Direct whole genome sequencing of avian poxvirus using Nanopore MinION

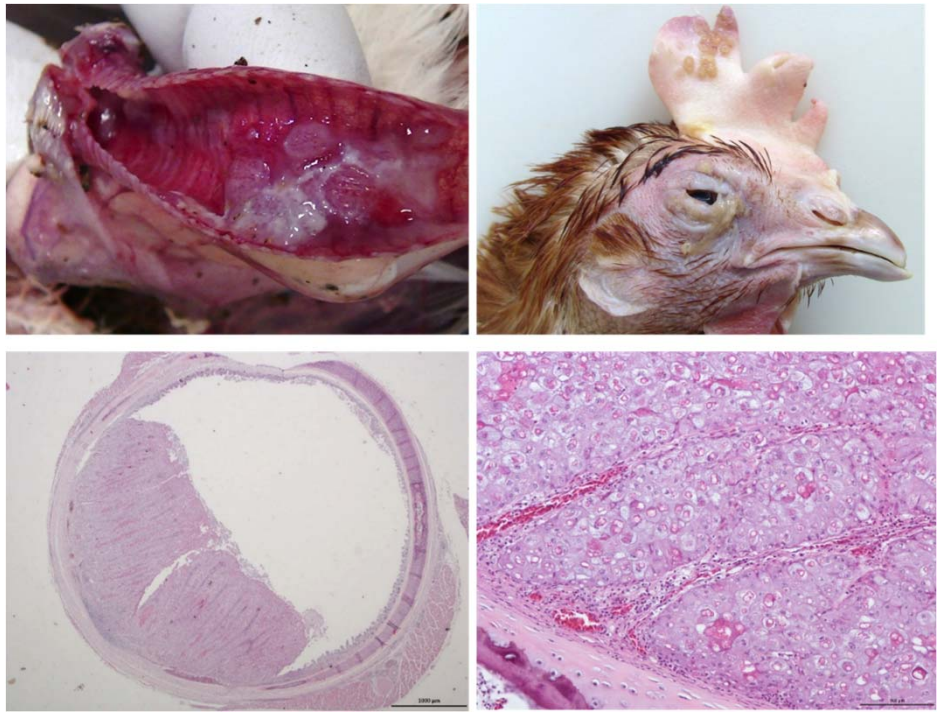
Guillaume Croville
UMR 1225 IHAP - INRA/ENVT

http://get.genotoul.fr
get@genotoul.fr
@get_genotoul

Clinical case

Aim of the project:

- Direct whole genome sequencing from lesion
- Avoid amplification bias
- Long-read for easy genome assembly



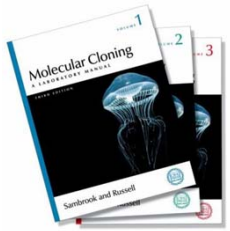
Long-read sequencing

You will…

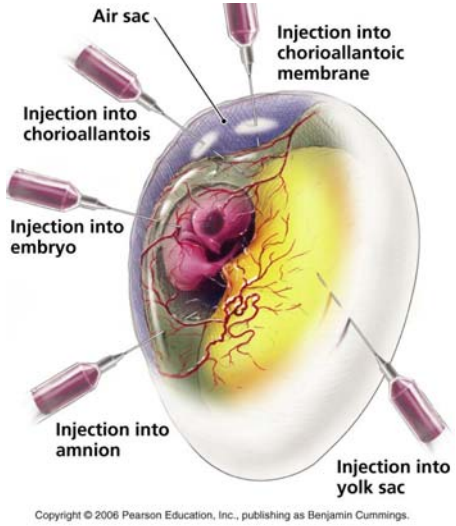
- Need good-quality sample
- Not use column for DNA extraction → Home-made lysis buffer:

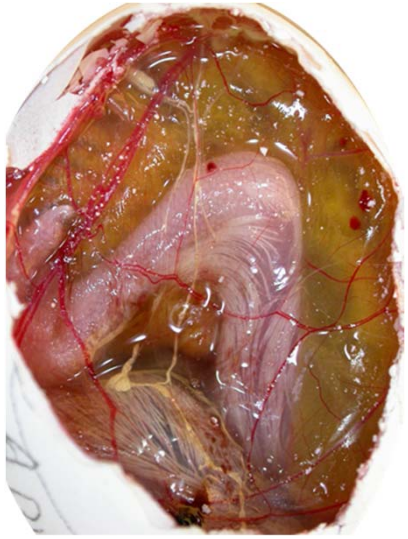
  SDS , NaCl, EDTA, Tris, PK

  Phenol:chloroform:isoamyl alcohol
- Not use vortex

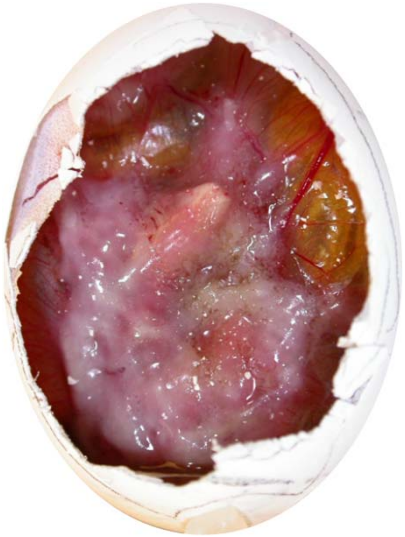


Protocol validation

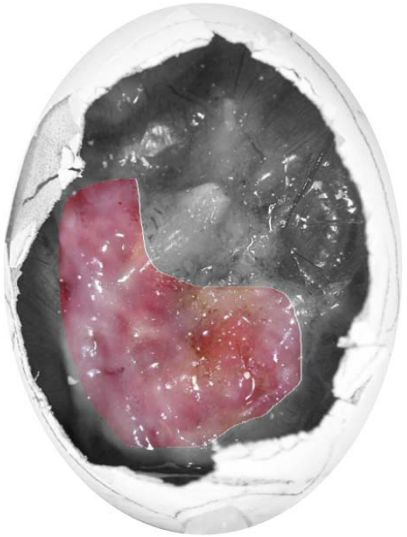




Negative control



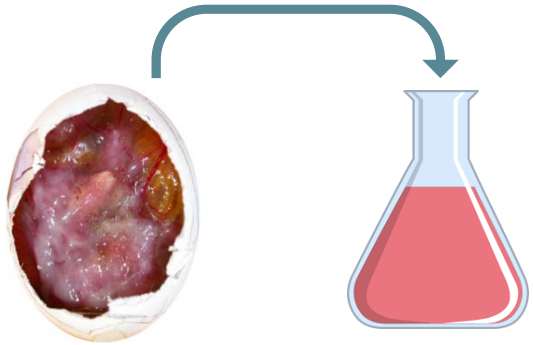
Infected



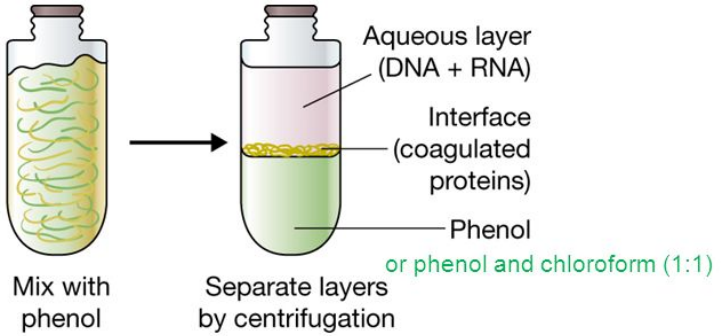
Infected

Virus propagated on chorioallantoic membrane

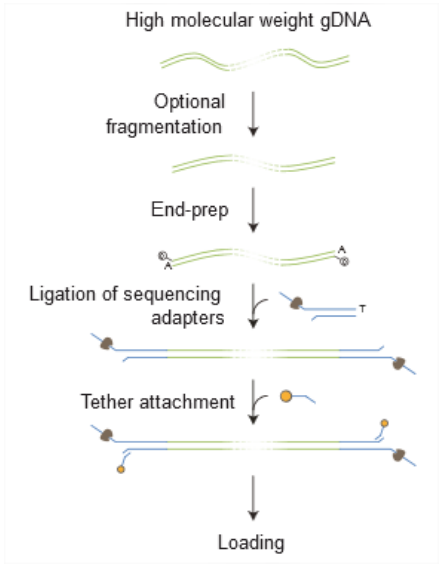
Protocol validation



O/N digestion with lysis buffer @ 55°C



Lib prep



Results from propagated virus

Total reads	Filtered sequences	Fowlpox sequences	Coverage	Sequencing depth
785 838	502 171	39 625	100%	638 X

```
Read 39625 items
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
     61    1533    4033    4968    7576   40360
```

