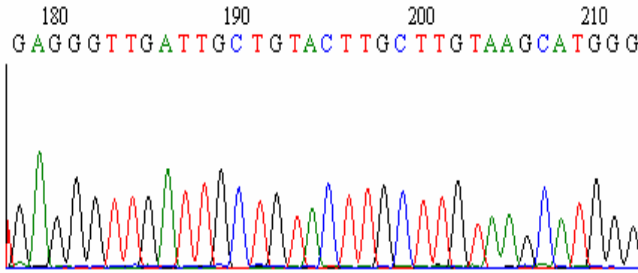
High Throughput Mass Spectrometry-Derived Base Compositions for the Detection and Strain Typing of Bacterial and Viral Pathogens

Steven A. Hofstadler, Ph.D.
Vice President of Research
Ibis Division of ISIS Pharmaceuticals
shofstad@isisph.com

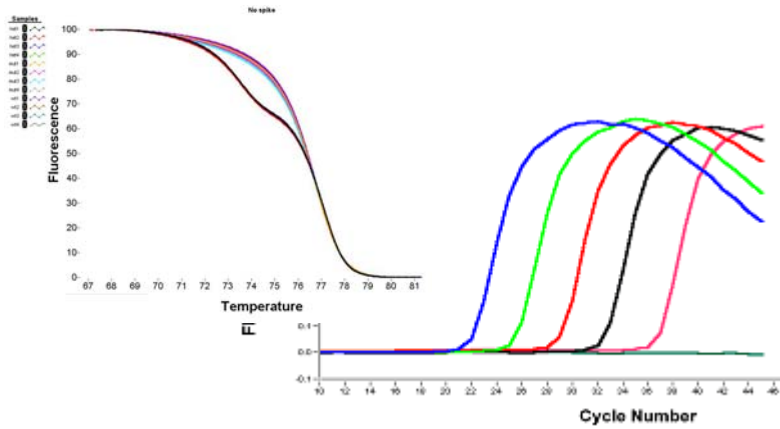
Outline

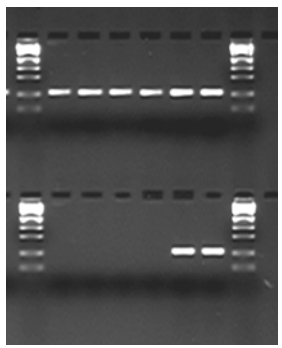
- Mass Spectrometry of DNA - 101
- Technology Overview
  - Principle of operation
- Examples:
  - Bacteria
    - Direct throat swab analysis for pathogen ID
    - Rapid “MLST” strain typing of GAS
    - Acinetobacter baumanii
    - Staphylococcus aureus
  - Viral
    - Pan-Influenza detection and strain typing
- Ibis T5000 System Components

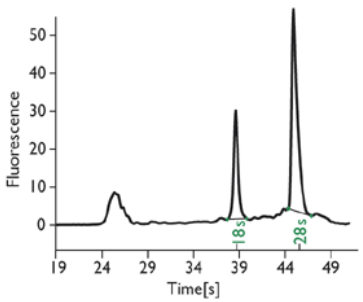
Interrogation of Amplified Nucleic Acids



- Sequencing
  - "Gold Standard"
- Fluorescent intercalating dye
- Hybridization
  - Specific probe with FRET pair
  - DNA microarray
- Melting profiles
- Electrophoresis
  - Slab gels
  - Capillary gel electrophoresis
- WHAT ABOUT MASS?



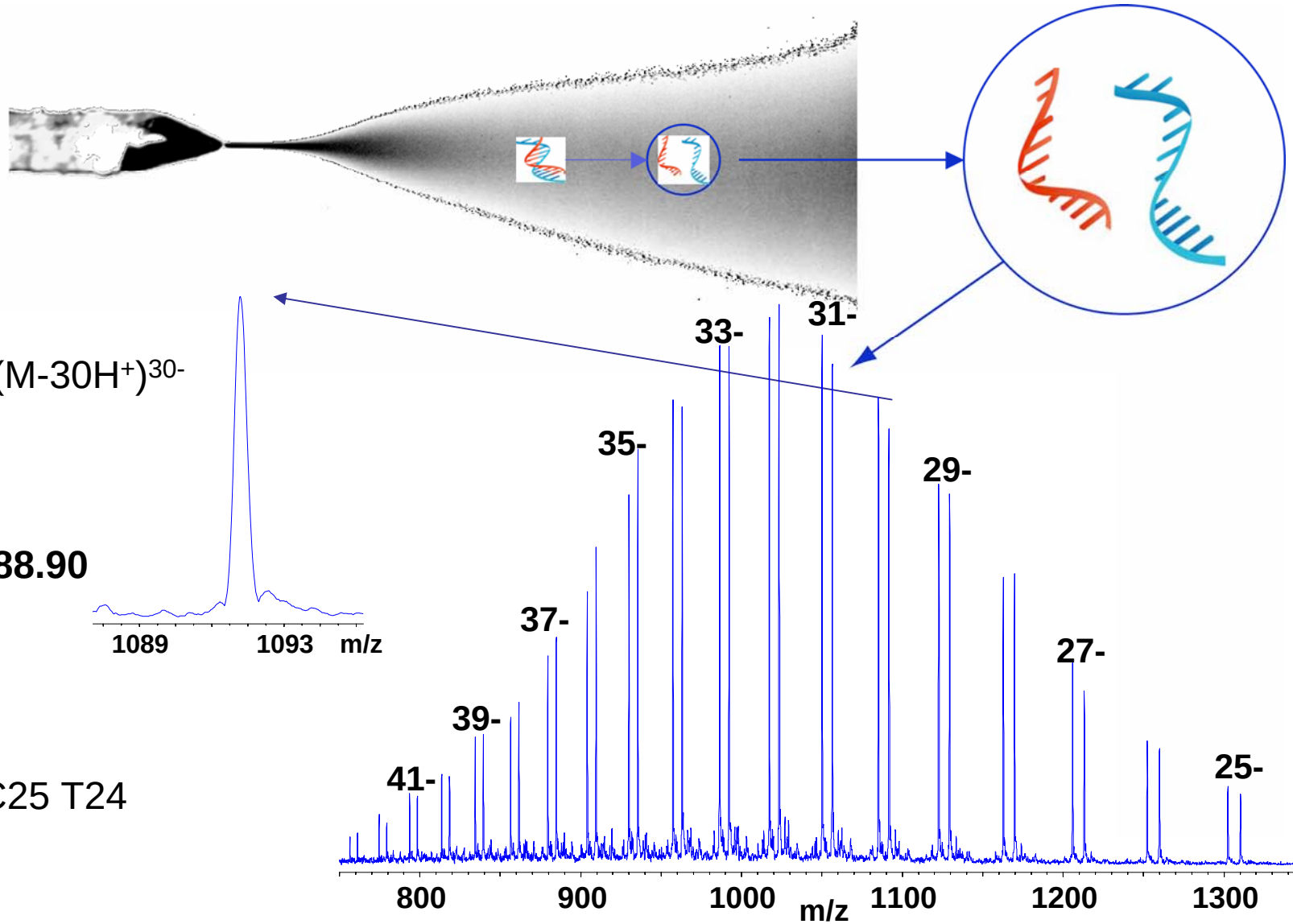




Mass Spectrometry of Nucleic Acids?

- Information content
  - From precise mass measurements unambiguous base compositions are derived [A10 G23 C32 T17] = [10 23 32 17]
- Speed
  - ≤ 1 minute/sample
- Applicability to mixtures
  - Dynamic range is > 100:1
  - MS succeeds where sequencing fails (e.g. mixtures)
- Automation
  - End-to-end process can be automated (including spectral processing/interpretation)
- Sensitivity
  - Single copy detection demonstrated with PCR front-end
- Breadth
  - Need not know what you're looking for to look for it…

Electrospray Ionization does not break DNA



The Intrinsic Mass Label

- **We usually refer to the "intrinsic mass label" from the perspective of a elemental composition**
  - **For small molecules an exact mass measurement can be used to back-calculate a list of elemental compositions consistent with the measured molecular weight**
- **The same holds true for DNA with respect to base composition except there are only four "letters".**
  - **A = 313.0576**
  - **G = 329.0526**
  - **C = 289.0464**
  - **T = 304.0461**

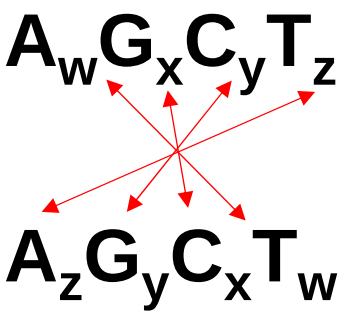
Single Strand: 32889.450 Da
(± 25 ppm or 0.75 Da): 928 base comps
(± 1 ppm or 0.03 Da): 82 base comps

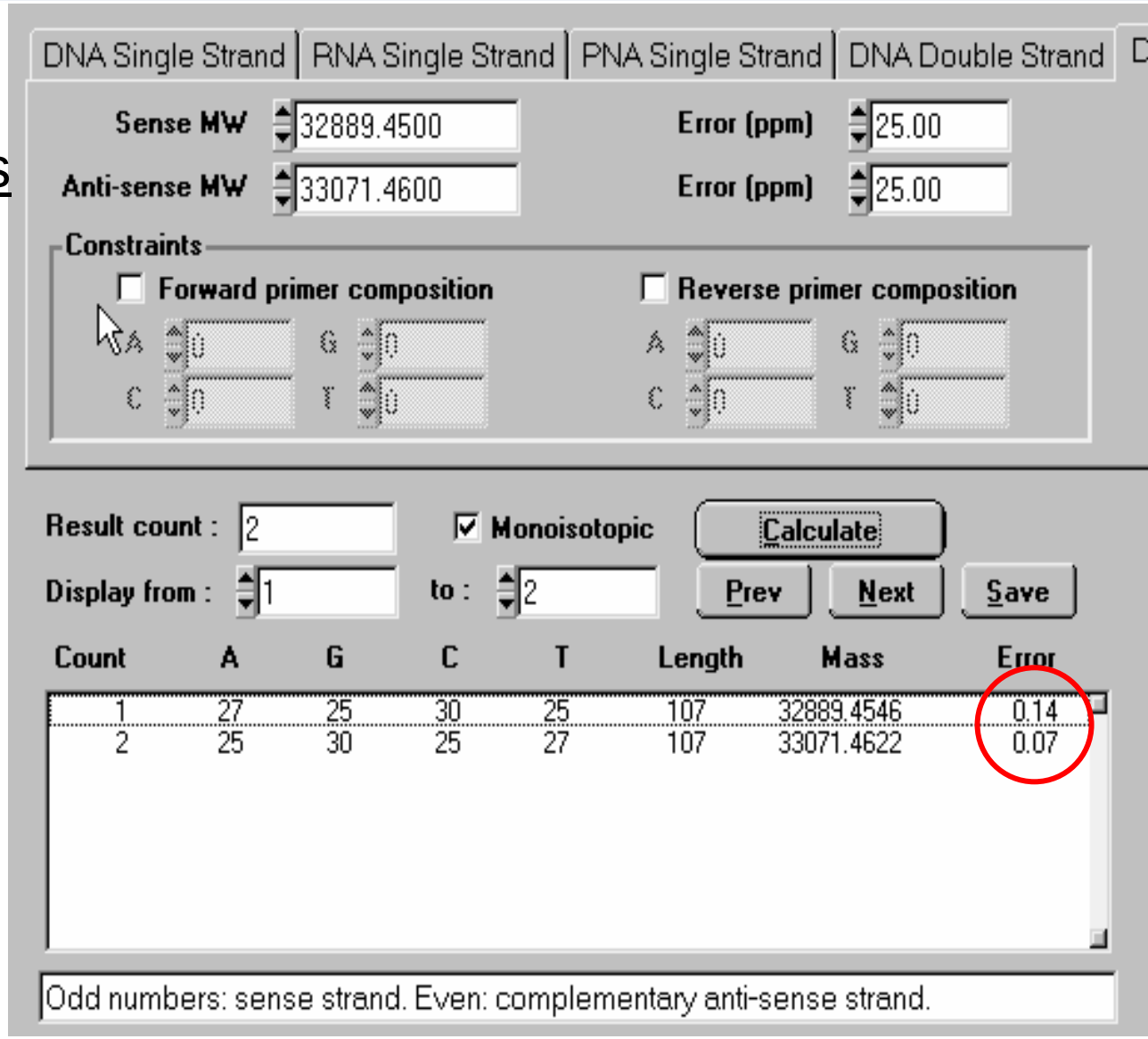
Single Strand: 33071.462 Da
(± 25 ppm or 0.75 Da): 948 base comps
(± 1 ppm or 0.03 Da): 95 base comps

Da: Dalton (atomic mass unit) **ppm: part per million**

Exact Mass Measurements of Both Strands Yields Unambiguous Base Composition

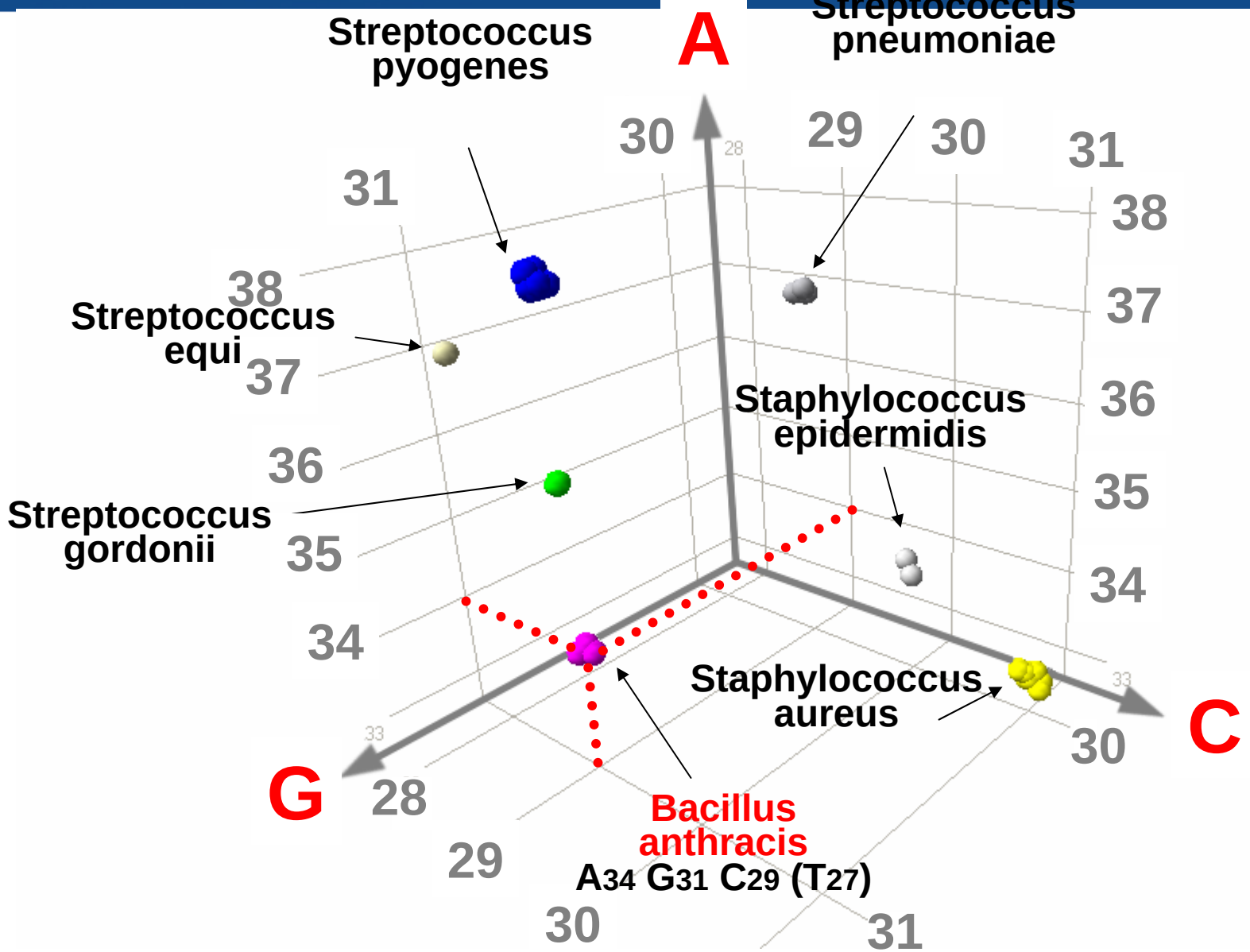
ppm	# comp pairs
0-25	1
50	13
100	66
250	378
500	1447





Derived and Expected Base Compositions

Primer 356 (RplB) Expected Products



Identification and Strain Typing of Bacterial and Viral Pathogens using High Performance Mass Spectrometry: The T·I·G·E·R Concept*

Triangulation Identification for Genetic Evaluation of Risk

The Bioweapons Arena:

- Biological weapons defense is not just about anthrax

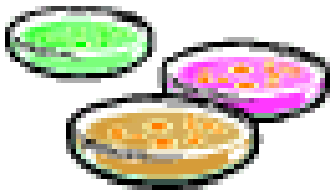
- Over 1000 agents known to infect humans*
  - 217 virus species
  - 538 bacterial species
  - 307 fungi
  - 66 parasitic protozoa

- Additional plant and animal pathogens not counted
- Numerous strain variations
- Potential bio-engineered organisms

**Taylor et al, Phil. Trans. R. Soc. Lond. B (2001) 356, 983-989*

Mainstream Bioagent Detection Today

- Culture techniques
  - Detects a subset of all pathogens
  - Can take multiple days (weeks)



- Single Agent Nucleic Acid Tests
  - One test for each agent (smallpox, anthrax, plague, etc.)
  - Need too many tests
  - Fail to detect newly emergent pathogens

Bacterial Threat Symbols

Microbial Rosetta Stone Database

NIAID Category A Priority Pathogen

NIAID Category B Priority Pathogen

NIAID Category C Priority Pathogen

HHS Select Agent

USDA High Consequence Animal Pathogen

USDA High Consequence Plant Pathogen

Validated Biological Weapon

Potential Biological Weapon

Validated Biocrime Agent

Globally Important Human Pathogen

Medically Important Human Pathogen

Important Animal Pathogen

Important Plant Pathogen

High Potential For Bioengineering

Zoonotic Agent

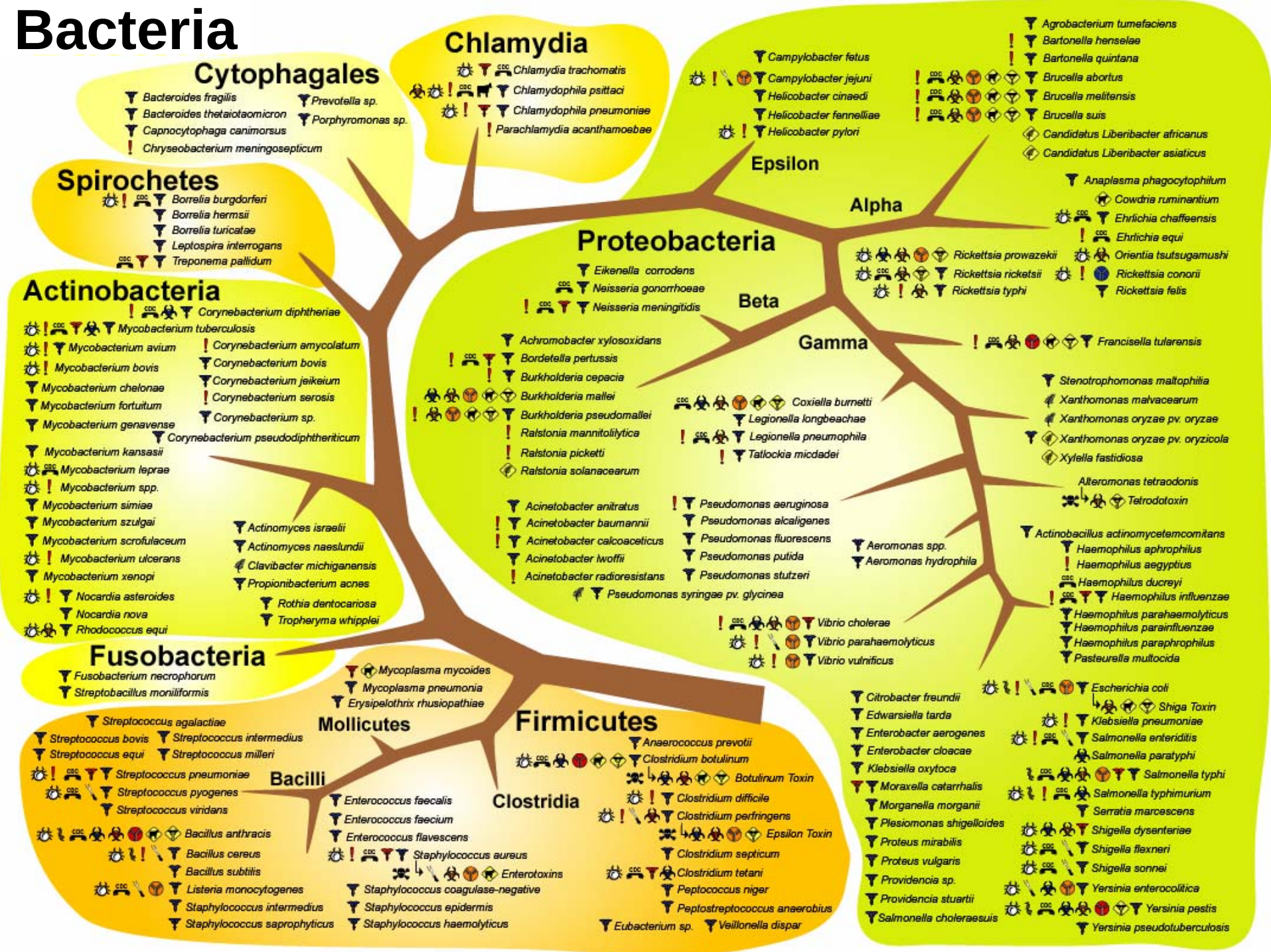
Toxin

CDC *CDC Notifiable Agent*

Principal Foodborne Pathogen

Emerging Infectious Agent

Bacteria


Cytophagales
Bacteroides fragilis
Bacteroides thetaiotaomicron
Capnocytophaga canimorsus
Chryseobacterium meningosepticum
Prevotella sp.
Porphyromonas sp.
Chlamydia
Chlamydia trachomatis
Chlamydophila psittaci
Chlamydophila pneumoniae
Parachlamydia acanthamoebae
Spirochetes
Borrelia burgdorferi
Borrelia hermsii
Borrelia turicatae
Leptospira interrogans
Treponema pallidum
Proteobacteria
Epsilon
Campylobacter fetus
Campylobacter jejuni
Helicobacter cinaedi
Helicobacter fennelliae
Helicobacter pylori
Alpha
Agrobacterium tumefaciens
Bartonella henselae
Bartonella quintana
Brucella abortus
Brucella melitensis
Brucella suis
Candidatus Liberibacter africanus
Candidatus Liberibacter asiaticus
Anaplasma phagocytophilum
Cowdria ruminantium
Ehrlichia chaffeensis
Ehrlichia equi
Orientia tsutsugamushi
Rickettsia prowazekii
Rickettsia ricketsii
Rickettsia typhi
Rickettsia conorii
Rickettsia felis
Beta
Eikenella corrodens
Neisseria gonorrhoeae
Neisseria meningitidis
Achromobacter xylosoxidans
Bordetella pertussis
Burkholderia cepacia
Burkholderia mallei
Burkholderia pseudomallei
Ralstonia mannitolilytica
Ralstonia picketti
Ralstonia solanacearum
Gamma
Francisella tularensis
Coxiella burnetti
Legionella longbeachae
Legionella pneumophila
Tatlockia micdadei
Stenotrophomonas maltophilia
Xanthomonas malvacearum
Xanthomonas oryzae pv. oryzae
Xanthomonas oryzae pv. oryzicola
Xylella fastidiosa
Alteromonas tetraodonis
Tetrodotoxin
Acinetobacter anitratus
Acinetobacter baumannii
Acinetobacter calcoaceticus
Acinetobacter lwoffii
Acinetobacter radioresistans
Pseudomonas aeruginosa
Pseudomonas alcaligenes
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas stutzeri
Pseudomonas syringae pv. glycinea
Aeromonas spp.
Aeromonas hydrophila
Actinobacillus actinomycetemcomitans
Haemophilus aphrophilus
Haemophilus aegyptius
Haemophilus ducreyi
Haemophilus influenzae
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus paraphrophilus
Pasteurella multocida
Vibrio cholerae
Vibrio parahaemolyticus
Vibrio vulnificus
Citrobacter freundii
Edwarsiella tarda
Enterobacter aerogenes
Enterobacter cloacae
Klebsiella oxytoca
Moraxella catarrhalis
Morganella morganii
Plesiomonas shigelloides
Proteus mirabilis
Proteus vulgaris
Providencia sp.
Providencia stuartii
Salmonella choleraesuis
Escherichia coli
Shiga Toxin
Klebsiella pneumoniae
Salmonella enteriditis
Salmonella paratyphi
Salmonella typhi
Salmonella typhimurium
Serratia marcescens
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Yersinia enterocolitica
Yersinia pestis
Yersinia pseudotuberculosis
Actinobacteria
Corynebacterium diphtheriae
Mycobacterium tuberculosis
Mycobacterium avium
Mycobacterium bovis
Mycobacterium chelonae
Mycobacterium fortuitum
Mycobacterium genavense
Mycobacterium kansasii
Mycobacterium leprae
Mycobacterium spp.
Mycobacterium simiae
Mycobacterium szulgai
Mycobacterium scrofulaceum
Mycobacterium ulcerans
Mycobacterium xenopi
Nocardia asteroides
Nocardia nova
Rhodococcus equi
Corynebacterium amycolatum
Corynebacterium bovis
Corynebacterium jeikeium
Corynebacterium serosis
Corynebacterium sp.
Corynebacterium pseudodiphtheriticum
Actinomyces israelii
Actinomyces naeslundii
Clavibacter michiganensis
Propionibacterium acnes
Rothia dentocariosa
Tropheryma whipplei
Fusobacteria
Fusobacterium necrophorum
Streptobacillus moniliformis
Mycoplasma mycoides
Mycoplasma pneumonia
Erysipelothrix rhusiopathiae
Mollicutes
Firmicutes
Bacilli
Streptococcus agalactiae
Streptococcus bovis
Streptococcus equi
Streptococcus intermedius
Streptococcus milleri
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus viridans
Bacillus anthracis
Bacillus cereus
Bacillus subtilis
Listeria monocytogenes
Staphylococcus intermedius
Staphylococcus saprophyticus
Enterococcus faecalis
Enterococcus faecium
Enterococcus flavescens
Staphylococcus aureus
Enterotoxins
Staphylococcus coagulase-negative
Staphylococcus epidermis
Staphylococcus haemolyticus
Clostridia
Anaerococcus prevotii
Clostridium botulinum
Botulinum Toxin
Clostridium difficile
Clostridium perfringens
Epsilon Toxin
Clostridium septicum
Clostridium tetani
Peptococcus niger
Peptostreptococcus anaerobius
Eubacterium sp.
Veillonella dispar

