

# Rapid whole-genome sequencing of SAV using the MinION sequencer

**Michael Gallagher**

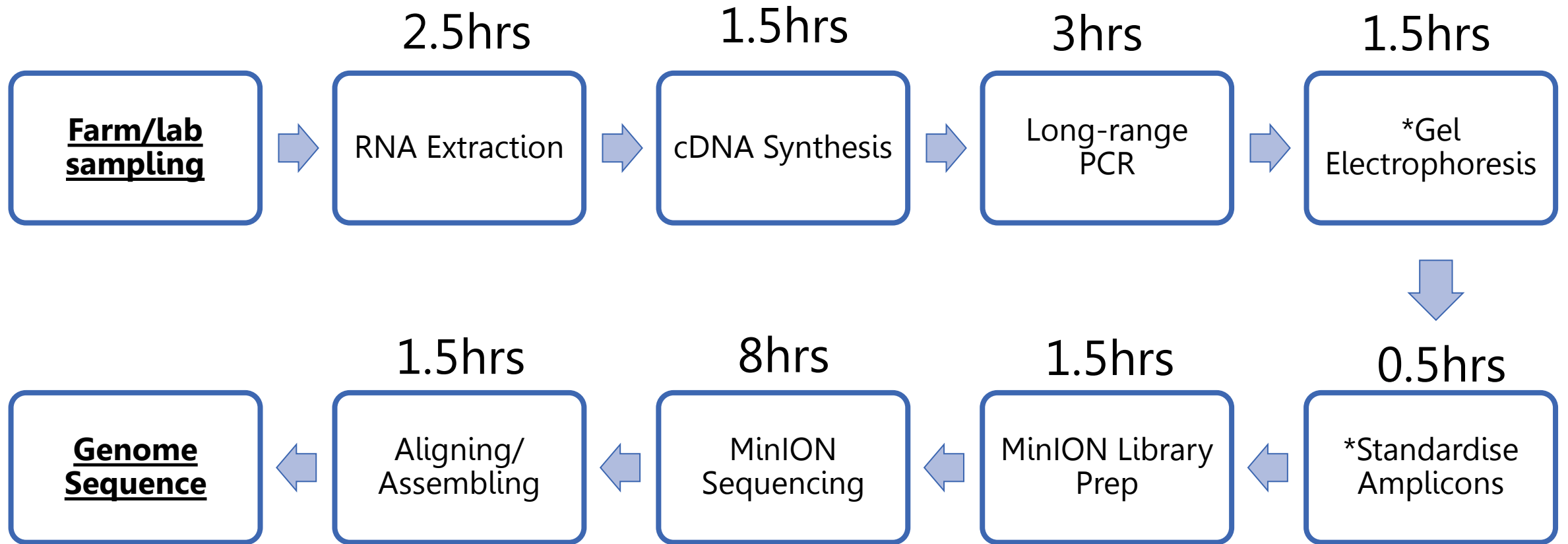
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# MinION - Oxford Nanopore Technologies

