

# An introduction to genome assemblies

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# An introduction to genome assemblies

## 1.0 Preface

In this genome assembly tutorial we will use our skill on the command-line interface to deal with the task of a genome assembly. You will learn how to judge the sequencing quality of a next generation sequencing (NGS) run and assemble the genome based on a collection of short sequences.

You will encounter some **To-do** sections at times. Write the solutions and answers into a text-file.

A PDF-version of this tutorial can be downloaded [here](#) or at [http://sschmeier.github.io/bioinf-workshop/genome-assembly/doc/GenomeAssembly\\_sschmeier.pdf](http://sschmeier.github.io/bioinf-workshop/genome-assembly/doc/GenomeAssembly_sschmeier.pdf)

## 1.1 Learning outcomes

1. Being able to compute, investigate and evaluate the quality of sequence data from a sequencing experiment.
2. Being able to compute, interpret and evaluate a whole genome assembly.
3. Being able to judge the quality of a genome assembly.

## 1.2. The data

### 1.2.1 Get the data

You can download the data-file [here](#) (or at <http://sschmeier.github.io/bioinf-workshop/genome-assembly/data/eli.low10paired.fastq.gz>). I will also bring the data on a USB drive, please copy it onto your system should the download not work.

The data is a down-sampled (randomly selected) small portion of the original sequencing data-set. This has been done because the amount of data produced was too high for the exercises today. Also, the original data was paired-end data (see *Figure 1*), thus we had two files, one for each end of the read. However, our assembler expects an read-interleaved file, which has been already created to make things easier.

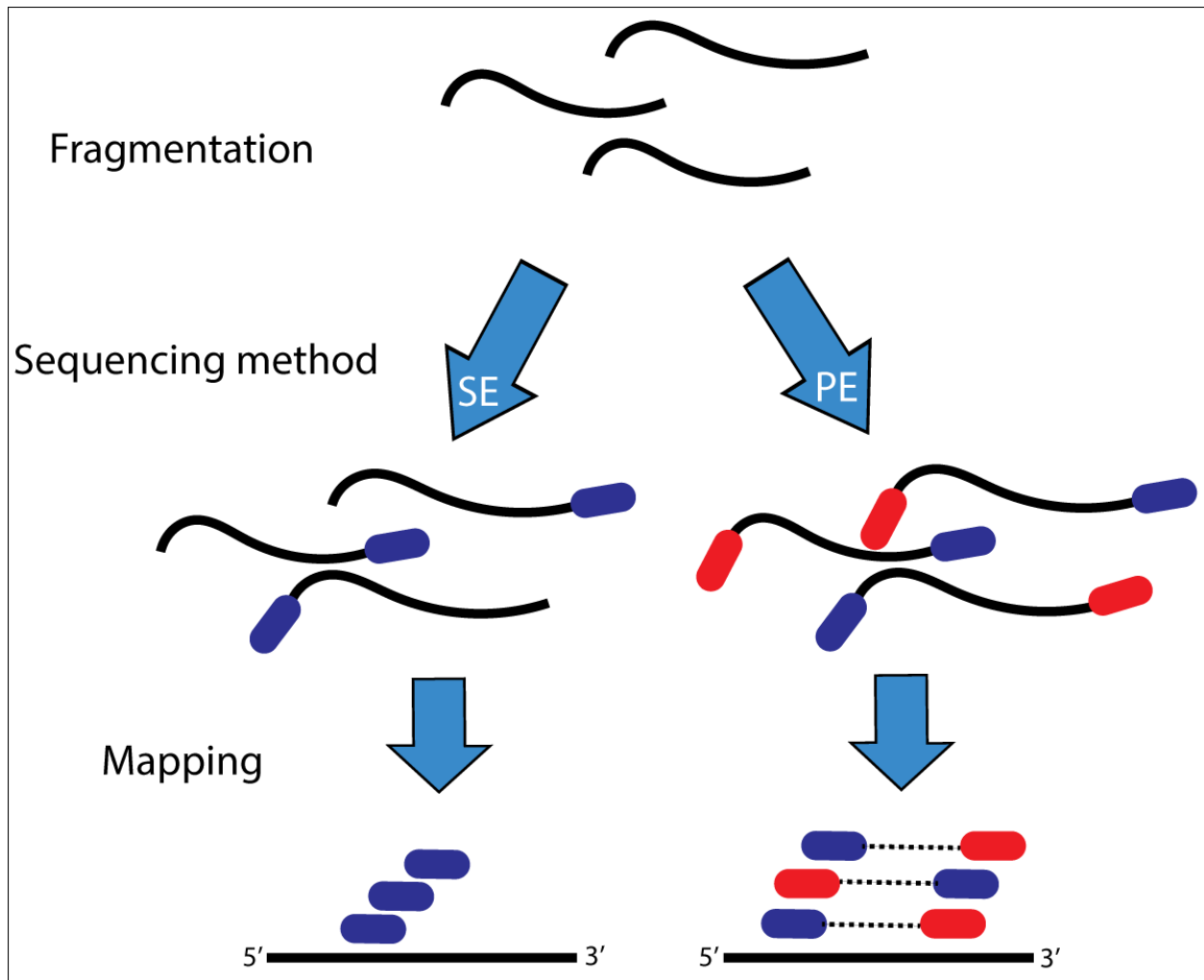


Figure 1: Illustration of single-end versus paired-end sequencing.

