

4th HEPATITIS C
TECHNICAL ADVISORY
GROUP
TAG Meeting

**BUILDING OF NEXT GENERATION
SEQUENCING (NGS) CAPACITY FOR
HEPATITIS C SURVEILLANCE**

Adam Kotorashvili PhD
Head of Genome Center
Lugar Center at National Center for Disease Control and Public Health Tbilisi Georgia

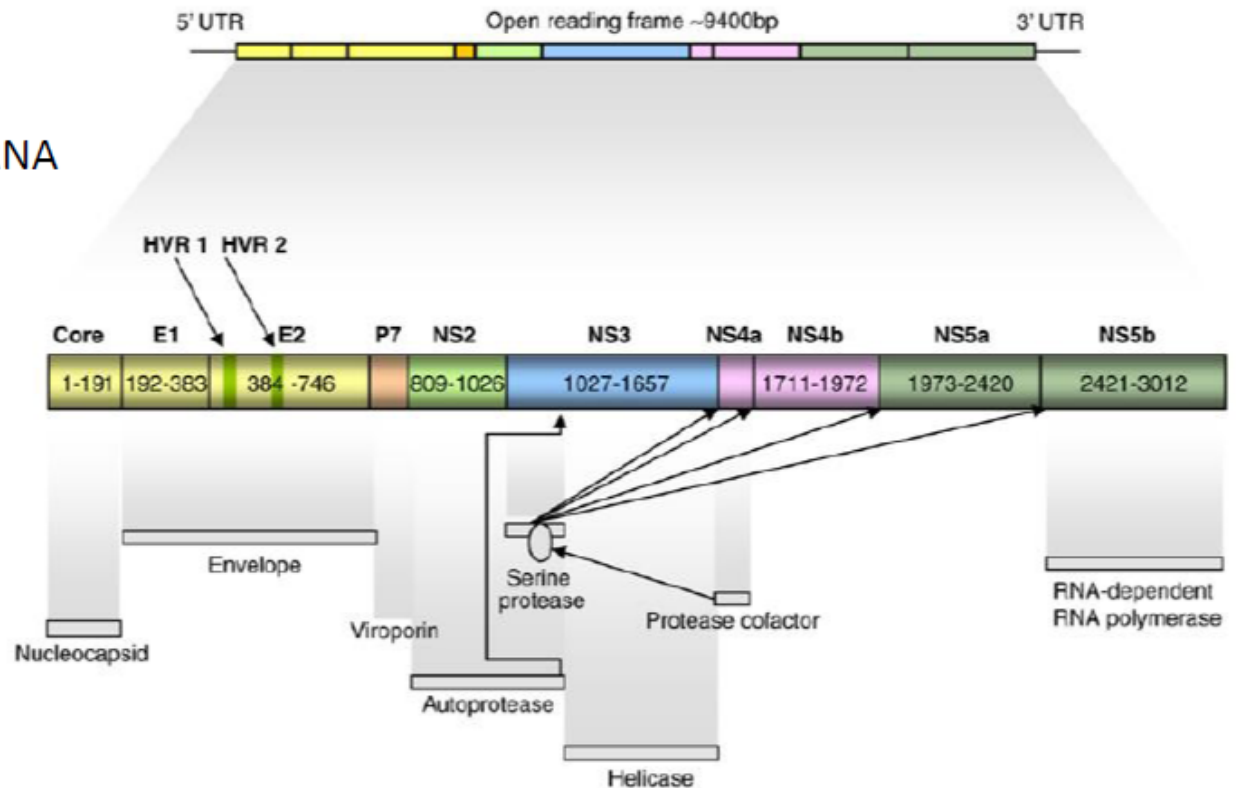
Hepatitis C Virus

Genome:

- Single-stranded, (+) strand RNA
- ~9,400 nt

Proteins:

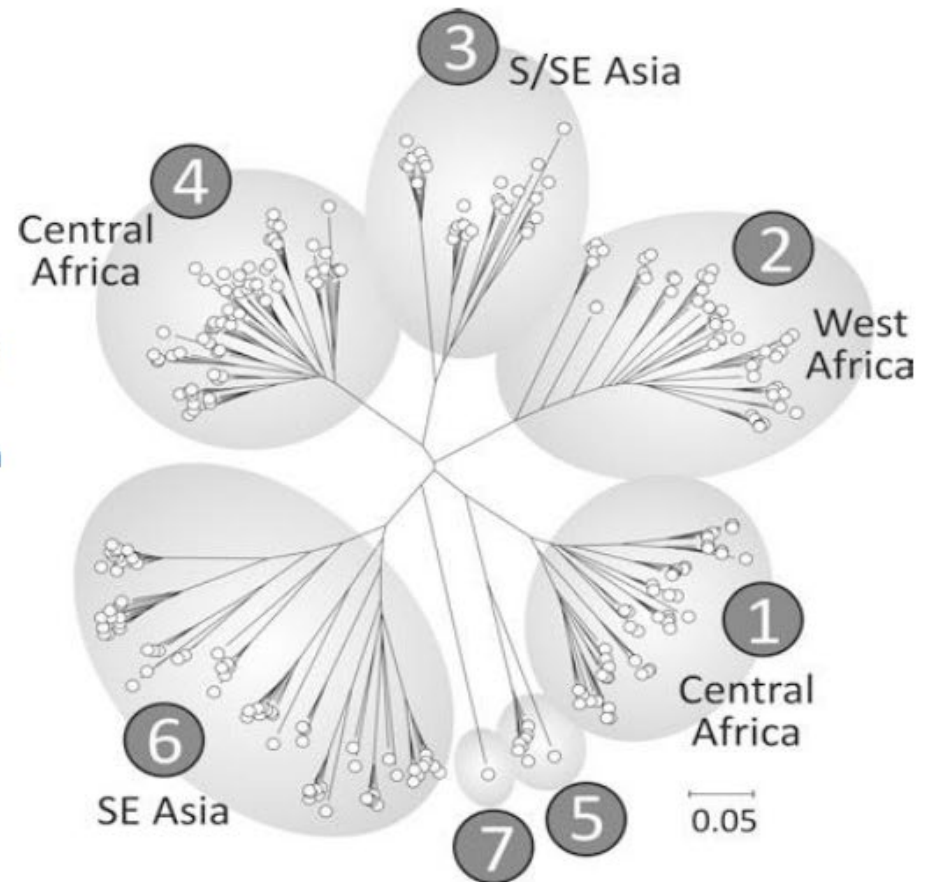
- ~3,000 aa polyprotein
- 10 individual proteins
 - 3 structural (C, E1, E2)
 - 7 non-structural



HCV is genetically heterogynous

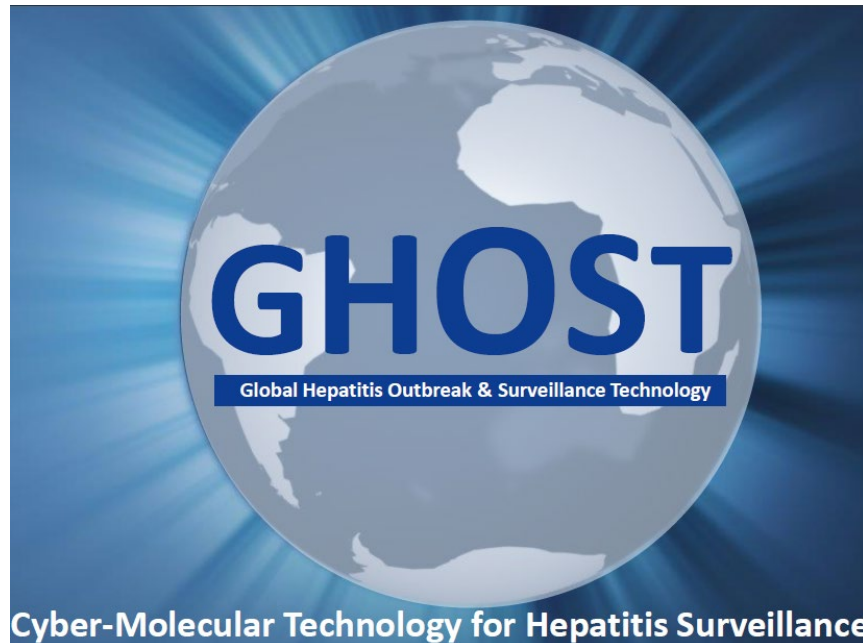
HCV strains are classified into several types

- **7 genotypes**
 - Each has specific geographic distribution
 - Genotype 1 predominant in the USA
- **67 subtypes**
 - Subtype 1A predominant in the USA



GHOST (Global Health Outbreaks and Surveillance Technology)

GHOST technology, will be used for hepatitis C (and other Hepatitis) in the global surveillance.



Advantages of GHOST

- Massive
- Real-Time
- Cost-effective
- Simple to use
- Expandable to other Pathogens

NCDC/Lugar Genome Center became the **first Regional Centre for the GHOST** Technology in Caucasus region, which will further expand our genome center's opportunities at NCDC/Lugar Center and will enrich GHOST database at CDC.

