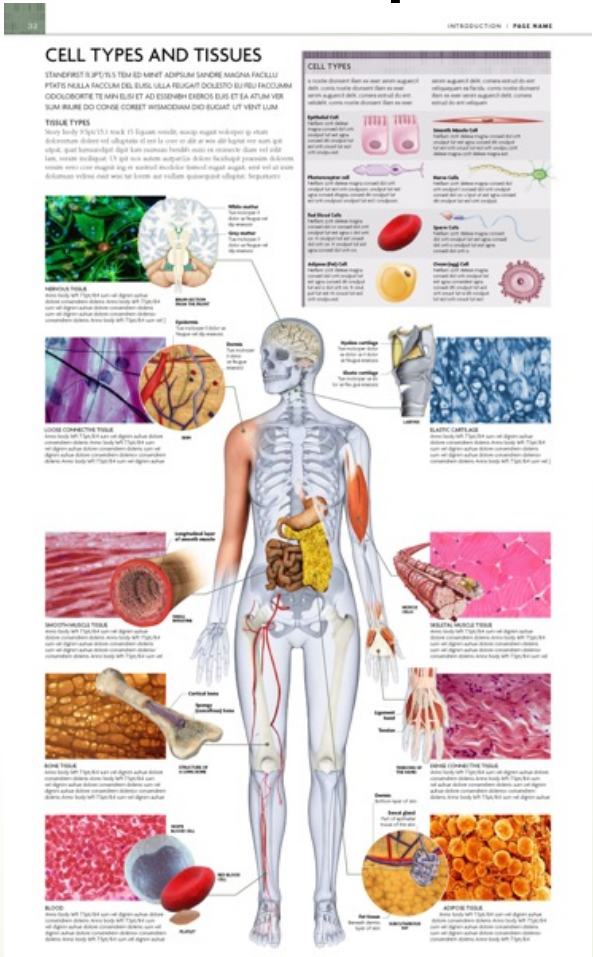
RNA-seq Introduction

Promises and pitfalls

Enabler for Life Sciences

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

-Cells

RNA gives information on which genes that are expressed

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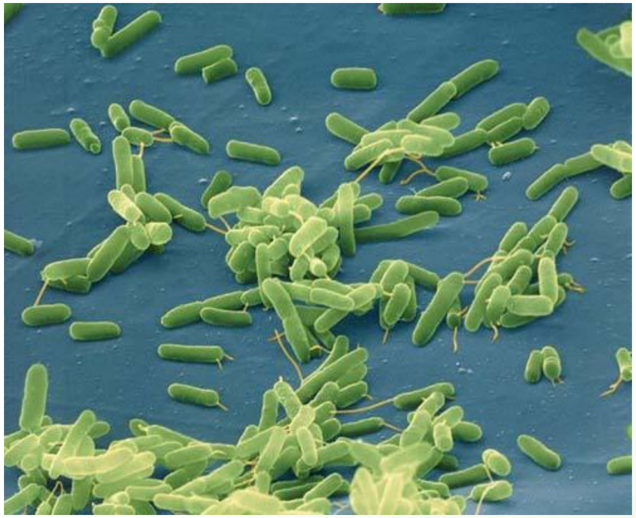
RNA gives information on which genes that are expressed



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

~~-Tissues~~

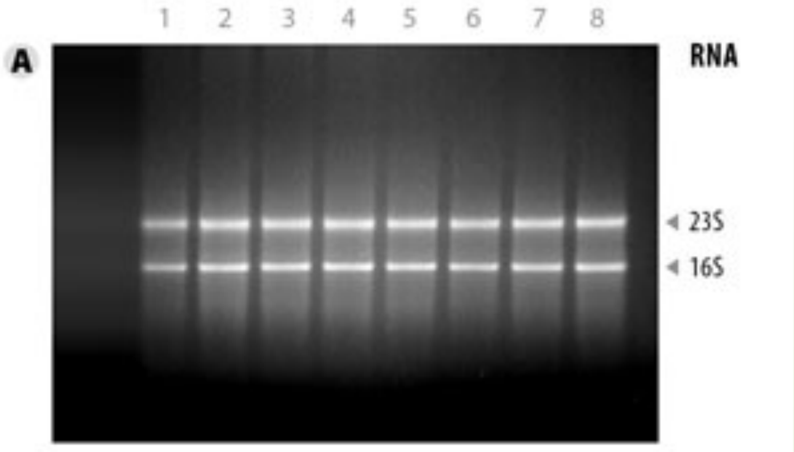
-~~Cell types~~

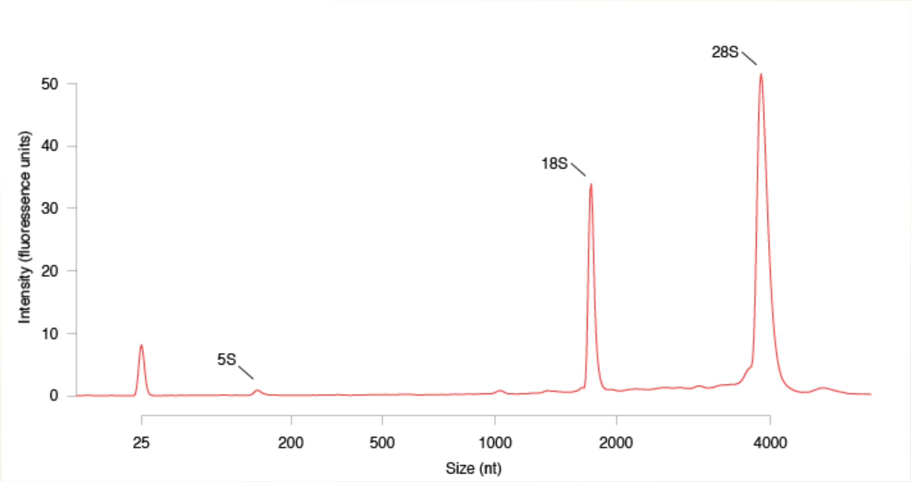
-Cell states

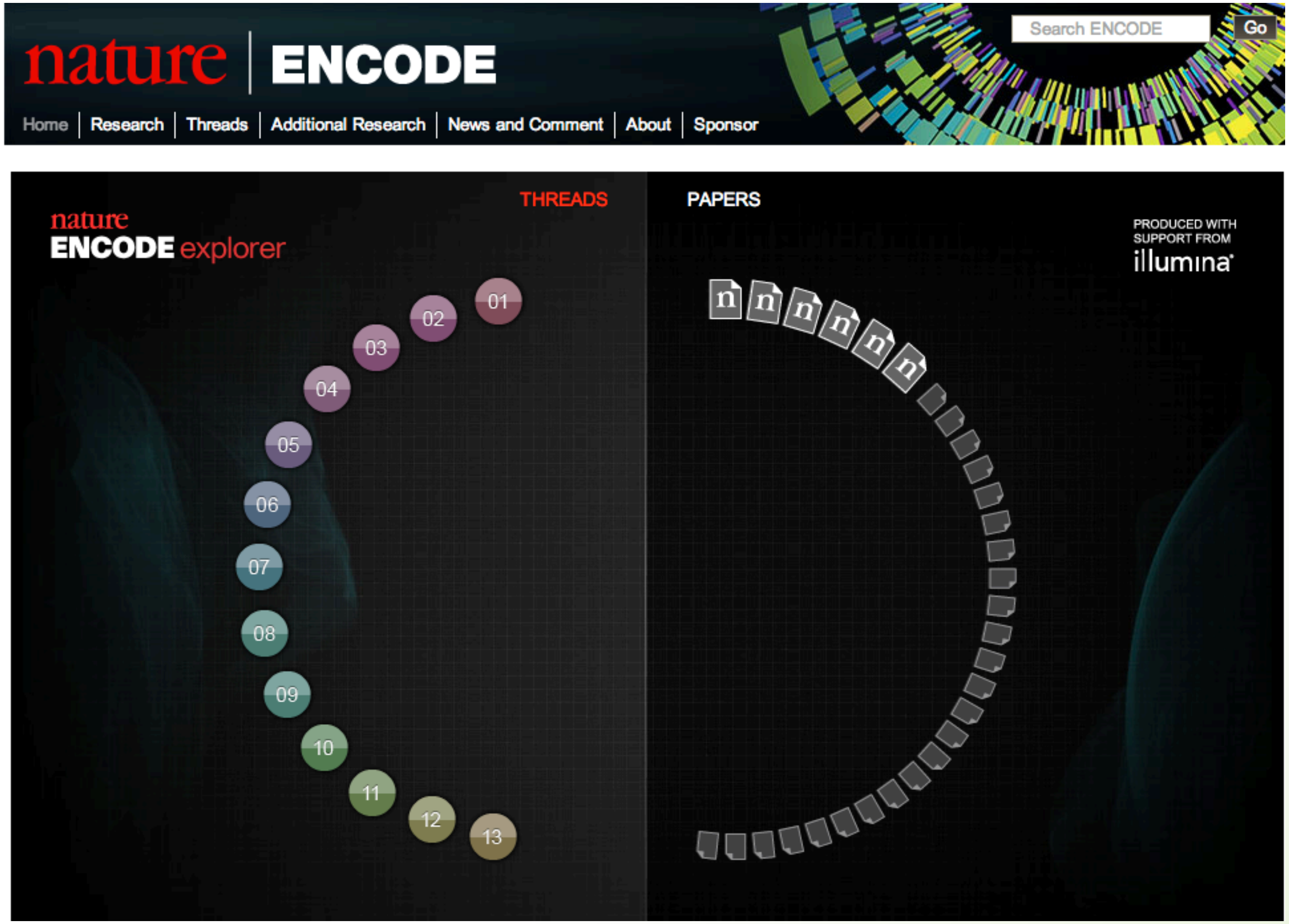
-Individuals

RNA flavors
(pre sequencing era)

- House keeping RNAs
  - rRNAs, tRNAs, snoRNAs, snRNAs, SRP RNAs, catalytic RNAs (RNAse E)
- Protein coding RNAs
  - (1 coding gene ~ 1 mRNA)
- Regulatory RNAs
  - Few rare examples

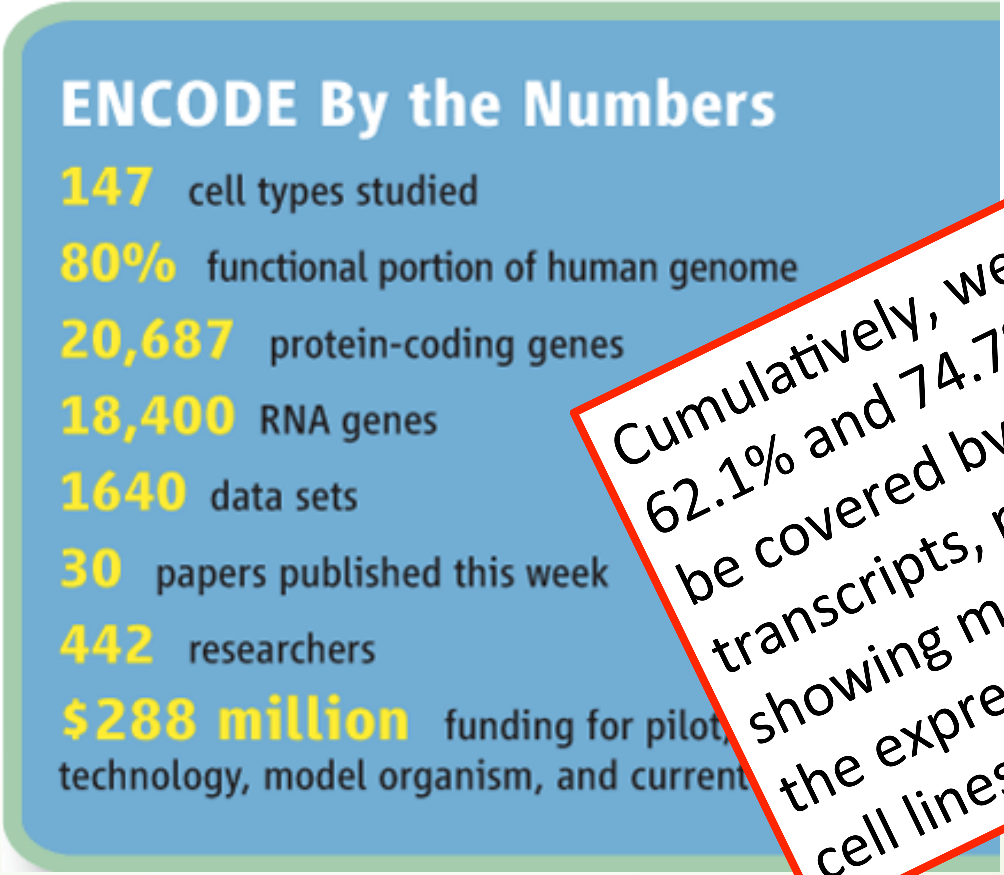






ENCODE, the Encyclopedia of DNA Elements, is a project funded by the National Human Genome Research Institute to identify all regions of transcription, transcription factor association, chromatin structure and histone modification in the human genome sequence.

