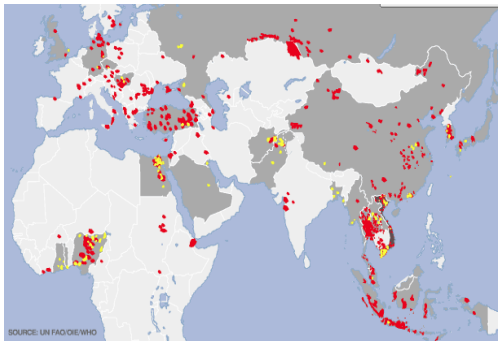
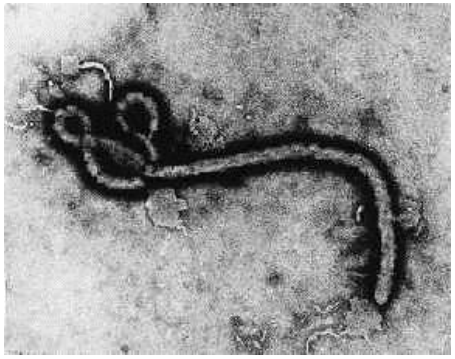
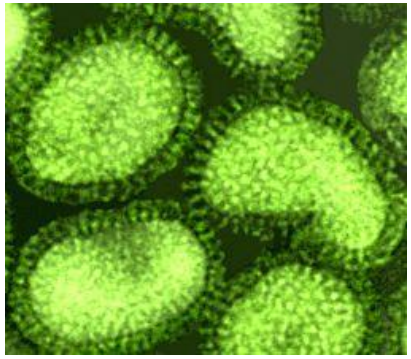
Overview of viral projects: technology, microbiome, phylogenetics, zoonotic infections

David E. Wentworth,
Dir. of Viral Programs







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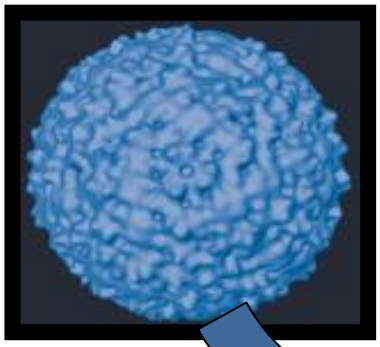
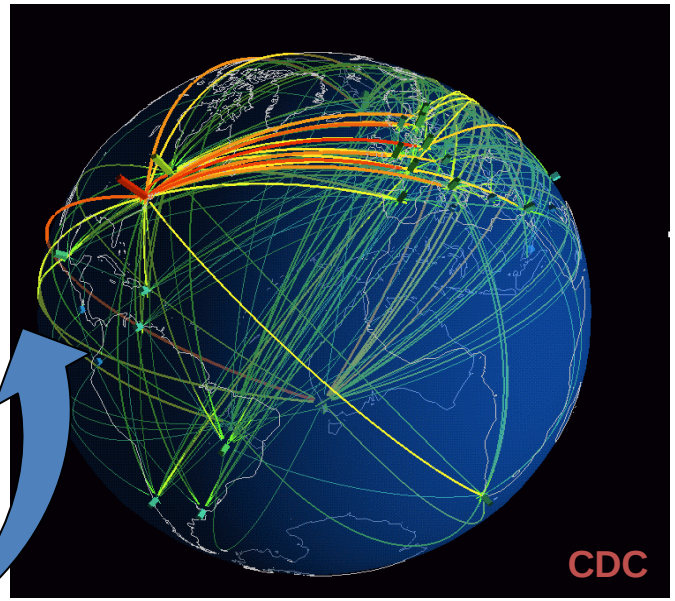
Outline

- NIH/NIAID Genomic Sequencing Centers for Infectious Diseases
- Zoonosis and emerging diseases
- Viral Sequencing Projects and Pipeline
  - Influenza virus genomics
  - Rotavirus and microbiome





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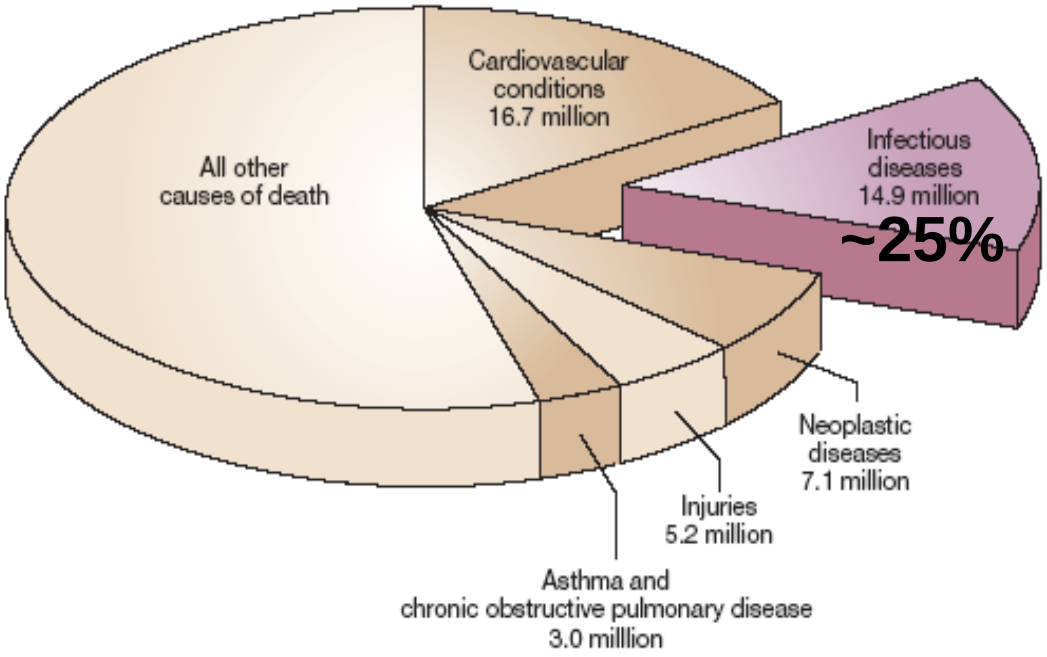
Infectious Diseases Worldwide

"a leading cause of death"



Infectious diseases	Annual deaths (million)
Respiratory infections	3.96
HIV/AIDS	2.77
Diarrhoeal diseases	1.80
Tuberculosis	1.56
Vaccine-preventable childhood diseases	1.12
Malaria	1.27
STDs (other than HIV)	0.18
Meningitis	0.17
Hepatitis B and C	0.16
Tropical parasitic diseases	0.13
Dengue	0.02
Other infectious diseases	1.76

Figure 2 Leading causes of death worldwide. About 15 million (>25%) of 57 million annual deaths worldwide are the direct result of infectious disease. Figures published by the World Health Organization (see http://www.who.int/whr/en and ref. 7).

Morens et al. (2004) Nature vol. 430 p 242-249

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NIH/NIAID Genomics Sequencing Centers for Infectious Disease

Provide services for rapid and cost efficient production of high-quality, genome sequences and high-throughput genotyping of NIAID Category A-C priority pathogens, microorganisms responsible for **emerging and re-emerging infectious diseases and their hosts**, related organisms, clinical isolates, and invertebrate vectors of infectious diseases.

- Themes:
  - Evolution of pathogenicity
  - Microbiome and infectious diseases
  - Vaccine development
  - Host-pathogen interactions
  - Genotype-phenotype association
  - Drug resistance

Services are provided by the J. Craig Venter Institute (JCVI), the Broad Institute, and the Institute for Genome Sciences at the University of Maryland School of Medicine

http://www.niaid.nih.gov/labsandresources/resources/dmid/gsc/Pages/default.aspx

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Current JCVI GSC Portfolio

Sequencing Projects

- *Adenovirus, Arbovirus, Coronavirus, Influenza, Measels, Mumps, Norovirus, Paramyxovirus, Rotavirus, Rubella, Varicella*
- *A. baumannii, Burkholderia spp., E. coli, Leptospira spp., S. aureus, Streptococcus spp., Y. pestis, Bordetella spp.*
- *A. fumigatus, Entamoeba spp., G. niphandrodes, H. hammondi, Toxoplasma spp., Malaria, C. gattii*

Other Sequencing Projects

- Nasal Microbiome
- Influenza Microbiome
- Ferret Metagenomics

Software Projects

- Primer Designer
- Cloud enabled genomic tools

Methods/Tech, Reagent Projects

- Uncultivable
- Synfluenza
- Synthetic tools for drug discovery

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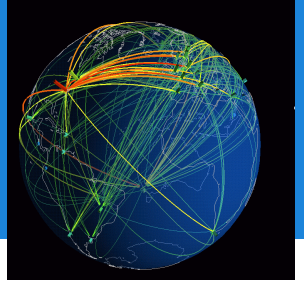
Definitions



- **Endemic (enzootic) disease**
  - **Pathogen present in human (animal) population**
- **Epidemic (epizootic)**
  - **Increased incidence of disease in humans (animals) or “outbreak”**
- **Pandemic**
  - **Worldwide increased incidence of disease**
- **Zoonosis**
  - **Disease transmitted from animals to humans , called “zoonotic transmission”**

Zoonosis and Emerging Infections

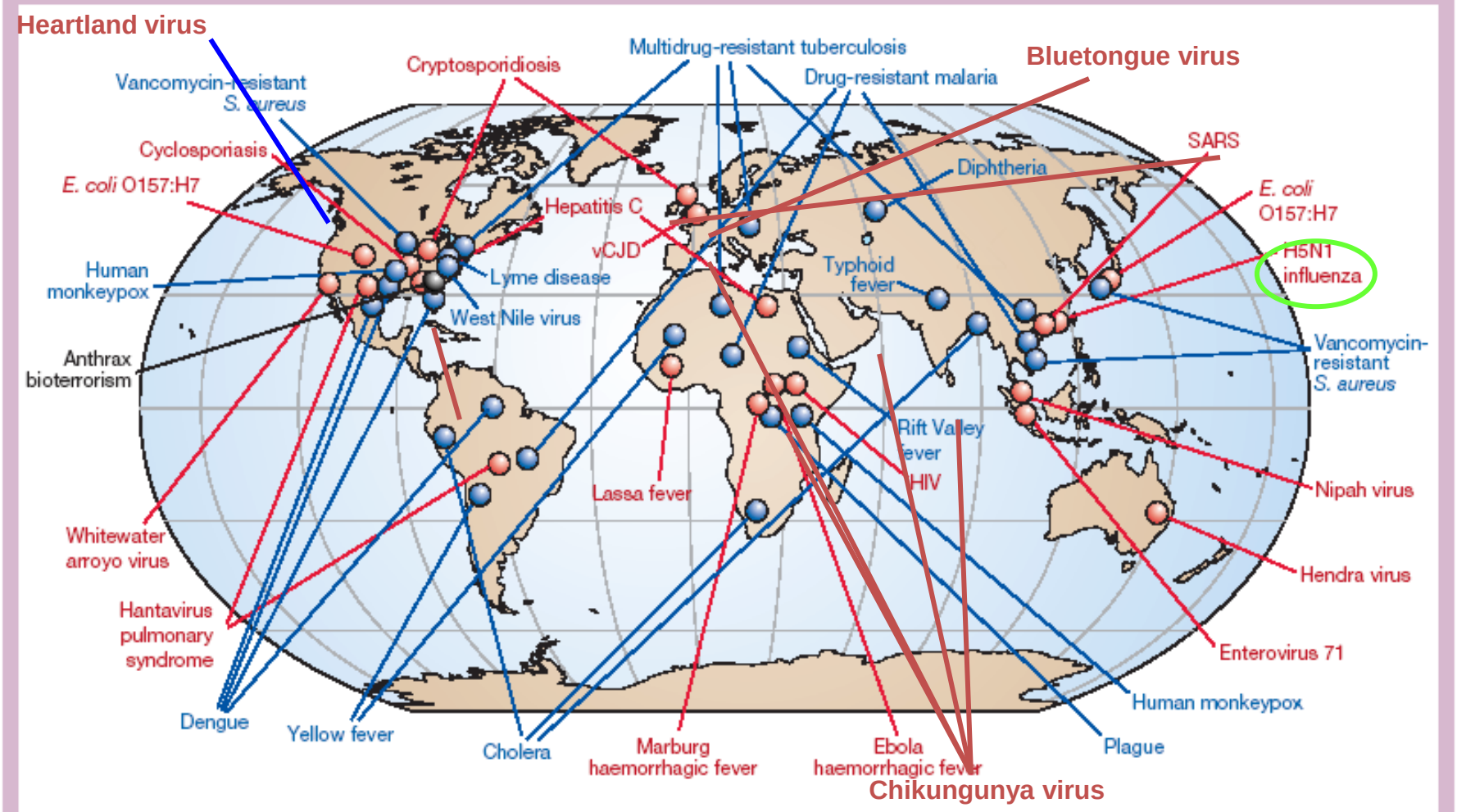


Figure 1 Global examples of emerging and re-emerging infectious diseases, some of which are discussed in the main text. Red represents newly emerging diseases; blue, re-emerging/resurging diseases; black, a 'deliberately emerging' disease. Adapted, with permission, from ref. 23.