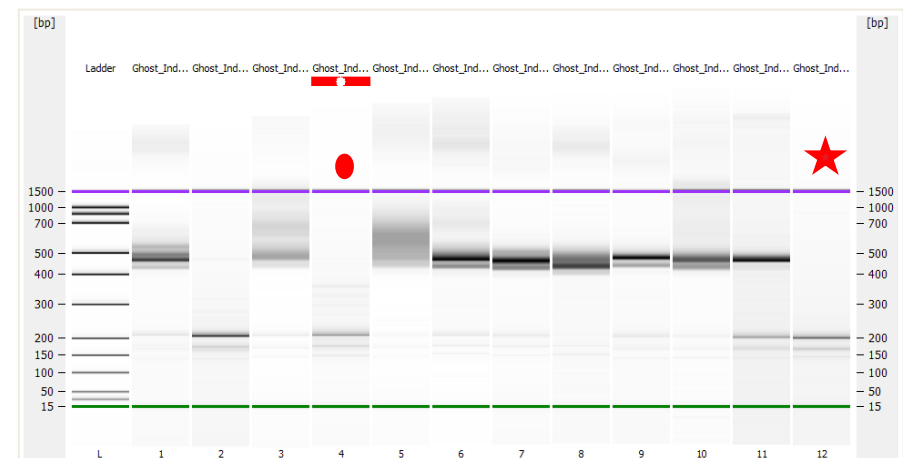
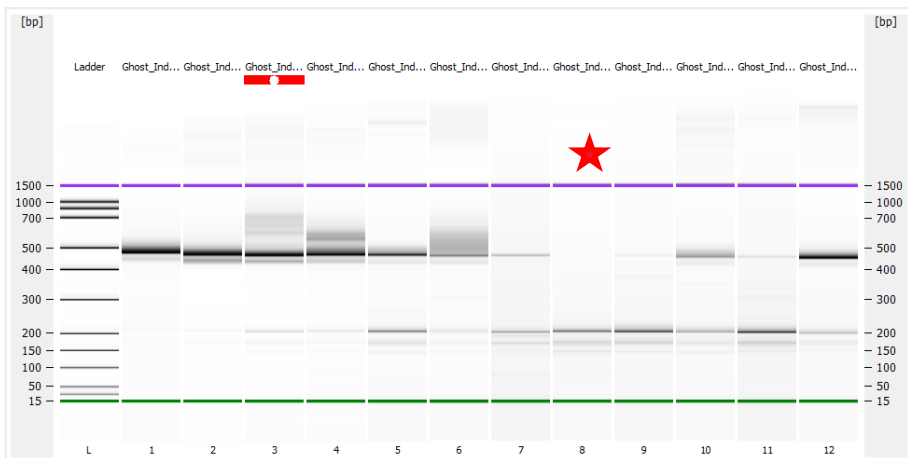
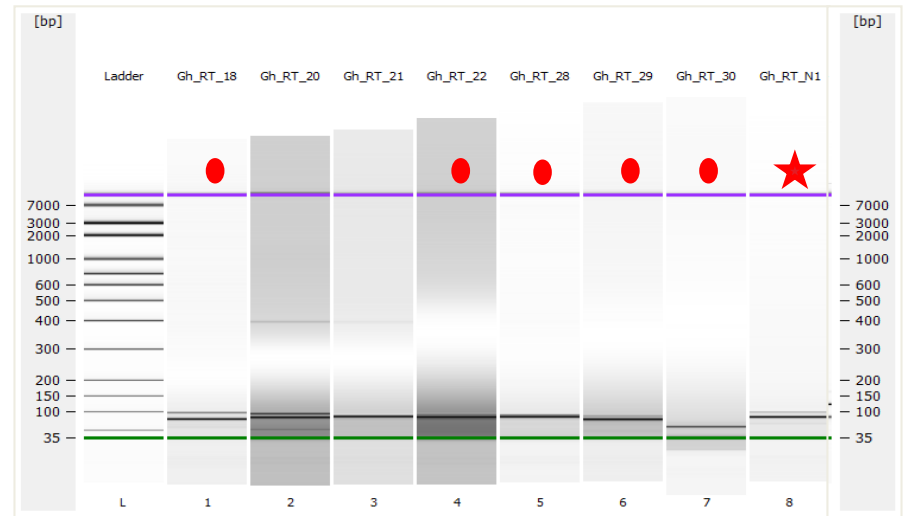
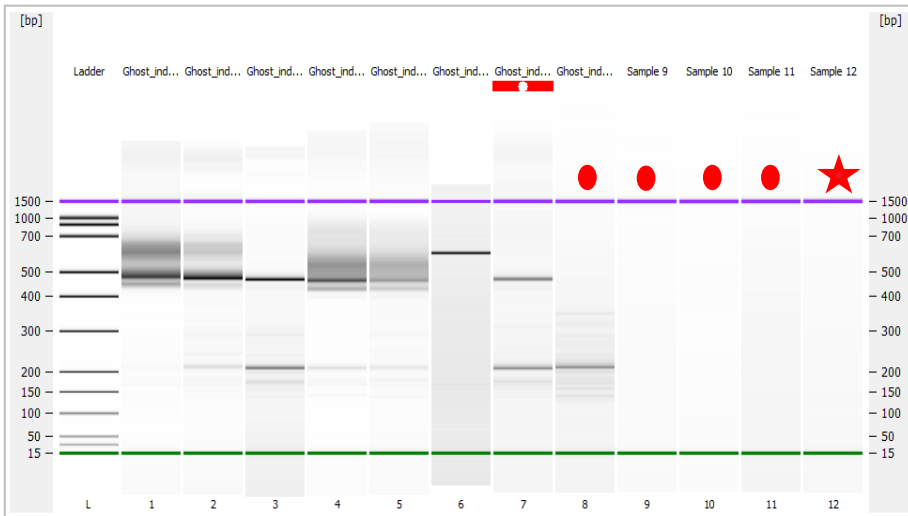
Objectives

