# Next Generation Sequencing & viral surveillance in blood donors

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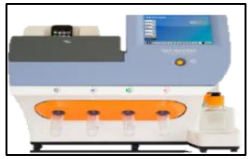
# Next generation sequencing

## 2nd generation

HiSeq
(Illumina)



PGM
(Ion Torrent)

Proton
(Ion Torrent)

## 3rd generation


MinION Mk1B
(Oxford Nanopore Technologies)

PACBIO

High throughput
Deep sequencing
Millions of sequence reads in one run

Amplification step of short DNA fragments needed
(50-400 bp)

Sequencing of individual molecules
(> 10,000 bp)
Real-time sequencing

# NGS applications & blood safety

- **Viral metagenomics: complementary diagnostic tool?**
  - Increasing chances to detect rare viral strains through unbiased sequencing
  - Methodological limitations
    - Low viral DNA content --> viral particle enrichment, viral nucleic acid amplification
    - Abundance of host and mitochondrial DNA --> digestion of host nucleic acids
    - Lack of bio-informatic specific tools to « polish » raw viral sequence reads
      - absence of genes that are conserved among all viruses
      - variability affecting reads assembly, alignment, and mapping
    - Confirmation with specific NAT needed

- **Pro-active surveillance tool for the animal reservoir**
  - Arthropods, bats, rodents,...

# NGS applications & blood safety

- **Identification of new viruses in blood donors**

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short Communication

Marseillevirus prevalence in multitransfused patients suggests blood transmission

Nikolay Popgeorgiev[a], Philippe Colson[a], Isabelle Thuret[b], Jacques Chiarioni[c], Pierre Gallian[d], Didier Raoult[a], Christelle Desnues[a,*]

PLoS Pathog. 2016 Jan;12:e1005386

Discovery of a Novel Human Pegivirus in Blood Associated with Hepatitis C Virus Co-Infection

Michael G. Berg[1☯], Deanna Lee[2,3☯], Kelly Coller[1], Matthew Frankel[1], Andrew Aronsohn[4], Kevin Cheng[1], Kenn Forberg[1], Marilee Marcinkus[1], Samia N. Naccache[2,3], George Dawson[1], Catherine Brennan[1], Donald M. Jensen[4], John Hackett, Jr.[1], Charles Y. Chiu[2,3,5*]

**Transfusion-transmission infection risk?**

J Infect Dis. 2014;210:2017-8.

**No Evidence of Marseillevirus-like Virus presence in Blood Donors and Recipients of Multiple Blood Transfusions**

Virginie Sauvage,[1] Audrey Livartowski,[1] Laure Boizeau,[1] Annabelle Servant-Delmas,[1] François Lionnet,[2] Jean-Jacques Lefrère,[1,3] and Syria Laperche[1]

Ann Intern Med 2018;168:158.

**Reply to Kandathil *et al*: No evidence of novel human pegivirus 2 active infection in HCV-infected blood donors from France, China and sub-Saharan Africa.**

*D Candotti, X Deng, T Li, Sy Laperche, V Sauvage.*

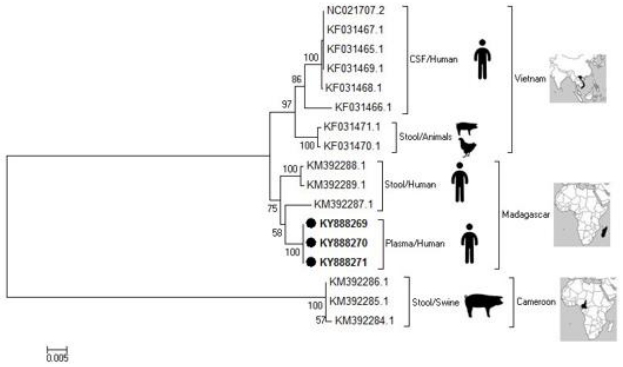
Transfusion. 2015;55:1256-62.

