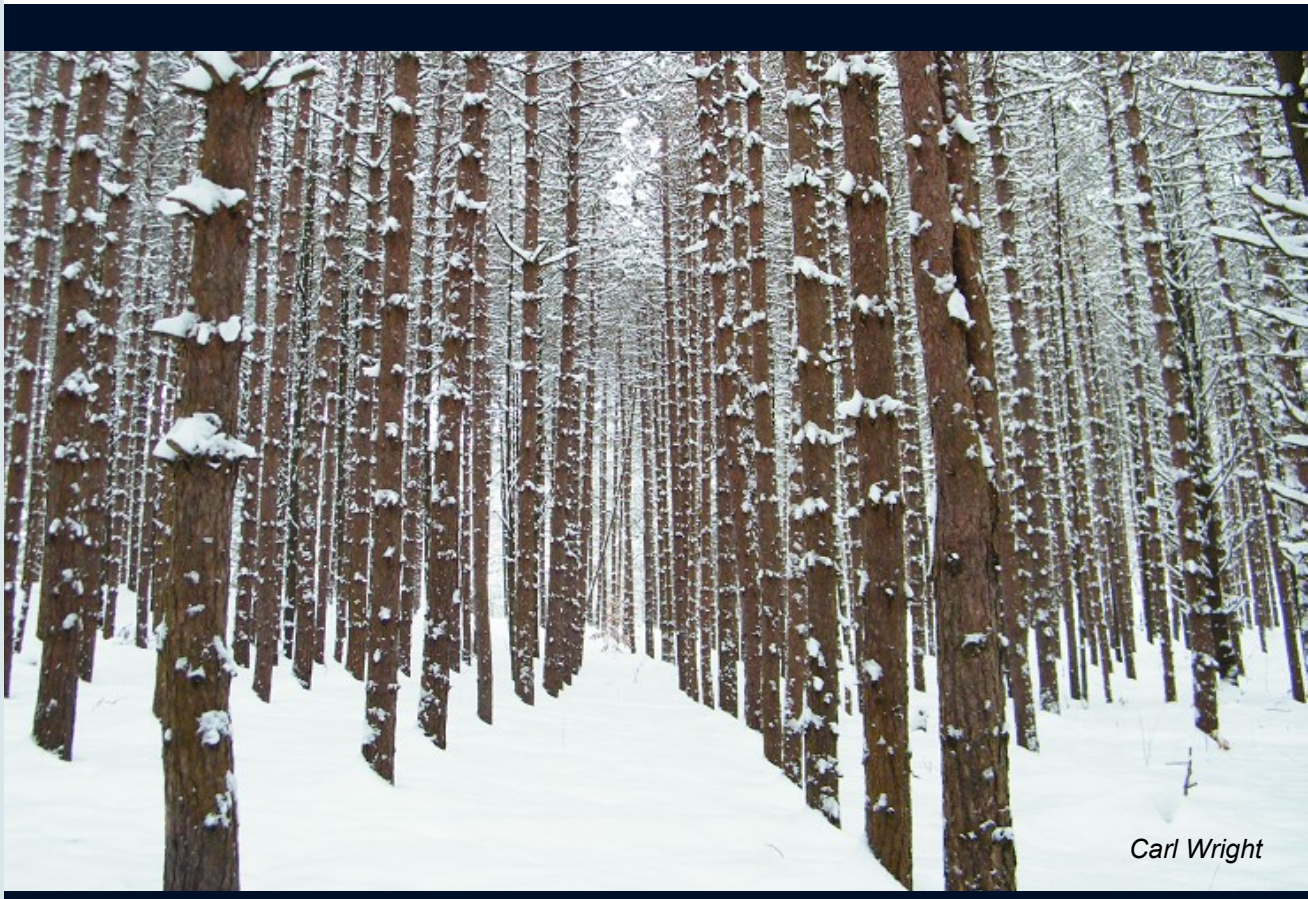
PNWTIRC

# Pacific Northwest Tree Improvement Research Cooperative

# Annual Report 2016-2017

Oregon State University College of Forestry
Department of Forest Ecosystems and Society

Glenn Howe, Scott Kolpak, Jennifer Kling, Susan McEvoy, Anna Magnuson, Erda Çeler



*Carl Wright*

http://pnwtirc.fsl.oregonstate.edu/

# PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Oregon State University College of Forestry
Department of Forest Ecosystems and Society


2016-2017

## Annual Report

Report editors
*Glenn Howe* *Jennifer Kling* *Anna Magnuson*
*Scott Kolpak* *Susan McEvoy* *Erda Çeler*

*Cover photo by Carl Wright, http://theoldfellowgoesrunning.com*

For information
*Glenn.Howe@oregonstate.edu*
*phone 541-737-9001, fax 541-737-1393*

# CONTENTS

## Introduction



## Research Presentations



## Appendices

# Pacific Northwest Tree Improvement Research Cooperative

## About the PNWTIRC

The Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) was formed in 1983 to conduct research in support of operational tree improvement in the Pacific Northwest. Emphasis is on region-wide topics dealing with major coniferous species. Membership has included representatives from public agencies and private forestry companies in western Oregon, western Washington, and coastal British Columbia.

## OUR MISSION IS TO:

- Create a knowledge base concerning genetic improvement and breeding of Pacific Northwest tree species
- Develop reliable, simple, and cost-effective genetic improvement methods and apply these methods to solve tree-breeding problems
- Promote effective collaboration and communication among public agencies and private industries engaged in tree improvement in the region

All participants provide guidance and receive early access to research results. Regular and Associate members provide financial and in-kind support and are represented on the Policy/Technical Committee. This committee is responsible for making decisions on program strategy and support, identifying research problems, establishing priorities, and assisting in the planning, implementation, and evaluation of studies. Because Contractual Participants provide less financial support, they have no voting rights on the Policy/Technical Committee. Liaison Members provide no financial support and have no voting rights. The PNWTIRC is housed in the Department of Forest Ecosystems and Society at Oregon State University.

# PNWTIRC PARTICIPANTS

## Regular Members

Bureau of Land Management
Cascade Timber Consulting
Green Diamond Resource Company
Hancock Timber Resource Group
Olympic Resource Management
Oregon Department of Forestry
Oregon State University
Port Blakely Tree Farms
Rayonier Forest Products
Roseburg Forest Products
Stimson Lumber Company
Washington State Department of Natural Resources
Weyerhaeuser

## Associate Members

Starker Forests

## Contractual Participants

Lone Rock Timber Company

## Liaison Members

Inland Empire Tree Improvement Cooperative
Northwest Tree Improvement Cooperative
USDA Forest Service, Pacific Northwest Research Station

# HIGHLIGHTS OF 2016-2017

- We published a PNWTIRC report entitled "*A high-density Affymetrix Axiom genotyping array for genomic selection in Douglas-fir*." This was a collaboration between the Pacific Northwest Tree Improvement Research Cooperative and the Northwest Advanced Renewables Alliance. The report authors were Glenn Howe, Keith Jayawickrama, Scott Kolpak, Jennifer Kling, Matt Trappe, Valerie Hipkins, Terrance Ye, Stephanie Guida, Rich Cronn, Sam Cushman, and Susan McEvoy.
- We published a PNWTIRC report entitled "*Genomic Selection Workplan*." This plan, written by the PNWTIRC and Northwest Tree Improvement Cooperative (Keith Jayawickrama and Terrance Ye), describes planned research that will focus on reducing the costs of genomic selection in Douglas-fir.
- Erda Çeler published her M.S. thesis on the Drought Hardiness Study. The title of her thesis is "*Douglas-fir seedlings in the Pacific Northwest: The genetics of drought adaptation*." Erda's project was a collaboration with Keith Jayawickrama, BLM, Weyerhaeuser, Silver Butte, and the Washington DNR. Erda was supported by the Turkish government, and now works as a liaison for international forestry research projects in Turkey.
- Scott Kolpak presented a talk at the 2017 meeting of the Western Forest Genetics Association and Canadian Forest Genetics Association. The title of his talk was "*Development of a high-density Affymetrix Axiom genotyping array for genomic selection in Douglas-fir*."
- Oguz Urhan continued to develop breeding strategies for western white pine in collaboration with Marc Rust, Richard Sniezko and others.
- Susan McEvoy was hired as a Bioinformatician, focusing on the assembly of the western white pine transcriptome.
- We hosted the annual meeting of the NSF Center for Advanced Forestry Systems (CAFS) from May 2-4, 2017, in Portland, Oregon.
- We increased PNWTIRC dues from $8,000 to $10,000 for Regular members. This was the first dues increase in over two decades.
- Former PNWTIRC graduate student and staff member, Lauren Magalska, took a position as the genetics coordinator for Port Blakely.
- Lauren Magalska was elected as the new Policy/Technical Committee Chair and Brian Baltunis will continue as the CAFS representative for OSU.

# MESSAGE FROM THE DIRECTOR

During the preparation of our Five-year Plan in 2015-16, the PNWTIRC decided to focus future research on genomic selection, a marker-based approach for improving the efficiency of tree breeding. Last year's major achievement was the publication of a PNWTIRC report that describes the development and performance of a SNP chip (genotyping array) that can be used for genomic selection in Coastal Douglas-fir. This report shows that we can genotype as many as ~28,000 SNPs in 10,000 to 15,000 genes—which should be more than enough to practice genomic selection in Douglas-fir breeding programs. We also showed that the Axiom array works for Interior Douglas-fir. Thus, genomic selection could be used in Douglas-fir breeding programs in British Columbia and the Inland Empire. This work was made possible by funding from the Northwest Advanced Renewables Alliance (NARA) and collaborations with Keith Jayawickrama and Terrance Ye. We then used the data from the Axiom array to conduct preliminary analyses of genomic selection. Although results are promising, genomic selection is also expensive, primarily because of high genotyping costs. Although genotyping costs will decline, current costs of SNP genotyping are almost five times the costs of progeny testing. Thus, future PNWTIRC research will focus on various strategies to reduce the costs of genomic selection. At the annual meeting in October, we distributed our research plan, "Genomic Selection Workplan: A Joint project between the PNWTIRC and NWTIC." This document describes a multi-year plan to reduce genomic selection costs and train tree breeders on the applied use of genomic selection.

Although we're focusing on genomic selection, we also completed the Drought Hardiness Study last year, and are continuing our research on western white pine. Erda Çeler graduated and published her M.S. thesis, "*Douglas-fir seedlings in the Pacific Northwest: The genetics of drought adaptation.*" Erda is now back in Turkey, working as a liaison for international forestry research projects. We're also continuing our collaborative research on western white pine. Based on the success of the Douglas-fir genotyping array, we are now developing a similar array to be used for improving blister rust resistance in western white pine. Susan McEvoy began as an OSU employee in spring 2017, and is now conducting the bioinformatic analyses needed to design a SNP chip for western white pine.

Finally, although we increased PNWTIRC dues this year (for the first time in over 20 years!), budget challenges are never far away. Things that could impact future budgets for research include the loss of external funds (e.g., completion of the CAFS project), an expected increase in indirect costs to OSU cooperatives, increases in PNWTIRC salaries, and changes in PNWTIRC membership. We'll track each of these changes, and then adjust as necessary.

Genetic markers have been used in Douglas-fir breeding programs since the early 1980s. This began with the seed orchard and population genetic studies conducted by Tom Adams and colleagues, and continued with the development of SSR markers by Gancho Slavov in the mid-2000s. However, the number of markers now available to breeders has increased by orders of magnitude, making new breeding strategies possible. Now, with our ongoing research on genomic selection in Douglas-fir, the PNWTIRC has firmly entered the genomics era.

Glenn Howe, PNWTIRC Director

# AGENDA – THURSDAY OCTOBER 19, 2017

# – ANNUAL MEETING –
# PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE (PNWTIRC)

**START TIME** 9:00 AM for coffee; 9:30 AM for presentations
**LOCATION** Community Room, Mt. Scott Fire Station Five
9339 SE Causey Ave., Happy Valley, OR
**CONTACT TEL** 541-730-3400 (Glenn)
**LUNCH** Lunch provided

| Time | Topic | Responsibility |
|---|---|---|
| 9:00-9:30 | Coffee | |
| 9:30-9:40 | Welcome and introductions | Sara Lipow |
| 9:40-10:00 | Overview<br>• *PNWTIRC accomplishments for 2016-17*<br>• *PNWTIRC plans for 2017-18* | Glenn Howe |
| 10:00-10:30 | A SNP chip for western white pine – Bioinformatic steps | Susan McEvoy<br>Glenn Howe |
| 10:30-10:50 | Break | |
| 10:50-11:40 | Axiom SNP chip – Final report | Glenn Howe |
| 11:40-12:00 | Drought Hardiness Study – Next steps | Scott Kolpak |
| 12:00-1:00 | Lunch | |
| 1:00-2:00 | Genomic selection work plan | Glenn Howe<br>Jennifer Kling |
| 2:00-2:20 | Break | |
| 2:20-2:40 | Budget and other business<br>• *Budget presentation and vote*<br>• *Elect new Policy/Technical Committee Chair* | Glenn Howe<br>Sara Lipow |
| 2:40-2:55 | Update - Seedlot Selection Tool/Species Potential Habitat Tool | Glenn Howe |
| 3:00 | Wrap-up and adjourn | Glenn Howe |

## Overview – 2016/2017

By Glenn Howe

Glenn Howe began this year's annual meeting by presenting an overview of PNWTIRC personnel, highlights, collaborations, and grants for 2016 – 2017. Current PNWTIRC staff include Glenn Howe (Director), Scott Kolpak (Research Coordinator), Jennifer Kling (Research Scientist), Anna Magnuson (Program Manager), and Susan McEvoy (Bioinformatician). Oguz Urhan and Erda Çeler served as PNWTIRC graduate students, Sara Lipow (Roseburg Forest Products) served as the Policy/Technical Committee Chair, and Brian Baltunis (Weyerhaeuser) served as the CAFS representative for OSU. In June 2017, Erda Çeler received with her M.Sc. degree, and is now working for the Turkish Government as a liaison for international research projects. Susan is a new PNWTIRC staff member focusing on the western white pine project. Glenn presented an overview of the day's presentations: *A SNP chip for western white pine – Bioinformatic steps* by Susan McEvoy, *Axiom SNP chip – Final report* by Glenn Howe, *Drought hardiness study – Next steps* by Scott Kolpak, *Genomic selection work plan* by Glenn Howe and Jennifer Kling, and *Update – Seedlot Selection Tool/Species Potential Habitat Tool* by Glenn Howe. Glenn presented highlights of last year's research and outreach activities and a brief overview of external collaborations and grants. Just before the meeting adjourned, Susan McEvoy presented the new PNWTIRC website (http://pnwtirc.forestry.oregonstate.edu/).

# PNWTIRC Annual Meeting 2017
# October 19, 2017

**Glenn Howe**

*Pacific Northwest Tree Improvement Research Cooperative*
*Department of Forest Ecosystems and Society*
*Oregon State University*

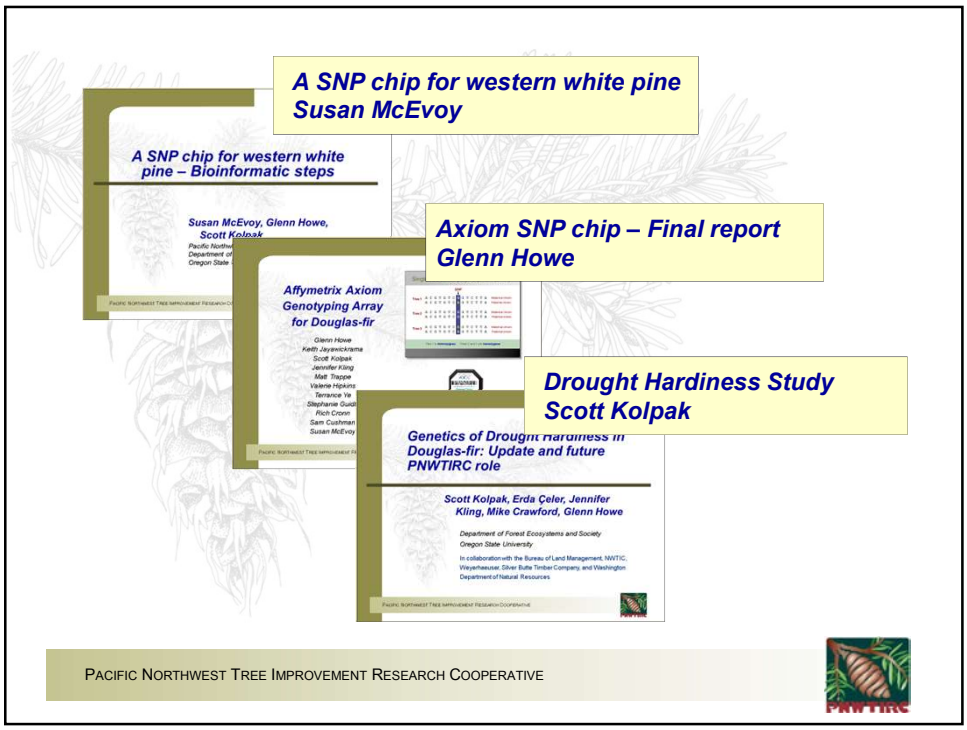
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

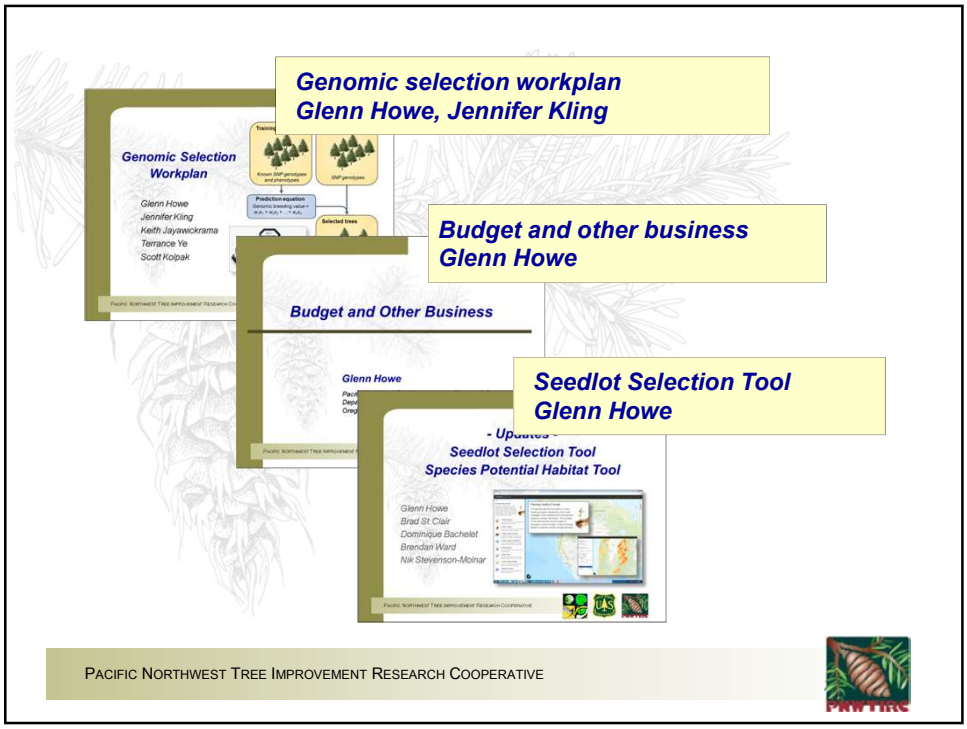
## PNWTIRC personnel

### 2016-2017

- Director – ***Glenn Howe***
- Research Coordinator – ***Scott Kolpak***
- Research Scientist – ***Jennifer Kling***
- Bioinformatician – ***Susan McEvoy***
- Program Manager – ***Anna Magnuson***
- Graduate students – ***Oguz Urhan, Erda Çeler***
- Faculty Research Assistant – ***Lauren Magalska***
- Policy/Technical Committee Chair – ***Sara Lipow***
- CAFS representative – ***Brian Baltunis***

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


A SNP chip for western white pine
Susan McEvoy
A SNP chip for western white pine – Bioinformatic steps
Axiom SNP chip – Final report
Glenn Howe
Affymetrix Axiom Genotyping Array for Douglas-fir
Drought Hardiness Study
Scott Kolpak
Genetics of Drought Hardiness in Douglas-fir: Update and future PNWTIRC role
Scott Kolpak, Erda Çeler, Jennifer Kling, Mike Crawford, Glenn Howe
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


Genomic selection workplan
Glenn Howe, Jennifer Kling
Genomic Selection Workplan
Glenn Howe
Jennifer Kling
Keith Jayawickrama
Terrance Ye
Scott Kolpak
Budget and other business
Glenn Howe
Budget and Other Business
Glenn Howe
Seedlot Selection Tool
Glenn Howe
Seedlot Selection Tool
Species Potential Habitat Tool
Glenn Howe
Brad St Clair
Dominique Bachelet
Brendan Ward
Nik Stevenson-Molnar
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## PNWTIRC highlights for 2016-2017

- **We published a PNWTIRC report on the Douglas-fir SNP chip**
  - *Collaboration with Keith Jayawickrama and the Northwest Advanced Renewables Alliance (NARA)*
- **Scott presented a talk at the 2017 meeting of the Western Forest Genetics Association and Canadian Forest Genetics Association**
- **We published the Genomic Selection Workplan**
  - *Collaboration with Keith Jayawickrama and Terrance Ye*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## PNWTIRC highlights for 2016-2017

- **Erda Çeler published her thesis on the Drought Hardiness Study**
  - *Collaboration with Keith Jayawickrama, BLM, Weyco, Silver Butte, WDNR*
  - *Erda Çeler was supported by a scholarship from the Turkish government*
- **Oguz Urhan continued to develop breeding strategies for WWP**
  - *Collaboration with Marc Rust, Richard Sniezko, and others*
  - *Oguz Urhan is being supported by a scholarship from the Turkish government*
- **We hosted the annual meeting of the NSF Center for Advanced Forestry Systems (CAFS)**
- **We increased PNWTIRC dues**

## Highlights of 2016-2017

### *Publications by PNWTIRC personnel*

- Çeler, E. 2017. Douglas-fir seedlings in the Pacific Northwest: The genetics of drought adaptation. M.S. thesis, Oregon State University. 152pp.
- Howe, G.T. 2017. Cooperative brings life to tree breeding tools and approaches. Western Forester 62(3):18-19.
- Howe, G.T. and Strauss, S.H. 2017. Biotechnology research is developing new tools for tree breeders. Western Forester 62(3):26-27.
- Frank, A., Howe, G.T., Sperisen, C., Brang, P., St.Clair, J.B., Schmatz, D.R., and Heiri, C. 2017. Risk of genetic maladaptation due to climate change in three major European tree species. Glob Change Biol 2017:1-14. DOI: 10.1111/gcb.13802.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Highlights of 2016-2017

### *Publications by PNWTIRC personnel (cont)*

- Frank, A., Pluess, A.R., Howe, G.T., Sperisen, C., Heiri, C. 2017. Quantitative genetic differentiation and phenotypic plasticity of European beech in a heterogeneous landscape: Indications for past climate adaptation. Perspect Plant Ecol Evol Syst 26:1-13.
- Frank, A., Sperisen, C., Howe, G.T., Brang, P., Walthert, L., St.Clair, J.B., and Heiri, C. 2017. Distinct genecological patterns in seedlings of Norway spruce and silver fir from a mountainous landscape. Ecology 98:211-227.
- St.Clair, J.B. and Howe, G.T. 2017. Building on a century of forest genetics research. Western Forester 62(3):16-17.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Highlights of 2016-2017

### *Presentations by PNWTIRC personnel*

- Çeler, E. and Howe, G.T. 2017. Douglas-fir seedlings in the Pacific Northwest: The genetics of drought adaptation. *Poster presentation In*: Proceedings, Forest Regeneration in Changing Environments, International Meeting of IUFRO Unit 1.01.04, Forest Establishment and Early Growth Dynamics, July 11-13, 2017, Corvallis, OR.
- Howe, G.T. 2017. Integrating traditional and molecular breeding for blister rust resistance in western white pine. *Abstract In*: Proceedings, Planting the Future, 44th Annual Meeting of the Inland Empire Tree Improvement Cooperative, March 8, 2017, Spokane Valley, Washington.
- Howe, G.T. 2017. Genomics and breeding of Douglas-fir. *Presentation In*: Douglas-fir Breeding Seminar, April 6, 2017, University of Canterbury, Christchurch, New Zealand.
- Howe, G.T. 2017. Genetics of trees. *Presentation In*: Reforestation Matters, USDA Forest Service National Silviculture Meeting, April 13, 2017, Portland, OR.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


## Highlights of 2016-2017

### *Presentations by PNWTIRC personnel (cont)*

- Howe, G.T. 2017. Trees on the move: Migration of tree species in response to climate change. *Presentation In*: Oregon State University Tree School, April 22, 2017, Rogue Community College, Grants Pass, OR.
- Howe, G.T. and Jayawickrama, K.J. 2016. Genomic selection for Douglas-fir tree improvement. *Presentation In*: Center for Advanced Forestry Systems Annual Meeting, May 2-4, 2017, Portland, Oregon.
- Howe, G.T. 2017. Genetic considerations for reforestation in the face of global climate change. *Abstract In*: Proceedings, Forest Regeneration in Changing Environments, International Meeting of IUFRO Unit 1.01.04, Forest Establishment and Early Growth Dynamics, July 11-13, 2017, Corvallis, OR.
- Howe, G.T. 2017. Adapting forests to climate change: The role of forest genetics. *Presentation In*: Mt. Hood Community College, September 7, 2017.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Highlights of 2016-2017

## *Presentations by PNWTIRC personnel (cont)*

- Kolpak, S.E., Jayawickrama, K., Kling, J., Trappe, M., Hipkins, V., Ye, T., Guida, Stephanie, Cronn, R., Cushman, S.A., McEvoy, S., and Howe, G.T. 2017. Development of a high-density Affymetrix Axiom genotyping array for genomic selection in Douglas-fir. *Abstract In*: Forest Genetics 2017: Health and Productivity under Changing Environments, Proceedings of the Joint Meeting of Western Forest Genetics Association and Canadian Forest Genetics Association, Edmonton, AB, June 26-29, 2017.
- Urhan, O., Rust, M.L., Davis, A., Howe, G.T., Hipkins, V. 2016. Development of genetic markers for western white pine and Douglas-fir. *Presentation In*: Center for Advanced Forestry Systems Annual Meeting, May 2-4, 2017, Portland, Oregon.

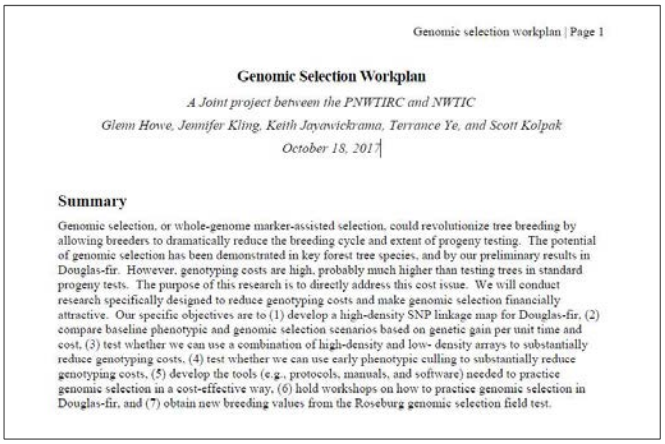
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Collaborations and grants

- **CAFS.** *Center for Advanced Forestry Systems – Phase II.* Howe, G.T., Maguire, D.A., and Strauss, S.H. National Science Foundation Industry/University Cooperative Research Center Program, 2012-2018, $300,000 (OSU).
- **USFS Forest Health Protection, Special Technology Development Program.** *Genetic markers for western white pine (WWP): Enabling molecular breeding for resistance to white pine blister rust.* Howe, G.T., Davis, A., Hipkins, V., Liu, J.-J., Mahalovich, M.F., Rust, M., and Sniezko, R., 2014-2018, $99,500.
- **USFS Pacific Northwest Research Station.** *Meta-analysis of Douglas-fir provenance tests to estimate responses to seed transfer and climate change.* Howe, G.T. and St.Clair, J.B. USDA-Forest Service Joint Venture Agreement, 2013-2018, $100,000.
- **USFS Pacific Northwest Research Station.** *Evaluating assisted migration options for adapting to climate change.* Howe, G.T. and St.Clair, J.B. USDA-Forest Service Joint Venture Agreement, 2013-2019, $40,000.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Plans for 2017-2018



Genomic selection workplan | Page 1

**Genomic Selection Workplan**

*A Joint project between the PNWTIRC and NWTIC*

*Glenn Howe, Jennifer Kling, Keith Jayawickrama, Terrance Ye, and Scott Kolpak*

*October 18, 2017*

**Summary**

Genomic selection, or whole-genome marker-assisted selection, could revolutionize tree breeding by allowing breeders to dramatically reduce the breeding cycle and extent of progeny testing. The potential of genomic selection has been demonstrated in key forest tree species, and by our preliminary results in Douglas-fir. However, genotyping costs are high, probably much higher than testing trees in standard progeny tests. The purpose of this research is to directly address this cost issue. We will conduct research specifically designed to reduce genotyping costs and make genomic selection financially attractive. Our specific objectives are to (1) develop a high-density SNP linkage map for Douglas-fir, (2) compare baseline phenotypic and genomic selection scenarios based on genetic gain per unit time and cost, (3) test whether we can use a combination of high-density and low- density arrays to substantially reduce genotyping costs, (4) test whether we can use early phenotypic culling to substantially reduce genotyping costs, (5) develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way, (6) hold workshops on how to practice genomic selection in Douglas-fir, and (7) obtain new breeding values from the Roseburg genomic selection field test.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## A SNP chip for western white pine – Bioinformatic steps

By Susan McEvoy, Glenn Howe, and Scott Kolpak

We are developing single nucleotide polymorphism (SNP) genetic markers for western white pine (WWP). Our long-term goal is to lay the foundation for using genomic selection to enhance resistance to white pine blister rust. Our specific objectives are to (1) use bioinformatics to assemble a WWP reference transcriptome to use for discovering SNP markers; (2) design a high-density Axiom genotyping array; and (3) design a plant breeding strategy for testing genomic selection in WWP. Key milestones for transcriptome assembly and SNP discovery include tissue sampling, RNA sequencing, sequence cleaning, transcriptome assembly, assembly cleaning, and assembly evaluation.

