Galaxy RNAseq tutorial - GCC2014

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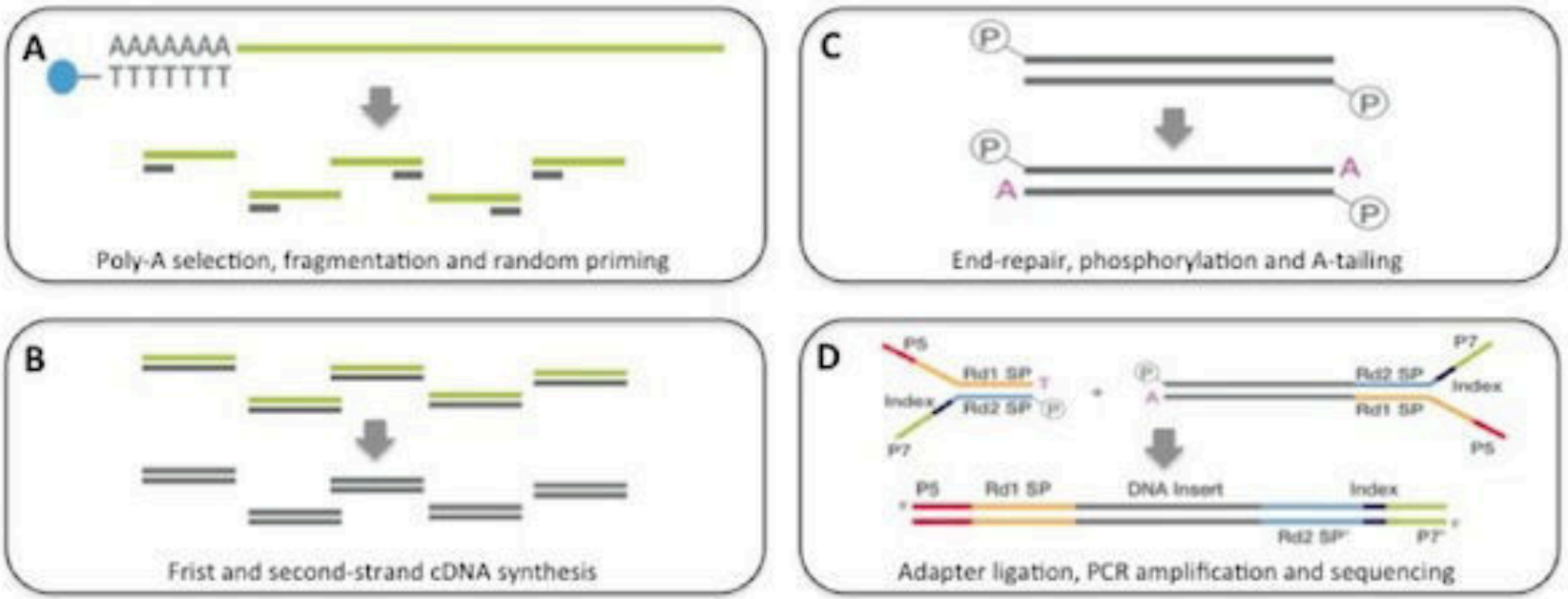
June 29, 2014

RNA-seq protocol

Illumina Tru-Seq RNA-seq protocol



Library prep begins from 100ng-1ug of Total RNA which is poly-A selected (A) with magnetic beads. Double-stranded cDNA (B) is phosphorylated and A-tailed (C) ready for adapter ligation. The library is PCR amplified (D) ready for clustering and sequencing.

http://bitesizebio.com/13542

Sequencers: HiSeq

Figure : HiSeq 2000.

Characteristics:

- High throughput.
- Paired end.
- High accuracy.
- Read length 2×150bp.
- Relatively long run time.
- Relatively expensive.

Sequencers: Ion Proton

Figure : Ion torrent.

