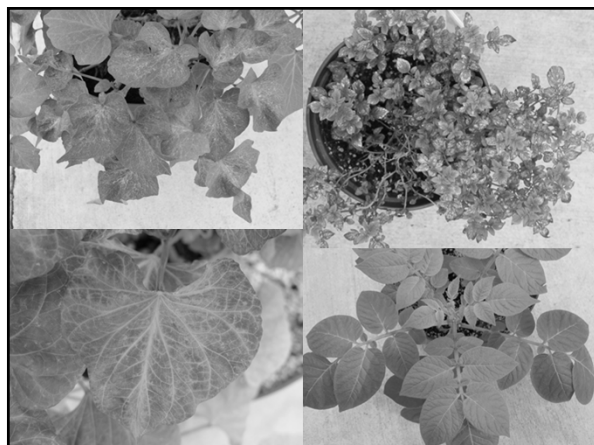


Is it practical to implement NGS technologies in NCPDN labs for virus detection

Dimitre Mollov

USDA ARS National Germplasm Resources Laboratory

What is NGS and how does it work?



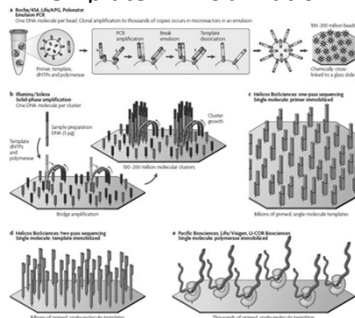
Next Generation Sequencing Technology

- Routine detection of viruses and other pathogens
- Extremely sensitive
- Highly reliable
- Identify unknown or poorly characterized viruses
- Whole genomes sequencing (most feasible approach)
 - Pathogen diversity; mutations; new strains
 - Ability to improve detection techniques

Template immobilization

NGS platforms

- 454
- Illumina
- SOLiD
- Polonator
- Helicos



Metzker, 2010

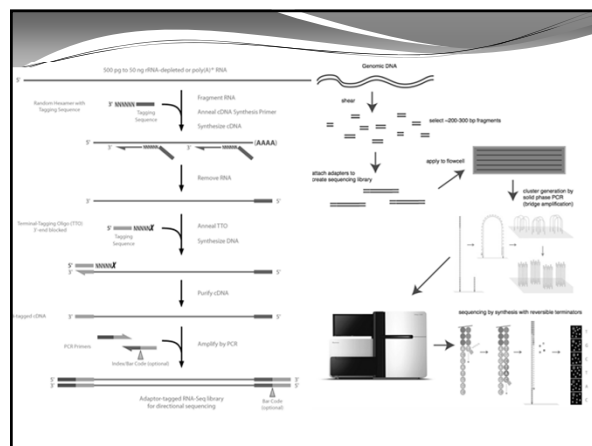
NGS target

- Total RNA (or mRNA) or DNA
- siRNA
- dsRNA
- Virion Nucleic Acid

Materials and methods



RNA
Extraction



NGS data

- *De novo* assembly
- NCBI database
 - Nucleotide
 - Protein
- E-probes
 - EDNA
 - Custom database
 - Nucleotide
 - Protein
- Mapping
 - Raw reads
 - Contigs

Materials and methods



Data analysis

Lab
conformation

Workflow

Sequence reads	Millions
Contig assembly	CLC Workbench
BLAST	NCBI Local database; BLASTN, BLASTX
Viral contigs	Computer vs. biology
Lab validation	PCR, Sequence
Map to reads	Final assembly

Sequence reads

25-50 million reads per sample,
 ~5-10% removed due to low quality
 Length of reads: ~100 (<50-600)

Contig assembly

<100,000 to >750,000 contigs per sample
 Why so many?

Not all are useful: 500-10,000 nt long =>
 10-20,000 contigs

NCBI Local database; BLASTN, BLASTX



I appreciate your dedication,
 but genome browsers just aren't
 really designed for phones.



