LEIDEN UNIVERSITY MEDICAL CENTER

RNA-seq using Galaxy

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Introduction

Sequencers: HiSeq

Figure 1 : HiSeq 2000.

Characteristics:

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

Introduction

Sequencers: Ion Torrent

Figure 2 : Ion torrent.

Characteristics:

- Moderate throughput.
- Single end (for now).
- High accuracy.
- Read length ±200bp.
- Short run time.
- Cheap runs.

Introduction

General layout of an RNA-seq pipeline.

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

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3. Expression (gene, transcripts) analysis.
    - Known transcripts.

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    - QC.
    - Data cleaning.
2. Alignment.
    - Use a specialised (RNA) aligner.
3. Expression (gene, transcripts) analysis.
    - Known transcripts.
4. Transcript assembly.
    - New transcripts, alternative splicing, etc.

Pre-alignment

Sickle / FastQC.

We use the Sickle for data cleaning.
For adapter clipping, we use Trimmomatic or the FastX toolkit (not in this practical).

- Remove linker sequences.
- Clip 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.
- ...

Pre-alignment

FastQC report.

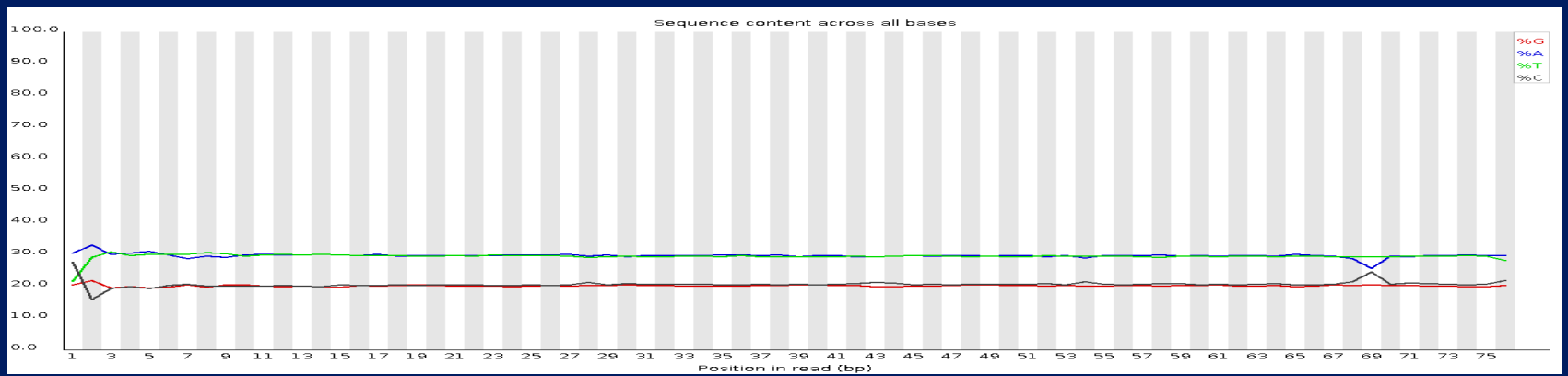

Figure 3 : Per base sequence content.

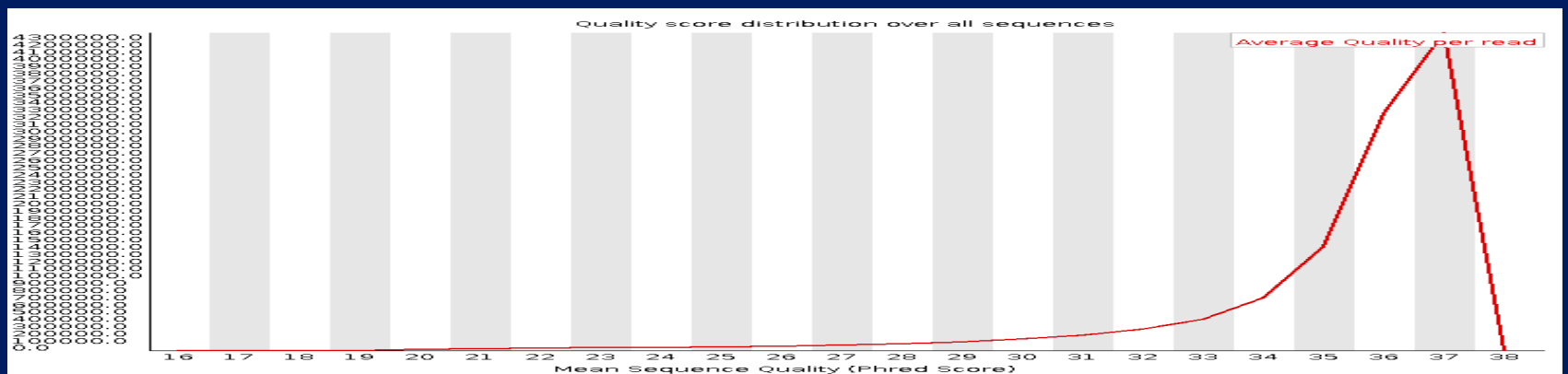

Figure 4 : Per sequence quality.

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RNA aligners.

Difference with DNA:

- Splicing.

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This affects:

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Available tools:

- Tophat.
- Gmap / Gsnap.
- PASSion.
- MapSplice.
- HMMSplicer.
- . . .

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Choose your aligner carefully.

If you work with pre-mRNA, the options are limited.

- Some tools find exons first, then use this to break up reads.
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Some tools heavily rely on annotation.

- A list of known splice sites.
- Motifs (canonical splice sites).

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

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Some features:

- Split read alignment.
  - Split both ends.
  - Split a read into many pieces.
- Fast.
- Memory efficient.
  - No limit on intron size.

