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Advanced Sequencing Technologies & Applications

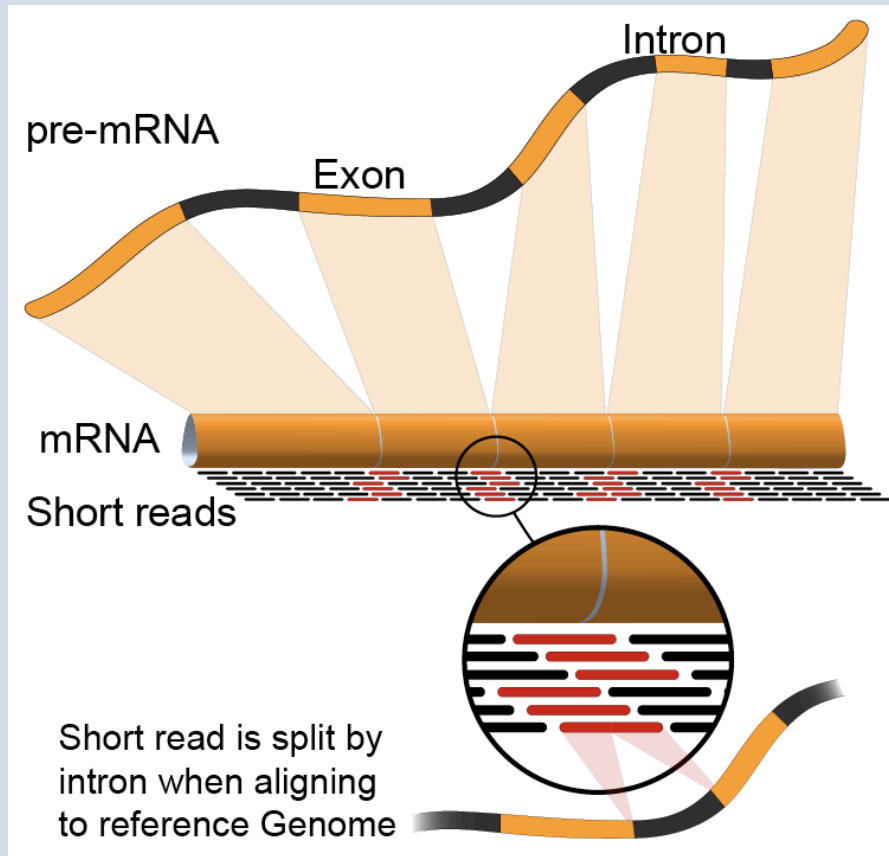
<http://meetings.cshl.edu/courses.html>



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RNA-Seq Module 4 Isoform Discovery and Alternative Expression (lecture)

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Advanced Sequencing Technologies & Applications
November 10 - 22, 2015



Learning objectives of the course

- Module 1: Introduction to RNA Sequencing
- Module 2: Alignment and Visualization
- Module 3: Expression and Differential Expression
- **Module 4: Isoform Discovery and Alternative Expression**

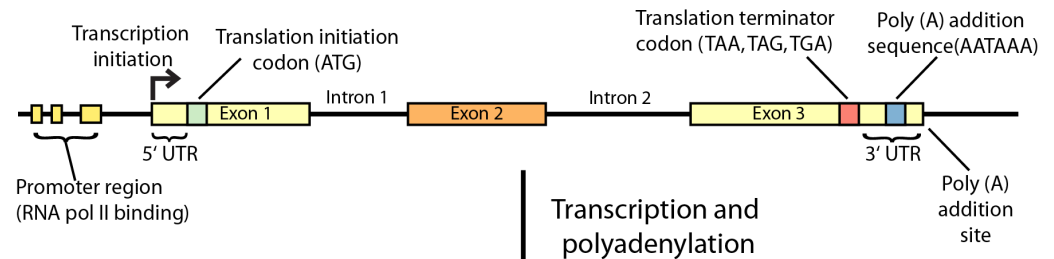
- Tutorials
 - Provide a working example of an RNA-seq analysis pipeline
 - Run in a ‘reasonable’ amount of time with modest computer resources
 - Self contained, self explanatory, portable

Learning Objectives of Module

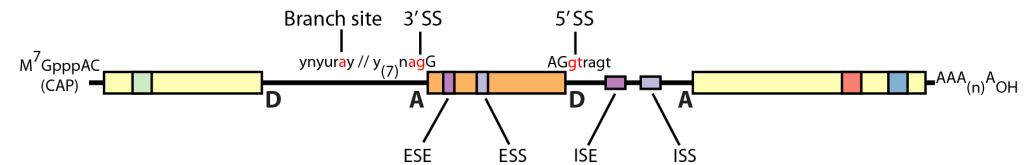
- Explore use of Cufflinks in reference annotation based transcript (RABT) assembly mode and 'de novo' assembly mode.
 - Both modes require a reference genome sequence...