**Absence of giant blood Marseille-like virus DNA detection by polymerase chain reaction in plasma from healthy US blood donors and serum from multiply transfused patients from Cameroon**

*Tung Gia Phan,[1,2] Christelle Desnues,[3] William M. Switzer,[4] Cyrille F Djoko,[5] Bradley S. Schneider,[6] Xutao Deng,[1,2] and Eric Delwart[1,2]*

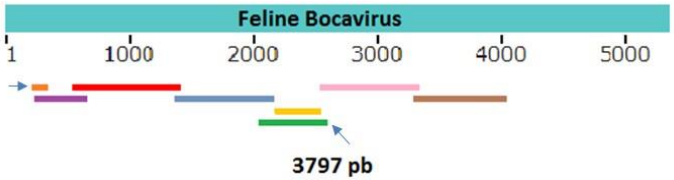
# NGS applications & blood safety

- **Identification of known viruses not expected to be present in blood**
  - **Cyclovirus-Vietnam (CyCV-VN)** detected in 43% blood donors from Madagascar
    - 1st identification in CSF of patients with encephalitis of unknown etiology
    - Present in animal and human stools in Madagascar
    - Detected in plasmas of italian blood donors co-infected with HBV, HCV or HIV




Sauvage V *et al*. High prevalence of Cyclovirus Vietnam (CyCV-VN) in plasma samples from Madagascan healthy blood donors. Infect Genet Evol. 2018;66:9-12.

- **Feline Bocavirus (FBoV) genotype 2** sequences detected in 2 (2.4%) blood donors from Mauritania
  - 1st identification in human
  - Domestic cat infections in Portugal, USA, Japon, China




**Transfusion-transmission & clinical relevance?**

# Third generation sequencer

# Nanopore MinION technology & HBV sequencing

- **Why?**

  - Surveillance of viral diversity in the blood donor

  - Analysis of the molecular complexity of the viral population infecting an individual

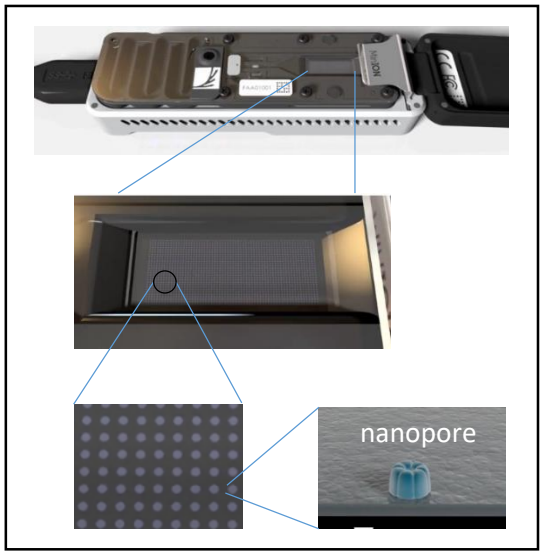
    - Quasispecies distribution
    - Multiple genotypes co-infections
    - Recombinant viruses
    - Individual sequences of the complete viral genome --> linkage between mutations

