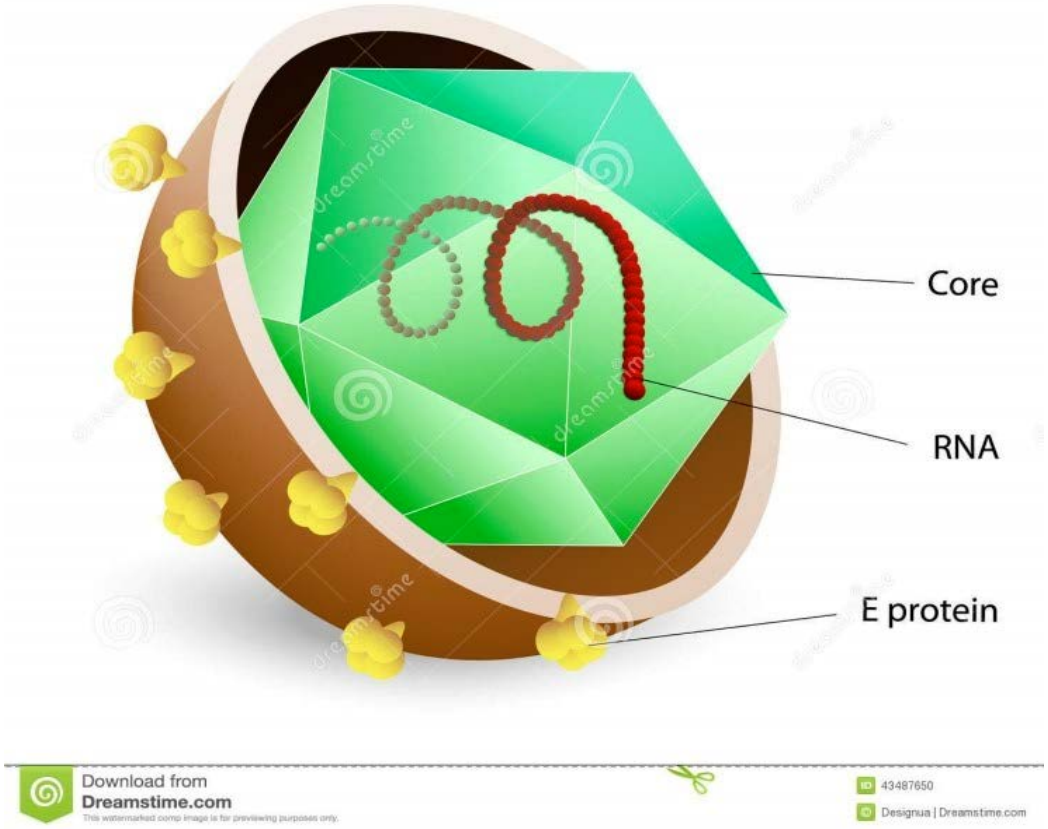
Application of Next Generation Sequencing for HCV

Kevin M. Rodeman
MDHHS Bureau of Laboratories

Hepatitis C virus



- HCV infects nearly 3% of the world's population.
- In the U.S. HCV is the most common chronic blood-borne infection and the leading cause of liver transplants.
- Since 2007 in U.S., HCV caused greater annual deaths than HIV infection.

- Estimated 3.9 Million Live with a Past or Current HCV Infection
- Estimated 101,200 People in Michigan Live with a Past or Current HCV Infection

Approximately, out of every 100 persons infected with HCV:

- 15–25 will clear the virus from their bodies
- 75–85 will go on to develop chronic infection
- 60–70 will go on to develop chronic liver disease
- 5–20 will go on to develop cirrhosis over a period of 20–30 years
- 1–5 will die from the consequences of chronic infection (liver cancer or cirrhosis)

