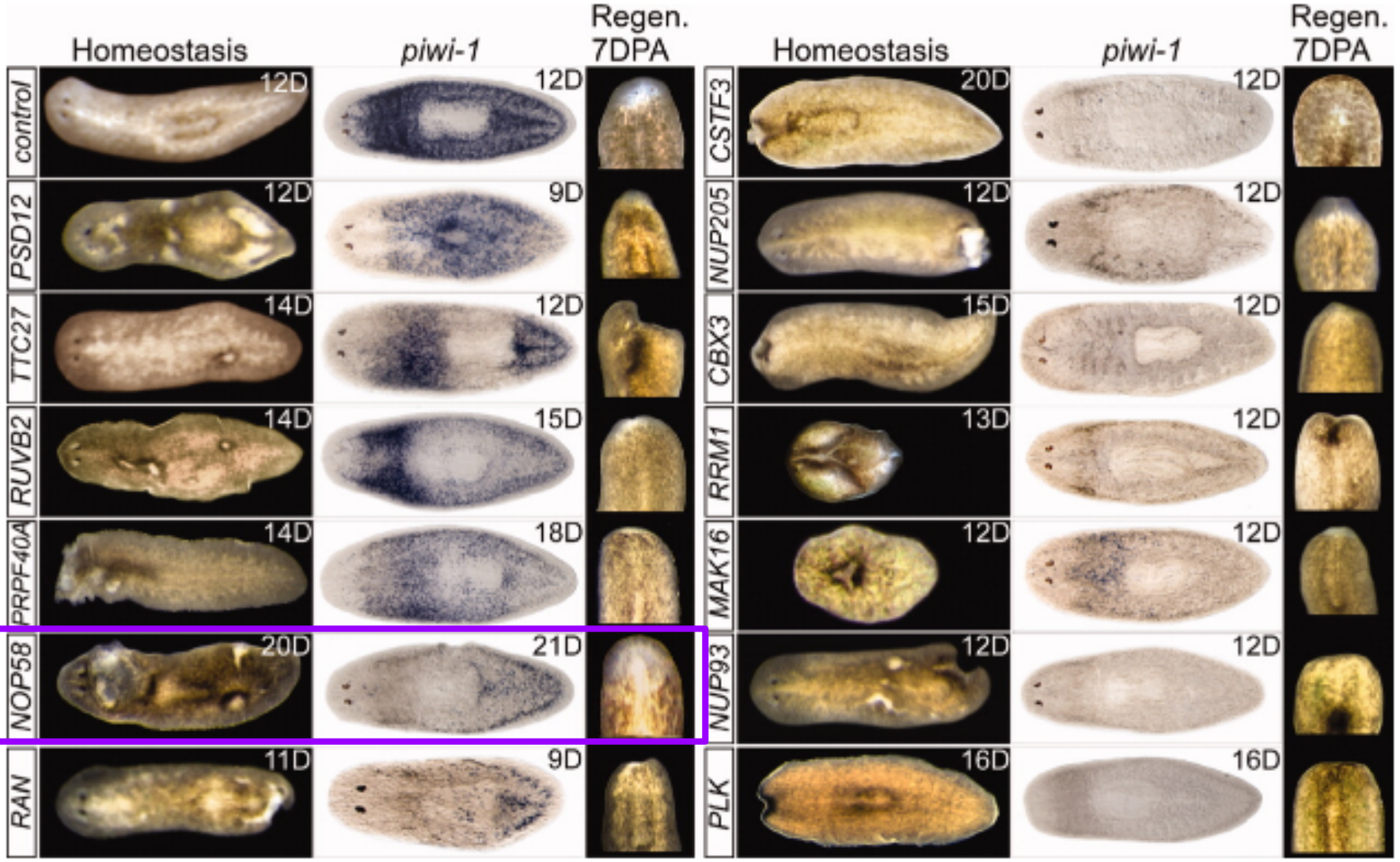
Do gene KOs affect stem cell expression and function?



RNAi induced KO of 1:1:1 planarian gene orthologs. These genes are only differentially expressed in planarian stem cells **not** mammalian embryonic stem cells.

A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals

Do gene KOs affect stem cell expression and function?

