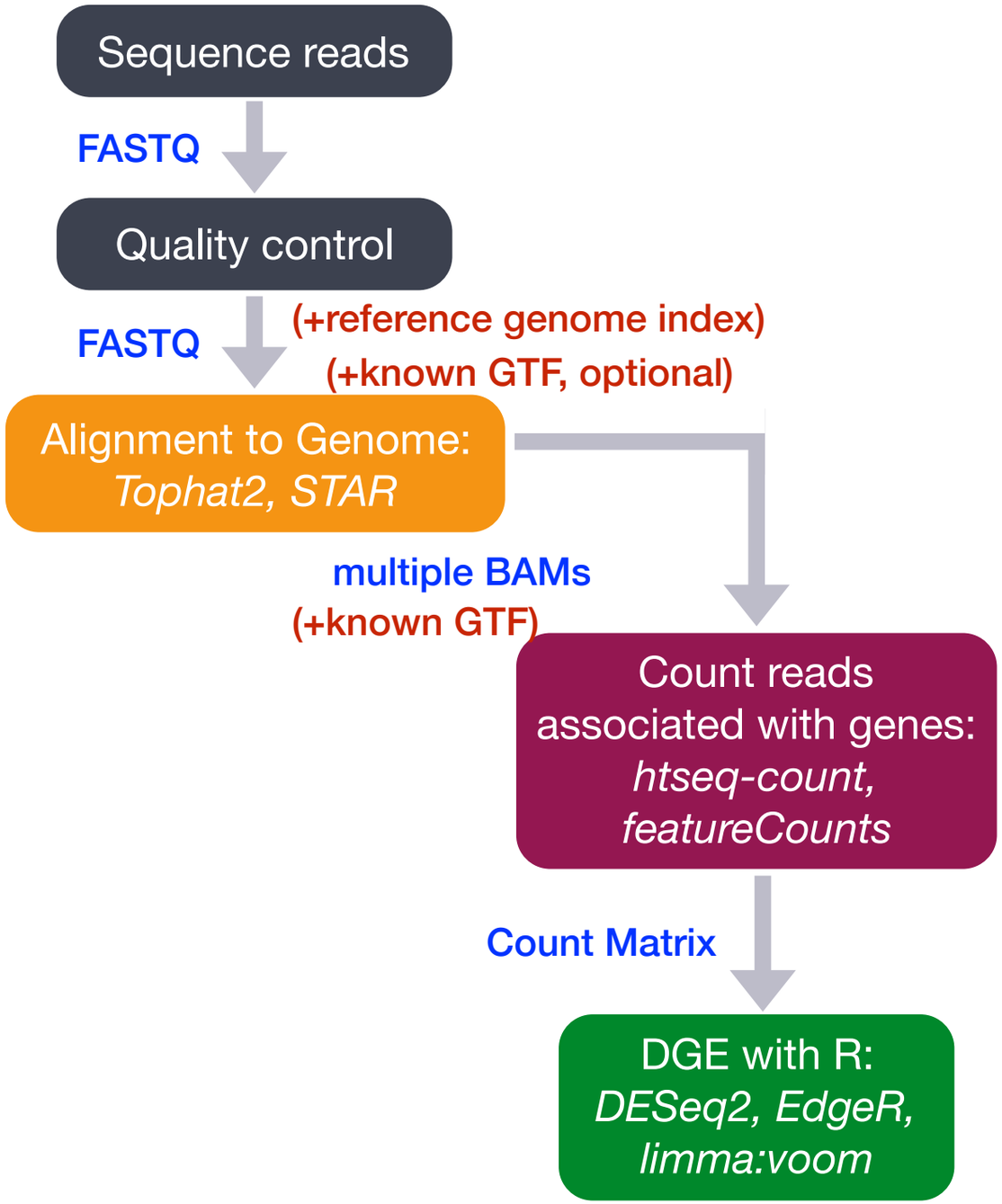
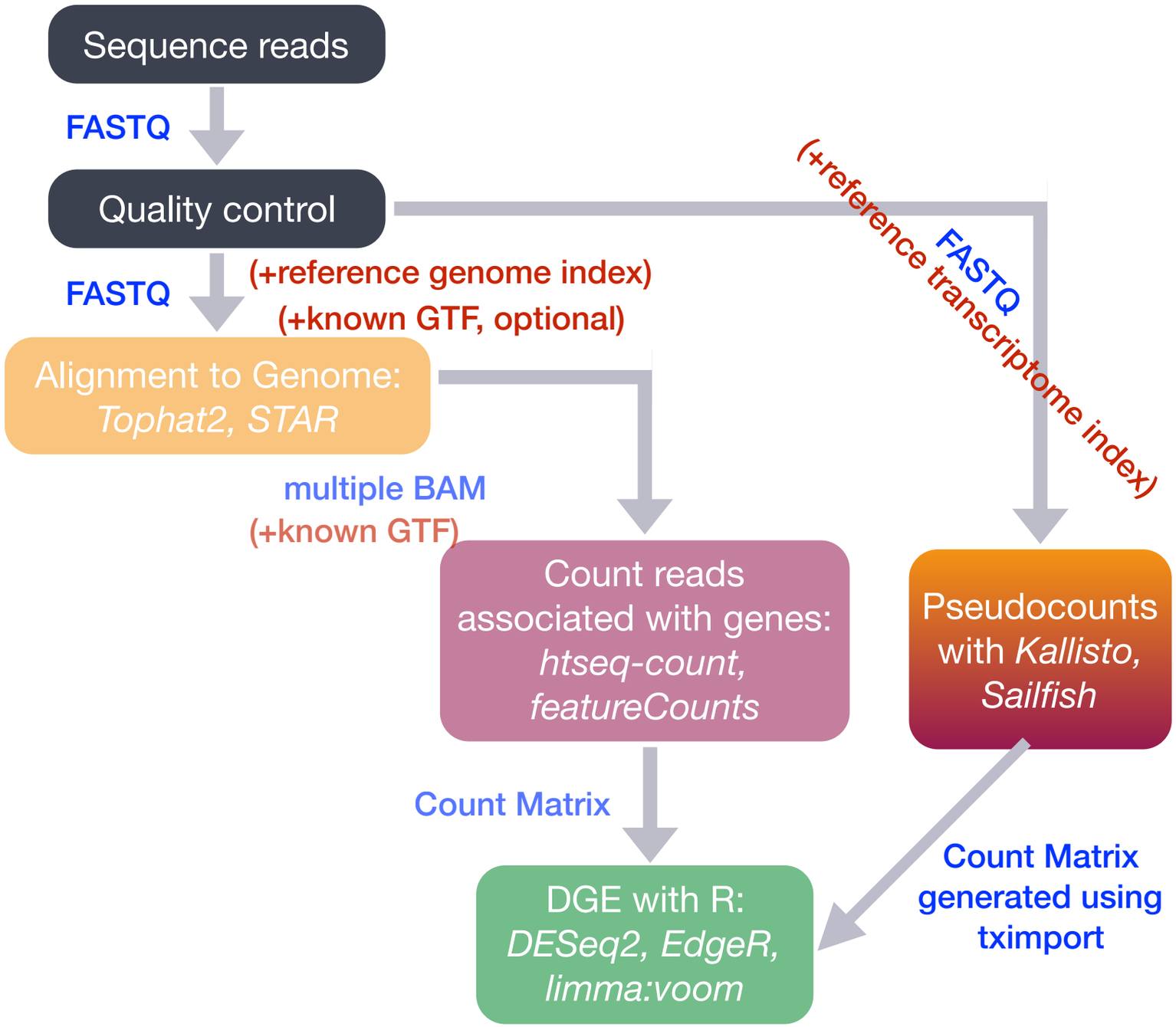