We pooled tree tissues from needles, branches, stems, roots, and buds collected from tens to hundreds of families or genotypes from three WWP breeding programs in western North America. Twelve RNA samples were extracted from pooled tissue samples, and then used to create two replicate RNA samples (normalized and non-normalized) for sequencing using the Illumina HiSeq 2500 platform. Each of these two samples produced 66–73 million reads of ~250 nt each. We downloaded additional WWP sequences (373 million reads of 74 to 101 nt each) from the European Nucleotide Archive (ENA), and then combined these with our HiSeq 2500 sequences. These ENA sequences were derived from primary needles and shoot-tip tissues collected from resistant and susceptible families. Prinseq was used to clean the sequences prior to assembly, including (1) removing short, low quality, low complexity, and duplicated sequences and (2) trimming sequence 'ends' containing low quality bases, poly-AT tails, or repeats of single-, di-, or tri-nucleotides.

The first draft of the reference transcriptome was built using *de novo* assembly with the Trinity RNA-Sequence Assembler. The draft WWP transcriptome consists of 210 Mb, with a median contig length of 529 nt. We are currently cleaning and filtering the draft assembly using the Deconseq and enTAP programs and curated databases of potentially contaminating sequences. These databases contain adaptor sequences, non-nuclear conifer sequences (i.e., cpDNA and mtDNA), highly repetitive conifer sequences (e.g., rDNA and retrotransposons), and sequences from potentially contaminating organisms (e.g., fungi and bacteria). After cleaning the assembly, we will begin SNP discovery, ultimately choosing the best SNPs to use for constructing a high-density Axiom array. We will seek additional funds and partnerships to construct the Axiom array, and eventually conduct a proof-of-concept trial in WWP breeding programs.

# A SNP chip for western white pine – Bioinformatic steps

**Susan McEvoy, Glenn Howe, Scott Kolpak**

*Pacific Northwest Tree Improvement Research Cooperative*
*Department of Forest Ecosystems and Society*
*Oregon State University*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Background

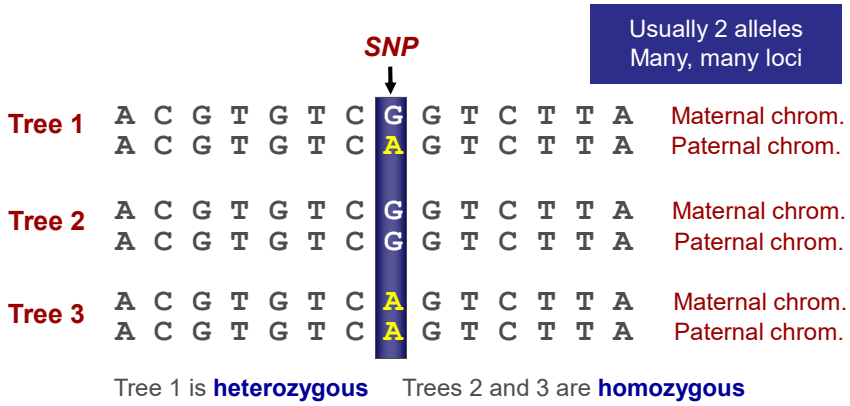
- Objectives
  - *Design an Axiom genotyping array for western white pine*
  - *Use the array as a foundation to attract funds to manufacture the array*
  - *Ultimately, use genomic selection to breed for resistance to white pine blister rust*
- Funding
  - *USFS Special Technology Development Program (STDP)*
  - *Center for Advanced Forestry Systems (CAFS)*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## What are SNPs and why do we need them?

- Single Nucleotide Polymorphism (SNP)
- Allelic sequences differ in one nucleotide (A, T, C, G)
- Track desirable genes (alleles) in a pedigree

Usually 2 alleles
Many, many loci




Tree 1 is **heterozygous** Trees 2 and 3 are **homozygous**

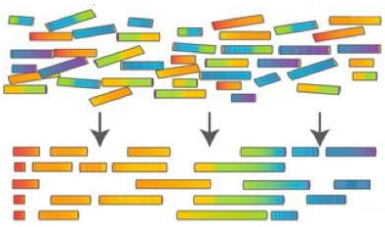
## Assembly in a nutshell

Sequences 'reads' are joined into longer sequences (contigs) using overlaps

Illumina
HiSeq 2500

Sequence Reads
TATCACGATCTCTCTGATTTCCG

de novo



reference-guided

## Steps

- Tissue samples
- RNA sequences
- Sequence cleaning
- Assembly
- Assembly cleaning
- Assembly evaluation
- SNP discovery
- Array design
- Genomic selection

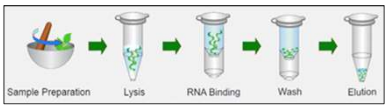
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Tissue samples

- Tissues included in transcriptome
  - *Needles, branches, stems, roots, buds*
- Three WWP breeding programs[1]
  - *USFS DGRC*
  - *USFS / IETIC*
  - *BC Ministry of Forests*
- Adjusted the final RNA pool to maximize the variety of genes

**Table 1.** Western white pine tissues were collected from the greenhouse or field, and then used for extracting RNA for high-throughput sequencing. The numbers in parentheses represent the approximate numbers of unrelated trees, full-sib families, or half-sib families in the tissue collection.

| | Greenhouse | | Field | | | |
|---|---|---|---|---|---|---|
| Tissue | November | September | Oct/Nov | Oct/Nov | November | November |
| Secondary needle | Seedlings Dorena (3) | Saplings Dorena (573+) | Saplings Dorena (9) | Mature trees Bingham (76) | Seedlings Tyrell (230+) | Mature trees BC (31) |
| Primary needle | — | — | — | — | Seedlings Tyrell (192+) | — |
| Branch | — | — | Mature trees Dorena (4) | — | — | — |
| Stem | Seedlings Dorena (3) | — | — | Mature trees Bingham (76) | — | — |
| Root | Seedlings Dorena (3) | — | — | — | — | — |
| Bud | — | — | — | Mature trees Bingham (76) | — | — |




[1] DGRC = Dorena Genetic Resource Center, IETIC = Inland Empire Tree Improvement Cooperative

# OSU RNA Sequences

RNA sequencing

- Submitted two replicate samples to Carver BioTech
  - *Non-normalized*
  - *Normalized*
- 250 base pair reads

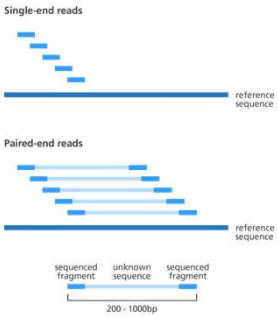
Illumina HiSeq 2500

**Table 2.** Western white pine RNAseq libraries and numbers of 250 nt reads.

| Sample | Name of fastq file | No. of reads |
|---|---|---|
| OSU_WWP_3_15_16 | OSU_WWP_3_15_16_ACAGTGAT_L001_R1_001 | 72,564,364 |
| OSU_WWP_3_15_16 | OSU_WWP_3_15_16_ACAGTGAT_L001_R2_001 | 72,564,364 |
| OSU_WWP_3_15_16_norm | OSU_WWP_3_15_16_norm_GTGAAACG_L001_R1_001 | 65,941,515 |
| OSU_WWP_3_15_16_norm | OSU_WWP_3_15_16_norm_GTGAAACG_L001_R2_001 | 65,941,515 |
| | 66 to 73 million reads produced | **277,011,758** |

# RNA Sequence Terminology

- Normalization – the process of equalizing the numbers of reads among genes.
  - *Some genes (e.g., photosynthetic genes) are expressed at very high levels. Thus, have many more reads than other genes. Normalization gets rid of the 'extra' copies of these highly expressed genes.*
- Paired-end reads – the fragment is sequenced from both ends to generate reads from both directions
  - *Both ends are sequenced*
  - *Distance between both ends is known*
  - *More likely to align than a single-end read*



# OSU RNA Sequences

## RNA sequencing

- Submitted two replicate samples to Carver BioTech
  - *Non-normalized*
  - *Normalized*
- 250 base pair reads

Illumina HiSeq 2500

**Table 2.** Western white pine RNAseq libraries and numbers of 250 nt reads.

| Sample | Name of fastq file | No. of reads |
|---|---|---|
| OSU_WWP_3_15_16 | OSU_WWP_3_15_16_ACAGTGAT_L001_R1_001 | 72,564,364 |
| OSU_WWP_3_15_16 | OSU_WWP_3_15_16_ACAGTGAT_L001_R2_001 | 72,564,364 |
| OSU_WWP_3_15_16_norm | OSU_WWP_3_15_16_norm_GTGAAACG_L001_R1_001 | 65,941,515 |
| OSU_WWP_3_15_16_norm | OSU_WWP_3_15_16_norm_GTGAAACG_L001_R2_001 | 65,941,515 |
| | 66 to 73 million reads produced | **277,011,758** |

# Canadian Forest Service sequences

## Collaborator is Jun-Jun Liu

- Take advantage of existing sequences to improve assembly
- Retrieved from the European Nucleotide Archive (ENA)
- Tissues – *Pinus monticola* primary needles and shoot-tip cDNA libraries

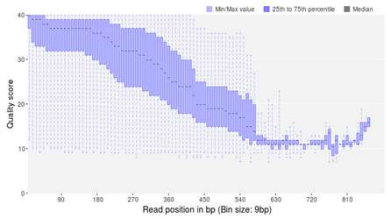
ENA European Nucleotide Archive

| Run accession | Tax ID | Scientific name | Instrument model | Library layout | FASTQ files (FTP) | FASTQ files (Galaxy) |
|---|---|---|---|---|---|---|
| SRR3273237 | 3345 | Pinus monticola | Illumina HiSeq 2000 | PAIRED | File 1 File 2 | File 1 File 2 |

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## RNA Sequences need to be cleaned

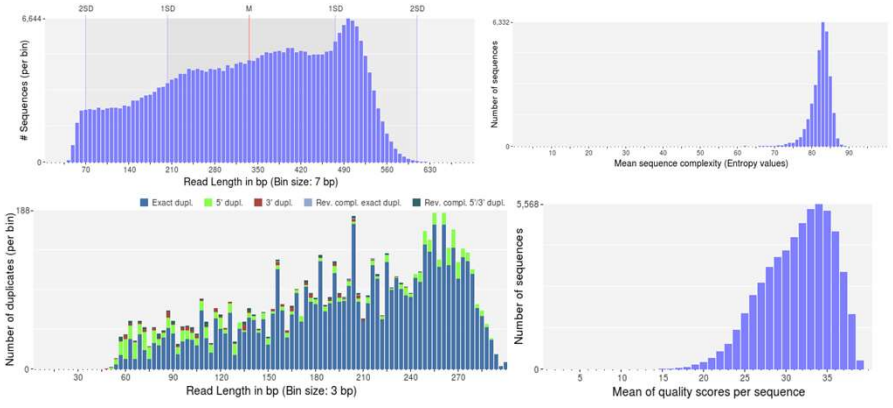
- Minimum length, low quality, duplicates, trimming of poly-A/T tails, N's
- Various tools are available
  - *Sickle for minimum length and low quality trimming of ends*
  - *SolexaQA Lengthsort for separating pairs, singles, and discards based on minimum length*
  - *Prinseq = many different cleaning options in one package:*




```
perl prinseq-lite.pl
-fastq OSU_WWP_3_15_16_norm_GTGAAACG_L001_R1_001.fastq
-fastq2  OSU_WWP_3_15_16_norm_GTGAAACG_L001_R2_001.fastq
-min_len 40                  #filter sequence shorter than 40
-min_qual_mean 20            #filter seq with quality score mean below 20
-trim_qual_left 25
-trim_qual_right 25          #trim ends based on quality score of 25
-trim_tail_left 5
-trim_tail_right 5           #trim poly-A/T tails with a min length of 5
-trim_ns_left 5
-trim_ns_right 5             #trim N tails with a min length of 5
-ns_max_p 1                  #filter sequence with more than 1% Ns
-derep 12                    #types of duplicates to filter
-lc_method entropy           #low complexity filter method
-lc_threshold 70             #low complexity threshold
```

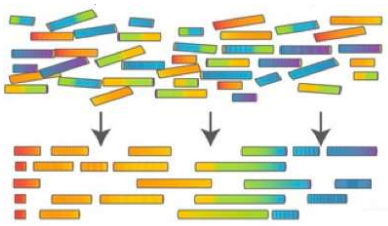
## RNA Sequences need to be cleaned

### Prinseq graphs



## Assembling the transcriptome

- Trinity RNA-Sequence Assembly
- Input: norm/non-norm, additional pine reads
- Output: FASTA file containing contigs (assembled reads)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Assemblies need to be cleaned

- Contaminant filtering using selected sequences
  - *Remove pine species from chloroplasts, mitochondria, rRNA, plus adapters (sequencing artifacts)*
  - *Deconseq*
- Contaminant filtering by taxonomy
  - *Fungi, bacteria, etc are common contaminants*
  - *NCBI's RefSeq Plant Protein Database*
  - *RefSeq all available proteins*
  - *EnTAP*

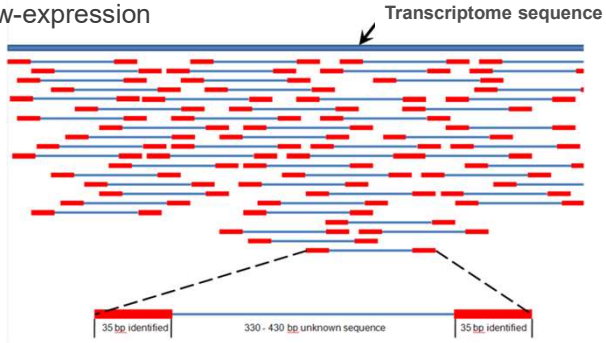
**Name**

README
RELEASE_NUMBER
announcements/
archaea/
bacteria/
complete/
fungi/
invertebrate/
mitochondrion/
other/
plant/
plasmid/
plastid/
protozoa/
release-catalog/
release-error-notice/
release-notes/
release-statistics/
vertebrate_mammalian/
vertebrate_other/
viral/

## How do we evaluate the assembly?

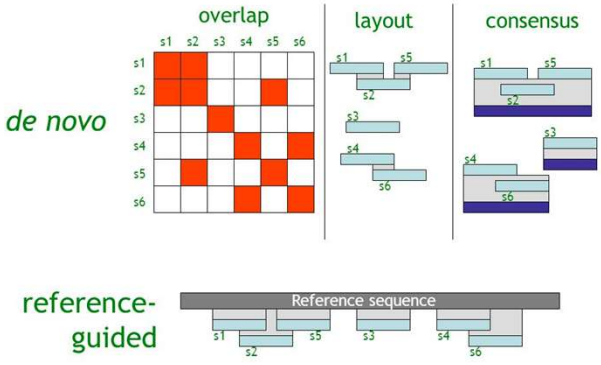
- Open reading frames
  - *Section of contigs with no stop signals*
  - *Length indicates potential to be a transcript*
- Read mapping on contigs
- Optional low-expression filtering

- Possible tools:
  - *Drap (De novo RNA-seq Assembly Pipeline)*
  - *CAP3*
  - *Novoalign*



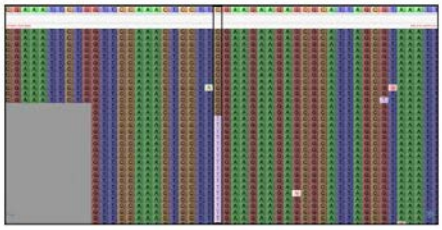

## Reference guided assembly

- *De novo* assembly – join sequences to form a transcriptome as described above
- Reference guided assembly – map sequences to a known transcriptome
- Loblolly pine will be our reference



## SNPs discovery

- Mapping original reads to the assembly gives us a pileup
- Scripts look for SNPs in pileup
- Differences can be SNPs or sequencing errors – the more reads the better




## SNP array design

- Affymetrix Axiom array (high density)
- Glenn will talk about this for Douglas-fir
- Axiom array will be used for genomic selection to improve resistance to white pine blister rust

# Acknowledgements

Thanks to….

- *Mike Crawford, BLM, Tyrell Seed Orchard*
- *Alvaro Hernandez, University of Illinois, Roy J. Carver Biotechnology Center*
- *Angelia Kegley, USFS, Dorena Genetic Resource Center*
- *John King, British Columbia Ministry of Forests and Range*
- *Marc L. Rust, University of Idaho, Inland Empire Tree Improvement Cooperative*
- *Richard Sniezko, USFS, Dorena Genetic Resource Center*
- *Nicholas Ukrainetz, British Columbia Ministry of Forests and Range*
- *Oguz Urhan, Oregon State University, PhD candidate*
- *USDA Forest Service Health Protection–Special Technology Development Program (STDP)*
- *CAFS, Center for Advanced Forestry Systems*
- *Center for Genome Research and Biocomputing, Oregon State University*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Axiom SNP chip – Final report

By Glenn Howe, Keith Jayawickrama, Scott Kolpak, Jennifer Kling, Matt Trappe, Valerie Hipkins, Terrance Ye, Stephanie Guida, Rich Cronn, Sam Cushman, and Susan McEvoy

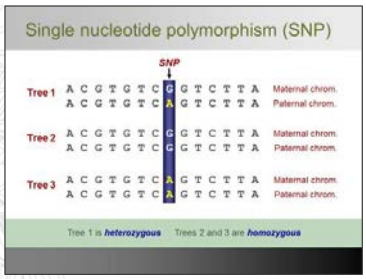
Plant and animal breeding programs are increasingly using large numbers of single nucleotide polymorphism markers (SNPs) to increase the efficiencies of breeding programs via genomic selection. We previously used transcriptome sequencing to identify 278,979 potential SNPs in ~20,000 Douglas-fir genes. We then tested a subset of these SNPs (N = 8067) using an Illumina Infinium® genotyping array. Here, we report on the design and testing of a new, larger-scale Affymetrix Axiom® genotyping array for 55,776 SNPs. Ultimately, this array will be used to conduct a rigorous test of genomic selection in Douglas-fir.

We tested the Axiom array on ~2,300 related and unrelated Coastal Douglas-fir trees (*Pseudotsuga menziesii* var. *menziesii*) from Oregon and Washington, and 11 trees of Interior Douglas-fir (*P. menziesii* var. *glauca*). Using the default Affymetrix quality control criteria (e.g., 97% call rate), 20,669 SNPs were reliably genotyped and polymorphic (i.e., are 'successful' SNPs). To increase the number of SNPs and improve genome coverage, we developed protocols to 'rescue' SNPs that did not pass the default Affymetrix quality control criteria. Lowering the call rate threshold from 97% to 60% increased the number of successful SNPs from 20,669 to 28,092. BLASTN alignment searches of the successful SNP sequences to version 1.0 of the Douglas-fir reference genome were associated with 15,037 putative transcripts.

We used a subset of 395 unrelated trees to calculate SNP population genetic statistics for Coastal Douglas-fir. Over a range of call rate thresholds (97% to 60%), the median call rate for SNPs in Hardy-Weinberg equilibrium ranged from 99.2% to 99.7%, and the median minor allele frequency ranged from 0.189 to 0.220. The successful SNPs also worked well on Interior Douglas-fir. The Axiom genotyping array will serve as an excellent foundation for studying the population genomics of Douglas-fir and for implementing genomic selection.

## *Affymetrix Axiom Genotyping Array for Douglas-fir*

*Glenn Howe*
*Keith Jayawickrama*
*Scott Kolpak*
*Jennifer Kling*
*Matt Trappe*
*Valerie Hipkins*
*Terrance Ye*
*Stephanie Guida*
*Rich Cronn*
*Sam Cushman*
*Susan McEvoy*





PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## PNWTIRC report

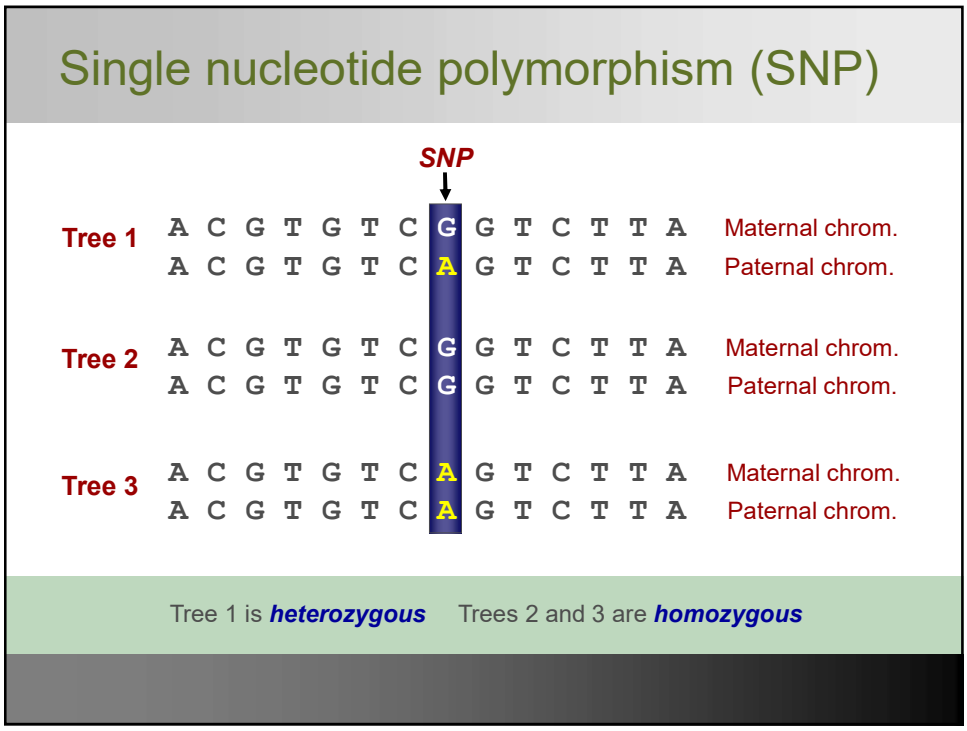
**A high-density Affymetrix Axiom genotyping array for genomic selection in Douglas-fir**

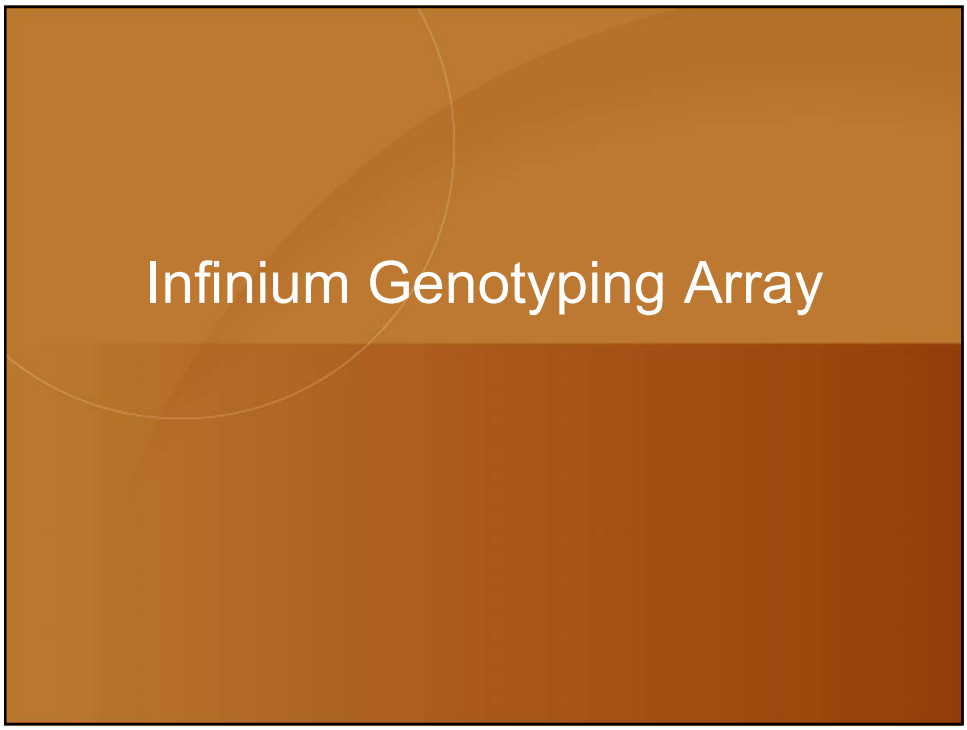
*Glenn Howe[1], Keith Jayawickrama[2], Scott E. Kolpak[1], Jennifer Kling[1], Matt Trappe[2], Valerie Hipkin[3], Terrance Ye[2], Stephanie Guida[4], Richard Cronn[5], Samuel A. Cushman[6], and Susan McEvoy[1]*

*October 19, 2017*

**CONFIDENTIAL - DO NOT DISTRIBUTE**

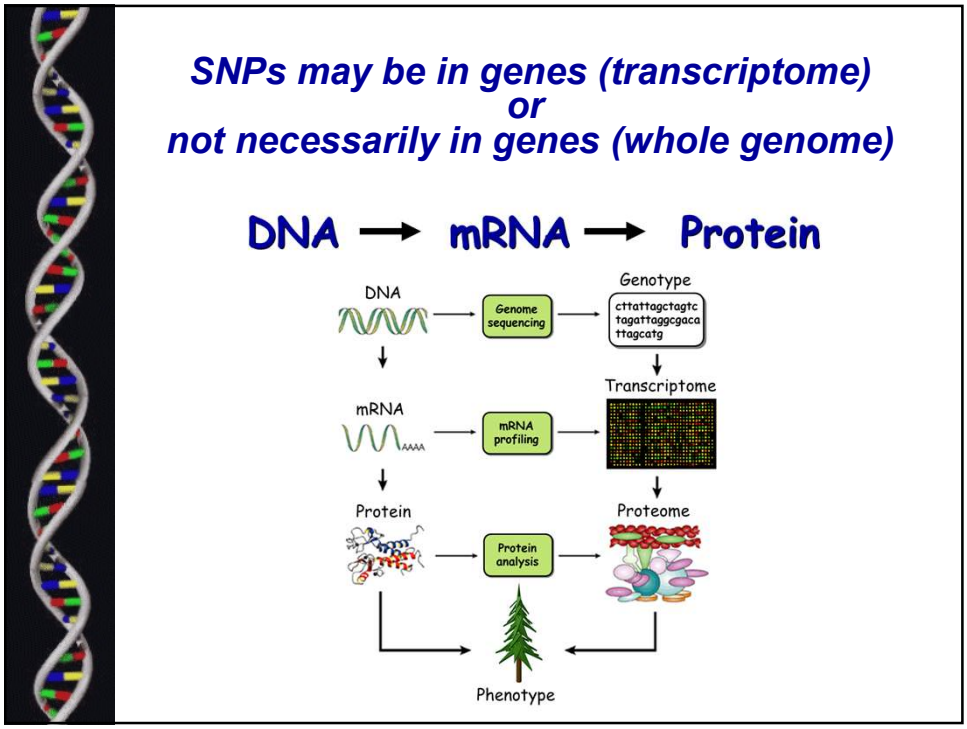
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


Single nucleotide polymorphism (SNP)
SNP
Tree 1
A C G T G T C G G T C T T A Maternal chrom.
A C G T G T C A G T C T T A Paternal chrom.
Tree 2
A C G T G T C G G T C T T A Maternal chrom.
A C G T G T C G G T C T T A Paternal chrom.
Tree 3
A C G T G T C A G T C T T A Maternal chrom.
A C G T G T C A G T C T T A Paternal chrom.
Tree 1 is heterozygous Trees 2 and 3 are homozygous


Infinium Genotyping Array

## Conifer Translational Genomics Network


PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE



## DF transcriptome assembly

| Statistic | Number |
|---|---|
| Total reads | 2,764,549 |
| Assembled reads | 2,544,087 |
| Total assembled | 2,741,911 |
| Singletons | 102,623 |
| Isogroups (genes) | 25,002 |
| Isotigs | 38,589 |
| One isotig/isogroup | 18,774 |
| Mean length of isotig | 1,390 |
| N50 | 1,883 |
| Total consensus nucleotides | 72,302,278 |

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Potential SNP markers in Douglas-fir

***278,979 SNPS detected in Douglas-fir genes***

***1 isotig/isogroup***

***Longest isotig/isogroup***

| Douglas-fir variety | No. of SNPs | No. of genes with SNPs |
|---|---|---|
| Coastal | 203,231 | 19,329 |
| Interior | 226,124 | 19,274 |
| Both (in common) | 151,014 | 17,361 |

***Conclusion = lots of SNP markers to choose from!***

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Douglas-fir SNP chip (Illumina Infinium)

## Douglas-fir SNP chip is available

- 7256 SNPs can be assessed
- Many more potential SNPs are available

Numbers and percentages of putative Douglas-fir SNPs attempted and assayed with an Illumina Infinium SNP array (n = 260 trees).

| | |
|---|---|
| SNPs attempted | 8769 |
| SNPs assayed by Illumina | 8067 |
| **Percent of SNPs (assayed/attempted)** | **92.0** |
| SNPs assayed by Illumina | 8067 |
| SNPs called (call frequency ≥ 0.85) | 7256 |
| **Percent of SNPs (called/assayed)** | **82.7** |
| SNPs called (call frequency ≥ 0.85) | 7256 |
| SNPs called that are polymorphic (MAF ≥ 0) | 5847 |
| **Percent SNPs (called MAF > 0/called)** | **80.6** |
| SNPs attempted | 8769 |
| SNPs called that are polymorphic (MAF ≥ 0) | 5847 |
| **Percent SNPs (called MAF > 0/attempted)** | **66.7** |

MAF = minor allele frequency. MAF > 0 means there's more than 1 allele

# Axiom Genotyping Array

## Affymetrix Axiom array is cheaper

***Large-scale genotyping service from GeneSeek***



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

NARA

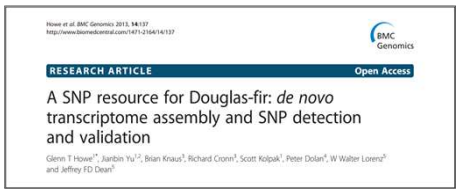
## Northwest Advanced Renewables Alliance

