3. Les NNGS : 3è génération de séquenceurs

Ion Torrent (Life technologies)

ion torrent
by life technologies

- Amplification sur billes (emPCR)
- Support : puce semi-conductrice
- Séquençage par synthèse
- Détection : Δ pH

INSTRUMENTS

Sequencing Instrument

Ion OneTouch™ Instruments

Emulsion PCR and Enrichment

REAGENTS

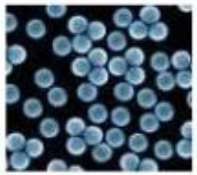
Semiconductor Chip

Sequencing Chemistry

- Natural nucleotides
- Natural enzymes

Sample Prep

- Libraries
- Clonal beads



DATA ANALYSIS

Torrent Server

➢ **Principe of sequencing chemistry : The first post-Light sequencing technology,** a complementary metal-oxide semiconductor (CMOS) integrated circuit for sequencing .

Sequence data are obtained by directly sensing the **hydrogen ions** produced by template-directed DNA polymerase **synthesis using all-natural nucleotides** on this massively parallel semiconductor-sensing device or ion chip.

$$DNA(n) + nucl. = DNA(n+1) + PPi \rightarrow PPi + H+$$

Lors de la formation de la liaison phospho-diester il y a relargage d'un ion H+ et de PPi

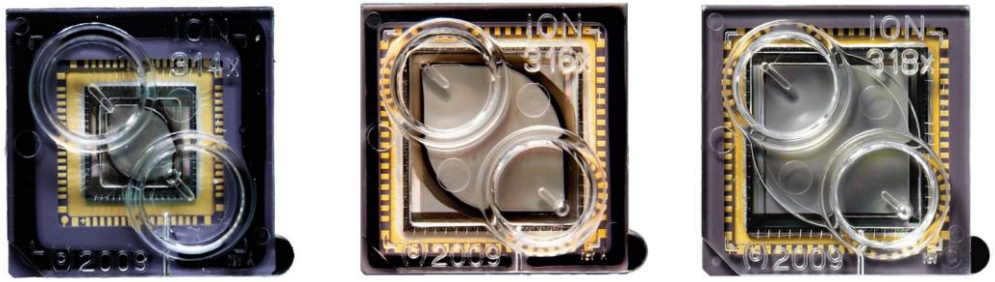
An integrated semiconductor device enabling non-optical genome sequencing

Jonathan M. Rothberg[1], Wolfgang Hinz[1], Todd M. Rearick[1], Jonathan Schultz[1], William Mileski[1], Mel Davey[1], John H. Leamon[1], Kim Johnson[1], Mark J. Milgrew[1], Matthew Edwards[1], Jeremy Hoon[1], Jan F. Simons[1], David Marran[1], Jason W. Myers[1], John F. Davidson[1], Annika Branting[1], John R. Nobile[1], Bernard P. Puc[1], David Light[1], Travis A. Clark[1], Martin Huber[1], Jeffrey T. Branciforte[1], Isaac B. Stoner[1], Simon E. Cawley[1], Michael Lyons[1], Yutao Fu[1], Nils Homer[1], Marina Sedova[1], Xin Miao[1], Brian Reed[1], Jeffrey Sabina[1], Erika Feierstein[1], Michelle Schorn[1], Mohammad Alanjary[1], Eileen Dimalanta[1], Devin Dressman[1], Rachel Kasinskas[1], Tanya Sokolsky[1], Jacqueline A. Fidanza[1], Eugeni Namsaraev[1], Kevin J. McKernan[1], Alan Williams[1], G. Thomas Roth[1] & James Bustillo[1]

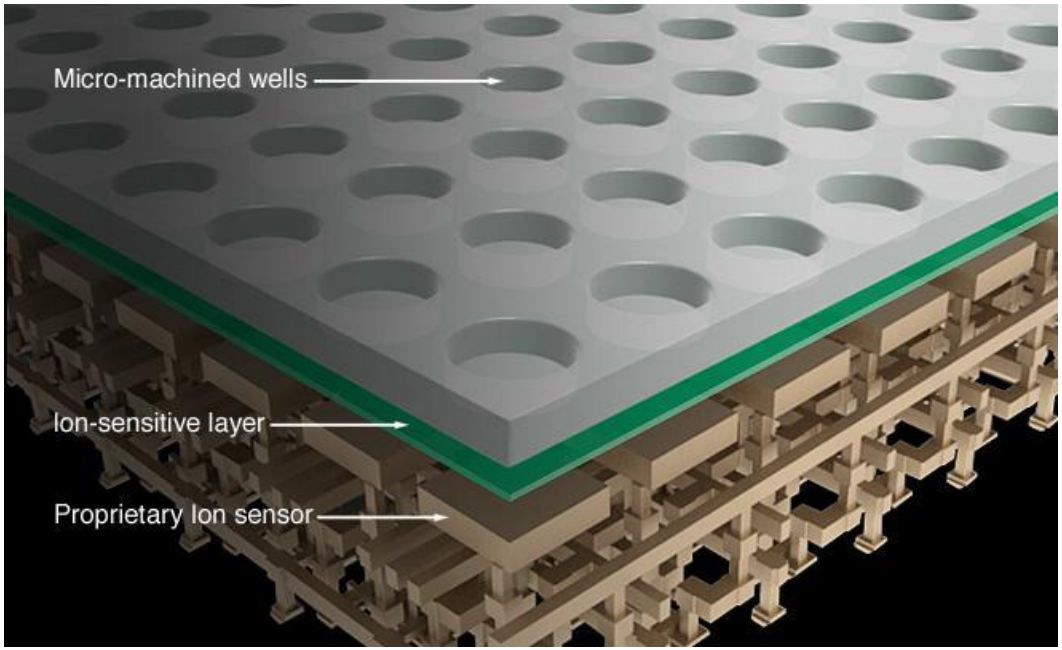
The seminal importance of DNA sequencing to the life sciences, biotechnology and medicine has driven the search for more scalable and lower-cost solutions. Here we describe a DNA sequencing technology in which scalable, low-cost semiconductor manufacturing techniques are used to make an integrated circuit able to directly perform non-optical DNA sequencing of genomes. Sequence data are obtained by directly sensing the ions produced by template-directed DNA polymerase synthesis using all-natural nucleotides on this massively parallel semiconductor-sensing device or ion chip. The ion chip contains ion-sensitive, field-effect transistor-based sensors in perfect register with 1.2 million wells, which provide confinement and allow parallel, simultaneous detection of independent sequencing reactions. Use of the most widely used technology for constructing integrated circuits, the complementary metal-oxide semiconductor (CMOS) process, allows for low-cost, large-scale production and scaling of the device to higher densities and larger array sizes. We show the performance of the system by sequencing three bacterial genomes, its robustness and scalability by producing ion chips with up to 10 times as many sensors and sequencing a human genome.

Rothberg et al. (2011) "An integrated semiconductor device enabling non-optical genome sequencing" Nature 475, 348 - 352

➢ Reagents : The Ion Semi-conductor Chip



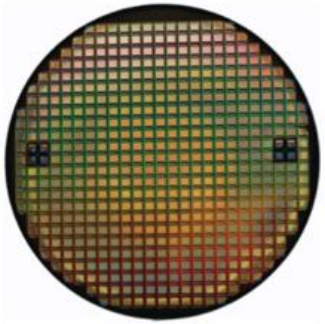
La puce semi-conductrice est un nano composant constitué de trois couches :



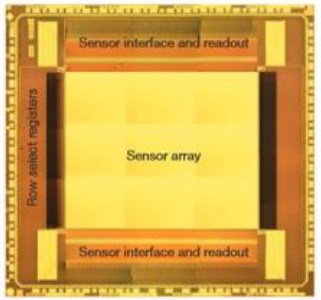
- La plaque supérieure est composée de puits - **Micro-machined wells**. C'est dans ces puits que les fragments **d'ADN simples brins** sont **polymérisés par une ADN polymérase**.
- La sous couche Ion-sensitive layer est une couche de métal oxydé, qui permet de traduire la variation de pH en Tension (V).
- Un capteur Ion-sensor qui permet l'acquisition du signal correspondant à un puit.

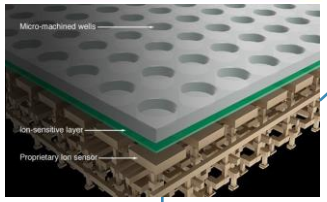
Chip Manufacture

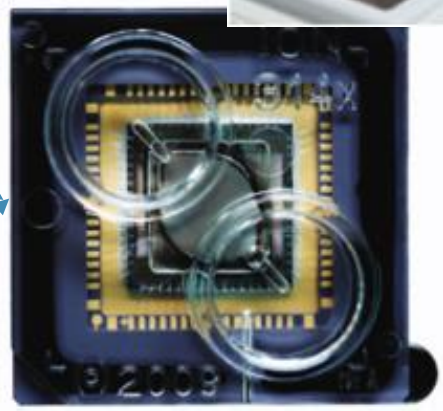
Semi-conductor manufacturing: **wafer** containing 200 individual ion sensors



Individual ion sensor and his ceramic package: contains millions of uniform wells



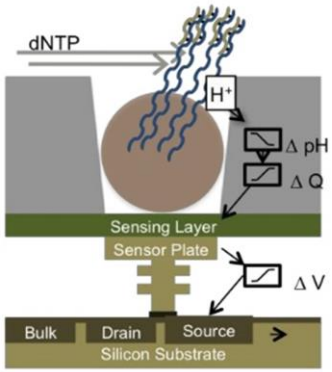




Chip: Semi-conductor packaging and loading

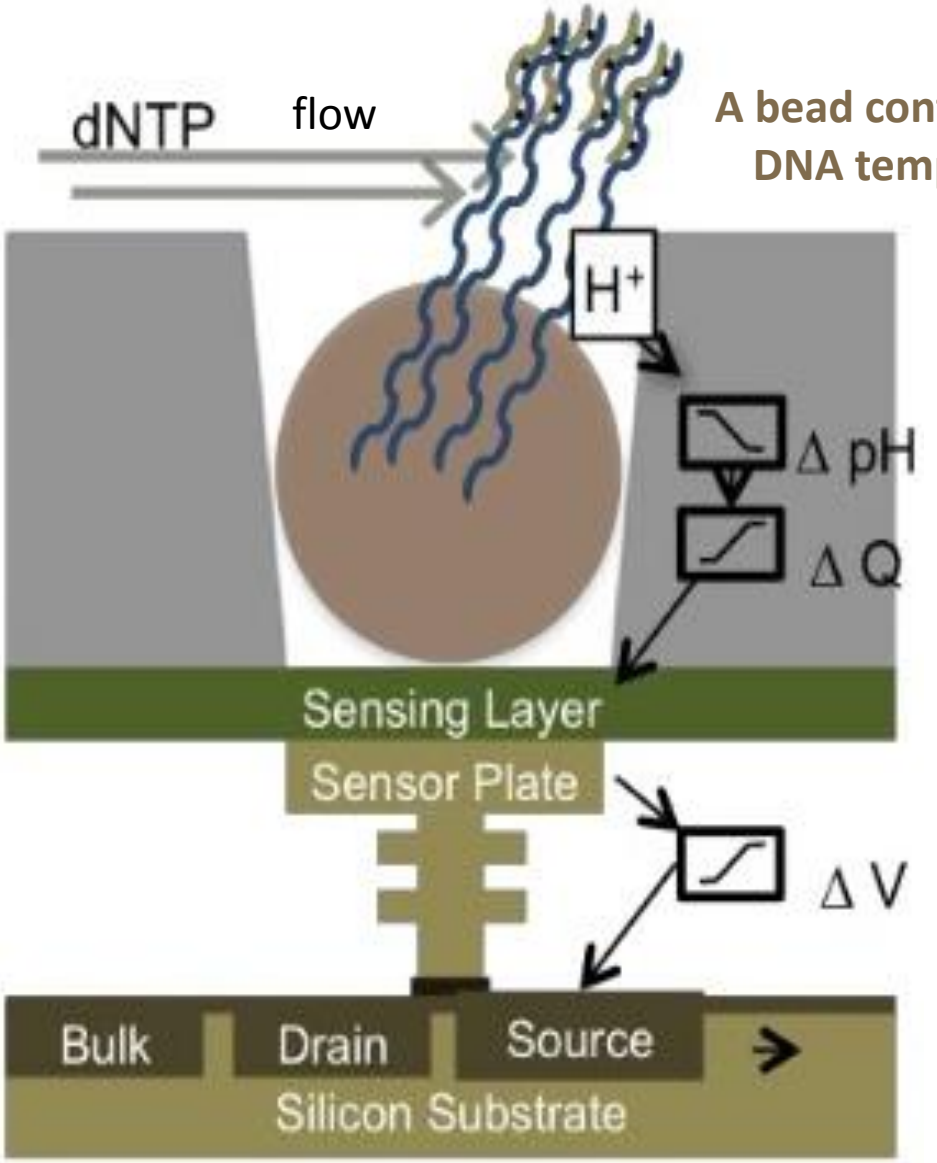
Individual micro-machined wells : 3.5 microm-diameter

