



GeT
Génome et
Transcriptome

Toulouse Genomic Core facility

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Next Generation Sequencing Lab Manager

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<http://get.genotoul.fr>

@GeT_Genotoul

Who are we?

- **Genomics and transcriptomics core facility spreads on 5 sites GeT in Toulouse**
- **National Infrastructure within the « France Génomique » program**
- **IBISA Label**
- **INRA strategic core-facility**
- **ISO9001 et NFX50-900 Certification**



The Core facility missions

- **To provide innovating technologies for genome analysis to the scientific community**
 - Sequencing / Genotyping
 - Gene expression
- **To Develop new protocols, new methodologies, acquire expertise and train in those technologies**
- **To animate workshop for user network**



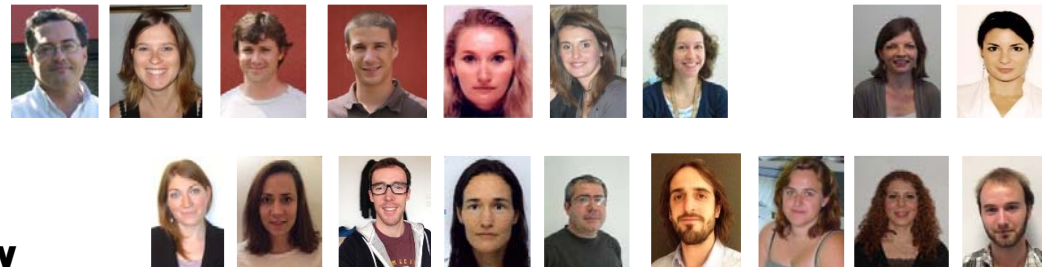
Team and Expertise

- **A team of 30 peoples with:**

- **Competence in biology, bioinformatics, biostatistics**
- **Experts in Agronomy, Environment, Microbiology, Health**
- **Technical Specialty and scientific community by site**

- **GeT- PlaGe (INRA Auzeville):**

- **NGS, 2nd et 3rd Generation**
- **Bioinformatics**
- **Agronomy, Environment, Ecology**



- **GeT-Biopuces (INSA Ranguueil):**

- **Microarray, NGS 2nd Generation**
- **Bioinformatics, Biostatistics**
- **Microbiology**



Team and Expertise

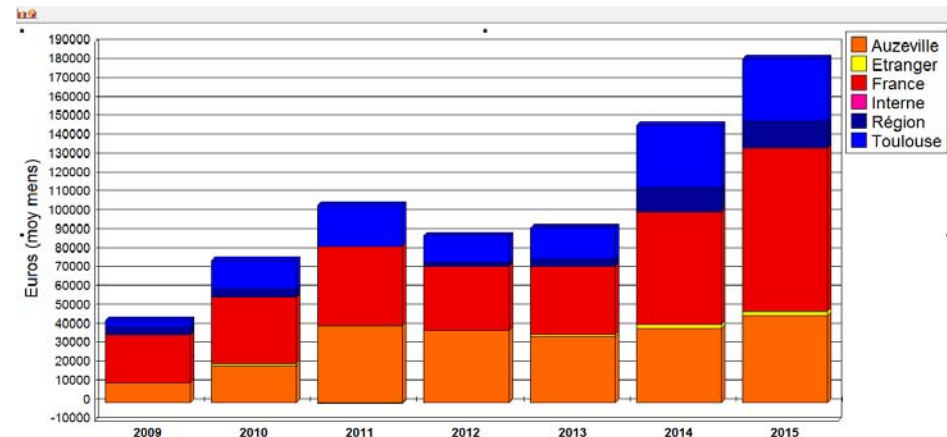
- **GeT-TRiX (INRA Saint Martin) :**
 - **Microarray,**
 - **Bioinformatics, Biostatistics**
 - **Toxicology**
- **GeT-TQ (CHU Rangueil):**
 - **QPCR, single cell,**
 - **Health**
- **GeT-Purpan (CHU Purpan) :**
 - **Sanger, NGS 2nd Generation**
 - **Health**



ACTIVITY

Activity per year

- **15 R&D projects**
 - Mate paired, chipseq, methylation, GBS, 3G NGS, capture, HiC...
- **More than 100 laboratories (INRA, CNRS, INSA, INSERM, CHU, CIRAD ...)**
 - More than 160 research teams
 - More than 250 projects
 - 2M€ of activity



Collaboration and partnership

- **17 projects in collaboration (France – Europe)**

- **CARTOSEQ** (2010-2014) INRA : Identification en masse des variants génétiques influençant les caractères d'élevage chez les trois principales races laitières françaises
- **FUNHYMAT** (2011-2015) Université Pau: Structure et fonctionnement de tapis microbiens contaminés avec des hydrocarbures
- **Domestichick** (2013-2016) INRA: De la génomique du genre Gallus à l'histoire de la domestication du poulet
- **EFFECTOORES** (2013-2016) : Exploitation des connaissances sur les effecteurs des Oomycetes pour la recherche de résistances durables aux maladies chez les plantes cultivées
- **IMPACT** (2014-2016) INRA: Identification of Matrix Proteins Affecting CalciteTexture in chicken and guinea fowls eggshells
- **PigLetBiota** (2014-2019): Une étude de biologie intégrative de l'influence du microbiote intestinal sur la robustesse des porcelets
- **AgENCODE** (2015-2016): a French pilot project to enrich the annotation of livestock genomes
- **Treasure** (2015-2019- H2020): Treatment and Sustainable Reuse of Effluents in semiarid climates
- **Bovano** (2015-2017): IDENTIFICATION AND FUNCTIONAL STUDY OF CATTLE DELETERIOUS MUTATIONS
- **Feed-A-Gene** (2015-2020 – H2020) : projet européen pour améliorer l'efficacité alimentaire des monogastriques
- **Vaisseaux et Cancer** (2013-2016 - INCA) : caractérisation moléculaire des vaisseaux qui contribuent à l'inhibition de la croissance tumorale
- **SELGEN - GenSSeq**: développement et la mise en œuvre des méthodes à haut débit permettant d'estimer la valeur génétique des animaux et végétaux
- **Agri-Métatranscriptomique** – diversité: Nouvelles perspectives dans l'étude des communautés microbiennes complexes
- **HeliOr** (2015-2018) : Séquençage du Génome de l'Orobanche et 2^{ème} génotype Tournesol dans le cadre de SUNRISE
- **Meta-Pac** (2015-2016): Mise au point des analyses de Metagénomique long read
- **B-TB** (2013 – 2016 ANR) Role of B cells tuberculosis immunity and inflammation.



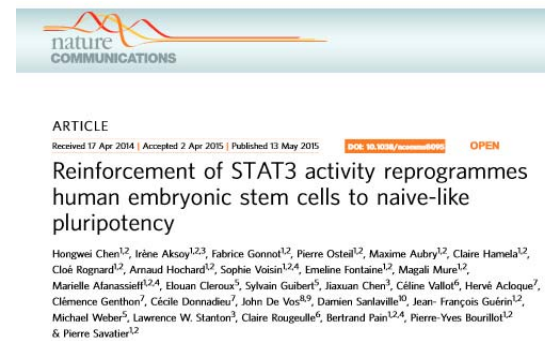
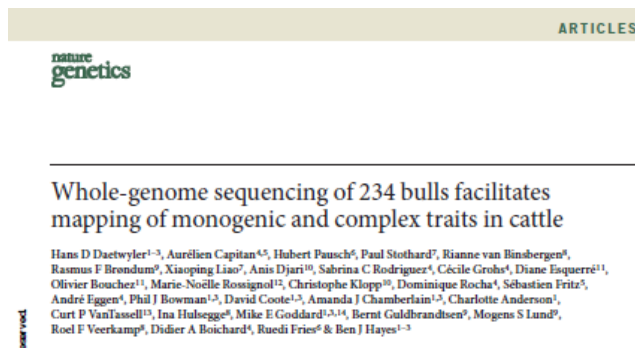
Collaboration and partnership

- **46 publications as co-author since 2012**

2012 (14) : PLoS ONE, NAR, Gene, Current Biology, Developmental and Comparative Immunology, Gene 500, Ecotoxicology, N, Biochimieew Phytologist, FEMS, Ecotoxicology, Nucleic Acids Research, PLOS Genetics, Hepatology

2013 (13) : Biotechnology for biofuel, BMC Genomics, Mol Ecol ressources, infection genetics and evolution, PLoS pathogene, BMC, molecular ecology, genome amouncements, molecular phyloenetics and evolution, FEMS, Gastroenterology & Hepatology, Biochem Biophys Res Commun, Front. Microbio

2014 (19): nature genetics, BMC Genomics, JAS, Exp and molecular pathology, Gene Nar, Molecular Biology ressources, EMBOJ, Emerging Infectious Diseases, molecular cell, PLoS ONE, PLoS Biol, Poultry science, FEMS, Biochemistry & Molecular Biology, Toxicology, Medecine, Research & Experimental, J Am Soc Nephrol, Pathologie Biologie, Journal of Biotechnology



TECHNOLOGIES

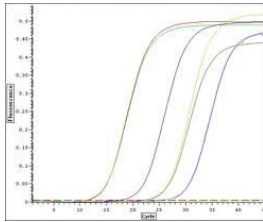
Tools to improve the activity

- **Sample and library quality controls**
- **Single cell capture (C1 Fluidigm)**
- **Pipetting platforms for sample preparation**
 - Partnership with Tecan (4 Evo), Agilent Bravo
 - Access array (fluidigm)



Tools to analyse gene expression and to genotype

PCR quantitative



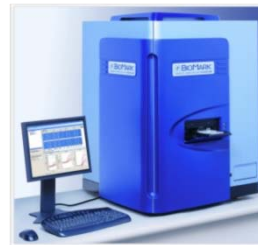
**ViiA7, QuantStudio,
ABI7900HT, ...**



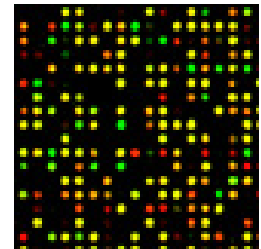
PCR quantitative microfluidic



BioMark (Fluidigm)



