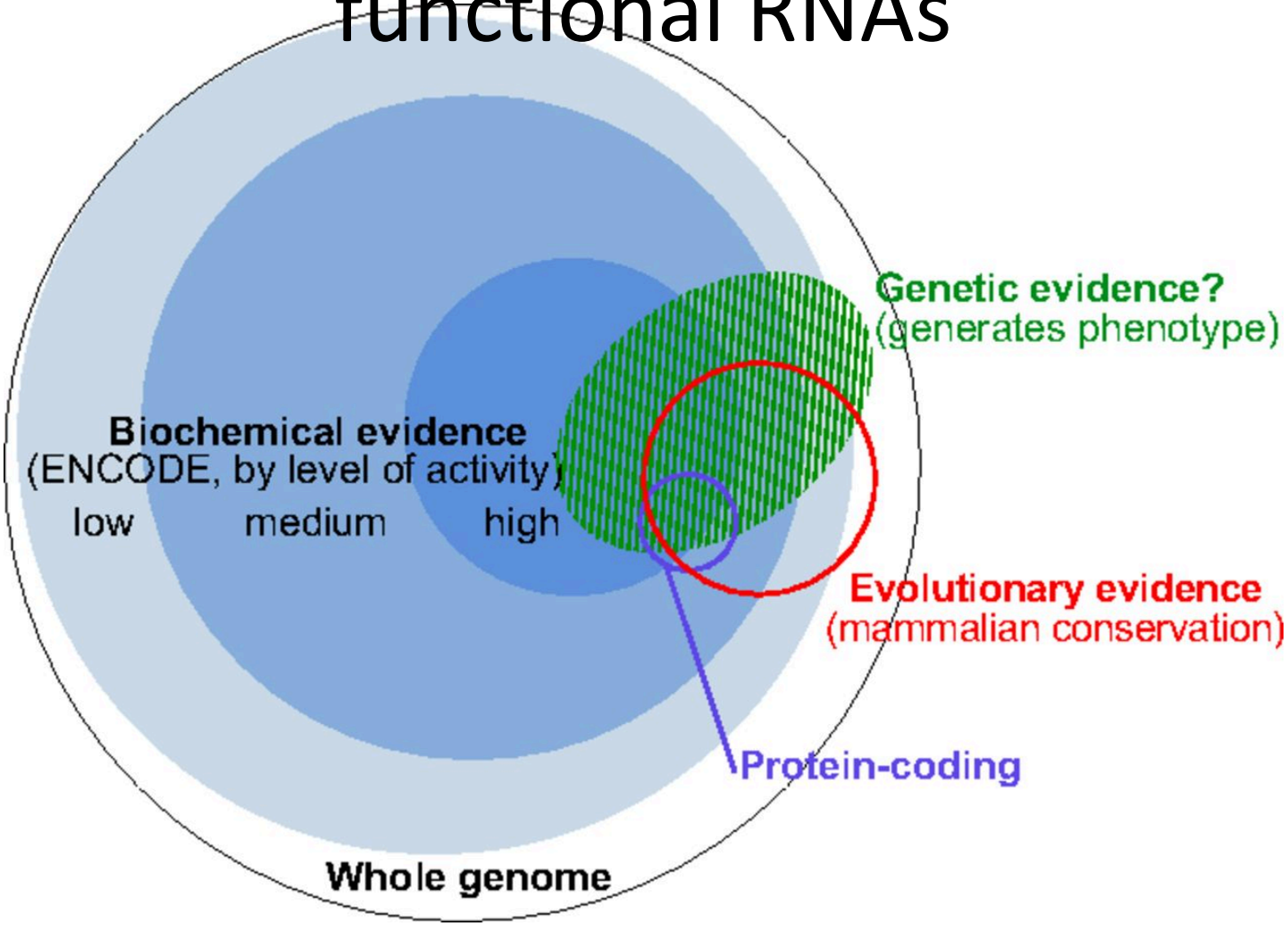
RNAseq analysis

-it's complicated

November 2017

RNA reads are not enough to identify functional RNAs



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

All the steps will affect the results

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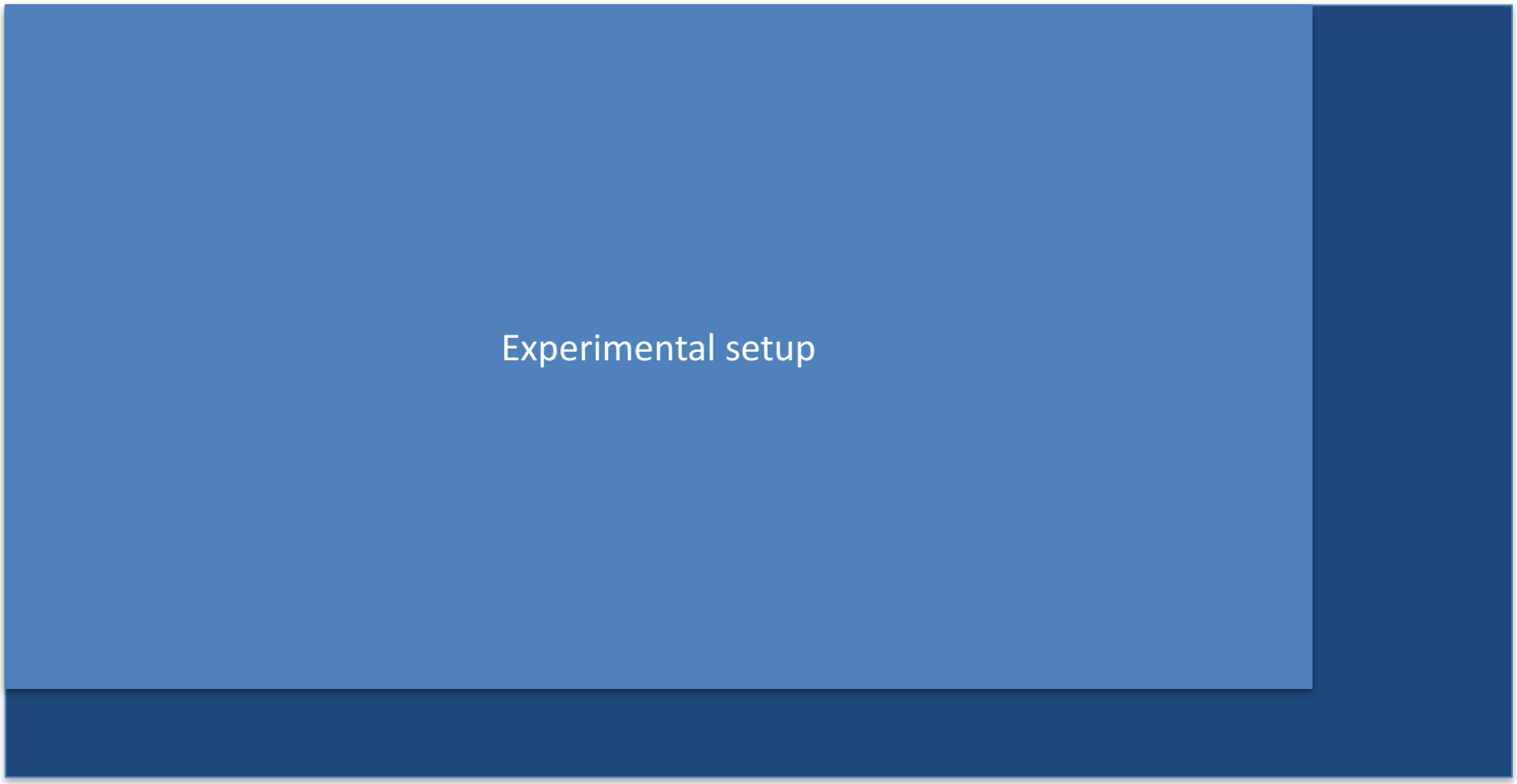