- A bead containing DNA template
- Translation of a proton signal to digital format



➢ Reagents : Sequencing by synthesis, detection of proton

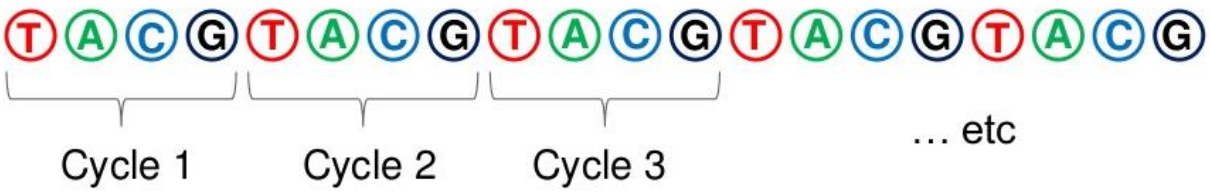
Individual micro-machined wells

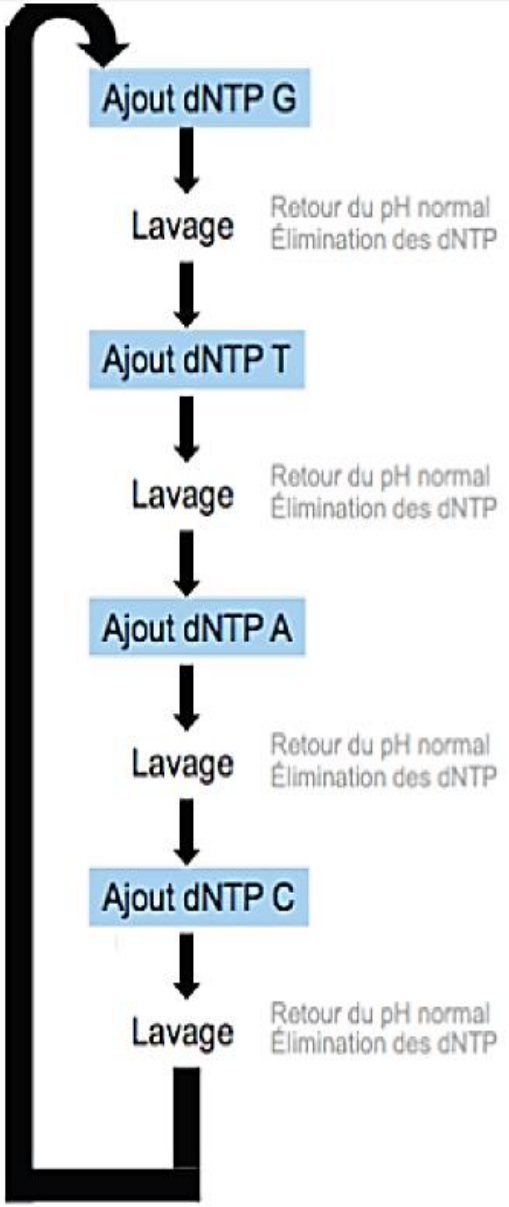


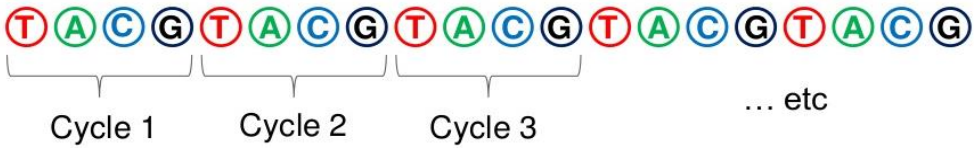
Flows and cycles

Sequencing reaction is cyclic

- **A flow** is the event of exposing the chip to one particular dNTP) , floowed by a washing step.
- **A cycle** is four consecutive dNTP flow with T-A-C-G.





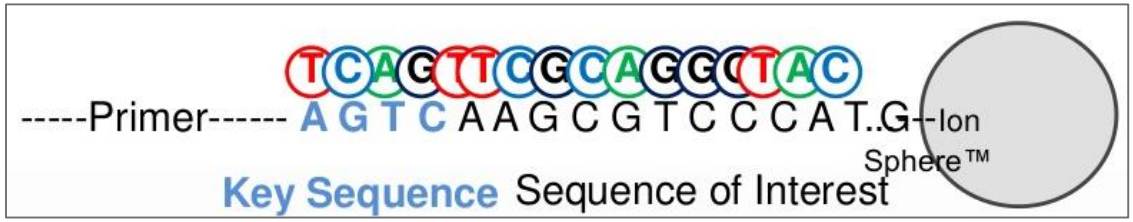


Cycle 1

Cycle 2

Cycle 3 + cycle 4

And so on…



➢ Reagents : signal acquisition

La variation du pH (ΔpH) due à la polymérisation du dNTP entraîne un changement du potentiel de la membrane-Ion-sensitive layer (ΔQ), inversement proportionnel à ΔpH. Le capteur traduit ΔQ en Volt (ΔV).

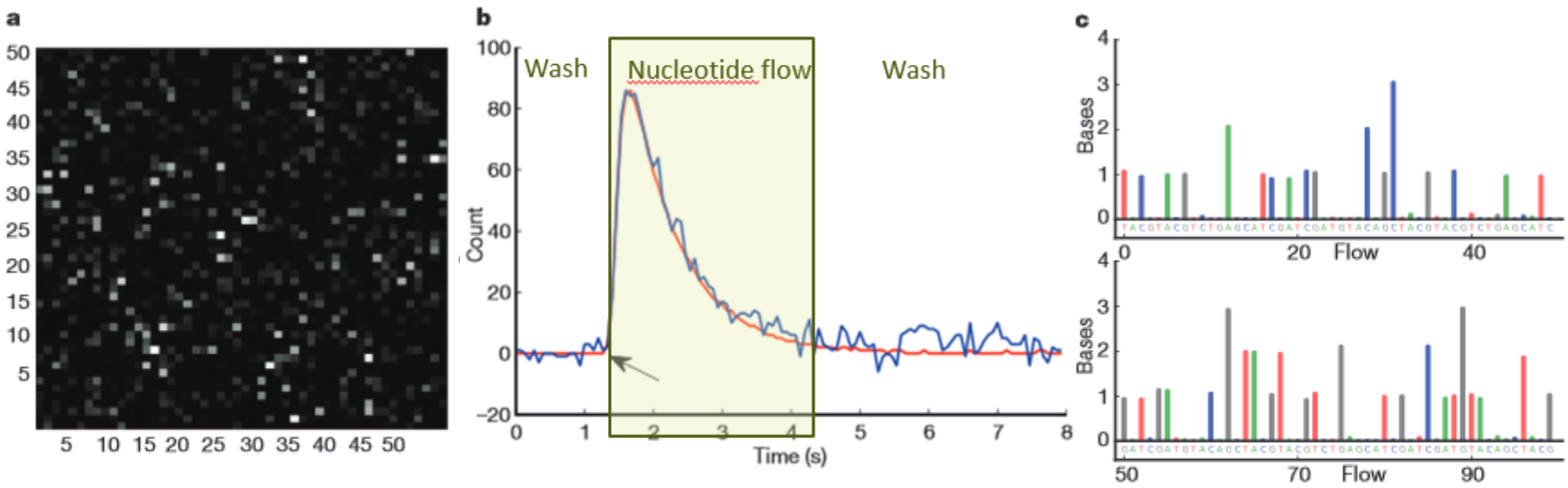


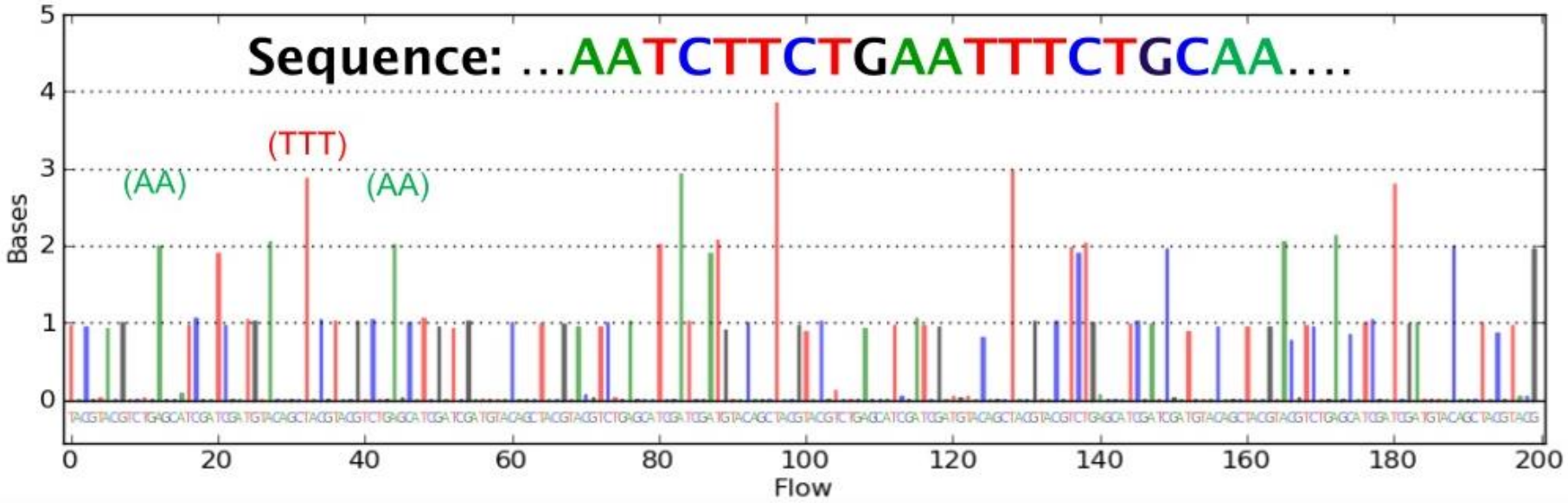
Figure 3 | Data collection and base calling. **a**, A 50 × 50 region of the ion chip. The brightness represents the intensity of the incorporation reaction in individual sensor wells. **b**, 1-nucleotide incorporation signal from an individual sensor well; the arrow indicates start of incorporation event, with the physical model (red line) and background corrected data (blue line) shown. **c**, The first 100 flows from one well. Each coloured bar indicates the corresponding number of bases incorporated during that nucleotide flow.

3 | VOL 475 | 21 JULY 2011

Modifié de

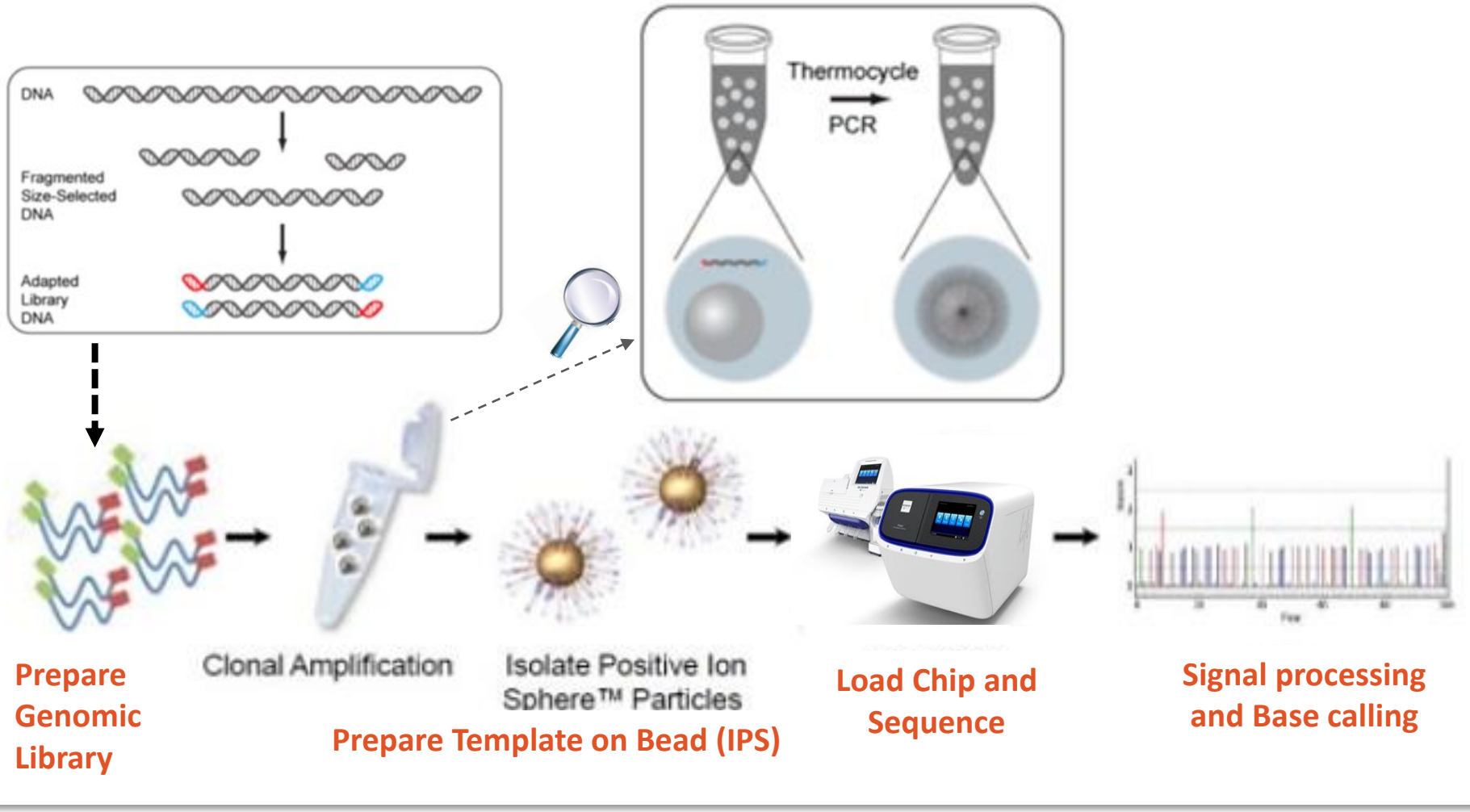
Data output : Ionogram

- Must be read “up-and-down” along with “left-to-right”
- Height of bar indicates how many nucleotides incorporated during flow



