

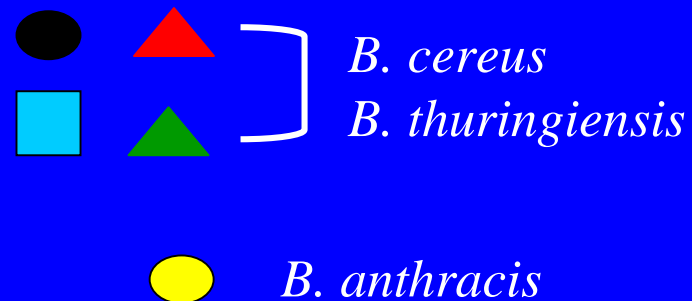
Importance of *Bacillus anthracis* molecular subtyping during the recent multi-state bioterrorism-associated anthrax outbreak in the United States

Alex R. Hoffmaster, Ph.D.

Epidemiologic Investigations Laboratory
Meningitis and Special Pathogens Branch
Centers for Disease Control and Prevention

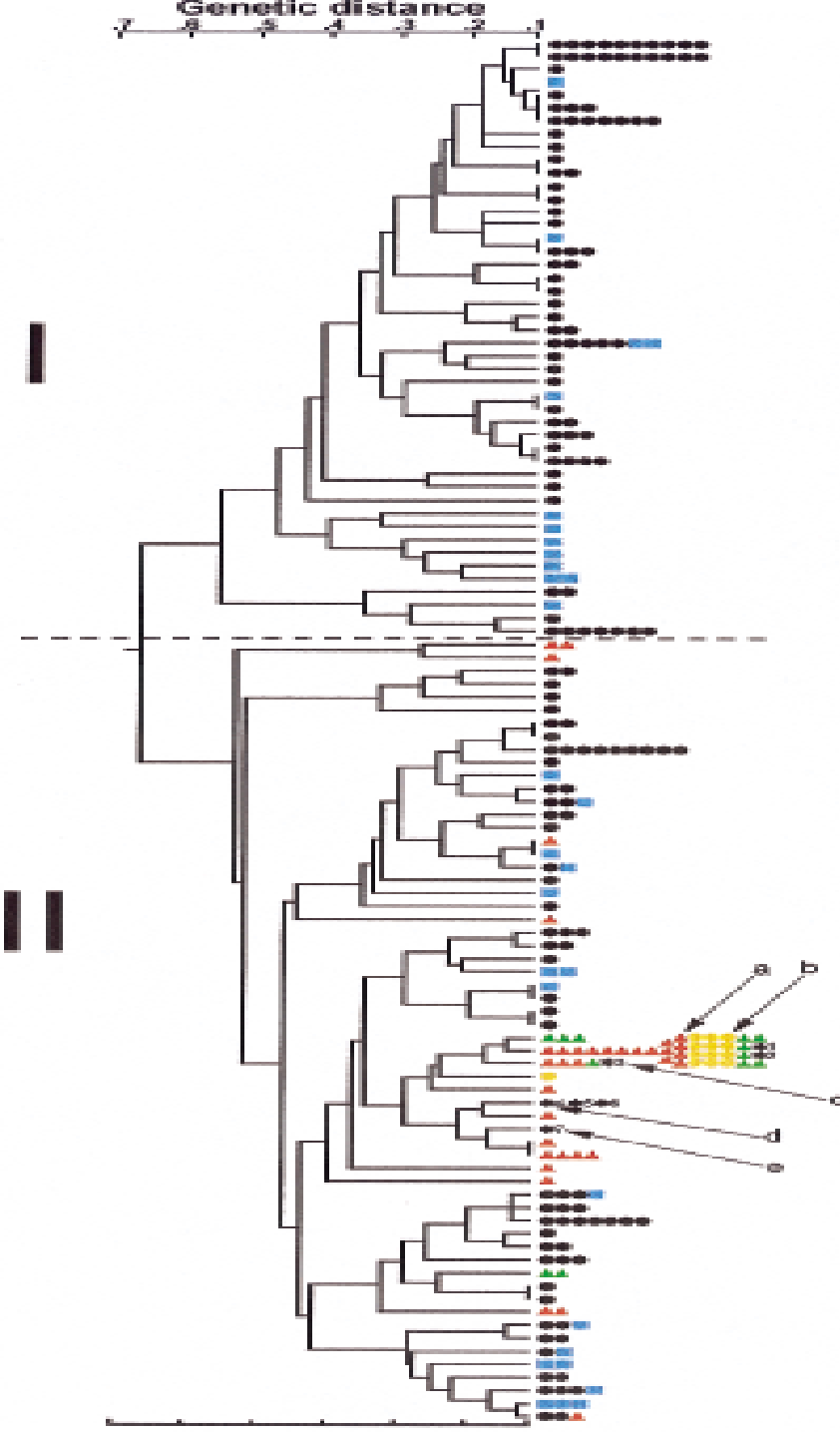


