# Lecture 3: Transcriptomics and RNA-seq

BioSci D132

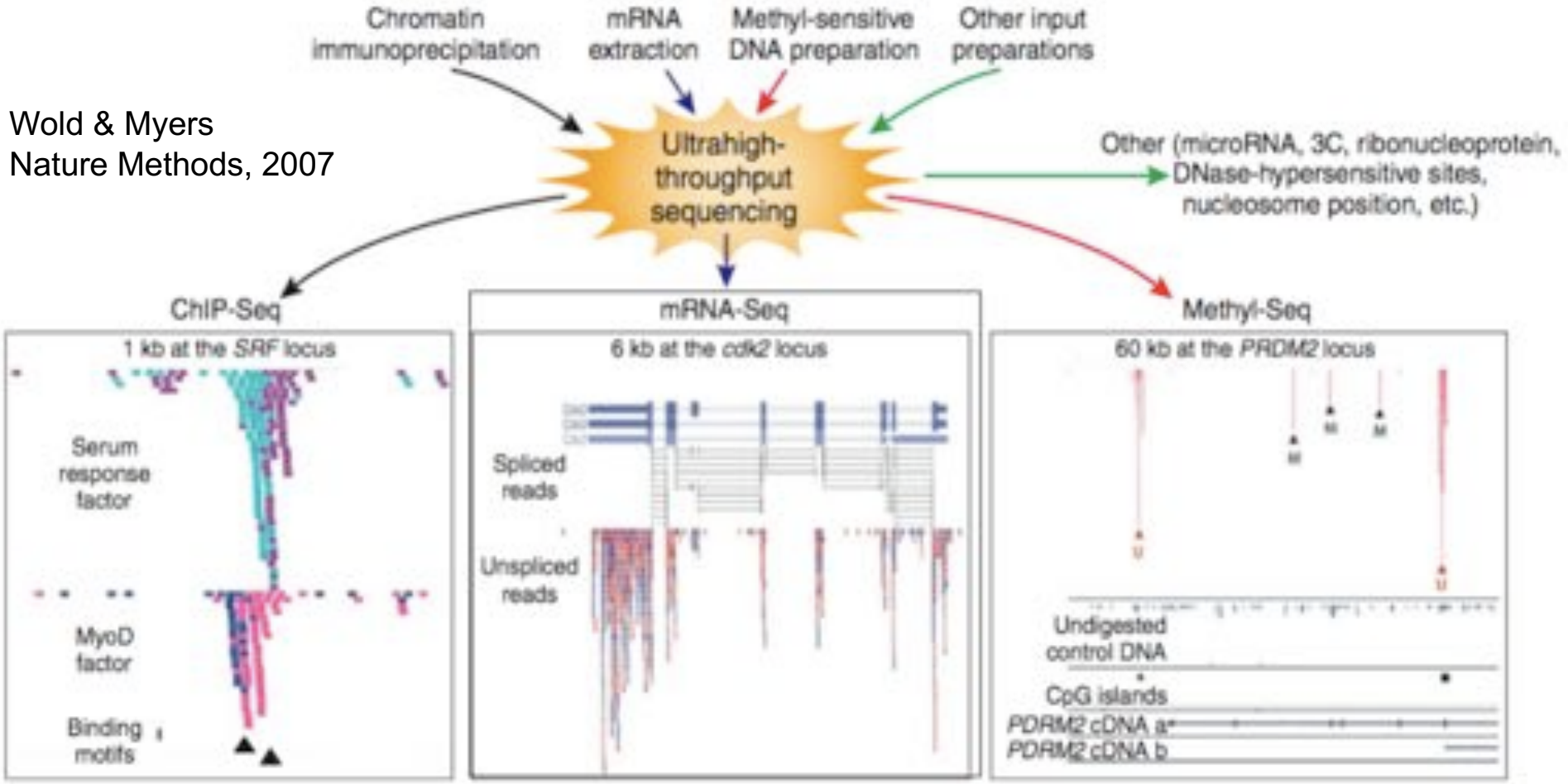
Oct 4, 2018

## Learning Objectives

- Describe RNA-seq workflow
- Calculate gene expression abundances using RNA-seq
- Understand the basics of Hierarchical and K-means clustering
- Describe the structure and applications of Gene Ontology and pathway analysis

# Functional genomics using sequencing

Wold & Myers
Nature Methods, 2007




For all sequence-counting assays, the more reads, the better

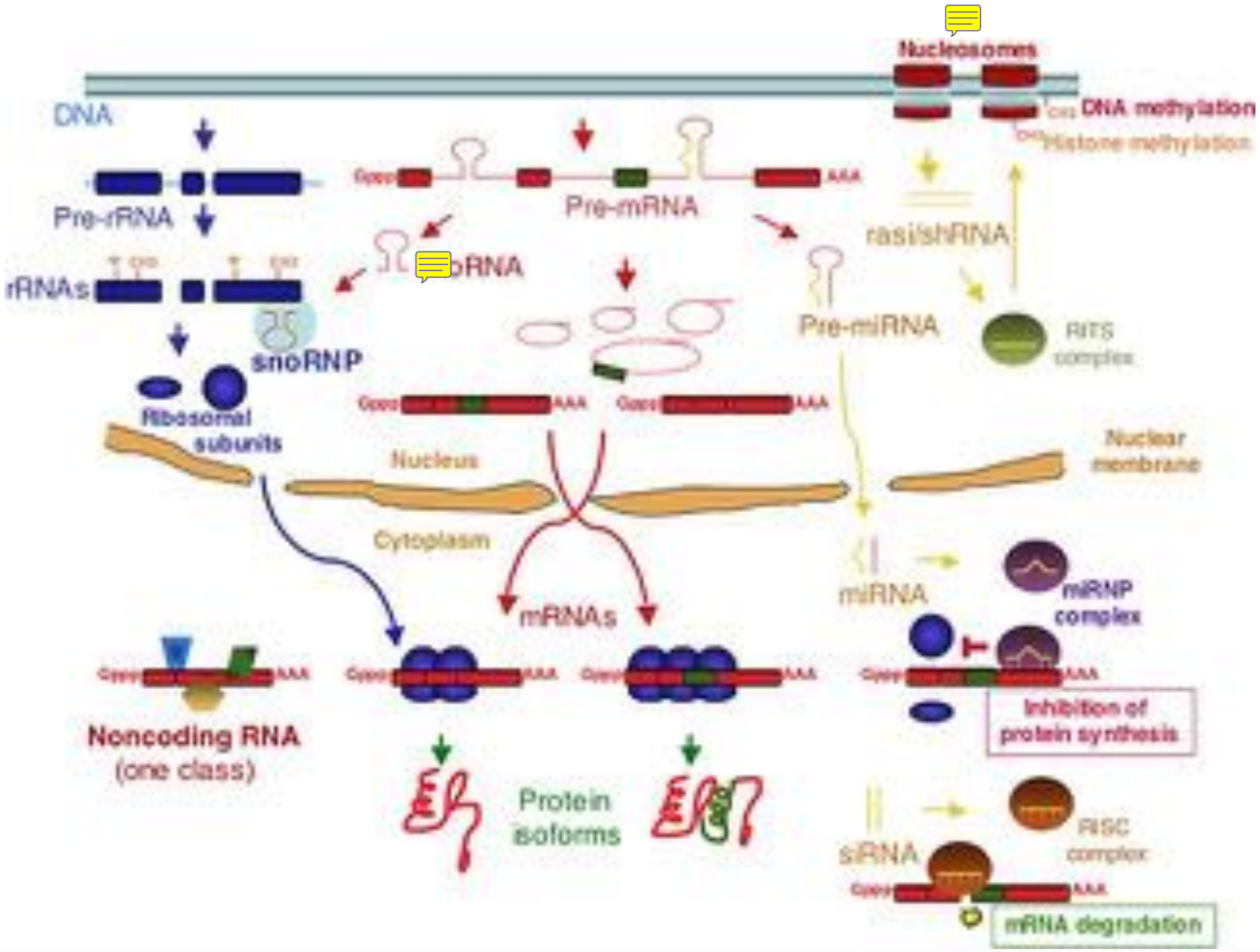
About half of the worldwide current generation of sequencing capacity is dedicated to these assays.

## The transcriptome is a signature of the identity of a cell

- Complete set of transcripts in a cell, tissue or animal.
- Includes:
  - Coding transcripts, mRNA
  - Non-coding Transcripts e.g. lnRNA, siRNA, miRNA
  - rRNA, tRNA (most of the RNA in the cell)
- Dictates the activities of a cell.
- An integral part of functional genomics.

- We will focus primarily on mRNA!

# There are many classes of RNA




(Soares, 2006)

# Estimate of total RNA content in mammalian cells

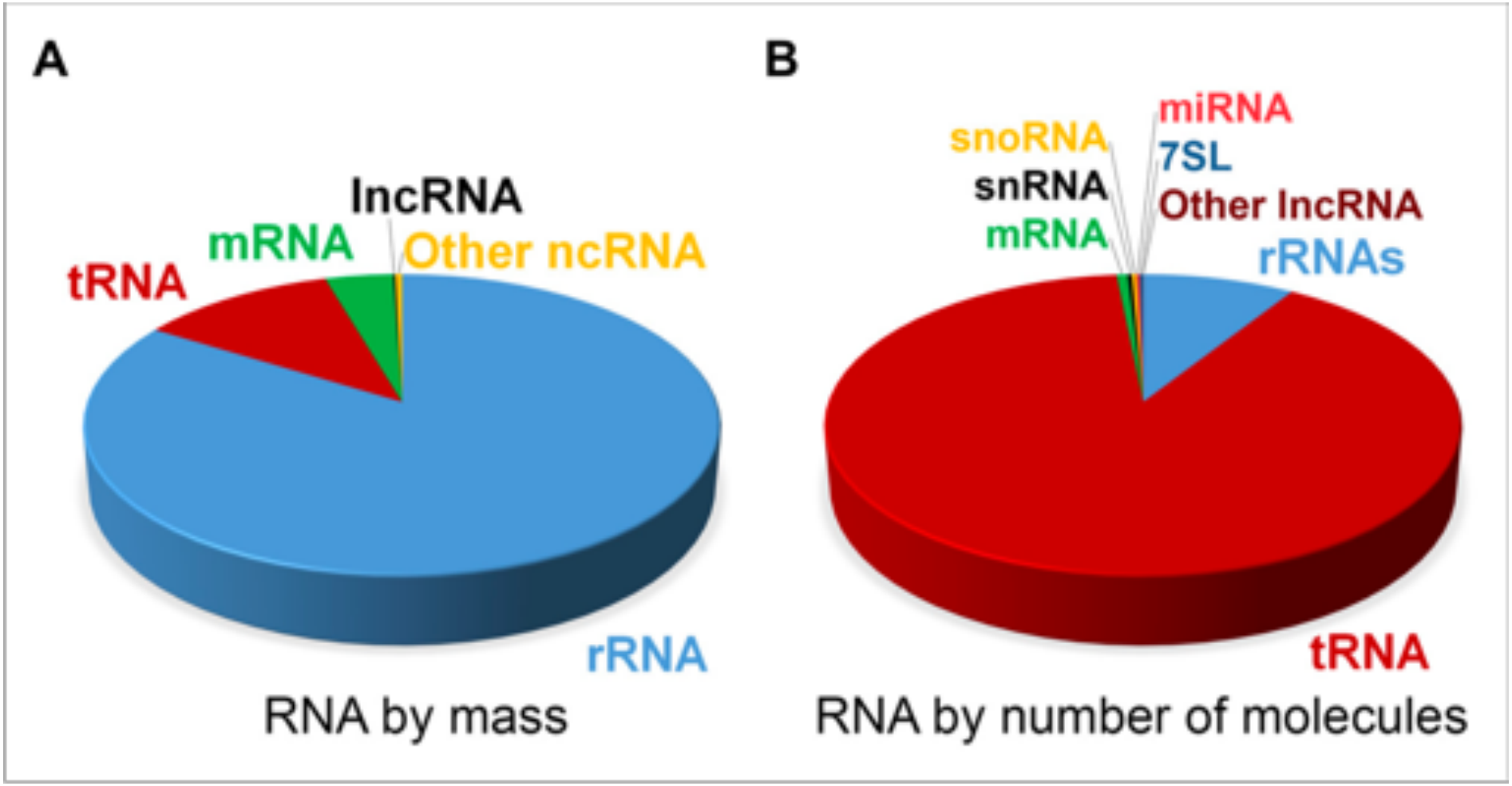
| Type | Percent of total RNA by mass | Molecules per cell | Average size (kb) | Total weight picograms/cell | Notes | Reference |
|---|---|---|---|---|---|---|
| rRNAs | 80 to 90 | 3–10 × $10^6$ (ribosomes) | 6.9 | 10 to 30 | | Blobel and Potter (1967), Wolf and Schlessinger (1977), Duncan and Hershey (1983) |
| tRNA | 10 to 15 | 3–10 × $10^7$ | <0.1 | 1.5 to 5 | About 10 tRNA molecules /ribosome | Waldron and Lacroute (1975) |
| mRNA | 3 to 7 | 3–10 × $10^5$ | 1.7 | 0.25 to 0.9 | | Hastie and Bishop (1976), Carter et al. (2005) |
| hnRNA (pre-mRNA) | 0.06 to 0.2 | 1–10 × $10^3$ | 10* | 0.004 to 0.03 | Estimated at 2–4% of mRNA by weight | Mortazavi et al. (2008), Menet et al. (2012) |
| Circular RNA | 0.002 to 0.03 | 3–20 × $10^3$ | ~0.5 | 0.0007 to 0.005 | Estimated at 0.1–0.2% of mRNA** | Salzman et al. (2012), Guo et al. (2014) |
| snRNA | 0.02 to 0.3 | 1–5 × $10^5$ | 0.1–0.2 | 0.008 to 0.04 | | Kiss and Filipowicz (1992), Castle et al. (2010) |
| snoRNA | 0.04 to 0.2 | 2–3 × $10^5$ | 0.2 | 0.02 to 0.03 | | Kiss and Filipowicz (1992), Cooper (2000), Castle et al. (2010) |
| miRNA | 0.003 to 0.02 | 1–3 × $10^5$ | 0.02 | 0.001 to 0.003 | About $10^5$ molecules per 10 pg total RNA | Bissels et al. (2009) |
| 7SL | 0.01 to 0.2 | 3–20 × $10^4$ | 0.3 | 0.005 to 0.03 | About 1–2 SRP molecules/100 ribosomes | Raue et al. (2007), Castle et al. (2010) |
| Xist | 0.0003 to 0.02 | 0.1–2 × $10^3$ | 2.8 | 0.0001 to 0.003 | | Buzin et al. (1994), Castle et al. (2010) |
| Other lncRNA | 0.03 to 0.2 | 3–50 × $10^3$ | 1 | 0.002 to 0.03 | Estimated at 1–4% of mRNA by weight | Mortazavi et al. (2008), Ramsköld et al. (2009), Menet et al. (2012) |

*The size for the average unspliced pre-mRNA is 17 kb; however, most pre-mRNAs are partially spliced at any given time, and the average size of hnRNA is estimated at 10 kb (Salditt-Georgieff et al., 1976).*

***Based on the finding that 1–2% of all mRNA species generate circular RNA, which is present at 10% of the level of the parental mRNA.*

(Palazzo and Lee, 2015)

# messengerRNAs are a small portion of the RNA of a cell



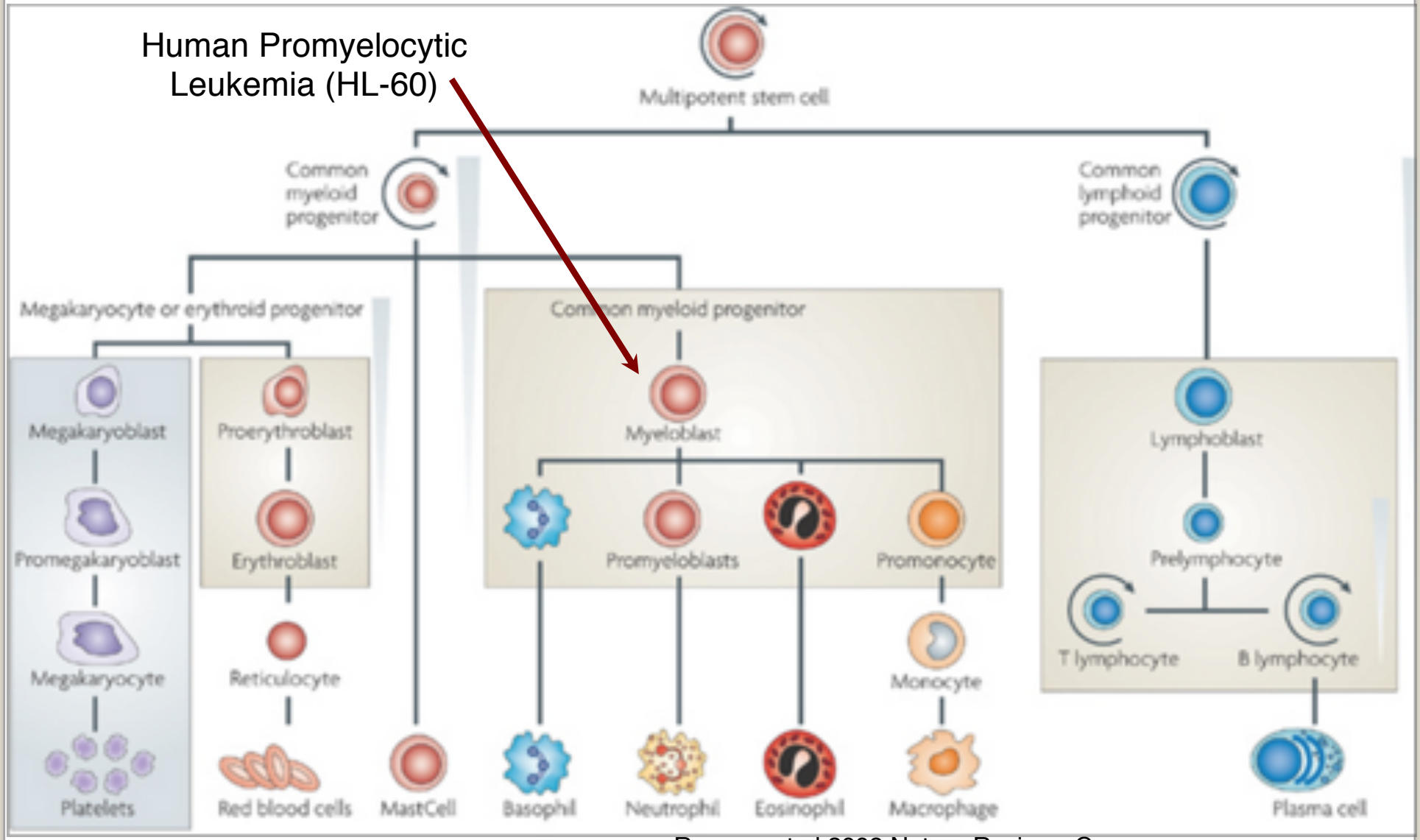

(Palazzo and Lee, 2015)

# Transcriptomics

- For a cell or tissue, transcriptomics entails:
  - identification
  - characterization
  - and cataloguing of all transcripts
  - time point and/or treatment

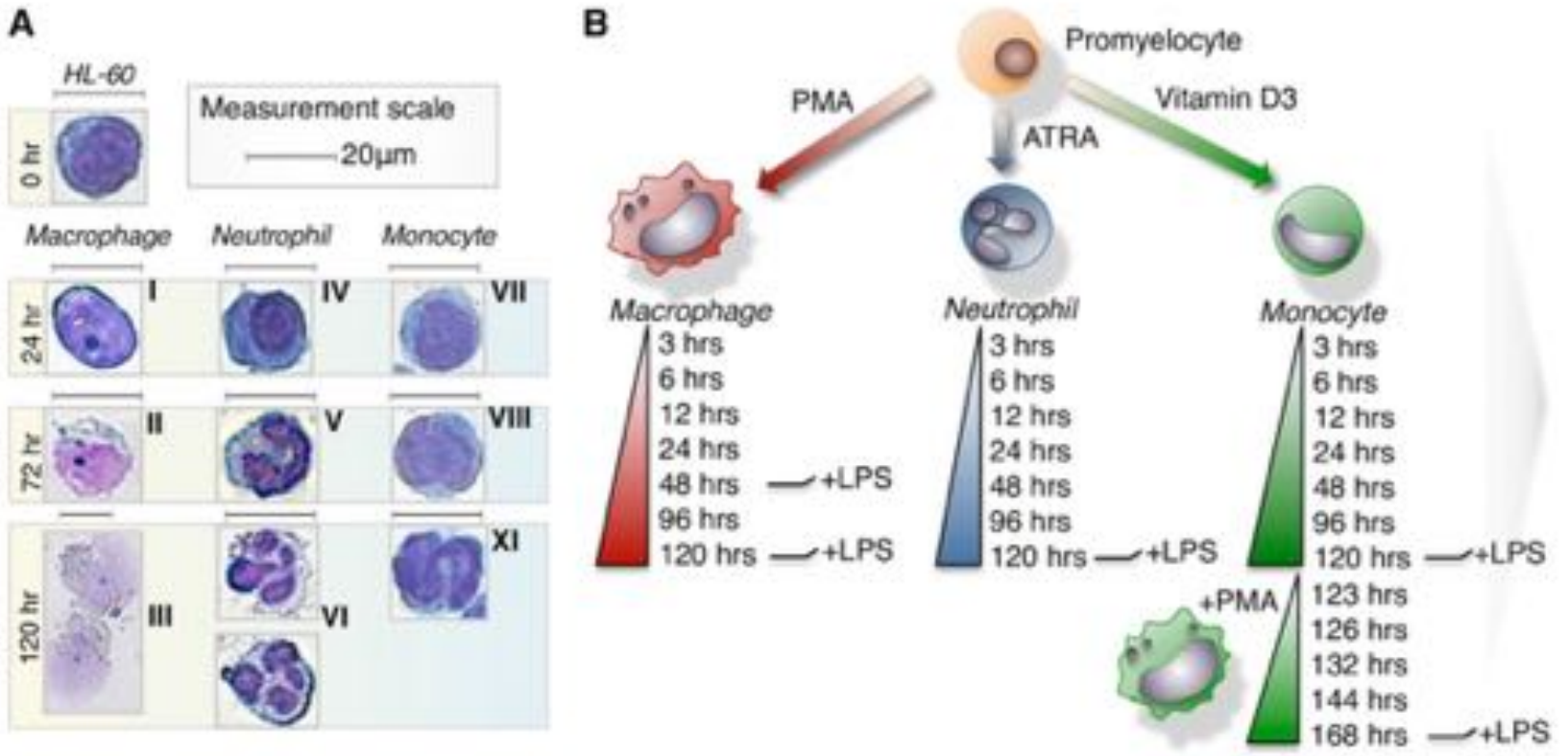
- Applications
  The big picture:
  - What genes are expressed?
  - Which genes are differentially expressed?

