Introduction to high-throughput deep-sequencing platforms: 454 (Roche), SOLiD (ABI) en Solexa (Illumina)

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Basic Problem

Researcher:	which mutations in a genomic region "REG" cause disease "DIS" ?
Experiment:	?? … … how do we collect data about the DNA sequence?

Inspiration from cell biology: *http://www.youtube.com/watch?v=983lhh20rGY*

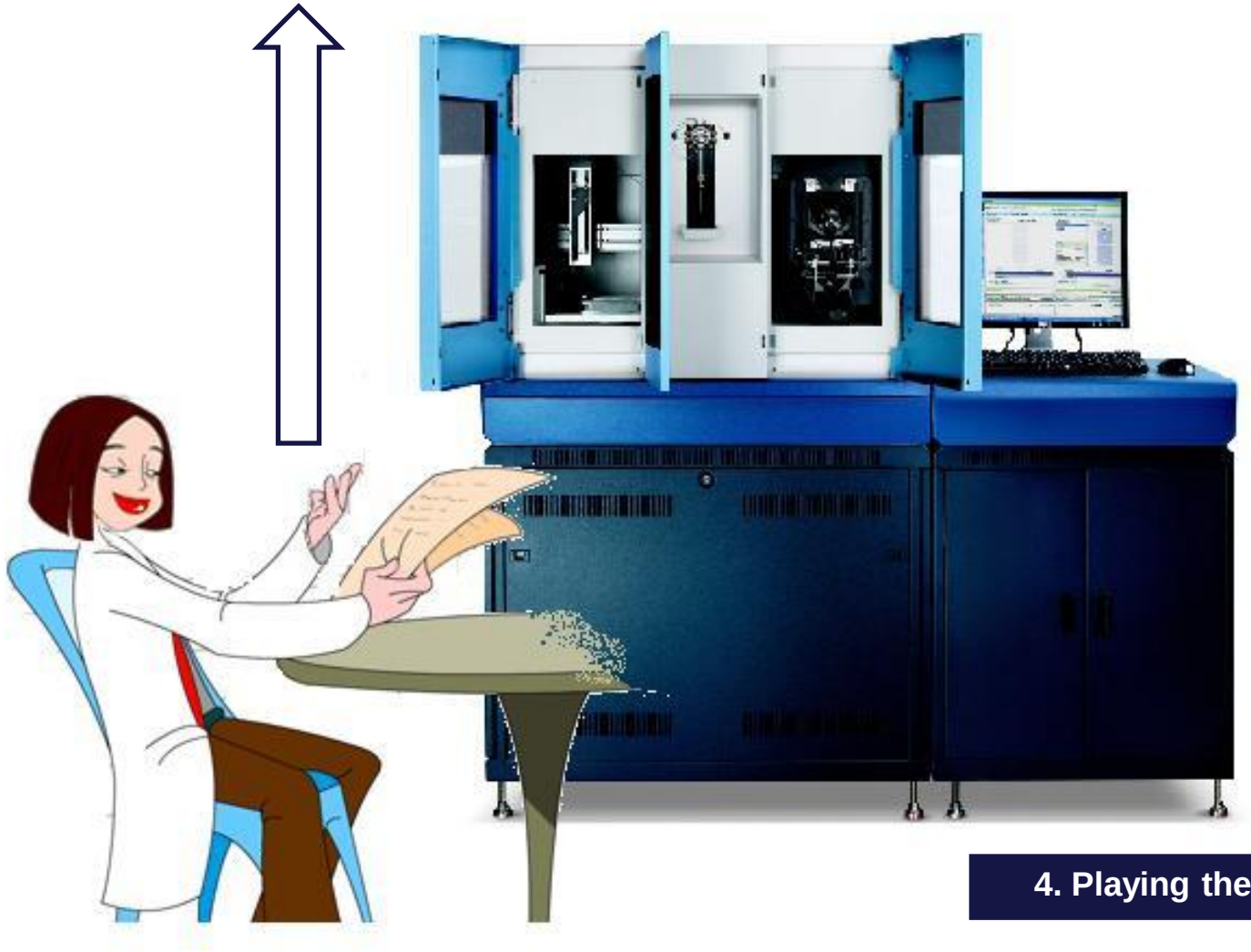
↓

Sequencing-by-synthesis:

Can we also read the genome base-by-base and output not mRNA but a text-file?