- ❑ Setting up a GHOST (Global Hepatitis Outbreak and Surveillance Technology) center at Lugar Center
- ❑ Study molecular epidemiology and transmission patterns of HCV infection among risk groups as part of the Elimination Program
- ❑ Support HCV surveillance in Georgia
- ❑ Contribute with regional data for GHOST global database

Methods

- ❑ Technology used for the project - GHOST (Global Hepatitis Surveillance Technology)
- ❑ Samples used for the capacity building
 - **HCV serosurvey** (20 specimens; 2015)
 - **Harm Reduction center** (24 specimens; 2017)
- ❑ RNA extraction – QIAamp Viral RNA mini
- ❑ Library Preparation – NEBnext Ultra RNA library
- ❑ Data QC and Analysis – GHOST tools

WETLAB - Gel electrophoreses generated for GHOST



- ★ Negative control
- No specific band detected

GHOST Network

SAMS secure access management services

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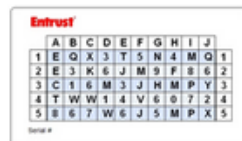
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GHOST Network



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Task ID	Name	Description	Task Type	Country	State	Status	Date	Data Sets	Owner
1258	combined		Analysis			Complete	11/07/2018	CLEAN(31) ANALYSIS(5)	Kotorashvili, Ad
1258	HCV		Analysis			Complete	11/06/2018	CLEAN(11) ANALYSIS(5)	Kotorashvili, Ad
1188	HR		Analysis			Complete	10/12/2018	CLEAN(4) ANALYSIS(4)	Kotorashvili, Ad
1182	HR		Quality Control	Georgia		Warning	10/10/2018	RAW(48) CLEAN(4)	Kotorashvili, Ad
1033	sero survey		Quality Control	Georgia		Warning	07/09/2018	RAW(48) CLEAN(11)	Kotorashvili, Ad
1019	QC		Quality Control	Georgia		Warning	06/27/2018	RAW(48)	Kotorashvili, Ad
982	test		Analysis			Complete	05/29/2018	CLEAN(9) ANALYSIS(5)	Kotorashvili, Ad
961	first run	test run	Quality Control	Georgia		Warning	05/29/2018	RAW(40) CLEAN(9)	Kotorashvili, Ad
718	analysis workshop	test	Analysis	Georgia		Complete	11/03/2017	CLEAN(7) ANALYSIS(5)	Kotorashvili, Ad
692	test workshop	test	Quality Control	Georgia		Complete	11/03/2017	RAW(14) CLEAN(7)	Kotorashvili, Ad

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Global Health Outbreak and Surveillance Technology (GHOST) - Version 0.7.0 (Build 001)
 For technical issues, please send e-mail to ghost@cdc.gov.