# Hematopoeisis – the generation of white blood cells




Ramsay et al 2008 Nature Reviews Cancer

# Time courses of gene expression

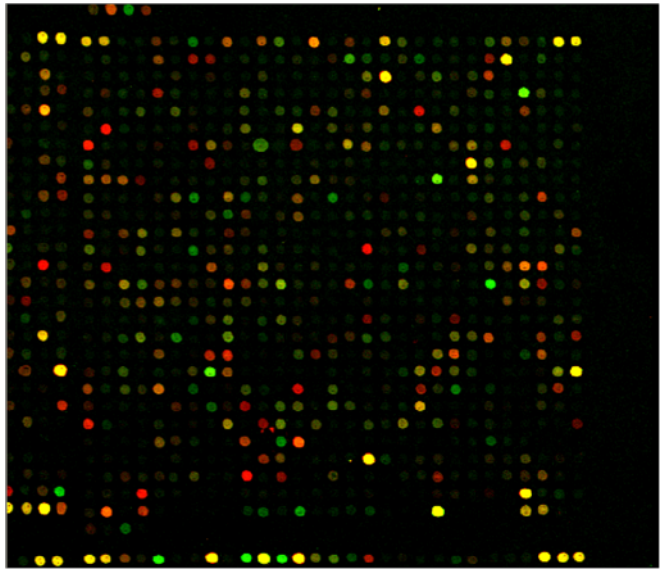



Measure RNA expression as cells are differentiating

(Ramirez, 2017)

# (Obsolete) technologies for transcriptomics

- Microarrays:
  - Was the main technique used for genome-wide functional assays for about 10 years.
  - Based on hybridization to pre-selected probes.
  - Limited dynamic range compared to RNA-seq.
  - High background noise compared to RNA-seq.
  - Lower reproducibility rate than RNA-seq.



http://www.microarray.org/sfgf/

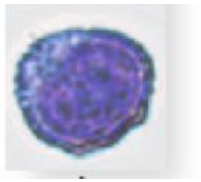
# Technologies for transcriptomics

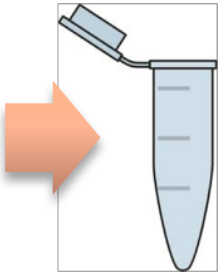
RNA-seq:

- A direct method for ultra-high-throughput sequencing of cDNA using millions of reads.

- Unless noted otherwise, this is really about sequencing mRNA

# Overview of RNA-seq



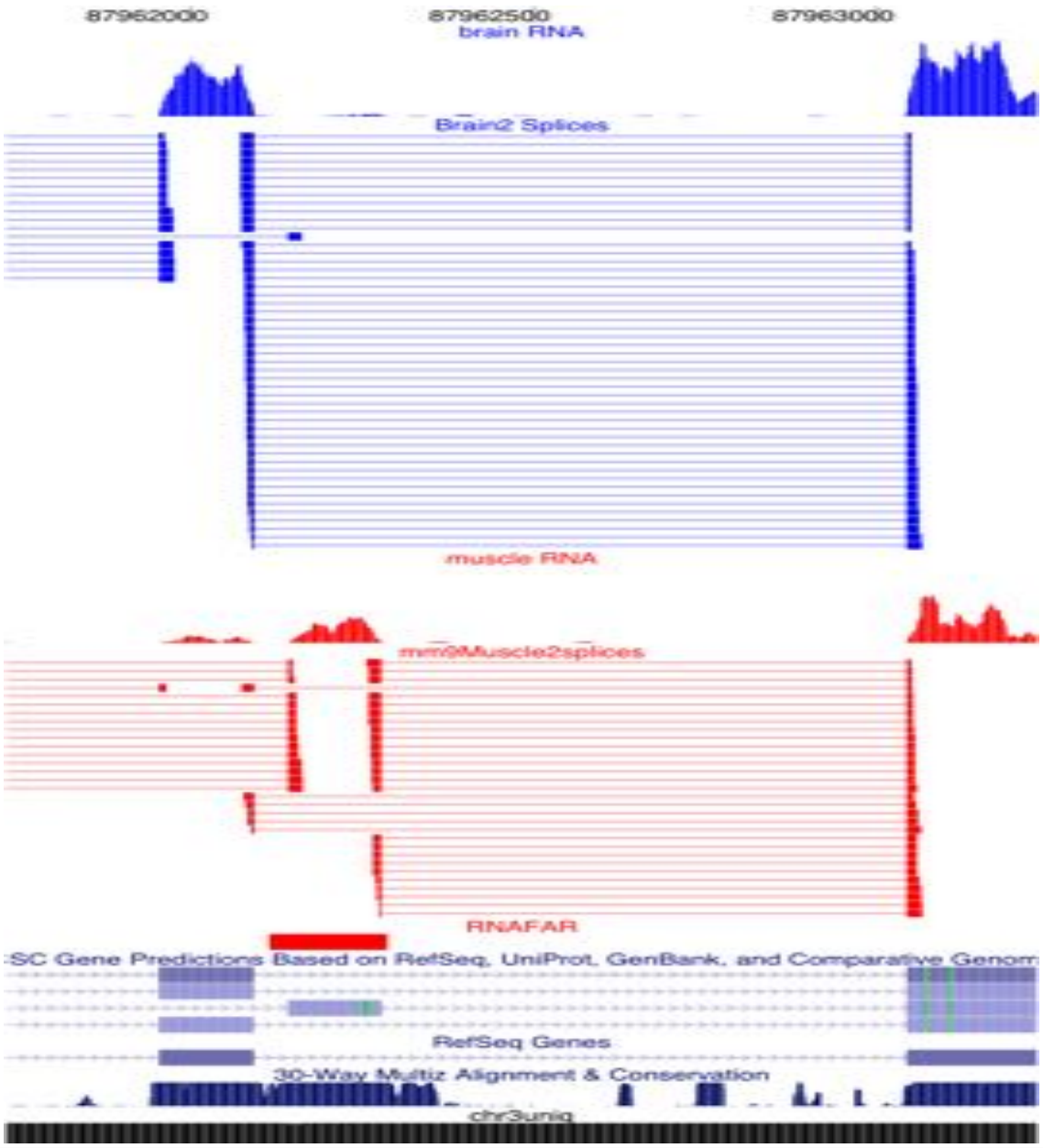



Cell/tissue RNA Sequencer RNA-seq reads

10-200 Million reads

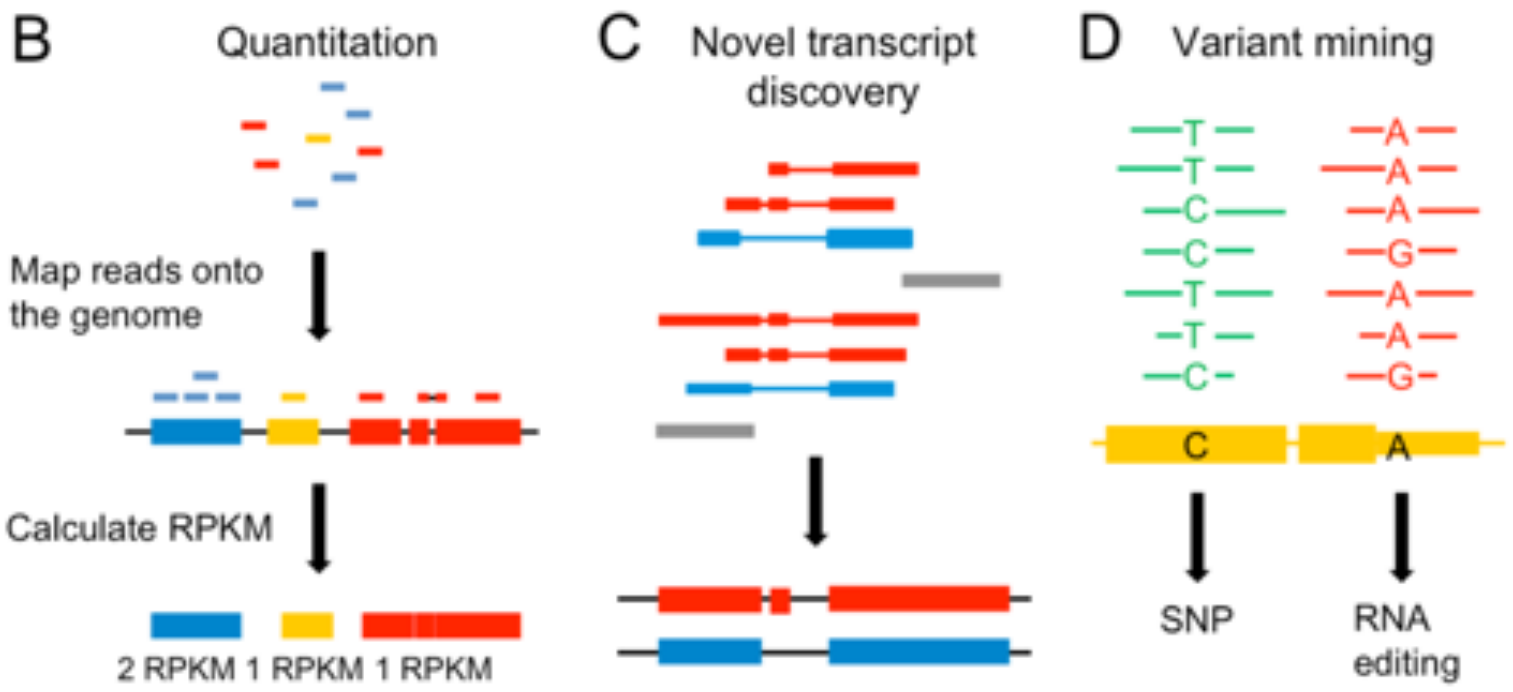
0.5-30 Gbp of sequence

# Detecting alternative splicing



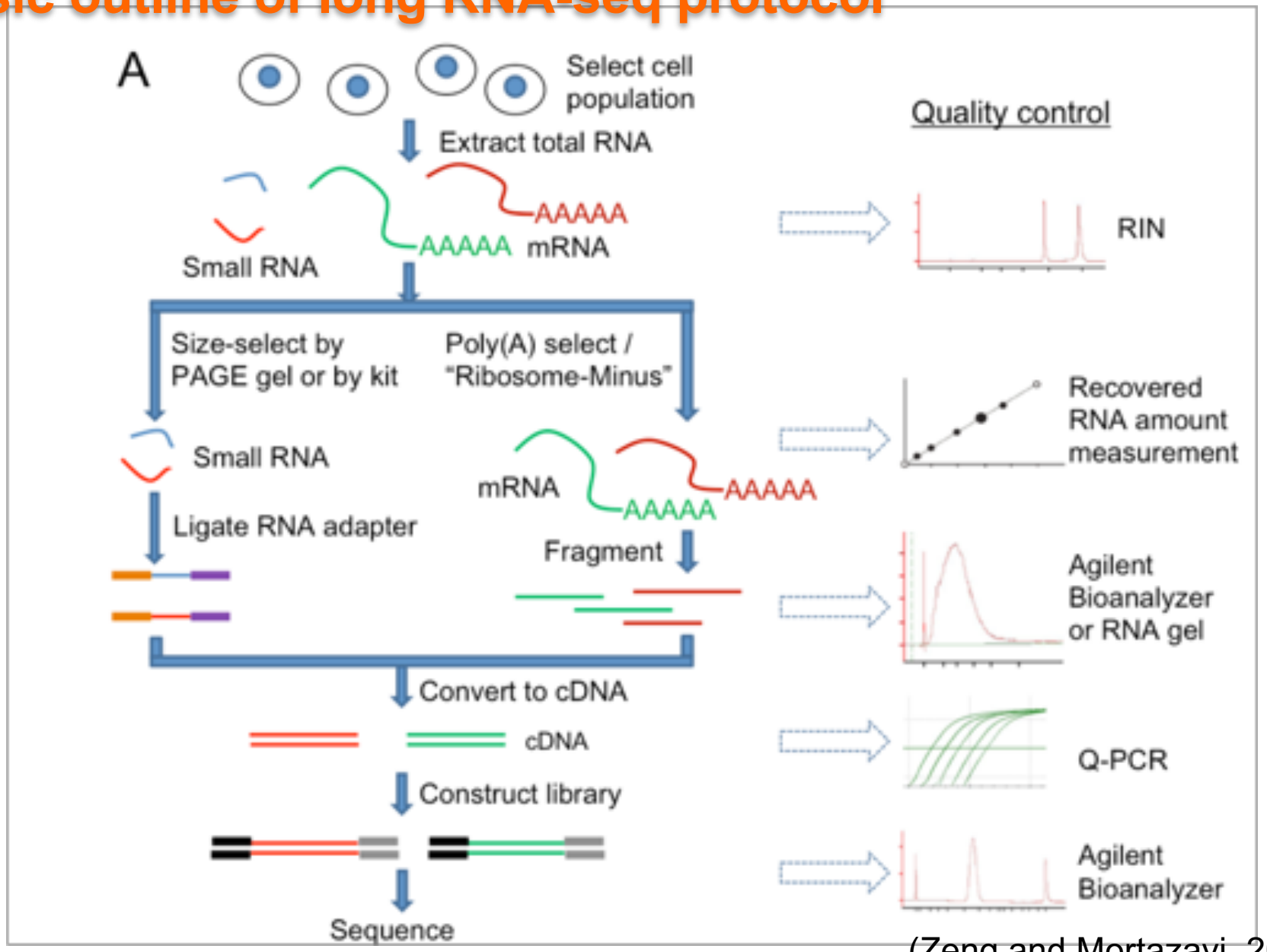

(Mortazavi, 2008)

# Applications of RNA-seq




(Zeng and Mortazavi, 2012)

# Basic outline of long RNA-seq protocol



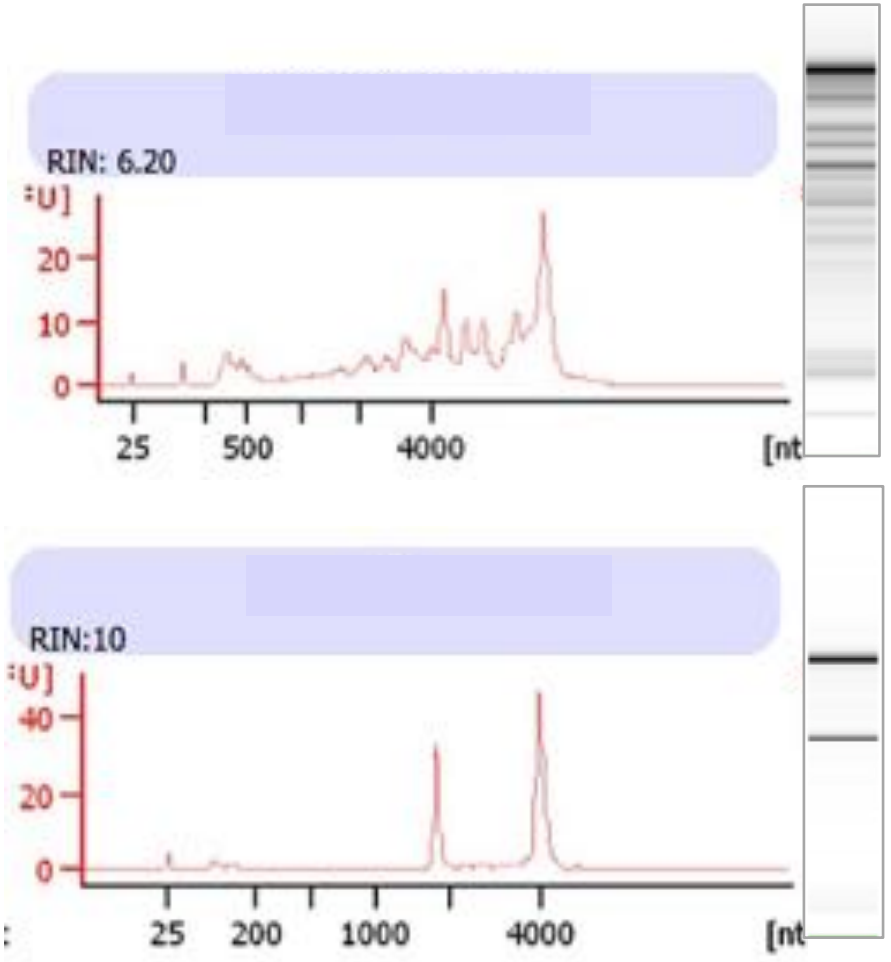

(Zeng and Mortazavi, 2012)

# Quality Control (QC) of RNA samples before sequencing

- Best results obtained when RNA isolated from a homogenous cell population or pooled samples.
- In case of extra step with PCR for amplification of low RNA quantities, the quality of RNA should be monitored with Q-PCR for any distortions introduced by PCR amplification.
- The quality of RNA should be quantitatively checked with Bioanalyzer before sequencing.

# RNA Integrity number

- Bioanalyzer, a microfluidics platform, can be used for sizing, quantification and quality control of RNA, DNA, proteins and cells.
- RNA integrity number (RIN) is a quantitative measure of RNA integrity on a scale of 1-10 (most-least degraded).
- Any sample with RNA integrity score of 8 or higher is suitable for sequencing.