Adapted from Morens et al. (2004) Nature, vol. 430 p 242-249

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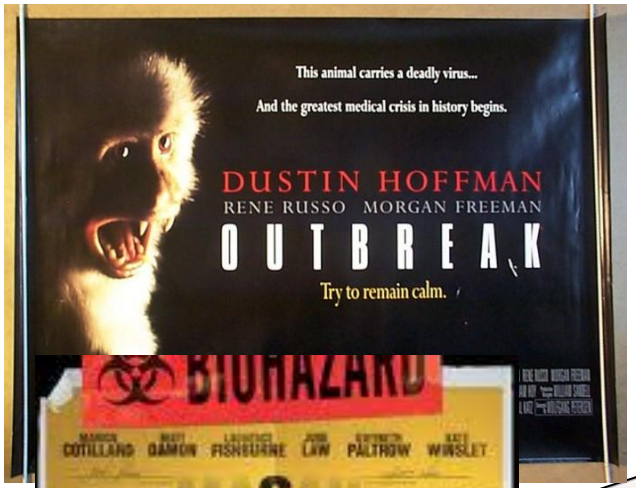
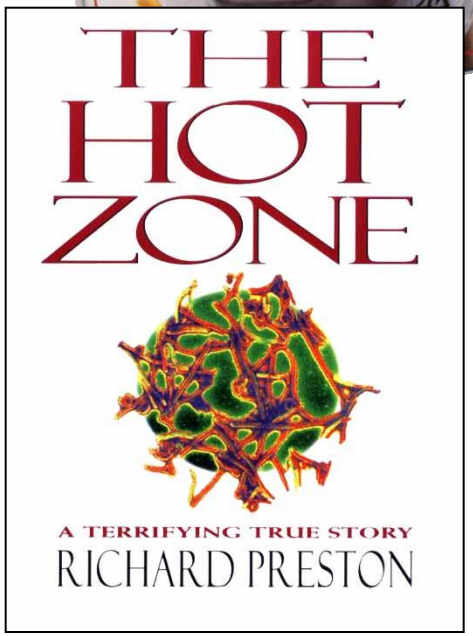
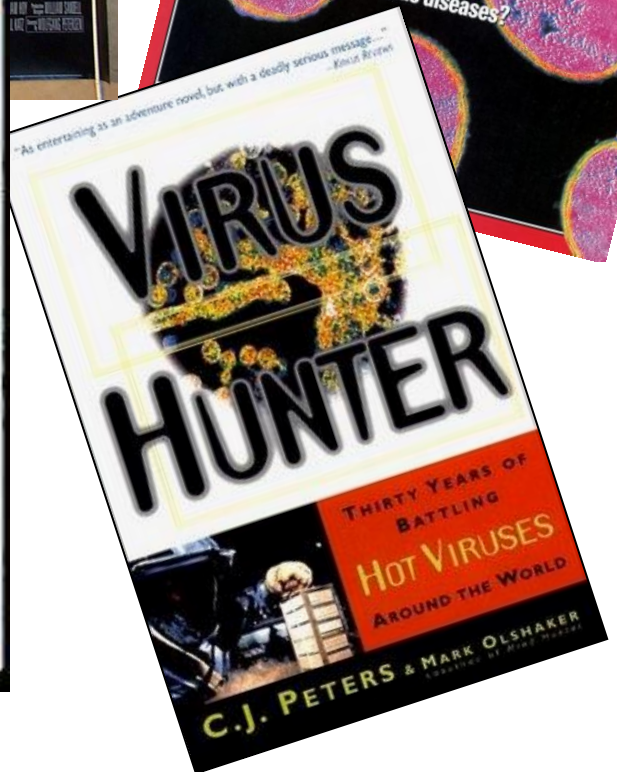
Emerging Viruses in the Press & Popular Culture



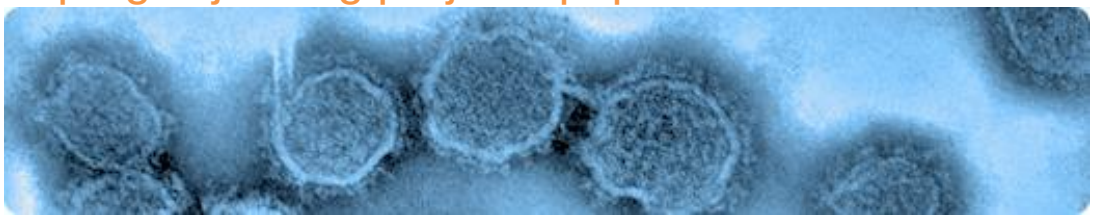




NIH/NIAID GSC Sponsored Viral Sequencing Projects at JCVI

- + RNA
  - Coronavirus
  - Norovirus
  - Venezuelan Equine Encephalitis Virus
  - Japanese Encephalitis Virus
  - Yellow fever virus
  - Rhinovirus
  - Enterovirus 71
- -RNA
  - Influenza A and B
  - Metapneumovirus
  - Respiratory Syncytial Virus
  - Parainfluenza
  - Measels
  - Mumps
  - Rubella
  - Filoviruses
- dsRNA
  - Rotavirus
- DNA
  - Varicella Zoster
  - Adenovirus
- Microbiome/ Metagenome
  - Influenza virus
  - Rotavirus
- Role of UTR's
  - Influenza virus
- Synfluenza
  - Synthesis of HA's and NA's

http://gsc.jcvi.org/projects.php



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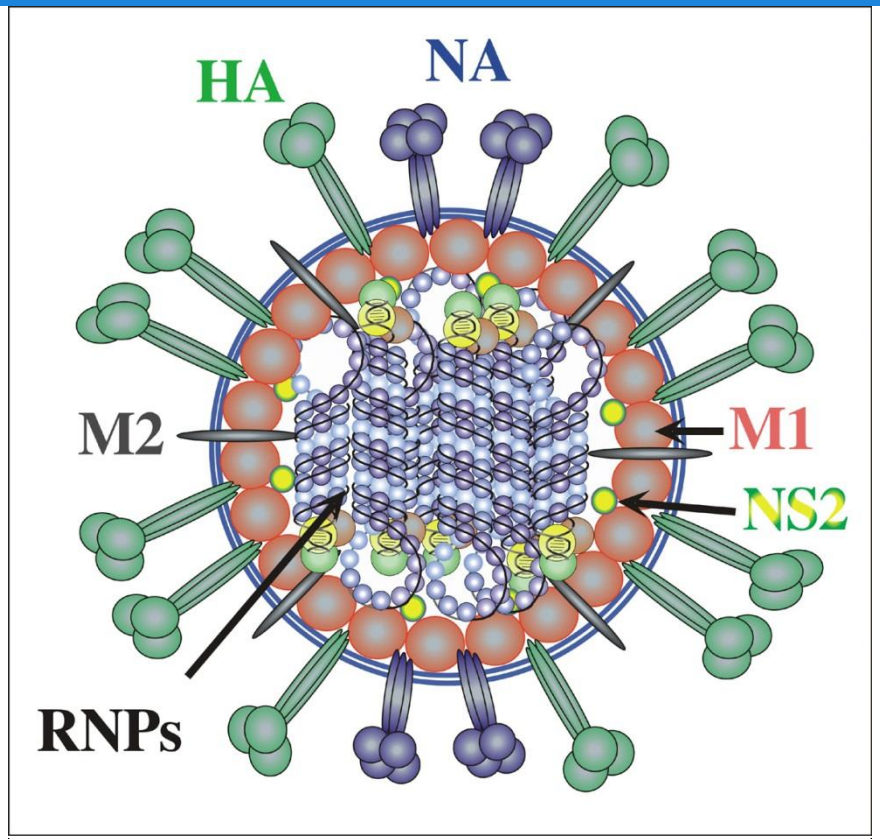
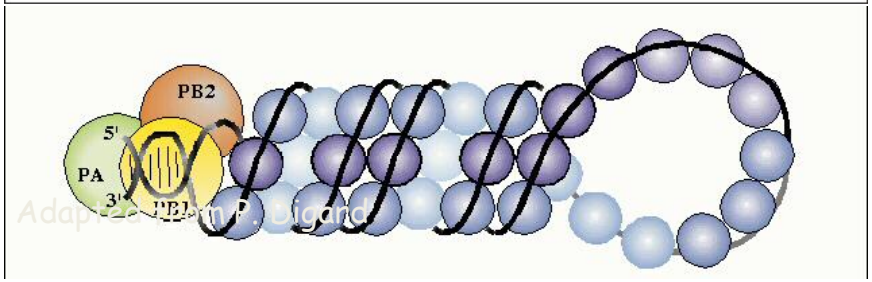
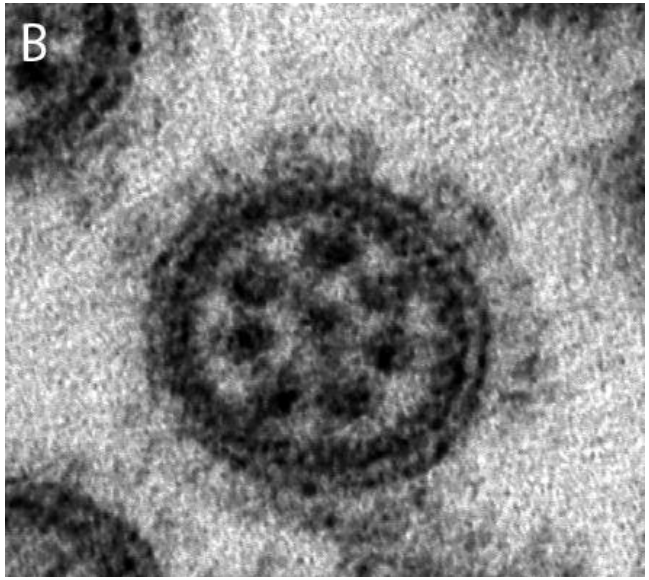
Influenza A Virus





Modified from P. Digard

- 16 distinct HA's +1
  - (H1-H16)
- 9 distinct NA's +1
  - (N1-N9)
- Nomenclature
  - A/Chicken/WI/5/78 (H7N7)



Thin Section EM. T. Noda, et al, Nature 439 (7075):490-492, 2006.

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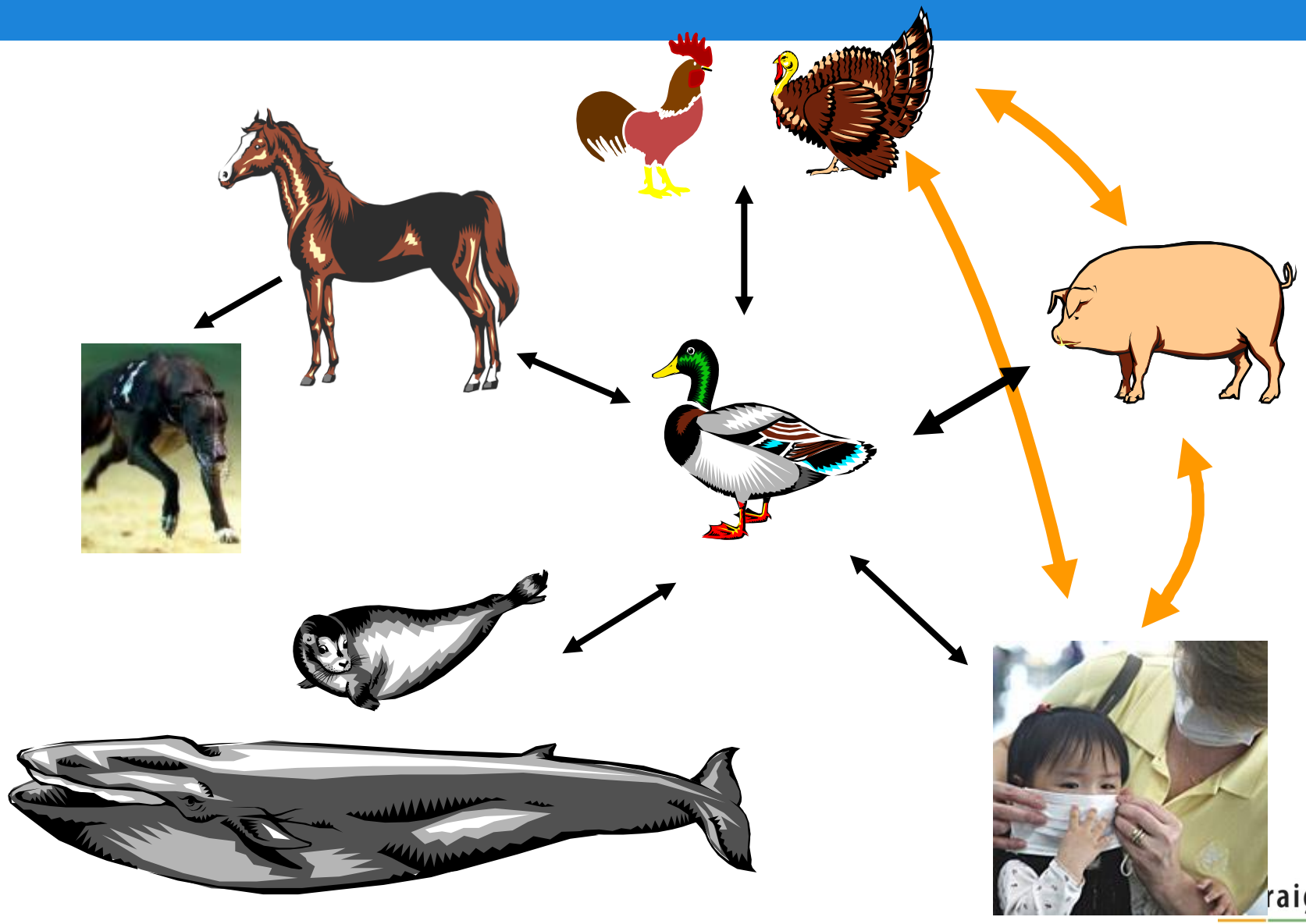
Influenza A Ecology, and Zoonosis



