

**Complete chloroplast genome sequence of a white spruce (*Picea glauca*)  
genotype from eastern Canada**

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### **Abstract**

Here we present the complete chloroplast genome sequence of white spruce (*Picea glauca*, genotype WS77111), a coniferous tree widespread in the boreal forests of North America. This sequence contributes to genomic and phylogenetic analyses of the *Picea genus*, part of ongoing research to understand their adaptation to environmental stress.

## Genome Announcement

Over tens of millions of years, conifers such as the white spruce (*Picea glauca*) have evolved to cope with adverse environmental conditions (1, 2), such as prolonged drought and increased pressure from forest insect pests (3). Plants have three different genomes: a nuclear, a mitochondrial, and a plastid (i.e. chloroplast) genome. In general, chloroplast genomes are derived from the ancestral genomes of the microbial endosymbiont from which these organelles originated (4). The nuclear genome of *P. glauca* (WS77111) was published in 2015 (5).

A *P. glauca* (genotype WS77111) needle tissue sample was collected in southeastern Ontario (44°19'48"N, 78°9'0"W; elevation: 250m). Genomic DNA was extracted from 60 gm tissue by BioS&T, using an organelle exclusion method yielding 300µg of high quality purified nuclear DNA, as previously described (6). The sample was sequenced at Canada's Michael Smith Genome Sciences Centre (GSC). Here we report on the assembled and annotated chloroplast genome of this genotype.

To sequence the sample, genomic DNA libraries were constructed according to the plate-based and paired-end library protocols at the GSC on a Microlab NIMBUS liquid handling robot (Hamilton, USA). Briefly, 1µg of genomic DNA was sonicated (Covaris LE220) in 62.5µL to 400-bp, purifying with PCRClean DX magnetic beads (Aline Biosciences). Illumina sequencing adapters were ligated overnight at 16°C. Pooled libraries were sequenced with paired-end 250-bp reads on an Illumina HiSeq2500 instrument in rapid mode. Using this protocol, four libraries were generated, sequencing approximately 400 million reads from each.

To assemble the chloroplast genome, we generated various random subsamples of read pairs from one lane of one library (i.e. 42,881,319 read pairs) producing subsets with 21X, 43X, 88X, 172X, 345X, 711X, 1219X, and 5619X coverage of the chloroplast genome. Each subset was assembled with ABySS v2.1.0 (7) ( $k = 128$ ,  $kc = 3$ ). Due to the large number of chloroplasts per cell, the chloroplast genome will be sequenced at a very high coverage over a full lane of data. Therefore, by subsampling the full dataset, the coverages of the nuclear and mitochondrial genomes were lowered to a level where these sequences do not assemble well, while the coverage of the chloroplast genome was still sufficient enough for a high quality assembly. The 43X, 88X, and 172X subsets produced the best ABySS chloroplast genome assemblies (N50 = 3692, 1313, 949, respectively), as determined by a QUAST analysis (v5.0.0) (8). For comparison, we used the white spruce admix (PG29) chloroplast genome (NCBI accession NC\_028594.1; (9)), the most closely related published chloroplast genome to the WS77111 genotype. The use of this admix as a reference has been established previously (10), as it is a naturally occurring ingress of *Picea glauca*, *Picea engelmannii*, and *Picea sitchensis* (5). We then performed additional ABySS assemblies with

varying  $k$  and  $kc$  parameters using these three subsets ( $k = 96, 112, 128, 144, 160, kc = 3, 4$ ). The assembly with the fewest aligning contigs ( $n = 14$ ) and fewest misassemblies (43X,  $k = 96, kc = 3$ ) was chosen for further scaffolding. Scaffolding the assembly using LINKS v1.8.5 (11) and the PG29 chloroplast genome joined the contigs into one piece. We then used Sealer v2.1.0 (12) to close the scaffold gaps. We modified the start position of our assembly to match the PG29 reference using BLAST v2.7.1 (13), and polished the final assembly with Pilon v1.22 (14), using BWA v0.1.7 (15) for read alignment.

The complete WS77111 chloroplast genome is 123,421 bp long, with 38.74% GC content. Using GeSeq v1.65 (16) with several other *Picea* chloroplast genomes as reference (9, 10), we annotated 114 genes: 74 protein-coding, 36 tRNA-coding, and four rRNA-coding genes. Only *rps12*, *petB*, *petD*, *rpl16*, and *psbZ* required manual annotation. The genome map in Figure 1 was generated using OGDRAW v1.2 (17).