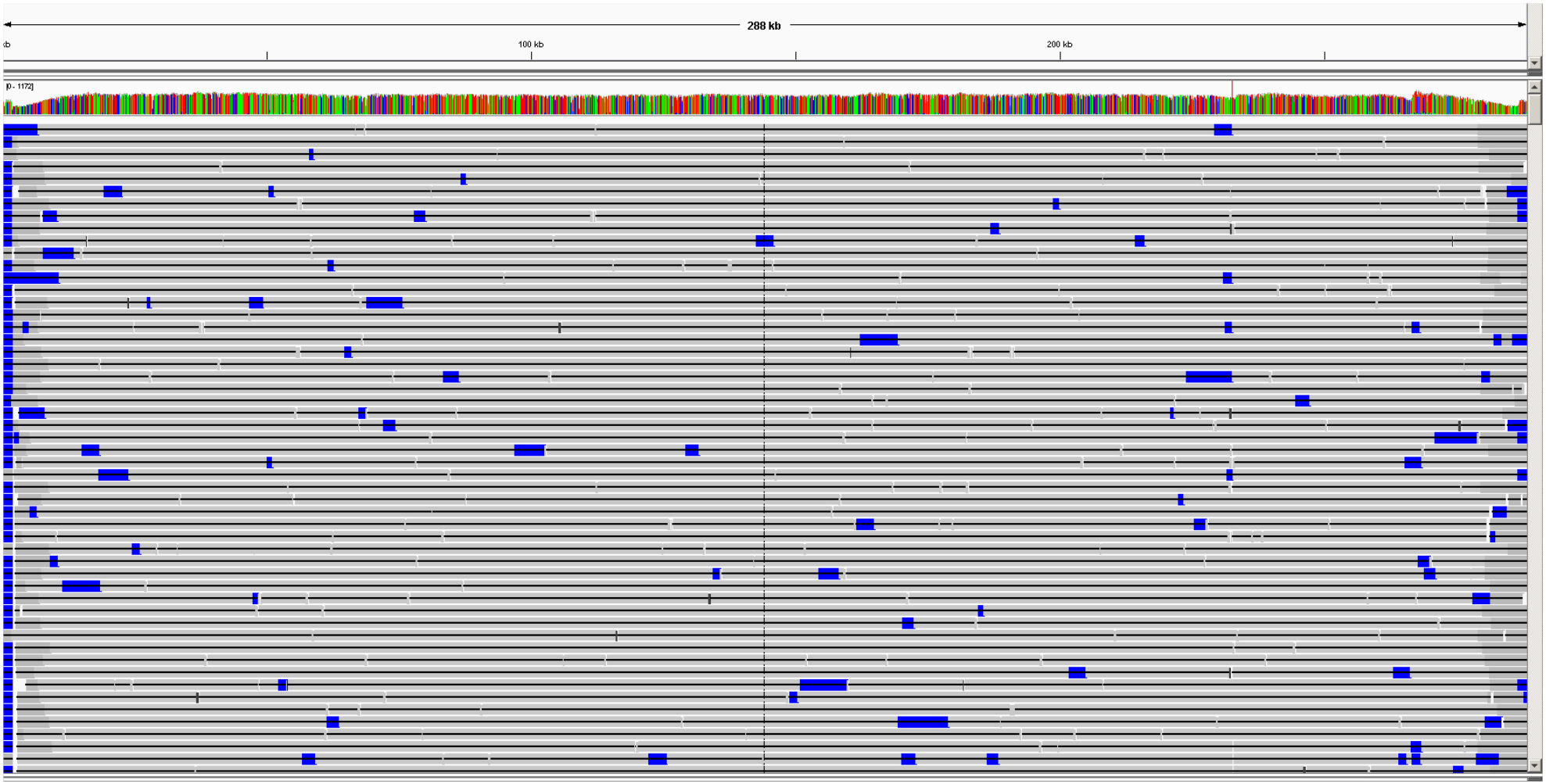
```
          (77,063 letters)

Database: viraldb
           933 sequences; 46,380,173 total letters

Searching.....................................................done



                                                                  Score    E
Sequences producing significant alignments:                       (bits) Value

ref|NC_002188.1| Fowlpox virus, complete genome                     2960   0.0
```

Bioinformatics

```
bwa index -a is fowlpox.fasta

bwa mem -x ont2d fowlpox.fasta virusRapid_nanonet.fastq > avipoxeq.sam

samtools sort avipoxeq1drapid.bam avipoxeq1drapid.sort

samtools index avipoxeq1drapid.sort.bam

samtools flagstat avipoxeq1drapid.sort.bam
```

```
gcroville@genotoul2 ~/work/minion/1D $ samtools flagstat avipoxeq1D.sort.bam
502171 + 0 in total (QC-passed reads + QC-failed reads)
0 + 0 secondary
1700 + 0 supplementary
0 + 0 duplicates
39625 + 0 mapped (7.89% : N/A)
```