Review of gene expression

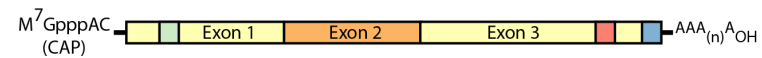
Double-stranded genomic DNA template



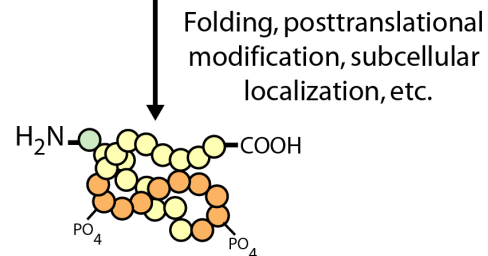
Single-stranded pre-mRNA (nuclear RNA)



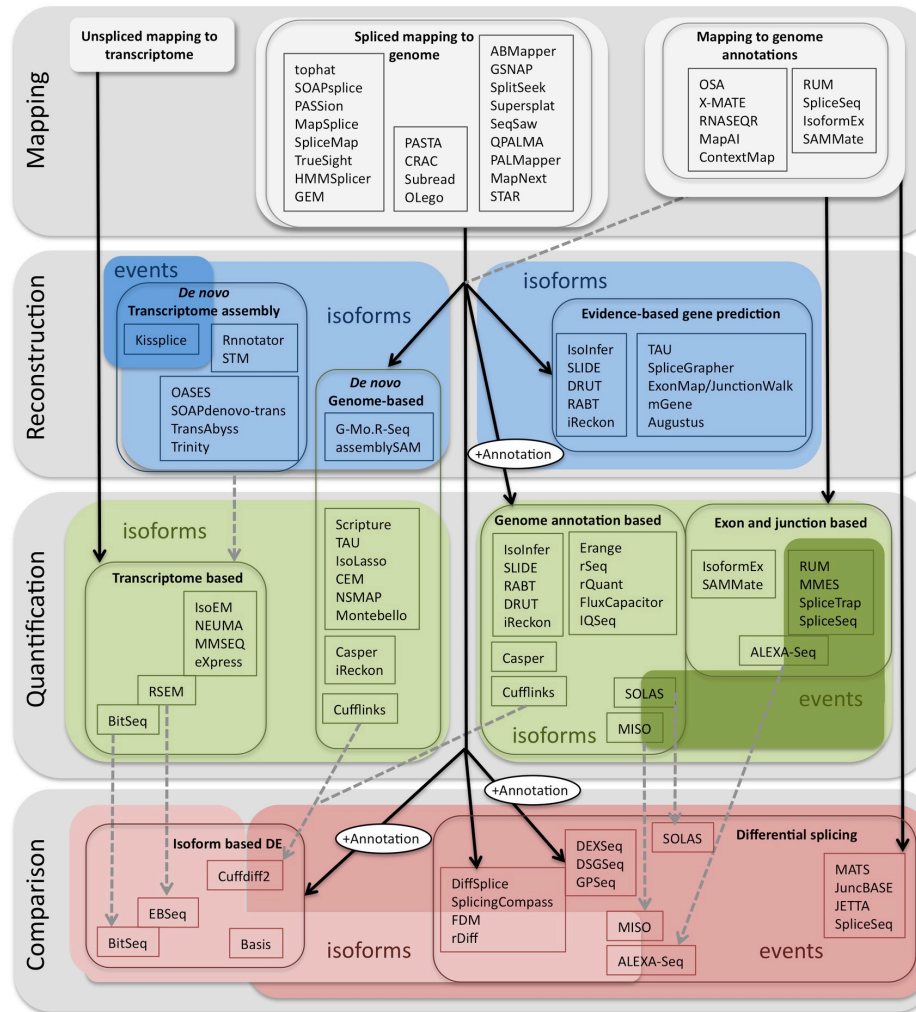
Mature mRNA



Protein (amino acid sequence)