The assembly of this new chloroplast genome will enable further analysis of *Picea* phylogeny and genetics.

**Accession number(s).** The complete chloroplast genome sequence of *Picea glauca*, genotype WS77111 is available from Genbank under accession MK174379, and the raw reads are in the SRA under SRX525336 and SRR1259605. The annotations used as references were from *Picea abies* (NC\_021456), *Picea asperata* (NC\_032367), *Picea glauca* genotype PG29 (NC\_028594), *Picea morrisonicola* (NC\_016069), and *Picea sitchensis* (NC\_011152, KU215903).

## Acknowledgements

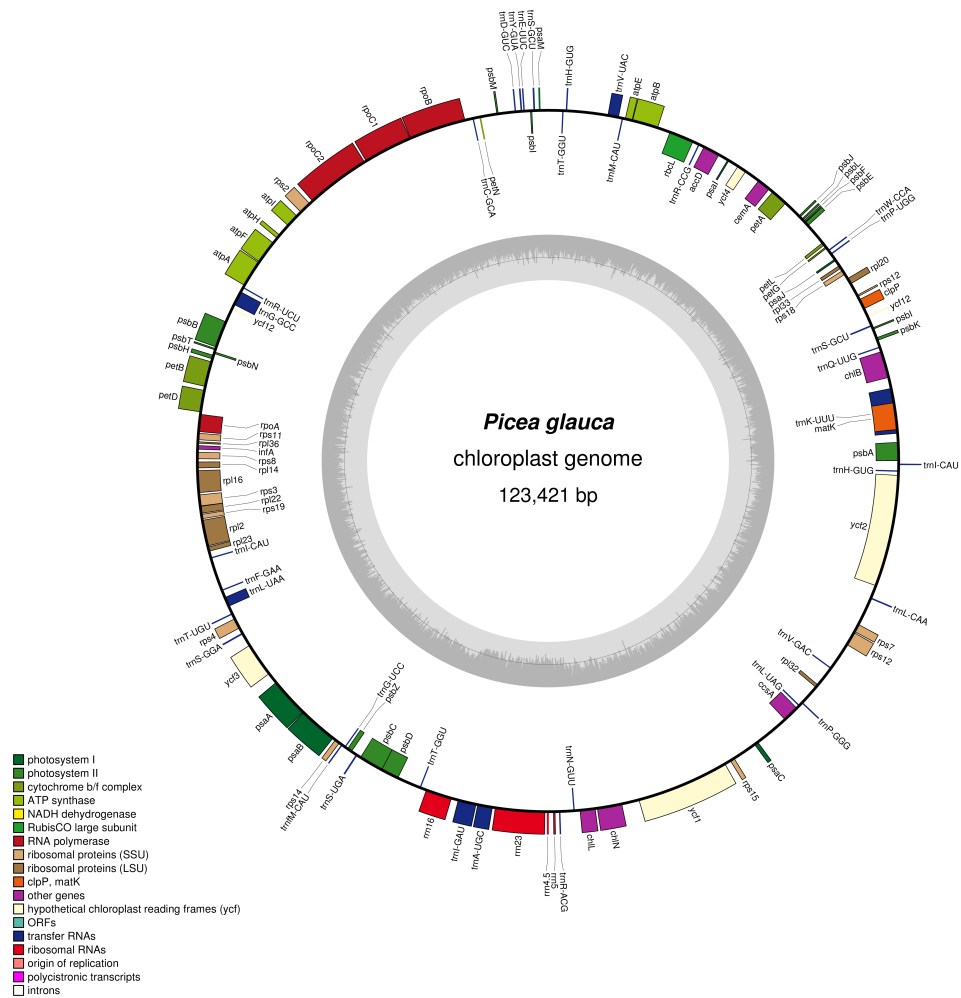
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## References

1. Li P, Beaulieu J, Bousquet J. 1997. Genetic structure and patterns of genetic variation among populations in eastern white spruce (*Picea glauca*). Can J For Res 27:189-198.
2. Bouillé M, Bousquet J. 2005. Trans-species shared polymorphisms at orthologous nuclear gene loci among distant species in the conifer *Picea* (*Pinaceae*): implications for the long-term maintenance of genetic diversity in trees. Am J Bot 92:63-73

