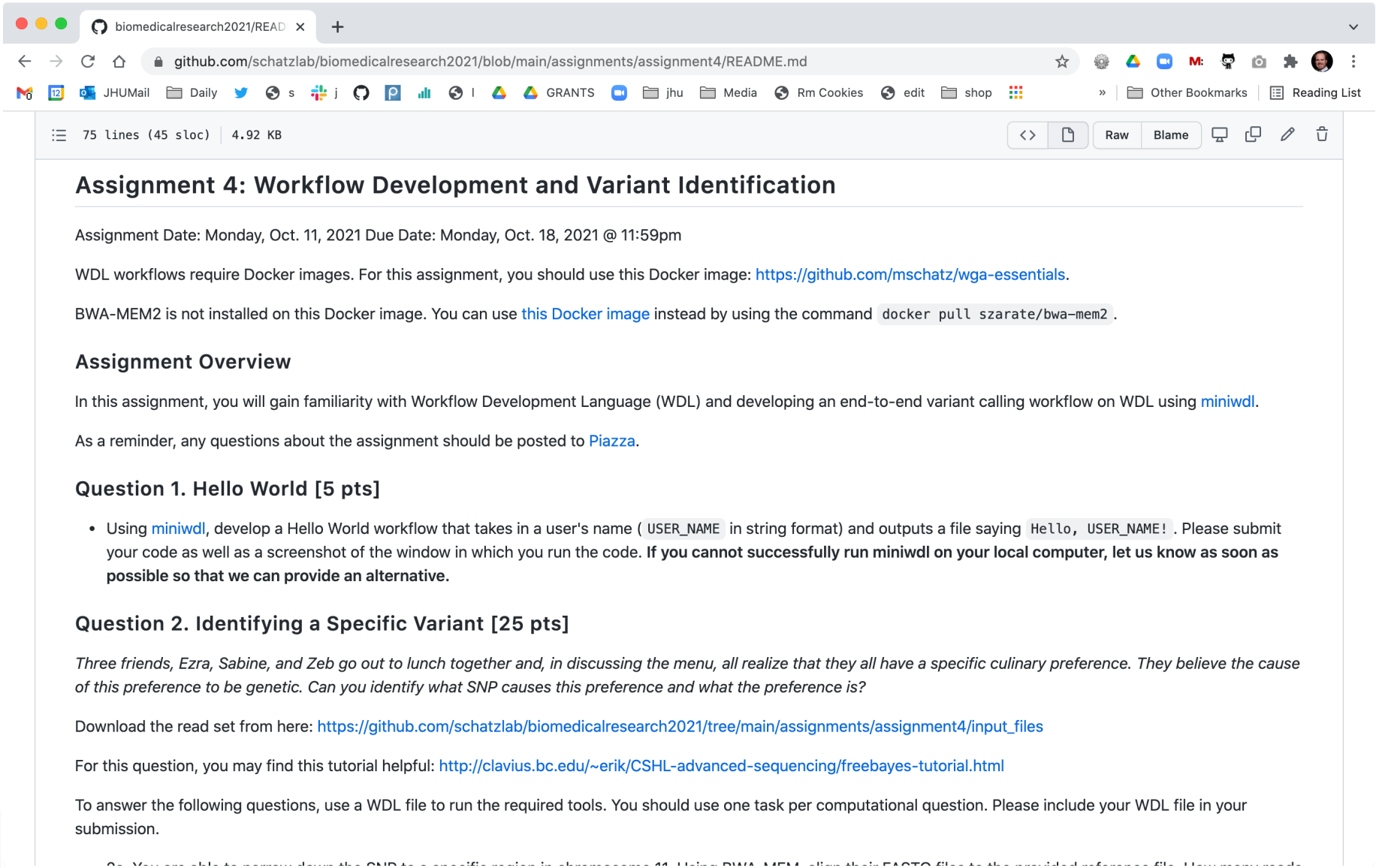
Lecture 14. RNAseq

Michael Schatz

October 18, 2021

Advanced Biomedical Research

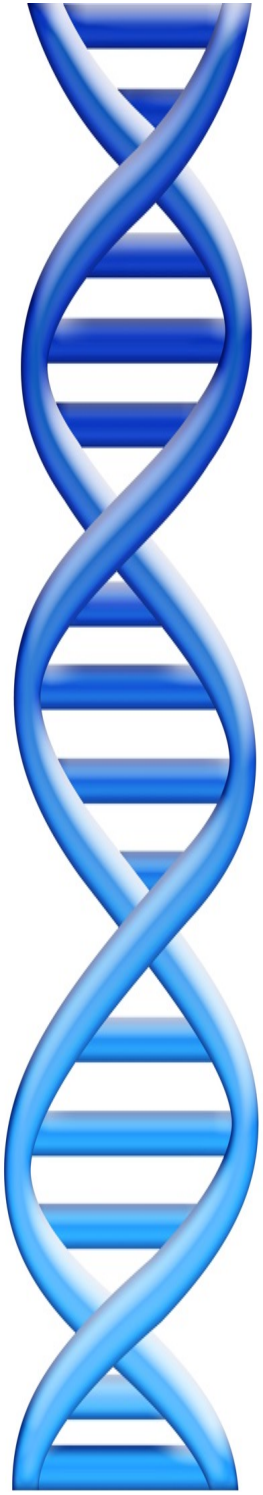
Assignment 4: WDLs
Due Oct 18 @ 11:59pm



https://github.com/schatzlab/biomedicalresearch2021

Goal: Genome Annotations

Gene!



Outline

1. Alignment to other genomes
2. Prediction aka "Gene Finding"
3. Experimental & Functional Assays

Basic Local Alignment Search Tool

- Rapidly compare a sequence Q to a database to find all sequences in the database with an score above some cutoff S.
  - Which protein is most similar to a newly sequenced one?
  - Where does this sequence of DNA originate?

- Speed achieved by using a procedure that typically finds "most" matches with scores > S.
  - Tradeoff between sensitivity and specificity/speed
    - Sensitivity – ability to find all related sequences
    - Specificity – ability to reject unrelated sequences

(Altschul et al. 1990)

Very Similar Sequences

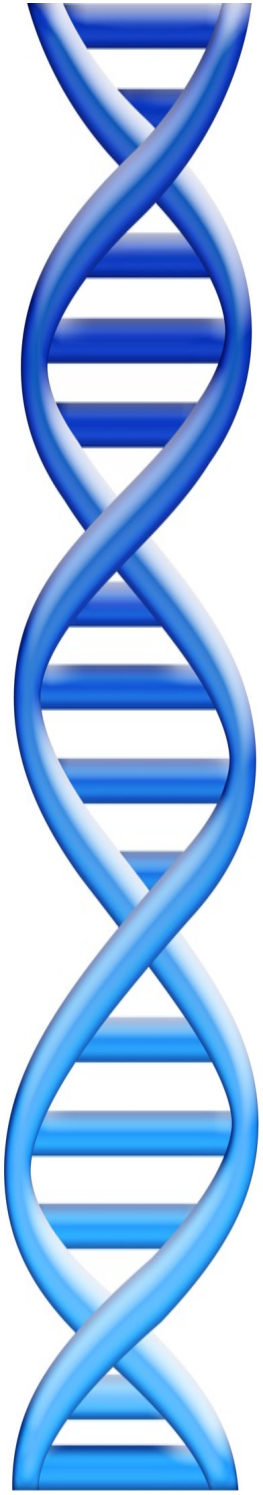
```
Query: HBA_HUMAN Hemoglobin alpha subunit
Sbjct: HBB_HUMAN Hemoglobin beta subunit

Score =  114 bits (285),  Expect = 1e-26
Identities = 61/145 (42%), Positives = 86/145 (59%), Gaps = 8/145 (5%)

Query  2   LSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHF------DLSHGSAQV 55
           L+P +K+ V A WGKV  +  E G EAL R+ + +P T+ +F  F      D   G+ +V
Sbjct  3   LTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKV 60

Query  56  KGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPA 115
           K HGKKV  A ++ +AH+D++    + LS+LH  KL VDP NF+LL + L+  LA H
Sbjct  61  KAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGK 120

Query  116 EFTPAVHASLDKFLASVSTVLTSKY 140
           EFTP V A+  K +A V+  L  KY
Sbjct  121 EFTPPVQAAYQKVVAGVANALAHKY 145
```



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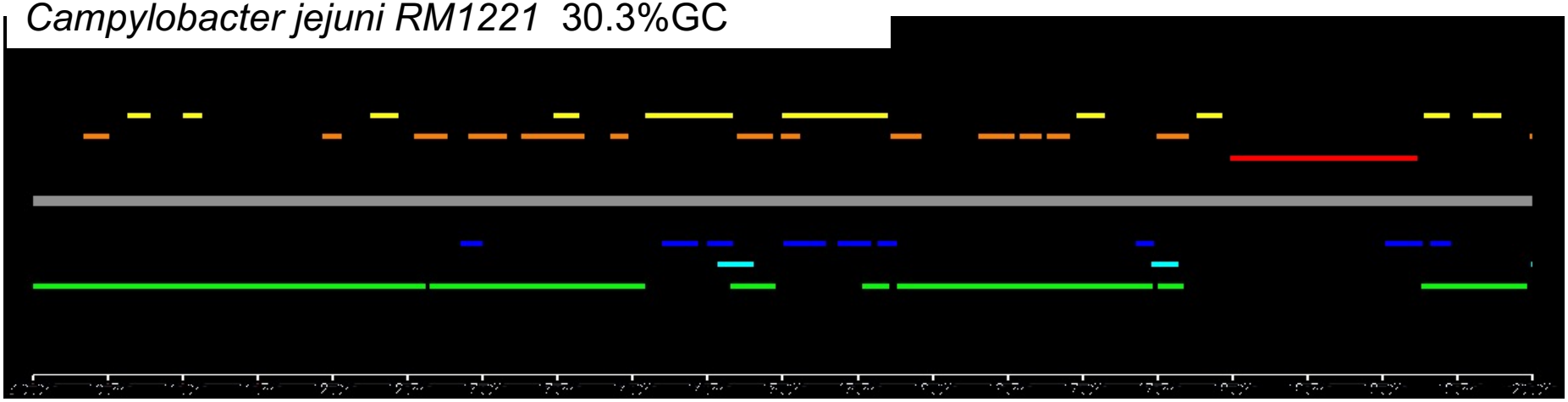
Campylobacter jejuni RM1221 30.3%GC



