

# ΙΝΣΤΙΤΟΥΤΟ ΕΦΑΡΜΟΣΜΕΝΩΝ ΒΙΟΕΠΙΣΤΗΜΩΝ



# INAB & Next Generation Sequencing



MiSeq

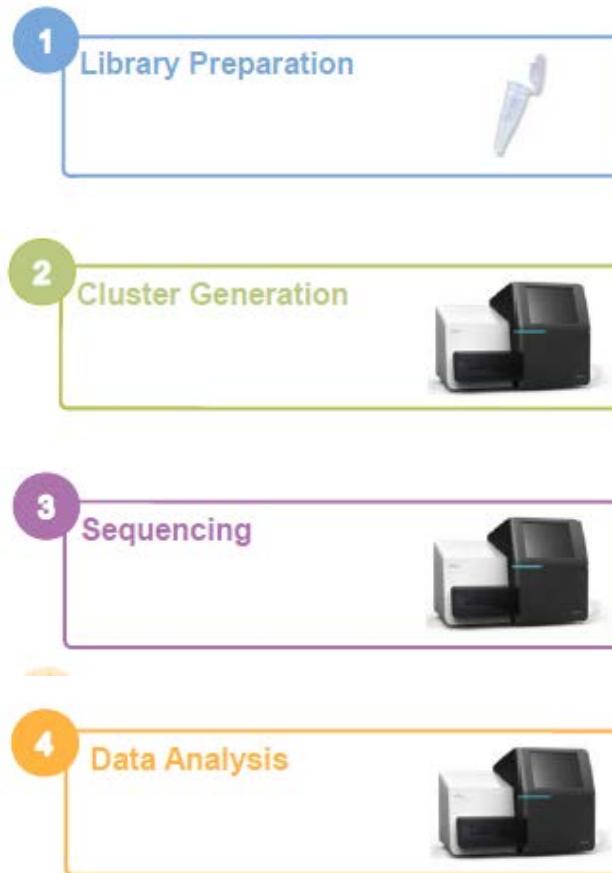


NextSeq500

illumina®

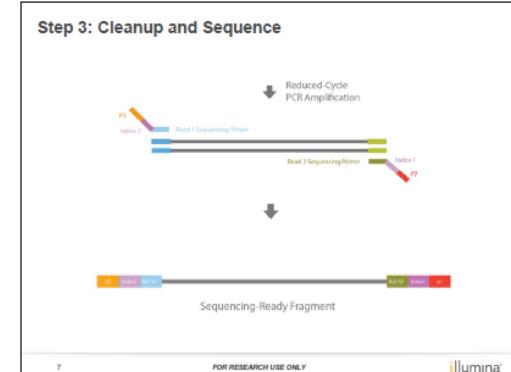
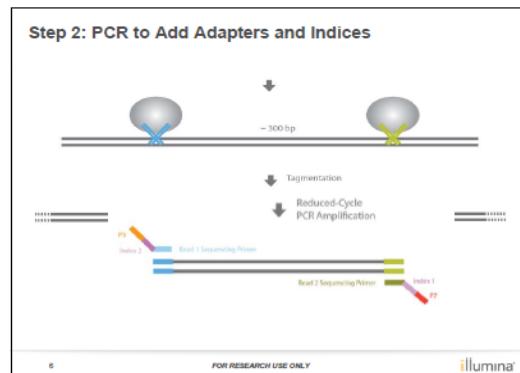
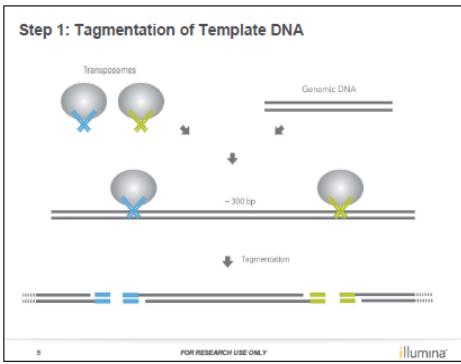


