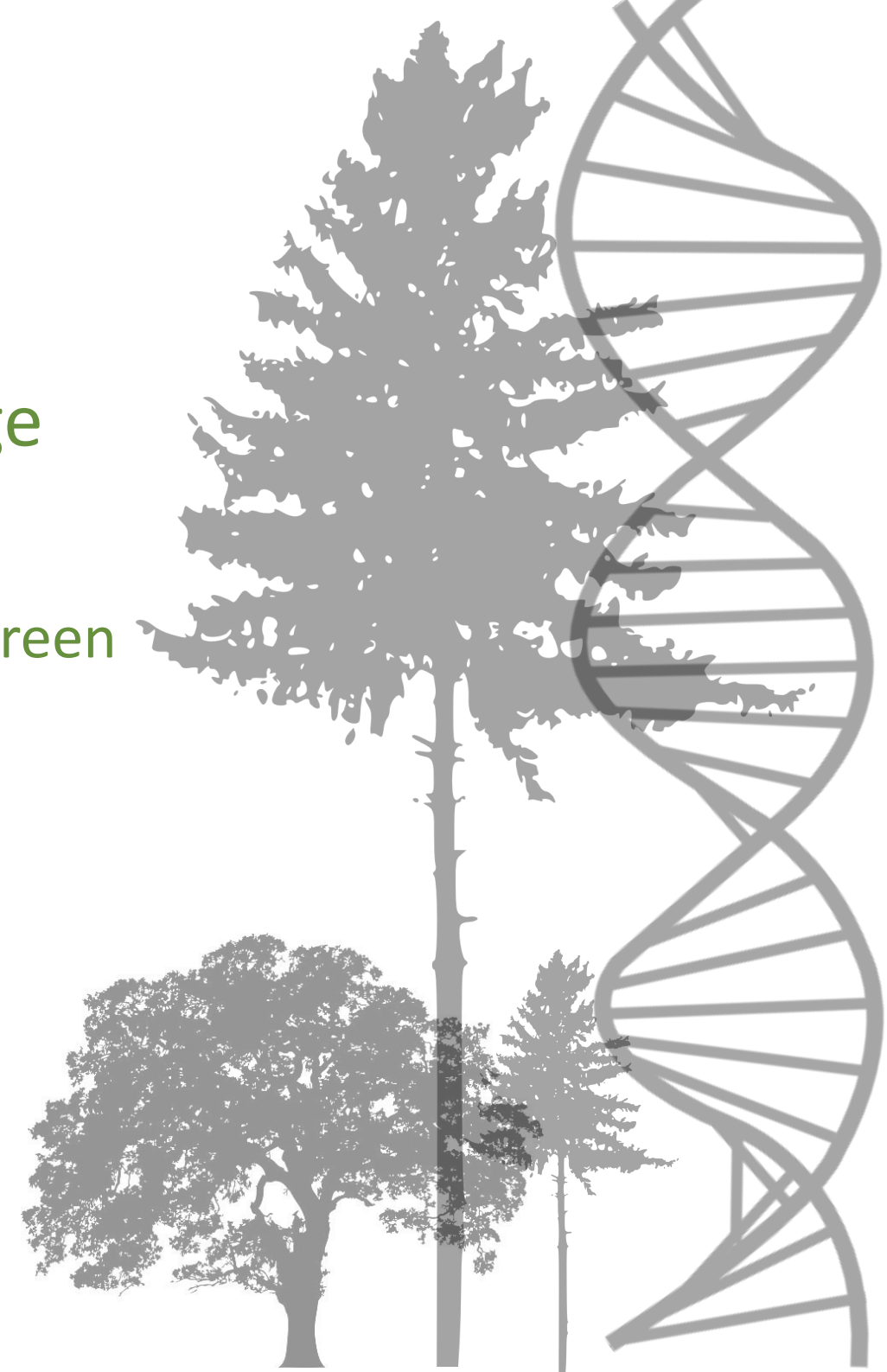
University of Oxford
Department of Biology

Genotyping strategies for large genome conifers -

How genetically diverse are UK evergreen woodlands?