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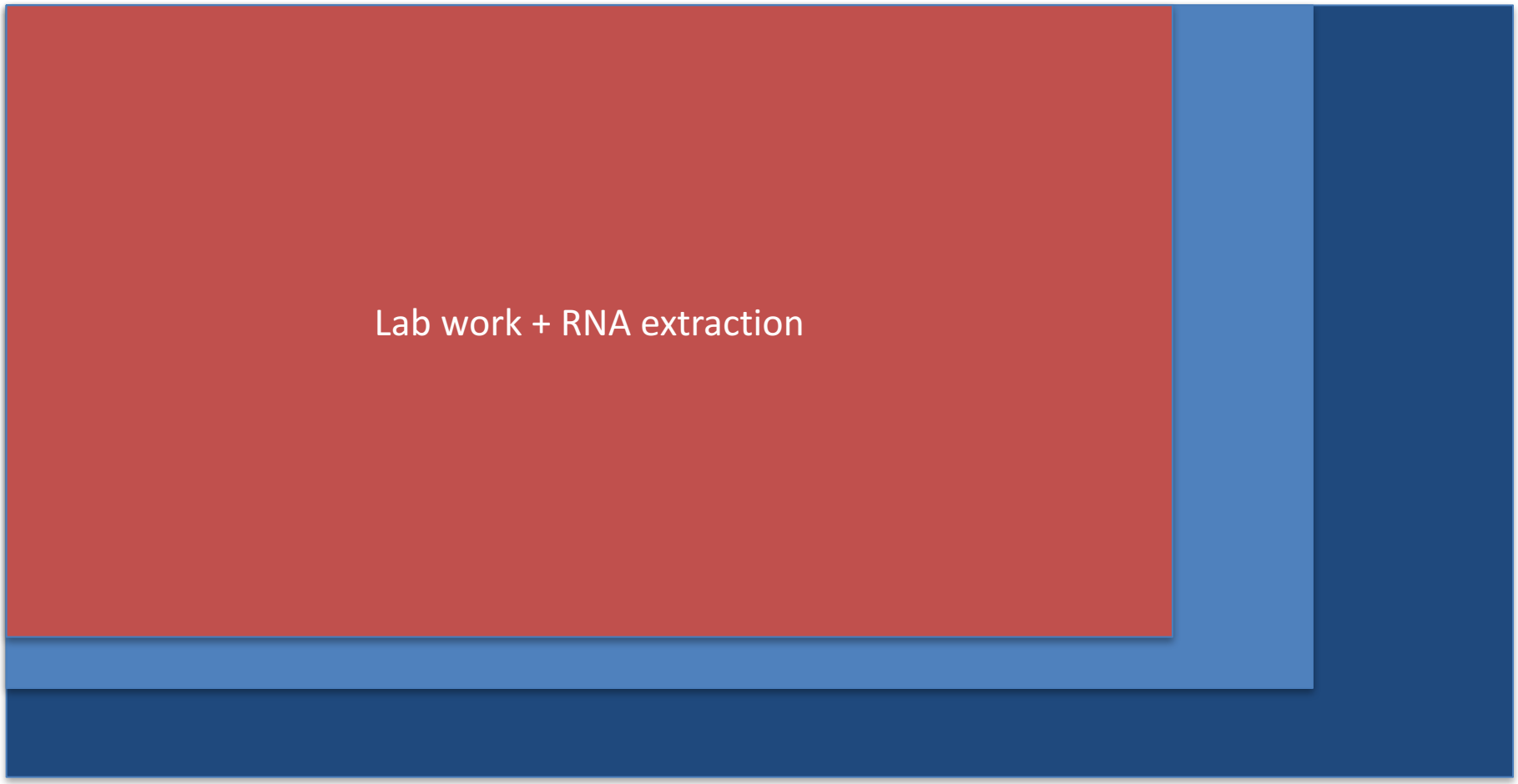

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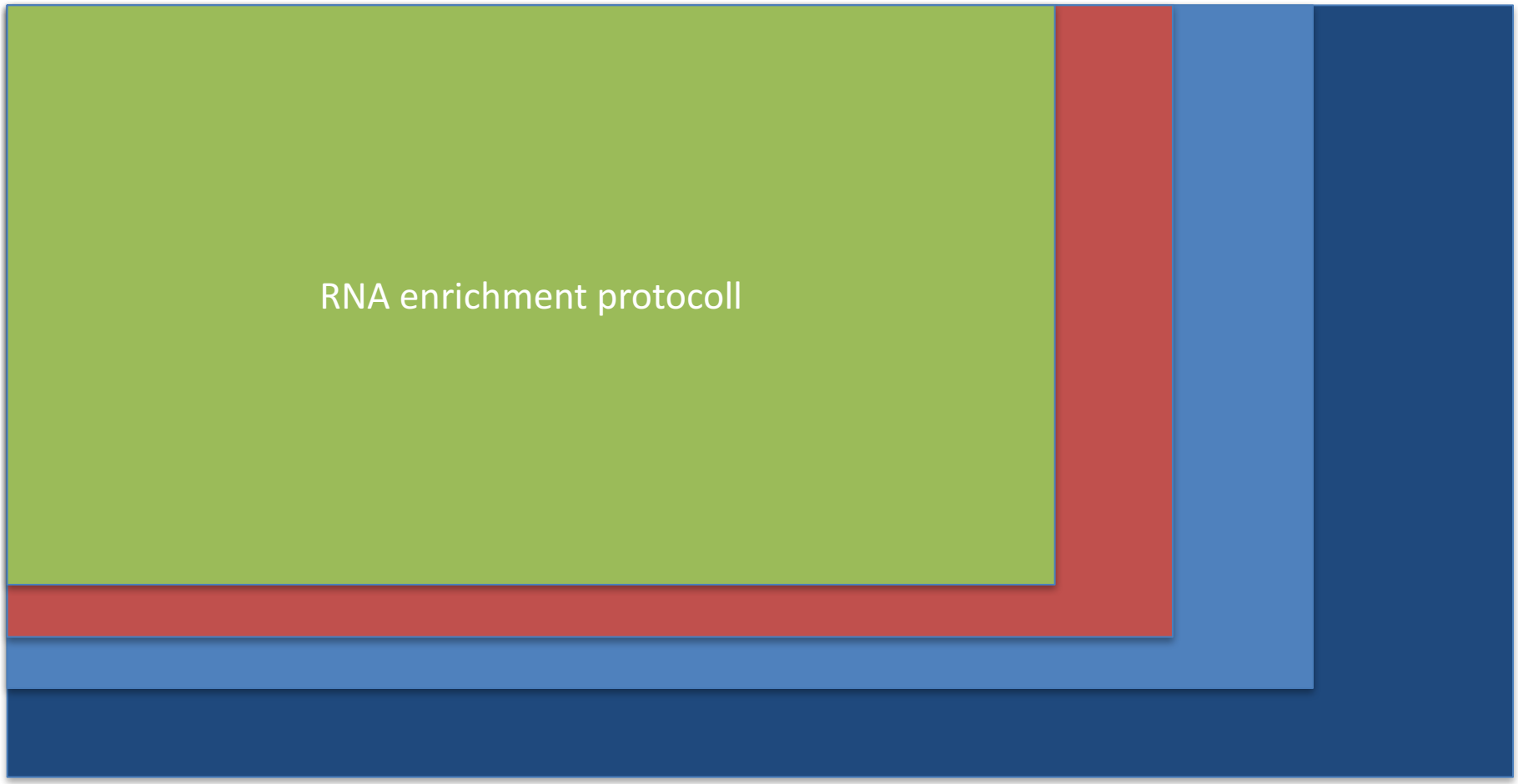

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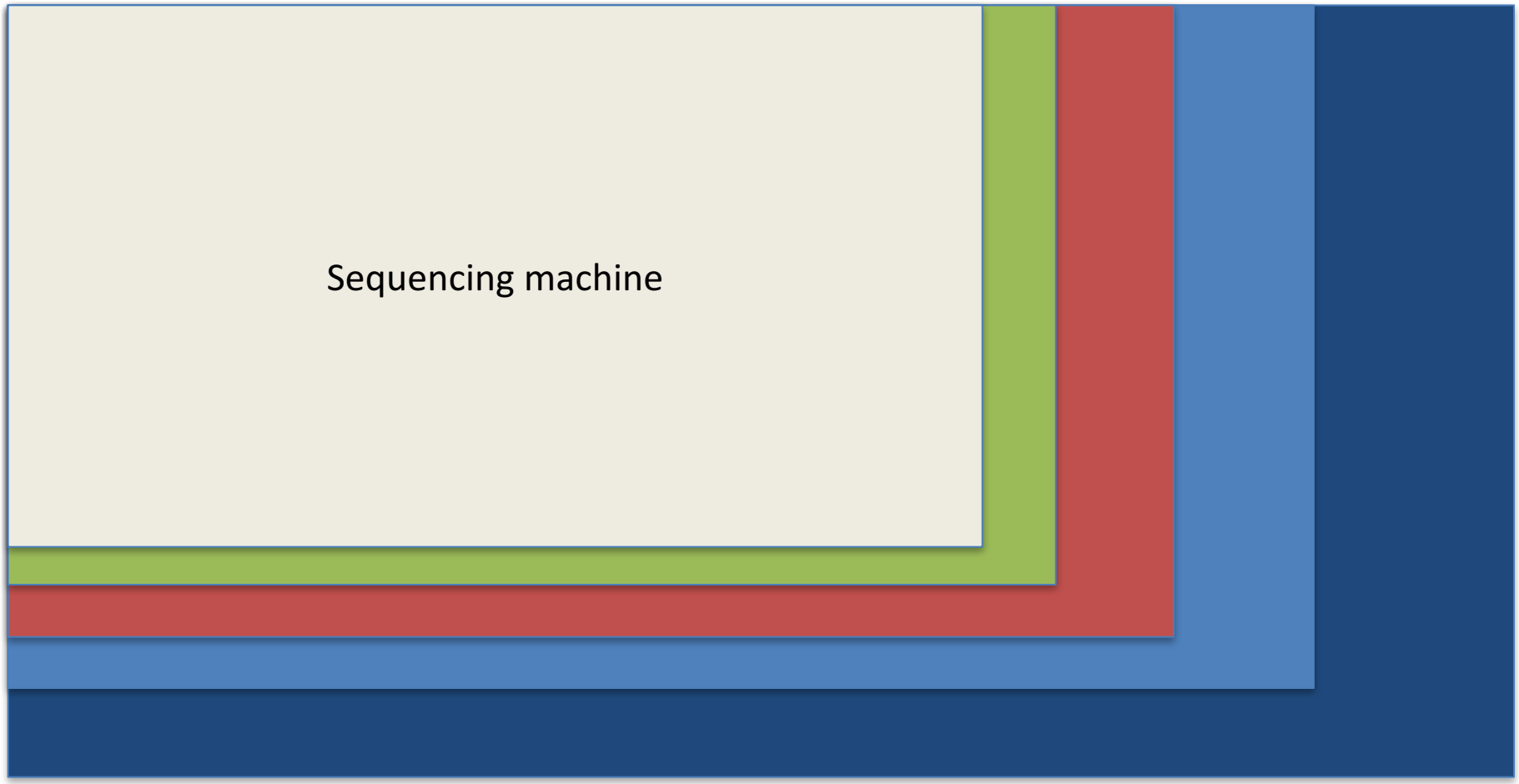

