

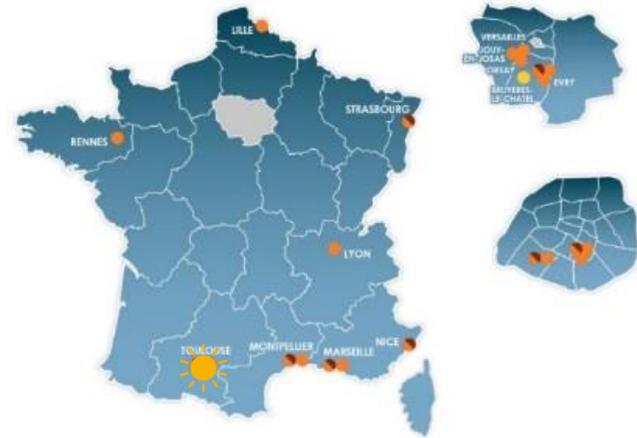
How does Nanopore technology meet the needs of our users ?

Workshop NanoSeq-2017

09 Octobre 2017

Catherine Zanchetta

⑤ **Genomics and Transcriptomics (GeT) Core Facility**
of Genotoul hosted by  **INRA**
SCIENCE & IMPACT



⑤ **A strong partnership with a bioinformatics core facility** 

⑤ **A node of the National Distributed Infrastructure « France Génomique »**



⑤ **Quality certifications ISO9001 & NFX 50 900, Propel**



Team and expertise

35 people on 5 sites

- Experts in Agronomy, the Environment, Microbiology, Health
- Competences in biology, bioinformatics, biostatistics



GeT-PlaGe : more than 140 laboratories in 2016 (INRA, CNRS, INSA, INSERM, CHU, CIRAD ...)

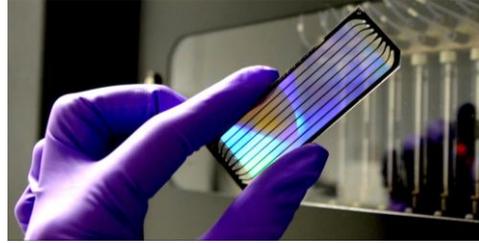
- More than 240 research teams
- More than 360 projects

GeT-PlaGe

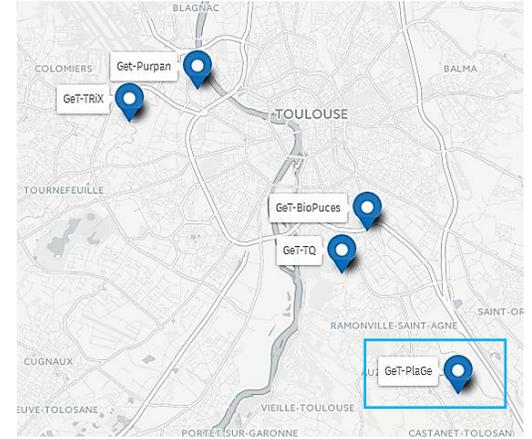
NGS technologies



Short reads



Illumina : MiSeq + HiSeq 3000

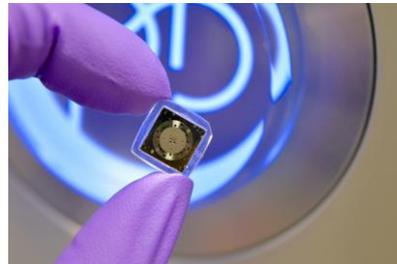


Synthetic long reads



10x genomics : Chromium

Long reads



Pacbio : RSII



ONT : MinION + GridION

How does Nanopore meet the needs of our users ?

(DNA studies)



Nanopore for genome assembly (vs Pacbio)