ENCyclopedia Of Dna Elements



ENCODE By the Numbers

147 cell types studied

80% functional portion of human genome

20,687 protein-coding genes

18,400 RNA genes

1640 data sets

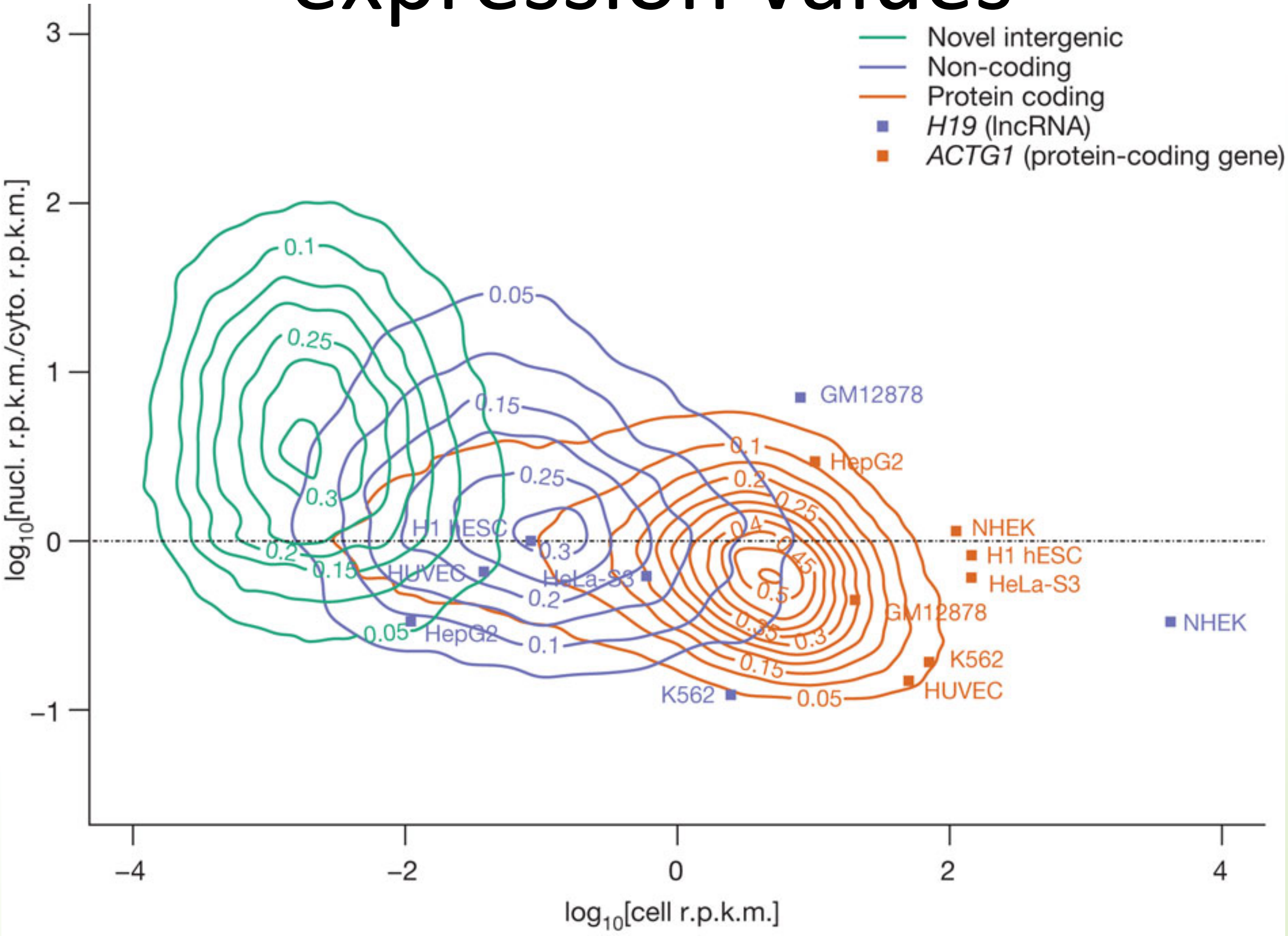
30 papers published this week

442 researchers

$288 million funding for pilot, technology, model organism, and current

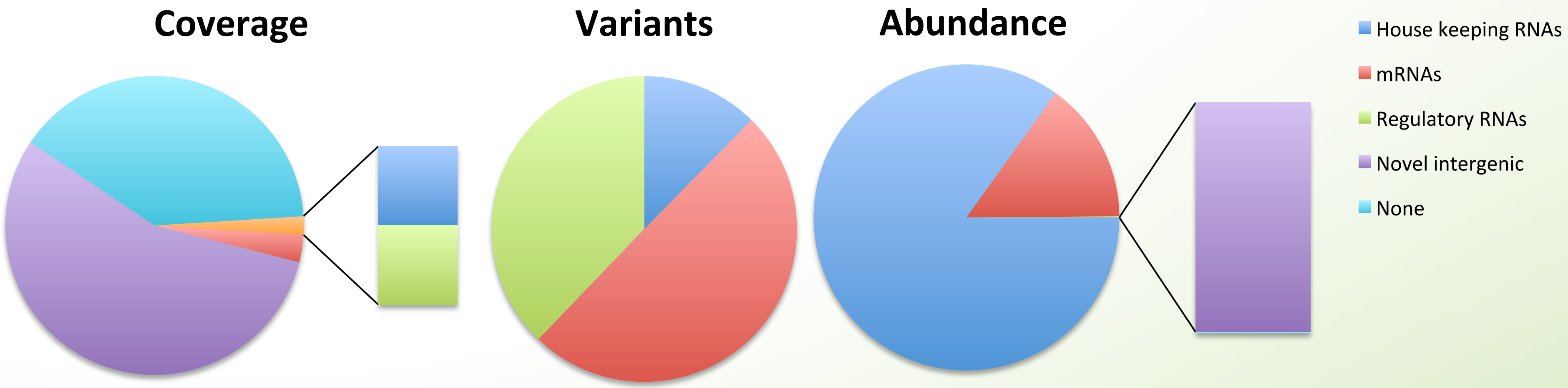
Cumulatively, we observed a total of 62.1% and 74.7% of the human genome to be covered by either processed or primary transcripts, respectively, with no cell line showing more than 56.7% of the union of the expressed transcriptomes across all cell lines.

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



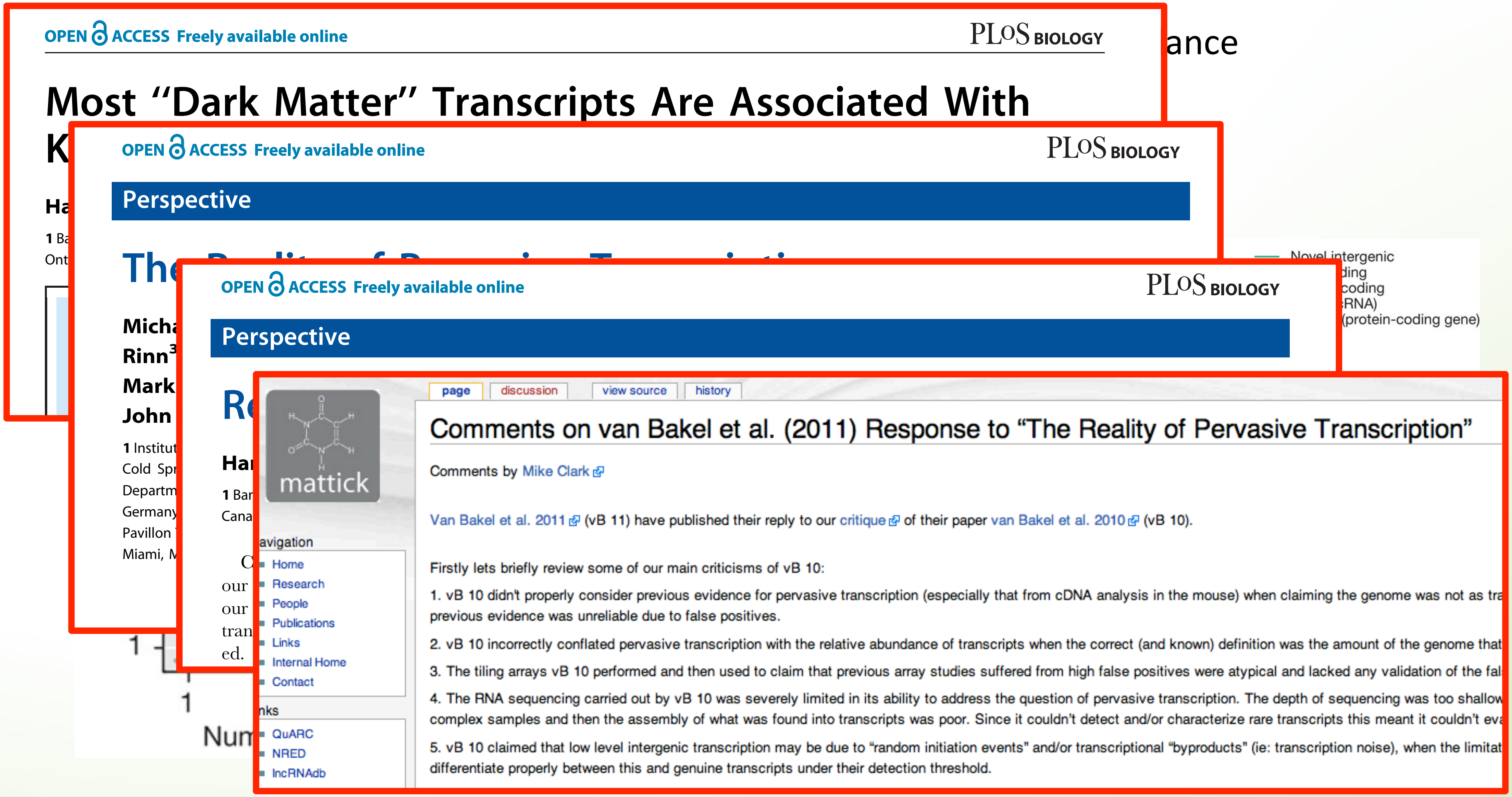
Adapted from **Landscape of transcription in human cells,** S Djebali *et al.* ***Nature 2012***

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.

This is of course not without an debate



ance

OPEN ACCESS Freely available online

PLoS BIOLOGY

Most "Dark Matter" Transcripts Are Associated With K

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PLoS BIOLOGY

Perspective

The

Micha
Rinn[3]
Mark
John

1 Institut
Cold Spr
Departm
Germany
Pavillon
Miami, M

OPEN ACCESS Freely available online

PLoS BIOLOGY

Perspective

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Comments on van Bakel et al. (2011) Response to "The Reality of Pervasive Transcription"

Comments by Mike Clark

Van Bakel et al. 2011 (vB 11) have published their reply to our critique of their paper van Bakel et al. 2010 (vB 10).