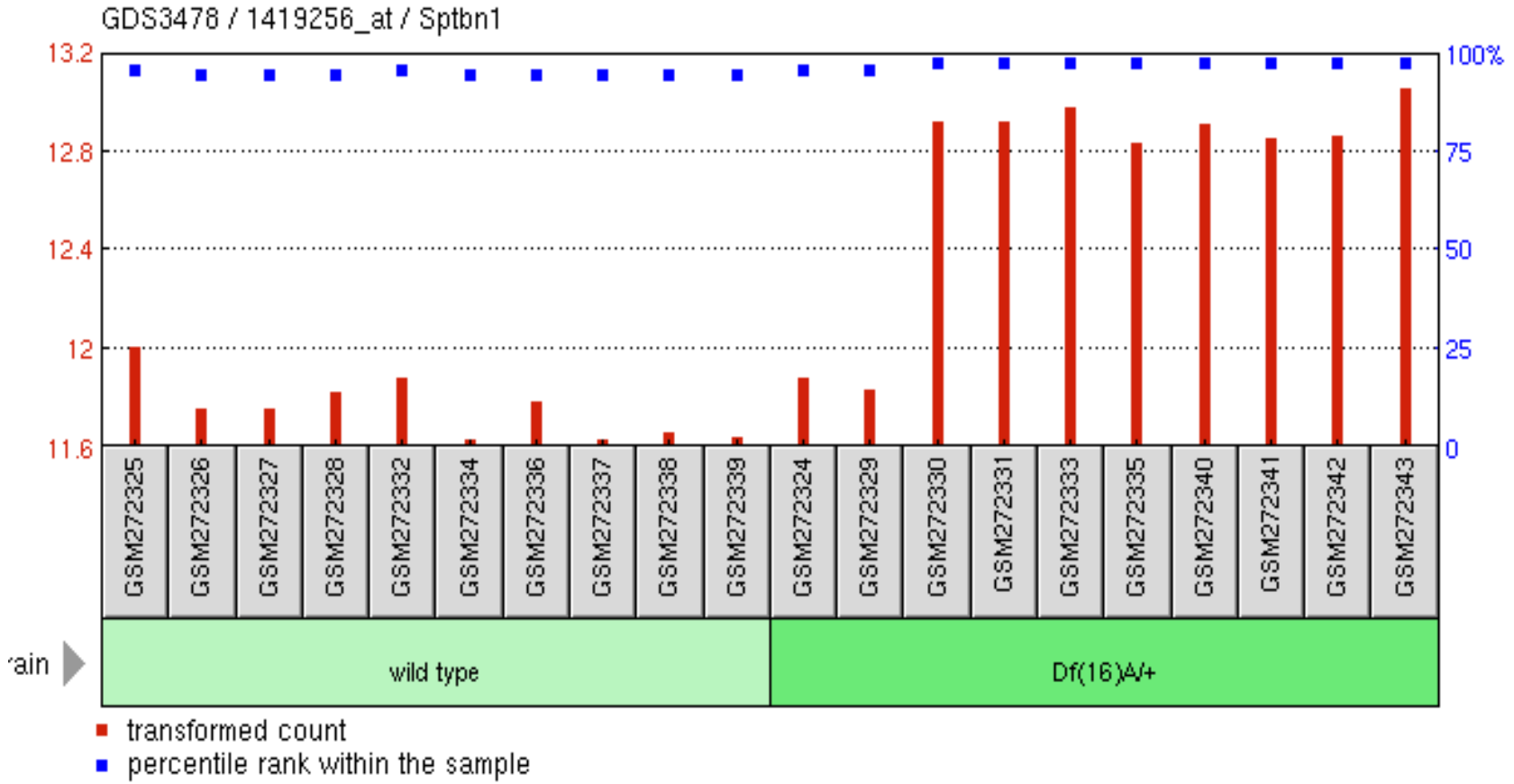


Planarian data now available

GEO: public functional genomics data repository

Gene expression omnibus

http://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS3478:1419256_at



What is the importance of studying stem cells in planaria?

Importance of studying stem cells in planaria

-Comparative between invertebrates and mammals

-Recognize conserved genetic programs

both mammals and planarians represent core, stem cell networks

-Functions and circuitry in stem cells that control self-renewal and pluripotency in multiple organisms https://macrocritters.wordpress.com/tag/planaria/