Characteristics:

- Moderate throughput.
- High accuracy.
- Read length 200bp.
- Short run time
- Cheap runs.
- Homopolymer issue.

General layout of an RNA-seq pipeline.

1. Pre-alignment.
   - QC.
   - Data cleaning.

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4. Transcript assembly.
   - New transcripts, alternative splicing, etc.

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We use Sickle for data cleaning.

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For adapter clipping, we can use Cutadapt, Trimmomatic or the FastX toolkit (not in this practical).

- Remove linker/adapter sequences.
- Trim low quality reads at the end of the read.
- Evaluate the part of the read that is left.

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The FastQC tool kit is used for quality control (both before and after the data cleaning step).

- GC content.
- GC distribution.
- Quality scores distribution.

FastQC report

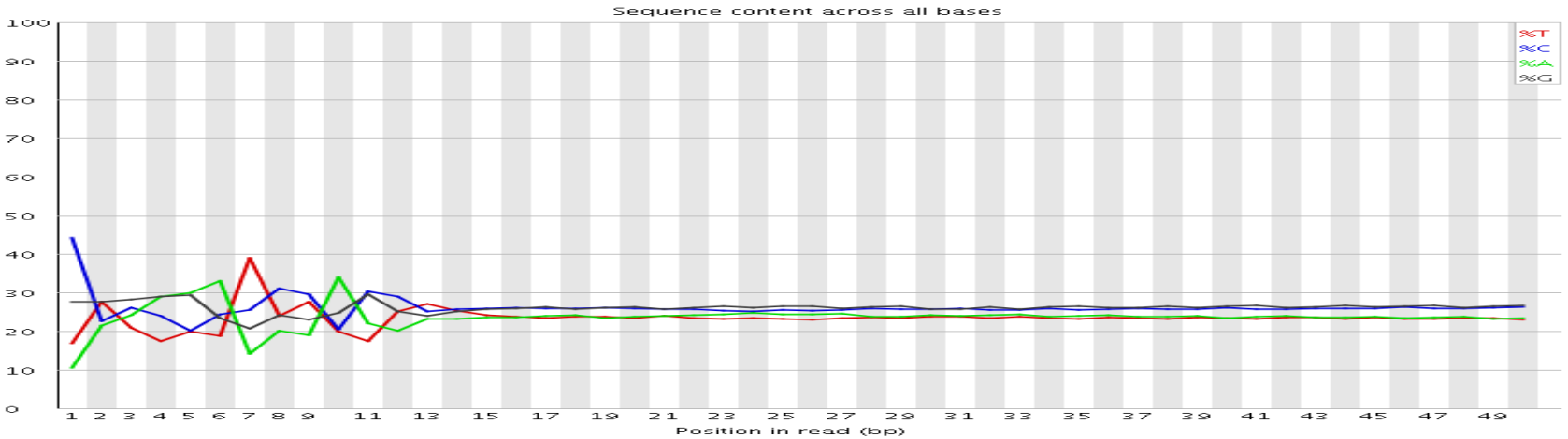


Figure : Per base sequence content. (ref. PMC2896536)