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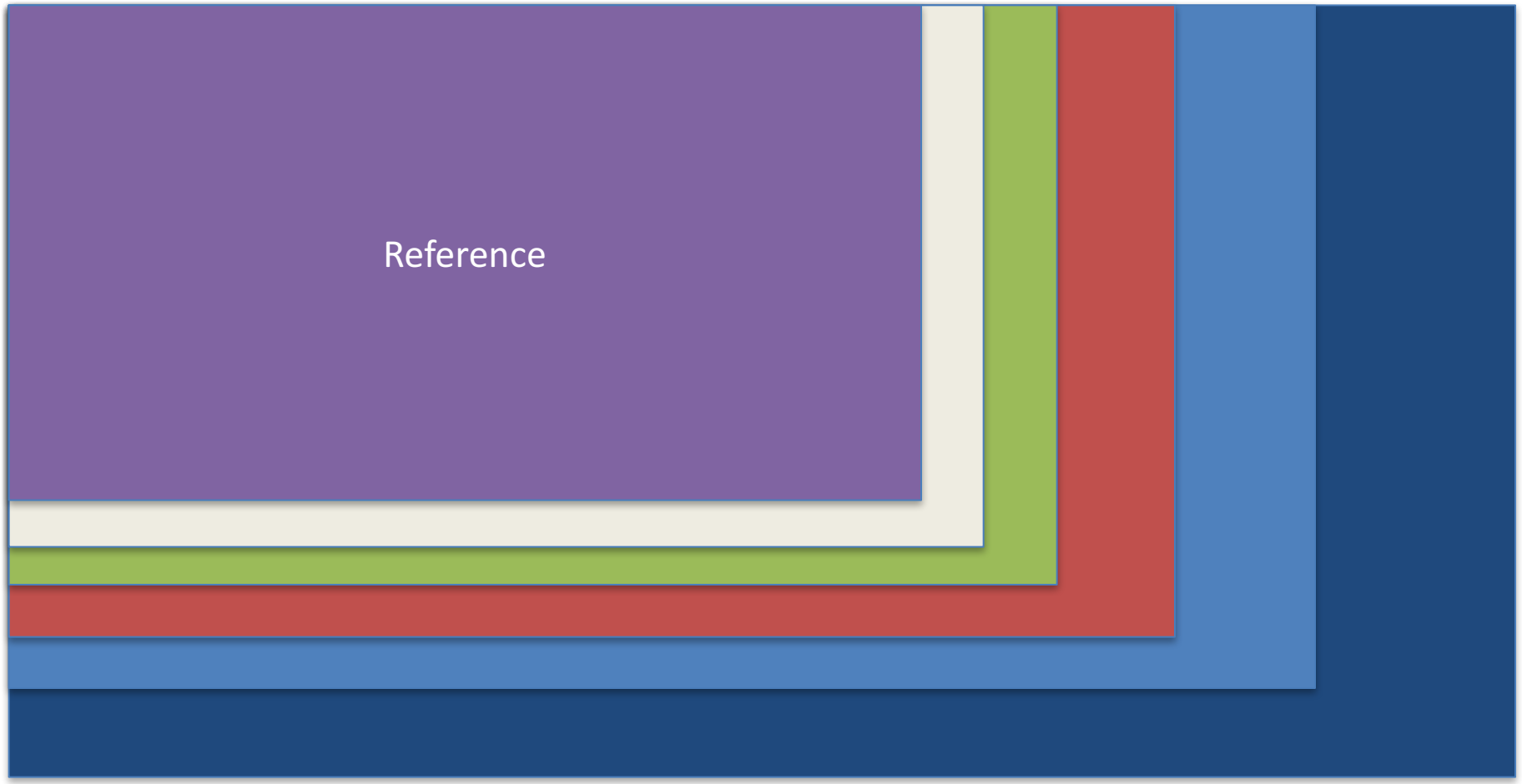

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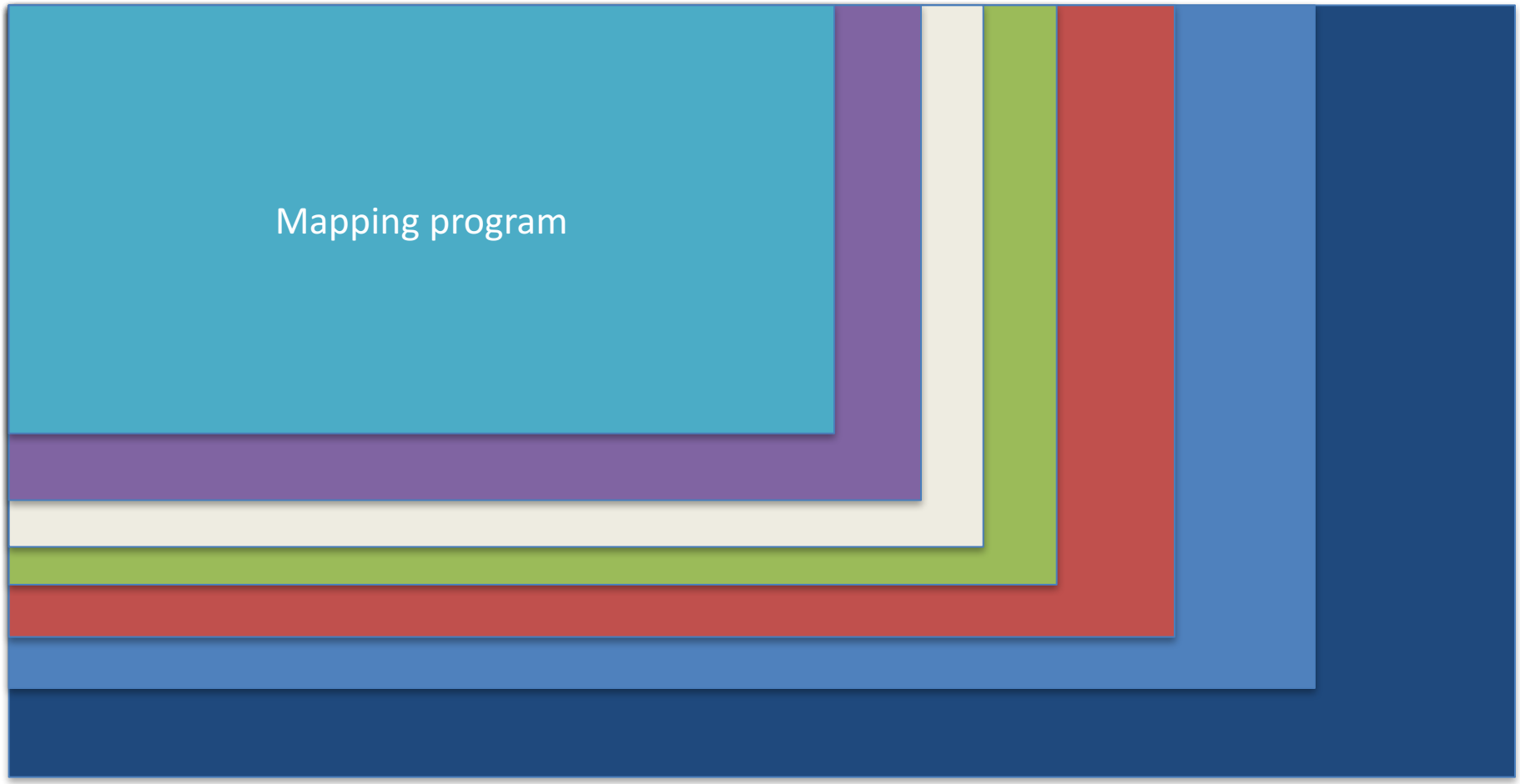