# NGS Experiments



# Library Preparation

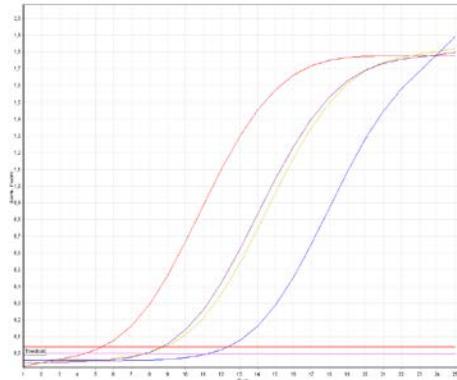
## NexTera DNA Sample Prep Kit



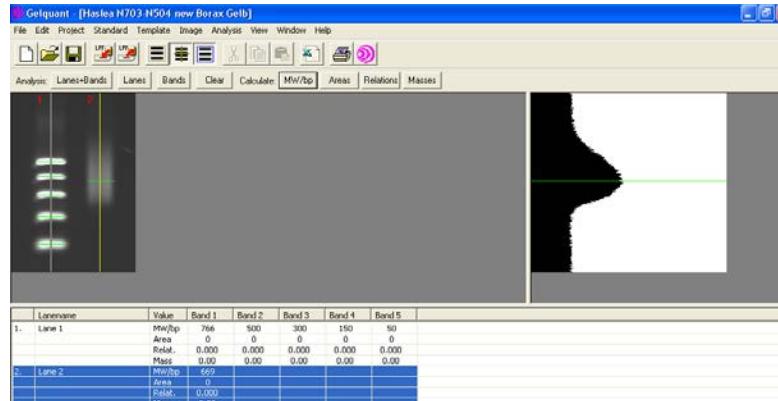
# Library validation methods



Quantification



Real time qPCR



LaneName	Value	Bond 1	Bond 2	Bond 3	Bond 4	Bond 5
1. Lane 1	Mw/bp	766	500	380	150	50
	Area	0	0	0	0	0
	Relat.	0.000	0.000	0.000	0.000	0.000
	Mass	0.00	0.00	0.00	0.00	0.00
2. Lane 2	Mw/bp	659				
	Area	0				
	Relat.	0.000				
	Mass	0.00				

Gel based analysis

# NGS Experiments

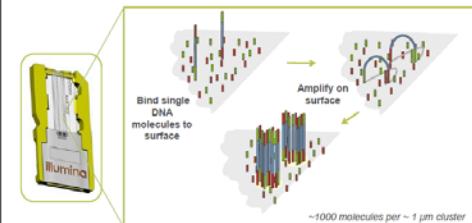
## 1 Library Preparation



## 2 Cluster Generation



### Cluster Generation

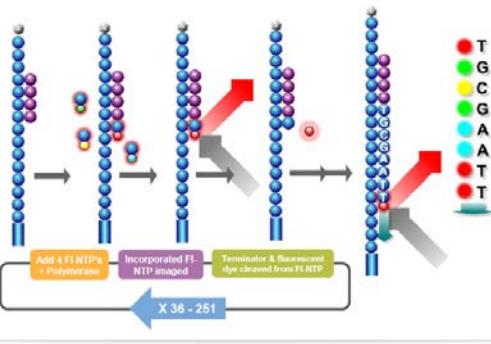


10

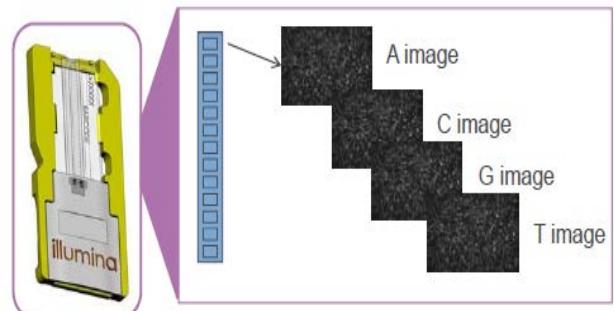
FOR RESEARCH USE ONLY

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## Sequencing by Synthesis



## 3 Sequencing



<http://support.illumina.com/>

## MiSeq Resources

### Online Training

- MiSeq: Introduction to the MiSeq System
- MiSeq: Sequencing Chemistry
- MiSeq: Getting Started
- MiSeq: How to Start a Run
- MiSeq: Instrument Washes
- MiSeq: Imaging and Base calling
- MiSeq: Data analysis overview