En absence de polymérisation du nucléotide on observe aucune variation de tension. La variation de tension est directement proportionnelle au nombre de nucléotide polymérisés (en bleu) .

➢ Reagents : Preparation of DNA template for Ion torrent sequencing



Modifié de

➢ Instrument and Bioinformatics solution

Prepare Genomic Library

Prepare Template on Bead

Load Chip and Sequence

Signal processing and Base calling

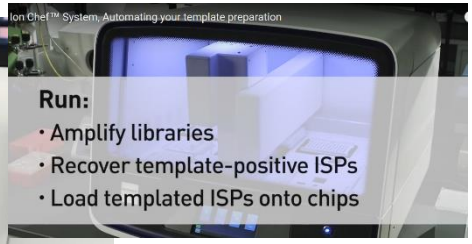
Ion Sequencing Kit

The AB Library Builder™ System

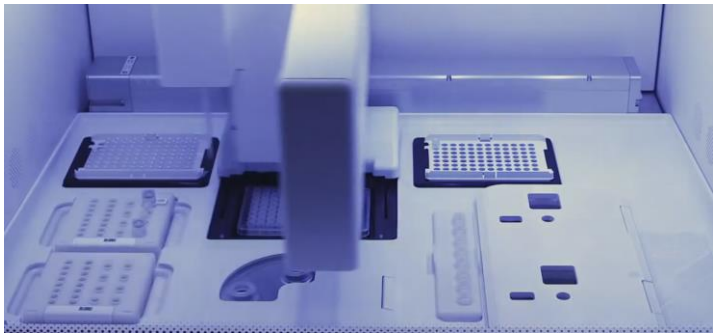
The Ion Chef™ System and the Chip

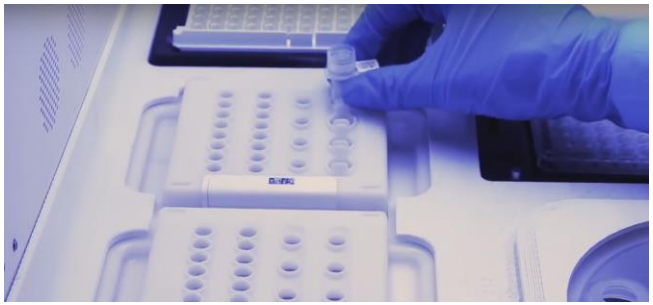
Ion Torrent™ technology : Ion Proton ™ and Ion PGM ™

Torrent Suite™ Software and Torrent Server, Plugins

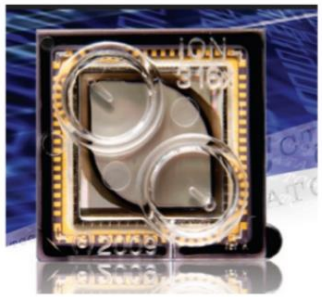


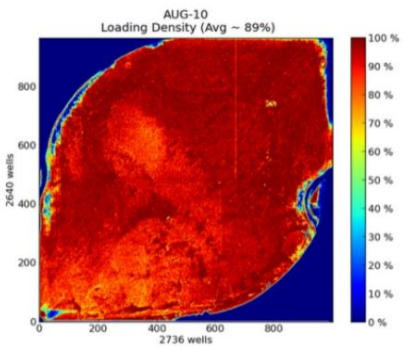
The Ion Chef™ System





Loading density on 316 chip





➢ Instrument : Specifications of Ion Torrent platform

Personal Genome Machine™

	Ion Torrent PGM			Ion Torrent Proton	
Chip Type	PGM 314	PGM 316	PGM 318	Proton I	Proton II (~July-2013)
# wells	1.3M	6.3M	11M	165M	660M
Total output	10-40Mb	100-400Mb	~1Gb	~10Gb	~100Gb
Run time	1-2 hrs	1-2 hrs	1-2 hrs	2.5 hrs	2.5 hrs
Read length	up to 400bp	~200bp	up to 400bp	~200bp	~200bp
Total reads	up to 0.6M	up to 3M	up to 6M	60-80M	240-330M

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Life Technologies Ion Torrent Personal Genome Machine PGM + Ion Torrent Server See original listing

Item condition: **Used**

"*Excellent condition. Unit powers up. Previously maintained under service contract. Just set up, add*" ... Read more

Ended: Jul 05, 2015, 4:38PM

Price: ~~US $18,000.00~~

Best offer accepted

Shipping: **$932.15** UPS Worldwide Saver

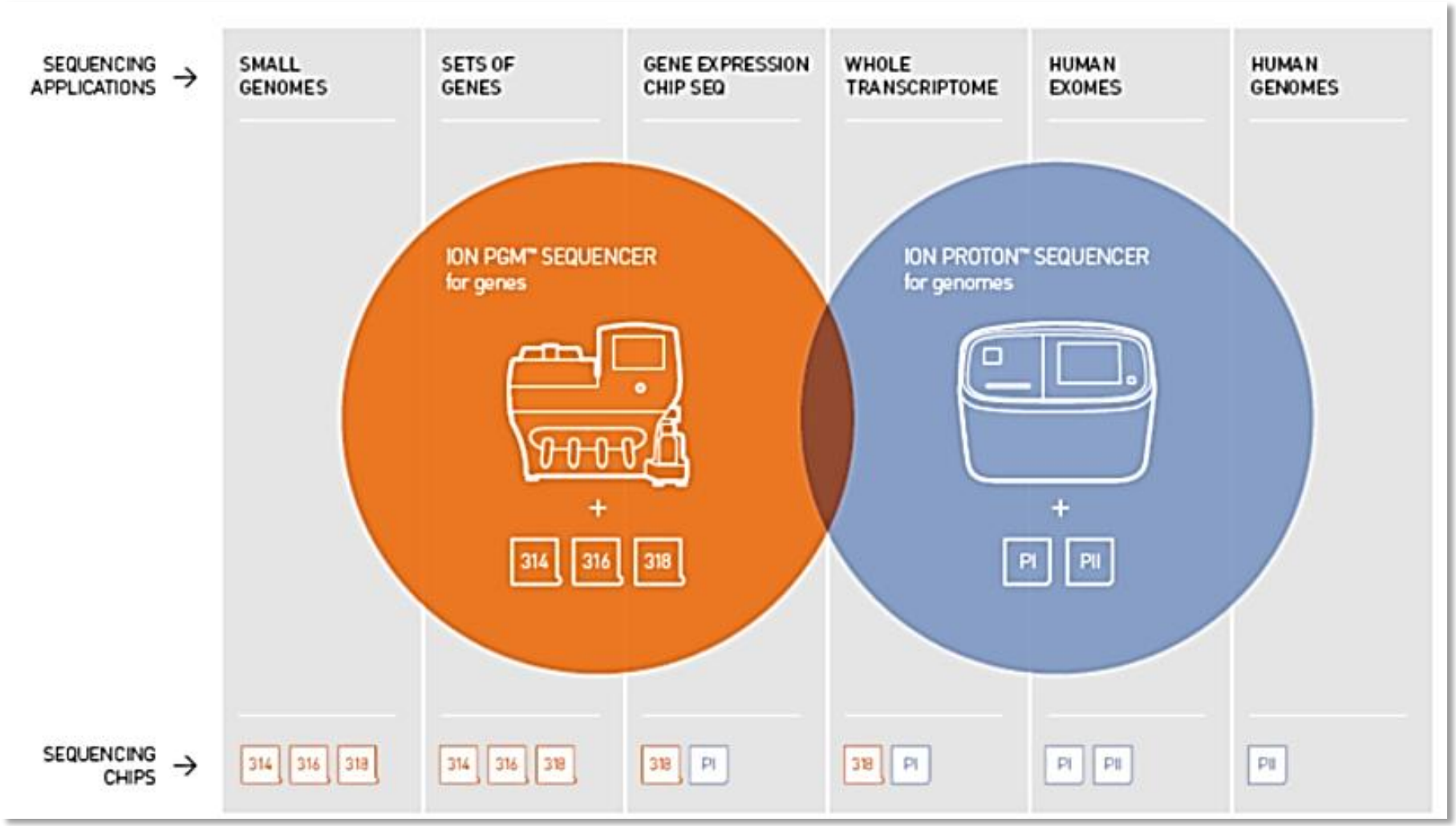
Item location: Newman, California, United States

Seller: **cisl_company** (888 ★) me | Seller's other items

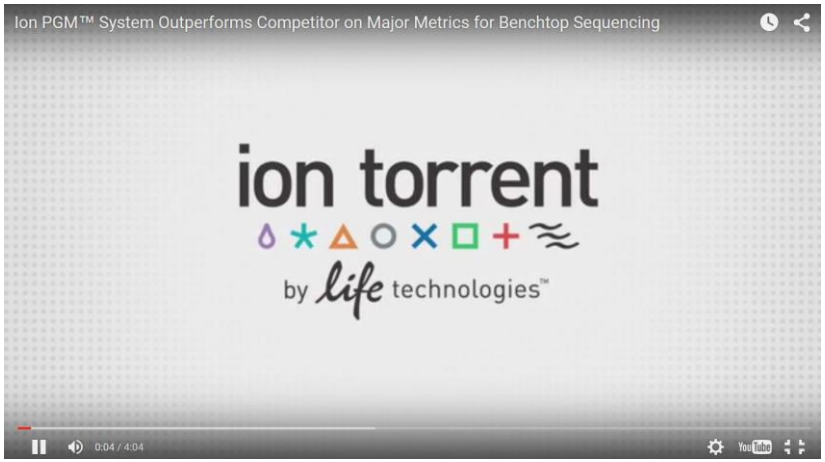
Advantages of CMOS integrated circuit for sequencing

- **Low-cost** semiconductor manufacturing techniques.
- **Scalability** : millions of uniform wells provide confinement and massively parallel, simultaneous detection of independent sequencing reactions.
- **Low power requirement** in comparison to a sensor designed for the detection of photons, no optical detection.
- **Flexible**: Flexible scaled chips on PGM™ (Personal Genome Machine™) and Proton systems for different throughput needs.
- **Fast and HT :** 1 - 2hrs run using an ion chip with 1.3M to 660M sensors generates approximately 1M-330M bases
- **Read length** : from 200bp to 400bp
- **High accuracy** : > 99,9 %

Applications fiels of Ion PGM & Proton Sequencer

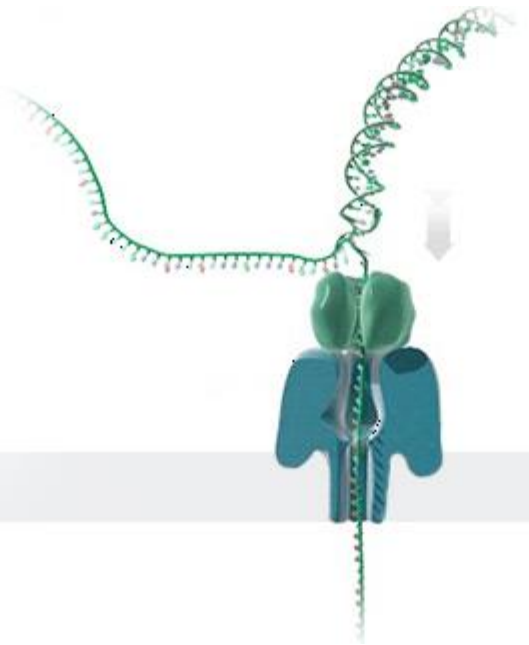


Ion torrent videos



Nanopore (Oxford, IBM)

- Pas d'amplification
- Support : membrane avec nanopores
- Séquençage par dégradation
- Détection : mesure de courant



a protein nanopore in an electrically resistant membrane bilayer.