Microarray



**Affymetrix – Agilent
iScan**



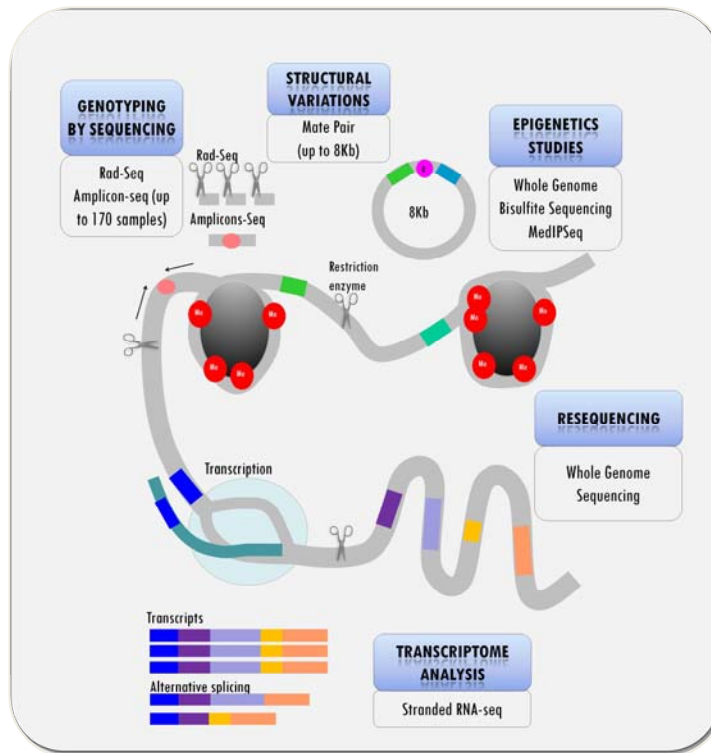
From Sanger to short read NGS revolution



400 pb
1Gb



200 pb
13 Gb



2x 300 pb
15 Gb



2x150 pb
700 Gb

From 1 human genome sequencing to....

... « 1000 genomes » projects for all species

From NGS short reads to long reads

- **First PACBIO installed in France**
- **SUNRISE project to acquire expertise**
 - **To validate quality of DNA**
 - **To improve library preparations**
 - **To increase the number of reads**
 - **To increase the length of reads**



IGM (San Diegao, USA)	202 SMR cells							
		NUM	MAX	N50 BP	N50 NUM	MEAN	MEDIAN	BP/SMRTcell
	moyenne	98666	45457	12211	28413	9176	9032	0,906 M
	max	146374	52725	12981	41602	9997	9809	1,36Gb
Lausanne University (Swiss)	59 SMRT cells							
	moyenne	106800	46800	15172	28371	10773	9821	1,15Gb
	max	144358	53253	16132	38325	11436	10568	1,6GB
Get-PlaGe (France)	146 SMRT cells							
	moyenne	77086,6301	52317,4932	15365,4795	19705,5822	10326,6773	9152,5137	800Mb
	max	126777	80974	20507	33133	13635	12295	1,3Gb

Improvements of the molecular biology steps have increased the length of the Pacio Sequences
 (B. Mayjonade)

From NGS short reads to long reads

- **Fifteen projects to develop new applications :**
 - **Whole genome sequencing on different species**
 - **Targeted sequencing**
 - **Complex population & metagenomics**
 - **RNA sequencing**
 - **Epigenetic**





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Focus on Next Generation Sequencing

From Sanger to 3rd generation

Unique sequence

Sanger



16 or 48 capillaries

Next Generation Sequencing

NGS - short reads

Ion Torrent

Ion PGM
2 Gb
400 pb



Ion S5
15 Gb
200 pb



Illumina

2xHiSeq 3000
700 Gb
2 x 150 pb



3 x MiSeq
15 Gb
2 x 300 pb



3G - long reads

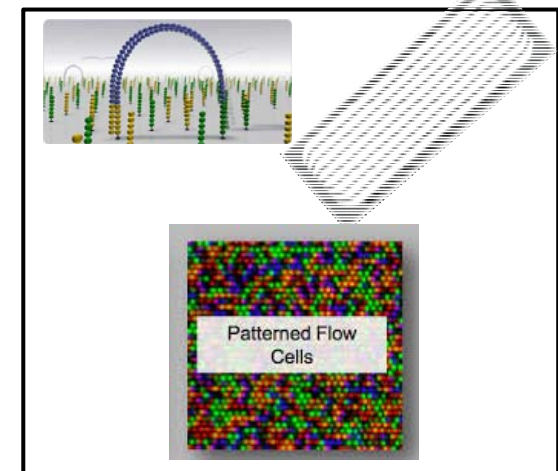
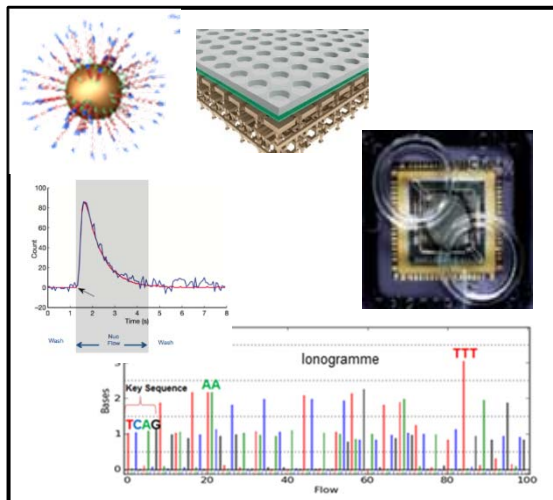
PacBio RsII
70 000 reads - 20 kb



NGS : Ion Torrent et Illumina



Ion Torrent	Illumina
Scalable: different chips ↔ output: 50 Mb to 15 Gb	Output: 2 Gb to 90 Gb / lane
Reads : 1 x 200 pb or 1 x 400 pb	Reads: 1 x 50 pb to 2 x 300 pb
Bioinformatic tools pre-defined	Many developpements ongoing Data stored on the bioformatic core facility's clusters
New: S5 sequencer	New: 2nd HiSeq3000



3rd generation: PacBio RsII

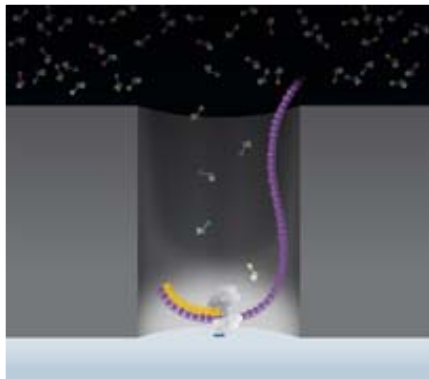


- + Single molecule real time sequencing
- + No PCR
- + Reads: 15-20 kb \Rightarrow 40 kb

- High error rate (>10%)

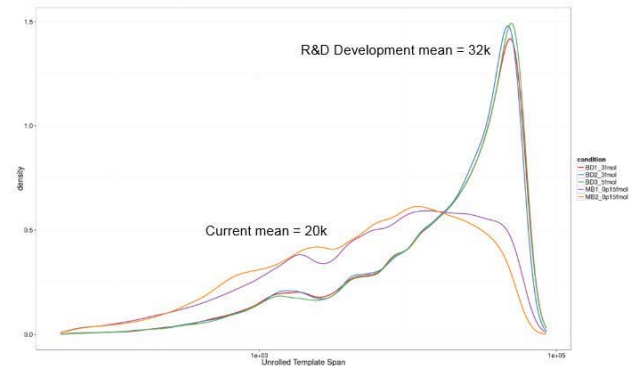


Random errors \Rightarrow corrected thanks to sequencing depth




 PACBIO

SAMPLE DATA FROM R&D



Targeting 2016 Release

NGS: which applications ?

Genomics Re-Sequencing

- **Whole Genome**
- **Whole Exome**
- **Gene panels**
- **Hotspots**

Transcritomics Expression

- **Whole transcriptome**
- **Full Length Transcripts**
- **Small RNA**
- **Targeted RNAseq**

Epigenetics

- **Whole Genome**
- **Targeted**

Metagenomics

- **16S**
- **Amplicons**

Genomics – Re-sequencing/ *de novo* sequencing

➤ Whole Genome

Available:

- "PCR-free " librairies for Illumina
- 1 HiSeq3000 run = 8 genomes at 30X \approx 2,5 days
- Ion S5 for small genomes
- GBS

⇒ mutations screening

Other applications :

- Sequencing on PacBio RSII: 100 SMRT cells \Leftrightarrow 3 Gb genome at 30X
- Sequencing on PacBio RSII: 1 SMRT cell \Leftrightarrow 1 bacterial genome at 100X

⇒ *De novo* sequencing, screening for chromosome modifications, haplotype detection, SNP identification...