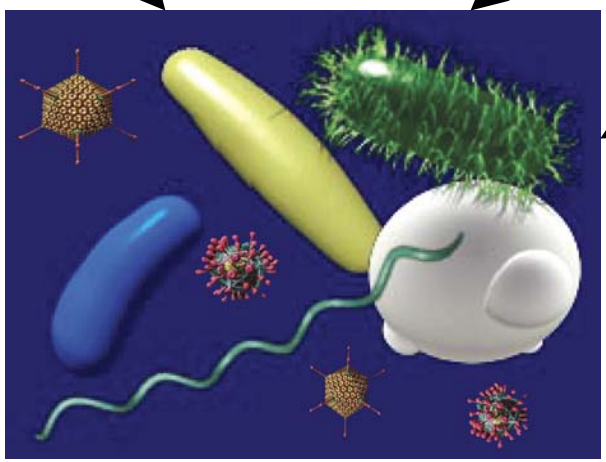
Concept of Operations

Animal Reservoirs of Infectious Agents

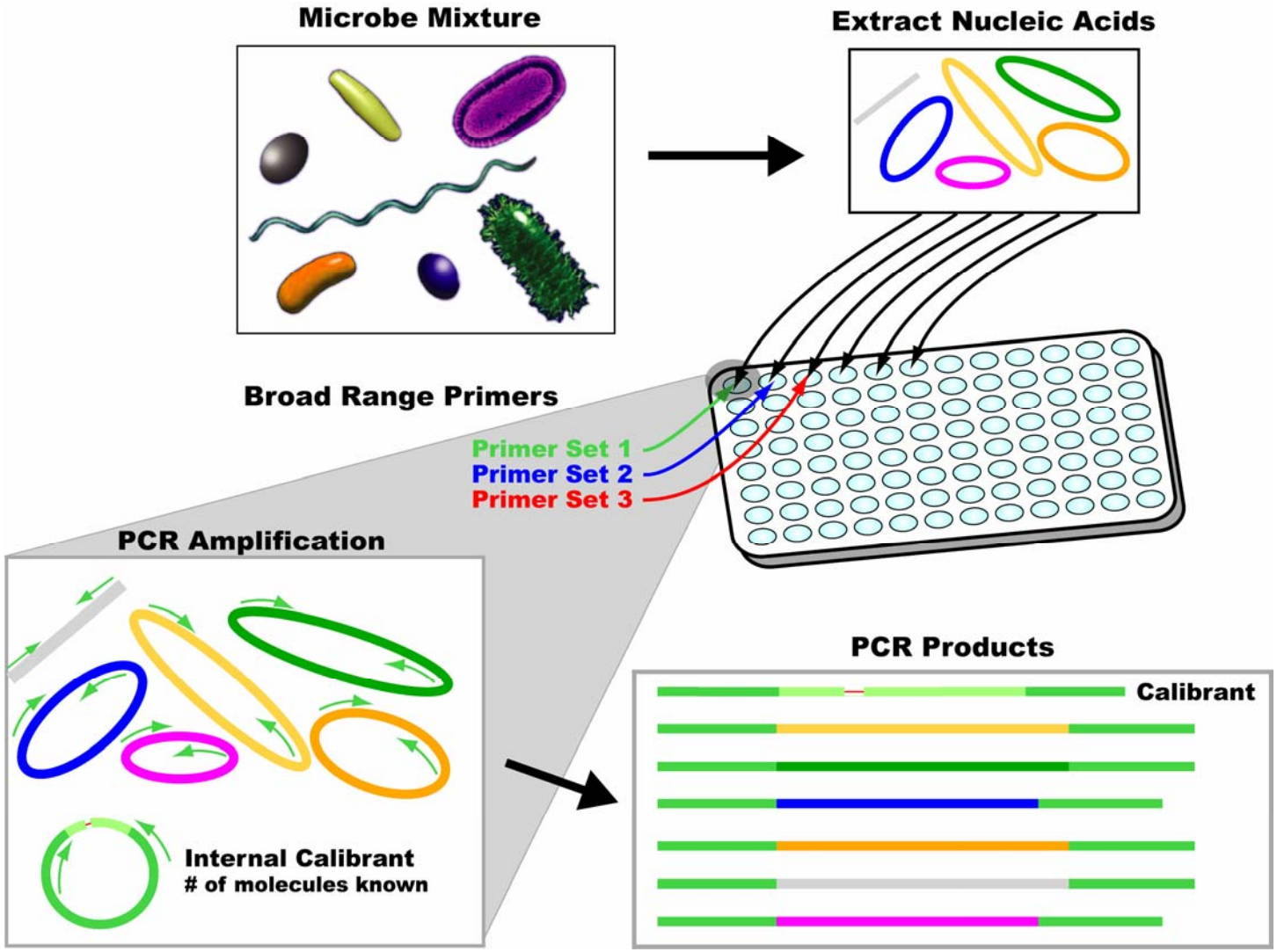
Environmental Surveillance of Public Places

Clinical Diagnostics/ Biosurveillance

Water Quality Biosurveillance

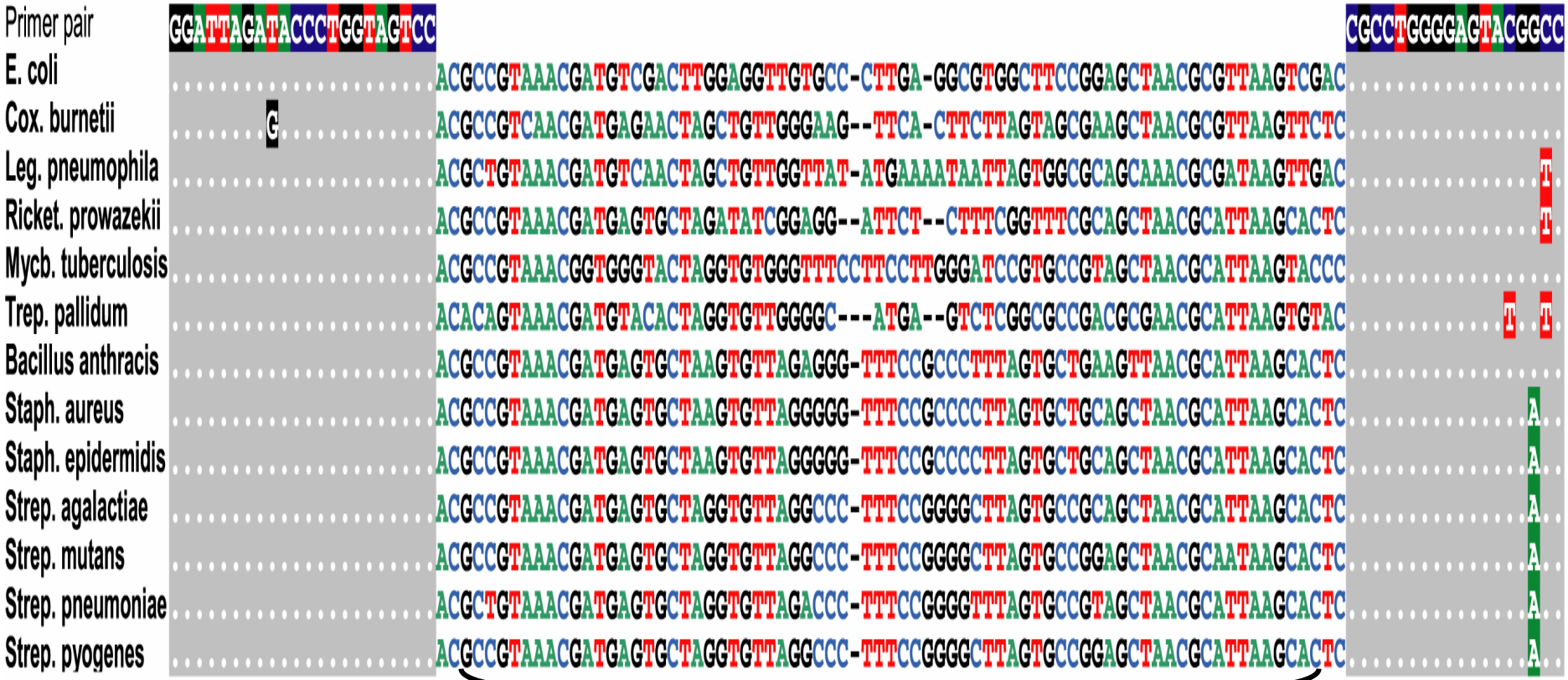


Part 1:
Sample Preparation and Broad Range PCR



Broad Range Priming in Bacteria

Primers bind to conserved regions present in ALL (or groups of) bacteria

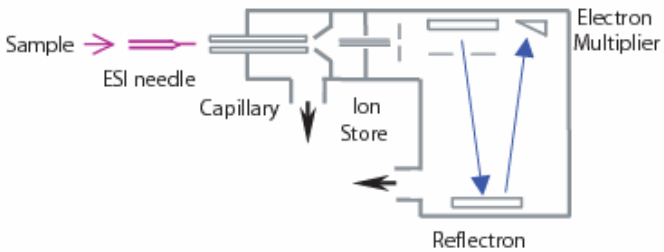


Region varies in different kinds of bacteria ⟹ $\Delta\ [A_w G_x C_y T_z]$

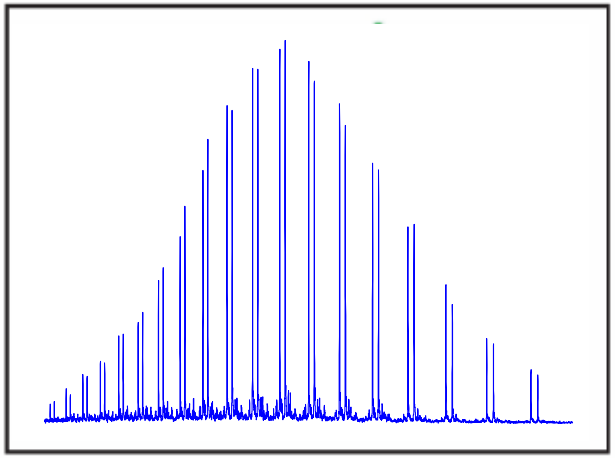
Part 2:

MS Analysis and Signal Processing

Mass Spectrometer



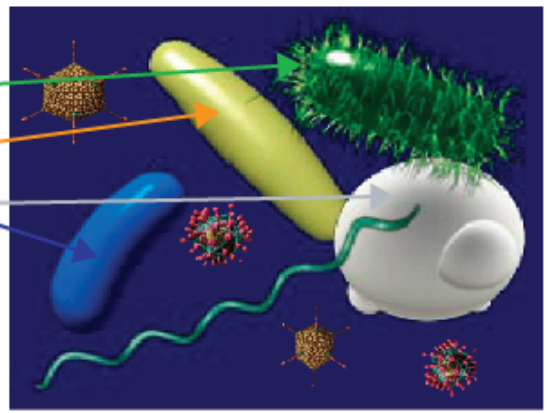
Spectral Signal



Signal Processing
Masses to Base Compositions

Organism	Mass	Base Count
E. coli	18234.970	$A_{12}G_{17}C_{17}T_{13}$
S. milleri	17948.926	$A_{14}G_{14}C_{12}T_{18}$
M. leprae	18610.017	$A_{11}G_{19}C_{15}T_{15}$
Unknown brucella	17936.912	$A_{11}G_{17}C_{16}T_{14}$

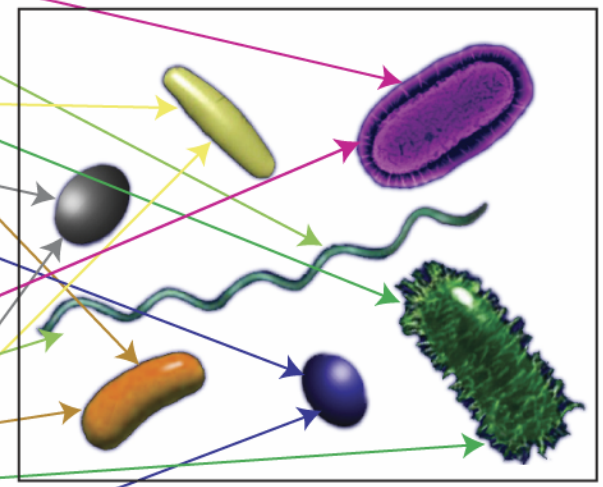
Base Compositions Map to Microbes



Part 3:
Triangulation Using Multiple Primer Pairs

ORGANISM	Mass	Base Count
Bacillus anthracis	35278.823	A26 G34 C27 T27
Borrelia burgdorferi	33770.606	A29 G31 C23 T26
Clostridium botulinum	35843.944	A29 G33 C30 T24
Escherichia coli	35641.855	A22 G39 C29 T25
Staph aureus	35240.807	A24 G35 C30 T25
Strep pneumoniae	35270.806	A24 G35 C28 T27
Strep pyogenes (Group A Strep)	35281.808	A23 G37 C30 T24

ORGANISM	Mass	Base Count
Bacillus anthracis	35174.799	A25 G32 C30 T27
Borrelia burgdorferi	36161.973	A30 G29 C23 T35
Clostridium botulinum	35576.881	A27 G34 C28 T26
Escherichia coli	35870.920	A27 G33 C27 T29
Staph aureus	35743.918	A29 G29 C30 T28
Strep pneumoniae	35180.787	A24 G32 C29 T29
Strep pyogenes (Group A Strep)	35155.781	A24 G31 C29 T30



Bacterial Primers for Surveillance for BW Agents - Anthrax Confirmation

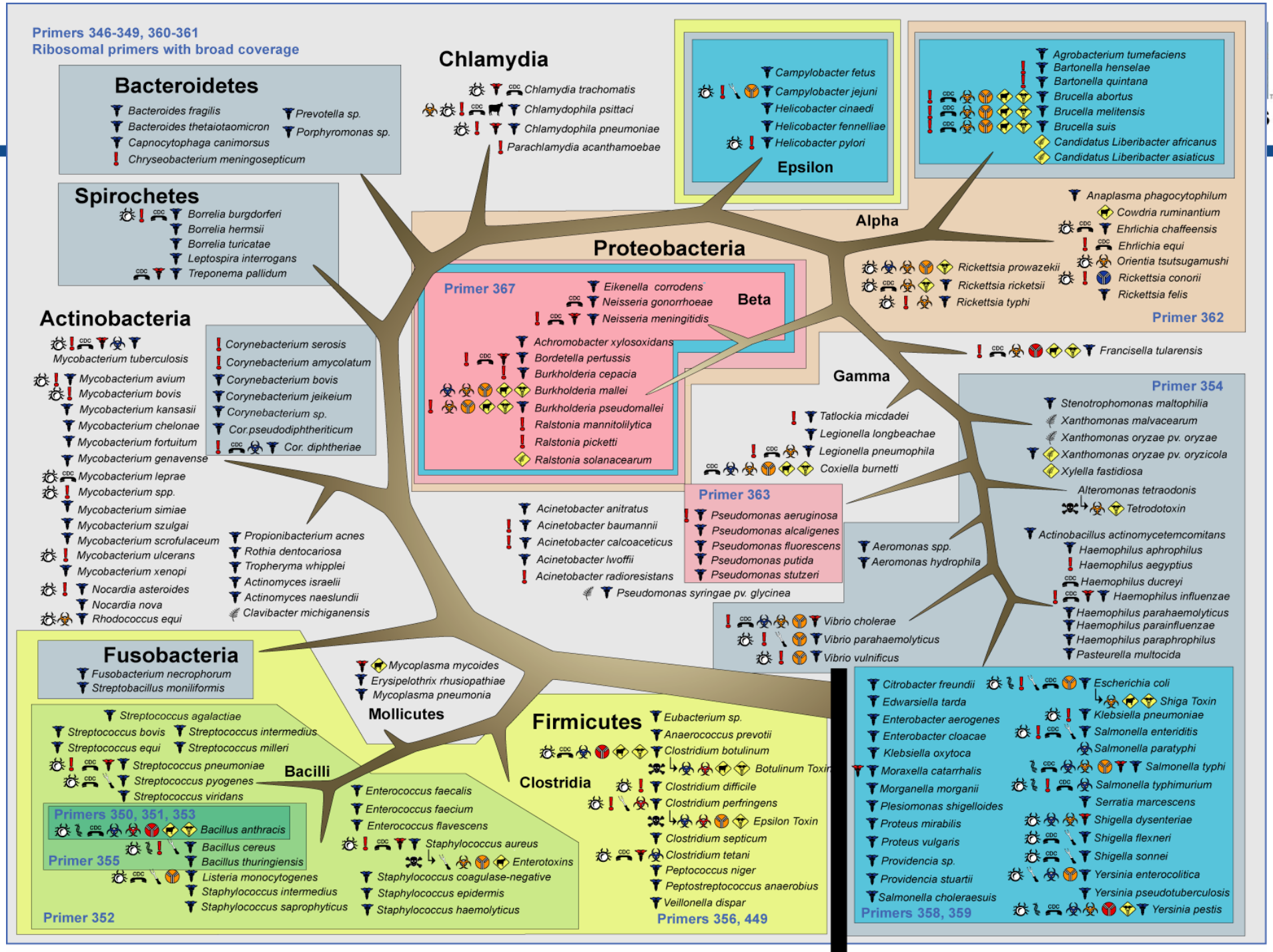
ibis Biosciences

Survey

Primer #	Gene target	Bacterial target	Primer specificity
346, 347, 348, 361	16S rDNA	ALL	Universally conserved ribosomal genes
349, 360	23S rDNA		
354	RNA polymerase, β' subunit (rpoC)	Bacteroidetes, Fusobacteria, Spirochaetes, Proteo, Bacilli	Division-wide Housekeeping genes
358	ValyI-tRNA synthetase (valS)	Proteobacteria (γ: Enterobacteria)	
359	RNA polymerase, β subunit (rpoB)	Proteobacteria (γ: Enterobacteria)	
362	RNA polymerase, β subunit (rpoB)	Proteobacteria (α, β)	
363	RNA polymerase, β' subunit (rpoC)	Proteobacteria (β, γ)	
367	Elongation factor EF-Tu (tufB)	Proteobacteria (β)	
356, 449	Ribosomal protein L2 (rplB)	Clostridia. Fusobacteria Bacilli, Proteobacteria (ε)	
352	Protein chain initiation factor (infB)	Bacilli	
355	Spore protein (sspE)	*Bacillus cereus* clade	Clade-specific genes

Drill-Down

350	Capsule biosynthesis protein (capC)	*Bacillus anthracis* pXO2 plasmid	Virulence plasmid
351	Adenylate cyclase (cyaA)	*Bacillus anthracis* pXO1 plasmid	Virulence plasmid
353	Lethal factor subunit (lef)	*Bacillus anthracis* pXO1 plasmid	Virulence plasmid


Primers 346-349, 360-361
Ribosomal primers with broad coverage
Bacteroidetes
Bacteroides fragilis
Bacteroides thetaiotaomicron
Capnocytophaga canimorsus
Chryseobacterium meningosepticum
Prevotella sp.
Porphyromonas sp.
Chlamydia
Chlamydia trachomatis
Chlamydophila psittaci
Chlamydophila pneumoniae
Parachlamydia acanthamoebae
Campylobacter fetus
Campylobacter jejuni
Helicobacter cinaedi
Helicobacter fennelliae
Helicobacter pylori
Epsilon
Agrobacterium tumefaciens
Bartonella henselae
Bartonella quintana
Brucella abortus
Brucella melitensis
Brucella suis
Candidatus Liberibacter africanus
Candidatus Liberibacter asiaticus
Spirochetes
Borrelia burgdorferi
Borrelia hermsii
Borrelia turicatae
Leptospira interrogans
Treponema pallidum
Proteobacteria
Alpha
Anaplasma phagocytophilum
Cowdria ruminantium
Ehrlichia chaffeensis
Ehrlichia equi
Orientia tsutsugamushi
Rickettsia conorii
Rickettsia felis
Rickettsia prowazekii
Rickettsia ricketsii
Rickettsia typhi
Primer 362
Primer 367
Beta
Eikenella corrodens
Neisseria gonorrhoeae
Neisseria meningitidis
Achromobacter xylosoxidans
Bordetella pertussis
Burkholderia cepacia
Burkholderia mallei
Burkholderia pseudomallei
Ralstonia mannitolilytica
Ralstonia picketti
Ralstonia solanacearum
Actinobacteria
Mycobacterium tuberculosis
Mycobacterium avium
Mycobacterium bovis
Mycobacterium kansasii
Mycobacterium chelonae
Mycobacterium fortuitum
Mycobacterium genavense
Mycobacterium leprae
Mycobacterium spp.
Mycobacterium simiae
Mycobacterium szulgai
Mycobacterium scrofulaceum
Mycobacterium ulcerans
Mycobacterium xenopi
Nocardia asteroides
Nocardia nova
Rhodococcus equi
Corynebacterium serosis
Corynebacterium amycolatum
Corynebacterium bovis
Corynebacterium jeikeium
Corynebacterium sp.
Cor.pseudodiphtheriticum
Cor. diphtheriae
Propionibacterium acnes
Rothia dentocariosa
Tropheryma whipplei
Actinomyces israelii
Actinomyces naeslundii
Clavibacter michiganensis
Gamma
Francisella tularensis
Tatlockia micdadei
Legionella longbeachae
Legionella pneumophila
Coxiella burnetti
Primer 354
Stenotrophomonas maltophilia
Xanthomonas malvacearum
Xanthomonas oryzae pv. oryzae
Xanthomonas oryzae pv. oryzicola
Xylella fastidiosa
Alteromonas tetraodonis
Tetrodotoxin
Primer 363
Pseudomonas aeruginosa
Pseudomonas alcaligenes
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas stutzeri
Acinetobacter anitratus
Acinetobacter baumannii
Acinetobacter calcoaceticus
Acinetobacter lwoffii
Acinetobacter radioresistans
Pseudomonas syringae pv. glycinea
Aeromonas spp.
Aeromonas hydrophila
Actinobacillus actinomycetemcomitans
Haemophilus aphrophilus
Haemophilus aegyptius
Haemophilus ducreyi
Haemophilus influenzae
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus paraphrophilus
Pasteurella multocida
Vibrio cholerae
Vibrio parahaemolyticus
Vibrio vulnificus
Fusobacteria
Fusobacterium necrophorum
Streptobacillus moniliformis
Mycoplasma mycoides
Erysipelothrix rhusiopathiae
Mycoplasma pneumonia
Mollicutes
Streptococcus agalactiae
Streptococcus bovis
Streptococcus intermedius
Streptococcus equi
Streptococcus milleri
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus viridans
Bacilli
Primers 350, 351, 353
Bacillus anthracis
Bacillus cereus
Bacillus thuringiensis
Primer 355
Listeria monocytogenes
Staphylococcus intermedius
Staphylococcus saprophyticus
Primer 352
Enterococcus faecalis
Enterococcus faecium
Enterococcus flavescens
Staphylococcus aureus
Enterotoxins
Staphylococcus coagulase-negative
Staphylococcus epidermis
Staphylococcus haemolyticus
Firmicutes
Eubacterium sp.
Anaerococcus prevotii
Clostridium botulinum
Botulinum Toxin
Clostridia
Clostridium difficile
Clostridium perfringens
Epsilon Toxin
Clostridium septicum
Clostridium tetani
Peptococcus niger
Peptostreptococcus anaerobius
Veillonella dispar
Primers 356, 449
Citrobacter freundii
Edwarsiella tarda
Enterobacter aerogenes
Enterobacter cloacae
Klebsiella oxytoca
Moraxella catarrhalis
Morganella morganii
Plesiomonas shigelloides
Proteus mirabilis
Proteus vulgaris
Providencia sp.
Providencia stuartii
Salmonella choleraesuis
Escherichia coli
Shiga Toxin
Klebsiella pneumoniae
Salmonella enteriditis
Salmonella paratyphi
Salmonella typhi
Salmonella typhimurium
Serratia marcescens
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Yersinia enterocolitica
Yersinia pseudotuberculosis
Yersinia pestis
Primers 358, 359

Broad Pathogen Detection

Instead of asking; “Is pathogen X in my sample?”, we ask: “Which pathogen, or pathogens, are in my sample?”

