# The National Forest Genetics Laboratory (NFGEL)

## Annual Report FY2015

*Inside*



ABOVE: Whitebark pine located at Crater Lake, Oregon. (istock.com).

### MESSAGE FROM THE DIRECTOR

During Fiscal Year 2015 (October 1, 2014 – September 30, 2015), NFGEL completed projects that identified hybrids in native species (our *Sisyrinchium* and butternut projects), provided guidance for species conservation (our Baker cypress and Torrey pine work), and maintained the integrity of reforestation material (in our Douglas-fir, slash pine, loblolly pine, limber pine, and sugar pine projects).

A large suite of people made all this work possible. The interesting questions that NFGEL are asked to address come from engaged, dedicated people committed to managing resources using the best available science. My thanks to all our project partners, both within the Forest Service and those from our collaborating organizations, who continue to use molecular genetics to ensure that we have healthy, sustainable, and resilient forests and grasslands. It also takes talented and enthusiastic people in the lab to work tirelessly to process thousands of samples received in the course of the year. An enormous thank you to the 21 people who worked at NFGEL this last year who generated data, analyzed results, and interpreted information for our managers. Their passion for their work and commitment to the NFGEL and Agency missions are greatly appreciated! And finally, our work could not be completed without the continued support from the Forest Service Washington Office Forest Management staff and guidance of the NFGEL Steering Committee. Thank you everyone for another successful year at NFGEL!

*- Valerie Hipkins*
*October 5, 2015*

NFGEL projects were processed to meet a variety of management objectives. Project results were used to guide restoration and conservation projects, and assist in silviculture and tree improvement activities. Eighteen project summaries are included in this Annual Report.

**Project # and Title**



# NFGEL PROJECTS

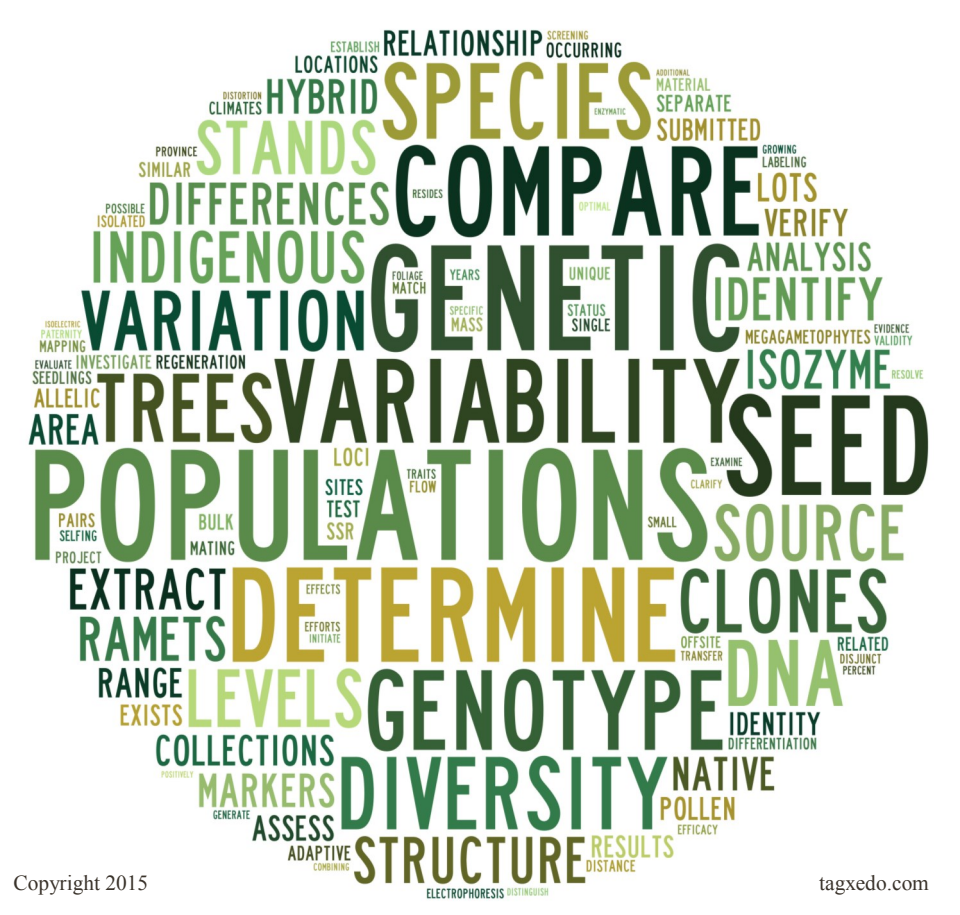
NFGEL develops reliable, effective, low-cost, and time-efficient technologies for characterizing genetic variation in all plant species to aid in adaptive management and planning efforts on forest and rangelands throughout the Nation. Projects are prioritized using a set of seven criteria. Once proposals have been accepted, projects are subject to a ranking within the laboratory scheduling queue dependent on various factors.

**NFGEL Project Prioritization Criteria**

Strategic Alignment
Value to Customer
Availability of Existing Technology
Availability of Sample Material
Importance to Risk Mitigation
Leverage Potential
Project Costs

**NFGEL Project Scheduling Factors**

Date of total sample receipt
Total project sample size
Availability of markers
Availability of operating and appropriate laboratory equipment
Availability of laboratory staff (professional and technician)
Purchase and availability of materials and supplies
Completion of a signed Contract, Agreement, or funds transfer document
Compatibility with other projects in the lab (species, size, laboratory protocols)



# MOLECULAR GENETIC VARIATION DOES NOT CORRESPOND TO SPECIES IDENTIFICATION IN THE *SISYRINCHIUM SARMENTOSUM* – *S. IDAHOENSE* COMPLEX IN OREGON AND WASHINGTON.

Project 258. Partner: USFS—Region 6 (Andy Bower)

*Sisyrinchium sarmentosum* Suksd. ex Greene (Iridaceae), endemic to southern Washington and northern Oregon, is considered a sensitive species by the USDA Forest Service and is currently under consideration for protection under the US Endangered Species Act. A widespread congener, *S. idahoense* E.P. Bicknell, occurs sympatric with *S. sarmentosum* at several occurrences. While previous taxonomic reports have delineated the two as distinct species, ongoing field census and management efforts indicate variable morphology and potential hybridization complicate positive identifications based on morphology. As such, the National Forest Genetics Lab (NFGEL) had been requested to conduct four genetic studies in the past fourteen years in order to inform management efforts.

In NFGEL Project 258, these four studies are reviewed in context of additional phylogenetic studies and government reports in order to summarize the current understanding of evolutionary relationships among *Sisyrinchium* populations. While the number of samples, genetic markers, and study objectives varied from study to study, the interspecific or interpopulation relationships among *Sisyrinchium* collections were consistently complex, and no genetic marker was identified to distinguish between *S. sarmentosum* and *S. idahoense* from the same region, although *S. bellum* was easily distinguished from the other two species.