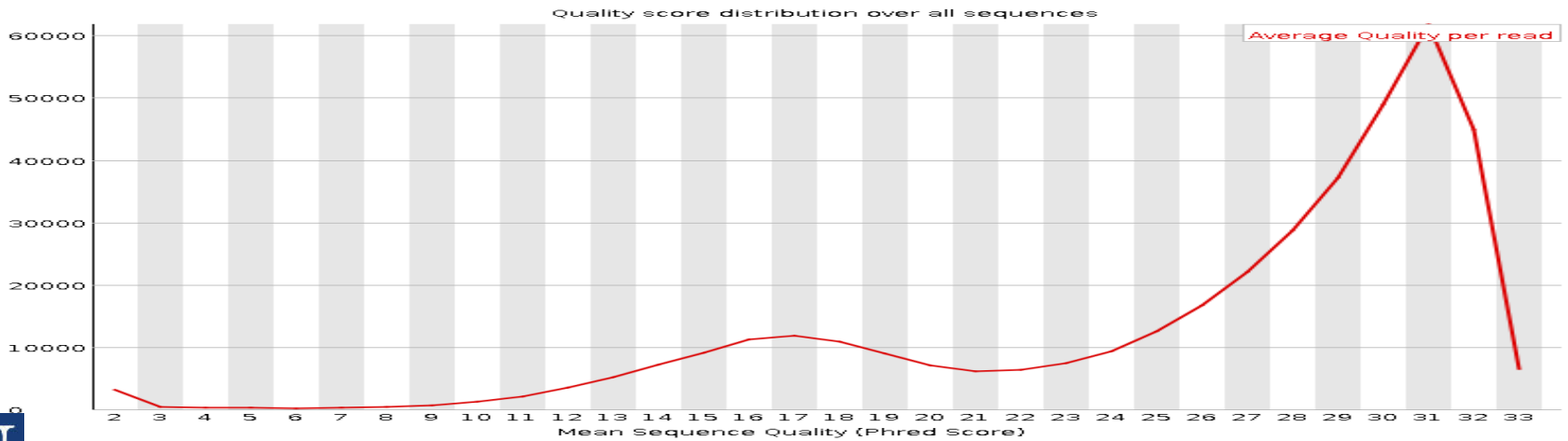
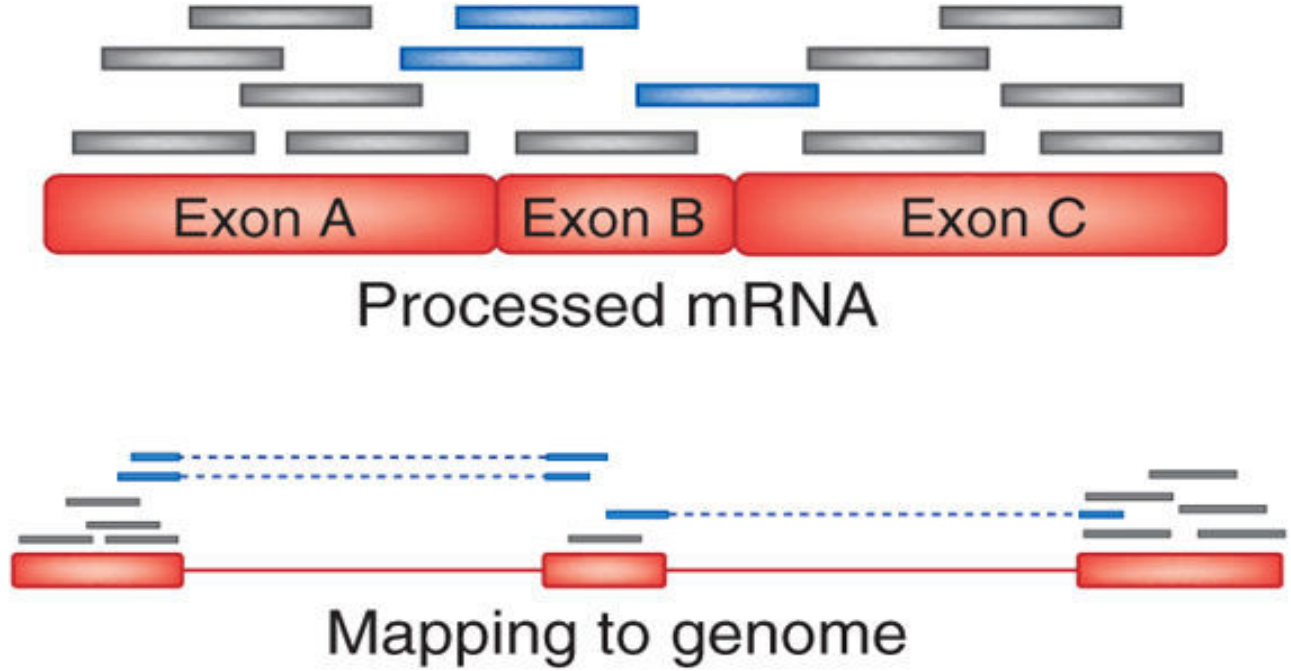


Figure : Per sequence quality.