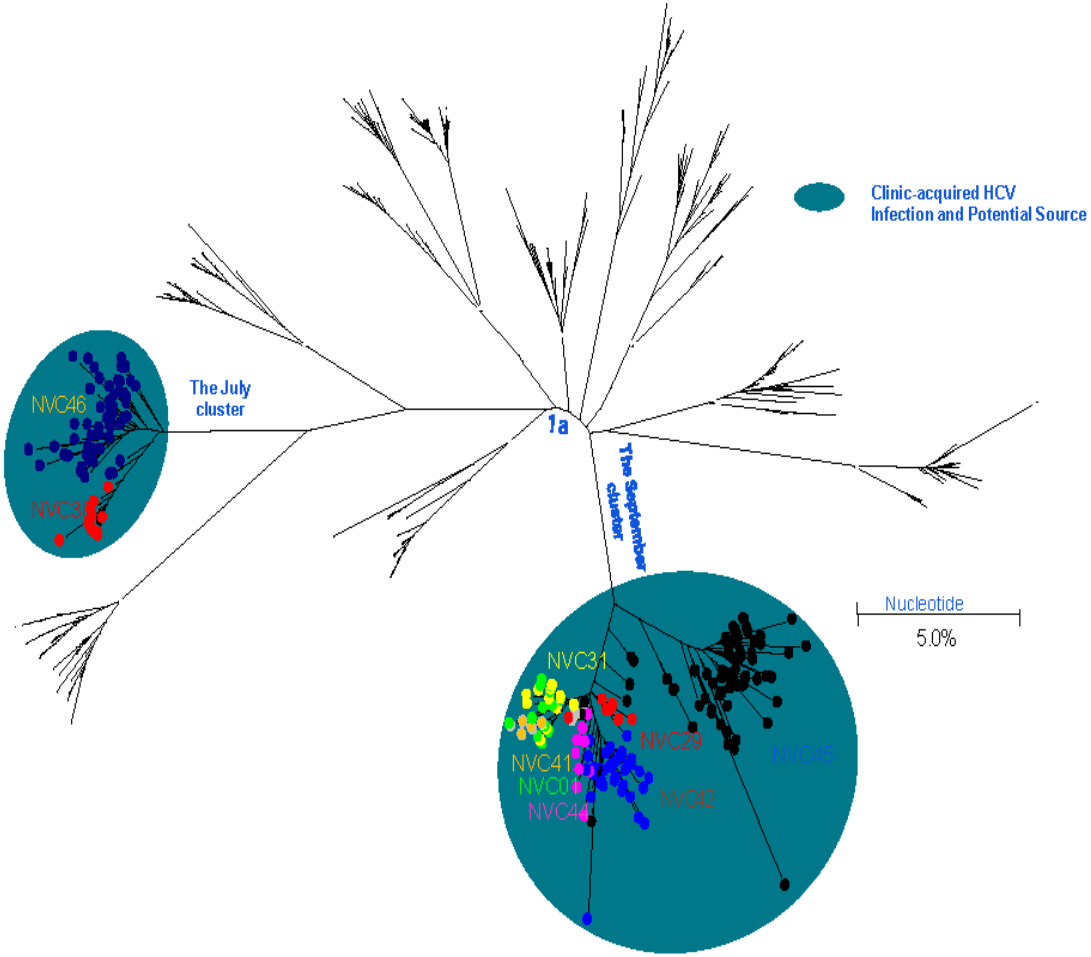
HCV Outbreaks Associated with the Following:

- Unsafe Injection Practices
- Drug Diversion
- Exposure to Blood and Blood Products
- MSM

How to Track Outbreaks?

- Sanger Sequencing of Each Infected Case to Yield a Consensus Sequence
- Consensus Sequences are not Satisfactory for accurate ID of viral strains-Quasispecies
- End-Point Limiting Dilution Technique
- Next Generation Sequencing
- Create a Phylogenetic Tree of Cases

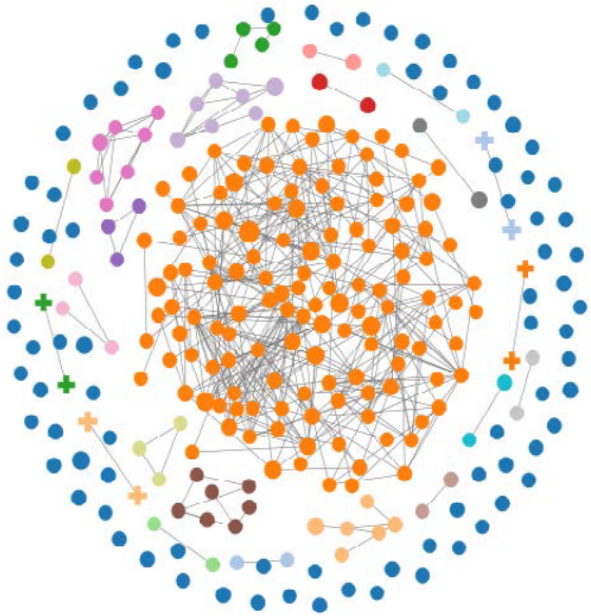
Traditional Phylogenetic Analysis



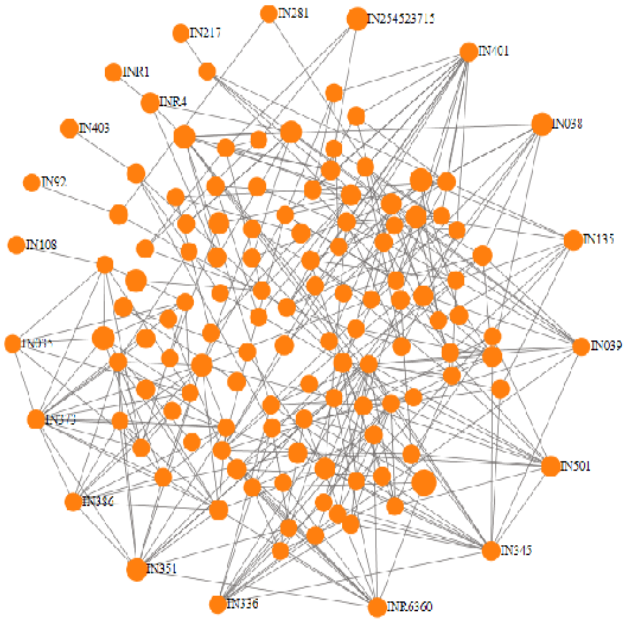
What is GHOST

- Global Health Outbreak and Surveillance Technology (GHOST) is a new web-based system that harnesses the power of novel bioinformatics technology and automatically performs a comprehensive analysis at the click of a button. Once a user inputs an HCV sample's unique fingerprint, GHOST does a quality control check, identifies transmission links between other virus samples, and automatically creates a simple graph that shows plainly which cases are linked by transmission.

