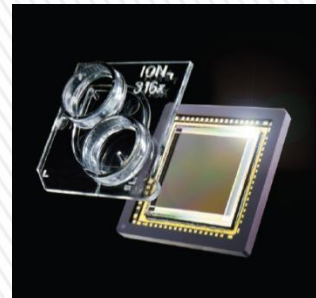




Ion Torrent

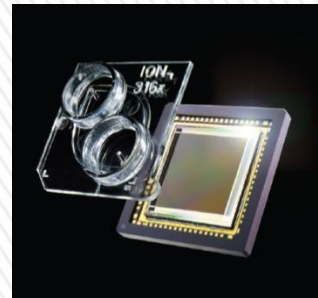
The chip is the machineTM
 The chip is the machine



Introduction

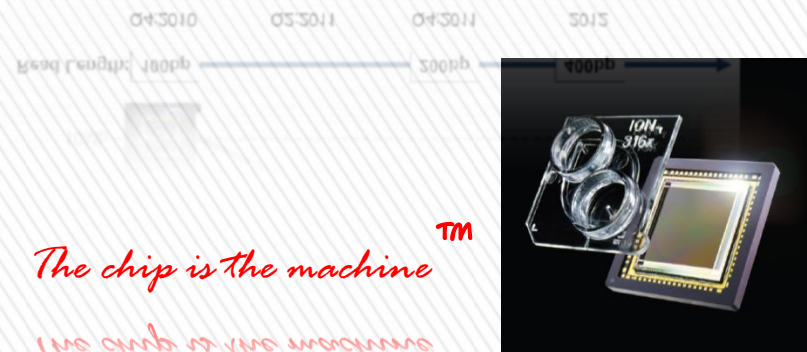
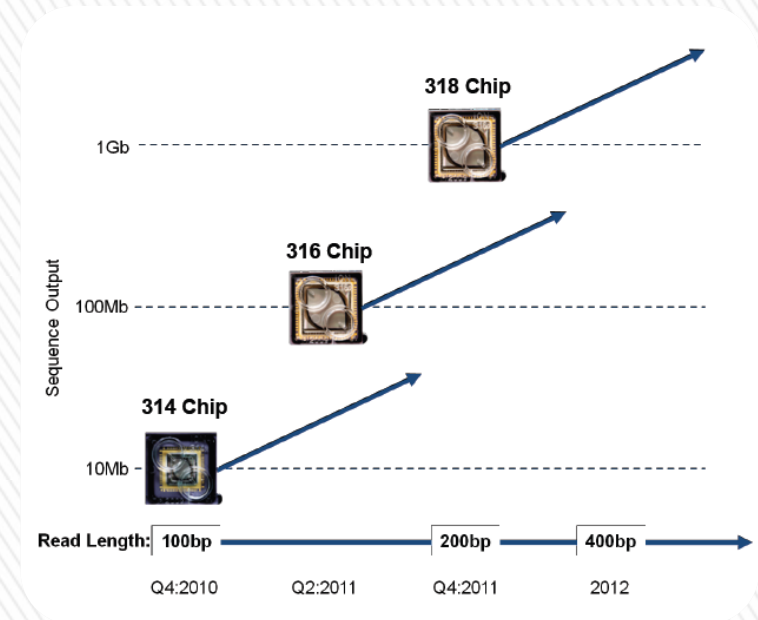
- The Ion Personal Genome Machine [PGM] is simple, more cost-effective, and more scalable than any other sequencing technology.
- Founded in 2007 by Jonathan Rothberg.
- Part of Life Technologies.
- Launched December 2010.
- First Postlight™ technologies for sequencing.
- The company's goal is to democratize sequencing and make this technology available to every lab.

The chip is the machine™
(the chip is the machine)

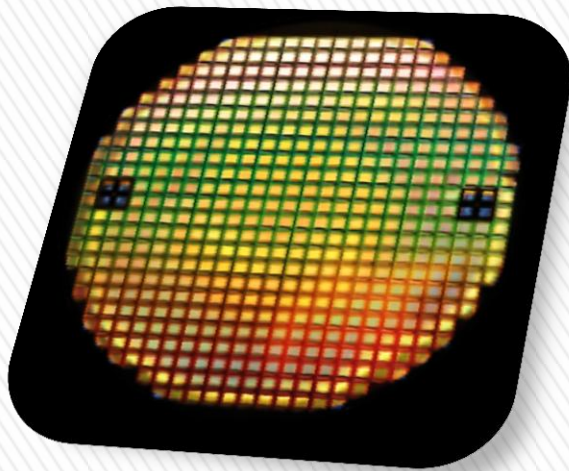


The chip is the machine TM

- *Simplicity*
 - Simple natural chemistry
 - Fast direct detection
- *Speed*
 - 10X faster workflow for any project
- *Scalability*
 - 10MB to 1GB in one year (100X)



How is it made?



- Wafers are cut from a silicon boule.
- The transistors and circuits are then pattern-transferred and subsequently etched onto the wafers using photolithography.
- The upper process is repeated 20 times or more, creating a multi-layer system of circuits.

		
CHIP 314	316	318*
DATE JAN:2011	JUL:2011	Q4:2011
OUTPUT 10 Mb	100 Mb	1Gb
READ LENGTH 100 bp	100 bp	200 bp

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How does it work?

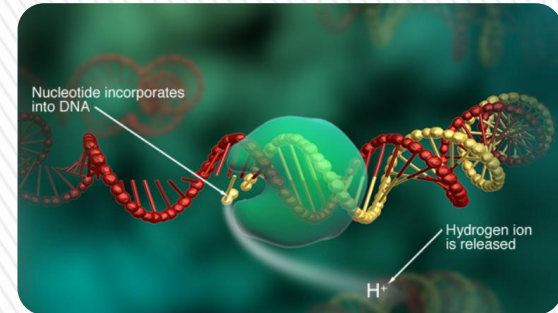
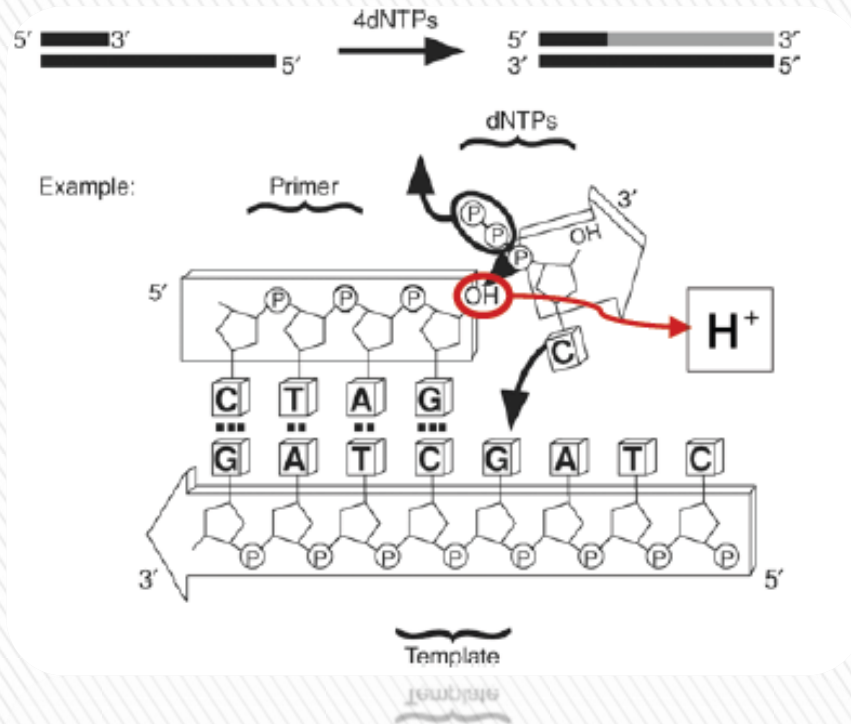
- Simple natural chemistry.
- Fast direct detection.