Firstly lets briefly review some of our main criticisms of vB 10:

1. vB 10 didn't properly consider previous evidence for pervasive transcription (especially that from cDNA analysis in the mouse) when claiming the genome was not as tra previous evidence was unreliable due to false positives.

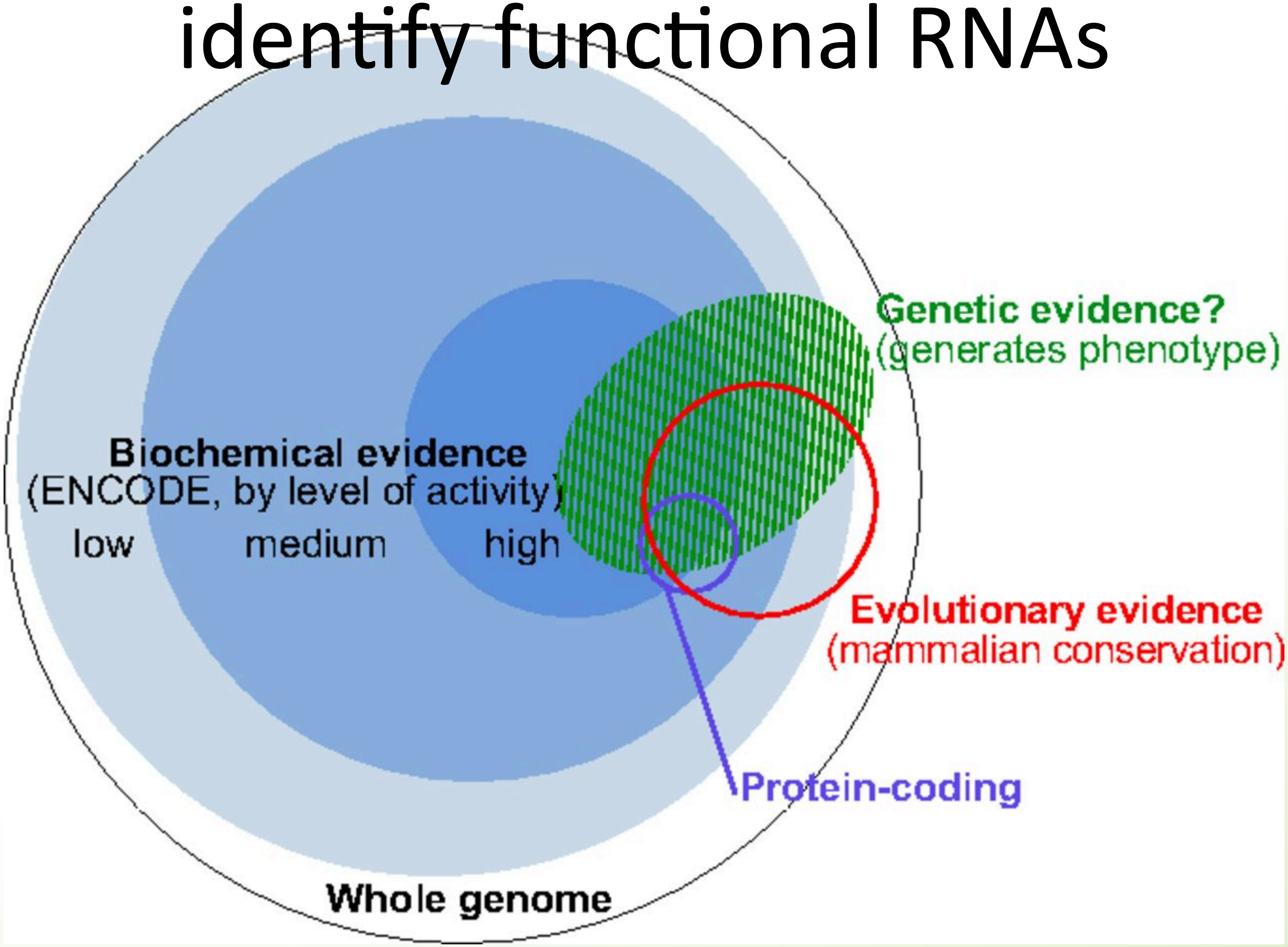
2. vB 10 incorrectly conflated pervasive transcription with the relative abundance of transcripts when the correct (and known) definition was the amount of the genome that

3. The tiling arrays vB 10 performed and then used to claim that previous array studies suffered from high false positives were atypical and lacked any validation of the fal

4. The RNA sequencing carried out by vB 10 was severely limited in its ability to address the question of pervasive transcription. The depth of sequencing was too shallow complex samples and then the assembly of what was found into transcripts was poor. Since it couldn't detect and/or characterize rare transcripts this meant it couldn't eva

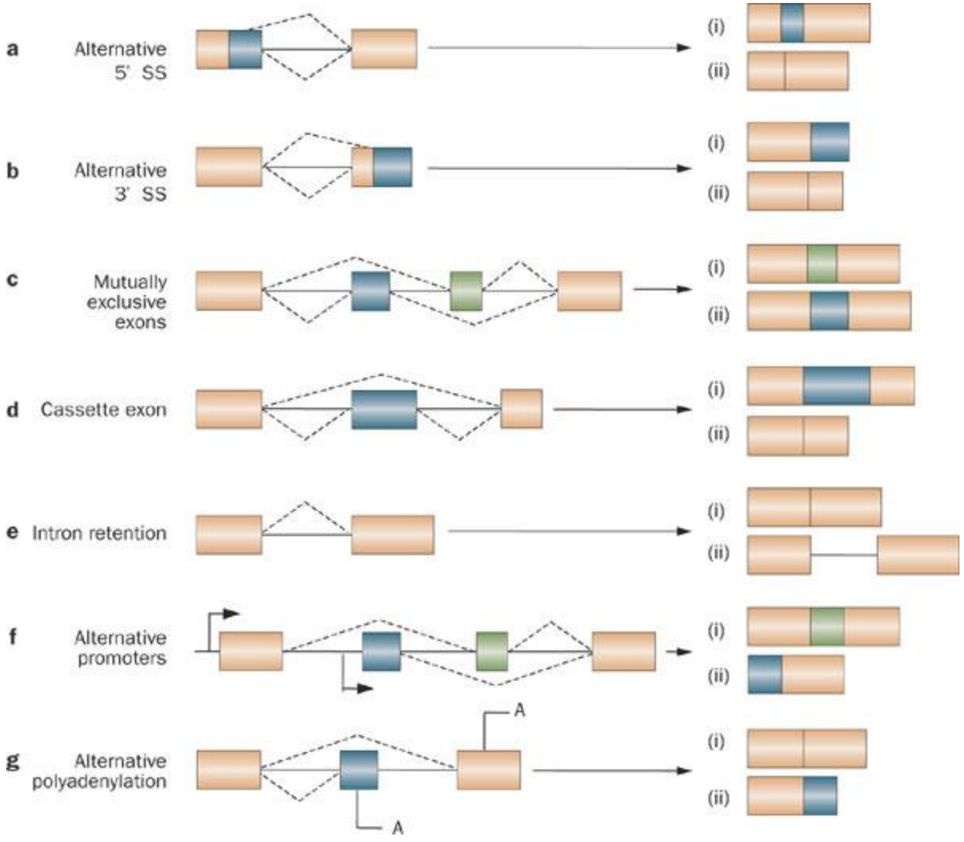
5. vB 10 claimed that low level intergenic transcription may be due to "random initiation events" and/or transcriptional "byproducts" (ie: transcription noise), when the limitat differentiate properly between this and genuine transcripts under their detection threshold.

Biochemical evidence not enough to identify functional RNAs



Defining functional DNA elements in the human genome
Kellis M et al. PNAS 2014;111:6131-6138

One gene many different mRNAs

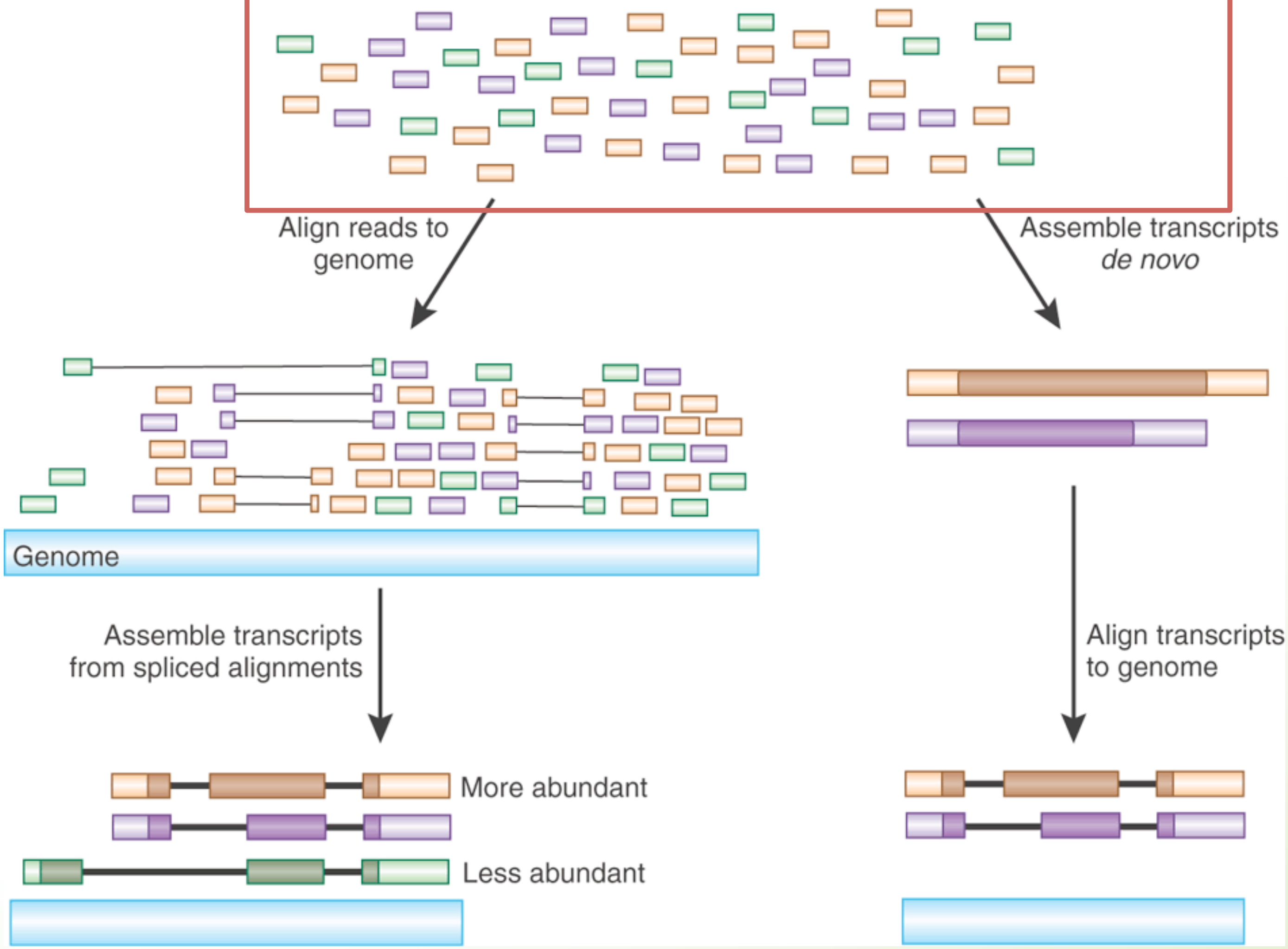


- RNA seq course

The RNA seq course

- From RNA seq to reads
- Mapping reads programs
- Transcriptome reconstruction using reference
- Transcriptome reconstruction without reference
- QC analysis
- sRNA analysis
- Differential expression analysis
  - mRNAs
  - miRNAs
- Genome annotation using RNA and other sources
- Differential expression using multi-variate analysis
- RNA long read analysis

