

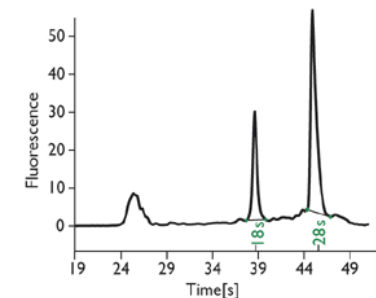
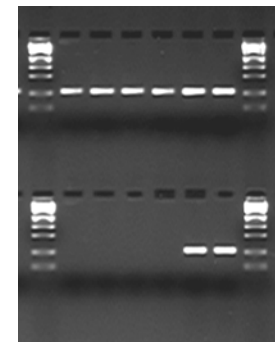
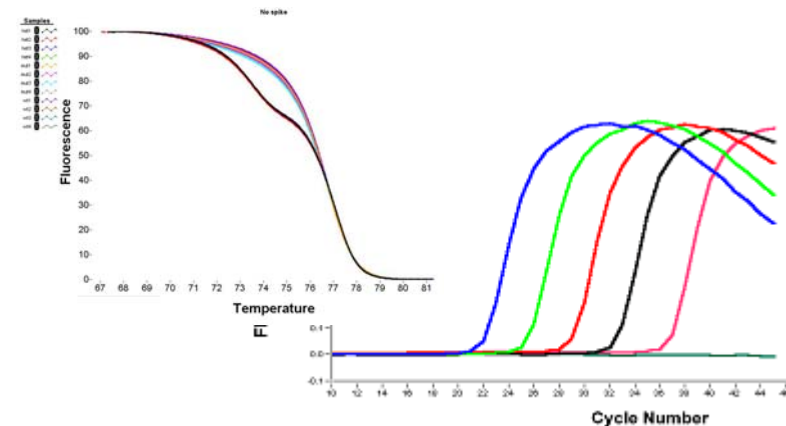
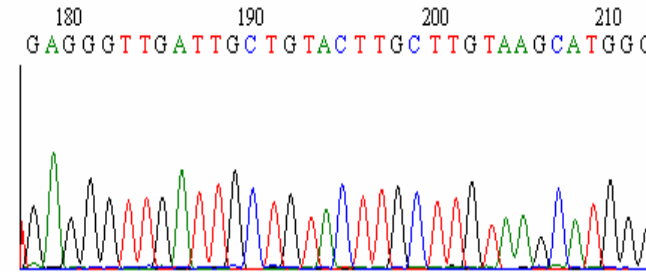
High Throughput Mass Spectrometry- Derived Base Compositions for the Detection and Strain Typing of Bacterial and Viral Pathogens

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Pharmaceuticals
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- **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 baumannii*
 - *Staphylococcus aureus*
 - **Viral**
 - Pan-Influenza detection and strain typing
- **Ibis T5000 System Components**

Interrogation of Amplified Nucleic Acids

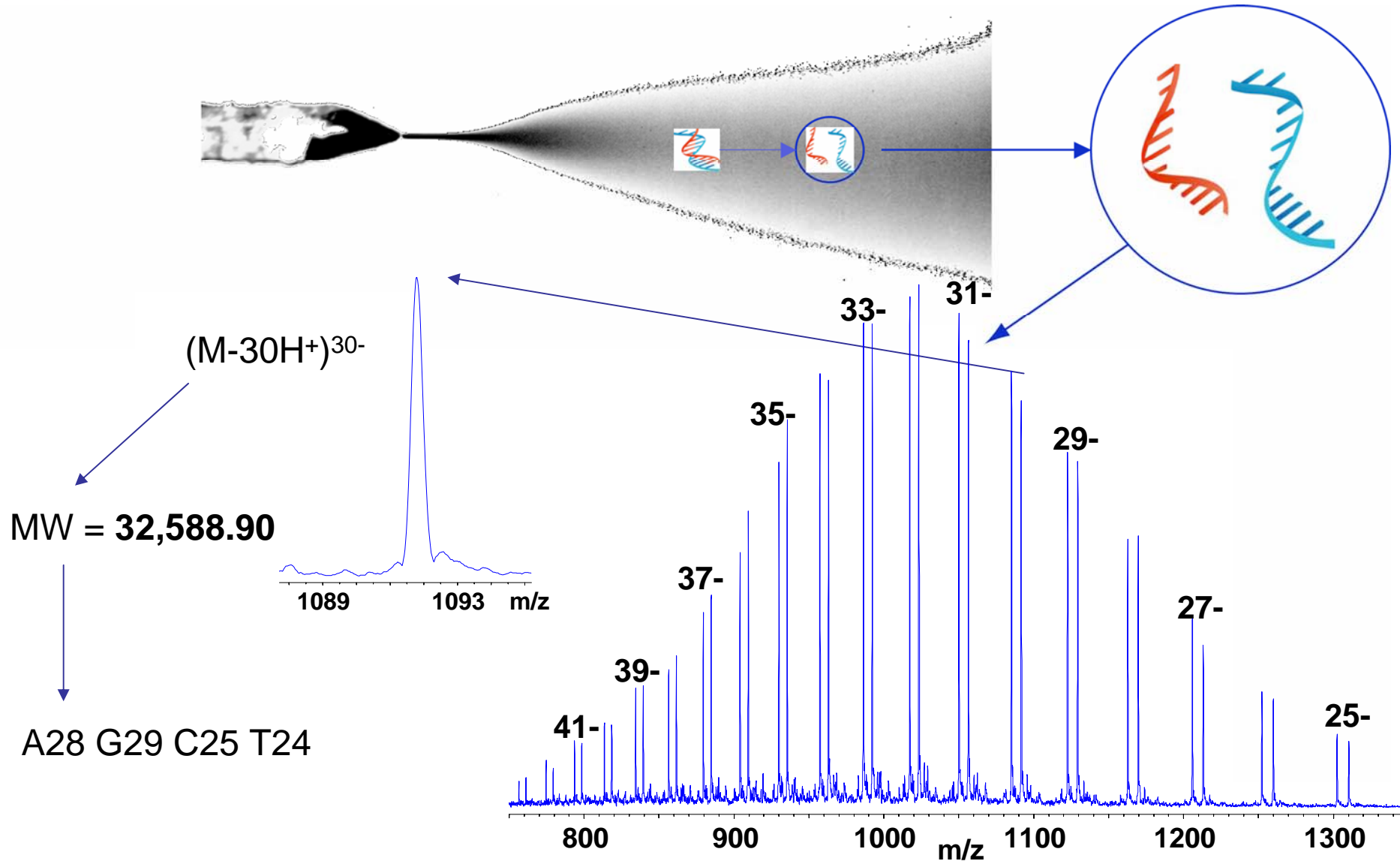
- Sequencing
 - “Gold Standard”
- Fluorescent intercalating dye
- Hybridization
 - Specific probe with FRET pair
 - DNA microarray
- Melting profiles
- Electrophoresis
 - Slab gels
 - Capillary gel electrophoresis
- WHAT ABOUT MASS?



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

(\pm 25 ppm or 0.75 Da): 928 base comps

(\pm 1 ppm or 0.03 Da): 82 base comps

Single Strand: 33071.462 Da

(\pm 25 ppm or 0.75 Da): 948 base comps

(\pm 1 ppm or 0.03 Da): 95 base comps

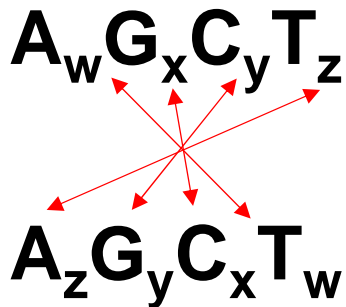


Da: Dalton (atomic mass unit)

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



DNA Single Strand | RNA Single Strand | PNA Single Strand | DNA Double Strand

Sense MW: 32889.4500 Error (ppm): 25.00
 Anti-sense MW: 33071.4600 Error (ppm): 25.00

Constraints

Forward primer composition Reverse primer composition

A: 0 G: 0 A: 0 G: 0
 C: 0 T: 0 C: 0 T: 0

Result count: 2 Monoisotopic **Calculate**

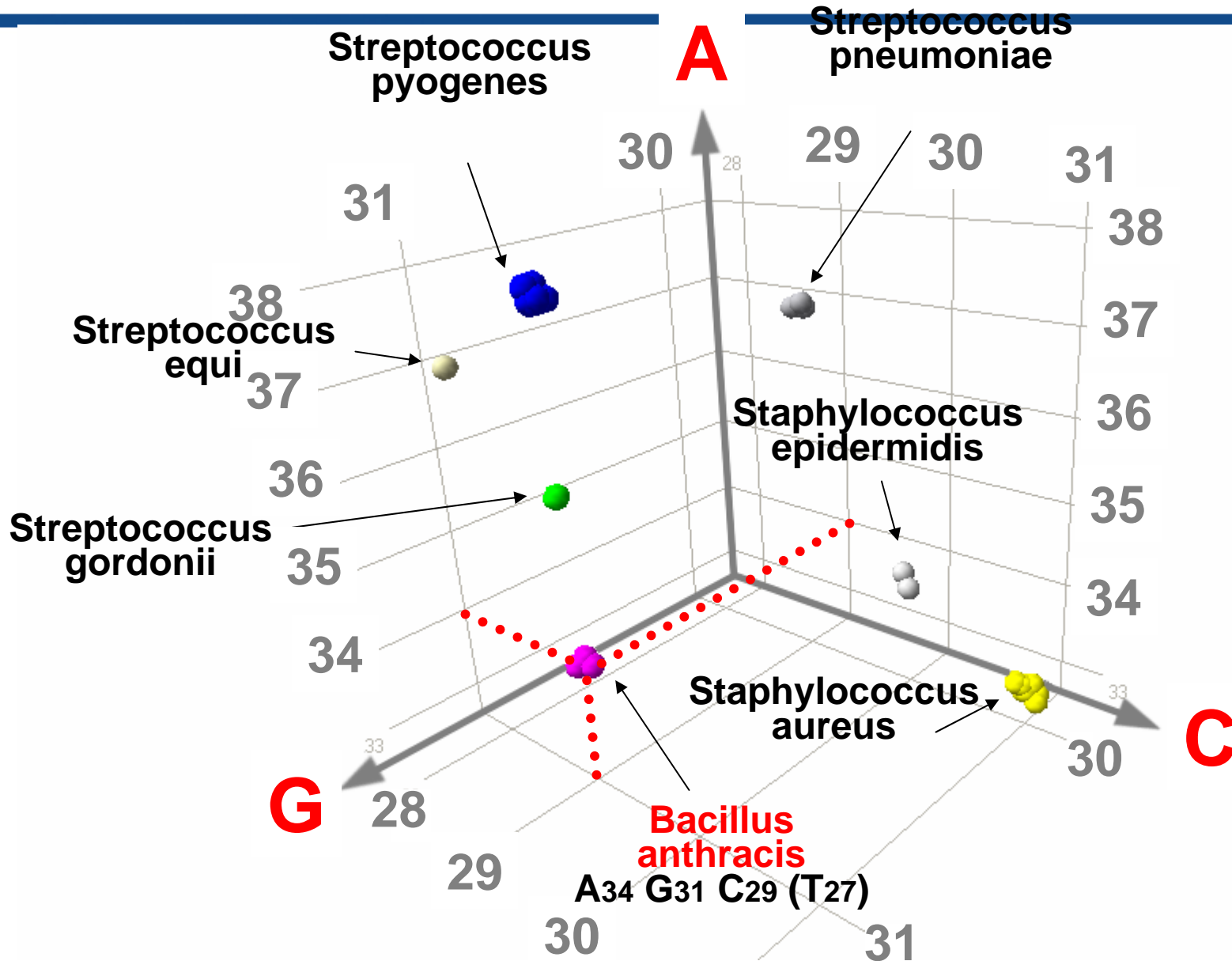
Display from: 1 to: 2 **Prev** **Next** **Save**

Count	A	G	C	T	Length	Mass	Error
1	27	25	30	25	107	32889.4546	0.14
2	25	30	25	27	107	33071.4622	0.07

Odd numbers: sense strand. Even: complementary anti-sense strand.

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***

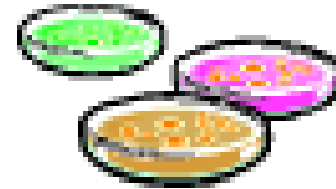
The Bioweapons Arena:

- **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*

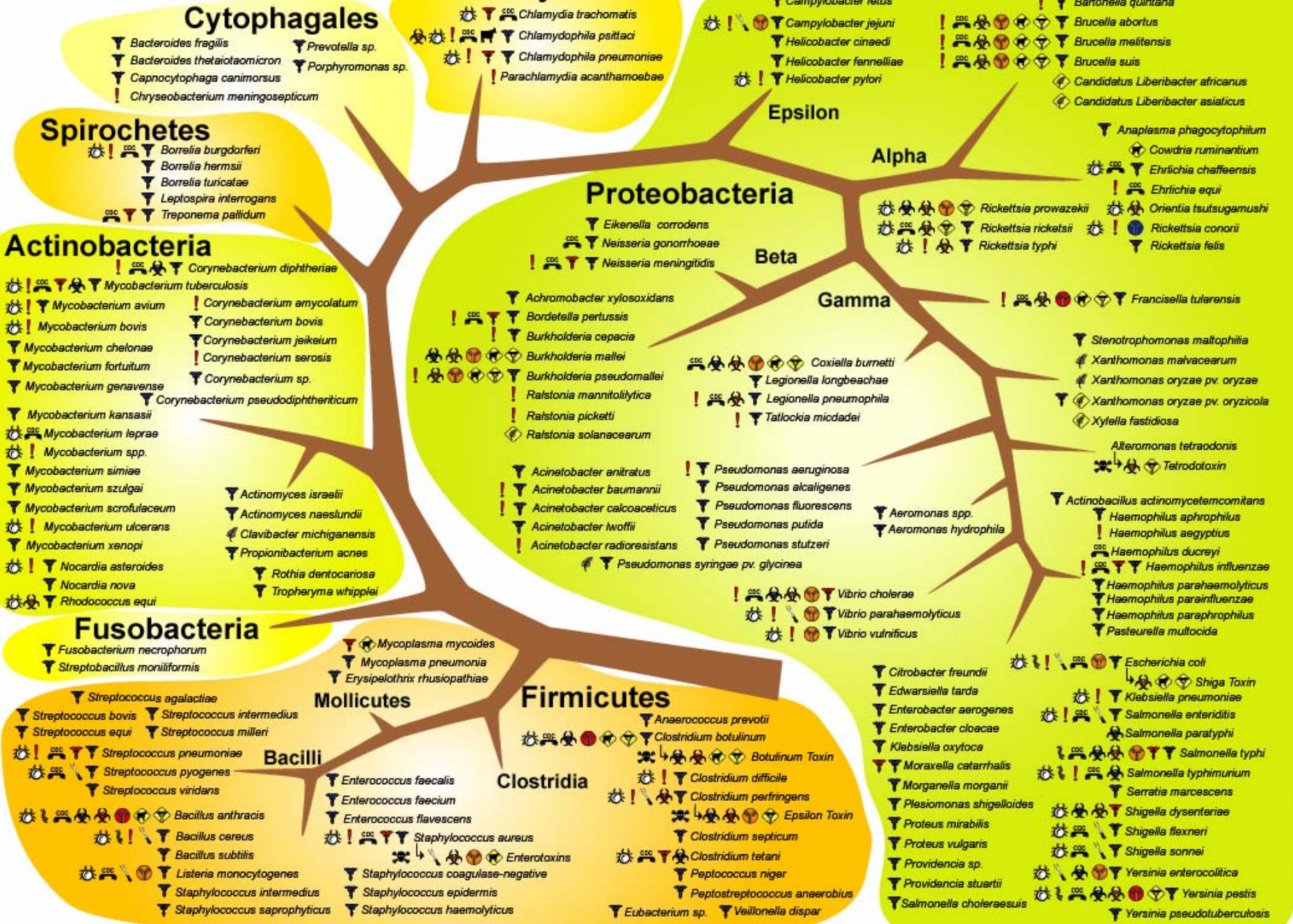
 *Toxin*

 *CDC Notifiable Agent*

 *Principal Foodborne Pathogen*

 *Emerging Infectious Agent*

Bacteria



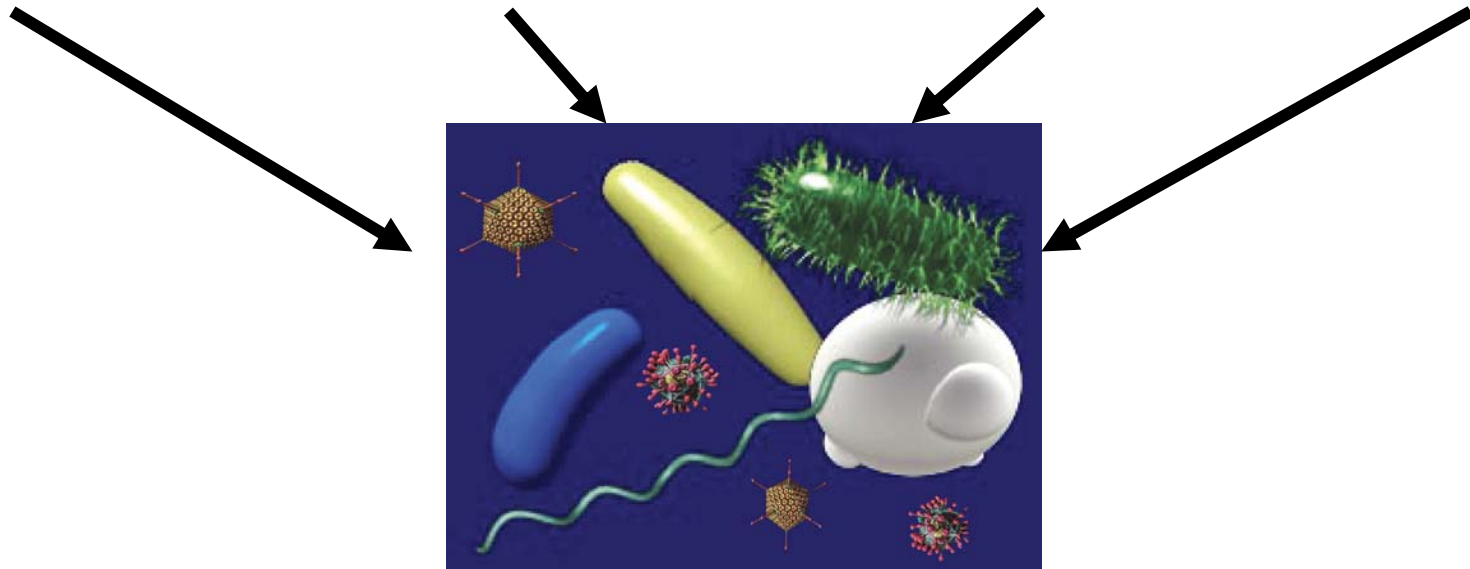
Concept of Operations

Animal Reservoirs
of Infectious
Agents

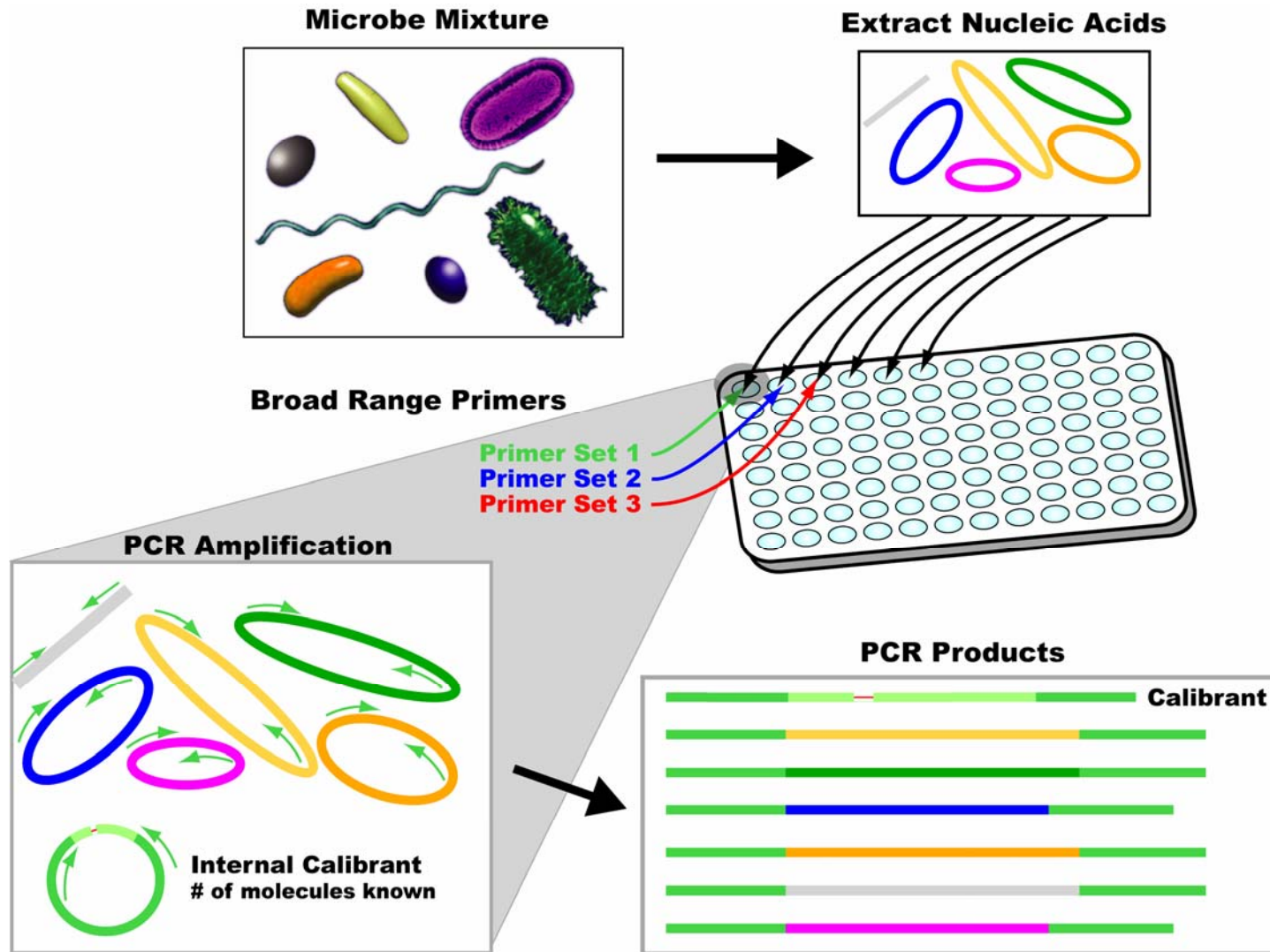
Environmental
Surveillance of
Public Places

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

Primer pair

GGATTAGATACCCCTGGTACTCC

CGCCCTGGGGAGTACGGCC

E. coli

ACGCCGTAAACGATGTCGACTTGGAGGTTGTGCC-CTTGA-GGCGTGGCTTCCGGAGCTAACCGGTTAAGTCGAC

Cox. burnetii

C

ACGCCGTCAACGATGAGAACTAGCTGTTGGGAAG--TTCA-CTTCTTAGTAGCGAAGCTAACCGGTTAAGTTCTC

Leg. pneumophila

ACGCTGTAACGATGTCAACTAGCTGTTGGTTAT-ATGAAAAAATATTAGTGGCGCAGCAAACCGGATAAGTTGAC

Ricket. prowazekii

ACGCCGTAAACGATGAGTGCTAGATATCGGAGG--ATTCT--CTTTCGGTTTCGCAGCTAACGCATTAAGCACTC

Mycb. tuberculosis

ACGCCGTAAACGGTGGGTACTAGGTGTTGGGTTTCCTTCCTTGGGATCCGTGCCGTAGCTAACGCATTAAGTACCC

Trep. pallidum

ACACAGTAAACGATGTACACTAGGTGTTGGGGC---ATGA--GTCCTCGGCGCCGACCGAACGCATTAAGTGAC

Bacillus anthracis

ACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGG-TTTCGCCCTTATGCTGAAGTTAACGCATTAAGCACTC

Staph. aureus

ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGG-TTTCGCCCTTATGCTGCAGCTAACGCATTAAGCACTC

Staph. epidermidis

ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGG-TTTCGCCCTTATGCTGCAGCTAACGCATTAAGCACTC

Strep. agalactiae

ACGCCGTAAACGATGAGTGCTAGGTGTTAGGCC-TTTCGGGGCTTATGCTGCCGAGCTAACGCATTAAGCACTC

Strep. mutans

ACGCCGTAAACGATGAGTGCTAGGTGTTAGGCC-TTTCGGGGCTTATGCTGCCGAGCTAACGCATTAAGCACTC

Strep. pneumoniae

ACGCTGTAACGATGAGTGCTAGGTGTTAGACC-TTTCGGGGTTATGCTGCCGTAGCTAACGCATTAAGCACTC

Strep. pyogenes

ACGCCGTAAACGATGAGTGCTAGGTGTTAGGCC-TTTCGGGGCTTATGCTGCCGAGCTAACGCATTAAGCACTC

Region varies in different

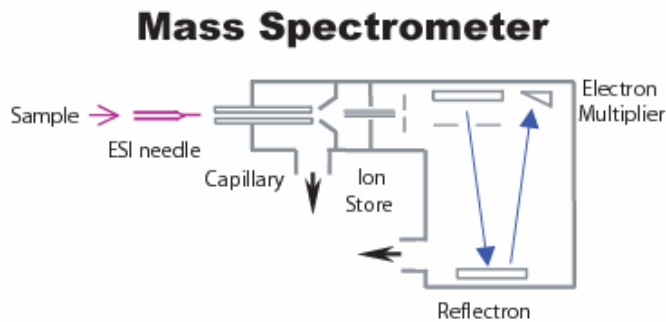
kinds of bacteria



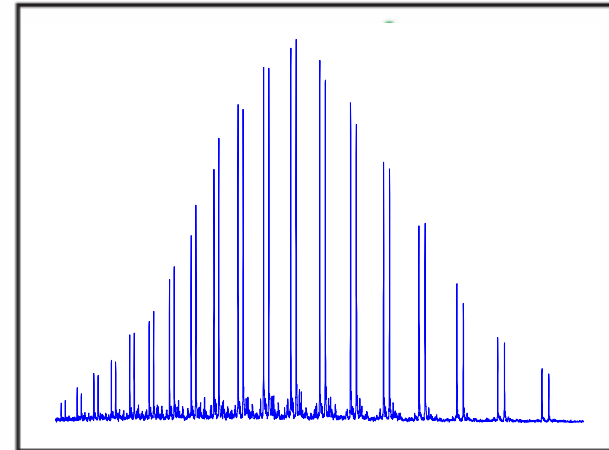
$\Delta [A_w G_x C_y T_z]$

Part 2:

MS Analysis and Signal Processing



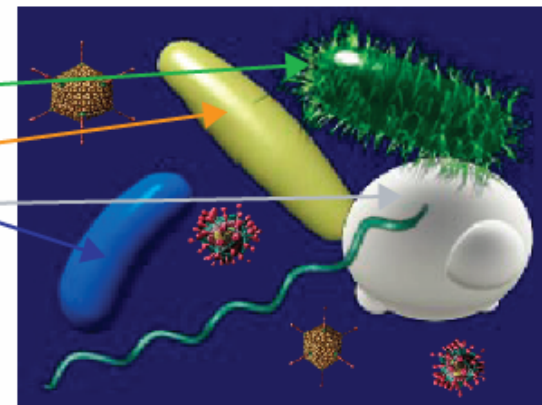
Spectral Signal



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

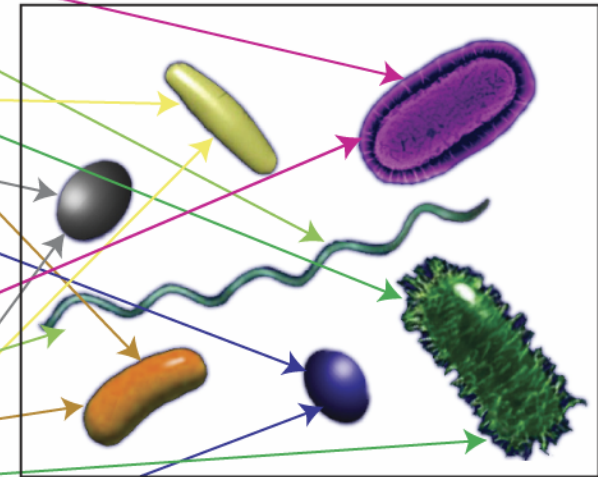
Base Compositions Map to Microbes



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

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	Valyl-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)	<i>Bacillus cereus</i> clade		Clade-specific genes

Drill-Down

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

Bacteroidetes

- ▼ *Bacteroides fragilis*
- ▼ *Bacteroides thetaiotaomicron*
- ▼ *Capnocytophaga canimorsus*
- ▼ *Chryseobacterium meningosepticum*
- ▼ *Prevotella sp.*
- ▼ *Porphyromonas sp.*

Spirochetes

- ▼ *Borrelia burgdorferi*
- ▼ *Borrelia hermsii*
- ▼ *Borrelia turicatae*
- ▼ *Leptospira interrogans*
- ▼ *Treponema pallidum*

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*
- ▼ *Propionibacterium acnes*
- ▼ *Rothia dentocariosa*
- ▼ *Tropheryma whippelii*
- ▼ *Actinomyces israelii*
- ▼ *Actinomyces naeslundii*
- ▼ *Actinomyces novae*
- ▼ *Clavibacter michiganensis*
- ▼ *Corynebacterium serosis*
- ▼ *Corynebacterium amycolatum*
- ▼ *Corynebacterium bovis*
- ▼ *Corynebacterium jeikeium*
- ▼ *Corynebacterium sp.*
- ▼ *Cor.pseudodiphtheriticum*
- ▼ *Cor. diphtheriae*

Fusobacteria

- ▼ *Fusobacterium necrophorum*
- ▼ *Streptobacillus moniliformis*

- ▼ *Mycoplasma mycoides*
- ▼ *Erysipelothrix rhusiopathiae*
- ▼ *Mycoplasma pneumonia*

Mollicutes

- ▼ *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*
- ▼ *Clostridium difficile*
- ▼ *Clostridium perfringens*
- ▼ *Epsilon Toxin*
- ▼ *Clostridium septicum*
- ▼ *Clostridium tetani*
- ▼ *Peptococcus niger*
- ▼ *Peptostreptococcus anaerobius*
- ▼ *Veillonella dispar*

Clostridia

- ▼ *Streptococcus agalactiae*
- ▼ *Streptococcus bovis*
- ▼ *Streptococcus intermedius*
- ▼ *Streptococcus equi*
- ▼ *Streptococcus milleri*
- ▼ *Streptococcus pneumoniae*
- ▼ *Streptococcus pyogenes*
- ▼ *Streptococcus viridans*

Bacilli

- ▼ *Bacillus anthracis*
- ▼ *Bacillus cereus*
- ▼ *Bacillus thuringiensis*
- ▼ *Listeria monocytogenes*
- ▼ *Staphylococcus intermedius*
- ▼ *Staphylococcus saprophyticus*

Primer 352

Chlamydia

- ▼ *Chlamydia trachomatis*
- ▼ *Chlamydia pneumoniae*
- ▼ *Chlamydia psittaci*
- ▼ *Chlamydia felis*
- ▼ *Parachlamydia acanthamoebae*

Epsilon

- ▼ *Campylobacter fetus*
- ▼ *Campylobacter jejuni*
- ▼ *Helicobacter cinaedi*
- ▼ *Helicobacter fennelliae*
- ▼ *Helicobacter pylori*

Proteobacteria

Primer 367

- ▼ *Eikenella corrodens*
- ▼ *Neisseria gonorrhoeae*
- ▼ *Neisseria meningitidis*
- ▼ *Achromobacter xylosoxidans*
- ▼ *Bordetella pertussis*
- ▼ *Burkholderia cepacia*
- ▼ *Burkholderia mallei*
- ▼ *Burkholderia pseudomallei*
- ▼ *Ralstonia mannitolilytica*
- ▼ *Ralstonia picketti*
- ▼ *Ralstonia solanacearum*

Primer 363

- ▼ *Acinetobacter anitratus*
- ▼ *Acinetobacter baumannii*
- ▼ *Acinetobacter calcoaceticus*
- ▼ *Acinetobacter lwoffii*
- ▼ *Acinetobacter radioresistans*
- ▼ *Pseudomonas aeruginosa*
- ▼ *Pseudomonas alcaligenes*
- ▼ *Pseudomonas fluorescens*
- ▼ *Pseudomonas putida*
- ▼ *Pseudomonas stutzeri*
- ▼ *Pseudomonas syringae pv. glycinea*

Alpha

- ▼ *Rickettsia prowazekii*
- ▼ *Rickettsia rickettsii*
- ▼ *Rickettsia typhi*
- ▼ *Anaplasma phagocytophilum*
- ▼ *Cowdria ruminantium*
- ▼ *Ehrlichia chaffeensis*
- ▼ *Ehrlichia equi*
- ▼ *Orientia tsutsugamushi*
- ▼ *Rickettsia conorii*
- ▼ *Rickettsia felis*

Primer 362

Gamma

- ▼ *Tatlockia micdadei*
- ▼ *Legionella longbeachae*
- ▼ *Legionella pneumophila*
- ▼ *Coxiella burnetii*

Primer 354

- ▼ *Francisella tularensis*
- ▼ *Stenotrophomonas maltophilia*
- ▼ *Xanthomonas malvacearum*
- ▼ *Xanthomonas oryzae pv. oryzae*
- ▼ *Xanthomonas oryzae pv. oryzicola*
- ▼ *Xylella fastidiosa*
- ▼ *Alteromonas tetradonis*
- ▼ *Tetradotoxin*
- ▼ *Actinobacillus actinomycetemcomitans*
- ▼ *Haemophilus aphrophilus*
- ▼ *Haemophilus aegyptius*
- ▼ *Haemophilus ducreyi*
- ▼ *Haemophilus influenzae*
- ▼ *Haemophilus parahaemolyticus*
- ▼ *Haemophilus parainfluenzae*
- ▼ *Haemophilus paraphrophilus*
- ▼ *Pasteurella multocida*

- ▼ *Citrobacter freundii*
- ▼ *Edwardsiella 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 enteritidis*
- ▼ *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?”**

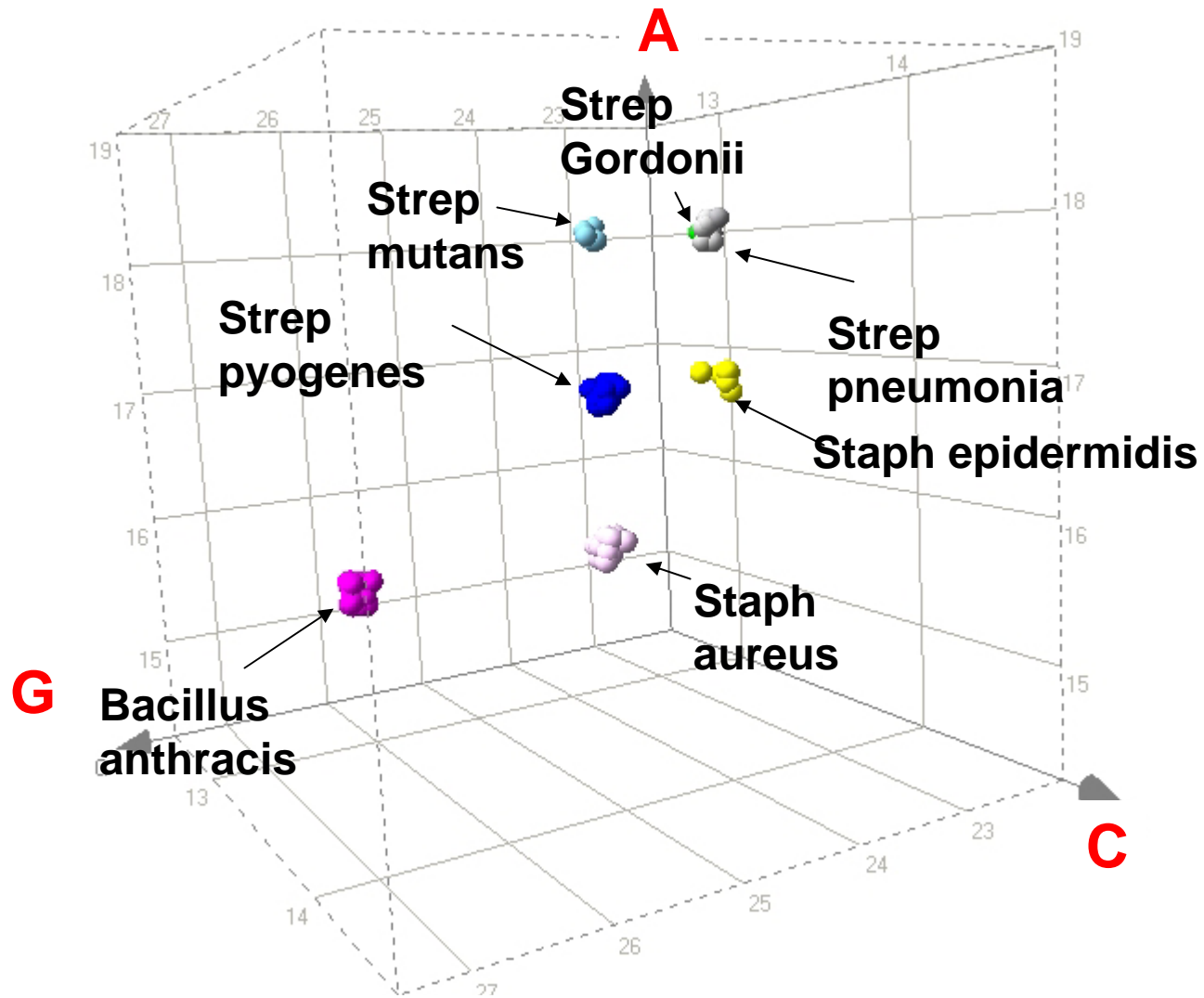
Group A Streptococcus (GAS) Outbreaks in Military Settings