Methods to study splicing by RNA-seq



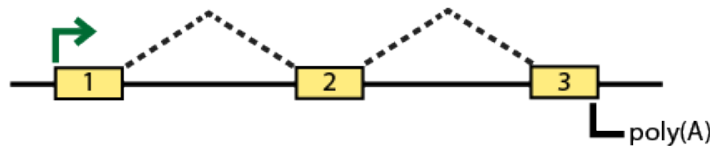
<http://www.rna-seqblog.com/data-analysis/splicing-junction/methods-to-study-splicing-from-rna-seq/>
<http://arxiv.org/ftp/arxiv/papers/1304/1304.5952.pdf>

Useful resources and discussion

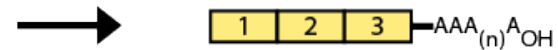
- Best approach to predict novel and alternative splicing events from RNA-seq data
 - <http://www.biostars.org/p/68966/>
 - <http://www.biostars.org/p/62728/>
- Alternative splicing detection
 - <http://www.biostars.org/p/65617/>
 - <http://www.biostars.org/p/11695/>
- Identifying genes that express different isoforms in cancer vs normal RNA-seq data
 - <http://www.biostars.org/p/50365/>
- Cufflinks / Cuffdiff Output - How are tests different?
 - <http://www.biostars.org/p/13525/>
- Visualization of alternative splicing events using RNA-seq data
 - <http://www.biostars.org/p/8979/>

Types of alternative expression - part 1

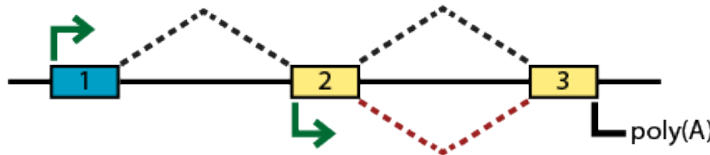
Simple transcription



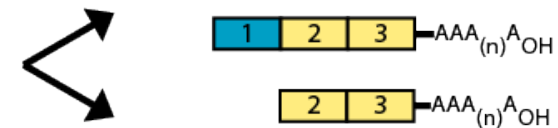
Canonical isoform



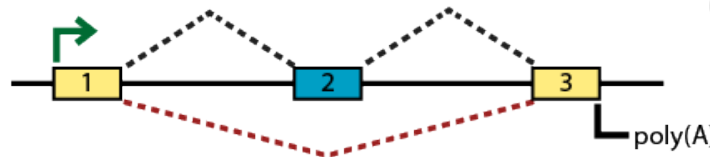
Alternative transcript initiation



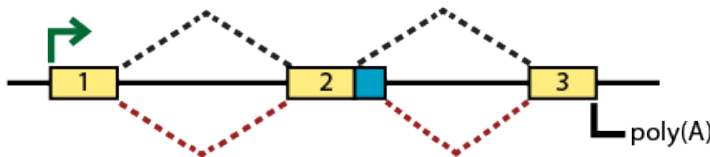
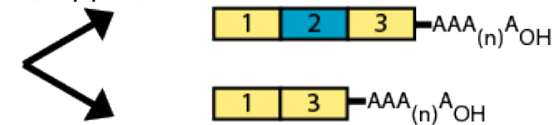
Alternate start sites



