



Cold
Spring
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Laboratory

Advanced Sequencing Technologies & Applications

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

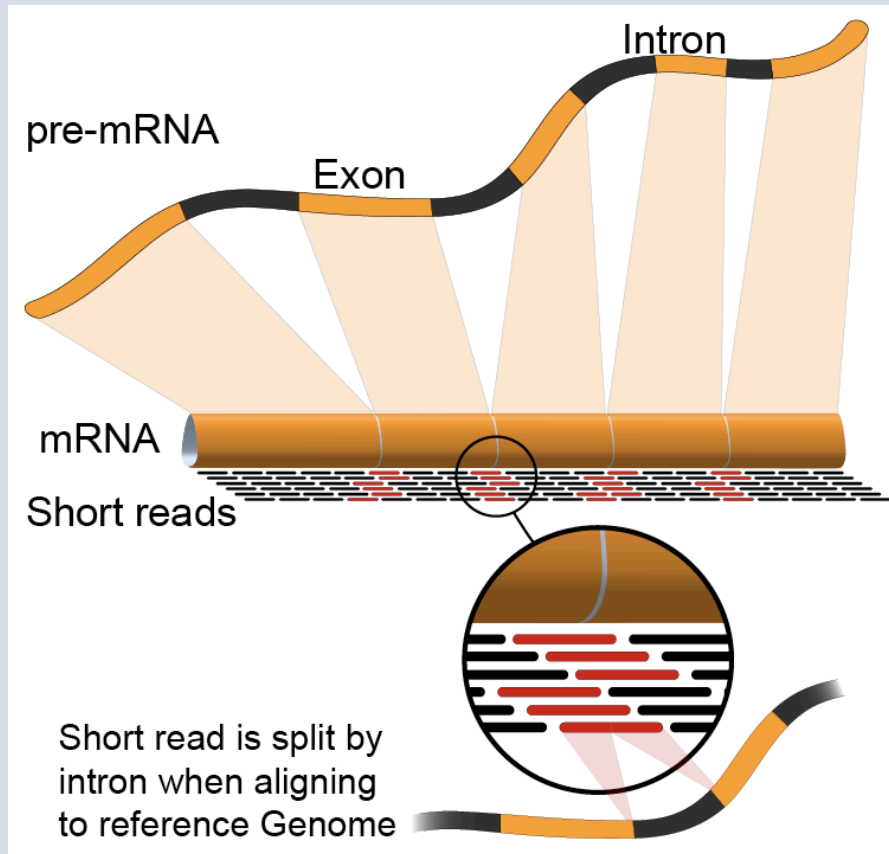


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RNA-Seq Module 4 Discovery and Alternative Expression (lecture)

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Alex Wagner, Jason Walker

Advanced Sequencing Technologies & Applications
November 6- 18, 2018



Learning objectives of the course

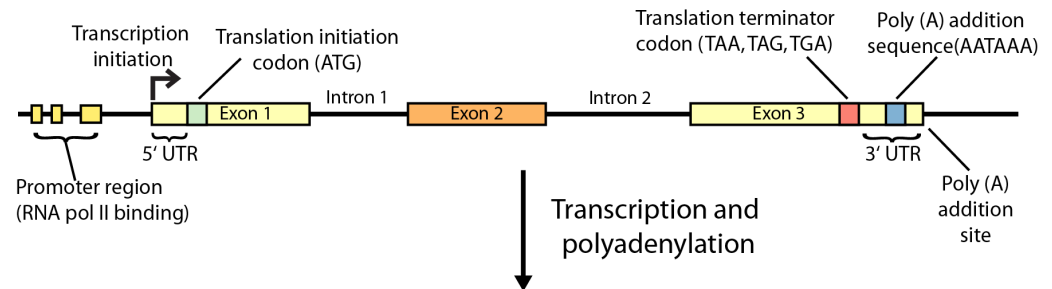
- Module 1: Introduction to RNA Sequencing
- Module 2: Alignment and Visualization
- Module 3: Expression and Differential Expression
- **Module 4: Isoform Discovery and Alternative Expression**
- Tutorials
 - Provide a working example of an RNA-seq analysis pipeline
 - Run in a ‘reasonable’ amount of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

Learning objectives of module 4

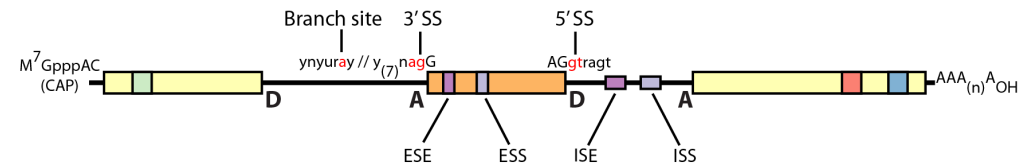
- Explore use of StringTie in modes that facilitate transcript/isoform discovery.
 - This still requires a reference genome sequence...