From RNA to short reads


Align reads to genome
Assemble transcripts *de novo*
Genome
Assemble transcripts from spliced alignments
Align transcripts to genome
More abundant
Less abundant

Sequencing platforms

PACIFIC BIOSCIENCES

	ABI 3730xl Sanger Sequencing	454 Life Sciences pyrosequencing	SOLiD + Illumina	Pacific Biosciences, Oxford Nanopore etc Single-molecule sequencing
Length/read	800 bp	400 bp	100 bp	20 000+ bp
Reads/run	96	1 million	2 billion	5 million
Bases/run	60 kbp	400 Mbp	500 Gbp	100 Gbp
Speed	10 years/HG	1 month/HG	1 day/HG	10 min/HG
	“Old school”	“2nd gen”		“3rd gen”

Promises and pitfalls

Sanger

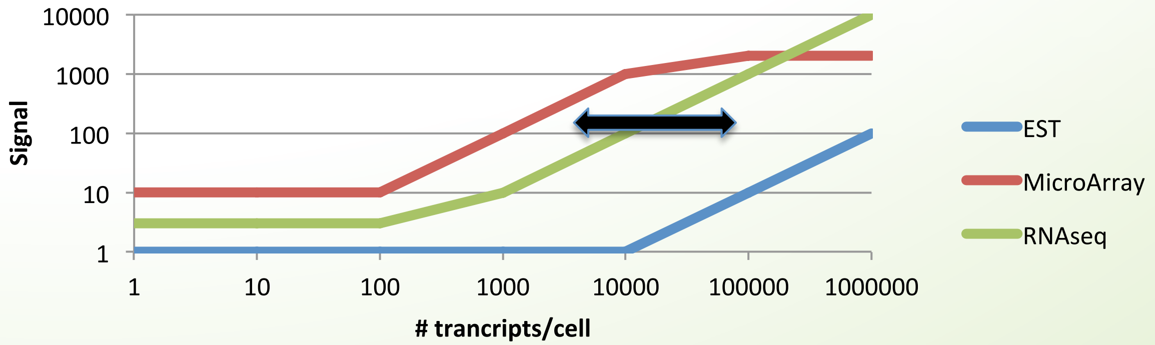
- Low throughput (-)
- Complete transcripts (+)
- Only highly expressed genes (--)
- Expensive (-)
- Low background noise (+)
- Easy downstream analysis (+)

Micro Arrays

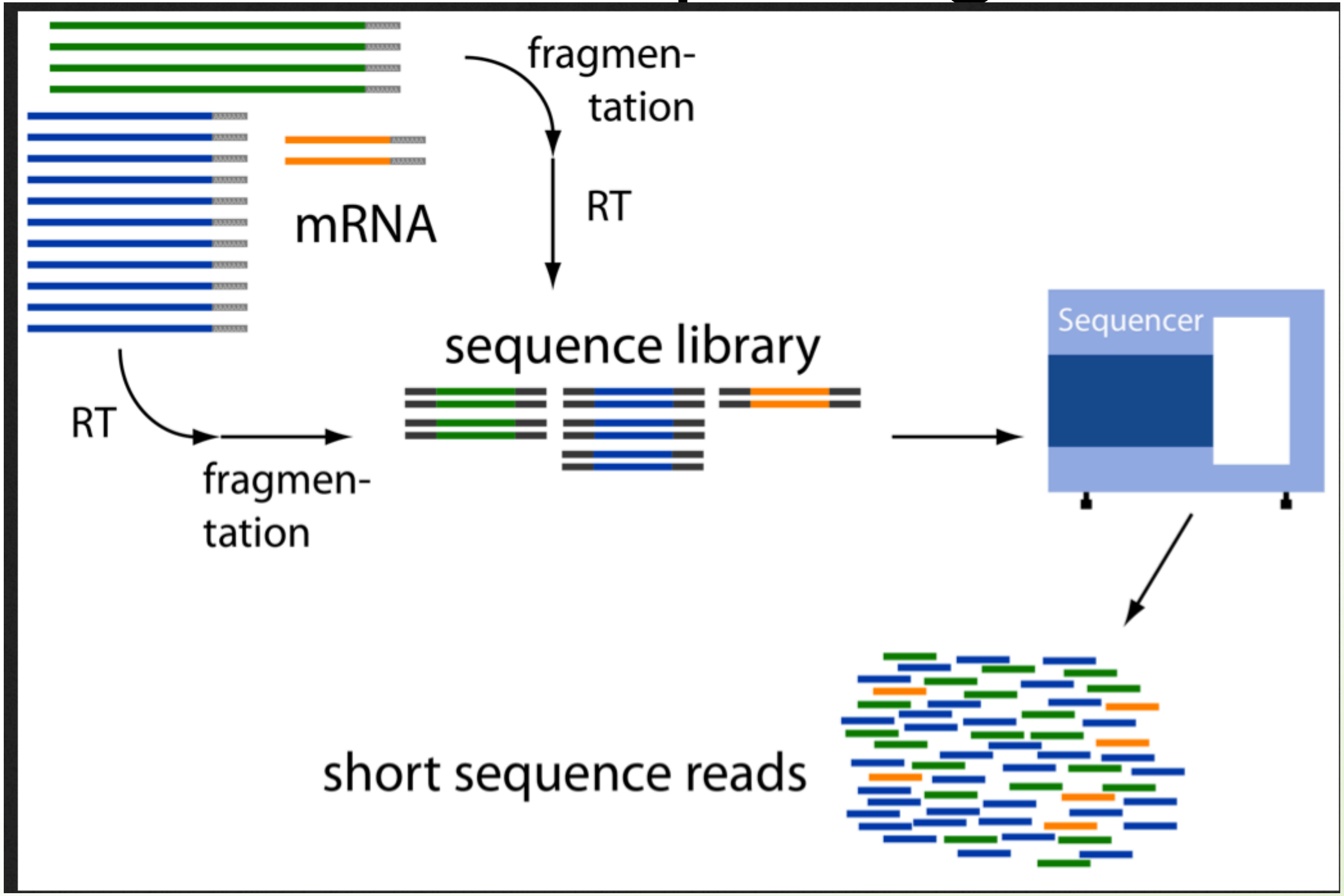
- High throughput (+)
- Only known sequences (-)
- Limited dynamic range (-)
- Cheap (+)
- High background noise (-)
- Not strand specific (-)
- Well established downstream methods (+)

RNAseq

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

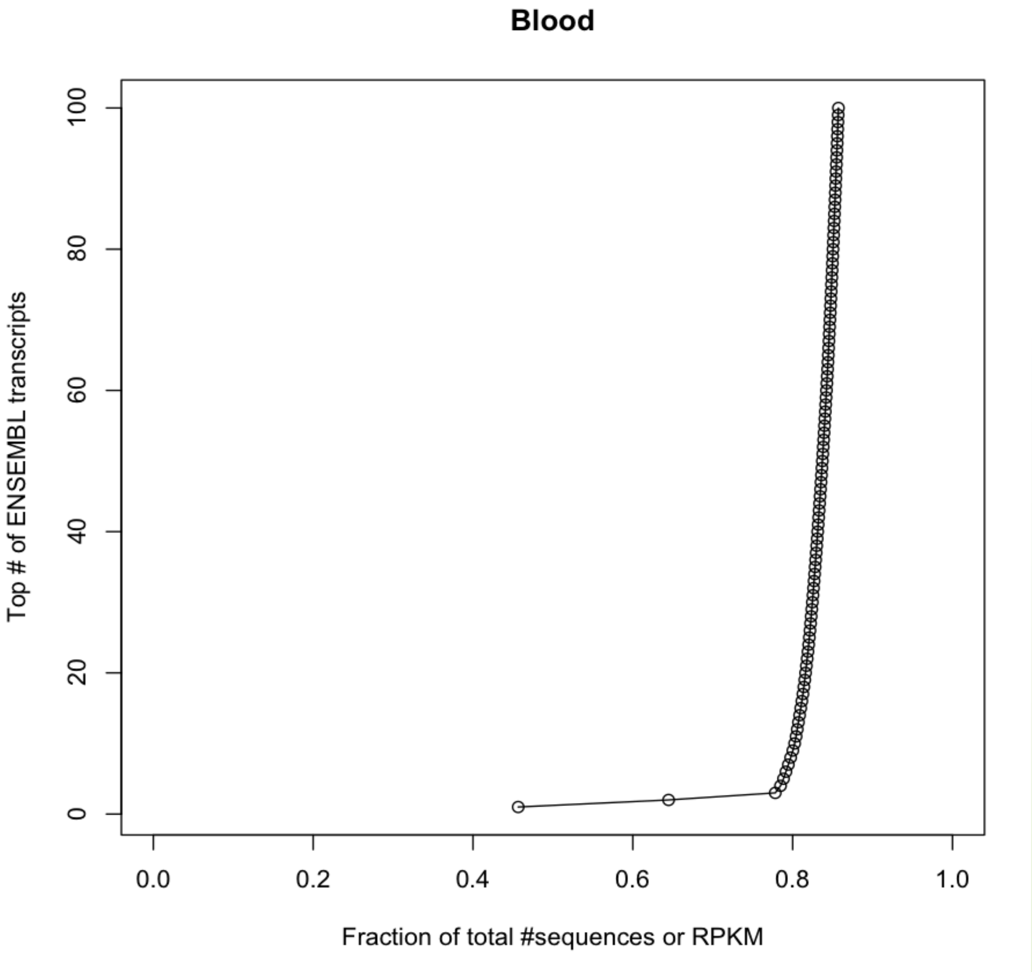


How are RNA-seq data generated?



