Transcriptomics

Mikayla Gallenberger
Micaela Zywicki

What is transcriptomics?

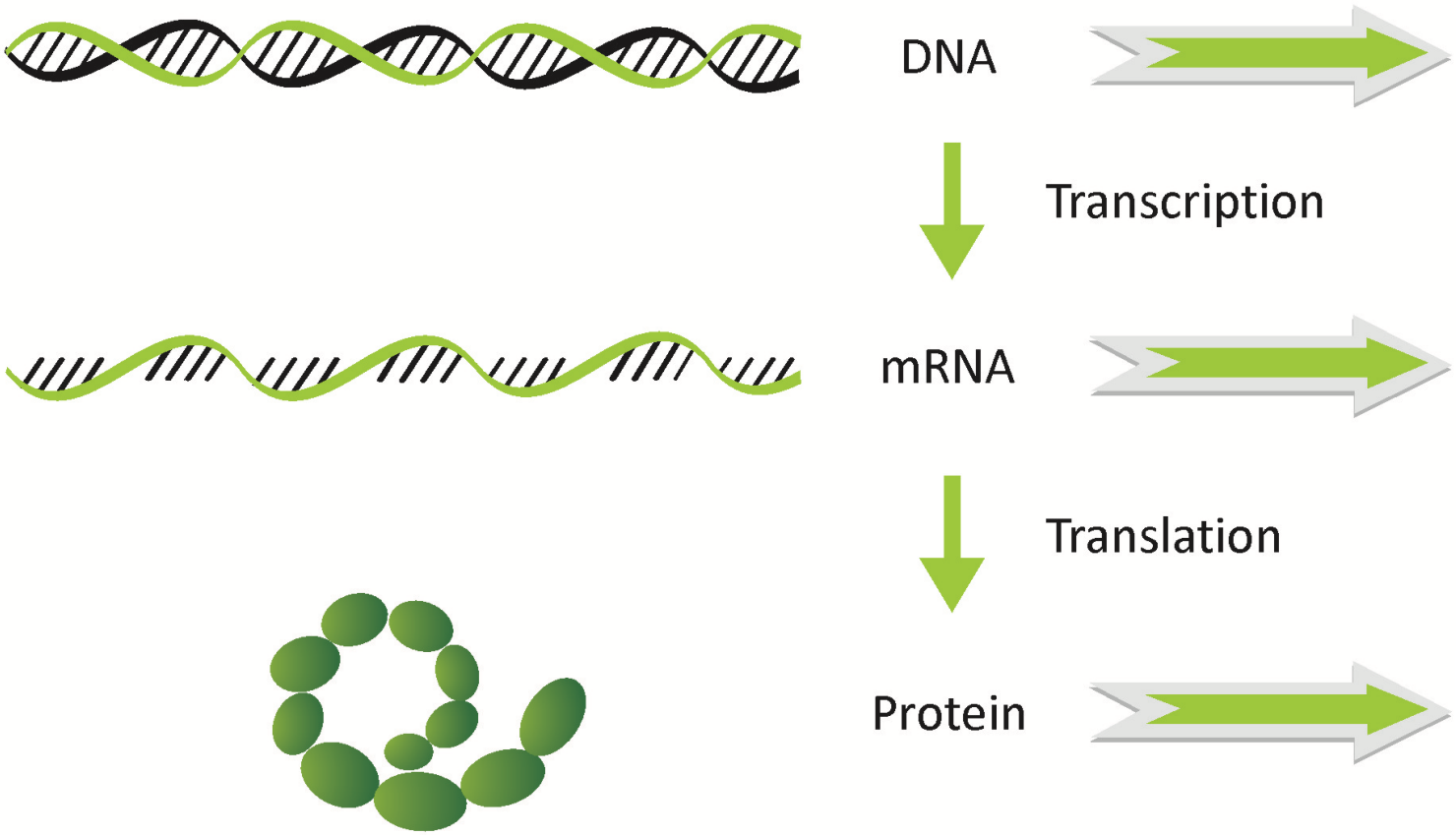
RNA-Seq

Planarians as model organisms

Stem cells

Labbe *et al.*

What is transcriptomics?



http://www.personome.com/transcriptomics.html

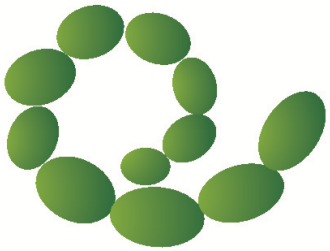
What is transcriptomics?

DNA → Genome

Transcription

mRNA → Transcriptome

Translation



Protein → Proteome

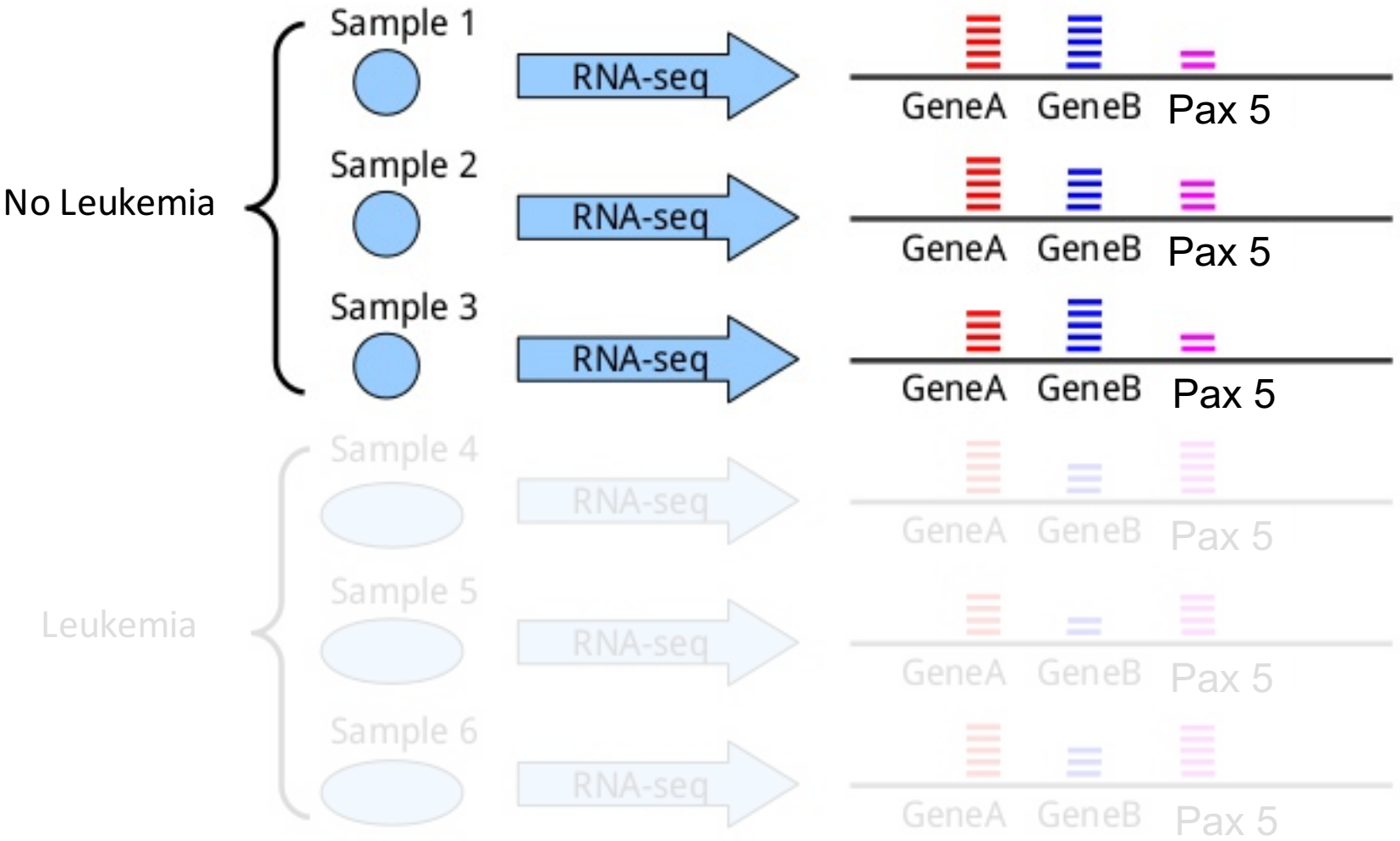
http://www.personome.com/transcriptomics.html

How can you quantify and profile mRNAs?

How can you quantify and profile mRNAs?

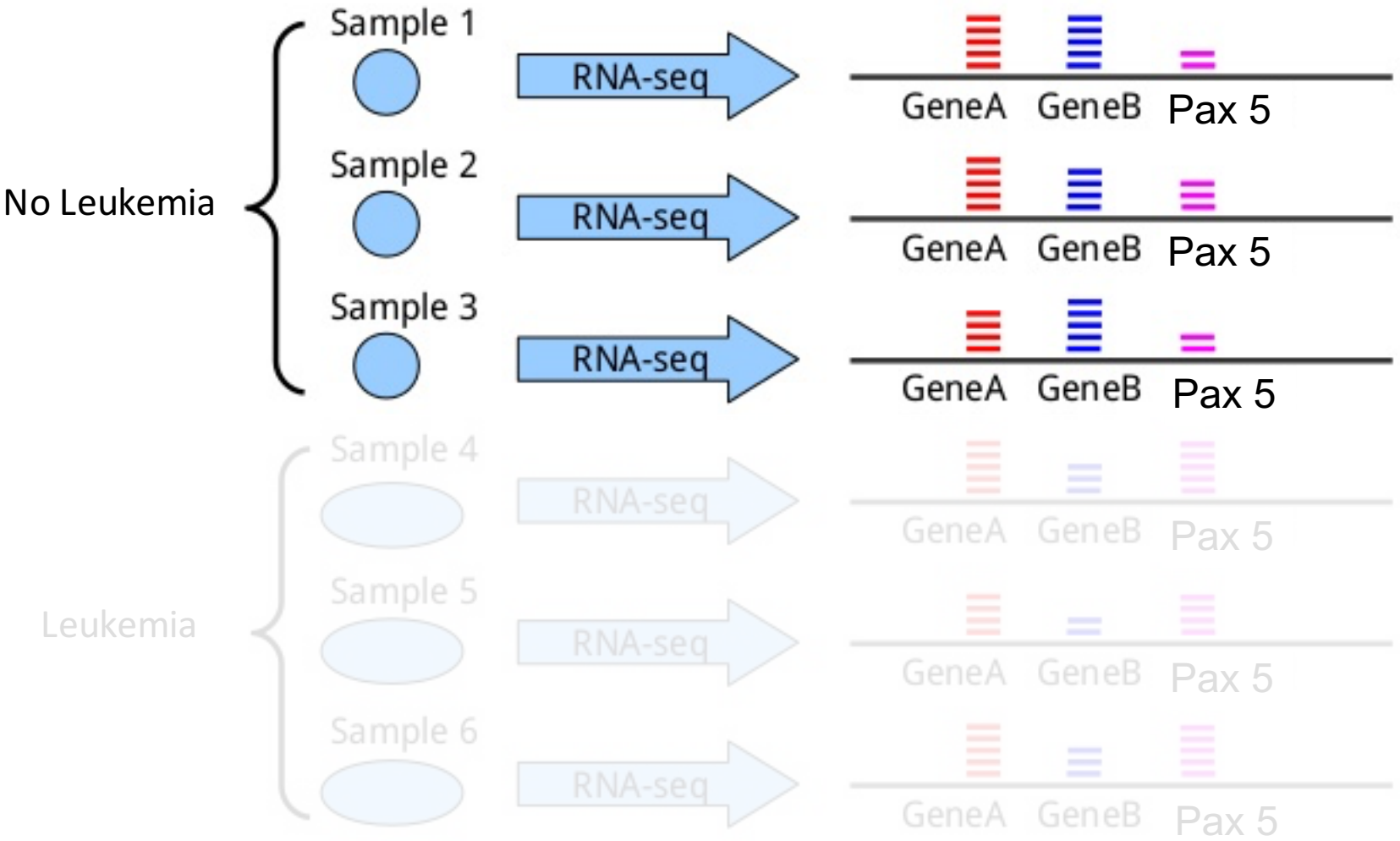
RNA-Seq

What does RNA-Seq measure?



http://www.slideshare.net/joachimjacob/1rna-seqpart1working-tothegoal

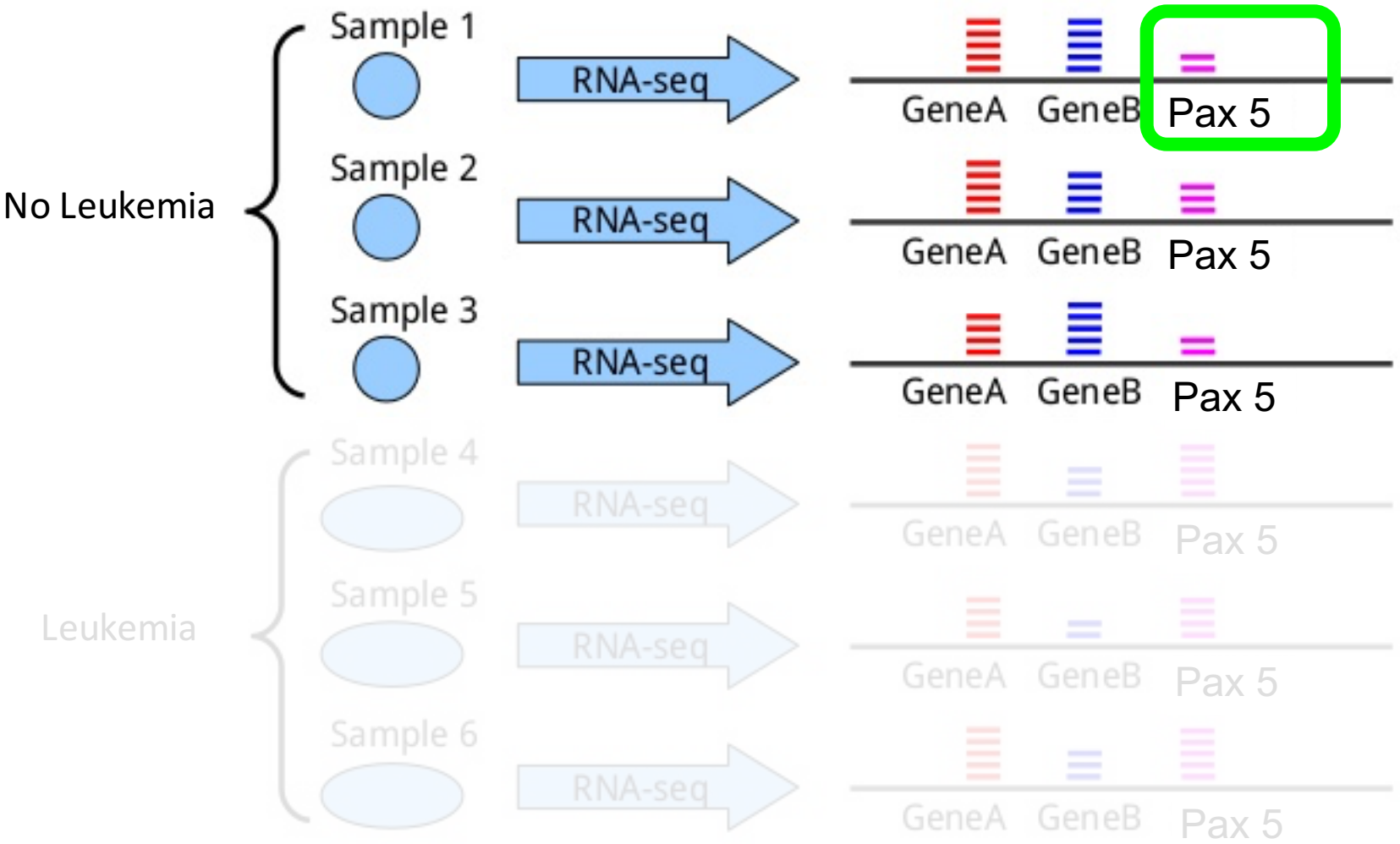
What does RNA-Seq measure?



