INTRODUCTION
What is Kefir?
Kefir is a type of fermented milk that is cultured with Kefir grains. Kefir grains make up the probiotic culture used to produce Kefir. These cauliflower-like grains contain mostly lactobacilli bacteria and yeasts held together by Kefiran, an exopolysaccharide structure (Korsak et al. 2014). Kefir grains are Mesophilic. Mesophilic starters are cultured at room temperature, which allows the probiotics to remain active when consumed. Properties of mesophilic starters also give Kefir the liquid consistency it is known for (Sarah, 2016). Kefir originated in the Caucasus Mountains thousands of years ago, the name coming from the Turkish word Kefiy meaning “good feeding.” Kefir is well known for its health benefits, which can be attributed to its probiotics. Probiotics are foods that contain beneficial bacteria and yeasts for the human body (Otles & Cagindi, 2003).

Kefir as a product
Rising awareness in American culture for maintaining good health is now driving up the demand for the Probiotic Food and Beverages Market ("Probiotics Market Analysis", 2015). In a highly competitive consumer Probiotic Food and Beverages Market, producers are constantly challenging to keep the quality of their product high while maintaining an affordable price for their product. In the United States Kefir is marketed as a yoghurt-like probiotic beverage. Therefore, the quality of Kefir can be determined by the viability of its probiotics and how effective they are at delivering health benefits to the human body. (Kaufman, 2015). This study aims to discover if Kefir products, bought at a supermarket, can produce enough viable probiotics to be extracted and cultured. If successful, can a dominant lactobacilli species be determined for each Kefir product tested, using standard barcoding methods.

HYPOTHESIS
Hypothesis # 1
If seven selected Kefir products are advertised to contain health beneficial probiotics, then these probiotics can be extracted from the Kefir products and cultured onto agar plates containing an MRS medium.

Hypothesis # 2
If probiotics from seven selected Kefir brands are successfully cultured onto an MRS medium, then the dominant lactobacilli species of each brand of Kefir can be determined using standard barcoding methods.

METHODS
Experimental Design
The following brands of Kefir were chosen: Dahlicious Lassi, Green Valley Organics, Helios Organic Greek, Wallaby, Maple Hill Creamery, Redwood Hill Farm, and Lifeway. These products were chosen based on the availability of Kefir brands at local supermarkets.

Protocols
Samples were obtained from all seven brands of Kefir. Samples were cultured with an MRS medium, which favors the growth of Lactobacillus bacteria. A 1:10 dilution "MRS Medium" and a 1:100 dilution "MRS Dilute Medium" were prepared for all of the seven Kefir samples, a total of fourteen plates. Samples were incubated at 37°C for 24 hours. Colonies were extracted from each plate by scraping a cross shape on the agar plate with a heat wire loop. The MRS medium sample was combined with the "MRS medium dilute" sample to create one sample for each Kefir brand, a total of seven samples. These samples were then used for DNA extraction. DNA extraction of the samples was done using SurePrep Soil DNA Isolation Kit. A Quant-iT kit was used to quantify the DNA and check the DNA extraction worked. The primers used for PCR amplification are EUB 338, the forward primer, and EUB 518, the reverse primer, these bacterial primers amplify the 16S rRNA gene. To purify the DNA, an ExoSap Treatment was used to remove primers and unincorporated nucleotides. Then the primers were amplified using a DNA Polymerase to produce a barcode. The barcodes were analyzed using BLAST database. The seven databases were then put into PhyIoT database to create a phylogenetic tree.

RESULTS

<table>
<thead>
<tr>
<th>KEFIR BRAND</th>
<th>PROBIOTICS LISTED ON LABEL</th>
<th>PROBIOTICS DETECTED</th>
<th>OUR CULTURES</th>
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</thead>
<tbody>
<tr>
<td>Dhalicous Lassi</td>
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<td></td>
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<tr>
<td>Green Valley Organics</td>
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<td></td>
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<td>Helios Greek</td>
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<td>Maple Hill</td>
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<td>Lifeway</td>
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<td>Redwood Hill Farm</td>
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<td>Wallaby</td>
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</table>

PHYLGENETIC TREE OF DOMINANT BACTERIA

<table>
<thead>
<tr>
<th>DOMINANT PROBIOTIC</th>
<th>E-VALUE</th>
<th>MRS</th>
<th>MRS Coeni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus casei</td>
<td>8e-73</td>
<td>MRS</td>
<td>MRS Coeni</td>
</tr>
<tr>
<td>L. casei</td>
<td>1e-75</td>
<td>MRS</td>
<td>MRS Coeni</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>8e-68</td>
<td>MRS</td>
<td>MRS Coeni</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>6e-73</td>
<td>MRS</td>
<td>MRS Coeni</td>
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<tr>
<td>L. rhamnosus</td>
<td>5e-75</td>
<td>MRS</td>
<td>MRS Coeni</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>5e-75</td>
<td>MRS</td>
<td>MRS Coeni</td>
</tr>
</tbody>
</table>

"WHICH LACTOBACILLUS SPECIES IS DOMINANT FOR EACH KEFIR PRODUCT TESTED?"

DISCUSSION
Hypothesis #1 and Hypothesis #2 cannot be rejected. All seven Kefir products do contain viable probiotics which can be extracted and cultured successfully. A successful barcode can be obtained from extracting DNA from the dominant Lactobacillus species of each brand. The lowest e-value found on the BLAST database determined the dominant Lactobacillus species of each brand. This study found that the majority of Kefir brands, Green Valley Organics, Helios Greek, Lifeway, Redwood Hill Farm, and Wallaby, presented with a dominant Lactobacillus species that was not found on the product’s label. The Kefir brands, Dahlicious Lassi, and Maple Hill were the only Kefir brands to advertise the dominant species of lactobacilli on their product’s label. Confusion in the taxonomic name of the probiotics could be the result of mislabeling. Lactobacillus zeae was previously classified as Lactococcus rhamnous and Lactococcus casei. (Dicks et al., 1996). Companies could still be using the old taxonomic names for the labeling of their probiotics for marketing purposes. A phylogenetic tree of the dominant species of lactobacilli shows the similarity in ancestry. Contamination of the Kefir grains by the dominant Lactobacillus species could also lead to mislabeling of Kefir products. This study only targeted lactobacilli species of bacteria and favored aerobic conditions of growth therefore a bias exists showing only the dominant Lactobacillus species in the Kefir product.

FUTURE STUDIES
Psychobiotics studies the ability of probiotics to produce and deliver neuroactive substances, such as GABA, to the human body (Diran et al., 2015). GABA is an important inhibitory neurotransmitter in the mammalian central nervous system. The metabolic process of GABA produces energy for the brain. GABA also controls, “the regulation of sleep-awake cycle, motor activity, vascular tone, as well as in maintaining a high seizure threshold, memory formation and cognition.” (Yunes et al., 2016). Lactobacillus and Bifidobacterium are both studied for their efficiency at producing GABA. (Yunes et al., 2016). Both probiotics can be found in Kefir. If Kefir contains cultivated lactobacilli and Bifidobacterium strains are found to produce GABA then Kefir producers can improve the quality of their Kefir by adding these strains of probiotics to their products.

REFERENCES
"WHICH LACTOBACILLUS SPECIES IS DOMINANT FOR EACH KEFIR PRODUCT TESTED?"

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GOT BUGS IN YOUR KEFIR?

"CAN ACTIVE PROBIOTICS BE EXTRACTED FROM KEFIR PRODUCTS FOUND AT SUPERMARKETS?"