

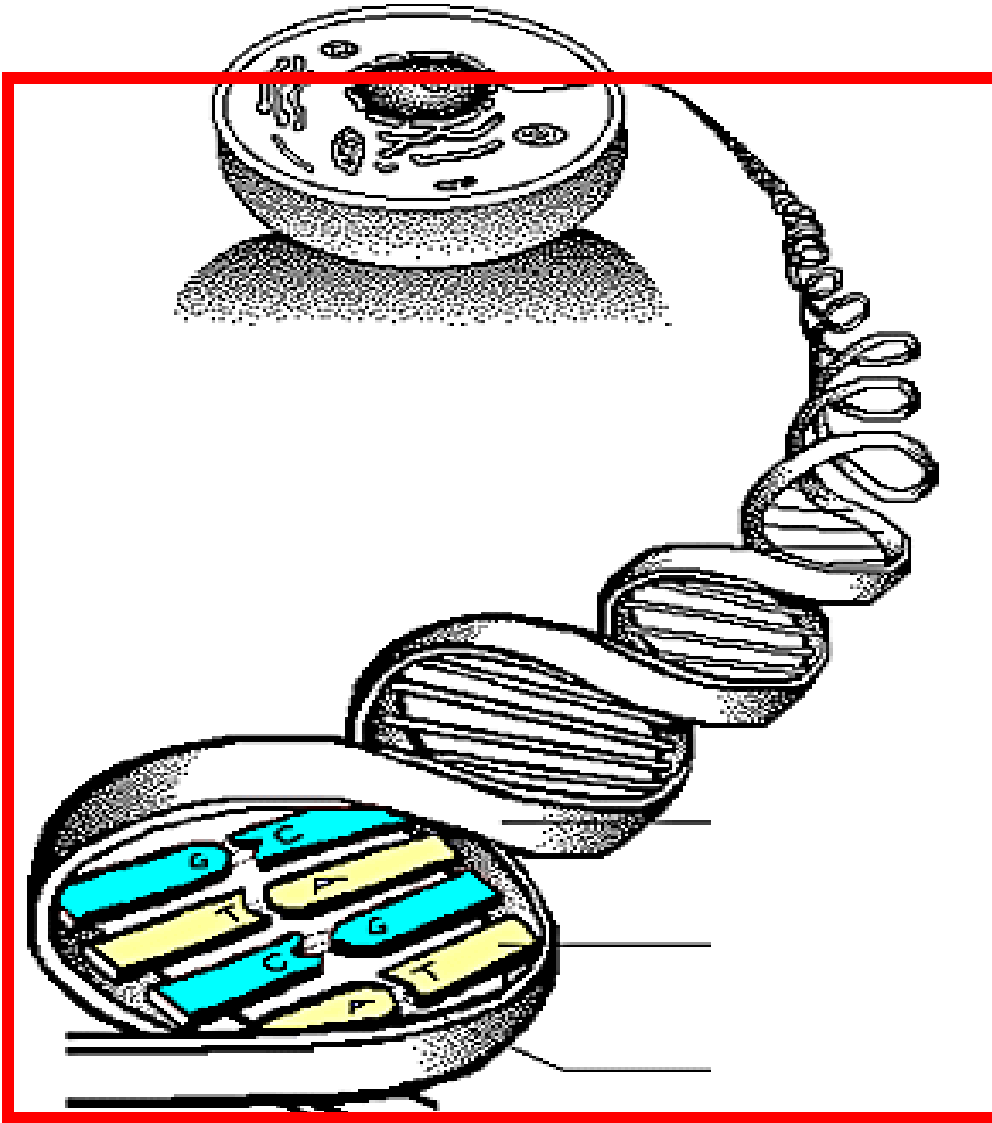
Pulse Field Gel Electrophoresis (PFGE) Explanation