- **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**

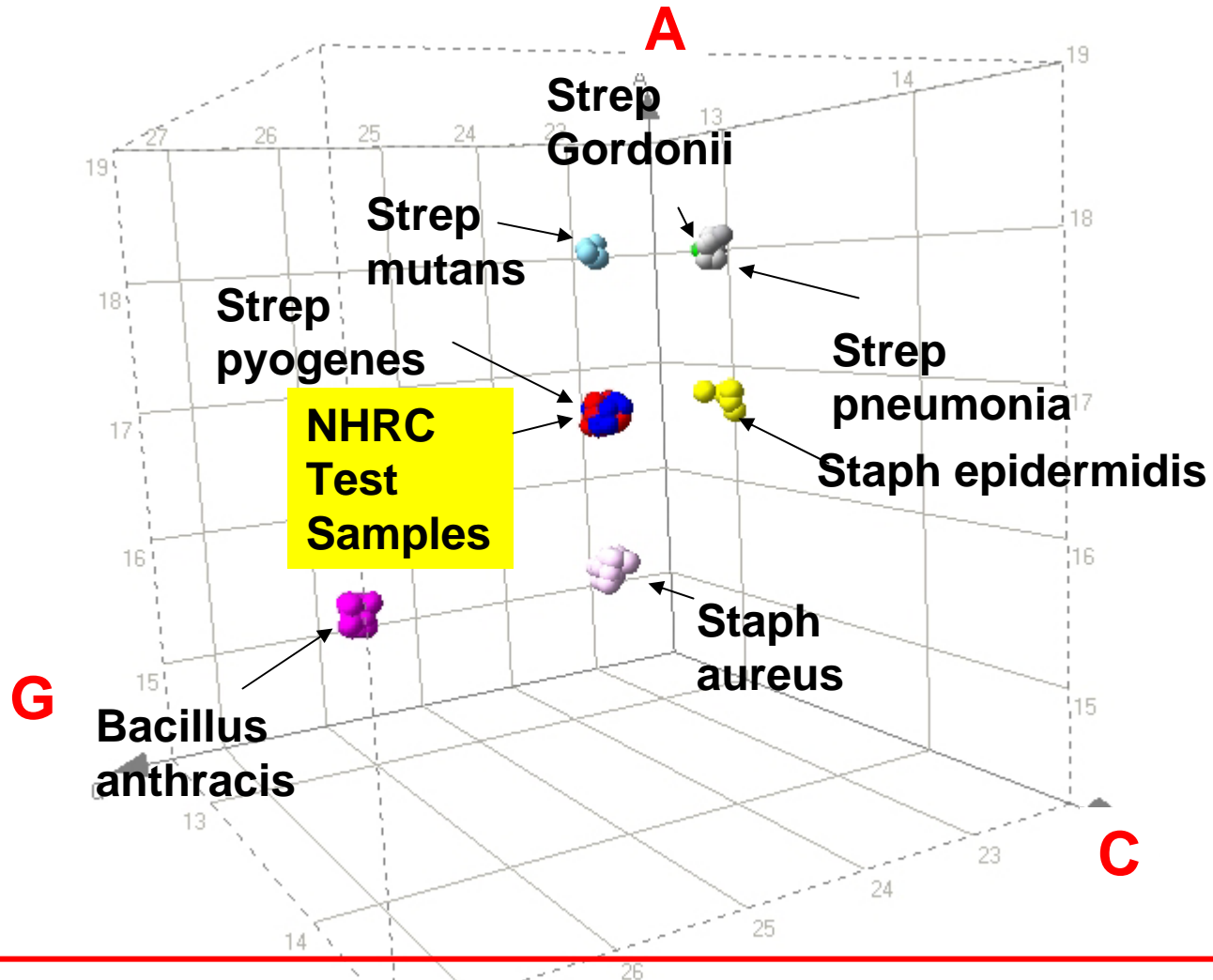


- **Confirm the causative agent as *Streptococcus pyogenes***
- **Provide tool for epidemiologic tracking**
 - Identify a unique signature of the outbreak strain
- **BONUS:**
 - Determine the outbreak strain type (Emm-type)

Primer 17 (a 23S primer) Expected Products



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

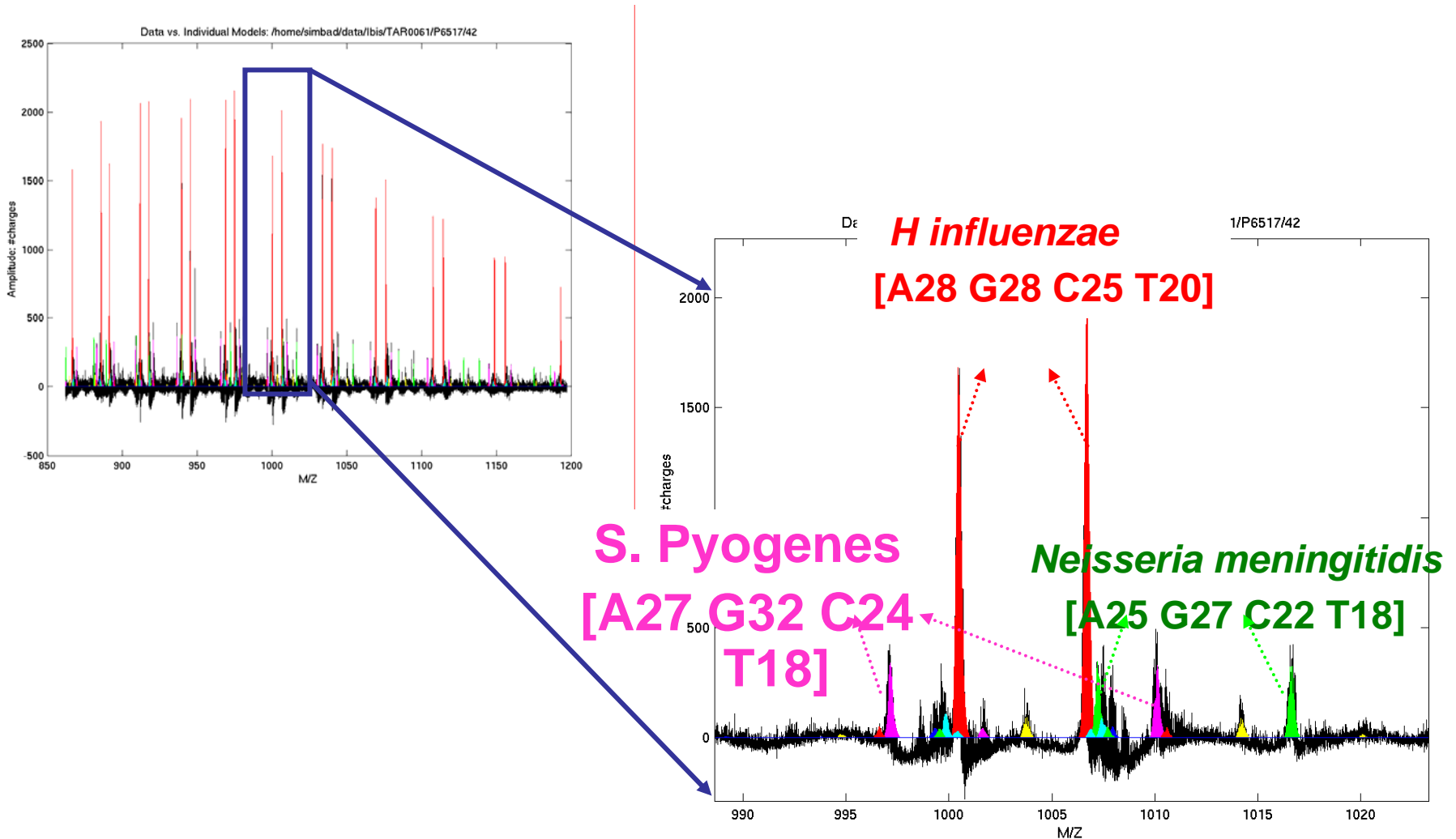


All primers of all samples consistent with *S. pyogenes*

Direct Analysis of Throat Swab*

*Repeat swab positive on culture for *Streptococcus pyogenes*

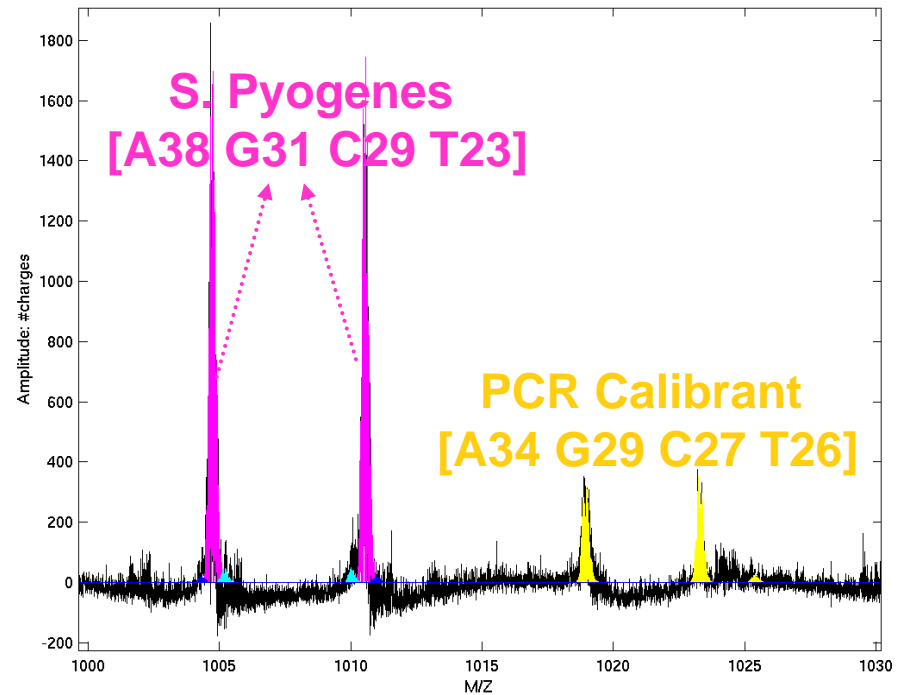
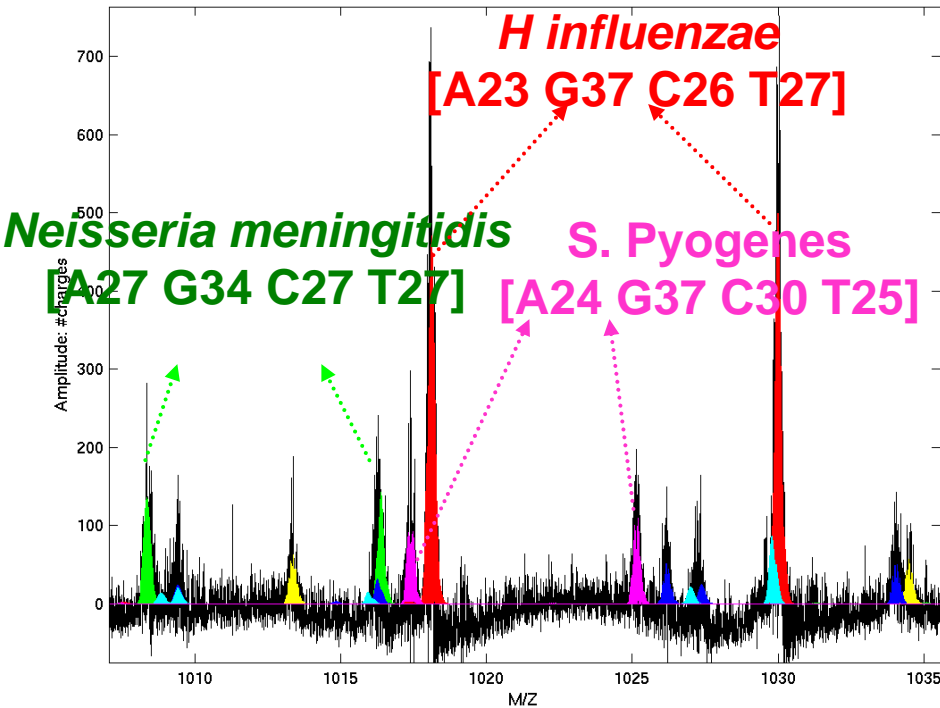
Primer 349: 23S rDNA 1826-1924 From an Interesting Throat Swab



Multi-primer Triangulation

Primer 347: 16S rDNA

Primer 356: rplB



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

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

Genotyping applications

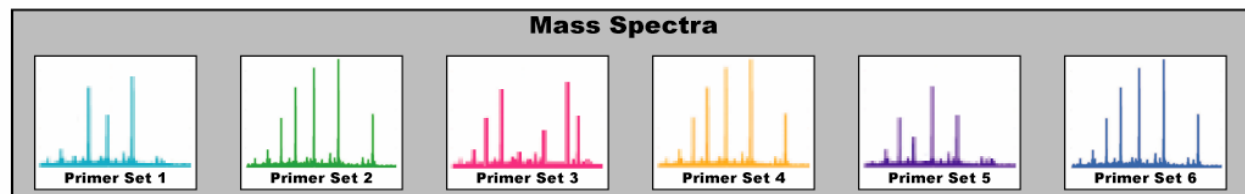
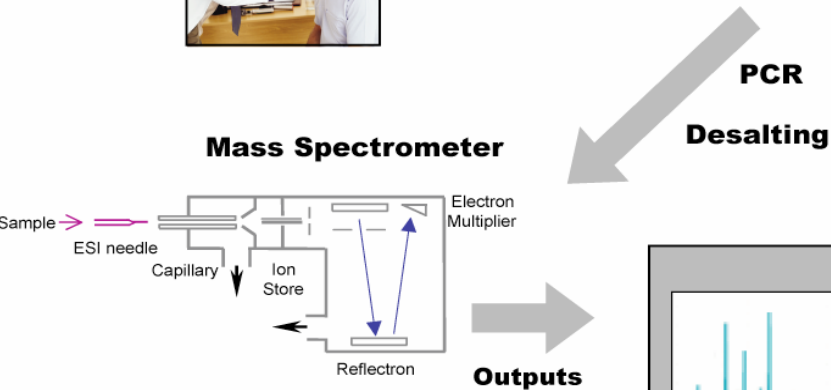
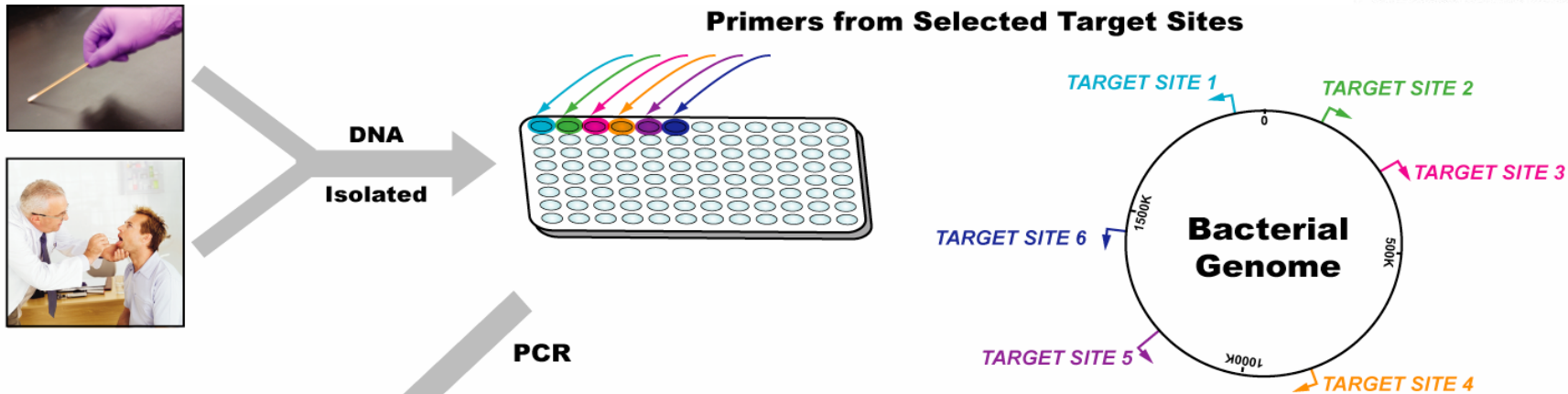
- Microbial forensics
- Epidemiology of disease outbreak
- Nosocomial strain tracking
- Food pathogen monitoring

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

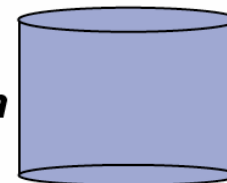
Rapid Strain Typing by Base Composition: *S. pyogenes*: our maiden genotyping voyage

