

NGS data analysis course

Quality control & Data Preprocessing

Ignacio Medina
David Montaner & Marta Bleda



PRINCIPE FELIPE
CENTRO DE INVESTIGACION

FastQ Format

- Standard Format for NGS data
- Conversion can be done from *sff*, *fasta* + *qual*, ...
- Extension of the Fasta format
- Text-based formats (easy to use!)
- If not compressed, it can be huge

http://en.wikipedia.org/wiki/FASTQ_format

Quality measurements

Base-calling **error probabilities** are reported by sequencers.

Usually in **Phred** (quality) score.

Usually coded by ASCII characters

Phred score

$$Q = -10 \log_{10} P$$

$$P = 10^{\frac{-Q}{10}}$$

[http://en.wikipedia.org/wiki/Phred_quality_score#
Definition](http://en.wikipedia.org/wiki/Phred_quality_score#Definition)

NGS Data Preprocessing Steps

- File parsing: convert to **fastq** format from **sff**, **fasta** + **qual**
...
- Split **multiplex** samples.
- Quality Control of the raw data.
- Filtering and trimming reads by quality.
- Adapter trimming
- Quality Control of the trimmed and filtered reads

- **FastQC:**

- quality control
- some filtering ...

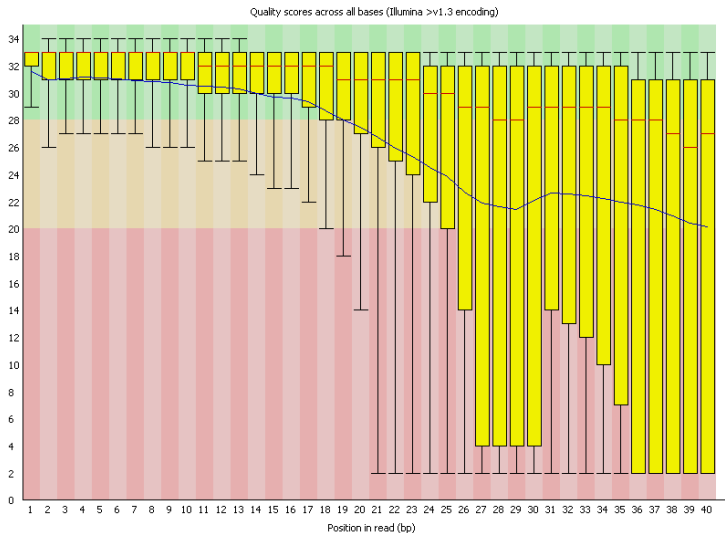
www.bioinformatics.babraham.ac.uk/projects/fastqc

- **Cutadapt:**

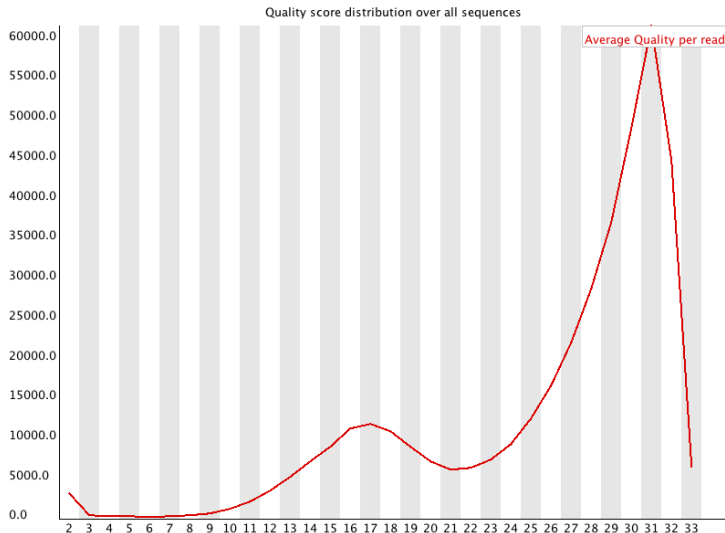
- adapter trimming
- filter reads by length (short, long)
- filter reads by quality