B. anthracis diversity?



← *B. anthracis*

Helgason *et al.*
Appl. Environ. Microbiol.
June 2000. 66: 2627-2630

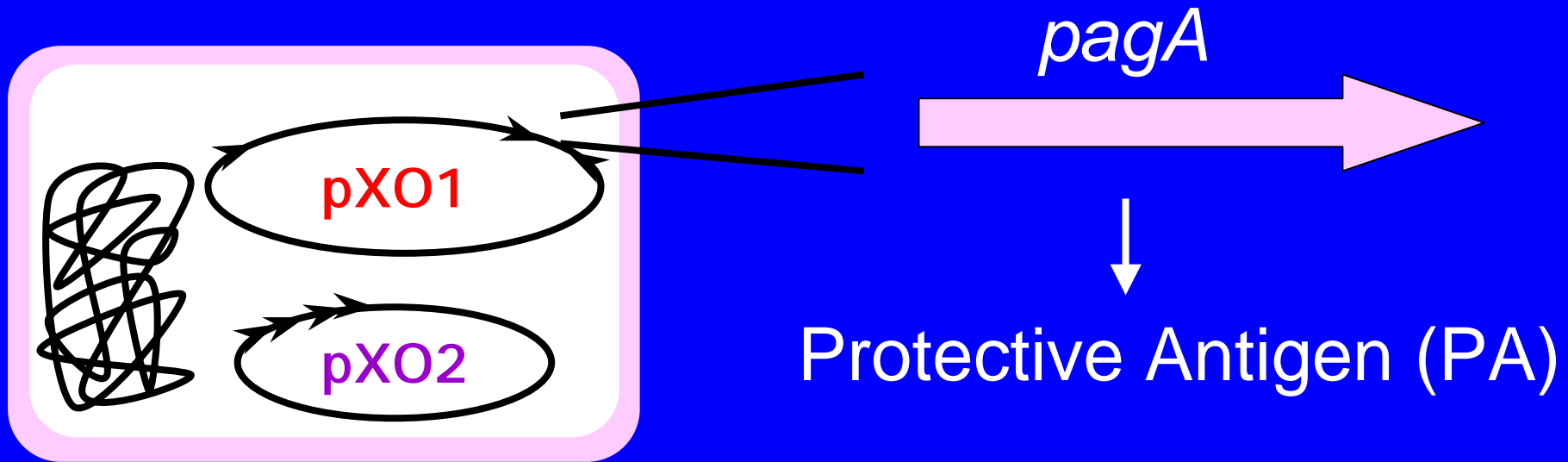


Molecular Subtyping Analysis

- *pagA* Sequencing
- Multiple-locus Variable-Number Tandem Repeat Analysis (MLVA)



pagA sequencing = PA genotype



- *pagA* 2.3 kb

- 6 PA genotypes

(Price *et al.* 1999. J. Bacteriol. 181:2358-2362)

