ΙΝΣΤΙΤΟΥΤΟ ΕΦΑΡΜΟΣΜΕΝΩΝ ΒΙΟΕΠΙΣΤΗΜΩΝ

INAB & Next Generation Sequencing

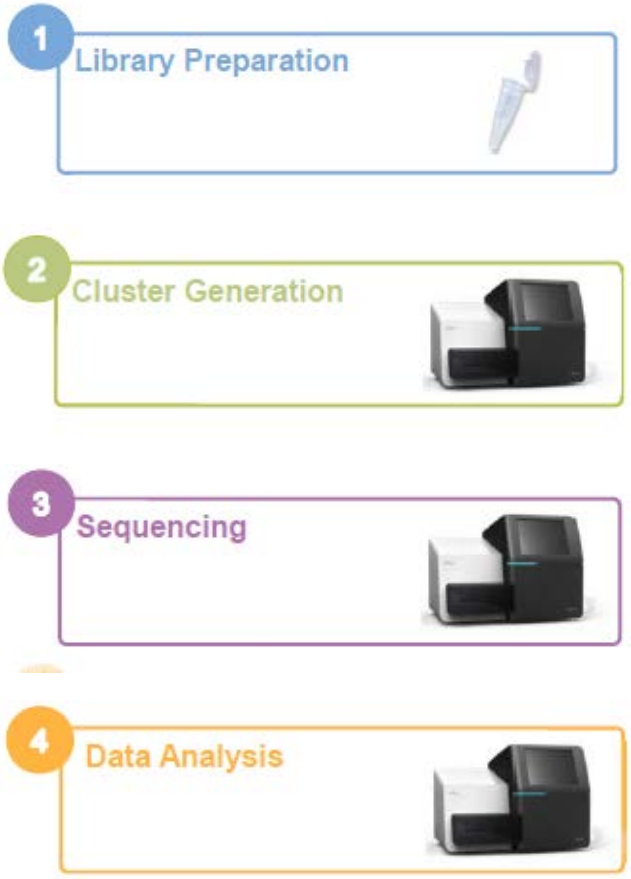
MiSeq

NextSeq500

illumina®

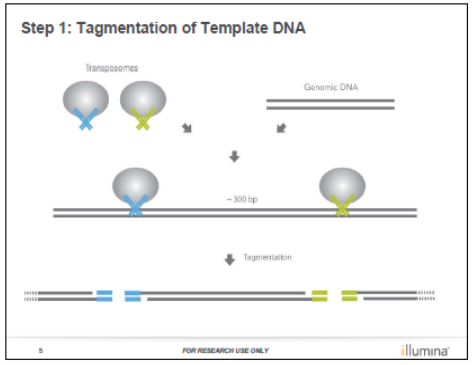
SafeBlood BioAnalytica

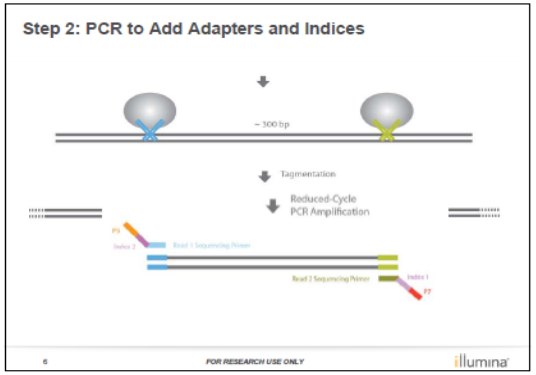
NGS Experiments

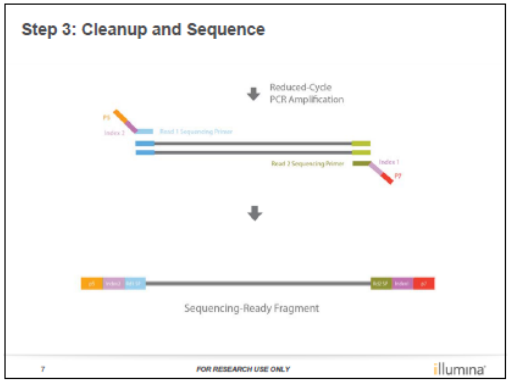


Library Preparation

Nextera DNA