gene expression levels

http://www.slideshare.net/joachimjacob/1rna-seqpart1working-tothegoal

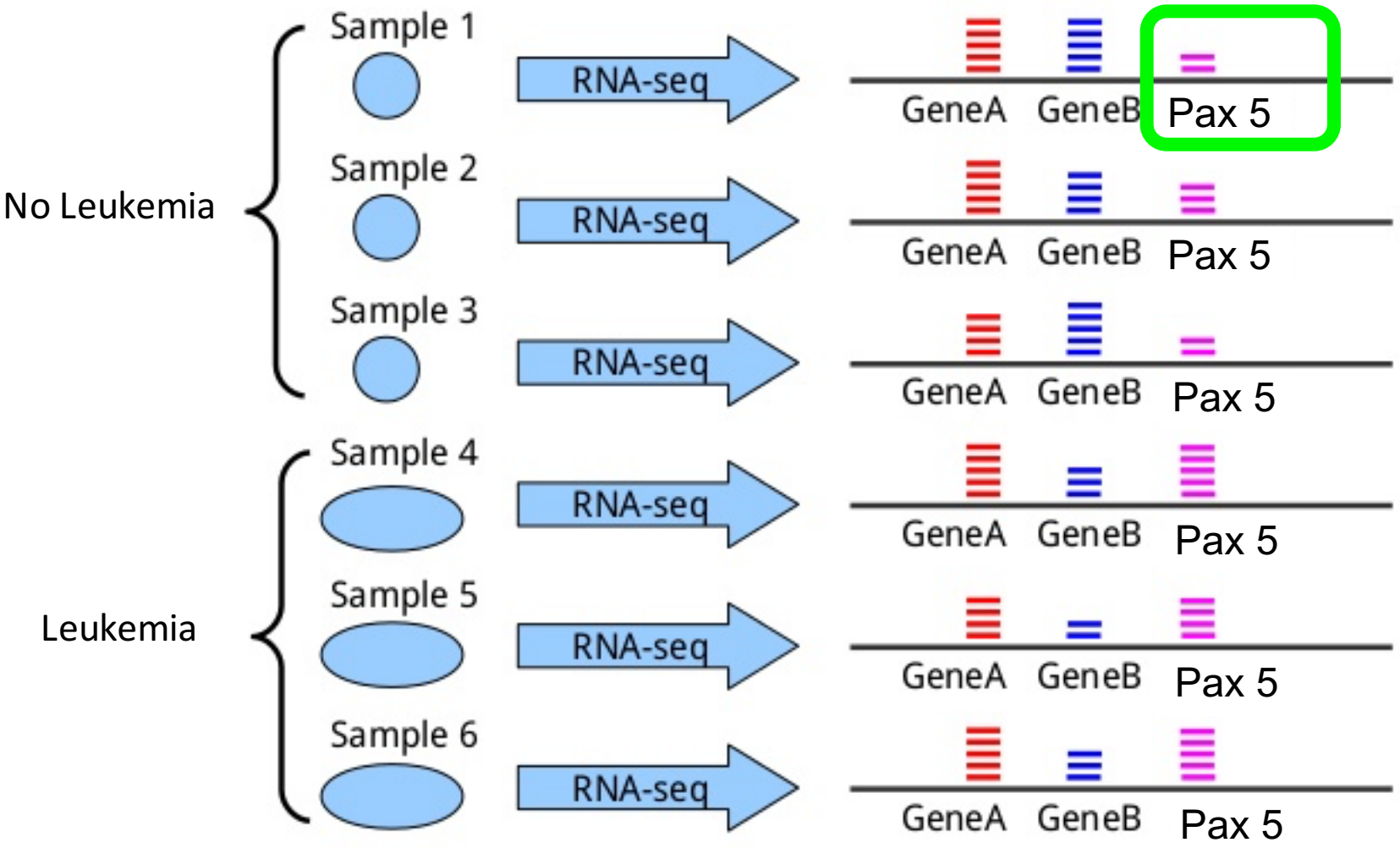
What does RNA-Seq measure?



gene expression levels

http://www.slideshare.net/joachimjacob/1rna-seqpart1working-tothegoal

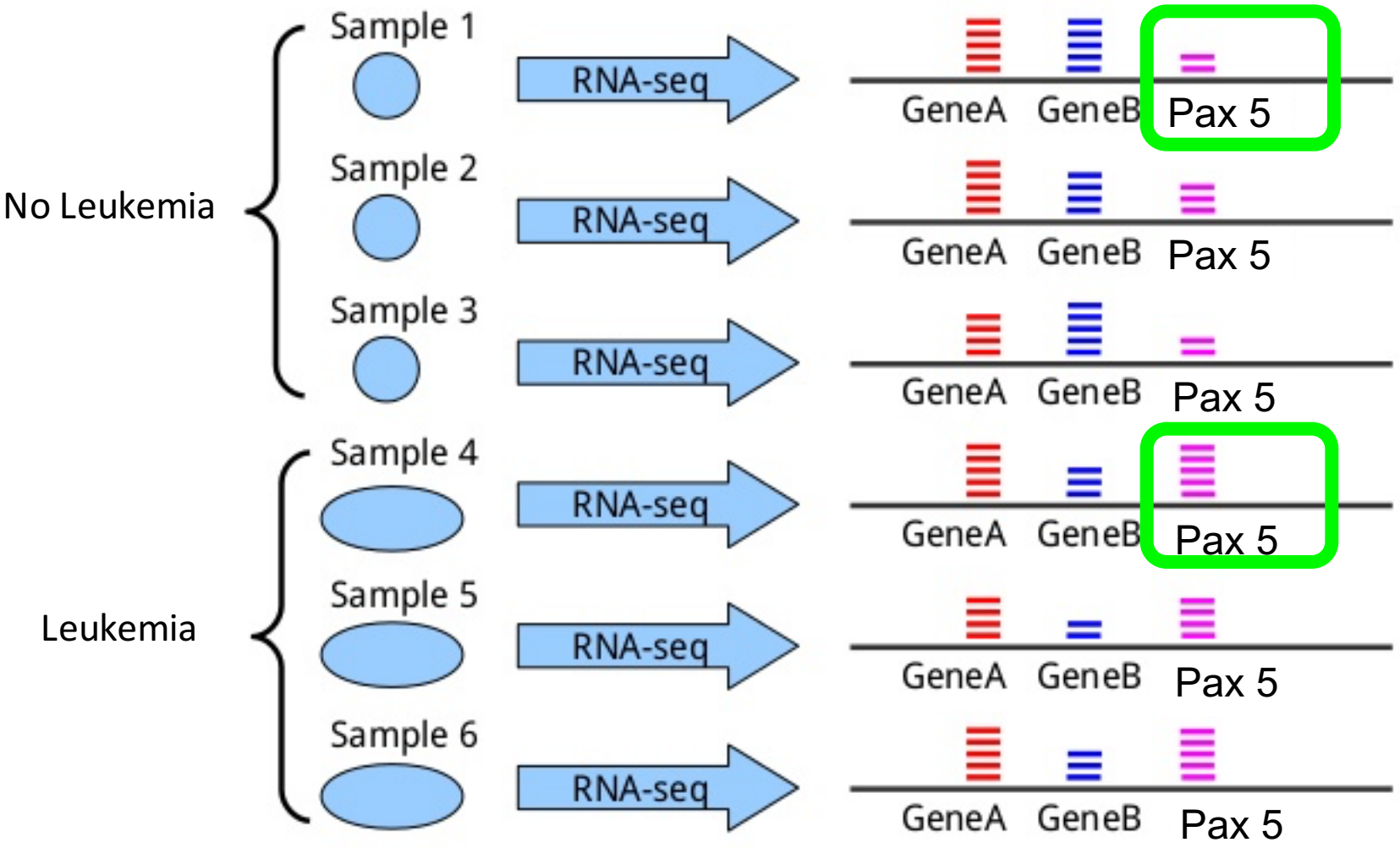
What does RNA-Seq measure?



gene expression levels

http://www.slideshare.net/joachimjacob/1rna-seqpart1working-tothegoal

What does RNA-Seq measure?

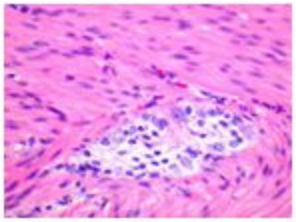


gene expression levels

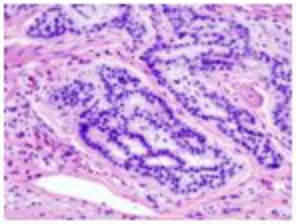
http://www.slideshare.net/joachimjacob/1rna-seqpart1working-tothegoal

How is RNA-Seq performed?

Samples of interest



No Leukemia
Normal Bone Marrow



Leukemia
Diseased Bone Marrow

Isolate RNAs

Generate cDNA, fragment, size select, add linkers

Sequence ends

100s of millions of paired reads
10s of billions bases of sequence

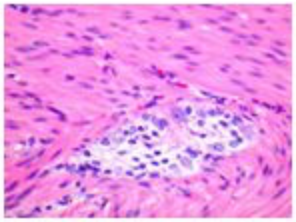
Map to genome, transcriptome, and predicted exon junctions

Downstream analysis

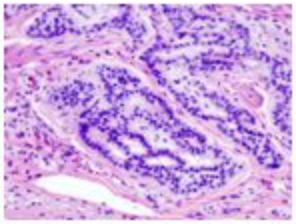
http://slideplayer.com/slide/1455873/

How is RNA-Seq performed?

Samples of interest

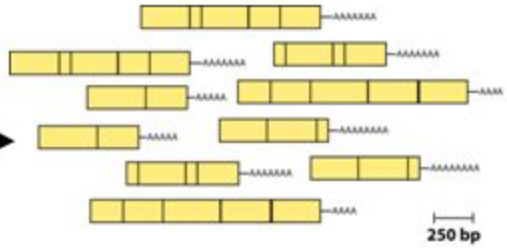


No Leukemia
Normal Bone Marrow



Leukemia
Diseased Bone Marrow

Isolate RNAs



Generate cDNA, fragment, size select, add linkers

Sequence ends

100s of millions of paired reads
10s of billions bases of sequence

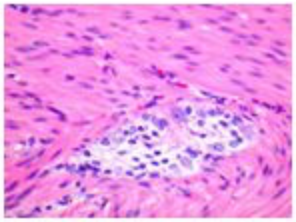
Map to genome, transcriptome, and predicted exon junctions

Downstream analysis

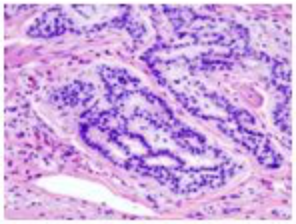
http://slideplayer.com/slide/1455873/

How is RNA-Seq performed?

Samples of interest

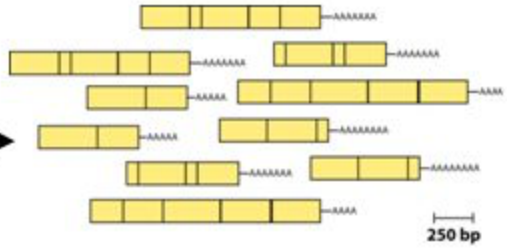


No Leukemia
Normal Bone Marrow

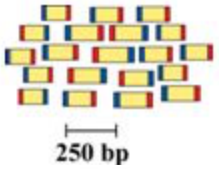


Leukemia
Diseased Bone Marrow

Isolate RNAs



Generate cDNA, fragment, size select, add linkers



Sequence ends

100s of millions of paired reads
10s of billions bases of sequence

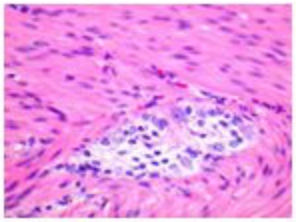
Map to genome, transcriptome, and predicted exon junctions

Downstream analysis

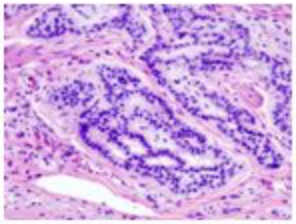
http://slideplayer.com/slide/1455873/

How is RNA-Seq performed?

Samples of interest

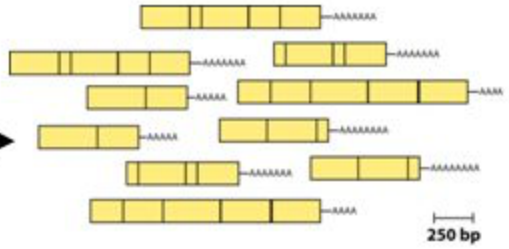


No Leukemia
Normal Bone Marrow

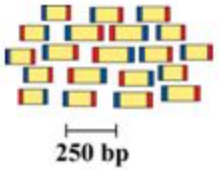


Leukemia
Diseased Bone Marrow

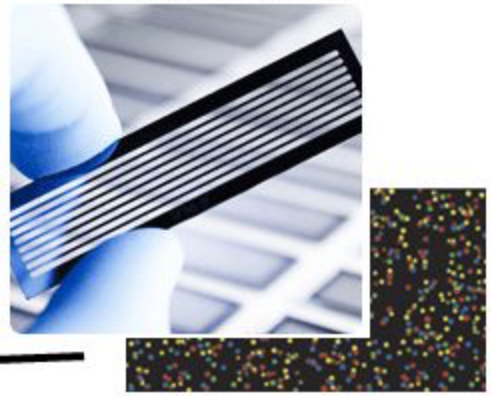
Isolate RNAs



Generate cDNA, fragment, size select, add linkers



Sequence ends



100s of millions of paired reads
10s of billions bases of sequence

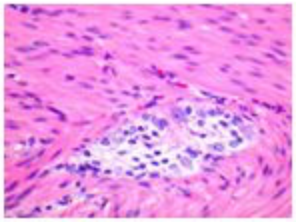
Map to genome, transcriptome, and predicted exon junctions

Downstream analysis

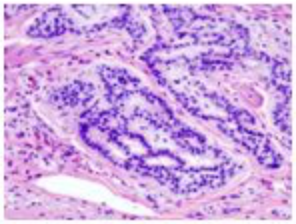
http://slideplayer.com/slide/1455873/

How is RNA-Seq performed?