Illumina HiSeq 3000



PacBio RSII



Ion S5



Genomics – Re-sequencing

➤ Whole Exome (human)

⇒ Mutation/Biomarker identification

- **Under development : AmpliSeq Exome Kit (Life Tech)**
 - + : easy and fast, cheaper than capture,
Dedicated bioinformatic pipelines,
CNV detection
 - : only CDS, less uniforme than capture kits,
small to medium groups
- **Possible developments: Nextera Rapid Capture exome**
 - + : "expanded" version with UTRs and miRNA,
only 50 ng input
 - : 8 Gb in 2x75 pb needed,
Dedicated to large groups

Ion S5



Illumina HiSeq



Genomics – Re-sequencing

➤ Targeted re-sequencing (panel, hotspots)

⇒ Mutation / biomarker screening on regions of interest

Already available:

- **AmpliSeq (Life Tech) Custom Panel**
- **AmpliSeq (Life Tech) Ready-to-use Panel**
Ex : Comprehensive Cancer Panel
- **TruSight (Illumina) kits**
Ex : TruSight Amplicon Cancer Panel
- **TruSeq Custom Amplicon Kit**

Ion PGM



Ion S5



Illumina MiSeq



Transcriptomics - Expression

➤ Whole transcriptome sequencing

⇒ Identification / quantification

Available:

Stranded RNAseq (HiSeq3000, 2 x 150 pb)

➤ Full length transcripts sequencing

Under development on PacBio RslI

⇒ Fusion transcripts, alternative transcripts

➤ small RNA sequencing

Available on Ion S5 sequencing

< 100 pb – stranded sequencing

ILLUMINA HiSeq



PacBio RslI



ION S5



Transcriptomics - Expression

➤ Targeted RNAseq ⇒ Expression

**Design possible for custom panels:
On Ion Torrent and Illumina**

➤ Targeted RNAseq - gene fusion

On Ion Torrent and Illumina :

- RNA apoptose / RNA cancer panel
- TruSight RNA Pan-Cancer...
- custom

Ion PGM



Ion S5



Illumina MiSeq



➤ Bisulfite conversion

Available for whole genome on HiSeq
RRBS (Reduced Representation Bisulfite Sequencing)

➤ lncRNA/small RNA

RNAseq protocols modifications (ribodepletion vs poly-A selection)

➤ ChIP-seq/MedIP-seq

Illumina compatible

➤ "direct« methylome

Available on PacBio RslI for small genomes

Illumina HiSeq



Ion S5



PacBio RslI



Metagenomics - Metabarcoding

➤ 16S – commercial kit

- 7 variable regions on 9
- Analysis on Ion Reporter
- Low throughput

➤ 16S full length

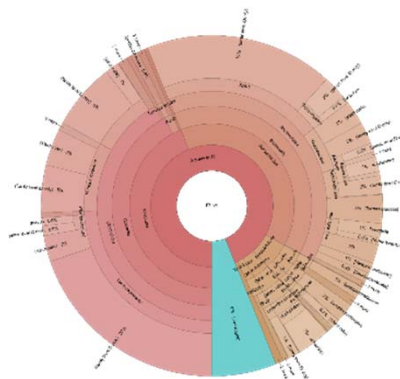
- Available PacBio RsII

➤ Custom Amplicons

- Available on Illumina – fully automated
- > 300 barcodes available
- 1 region 300-400 pb