Keith Jayawickrama

Northwest Advanced Renewables Alliance

USDA NIFA

## Resources for genomic selection



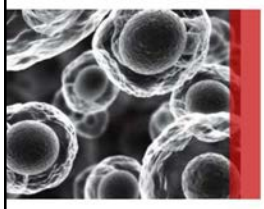

*"Our SNP database may contain as many as ~200,000 true SNPs, and as many as ~69,000 SNPs that could be genotyped at ~20,000 gene loci"*


*"A total number of 187,653 single nucleotide polymorphisms (SNPs) were detected by three SNP detection tools"*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## NFGEL helped with DNA isolation

affymetrix
Biology for a better world



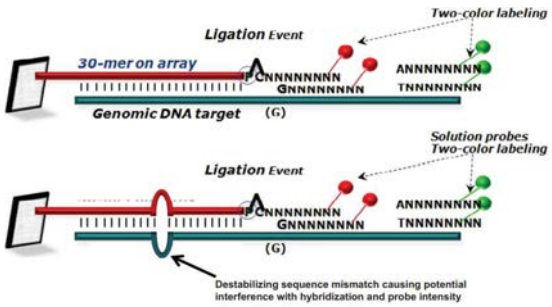
Affymetrix

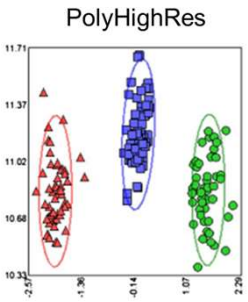
2

eBioscience GeneChip USB

## Axiom array – Two-color system

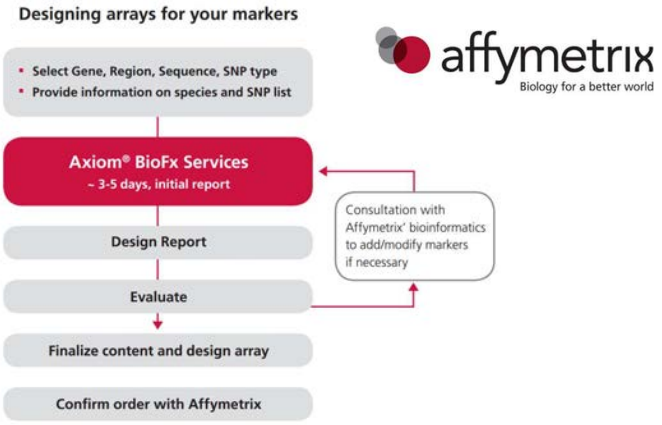
**Red** = **Red** + **Red** = **AA**
**Green** = **Green** + **Green** = **BB**
**Blue** = **Red** + **Green** = **AB**



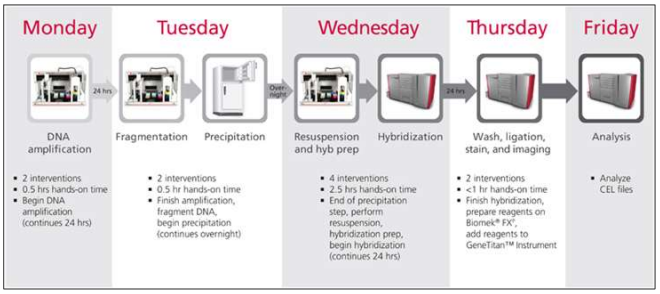

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Design array with Affymetrix

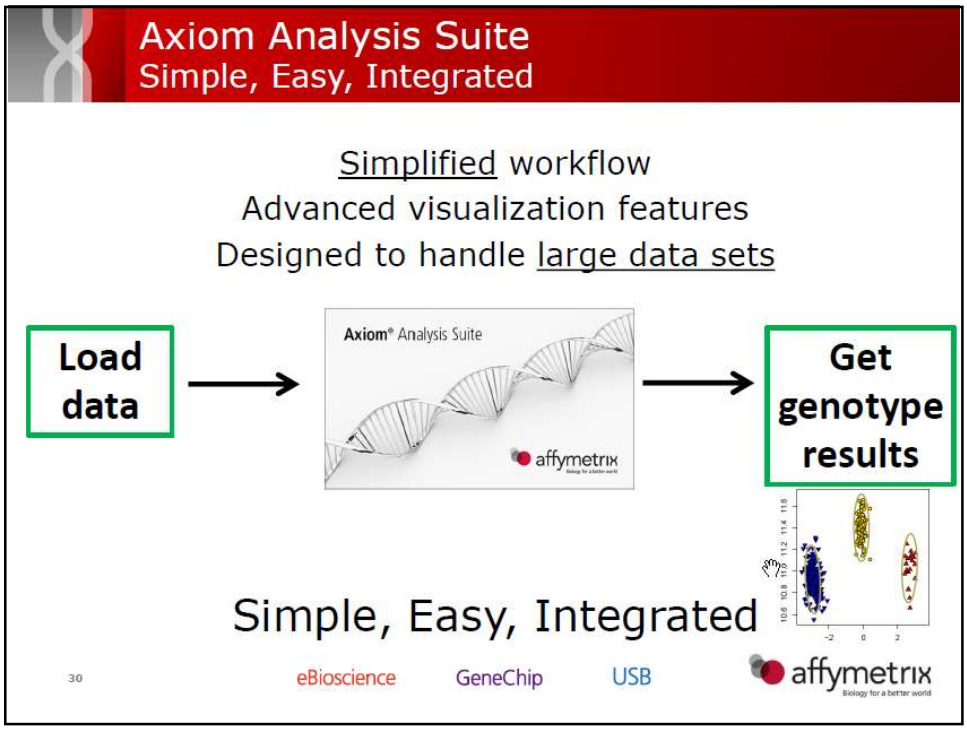


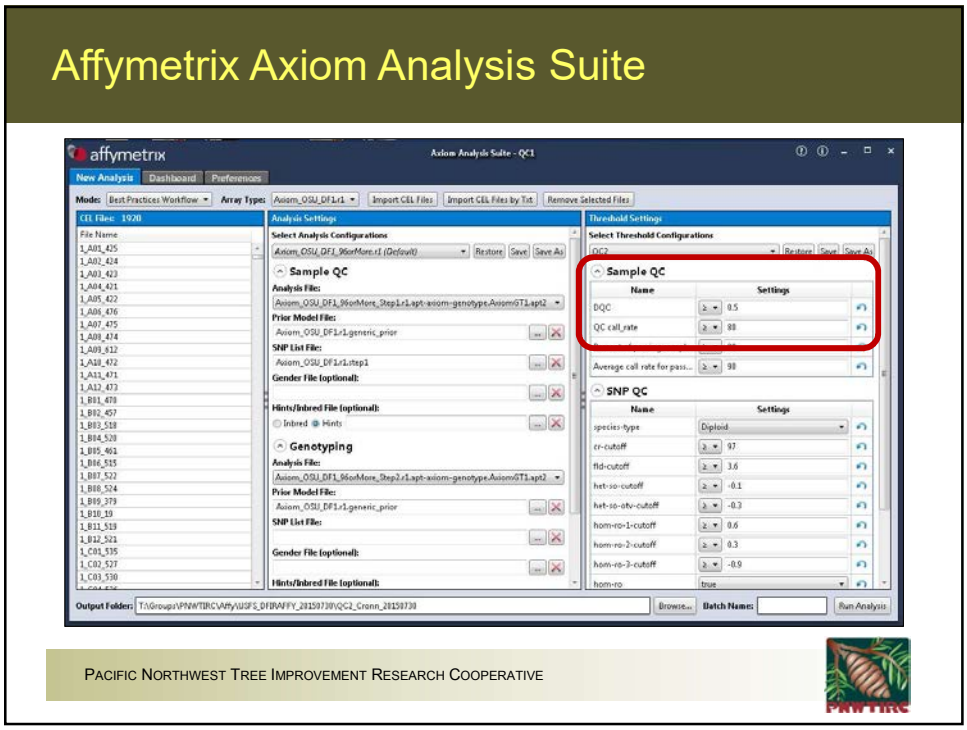

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Genotyping by GeneSeek

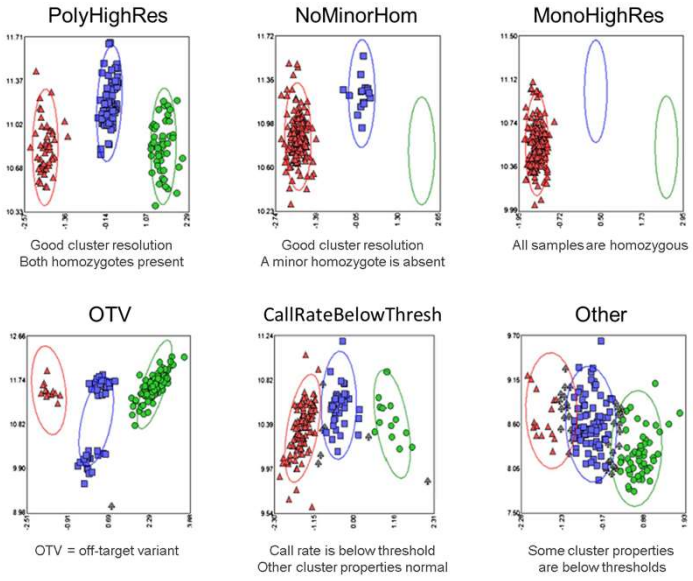



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


Axiom Analysis Suite
Simple, Easy, Integrated
Simplified workflow
Advanced visualization features
Designed to handle large data sets
Load data
Axiom® Analysis Suite
affymetrix
Get genotype results
Simple, Easy, Integrated
30
eBioscience
GeneChip
USB
affymetrix
Biology for a better world

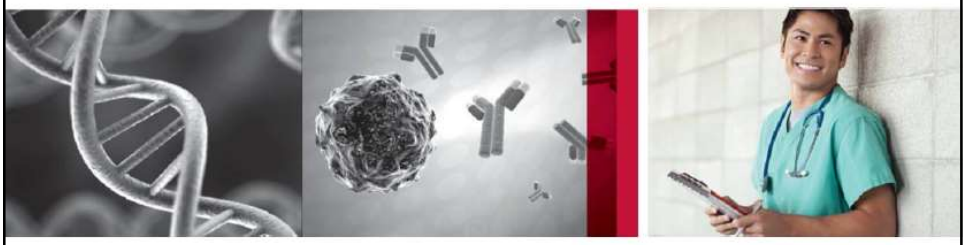

Affymetrix Axiom Analysis Suite
affymetrix
Axiom Analysis Suite - QC1
New Analysis
Dashboard
Preferences
Sample QC
SNP QC
Genotyping
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE
PNWTIRC

## SNP classifications




# Analytical Procedures Needed for Conifers

affymetrix
Biology for a better world



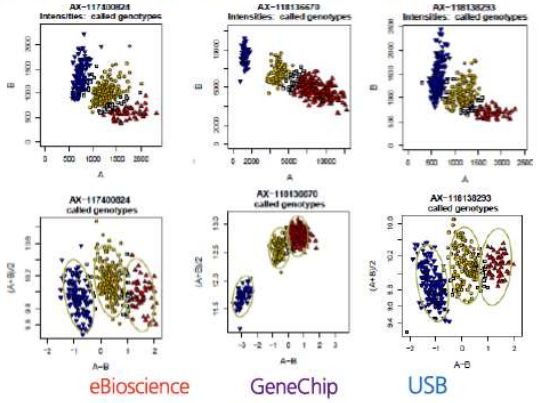
# OSU_DouglasFir

Advanced Filters

eBioscience GeneChip USB

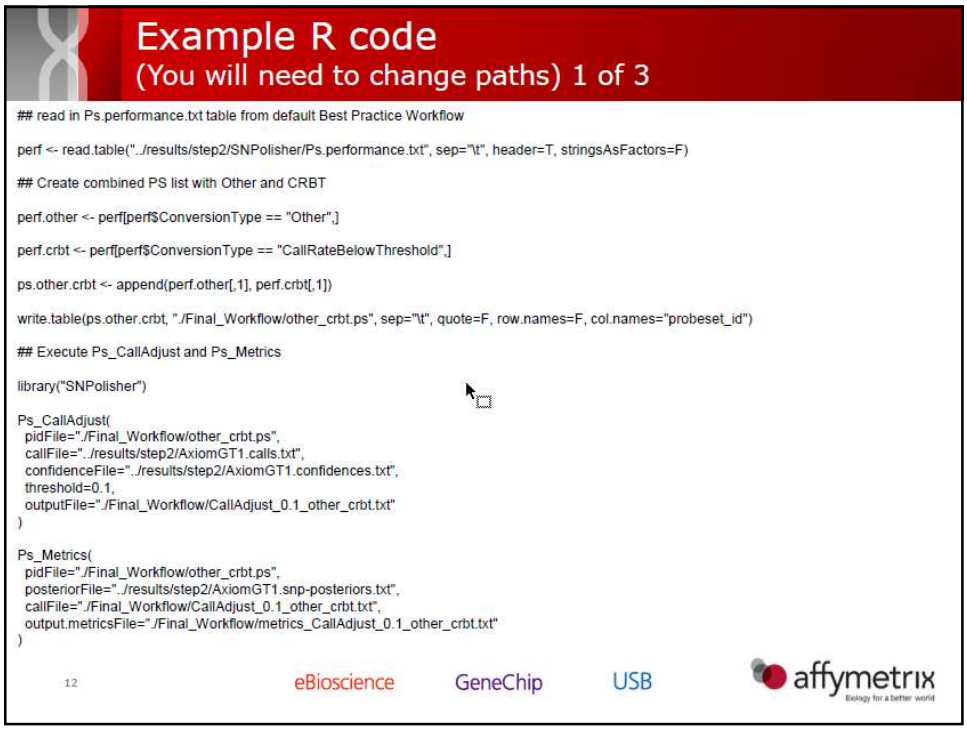
## Results – 3 cluster rescue

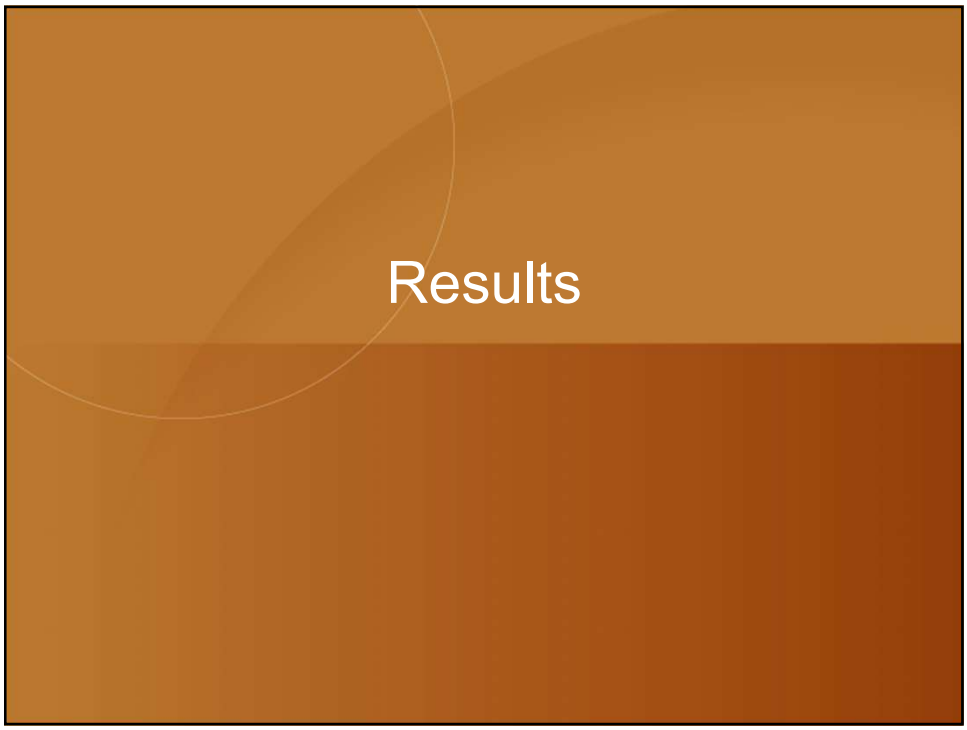
- Performing the probeset rescue operation on the combined 'Other' and 'CallRateBelowThreshold' probesets results in 5802 probesets passing the advanced filter thresholds.
  - These probesets are lower resolution (clusters are closer together)
  - SNPs are still likely polymorphic and probesets are providing accurate calls
  - Must use Ps_CallAdjust-ed calls table to increase stringency on call confidence



eBioscience GeneChip USB

affymetrix
Biology for a better world

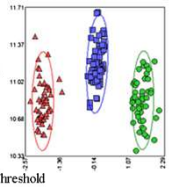

Example R code
(You will need to change paths) 1 of 3
## read in Ps.performance.txt table from default Best Practice Workflow
perf <- read.table("../results/step2/SNPolisher/Ps.performance.txt", sep="\t", header=T, stringsAsFactors=F)
## Create combined PS list with Other and CRBT
perf.other <- perf[perf$ConversionType == "Other",]
perf.crbt <- perf[perf$ConversionType == "CallRateBelowThreshold",]
ps.other.crbt <- append(perf.other[,1], perf.crbt[,1])
write.table(ps.other.crbt, "./Final_Workflow/other_crbt.ps", sep="\t", quote=F, row.names=F, col.names="probeset_id")
## Execute Ps_CallAdjust and Ps_Metrics
library("SNPolisher")
Ps_CallAdjust(
pidFile="./Final_Workflow/other_crbt.ps",
callFile="../results/step2/AxiomGT1.calls.txt",
confidenceFile="../results/step2/AxiomGT1.confidences.txt",
threshold=0.1,
outputFile="./Final_Workflow/CallAdjust_0.1_other_crbt.txt"
)
Ps_Metrics(
pidFile="./Final_Workflow/other_crbt.ps",
posteriorFile="../results/step2/AxiomGT1.snp-posteriors.txt",
callFile="./Final_Workflow/CallAdjust_0.1_other_crbt.txt",
output.metricsFile="./Final_Workflow/metrics_CallAdjust_0.1_other_crbt.txt"
)
12
eBioscience
GeneChip
USB
affymetrix


Results

# SNP categories – Table 1

| | Final SNP call rate threshold[#] | | | | | |
|---|---|---|---|---|---|---|
| | Default | Rescue | | | | |
| SNP category | 97% | 90% | 80% | 70% | 60% | Affymetrix abbreviation |
| Off-target variant | 1 | 1 | 1 | 1 | 1 | OTV |
| Other | 31 | 30 | 27 | 24 | 23 | Other |
| Call rate below threshold | 8 | 3 | 2 | 2 | 2 | CallRateBelowThreshold |
| Not Converted | 40 | 34 | 30 | 27 | 27 | OTV + Other + CallRateBelowThreshold |
| No minor homozygote | 12 | 12 | 12 | 12 | 12 | NoMinorHom |
| Monomorphic high resolution | 16 | 16 | 16 | 16 | 16 | MonoHighResolution |
| Polymorphic high resolution | 31 | 31 | 31 | 31 | 31 | PolyHighResolution |
| Rescued[#] | – | 6 | 10 | 13 | 14 | Rescued from Other and CallRateBelowThreshold |
| Converted[‡] | 60 | 66 | 70 | 73 | 73 | PolyHighResolution + NoMinorHom + MonoHighResolution + Rescued |
| Percent successful (population ave) | 31.5 | 37.5 | 41.7 | 44.1 | 45.0 | PolyHighResolution + Rescued |
| Number successful (population ave) | 17,542 | 20,926 | 23,252 | 24,601 | 25,104 | PolyHighResolution + Rescued |
| Percent successful (population sum) | 37.1 | 42.9 | 46.9 | 49.5 | 50.4 | PolyHighResolution + Rescued |
| Number successful (population sum) | 20,669 | 23,917 | 26,180 | 27,616 | 28,092 | PolyHighResolution + Rescued |

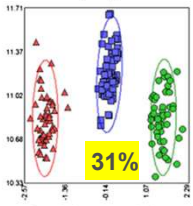
PolyHighRes



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

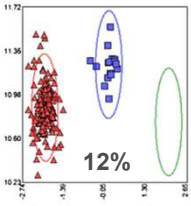
# SNP classifications – Default 97% call rate

PolyHighRes



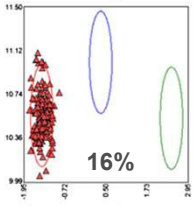

Good cluster resolution
Both homozygotes present

NoMinorHom




Good cluster resolution
A minor homozygote is absent

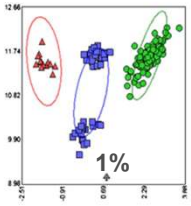
MonoHighRes




All samples are homozygous

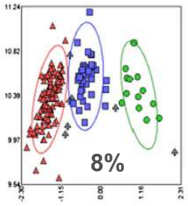
***Successful SNPs = 20,669***

OTV



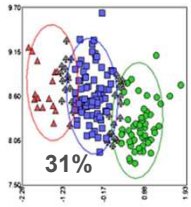

OTV = off-target variant

CallRateBelowThresh



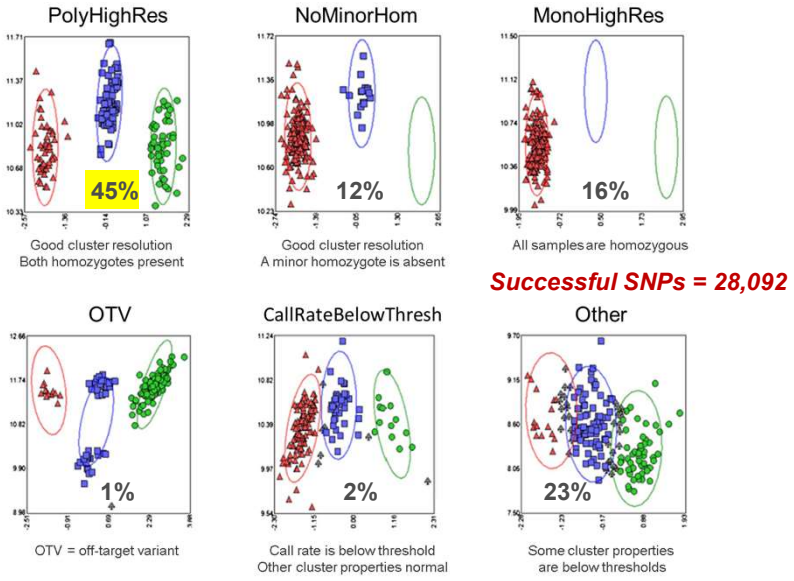

Call rate is below threshold
Other cluster properties normal

Other




Some cluster properties
are below thresholds

# SNP classifications – 60% call rate




***Successful SNPs = 28,092***

# Predictors of SNP success – Table 2

## Array Design Variables

Various criteria (variables) were used to select SNPs for the Axiom array (Final rank)

These variables were associated with SNP success

***Final rank***
**Q1 = 61.5% SNP success**
**Q3 = 46.3% SNP success**

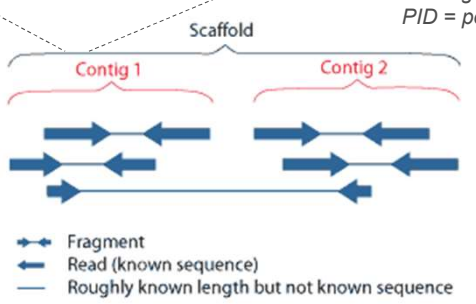
| Variable | No. of obs. | Category or mean | Percent or mean | | Number | |
|---|---|---|---|---|---|---|
| | | | Success | Fail | Success | Fail |
| ***Transcript ranking variables‡*** | | | | | | |
| No. of hits to scaffolds* (transcript mean) (v0.5) | 58350 | 1 | 58.5 | 41.5 | 18745 | 13286 |
| | | >1 | 41.5 | 58.5 | 9401 | 13244 |
| | | 0 | 27.5 | 72.5 | 1011 | 2663 |
| Transcript confidence score* (absent for HU SNPs) | 54625 | C1-C3 | 55.8 | 44.2 | 13986 | 11088 |
| | | C4-C7 | 49.6 | 50.4 | 14662 | 14889 |
| No. of SNPs per transcript# | 58350 | **Mean** | **12.00** | **10.36** | **29157** | **29193** |
| | | Q3 | 56.2 | 43.8 | 9201 | 7174 |
| | | Q1 | 43.5 | 56.5 | 7375 | 9570 |
| Combined transcript rank# | 58350 | **Mean** | **27252.2** | **31096.5** | **29157** | **29193** |
| | | Q1 | 52.5 | 47.5 | 7659 | 6930 |
| | | Q3 | 35.7 | 64.3 | 5212 | 9377 |
| ***Probeset-within-transcript ranking variables*** | | | | | | |
| Infinium success† | 6173 | Infinium | 74.5 | 25.5 | 4598 | 1575 |
| Probability of flanking SNPs* | 58350 | 1 | 50.8 | 49.2 | 27731 | 26845 |
| | | 2 | 37.8 | 62.2 | 1426 | 2348 |
| No. of perfect alleles* (percent identity = 100%)(v0.5) | 58350 | 1 | 53.5 | 46.5 | 23915 | 20800 |
| | | 2 | 25.5 | 74.5 | 200 | 583 |
| | | 0 | 39.2 | 60.8 | 5042 | 7810 |
| pConvert# | 57381 | **Mean** | **0.615** | **0.595** | **28506** | **28875** |
| | | Q3 | 57.7 | 42.3 | 8319 | 6087 |
| | | Q1 | 41.5 | 58.5 | 6429 | 9059 |
| Target SNP probability* (OSU probesets) | 53958 | $P < 0.0001$ | 55.0 | 45.0 | 24598 | 20140 |
| | | $P < 0.001$ | 39.7 | 60.3 | 3658 | 5562 |
| Target SNP probability* (HU probesets) | 3725 | 3 programs | 23.3 | 76.7 | 128 | 422 |
| | | 2 programs | 12.0 | 88.0 | 381 | 2794 |
| Final rank‡ | 58350 | **Mean** | **27891.8** | **30457.6** | **29157** | **29193** |
| | | Q1 | 61.5 | 38.5 | 8966 | 5622 |
| | | Q3 | 46.6 | 53.4 | 6798 | 7790 |
| ***Other variables§*** | | | | | | |
| Recommendation* | 57296 | Recommend | 54.7 | 45.3 | 17778 | 14749 |
| | | Neutral | 43.2 | 56.8 | 10690 | 14079 |

## BLAST – Compare SNPs to the reference genome

### Scaffolds are sections of the reference genome

GATGCAGTGGCATT - [A/C] – AGCGATGTAAGCGAT = **SNP sequence**

*Compare SNP sequence to DF reference genome (scaffolds)*
*PID = percent identity*




***If the SNP sequence matches multiple locations = not good!***

## BLAST variables

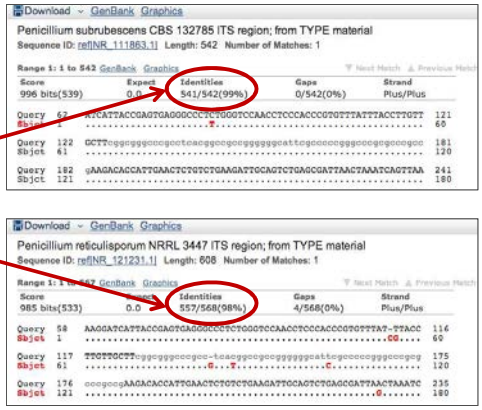
### Basic Local Alignment Search Tool

Searches for matches between the SNP sequence and the reference genome (scaffolds)

*Query = SNP sequence*
*Subject = reference genome (scaffold)*

***Example***
*Best hit = 99%*
*Second best hit = 98%*
*Difference = 2%*

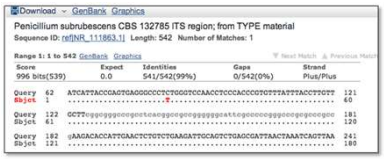
***Douglas-fir***
*Successful SNPs diff = 16%*
*Failed SNPs diff = 11%*
*(Table 2)*



# BLAST variables – Table 3

## Basic Local Alignment Search Tool

*Query = SNP sequence*
*Subject = reference genome (scaffold)*




***Scaffold PID***
***(best hit – second-best hit)***
Q3 = 61.9% SNP success
Q1 = 25.3% SNP success

| Variable | No. of obs. | Category or mean | Percent or mean | | Number | |
|---|---|---|---|---|---|---|
| | | | Success | Fail | Success | Fail |
| ***Percent identity (PID)**** | | | | | | |
| Scaffold PID (best hit) | 55766 | **Mean** | **99.0** | **98.4** | **28092** | **27674** |
| | | Q1 | 55.3 | 44.7 | 15491 | 12514 |
| | | Q3 | 51.2 | 48.8 | 12514 | 11945 |
| Scaffold PID (second-best hit) | 55766 | **Mean** | **83.0** | **87.4** | **28092** | **27674** |
| | | Q1 | 59.9 | 40.1 | 22775 | 15218 |
| | | Q3 | 27.1 | 72.9 | 3787 | 10190 |
| Scaffold PID (best-hit – second-best hit) | 55766 | **Mean** | **16.0** | **11.0** | **28092** | **27674** |
| | | Q3 | 61.9 | 38.1 | 9963 | 6144 |
| | | Q1 | 25.3 | 74.7 | 3486 | 10304 |
| ***Number of hits**** | | | | | | |
| Number of hits to scaffolds | 55766 | 1 | 60.9 | 39.1 | 22946 | 14753 |
| | | > 1 | 29.1 | 70.9 | 4978 | 12117 |
| | | 0 | 17.3 | 82.7 | 168 | 804 |
| Number of hits to singletons | 55766 | 1 | 11.8 | 88.2 | 79 | 589 |
| | | > 1 | 10.9 | 89.1 | 93 | 764 |
| | | 0 | 51.5 | 48.5 | 27920 | 26321 |
| Number of hits to gene models | 55766 | 1 | 55.8 | 44.2 | 10759 | 8523 |
| | | > 1 | 24.6 | 75.4 | 1126 | 3445 |
| | | 0 | 50.8 | 49.2 | 16207 | 15706 |
| Number of hits to transcripts | 55766 | 1 | 54.1 | 45.9 | 12389 | 10529 |
| | | > 1 | 47.9 | 52.1 | 3618 | 3943 |
| | | 0 | 47.8 | 52.2 | 12085 | 13202 |

