

# PFGE Shortcuts that Save a Penny but Cost a Dollar

2013 InFORM Meeting

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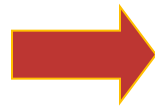
# Objectives

- ❑ Identify critical steps in PulseNet PFGE protocols
- ❑ Summarize costs associated with performing PFGE
- ❑ Describe impacts associated with ineffective cost-cutting measures

# PFGE Workflow



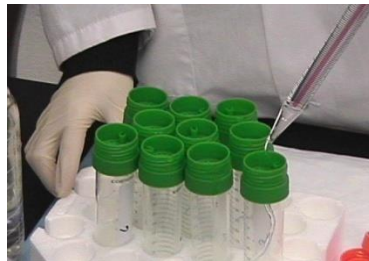
patient specimen  
collection



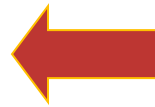
specimen



grow isolated  
colony



cell lysis and  
plug washing



cells trapped  
in plug

=



agarose

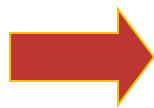
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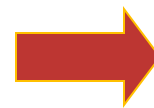
cell  
suspension



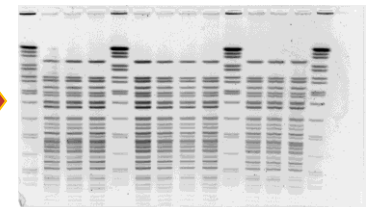
enzyme digestion



electrophoresis



imaging



tiff for analysis

# Reagent Costs – *E. coli* and *Salmonella*

Organism	Reagent	*Cost - USD	Price / unit - USD	Amt / isolate	Amt / 10 isolates	Amt / 15 isolates	†Cost / 10 well gel	††Cost / 15 well gel	# of isolates	# of gels
ALL	SeaKem Gold - 125g	\$600	5 USD / g	0.004 g	1.04 g	1.56 g	\$5.20	\$7.80	1000	about 75
ALL	Proteinase K - 5ml	\$135	27 USD / ml	0.045 ml	0.45 ml	0.675 ml	\$12.15	\$18.23	110	7 to 10
ALL	1L water - plug washes			30 ml	300 ml	450 ml			30	2 or 3
ALL	1L TE - plug washes			60 ml	600 ml	900 ml			15	1 or 2
ALL	NEB BSA - 12 mg / 0.6ml	\$24	40 USD/ml	0.002 ml	0.02 ml	0.03 ml	\$0.80	\$1.20	300	20 to 30
EC, SALM	Roche Buffer H - 5ml	\$38	6.4 USD/ml	0.04 ml	0.4 ml	0.6 ml	\$2.56	\$3.84	125	30 to 60
EC, SALM	Roche XbaI - 5,000 U	\$126	0.025 USD/U	50 U	500 U	750 U	\$12.50	\$18.75	100	25 to 30
EC, SALM	Roche BlnI - 1,000 U	\$364	0.364 USD/U	30 U			\$76.44	\$120.12	125	11 to 17
ALL	10X TBE - 4L Sigma	\$114	29 USD / L		0.12 L	0.12 L	\$3.48	\$3.48	300	20 to 30

<i>Salmonella</i> and <i>E. coli</i>	XbaI only	Total cost =	\$36.69	\$53.30
	XbaI + BlnI (2 gels)	Total cost =	\$128.92	\$194.74

- \* Before PulseNet discount, if applicable
- † 3 standards + 7 isolates per gel
- †† 4 standards + 11 isolates per gel

