

# Genomic survey sequences and the structure of the *Rubus chamaemorus* L. genome as determined by ddRAD tags



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## Introduction

Cloudberry (*Rubus chamaemorus*) is an economically important Nordic berry species. However *Rubus chamaemorus* is largely undomesticated and demand for fruit often exceeds supply. Further complications arise by the fact that *Rubus chamaemorus* usually produces male and female plants that take several years to flower and bear fruit, and these can often be variable in quality. On the other hand, considering that the diploid *Rubus ideaus* has breeding programs that are particularly advanced, and that much more is known about the biological significance associated with DNA markers or beneficial bioactive compounds, it would be useful to determine the relationship of the genomic structure between these two species.



## What do we already know about the cloudberry genome?

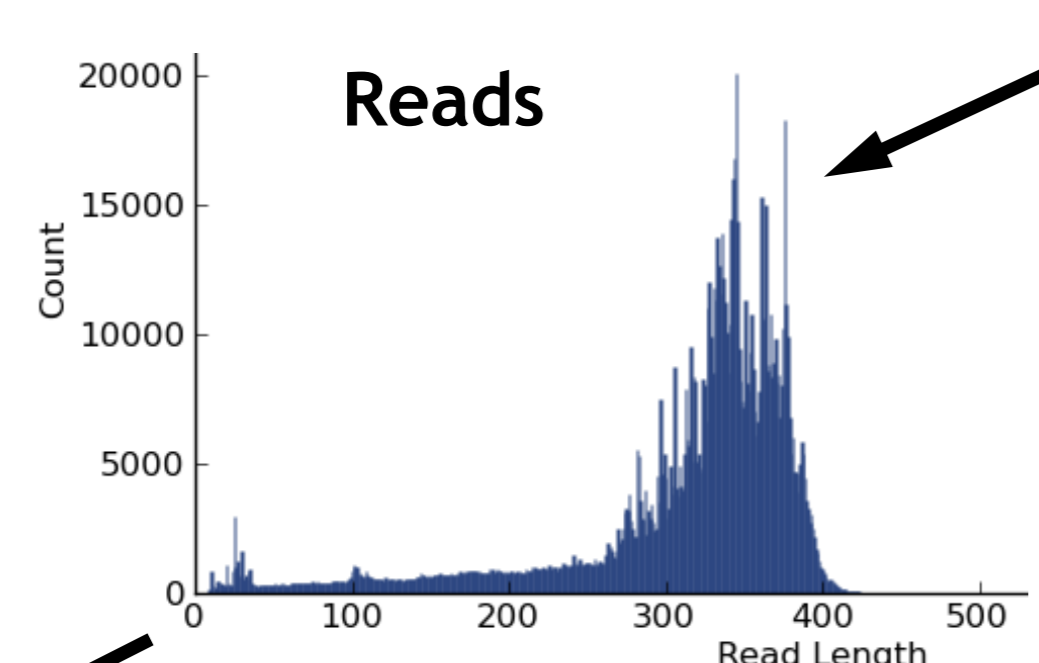
- Genome size of ca. 1.2 Gb
- Octoploid (2n = 56), however the ploidy type has not been determined
- *R. chamaemorus* may be an allopolyploid with an ancestral relationship to *R. lasiococcus* and *R. pedatus*, while *R. articus* might appear as an ancestral sister species (Micheal, 2006)
- However there is a paucity of DNA sequence information as NCBI has only 27 sequence accessions.

Controlled crosses between stable *Rubus chamaemorus* varieties - performed several years ago:

Fjellgull x Apollen  
Fjellgull x Nyby  
Nyby x Apollen

Fjellgull  
Apollen  
Fjordgull  
Kautokeino

The ddRAD protocol (Peterson et al., 2012) was modified to run on the Ion Torrent platform.



