Culture-independent detection of *Campylobacter* by metagenomic sequencing of faecal samples

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**Our task**
- To use open-source software to detect *Campylobacter* in metagenomic datasets from sequencing of artificially inoculated faecal samples from chicken and humans
- To define a detection limit using this method
- To look at diversity among identical samples spiked with different levels of *Campylobacter*

**Our conclusion**
- High detection limits – 10⁴-10⁷ CFU/g
- Detection limits are lower in chicken faecal samples than in human faecal samples
- Kraken is slightly better at detection than BLAST
- Chicken faecal samples derived from same matrix and spiked with different levels of *Campylobacter* are 84-99% similar and the most abundant genera are *Lactobacillus*, *Escherichia*, and *Bacteroides*

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**Figure 1 – Hits to *Campylobacter* found using BLAST (red) and Kraken (blue)**
Detection of *Campylobacter* in human faecal samples is possible from 10⁷ CFU/g using BLAST (red bars) and from 10⁶ CFU/g using Kraken (blue bars). For BLAST results hits are number of contigs matching *Campylobacter* in proportion to the total number of contigs. For Kraken results hits are number of reads assigned to *Campylobacter* in proportion to the total number of reads.

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**Figure 2 – Hits to *Campylobacter* found using BLAST (red) and Kraken (blue)**
Detection of *Campylobacter* in chicken faecal samples is possible from 10⁶ CFU/g using BLAST (red bars) and from 10⁴ CFU/g using Kraken (blue bars). For BLAST results hits are number of contigs matching *Campylobacter* in proportion to the total number of contigs. For Kraken results hits are number of reads assigned to *Campylobacter* in proportion to the total number of reads.

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**Figure 3 – Similarity in diversity**
84-99% similarity is seen in diversity among the chicken faecal samples. We observe a lower similarity in the faecal sample composition than expected, since they derive from the same faecal matrix. We speculate that this is due to heterogeneity within the matrix.

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**Figure 4 – Most abundant genera**
Most abundant genera in the chicken faecal samples are *Lactobacillus*, *Escherichia*, and *Bacteroides*. There are more variation among the samples than we expected as they derive from same faecal matrix. Even the most abundant genus is not the same in all samples.

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**What we did**
- DNA extraction
- *Campylobacter* inoculation
- Sequencing on Illumina MiSeq

**Next steps**
- Try other software programs for detection
- Find a software solution for typing
- Look for other pathogenic bacteria
- End goal: To replace culturing and molecular analyses by diagnostic metagenomics used for detection and typing in surveillance and outbreak investigation