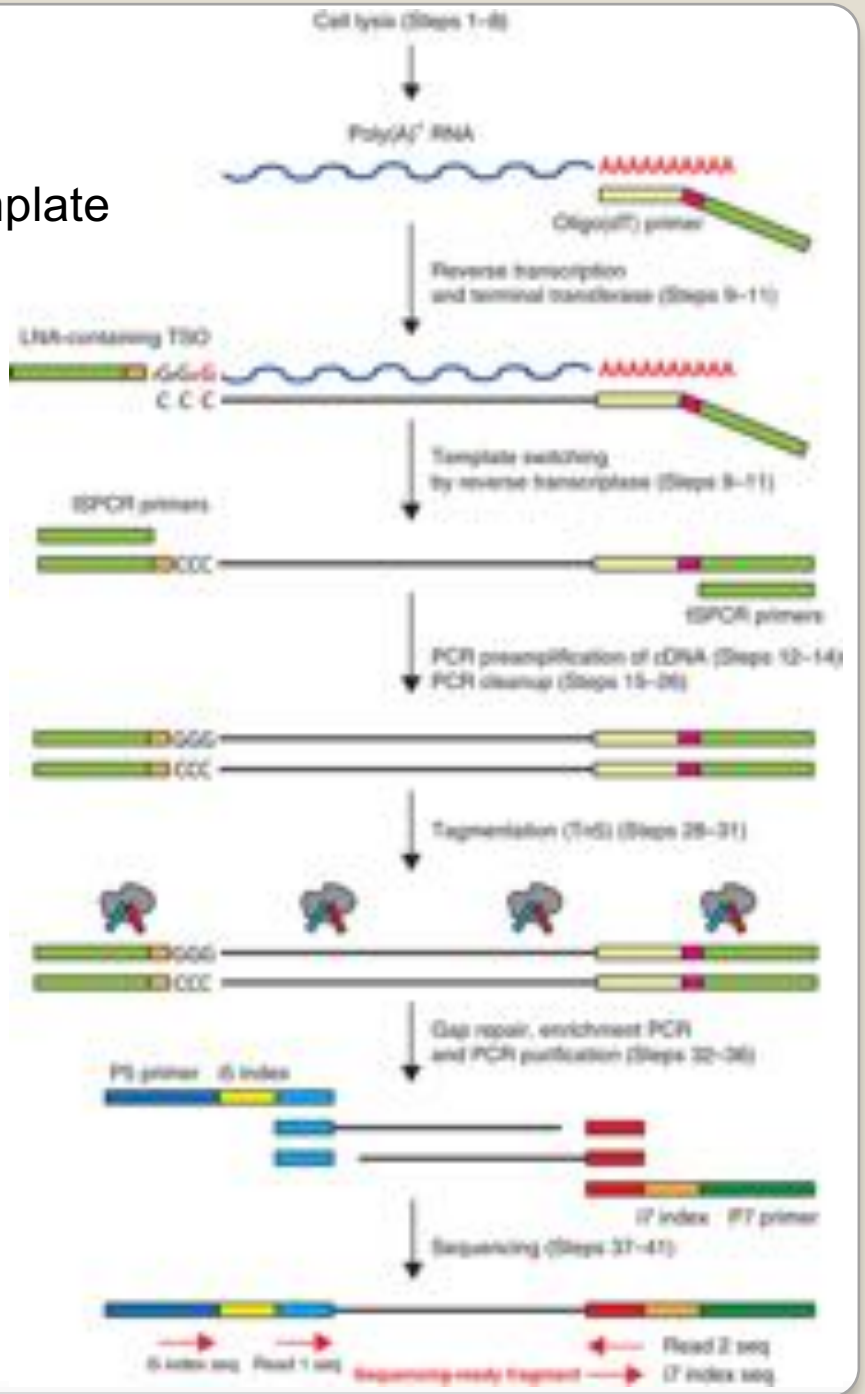


# 2013: Smart-seq2

SMART: **S**witching **M**echanism **a**t 5' of **R**NA **T**emplate

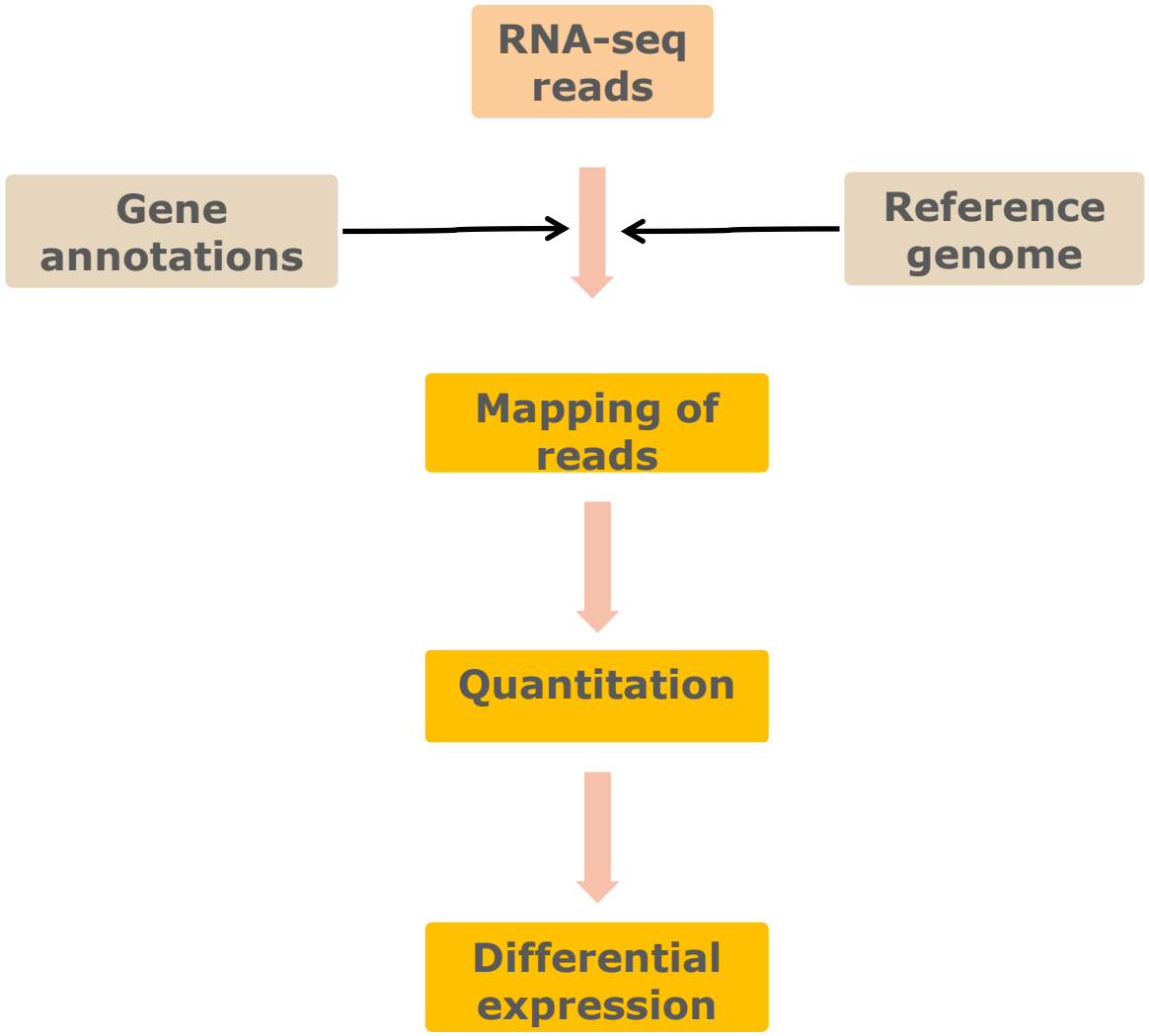
Developed for Full length RNA transcription

Optimized for small amounts of RNA




(Picelli, 2014)

# Overview of RNA-seq data analysis

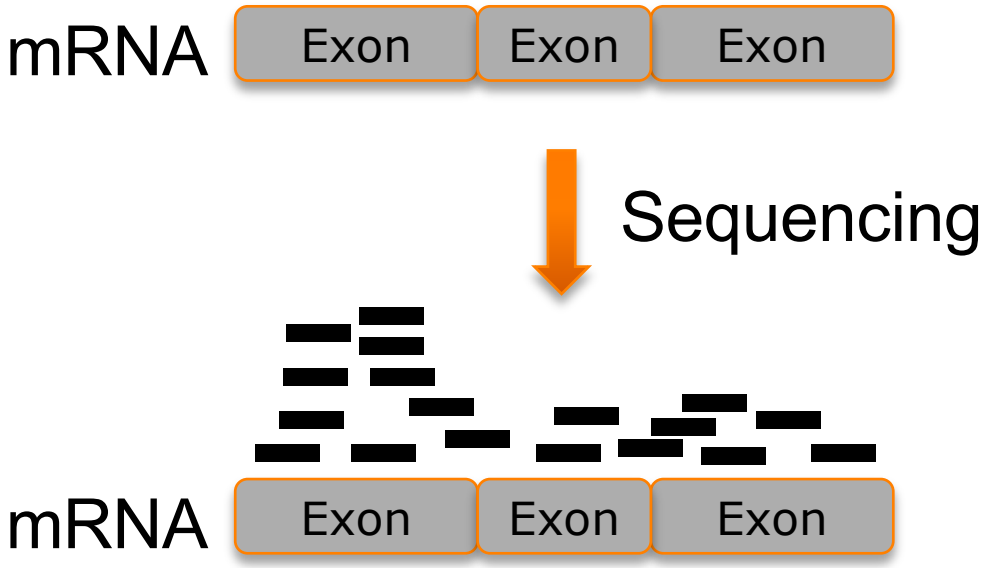


## Computational challenges posed by RNA-seq

- Reliable and efficient mapping of short reads to genome.
- Reliable mapping of reads across splice junctions.
- Reliable transcript assembly.
- More difficult in larger, complex genomes due to orthologs, repeats, and pseudo-genes.

## Uneven coverage levels

- Transcripts coverage levels differ along their lengths depending on library construction procedure.
  - Fragmentation before or after reverse transcription.



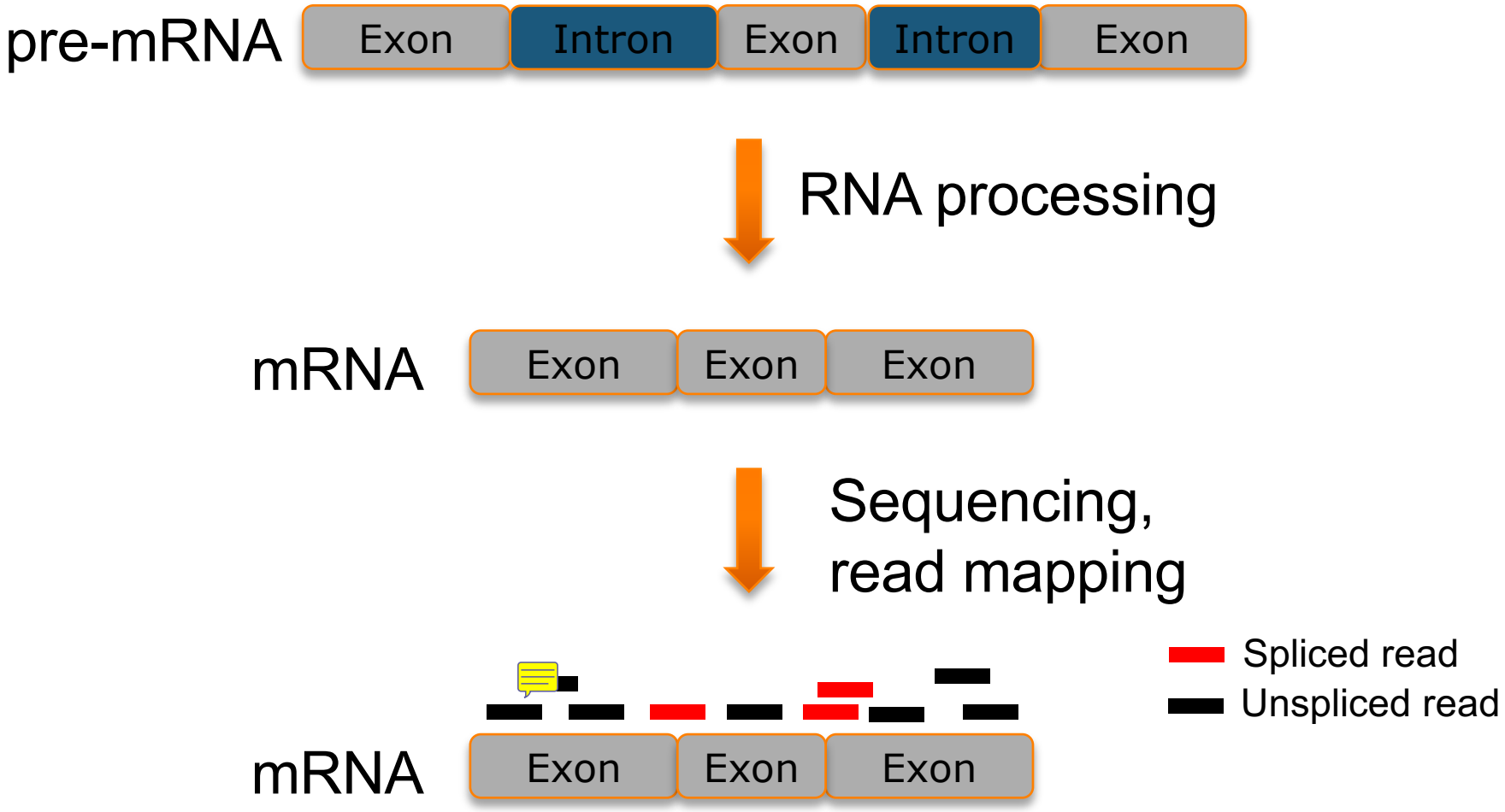
## Sequencing bias

- GC-rich and GC-poor regions are under-sampled during sequencing by some chemistries (e.g. Illumina).
- Random priming causes bias against common hexamer e.g. (AAAAAA).
- Not so random priming.

# Mapping RNA-seq reads

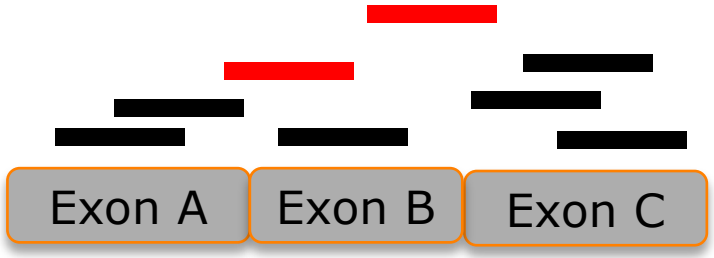
- Mapping reads:
  - determining the position of a read within the reference genome
- “Read mapping” problem:
  - Millions to billions of short reads.
  - Large reference genomes.
  - How to efficiently achieve mapping.
  - Deal with reads from repetitive regions.
  - Deal with sequencing errors .

# Spliced and unspliced reads



# Alternative mapping strategies

- Mapping on the transcriptome

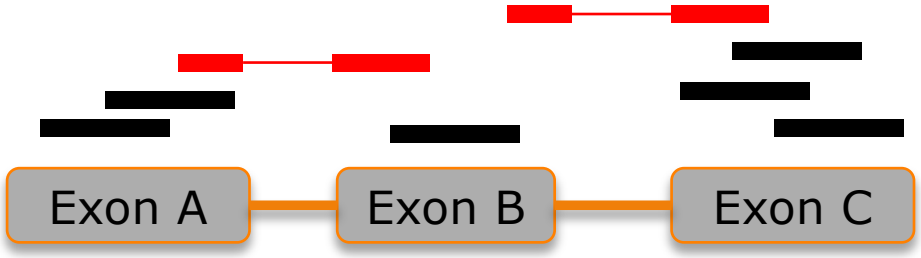



Junction spanning read

Aligned read

- Must provide the mRNAs
- Faster
- No discovery

- Mapping on the genome



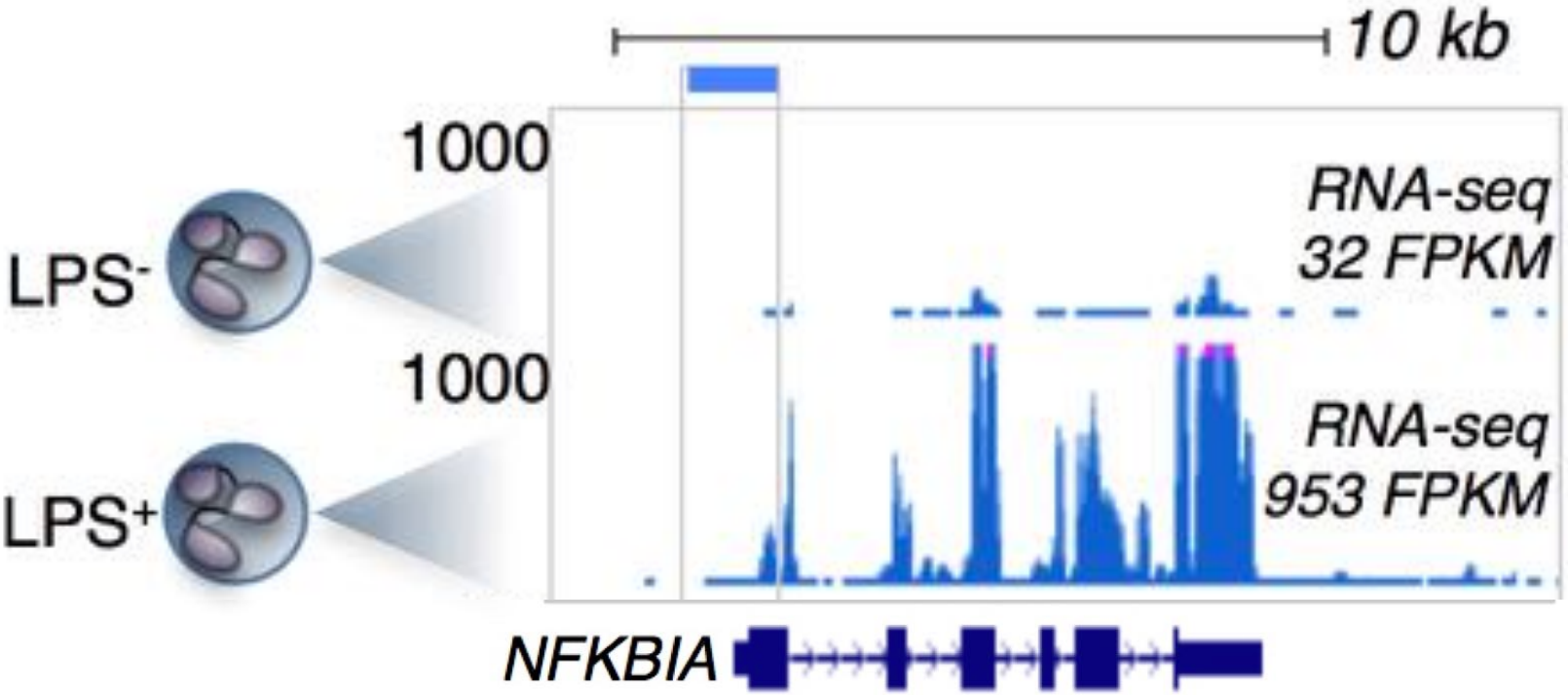

Intron-spanning read

Aligned read

- Must provide genome
- Slower, more computation
- Can discover new mRNAs

# Quantifying gene expression

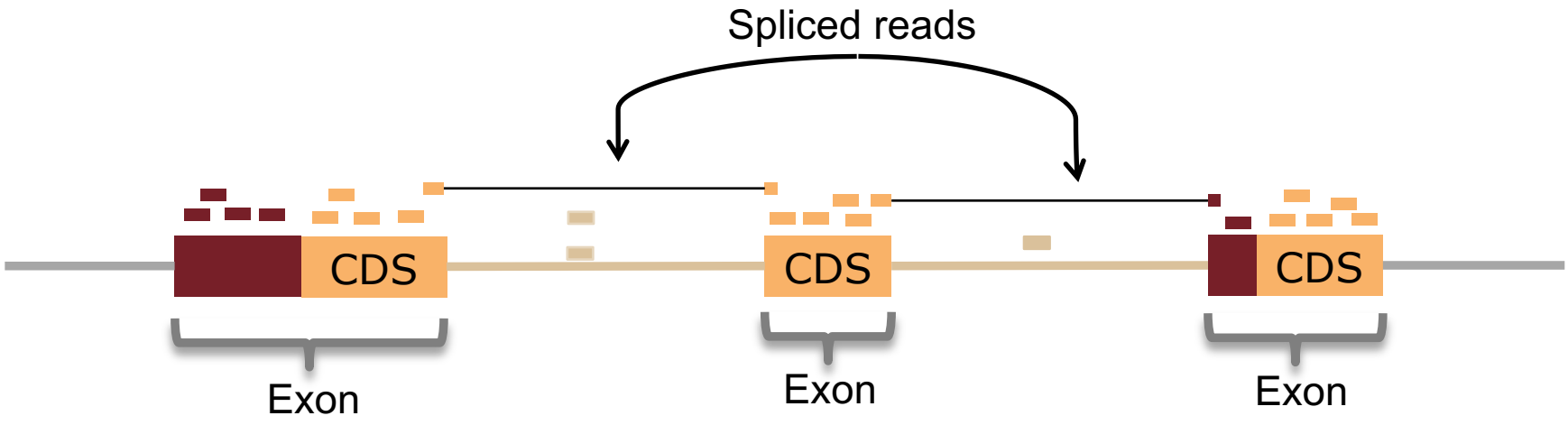
- Read count or read density can be used to estimate expression levels.
- More “normalized signal” → More expression




(Ramirez, 2017)

# Summarizing read counts

- Summarize over a biologically meaningful unit e.g. exon, CDS, transcript, or gene.
- Summarizing over the entire gene length also captures reads mapped to introns.