### Documentation

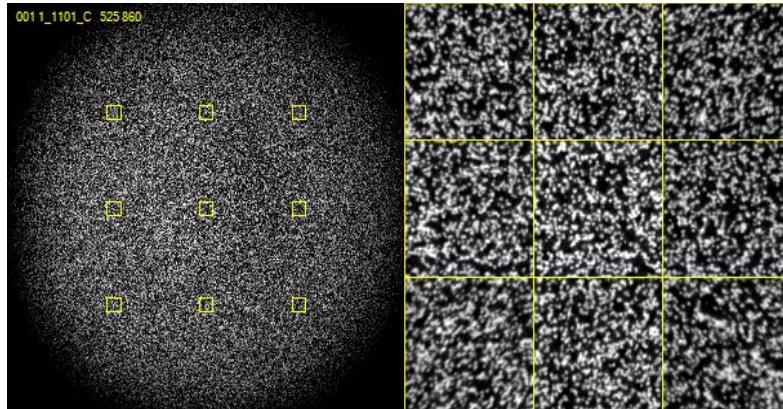
- MiSeq User Guide

79

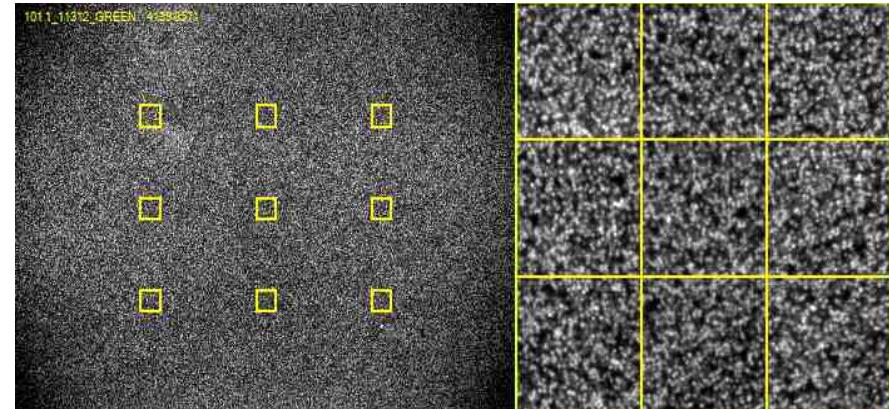
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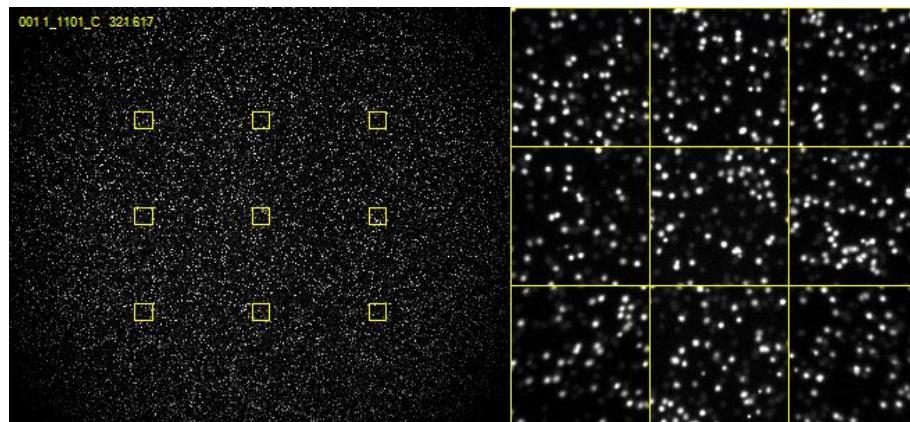
# First images



