

Advances in detecting pathogens in foods –



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Test Methods - why are they important ?

- Everything we know about the microbiology of our food—is based on testing
- We make very big decisions based on test results
- Our knowledge about the microbiology of our foods/ingredients and environment
- All based on the use of robust test methods

Some of the Issues we face

- Slow speed of obtaining results
- “New” pathogens - do we have methods?
- New techniques opening new opportunities

New / Unusual Pathogens

- What happens if something new comes up
- No method = No information
- The need to detect “new” pathogens has resulted in development of new methods.
 - Good examples
 - Viruses
 - Shiga Toxin Producing *E.coli* (STEC)

Viruses

- Are not bacteria
- Norovirus, Hepatitis A, Hepatitis E
- They can't grow in food
- We can't culture / grow them in the lab

- Are they a problem?
 - Yes - it's suggested that Norovirus causes 3 million cases of disease each year in UK
 - Sources: person to person; environmental; shellfish; fresh produce
- How do we detect them?

Polymerase Chain Reaction (PCR) for detection of viruses

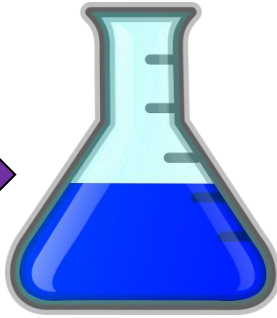
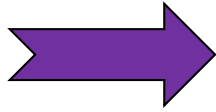
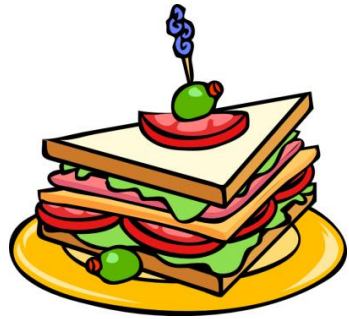
- Take the food
- Release virus particles
- Release viral nucleic acid (RNA)
- Amplify RNA quickly using PCR
- Detect amplified RNA
- Campden BRI are UKAS accredited

- Note: we detect the viral nucleic acid
- Expert interpretation required

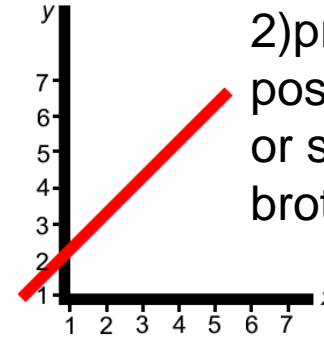
Shiga Toxin Producing E.coli (STEC)

- Nasty pathogens
- Linked to raw meats, fresh produce, raw milk and dairy products
- This group have required a re-think in method development
 - We are not looking for a species of bacterium
 - We are not looking for the presence of a toxin
 - We are looking for a particular species of bacterium that has the capacity to produce a toxin under certain conditions.
- Hazard Group 3 Pathogens
- UKAS accreditation

The Test



Test broth for STEC by PCR



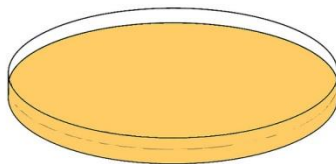
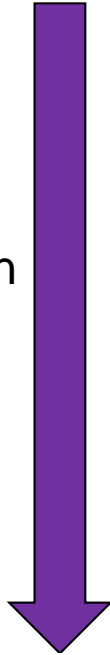
Result :