GHOST Analysis

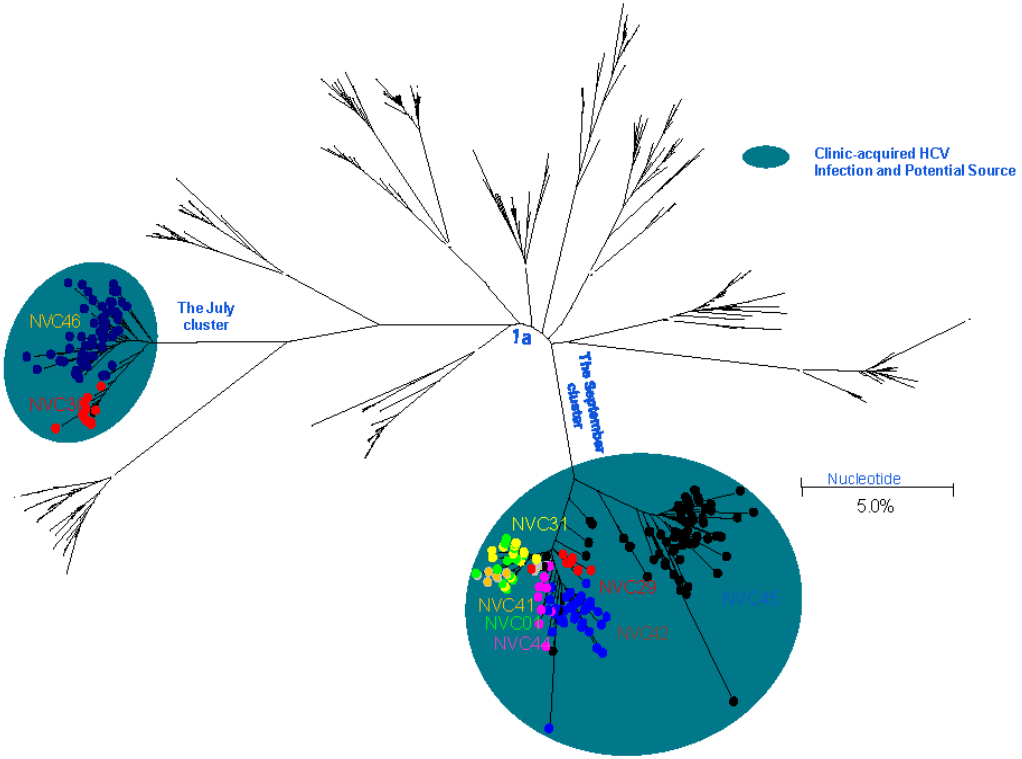


Major Cluster

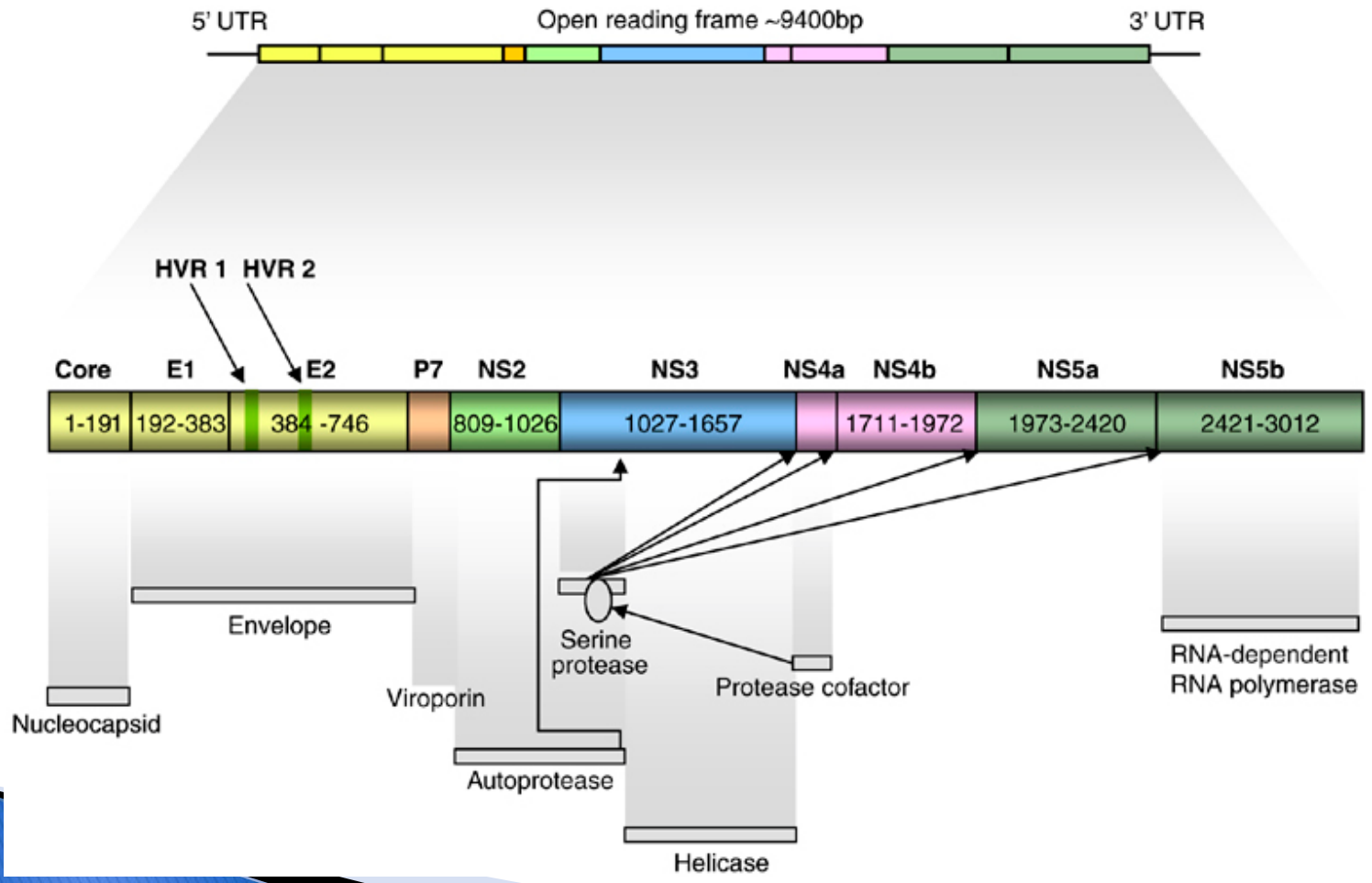


GHOST was an essential part of the "The Indiana Injection Drug Use-Associated HIV/HCV Outbreak Response Team", which was selected as the winner of the 2015 CDC/ATSDR Honor Award for Excellence in Emergency Response – Domestic.

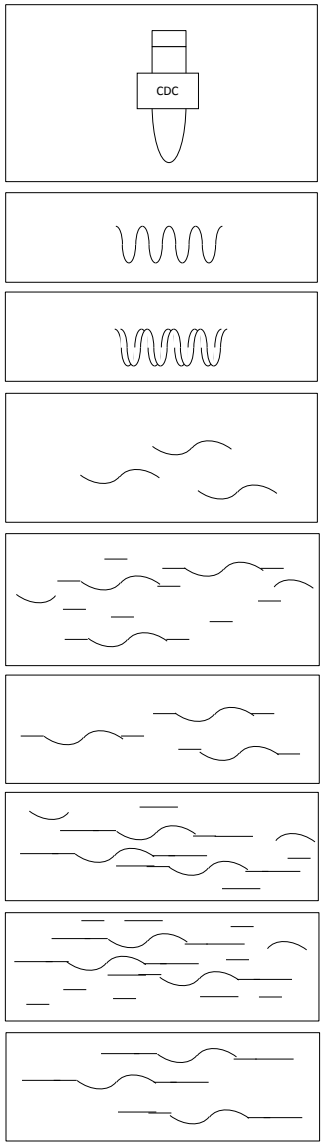
Traditional Phylogenetic Analysis



HCV Viral Genome



GHOST Lab Methodology Workflow



Patient specimen
HCV positive

↓

RNA extraction

↓

cDNA synthesis

↓

1st PCR (HVR1 amplification)

↓

2nd PCR (Barcode MID tags)

↓

Product purification

↓

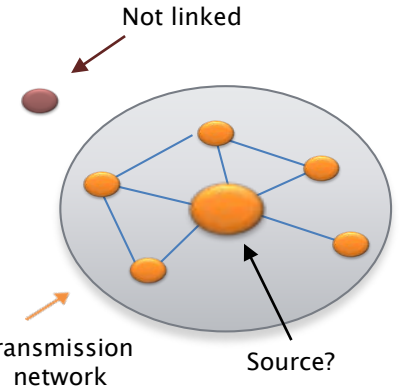
Index PCR (Illumina index tags)