# Predictors of SNP success – Table 4

**Table 4. Variables associated with genotyping success using an Axiom array.** Array design variables included variables calculated using v0.5 of the Douglas-fir reference genome. After genotyping, alternative variables were calculated using v1.0 of the reference genome. Variables were ranked using stepwise regression and the SAS LOGISTIC procedure ($p < 0.05$). DF is degrees of freedom. Step is the order in which the variables entered the model based on Chi-square test probabilities (Prob). Successful SNPs were those that had a call rate > 60% and were polymorphic.

| Variable | DF | Array design variables (ROC area = 0.6447)** | | | Final selected variables (ROC area = 0.6779)** | | |
|---|---|---|---|---|---|---|---|
| | | Step | Chi-square | Prob | Step | Chi-square | Prob |
| Scaffold PID (best-hit – second-best hit) (v1.0)# | 1 | – | – | – | 1 | 4689.82 | < 0.0001 |
| No. of hits to scaffolds (transcript mean) (v0.5)‡ | 2 | 1 | 1585.65 | < 0.0001 | – | – | – |
| Target SNP probability | 1 | 3 | 658.57 | < 0.0001 | 2 | 604.62 | < 0.0001 |
| pConvert | 1 | 2 | 739.16 | < 0.0001 | 3 | 296.89 | < 0.0001 |
| Number of perfect alleles (PID = 100%) (v0.5) | 2 | 4 | 332.17 | < 0.0001 | – | – | – |
| Number of SNPs per transcript† | 66 | 5 | 269.55 | < 0.0001 | – | – | – |
| Number of hits to singletons (v1.0) | 2 | – | – | – | 4 | 145.62 | < 0.0001 |
| Number of hits to gene models (v1.0) | 2 | – | – | – | 5 | 84.38 | < 0.0001 |
| Number of hits to scaffolds (v1.0) | 2 | – | – | – | 6 | 34.80 | < 0.0001 |
| Probability of flanking SNPs | 1 | 6 | 51.01 | < 0.0001 | 7 | 27.04 | < 0.0001 |
| Scaffold second-best hit PID (v1.0) | 1 | – | – | – | 8 | 19.90 | < 0.0001 |
| Transcript confidence score | 1 | 7 | 6.69 | 0.0097 | 9 | 11.44 | 0.0007 |
| No. of hits to reference transcripts (v1.0) | 2 | – | – | – | 10 | 13.50 | 0.0012 |

## Predictors of SNP success

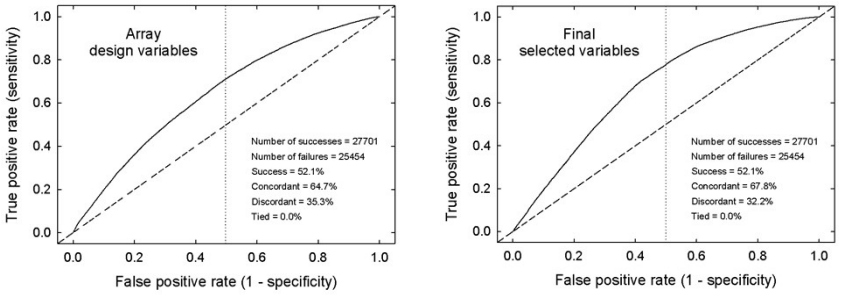
### Top three variables

- ***BLAST difference*** – Difference in percent identity between best hit and second-best hit
- ***Target SNP probability*** – SNPs detected using bioinformatics are highly likely to be real SNPs and not sequencing errors
- ***pConvert*** – An Affymetrix variable
  - *Probe thermodynamics*
  - *Number of 16-mer matches to the genome*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Predictors of SNP success

### Receiver operating characteristic (ROC) curves




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Population genetics – Appendix 1

**Appendix 1.** Population genetic statistics for successful SNPs genotyped in 395 Coastal Douglas-fir trees. Successful SNPs are those that were polymorphic and had a call rate (CR) that exceeded the indicated CR threshold after one or two phases of analysis with alternative quality control (QC) thresholds. $N_{indiv}$ shows the range in n umber of trees analyzed for each statstic, $N_{allele}$ is the number of alleles, CR is the measured call rate, MAF is minaor allele frequency, $HET_{obs}$ and $HET_{exp}$ are the observed and expected heterozygosities, DIV is the diversity statistic, and PIC is polymorphic information content as measured using SAS Proc Allele (SAS version 9.4).

| | Non-polymorphic | | | | Polymorphic/Non-HWE | | | | Polymorphic/HWE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pop. Statistic | Min | Mean | Median | Max | Min | Mean | Median | Max | Min | Mean | Median | Max |
| CR = 60% | No. of SNPs = 395 | | | | No. of SNPs = 3125 | | | | No. of SNPs = 24574 | | | |
| $N_{indiv}$ (trees) | 107.0 | 112.3 | 112.0 | 283.0 | 57.0 | 318.6 | 325.0 | 395.0 | 59.0 | 348.8 | 390.0 | 395.0 |
| $N_{allele}$ | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| CR(%) | 95.5 | 99.9 | 100.0 | 100.0 | 50.9 | 92.0 | 98.2 | 100.0 | 52.7 | 95.7 | 99.2 | 100.0 |
| MAF | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.188 | 0.152 | 0.500 | 0.002 | 0.236 | 0.220 | 0.500 |
| $HET_{obs}$ | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.223 | 0.189 | 0.878 | 0.004 | 0.319 | 0.338 | 0.635 |
| $HET_{exp}$ | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.305 | 0.258 | 0.500 | 0.004 | 0.360 | 0.343 | 0.500 |
| DIV | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.252 | 0.258 | 0.500 | 0.004 | 0.323 | 0.343 | 0.500 |
| PIC | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.203 | 0.225 | 0.375 | 0.004 | 0.261 | 0.284 | 0.375 |
| CR = 97% | No. of SNPs = 394 | | | | No. of SNPs = 1771 | | | | No. of SNPs = 18497 | | | |
| $N_{indiv}$ (trees) | 107.0 | 112.3 | 112.0 | 283.0 | 103.0 | 330.1 | 283.0 | 395.0 | 102.0 | 350.9 | 393.0 | 395.0 |
| $N_{allele}$ | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| CR(%) | 95.5 | 99.9 | 100.0 | 100.0 | 92.0 | 99.4 | 99.7 | 100.0 | 91.1 | 99.3 | 99.7 | 100.0 |
| MAF | 0.000 | 0.000 | 0.000 | 0.000 | 0.003 | 0.092 | 0.033 | 0.500 | 0.002 | 0.215 | 0.189 | 0.500 |
| $HET_{obs}$ | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.117 | 0.046 | 0.862 | 0.004 | 0.298 | 0.304 | 0.615 |
| $HET_{exp}$ | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.166 | 0.064 | 0.500 | 0.004 | 0.338 | 0.306 | 0.500 |
| DIV | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.139 | 0.064 | 0.500 | 0.004 | 0.301 | 0.306 | 0.500 |
| PIC | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.118 | 0.062 | 0.375 | 0.004 | 0.245 | 0.259 | 0.375 |

| Stat | CR 60% | CR 97% |
|---|---|---|
| No. SNPs | 27,699 | 20,268 |
| MAF | 0.236 | 0.215 |
| $Het_{obs}$ | 0.319 | 0.298 |

*SNPs are highly variable*

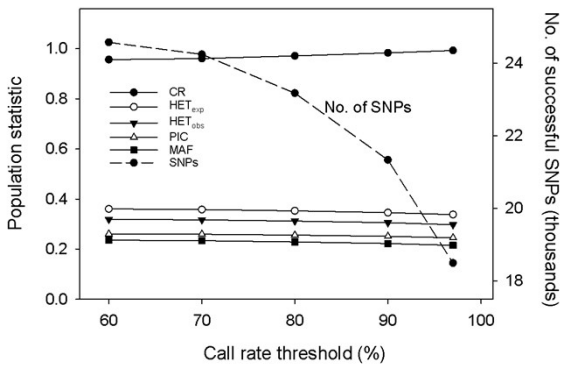
*Good for genomic selection*



# Call rate threshold (CR)

Lower CR threshold results in more SNPs

CR threshold has little effect on population genetic stats



## Axiom SNP characteristics (CR = 60%)

***Unrelated Coastal Douglas-fir only***

***55,766 SNPs attempted***

***27,699 SNPs polymorphic and 'called'***

***24,574 SNPs = polymorphic, 'called', HWE***

| Statistic | Mean | Median | Min | Max |
|---|---|---|---|---|
| Call rate (%) | 95.7 | 99.2 | 52.7 | 1.000 |
| Polymorphic information content | 0.261 | 0.284 | 0.004 | 0.375 |
| Heterozygosity | 0.319 | 0.338 | 0.004 | 0.635 |
| Minor allele frequency | 0.236 | 0.220 | 0.002 | 0.500 |

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Next steps - Third-generation SNP chip

### Lower the costs of SNP genotyping

- Only include successful SNPs on the array (28K instead of 50K)
- Only include enough SNPs needed for genomic selection (5K?)
- Use lower-cost, low-density SNP arrays
  - *Sequenom*
  - *Smaller Axiom arrays*
- Combine low-density and high-density arrays for genomic selection
- Genomic selection workplan

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## PNWTIRC report

**A high-density Affymetrix Axiom genotyping array for genomic selection in Douglas-fir**

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

*October 19, 2017*

**CONFIDENTIAL - DO NOT DISTRIBUTE**

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Drought Hardiness Study – Next steps

By Scott Kolpak, Erda Çeler, Jennifer Kling, Mike Crawford, and Glenn Howe

Scott Kolpak presented a summary of the Douglas-fir Drought Hardiness Study (DHS) that was initiated by the BLM in 2008-2009 and planted in 2015 at three hot and dry sites in Southern Oregon. Erda Çeler, a graduate student from Turkey, conducted first-year measurements and analyses on two of the sites for her M.S. thesis. Details on Erda's measurements and research findings can be found in her thesis (http://pnwtirc.forestry.oregonstate.edu/douglas-fir-seedlings-pacific-northwest-genetics-drought-adaptation).

Scott Kolpak and Mike Crawford visited all three sites in September 2017 to assess current management needs and evaluate potential research activities. A few key needs include (1) measuring the presence and causes of mortality at the Sprague site, (2) measuring base-line growth, mortality, and herbicide damage at Mill Pond (similar to measurements taken at Sprague and Lost Creek), and (3) removing competing brush in some replications at Lost Creek. We also presented a range of potential research activities and collaborations. Finally, Glenn led a discussion to consider PNWTIRC's role in the DHS in the future.

# Genetics of Drought Hardiness in Douglas-fir: Update and future PNWTIRC role

**Scott Kolpak, Erda Çeler, Jennifer Kling, Mike Crawford, Glenn Howe**

*Department of Forest Ecosystems and Society*
*Oregon State University*

In collaboration with the Bureau of Land Management, NWTIC, Weyerhaeuser, Silver Butte Timber Company, and Washington Department of Natural Resources

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## What is the future role of drought hardiness research by the PNWTIRC?

### Draft Five-Year Plan – Core Research


"We will evaluate whether to continue working with this research project after Erda completes her M.S. thesis, and after the trees have completed their third growing season."

Erda defended her M.S. thesis on May 25, 2017

"Douglas-fir Seedlings in the Pacific Northwest:
The Genetics of Drought Adaptation"

## Erda Çeler's thesis questions

- Are Douglas-fir drought adaptation traits heritable?
- Are drought adaptation traits associated with the climatic origin of the Douglas-fir families?
- Is there an association between drought adaptation traits and seedling characteristics at the time of planting?
- Is early bud flush associated with other drought adaptation traits?

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Drought hardiness study - background

- Initiated by Jeannette Griese at the BLM in 2008 – 2009
  - *How will BLM's genetic resources respond to drought stress?*
- Has been managed as a collaboration among:
  - *Northwest Tree Improvement Cooperative (Keith Jayawickrama)*
  - *Bureau of Land Management (Mike Crawford, Jeannette Griese and George McFadden)*
  - *Washington Department Natural Resources (Jeffrey DeBell)*
  - *Plum Creek Timber Company (Jim Smith)*
  - *Weyerhaeuser (Brian Baltunis, James Benson)*
  - *Silver Butte Timber Company (Darin McMichael, Whitney Schimke)*

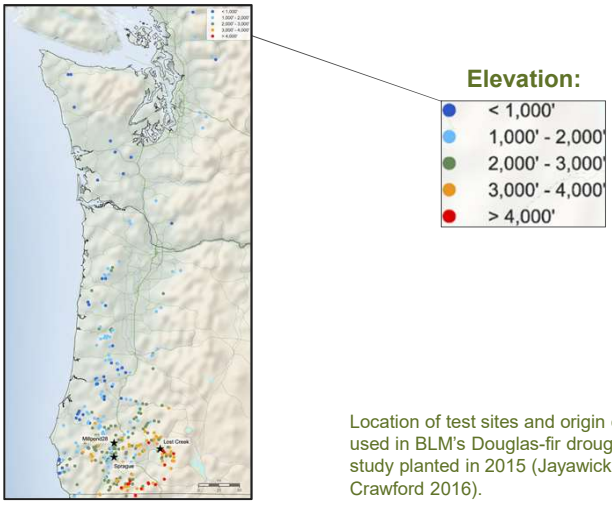
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Drought hardiness study - germplasm

- 429 families from elite parents in western Oregon and Washington planted at three sites: Lost Creek, Sprague, and Millpond
  - Seedlots
    - *Most are OP seed from first-generation parents in orchards*
    - *Some are half-sib families created by pooling full-sib families*
    - *Two are woods-run seedlots from southern Oregon*
- Seedlings were grown in the BLM Sprague greenhouse

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Drought hardiness study – germplasm

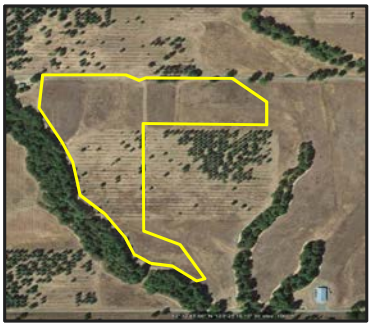



Location of test sites and origin of parents used in BLM's Douglas-fir drought hardiness study planted in 2015 (Jayawickrama and Crawford 2016).

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Drought hardiness study – planting sites

### Sprague site – BLM

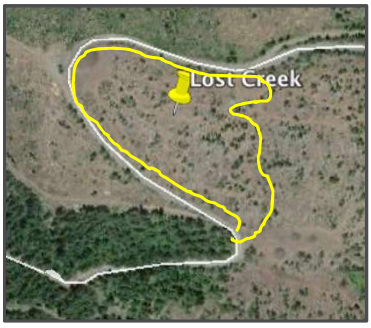


- 6722 Douglas-fir seedlings
- 384 families
- RBD with 23 blocks
- Single-tree plots

**2015**

## Drought hardiness study – planting sites

### Lost Creek site - Weyerhaeuser




- 3591 Douglas-fir seedlings
- 304 families
- RBD with 17 blocks
- Single-tree plots

**2015**

## Drought hardiness study – planting sites

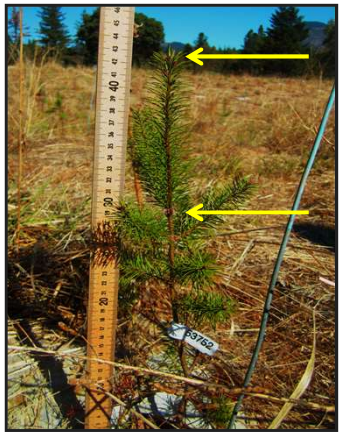
### Mill Pond site – Silver Butte


- 8381 Douglas-fir seedlings
- 389 families
- RBD with 22 blocks
- Single-tree plots

**2015**

## Drought hardiness study – traits measured

- **Height**
  - ***Ht14*** *is total growth in the greenhouse at year 2014*
  - ***Ht15*** *is height in the field at year 2015*
  - ***Htinc*** *growth in the field (2014-2015)*
- **Second flushing (Sflush)**
  - *Presence/absence*



**Height measurements**

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Drought hardiness study – traits measured

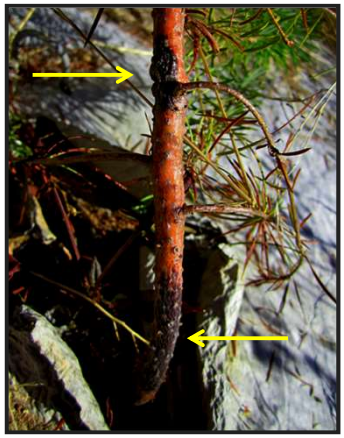
- **Bud flush (Flush)**
  *Five categories to classify timing of bud flush*
  1 = the bud was closed, tight, and dark
  2 = the bud was closed, swollen, light colored
  3 = the bud was just beginning to burst through tip
  4 = the bud was open, needles around 1 cm long
  5 = the bud was fully open, needles fully elongated
- **Foliage damage (FD)**
  – *Percentage of dead foliage*

**Buds were fully open**

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Drought hardiness study – traits measured

- **Stem damage (SD)**
  – *Percentage of stem damaged by sunscald*
- **Leader damage (LD)**
  – *Presence/absence*
- **Mortality (Mort)**
  – *Dead/alive*



**Stem with sun-scald**

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Climate data

- ClimateNA software
  - *ClimateNA can predict climate variables for specific locations based on latitude, longitude, and elevation based on historical averages*
  - *Historical climate data can be used to understand adaptation of parents to their native climates*
- Weather station data
  - *Sites have weather stations*
  - *Batteries are replaced every 9-months, typically weather downloaded every 6-months*

**Weather station**

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

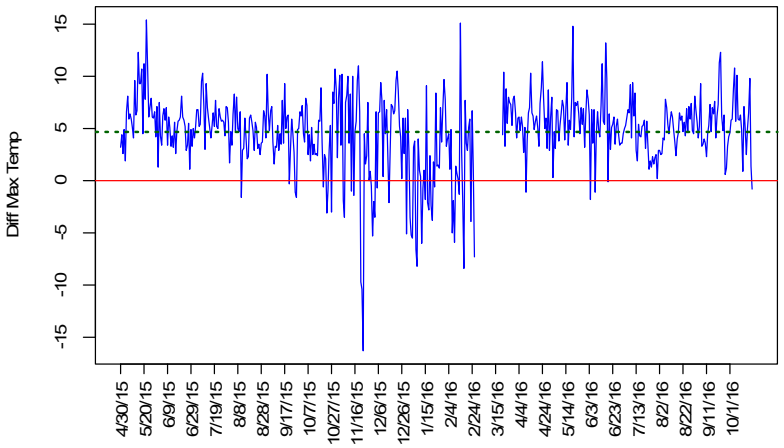
## Results and discussion - Çeler's thesis

- Herbicide damage at the Mill Pond site – these trees were not included in Erda's thesis
- The following short-term DHS results only include the Lost Creek and Sprague sites

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Weather station data

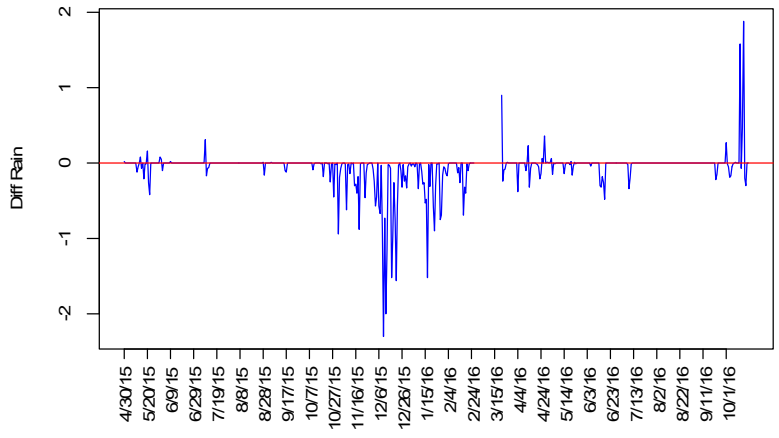
### Sprague had greater maximum temperatures




Differences in maximum temperatures at Sprague and Lost Creek, April 2015 – Dec 2016

## Weather station data

### Sprague had less rain than Lost Creek




Differences in rainfall at Sprague and Lost Creek, April 2015 – Dec 2016

## Heritability

- Generally low heritabilities – first growing season in the field
- Heritabilities differed widely among traits
- High heritability for bud flush and height

$$h_i^2 = \sigma_A^2 / \sigma_P^2$$

| | Ht14 | Ht15 | Htinc | Flush | SFlush | FD_bin | SD_bin | LD_bin | Mort |
|---|---|---|---|---|---|---|---|---|---|
| **Sprague Heritabilities $h^2$** | | | | | | | | | |
| Individual heritabilities | 0.96 | 0.91 | 0.13 | 0.62 | 0.05 | 0.05 | 0.02 | 0.09 | 0.07 |
| Family heritabilities | 0.85 | 0.84 | 0.34 | 0.75 | 0.16 | 0.16 | 0.06 | 0.26 | 0.21 |
| **Lost Creek Heritabilities $h^2$** | | | | | | | | | |
| Individual heritabilities | 0.93 | 0.99 | 0.20 | 0.83 | 0.13 | 0.08 | 0.02 | 0.06 | 0.12 |
| Family heritabilities | 0.83 | 0.85 | 0.45 | 0.81 | 0.34 | 0.23 | 0.06 | 0.19 | 0.31 |
| **Sprague and Lost Creek Heritabilities $h^2$** | | | | | | | | | |
| Individual heritabilities | **0.72** | **0.72** | 0.08 | **0.64** | 0.02 | 0.00 | 0.02 | 0.05 | 0.03 |
| Family heritabilities | **0.78** | **0.79** | 0.34 | **0.82** | 0.09 | 0.00 | 0.16 | 0.26 | 0.15 |

## Genetic correlations

- Large height differences in the greenhouse persisted in the field
- Low correlation between growth in the greenhouse and drought adaptation traits
- Low correlation between field growth and other drought adaptation traits

| | Ht14 | Ht15 | Htinc | Flush | SFlush | FD_bin | SD_bin | LD_bin | Mort |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Sprague | | | |
| **Ht14** | | **0.97** | 0.06 | -0.13 | -0.18 | -0.13 | 0.11 | -0.25 | 0.11 |
| **Ht15** | **0.97** | | 0.28 | -0.20 | -0.18 | -0.17 | 0.11 | -0.3 | 0.09 |
| **Htinc** | 0.23 | 0.45 | | -0.31 | -0.05 | -0.19 | -0.01 | -0.27 | -0.10 |
| **Flush** | -0.20 | -0.17 | 0.04 | | 0.29 | -0.04 | 0.00 | 0.45 | -0.18 |
| **SFlush** | 0.34 | 0.41 | 0.42 | 0.05 | | -0.08 | -0.05 | 0.29 | -0.20 |
| **FD_bin** | 0.06 | -0.07 | -0.48 | -0.04 | -0.23 | | 0.01 | 0.09 | 0.62 |
| **SD_bin** | 0.19 | 0.20 | 0.11 | -0.16 | -0.01 | 0.06 | | -0.03 | 0.01 |
| **LD_bin** | 0.00 | 0.00 | 0.00 | 0.28 | 0.06 | 0.02 | -0.05 | | -0.14 |
| **Mort** | -0.07 | -0.21 | -0.57 | -0.02 | -0.29 | 0.81 | 0.01 | -0.07 | |
| | | | Lost Creek | | | | | | |

## Genetic correlations

- Taller trees at the time of planting did not have higher mortality
- Families with more foliage damage had greater mortality

| | Ht14 | Ht15 | Htinc | Flush | SFlush | FD_bin | SD_bin | LD_bin | Mort |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Sprague | | | |
| Ht14 | | 0.97 | 0.06 | -0.13 | -0.18 | -0.13 | 0.11 | -0.25 | **0.11** |
| Ht15 | 0.97 | | 0.28 | -0.20 | -0.18 | -0.17 | 0.11 | -0.3 | 0.09 |
| Htinc | 0.23 | 0.45 | | -0.31 | -0.05 | -0.19 | -0.01 | -0.27 | -0.10 |
| Flush | -0.20 | -0.17 | 0.04 | | 0.29 | -0.04 | 0.00 | 0.45 | -0.18 |
| SFlush | 0.34 | 0.41 | 0.42 | 0.05 | | -0.08 | -0.05 | 0.29 | -0.20 |
| FD_bin | 0.06 | -0.07 | -0.48 | -0.04 | -0.23 | | 0.01 | 0.09 | **0.62** |
| SD_bin | 0.19 | 0.20 | 0.11 | -0.16 | -0.01 | 0.06 | | -0.03 | 0.01 |
| LD_bin | 0.00 | 0.00 | 0.00 | 0.28 | 0.06 | 0.02 | -0.05 | | -0.14 |
| Mort | **-0.07** | -0.21 | -0.57 | -0.02 | -0.29 | **0.81** | 0.01 | -0.07 | |
| | | | Lost Creek | | | | | | |

## Correlations between breeding values and climate

- Greenhouse growth was positively associated with temperature
- Field growth was not associated with temperature

| | Ht14 | Htinc |
|---|---|---|
| Mean annual temperature (MAT) | **0.45** | **0.08** |
| Mean annual precipitation (MAP) | **0.02** | **0.03** |
| Mean summer precipitation (MSP) | **0.05** | **0.11** |
| Number frost free days (NFFD) | **0.38** | **-0.02** |
| Frost free period (FFP) | **0.26** | **-0.07** |
| Precipitation as snow (PAS) | **-0.36** | **-0.05** |
| Extreme minimum temperature (EMT) | **0.26** | **-0.05** |
| Extreme maximum temperature (EXT) | **0.43** | **0.18** |

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Correlations between breeding values and climate

- Early flushing was associated with warmer (NFFD) and drier (MSP) climates
- SFlush was associated with warmer climates

| | Flush | SFlush |
|---|---|---|
| Mean annual temperature (MAT) | 0.19 | **0.26** |
| Mean annual precipitation (MAP) | -0.12 | 0.08 |
| Mean summer precipitation (MSP) | **-0.23** | 0.05 |
| Number frost free days (NFFD) | **0.23** | **0.23** |
| Frost free period (FFP) | 0.18 | 0.15 |
| Precipitation as snow (PAS) | -0.20 | -0.20 |
| Extreme minimum temperature (EMT) | 0.30 | 0.19 |
| Extreme maximum temperature (EXT) | 0.09 | **0.26** |

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Experimental conclusions (Çeler's thesis)

- Drought adaptation of Douglas-fir seedlings is partly determined by genetics
- Natural selection for drought hardiness has been stronger in areas that are warmer and drier
  - *Drought adaptation traits and climate variables are correlated*
- The Sprague site should be particularly good for screening drought adaptation traits
  - *Sprague was hotter and drier than Lost Creek, and this adversely affected growth, damage, and survival*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Experimental conclusions (Çeler's thesis)

- Early bud flush is a genetically controlled drought avoidance strategy
  - *Early flushing was associated with warmer (NFFD) and drier (MSP) climates*
  - *Genotypes (families) from areas with warmer and drier climates were more likely to flush early*
- There is no relationship between height at the time of planting (Ht14) and mortality either at Sprague or at Lost Creek
  - *Low correlation between Ht14 and Mort*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Research implications (Çeler's thesis)

- There were large differences in height among families in the greenhouse (heritabilities in the greenhouse were unusually high)
- Variation in height after one year in the field largely reflected differences in growth that occurred in the greenhouse
  - *Height increment is more relevant for understanding the genetics of field growth and drought hardiness*
- Initial measurements can be used as covariates in later analyses
  - *Either Ht14 or Ht15 can be used as an "initial height" with later height measurements to understand drought adaptation in the field*
- Bud flush ('*Flush')* was highly heritable
  - *Flush would be amenable to genetic improvement*
  - *Flush would be a good trait to use for practicing assisted migration*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Site visit update

## Sprague site - BLM

- Tree health is good and mortality is low
- Trees are likely to grow well next growing season

Site visit update

## Sprague site - BLM

- Drought related leader damage measured for Celer's thesis has affected seedling form
- New drought related leader damage is minor

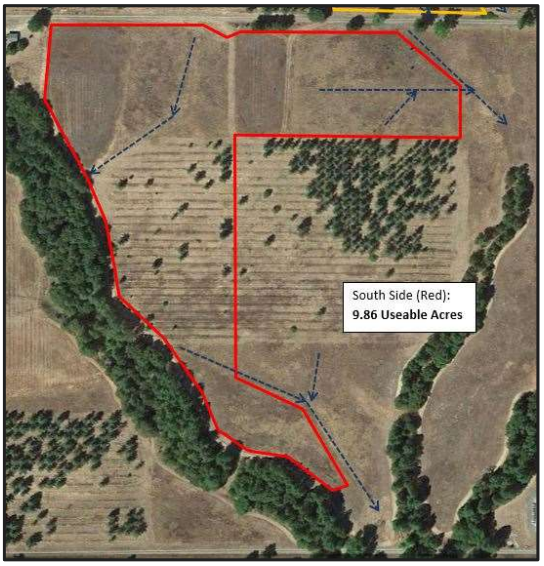
Site visit update

## Sprague site - BLM

- Mortality has continued in some areas
  - *Likely related to particularly wet areas*
  - *Collect new mortality data to capture ALL mortality*

Site visit update

## Sprague site - BLM




- Mapped intermittent streams prior to planting

Site visit update

## Sprague site - BLM

- Mortality has continued in some areas
  - *Likely related to particularly wet areas*
  - *Collect new mortality data to capture ALL mortality*

Site visit update

## Sprague site - BLM

- Mike Crawford 'mapped' the wet areas this winter
  - *Add these data to Erda's files to use in later analyses*

Site visit update

## Lost Creek site - Weyerhaeuser

- Tree health is good and mortality is low
- Leader growth was high last growing season

Site visit update

## Lost Creek site - Weyerhaeuser

- Tree health is good and mortality is low
- Leader growth was high last growing season

Site visit update

## Lost Creek site - Weyerhaeuser

- Brush removal in some areas of the site would be helpful

Site visit update

## Lost Creek site - Weyerhaeuser

- Brush removal in some areas of the site would be helpful

Site visit update

## Mill Pond site – Silver Butte

- Tree health is good and mortality is low
- Trees are likely to grow well next growing season

Site visit update

## Mill Pond site – Silver Butte

Ht14
Ht15
Htinc
Flush
Sflush
FD_bin
SD_bin
LD_bin
Mort

**Herbicide damage 2015**

- Measure similar traits as for Sprague and Lost Creek
  - *Establish base-line measurements for all sites*