In this most recent NFGEL study (Project 258) we worked together with Richard Cronn, USFS Pacific Northwest Research Station to examine whole plastid sequence variation in order to determine whether *S. sarmentosum* is a genetically distinct species and whether hybrids with *S. idahoense* are detectable. Whole-plastid sequence variation was assessed by using a hybrid capture technique. The hybrid capture genome reduction technique efficiently captured the plastid genome in the *Sisyrinchium* samples. In general, millions of reads were produced for each individual sample in the multiplexed libraries, with one sample generating nearly 20 million reads. The *Sisyrinchium* plastid genome was estimated to be 145 kbp from the assembled data, with over 103 kbp assessed for variation (the invariant repeat regions were excluded). Genetic variants (SNP's and indels) identified from the assembled sequences revealed distinct variation in *S. bellum* but not between putative *S. idahoense* and *S. sarmentosum*. A total of 205 variants were identified in 35 haplotypes in the complete data set. Low levels of variation were observed within populations.

ABOVE: Common garden planting of *Sisyrinchium* growing at the University of Washington, Seattle, WA, showing variability in flower morphology.

Whole plastid sequence variation failed to resolve species level differences, likely due to any or all of three processes: 1) recent divergence resulting in persistent shared variation; 2) ongoing hybridization producing a species complex rather than distinct lineages; or 3) misidentification based on field morphology upon collection for genetic analyses. Based on NFGEL studies using three data types over 14 years, it is unclear whether the *S. idahoense* and *S. sarmentosum* from the Pacific Northwest constitute two distinct species, one species with two (or more) morphotypes, or a species complex with multiple introgression events. Given the lack of genetic divergence between these putative species, common garden, controlled crosses, and demographic studies are recommended to better inform conservation plans.

## GENETIC DIVERSITY AND POPULATION STRUCTURE IN THE RARE, ENDEMIC BAKER CYPRESS (CUPRESSUS BAKERII): UPDATE.

Project 269. Partner: USFS—Region 6 (Andy Bower)

Preliminary results from an isozyme study characterizing genetic variation in Baker cypress was presented in the FY13 NFGEL Annual Report. Andy Bower (US Forest Service, Region 6 and project lead) has prepared a manuscript of project findings that will be submitted to a peer reviewed journal.

[*Abstract as presented at the WFGA Annual Meeting in Seattle WA by A. Bower*].
Baker cypress (*Cupressus bakerii*) is one of ten species of cypress found on the west coast of North America. It is restricted to a small number of highly disjunct, isolated populations, making it particularly vulnerable to the influences of genetic drift, inbreeding, and reduced gene flow. Baker cypress is fire adapted and its serotinous cones require the heat of a fire to open and release their seeds. Altered fire regimes in some areas have negatively impacted the health and vigor of some populations and lower levels of genetic diversity may make this species more susceptible to the impacts of predicted future climate change. Previously, no information on Baker cypress genetics was available. We used 12 polymorphic allozyme loci to assess genetic diversity and population structure for eight of the 11 known populations of Baker cypress. Overall mean observed heterozygosity (Ho) was 0.178 and expected heterozygosity (He) was 0.204, values higher than for other cypress species and other fire-adapted conifers. Despite the relatively high levels of genetic diversity, in many populations $F > 0$ indicating a deficiency of heterozygotes, most likely due to inbreeding and possibly a Wahlund effect. Population differentiation among seven of the eight populations (the northernmost population excluded) was 9%, considerably lower than for other conifers with disjunct populations. Our results indicate that the current population structure of the species is likely a fairly recent reduction from a formerly widespread distribution.

## RAMET IDENTIFICATION AND PARENTAL VERIFICATION IN DOUGLAS-FIR (PSEUDOTSUGA MENZIESII) CLONES.

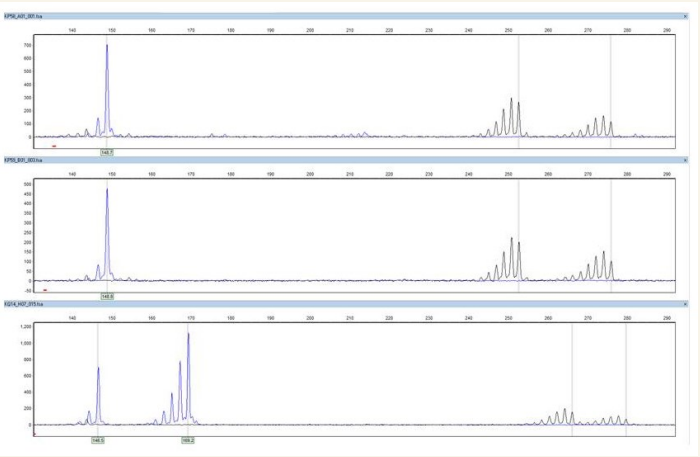
Project 277. Partner: Northwest Tree Improvement Cooperative, Oregon State University.

Members of the Northwest Tree Improvement Cooperative (NWTIC) wished to use molecular markers to verify parentage of forward selections which are being deployed in 2$^{nd}$-cycle production and co-operative 3$^{rd}$-cycle Douglas-fir breeding orchards. Microsatellite data at six loci were used to genotype orchard material to:

1. Confirm clonal identity of submitted ramets.
2. Confirm parentage of forward selections.

NWTIC serves and advises nine smaller, regional advanced-generation cooperative groups. NFGEL received samples for genetic testing from three coop members. Branch tips from a total of 3,691 Douglas-fir trees representing 907 clones (parents/forward selections/etc) were received by the lab. All samples were genotyped at six microsatellite (SSR) loci.

DNA was extracted from all needle samples and genotypes determined at six microsatellite markers developed by the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC), Oregon State University. DNA fragments were analyzed on ABI-3130xl instruments.



Microsatellite data for three individuals of Douglas-fir at two loci.

Clonal identity inconsistencies (mis-labeled ramets or overgrown root-stock) were discovered in approximately 3% of the trees analyzed. Parentage problems were discovered in approximately 15% of forward selections. Parental discrepancies ranged from indicated mothers and/or fathers not being possible parents in some selections, to the identification of the correct genotype of a parent involved in ramet mislabeling within a parental clone.

## DNA FINGERPRINTING ELITE POPULUS CLONES.

Project 285. Partner: Greenwood Resources.

Poplar. Dave Powell, USDA Forest Service (retired), Bugwood.org.

Molecular markers have great utility for fingerprinting elite clonal material. Here, nine microsatellite markers were used to obtain genotypes among 91 poplar samples.