Review of gene expression

Double-stranded genomic DNA template

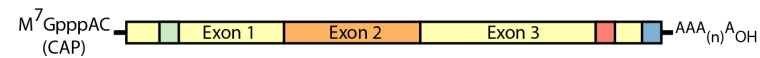


Single-stranded pre-mRNA (nuclear RNA)



RNA processing

Mature mRNA

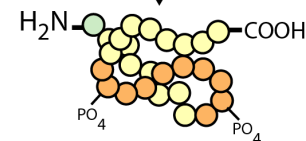


Export to cytoplasm and translation

Protein (amino acid sequence)

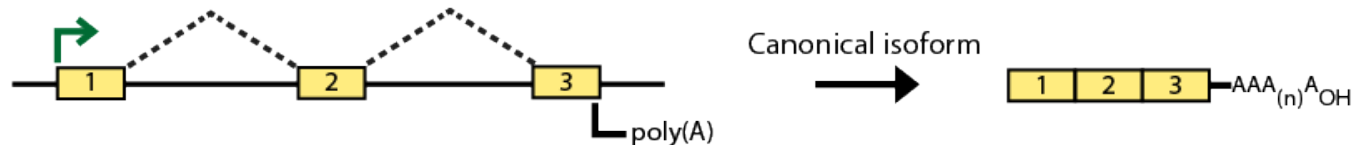


Folding, posttranslational modification, subcellular localization, etc.

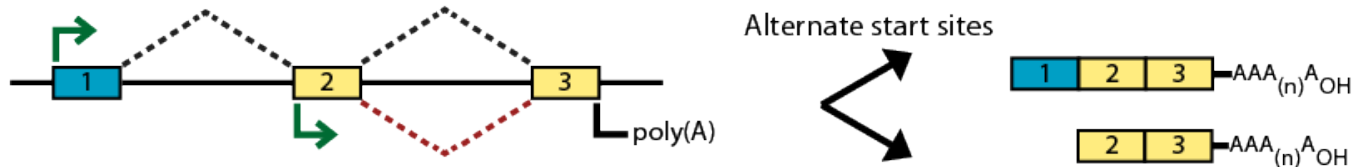


Types of alternative expression - part 1

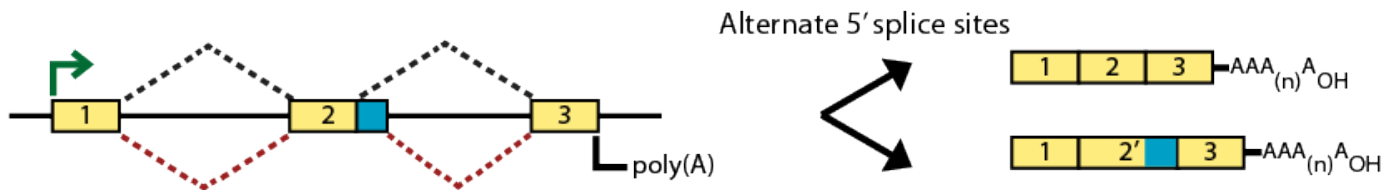
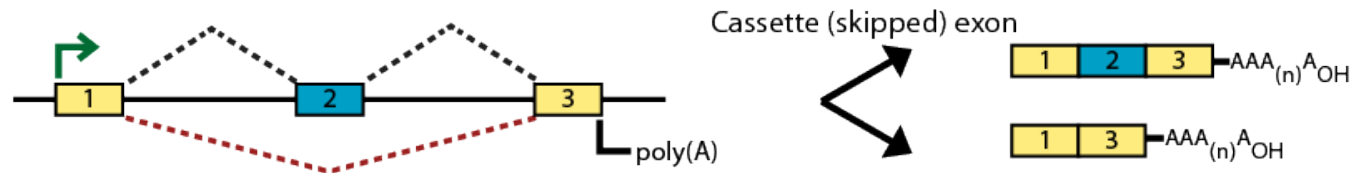
Simple transcription



