Introduction to read alignment pipelines and gene expression estimates

The Counts They Are a-Changin'

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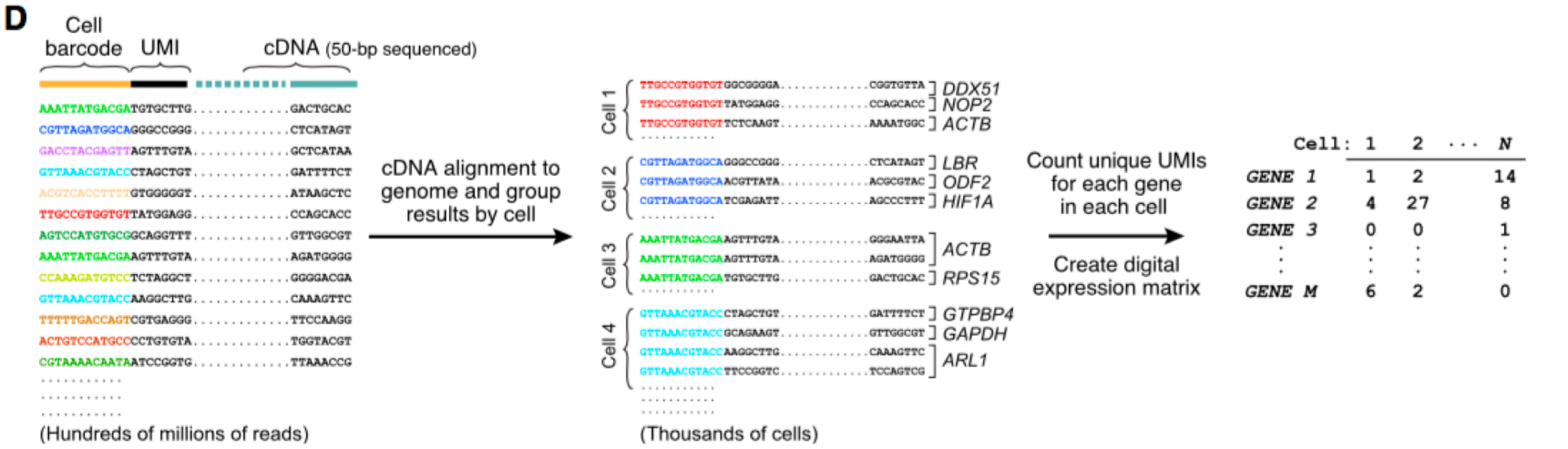
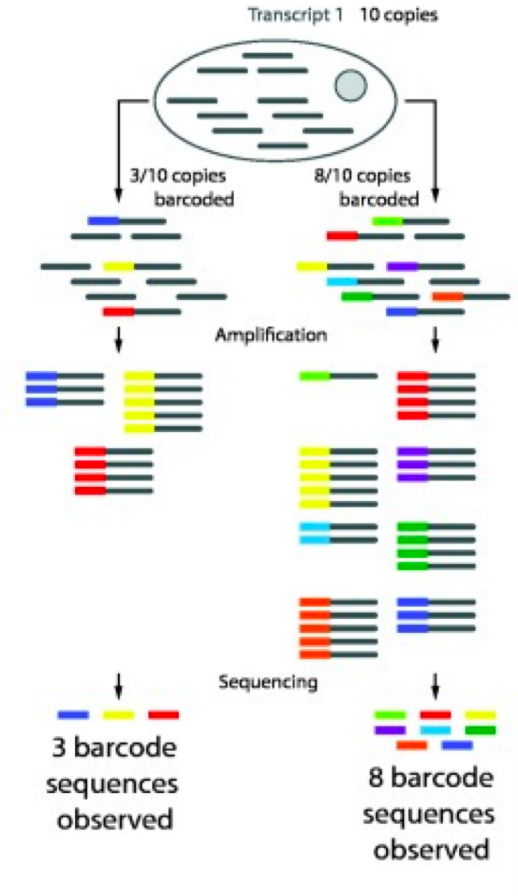
How to get from hundred of million of reads to a count table

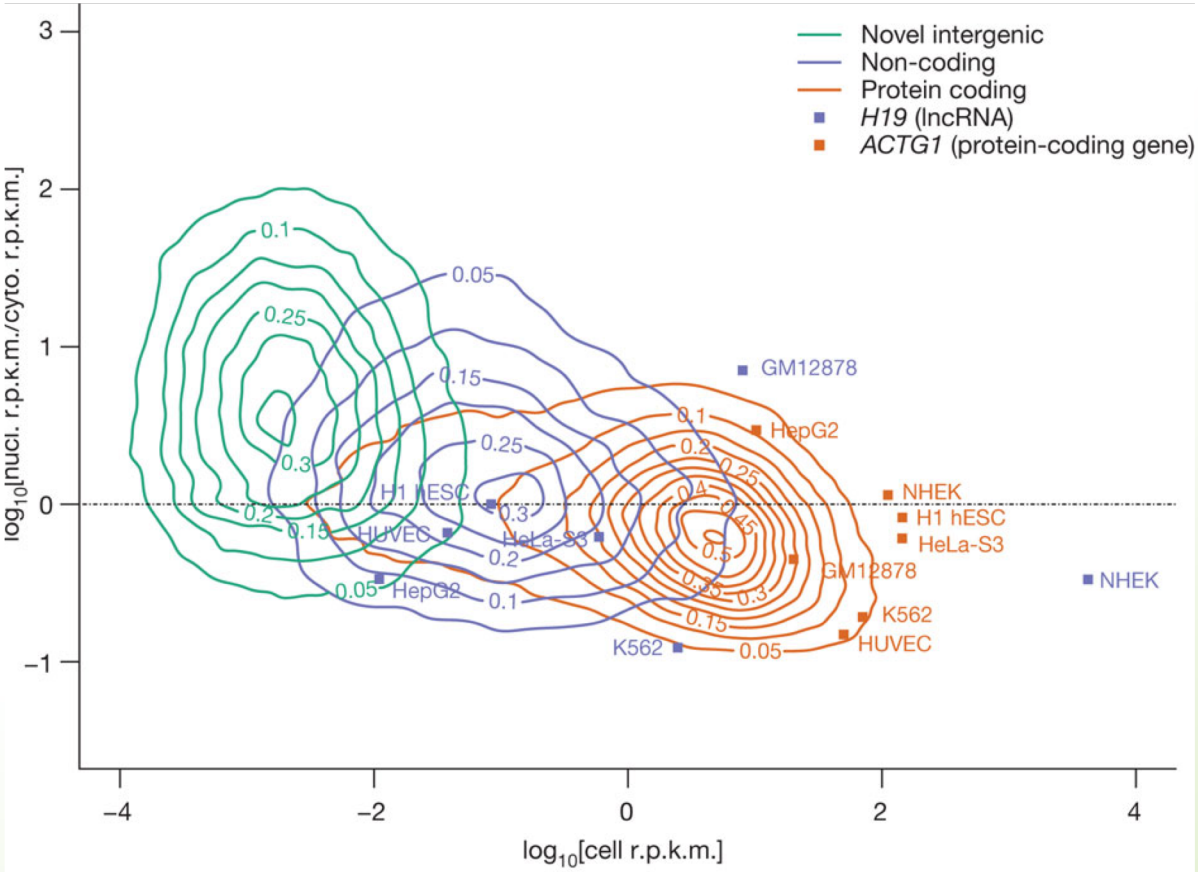


UMI (Unique molecular identifiers) will make sure that one fragment is counted as one read



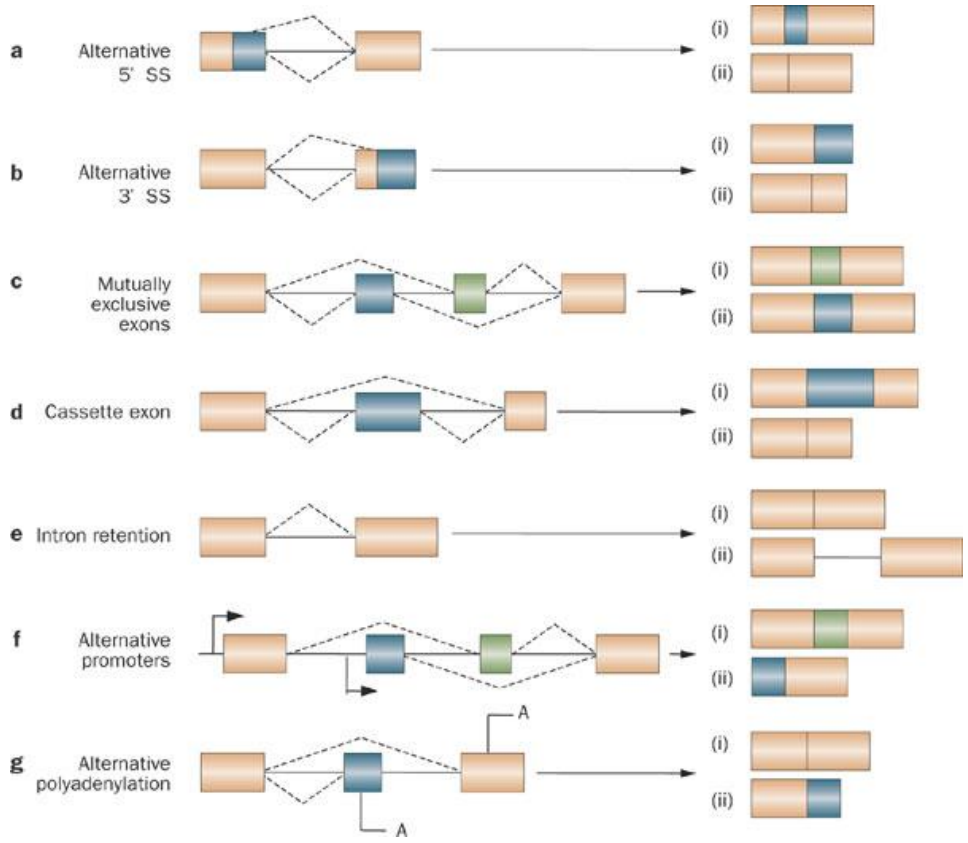
- Will remove erorrs that occur during the amplification step.
- Will not handle sampling bias