If you need to refresh how Illumina paired-end sequencing works have a look at the [Illumina webpage](http://www.illumina.com/technology/next-generation-sequencing/paired-end-sequencing_assay.html) ([http://www.illumina.com/technology/next-generation-sequencing/paired-end-sequencing\\_assay.html](http://www.illumina.com/technology/next-generation-sequencing/paired-end-sequencing_assay.html)) and this [video](https://youtu.be/HMyCqWhwB8E) at <https://youtu.be/HMyCqWhwB8E>.

### 1.2.2 Investigate the data

Make use of your newly developed skills on the command-line to investigate the files in two folders.

#### To-do

1. Unzip the data using `gzip`.
2. Use the command-line to get some ideas about the file.
3. What kind of files are we dealing with?
4. How many sequence reads are in the file?

### 1.3 Quality assessment of sequencing reads (SolexaQA++)

To assess the sequence read quality of the Illumina run we make use of a program called [SolexaQA](http://solexaqa.sourceforge.net/) (<http://solexaqa.sourceforge.net/>) [Cox2010]. This was originally developed to work with Solexa data (since bought by

ILLUMINA), but long since working with ILLUMINA data. It produces nice graphics that intuitively show the quality of the sequences. It is also able to dynamically trim the bad quality ends off the reads.

From the webpage:

“SolexaQA calculates sequence quality statistics and creates visual representations of data quality for second-generation sequencing data. Originally developed for the ILLUMINA system (historically known as “Solexa”), SolexaQA now also supports Ion Torrent and 454 data.”

### 1.3.1 Download/install SolexaQA++

Download [SolexaQA](#) from the developer webpage [here](#).

Note! Emergency link [here](#) or at [http://sschmeier.github.io/bioinf-workshop/genome-assembly/apps/SolexaQA++\\_v3.1.3.zip](http://sschmeier.github.io/bioinf-workshop/genome-assembly/apps/SolexaQA++_v3.1.3.zip).

```
# unzip the archive
$ unzip SolexaQA++_v3.1.3.zip
# make the file executable
$ chmod a+x SolexaQA++_v3.1.3/Linux_x64/SolexaQA++
# copy the executable to my working directory
$ cp ./SolexaQA++_v3.1.3/Linux_x64/SolexaQA++ .
# run the program
$ ./SolexaQA++
# should now run the program
```

### 1.3.2 Understand SolexaQA++

SolexaQA++ has three modes that can be run. Type:

```
$ ./SolexaQA++
```

```
SolexaQA++ v3.1.3
Released under GNU General Public License version 3
C++ version developed by Mauro Truglio (M.Truglio@massey.ac.nz)

Usage: SolexaQA++ <command> [options]

Command: analysis      quality analysis and graphs generation
         dynamictrim   trim reads using a chosen threshold
         lengthsort    sort reads by a chosen length
```

The three modes are: analysis, dynamictrim, and lengthsort

analysis - the primary quality analysis and visualization tool. Designed to run on unmodified FASTQ files obtained directly from ILLUMINA, Ion Torrent or 454 sequencers.

dynamictrim - a read trimmer that individually crops each read to its longest contiguous segment for which quality scores are greater than a user-supplied quality cutoff.

lengthsort - a program to separate high quality reads from low quality reads. LengthSort assigns trimmed reads to paired-end, singleton and discard files based on a user-defined length cutoff.

### 1.3.3 Run SolexaQA++ analysis on untrimmed data

#### To-do

1. Create a directory for the result-files -> **qa\_untrimmed/**.
2. Run SolexaQA++ with the untrimmed data, and submit result-directory **qa\_untrimmed/**.
3. Investigate the result-files in **qa\_untrimmed/**.
4. Compare your results to these examples of a particularly bad MiSeq run (Figure 2-5, taken from <http://solexaqa.sourceforge.net/>). Write down your observations.
5. What elements in these example figures (Figure 2-5) show that we are dealing with a bad run? Write down your explanations.

Hint! Should you not get 1 and/or 2 right, try these commands [here](#) or at <http://sschmeier.github.io/bioinf-workshop/genome-assembly/code/solexaqa1.txt>.