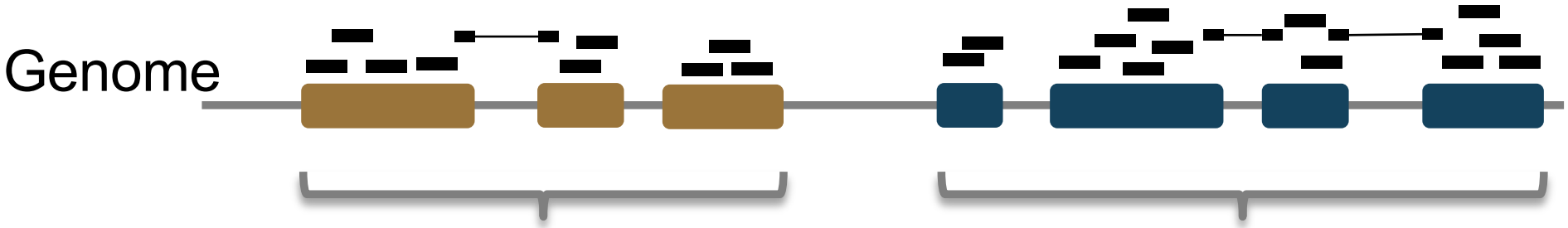



(Adapted from Oshlack, 2010)

# RPKM Reads Per Kilobase of exon per Million reads

$$RPKM = \frac{Read\ counts\ for\ transcript}{\frac{N}{1000000}\ \frac{L}{1000}}$$

Where N = Total reads mapped
L = Length of region (bp)




Gene A
Length = 1000 bp

Gene B
Length = 1500 bp

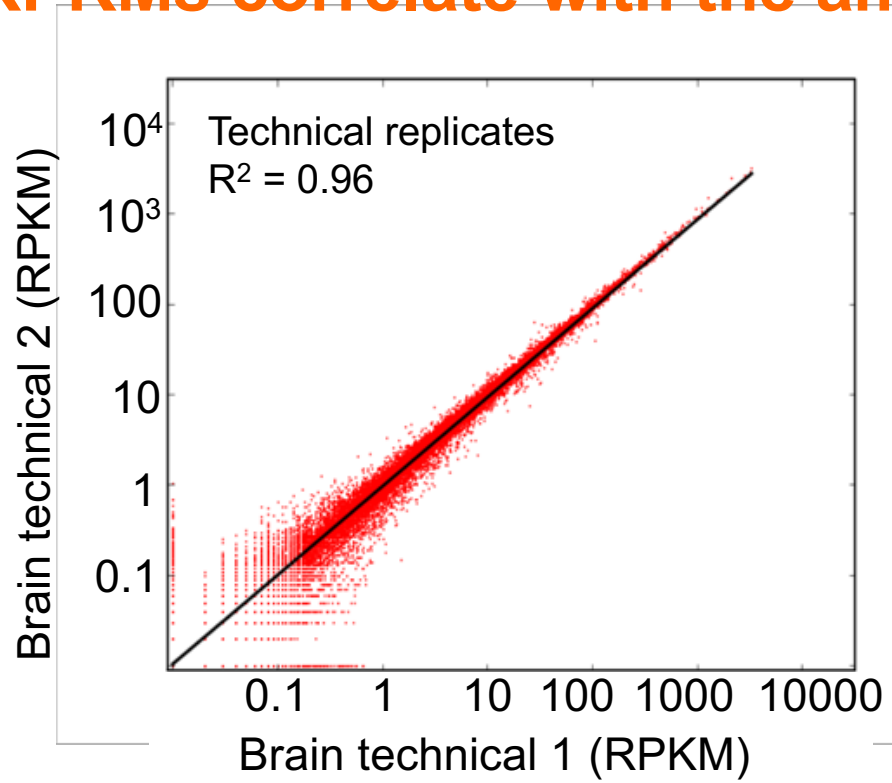
Assuming 1 million reads mapped

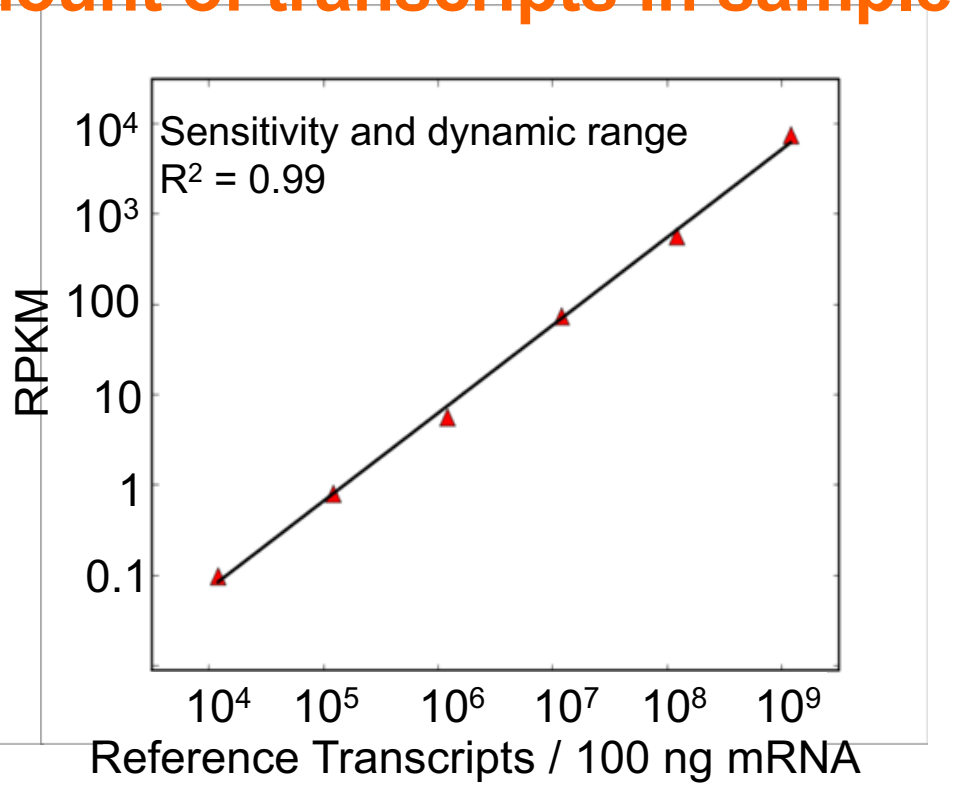
RPKM = 10/1kb = 10

RPKM = 15/1.5kb = 10

If paired-end reads, then Fragments Per Kilobase per Million of mapped reads (FPKM). I will use RPKM and FPKM interchangeably, but there are differences – see video.

# RPKMs correlate with the amount of transcripts in sample

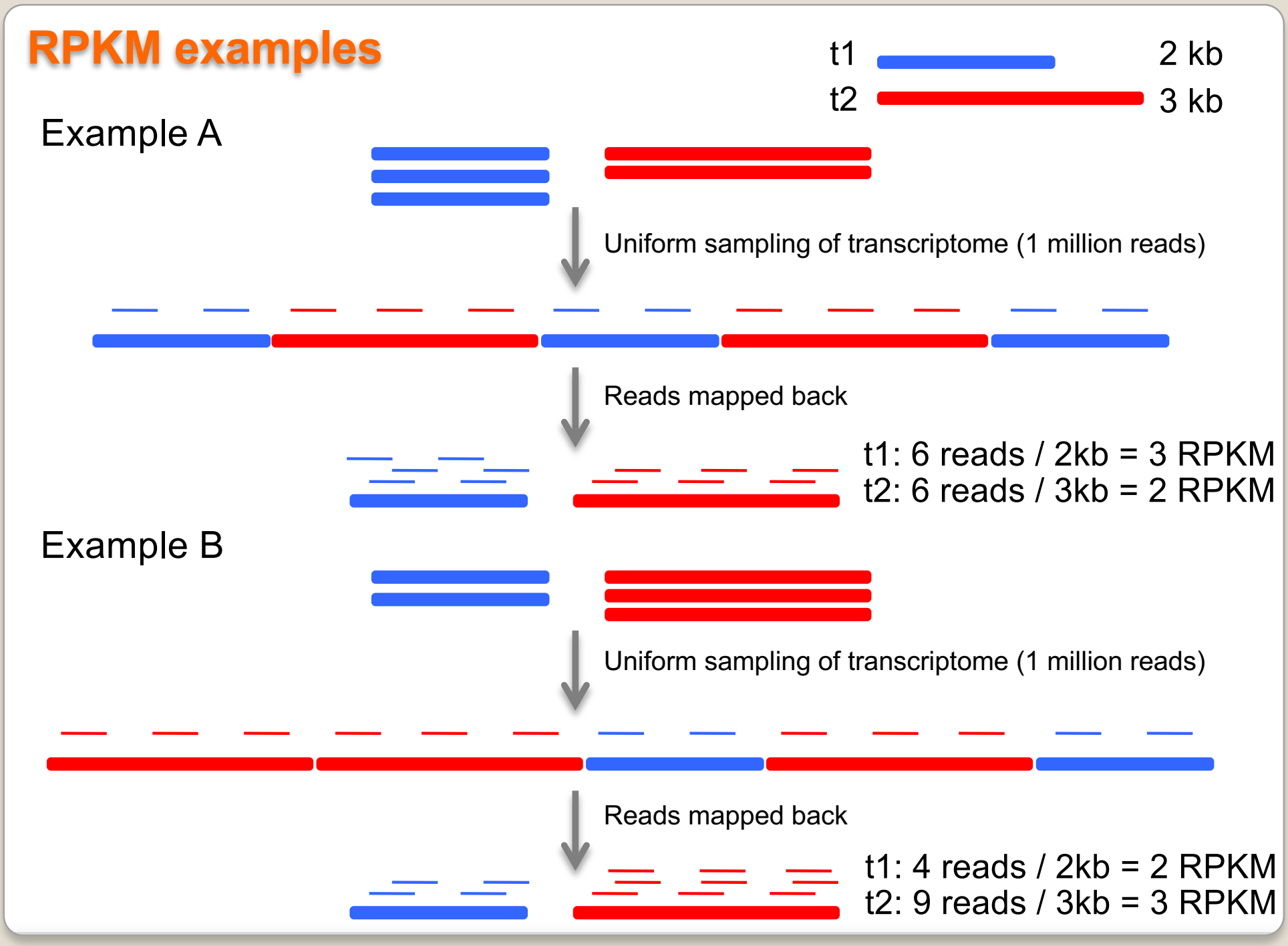


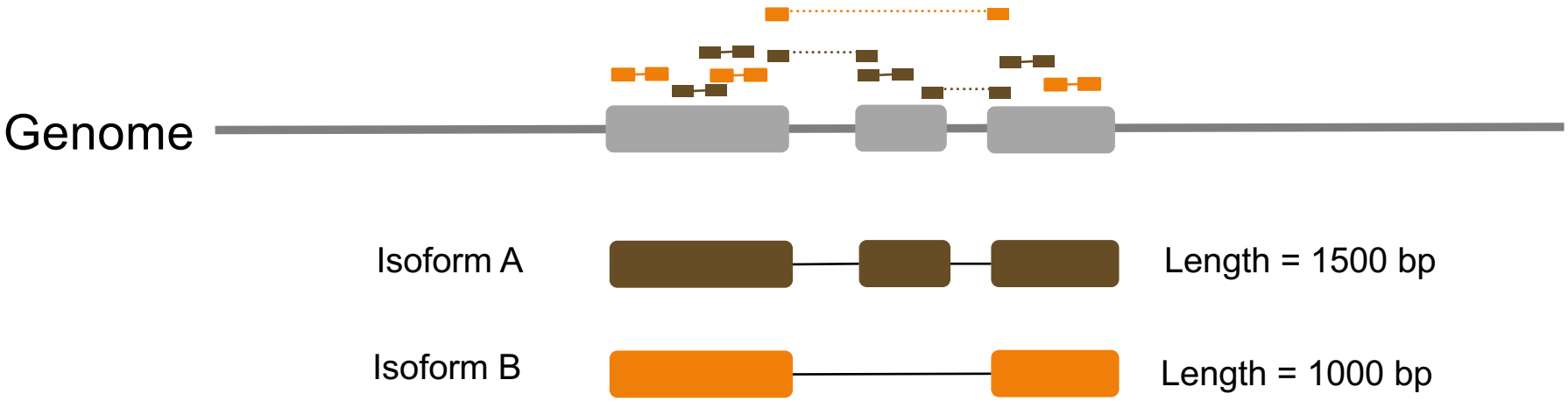

Copies of known transcripts added at known quantities

(Mortazavi, 2008)

# RPKM examples



# Adding transcript FPKMs to get gene FPKMs




Assuming 1 million reads mapped → **FPKM = $FPKM_A$ + $FPKM_B$** = 6/1.5kb + 4/1kb = 8

## Yet another quantity - TPM: Transcript Per Million

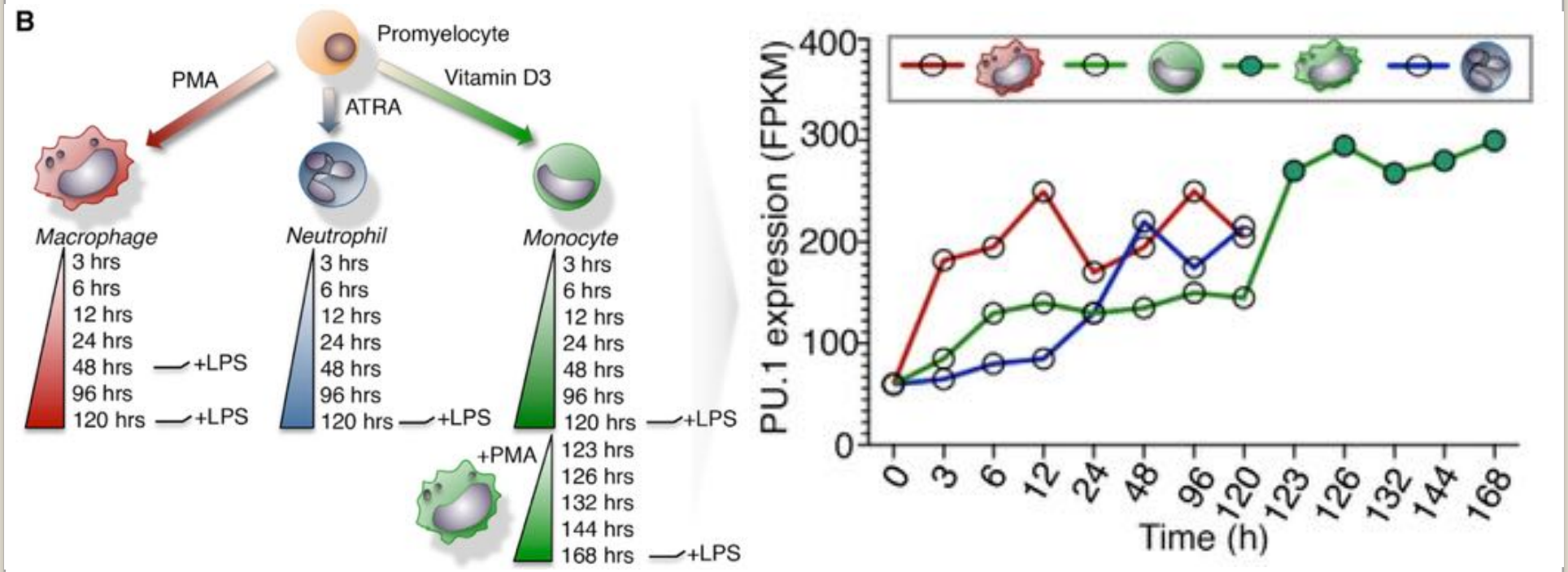
$$TPM_i = \left( \frac{FPKM_i}{\sum_j FPKM_j} \right) * 10^6$$

More stable if there is a big difference in distribution of transcripts and transcripts lengths – for example comparing very different cell types

## Quantitation of transcript expression

- Some caveats:
  - Uneven read coverage across the length of a transcript.
  - Single-end reads have less information and are harder to assemble. Paired-end reads are better.
  - Unstable solutions that are sensitive to the number of reads mapped.
  - Biological replicates are very important. Count-based analysis tools such as edgeR best used with replicates.

# Time courses of gene expression




PU.1 is an important transcription factor controlling myeloid cell differentiation

(Ramirez, 2017)

# Differential expression analysis

- **Goal:**
  To determine the set of differentially expressed genes, transcripts between two conditions or time points in a time-course experiment.

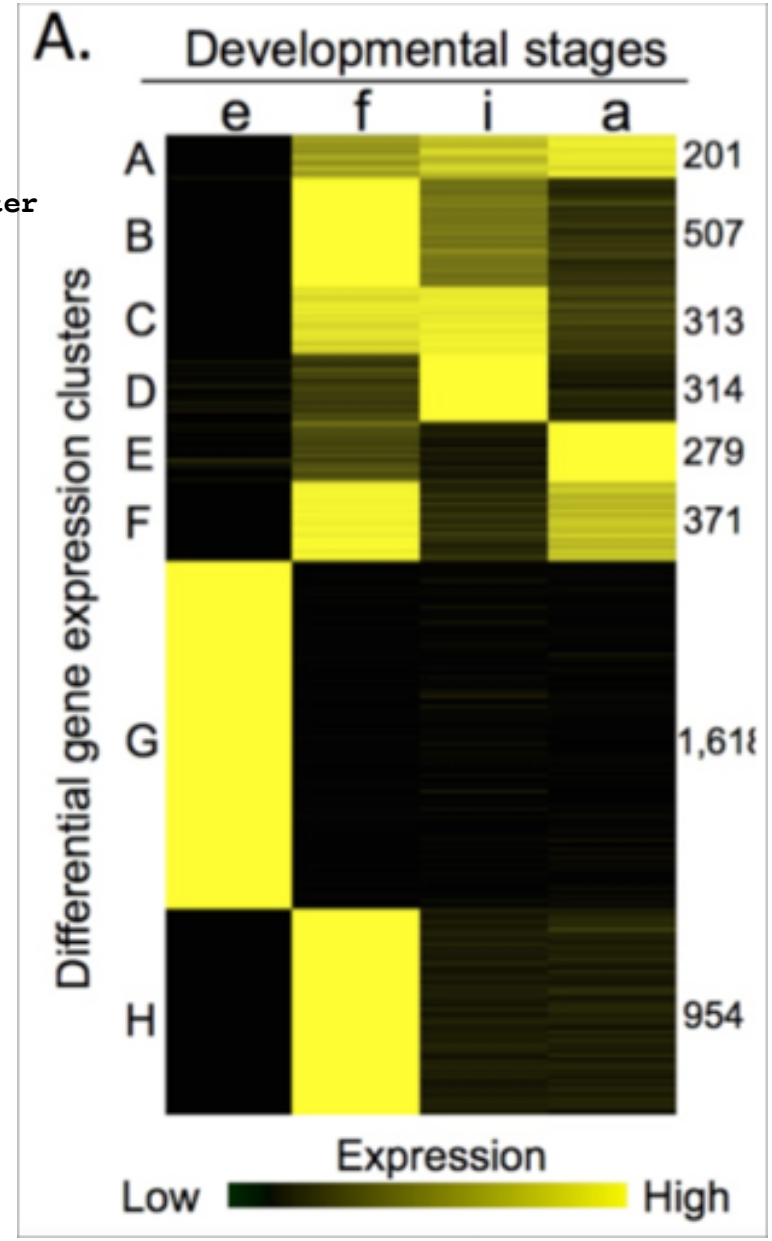
- **Approach:**

1. Determine gene, transcript abundances.
2. Test for differentially expressed genes and transcripts e.g. test for statistical significance of differences between the expressions of genes from different conditions, time points.