- 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

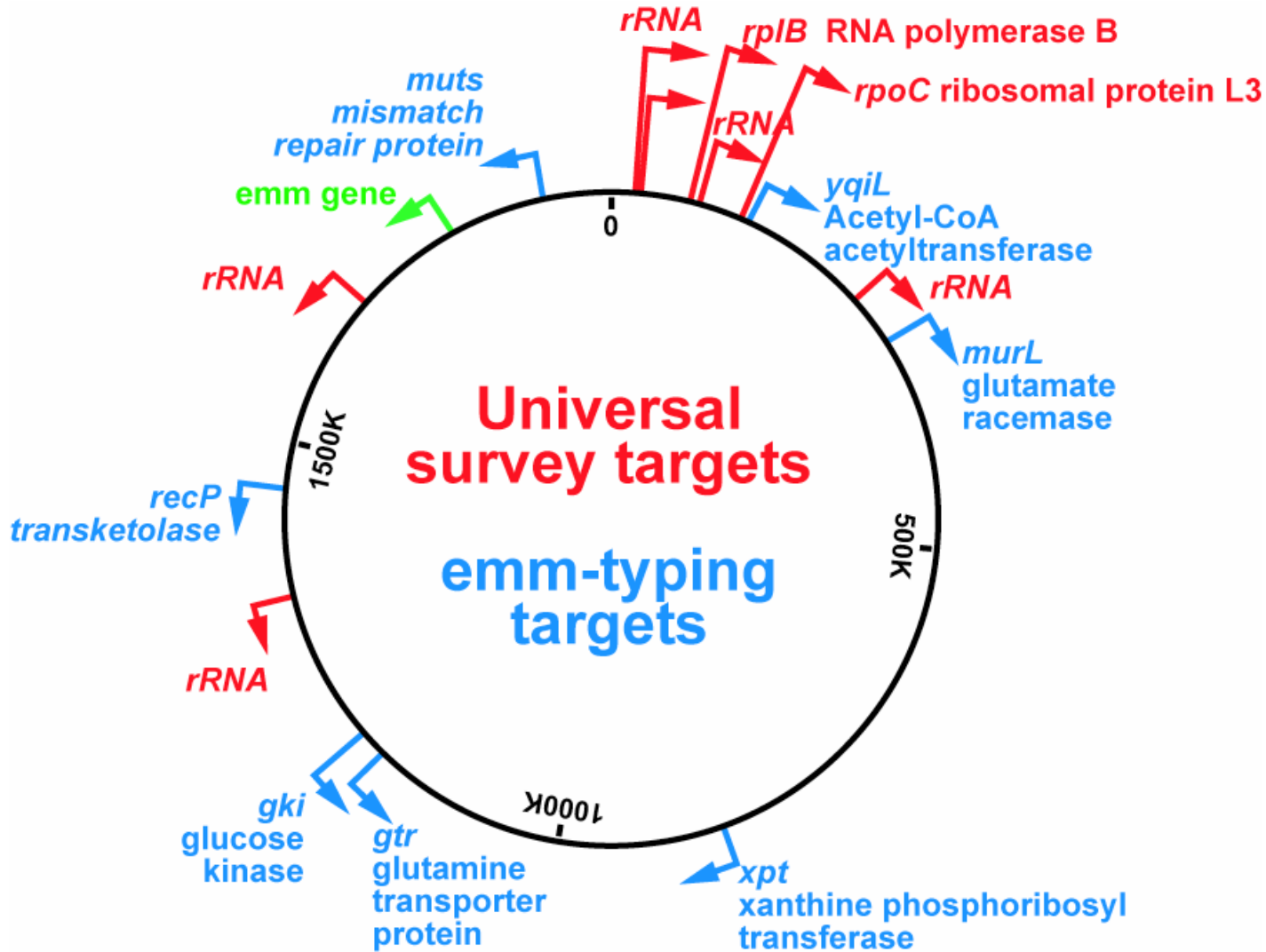


Isolate	Primer Set 1	Primer Set 2	Primer Set 3	Primer Set 4	Primer Set 5	Primer Set 6
Isolate 1	A39 G25 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A32 G35 C17 T32	A39 G28 C16 T32
Isolate 2	A40 G24 C20 T34	A38 G27 C23 T33	A30 G36 C20 T36	A40 G29 C19 T31	A31 G35 C17 T33	A39 G28 C15 T33
Isolate 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

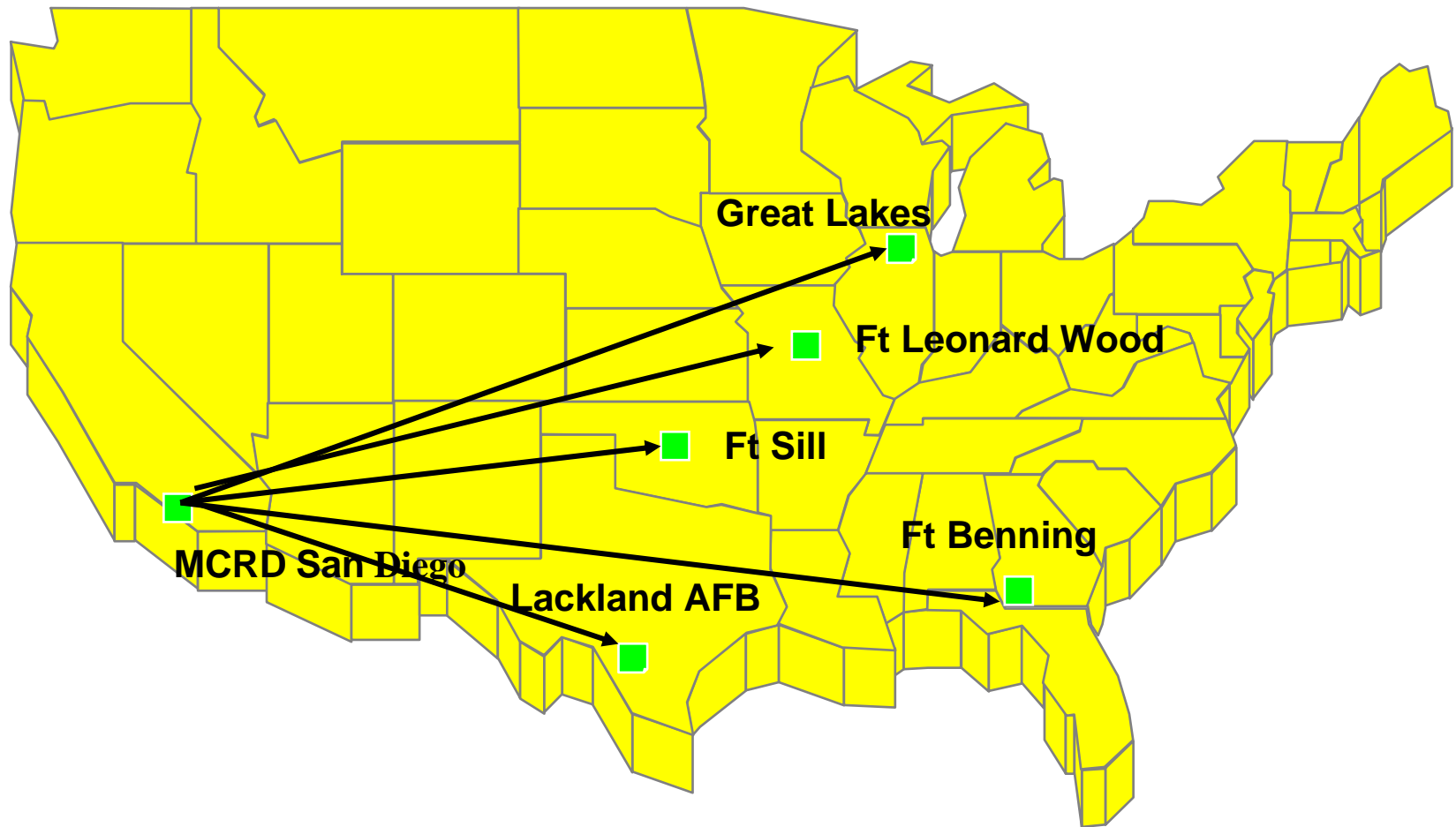


Archive NHRC Samples

Sample ID	EMM-type determination			Base Compositions					
	MLST mass spectrometry	emm-gene sequencing	MLST sequencing	murl	mutS	xpt	yqil	gki	gtr
52	3	3	MB	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	MB	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

Archive Samples

Genotyping *Streptococcus pyogenes* Outbreaks at Military Bases in 2003



Lackland AFB

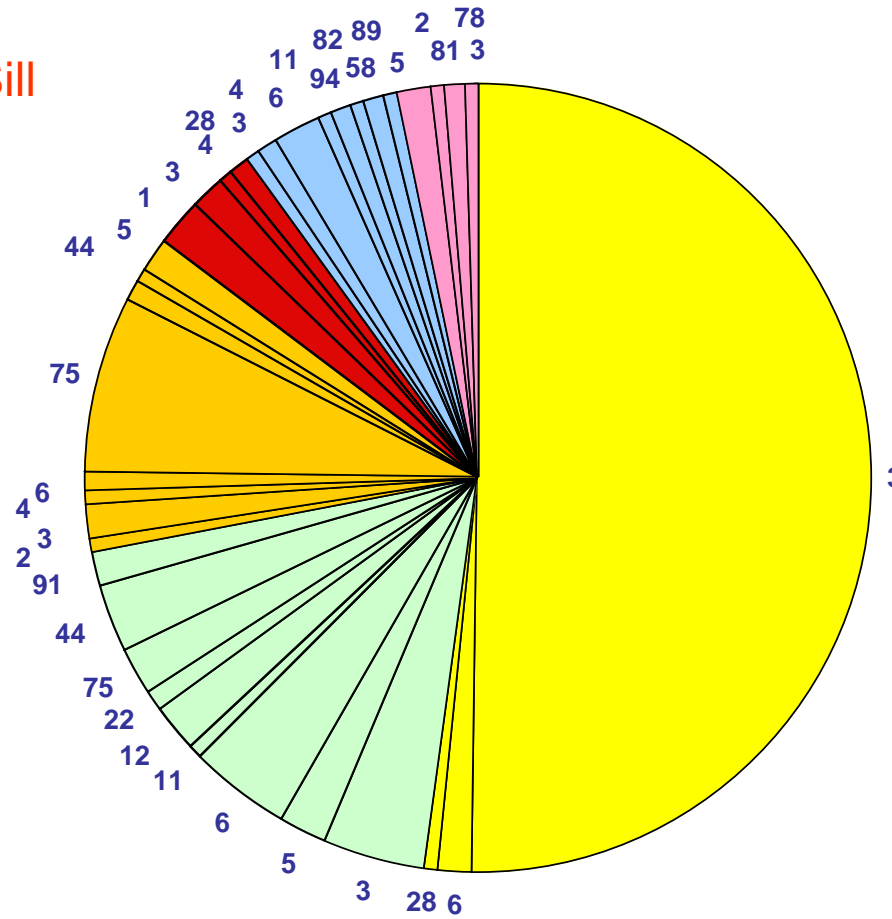
Ft. Benning

Ft. Sill

MCRD
San Diego

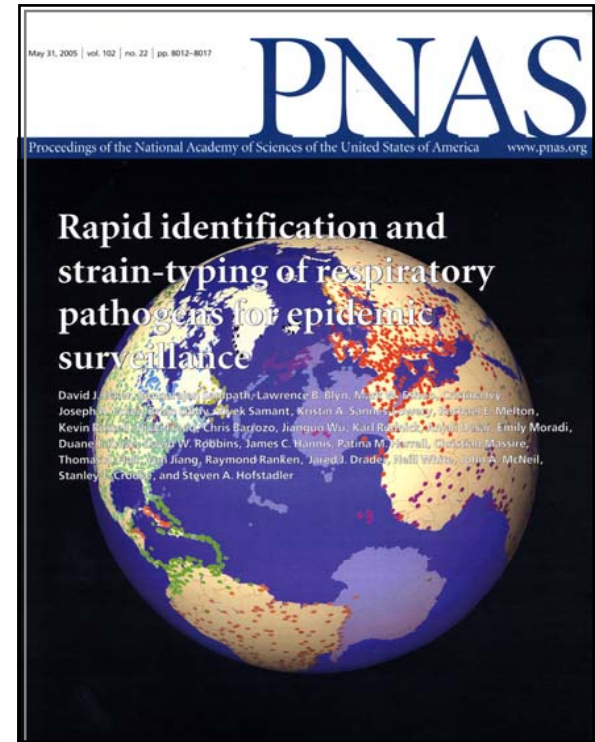
Ft. Leonard
Wood

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**

***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

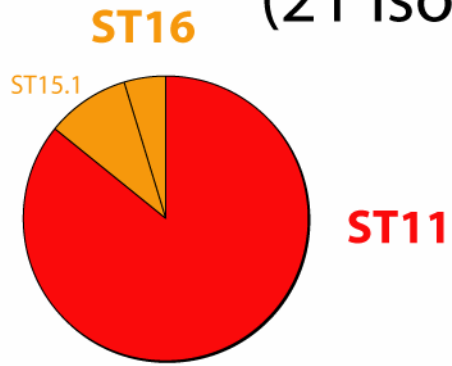


28th Combat Support Hospital (CSH) in Iraq

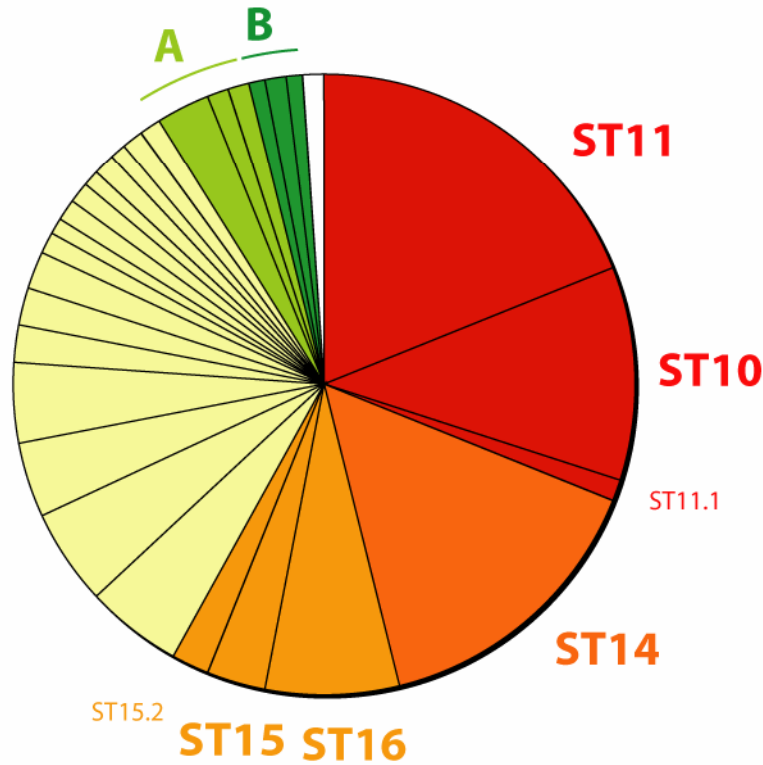
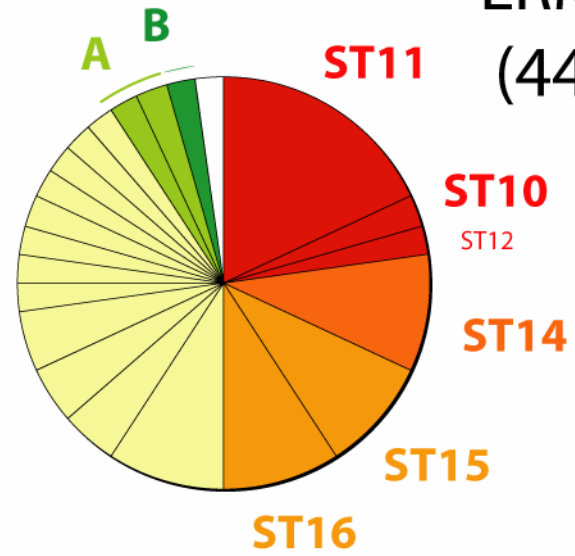
- **Selected 6 target genes: efp, trpE, adk, mutY, fumC, ppa**
 - **1679 nucleotides of DNA**
- **8 PCR primers/mass spectrometry analysis**
- **Identified the genotype of all 216 OIF isolates**
 - **61 Unique genotypes**

Species	ST	#	ref. strain	1151-trpE	1156-adt	1158-mutY	1160-mutY	1165-fumC	1167-fumC	1170-fumC	1171-ppa	
<i>Acinetobacter baumannii</i>	2	1	ATCC9955	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		A44G35C21T42	A44G32C26T38	A27G21C25T22	A32G35C28T34	A39G33C30T37	A40G35C32T40	A38G27C20T51	A36G35C31T47	
	1	1		LUH6024 (II)	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	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		

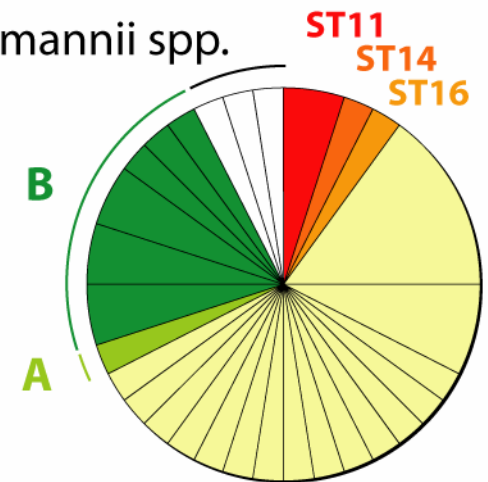
USNS Comfort
(21 isolates)



LRMC
(44 isolates)



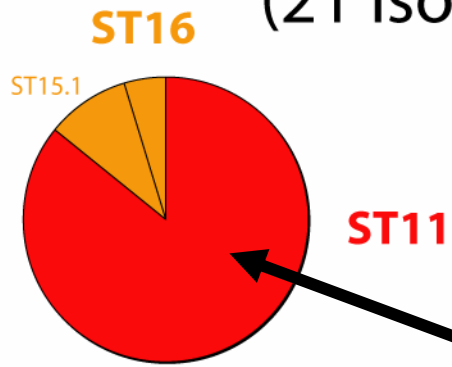
other non-*baumannii* spp.