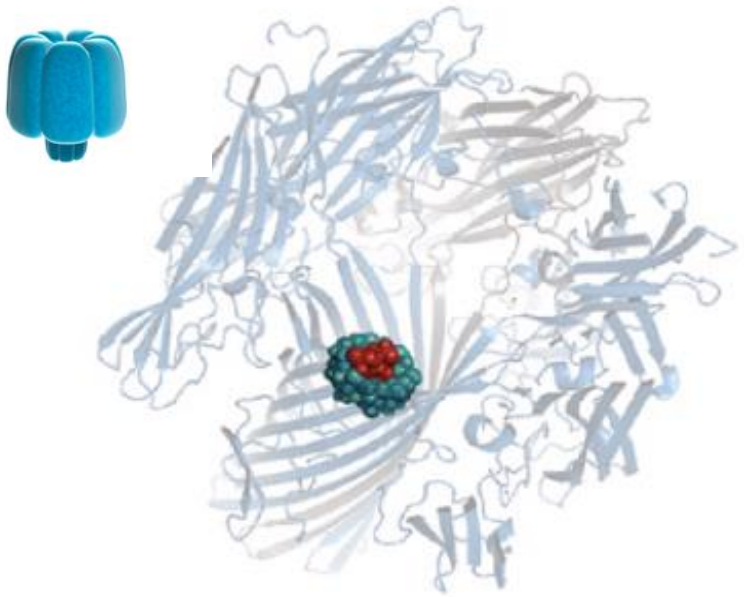
https://nanoporetech.com/science-technology/how-it-works

➢ Nanopore fabrication

A nanopore is, essentially, a nano-scale hole. This hole may be:

- biological: formed by a pore-forming protein in a membrane such as a lipid bilayer;
- solid-state: formed in synthetic materials such as silicon nitride or graphene;
- hybrid: formed by a pore-forming protein set in synthetic material.

Biological nanopores : La protéine naturelle formant le Nanopore est l'**alpha-hemolysine** (heptamère de 1nm) elle n'est pas capable de différencier les bases. Oxford Nanopore utilise les techniques de protein engineering pour adapter le Nanopore à la détection de dNMP ; **une cyclodextrine** qui possède une affinité différenciée pour les bases nucléotidiques

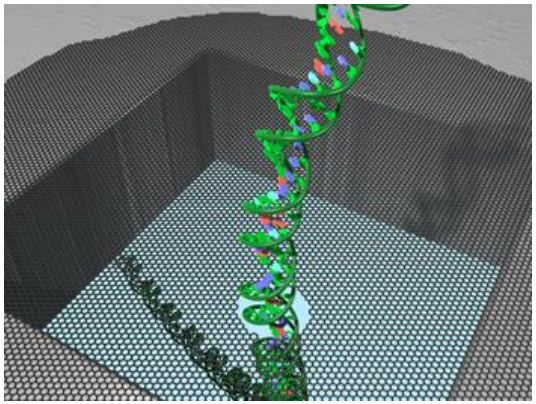


α-hemolysin nanopore (ribbon diagram) with covalently attached cyclodextrin (teal blue) transiently binds a base (red) traversing the pore.

Solid-state nanopore sensing platforms in a variety of forms **including silicon nitride (SiNx or SiO),** graphene, and modifications to these solid-state materials for the sensing process.

Graphene membrane

- Graphene is a robust, single-atom-thick lattice of carbon with high electrical conductivity. It is also chemically inert
- The fine depth of the graphene membrane provides optimal spatial resolution along the DNA.
- Graphene was use to separate two chambers containing ionic solutions, and created a nanopore in the graphene. trans-electrode, measuring a current flowing through the nanopore between two chambers



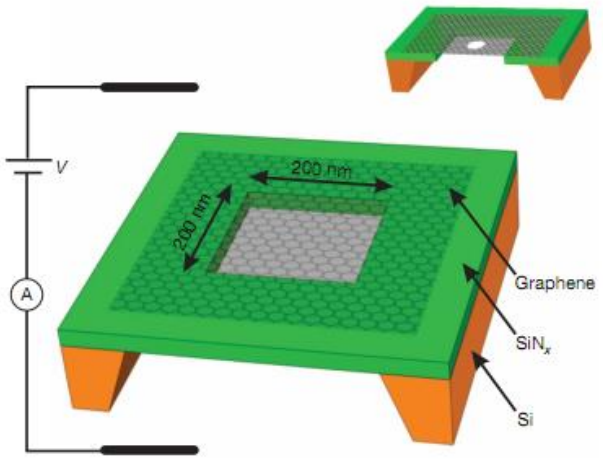


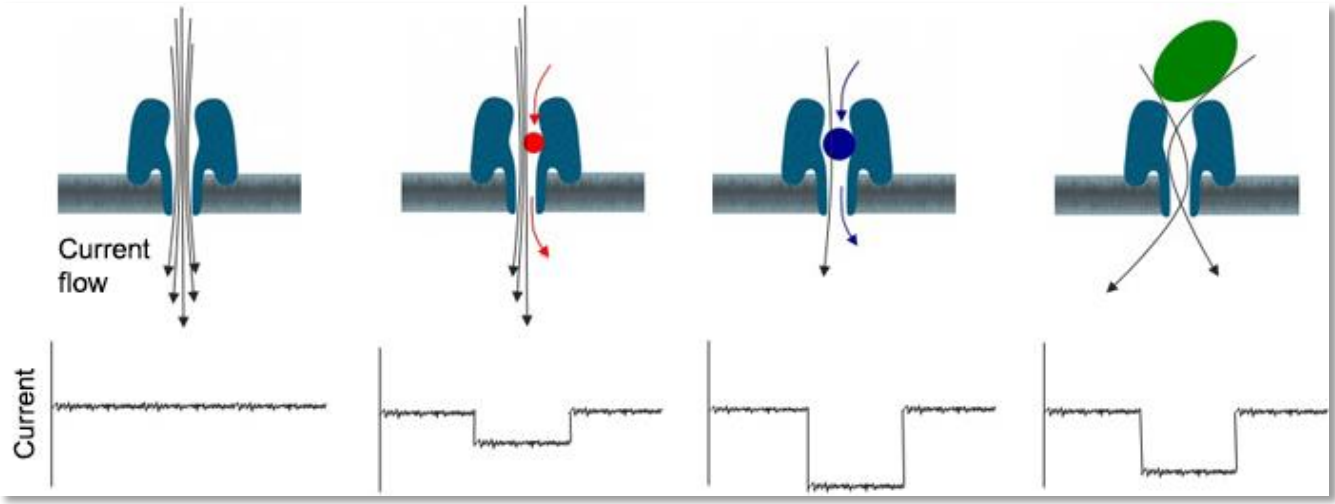
Figure 1 | Diagram of our experiments. A graphene membrane was mounted over a 200 × 200 nm aperture in SiN_x suspended across a Si frame (not to scale). The membrane separates two ionic solutions (not shown) in contact with Ag/AgCl electrodes (thick lines top and bottom, connected via a voltage source and a sensitive ammeter, A). Inset, cross-section through the Si frame, SiN_x aperture, and the graphene membrane through which a nanopore has been drilled.

Graphene as a subnanometre trans-electrode membrane, Garaj S et al., Nature 467 (7312), 190–193 (2010)

➢ Nanopore sensing

A nanopore may be used to identify a target analyte as follows:

- A protein nanopore set in an electrically resistant membrane bilayer. An **ionic current** is passed through the nanopore **by setting a voltage** across this membrane.

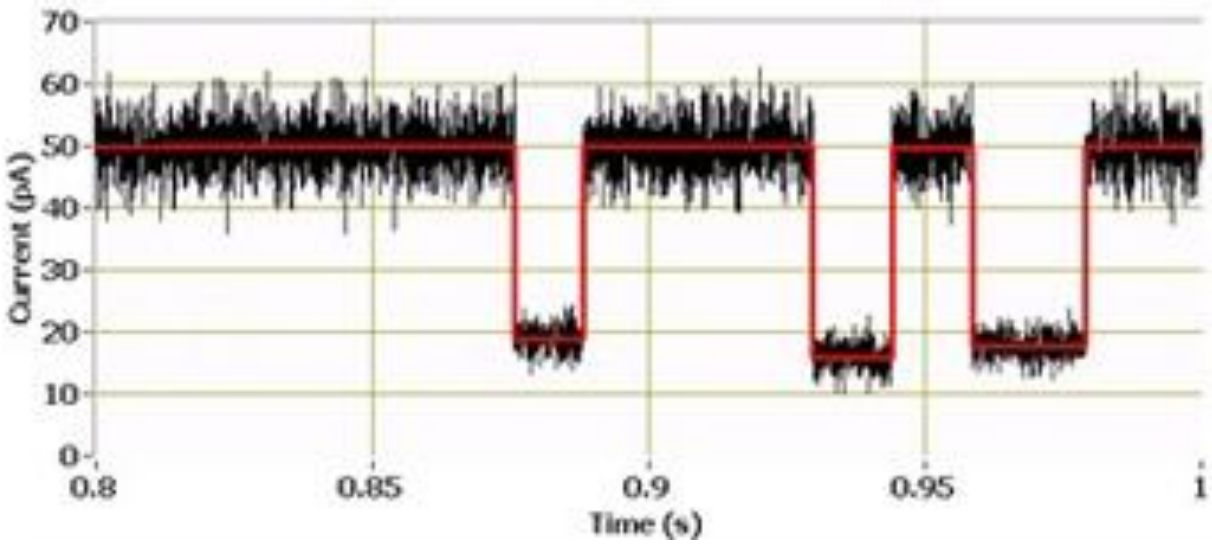


- **Electrical signature** : If an analyte passes through the pore or near its aperture, this event creates **a characteristic disruption in current**. Measurement of that current makes it possible to identify the molecule in question.

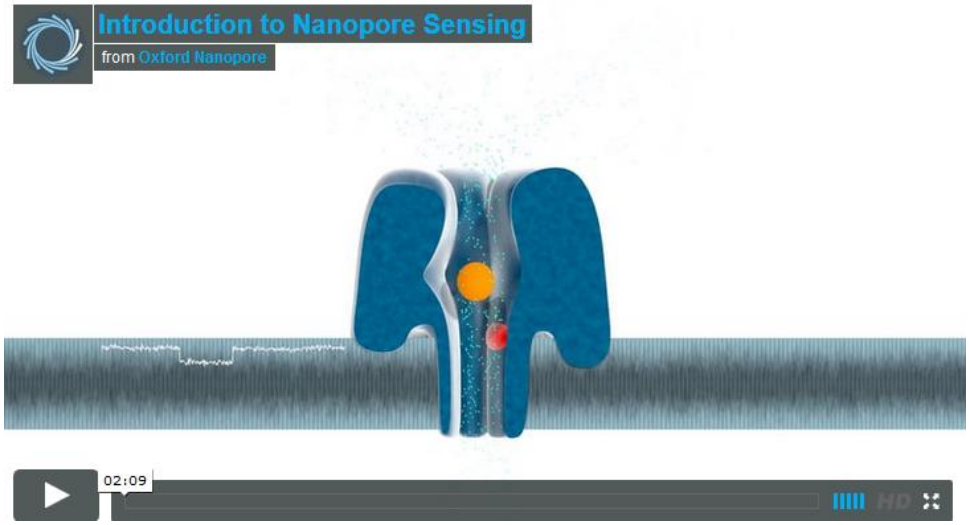
For example, this system can be used to **distinguish between the four standard DNA bases G, A, T and C, and also modified bases**. It can be used to identify target **proteins and small molecules,** or to gain **rich molecular information**, for example to distinguish between the enantiomers of ibuprofen or study molecular binding dynamics

A typical single nanopore 'trace' ('squiggle') as measured in a single nanopore experiment with a single analyte.

- The current is measured at very high frequency
- Each point on the black line represents a single measurement.
- As an analyte interacts with the nanopore the current is disrupted, as shown by the stepped drops in current – this is an 'event'.
- 'Data reduction' is shown as a red line overlaid onto the squiggle.