↓

Pooling final products

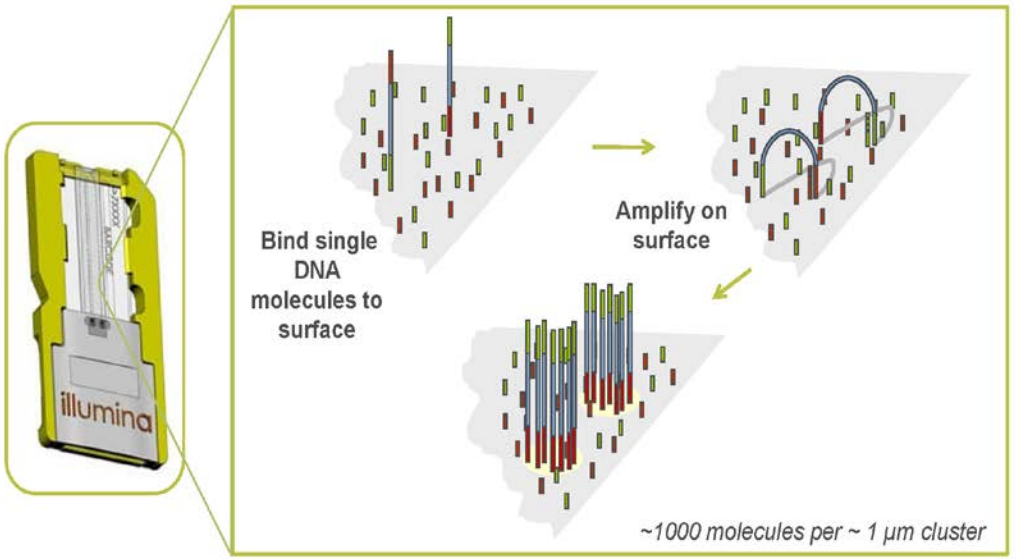
↓

Library purification → Sequencing Illumina MiSeq → Fastq files GHOST portal upload →