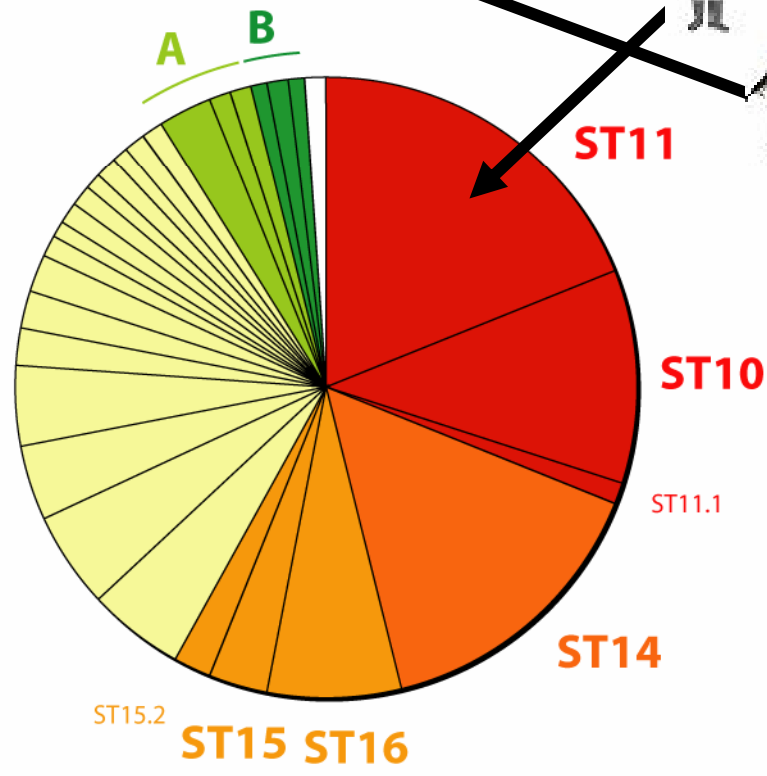
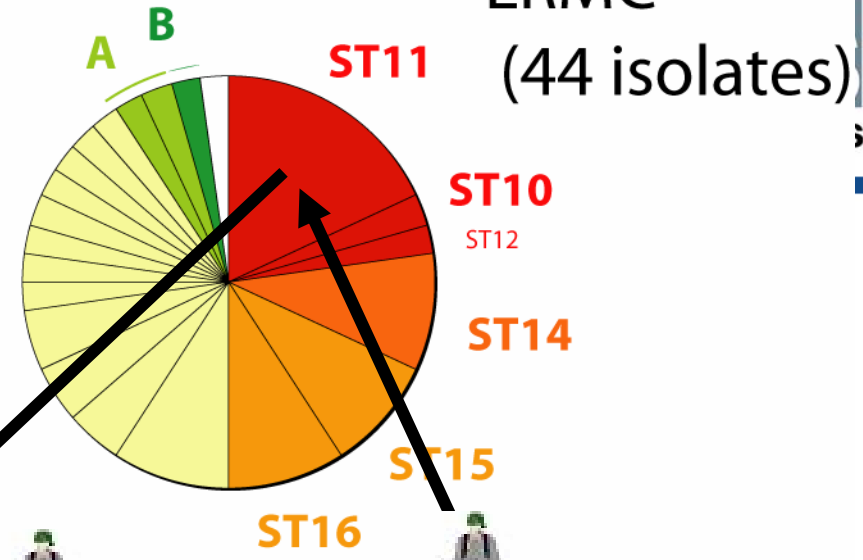
WRAMC
(100 isolates)

Field locations
(40 isolates)

USNS Comfort
(21 isolates)

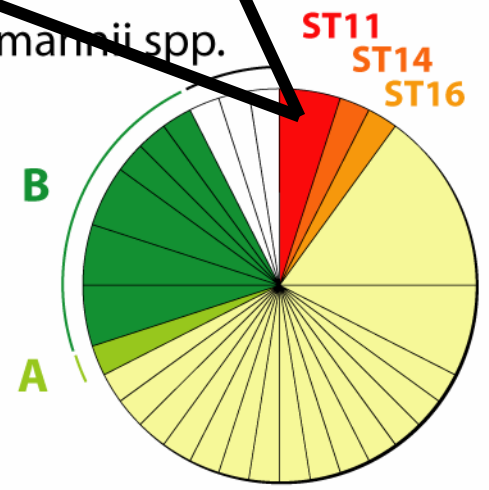


LRMC
(44 isolates)



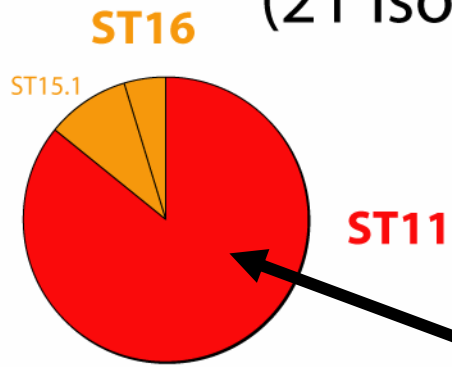
WRAMC
(100 isolates)

other non-*baumannii* spp.

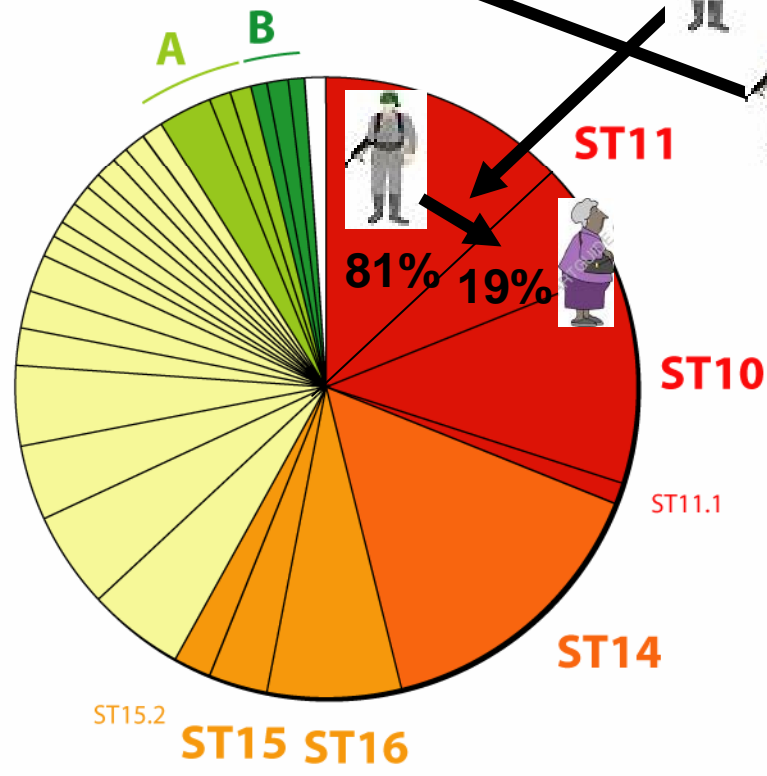
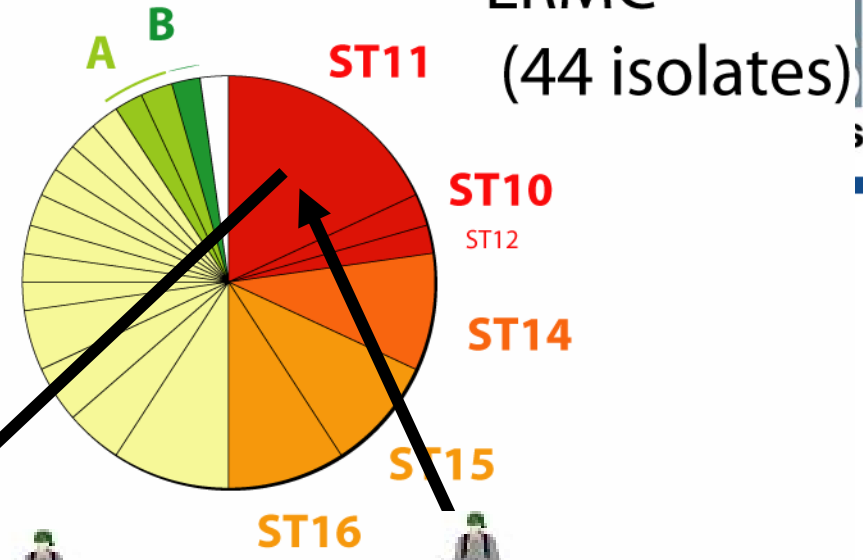


Field locations
(40 isolates)

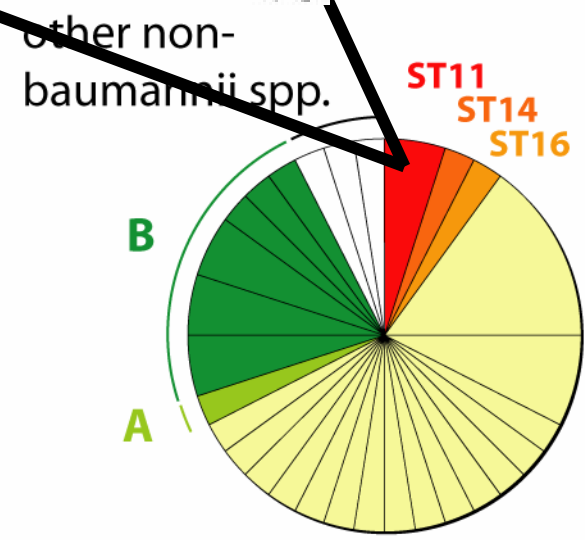
USNS Comfort (21 isolates)



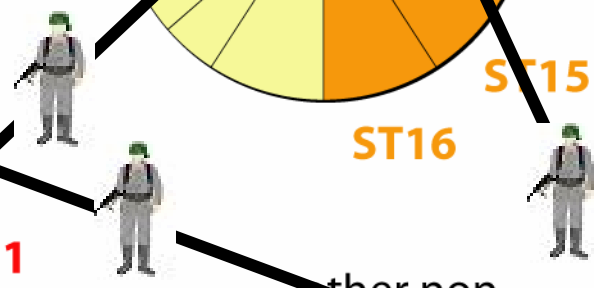
LRMC (44 isolates)



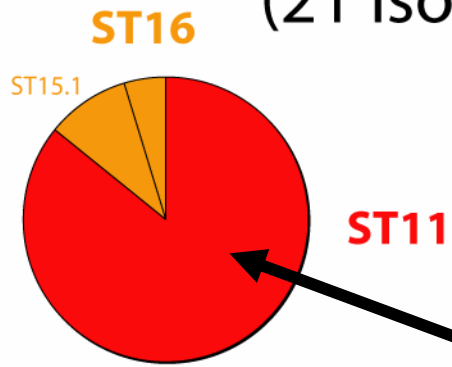
WRAMC (100 isolates)



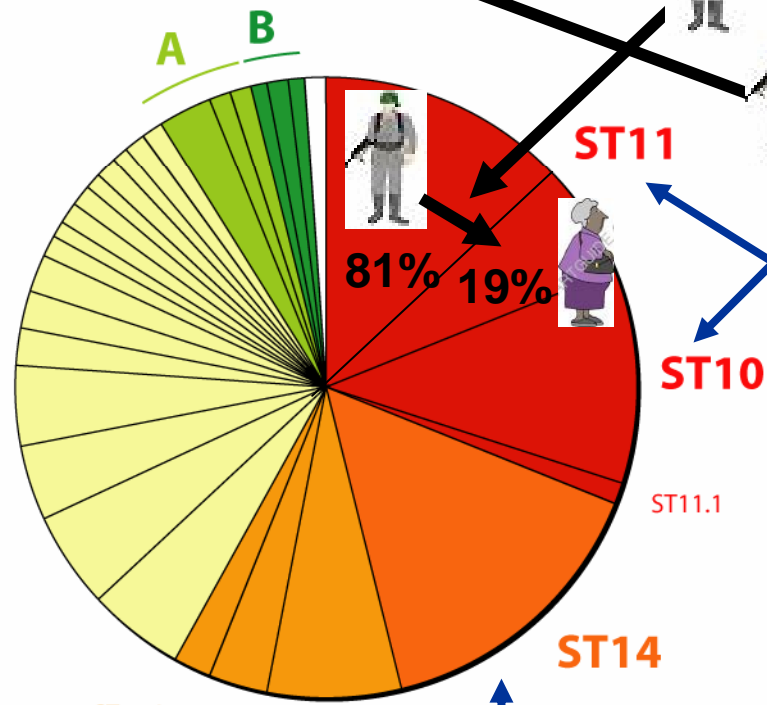
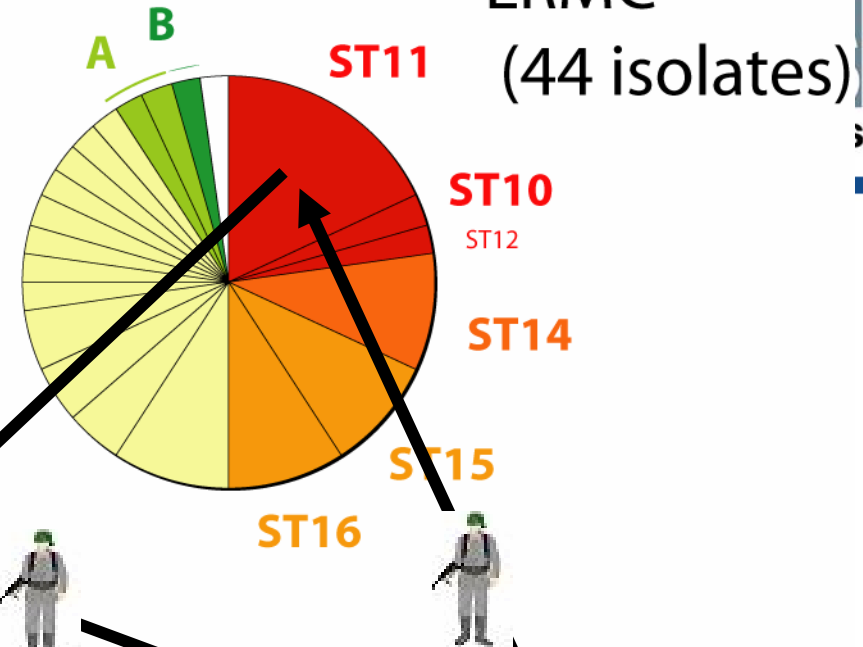
Field locations (40 isolates)



USNS Comfort
(21 isolates)



LRMC
(44 isolates)

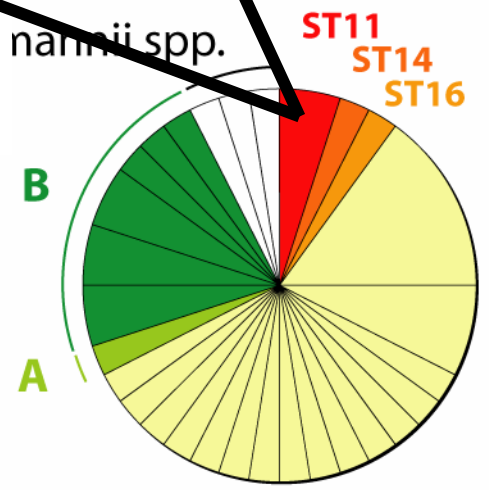


European Clone II

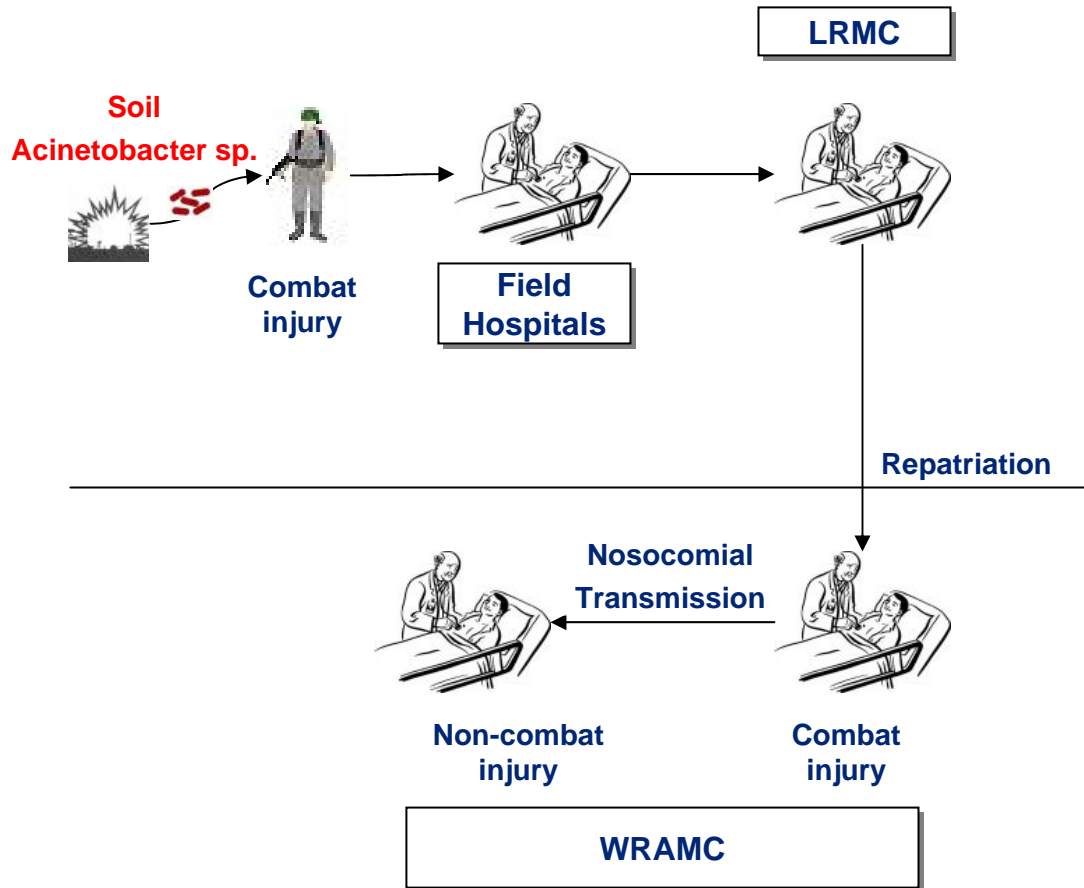
WRAMC

ST15.2
ST15 ST16
European Clone I
European Clone III

Field locations
(40 isolates)