Overview

1. Speaking the Language	2. Mastering the Lab	3. Understanding Data Files

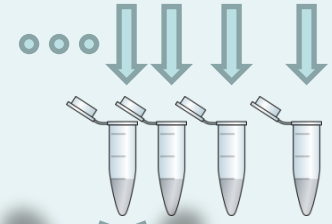


4. Playing the Game!

Speaking the language (1/2)

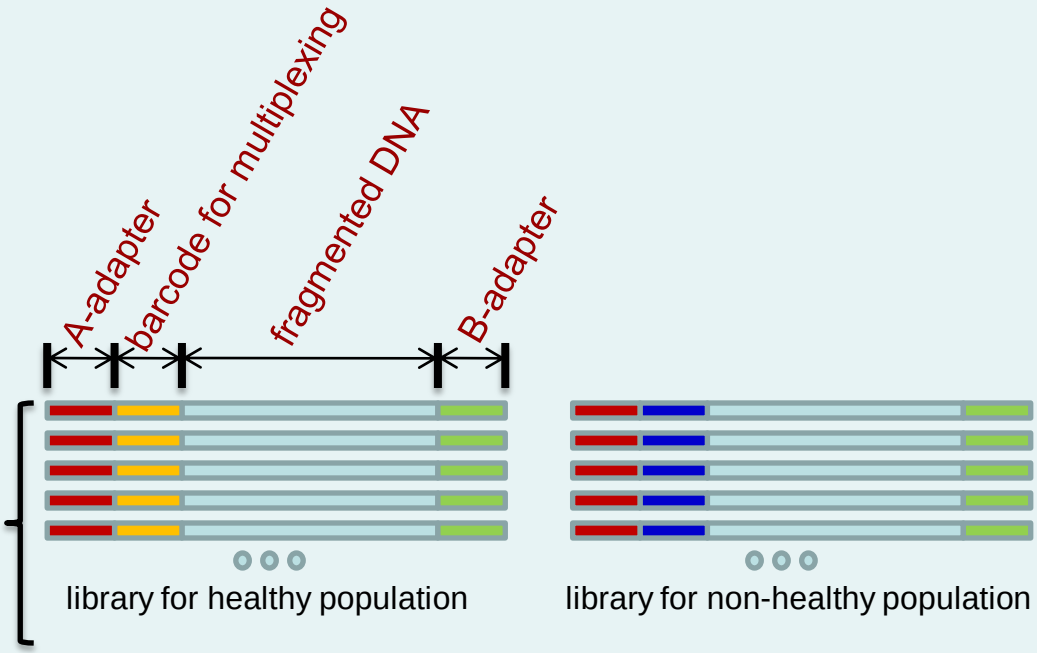
Sample preparation

biological replicates



technical replicates

Library preparation

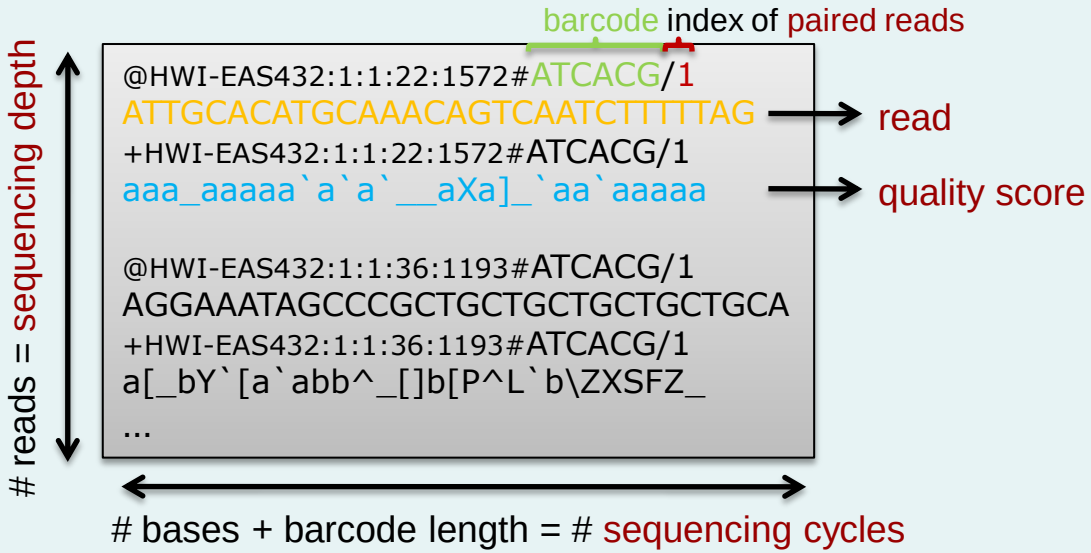


Speaking the Language (2/2)