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NIAID Sponsored Influenza Genome Sequencing Project Goals

Increase genome knowledge base

- Improve understanding
  - Evolution, spread, antiviral resistance, and disease
- Aid in the development of:
  - Vaccines, Therapies, Diagnostics
- Data generated is publicly available
  - GenBank
  - Analysis tools -> NCBI, IRD

Mitigate the impact influenza epidemics/pandemics

http://www.niaid.nih.gov/LabsAndResources/resources/dmid/gsc/Influenza/Pages/overview.aspx

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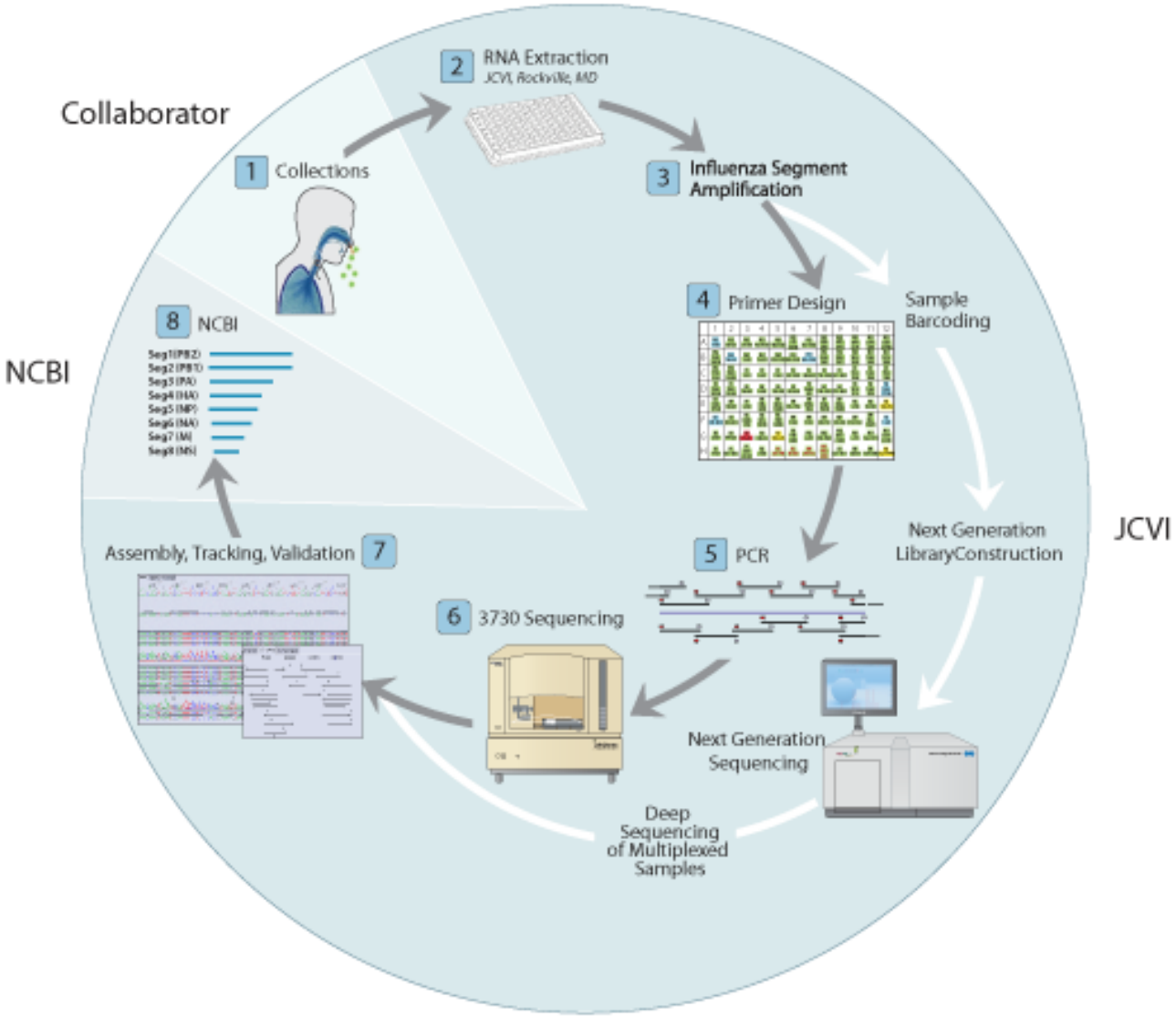
Virus Sequencing Pipelines



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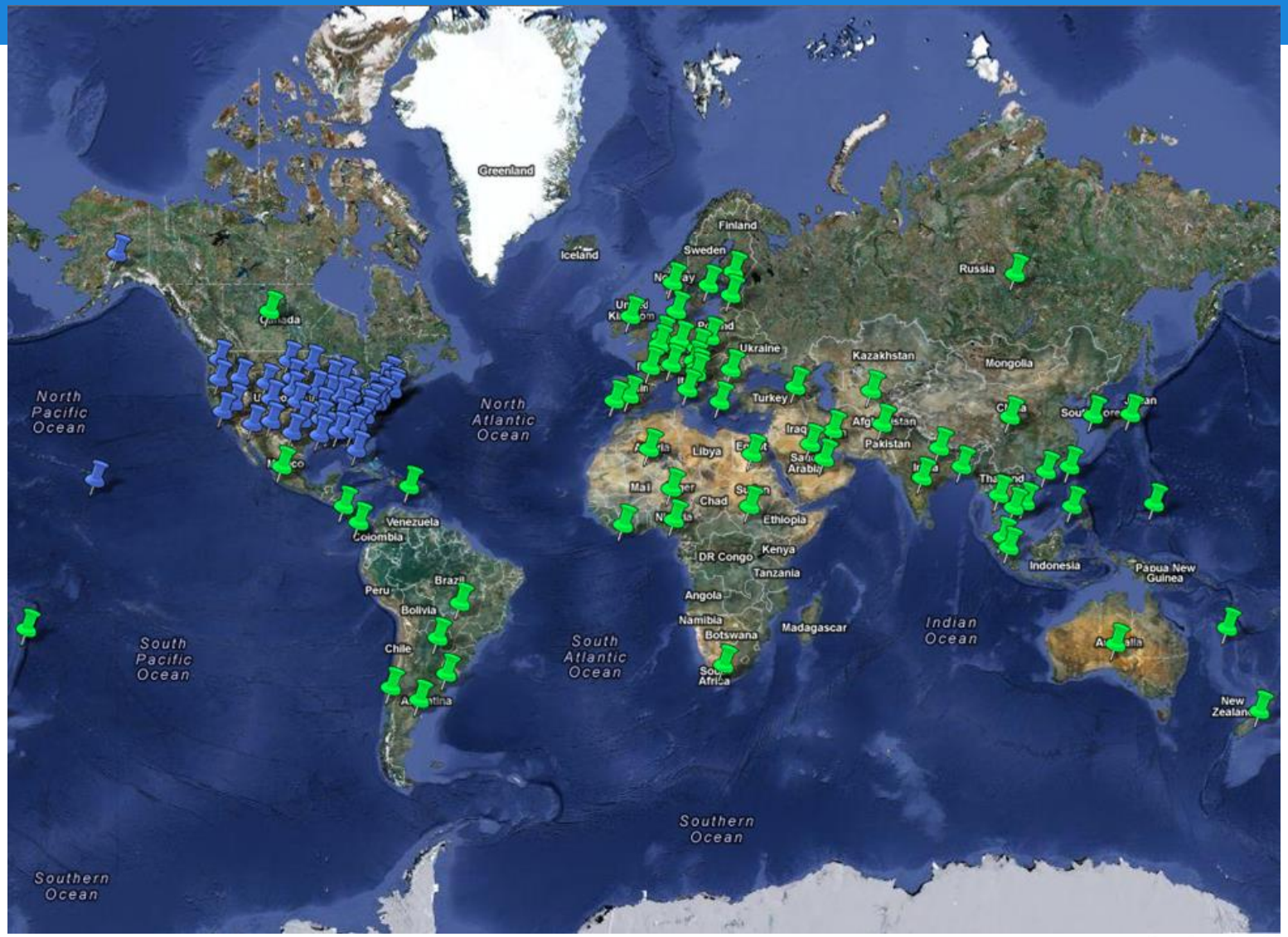
Influenza Genome Sequencing Project Collaborators



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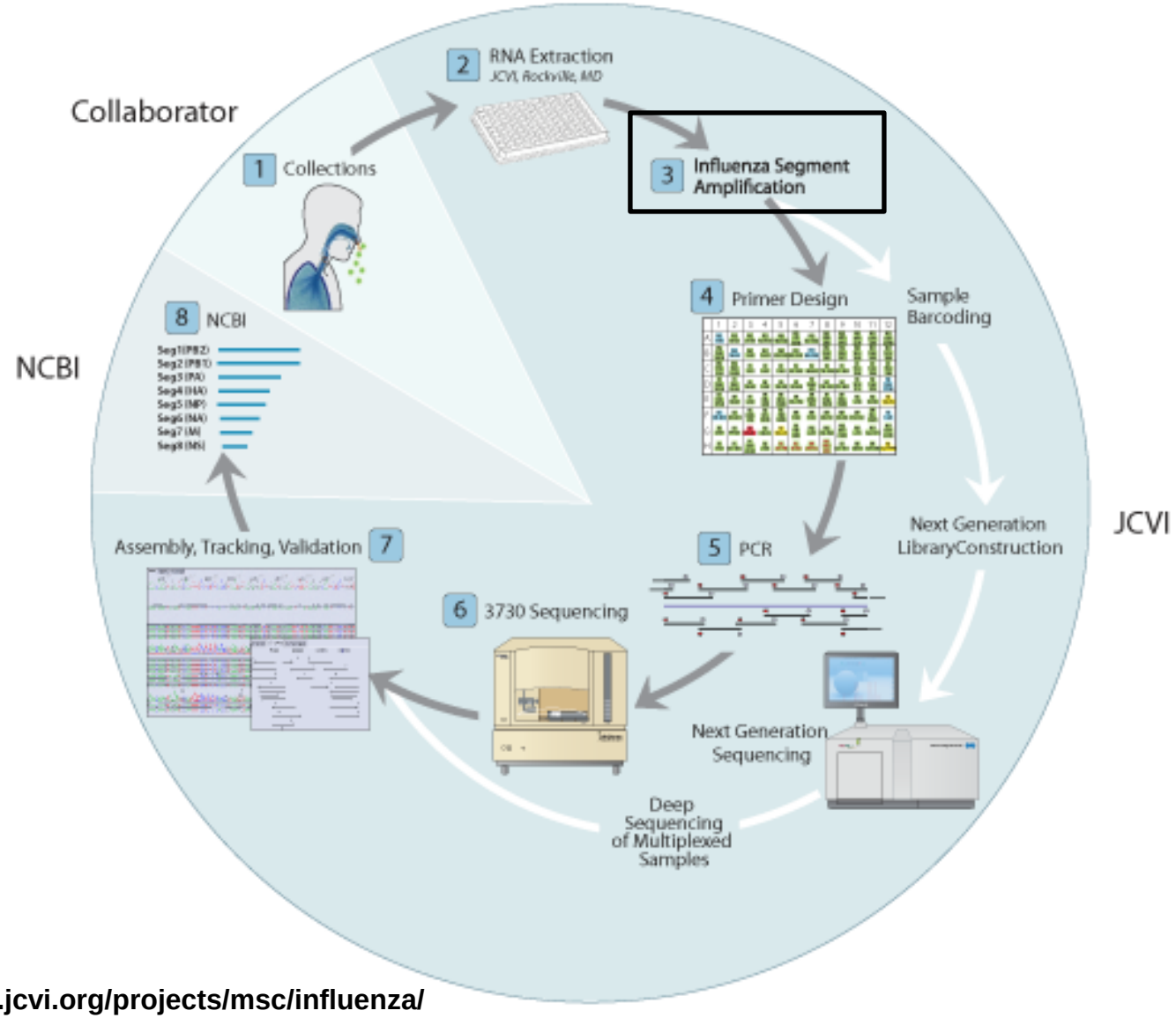
Influenza Virus Sequencing Pipeline



