Isolation, Purification and Analyses of Mice Gut Microbiome

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Background

- Colorectal cancer is the among the deadliest cancers – being the second most common cancer-related cause of death worldwide.
- A primary risk factor for Colitis-Associated Colorectal Cancer (CAC) is inflammatory bowel disease, with approximately 6.8 million people affected globally.
- Etiological factors affecting colorectal cancer include chronic inflammation, the microbiome, diet, genetics, and other epigenetic factors.
- Animal models are an effective tool to observe the effects of diet on inflammation and the microbiome in a controlled environment.

Objective

Determine the efficacy of dietary intervention with black raspberries on colitis, colon tumorigenesis and gut microbiome of mice consuming either a standard diet or a western type diet that emulates typical us nutrient intakes.

Model

Diet Groups

- **AIN93G – standard diet promoting health**
- **AIN93G + 10% Black Raspberries (BRB)**
- **Total Western Diet (TWD) – Promotes inflammation-associated colorectal carcinogenesis**
- **TWD + 10% BRB**

Rodent Subjects

- **C57Bl6/J Mice**
- **5 months old**
- **Male**

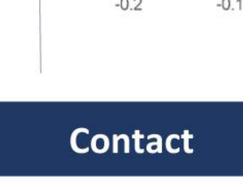
Fecal Collection

- **Day 0: basal**
- **Day 21: treatment**
- **Day 33: Colitis**
- **Day 47: Recovery**
- **Day 112: Endpoint**

Day 0
Start of Diet

Day 21
AOM injection (a carcinogen) to promote tumorigenesis and administration of DSS for 10 days to induce inflammation.

Day 33
Colitis: Assessment of inflammation of the colon



Day 47
Recovery: Assessment of colitis after recovery period

Day 112
End point: Assessment of end points

Protocol

Isolation

- Fecal samples are homogenized with various solutions in order to break down and isolate the DNA in the bacteria present
- Spectrophotometer is used to measure the concentration of nucleic acid in the sample.

Amplification

- Polymerase Chain Reaction is utilized to amplify specific regions of the bacterial DNA.
- For PCR, combine DNA samples with primers, DNA polymerase, and DNA complementary bases.

Barcoding

- Barcodes are short sequences used to identify the bacteria that is present.
- A second round of PCR is done to attach specific barcodes to specific samples.

Purification

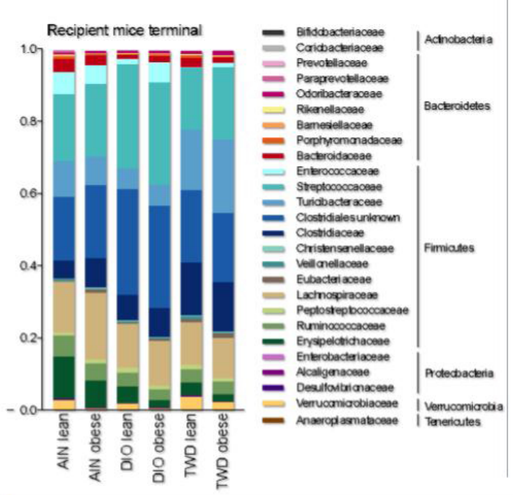
- Magnetic beads are utilized to bind DNA in a pH-dependent manner. DNA can the be purified from rest of sample.
- The beads have a positive charge, and bind DNA at a low pH

Analysis Using QIIME

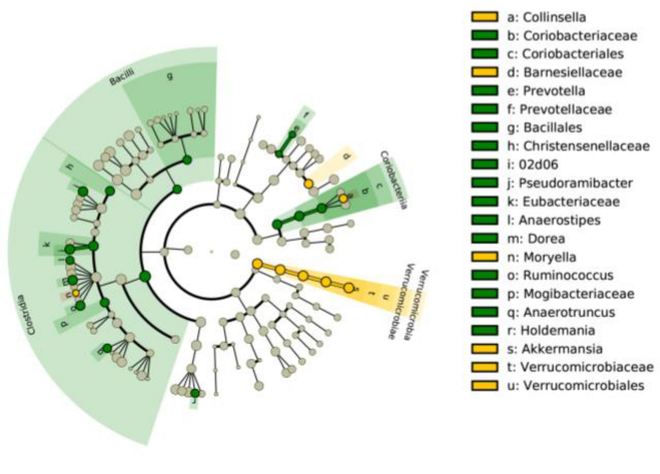
- Barcode is removed
- Using Operational Taxonomic Units (OTUs) QIIME will assign taxonomic identities and align the sequences to a database, identifying the bacteria present and their abundance in each sample.
- Other analyses include PCA plots, HCC plots, LEfSe plots and more.

Results: examples of analyses

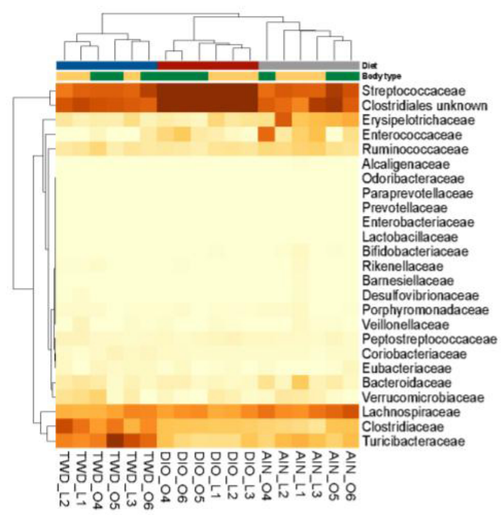
Taxonomy graphs



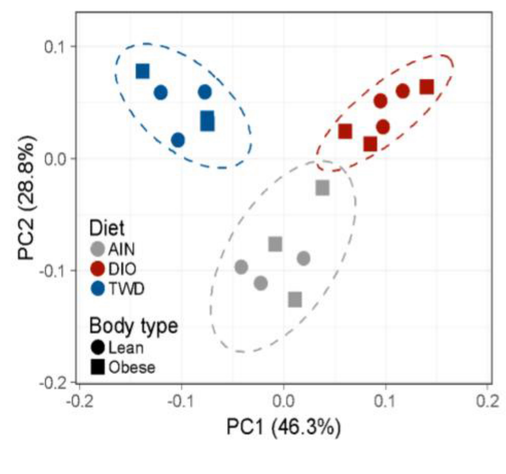
LEfSe plots



HCC plots



PCA Plots



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