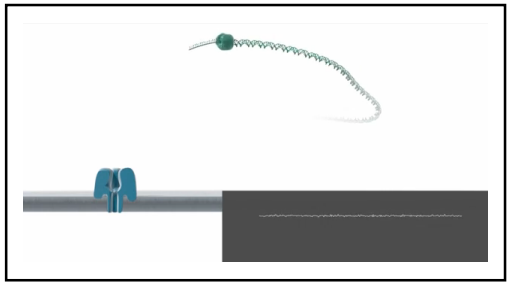
# Third generation sequencer

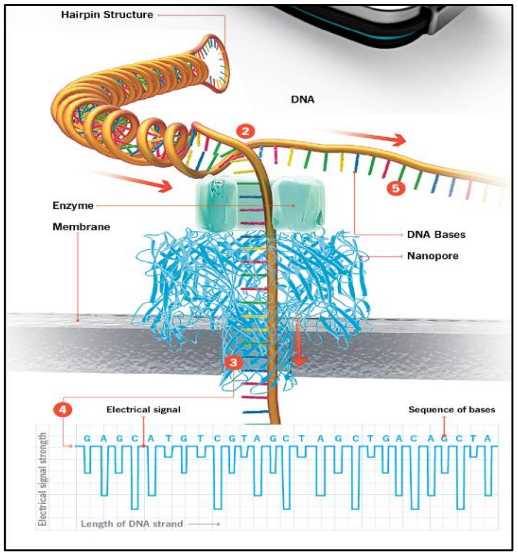
# Nanopore MinION technology & HBV sequencing

- **How?**

  - Voltage applied on membrane with nanopores (n=2,080) to drive DNA through the pore and an ion flow measured by sensor (several thousand times/sec)
  - Change in current pattern or magnitude when DNA molecule passes through the nanopore
  - Data streams passed to a microchip (ASIC)
  - Data acquisition and analysis carried out by specific software



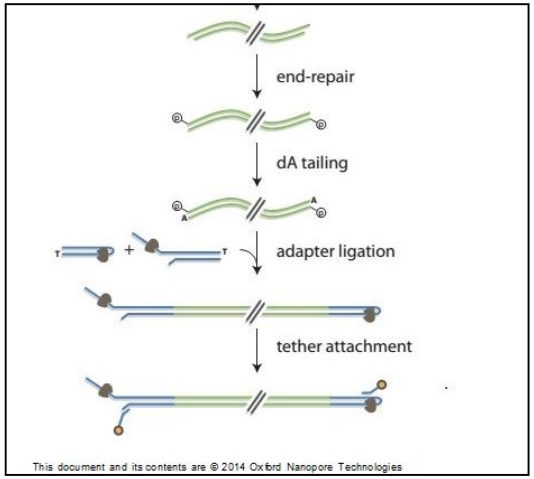





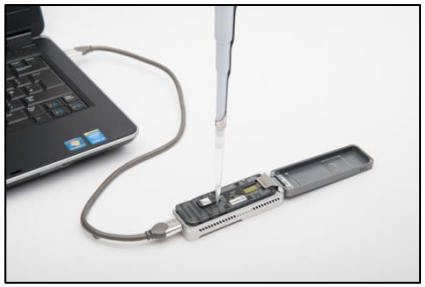
# MinION workflow

## 1. Library construction (~120 min)

- «full-length» HBV PCR product

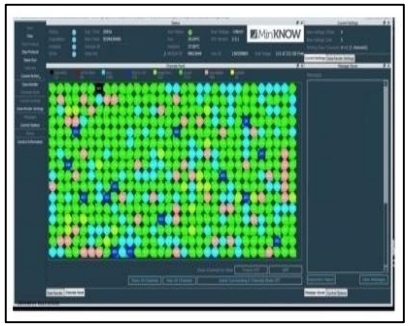



## 2. Initialisation & flow-cell loading(~40 min)

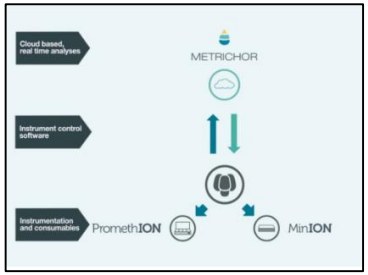


- USB3 port
- SSD
- >8 GB RAM
- 1 terabytes hard disk space
- QC assessment plateform: nb active pores in flow-cell