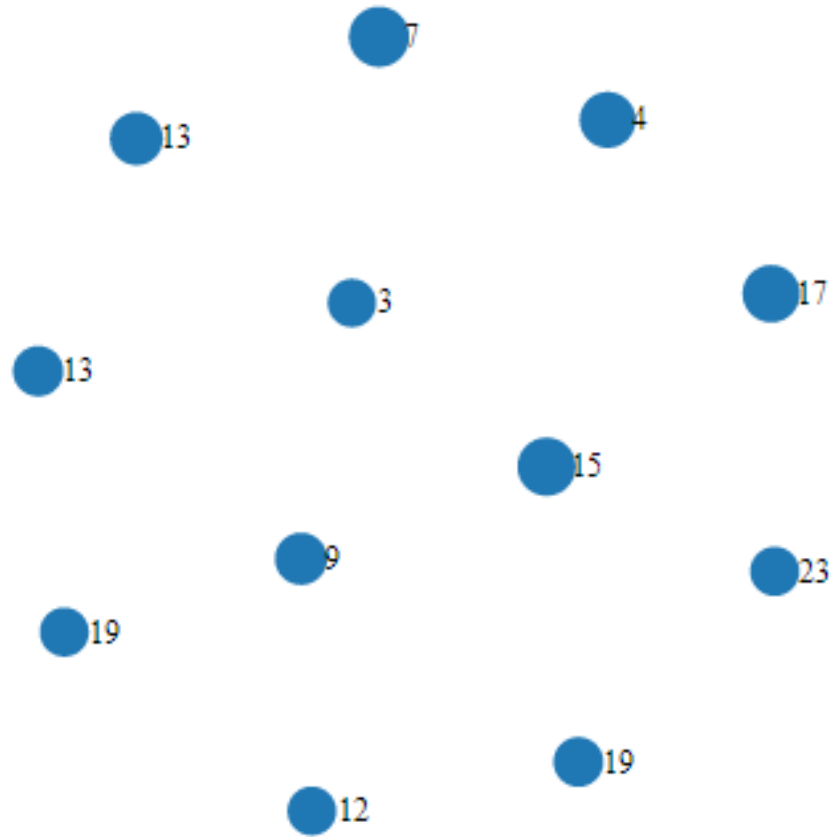


Results - GHOST all_samples_info_CSV

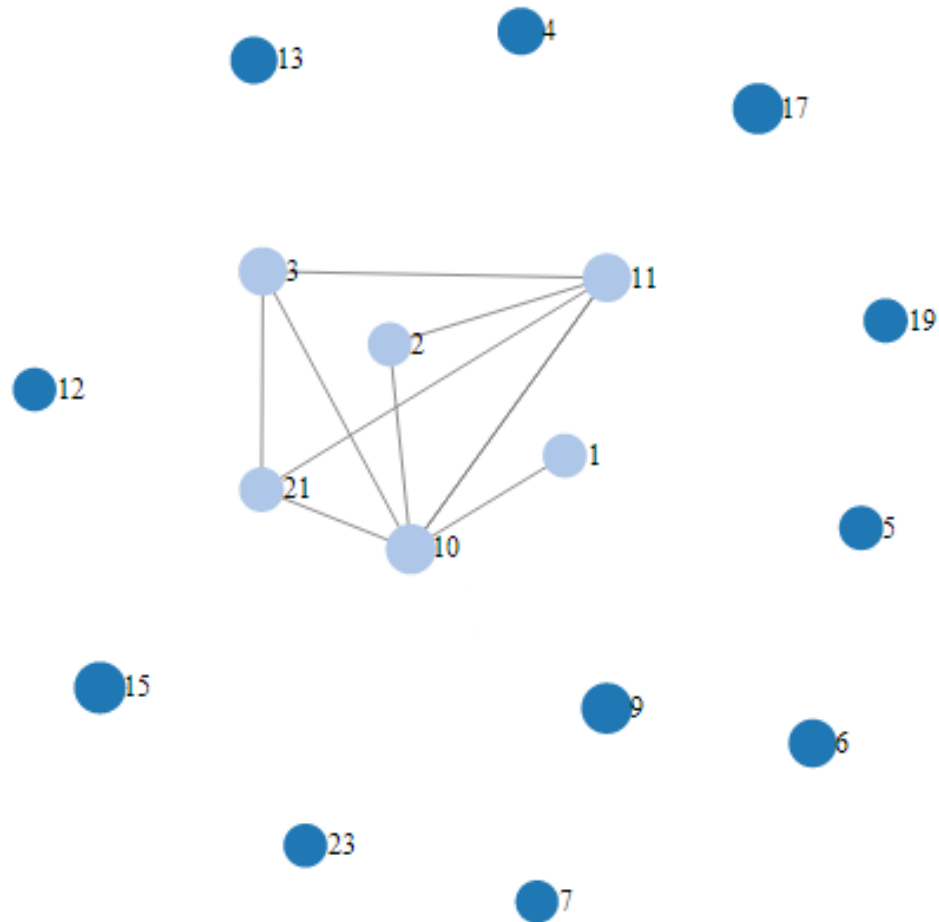
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9	3a	2790	11672	0.032228001	3015
21	2k1b	4050	13311	0.01823572	3873
23	3a	3703	12796	0.010599717	3191
10	1b	1601	2435	0.062342798	151
10	2k1b	417	825	0.048298433	79
10	3a	18	43	0.006903814	26
20	1b	4850	8438	0.059578341	230
13	2k1b	4216	13907	0.019283246	4694
12	2k1b	3275	15880	0.008587333	6596
19	1b	1735	2964	0.01514224	464
3	2k1b	4252	11424	0.049314101	1721
5	3a	5010	18385	0.015416476	2770
11	1b	2706	5865	0.057966237	257
19	2k1b	2169	7727	0.009715996	2846
7	2k1b	4918	13534	0.090491742	740