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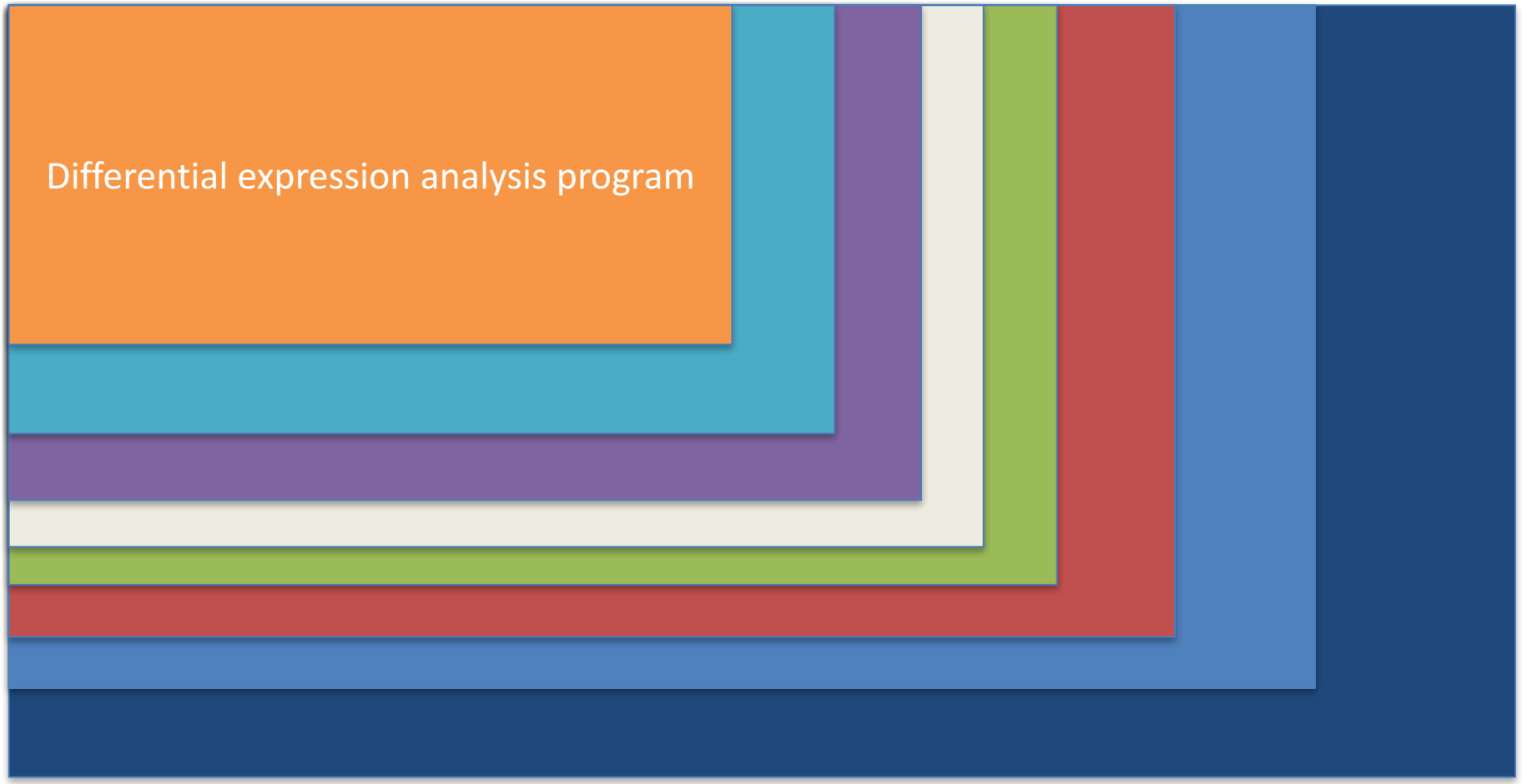
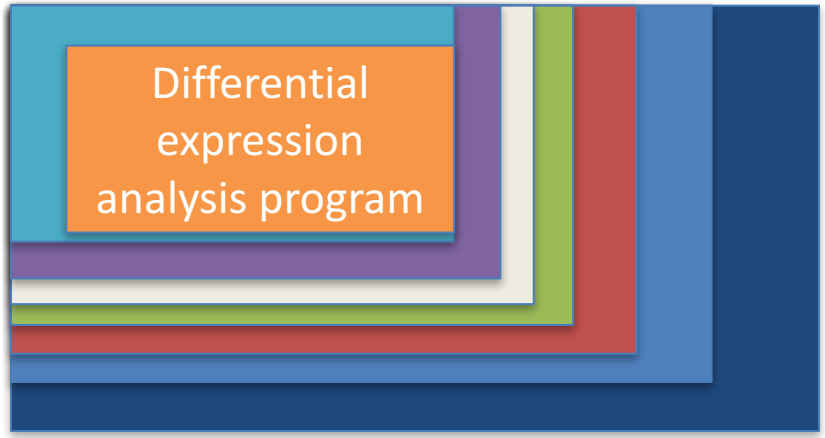
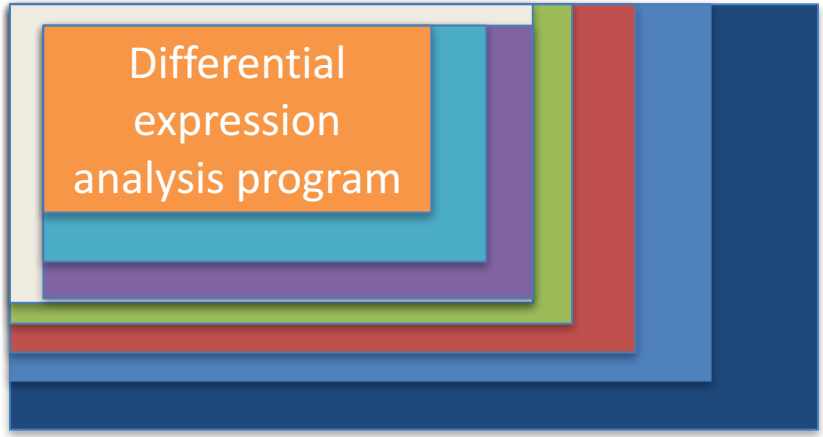
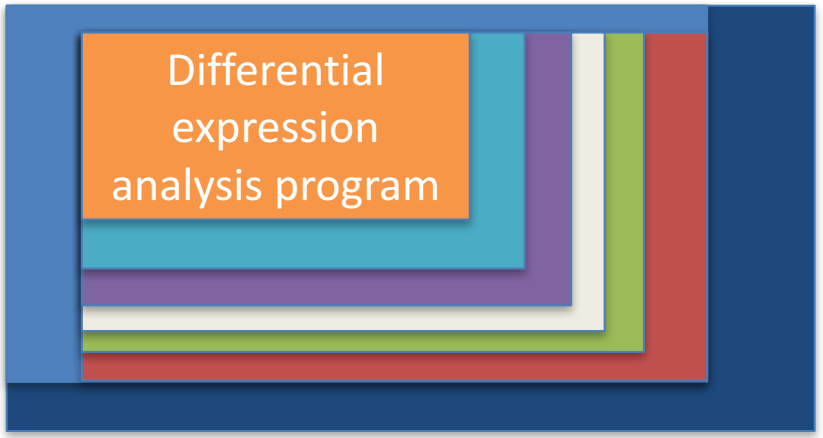


Try to be as consistent as possible

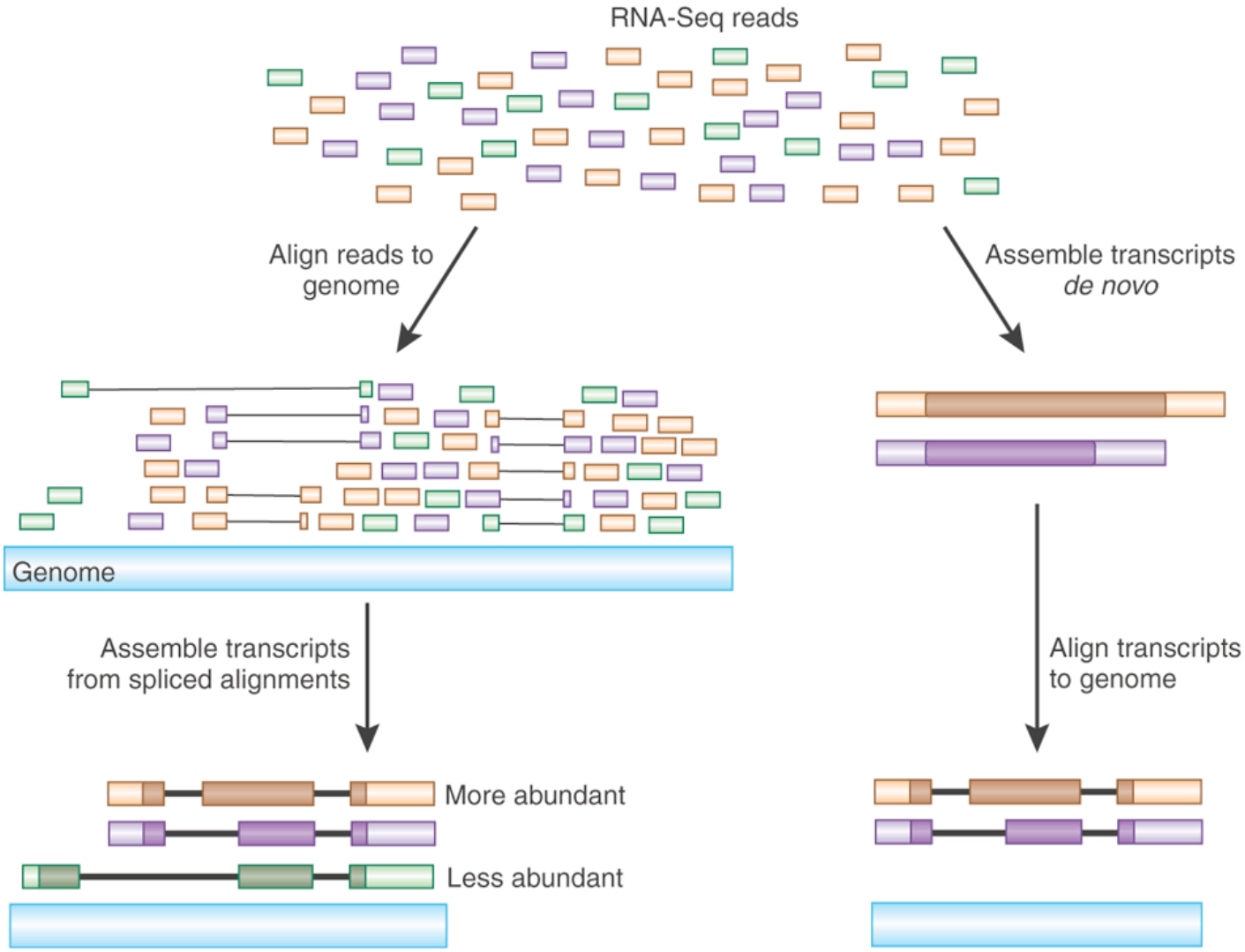






Use a pipeline!