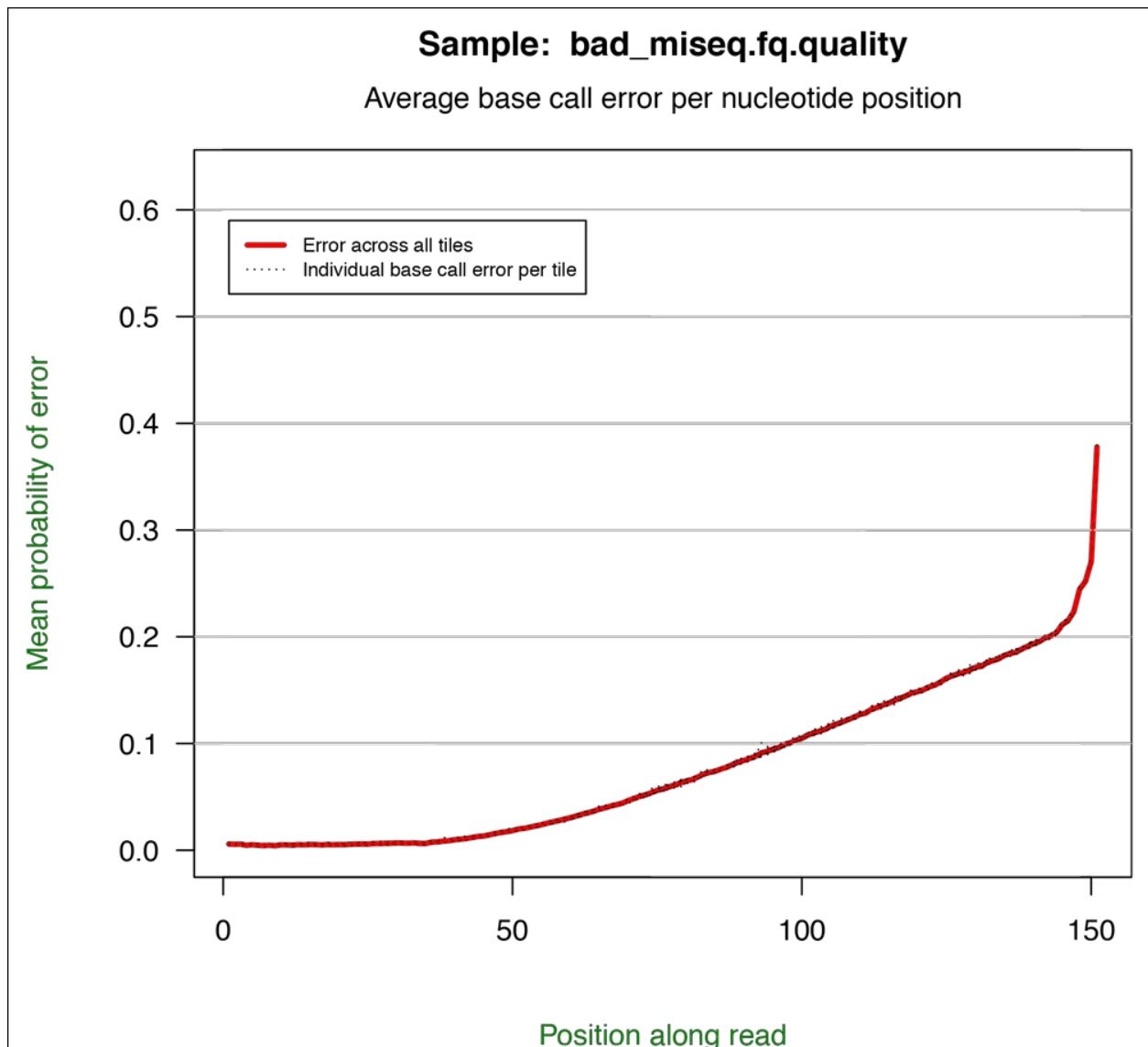


Figure 2: SolexaQA++ example quality plot along reads of a bad MiSeq run.

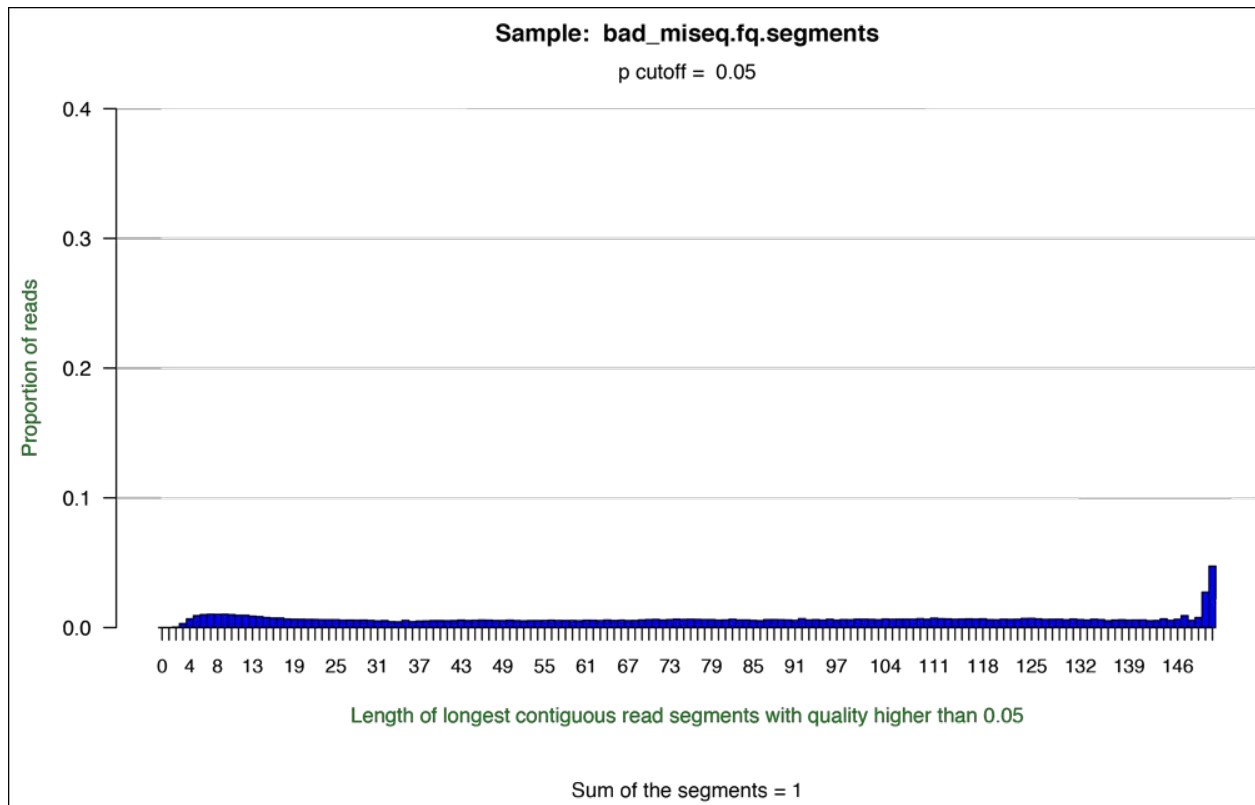


Figure 3: SolexaQA++ example histogram plot of a bad MiSeq run.

