RNA-seq Introduction

Promises and pitfalls

Enabler for Life Sciences

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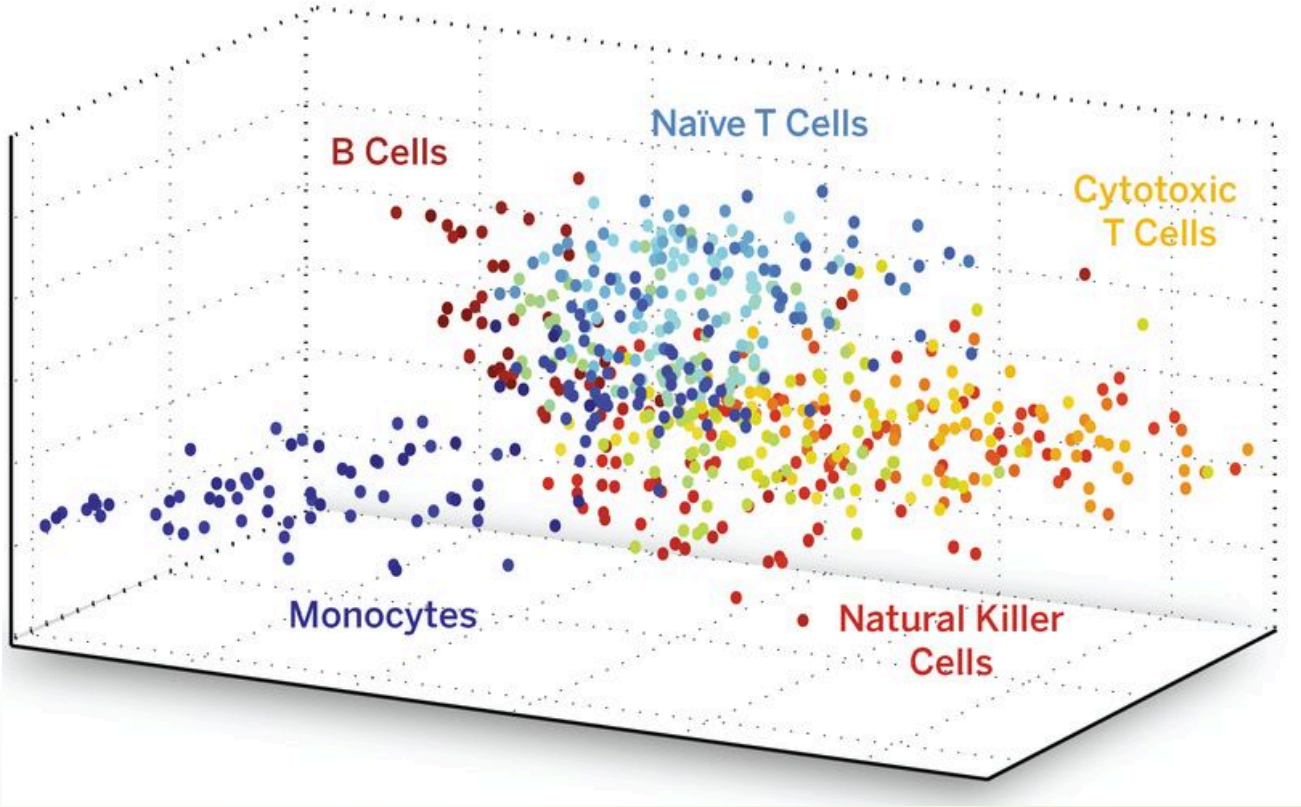
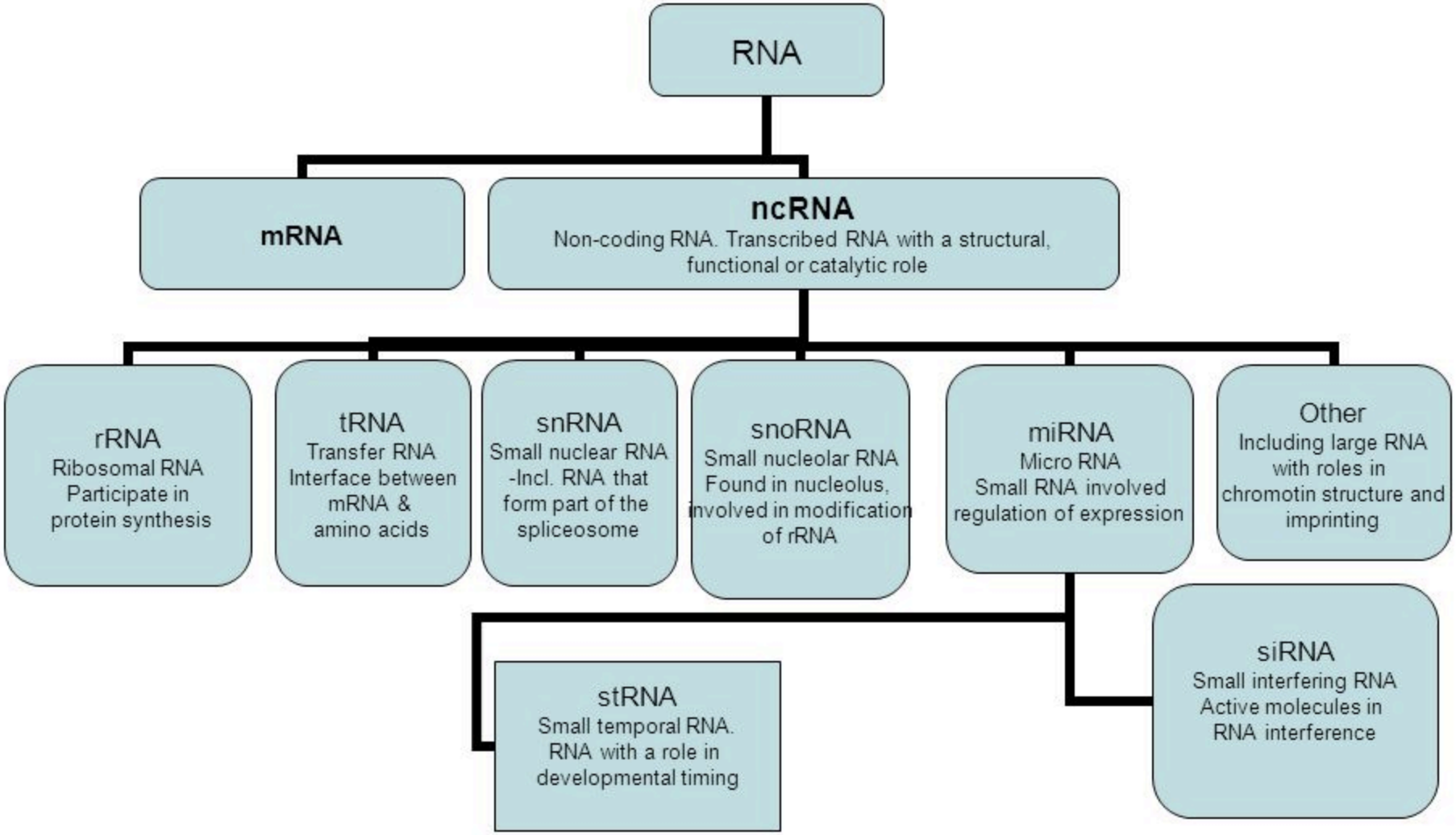
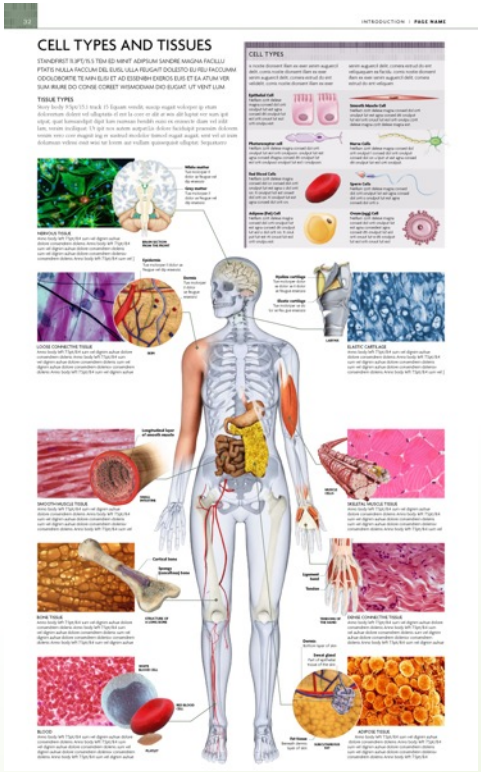
DNA is the same in all cells but which RNAs that is present is different in all cells