Gene and Isoform detection



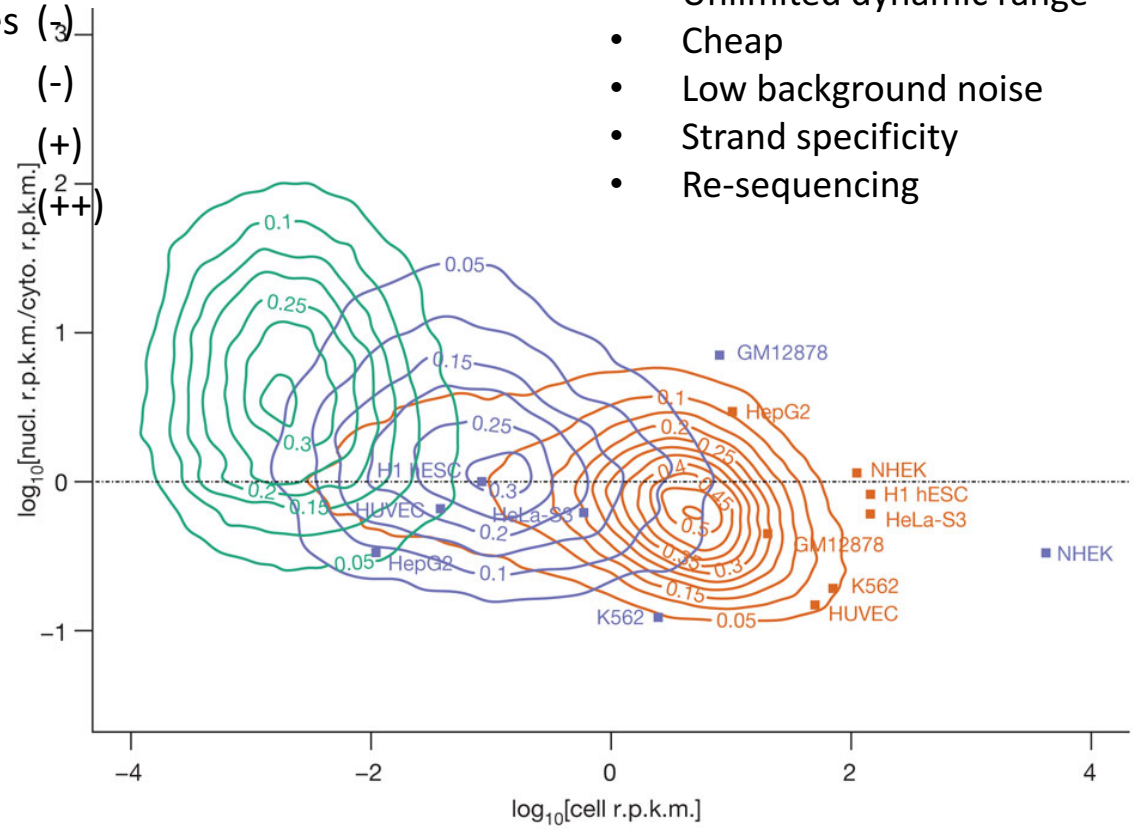
Promises and pitfalls

Long reads

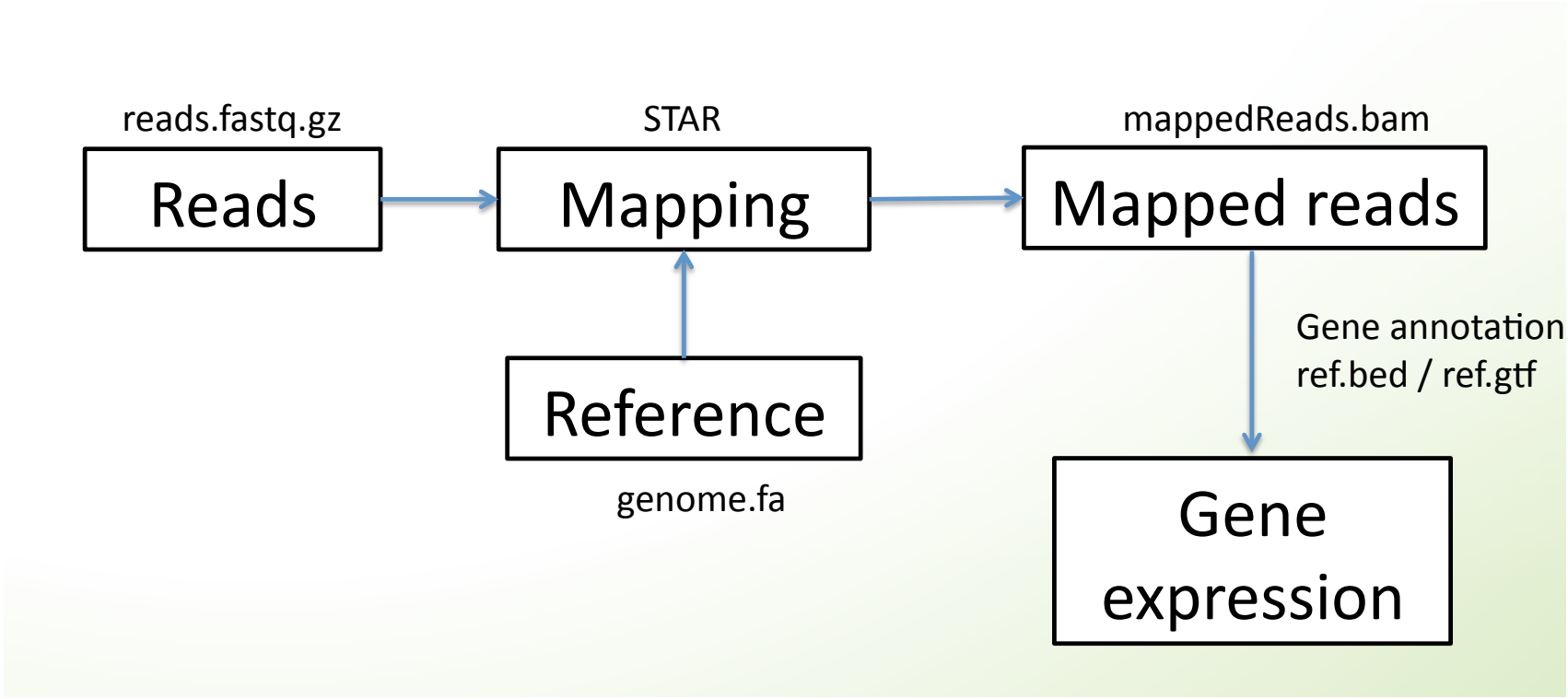
- Low throughput (-)
- Complete transcripts (++)
- Not quantitative (-)
- Only highly expressed genes (-)
- Expensive (-)
- Low background noise (+)
- Easy downstream analysis (++)

short reads

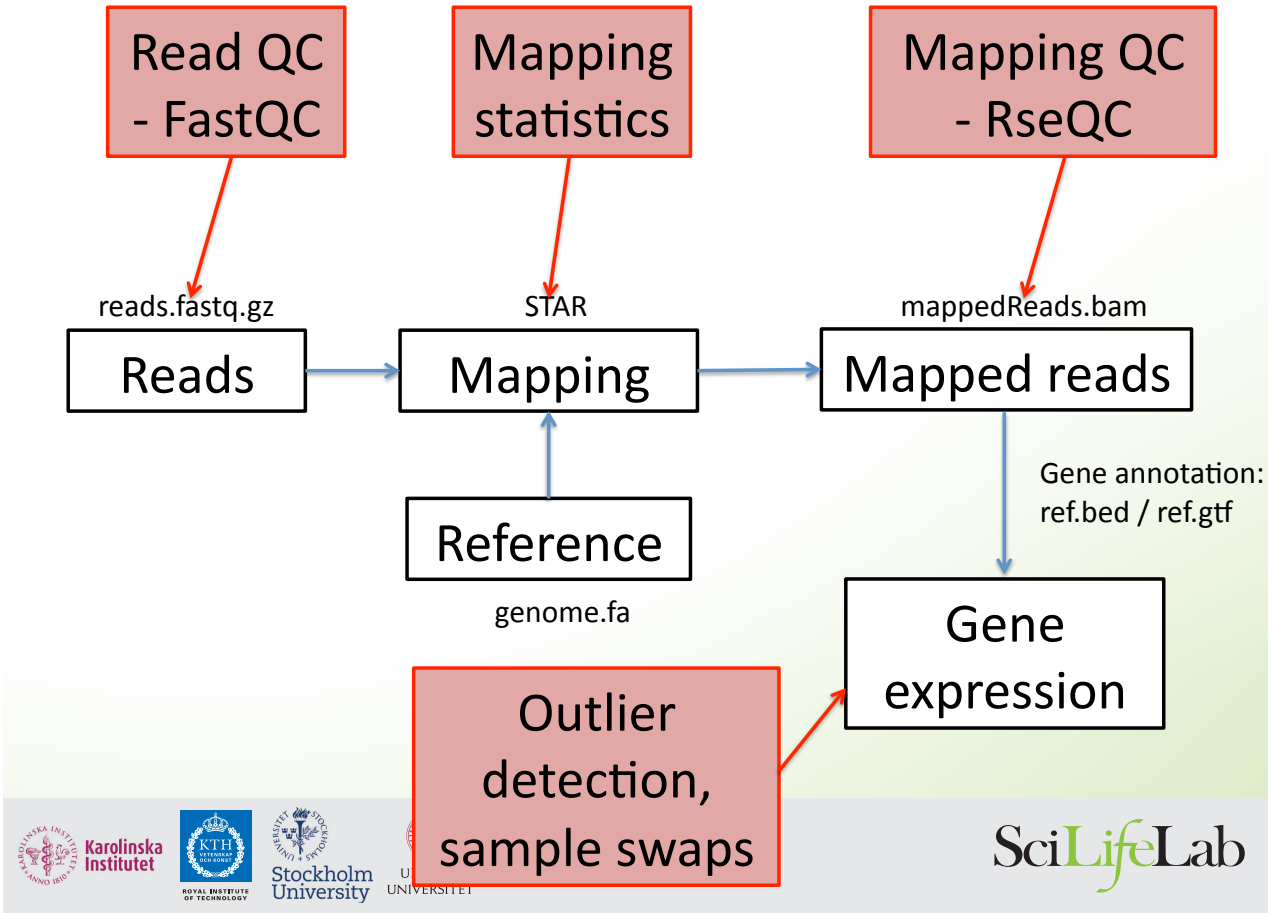
- High throughput (++)
- Quantitative (++)
- Fractions of transcripts (-)
- Full dynamic range (+-)
- Unlimited dynamic range (+)
- Cheap (+)
- Low background noise (+)
- Strand specificity (+)
- Re-sequencing (+)



