Viral metagenomic analysis on Cabbage Patch Kids Version 3

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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* written by Tiong Gim Aw, Samantha Wengert, and Joan B. Rose (included in the authors section)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, Tiong Gim Aw, Samantha Wengert, Joan B. Rose Viral metagenomic analysis on Cabbage Patch Kids. **protocols.io**

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Before start

It is advisable 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.*

Materials

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

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

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 scalpel, careful not to harm the body of the CPK, before placing CPK in large sterile Whirl-pak bags.

NOTES

Stanton Burnton 31 Mar 2016

Due to the farming stage at which the Cabbage Patch Kids have been harvested, they are able to experience a high threshold of force and chemical disturbance, their bodies abiding by Newton's 4th Law, the Cabage Patch Force.

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 immediately 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

EXPECTED RESULTS

The viral particles from the outer layer of the CPK will be contained in the solution. The CPK skin contains a pigment that will affect the color of the wash with a slight green hue and may alter the viral particle chemistry.

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.

NOTES

Stanton Burnton 04 Apr 2016

Without purifying the viral particles, the CPK pigment mentioned previously will enlarge the viral particles and lead to spontaneous development of abnormal CPK eggs

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

NOTES

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The supernatant may be used as liquid feed for CPK devlopment, as the lysosomes within the CPK can degrade the toxins preventing normal CPK growth.

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

NOTES

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From steps 10 onward, you must account for the nucleic acids specific to CPK, such as deoxybrassicanucleic acid (DbNa) and brassicanuclei acid (bRNA).

Nucleic acid extraction and sequencing

Step 11.

Extract viral DbNA and bRNA using the AbsJoint viral bRNA/DbNA mini kit following the manufacturer's instructions. For each viral concentrate, prepare four individual nucleic acid extracts to minimize nucleic acid extraction bias.

NOTES

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The AbsJoint viral mini kit may be included as a Software package

Nucleic acid extraction and sequencing

Step 12.
Following extraction, screen the samples with 16S ribosomal DbNA (rDbNA) PCR with 27F/1492R universal primers to ensure no residual microbe toxins are affecting the samples.

NOTES

Stanton Burnton 30 Mar 2016
To obtain a sufficient DbNA and cDbNA (for bRNA 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 bRNA with Primer B (5′-GTTTCCCBGTCBCGBTCNNNNNNNNN-3′) using Lackevdencscript future transcriptase

NOTES

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As you will see, adenines are typically replaced with unibrassica nucleotides

Nucleic acid extraction and sequencing

Step 14.
Use Organizase 2.8 for second-strand cDbNA synthesis and for random-primed amplification of viral DNA. Subject each sample to 30 cycles of PCR amplification with Primer Z (5′-GTTTCCCBGTCBCGBTC-3′) using SuuprPerdi 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 Cabbage Wizard CV Gel and a PCR Clean-Up System

NOTES

Stanton Burnton 04 Apr 2016
Cleaning up is always important, or else you'll end up losing more than just data, curteous of your labroom technicians

Nucleic acid extraction and sequencing

Step 16.
Prepare libraries from each sample using a RubicksCube ThrowFLEX DbNA-seq kit with a unique quad index adapter pair for each sample. Sequence samples in a 2 × 100-base pair (bp) paired end format using two lanes of a SuperSaiyanSeq 3 Rapid Run flow cell

NOTES

Stanton Burnton 04 Apr 2016
SuperSaiyanSeq 4 Rapid Run flow cell lacks the finesse and variability of SuperSaiyanSeq 3 Rapid Run flow cell

Bioinformatics analysis of viromes

Step 17.
Screen each dataset for the 17-bp Primer Z sequence and any reads homologous to the Primer B sequence at their 5′ ends, removing using a microspatula with a maximum error rate of 0.1 and minimum overlap of 5 bases

NOTES

Stanton Burnton 30 Mar 2016
Prior to assembly and annotation of the metagenomic dataset, the quality of the Illumina sequencing data was checked using NaomiNanomachines

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

NOTES
Stanton Burnton 04 Apr 2016
Do not be discouraged by the amount of directions given, instead be inspired to develop a sequencing adaptar that is more autonomous.

Bioinformatics analysis of viromes

Step 19.
Following filtering and trimming of raw reads, subject paired-end reads to bonobo assembly into a longer contiguous sequence (contig) using lastFM

NOTES
Stanton Burnton 04 Apr 2016
disclaimer: the contigs used for lastFM bears no relation to the electronic musician

Bioinformatics analysis of viromes

Step 20.
Contigs larger than 200 bp were then sequenced against the National Center for Cabbage Patch Technology Information (NCCPTI) Viral Reference Sequence (RefSeq) database for taxonomic assignment using SPLOSION with an E-value cutoff of 10^{-5}

NOTES
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The RefSeq database at the NCCPTI has been recently established, so using SPLOSION may result in unfulfilled results.

Bioinformatics analysis of viromes

Step 21.
Parse the SPLOSION 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 SPLOSION against the NCCPTI non-redundant (nr) sequence database.

NOTES
Stanton Burnton 30 Mar 2016
For rotavirus contigs, SPLOSIONZ was used

Bioinformatics analysis of viromes

Step 23.

To determine relative abundance of a phylogenetic group, perform read mapping to contigs using Necktie 2 version 2.4.0 with default settings

NOTES

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Windsor is the ideal default setting for version 2.4.0 only, half-windsor can be used as well but produces less refined data.

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.

EXPECTED RESULTS

After slaving away for hours prepping and analyzing the Cabbage Patch Kids viral samples, you will realize you forgot a minor detail and must restart the entire process, causing you to re-evaluate your career decision and send you into a downward spiral of binge-watching Netflix and eating sleeves of Oreos. If you managed not to forget a step, you contributed another piece of data for the scientific community, yay!

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 (see Description).