Sample Prep Kit

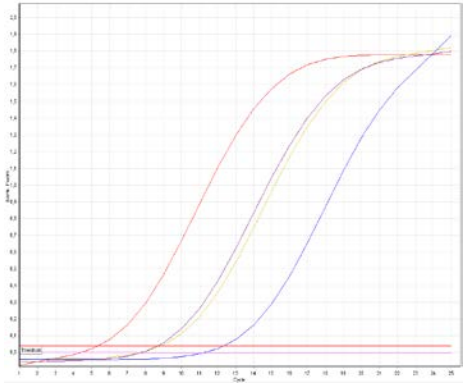




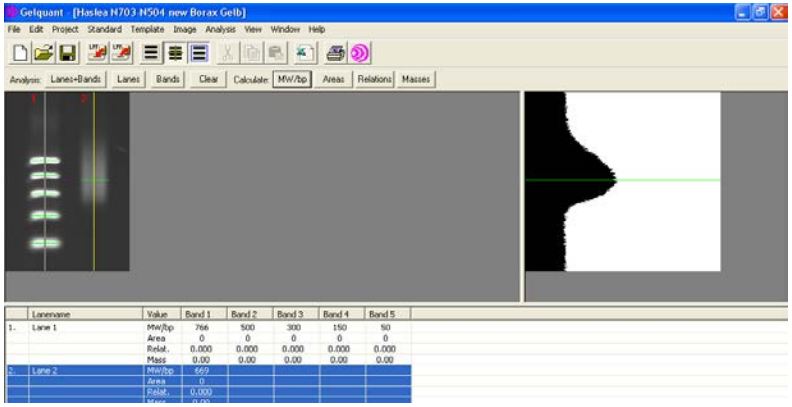


Library validation methods

Quantification



Real time qPCR

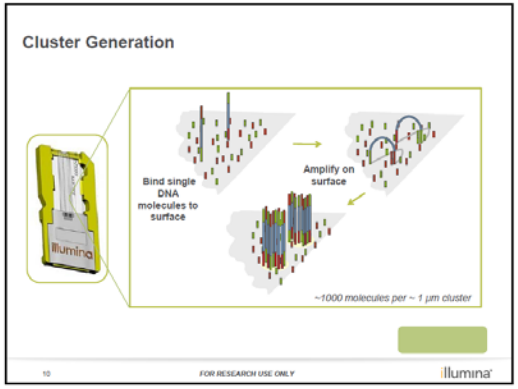


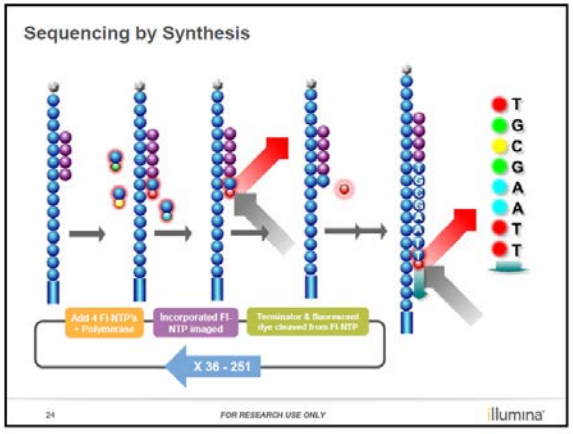
Gel based analysis

NGS Experiments

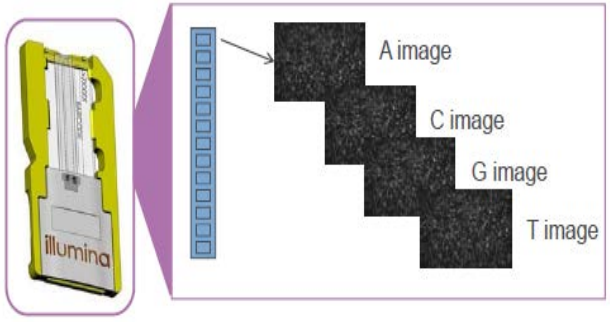