DNA was isolated from desiccated leaf tissue following NFGEL Standard Operating Procedures. The average DNA yield per tree was 6.8 ug. Results at the nine SSR loci indicate distinct genotypes among 89 of the trees analyzed. Two of the 91 trees share the same genotype. The Probability of Identity within the 91 trees sampled (the average probability that two unrelated individuals, drawn from the same population, will by chance have the same multilocus genotype) is $2.7 \times 10^{-13}$.

The number of alleles per locus ranged from four to 32. Three alleles were detected at some loci in some clones. The presence of three or more alleles at a locus indicates polyploidy or gene/chromosome duplications, which are well documented conditions in *Populus*. A Principal Component Analysis (PCA) was conducted in order to find and plot major patterns within the dataset. PCA based on genetic distances between pairs of 'populations' indicate that genetic differences strongly correspond to taxon identity, with the first two coordinates explaining 96.1% of the total variation. All taxonomic groups are well separated except for the TxD and DxT hybrids that group closely together.

# IS A TORREY PINE STAND FOUND IN BOLINAS, CA NATURAL OR PLANTED?

Project 300. Partner: USFS—Pacific Southwest Research Station (Tom Ledig)

Project Background and Objectives

A putative natural population of Torrey pine was found in Bolinas, CA. It is doubtful that it is natural, but if it is, it would certainly be worth noting. About six seed were collected from this Bolinas stand.

The objective of the project is to generate isozyme data on the Bolinas seed and compare the variation to Torrey pine seeds from La Jolla and Santa Rosa Island. The La Jolla trees are completely homozygous at 59 putative isozyme loci (Ledig and Conkle, 1983). Likewise, pines on Santa Rosa Island are also homozygous. However, the two populations are fixed for different alleles at a malate dehydrogenase locus and a shikimate dehydrogenase locus. The US Forest Service Institute of Forest Genetics has seeds of Torrey pine that were collected back in the 1980's that could be run in comparison to the Bolinas material. If the Bolinas stand is a natural population, it would likely be polymorphic for those two loci – or at least different from either of the two known populations. More likely, it will be identical to the La Jolla population, a result of planting (the La Jolla, mainland, subspecies is in the nursery trade and used in landscaping around San Diego).

Material and Methods

NFGEL received seven seed from the Bolinas, CA stand on 3/28/2014. NFGEL also obtained seed from 16 trees from Santa Rosa Island and Torry Pine State Reserve, La Jolla from the US Forest Service Placerville Nursery seed bank on 7/16/2014.

Seed was soaked overnight in 1% $H_2O_2$ and plated on 1% $H_2O_2$ soaked kimpack in a petrie dish. Seed was refrigerated for up to 30 days. After stratification, seed was placed in a germinator at standard conifer seed settings used at NFGEL. [Bolinas soak – 6/26/14; strat start 6/27/14; germinator start 7/25/14. Santa Rosa and La Jolla soak – 7/16/14; start strat 7/18/14; germ start 8/18/14.]

Megagametophyte/embryo pairs were prepared for isozymes from each seed by submerging approximately 50mg of tissue in 100ul 0.2 M phosphate buffer, pH 7.5. Isozyme preparations were made on 8/15/14 and 8/29/14. A total of six seed germinated and were prepared from the Bolinas stand, 7 seed from the Santa Rosa stand, and 8 seed from the La Jolla stand. Each seed from the Santa Rosa and La Jolla stands were from different mother trees.

On 10/16/2014, the 21 meg/embryo pairs were run on 11% starch gels and stained for 15 enzyme systems using three gel buffers (LB, SB, MC8) following NFGEL Standard Operating Procedures. Samples were scored at 23 loci (PGM, PGI1, PGI2, ADH, LAP1, LAP2, FEST, CAT, UGPP, TPI, AAT1, AAT2, AAT3, SOD, IDH, MDH1, MDH2, MDH3, SKD1, SKD2, 6PGD1, 6PGD2, and DIA).

Results and Discussion

We ran 6 seed from Bolinas, 7 seed from Santa Rosa Island, and 8 seed from Torrey Pine State Reserve in La Jolla. Our results followed the Evolution paper in that all samples from each of the three stands were homozygous, with the malic dehydrogenase and shikimate dehydrogenase loci homozygous for difference alleles. At 21 loci, all 21 seed shared the same monomorphic genotype (homozygous at all loci for a single allele). At the MDH1 locus, the Bolinas and La Jolla seed shared the same homozygous genotype, while the Santa Rosa seed were homozygous for an alternate allele. This is also true for the SKD1 locus: the Bolinas and La Jolla seed shared the same homozygous genotype, while the Santa Rosa seed were homozygous for an alternate allele. Therefore, the Bolinas seed was identical to that from La Jolla, and indicate that the Bolinas stand is likely planted and not natural in origin.

Reference: Ledig, FT and MT Conkle. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex Carr.). Evolution 37(1): 79-85.

# SPECIES IDENTIFICATION OF *CRONARTIUM*.

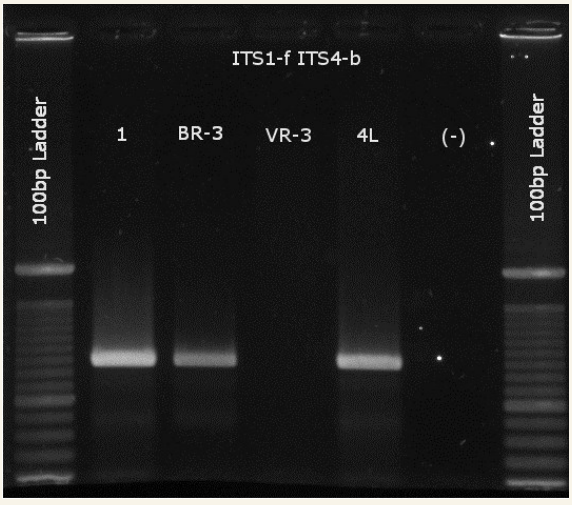
Project 301. Partner: USFS—Pacific Southwest Research Station (Detlev Vogler)

Spores were collected from either whitebark or limber pine (*Pinus albicaulis* and *P. flexilis*) in northeastern Nevada on Pine Mountain, approx. 8600 feet elevation. These are typical isolated desert mountain distributions, with pines only at the tops of the higher peaks. The spores were collected, sieved, and delivered to NFGEL in laboratory tubes.

