Listeria Sequencing in Real Time

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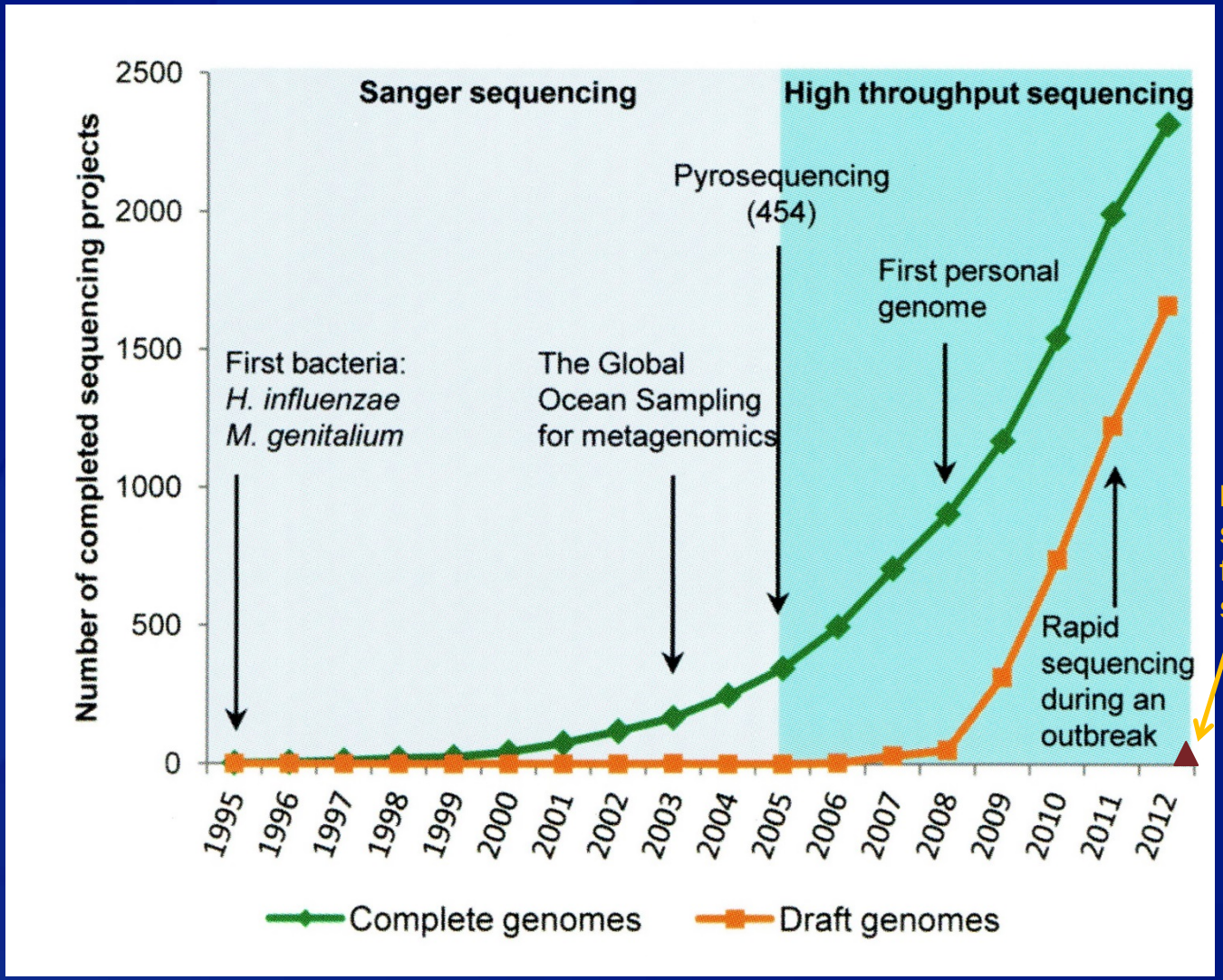
Division of Foodborne, Waterborne, and Environmental Diseases