Samples of interest

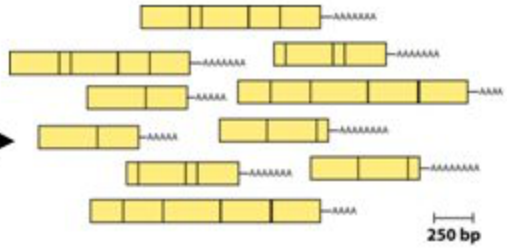


No Leukemia
Normal Bone Marrow

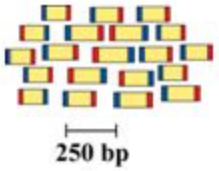


Leukemia
Diseased Bone Marrow

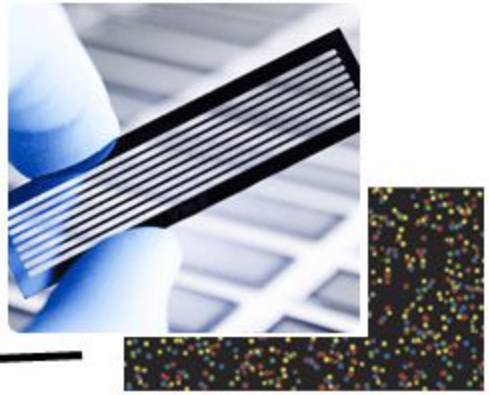
Isolate RNAs



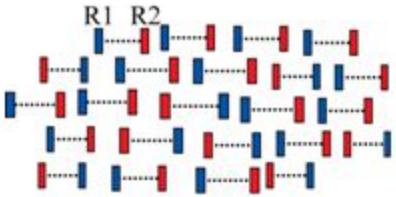
Generate cDNA, fragment, size select, add linkers



Sequence ends



100s of millions of paired reads
10s of billions bases of sequence



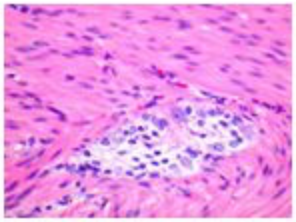
Map to genome, transcriptome, and predicted exon junctions

Downstream analysis

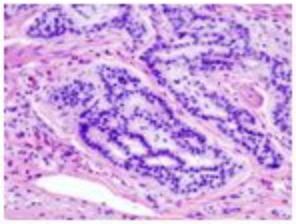
http://slideplayer.com/slide/1455873/

How is RNA-Seq performed?

Samples of interest

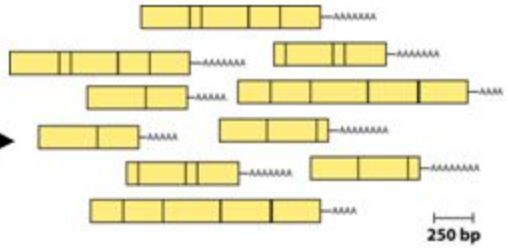


No Leukemia
Normal Bone Marrow

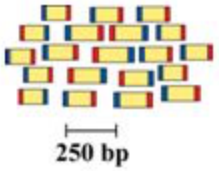


Leukemia
Diseased Bone Marrow

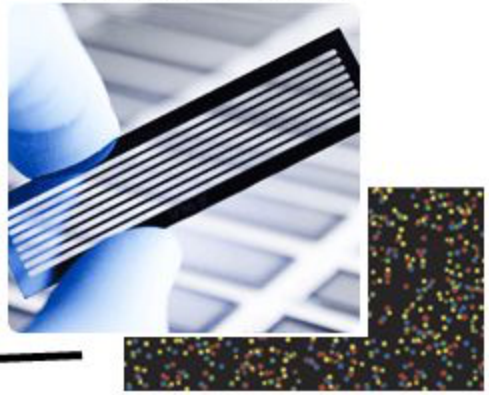
Isolate RNAs



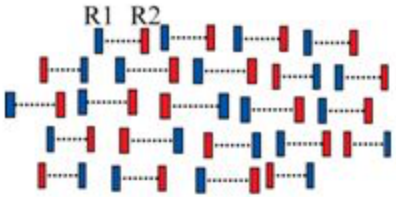
Generate cDNA, fragment, size select, add linkers



Sequence ends



100s of millions of paired reads
10s of billions bases of sequence



Map to genome, transcriptome, and predicted exon junctions

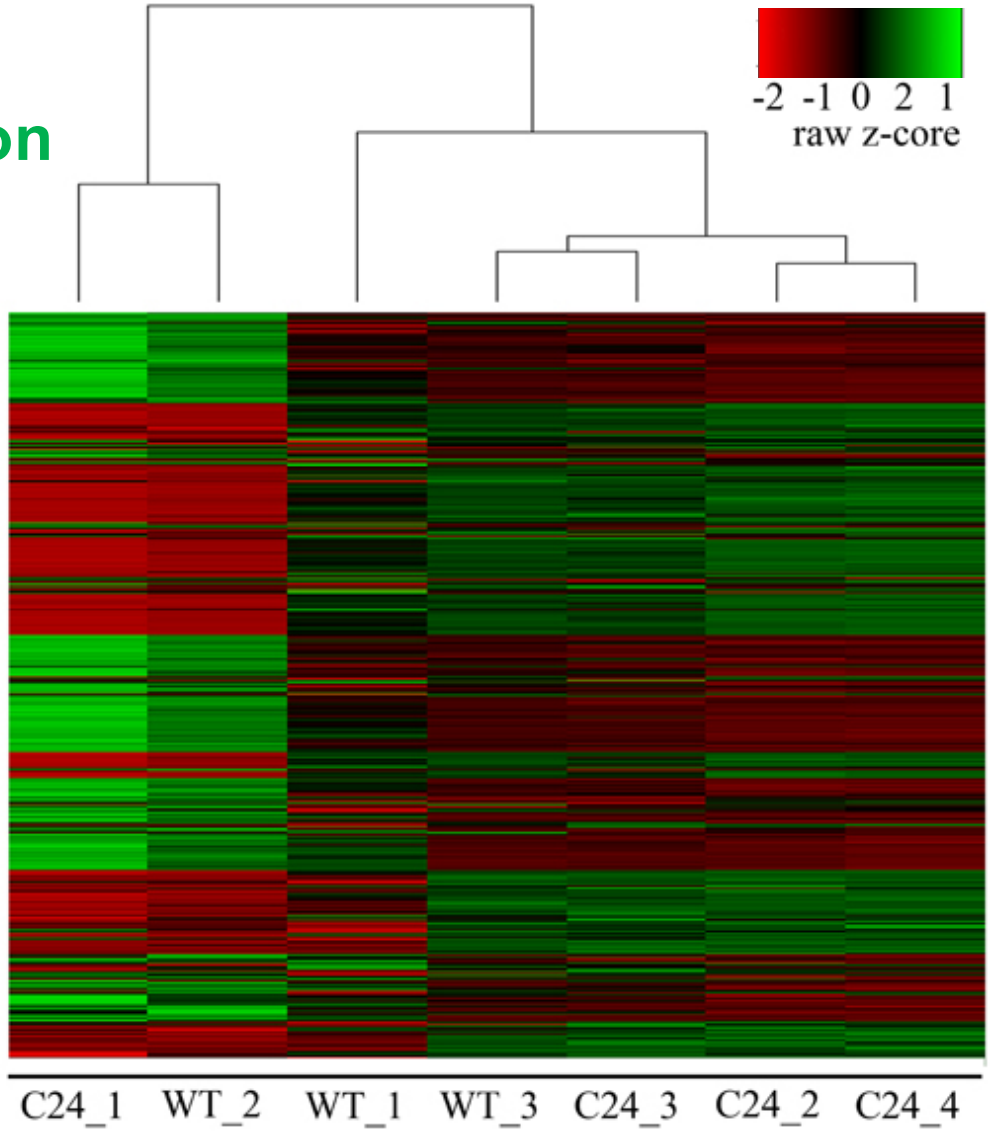
Downstream analysis

http://slideplayer.com/slide/1455873/

How do you analyze RNA-seq data?

Green=High Expression

Red=Low Expression



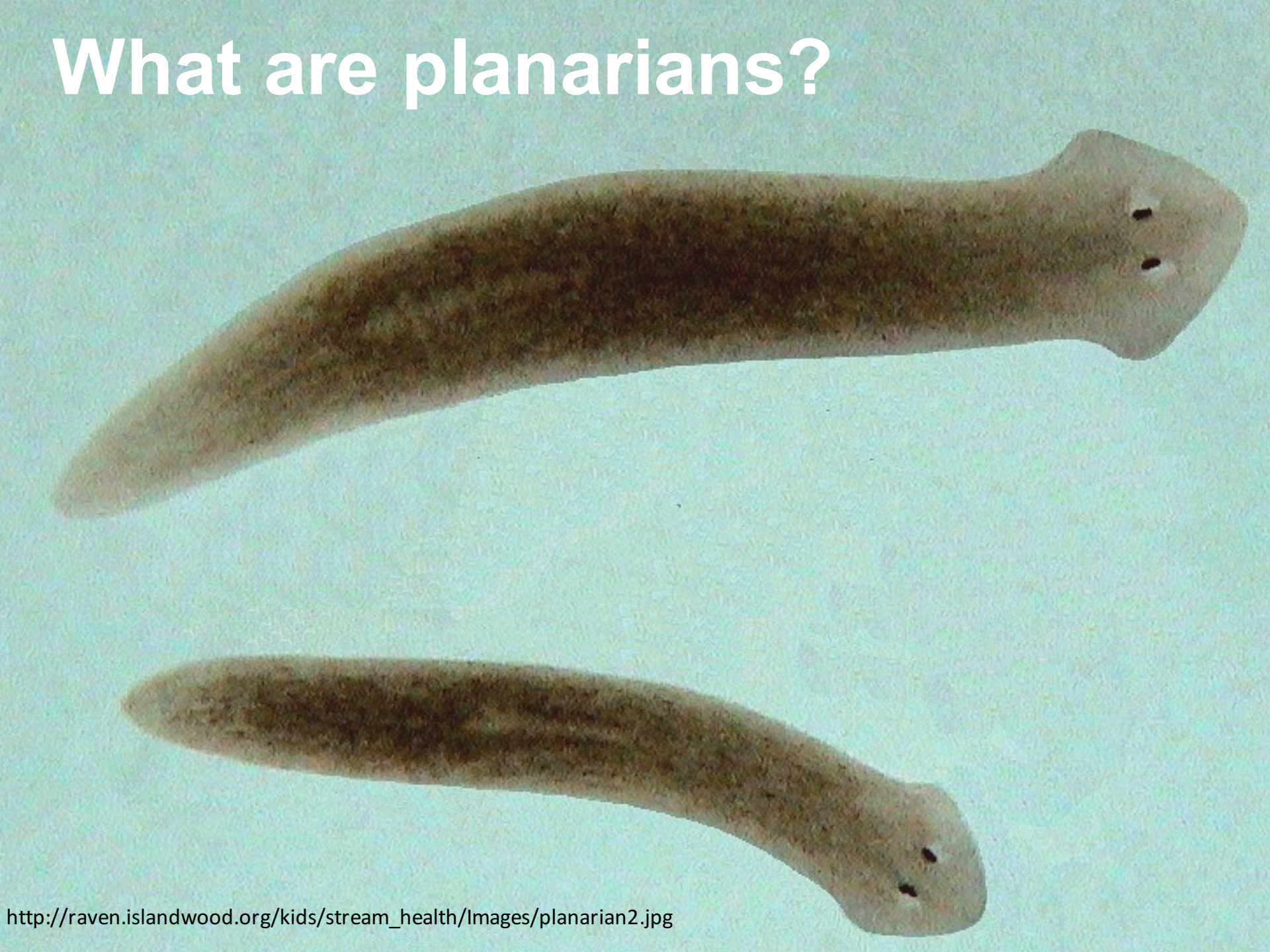
http://journal.frontiersin.org/article/10.3389/fgene.2014.00043/full

Why bother with RNA-seq?

Technology	Tiling microarray	cDNA or EST sequencing	RNA-Seq
Technology specifications			
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
Application			
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
Practical issues			
Required amount of RNA	High	High	Low
Cost for mapping transcriptomes of large genomes	High	High	Relatively low

How can you apply RNA-seq to your project?

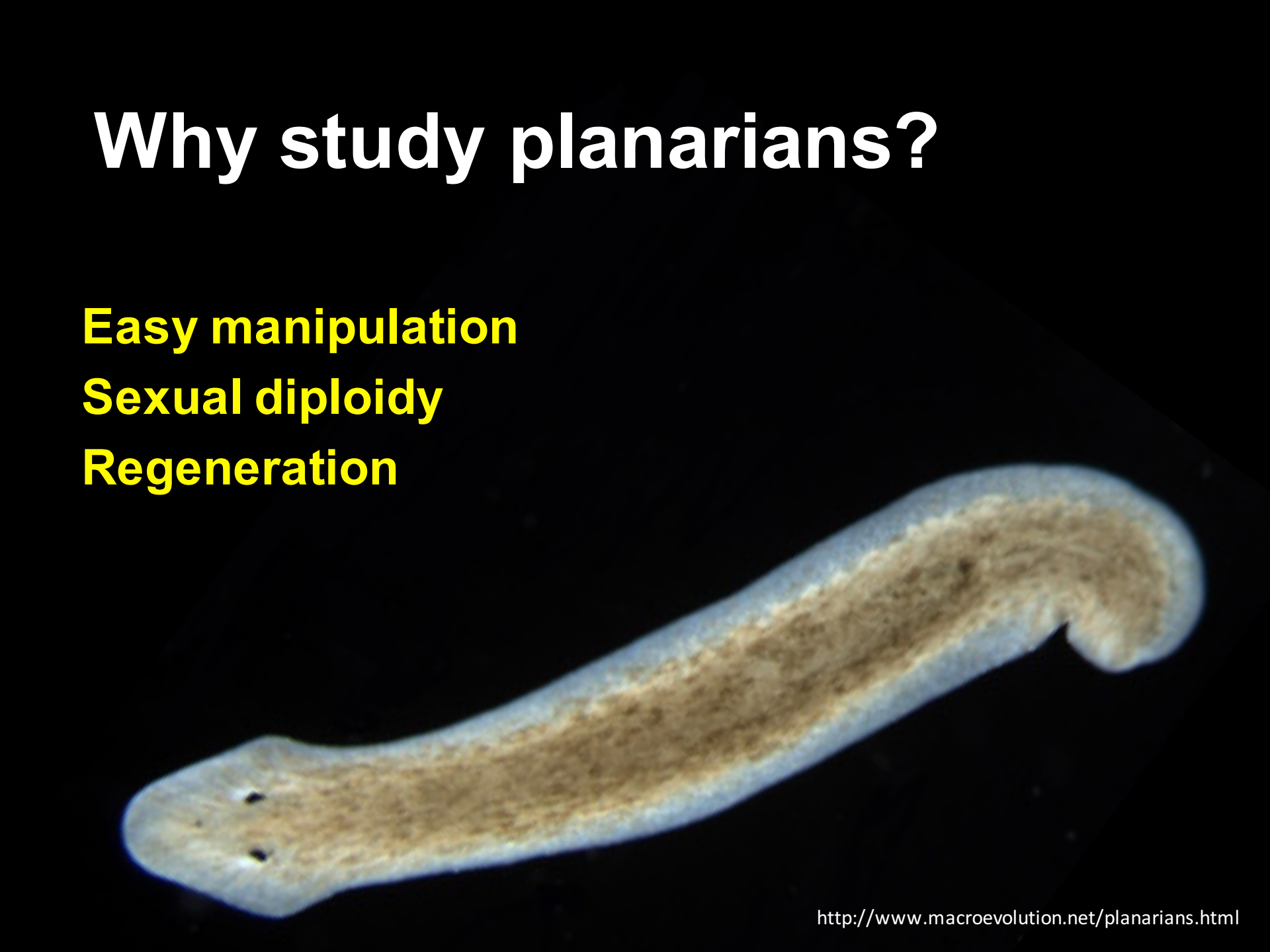
What are planarians?



http://raven.islandwood.org/kids/stream_health/Images/planarian2.jpg

Why study planarians?

Easy manipulation
Sexual diploidy
Regeneration



http://www.macroevolution.net/planarians.html

How are planarians grown in lab?