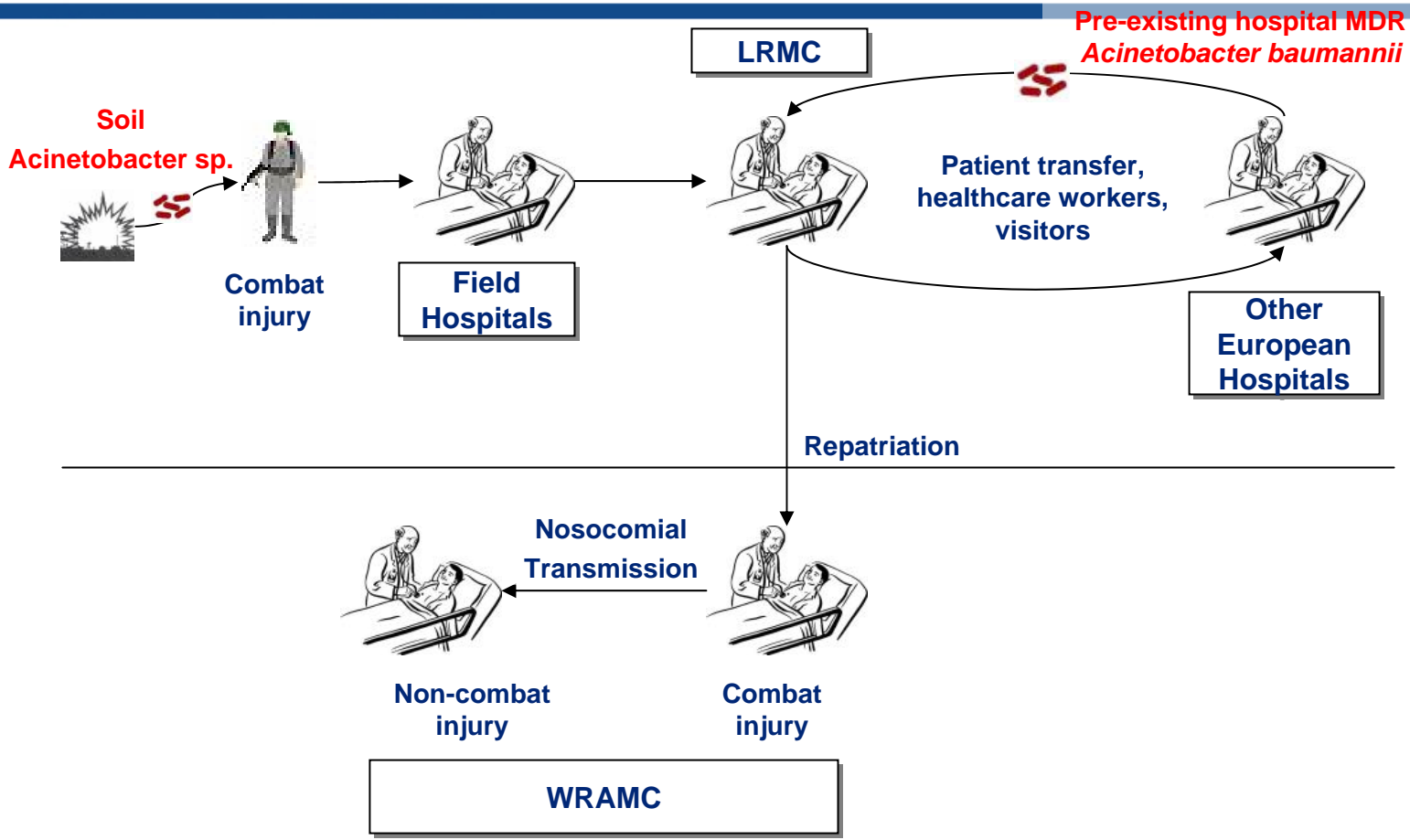


A.) *De novo* soil infection model



A.) De novo soil infection model

B.) Hospital Contamination Model

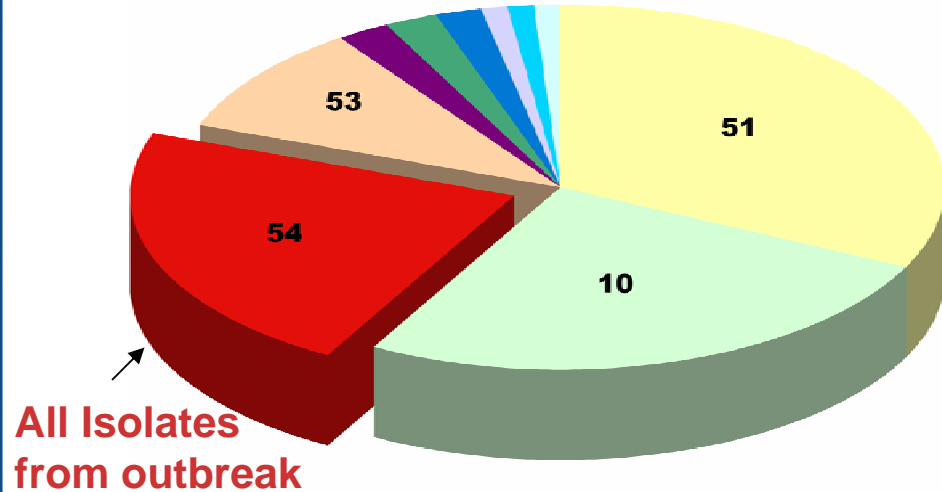


Summary/Lessons Learned

- **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**

Northwestern Memorial Hospital Acinetobacter Outbreak

- Outbreak April-December, 2005
- High-level carbapenam 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



Organism	Genotype	# of isolates	T5000 Cipro
<i>A. baumannii</i>	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	
<i>A. species 3</i>	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



➤ Develop and validate ESI-MS assays for characterizing *S. aureus* in nosocomial settings

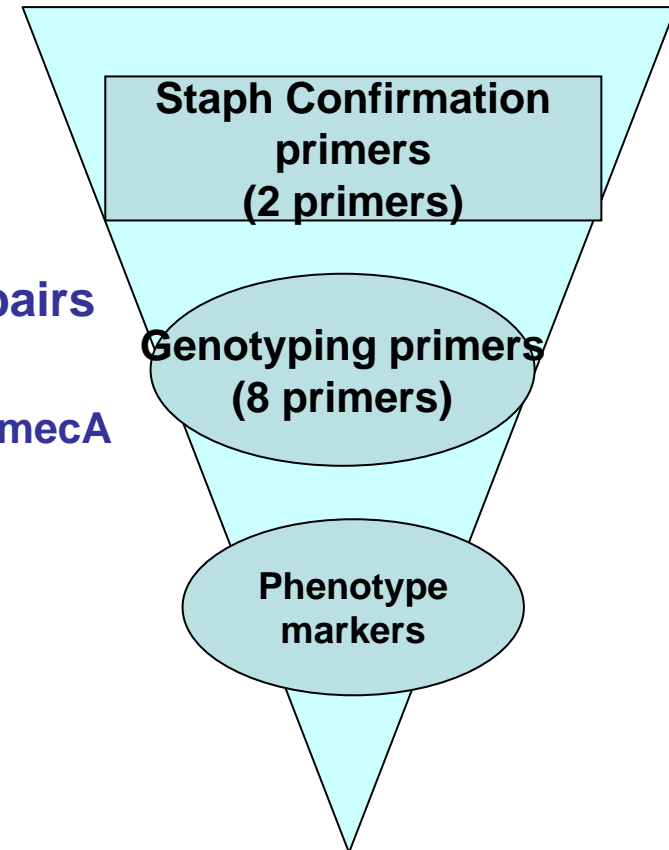
- Identify the organism as *S. aureus*
- Determine drug-resistance profile (methicillin, erythromycin, mupirocin)
- Determine virulence phenotype markers
- Genotype to identify strain

➤ 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

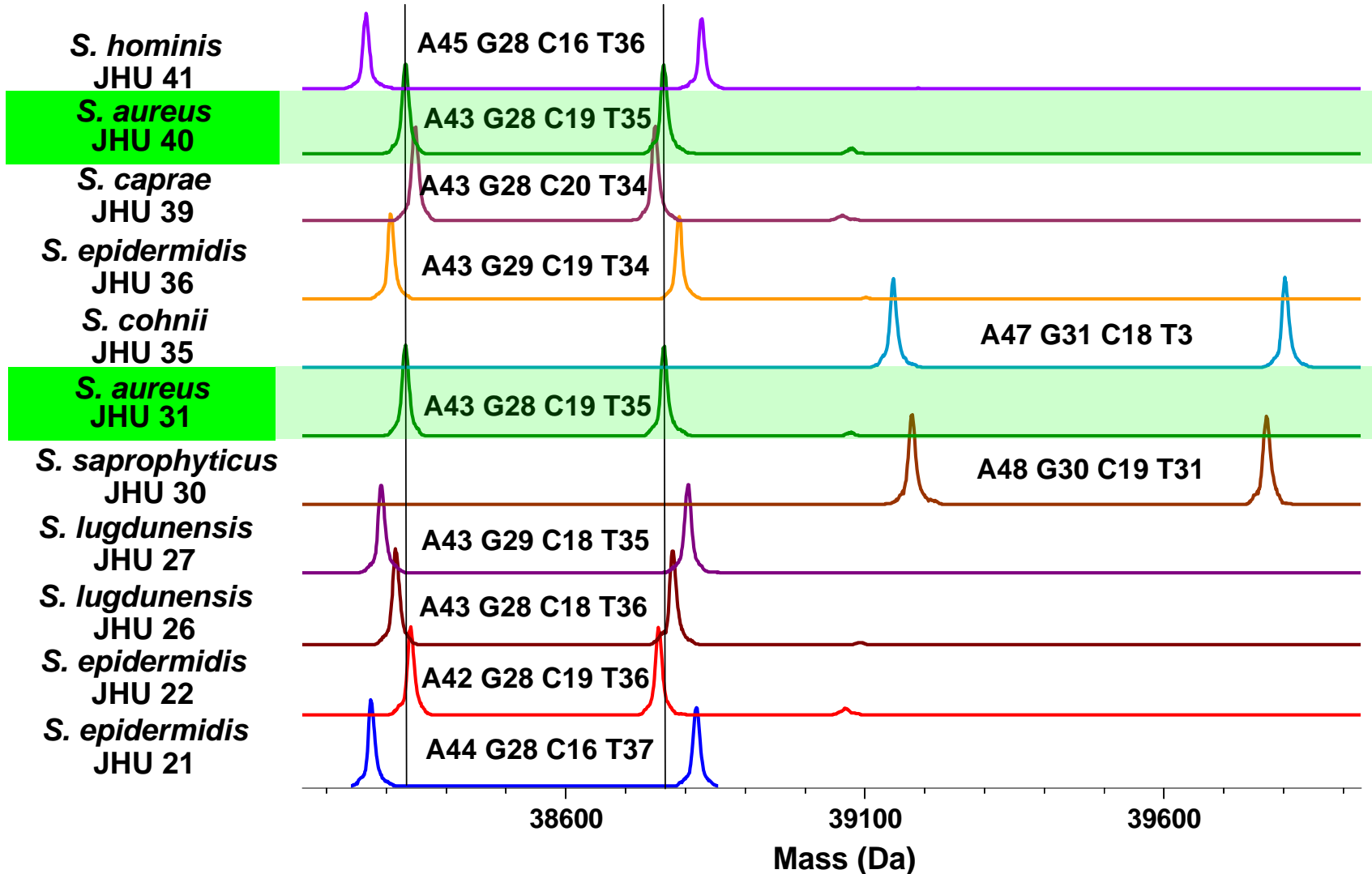
Staphylococcus aureus confirmation / drug-resistance / virulence factor panel

- ❑ **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

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

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

PVL + by Standard
lucs/lukF PCR Assays

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

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
	CDCVRS A1	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
	CDCVRS A2	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

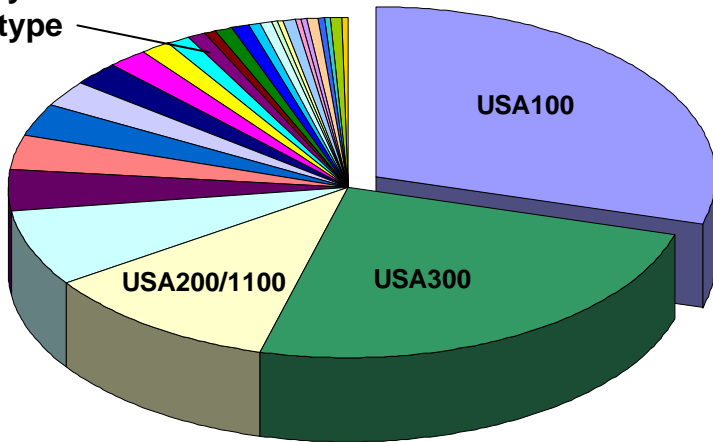


CDC0031 was *Staphylococcus schleiferi*

Survey of 326 *Staphylococcus aureus* clinical isolates

Genotyping, strain identification

Extremely virulent type USA400



- 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

# of isolates	Identification		Virulence	Antibiotic Resistance				
	<i>tufB</i>	<i>nuc</i>	PVL	Methicillin		Erythromycin		Mupirocin
				<i>mecA</i>	<i>mecI-R</i>	<i>ermA</i>	<i>ermC</i>	<i>mupR</i>
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



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



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



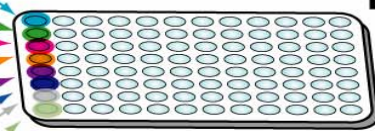
T5000 Influenza Virus Assay



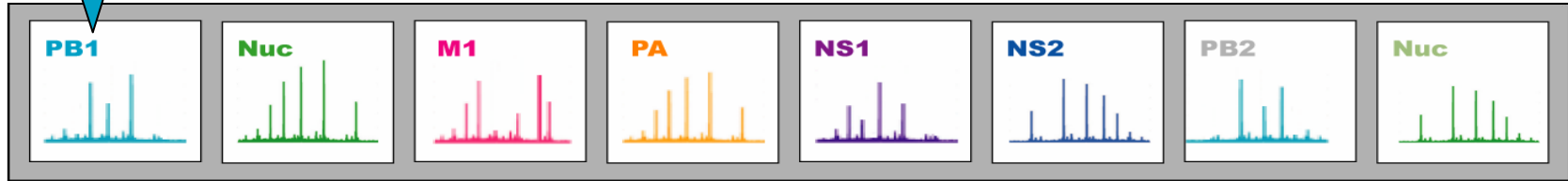
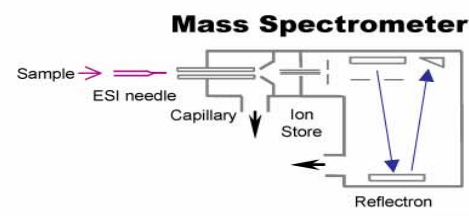
Isolated RNA

Broad Primer

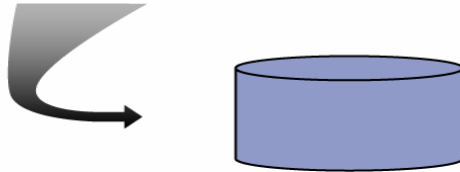
- Pan Influenza Primer **PB1**
- Influenza A Primers
 - Nuc**
 - M1**
 - PA**
 - NS1**
 - NS2**
- Influenza B Primers
 - PB2**
 - Nuc**



PCR Desalting

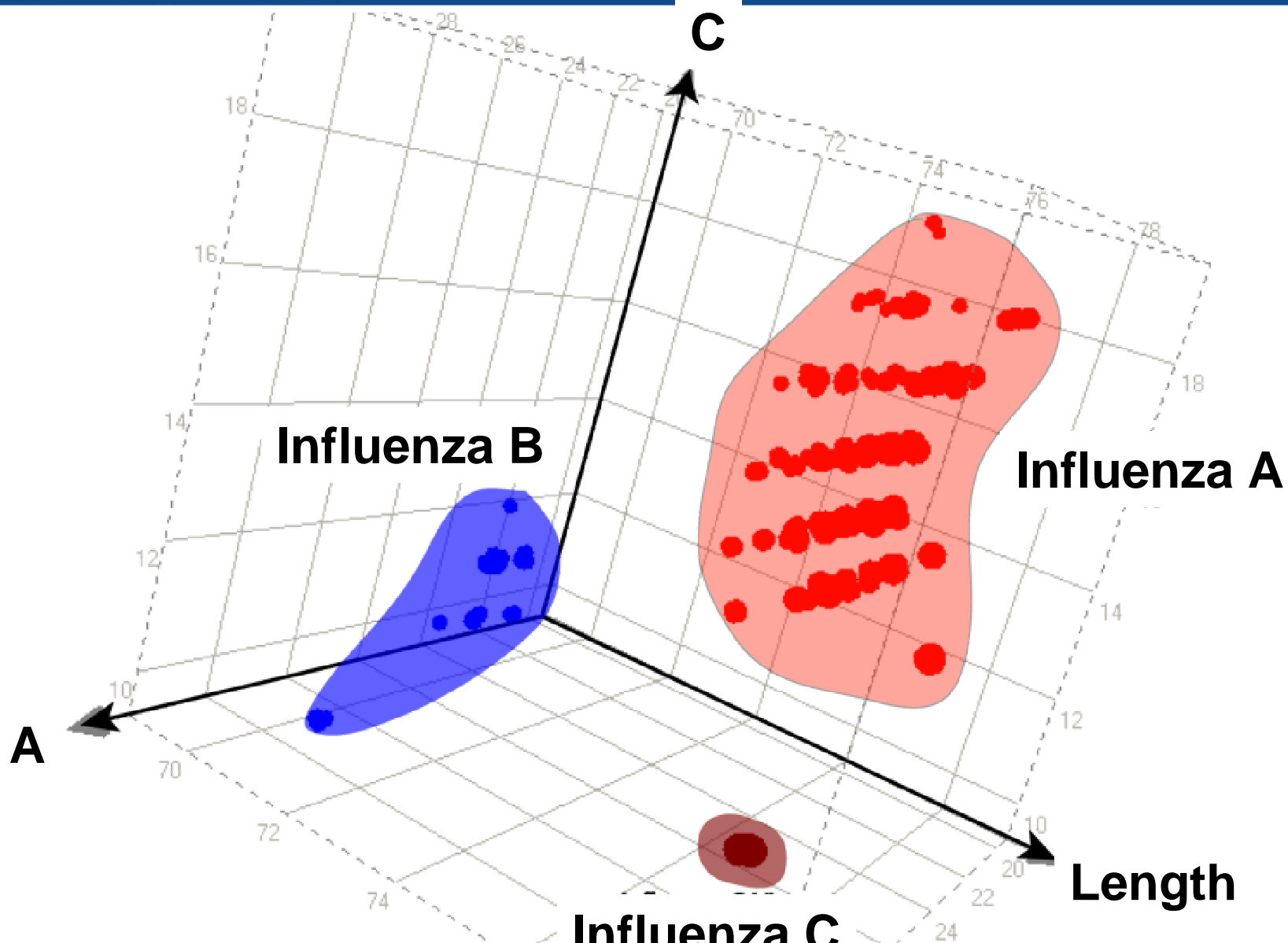


Influenza A	A39 G32 C23 T34	A32 G23 C20 T26	A24 G30 C23 T28	A37 G23 C27 T25	A28 G21 C32 T38	A35 G27 C17 T26		
Influenza B	A37 G31 C20 T34						A23 G27 C19 T22	A36 G17 C36 T11
Mixture	A39 G32 C23 T34	A32 G23 C20 T26	A24 G30 C23 T28	A37 G23 C27 T25	A28 G21 C32 T38	A35 G27 C17 T26	A23 G27 C19 T22	A36 G17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