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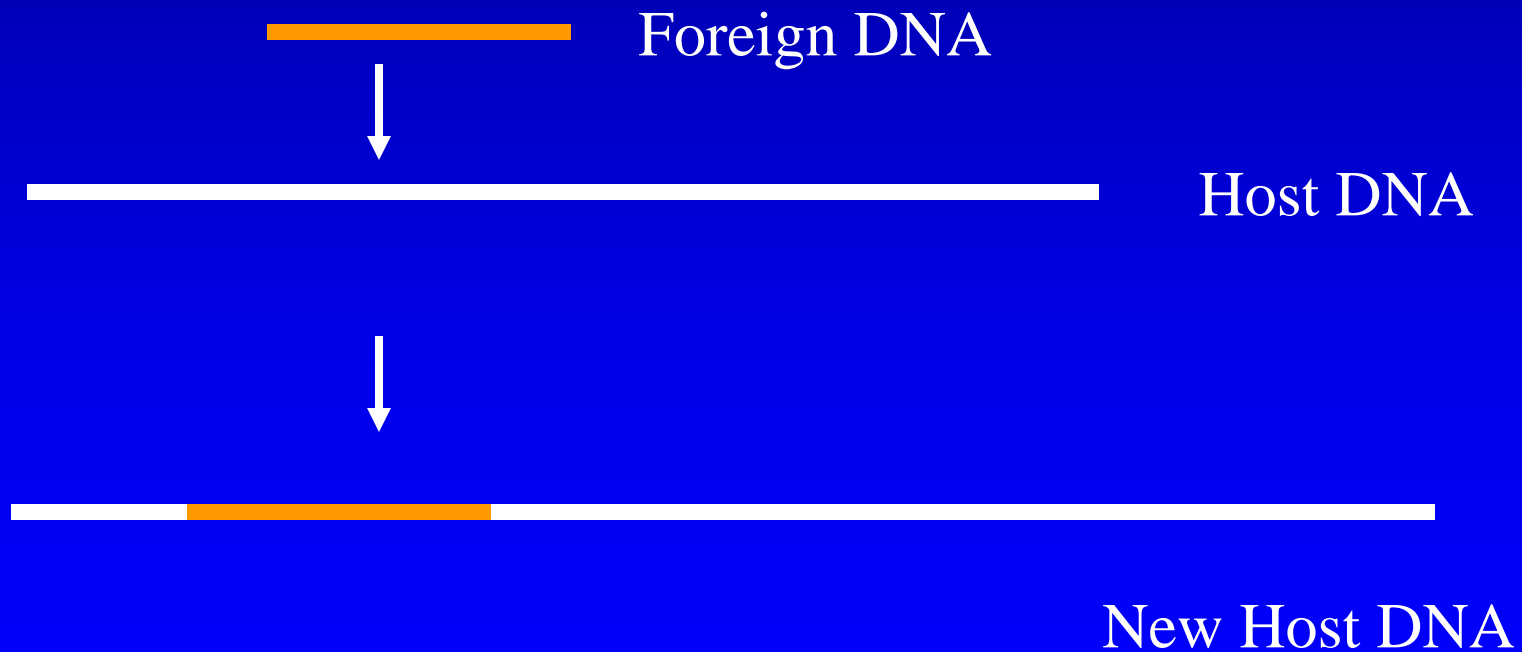
E. coli O157:H7



