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Title: Comparing the colorectal cancer microbiome of stool and tissue samples

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Sponsor: Cancer Society of New Zealand, Canterbury-West Coast Division Inc, Cancer Society Hurunui & Greymouth Groups

Introduction: The microbiome is the community of microbes that live on and inside of us. The gut microbiome is important for health and plays important roles in metabolism with vitamin synthesis, energy production, and protection, e.g. stimulating the immune system and protecting against pathogens. Disruption of the normal gut microbiome (dysbiosis) is linked with inflammation, metabolic disorders and cancer. Colorectal cancer (CRC) is the 2nd most deadly cancer in NZ, with 1200 diagnoses each year. When detected early, current treatment options result in a positive outcome for up to 90% of patients. It has been recently shown that changes in the bacterial communities in the gut are linked to the development of CRC, as well as tumour location, tumour subtype and response to therapy. It is now possible to use next generation sequencing to identify and characterise the bacteria that exist in these communities and link them to clinical patient characteristics.

Aim: The aim of the project was to first to compare a recently developed bench-top sequencing technology to an established method for sequencing CRC tissue microbiomes. This is the first step in determining the applicability of using this technology to characterise the microbiomes of CRC stool samples.

Impact: The MinION sequencers (Oxford Nanopore Technology) are small, relatively inexpensive gene sequencers that can feasibly be used for rapid identification of bacterial communities. It can be operated with any computer and gives near immediate results. Classification of microbial communities and potential pathogens can be performed rapidly for multiple samples. The bioinformatic pipeline and bacterial classification tool Centrifuge can be used to provide accurate species level classification in minutes, and therefor is a potentially powerful diagnostic tool. By relating the microbiomes of stool samples to tumour tissue samples from the same patients, we can assess whether non-invasive stool samples can be used as a proxy for testing tissue samples that can give clinically useful information.

Method: DNA and RNA were extracted from each tumour sample. The RNA was analysed with an Illumina HiSeq sequencer while the DNA was analysed with an ONT MinION sequencer. A similar extraction process was carried out on corresponding fecal and luminal stool samples. The resulting sequence data from each platform was reviewed for quality control. The processed sequence data was analysed by the sequence classification software. The database used for the analysis contained more than 8000 bacterial species.

Results: The MinION sequencer was found to give comparable results to the established method (RNA sequencing), and sufficient overlap with the detection ability of RNA sequencing to validate the pipeline. We could achieve high accuracy classification despite the relatively high error rate of the nanopore sequencer (fig 1). At the genus taxonomic level, the sequencer was shown to have an overlap of 85% compared to RNA sequencing. At the species level, the overlap was 76.7%.

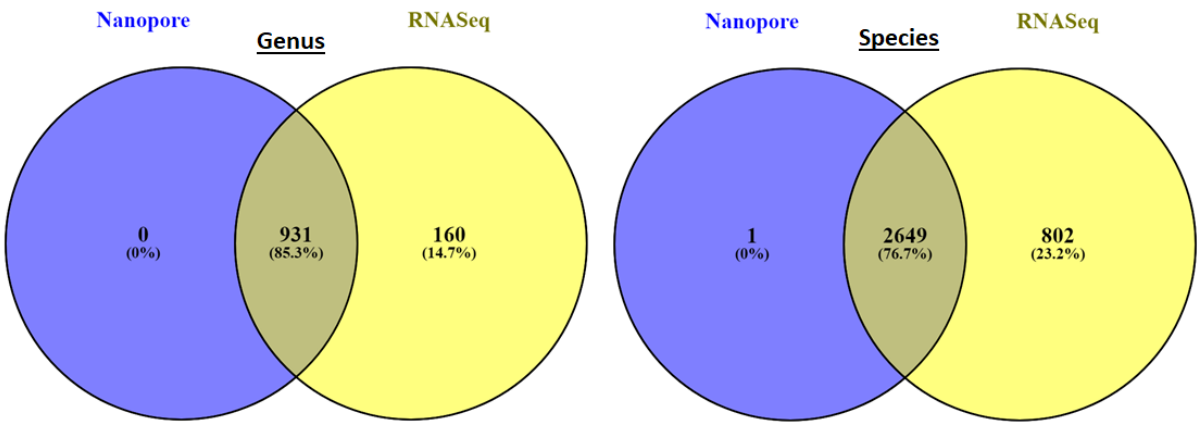

Fig 1: Taxonomic classification differences between platforms.

When we looked at specific bacteria that have been implicated in the development of CRC, such as *Fusobacterium nucleatum* and *Bacteriodes fragilis*, our analysis using the MinION sequencer was able to detect these species in the DNA from tumour tissue (fig 2).

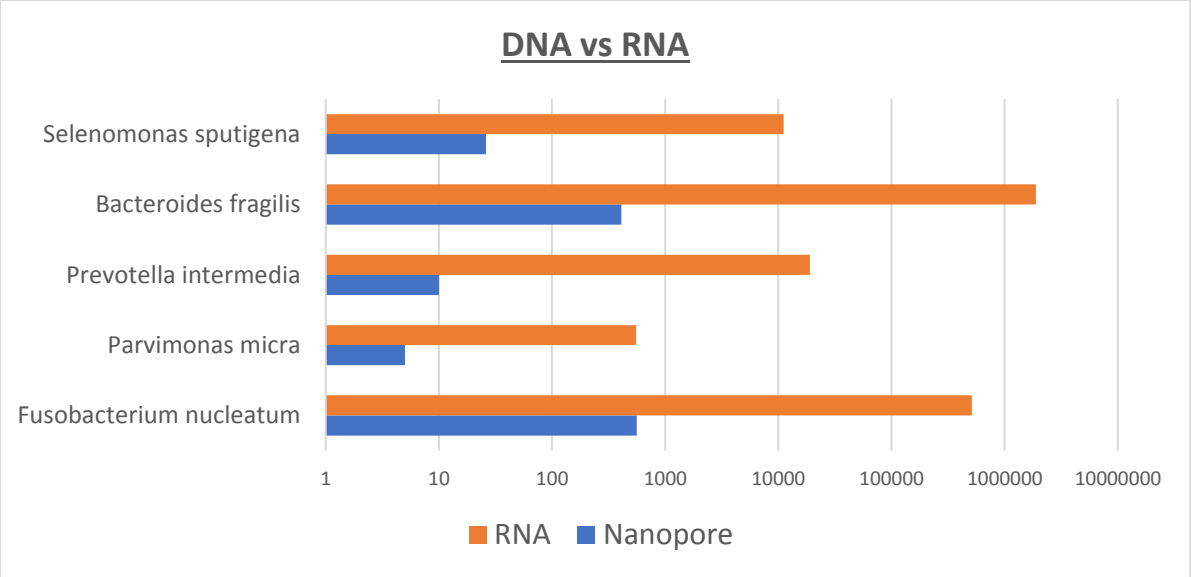

Fig 2: Differential detection in number of reads in logarithmic scale, between platforms.

Conclusions and Future Directions:

MinION sequencing, although still in development is a promising technology for clinical genomic diagnostics. The software available for the processing of this data is still currently being developed, making bioinformatic analysis of the data time consuming in comparison to established technologies. With the bioinformatics pipeline that we established, we can demonstrate that MinION can be used for the analysis of CRC tumour microbiome communities.

The next step, is to analyse this on a much higher level and further mine the data utilizing matched stool samples. This has become the principle component of my upcoming Masters studies. We will attempt to further develop the workflow to characterise differences in the microbiome of stool samples that related to specific phenotypes, such as poor prognosis and response to therapy.