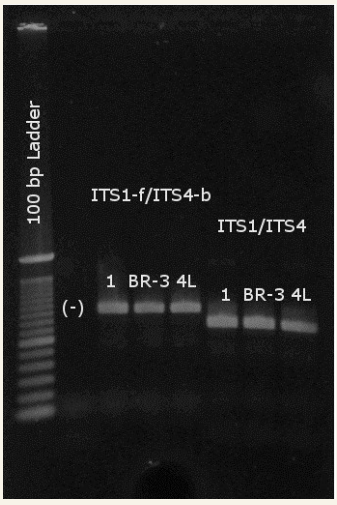
The project objective was to confirm *Cronartium ribicola* as the pathogen from at least one representative of each host type through DNA sequencing of the ITS region. This sequencing was meant to document the first new find of blister rust in northeastern Nevada since Vogler and Charlet reported the fungus on whitebark pine in the Jarbidge Mountains in 2004. This new find would be 7 air miles WSW of the former find. Unlike elsewhere where pine host type is generally continuous, in these desert island ranges the white pine hosts are spotty and scattered atop a few isolated mountain tops, and the distributions of the obligate alternate hosts (species of *Ribes,* gooseberry and currant) are similarly disjunct. Nevertheless, the pathogen seems to have an uncanny ability to get around by wind-blown spores, despite its hosts being distributed sparsely. (Provided by D. Vogler)

DNA was extracted from the spore samples using a modified Qiagen DNeasy plant kit protocol. ITS amplification was achieved using Phusion taq and master mix. The amplification product was separated on a 1% agarose gel, and the ~900bp band excised and purified. The cleaned DNA fragments were sequenced using Big Dye 3.1 Ready mix and one of four ITS primers on ABI-3130xl instruments. All sequences were examined for sequence quality, aligned, and then trimmed, eliminating fringe areas of low quality near primer attachment loci typical in Sanger sequencing. After trimming, all three individual samples were determined to be identical and a consensus sequence for the group was extracted from the data set. Top blast hits for the consensus sequence are all *Cronartium ribocola* sequences confirming that *Cronartium ribicola* is the pathogen from the collected spores.

Final sample preps:




PCR1 for gel excision




PCR2 for sequencing and sizing of region of interest

# DISTINGUISHING BUTTERNUT (JUGLANS CINEREA) FROM JAPANESE WALNUT (J. AILANTIFOLIA) AND THEIR HYBRIDS.

Project 302. Partner: USFS—Region 8 (Barbara Crane)
Project 306. Partner: USFS—Region 9 (Scott Rogers and Paul Berrang)

The taxonomic status and identification of butternuts and their hybrids can be complicated at the phenotypic and genotypic levels. Many butternut hybrids are likely $F_2$'s, products of backcrosses, or other, even more complex hybridization events. In FY15, NFGEL completed two projects to assess the species and hybrid identity of submitted putative butternut samples. One project contained tree samples submitted by Region 8 (Project #302) and the other project originated from Region 9 (Project #306).

This work used three molecular markers (markers *trnT-F*, ITS, and 22-5) from Zhao and Woeste (2010) to assess the species and hybrid identity of submitted putative butternut samples. Methodology followed protocols developed by Dr. Keith E. Woeste, US Forest Service, Hardwood Tree Improvement and Regeneration Center, Purdue University, West Lafayette, IN.

The molecular markers developed by Zhao and Woeste (2010) do not "prove that a specimen is a butternut; (it) can only show or fail to show evidence that it is a hybrid." These markers recognize introgressed genomic fragments from Japanese Walnut (genomic introgression from Japanese Walnut into butternut, or in other words, past hybridization of Japanese Walnut and butternut). At these three genetic markers used in this project, Zhao and Woeste did find some known hybrids that shared the same markers with butternut. To distinguish these groups, further markers would have to be run: 15R-8 (though the Type 2 error is high in this marker), 39-6, and/or 40-1 (NFGEL could not reproduce this marker in our lab).

**Methods**

Leaf tissue was received from 50 trees from Region 9 and 264 trees from Region 8 for a total of 314 samples. DNA was extracted from all samples using the Qiagen DNEasy 96-well standard protocol, following the NFGEL liquid nitrogen extraction procedure. Yields were estimated using a Gemini XPS 96-well plate fluorometer and pico green fluorescence and ranged from 0.7 to 14.1 ug per sample (average yield = 6.4ug). All samples were amplified using three markers (Zhao and Woeste 2010): 22-5 (sequence characterized amplified region (SCAR) of nuclear DNA), *trnT-F* (a chloroplast marker), and ITS (internal transcribed spacer region of nuclear DNA). Markers were either visualized on an ABI-3130xl or on 1.4% agarose gels stained with gel red under UV light.


*Juglans cinerea* (butternut). Rob Routledge, Sault College, Bugwood.org

**Results and Discussion**

Analysis of the DNA data was completed by assigning a haplotype or pattern per locus to each individual based on the presence of specific bands. Amplification of the *trnT-F* locus followed by digestion with MboII produced two haplotypes: haplotype 1 which contained one band at 460bp, and haplotype 2 which contained one band at 390bp. The amplification of the ITS region and subsequent digestion with BSiEI produced two distinct patterns: pattern A was distinguished by two bands at 430 bp and 305 bp, and pattern B contained three bands at 305 bp, 240 bp, and 195 bp. And finally, amplification of the 22-5 locus produced up to five patterns: pattern A contained a single fragment at 651bp, pattern B contained a single fragment at 698bp, pattern C contained one 702bp fragment, pattern D contained a 688bp fragment, and pattern E contained a 640bp fragment. Using control DNA from prior NFGEL butternut identification projects, we confirmed that all three molecular markers revealed fixed differences between *Juglans cinerea*, *J. ailantifolia*, and *J. nigra*. Possible marker phenotypes for *J. cinerea*, *J. ailantifolia,* and butternut hybrids were also identified by Zhao and Woeste (2010). One multilocus phenotype is possible for all *J. cinerea* individuals, and a different multilocus genotype is found among all *J. ailantifolia* trees. Hybrid trees have been characterized by six different phenotypes and provide evidence of Japanese walnut introgression into butternut.

**Conclusion**

For Region 9 material:

Fifty samples were submitted for analysis. Genetic data confirmed that 46 of these samples are *J. cinerea*, and four are *J. cinerea* hybrids (two of which were pre-identified as hybrids at the time of sample submission).

For Region 8 material:

Of the 264 total trees submitted for analysis, 15 of the trees are consistent as being Japanese Walnut or Black Walnut,

82 of the trees are products of a variety of simple and complex hybridization events of *J. cinerea* with Japanese Walnut (or possibly other compatible *Juglans* species),

and 167 trees are consistent with butternut identity (no evidence of hybridization with another species was detected).

