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# Advanced Sequencing Technologies & Applications

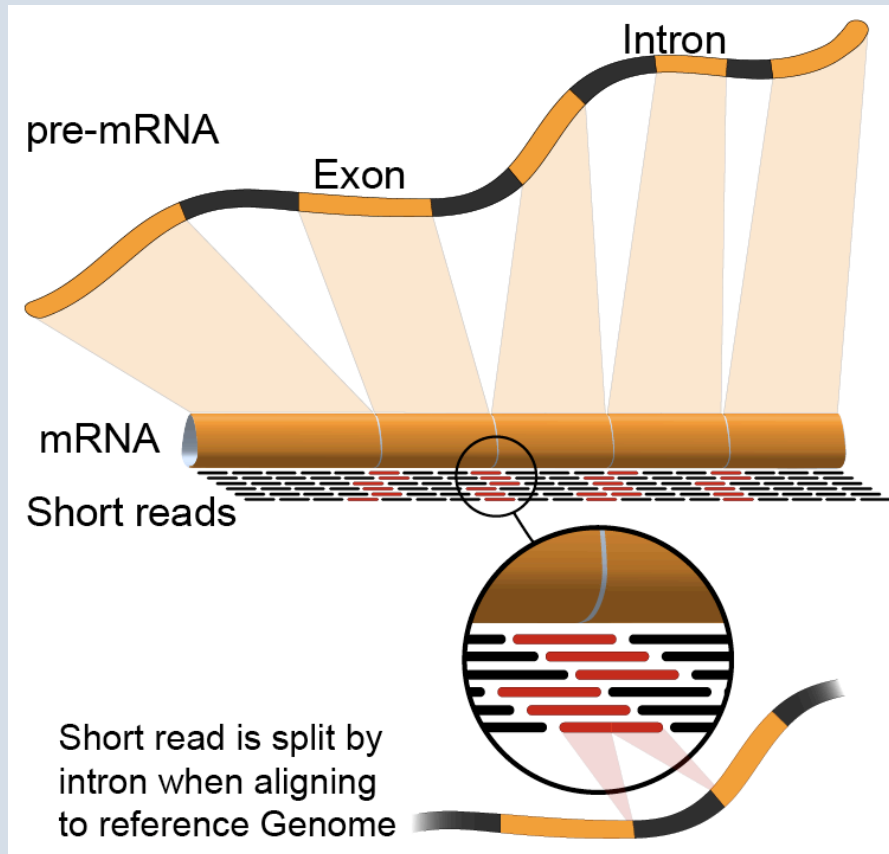
<http://meetings.cshl.edu/courses.html>



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

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Advanced Sequencing Technologies & Applications  
November 7 - 20, 2016



# Learning objectives of the course

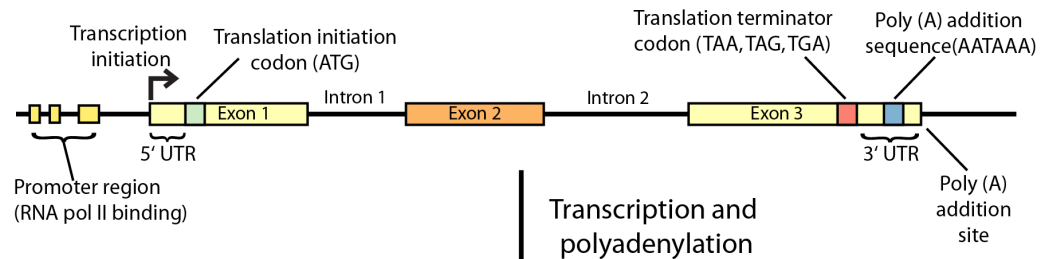
- Module 1: Introduction to RNA Sequencing
- Module 2: Alignment and Visualization
- Module 3: Expression and Differential Expression
- **Module 4: Isoform Discovery and Alternative Expression**
  
- Tutorials
  - Provide a working example of an RNA-seq analysis pipeline
  - Run in a ‘reasonable’ amount of time with modest computer resources
  - Self contained, self explanatory, portable

# Learning objectives of module 4

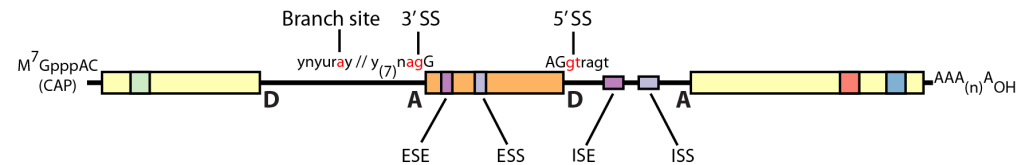
- Explore use of StringTie in modes that facilitate transcript/isoform discovery.
  - This still requires a reference genome sequence...