Sequence reads and contigs

Virus related contigs: 0 to >100
 Plant virus related contigs

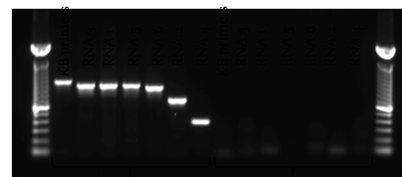
How about uncharacterized viruses

Computer vs. biology



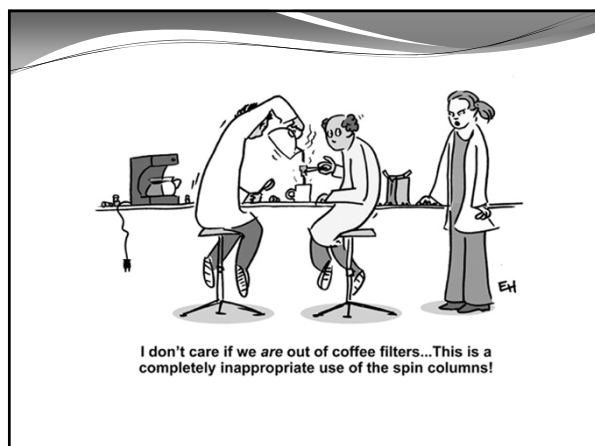
I don't care if it is all bioinformatics. I'm a *biologist*, I'm *doing*
 an experiment, so I'm wearing my safety glasses!

Lab validation



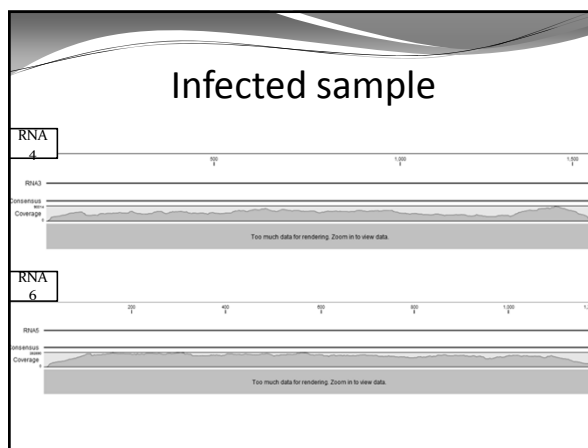
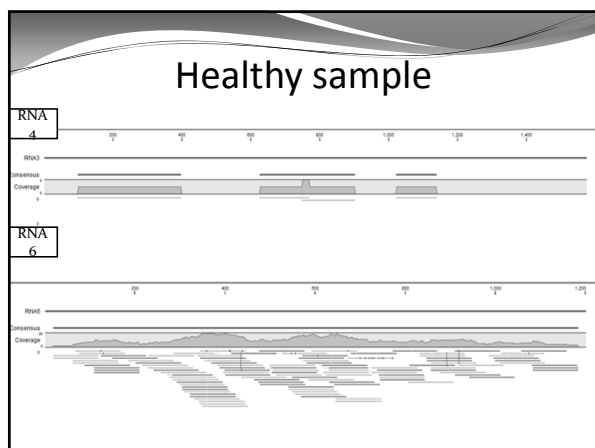
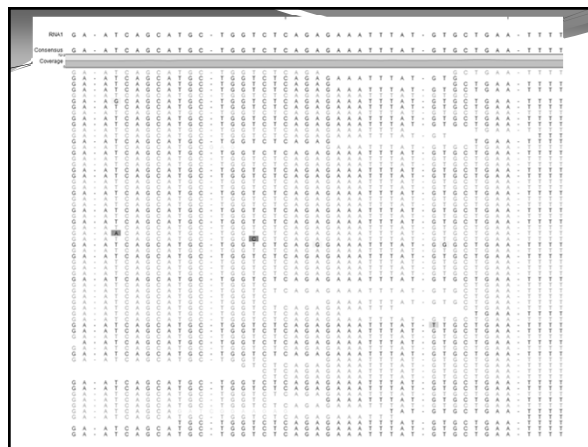
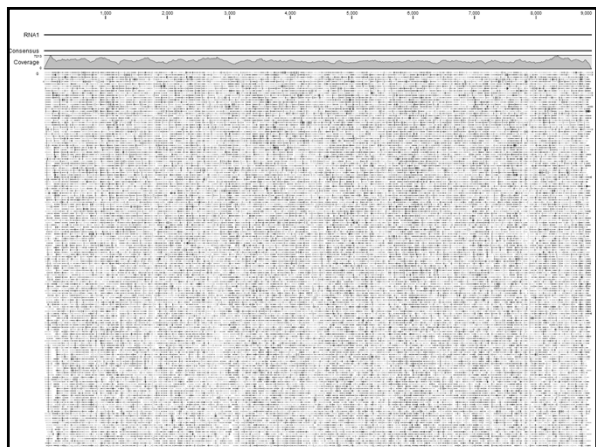
Infected Healthy

