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Development of a new tool (4TREE) for adapted genome selection in European tree species

Authors

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Objective of the 4TREE array: To create a useful, affordable, high-throughput, robust (cross platform utilization), user-friendly and largely reusable genotyping tool for *Populus sp*, *Fraxinus sp*, *Pinus pinaster* and *Pinus pinea*, fostering collaboration between breeding programs across Europe.

Uses: Genome wide association studies, genomic predictions, monitoring and characterizing novel sources of variation.

This array will be used in B4EST for genotyping trees for multi-trait genotype-phenotype and genotype-environment association analyses to pinpoint genome regions related to the performance and adaptation in breeding.

The development of this Axiom array is a two step process: one step is the design of a screening array (450K SNPs, 480 individuals) to identify robust SNPs for the construction of one final array (12.5K SNPs/species) in a second step. This poster presents the screening array.

Pinus pinea

1

- RNA sequencing of 5 individuals from 5 countries
- Reference : Pinus taeda V2.0 genome, Zimin et al. 2017, doi: 10.1093/gigascience/gix072

2

- SNPs calling with GATK V4.0.3.0
- Min coverage: 8
- Min % of reads supporting the alternative allele: 20%
- No interspecific SNPs
- Positions informative in at least 2 samples

3

SNPs submitted	128,279
Recommended	3,495
Neutral	3,212
Not recommended	121,572

4

- Priorities:** Recommended and neutral markers
- Filters:**
- Probability of conversion
 - Unique probes
 - No interfering polymorphisms in the flanking sequences

36,784 SNPs

Populus sp.

1/2

- Available data ***
- Populus nigra**
- Infinium array, Faivre-Rampant et al. 2016, doi: 10.1111/1755-0998.12513
 - RNAseq, Rogier et al. 2018, doi: 10.1186/s12864-018-5239-z
 - WGS, Pégard et al. 2019, doi: 10.1186/s12864-019-5660-y
 - GWAS candidates, V. Segura unpublished data
- Populus deltoides**
- WGS of 5 individuals, Pinosio et al. 2016, doi: 10.1093/molbev/msw161, unpublished data
 - Exome capture, Fahrenkrog et al. 2017, doi: 10.1002/ece3.3466

3

SNPs submitted	2,213,994
Recommended	1,738,409
Neutral	196,596
Not recommended	278,989

4

- Priorities and filters:**
- Intraspecific SNPs to one or both species with a MAF > 5%
 - Biallelic interspecific SNPs (substitutions)
 - SNPs from the existing Infinium array
 - Candidate SNPs from GWAS for wood quality
 - Homogeneous coverage over the genome
 - 60% of SNPs in genes

137,545 SNPs

* No SNPs detection inside the project

Axiom screening array

1

Genomic resources

2

SNPs detection

3

Affymetrix scoring

- **"Recommended":**
 - Probability of conversion > 0.6
 - No interfering polymorphisms within 24 bases
 - 24 bases flanking sequences not shared with another SNP
- **"Not recommended":**
 - SNP and its flanking sequences duplicated in the list
 - Flanking sequences shared with another SNP
 - Probability of conversion < 0.4
 - Interfering polymorphisms in the flanking sequences
 - Homology problems
- **"Neutral":** All the other markers

4

SNPs selection

449,688 SNPs

Evaluation of SNPs selection

<i>Pinus pinea</i>	108 individuals
<i>Pinus pinaster</i>	119 individuals
<i>Populus sp</i>	118 individuals
<i>Fraxinus sp</i>	135 individuals

Final selection

Final array 4TREE
12.5K SNPs/species

Commercially available on demand
in summer 2020

Pinus pinaster

1

- Available data ***
- Infinium array, Plomion et al. 2016, doi: 10.1111/1755-0998.12464
- Unpublished GenTree data** (S. Scalabrin)
- Exome capture on 163 individuals
 - Reference: exome *de novo* assembly

2

- SNPs calling with GATK V4.0.3.0
- GATK: QD < 2, MQ < 40, MQRankSum < -12.5
- Min coverage: 8
- Min % of reads supporting the alternative allele: 20%
- Positions informative in at least 30 samples

3

SNPs submitted	191,785
Recommended	6,562
Neutral	7,898
Not recommended	177,325

4

- Priorities:** Markers from the Infinium array
- Filters:**
- Recommended or neutral
 - Probability of conversion
 - Unique probes
 - No interfering polymorphisms in the flanking sequences

137,321 SNPs

Fraxinus sp.

1/2

- Available data ***
- RNAseq, Harper et al. 2016, doi: 10.1038/srep19335
 - WGS, Sollars et al. 2017, doi: 10.1038/nature20786
 - WGS, dieback disease, Stocks et al. 2019, doi: 10.1101/626234
 - WGS, ash borer, Kelly et al. 2019, doi: 10.1101/772913

3

SNPs submitted	327,064
Recommended	279,337
Neutral	26,059
Not recommended	21,668

4

- Priorities:** Candidates associated with diseases and incompatibility (up to 8 SNPs per gene or transcript)
- Filters:**
- SNPs from associative transcriptomics
 - Up to 8 SNPs per gene
 - MAF > 5%
 - MAF < 5%
 - Recommended
 - Coverage

138,038 SNPs



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