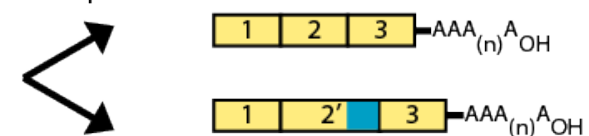
Alternative splicing



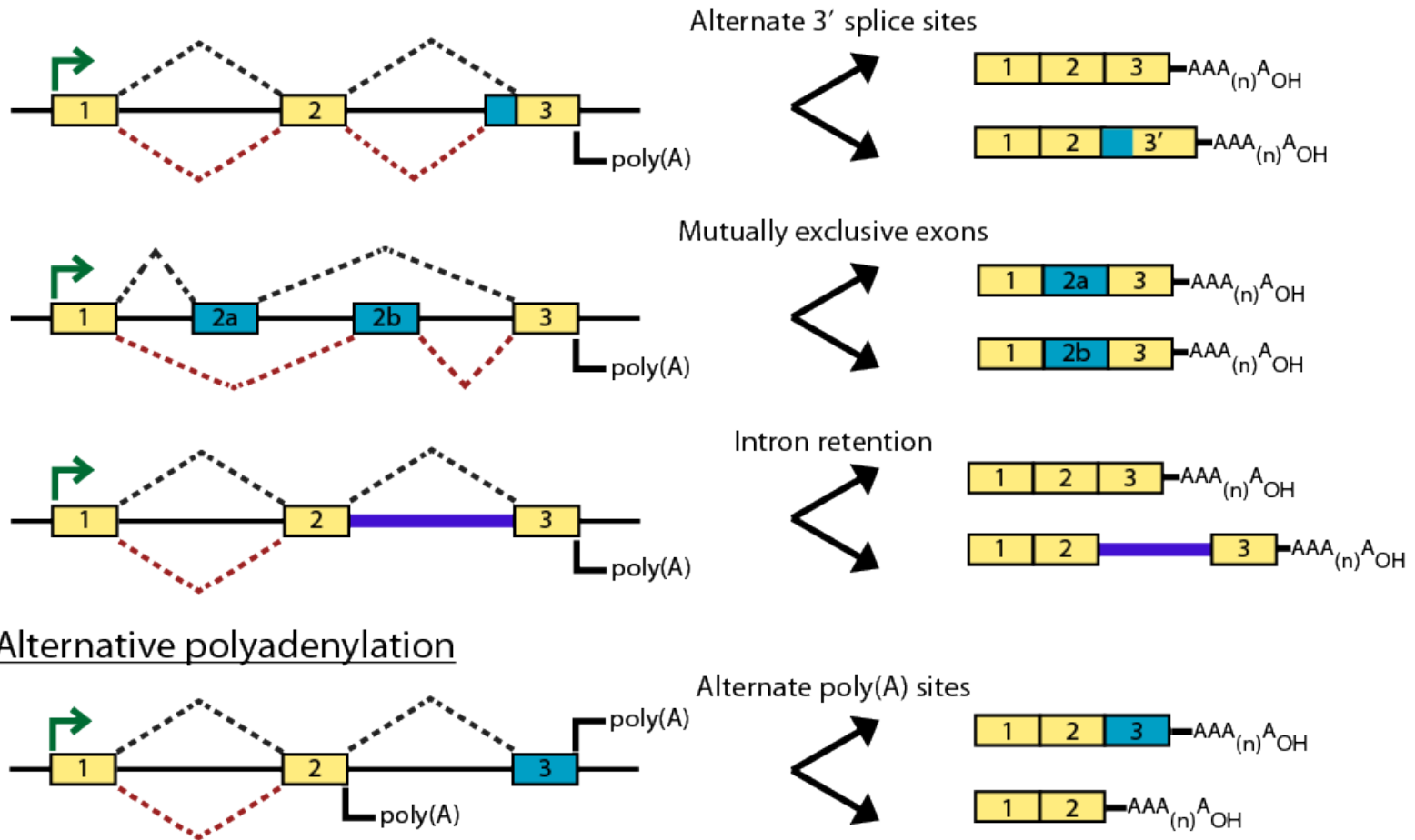
Cassette (skipped) exon



