

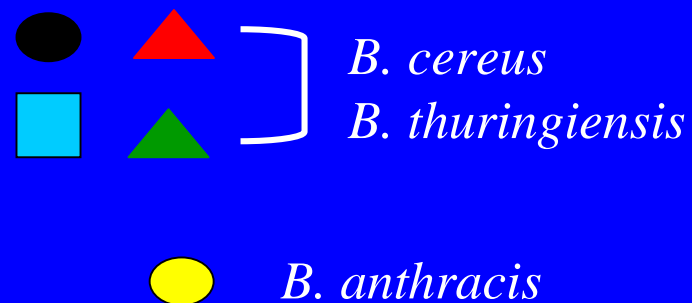
# Importance of *Bacillus anthracis* molecular subtyping during the recent multi-state bioterrorism-associated anthrax outbreak in the United States

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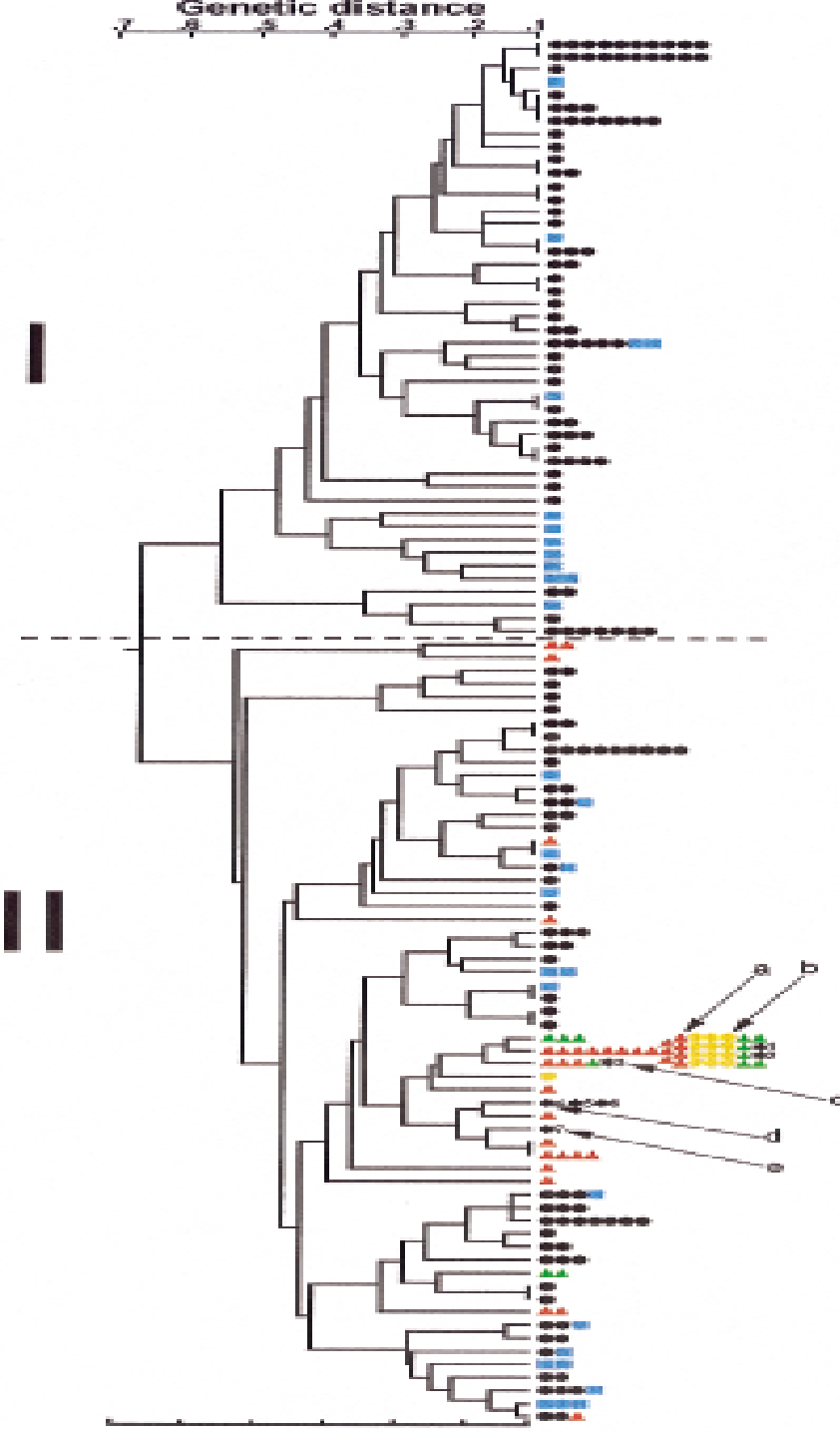