MiSeq DNA samples

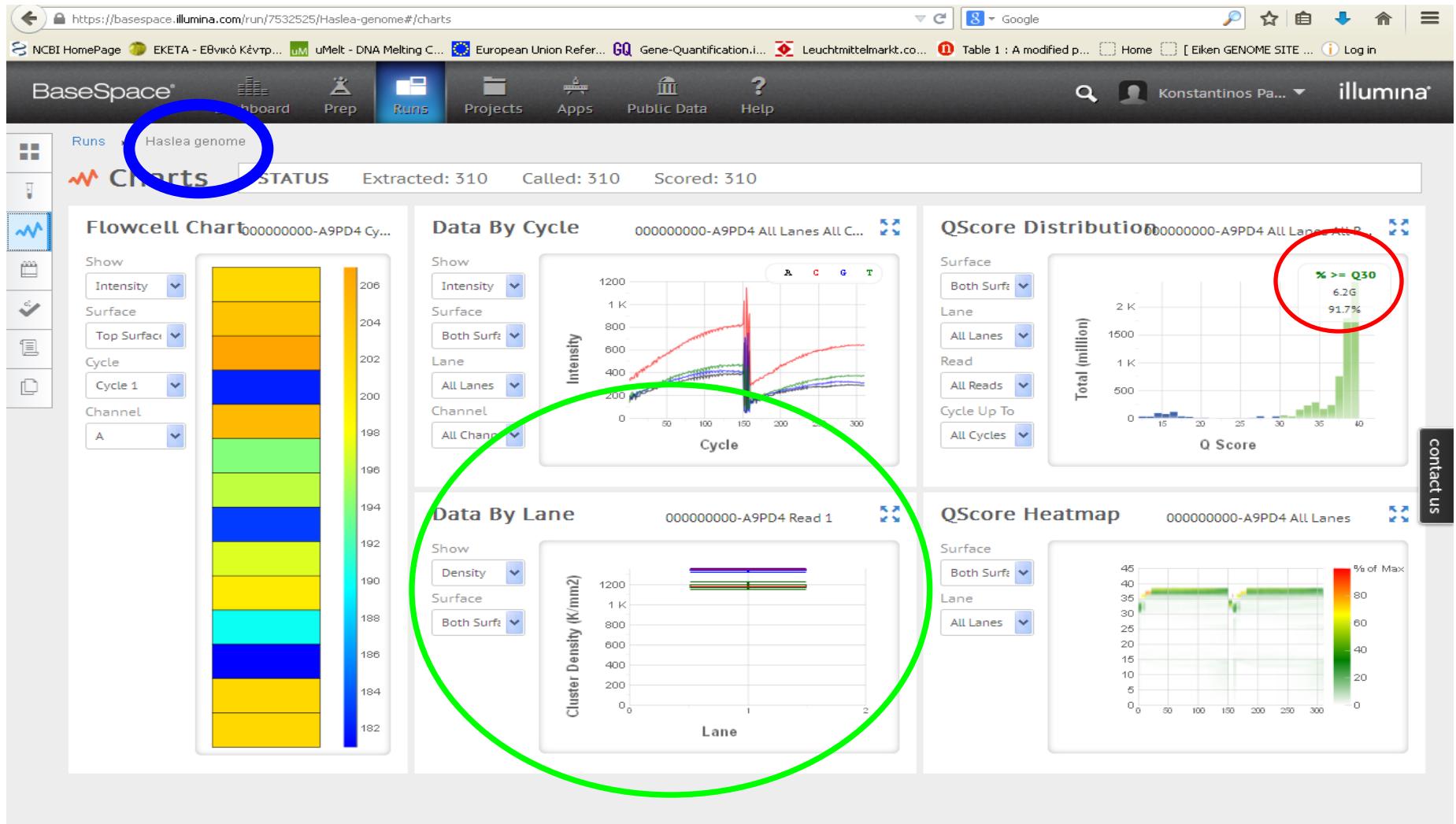


NextSeq500 RNA samples



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 x 36 bp		~4 hrs	540-610 Mb
2 x 25 bp		~5.5 hrs	750-850 Mb
2 x 150 bp		~24 hrs	4.5-5.1 Gb
2 x 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 x 36 bp
> 90% bases higher than Q30 at 2 x 25 bp
> 80% bases higher than Q30 at 2 x 150 bp
> 75% bases higher than Q30 at 2 x 250 bp

MyIllumina: Dashboard Run Detail CLL - Pasteur - Bas... +

https://basespace.illumina.com/run/14865891/CLL-Pasteur#/sampleqc

NCBI HomePage EKETA - EBVnö Kévp... uMelt - DNA Melting C... European Union Refer... Gene-Quantification.i... Leuchtmittelmarkt.co... Table 1 : A modified p... Home Eiken GENOME SITE ... Log in

BaseSpace® Dashboard Prep Runs Projects Apps Public Data Help

Runs > CLL - Pasteur

### Indexing QC

Lane 1

#### Reads Mapped to index ID

Total Reads	PF Reads	% Reads Identified (PF)	CV	Min	Max
21259716	18803113	97.8785	0.7241	3.5036	24.9153