# Reagent Costs – *Listeria monocytogenes*

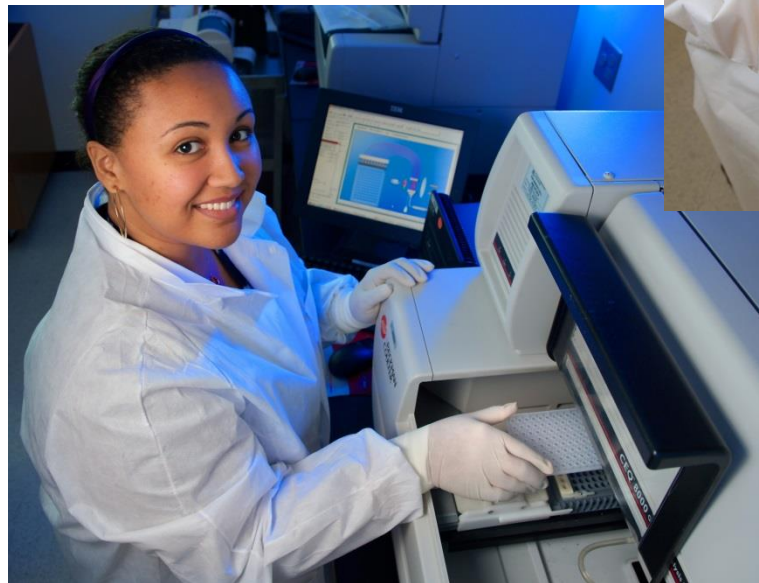
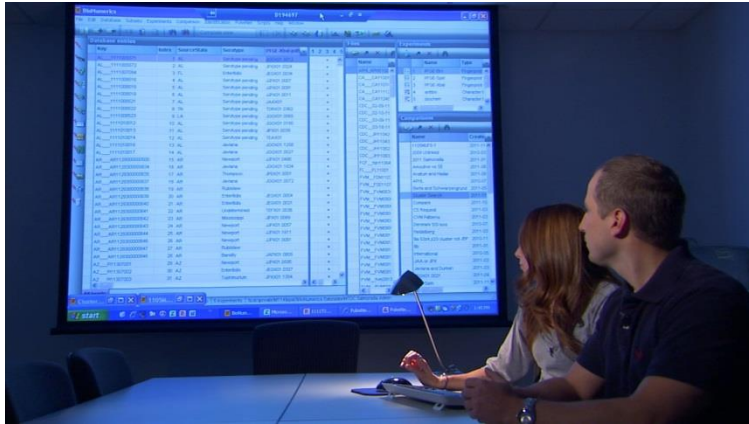
Organism	Reagent	*Cost - USD	Price / unit - USD	Amt / isolate	Amt / 10 isolates	Amt / 15 isolates	†Cost / 10 well gel	††Cost / 15 well gel	# of isolates	# of gels
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ALL	Proteinase K - 5ml	\$135	27 USD / ml	0.045 ml	0.45 ml	0.675 ml	\$12.15	\$18.23	110	7 to 10
Listeria	Lysozyme – L6876-1G	\$45	45 USD / g	0.0004 g	0.004 g		\$0.20		> 300	> 100
ALL	1L water - plug washes			30 ml	300 ml	450 ml			30	2 or 3
ALL	1L TE - plug washes			60 ml	600 ml	900 ml			15	1 or 2
ALL	NEB BSA - 12 mg / 0.6ml	\$24	40 USD/ml	0.002 ml	0.02 ml	0.03 ml	\$0.80	\$1.20	300	20 to 30
EC, SALM	Roche Buffer H - 5ml	\$38	6.4 USD/ml	0.04 ml	0.4 ml	0.6 ml	\$2.56	\$3.84	125	30 to 60
Listeria	NEB CutSmart Buffer – 5ml	\$17	3.4 USD / ml	0.04 ml	0.4 ml		\$0.95	\$1.50	125	30 to 60
ALL	Roche XbaI - 5,000 U	\$126	0.025 USD/U	50 U	500 U	750 U	\$12.50	\$18.75	100	25 to 30
Listeria	NEB Ascl – 2,500 U	\$252	0.101 USD/U	25 U	250 U			\$17.68	100	9 to 14
Listeria	NEB ApaI – 5,000 U	\$65	0.013 USD/U	25 U	250 U			\$2.28	200	18 to 28
ALL	10X TBE - 4L Sigma	\$114	29 USD / L		0.12 L	0.12 L	\$3.48	\$3.48	300	20 to 30