Same analysis pipeline as used for 454 data



Integrative Genomics Viewer

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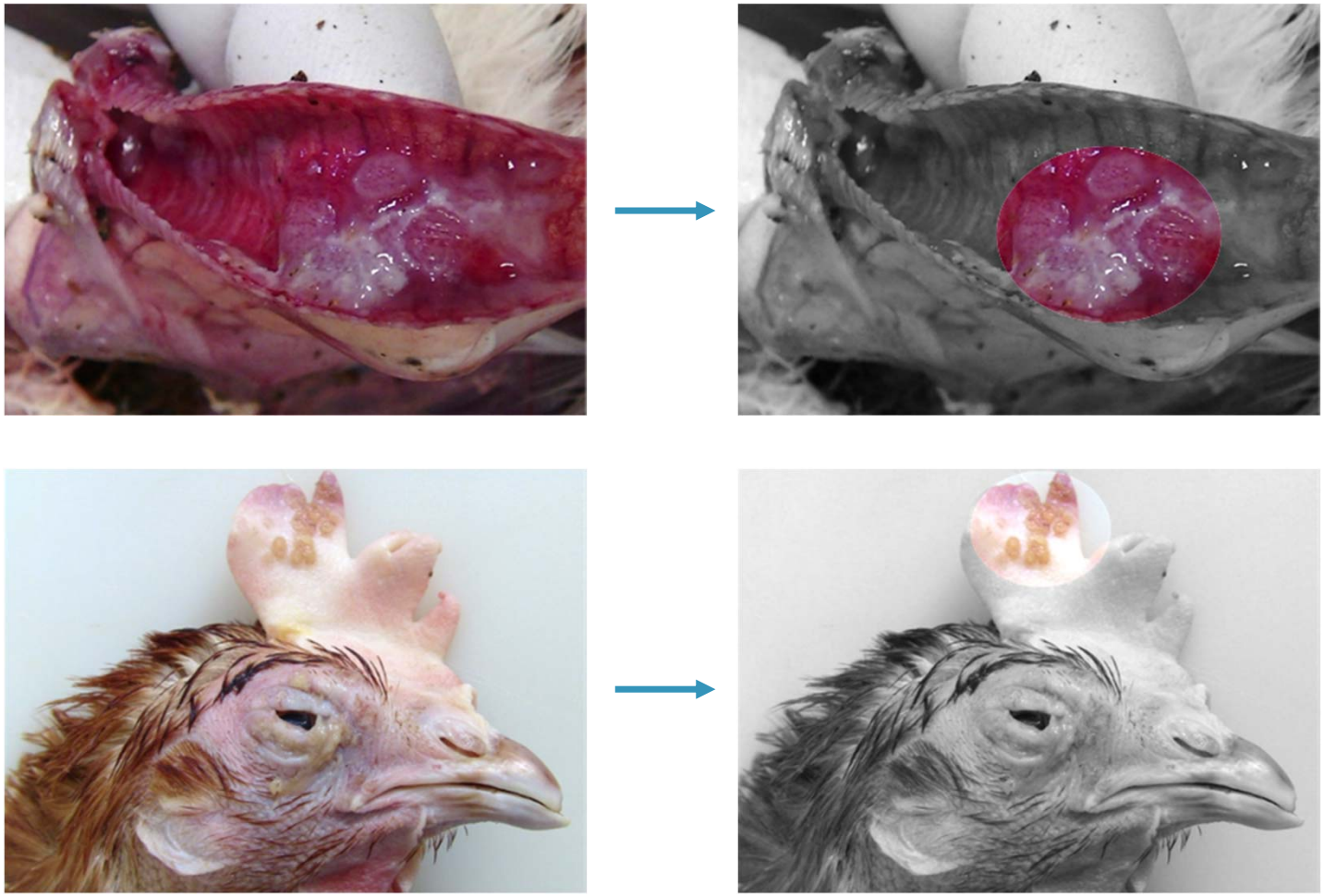
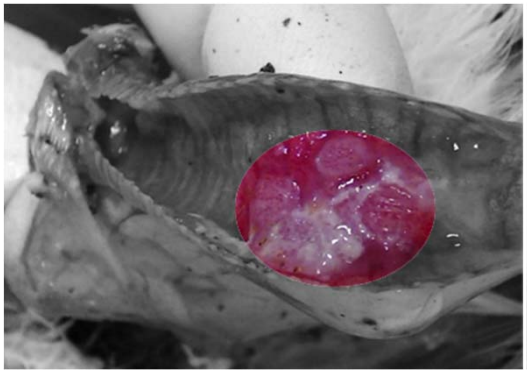
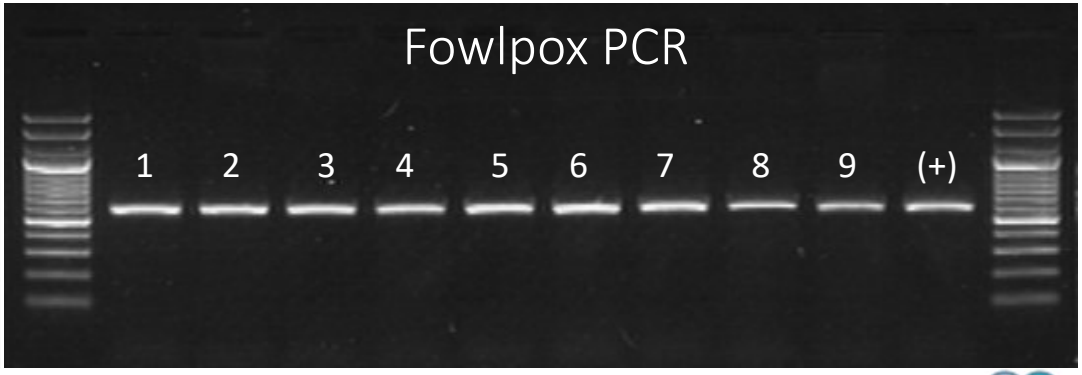
Direct sequencing from clinical lesions



