Special Topics in Biostatistics and Bioinformatics
Week IX

Ege Ülgen, M.D., Ph.D.

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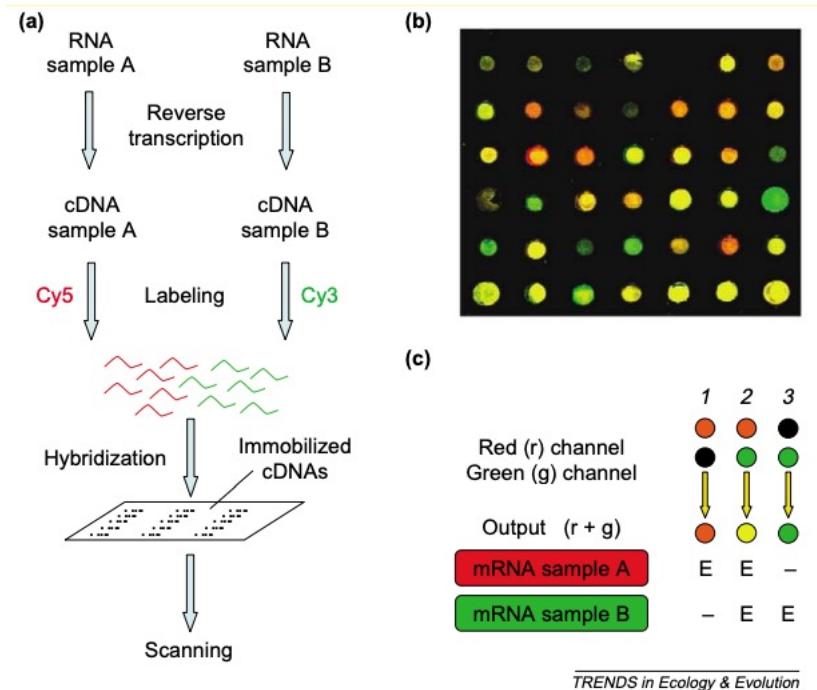
Gene Expression Analysis

- **Microarray**
- RNAseq

Microarray Analysis Workflow

(a) RNA sample A / RNA sample B → Reverse transcription → cDNA sample A / cDNA sample B → Cy5 / Labeling / Cy3 → Hybridization / Immobilized cDNAs → Scanning

(b)

(c) Red (r) channel / Green (g) channel / Output (r + g) / mRNA sample A / mRNA sample B

TRENDS in Ecology & Evolution

- Image Analysis
- Raw Data
- Diagnostic Plots
- Normalization
- Filtering
- Differential gene expression analysis
- Annotation*