Index Number	Sample ID	Project	Index 1 (I7)	Index 2 (I5)	% Reads Identified (PF)
1	1	NA	TTAGGC		3.5036
2	2	NA	ACAGTG		5.1445
3	3	NA	TGACCA		3.8871
4	4	NA	GCCCAT		4.5575
5	5	NA	GATCAG		20.8940
6	6	NA	TAGCTT		18.7601
7	7	NA	GGCTAC		16.2164
8	8	NA	CTTGTA		24.9153

Index Number

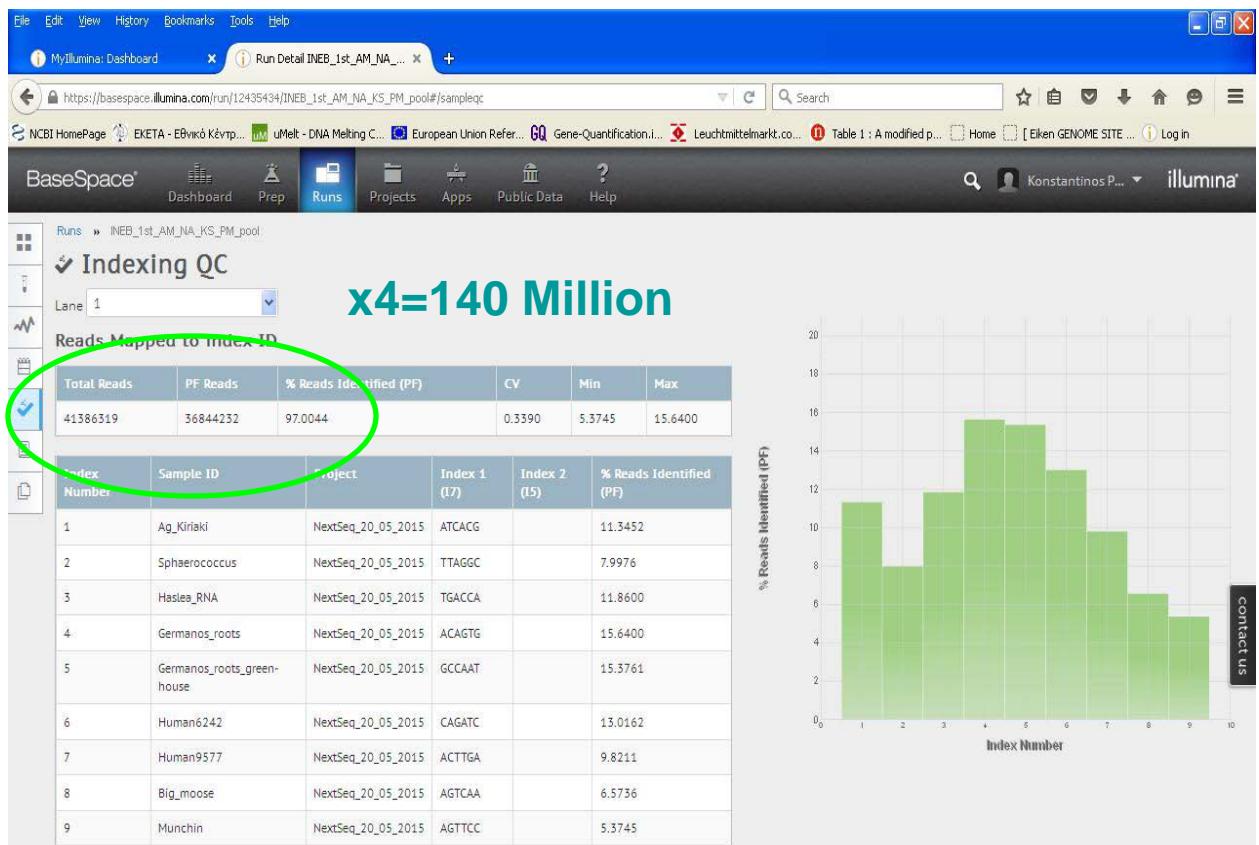
Results Identified (PF)

# Results NextSeq500 experiment

NEXTSEQ SERIES MID OUTPUT KIT *		
READ LENGTH	TOTAL TIME†	OUTPUT
2 x 150 bp	26 hrs	32.5-39 Gb
2 x 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