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Simple natural chemistry

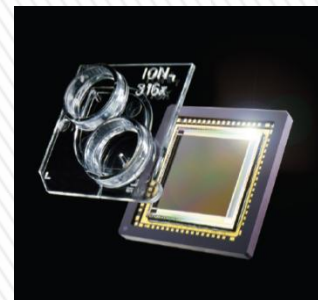


When a nucleotide is incorporated into a strand of DNA by a polymerase, a hydrogen ion is released as a byproduct

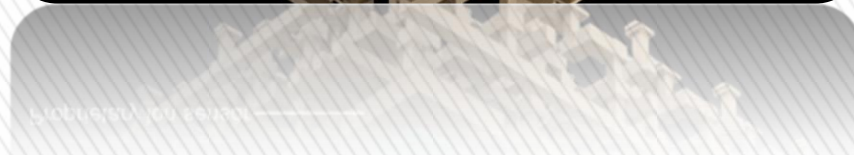
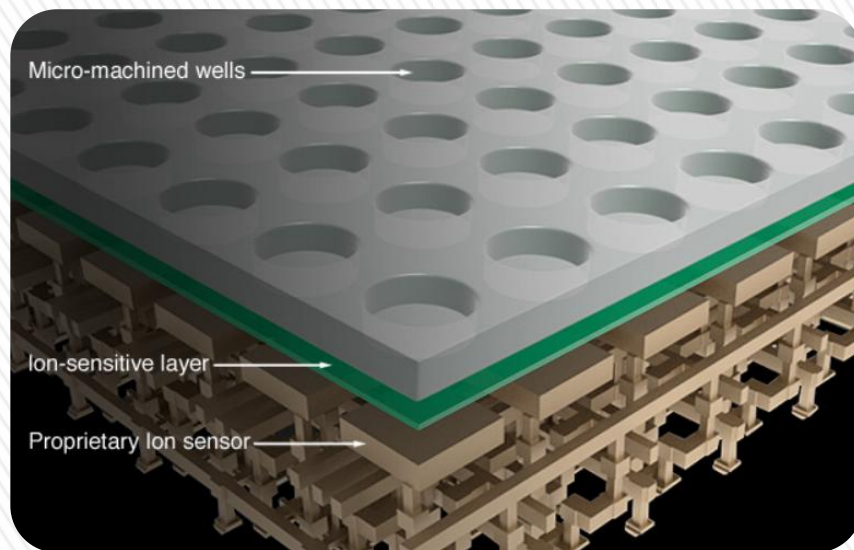


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Fast direct detection

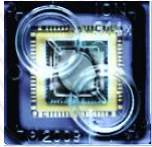


The chip is the machine TM

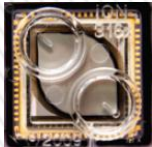
The chip is the machine

			
CHIP	314	316	318*
DATE	JAN:2011	JUL:2011	Q4:2011
OUTPUT	10 Mb	100 Mb	1 Gb
READ LENGTH	100 bp	100 bp	200 bp

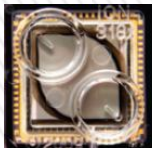
Micro-machined wells



- 1 million sensors in the first-generation Ion 314™ chips



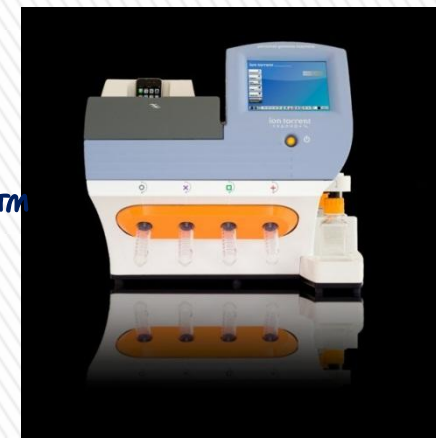
- 7 million sensors in the second-generation Ion 316™ chips



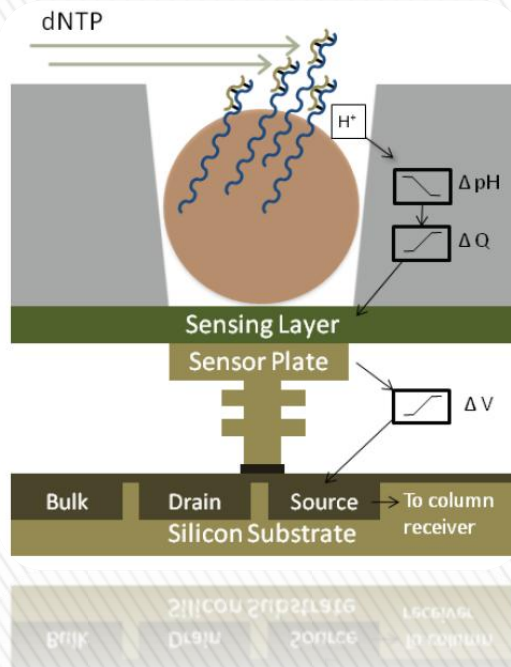
- 12 million sensors in the third-generation Ion 318™ chips

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The chip is the machine



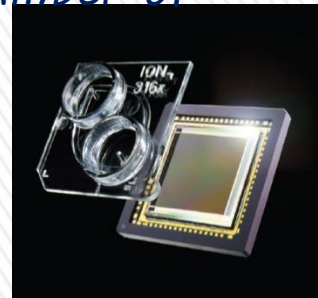
Fast direct detection



1. Nucleotide bases (dNTP's) are sequentially flowed into well one at a time.
2. Upon incorporation, the nucleotide releases a hydrogen ions which creates a pH change.
3. Sensing layer binds to the hydrogen ions.
4. Sensing plate transmits ions to the field effective transistor (FET) gate.
5. Gate registers voltage change between source and drain proportional to number of bases incorporated.