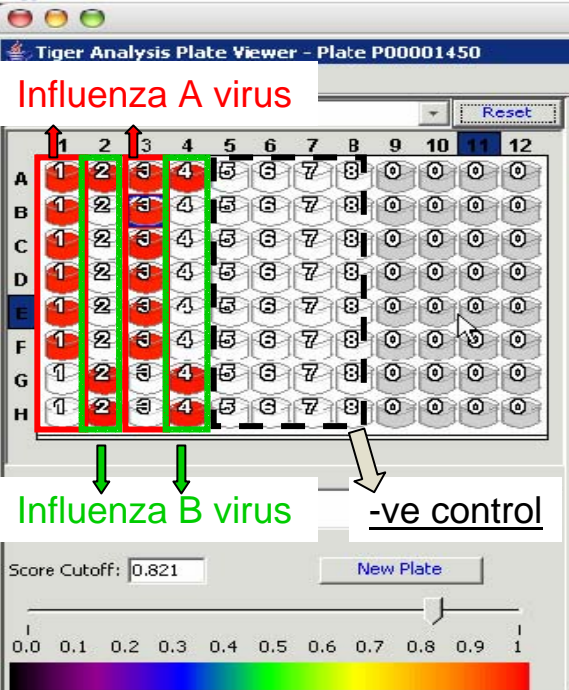


Influenza species, subtype, clade type determined by comparison with database

Pan-influenza Primer Polymerase PB1 Primer PP2798 - Base Composition



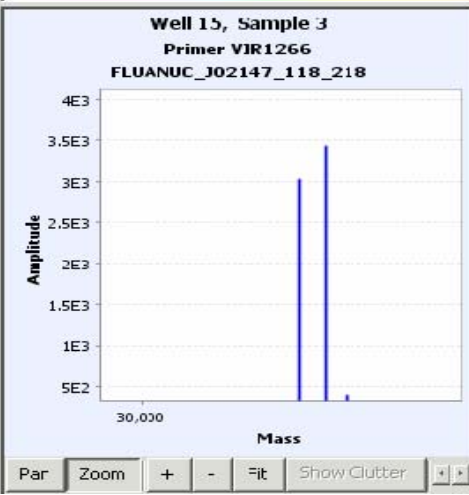
Influenza Virus Assay Results Viewer



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	Peak2 Abundance
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

- **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

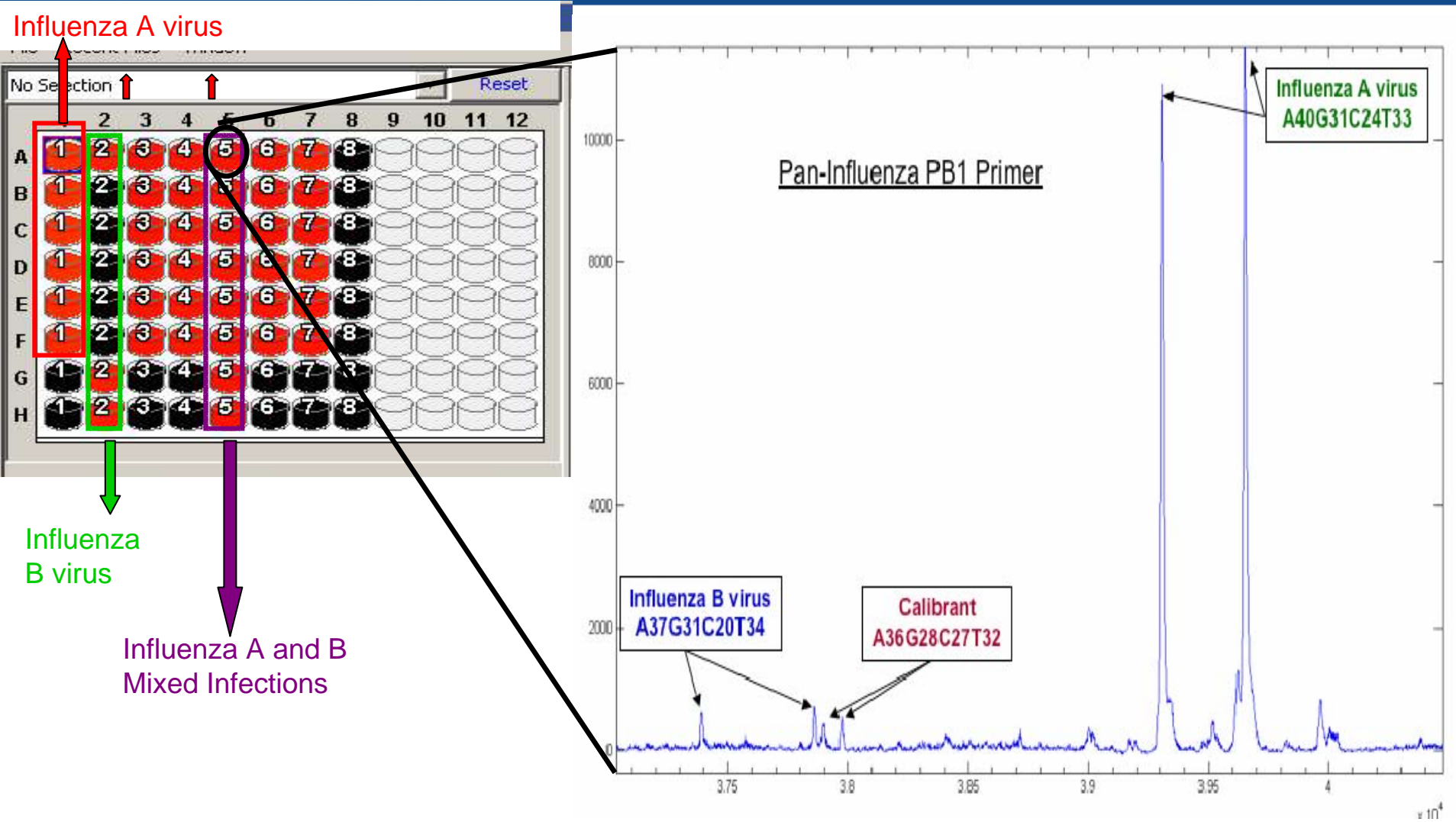
Source	Location	Collection Dates	Sample type	No. of Samples
Naval Health Research Center	MCRD, San Diego Ft. Leonard Wood, Ft. Sill, Ft. Benning, Lackland AFB	1999-2005	Throat swabs, nasal swabs, nasal washes	317
Johns Hopkins University Medical Center	Baltimore, MD	2003-2005	Nasal aspirates	229
NY State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

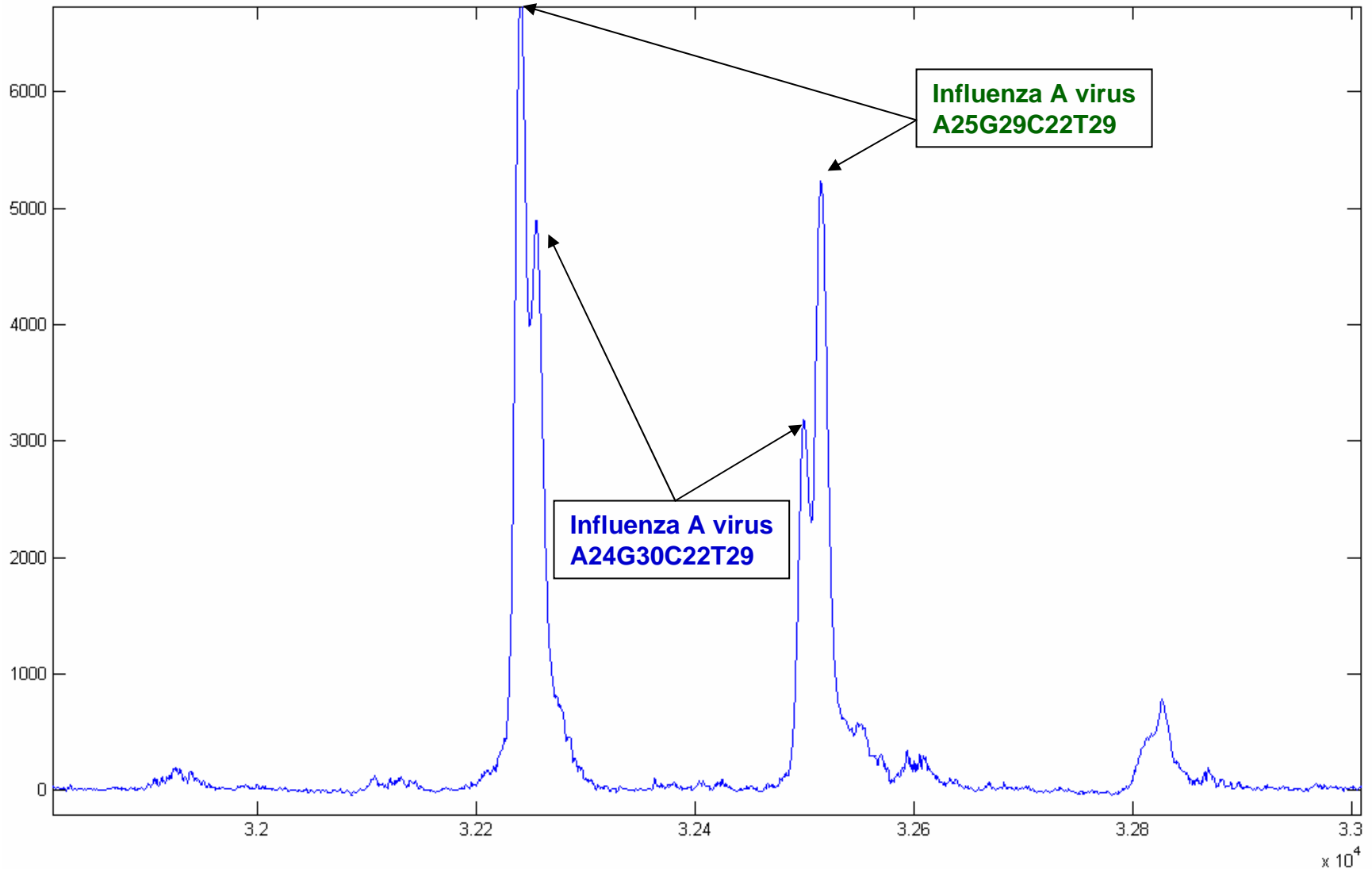
		Culture/Real Time PCR		Total
		Positive	Negative	
T5000	Positive	243	10	253
	Negative	8	395	403
Total		251	405	656
		<i>Sensitivity</i>	96.8	
		<i>Specificity</i>	97.5	
		<i>PPV</i>	96.0	
		<i>NPV</i>	98.0	

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

Detection of Mixed Infections

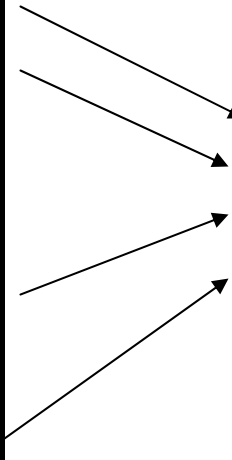


Detection of Multiple Influenza A Contributors



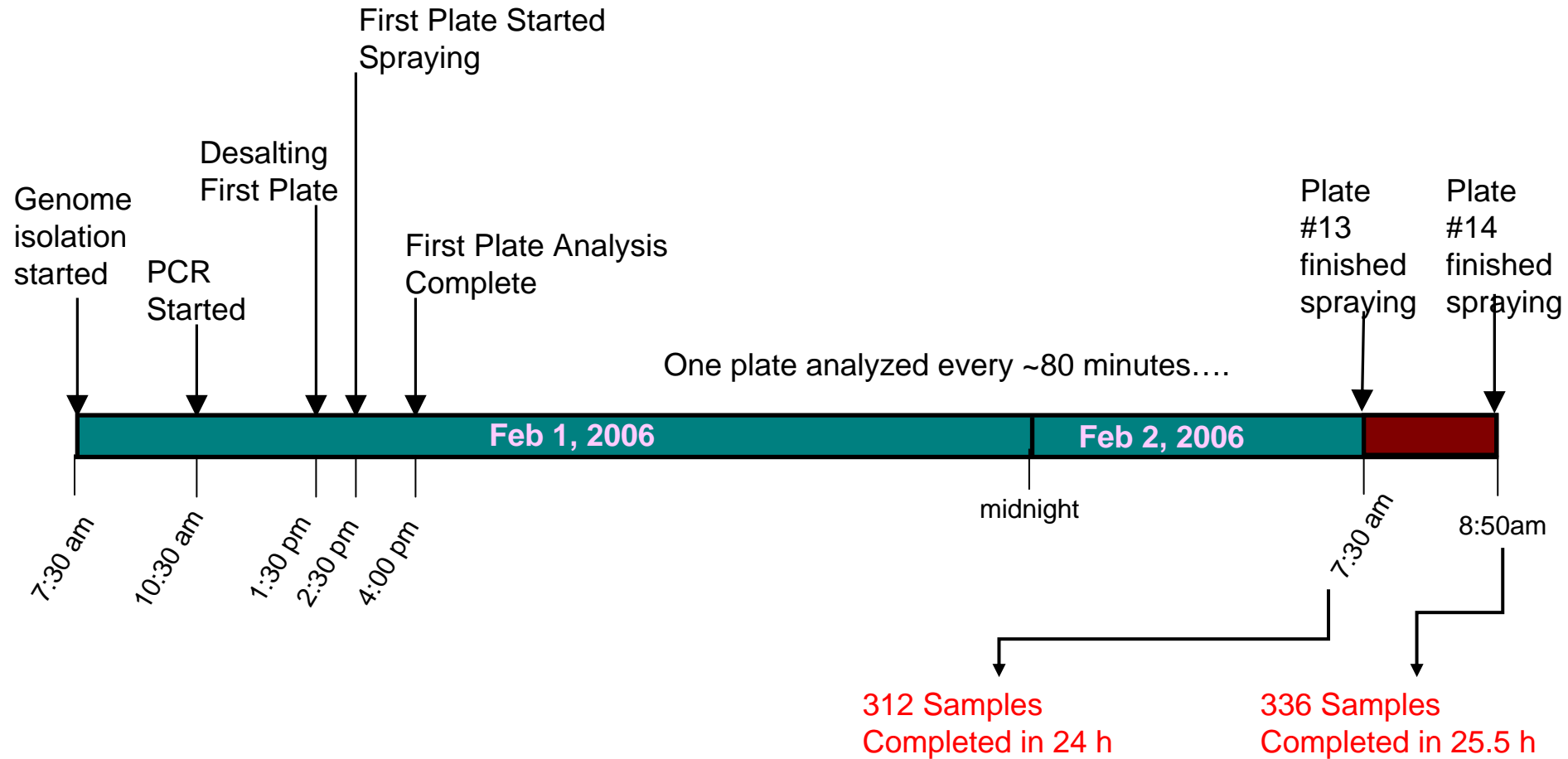
High Throughput 4 Primer Format

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



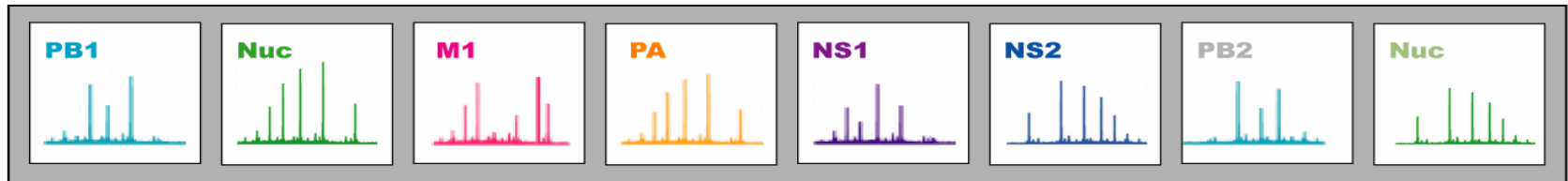
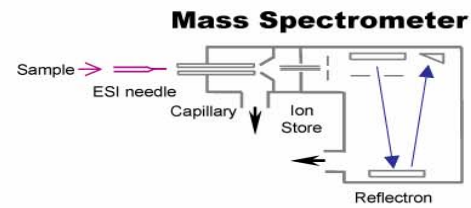
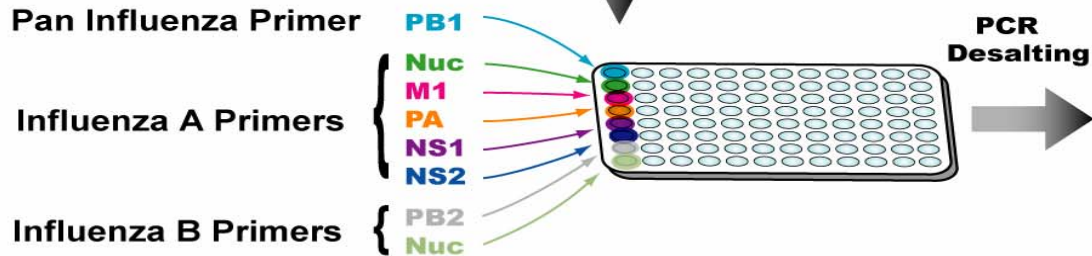
Avian Influenza Virus Detection: University of Georgia Samples

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

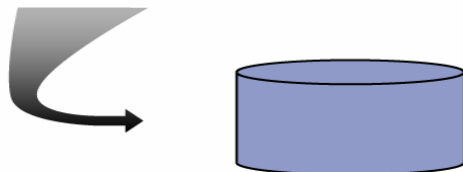
- Different host species: mallard, seagull, teal,...
- Different combinations of H and N subtypes: H12N4, H3N8...