- 1) negative or
- 2) presumptive positive-stx, eae or serogroups in broth

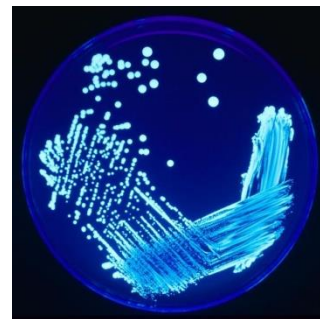
Sample

Enrichment in broth

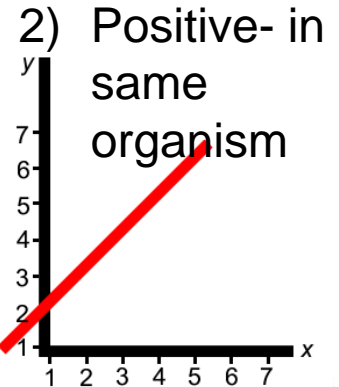
Streak presumptives on agar



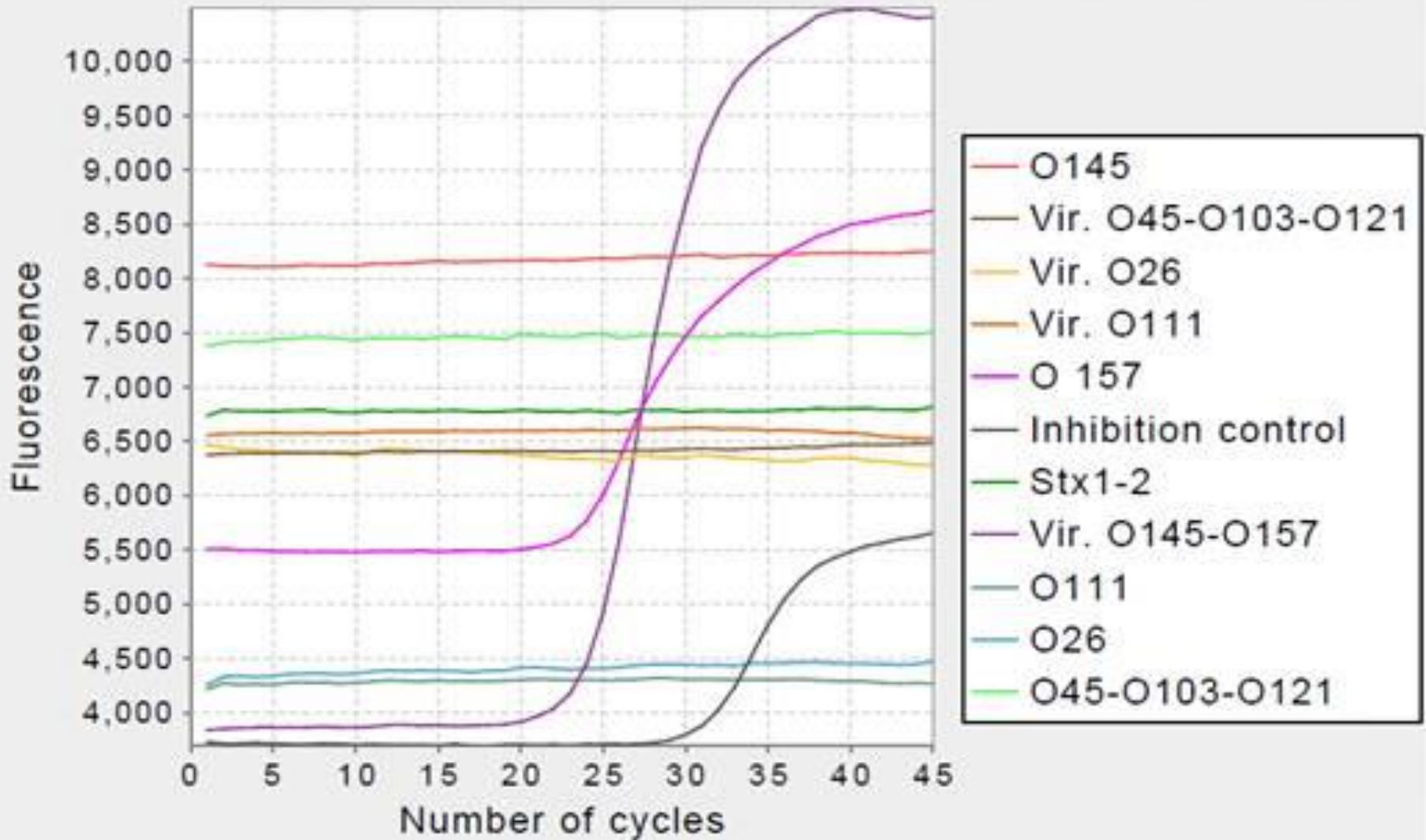
Test individual colonies by STEC PCR



- Result:
- 1) Negative stx, eae. Serogroups in different organisms
 - 2) Positive- in same organism