# Review of gene expression

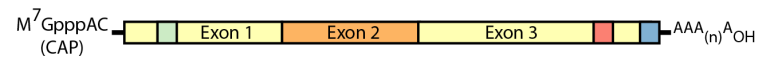
## Double-stranded genomic DNA template



## Single-stranded pre-mRNA (nuclear RNA)



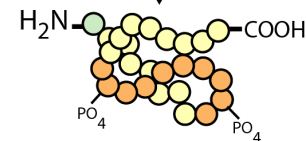
## Mature mRNA



## Protein (amino acid sequence)

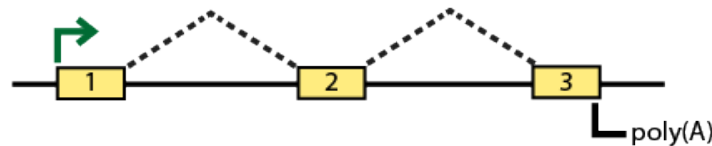


Folding, posttranslational modification, subcellular localization, etc.

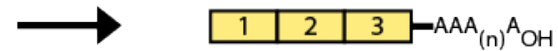


# Types of alternative expression - part 1

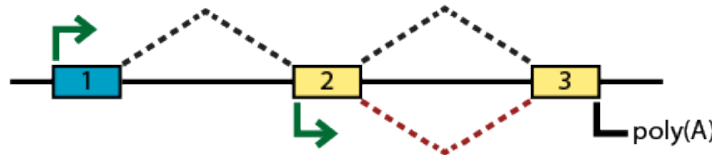
## Simple transcription



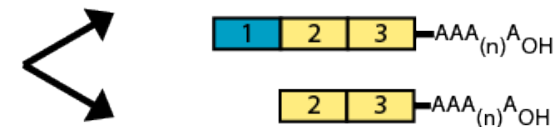
Canonical isoform



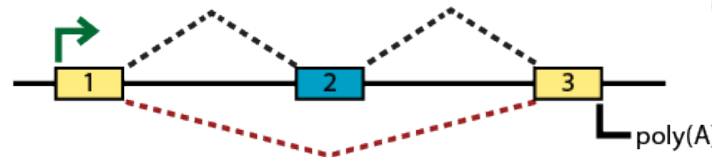
## Alternative transcript initiation



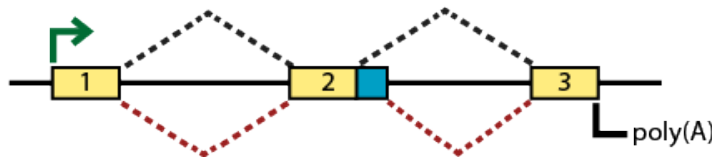
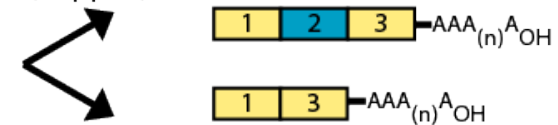
Alternate start sites



