2010 JIFSAN Advisory Council Spring Symposium

Advances in Rapid Microbiology Testing Methods

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Office of Regulatory Science, Center for Food Safety & Applied Nutrition
U.S. Food & Drug Administration

March 24, 2010

Major Outbreaks Over the Last 3 Years

YEAR	AGENT	VEHICLE	CASES	SOURCE	STATE
2008	Salmonella Saintpaul	jalapeno/serrano peppers	1442**	Mexico	ML
2008	Salmonella Typhimurium	Peanut butter/peanut paste	677	GA	ML
2006	Salmonella Tennessee	peanut butter	628	GA	ML
2009	*Salmonella* Saintpaul	Sprouts	235		ML
2006	E coli O157:H7	spinach	204	CA	ML
2006	Salmonella Typhimurium	tomatoes	190	OH	ML
2009	Bacillus Cereus	Macaroni and cheese	150	CT	ML
2009	*Salmonella* Typhimurium	Cucumber (suspected*)	118		ML
2006	Salmonella Newport	tomatoes	107	VA	ML
2006	E coli O157:H7	lettuce	81	CA	ML

Serotypes found during Saintpaul pepper investigation - 2008

- Abaetetuba
- Albuquerque
- Anatum
- Braenderup
- Cerro
- Derby
- Give
- Huvudsta
- Infantis
- Javiana
- Koumra
- Madelia
- Mbandaka
- Michigan
- Minnesota
- Muenchen
- Newport
- Ohio
- Oranienburg
- Pomona
- Saintpaul
- Sandiego
- Soahanina
- Tallahassee
- Tucson
- Typhimurium
- Weltevreden
- *S. enterica* subsp. *arizonae*
- *S. enterica* subsp. *diarizonae*
- *S. enterica* subsp. *houtenae*

Additional Significant Outbreaks (last 3 years)

2009	*Salmonella* Orianburg	Round tomatoes	79		ML
2009	E. Coli 0157:H7	Cookie Dough	77		ML
2009	*Salmonella* Rissen	White Pepper Spice (suspected)	72		ML
2009	*Salmonella* Newport	Unknown	72		ML
2006	E coli O157:H7	lettuce	71	CA	ML
2007	Salmonella Wandsworth	veggie booty	69	China/Egypt	ML
2007	Salmonella Newport	tomatoes	65	VA	ML
2008	Salmonella Litchfield	cantaloupe	57	Honduras	ML

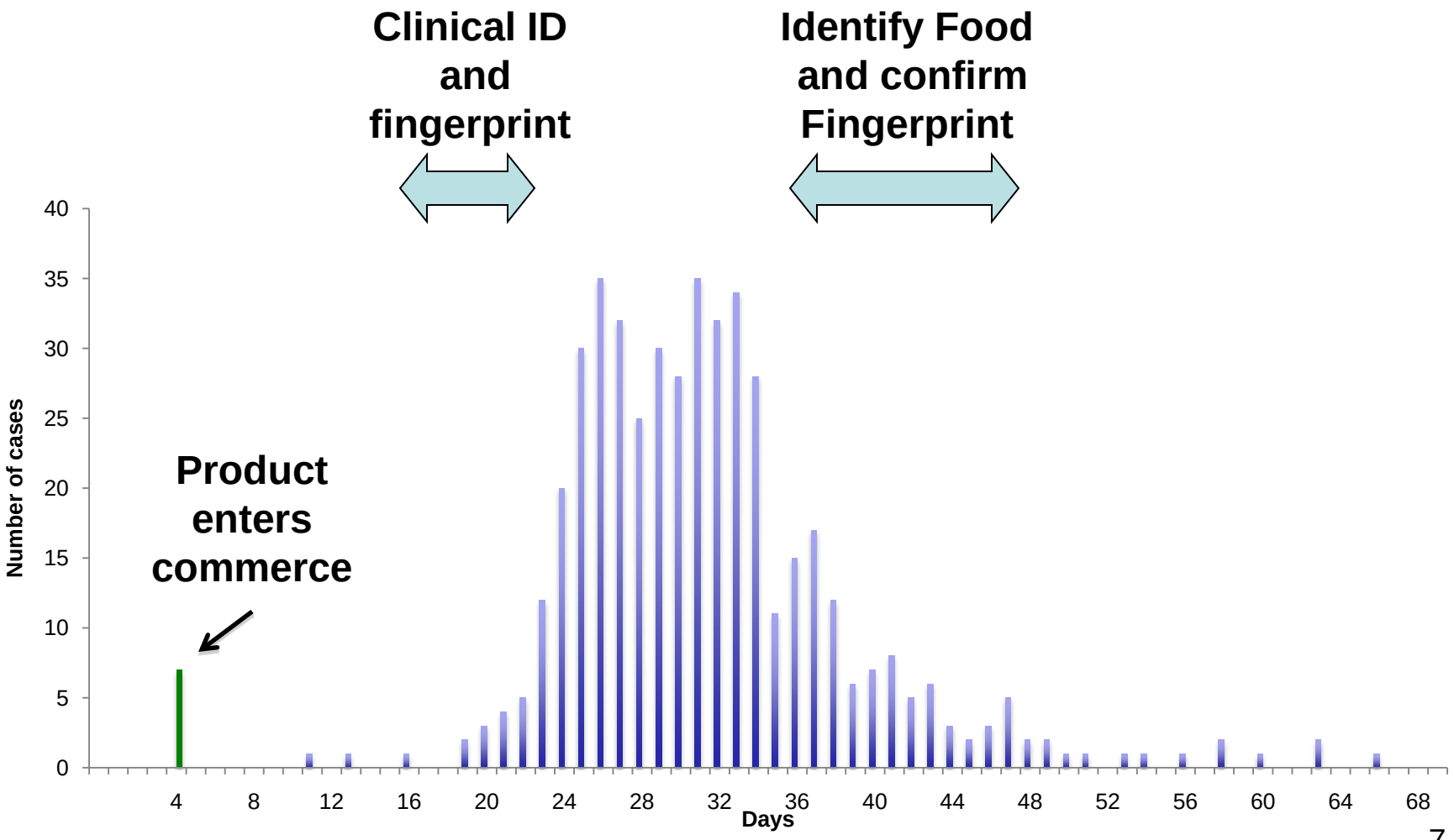
Recent Outbreaks
- Part of an increasing trend

- **There are several possible explanations for the apparent increase**
  - Better and more rapid detection of outbreaks
  - Increase in sale of fresh-cut produce
  - Wider distribution from a more limited production area
  - Globalization of the produce supply
  - Increase in the numbers of consumers at high risk for foodborne illnesses

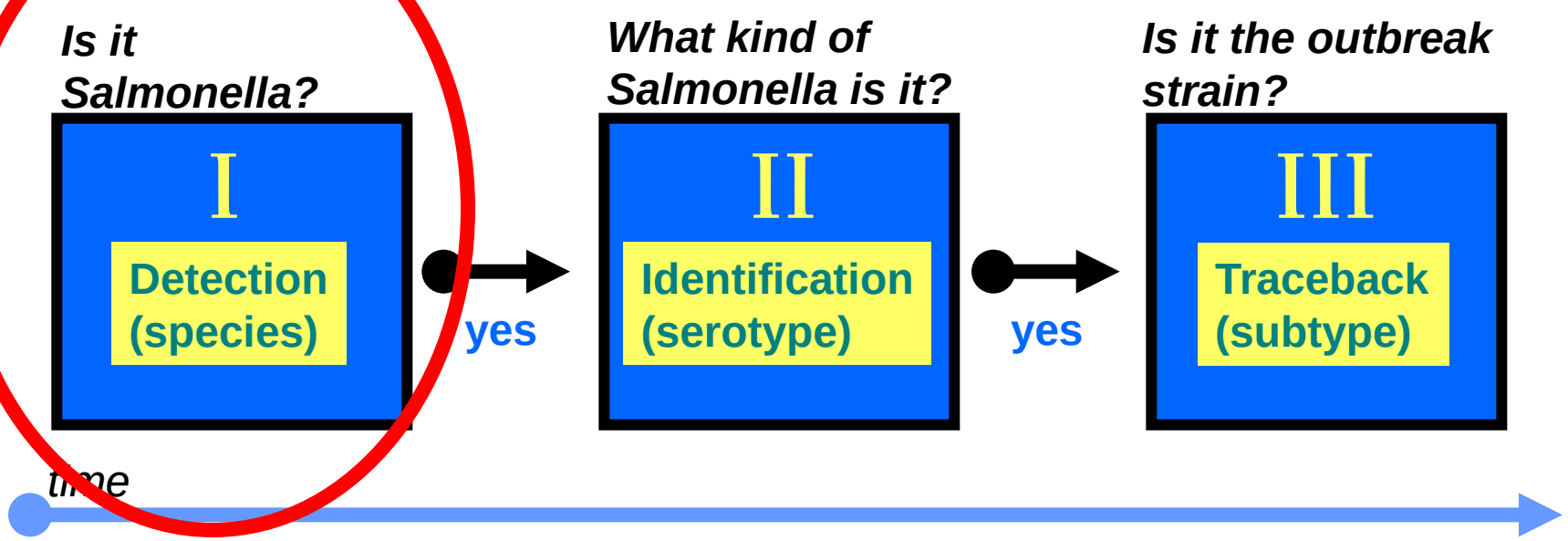
We Need Rapid Methods

- Increase number of samples analyzed for routine surveillance of domestic and imported products
- Tracking/identifying sources of outbreaks (molecular epidemiology)
- Problem 1: Improved sampling and recovery of contaminants
- Problem 2: Rapid fingerprinting of micro-organisms or identification of new chemical toxins

Why do we need rapid methods?