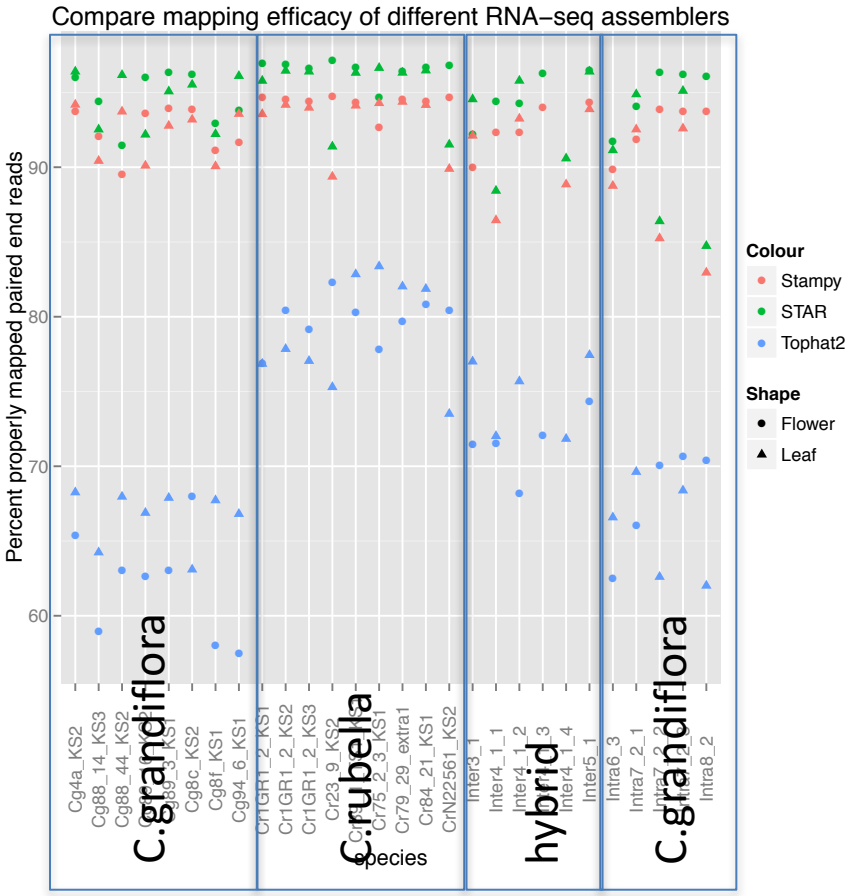
RNA-seq analysis workflow



Do a lot of QC

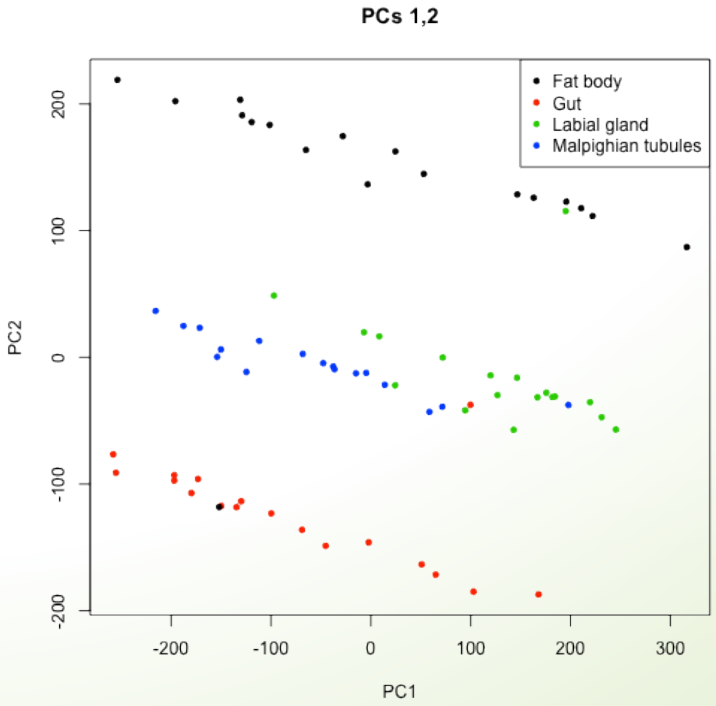

Read QC
- FastQC
Mapping
statistics
Mapping QC
- RseQC
reads.fastq.gz
STAR
mappedReads.bam
Reads
Mapping
Mapped reads
Gene annotation:
ref.bed / ref.gtf
Reference
genome.fa
Gene
expression
Outlier
detection,
sample swaps
Karolinska
Institutet
Stockholm
University
SciLifeLab

More variation when using top hat 2 with default settings than when using STAR or Stampy with default setting