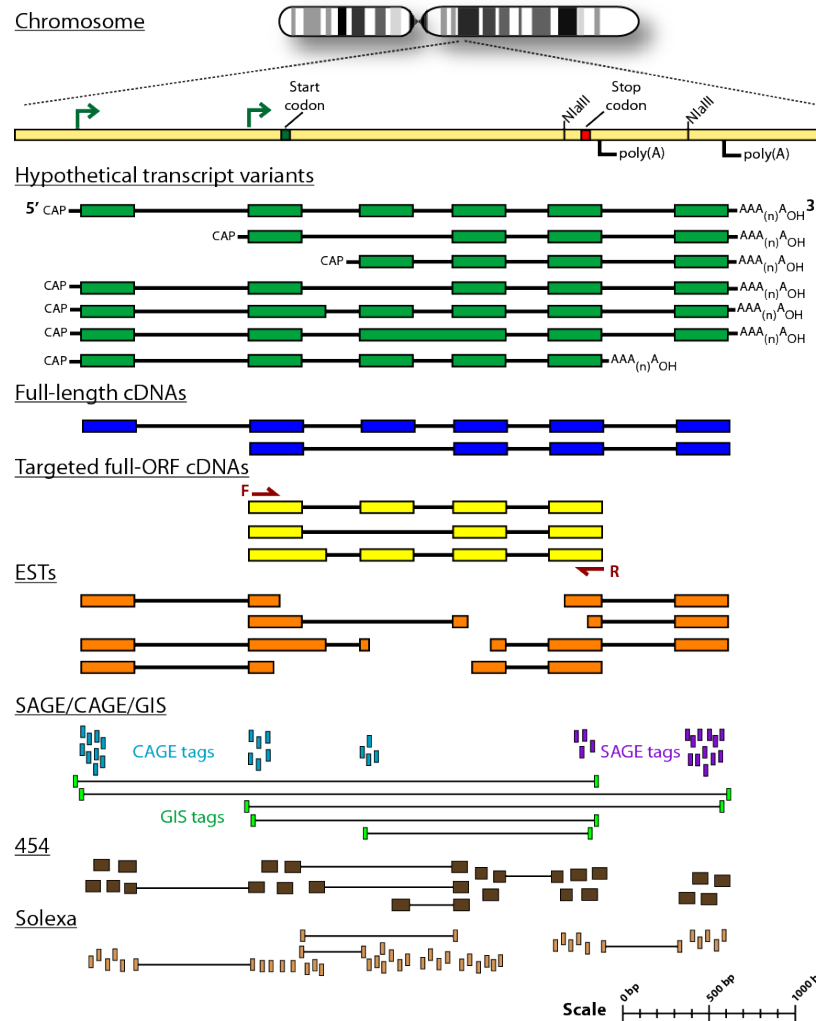
Alternate 5' splice sites



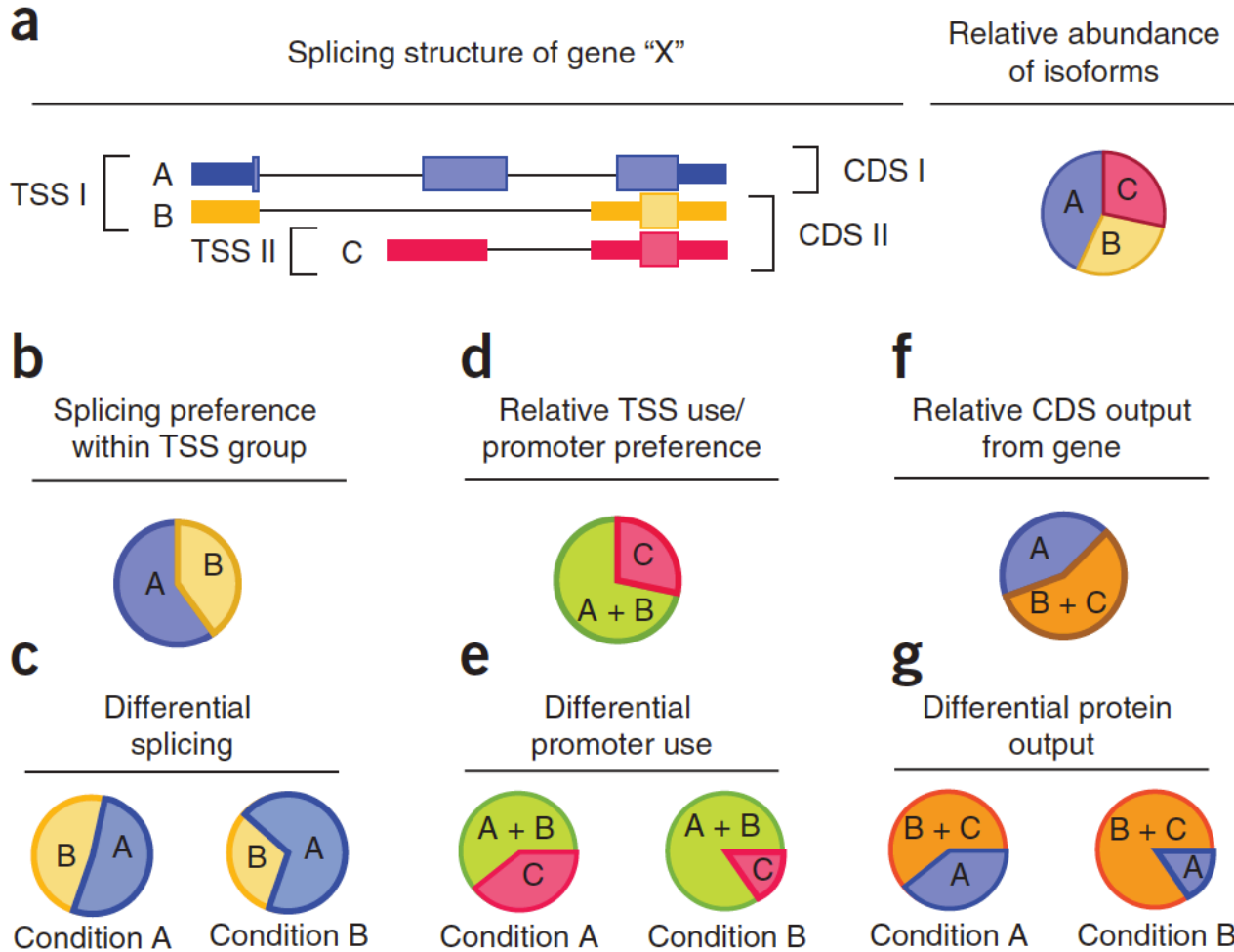
Types of alternative expression – part 2



