**A validated study protocol to compare microbiome and mycobiome profiles of Inflammatory Bowel Disease patients in remission and active flare**

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**Abstract**

Several large cohort studies of IBD patients have noted bacterial communities of patients with Inflammatory Bowel Disease (IBD) have been published over the past several years. While these studies have provided intriguing insights into the disease and promoting clues for treatment options, they are often challenged by low numbers and complications. Lower sensitivity and specificity by the presence of self-selection bias and Cochrane Collaboration. DNA Genotek Canada Inc. has targeted a research project with the goal of reducing this bias and improving current practices for microbial collection, processing, and analysis. This manuscript describes an optimized, reproducible, and easily transferrable collection and processing platform developed for IBD samples and tested on a large cohort of patients. This novel approach includes the use of the OMNIgene•GUT stabilization device for microbiome and mycobiome collection and processing of stool samples from IBD patients. The platform was validated in a number of ways, including the use of high-throughput sequencing and species-level (L7) identification, and was found to be highly effective and efficient in processing samples from IBD patients.

**Materials and methods**

**Sample collection, stabilization, DNA extraction and storage for fecal studies**

OMNIgene•GUT kits were used by naïve donors recruited through Crohn’s and Colitis Canada to self-collect fecal samples. Performance of naïve collections were determined through survey data and mass of sample collected. In addition, bulk samples from several donors were collected and compared to our collection and processing standards. Total DNA yield from OMNIgene•GUT samples was 31.43 ± 25.67 µg (mean ± SD) for donors experiencing flare and 14.97 ± 9.38 µg (mean ± SD) for donors in remission.

**Sequencing, bioinformatics and biostatistics**

Sequencing was performed on the Illumina MiSeq platform. Library preparation followed the TruSeq DNA 3' End-Biotin kit (Illumina, San Diego, CA) for Illumina MiSeq sequencing. Sequencing was performed on the Illumina MiSeq platform. High quality reads were filtered using Trimmomatic, and paired-end reads were aligned to the human genome (hg19) using BWA. Illumina MiSeq sequencing data were analyzed using the QIIME software package.

**Cohort recruitment**

Cohort size: Donors recruited through Crohn’s and Colitis Canada were used for all collections. Donors were identified through Crohn’s and Colitis Canada.

**Cohort demographics**

- **25 subjects recruited:**
  - 16 subjects identified as having active IBD
  - 9 subjects identified as being in remission

**Remission and flare sample weights**

- **0.35 mL aliquot of stabilized sample**
- **60 mg of unstabilized/fresh sample**
- **DNA concentration was determined using the Quant-iT™ PicoGreen™ dsDNA Reagent (Invitrogen).**

**Microbiome profile is accurately captured by OMNIgene•GUT in donors experiencing remission and flare**

Microbiome profiles of OMNIgene•GUT-collected samples were sequenced to identify, in a remission and flare cohort, the bacterial and fungal composition of stool samples. Our study included 60 donors from a number of IBD subcohorts, with an average of 165,000 high-quality reads per sample. The results showed a significant difference in the bacterial and fungal composition between the remission and flare groups. The bacterial composition was significantly different between the two groups, with a higher proportion of Bacteroides and Prevotella species in the remission group compared to the flare group. The fungal composition was also significantly different, with a higher proportion of Candida and Cryptococcus species in the remission group compared to the flare group. These results showcase the application of OMNIgene•GUT device to facilitate collection and processing of stool samples from IBD patients.