<i>Listeria</i>	Ascl + ApaI (2 gels)	Total cost =	\$80.43	\$89.58
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- \* Before PulseNet discount, if applicable
- † 3 standards + 7 isolates per gel
- †† 4 standards + 11 isolates per gel



# Don't overlook personnel costs!

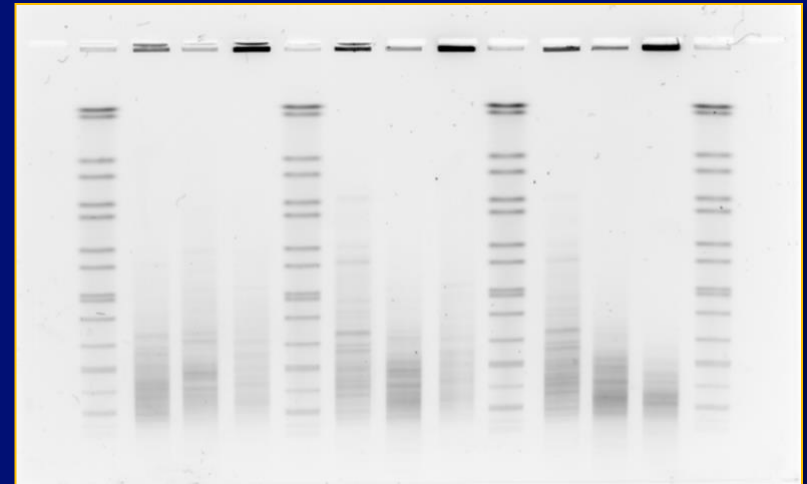


**USING OLD OR EXPIRED  
REAGENTS IS FINE –  
UNTIL IT ISN'T...**

# Using old or expired reagents costs \$\$

- ❑ date / label all reagents
- ❑ track lot numbers
- ❑ discard expired reagents

## *Listeria monocytogenes*



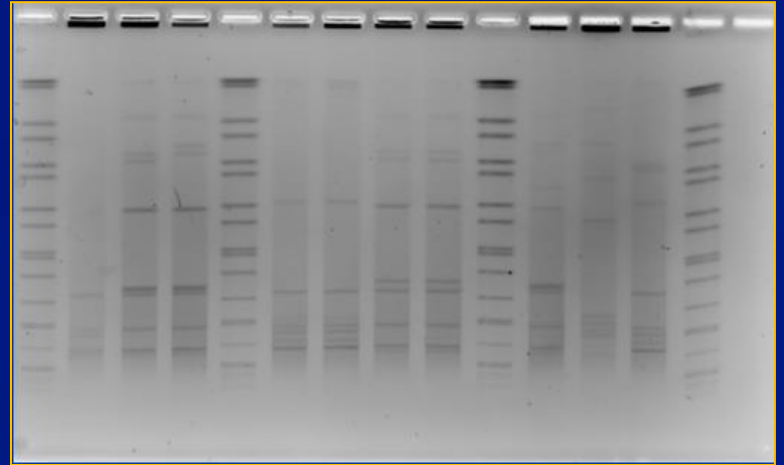
➤ TE made with expired  
1M Tris-HCl



# Using old or expired reagents costs \$\$

- ❑ some reagents are more sensitive than others
- ❑ some organisms more sensitive than others