Sequencing

- Amplification
- Sequencing chemistry
- Image processing
- Base calling

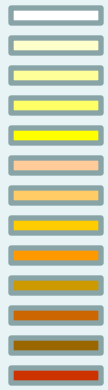
Fastq-output



De novo assembly

contigs

Alignment



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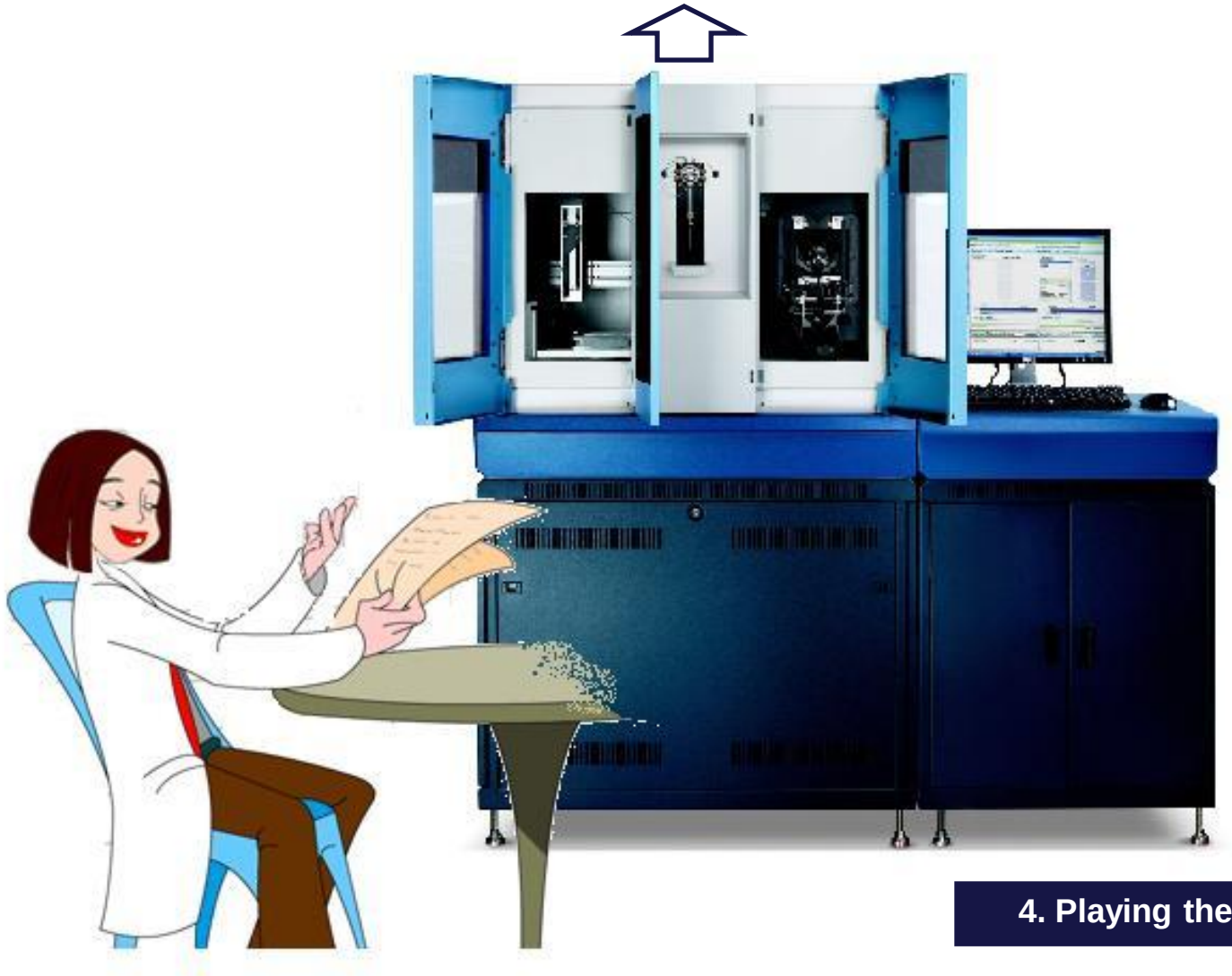