Direct sequencing from clinical lesions

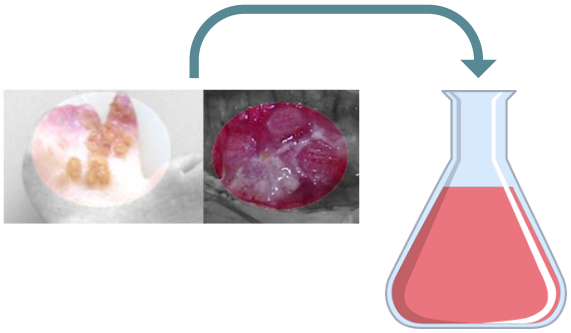
	dsDNA Concentration	260 / 280 ratio	260 / 230 ratio
Cutaneous lesion	1,8 µg/µL	1,77	2,13
Tracheal lesion	1,5 µg/µL	1,79	2,08
Tracheal lesion	1,1 µg/µL	1,81	2,03





Genotoul GeT

Protocol validation



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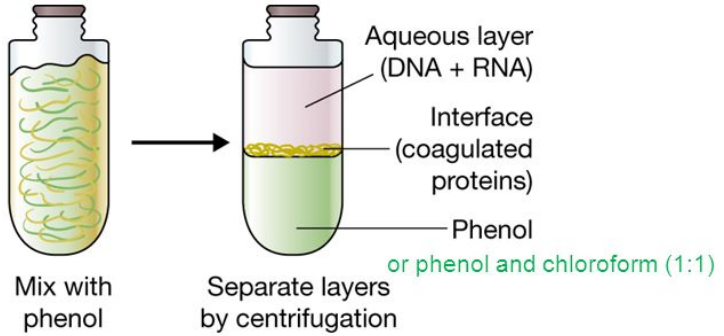
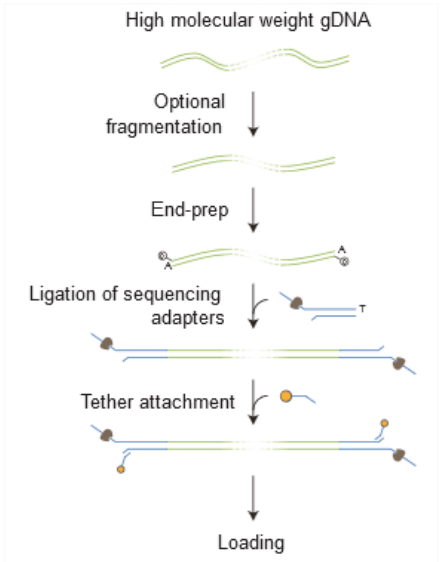