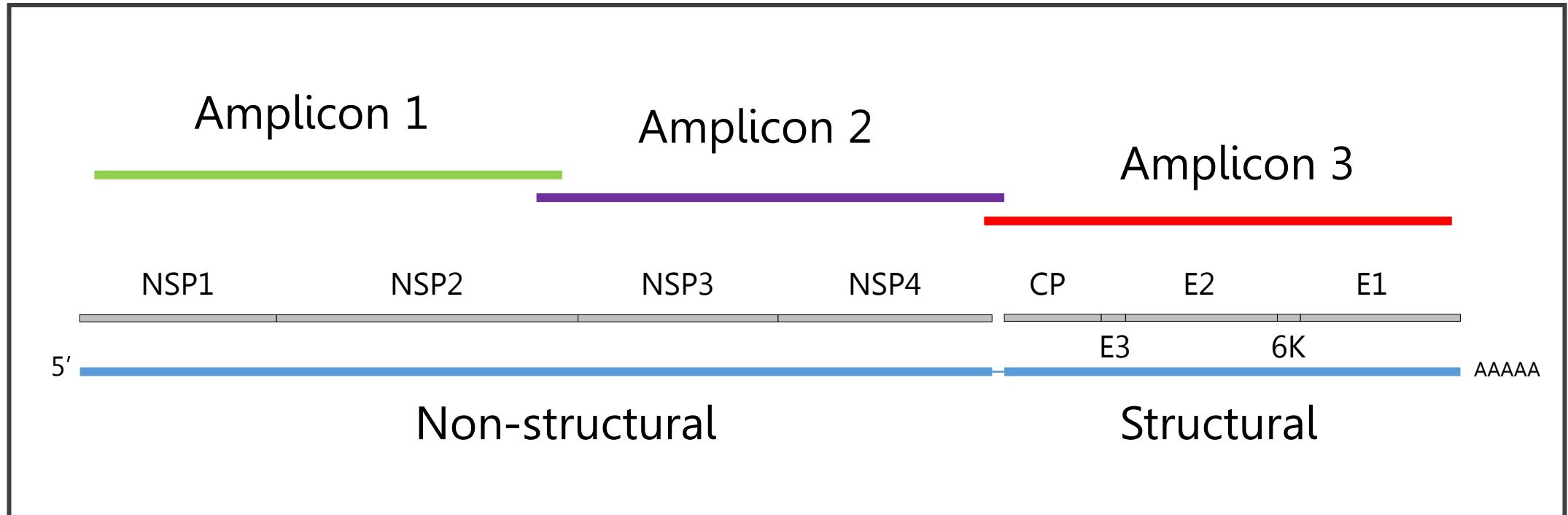
- Portable DNA and RNA sequencer capable of sequencing ultra-long reads
- Pros
  - Cheap infrastructure and portable
  - Real-time results possible
- Cons
  - Low per-base accuracy
  - Lower output than rival platforms (e.g. Illumina)