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	Misphillion 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	Misphillion 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



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

		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	
	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)

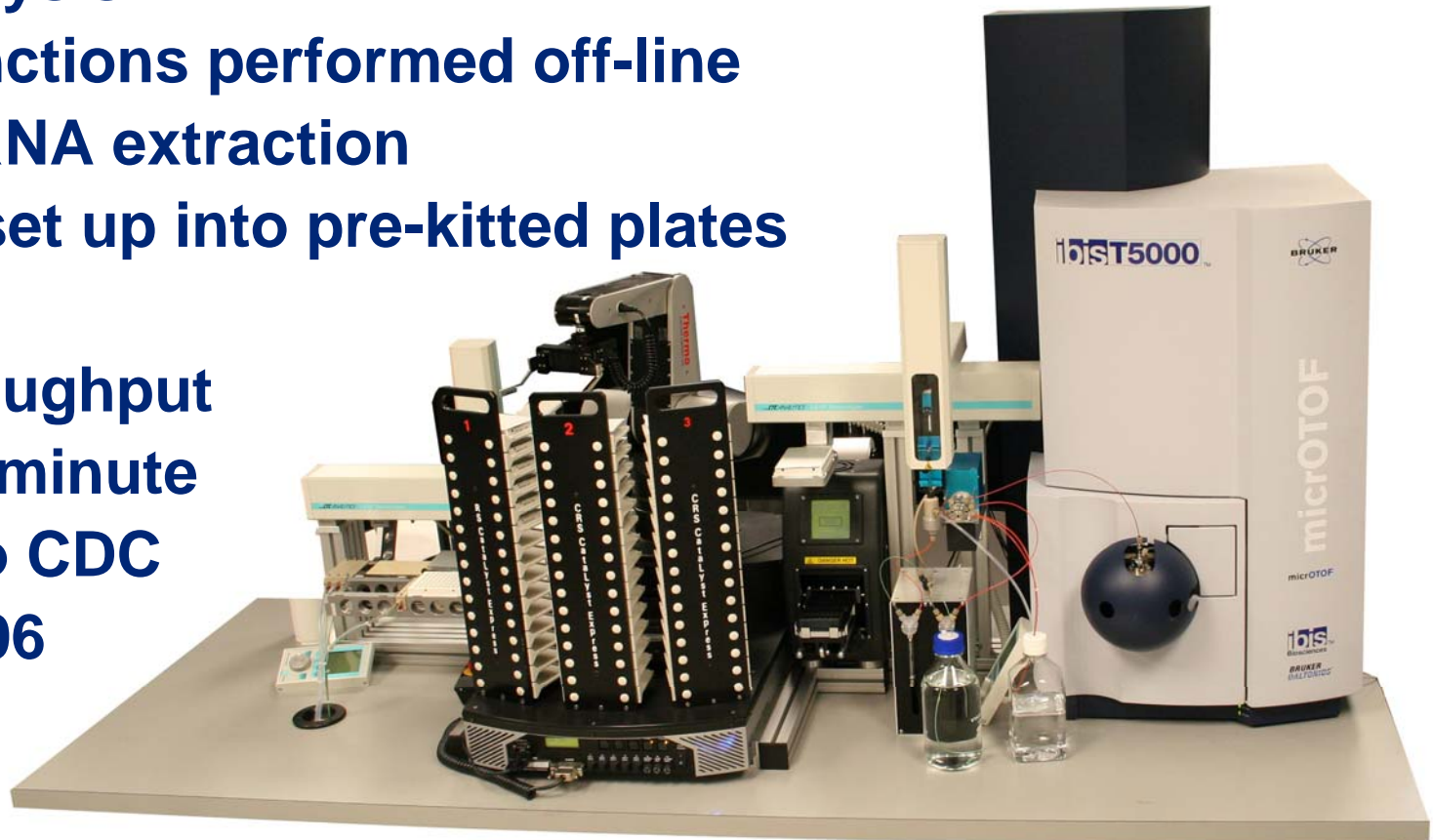
(Johns Hopkins)



Hospital infection control ('07)

Ibis T5000

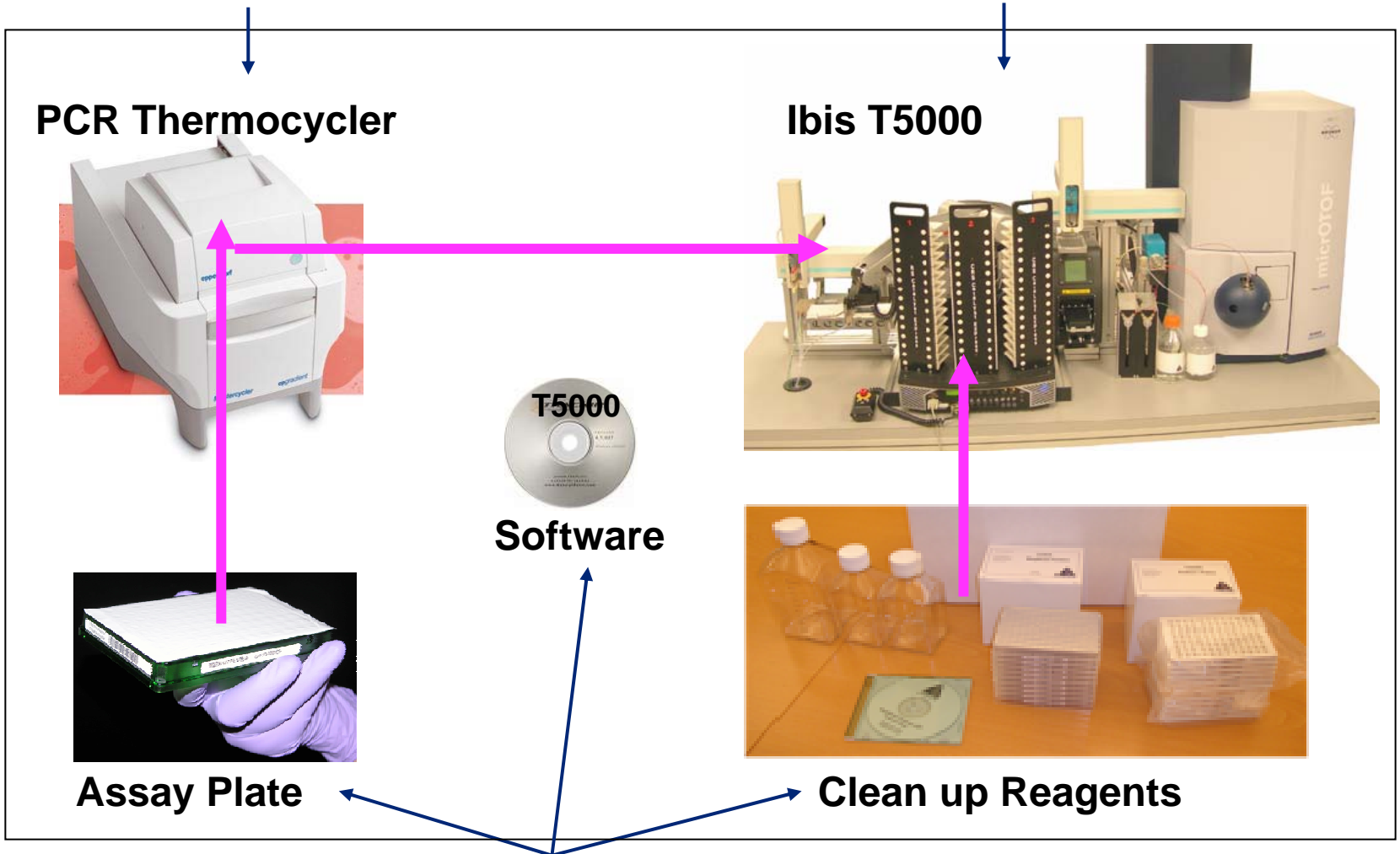
- Amplicon purification
- Automated ESI-TOF analysis
 - Robotic arm moves plates for unattended operation
- Data analysis
- Other functions performed off-line
 - DNA/RNA extraction
 - Plate set up into pre-kitted plates
 - PCR
- High throughput
 - 1 well/minute
- Deploy to CDC
 - Sept '06



T5000 System and Consumables

Customer buys from third party

Bruker will manufacture in 2007



Ibis makes

Ibis Research Team



MS & Automation

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Len Cummins
Jose Gutierrez
Abel Gutierrez
Yun Jiang
Kristin Lowery
Sheri Manalili
Amy Schink
James Hannis

Computational Genomics

Dave Ecker
Ranga Sampath
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Harold Levene
Feng Li
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Joe Ecker
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Rachael Melton
Ray Ranken
Brian Libby
Thuy Pennella
David Duncan
Nancy Fan

Software/Database

Michael Pear
Neill White
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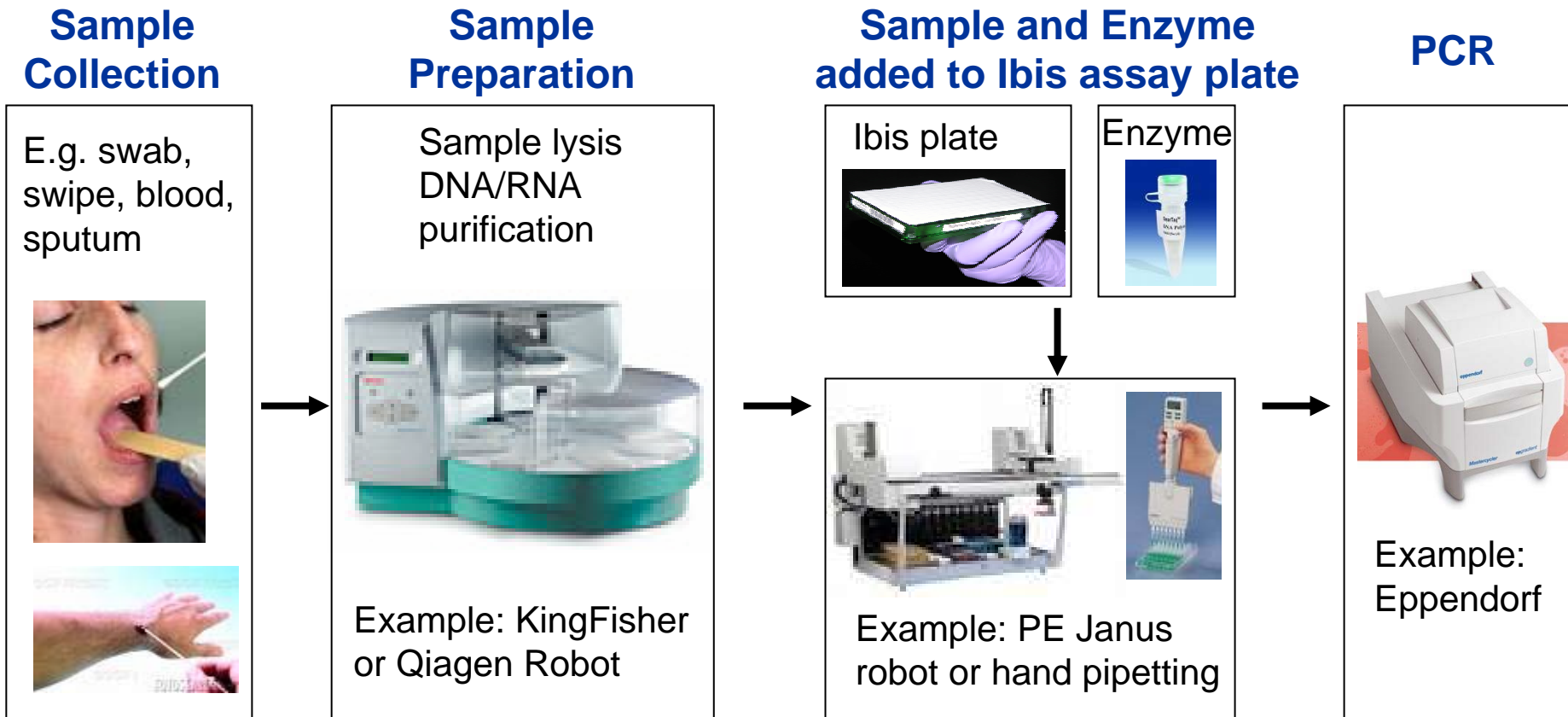
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Terry Leighton
Karl Rudnick
Karen Studarus
Contractors
Fred Hajjar
Robert Palmer



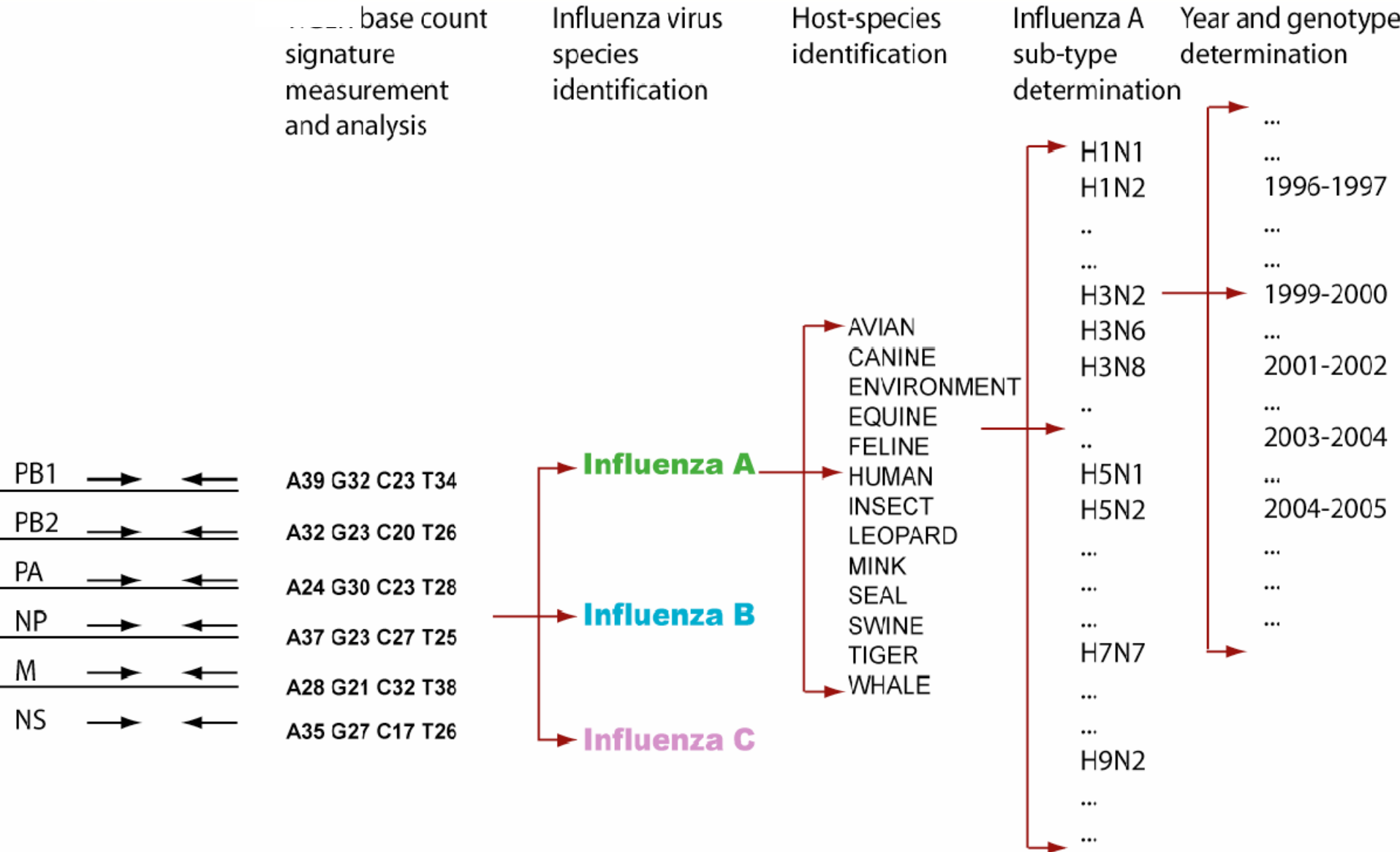
Backup Material

Steps Required Before T5000 Analysis

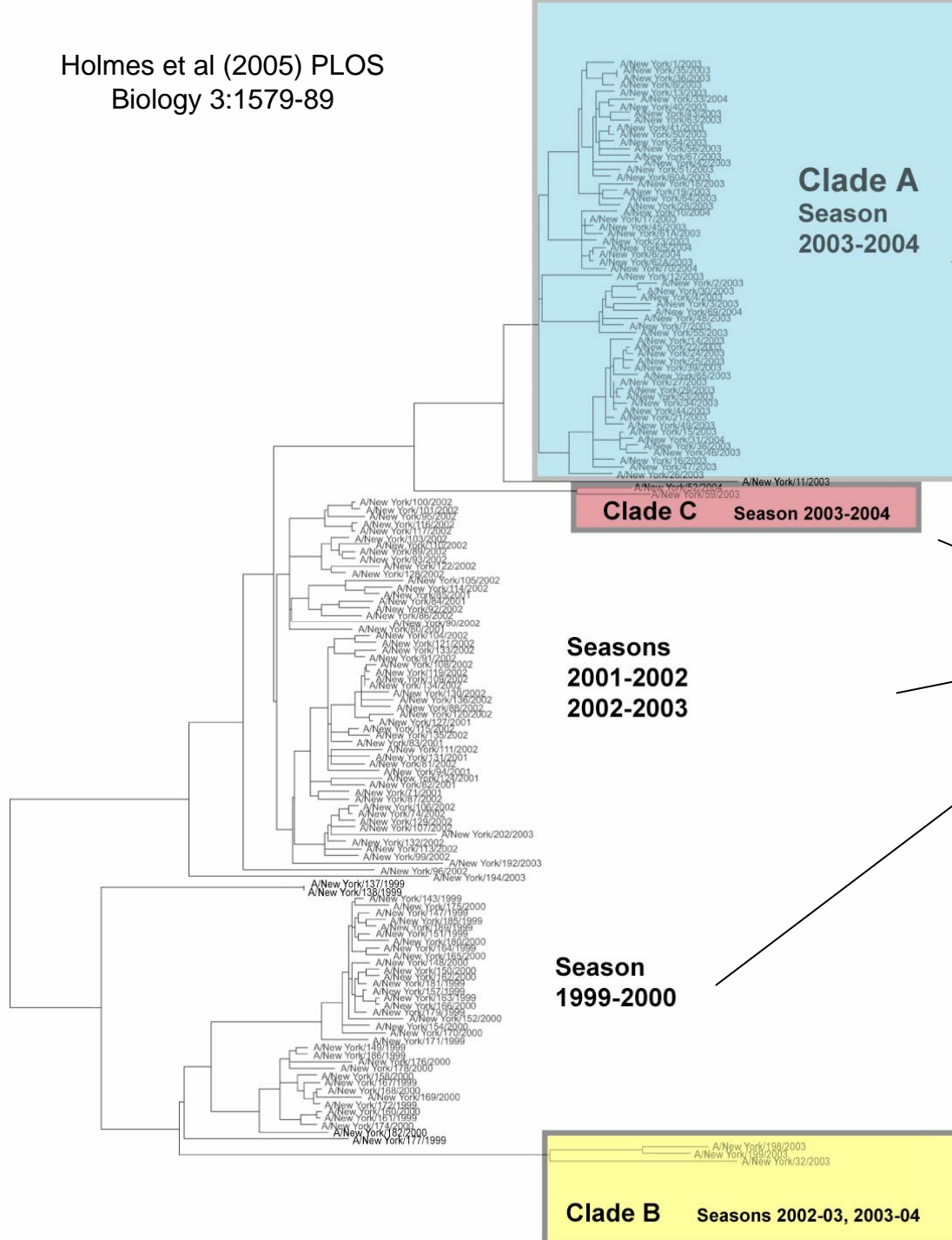


Ibis provides protocols and qualifies enzymes/equipment for use with T5000

Characterization of Influenza Virus



Evolutionary relationship of H3N2 samples 1999-2004: Whole-genome Analysis



**Cladistic
distribution of
circulating H3N2
isolates**

Evolutionary relationship of H3N2 samples 1999-2004:

6-primer Base-composition Analysis

T5000 signatures distinguish H3N2 clades