How does the environment affect planarian growth?

Nature Reviews | Genetics

http://prometheus.med.utah.edu/~marclab/Newmark_Sanchez_Nature_2002.pdf

Why are planarians a good model to study regeneration?

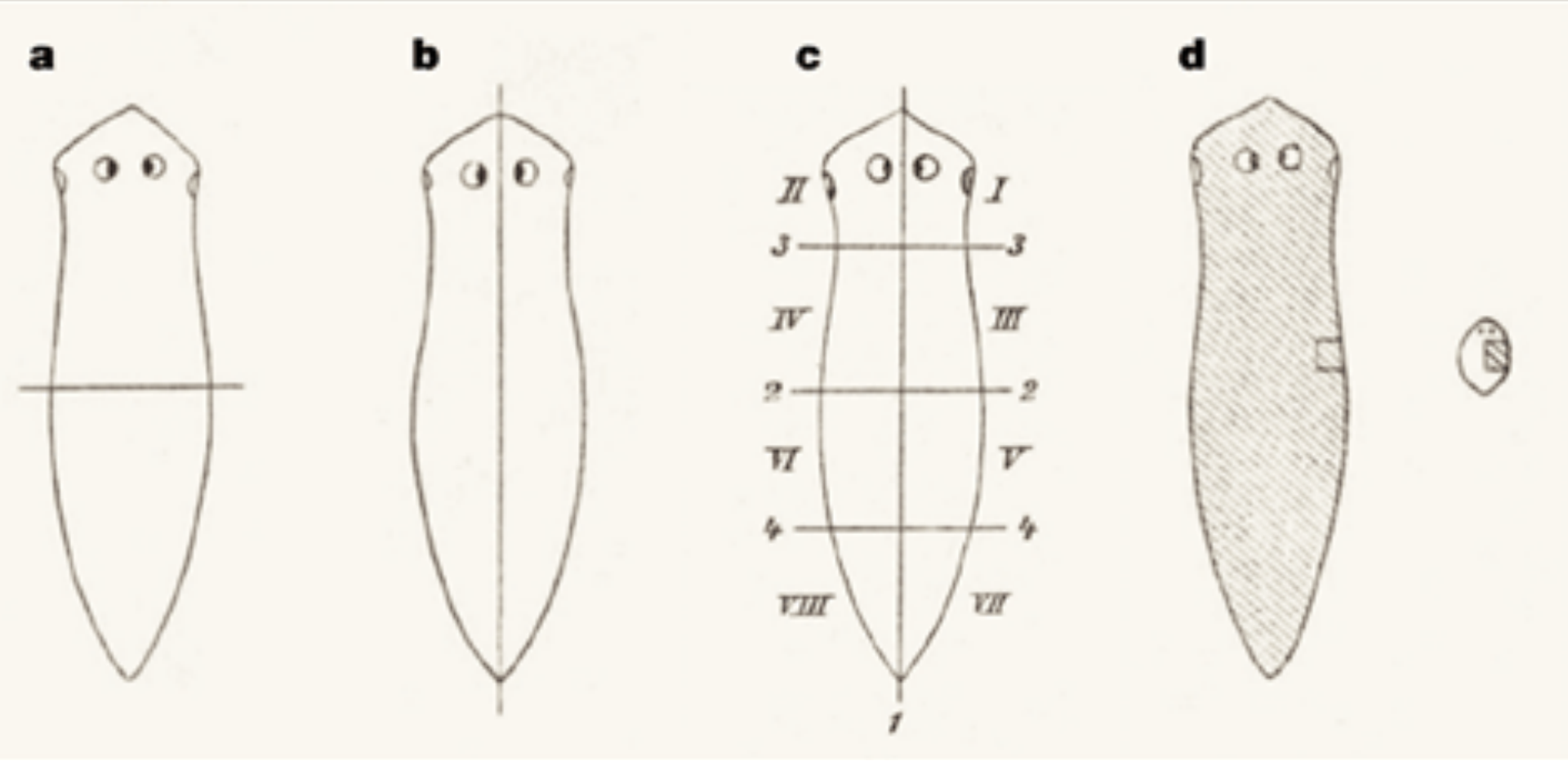


FIGURE 2 | The planarian's regenerative and remodelling abilities.

Do you believe us?

https://www.youtube.com/watch?v=vXN_5SPBPtM

What are stem cells?

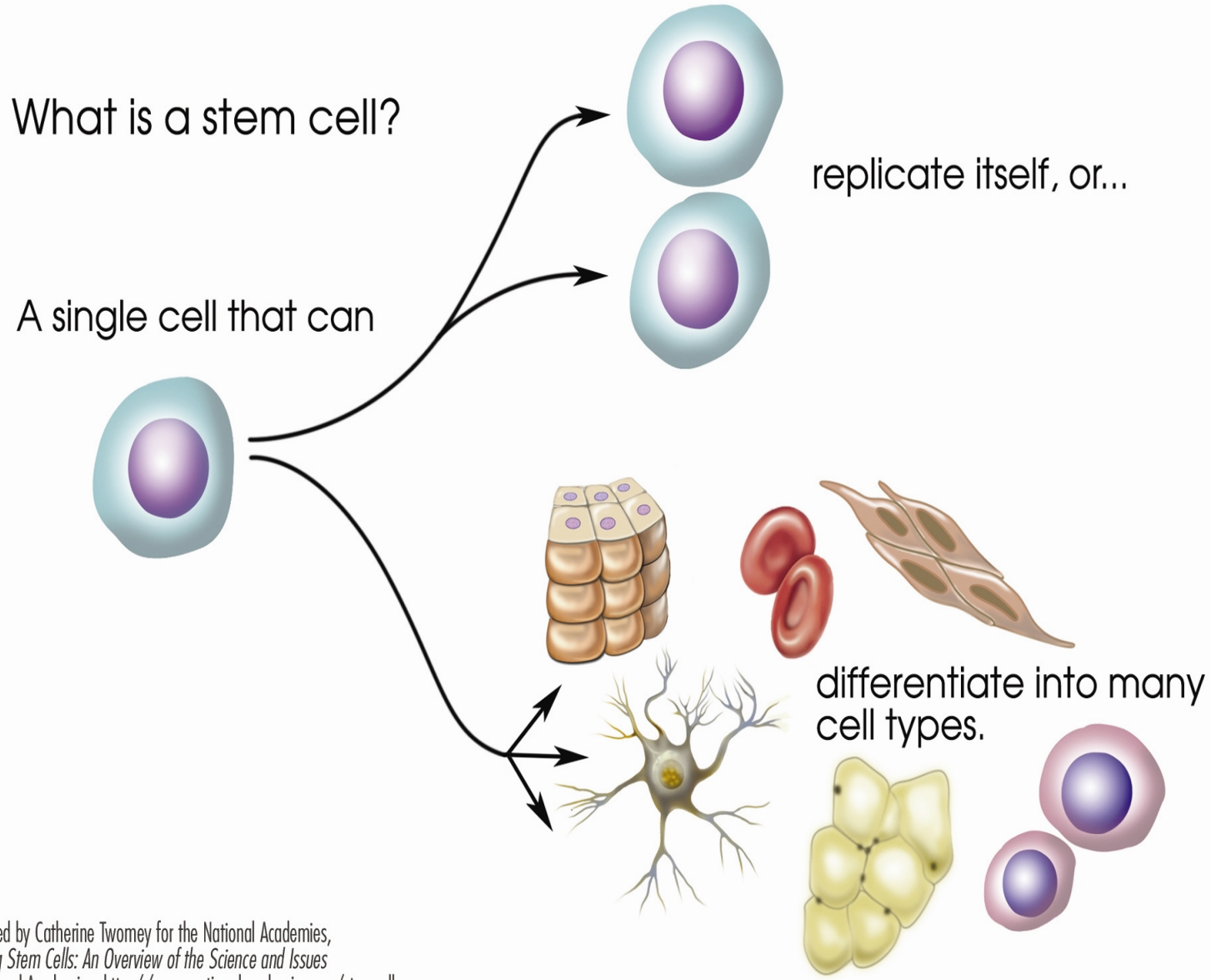


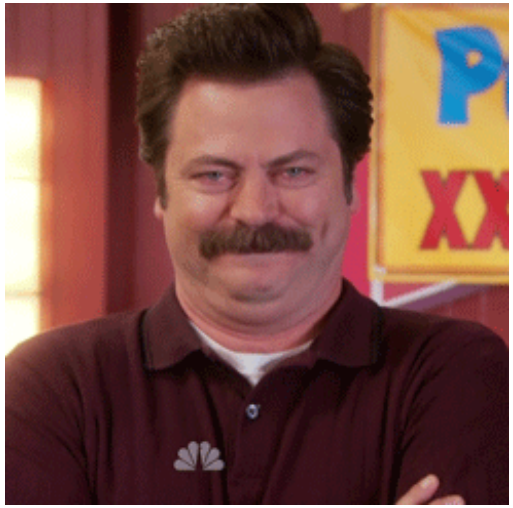
Image prepared by Catherine Twomey for the National Academies,
Understanding Stem Cells: An Overview of the Science and Issues
from the National Academies, http://www.nationalacademies.org/stemcells.
Academic noncommercial use is permitted.

http://nas-sites.org/stemcells/stem-cell-basics/download-stem-cell-figure

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

Labbé, et al.

What species did the paper compare?

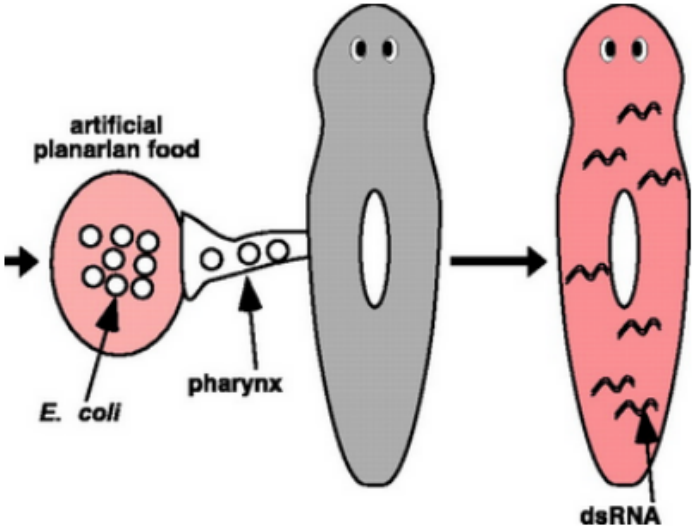


http://www.biologyjunction.com/planarian_regeneration_by_lora_b.htm

http://favim.com/image/251684/

http://replygif.net/t/ron-swanson

How were the species compared?