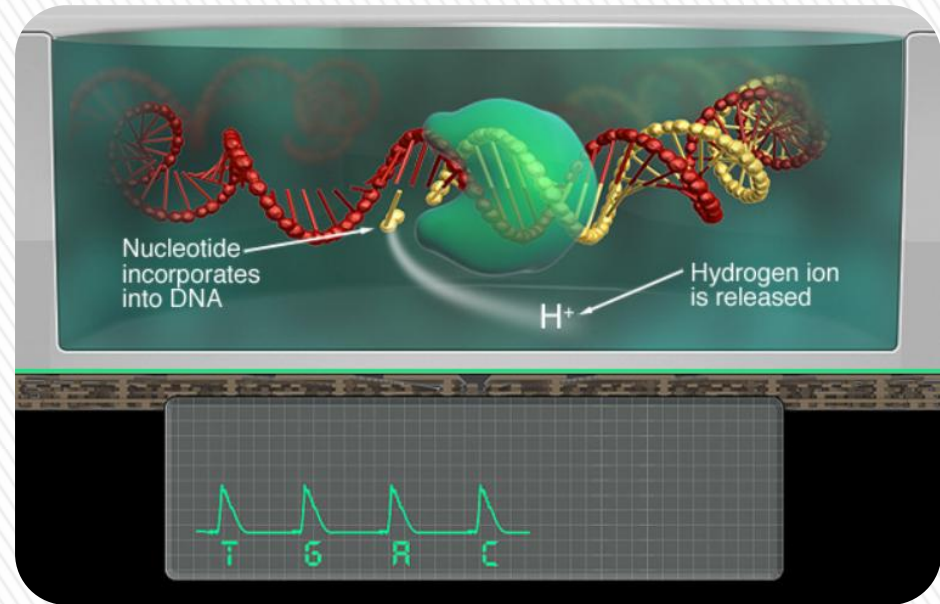
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Fast direct detection (situation 1)

The nucleotide floods the chip with one nucleotide after another.



			
CHIP	314	316	318*
DATE	JAN:2011	JUL:2011	Q4:2011
OUTPUT	10 Mb	100 Mb	1 Gb
READ LENGTH	100 bp	100 bp	200 bp

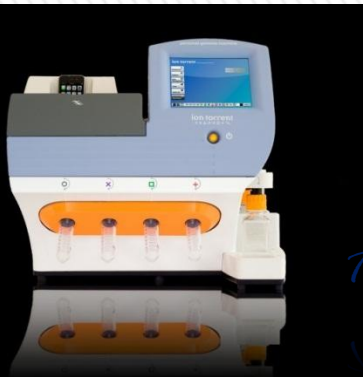
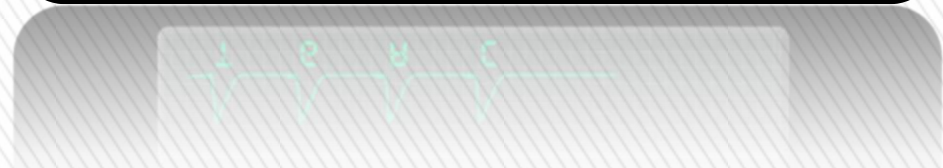
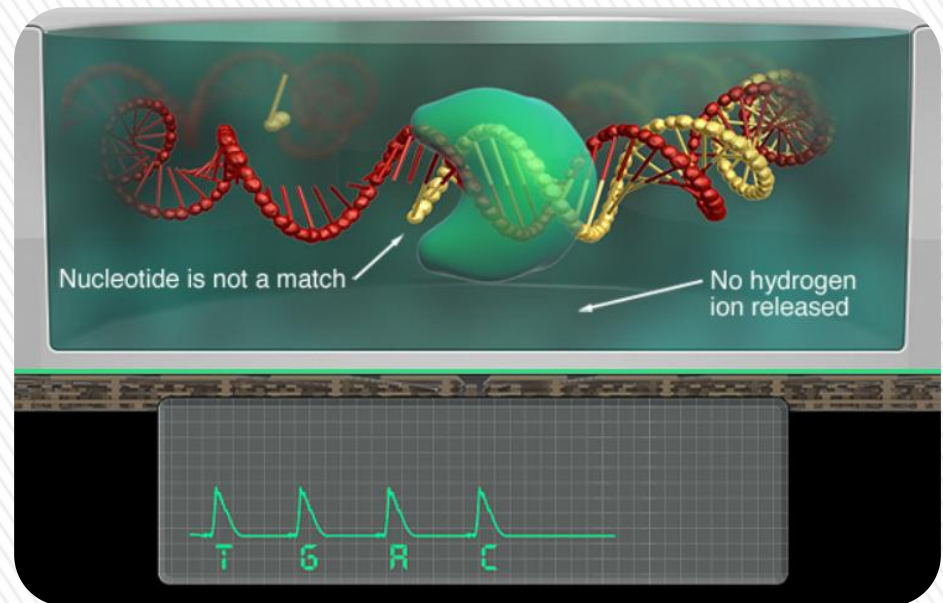
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Fast direct detection (situation 2)

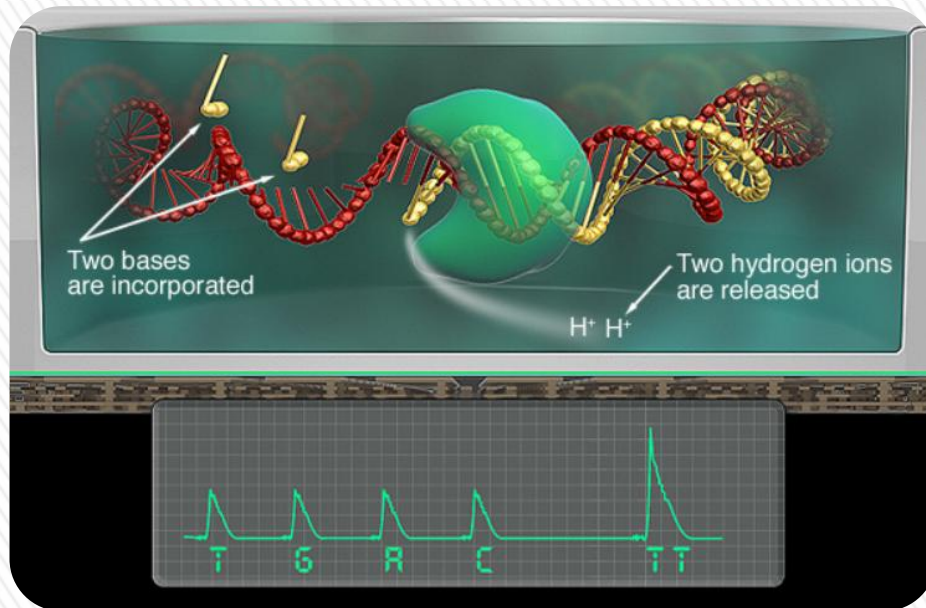
If the next nucleotide that floods the chip is not a match, no voltage change will be recorded and no base will be called.