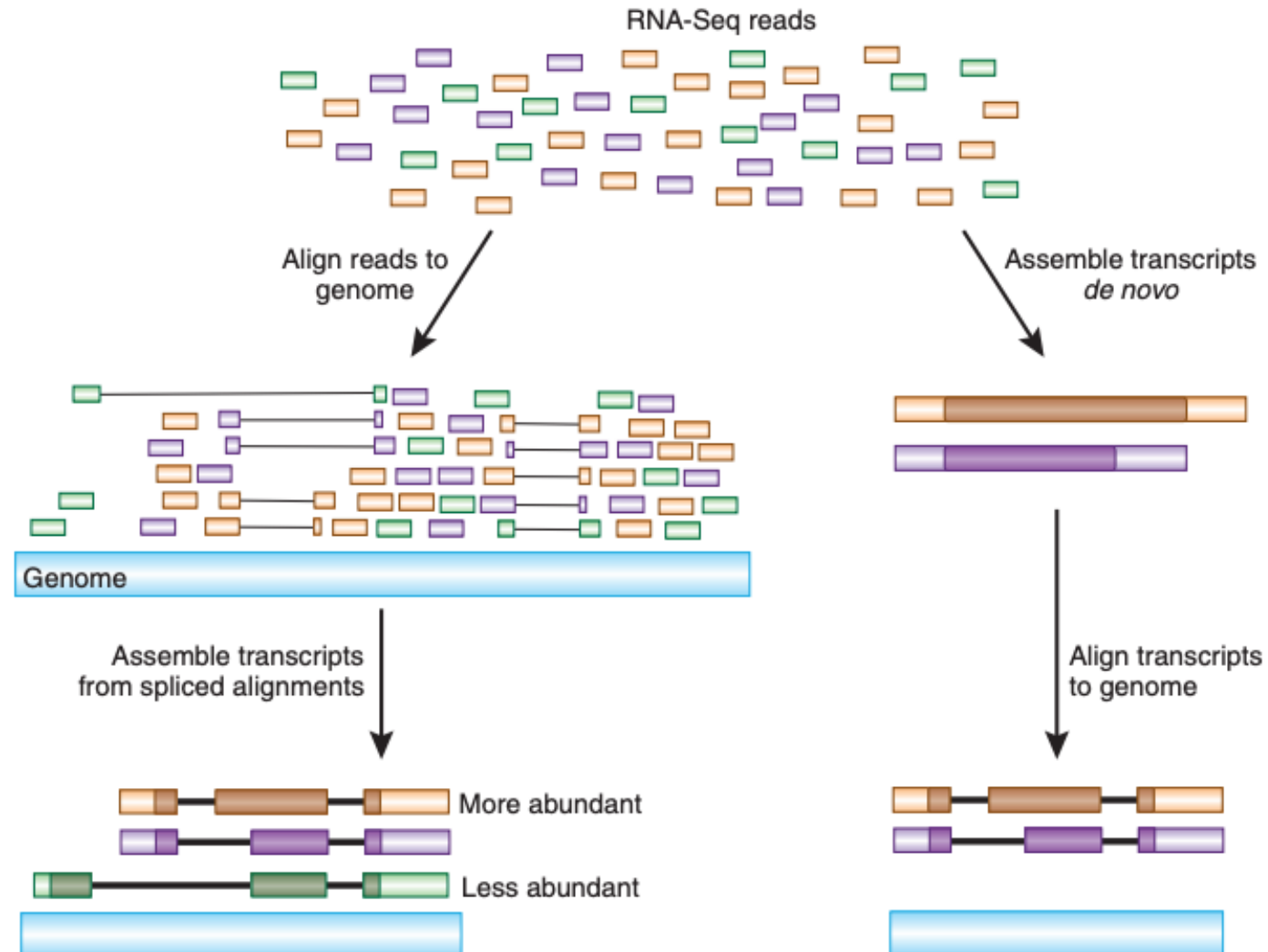# Microarray Analysis Workflow
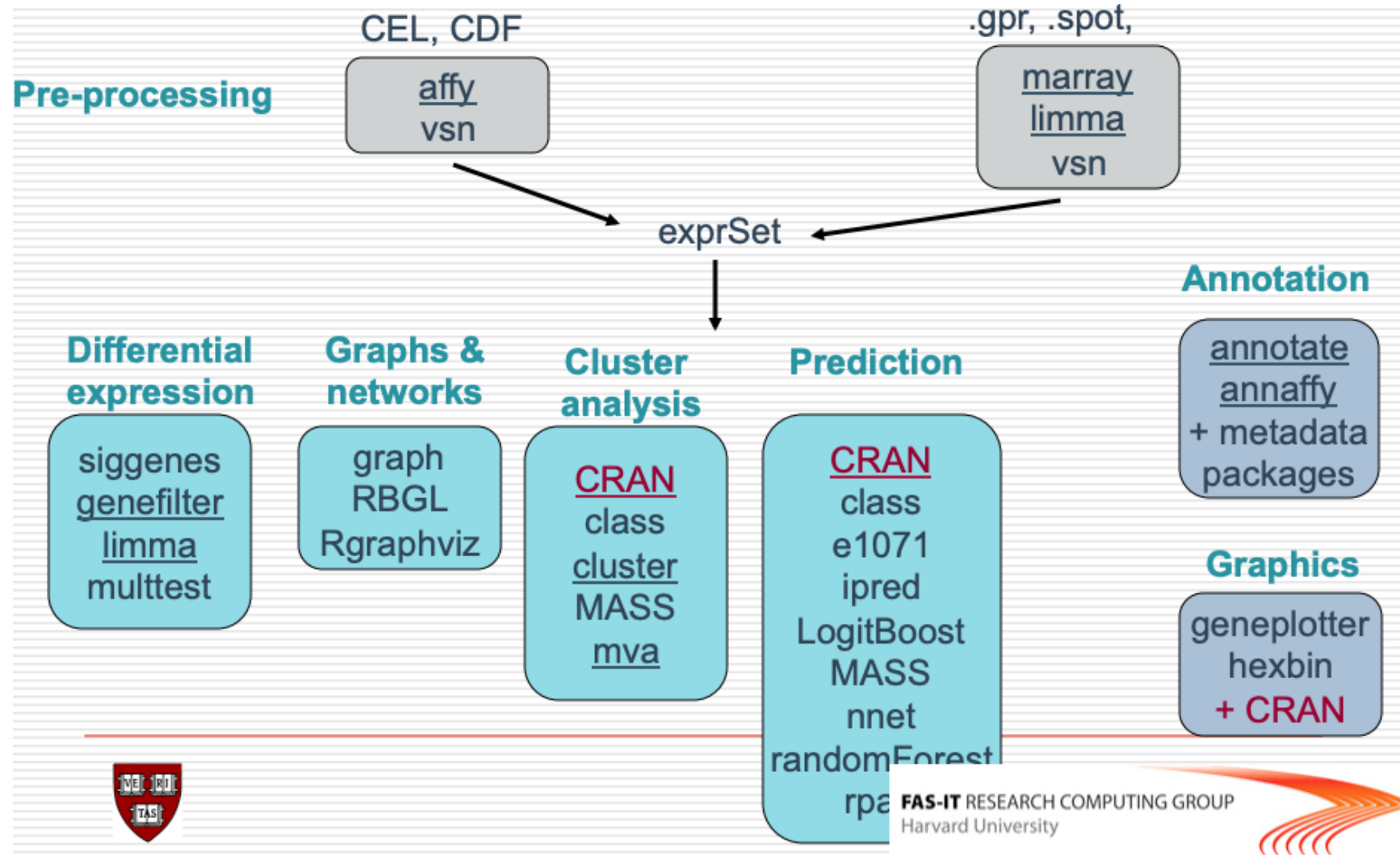
Gene Expression Analysis

- Microarray
- RNAseq

RNAseq Analysis Steps

1. Align
2. Count/Quantify
3. Normalize
4. Statistical Test

Align

STAR
hisat2
Rail
Tophat2

Trinity
SPAdes
Trans-
ABySS
Bridger

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

Count/Quantify

- SALMON
- HTSeq
- featureCounts
- Kallisto
- derfinder

Assemble and Quantify

- StringTie
- Cufflinks
- Trinity
- RSEM

Normalize

- DESeq2/edgeR
- EDAseq
- Cqn
- Ballgown
- derfinder

Statistical Tests

- limma
- DESeq2/edgeR
- Ballgown
- derfinder