# *B. anthracis* diversity?



← *B. anthracis*

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

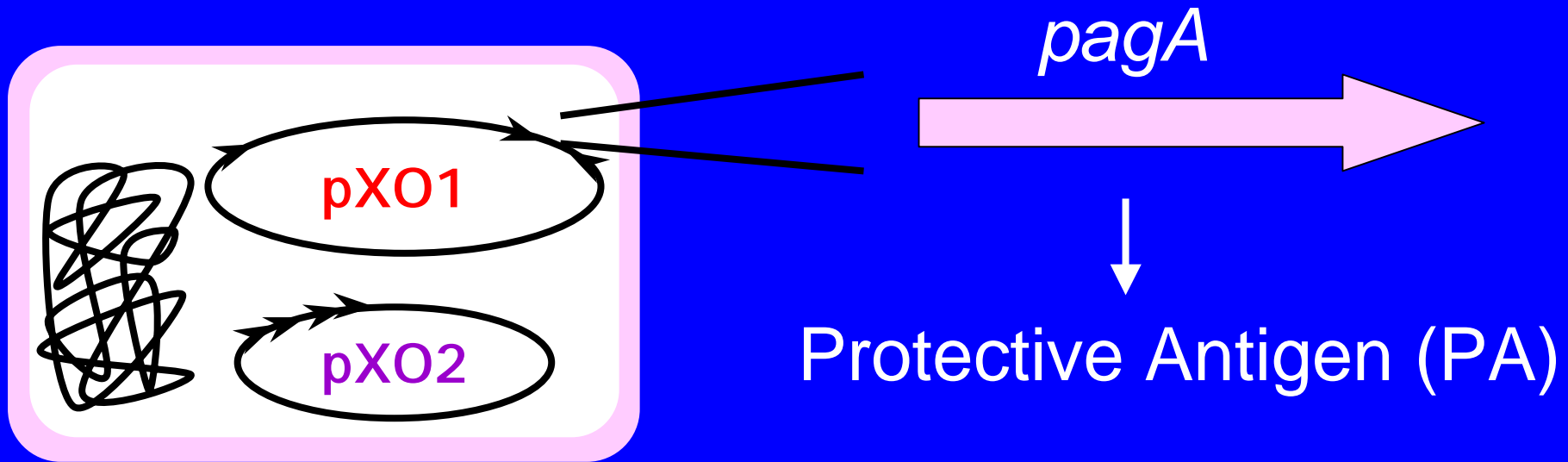


# Molecular Subtyping Analysis

- *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*<sub>1</sub>, *vrrB*<sub>2</sub>, *vrrC*<sub>1</sub>, *vrrC*<sub>2</sub>, CG3
- Two plasmid markers:  
pXO1, pXO2