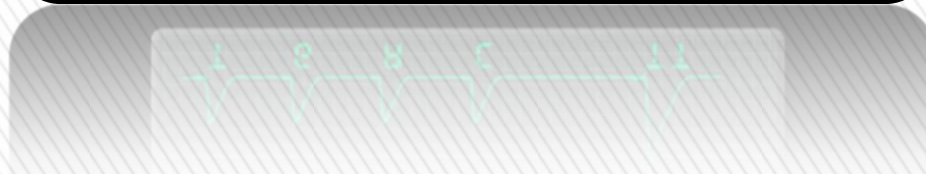
The chip is the machine™
The chip is the machine



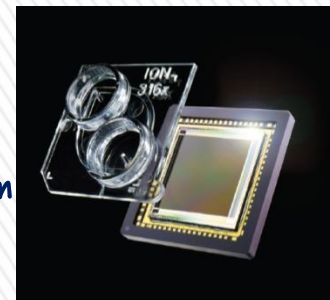
Fast direct detection (situation 3)



If there are two identical bases on the DNA strand, the voltage will be double, and the chip will record two identical bases called.



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How does it perform

- *Speed*
- *Highly accurate sequence*



CHIP	314	316	318*
DATE	JAN:2011	JUL:2011	Q4:2011
OUTPUT	10 Mb	100 Mb	1 Gb
READ LENGTH	100 bp	100 bp	200 bp

The chip is the machine TM

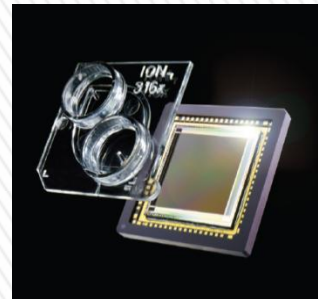
The chip is the machine



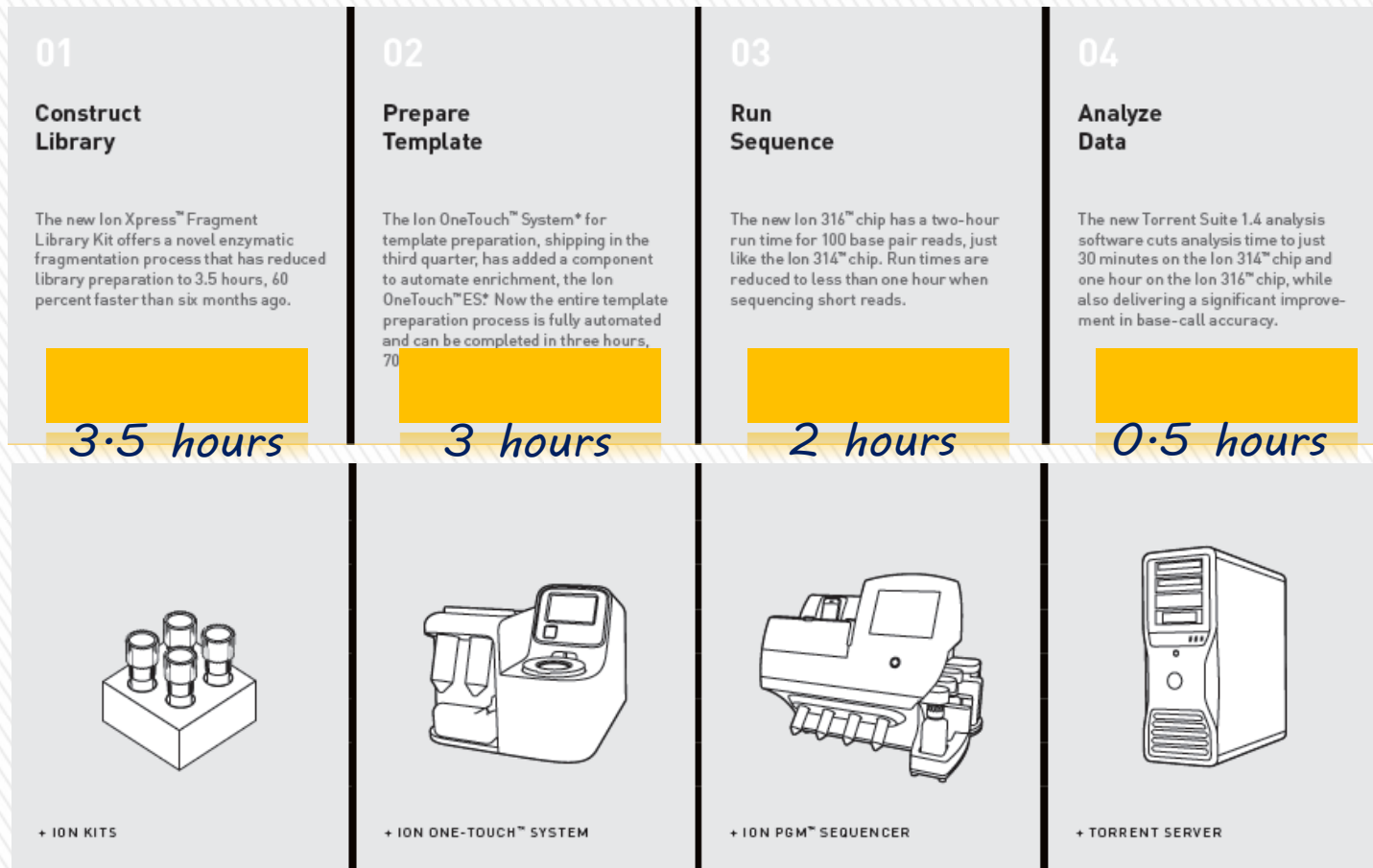
Speed

- Fastest sequencing workflow
- Two hour sequencing run for up to 200bp reads
- Fully prep 8 samples in parallel in less than 6 hours

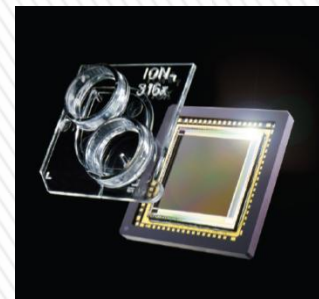
The chip is the machine™
The chip is the machine



Fastest sequencing workflow



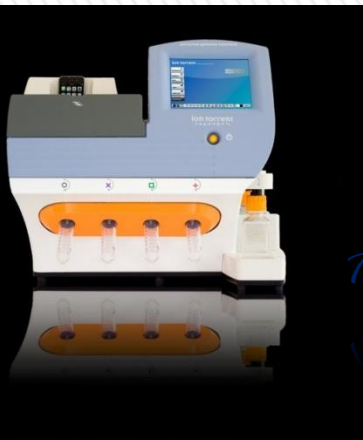
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The Ion OneTouch™ System



System comprises two modules: the Ion OneTouch™ Instrument and the Ion OneTouch™ ES (enrichment system).



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Ion OneTouch™ System Workflow

01

Set up instrument

Load amplification plate and tubes containing oil/recovery solution. Prepare centrifuge.



02

Set up amplification reaction

Combine library, amplification mix, enzyme, and Ion Sphere™ particles. Attach filter.



03

Amplify

Load amplification reaction and filter assembly. Initiate run from touch screen.



04

Set up ES

Load enrichment reagents into the 8-well strip (provided), and attach liquid handling tip provided.



05

Retrieve sample

Recover sample from OneTouch™ Instrument. Remove recovery solution. Resuspend.



06

Enrich

As noted in step 2, load sample in one well and initiate enrichment run.