4. Playing the Game!

Mastering the Lab

	GS FLX Titanium	GA IIx Solexa	SOLiD 3 Plus
Sample requirements	>5 µg of dsDNA in a 10 µL volume	0.1-1µg of dsDNA ; 10µg for mate pairs	10ng-5µg of dsDNA ; 5µg-20µg for mate pairs
Multiplexing	16 regions x 12 barcodes	8 lanes x 12 barcodes	2 cells x 8 samples/cell x 16 barcodes
Paired ends separation	3kb-20kb (half of the read length for each end)	200bp-10kb	600bp-10kb
Amplification	Emulsion PCR	Bridge amplification	Emulsion PCR
Sequencing chemistry	Pyrosequencing	Polymerase based sequencing-by-synthesis	Sequencing by ligation
Read length	*400 bp average - 500 bp modal (variable)*	2 x 35, 50, 75 and 100bp (fixed)	35 and 50 (fixed)
Reads per run	*> 1 M*	225-250M	>1000M
Mbp per run	*400 Mbp – 600 Mbp*	25 Gbp	60Gbp
Accuracy	*99% accuracy at the 400th base and higher for preceding bases*	99,9% accuracy and higher for more than 70% of the bases;	99,9% accuracy and higher for more than 80% of the bases
Specifics	no complex optics or lasers		data is recorded in ABI color space

SOLiD (ABI)

Introduction video	*http://www.appliedbiosystems.com/solid4*

	SOLiD 3 Plus
Sample requirements	10ng-5µg of dsDNA ; 5µg-20µg for mate pairs
Multiplexing	2 cells x 8 samples/cell x 16 barcodes
Paired ends separation	600bp-10kb
Amplification	Emulsion PCR
Sequencing chemistry	Sequencing by ligation
Read length	35 and 50 (fixed)
Reads per run	>1000M
Mbp per run	60Gbp
Accuracy	99,9% accuracy and higher for more than 80% of the bases
Specifics	data is recorded in ABI color space

Solexa (Illumina)

Introduction video	*http://www.illumina.com/technology/sequencing_technology.ilmn*

	GA IIx Solexa
Sample requirements	0.1-1µg of dsDNA ; 10µg for mate pairs
Multiplexing	8 lanes x 12 barcodes
Paired ends separation	200bp-10kb
Amplification	Bridge amplification
Sequencing chemistry	Polymerase based sequencing-by-synthesis
Read length	2 x 35, 50, 75 and 100bp (fixed)
Reads per run	225-250M
Mbp per run	25 Gbp
Accuracy	99,9% accuracy and higher for more than 70% of the bases;
Specifics	

454 platform (Roche)