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Tophat

Tophat: A spliced read mapper for RNA-Seq.

http://tophat.cbcb.umd.edu

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Approach:

- Identify potential exons.
  - Split all reads into small segments, align independently.
- Make a database of splice junctions.
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Some considerations:

- The software is optimized for reads 75bp or longer.
- Mixing paired- and single- end reads together is not supported.

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Expression analysis and transcript assembly

Cufflinks.

Input:

- Aligned reads.
  - Gmap / Gsnap.
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- Assemble transcripts.
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Differential expression and regulation (`Cuffcompare`).

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- Use predefined transcripts.
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When to use:

- Only interested in expression.
- Alternative splicing.

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Variant calling

Principle of variant calling

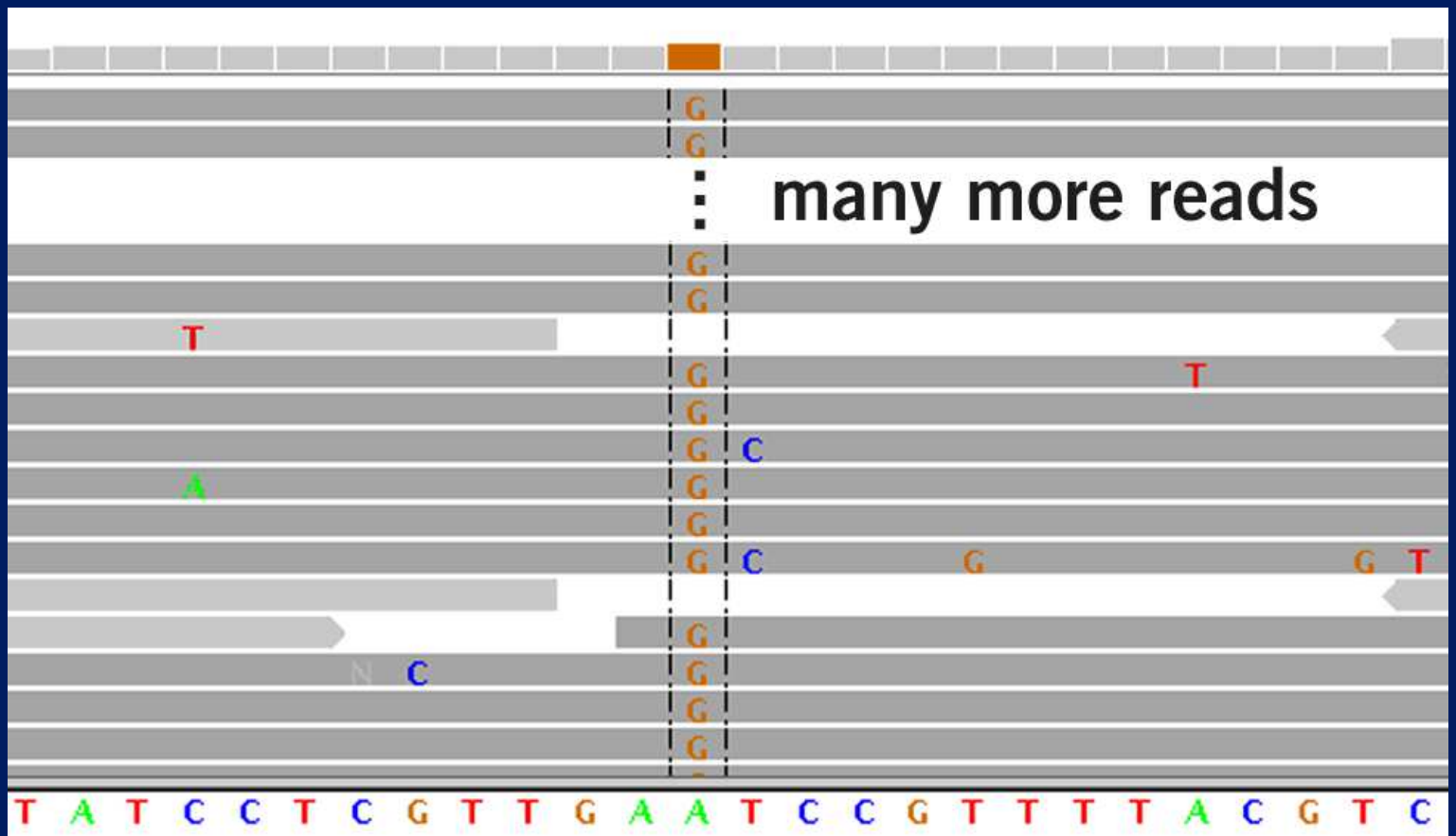

Figure 5 : Result of an alignment.

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Variant callers can use:

- Fixed settings.
- Statistical models.

Variant calling

Some considerations

Things a variant caller might take into account:

- Strand balance.
- Base quality.
- Mapping quality.
  - Distribution within the reads.
- Ploidity of the organism in question.

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- RNA editing.
  - Some variants will not be present on DNA.
- Strand specific sampleprep.

Pipelines

Combining tools in a pipeline.

```
1  bwa aln -t 8 $reference $i > $i.sai
2  bwa samse $reference $i.sai $i > $i.sam
3  samtools view -bt $reference -o $i.bam $i.sam
```

Listing 1 : Shell script.

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Listing 1 : Shell script.

```
1  %.sai: %.fq
2    $(BWA) aln -t $(THREADS) $(call MKREF, $@) $< > $@
3
4  %.sam: %.sai %.fq
5    $(BWA) samse $(call MKREF, $@) $^ > $@
6
7  %.bam: %.sam
8    $(SAMTOOLS) view -bt $(call MKREF, $@) -o $@ $<
```

Listing 2 : Makefile.

Galaxy

Outline of the practical

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

Questions?

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