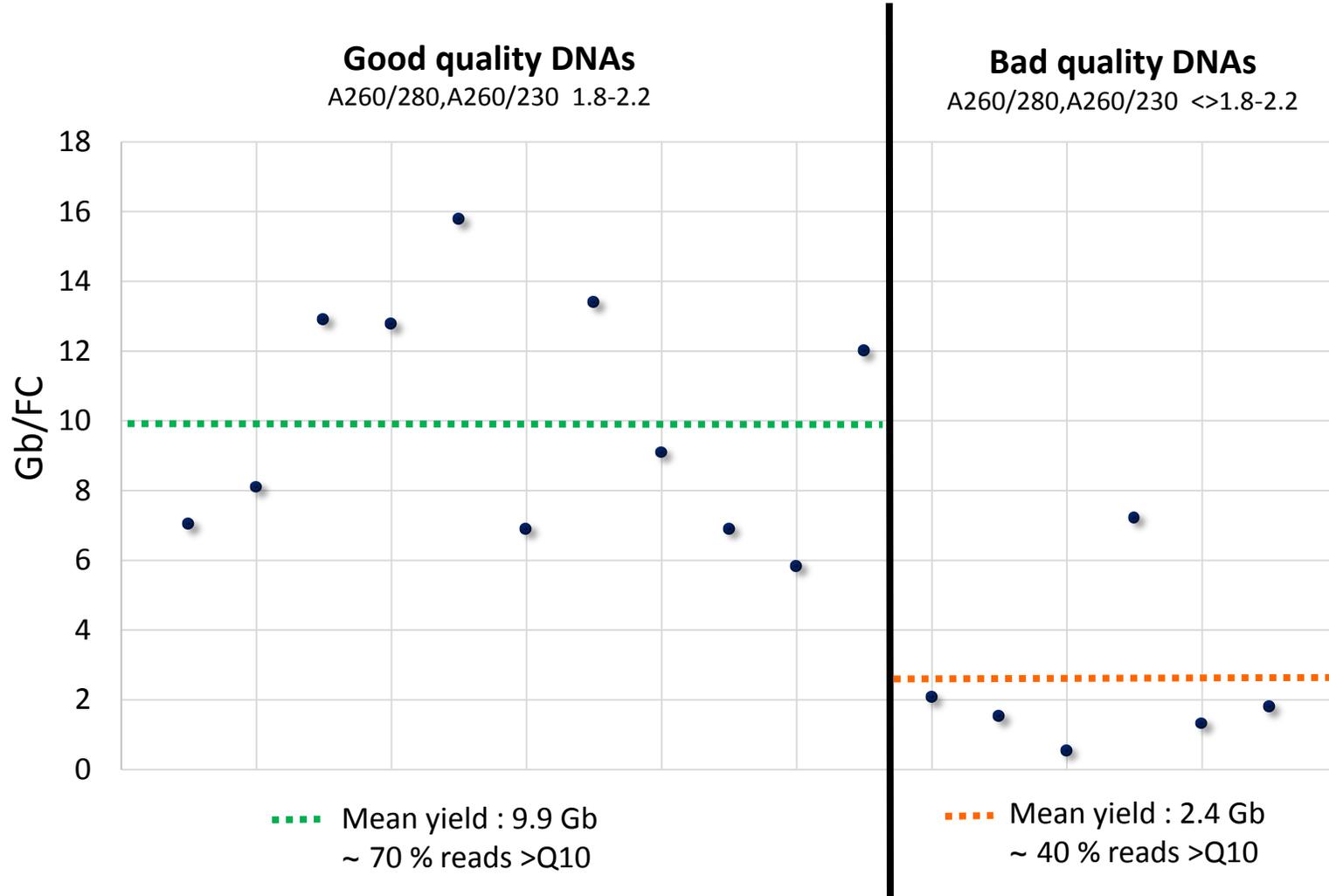
Sequencing of Fowlpoxviruses in clinical samples

Genome assembly using Nanopore technology

Yield



● = 1 DNA



Genome assembly using Nanopore technology

Higher yields

- Have a good quality DNA



Nanodrop

A260/280 = 1.8-2.2

A260/230 = 1.8-2.2

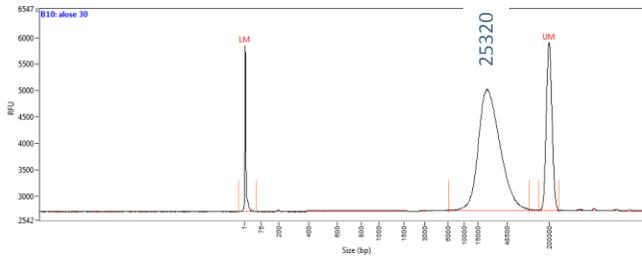
« le test des billes »



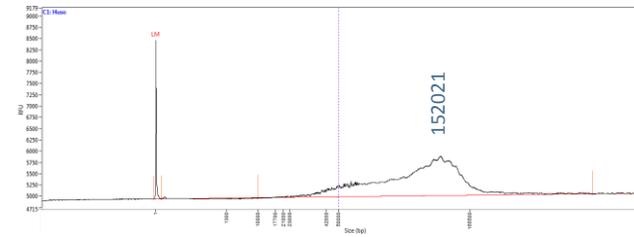
- Start the library prep with the right number of molecules



Qubit



Fragment analyzer
DNA < 40 kb



FEMTO
DNA > 40 kb



- Make a suitable library prep



Megaruptor shearing
(improves the yield ; if necessary)



BluePippin
(Removes small fragments ; if necessary)

Genome assembly using Nanopore technology

Assemblies improvement (small genomes)