1 Library Preparation

2 Cluster Generation

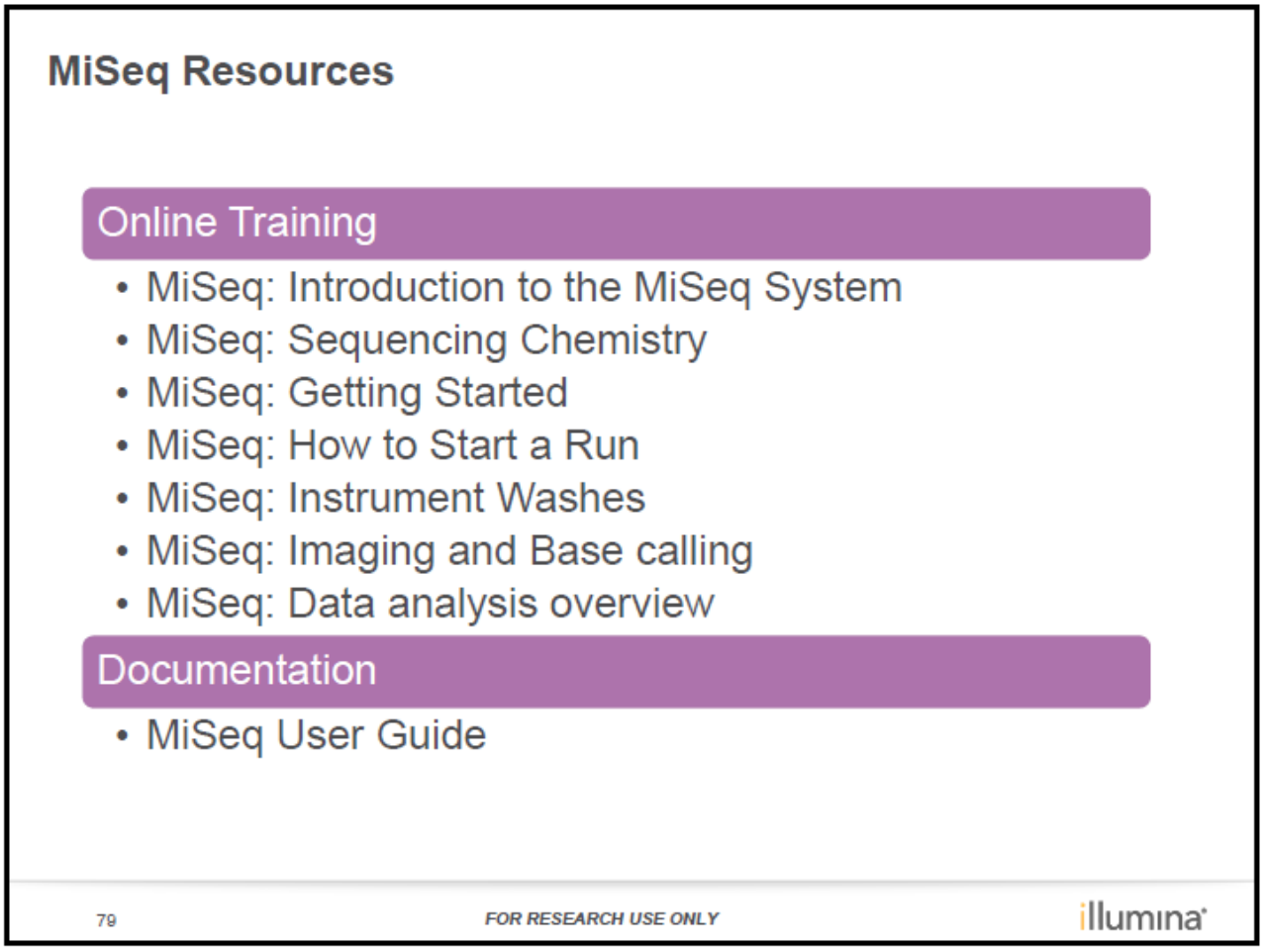




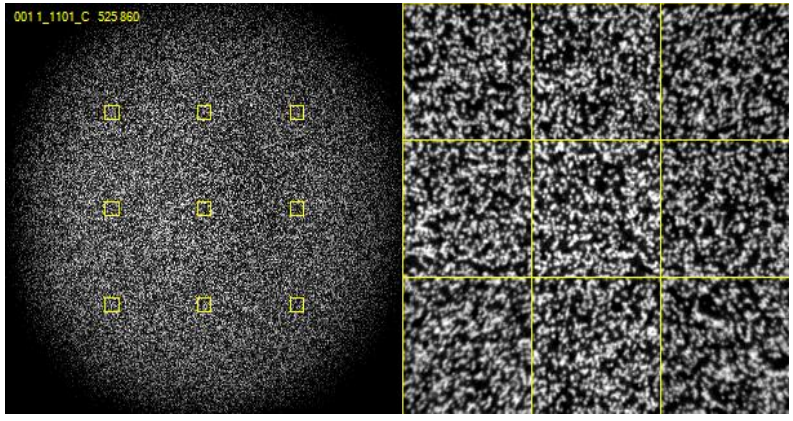
3 Sequencing



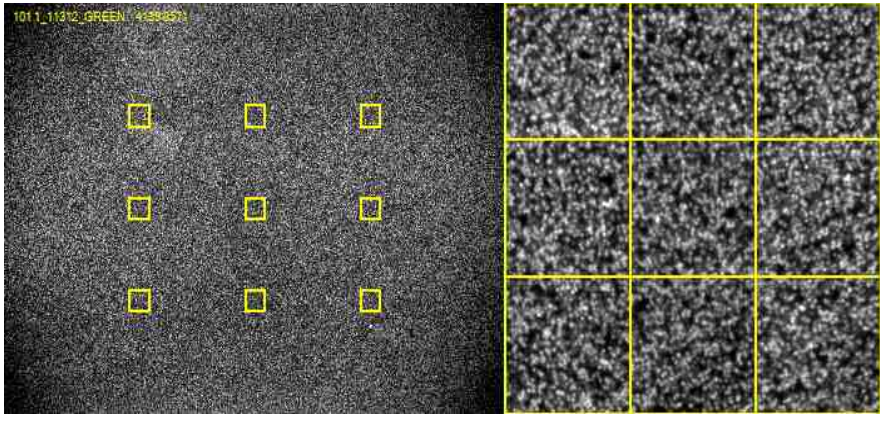
http://support.illumina.com/



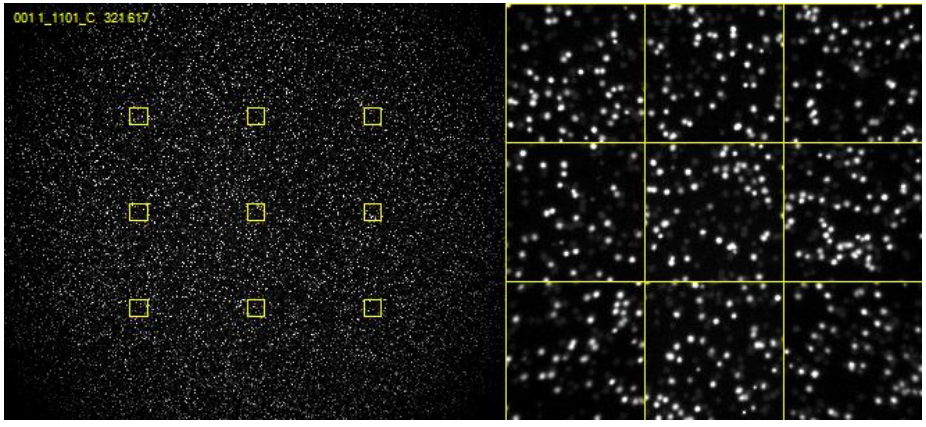
First images



MiSeq DNA samples

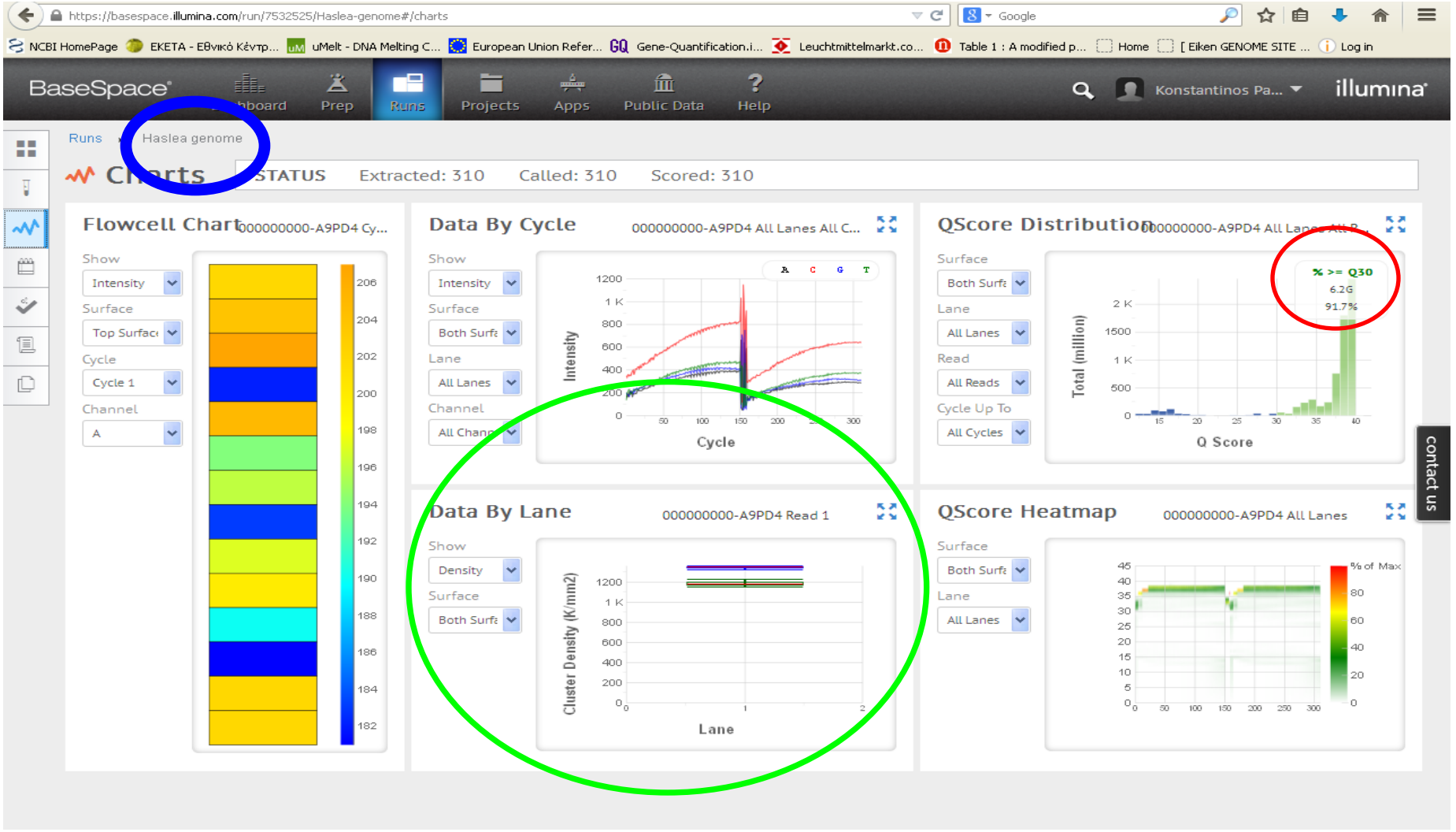


NextSeq500 RNA samples



MiSeq first DNA samples

DNA sequencing run



Sofia Michailidou

Results MiSeq experiment

MiSeq Specifications

Cluster Generation and Sequencing

MISEQ REAGENT KIT V2

READ LENGTH	TOTAL TIME*	OUTPUT
1 × 36 bp	~4 hrs	540-610 Mb
2 × 25 bp	~5.5 hrs	750-850 Mb
2 × 150 bp	~24 hrs	4.5-5.1 Gb
2 × 250 bp	~39 hrs	7.5-8.5 Gb