	CHIP	314	316	318*
	DATE	JAN:2011	JUL:2011	Q4:2011
OUTPUT		10 Mb	100 Mb	1Gb
READ LENGTH		100 bp	100 bp	200 bp

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60% reduction of total time compared to standard methods



Ion OneTouch™ System specifications

System	Ion OneTouch™ System (Cat No. 4470001) includes: <ul style="list-style-type: none"> • Ion OneTouch™ Instrument • Ion OneTouch™ ES
Dimensions and weight	<ul style="list-style-type: none"> • Ion OneTouch™ Instrument: (12 in x 16 in x 14 in, 23 lb; 30 cm x 41 cm x 36 cm, 10.4 kg) • Ion OneTouch™ ES: (9.5 in x 12.5 in x 11 in, 12 lb; 24 cm x 32 cm x 28 cm, 5.4 kg)
System run time	4 hours total time, minutes of hands-on time
Throughput	Supports template preparation for Ion 314™ chips, Ion 316™ chips, and Ion 318™ chips*
Library types	Supports template preparation with a broad range of libraries used for various applications: <ul style="list-style-type: none"> • Genomic DNA (fragment and mate-paired) • Amplicon • RNA (cDNA)
Operating environment	Temperature: 15–25°C; humidity: 20–80%, noncondensing
Consumables	Ion OneTouch™ System Template Kit (Cat No. 4468660)
Power requirements	110/220 V (US/International)
Multiplexing	Up to 384 barcoded libraries for DNA- or RNA-based applications



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Torrent server



Processors

Two Six-core processors

RAM 48 GB RAM

Storage

Eight 2 TB Hard drives in RAID 5 with
12 TB usable

Network

Quad port gigabit NIC

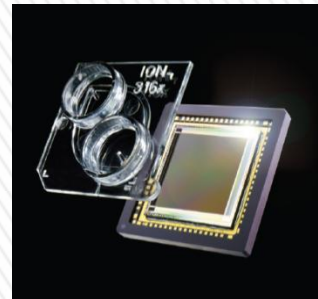
GPU

NVIDIA Graphic Processor Unit

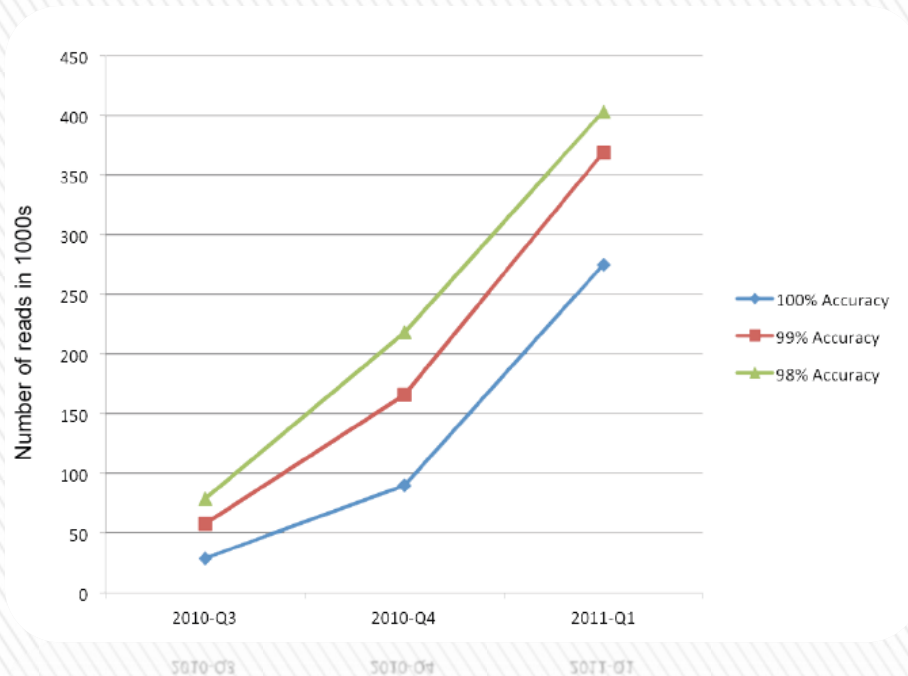
Chassis Dell Precision T7500 tower

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Highly accurate sequence



This figure shows the number of reads from single sequencing runs of *E. coli* on 314 Ion sequencing chips, Increasing yield of number of reads across the last 3 quarters. X axis is the number of 100 base reads in thousands.

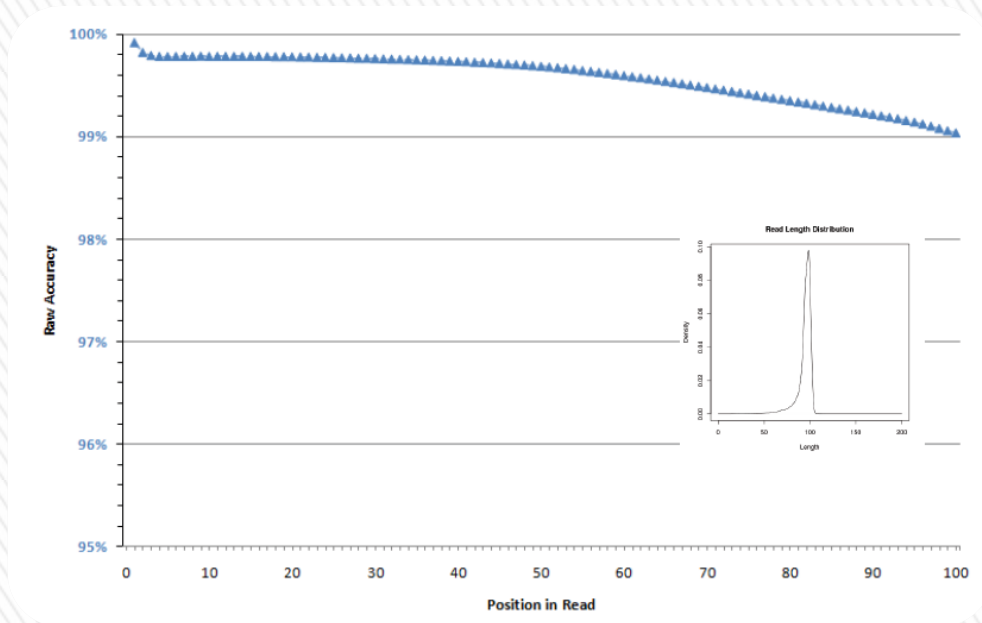
The number of 100 base reads with 0 or 1 errors (99% accuracy) has increased from 58K to 166K to 369K over the last three quarters of development effort.