http://gsc.jcvi.org/projects/msc/influenza/

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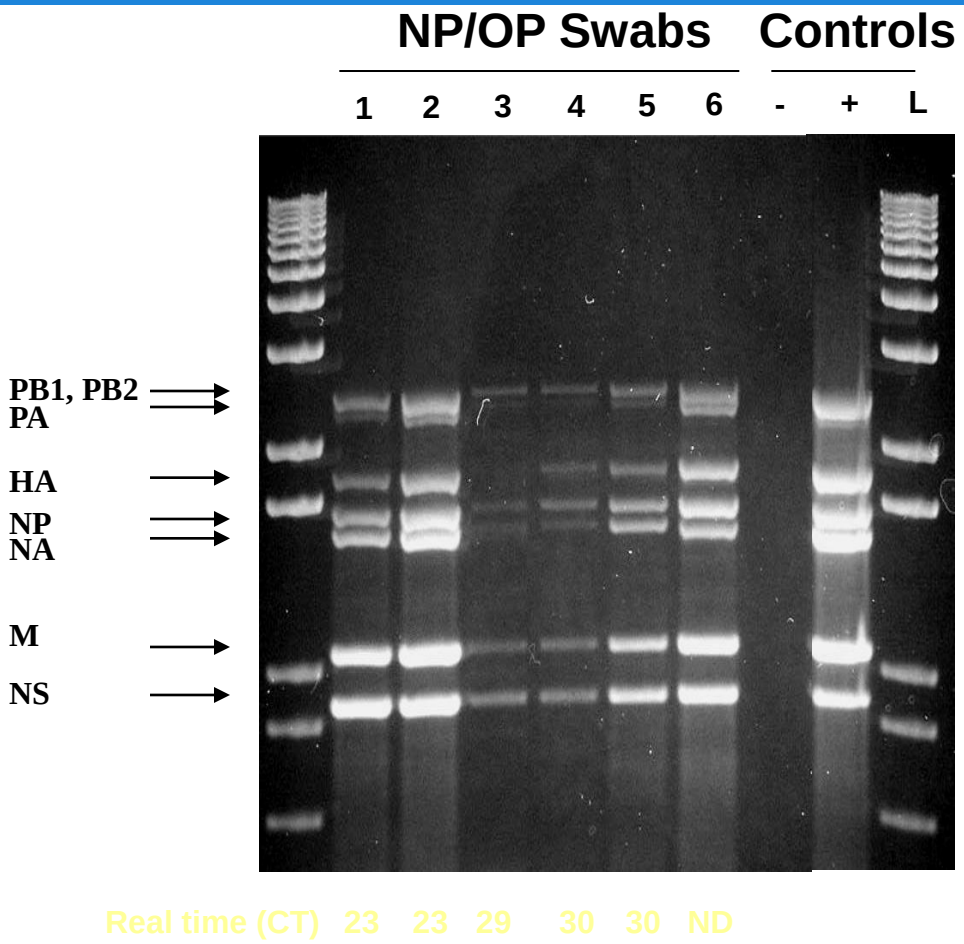
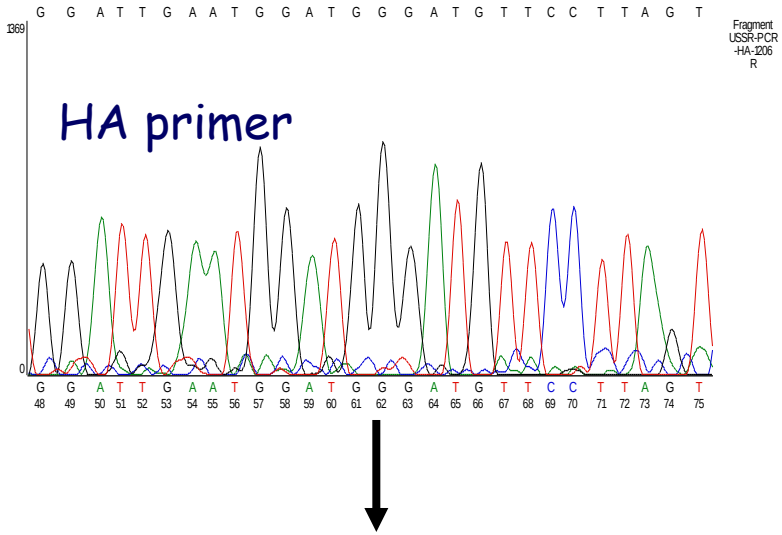
Genomic Amplification Directly From Clinical Specimens



Sequence M-RTPCR Amplicons



Genetic/Molecular Analysis

- Phylogeny
- Virulence Determinants
- Used in NIAID/JCVI influenza sequencing pipeline

Zhou, B., M. E. Donnelly, D. T. Scholes, K. St.George, M. Hatta, Y. Kawaoka, and D. E. Wentworth. 2009. J.Virol. 83:10309-10313.

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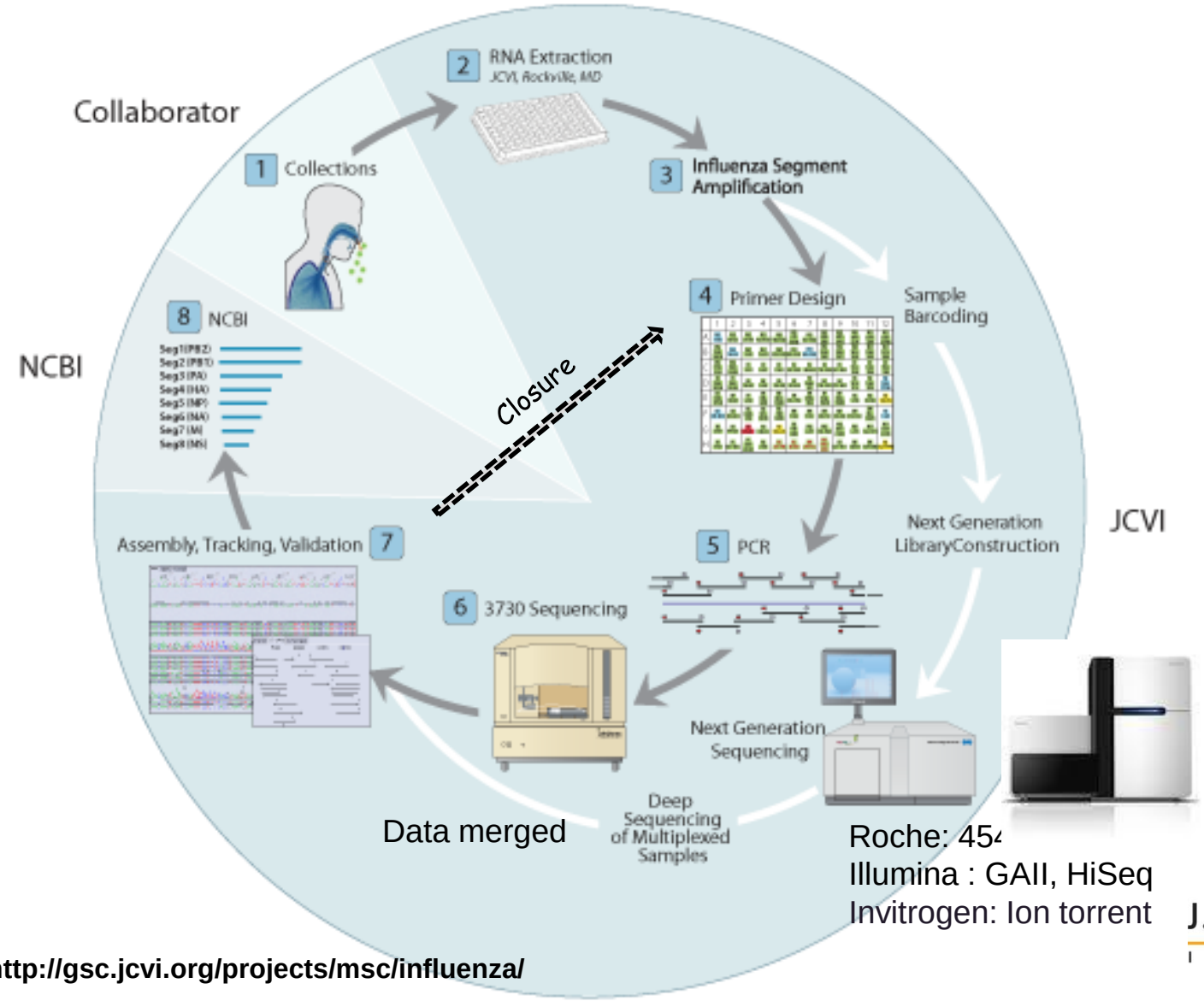
JCVI Sequencing, Assembly, and Submission



http://gsc.jcvi.org/projects/msc/influenza/

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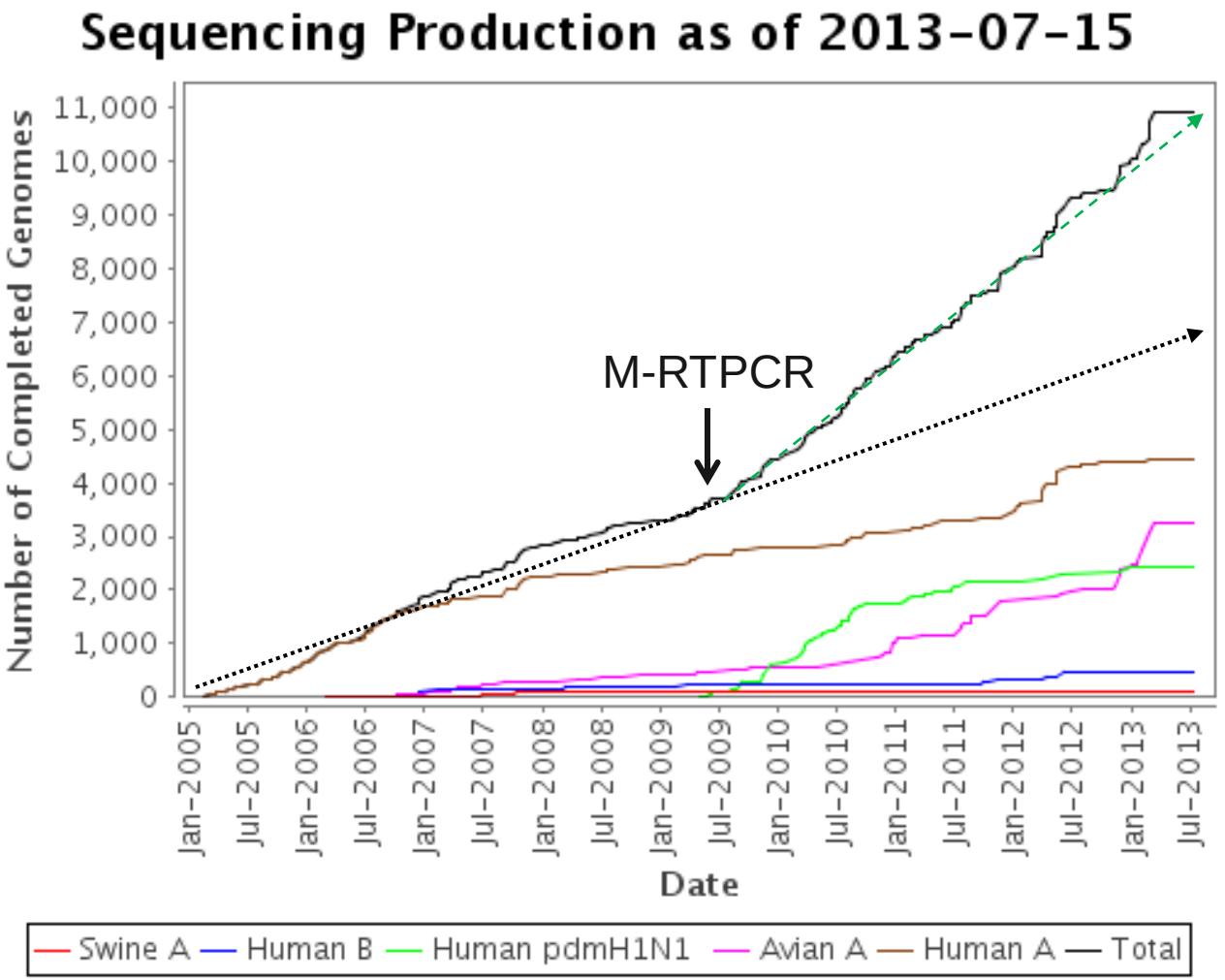
Influenza Status



