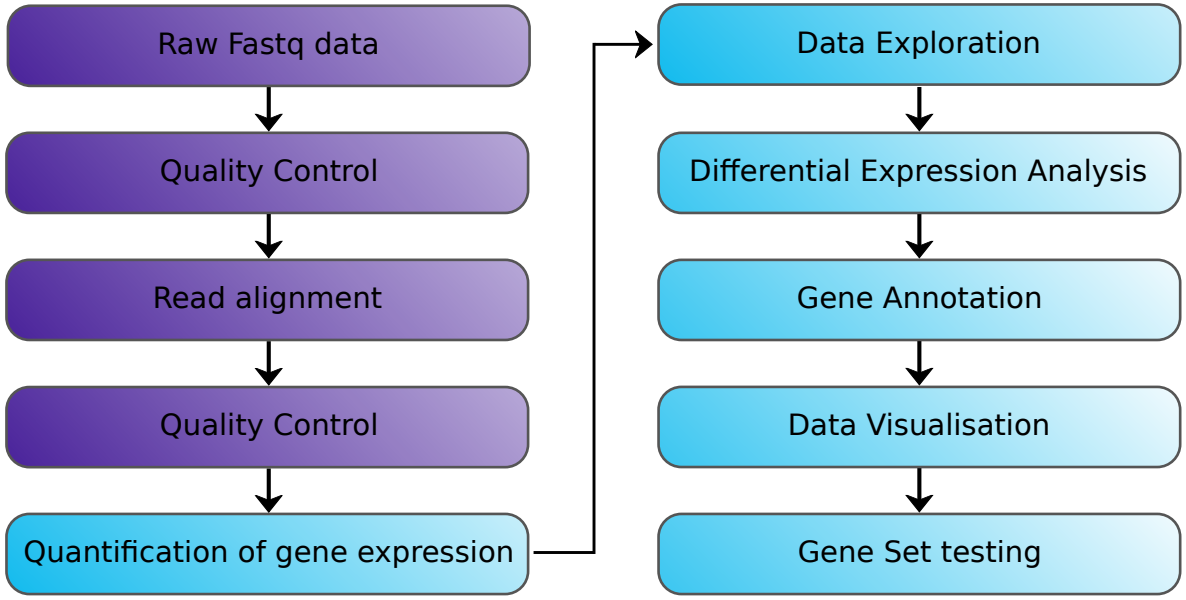
CANCER RESEARCH UK | CAMBRIDGE INSTITUTE