There is a wide variety of different functional RNAs



Which RNAs (and sometimes then translated to proteins) varies between samples



-Tissues

-Cell types

-Cell states

-Individuals

-Cells

RNA gives information on which genes that are expressed

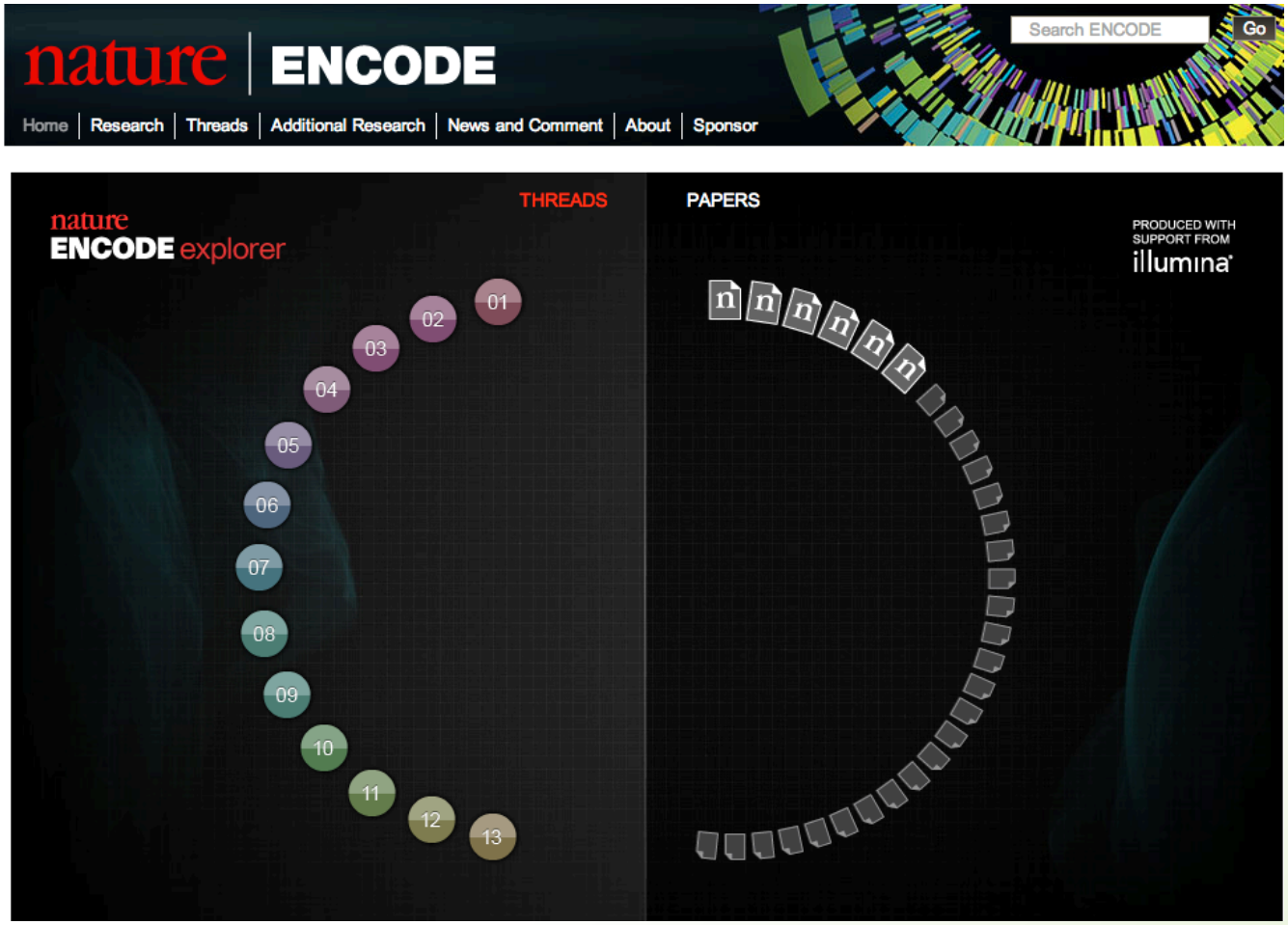
How DNA get transcribed to RNA (and sometimes then translated to proteins) varies between e. g.

-Tissues

-Cell types

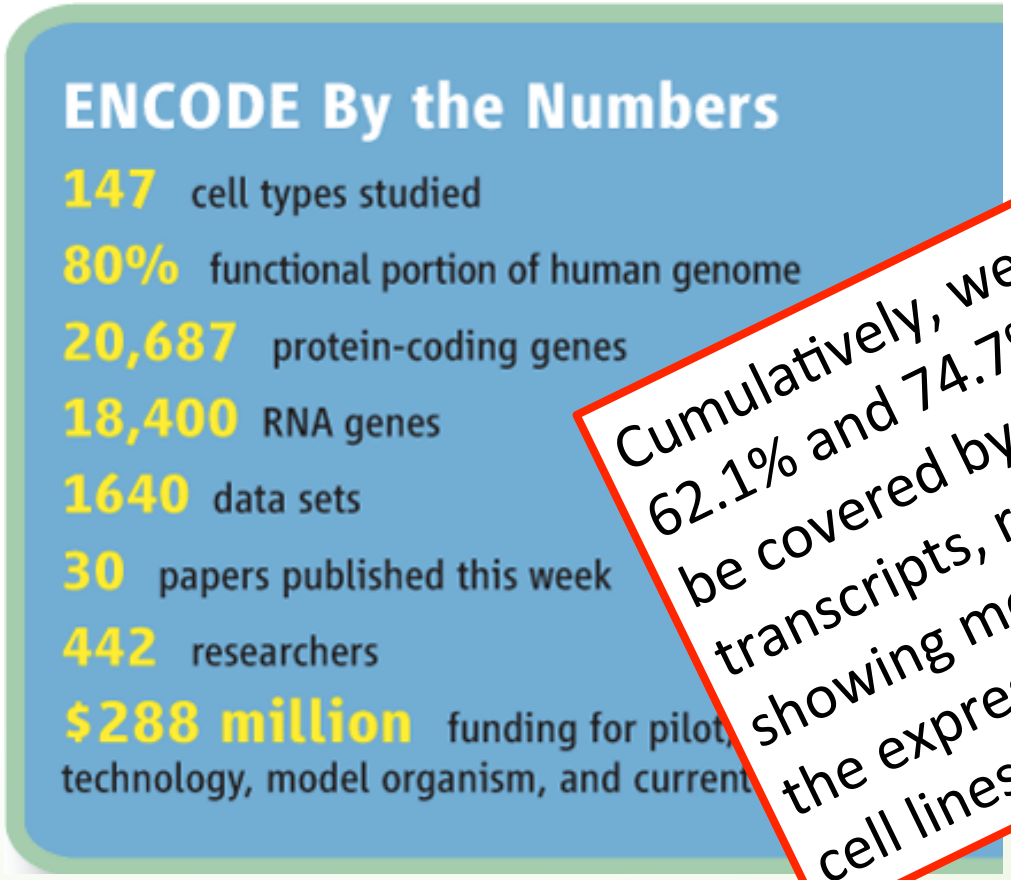
-Cell states

-Individuals



ENCODE, the Encyclopedia of DNA Elements, is a project funded by the National Human Genome Research Institute to identify all regions of transcription, transcription factor association, chromatin structure and histone modification in the human genome sequence.

ENCyclopedia Of Dna Elements



Cumulatively, we observed a total of 62.1% and 74.7% of the human genome to be covered by either processed or primary transcripts, respectively, with no cell line showing more than 56.7% of the union of the expressed transcriptomes across all cell lines.

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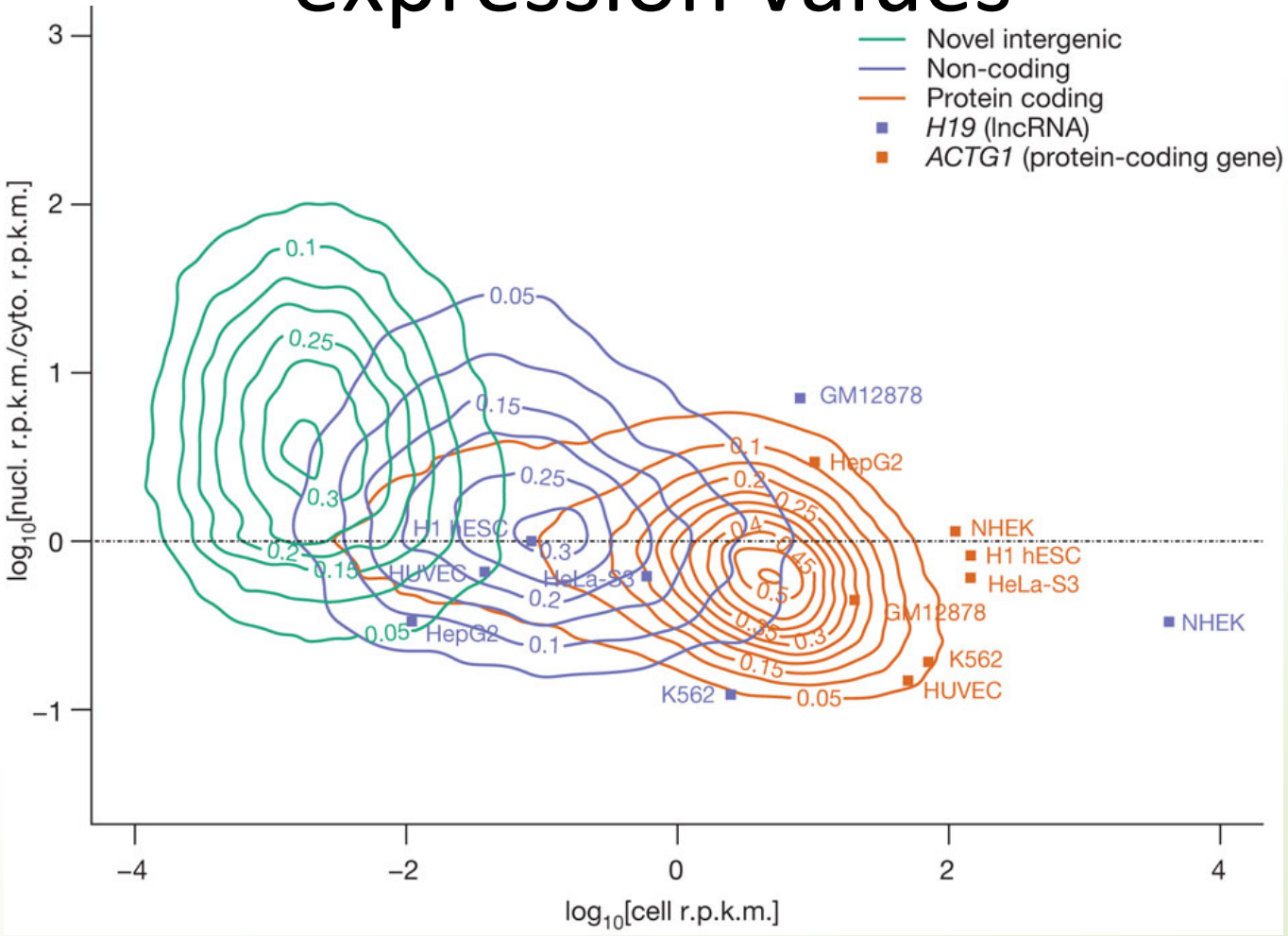
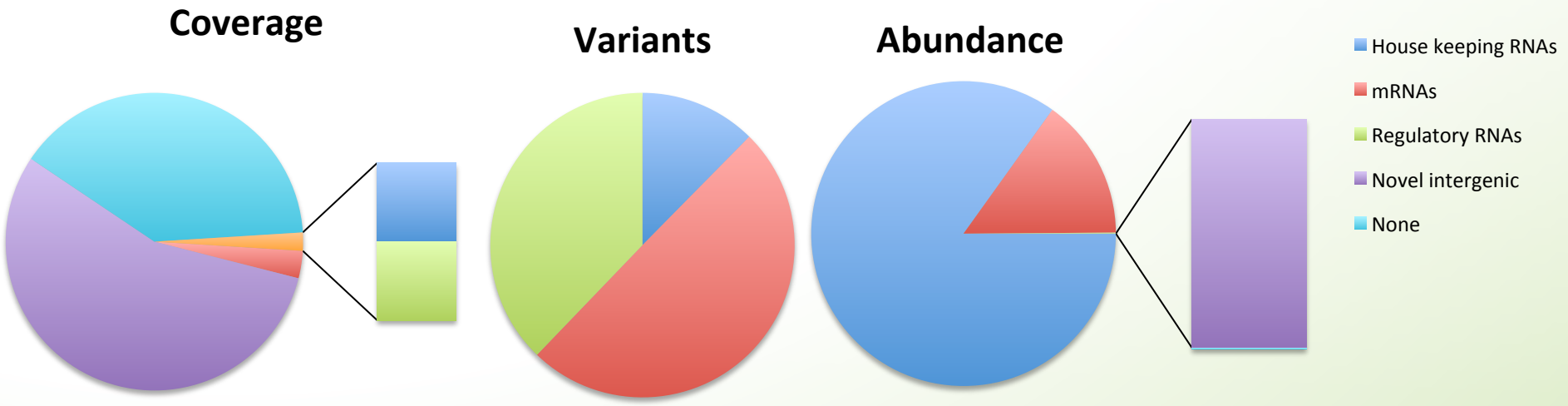
Different kind of RNAs have different expression values