Plant Genomes Online Conference
Laura Guillardin
April 2022



Outline

BACKGROUND
GENERAL AIMS OF RESEARCH
EXPERIMENTAL APPROACH & RESULTS

Outline

BACKGROUND

CHALLENGES IN THE 21st-CENTURY FOREST MANAGEMENT

Climate change

Extreme temperatures

Species switch

Pests & diseases

Drought & Fires

Continuous Cover Forestry approach

Multi-purpose forests

Timber production

Biodiversity

Landscape

Public access, safety and recreation

Water quality and flooding risk

Carbon management – both in the soil and in standing timber

Cultural values – including archaeology, history and community interest

CONTINUOUS COVER FORESTRY APPROACH

Principles:

Ecosystem management

Natural regeneration and disturbances

Work with site limitations

Irregular stand structure with a mixture of ages and species

Development:

Even-aged plantations

Thinnings
...

First stages of irregular stands

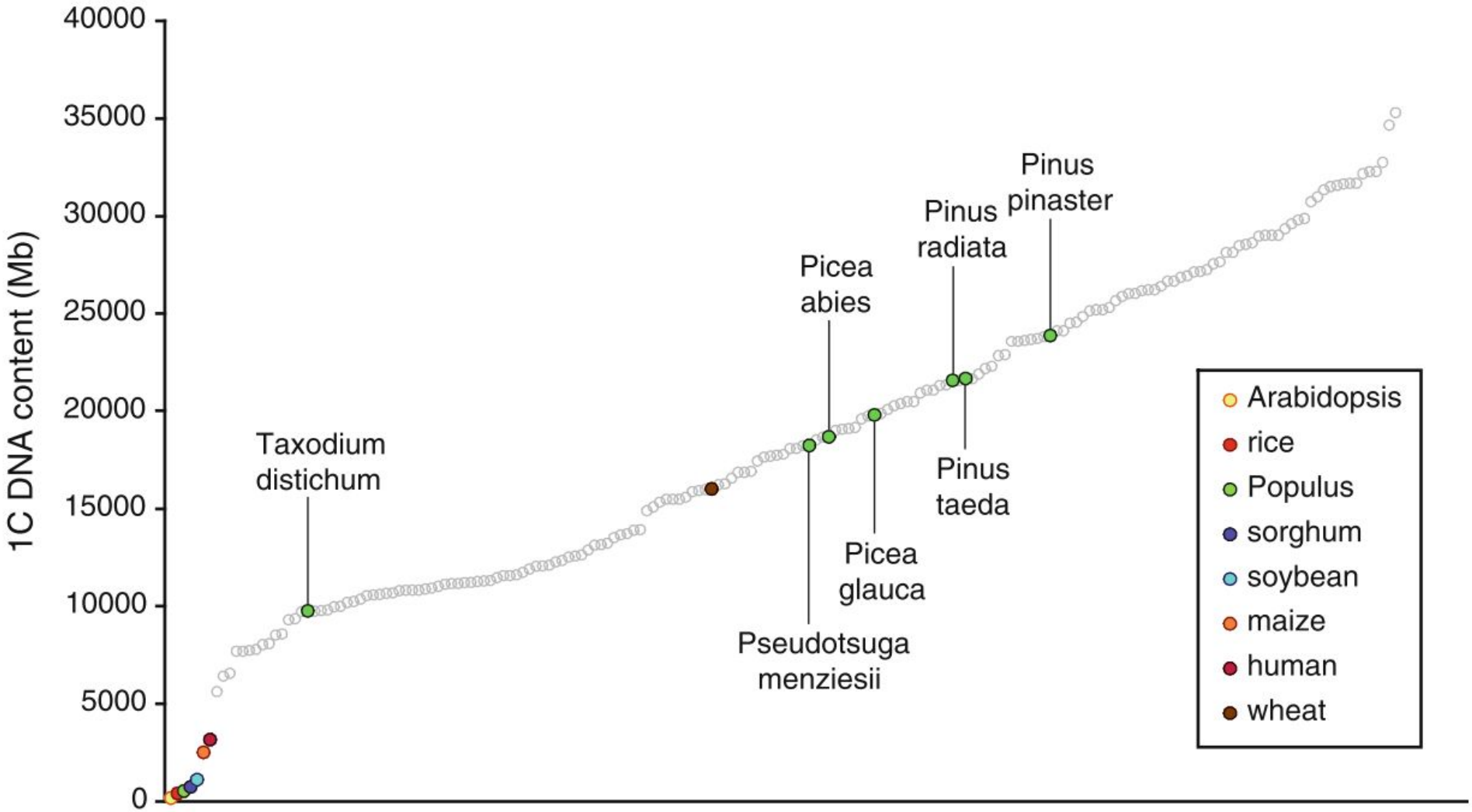
Planting
Selection thinnings

Irregular, mixed stand

Background

UK POPULATIONS

Background



MacKay et al. 2012

Outline

GENERAL AIMS OF RESEARCH

General aims of research

Hypothesis

The planted trees that composed the UK forests may **not hold** enough **genetic diversity** to face the current and future disturbances.

So, how the **gene pool** is being transmitted to the **offspring**?

Objectives

Asses genetic diversity in **canopy trees** and compare it with the genetic diversity that appears in **natural regeneration** seedling and saplings.