A series of electrical signals, recorded at a rate of tens of thousands per second


Introduction to Nanopore Sensing
from Oxford Nanopore
02:09
HD

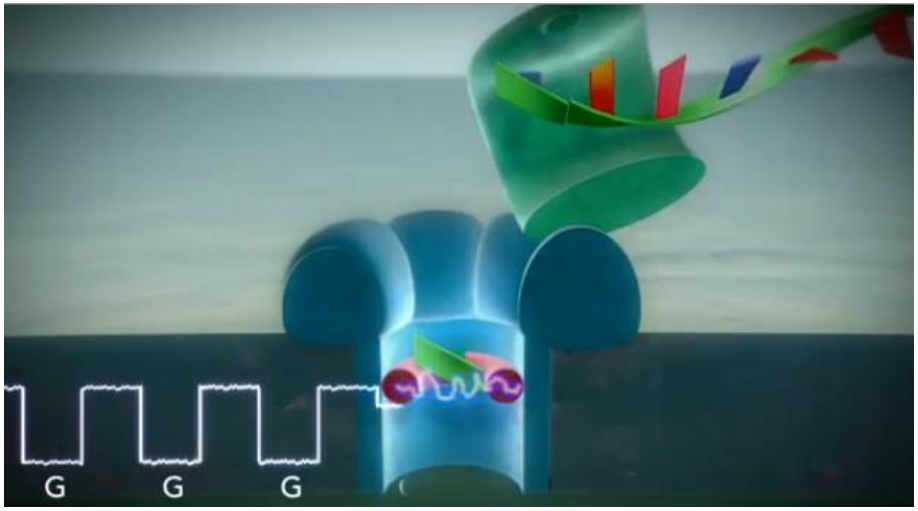
➢ Application to ‘strand’ DNA sequencing

'Strand sequencing' is a technique that passes intact DNA polymers through a protein nanopore, sequencing in real time as the DNA translocates the pore

Séquençage par dégradation : exonucléase

- Combinaison du Nanopore avec une exonucléase qui clive le brin d'ADN base par base.

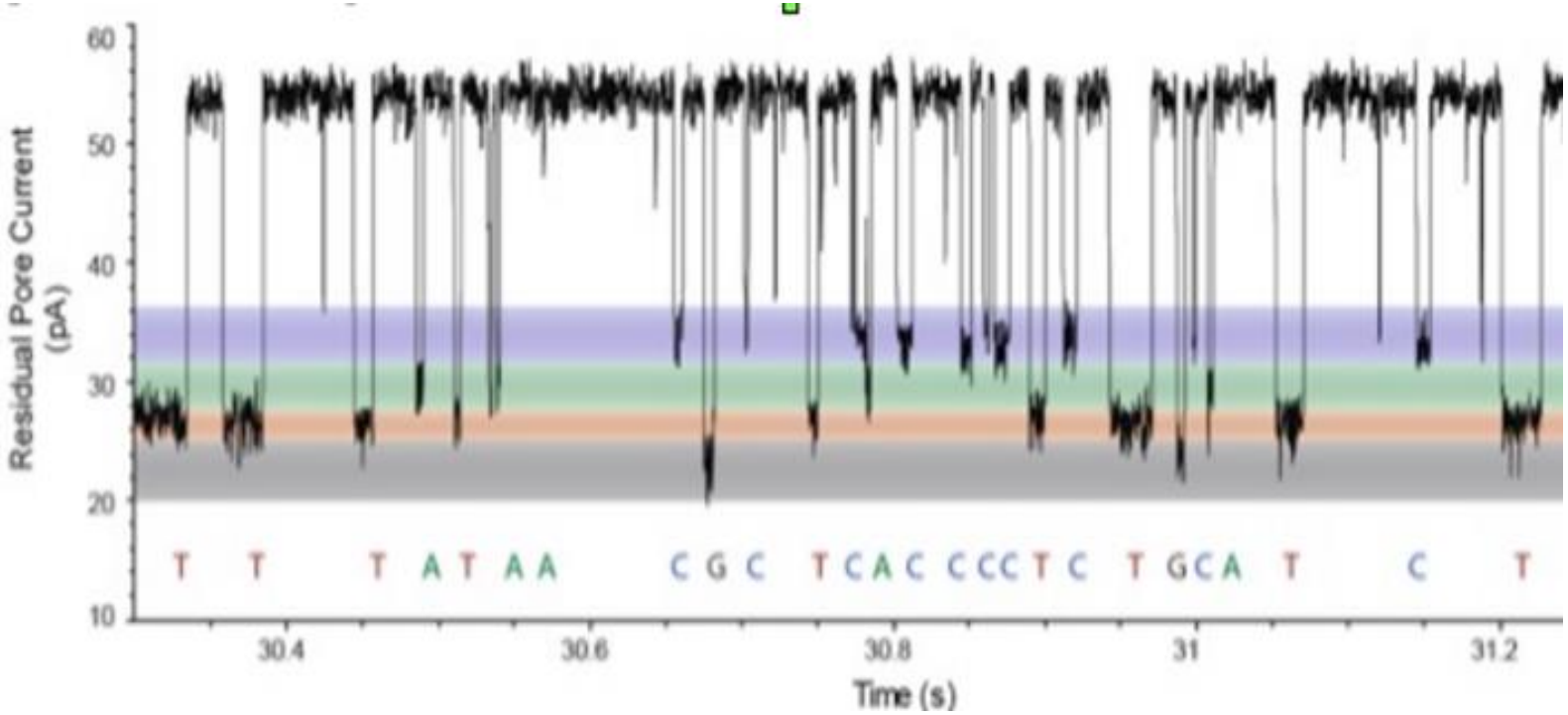
- La bicouche lipidique avec le nanopore forme 2 compartiments dans le puits qui contiennent chacun une électrode. L’échantillon d’ADN est placé dans le compartiment du haut, pris en charge par l’exonucléase et est clivé base par base.

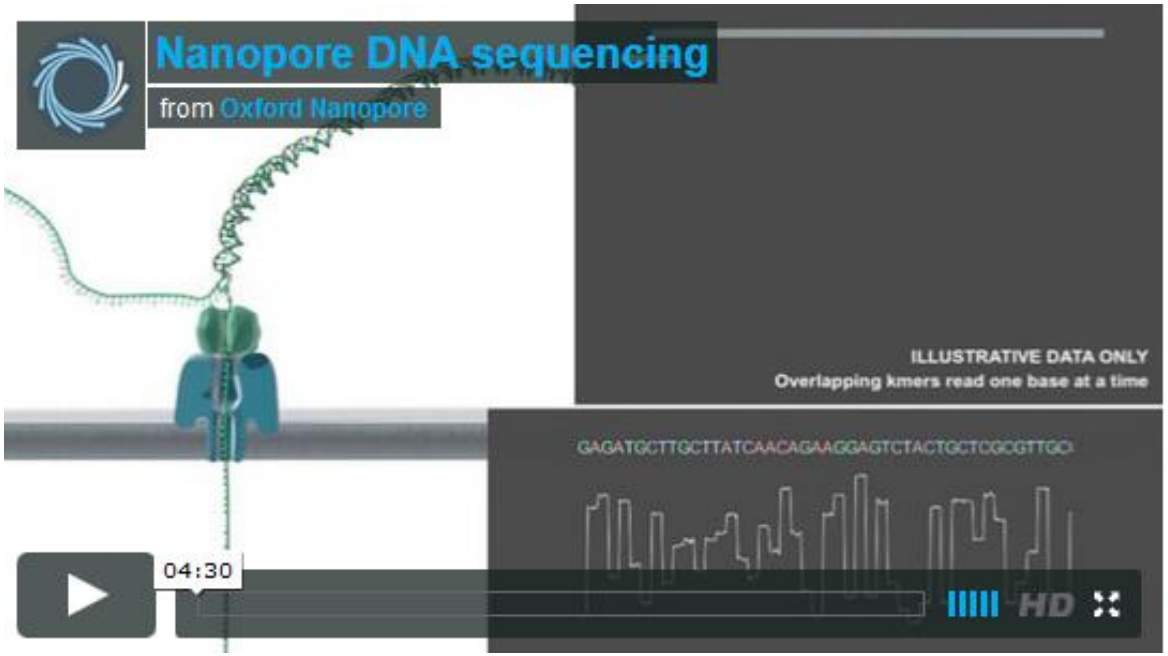


Signature électrique du passage de la base clivée par l'exonucléase dans le nanopore, G ici.

➢ Acquisition du signal signature

Les bases se fixent temporairement sur l'adaptateur cyclodextrine. Chaque passage d'une base à travers le pore entraîne un encombrement stérique dans le pore diminuant le flux d'électrolytes traduit par une diminution brutale de l'intensité électrique (de 55 pA à 20-35 pA). Le minimum d'intensité mesuré correspond à une base en particulier : **signature électrique**.




Nanopore DNA sequencing
from Oxford Nanopore
ILLUSTRATIVE DATA ONLY
Overlapping kmers read one base at a time
GAGATGCTTGCTTATCAACAGAAGGAGTCTACTGCTCGCGTTGCI
04:30
HD

Instrument : Nanopore Platform

Scaled according to need; USB device MinION™ or the desktop PromethION™.

MinION™

Real time biological analyses in a pocket-sized portable device

PromethION™

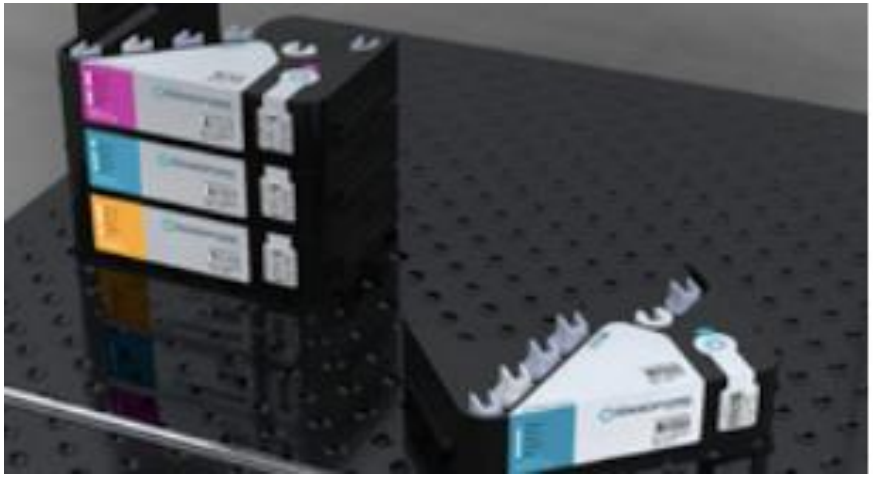
Benchtop high throughput analyses for one or many samples

GridION™

Modular installation for all scales of analyses


Scaling of nanopore devices: Oxford Nanopore
from Oxford Nanopore
04:32
HD

Des cartouches et des « nodes » en cluster



Une cartouche contient tous les réactifs nécessaires pour réaliser la réaction de séquençage. Elles sont usinées au préalable pour un type d'expérience et sont prêtes à l'emploi. On insert l'échantillon dans la cartouche puis la cartouche dans le « node.

Chaque **unité de base est appelée un « node »**. Chacun de ces « nodes » contient un lecteur et un système informatique autonome (hardware).

