2012 PulseNet PFGE Laboratory Update: Everything Old Is New Again

Grand Hyatt, Atlanta, CA
August 27th – 30th, 2012

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Enteric Diseases Laboratory Branch, CDC

OBJECTIVES

- Identify how pattern quality may be negatively affected by variations in enzyme quality
- Recognize how gel quality may be negatively impacted by variations in agarose quality
- Describe the network-wide impact of reagent quality on gel and image quality

PFGE Reference Laboratory

- Efrain Ribot, PhD – Team Lead
- Molly Freeman, PhD – Unit Chief
- Maurice Curtis, BS
- Jessica Halpin, MS

PFGE Reference Laboratory – What do we do?

- **In the lab…**
  - Test difficult to type isolates, triage during outbreaks
  - PFGE training – annual lab training in Spring
  - PFGE protocol development, reagent testing, validation studies
  - Provide technical support, customer service
  - Projects – epi studies, WGS studies, etc…
  - PFGE troubleshooting – PFGE@cdc.gov

Troubleshooting approaches

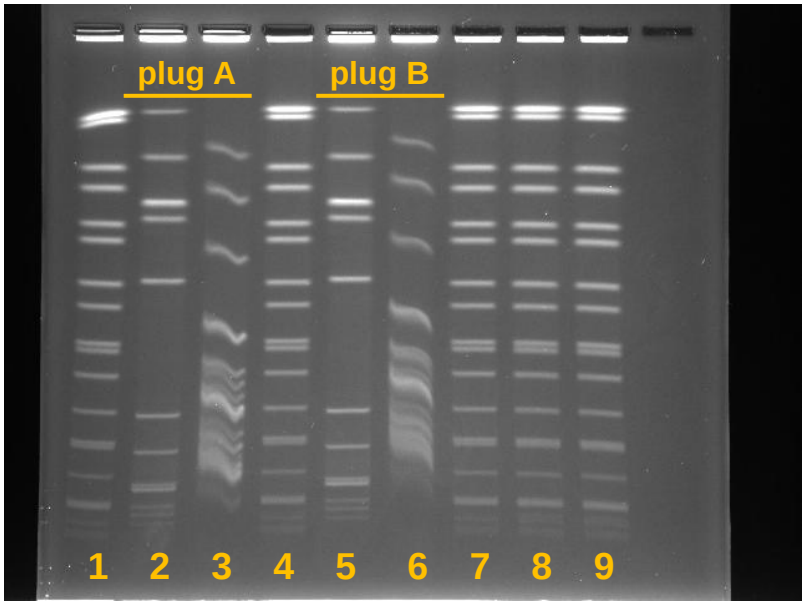
- **Study the protocol**
- **What has changed since the last "good" gel?**
  - Equipment, reagent, person, isolate / strain, etc…
  - Change in lab procedures / new SOPs, new vendor, contamination / decontamination event, etc…
- **Examine the gel closely**
  - Is the problem:
    - in all lanes, only the isolates, only the standards, only one enzyme?
    - apparent with multiple lab personnel?
    - apparent with multiple enzymes or multiple organisms?
    - associated with a specific mapper?
- **Maintain notebook of troubleshooting findings, gels**

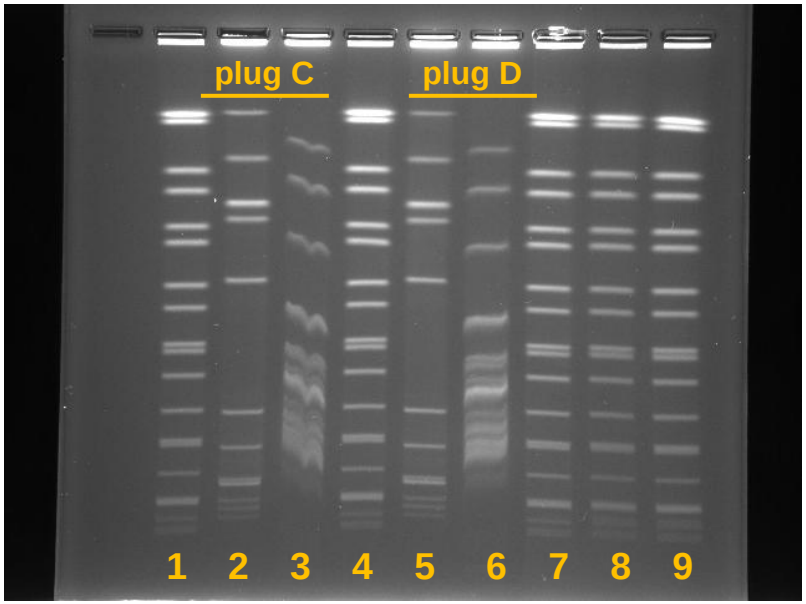
→ **What is in common and what has changed?**

“Wavy” bands

CASE STUDY #1

Listeria Gels – NH Lab, Winter 2012





- same isolates in lanes 2, 3, 5 and 6
- different techs, different plugs, different master mixes
- *Asc*I (NEB) – lanes 2 and 5
- *Apa*I (NEB lot# 0421103) – lanes 3 and 6, 125units (2.5µL of 50u/µl)
- BSA included

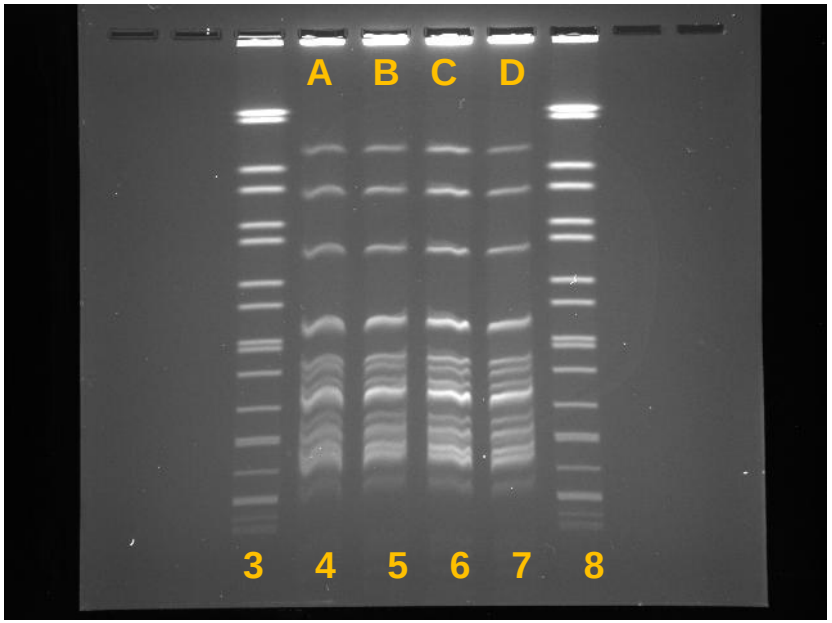
What's going on?