<http://code.google.com/p/cutadapt>

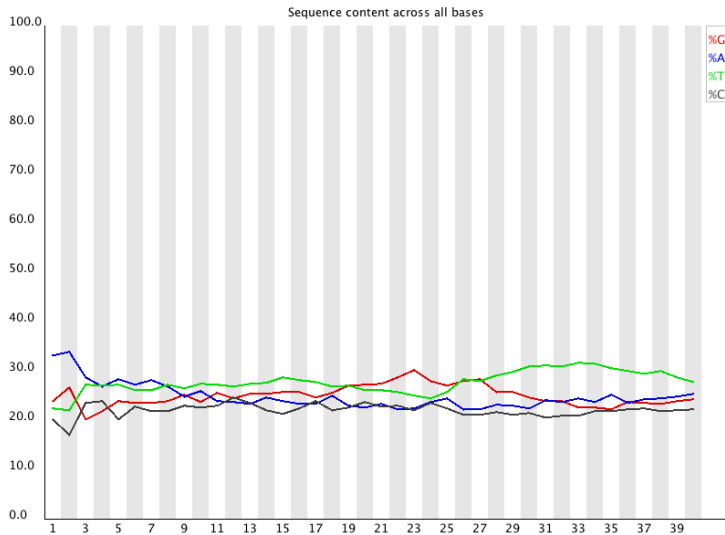
Per Base Sequence Quality



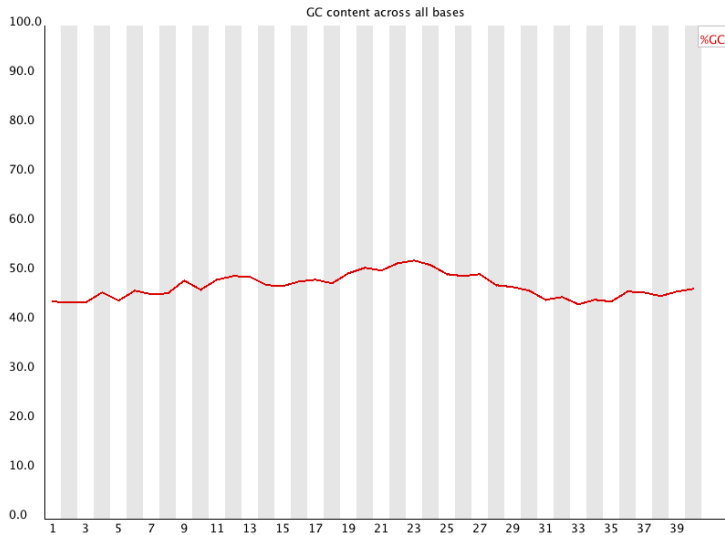
Per Sequence Quality



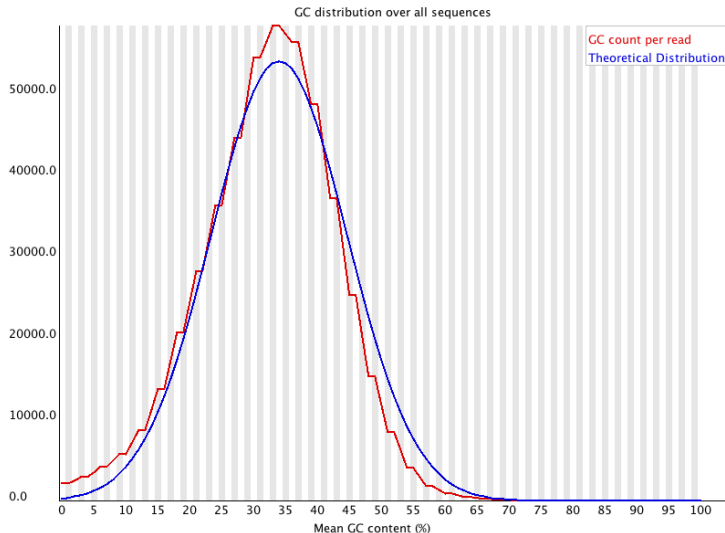
Per Base Sequence Content



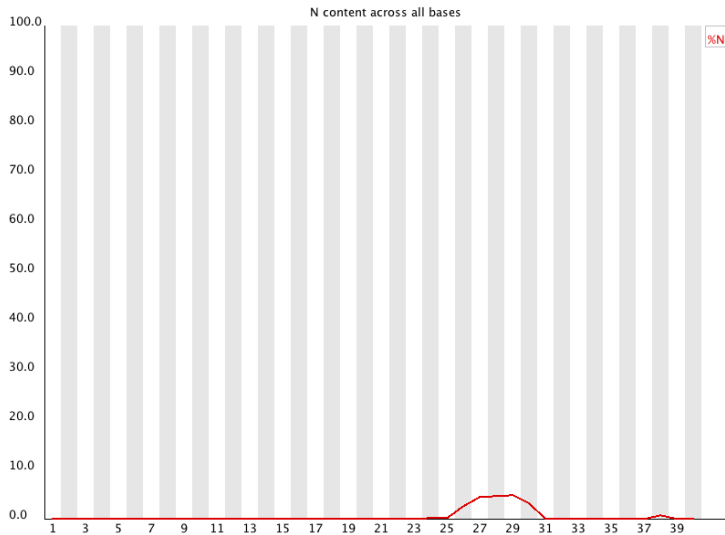
Per Base GC Content



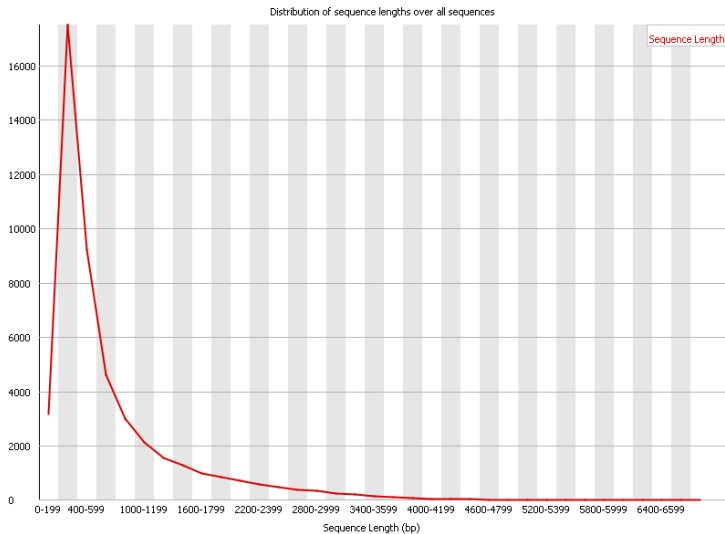
Per Sequence Nucleotide Content



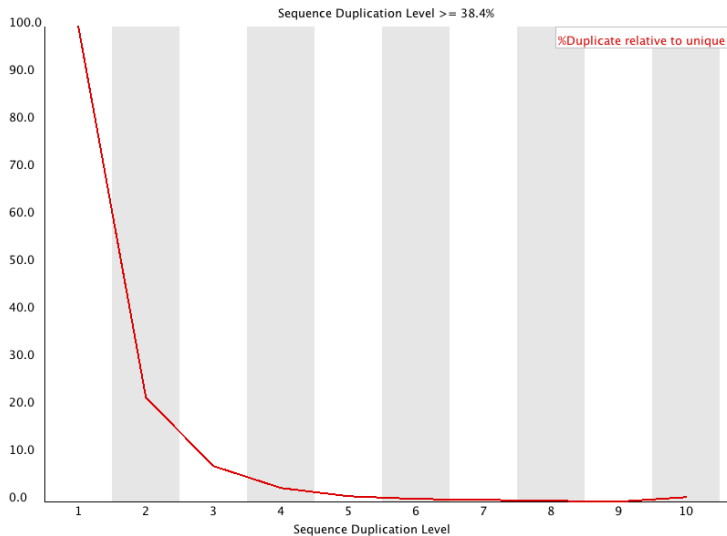
Per Base N Content



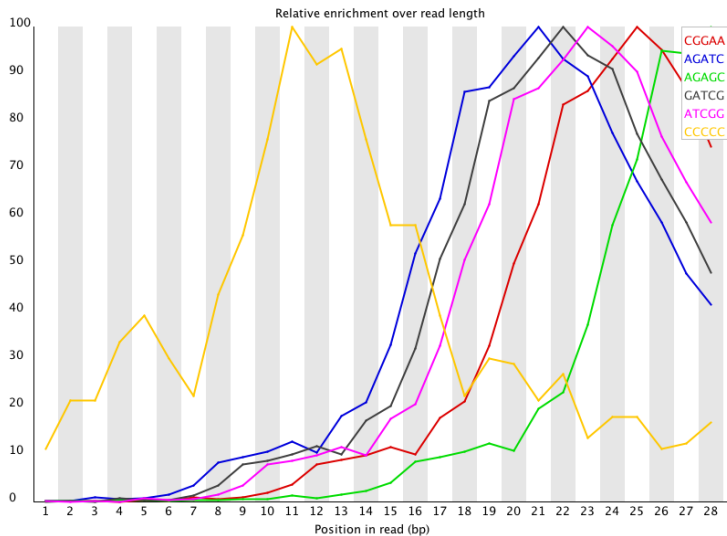
