Viral metagenomic analysis on Cabbage Patch Kids

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Abstract

This protocol is a parody of an existing scientific journal article and is intended to showcase the features available on the protocols.io platform in order for VERVE Net members to develope future protocols. While the viral metagenomic analysis on Cabbage Patch Kids is impossible to perform, due to the real-world non-existant nature of Cabbage Patch kids, the sourced article is *Metagenomic analysis of viruses associated with field-grown and retail lettuce identifies human and animal viruses* and the "Material and methods" section has been adapted from using actual lettuce data to a metagenomic protocol for Cabbage Patch Kids.

Citation: Stanton Burnton Viral metagenomic analysis on Cabbage Patch Kids. **protocols.io** dx.doi.org/10.17504/protocols.io.eqtbdwn
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Before start

It is adviseable to obtain all necessary materials prior to obtaining field Cabbage Patch Kids. Cabbage Patch Kids mutate quickly from field removal and must be maintained in an adequate environment to prevent a syndrome what has recently been described as *Ravage Patch Kids.*

Protocol

Cabbage Patch Kid (CPK) collection for metagenomic analysis

Step 1.
Collect Cabage Patch Kids (CPK) at varying stages of farming production, including:

- hand-cut by field workers and packaged
- washed and cut by processing workers
- hand-cut by researchers using sterile equipment
- produce distribution centers

Cabbage Patch Kid (CPK) collection for metagenomic analysis

Step 2.
Use sterile gloves and cut outer leaflets off using a scapel, careful not to harm the body of the CPK, before placing CPK in large sterile Whirl-pak bags.

Virus recovery from CPK

Step 3.
Wash each sample in the Whirl-pak bag with 250 ml sterile 100 mM Tris — 50 mM glycine buffer at a pH of 9.5 and gently mix for 20 min at room temperature. Recover the wash solution immediatly and adjust the pH to neutral 7.2 ± 0.2

AMOUNT
250 ml Additional info:

DURATION
00:20:00

PROTOCOL

. Tris-glycine buffer

CONTACT: Stanton Burnton
Step 3.1.
Mix 100mM Tris with 50mM glycine to yield a buffer

AMOUNT
250 ml Additional info:

Virus recovery from CPK

Step 4.
Use polyethylene glycol (PEG) precipitation to concentrate and purify the viral particles contained in the wash solution.

Virus recovery from CPK

Step 5.
Mix samples with 10% (weight/volume) PEG 8000 and .3 M NaCl (weight/volume), then incubate at 4 °C for 18 hours before centrifuging the samples at 10,800 × *g* (8000 rpm) for 30 min at 4 °C.

DURATION
18:30:00

Virus recovery from CPK

Step 6.
Pour off the supernatant and dissolve the pellet in 20 mL of sterile phosphate buffered saline, letting soak for 1 hour at room temperature

AMOUNT
20 ml Additional info:

REAGENTS
1X PBS (Phosphate-buffered saline) by Contributed by users

DURATION
01:00:00

Virus recovery from CPK

Step 7.
Add an equal volume of chloroform to each PEG precipitate to remove the PEG and purify the sample.

REAGENTS
Chloroform by Contributed by users

Virus recovery from CPK

Step 8.
Vortex the solutions for 30 seconds and centrifuge at 3000 × *g* (4300 rpm) for 15 min at 4 °C to collect the supernatant containing virus particles.

DURATION
00:15:30

Virus recovery from CPK

Step 9.
Pass the remaining supertanant through 0.45 and 0.22 µm filters and further concentrate to approximately 1 mL by Amicon centrifugal ultrafiltration (30 kDa)

Nucleic acid extraction and sequencing

Step 10.

Treat the final 1 mL concentrates with 100 units of DNase-I for 1 hour at 37 °C before nucleic acid extraction to remove free nucleic acids from the concentrated virus samples.

REAGENTS

deoxyribonuclease I (DNase I, 100 U/ml) D 4263 by Sigma Aldrich

DURATION

01:00:00

Nucleic acid extraction and sequencing

Step 11.

Extract viral DNA and RNA using a PureLink viral RNA/DNA mini kit (Life Technologies) following the manufacturer's instructions. For each viral concentrate, prepare three individual nucleic acid extracts to minimize nucleic acid extraction bias.