Introduction video	*http://www.youtube.com/watch?v=bFNjxKHP8Jc*

	GS FLX Titanium
Sample requirements	>5 µg of dsDNA in a 10 µL volume
Multiplexing	16 regions x 12 barcodes
Paired ends separation	3kb-20kb (half of the read length for each end)
Amplification	Emulsion PCR
Sequencing chemistry	Pyrosequencing
Read length	*400 bp average - 500 bp modal (variable)*
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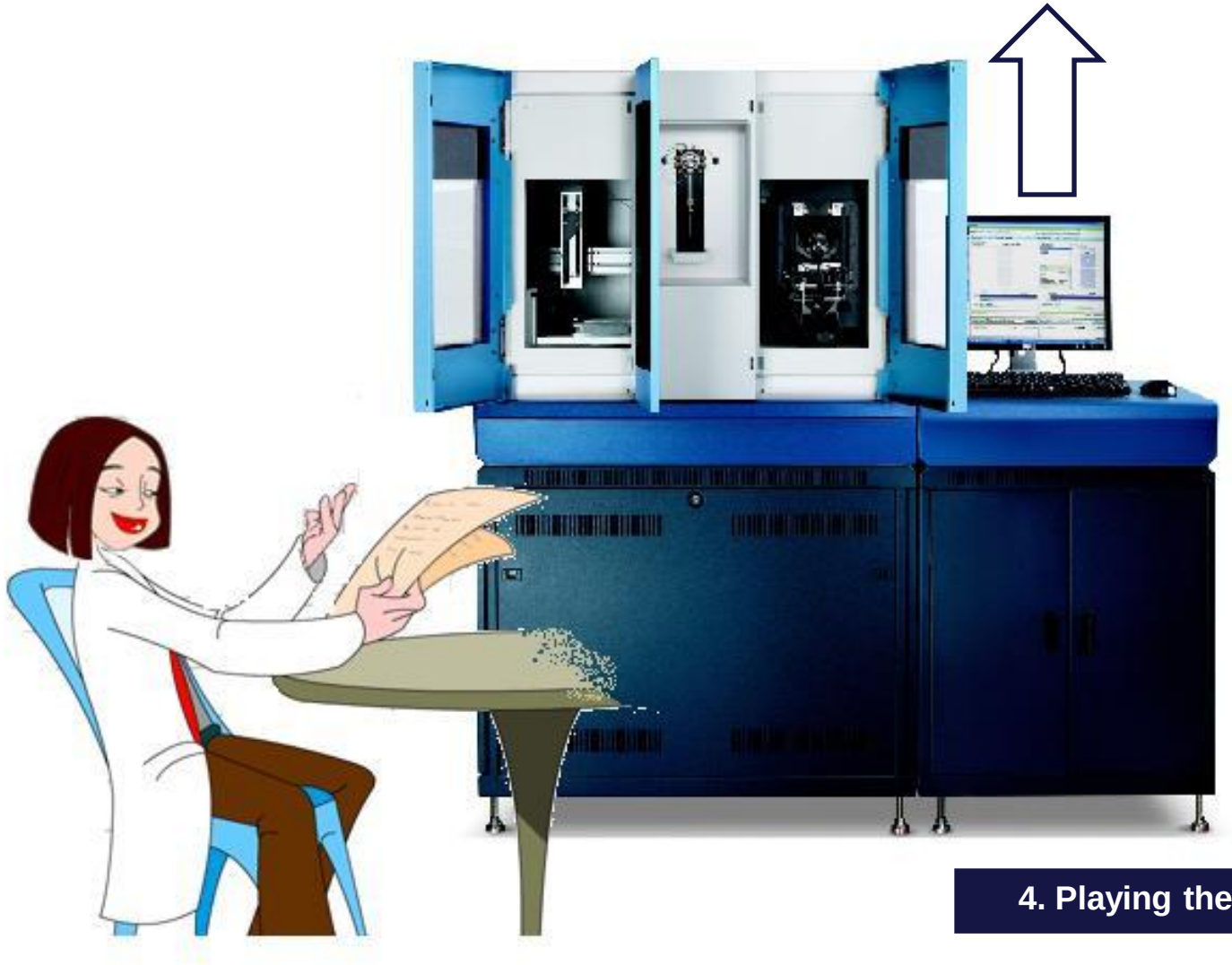
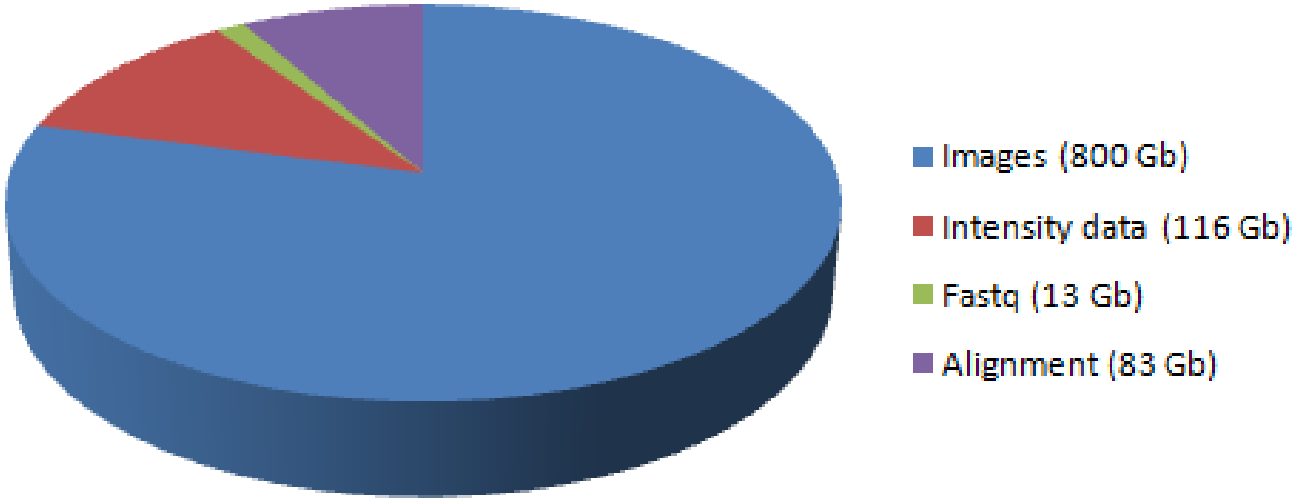