**Target
region
for
PFGE**

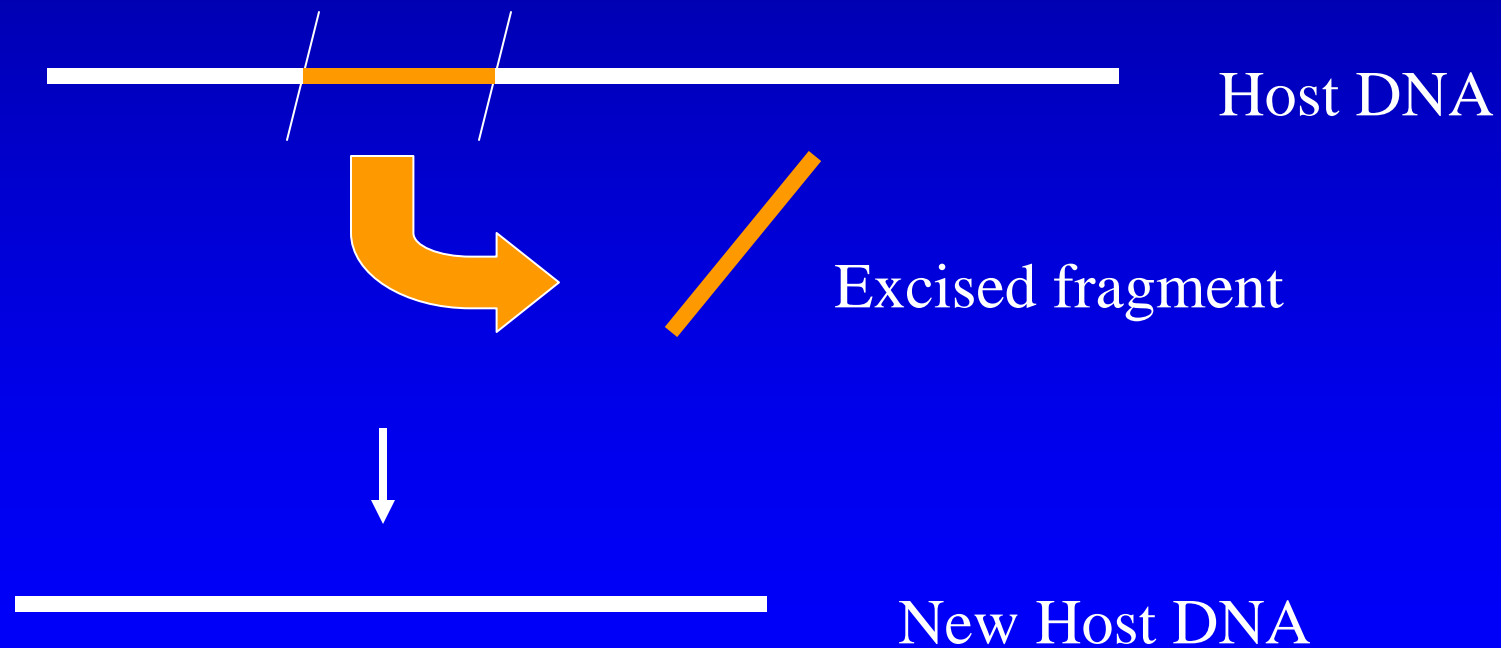
Principle: Strain diversity

Insertion event



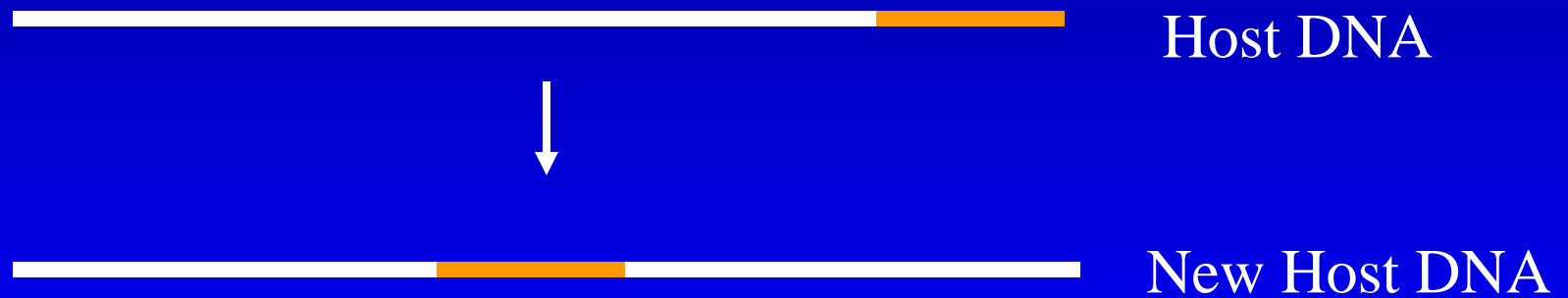
Principle: Strain diversity

Deletion event



Principle: Strain diversity

Rearrangement event



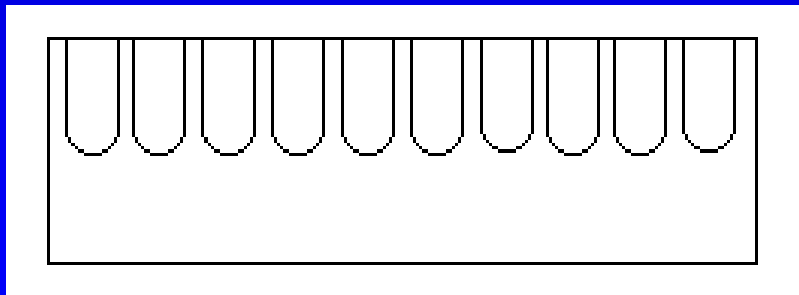
Principle: PFGE Subtyping

- **Strain diversity (insertions, deletions & rearrangements) give rise to changes in DNA fragments.**