Sequencing Application Evolution



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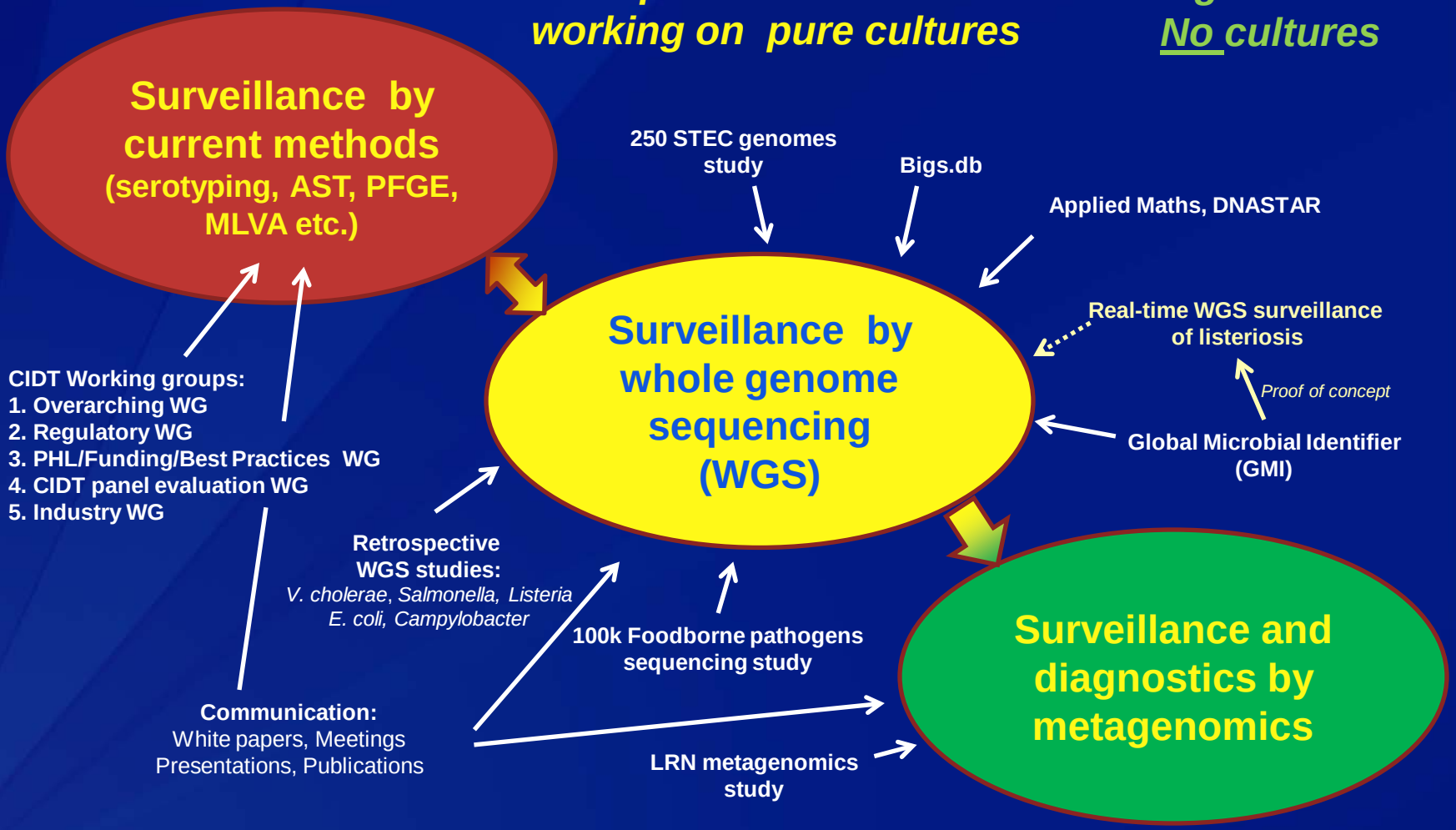
Laboratory Strategy to Meet The Challenge of Culture Independent Diagnostic Methods (CIDT)



Proof-of-Concept on the Use of Real-Time Whole Genome Sequencing in Conjunction with Enhanced Surveillance for Listeriosis

- Collaboration among the public health departments in the states, CDC, FDA, USDA, and NCBI
- International component: Developing and refining bioinformatics ‘pipelines’ with partners in Canada, England, France, Denmark and Australia

WGS For Surveillance

- **WGS has been used retrospectively but rarely to guide public health action in an outbreak investigation and never for routine surveillance**
- **Need to prove the public health impact of WGS to move the technology forward**
  - Can outbreaks be detected and solved faster, with fewer cases?

Why *Listeria monocytogenes?*

- **Listeriosis is a foodborne disease that is serious, fairly rare, and commonly associated with outbreaks**
  - Low cost and potentially high public health impact
- **Current subtyping methods are not ideal**
  - No high-discriminatory alternative to PFGE
  - WGS provides information about the evolutionary relationship between isolates
- **The epidemiological surveillance component is very strong with the *Listeria* Initiative**
- **The food regulatory component of *Listeria* control is strong**
- **The *Listeria* genome is fairly small and relatively easy to sequence and analyze**

What do we want to do?