Microbiological investigations of *Salmonella* are a 3-step process:



Mitigation strategies include:

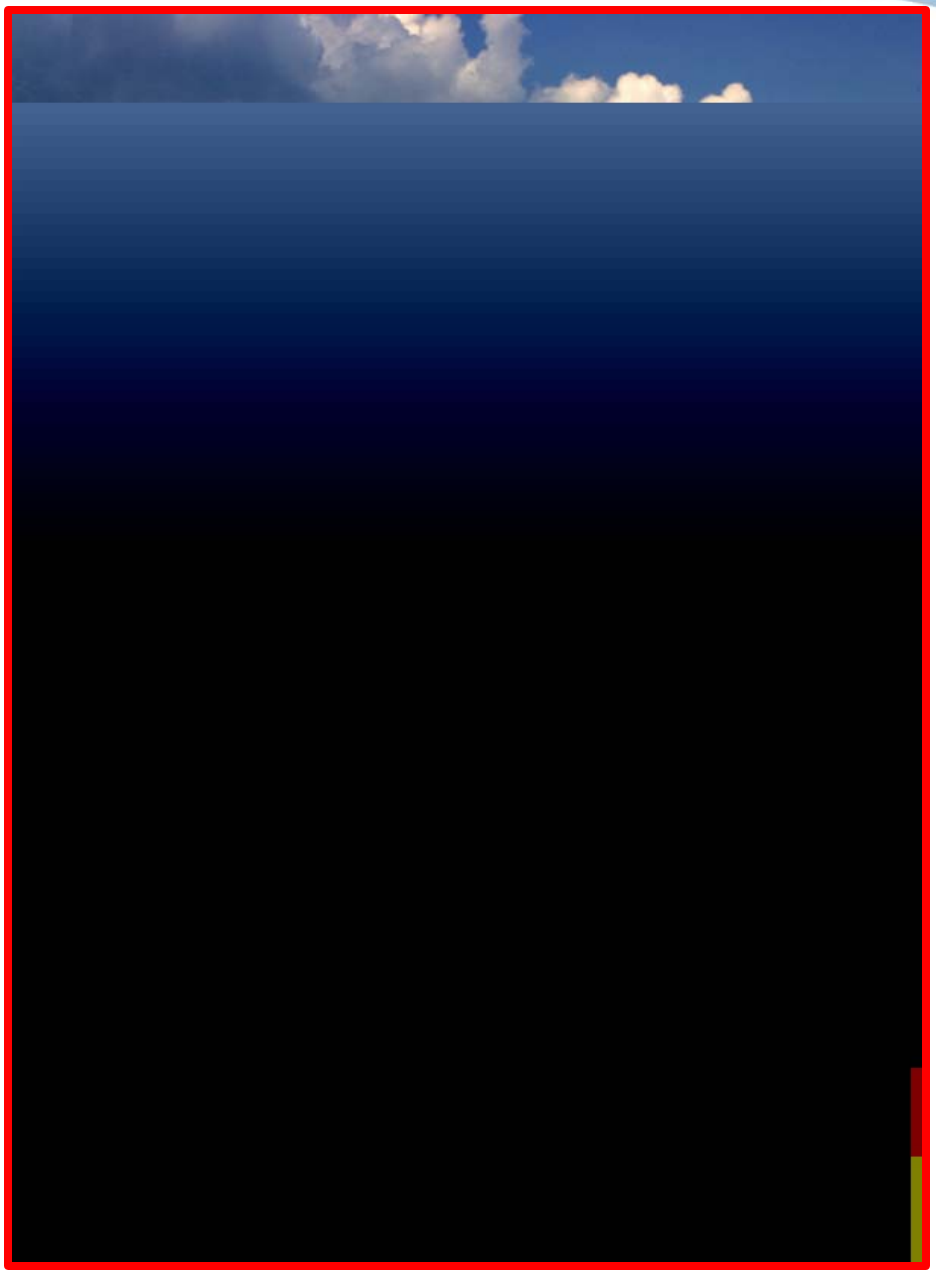
- **Any method that combines two of the three boxes—particularly boxes I and II.**
- **Any method(s) that shrink the width (*i.e.*, timespan) of one or more boxes**

"The Smokin' Jalapeno"

(Firm ran out of plastic cover for bedding-Sample collected scored positive for *Salmonella* Saintpaul)

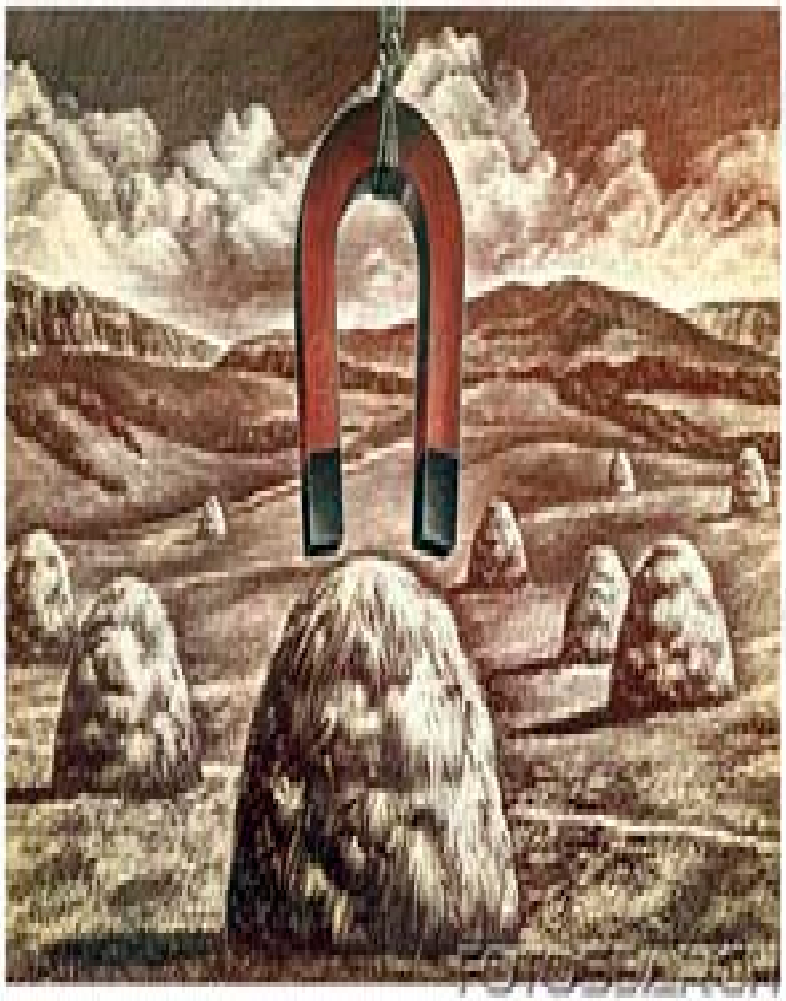
Another Kind of 'Risk Assessment'

- **Indigenous microbial population on produce- 10^2 to 10^{10} cfu/g**

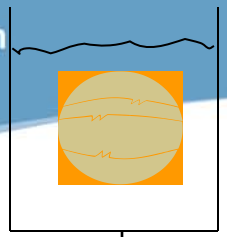


Needle in the Haystack

- *Escherichia coli 0157:H7*
- *STEC*
- *Listeria*
- *Salmonella*
- *Shigella*
- *Enterobacter (Chronobacter) sakazakii*
- Fungi
- *Staphylococcus aureus* (enterotoxin)
- *Clostridium (toxins)*
- *Francisella tularensis*
- *Bacillus anthracis, B. cereus*



An Improved Soak Method for Canteloupes suspect for Salmonella



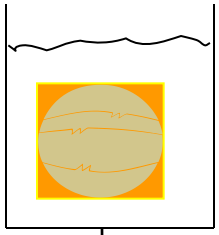
Cantaloupe in bag with preenrichment broth

Shake, 100 rpm 5 min

Soak

Rinse (25 mL)

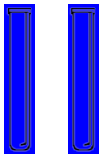
Pre-enrichment

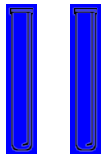


24 h, 35ºC

24 h, 35ºC

Selective enrichment

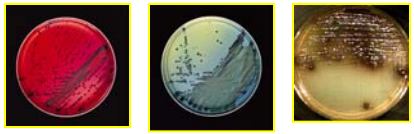


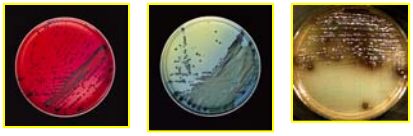


24 h, 35ºC and 42ºC

24 h, 35ºC and 42ºC

Selective plating





Schematic of Revised Method - ESAK

PIF

25 g

Incubate 6 hrs
@ 37ºC

225 ml H_2O/BPW

10 ml

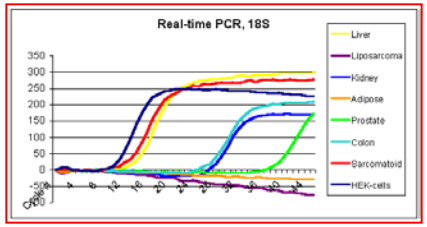
(concurrently)
continue PIF/H_2O
Incubation O/N
at 37ºC

Centrifuge 5000 rpm for 10 min
Decant supernatant
Resuspend pellet in 100 ul H_2O/BPW

Plate on Chromogenic Agar

Incubate
Overnight
@ 37ºC

Confirm with RT-PCR, RapidID 32 E, Class Fatty Acid Analysis



Molecular Detection Methods

Conventional and Real-Time PCR allows us to target specific bacterial pathogens despite the background microbial load

BioRad
IQ5

Roche
Light Cycler

Corbett
Rotorgene

Cepheid
Smart Cycler

Roche TaqMan

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2009, p. 3714–3720 Vol. 75, No. 11
0099-2240/09/$08.00+0 doi:10.1128/AEM.02686-08

Detection of Live *Salmonella* sp. Cells in Produce by a TaqMan-Based Quantitative Reverse Transcriptase Real-Time PCR Targeting *invA* mRNA▽†

Narjol González-Escalona,* Thomas S. Hammack, Mindi Russell, Andrew P. Jacobson, Antonio J. De Jesús, Eric W. Brown, and Keith A. Lampel

Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland

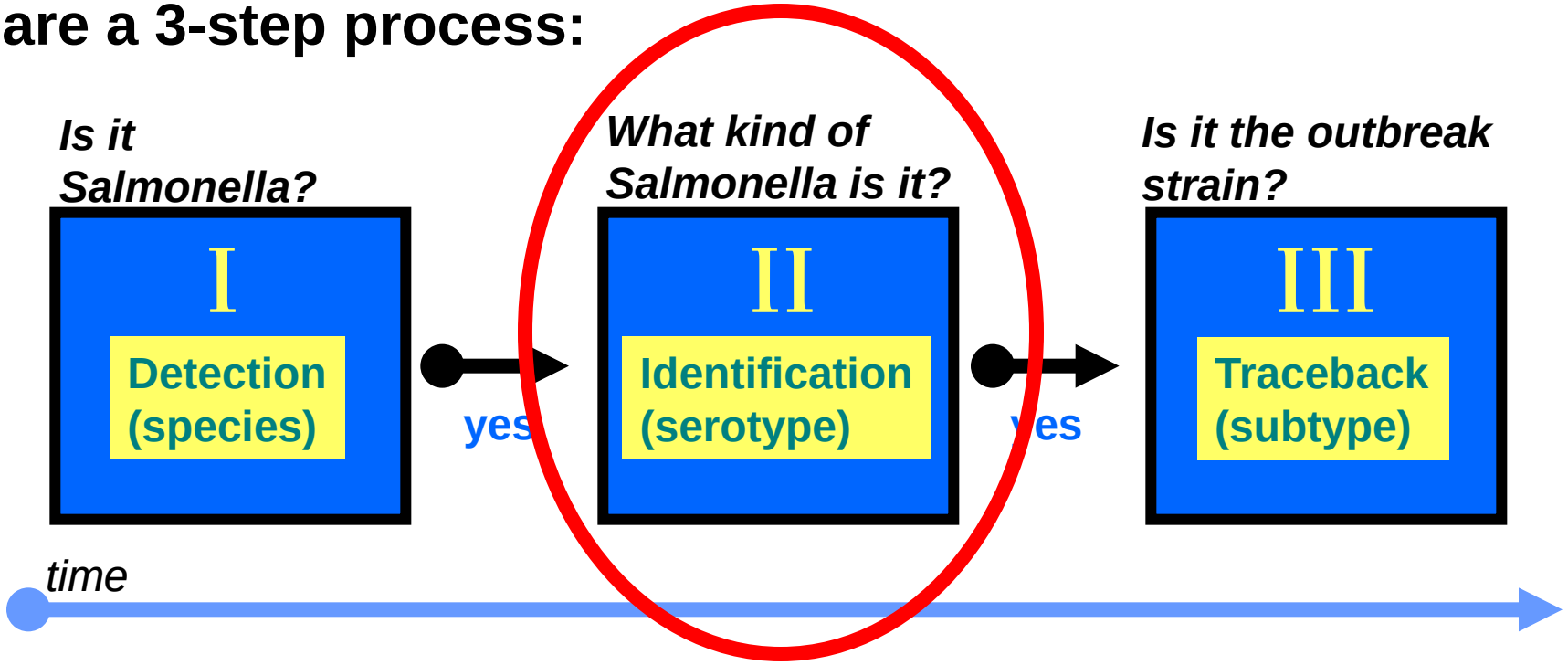
Received 24 November 2008/Accepted 3 April 2009

Salmonella enterica **contamination in foods is a significant concern for public health. When DNA detection methods are used for analysis of foods, one of the major concerns is false-positive results from the detection of dead cells. To circumvent this crucial issue, a TaqMan quantitative real-time RT-PCR (qRT-PCR) assay with an RNA internal control was developed. *invA* RNA standards were used to determine the detection limit of this assay as well as to determine *invA* mRNA levels in mid-exponential-, late-exponential-, and stationary-phase cells. This assay has a detection limit of 40 copies of *invA* mRNA per reaction. The levels of *invA* mRNA in mid-exponential-, late-exponential-, and stationary-phase *S. enterica* cells was approximately 1 copy per 3 CFU, 1 copy per CFU, and 4 copies per 10^3 CFU, respectively. Spinach, tomatoes, jalapeno peppers, and serrano peppers were artificially contaminated with four different *Salmonella* serovars at levels of 10^5 and less than 10 CFU. These foods were analyzed with qRT-PCR and with the FDA's *Bacteriological Analytical Manual Salmonella* culture method (W. A. Andrews and T. S. Hammack, *in* G. J. Jackson et al., ed., Bacteriological analytical manual online, http://www.cfsan.fda.gov/~ebam/bam-5.html, 2007). Comparable results were obtained by both methods. Only live *Salmonella* cells could be detected by this qRT-PCR assay, thus avoiding the dangers of false-positive results from nonviable cells. False negatives (inhibition of the PCR) were also ruled out through the use of an RNA internal control. This assay allows for the fast and accurate detection of viable *Salmonella* spp. in spinach, tomatoes, and in both jalapeno and serrano peppers.**

Salmonella enterica contamination in various foods is a significant public health concern, domestically and internationally (22, 29, 37). *Salmonella* infects millions of people every year, accounting for an estimated 9.7%, 25.6%, and 30.6% of illnesses, hospitalizations, and deaths, respectively, of the total U.S. food-borne diseases caused by known food-borne pathogens (29). Consumption of fresh fruits and produce increased almost 50% between 1979 and 1994 (38). Fresh produce is exists for the development of faster culture-independent screening and detection methods for this pathogen in produce.

In recent years, a plethora of new molecular methods based on *Salmonella* DNA detection (e.g., *invA* gene) either by conventional or real-time PCR have been developed (23, 27, 41). Real-time PCR (quantitative PCR [qPCR]) is faster and more sensitive than conventional PCR and provides real-time data,

Microbiological investigations of *Salmonella* are a 3-step process:



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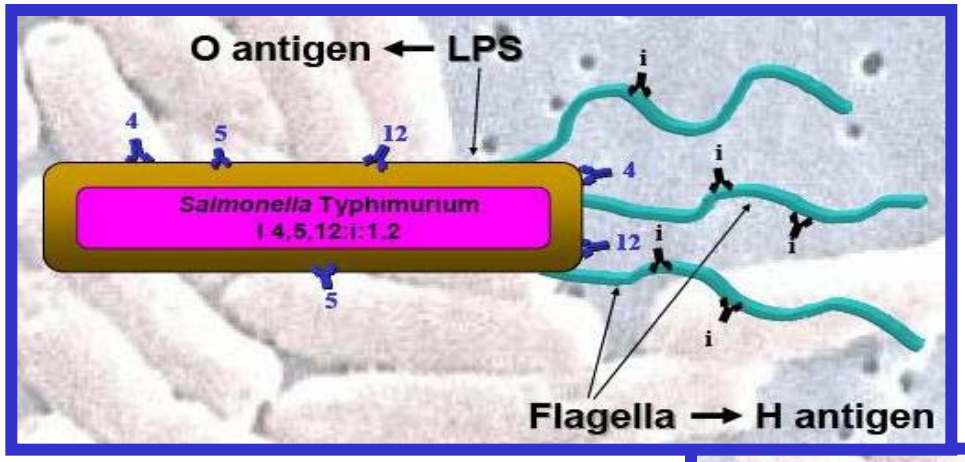
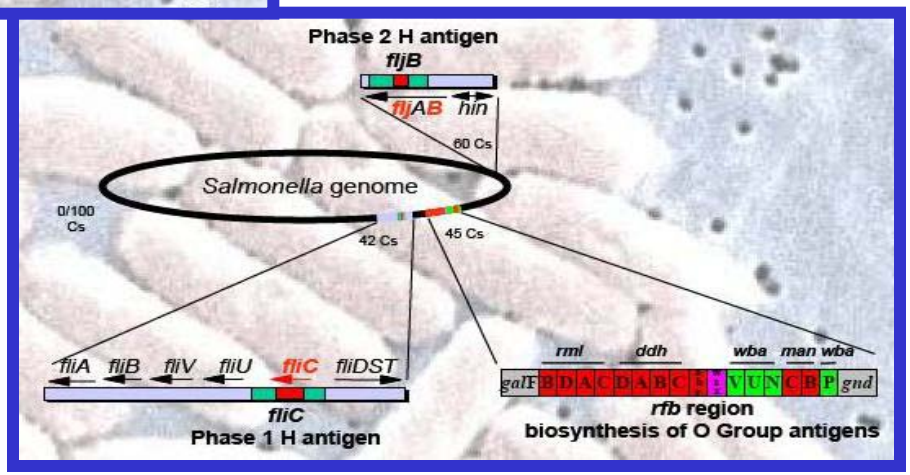
CDC Molecular Serotyping Protocol



