Rapid whole-genome sequencing of SAV using the MinION sequencer

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• 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

Farm/lab sampling → RNA Extraction → cDNA Synthesis → Long-range PCR

2.5hrs 1.5hrs 3hrs

Aligning/Assembling → MinION Sequencing → MinION Library Prep

1.5hrs 8hrs 1.5hrs

*Gel Electrophoresis → *Standardise Amplicons

0.5hrs
Whole Genome Amplification

Amplicon 1

Amplicon 2

Amplicon 3

Non-structural

Structural

NSP1 NSP2 NSP3 NSP4 CP E2 E1

E3 6K

5’ AAAAA
‘Tissue-to-Genome’ Workflow ~20 hours

1. Farm/lab sampling
   - RNA Extraction: 2.5hrs
   - cDNA Synthesis: 1.5hrs
   - Long-range PCR: 3hrs
   - *Gel Electrophoresis: 1.5hrs

2. Genome Sequence
   - Aligning/Assembling: 1.5hrs
   - MinION Sequencing: 8hrs
   - MinION Library Prep: 1.5hrs
   - *Standardise Amplicons: 0.5hrs
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

![Price Per Sample vs Level of Multiplexing Graph]

1 Virus per Run
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, ≥500x coverage achieves >99.9% accurate consensus.

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SAV1 MinION to SAV1 Reference
SAV Subtype 6 Whole Genome

<table>
<thead>
<tr>
<th>Gene</th>
<th>% nuc identity</th>
<th>% AA identity</th>
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<tbody>
<tr>
<td>NSP1</td>
<td>91.8</td>
<td>94.7</td>
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<tr>
<td>NSP2</td>
<td>89.9</td>
<td>95.6</td>
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<tr>
<td>NSP3</td>
<td>83.8</td>
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<td>NSP4</td>
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<td>CP</td>
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<tr>
<td>E1</td>
<td>91.4</td>
<td>96.7</td>
</tr>
<tr>
<td>Total genome</td>
<td>89.2</td>
<td>93.9</td>
</tr>
</tbody>
</table>

- 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
Potential Uses of MinION Sequencing

- Identifying virulence markers
- Hypervariable regions

Hammoumi et al. 2016
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