

**Complete chloroplast genome sequence of an Engelmann spruce (*Picea engelmannii*)
genotype from western Canada**

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ABSTRACT

Engelmann spruce (*Picea engelmannii*) is a conifer found primarily on the west coast of North America. Here, we present the complete chloroplast genome sequence of *Picea engelmannii*, genotype Se404-851. This chloroplast sequence will benefit future conifer genomic research, and contribute resources to further species conservation efforts.

GENOME ANNOUNCEMENT

We sequenced, assembled, and annotated the complete chloroplast genome of Engelmann spruce (*Picea engelmannii*, genotype Se404-851). Engelmann spruce dominates much of the large spruce forests of interior British Columbia, where it has been reported to hybridize with *Picea glauca* and *Picea sitchensis* (1), and its range extends southward to New Mexico. The tree has three different genomes, a nuclear genome, a mitochondrial genome, 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 (2).

A needle tissue sample was collected from a 13-year-old Engelmann spruce grown at the Kalamalka Forestry Centre in British Columbia (50°14'38.4"N 119°16'40.8"W; elevation of 450m), planted from a seed from Don Fernando Mountain, New Mexico (36°17'60"N 105°24'0"W; elevation: 2987m). 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 (3). The sample was sequenced at Canada's Michael Smith Genome Sciences Centre.

To sequence the sample, a 900 bp whole genome library was constructed following the previously described protocol (4-5) with minor modifications. Briefly, 5 µg of genomic DNA was subjected to shearing by sonication (Covaris LE220) using a Duty Factor of 5 and Peak Incident Power of 450 for 70 seconds. The sonicated DNA products were fractionated in a 6% PAGE gel to recover fragments greater than 700 bp for library preparation. These PCR-Free libraries were sequenced with paired-end 150 base reads on the Illumina HiSeqX platform using V4 chemistry according to manufacturer recommendations. Using this protocol, four libraries were generated, sequencing approximately 200 million reads from each.

To assemble the chloroplast genome, we subsampled the whole genome shotgun sequencing reads of one lane of one library (i.e. 41,748,620 read pairs) to subsets of 0.75, 1.5, 3, 6, 12, 25, 41 million read pairs, then assembling each subset with ABySS v2.1.1 (6) ($k=128$, $kc=3$). The ABySS assembly of the 3M read pair subset resulted in a single 123,601 contig that aligned to the reference chloroplast sequence (*Picea glauca* admix genotype PG29, NCBI accession NC_028594; (7)), with zero misassemblies and internal gaps, based on QUAST v5.0.0 (8) analysis.

Using BLAST v2.7.1 (9), we aligned our assembly to the reference chloroplast sequence (PG29), modifying start and stop positions for consistency with previously published conifer chloroplast genomes. To ensure there were no missing sequences at the ends of our assembly we introduced a gap at the end, circularized the sequence, and ran Sealer v2.1.1 (10), closing the ‘end’ gap, and removing overlapping sequences, as previously done (11). Finally, the resulting assembly was polished using Pilon v1.22 (12), using the 3M subset of read pairs aligned with BWA v0.1.7 (13).

The complete *P. engelmannii* Se404-851 chloroplast genome is 123,542 bp long, with 38.74% GC content. Using GeSeq v1.65 (14) and other *Picea* chloroplast genomes as references (6, 10), we annotated 114 genes: 74 protein-coding, 36 tRNA-coding and 4 rRNA-coding. We note that four genes (*rps12*, *petB*, *petD*, and *rpl16*) in this list were manually annotated. We used OGDRAW v1.2 (15) to generate the map in Figure 1.

The introduction of this new chloroplast genome will benefit conifer genomic research, and inform future evolutionary studies.

Accession number(s). The complete chloroplast genome sequence of *Picea engelmannii*, genotype Se404-851 is available under Genbank accession [MK241981](#), and the raw reads in the SRA under [SRX5070635](#) and [SRR8252852](#). 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](#)).

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Figure 1. The complete chloroplast genome of *Picea engelmannii* genotype Se404-851

The *Picea engelmannii* chloroplast genome was annotated using GeSeq v1.65 (14), and plotted using OGDRAW v1.2 (15). The inner grey circle illustrates the GC content of the genome.

Picea engelmannii
chloroplast genome
123,542 bp

- photosystem I
- photosystem II
- cytochrome b/f complex
- ATP synthase
- NADH dehydrogenase
- RubisCO large subunit
- RNA polymerase
- ribosomal proteins (SSU)
- ribosomal proteins (LSU)
- clpP, matK
- other genes
- hypothetical chloroplast reading frames (ycf)
- ORFs
- transfer RNAs
- ribosomal RNAs
- origin of replication
- polycistronic transcripts
- introns