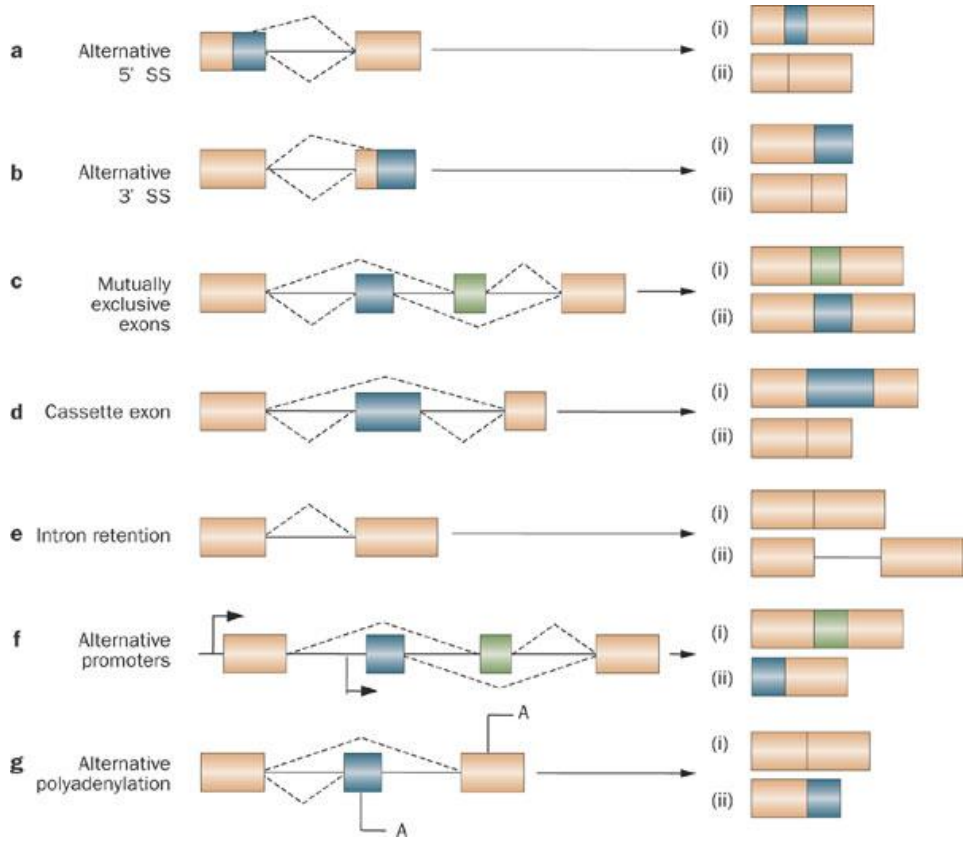


Landscape of transcription in human cells, S Djebali *et al.* ***Nature 2012***

What defines RNA depends on how you look at it



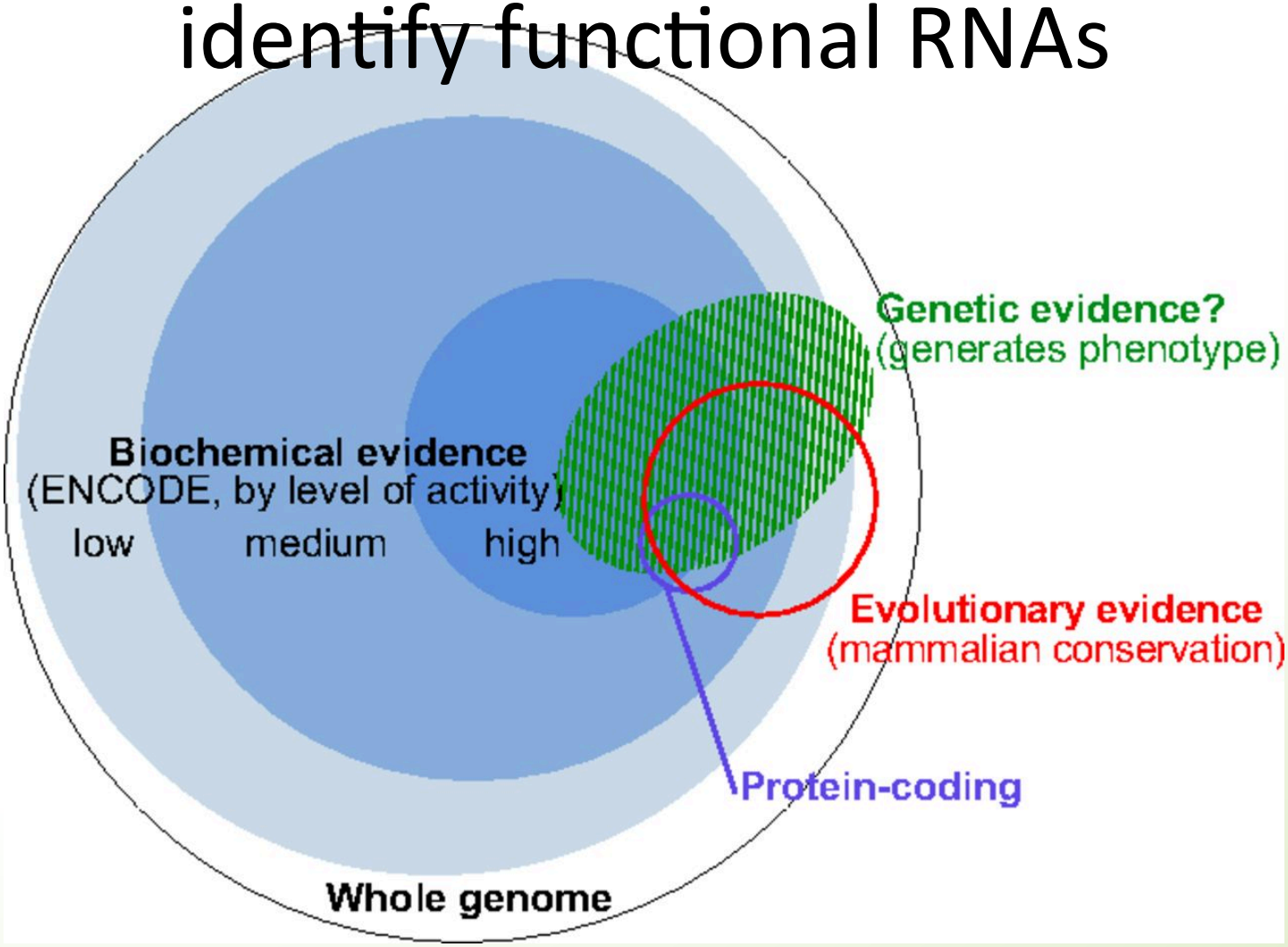
One gene many different mRNAs



Defining functional DNA elements in the human genome

- Statement
  - A priori, we should not expect the transcriptome to consist exclusively of functional RNAs.
- Why is that
  - Zero tolerance for errant transcripts would come at high cost in the proofreading machinery needed to perfectly gate RNA polymerase and splicing activities, or to instantly eliminate spurious transcripts.
  - In general, sequences encoding RNAs transcribed by noisy transcriptional machinery are expected to be less constrained, which is consistent with data shown here for very low abundance RNA
- Consequence
  - Thus, one should have high confidence that the subset of the genome with large signals for RNA or chromatin signatures coupled with strong conservation is functional and will be supported by appropriate genetic tests.
  - In contrast, the larger proportion of genome with reproducible but low biochemical signal strength and less evolutionary conservation is challenging to parse between specific functions and biological noise.