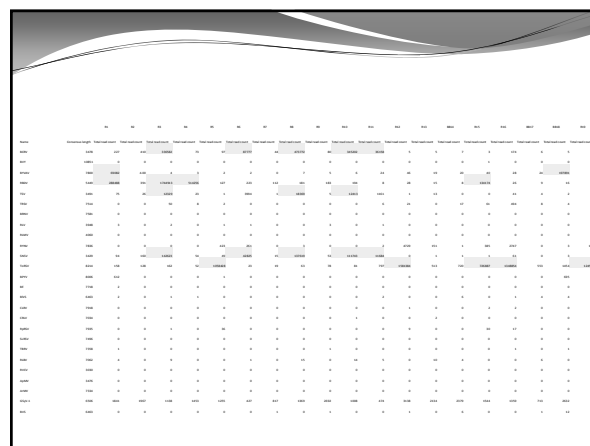
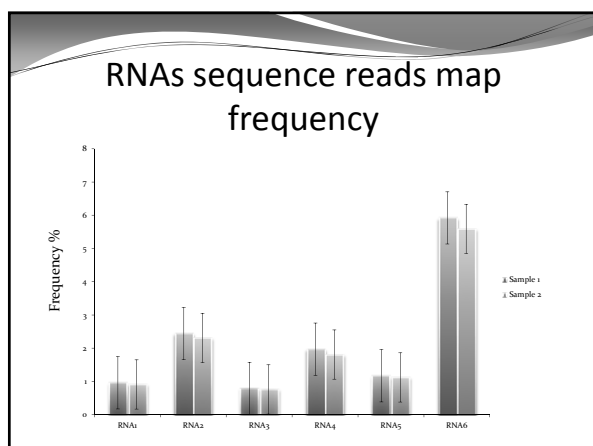
PCR, Sequence



Sequence reads mapping

	Sample 1		Sample 2		Healthy control
	number	percent	number	percent	number
RNA1	373,519	0.96	340,701	0.91	26
RNA2	952,707	2.44	870,223	2.31	66
RNA3	309,844	0.79	285,005	0.76	11
RNA4	767,414	1.97	681,795	1.81	16
RNA5	455,429	1.17	420,168	1.12	26
RNA6	2,306,848	5.92	2,103,686	5.59	109





Sample	NC-1	NC-2	NC-3	NC-4	NC-5	NC-6	NC-7	NC-8	NC-9	NC-10	NC-11	NC-12	NC-13	NC-14
Concentrate	Read Count	Read Count	Read Count	Read Count	Read Count	Read Count	Read Count	Read Count	Read Count	Read Count	Read Count	Read Count	Read Count	Read Count
NC-1	4532	35	330	18	3224	24	324	4444287	957882	180	635	52	4204538	108954
NC-2	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-3	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-4	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-5	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-6	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-7	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-8	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-9	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-10	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-11	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-12	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-13	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-14	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-15	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-16	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-17	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-18	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-19	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-20	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-21	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-22	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-23	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-24	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-25	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-26	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-27	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-28	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-29	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-30	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-31	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-32	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-33	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-34	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-35	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-36	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-37	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-38	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-39	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-40	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-41	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-42	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-43	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-44	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-45	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-46	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-47	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-48	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-49	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-50	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-51	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-52	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-53	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-54	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-55	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-56	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-57	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-58	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-59	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-60	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-61	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-62	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-63	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-64	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-65	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-66	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-67	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-68	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-69	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-70	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-71	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-72	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-73	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-74	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-75	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-76	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-77	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-78	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-79	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-80	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-81	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-82	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-83	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-84	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-85	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-86	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-87	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-88	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-89	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-90	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-91	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-92	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-93	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-94	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-95	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-96	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-97	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-98	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-99	3820	1	4	3	0	4	2	1	0	2	7	2	0	0
NC-100	3820	1	4	3	0	4	2	1	0	2	7	2	0	0

Do I see NCPDN using NGS?

- RNA extraction
- DNase treatment ~\$50
- rRNA depletion ~\$100
- Library preparation ~\$100-200
- Sequence run ~200
- Total cost ~\$525-650 per sample
- Bioinformatics support
- Lab validation – PCR and Sequence

Next Generation Sequencing Technology: Why do it?

- Routine detection of viruses and other pathogens
- Extremely sensitive
- Highly reliable