Different kind of RNAs have different expression values

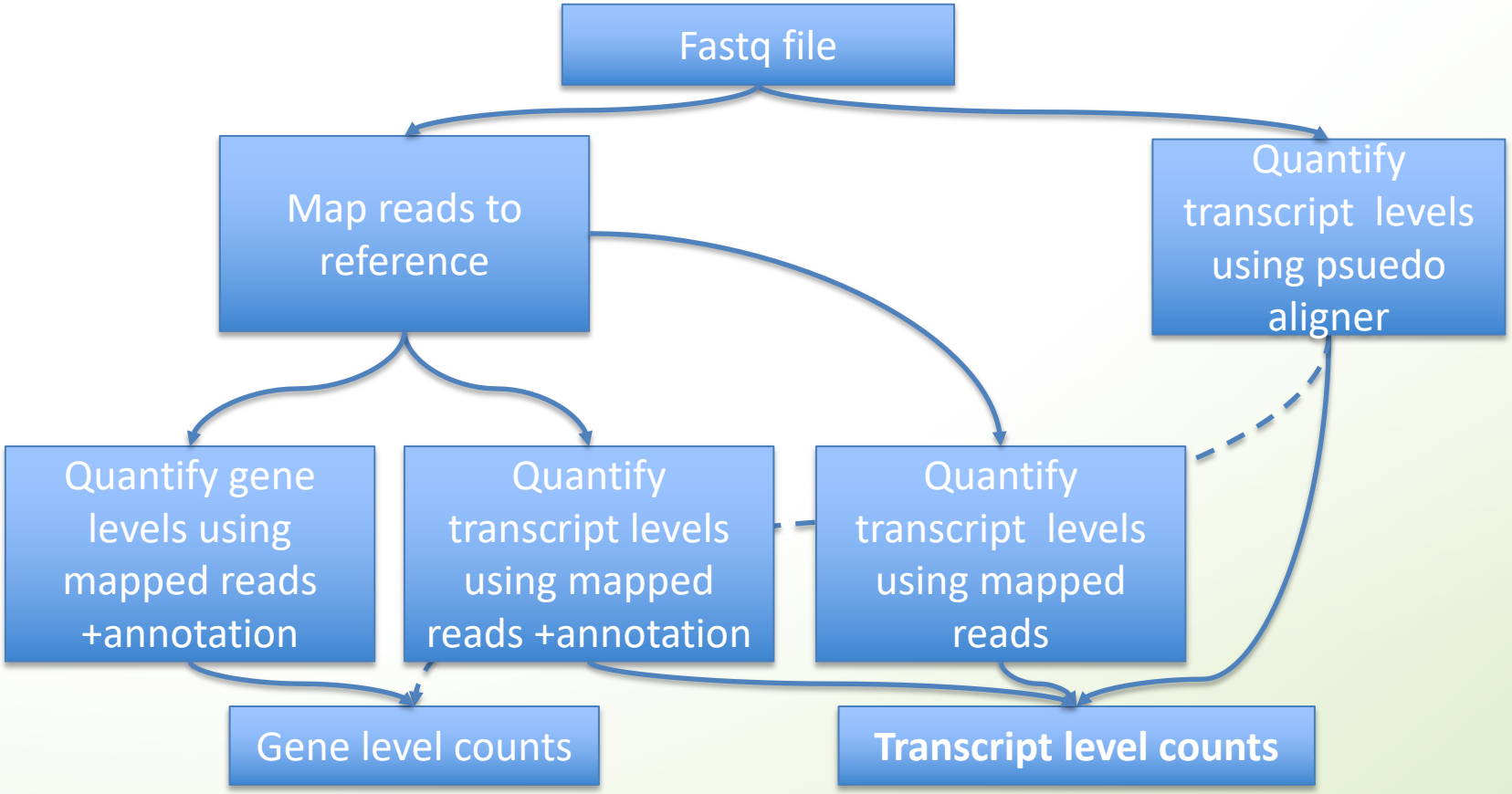


Landscape of transcription in human cells, S Djebali *et al.* ***Nature 2012***

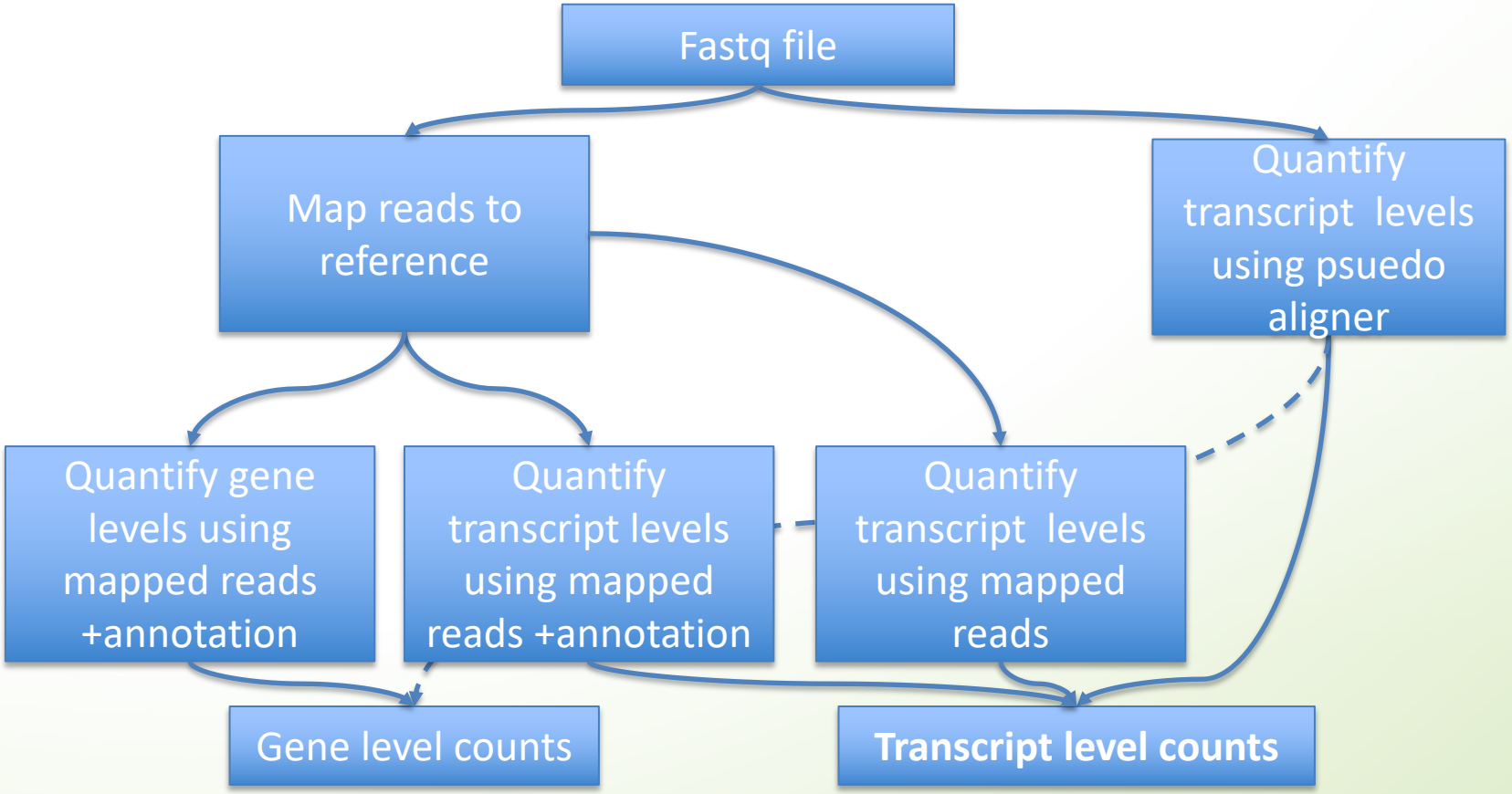
One gene many transcripts



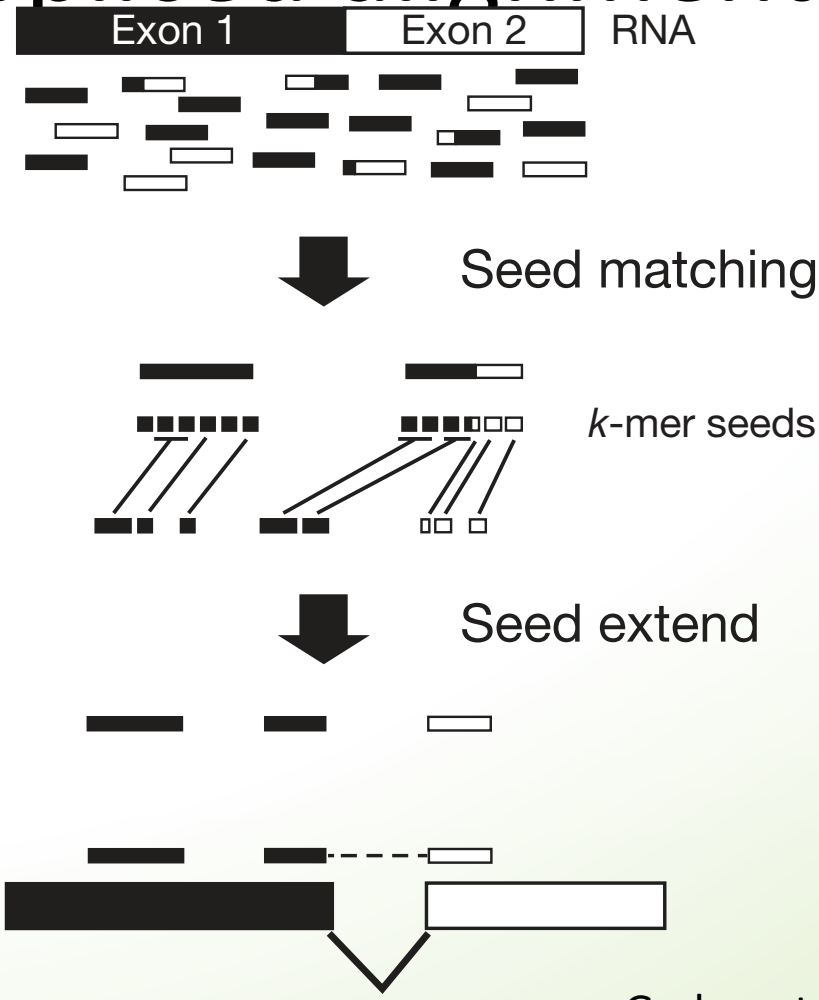
Different paths to get a count table



Good news is that they are all working very well!!