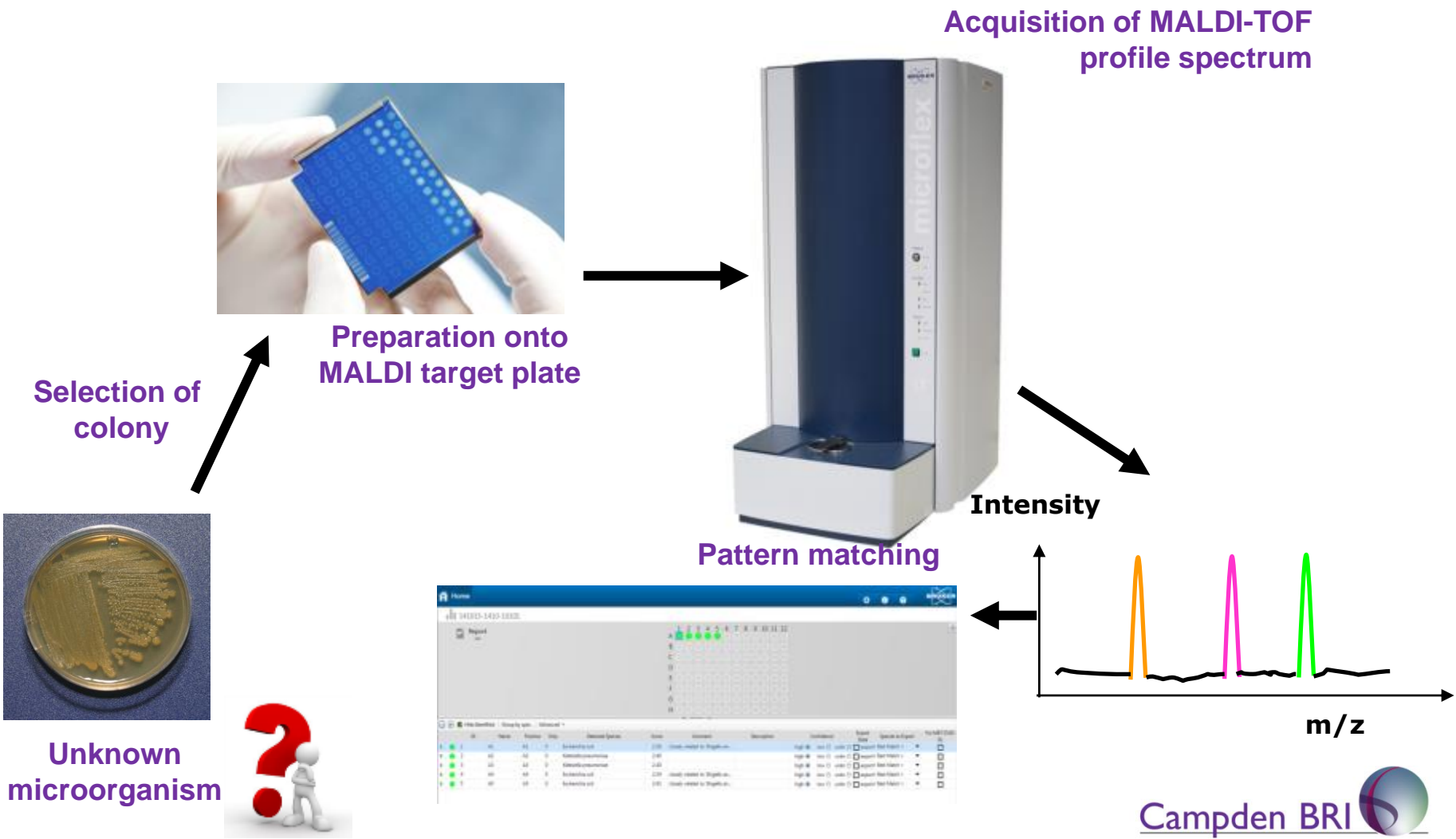
Some Results



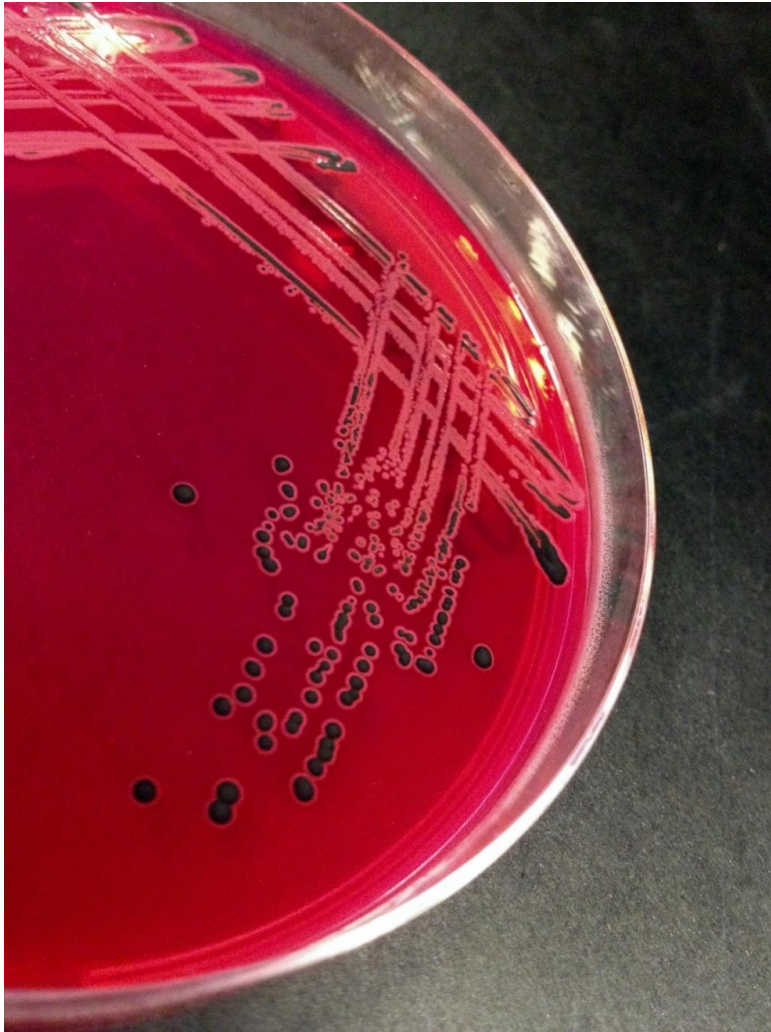
New techniques - new opportunities

- MALDI ToF
- Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectroscopy
- A Mass Spec procedure that can be used to identify microbial cultures
- Campden BRI use MALDI to identify Salmonella, Listeria, Campylobacter and a range of spoilage organisms

Example MALDI-ToF work flow – MALDI Biotyper

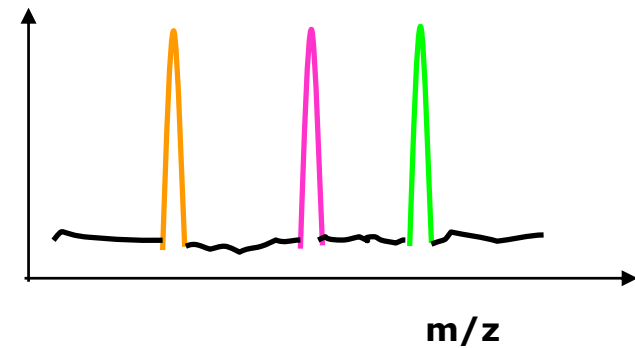


MALDI- Time Difference



Conventional identification time from a microbial colony - 24h to 48h

Intensity



Maldi Identification time 1-10 minutes.