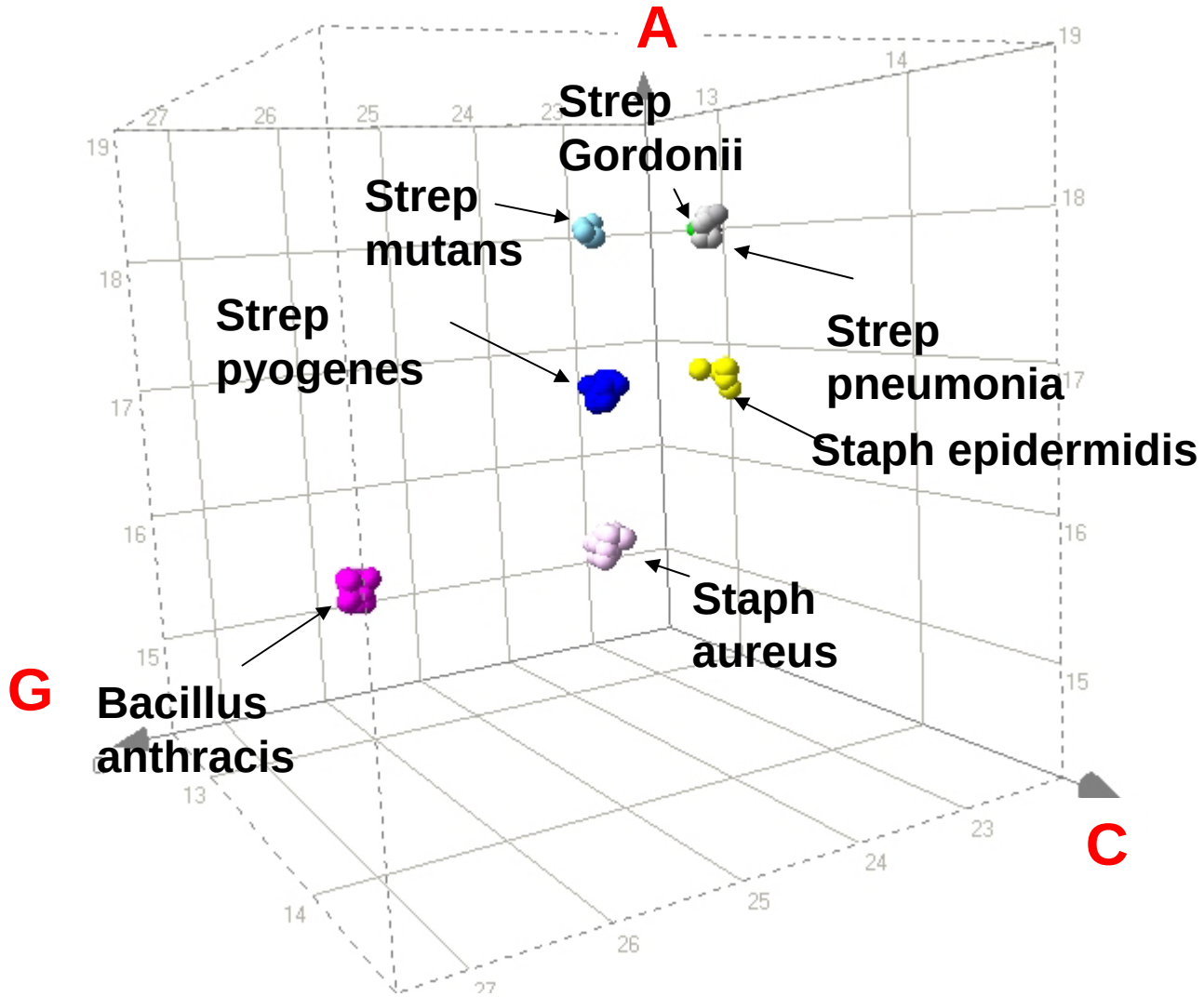
Group A Streptococcus (GAS) Outbreaks in Military Settings

- Outbreaks of Group A strep at MCRC 2002/2003
  - Highly virulent strain
  - One death, 160 hospitalized
  - Training activities suspended
- Initial analysis of post-culture samples
  - 80 samples sent from NHRC, Dr. Kevin Russell, December 20, 2002
  - "Hijacked" some BW air surveillance plates
- Follow up surveillance at multiple military bases
- Direct analysis of throat swabs without culture

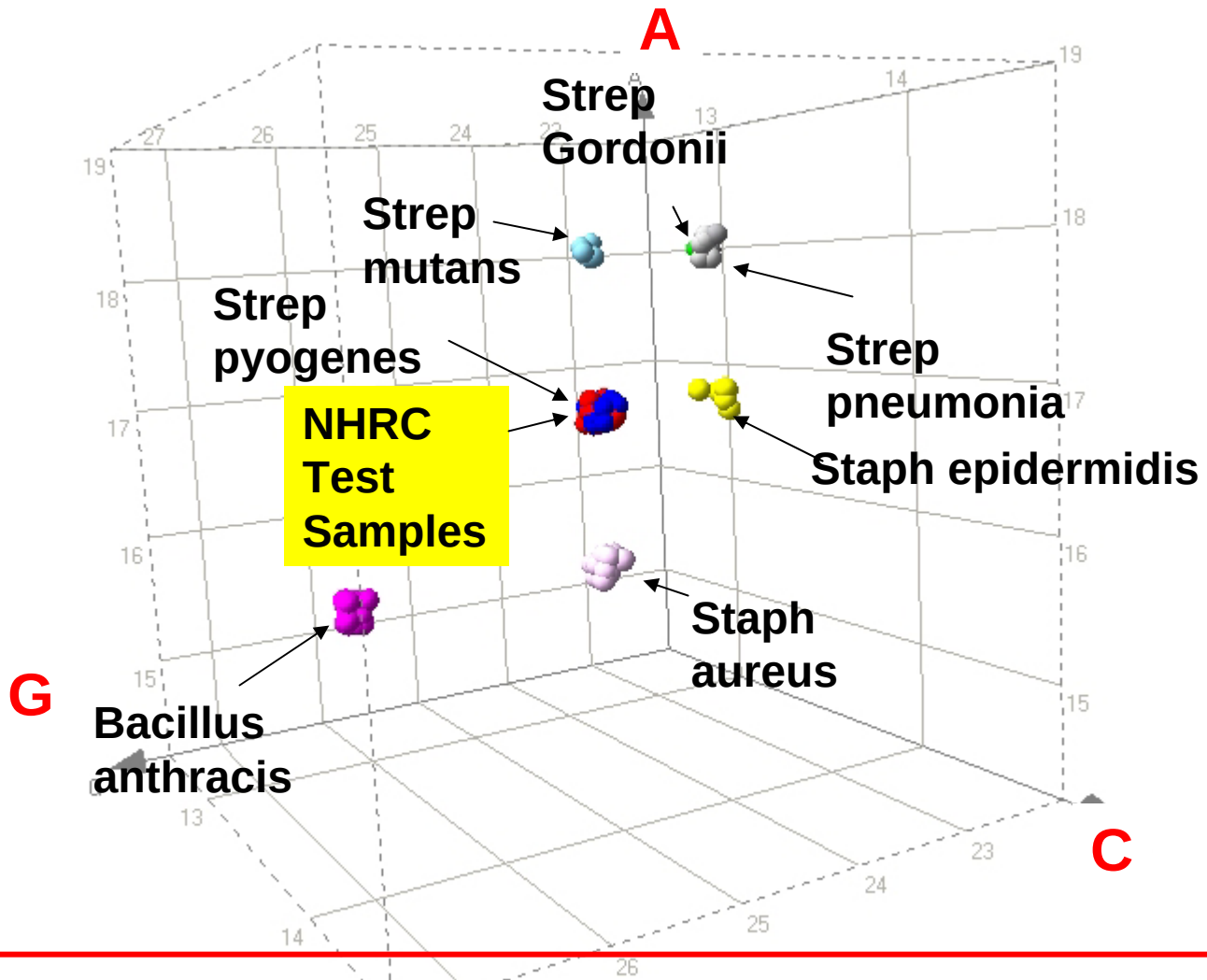
- Confirm the causative agent as *Streptococcus pyogenes*
- Provide tool for epidemiologic tracking
  - Identify a unique signature of the outbreak strain
- BONUS:
  - Determine the outbreak strain type (Emm-type)

Ecker et al. *Proc. Nat. Acad. Sci. USA,* (2005) 102(22), 8012-17

Primer 17 (a 23S primer)
Expected Products



Primer 17 (a 23S primer)
Observed Products (from culture)

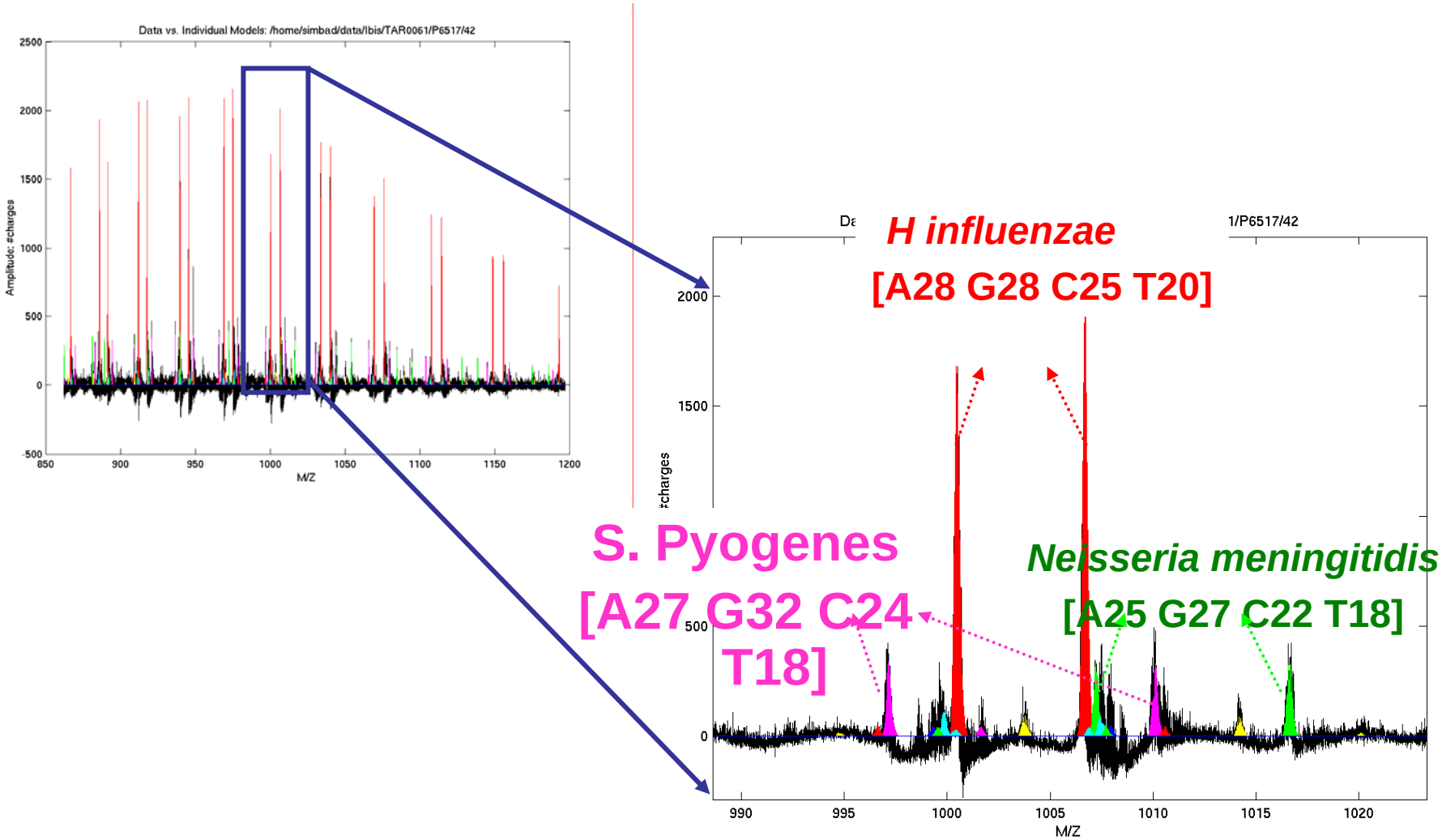


All primers of all samples consistent with S. pyogenes

Direct Analysis of Throat Swab*

Repeat swab positive on culture for *Streptococcus pyogenes

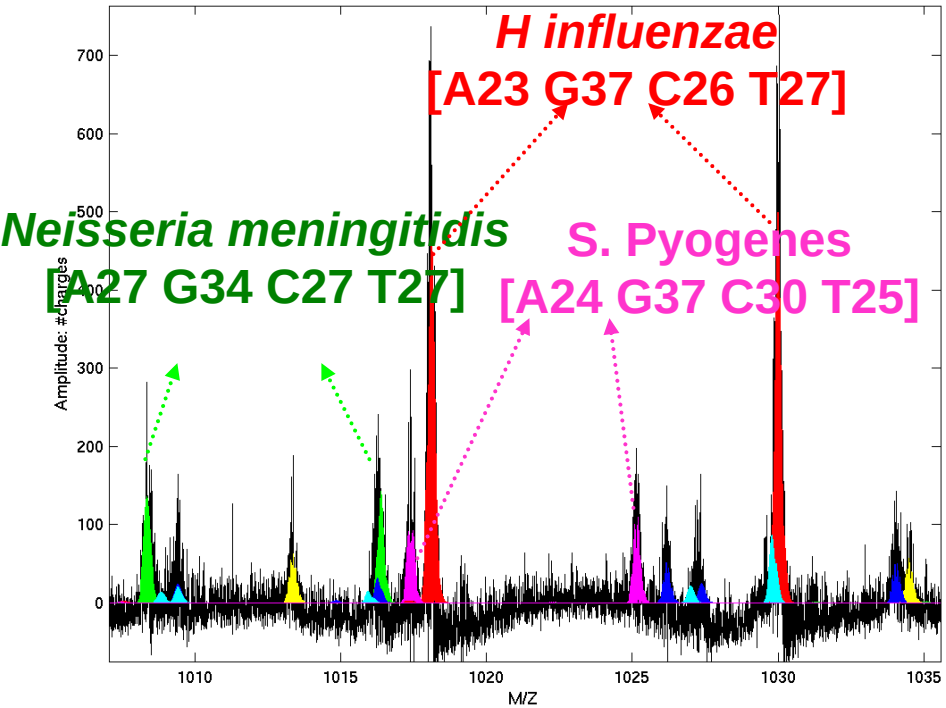
Primer 349: 23S rDNA 1826-1924 From an Interesting Throat Swab



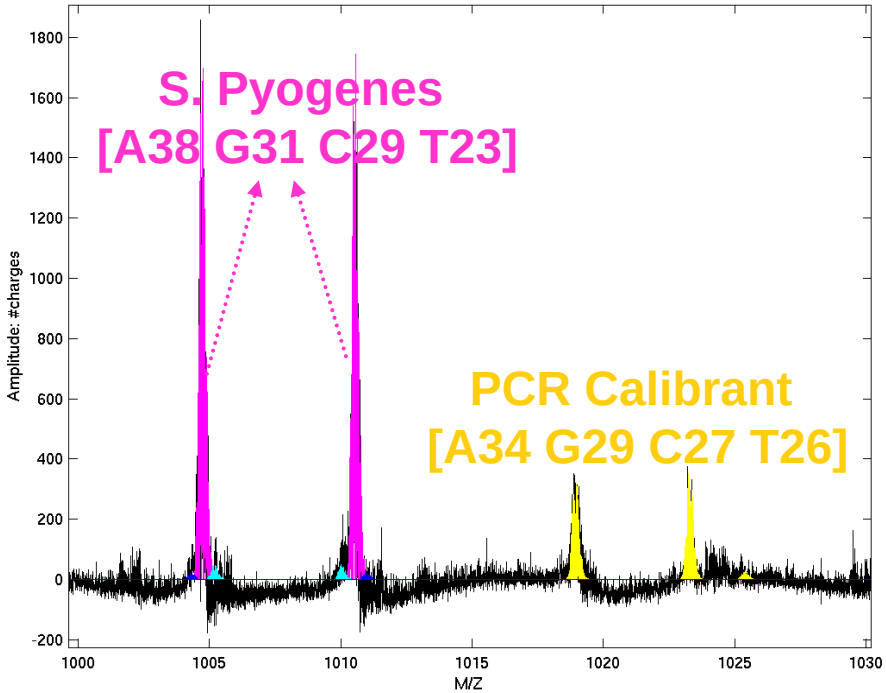
Ecker et al. *Proc. Nat. Acad. Sci. USA*, (2005) 102(22), 8012-17

Multi-primer Triangulation

Primer 347: 16S rDNA



Primer 356: rplB

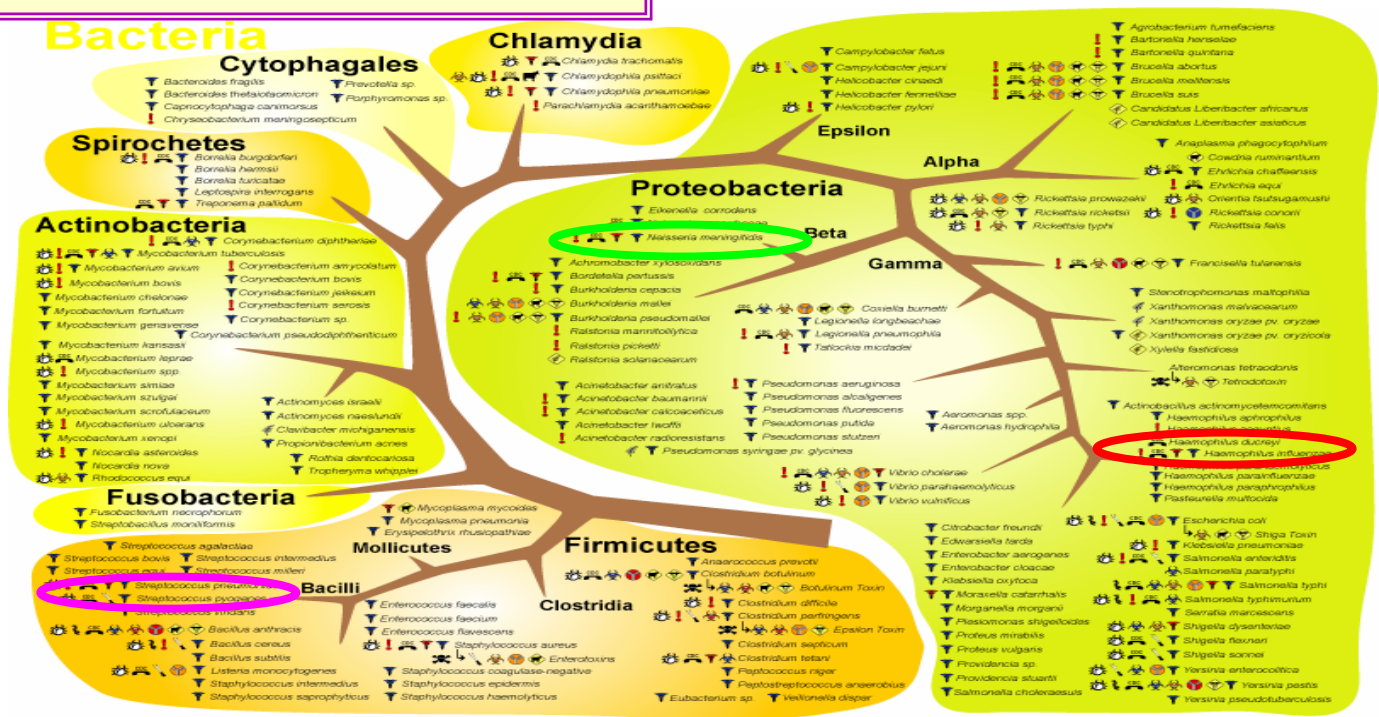


Organism	Cumulative Estimate of Genomes/Swab	Relative Abundances
Haemophilus influenzae	7.38E+05	1.00
Neisseria meningitidis	3.77E+05	0.51
Streptococcus pyogenes	1.89E+05	0.26

Ecker et al. *Proc. Nat. Acad. Sci. USA*, (2005) 102(22), 8012-17

Haemophilus influenzae, *Neisseria meningitidis*, *Streptococcus pyogenes* (Ratio 4/2/1, 1.5 X 10^6 genomes/swab)

Primer #1	Mass	Base Count
Blue	18234.970	$A_{12}G_{17}C_{17}T_{13}$
Blue	17948.926	$A_{14}G_{14}C_{12}T_{18}$
Blue	18610.017	$A_{11}G_{19}C_{15}T_{15}$
Blue	17936.912	$A_{11}G_{17}C_{16}T_{14}$
Blue	18877.118	$A_{18}G_{15}C_{15}T_{13}$



Strain Differentiation

Instead of asking; "Is strain X in my sample?", we ask: "Which strain(s) are in my sample.

Genotyping applications

- Microbial forensics
- Epidemiology of disease outbreak
- Nosocomial strain tracking
- Food pathogen monitoring

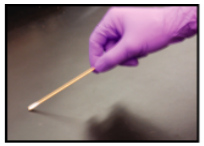
Advantages

- Same technology platform as for broad identification
- Speed: Time to 1st answer < 4 hours
- Throughput - Approximately 200 samples/24 hours
- No Culture Step - Direct environmental or clinical samples can be analyzed
- Robust to Mixtures

Rapid Strain Typing by Base Composition: *S. pyogenes*: our maiden genotyping voyage

- Developed MS-based *Multi Locus Sequence Typing* strategy
- Analyzed housekeeping genes for sites that differentiate alleles (sequence types) and identify emm-types using base composition
- Tested 24 primer pairs, selected 6 most informative pairs
- Processed 80 NHRC samples end to end (blinded)
  - 51 from outbreak
  - 29 archived samples from various outbreaks

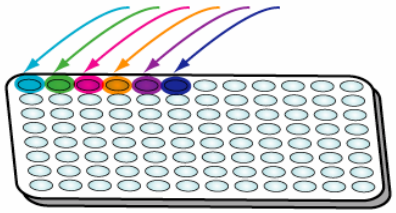
ibis Biosciences

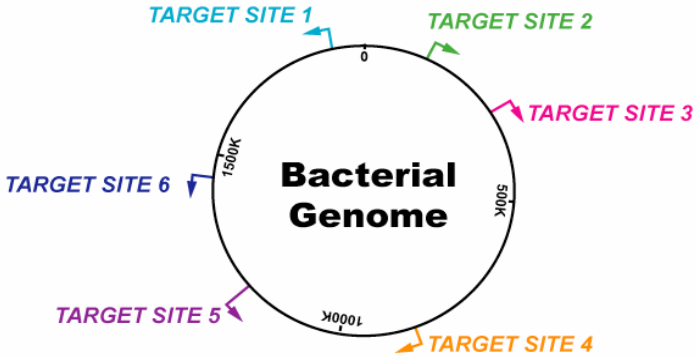


DNA

Isolated

Primers from Selected Target Sites

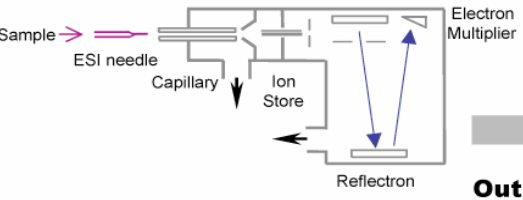




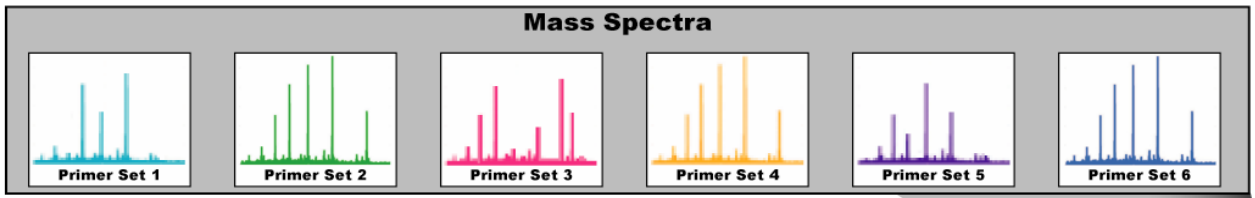
PCR

Desalting

Mass Spectrometer

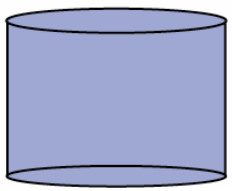


Outputs

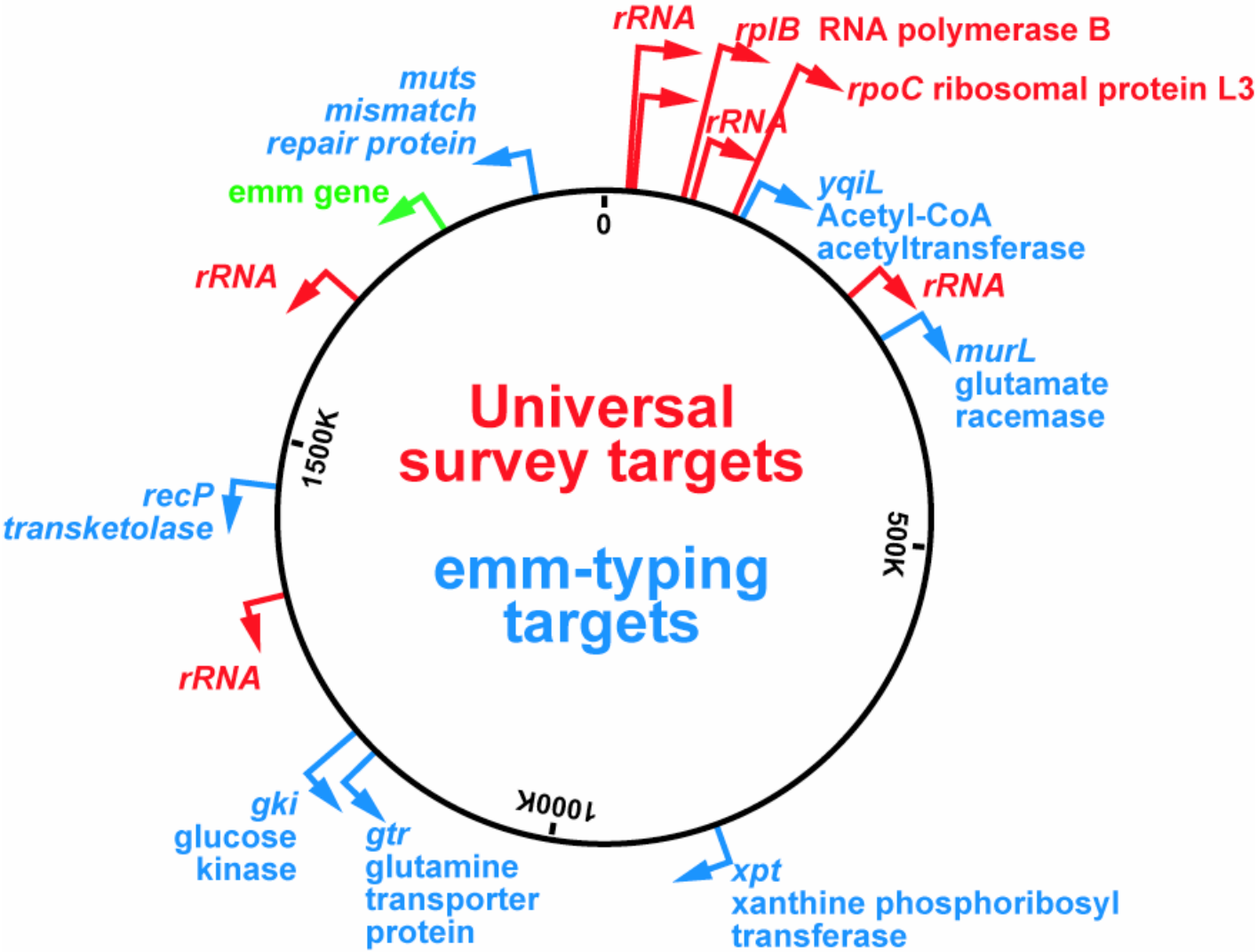


Isolate 1	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
Isolate 2	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
Isolate 3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A41 G28 C18 T32	A30 G36 C17 T33	A39 G28 C16 T32