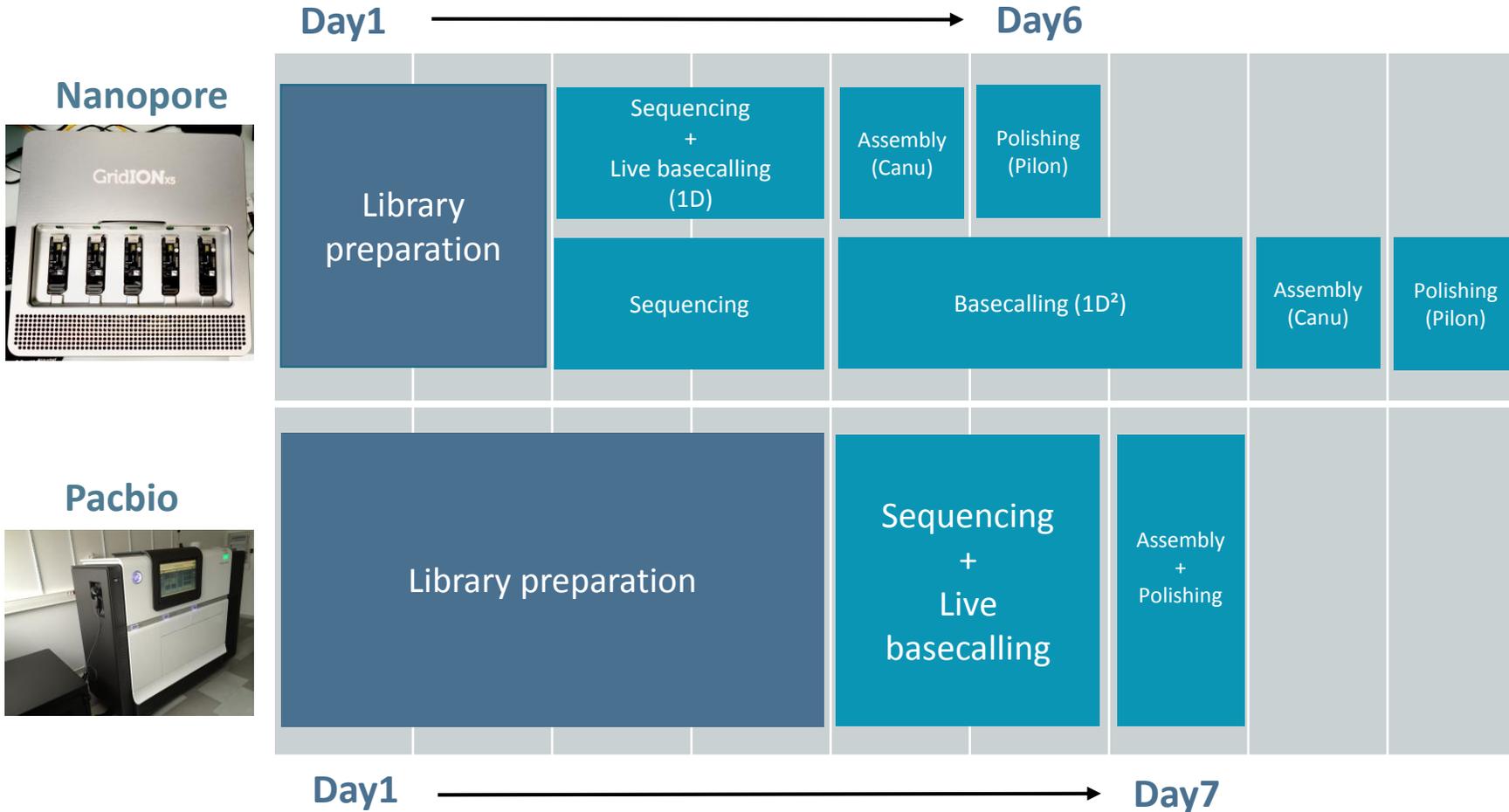
Complexity →

Data	<i>Xanthomonas campestris</i> (5Mb)	<i>Arabidopsis thaliana</i> (120Mb)	<i>Ganoderma boninense</i> (50Mb)
Illumina	50-100 contigs	> 1000 contigs	> 10 000 contigs
	+ 1FC (>>>80X Nanopore)	+ 1FC (80X Nanopore)	+ 1FC (100X Nanopore)
Illumina + Nanopore	1 contig	140 contigs	525 contigs

Pacbio vs Nanopore

Lib prep and bioinformatics

Example of a bacterial genome assembly :
Same quantity of data / Same informatics resources



Pacbio vs Nanopore

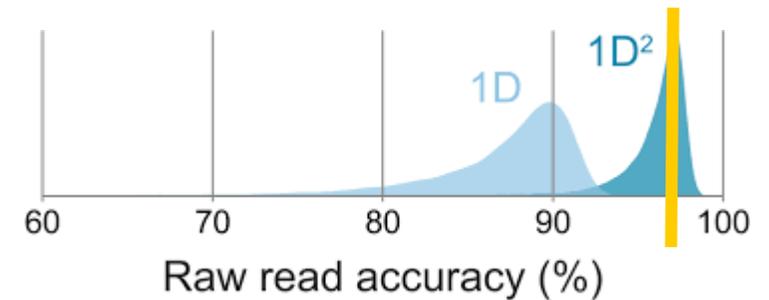
Accuracy and correction

Example of a bacterial genome assembly :
Same quantity of data / Same informatics resources