Biochemical evidence not enough to identify functional RNAs

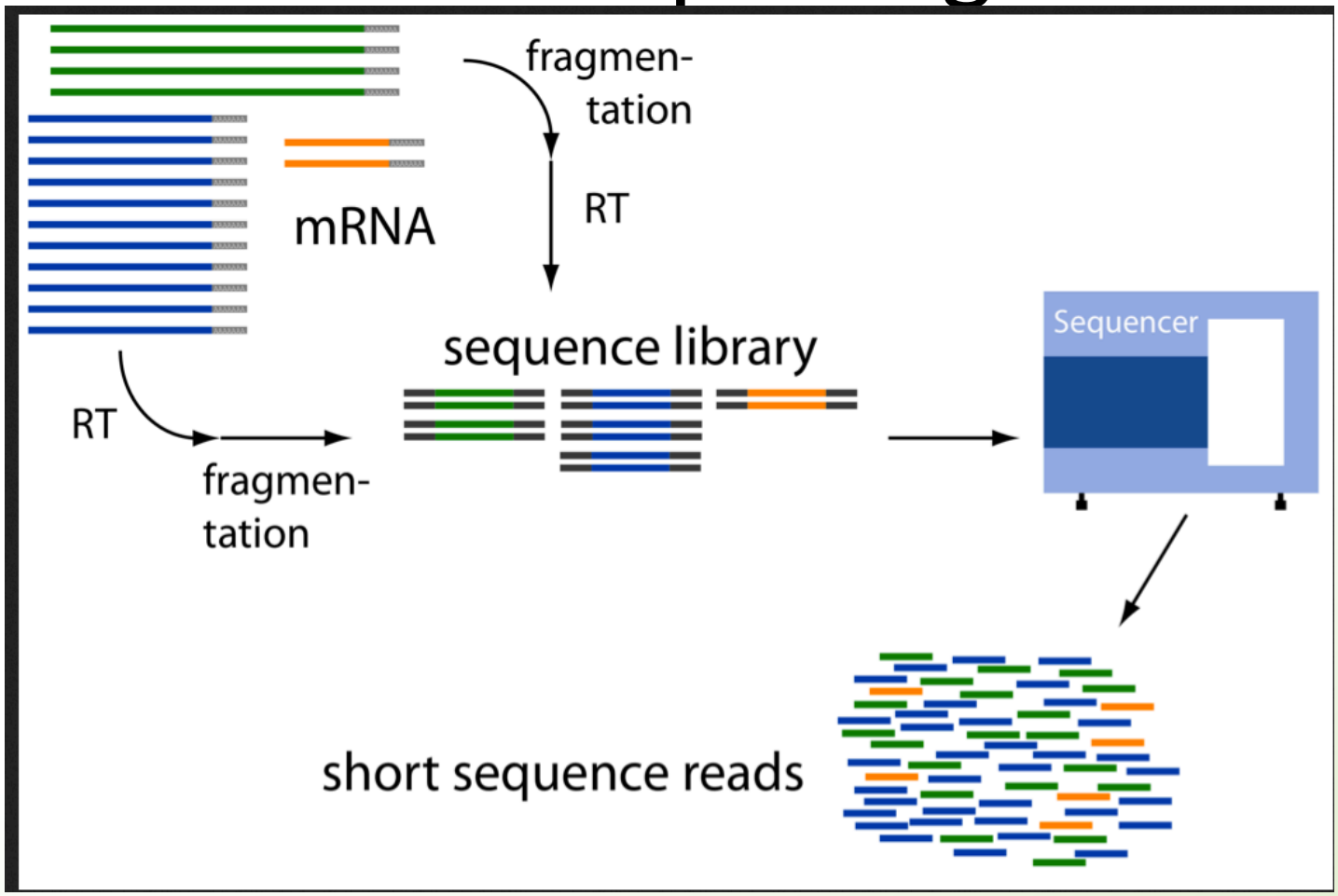


- RNA seq course

The RNA seq course

- From RNA seq to reads (Introduction)
- Mapping reads programs (Monday)
- Transcriptome reconstruction using reference (Monday)
- Transcriptome reconstruction without reference (Monday)
- QC analysis (Tuesday)
- Differential expression analysis (Tuesday)
- miRNA analysis (Tuesday)
- Gene set analysis (Wednesday)
- Long RNA seq analysis(Thursday)
- Single cell analysis (Wednesday)

How are RNA-seq data generated?



Sampling process

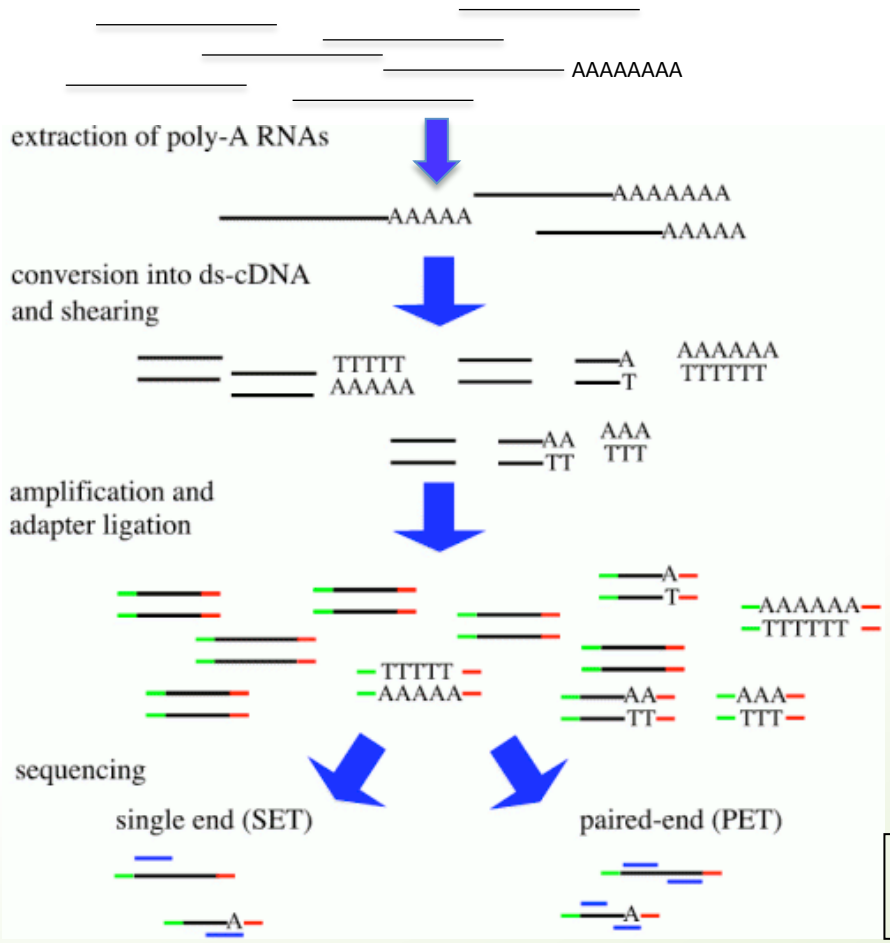
Depending on the different steps you will get different results