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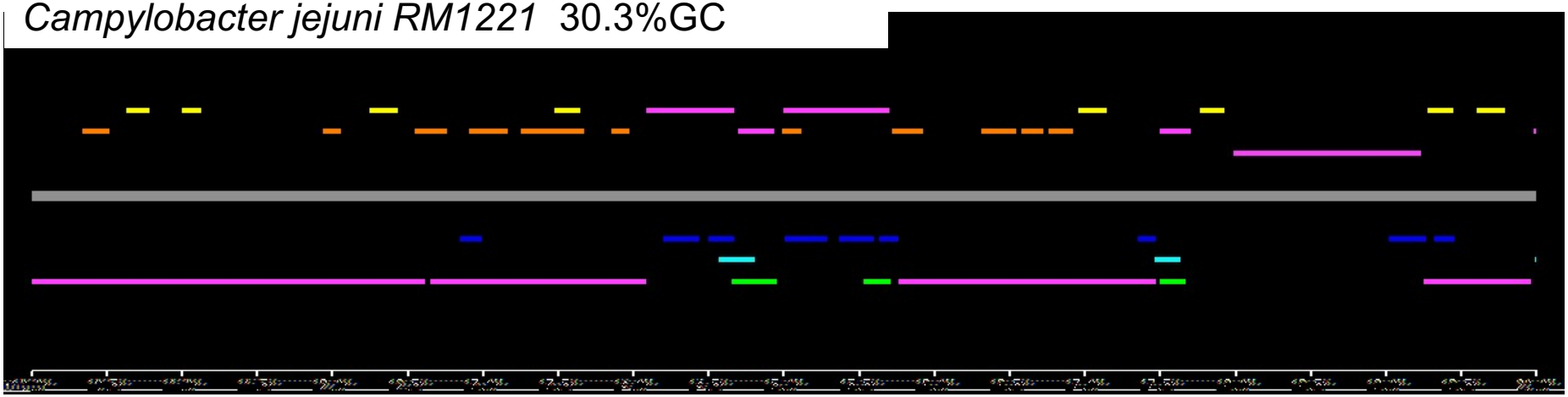
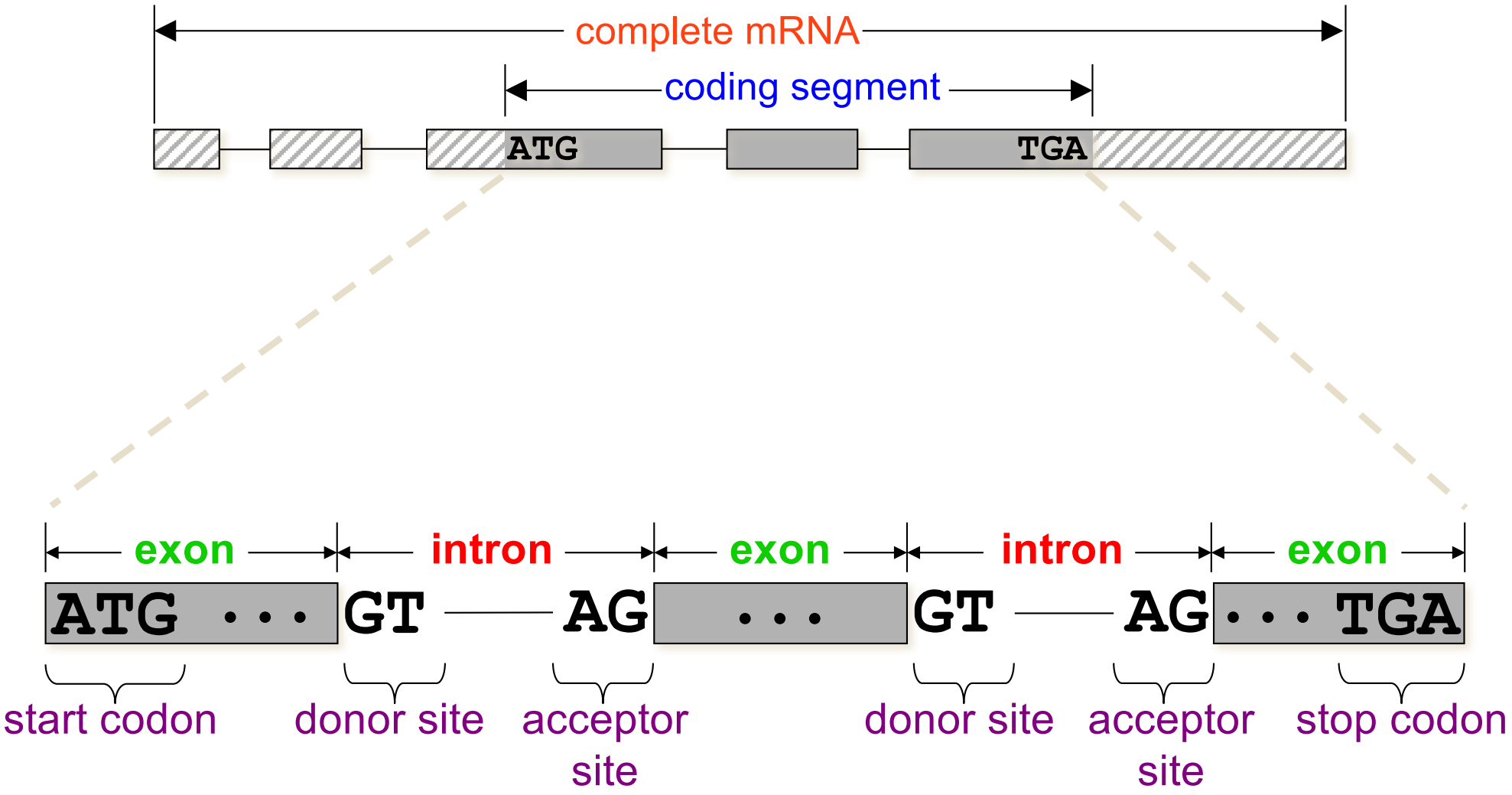


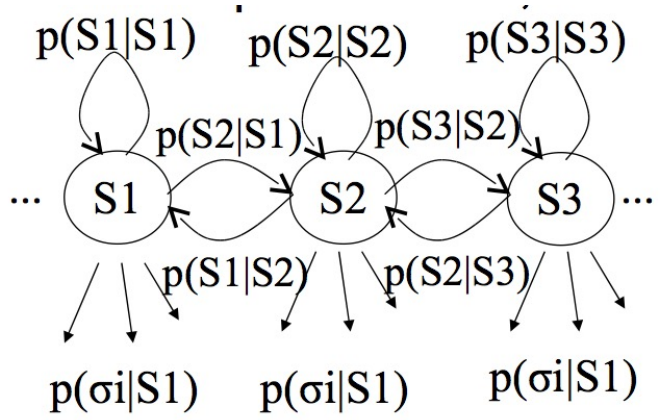
Eukaryotic Gene Syntax



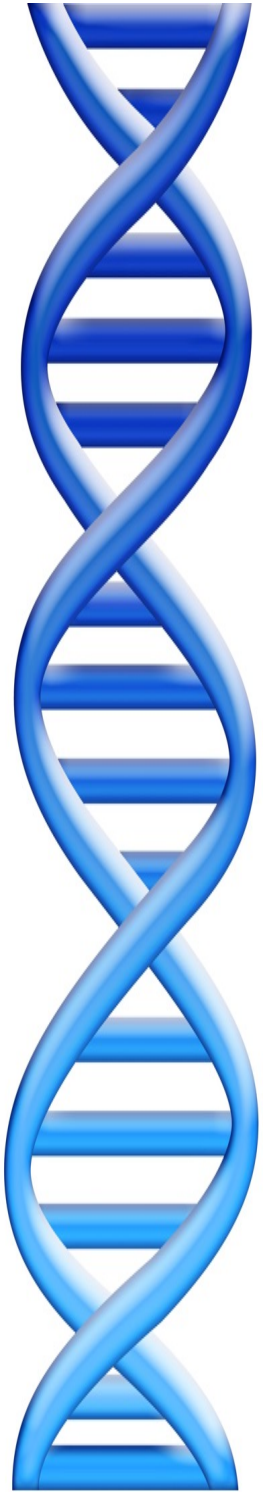
Regions of the gene outside of the CDS are called *UTR*'s (*untranslated regions*), and are mostly ignored by gene finders, though they are important for regulatory functions.

HMMs for Gene Finding

- Similar to Markov models used for prokaryotic gene finding, but system may transition between multiple models called states (gene/non-gene, intergenic/exon/intron)
- Observers can see the emitted symbols of an HMM (i.e., nucleotides) but have no ability to know which state the HMM is currently in.
  - But we can ***infer*** the most likely hidden states of an HMM based on the given sequence of emitted symbols.