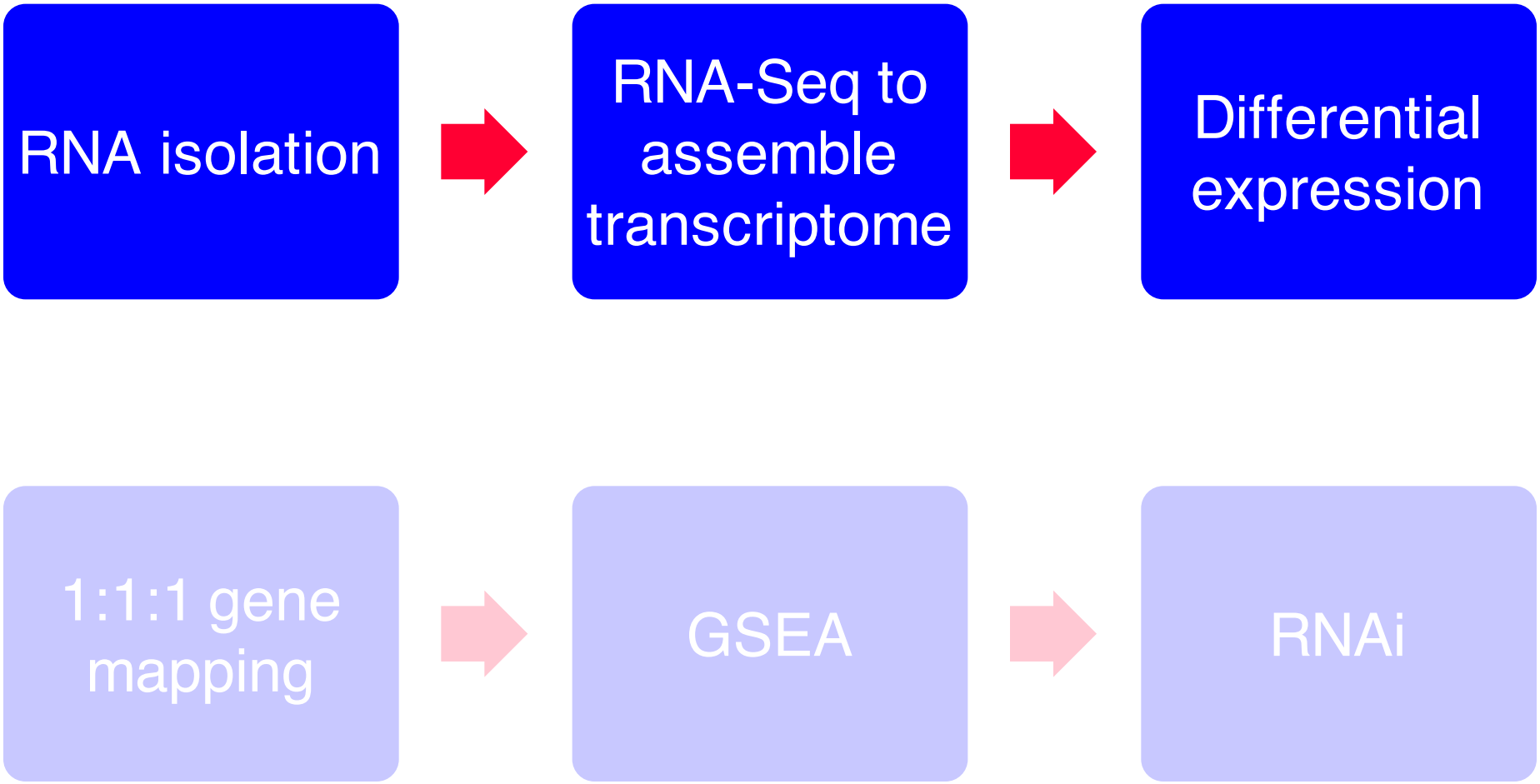


http://www.illumina.com/

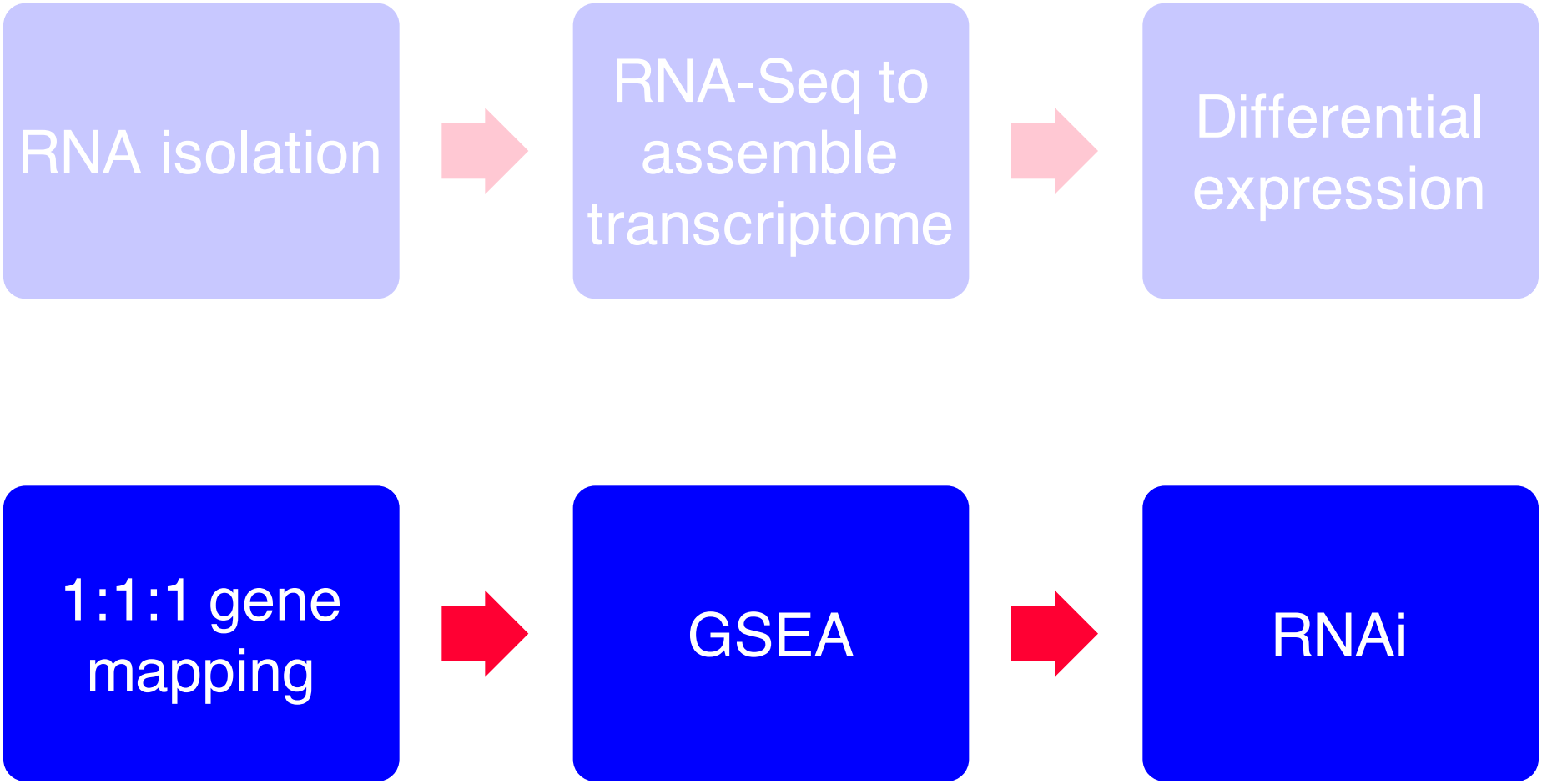
http://www.bioinformatics-made-simple.com/2012/02/5-server-for-batch-blast-

http://genetics564.weebly.com/uploads/8/6/5/7/865764/ranscriptomics3-3-15kellyandtony.pdf

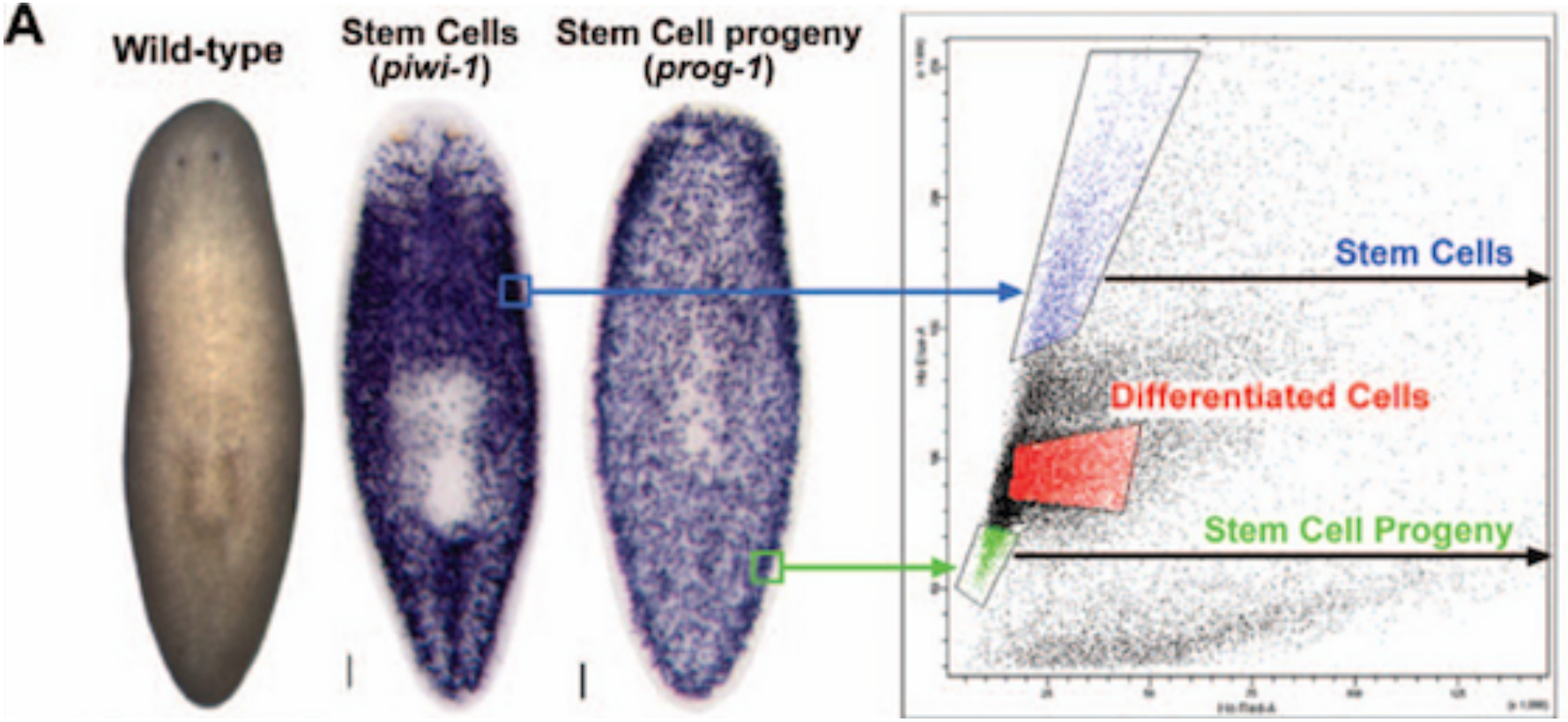
What was their plan?



What was their plan?



How did they isolate RNA?



Fluorescence-Activated Cell Sorting of irradiated and non-irradiated planarians

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

How did they determine differential expression (DE)?

Stem cells RNA

Stem cell progeny RNA

Differentiated cells RNA

http://www.illumina.com/

What did RNA-Seq output show?

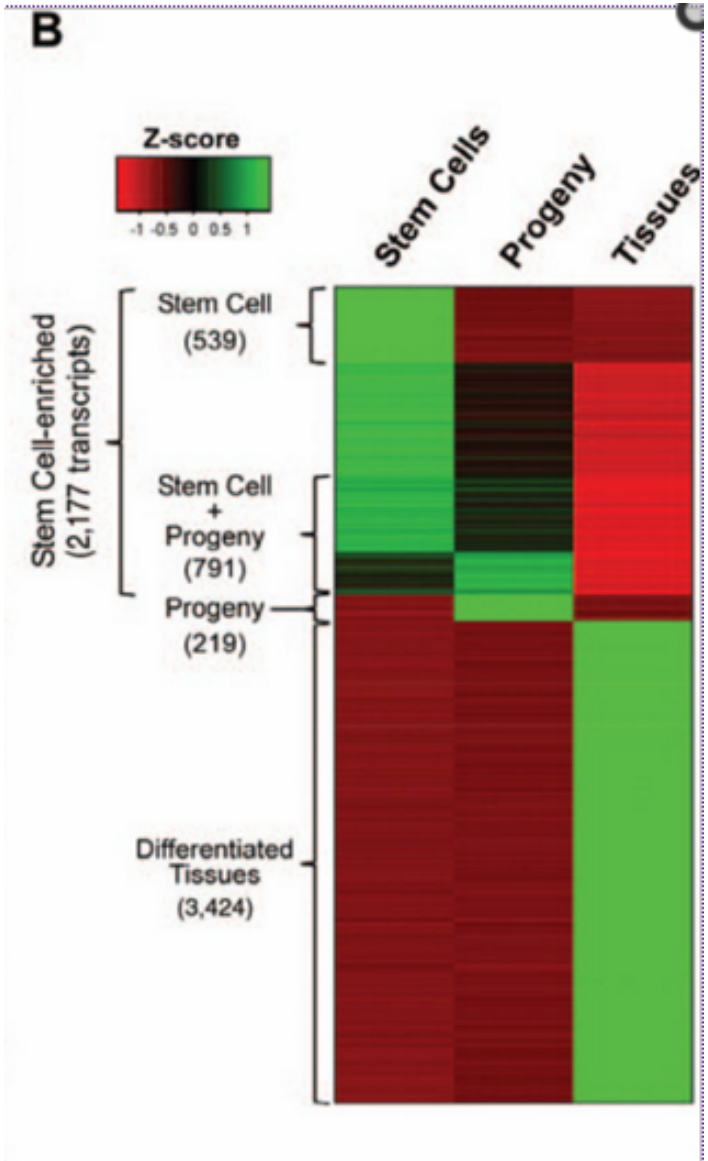
Green=High Expression

Red=Low Expression

2,177 transcripts DE in stem cell compartment

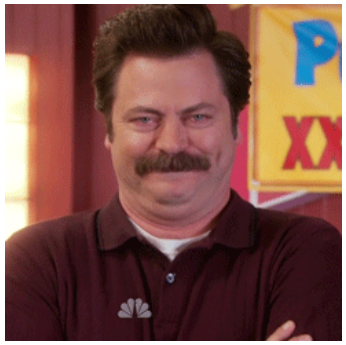
539 transcripts DE in stem cells

219 transcripts DE in progeny



Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

How did they compare gene expression to mammalian ESCs?

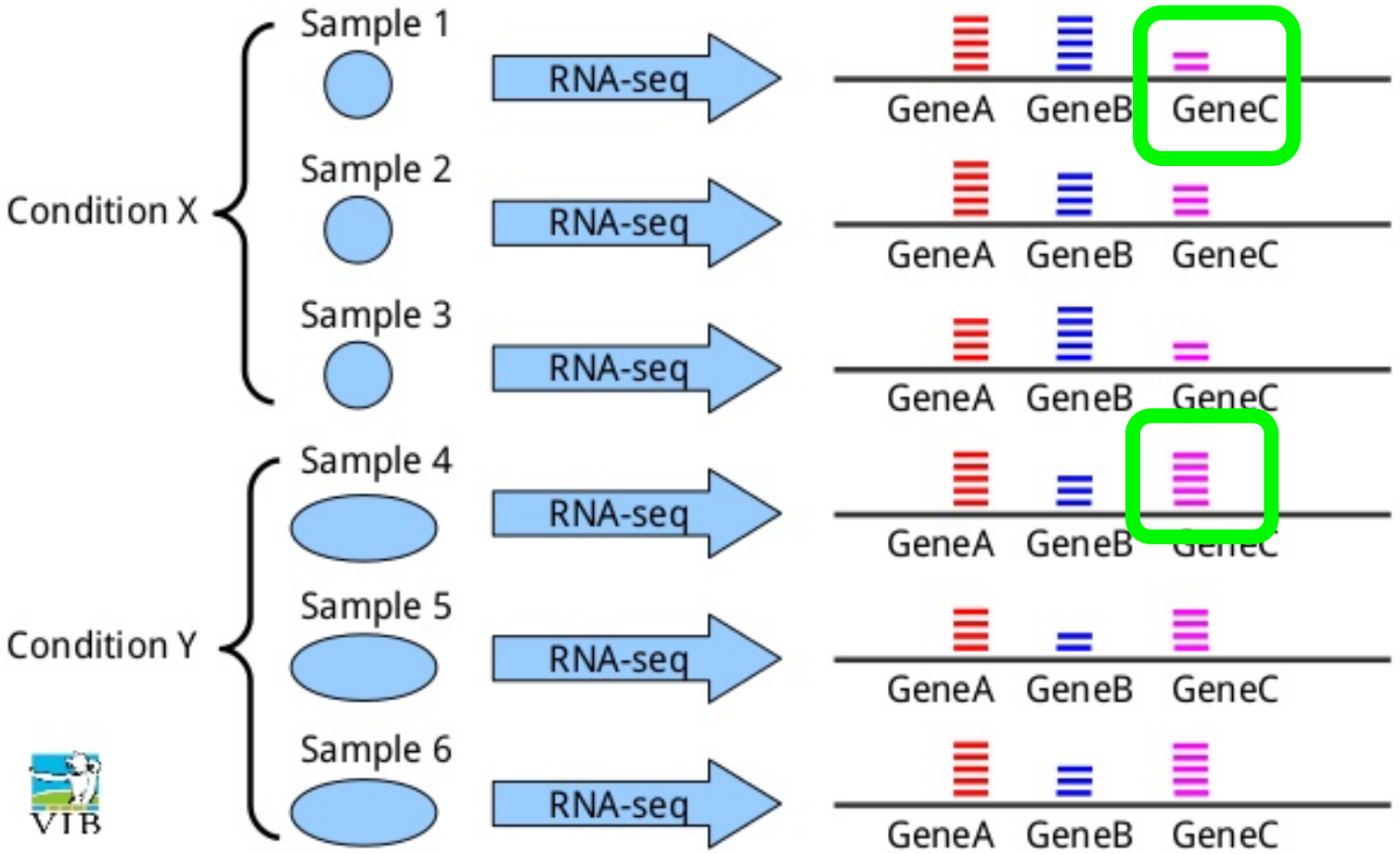


e!Ensembl

>5 fold cRPKM values= DE

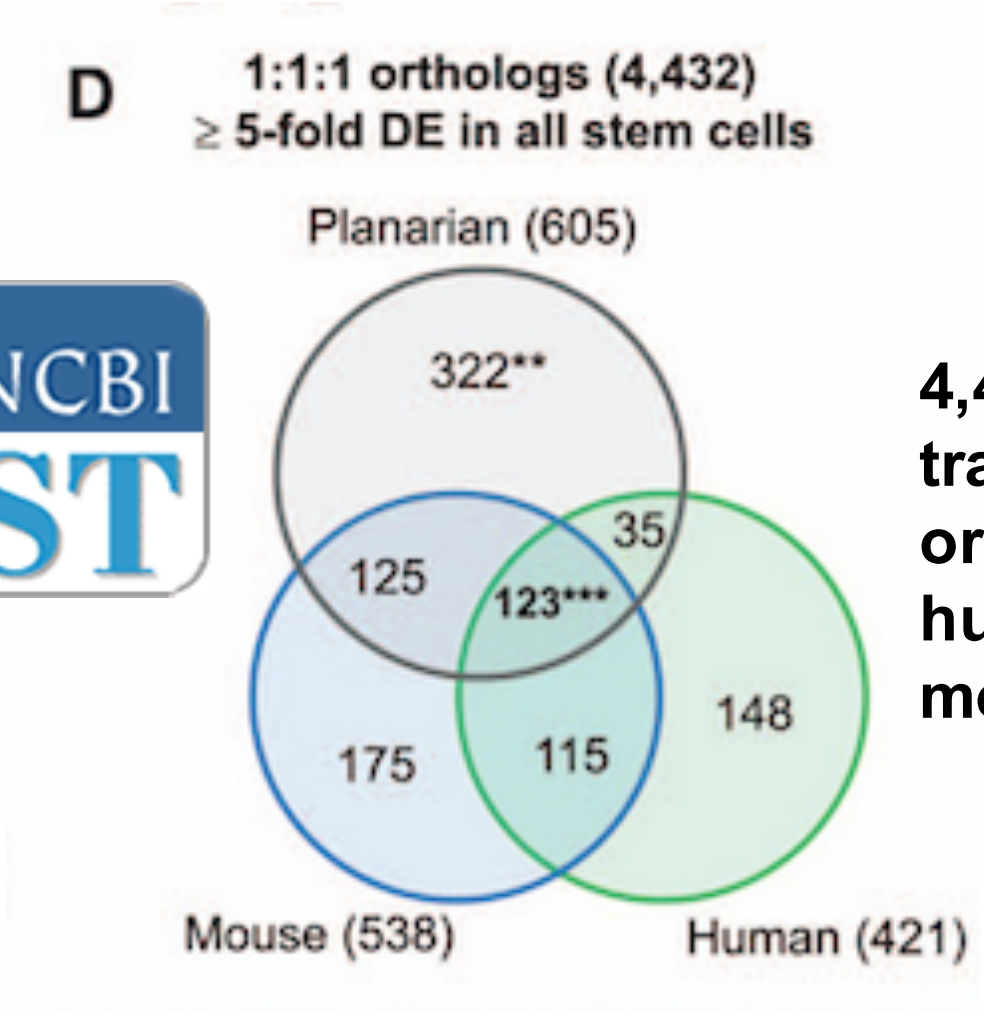
http://77.235.253.122/ensembl-protists/

What is cRPKM?