Raw read accuracy

Technology	Accuracy
Pacbio P6C4	81.3 %
Nanopore Albacore V0	81.0 %
Nanopore Albacore V1	86.3 %
Nanopore Albacore V2 1D	86.4 %
Nanopore Albacore V2 1D²	90.4 %
Illumina	99.7 %

Oxford Nanopore announcement



Assemblies assesment (BUSCO)

Methods	% Complete genes	% Fragmented genes	% Missing genes
Nanopore (1D ; R9.4) Albacore V1	12.2	27.0	60.8
Nanopore (1D ; R9.4) Albacore V1 Nanopolish	71.6	15.5	12.9
Nanopore (1D², R9.5) Albacore V2 Racon x2	77.0	7.4	15.6
Pacbio P6C4	95.3	0	4.7

Pacbio vs Nanopore

Comparison

	Nanopore		Pacbio	
			RSII	SEQUEL (expected)
Sensitivity (DNA quality)	Sensitive		Very sensitive	
Lib prep	2 days		4 days	
Yield	7.5Gb/FC		1-2Gb/SMRT	5Gb/SMRT
Bioinformatic analysis	3-6 days		3 days	
Methylations			« Included »	
Assembly quality			+1	
Price	+1			

Pacbio vs Nanopore

Comparison

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Methylations			« Included »	
Assembly quality			+1	
Price	+1			
DNA quantity	1.8µg/Gb (5.4 for 1D ²)		1µg/Gb	70ng/Gb

What am I going to talk about for the next 20 minutes ?



Nanopore for genome assembly (vs Pacbio)



Sequencing of Fowlpoxviruses in clinical samples

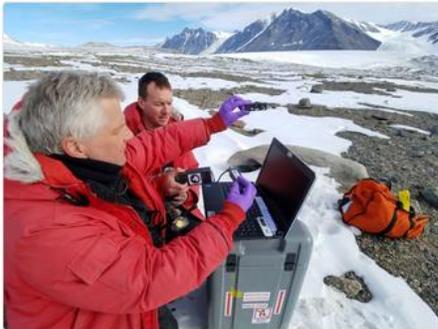
Portable device

- Shipment for samples with a biological risk : 500-1000€
- Biosecurity : better to keep the sample on site

From Ebola to Zika, tiny mobile lab gives real-time DNA data on outbreaks

While the elves are busy at North Pole, Extreme Microbiome Project team is busy in Antarctica.

À l'origine en anglais



08:09 - 25 déc. 2016

41 Retweets 80 J'aime



A genomic surveillance system which fits in a suitcase can help health workers to quickly understand the spread of viruses and break the chain of infection



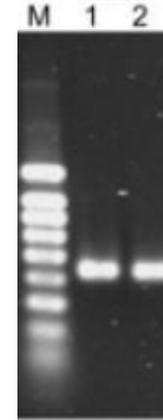
The MinION device, which weighs less than 100g, takes frequent electrical current measurements as a single strand of DNA passes through one of 2,000 pores in a plastic membrane. Photograph: Tommy Trenchard/EMLabs