AAAGCATGCATTTAACGTGAGCACAATAGATTACA



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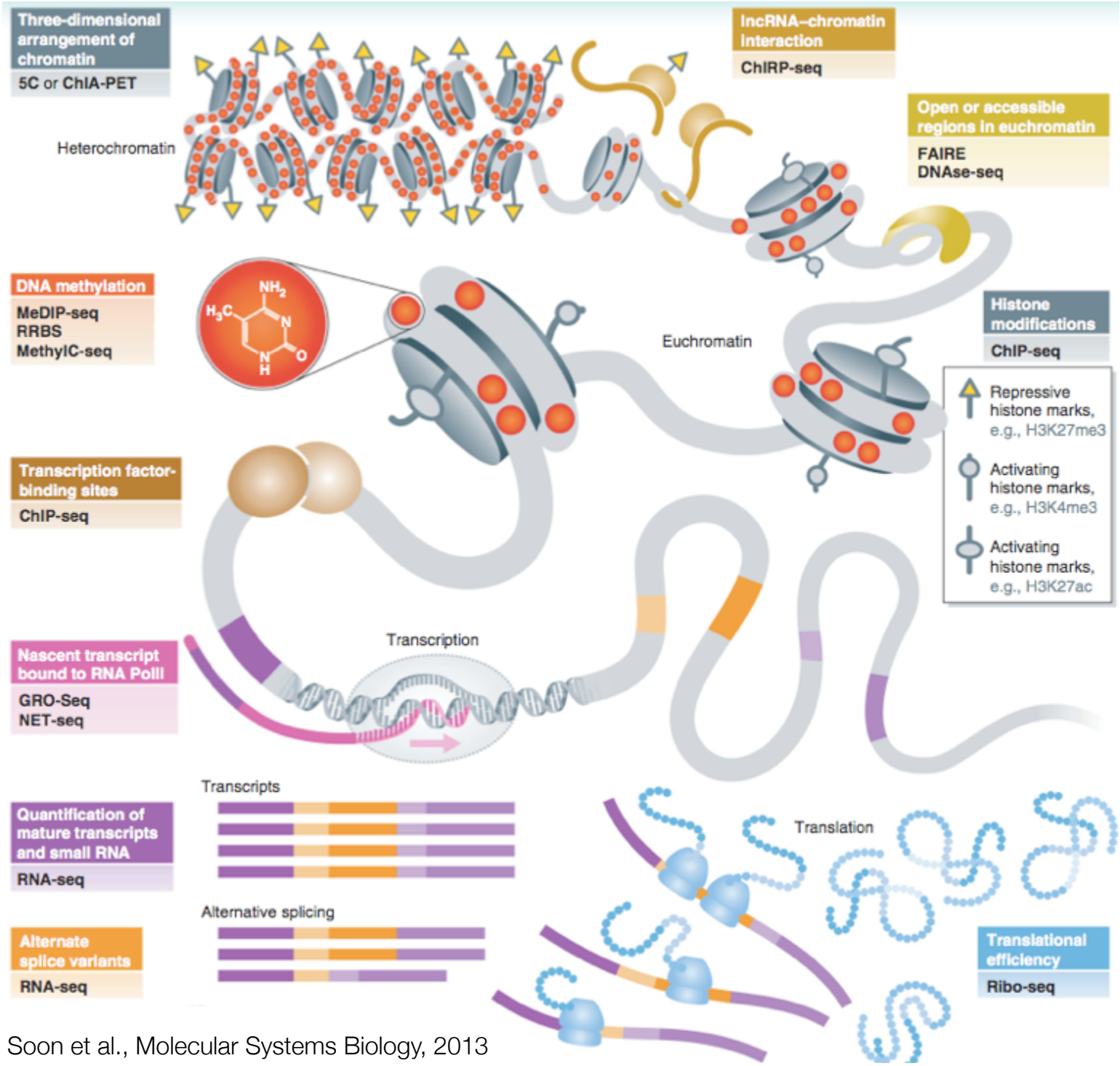
Sequencing Assays

The *Seq List (in chronological order)

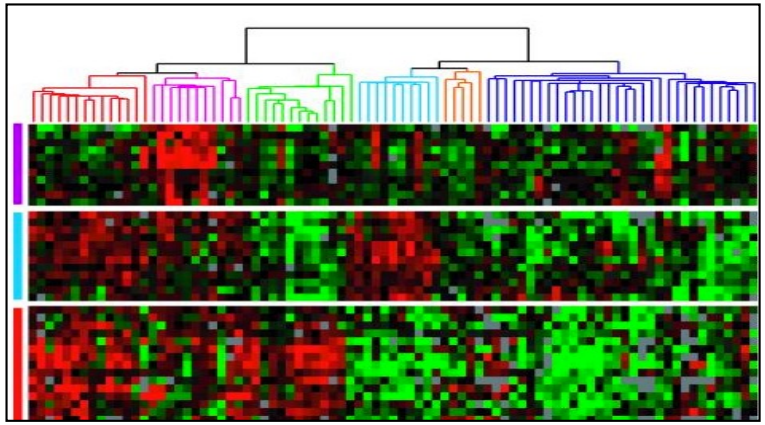
1. Gregory E. Crawford et al., "Genome-wide Mapping of DNase Hypersensitive Sites Using Massively Parallel Signature Sequencing (MPSS)," Genome Research 16, no. 1 (January 1, 2006): 123–131, doi:10.1101/gr.4074106.
2. David S. Johnson et al., "Genome-Wide Mapping of in Vivo Protein-DNA Interactions," Science 316, no. 5830 (June 8, 2007): 1497–1502, doi:10.1126/science.1141319.
3. Tarjei S. Mikkelsen et al., "Genome-wide Maps of Chromatin State in Pluripotent and Lineage-committed Cells," Nature 448, no. 7153 (August 2, 2007): 553–560, doi:10.1038/nature06008.
4. Thomas A. Down et al., "A Bayesian Deconvolution Strategy for Immunoprecipitation-based DNA Methylome Analysis," Nature Biotechnology 26, no. 7 (July 2008): 779–785, doi:10.1038/nbt1414.
5. Ali Mortazavi et al., "Mapping and Quantifying Mammalian Transcriptomes by RNA-Seq," Nature Methods 5, no. 7 (July 2008): 621–628, doi:10.1038/nmeth.1226.
6. Nathan A. Baird et al., "Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers," PLoS ONE 3, no. 10 (October 13, 2008): e3376, doi:10.1371/journal.pone.0003376.
7. Leighton J. Core, Joshua J. Waterfall, and John T. Lis, "Nascent RNA Sequencing Reveals Widespread Pausing and Divergent Initiation at Human Promoters," Science 322, no. 5909 (December 19, 2008): 1845–1848, doi:10.1126/science.1162228.
8. Chao Xie and Martti T. Tammi, "CNV-seq, a New Method to Detect Copy Number Variation Using High-throughput Sequencing," BMC Bioinformatics 10, no. 1 (March 6, 2009): 80, doi:10.1186/1471-2105-10-80.
9. Jay R. Hesselberth et al., "Global Mapping of protein-DNA Interactions in Vivo by Digital Genomic Footprinting," Nature Methods 6, no. 4 (April 2009): 283–289, doi:10.1038/nmeth.1313.
10. Nicholas T. Ingolia et al., "Genome-Wide Analysis in Vivo of Translation with Nucleotide Resolution Using Ribosome Profiling," Science 324, no. 5924 (April 10, 2009): 218–223, doi:10.1126/science.1168978.
11. Alayne L. Brunner et al., "Distinct DNA Methylation Patterns Characterize Differentiated Human Embryonic Stem Cells and Developing Human Fetal Liver," Genome Research 19, no. 6 (June 1, 2009): 1044–1056, doi:10.1101/gr.088773.108.
12. Mayumi Oda et al., "High-resolution Genome-wide Cytosine Methylation Profiling with Simultaneous Copy Number Analysis and Optimization for Limited Cell Numbers," Nucleic Acids Research 37, no. 12 (July 1, 2009): 3829–3839, doi:10.1093/nar/gkp260.
13. Zachary D. Smith et al., "High-throughput Bisulfite Sequencing in Mammalian Genomes," Methods 48, no. 3 (July 2009): 226–232, doi:10.1016/j.ymeth.2009.05.003.
14. Andrew M. Smith et al., "Quantitative Phenotyping via Deep Barcode Sequencing," Genome Research (July 21, 2009).



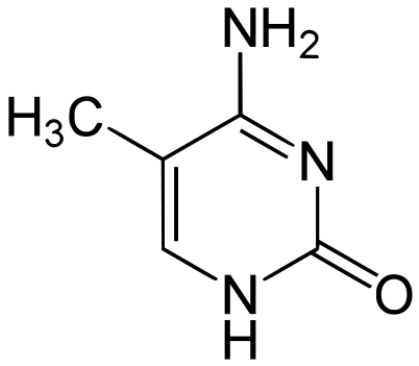
Soon et al., Molecular Systems Biology, 2013