**Reference:** Zhao, P and KE Woeste. 2010. DNA markers identify hybrids between butternut (*Juglans cinerea* L.) and Japanese walnut (*Juglans ailantifolia* Carr.). Tree Genetics & Genomes DOI 10.1007/s11295-010-0352-4

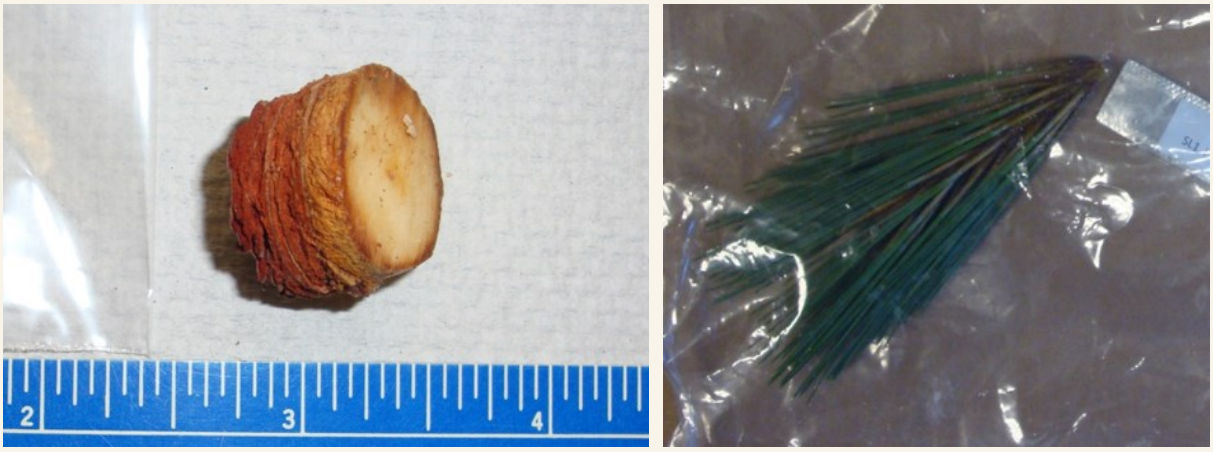
Table: Project 302 data at three molecular markers used for hybrid identification.

| 22-5 Pattern | *trnT-F* Haplotype | ITS Pattern | # Samples (Trees) | Identification Call |
|---|---|---|---|---|
| A | 1 | A | 167 | Butternut |
| C | 2 | B | 11 | Japanese Walnut |
| B | 1 | B | 4 | Black Walnut |
| AC | 2 | AB | 17 | Hybrid |
| A | 2 | A | 14 | Hybrid |
| AC | 2 | B | 11 | Hybrid |
| AC | 2 | A | 10 | Hybrid |
| A | 2 | AB | 8 | Hybrid |
| C | 2 | AB | 5 | Hybrid |
| A | 2 | B | 5 | Hybrid |
| C | 2 | A | 4 | Hybrid |
| AC | 1 | AB | 3 | Hybrid |
| C | 1 | AB | 2 | Hybrid |
| A | 1 | AB | 1 | Hybrid |
| CD | 2 | AB | 1 | Hybrid |
| AE | 1 | A | 1 | Hybrid (or possibly previously undetected variation within butternut) |

## RAMET IDENTIFICATION AND PARENTAL VERIFICATION IN SLASH AND LOBLOLLY PINE CLONES.

Project 303. Partner: Private Company

Molecular markers were used to identify ramets and verify parents from a total of 1,270 slash and loblolly pine trees. DNA was obtained from either needle tissue or from plugs of wood taken from tree trunks. Six microsatellite loci were used to assess DNA variability among the slash and loblolly pine study samples. These markers were developed by Dr. Craig Echt and Sedley Josserand, US Forest Service, Southern Institute of Forest Genetics, Saucier, MS. Genetic data was able to determine that 3.4% of the ramets submitted for analysis were mislabeled (mislabeling of the ramet, overgrown rootstock, etc). In addition, the genetic data did not support parental identities for some progeny within seven families of loblolly pine.

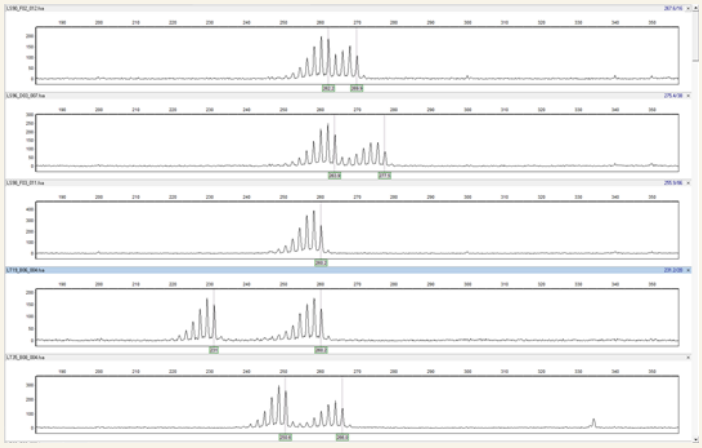


Sample material used for DNA extraction in genetic testing of ramets and progeny.

## GENETIC FINGERPRINTING: RAMET IDENTIFICATION IN DOUGLAS-FIR (PSEUDOTSUGA MENZIESII) CLONES.

Project 307. Partner: Private Company

Six microsatellite loci developed by the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) were used to assess DNA variability among 403 Douglas-fir study samples for the purpose of confirming clonal identity. The SSR data were able to resolve 20 unique genotypes among the individual samples. Each of the 19 clones had unique genotypes except for Clone 1 that had two detected genotypes among four ramets submitted. Three of the four ramets shared a genotype that matched no other in the dataset. The fourth ramet had a different genotype that also matched no other genotype in the dataset. Therefore, only 1 out of 403 trees (0.2%) analyzed contained mislabeling (mislabeling of the ramet, overgrown rootstock, etc).



Microsatellite data for five Douglas-fir trees at one locus.

## LIMBER PINE FAMILY IDENTIFICATION.

Project 308. Partner: USFS—Rocky Mountain Research Station (Anna Schoettle)

Genetic testing was requested to confirm the identity of two limber pine families. The two families were being used to test the association of stress tolerance traits with rust resistance. The correct identities were needed to improve our understanding of (1) rust resistance mechanisms, (2) ecological costs of rust resistance, and (3) seed transfer for restoration plantings.


Dave Powell, USDA Forest Service (retired), Bugwood.org

NFGEL conducted an allozyme analysis on 105 seed and vegetative bud samples. Resulting allozyme variation among samples was low so conclusions were limited. The two mother trees only varied at three isozyme loci and shared the common allele at the other 18 loci assessed. Open pollinated progeny were assessed for family identity at these three diagnostic isozyme loci, though they were genotyped at all 21 loci. Even given the shared allozyme alleles among the two mothers, we were still able to determine that some progeny were misidentified. More variable molecular markers would have allowed for a more definitive family identification of progeny. Unused tissue from each sample has been saved in the NFGEL -80C freezers for possible DNA extraction and analysis at more variable markers if desired in the future.

## DNA EXTRACTION FROM DOUGLAS-FIR FOR SNP DEVELOPMENT.

Project 309. Partner: USFS—Pacific Northwest Research Station (Richard Cronn)

The objective of Project 309 was to extract DNA from submitted samples of Douglas-fir for SNP marker development.