Quantification of Gene Expression with Salmon

March 2021

Differential Gene Expression Analysis Workflow

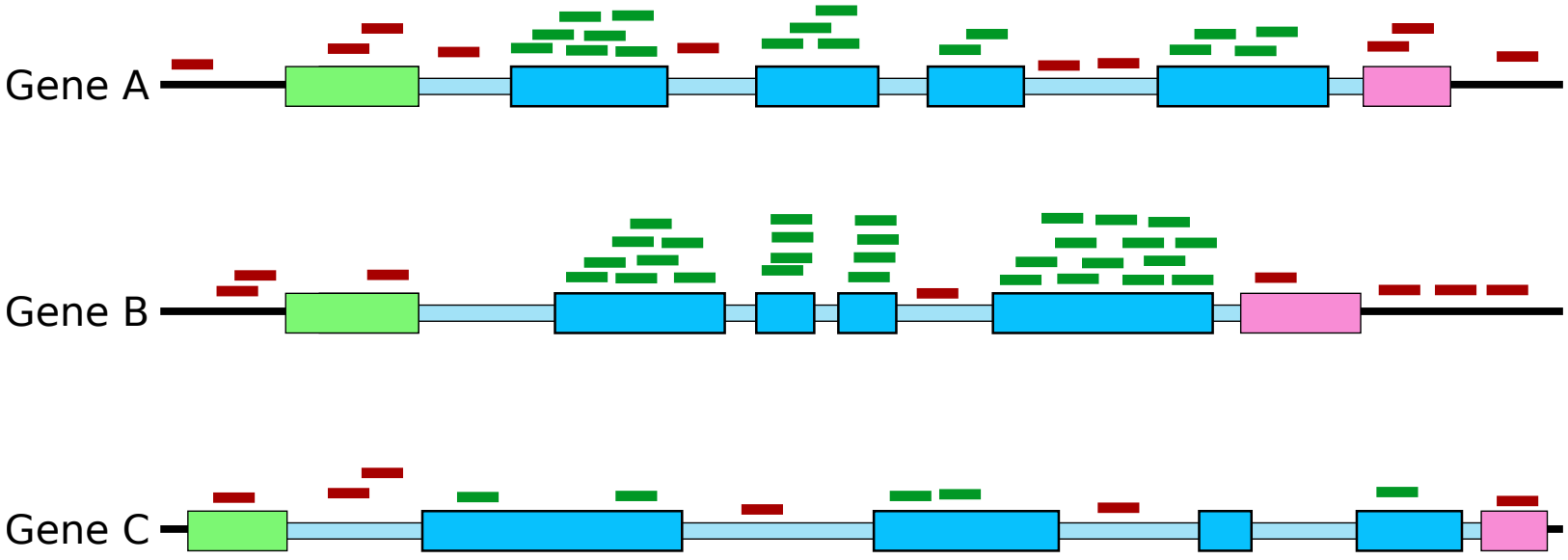


A Simple Counting Approach

Align to the genome to get the locations of our reads.

Gene annotation references give us the locations of exons of genes on the genome.

So the simplest approach is to count how many reads overlap each gene.

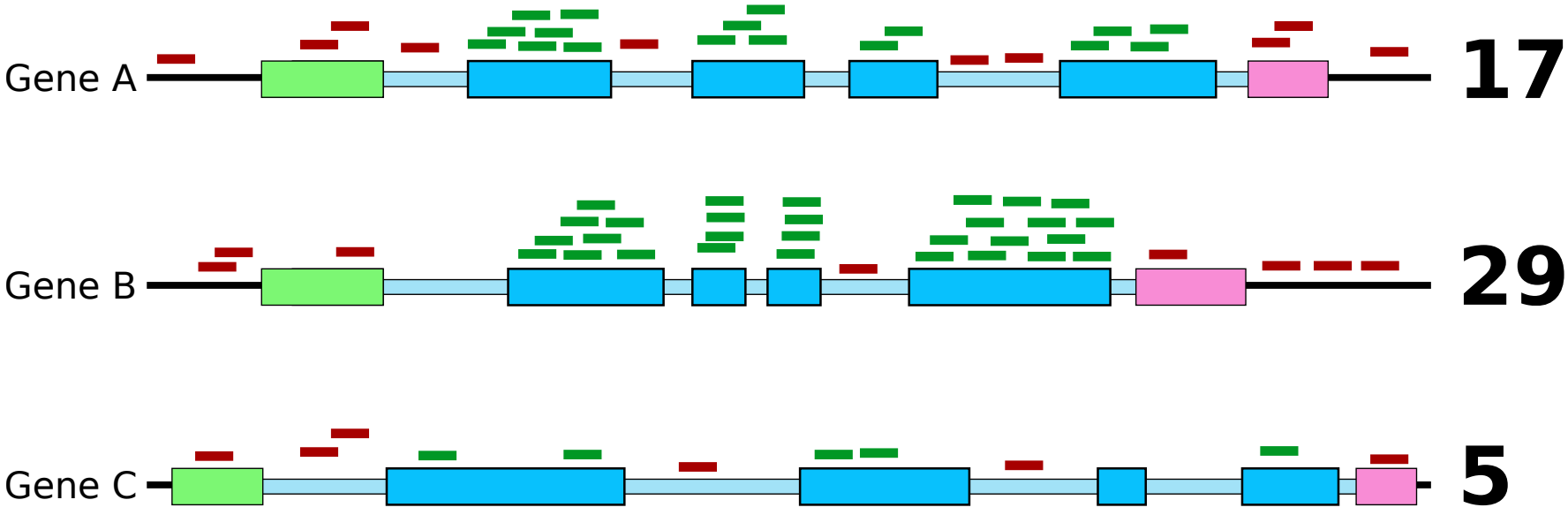


A Simple Counting Approach

We now have the locations of our reads on the genome.