➤ Whole metagenome / transcriptome

- Available on HiSeq



Ion PGM



PacBio RsII



Illumina MiSeq



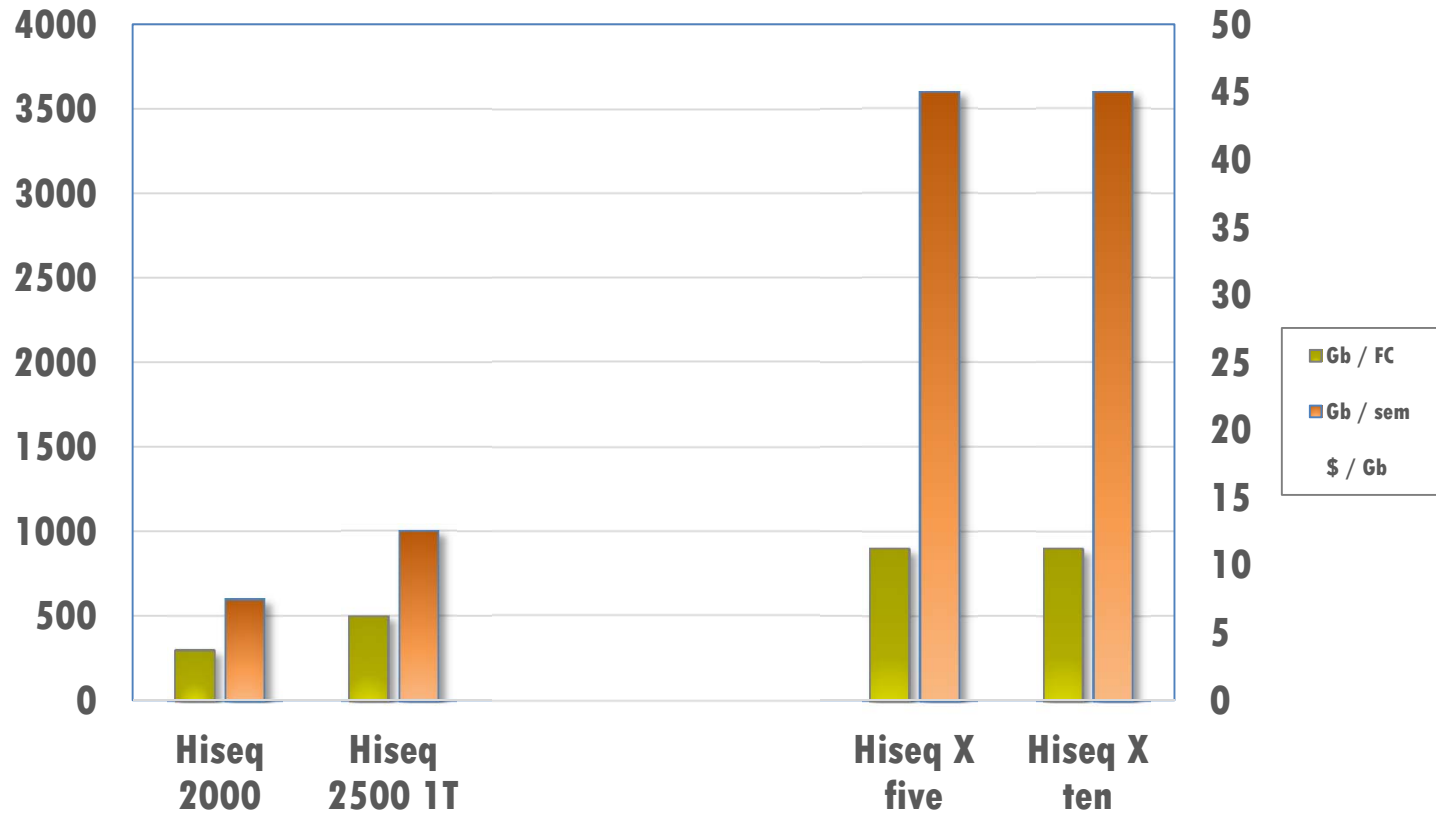
Ion S5



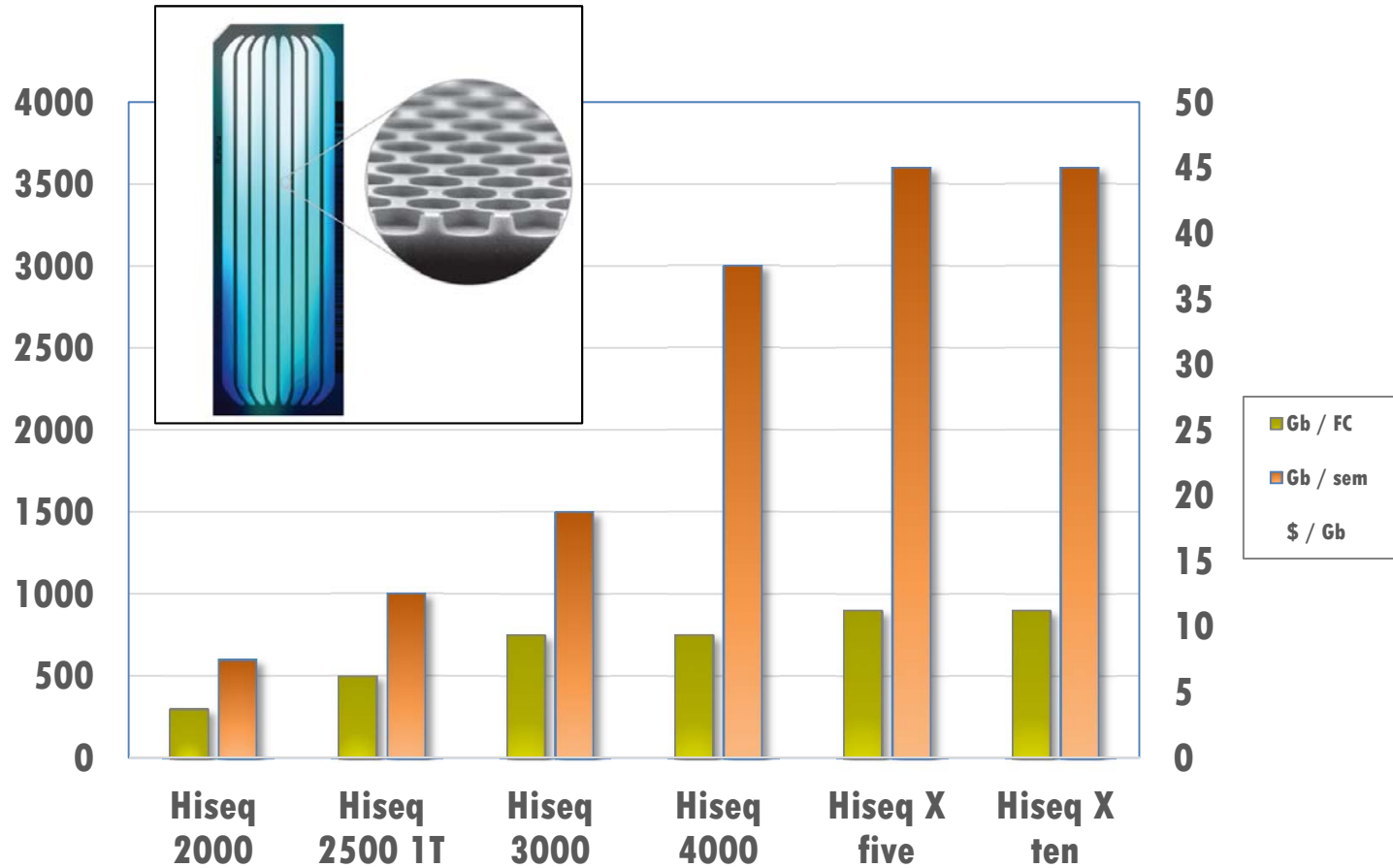
Illumina HiSeq



HiSeq productivity and costs



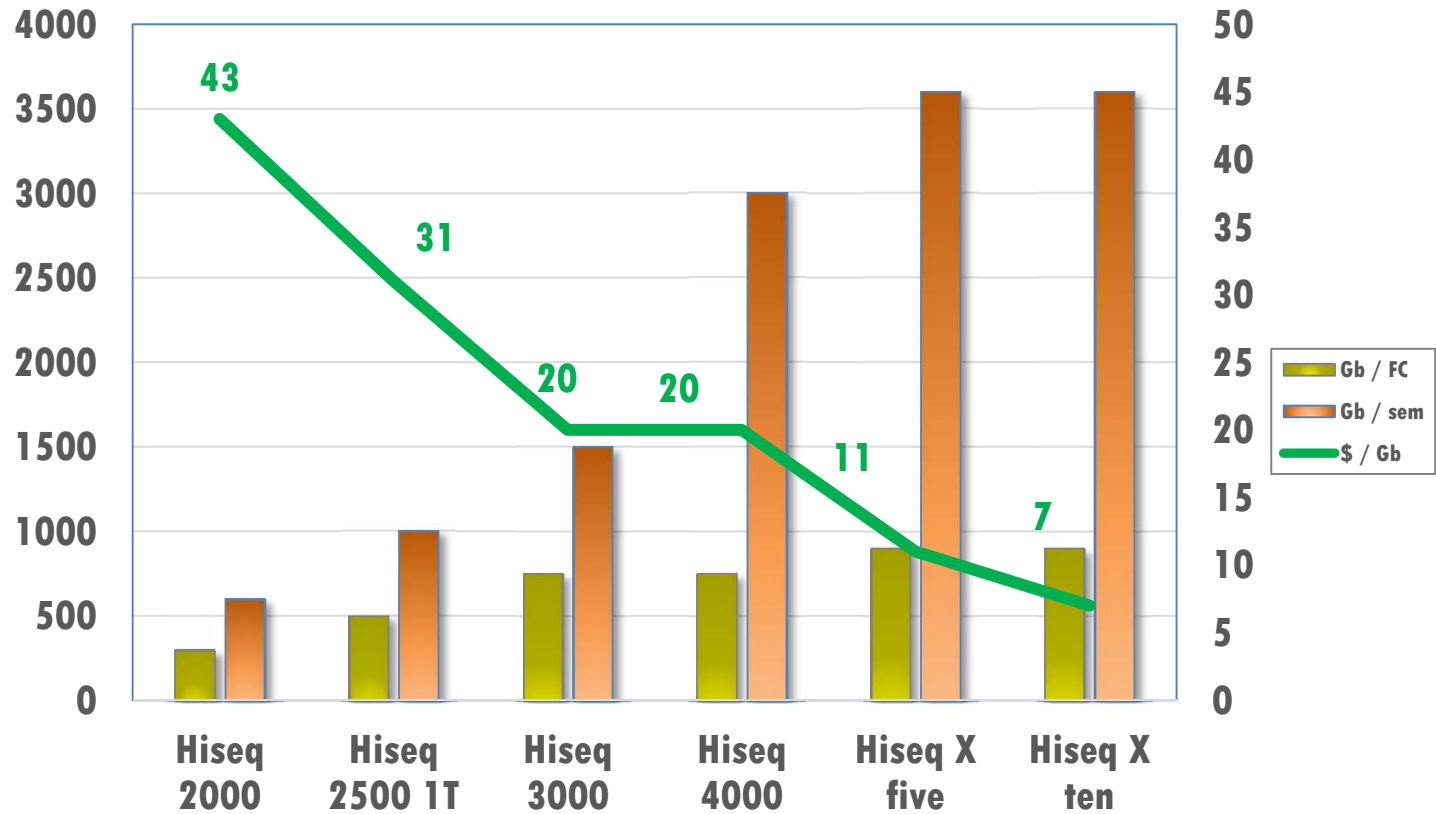
HiSeq productivity and costs



January 2015 : announcement of HiSeq3000 & HiSeq4000
 (same Flow Cells as HiSeq X)

HiSeq productivity and costs

Illumina reagent costs \$ / Gb



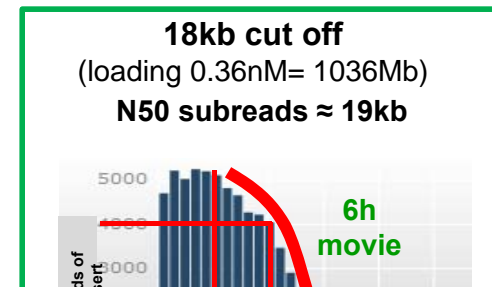
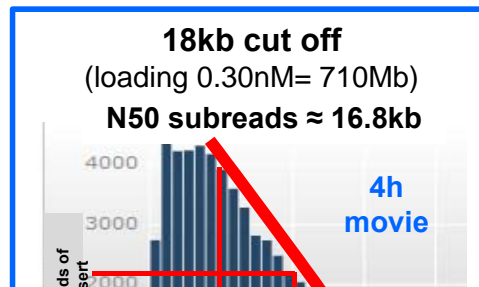
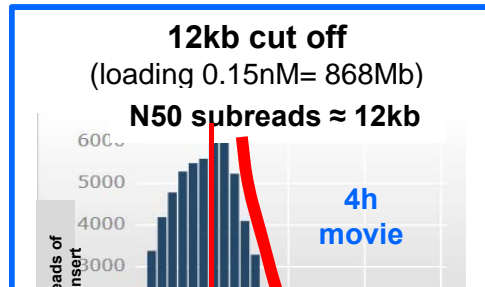
October 2015 : HiSeqX open to non humain (30x coverage) but 5 HiSeqX at least...



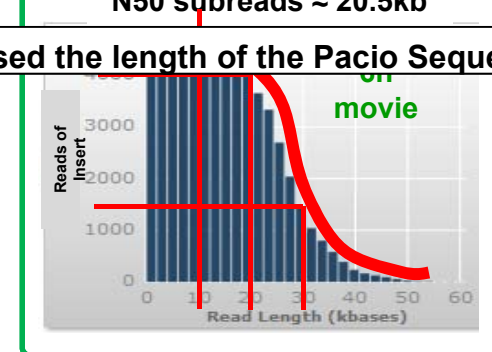
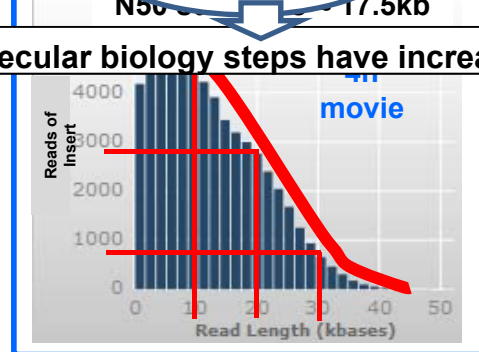
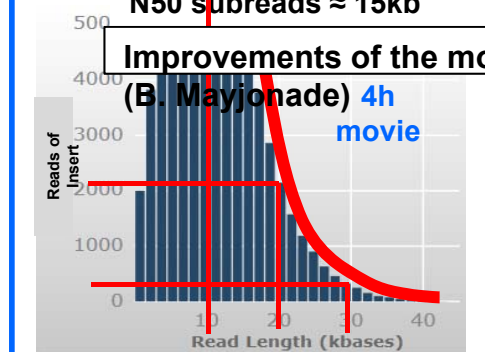
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Example of PacBio sequencing projects

Read length increase



	NUM	MAX	N50 BP	N50 NUM	MEAN	MEDIAN	BP/SMRTcell
IGM (San Diego, USA) 202 SMRT cells							
moyenne	98666	45457	12211	28413	9176	9032	0,906 M
max	146374	52725	12981	41602	9997	9809	1,36Gb
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max	126777	80974	20507	33133	13635	12295	1,3Gb



Improvements of the molecular biology steps have increased the length of the Pacio Sequences

(B. Mayjonade)

Read length increase

147 SMRT on 1st sunflower genome:

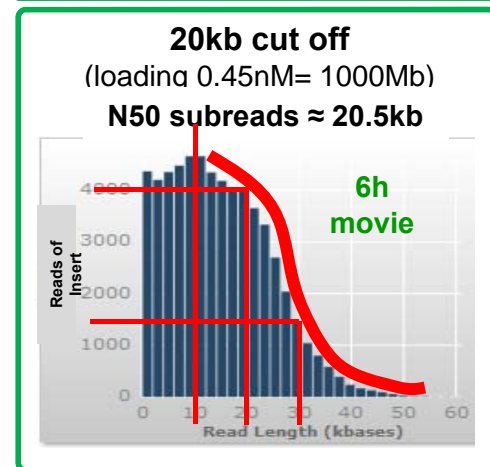
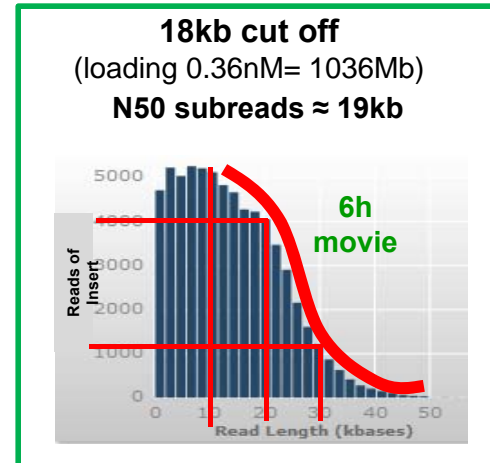
- **N50 15365**
- **800 Mb / SMRT cell**

103 SMRT on 2nd sunflower genome:

- **N50 18510**
- **1 041 Mb / SMRT cell (max 1 445 Mb)**

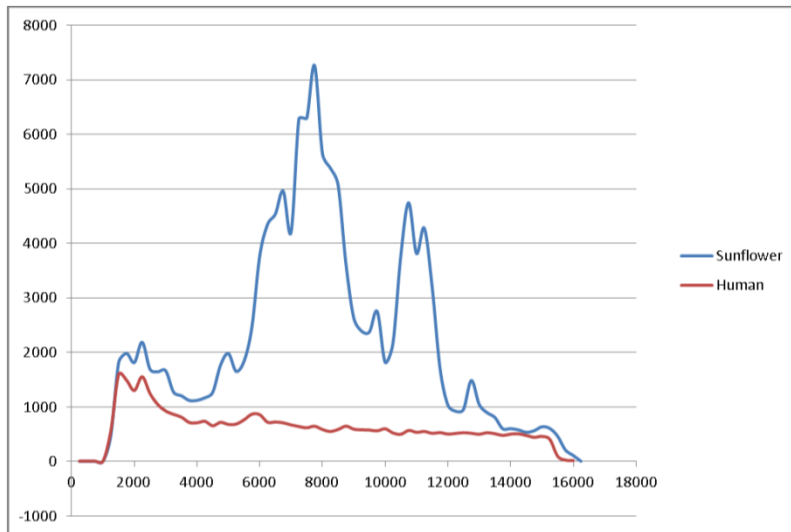
Top 10 of our longest subreads

80974 bp
 79860 bp
 79834 bp
 78105 bp
 77481 bp
 76881 bp
 76558 bp
 76355 bp
 75569 bp
 75559 bp