RNA->

enrichments ->

library ->

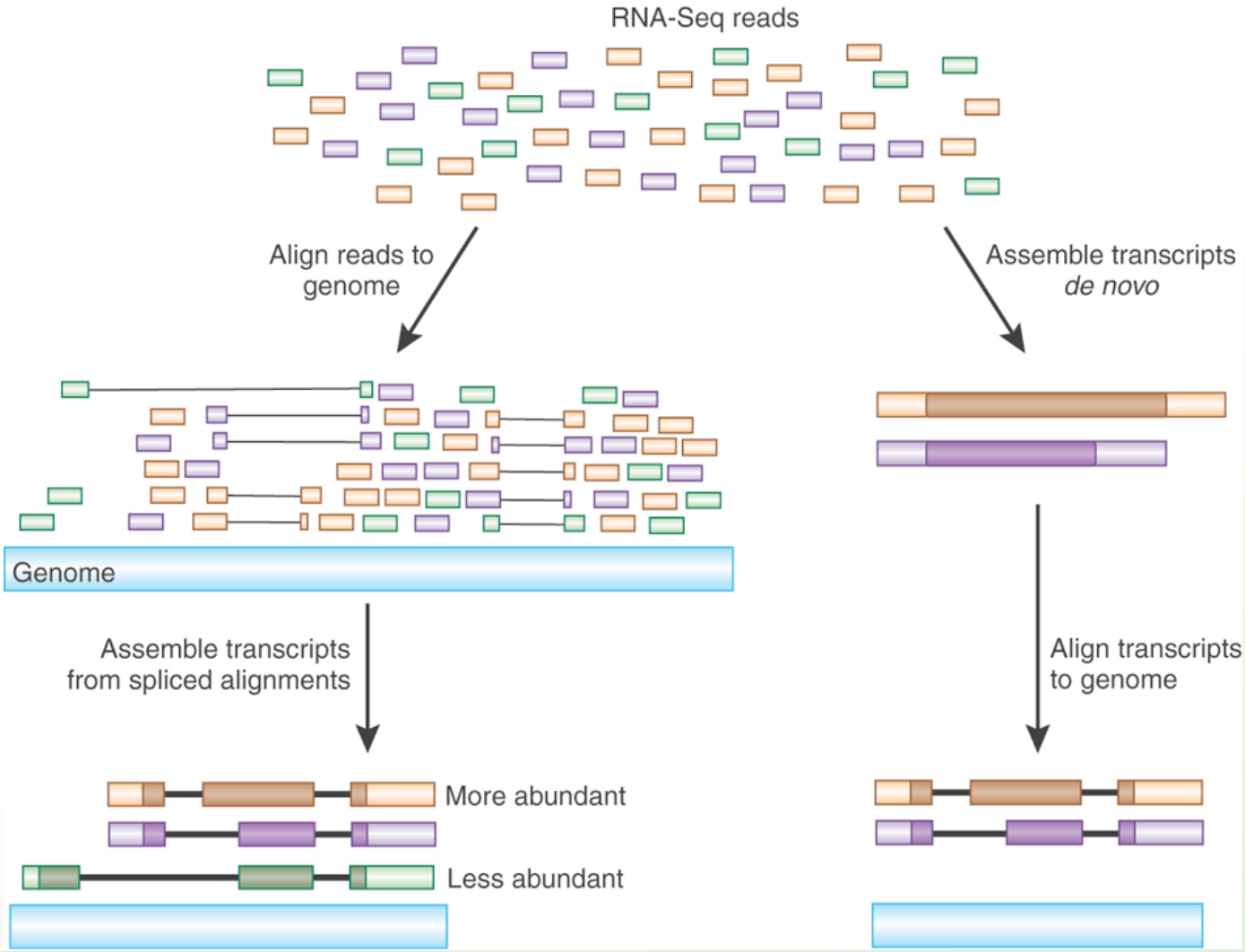
reads ->



PolyA	(mRNA)
RiboMinus	(- rRNA)
Size <50 nt	(miRNA)
…..	

Size of fragment
Strand specific
5’ end specific
3’ end specific
…..

Single end (1 read per fragment)
Paired end (2 reads per fragment)


RNA-Seq reads
Align reads to genome
Assemble transcripts de novo
Genome
Assemble transcripts from spliced alignments
Align transcripts to genome
More abundant
Less abundant

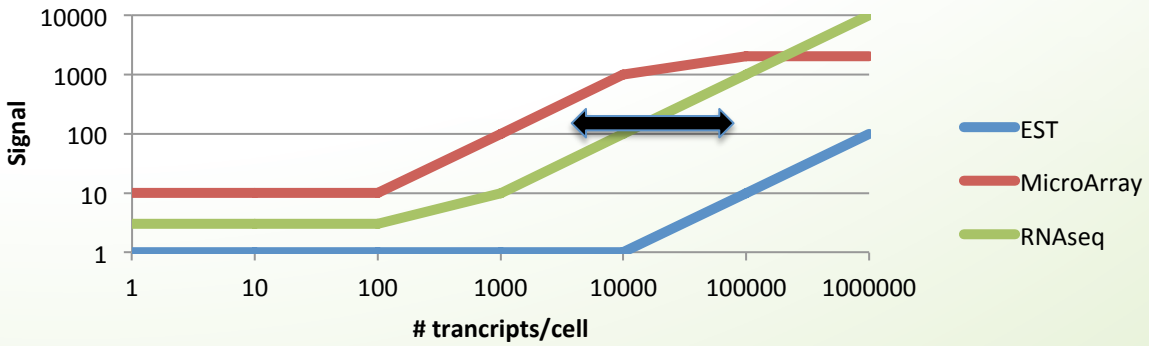
Promises and pitfalls

Long reads

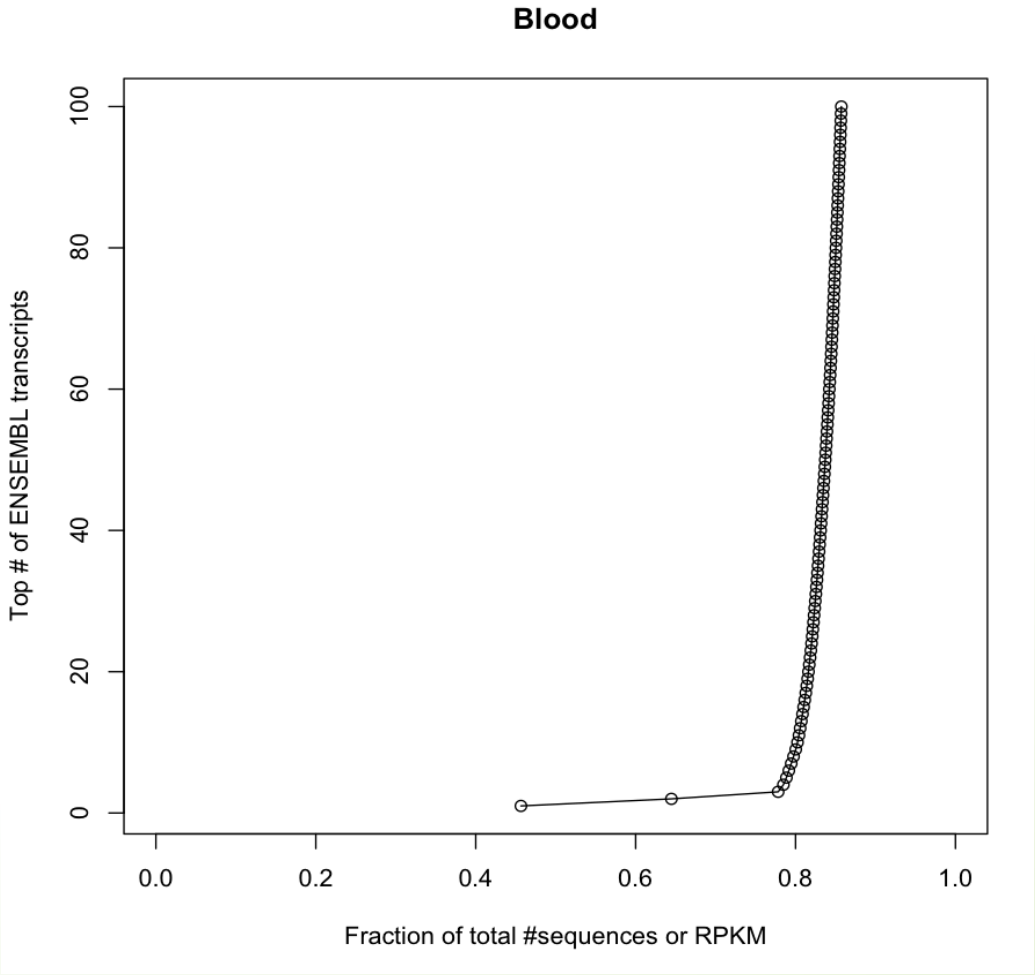
- Low throughput (-)
- Complete transcripts (+)
- Only highly expressed genes (--)
- Expensive (-)
- Low background noise (+)
- Easy downstream analysis (+)

short reads

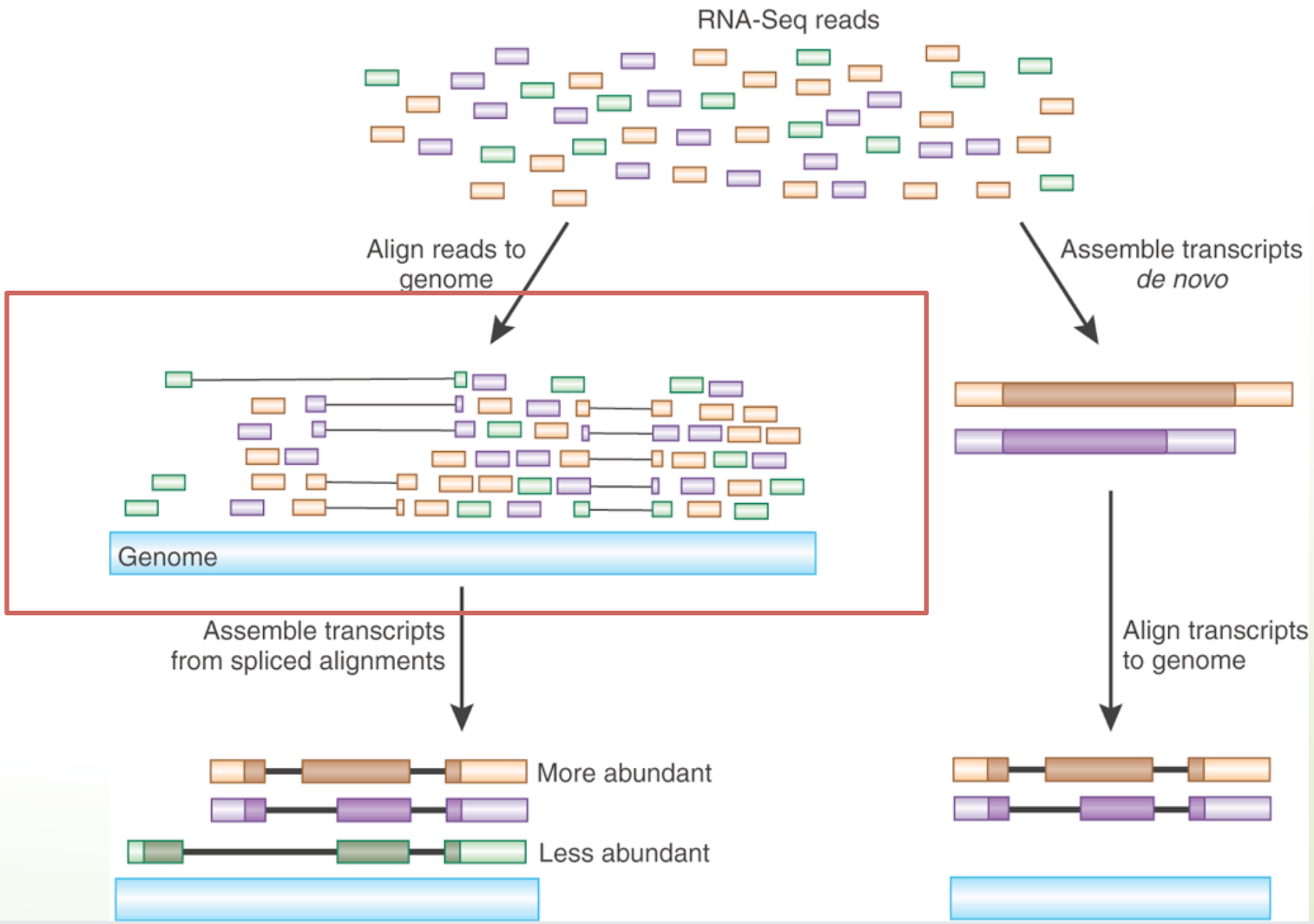
- High throughput (+)
- Fractions of transcripts (-)
- Full dynamic range (+-)
- Unlimited dynamic range (+)
- Cheap (+)
- Low background noise (+)
- Strand specificity (+)
- Re-sequencing (+)