- De novo assembly
- Read map to *Rubus ideaus* BACs
- Read map to *Rubus chamaemorus* cv Fjellgull reference contigs

create ddRAD tags  
SbfI and NdeI digestion  
(multiplex)

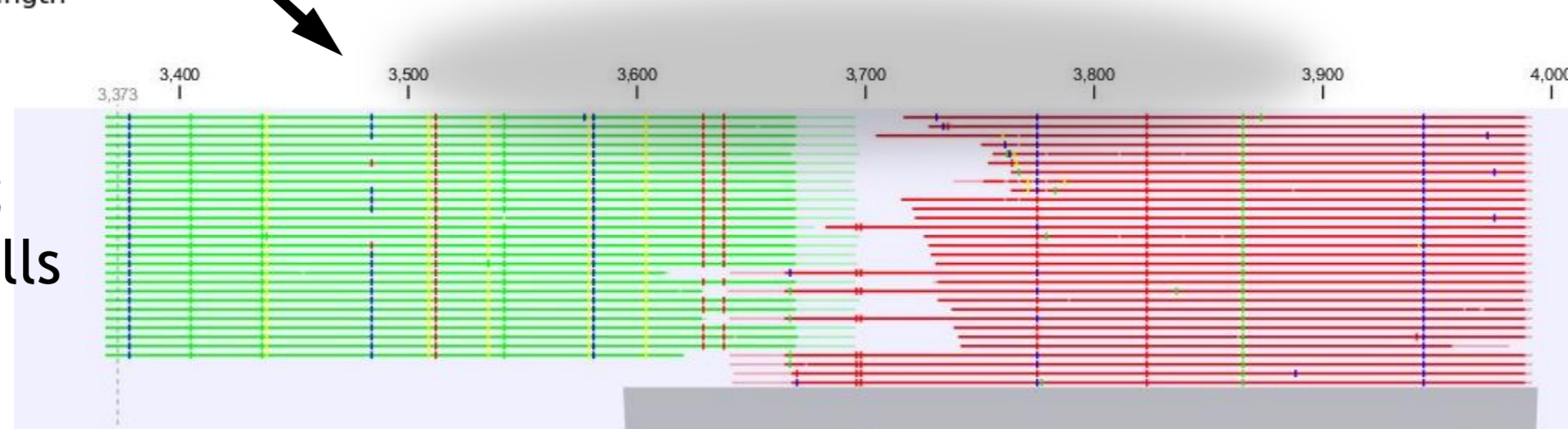
sequencing chip

Semiconductor sequencing

Ion Torrent PGM



Alignment; variant calls



Two ddRAD tags from *Rubus chamaemorus* and their alignment to a segment of the *Rubus ideaus* sequence BAC38J3 (59 k reads could be mapped to 1.4 Mb)

A reference genome of *Rubus ideaus* 275 Mbp (2n = 14) is being assembled and is thought to be ideal for comparative genomics with *R. chamaemorus*.

Using the Ion Torrent sequencing platform *Rubus chamaemorus* was sequenced with ddRAD tags. Our preliminary analysis obtained 335 Mb of sequence distributed in 1.08 million reads, with a mean length of 309 bases. Following assembly, 16500 unique genomic tags were identified representing 4.8 Mb of the *Rubus chamaemorus* genome (0.4%). Sequencing however, did not yet show distinct homeologous segments and further sequence depth is required to resolve the ancestral route of polyploidization, and call SNPs and variants with confidence.

Knowing the type of polyploid genome structure will greatly assist in future breeding programs. The Ion Torrent sequencer provides a scalable and cheap platform to accurately sequence a small fraction of a genome.

## ddRAD applications:

- De novo SNP discovery
- Genomic survey
- Phylogeny
- Pedigree analysis
- Quantitative Trait Loci
- Genome Wide Association Studies

## Where too next?

- Increase depth of coverage sufficient to call alleles in sister chromatids
- Estimate heterozygosity
- Mapping and mapping syntenic regions in the Rosaceae
- Apply Genomic Selection (GS) and begin breeding with Cloudberry
- Comparative genomics; derive an understanding of the extent in which beneficial trait development can be applied across *Rubus*

## References:

Micheal, K. (2006). Clarification of the basal relationships in *Rubus* (Rosaceae) and the origin of *Rubus chamaemorus*. PhD thesis. Western Kentucky University.  
Peterson et al., (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLoS ONE vol. 7 (5) p. 11