Sunflower genome sequencing

Sunflower : 30 % repeated sequences(LTR)
Homme : 8.8 % repeated sequences



The Challenge: obtention of sequences longer than the repeats

- INRA (Sunflower team):**
 Hiseq, 127 X
 → **43 % genome covered**
- International consortium:**
 454, Hiseq, Genetic & Physic maps (BAC)
 → **63 % genome covered**
- INRA (Sunflower team & GeT-PlaGe) :**
 PacBio, 107 X (407 SMRT)
 → **84 % genome covered**

#ctg	MAX	N50 BP	# > N50	MEDIAN	Gb
13 124	4.4M	498 kb	1700	118 kb	3.03

Direct methylation analysis on PacBio

Goal: identification of different methylation patterns on evolved strains of the bacteria *Ralstonia solanacearum* from PacBio sequencing

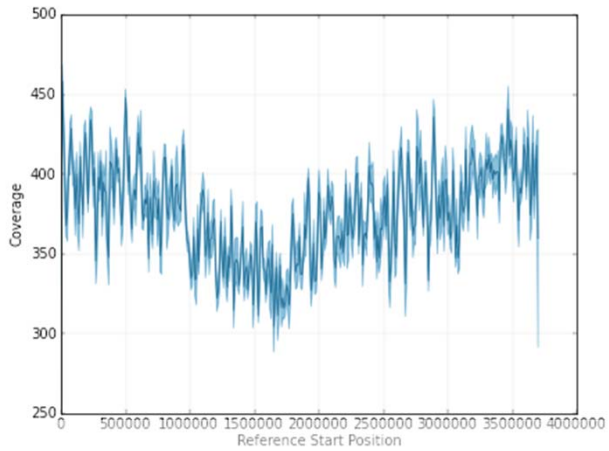
2 strains: ancestral strain & evolved strain

3 SMRTcells/strain

Direct methylation identified from PacBio sequencing:

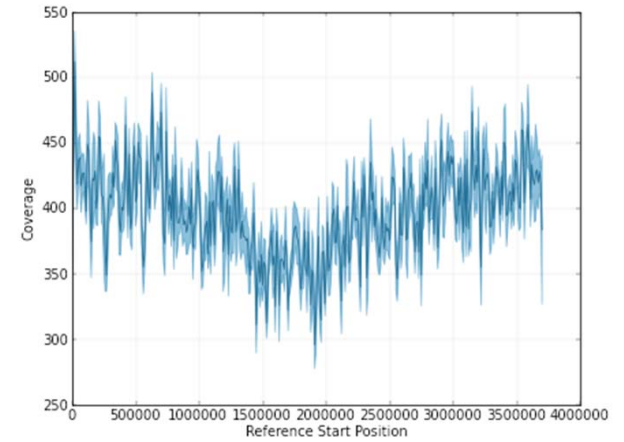
- ✓ **6mA @50X**
- ✓ **4mC @50X**
- ✓ **5mC @500X**

Ancestral strain

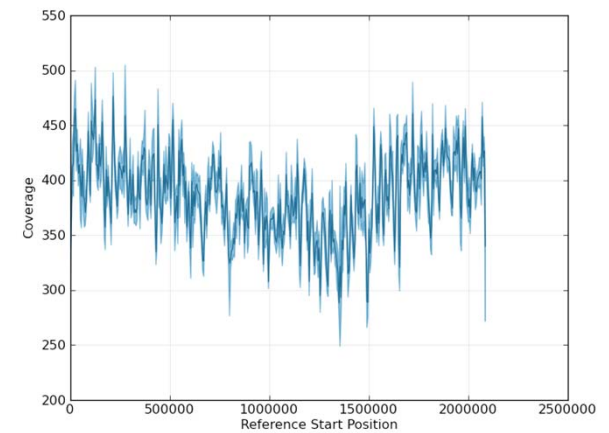
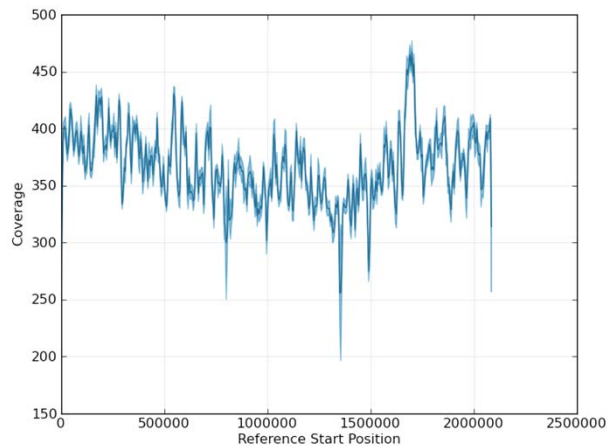


Chromosome

Evolved strain



Plasmid

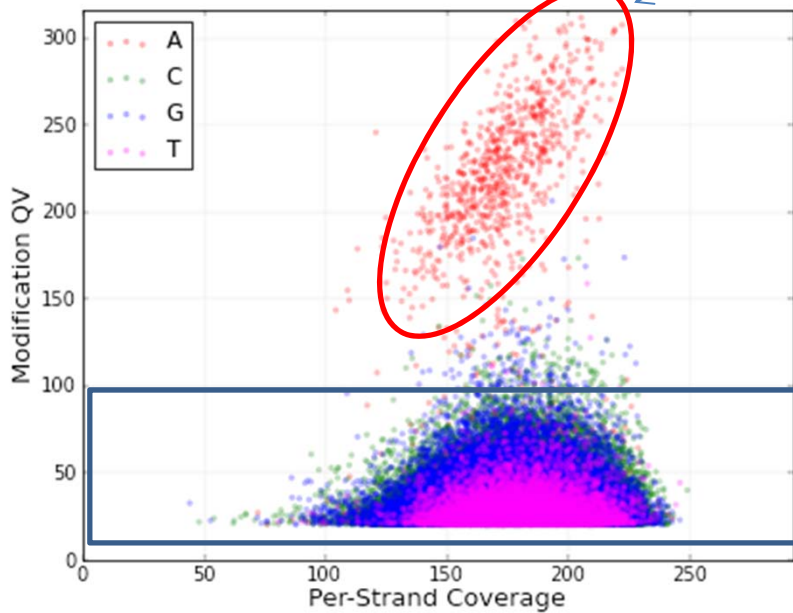


Modifications identified

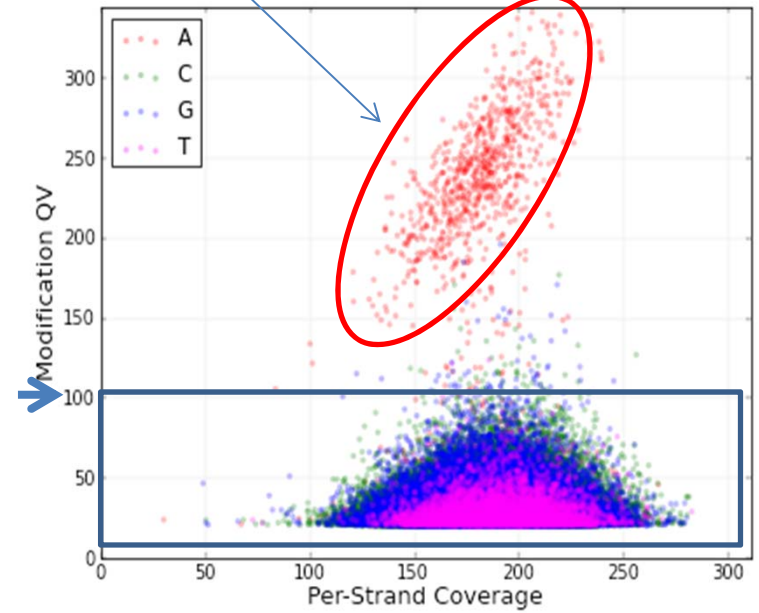
Ancestral strain

Evolved strain

6mA methylation signal



Threshold
QV 100
Noise



Preliminary results

6mA pattern: GTWWAC

784 GTWWAC patterns in the genome

>98% of 6mA with a méthylation at a QV >100

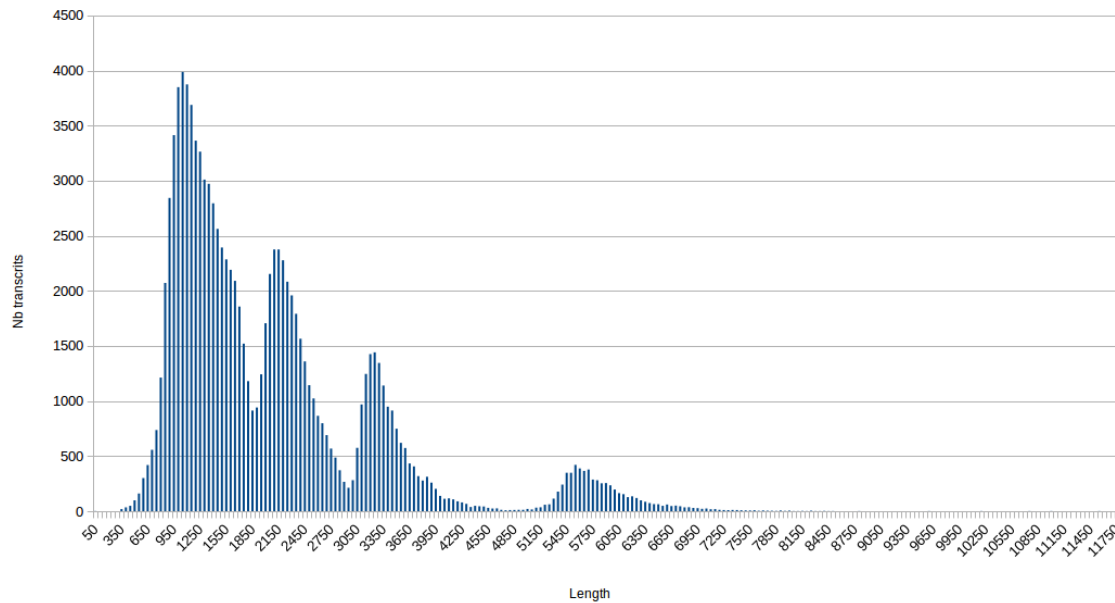
>98% 6mA -> typical pattern for bacteria