RNAseq alignment



http://www.nature.com/nbt/journal/v27/n5/full/nbt0509-455.html

RNAseq alignment

Aligning RNAseq reads to reference genome

- Alternative splicing.

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This affects:

- Insert sizes.
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Available RNAseq aligners

- Tophat.
- Gmap / Gsnap.
- STAR.
- GEM.
- HMMSplicer.

http://www.nature.com/nmeth/journal/v10/n12/full/nmeth.2722.html

Tophat

http://ccb.jhu.edu/software/tophat/

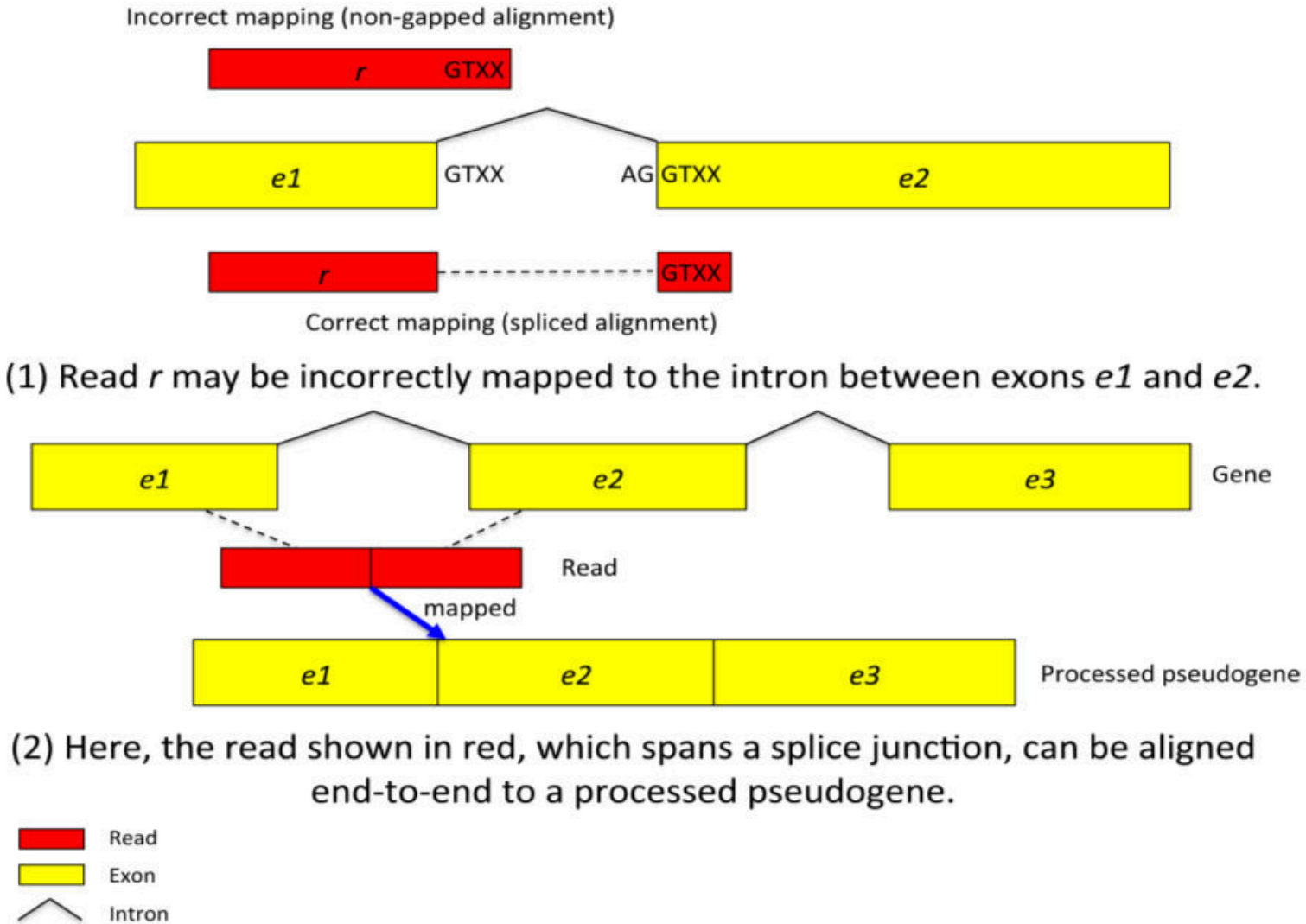
Two-step approach:

- (optional) Align to transcriptome first.
- Use bowtie to align whole reads, identify potential exon.
- Split left-over reads into small segments, align independently.
  - Make a database of splice junctions.
  - Map the reads to confirm the splice junctions.

Some considerations:

- Does not support soft-clipping

Tophat alignment



http://genomebiology.com/2013/14/4/R36

STAR

https://code.google.com/p/rna-star/

- Selected by many large sequencing consortium, e.g., Geuvadis.
- High alignment yield with good alignment quaility (close to tophat).
- Very very fast.

Some considerations:

- Require large amount of memory (more than 30GB).

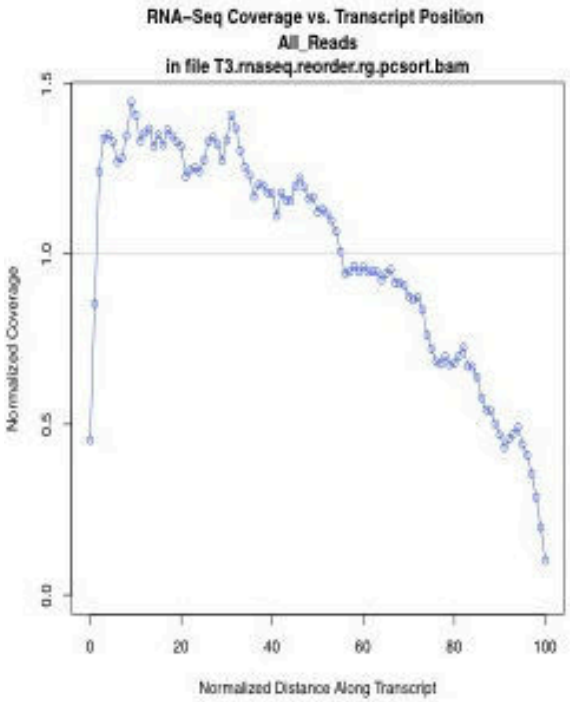
Gmap/Gsnap

http://research-pub.gene.com/gmap/

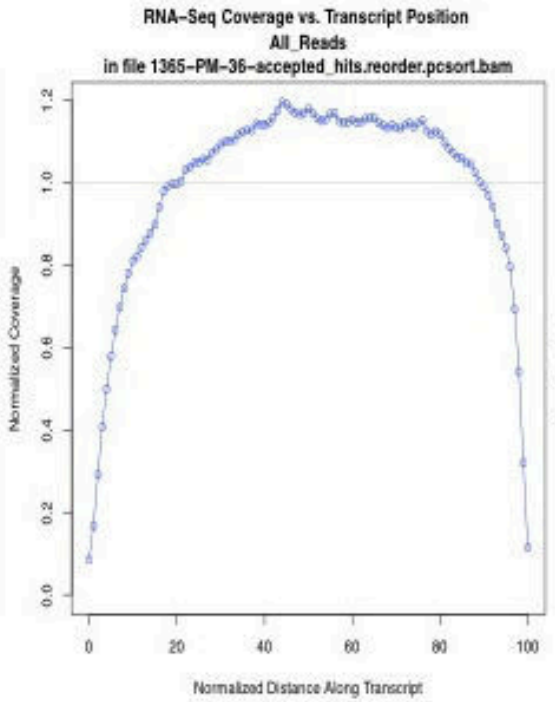
- Before tophat2 and STAR
  - Split both ends.
  - Split a read into many pieces.
- Fast.
- Memory efficient.