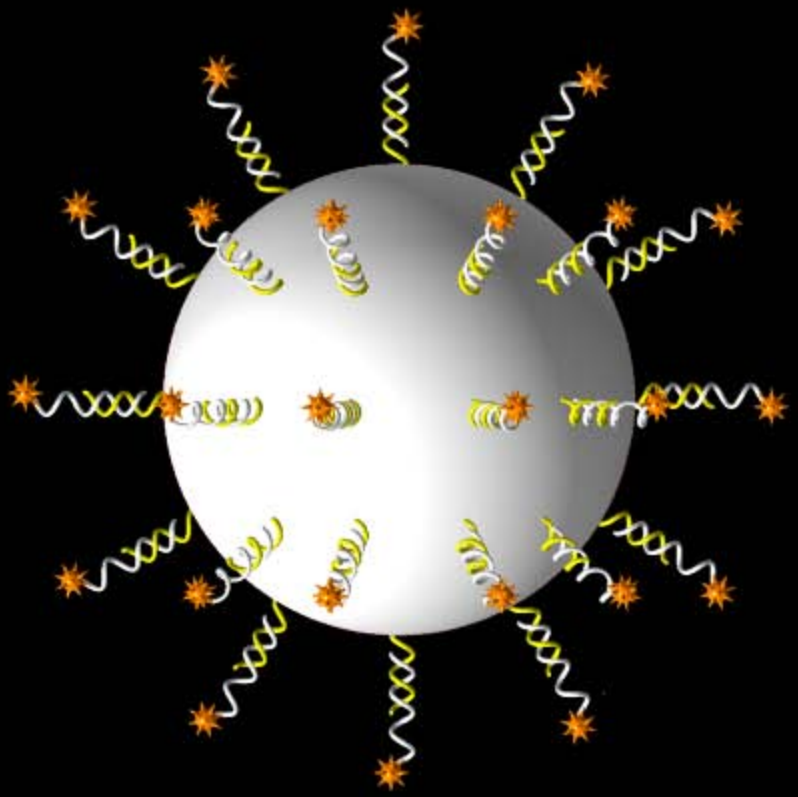
Molecular serotyping using xMAP

Identification of major *Salmonella* serotypes -CDC



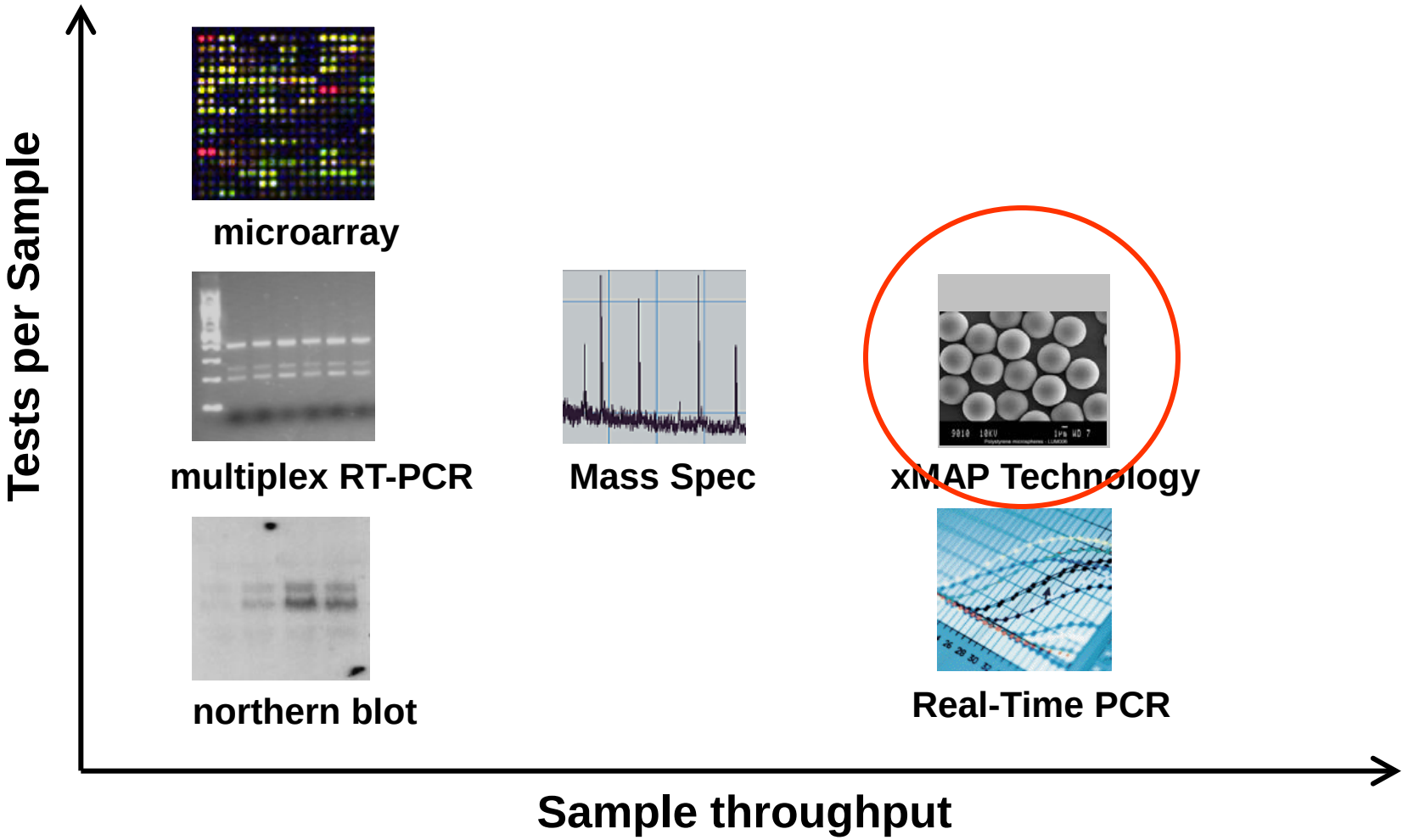
Bioplex – based serological identification

X-MAP TECHNOLOGY



BIOPLEX/LUMINEX PLATFORM

New Frontiers in Molecular Epidemiology

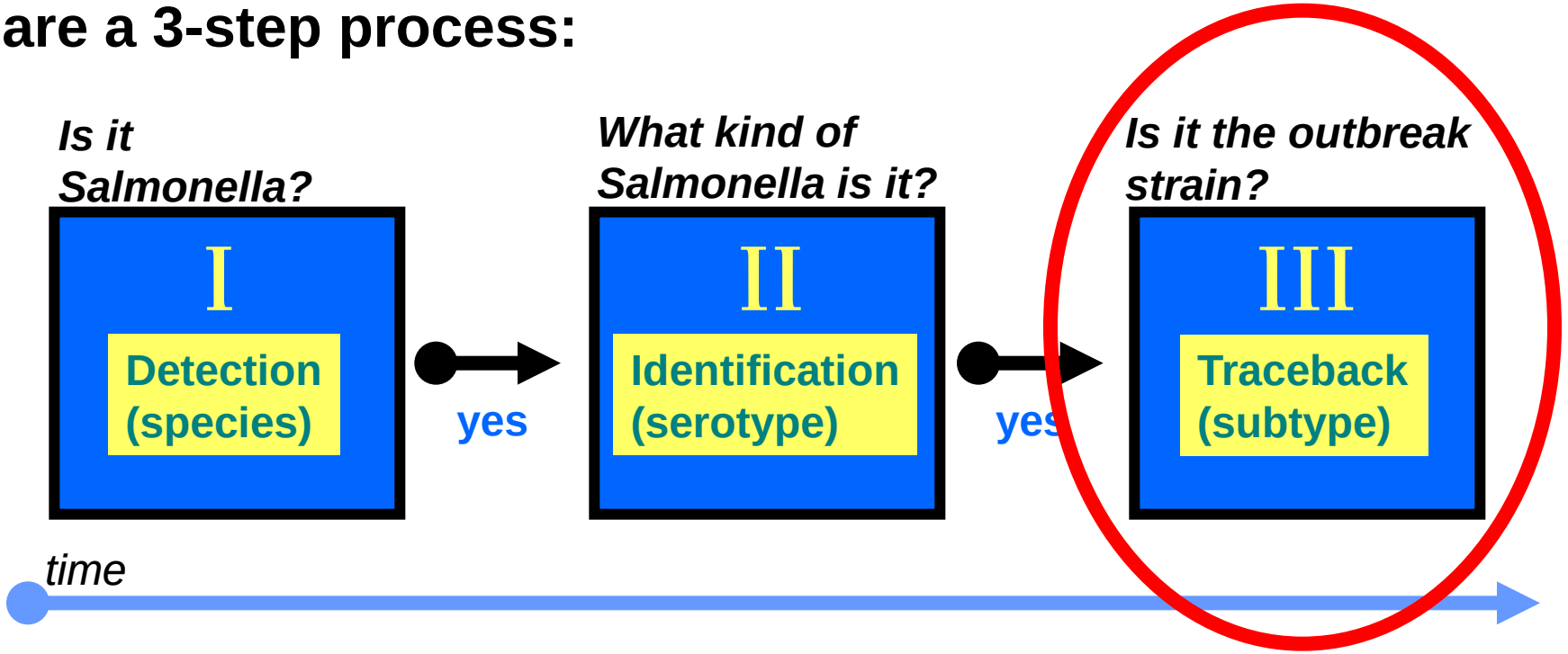


qPremi® Test Salmonella

- RT-PCR mini-array
- Portable and rapid (<2 hrs)
- Digital signal processing
- Traditional qPCR chemistry

PREMITEST

Microbiological investigations of *Salmonella* are a 3-step process:



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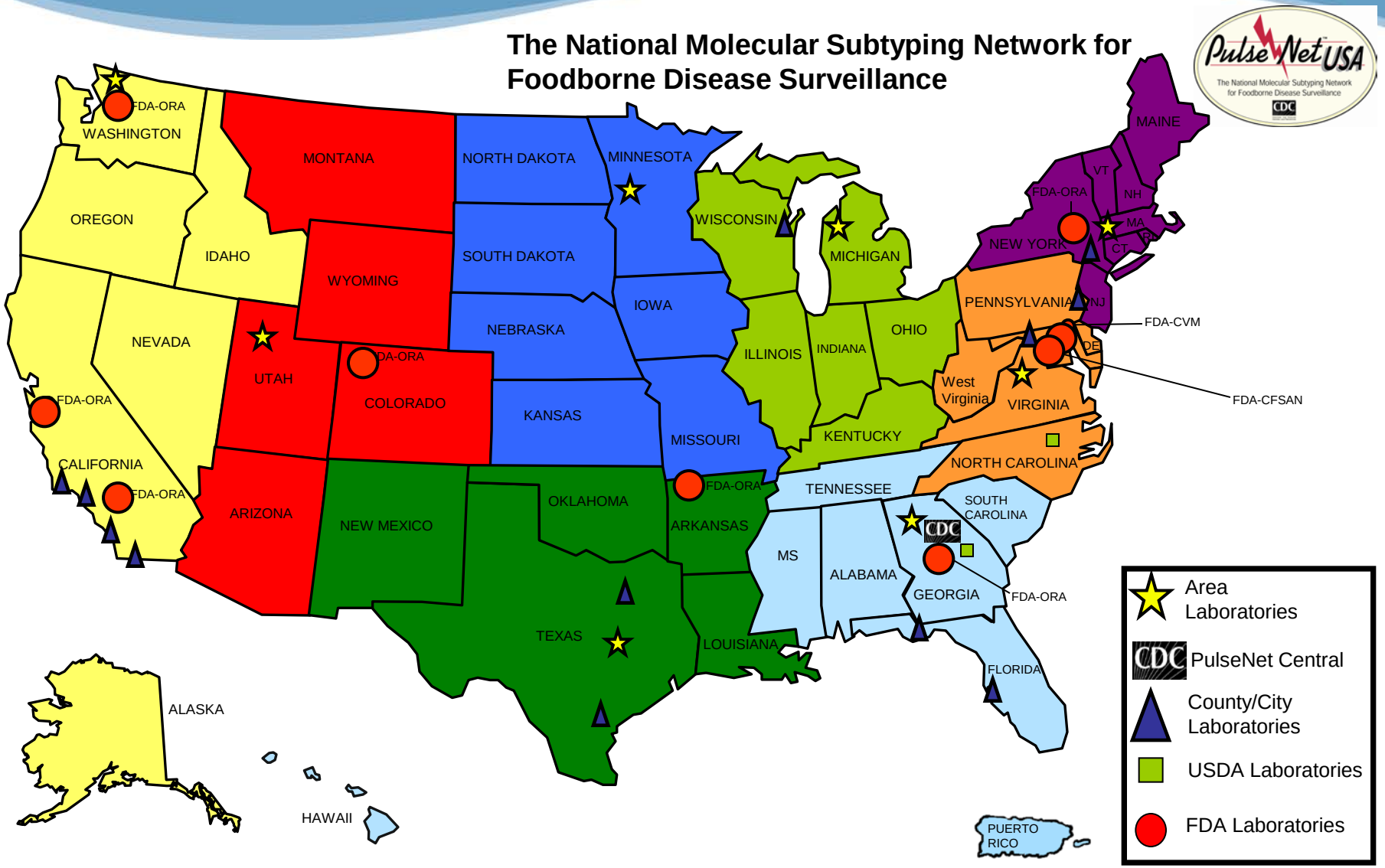
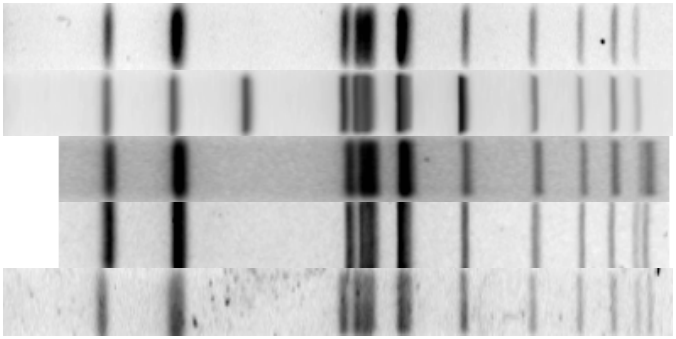
The National Molecular Subtyping Network for Foodborne Disease Surveillance



West | Mountain | South Central | North Central | Midwest | Mid-Atlantic | Southeast | Northeast

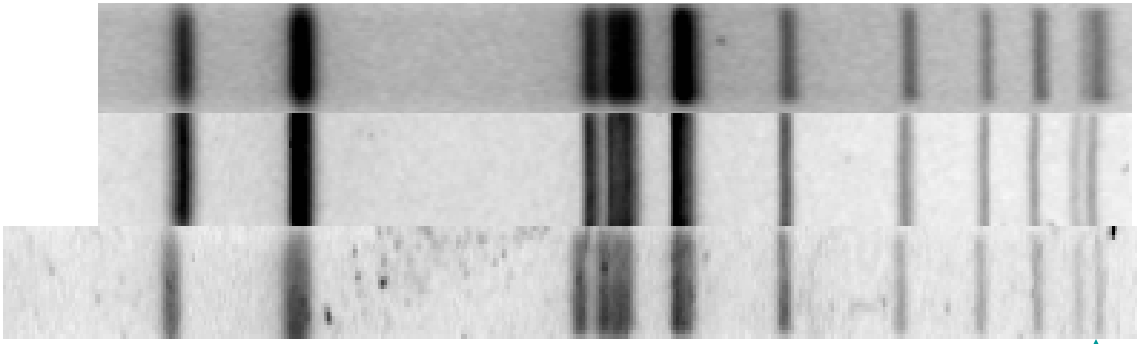
May 2005 *S*. Enteritidis food-related clusters



JEGX01.0004	NC and VT (eggs?)
JEGX01.0005	UT food
JEGX01.0018	GA hospital (eggs)
JEGX01.0021	SC buffet restaurant (eggs)
JEGX01.0056	GA hospital (eggs)

Pattern JEGX01.0004 makes up 40% of all of the *S*. Enteritidis seen in the PulseNet database.

Three very common *S*. Enteritidis patterns that are difficult to differentiate. They are often found together or in concurrent outbreaks.



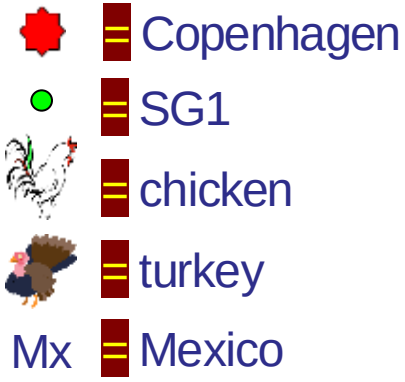
JEGX01.0018

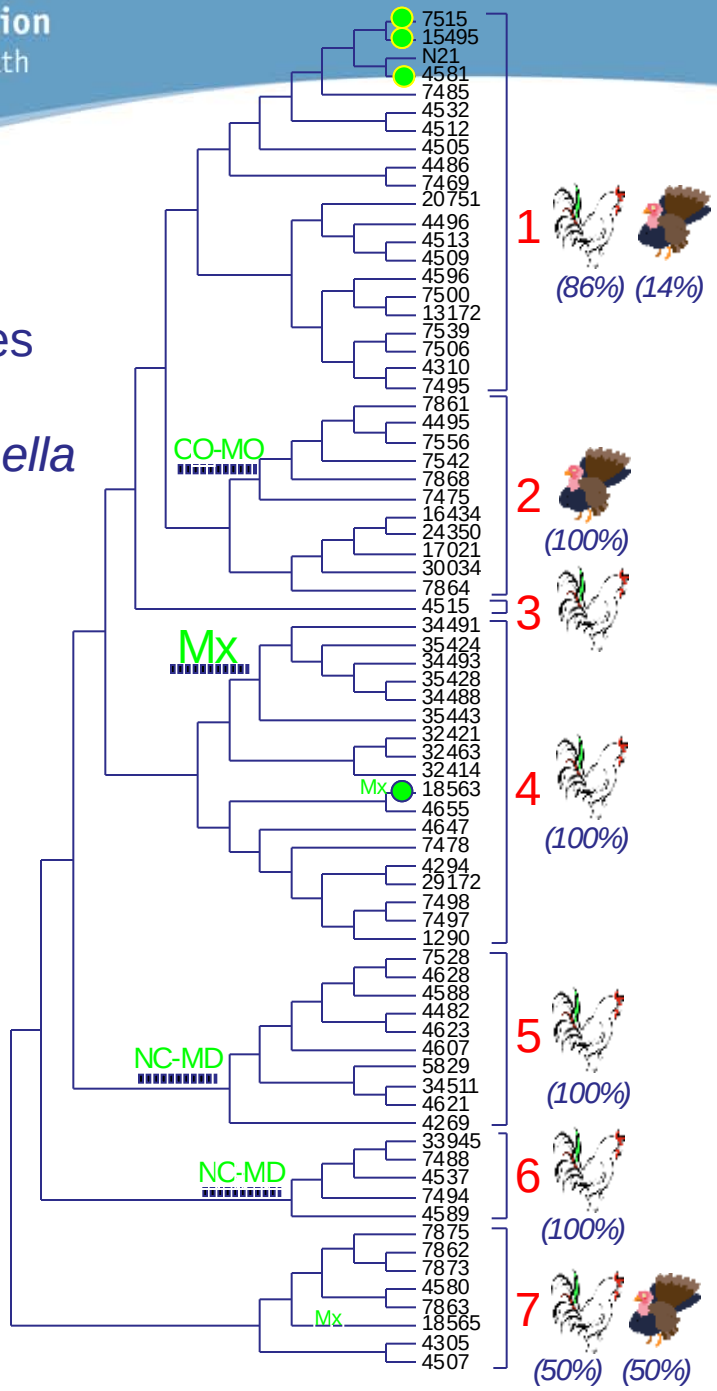
JEGX01.0021

JEGX01.0056

Difference is in a band-shift at ~40 kb

"Simultaneous analysis of combined enzymes provides insight into sources and lineages of epidemic clones of *Salmonella* Typhimurium"