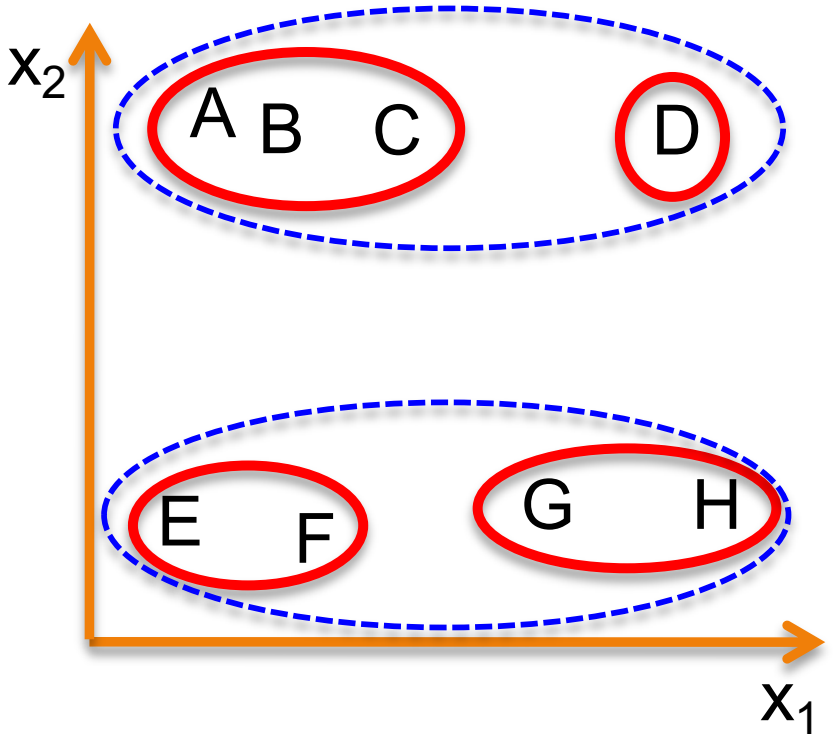
# Visualizing differential expression

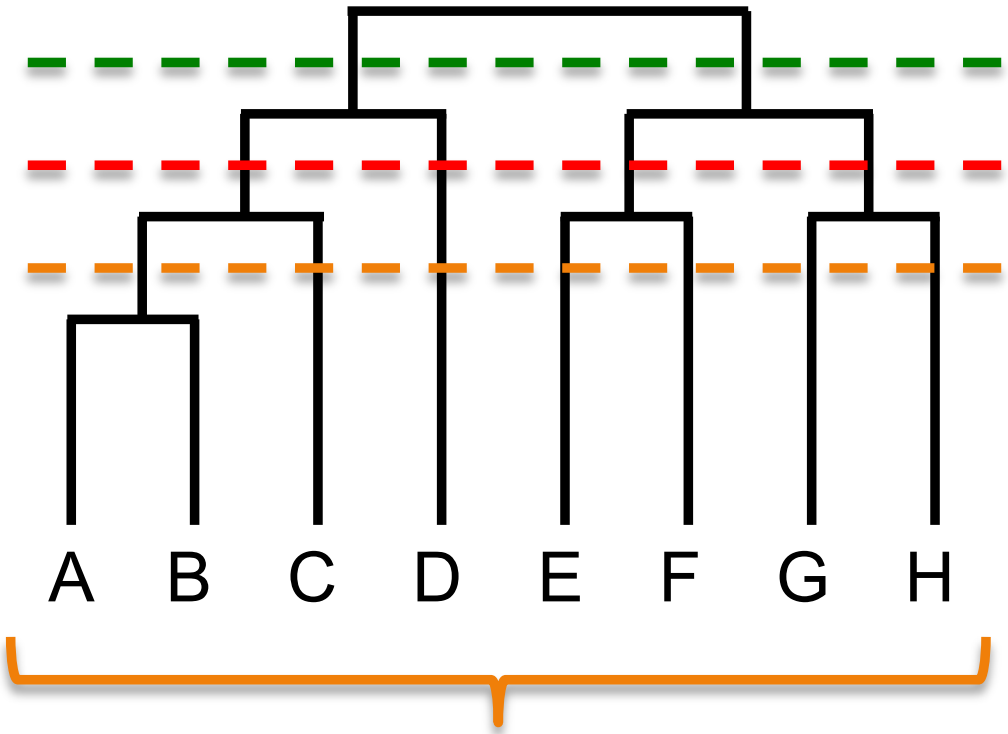
| carpoID | emb | L1 | IJ | adult | cluster |
|---|---|---|---|---|---|
| L596_g9169 | 0.0 | 63.4 | 291.97 | 350.93 | A |
| L596_g9943 | 1.73 | 66.395 | 57.695 | 71.27 | A |
| L596_g23550 | 3.365 | 90.53 | 114.275 | 88.255 | A |
| L596_g14277 | 5.515 | 199.63 | 388.89 | 403.54 | A |
| L596_g16663 | 0.0 | 11.02 | 33.11 | 25.85 | A |
| L596_g12422 | 4.835 | 665.535 | 492.87 | 463.355 | A |
| L596_g19270 | 0.765 | 66.185 | 66.815 | 81.23 | A |
| L596_g25810 | 0.83 | 47.095 | 71.08 | 87.015 | A |
| L596_g17081 | 1.775 | 263.21 | 252.68 | 399.955 | A |
| L596_g9668 | 0.065 | 6.125 | 8.415 | 6.34 | A |
| L596_g13068 | 0.0 | 14.755 | 20.515 | 14.225 | A |
| L596_g26347 | 11.95 | 0.63 | 14.49 | 19.045 | A |
| L596_g27125 | 0.0 | 32.415 | 22.06 | 24.805 | A |
| L596_g1905 | 1.125 | 44.54 | 27.73 | 62.905 | A |
| L596_g6738 | 1.355 | 107.825 | 95.11 | 91.515 | A |
| L596_g25011 | 2.6 | 76.515 | 51.075 | 99.53 | A |
| L596_g25018 | 0.21 | 13.01 | 21.8 | 36.64 | A |
| L596_g27623 | 1.335 | 45.795 | 38.37 | 61.055 | A |
| L596_g24065 | 0.055 | 18.81 | 37.135 | 29.335 | A |
| L596_g24064 | 0.0 | 23.8 | 171.315 | 121.575 | A |
| L596_g10859 | 2.765 | 121.065 | 109.31 | 147.47 | A |

We can visualize major groups of genes using clustering



# Clustering: Hierarchical & K-means




K-means clustering

K=2 ABCD EFGH

K=4 ABC D EF GH

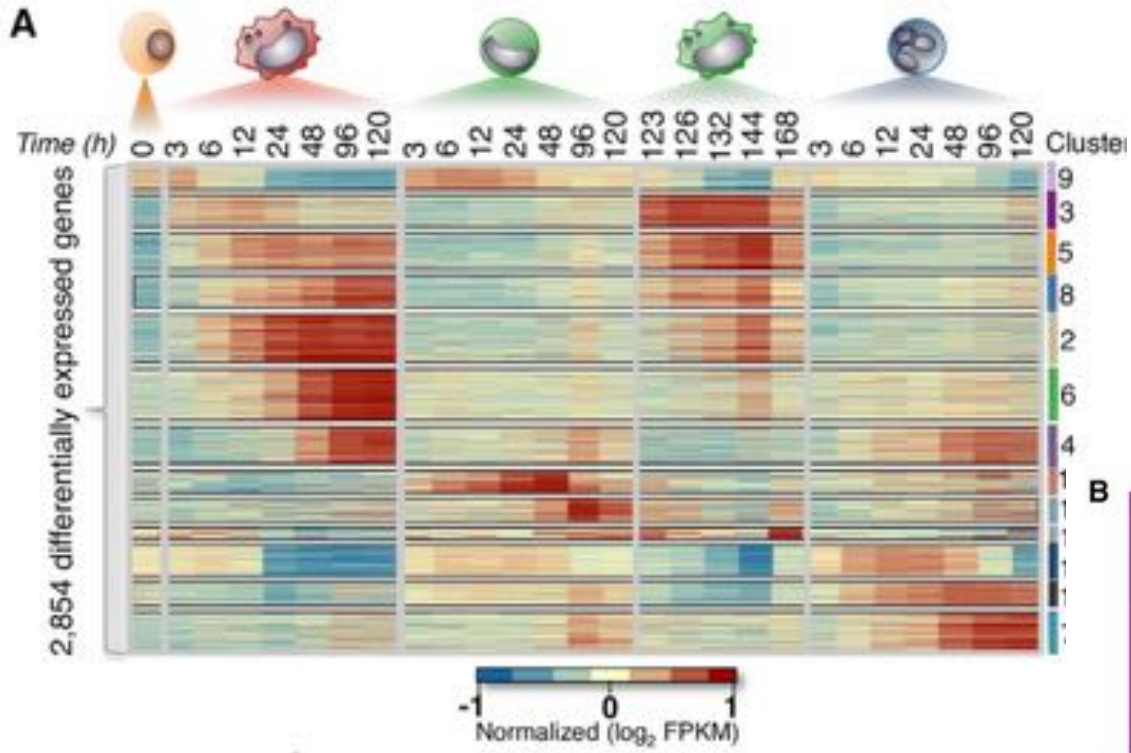
Hierarchical clustering

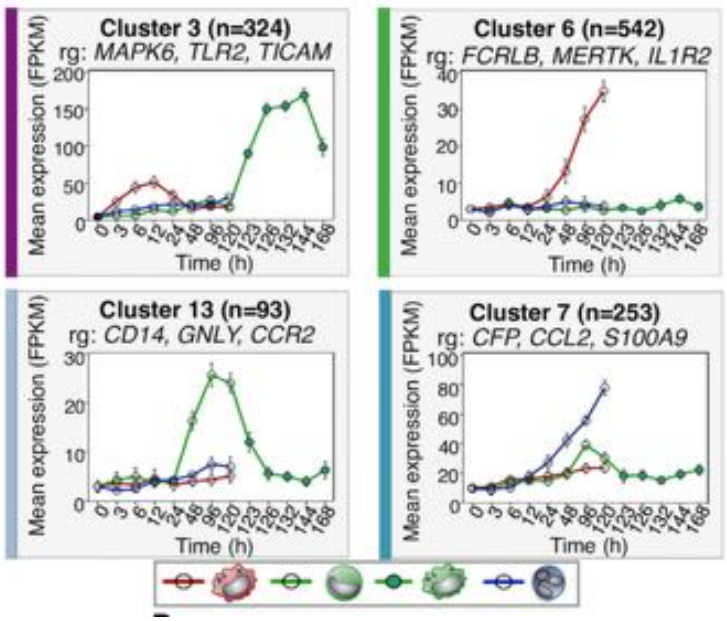
AB C D E F G H

ABC D EF GH

ABCD EFGH

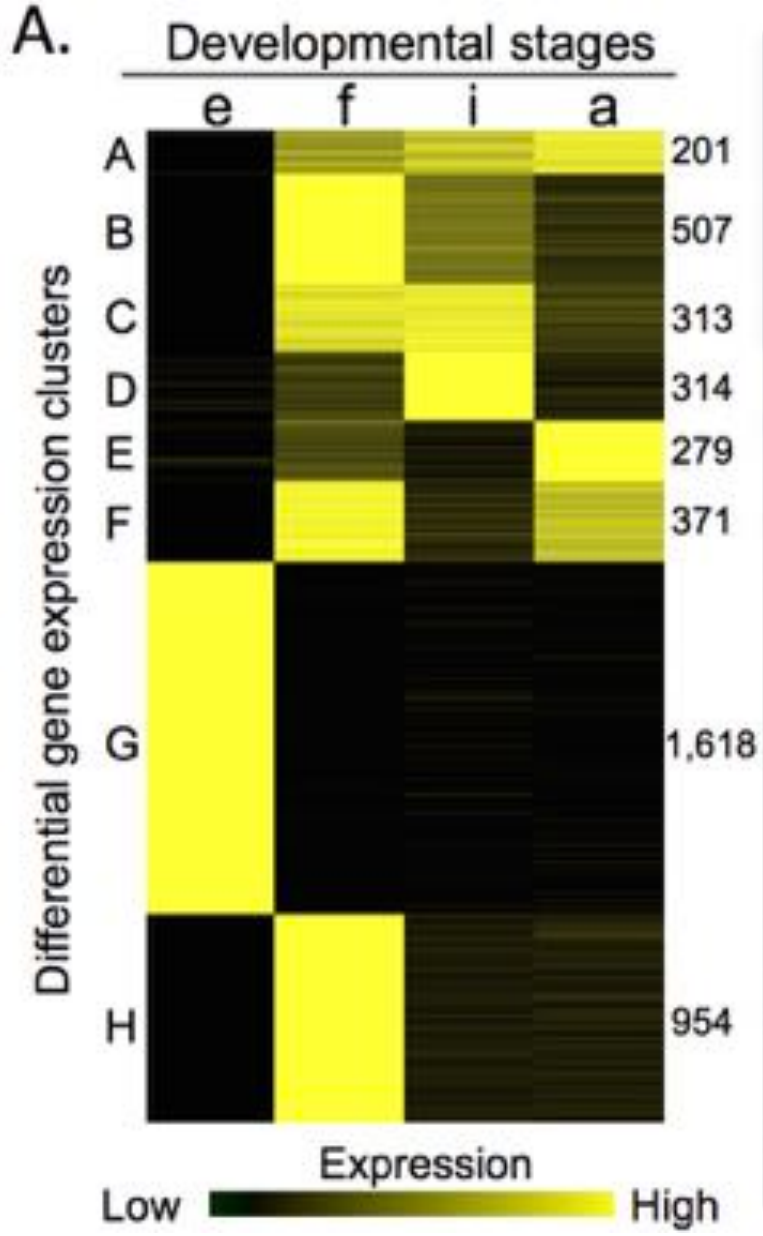
# Heatmaps of gene expression




(Ramirez, 2017)

# Going from differential expression to function




| |
|---|
| cytosolic ribosomal subunit (small and large), nematode larval development. translation initiation, growth |
| GPCR activity, TF activity, dopamine receptor signaling pathway, response to food |
| Sarcomere, metal ion binding |
| Extracellular space |
| Post-embryonic body morphogenesis |
| No significant enrichment results |
| Regulation of ubiquitin-protein transferase activity, mitotic interphase, mitotic G2 DNA damage checkpoint, SCF ubiquitin ligase complex, nucleosome, protein neddylation |
| Regulation of muscle contraction, neuron fate specification, regulation of pharyngeal pumping, acetylcholine-gated channel complex, post synaptic membrane, sarcomere, action potential |

(Dillman, in press)

# Using sequence to derive function

## Assume that a gene is differentially expressed

| carpoID | emb | L1 | IJ | adult | DE_cluster |
|---|---|---|---|---|---|
| L596_g19086 | 451.88 | 0.82 | 0.0 | 0.32 | G |
| L596_g28254 | 294.365 | 1.125 | 0.345 | 0.12 | G |
| L596_g22582 | 316.345 | 0.54 | 0.04 | 0.805 | G |

## And that it's sequence is:

>L596_g19086.t1
CCACCTCTCACCTACCCAGAAATCCAAAACCCTTCCACCATGTACATTCCATCTGTGAGC
ATAAATTCAACCGGAACCTCCCTCTGCTACGGCGACATCGACGGTTACTTTGTCTACAGC
CTCAACGACATCAAGCAGAAGAATTTCTGTGAGAAATTCGATCAATGGGATCCGTCTACA
GAGCTCTACTTGGCCGAACGTCTCAACACTTCTAAACTTCTGCTGTGCTGTTCCCACAAG
GACAGTCGTGAGGTTCTGATGATCGGCTTAAAGGCTAAGCAGGTCATTATGAAGCTCGAC
ATGCCTTCGGATGTTCTTAGTGTTAGGATTAACAATGAGAGCCTAATCATCTGCATGCAG
CACATGATCCGAATCTTCATCCCCCAGACAATGCAGGCCATCCACACCATCAACGACATC
CCTTCAAACCCTAACGGCGCTGCTGACCTCTCCCAAACCACCTCTATGATCGCATATCCA
ACTTTCGAAAACGGCGGAAAAGTGGTGATATACGACGGAAAGAACCTGAAAGCCGTTCGG
GTCATTGACGCCCATGATGGACCCGTTGTGGTTCTGAAATTCAACAAGCAAGGAACCCTC
ATAGCTACTGCCTCAGACAAGGGTACTGTAATCAGAGTCTTCAGCGTGGAAACGGGCGTT
CTTGTCCATGAATTTCAACGAGGCGTCACACGATTTGCGACGATTTACTCTATCGCATTT
TCTGAAGATTCTCAGTACCTCGCCTGCACCAGTAATACGGGTACCGTACACCTCTTCCAT
TTGTCTCCACGTGAAAATGAGCCATGCTTCCCTAAAGATAGTAATCCTATCAGTGACCTC
GTCAGCTATTTGTGGAAAAGCGCTGAGGCCTACACACCTGCCGTGGTGAGACCCAAATCT
ACGTCCTCCTGTGTGCTGCCAGTCGGGGCTAACAGTTTCGCAGCCTGTGCGCTGAGGATT
ATGAATAACAGGCTGCATTTGATTGTGGCTACTTCGGAGAAGTATTTGTTTGTTTATGAG
TTTGACCCTTATAAGTTAGAGCTGAGTTTGAAGAGTCAGTTCGAGTTGGGTAGGGAGGAA
GAAGAGAAGGGAGTTGGTTTGATGAACTCGTTTTGAAGGTTGGATAGAAGTGTTTTTGAC
TATAAAGATTGTTTTAAGAGAGATTTAAGTGTGAAAAACACAACTTAAGGTTAATACATA
AAAGGCTGCAAA

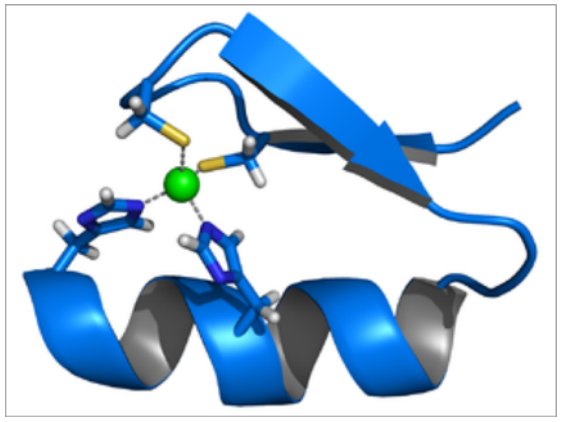
What is its Function?

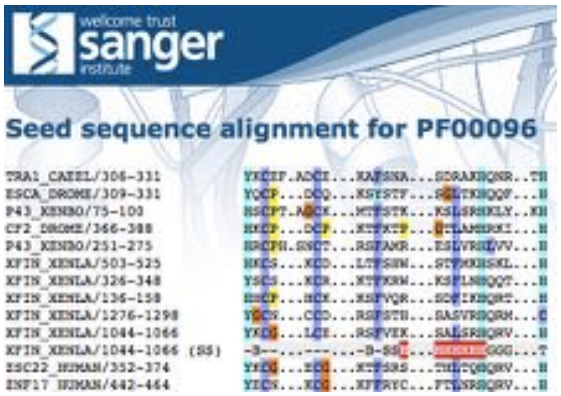
# Annotate protein domains from sequence

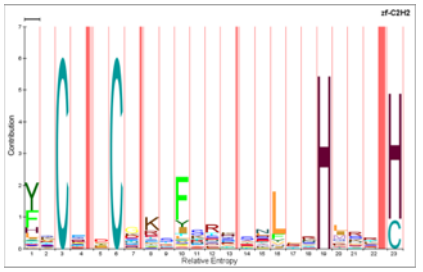
- Proteins typically have functional domains, such as
    - DNA binding
    - Transmembrane

- We can use profile-HMMs of these domains to annotate novel genes using a combination of :
    - HMMer
    - The Pfam database of protein families

A famous protein domain – C2H2 Zinc fingers





# Using PFAM to annotate protein domains

wellcome trust sanger institute

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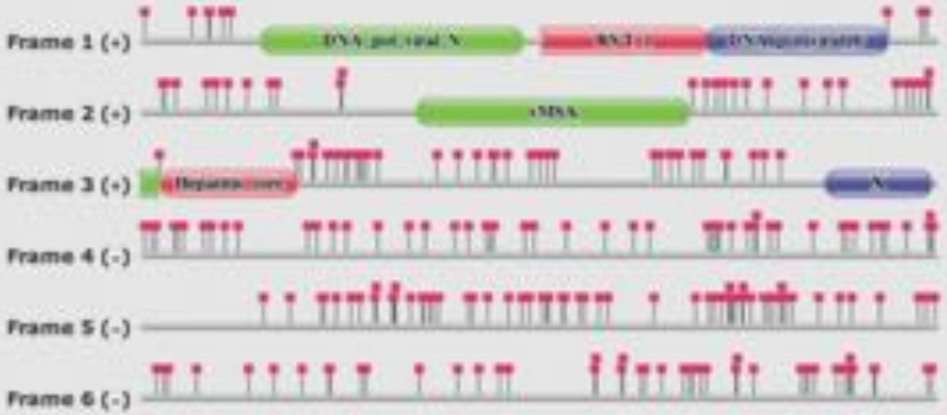
Pfam

## DNA sequence search results

This page shows the results of searching your **DNA** sequence for Pfam-A matches. To do this we perform a six-frame translation to generate a set of **protein** sequences, which we then search using the normal Pfam-A HMMs and **GA** cut-offs.