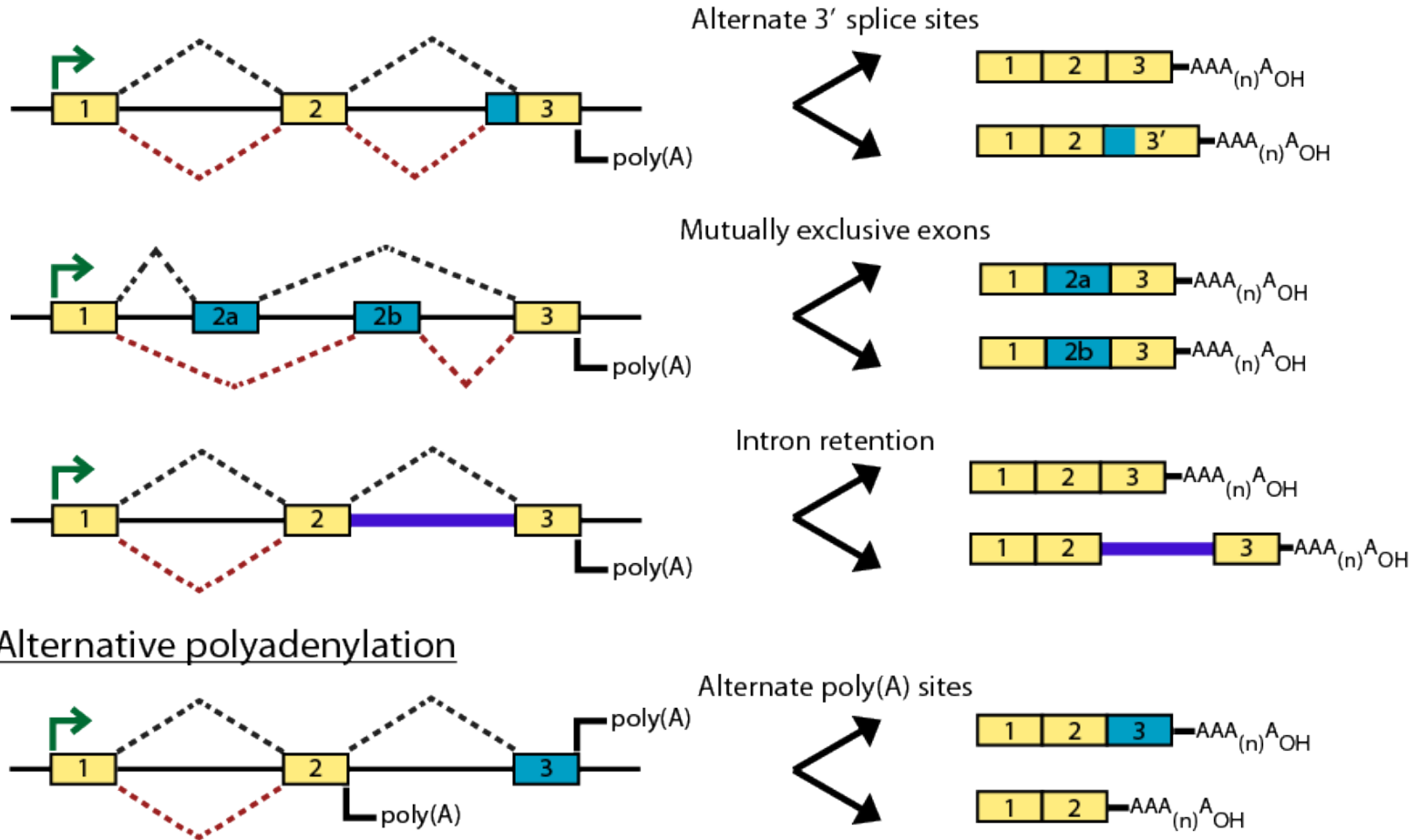
Alternative transcript initiation



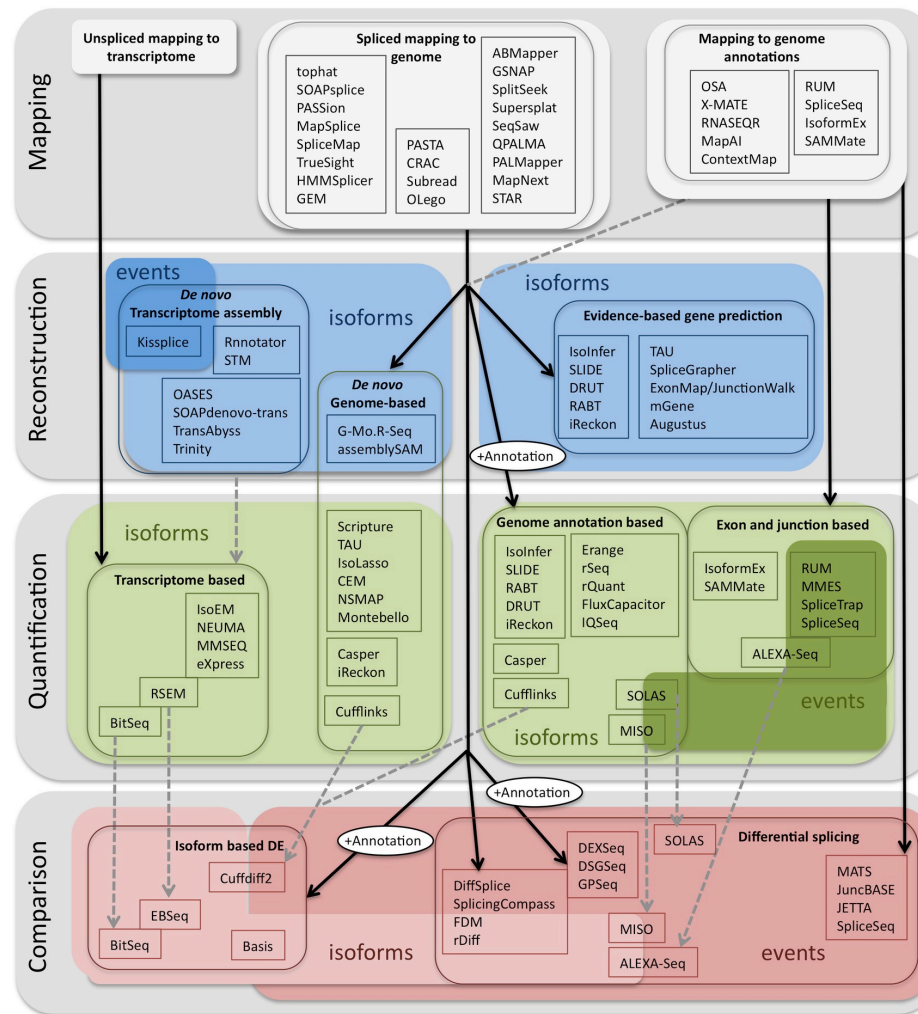
Alternative splicing



Types of alternative expression – part 2