Identification without *a priori*



- Symptoms
- PCR



• Histology

Reticuloendotheliosis virus strain 104865, complete genome

GenBank: KJ756349.1

[GenBank](#) [Graphics](#)

```
>KJ756349.1 Reticuloendotheliosis virus strain 104865, complete genome
AATGTGGAGGGAGCTCCGGGGAAATGTGGAGGGAGCTCCGGGGGAAATAGCCCTGGCTCCTAAGT
CCGATTAAGCTTGTAAACAGCTTCTGGCTTGGCCGCACTTACTTGGATATATTTGGCGATATG
ATTTCCGAAATCCGATCCAGAGCGGGTCTAAACCAATAAAGAAATTTTGTGGCCAGCAATC
AGACCCTTGCACATCCAATCACGAACAACAGAGATCGAACTATCATCTAGCCCAATGGTGTAAA
GGCAGATGCTATCTCAATGGAGAAATGTCAATGCAATCTCTGTAAGCGCTATATAAGCCAGGTG
CATCTCTGCTGGGGTGGCGTCTAGACATGTTGTAGCTGGCCGCAATTCGAACTGTAAATAAA
AGCTTTTCTATATCTCAGATGGCAGATGAGAGATTTTGTCTGTGTTGGCTGGCCCTACTG
GGTGGGTAGGGATCCGACTGAAATCCATGATTTCCGATACACATTTGGGGCTCCTCCGGATCCT
CCCATCGCAGAGTCCCTACTGTTTCTGAACTCCGGCCCGGTAAAGTACTGATTTTGGTAC
CTCCGAGGTTTGGAGATCGAGTGGCGGACCTCCGGGAAGCTCCACTCCCTCAGCAGGGGA
CCGCTCTGTGAGCTCTGTGATCTGTGTGAACTCTGTAGAGCGGTGATATAGTGG
TGGTGTGTTTGTGTTTACCTGTGTTTGTCTCACTGTGCAAGCCGCTCCGAAATGGTGA
CCACCCGCGCGCTTGGCAATAACTTTGGAGAGTCTTTGGCTCAGTGTCTTCGGTTTACTCG
TCTCCTCTCCTCTCCGGCGGATGGACAGGATCGAGGGCTTTTAACCCCTTAGAGTGCA
TTCTGAAGACTTCTCTGATTTAGAGAGGGCGGAGATGGGGAGTGGATTTGTTTACCT
GGCAGATTATGTAGTTGAAATGGCCAGCTTTGGCTGGGTGGCCAGAGAGGAGCTTAGACTTT
AGGTTGATGCCCGGTGAGAAATAGTTTTGGGAATCCAGGCAATCCAGACAGTAAATATATAA
CCCTGTGATAGATTAACCATAGAAAGGCTAAATGTTGAAGATGGGGGTAAACCCAGCCAGGAC
CTCTAAGTCTTTAGTCCAAAGATTAATCCAGGGGGCGCTGCTCCCTCAGCCAGAAAC
CCCCCTGATGAGGAGGCTCAATTCCTGATGAGACCTCTCTCCGGCCAGCCCTCCCACTC
CATATCTGAGGTACTGCCATTGCAGAGAAAGTGGAGGGGCAACACAGACTCTACTGTAAAGTC
```

Nanopore in the veterinary field

Context



- Mortality : 2% daily
- dsDNA virus
- ~ 290 kb

→ **Proof of concept of the identification of Fowlpoxviruses without enrichment or isolation step**

⚠ Sample not necessary dedicated to sequencing (especially long-read sequencing)

Nanopore in the veterinary field

Samples

Total = 10 samples

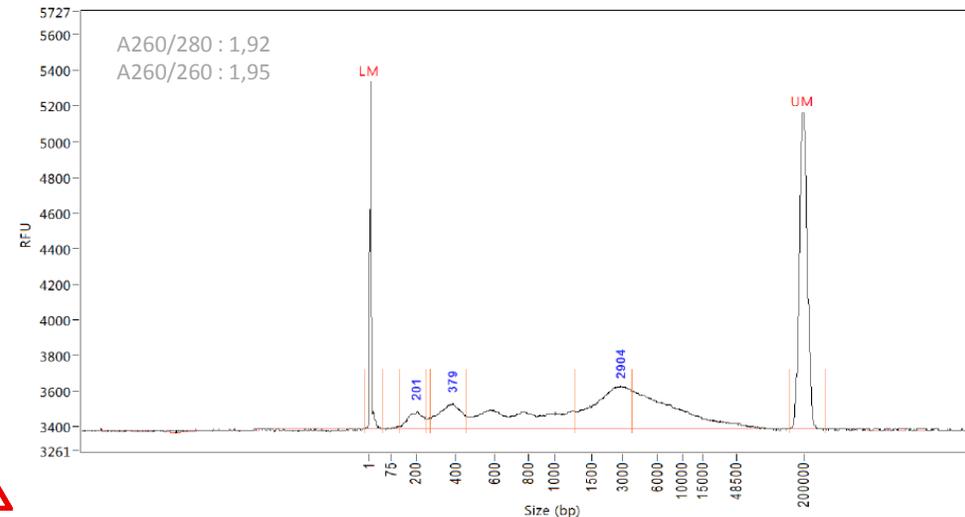
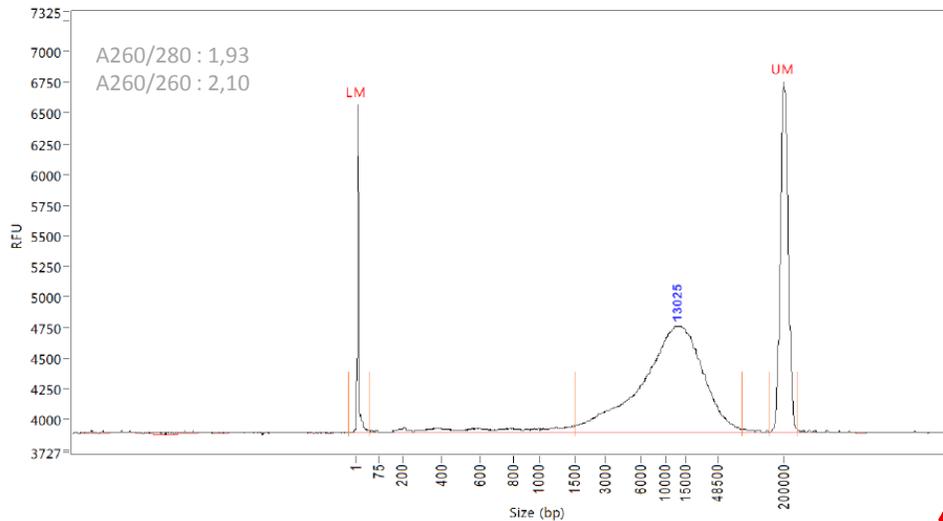
Fowlpox virus confirmed : PCR p4b locus and *fpv140* gene