name	genotype	nhap	nreads	totaldiv	maxfreq
1	3a	5540	17556	0.013784677	3307
4	1b	5163	8813	0.059405673	211
6	3a	8774	16442	0.046326288	450
9	3a	1163	5116	0.067976792	1580
2	1b	4382	10289	0.013190268	2023
1	2k1b	7691	15935	0.056094031	1753
2	2k1b	5703	14710	0.04861738	2966
7	3a	2759	17830	0.0046085	10393
14	2k	118	280	0.03376836	139
14	2k1b	6403	15157	0.068451302	850
4	1b	5742	14195	0.039691961	906
13	3a	226	304	0.03799929	30
22	2k	4071	6159	0.096426725	546
22	2k1b	3113	6098	0.044196099	1033
17	1b	2658	2958	0.069505793	24
11	1b	41	89	0.00810848	27
11	2k1b	1753	5207	0.050311955	923
11	3a	471	1020	0.016161674	163

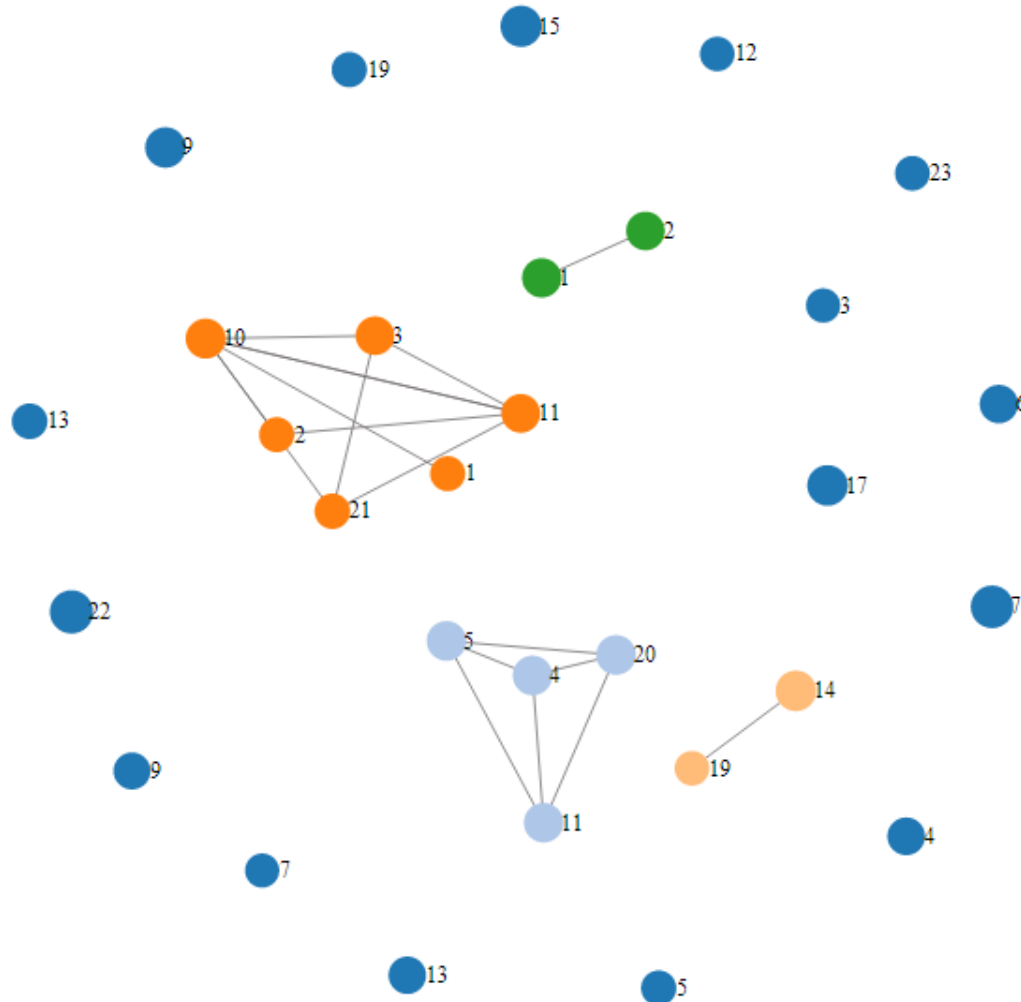
Results – GHOST minimal distance HCV serosurvey