Site visit update

## Mill Pond site – Silver Butte

Herbicide damage 2015

- Most trees have recovered from herbicide damage
  - *But, it can still be measured*

Site visit update

## Mill Pond site – Silver Butte

Herbicide damage 2015

- Most trees have recovered from herbicide damage
  - *But, it can still be measured*

## Drought hardiness study (DHS)

### Potential future directions

- Sprague
  - *Record updated mortality, and characterize causes of mortality*
  - *Add wet microsite areas Mike Crawford collected to existing data files*
  - *Use spatial analysis for mortality data*
- Mill Pond
  - *Record base-line growth, mortality, and herbicide damage*
- Archive and distribute existing base-line data to DHS collaborators
- Update the new weather station data and examine indicators of drought at the southern Oregon sites
  - *2015 – 2016 vs 2017*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Drought hardiness study (DHS)

### Potential future directions

- Collaborate with VMRC (Carlos Gonzalez) to take drought stress physiology measurements
- Take DHS sites measurements on a regular schedule (e.g., 5-year, 10-year, 15-year)
  - *On older trees ( > 10-years), other measurements are possible (e.g., growth ring analysis from increment cores)*
- Beyond 15 years, the cumulative drought hardiness measurements (e.g., growth, mortality) will also be important

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

Oregon State University

Vegetation Management Research Cooperative

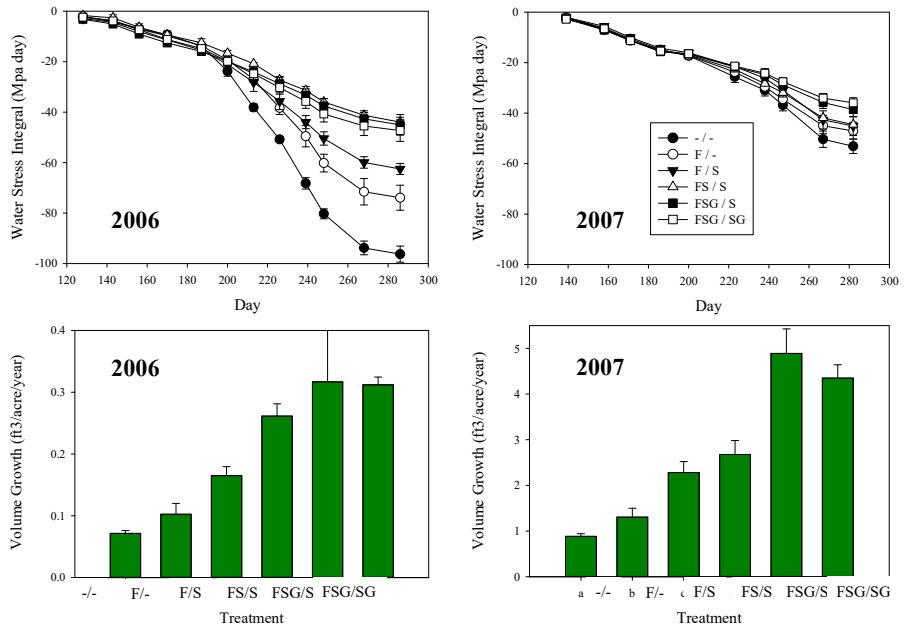
# Vegetation Management Effects on

## Soil/Plant Water Relations, Survival and Growth

Carlos A. Gonzalez-Benecke, Assistant Professor

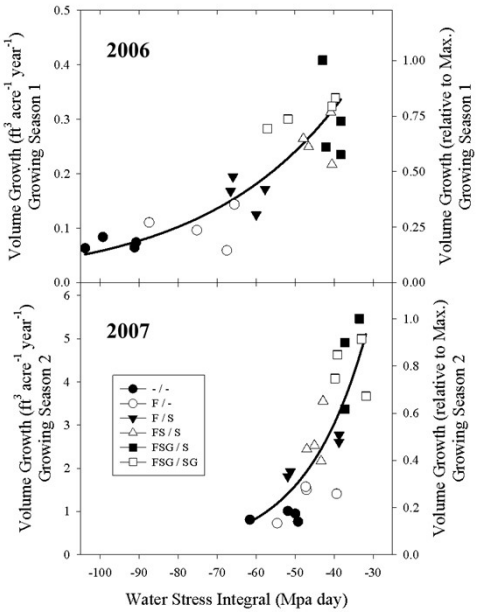
Department of Forest Engineering, Resources & Management
Oregon State University

## Water Stress Integral



# Water Stress Integral

Correlation between WSI and Douglas-fir productivity (volume growth)




## Acknowledgments


- DHS Collaborators
  - *Keith Jayawickrama (NWTIC)*
  - *Mike Crawford, Jeannette Griese, and George McFadden (BLM)*
  - *Jim Smith (Plum Creek)*
  - *Brian Baltunis and James Benson (Weyerhaeuser)*
  - *Darin McMichael and Whitney Schimke (Silver Butte)*
  - *Jeffrey DeBell (Washington DNR)*
- Field measurements: Scott Kolpak, Kori Ault, Anna Magnuson, Oguz Urhan, Lauren Yap, Jennifer Kling, Glenn Howe


## DHS – future directions

### Discussion

- Are PNWTIRC members interested in continuing working on the DHS?
- What sources of funding should be used for new work?
- Who would be responsible?
  - PNWTIRC, BLM, NWTIC, in-kind from PNWTIRC members?
  - Overall project
  - Site maintenance?
  - Weather station data?
  - Measurements?
  - Data analyses?
  - Publications?

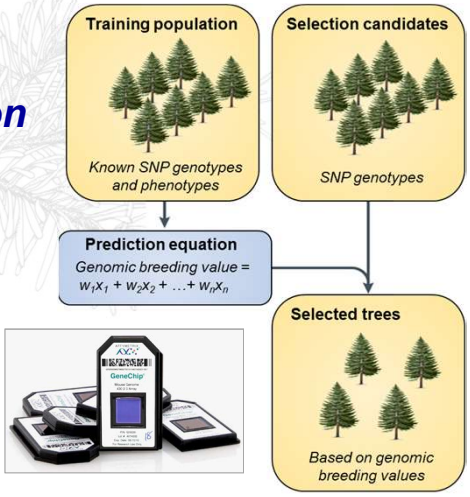
## Genomic selection work plan

By Glenn Howe, Jennifer Kling, Keith Jayawickrama, Terrance Ye, and Scott Kolpak

Glenn Howe and Jennifer Kling presented our proposed *Genomic Selection Work Plan*. This document is intended to guide PNWTIRC research into the use of genomic selection in Douglas-fir breeding programs. Genomic selection, or whole-genome marker-assisted selection, could revolutionize tree breeding by allowing breeders to dramatically reduce the breeding cycle and extent of progeny testing. The potential of genomic selection has been demonstrated in key forest tree species, and by our preliminary results in Douglas-fir. However, genotyping costs are high, probably much higher than testing trees in standard progeny tests. The purpose of this research is to directly address this cost issue. We will conduct research specifically designed to reduce genotyping costs and make genomic selection financially attractive. Our specific objectives are to (1) develop a high-density SNP linkage map for Douglas-fir, (2) compare baseline phenotypic and genomic selection scenarios based on genetic gain per unit time and cost, (3) test whether we can use a combination of high-density and low-density arrays to substantially reduce genotyping costs, (4) test whether we can use early phenotypic culling to substantially reduce genotyping costs, (5) develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way, (6) hold workshops on how to practice genomic selection in Douglas-fir, and (7) obtain new breeding values from the Roseburg genomic selection field test.

## Genomic Selection Workplan

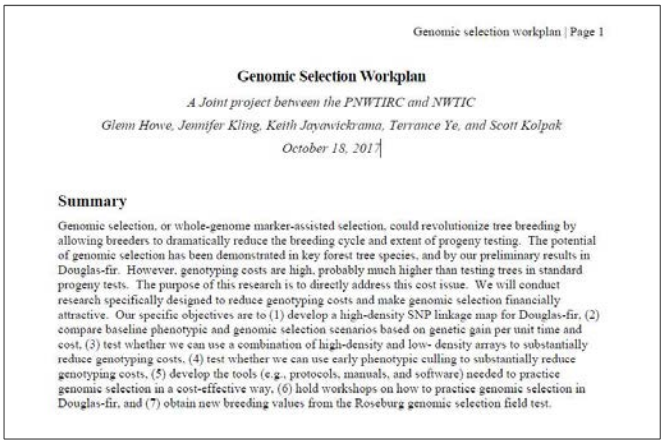
*Glenn Howe*
*Jennifer Kling*
*Keith Jayawickrama*
*Terrance Ye*
*Scott Kolpak*






PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Genomic selection workplan



Genomic selection workplan | Page 1

**Genomic Selection Workplan**

*A Joint project between the PNWTIRC and NWTIC*

*Glenn Howe, Jennifer Kling, Keith Jayawickrama, Terrance Ye, and Scott Kolpak*

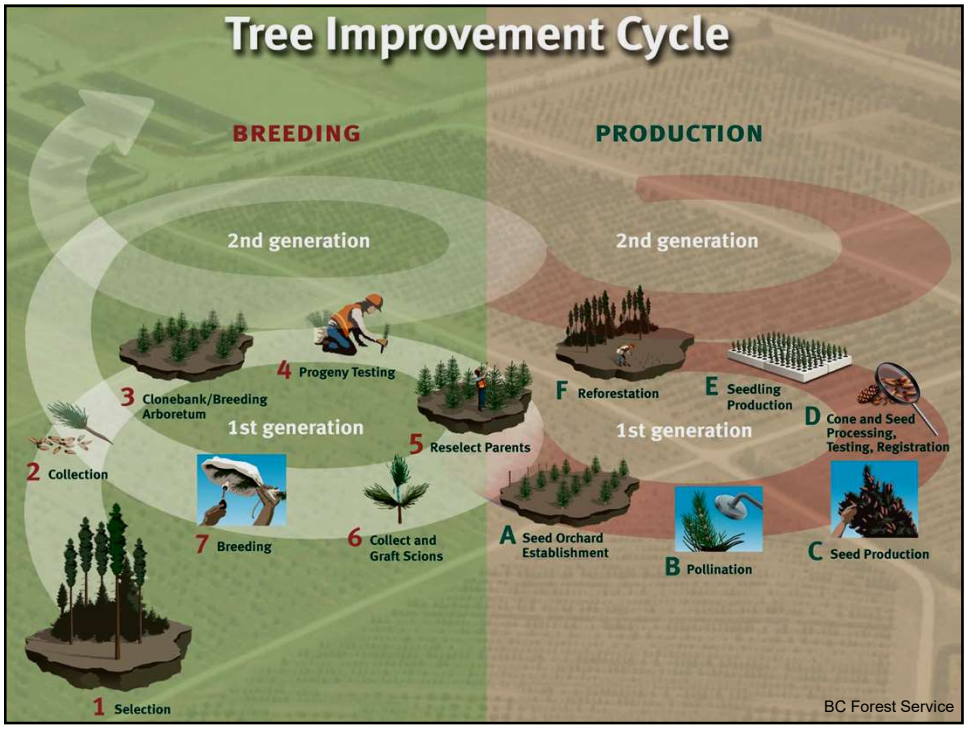
*October 18, 2017*

**Summary**

Genomic selection, or whole-genome marker-assisted selection, could revolutionize tree breeding by allowing breeders to dramatically reduce the breeding cycle and extent of progeny testing. The potential of genomic selection has been demonstrated in key forest tree species, and by our preliminary results in Douglas-fir. However, genotyping costs are high, probably much higher than testing trees in standard progeny tests. The purpose of this research is to directly address this cost issue. We will conduct research specifically designed to reduce genotyping costs and make genomic selection financially attractive. Our specific objectives are to (1) develop a high-density SNP linkage map for Douglas-fir, (2) compare baseline phenotypic and genomic selection scenarios based on genetic gain per unit time and cost, (3) test whether we can use a combination of high-density and low- density arrays to substantially reduce genotyping costs, (4) test whether we can use early phenotypic culling to substantially reduce genotyping costs, (5) develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way, (6) hold workshops on how to practice genomic selection in Douglas-fir, and (7) obtain new breeding values from the Roseburg genomic selection field test.

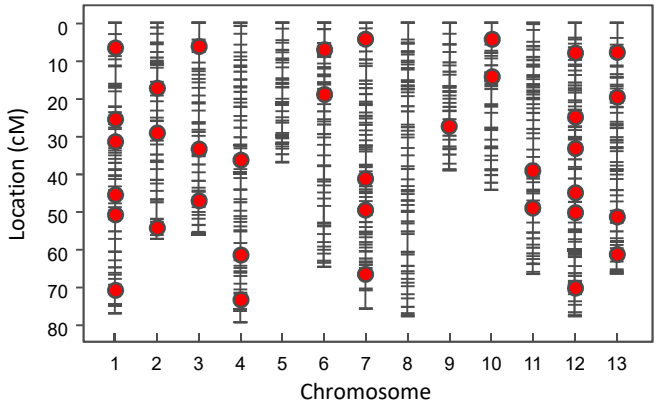
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Genomic Selection



## Genomic selection

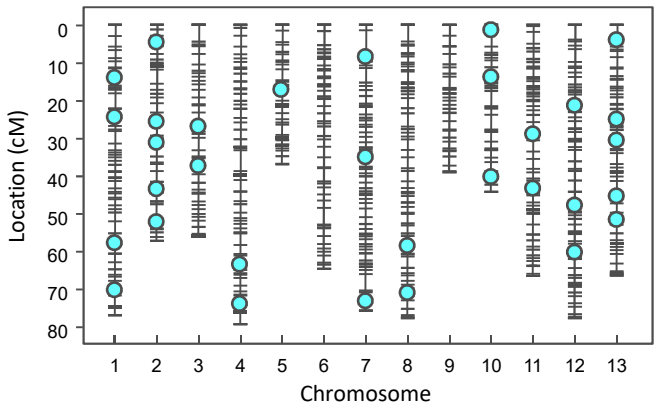
Relies on markers linked to quantitative trait loci




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Genomic selection

Relies on markers linked to quantitative trait loci

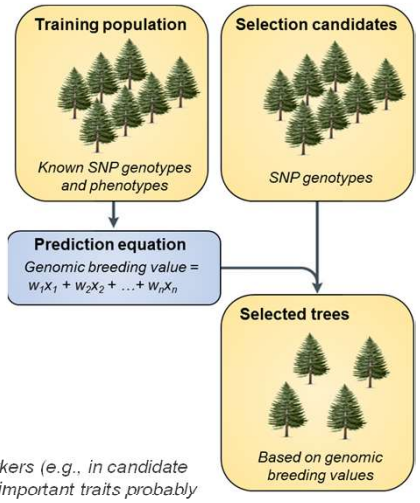



PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Genomic selection

### *More promising than association genetics*[1]

- Objective is to predict breeding values using a genome-wide set of markers (e.g., tens of thousands of SNPs)
- With enough markers, at least one marker will be linked to each important gene
- No need to identify which specific genes or markers are important
- Highly effective in livestock breeding




[1]*The objective of association genetics is to find key markers (e.g., in candidate genes) that are associated with the trait of interest. But important traits probably controlled by tens to hundreds of genes with small effects.*

## Genomic selection

***Unlike candidate gene approaches, genomic selection markers will work for any measured trait***

***Growth***
- *Height, diameter, volume growth*

***Adaptability***
- *Cold hardiness*
- *Spring bud flush*

***Stem form***
- *Ramicorn branches and forks*
- *Sinuosity*

***Wood stiffness***

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

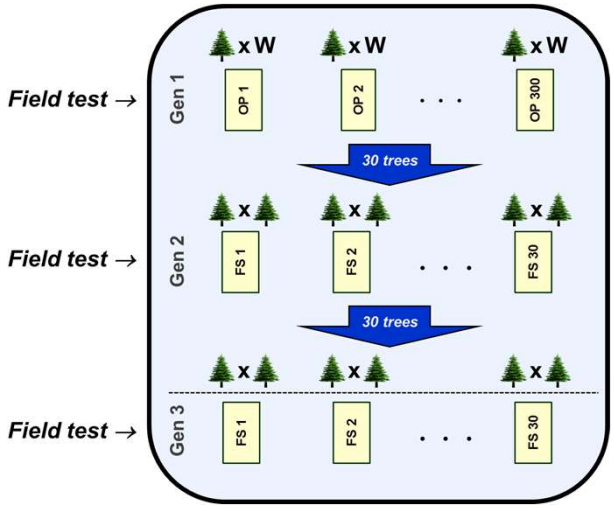
## Genomic selection

*Field tests are costly*

*Testing and selection take a long time*

*Low heritability traits*

*Mature traits*




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

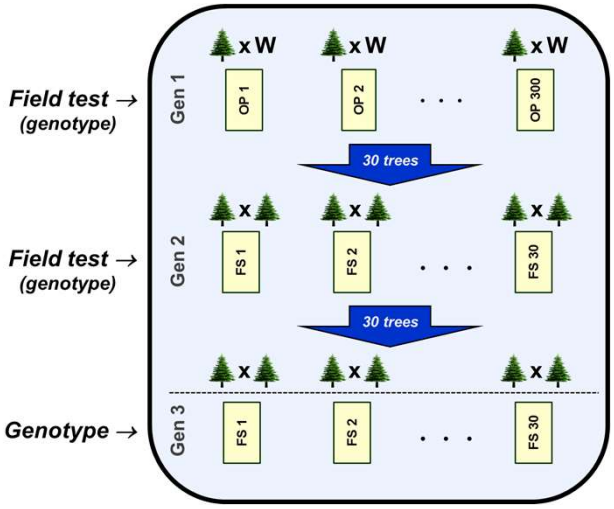
## Genomic selection

*Skip field testing*

*Make selections early*

*Increase heritabilities*

*Select for difficult to measure traits*




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Genomic selection
## Valuable for within-family selection

***Parent 1*** **x** ***Parent 2***

***offspring 1***
***offspring 2***
***offspring 3***
***etc***

- All offspring have the same expected phenotype (= parental average)
- Field testing is used to find which offspring are superior
- Genomic selection could be used instead

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Potential advantages of genomic selection

- Skip entire cycles of field testing
- Reduce the size of field tests by using genomic selection for early culling
- Shorten the generation interval
- Increase heritabilities
- Select for difficult to measure traits (e.g., mature traits at an early age)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Preliminary Results

## Performance of genomic selection

***Predictive ability is the correlation between breeding values estimated from phenotypes versus SNPs***

**Table 4. Performance of genomic selection in Douglas-fir.** Predictive ability (PA) was calculated using rrBLUP and 22,458 SNP markers. PA is the correlation between breeding values estimated from phenotypic measurements versus SNP markers using 10-fold cross-validation.

| Trait (age 12) | Predictive ability (PA) |
|---|---|
| Height | 0.698 |
| DBH | 0.655 |
| Volume | 0.612 |
| Ramicorn branching | 0.874 |
| Forking | 0.887 |
| Sinuosity | 0.852 |
| Specific gravity | 0.632 |

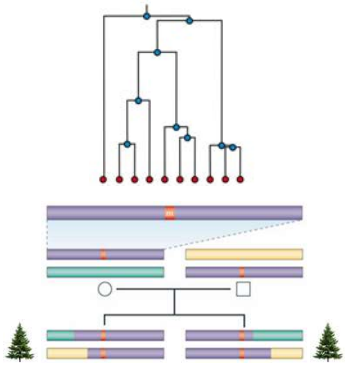
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## How many SNPs are needed?

Fewer SNPs are needed if the breeding population is small

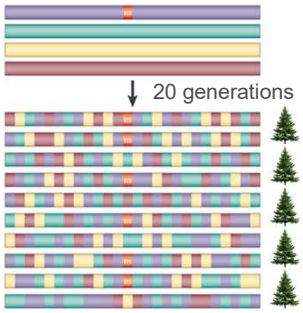
**Recent common ancestors**

- *Small $N_e$*
- *Large linkage blocks*
- *Fewer SNPs needed*



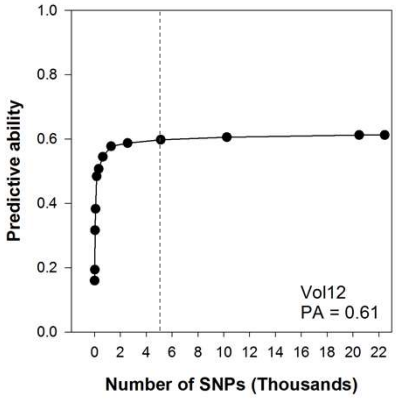
**Distant common ancestors**

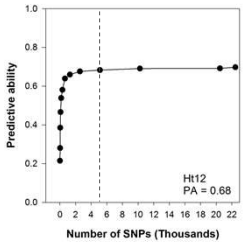
- *Large $N_e$*
- *Small linkage blocks*
- *Many SNPs needed*

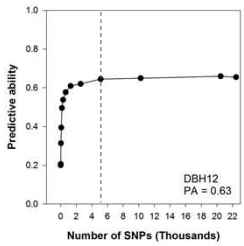



*Cardon and Bell (2001) Nature Reviews Genetics 2:91-99*

## How many SNPs are needed?

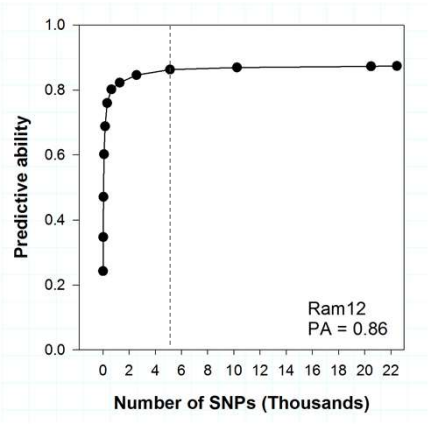


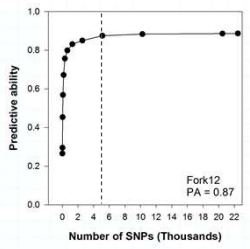



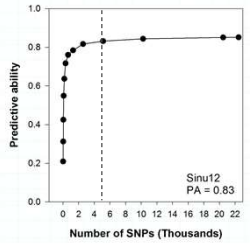




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## How many SNPs are needed?




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Axiom genotyping arrays

### Different formats – different costs

| Name | Number of SNPs | Minimum order size | Samples/run |
|---|---|---|---|
| 384HT Custom Array | 1.5-50K | 1920 | 384 |
| TG Array | 1-90K | 480 | 24 or 96 |
| TG Array | 90-200K | 480 | 24 or 96 |
| GW Array Plate | 200-675K | 480 | 24 or 96 |
| GW Array Plate | 675-1300K | 480 | 96 |
| GW Array Plate | 1300-2000K | 480 | 96 |
| GW Array Plate | 2000-2600K | 480 | 96 |

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Low density arrays are cheaper

| Statistic | Number of SNPs | Percent |
|---|---|---|
| SNPs assayed | 40 | 100.00 |
| Called SNPs (frequency > 0.85) | 36 | 90.00 |
| Called SNPs that are polymorphic | 36 | 100.00 |

| Statistic | Mean | Median | Range |
|---|---|---|---|
| Call frequency | 0.99 | 1.00 | 0.93 - 1.00 |
| Minor allele frequency (MAF) | 0.40 | 0.41 | 0.22 - 0.50 |
| Heterozygosity (observed) | 0.47 | 0.44 | 0.27 - 0.96 |
| Heterozygosity (expected) | 0.47 | 0.49 | 0.35 - 0.50 |


## Collaborators can reduce costs

Potential collaborators for economies of scale…

Pacific Northwest Tree Improvement Research Cooperative

PNWTIRC

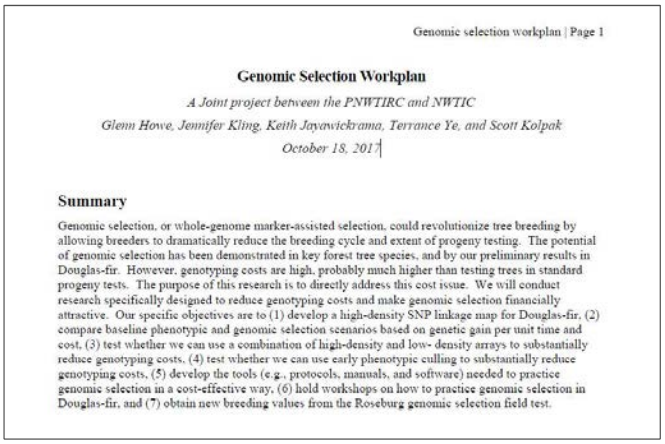
OSU Oregon State College of Forestry Northwest Tree Improvement Cooperative

Genome Canada LSARP Proposal: CoAdapTree: Healthy Trees for New Climates

Project Leaders: Sally Aitken, UBC; Sam Yeaman, University of Calgary; and Richard Hamelin, Laval University and UBC

SCION forests · products · innovation

## Genomic selection workplan



Genomic selection workplan | Page 1

**Genomic Selection Workplan**

*A Joint project between the PNWTIRC and NWTIC*

*Glenn Howe, Jennifer Kling, Keith Jayawickrama, Terrance Ye, and Scott Kolpak*

*October 18, 2017*

**Summary**

Genomic selection, or whole-genome marker-assisted selection, could revolutionize tree breeding by allowing breeders to dramatically reduce the breeding cycle and extent of progeny testing. The potential of genomic selection has been demonstrated in key forest tree species, and by our preliminary results in Douglas-fir. However, genotyping costs are high, probably much higher than testing trees in standard progeny tests. The purpose of this research is to directly address this cost issue. We will conduct research specifically designed to reduce genotyping costs and make genomic selection financially attractive. Our specific objectives are to (1) develop a high-density SNP linkage map for Douglas-fir, (2) compare baseline phenotypic and genomic selection scenarios based on genetic gain per unit time and cost, (3) test whether we can use a combination of high-density and low- density arrays to substantially reduce genotyping costs, (4) test whether we can use early phenotypic culling to substantially reduce genotyping costs, (5) develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way, (6) hold workshops on how to practice genomic selection in Douglas-fir, and (7) obtain new breeding values from the Roseburg genomic selection field test.