1

Library Preparation



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

# Conclusions

2

Cluster Generation



3

Sequencing



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



# NGS Experiments at INAB



Sphaerococcus  
Transcriptome



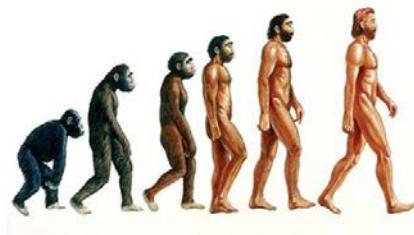
Sludge  
Metatranscriptome



Fish  
16S Metagenomics



Tomato/SNP



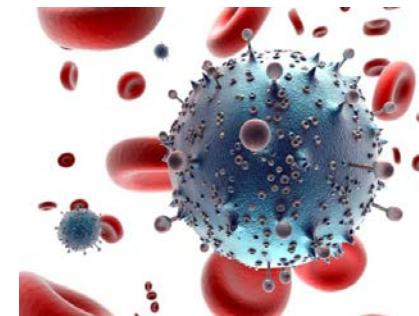
Human RNA/DNA  
Transcriptome/  
Amplicon analysis



Kefir  
16S-ITS Metagenomics

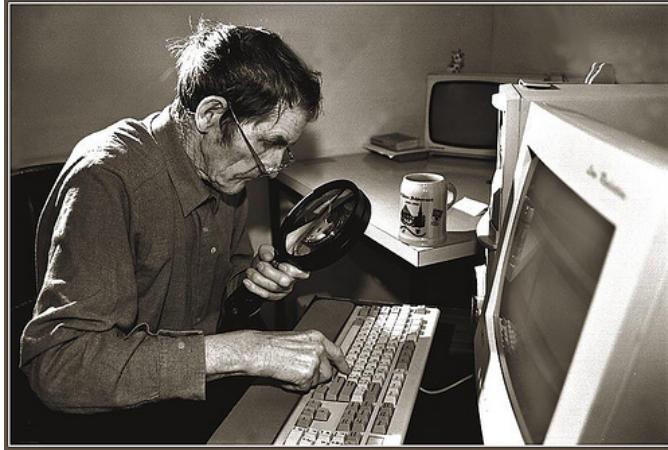


Haslea ostrearia  
Genomic, de novo



Influenza  
Virus mutations

# Data Analysis



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

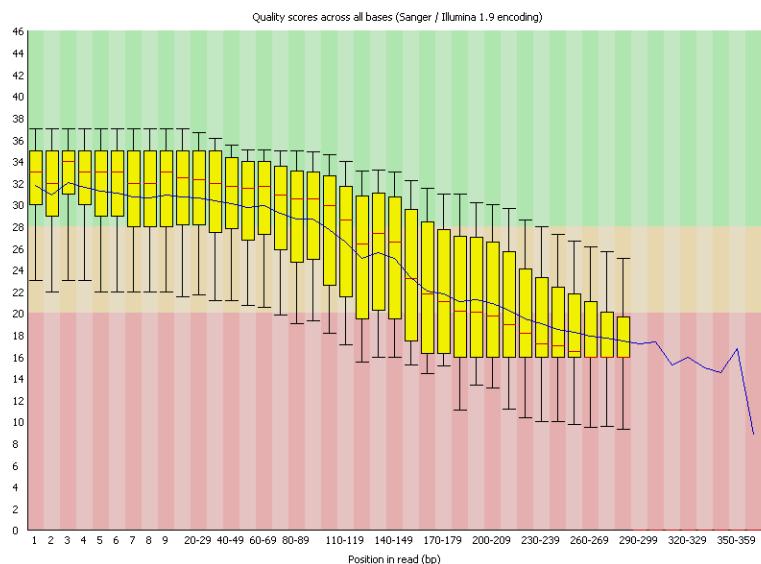


# Comparison of NGS platforms

Quality analysis of sequencing runs from the same RNA sample!

