Direct whole genome sequencing of poxvirus using Oxford Nanopore MinION

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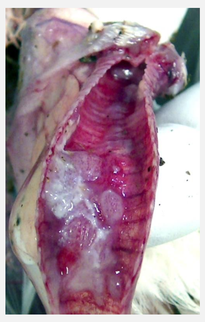
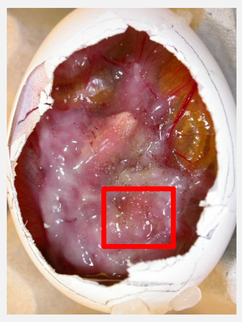
Material & methods



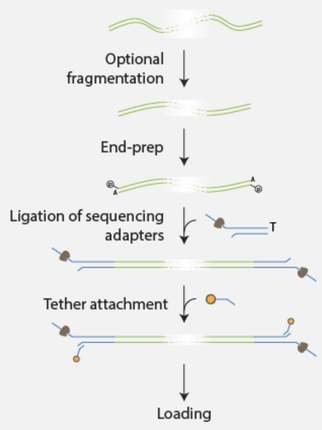


From tracheal lesion to embryonnated egg culture on the chorioallantois

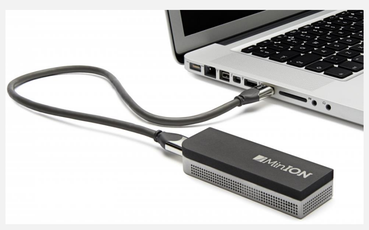
Tissue lysis O/N @ 55°C using SNET/PK buffer[1]

DNA extraction using Phenol:Chloroform: Isoamyl Alcohol[1]

1D Library prep



Sequencing on a R 9.4 Flowcell



Basecalling

METRICHOR
An Oxford Nanopore Company

Sequence alignment with Burrows-Wheeler Aligner[2]