Sequence type identified by database comparison



Housekeeping Genes used in Strep pyogenes MLST Strain Typing



Signatures from MCRD San Diego, December 2002

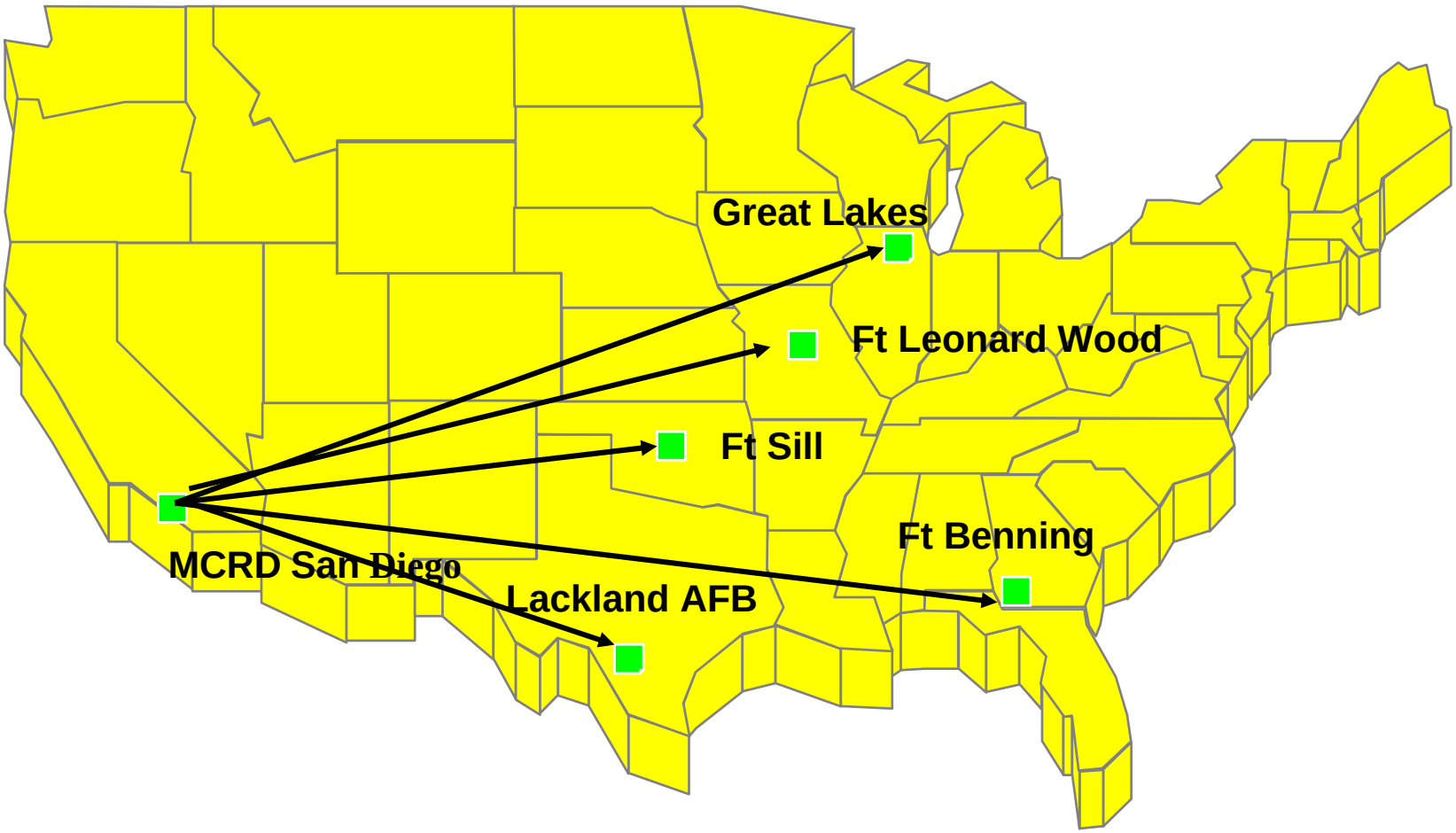
		EMM-type determination		Base Compositions					
	Sample	TIGER emm	Sequencing-emm	murI	mutS	xpt	yqiI	gki	gtr
Outbreak Samples	1	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	2	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	3	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	4	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	5	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	6	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	7	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	8	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	9	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	10	6	6	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	11	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	12	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	13	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	14	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	15	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	16	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	17	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	18	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	19	6	6	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	20	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	21	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	22	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	23	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	24	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	25	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	26	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	27	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	28	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	29	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	30	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	31	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	32	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	33	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	34	28	28	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A41 G28 C18 T32	A30 G36 C17 T33	A39 G28 C16 T32
	35	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	36	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	37	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	38	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	39	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	40	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	41	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	42	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	43	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	44	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	45	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	46	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	47	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	48	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	49	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	50	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	51	3	3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32

Archive NHRC Samples

Biosciences

		EMM-type determination			Base Compositions					
	Sample ID	MLST mass spectrometry	emm-gene sequencing	MLST sequencing	murI	mutS	xpt	yqiI	gki	gtr
Archive Samples	52	3	3	M3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	53	3	3	ND	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	54	44/61,82,9	44/61	ND	A40 G24 C20 T34	A38 G26 C24 T33	A30 G36 C20 T36	A41 G28 C19 T31	A30 G36 C18 T32	A39 G28 C15 T33
	55	11	11	ND	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A41 G28 C19 T31	A30 G36 C20 T30	A39 G28 C16 T32
	56	25,75	75	ND	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A30 G36 C17 T33	A39 G28 C15 T33
	57	6	6	ND	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	58	25,75	75	ND	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A30 G36 C17 T33	A39 G28 C15 T33
	59	12	12	ND	A40 G24 C20 T34	A38 G26 C24 T33	A30 G36 C19 T37	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	60	25,75	75	ND	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A30 G36 C17 T33	A39 G28 C15 T33
	61	6	6	ND	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	62	3	3	ND	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	63	6	6	ND	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	64	5,58	5	ND	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A30 G36 C20 T30	A39 G28 C15 T33
	65	6	6	ND	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	66	5,58	5	ND	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A30 G36 C20 T30	A39 G28 C15 T33
	67	3	3	ND	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	68	44/61,82,9	44/61	ND	A40 G24 C20 T34	A38 G26 C24 T33	A30 G36 C20 T36	A41 G28 C19 T31	A30 G36 C18 T32	A39 G28 C15 T33
	69	5,58	5	M5	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A30 G36 C20 T30	A39 G28 C15 T33
	70	3	3	M3	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	71	53,91	91	M91	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C19 T37	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	72	44/61,82,9	44/61	M44/61	A40 G24 C20 T34	A38 G26 C24 T33	A30 G36 C20 T36	A41 G28 C19 T31	A30 G36 C18 T32	A39 G28 C15 T33
	73	6	6	M6	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	74	6	6	ND	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	75	12	12	ND	A40 G24 C20 T34	A38 G26 C24 T33	A30 G36 C19 T37	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	76	44/61,82,9	44/61	ND	A40 G24 C20 T34	A38 G26 C24 T33	A30 G36 C20 T36	A41 G28 C19 T31	A30 G36 C18 T32	A39 G28 C15 T33
	77	3	3	ND	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
	78	12	12	ND	A40 G24 C20 T34	A38 G26 C24 T33	A30 G36 C19 T37	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
	79	22	22	M22	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A38 G29 C15 T33
	80	53,91	ND	M91	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C19 T37	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32

Genotyping *Streptococcus pyogenes* Outbreaks at Military Bases in 2003



Lackland AFB

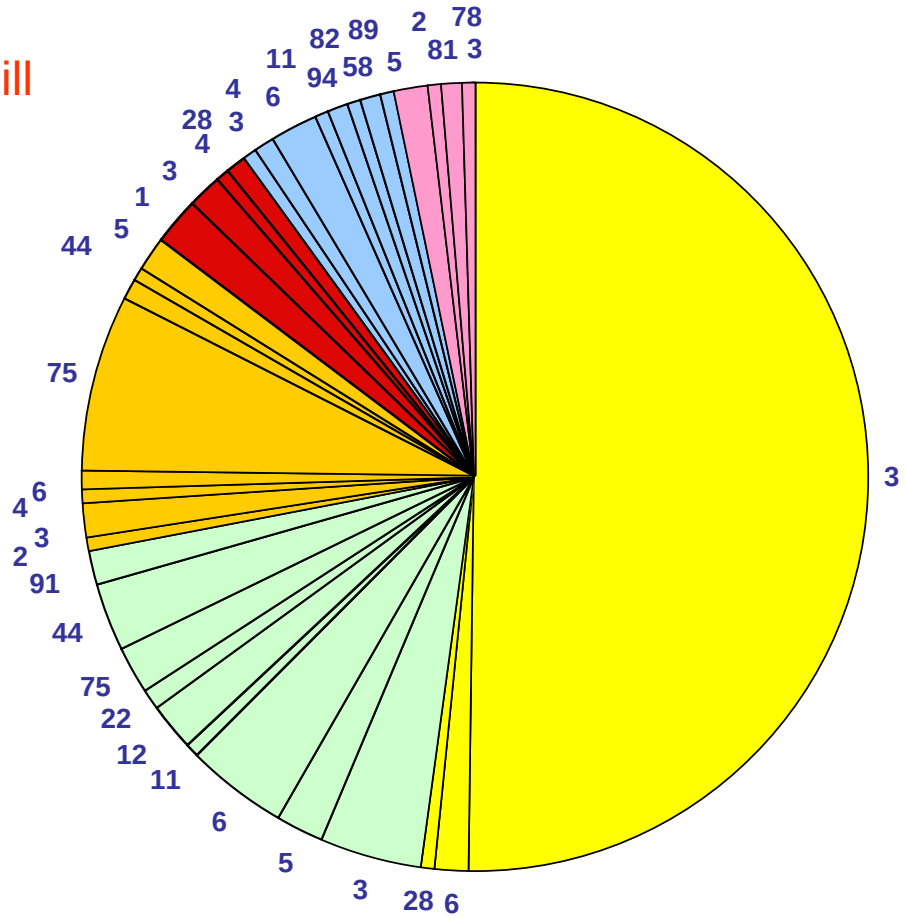
Ft. Benning

Ft. Sill

MCRD
San Diego

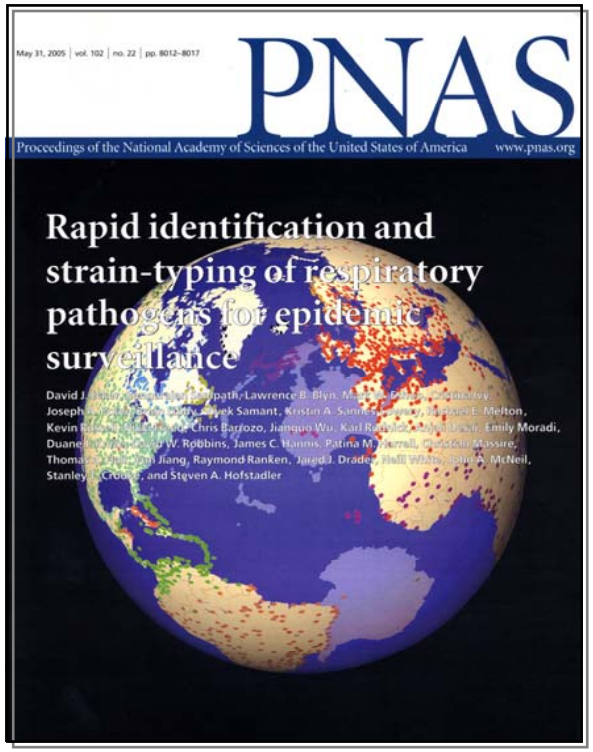
Ft. Leonard
Wood

NHRC
San Diego



Conclusions of Pneumonia Study*

- **Primary pathogen:**
  - ***Streptococcus pyogenes* (GAS)**
  - **known virulent strain**
- **Secondary pathogens:**
  - **Haemophilus influenzae**
  - **Neisseria meningitidis**
- **5 other military facilities**
  - **Determined these sites had a mixture of strain types**
- **Throughput**
  - **>200/samples per day**



***Ecker et al. (2005) Proc. Natl. Acad. Sci. Vol. 102: p8012-8017**

Hospital-acquired Infections

Acinetobacter Infections in Military Hospitals

Walter Reed Army Medical Center (WRAMC):

COL Tim Endy
MAJ Paul Scott
COL David Craft

Landstuhl Regional Medical Center, Landstuhl, Germany (LRMC)

MAJ Greg Deye

Louis Stokes Cleveland Department of Veterans Affairs Medical Center

Robert A. Bonomo

Acinetobacter baumannii: The Problem

ibis Biosciences

***Acinetobacter baumannii* is problematic bacteria for wounded soldiers**

- **Found in soil - blast wounds**
- **Spreads in hospitals**
- **New "Superbug" - resistant to nearly all antibiotics**

Epidemiological Survey

- Received 216 bacterial isolates Operation Iraqi Freedom (OIF)
- Patients and their environment from:
  - Combat support hospital in Iraq
  - USNS Comfort (hospital ship)
  - Landstuhl Regional Medical Center - Germany
  - Walter Reed Army Medical Center - Maryland USA

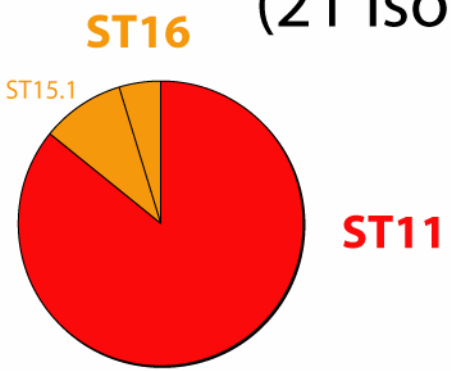
28th Combat Support Hospital (CSH) in Iraq

Acinetobacter genotyping

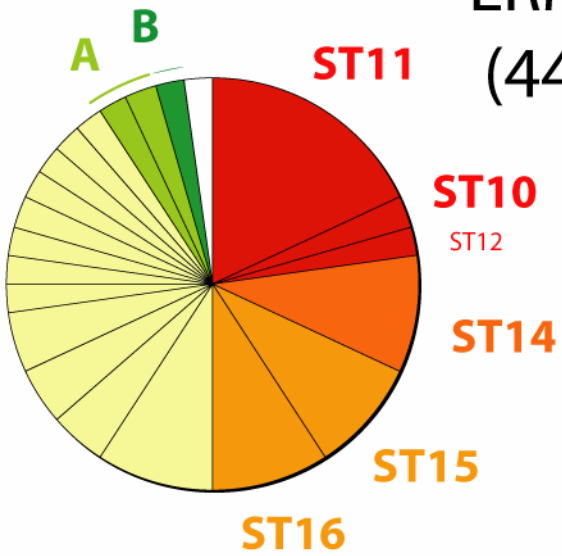
- Selected 6 target genes: efp, trpE, adk, mutY, fumC, ppa
  - 1679 nucleotides of DNA
- 8 PCR primers/mass spectrometry analysis
- Identified the genotype of all 216 OIF isolates
  - 61 Unique genotypes

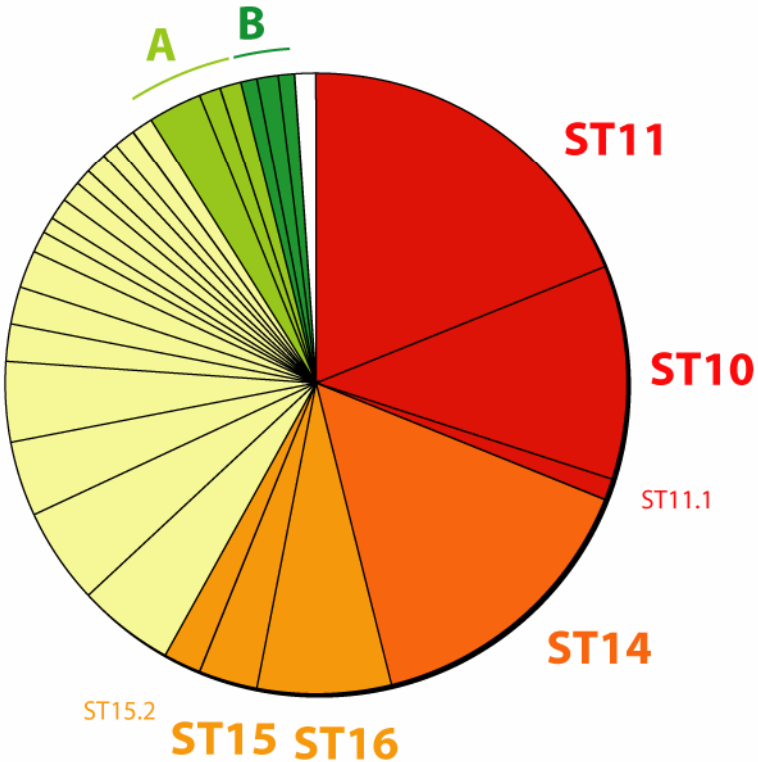
Species	ST	#	ref. strain	1151-trpE	1156-adk	1158-mutY	1160-mutY	1165-fumC	1167-fumC	1170-fumC	1171-ppa
Acinetobacter baumanii	2	1		A45G34C21T42	A44G32C25T39	A27G21C26T21	A32G35C28T34	A40G33C30T36	A41G34C35T37	A38G27C20T51	A35G37C32T45
	3	6		A44G35C22T41	A44G32C26T38	A27G20C27T21	A32G35C28T34	A40G33C30T36	A41G34C35T37	A38G27C20T51	A35G37C31T46
	4	5		A44G35C22T41	A44G32C27T37	A27G20C27T21	A32G35C28T34	A39G33C30T37	A40G35C32T40	A38G27C21T50	A35G37C30T47
	5	1		A44G35C22T41	A44G32C27T37	A27G21C26T21	A32G35C28T34	A39G33C30T37	A40G35C32T40	A38G27C21T50	A35G37C30T47
	6	1		A44G35C22T41	A44G32C27T37	A27G21C25T22	A32G35C28T34	A40G33C30T36	A40G35C35T37	A38G27C21T50	A35G37C33T44
	7	7		A44G35C22T41	A44G32C25T39	A27G21C25T22	A32G35C29T33	A40G33C30T36	A41G34C35T37	A38G27C20T51	A35G37C33T44
	9	8	ATCC9955	A44G35C21T42	A44G32C26T38	A27G21C25T22	A32G35C28T34	A39G33C30T37	A40G35C32T40	A38G27C20T51	A36G35C31T47
	1	1		A44G35C21T42	A44G32C26T38	A27G21C25T22	A32G35C29T33	A40G33C30T36	A41G34C34T38	A38G27C21T50	A35G37C33T44
	10	12		A44G35C21T42	A44G32C26T38	A27G21C26T21	A32G35C28T34	A40G33C30T36	A41G34C34T38	A38G27C21T50	A35G37C33T44
	11	51		A44G35C21T42	A44G32C26T38	A27G21C25T22	A32G34C28T35	A40G33C30T36	A41G34C34T38	A38G27C21T50	A35G37C33T44
	12	9	LUH6024 (II)	A44G35C21T42	A44G32C26T38	A27G21C26T21	A32G34C29T34	A40G33C30T36	A41G34C34T38	A38G27C21T50	A35G37C33T44
	12.1	1	LUH6011 (II)	A44G35C21T42	A44G32C26T38	A27G21C26T21	A32G34C29T34	A40G33C30T36	A41G34C34T38	A38G27C21T50	A35G37C33T44
	12.2	1		A44G35C21T42	A44G32C26T38	A27G21C26T21	A32G34C29T34	A40G33C30T36	A41G34C34T38	A38G27C21T50	A35G37C33T44
	13	8		A44G35C22T41	A44G32C26T38	A27G21C25T22	A32G35C28T34	A40G33C29T37	A40G35C33T39	A38G27C21T50	A35G37C33T44
	14	28	LUH6030 (III)	A44G35C22T41	A44G32C27T37	A27G21C25T22	A31G36C28T34	A40G33C29T37	A40G35C34T38	A38G27C21T50	A35G37C30T47
	8	1	LUH6035 (III)	A44G35C22T41	A44G32C27T37	A27G21C25T22	A31G36C28T34	A40G33C29T37	A40G35C34T38	A38G27C20T51	A35G37C30T47
	15	12	LUH6013 (I)	A44G35C22T41	A44G32C26T38	A29G19C26T21	A31G35C29T34	A40G33C29T37	A41G35C32T39	A37G28C20T51	A35G37C30T47
	45	2		A44G35C22T41	A44G32C26T38	A29G19C26T21	A31G35C29T34	A40G33C29T37	A41G35C32T39	A37G28C20T51	A35G37C32T45
	46	2		A44G35C22T41	A44G32C27T37	A29G19C26T21	A31G35C29T34	A40G33C29T37	A41G35C32T39	A37G28C20T51	A35G37C30T47
	16	13		A44G35C22T41	A44G32C27T37	A29G19C26T21	A31G35C29T34	A40G34C29T36	A41G35C32T39	A37G28C20T51	A35G37C30T47
	17	1		A44G35C20T43	A44G32C27T37	A27G21C25T22	A32G35C28T34	A40G33C29T37	A41G34C35T37	A38G27C21T50	A35G36C32T46
	18	1		A43G36C21T42	A44G32C27T37	A27G21C26T21	A31G36C28T34	A40G33C29T37	A41G35C32T39	A37G28C20T51	A35G37C31T46
	19	1		A43G36C21T42	A44G32C26T38	A27G21C25T22	A32G35C28T34	A40G33C29T37	A41G35C32T39	A37G28C20T51	A35G37C31T46
	20	1		A43G36C20T43	A44G32C27T37	A28G21C24T22	A32G35C29T33	A40G33C29T37	A40G35C33T39	A37G28C20T51	A35G37C30T47
	21	2		A43G36C20T43	A44G32C27T37	A28G21C24T22	A32G35C29T33	A39G33C30T37	A40G35C32T40	A38G27C20T51	A35G37C33T44
	22	1		A43G36C20T43	A44G32C27T37	A27G21C25T22	A31G36C28T34	A40G33C30T36	A41G34C35T37	A38G27C20T51	A35G37C33T44
	23	1		A43G36C20T43	A44G32C27T37	A27G21C26T21	A32G34C29T34	A39G33C30T37	A40G35C32T40	A38G27C21T50	A35G37C31T46
	24	7		A43G36C20T43	A44G32C27T37	A27G21C26T21	A32G35C28T34	A40G33C30T36	A40G35C34T38	A39G26C22T49	A35G37C33T44
	25	5		A43G36C20T43	A44G32C27T37	A27G21C25T22	A32G35C29T33	A40G33C30T36	A40G35C34T38	A39G26C22T49	A35G37C33T44
	26	3		A43G36C20T43	A44G32C26T38	A27G21C26T21	A32G35C28T34	A40G33C29T37	A40G35C34T38	A38G27C21T50	A35G37C33T44
	27	1		A44G35C19T44	A44G32C27T37	A27G21C25T22	A32G35C28T34	A39G33C30T37	A40G35C33T39	A38G27C20T51	A35G37C33T44
	28	1		A44G35C22T41	A44G32C27T37	A27G21C25T22	A32G34C28T35	A40G33C30T36	A42G34C33T38	A38G27C20T51	A35G37C31T46
	29	3	LUH6049 (I)	A44G35C21T42	A44G32C27T37	A27G21C25T22	A32G34C28T35	A39G33C30T37	A40G35C32T40	A38G27C20T51	A35G36C29T49
	30	6		A44G35C19T44	A44G32C27T37	A27G21C25T22	A32G35C28T34	A39G33C30T37	A40G35C32T40	A38G27C21T50	A35G36C29T49
	31	3	ATCC15308	A44G35C22T41	A44G32C27T37	A27G21C25T22	A31G36C28T34	A40G33C29T37	A41G35C32T39	A37G28C20T51	A35G37C31T46
	32	1	ATCC15150	A43G36C20T43	A44G32C27T37	A27G21C25T22	A31G36C28T34	A40G33C29T37	A41G35C33T38	A38G27C20T51	A35G37C33T44
	33	1	ATCC27227	A43G36C20T43	A44G32C27T37	A27G21C25T22	A31G36C28T34	A40G33C30T36	A41G34C35T37	A38G27C20T51	A35G37C31T46
	34	1	ATCC15151	A44G35C22T41	A44G32C26T38	A27G21C25T22	A31G36C28T34	A39G33C30T37	A40G35C32T40	A39G26C20T51	A35G37C31T46
	35	1	ATCC15149	A44G35C22T41	A44G32C27T37	A27G21C25T22	A32G35C28T34	A39G33C30T37	A40G35C33T39	A38G27C20T51	A35G37C31T46
	36	1	ATCC17904	A44G35C22T41	A44G32C27T37	A27G20C27T21	A32G35C28T34	A40G33C30T36	A41G34C35T37	A38G27C20T51	A35G37C30T47
	37	1		A44G35C21T42	A44G32C26T38	A27G20C27T21	A32G35C28T34	A40G33C29T37	A41G35C33T38	A38G27C20T51	A35G37C32T45
	38	1		A44G35C22T41	A44G32C27T37	A27G21C25T22	A32G34C28T35	A40G33C30T36	A42G34C33T38	A38G27C20T51	A35G36C29T49
	39	1		A44G35C22T41	A44G32C26T38	A27G21C26T21	A32G35C28T34	A39G33C30T37	A40G35C33T39	A38G27C20T51	A35G37C30T47
	40	1		A44G35C22T41	A44G32C26T38	A28G20C25T22	A32G35C28T34	A39G33C30T37	A40G35C32T40	A38G27C20T51	A35G37C33T44
	41	1		A44G35C21T42	A44G32C27T37	A28G20C25T22	A32G34C28T35	A40G33C30T36	A40G35C34T38	A38G27C21T50	A35G37C31T46
	42	1		A44G35C22T41	A44G32C26T38	A27G21C25T22	A31G36C28T34	A40G33C30T36	A41G35C33T38	A38G27C20T51	A35G37C29T48
	43	1		A45G34C20T43	A44G32C27T37	A28G20C25T22	A31G36C29T33	A40G33C30T36	A40G35C34T38	A39G26C22T49	A35G37C31T46
	44	2		A44G35C21T42	A44G32C27T37	A27G21C26T21	A32G35C28T34	A39G33C30T37	A40G35C33T39	A38G27C20T51	A35G37C32T45

USNS Comfort
(21 isolates)



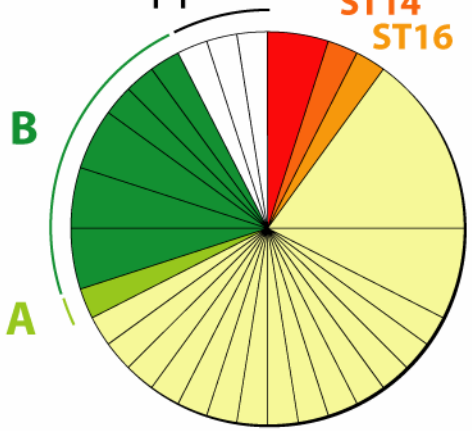
LRMC
(44 isolates)