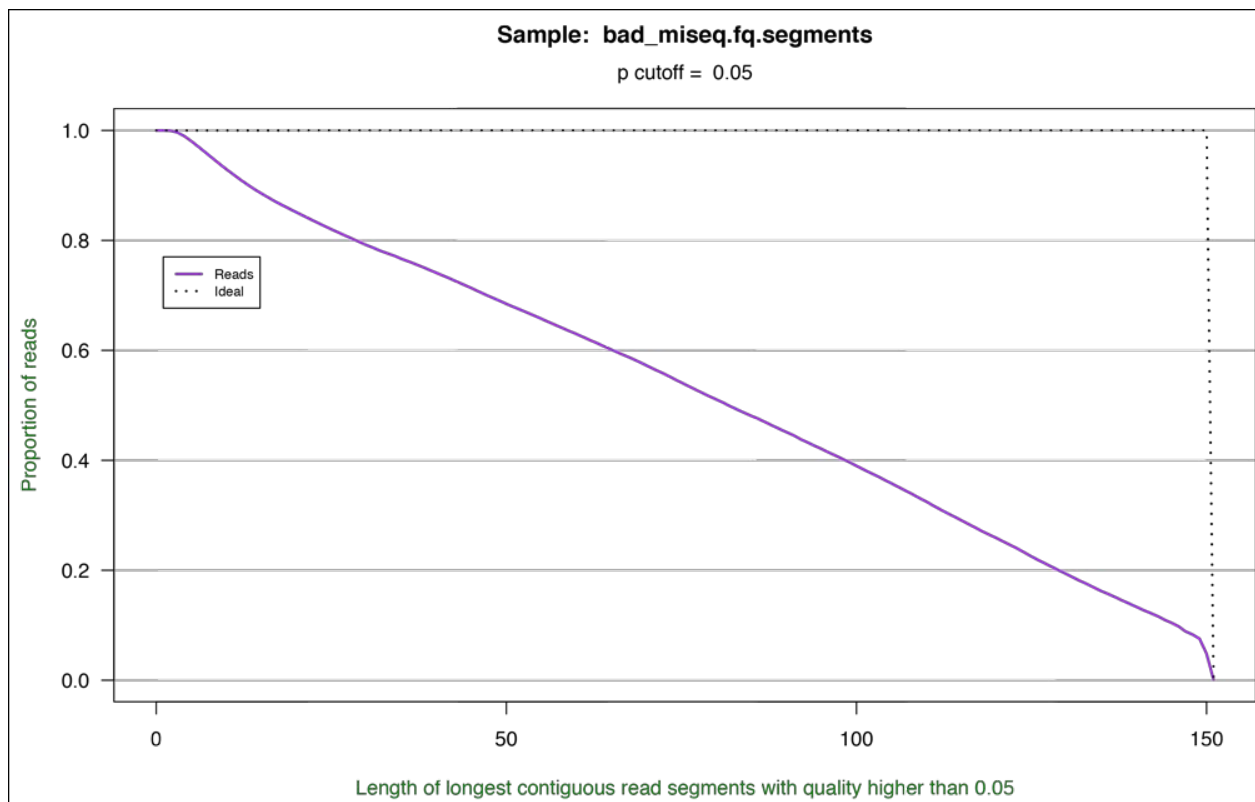


Figure 4: SolexaQA++ example cumulative plot of a bad MiSeq run.

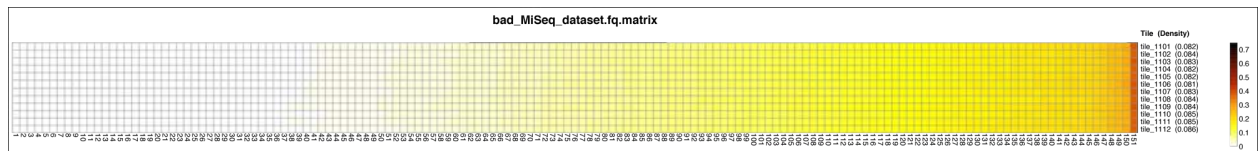


Figure 5: SolexaQA++ example quality heatmap of a bad MiSeq run.

### 1.3.4 Use SolexaQA++ to dynamic trim the data

Despite what you may have found out about the untrimmed data, it is a good idea to trim the data before further analyses.

#### To-do

1. Create a directory for the result-files -> **qa\_toTrimmed/**
2. Use SolexaQA++ to trim the reads based on quality and a probability cutoff of 0.01.

Hint! Should you not get 1 and/or 2 it right, try these commands [here](#) or at <http://sschmeier.github.io/bioinf-workshop/genome-assembly/code/solexaqa2.txt>.

### 1.3.5 Run SolexaQA++ analysis on trimmed data

#### To-do

1. Create a directory for the result-files -> **qa\_trimmed/**.
2. Do the quality assessment again with the trimmed data-set.
3. Compare the results in **qa\_trimmed/** to the untrimmed results in **qa\_untrimmed/**. Can you say something about the differences in the untrimmed and trimmed data sets? Write down your observations.

Hint! Should you not get 1 and/or 2 it right, try these commands [here](#) or at <http://sschmeier.github.io/bioinf-workshop/genome-assembly/code/solexaqa3.txt>.

## 1.4 Quality assessment of sequencing reads (FastQC)

### 1.4.1 Download/install FastQC (might already be installed)

Download **FastQC** (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) from the developer webpage [here](#).