## *Listeria monocytogenes*



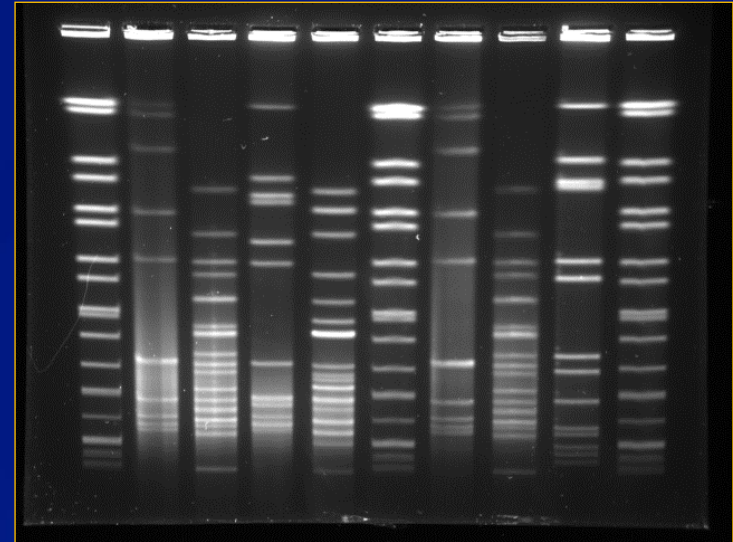
➤ Plugs made using *Listeria monocytogenes* grown on expired BHI plates

# Improperly storing reagents costs \$\$

- Effective cost-saving strategy → make smaller batches or aliquots to avoid contamination and reduce waste

- plug agarose –  
1% SKG + 0.5% SDS
- lysozyme
- about 25 cents per  
150 µl aliquot  
of 20 mg/ml lysozyme

## *Listeria monocytogenes*



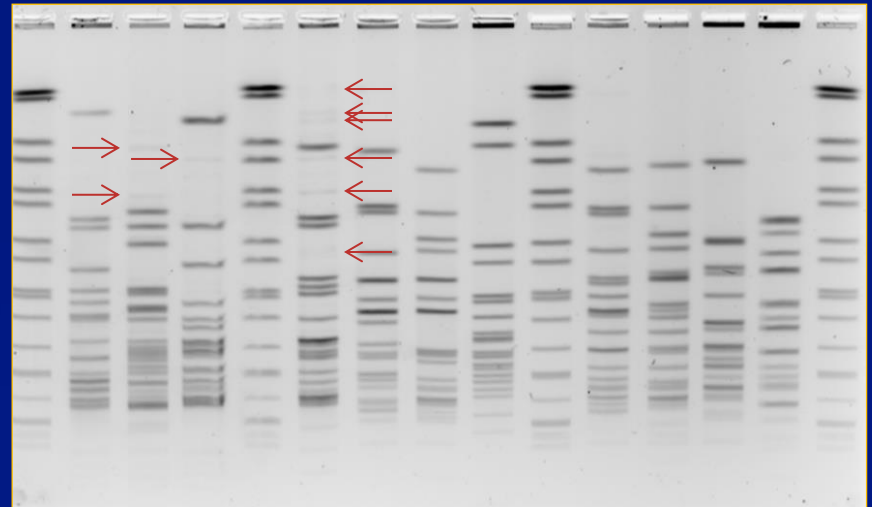
- *Listeria monocytogenes* plugs made using re-heated plug agarose and with lysozyme that had gone through multiple freeze-thaw cycles

# Including BSA is a cheap insurance step

## □ Always include BSA in the enzyme master mix

- dilute stock to 0.1 mg/ml final concentration
- certain enzymes and organisms or serotypes are more sensitive
- cost ~ 10 cents per reaction