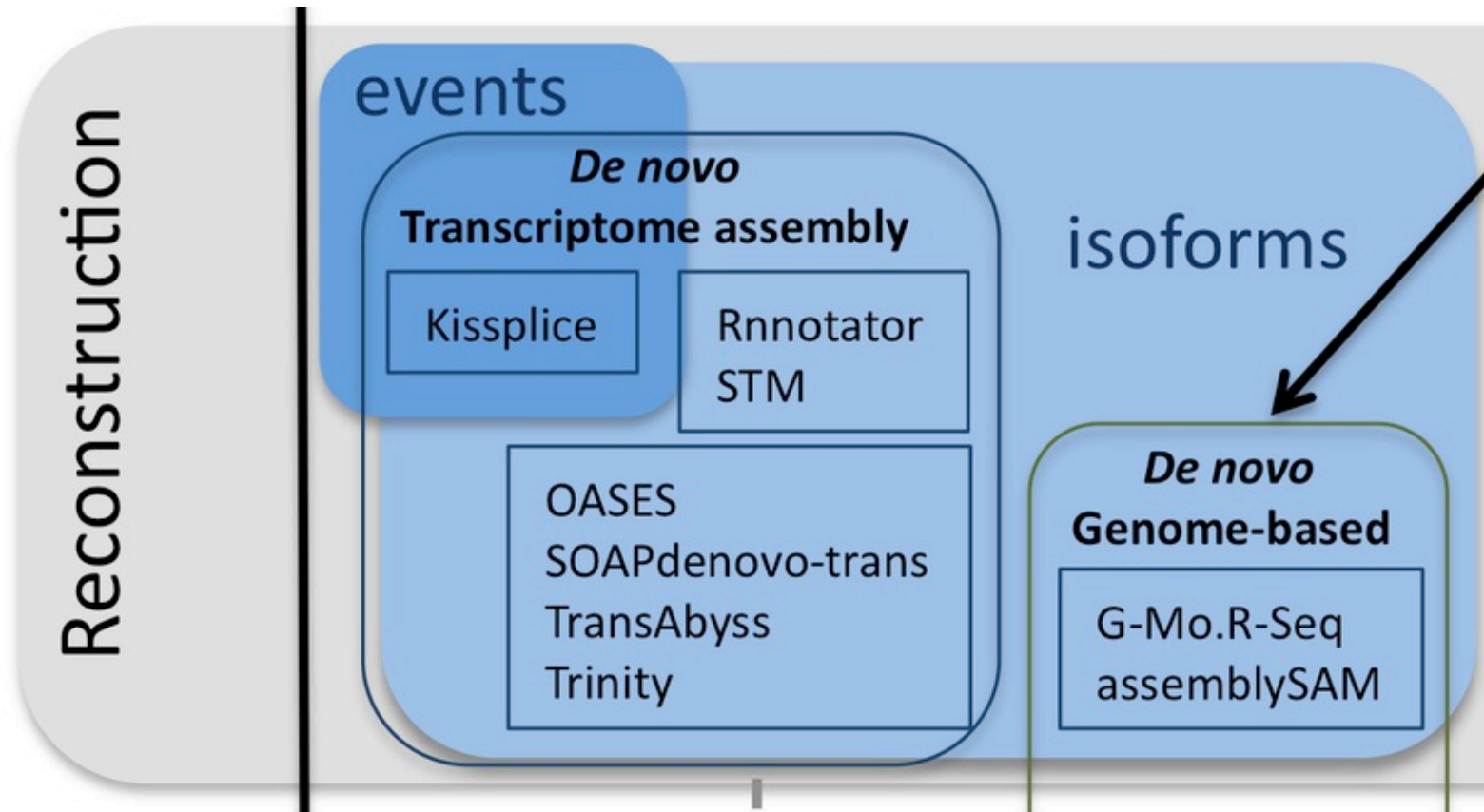


Methods to study splicing by RNA-seq



<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

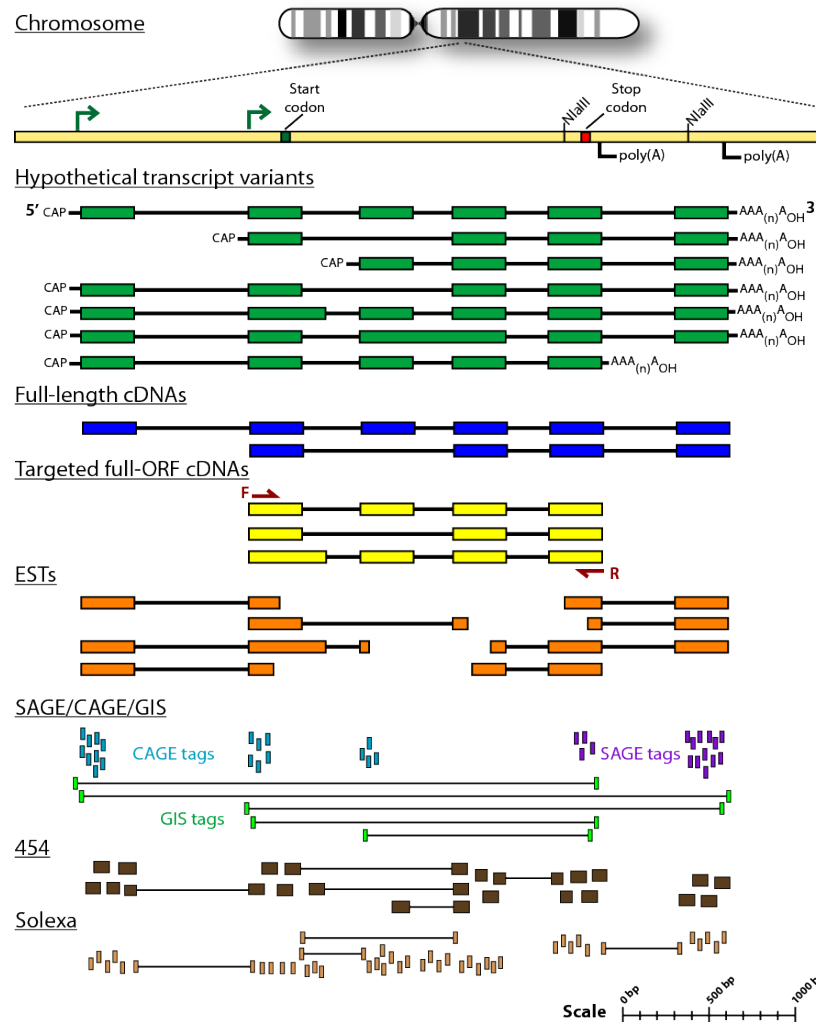


<http://www.rna-seqblog.com/data-analysis/splicing-junction/methods-to-study-splicing-from-rna-seq/>
<http://arxiv.org/ftp/arxiv/papers/1304/1304.5952.pdf>