NFGEL received either individual seedlings or mature needles of Douglas-fir between 9/30/14 and 10/2/2014. Two of the boxes arrived frozen and were placed at -20C upon receipt. The other three boxes were delayed for two days in shipment and arrived warm and were immediately placed at 4C.

DNA was isolated using the Qiagen DNeasy 96 format kit with the liquid nitrogen and proteinase-K modifications. Samples were eluted in 100ul AE buffer, concentration readings were determined with pico green, and DNA mailed to R. Cronn. Samples were eluted with a second volume of 100ul AE buffer and that DNA was retained at NFGEL frozen at -80C. The average DNA yield per sample was 11.0 ug from the first elution and 4.4 ug from the second elution. The DNA yields were twice as high from the seedlings compared to the needles (12.0 ug vs 6.6 ug, respectively (first elution)). DNA yields were not decreased in samples that arrived warm. However, we did not test for DNA quality in these samples. There were a total of 400 DNA extractions that included 398 samples (2 samples were extracted a second time because their collection tubes broke during homogenization in the mixer-mill). Plates of DNA were frozen at -80C and shipped on dry ice via overnight UPS on December 2, 2014. All remaining plant tissue was returned in the shipment.

# VERIFICATION OF CLONAL GENOTYPES IN DOUGLAS-FIR (PSEUDOTSUGA MENZIESII).

Project 311. Partner: Private Company

The objective of this project was to genotype Douglas-fir trees from a tree improvement program to verify their clonal genotypes.

Branch tips from a total of 60 Douglas-fir individuals were received by NFGEL. Ramets received were from 12 uniquely labeled clones. Four samples were duplicate collections resulting in 56 unique samples and 4 duplicate samples. After removing the duplicate samples, there was an average of 5 ramets/clone, ranging from 18 to 3 ramets/clone. DNA was extracted using the Qiagen DNeasy-96 format plant kit following NFGEL Standard Operating Procedures (SOPs) with the liquid nitrogen bench-top procedure. DNA was isolated from individual trees using approximately 50 mg of needle tissue per tree. Tissue was minced by hand into 1-2mm long pieces and placed in a Qiagen DNeasy-96 format plant kit collection tube. Tissue was ground to a powder by processing liquid nitrogen frozen needle pieces on the Qiagen Mixer-Mill with tungsten beads. All DNA samples were quantified using a Gemini XPS Microplate Spectrofluorometer (Molecular Devices, Sunnyvale, California, USA) with PicoGreen dsReagent (Invitrogen, Carlsbad, California, USA).

Douglas-fir. IStock.com

Six markers developed by the Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) were used to genotype the samples. PCR/ABI conditions followed NFGEL Standard Operating Procedures and DNA fragments were analyzed on an ABI-3130xl instrument. Peaks were sized and binned by hand, and then alleles were called using GeneMarker 1.95 (SoftGenetics, State College, PA), with GS-ROX as an internal size standard for each sample.

The SSR data were able to resolve 16 unique genotypes among the 60 samples. The Probability of Identity (PI) (the average probability that two unrelated individuals, drawn from the same population, will by chance have the same multilocus genotype) is $8.3 \times 10^{-8}$, indicating sufficient discriminatory power of the data for distinguishing unique genetic individuals. Genotypic matches and comparisons among samples were made and inferences regarding mislabeling were determined.

## DNA EXTRACTION FROM DESICCATED DOUGLAS-FIR NEEDLES.

Project 312. Partner: Northwest Tree Improvement Cooperative, Oregon State University.

An effort is underway at Oregon State Univeristy (OSU) to selectively breed Douglas-fir for traits amenable for biofuel use while retaining wood value for traditional uses. NFGEL is aiding this effort by isolating DNA from desiccated Douglas-fir needle samples and shipping samples to OSU. DNA was extracted from approximately 300 samples using the Qiagen DNEasy 96-well standard protocol, following the NFGEL liquid nitrogen extraction procedure with the addition of proteinaseK. Yields were estimated using a Gemini XPS 96-well plate fluorometer and pico green fluorescence. The average yield per sample was 8.9 ug (ranging from 0.9 to 46.8 ug). The average DNA concentration per sample was 49.5 ng/ul (ranging from 4.8 to 259.9 ng/ul).


Sample of desiccated Douglas-fir needle tissue.

## RAMET IDENTIFICATION AND PARENTAL VERIFICATION IN DOUGLAS-FIR (PSEUDOTSUGA MENZIESII) SAMPLES.

Project 313. Partner: Northwest Tree Improvement Cooperative, Oregon State University.

NFGEL completed the genotyping of 24 additional Douglas-fir samples provided to our lab as follow-up work to Project 277. There were two objectives for this work: (1) determine the correct clonal identify of 14 specific ramets and (2) determine if two specific trees are open pollinated siblings of a known female parent.

All methods for DNA isolation and microsatellite analysis follow those used in NFGEL Project 277. Genetic data was successfully able to clonally identify all submitted ramets. Data also determined that the two progeny trees can be open pollinated siblings of the same female parent.

# GENETIC FINGERPRINTING MYSTERY POPLARS.

Project 314. Partner: Greenwood Resources

Poplars. IStock.com

NFGEL completed the genotyping of 6 poplar samples provided to our lab on 2/25/2015 and 3/3/2015. These samples were genotyped at the same nine microsatellite loci that were used in our prior genotyping work from NFGEL Projects 261and 285. The objectives of this work was to determine if each submitted sample belonged to the same clone, and to identify the contaminant by comparison to the library of fingerprints generated in prior NFGEL Projects.

Total genomic DNA was isolated from the terminal bud or from cambial tissue using the Qiagen® DNEasy-96 plant kit following the liquid nitrogen procedure with the addition of proteinase-K. DNA concentrations were quantified using fluorometry with picogreen. DNA was extracted multiple times per sample. The average DNA yield per sample was 5.8 ug. Our reaction conditions, PCR programs, and ABI protocols follow those used in NFGEL Project 261. The results were confirmed by replicating amplification of all loci for all samples from two to three times each. Error rates for these data are near zero, except for loci 287 and 2731, which had error rates of 2.1% and 1.8%, respectively.

Results at nine microsatellite loci indicate that a single genotype is shared by the three samples received on 2/25/2015. A different genotype is shared by the three sample received on 3/3/2015. When the two new genotypes were compared to the library of fingerprints generated at NFGEL, we were able to match one of the two genotypes to a sample in the library. The second genotype was unique and not found in the library.

# SUGAR PINE BLISTER RUST RESISTANT SEEDLOT DNA EXTRACTION.

Project 315. USFS—Region 5 (Joan Dunlap and Arnaldo Ferreira)

White pine blister rust is a major threat to high elevation white pines, including sugar pine (*Pinus lambertiana*). Sugar pine contains a major gene that confers resistance to the disease. The hypersensitive reaction (HR) is a well-understood resistance-virulence system of the white pine blister rust pathosystem. The host R gene is associated with the HR response. However, molecular characterization of the gene involved in resistance is difficult due to the large size of the conifer genome.