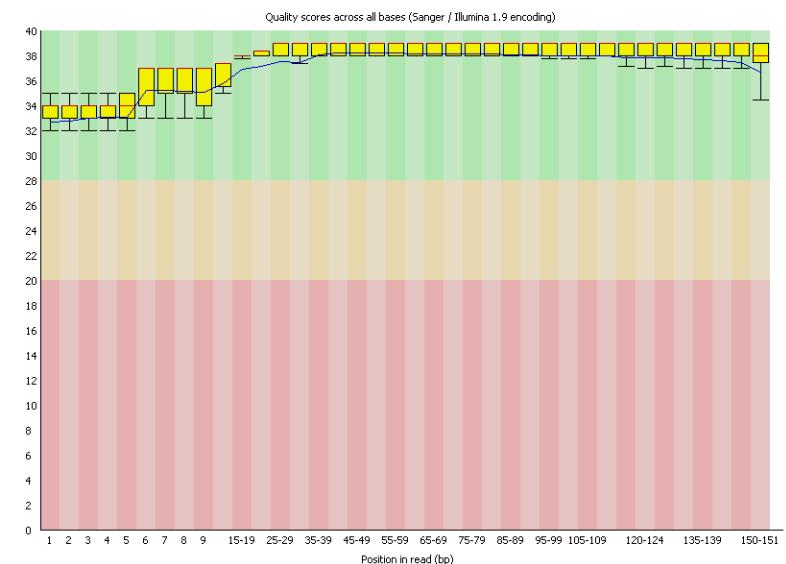
Ion Torrent

Reads:497466



MiSeq

Reads:849523

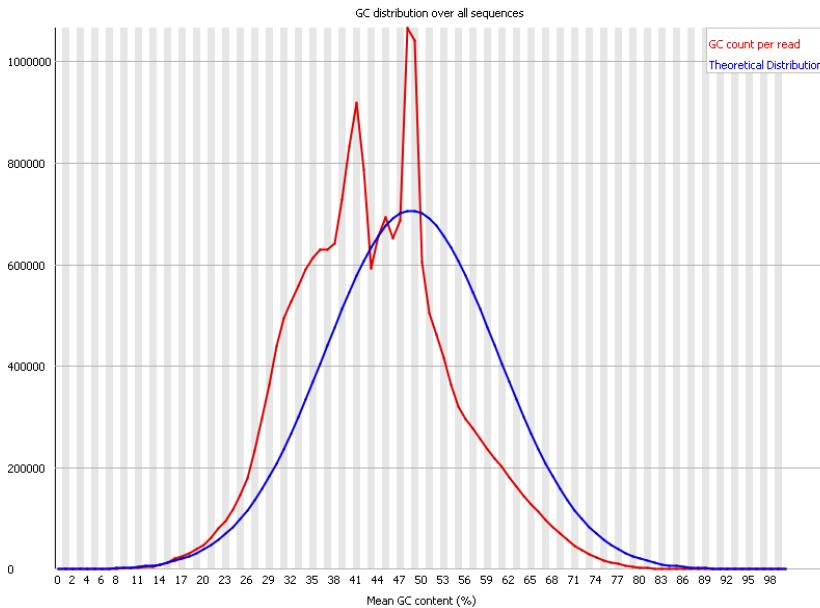


FastQC analysis

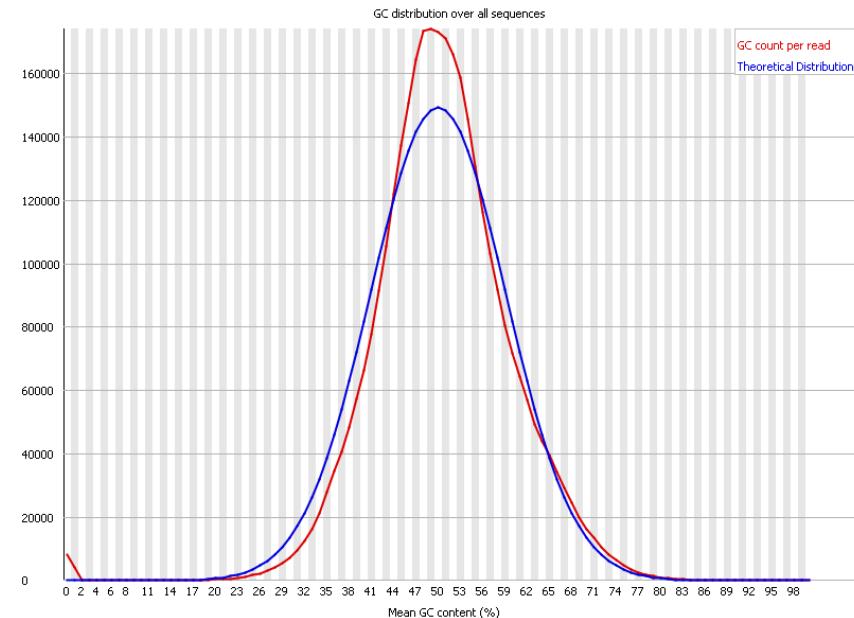
# Comparison of NGS providers

Quality analysis of sequencing runs from Red Algae RNA samples

Laurencia glandulifera- China