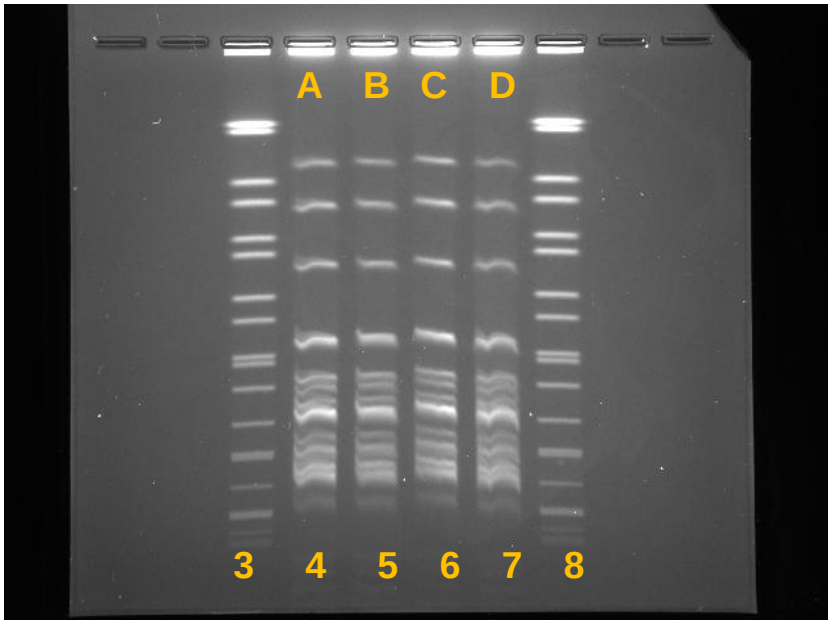
- **Wavy bands:**
  - Only in slices digested with *Apa*I
  - Occur in presence of BSA
  - Present for multiple lab personnel

- **Ideas:**
  - Damaged plug slice – bad spatula, bad razor, alcohol wipes, etc…
  - *Apa*I contaminant
  - Change in PulseNet protocol

→ **What is in common and what has changed?**

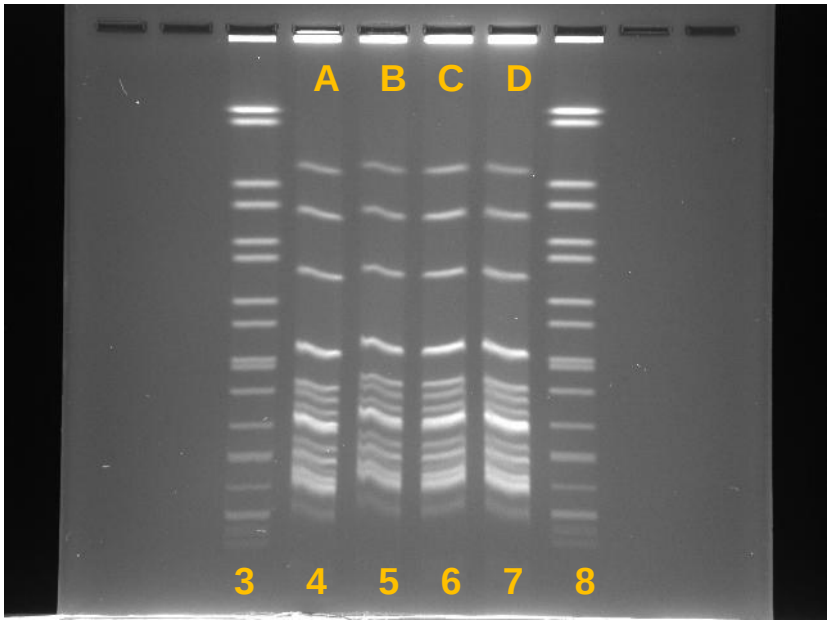
Listeria Gels – damaged plug slices?

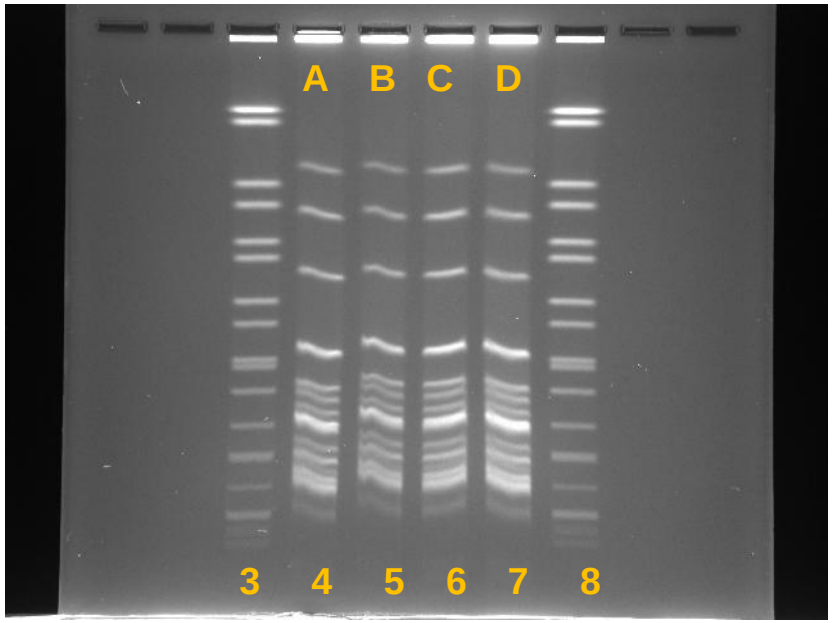




- **different razors, petri dishes, spatulas**
- same scientist
- same 4 plugs from previous slide
- *Apa*I (NEB lot# 0421103) – lanes 4 – 7, 125units (2.5μL)
- same master mix, BSA included

Listeria gels – *Apa*I enzyme?

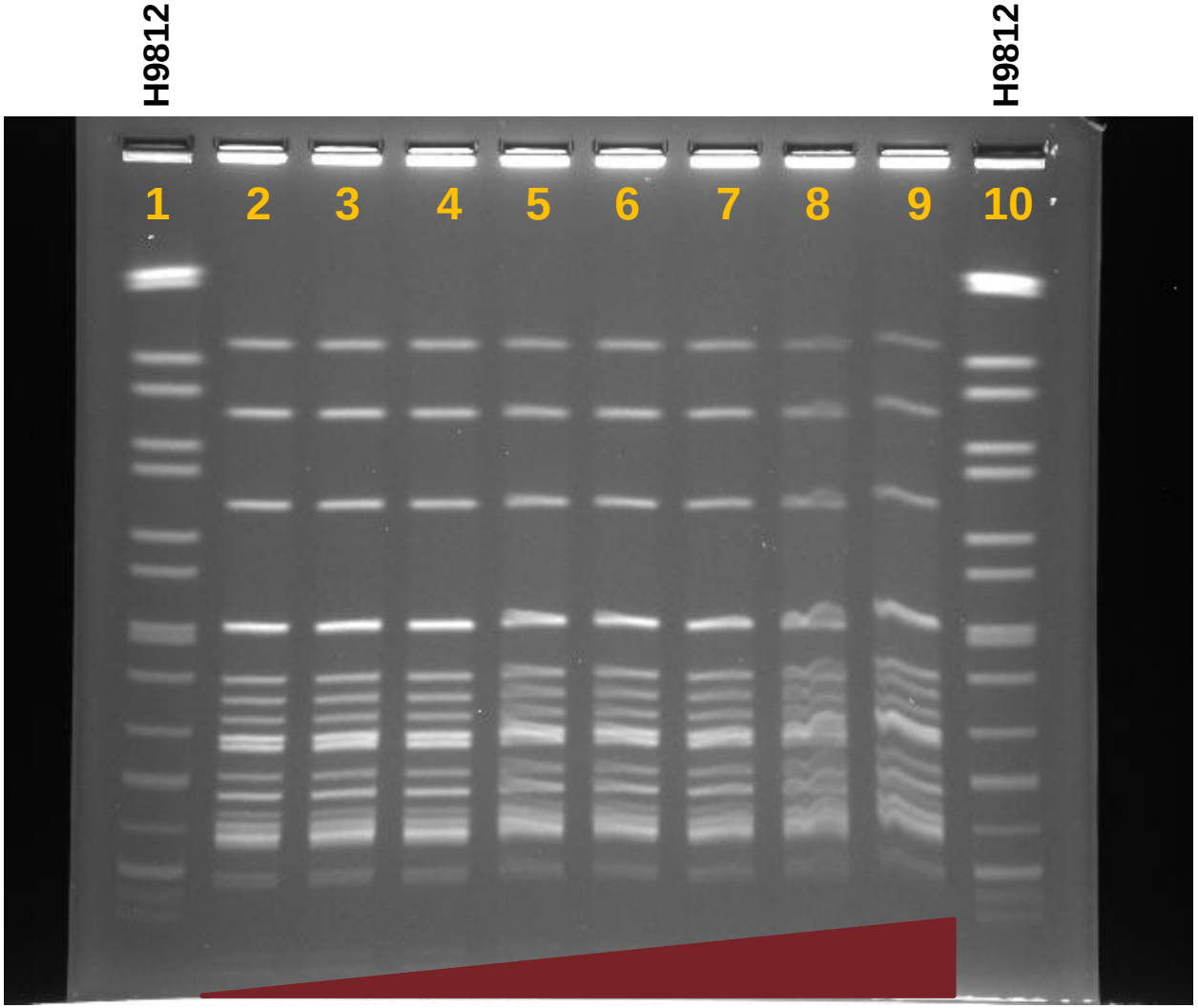




- same scientist
- same 4 plugs as gels from previous slides
- *Apa*I (NEB lot# 0431110) – lanes 4 – 7, 125units (2.5μL)
- same master mix for both gels BSA added

"The *Apa*I product manager thought it may be protein binding causing the issue."