	CHIP	314	316	318*
	DATE	JAN:2011	JUL:2011	Q4:2011
	OUTPUT	10 Mb	100 Mb	1 Gb
	READ LENGTH	100 bp	100 bp	200 bp

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Highly accurate sequence



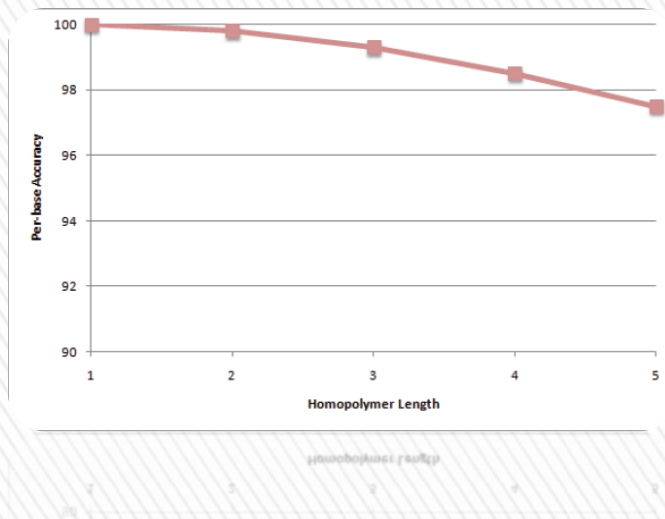
Accuracy versus total position from a single run of 314 using *E. coli* Dh10B. Base calls were generated with default parameters in v1.3.0 of the Torrent Suite software. After filtering based solely on the inherent signal properties of the data 475k reads were produced.



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Highly accurate sequence - Homopolymer



The red line - data got from a single run of an Ion 314 chip using E-coil DH10B.

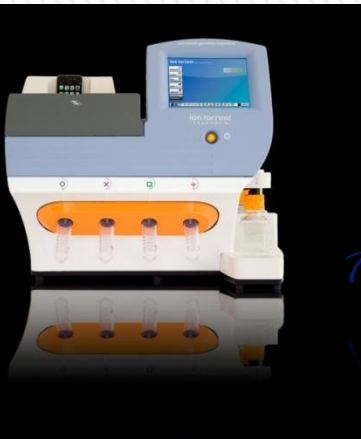
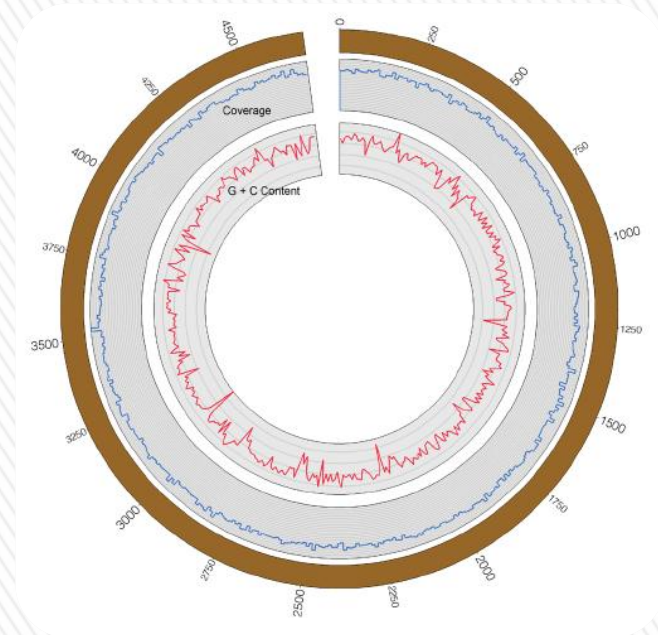


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Highly accurate sequence - Coverage

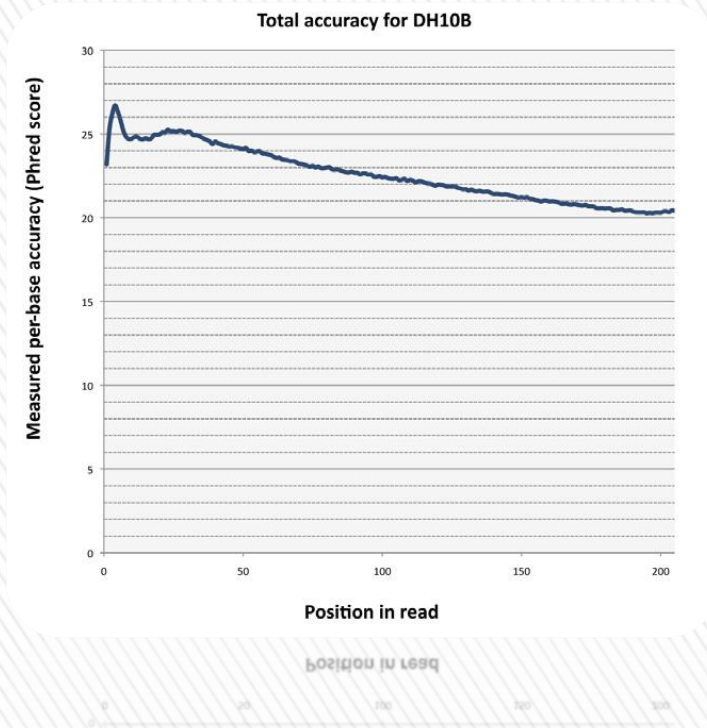
Circular plot shows percentage of G+C content in the inner red circle and regional coverage level in the outer blue circle



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New progress



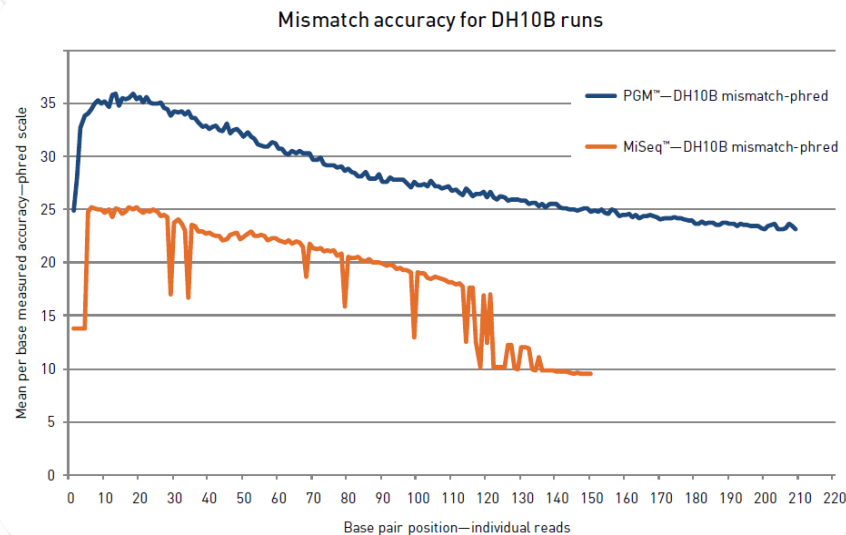
CHIP	314	316	318*
DATE	JAN:2011	JUL:2011	Q4:2011
OUTPUT	10 Mb	100 Mb	1 Gb
READ LENGTH	100 bp	100 bp	200 bp

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In October 2011, Ion Torrent launched a long read kit for the PGM sequencer, which can provide high quality read lengths of 225 bases. Furthermore, Reads longer than 500 bases are achievable, that have been demonstrated.