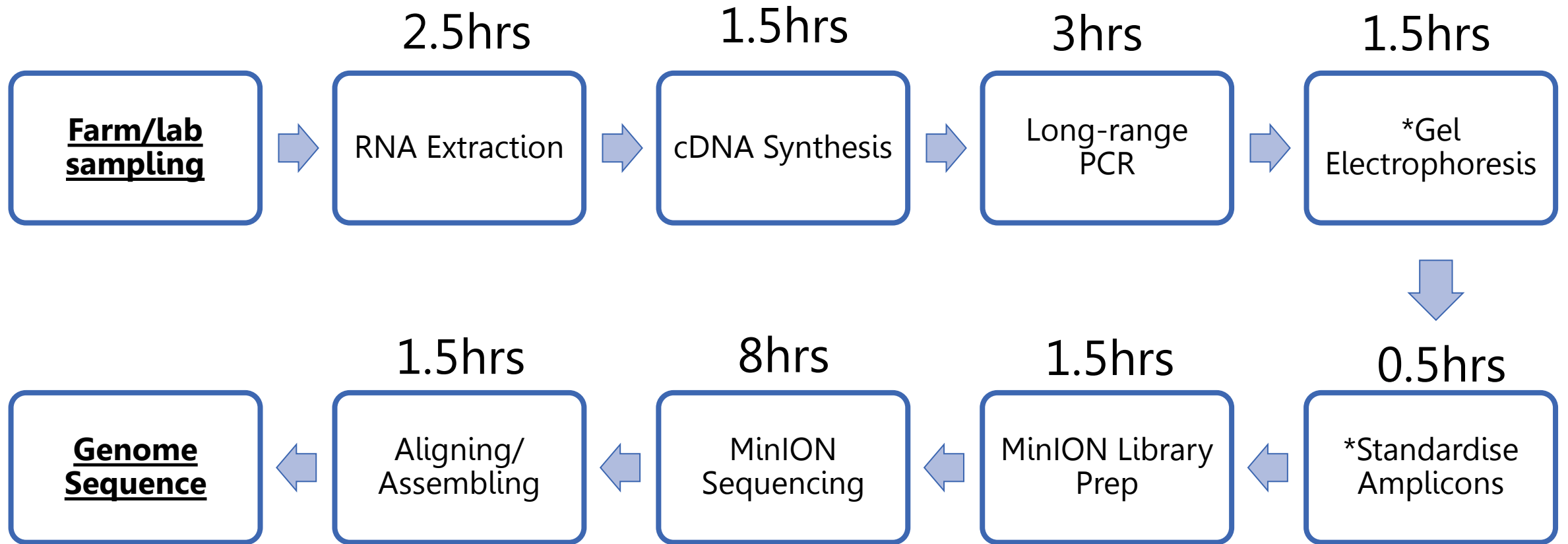
# 'Tissue-to-Genome' Workflow ~20 hours



# Whole Genome Amplification

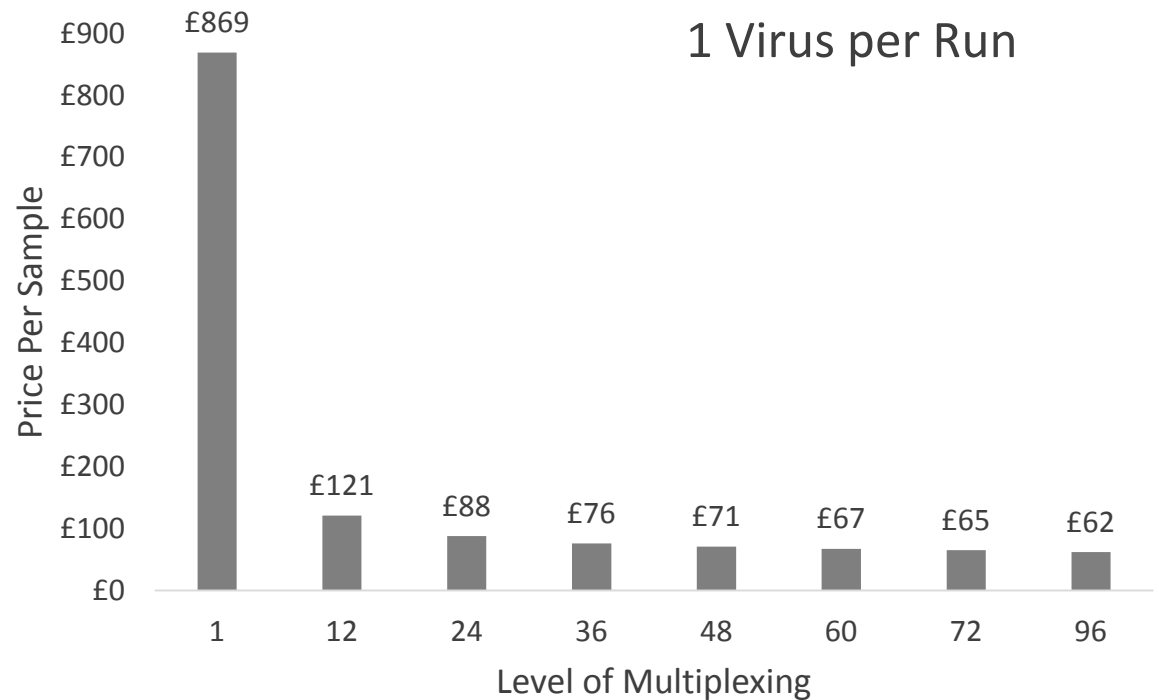


# 'Tissue-to-Genome' Workflow ~20 hours



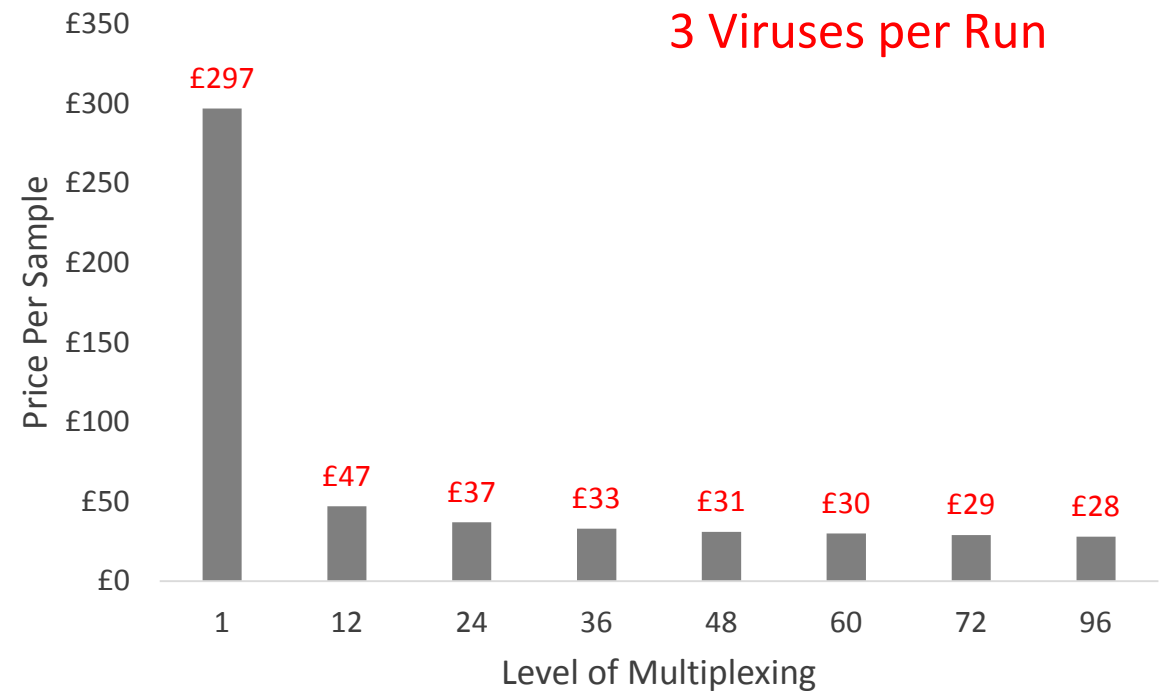
# Cost per SAV Genome Outline

- Initial set-up costs ~£1,500
- MinION cost per sample drops with multiplexing
- At an average sequencing rate ~7 hours required for 96 samples