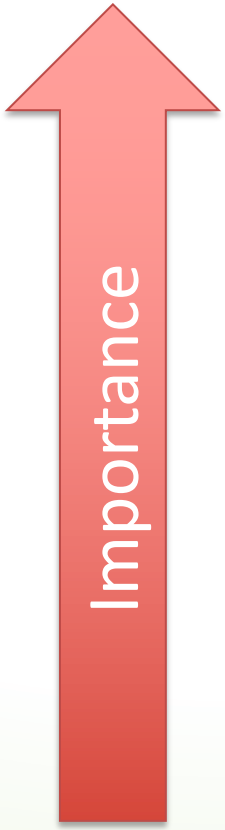
Spliced alignment



Garber et al. *Nature Methods* 2011

How important is mapping accuracy?

Depends what you want to do:



Identify novel genetic variants or RNA editing

Allele-specific expression

Genome annotation

Gene and transcript discovery

Differential expression

Current RNA-seq aligners

TopHat2	Kim et al. *Genome Biology* 2013
HISAT2	Kim et al. *Nature Methods* 2015
STAR	Dobin et al. *Bioinformatics* 2013
GSNAP	Wu and Nacu *Bioinformatics* 2010
OLego	Wu et al. *Nucleic Acids Research* 2013
HPG aligner	Medina et al. *DNA Research* 2016
MapSplice2	http://www.netlab.uky.edu/p/bioinfo/MapSplice2

Compute requirements

Program	Run time (min)	Memory usage (GB)
HISATx1	22.7	4.3
HISATx2	47.7	4.3
HISAT	26.7	4.3
STAR	25	28
STARx2	50.5	28
GSNAP	291.9	20.2
OLego	989.5	3.7
TopHat2	1,170	4.3

Run times and memory usage for HISAT and other spliced aligners to align 109 million 101-bp RNA-seq reads from a lung fibroblast data set. We used three CPU cores to run the programs on a Mac Pro with a 3.7 GHz Quad-Core Intel Xeon E5 processor and 64 GB of RAM.

Kim et al. *Nature Methods* 2015

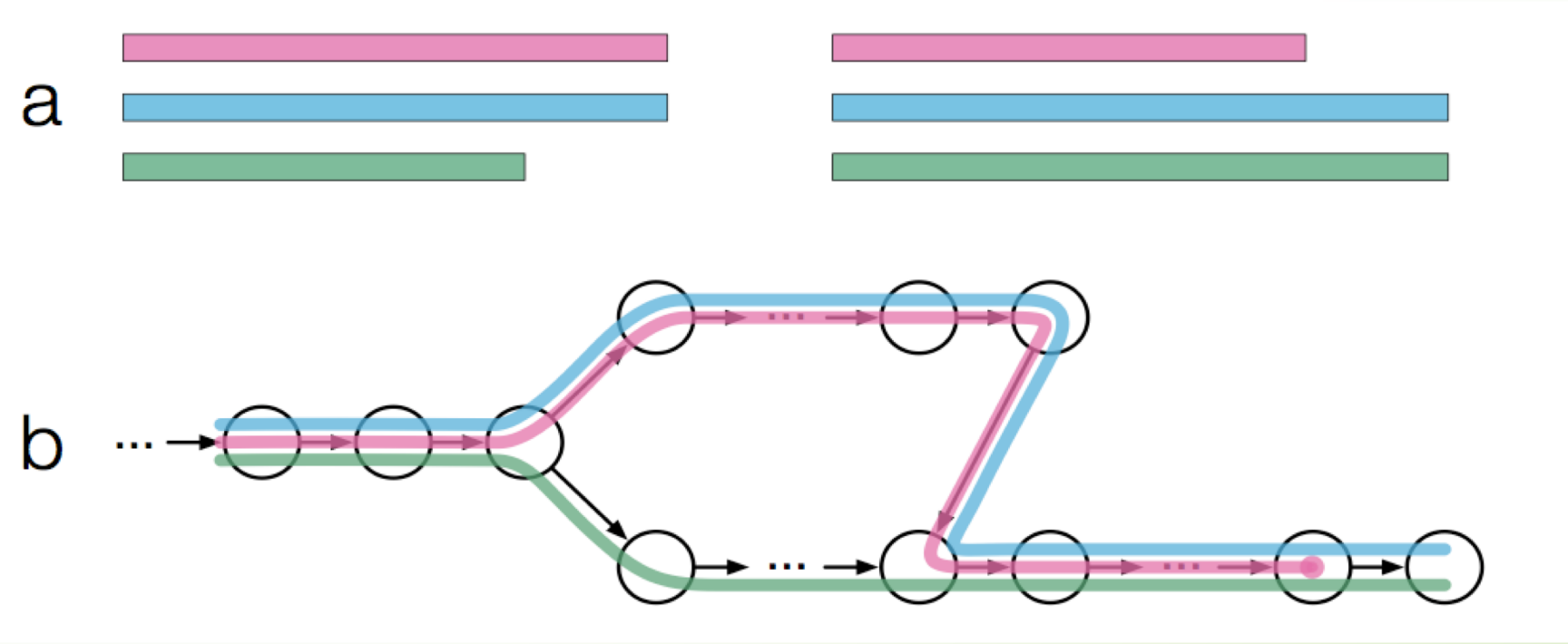
Innovations in RNA-seq alignment software

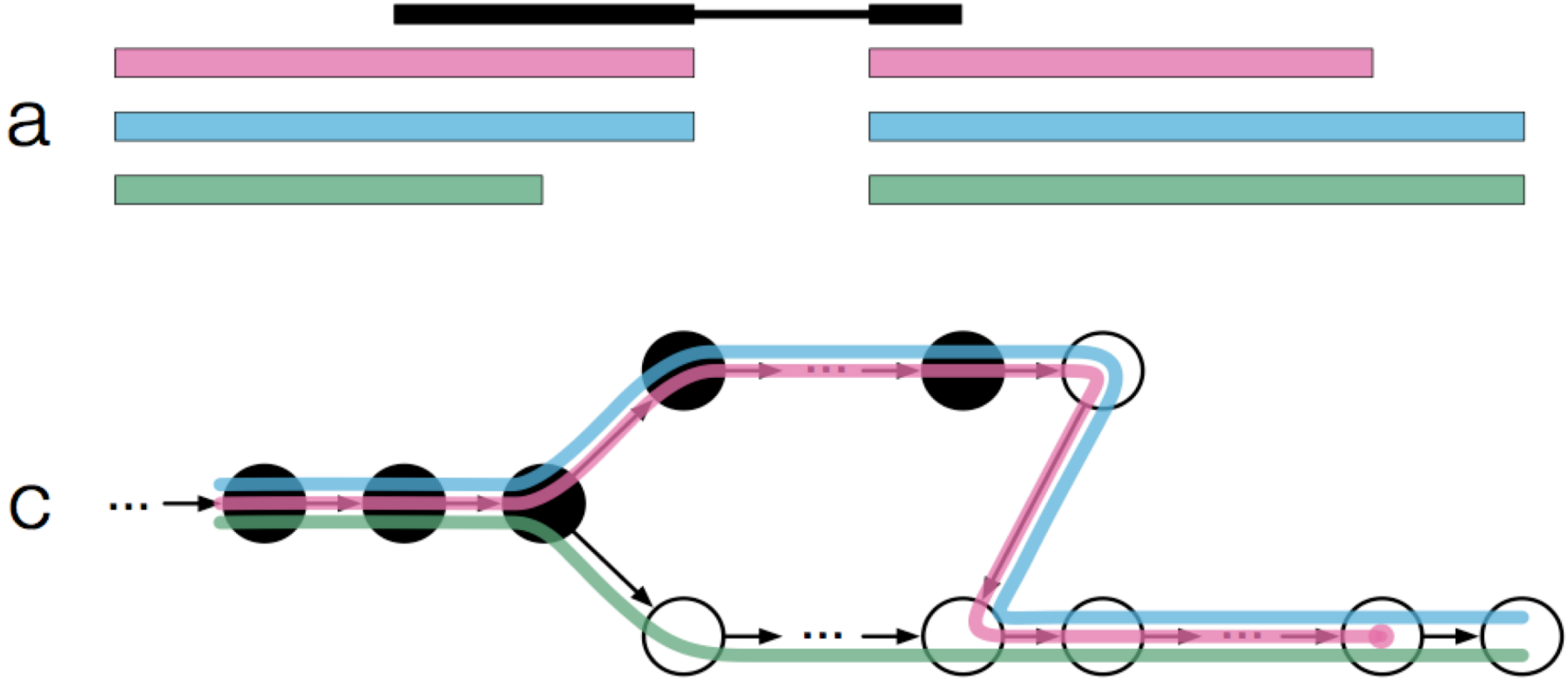
- Read pair alignment
- Consider base call quality scores
- Sophisticated indexing to decrease CPU and memory usage
- Map to genetic variants
- Consider junction annotation
- Two-step approach (junction discovery & final alignment)

Recommendations when using mapping programs

- Use STAR, HISAT2
- STAR and HISAT2 are the fastest
- HISAT2 uses the least memory
- Always check the results!