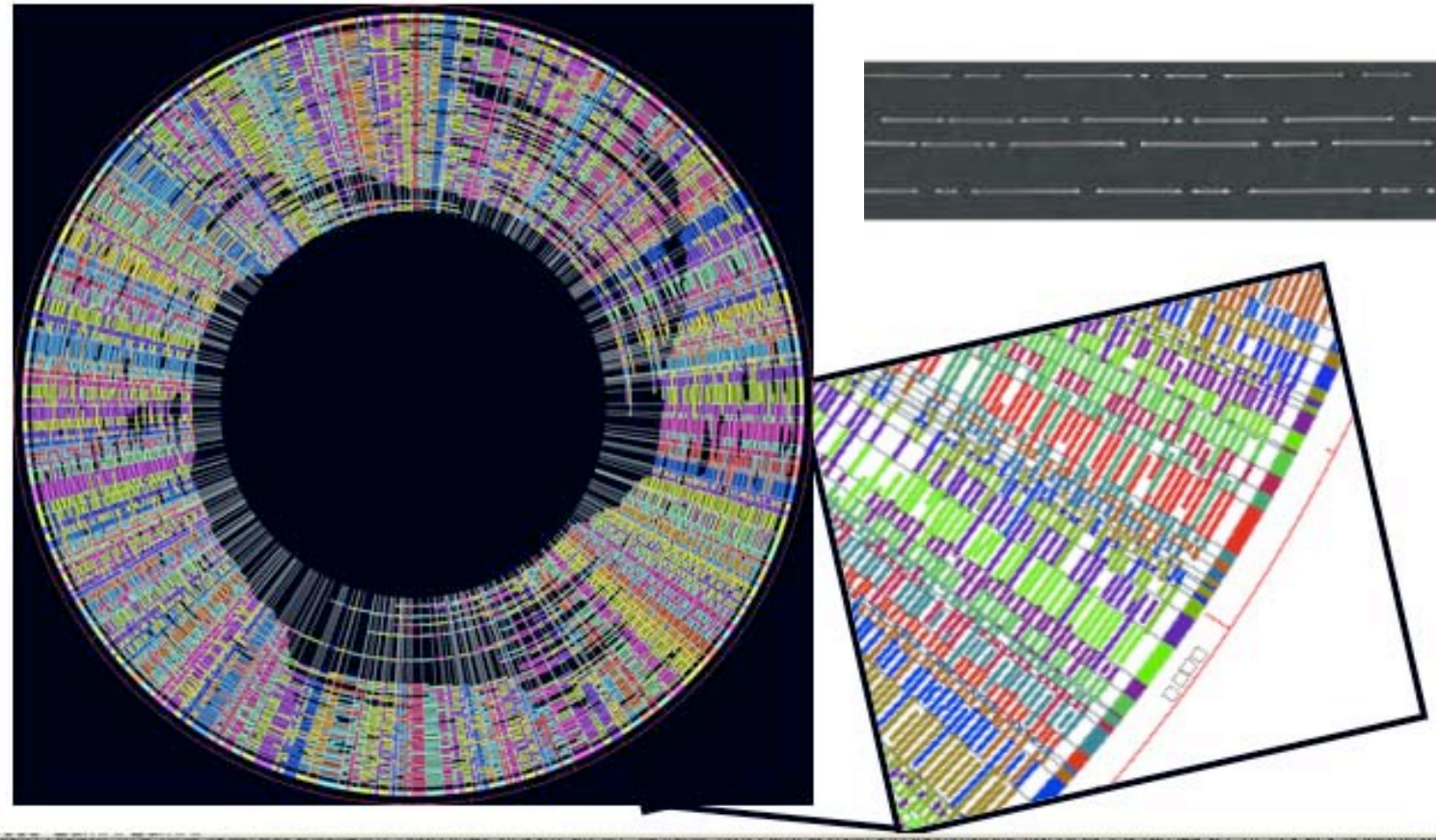




Optical Mapping

Courtesy of Dr.
Michael Kotewicz
CFSAN-OARSA

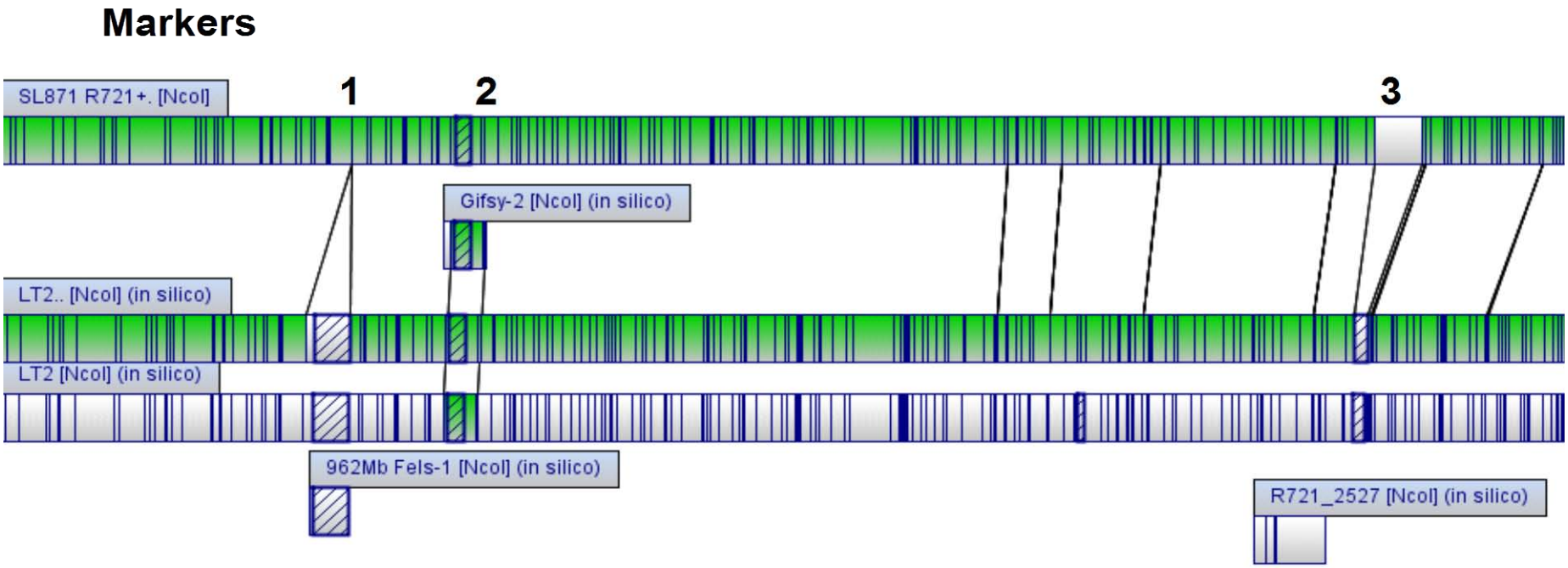
Multiple contigs; assembly



Linear representation of bacterial genome "consensus map"

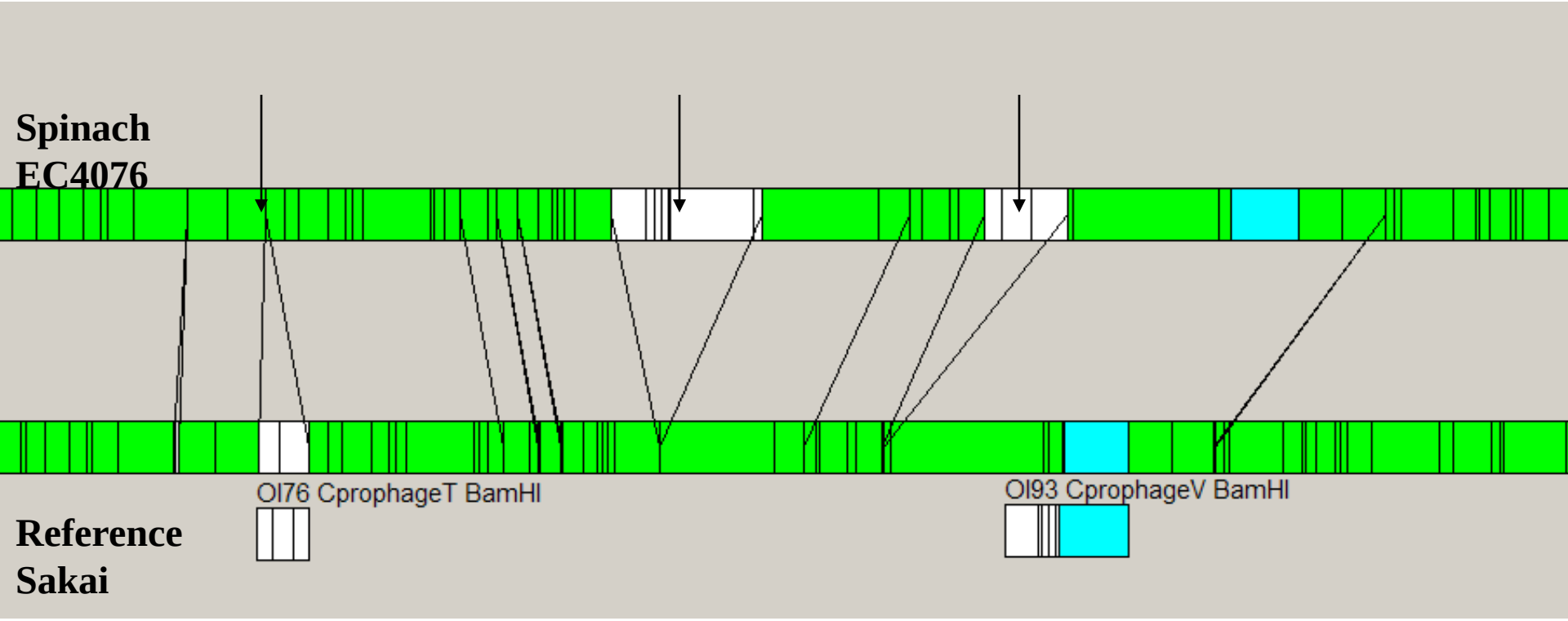
Optical Mapping

Peanut paste strain SL871 chromosomal markers 1-7
(PFGE type 0459, contains genes from plasmid R721)



Optical Mapping:
Using the right tool at the right time;