Nucleic acid extraction and sequencing

Step 12.

Following extraction, screen the samples with 16S ribosomal DNA (rDNA) PCR with 27F/1492R universal primers to ensure the absence of any contaminating microbial DNA.

NOTES

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To obtain a sufficient DNA and cDNA (for RNA viruses) for metagenomics sequencing, the viral nucleic acids were reverse transcribed and amplified as previously described

Nucleic acid extraction and sequencing

Step 13.

Reverse transcribe RNA with Primer A (5′-GTTTCCCAGTCACGATCNNNNNNNNN-3′) using Superscript III reverse transcriptase

Nucleic acid extraction and sequencing

Step 14.

Use Sequenase 2.0 for second-strand cDNA synthesis and for random-primed amplification of viral DNA. Subject each sample to 40 cycles of PCR amplification with Primer B (5′-GTTTCCCAGTCACGATC-3′) using AmpliTaq Gold

Nucleic acid extraction and sequencing

Step 15.

Perform three PCR reactions from the same nucleic acid extract to minimize amplification bias and pool the PCR products. Purify PCR products using Promega Wizard SV Gel and a PCR Clean-Up System

Nucleic acid extraction and sequencing

Step 16.

Prepare libraries from each sample using a Rubicon ThruPLEX DNA-seq kit with a unique dual index adapter pair for each sample. Sequence samples in a 2 × 100-base pair (bp) paired end format using two lanes of an Illumina HiSeq 2500 Rapid Run flow cell

Bioinformatics analysis of viromes

Step 17.

Screen each dataset for the 17-bp Primer B sequence and any reads homologous to the Primer B sequence at their 5′ ends, removing using cutadapt with a maximum error rate of 0.2 and minimum overlap of 10 bases

NOTES

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Prior to assembly and annotation of the metagenomic dataset, the quality of the Illumina sequencing data was checked using FastQC

Bioinformatics analysis of viromes

Step 18.

Use Trimmomatic for sequencing adapter removal and quality trimming with parameters including : a maximum mismatch count value of 2 allowed for a full match (seed mismatch), a palindrome clip threshold of 30, a simple clip threshold of 10, a minimum adapter length of 8 with both the forward and reverse read kept, removal of low quality leading and trailing bases below a quality of 3, a 4-base sliding window scan that cuts when the average quality is below 15, and removal of reads less than 30 bases long

Bioinformatics analysis of viromes

Step 19.

Following filtering and trimming of raw reads, subject paired-end reads to *de novo* assembly into a longer contiguous sequence (contig) using IDBA-UD

Bioinformatics analysis of viromes

Step 20.

Query Contigs larger than 200 bp were then against the National Center for Biotechnology Information (NCBI) Viral Reference Sequence (RefSeq) database for taxonomic assignment using BLASTX with an E-value cutoff of 10^{-5}

Bioinformatics analysis of viromes

Step 21.

Parse the BLASTX output using the MEtaGenome Analyzer (MEGAN) version 5.6.6 with the following parameters for the Lowest Common Ancestor (LCA) algorithm: min score = 50.0, max expected = 1.0 E^{-5}, top percent = 10.0, min support percent = 0.1, min support = 1, and LCA percent = 100.0

Bioinformatics analysis of viromes

Step 22.

Extract contigs identified as viral pathogens of human and animal and use them as the queries in BLASTX against the NCBI non-redundant (nr) sequence database.

NOTES

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For rotavirus contigs, BLASTN was used

Bioinformatics analysis of viromes

Step 23.

To determine relative abundance of a phylogenetic group, perform read mapping to contigs using Bowtie 2 version 2.1.0 with default settings

Bioinformatics analysis of viromes

Step 24.

Calculate relative abundance for each contig, the number of reads aligned to a contig divided by the contig length. Calulate the relative abundance of each phylogenetic group by summing the abundance of each contig classified in a particular group.

Warnings

Take care not to harvest live Cabbage Patch Kids, as legislation prohibits the possession, distribution, and sale of live Cabbage Patch Kids. Please refer to the government guidlelines set in place which detail the legalities of Cabbage Patch Kid farming and production.

This protocol is derived from a journal article in the International Journal of Food Microbiology about lettuce and not actually a protocol used to conduct a viral metagenomic analysis on Cabbage Patch Kids.