“Pseudoalignments” in calisto

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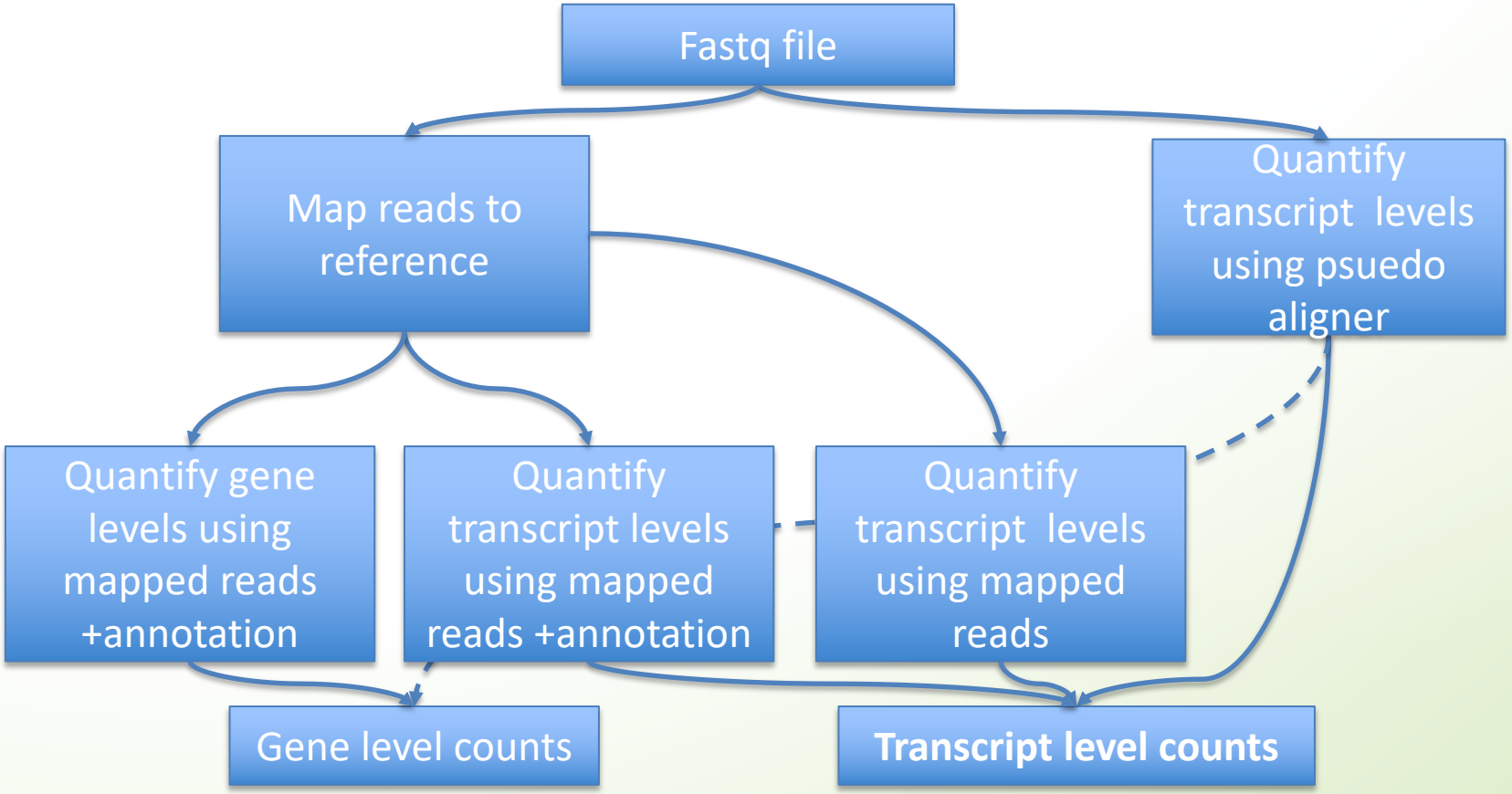
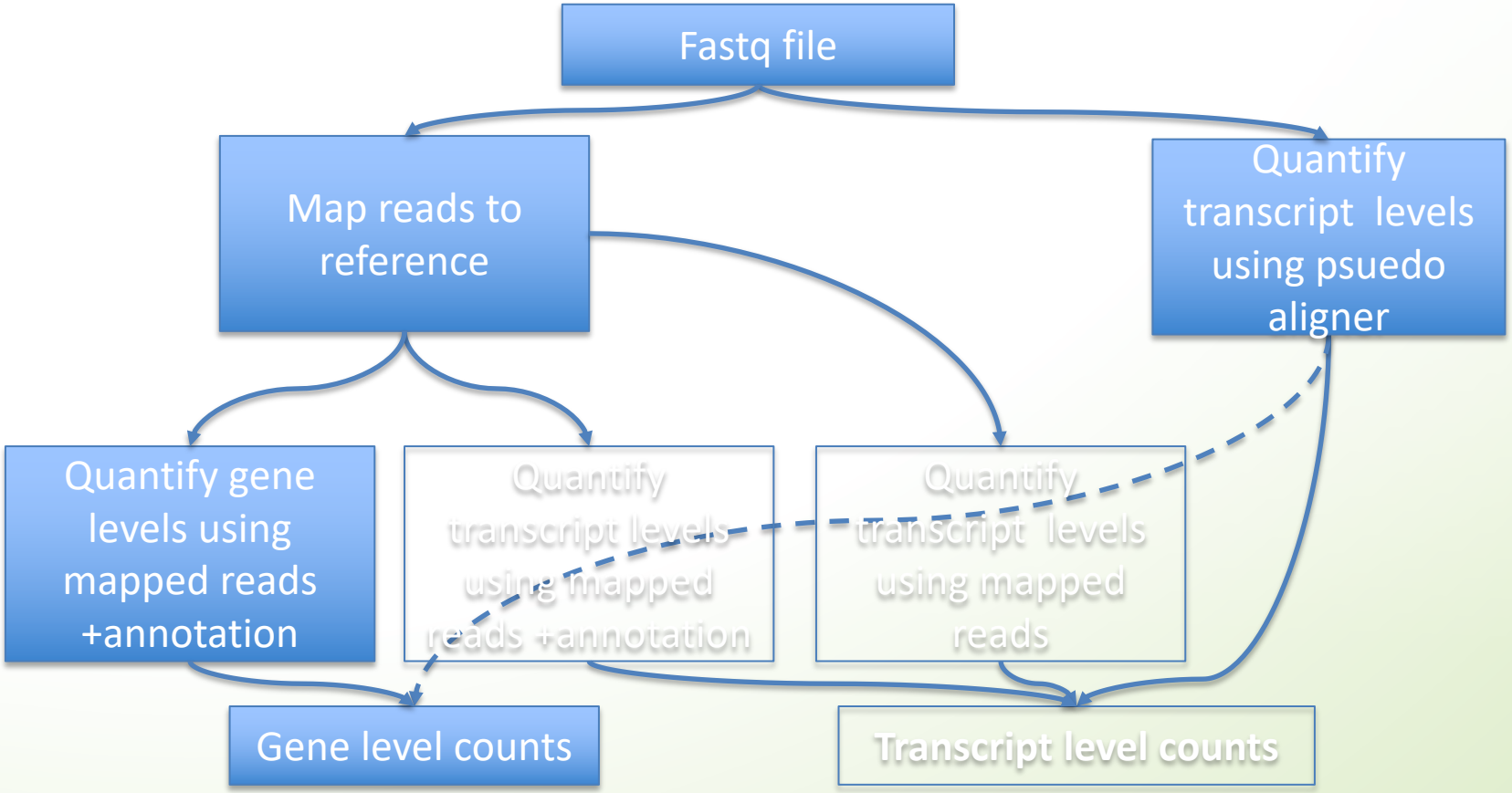
Different paths to get a count table



Gene expression estimates

- Expression estimates on gene level
- Expression estimates on transcript level

Gene level analysis



Gene level analysis

SCIENTIFIC REPORTS

OPEN

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Benchmarking of RNA-sequencing analysis workflows using whole-transcriptome RT-qPCR expression data

Celine Everaert[1,2,3], Manuel Luypaert[4], Jesper L. V. Maag[5], Quek Xiu Cheng[5], Marcel E. Dinger[5], Jan Hellemans[4] & Pieter Mestdagh[1,2,3]