We extracted DNA from known "R" and known 'r' sugar pine megagametophytes to serve as testing material that is available to researchers to aid in the dissection of the blister rust plant-pathogen system.

NFGEL received two seed from each of 81 sugar pine families for DNA extraction. The R5 Sugar Pine Rust Resistance Program determined that 40 of the mother trees are the "ss" genotype, 39 are "RR", and two are "Rr". Therefore, there were a total of 162 seed received (2 seed from each of 81 families).

All seed was soaked for 48 hrs in 1% $H_2O_2$ at room temperature. Megagametophytic tissue was dissected into Qiagen collection tube racks, avoiding the transfer of diploid material. Meg tissue from each seed was dissected into four replicate racks resulting in 8 total dissections for all sample seed (each seed was dissected four times; two seeds per family; 8 total dissections per family were completed). A seed was dissected into four smaller quantities due to limitations of the spin columns used during DNA extraction. An entire sugar pine meg would plug the filter resulting in little or no DNA yield. Therefore, each seed was dissected into four smaller amounts so as to not inhibit the functioning of the filter during extraction while maximizing DNA yield per seed. Also, each seed per family was handled and extracted separately, in case there are genotyping errors in the mothers.

DNA was isolated from two racks (dissections) per seed. The other two racks per seed containing dissected meg tissue are being held at -80C for possible future DNA extraction.

DNA extraction was conducted using Qiagen DNeasy 96-well format plant kits with the addition of a proteinase-K step. Meg tissue was ground to a powder under liquid nitrogen with a tungsten bead on the Qiagen Mixer-Mill before the addition of AP1 buffer. DNA concentrations were quantified using a Gemini XPS Microplate Spectrofluorometer (Molecular Devices, Sunnyvale, California, USA) with PicoGreen dsReagent (Invitrogen, Carlsbad, California, USA).

DNA was isolated from a total of 324 samples (81 families * 2 seed * 2 extractions). The average DNA yield per extraction was 17.8 ug. The average DNA yield per seed (the two extractions from one seed totaled together) was 35.6 ug. The average DNA yield per family (two extractions from each of two seed) was 71.2 ug.

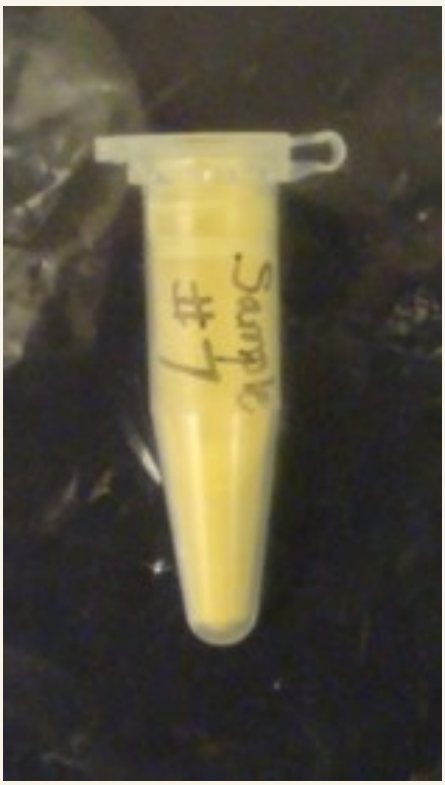
Sugar pine. IStock.com

## CLONAL IDENTIFICATION OF DOUGLAS-FIR SAMPLES.

Project 318. Partner: Northwest Tree Improvement Cooperative, Oregon State University.

The objective of this work was to verify the clonal identity of two samples of Douglas-fir pollen stored from untested trees. Two vials of Douglas-fir pollen were received on May 1, 2015. DNA was isolated two independent times from each pollen sample. Two negative DNA isolation samples were also prepared. DNA was extracted from between 5mg and 20mg of pollen per isolation.

Pollen was placed in Qiagen DNeasy-96 format plant kit collection tubes containing tungsten beads. Tissue was ground to a powder by processing liquid nitrogen frozen pollen on the Qiagen Mixer-Mill. A total of four DNA extractions were carried out following NFGEL SOPs using the liquid nitrogen bench-top procedure with the addition of Proteinase-K. DNA concentrations were quantified using a Gemini XPS Microplate Spectrofluorometer (Molecular Devices, Sunnyvale, California, USA) with PicoGreen dsReagent (Invitrogen, Carlsbad, California, USA). The average DNA yield was 8.5ug (7.1ug, 7.3ug, 9.1ug and 10.5ug for the four samples). Each DNA sample was genotyped at the six SSR loci, with two of the samples amplified a second time. Therefore, each pollen sample had two independent DNA extractions, and a total of three replicated amplifications. Genotypes among the three fingerprints per pollen sample were identical at all six SSR loci. Both pollen samples shared the same matching genotype and matched a genotype from our Douglas-fir genotype library.



Vial of conifer pollen.

# NFGEL REQUESTS FOR ASSISTANCE

In addition to accepting and processing Projects, NFGEL also fulfills requests for assistance. A Project is characterized by (1) the presence of a project plan with defined management objectives and sampling design, (2) the receipt of plant material needed to meet objectives, (3) a strategy for the laboratory methodology needed to meet objectives, and (4) the design for the interpretation and reporting of results. Significant work done by NFGEL Staff outside the Project definition is captured by the use of "Requests for Assistance (RAF)" numbers.

## 15-RAF011: Genetic banking of whitebark pine needle tissue.

Partner: USFS—R6 (Richard Sniezko)

NFGEL was requested to develop a bank for desiccated whitebark pine needles that could be used in the future for DNA studies. We received samples from October 10, 2014 through August 14, 2015. A total of 715 trees from 54 stands were received. Desiccant was added to many samples, and all samples were vacuum sealed and placed in the NFGEL drying cabinets. A database of sample information was built using Excel.

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## 15-RAF012: Identification of the origin of unusual, fragmented stands of aspen in the Willamette Valley.

Partner: Oregon State University

A graduate student in the Strauss Lab is working on a project to identify the origin of unusual, fragmented stands of aspen in the Willamette Valley. The hypothesis is that these aspens represent the living remnants of the catastrophic "Missoula floods" that inundated the Willamette Valley over 10,000 years ago. He will use a GBS (genotyping by sequencing) approach for DNA fingerprinting material, and is looking for aspen samples from the west that could provide context for phylogenetic interpretation.

DNA was requested from past NFGEL projects. We sent DNA from 4 genotypes from Walla Walla (Project 262); 5 genotypes from Fresno (Project 282); and 5 genotypes from Lassen (Project 150). DNA samples were transferred to 1.5ml microfuge tubes and frozen at -80C. Samples were mailed overnight on dry ice. We did not have 10ug from any one sample (tree). However, we did have DNA from multiple Individual sample tubes were labeled with "NFGEL #".