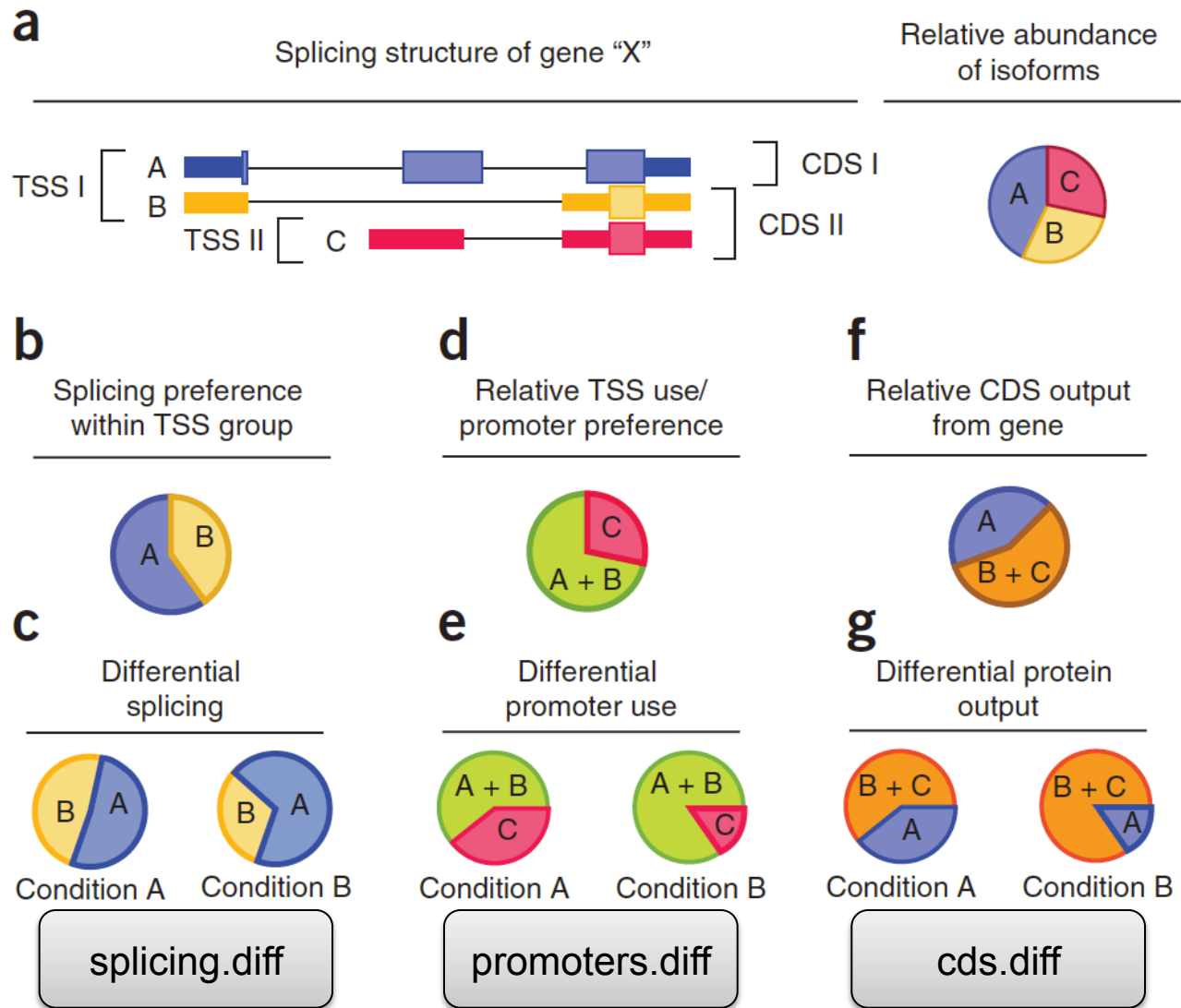
Sequencing methods for studying alternative isoforms