Sequence Length Distribution



Duplicate Sequences Distribution



Overrepresented Kmers



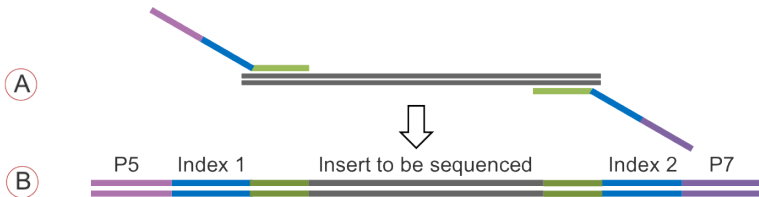
More FastQ examples and documentation

... may be found at [FastQ home page](#)

- Example Reports

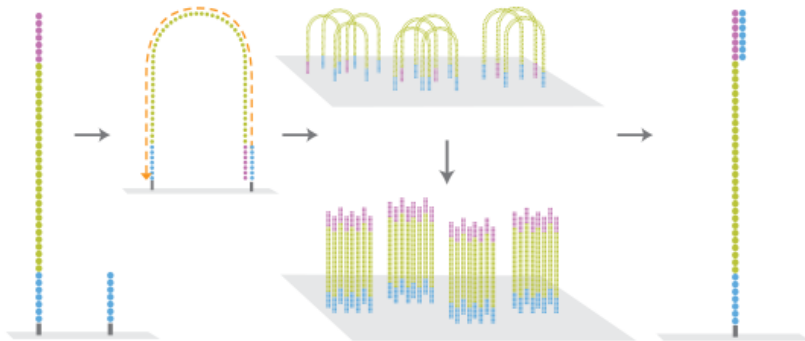
Sequencing process: PCR primers

One-step PCR Method



- A Target-specific PCR with indices and sequencing adaptors
- B Final amplicon ready to be sequenced

Sequencing process: PCR primers



NGS adaptors and Cutadapt