Insertions and deletions

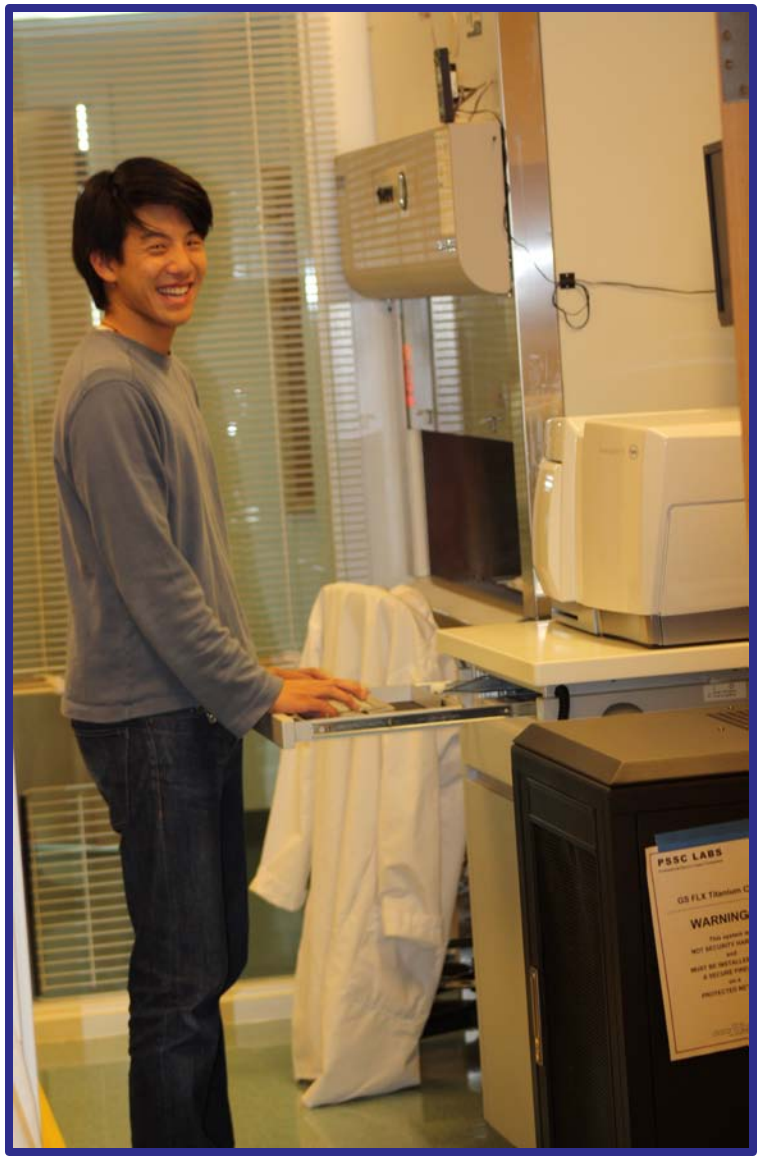


Whole Genome Sequencing

454 Technologies whole-genome sequencing systems:

- **allows for multiple coverage of a complete bacterial pathogen's genome in 3 days**
- **provides raw data for SNP discovery**
- **allows for identification of rapidly changing genetic markers**

From where and how are genotypic targets derived?

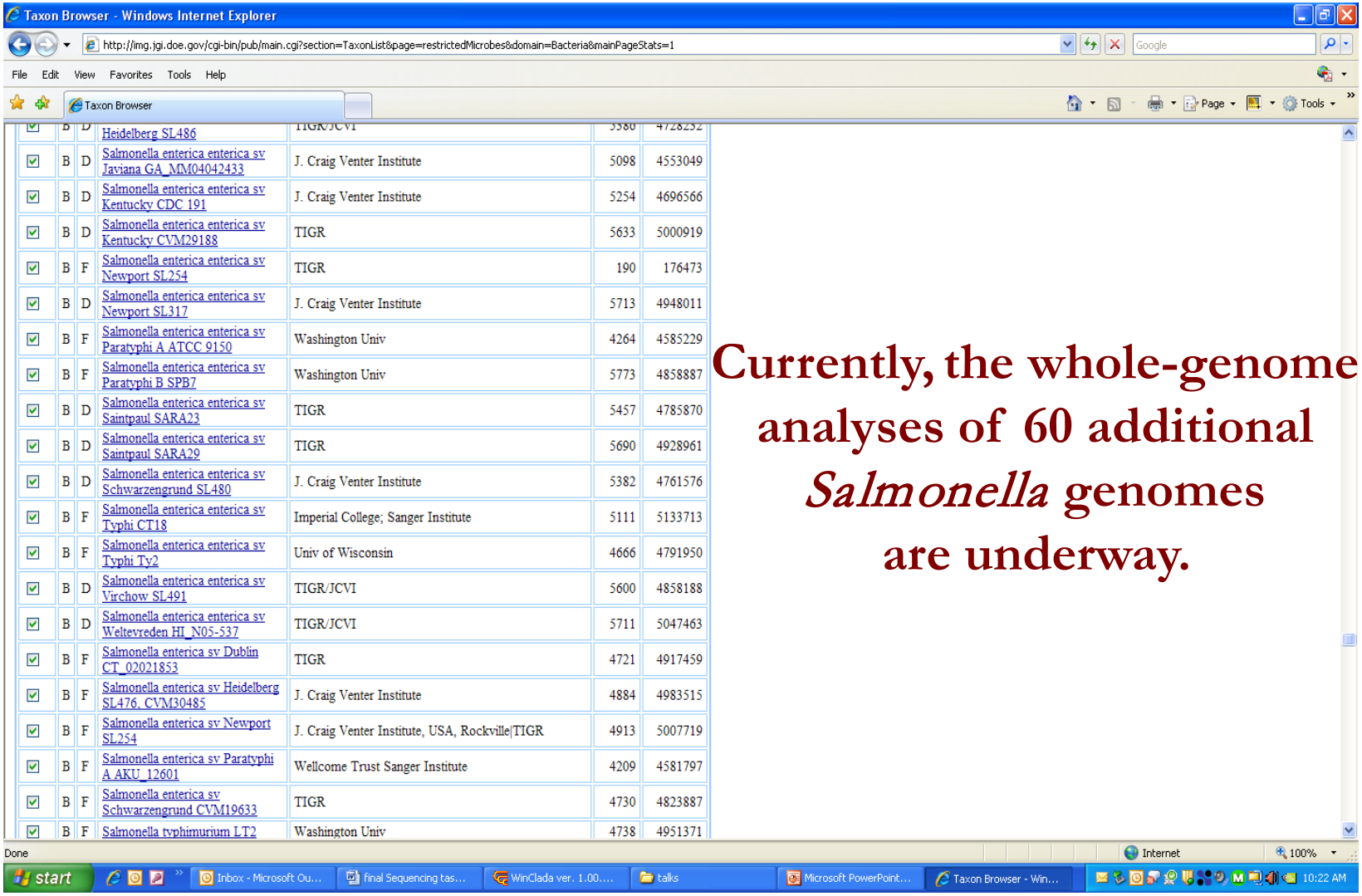


New instrumentation installed in Fall 2009

Ability to collect 4 microbial genomes per day.

Charlie Wang

Genome scanning strategies:



☑	B	D	Heidelberg SL486	TIGR/JCVI	5380	4728252
☑	B	D	Salmonella enterica enterica sv Javiana GA_MM04042433	J. Craig Venter Institute	5098	4553049
☑	B	D	Salmonella enterica enterica sv Kentucky CDC 191	J. Craig Venter Institute	5254	4696566
☑	B	D	Salmonella enterica enterica sv Kentucky CVM29188	TIGR	5633	5000919
☑	B	F	Salmonella enterica enterica sv Newport SL254	TIGR	190	176473
☑	B	D	Salmonella enterica enterica sv Newport SL317	J. Craig Venter Institute	5713	4948011
☑	B	F	Salmonella enterica enterica sv Paratyphi A ATCC 9150	Washington Univ	4264	4585229
☑	B	F	Salmonella enterica enterica sv Paratyphi B SPB7	Washington Univ	5773	4858887
☑	B	D	Salmonella enterica enterica sv Saintpaul SARA23	TIGR	5457	4785870
☑	B	D	Salmonella enterica enterica sv Saintpaul SARA29	TIGR	5690	4928961
☑	B	D	Salmonella enterica enterica sv Schwarzengrund SL480	J. Craig Venter Institute	5382	4761576
☑	B	F	Salmonella enterica enterica sv Typhi CT18	Imperial College; Sanger Institute	5111	5133713
☑	B	F	Salmonella enterica enterica sv Typhi Ty2	Univ of Wisconsin	4666	4791950
☑	B	D	Salmonella enterica enterica sv Virchow SL491	TIGR/JCVI	5600	4858188
☑	B	D	Salmonella enterica enterica sv Weltevreden HI_N05-537	TIGR/JCVI	5711	5047463
☑	B	F	Salmonella enterica sv Dublin CT_02021853	TIGR	4721	4917459
☑	B	F	Salmonella enterica sv Heidelberg SL476, CVM30485	J. Craig Venter Institute	4884	4983515
☑	B	F	Salmonella enterica sv Newport SL254	J. Craig Venter Institute, USA, Rockville|TIGR	4913	5007719
☑	B	F	Salmonella enterica sv Paratyphi A AKU_12601	Wellcome Trust Sanger Institute	4209	4581797
☑	B	F	Salmonella enterica sv Schwarzengrund CVM19633	TIGR	4730	4823887
☑	B	F	Salmonella typhimurium LT2	Washington Univ	4738	4951371

Currently, the whole-genome analyses of 60 additional *Salmonella* genomes are underway.