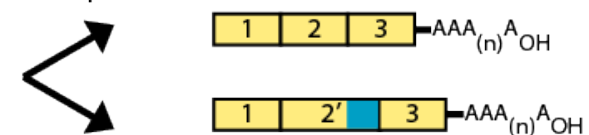
## Alternative splicing



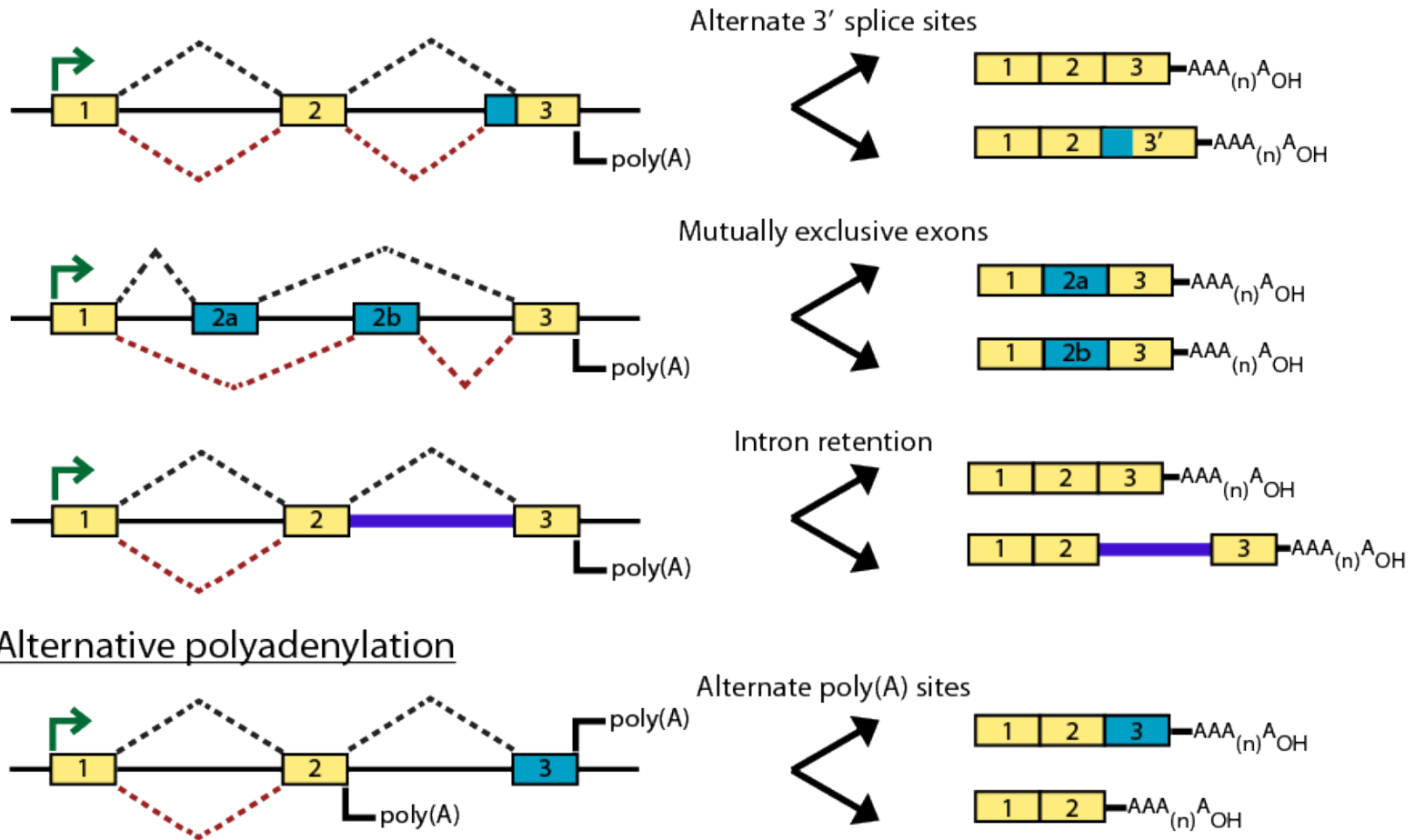
Cassette (skipped) exon



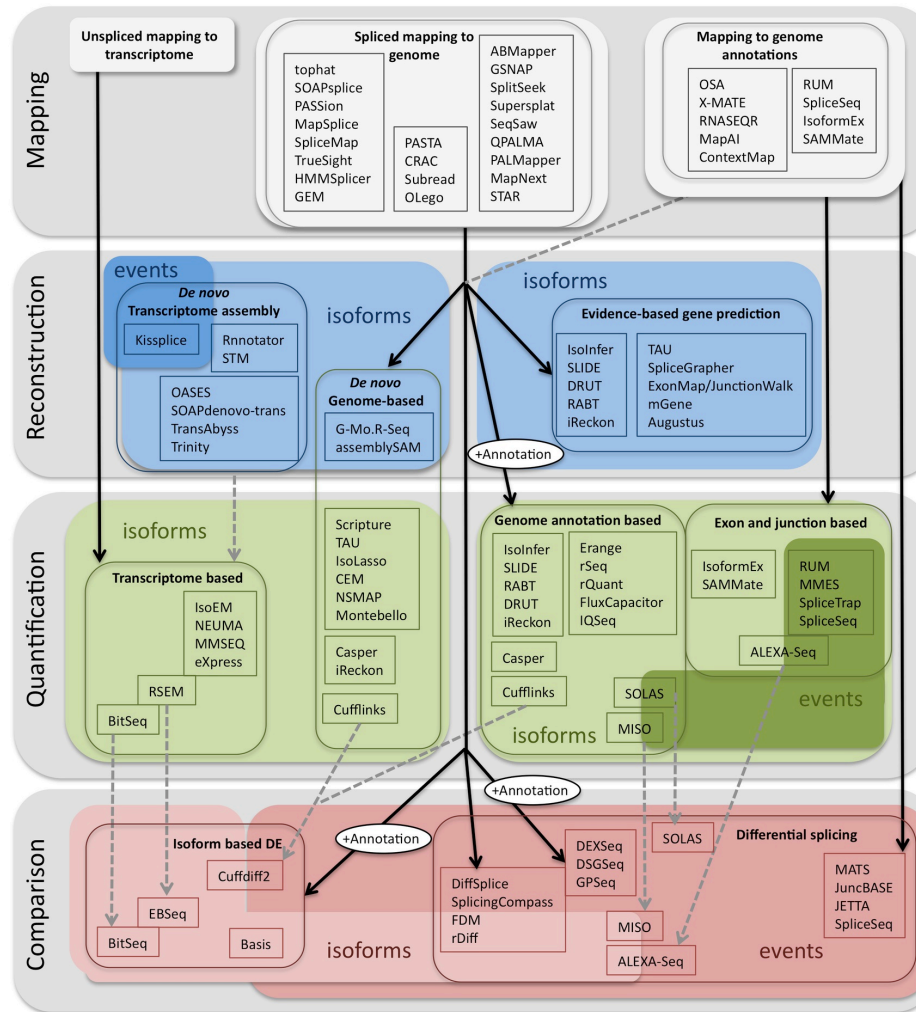
Alternate 5' splice sites