Multi-locus variable-number tandem repeat analysis (MLVA)

5'—3' GATCCCATCATCATGGATC 3'—5'

5'—3' GATCCCATCATCATCATCATGGATC 3'—5'

100 bp 

106 bp 

PCR

- Eight VNTR markers are combined in four multiplex PCR reactions
- Six chromosomal markers:
vrrA, *vrrB*₁, *vrrB*₂, *vrrC*₁, *vrrC*₂, CG3
- Two plasmid markers:
pXO1, pXO2

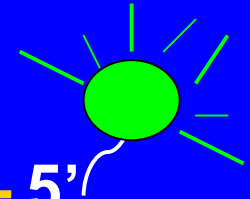


PCR

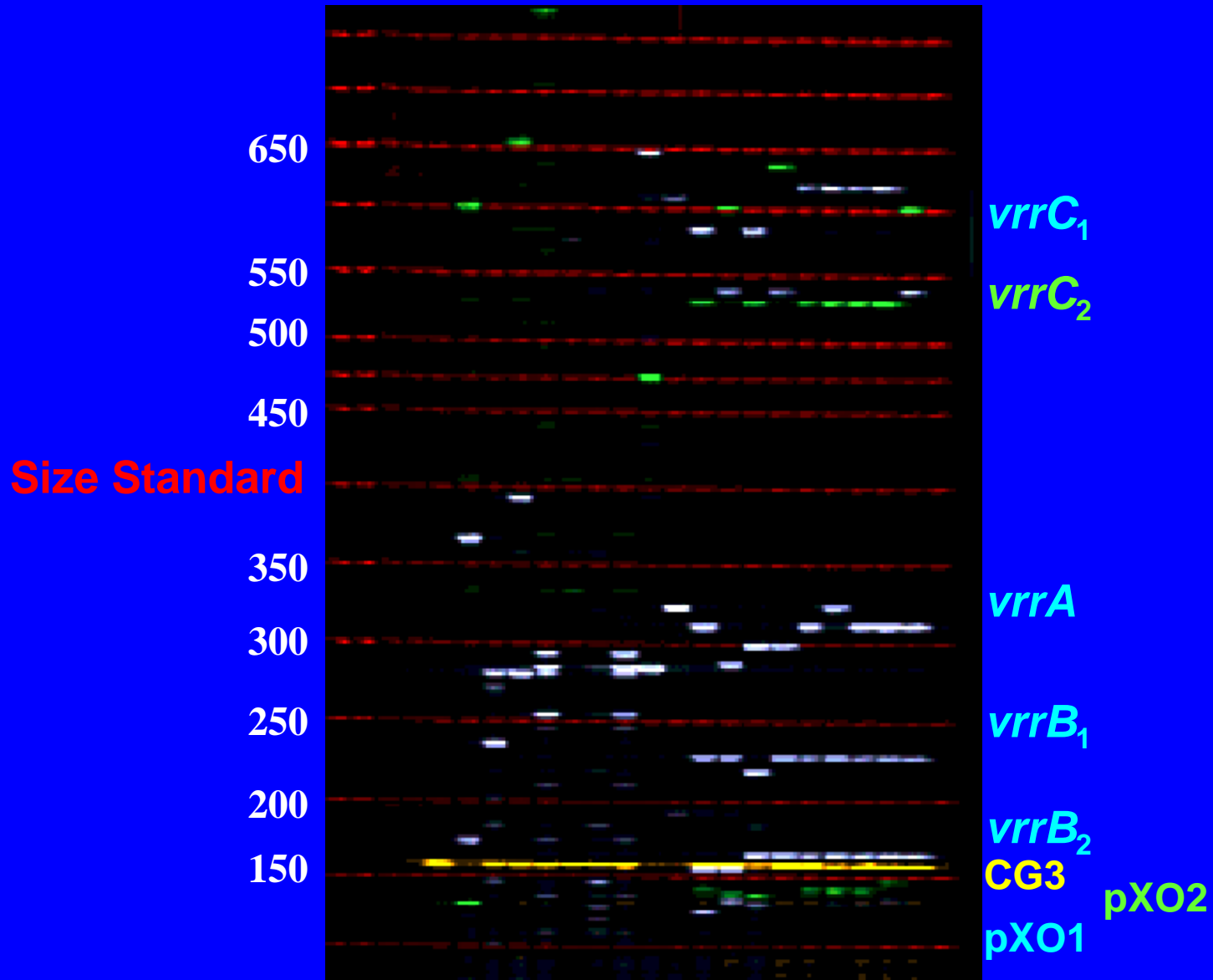
- PCR products are labeled with fluorescent primers using different colored dyes (**blue**, **green**, **yellow**)
- PCR products are pooled for visualization on an ABI Genescan gel