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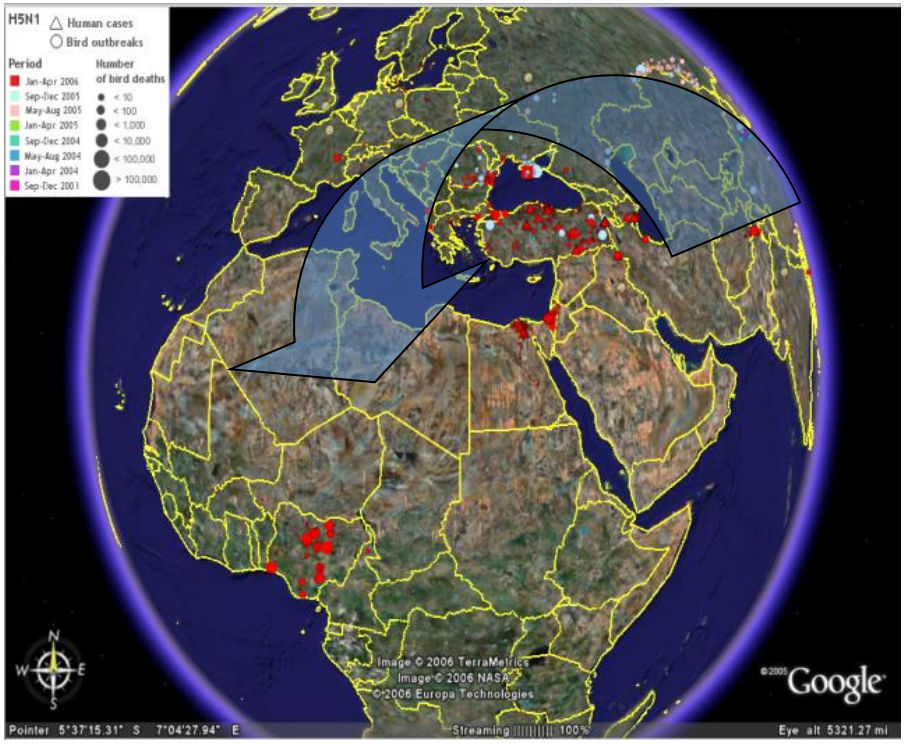
Viral Genomics Elucidates:

- **Global movement**
  - **Human**
  - **Avian**
    - **migratory pathways**
- Antigenic Shift
  - New viruses from reservoirs
  - Pandemic potential
- Genetic Determinants
  - Virulence (HPAI e.g., H5N1)
  - Drug Resistance
- Evolution of seasonal influenza
  - Antigenic Drift
    - Vaccine selection



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Viral Genomics Elucidates:

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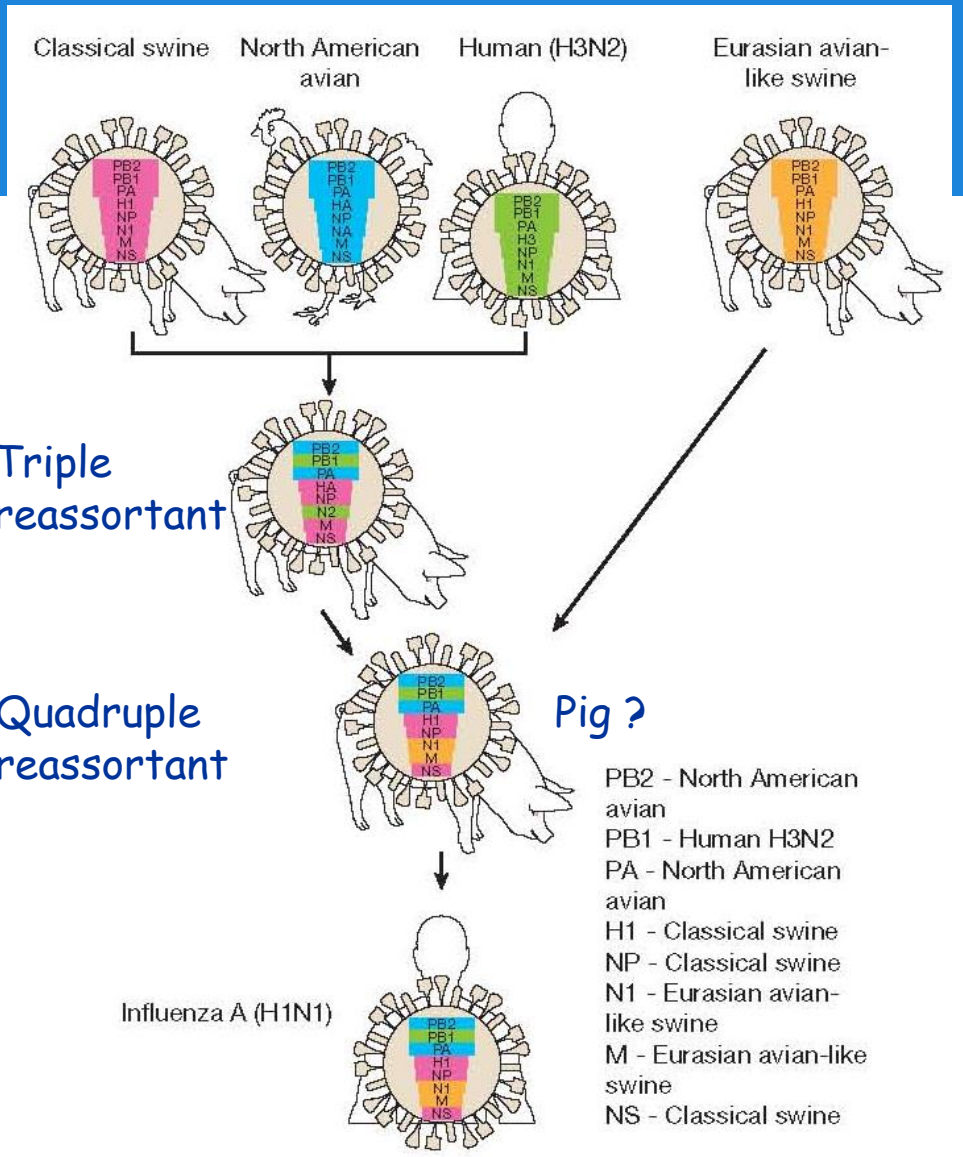

Figure 4 | Genesis of swine-origin H1N1 influenza viruses. In the late 1990s,

G. Neumann, T. Noda, and Y. Kawaoka. *Nature* 459 (7249):931-939, 2009.

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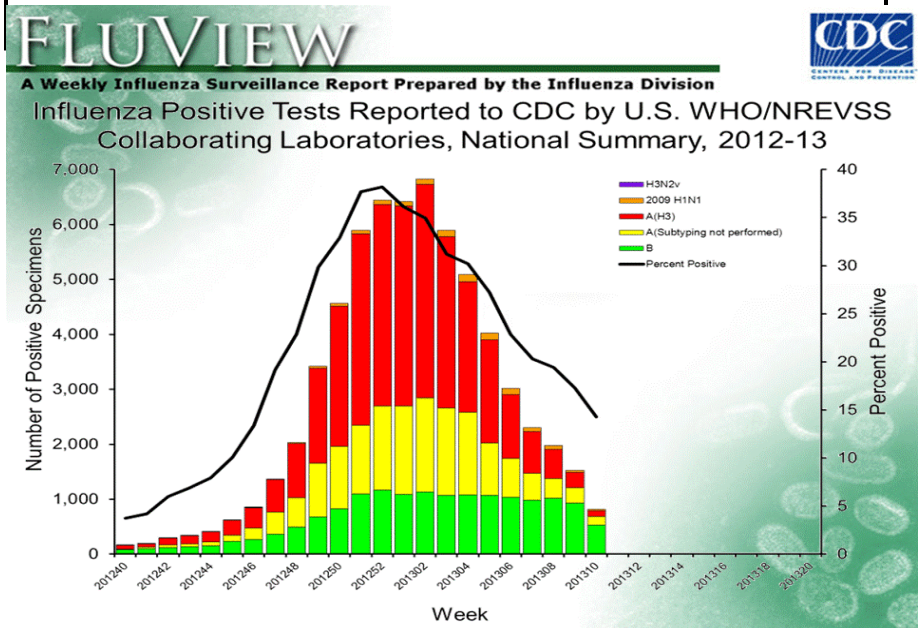
Viral Genomics Elucidates:

- **Evolution of seasonal influenza**
  - **Antigenic Drift**
    - **Vaccine selection**

Collaboration with J. Musser



Clade definitions from the Center for Disease Control (CDC)

Maximum-likelihood Phylogenetic Tree of HA

Clade 3D (newly proposed based on this study)

Purple: vaccine strains
Red: 2012-2013 samples
Black: reference H3N2 strains

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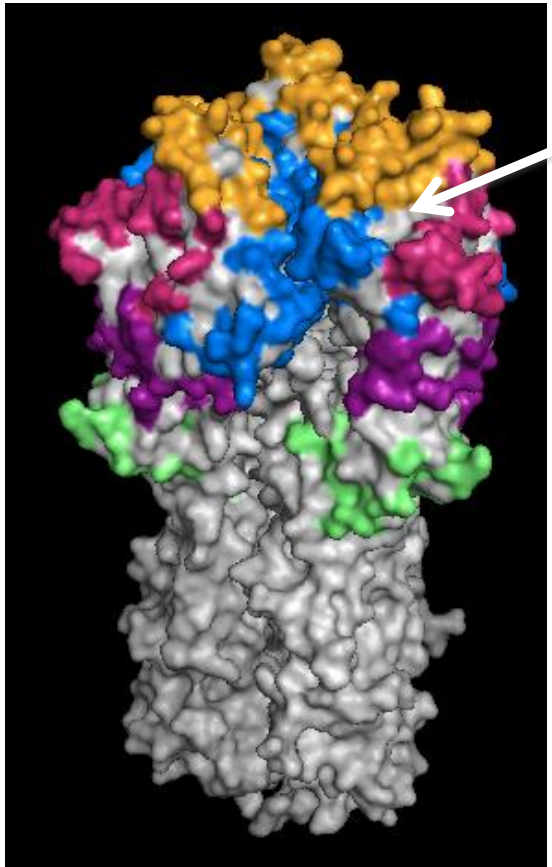
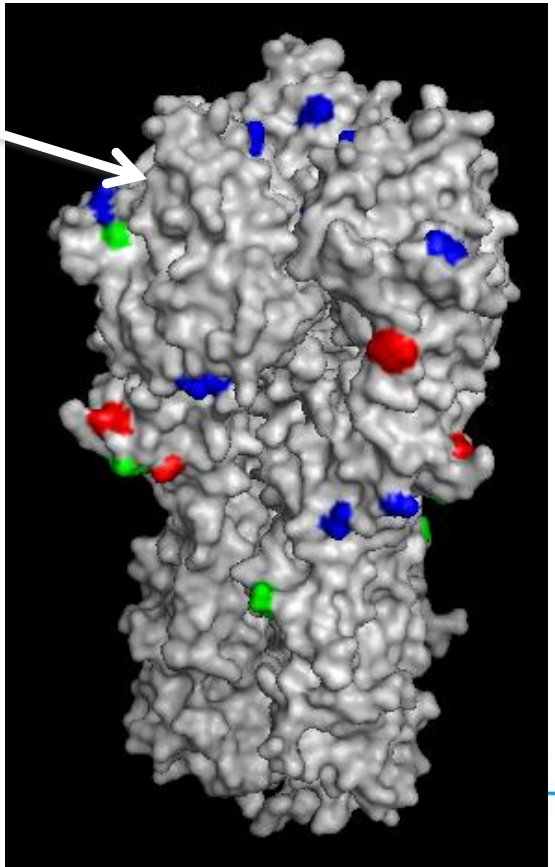
Key Amino Acid Substitutions in the 2012/13 H3N2 HA Structure

Name	Key Amino Acid Substitutions of Interest in 2012-2013 Samples									
	49	61/64* (Epitope C)	69 (Epitope C)	110 (Epitope E)	160/161* (Epitope A)	214 (Epitope B)	228 (Epitope D)	277 (Epitope E)	294 (Epitope C)	296 (Epitope C)
Pre-2012-2013 Virus	Q	N/I	D	Y	N/N	S	A	R	N	E
Clade 5	Q	S/T	N	H	N/N	A	A	R	N	A
Clade 3A	Q	S/T	D	Y	D/S	A	S	Q	N	E
Clade 3D	R	N/I	D	Y	N/S	S	A	R	K	E

*Changes a potential glycosylation site.