- **Subsequent generations of bacteria retain the parental DNA pattern until another diversity event (clonality).**

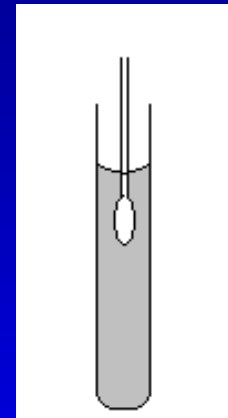
Practice



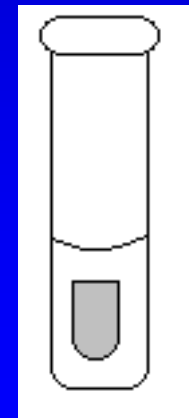
24 hour culture



Make agarose plugs



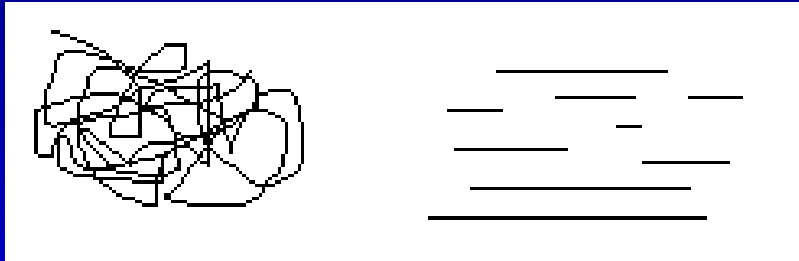
Cell suspension
abs ~1.8



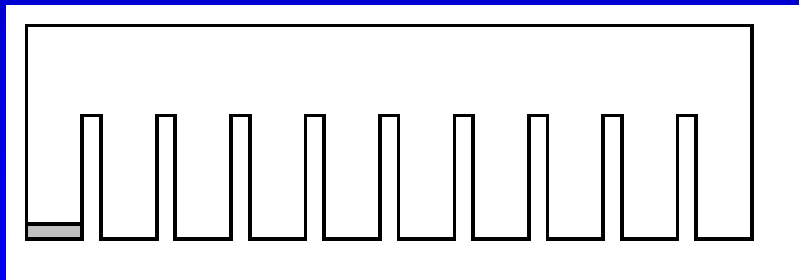
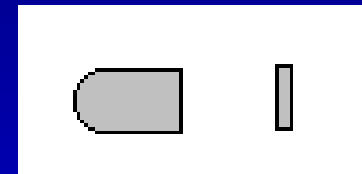
Lyse cells and wash
plugs