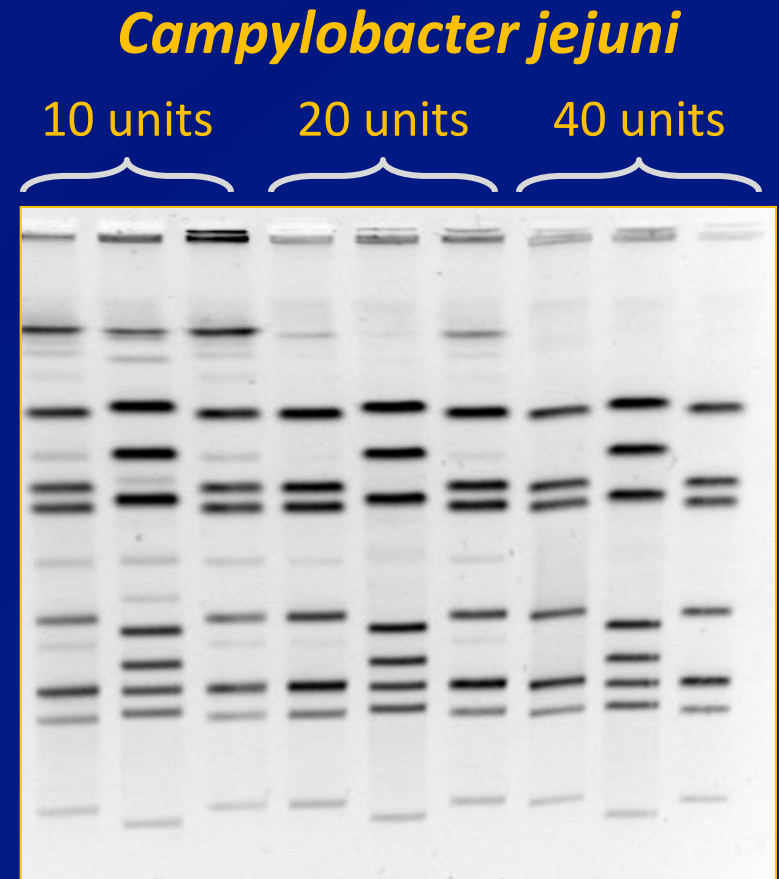
### *Listeria monocytogenes*



- *Listeria monocytogenes* plug slices digested with *Ascl* without BSA included in the master mix

# Decreasing enzyme increases ghost bands

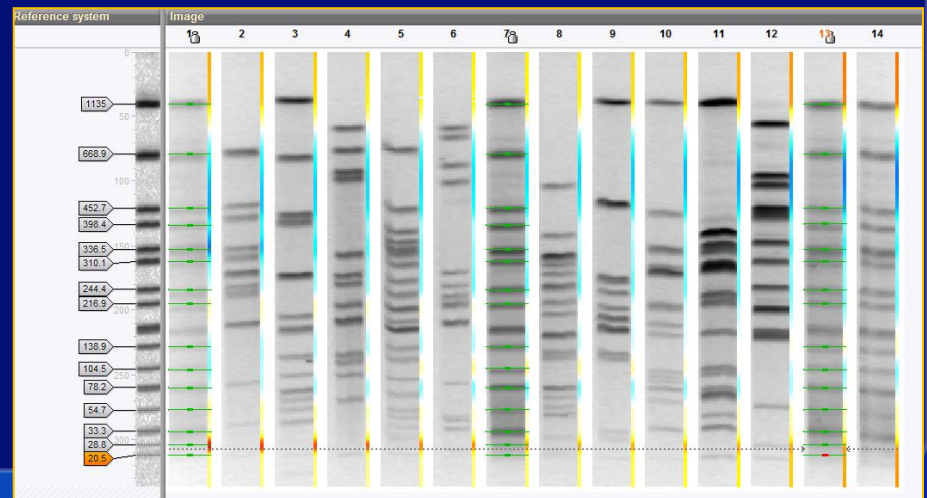
- ❑ PulseNet protocols are optimized to produce the cleanest patterns with the fewest units of enzyme
- ❑ Using fewer units of enzyme leads to
  - incomplete restriction and ghost bands
  - repeated isolates and gels
  - delays in reporting of data