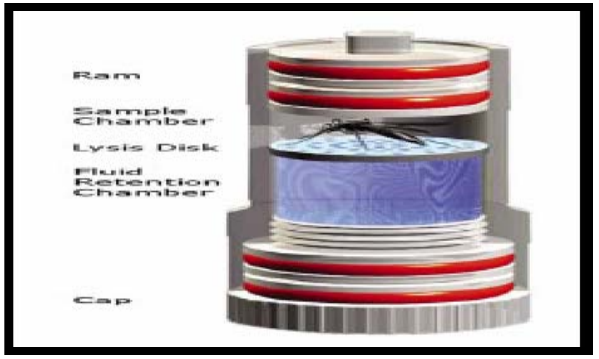
Genome scanning strategies:

Bioinformatic tools are now under development to meet these data density tools including alignment tools, search engines, and genome assembly software.
Dr. Kurt Lienau

Phenotypic typing strategies:

(1)Phenotype Microarray

(2)LC-MS Protein Profiling



(3)Antibiotic Resistance Profiling
~NARMS

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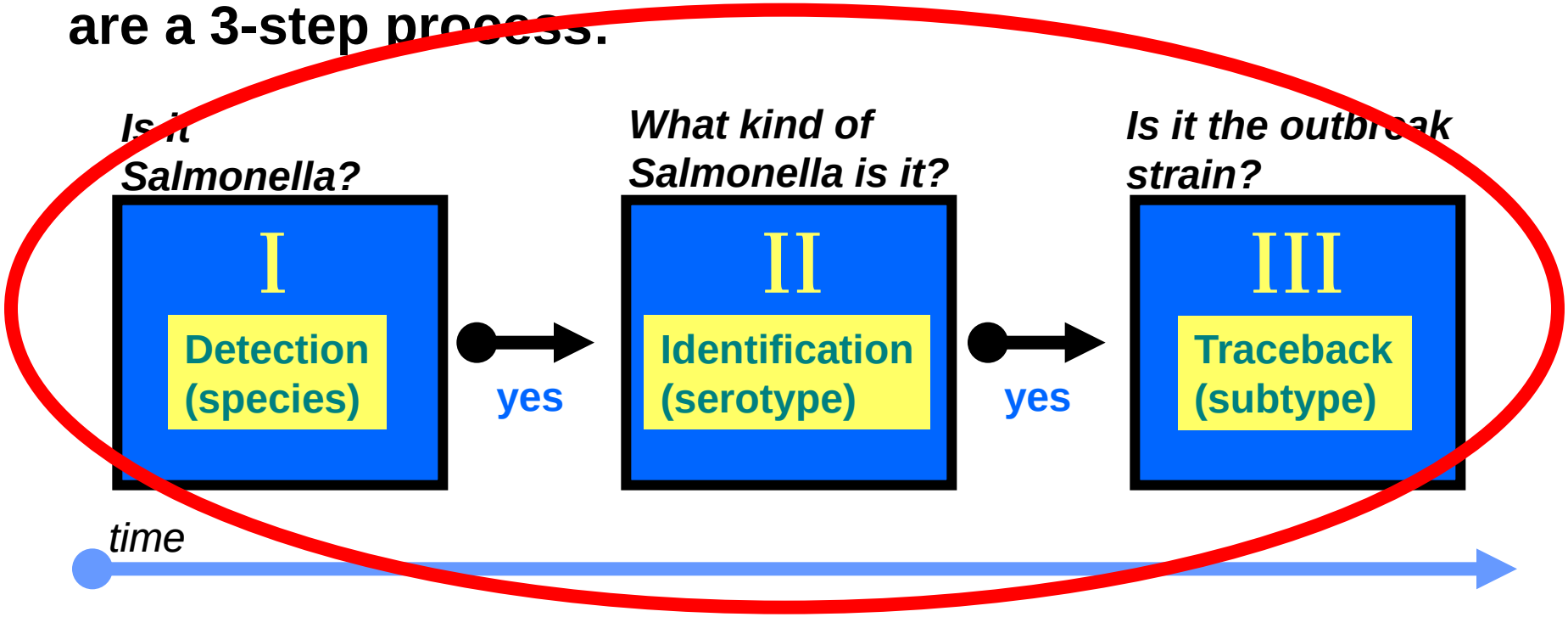

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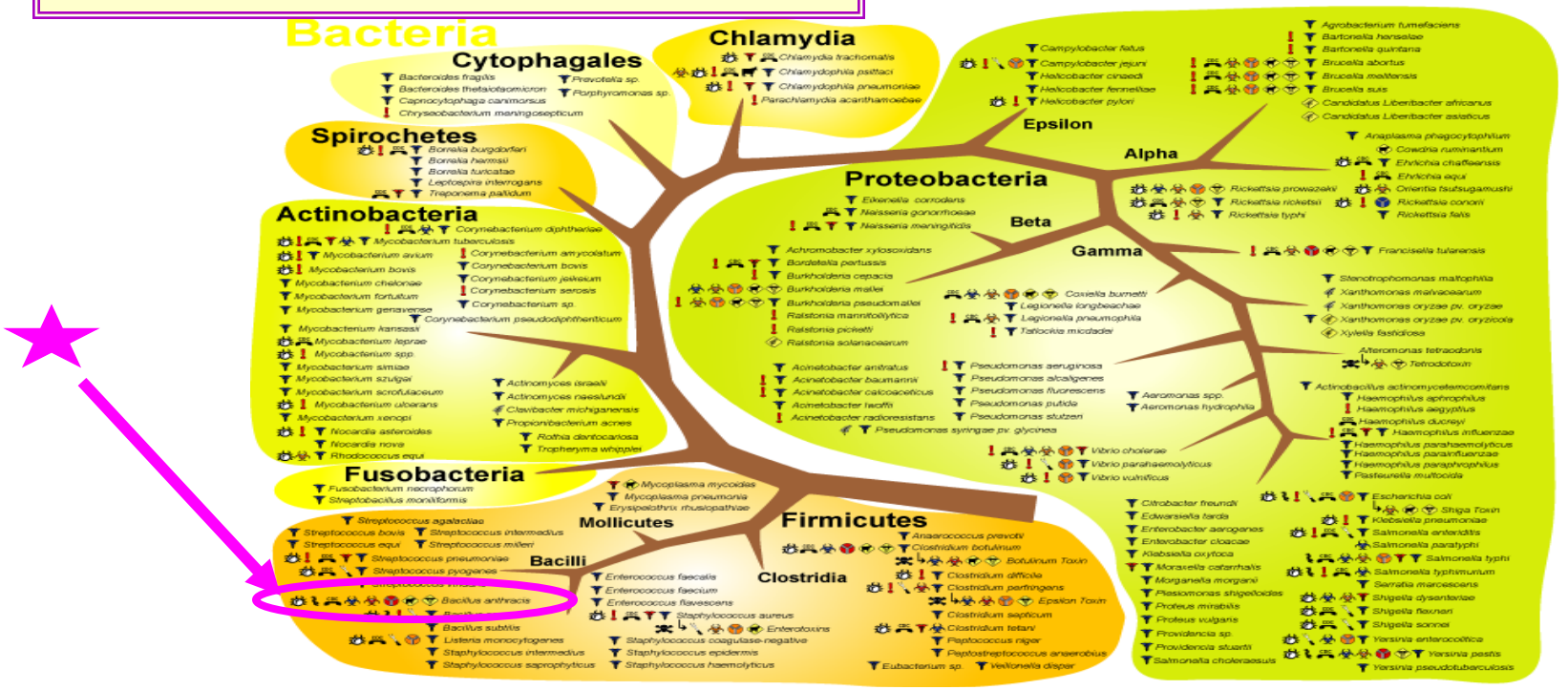
Objectives of Ibis T5000 Biosensor Technology

- Broad identification of all microbes
  - Bacteria, Viruses, Fungi, Protozoa
  - No culturing
  - No DNA sequencing
- Mixed populations of microbes
  - Quantitative
- High resolution genotyping, strain identification and antibiotic resistance determination
- Emerging infectious disease
- Cost effective, rapid, high throughput

Abbott Molecular

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	$A_{18}G_{15}C_{15}T_{13}$



Sample Collection and Processing

Sample Collection

6-24 hour indoor and outdoor air samples

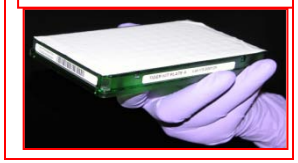
Sample Preparation

Sample lysis followed by DNA/RNA purification

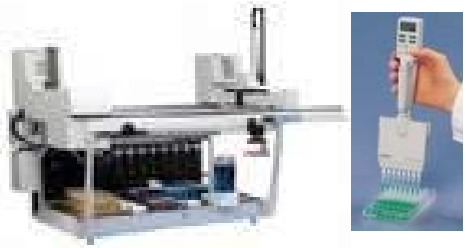
KingFisher

Sample and Enzyme added to Ibis assay plate

Ibis plate



Enzyme



PE Janus robot or hand pipetting

PCR

Eppendorf

FDAs Rules of the Game…

…Mastering the 4 R's of the Microbial Detection/Identification Method

Robustness: The technique must reveal enough genetic differences to effectively discriminate between closely related strains

Repeatability: The technique must be reliable enough to consistently yield the Same result across laboratories and at different times

Rapidity: The technique must be rapid enough to stay in tune with an investigation

Recognition: The technique must be recognized in court as a 'tried and true' method for science-based regulatory investigations

Summary

- **FDA is facing new analytical challenges as a result of the globalization of the food supply and changes in many other areas, including consumer preferences and food industry practices**
  - Inexpensive rapid screening tools
  - Faster, selective sample cleanup and enrichment
  - Multi-analyte and/or multi-pathogen detectors
  - Broad range, non-targeted chemical and biochemical detectors
- **Protecting and promoting the public health remains our focus.**

- 1942
  - *Staphylococcus aureus*
  - *Salmonella*
  - *Clostridium botulinum*
  - Streptococci

Emergence of Foodborne Pathogens

- *Campylobacter jejuni*
- *Clostridium botulinum* (infant)
- *E. coli 0157:H7*
- *Listeria monocytogenes*
- *Salmonella* Enteritidis
- *Vibrio cholerae* (Latin America)
- *Vibrio vulnificus*
- *STECs*
- *4,5,12:i:- Salmonella*
- *Yersinia enterocolitica*
- *Norwalk and Norwalk-like viruses*
- *Rotavirus*
- *Cryptosporidium parvum*
- *Giardia lamblia*
- *Toxoplasma gondii*
- Bovine spongiform encephalopathy prion
- *V. Parahaemolyticus*
- *E. sakazakii*
- *MRSA*

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