Cufflinks alternative splicing tests

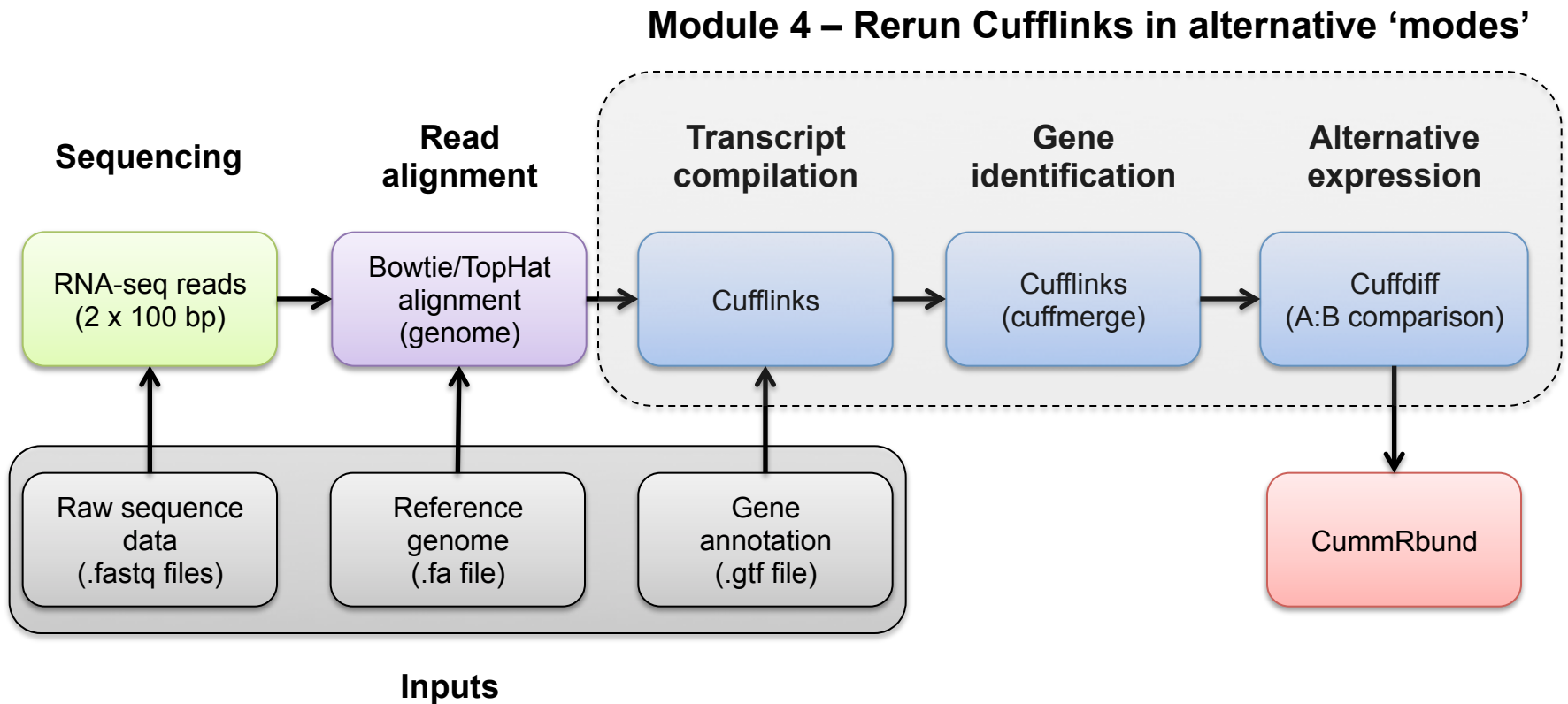


Cufflinks alternative splicing tests



Introduction to tutorial (Module 4)