Receptor Binding Site



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Genomics and Vaccine Informatics

- What should go into a vaccine?
  - Track the viral evolution
  - Determine/predict vaccine candidates protection
  - Combine the information

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Rotavirus Genomics and Microbiome Interactions

Significance

- Diarrheal diseases cause ~1.5 million annual fatalities
  - children under 5 years of age
- Rotaviruses contribute to ~500,000 annual pediatric deaths

Study Aims

- Advance understanding of pediatric diarrheal diseases in Africa
- Metagenomic approaches
  - evaluate enteric community relationships
    - rotaviruses, other pathogens, and commensal microbiota

Hypotheses

- Rotavirus infection has a direct impact on the gut microbiome
  - rotaviral genotype is likely to correlate with specific alteration(s) of the host microbiota.
- The rotaviral vaccination campaign in South Africa has/will alter pediatric enteric microbial communities

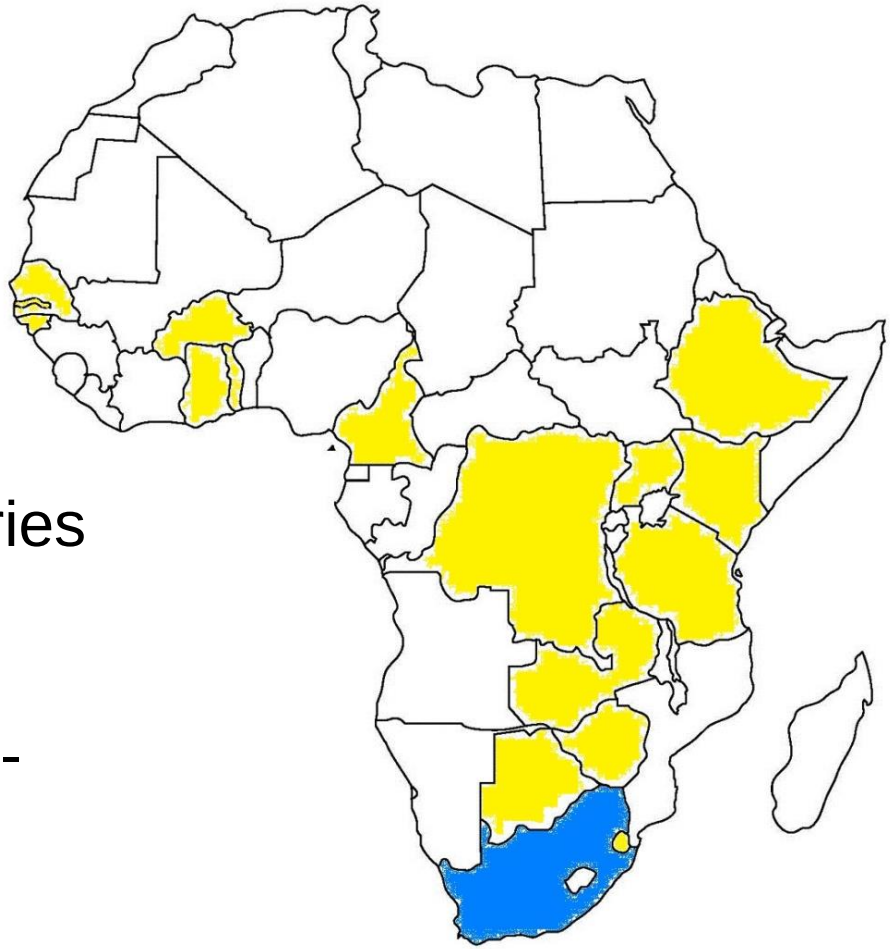
Collaboration with M. Jeffrey Mphahlele
Department of Virology, University of Limpopo

Rotavirus Samples & Locations

Samples

- Preliminary data:
  - 12 stool samples
    - South Africa
    - collected 2004-2010
- ~300 stool samples
  - multiple African countries
  - collected 1998-2010
  - children <5 years old
  - pre- and post-rotaviral-vaccination periods

Locations



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MRC SOUTH AFRICA

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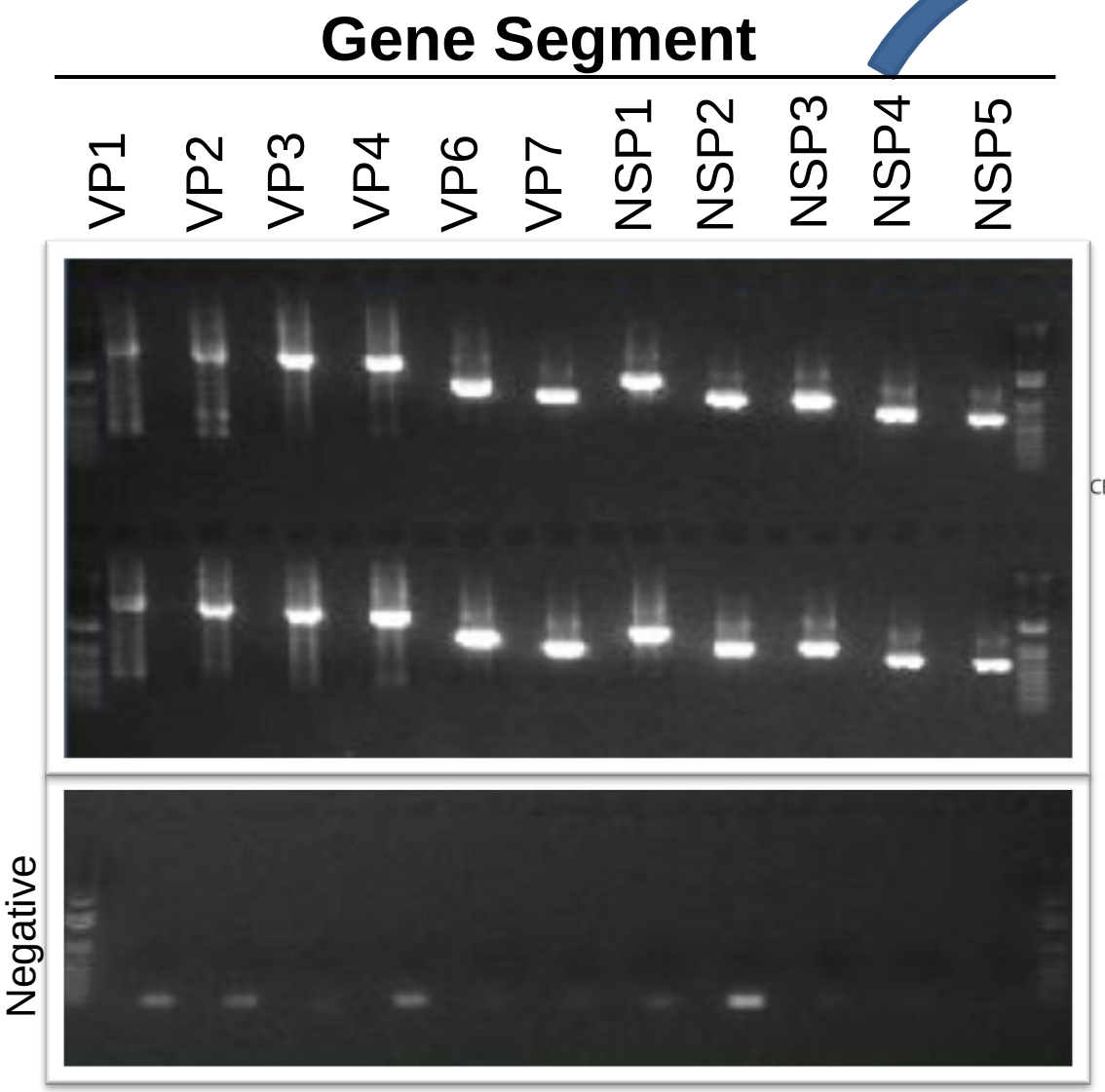
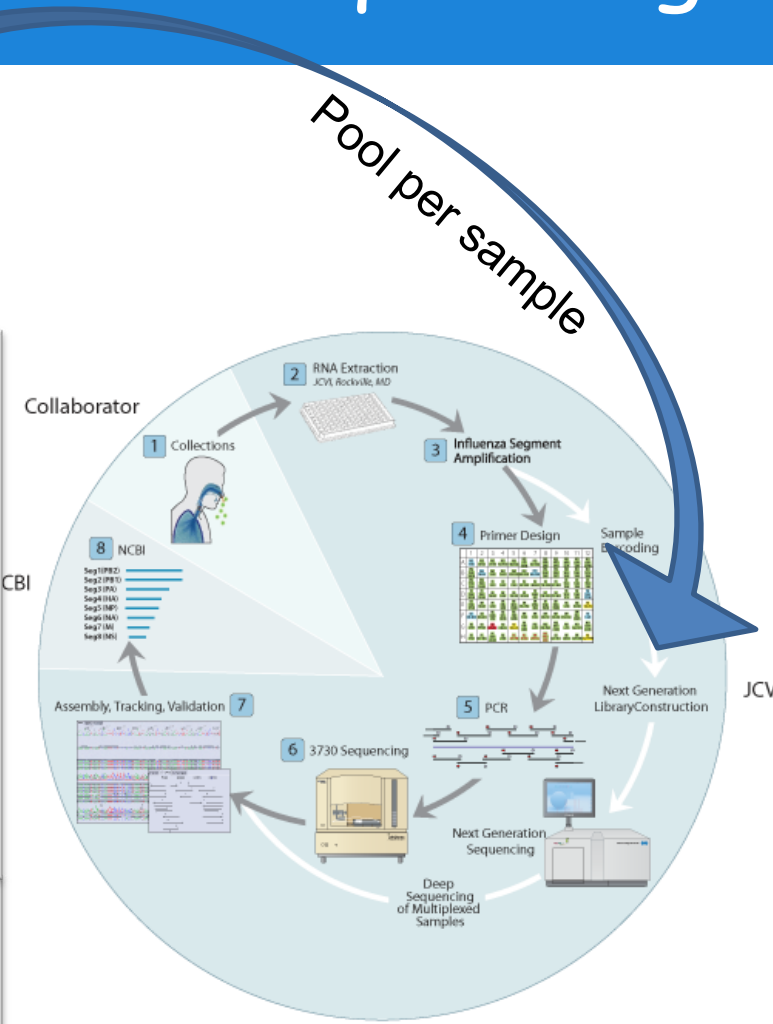
Rotavirus Amplification and Sequencing





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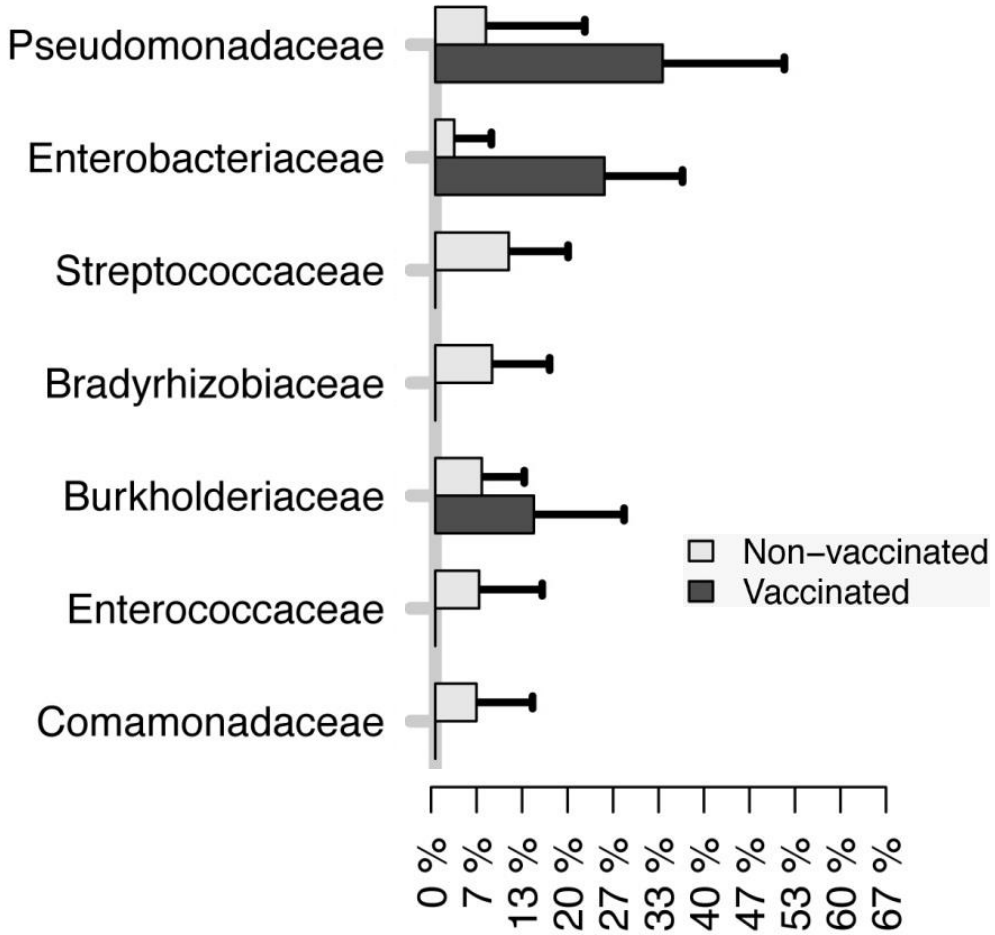
16S Sequencing and Changes in Top 7 Operational Taxonomic Units