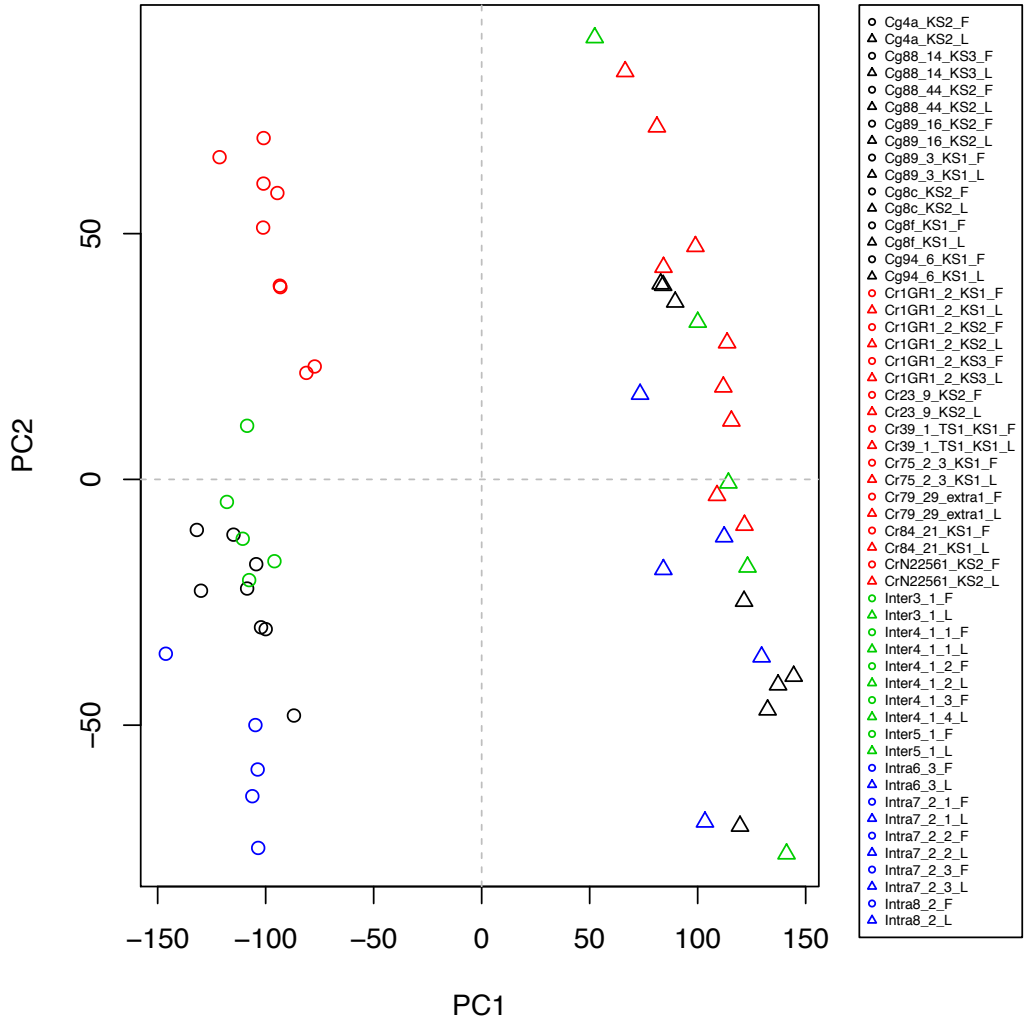


RNA QC

PCA analysis detected potential sample swaps



Principal component 1 separates samples from flowers and leaves



Expression levels are similar between RT-qPCR and RNA-seq data

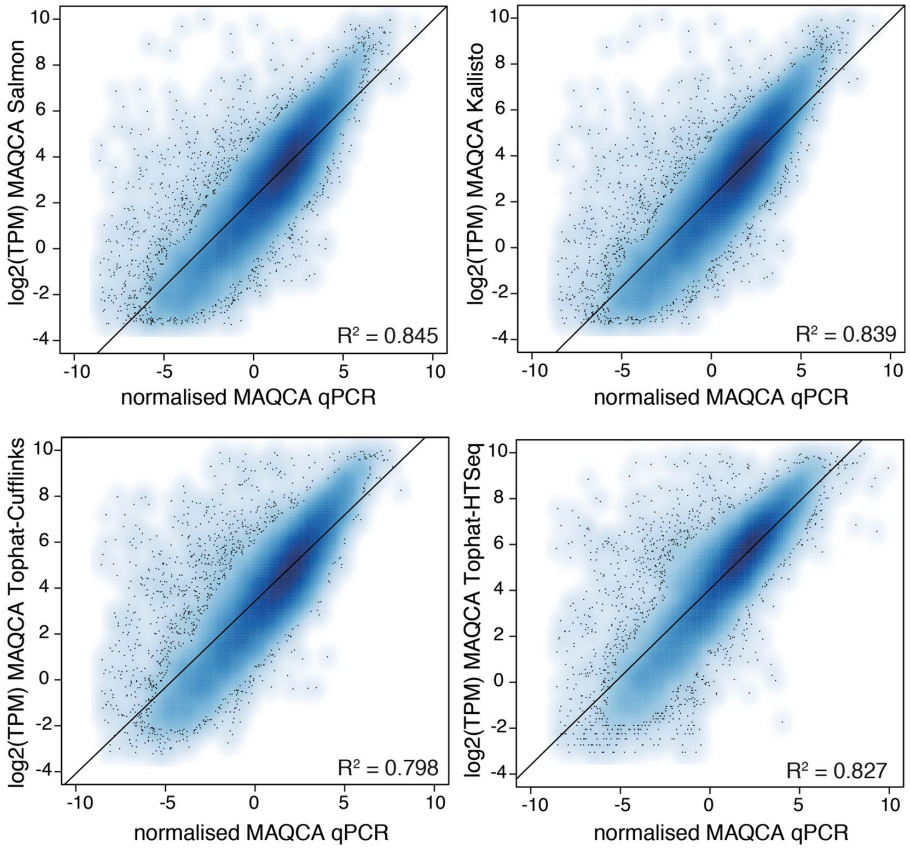
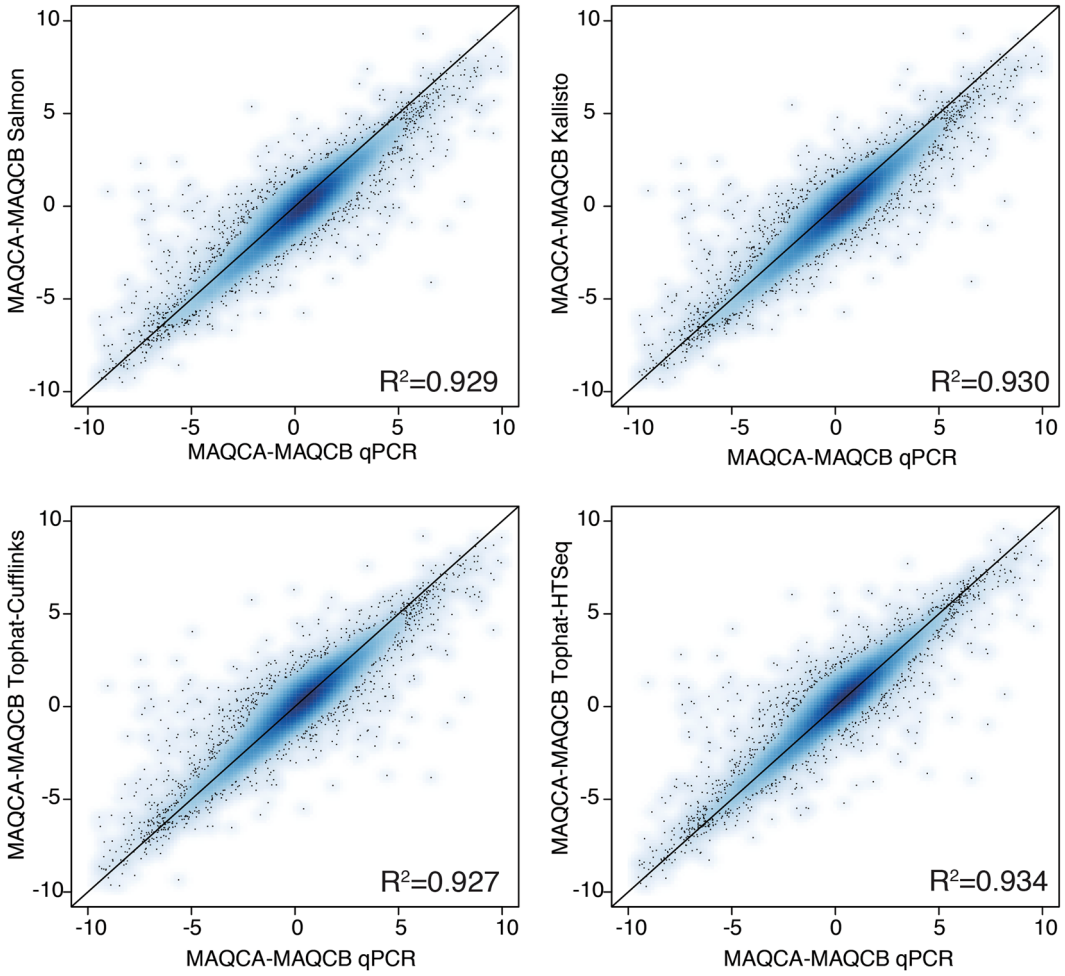
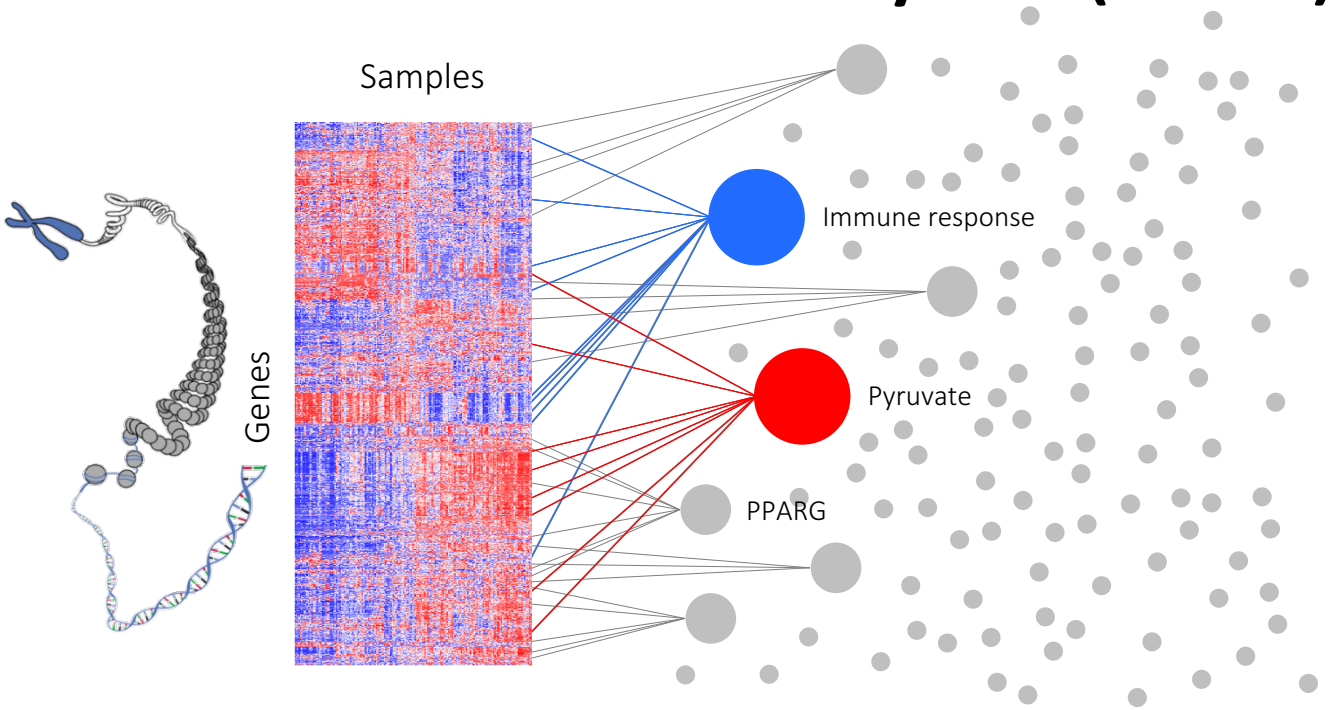


Figure 1. Gene expression correlation between RT-qPCR and RNA-seq data. The Pearson correlation coefficients and linear regression line are indicated. Results are based on RNA-seq data from dataset 1.