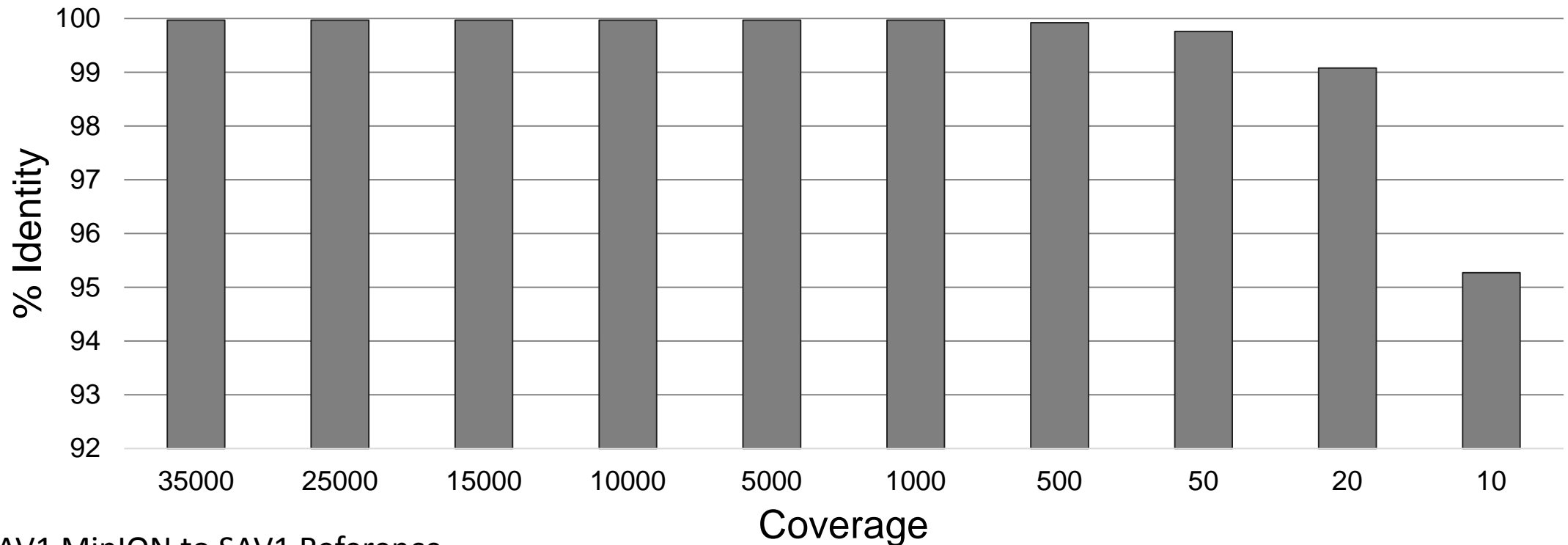
# Cost per SAV Genome Outline

- Initial set-up costs ~£1,500
- MinION cost per sample drops with multiplexing
- At an average sequencing rate ~21 hours required for 288 samples
- Potential to reduce costs by including multiple, distinct viruses
  - Separate out samples later



# High quality SAV genomes

- Despite a lower per-base accuracy than Illumina or Sanger,  $\geq 500x$  coverage achieves  $>99.9\%$  accurate consensus