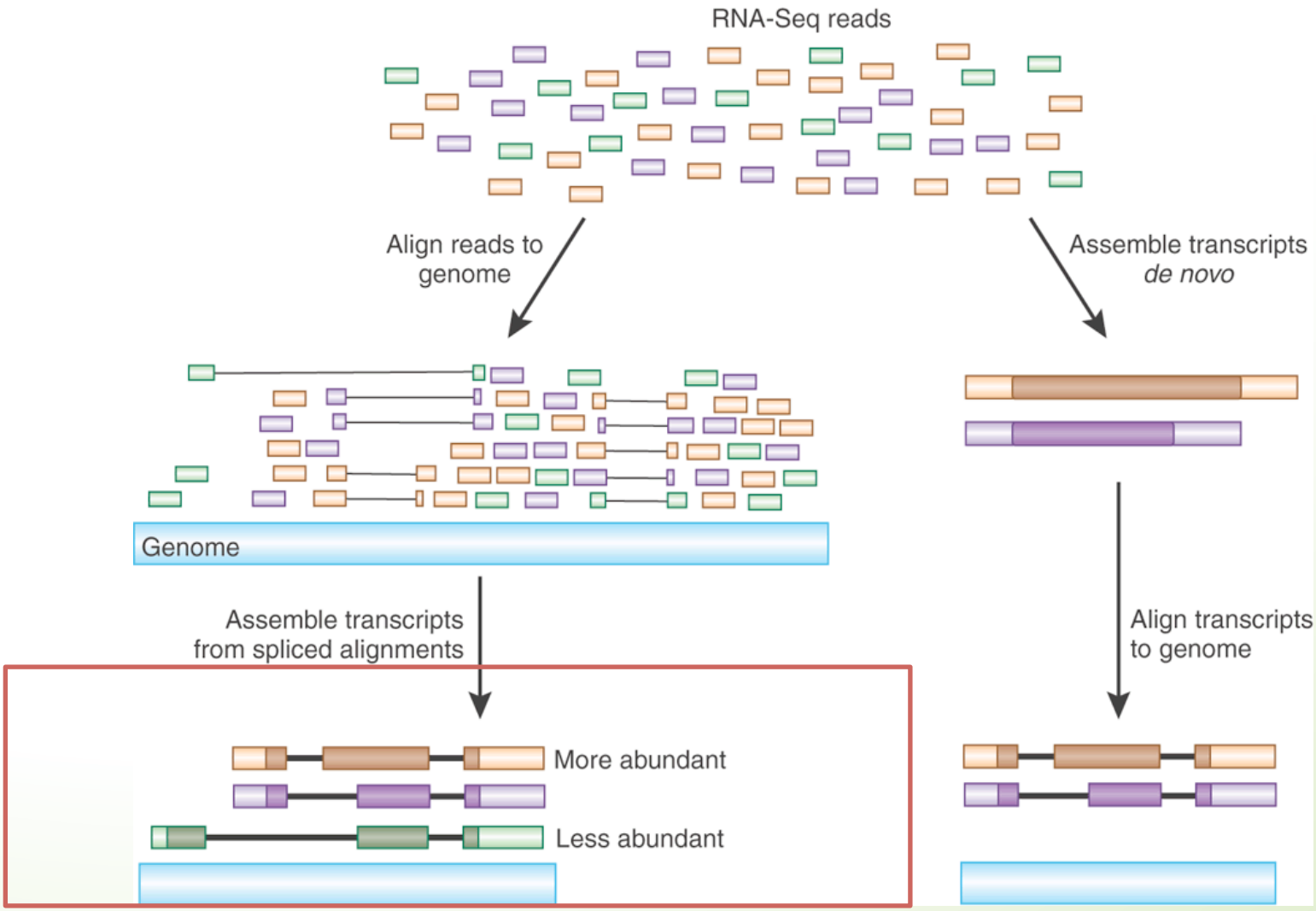
RNA seq reads correspond directly to abundance of RNAs in the sample