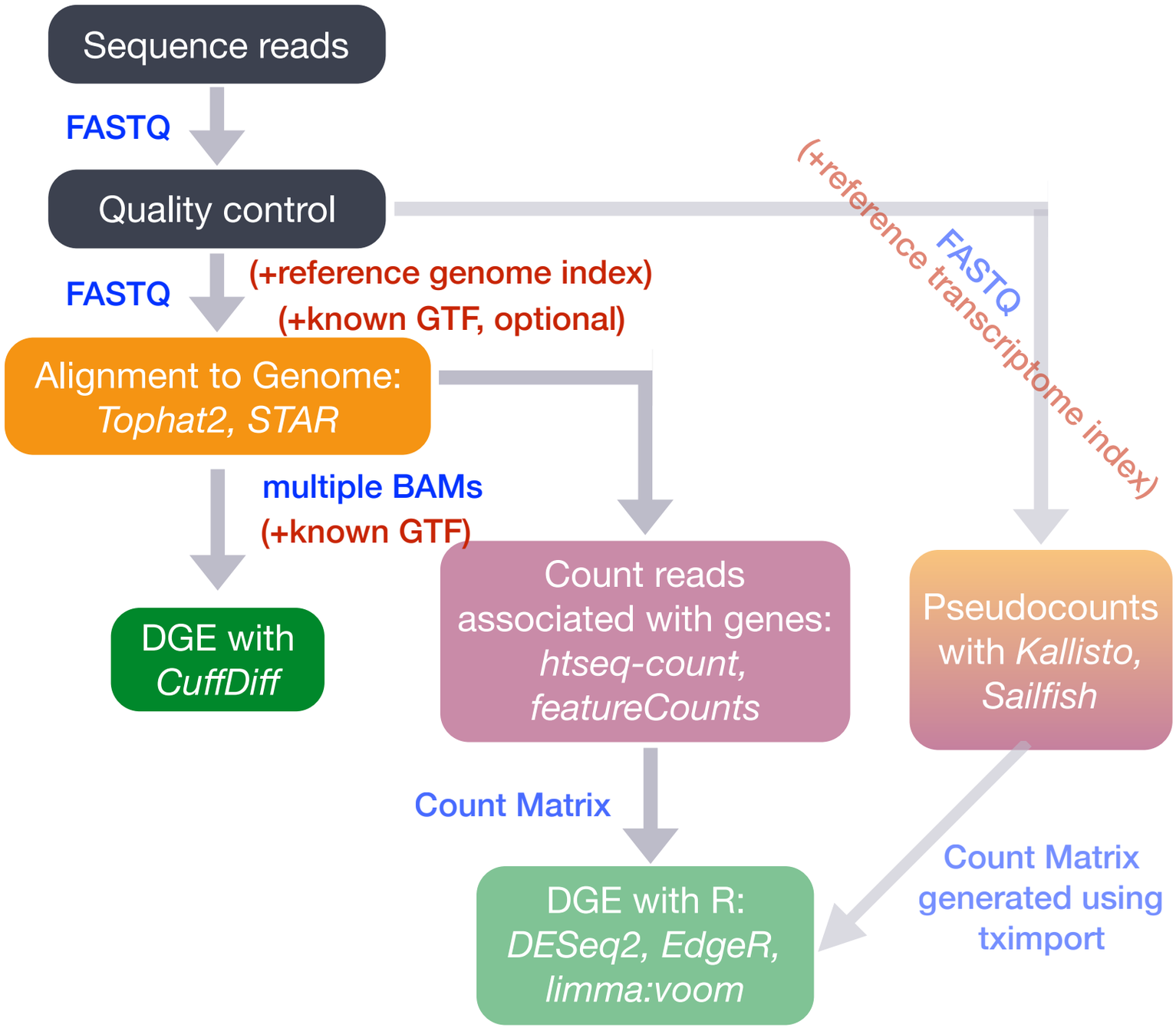
RNA-Seq: Analysis methods