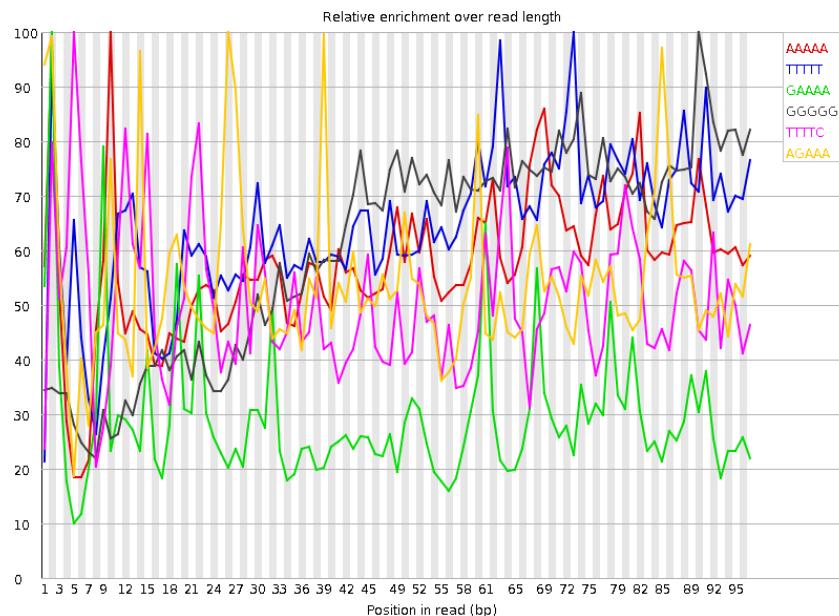
Laurencia MPO437-INAB



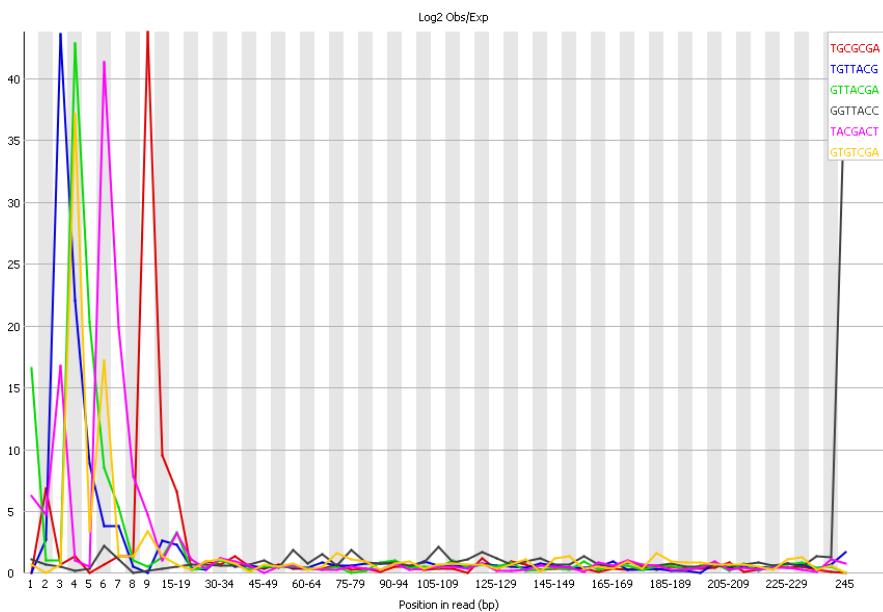
FastQC analysis

# Comparison of NGS quality plots

Mouse mRNA-Belgium



Human mRNA-INAB



FastQC Kmer Content

# NGS Team

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

# Questions ?