We also know the locations of exons of genes on the genome.

So the simplest approach is to count how many reads overlap each gene.



e.g. featureCounts or HTSeq

Problems with the Simple Counting Approach

- Genes have multiple transcripts, alternative splicing introduces ambiguity
- Traditional alignment is (relatively) slow and computationally intensive
- Read sampling is not uniform, there are biases

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More sophisticated approaches:

- CuffLinks - Trapnell *et al.* (2010) Nature Biotechnology doi:10.1038/nbt.1621
- RSEM - Li and Dewey (2011) BMC Bioinformatics doi:10.1186/1471-2105-12-323
- Sailfish - Patro *et al.* (2014) Nature Biotechnology doi:10.1038/nbt.2862
- Kallisto - Bary *et al.* (2016) Nature Biotechnology doi:10.1038/nbt.3519
- **Salmon** - Patro *et al.* (2017) Nature Methods doi:10.1038/nmeth.4197

Problems with the Simple Counting Approach

- Genes have multiple transcripts, alternative splicing introduces ambiguity

Count against the transcriptome instead.

Summarise to gene level for differential gene expression analysis.

Quasi-mapping/Pseudo-alignment

- Traditional alignment is (relatively) slow and computationally intensive

Switch to *quasi-mapping* or *pseudo-alignment* to transcriptome

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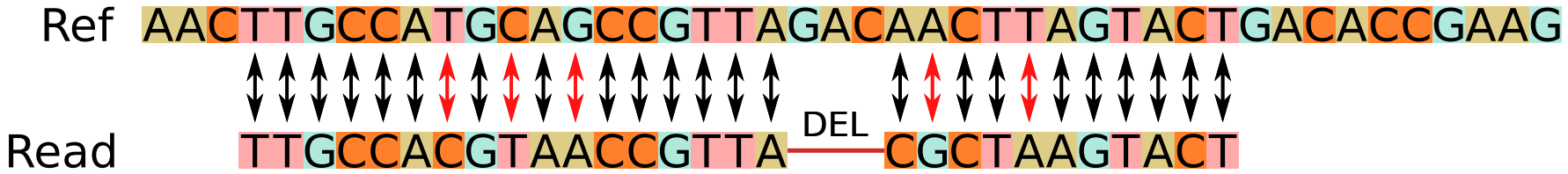
- Traditional alignment is (relatively) slow and computationally intensive