4. Playing the Game!

Understanding Data Files

Platform	**Solexa – GA II / GAP v1.3**
Single reads	1000 Gb for 8 lanes
Paired reads & 76 cycles	4000 Gb for 8 lanes



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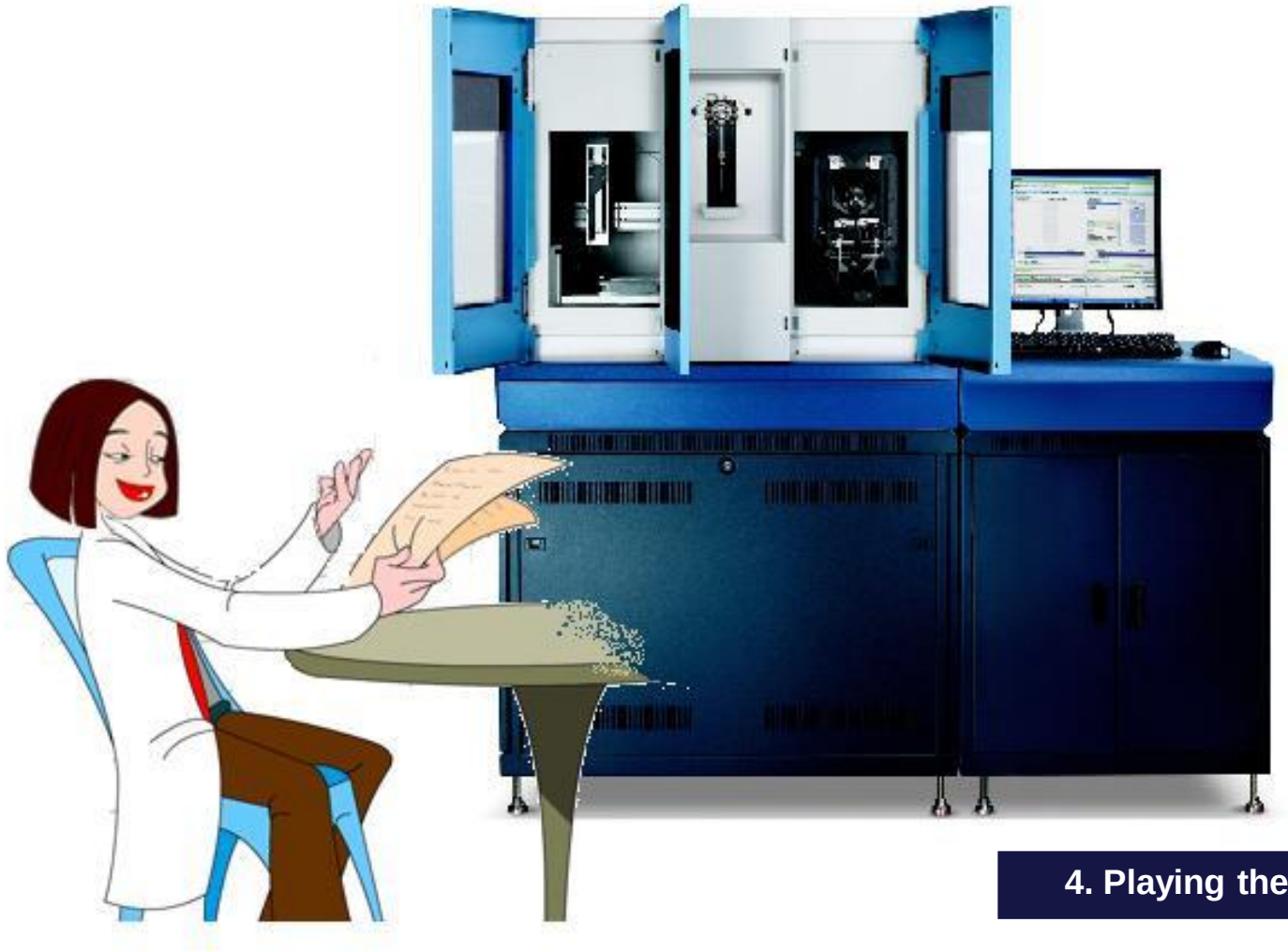