# Types of alternative expression – part 2



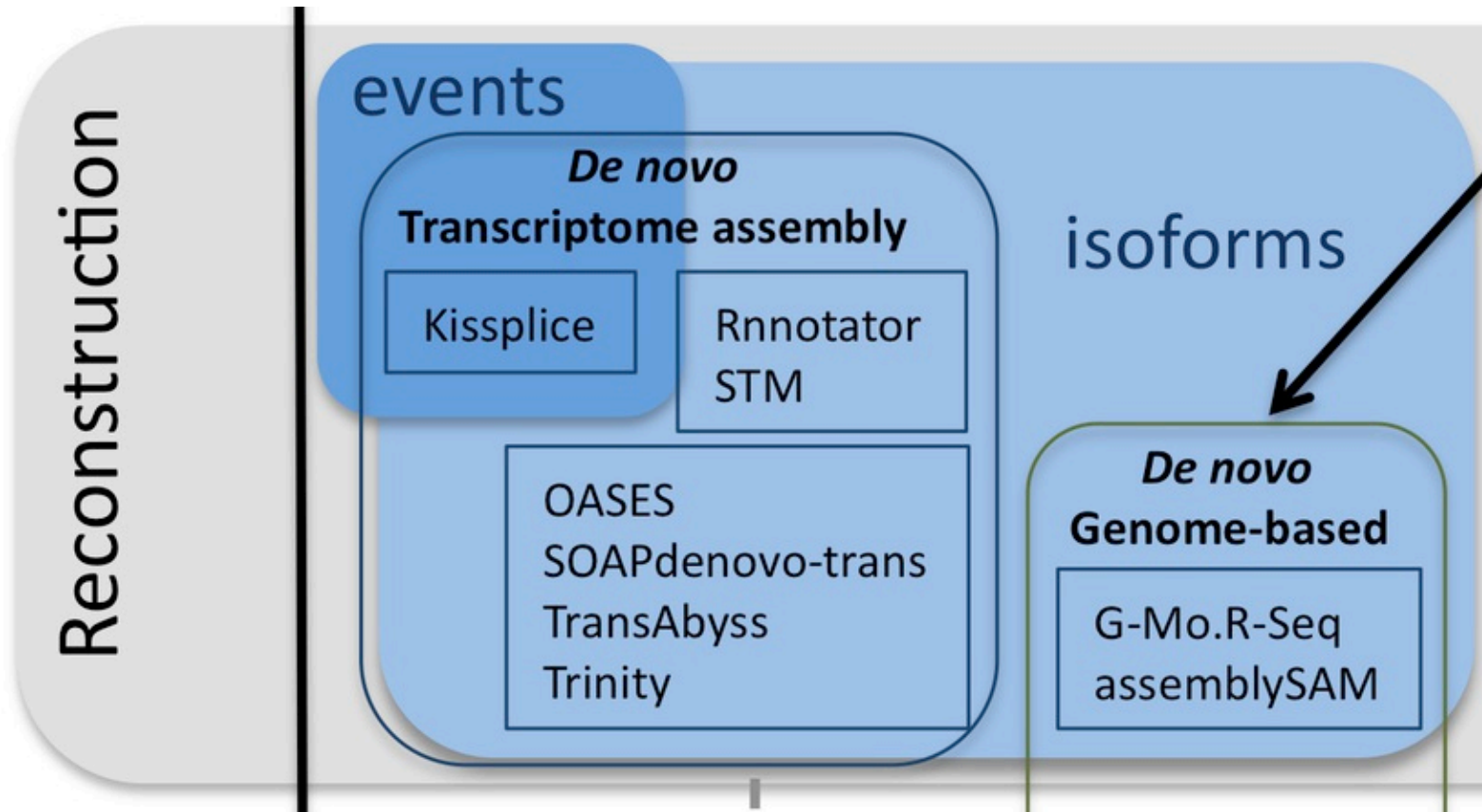
# Methods to study splicing by RNA-seq



<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>