Tissue lysis O/N @ 55°C using SNET/PK buffer¹

DNA extraction using Phenol:Chloroform: Isoamyl Alcohol¹
Rnase treatment

Quality control



Total = 7 samples for sequencing

Nanopore in the veterinary field

Runs

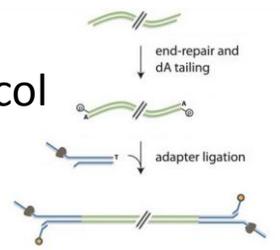


3 hours

8Kb gtube shearing



1D protocol



48 hours

3 Samples



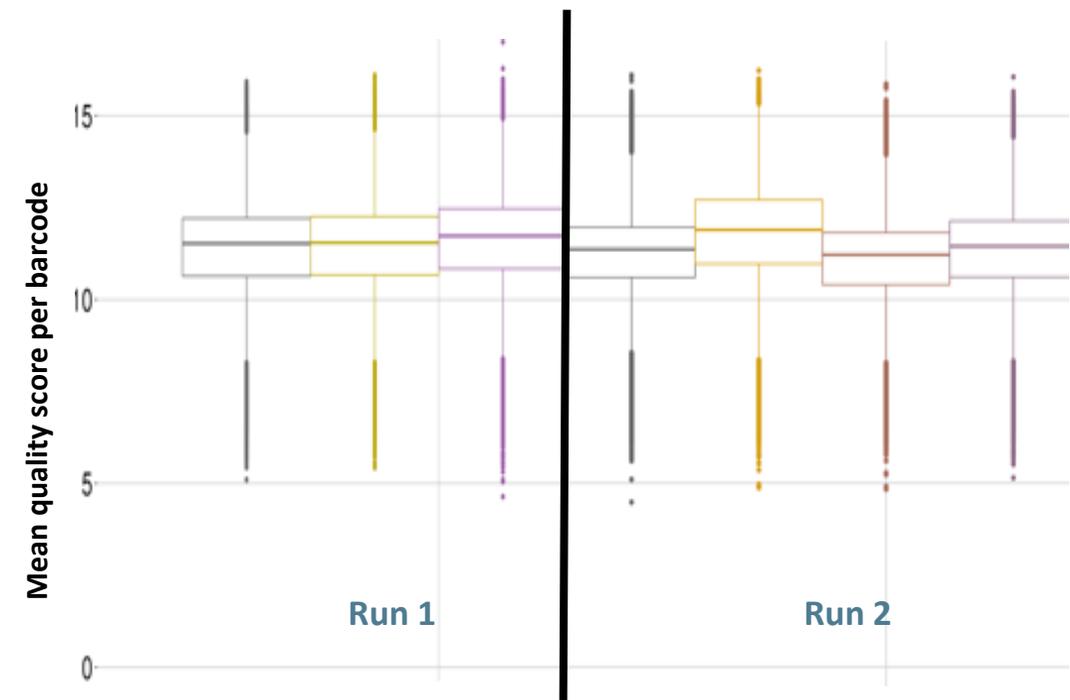
R9.4 FCs

4 Samples



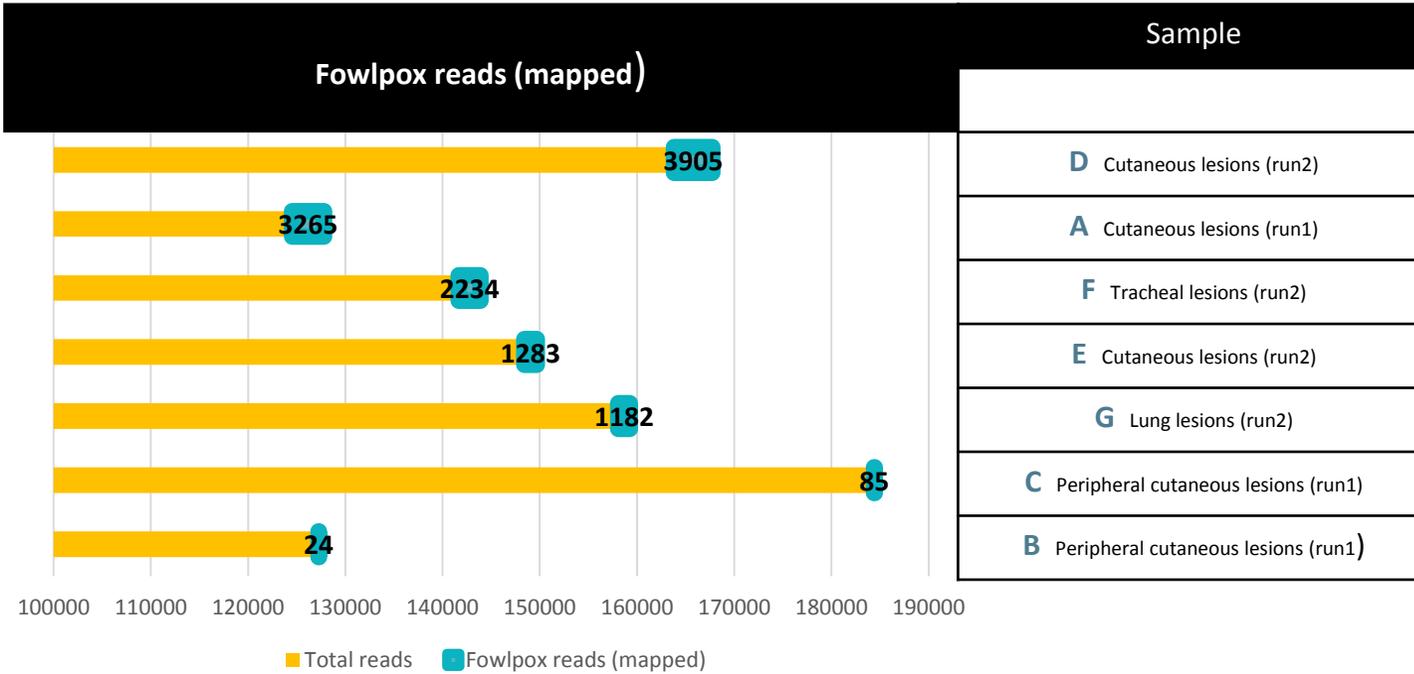
2.6Gb
All barcodes =
436 000 reads (65%)

3.6Gb
All barcodes =
634 000 reads (70%)



Nanopore in the veterinary field