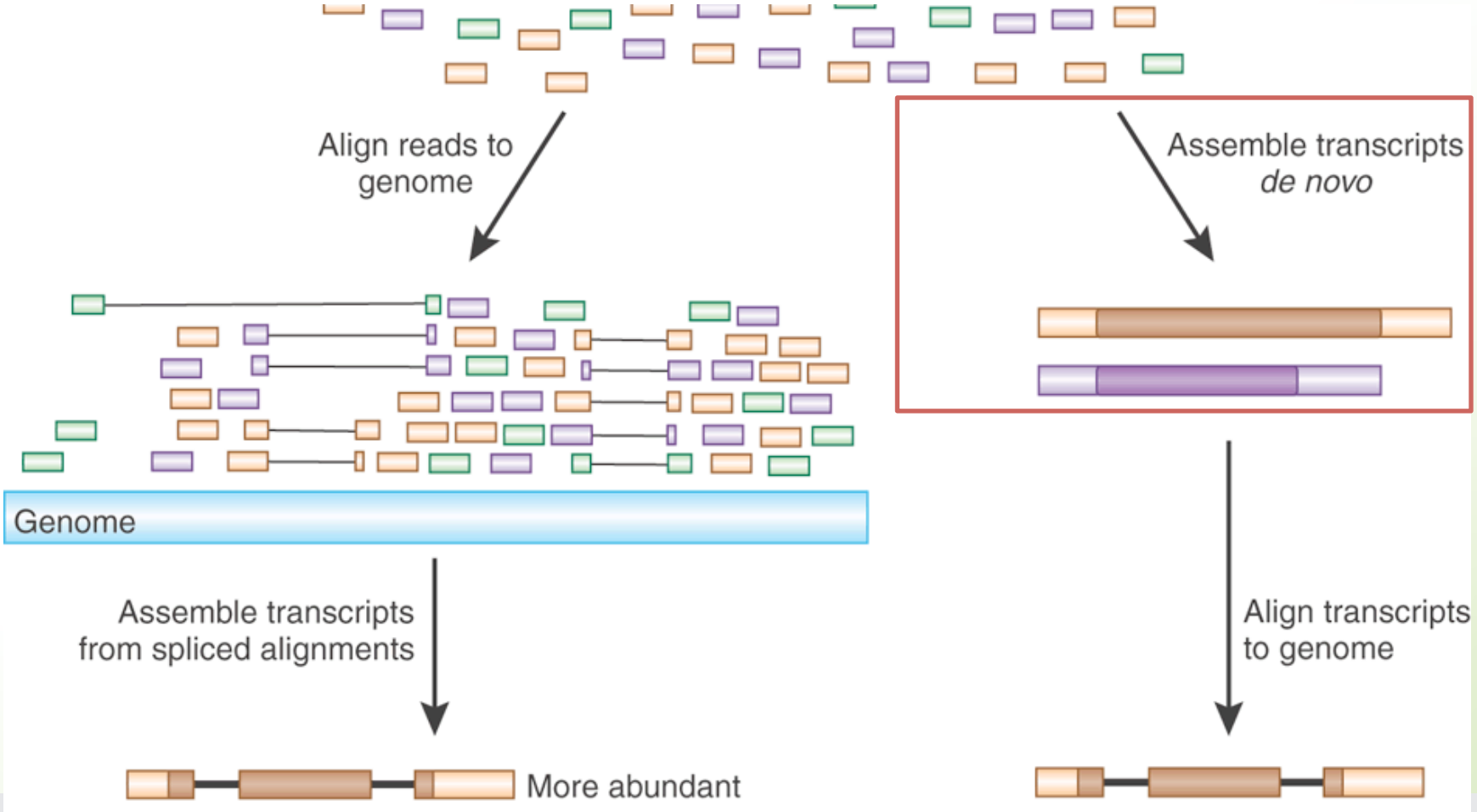
Map reads to reference

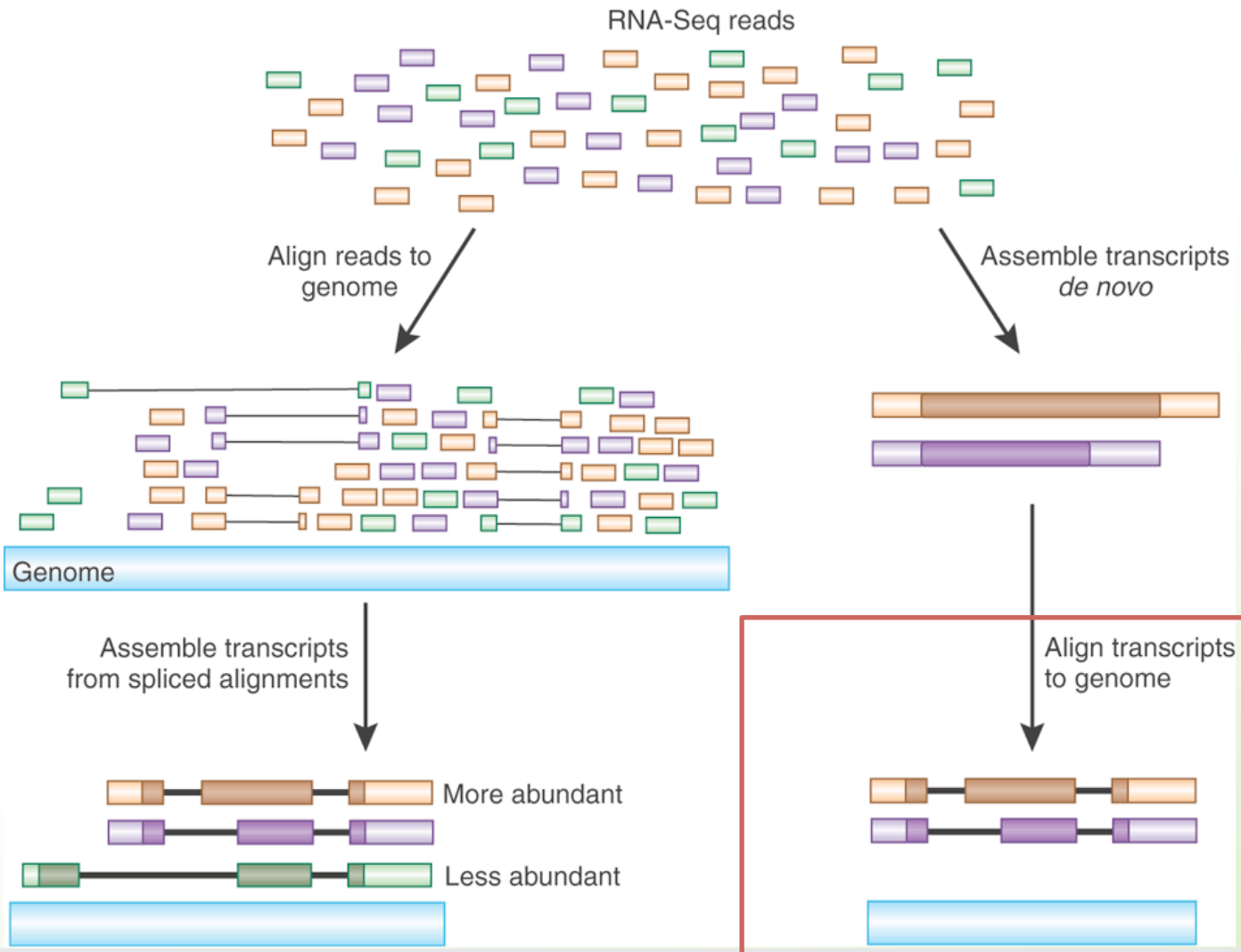


Transcriptome assembly using reference



Transcriptome assembly without reference




RNA-Seq reads
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Quality control

-samples might not be what you think they are

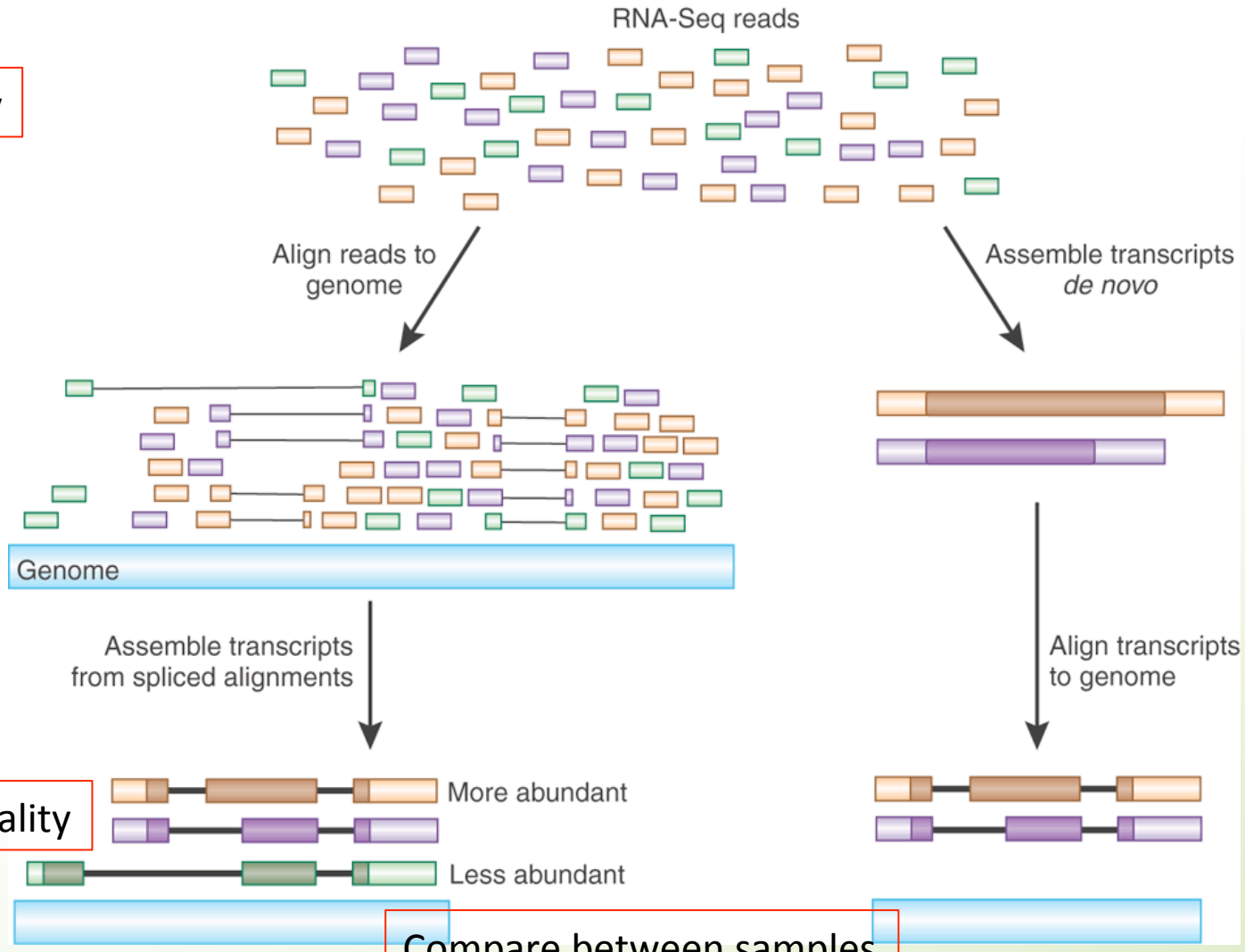
- Experiments go wrong
  - 30 samples with 5 steps from samples to reads has 150 potential steps for errors
  - Error rate 1/100 with 5 steps suggest that one of every 20 samples the reads does not represent the sample
- Mixing samples
  - 30 samples with 5 steps from samples to reads has ~24M potential mix ups of samples
  - Error rate 1/ 100 with 5 steps suggest that one of every 20 sample is mislabeled
- Combine the two steps and approximately one of every 10 samples are wrong