Enteric bacterial communities may be changing with the introduction of rotaviral vaccines in South Africa

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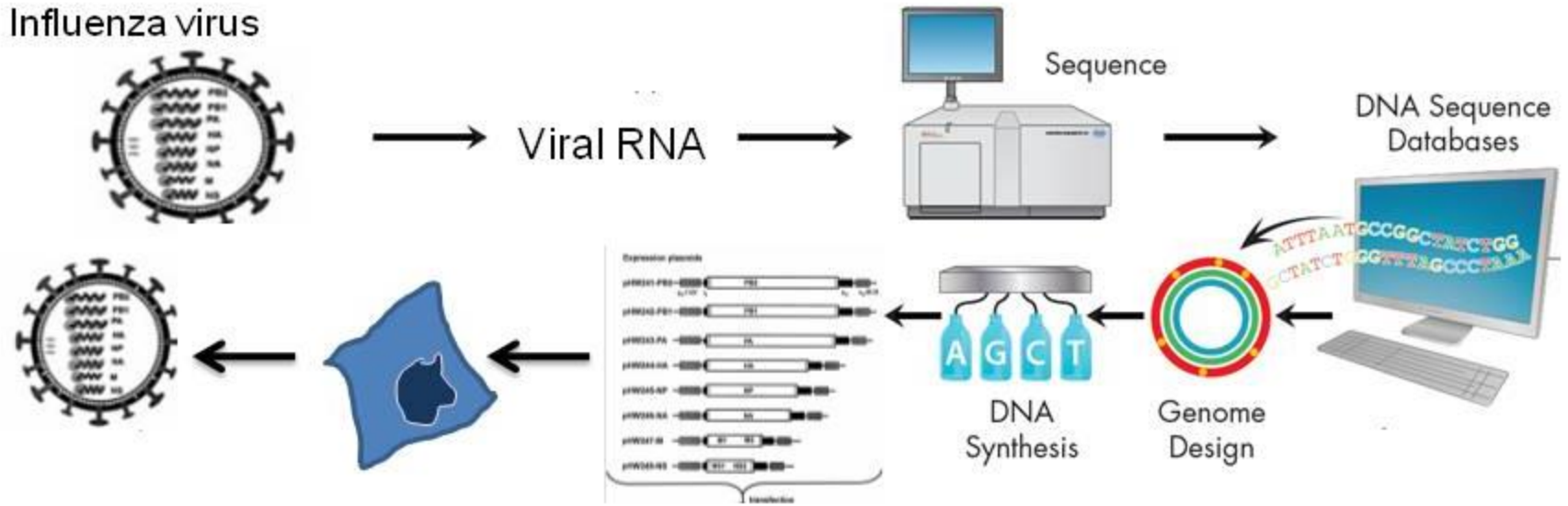
Viral Synthetic Genomics



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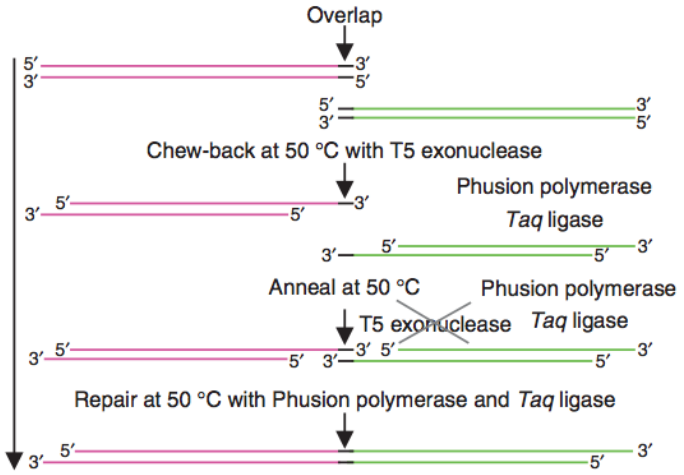
Synthetic Genomics Tools

Gibson Assembly



RESEARCH ARTICLE

Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome

Daniel G. Gibson, Gwynedd A. Bende
Holly Baden-Tillson, Jayshree Zaveri, T
Mikkel A. Algire, Chuck Merryman, Lei
Clyde A. Hutchison III, Hamilton O. Sm

genome, we needed to establish convenient and reliable methods for the assembly and cloning of much larger synthetic DNA molecules.

Strategy for synthesis and assembly. The native 580,076-bp *M. genitalium* genome sequence (*Mycoplasma genitalium* G37 ATCC 33530 genomic sequence; accession no. L43967) (*3*) was partitioned into 101 cassettes of approximately 5 to 7 kb in length (Fig. 1) that were individually synthesized, verified by sequencing,

PNAS

One-step assembly in yeast of 25 overlapping DNA fragments to form a complete synthetic *Mycoplasma genitalium* genome

Daniel G. Gibson[a,1], Gwynedd A. Benders[b], Kevin C. Axelrod[a], Jayshree Zaveri[a], Mikkel A. Algire[a], Monzia Moodie[a], Michael G. Montague[a], J. Craig Venter[a], Hamilton O. Smith[b], and Clyde A. Hutchison III[a,b,1]

[a]The J. Craig Venter Institute, Synthetic Biology Group, Rockville, MD 20850 and [b]The J. Craig Venter Institute, Synthetic Biology Group, San Diego, CA 92121

Contributed by Clyde A. Hutc

We previously reported *Mycoplasma genitalium* *myces cerevisiae* by reco ments to produce a 592-k demonstrating assembly lapping fragments in a sin greatly simplifies the ass synthetic and natural fra

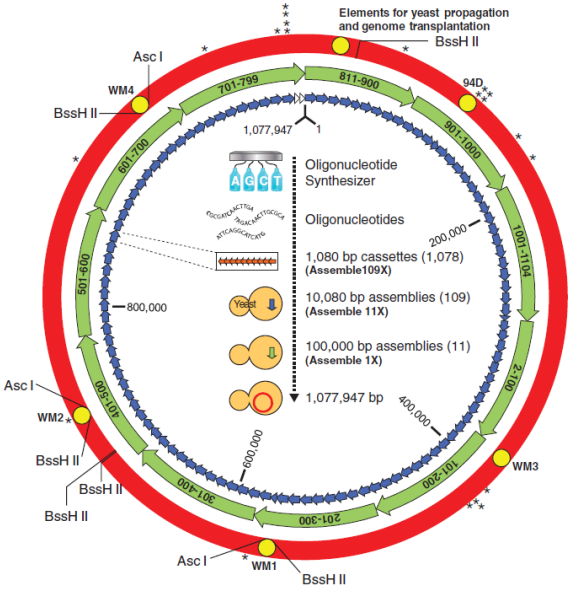
Enzymatic assembly of DNA molecules up to several hundred kilobases

Daniel G Gibson[1], Lei Young[1], Ray-Yuan Chuang[1], J Craig Venter[1,2], Clyde A Hutchison III[2] & Hamilton O Smith[2]

n method for

overlapping DNA molecules and then incubated at 50 °C for as few as 15 min (Online Methods). This approach dramatically simplifies the construction of large DNA molecules from constituent parts.

Exonucleases that recess double-stranded DNA from 5′ ends will not compete with polymerase activity. Thus, all enzymes required for DNA assembly can be simultaneously active in a single isothermal reaction. Furthermore, circular products can be enriched as they are not processed by any of the three enzymes in the reaction. We optimized a 50 °C isothermal assembly system using the activities of the 5′ T5 exonuclease (Epicentre), Phusion DNA polymerase (New England Biolabs (NEB)) and *Taq* DNA ligase (NEB) (**Fig. 1**). *Taq* DNA polymerase (NEB) can be used in place of Phusion DNA polymerase (data not shown), but the latter



Synthesis of DNA fragments in yeast by one-step assembly of overlapping oligonucleotides

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ABSTRACT

Here it is demonstrated th *myces cerevisiae* can take least 38 overlapping singl otides and a linear double- transformation event. Thes overlap by as few as 20 b as 200 nucleotides in lengt scheme for assembling oligonucleotides could be a synthetic DNA molecules.

RESEARCH ARTICLE

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

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We report the design, synthesis, and assembly of the 1.08–mega–base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including "watermark" sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

crude *M. mycoides* or *M. capricolum* extracts, or by simply disrupting the recipient cell's restriction system (*8*).

We now have combined all of our previously established procedures and report the synthesis, assembly, cloning, and successful transplantation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by this synthetic genome.

Synthetic genome design. Design of the *M. mycoides* JCVI-syn1.0 genome was based on the highly accurate finished genome sequences of two laboratory strains of *M. mycoides* subspecies *capri* GM12 (*8*, *9*, *11*). One was the genome donor used by Lartigue *et al*. [GenBank accession CP001621] (*10*). The other was a strain created by transplantation of a genome that had been cloned and engineered in yeast, YCpMmyc1.1-Δ*typeIIres* [GenBank accession CP001668] (*8*). This project was critically dependent on the accuracy of these sequences. Although we believe that both finished *M. mycoides* genome sequences are reliable, there are 95 sites at which they differ. We

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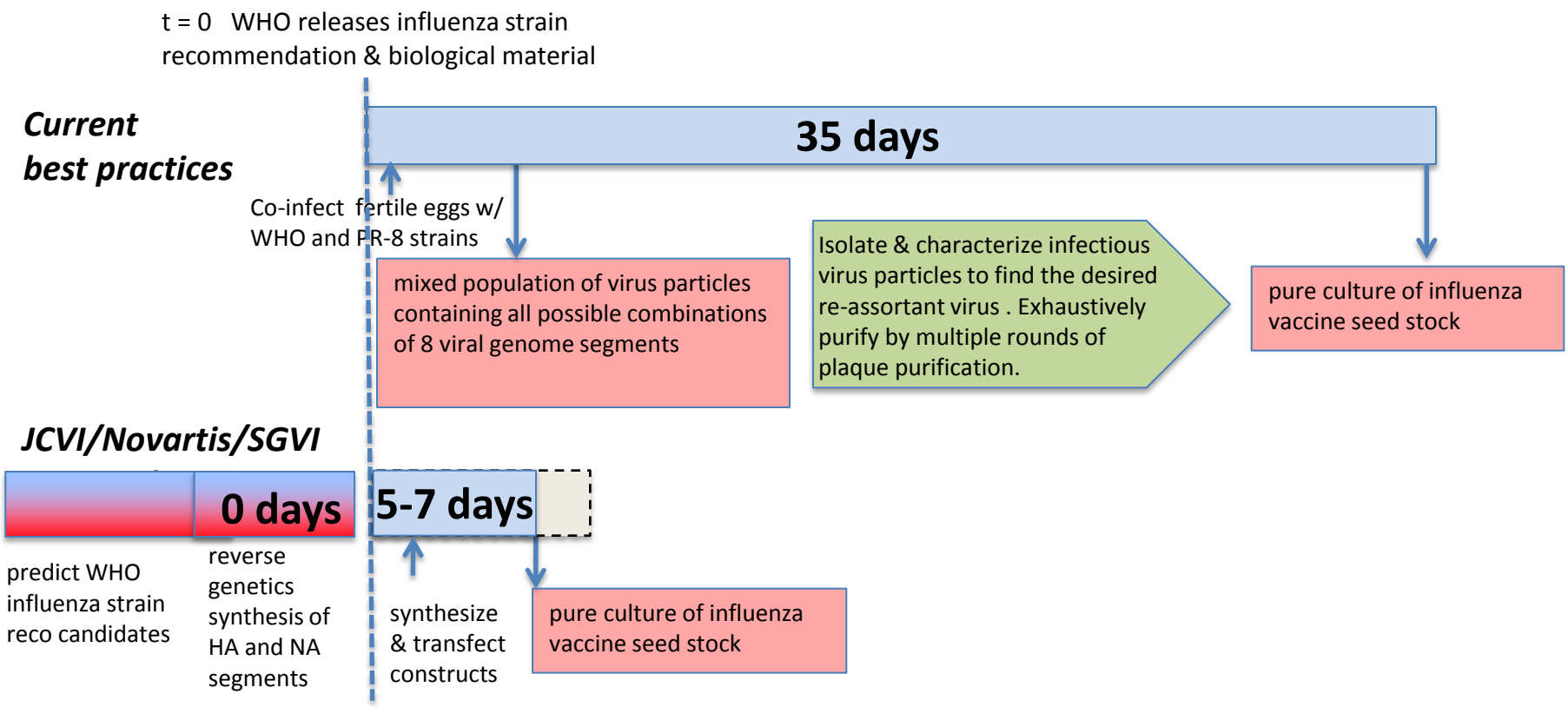
Accelerating Flu Vaccine Development



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Speeding Vaccine Seeds

A BARDA-funded collaboration between Novartis,* Synthetic Genomics Vaccines Inc. *(SGVI)/J. Craig Venter Institute (JCVI)

- Rapidly synthesize flu gene segments (HA and NA)
- Rescue recombinant viruses with optimized flu backbone

Milestone 1 (Sept. 2011): Demonstrate virus rescue within 7 days of receiving HA and NA sequence information

Status – Milestone surpassed

We were able to confirm rescue of an H7N9 virus within 5 days of initiating the process

Slide Modified from Peter Mason, Novartis

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Rapid Response to Zoonotic H7N9 Influenza Outbreak

- Identified unique H7N9 virus in people in late March
- Sequence of first viruses available April 1
- H7 and N9 genes synthesized and candidate vaccine viruses generated in less than a week.