Note! Emergency link [here](#) or at [http://sschmeier.github.io/bioinf-workshop/genome-assembly/apps/fastqc\\_v0.11.3.zip](http://sschmeier.github.io/bioinf-workshop/genome-assembly/apps/fastqc_v0.11.3.zip).

```
# Download file on the command-line
$ wget http://www.bioinformatics.babraham.ac.uk/projects/fastqc/fastqc_v0.11.3.zip
# Unzip the archive
$ unzip fastqc_v0.11.3.zip
# copy the program to the working directory
$ cp ./FastQC/fastqc .
# run the program
$ ./fastqc --help
# should now run the program
```



**FastQC** - A high throughput sequence QC analysis tool

#### SYNOPSIS

```
fastqc seqfile1 seqfile2 .. seqfileN
```

```
fastqc [-o output dir] [--(no)extract] [-f fastq|bam|sam]  
      [-c contaminant file] seqfile1 .. seqfileN
```

#### DESCRIPTION

**FastQC** reads a set of sequence files and produces from each one a quality control report consisting of a number of different modules, each one of which will help to identify a different potential type of problem in your data.

If no files to process are specified on the command line then the program will start as an interactive graphical application. If files are provided on the command line then the program will run with no user interaction required. In this mode it is suitable for inclusion into a standardised analysis pipeline.

### 1.4.2 Understand FastQC

FastQC is a very simple program to run that provides similar and additional information to SolexaQA++.

From the webpage:

FastQC aims to provide a simple way to do some quality control checks on raw sequence data coming from high throughput sequencing pipelines. It provides a modular set of analyses which you can use to give a quick impression of whether your data has any problems of which you should be aware before doing any further analysis.

The basic command looks like:

```
$ fastqc -o result-dir/ input-file.[txt/fa/fq] ...
```

- -o result-dir/ is the directory where the result files will be written
- input-file.[txt/fa/fq] is the sequence file to analyze, can be more than one file.

Hint! The result will be a HTML page per input file that can be opened in a web-browser.

### 1.4.3 Run FastQC on the untrimmed and trimmed data

#### To-do

1. Create a directory for the results -> **fastqc\_results/**
2. Run FastQC with both files
3. Compare the two result-files in a browser
4. Visit the [FastQC website](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and read about sequencing QC reports for good and bad Illumina sequencing runs.

5. Compare your results to these examples (Figure 6-8) of a particularly bad run (taken from <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Write down your observations.
6. What elements in these example figures (Figure 6-8) show that we are dealing with a bad run? Write down your observations.

Hint! Should you not get it right, try these commands [here](#) or at <http://sschmeier.github.io/bioinf-workshop/genome-assembly/code/fastqc.txt>.

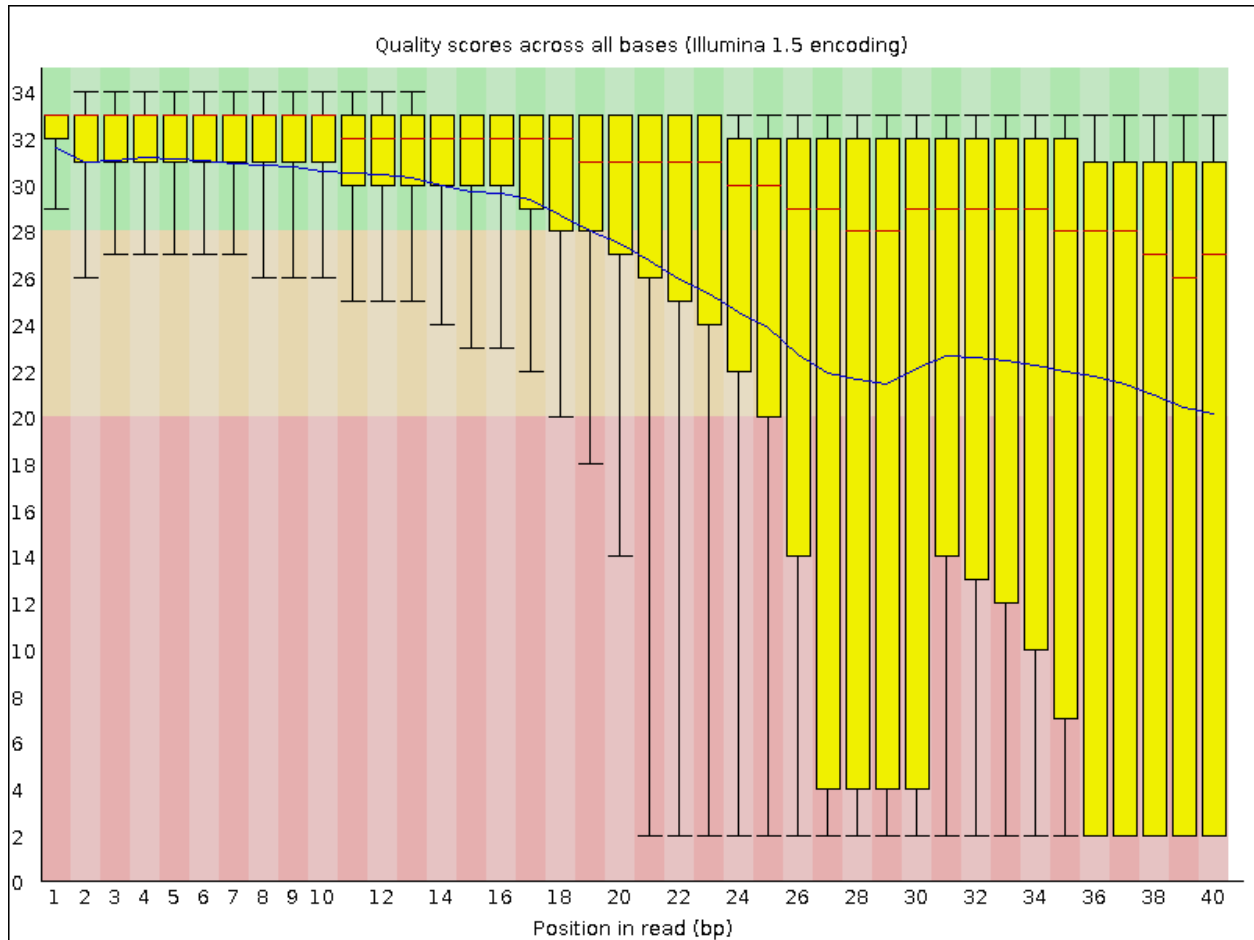


Figure 6: Quality score across bases.