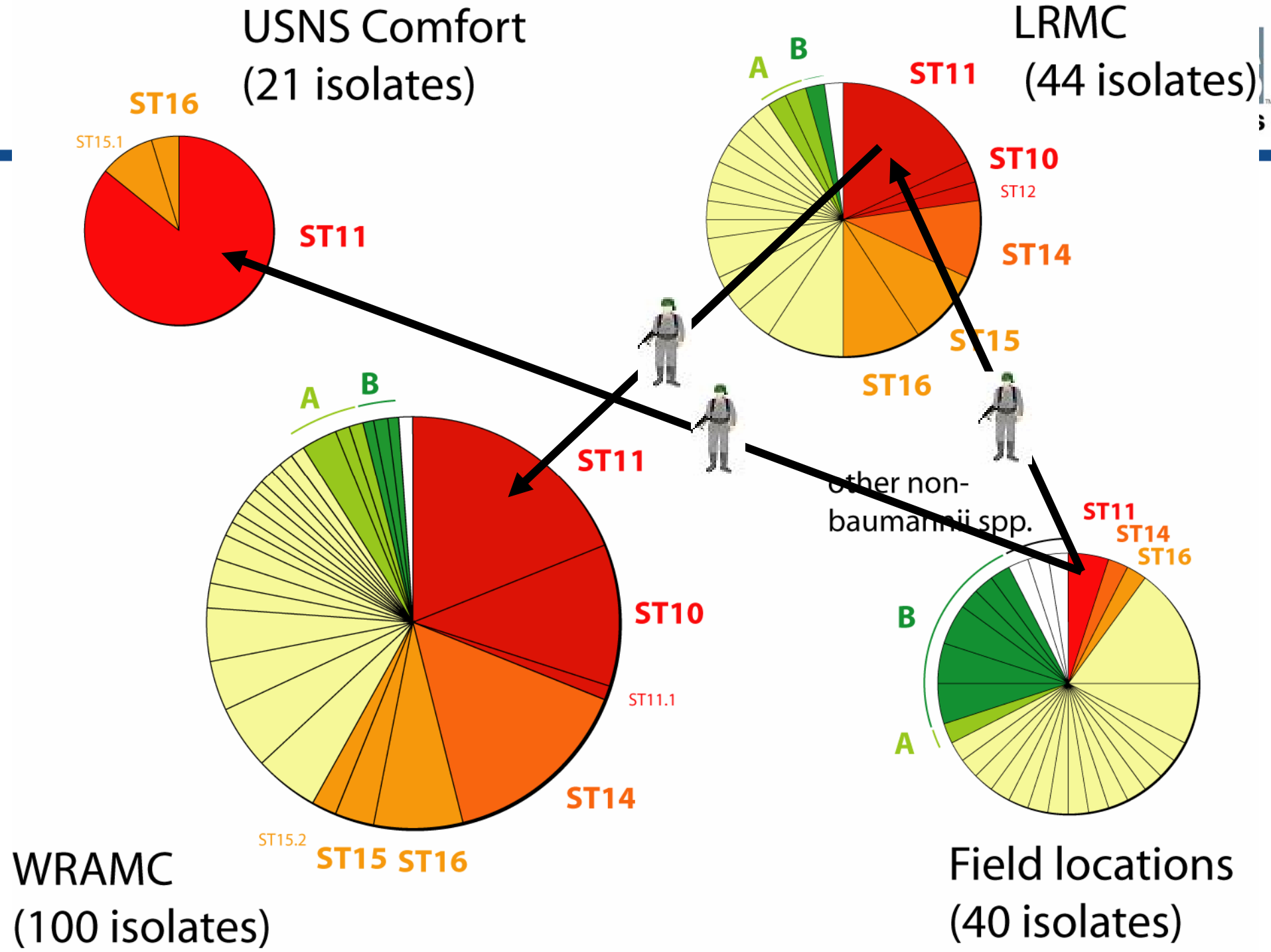


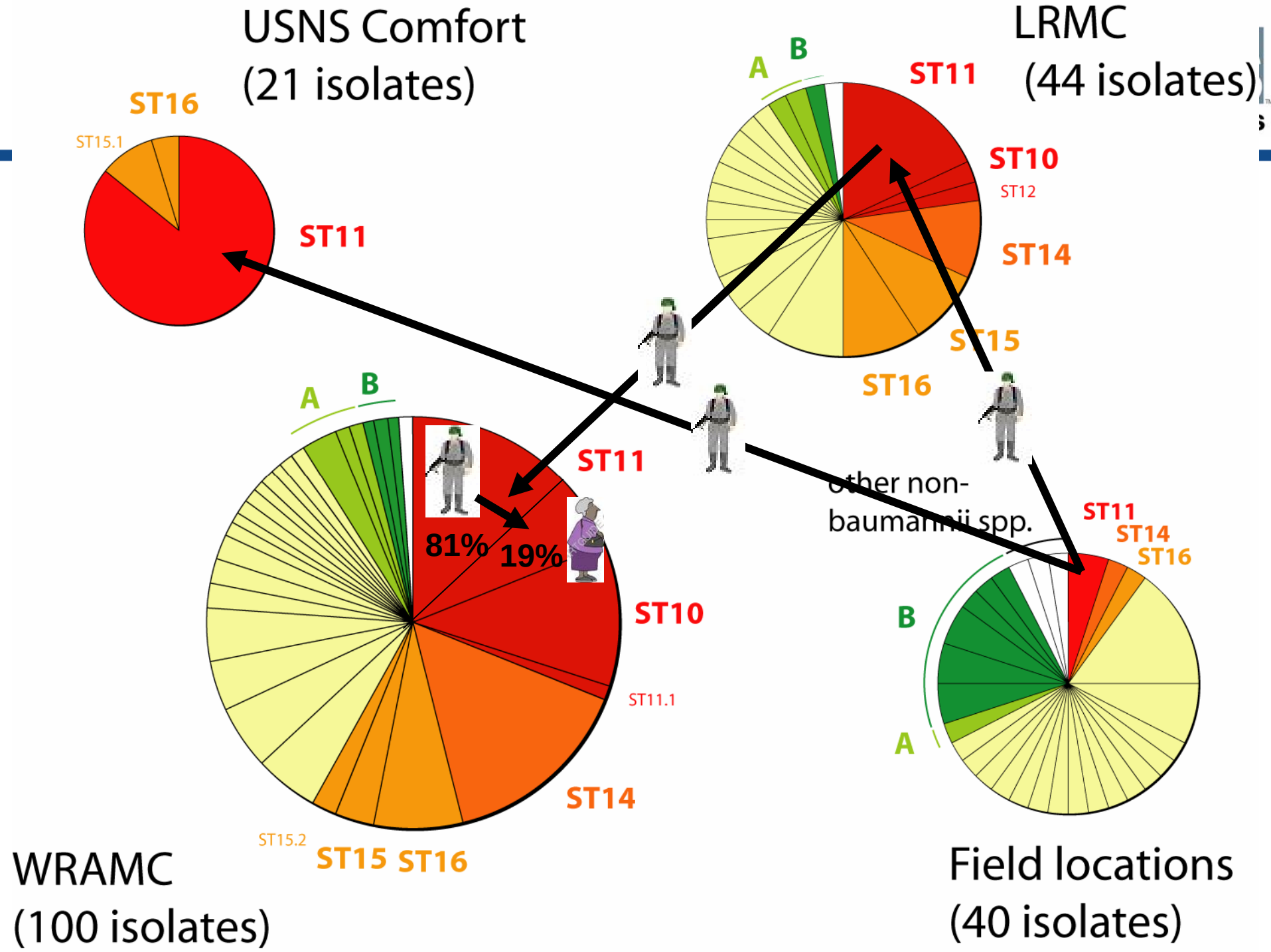
WRAMC
(100 isolates)

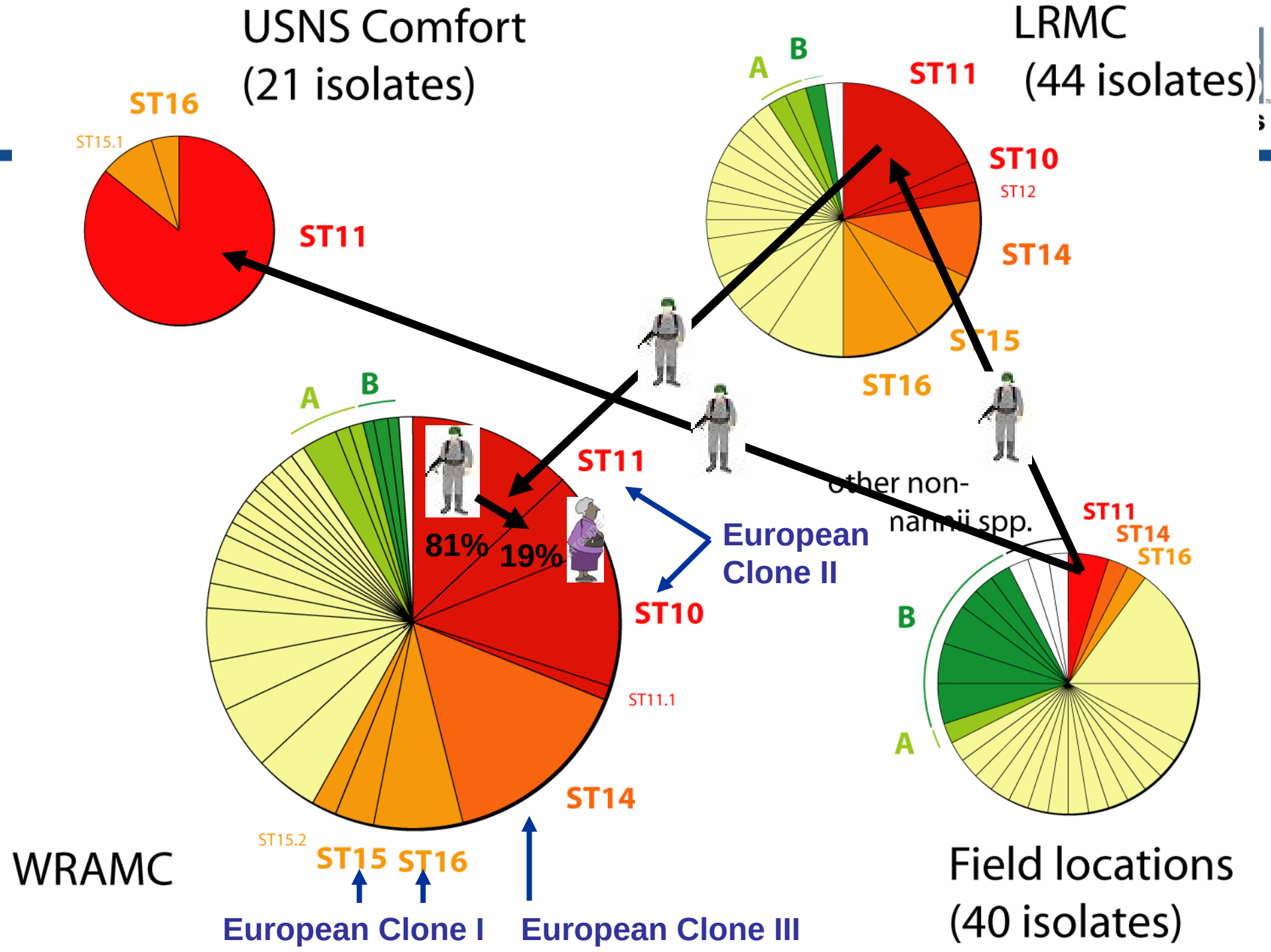
other non-
baumannii spp.



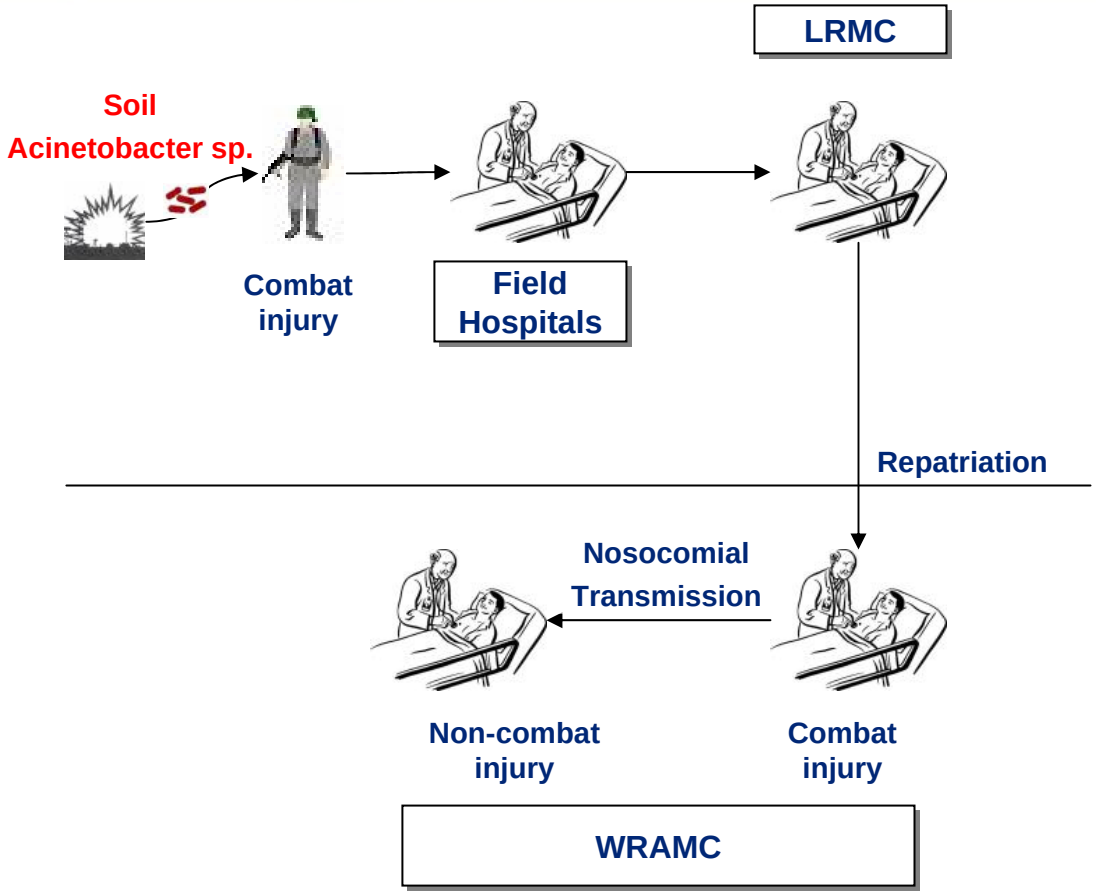
Field locations
(40 isolates)


USNS Comfort
(21 isolates)
ST16
ST15.1
ST11
LRMC
(44 isolates)
A
B
ST11
ST10
ST12
ST14
ST15
ST16
A
B
ST11
ST10
ST11.1
ST14
ST15.2
ST15
ST16
other non-
baumannii spp.
ST11
ST14
ST16
B
A
WRAMC
(100 isolates)
Field locations
(40 isolates)


USNS Comfort
(21 isolates)
ST16
ST15.1
ST11
LRMC
(44 isolates)
A
B
ST11
ST10
ST12
ST14
ST15
ST16
A
B
ST11
81%
19%
ST10
ST11.1
ST14
ST15.2
ST15
ST16
other non-
baumannii spp.
ST11
ST14
ST16
B
A
WRAMC
(100 isolates)
Field locations
(40 isolates)


USNS Comfort
(21 isolates)
ST16
ST15.1
ST11
LRMC
(44 isolates)
A
B
ST11
ST10
ST12
ST14
ST15
ST16
A
B
ST11
81%
19%
ST10
ST11.1
ST14
ST15.2
ST15
ST16
WRAMC
European Clone II
European Clone I
European Clone III
other non-
nannii spp.
ST11
ST14
ST16
B
A
Field locations
(40 isolates)

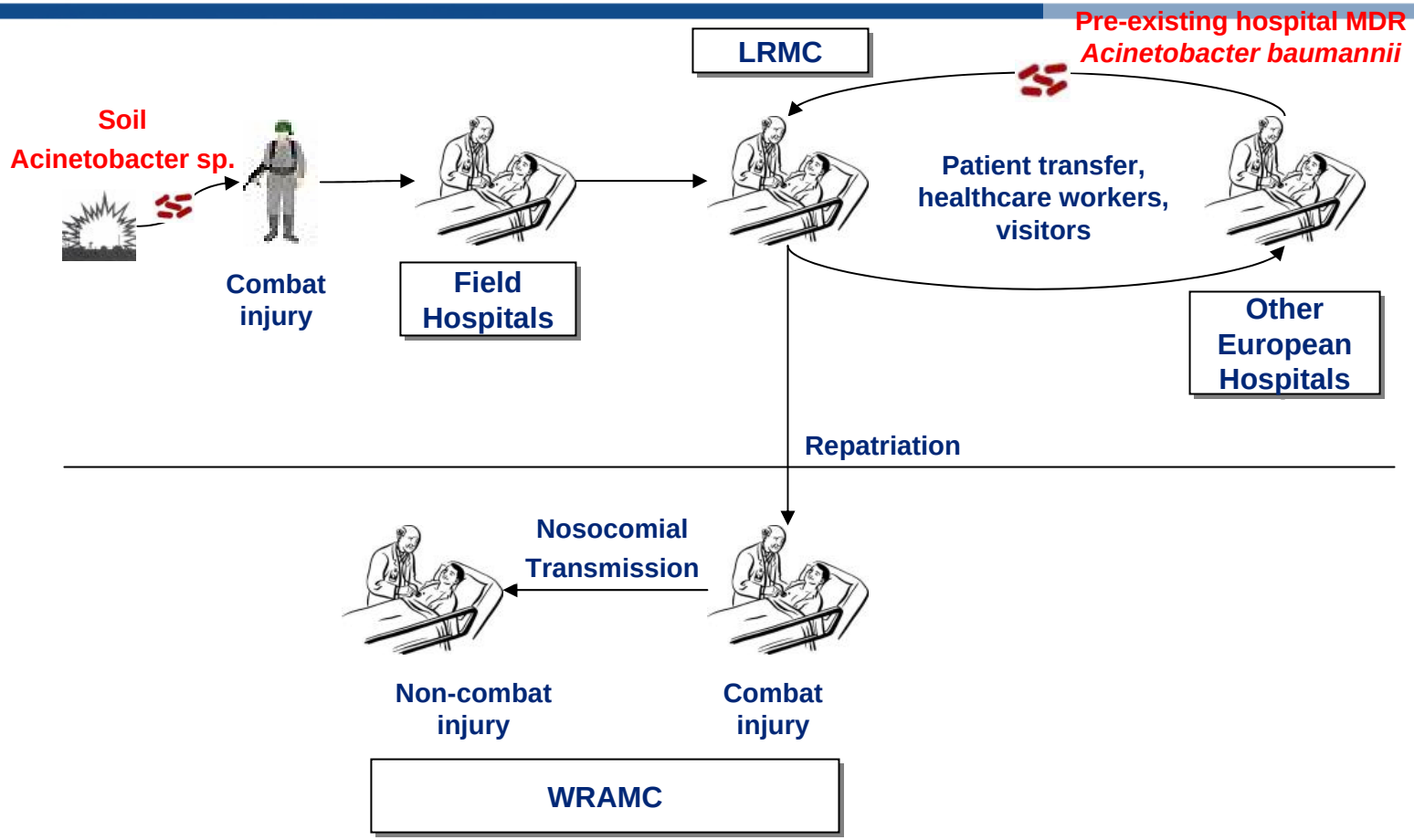
A.) *De novo* soil infection model



European Clones 190/216

A.) *De novo* soil infection model

B.) Hospital Contamination Model



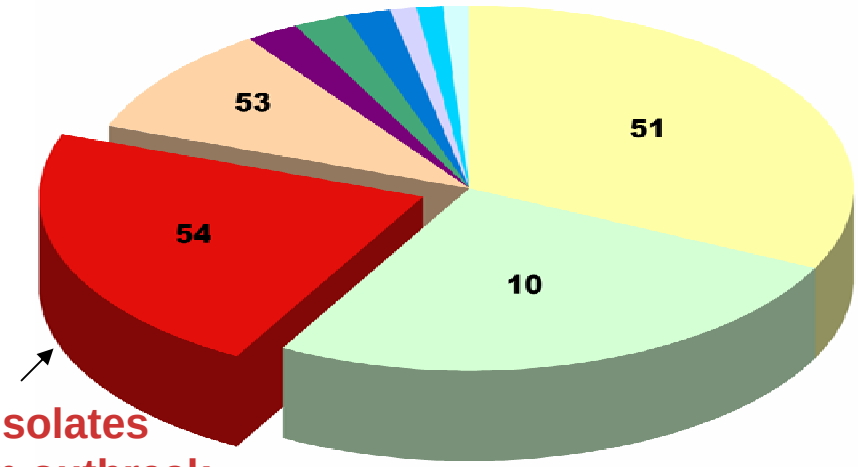
Summary/Lessons Learned

- 216 isolates from OIF
  - 61 unique genotypes
- Major clusters identical with European hospital strains
  - Explains rapid spread
  - Explains multi drug-resistance
- Survey of incoming patients from other healthcare facilities
  - Isolate and treat
- Survey the environment
  - Decontaminate

Methodology -- J. Clin. Micro (2006)Aug;44(8):2921-32.

Northwestern Memorial Hospital Acinetobacter Outbreak

- Outbreak April-December, 2005
- High-level carbepenam and fluoroquinolone resistance
- Similar infections city-wide in Chicago
- Ibis received 94 samples on 1/26/06 and returned a report to NMH 1/27/06



All Isolates from outbreak

Organism	Genotype	# of isolates	T5000 Cipro
A. baumannii	54	21	Resistant
	10	8	Resistant
		16	
	51	29	Resistant
		1	
	53	9	Resistant
	27	1	Resistant
		1	Sensitive
	3	2	Resistant
	14	1	Resistant
A. species 3	B7	1	Sensitive

- T5000 correctly identified the outbreak isolates
- T5000 drug resistance data correlated with classical resistance test
- 24 hour results

Methicillin Resistant *Staphylococcus aureus* (MRSA): Detection and Typing

Collaborators:

Karen Carroll
Johns Hopkins University Medical Center

Jean Patel, Brandy Limbago, Linda McDougal
CDC

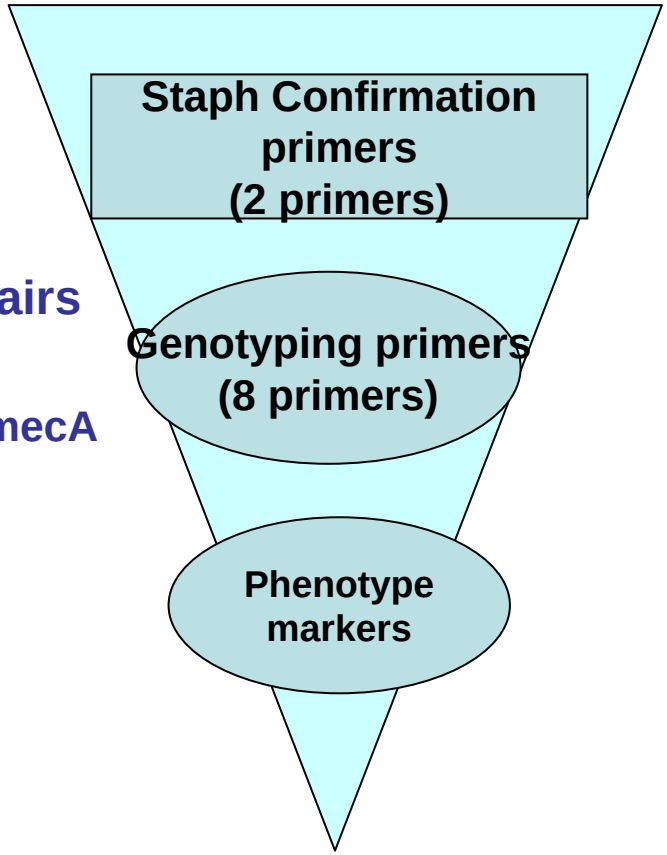
Donna Wolk
Tucson Arizona, VA Hospital

S. aureus Characterization

- **Develop and validate ESI-MS assays for characterizing *S. aureus* in nosocomial settings**
  - **Identify the organism as *S. aureus***
  - **Determine drug-resistance profile (methicillin, erythromycin, mupirocin)**
  - **Determine virulence phenotype markers**
  - **Genotype to identify strain**
- **Experiments**
  - **Testing of >250 JHU isolates**
  - **Testing of 31 *S. aureus* isolates from CDC *S. aureus* Lab**
  - **Testing of ~ 100 clinical isolates from Arizona VA Hospital**

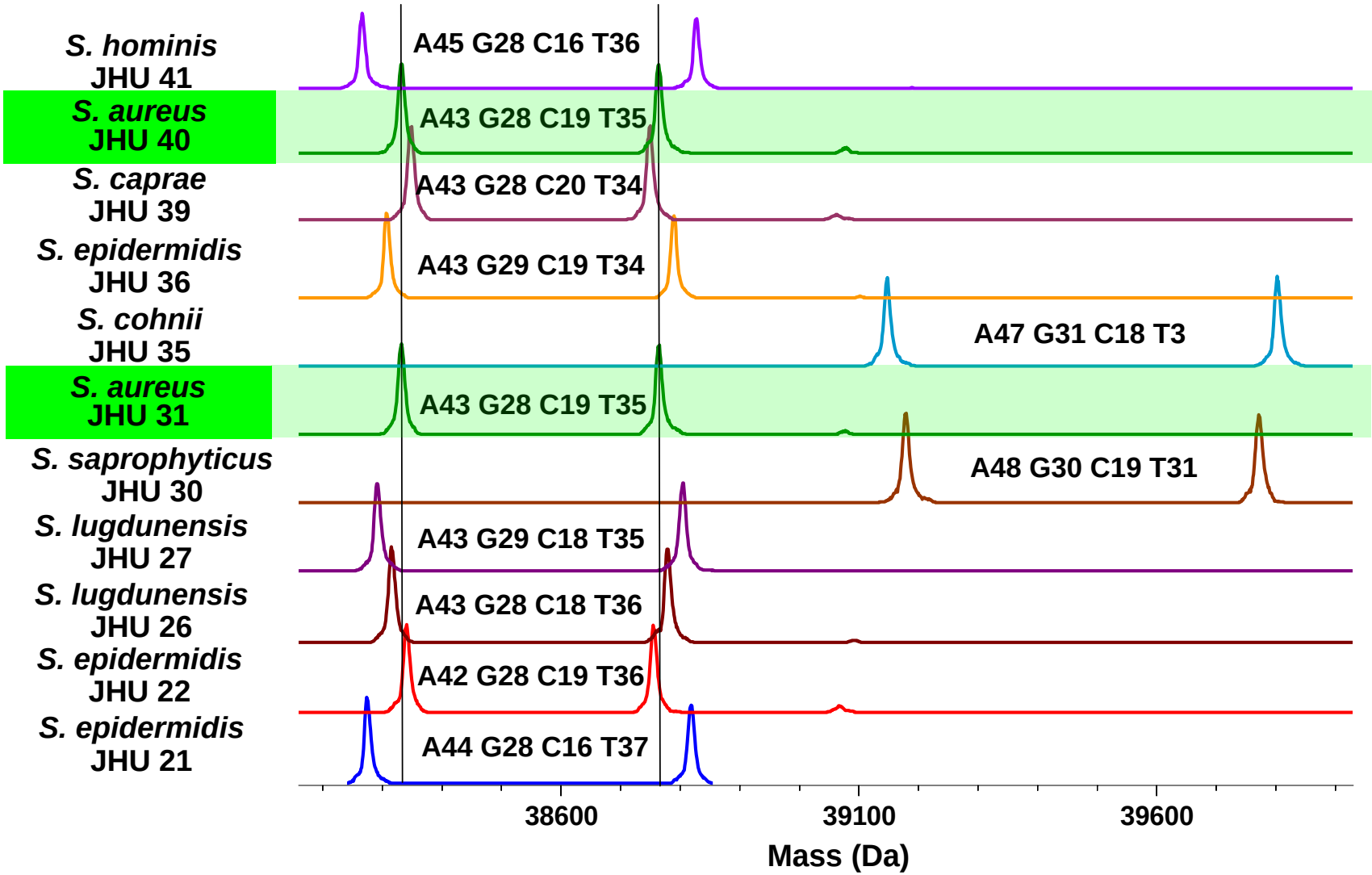
Staphylococcus aureus confirmation / drug-resistance / virulence factor panel

- *S. aureus* confirmation – 2 primer pairs
  - *nuc* -- *S. aureus*-specific marker
  - tufB - Staphylococcus genus marker –
    - Universal housekeeping gene
    - tufB primer amplifies known *Staphylococci*
- Drug resistance – 4 primer pairs
  - High-level methicillin resistance – 2 primer pairs
    - mecA – methicillin-resistant PBP2A gene
    - mecI-R – junction of regulatory region and mecA gene
  - Macrolide / erythromycin resistance
    - ermA
    - ermC
  - Mupirocin resistance
    - mupR
- Virulence – 1 primer pair
  - PVL – Panton-Valentine leukocidin
  - PVL homolog lukEv-lukDv (lukDv component- α- and γ-hemolysin)



tufB primer pair primes all *Staphylococcus* but gives unique signature for *S. aureus*

tufB, together with a positive *nuc* signal, provides confirmation of *S. aureus*