Tracing & tracking bacterial contamination

- We can differentiate between strains of the same species
- This can help us in determining where a contaminant came from
- Sequencing or Ribotyping-
 - Based on analysis of bacterial DNA

Automated Ribotyping at Campden BRI

- Automatic robotic analysis
- An unknown isolate
- To an identified and subtyped isolate
- 8 hours



RiboPrinter Results

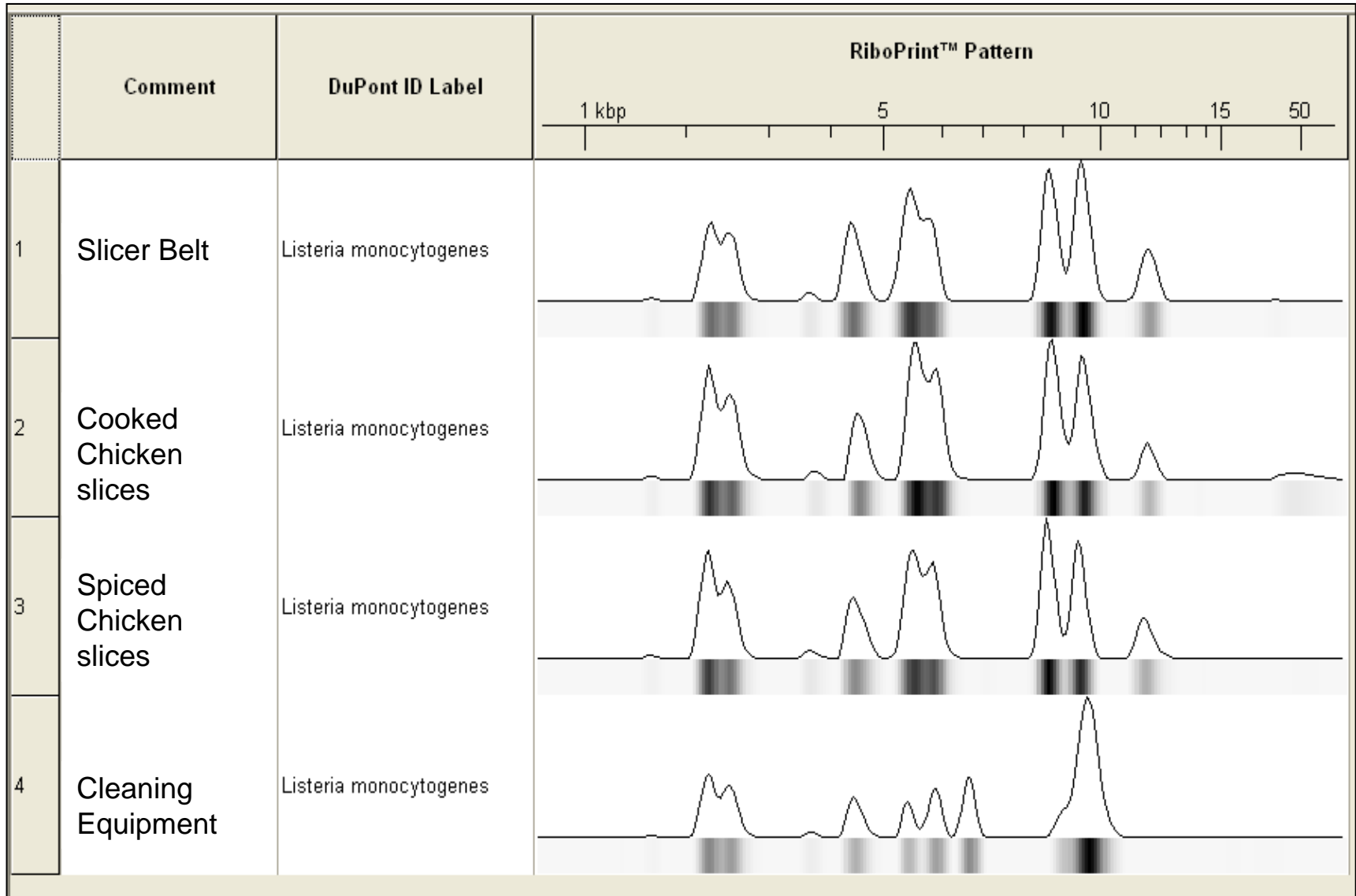