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

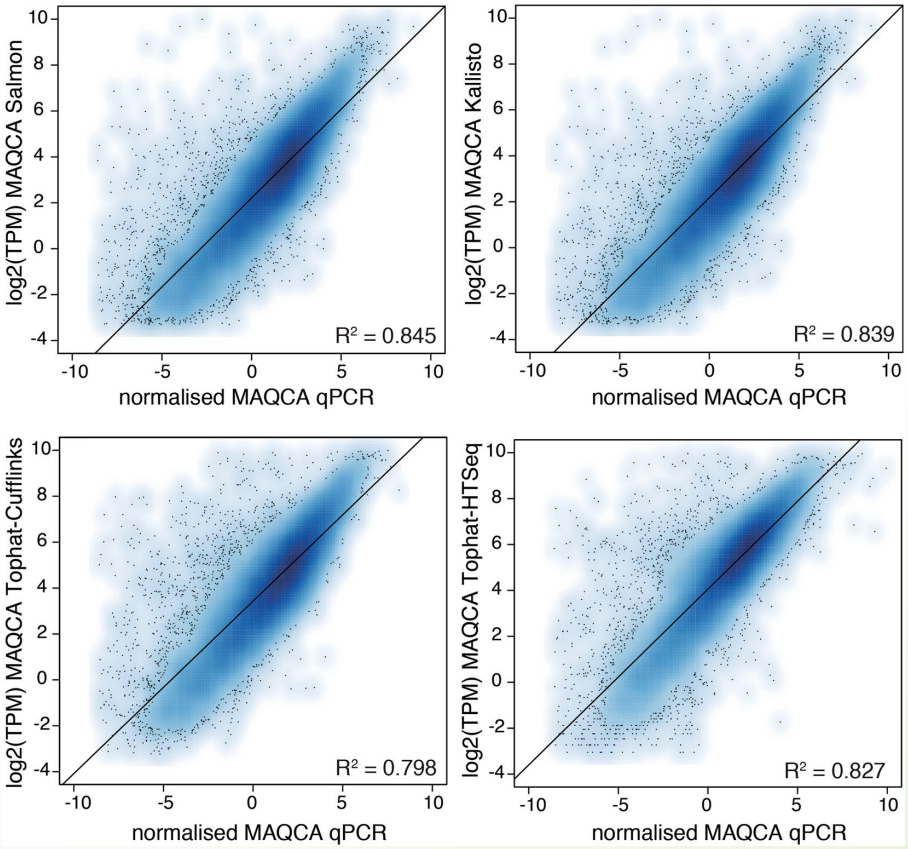
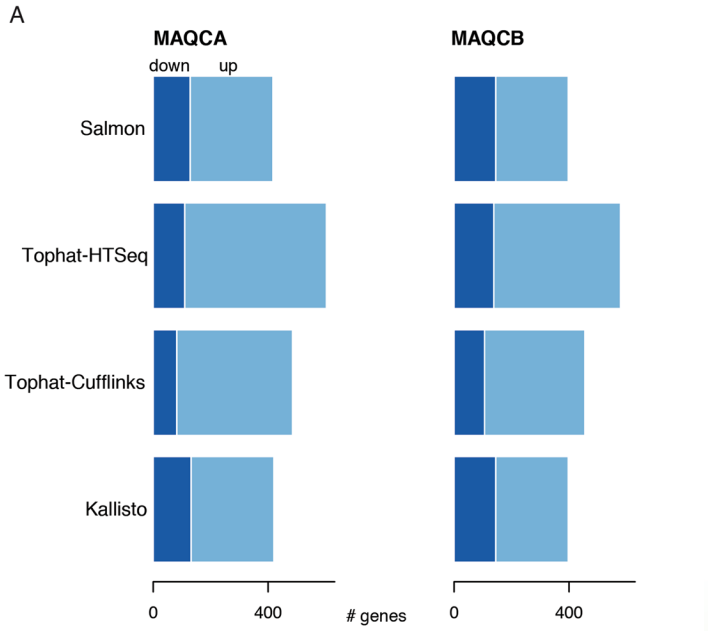
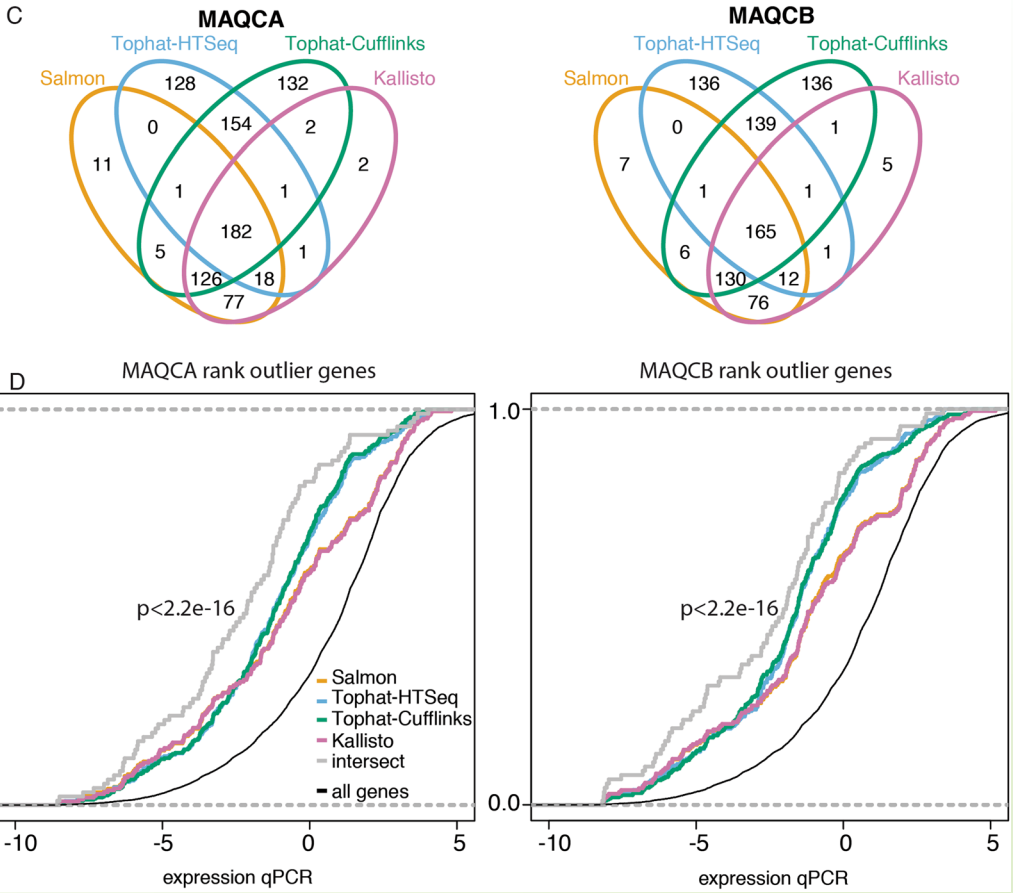


Figure 1. Gene expression correlation between RT-qPCR and RNA-seq data. The Pearson correlation coefficients and linear regression line are indicated. Results are based on RNA-seq data from dataset 1.