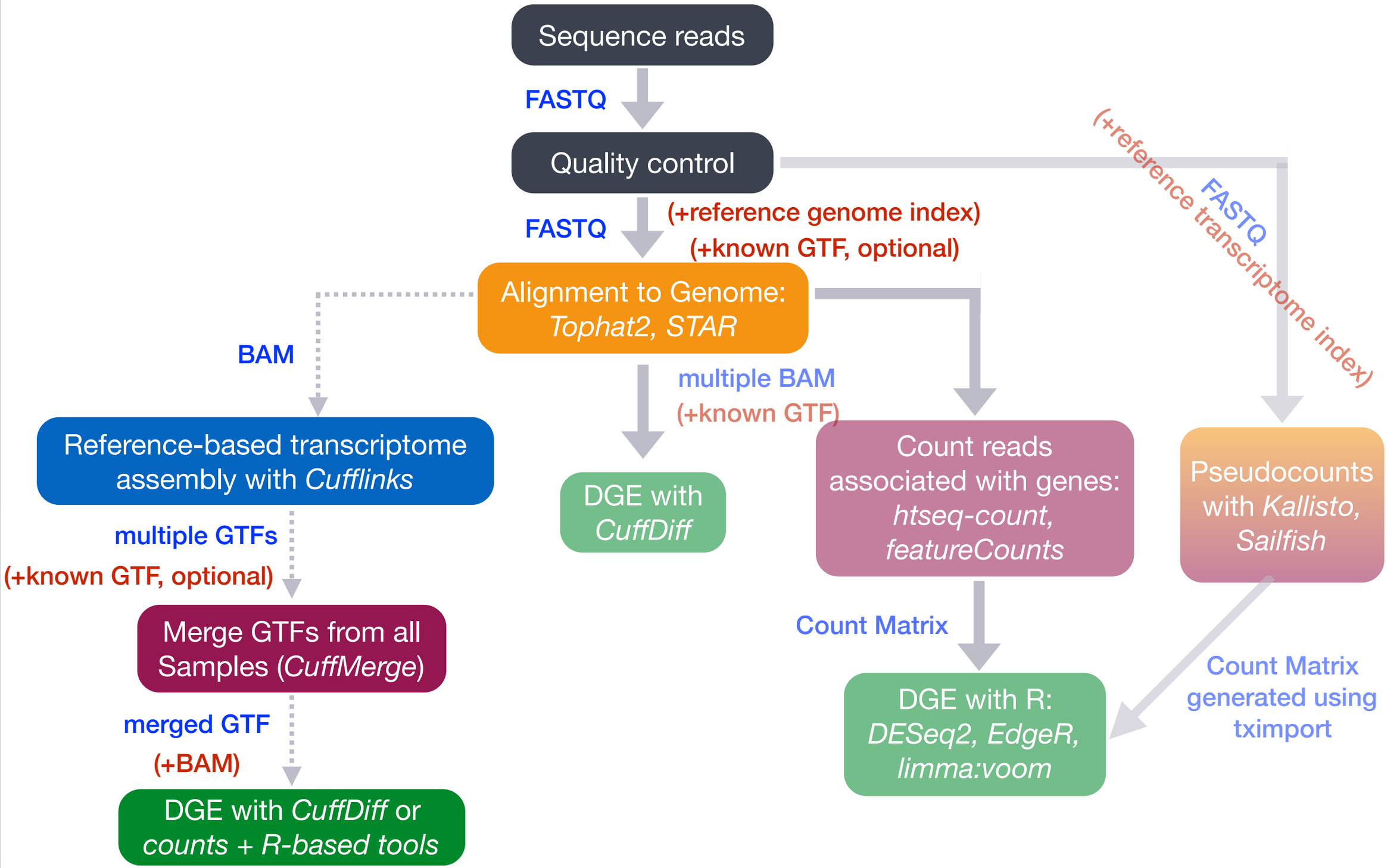
Differential Gene Expression Analysis Workflow