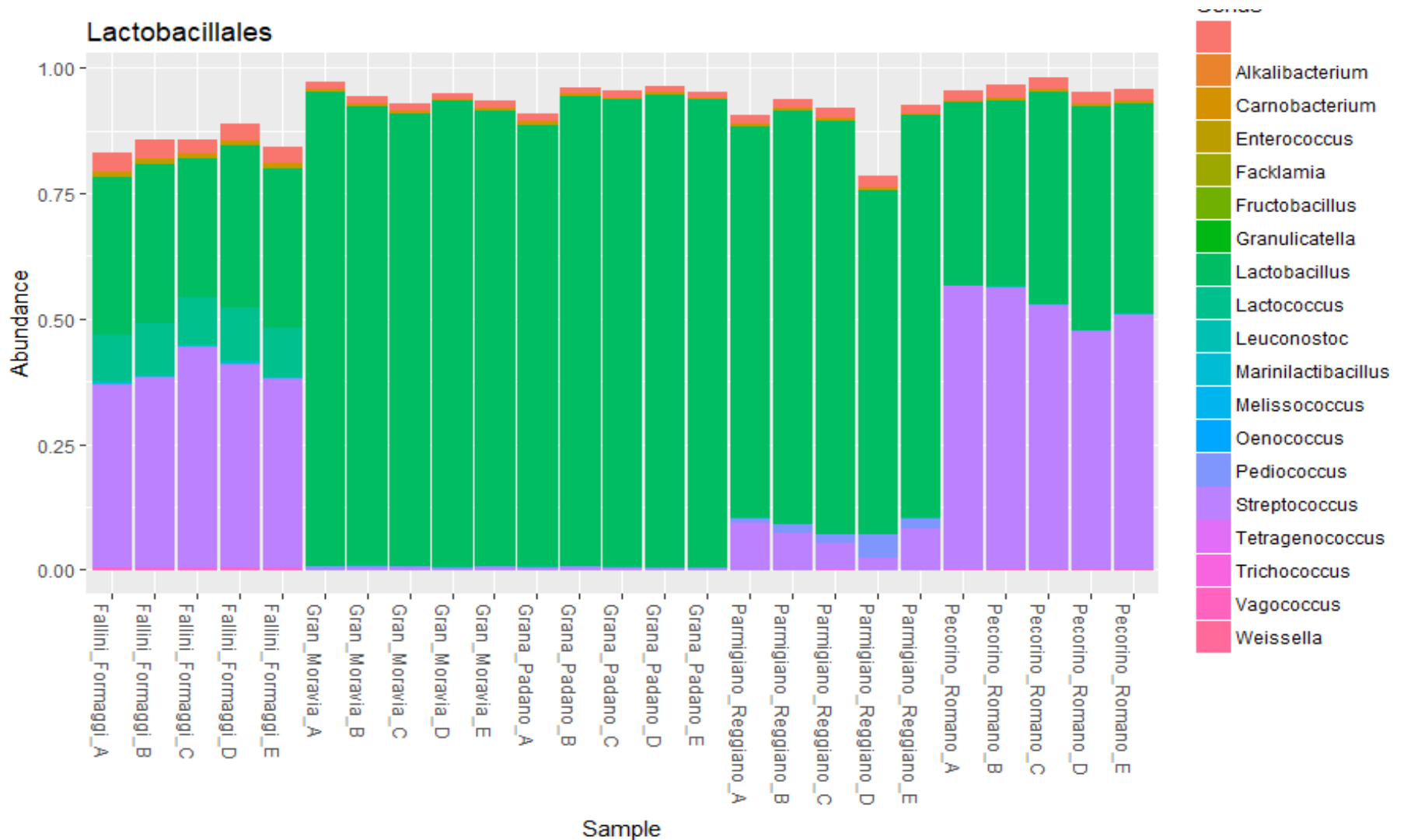


Image courtesy of Qualicon

Finally - a new technique – Ecology of Food

- Extract all of the DNA from a sample
- Amplifies parts of only bacterial DNA
- Sequence these parts and match to a database
- Identify all of the types of bacteria in the sample - in one go.
- We use it and call it **Advanced Microbial Profiling (AMP)**

Advanced Microbial Profiling



Contact

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