An enrichment score used to determine DE

How did they determine orthologs?



NCBI BLAST

4,432 planarian transcript orthologs to human and mouse genes

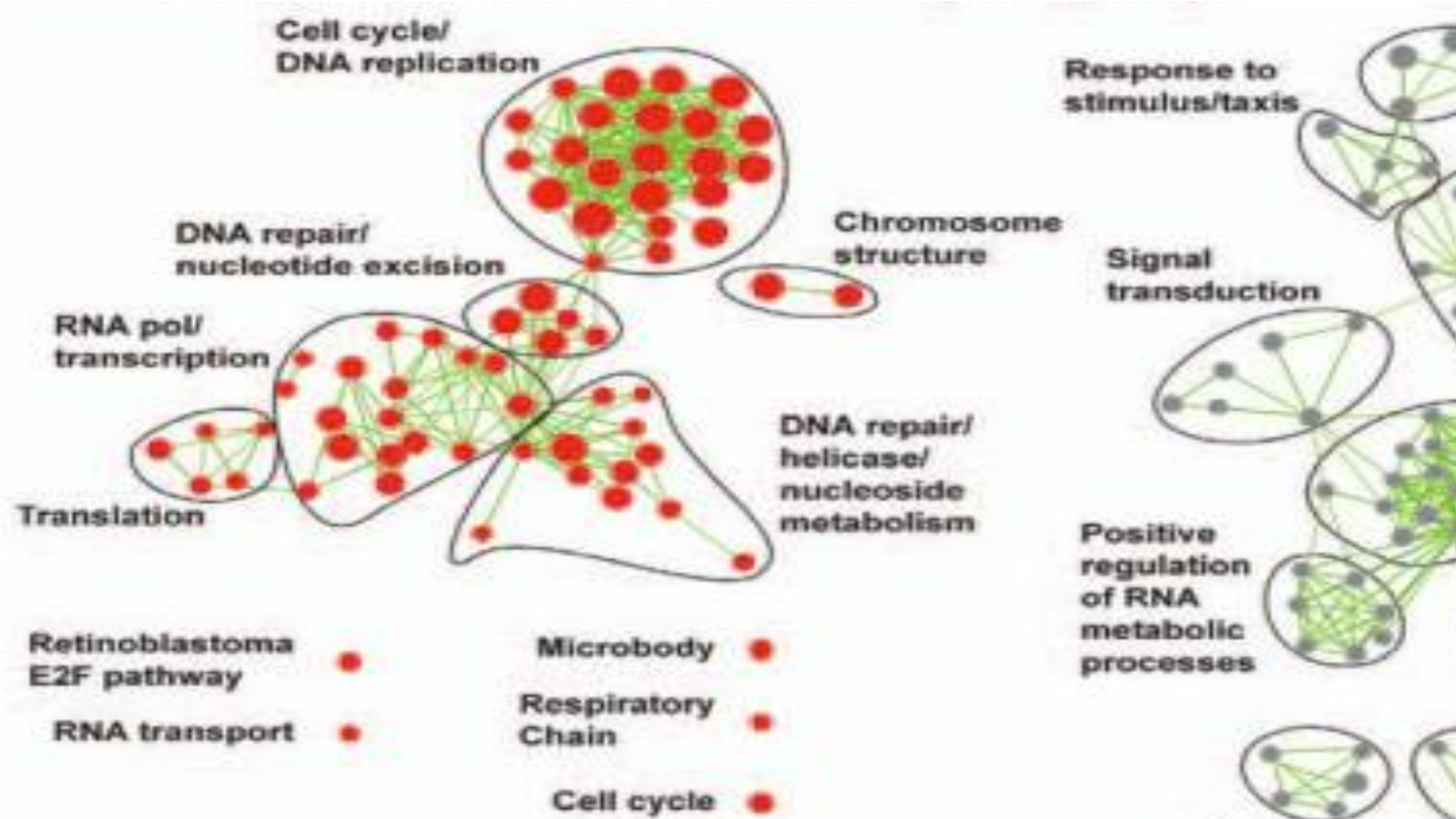
What can Gene Ontology (GO) tell you?

Cellular components

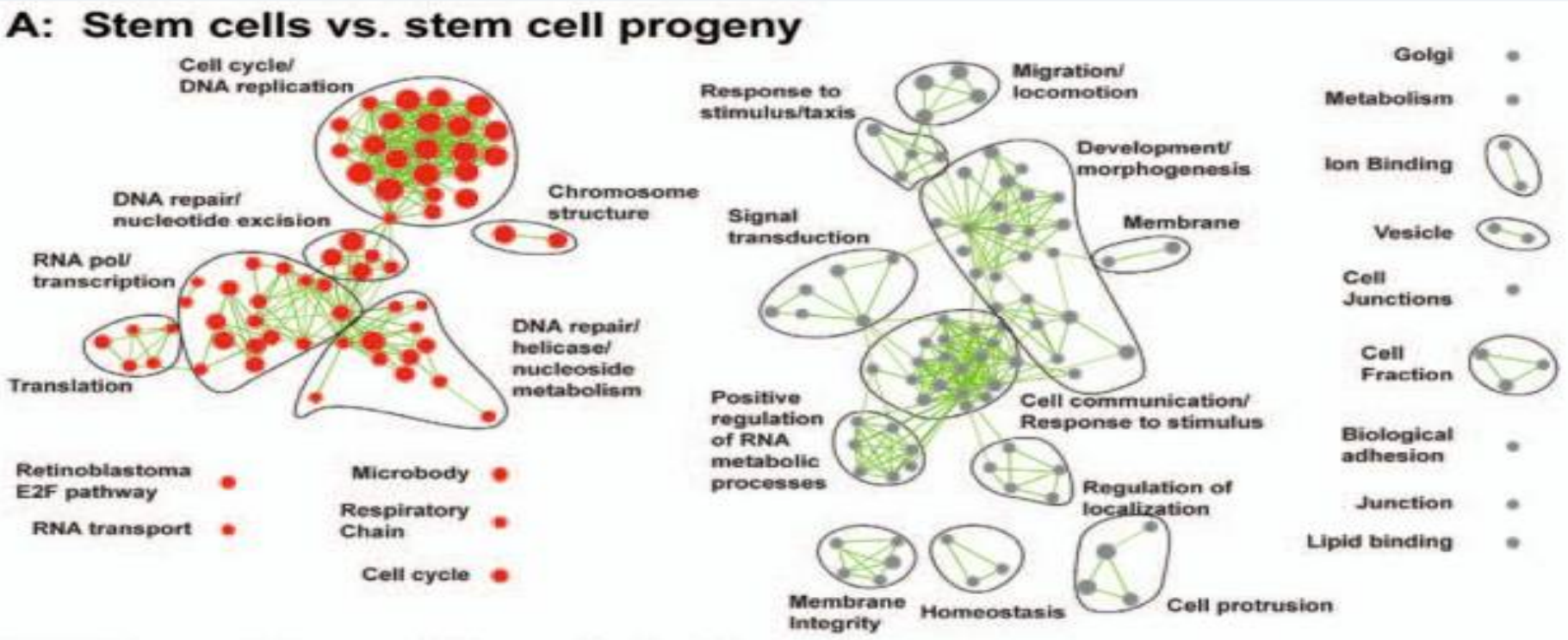
Molecular function

Biological process

What is Gene Set Enrichment Analysis (GSEA)?



Which pathways were enriched in stem cells?

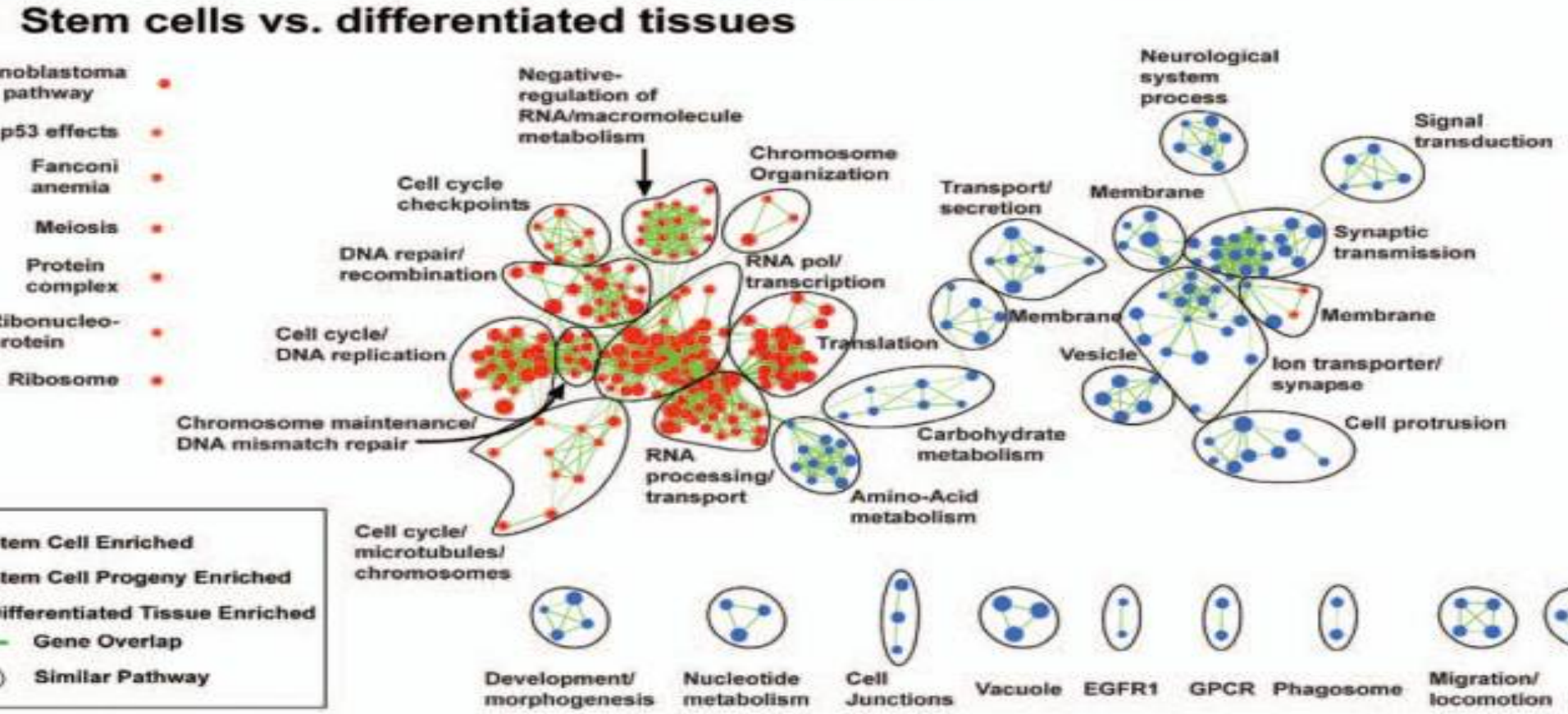


Stem cells: increased cell cycle and DNA replication

Progeny: development, differentiation, migration, etc.

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

Which pathways were enriched in differentiated tissues?

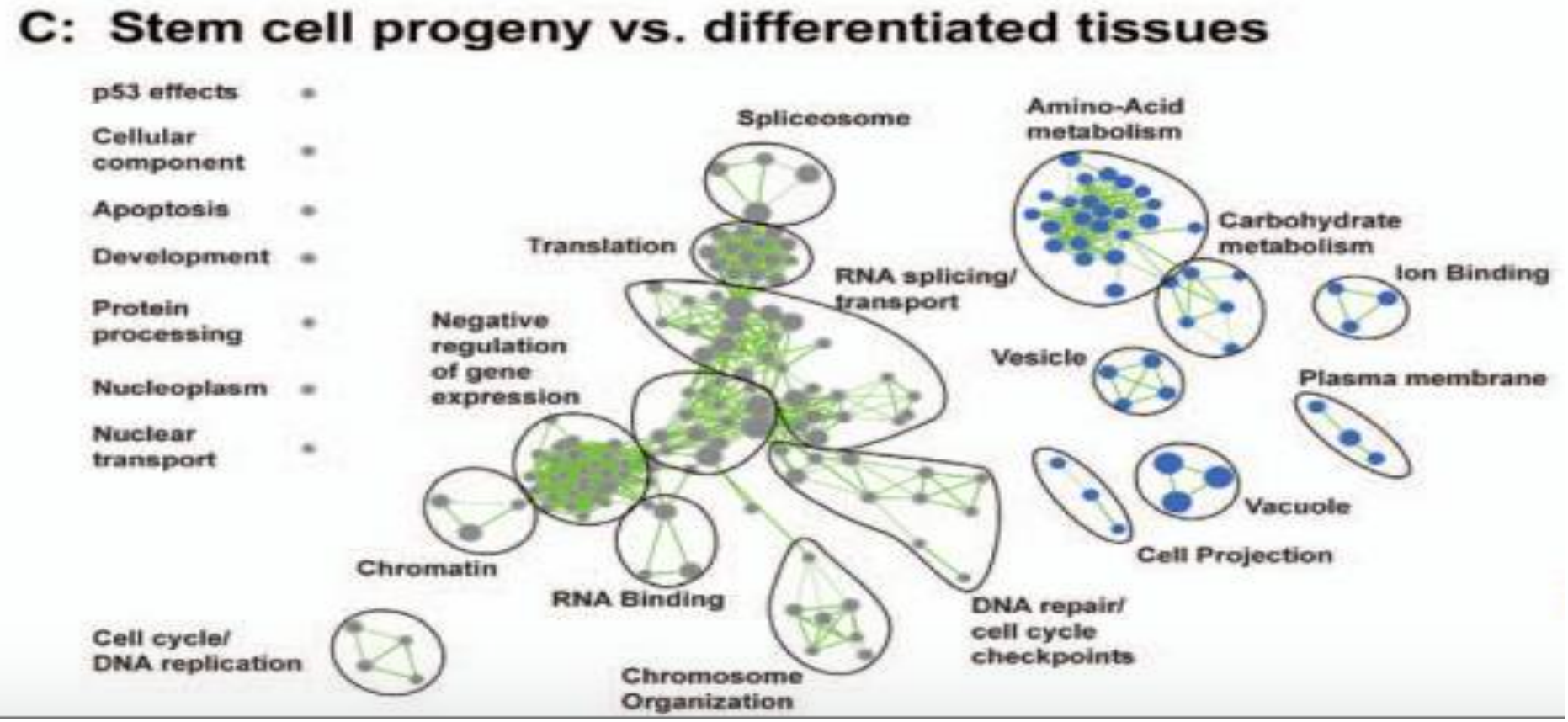


Stem cells: cell cycle, DNA repair, RNA processing/transport

Differentiated tissue: maintain nervous system

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

Which pathways were enriched in stem cell progeny?