➤ *Campy* plug slices digested with increasing amount of *KpnI*

# Agaroses besides SeaKem Gold

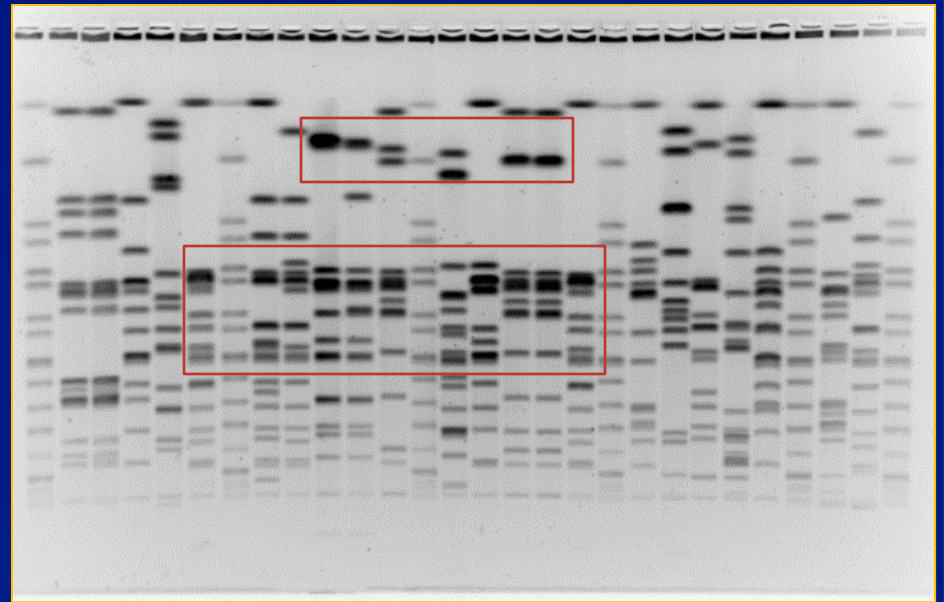
- ❑ SeaKem Gold (SKG) is only agarose approved for making plugs and running gels
  - Amresco III is acceptable for running gels, but not making plugs
  - BioRad Megabase is **no longer acceptable** due to inconsistent performance (varying run time, failing normalization)
- ❑ Consequences of short gel length and failed normalization?
  - band marking difficult
  - pattern naming / cluster detection compromised
  - gels need to be repeated



# Running too many isolates on a gel negatively impacts band resolution

- ❑ Band resolution and analysis are more difficult on narrow lanes
- ❑ Optimal
  - 10-well in 14 cm wide gel
  - 15-well in 21 cm wide gel
- ❑ Sub-optimal
  - 15-well in 14 cm wide gel
  - 20- or 30-well in 21 cm wide gel
- ❑ Isolates should be repeated