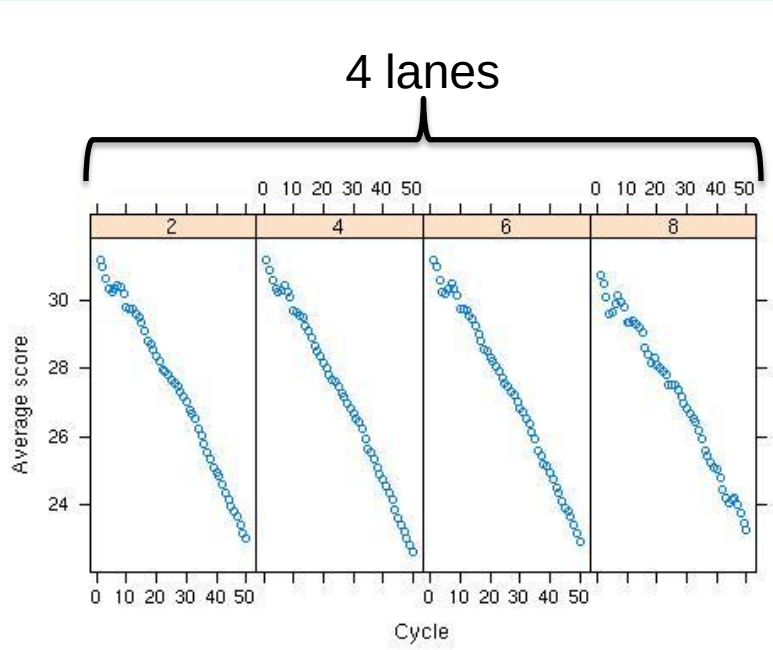
4. Playing the Game!

Playing the Game

Introduction video	*http://www.illumina.com/technology/sequencing_technology.ilmn*

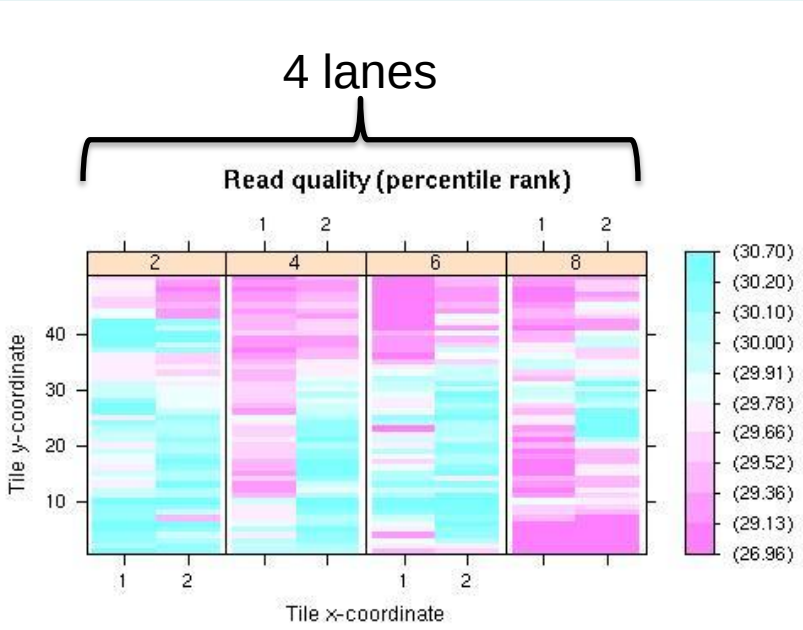
Quality Control Report

Base quality per cycle



50 cycles

Base quality per tile



CCD camera takes pictures of 1 tile at a time

Playing the Game

	GS FLX Titanium	GA IIx Solexa	SOLiD 3 Plus
Time per run	10 hours	2 days (2x35) - 9.5 days (2x100)	8.5 days (2x35) – 13 days (2x50)
Cost per run	9,000 EURO	10,000 EURO (1x35) - 35,000 EURO (2x75)	45,000 EURO (1x50)
Cost per Mbp	< 18 EURO	< 1.8 EURO	< 1.8 EURO