5'—3' GATCCATCATCATGGATC

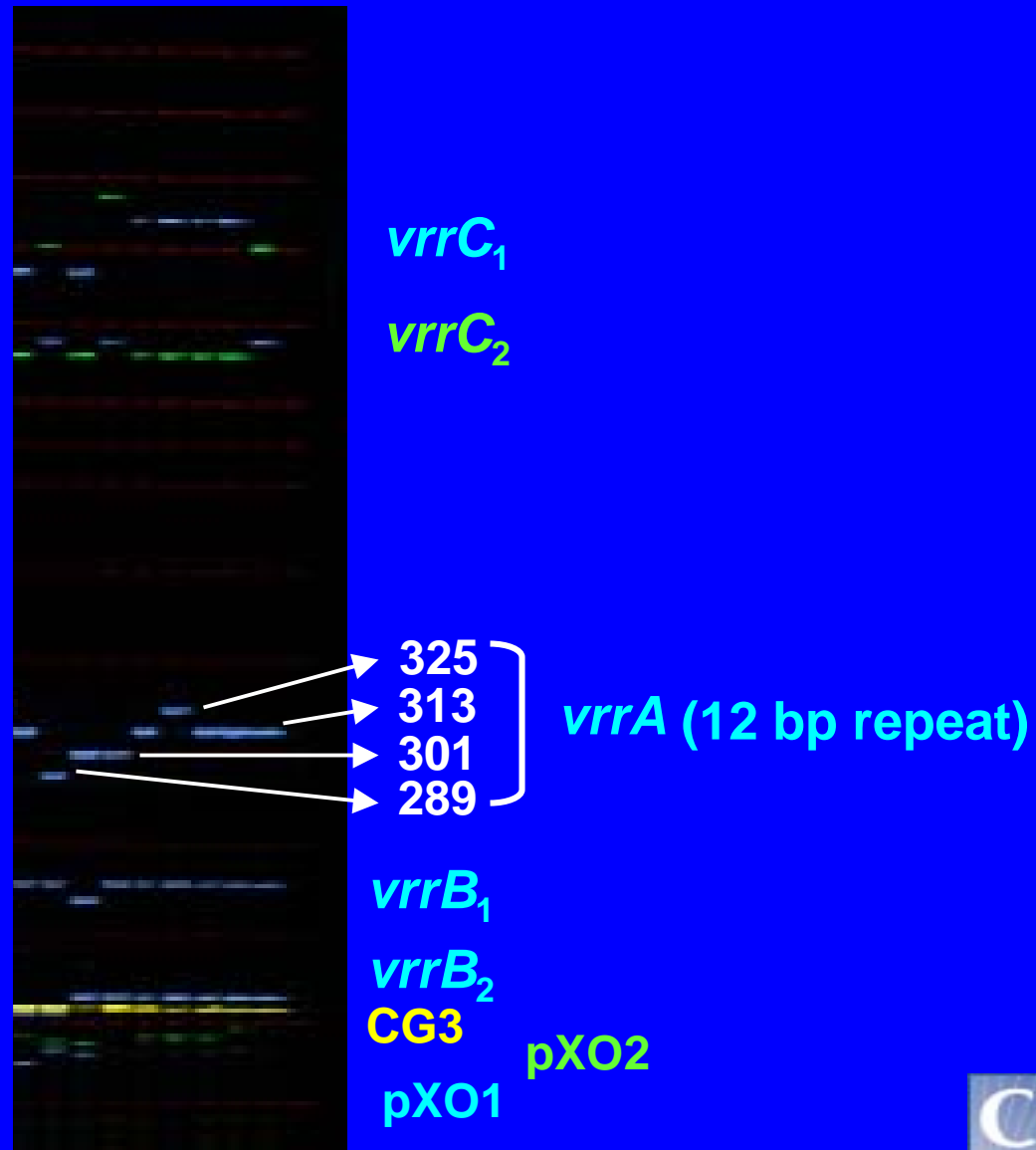
3'—5'



