Introduction to RNA-seq analysis

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Piotr Gawroński

Euroleague FOR LIFE SCIENCES

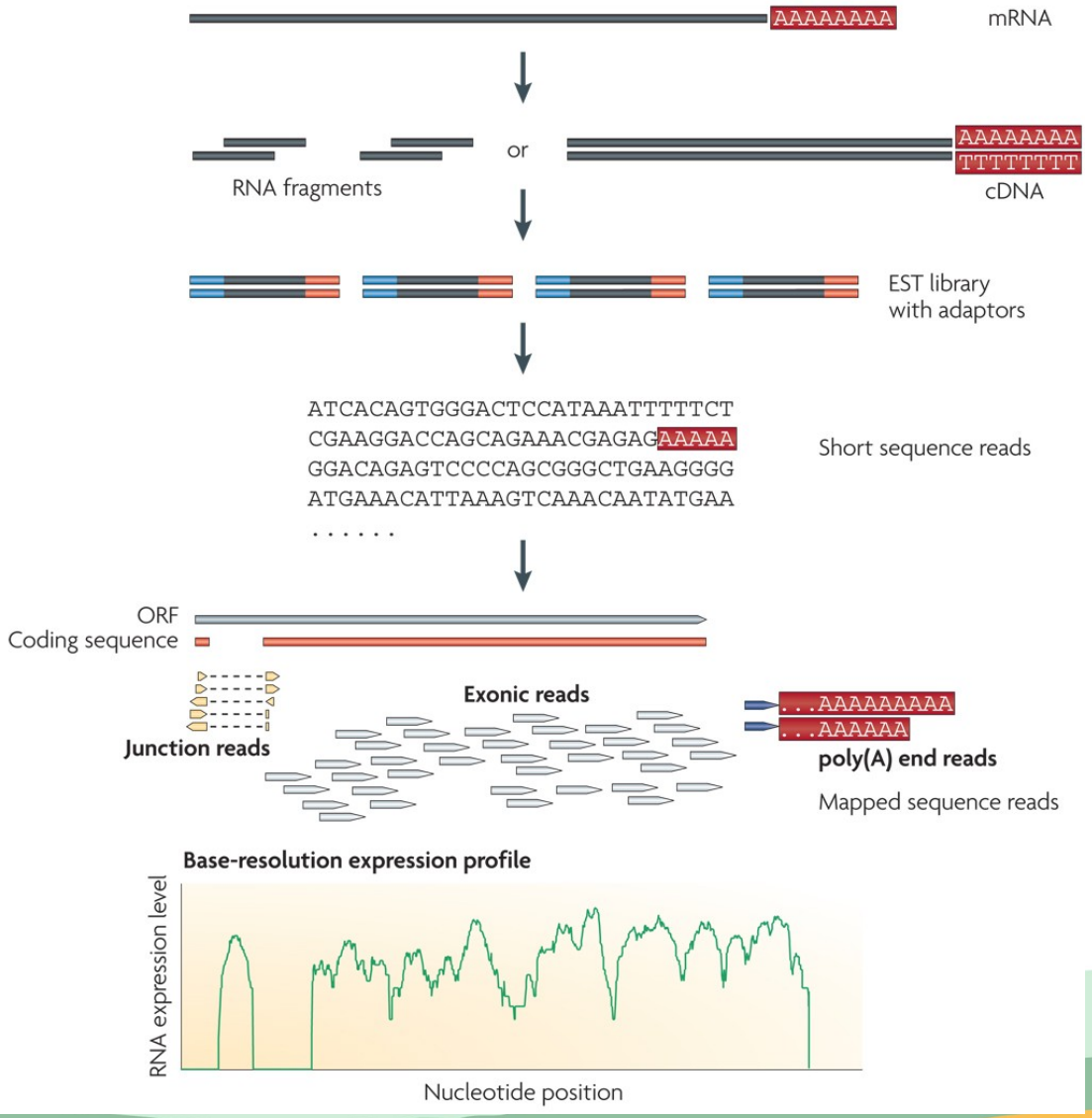
WARSAW UNIVERSITY OF LIFE SCIENCES - SGGW