RNA QC

Read quality

Mapping statistics

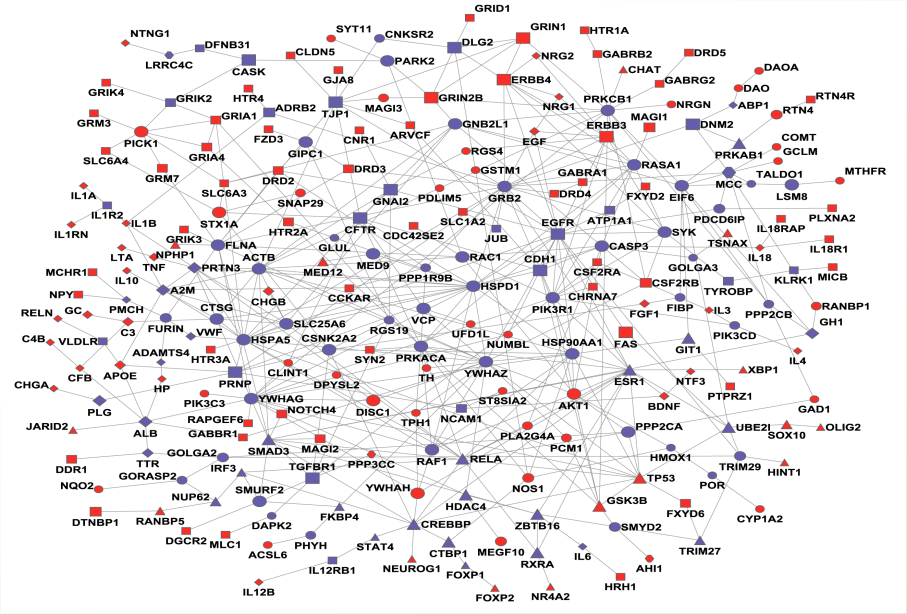
Transcript quality



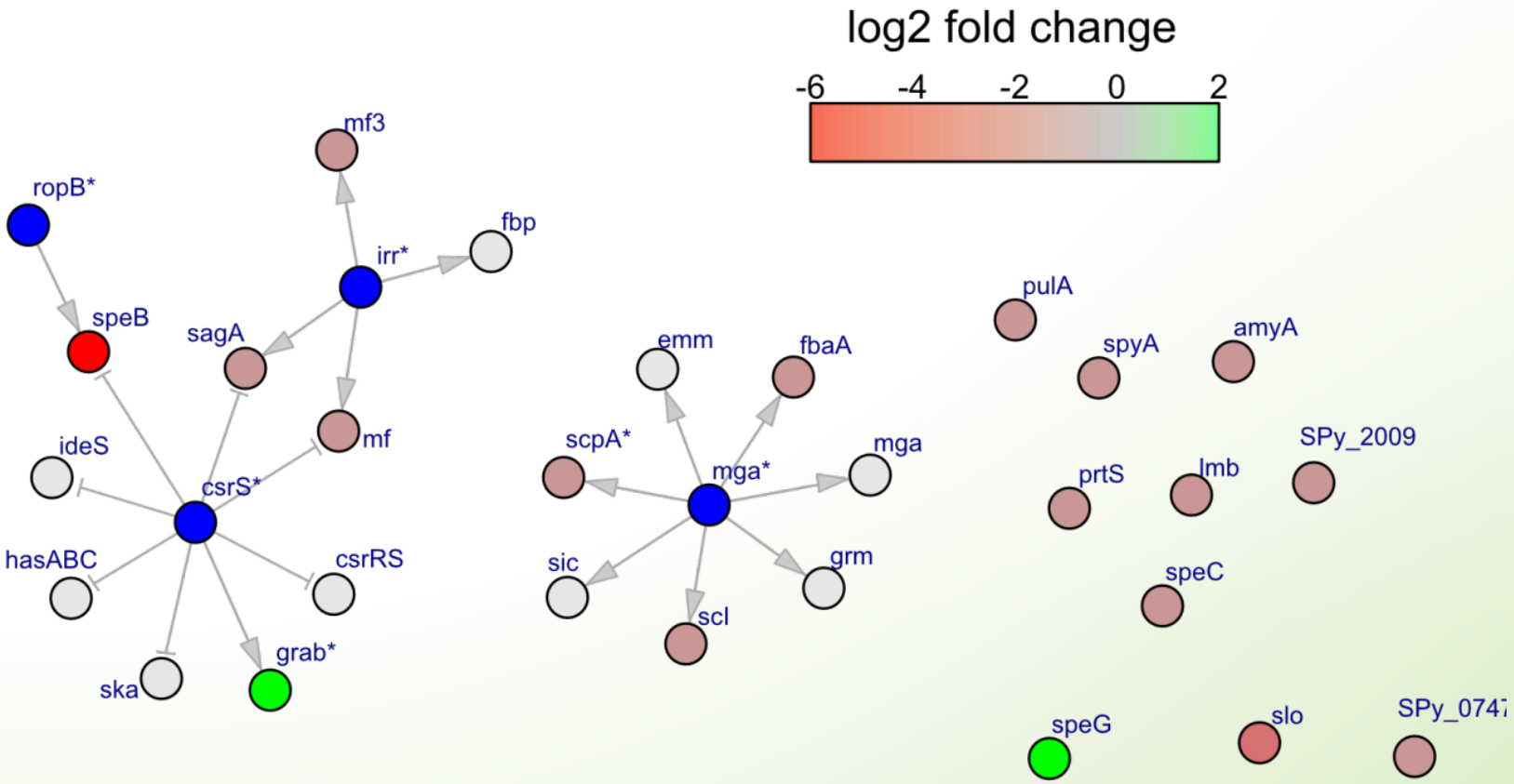
Compare between samples

Differential expression analysis using univariate analysis

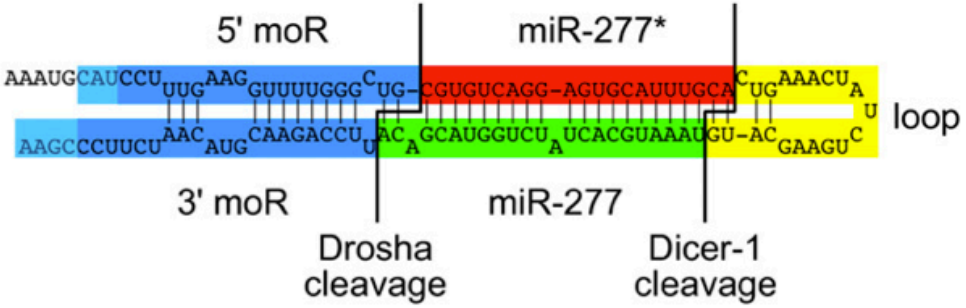
Typically **univariate** analysis (one gene at a time) – even though we know that genes are not independent

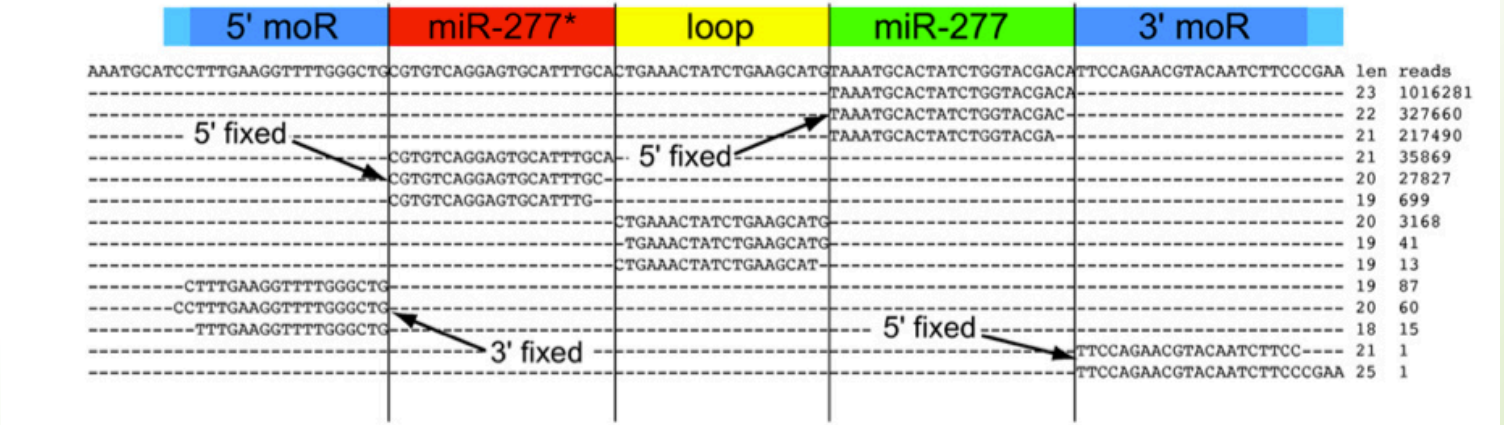


Gene set analysis and data integration



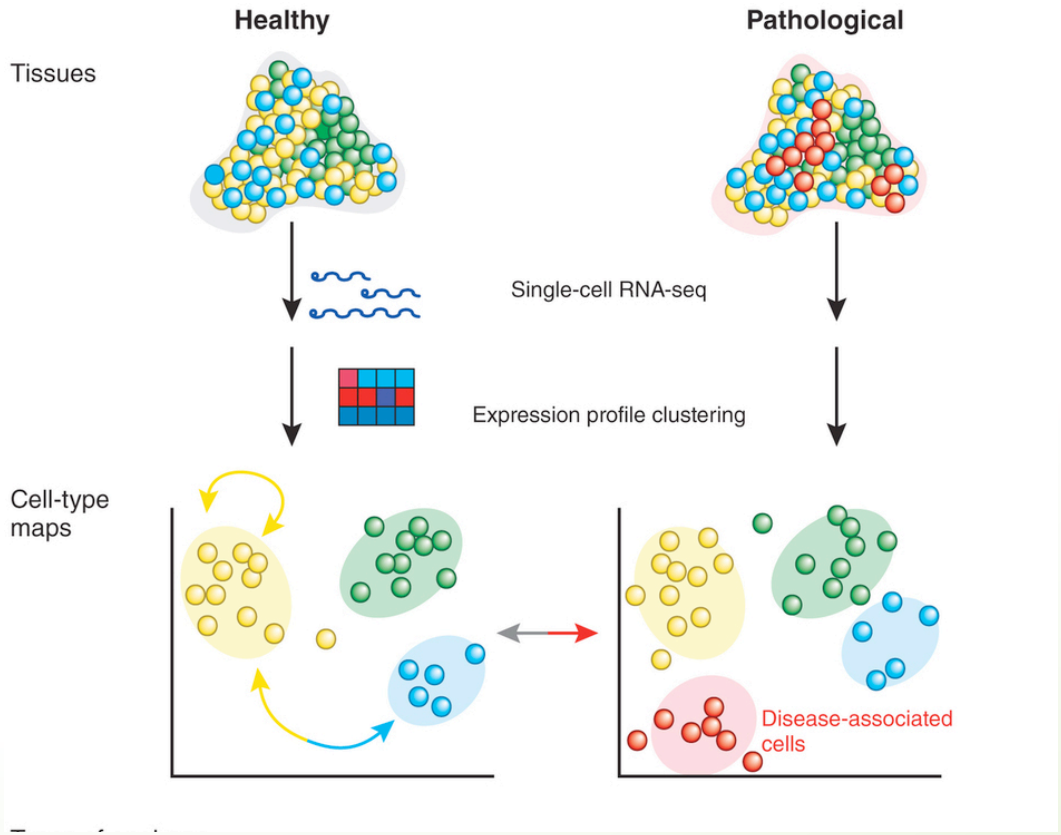
microRNA analysis (Johan)





(Berezikov et al. Genome Research, 2011.)

Single cell RNA-seq analysis



(Sandberg, Nature Methods 2014)