MISeq Flow Cell

Cluster Generation Overview



Skip Details

FOR RESEARCH USE ONLY

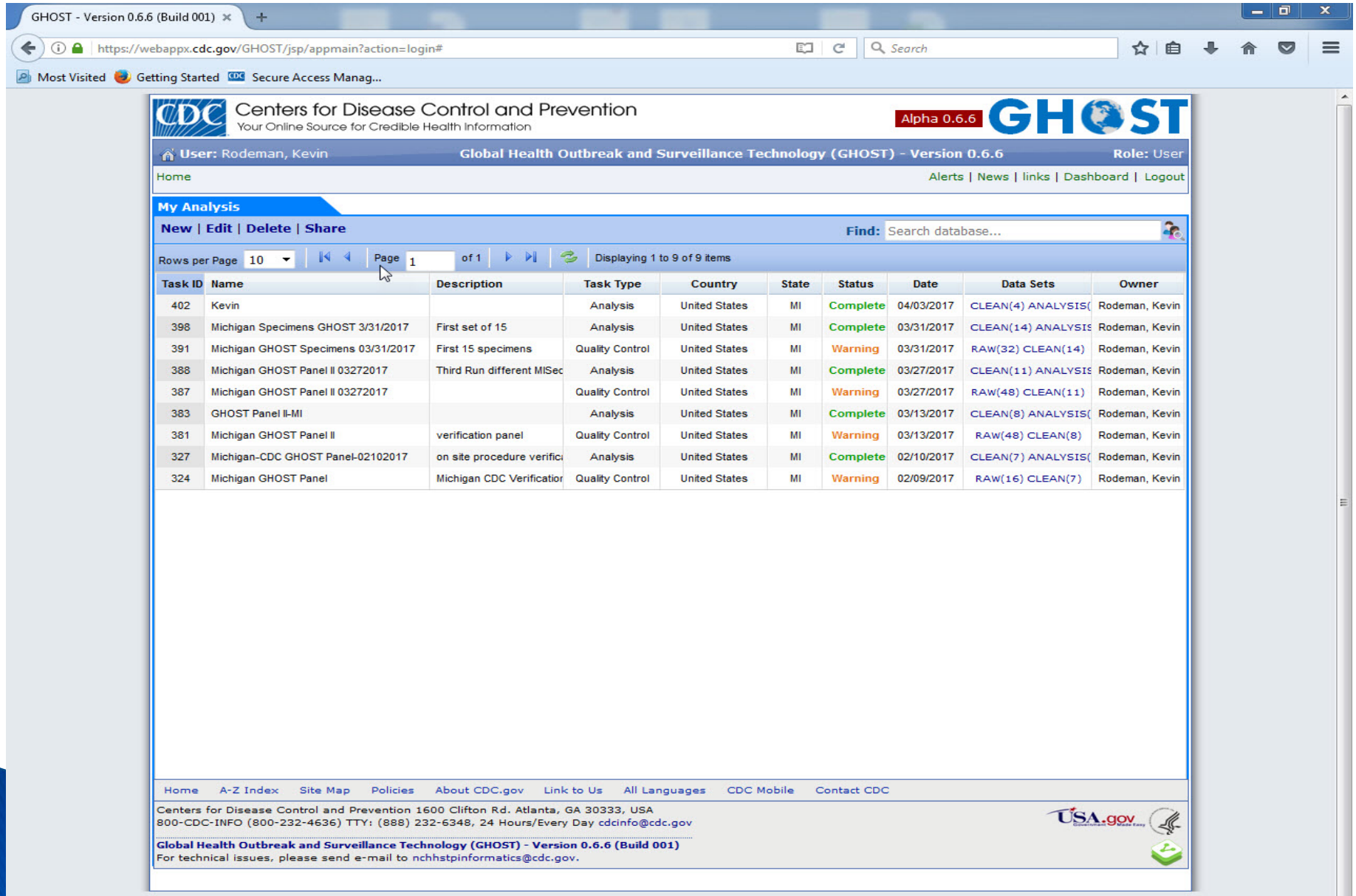
MiSeq™
illumina
Done

Data from MISEQ is a FastQ File

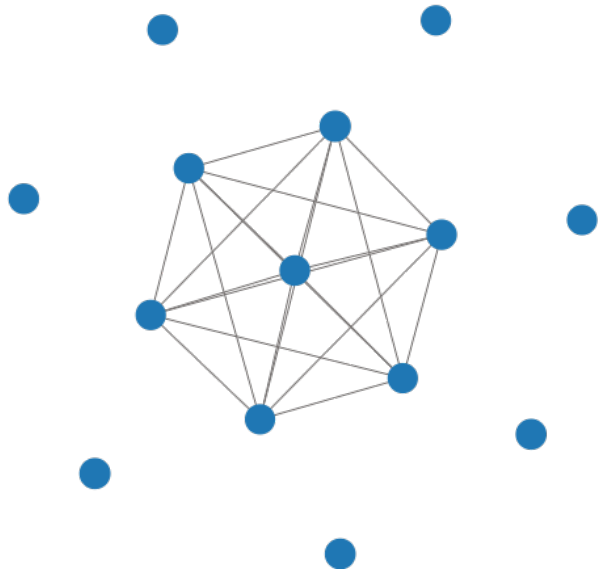
```
--->gzip -cd L2I_S1_L001_R1_001.fastq.gz | head
@M00805:5:000000000-A0VLL:1:1101:16473:1320 1:N:0:1
NTTGTCATCAGCTGAAGATGAAATAGGATGTAATCAGACGACACAGGAAGCAGATTTTGCTAAT
TTGGAACTAGGTCAGCTGAAGATCCTGTGAGCGAAGTTCCGGCAGTGTCACAGCAC
+
#55<<?BBDBDDDDDDFFFFFFHHHHHFHHAFHHHHHHHHHHBHHHHHFFFHHHHHHHHHDGDGHC
AFHFHHHHHHFGHDDHFBFHDFFHFFFHHHFFA=@BEEEED)@<B?BE3==?EEEE
@M00805:5:000000000-A0VLL:1:1101:15023:1321 1:N:0:1
NAGAAATCACAGACATACAAAGCAGTCTGTGTCCTTAGGTCCTGAGCAGCCTCCAGCACATTCT
AGCATCTGCCGTCACATTGTTCTGCACACACCGTCCTTGTCACTGCAGAAGACAGA
+
#55???BBDDEDDDDDGGGGGGIIIIIIIIIIIIIIIIIHIHIIHFGHHHIIIIIIIIIHIIII
HHHHHHHHHHHHHHHHHHHHHHHHHHHGGFGEGGGGGGGGGGGGGGGEGGGGCEGG>
@M00805:5:000000000-A0VLL:1:1101:14046:1321 1:N:0:1
NTTTCGTGGAAGTGGGTTACCTGACAGTGTGCACGCCCCCAGCAGGTTCACAATATTCTCGTGG