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Goals and Objectives

Our long-term goal is to substantially reduce the costs of genomic selection in Douglas-fir

## Objectives

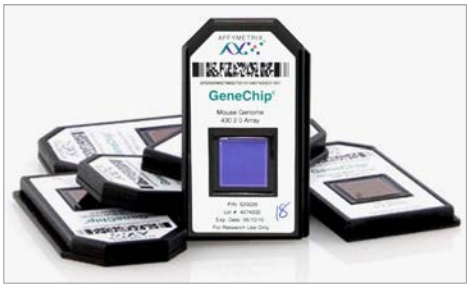
1. Develop a high-density SNP linkage map for Douglas-fir
2. Compare baseline phenotypic selection and genomic selection scenarios based on genetic gain per unit time and cost
3. Test whether we can use a combination of high-density and low-density arrays (HD/LD arrays) to substantially reduce genotyping costs
4. Test whether we can use multi-stage selection to substantially reduce genotyping costs
5. Develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way
6. Hold workshops on how to practice genomic selection in Douglas-fir
7. Obtain new breeding values from the Roseburg genomic selection field test

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


# General Methods

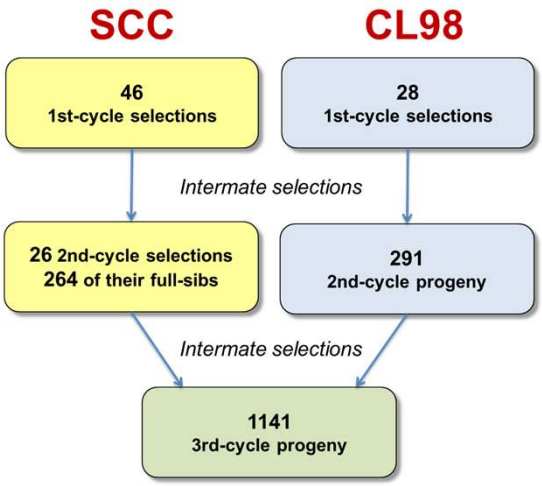
## Axiom genotyping array

***Large-scale genotyping service from GeneSeek***




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## NARA pedigree and empirical data




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## NARA site with full-sib progeny

Large full-sib families in the third cycle

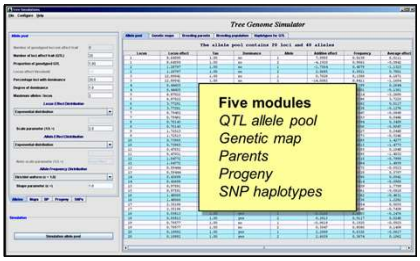

Rigorous test of genomic selection

| Internal_ID | geno_ID | plate_well_cel | female | male |
|---|---|---|---|---|
| 41602 | 573370-1234 | 4_B01 | 41408 | 41424 |
| 41602 | 573370-1235 | 11_E04 | 41408 | 41424 |
| 41602 | 573370-1236 | 14_B12 | 41408 | 41424 |
| 41602 | 573370-1237 | 12_D05 | 41408 | 41424 |
| 41603 | 573370-1241 | 13_F04 | 41419 | 41417 |
| 41603 | 573370-1242 | 14_H02 | 41419 | 41417 |
| 41603 | 573370-1243 | 14_B11 | 41419 | 41417 |
| 41603 | 573370-1244 | 14_D09 | 41419 | 41417 |
| 41603 | 573370-1245 | 13_G02 | 41419 | 41417 |
| 41603 | 573370-1246 | 13_H07 | 41419 | 41417 |
| 41603 | 573370-1247 | 15_G09 | 41419 | 41417 |
| 41603 | 573370-1248 | 22_H2 | 41419 | 41417 |
| 41603 | 573370-1249 | 13_G04 | 41419 | 41417 |
| 41603 | 573370-1250 | 23_E10 | 41419 | 41417 |
| 41603 | 573370-1251 | 15_G06 | 41419 | 41417 |
| 41603 | 573370-1252 | 14_A08 | 41419 | 41417 |
| 41603 | 573370-1253 | 15_F07 | 41419 | 41417 |
| 41603 | 573370-1254 | 20_A12 | 41419 | 41417 |
| 41603 | 573370-1255 | 13_F11 | 41419 | 41417 |
| 41603 | 573370-1256 | 20_E05 | 41419 | 41417 |
| 41603 | 573370-1257 | 20_A09 | 41419 | 41417 |
| 41603 | 573370-1258 | 15_E11 | 41419 | 41417 |
| 41603 | 573370-1259 | 13_G01 | 41419 | 41417 |
| 41603 | 573370-1260 | 15_H12 | 41419 | 41417 |
| 41603 | 573370-1261 | 14_D07 | 41419 | 41417 |
| 41603 | 573370-1262 | 15_E09 | 41419 | 41417 |
| 41603 | 573370-1263 | 20_C09 | 41419 | 41417 |
| 41603 | 573370-1264 | 20_D05 | 41419 | 41417 |
| 41603 | 573370-1265 | 13_F06 | 41419 | 41417 |
| 41603 | 573370-1266 | 20_C11 | 41419 | 41417 |
| 41603 | 573370-1267 | 22_D1 | 41419 | 41417 |
| 41603 | 573370-1268 | 15_H04 | 41419 | 41417 |
| 41603 | 573370-1269 | 15_E06 | 41419 | 41417 |
| 41603 | 573370-1270 | 13_G03 | 41419 | 41417 |
| 41603 | 573370-1271 | 13_H03 | 41419 | 41417 |
| 41603 | 573370-1272 | 13_G10 | 41419 | 41417 |
| 41603 | 573370-1273 | 13_H04 | 41419 | 41417 |
| 41603 | 573370-1274 | 13_H02 | 41419 | 41417 |
| 41603 | 573370-1275 | 15_F01 | 41419 | 41417 |
| 41603 | 573370-1276 | 15_G03 | 41419 | 41417 |
| 41603 | 573370-1277 | 15_H10 | 41419 | 41417 |
| 41603 | 573370-1278 | 20_H09 | 41419 | 41417 |
| 41603 | 573370-1279 | 13_H08 | 41419 | 41417 |
| 41603 | 573370-1280 | 15_E05 | 41419 | 41417 |
| 41603 | 573370-1281 | 13_G07 | 41419 | 41417 |
| 41603 | 573370-1282 | 13_G06 | 41419 | 41417 |
| 41603 | 573370-1283 | 22_F1 | 41419 | 41417 |
| 41603 | 573370-1284 | 20_F07 | 41419 | 41417 |
| 41603 | 573370-1285 | 13_F03 | 41419 | 41417 |
| 41603 | 573370-1286 | 22_E1 | 41419 | 41417 |
| 41603 | 573370-1287 | 15_F03 | 41419 | 41417 |
| 41603 | 573370-1288 | 15_F04 | 41419 | 41417 |
| 41603 | 573370-1289 | 13_H05 | 41419 | 41417 |
| 41603 | 573370-1290 | 15_E04 | 41419 | 41417 |
| 41603 | 573370-1292 | 15_H02 | 41419 | 41417 |
| 41603 | 573370-1293 | 15_G07 | 41419 | 41417 |
| 41603 | 573370-1294 | 13_H09 | 41419 | 41417 |
| 41603 | 573370-1295 | 14_H07 | 41419 | 41417 |
| 41603 | 573370-1296 | 14_C05 | 41419 | 41417 |
| 41603 | 573370-1297 | 15_H08 | 41419 | 41417 |
| 41603 | 573370-1298 | 14_D06 | 41419 | 41417 |
| 41603 | 573370-1299 | 13_F07 | 41419 | 41417 |
| 41603 | 573370-1300 | 14_A06 | 41419 | 41417 |
| 41604 | 573370-1301 | 13_D06 | 41451 | 41456 |
| 41604 | 573370-1302 | 14_H01 | 41451 | 41456 |
| 41604 | 573370-1303 | 15_G01 | 41451 | 41456 |

## Simulated data

Breeding program simulations

- Simulates SNPs
- Simulates phenotypes
- Various mating designs are available
- Will add the ability to use pedigrees as input




*Updated to account for the structure of NWTIC breeding programs*

*Being used to optimize sampling design for SNP genotyping*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Objective 1

Develop a high-density SNP linkage map for Douglas-fir

## Objective 1

Develop a high-density SNP linkage map for Douglas-fir

***Objectives***

1.1. Develop a high-density (>20K SNP) linkage map for Douglas-fir to use for guiding genomic selection

- Select the optimal subset of markers to use for genomic selection
- Use for imputing missing data (e.g., combined HD/LD arrays)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Objective 1

Develop a high-density SNP linkage map for Douglas-fir

***Methods***

1.1. Select high-quality markers from each family

1.2. Calculate the number of recombinants and non-recombinants for each family

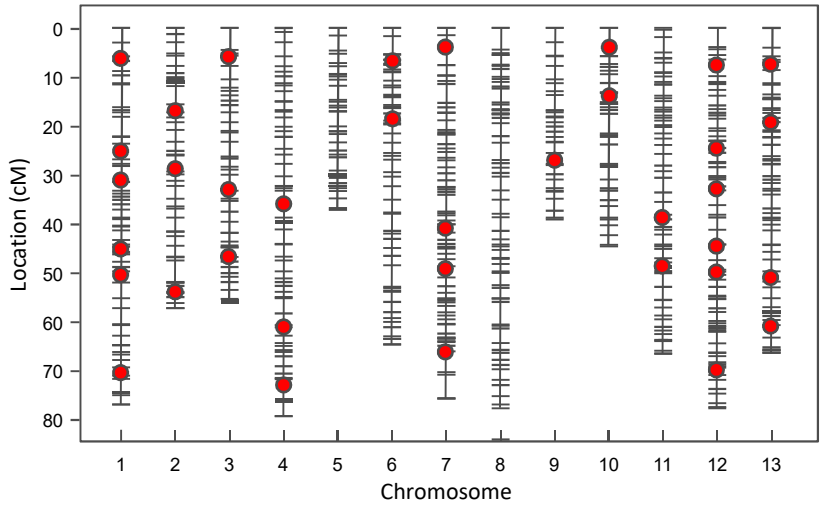
1.3. Calculate pairwise recombination frequencies and LOD scores across all families

1.4. Group the SNPs into linkage groups

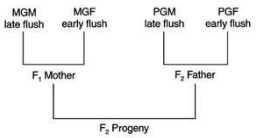
1.5. Order the markers within each linkage group

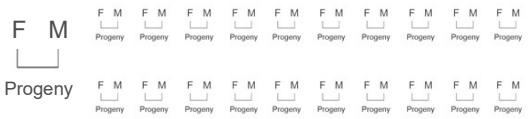
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

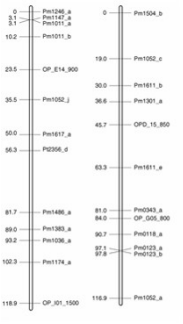
## Linkage map – Why?

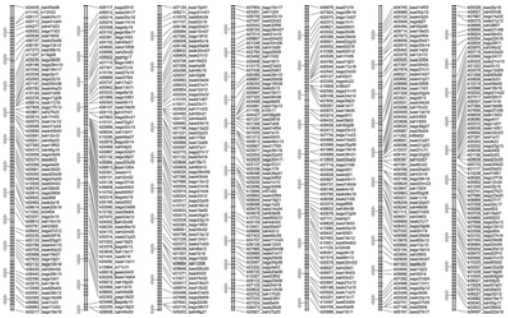


## Douglas-fir linkage map



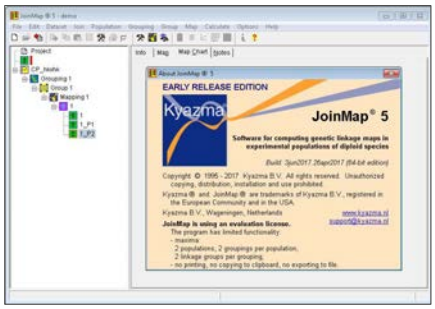


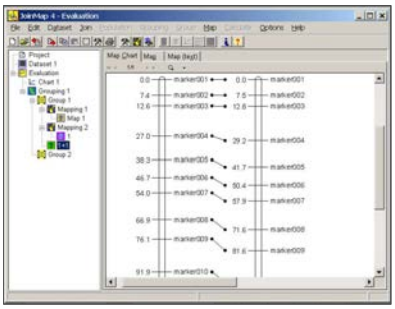
First-generation linkage map

*Jermstad et al (1998) Theor Appl Genet 97:76*

High-density linkage map

## We have been using JoinMap



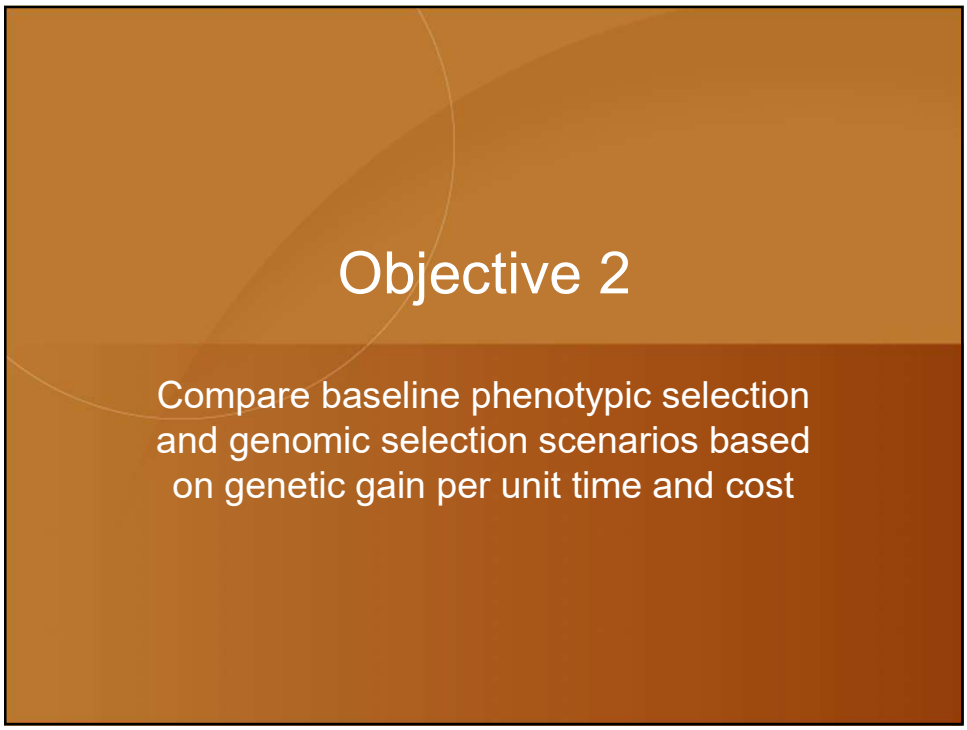



### Other approaches are needed too…

Van Ooijen, J.W. 2006. JoinMap ® 4, Software for the calculation of genetic linkage maps in experimental populations, Kyazma B.V., Wageningen, Netherlands, 57pp.

Strnadov-Neeley, V., Buluc, A., Chapman, J., Gilbert, J.R., Gonzalez, J., and Oliker, L. 2015. Efficient data reduction for large-scale genetic mapping. In Proceedings of the 6th ACM Conference on Bioinformatics, Computational Biology and Health Informatics. ACM, Atlanta, Georgia. pp. 126-135.

Preedy, K.F., and Hackett, C.A. 2016. A rapid marker ordering approach for high-density genetic linkage maps in experimental autotetraploid populations using multidimensional scaling. Theor Appl Genet 129:2117-2132.




## Objective 2

Compare baseline phenotypic selection and genomic selection scenarios based on genetic gain per unit time and cost

***Objectives***

2.1. Compare genomic selection versus phenotypic selection based on field tests

2.2. Determine the optimum number of SNPs and training population size for genomic selection

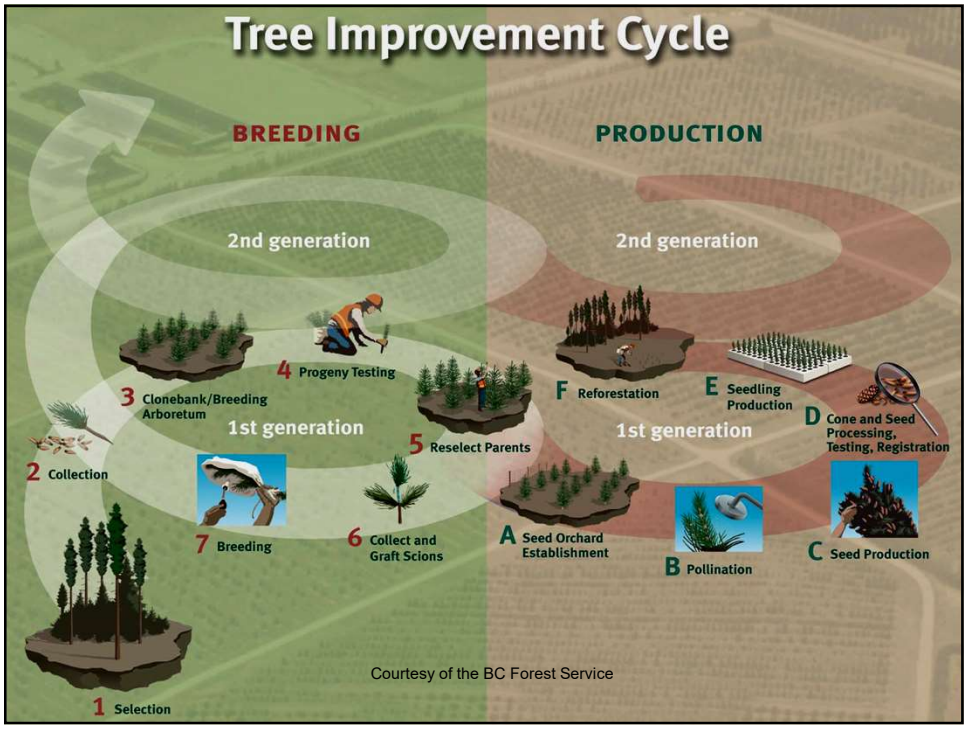
PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

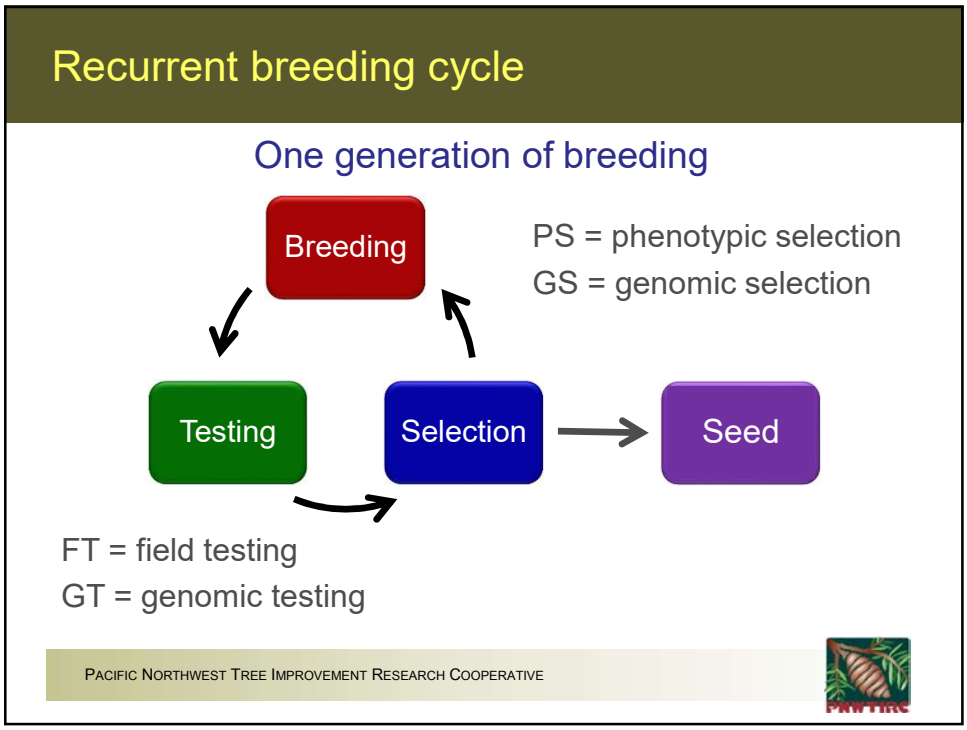
## Evaluation criterion

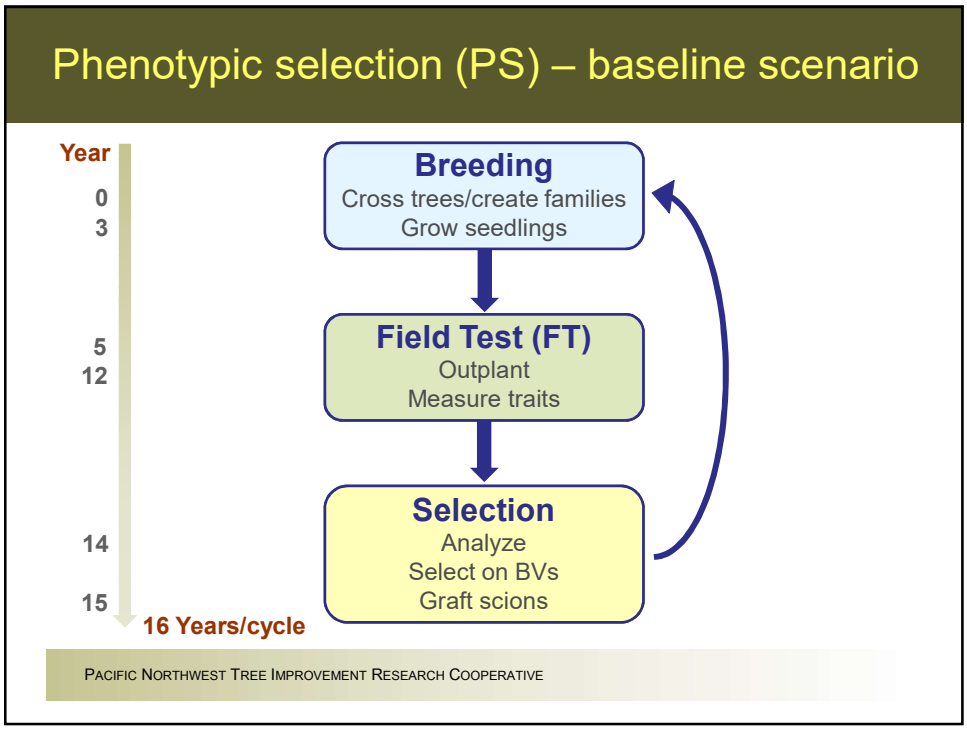
**Expected genetic gain per unit of time and cost**
**= Genetic gain / (time*cost)**

- Genetic gain per selection cycle
  - *Direct response to selection for target trait (BV)*
  - *Indirect response to selection for a correlated trait (e.g., GEBV)*
- Divide by the number of years/cycle of selection
- Divide by the total cost/cycle of selection

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE




Recurrent breeding cycle
One generation of breeding
Breeding
PS = phenotypic selection
GS = genomic selection
Testing
Selection
Seed
FT = field testing
GT = genomic testing
Pacific Northwest Tree Improvement Research Cooperative


Phenotypic selection (PS) – baseline scenario
Year
0
3
5
12
14
15
16 Years/cycle
Breeding
Cross trees/create families
Grow seedlings
Field Test (FT)
Outplant
Measure traits
Selection
Analyze
Select on BVs
Graft scions
Pacific Northwest Tree Improvement Research Cooperative

## Phenotypic selection (PS) – baseline scenario

### *NWTIC protocol for PS*

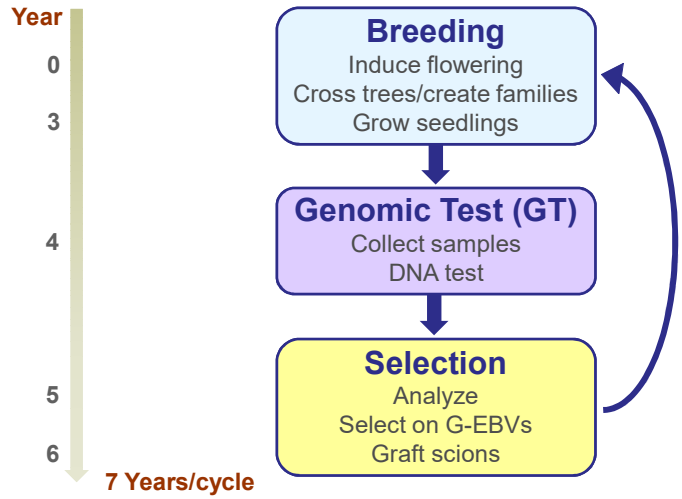
- Number of full-sib families (crosses) = 30
- Size of field test
  - *Number of trees/family = 50*
  - *10 single-tree plots/family at 5 sites*

### *Simulation studies*

- Mimic NWTIC protocol
- 30 full-sib families, 50 trees per family
- Select best tree in each family
- Calculate gain/(time*cost)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Genomic selection (GS) – baseline scenario




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Genomic selection (GS) – variables

- For baseline GS, determine optimum
  - *Size of training population*
  - *Number of SNPs*
- Use these values for later objectives that aim to reduce costs

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Baseline scenarios – cost comparison

| Activity | Phenotypic selection | Genomic selection |
|---|---|---|
| Make crosses | ? | ? |
| Seedling production | $2-$5/tree | $2-$5/tree |
| DNA genotyping | 0 | <<$55/sample |
| Site preparation | ? | ? |
| Site maintenance (age 2-12) | $10-$15/tree | 0 |
| Measurements (age 8-12) | ? | ? |
| Establish breeding arboretum | ? | ? |
| Subtotal | ? | ? |
| Cost per year | ? | ? |

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Objective 3

Test whether we can use a combination of high-density and low-density arrays (HD/LD arrays) to substantially reduce genotyping costs

## Objective 3

Test whether we can use high-density and low-density arrays (HD/LD arrays) to substantially reduce genotyping costs

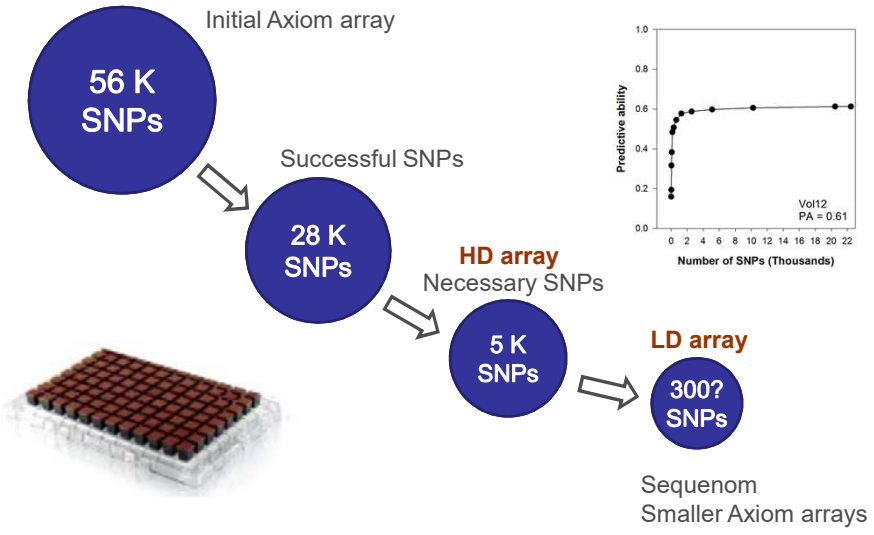
***Objectives***

3.1. Test whether HD/LD arrays can be used to reduce the costs of genomic selection

3.2. Determine the optimal combination of HD/LD arrays (e.g., numbers of SNPs and relative proportions of HD and LD arrays)



## High density (HD) vs low density (LD) arrays




## Combine LD and HD arrays

- Genotyping all trees with HD arrays is expensive
- Genotype a subset of trees with LD array (e.g., 300 SNPs) to reduce costs
- HD/LD arrays have been successful in salmon, pig, and other species
- Impute missing SNPs using information from HD arrays
- A genetic map will improve imputation accuracy

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

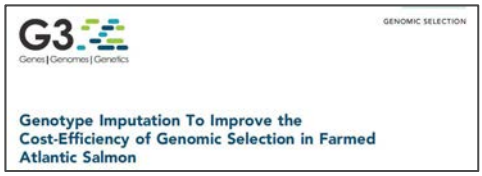
## Imputation

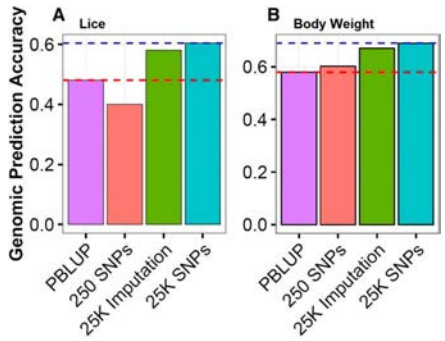
- Definition: predicting genotypes that are not directly measured in a sample
- Many applications
  - *Boost power in association studies*
  - *Fine map genes*
  - *Combine data sets from different genotyping platforms*
  - *Correct genotyping errors*
- Two basic approaches
  - *Population based – unordered, uses linkage disequilibrium*
  - *Pedigree based – uses linkage along ordered chromosomes*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## HD/LD example

- 624 salmon from 59 families
- Traits
  - *Sea lice count*
  - *Body weight*
- HD genotypes available for two generations
- LD panel has 250 SNPs
  - *All offspring at LD*
  - *75% LD / 25% HD*
- Imputation accuracies up to 0.90 with LD arrays
  - *HD data used for parents*
  - *25% of offspring at HD*



## LD/HD arrays – variables to investigate

- Number of SNPs on the LD array
- Imputation method
  - *Use standard measures of imputation accuracy*
- Number of progeny evaluated with LD vs HD arrays
  - *All parents genotyped at HD*
  - *Progeny genotyped using all LD to 25% LD / 75% HD*
- Evaluate alternatives using gain/(year*cost)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


## Objective 4

Test whether we can use multi-stage selection to substantially reduce genotyping costs

## Objective 4

Test whether we can use multi-stage selection to substantially reduce genotyping costs

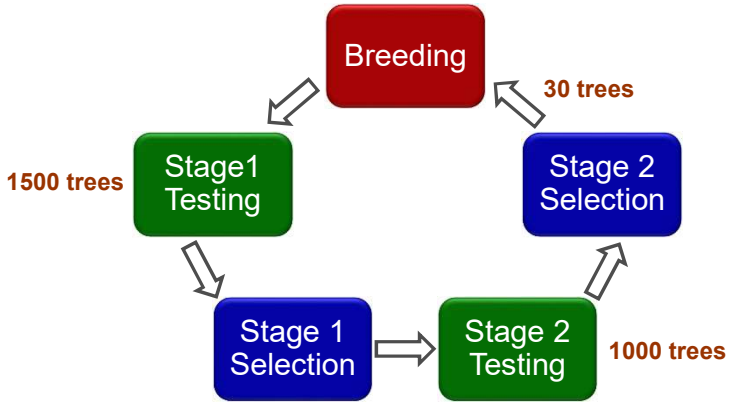
***Objectives***

4.1. Compare multi-stage selection scenarios to baseline scenarios (Objective 2) using our measure of relative genetic gain (gain/[year*cost])

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Multistage-stage selection

More than one testing and selection stage in one breeding cycle




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

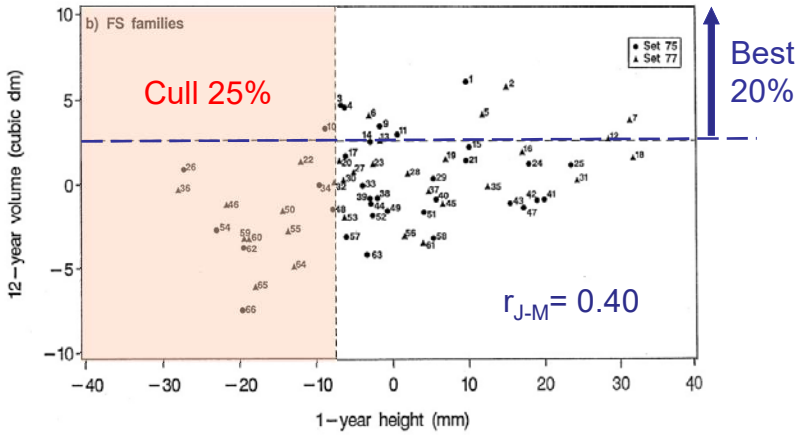
## What is early culling?