# Methods to study splicing by RNA-seq

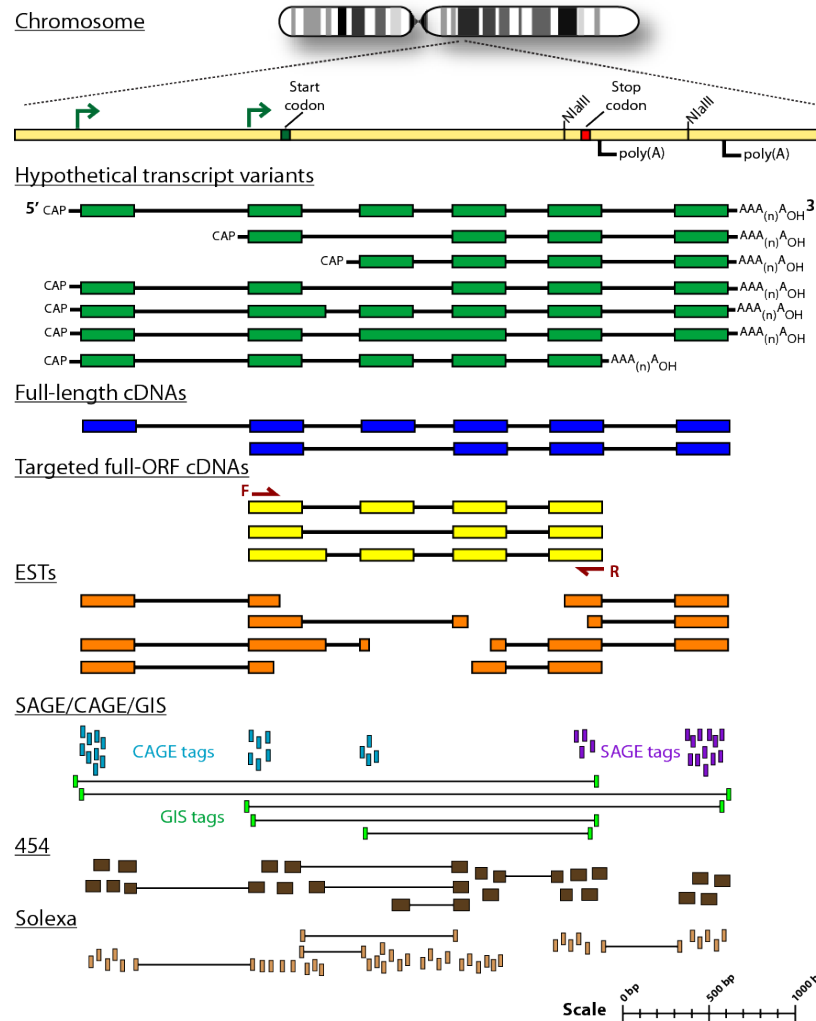


<http://www.rna-seqblog.com/data-analysis/splicing-junction/methods-to-study-splicing-from-rna-seq/>  
<http://arxiv.org/ftp/arxiv/papers/1304/1304.5952.pdf>