3. Kiss GK, Yanchuk AD. 1991. Preliminary evaluation of genetic variation of weevil resistance in interior spruce in British Columbia. *Can J For Res* 21:230–234.
4. Ku C, Nelson-Sathi S, Roettger M, Sousa FL, Lockhart PJ, Bryant D, Hazkani-Covo E, McInerney JO, Landan G, Martin WF. 2015. Endosymbiotic origin and differential loss of eukaryotic genes. *Nature* 524:427-432.
5. Warren RL, Keeling CI, Yuen MMS, Raymond A, Taylor GA, Vandervalk BP, Mohamadi H, Paulino D, Chiu R, Jackman SD, Robertson G, Yang C, Boyle B, Hoffmann M, Weigel D, Nelson DR, Ritland C, Isabel N, Jaquish B, Yanchuk A, Bousquet J, Jones SJM, Mackay J, Birol I, Bohlmann J. 2015. Improved white spruce (*Picea glauca*) genome assemblies and annotation of large gene families of conifer terpenoid and phenolic defense metabolism. *Plant J* 83:189-212
6. Birol I, Raymond A, Jackman SD, Pleasance S, Coope R, Taylor GA, Yuen MMS, Keeling CI, Brand D, Vandervalk BP, Kirk H, Pandoh P, Moore RA, Zhao Y, Mungall AJ, Jaquish B, Yanchuk A, Ritland C, Boyle B, Bousquet J, Ritland K, Mackay J, Bohlmann J, Jones SJ. 2013. Assembling the 20 Gb white spruce (*Picea glauca*) genome from whole-genome shotgun sequencing data. *Bioinformatics* 29:1492–1497.
7. Jackman SD, Vandervalk BP, Mohamadi H, Chu J, Yeo S, Hammond SA, Jahesh G, Khan H, Coombe L, Warren RL, Birol I. 2017. ABySS 2.0: resource-efficient assembly of large genomes using a Bloom filter. *Genome Res* 27:768-777.
8. Mikheenko A, Prjibelski A, Saveliev V, Antipov D, Gurevich A. 2018. Versatile genome assembly evaluation with QUAST-LG. *Bioinformatics* 34:i142–i150.
9. Jackman SD, Warren RL, Gibb EA, Vandervalk BP, Mohamadi H, Chu J, Raymond A, Pleasance S, Coope R, Wildung MR, Ritland CE, Bousquet J, Jones SJM, Bohlmann J, Birol I. 2016. Organellar genomes of white spruce (*Picea glauca*): Assembly and annotation. *Genome Biol Evol* 8:29–41.
10. Coombe L, Warren RL, Jackman SD, Yang C, Vandervalk BP, Moore RA, Pleasance S, Coope RJ, Bohlmann J, Holt RA, Jones SJM, Birol I. 2016. Assembly of the complete Sitka spruce chloroplast genome using 10X Genomics’ GemCode sequencing data. *PLoS One* 11.
11. Warren RL, Yang C, Vandervalk BP, Behsaz B, Lagman A, Jones SJM, Birol I. 2015. LINKS: Scalable, alignment-free scaffolding of draft genomes with long reads. *Gigascience* 4:35-35.
12. Paulino D, Warren RL, Vandervalk BP, Raymond A, Jackman SD, Birol I. 2015. Sealer: a scalable gap-closing application for finishing draft genomes. *BMC Bioinformatics* 16.

13. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403-410.
14. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PloS One* 9:e112963-e112963.
15. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754-1760.
16. Tillich M, Lehwarck P, Pellizzer T, Ulbricht-Jones ES, Fischer A, Bock R, Greiner S. 2017. GeSeq – versatile and accurate annotation of organelle genomes. *Nucleic Acids Res* 45.
17. Lohse M, Drechsel O, Kahlau S, Bock R. 2013. OrganellarGenomeDRAW—a suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. *Nucleic Acids Res* 41:W575-W581.



**Figure 1: The complete chloroplast genome of *Picea glauca*, isolate WS77111.** The *Picea glauca* chloroplast genome was annotated using GeSeq (16), and plotted using OGDRAW (17). The inner grey circle illustrates the GC content of the genome.