Listeria and *Apa*I – protein binding?



***Apa*I (units):**	25	50	75	100	125	150	200	250
***Apa*I (uL):**	0.5	1	1.5	2	2.5	3	4	5

Result:

- band distortion directly correlated with units of enzyme added to master mix

What's going on?

- **Ideas:**
  - ~~Damaged plug slice – bad spatula, bad razor, alcohol wipes, etc…~~
  - *Apa*I contaminant / protein binding
    - wavy bands appeared with **new lots** in January
    - wavy bands directly correlated with increasing units of enzyme
  - Change in PulseNet protocol
    - carrying out pre-digestion step (1X NEBuffer 4) and post-digestion step (0.5X TBE) had a small improvement (data not shown)
    - *Asc*I: 40 units / sample, 2hrs at 37°C
    - *Apa*I: 50 units / sample, 2hrs at 25°C
    - ***Asc*I: 25 units / sample, 2hrs at 37°C (new recommendation)**
    - ***Apa*I: 25 units / sample, 2hrs at 25°C (new recommendation)**

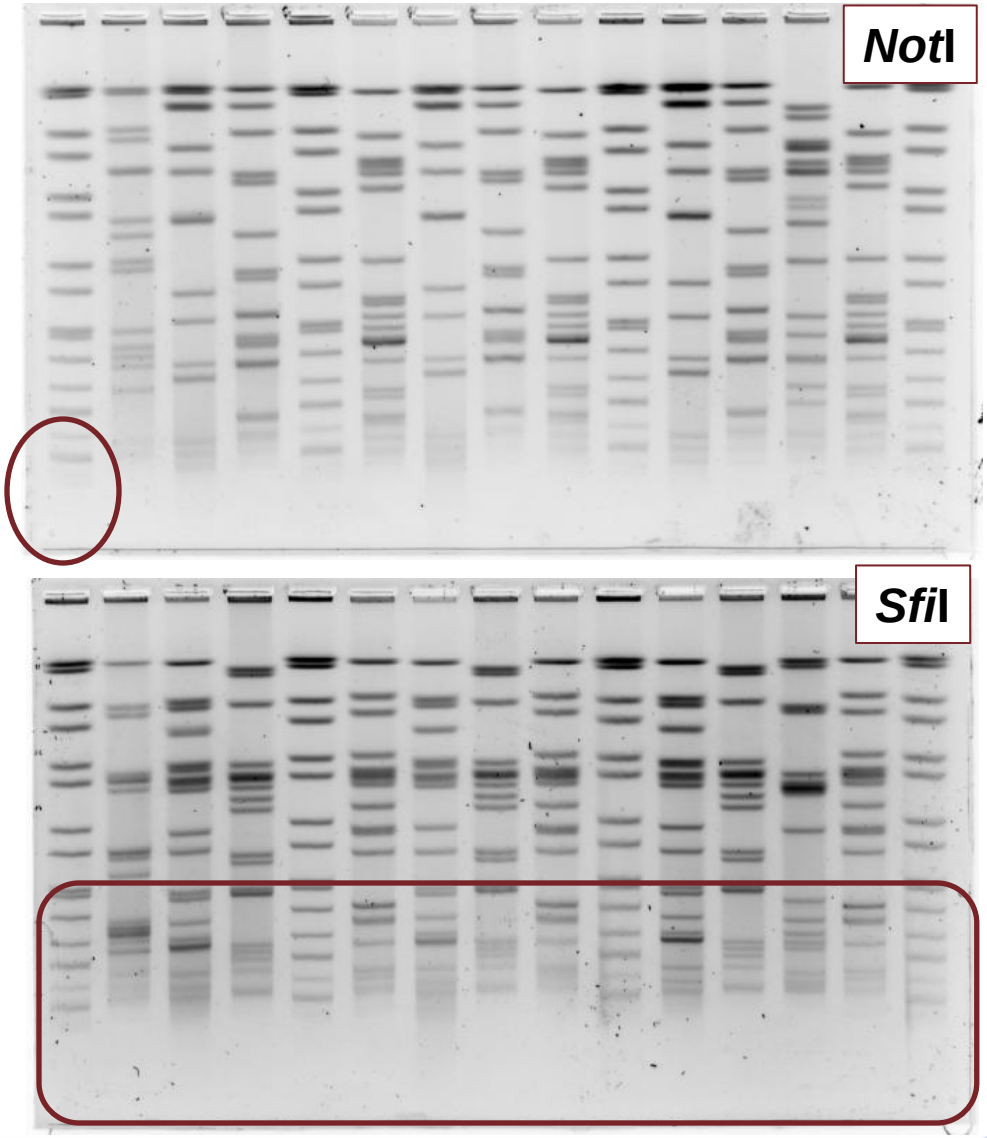
→ **What is in common and what has changed?**