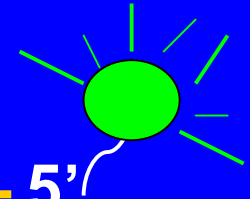


# PCR

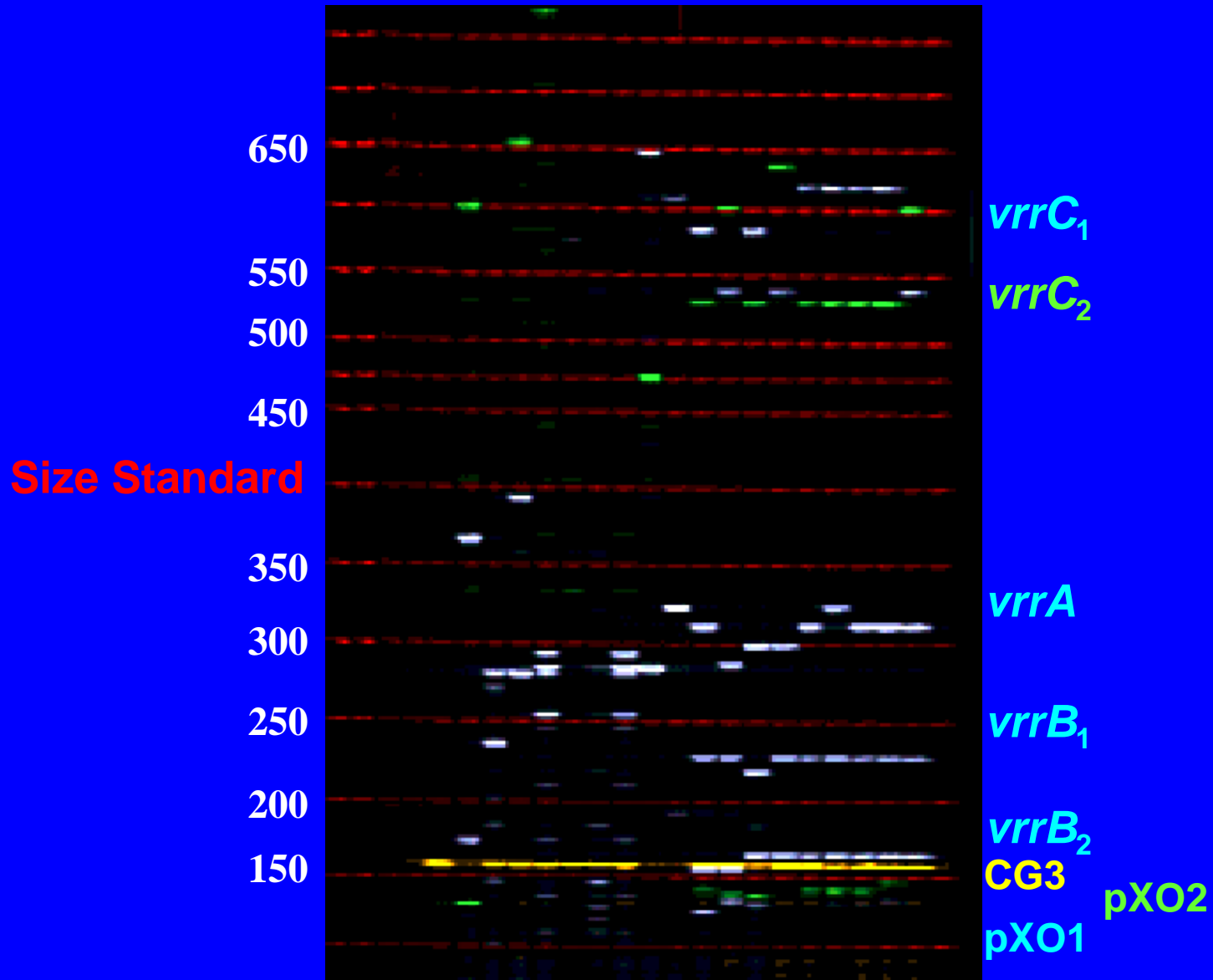
- PCR products are labeled with fluorescent primers using different colored dyes (**blue**, **green**, **yellow**)
- PCR products are pooled for visualization on an ABI Genescan gel