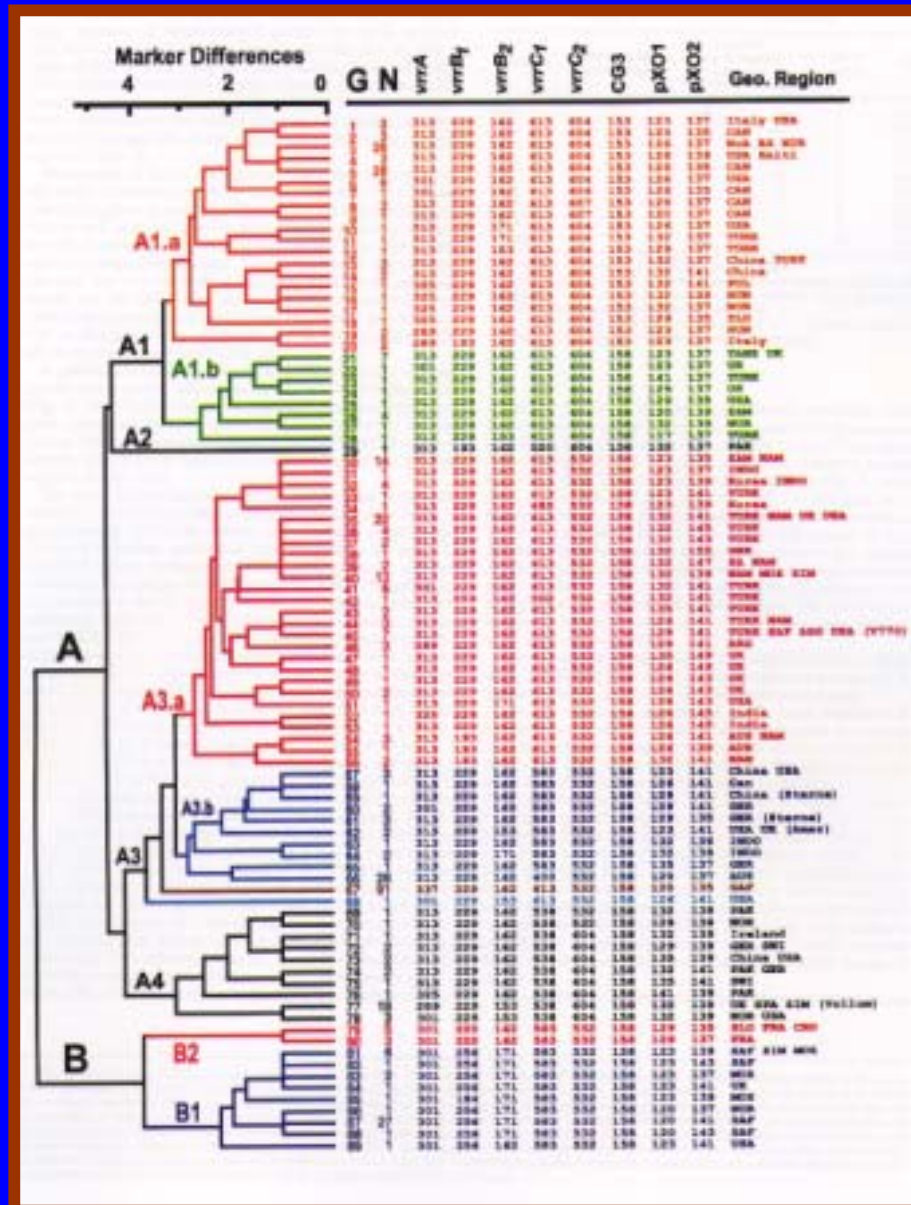
MLVA Gel Image



Genescan Gel Image



MLVA-based dendrogram



Keim *et al.* 2000. *J. Bacteriol.* 182:2928-2936.



Molecular Subtyping Analysis

<u>Strain/Isolate</u>	<u><i>vrrA</i></u>	<u><i>vrrB</i>₁</u>	<u><i>vrrB</i>₂</u>	<u><i>vrrC</i>₁</u>	<u><i>vrrC</i>₂</u>	<u>CG3</u>	<u>pXO1</u>	<u>pXO2</u>	<u>MLVA type</u>	<u>PA genotype</u>
Outbreak strain	313	229	153	583	532	158	123	141	62	I