Differential Gene Expression Analysis Workflow



Differential Gene Expression Analysis Workflow



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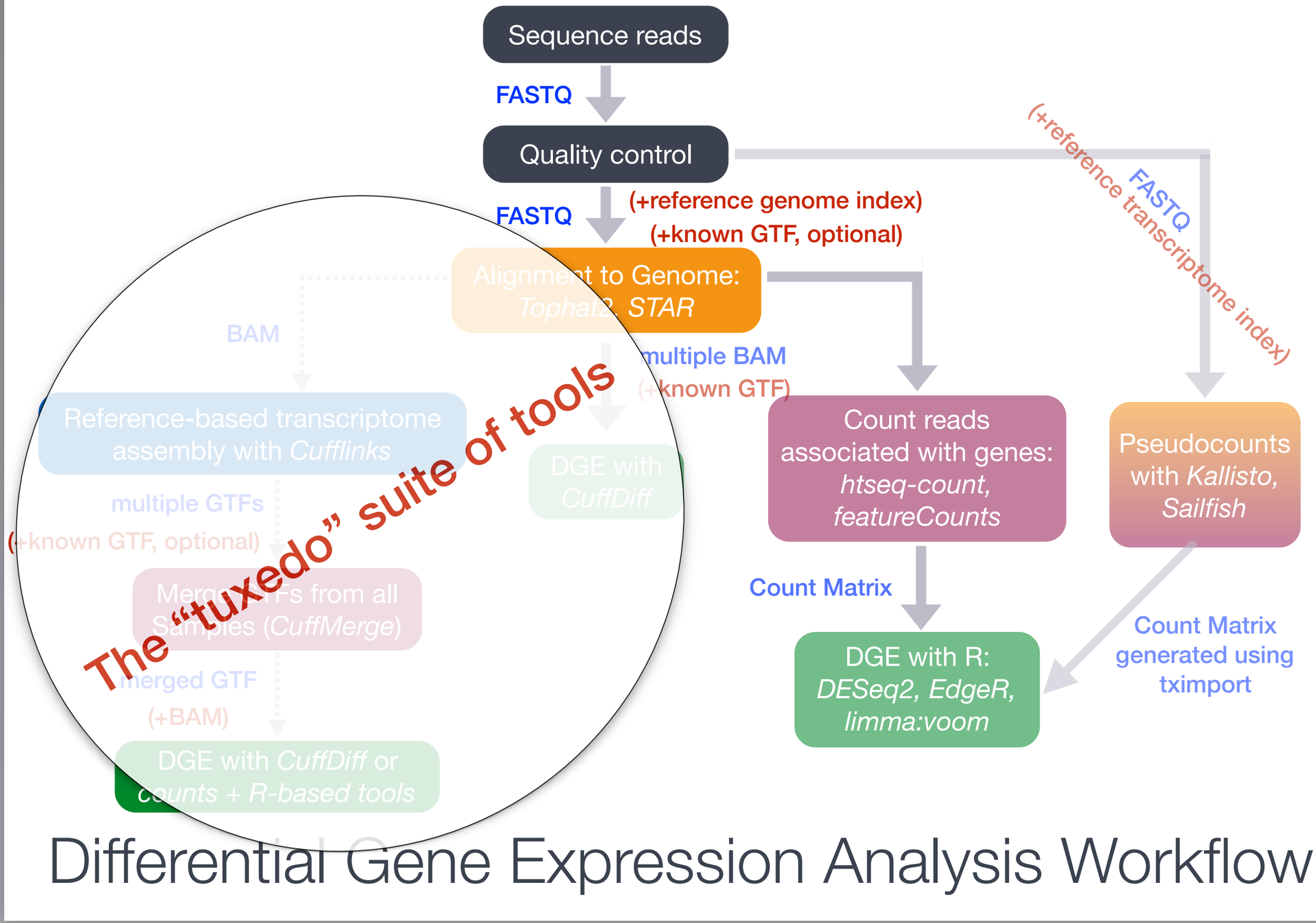
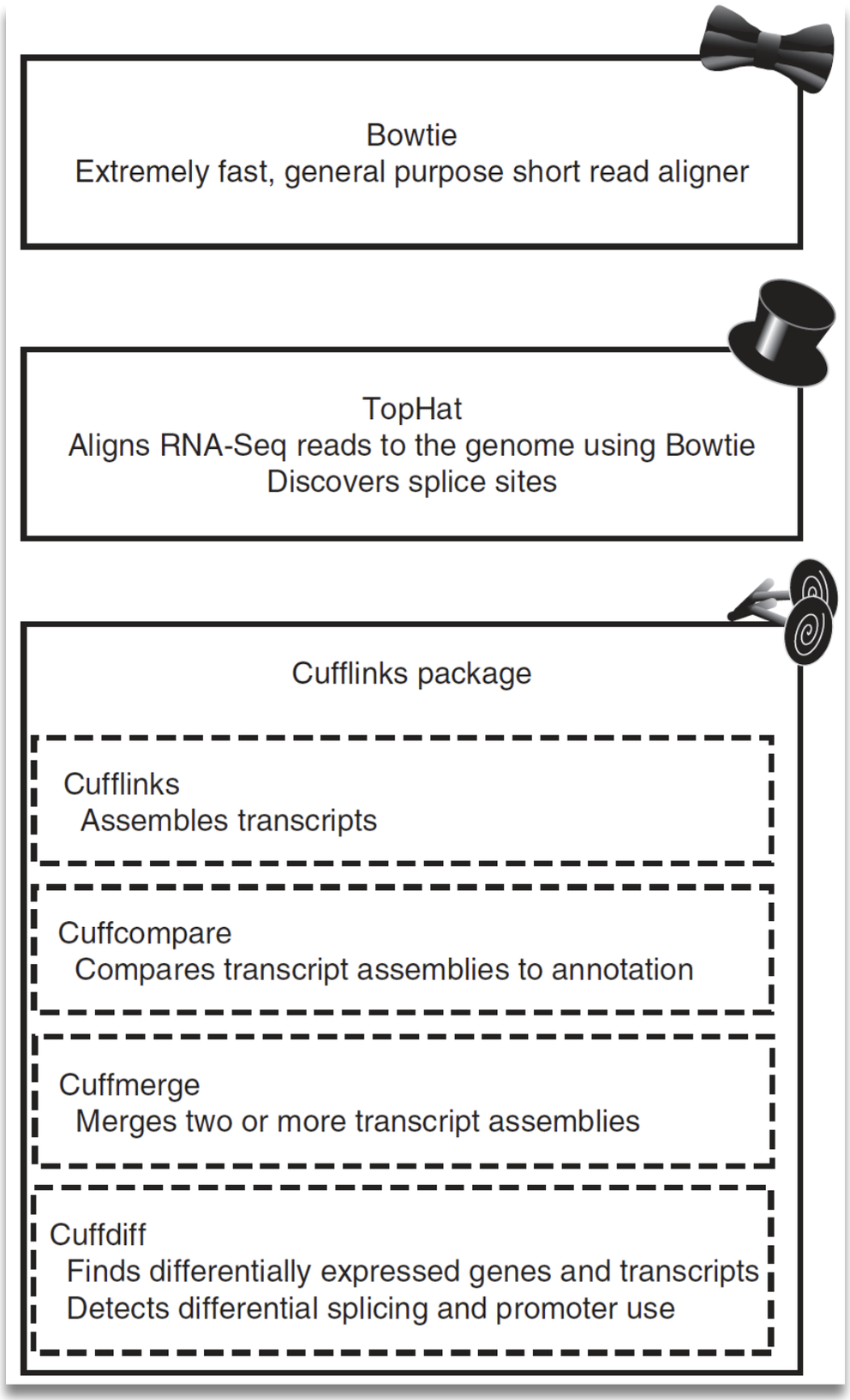