Sampling process

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



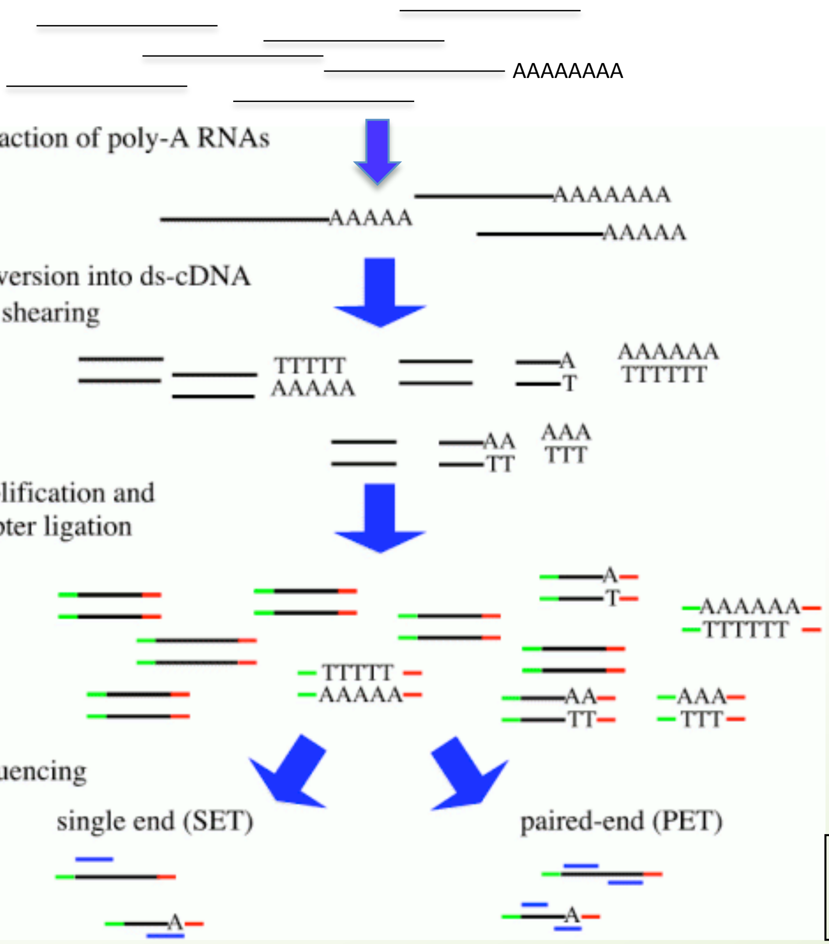
RNA to reads

RNA->

enrichments ->

library ->

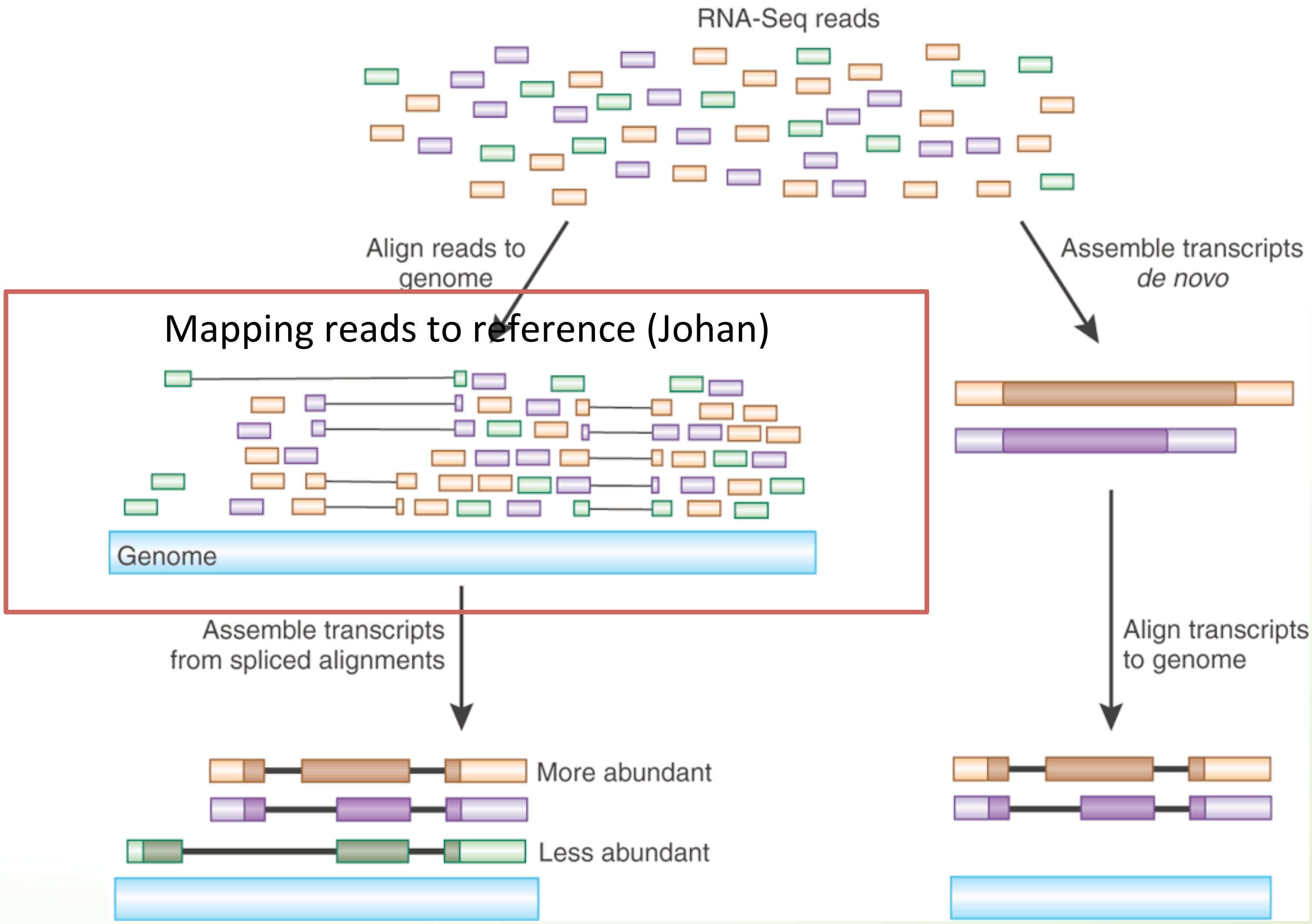
reads ->



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

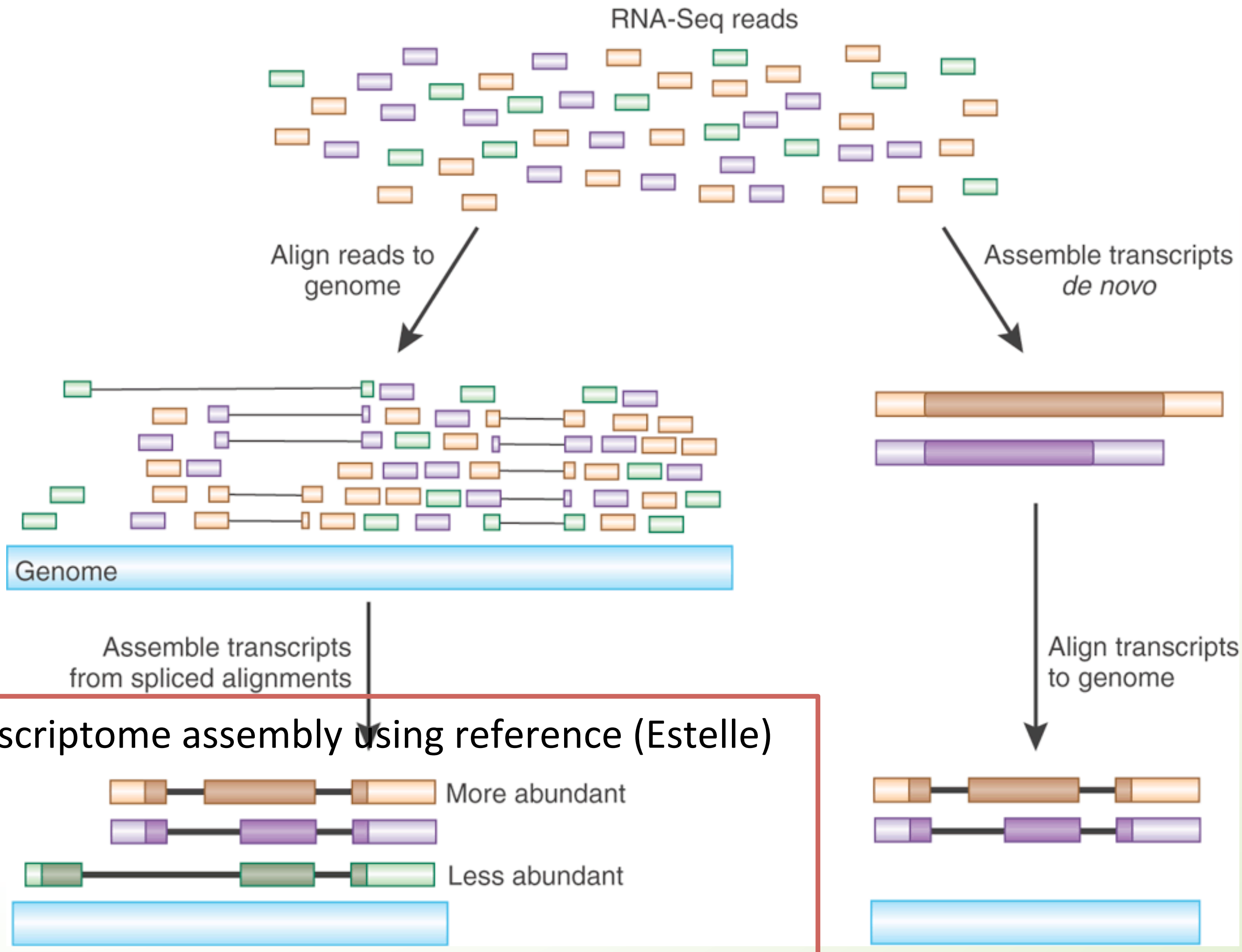
Size of fragment
Strand specific
5’ end specific
3’ end specific
…..

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



Mapping reads to reference (Johan)

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



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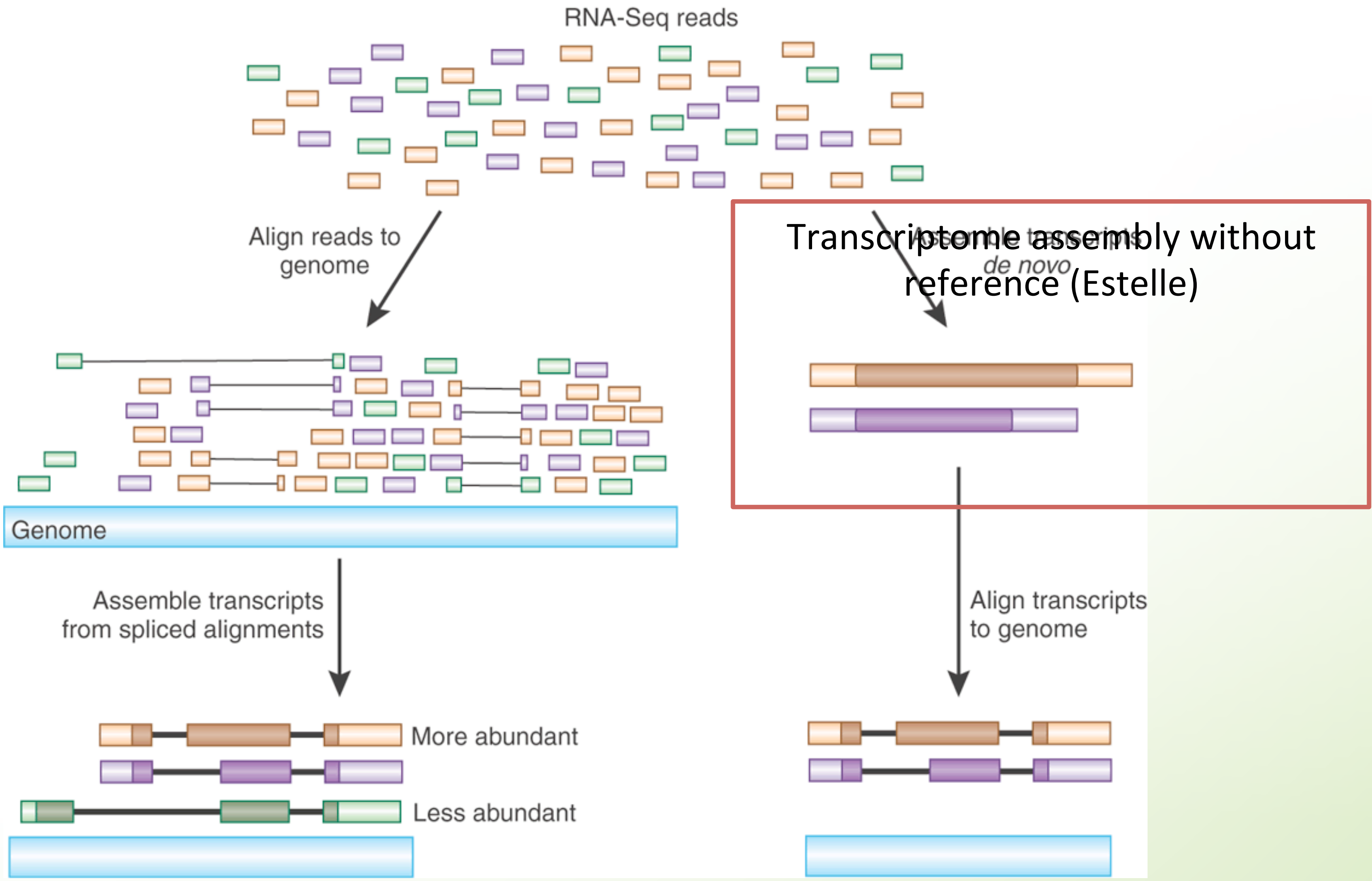
Transcriptome assembly using reference (Estelle)