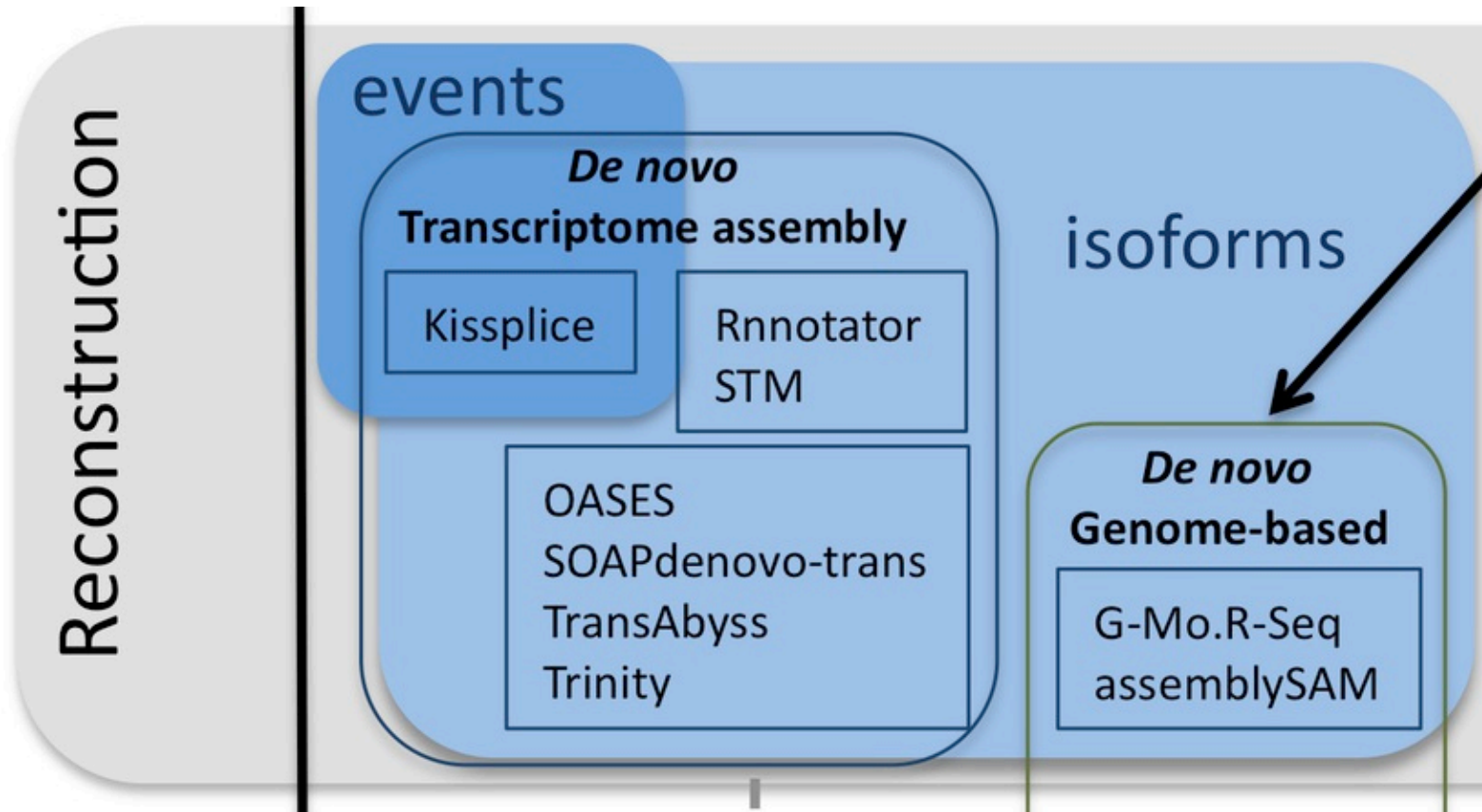
Bowtie/Tophat/Cufflinks/Cuffdiff RNA-seq Pipeline



What if I don't have a reference genome for my species?

- Have you considered sequencing the genome of your species?
- If that is not practical or you simply prefer a transcript discovery approach that does not rely on prior knowledge of the genome or transcriptome there are some tools available ...
 - Unfortunately de novo transcriptome assembly is beyond the scope of this workshop
 - The good news is that the skills you learn here will help you figure out how to install and run those tools yourself

Methods to study splicing by RNA-seq



<http://www.rna-seqblog.com/data-analysis/splicing-junction/methods-to-study-splicing-from-rna-seq/>
<http://arxiv.org/ftp/arxiv/papers/1304/1304.5952.pdf>

Break