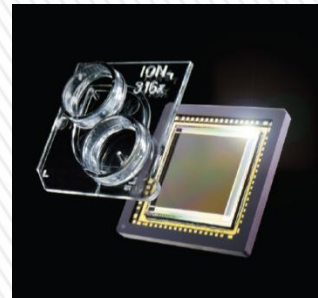
The comparison between Life Ion PGM and Illumina MiSeq™



This figure shows total per-base mismatch accuracy rate for complete DH10B runs on both the Ion PGM sequencer and Illumina MiSeq sequencer.

This plot represents errors that are seen as mismatched bases that lead to substitution errors rather than gapped extensions.

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The comparison between Life Ion PGM and Illumina MiSeq™

	Ion PGM™ sequencer long read—DH10B	Illumina MiSeq™—DH10B
Overall average coverage	10x	421x
Observed consensus substitutions	0	11
Percentage of total genome covered	99.98%	94.17%
Error rate at base 150 (all error types)	2.99%	11.2%
Average total per-base error rate	1.2%	2.8%

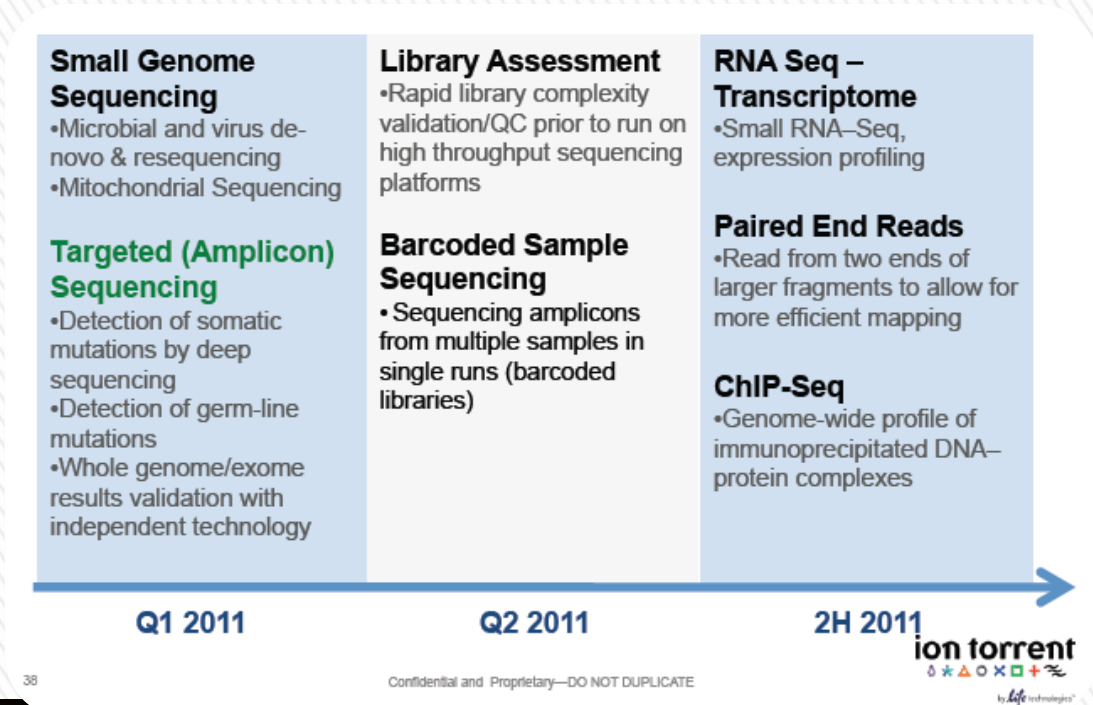
Comparison of selected features of the consensus sequence derived from the Ion PGM™ sequencer and MiSeq™ platforms.



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Applications



38

Confidential and Proprietary—DO NOT DUPLICATE

CHIP	314	316	318*
DATE	JAN:2011	JUL:2011	Q4:2011
OUTPUT	10 Mb	100 Mb	1 Gb
READ LENGTH	100 bp	100 bp	200 bp

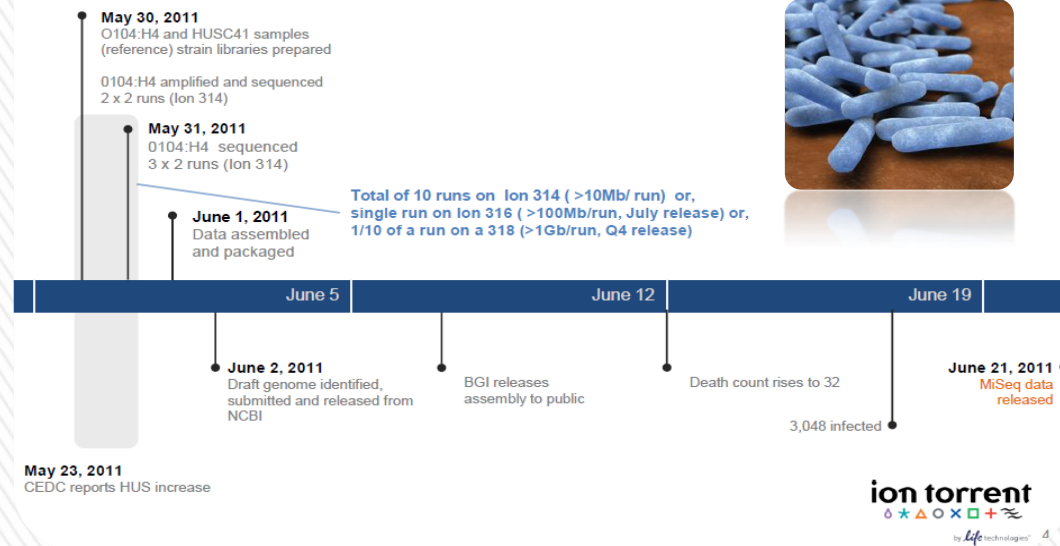
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Small genome sequencing

Speed: *E. coli* Outbreak Isolate Sequenced Across 2 Continents within 3 Days *Time to Results Matter*



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TM



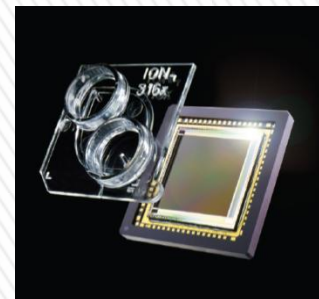
Target resequencing

Gene	Chromosome: position	Variant	Sample	Allele frequency		Total coverage
				Normal allele	Somatic allele	
GPR137B	chr1: 236343437	G>C	Tumor	81.8%	18.2%	6,359
			Normal	100.0%	0.0%	4,135
			Adjacent	100.0%	0.0%	1,514
IQCH	chr15: 67687770	G>C	Tumor	76.3%	23.7%	4,477
			Normal	99.9%	0.1%	3,770
			Adjacent	100.0%	0.0%	981
KLKB1	chr4: 187159516	A>G	Tumor	68.3%	31.7%	1,918
			Normal	99.8%	0.2%	3,011
			Adjacent	100.0%	0.0%	399
KRAS	chr12: 25398284	C>A	Tumor	76.1%	23.9%	3,866
			Normal	99.9%	0.1%	4,201
			Adjacent	100.0%	0.0%	2,136