Resistance / PVL profile for 32 blinded CDC samples

ibis Biosciences

One sample was *S. schleiferi* and was confirmed as non-aureus by tufB

ESI-MS MLST Group	CDC sample	2081 (ermA)	2086 (ermC)	2095 (pvLuk)	2256 (nuc)	2249 (tufB)	879 (mecA)	2056 (mecI-R)	2313 (mupR)
1	CDC0010	-	-	PVL- / lukD+	+	S. aureus	+	+	-
	CDC0015	-	-	PVL+ / lukD+	+	S. aureus	-	-	-
	CDC0019	-	+	PVL- / lukD+	+	S. aureus	+	+	-
	CDC0026	+	-	PVL- / lukD+	+	S. aureus	+	+	-
	CDC0030	+	-	PVL- / lukD+	+	S. aureus	+	+	-
	CDC004	-	-	PVL+ / lukD+	+	S. aureus	+	+	-
	CDC0014	-	+	PVL+ / lukD+	+	S. aureus	+	+	-
	CDC008	-	-	PVL- / lukD+	+	S. aureus	+	+	-
2	CDC001	+	-	PVL- / lukD+	+	S. aureus			-
	CDC0022	+	-	PVL- / lukD+	+	S. aureus			-
	CDC006	+	-	PVL- / lukD+	+	S. aureus			+
	CDC007	-	-	PVL- / lukD+	+	S. aureus			-
	CDCVRSA1	+	-	PVL- / lukD+	+	S. aureus			-
	CDCVRSA2	+	+	PVL- / lukD+	+	S. aureus			-
3	CDC0011	+	-	PVL- / lukD-	+	S. aureus			-
	CDC0012	-	-	PVL+ / lukD-	+	S. aureus			-
	CDC0021	+	-	PVL- / lukD-	+	S. aureus			-
4	CDC0023	+	-	PVL- / lukD+	+	S. aureus			-
	CDC0025	+	-	PVL- / lukD+	+	S. aureus			-
5	CDC005	-	-	PVL- / lukD-	+	S. aureus			-
	CDC0018	+	-	PVL+ / lukD-	+	S. aureus	+	+	-
6	CDC002	-	-	PVL- / lukD-	+	S. aureus	+	+	-
	CDC0028	+	-	PVL- / lukD-	+	S. aureus	+	+	-
7	CDC003	-	-	PVL- / lukD+	+	S. aureus	+	+	-
8	CDC0013	-	-	PVL+ / lukD+	+	S. aureus	+	+	-
9	CDC0016	-	-	PVL- / lukD+	+	S. aureus	+	+	-
10	CDC0027	+	-	PVL- / lukD+	+	S. aureus	+	+	-
11	CDC0029	-	-	PVL+ / lukD+	+	S. aureus	+	+	-
12	CDC0020	-	+	PVL- / lukD+	+	S. aureus	-	-	-
13	CDC0024	-	-	PVL- / lukD-	+	S. aureus	+	+	-
--	CDC0031	-	-	PVL- / lukD-	+	S. scleiferi	-	-	-

PVL + by Standard luks/lukF PCR Assays

Discrimination of 32 blinded CDC strains into 13 sequence groups by ESI-MS-multi-locus genotyping

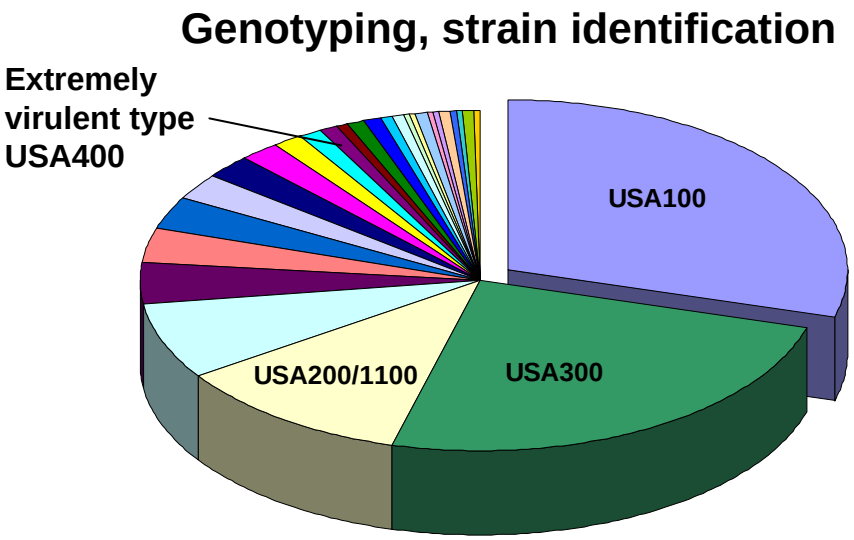
ibis Biosciences

Group	Sample	BCT2146	BCT2149	BCT2150	BCT2156	BCT2157	BCT2161	BCT2163	BCT2166	Percent of STs excluded
1	CDC0010	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	95.1
	CDC0015	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	95.1
	CDC0019	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	95.1
	CDC0026	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	95.1
	CDC0030	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	95.1
	CDC004	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	95.1
	CDC0014	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	95.1
	CDC008	A44 G24 C18 T29	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	95.1
2	CDC001	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36	94.1
	CDC0022	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36	94.1
	CDC006	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36	94.1
	CDC007	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36	94.1
	CDCVRSA1	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36	94.1
	CDCVRSA2	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36	94.1
3	CDC0011	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31	A32 G25 C23 T29	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37	92.7
	CDC0012	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31	A32 G25 C23 T29	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37	92.7
	CDC0021	A45 G24 C18 T28	A58 G24 C19 T51	A41 G36 C12 T43	A51 G29 C21 T31	A32 G25 C23 T29	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37	92.7
4	CDC0023	A45 G24 C18 T28	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	96.9
	CDC0025	A45 G24 C18 T28	A59 G24 C18 T51	A40 G36 C13 T43	A50 G30 C20 T32	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	96.9
5	CDC005	A44 G24 C18 T29	A59 G23 C19 T51	A40 G36 C14 T42	A51 G29 C21 T31	A32 G25 C24 T28	A51 G27 C21 T30	A42 G36 C22 T43	A37 G30 C18 T37	98.6
	CDC0018	A44 G24 C18 T29	A59 G23 C19 T51	A40 G36 C14 T42	A51 G29 C21 T31	A32 G25 C24 T28	A51 G27 C21 T30	A42 G36 C22 T43	A37 G30 C18 T37	98.6
6	CDC002	A46 G23 C20 T26	A58 G24 C19 T51	A42 G36 C12 T42	A51 G29 C20 T32	A33 G25 C23 T28	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37	97.6
	CDC0028	A46 G23 C20 T26	A58 G24 C19 T51	A42 G36 C12 T42	A51 G29 C20 T32	A33 G25 C23 T28	A50 G28 C22 T29	A42 G36 C22 T43	A37 G30 C18 T37	97.6
7	CDC003	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31	A32 G25 C23 T29	A51 G28 C22 T28	A41 G37 C22 T43	A37 G30 C18 T37	99.7
8	CDC0013	A45 G23 C20 T27	A59 G24 C18 T51	A40 G36 C13 T43	A51 G29 C21 T31	A32 G25 C23 T29	A51 G28 C22 T28	A42 G36 C22 T43	A36 G31 C19 T36	99.6
9	CDC0016	A45 G23 C18 T29	A58 G24 C19 T51	A40 G37 C13 T42	A51 G29 C21 T31	A32 G25 C24 T28	A50 G28 C21 T30	A42 G36 C22 T43	A37 G30 C18 T37	98.4
10	CDC0027	A45 G23 C20 T27	A58 G24 C18 T52	A40 G36 C13 T43	A51 G29 C21 T31	A33 G25 C22 T29	A50 G28 C22 T29	A43 G36 C21 T43	A36 G31 C19 T36	99.7
11	CDC0029	A45 G23 C20 T27	A58 G24 C19 T51	A40 G36 C13 T43	A50 G30 C20 T32	A33 G25 C22 T29	A50 G28 C22 T29	A42 G36 C22 T43	A36 G31 C19 T36	98.4
12	CDC0020	A44 G23 C21 T27	A59 G23 C18 T52	A40 G36 C13 T43	A50 G30 C20 T32	A33 G25 C22 T29	A50 G28 C21 T30	A42 G36 C22 T43	A36 G31 C18 T37	98.7
13	CDC0024	A45 G23 C20 T27	A57 G25 C19 T51	A40 G36 C13 T43	A51 G29 C22 T30	A33 G25 C22 T29	A51 G28 C22 T28	A42 G36 C22 T43	A37 G30 C18 T37	98.7
--	CDC0031	No product	No product	No product	No product	A34 G25 C25 T25	A51 G27 C24 T27	No product	No product	NA

CDC0031 was *Staphylococcus schleiferi*

Survey of 326 *Staphylococcus aureus* clinical isolates

Ibis Biosciences



- Isolates obtained from:
  - Centers for Disease Control
  - Johns Hopkins University
  - University of Arizona
- T5000 genotype grouping of isolates was consistent with PFGE/sequencing results
- T5000 drug resistance and virulence data was >99% concordant with current methods

	Identification		Virulence	Antibiotic Resistance				
# of isolates				Methicillin		Erythromycin		Mupirocin
	tufB	*nuc*	PVL	*mecA*	*mecI-R*	*ermA*	*ermC*	*mupR*
81	S. aureus	+	-	+	+	+	-	-
81	S. aureus	+	-	+	+	-	-	-
34	S. aureus	+	-	+	+	+	-	-
32	S. aureus	+	-	+	+	-	+	-
30	S. aureus	+	+	+	+	-	-	-
30	S. aureus	+	-	+	+	-	-	-
10	S. aureus	+	-	+	+	-	+	-
7	S. aureus	+	+	-	-	-	-	-
3	S. aureus	+	-	+	+	+	-	+

Influenza Virus Surveillance: Project Collaborators

CMDR Kevin Russell M.D., Naval Health Research Center, San Diego, CA

Kirsten St.George, MAppSc, PhD, New York State Department of Health, Slingerlands, NY

Charlotte Gaydos, Dr.P.H. and Rich Rothman, M.D. Johns Hopkins University, Baltimore, MD

Stan Lemon M.D. , University of Texas Medical Branch, Galveston, TX

Wendy Sessions. M, SV (ASCP), Texas Department of State Health Services

Dave Stallknecht and Ginger Goekjian, College of Veterinary Medicine, University of Georgia

T5000 Influenza Virus Assay

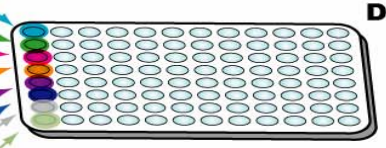
Isolated RNA

Broad Primer

Pan Influenza Primer: PB1

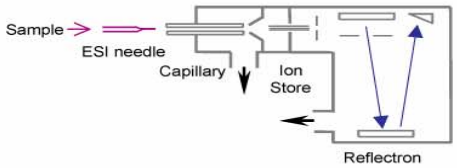
Influenza A Primers: Nuc, M1, PA, NS1, NS2

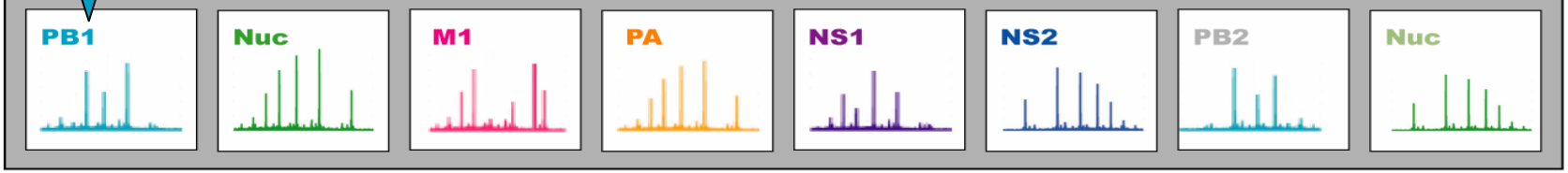
Influenza B Primers: PB2, Nuc



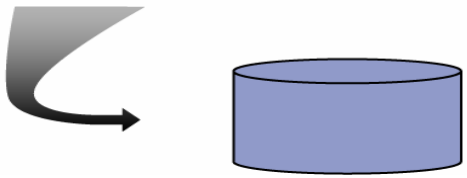
PCR Desalting

Mass Spectrometer





Influenza A	A39 G32 C23 T34	A32 G23 C20 T26	A24 G30 C23 T28	A37 G23 C27 T25	A28 G21 C32 T38	A35 G27 C17 T26		
Influenza B	A37 G31 C20 T34						A23 G27 C19 T22	A36 G17 C36 T11
Mixture	A39 G32 C23 T34	A32 G23 C20 T26	A24 G30 C23 T28	A37 G23 C27 T25	A28 G21 C32 T38	A35 G27 C17 T26	A23 G27 C19 T22	A36 G17 C36 T11



Influenza species, subtype, clade type determined by comparison with database

Pan-influenza Primer Polymerase PB1 Primer

Forward primer

Reverse primer

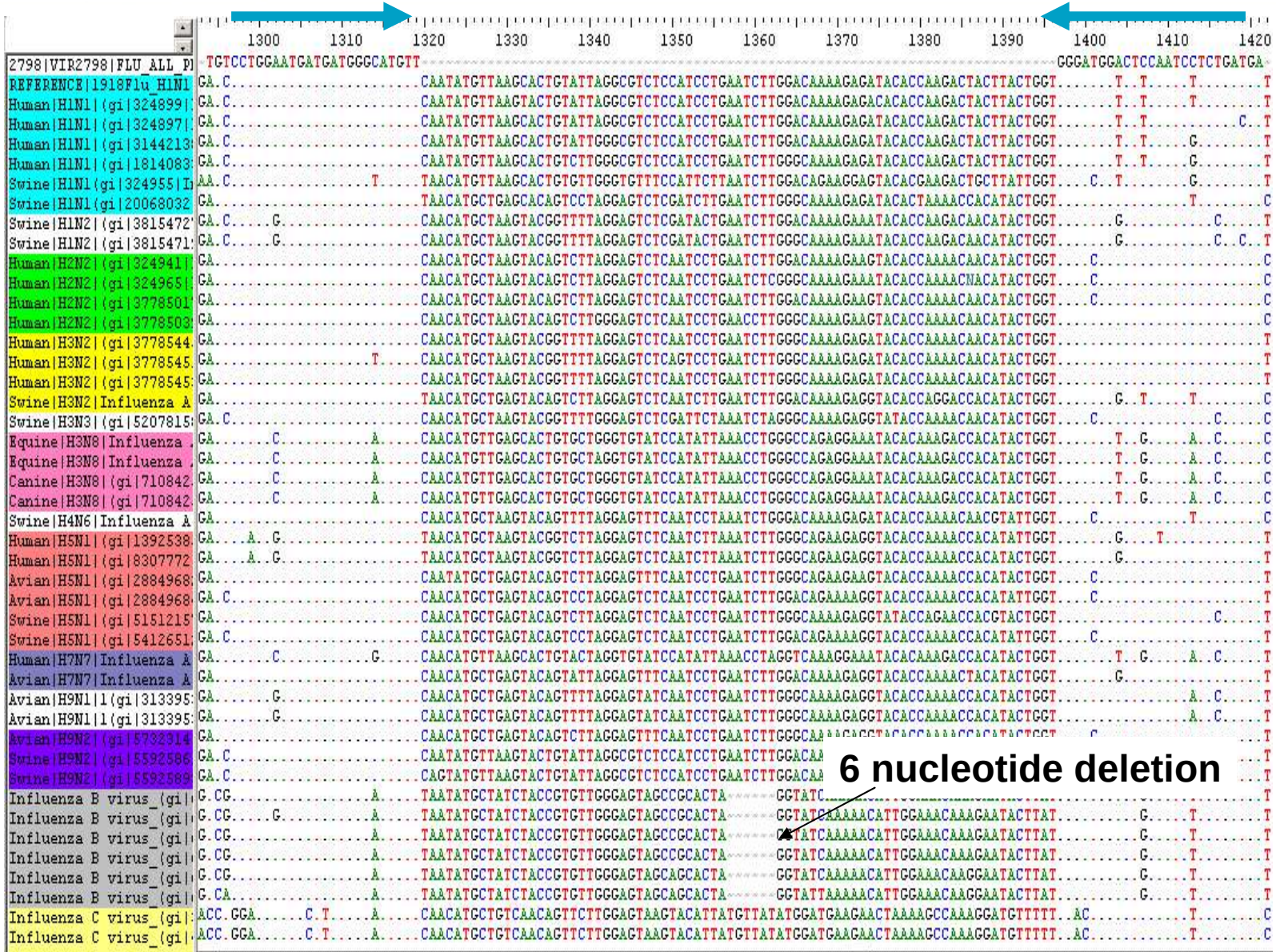
Human and Swine H1N1, H2N2, H3N2

Canine, Equine H3N8

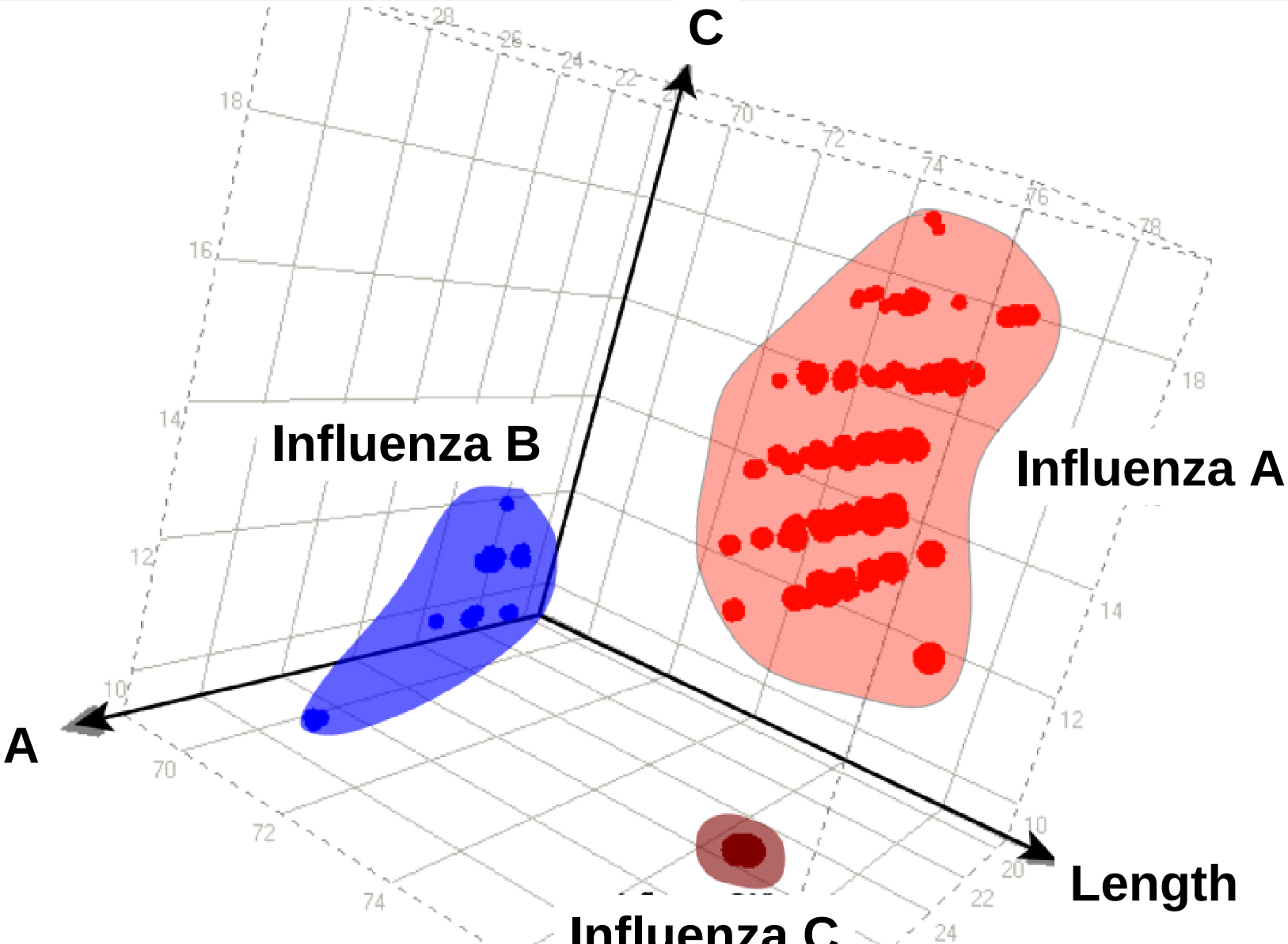
Human, Avian H5N1

Other Avian, Swine

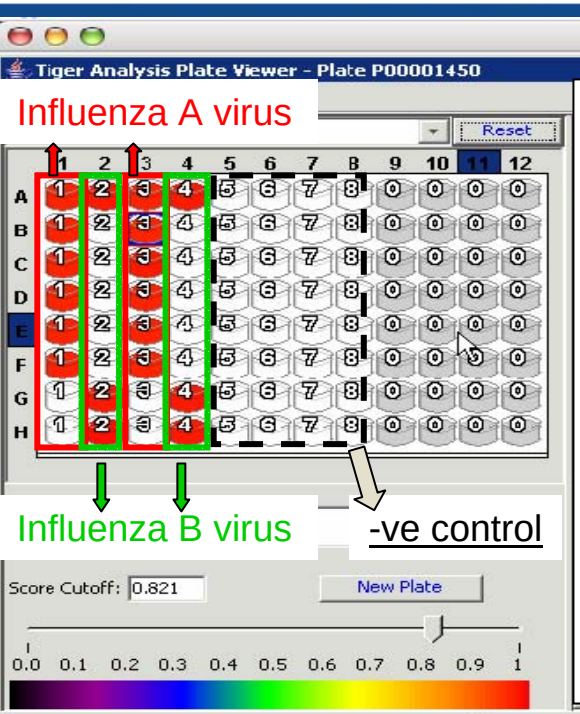
Influenza B and C



Pan-influenza Primer Polymerase PB1 Primer PP2798 - Base Composition



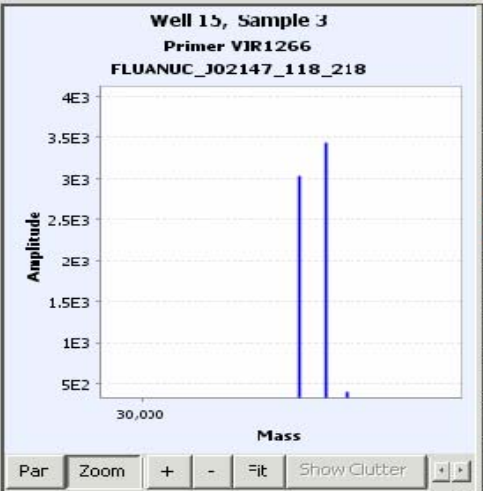
Influenza Virus Assay Results Viewer



Conclusion

Samples 1 & 3: Influenza A virus (H3N2: A/New York/../2003)

Samples 2 & 4: Influenza B virus



Organism	Sample Score	Basecount	Peak1 Mass	Peak1 Abundance	Peak1 Fit Error	Peak2 Mass	Pe Abun
Influenza A virus|A/NEW YORK/14/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/15/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/16/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/21/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/22/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/24/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/25/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/26/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/268/2003(H3N2)	0.96539	A26G20C23T32	31051.6 31364.1	3030.0 396.0	0.0 0.0	31225.7 31225.7	3440.0 3440.0
Influenza A virus|A/NEW YORK/27/2003(H3N2)	0.96539	A26G20C23T32	31051.6	3030.0	0.0	31225.7	3440.0

Validation Study Test Isolates

- **24 human influenza isolates**
  - 18 influenza A
  - 6 influenza B

- **63 avian influenza isolates**
  - 16 different avian species
    - Chicken, Duck, Goose, Egret, Teal,….
  - 28 distinct H/N types
    - *29 HIGHLY PATHOGENIC H5N1 isolates*
  - 8 worldwide geographical locations
    - North America, Africa, Asia

- **4 swine influenza isolates**

- **1 equine influenza isolate**

Human Influenza Trial - Blinded Samples

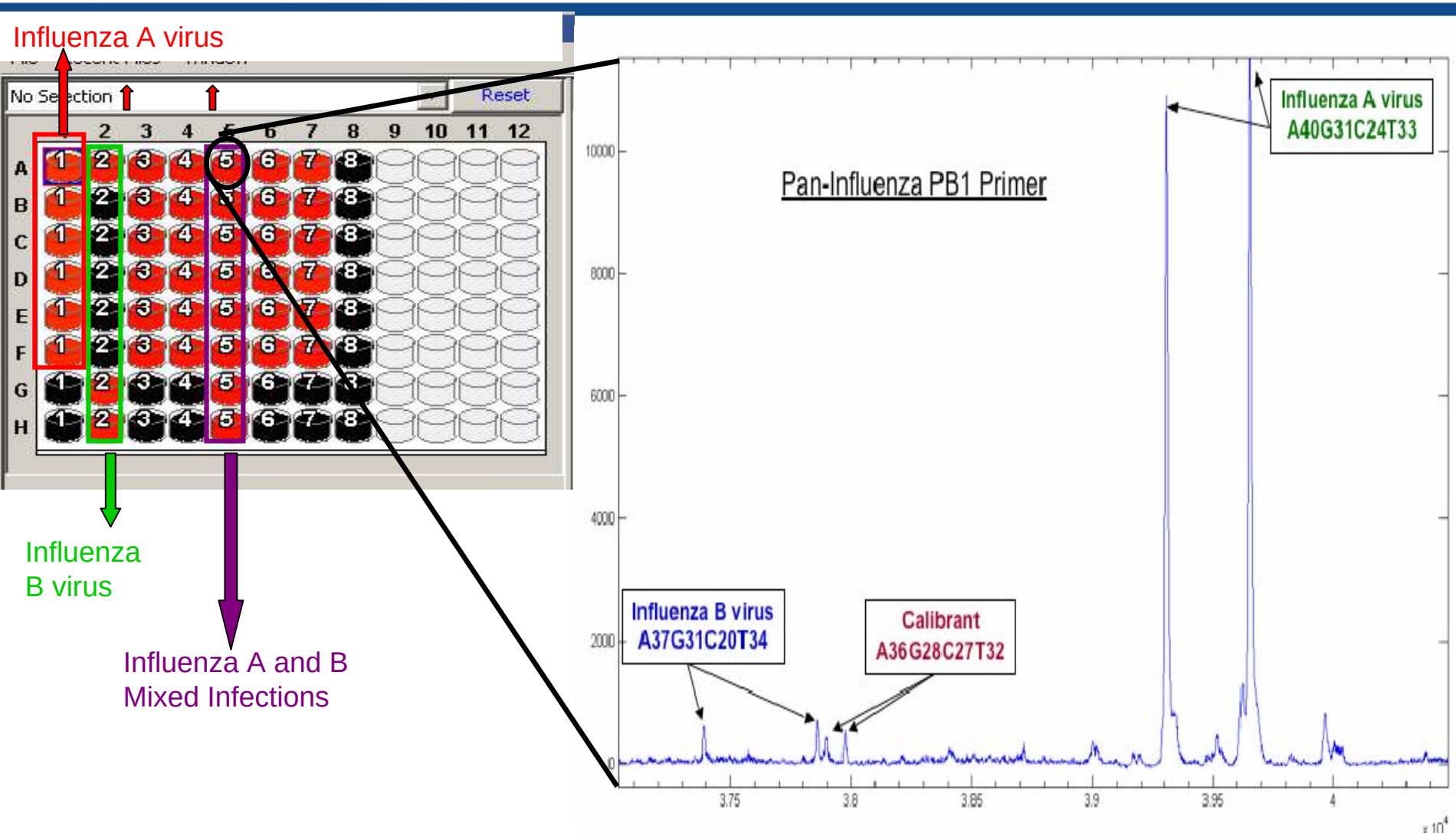
Source	Location	Collection Dates	Sample type	No. of Samples
Naval Health Research Center	MCRD, San Diego Ft. Leonard Wood, Ft. Sill, Ft. Benning, Lackland AFB	1999-2005	Throat swabs, nasal swabs, nasal washes	317
Johns Hopkins University Medical Center	Baltimore, MD	2003-2005	Nasal aspirates	229
NY State Dept. of Health	Throughout NY	1999-2005	Nasal aspirates, BAL, tracheal aspirates, throat swabs	100
TX State Dept. of Health	Throughout TX	2005-2006	Throat swabs, nasal washes	10
Total				656