Switch to *quasi-mapping* or *pseudo-alignment*



Quasi-mapping/Pseudo-alignment

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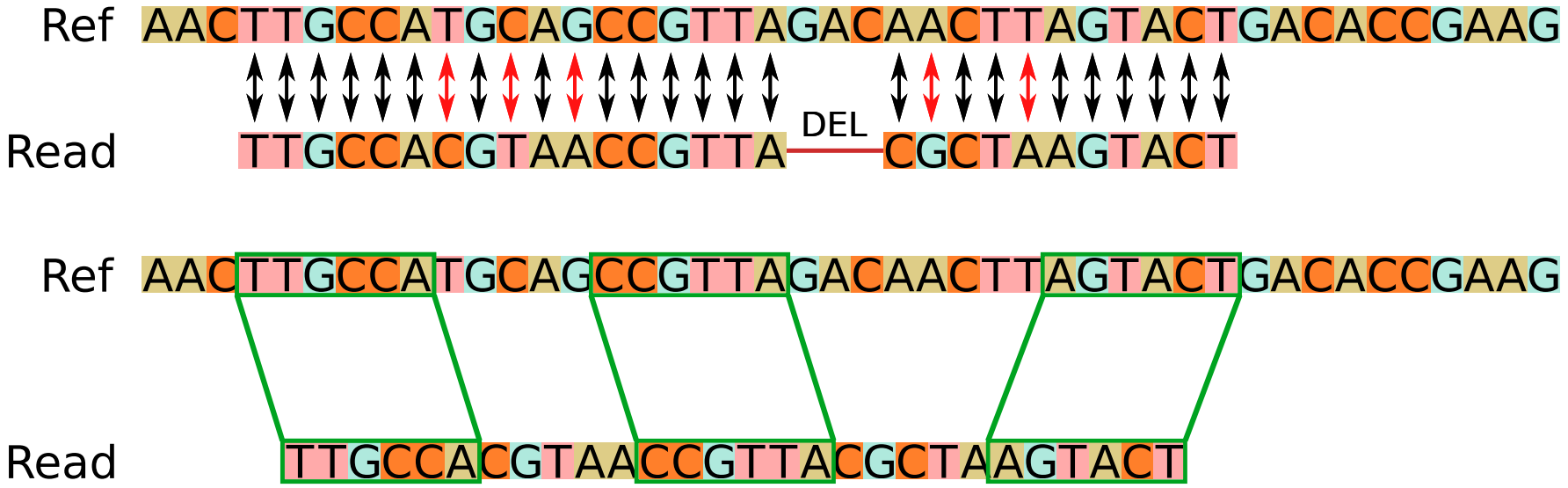
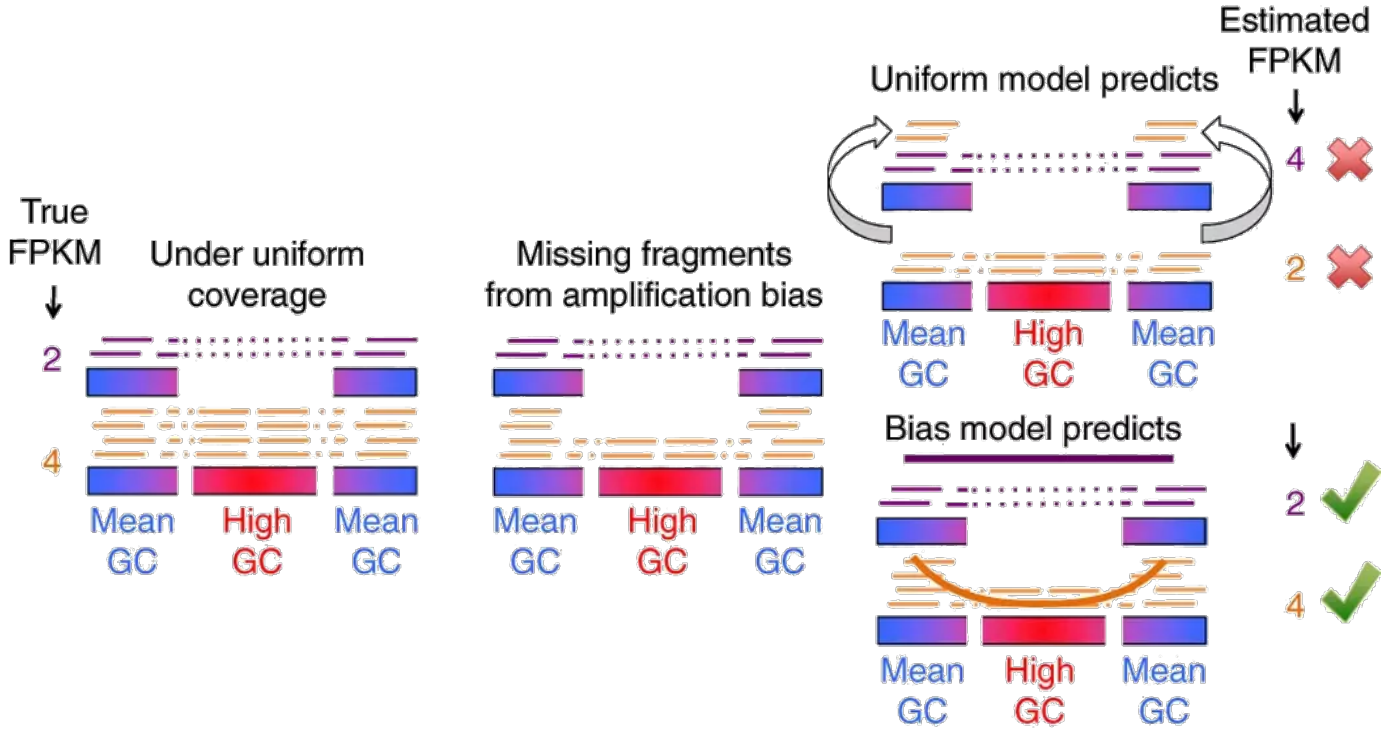
Switch to *quasi-mapping* or *pseudo-alignment*