Reads Passing Filter†

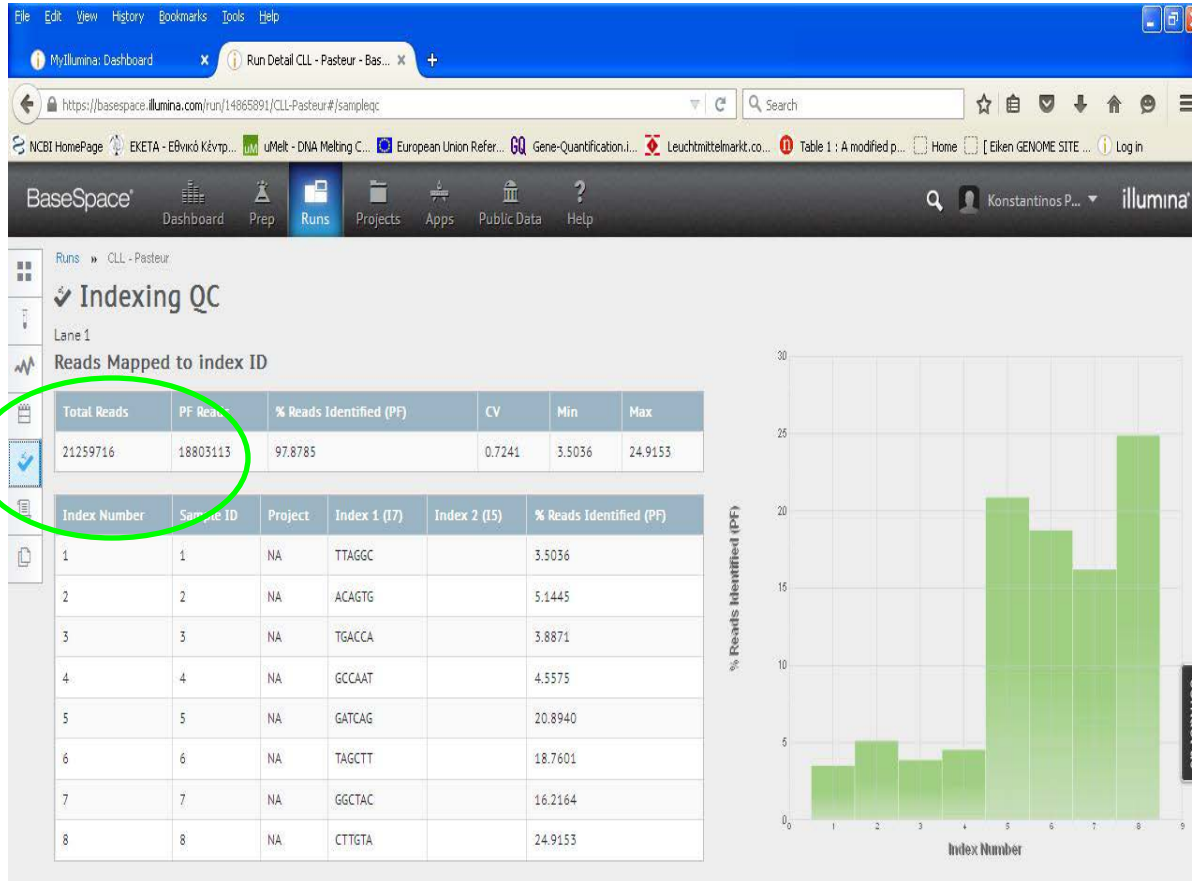
MISEQ REAGENT KIT V2

Single Reads	12-15 M
Paired-End Reads	24-30 M

Quality Scores††

MISEQ REAGENT KIT V2

> 90% bases higher than Q30 at 1 × 36 bp
> 90% bases higher than Q30 at 2 × 25 bp