Preliminary analysis:

- ✓ **GTWWAC pattern associated with 99,2% of the 6mA**
- ✓ **GCCGGC pattern associated with 33.2% of the 4mC**
- ✓ **5mC difficile à voir en PacBio**

~100 different patterns identified between ancestral and evolved strains => genes or intergenic regions, including many regulatory genes

Iso-seq = full length transcripts sequencing on PacBio RSII

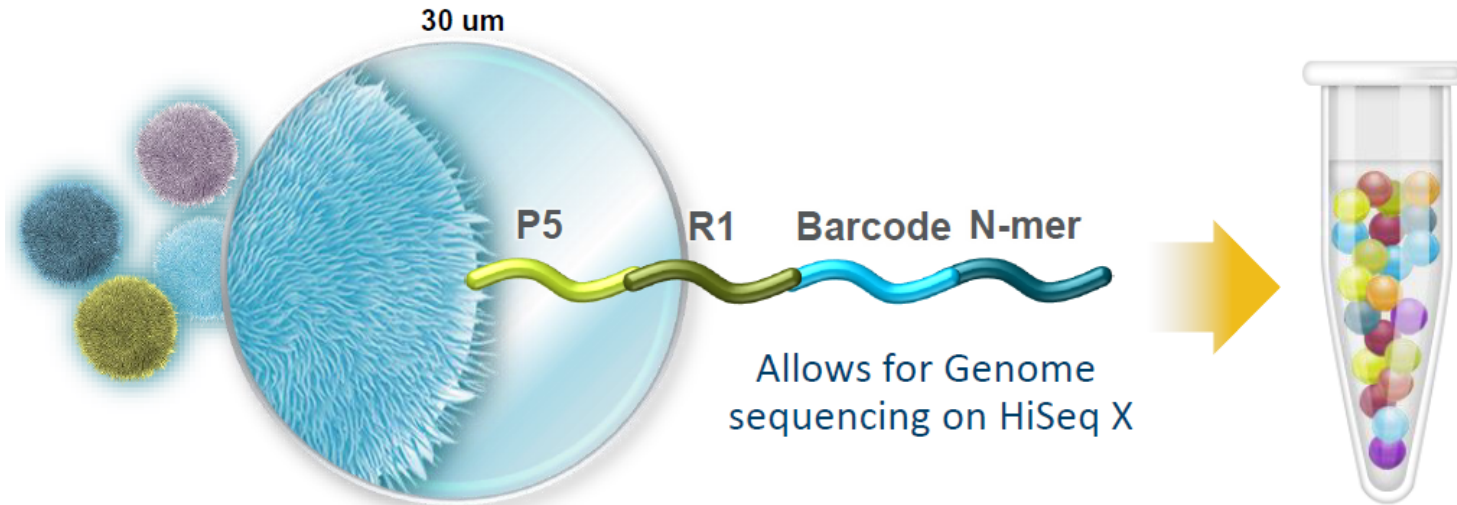


Preliminary results : ~110000 different transcripts identified

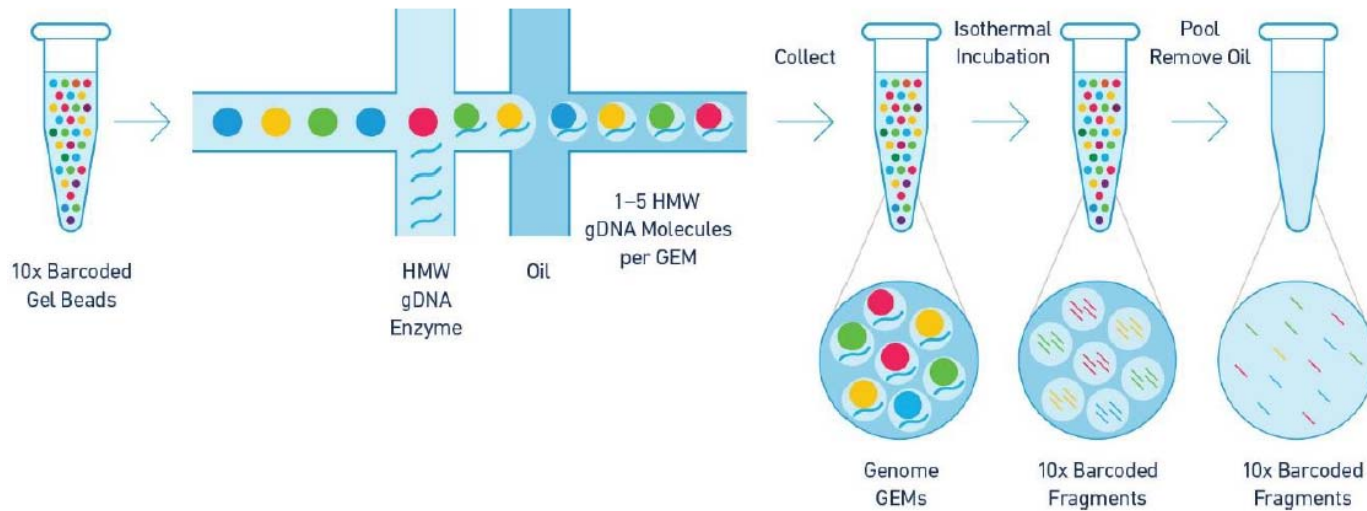
New technology available

Chromium (10X genomics)

- Library prep for Illumina sequencing
- Long range genomic (~100 kb), haplotyping
- Single cell analysis
- Exome sequencing



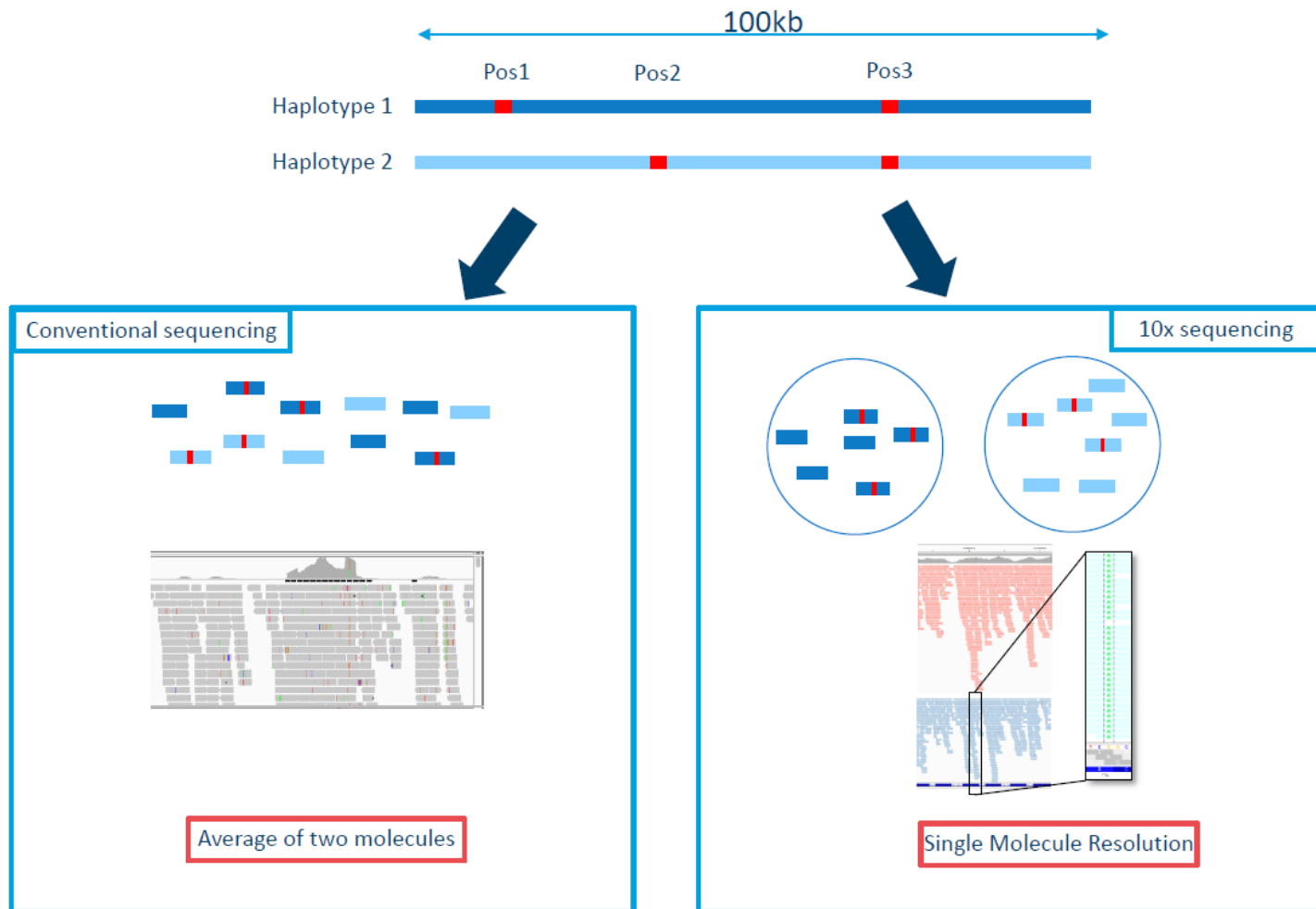
10X GENOMICS



Linked-Reads



10X GENOMICS



The future?