Practice

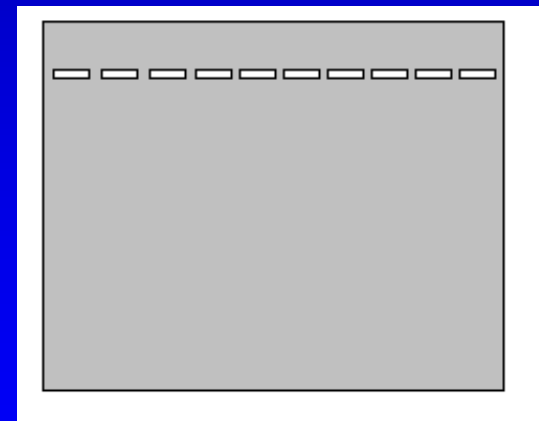
Restrict DNA in plugs



Slice 2mm piece of plug



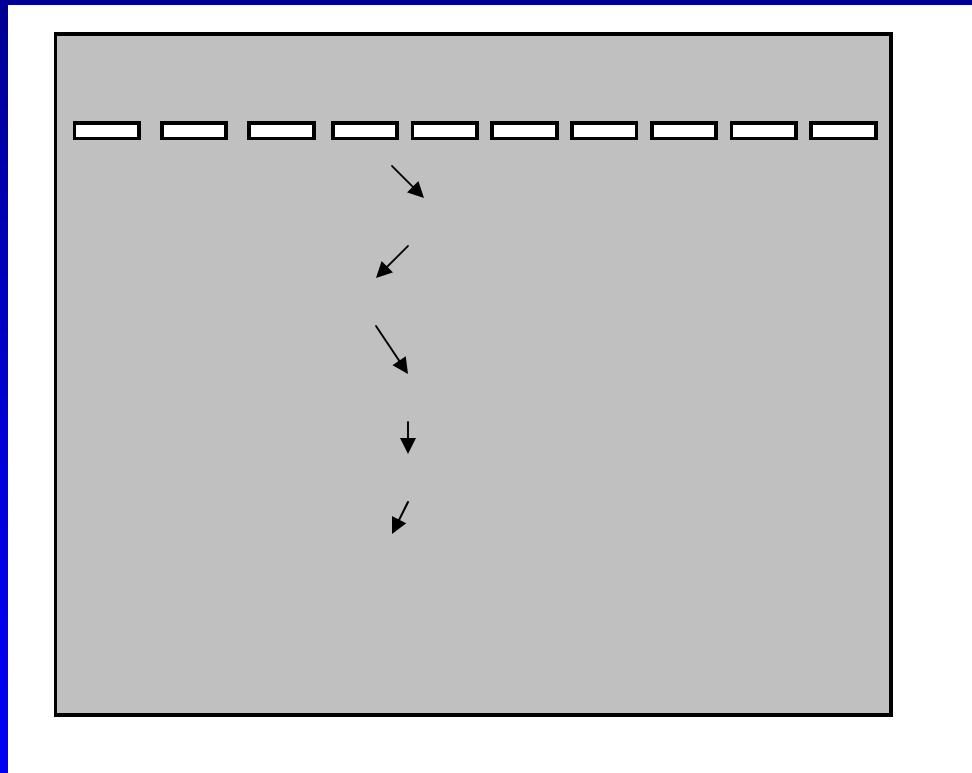
Load slices onto comb