*-seq in 4 short vignettes

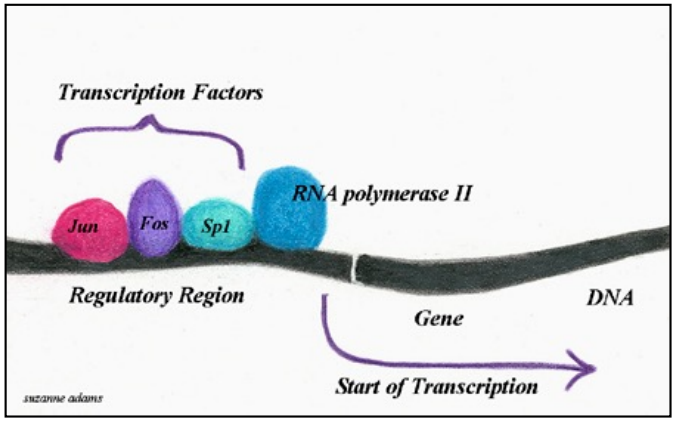
RNA-seq



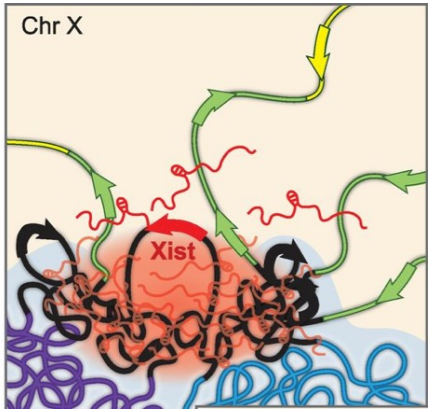
Methyl-seq



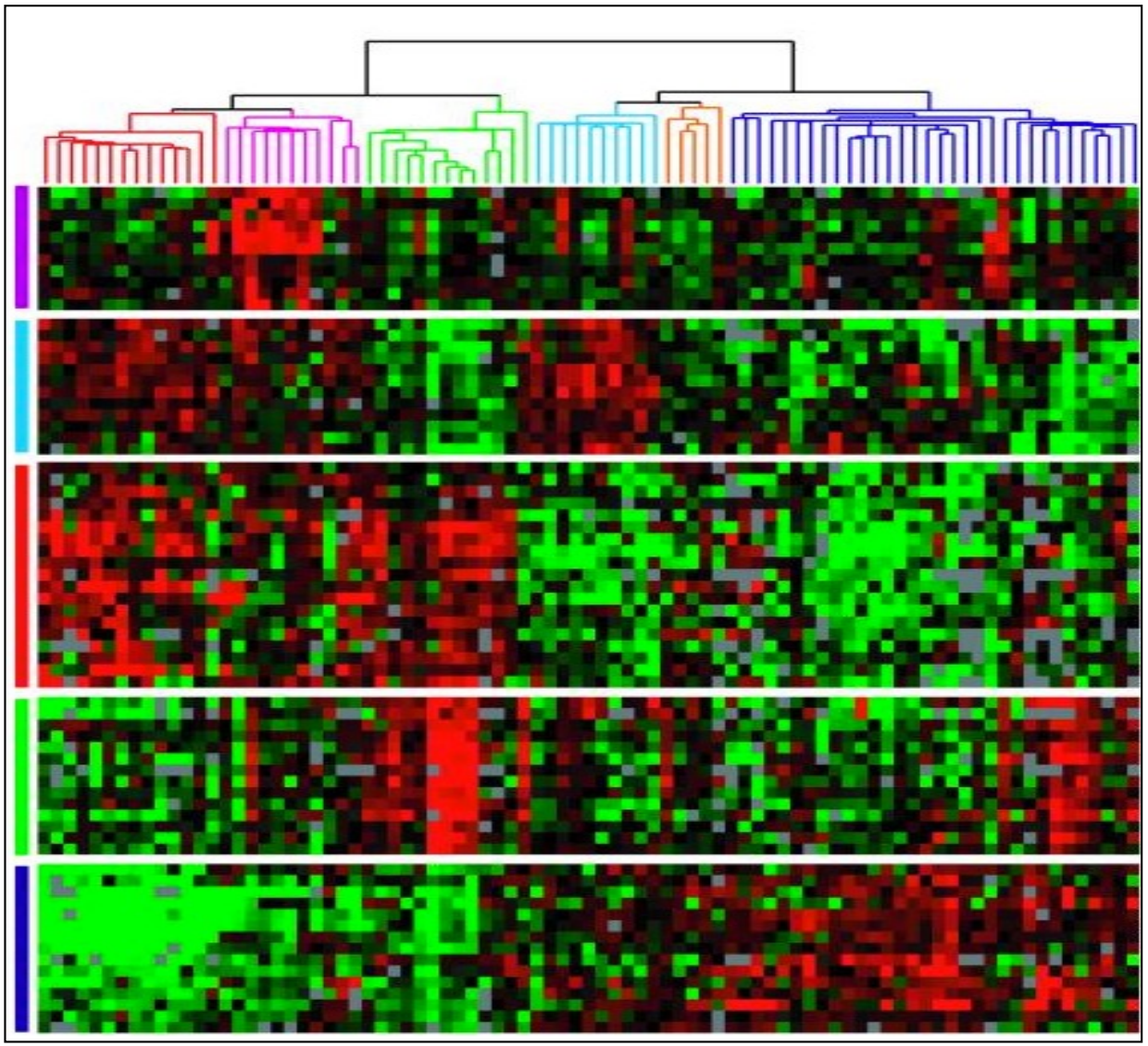
ChIP-seq



Hi-C

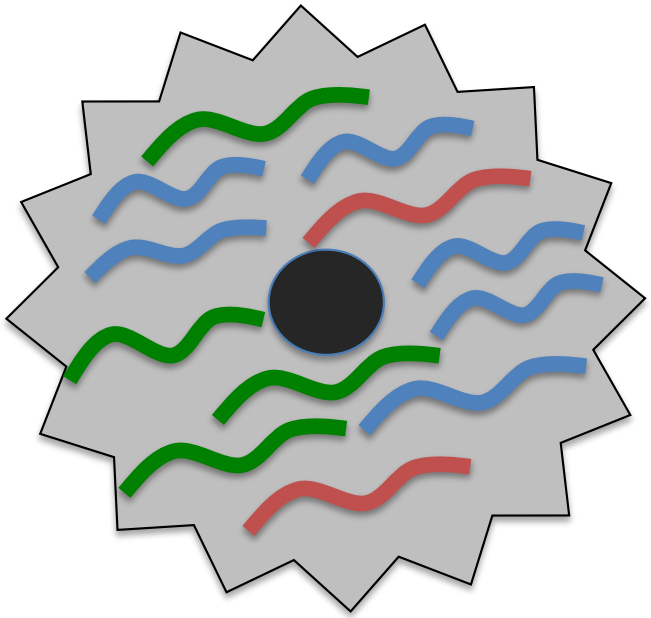


RNA-seq

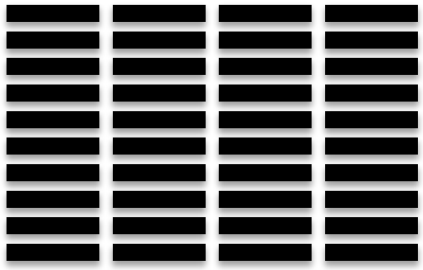


Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications.
Sørlie et al (2001) *PNAS*. 98(19):10869-74.