**Show** the detailed description of this results page.

We have found **7** significant hits and **0** insignificant hits in **3** frames.




**Show** the DNA and protein sequences, and the URL for bookmarking these results.

Return to the search form to look for Pfam domains on a new sequence.

### Significant Pfam-A Matches

Show or hide all alignments. Toggle between amino-acid and DNA sequence coordinates.

| Frame (sense) | Family | Description | Entry type | Clan | Envelope Start | Envelope End | Alignment Start | Alignment End | HMM From | HMM To | HMM length | Bit score | E-value | Predicted active sites | Show/hide alignment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 (+) | DNA_pol_viral_N | DNA polymerase (viral) N-terminal domain | Family | n/a | 163 | 514 | 163 | 514 | 1 | 379 | 379 | 573.7 | 1.7e-172 | n/a | Show |
| 1 (+) | RVT_1 | Reverse transcriptase (RNA-dependent DNA _ | Family | CL0027 | 538 | 762 | 539 | 762 | 2 | 214 | 214 | 206.6 | 2.7e-61 | n/a | Show |
| 1 (+) | DNA_pol_viral_C | DNA polymerase (viral) C-terminal domain | Family | n/a | 763 | 1005 | 763 | 1005 | 1 | 245 | 245 | 477.2 | 8.1e-144 | n/a | Show |
| 2 (+) | vMSA | Major surface antigen from hepadnavirus | Family | n/a | 372 | 737 | 372 | 737 | 1 | 364 | 364 | 563.4 | 2.2e-169 | n/a | Show |
| 3 (+) | Hep_core_N | Hepatitis core protein, putative zinc fi _ | Domain | n/a | 1 | 24 | 1 | 24 | 4 | 27 | 27 | 58.7 | 2.5e-16 | n/a | Show |
| 3 (+) | Hepatitis_core | Hepatitis core antigen | Domain | n/a | 28 | 209 | 28 | 209 | 1 | 187 | 187 | 315.3 | 1.1e-94 | n/a | Show |
| 3 (+) | X | Trans-activation protein X | Family | n/a | 923 | 1064 | 923 | 1064 | 1 | 142 | 142 | 268.1 | 1.4e-80 | n/a | Show |

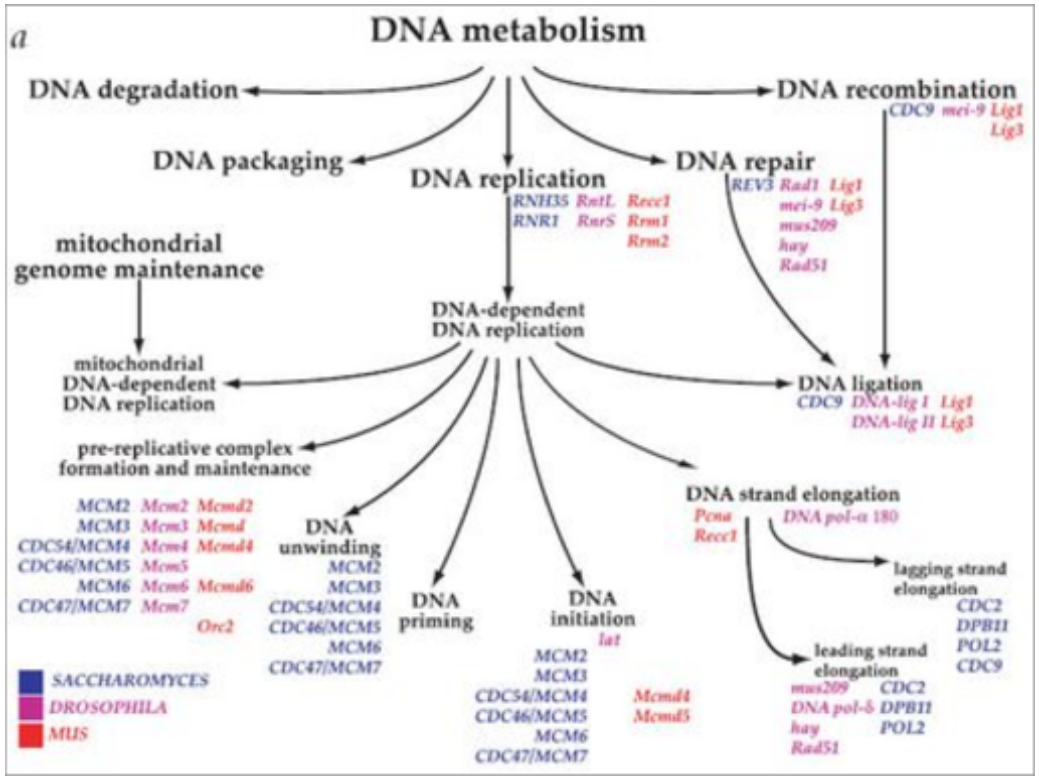
Comments or questions on the site? Send a mail to pfam-help@sanger.ac.uk. Our cookie policy.
The Wellcome Trust

(Finn, 2014)

# Annotate transcript function

- Once we know functional domains and/or orthologs, what can we say about a gene in relation to others

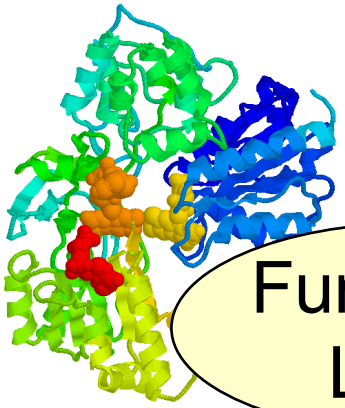
- To analyze a set of genes, a standardized structured vocabulary is helpful. Why would that be ?

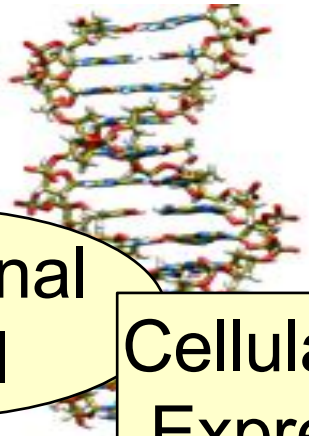



(Ashburner, 2000)

# To analyze gene enrichments, must standardize on vocabulary

The function is on the protein





Functional Label

Cellular Role
Expression
Interactions
.....

Controlled Vocabulary

High throughput

Accessible

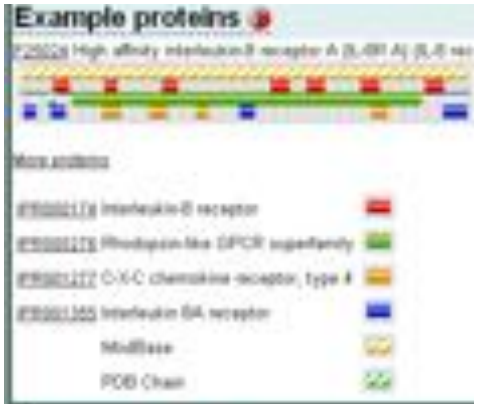
Slides courtesy of Ana Conesa

# Several databases are designed for data-mining

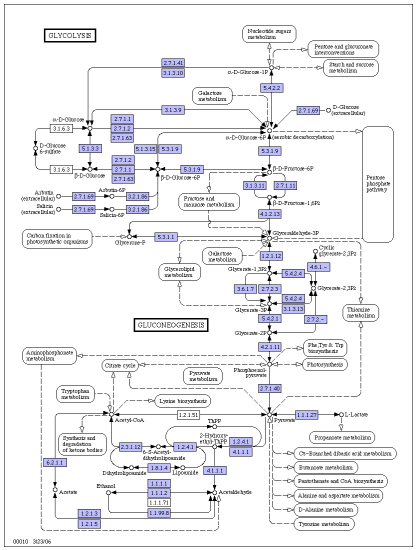

***Molecular Function***
***Biological Process***
***Cellular Component***



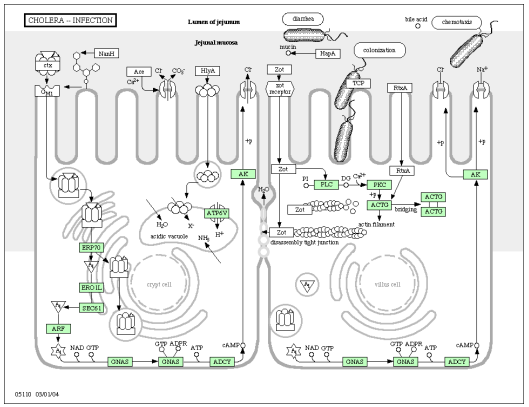
***Functional motifs***




***Metabolic pathways***




***KEGG orthologues***




Slides courtesy of Ana Conesa

## Gene Ontology (GO)

- ✓ Project developed by the Gene Ontology Consortium
- ✓ Provides a controlled vocabulary to describe gene and gene product attributes in any organism
- ✓ Lastest version more than 33,300 terms
- ✓ Includes both the development of the Ontology and the maintenance of a Database of annotations

http://www.geneontology.org/

Slides courtesy of Ana Conesa

# The three categories of GO

## Molecular Function

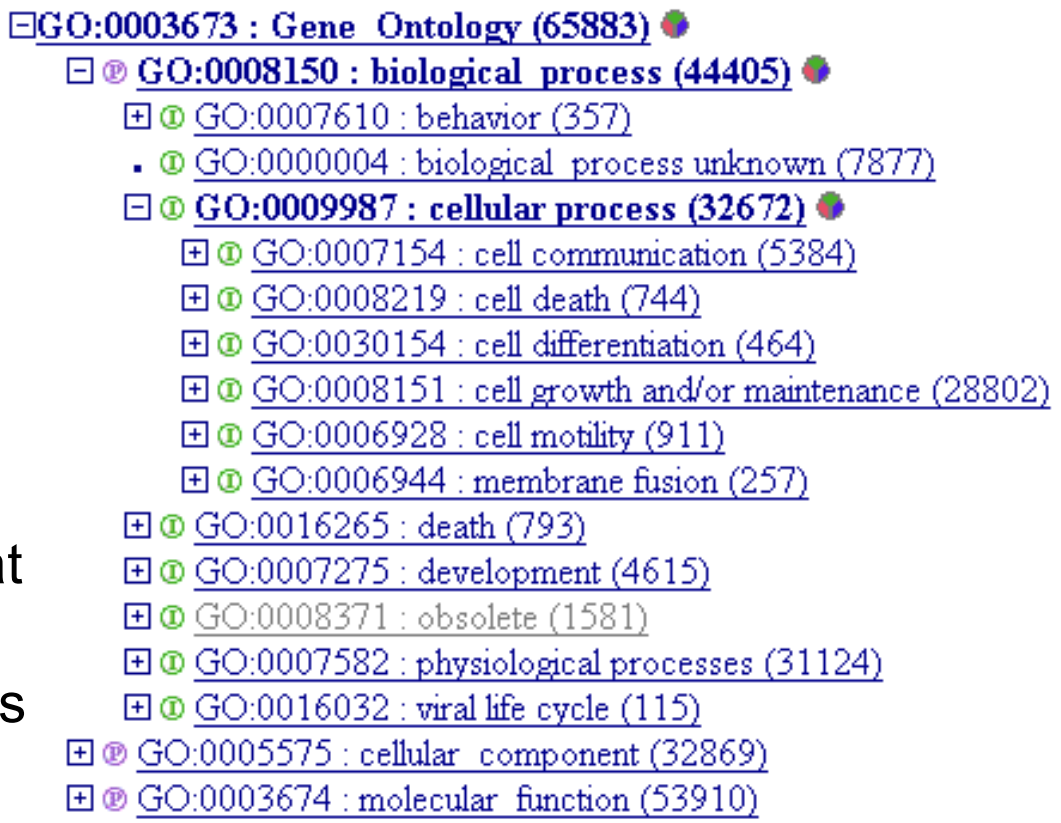
the tasks performed by individual gene products; examples are *transcription factor* and *DNA helicase*

## Biological Process

broad biological goals, such as *mitosis* or *purine metabolism*, that are accomplished by ordered assemblies of molecular functions

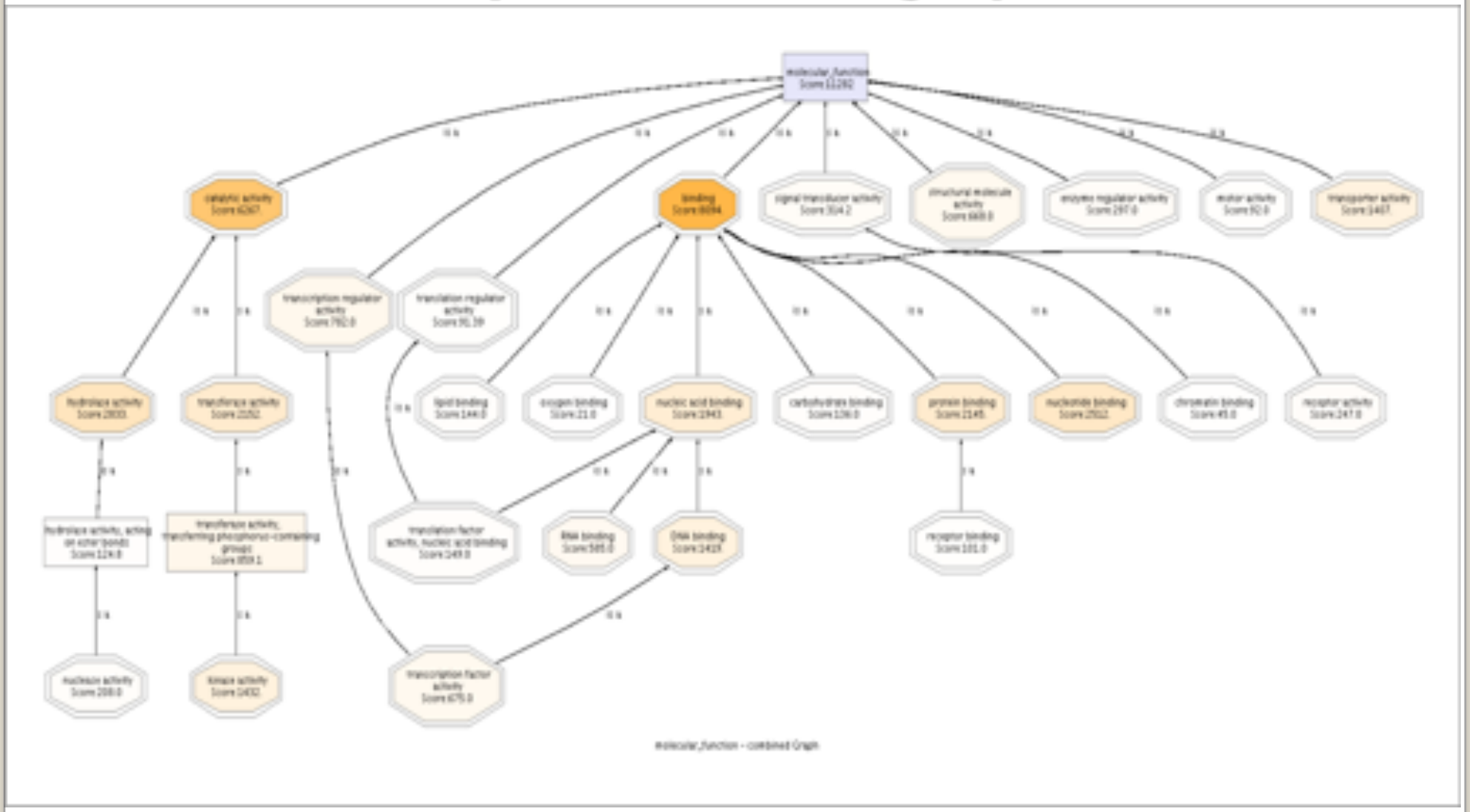
## Cellular Component

subcellular structures, locations, and macromolecular complexes; examples include *nucleus*, *telomere*, and *origin recognition complex*




Slides courtesy of Ana Conesa

# The GO hierarchy is a directed graph

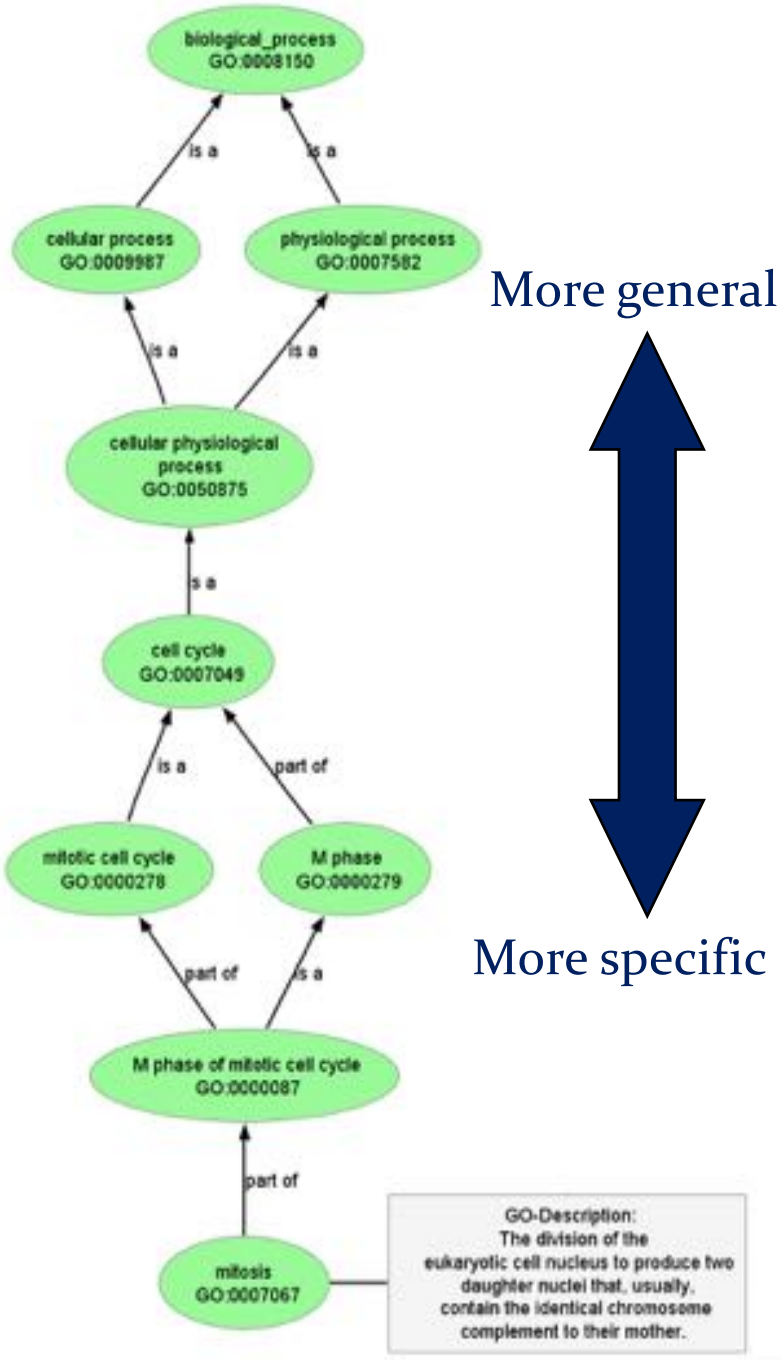


Slides courtesy of Ana Conesa

# The GO hierarchical levels

- ✓ Annotations are given to the most specific (low) level
- ✓ True path rule: annotation at a given term implies annotation to all its parent terms
- ✓ Annotation is given with an Evidence Code:
  - **IDA**: inferred by direct assay
  - **TAS**: traceable author statement
  - **ISS**: infered by sequence similarity
  - **IEA**: electronic annotation
  - ….

