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

Name: Abenaya Muralidharan

Program: Biotechnology

Faculty Sponsor: Dr. Douglas Johnson

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

- ✓Genus that consists of fruit-bearing shrubs.
- ✓Examples: blueberries, cranberries, huckleberries
- ✓They can be found in the cooler areas of the northern hemisphere.
- ✓About 16% of the world's *Vaccinium* grows in Canada.
- ✓Comprises of 450 species worldwide
- ✓Have high levels of compounds that can be used for medicinal purposes.
- ✓Bluberries (***Vaccinium corymbosium***) contain high level 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 barcode Vaccinium??

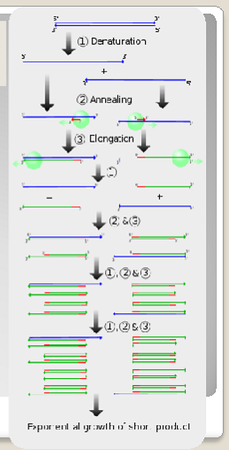
❖Barcoding chloroplast regions allows for a means of distinguishing between species and subspecies.

MAIN TECHNIQUES USED

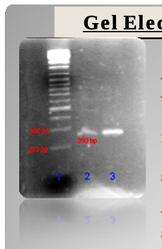
Polymerase Chain Reaction (PCR)

- ➢Cloning 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 2^{34} copies are made.
- ➢Since all clones differ in length, the ones of desired lengths need to be isolated using gel electrophoresis.

A thermal cycler for PCR

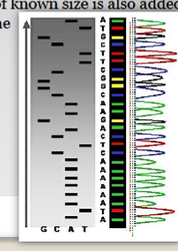


Gel Electrophoresis and DNA Sequencing



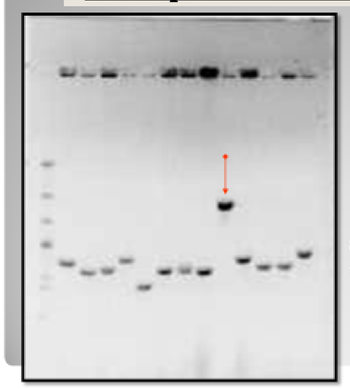
- ➢In gel electrophoresis, the samples (~5µL) are loaded in wells in an agarose gel. A buffer is also added to maintain the pH levels.
- ➢An electric field is used. The negatively charged DNA migrates through the gel towards the positive end.
- ➢The bigger DNA strands migrate less than the smaller strands.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

❖The regions of desired size are cloned.
❖Then, the sequence of the DNA is obtained (see example on the right).
❖The sequence shows the nucleotide sequence of the DNA strands which can be analyzed and compared to known sequences found in a genetic library.



RESULTS

Example: Gel run for *Vaccinium*



Cloning of *1.v.o* **trn-psb (625 base pairs)**

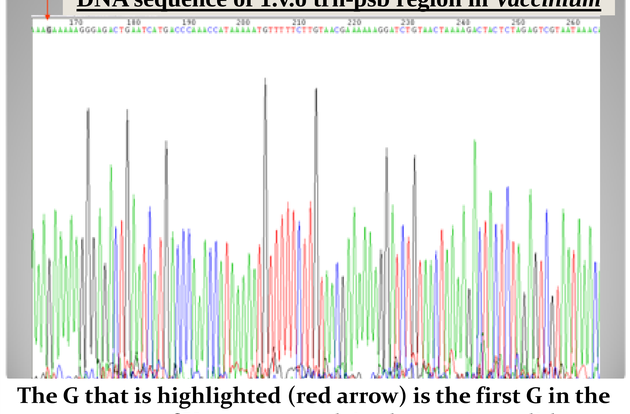
➢Only one of the samples (red arrow) was the right size of 625 bp.

CLUSTAL multiple sequence alignment

```
Vac_G3vo_trnpsb GAAAAAGGGAGACTGAATCATGAACCAAACCATAAAAATCTTTTTGTTGTAACGAAAAAA
Vac_1vo_trnpsb  GAAAAAGGGAGACTGAATCATGACCCAAACCATAAAAATGTTTTTCTTGTAACGAAAAAA
Vac_2vo_trnpsb  GAAAAAGGGAGACTGAATCATGACCCAAACCATAAAAATGTTTTTCTTGTAACGAAAAAA
                ********************** ********** ***** **************

Vac_G3vo_trnpsb CGATCTGTAACTAAAAGACTACTCTAG--TCGTAATAAAAAAAAAGAGAAGTAAAGGAGC
Vac_1vo_trnpsb  GGATCTGTAACTAAAAGACTACTCTAGAGTCGTAATAAACAAAAAGAGAAGTAAAGGAGC
Vac_2vo_trnpsb  GGATCTGTAACTAAAAGACTACTCTAGAGTCGTAATAAACAAAAAGAGAAGTAAAGGAGC
                 ************************  *********  ********************

Vac_G3vo_trnpsb AATGCACTCTTGATAGAACAAGAGGTGATTGCTCCCTTACTTTCAAAAACTCGTATACAC
Vac_1vo_trnpsb  AATGCACTCTTGATAGAACAAGAGGTGATTGCTCCCTTACTTTCAAAAACTCGTATACAC
Vac_2vo_trnpsb  AATGCACTCTTGATAGAACAAGAGGTGATTGCTCCCTTACTTTCAAAAACTCGTATACAC
                ************************************************************

Vac_G3vo_trnpsb TAGGACTAAAGTCTTATCCAGTTGTAGATGGAGCTTCAATAGCCGCTAGGTCTAGAGGGA
Vac_1vo_trnpsb  TAGGACTAAAGTCTTATCCAGTTGTAGATGGAGCTTCAATAGCCGCTAGGTCTAGAGGGA
Vac_2vo_trnpsb  TAGGACTAAAGTCTTATCCAGTTGTAGATGGAGCTTCAATAGCCGCTAGGTCTAGAGGGA
                ************************************************************

Vac_G3vo_trnpsb AGTTATGAGCATTACGTT-ATGCATAA
Vac_1vo_trnpsb  AGTTATGAGCATTACGTTCATGCATAA
Vac_2vo_trnpsb  AGTTATGAGCATTACGTTCATGCATAA
                ****************** ********
```

The * indicate the presence of the same base in all three accessions. By counting differences, the more similar and more distant sequences can be identified. Barcoding uses differences to infer relatedness.**

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



The G that is highlighted (red arrow) is the first G in the sequence of Vac_1vo_trnpsb in the previous slide.

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.

BIBLIOGRAPHY

Canadian centre for DNA barcoding. DNA barcoding [online]. July 2010 [cited 14 March 2011]. Available from World Wide Web: <http://www.ccdb.ca/pa/ge/news/scientific-publications#_587 >.

Consortium for the barcode of life. Plant working group [online]. November 2009 [cited 14 March 2011]. Available from World Wide Web: <http://www.barcoding.si.edu/plant_working_group.html>.

Janzen, Daniel H. A DNA barcode for land plants [online]. CBOL Plant working group. University of Pennsylvania, May 2009 [cited 14 March 2011]. Available from World Wide Web: <http://www.pnas.org/content/106/31/12794.full>.

KEW. Barcoding plants [online]. April 2010 [cited 14 March 2011]. Available from World Wide Web: <http://www.kew.org/barcoding/index.html>.

Whitlock , Barbara A., Hale, Amanda M. and Groff, Paul A. Intraspecific Inversions Pose a Challenge for the trnH-psbA Plant DNA Barcode [online]. July 2010 [cited 14 March 2011]. Available from World Wide Web: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2903610/>.