Results – GHOST minimal distance Harm Reduction

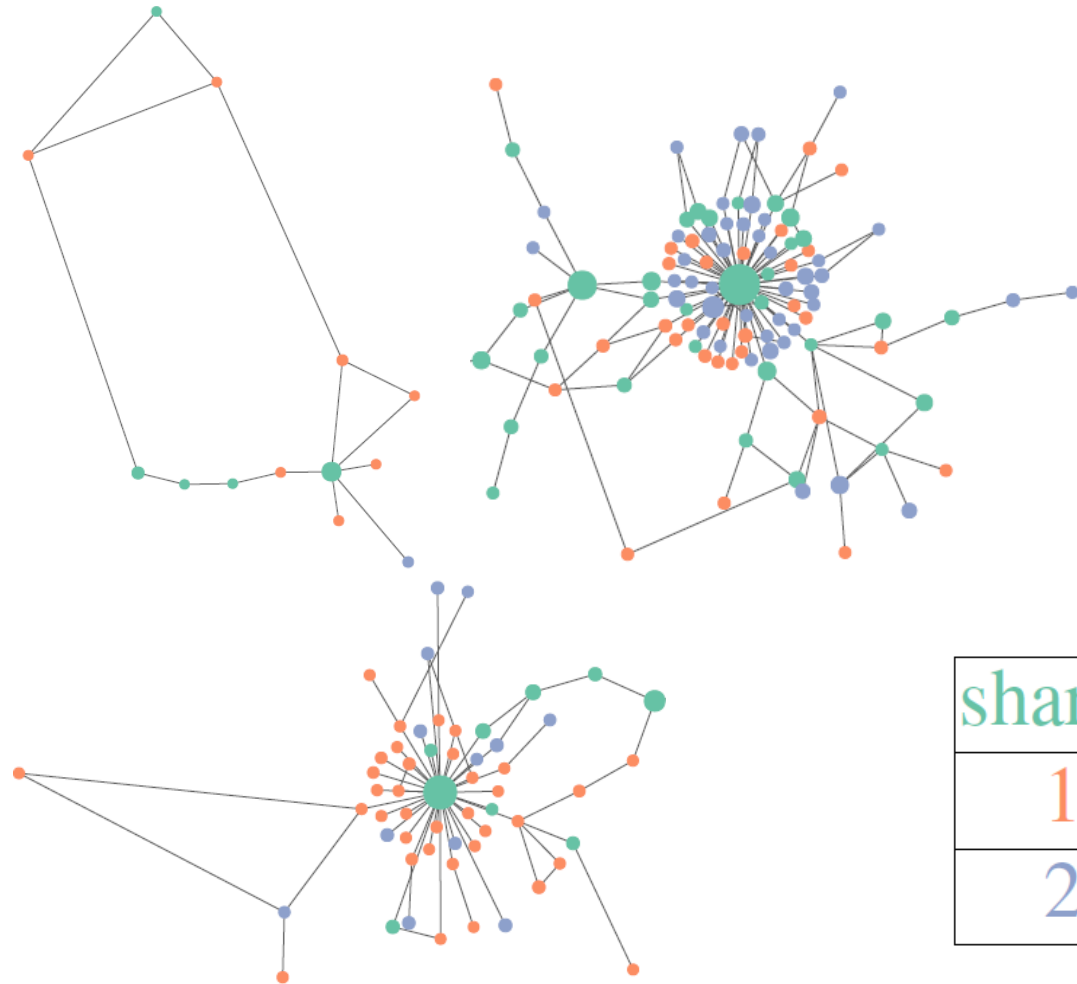


Results – GHOST minimal distance SS + HR



Results - GHOST based Kstep Networks

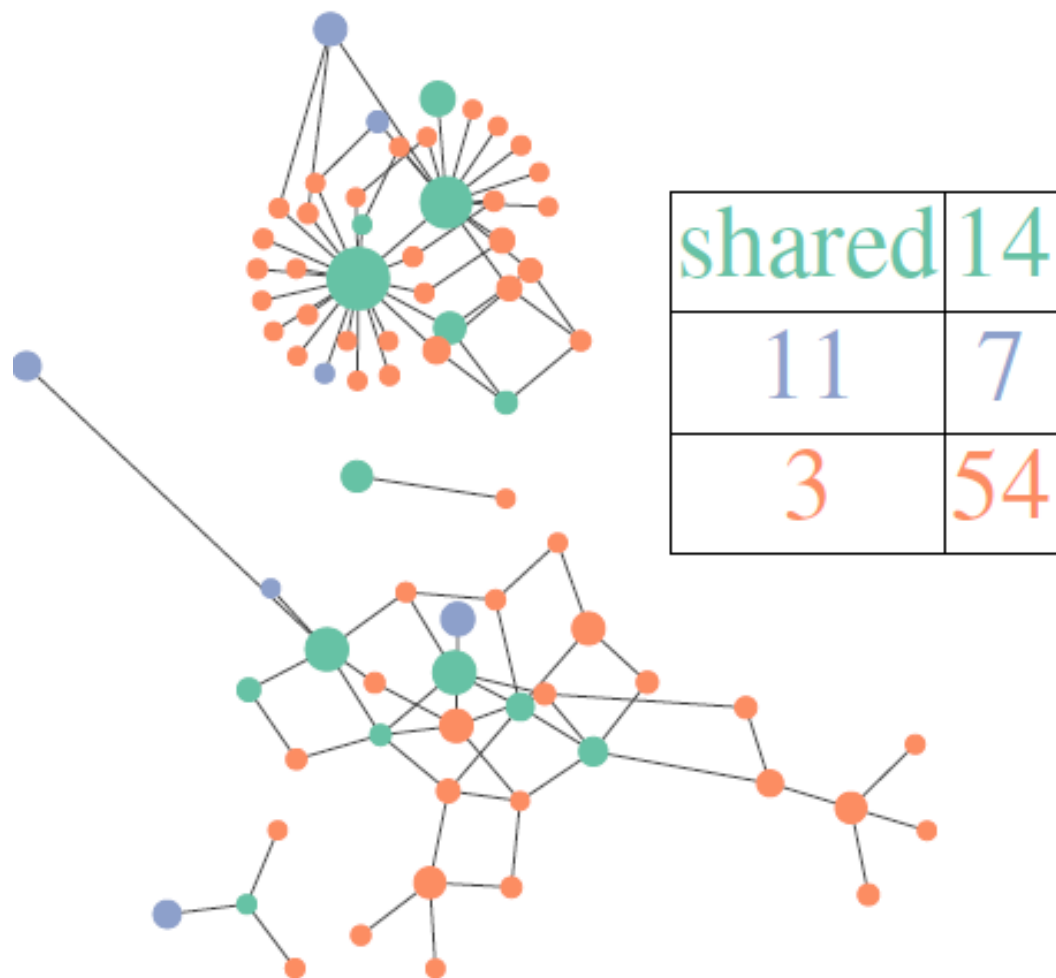
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17	1b	2658	2958	0.069505793	24
11	1b	41	89	0.00810848	27
11	2k1b	1753	5207	0.050311955	923
11	3a	471	1020	0.016161674	163



shared	56
1	89
2	69

Results - GHOST based Kstep Networks

name	genotype	nhap	nreads	totaldiv	maxfreq
1	3a	5540	17556	0.013784677	3307
4	1b	5163	8813	0.059405673	211
6	3a	8774	16442	0.046326288	450
9	3a	1163	5116	0.067976792	1580
2	1b	4382	10289	0.013190268	2023
1	2k1b	7691	15935	0.056094031	1753
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14	2k	118	280	0.03376836	139
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11	2k1b	1753	5207	0.050311955	923
11	3a	471	1020	0.016161674	163



Conclusions and Future focus

- ❑ NCDC/Lugar Center became **first regional lab** for GHOST technology
- ❑ GHOST is a powerful NGS based technology for studying molecular epidemiology and transmission patterns of HCV infection
- ❑ For next year we plan to analyze at list three hundred specimens from HR
- ❑ WETLAB System automation
- ❑ Involve specimens from regional countries
- ❑ Apply technology for other pathogens (**HAV, HBV**)
- ❑ Improve – enhance surveillance
- ❑ Enrich global GHOST database

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