## 3. Sequencing run & assessment of nanopore activity




## 4. Base calling & bioinformatics analysis

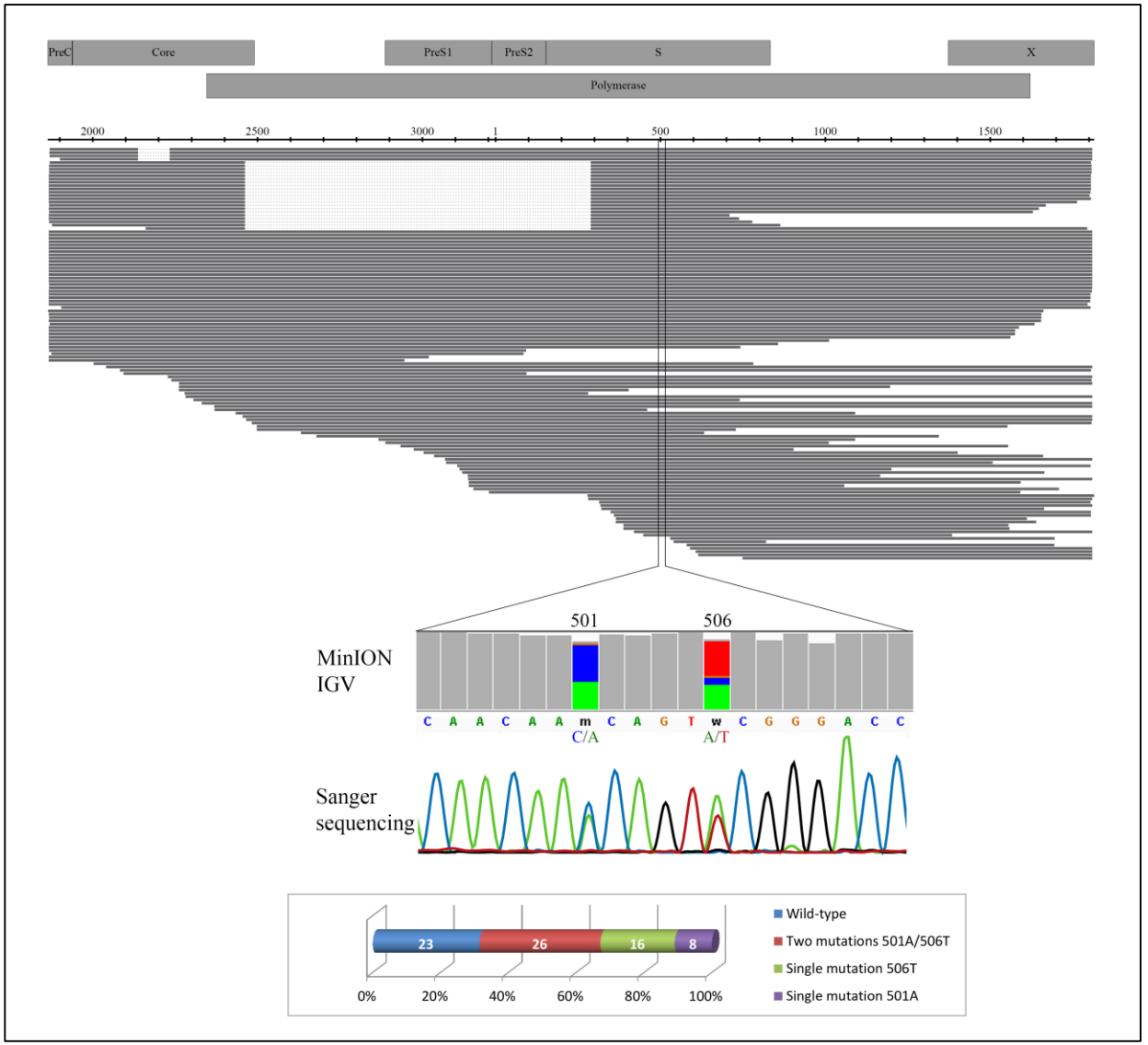




**Complete genome HBV consensus sequence: ~20 min**

# HBV variants in blood donors

- **Mixed population of wild type and single nucleotide mutants**
- **Unique deletions**
- **(Un)known spliced variants**
- **Recombinant viruses (absence of parental sequences)**




Sauvage V et al. Early MinION™ nanopore single-molecule sequencing technology enables the characterization of hepatitis B virus genetic complexity in clinical samples. PLoS One. 2018; 13:e0194366.