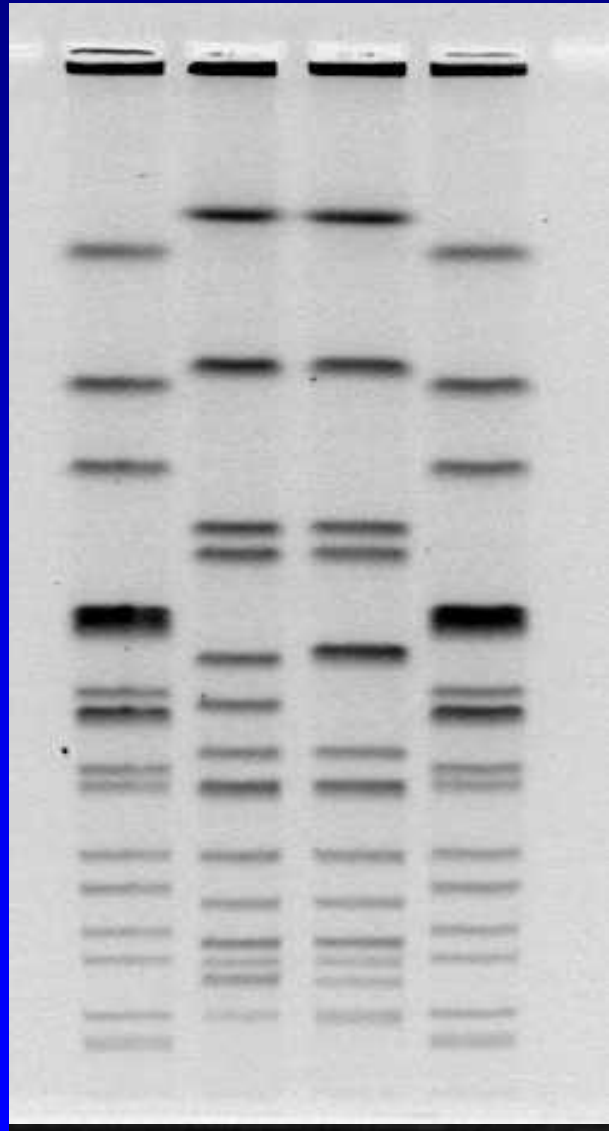
Pour gel and remove comb

electrodes

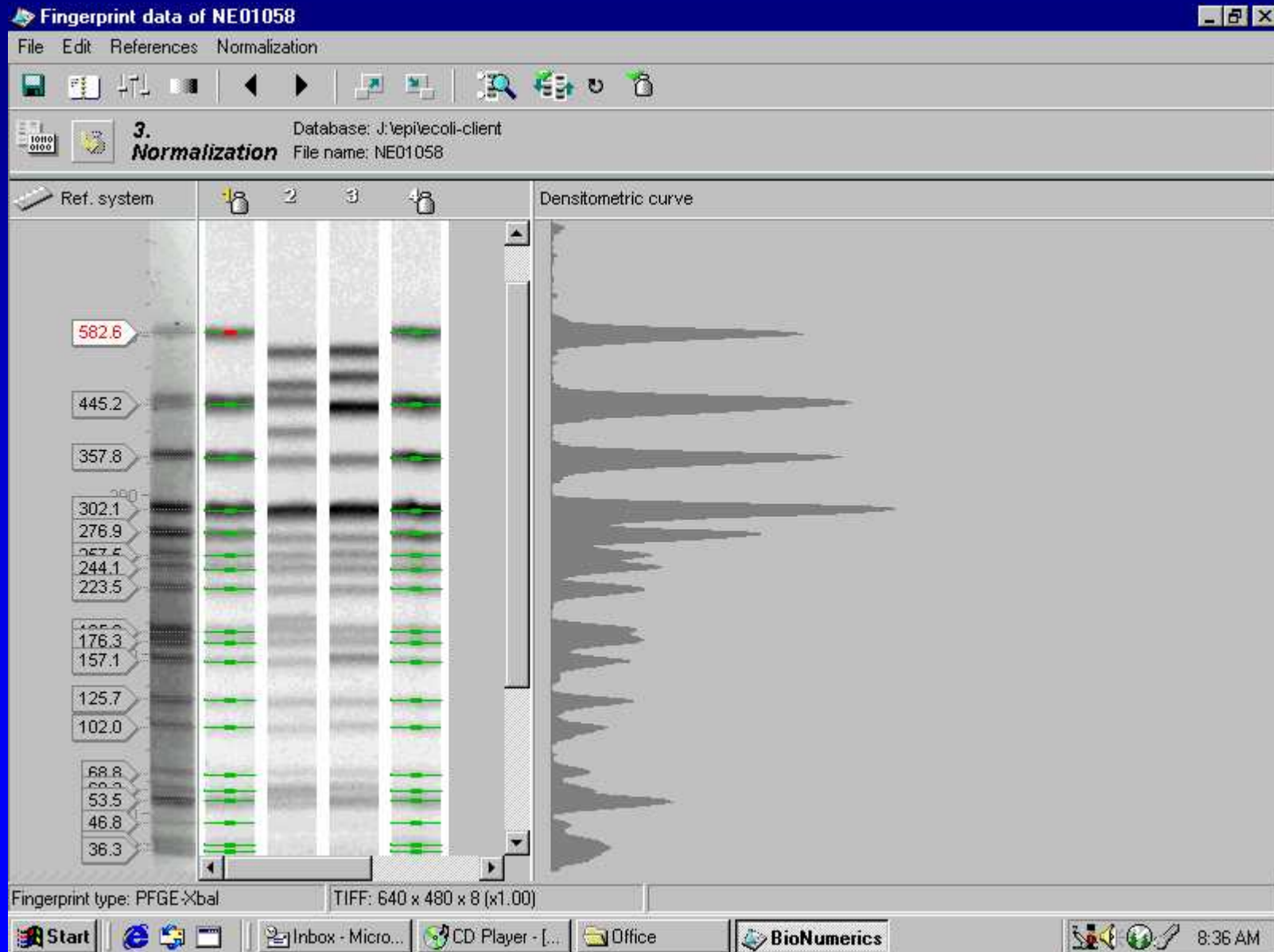
Electric current 18-20 hours
buffer 14 C