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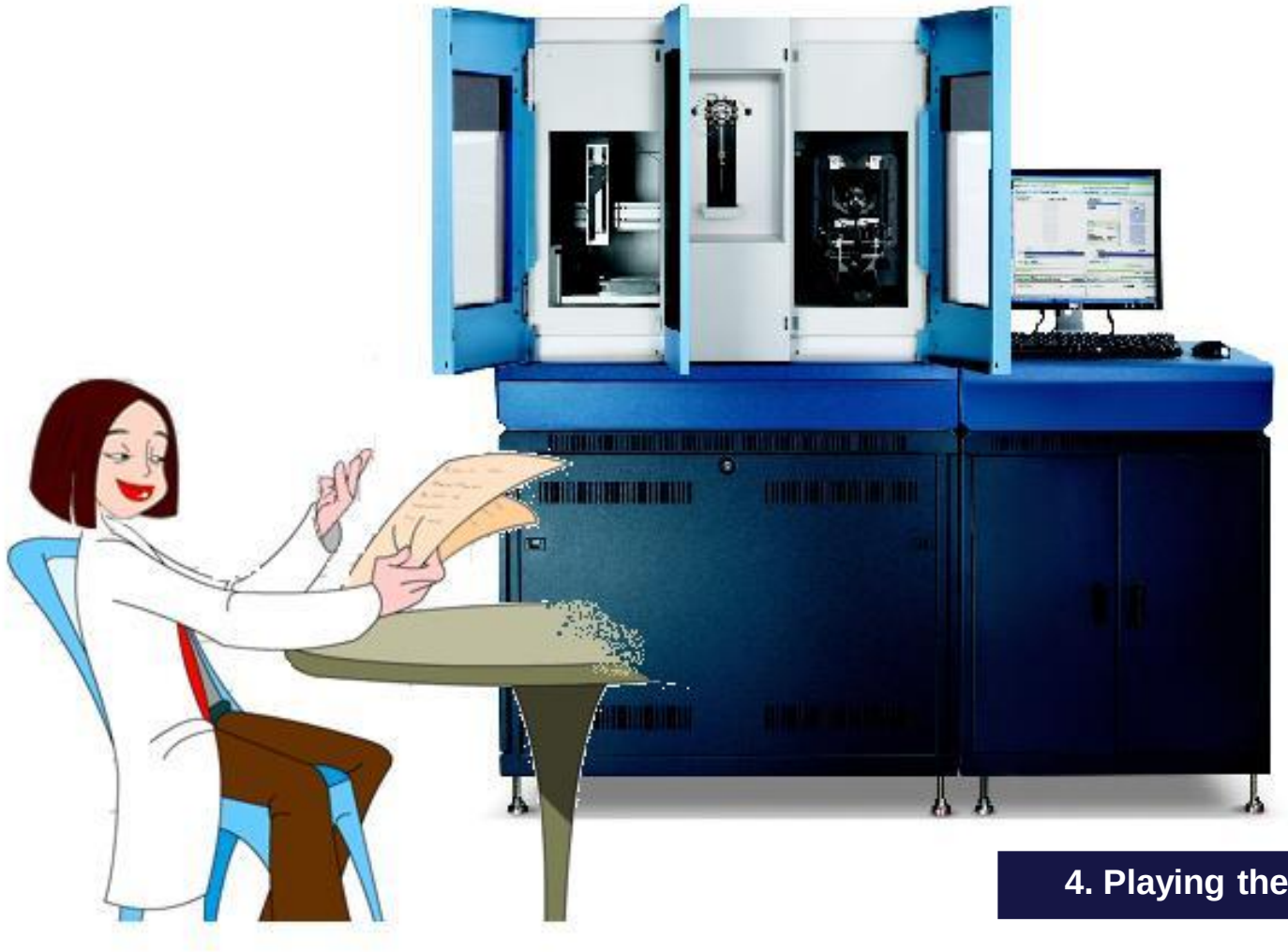

4. Playing the Game!

Thanks!

VIB Micro	Array Facility
Torik Ayoubi	Ruth Maes
Joke Allemeersch	Kizi Coeck
Rudy Van Eijsden	Lieze Wolput