Diffuse, faint and/or disappearing low molecular weight bands

CASE STUDY #2

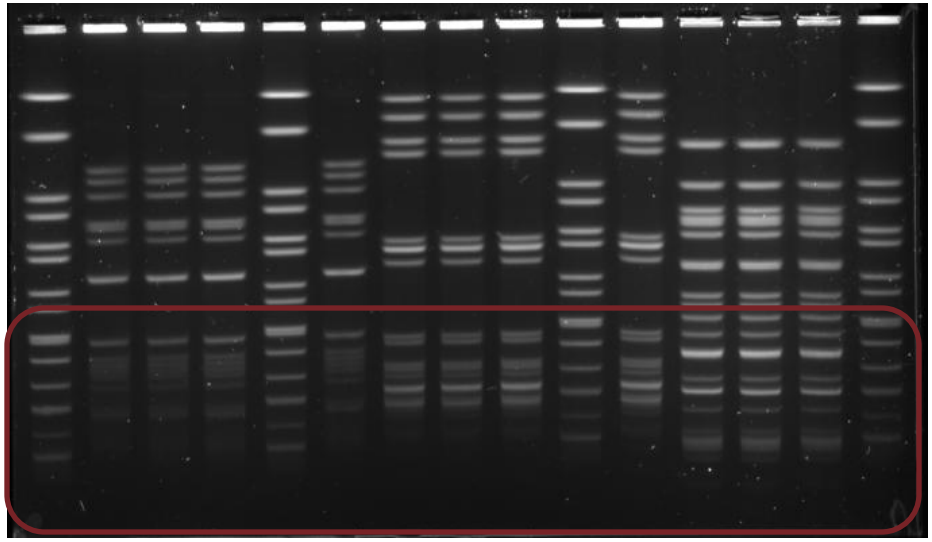
“Fuzzy” Gels – CDC, Fall 2011

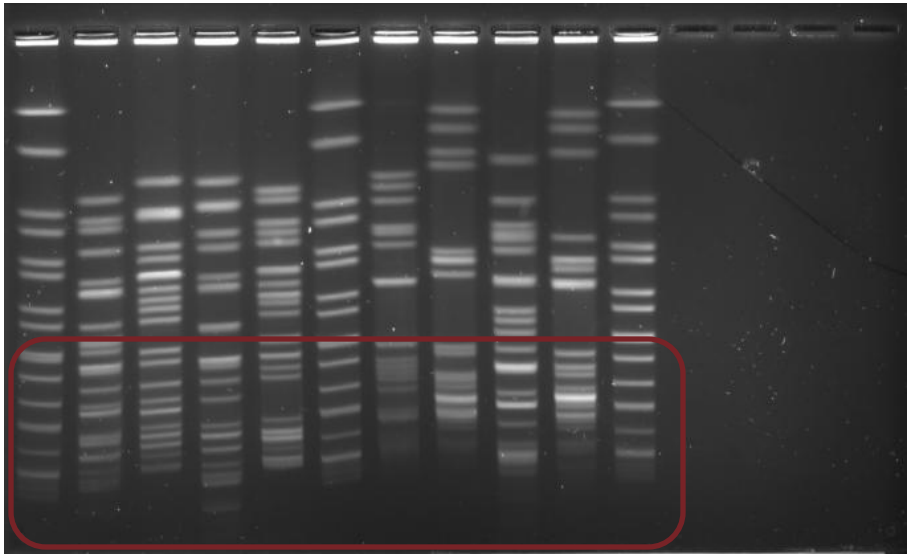
- SeaKem Gold (SKG) lot # 242048
- *Vibrio parahaemolyticus* conditions (10s – 35s)
- run time = 20 hours
- **could not be analyzed**
  - 1 hour short
  - bottom bands of standard (28.8 and 20.5kb) missing
  - some “tracking”



“Fuzzy” Gels – CDC, Fall 2011

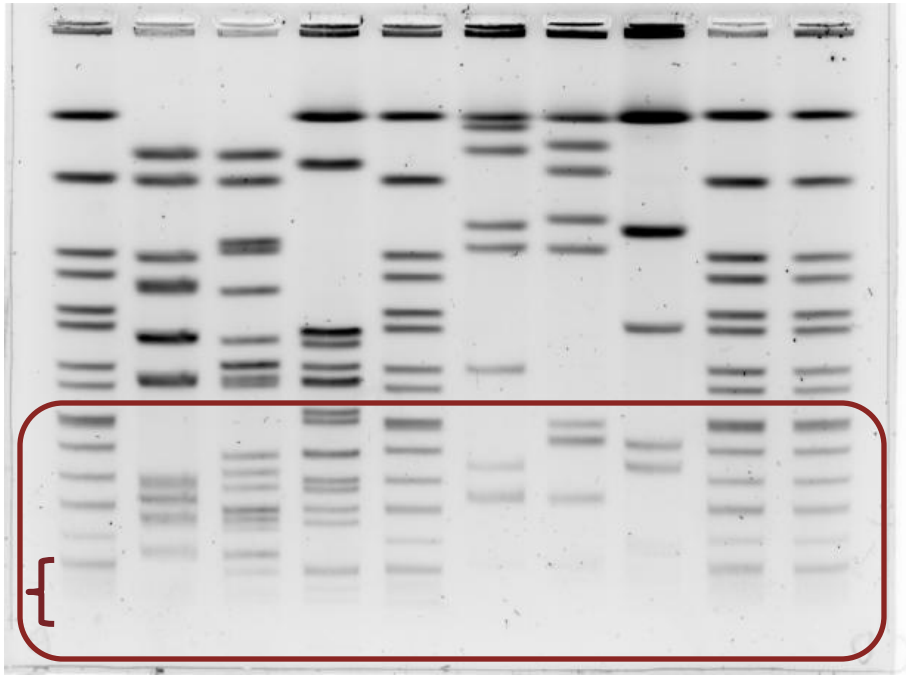
- SeaKem Gold (SKG) lot # 242048
- *E. coli* O157 conditions (2.16s – 54.17s)
- run time = 20.25 hours
- **could not be analyzed**
  - 1 hour short
  - bottom bands of standard (28.8 and 20.5kb) missing
  - some “tracking”





“Fuzzy” Gels – CDC, Fall 2011

- SeaKem Gold (SKG) lot # 242048
- *Salmonella* conditions (2.16s – 63.8s)
- run time = 18 hours
- **could not be analyzed**
  - ~30 minutes short
  - bottom bands of standard (28.8 and 20.5kb) indistinct
  - no “tracking”
  - stained with GelRed



What's going on?

- **Indistinct, diffuse low-molecular weight bands**
  - Observed in multiple PulseNet protocols
    - *E. coli*, *Salmonella*, *Vibrios*, etc…
    - *Xba*I, *Bln*I, *Sfi*I, *Not*I, etc…
    - Ethidium bromide, GelRed
  - Observed for different technicians in multiple labs

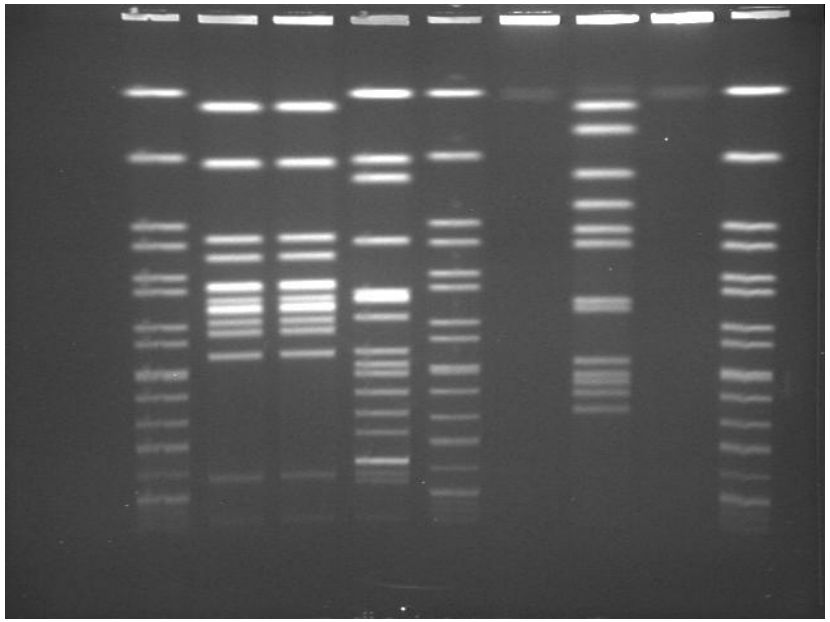
- **Ideas**
  - Contaminant in water
  - Contaminant or formulation change in TBE of one of the components
  - Contaminant or formulation change in agarose

→ **What is in common and what has changed?**

Comparison of lots of SKG (Lonza)

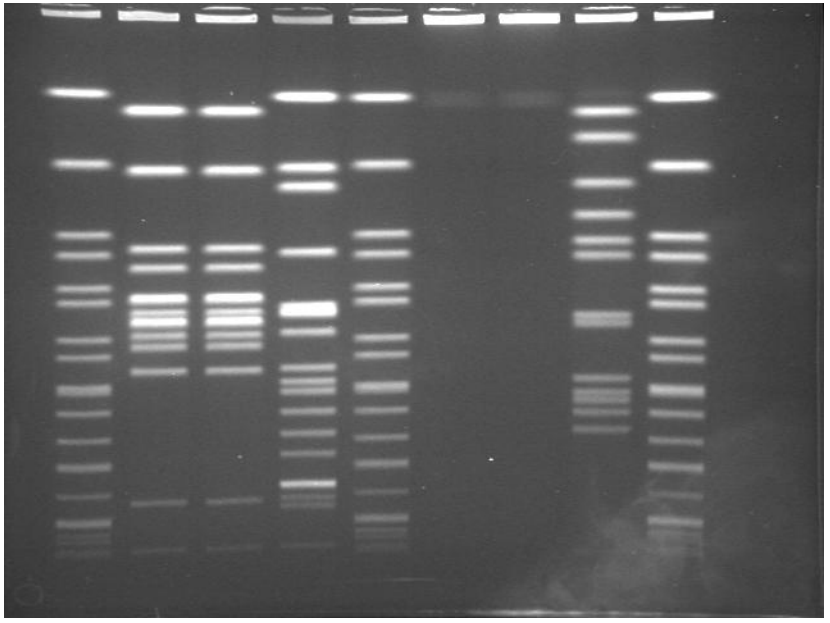
***Salmonella* conditions (2.16s – 63.8s)**