Differential Gene Expression Analysis Workflow



Bowtie/Bowtie2 both use Burrows-Wheeler indexing for aligning reads.

Tophat uses either Bowtie or Bowtie2 to align reads in a splice-aware manner and aids the discovery of new splice junctions

The Cufflinks package has 4 components, the 2 major ones are listed below -

Cufflinks does **reference-based transcriptome assembly**

Cuffdiff does statistical analysis and identifies differentially expressed transcripts in a **simple pairwise comparison**, and a series of pairwise comparisons in a time-course experiment

Trapnell et al., Nature Protocols, March 2012

When is it appropriate to use Cufflinks/CuffDiff?

You are looking to identify as yet unknown genes/isoforms

Your experiment is a pairwise comparison

You are looking for isoform-level differential expression

Cons:

Complex experimental designs are not supported

False positives are rampant with novel discovery methods

When is it appropriate to use R-based analysis methods using raw counts?

For almost all experiments where you are looking to identify differentially expressed genes (known genes), irrespective of the complexity of the experimental design

Cons:

A basic knowledge of R programming is required

Not useful for isoform-specific differential expression analysis

Undercounting due to discarded multi-mappers

Alternative methods: transcriptome assembly

Martin J.A. and Wang Z., Nat. Rev. Genet. (2011) 12:671–682

Alternative methods: transcriptome assembly

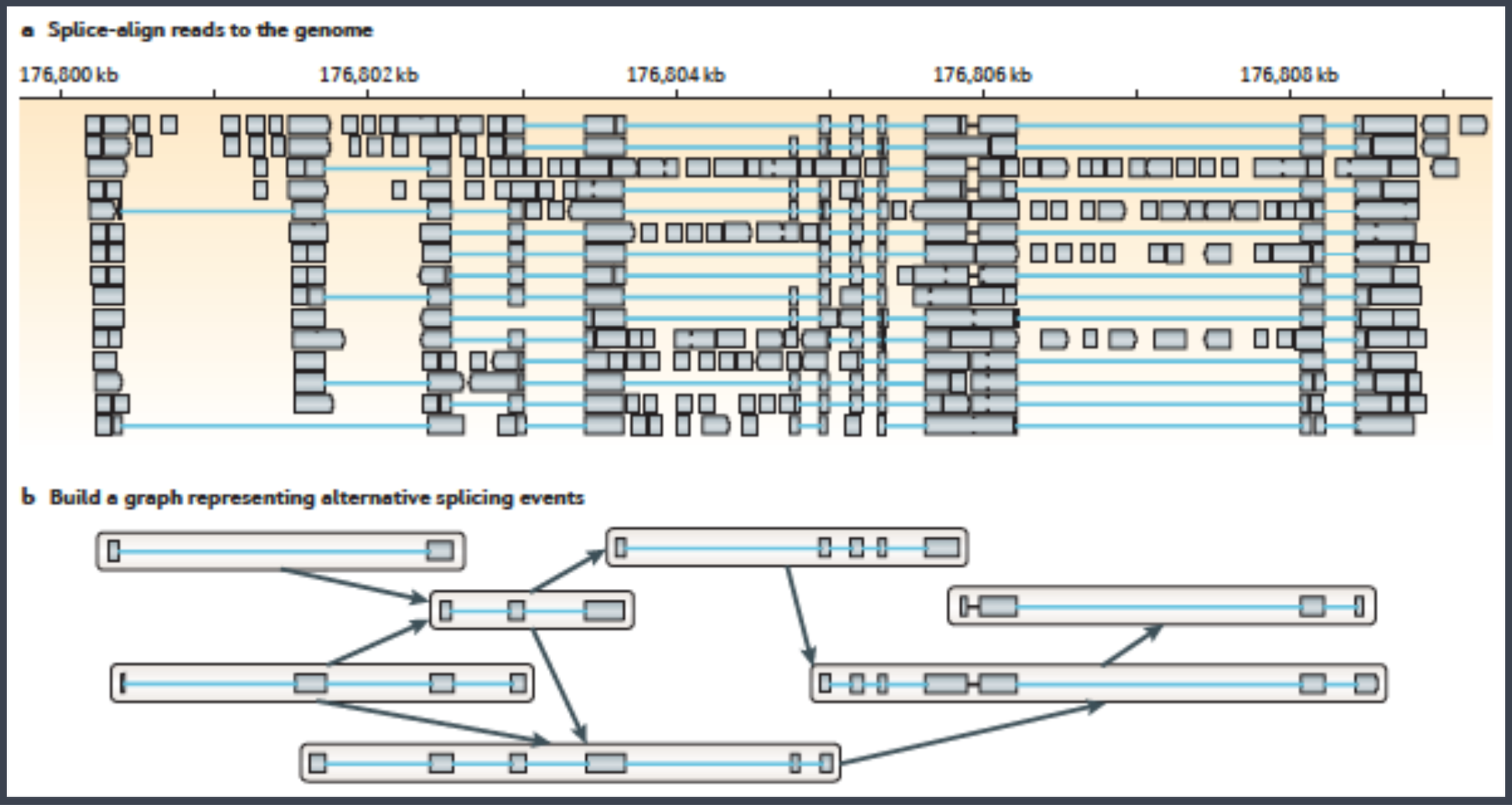
Reference-based assembly

This type of assembly is used when the genome sequence is known.

- Transcriptome data are not available
- Transcriptome information available is not good enough, i.e. missing isoforms of genes, or unknown non-coding regions
- The existing transcriptome information is for a different tissue type or state
- Cufflinks and Scripture are two reference-based transcriptome assemblers
- Annotation of any newly-discovered genes or isoforms will be performed downstream

Alternative methods: transcriptome assembly

Reference-based assembly



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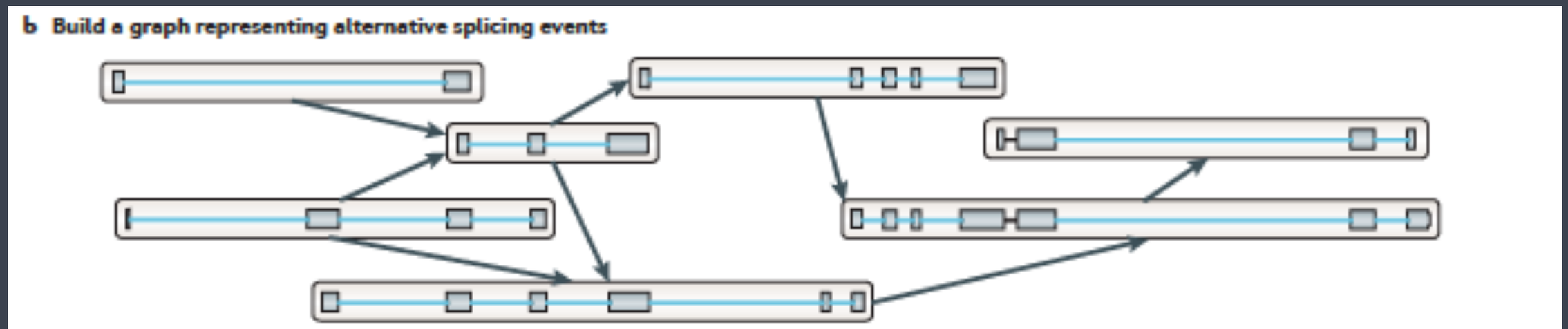
Alternative methods: transcriptome assembly

Reference-based assembly



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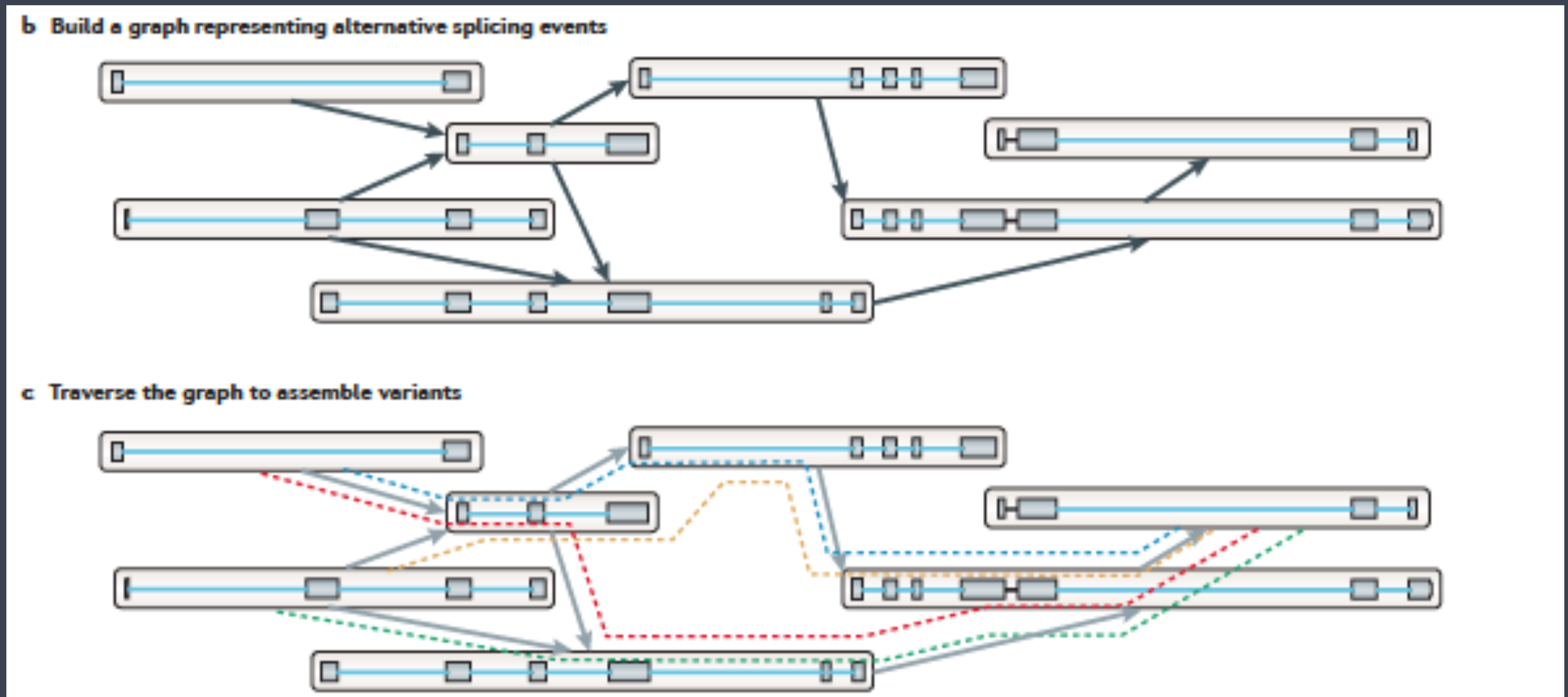
Alternative methods: transcriptome assembly

