VirusDetect pipeline
- virus detection with small RNA sequencing

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Big thanks to Jan Kreuze and Jari Valkonen!
Outline

- Small interfering RNA (siRNA) as an antiviral defense mechanism
- VirusDetect pipeline
  - central concepts
  - analysis steps
  - result files
- Using VirusDetect in Chipster
- Using VirusDetect on command line in the Taito cluster
RNA interference (RNAi)

- RNAi is an antiviral defense mechanism in eukaryotic organisms
  - Upon viral infection Dicer enzymes cut viral dsRNA to small interfering RNA (siRNA) molecules, which are 21-24 nucleotides long.
  - siRNAs associate with Argonaute proteins and guide the RNA-induced silencing complex (RISC) to degrade viral RNA
  - siRNAs are further amplified by RNA-dependent RNA polymerases (RdRP)

- We can sequence siRNAs and assemble virus genomes from the reads → virus detection and identification
Procedure

Extract RNA & run in 4% agarose gel

Cut and purify 20-30 nt band, send to sequencing provider for processing & sequencing on Illumina HiSeq 2000
VirusDetect – bioinformatics pipeline for detecting viruses in small RNA-seq data

- Combines several analysis tools in one tool
- Assembles sRNA reads to longer sequences (contigs) in two ways
  - Reference-guided assembly: match reads to known virus sequences and combine matching reads together
  - de novo assembly: match reads to each other
- Compares the contigs to known virus sequences
  - Uses BLAST for similarity search
  - If no similarity is found, reports siRNA size distribution profile

- Read more
  - http://virusdetect.feilab.net/cgi-bin/virusdetect/
Reference-guided assembly

Virus reference sequence, 1000 nt

Assembled 2 contigs, coverage is 80% (800/1000 nt)

Figure by Dr Jan Kreuze
Reference-guided assembly of contigs

- Align reads with the aligner BWA to reference virus database
- As a reference database VirusDetect uses GenBank virus sequences. They have been
  - Classified to 8 different host kingdoms
    - Vertebrate, invertebrate, plant, protozoa, algae, fungus, bacteria, archaea
  - Processed to remove redundancy (sequences that are more than 95% similar have been combined)
  - The same reference database is used later on for BLAST searches

- Perform reference-guided assembly of the aligned reads using Samtools
De novo assembly of contigs

- Remove host-derived small RNAs
  - Align reads to host genome with BWA, keep the unaligned reads

- Assemble de novo contigs with Velvet

- Remove host-derived contigs
  - Align contigs to host genome with BWA
VirusDetect steps for contig assembly

1. sRNA reads
   - Alignment to host genome (host organism)
   - Host derived sRNA reads
   - Unmapped sRNA reads
     - De novo assembly
     - Contigs
     - Host derived contigs
     - Alignment to host genome (host organism)
       - De novo contigs
     - Contigs
       - Virus reference based contigs
       - Virus reference database and reference-guided assembly
VirusDetect steps for virus identification

Contigs without hits → BLASTN against virus reference database. Result filtering* → BLASTN results (html, tsv, pdf, fasta, bam)

BLASTX against virus reference database. Result filtering* → Undetermined contigs

Undetermined contigs → siRNA size profiling

BLASTX results (html, tsv, pdf, fasta, bam) → Undetermined contigs (undetermined_contigs.fa, undetermined.html)

* Result filtering parameters:
- Minimum fraction of a contig covered by virus reference (0.75)
- Minimum fraction of virus reference covered by contigs (0.1)
- Minimum read depth (5)
Parameters for filtering BLAST results

- **Minimum fraction of a contig covered by virus reference**
  - The BLAST match must cover at least this fraction of the contig in order to make it significant for virus assignment. By default only contigs that match to the reference viruses for more than 75% of their length are considered.

- **Minimum fraction of virus reference covered by contigs**
  - Virus assignment is reported only if at least this percentage of the virus reference is covered with significant matches to contigs. The default is 10%.

- **Minimum read depth**
  - The average number of times each nucleotide of the reference sequence is covered by reads
VirusDetect log file and result tables

- **Vd.log**
  - Info on number of contigs assembled, reads that aligned, and viruses detected

- **blastn_matching_references.html**
  - Table listing reference viruses that have matching contigs identified by BLASTN.

- **Virus.bn.pdf**
  - Detailed BLASTN result for each virus reference match.

- **undetermined.html**
  - Table listing the length, siRNA size distribution and 21-22nt percentage of undetermined contigs. Potential virus contigs (21-22 nt > 50%) indicated in green.

- **undetermined_blast.html**
  - Table listing contigs that have hits in the virus reference database but not assigned to any reference viruses because they did not pass the 3 filtering criteria.