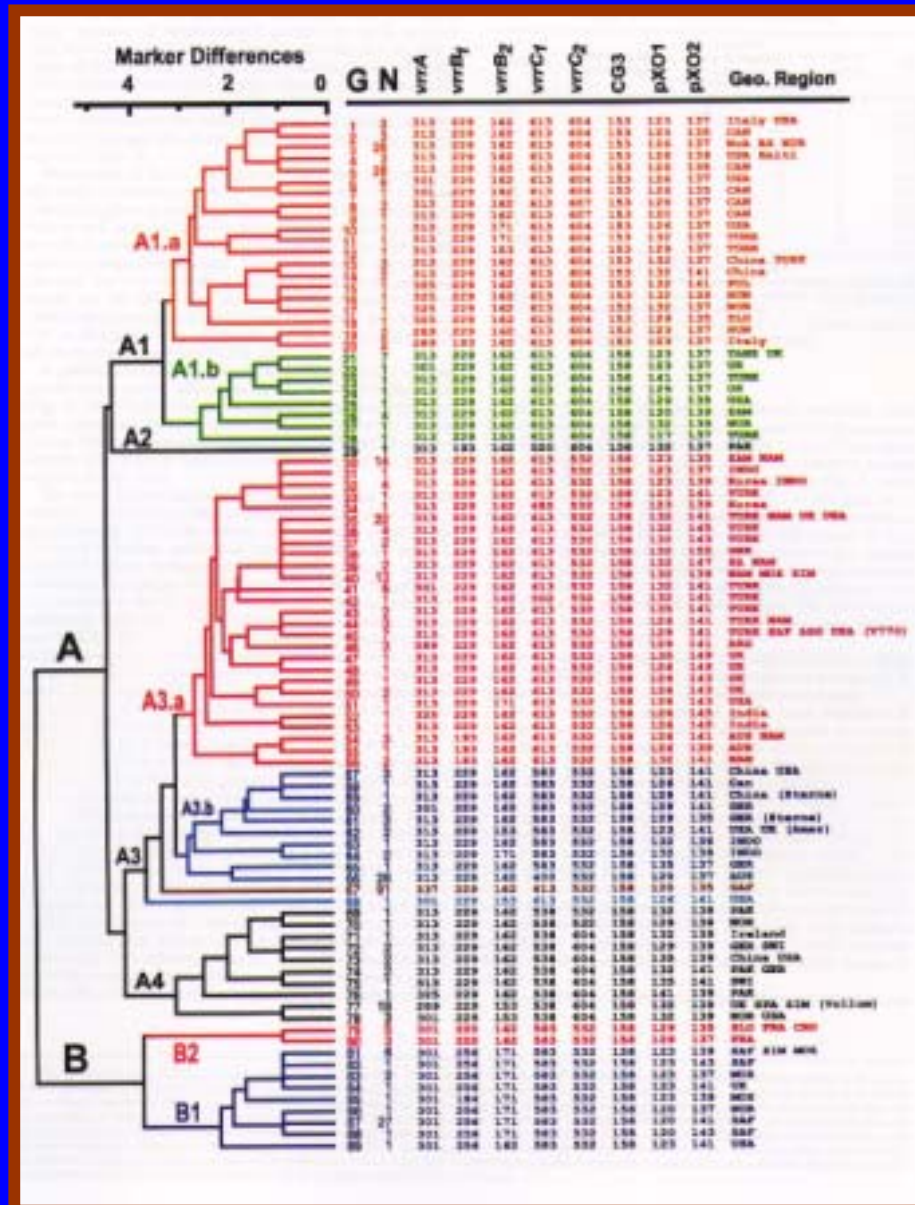
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Ames	313	229	153	583	532	158	123	141	62	I
NH (2000032764)	301	229	153	538	604	158	132	139	78	VI
NH (2000032760)	313	229	153	538	604	158	123	139	New	VI
RI (2000032763)	313	229	162	538	604	158	132	139	71	VI
RI (2000032761)	313	229	162	538	604	158	132	139	71	VI
MA (2000032762)	313	229	153	538	604	158	132	143	New	VI