Sequel (PacBio)

- Same chemistry as the PacBio RSII (SMRT),
- Throughput x 7
- Similar error rate
- Price/Gb /4



PromethION (Oxford Nanopore)

- Nanopore technology
- "MinION multiple"



The future?



GOUVERNEMENT.fr
 Recherche [] [] [] []
 L'ACTUALITÉ du Premier ministre | LES ACTIONS du Gouvernement | LE GOUVERNEMENT et les Institutions | PARTAGEZ et diffusez
 En un coup d'oeil | Toute l'actualité | En images | Espace presse | Agenda | Discours et rapports
 22 juin 2016 - Communiqué
Remise du rapport "France Médecine Génomique 2025" : faire entrer la France dans l'ère de la médecine génomique



Illumina HiSeq X ten : 18000 genomes/year, 1000\$ genome



genomeweb
 Business & Policy | Technology | Research | Clinical | Disease Areas | Applied Markets | Resources
 Home » Business, Policy & Funding » Research Funding » France Plans to Invest €670M in Genomics, Personalized Medicine
 Jun 23, 2016 | a GenomeWeb staff reporter
France Plans to Invest €670M in Genomics, Personalized Medicine
 NEW YORK (GenomeWeb) – The French government announced that it plans to invest €670 million (\$760.8 million) in a genomics and personalized medicine program meant to improve the diagnosis and prevention of disease in the country.

**Target: 230000 genomes/year (2020)
12 sites in France**

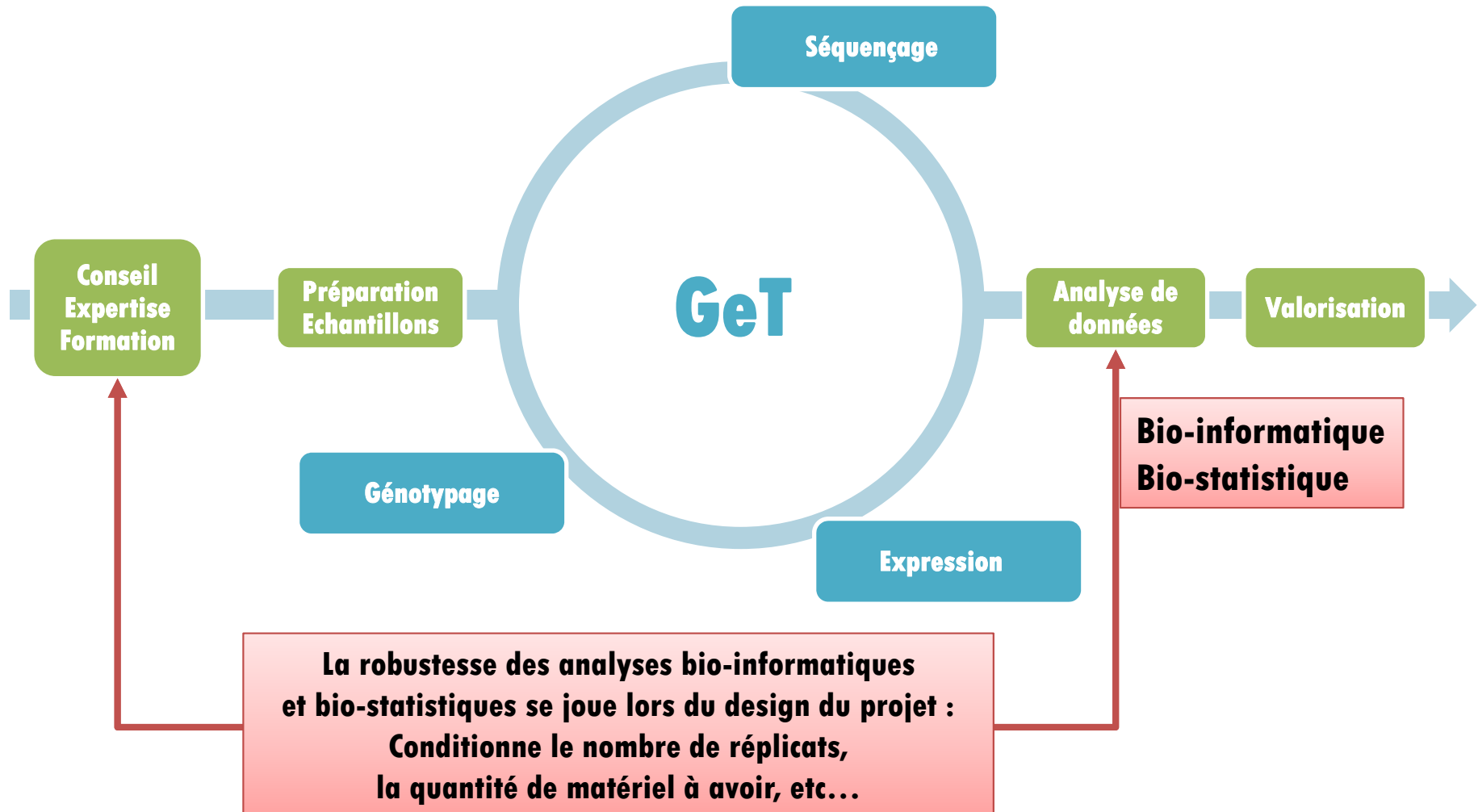


GET

Génome et
Transcriptome

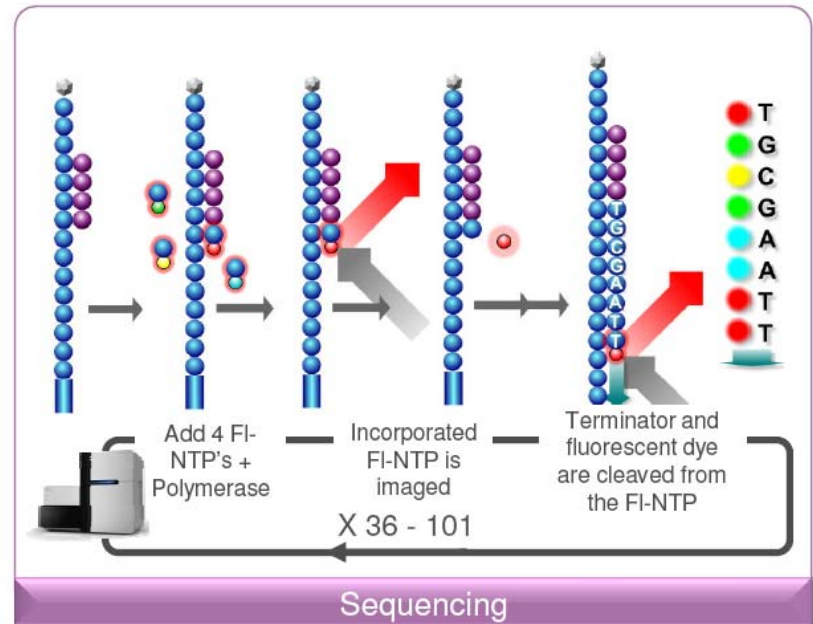
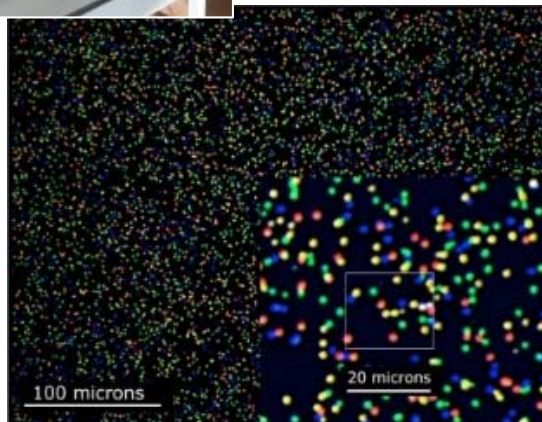
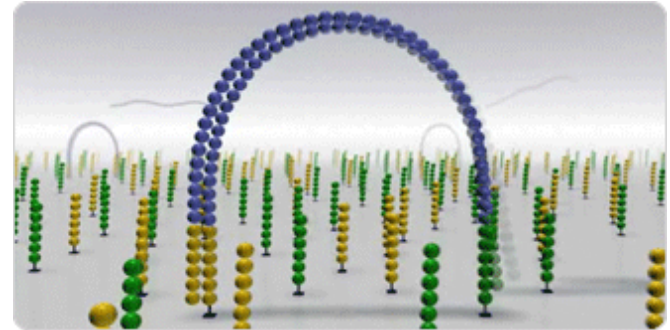
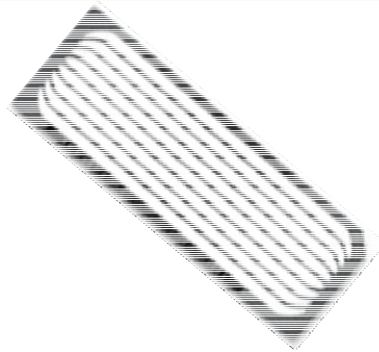
Thanks!

