IX International Course of Massive Data Analysis FOR GENOMICS

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Quality Control for Mapped Sequences

Introduction

SAMStat

QualiMap

Conclusions

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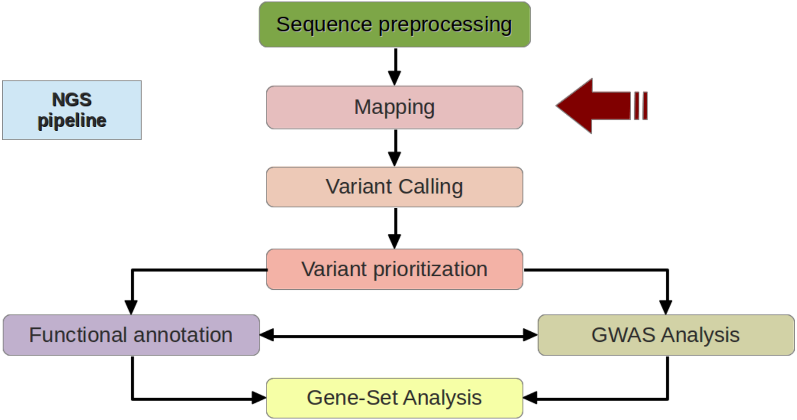
Where are we?


NGS pipeline
Sequence preprocessing
Mapping
Variant Calling
Variant prioritization
Functional annotation
GWAS Analysis
Gene-Set Analysis

Why QC on mapped sequences

Acknowledgment: Fernando García Alcalde

- The reads **may look OK** in QC analyses of **raw reads** but some **issues** only show up **once the reads are aligned**: low coverage, homopolymer biases, experimental artifacts, etc.
- These unwanted biases can be introduced by the selected:
  - Sample extraction process
  - Sequencing technology
  - Sample preparation protocol
  - Mapping algorithm

Why QC on mapped sequences

- SAM/BAM files usually contain information from tens to hundreds of millions of reads
- The **systematic detection** of such biases is a **non-trivial** task that is **crucial** to to drive appropriate downstream analyses.
- Look for big biases that really affect the analysis
- Difficult to provide guidelines: general trends

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SAMStat

Features

- Facilitates the indentification of sequencing error biases that may disturb the mapping process
- Provides a concise html page with statistics that highlight problems in the data processing:
  - Reads with an excesive proportion of mapping errors
  - Reads containing contaminants
  - Reads representing novel splice junctions/genomic regions
  - ...
- Easy-to-use command-line tool freely downloadable at:

http://samstat.sourceforge.net

Timo Lassmann, Yoshihide Hayashizaki, and Carsten O. Daub. SAMStat: monitoring biases in next generation sequencing data Bioinformatics (2011) 27(1): 130-131

Running SAMStat

- Input: a BAM/SAM file (other sequence files are also accepted such as fasta or fastq)
- Output: an html report

Run SAMStat with a .bam example

```
samstat /home/biouser/mda13/mqc-igv/test1.bam
```

- The html report will be saved at `/home/biouser/mda13/mqc-igv/test1.bam.html`. Use a web browser (e.g. Firefox) to open it

SAMStat report

Concepts

- Mapping quality: an integer in [0,254] representing $-10 \cdot \log_{10} P(\text{mapping error})$
- Calculated as a function of the quality of the read, and a score that indicates how well the read is aligned
- Algorithm-specific
- The higher it is, the better the alignment. ($\text{MAPQ} = 30 \Longrightarrow 0.001$ error rate)
- 255 indicates that the mapping quality is not available.

SAMStat report

Number of aligned reads and mapping quality

- Proportion of reads mapped in each mapping quality range.
- The “red part” should fill most of the pie chart area

SAMStat report

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SAMStat report

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- **Why?**

SAMStat report

Mean base quality

- Mean quality per read base in each mapping quality range
- Higher the base quality $\Longrightarrow$ higher mapping quality expected.

SAMStat report

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Error profiles

- Number of mismatches at each read position, segregated by the nucleotide causing the mismatch
- Should be more or less stable across read positions
- More errors are expected at the end of the reads since base qualities tend to be lower at that positions
- Nucleotide peaks at different positions may indicate experimental artifacts that disturb read mapping

SAMStat report

Over-represented di-nucleotides

- Over-representation scores for each possible di-nucleotide at each read position.
- Significant socores (p-value $<=$ 1e-100) appear in bold
- Over-represented di-nucleotides may indicate experimental artifacts that disturb read mapping

SAMStat report

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Error distribution

- Distribution of the number of errors (mismatches and indels) per read, segregated by mapping quality ranges
- No more than ~ 2 mismatches should be allowed for short ($\sim 75b$) reads

SAMStat report

Nucleotide composition

- Number of As,Cs,Gs and Ts appearing at each read position and segregated by mapping quality
- The counts and proportions should be almost invariant accross read positions

SAMStat report

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Length distribution

- Distribution of the number of bases per read

SAMStat report

Top 5 over-represented 2-mers

- Summary of the “Over-represented di-nucleotides”, including the top-5 2-mers in each position

SAMStat report

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Top 20 over-represented 10-mers

- The 20 most significant 10-mers per quality level

More on SAMStat

Hands-on

- Run SAMstat on `/home/biouser/mda13/mqc-igv/test2.bam` and `/home/biouser/mda13/mqc-igv/test3.bam`
- Interpret the results

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Qualimap

Aim

Provide an overall view of the data that helps to the detect biases in the sequencing and/or mapping of the data

Run QualiMap

```
qualimap
```

- BAM file needs to be sorted: `samtools sort <filename> <fileout>`
- File → New analysis → BAM/SAM file → /home/biouser/mda13/mqc-igv/HG00096.chrom20.bam

García-Alcalde, et al. Qualimap: evaluating next generation sequencing alignment data. Bioinformatics(2012) 28 (20): 2678-2679

Features

- Fast analysis accross the reference of genome coverage and nucleotide distribution
- Easy to interpret summary of the main properties of the alignment data
- Analysis of the reads mapped inside/outside of the regions provided in GFF format
- Insert size mean and median value calculation and plotting statistical distribution
- Analysis of the adequasy of the sequencing depth in RNA-seq experiments
- Clustering of epigenomic profiles

Hands on

- Open the online help
- Go through the examples
- Run qualimap over /home/biouser/mda13/mqc-igv/igv1.bam (with and without reference annotation http://reports.bioinfomgp.org/external-downloads/chr21.gtf)
- Run qualimap in the previous data (with and without reference annotation http://reports.bioinfomgp.org/external-downloads/refseqgenes.gtf)
- Have a look at http://reports.bioinfomgp.org/external-downloads/fullbam/qualimapReport.html
- Drive conclusions from what you get
- BONUS: Run qualimap via de command line

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- One should always perform QC on the mapped data
- The correct interpretation of the QC output may save a lot of time (and money) on downstream analyses
- The expected results are experiment-specific $\Longrightarrow$ Learn from experience