The End Result

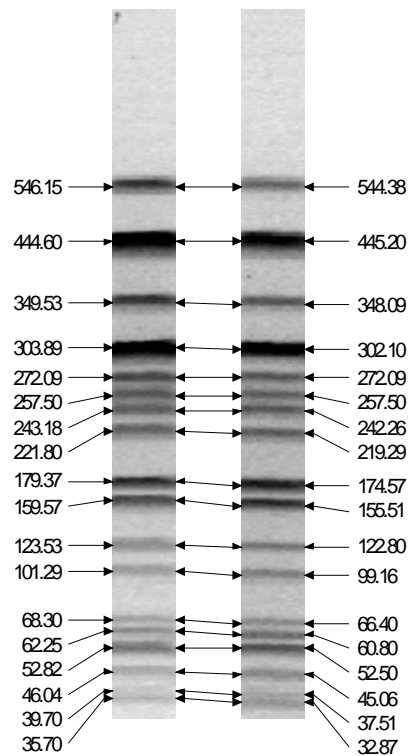


The Analysis Process: Normalization



The Analysis Process: Making Comparisons

Key	REF 1784	REF 1823
IsolatDate	2000-07-12	2000-07-07
Serotype	E. Coli O157:H7	E. Coli O157:H7
PFGE-XbaI-pattern	x.0055	x.0055



Similarity: 100.00%

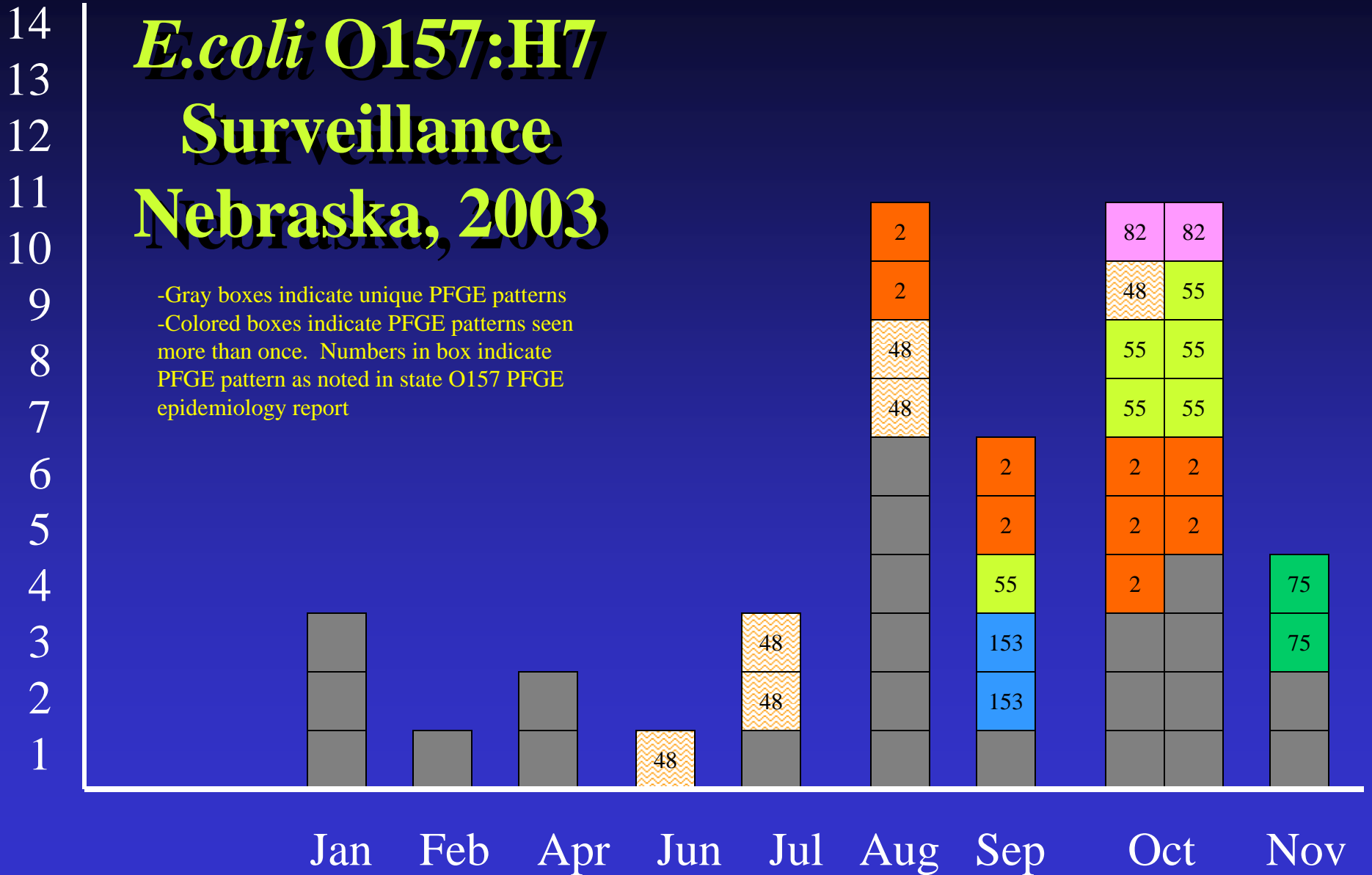
Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-98.3%]