Post alignment QC (5'-3' coverage bias)

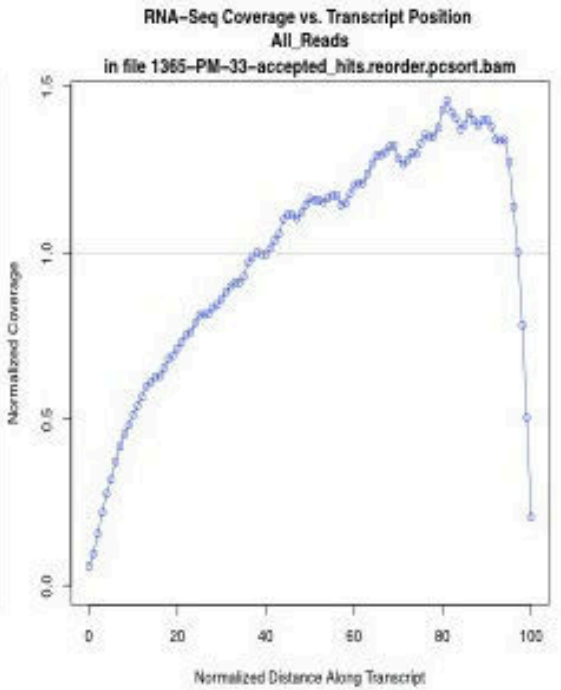
5' bias
(strand oriented protocol)



no bias
(low coverage at ends of transcript)



3' bias
(poly-A selection)



Zuojian Tang NYU Langone MEDICAL CENTER

https://www.biostars.org/p/102812/

Cufflinks

Input:

- Aligned reads.
  - Gmap / Gsnap.
  - Tophat.

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What it can do:

- Assemble transcripts.
- Estimate transcript abundance.

Cufflinks

Modes of operation:

- Use predefined transcripts.
- Assemble transcripts assisted by known transcripts.
- Assemble transcripts with no prior knowledge.

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When to use:

- Only interested in expression.
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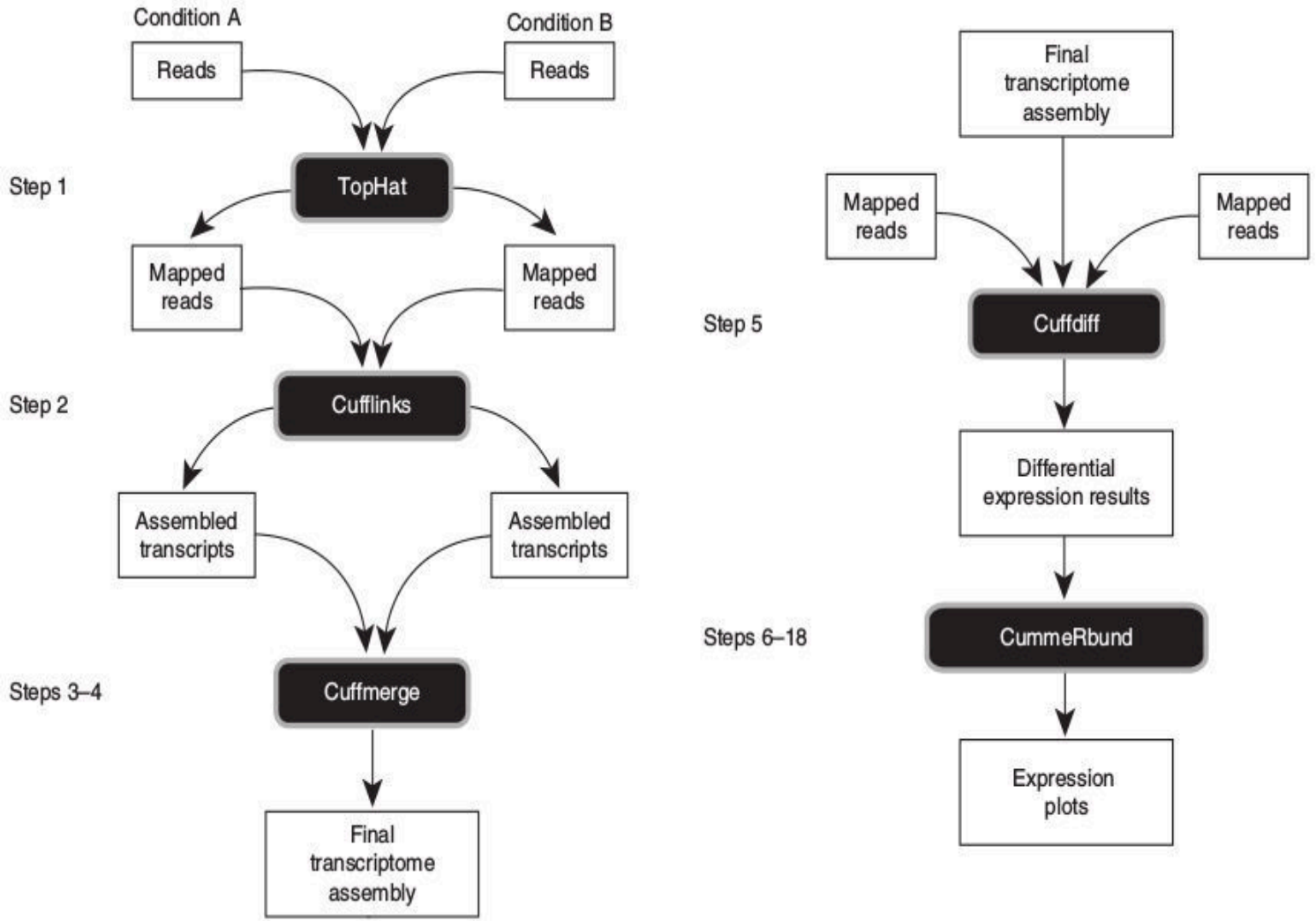
Discover new transcripts (Cuffcompare).

Cuffdiff

Find significant changes in transcript expression, splicing, and promoter use.

- Support multiple samples and replicates
- Models to estimate the distribution
  - pooled, per-condition, blind
- Output a number of statistic tests
  - fold change, p values, q values

Tuxedo pipeline



http://www.ncbi.nlm.nih.gov/pubmed/22383036

Combining tools in a pipeline

Listing 1 : Shell script

```
bwa mem -t 8 $reference $i > $i.sam
samtools view -bt $reference -o $i.bam
    $i.sam
```

Listing 2 : Makefile

```
%.sam: %.fq
  $(BWA) mem -t $(THREADS) $(call MKREF,
      $@) $< > $@

%.bam: %.sam
  $(SAMTOOLS) view -bt $(call MKREF, $@)
      -o $@ $<
```

Snakemake, GATK Queue

Snakemake

- Inspired by good features of makefiles
- Python like syntax

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GATK Queue

- Scala based
- full DRMAA support

Acknowledgements:

Jeroen Laros (LUMC)
Wibowo Arindrarto (LUMC)
Irina Pulyakhina (LUMC)
Peter-Bram 't Hoen (LUMC)
Johan den Dunnen (LUMC)
Andrew Stubbs (ErasmusMC)

Outline of the practical

1. Do a typical RNA-seq analysis.
   - Expression.
2. Workflows.
   - Rerun the analysis with no effort.
3. Differential expression analysis.