Lib prep

Multiplex



Direct sequencing from clinical lesions

	Filtered reads	Mean read size (bp)	Foxlpox reads	% identity vs ref. genome	Sequencing depth	Longest read
Cutaneous lesion	163 824	1 352	3 905	99,7	22X	61 912
Tracheal lesion	124 534	2 568	3 265	99,8	30X	49 439
Tracheal lesion	141 670	3 253	2 234	99,8	19X	37 780

Fowlpox whole genomes from 3 samples out of 10

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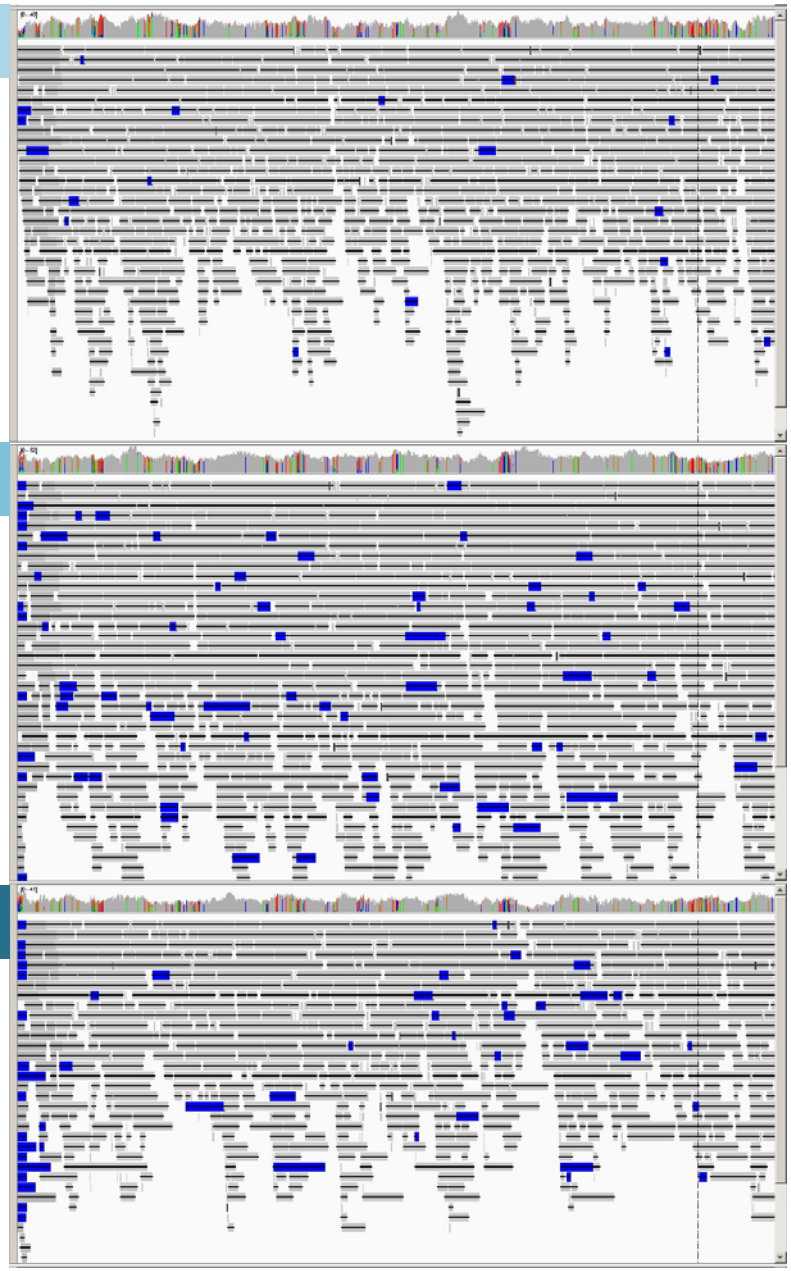
Cutaneous lesion

Tracheal lesion #1

Tracheal lesion #2



Feedback

- Whole genome sequencing from clinical lesion is therefore feasible.

- Sequencing fidelity and read size are adequate for genome assembly and virus identification.

A dream that became reality!

What's next?

- Whole-genome sequencing of other viruses from lesion:

  DNA, RNA viruses (e.g. influenza)

- Metagenomics project

- Generally speaking, ONT sequencing will be used for:

  Easy genome assembly, routine NGS sequencing in the lab,

  diagnostics? → waiting for « Flongle »

Acknowledgements