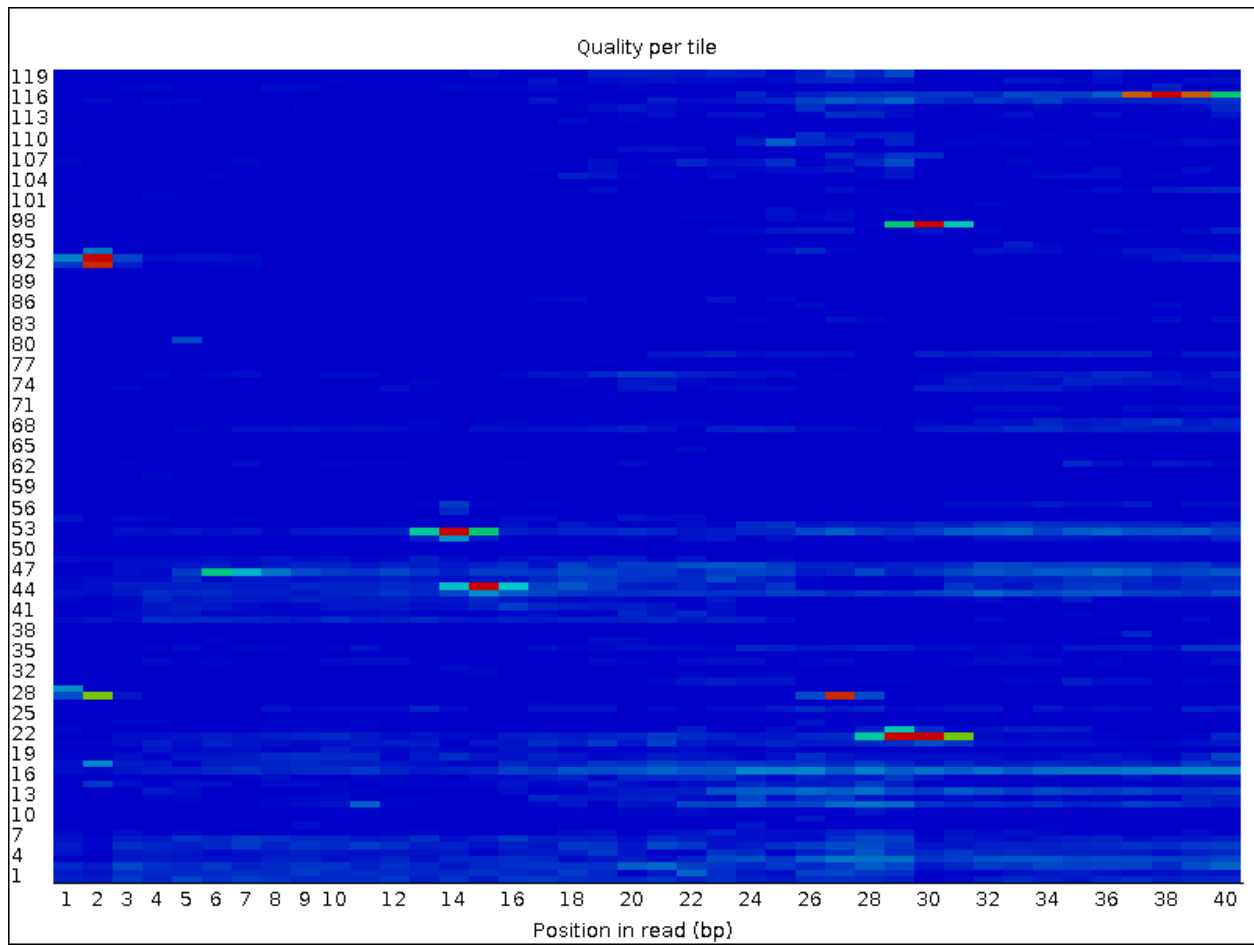


Figure 7: Quality per tile.