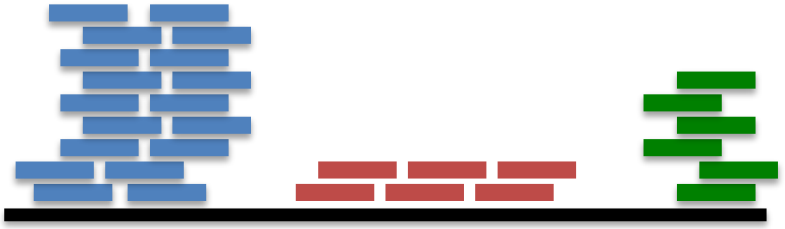
RNA-seq Overview



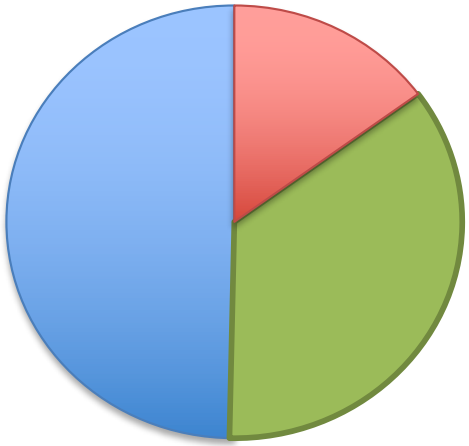
Sequencing



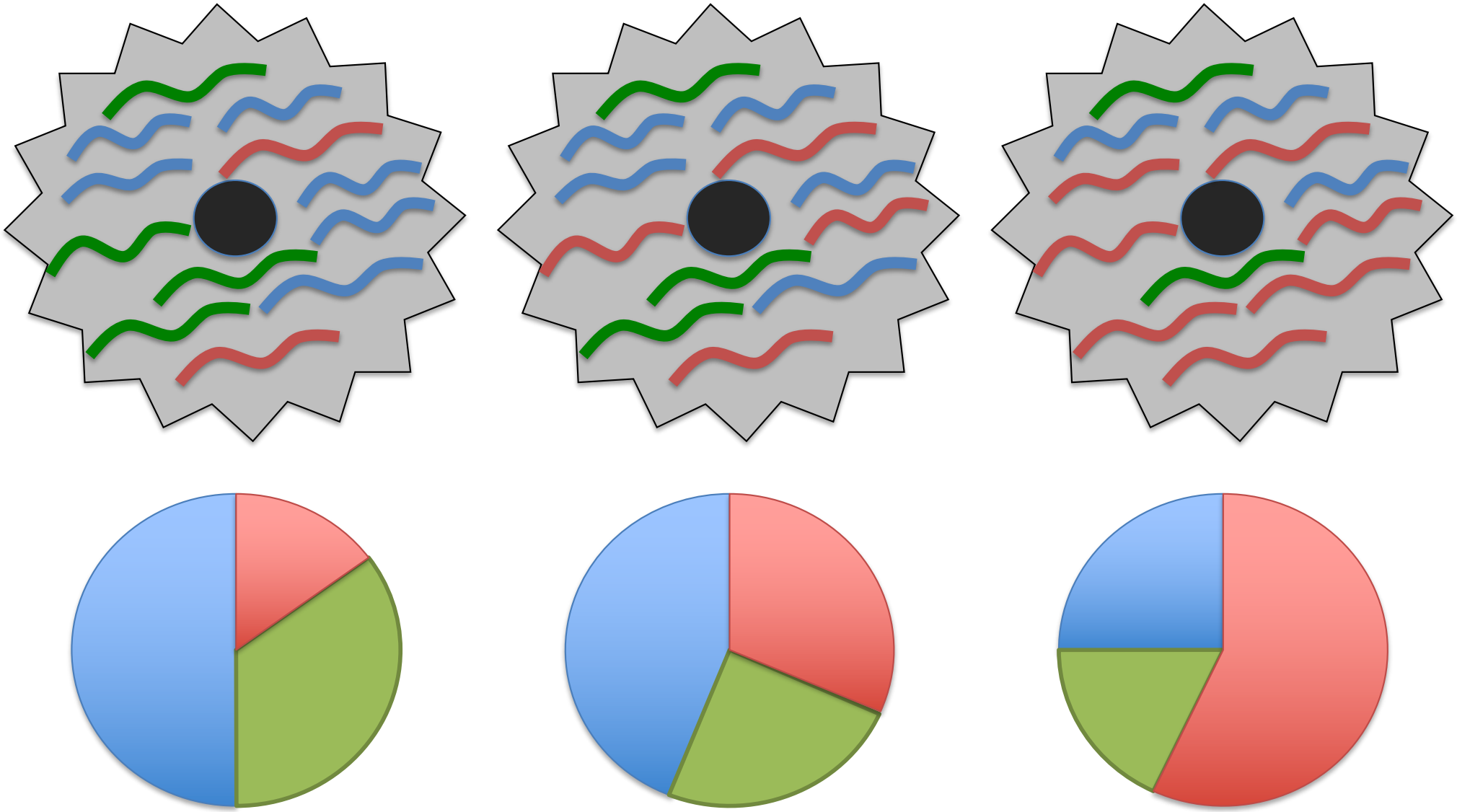
Mapping
& Assembly



Quantification



RNA-seq Overview



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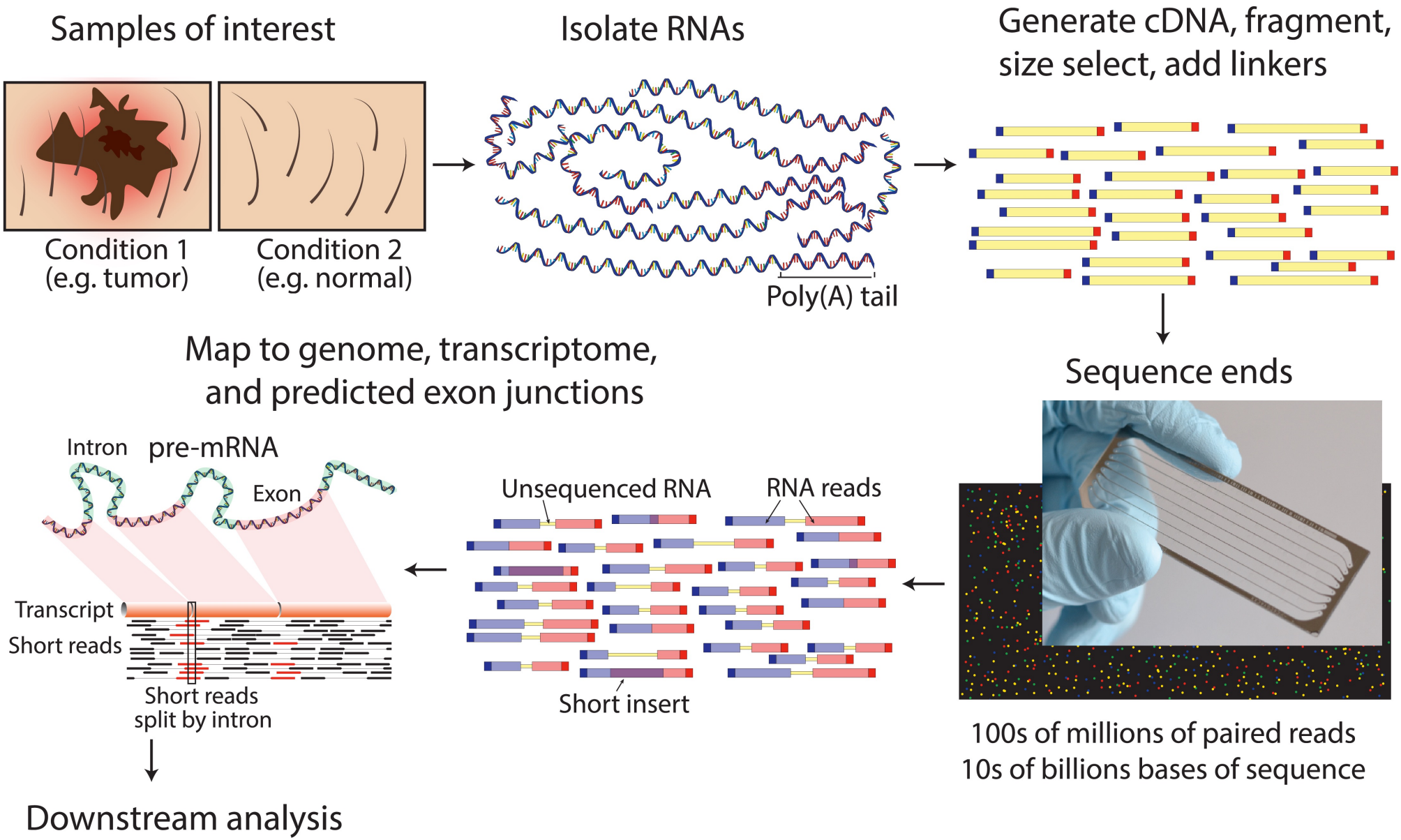
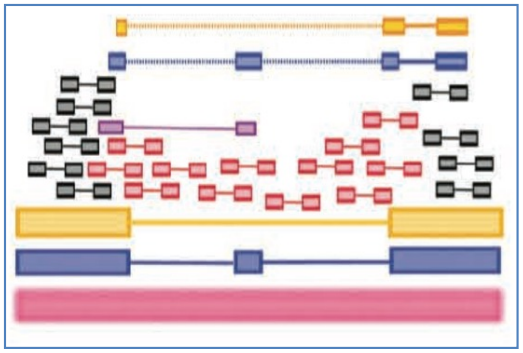


RNA-seq Challenges



Challenge 1: Eukaryotic genes are spliced

RNA-Seq Approaches

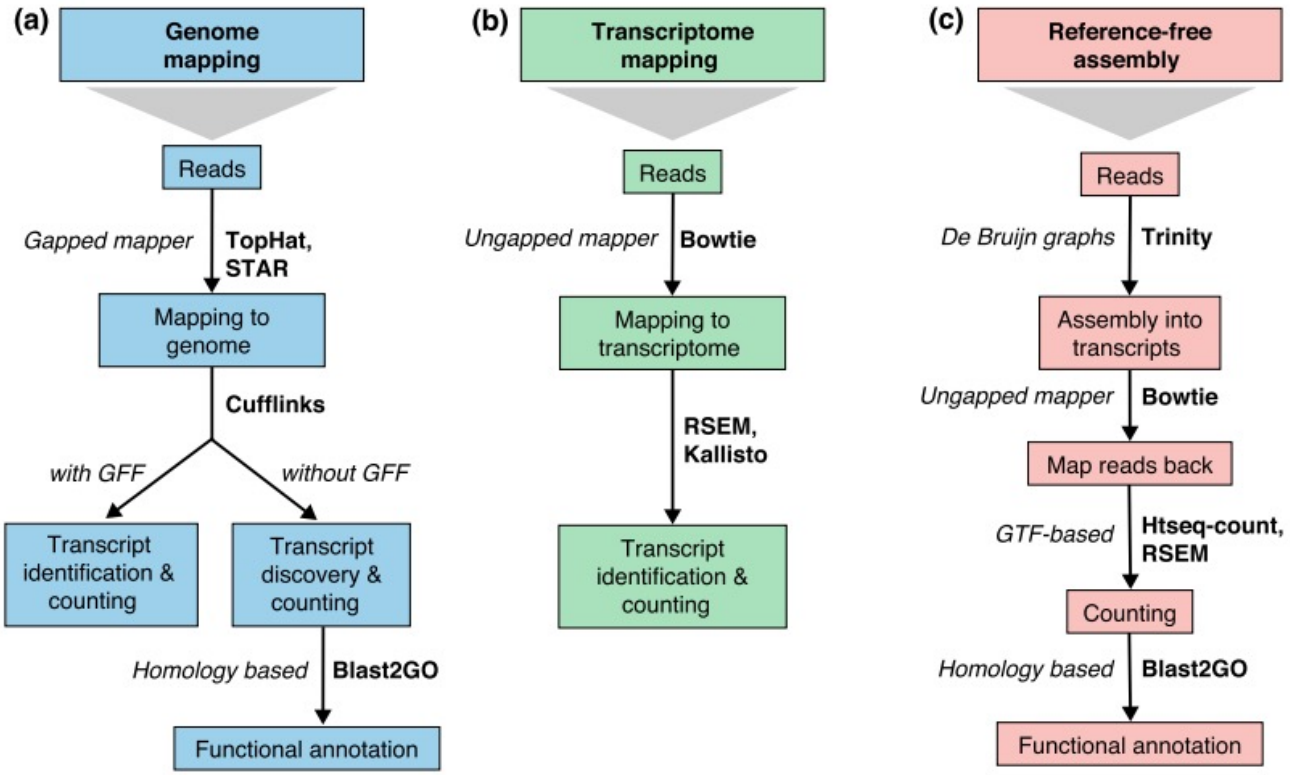
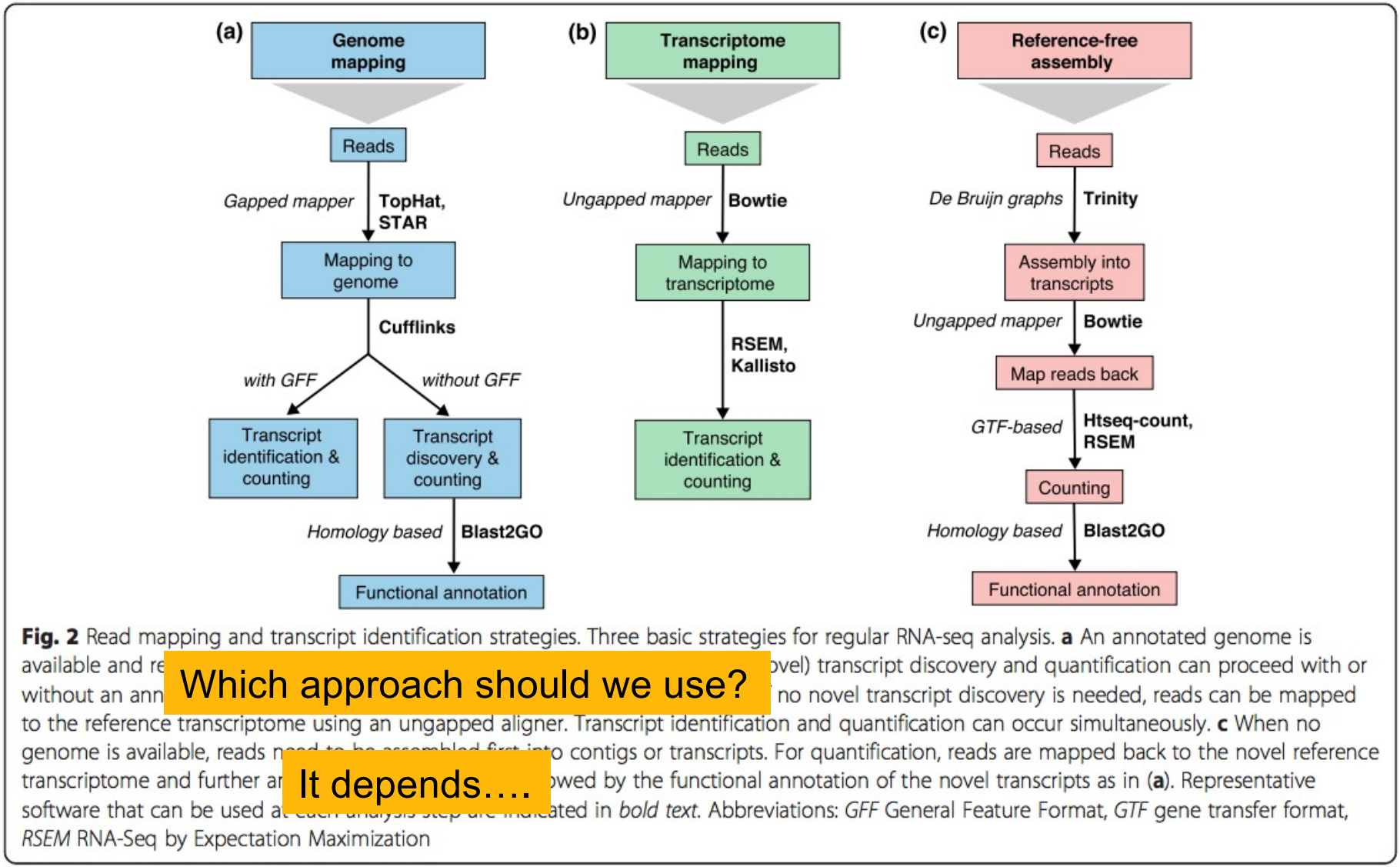