Lowly expressed genes are more problematic to identify using RNA seq





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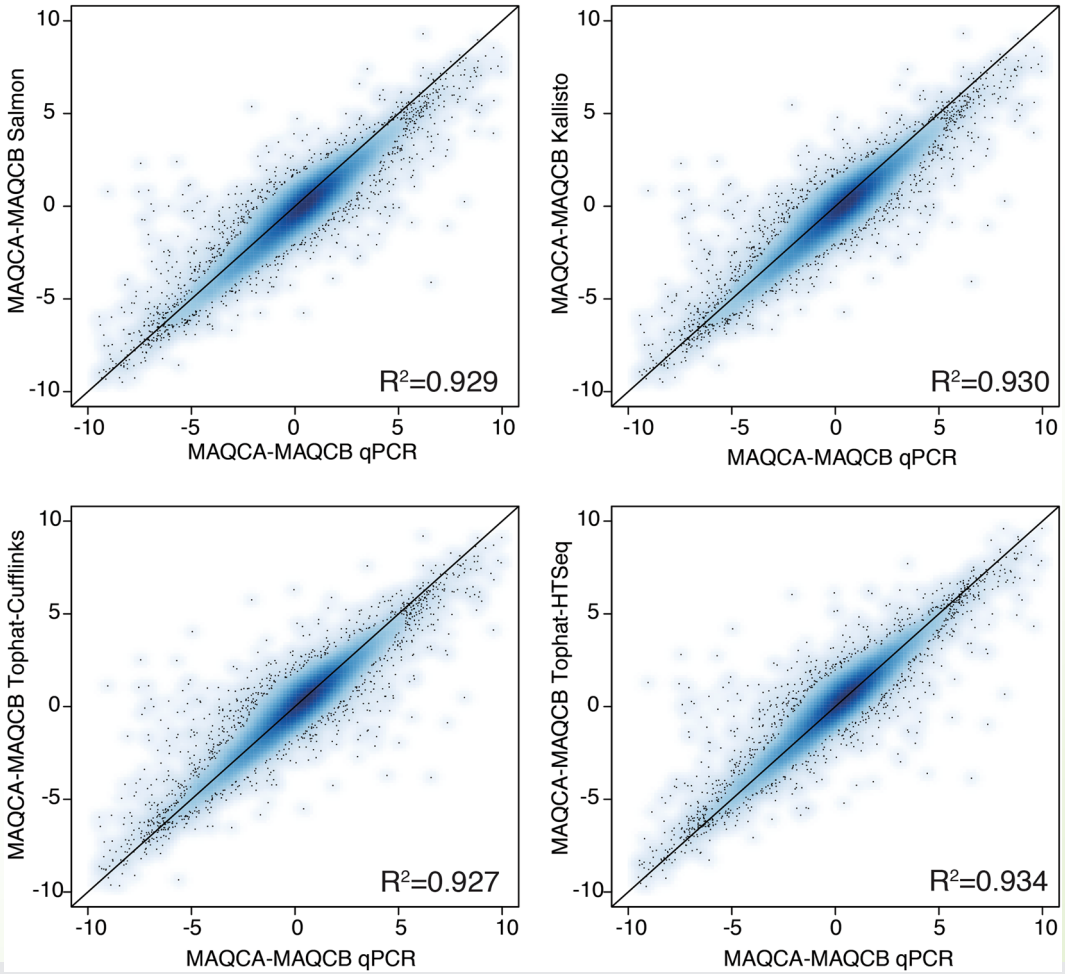
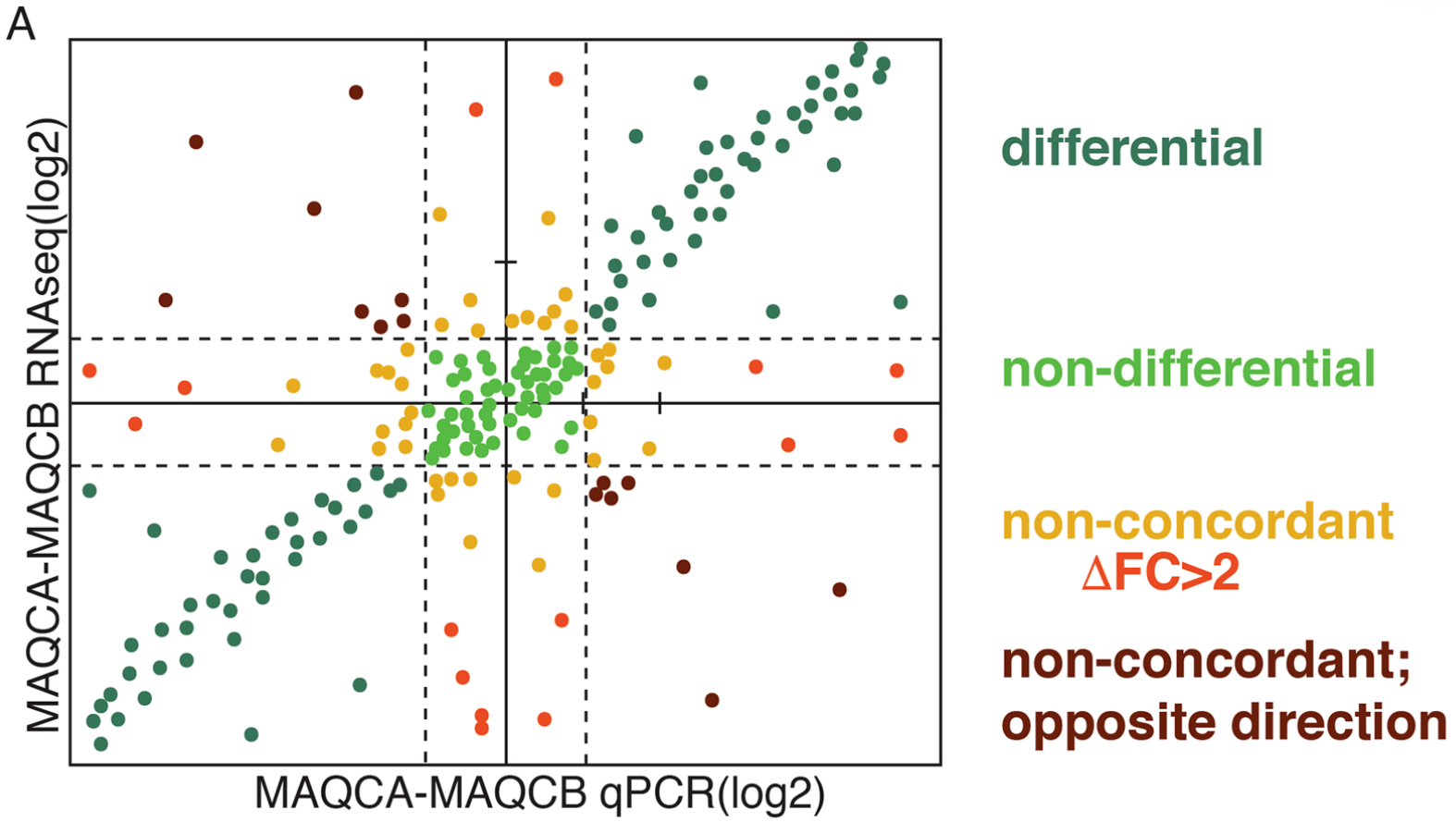
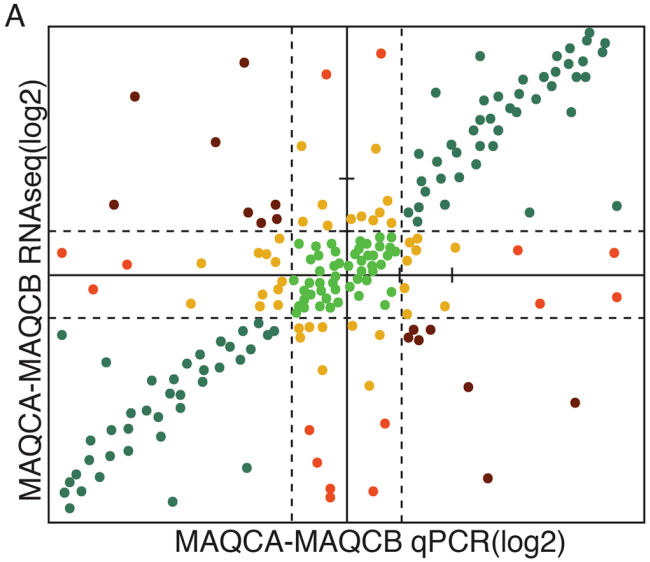
Most problems are consistent so they disappear when you do diff-exp analysis