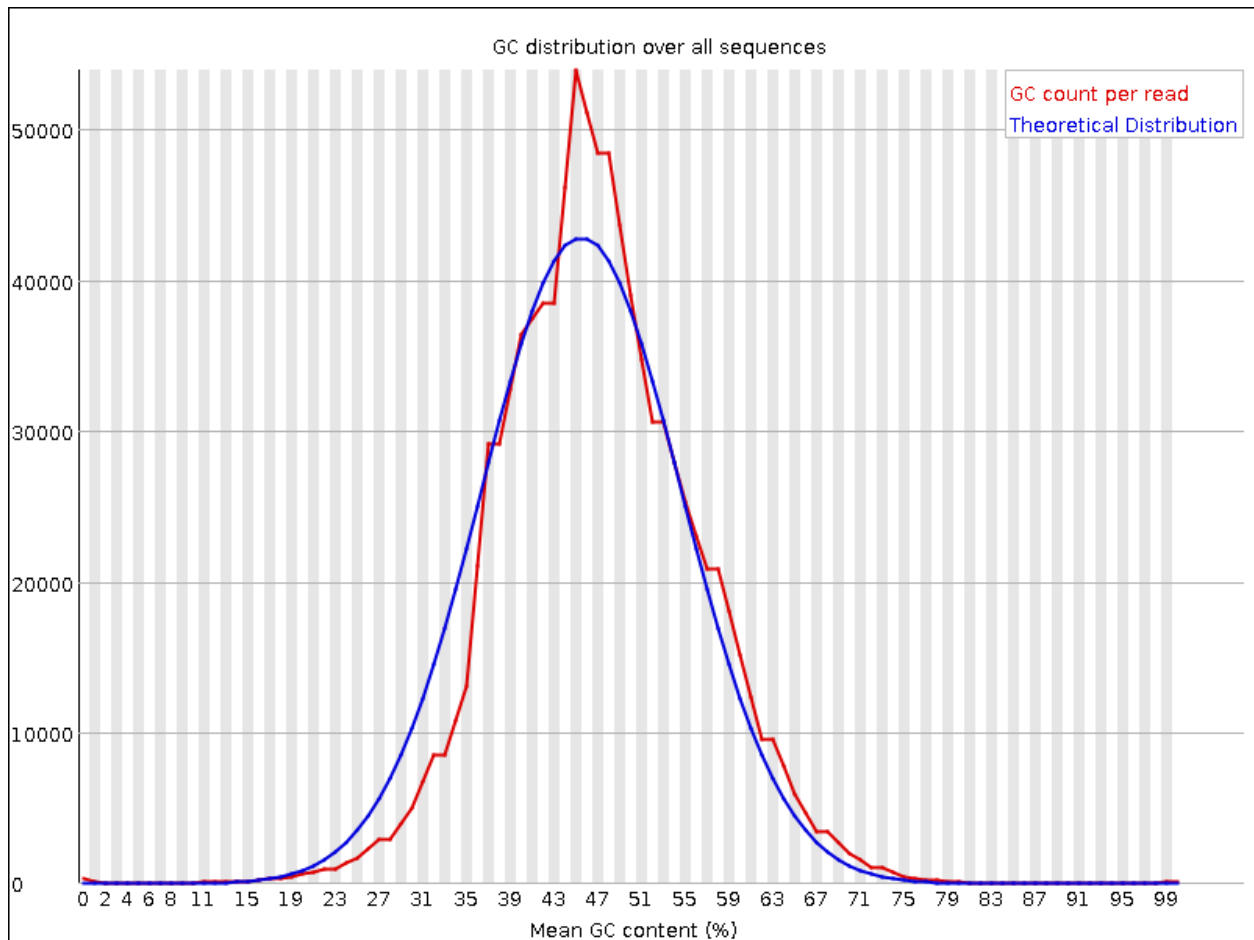


Figure 8: GC distribution over all sequences.

## 1.5 Whole genome assembly (velvet)

**Velvet** (<https://www.ebi.ac.uk/~zerbino/velvet/>) is a *de Bruijn* graph assembly program [Zerbino2008]. It is developed with the aim of assembling very short reads like in our case.

From the webpage:

Velvet is a *de novo* genomic assembler specially designed for short read sequencing technologies, such as Solexa or 454. Velvet currently takes in short read sequences, removes errors then produces high quality unique contigs. It then uses paired-end read and long read information, when available, to retrieve the repeated areas between contigs.

### 1.5.1 Download/install Velvet (might already be installed)

Download **Velvet** from the developer website ([https://www.ebi.ac.uk/~zerbino/velvet/velvet\\_1.2.10.tgz](https://www.ebi.ac.uk/~zerbino/velvet/velvet_1.2.10.tgz)).

Note! Emergency download-link [here](#) or at [http://sschmeier.github.io/bioinf-workshop/genome-assembly/apps/velvet\\_1.2.10.tgz](http://sschmeier.github.io/bioinf-workshop/genome-assembly/apps/velvet_1.2.10.tgz).

```
# unzip
$ gunzip velvet_1.2.10.tgz
# extract the archive
```

```
$ tar xvf velvet_1.2.10.tar
# change directory
$ cd velvet_1.2.10/
# compile program
$ make 'MAXKMERLENGTH=63'
# this will take a few seconds
# we have now two programs: velveth and velvetg

# copy these to your home directory
```

### 1.5.2 Understanding Velvet

Velvet uses two different programs sequentially to achieve the assembly. You have to use them one at a time. You should familiarize yourself with the structure of the program calls. Once you understand this it is very easy to change things around.

#### velveth

This program is a hashing program. It basically prepares your data for the assembler velvetg. An example program call looks like:

```
# Running velveth without parameters gives you a help page
$ ./velveth
# Here a complete example call
$ ./velveth results_directory/ 63 -fastq -shortPaired input_seqs.fastq
```

- `./velveth` the program including the full path where the program is located
- `result_directory/` the directory where you want the results to be saved
- `63` the kmer value
- `-fastq` specifies the input sequence-file type
- `-shortPaired` the type of the reads (here short paired reads)
- `input_seqs.fastq` the file-name of the input data

#### velvetg

This program is the core of velvet and the actual assembler. An example program call looks like this:

```
$ ./velvetg result_directory/ -cov_cutoff 10 -exp_cov 30
    -min_contig_lgth 500 -ins_length 300
```

- `./velvetg` the program including the full path where the program is located
- `result_directory/` the directory where you want the results to be saved
- `-cov_cutoff 10` removal of low coverage nodes AFTER tour bus or allow the system to infer it [do not worry about this]
- `-exp_cov 30` the expected coverage of unique regions or allow the system to infer it [do not worry about this]
- `-min_contig_lgth 500` the minimum size of contig to report back (to exclude tiny ones we only look at contigs >500bp)
- `-ins_length 300` expected distance between two paired-end reads in the respective short-read dataset

### 1.5.3 Run Velvet with untrimmed data

#### To-do

1. Make sure you have the untrimmed data and you know where it is.
2. Create a directory for the results -> `velvet_untrimmed/`
3. Run `velveth` with the data `eli.low10paired.fastq`
4. Run `velvetg`

Hint! Should you not get it right, try these commands [here](#) or at <http://sschmeier.github.io/bioinf-workshop/genome-assembly/code/velvet1.txt>.