Useful resources and discussion

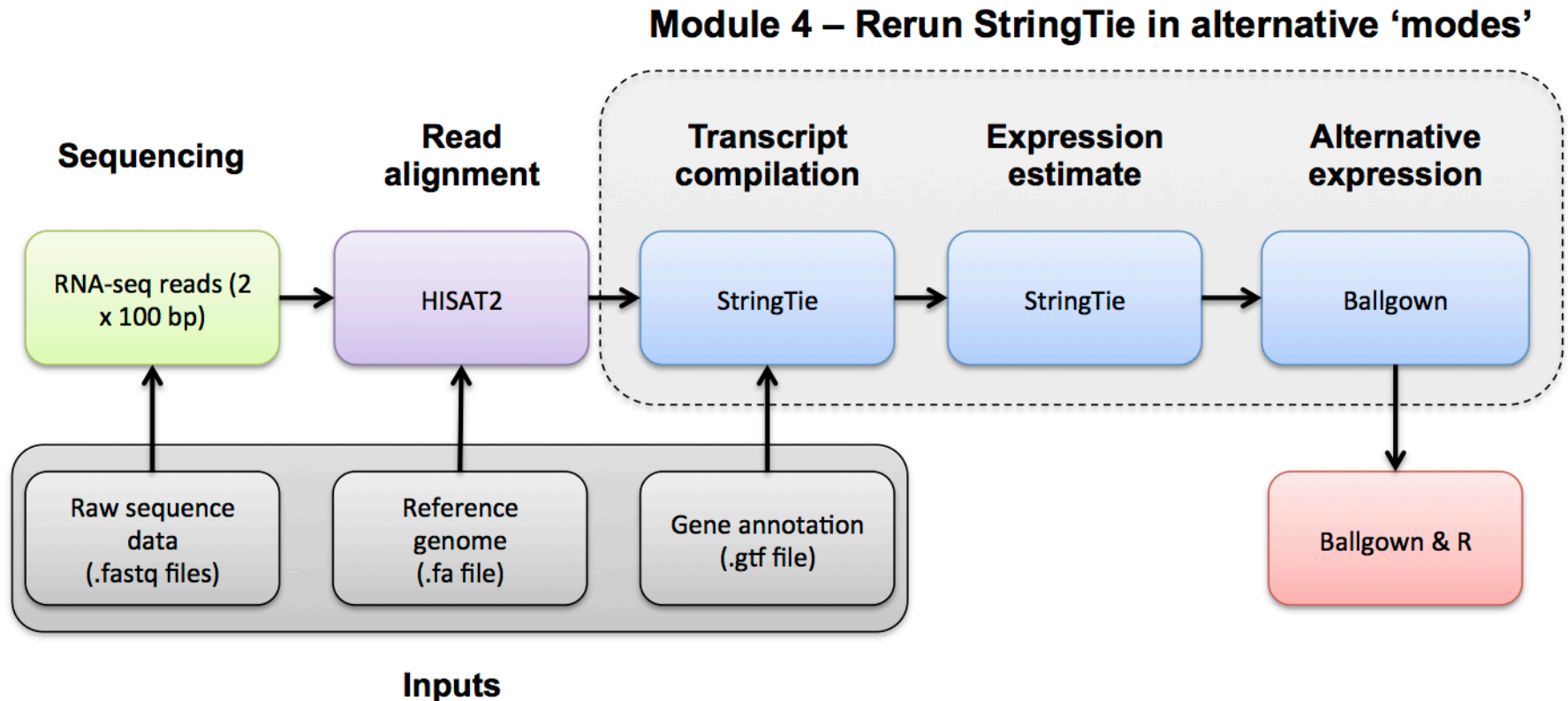
- Best approach to predict novel and alternative splicing events from RNA-seq data
 - <http://www.biostars.org/p/68966/>
 - <http://www.biostars.org/p/62728/>
- Alternative splicing detection
 - <http://www.biostars.org/p/65617/>
 - <http://www.biostars.org/p/11695/>
- Identifying genes that express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

HiSat/StringTie/Ballgown RNA-seq Pipeline



We are on a Coffee Break &
Networking Session