Reference-based assembly



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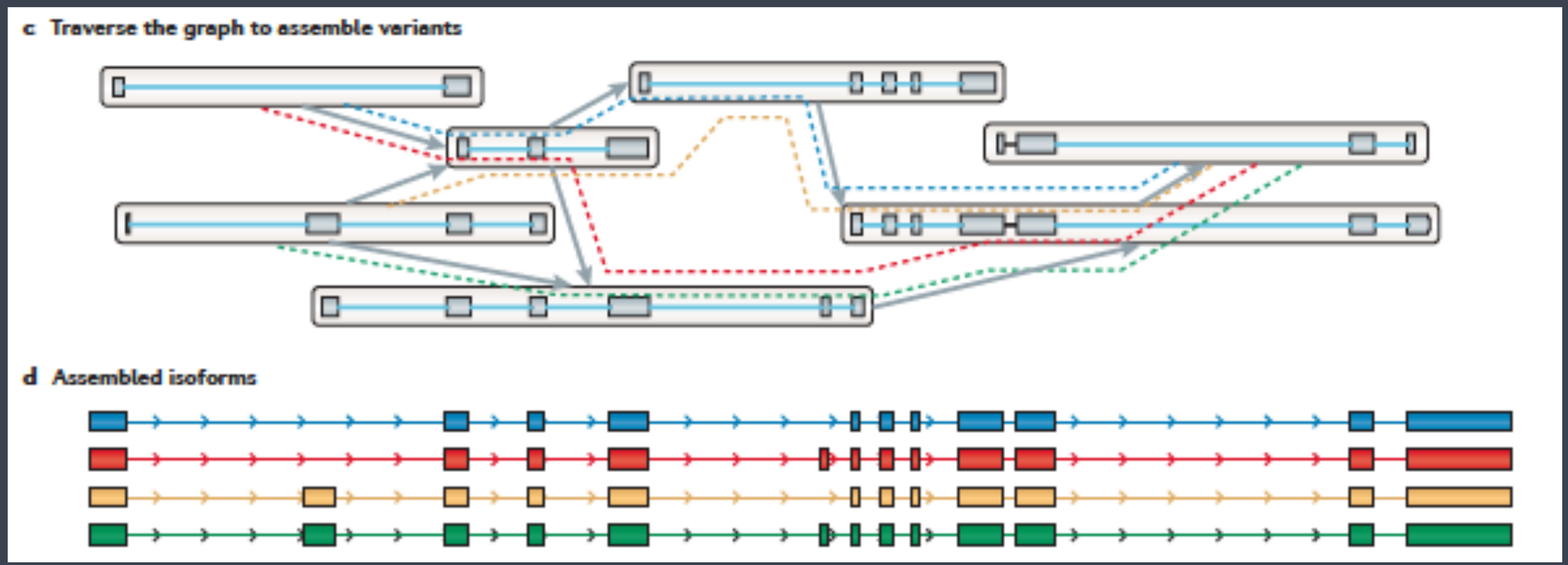
Alternative methods: transcriptome assembly

Reference-based assembly



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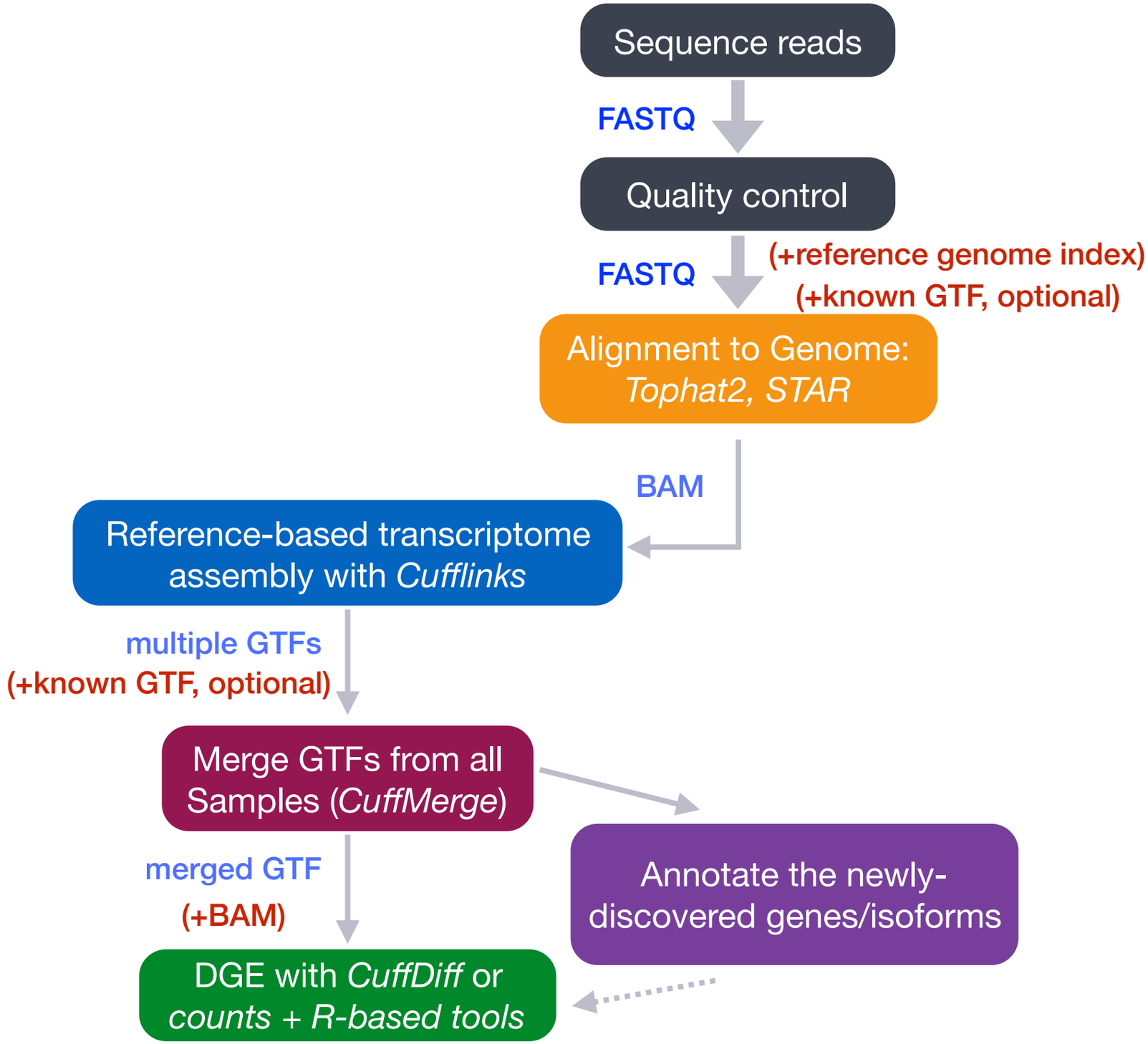