Clinical Samples Summary

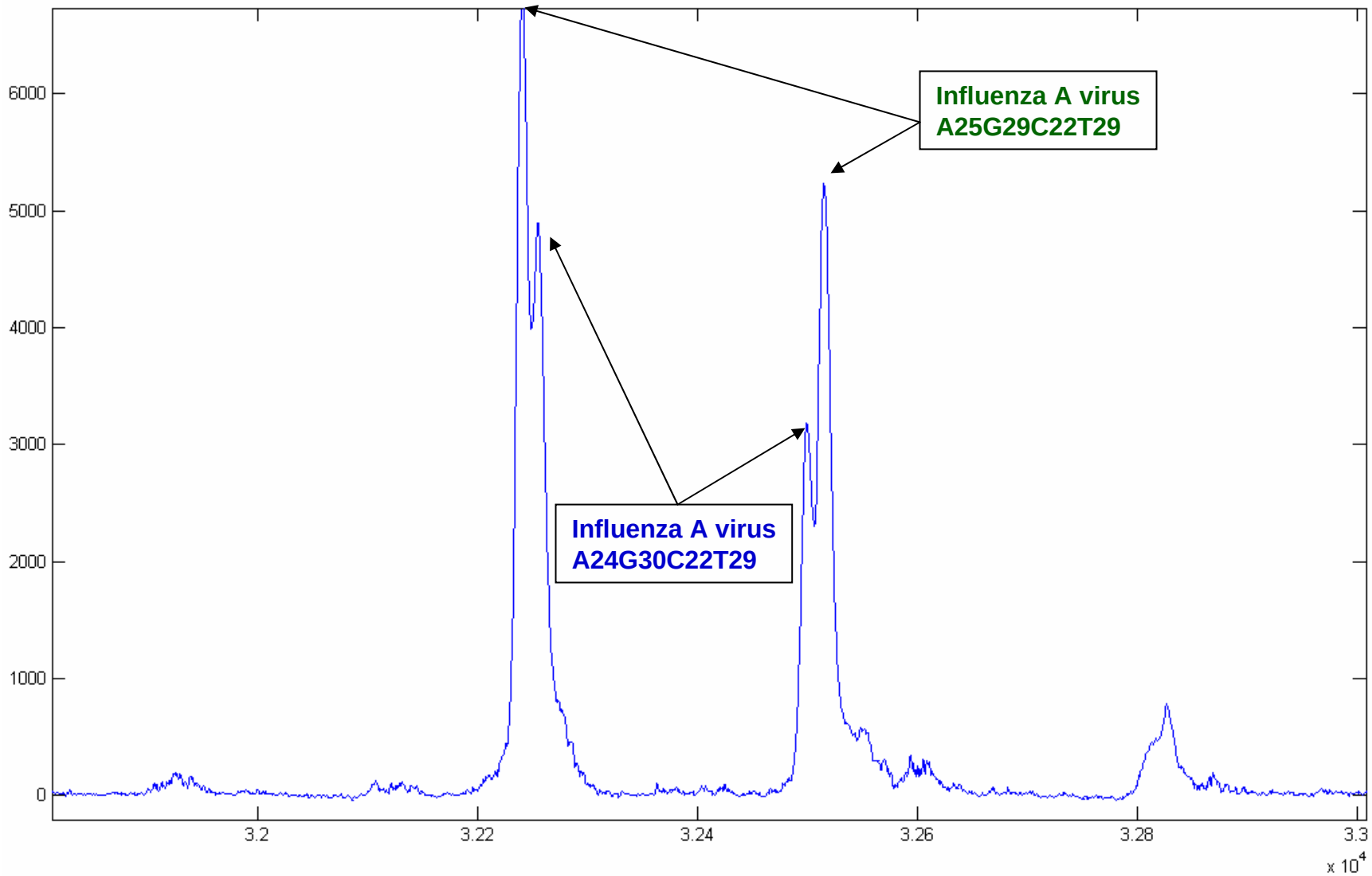
		Culture/Real Time PCR		
		Positive	**Negative**	**Total**
T5000	**Positive**	243	10	253
	Negative	8	395	403
	Total	251	405	656
		Sensitivity	*96.8*	
		Specificity	*97.5*	
		PPV	*96.0*	
		NPV	*98.0*	

Influenza A			**Influenza B**	**Total**
H3N2	H1N1	(H3/H1)N2		
149	34	3	67	253

Detection of Mixed Infections



Detection of Multiple Influenza A Contributors

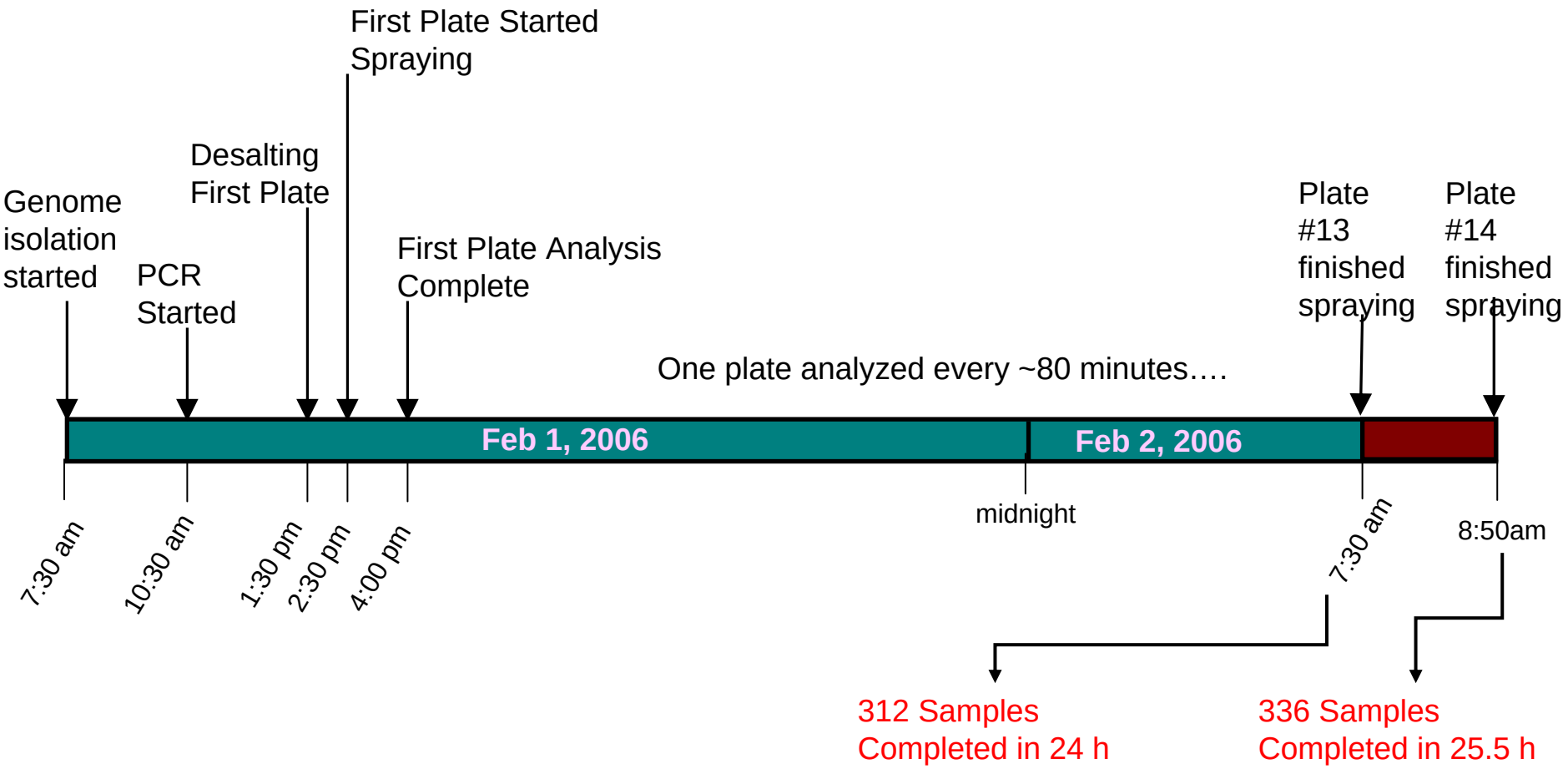


High Throughput 4 Primer Format

Primer Pair	Species	Segment
VIR2798	All Influenza	PB1
VIR1266	All Influenza A	Nuc
VIR1279	All Influenza A	M1
VIR1287	All Influenza A	PA
VIR2775	All Influenza A	NS1
VIR2777	All Influenza A	NS2
VIR1261	All Influenza B	PB2
VIR1275	All Influenza B	Nuc

Primer Pair	Species	Segment
VIR2798	Pan Influenza	PB1
VIR1266	Influenza A	Nuc
VIR2777	Influenza A	NS2
VIR1275	Influenza B	Nuc

Throughput Study: Time Line



Avian Influenza Virus Detection: University of Georgia Samples

ibis Biosciences

24 avian influenza virus isolates collected over a six-year period

- Different host species: mallard, seagull, teal,…
- Different combinations of H and N subtypes: H12N4, H3N8…

SCWDS ID#	Serotype	Species	Location	Date
AI00-1412	H6N1	REKN	Bower's Beach, DE	5/25/00
AI00-1794	H12N4	RUTU	Bower's Beach, DE	5/20/96
AI00-2150	H12N5	RUTU	Villas, NJ	5/15/96
AI00-629	H7N9	RUTU	Port Mahon, DE	5/19/96
AI02-262	H2N4	RUTU	Mispillion Harbor,DE	5/22/98
AI02-690	H2N9	RUTU	Reed's Beach, NJ	5/22/98
AI03-128	H9N7	RUTU	Reed's Beach, NJ	5/20/99
AI03-128	H9N7	RUTU	Reed's Beach, NJ	5/20/99
AI03-755	H9N5	RUTU	Mispillion Harbor,DE	5/20/99
AI04-127	H10N7	RUTU	Bower's Beach, DE	5/19/00
AI05-415	H3N8	RUTU	Fortescue Beach, NJ	5/21/01
AI05-415	H3N8	RUTU	Fortescue Beach, NJ	5/21/01
AI05-669	H11N8	RUTU	Reed's Beach, NJ	5/25/01
AI05-784	H11N6	RUTU	Reed's Beach, NJ	5/25/01
MN00-283	H5N2	MALL	Thief Lake, MN	9/10/96
MN00-382	H5N3	MALL	Thief Lake, MN	9/10/96
MN98-115	H4N8	MALL	Roseau Co., MN	09/ /1998
MN98-115	H4N8	MALL	Roseau Co., MN	09/ /1998
MN98-66	H6N5	MALL	Roseau Co., MN	09/ /1998
MN99-160	H4N6	MALL	Roseau Co., MN	/ /1999
MN99-17	H7N7	MALL	Roseau Co., MN	/ /1999
NC6412-009	H10N7	MALL	JM Futch, NC	12/20/00
NC675-075	H3N2	ABDU	Mattamuskeet, NC	12/21/00
TX01-32	H8N4	CITE	Brazoria Co., TX	2/11/97
TX01-7	H8N4	AGWT	Brazoria Co., TX	2/11/97
TX02-27	H1N4	BWTE	Brazoria Co., TX	2/18/98
TX02-75	H1N3	BWTE	Brazoria Co., TX	2/18/98

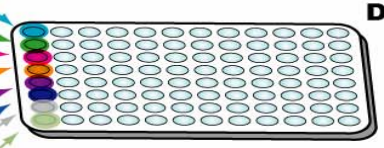
Avian Flu Detection: No Change in Assay or Primers

Isolated RNA

Pan Influenza Primer: PB1

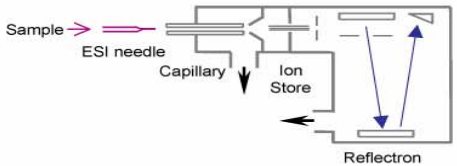
Influenza A Primers: Nuc, M1, PA, NS1, NS2

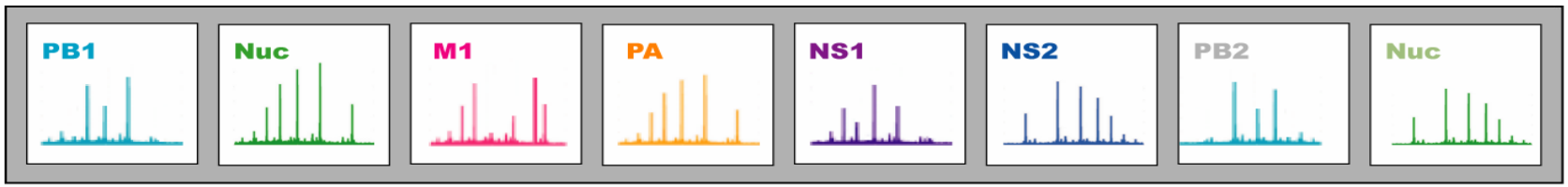
Influenza B Primers: PB2, Nuc



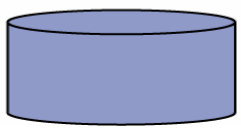
PCR Desalting

Mass Spectrometer





Influenza A	A39 G32 C23 T34	A32 G23 C20 T26	A24 G30 C23 T28	A37 G23 C27 T25	A28 G21 C32 T38	A35 G27 C17 T26		
Influenza B	A37 G31 C20 T34						A23 G27 C19 T22	A36 G17 C36 T11
Mixture	A39 G32 C23 T34	A32 G23 C20 T26	A24 G30 C23 T28	A37 G23 C27 T25	A28 G21 C32 T38	A35 G27 C17 T26	A23 G27 C19 T22	A36 G17 C36 T11



Influenza species, subtype, clade type determined by comparison with database

T5000 Discrimination of Diverse *Avian* Influenza Virus Isolates

*Same panel was used for human Influenza detection

Biosciences

Serotype	Species	Location	Date	2798	1266	1279	1287	2775	2777
H1N3	BWTE	Brazoria Co., TX	2/18/98	A37G33C24T34	A35G22C22T22	A23G30C25T27	A35G27C28T22	A40G30C20T29	A35G27C16T27
H9N7	RUTU	Reed's Beach, NJ	5/20/99	A37G33C25T33	A37G21C20T23	A22G30C26T27	A35G26C28T23	A37G32C22T28	A34G27C18T26
H3N8	RUTU	Fortescue Beach, NJ	5/21/01	A37G34C23T34	A34G23C23T21	A23G30C24T28	A34G27C28T23	A37G32C22T28	A35G27C16T27
H3N8	RUTU	Fortescue Beach, NJ	5/21/01	A37G34C23T34	A34G24C20T23	A23G30C24T28	A34G27C28T23	A37G32C22T28	A34G27C18T26
H3N2	ABDU	Mattamuskeet, NC	12/21/00	A37G34C23T34	A34G24C21T22	A21G32C24T28	A35G26C29T22	A37G32C22T28	A34G27C18T26
H11N8	RUTU	Reed's Beach, NJ	5/25/01	A38G33C22T35	A35G22C22T22	A22G31C25T27	A34G27C28T23	A40G30C20T29	A35G27C16T27
H11N6	RUTU	Reed's Beach, NJ	5/25/01	A38G33C22T35	A35G22C22T22	A23G30C24T28	A34G27C28T23	A40G30C20T29	A35G27C16T27
H9N5	RUTU	Mispillion Harbor,DE	5/20/99	A38G33C23T34	A37G21C20T23	A24G28C23T30	A35G26C28T23	A37G32C22T28	A34G27C18T26
H9N7	RUTU	Reed's Beach, NJ	5/20/99	A38G33C24T33	A34G24C21T22	A22G30C26T27	A35G26C28T23	A37G32C22T28	A34G27C18T26
H6N5	MALL	Roseau Co., MN	09/ /1998	A39G32C22T35	A36G21C22T22	A21G32C25T27	A34G27C28T23	A40G30C20T29	A35G27C16T27
H4N6	MALL	Roseau Co., MN	/ /1999	A39G32C23T34	A35G22C23T21	A22G31C27T25	A34G27C28T23	A37G32C22T28	A34G27C18T26
H5N2	MALL	Thief Lake, MN	9/10/96	A39G32C23T34	A36G21C22T22	A23G31C26T25	A35G26C27T24	A37G32C22T28	A34G27C18T26
H8N4	AGWT	Brazoria Co., TX	2/11/97	A39G32C23T34	A36G22C21T22	A24G28C25T28	A34G27C28T23	A37G32C22T28	A34G27C18T26
H7N7	MALL	Roseau Co., MN	/ /1999	A39G32C23T34	A36G22C22T21	A20G32C27T26	A34G27C29T22	A37G32C22T28	A34G27C18T26
H5N3	MALL	Thief Lake, MN	9/10/96	A39G32C24T33	A34G24C21T22	A21G32C25T27	A34G27C28T23	A37G32C22T28	A34G27C18T26
H2N4	RUTU	Mispillion Harbor,DE	5/22/98	A39G32C24T33	A35G22C22T22	A22G31C25T27	A35G26C29T22	A37G32C22T28	A34G27C18T26
H12N5	RUTU	Villas, NJ	5/15/96	A39G32C24T33	A36G21C22T22	A21G32C24T28	A35G26C29T22	A37G32C22T28	A34G27C18T26
H7N9	RUTU	Port Mahon, DE	5/19/96	A39G32C24T33	A36G21C22T22	A21G32C24T28	A34G27C29T22	A37G32C22T28	A34G27C18T26
H2N9	RUTU	Reed's Beach, NJ	5/22/98	A39G32C24T33	A36G21C22T22	A23G30C26T26	A35G26C29T22	A37G32C22T28	A34G27C18T26
H4N8	MALL	Roseau Co., MN	09/ /1998	A40G31C22T35	A29G24C19T29	A25G28C25T27	A34G27C28T23	A37G32C22T28	A34G27C18T26
H4N8	MALL	Roseau Co., MN	09/ /1998	A40G31C22T35	A34G24C21T22	A25G28C25T27	A34G27C28T23	A37G32C22T28	A34G27C18T26
H12N4	RUTU	Bower's Beach, DE	5/20/96	A40G31C23T34	A35G23C22T21	A23G30C26T26	A35G26C29T22	A37G32C21T29	A34G27C17T27
H10N7	RUTU	Bower's Beach, DE	5/19/00	A40G31C24T33	A33G25C21T22	A23G30C25T27	A35G27C27T23	A38G31C22T28	A34G27C18T26
H10N7	MALL	JM Futch, NC	12/20/00	A40G31C24T33	A35G22C21T23	A23G30C26T26	A34G27C29T22	A37G32C22T28	A34G27C18T26
H8N4	CITE	Brazoria Co., TX	2/11/97	A40G31C24T33	A35G22C22T22	A22G30C27T26	A34G27C29T22	A40G30C20T29	A35G27C16T27
H1N4	BWTE	Brazoria Co., TX	2/18/98	A40G31C24T33	A35G22C22T22	A24G28C25T28	A35G26C28T23	A40G30C20T29	A35G27C16T27
H6N1	REKN	Bower's Beach, DE	5/25/00	No Detection	No Detection	No Detection	No Detection	No Detection	No Detection

		Host	Serotype	PB1	NUC	M1	PA	NS1	NS2
Influenza A virus	Mammalian species	Human	H1N1	36 30 26 36	33 23 20 25	21 32 22 30	36 27 27 22	37 32 22 28	33 28 18 26
			H1N1	36 30 26 36	31 25 20 25	22 31 25 27	36 26 28 22	37 32 22 28	35 26 18 26
			H1N1	36 30 26 36	33 23 20 25	22 31 23 29	36 26 29 21	38 31 22 28	35 26 18 26
			H1N1	33 32 27 36	34 22 21 24	24 27 26 28	36 26 27 23	36 33 22 28	33 28 18 26
			H1N1	33 32 27 36	34 22 21 24	24 27 27 27	36 26 27 23	36 33 22 28	33 28 18 26
			H1N1	33 32 27 36	34 22 21 24	24 27 27 27	36 26 27 23	35 34 22 28	32 29 18 26
			H3N2	39 32 24 33	32 24 20 25	24 30 23 28	36 24 28 24	38 32 21 28	35 27 17 26
			H3N2	39 32 23 34	32 24 20 25	24 30 23 28	36 24 28 24	38 32 21 28	35 27 17 26
			H3N2	39 32 23 34	32 23 20 26	24 30 23 28	36 24 28 24	38 32 21 28	35 27 17 26
			H3N2	40 31 24 33	31 25 21 24	25 29 22 29	36 24 27 25	39 31 21 28	36 26 17 26
			H3N2	40 31 24 33	32 24 21 24	25 29 22 29	36 24 27 25	39 31 21 28	36 26 17 26
			H3N2	39 32 24 33	31 25 21 24	25 29 22 29	36 24 27 25	39 31 21 28	36 26 17 26
			H3N2	40 31 24 33	32 24 21 24	25 29 23 28	36 24 27 25	39 31 21 28	36 26 17 26
		Swine	H1N1	39 32 26 31	33 24 20 24	25 27 25 28	34 28 28 22	35 35 21 28	33 29 17 26
			New	39 32 25 32	35 22 21 23	22 28 26 29	35 27 27 23	35 36 21 27	33 30 17 25
		Equine	H7N7	39 32 23 34	38 20 21 22	25 27 21 32	35 27 27 23	40 29 21 29	35 26 17 27
	Avian Species	American Black Duck	H3N2	37 34 23 34	34 24 21 22	21 32 24 28	35 26 29 22	37 32 22 28	34 27 18 26
		American Green-winged Teal	H8N4	39 32 23 34	36 22 21 22	24 28 25 28	34 27 28 23	37 32 22 28	34 27 18 26
		Blue-winged Teal	H1N3	37 33 24 34	35 22 22 22	23 30 25 27	35 27 28 22	40 30 20 29	35 27 16 27
		Blue-winged Teal	H1N4	40 31 24 33	35 22 22 22	24 28 25 28	35 26 28 23	40 30 20 29	35 27 16 27
		CITE	H8N4	40 31 24 33	35 22 22 22	22 30 27 26	34 27 29 22	40 30 20 29	35 27 16 27
		Mallard	H10N7	40 31 24 33	35 22 21 23	23 30 26 26	34 27 29 22	37 32 22 28	34 27 18 26
		Mallard	H4N6	39 32 23 34	35 22 23 21	22 31 27 25	34 27 28 23	37 32 22 28	34 27 18 26
		Mallard	H4N8	40 31 22 35	34 24 21 22	25 28 25 27	34 27 28 23	37 32 22 28	34 27 18 26
		Mallard	H5N2	39 32 23 34	36 21 22 22	23 31 26 25	35 26 27 24	37 32 22 28	34 27 18 26
		Mallard	H5N3	39 32 24 33	34 24 21 22	21 32 25 27	34 27 28 23	37 32 22 28	34 27 18 26
		Mallard	H6N5	39 32 22 35	36 21 22 22	21 32 25 27	34 27 28 23	40 30 20 29	35 27 16 27
		Mallard	H7N7	39 32 23 34	36 22 22 21	20 32 27 26	34 27 29 22	37 32 22 28	34 27 18 26
		Ruddy Turnstone	H10N7	40 31 24 33	33 25 21 22	23 30 25 27	35 27 27 23	38 31 22 28	34 27 18 26
		Ruddy Turnstone	H11N6	38 33 22 35	35 22 22 22	23 30 24 28	34 27 28 23	40 30 20 29	35 27 16 27
		Ruddy Turnstone	H12N4	40 31 23 34	35 23 22 21	23 30 26 26	35 26 29 22	37 32 21 29	34 27 17 27
		Ruddy Turnstone	H12N5	39 32 24 33	36 21 22 22	21 32 24 28	35 26 29 22	37 32 22 28	34 27 18 26
		Ruddy Turnstone	H2N4	39 32 24 33	35 22 22 22	23 30 24 28	35 26 29 22	37 32 22 28	34 27 18 26
		Ruddy Turnstone	H2N9	39 32 24 33	36 21 22 22	24 29 25 27	35 26 29 22	37 32 22 28	34 27 18 26
		Ruddy Turnstone	H3N8	37 34 23 34	34 24 20 23	23 30 24 28	34 27 28 23	37 32 22 28	34 27 18 26
		Ruddy Turnstone	H7N9	39 32 24 33	36 21 22 22	21 32 24 28	34 27 29 22	37 32 22 28	34 27 18 26
		Ruddy Turnstone	H9N5	38 33 23 34	37 21 20 23	24 28 23 30	35 26 28 23	37 32 22 28	34 27 18 26
		Ruddy Turnstone	H9N7	38 33 24 33	37 21 20 23	22 30 26 27	35 26 28 23	37 32 22 28	34 27 18 26
		Teal	H5N1	37 32 25 34	34 23 21 23	23 29 24 29	35 25 29 23	37 32 21 29	34 27 18 26
		Chicken	H5N1	37 32 25 34	34 23 21 23	23 29 24 29	35 25 29 23	38 31 21 29	35 27 16 27
		Chicken	H5N1	37 32 25 34	34 23 21 23	23 29 24 29	35 25 29 23	37 32 21 29	34 28 16 27
		HUMAN	H5N1	37 32 26 33	34 23 21 23	23 29 24 29	35 25 29 23	37 32 21 29	34 28 16 27
		Goose/Chicken/Duck/HUMAN	H5N1	37 32 26 33	34 23 21 23	23 29 24 29	35 25 29 23	38 31 21 29	35 27 16 27
		Egret/Chicken/Duck/HUMAN	H5N1	37 32 26 33	34 23 21 23	23 29 25 28	36 24 29 23	37 32 21 29	34 28 16 27
		Pintail	H5N3	38 32 26 32	34 24 23 20	23 30 25 27	34 27 29 22	40 30 20 29	35 27 16 27
		Shoveler	NEW	37 33 24 34	36 23 19 23	24 29 22 30	33 27 30 22	43 30 20 26	34 28 16 27
		Shoveler	H10	39 31 26 32	34 24 22 21	23 30 25 27	34 27 29 22	37 32 22 28	34 27 18 26
		Shoveler	H10	38 32 26 32	35 23 22 21	24 30 26 25	35 26 29 22	37 32 22 28	34 27 18 26
		Teal	H10	38 32 26 32	34 24 22 21	24 29 25 27	34 27 29 22	37 32 22 28	34 27 18 26
		Teal	H11	37 33 26 32	34 24 23 20	24 29 25 27	34 27 29 22	37 32 22 28	34 27 18 26
		Teal	H5N2	38 32 26 32	34 24 23 20	24 29 25 27	34 27 29 22	40 30 20 29	35 27 16 27
		Teal	H9	39 31 26 32	33 25 21 22	24 30 25 26	34 27 29 22	37 32 21 29	34 27 17 27
		Goose	H7	38 32 26 32	34 24 22 21	23 30 25 27	34 27 28 23	39 31 21 28	35 27 17 26
		Shoveler	H9N2	37 32 26 33	34 24 22 21	23 30 25 27	33 28 29 22	37 32 22 28	34 27 18 26