- Reduce testing costs (FT or GT) by culling poor trees first (two-stage selection)
- Efficiency depends on $h^2$ and $r_A$ between seedling traits and final selection traits
- Requires experimental design (blocking) and data collection at the seedling stage

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Early culling – previous results

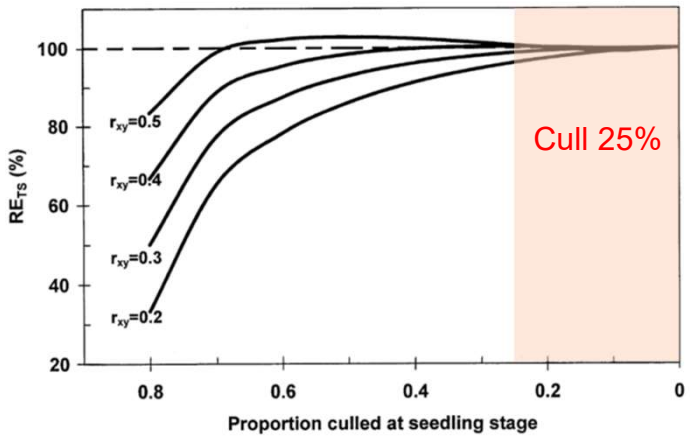
Full-sib families




*Adams et al (2001) Silvae Genetica 50:167-175*

## Early culling – impact of $r_{J\text{-}M}$ on efficiency

Relative efficiency of early culling




*Adams et al (2001) Silvae Genetica 50:167-175*

## Multi-stage selection – breeding scenarios

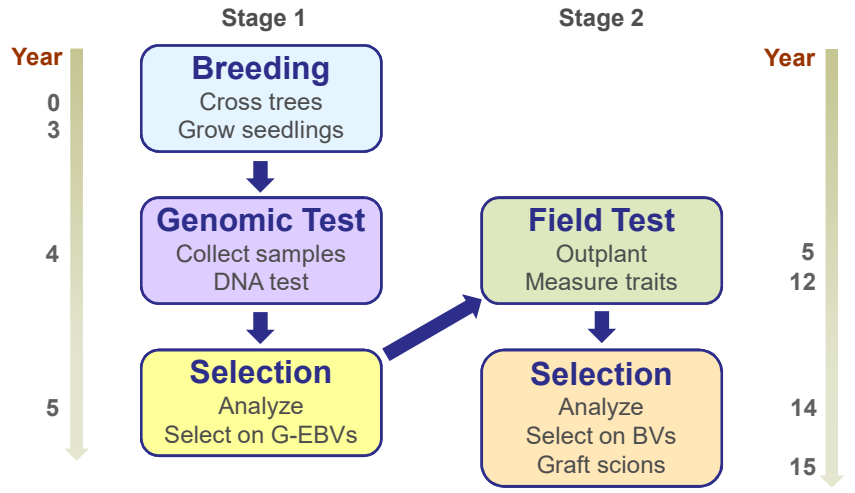
Genomic = genomic selection
Field = phenotypic selection based on field tests
Seedling = phenotypic selection based on nursery or greenhouse tests

---

- Genomic ⟶ Field
- Field ⟶ Genomic
- Seedling ⟶ Genomic
- Seedling ⟶ Field
- Seedling ⟶ Genomic ⟶ Field
- Seedling ⟶ Field ⟶ Genomic

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Genomic → Field (Two-stage selection)




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

**16 Years/cycle**

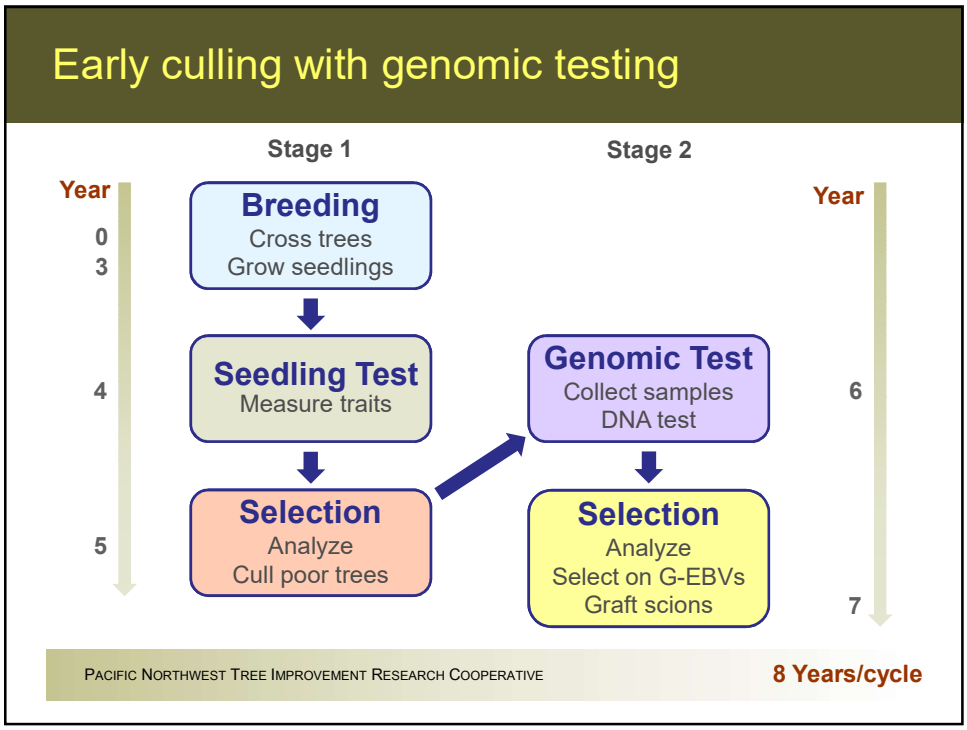
## Genomic → Field – variables

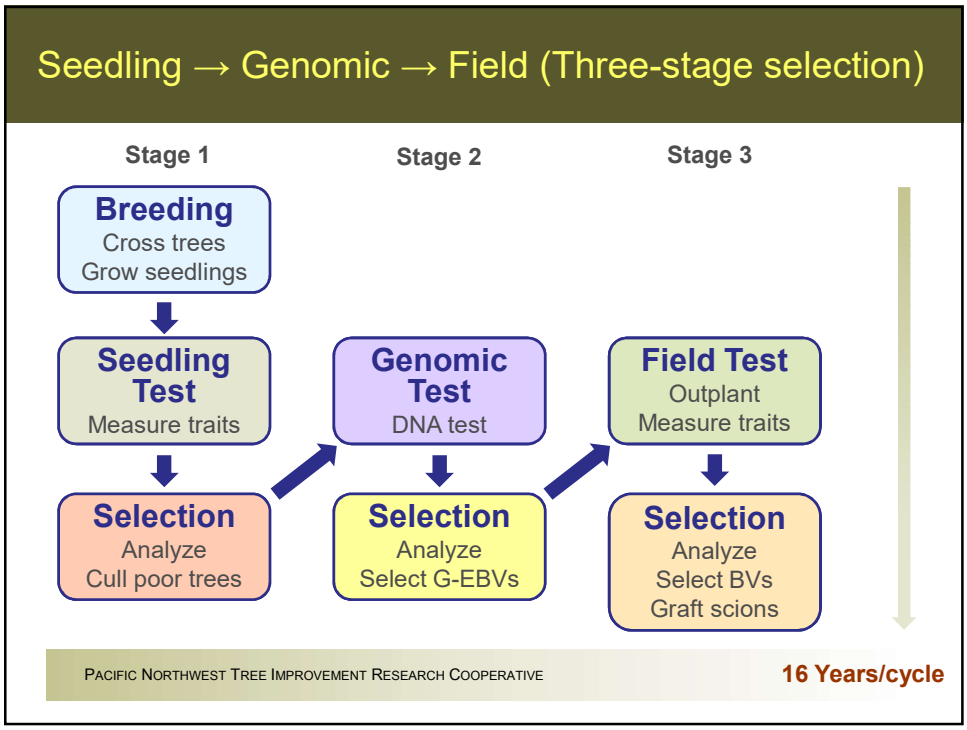
- Number of SNPs and training population size are already established

***Determine optimum***

- Selection intensity for the genomic test
- Number of trees to test in field trials
- Evaluate genetic gain/(years*cost)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


Early culling with genomic testing
Stage 1
Stage 2
Year
0
3
4
5
Breeding
Cross trees
Grow seedlings
Seedling Test
Measure traits
Selection
Analyze
Cull poor trees
Genomic Test
Collect samples
DNA test
Selection
Analyze
Select on G-EBVs
Graft scions
Year
6
7
Pacific Northwest Tree Improvement Research Cooperative
8 Years/cycle


Seedling → Genomic → Field (Three-stage selection)
Stage 1
Stage 2
Stage 3
Breeding
Cross trees
Grow seedlings
Seedling Test
Measure traits
Selection
Analyze
Cull poor trees
Genomic Test
DNA test
Selection
Analyze
Select G-EBVs
Field Test
Outplant
Measure traits
Selection
Analyze
Select BVs
Graft scions
Pacific Northwest Tree Improvement Research Cooperative
16 Years/cycle

## Early culling – variables

- Traits
  - *Focus on seedling height as early culling criterion*
  - *Stem volume at age 15 as the target trait*
- Proportion of progeny to cull based on the seedling test (phenotype)
- Selection intensity for the genomic test
- Number of trees to test in field trials
- Evaluate alternatives using gain/(year*cost)

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


## Objective 5

Develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way

## Objective 5

Develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way

***Objectives*** *We will develop…*

5.1. Written protocols for foliage collection, tracking, and storage

5.2. Written protocols for isolating DNA, including outsourcing options

5.3. Instructions for using existing and custom software for data analysis

5.4. Guidelines for data interpretation

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE


## Objective 6

Hold workshops on how to practice genomic selection in Douglas-fir

## Objective 6

Hold workshops on how to practice genomic selection in Douglas-fir

***Objectives***

6.1. Hold one or more workshops to provide training on implementing genomic selection

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Genomic selection workshops

- One or more workshops to provide training in GS
- Will use documentation from Objective 5
  - *Needle collection and storage*
  - *DNA isolation and genotyping*
  - *Data analysis and interpretation*
- Organized and presented by PNWTIRC and NWTIC staff

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

# Objective 7

Obtain new breeding values from the Roseburg genomic selection field test

## Objective 7

Obtain new breeding values from the Roseburg genomic selection field test

***Objectives***

7.1. Develop a measurement schedule for the NARA test plantation

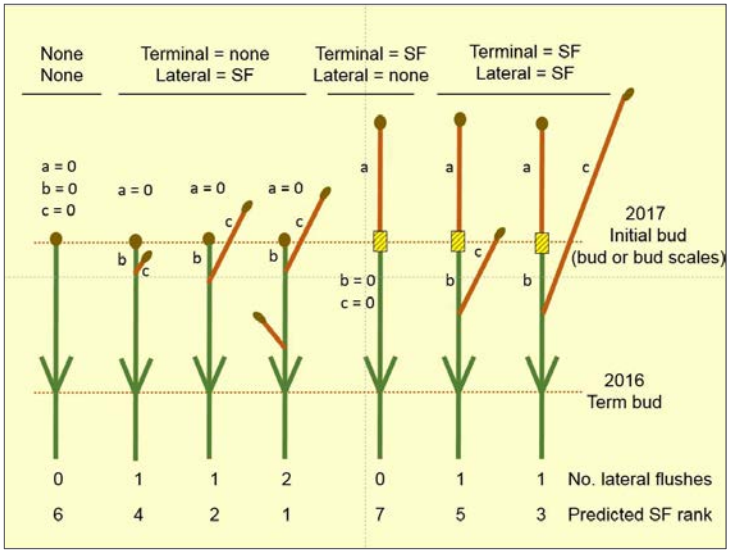
7.2. Take measurements according to the measurement schedule

7.3. Analyze the data to estimate breeding values and test genomic selection

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## GS validation – Roseburg field test

## Second flushing as a predictor of stem defect



## Potential activities – Discussion

- Verify genotype identities based on accession file
- Normal NWTIC measurements (who and when?)
- Additional measurements (who and when)
  - *Second flushing as a predictor of stem defect?*
  - *Spring bud flush?*
  - *Fall cold hardiness?*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Summary

Long-term goal is to reduce costs of GS in Douglas-fir

Objective 1 – linkage map
Objective 2 – baseline protocols for PS, GS
Objective 3 – combine LD/HD arrays
Objective 4 – multi-stage testing
Objective 5 – tools for GS – manuals, software
Objective 6 – GS workshops
Objective 7 – additional phenotypes for validation

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Budget

By Glenn Howe

Glenn Howe presented last year's budget (FY2016-2017) and the proposed budget for next year (FY2017-2018). During this portion of the annual meeting, we also elected a new Policy/Technical Committee Chair and OSU representative for the NSF Center for Advanced Forestry Systems (CAFS).

# Budget and Other Business

**Glenn Howe**

*Pacific Northwest Tree Improvement Research Cooperative*
*Department of Forest Ecosystems and Society*
*Oregon State University*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## Budget 2016-17

### Main points

- 2015-16 income = $102K
- 2016-17 income = $104K
- 2017-18 income = $117.5K
- Indirect = 13%

**Attachment #1**
**Financial Support Received in 2016-17**

| Organization | Financial Support |
|---|---|
| **Regular Members** | |
| Cascade Timber Consulting | 8,000 |
| Bureau of Land Management | 10,000 |
| Green Diamond Resource Company | 8,000 |
| Hancock Forest Management | 8,000 |
| Olympic Resource Management | 8,000 |
| Oregon Department of Forestry | 8,000 |
| Port Blakely Tree Farms | 8,000 |
| Rayonier | 8,000 |
| Roseburg Forest Products | 8,000 |
| Stimson Lumber Company | 8,000 |
| Washington State Dept. of Natural Resources | 8,000 |
| Weyerhaeuser | 8,000 |
| **Associate Members** | |
| Starker Forests | 4,000 |
| **Contractual Members** | |
| Lone Rock Timber Company | 2,000 |
| **Total** | **104,000** |

# Budget 2016-17

## *Main points*

- Summarizes personnel costs
- Personnel costs were covered by PNWTIRC members and OSU (Director)
- Carryover increased, but will decrease next year
- CAFS and STDP funds were used to pay some salaries
- The CAFS project is ending this year and most funds have already been spent

**Attachment #2**
**PNWTIRC Income and Expenditures by Source**
**FY 2016-2017**

| Income and Expenditures | OSU | Members | Total |
|---|---|---|---|
| **Income** | | | |
| OSU Forest Research Laboratory | 142,602 | 0 | 142,602 |
| Membership fees and contracts | 0 | 104,000 | 104,000 |
| Carryover from previous year | 0 | 133,617 | 133,617 |
| **Total income** | **142,602** | **237,617** | **380,219** |
| **Expenditures** | | | |
| Salaries and OPE* | | | |
| Director (0.45 FTE; OSU funded) | 71,643 | 0 | 71,643 |
| Program Manager | 0 | 11,932 | 11,932 |
| Research Coordinator | 0 | 27,000 | 27,000 |
| Research Scientist | 0 | 28,606 | 28,606 |
| Faculty Research Assistant | 0 | 2,278 | 2,278 |
| Graduate students | 0 | 0 | 0 |
| Student employees | 0 | 151 | 151 |
| OPE reimbursement | 0 | -20 | -20 |
| Supplies and Services | 0 | 7,300 | 7,300 |
| Travel | 0 | 1,466 | 1,466 |
| **Total direct costs (TDC)** | **71,643** | **78,712** | **150,355** |
| **Indirect costs**** | **70,959** | **10,233** | **81,192** |
| **Direct + Indirect Costs** | **142,602** | **88,945** | **231,547** |
| **Carryover to next year** | **0** | **148,672** | **148,672** |

# Budget 2016-17

## *Main points*

- Summarizes costs by project
- We allocated funds for SNP genotyping that were not spent
- PNWTIRC funds were allocated to the Drought Hardiness Study for the first time last year

**Attachment #3**
**Proposed and Actual PNWTIRC Budgets for 2016-2017***

| Income | Proposed (10/16) | Actual (7/17) |
|---|---|---|
| Members fees and contracts | 102,000 | 104,000 |
| Carryover from previous year | 133,617 | 133,617 |
| **Total income** | **235,617** | **237,617** |

| Expenses | Proposed (10/16) | Actual (7/17) |
|---|---|---|
| SNP marker assisted selection | 126,455 | 50,976 |
| New research (e.g., Drought) | 2,694 | 2,700 |
| Site characterization (CAFS) | 2,959 | 2,278 |
| WWP genetic markers (UI/CAFS) | 2,694 | 2,000 |
| Technology transfer | 0 | 0 |
| Administration | 21,305 | 20,759 |
| **Total direct costs (TDC)** | **156,108** | **78,712** |
| **Indirect costs**** | **20,294** | **10,233** |
| **Direct + Indirect costs** | **176,402** | **88,945** |
| **Carryover to next year** | **59,214** | **148,672** |

# Budget details for 2016-17

**Attachment #4**
**Expenditures of Cooperator Funds for Fiscal Year 2016-2017 by Project and Activity**

| Expense* | SNP MAS | Drought Hardiness | Site Char. (w/CAFS) | WWP | Tech Transfer | Admin. | Total |
|---|---|---|---|---|---|---|---|
| Director (funded by OSU) (approx. FTE) | 0<br>0.20 | 0<br>0.05 | 0<br>0.05 | 0<br>0.05 | 0<br>0.00 | 0<br>0.10 | 0<br>0.45 |
| Program Manager (approx. FTE) | 0<br>0.00 | 0<br>0.00 | 0<br>0.00 | 0<br>0.00 | 0<br>0.00 | 11,932<br>0.17 | 11,932<br>0.17 |
| Research Coordinator (approx. FTE) | 23,900<br>0.26 | 1,100<br>0.01 | 0<br>0.00 | 1,000<br>0.01 | 0<br>0.00 | 1,000<br>0.01 | 27,000<br>0.30 |
| Research Scientist (approx. FTE) | 25,006<br>0.19 | 1,600<br>0.01 | 0<br>0.00 | 1,000<br>0.01 | 0<br>0.00 | 1,000<br>0.01 | 28,606<br>0.22 |
| Faculty Research Assistant (approx. FTE) | 0<br>0.00 | 0<br>0.00 | 2,278<br>0.03 | 0<br>0.00 | 0<br>0.00 | 0<br>0.00 | 2,278<br>0.03 |
| Graduate students (approx. FTE)** | 0<br>0.00 | 0<br>0.00 | 0<br>0.00 | 0<br>0.00 | 0<br>0.00 | 0<br>0.00 | 0<br>0.00 |
| Undergraduate students | 151 | 0 | 0 | 0 | 0 | 0 | 151 |
| OPE reimbursement | 0 | 0 | 0 | 0 | 0 | -20 | -20 |
| **Personnel sub-total** | **49,057** | **2,700** | **2,278** | **2,000** | **0** | **13,912** | **69,947** |
| Supplies & Services | 1,919 | 0 | 0 | 0 | 0 | 5,381 | 7,300 |
| Travel | 0 | 0 | 0 | 0 | 0 | 1,466 | 1,466 |
| **Non-personnel sub-total** | **1,919** | **0** | **0** | **0** | **0** | **6,847** | **8,765** |
| **Total direct costs (TDC)** | 50,976 | 2,700 | 2,278 | 2,000 | 0 | 20,759 | 78,712 |
| **Indirect (13% of TDC)** | 6,627 | 351 | 296 | 260 | 0 | 2,699 | 10,233 |
| **Total costs** | **57,603** | **3,051** | **2,574** | **2,260** | **0** | **23,457** | **88,945** |

# Budget 2017-18

## *Main points*

- Summarizes proposed costs of personnel for 2017-2018
- Oguz Urhan is associated with the PNWTIRC, but is supported by the Turkish government
- Dues was increased for 2017-18
- BLM is not a member in 2017-18

**Attachment #5**

**Proposed Expenditures of Cooperator Funds for Fiscal Year 2017-2018**

| Income and Expenditures | FY 2016-17 | FY 2017-18 |
|---|---|---|
| **Income from Cooperators** | | |
| Membership fees and contracts | 104,000 | 117,500 |
| Carryover from previous year | 133,617 | 148,672 |
| **Total income** | **237,617** | **266,172** |
| **Expenditures** | | |
| Salaries and OPE* | | |
| Director (0.45 FTE; OSU funded) | 0 | 0 |
| Program Manager | 11,932 | 11,928 |
| Research Coordinator | 27,000 | 55,114 |
| Research Scientist | 28,606 | 27,052 |
| Faculty Research Assistant | 2,278 | 0 |
| Programmer | 0 | 7,500 |
| Student employees | 151 | 500 |
| OPE reimbursement | -20 | 0 |
| Supplies and Services | 7,300 | 4,000 |
| Travel | 1,466 | 4,000 |
| **Total direct costs (TDC)** | **78,712** | **110,094** |
| **Indirect costs**** | **10,233** | **14,312** |
| **Direct + Indirect Costs** | **88,945** | **124,406** |
| **Carryover to next year** | **148,672** | **141,766** |

# Budget 2017-18

## *Main points*

- Summarizes proposed costs by project for 2017-2018
- Focus on genomic selection
- Future of the Drought Hardiness Study is open for discussion
- Carryover will decrease

**Attachment #6**

**Proposed Expenditures of Cooperator Funds for Fiscal Year 2017-2018**

| Income | FY 2016-17 | FY 2017-18 |
|---|---|---|
| Members fees and contracts | 104,000 | 117,500 |
| Carryover from previous year | 133,617 | 148,672 |
| **Total income** | **237,617** | **266,172** |
| **Expenses** | **FY 2016-17** | **FY 2017-18** |
| SNP marker assisted selection | 50,976 | 80,580 |
| Drought hardiness | 2,700 | 6,593 |
| Site characterization (CAFS) | 2,278 | 0 |
| WWP genetic markers (UI/CAFS) | 2,000 | 0 |
| Technology transfer | 0 | 0 |
| Administration | 20,759 | 22,921 |
| **Total direct costs (TDC)** | **78,712** | **110,094** |
| **Indirect costs*** | **10,233** | **14,312** |
| **Direct + Indirect costs** | **88,945** | **124,406** |
| **Carryover to next year** | **148,672** | **141,766** |

# Budget details for 2017-18

**Attachment #7**

**Proposed Expenditures of Cooperator Funds for Fiscal Year 2017-2018**

| Expense* | SNP MAS | Drought Hardiness | Site Char. (w/CAFS) | WWP | Tech Transfer | Admin. | Total |
|---|---|---|---|---|---|---|---|
| Director (funded by OSU) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| (approx. FTE) | 0.15 | 0.05 | 0.05 | 0.05 | 0.00 | 0.15 | 0.45 |
| Program Manager | 0 | 0 | 0 | 0 | 0 | 11,928 | 11,928 |
| (approx. FTE) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 | 0.13 |
| Research Coordinator | 45,928 | 4,593 | 0 | 0 | 0 | 4,593 | 55,114 |
| (approx. FTE) | 0.50 | 0.05 | 0.00 | 0.00 | 0.00 | 0.05 | 0.60 |
| Research scientist | 27,052 | 0 | 0 | 0 | 0 | 0 | 27,052 |
| (approx. FTE) | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 |
| Faculty Research Assistant | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| (approx. FTE) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Programmer | 7,500 | 0 | 0 | 0 | 0 | 0 | 7,500 |
| (contract) | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| Student employees | 100 | 0 | 0 | 0 | 0 | 400 | 500 |
| (proportion of expense) | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.80 | 1.00 |
| **Personnel sub-total** | **80,580** | **4,593** | **0** | **0** | **0** | **16,921** | **102,094** |
| Supplies & Services | 0 | 0 | 0 | 0 | 0 | 4,000 | 4,000 |
| Travel | 0 | 2,000 | 0 | 0 | 0 | 2,000 | 4,000 |
| **Non-personnel sub-total** | **0** | **2,000** | **0** | **0** | **0** | **6,000** | **8,000** |
| **Total direct costs (TDC)** | 80,580 | 6,593 | 0 | 0 | 0 | 22,921 | 110,094 |
| **Indirect (13% of TDC)** | 10,475 | 857 | 0 | 0 | 0 | 2,980 | 14,312 |
| **Total costs** | **91,056** | **7,450** | **0** | **0** | **0** | **25,901** | **124,406** |

## Budget and other business

***Vote on budget***

***Elect new Policy/Technical Committee Chair***

***Elect new CAFS OSU Site Representative***

***Other business?***

## Update – Seedlot Selection Tool / Species Potential Habitat Tool

By Glenn Howe, Brad St.Clair, Dominique Bachelet, Brendan Ward, and Nik Stevenson-Molnar

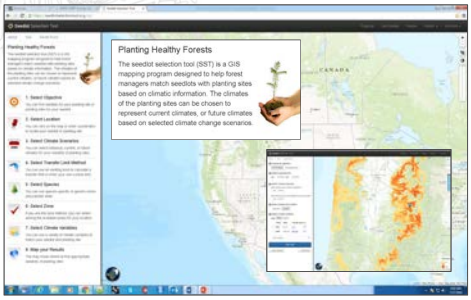
The Seedlot Selection Tool (SST) continues to be developed and expanded as a collaboration between Glenn Howe (OSU, PNWTIRC), Brad St.Clair (US Forest Service, Pacific Northwest Research Station), Dominique Bachelet (OSU), and staff at the Conservation Biology Institute (Brendan Ward and Nik Stevenson-Molnar). The SST is available online at https://seedlotselectiontool.org/sst.

The SST is a GIS mapping tool designed to help forest managers match seedlots with planting sites based on climatic information. The climates of the planting sites can be chosen to represent current climates, or future climates based on selected climate change scenarios. Key updates to the SST for 2016-2017 include increasing the geographic scope to include Alaska and Canada, adding seed zones for Ontario and the Canadian Maritime provinces, and adding the ability to use the SST across different regions. Next year, we will add more regions (i.e., Central US, Eastern US, Mexico), more seed zones and breeding zones (including NWTIC breeding zones), and more functions that can be used to customize the mapped results.

We are also developing new tools with funding from the USDA Forest Service. A Climate Smart Restoration Tool (CSRT) is being developed that uses the same methods as the SST, but this tool targets non-tree restoration species, particularly species of concern to managers in the Great Basin (https://consbio.org/products/projects/climate-smart-restoration-tool). A second tool, the Species Potential Habitat Tool (SPHT), is being developed to allow users to identify suitable species for sites under current or future climates (https://consbio.org/products/projects/species-potential-habitat-tool). Together, the SST and SPHT will allow users to examine assisted migration at both the within-species and species levels.

# - Updates -
# Seedlot Selection Tool
# Species Potential Habitat Tool

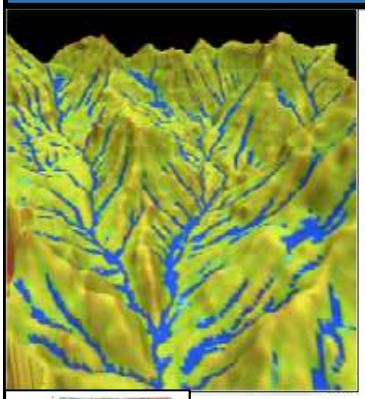
*Glenn Howe*
*Brad St.Clair*
*Dominique Bachelet*
*Brendan Ward*
*Nik Stevenson-Molnar*




PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE

## My Forest Today and tomorrow …

**Vulnerability** | **Species and seedlots** | **Stand management**

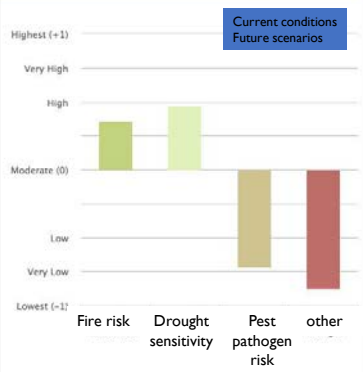


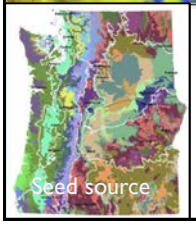
**Current**

1. Douglas fir
2. Lodgepole pine
3. ..
4. …

**Future**

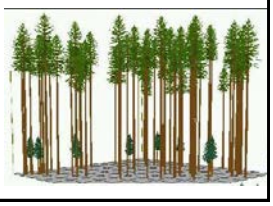
1. Douglas fir
2. Lodgepole pine
3. ..
4. …




Summary statistics

| Site Index | Age | Trees/ acre | Volume per acre: Total volume | Volume per acre: Merchant-able volume | Volume per acre: Merchant-able volume | Removals per acre: Trees/ acre | Removals per acre: Total volume | Removals per acre: Merchant-able volume | Removals per acre: Merchant-able volume | Removals per acre: BA/ acre | CCF | Top height | Growth: PRD | Growth: ACC | Growth: MORt/ year | D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ft³ | Ft³ | | Bd ft | | Ft³ | Ft³ | Bd ft | Ft² | Ft | Yr | | Ft³ | Ft³ |
| 50 | 5 | 500 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 5 | 0 | 0 | 0.0 |
| | 10 | 447 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 6 | 10 | 1 | 0 | 0.6 |
| | 20 | 371 | 13 | 0 | 0 | 121 | 1 | 0 | 0 | 3 | 7 | 12 | 10 | 6 | 0 | 1.4 |
| | 30 | 216 | 70 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 19 | 21 | 10 | 21 | 0 | 2.8 |
| | 40 | 204 | 278 | 57 | 189 | 0 | 0 | 0 | 0 | 24 | 44 | 29 | 10 | 51 | 1 | 4.7 |
| | 50 | 194 | 776 | 458 | 1617 | 94 | 557 | 422 | 1499 | 17 | 29 | 31 | 10 | 36 | 1 | 5.6 |

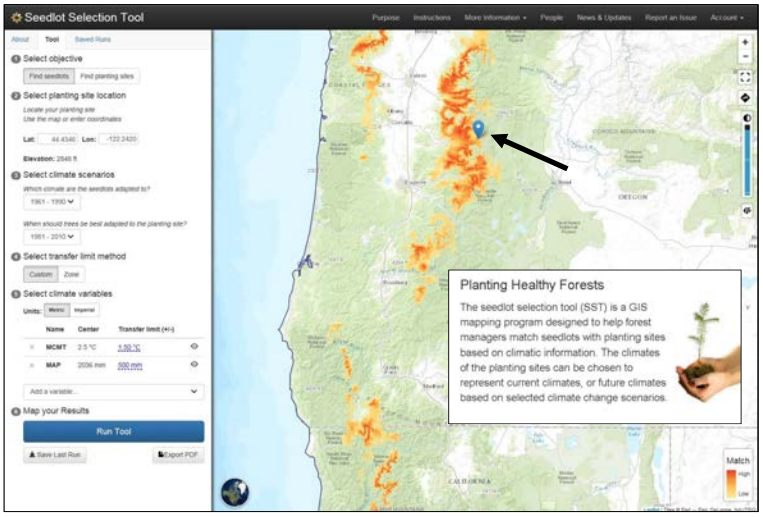


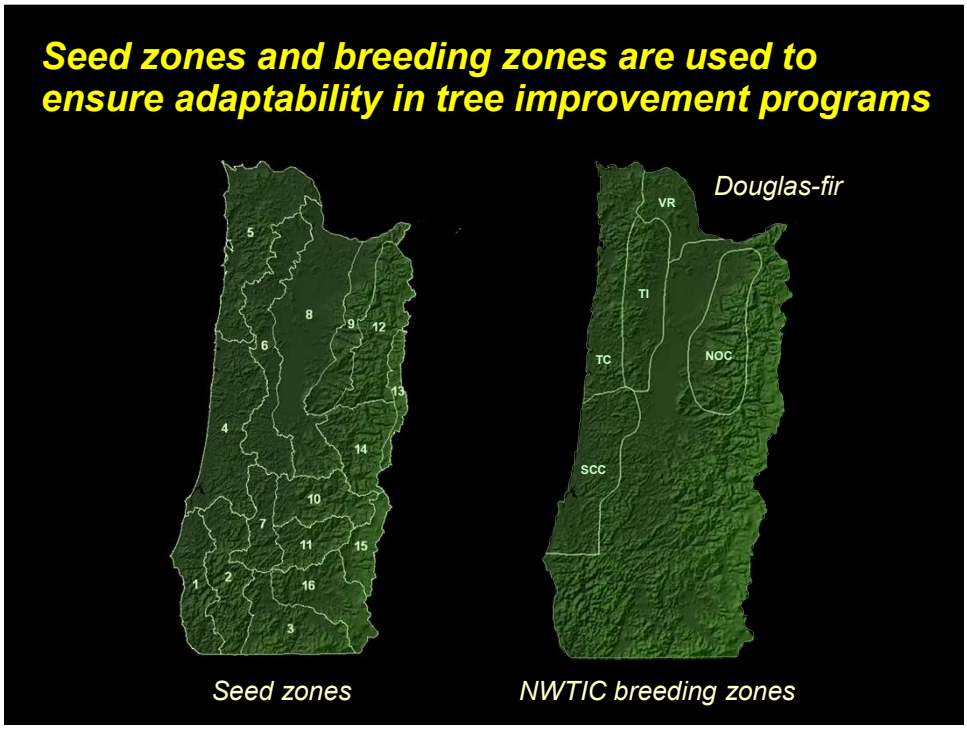
# Seedlot Selection Tool (SST)