Assess the level of genetic variability **across the study species** (*Pseudotsuga menziesii* & *Thuja plicata)* by comparing them to **provenance trials data**.

Evaluate differences in the genetic diversity at **different stages of CCF** plantations in both canopy trees and natural regeneration.

Outline

EXPERIMENTAL APPROACH & RESULTS

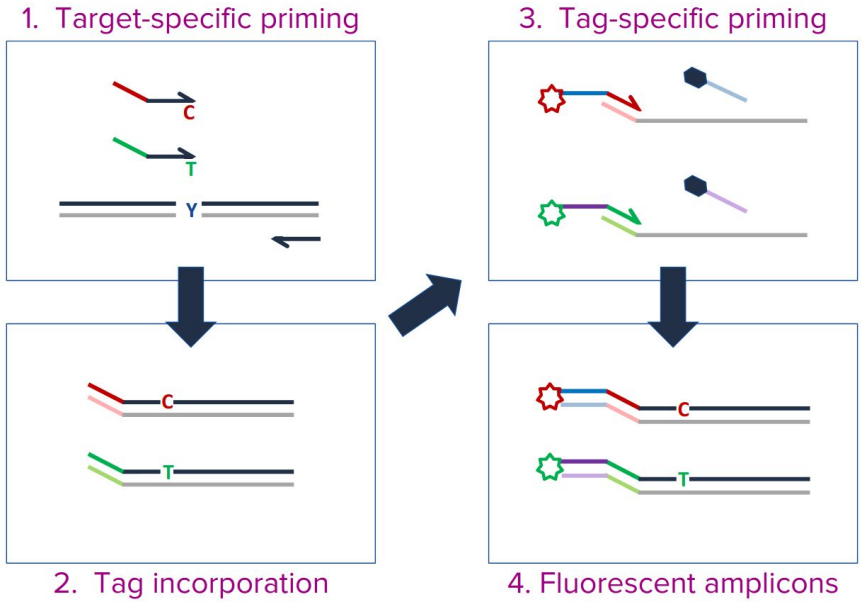
**Focus on Genomic Strategies to genotype individuals

Experimental approach & results

1 How SNPtype assay genotyping works?

FLUIDIGM®

SNP Type™ Chemistry



Based on allele-specific PCR. Three primers and two universal probes to distinguish between two alleles.

In the first two rounds of PCR, the two forward allele-specific primers (ASP1 and ASP2), in combination with the reverse locus-specific primer (LSP), amplify each allele (1) and incorporate the priming site for the fluorophore-labeled probe (2).

ASP1 contains the FAM tag sequence on the 5' end and locus-specific sequence terminating in one of the SNP alleles at the 3' end.

The fluorophores get attached.

ASP2 contains the HEX tag sequence on the 5' end and locus-specific sequence (4) ending in the other SNP allele at the 3' end.

The fluorescent amplicons (4) can be detected on the IUNO™ or Biomark HD™ Systems.

The LSP (locus-specific primer) is an unlabeled reverse primer specific to the locus.