Fig. 2 Read mapping and transcript identification strategies. Three basic strategies for regular RNA-seq analysis. **a** An annotated genome is available and reads are mapped to the genome with a gapped mapper. Next (novel) transcript discovery and quantification can proceed with or without an annotation file. Novel transcripts are then functionally annotated. **b** If no novel transcript discovery is needed, reads can be mapped to the reference transcriptome using an ungapped aligner. Transcript identification and quantification can occur simultaneously. **c** When no genome is available, reads need to be assembled first into contigs or transcripts. For quantification, reads are mapped back to the novel reference transcriptome and further analysis proceeds as in (**b**) followed by the functional annotation of the novel transcripts as in (**a**). Representative software that can be used at each analysis step are indicated in *bold text*. Abbreviations: *GFF* General Feature Format, *GTF* gene transfer format, *RSEM* RNA-Seq by Expectation Maximization

A survey of best practices for RNA-seq data analysis
Conesa et al (2016) Genome Biology. doi 10.1186/s13059-016-0881-8

RNA-Seq Approaches



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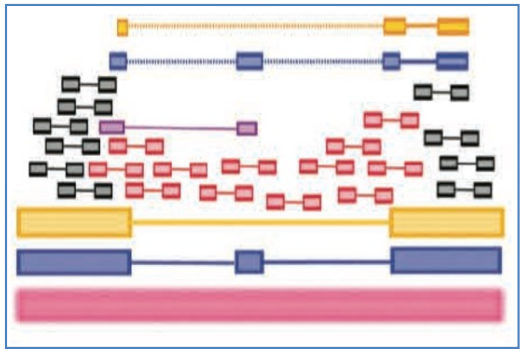
Which approach should we use?

It depends….

A survey of best practices for RNA-seq data analysis

Conesa et al (2016) Genome Biology. doi 10.1186/s13059-016-0881-8

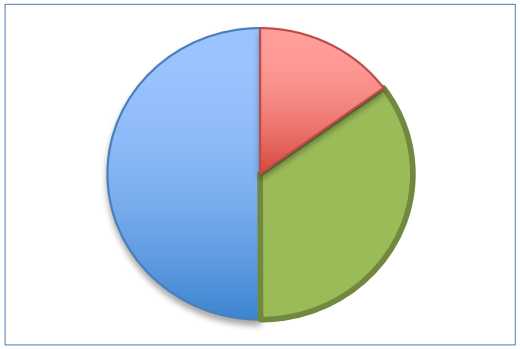
RNA-seq Challenges



Challenge 1: Eukaryotic genes are spliced

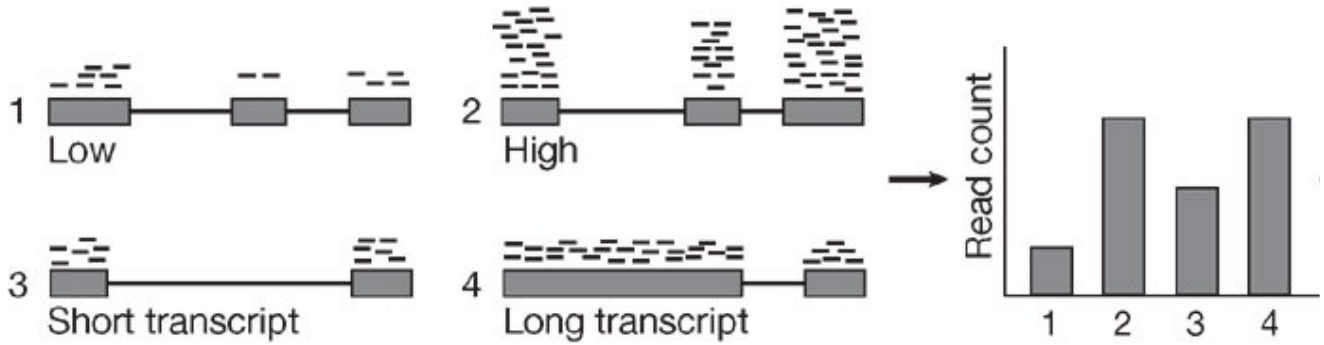
Solution: Use a spliced aligner, and assemble isoforms

TopHat: discovering spliced junctions with RNA-Seq.
Trapnell et al (2009) *Bioinformatics*. 25:0 1105-1111



Challenge 2: Read Count != Transcript abundance

RPKM, FPKM, TPM

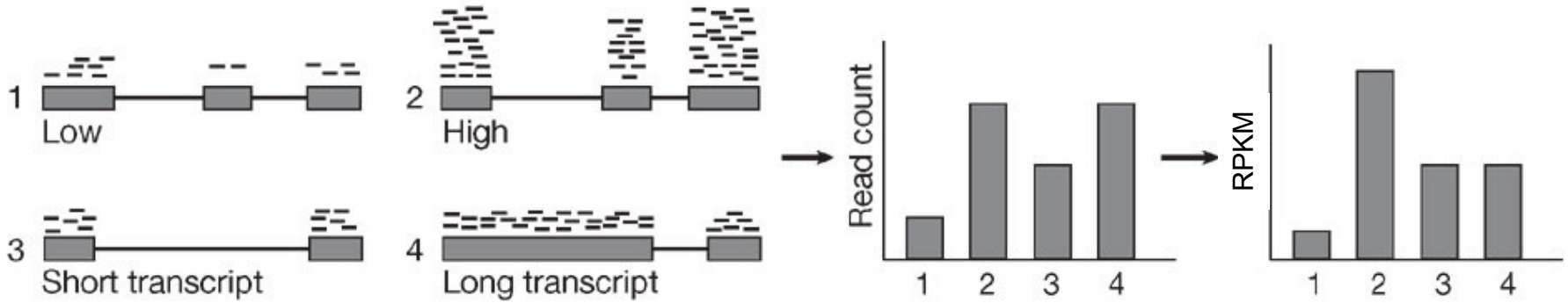


Counting Reads that align to a gene DOESN'T work!