Most problems are consistent so they disappear when you do diff-exp analysis



Gene-set analysis (GSA)

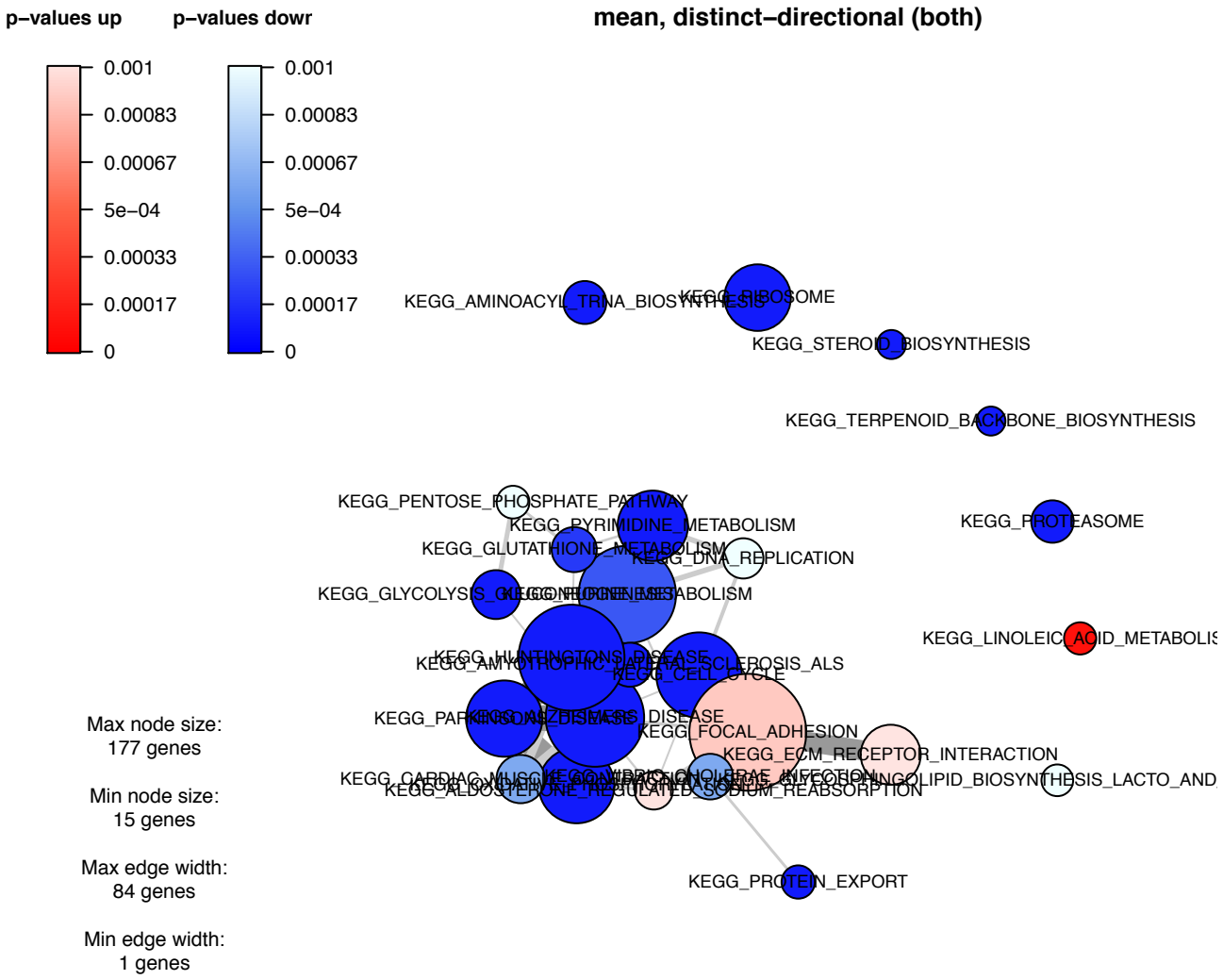


GO-terms
Pathways
Chromosomal locations
Transcription factors
Histone modifications
Diseases
etc…

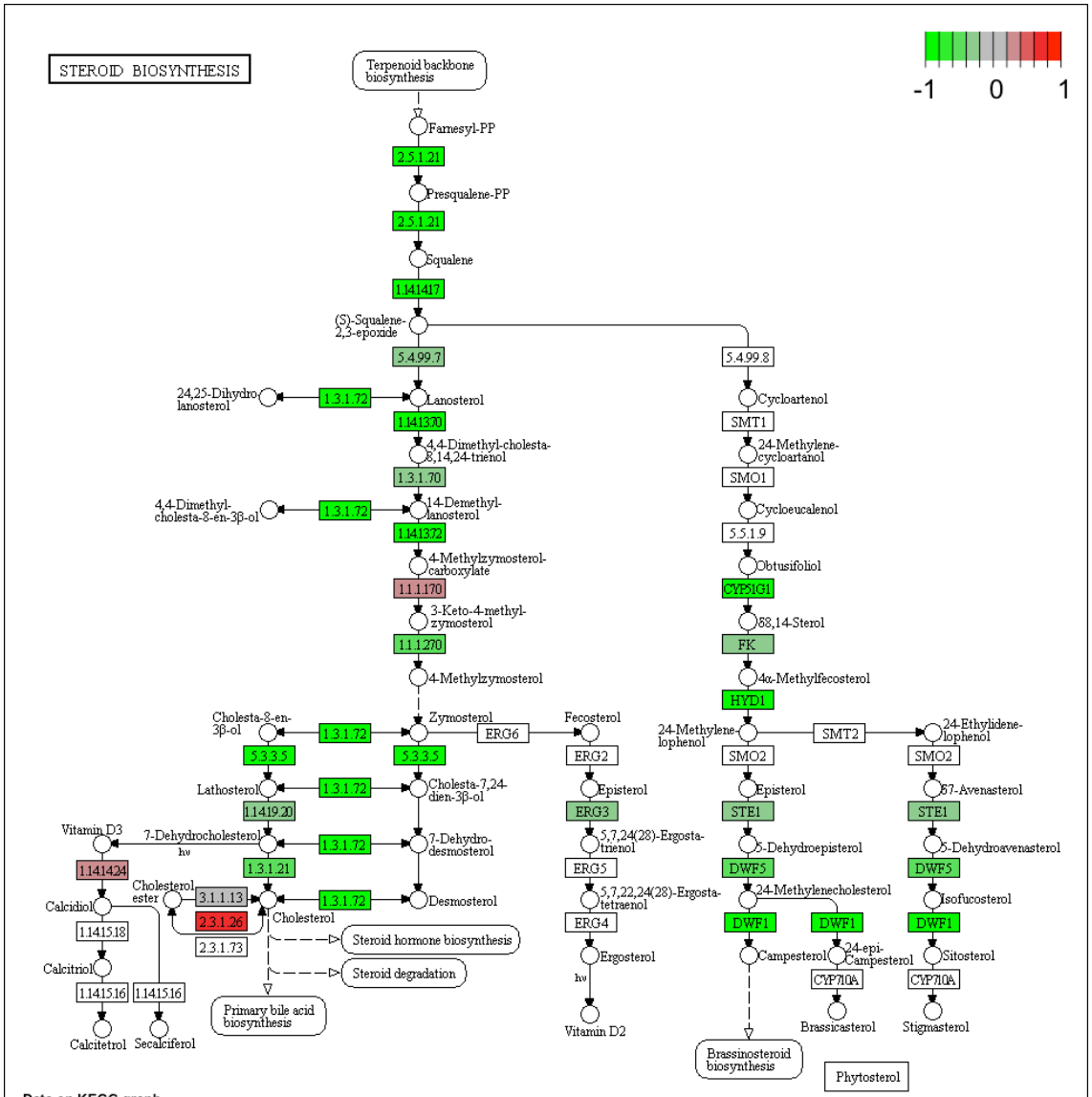
Gene-level data → Gene-set analysis → Gene-set data (results)

We will focus on transcriptomics and differential expression analysis
However, GSA can in principle be used on all types of genome-wide data.

Analysis regarding Type II Diabetes



Expression of genes on pathway



Exercises

- Mapping
  - STAR
  - HISAT2
- Tutorial for reference guided assembly
  - Cufflinks
  - Stringtie
- Tutorial for de novo assembly
  - Trinity
- Visualise mapped reads and assembled transcripts on reference
  - IGV
- RNA quality controll
  - Tutorial for RNA seq Quality Control
- Differential expression analysis
  - DEseq2
  - Calisto and Sleuth
  - multi variate analysis in SIMCA
- small RNA analysis
  - miRNA analysis

- **Introductory**
  - Introduction to the RNA seq data provided
  - Short introduction to R
  - Short introduction to IGV
- **Beta labs**
  - Single cell RNA PCA and clustering
  - Gene set analysis
- **UPPMAX**
  - sbatch script example

Need help??

- We are here for you. Apply for help.