ACATGAGTGCCTCTCTTTCAGAGCTGTCTGCTTTTTCTGTCAAAGAAAGGAGCATT
```

Converting FastQ to FastA File

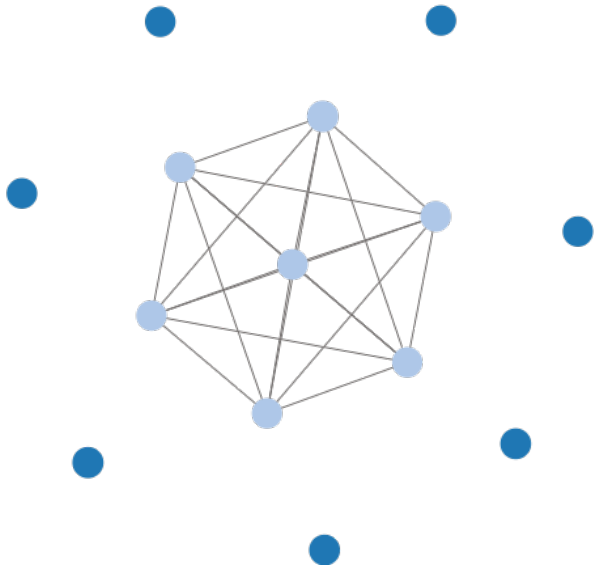
```
>Sequence_1 assembly1
CCCTAAACCCTAAACCCTAAACCCTAAACCTCTGAATCCTTAATCCCTAAATCCCTAAAT
CTTTAAATCCTACATCCATGAATCCCTAAATACCTAATTCCCTAAACCCGAAACCGGTTT
CTCTGGTTGAAAATCATTGTGTATATAATGATAATTTTATCGTTTTTATGTAATTGCTTA
TTGTTGTGTGTAGATTTTTTAAAAATATCATTTGAGGTCAATACAAATCCTATTTCTTGT
GGTTTTCTTTCCTTCACTTAGCTATGGATGGTTTATCTTCATTTGTTATATTGGATACAA
GCTTTGCTACGATCTACATTTGGGAATGTGAGTCTCTTATTGTAACCTTAGGGTTGGTTT
ATCTCAAGAATCTTATTAATTGTTTGGACTGTTTATGTTTGGACATTTATTGTCATTCTT
>Sequence_2
CCCTAAACCCTAAACCCTAAACCCTAAACCTCTGAATCCTTAATCCCTAAATCCCTAAAT
CTTTAAATCCTACATCCATGAATCCCTAAATACCTAATTCCCTAAACCCGAAACCGGTTT
CTCTGGTTGAAAATCATTGTGTATATAATGATAATTTTATCGTTTTTATGTAATTGCTTA
TTGTTGTGTGTAGATTTTTTAAAAATATCATTTGAGGTCAATACAAATCCTATTTCTTGT
GGTTTTCTTTCCTTCACTTAGCTATGGATGGTTTATCTTCATTTGTTATATTGGATACAA
GCTTTGCTACGATCTACATTTGGGAATGTGAGTCTCTTATTGTAACCTTAGGGTTGGTTT
ATCTCAAGAATCTTATTAATTGTTTGGACTGTTTATGTTTGGACATTTATTGTCATTCTT
```