Un cluster de »nodes »

Technology within the GridION™ system

A single node as a desktop installation

Nodes may be clustered through a network for larger throughput installation

Each GridION node operates with a single disposable cartridge

- Nombre d'expérience en même temps : 2,000 nanopore experiments at one time (or more)
- Quantité de données générées : tens of Gb per day
- Longueur : tens of kb in a single read
- Recent announcement of accuracy of 96 % (bonne résolution des signatures électriques)
- Durée : There is no fixed run time; the system may be run for a short or long period of time according to the experimental need.
- Coût : "the cost per base will also be lower than other systems"
- No accompanying server will be needed as each node contains the required local computing hardware.
- Types de molécules : **séquençage ADN, ARN et protéines possibles + analyse de petites molécules (cartouches spécifiques)**

20 « nodes » in parallel to sequence a human genome at 15x in 15 minutes for less than $10/Gb (AGBT, Feb 2012)

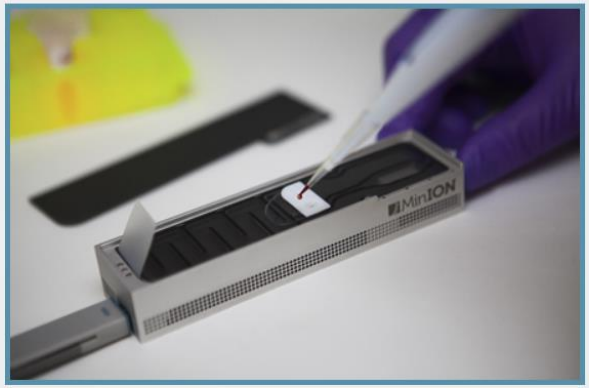
Données octobre 2013

Technology within the MinION™ system

The MinION device is a miniaturised single molecule analysis system, designed for single use and to work through the USB port of a laptop or desktop computer

The MinION device is designed to sense from complex samples such as blood/serum

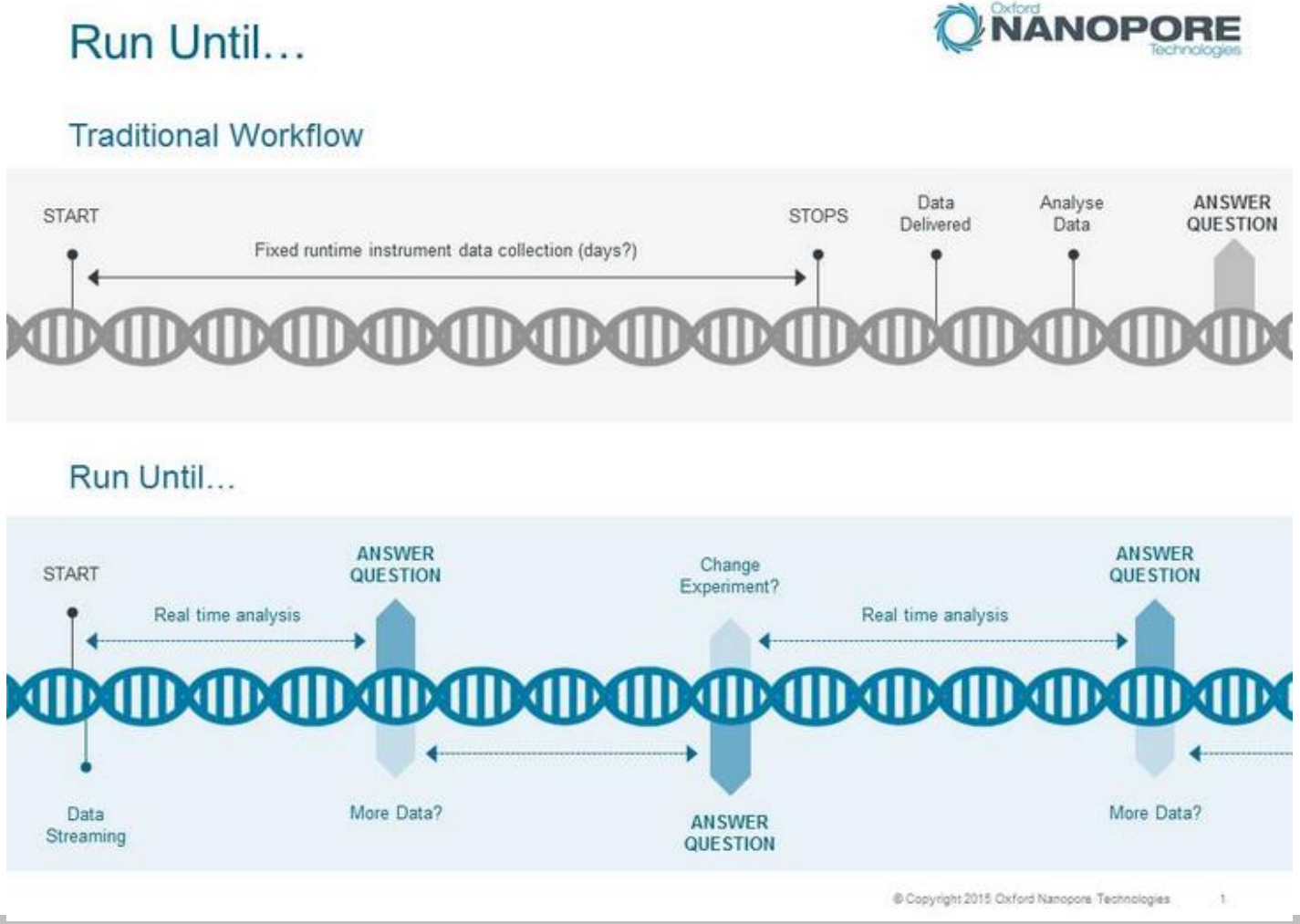
- 1Gb/day
- 500 nanopores
- A single MinION device is expected to retail at under $900
- MinION devices will be operable directly with a PC/laptop

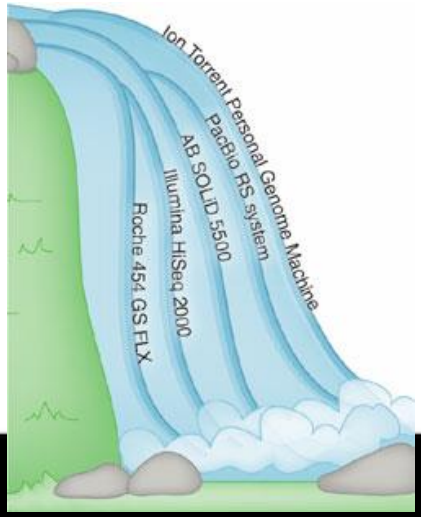


* all images are of an alpha prototype of the MinION device, final device expected to be at least half this size

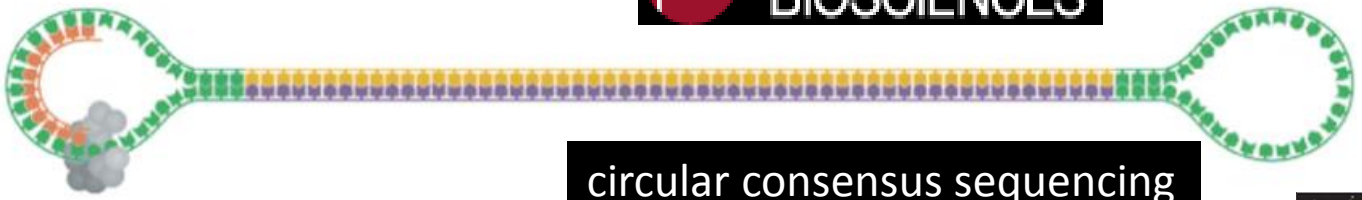
➢ Run until…

A key feature of the MinION™ device, the PromethION™ and the GridION™ system is that there is **no fixed run time**; a user can run any of the systems for a short or long period of time as data is streamed in real time. This can enable real-time analyses so that the user can predetermine an experimental endpoint and run the system for as long as it takes to collect sufficient data to address that question

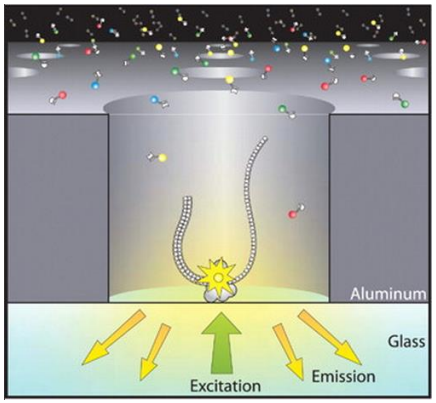




	Run Time	Sequence Length	Reads/Run	Total nucleotides sequenced per run
Capillary Sequencing (ABI37000) Sanger	~2.5 h	800 bp	386	0.308 Mb
454 Pyrosequencing (GS FLX Titanium XL+)	~23 h	700 bp	1,000,000	700 Mb (0.7 Gb)
Illumina (HiSeq X Ten)	72 h (3 days)	2 x 150 bp	6,000,000,000	1,600,000,000 Mb (1,600 Gb)
Illumina (MiSeq)	65 h	2 x 300 bp	2 x 22,000,000	13,500 Mb (13.5 Gb)
SOLID (5500xl system)	120 h (7 days)	2 x 60 bp	400,000,000	300,000 Mb (300 Gb)
Ion Torrent (Ion Proton I)	2 h	200 bp	60,000,000	10,000 Mb (10 Gb)
PacBio (PacBioRS II)	1.5 h	~8,500 bp	50,000	375 Mb (0.37 Gb)



circular consensus sequencing

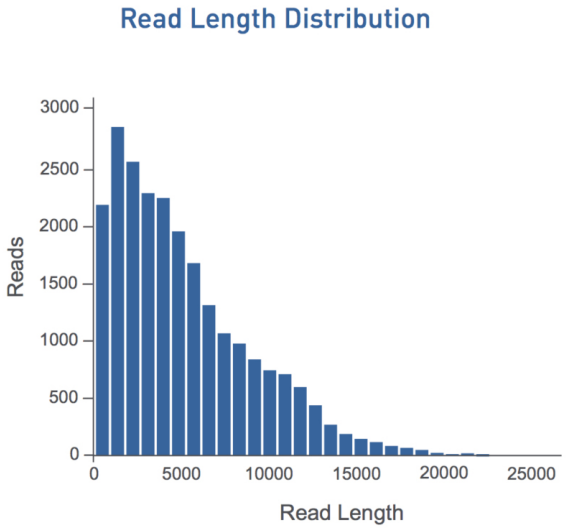


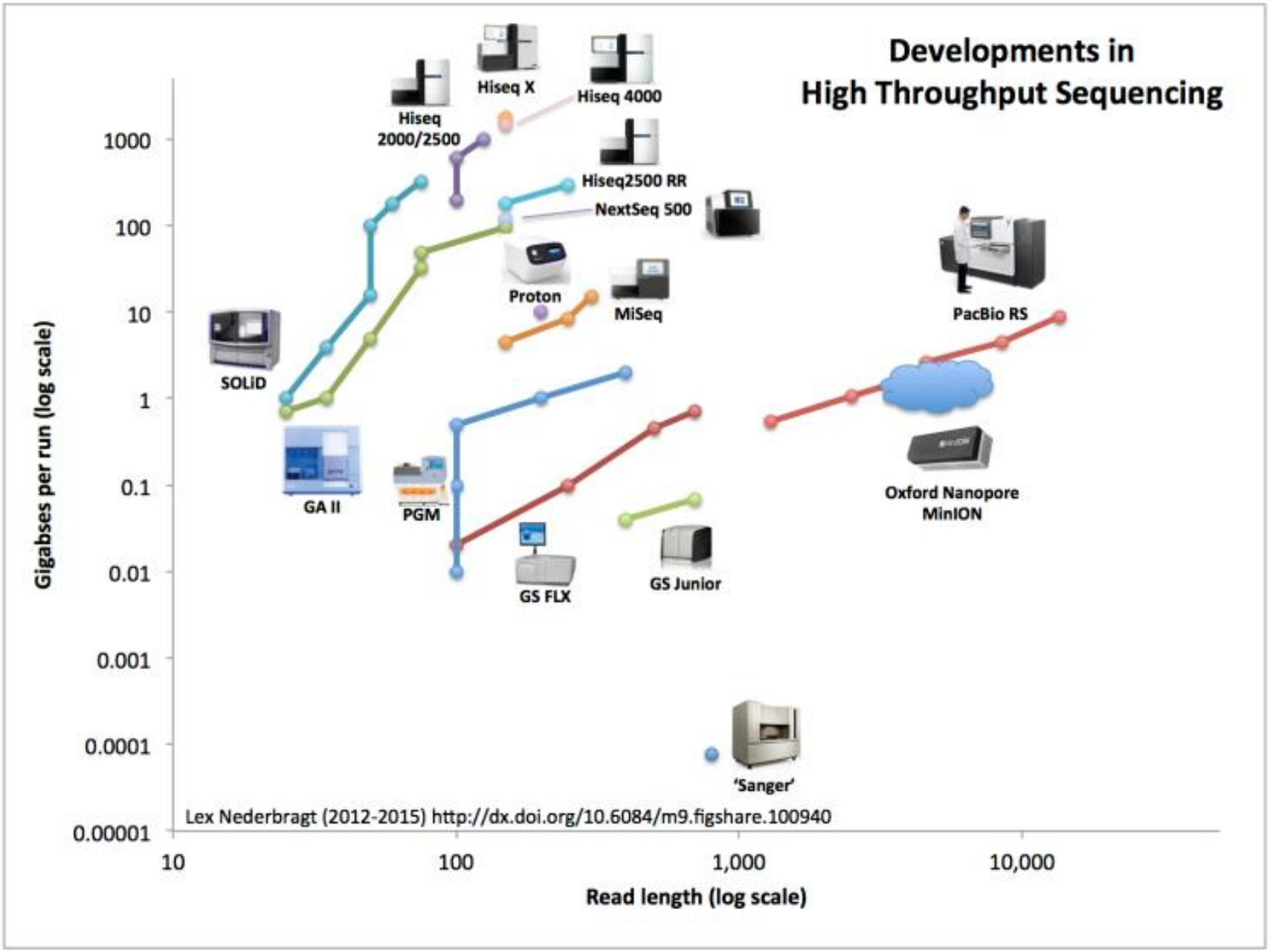
- Schematic representation of the **SMRTBell template** for PacBio sequencing : ligating hairpin adaptors to both ends of double-stranded DNA molecules. From http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2926623/

