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# Shotgun-sequencing of plant DNA: selection of material and methods

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## Abstract

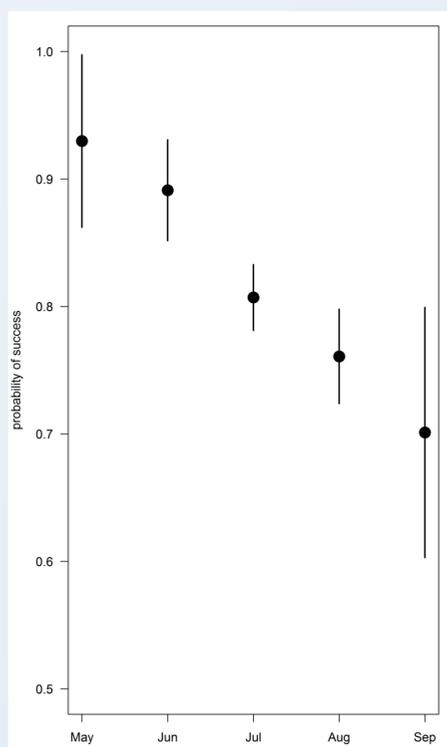
- Initially, rbcLa and ITS2 were PCR amplified and Sanger sequenced for 564 species from 1900 specimens
- To increase the amount of the genome covered, shotgun sequencing of 1670 species is in progress
- Our Sanger analyses had a success rate of 74% and 85% for ITS2 and rbcLa, respectively.
- Overall success rate of Sanger sequencing decreased with later collection date in the year.
- Preliminary results of the shotgun sequencing and assembly of 277 specimens gives a success rate of 82% and 72% for rbcLa and matK, respectively.
- Constructing a reference sequence library consisting of not only a few barcode loci, but full plastid and ribosomal sequences is beneficial as it provides a valuable resource for novel research avenues (tabel 1).

Barcode marker	Length (bp)
ITS2	200
MatK	600
RbcLa	500
Genome skimming	880 000 000

**Tabel 1:** Tabel showing length in basepairs on standards barcode markers and amount of basepairs obtained using shotgun sequencing.

## Introduction

- The goal of the Norwegian Barcode of Life (NorBOL) project is to make standardize barcodes of 20 000 species available in the Barcode of Life Data System (BOLD).
- Initially, 1900 specimens of 564 species were sequenced using Sanger sequencing and an additional 1814 specimens of 1670 species were selected for shotgun sequencing to obtain a higher taxonomic resolution.



**Figure 1:** Specimens of vascular plants collected early in the growing season have a significantly higher rate of success.

## Conclusion

- Collecting vascular plants early in the season gives a significantly higher probability of success.
- The larger reference sequence datasets obtained with shotgun sequencing allow for a greater range of research questions to be addressed, compared to the limited datasets derived from Sanger sequencing.



**Figure 2:** showing success rate for Sanger sequencing and success rate and those still in progress for Shotgun sequencing.

## Result

Sequencing success rate is linked to the month in which the specimens were originally collected (Figure 1). Specimens collected early in the growing season have a significantly higher success rate.

The preliminary results for the 277 shotgun sequenced species are: the full chloroplast was obtained for 55% of the specimens, an additional 23% specimens had a partial chloroplast and the ribosomal DNA was obtained for 88% of the specimens (figure 2).

Preliminary data shows that good quality sequences are obtained on all taxonomic classes by using shotgun sequencing while this is not obtained for Sphenopsida, Psilotopsida and Lycopsida by using Sanger sequencing.