Slides courtesy of Ana Conesa



# GO example: NRSF/REST (part 1)

**Gene Ontology (GO): 11 molecular function terms** (see first 5): *About this table*

| GO ID | Qualified GO term | Evidence | PubMed IDs |
|---|---|---|---|
| GO:0001046 | core promoter sequence-specific DNA binding | IDA | 8568247 |
| GO:0001047 | core promoter binding | IDA | 17984088 |
| GO:0001078 | RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in negative regulation of transcription | IDA | 10449787 |
| GO:0003677 | DNA binding | -- | -- |
| GO:0003682 | chromatin binding | ISS | -- |
| GO:0003700 | sequence-specific DNA binding transcription factor activity | IDA | 19342457 |
| GO:0005515 | protein binding | IPI | 10449787 |
| GO:0008134 | transcription factor binding | IPI | 17130167 |
| GO:0015271 | outward rectifier potassium channel activity | IMP | 18570921 |
| GO:0044212 | transcription regulatory region DNA binding | IDA | 17555596 |
| GO:0046872 | metal ion binding | IEA | -- |

**Gene Ontology (GO): 4 cellular component terms**: *About this table*

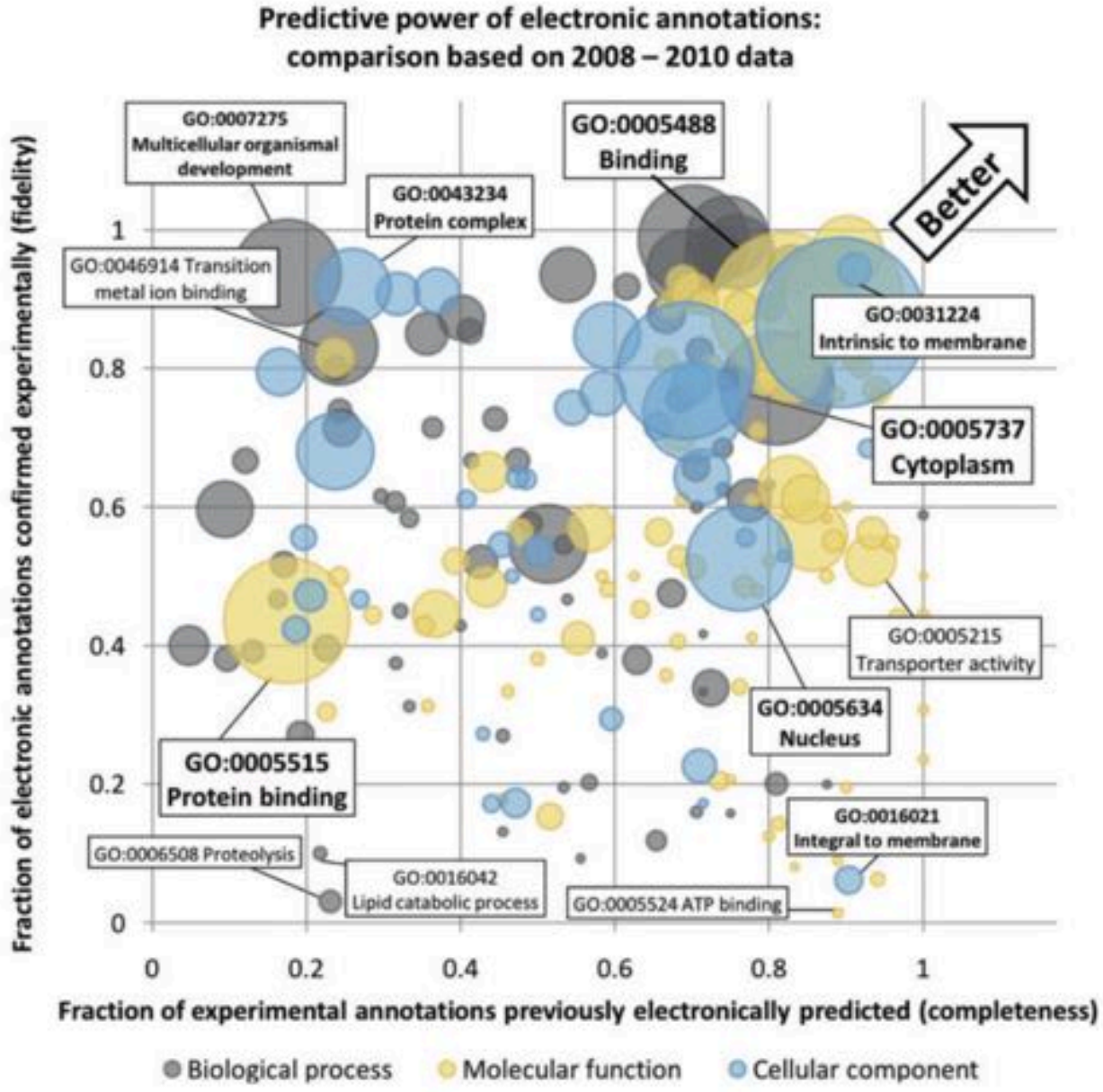
| GO ID | Qualified GO term | Evidence | PubMed IDs |
|---|---|---|---|
| GO:0005634 | nucleus | NAS | 7871435 |
| GO:0005737 | cytoplasm | -- | -- |
| GO:0005829 | cytosol | IEA | -- |
| GO:0017053 | transcriptional repressor complex | IDA | 10734093 |

# GO example: NRSF/REST (part 2)

**Gene Ontology (GO): 24 biological process terms** (see first 5): ***About this table***

| GO ID | Qualified GO term | Evidence | PubMed IDs |
|---|---|---|---|
| GO:0000122 | negative regulation of transcription from RNA polymerase II promoter | TAS | 7697725 |
| GO:0006355 | regulation of transcription, DNA-templated | NAS | 7871435 |
| GO:0008285 | negative regulation of cell proliferation | IMP | -- |
| GO:0010629 | negative regulation of gene expression | IMP | -- |
| GO:0032348 | negative regulation of aldosterone biosynthetic process | IMP | 19342457 |
| GO:0035690 | cellular response to drug | IMP | -- |
| GO:0043065 | positive regulation of apoptotic process | IMP | -- |
| GO:0043280 | positive regulation of cysteine-type endopeptidase activity involved in apoptotic process | IMP | -- |
| GO:0043922 | negative regulation by host of viral transcription | IDA | 17555596 |
| GO:0045665 | negative regulation of neuron differentiation | IMP | 18570921 |
| GO:0045892 | negative regulation of transcription, DNA-templated | NAS | 7871435 |
| GO:0045893 | positive regulation of transcription, DNA-templated | IDA | 17984088 |
| GO:0045955 | negative regulation of calcium ion-dependent exocytosis | ISS | -- |
| GO:0046676 | negative regulation of insulin secretion | IMP | -- |
| GO:0050768 | negative regulation of neurogenesis | ISS | -- |
| GO:0060379 | cardiac muscle cell myoblast differentiation | ISS | -- |
| GO:0070933 | histone H4 deacetylation | IDA | 17555596 |
| GO:0071257 | cellular response to electrical stimulus | IMP | 18570921 |
| GO:0071385 | cellular response to glucocorticoid stimulus | IDA | 17984088 |
| GO:0071805 | potassium ion transmembrane transport | IMP | 18570921 |
| GO:2000065 | negative regulation of cortisol biosynthetic process | IMP | 19342457 |
| GO:2000706 | negative regulation of dense core granule biogenesis | ISS | -- |
| GO:2000740 | negative regulation of mesenchymal stem cell differentiation | IMP | 18570921 |
| GO:2000798 | negative regulation of amniotic stem cell differentiation | IMP | -- |

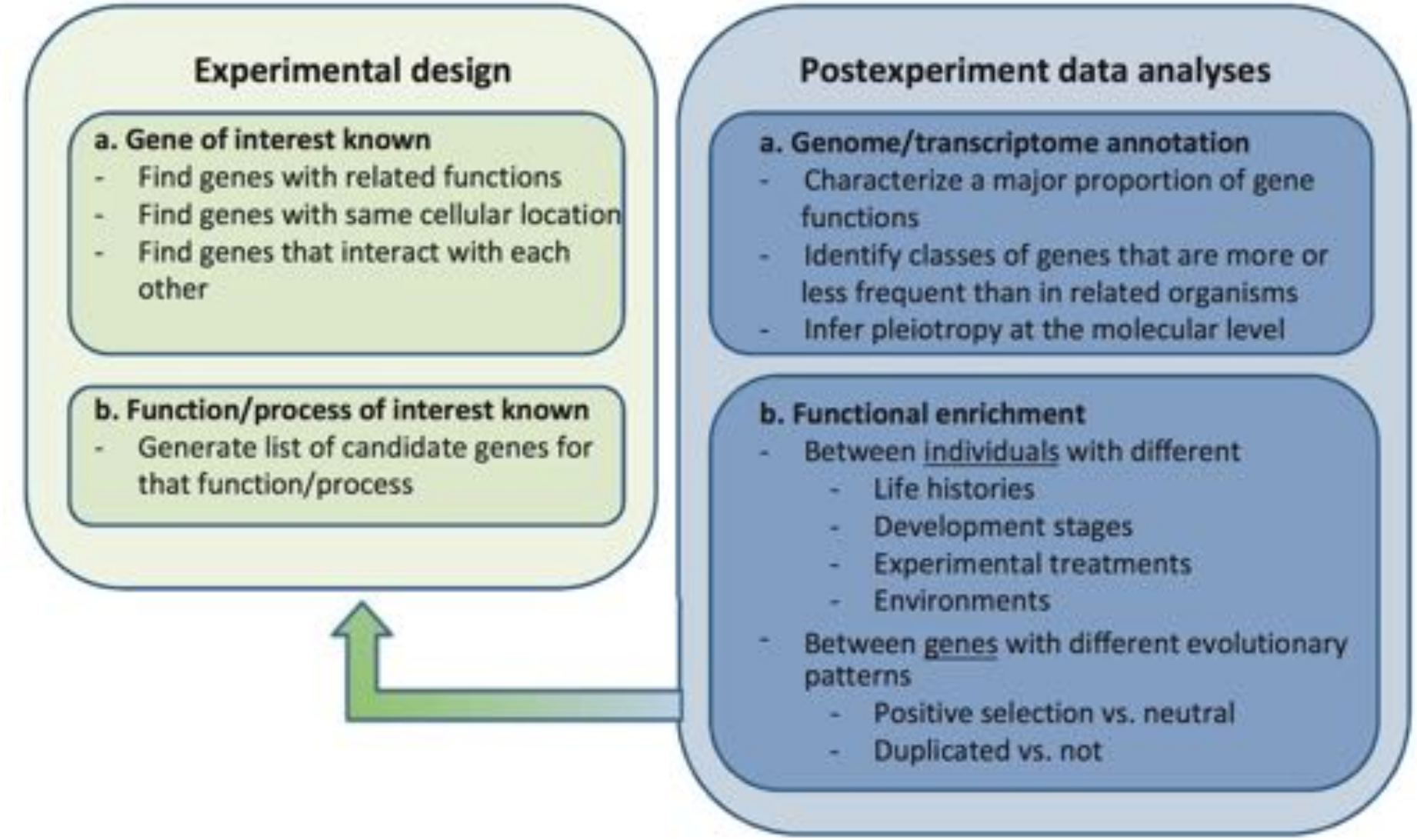
# Some computationally predicted GO terms are more likely to be experimentally validated




(du Plessis, 2011)

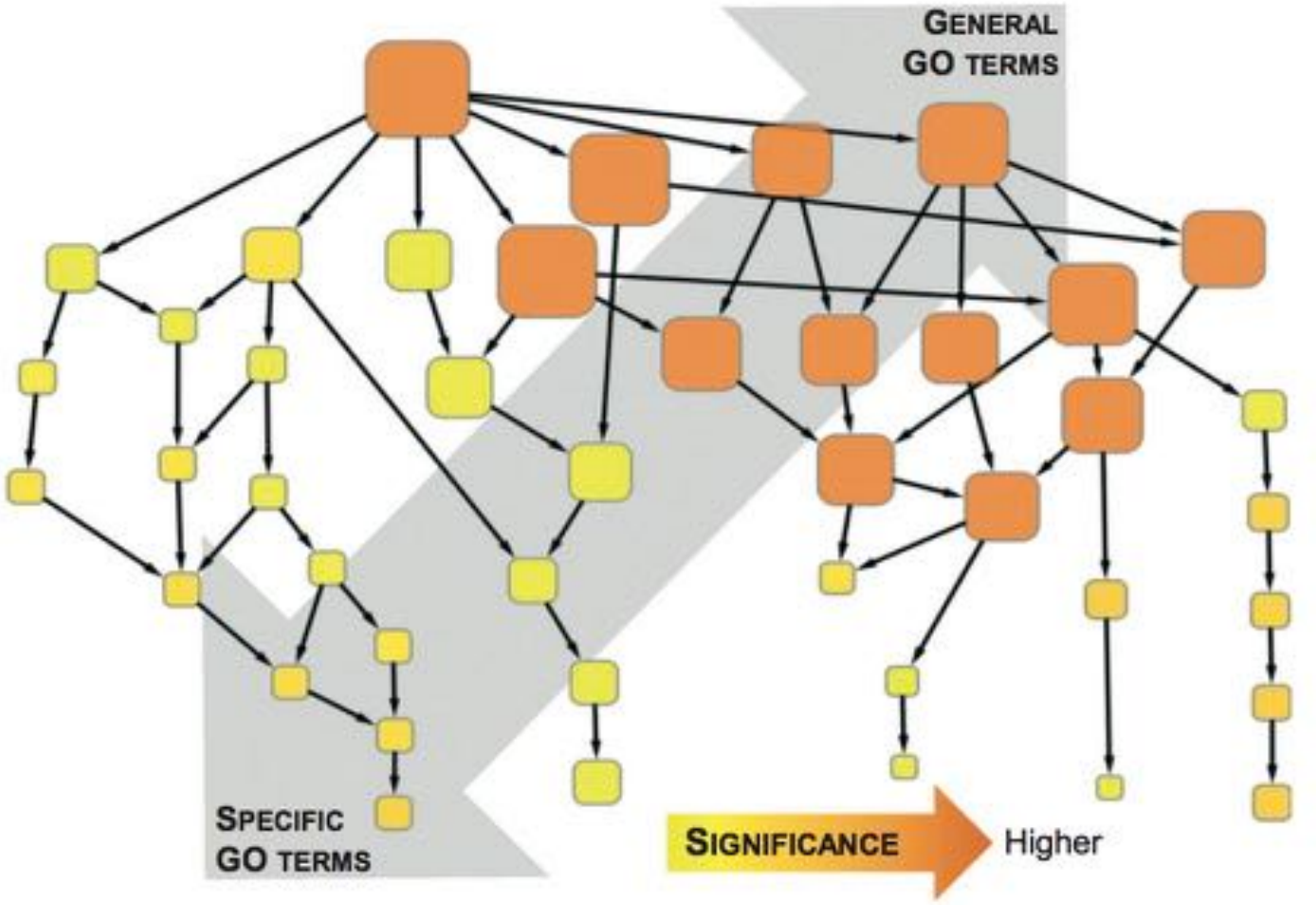
# Applications of GO

Can be applied to analysis of samples from annotated genomes or unannotated genomes



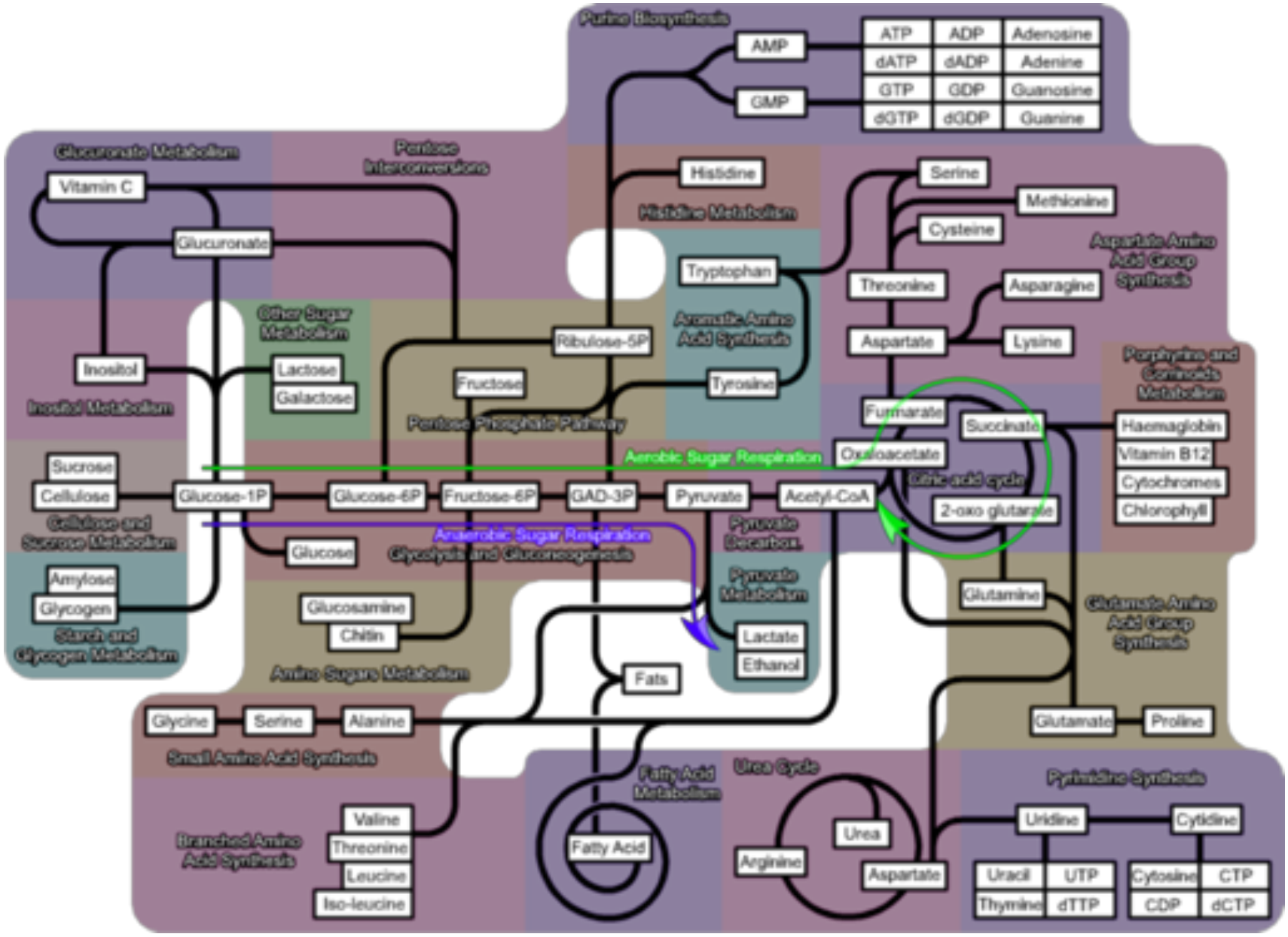

(Primmer, 2013)