- **Sequence all clinical isolates in the U.S. during one year as close to real-time as possible in parallel with current surveillance**
  - PulseNet PFGE, strain characterization at CDC, interview of case-patients
- **Upload sequences to NCBI (Genbank), a public database, as the sequences are generated**
  - With metadata that do NOT identify state or isolation date but with link to the PulseNet database
- **Evaluate data on a weekly basis**
- **Follow-up on clusters detected**
  - Both PFGE and WGS defined clusters

Technical Questions

- Is it possible to sequence and analyze the data in real-time?
- What problems will we encounter?
- Which targets has the strongest epidemiological concordance?
  - Core SNPs
  - Kmer SNPs
  - wgMLST
- How do we define clusters?
- Do we need to define clusters?
- How do we track/monitor clusters in real time?
- How can we compare strains/isolates over time?
- What bioinformatics tools and approaches works most efficiently for outbreak detection, delineation and control?
  - Speed ~ accuracy
- How does it work compared to PFGE?

Experience So Far

- **Exciting technology**
- **All PFGE clusters do also cluster by WGS**
  - Some are split
  - More accurate case definition
- **One cluster identified by WGS that was not recognized by PFGE**

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- **Exciting technology**
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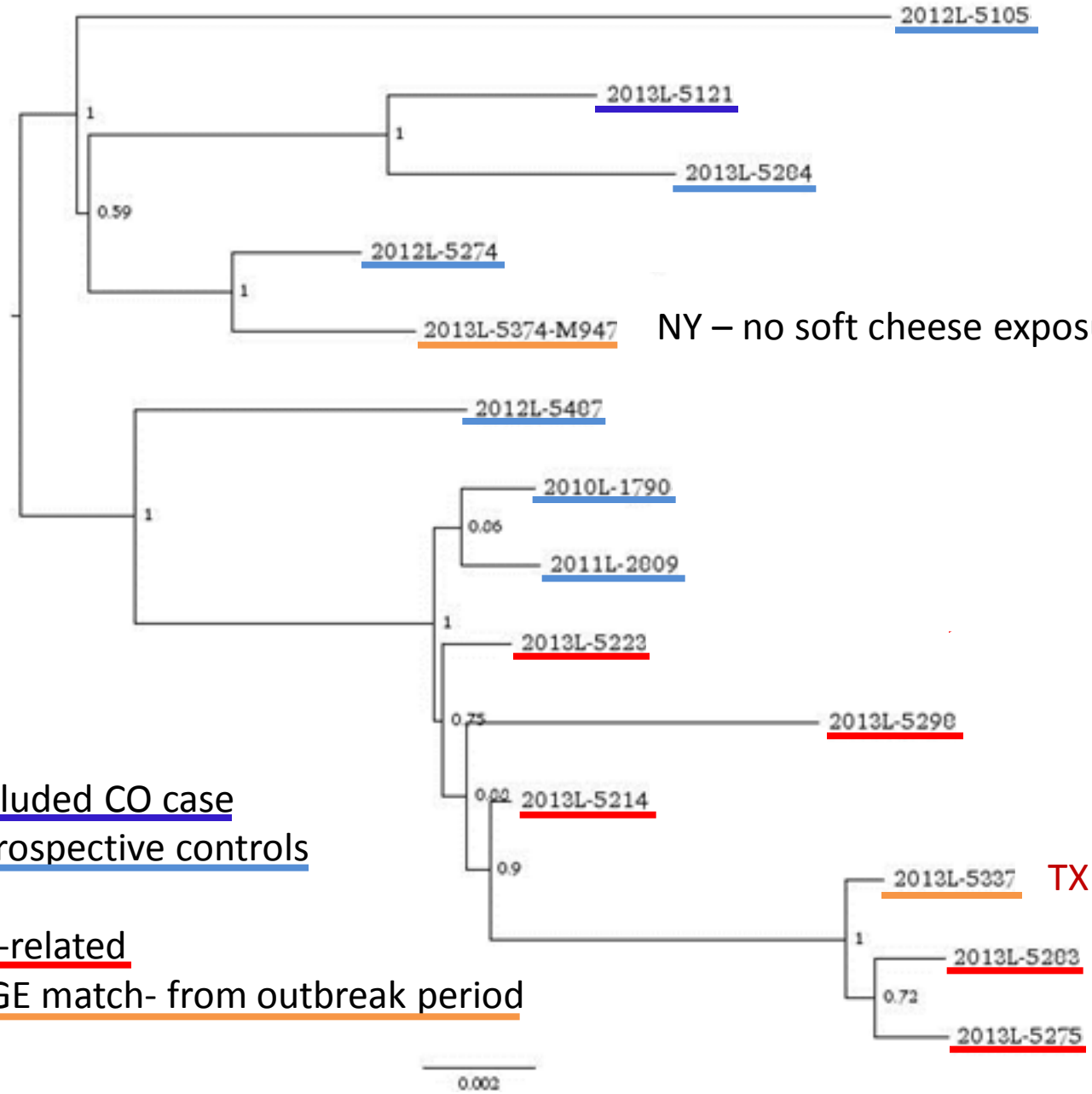
- **One cluster identified by WGS that was not recognized by PFGE**
- **Resource intensive**
  - Not been able to test the performance of different clustering tools in real-time