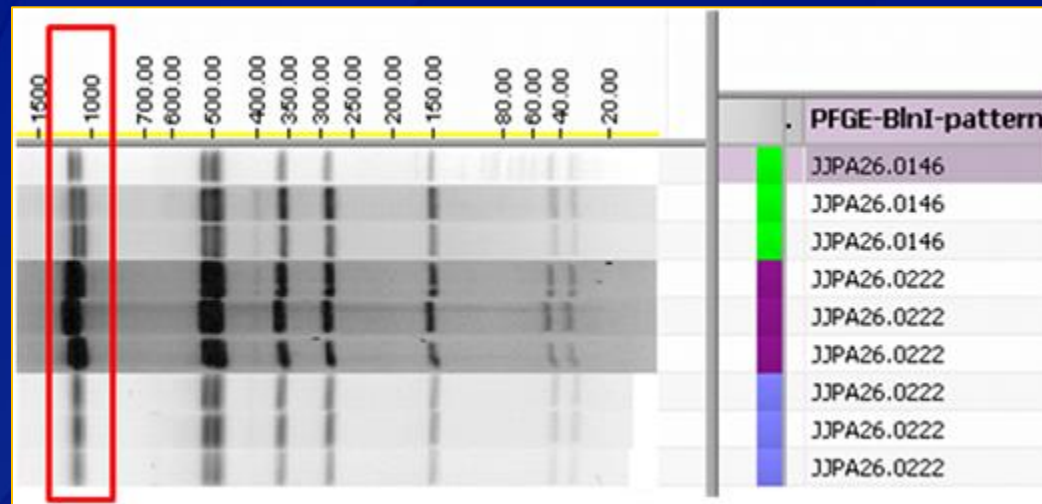
*Salmonella*





# Impact of band resolution on pattern naming / cluster detection

## *Salmonella* ser Newport

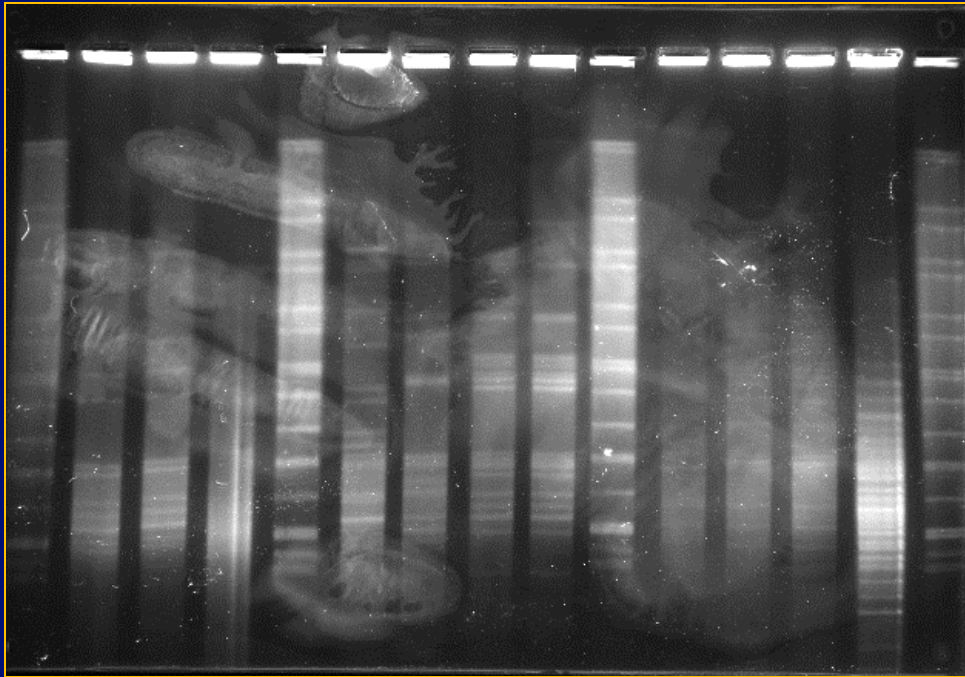


- Oversaturated single band or true doublet?
- “How close are these patterns even though they are considered different? Could the differences be due to a genetic event or shift during the course of this outbreak?”



# A picture is worth a thousand words (and hundreds of dollars)

*C. botulinum*



Cost	
"Bad" gloves	\$9 / 100 gloves
"Good" gloves	\$22 / 100 gloves
Repeating a gel	~\$100 / gel + 1 – 2 days

# Money saving tips that work

- ❑ Taking advantage of PulseNet discounts – BioRad, Roche, Lonza, Amresco
- ❑ Making small batches of reagents – plug agarose, CSB, etc...
- ❑ Making small aliquots of lysozyme
- ❑ Cutting 2 slices of H9812 in 200  $\mu$ l *Xba*I master mix or 3 or 4 slices in 400  $\mu$ l of *Xba*I master mix
- ❑ Using only 25 units of *Apa*I and *Asc*I
- ❑ Staining multiple gels in EtBr or GelRed
- ❑ Others?

# Acknowledgements

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## Thank you!

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

National Center for Emerging and Zoonotic Infectious Diseases

Division of Foodborne, Waterborne and Environmental Diseases