- **Very long reads**

- **High error rate** > 10%

- CCS ; but the multi-pass sequencing allows for **calling a consensus** of the sequence of the insert, overcoming the high single-pass error-rate of the technology





https://flxlexblog.wordpress.com/

Les plateformes de 3è génération ou NNGS : **SMRT and/or non optical detection**

- pas de clonage en vecteur
- amplification clonale sur support pré-séquençage
- fragment courts, jusqu'à 1000b
- temps de séquençage en jours
- séquençage par synthèse
- signal fluorescent ou lumineux

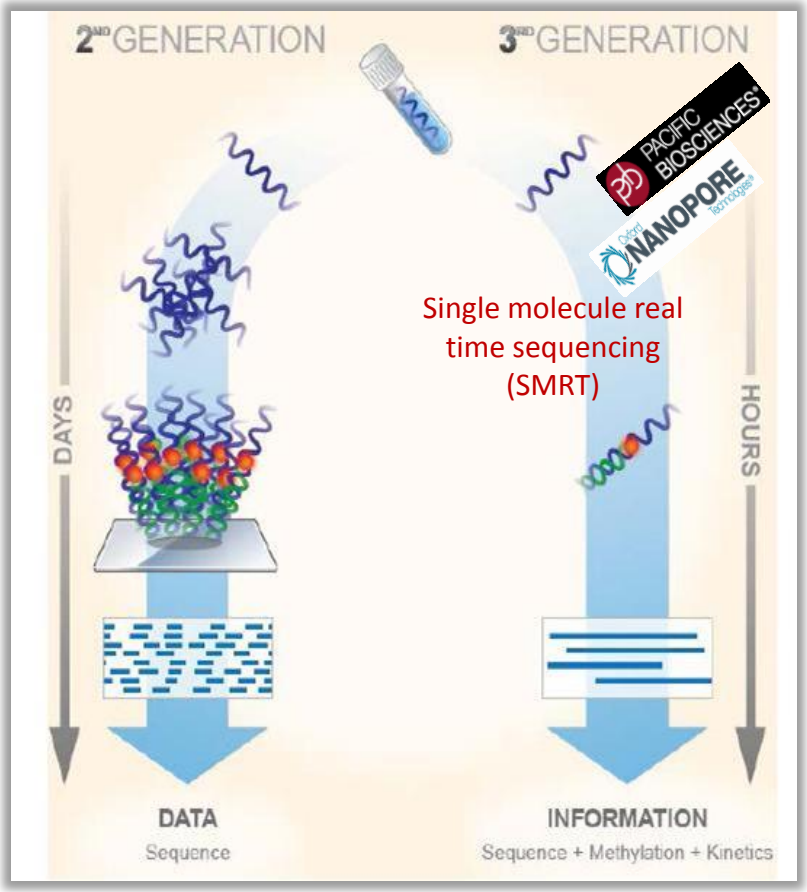
- pas de clonage en vecteur
- pas d'amplification (SMRT) Pacbio et Helicos †, Nanopore
- de 200b à 10.000kb ("run until")
- temps de séquençage en heures
- séquençage par synthèse ou dégradation
- signal fluorescent ou électrique

Synthèse

life technologies | applied biosystems

illumina

454 SEQUENCING | Roche



Synthèse

PACIFIC BIOSCIENCES

Helicos BioSciences Corporation

SMRT fluorescence

life technologies | ion torrent — Semiconductor Sequencing for Life™

courant

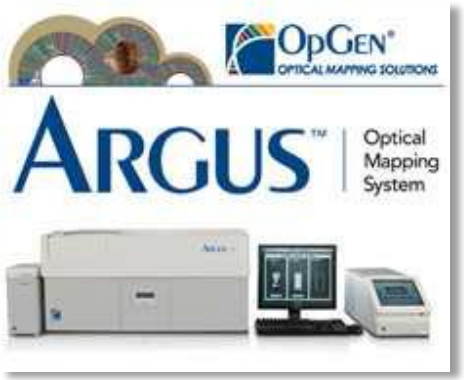
Dégradation

SMRT courant

Oxford NANOPORE Technologies

Helicos BioSciences Corporation
LaserGen, Inc.
OpGen
mobious biosystems
ELECTRON OPTICA
BIONANO GENOMICS
Electronic BIOSCIENCES
gnubio
LIGHTSPEED GENOMICS
NABsys
STRATOS genomics inc.
dnae
Reveo
IBM
Noblegen
Complete genomics
genia
HALCYON MOLECULAR
QuantuMD
ZS Genetics, Inc. Working At The Scale Of Life
NANOPORE

4. Next-Generation Mapping (NGM)

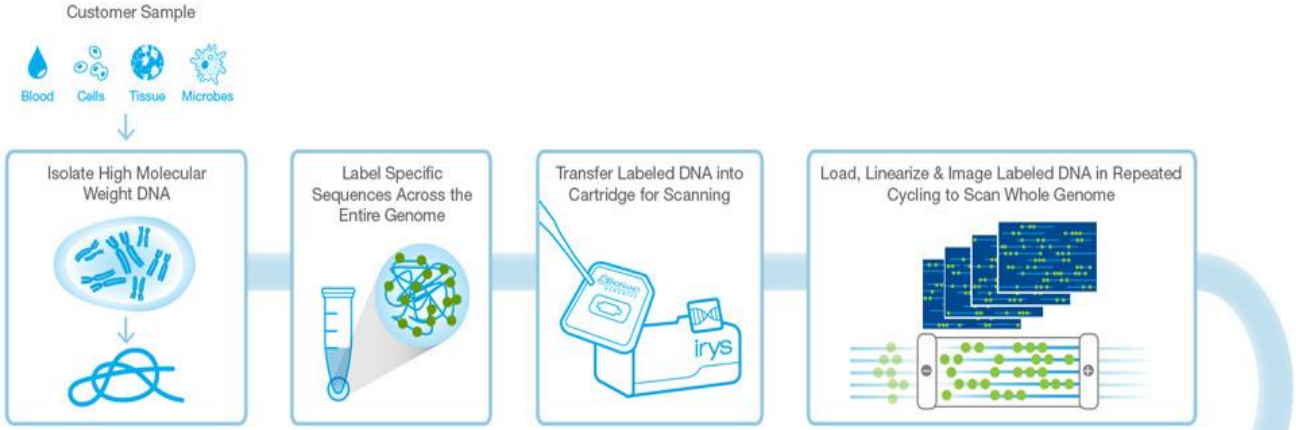


BIONANO GENOMICS

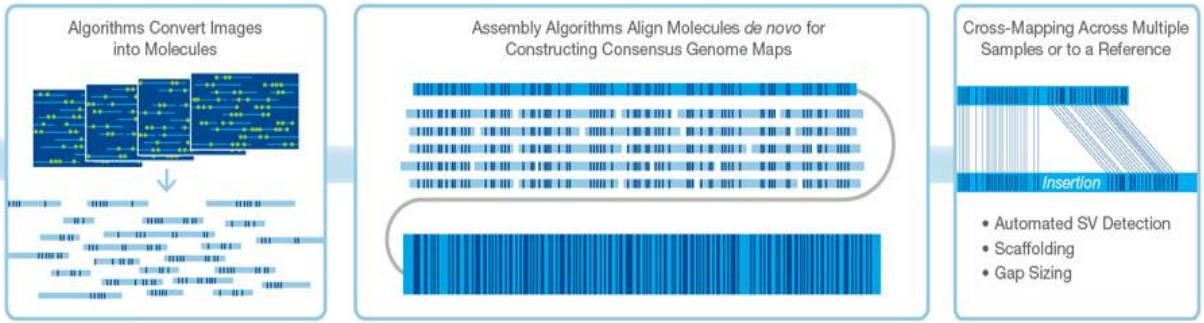
THE MAP IS BACK

Irys® single-molecule next-generation mapping (NGM) brings physical genome maps back to your lab. Accelerate your research by rapidly generating whole genome maps for any organism.

Learn More

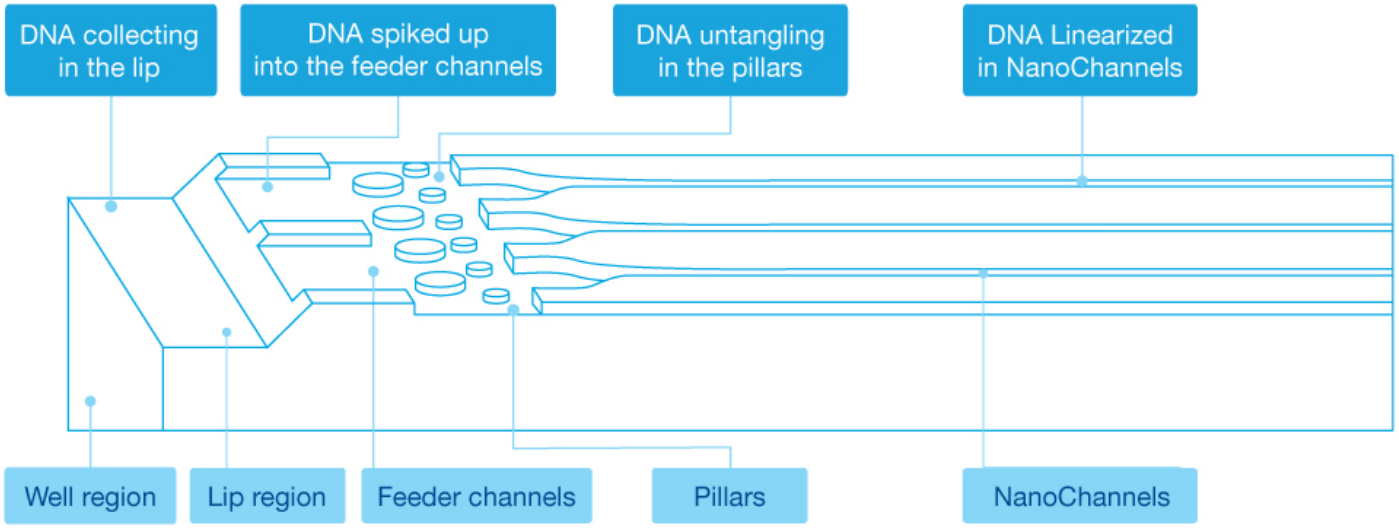


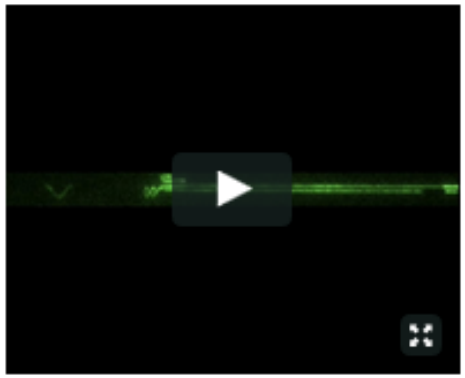
High-Throughput, High-Resolution Imaging Gives Contiguous Reads up to Mb Length



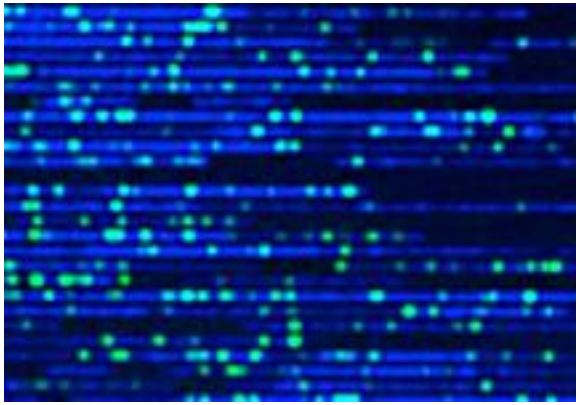

irysPrep™
Reagents
+
irysChip™
Consumable
+
irys™
Instrument
+
irysView™
Software

irysChip™ Schematic





Molecule Linearizing in NanoChannel (400 kb)



- A **Whole Genome Map (WGM)** is a high-resolution, ordered, restriction map that spans the genome.
- Resembling a barcode, the Whole Genome Map is unique to the organism and provides a complete, **structural view of the genome** that reveals its architecture in a single, easy-to-interpret image.

Applications

1. Whole genome mapping
2. Comparative genomics
3. Strain typing
4. Physical map position and correction
5. Structural variation analysis

A New Paradigm for Reference Genome Assembly

- Joins scaffolds to close gaps–more complete view for comparative genomics studies
- Defines order and orientation for more than 90% of sequence scaffolds–more confidence in accuracy
- Completes assembly in 2 weeks–faster projects and publications

especially for larger genomes–plants, animals and humans.