Results : Fowlpox identification

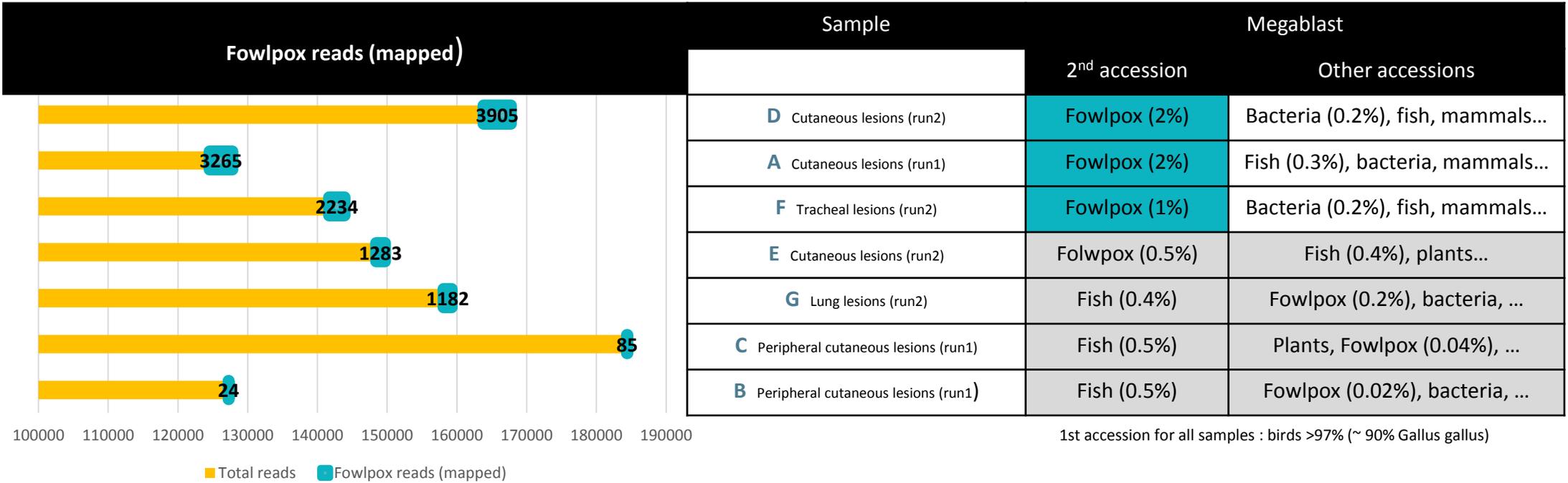


Fowlpox reads found in all samples

- % of fowlpox sequences depends on the « taking zone »
- Preferred zones : cutaneous lesions or tracheal lesions

Nanopore in the veterinary field

Results : Fowlpox identification



→ Proof of concept of the identification of the Fowlpox in all samples

+ Fowlpox detected in 3 samples without *a priori* and without enrichment or isolation step



Nanopore in the veterinary field

Assembly and what's next ?