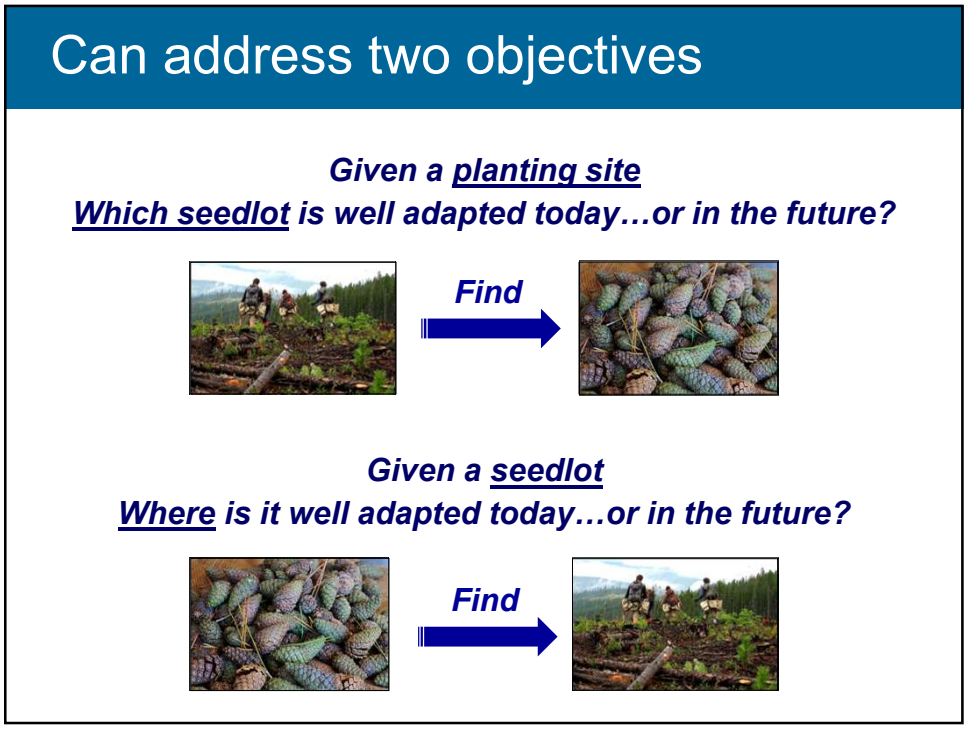
https://seedlotselectiontool.org/sst/



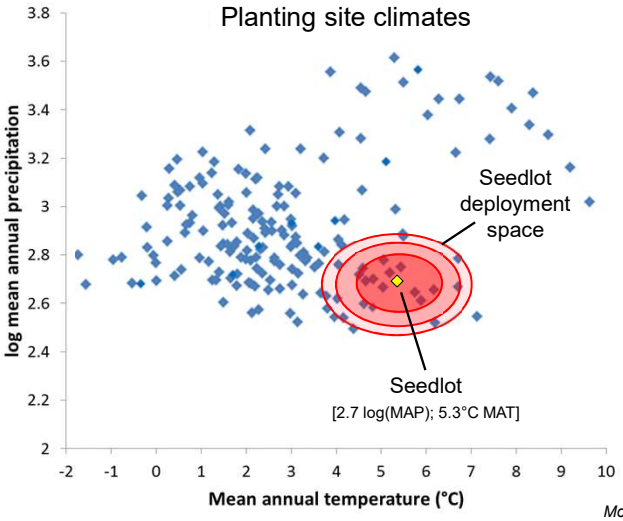
# The SST is a climate mapping tool




Seed zones and breeding zones are used to ensure adaptability in tree improvement programs
Douglas-fir
5
8
9
12
6
13
4
14
10
7
11
15
1
2
16
3
VR
TI
TC
NOC
SCC
Seed zones
NWTIC breeding zones


Can address two objectives
Given a planting site
Which seedlot is well adapted today…or in the future?
Find
Given a seedlot
Where is it well adapted today…or in the future?
Find

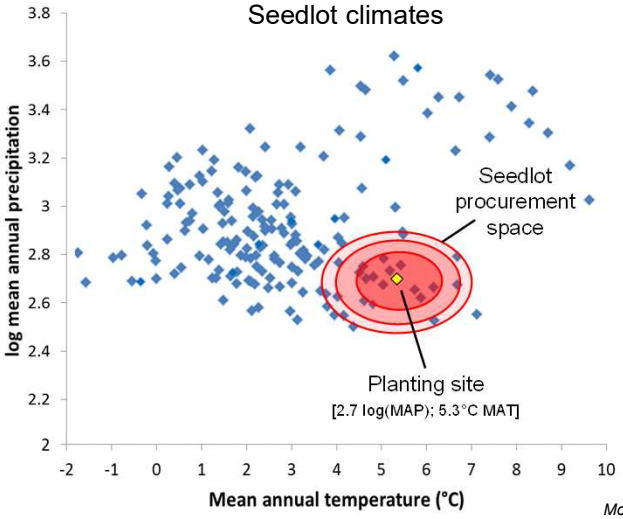
# Find planting sites for my seedlot




*Modified from Greg O'Neill*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE - ANNUAL MEETING Glenn T Howe | Slide 7

# Find seedlots for my planting site

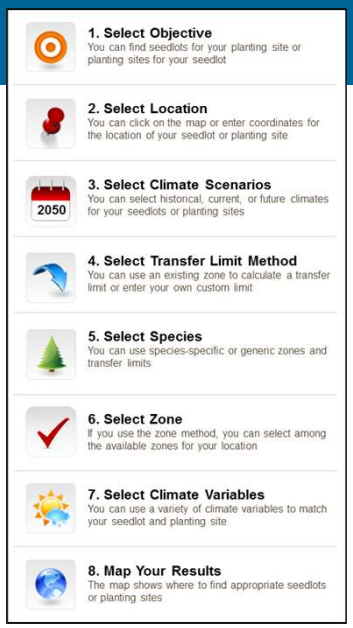



*Modified from Greg O'Neill*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE - ANNUAL MEETING Glenn T Howe | Slide 8

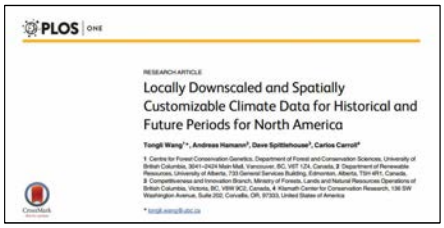
## How the tool works

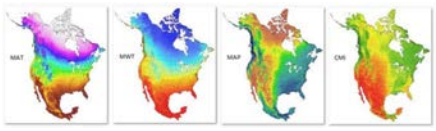
- Select objective
- Select location
- Select climate scenarios
- Select transfer limit method
- Select species
- Select zone
- Select climate variables
- Map your results





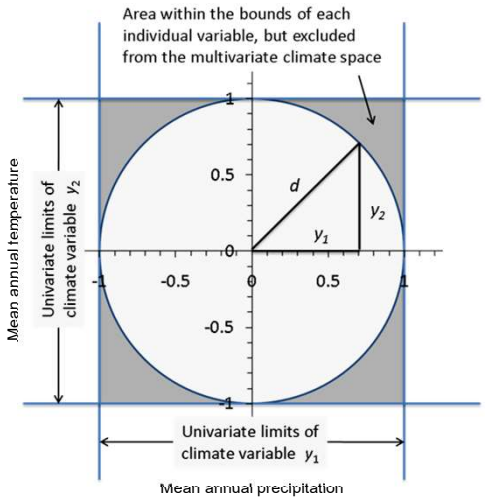

## ClimateNA – Climate interpolation



SST resolution is 15 arc-seconds
~327 meters at 45° latitude

# Climate space and transfer limit




*Transfer limit = radius*

*On the standardized scale, the transfer limit = 1.0*

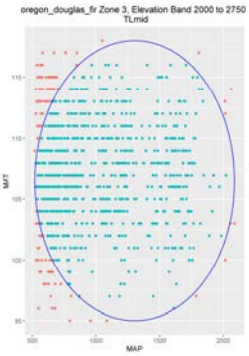
*Transfer distance = d*

$$d = \sqrt{(y_1^2 + y_2^2)}$$

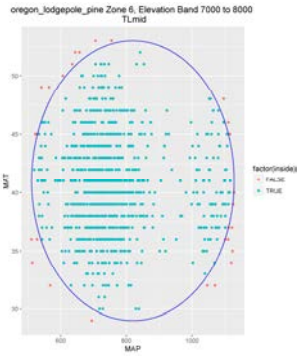


# Seed zone climate space

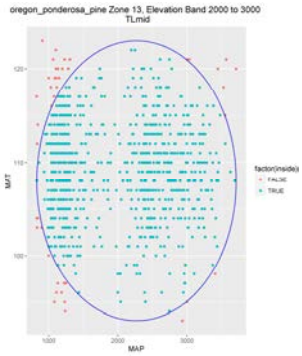
Douglas-fir (2000-2750')

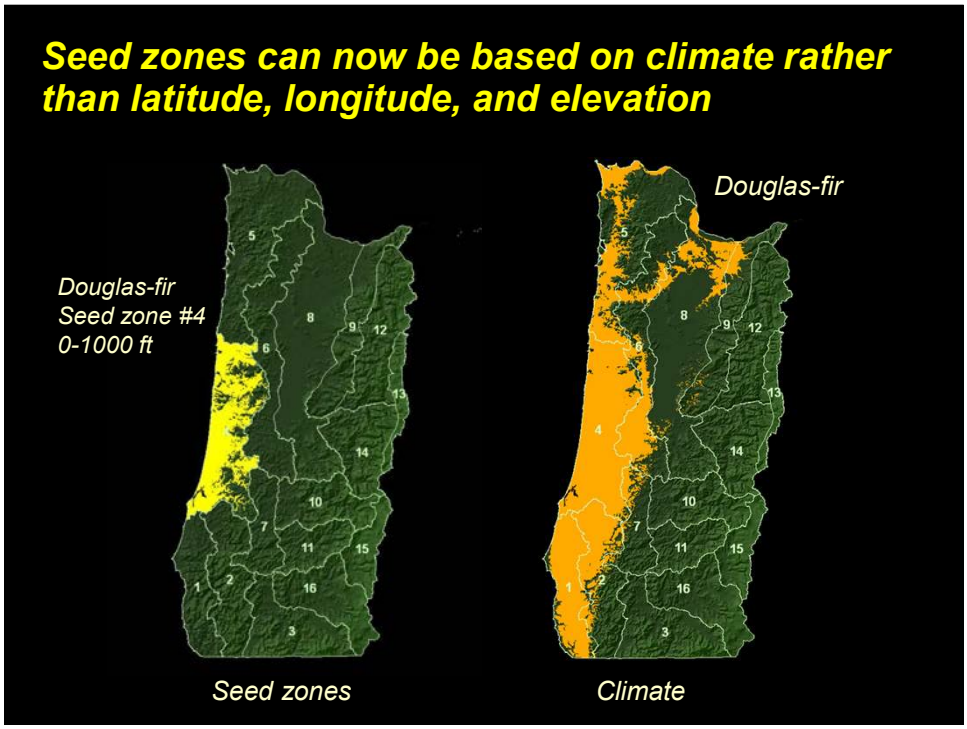


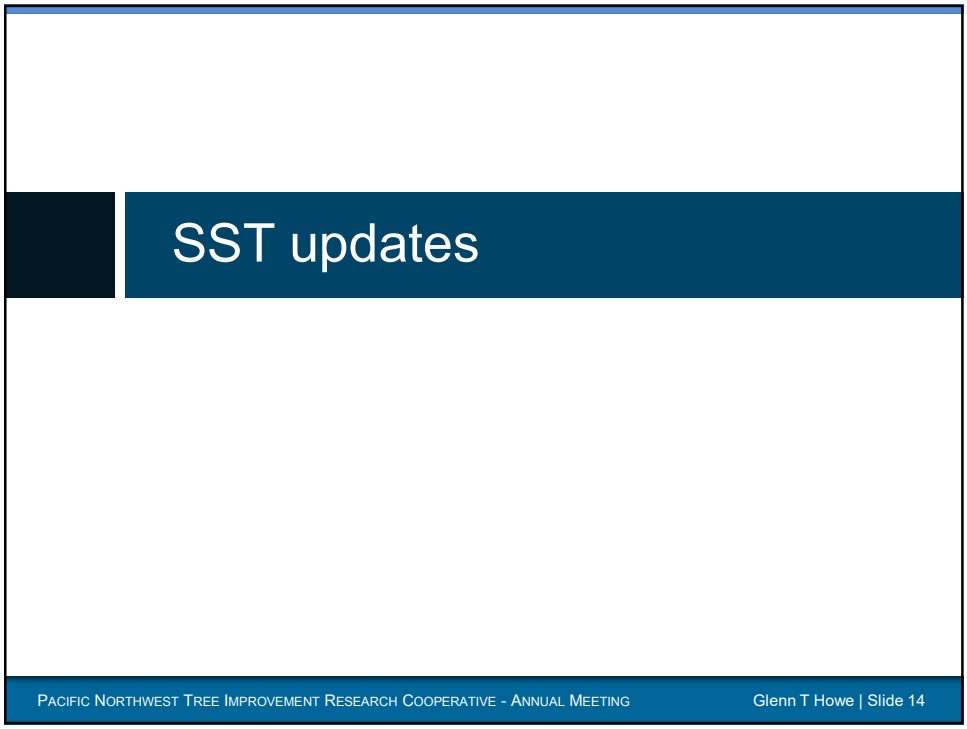

Lodgepole pine (7000-8000')




Ponderosa pine (2000-3000')




Seed zones can now be based on climate rather than latitude, longitude, and elevation
Douglas-fir
Seed zone #4
0-1000 ft
Douglas-fir
Seed zones
Climate


SST updates
Pacific Northwest Tree Improvement Research Cooperative - Annual Meeting
Glenn T Howe | Slide 14

## Updates to the SST: 2016-2017

- New regions
  - *Alaska and western Canada*
  - *North central Canada*
  - *Northeast Canada*
- Added the ability to map the climate for a location in one region into another region
- Seed zones were added for Ontario and the Canadian Maritime provinces
- We added ability to export results to a pdf or PowerPoint slide

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE - ANNUAL MEETING Glenn T Howe | Slide 15

## Updates to the SST: 2017-2018

- New regions
  - *Central United States*
  - *Eastern United States*
  - *Mexico*
- New functions
  - *More seed zones and breeding zones*
  - *Ability to export results to GIS file formats (GeoTiff)*
  - *Ability to apply constraints to the mapped area (photoperiod, elevation, latitude, longitude, distance)*
- We would like to add NWTIC breeding zones
  - *Need breeding zones boundaries (shape files)*
  - *Elevational limits*

PACIFIC NORTHWEST TREE IMPROVEMENT RESEARCH COOPERATIVE - ANNUAL MEETING Glenn T Howe | Slide 16

# New tools – USFS funded

***Climate Smart Restoration Tool***

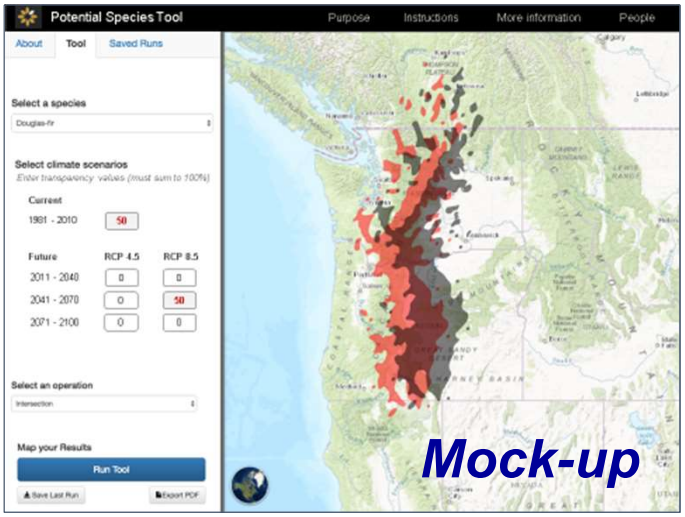
- SST version appropriate for restoration species
- Sagebrush and others

***Species Potential Habitat Tool (SPHT)***

- Will deliver potential species habitats for current and future climates
- Will interact with the SST (can be used to constrain SST output)



# Species Potential Habitat Tool (SPHT)




danke
謝謝
ngiyabonga
teşekkür ederim
спасибо
dank je
thank you
gracias
tapadh leat
mochchakkeram
bedankt
hvala
mauruuru
dziękuję
go raibh maith agat
obrigado
sagolun
sukriya
kop khun krap
arigatō
takk
dakujem
grazie
мерси
terima kasih
감사합니다
merci
ευχαριστώ

APPENDIX I

## Literature Cited

Cardon, L.R. and Bell, J.I. 2001. Association study designs for complex diseases. Nature Reviews Genetics 2:91.

Çeler, E. 2017. Douglas-fir seedlings in the Pacific Northwest: The genetics of drought adaptation. M.S. thesis, Oregon State University. 152pp.

Howe, G.T., Yu, J., Knaus, B., Cronn, R., Kolpak, S., Dolan, P., Lorenz, W.W. and Dean, J.F. 2013. A SNP resource for Douglas-fir: de novo transcriptome assembly and SNP detection and validation. BMC Genomics 14(1):137.

Müller, T., Ensminger, I., and Schmid, K.J. 2012. A catalogue of putative unique transcripts from Douglas-fir (*Pseudotsuga menziesii*) based on 454 transcriptome sequencing of genetically diverse, drought stressed seedlings. BMC Genomics 13(1):673.

Preedy, K.F., and Hackett, C.A. 2016. A rapid marker ordering approach for high-density genetic linkage maps in experimental autotetraploid populations using multidimensional scaling. Theor Appl Gen 129:2117-2132.

Strnadov-Neeley, V., Buluc, A., Chapman, J., Gilbert, J.R., Gonzalez, J., and Oliker, L. 2015. Efficient data reduction for large-scale genetic mapping. In Proceedings of the 6th ACM Conference on Bioinformatics, Computational Biology and Health Informatics. ACM, Atlanta, Georgia. pp. 126-135.

Tsai, H.-Y., Matika O., Edwards, S.M., Antolín-Sánchez, R., Hamilton, A., Guy, D.R., Tinch, A.E., Gharbi K., Stear, M.J., Taggart, J.B., Bron, J.E., Hickey, J.M., Houston, R.D. 2017. Genotype imputation to improve the cost-efficiency of genomic selection in farmed Atlantic salmon. G3: Genes|Genomes|Genetics 7(4):1377-1383.

Van Ooijen, J.W. 2006. JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations, Kyazma B.V., Wageningen, Netherlands, 57pp.

Wang, T., Hamann, A., Spittlehouse, D., Carroll, C. 2016. Locally downscaled and spatially customizable climate data for historical and future periods for North America. PLoS ONE 11(6):e0156720.

APPENDIX II

## Publications by PNWTIRC personnel 2016-2017

Çeler, E. 2017. Douglas-fir seedlings in the Pacific Northwest: The genetics of drought adaptation. M.S. thesis, Oregon State University. 152pp.

Frank, A., Howe, G.T., Sperisen, C., Brang, P., St.Clair, J.B., Schmatz, D.R., and Heiri, C. 2017. Risk of genetic maladaptation due to climate change in three major European tree species. Glob Change Biol 2017:1-14. DOI: 10.1111/gcb.13802.

Frank, A., Pluess, A.R., Howe, G.T., Sperisen, C., Heiri, C. 2017. Quantitative genetic differentiation and phenotypic plasticity of European beech in a heterogeneous landscape: Indications for past climate adaptation. Perspect Plant Ecol Evol Syst 26:1-13.

Frank, A., Sperisen, C., Howe, G.T., Brang, P., Walthert, L., St.Clair, J.B., and Heiri, C. 2017. Distinct genecological patterns in seedlings of Norway spruce and silver fir from a mountainous landscape. Ecology 98:211-227.

Howe, G.T. 2017. Cooperative brings life to tree breeding tools and approaches. Western Forester 62(3):18-19.

Howe, G.T. and Strauss, S.H. 2017. Biotechnology research is developing new tools for tree breeders. Western Forester 62(3):26-27.

St.Clair, J.B. and Howe, G.T. 2017. Building on a century of forest genetics research. Western Forester 62(3):16-17.

APPENDIX III

## Workshops, Presentations, and Abstracts by PNWTIRC personnel 2016-2017

Çeler, E. and Howe, G.T. 2017. Douglas-fir seedlings in the Pacific Northwest: The genetics of drought adaptation. Poster presentation In: Proceedings, Forest Regeneration in Changing Environments, International Meeting of IUFRO Unit 1.01.04, Forest Establishment and Early Growth Dynamics, July 11-13, 2017, Corvallis, OR.

Howe, G.T. 2017. Integrating traditional and molecular breeding for blister rust resistance in western white pine. Abstract In: Proceedings, Planting the Future, 44th Annual Meeting of the Inland Empire Tree Improvement Cooperative, March 8, 2017, Spokane Valley, Washington.

Howe, G.T. 2017. Genomics and breeding of Douglas-fir. Presentation In: Douglas-fir Breeding Seminar, April 6, 2017, University of Canterbury, Christchurch, New Zealand.

Howe, G.T. 2017. Genetics of trees. Presentation In: Reforestation Matters, USDA Forest Service National Silviculture Meeting, April 13, 2017, Portland, OR.

Howe, G.T. 2017. Trees on the move: Migration of tree species in response to climate change. Presentation In: Oregon State University Tree School, April 22, 2017, Rogue Community College, Grants Pass, OR.

Howe, G.T. and Jayawickrama, K.J. 2016. Genomic selection for Douglas-fir tree improvement. Presentation In: Center for Advanced Forestry Systems Annual Meeting, May 2-4, 2017, Portland, Oregon.

Howe, G.T. 2017. Genetic considerations for reforestation in the face of global climate change. Abstract In: Proceedings, Forest Regeneration in Changing Environments, International Meeting of IUFRO Unit 1.01.04, Forest Establishment and Early Growth Dynamics, July 11-13, 2017, Corvallis, OR.

Howe, G.T. 2017. Adapting forests to climate change: The role of forest genetics. Presentation In: Mt. Hood Community College, September 7, 2017.

Kolpak, S.E., Jayawickrama, K., Kling, J., Trappe, M., Hipkins, V., Ye, T., Guida, S., Cronn, R., Cushman, S.A., McEvoy, S., and Howe, G.T. 2017. Development of a high-density Affymetrix Axiom genotyping array for genomic selection in Douglas-fir. Abstract In: Forest Genetics 2017: Health and Productivity under Changing Environments, Proceedings of the Joint Meeting of Western Forest Genetics Association and Canadian Forest Genetics Association, Edmonton, AB, June 26-29, 2017.

Urhan, O., Rust, M.L., Davis, A., Howe, G.T., Hipkins, V. 2016. Development of genetic markers for western white pine and Douglas-fir. Presentation In: Center for Advanced Forestry Systems Annual Meeting, May 2-4, 2017, Portland, Oregon.

APPENDIX IV

## Collaborations and Grants 2016-2017

CAFS. Center for Advanced Forestry Systems – Phase II. Howe, G.T., Maguire, D.A., and Strauss, S.H. National Science Foundation Industry/University Cooperative Research Center Program, 2012-2018, $300,000 (OSU).

USFS Forest Health Protection, Special Technology Development Program. Genetic markers for western white pine (WWP): Enabling molecular breeding for resistance to white pine blister rust. Howe, G.T., Davis, A., Hipkins, V., Liu, J.-J., Mahalovich, M.F., Rust, M., and Sniezko, R., 2014-2018, $99,500.

USFS Pacific Northwest Research Station. Meta-analysis of Douglas-fir provenance tests to estimate responses to seed transfer and climate change. Howe, G.T. and St.Clair, J.B. USDA-Forest Service Joint Venture Agreement, 2013-2018, $100,000.

USFS Pacific Northwest Research Station. Evaluating assisted migration options for adapting to climate change. Howe, G.T. and St.Clair, J.B. USDA-Forest Service Joint Venture Agreement, 2013-2019, $40,000.

APPENDIX V

## Annual Meeting Minutes

October 19, 2017, Mt. Scott Fire Station 5, Happy Valley, OR

**I. Attendees**

Jeannette Griese – Bureau of Land Management
Michael Crawford – Bureau of Land Management
Darian Domes – Cascade Timber Consulting
John Jayne – Cascade Timber Consulting
Florian Deisenhofer – Hancock Forest Management
Keith Jayawickrama – NWTIC, OSU
Terrance Ye – NWTIC, OSU
Dan Cress – Olympic Resource Management
Andrew Wodnik – Olympic Resource Management
Brad St.Clair – PNW Research Station, USFS
Glenn Howe – PNWTIRC, OSU
Jennifer Kling – PNWTIRC, OSU
Anna Magnuson – PNWTIRC, OSU
Scott Kolpak – PNWTIRC, OSU
Susan McEvoy – PNWTIRC, OSU
Oguz Urhan – PNWTIRC, OSU
Lauren Magalska – Port Blakely Tree Farms
Josh Sherrill – Rayonier Forest Resources
Sara Lipow – Roseburg Forest Products
Margaret Banks – Stimson Lumber Co.
Jeff DeBell – Washington State DNR
Brian Baltunis – Weyerhaeuser

**II. Welcome**

Sara Lipow, PNWTIRC Policy/Technical Chair, called the meeting to order at 9:27 am.

**III. PNWTIRC highlights for 2016-2017**

Glenn Howe presented an overview of major accomplishments for 2016-17.

1. Administration and members
   - Director – Glenn Howe
   - Research Coordinator – Scott Kolpak
   - Program Manager – Anna Magnuson
   - Graduate student – Erda Çeler, Oguz Urhan
   - Faculty Research Assistant – Lauren Magalska
   - Policy/Technical Committee Chair – Sara Lipow
   - CAFS representative – Brian Baltunis
2. Research
3. Publications
4. Presentations
5. Collaborations and grants

**IV. PNWTIRC plans for 2017-18**

Glenn Howe presented plans for 2017-2018. Discussions were based around the Genomic Selection Workplan. Specific objectives include:

- Develop a high-density SNP linkage map for Douglas-fir
- Compare baseline phenotypic and genomic selection scenarios based on genetic gain per unit time and cost
- Test whether we can use a combination of high-density and low-density arrays to substantially reduce genotyping costs

- Test whether we can use early phenotypic culling to substantially reduce genotyping costs
- Develop the tools (e.g., protocols, manuals, and software) needed to practice genomic selection in a cost-effective way
- Hold workshops on how to practice genomic selection in Douglas-fir
- Obtain new breeding values from the Roseburg genomic selection field test

**V. PNWTIRC research presentations**

1. *A SNP chip for western white pine—Bioinformatic steps.* Susan McEvoy, Glenn Howe
2. *Axiom SNP chip—Final report.* Glenn Howe
3. Drought Hardiness Study—Next steps. Scott Kolpak
   Decisions: Glenn asked if there was interest in continuing this project in the future. There was some discussion about the original intentions of the project. Currently, there isn't strong interest or a plan prepared, so we will focus on genomic selection instead. In the future, there might be opportunities to collaborate with others who are interested in drought hardiness.
4. *Genomic Selection Workplan.* Glenn Howe, Jennifer Kling
   Decisions: This written plan is a first pass at a concept. Detailed costs and methods will be worked out later. The NARA project will be a good proof-of-concept to show value and help get funding from other external grants or PNWTIRC members. Repeating the experiment in another breeding population would complement the NARA experiment and the Roseburg test site, thereby increasing confidence in genomic selection.
5. *Update – Seedlot Selection Tool/Species Potential Habitat Tool.* Glenn Howe

**VI. Budget**

Glenn Howe presented the budget for FY 2016-2017. The proposed budget for FY 2017-2018 was also presented. A motion to approve the budgets was offered by Sara Lipow, and approved by unanimous voice vote.

**VII. Policy/Technical Committee Chair**

Lauren Magalska was nominated as the new Policy/Technical Committee Chair by Dan Cress. The nomination was seconded by Brian Baltunis and approved by unanimous voice vote.

**VIII. CAFS representative**

Brian Baltunis was nominated to continue as the OSU CAFS Site Representative. The nomination was seconded and approved by unanimous voice vote.

**IX. Planning 2018 meeting**

Next year's meeting will be held Thursday, Oct 18, 2018

**X. PNWTIRC website**

Susan McEvoy shared the new PNWTIRC website.

**IX. Meeting adjourned**

The meeting adjourned at 3:15 pm.

APPENDIX VI

## Financial Statement 2016-2017

| PNWTIRC Financial Support for Fiscal Year 2016-2017 | |
|---|---|
| Regular members[1] | 98,000 |
| Associate members[1] | 4,000 |
| Contracts | 2,000 |
| Forest Research Laboratory, Oregon State University[2] | 142,602 |
| Total | 246,602 |

[1] Each Regular Member contributed $8,000 or $10,000 and each Associate Member contributed $4,000 excluding in-kind contributions of labor, supplies, etc.

[2] The contribution from Oregon State University includes salaries, facility costs, and administrative support.