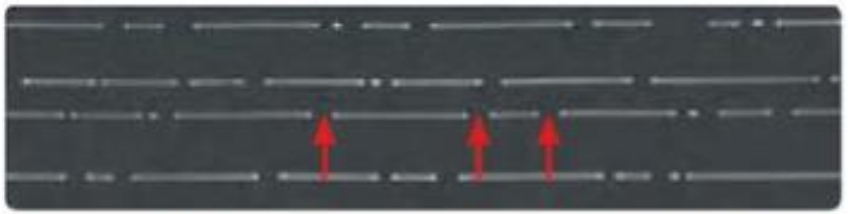
1. Extract

Using OpGen's optimized DNA extraction kit, high molecular weight DNA needed to produce Whole Genome Maps can be extracted in as little as two hours.

2. Immobilize & Digest

Single DNA molecules are flowed through microfluidic channels and immobilized on a charged glass surface. The immobilized DNA is digested, maintaining the fragment order.

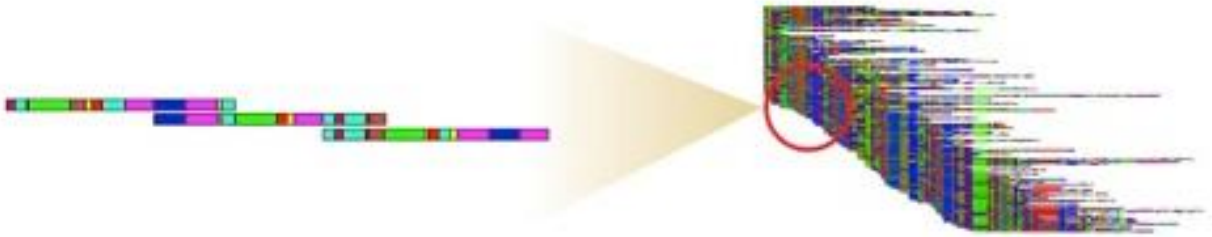
Genomic DNA, captured as single DNA molecules from random breakage of intact chromosomes, is loaded into microchannels, immobilized electrostatically, then digested with a restriction endonuclease.



Digestion reveals cleavage sites. The restriction fragment order is maintained for each molecule.

3. Measure & Assemble

The DNA fragments are stained with fluorescent dye; fragment length is proportional to fluorescence intensity. By overlapping fragment patterns, the single-molecule maps are assembled to produce a Whole Genome Map that provides a minimum 30 X coverage.

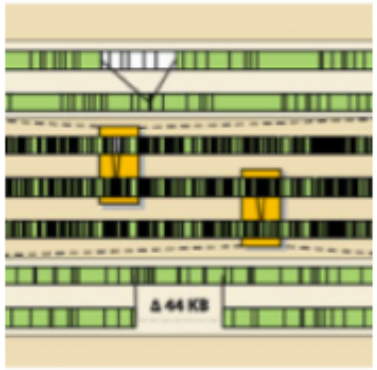


Overlapping Single-molecule Maps are assembled to produce a highly accurate, Whole Genome Map. The red circle indicates 30x coverage, ensuring accuracy of the consensus Whole Genome Map.

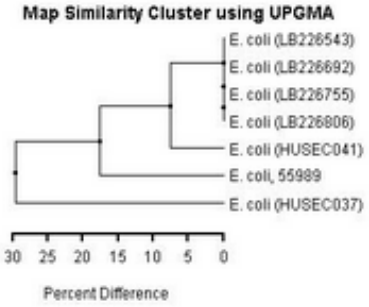
4. Analyze

The MapSolver™ Analysis tool provides powerful features to evaluate and compare Whole Genome Maps. Discover genetic variation, perform high-resolution epidemiology, or accelerate whole-genome sequencing.

- View and compare genomes
- Identify important genetic variations
- Upload sequence contig data to align contigs against an ordered whole-genome scaffold
- Use the Whole Genome Map as an independent source to guide sequence placement, confirm assemblies, identify misassemblies and gaps, and finish *de novo* sequences

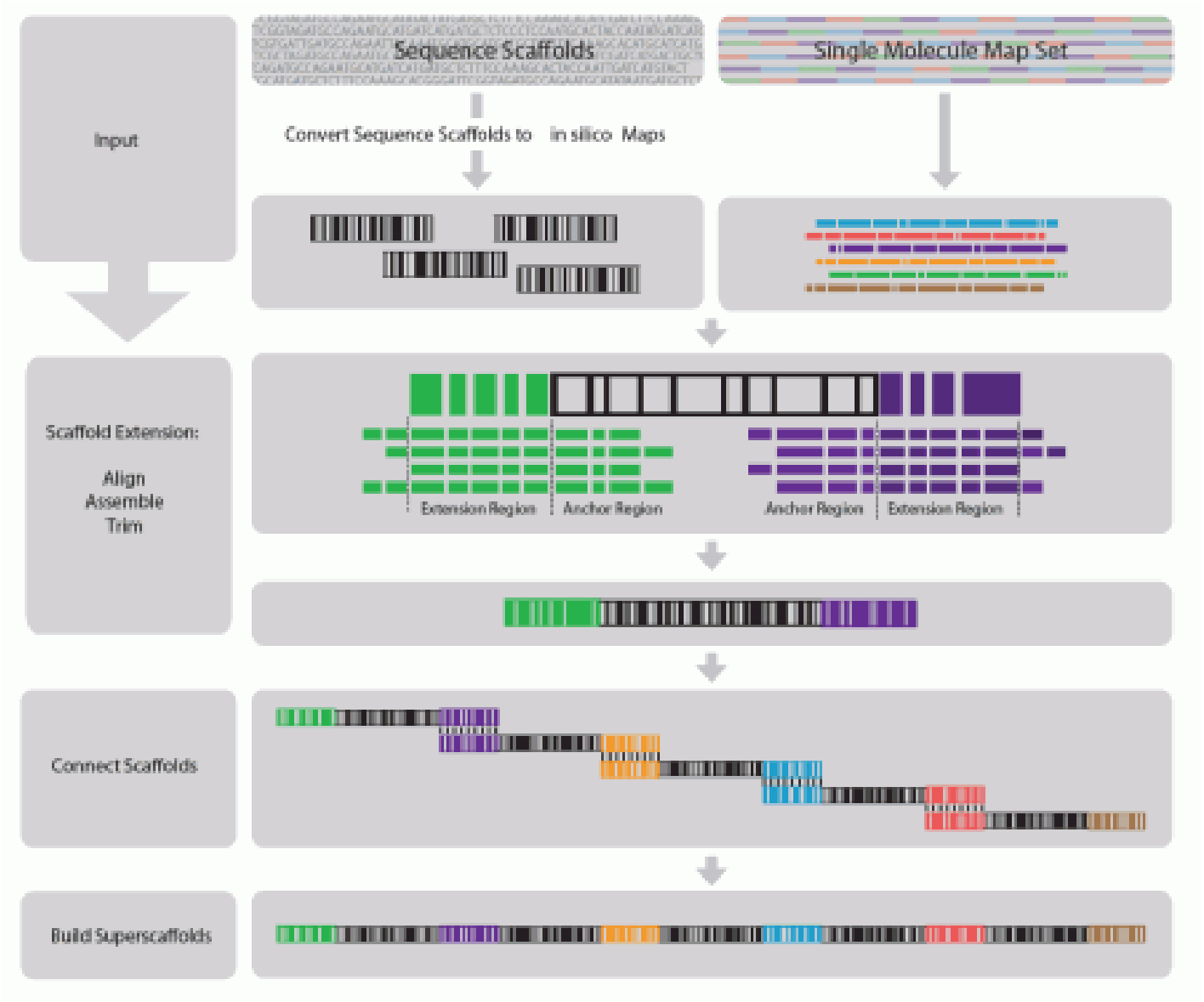


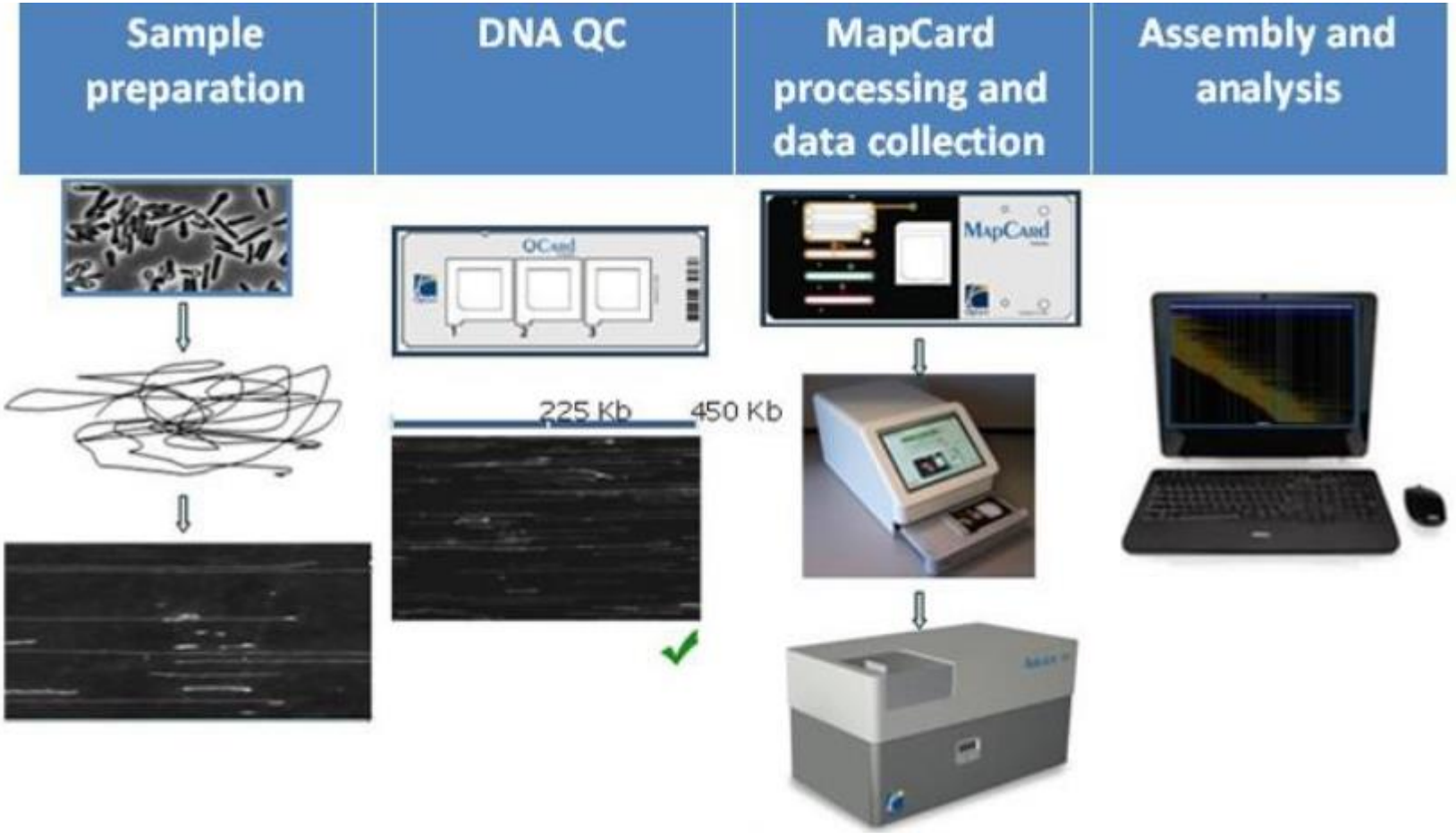
Directly compare maps to discover insertions, deletions, and other genetic elements that other technologies miss. Differences and similarities are highlighted for at-a-glance discovery.



Map-based similarity clustering accurately distinguishes strains and describes relatedness between isolates.

Align sequence contigs with Whole Genome Maps to quickly orient contigs, locate gaps and correct misassemblies.


Input
Sequence Scaffolds
Single Molecule Map Set
Convert Sequence Scaffolds to in silico Maps
Scaffold Extension:
Align
Assemble
Trim
Extension Region
Anchor Region
Anchor Region
Extension Region
Connect Scaffolds
Build Superscaffolds



- Whole Genome Maps are generated from single DNA molecules, so they are de novo, independent of sequence information, and *do not require amplification or PCR steps*.
  - Intact single molecule DNA (250kb-2.5Mbp) is collected and analyzed to create a high definition map of the genome. Important positional changes are evident that sequencing alone cannot detect.
  - Thousands of single molecule restriction map reads are assembled to create the Whole Genome Map.

Genomics

I. Whole genome sequence acquisition

From Sanger to NGS

1. Whole genome sequencing : different strategies
2. Genome data resources: Genome Browsers, reference genome databases
3. NGS technologies
4. DNA-seq applications