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bwa mem -x pacbio
```

Alignment display with IGV[3]

igv

[1]**Sambrook J, Russell DW**. 2006. Preparation of genomic DNA from mouse tails and other small samples. CSH Protoc.
[2]**Li H**, *et al.* 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 25:1754–1760.
[3]**Robinson JT**, *et al*. 2011. Integrative genomics viewer. Nat. Biotechnol. 29:24–26.

Introduction and objectives

During fall 2015, two independent cases of fowlpox were diagnosed in commercial layer farms in western France. This disease is caused by an avian poxvirus, whose genome is linear and reaches 288 kb in length. They belong to the *Poxviridae* family (**Figure 1**), like the causative agent of variola.

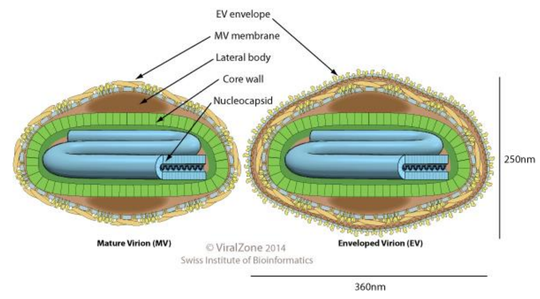

Figure 1. *Poxviridae* virion.

Diseased birds showed severe dyspnea and suffocation before death. The main macroscopic lesion was a subacute tracheitis with severe thickening of the mucosa. Almost no cutaneous lesion could be observed in any bird. All tracheal swabs and tissues sampled in both farms tested positive for PCR targeting p4b gene shared by all avipoxviruses.
The aim of this proof-of-concept study was to generate a full genome sequence, directly from the isolate. The genomes of poxviruses show repeated regions, which may be challenging for genome assembly from short reads. MinION sequencing was therefore assessed to obtain an easy *de novo* genome assembly.

Results

A total of 785 838 reads were obtained, of which 610 797 were filtered for quality and analyzed using Metrichor® (Oxford Nanopore Company). The average sequence length was 3.63 kb (**Figure 2**). A total of 39 625 viral reads were aligned on a reference fowlpox genome sequence using the Burrows-Wheeler Aligner software package[2] and a consensus sequence of 288 539 bp was obtained with a mean depth of 638 X (**Figure 3**).

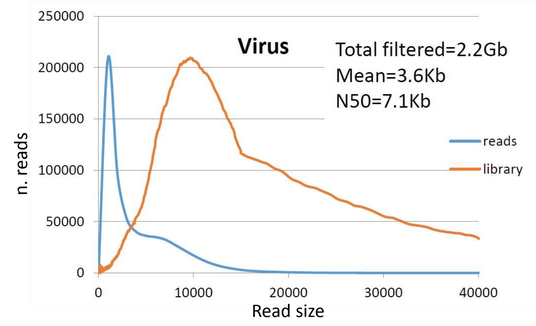

Figure 2. Size profiles of library submitted to and reads generated with the 1D sequencing kit.

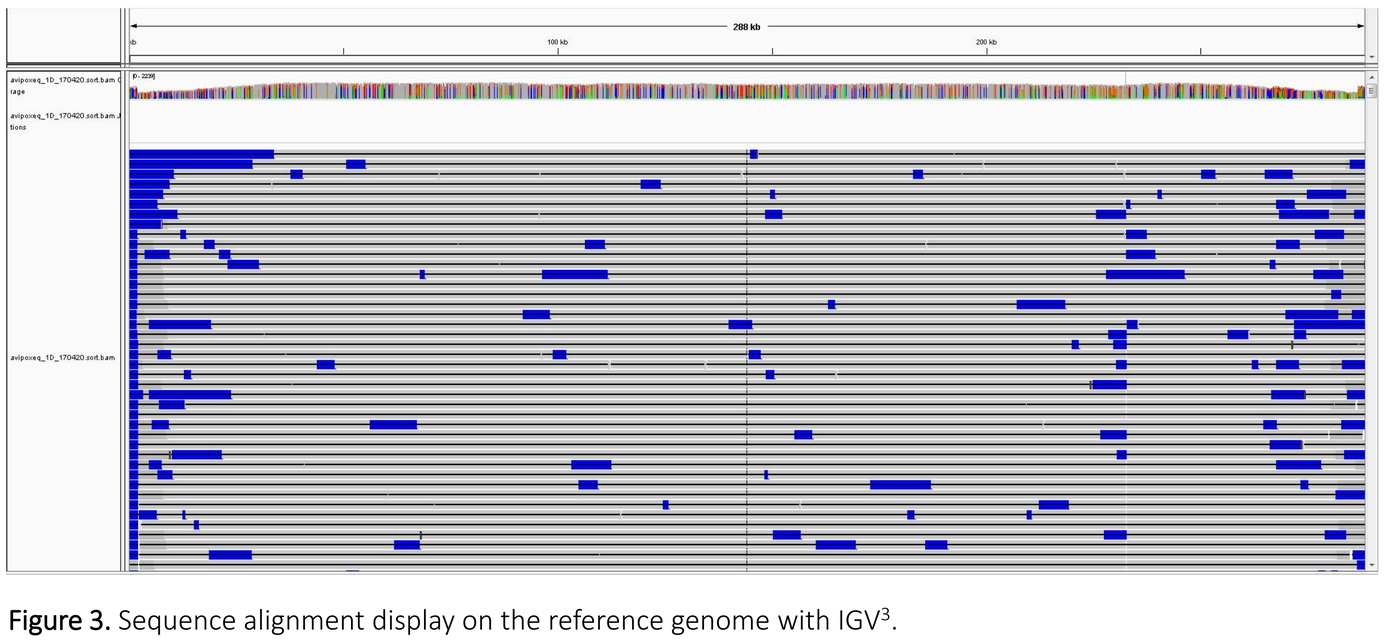

Figure 3. Sequence alignment display on the reference genome with IGV[3].

Discussion

This proof-of-concept study was performed to assess the suitability of MinION sequencing for surveillance of poxviruses of both medical and veterinary importance.
Using the 1D sequencing kit, we were able to readily generate the full genome sequence of a fowlpoxvirus.
The size of viral reads ranged from several hundred bp to 77kb in length, allowing an easy assembly.
The complete genome analysis confirmed that this fowlpox virus is clustered within clade A1 and hosts a full length reticuloendotheliovirus (REV) insert. The complete genomic features of this virus remain to be thoroughly investigated.
Further sequencing assays are being performed directly from tissues without propagation and the preliminary results are consistent with the data presented here.
In conclusion, MinION sequencing is suitable for rapid diagnostics of clinical samples and *de novo* assembly of poxvirus genomes.