Conclusions

- Base compositions derived from broad range primers can be used to triangulate to microbial identification
- "Drill-Down" primers can be used to rapidly genotype strain variants and/or determine drug resistance
- Platform enables broad range Influenza surveillance
  - Broad range human and avian in same assay
- Demonstrated for bacteria and viruses without culture
  - Also applicable to fungi, protozoa, and humans

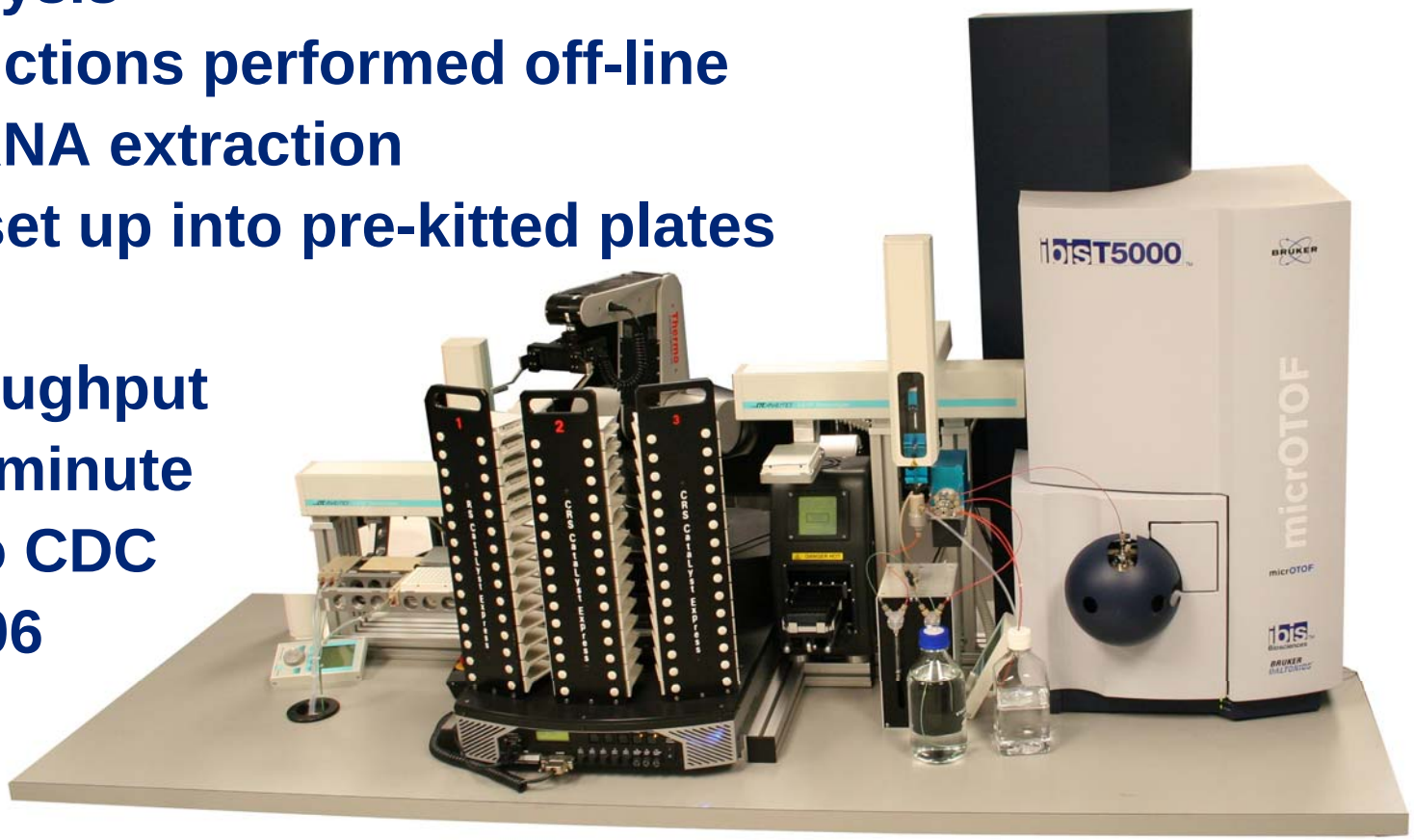
Instead of asking; "Is pathogen X in my sample?", we ask: "Which pathogen(s) are in my sample.

Deployments

Site	***Purpose***
USAMRIID	**Bioweapons Detection (Deployed)**
Dept. Homeland Security	**National Bioforensic Analysis Center (Deployed)**
Naval Health Research Center	**Respiratory Disease Surveillance in Operational Forces (Deployed)**
CDC	**Staph. & Strep genotyping (Sept 06)**
(Johns Hopkins)	**Hospital infection control ('07)**

Ibis T5000

- Amplicon purification
- Automated ESI-TOF analysis
  - Robotic arm moves plates for unattended operation
- Data analysis
- Other functions performed off-line
  - DNA/RNA extraction
  - Plate set up into pre-kitted plates
  - PCR
- High throughput
  - 1 well/minute
- Deploy to CDC
  - Sept '06



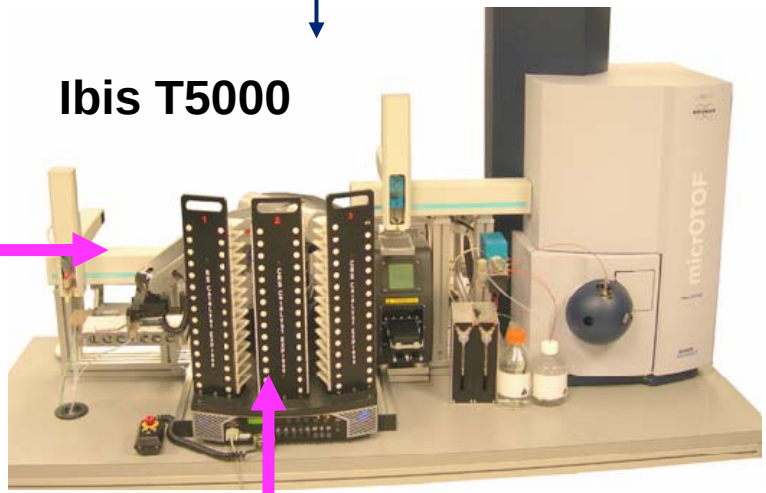
T5000 System and Consumables

Customer buys from third party

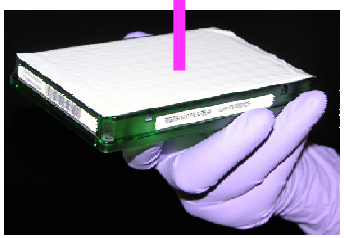
Bruker will manufacture in 2007

PCR Thermocycler

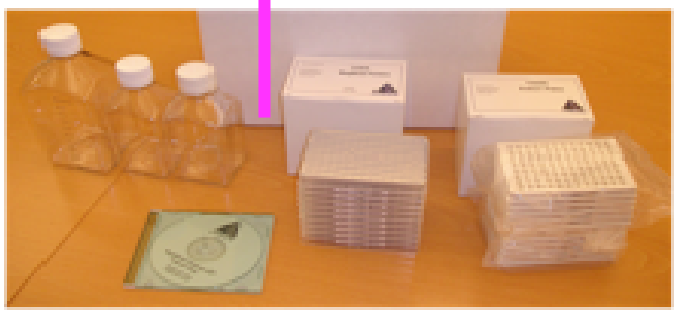
Ibis T5000



Software



Assay Plate



Clean up Reagents

Ibis makes

Ibis Research Team

MS & Automation
Jared Drader
Len Cummins
Jose Gutierrez
Abel Gutierrez
Yun Jiang
Kristin Lowery
Sheri Manalili
Amy Schink
James Hannis

Computational Genomics
Dave Ecker
Ranga Sampath
Tom Hall
Christian Massire
Harold Levene
Feng Li
Vanessa Harpin

Biology
Larry Blyn
Mark Eshoo
Cristina Ivy
D. J. Walcott
Joe Ecker
Kristin Lowery
Rachael Melton
Ray Ranken
Brian Libby
Thuy Pennella
David Duncan
Nancy Fan

Software/Database
Michael Pear
Neill White
Thomas Morton

SAIC
Dino Sofianos
Dave Robbins
Duane Knize
Terry Leighton
Karl Rudnick
Karen Studarus

Contractors
Fred Hajjar
Robert Palmer

Backup Material

Steps Required Before T5000 Analysis

Ibis Biosciences

Sample Collection

E.g. swab, swipe, blood, sputum

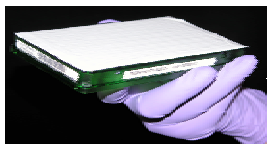
Sample Preparation

Sample lysis DNA/RNA purification

Example: KingFisher or Qiagen Robot

Sample and Enzyme added to Ibis assay plate

Ibis plate



Enzyme

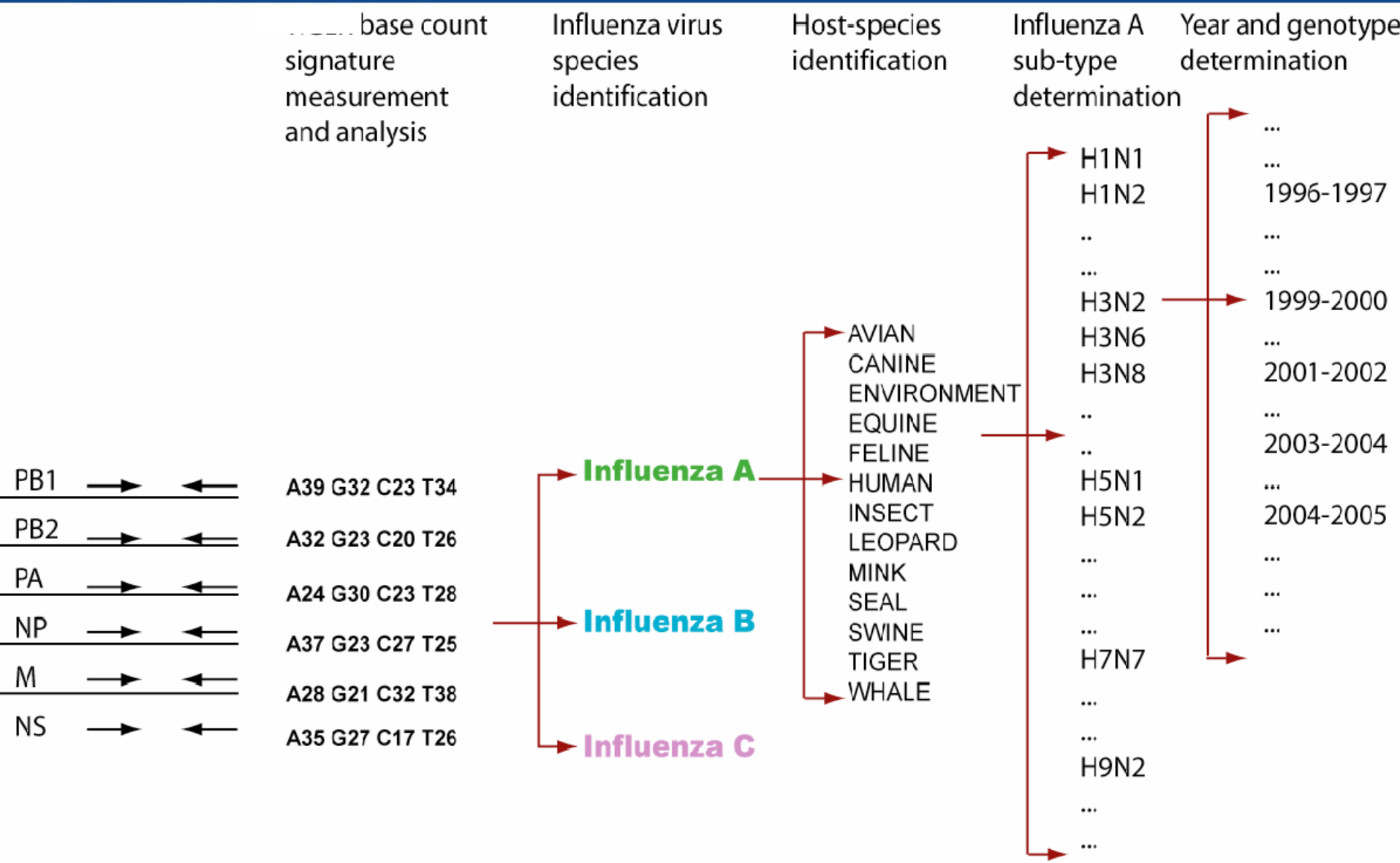
Example: PE Janus robot or hand pipetting

PCR

Example: Eppendorf

Ibis provides protocols and qualifies enzymes/equipment for use with T5000

Characterization of Influenza Virus



Study Results: Influenza A Virus Base Composition Signatures…

Closest Match To Database Strain	PB1	Nuc	M1	PA	NS1	NS2
A/NEW YORK/26/2003(H3N2)	A39G32C23T34	A32G23C20T26	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/26/2003(H3N2)	A39G32C23T34	A32G23C20T26	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/26/2003(H3N2)	A39G32C23T34	A32G23C20T26	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/26/2003(H3N2)	A39G32C23T34	A32G23C20T26	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
Variant of A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C27T25	A38G32C21T28	A35G27C17T26
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/317/1999(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26
A/NEW YORK/153/1999(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C23T28	A36G24C28T24	A39G31C21T28	A36G26C17T26
A/NEW YORK/153/1999(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C23T28	A36G24C28T24	A39G31C21T28	A36G26C17T26
VARIANT OF A/CANTERBURY/129/2005(H3N2) strains	A40G31C24T33	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
VARIANT OF A/CANTERBURY/220/2005	A38G32C24T34	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
A/CANTERBURY/129/2005(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
A/CANTERBURY/129/2005(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
A/CANTERBURY/129/2005(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
VARIANT OF A/CANTERBURY/127/2005(H3N2)	A38G33C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
VARIANT OF A/CANTERBURY/127/2005(H3N2)	A39G32C23T34	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
A/CANTERBURY/127/2005(H3N2)	A39G32C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
A/CANTERBURY/127/2005(H3N2)	A39G32C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
A/CANTERBURY/127/2005(H3N2)	A39G32C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
A/CANTERBURY/127/2005(H3N2)	A39G32C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
A/NEW YORK/373/2005(H3N2)	A39G32C24T33	A31G25C21T24	A24G30C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
A/NEW YORK/373/2005(H3N2)	A39G32C24T33	A31G25C21T24	A24G30C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
A/CANTERBURY/101/2004(H3N2)	A40G31C24T33	A32G24C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
VARIANT OF A/NEW YORK/387/2004(H3N2)	A40G31C24T33	A32G24C21T24	A25G29C23T28	A36G24C27T25	A39G31C21T28	A36G26C17T26
A/NEW YORK/244/2004(H3N2)	A40G31C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26
VARIANT OF A/NEW YORK/387/2004(H3N2)	A40G31C24T33	A32G24C21T24	A25G29C23T28	A36G24C27T25	A39G31C21T28	A36G26C17T26

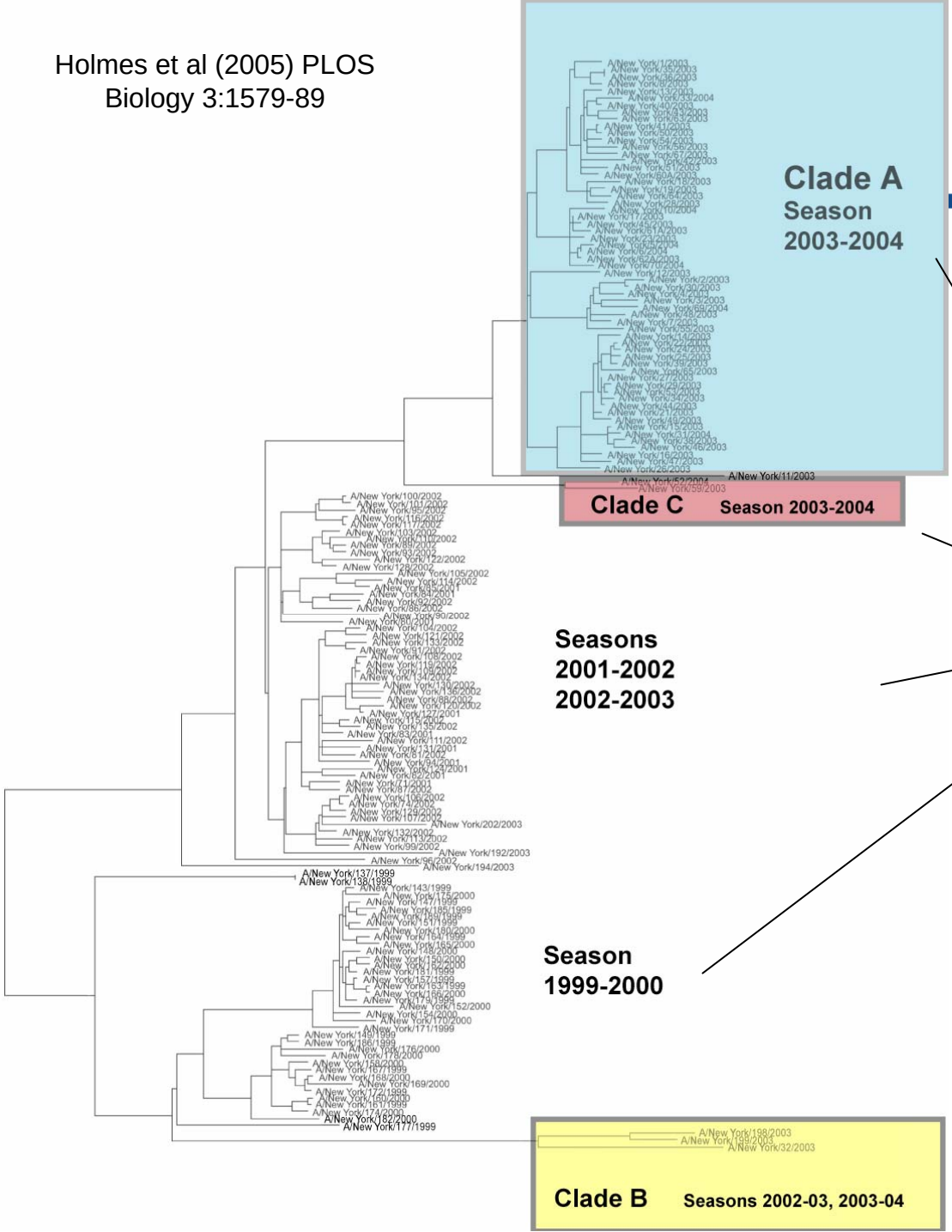
...Correlations to Circulating Clades

Closest Match To Database Strain	PB1	Nuc	M1	PA	NS1	NS2	Clade Designation
A/NEW YORK/26/2003(H3N2)	A39G32C23T34	A32G23C20T26	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_1
A/NEW YORK/26/2003(H3N2)	A39G32C23T34	A32G23C20T26	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_1
A/NEW YORK/26/2003(H3N2)	A39G32C23T34	A32G23C20T26	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_1
A/NEW YORK/26/2003(H3N2)	A39G32C23T34	A32G23C20T26	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_1
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_5
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_5
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_5
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_5
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_5
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_5
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_5
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_5
A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Clade A_5
Variant of A/NEW YORK/50/2003(H3N2)	A39G32C23T34	A32G24C20T25	A24G30C23T28	A36G24C27T25	A38G32C21T28	A35G27C17T26	Clade A_5_SNP_PA
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Seasons 2001-03
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Seasons 2001-03
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Seasons 2001-03
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Seasons 2001-03
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Seasons 2001-03
A/NEW YORK/95/2002(H3N2)	A39G32C24T33	A32G24C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	Seasons 2001-03
A/NEW YORK/317/1999(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C23T28	A36G24C28T24	A38G32C21T28	A35G27C17T26	1998-2000 worldwide collection
A/NEW YORK/153/1999(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C23T28	A36G24C28T24	A39G31C21T28	A36G26C17T26	1999-2000 Season
A/NEW YORK/153/1999(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C23T28	A36G24C28T24	A39G31C21T28	A36G26C17T26	1999-2000 Season
VARIANT OF A/CANTERBURY/129/2005(H3N2) strains	A40G31C24T33	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_PB1_SNP_2005
VARIANT OF A/CANTERBURY/220/2005	A38G32C24T34	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_PB1_SNP_2005
A/CANTERBURY/129/2005(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_SNP_2005
A/CANTERBURY/129/2005(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_SNP_2005
A/CANTERBURY/129/2005(H3N2)	A39G32C24T33	A31G25C20T25	A24G30C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_SNP_2005
VARIANT OF A/CANTERBURY/127/2005(H3N2)	A38G33C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_M1_PB1_SNP_2005
VARIANT OF A/CANTERBURY/127/2005(H3N2)	A39G32C23T34	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_M1_PB1_SNP_2005
A/CANTERBURY/127/2005(H3N2)	A39G32C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_M1_SNP_2005
A/CANTERBURY/127/2005(H3N2)	A39G32C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_M1_SNP_2005
A/CANTERBURY/127/2005(H3N2)	A39G32C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_M1_SNP_2005
A/CANTERBURY/127/2005(H3N2)	A39G32C24T33	A31G25C20T25	A25G29C22T29	A37G24C27T24	A39G31C21T28	A36G26C17T26	Clade B_PA_M1_SNP_2005
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	Clade B NP_M1_SNP
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	Clade B NP_M1_SNP
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	Clade B NP_M1_SNP
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	Clade B NP_M1_SNP
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	Clade B NP_M1_SNP
A/NEW YORK/386/2004(H3N2)	A39G32C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	Clade B NP_M1_SNP
A/NEW YORK/373/2005(H3N2)	A39G32C24T33	A31G25C21T24	A24G30C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	Clade B NP_SNP
A/NEW YORK/373/2005(H3N2)	A39G32C24T33	A31G25C21T24	A24G30C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	Clade B NP_SNP
A/CANTERBURY/101/2004(H3N2)	A40G31C24T33	A32G24C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	2004-05 worldwide
VARIANT OF A/NEW YORK/387/2004(H3N2)	A40G31C24T33	A32G24C21T24	A25G29C23T28	A36G24C27T25	A39G31C21T28	A36G26C17T26	2004-05 worldwide
A/NEW YORK/244/2004(H3N2)	A40G31C24T33	A31G25C21T24	A25G29C22T29	A36G24C27T25	A39G31C21T28	A36G26C17T26	2004-05 worldwide
VARIANT OF A/NEW YORK/387/2004(H3N2)	A40G31C24T33	A32G24C21T24	A25G29C23T28	A36G24C27T25	A39G31C21T28	A36G26C17T26	2004-05 worldwide

Holmes et al (2005) PLOS Biology 3:1579-89

Evolutionary relationship of H3N2 samples 1999-2004:

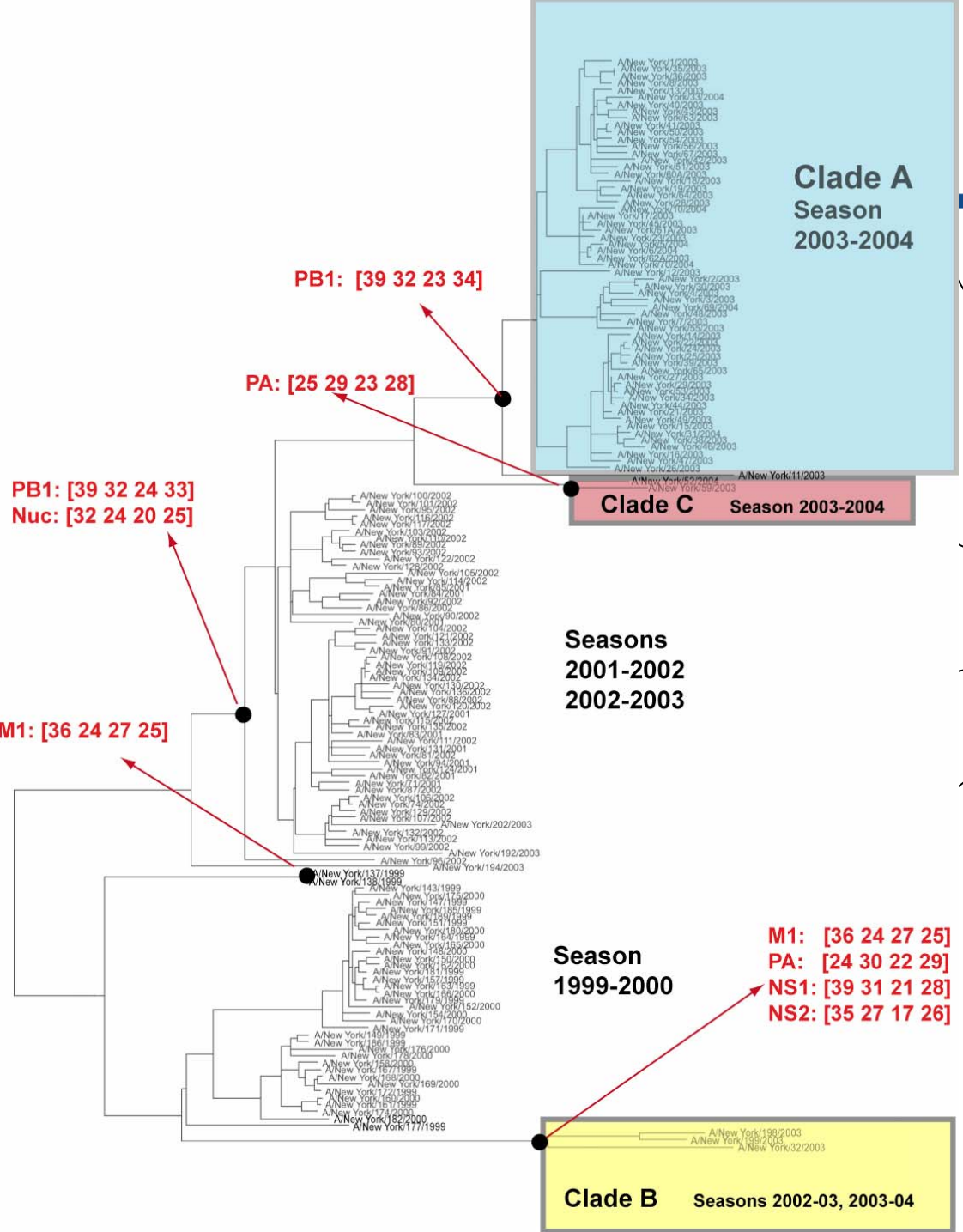
Whole-genome Analysis



Cladisitic distribution of circulating H3N2 isolates

Evolutionary relationship of H3N2 samples 1999-2004:

6-primer Base-composition Analysis



T5000 signatures distinguish H3N2 clades