SKG lot# 252573



- SKG agarose clumped in the flask before microwaving, took longer to melt
- length is ~1hr short
- lower MW bands are faint and diffuse
- whole image is not as sharp as usual

SKG lot# 209453



- SKG agarose appearance and melting was normal
- good length
- all bands are crisp and distinct

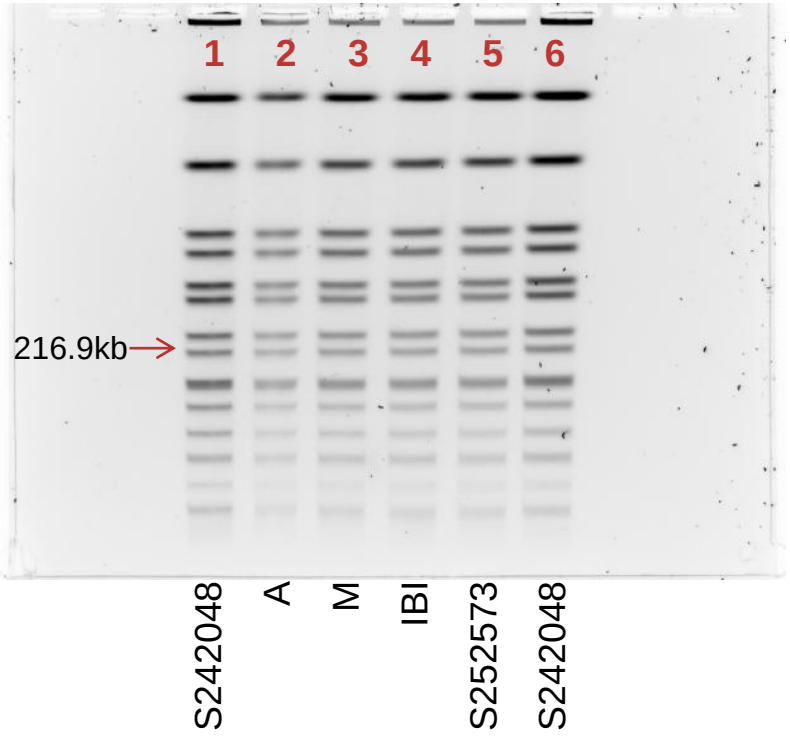
Agarose Testing

- **Hypothesis:** Poor gel quality is due to bad lot(s) of SeaKem Gold used for running agarose

- **Plug agarose**
  - Amresco LF™, Bio-Rad Megabase, IBI (not validated), SKG lot # 242048, SKG lot # 252573
  - All plugs made on the same day with the same suspension of H9812 cells
- **Running agarose**
  - Amresco LF™, Bio-Rad Megabase, IBI (not validated), SKG lot # 242048, SKG lot # 252573, SKG Lot# 169896
  - All gels run by the same scientists using the same instrument for 18 hours, 14°C, *Salmonella* switch times

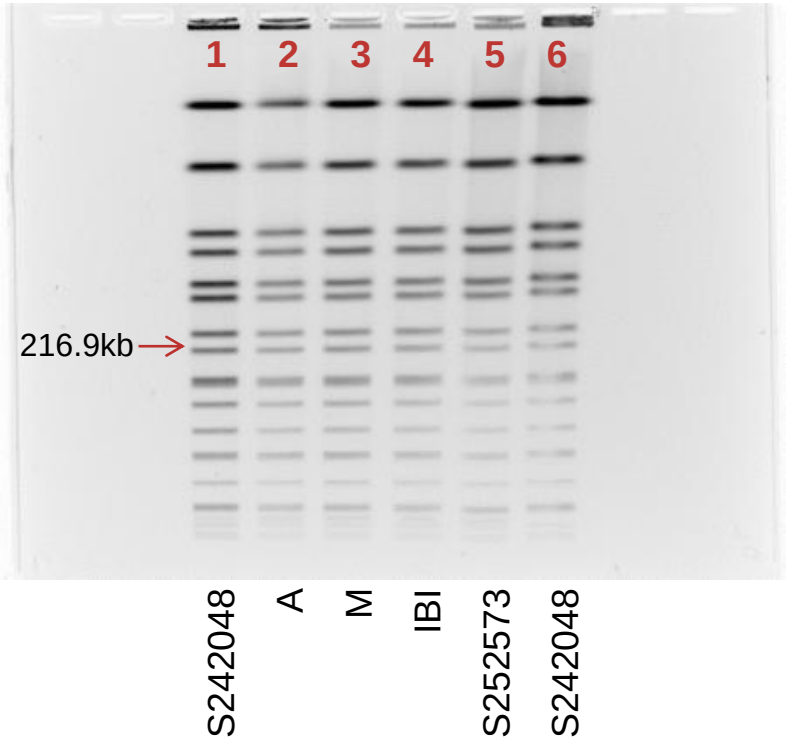
Comparison of brand and lots of plug and running agarose

SKG lot # 252573



- indistinct, faint bands observed regardless of plug agarose used

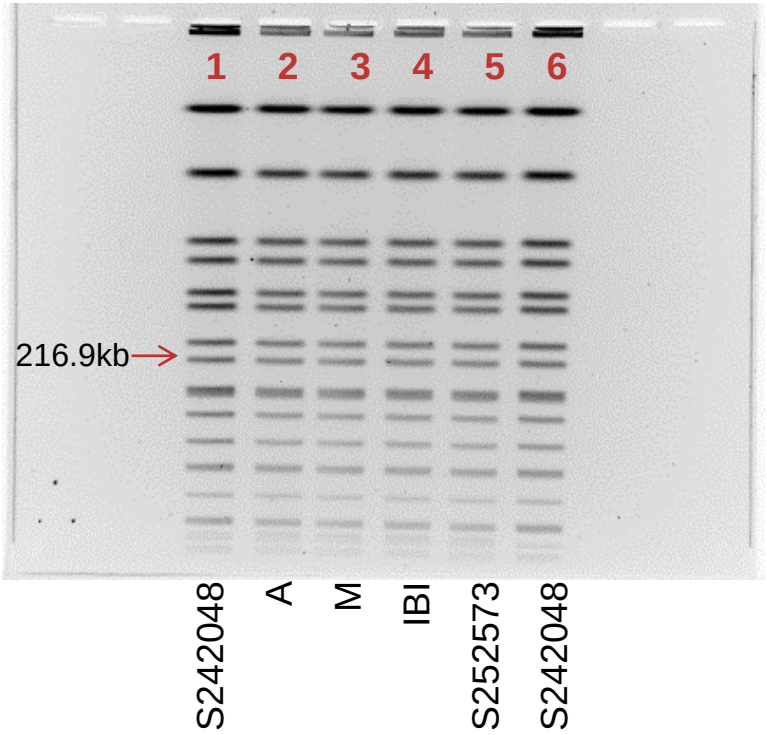
Amresco LF™ lot# 2899B228



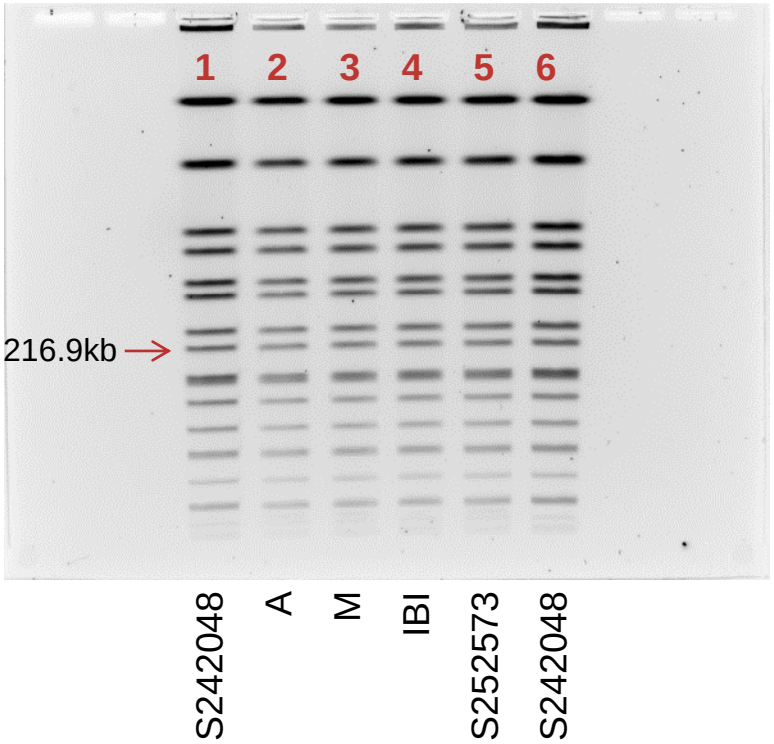
- crisp bands at all molecular weights, all plug agaroses
- some “tracking” observed