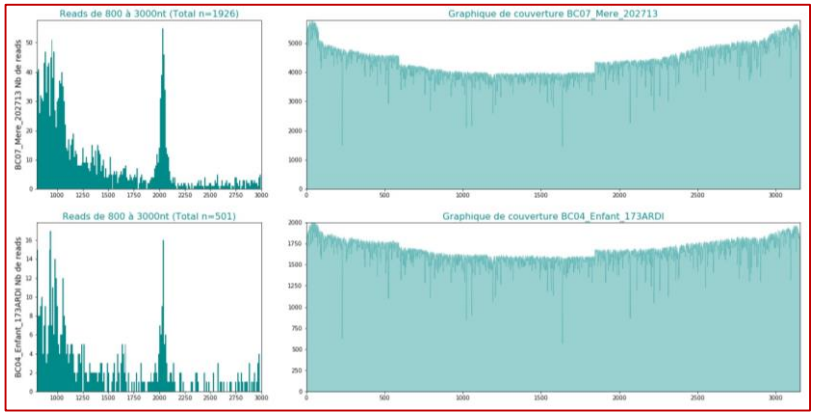
# HBV transmission

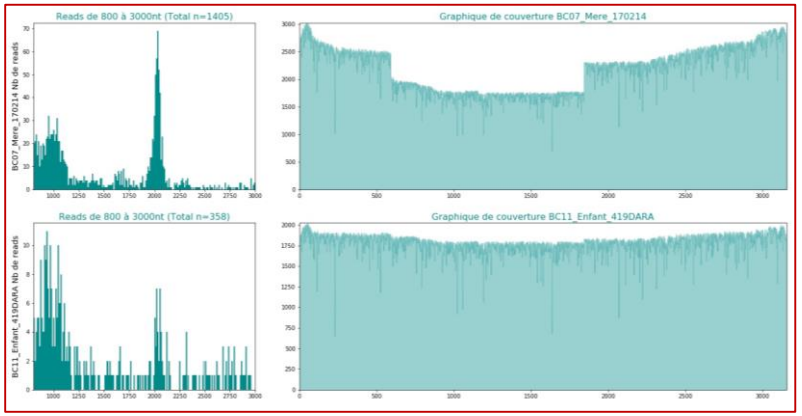
## Mother-to-child transmission of HBV genotype E in Cameroon

- **Aim: to obtain genetic evidence of HBV transmission based on whole genome analysis**
  - Limited genetic variability of $HBV_E$ --> analysis of long sequences needed
- **26 mother/child pairs analyzed --> 25 $HBV_E$ and 1 $HBV_D$**
- **Identical mother and child consensus sequences in 25 pairs**
  - Same variants present in corresponding mother and child sequences
- **21 nucleotide differences observed in 1 mother/child pair**
- **HBV vertical transmission supported in 25/26 (96%) pairs**

Mother

Child



# Conclusions

- **Potential contribution of 3rd generation sequencing to blood safety through viral metagenomics & deep genetic characterization of viral strains infecting blood donors and recipients**
  - Proactive surveillance of blood donor population
  - Identification of new viruses or emerging variants of known viruses
  - Resolution of difficult cases of transfusion-transmission

- **Advantages**
  - Method without a priori
  - Portability and affordability
  - Speed in data production: i.e. 20 min for HBV 3.2kb genome
  - Long single molecule sequencing
  - Immediate identification of recombinant viruses & large multiple in-frame deletions

- **Limitations**
  - Analytical sensitivity
  - Sequencing error rate potentially challenging single-nucleotide resolution but improving
  - Still not suitable for high throughput testing
  - Data processing & bioinformatics: development of specific softwares for viral sequences

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