Unmatched bands: 0

Cases

E. coli O157:H7 Surveillance Nebraska, 2003

-Gray boxes indicate unique PFGE patterns
-Colored boxes indicate PFGE patterns seen more than once. Numbers in box indicate PFGE pattern as noted in state O157 PFGE epidemiology report

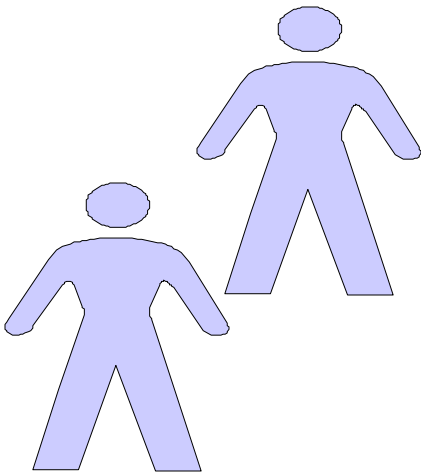


Nebraska *E. coli* Database

- 308 isolates typed since 1999
- 170 pfge patterns
- 138 (44%) patterns observed only once
- Most common pattern (x.0002) seen 32 times = 10% database

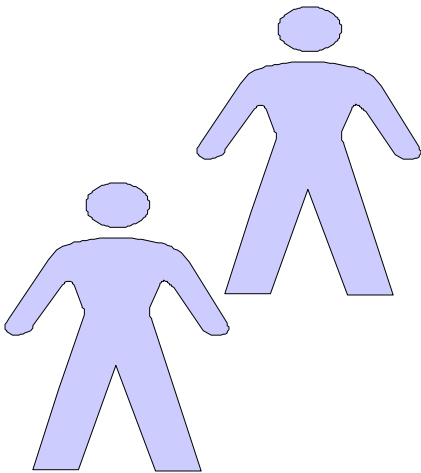
Microbial Subtyping: What it doesn't mean

- A match does NOT mean the cases are DEFINATELY related
- A non-match does not mean that the cases are definitely NOT related.



Microbial Subtyping: What it does mean

- A match means the cases are **MORE LIKELY** to have a common source than if they didn't match
- A non-match means the cases are **LESS LIKELY** to have a common source than if they did match



Any questions??

Contact information

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