Comparison of brand and lots of plug and running agarose

SKG lot # 169896 – “old”



SKG lot # 267744 – “replacement”



- crisp bands at all molecular weights regardless of plug agarose used

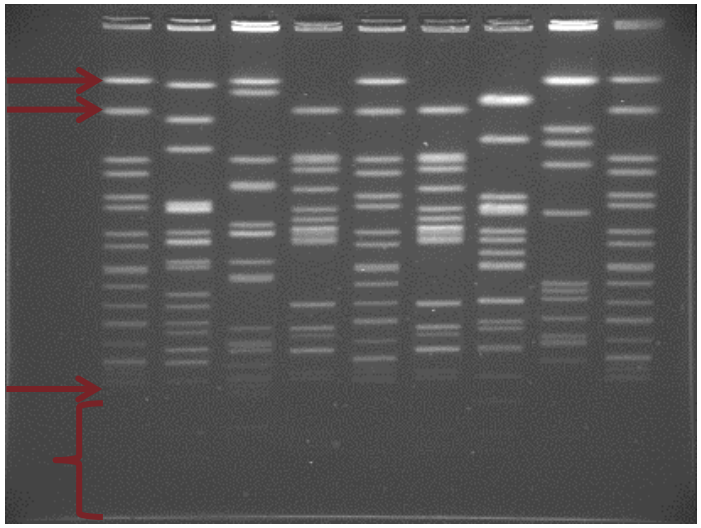
Conclusions and Recommendations – SeaKem Gold (Lonza)

- **SeaKem Gold (SKG) lots #s 242048, 252573, 255693, and 262031 should not be used for plug or running agarose**
  - All 4 lots were linked to a single "bulk batch" at Lonza
  - Bulk batch and individual lots passed all QA/QC tests at Lonza
    - ✓ **Smallest band on QA/QC gels was 200kb**
  - No change in production process or formulation of SKG was identified
- **Please contact Lonza for a possible replacement**
  - Will replace at their discretion
  - New, replacement lots produce good quality gels
- **Continue to monitor agarose characteristics and gel quality**

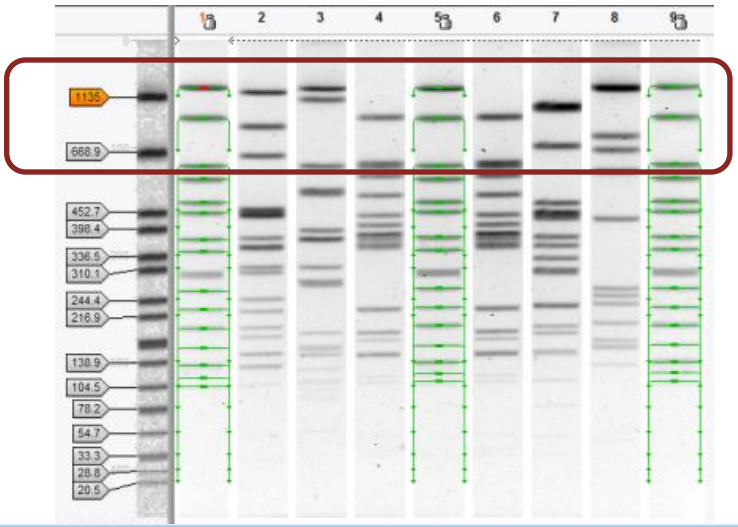
Short gels / long run times, bad normalization

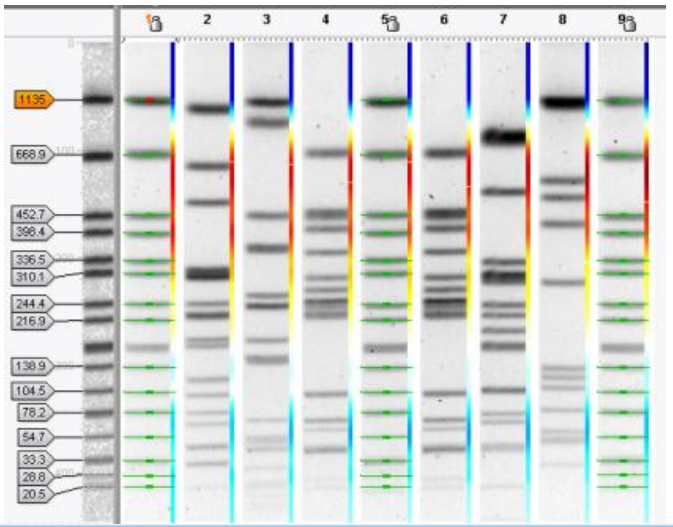
CASE STUDY #3

Characterization of poor quality gels run with Megabase (Bio-Rad)

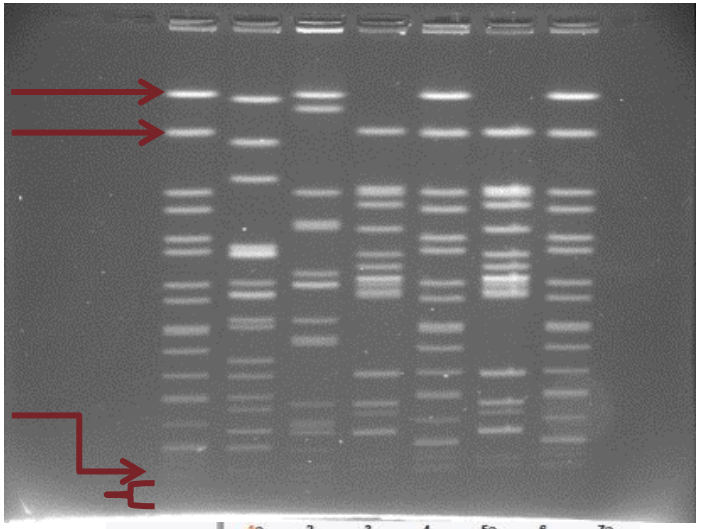


- Megabase lot# 45100031
- *Salmonella* conditions (2.16s – 63.8s)
- run time – 18.25 hours, 4hrs short
- failed normalization