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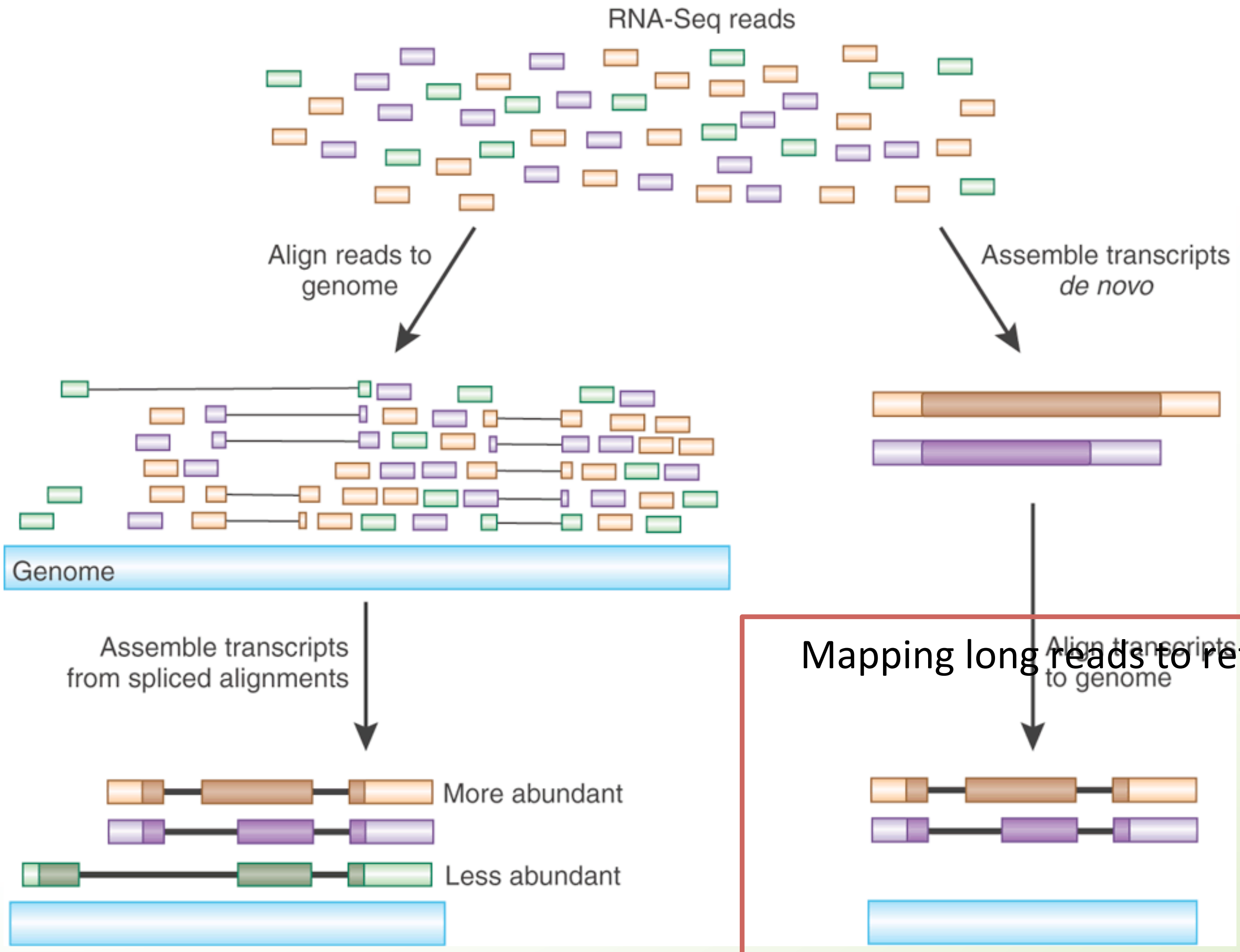
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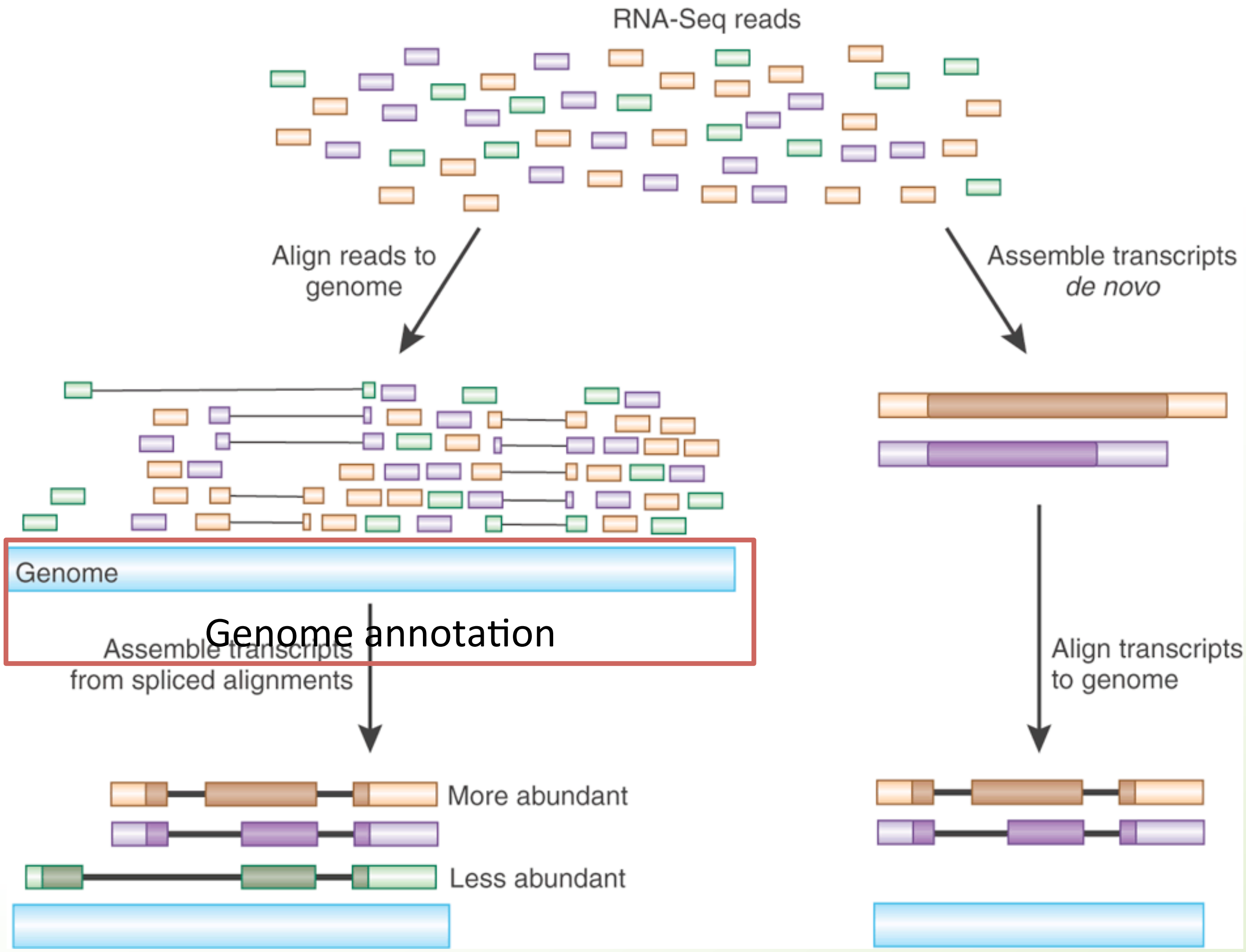
Mapping long reads to reference