Differential Gene Expression Analysis Workflow

Alternative methods: transcriptome assembly

De novo assembly

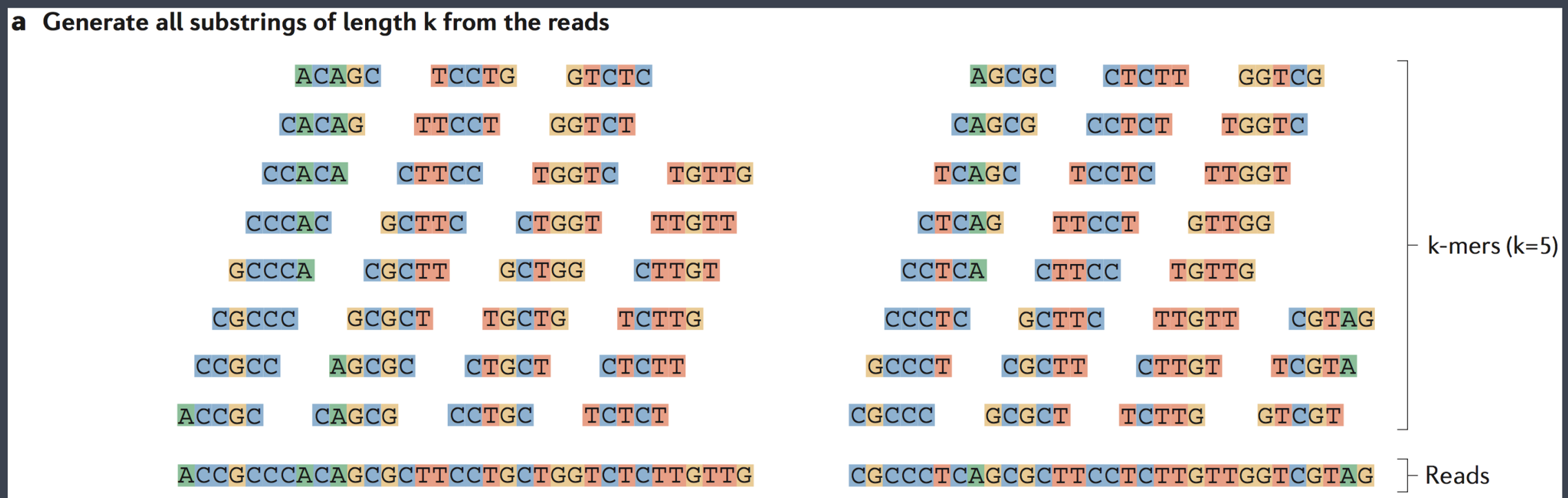
This type of assembly is used when very little information is available for the genome

- An assembly of this type is often the first step in putting together information about an unknown genome
- Amount of data needed for a good de novo assembly is higher than what is needed for a reference-based assembly
- Assemblies of this sort can be used for genome annotation, once the genome is assembled
- Oases, TransABySS, Trinity are examples of well-regarded transcriptome assemblers, especially Trinity
- Annotation of any newly-discovered genes or isoforms will be performed downstream

It is not uncommon to use both methods and compare and combine the assemblies, even when a genome sequence is known, especially for a new genome.

Alternative methods: transcriptome assembly

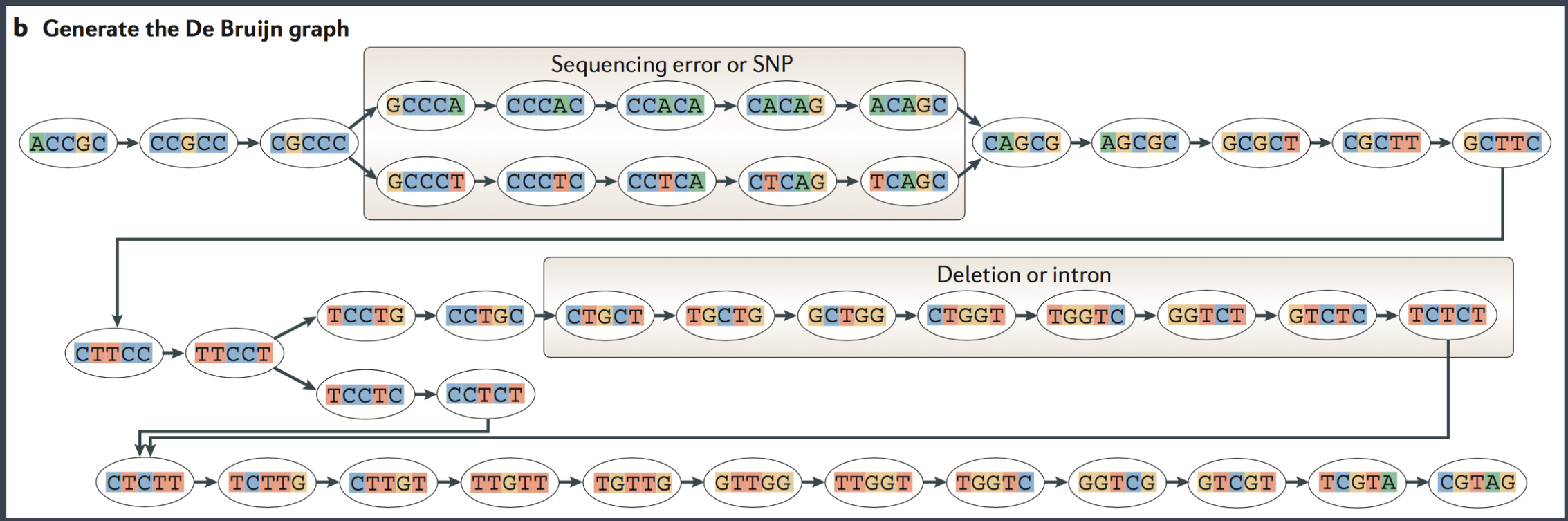
De novo assembly



Martin J.A. and Wang Z., Nat. Rev. Genet. (2011) 12:671–682

Alternative methods: transcriptome assembly

De novo assembly (De Bruijn graph construction)