Bias models

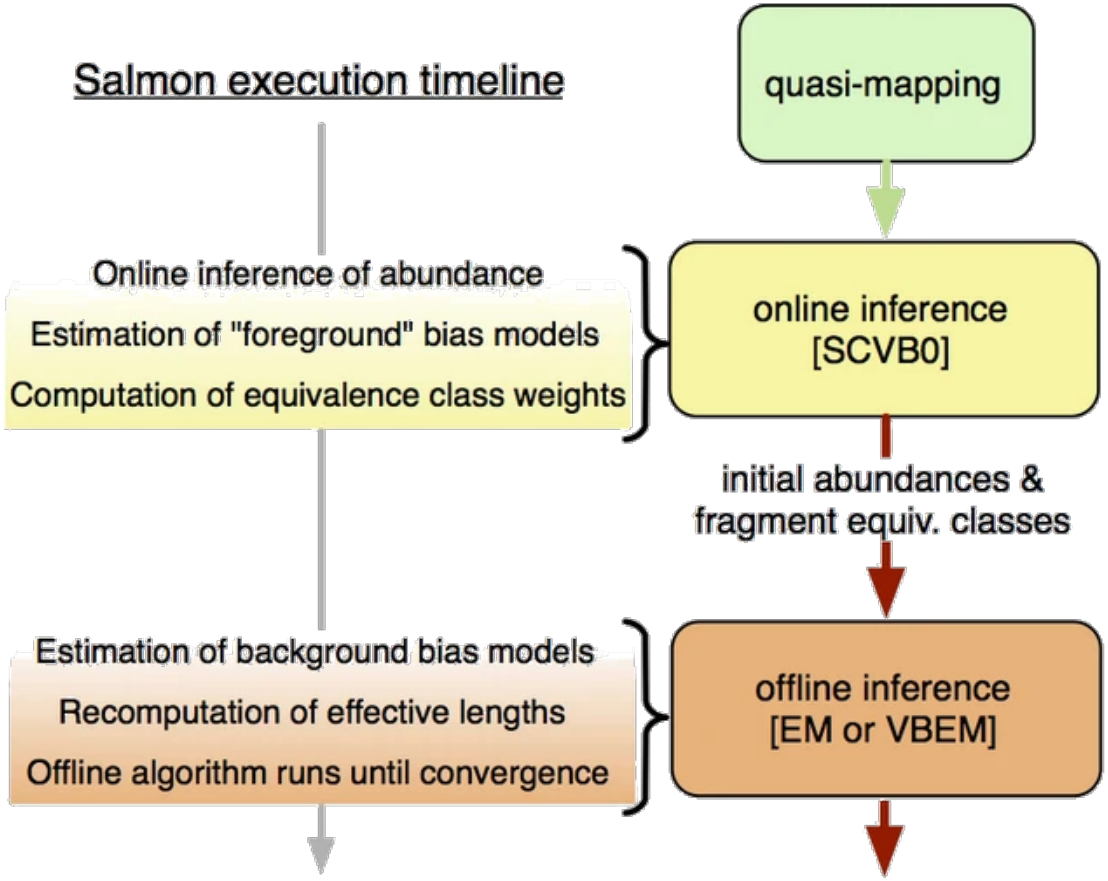
- Read sampling is not uniform, there are biases