Toy example of differences between to methods that can arise



Non-concordant results are often found in lowly expressed genes

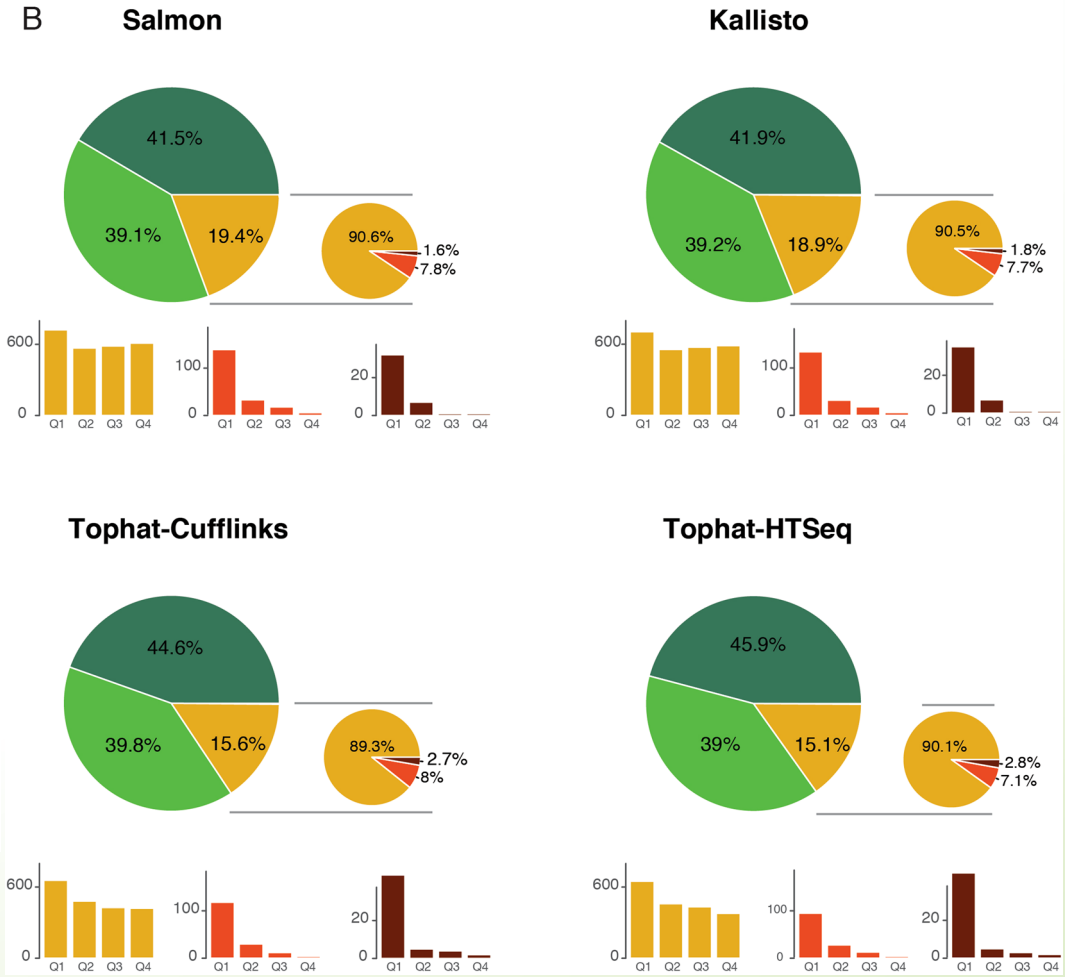


differential

non-differential

non-concordant
ΔFC>2

non-concordant;
opposite direction



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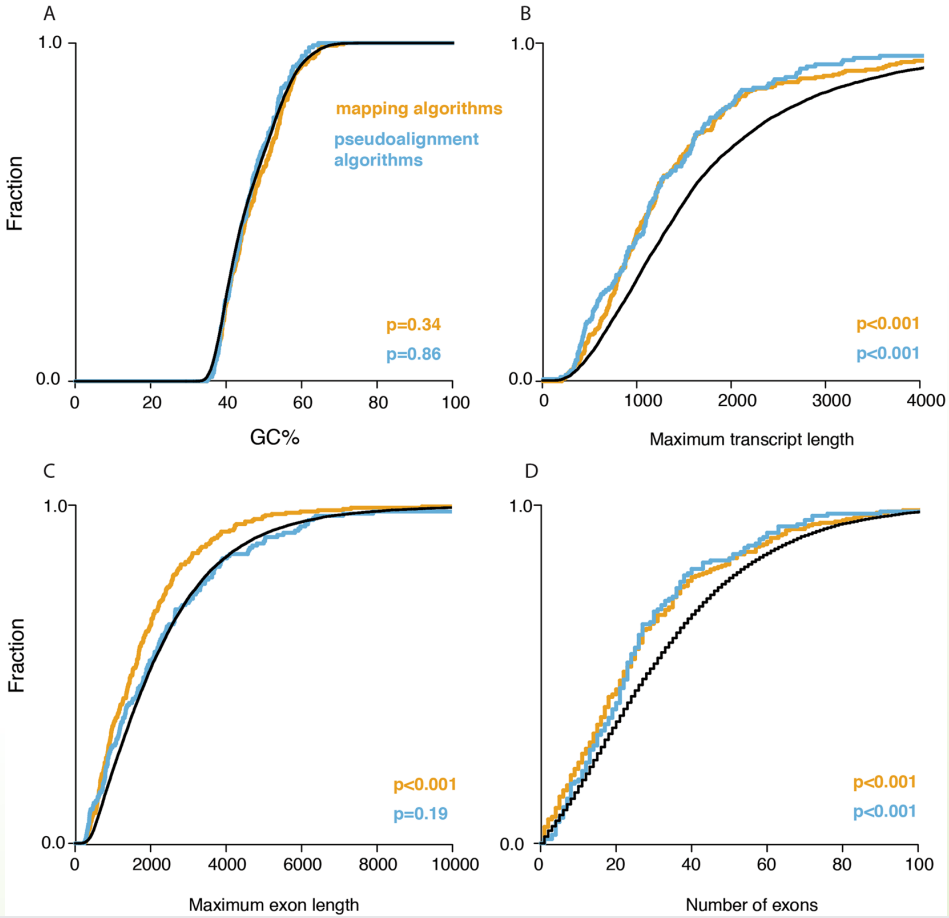
Small transcripts are harder to to get correct values for



Transcript level analysis

Zhang *et al. BMC Genomics* (2017) 18:583
DOI 10.1186/s12864-017-4002-1

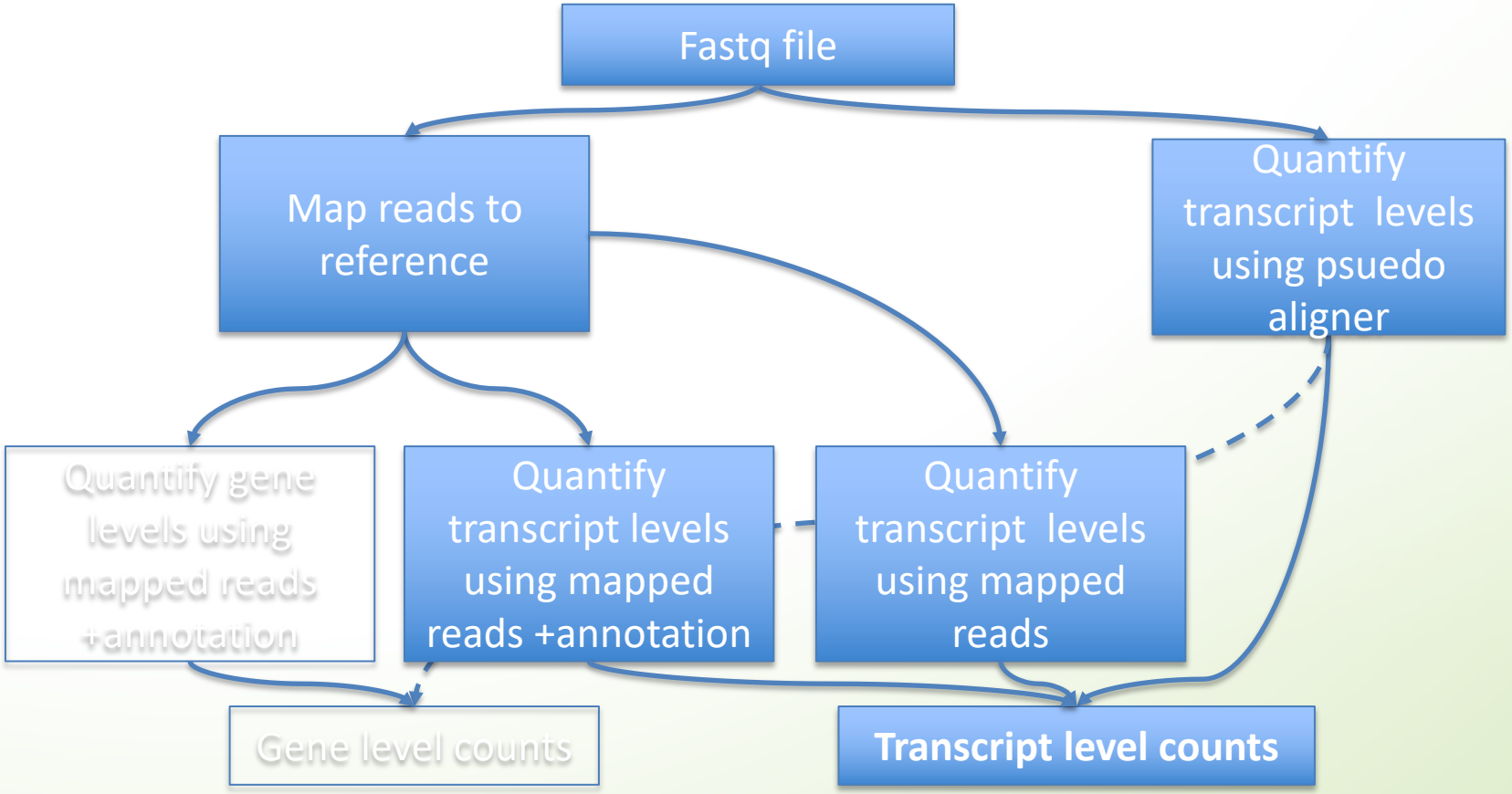
BMC Genomics

RESEARCH ARTICLE Open Access

Evaluation and comparison of computational tools for RNA-seq isoform quantification

Chi Zhang[1], Baohong Zhang[1], Lih-Ling Lin[2] and Shanrong Zhao[1*]

Transcript level analysis



Methods used in paper

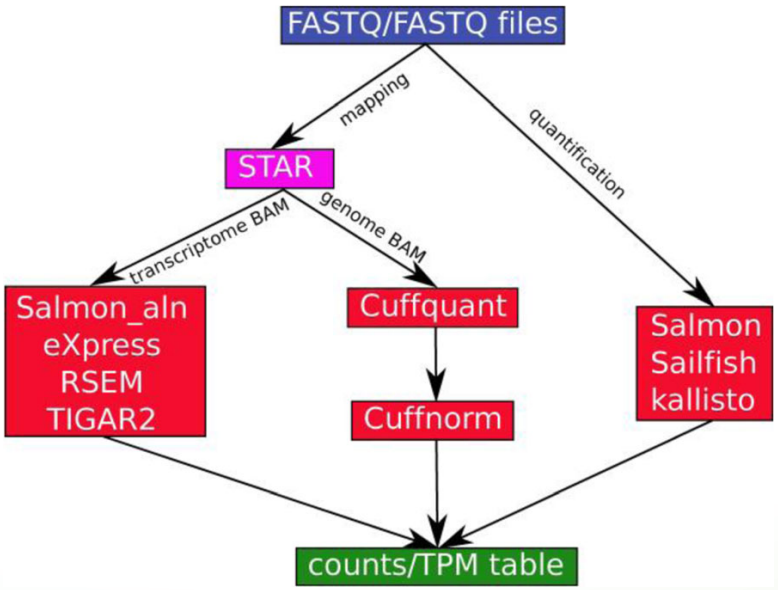


Table 1 Run time metrics of each method on 50 million paired-end reads of length 76 bp in an high performance computing cluster

	Memory (Gb)	Run time (min)	Algorithm	Multi-thread
Cufflinks	3.5	117	ML	Yes
RSEM	5.6	154	ML	Yes
eXpress	0.55	30	ML	No
TIGAR2	**28.3**	**1045**	VB	Yes
kallisto	3.8	7	ML	Yes
Salmon	6.6	6	VB/ML	Yes
Salmon_aln	3	7	VB/ML	Yes
Sailfish	6.3	5	VB/ML	Yes

For methods that support multi-threading, eight threads were used. For alignment-free methods (Kallisto, Salmon and Sailfish), a mapping step was included. The best performer in each category is underlined and the worst performer is in bold
ML Maximum Likelihood, *VB* Variational Bayes