5'—3' GATCCATCATCATGGATC

3'—5'

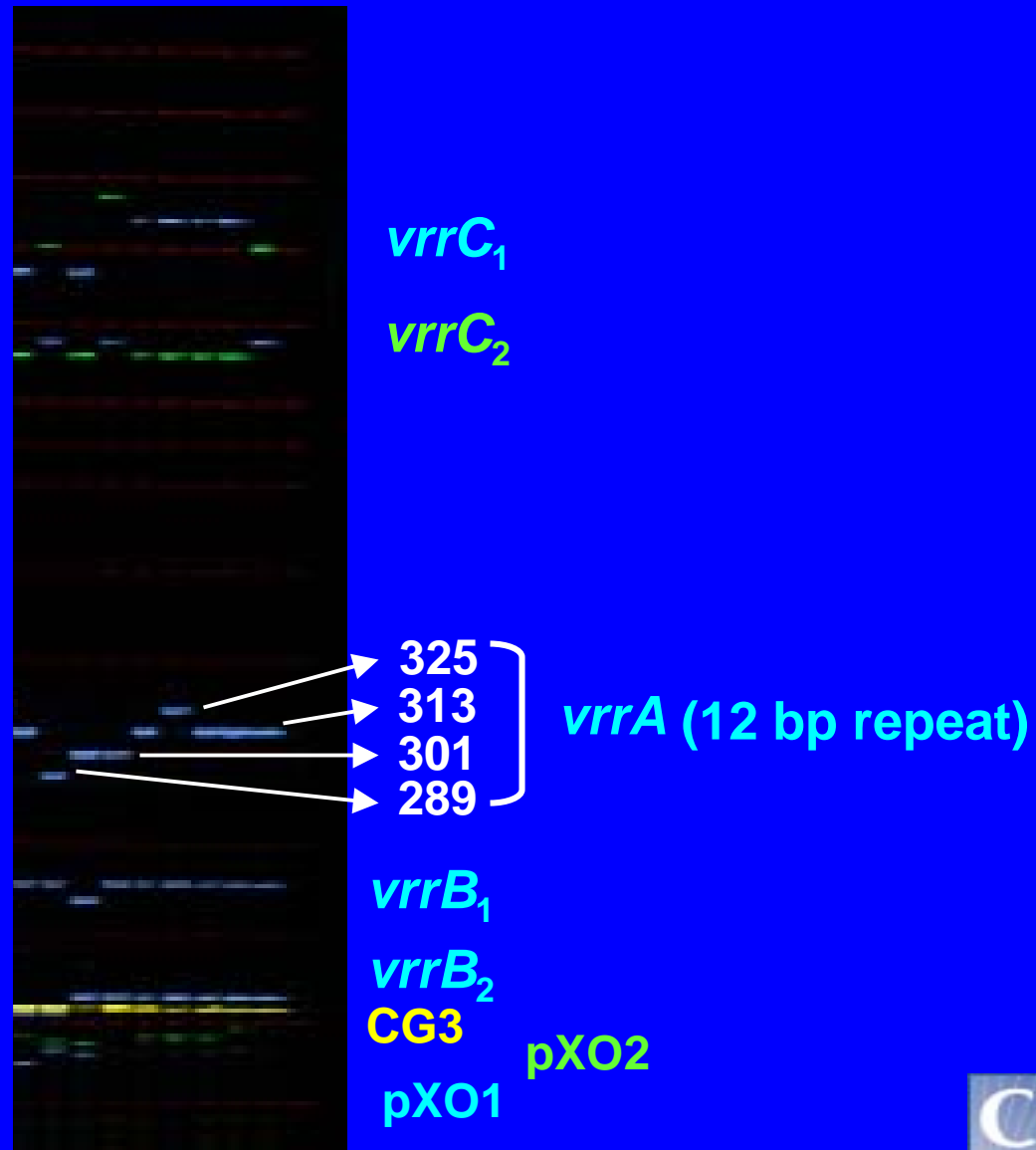


# MLVA Gel Image





# Genescan Gel Image





# Molecular Subtyping Analysis

<b><u>Strain/Isolate</u></b>	<b><u><i>vrrA</i></u></b>	<b><u><i>vrrB</i><sub>1</sub></u></b>	<b><u><i>vrrB</i><sub>2</sub></u></b>	<b><u><i>vrrC</i><sub>1</sub></u></b>	<b><u><i>vrrC</i><sub>2</sub></u></b>	<b><u>CG3</u></b>	<b><u>pXO1</u></b>	<b><u>pXO2</u></b>	<b><u>MLVA type</u></b>	<b><u>PA genotype</u></b>
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
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Pasteur (LRN)	313	229	162	613	604	153	--	137	Pasteur*	--
<b>YY(2002007581/82)</b>	<b>313</b>	<b>229</b>	<b>162</b>	<b>613</b>	<b>532</b>	<b>158</b>	<b>129</b>	<b>141</b>	<b>45</b>	<b>I</b>
<b>YY(2002007648)</b>	<b>313</b>	<b>229</b>	<b>162</b>	<b>613</b>	<b>532</b>	<b>158</b>	<b>129</b>	<b>141</b>	<b>45</b>	<b>I</b>
