Identification of *Vaccinium* species by DNA barcoding:
Linking DNA sequence to phytochemistry

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### EXPERIMENTAL OUTLINE
- Collect *Vaccinium* samples from the Ottawa region  
- Isolate DNA from the samples  
- Using PCR, amplify sequence of interest  
- Clone sequence  
- Do PCR and gel electrophoresis to confirm the success of the clones  
- Sequence the clones  
- Analyze the sequences

### BACKGROUND INFORMATION
**Vaccinium**
- Genes that consist of fruit-bearing shrubs.  
- Examples: blueberries, cranberries, huckleberries  
- They are found in the cooler areas of the northern hemisphere.  
- About 30% of the world’s Vaccinium grows in Canada.  
- Consist of 150 species worldwide  
- Have high levels of compounds that can be used for medicinal purposes.  
- Riberines (*Vaccinium corymbosum*) contain high levels of antioxidants that can be used to protect against cancer, heart and vascular disease, urinary tract infections, diabetes, cataracts and other neurological diseases.  
- Recently Vaccinium species have been used to treat Type 2 Diabetes.

Why do we need to barocode Vaccinium?  
- Barcoding chloroplast regions allows for a means of distinguishing between species and subspecies.

### MAIN TECHNIQUES USED
- **Polymerase Chain Reaction (PCR)**
  - Closing of desired region of DNA  
  - DNA is denatured using high temperatures resulting in two single strands  
  - The forward and reverse primers create complementary strands of varying lengths using the single strands as templates.  
  - This process repeats until about 20 copies are made  
  - Since all clones differ in length, the ones of desired lengths need to be isolated using gel electrophoresis.

- **Gel Electrophoresis and DNA Sequencing**
  - In gel electrophoresis, the samples (~5µl) are loaded in wells in an agarose gel. A buffer is also added to maintain the pH levels.
  - An electric field is used. The negatively charged DNA migrates through the gel towards the positive end.
  - The bigger DNA strands migrate less than the smaller strands.
  - A marker containing strands of known size is also added to estimate the relative size of the strands nearby.

### RESULTS

### BIBLIOGRAPHY

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**Example: Gel run for Vaccinium**

**CLUSTAL multiple sequence alignment**

**DNA sequence of 1.v.o trn-psb region in Vaccinium**

**Analyzing sequences**

In which species/accessions are similarities found?  
What are the similarities?  
What does that mean?

**RESULTS ARE NOT IN YET!!!!**

We have cloned several sequences that will be analyzed. Once the sequences are acquired, the above questions can be answered.