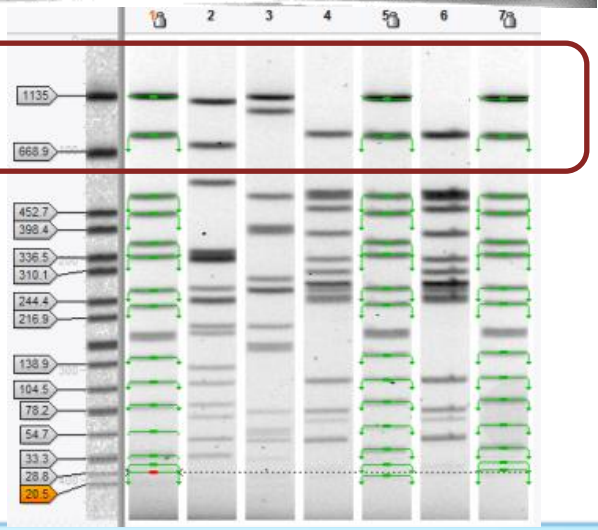


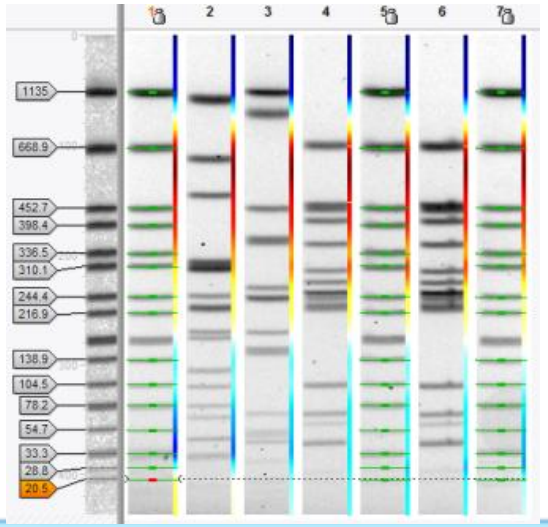


Characterization of poor quality gels run with Megabase (Bio-Rad)



- Megabase Lot# 45100031
- *Salmonella* conditions (2.16s – 63.8s)
- run time – 22.25 hours, unreasonably long time for proper length
- failed normalization





What's going on?

- **Failing normalization – gap between 1135kb and 668.9kb bands was smaller than expected**
- **Increased running time for bottom band of standard (20.5kb) to reach 1cm from bottom of gel**
  - Observed in multiple PulseNet protocols
  - Observed for different technicians in multiple labs on multiple instruments
  - Additional run time did not improve normalization
- **Ideas**
  - Machine malfunction
  - Contaminant or formulation change in agarose

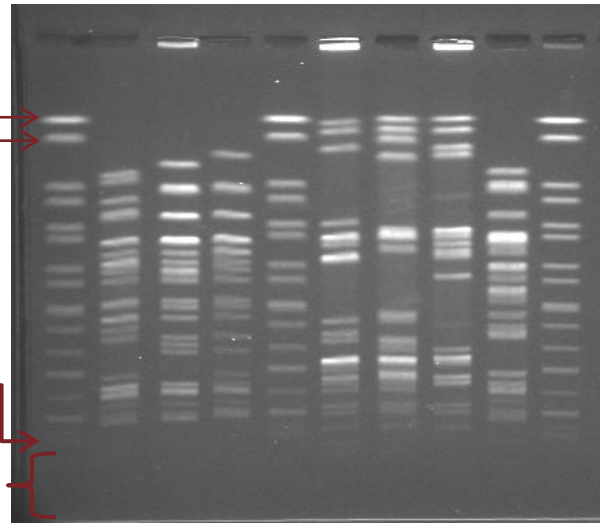
→ **What is in common and what has changed?**

Agarose Testing

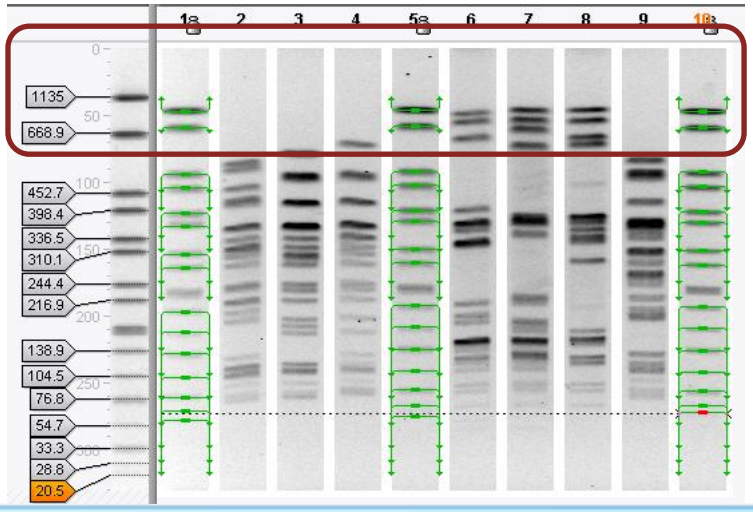
- **Hypothesis:** Poor gel quality is due to bad lot(s) of Megabase, performing similarly to “old” formulation

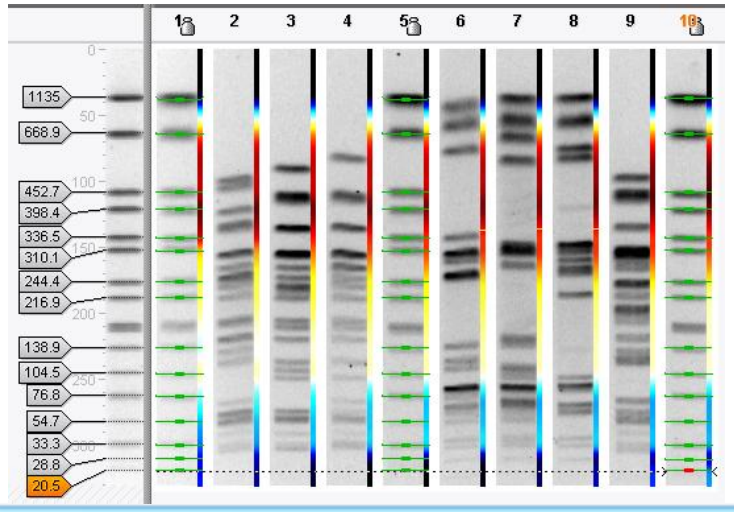
- **Running agarose**
  - Megabase, “old” formulation
  - Megabase 037, “test lot”
  - Megabase lot# 45100031, “new” formulation

Characterization of gels run with “old” formulation of Megabase (Bio-Rad)

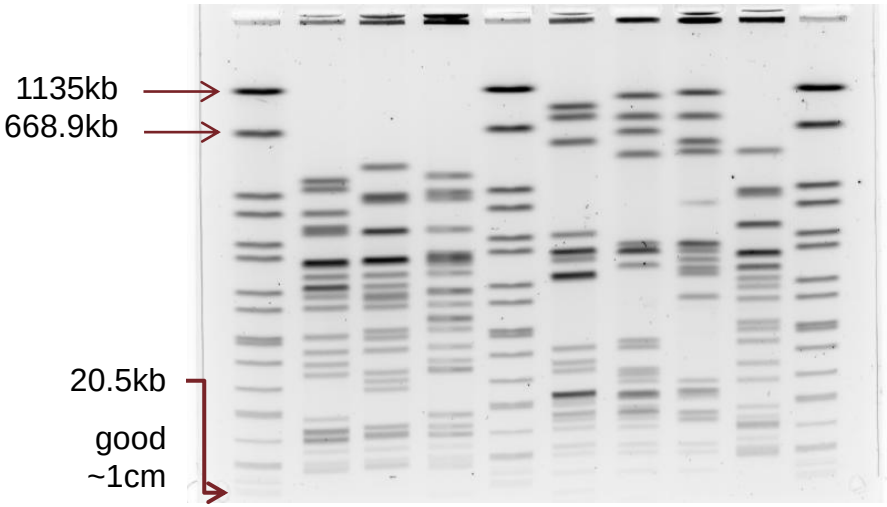


- Megabase – “old” formula
- tested in 2008
- *E. coli* switch times (2.16s – 54.17s)
- run time = 19 hours, ~2hrs short
- failed normalization