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<b>YY(2002007650)</b>	<b>313</b>	<b>229</b>	<b>162</b>	<b>583</b>	<b>532</b>	<b>158</b>	<b>129</b>	<b>-</b>	<b>Sterne*</b>	<b>I</b>
<b>YY(2002007651)</b>	<b>313</b>	<b>229</b>	<b>162</b>	<b>583</b>	<b>532</b>	<b>158</b>	<b>129</b>	<b>-</b>	<b>Sterne*</b>	<b>I</b>



# Clinical Samples

## Case # 10

## MLVA loci detected

## *pagA*

Pleural fluid

*vrrA*, *vrrB*<sub>1</sub>, *vrrB*<sub>2</sub>, *vrrC*<sub>1</sub>, *vrrC*<sub>2</sub>, CG3, pXO1, pXO2

Yes

Blood

Negative

No

CSF

Negative

No

Lung

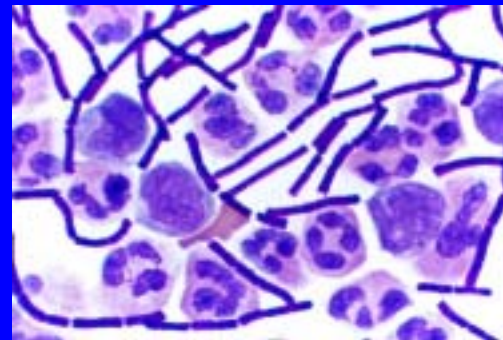
*vrrA*, CG3

No

Lymph Node

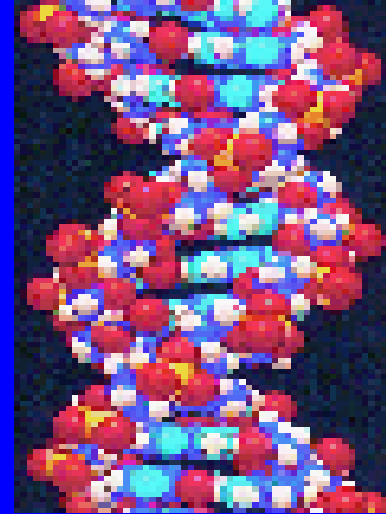
*vrrA*, *vrrB*<sub>1</sub>, *vrrB*<sub>2</sub>, *vrrC*<sub>1</sub>, *vrrC*<sub>2</sub>, CG3, pXO1, pXO2

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....