- Overall Coverage: 1M reads in experiment 1 vs 10M reads in experiment 2
- Gene Length: gene 3 is 10kbp, gene 4 is 100kbp

1. RPKM: Reads Per Kilobase of Exon Per Million Reads Mapped (Mortazavi et al, 2008)

RPKM, FPKM, TPM



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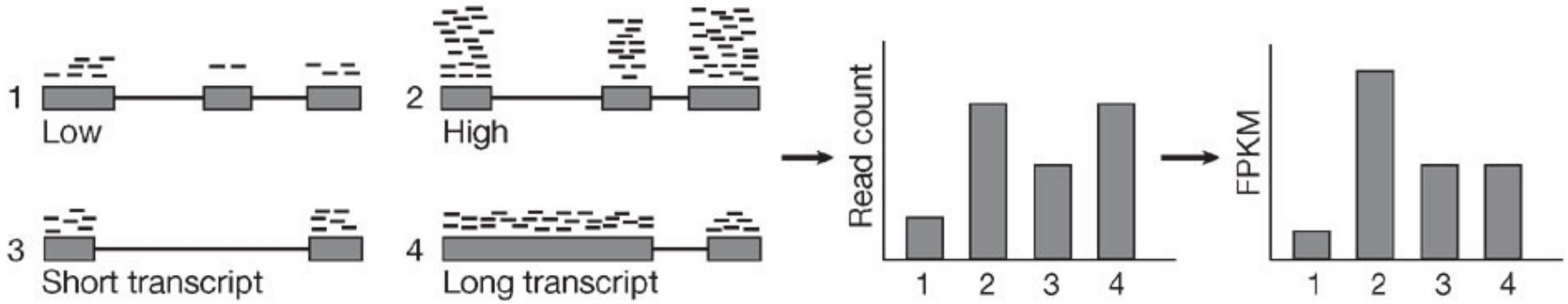
1. RPKM: Reads Per Kilobase of Exon Per Million Reads Mapped (Mortazavi et al, 2008)

(Count reads aligned to gene) / (length of gene in kilobases) / (# millions of read mapped)

=> Wait a second, reads in a pair arent independent!

RPKM, FPKM, TPM



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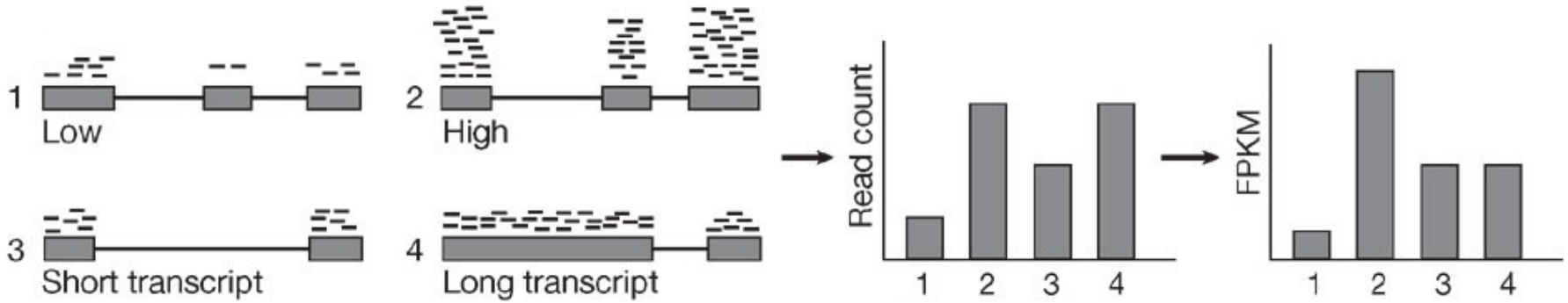
=> Wait a second, reads in a pair arent independent!

2. FPKM: Fragments Per Kilobase of Exon Per Million Reads Mapped (Trapnell et al, 2010)

$\Rightarrow$ Does a much better job with short exons & short genes by boosting coverage

$\Rightarrow$ Wait a second, FPKM depends on the average transcript length!

RPKM, FPKM, TPM



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2. FPKM: Fragments Per Kilobase of Exon Per Million Reads Mapped (Trapnell et al, 2010)

=> Wait a second, FPKM depends on the average transcript length!

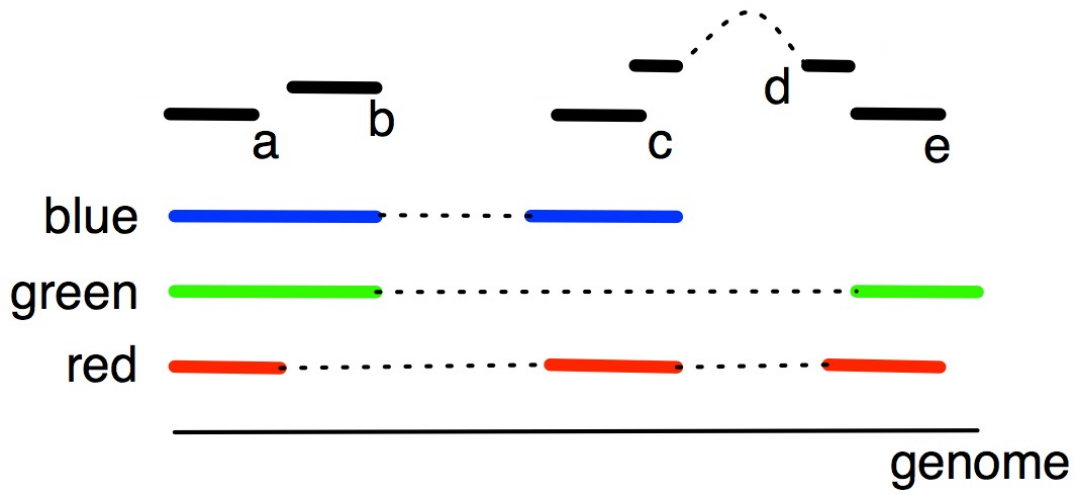
3. TPM: Transcripts Per Million (Li et al, 2011)

⇒ If you were to sequence one million full length transcripts, TPM is the number of transcripts you would have seen of type i, given the abundances of the other transcripts in your sample

=> Recommend you use TPM for all analysis, easy to compute given FPKM

$$\mathrm{TPM}_i = \left(\frac{\mathrm{FPKM}_i}{\sum_j \mathrm{FPKM}_j} \right) \cdot 10^6$$

Multi-mapping? Isoform ambiguity? Expectation Maximization to the Rescue



The gene has three isoforms (red, green, blue) of the same length.
Our initial expectation is all 3 isoforms are equally expressed

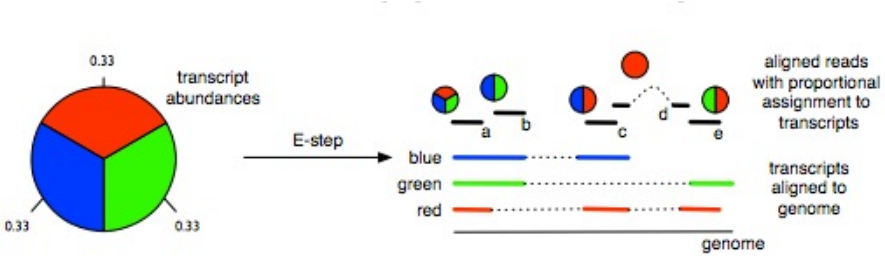
There are five reads (a,b,c,d,e) mapping to the gene.

- Read a maps to all three isoforms
- Read d only to red
- Reads b,c,e map to each of the three pairs of isoforms.

What is the most likely expression level of each isoform?

Models for transcript quantification from RNA-seq
Pachter, L (2011) arXiv. 1104.3889 [q-bio.GN]

Multi-mapping? Isoform ambiguity? Expectation Maximization to the Rescue



The gene has three isoforms (red, green, blue) of the same length. Initially every isoform is assigned the same abundance (red=1/3, green=1/3, blue=1/3)

There are five reads (a,b,c,d,e) mapping to the gene. Read a maps to all three isoforms, read d only to red, and the other three (reads b,c,e) to each of the three pairs of isoforms.

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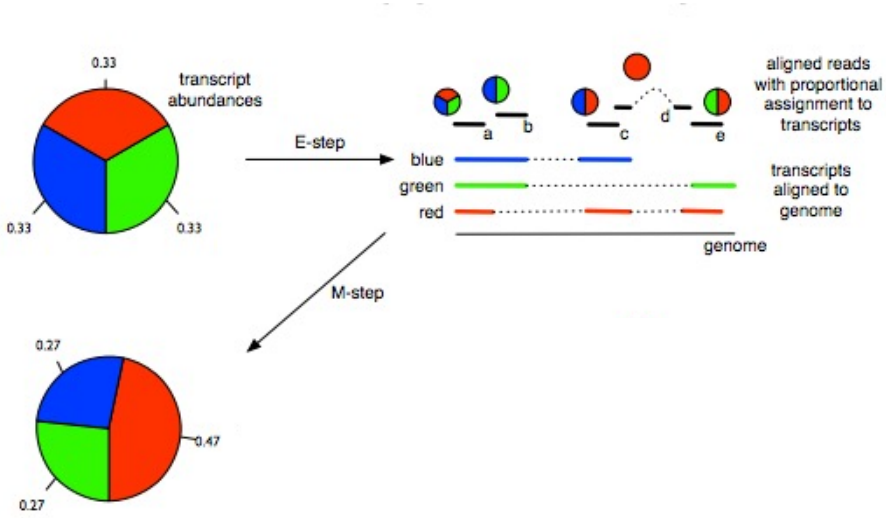
Models for transcript quantification from RNA-seq
Pachter, L (2011) arXiv. 1104.3889 [q-bio.GN]

Multi-mapping? Isoform ambiguity? Expectation Maximization to the Rescue



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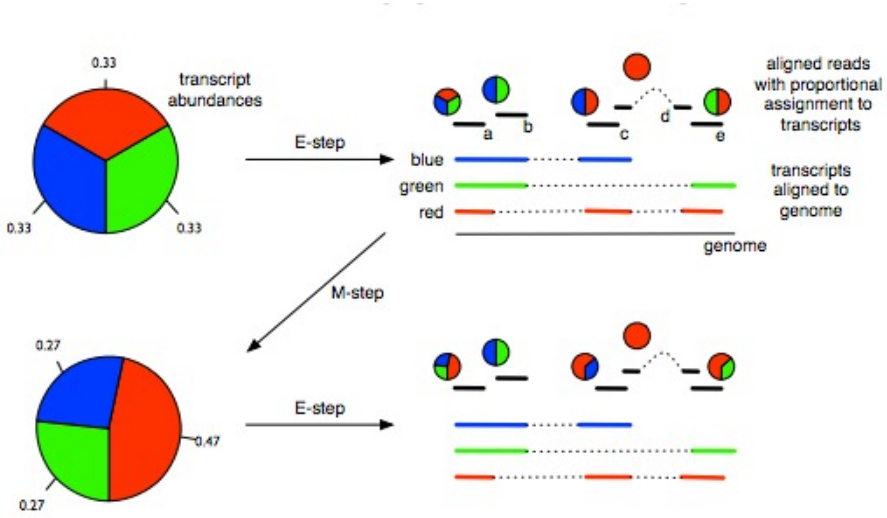