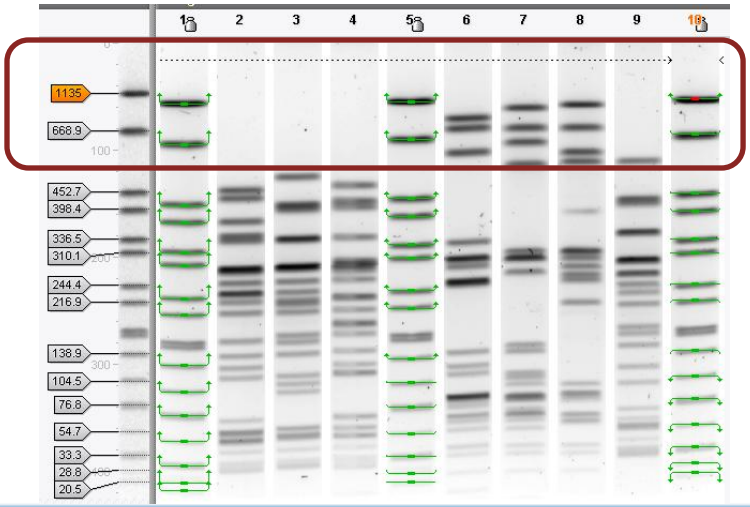


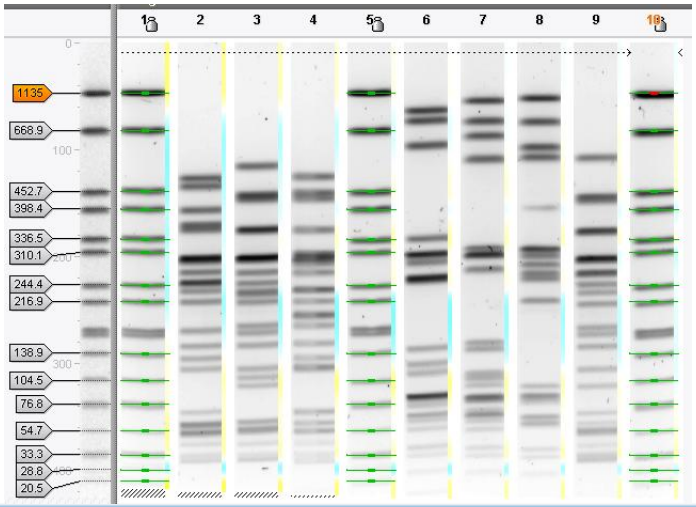


Characterization of gels run with “new” formulation of Megabase (Bio-Rad)

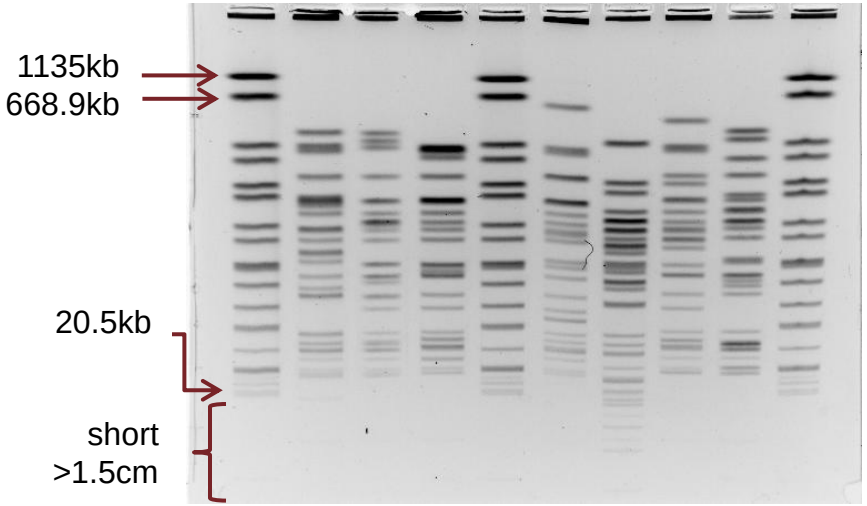


- Megabase – 037 “test lot”
- tested in 2009
- *E. coli* switch times (2.16s – 54.17s)
- run time = 18.25 hours; good length in a reasonable run time
- good normalization

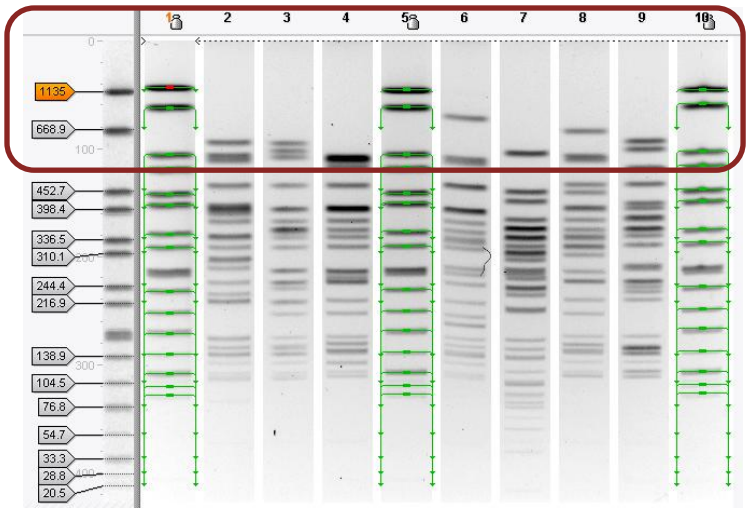


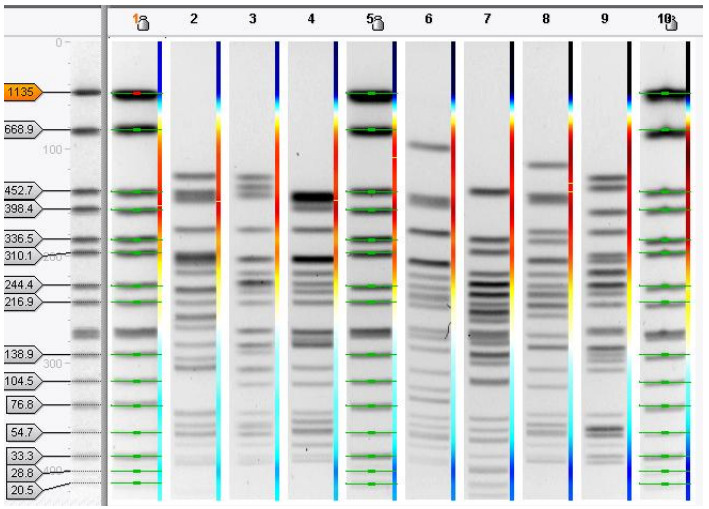


Characterization of gels run with “new” formulation of Megabase (Bio-Rad)



- Megabase lot# 45100031
- *E. coli* switch times (2.16s – 54.17s)
- run time = 18 hours, >2 hours short
- failing normalization





Conclusions and Recommendations – Megabase (Bio-Rad)

- **Megabase from all lots should not be used for plug or running agarose**
  - No change in production process or formulation of Megabase was identified, low end of specification range
  - PulseNet protocols will be updated to remove language suggesting Megabase as an acceptable alternative to SKG
  - Additional lots or new formulations will be tested and recommendations regarding using Megabase in PulseNet protocols revised
- **Continue to monitor agarose characteristics and gel quality**

Sources of Variation within Standardized Protocols

- **Interpretation of instructions**
  - legacy – “but we’ve always done it that way…”
  - protocol drift vs. protocol shift
- **Reagents**
  - lot-to-lot from one vendor
  - vendor-to-vendor
  - in-house prepared buffers vs. commercial, purchased buffers
  - water quality
- **PFGE equipment**
  - models, electrical supply, etc…
- **Image acquisition**
  - staining, imaging system, camera, etc…

Conclusions

- **Communication was key to identifying the cause of poor quality gels in these examples**
  - Reagents used across the entire network will impact data network-wide
  - Difficult to isolate variable or identify cause of poor quality gels if look at these phenomenon individually
  - Time to identify and resolve issues reduced due to cooperation and network-wide communication
- **Reagents will continue to have network-wide impact as production processes change, outsourcing, etc…**

Acknowledgements

All PulseNet participants at CDC, FDA, USDA, and in the State Public Health Laboratories

The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention

For more information please contact Centers for Disease Control and Prevention
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E-mail: cdcinfo@cdc.gov Web: www.cdc.gov