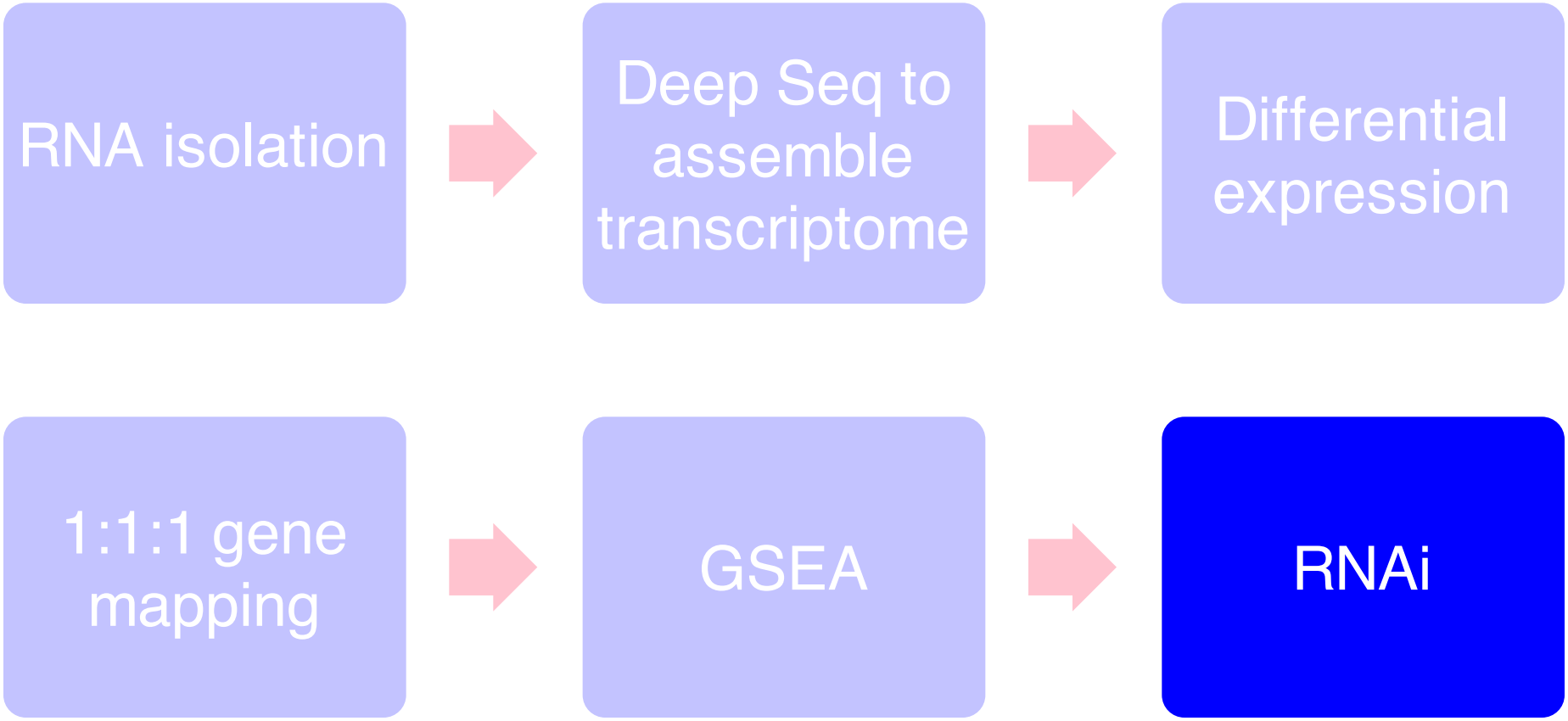


Progeny: spliceosome, chromatin modification, p53

No enrichment of nervous system pathway

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

How did they test the functions of these genes?



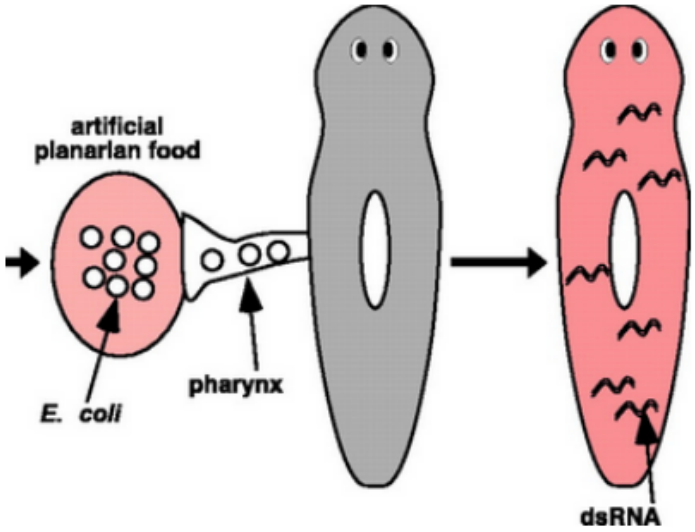
How did they do RNAi in planarians?

F1 D0 | F2 D3 | F3 D6 | F4 D9 | A D12 | 7DPA D19

Group A: no ortholog, DE in planarians

Group B: ortholog, no DE in mammals

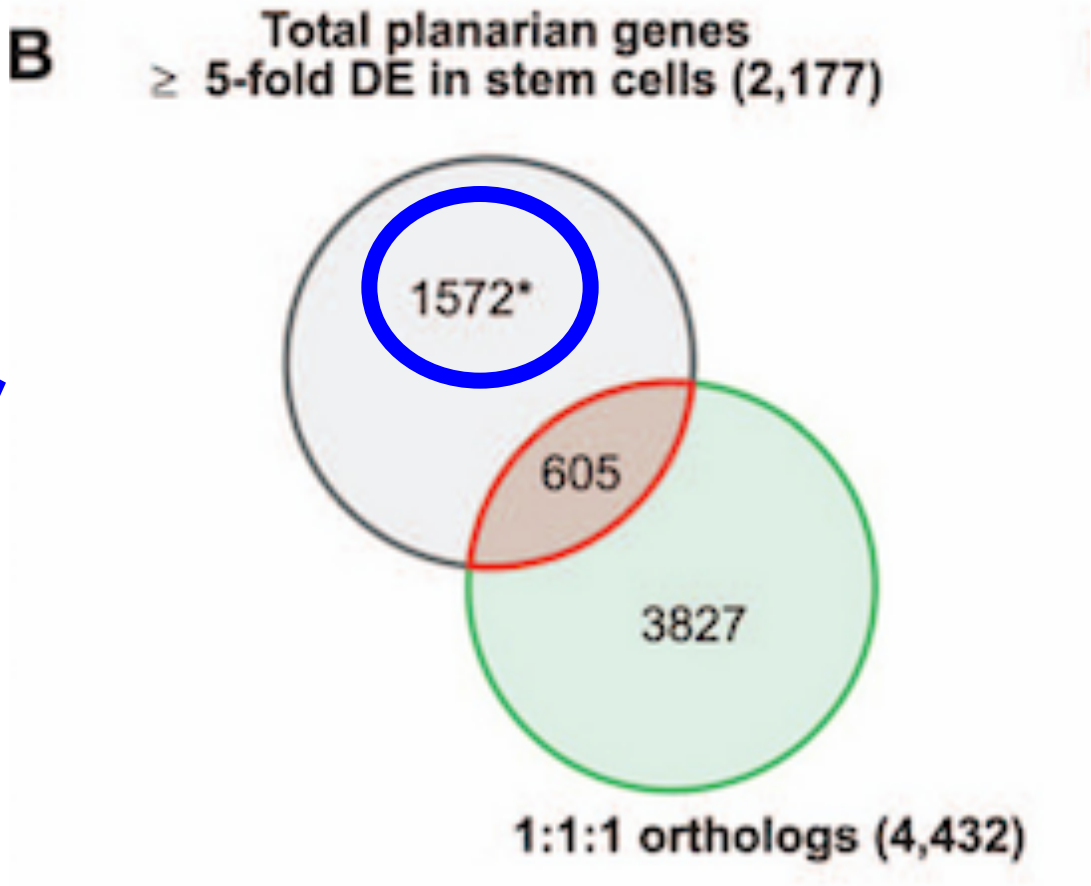
Group C: ortholog, DE in mammals



http://genetics564.weebly.com/uploads/8/6/5/7/865764/transcriptomics3-3-15kellyandtony.pdf

What genes were DE in planarians?

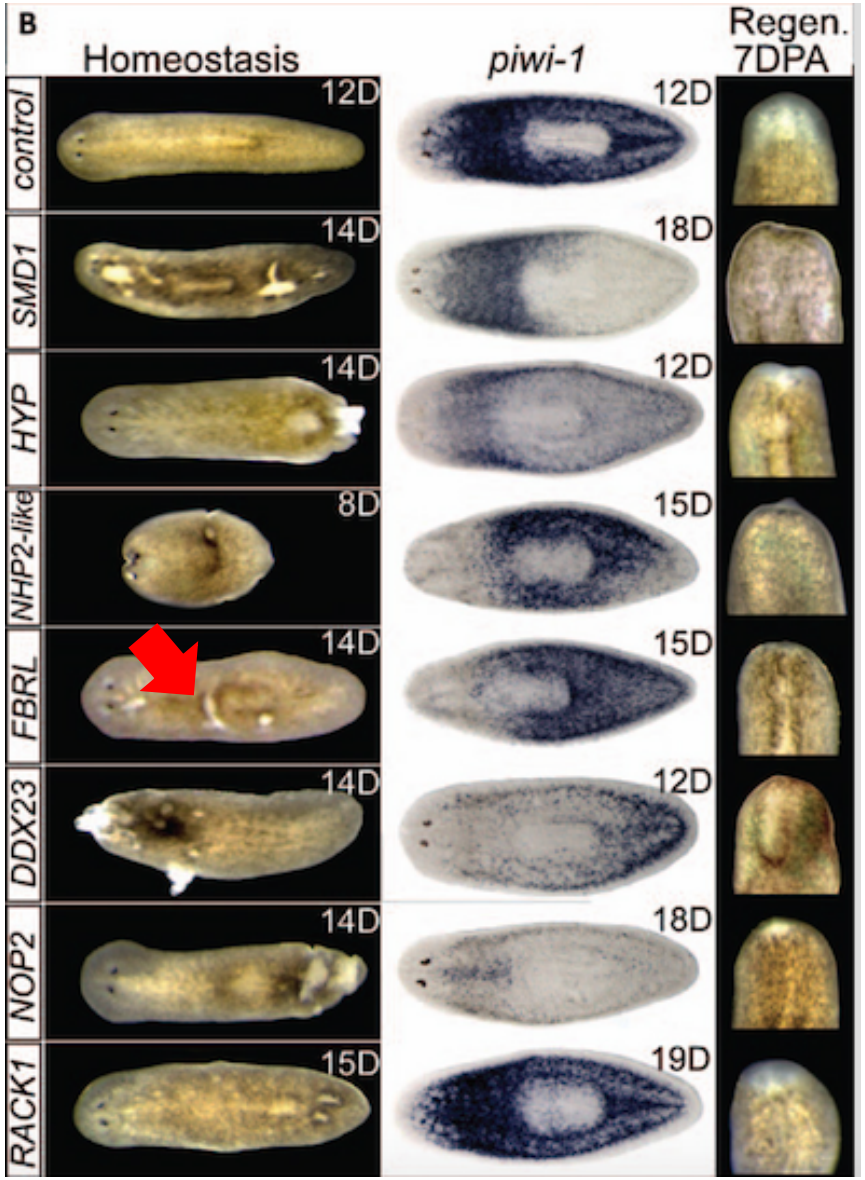
Group A: no ortholog, DE in planarians



1572 planarian DE stem cell genes without a 1:1:1 ortholog

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

What happened when Group A genes were knocked out?



Group A: no ortholog, DE in planarians

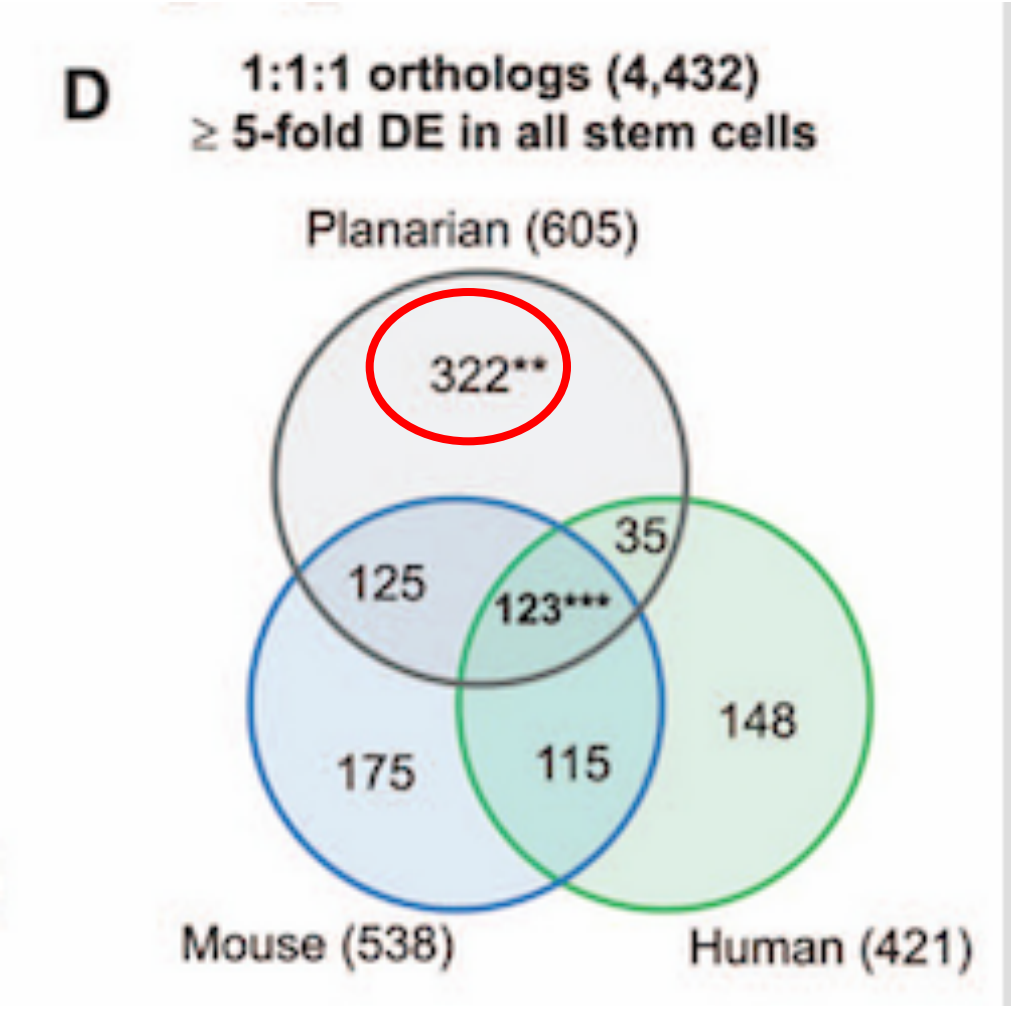
Dorsal lesions

SC population decreased except with RACK1 knockdown

Head regeneration ability severely diminished

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

Which genes had orthologs but no DE?