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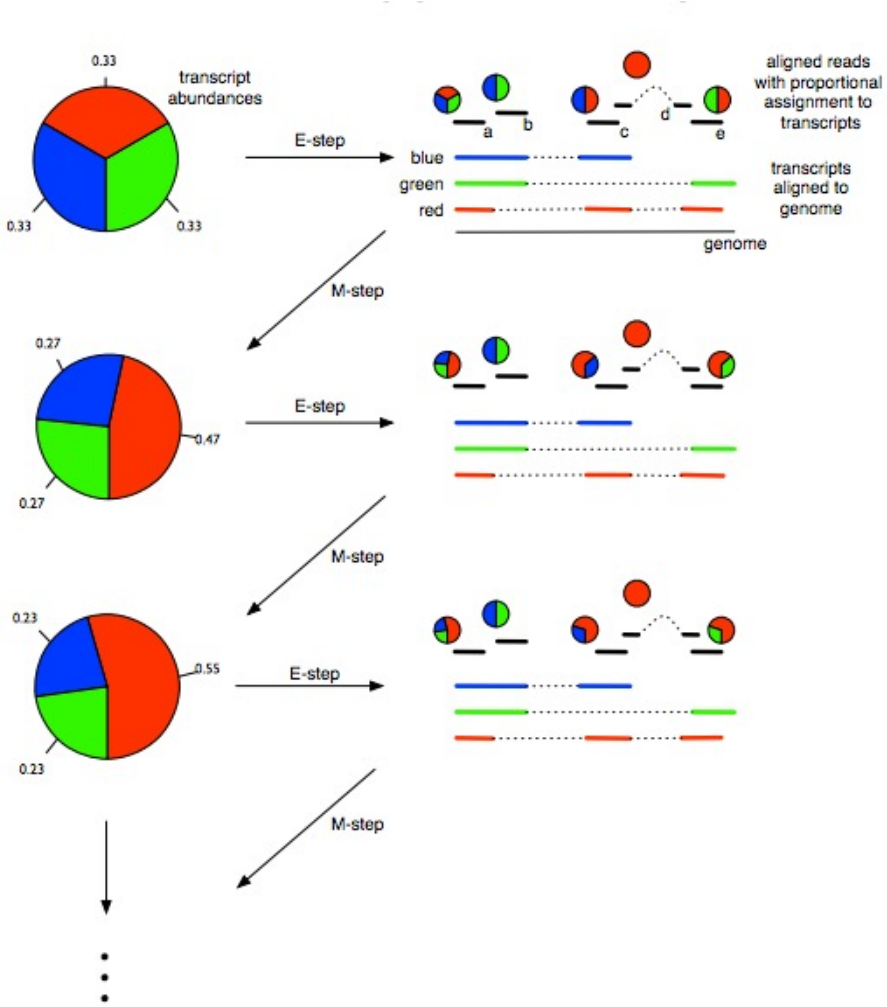

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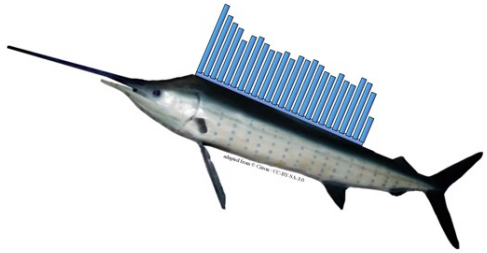
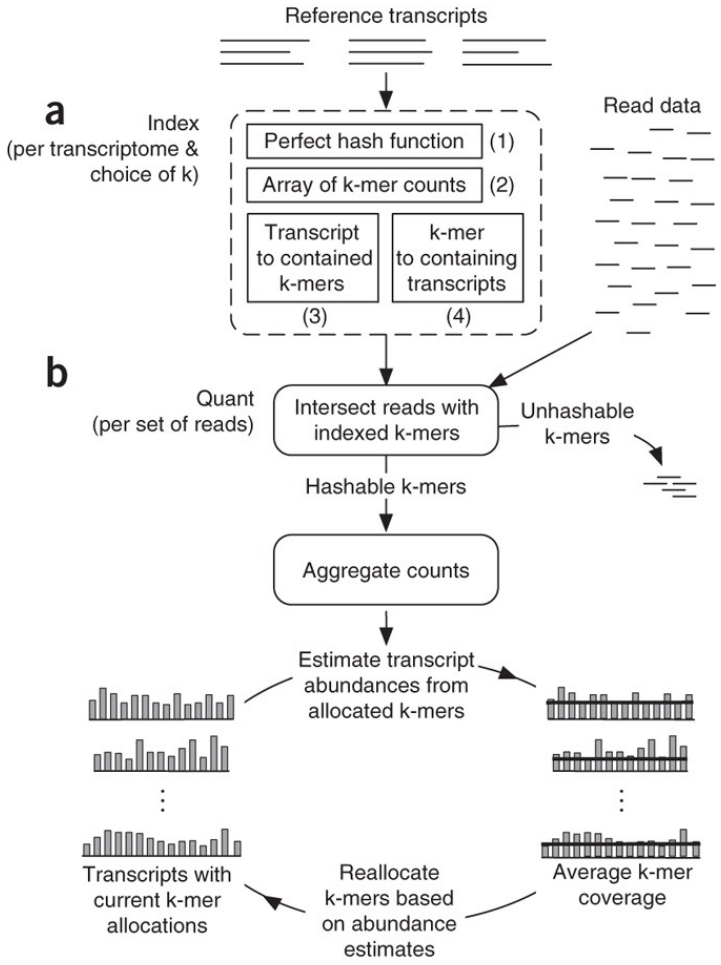
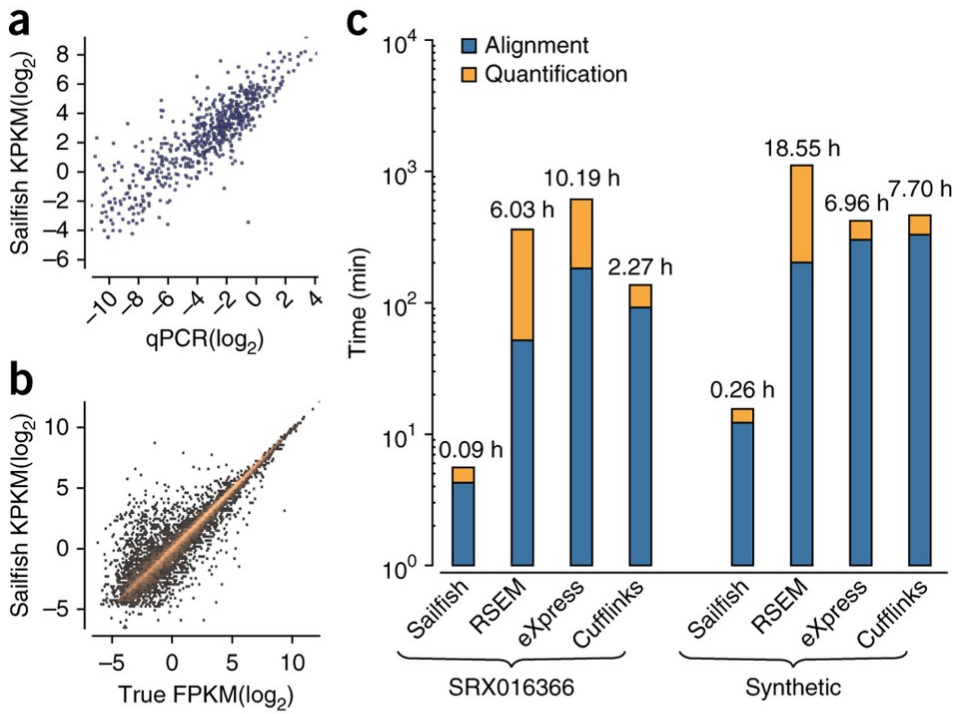
Sailfish: Fast & Accurate RNA-seq Quantification







d

	Human brain tissue				Synthetic			
	Sailfish	RSEM	eXpress	Cufflinks	Sailfish	RSEM	eXpress	Cufflinks
Pearson	0.85	0.82	0.85	0.85	0.96	0.96	0.95	0.94
Spearman	0.84	0.80	0.85	0.85	0.76	0.77	0.77	0.75
RMSE	–	–	–	–	6.06	8.90	8.83	10.05
medPE	–	–	–	–	6.50	12.48	14.06	12.42

Sailfish enables alignment-free isoform quantification from RNA-seq reads using lightweight algorithms
Patro et al (2014) Nature Biotechnology 32, 462–464 doi:10.1038/nbt.2862

Annotation Summary

- Three major approaches to annotate a genome
  1. Alignment:
     - Does this sequence align to any other sequences of known function?
     - Great for projecting knowledge from one species to another
  2. Prediction:
     - Does this sequence statistically resemble other known sequences?
     - Potentially most flexible but dependent on good training data
  3. Experimental:
     - Lets test to see if it is transcribed/methylated/bound/etc
     - Strongest but expensive and context dependent

- Many great resources available
  - Learn to love the literature and the databases
  - Standard formats let you rapidly query and cross reference
  - Google is your number one resource ☺