Démarche construction projets



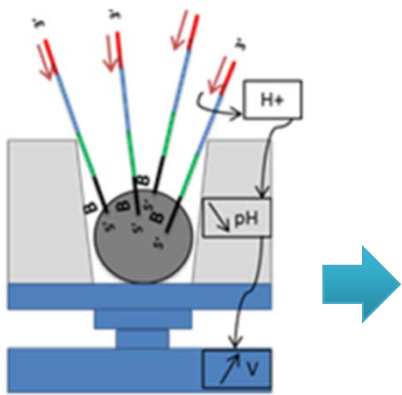
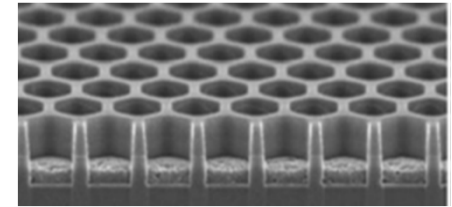
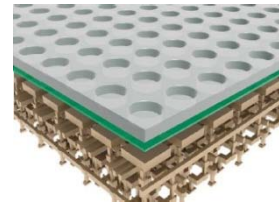
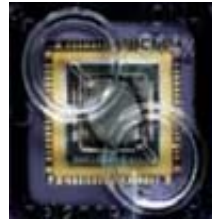
Chimie Illumina en détails

- Séquençage par Synthèse
- Détection par fluorescence

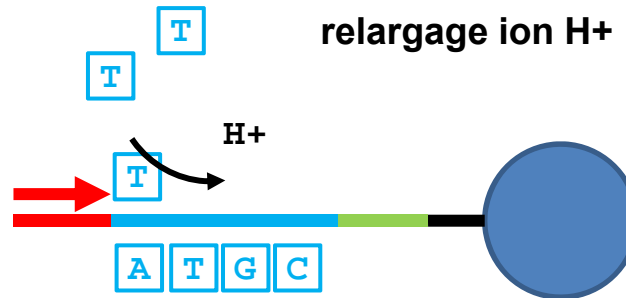


Chimie Ion Torrent en détails

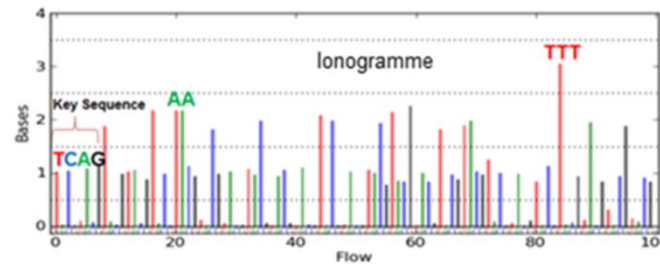
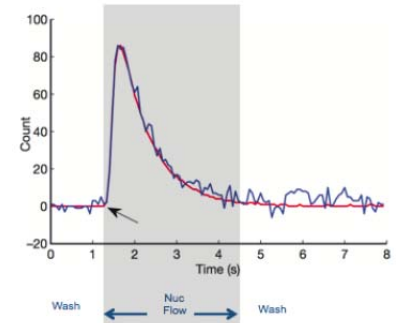
- Séquençage par Synthèse
- Par semi-conduction



Flow de dNTP
relargage ion H⁺



différence de potentiel
détectée par la machine

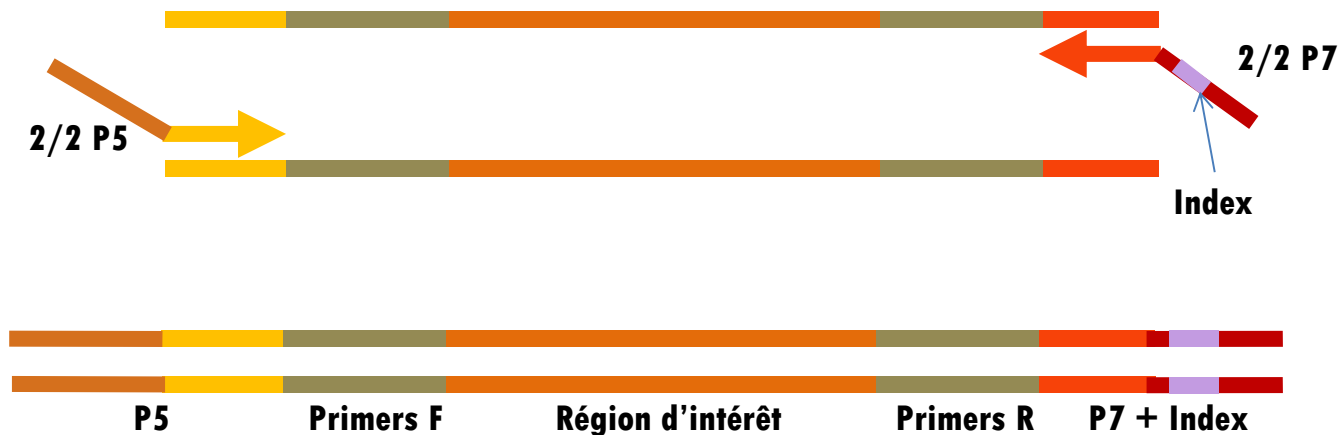


Séquençage amplicons sur Illumina

PCR1 (classique, 30 à 35 cycles) : amplification région cible

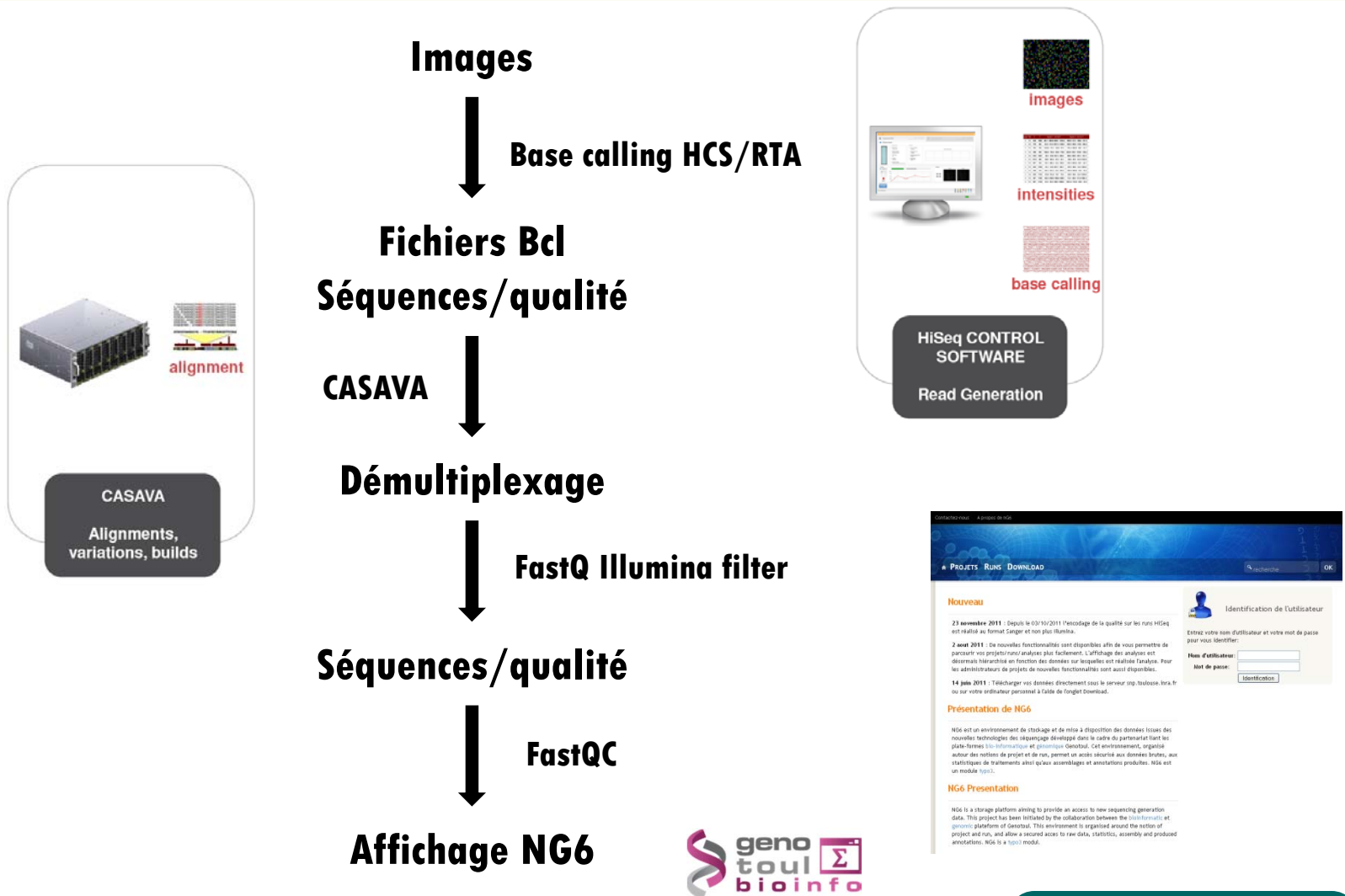


PCR2 (réduite, 10 à 12 cycles) : ajout adaptateurs + index



1 Librairie = 2 PCRs, Réalisable en plaque 96 puits, Automatisée

Analyses qualitatives des données Illumina



Génomique - Reséquençage

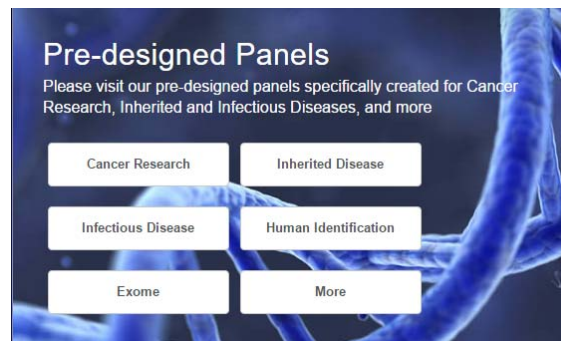
➤ Séquençage ciblé (panel, hotspots)

- ⇒ Recherche / typage mutations sur régions d'intérêt
- ⇒ Possibilité de recherche en profondeur

Peut être réalisé :

- **Sur Ion Torrent : tout type de panels AmpliSeq**
 - Custom - humain et souris - 200 ou 400 pb
 - Communautaires - 200 pb
 - Ready-to-use : panels dédiés cancérologie - 200 pb
 - Cancer Hotspot Panel v2 *FFPE
 - Comprehensive Cancer Panel *FFPE

⇒ www.ampliseq.com



Technologies compatibles

Ion Torrent



Génomique - Reséquençage

➤ Séquençage ciblé (panel, hotspots)

- ⇒ Recherche / typage mutations sur régions d'intérêt
- ⇒ Possibilité de recherche en profondeur

Des développements peuvent être envisagés:

- **Sur Illumina 2x75 ou 2x150pb**
 - TruSight Tumor 15
 - TruSight Myeloid
 - TruSight Amplicon Cancer Panel
- TruSeq Custom Amplicon Kit
existe une version low input - FFPE
⇒ DesignStudio Illumina

Technologies compatibles

Illumina MiSeq



Illumina MiniSeq ?