> 80% bases higher than Q30 at 2 × 150 bp
> 75% bases higher than Q30 at 2 × 250 bp



Results NextSeq500 experiment

NEXTSEQ SERIES MID OUTPUT KIT*

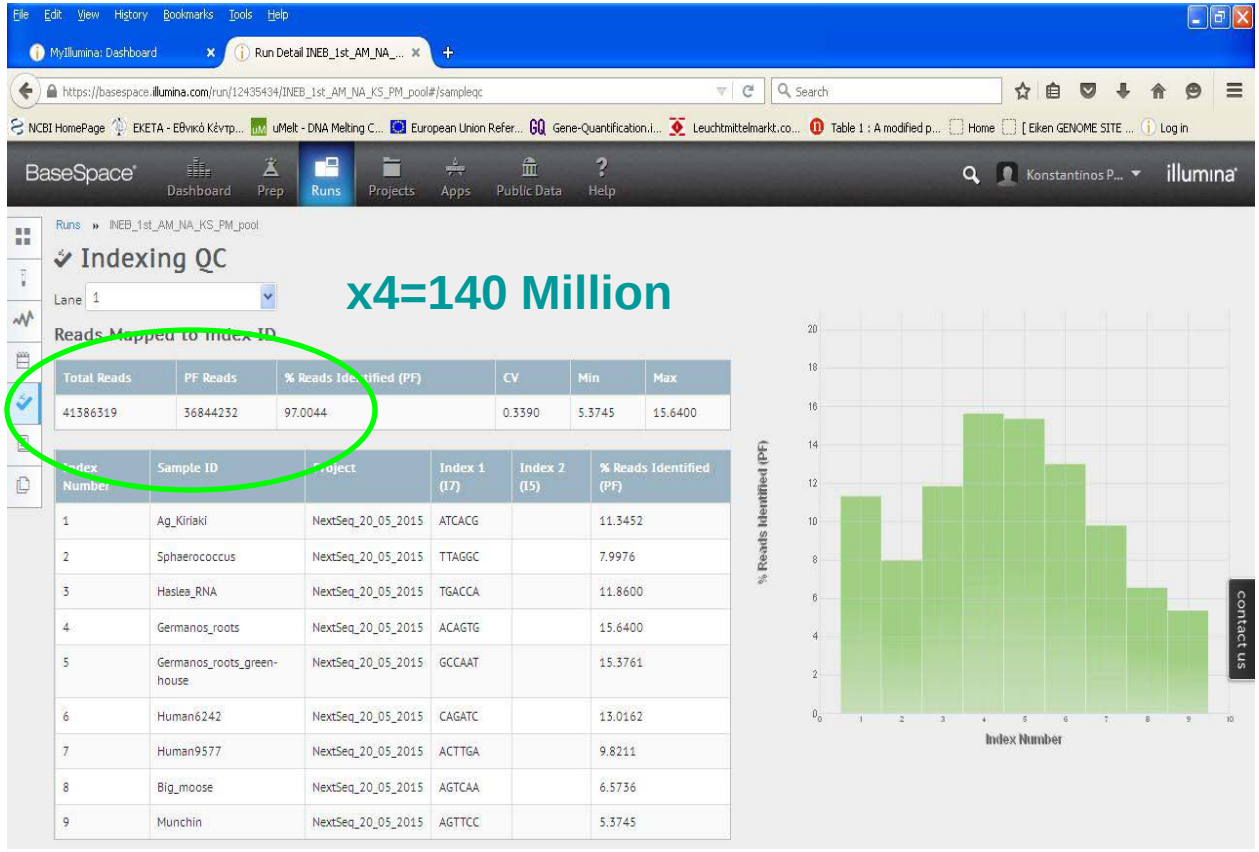
READ LENGTH	TOTAL TIME†	OUTPUT
2 × 150 bp	26 hrs	32.5-39 Gb
2 × 75 bp	15 hrs	16.25-19.5 Gb