Include modelling for GC bias, positional bias and sequence bias in the quantification algorithm



Love *et al.* (2016) Nature Biotechnology doi:10.1038/nbt.3682

Salmon workflow



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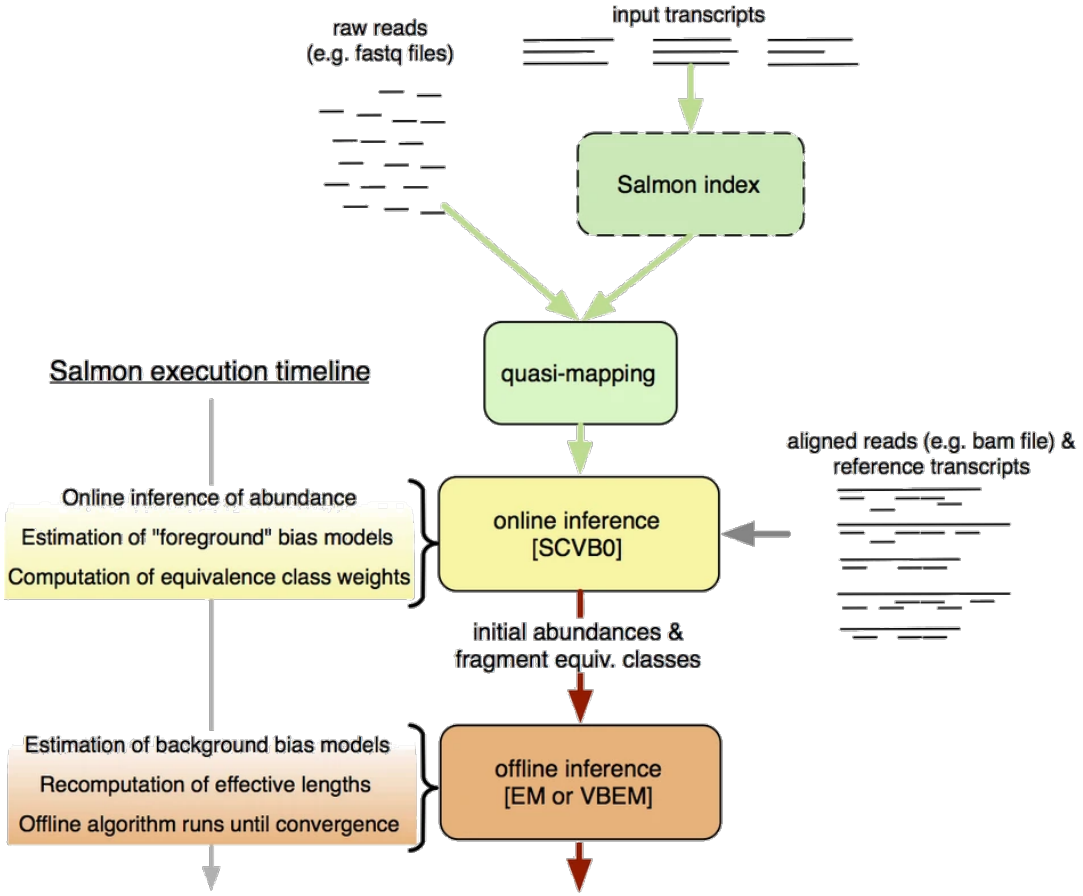
Salmon workflow



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Practical

1. Create and index to the transcriptome with Salmon
2. Quantify transcript expression using Salmon