GHOST - Version 0.6.6 (Build 001)
https://webappx.cdc.gov/GHOST/jsp/appmain?action=login#
Search
Most Visited
Getting Started
Secure Access Manag...
Centers for Disease Control and Prevention
Your Online Source for Credible Health Information
Alpha 0.6.6
GHOST
User: Rodeman, Kevin
Global Health Outbreak and Surveillance Technology (GHOST) - Version 0.6.6
Role: User
Home
Alerts | News | links | Dashboard | Logout
My Analysis
New | Edit | Delete | Share
Find: Search database...
Rows per Page 10
Page 1 of 1
Displaying 1 to 9 of 9 items
Task ID | Name | Description | Task Type | Country | State | Status | Date | Data Sets | Owner
402 | Kevin | | Analysis | United States | MI | Complete | 04/03/2017 | CLEAN(4) ANALYSIS(| Rodeman, Kevin
398 | Michigan Specimens GHOST 3/31/2017 | First set of 15 | Analysis | United States | MI | Complete | 03/31/2017 | CLEAN(14) ANALYSIS | Rodeman, Kevin
391 | Michigan GHOST Specimens 03/31/2017 | First 15 specimens | Quality Control | United States | MI | Warning | 03/31/2017 | RAW(32) CLEAN(14) | Rodeman, Kevin
388 | Michigan GHOST Panel II 03272017 | Third Run different MISec | Analysis | United States | MI | Complete | 03/27/2017 | CLEAN(11) ANALYSIS | Rodeman, Kevin
387 | Michigan GHOST Panel II 03272017 | | Quality Control | United States | MI | Warning | 03/27/2017 | RAW(48) CLEAN(11) | Rodeman, Kevin
383 | GHOST Panel II-MI | | Analysis | United States | MI | Complete | 03/13/2017 | CLEAN(8) ANALYSIS(| Rodeman, Kevin
381 | Michigan GHOST Panel II | verification panel | Quality Control | United States | MI | Warning | 03/13/2017 | RAW(48) CLEAN(8) | Rodeman, Kevin
327 | Michigan-CDC GHOST Panel-02102017 | on site procedure verifica | Analysis | United States | MI | Complete | 02/10/2017 | CLEAN(7) ANALYSIS(| Rodeman, Kevin
324 | Michigan GHOST Panel | Michigan CDC Verificatior | Quality Control | United States | MI | Warning | 02/09/2017 | RAW(16) CLEAN(7) | Rodeman, Kevin
Home | A-Z Index | Site Map | Policies | About CDC.gov | Link to Us | All Languages | CDC Mobile | Contact CDC
Centers for Disease Control and Prevention 1600 Clifton Rd. Atlanta, GA 30333, USA
800-CDC-INFO (800-232-4636) TTY: (888) 232-6348, 24 Hours/Every Day cdcinfo@cdc.gov
USA.gov
Global Health Outbreak and Surveillance Technology (GHOST) - Version 0.6.6 (Build 001)
For technical issues, please send e-mail to nchhstpinformatics@cdc.gov.

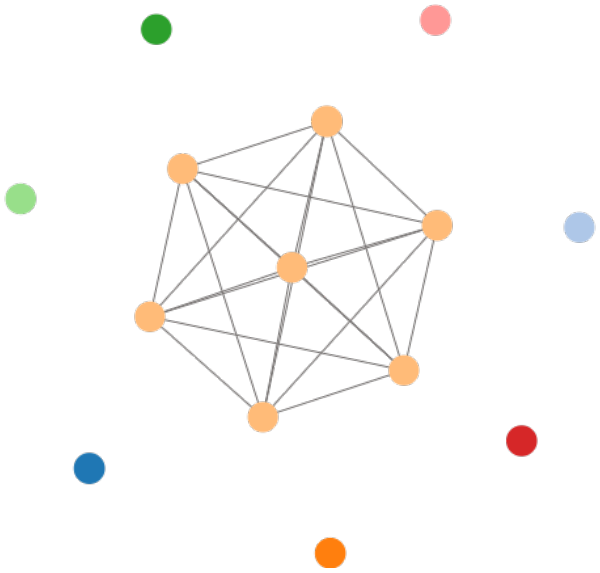
HCV Specimens Analyzed by GHOST-Genotype