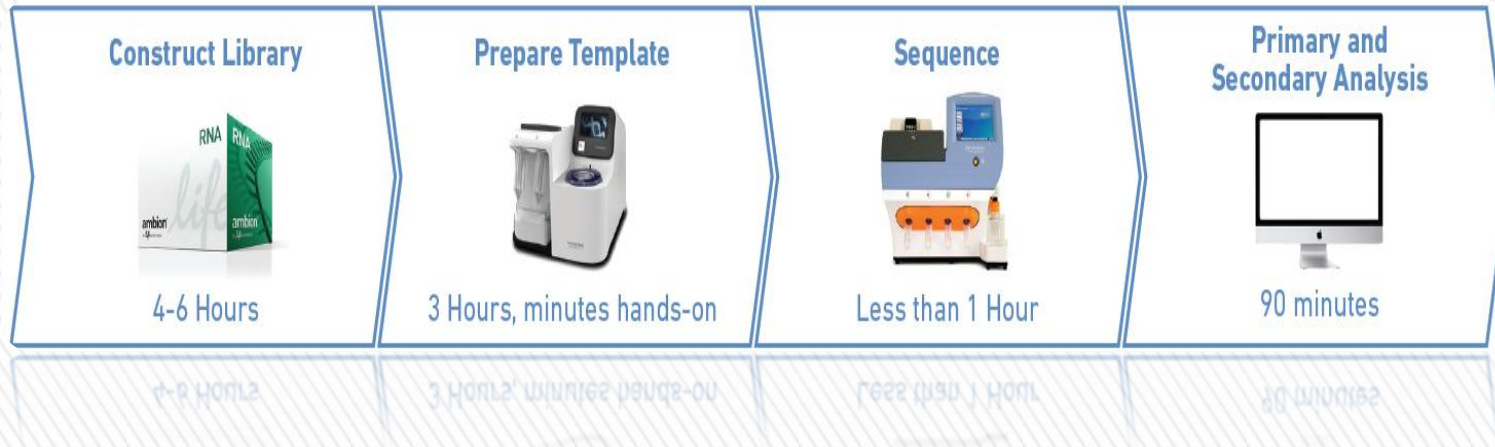
Data courtesy of Sean Grimmond, Queensland Centre of Medical Genomics

Table confirmed somatic mutations on the Ion
PGM™ system

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Ion RNA-Seq solution



CHIP	314 316 318*
DATE	JAN:2011 JUL:2011 Q4:2011
OUTPUT	10 Mb 100 Mb 1Gb
READ LENGTH	100 bp 100 bp 200 bp

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Ion Torrent PGM is the machine



Expression data comparison between PGM and microarray

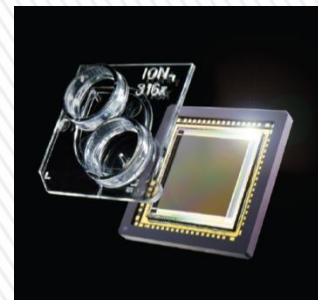
	Total Assays	Intersection Assays	Intersection Detected
TaqMan®	748	708	308
GeneChip® Array	847	708	216
Ion 314™	904	708	230
Ion 316™*	904	708	358

The figure illustrates that the sensitivity with the Ion 316 sequencing chip surpasses the sensitivity of detecting miRNA species

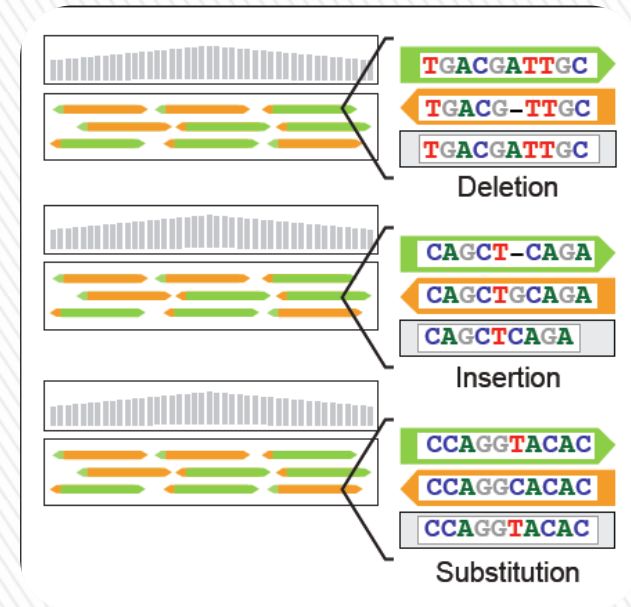
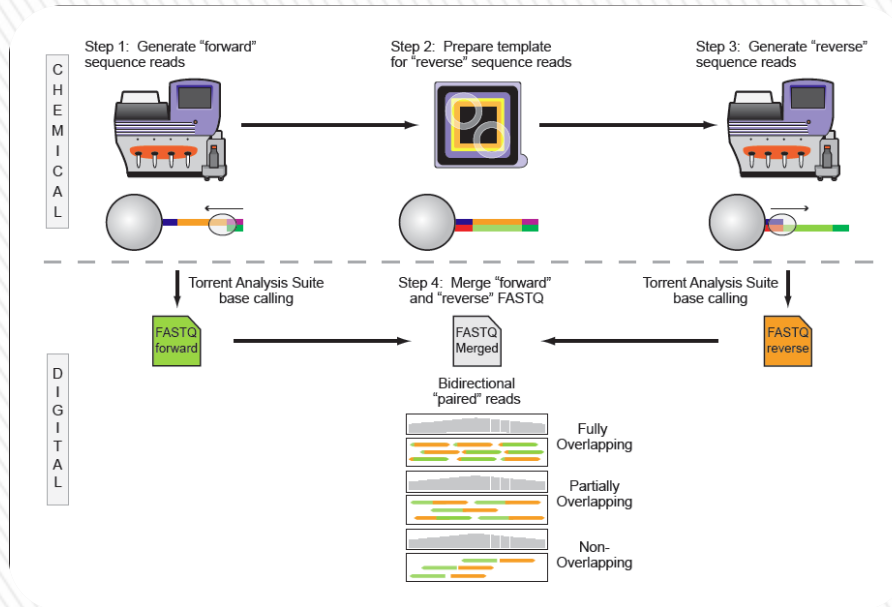
The first column shows the total number of miRNAs that can theoretically be detected by each platform based on their respective content. PGM™ detection threshold is not content dependent and the number represents the total number of miRNAs included in the miRBase up to date. Detection by TaqMan® is calculated at Ct<40. The number of RNAs that all 3 platforms can theoretically detect is shown in the second column and the actual number of miRNAs detected by each platform is indicated on the last column.

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Paired end sequencing



CHIP	314	316	318*
DATE	JAN:2011	JUL:2011	Q4:2011
OUTPUT	10 Mb	100 Mb	1 Gb
READ LENGTH	100 bp	100 bp	200 bp

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TM

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