- Part 1 (mRNA): Piotr, ~1.5 h
- Part 2 (miRNA): Marek, ~2.5 h

RNA-seq

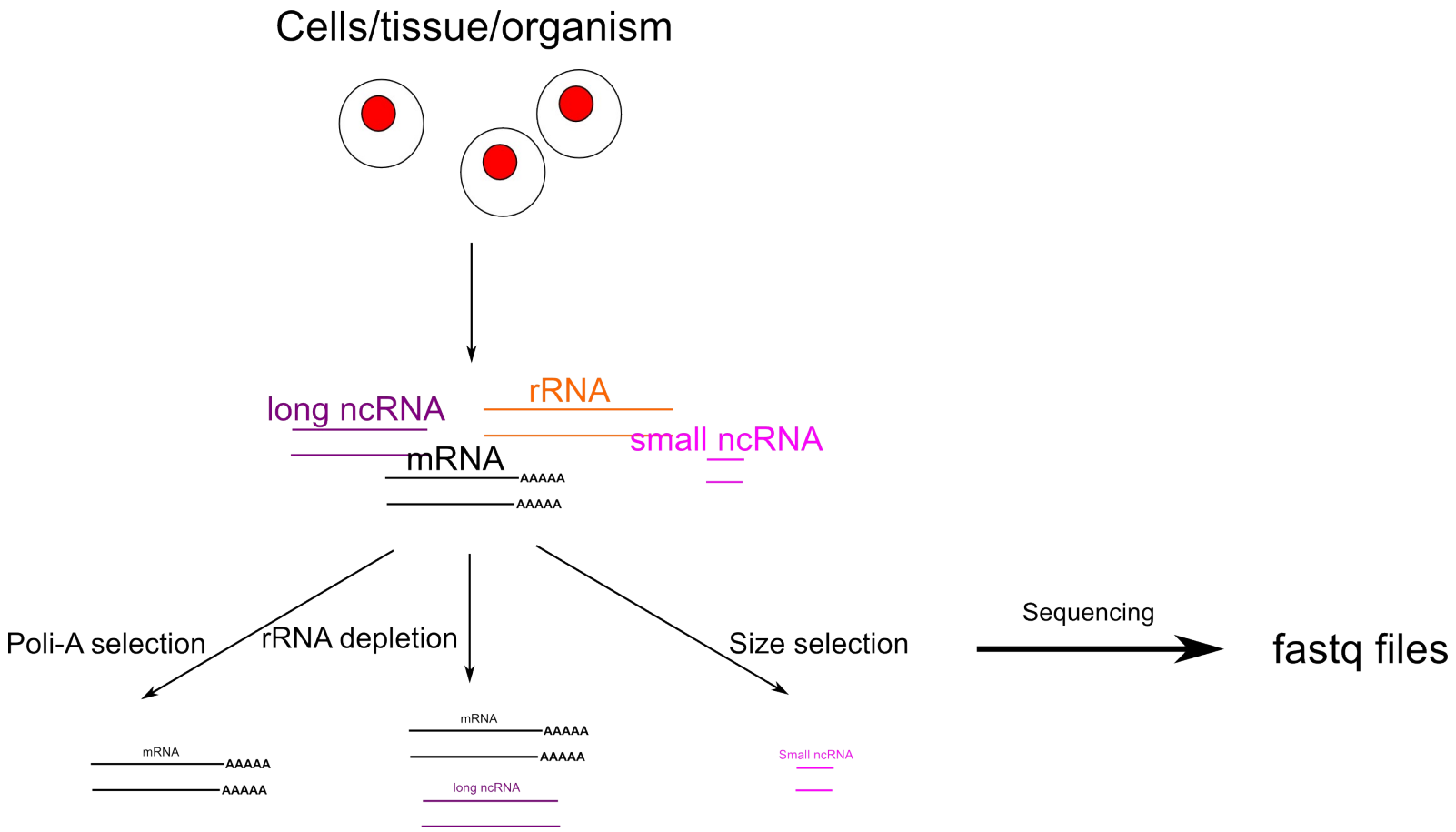
- Comprehensive view of the transcriptome
- Higher sensitivity and increased dynamic range than microarrays
- Possible detection of SNVs and splicing variants
- Data analysis being actively developed → multiple algorithms and programs

Typical RNA-Seq experiment



Wang et al., 2009; 10.1038/nrg2484

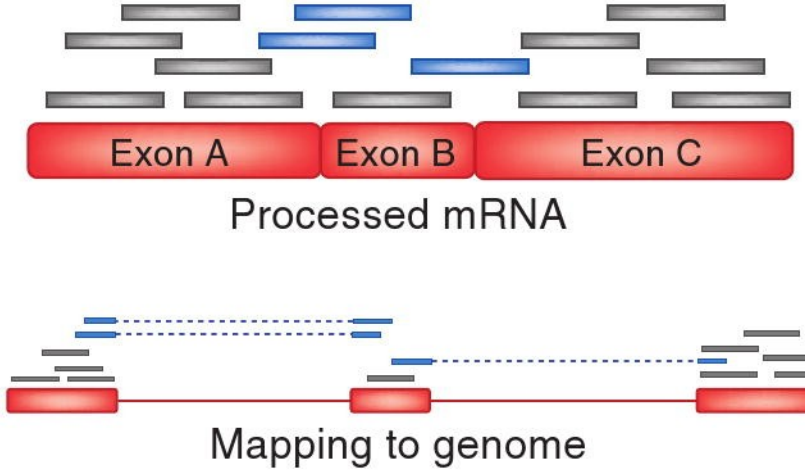
RNA types


Cells/tissue/organism
long ncRNA
rRNA
small ncRNA
mRNA
AAAAA
AAAAA
Poli-A selection
rRNA depletion
Size selection
Sequencing
fastq files
mRNA
AAAAA
AAAAA
mRNA
AAAAA
AAAAA
long ncRNA
Small ncRNA