### 1.5.4 Run Velvet with trimmed data

#### To-do

1. Make sure you have the trimmed data and you know where it is.
2. Create a directory for the results -> `velvet_trimmed/`
3. Run `velveth` with the data `qa_toTrimmed/eli.low10paired.fastq.trimmed`
4. Run `velvetg`

Hint! Should you not get it right, try these commands [here](#) or at <http://sschmeier.github.io/bioinf-workshop/genome-assembly/code/velvet2.txt>.

### 1.5.5 Evaluate assemblies

#### To-do

1. Look at the Log files from trimmed and untrimmed assemblies.
2. Look at the `stat.txt` files from trimmed and untrimmed assemblies.
3. Look at the `contigs.fa` files of the trimmed and untrimmed assemblies.
4. What can we say about the the assemblies?
5. How does untrimmed and trimmed compare?
6. What can you say about the trimming procedure in light of assembling sequences?

### 1.5.6 Assembly quality assessment

**Quast** (QQuality ASsesment Tool, <http://quast.bioinf.spbau.ru/>) [Gurevich2013], evaluates genome assemblies by computing various metrics, including:

- N50: length for which the collection of all contigs of that length or longer covers at least 50% of assembly length
- NG50: where length of the reference genome is being covered
- NA50 and NGA50: where aligned blocks instead of contigs are taken
- missassemblies: misassembled and unaligned contigs or contigs bases
- genes and operons covered

The program can be used on their website (<http://quast.bioinf.spbau.ru/>) or downloaded for local use at this direct [link](#).

### 1.5.7 Visualize assemblies

**Bandage** (Bioinformatics Application for Navigating De novo Assembly Graphs Easily, <https://rrwick.github.io/Bandage/>) is a program that visualises an assembly as a graph [Wick2015].

## 1.6 Genome annotation

Geneome annotation can be facilitated for example with these programs:

- Genome annotation pipeline **RAST** (<http://rast.nmpdr.org/>) [Aziz2008]
- Phage-specific annotation pipeline **PHAST** (<http://phast.wishartlab.com/>) [Zhou2011]

## 1.7 What's next?

Next steps could include:

- Map all reads to the “new” genome
- Look at the aligned reads in a genome viewer
- Predict genes in the “new” genome
- Overlay gene information with aligned reads in the genome viewer
- etc.

## 1.8 Further reading

*Background on Genome Assemblies:*

- [Compeau2011](#)
- [Nagarajan2013](#)

*Evaluation of Genome Assembly tools:*

- [Salzberg2012](#)

## 1.9 References

Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. **The RAST Server: rapid annotations using subsystems technology.** *BMC Genomics* 2008 Feb 8;9:75

Compeau PE, Pevzner PA, Tesler G. **How to apply de Bruijn graphs to genome assembly.** *Nat Biotechnol.* 2011 Nov 8;29(11):987-91

Cox MP, Peterson DA and Biggs PJ. **SolexaQA: At-a-glance quality assessment of Illumina second-generation sequencing data.** *BMC Bioinformatics* 2010, 11:485

Gurevich A, Saveliev V, Vyahhi N and Tesler G. **QUAST: quality assessment tool for genome assemblies.** *Bioinformatics* 2013, 29(8), 1072-1075

Nagarajan N, Pop M. **Sequence assembly demystified.** *Nat Rev Genet.* 2013 Mar;14(3):157-67

Salzberg SL, Phillippy AM, Zimin A, Puiu D, Magoc T, Koren S, Treangen TJ, Schatz MC, Delcher AL, Roberts M, Marçais G, Pop M, Yorke JA. **GAGE: A critical evaluation of genome assemblies and assembly algorithms.** *Genome Res.* 2012 Mar;22(3):557-67

Wick RR, Schultz MB, Zobel J and Holt KE. **Bandage: interactive visualization of de novo genome assemblies.** *Bioinformatics* 2015, 10.1093/bioinformatics/btv383

Zerbino DR and Birney E. **Velvet: algorithms for de novo short read assembly using de Bruijn graphs.** *Genome Res.* 2008, 18:821-829.

Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. **PHAST: a fast phage search tool.** *Nucleic Acids Res.* 2011 Jul;39(Web Server issue):W347-52.

## 2.0 Web links

This tutorial: <http://sschmeier.github.io/bioinf-workshop/genome-assembly/>

Illumina paired-end sequencing: [http://www.illumina.com/technology/next-generation-sequencing/paired-end-sequencing\\_assay.html](http://www.illumina.com/technology/next-generation-sequencing/paired-end-sequencing_assay.html)

FastQC: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

SolexaQA: <http://solexaqa.sourceforge.net/>

Velvet: <https://www.ebi.ac.uk/~zerbino/velvet/>

Quast: <http://quast.bioinf.spbau.ru/>

Bandage: <https://rrwick.github.io/Bandage/>

PHAST: <http://phast.wishartlab.com/>

RAST: <http://rast.nmpdr.org/>

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