# Enrichment analyses of GO terms favors more general terms



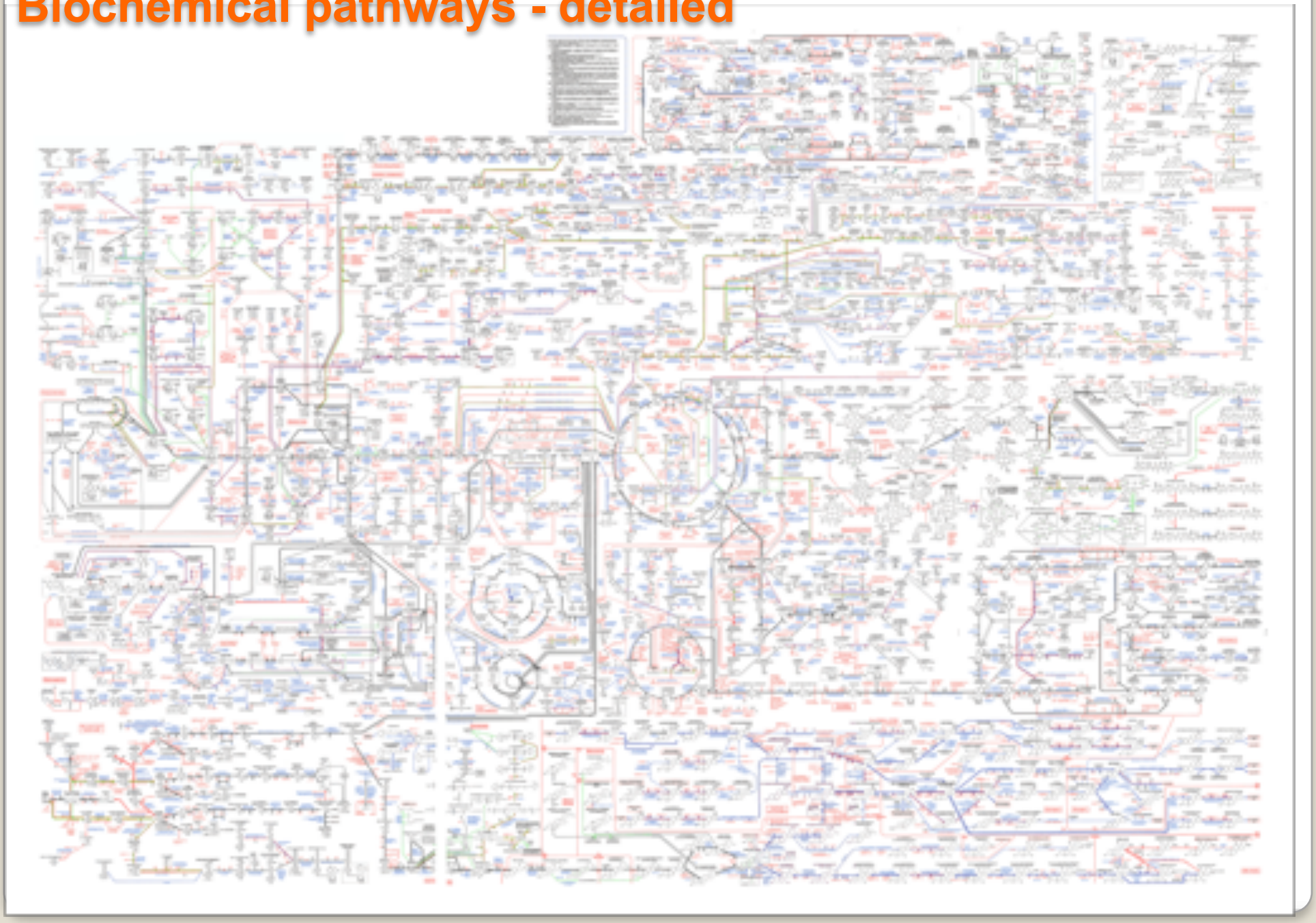

(Primmer, 2013)

# Biochemical pathways - simplified




wikipedia

# Biochemical pathways - detailed



http://biochemicalpathwayswallchart.blogspot.com/2010/10/roche-biochemical-pathways-wall-chart.html

# Known biochemical networks are catalogued by KEGG


**KEGG PATHWAY** is a collection of manually drawn pathway maps representing our knowledge on the molecular interaction and reaction networks for:

1. **Metabolism**
   Global/overview Carbohydrate Energy Lipid Nucleotide Amino acid Other amino Glycan
   Cofactor/vitamin Terpenoid/PK Other secondary metabolite Xenobiotics Chemical structure
2. **Genetic Information Processing**
3. **Environmental Information Processing**
4. **Cellular Processes**
5. **Organismal Systems**
6. **Human Diseases**

and also on the structure relationships (KEGG drug structure maps) in:

7. **Drug Development**

1. Metabolism

1.1 Carbohydrate Metabolism
- Glycolysis / Gluconeogenesis
- Citrate cycle (TCA cycle)
- Pentose phosphate pathway
- Pentose and glucuronate interconversions
- Fructose and mannose metabolism
- Galactose metabolism
- Ascorbate and aldarate metabolism
- Starch and sucrose metabolism
- Aminosugars metabolism
- Nucleotide sugars metabolism
- Pyruvate metabolism
- Glyoxylate and dicarboxylate metabolism
- Propanoate metabolism
- Butanoate metabolism
- C5-Branched dibasic acid metabolism
- Inositol metabolism
- Inositol phosphate metabolism

1.2 Energy Metabolism
- Oxidative phosphorylation
- Photosynthesis
- Photosynthesis - antenna proteins
- Carbon fixation
- Reductive carboxylate cycle (CO2 fixation)
- Methane metabolism
- Nitrogen metabolism
- Sulfur metabolism

1.3 Lipid Metabolism
- Fatty acid biosynthesis
- Fatty acid elongation in mitochondria
- Fatty acid metabolism
- Synthesis and degradation of ketone bodies
- Biosynthesis of steroids
- Bile acid biosynthesis

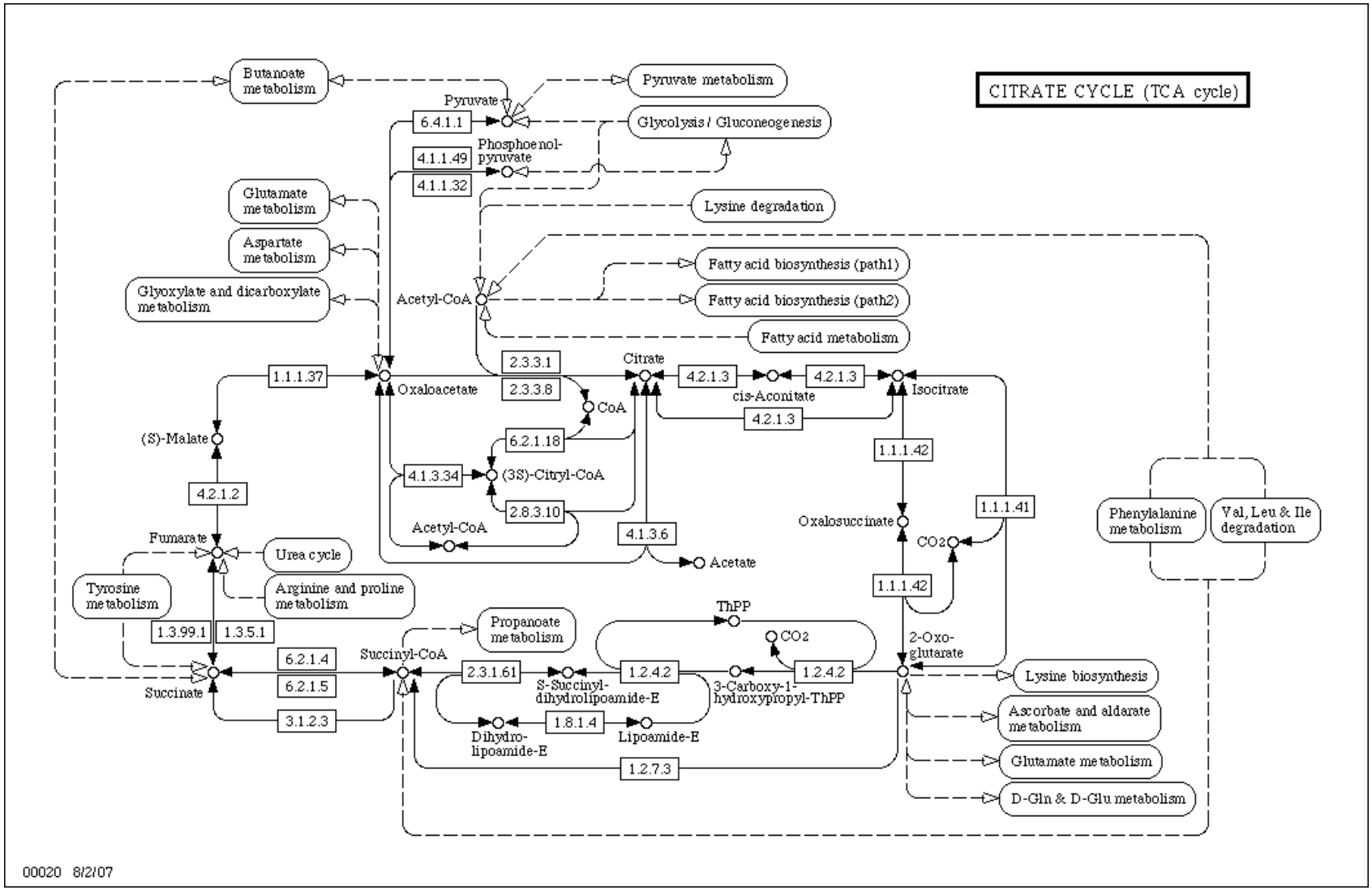
## Current Statistics

**KEGG Database** as of 2015/7/17

| | | |
|---|---|---|
| Systems information | | |
| KEGG PATHWAY | Pathway maps, reference (total) | 475 (402,444) |
| KEGG BRITE | Functional hierarchies, reference (total) | 205 (138,436) |
| KEGG MODULE | KEGG modules, reference (total) | 708 (325,410) |
| Genomic information | | |
| KEGG ORTHOLOGY | KEGG Orthology (KO) groups | 18,879 |
| KEGG GENOME | KEGG Organisms | 3,985 |
| KEGG GENES | Genes in high-quality genomes (312 eukaryotes, 3445 bacteria, 211 archaea) | 17,556,548 |

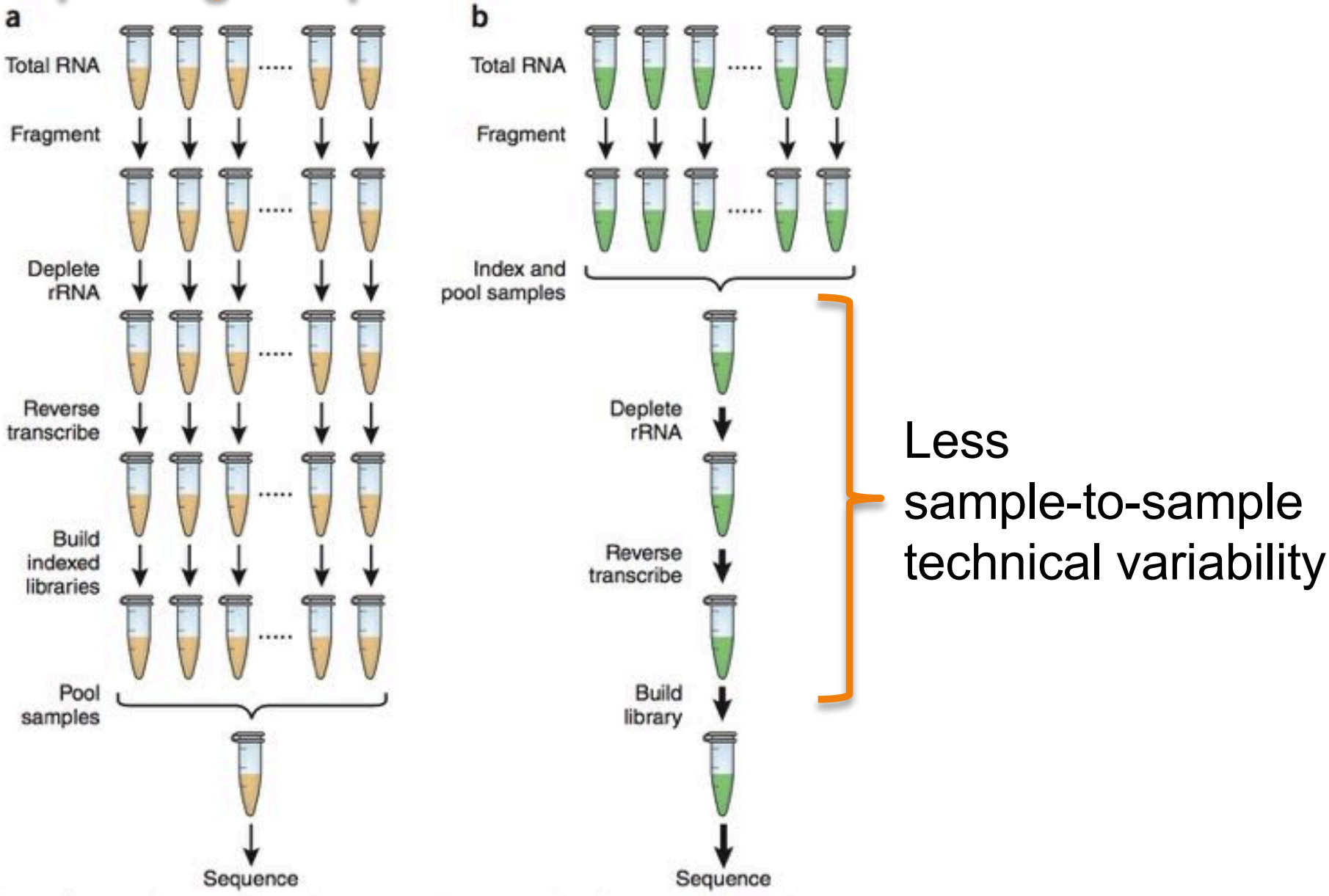
http://www.genome.jp/kegg/

# A well-known biochemical pathway: the TCA cycle



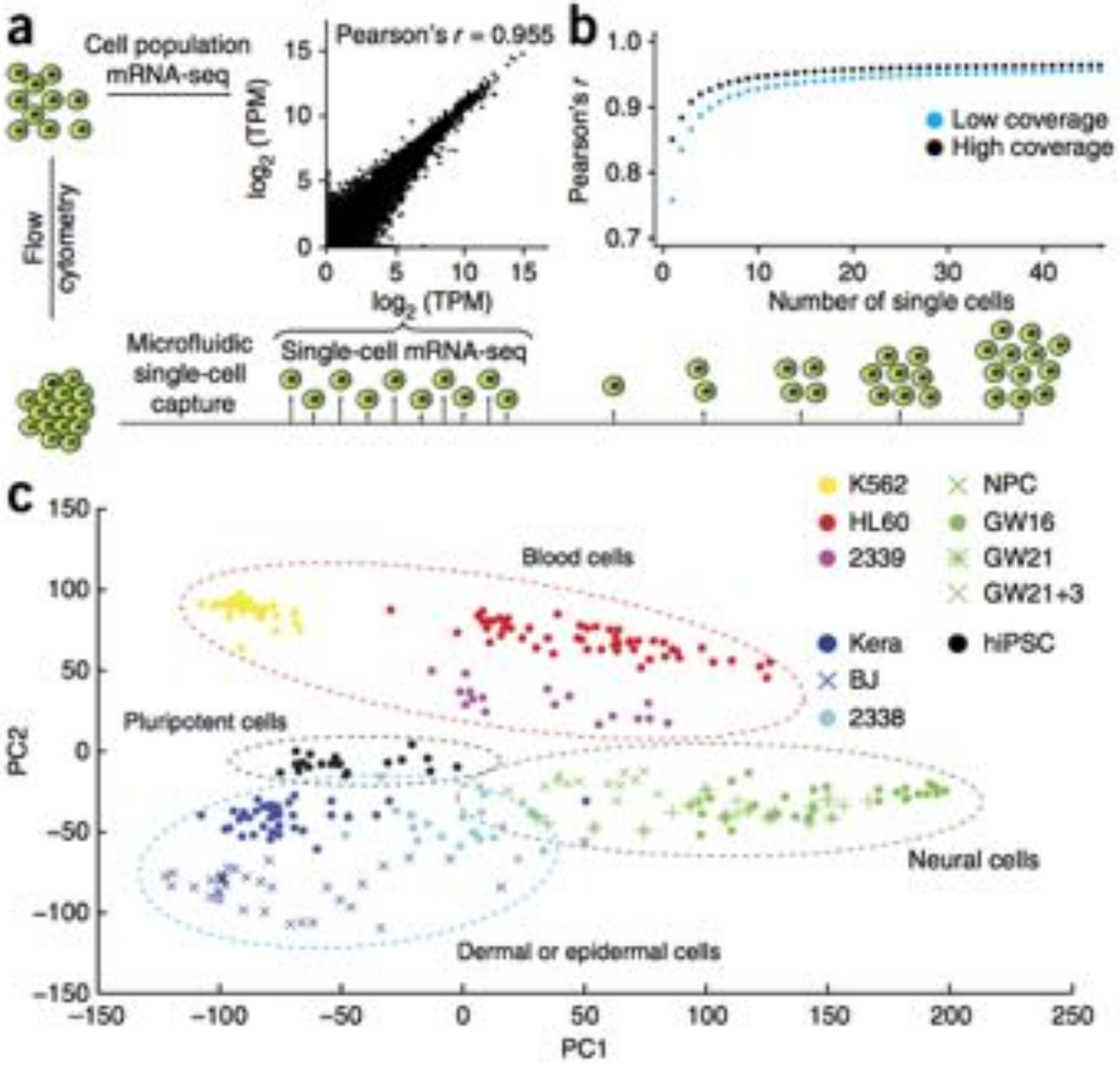

Are several of these genes differentially expressed ?

# Multiplexing samples



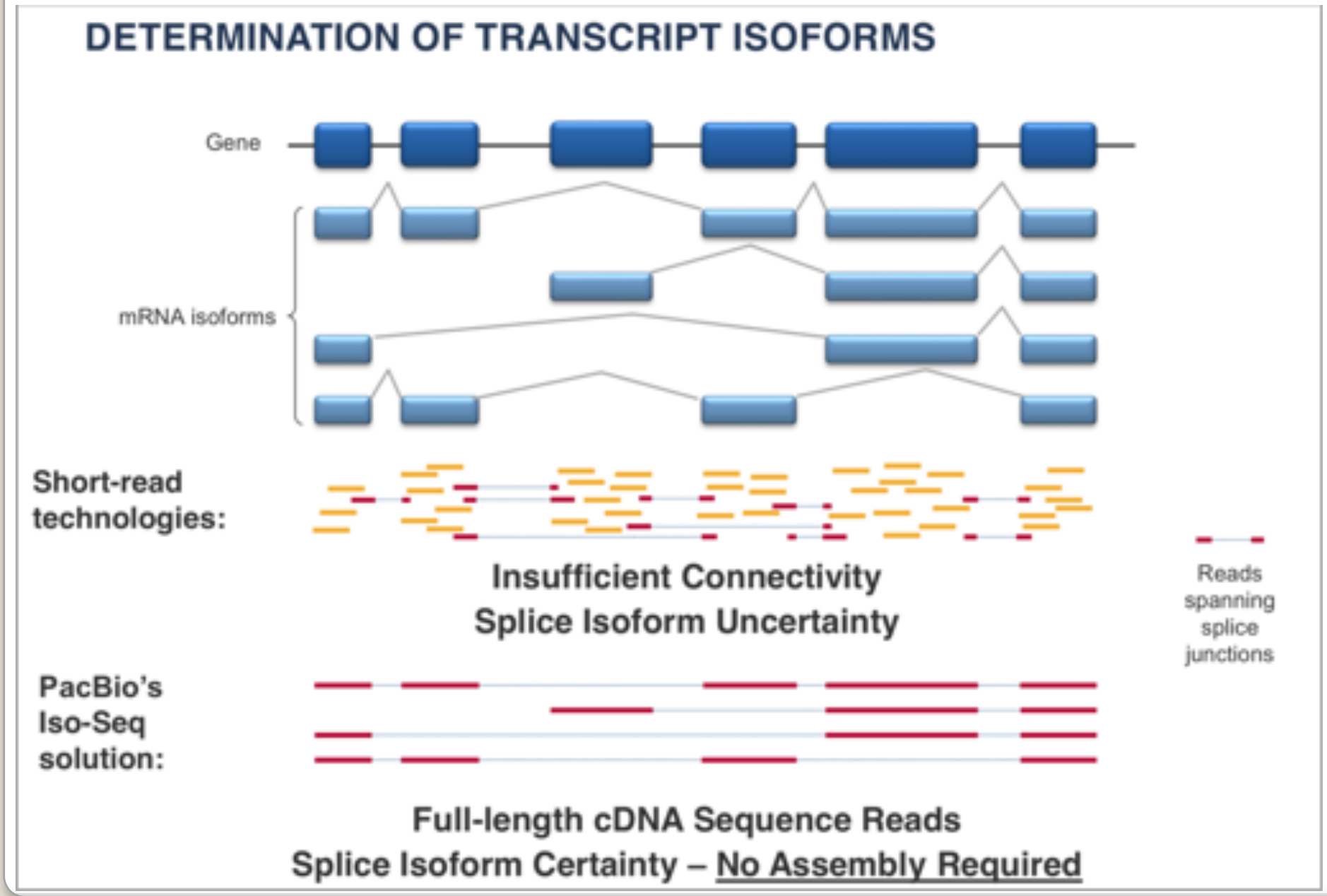

(Mortazavi, 2015)

# Single-cell RNA-seq captures population behavior




(Pollen, 2014)

# Using long-reads to sequence entire mRNAs end-to-end




PacBio

# Online videos

RNA-seq:

https://www.youtube.com/watch?v=tlf6wYJrwKY

RPKM vs FPKM vs TPM:

https://www.youtube.com/watch?v=TTUrtCY2k-w

Gene Ontology:

https://www.youtube.com/watch?v=3EUaurjK7u8