NEXTSEQ SERIES MID OUTPUT KIT

Single Reads	Up to 130 Million
Paired-End Reads	Up to 260 Million

NEXTSEQ SERIES MID OUTPUT KIT

≥ 75% bases higher than Q30 at 2 x 150 bp
≥ 80% bases higher than Q30 at 2 x 75 bp



Output: fastq files!

Conclusions

- NGS Library preparation is research oriented
- Solutions on the market
- Flexible

Conclusions

- Success depends not only on accurate library quantification.
- Characteristics of the Library (diversity, size, etc) are important.

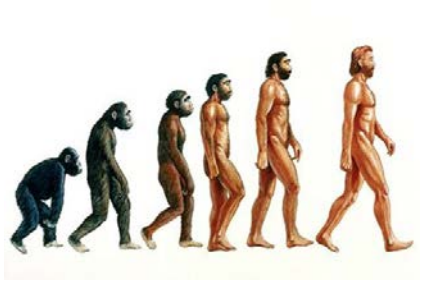
NGS Experiments at INAB

Sphaerococcus
Transcriptome

Sludge
Metatranscriptome

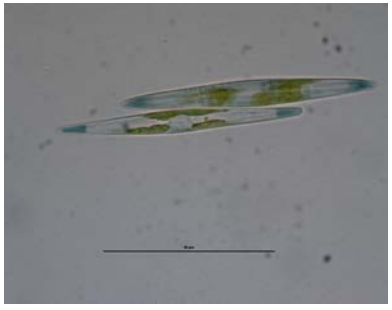
Fish
16S Metagenomics

Tomato/SNP

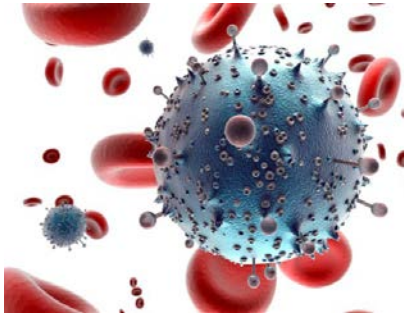


Human RNA/DNA
Transcriptome/
Amplicon analysis

Kefir
16S-ITS Metagenomics

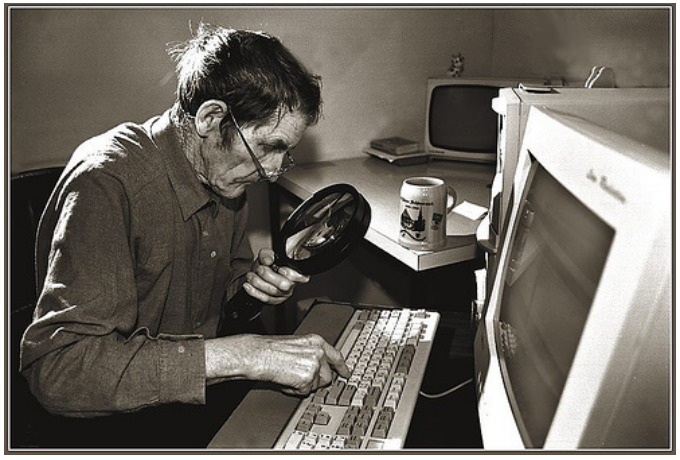


Haslea ostrearia
Genomic, de novo



Influenza
Virus mutations

Data Analysis

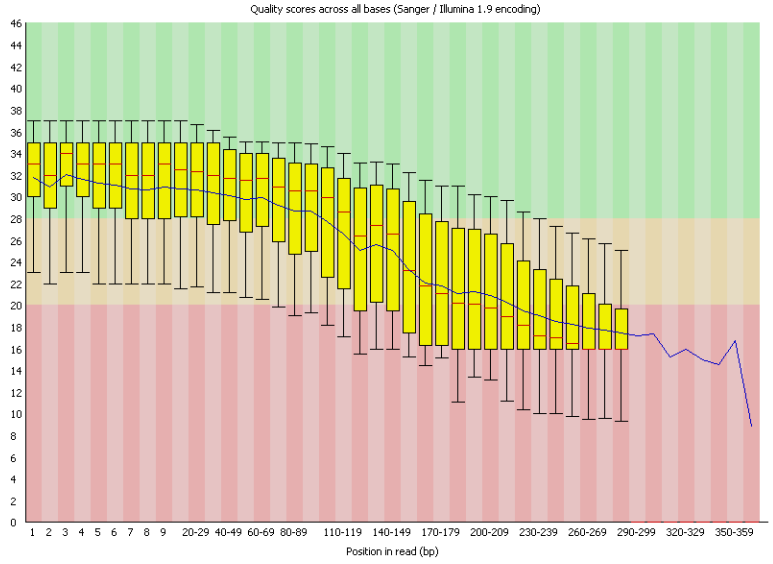


Bioinformatics skills essential for troubleshooting, contamination monitoring, library validation, etc.

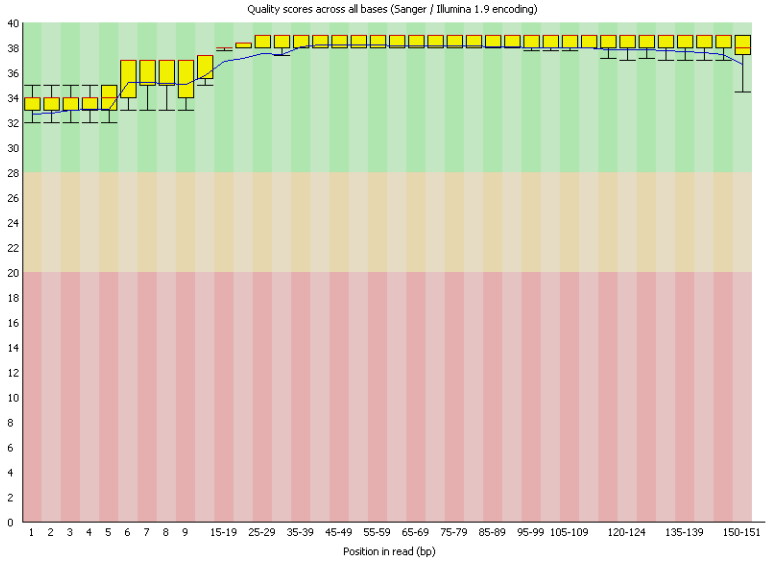
Comparison of NGS platforms

Quality analysis of sequencing runs from the same RNA sample!