	D sample
Coverage	30X
Number of contigs	2
Total length	288kb
% mapped on ref (288kb)	98 %
Accuracy	96.5 %

Identification of the REV sequence (full length : putatively induces an increase of the virulence)

Genes and proteins analysis (combination with Illumina data ?)

Nanopore in the veterinary field

Not « a diagnostic tool » but a « confirmation tool »

Provide informations for epidemiology

Expectations : **Faster & Cheaper**



FLONGLE
("flow cell dongle")

ADAPTS MINION TO SMALL FLOW CELLS

- Low cost, smaller, 128/256 channel flow cell
- Supports all current ONT chemistries
 - R9.5 DNA chemistry @ 450 b/s
 - With 256 channels → ~400 Mb/hr



Flow cell prototype with 128 channels

@NanoporeConf | #NanoporeConf



LC

APPLICATIONS

- Suitable for frequent, low cost DNA analysis
- Sample and strain identification
- Enriched DNA panels, hypothesis driven analysis
- Currently pursuing regulatory approval for diagnostics

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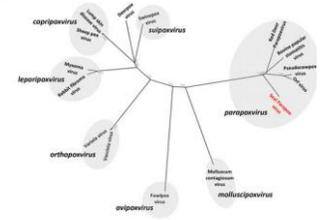
SCIENTIFIC REPORTS

OPEN Recovery of the first full-length genome sequence of a parapoxvirus directly from a clinical sample

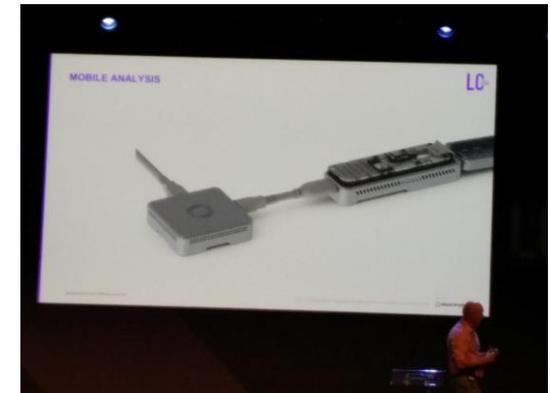
Received: 10 January 2017
Accepted: 9 May 2017
Published online: 16 June 2017

Thomas Günther¹, Ludwig Haas¹, Malik Alawi^{1,2}, Peter Wohlsein¹, Jerzy Marks¹, Adam Grundhoff^{1,3}, Paul Becher^{1,2} & Nico

We recovered the first full-length ooxovirus



Phylogenetic tree analysis based on 47 proteins. Protein sequences were considered being

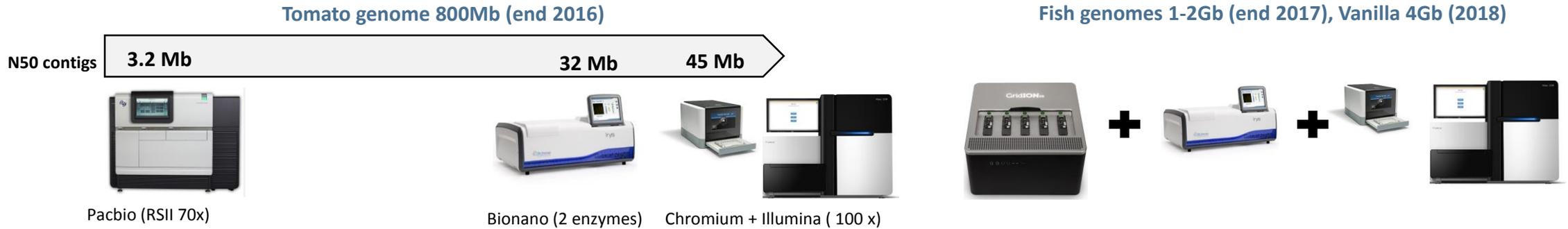


→ Next step : confirm with other DNA viruses & RNA viruses



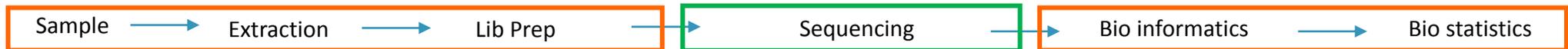
To conclude !!!

- ⌘ To go further and improve genomes assembly : scaffolding technologies (10X Genomics, BioNano...)



- ⌘ ONT has opened the door to a wide range of applications

- ⌘ Nanopore technology : Long DNA fragments = long reads



Remerciements



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