Experimental approach & results

2 What do I need to make SNPtype assay genotyping to work?

SNPs sequences, basically

Howe *et al. BMC Genomics* (2020) 21:9
https://doi.org/10.1186/s12864-019-6383-9

BMC Genomics

RESEARCH ARTICLE Open Access

An Axiom SNP genotyping array for Douglas-fir

Glenn T. Howe[1*], Keith Jayawickrama[2], Scott E. Kolpak[1], Jennifer Kling[1], Matt Trappe[2], Valerie Hipkins[3], Terrance Ye[2], Stephanie Guida[4], Richard Cronn[5], Samuel A. Cushman[6] and Susan McEvoy[1]

Howe et al. 2020

28,095 SNPs from 2 databases both from transcripts (exons)

How does the SNP seq look like?

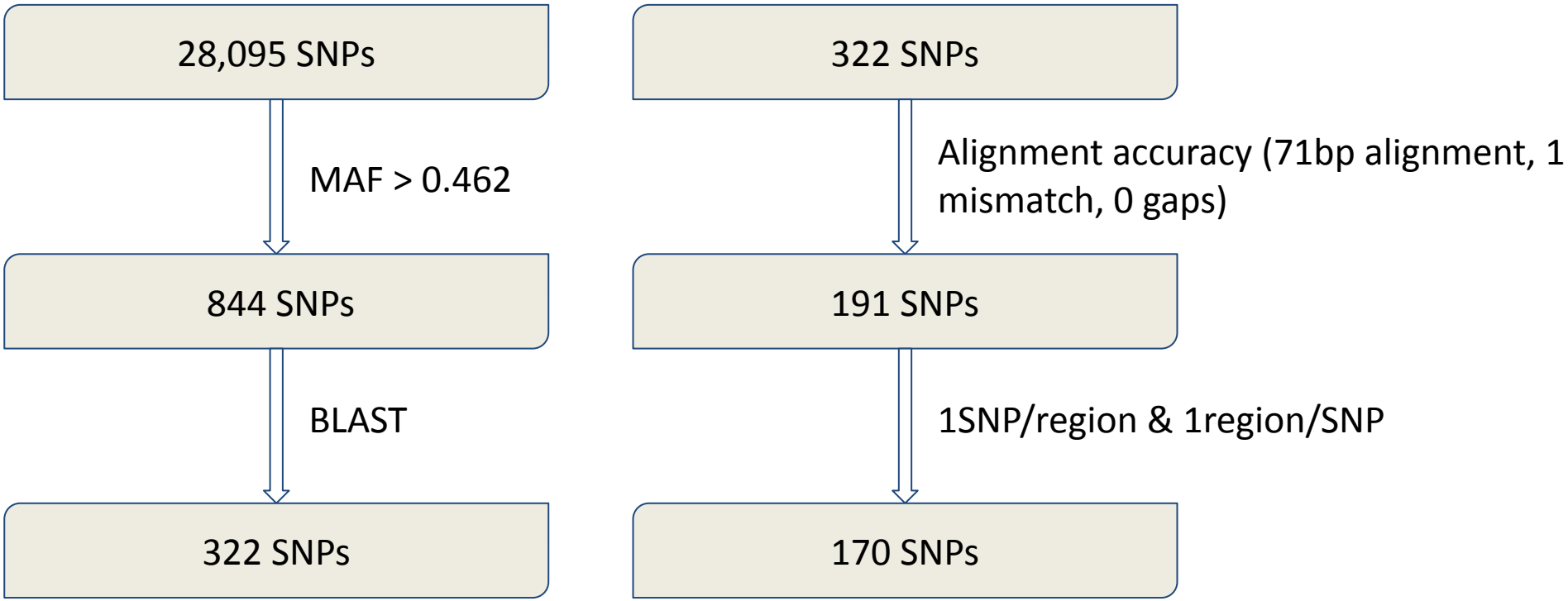
35bp...[SNP]...35bp

Too many SNPs & Too short seq

Experimental approach & results

P. menziesii

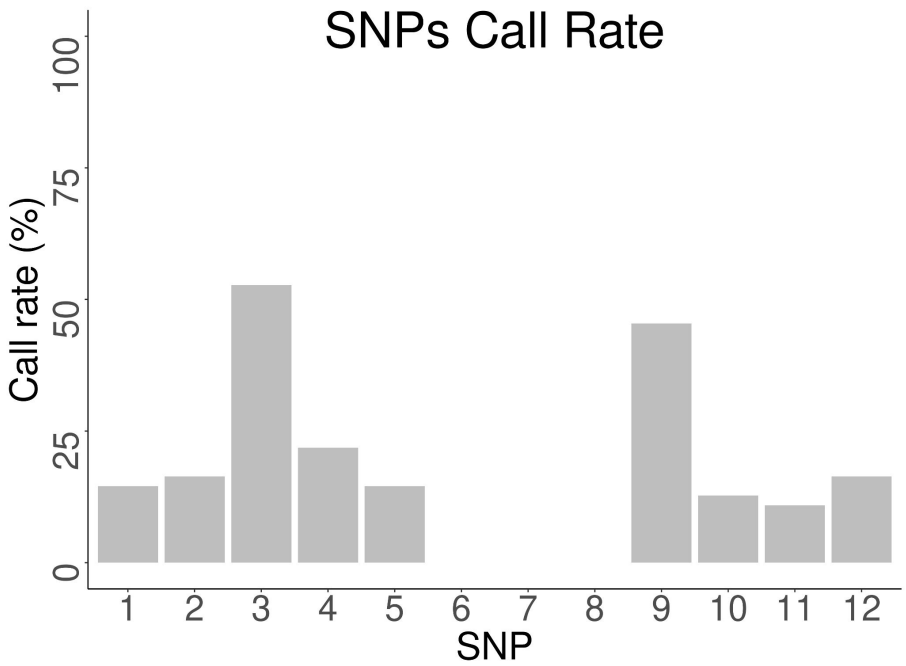
3 SELECT SNPs by performing different filterings