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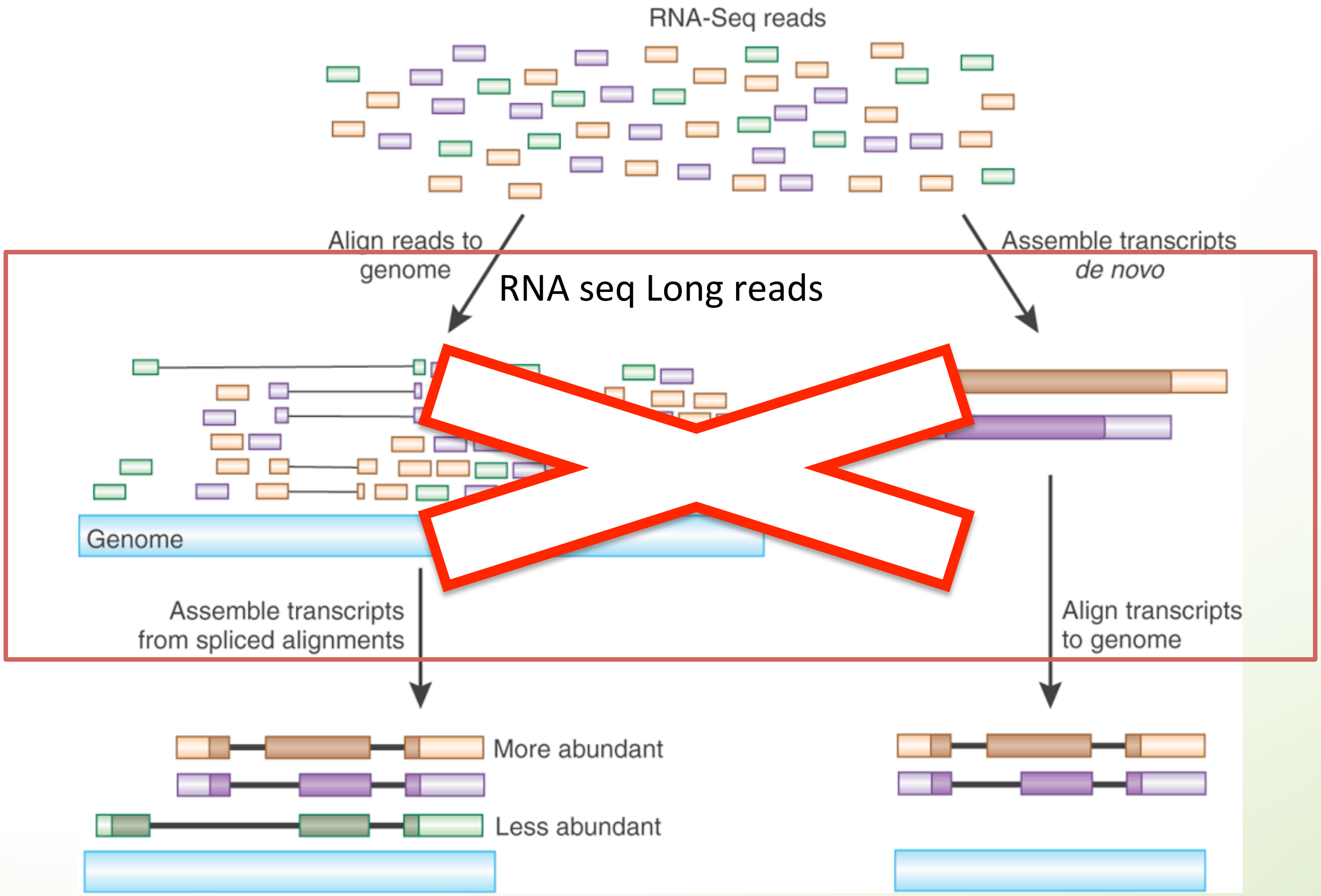
Genome annotation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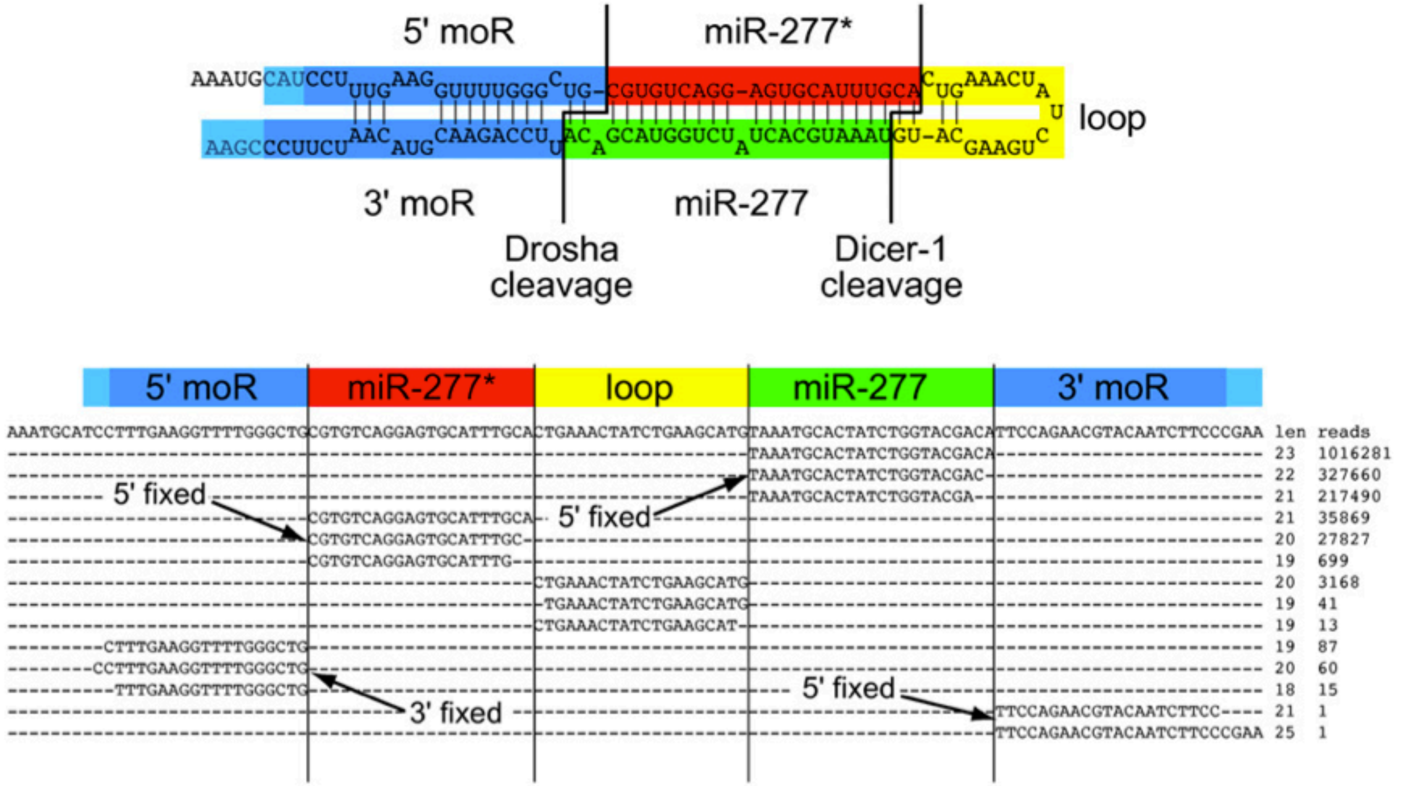
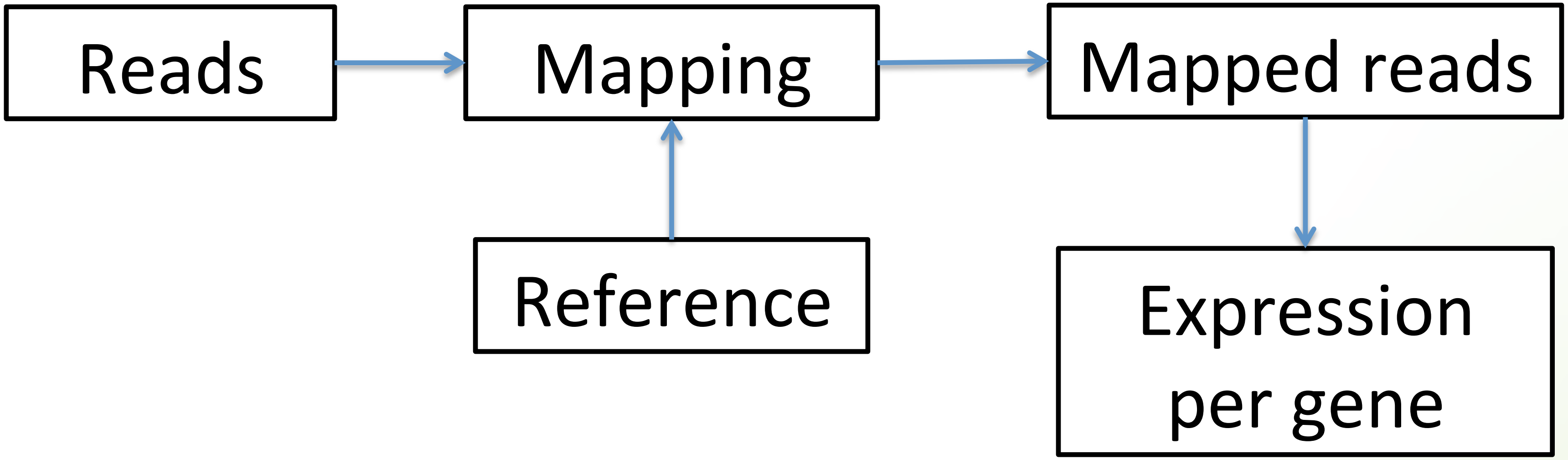
RNA seq Long reads

microRNA analysis (Jakub)



```
AAATGCATCCTTTGAAGGTTTTGGGCTGCGTGTCAGGAGTGCATTTGCACTGAAACTATCTGAAGCATGTAAATGCACTATCTGGTACGACATTCCAGAACGTACAATCTTCCCGAA len reads
---------------------------------------------------------------------TAAATGCACTATCTGGTACGACA------------------------- 23  1016281
---------------------------------------------------------------------TAAATGCACTATCTGGTACGAC-------------------------- 22  327660
---------------------------------------------------------------------TAAATGCACTATCTGGTACGA--------------------------- 21  217490
----------------------------CGTGTCAGGAGTGCATTTGCA------------------------------------------------------------------- 21  35869
----------------------------CGTGTCAGGAGTGCATTTGC-------------------------------------------------------------------- 20  27827
----------------------------CGTGTCAGGAGTGCATTTG--------------------------------------------------------------------- 19  699
-------------------------------------------------CTGAAACTATCTGAAGCATG------------------------------------------------ 20  3168
--------------------------------------------------TGAAACTATCTGAAGCATG------------------------------------------------ 19  41
-------------------------------------------------CTGAAACTATCTGAAGCAT------------------------------------------------- 19  13
---------CTTTGAAGGTTTTGGGCTG----------------------------------------------------------------------------------------- 19  87
--------CCTTTGAAGGTTTTGGGCTG----------------------------------------------------------------------------------------- 20  60
----------TTTGAAGGTTTTGGGCTG----------------------------------------------------------------------------------------- 18  15
--------------------------------------------------------------------------------------------TTCCAGAACGTACAATCTTCC---- 21  1
--------------------------------------------------------------------------------------------TTCCAGAACGTACAATCTTCCCGAA 25  1
```

(Berezikov et al. Genome Research, 2011.)



Reads

Mapping

Reference

Mapped reads

Expression per gene

ANOTHER WAY OF LOOKING AT IT

Quality control

-samples might not be what you think they are

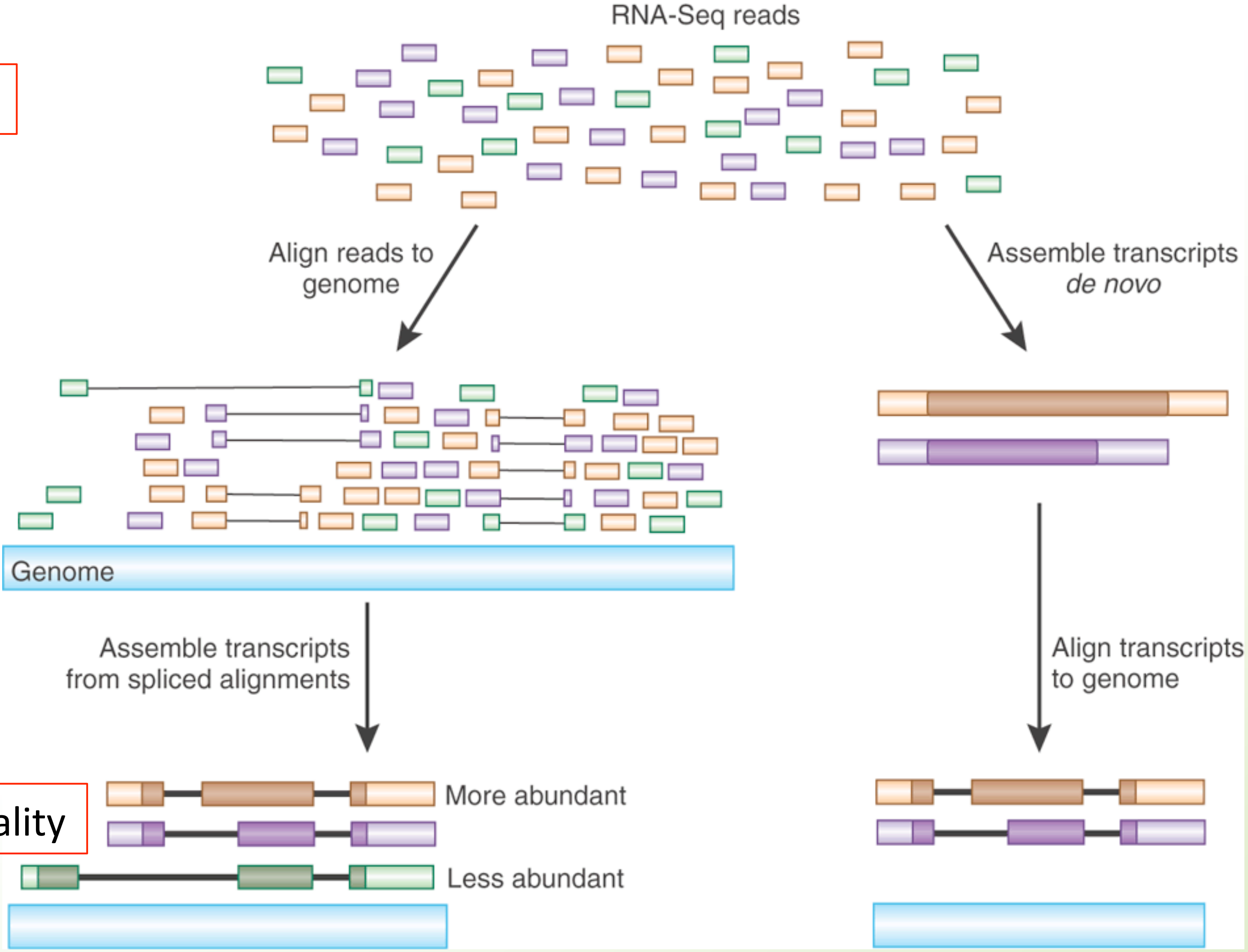
- Experiments go wrong
  - 30 samples with 5 steps from samples to reads has 150 potential steps for errors
  - Error rate 1/100 with 5 steps suggest that one of every 20 samples the reads does not represent the sample
- Mixing samples
  - 30 samples with 5 steps from samples to reads has ~24M potential 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 (Åsa)