Whole Genome Sequencing of *Listeria monocytogenes* during the Crave Brothers Cheese outbreak

High confidence core SNP



NY – no soft cheese exposure

TX - no exposure info

excluded CO case

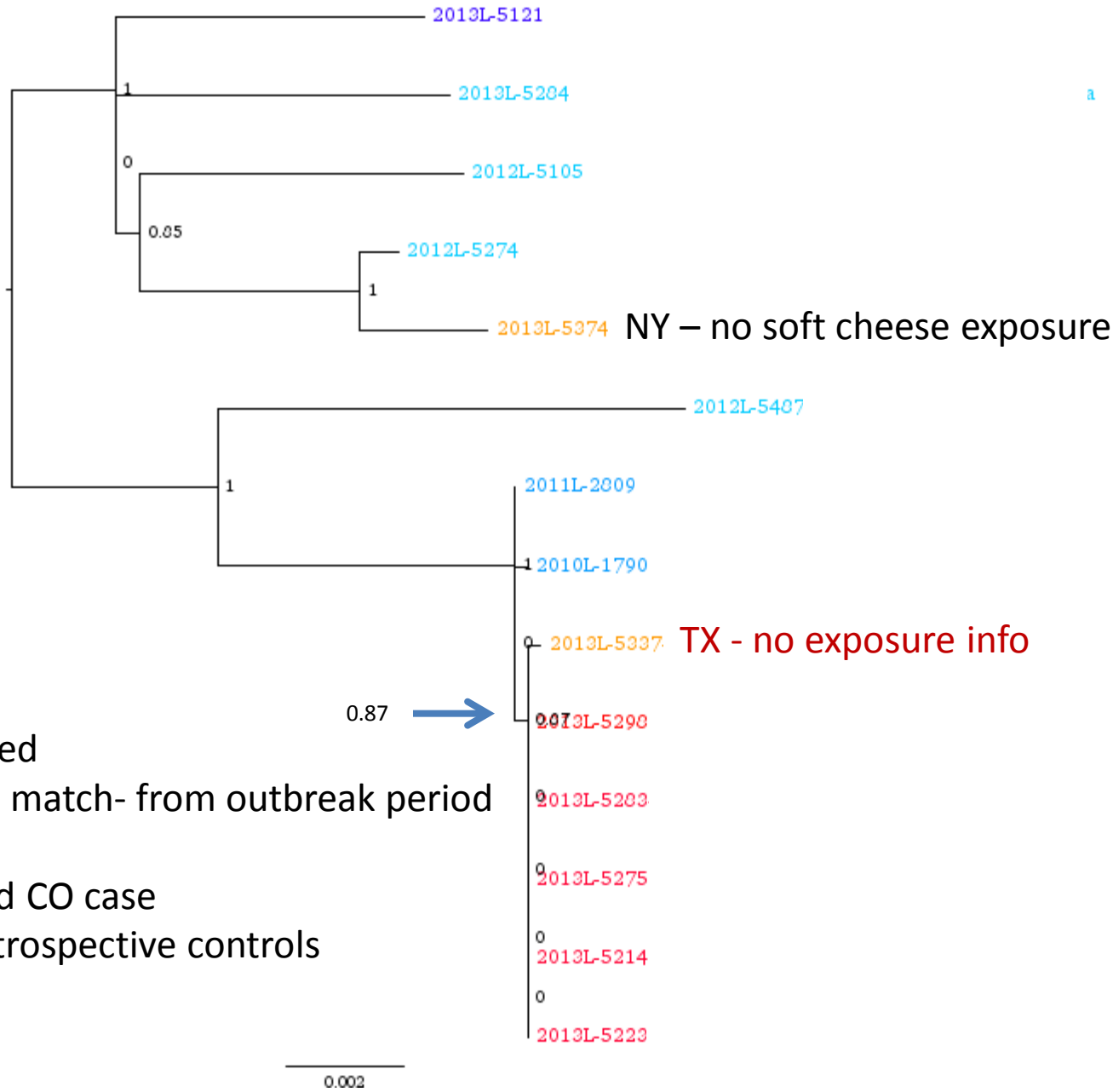
retrospective controls

Epi-related

PFGE match- from outbreak period

Whole Genome Sequencing of *Listeria monocytogenes* during the Crave Brothers Cheese outbreak

kSNP



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Red= epi-related

Orange= PFGE match- from outbreak period

Blue= excluded CO case

Light blue= retrospective controls

Acknowledgements

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Disclaimers:

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