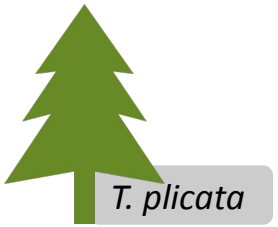
Experimental approach & results

P. menziesii

3 Preliminary results

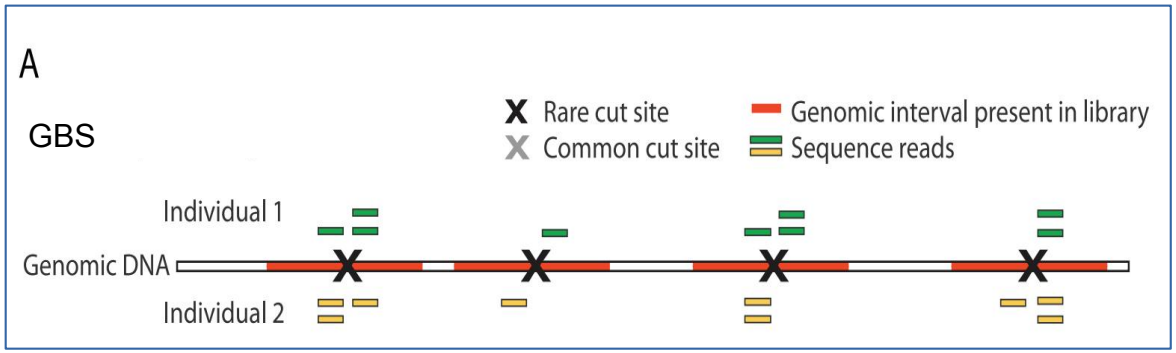


Experimental approach & results



1 How to develop molecular markers?

Genotyping By Sequencing (GBS)



(Peterson *et al.*, 2012)

Sequencing
Illumina NextSeq 6000 S4 platform

↓

Raw output reads

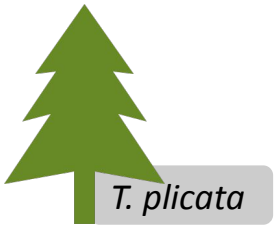
↓

Bioinformatic processing

↓

Analysis

Experimental approach & results



2 How to choose the restriction enzymes?

Simulating the DNA digestion using the reference genome and ddRADseqtools (Mora-Marquez et al. 2016)

Enzyme	Fragments 126_400	Fragments 126_300
ApeKI	819147	565079
BfaI	10543949	7962110
PstI_MspI	164850	112191
NsiI_MspI	480844	319908
SbfI_MspI	10214	8236

3 Analyse the sequenced reads

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