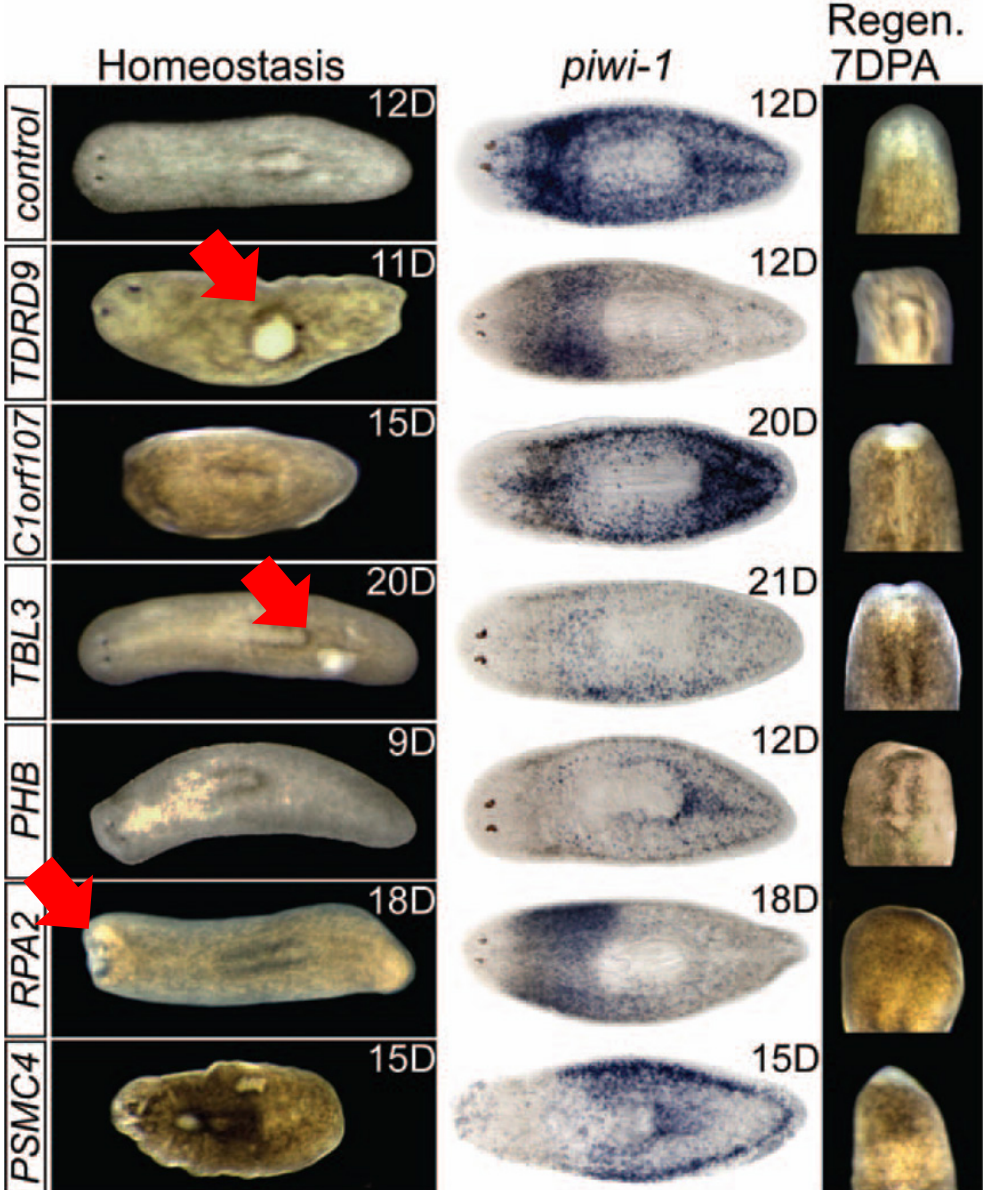


Group B: ortholog, no DE in mammals

322 planarian stem cell genes with a 1:1:1 ortholog but no DE in mammalian ESCs

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

What happened when Group B genes were knocked out?



Dorsal lesions and head regression

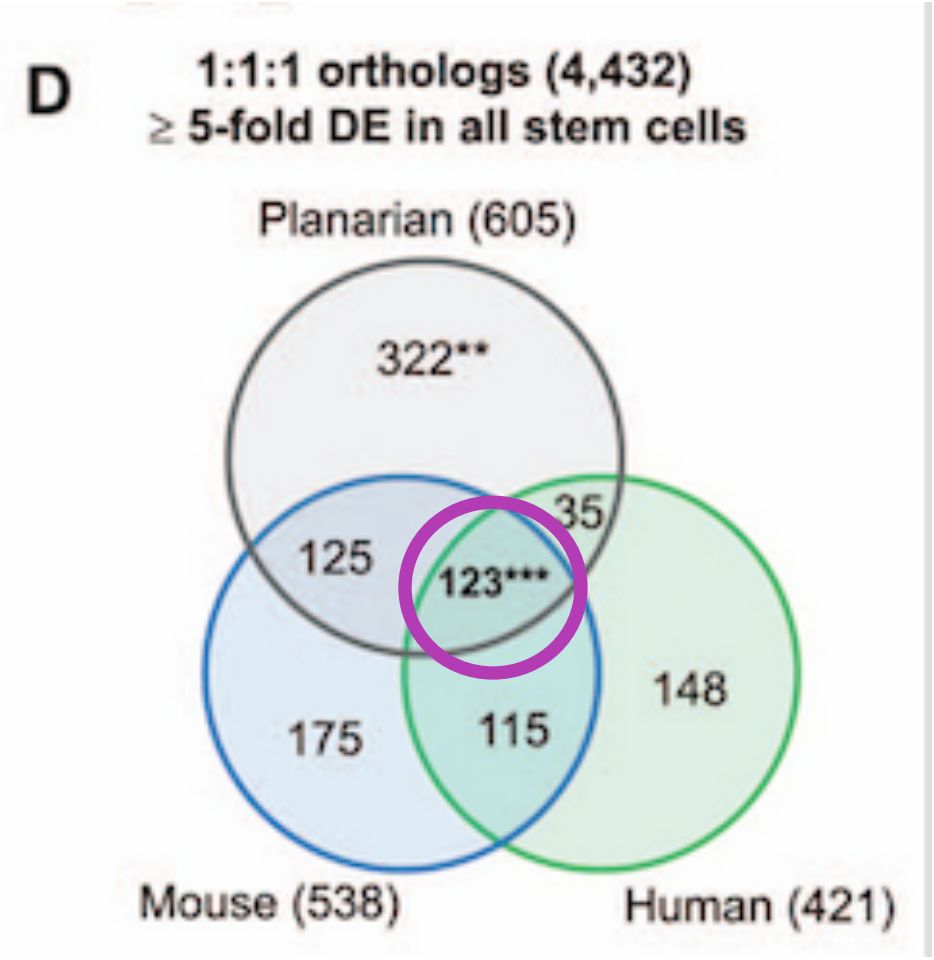
Loss of stem cell population (piwi-1)

Decreased regenerative ability in all

Group B: ortholog, no DE in mammals

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

Which genes were DE and had orthologs?

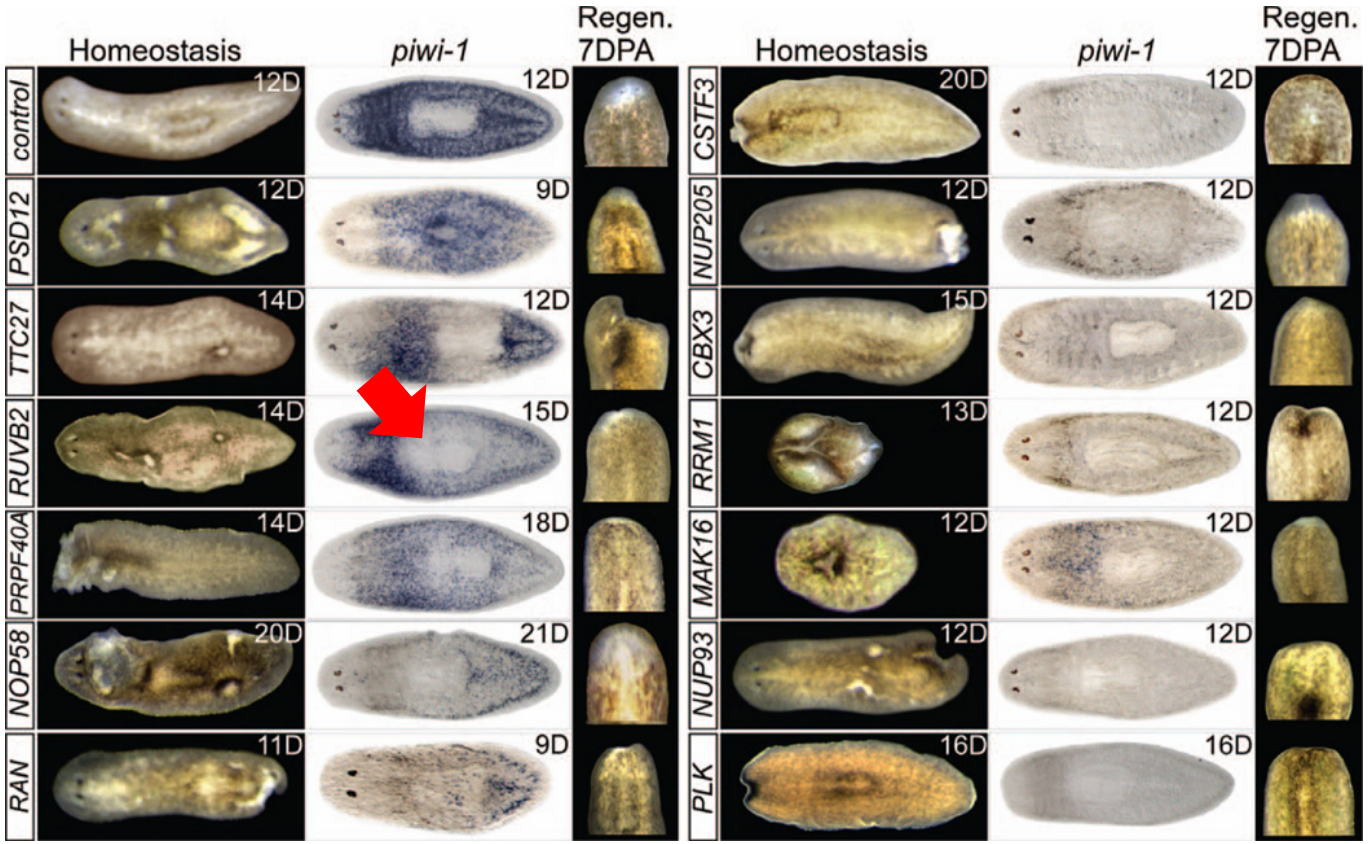


Group C: ortholog, DE in mammals

123 planarian stem cell genes with 1:1:1 ortholog and DE in mouse, human and planarians SCs

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

What happened when Group C genes were knocked out?



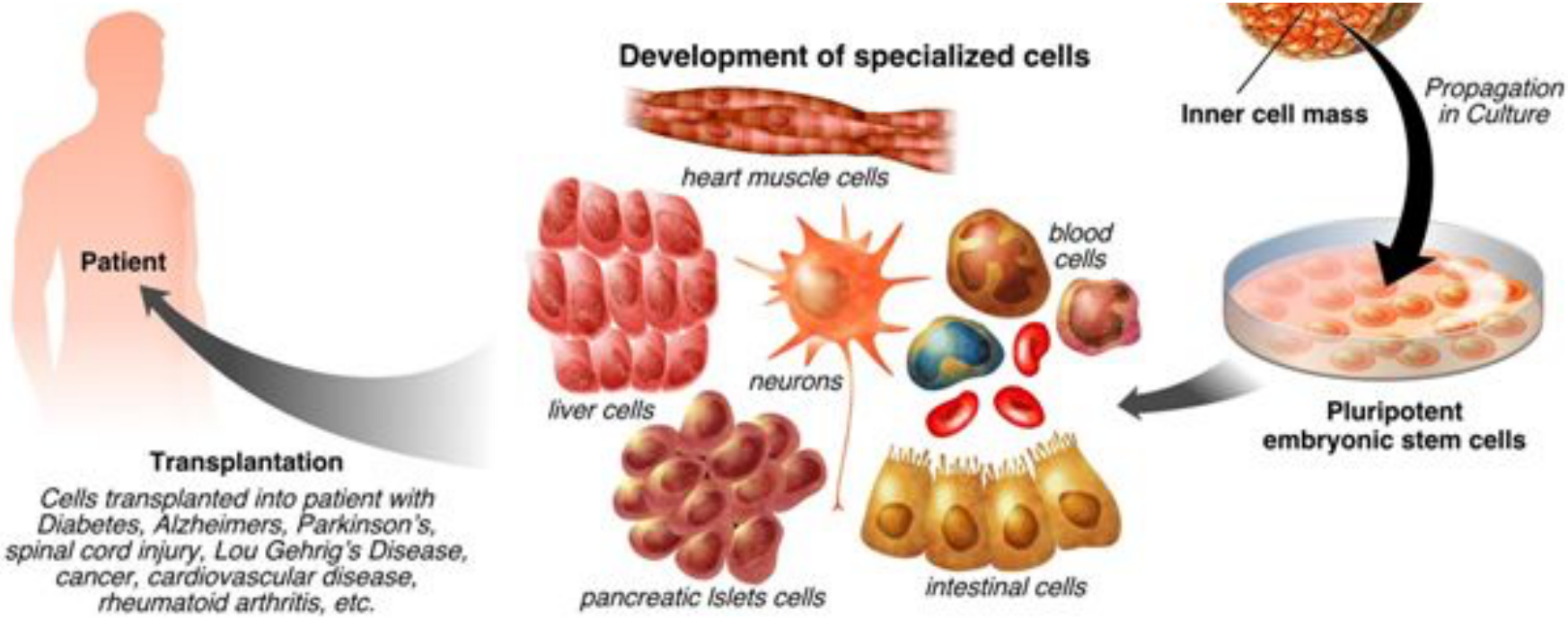
Group C: ortholog DE in mammals

Dorsal lesions and head regression
Spatial-specific losses of SC population
No head regeneration in any RNAi treatments

Labbe, et al. (2012) *Stem Cells* 30(8):1734-1745.

How is this information useful?

How is this information useful?



http://klinikong.com/stemcelltherapy/stemcells.php

Summary

Planarians are a novel tool for understanding stem cell biology

Discovered conserved factors in stem cell biology

Future direction: test these genes in human/mouse

Thank you for your attention!

https://macrocritters.files.wordpress.com/2012/10/planaria-copyright-ernie-cooper-2012.jpg
https://www.youtube.com/watch?v=vXN_5SPBPtM