MLVA-based dendrogram



Keim *et al.* 2000. *J. Bacteriol.* 182:2928-2936.



Molecular Subtyping Analysis

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XX (2002017388/90)	313	229	162	613	604	153	--	137	Pasteur*	--
Pasteur (LRN)	313	229	162	613	604	153	--	137	Pasteur*	--
YY(2002007581/82)	313	229	162	613	532	158	129	141	45	I
YY(2002007648)	313	229	162	613	532	158	129	141	45	I
YY(2002007649)	313	229	162	613	604	153	-	137	Pasteur*	--
YY(2002007650)	313	229	162	583	532	158	129	-	Sterne*	I
YY(2002007651)	313	229	162	583	532	158	129	-	Sterne*	I

Clinical Samples

Case # 10

MLVA loci detected

pagA

Pleural fluid

vrrA, *vrrB*₁, *vrrB*₂, *vrrC*₁, *vrrC*₂, CG3, pXO1, pXO2

Yes

Blood

Negative

No

CSF

Negative

No

Lung

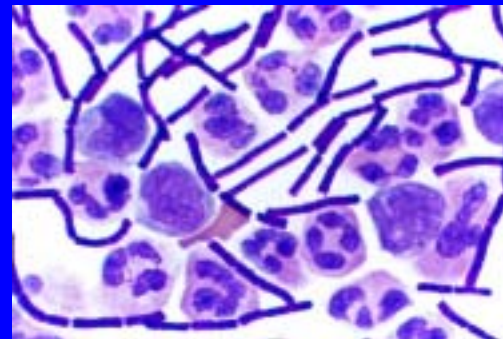
vrrA, CG3

No

Lymph Node

vrrA, *vrrB*₁, *vrrB*₂, *vrrC*₁, *vrrC*₂, CG3, pXO1, pXO2

Yes



Future

- Homomeric repeats
- SNP-Microarray
- Genomic Identity Review by Annealing of Fractionated Fragments (GIRAFF)



Conclusions

- Subtyping linked isolates from patients, buildings, mail, and powder
- No genotype other than GT62 was associated with inhalational or cutaneous anthrax cases
- Speed of MLVA allowed for same day results
- Current state of the art methods worked well but....

Acknowledgments

Tanja Popovic

Leonard Mayer
Collette Fitzgerald
Efrain Ribot

And everybody and their brother who

CDC

LRN

APHL

Clinicians

Epidemiologists

and more....