Fastq files

```
@FCH35MWBBXX:6:1101:3365:1562#0/1 t5254543
TGACTGTGACAGGACCACCTAGAGATGCTGTTGGAATGAGTAGAATTCT
+
`[[e[[``KV[K``VVejjjVVeVV[VKVKV[KVee[KKK`KKV[VeVV
```

- Fastq files contain read sequence and quality
- Huge files (20 mio reads, 2x100 bp: ~10 Gb)
- Need efficient bioinformatics software



Data analysis
(starting from fastq)

1. Trimming adapter sequences (*TrimGalore!*)

2. Read quality check (*FastQC*)

3. Mapping (*TopHat2*, *STAR*)

4. Counting reads and DGE analysis (*DESeq2*, *edgeR*)

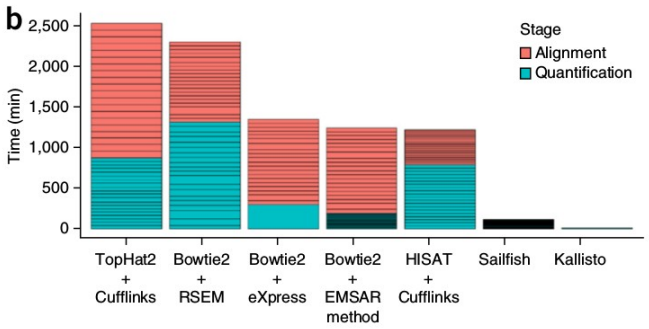
Hours/days!

Data analysis
(starting from fastq)

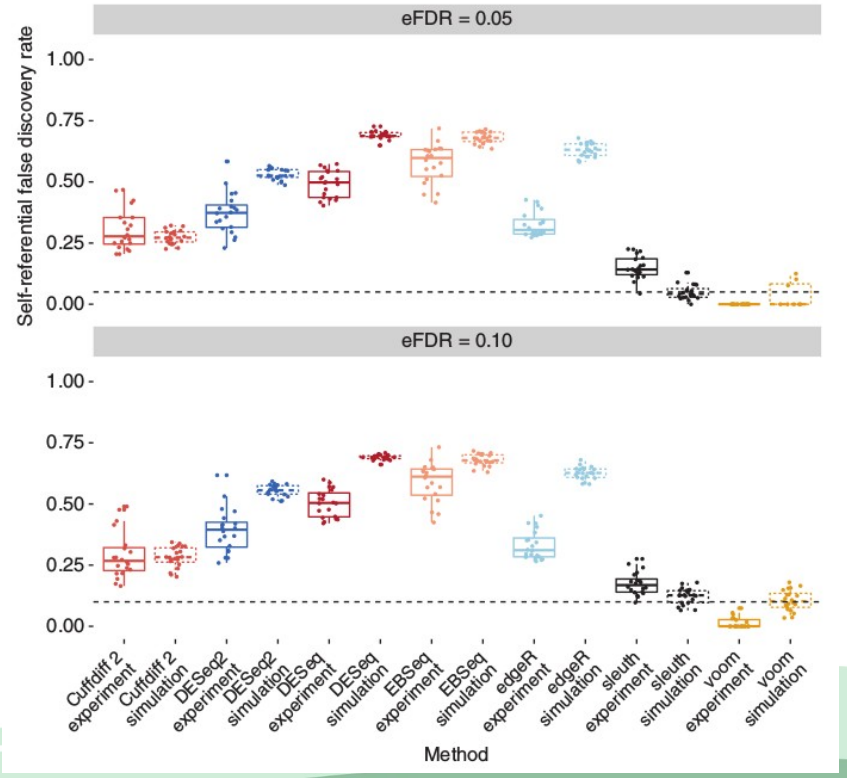
1. Build and index (*kallisto*)

2. Quantify reads against the index (*kallisto*)

3. Analyze results – DGE (*sleuth*)



Bray et al., 2015; 10.1038/nbt.3519



Pimentel et al., 2017; 10.1038/nmeth.4324

Our plan

1. Build and index (*kallisto*)
2. Quantify reads against the index (*kallisto*)
3. **Analyze results – DGE (*sleuth*)**

Experimental setup

Folder name	Treatment	Type	Organism
col_LL_01	cL	mRNA	Arabidopsis
col_LL_02	cL	mRNA	Arabidopsis
col_LL_03	cL	mRNA	Arabidopsis
col_HL_01	HL	mRNA	Arabidopsis
col_HL_02	HL	mRNA	Arabidopsis
col_HL_03	HL	mRNA	Arabidopsis

Aim: find regulated transcripts (HL vs. cL)

http://pachterlab.github.io/kallisto/

http://pachterlab.github.io/sleuth/

Questions?

Thank you for your attention!