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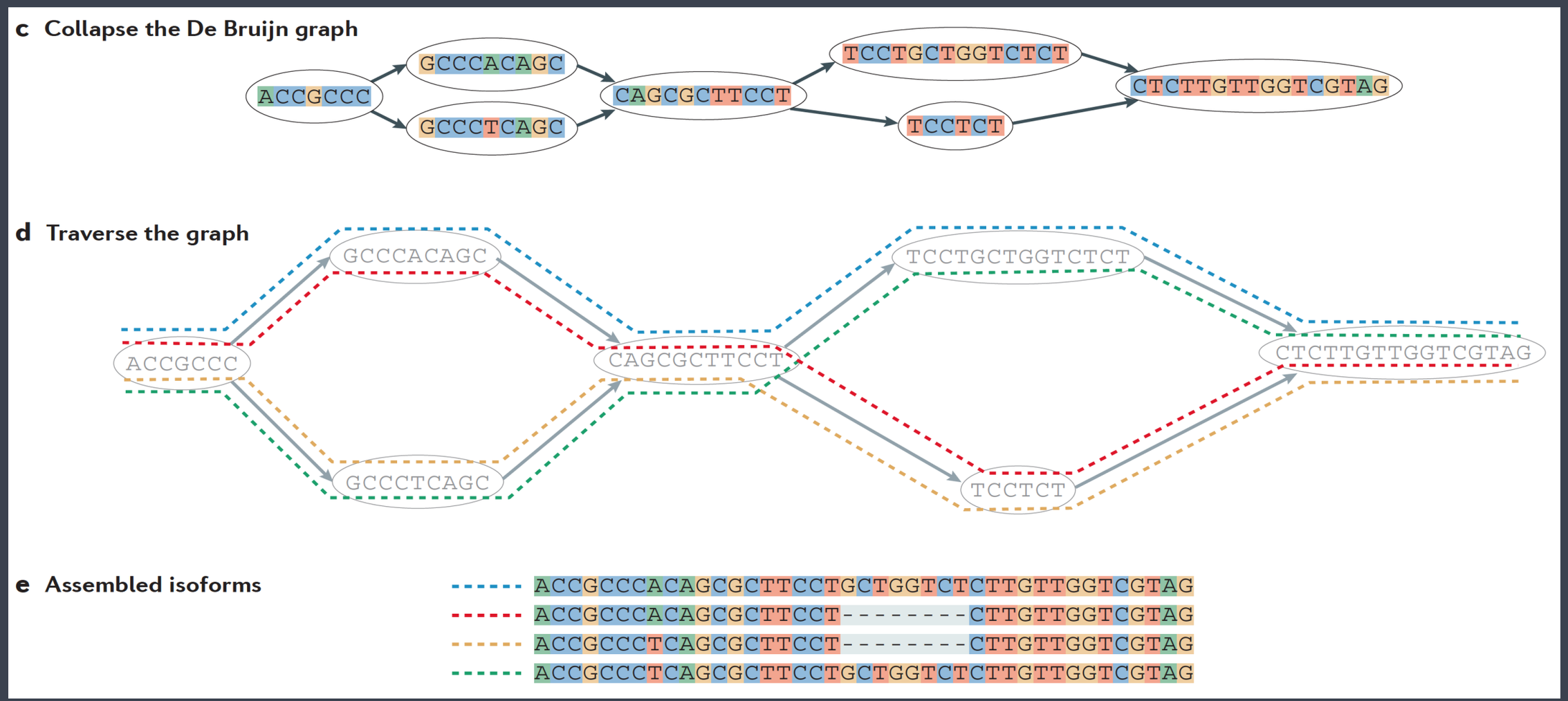

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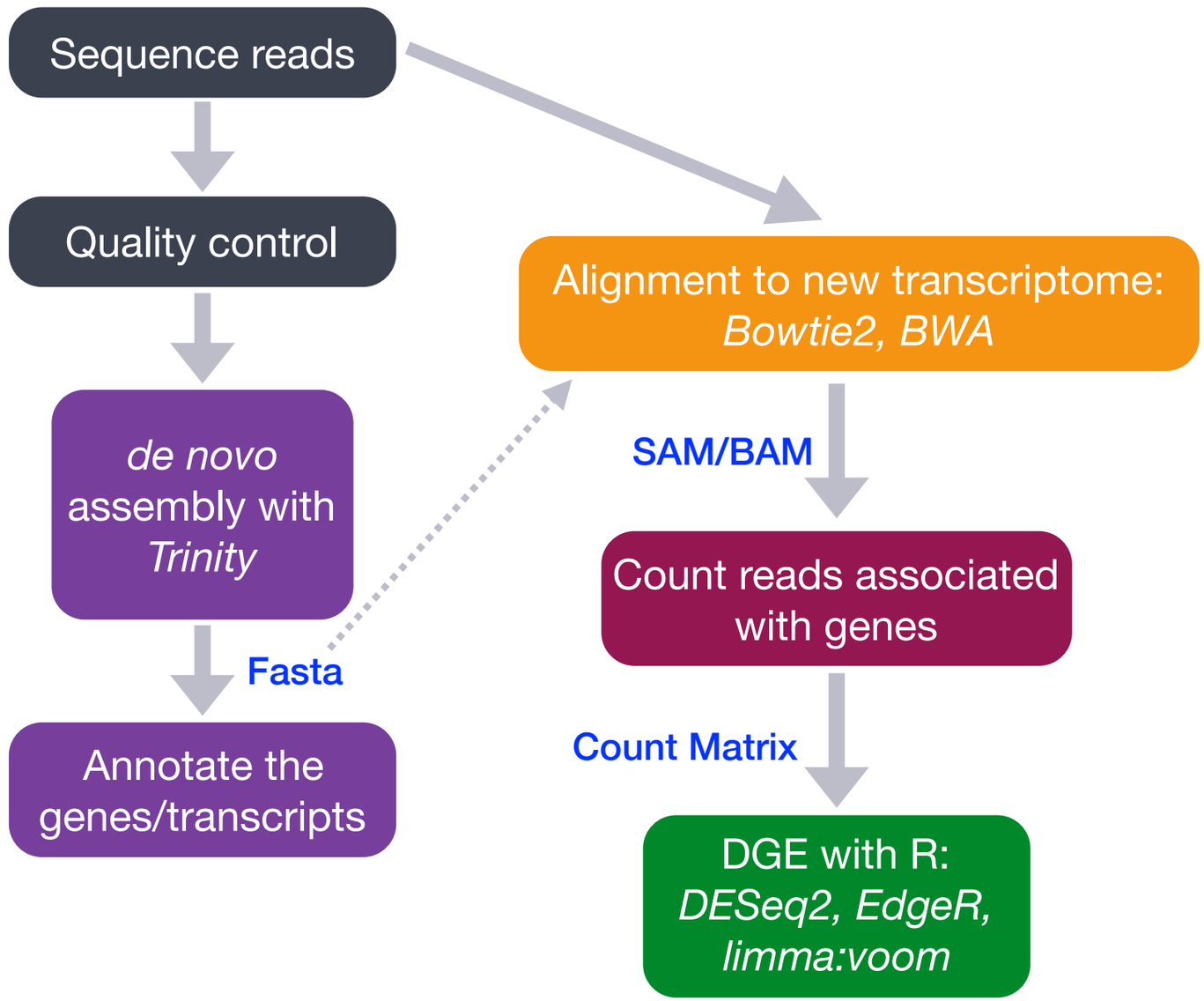

Differential Gene Expression Analysis Workflow

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