SAV1 MinION to SAV1 Reference



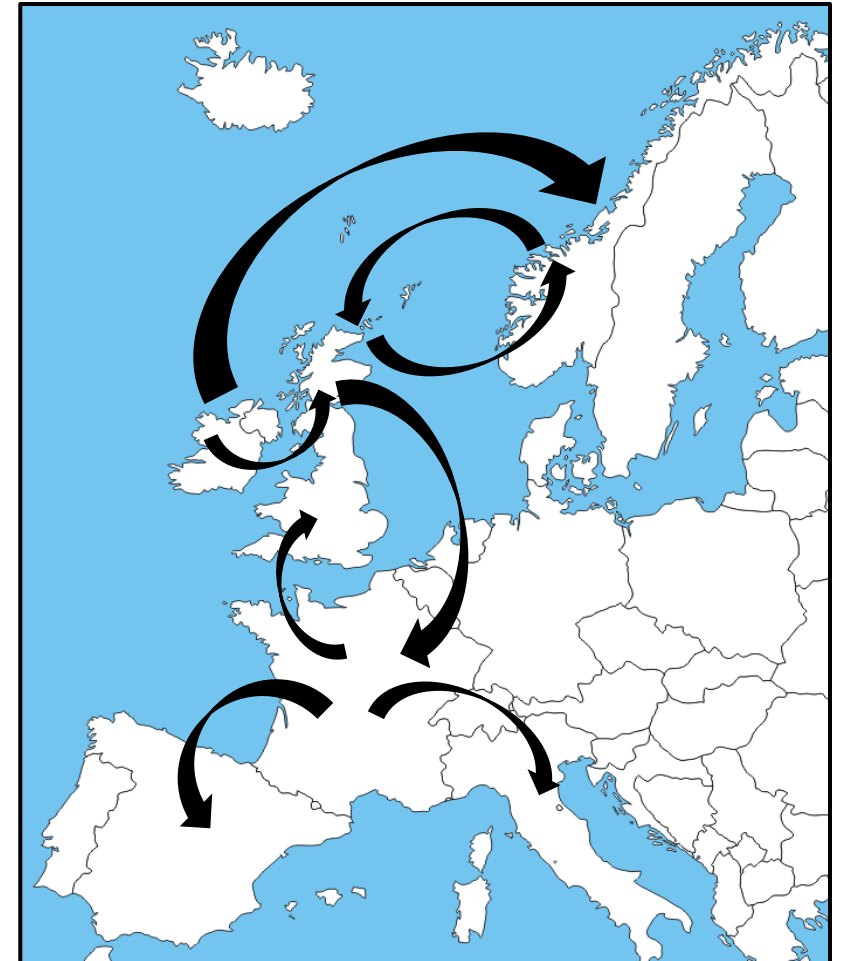
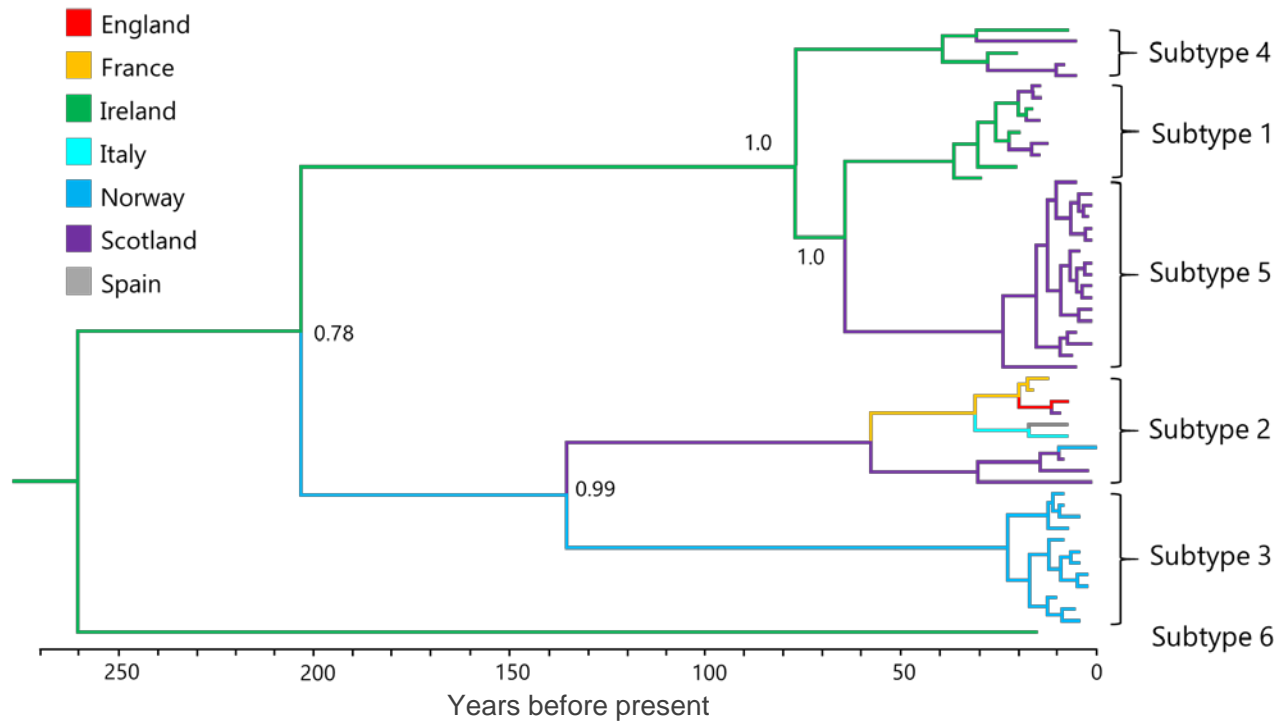
# SAV Subtype 6 Whole Genome

<u>Gene</u>	<u>% nuc identity</u>	<u>% AA identity</u>
NSP1	91.8	94.7
NSP2	89.9	95.6
NSP3	83.8	88.9
NSP4	87.9	95.6
CP	90.2	91.8
E3	88.7	93.0
E2	87.8	92.7
6K	91.2	95.6
E1	91.4	96.7
<u>Total genome</u>	<u>89.2</u>	<u>93.9</u>

- First whole genome sequence for subtype 6
- 100% identity to NsP3 and E2 fragments
- Sequenced in 2 days from cell culture RNA
- 89.2% nucleotide similarity to subtype 1

# Potential Uses of MinION Sequencing

- Pathogen origins and transmission dynamics



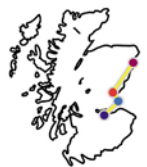


# Acknowledgements

- Dan Macqueen and lab group
- Iveta Matejusova and Marine Science Scotland
- Neil Ruane – SAV6 sample
- Lien Nguyen – Lab work help



**marinescotland**



**eastbio**  
the East of Scotland Bioscience Doctoral Training Partnership



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