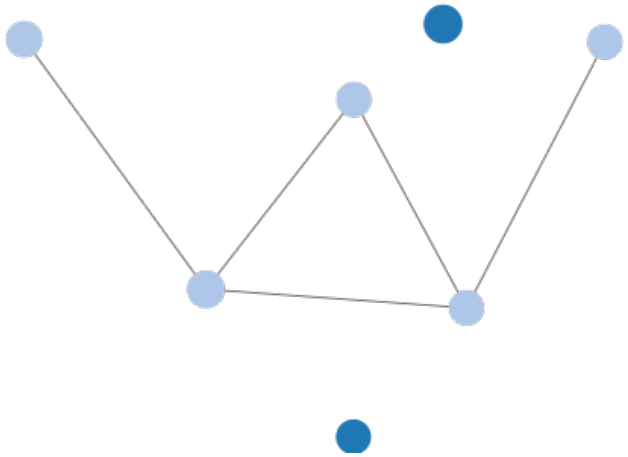
HCV Specimens Analyzed by GHOST-Cluster



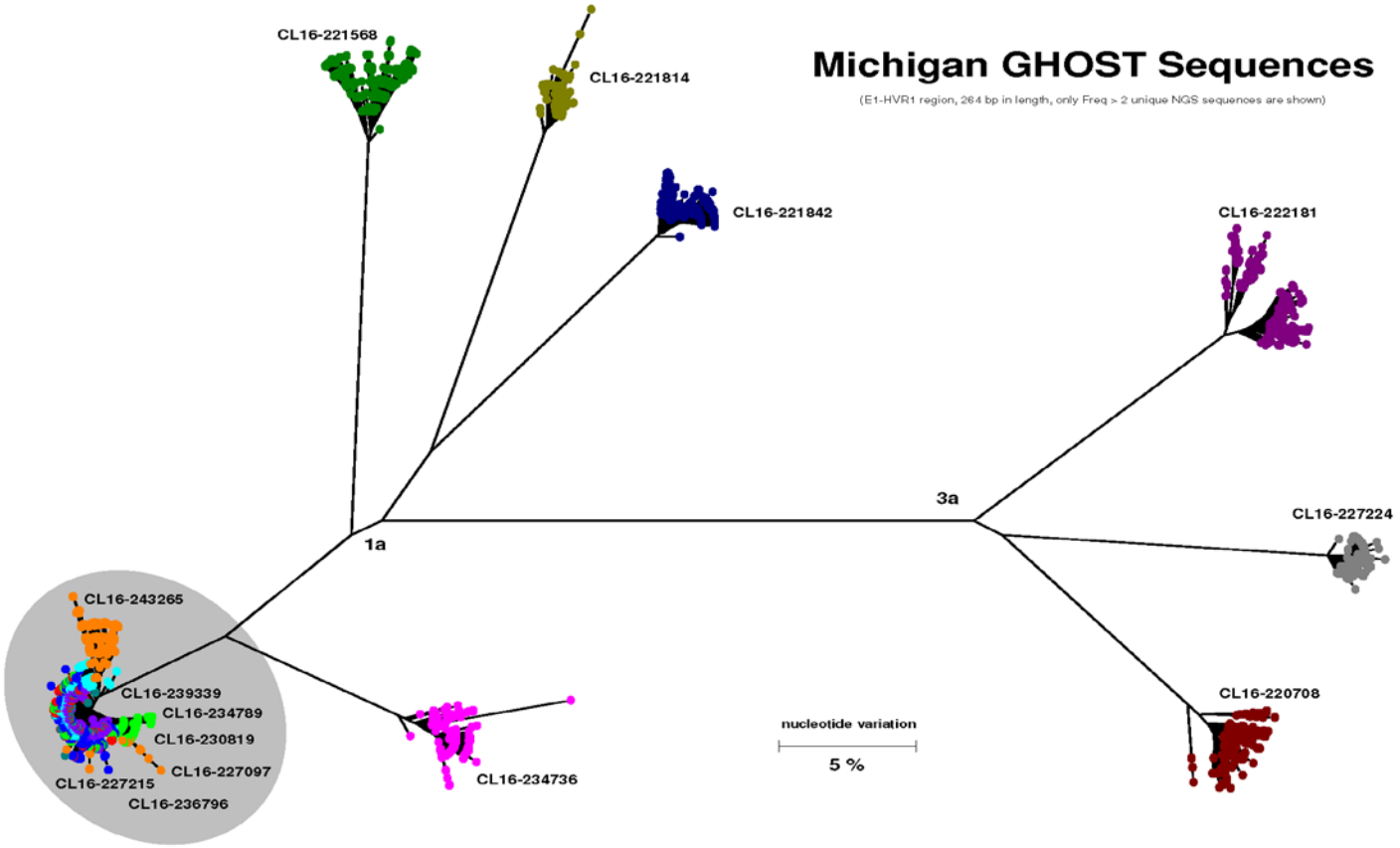
HCV Specimens Analyzed by GHOST-Strain



Unable to Determine Direction of Infection



Phylogenetic Tree MI GHOST Sequences



It's Modular

- We are currently testing new HCV modules that
  - Detect Persons Who Inject Drugs
  - Estimate duration of infection
  - Monitor for drug resistance
- Modules to investigate Hepatitis A and B are in the works
- Discussions with Malaria and HIV Divisions to integrate these other pathogens are in progress