	February		March		April		May		June		July		unknown month of onset		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Total	2	2	30	12	88	7	3	0	0	0	2	0	10	23	135	44

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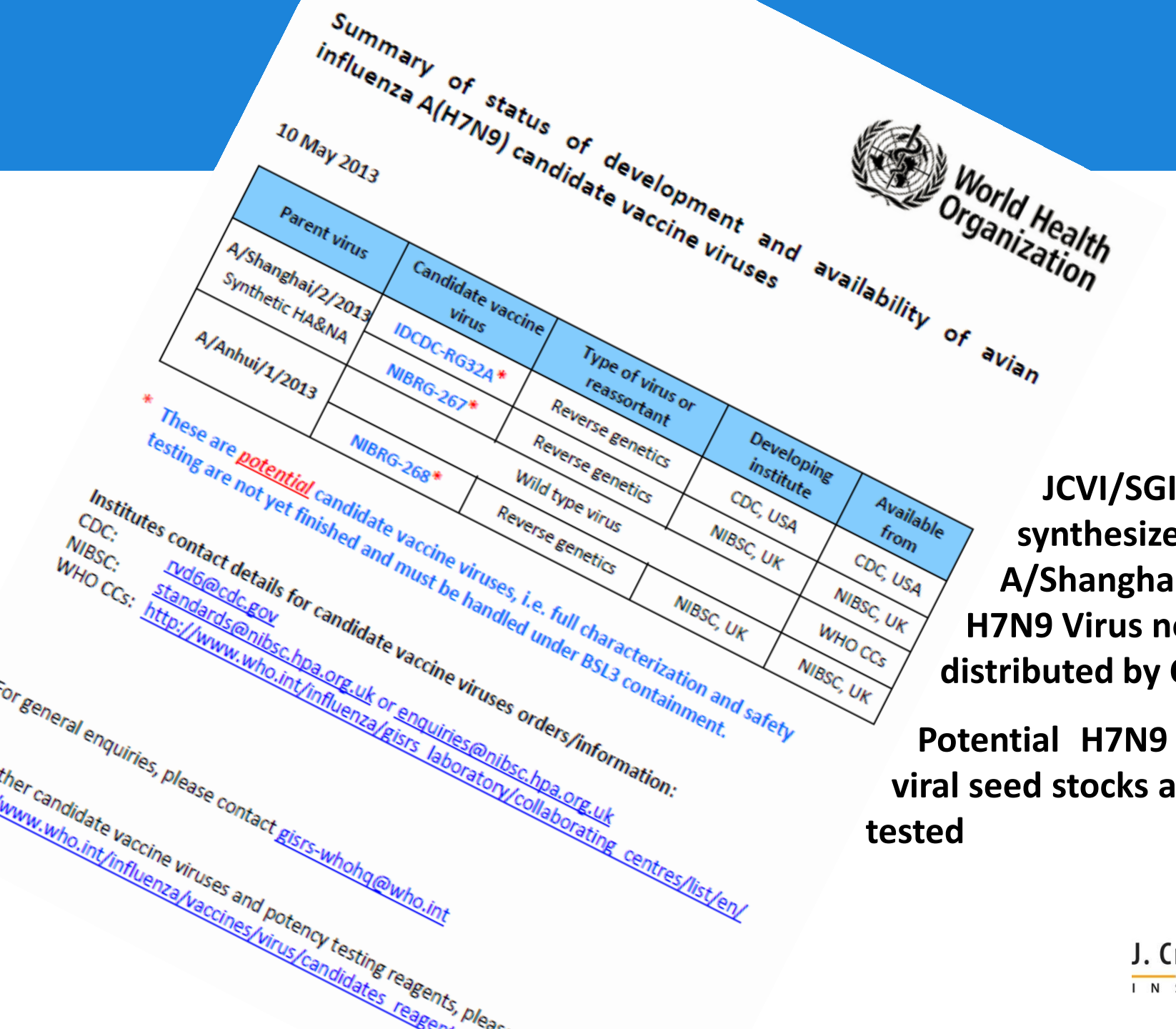

Summary of status of development and availability of avian influenza A(H7N9) candidate vaccine viruses

World Health Organization

10 May 2013

Parent virus	Candidate vaccine virus	Type of virus or reassortant	Developing institute	Available from
A/Shanghai/2/2013 Synthetic HA&NA	IDCDC-RG32A*	Reverse genetics	CDC, USA	CDC, USA
	NIBRG-267*	Reverse genetics	NIBSC, UK	NIBSC, UK
A/Anhui/1/2013		Wild type virus		WHO CCs
	NIBRG-268*	Reverse genetics	NIBSC, UK	NIBSC, UK

* These are potential candidate vaccine viruses, i.e. full characterization and safety testing are not yet finished and must be handled under BSL3 containment.

Institutes contact details for candidate vaccine viruses orders/information:
CDC: rvd6@cdc.gov
NIBSC: standards@nibsc.hpa.org.uk or enquiries@nibsc.hpa.org.uk
WHO CCs: http://www.who.int/influenza/gisrs_laboratory/collaborating_centres/list/en/

For general enquiries, please contact gisrs-whohq@who.int

ther candidate vaccine viruses and potency testing reagents, plea
www.who.int/influenza/vaccines/virus/candidates_reagen

JCVI/SGI/Novartis synthesized A/Shanghai/2/2013 H7N9 Virus now being distributed by CDC

Potential H7N9 vaccine viral seed stocks are being tested

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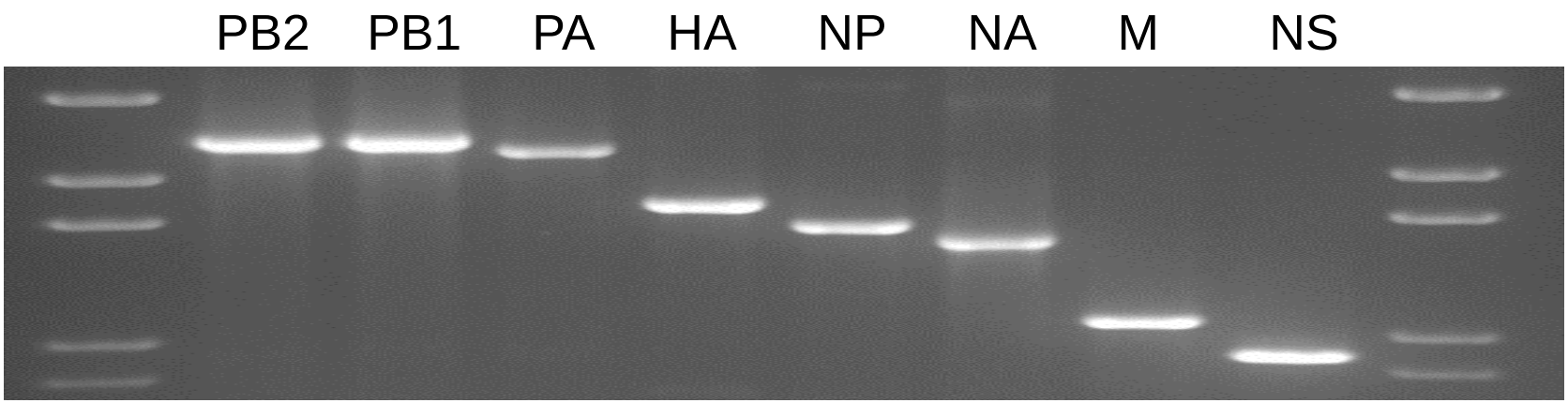
Emerging Viral Genome Synthesis

- Synthesized the H7N9 genome (Wentworth, A/Anhui/1-JCVI.1/2013)



- Research and Experimental LAIV Production

- Bat influenza
- Coronaviruses
  - MERS
  - HKU1
- Morbillivirus

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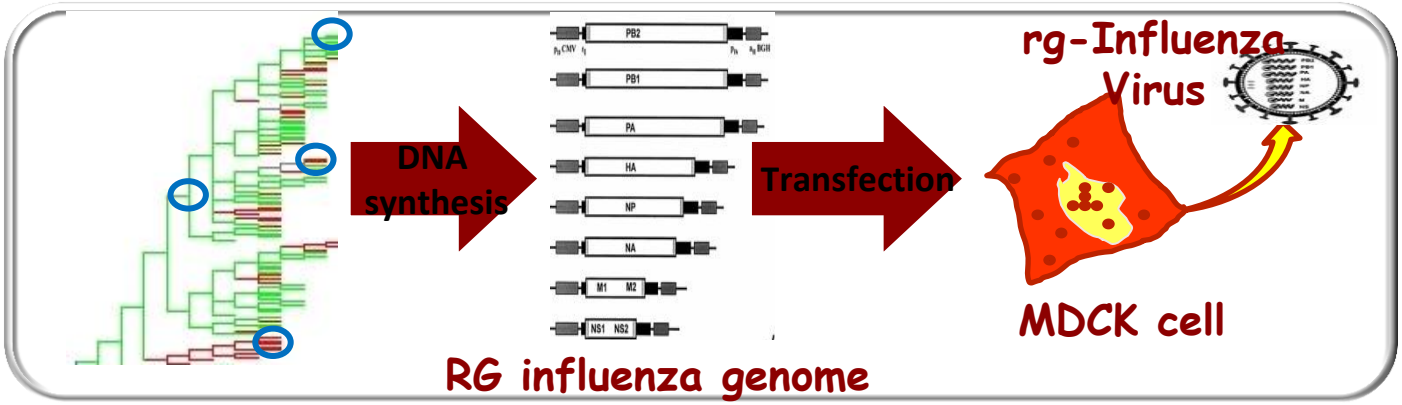
Summary

- **Infectious Diseases:**
  - Leading causes of death
  - Zoonosis and emerging viruses
    - Pandemic threats
- **High throughput genomic surveillance**- circulating viruses, drift variants, drug resistance, pandemic threats
  - Evolution/phylogenetics, vaccines, antivirals
- **Synthetically engineered genomes as TIVs/LAIVs**
  - Inactivated vaccines (TIV)
  - Basic research



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  - Hon Ip

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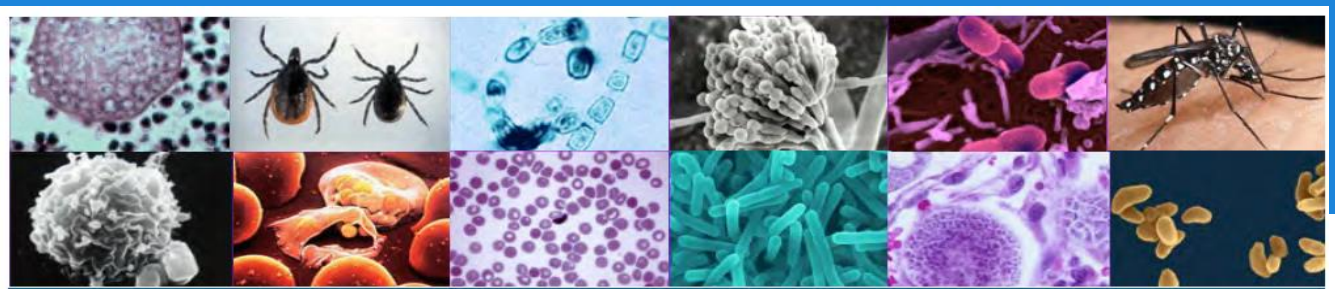

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National Institute of Allergy and Infectious Diseases
Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.

Biomedical Advanced Research & Development Authority
Assistant Secretary for Preparedness & Response
U.S. Department of Health & Human Services

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