Isoform quantification problematic for genes with many isoforms

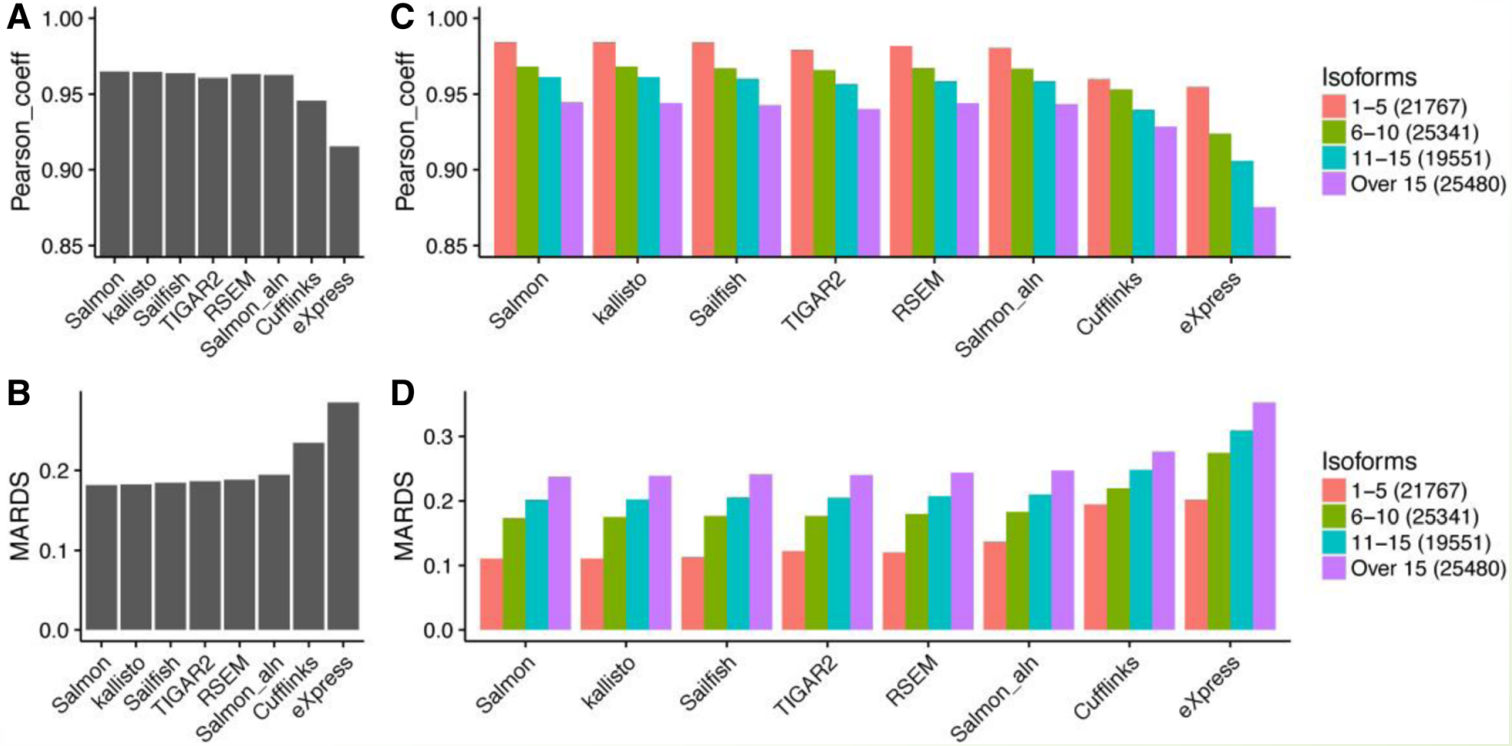


Fig. 2 Comparisons of the overall performance among different methods and the impact of the number of transcripts on the accuracy of isoform quantification. **a** Pearson correlation coefficient. **b** mean absolute relative differences and **c-d**) The above metrics were broken into separate groups according to the number of annotated transcript isoforms for each gene. The number of transcripts in each group is shown in figure legends. The accuracy metrics were calculated by comparing the estimated counts with the "ground truths" in simulated dataset

Results are very similar between methods

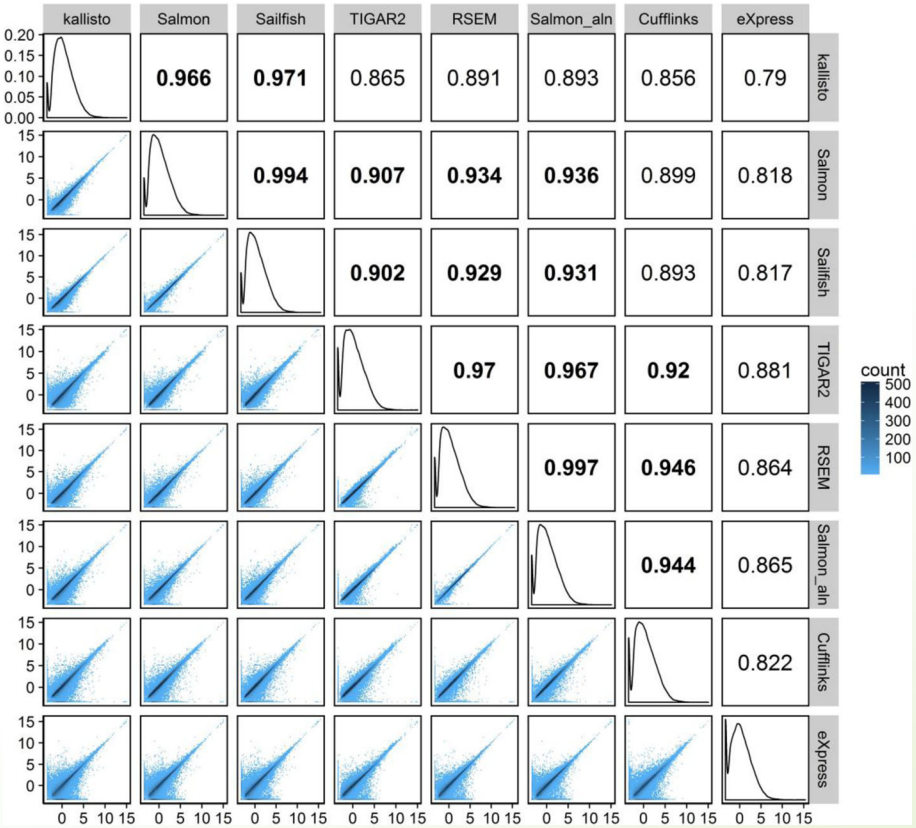
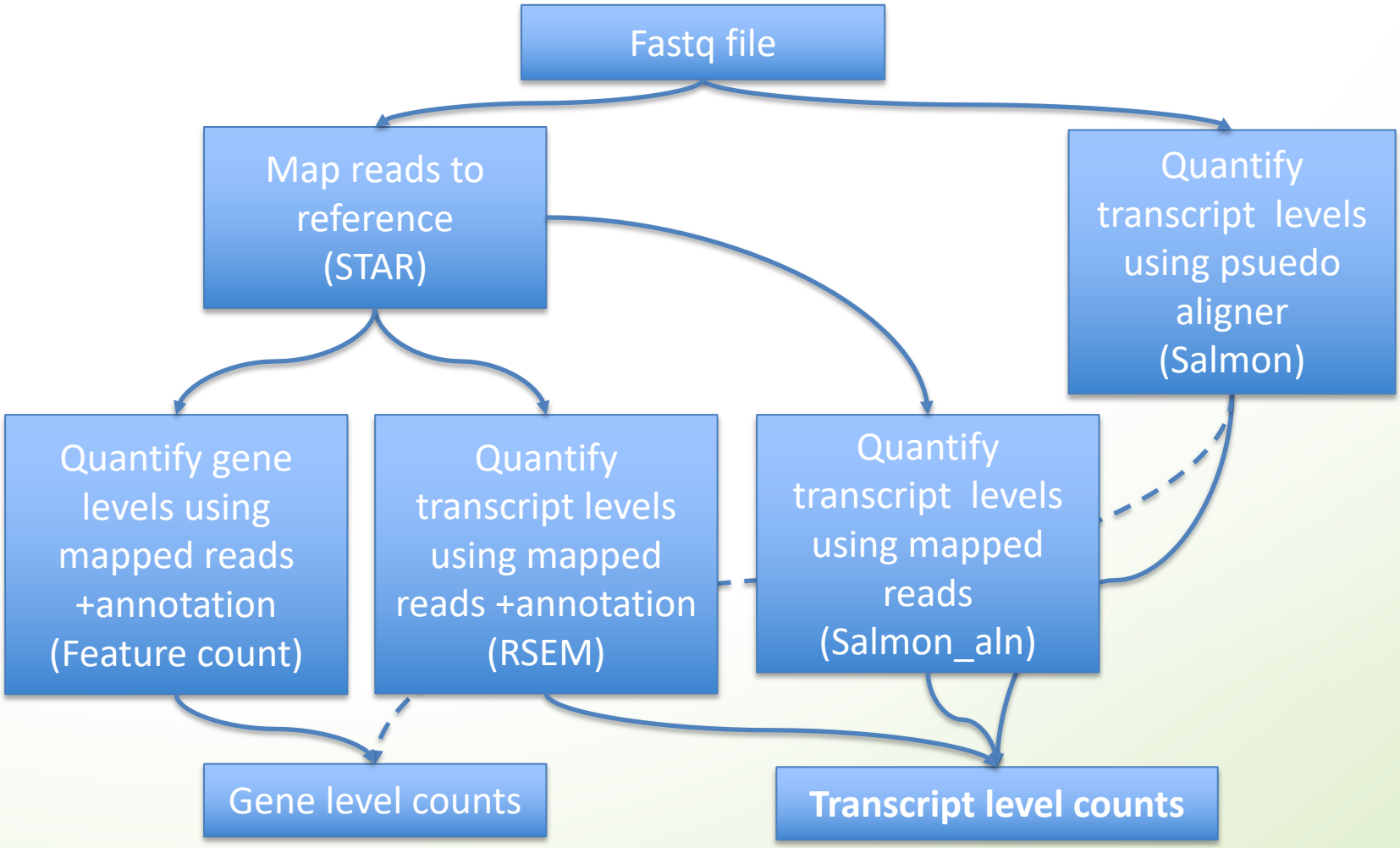


Fig. 5 Pairwise correlation of estimated TPM values for all transcripts between methods for the HBRR-C4 sample. The distribution of transcripts' TPMs from each method was plotted on the diagonal panels. Pairwise density plots and R^2 values are shown in the lower and upper triangular panels, respectively. R^2 values over 0.9 are in *bold*. Methods are grouped using hierarchical clustering

What to choose?
My personal choices



Good luck!