High-throughput QIAxcel® based subtyping: Enhanced epidemiologic surveillance of *Campylobacter jejuni*

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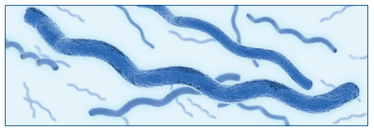
C. jejuni — a significant public health concern

- *C. jejuni* is the leading cause of foodborne bacterial enteritis in Canada and worldwide.
- Approximately 90% of human foodborne infections are caused by *C. jejuni*.
- Campylobacteriosis causes symptoms such as fever, abdominal pain, nausea, vomiting, and diarrhea.
- In Canada, there are probably ~300,000 cases a year, costing in total ~$300 million.

Challenges in *C. jejuni* subtyping

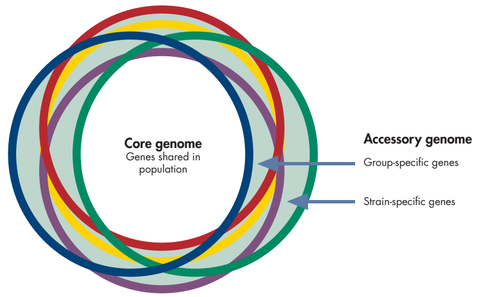


Campylobacter jejuni.

- **Pulsed-field gel electrophoresis (PFGE)** is a gold standard method for many bacterial foodborne pathogens, but not optimal for *C. jejuni* due to fingerprint "instability" (Barton et al., 2006).
- **flaA typing** through RFLP or sequencing of flaA alleles is rapid and inexpensive. However, high rates of recombination in *C. jejuni* can lead to erroneous genotypic clusters.
- **Multi locus sequence typing (MLST)** is a gold standard for *C. jejuni* subtyping and has several advantages, such as unambiguous and highly portable sequence data. However, MLST is a low-throughput method, lacks resolution in some epidemiological contexts, and is costly to implement.

Comparative genomic fingerprinting (CGF)

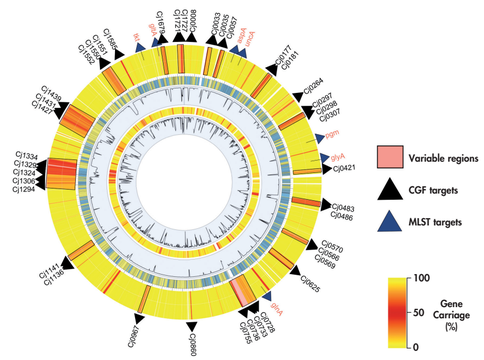
- Pan-genome = core genes + accessory genes of a species
- Accessory genes are responsible for many 'major phenotypic traits' of importance
- The accessory genome houses most of the genetic variability. Its diversity can be utilized for strain subtyping.
- The 'true' gold standard is the whole-genome phylogeny and a good subtyping method should compare favorably to the whole-genome phylogeny.



The pan-genome concept.

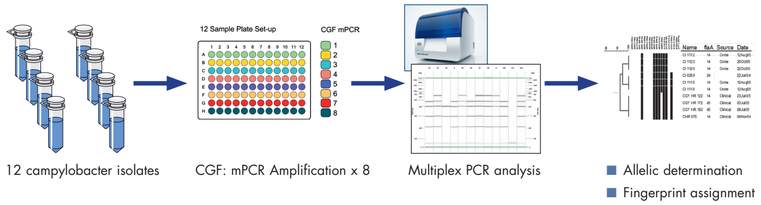
C. jejuni diversity and the CGF scheme

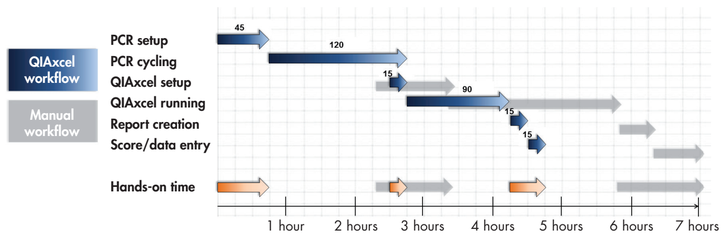
- The method is based on presence/absence of accessory genes.
- Each marker has intermediate carriage in the *C. jejuni* population = 'random' binary marker.
- Multi-marker profile: 40 markers → 2^{40} possible fingerprints.
- SNP-free PCR primer design → multiplex PCR → 8 x multiplex PCRs = a comparative genomic fingerprint.



Genomic diversity in *C. jejuni*, containing 16 hypervariable regions with accessory genes.

CGF workflow

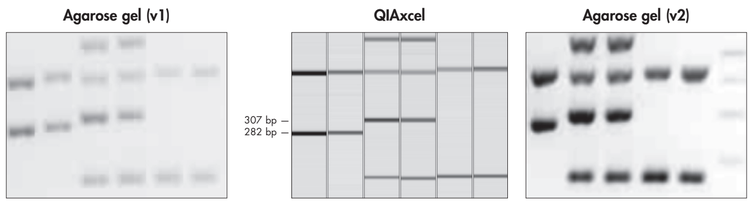




CGF workflow timeline. Complete CGF fingerprint analysis of 12 isolates in less than 5 hours, with minimal hands-on time.

QIAxcel Advanced improves CGF data analysis and workflow

Improved accuracy of CGF band sizing analysis: fewer falsely detected or misclassified bands.



As opposed to the standard gel electrophoresis, the QIAxcel platform enables automated streamlined analysis of CGF mPCRs, thereby facilitating high-throughput analysis and improving the discriminatory power.

Advantages:

- Automated analysis
- Automated data reporting
- Decreased time for sample-answer (~48 isolates per work day)
- Reduced costs (~$7 USD per isolate)

Conclusion

- There are a number of challenges with existing methods of subtyping *Campylobacter jejuni*.
- CGF exploits significant differences in accessory genome content in *C. jejuni* and thereby has a greater discriminatory power than MLST.
- Adoption of the QIAxcel Advanced System has improved CGF data analysis and facilitated method assessment/deployment, resulting in:
  - ☐ Improved sensitivity and accuracy
  - ☐ A simplified, automated workflow
  - ☐ Automated data reporting
  - ☐ Increased throughput.
- A common method for subtyping has made it feasible to envision national networks for campylobacter surveillance, involving medical research and environmental settings, as well as the food production and agricultural industries.
- Comprehensive human, farm-to-fork, and source-to-tap surveillance (under a national database), will lead to:
  - ☐ Improved understanding of campylobacter epidemiology
  - ☐ The development and assessment of intervention strategies to mitigate risk.

The applications presented here are for molecular biology applications. They are not intended for the diagnosis, prevention or treatment of a disease.

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1082042 04/2014