Read quality

Mapping statistics

Transcript quality



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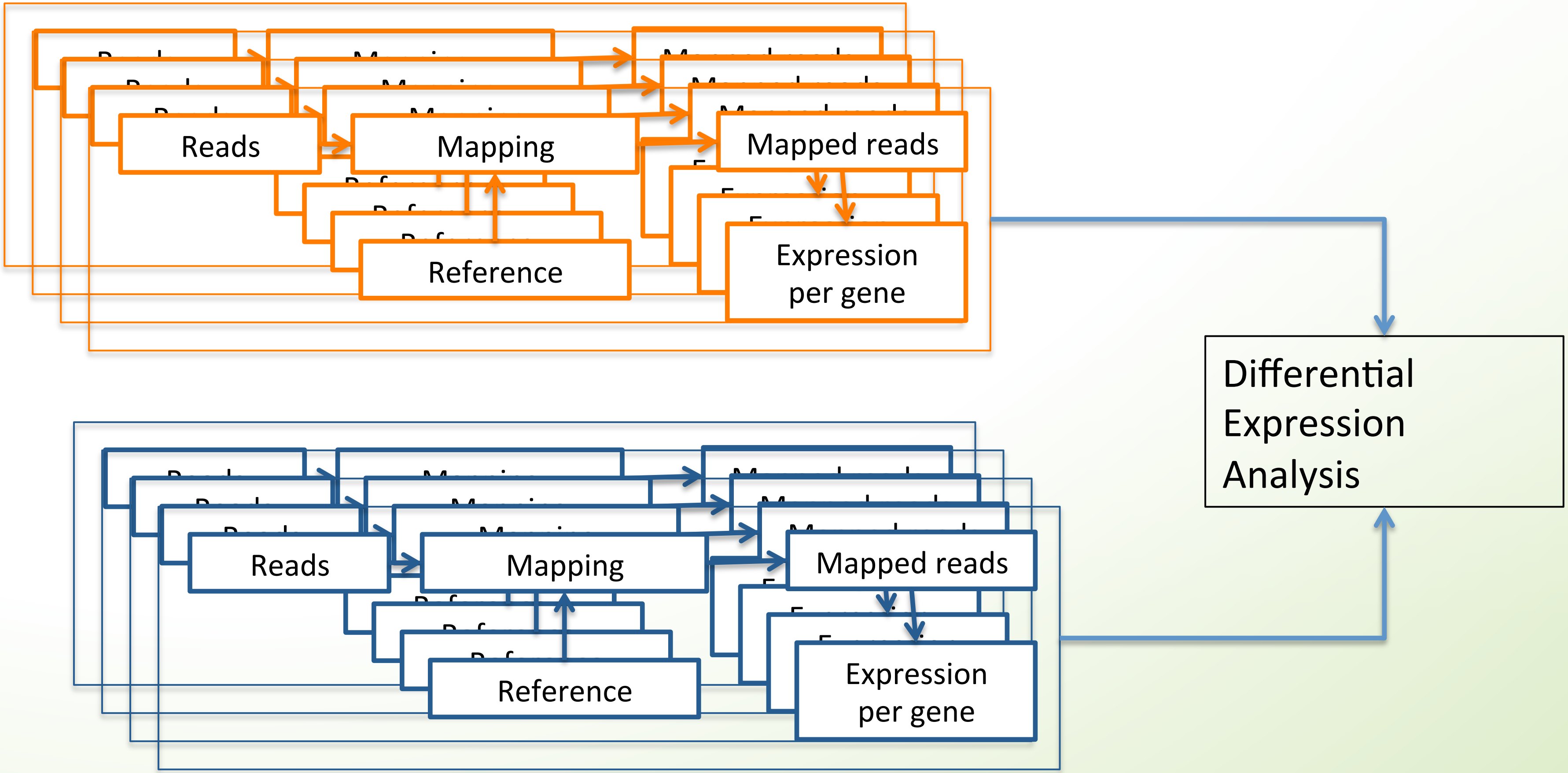
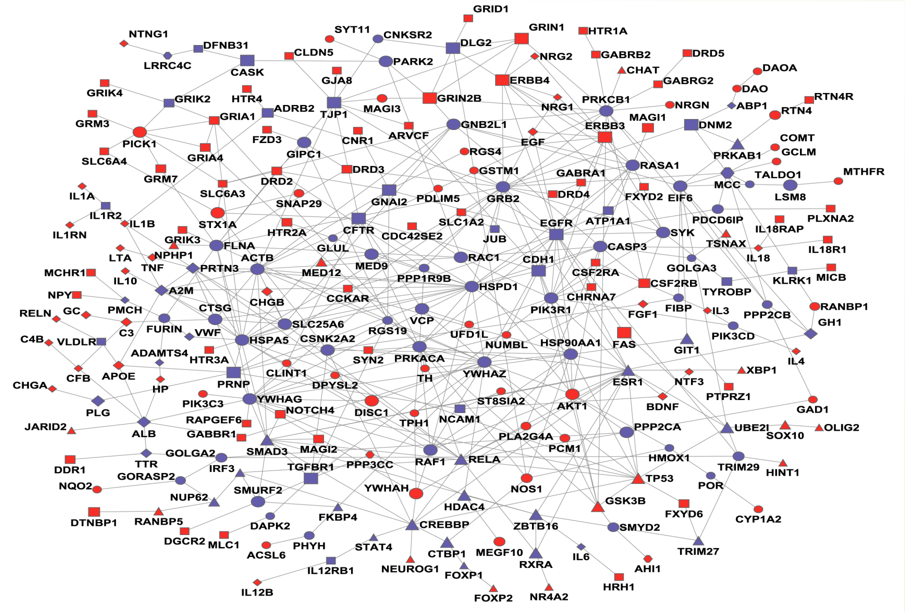
Compare expression between different samples (Åsa)



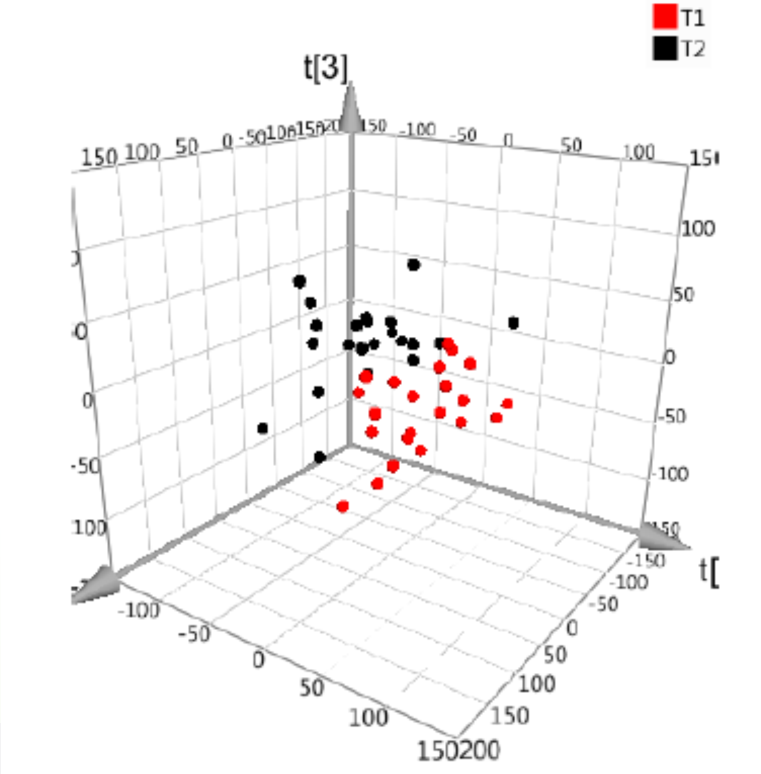
Differential expression analysis using univariate analysis (Åsa)

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

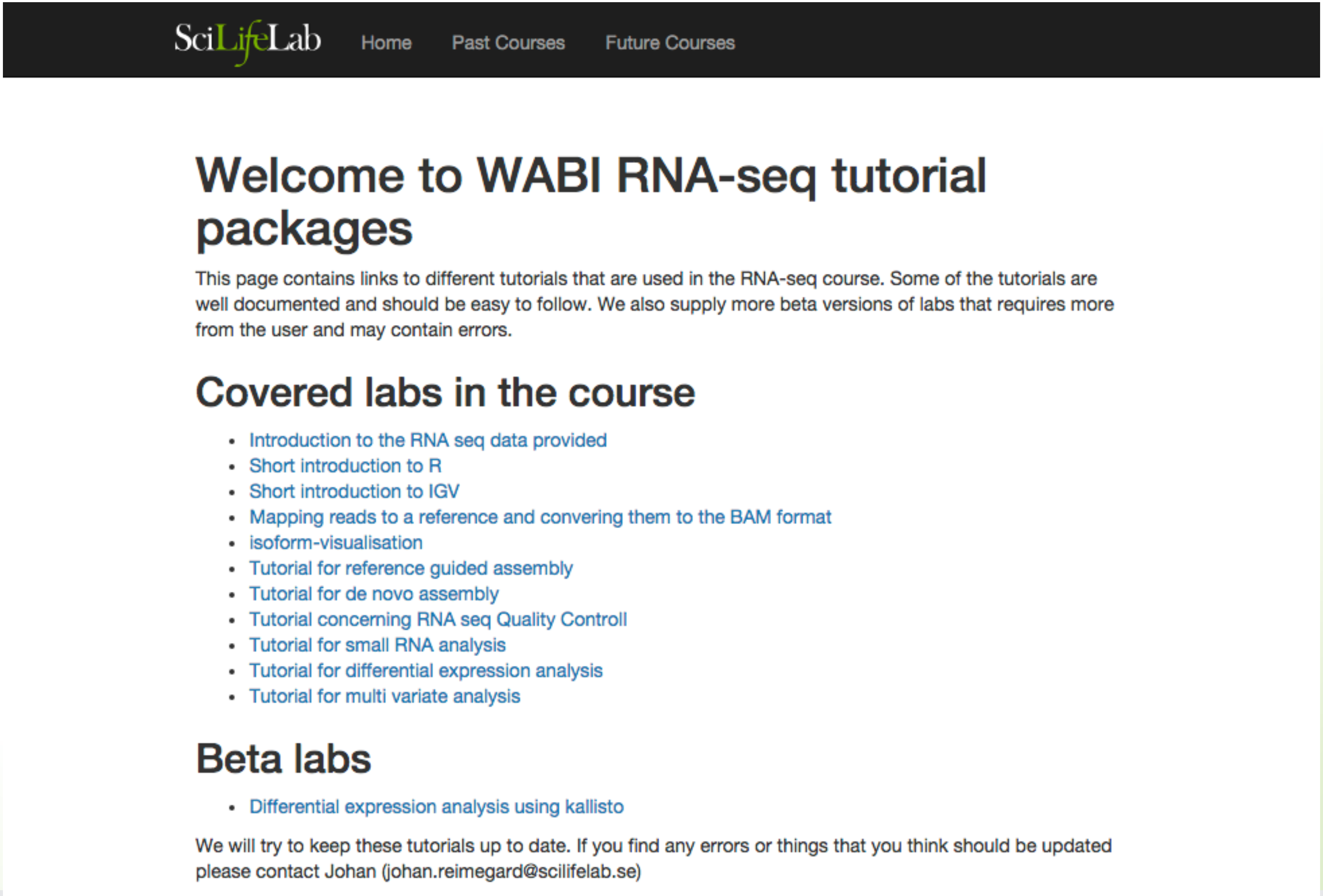


Multi variate differential expression analysis (Sanela)

Multivariate methods such as PCA (unsupervised) or PLS (supervised) can be used to obtain loadings for features (genes/transcripts/…) that contribute to separation of groups



The loading scores can be used as a different kind of measure of which genes are interesting



Welcome to WABI RNA-seq tutorial packages

This page contains links to different tutorials that are used in the RNA-seq course. Some of the tutorials are well documented and should be easy to follow. We also supply more beta versions of labs that requires more from the user and may contain errors.

Covered labs in the course

- Introduction to the RNA seq data provided
- Short introduction to R
- Short introduction to IGV
- Mapping reads to a reference and convering them to the BAM format
- isoform-visualisation
- Tutorial for reference guided assembly
- Tutorial for de novo assembly
- Tutorial concerning RNA seq Quality Controll
- Tutorial for small RNA analysis
- Tutorial for differential expression analysis
- Tutorial for multi variate analysis

Beta labs

- Differential expression analysis using kallisto

We will try to keep these tutorials up to date. If you find any errors or things that you think should be updated please contact Johan (johan.reimegard@scilifelab.se)