Ion Torrent
Reads:497466



MiSeq
Reads:849523

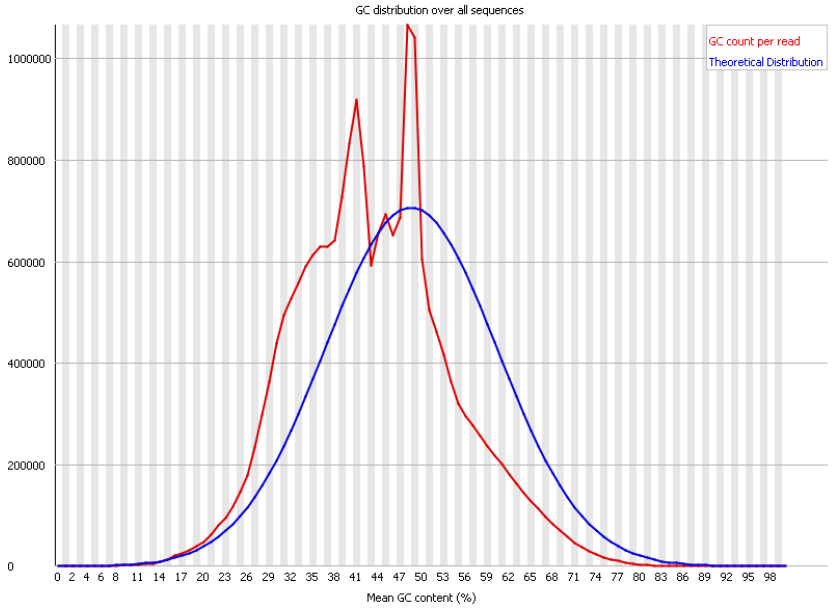


FastQC analysis

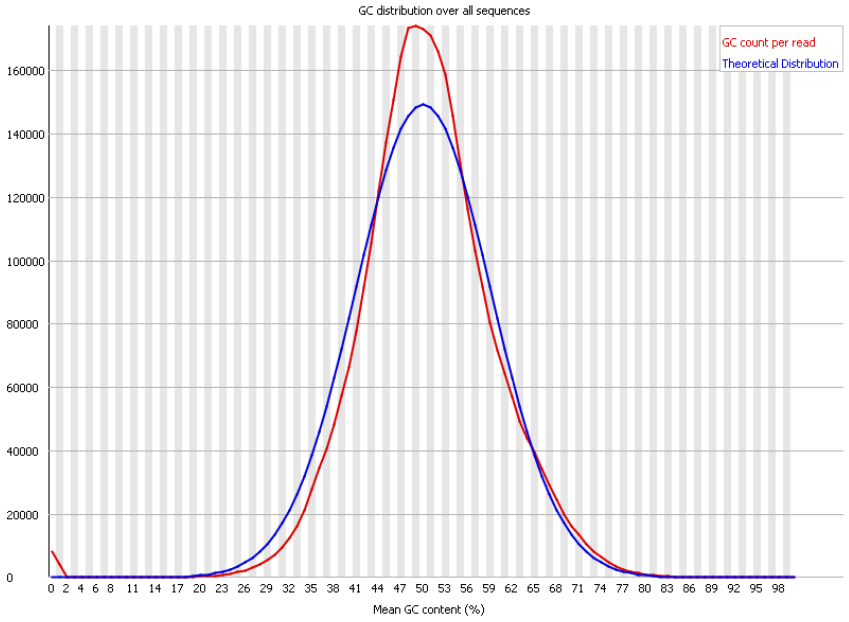
Comparison of NGS providers

Quality analysis of sequencing runs from Red Algae RNA samples

Laurencia glandulifera- China



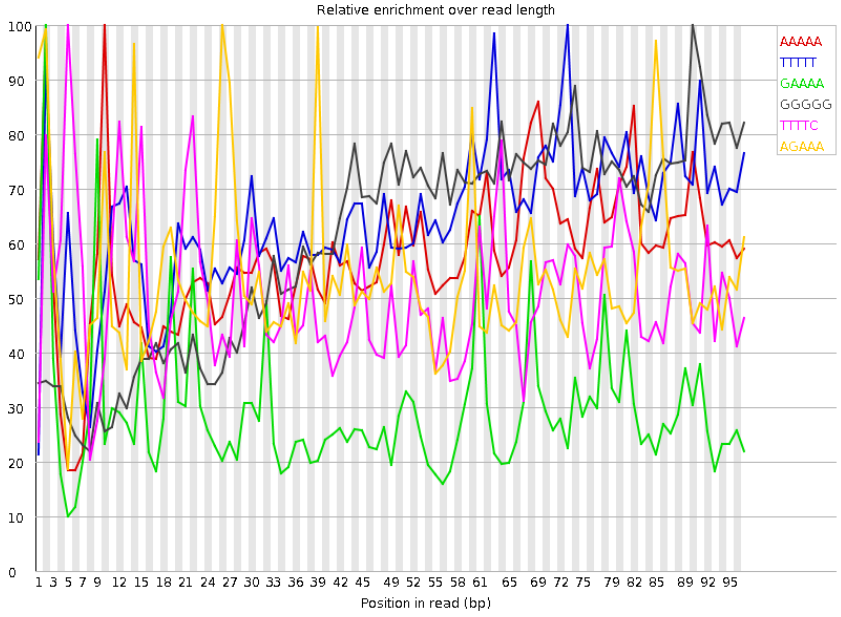
Laurencia MPO437-INAB



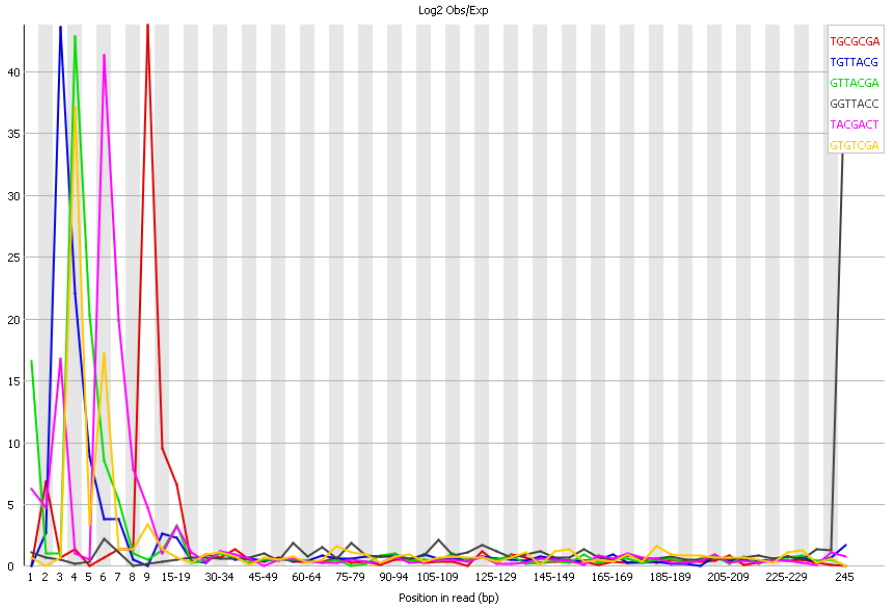
FastQC analysis

Comparison of NGS quality plots

Mouse mRNA-Belgium



Human mRNA-INAB



FastQC Kmer Content

NGS Team

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- Notis Argiriou
- Anastasia Chatzidimitriou
- Sofia Michailidou
- Evangelia Mouchtaropoulou
- Evangelia Stalika
- Mata Laidou
- Kostas Pasentsis

Questions ?