- **blastn_matches.tsv**
  - Table listing contigs listing that have hits in the virus reference database.
Vd.log

rocess sample inputseq (total read: 50000)
[11/01/17 07:16:10] Align reads to reference virus sequence database
  10987 reads aligned
  62 unique contigs were generated
  30315 reads aligned
  51 contigs were assembled
  No host-derived contig was removed
  39 unique contigs were generated
[11/01/17 07:16:46] Remove redundancies in virus contigs
  100 contigs were assembled
  No host-derived contig was removed
  70 unique contigs were generated
[11/01/17 07:16:54] Virus identification
  2 viruses were identified by nucleotide similarity (BLASTN)
  No virus was identified by translated protein similarity (BLASTX)
  Contigs having enrichment of 21-22nt sRNAs were identified as potential virus sequences.
Please check undetermined.html

###############################################################
Following output files were collected:

- **Sample_7_virusdetect_contigs.fa**
  - Sequences of non-redundant contigs derived through reference-guided and de novo assemblies.

- **Sample_7_contigs_with_blastn_matches.fa**
  - Sequences of contigs that match to virus references by BLASTN.

- **Sample_7_undetermined_contigs.fa**
  - Sequences of contigs that do not match to virus references.

- **Sample_7_blastn_matching_references.html**
  - Table listing reference viruses that have corresponding virus contigs identified by BLASTN.

  In addition, a pdf formatted report file is returned for each match.

- **Sample_7_blastn_matches.tsv**
  - Table of BLASTN matches to the reference virus database.

- **Sample_7_undetermined.html**
  - Table listing the length, siRNA size distribution and 21-22nt percentage of undetermined contigs.
  - Potential virus contigs are indicated in green.

- **Sample_7_undetermined_blast.html**
  - Table listing contigs having hits in the virus reference database but not assigned to any reference viruses because they did not meet the coverage or depth criteria.

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```
 total 320K
-rw-r--r-- 1 chipster chipster 24K Nov 1 07:17 Sample_7_blastn_matches.tsv
-rw-r--r-- 1 chipster chipster 2.7K Nov 1 07:17 Sample_7_blastn_matching_references.html
-rw-r--r-- 1 chipster chipster 14K Nov 1 07:17 Sample_7_contigs_with_blastn_matches.fa
-rw-r--r-- 1 chipster chipster 104K Nov 1 07:17 Sample_7_KJ534601.bn.pdf
-rw-r--r-- 1 chipster chipster 144K Nov 1 07:17 Sample_7_M72416.bn.pdf
-rw-r--r-- 1 chipster chipster 1.2K Nov 1 07:17 Sample_7_undetermined_blast.html
-rw-r--r-- 1 chipster chipster 129 Nov 1 07:17 Sample_7_undetermined_contigs.fa
-rw-r--r-- 1 chipster chipster 987 Nov 1 07:17 Sample_7_undetermined.html
-rw-r--r-- 1 chipster chipster 15K Nov 1 07:16 Sample_7_virusdetect_contigs.fa
```

Results have been collected to a single tar formatted archive file.
You can use tool: Extract .tar or .tar.gz file in Utilities folder to extract result files from the tar archive.
coverage = bases (percentage) of the reference that is covered by contigs
depth = the average number of times each reference base is covered by reads
depth norm = normalized depth: the average number of times each reference base is covered by reads, per million of total reads
% identity = the average percentage of sequence identity to the reference of all contigs aligned to that reference
% iden max = maximum percentage of sequence identity of the contigs to the reference
Good match has high coverage and high depth
• Blue = virus sequence
• Red = contigs (lighter color indicates lower identity)
Green color indicates potential virus contigs (21-22nt >50 %)

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<th>19</th>
<th>20</th>
<th>21</th>
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<th>32</th>
<th>33</th>
<th>21-22 (%)</th>
<th>Depth</th>
<th>Depth (Norm)</th>
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</tbody>
</table>
VirusDetect sequence files and BAM

- **virusdetect_contigs.fa**
  - Sequences of non-redundant contigs derived through reference-guided and *de novo* assemblies.

- **contigs_with_blastn_matches.fa**
  - Sequences of contigs that match to virus references by BLASTN.

- **undetermined_contigs.fa**
  - Sequences of contigs that do not match to virus references.

- **blastn_matching_references.fa and .fai.**
  - Virus reference sequences that produced BLASTN hits, and fasta index file

- **blastn_matches.bam and .bai.**
  - BAM file containing the BLASTN alignment of each contig to its corresponding virus reference sequence, and BAM index file.
How many reads should I have?

More reads give better reference coverage, longer contigs and more depth

Figure from Zheng Y et al (2017) VirusDetect: An automated pipeline for efficient virus discovery using deep sequencing of small RNAs. Virology 500:130-138
VirusDetect in Chipster

- Two tools in the “Small RNA-seq” category
  - VirusDetect
  - VirusDetect with own host genome

- As a result you get the Vd.log file and a tar package file
  - The tar package contains all the other result files. You can extract them using the tool “Utilities / Extract tar file”
  - If you used the tool “VirusDetect with own host genome”, the tar package contains also an index file for your host genome. Use it in the next analysis instead of the fasta genome file (genome indexing can take a couple of hours)

- You can practise with example sessions
  - course_VirusDetect_potato_DONE
  - course_VirusDetect_raspberry_DONE