# Useful resources and discussion

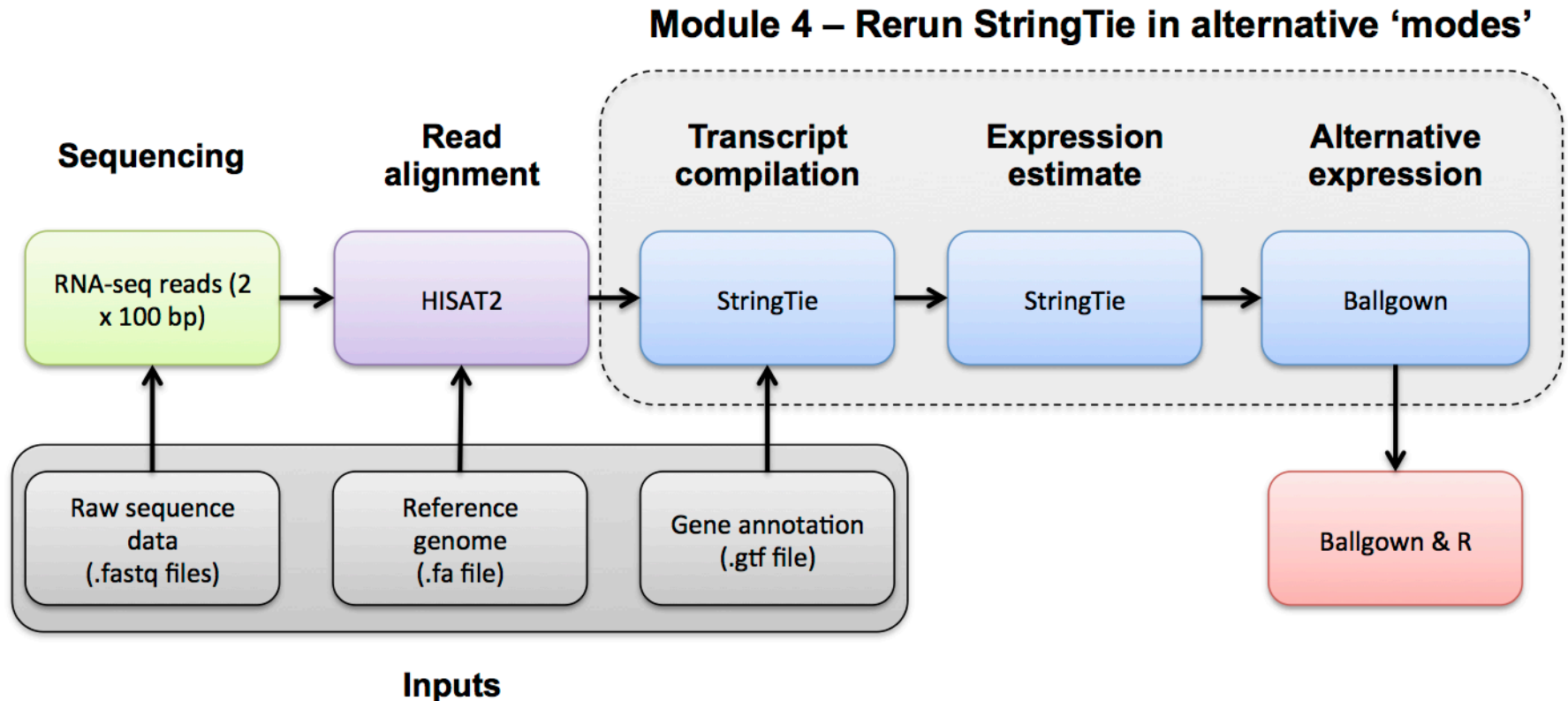
- Best approach to predict novel and alternative splicing events from RNA-seq data
  - <http://www.biostars.org/p/68966/>
  - <http://www.biostars.org/p/62728/>
- Alternative splicing detection
  - <http://www.biostars.org/p/65617/>
  - <http://www.biostars.org/p/11695/>
- Identifying genes that express 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/>

# Sequencing methods for studying alternative isoforms



# **Introduction to tutorial (Module 4)**

# Bowtie/Tophat/Cufflinks/Cuffdiff RNA-seq Pipeline



Break