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## 15-RAF013: Testing moisture content of Pongamia seeds.

Partner: TerViva

We were requested to test the residual moisture content in some recently collected Pongamia seeds. Pongamia is an oilseed species used in industrial products and biofuels. Moisture content of 26 seedlots needed to be checked. Moisture content was checked with the Placerville Nursery's Ro-Tronic Hygropalm 22. Seed was dried to 6– 9% moisture content in a room @ +- 39-44% relative humidity and 69° to 74°F.

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## 15-RAF014: Genetic banking of sugar pine needle tissue.

Partner: USFS—R6 and R5 (Richard Sniezko and Joan Dunlap, respectively)

NFGEL was requested to develop a bank for desiccated sugar pine needles that could be used in the future for DNA studies. We received samples from May 2015 through September 2015. About 2 to 10 fascicles per tree were put into individual coin envelopes, each envelope containing about 1 to 2 Tbsp of Fisher silica desiccant. After checking sample information into the excel database, needle tissue was vacuum sealed and placed in the NFGEL drying cabinets.

# NFGEL Staffing and Organization

## NFGEL Steering Committee

NFGEL is guided by a Steering Committee made up of Agency professionals with an interest in the genetic assessment of our nation's resources. Steering Committee members:

1. oversee and ensure the accomplishments of the agreed upon work of NFGEL,
2. assist in setting national priorities for NFGEL workload, and
3. assist in securing necessary resources to accomplish the program of work.

| Member | Position | Location |
|---|---|---|
| **John Crockett, Chair** | Assistant Director, Forest Management | Washington Office, Washington, DC |
| **Tom Blush** | Regional Geneticist | Region 5, Placerville, CA |
| **Barbara Crane** | Regional Geneticist | Region 8, Atlanta, GA |
| **Keith Woeste** | Molecular Geneticist and Hardwood Breeding | Northern Research Station, West Lafayette, IN |
| **Gary Man** | Forest Health Specialist, State and Private Forestry | Washington Office, Washington, DC |
| **David Pivorunas** | Assistant Threatened, Endangered, and Sensitive (TES) Species Program Leader; Watershed, Fish, Wildlife, Air, and Rare Plants | Washington Office, Washington, DC |
| **Larry Stritch** | National Botanist, Rangelands Management & Vegetation Ecology | Washington Office, Washington, DC |
| **David Gwaze** | National Silviculturist and Genetics Lead | Washington Office, Washington, DC |

# NFGEL Staff

NFGEL work in FY15 was conducted by 22 talented people. The total full-time equivalents (FTEs) among employees was 4.21 (2.65 permanent FTEs including 3 employees and 1.56 temporary FTEs comprising 19 staff members). Temporary staff included those on contract, college and high school interns, volunteers, and 1039 positions.

| EMPLOYEE | POSITION | TOUR (% FTE for year) | DATES |
|---|---|---|---|
| **Valerie Hipkins** | Director | Permanent (100%) | 10/1/14 – 9/30/15 |
| **Jennifer DeWoody** | Geneticist | Permanent (100%) | 10/1/14 – 9/30/15 |
| **Randy Meyer** | Lab Biotechnician | Permanent (65%) | 10/1/14 – 9/30/15 |
| **Jacob Snelling** | Lab Biotechnician | Contract (46%) | 3/2/15 –8/14/15 |
| **Anne Russell** | Lab Biotechnician | Temp-1039 (31%) | 1/12/15 – 4/24/15 |
| **Garrett Short** | Lab Biotechnician | Temp-1039 (15%) | 6/22/15 – 8/14/15 |
| **Jian Alsarraj** | Lab Biotechnician | Temp-1039 (15%) | 6/24/15 – 9/30/15 |
| **Andrew Jackson** | Lab Biotechnician | Contract (10%) | 6/22/15 – 8/27/15 |
| **Keenan Raleigh** | Lab Biotechnician | Contract (10%) | 5/19/15 – 7/17/15 |
| **James Boom** | Lab Biotechnician | Temp-1039 (5%) | 11/3/14 – 4/30/15 |
| **Courtney Canning** | Lab Biotechnician | Temp-1039 (3%) | 10/1/14 – 1/5/15 |
| **Reginald King** | Lab Biotechnician | Temp-1039 (3%) | 10/1/14 – 1/5/15 |
| **Edward Sprigg** | Lab Biotechnician | Temp-1039 (3%) | 8/6/15 – 9/24/15 |
| **Amanda Cutler** | Lab Biotechnician | Temp-1039 (4%) | 8/25/15 – 9/30/15 |
| **Nick Freij** | Intern | American River College (2%) | 6/17/15 –7/17/15 |
| **Lee Ann Norton** | Intern | American River College (1%) | 3/23/15 –4/6/15 |
| **Brendan Aherns** | Intern | American River College (2%) | 10/1/14 –12/5/14 |
| **Bailey Jones** | Volunteer | Sonoma State University (1%) | 5/19/15 –5/28/15 |
| **Laura Polkinghorn** | Volunteer | El Dorado High School (1%) | 10/1/14 –12/8/14 |
| **Morgan Linville** | Volunteer | El Dorado High School (2%) | 10/1/14 –2/20/15 |
| **Aubrey Carr** | Lab Biotechnician | Contract (1%) | 9/18/15 –9/30/15 |
| **Anika Morkowski** | Lab Biotechnician | Contract (1%) | 9/18/15 –9/30/15 |

# STAFF ACTIVITIES

## NFGEL Publications

- Potter, KM, VD Hipkins, MF Mahalovich, and RE Means. 2015. Nuclear genetic variation across the range of ponderosa pine (*Pinus ponderosa*): Phylogeographic, taxonomic and conservation implications. Tree Genetics and Genomes 11:38. 23p.

- Shinneman, DJ, RE Means, KM Potter, and VD Hipkins. 2015. Exploring climate niches of ponderosa pine (*Pinus ponderosa* Douglas ex Lawson) haplotypes in the western United States: implications for evolutionary history and conservation. PLOS ONE (In Review)

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## Presentations

- Hipkins, VD. Linking science to management: using DNA to support resource decisions. Sustainable Landscape Management Board of Directors (SLMBOD) Meeting. US Forest Service, Washington Office. Washington DC (presentation by virtual attendance). October 1, 2014.

- Hipkins, VD. Timber related forensics using laboratory genetics in the US Forest Service. Expert Group Meeting on Forensic Analysis in Support of Law Enforcement Operations Related to Forest Crime. United Nations, Vienna, Austria. December 10 – 12, 2014.

- Hipkins, VD. Linking science to management: using genetics to support resource decisions. Forest Management Staff Retreat. US Forest Service, Washington Office, Washington DC. January 21, 2015.

- DeWoody, J. Highlights of Plant and Animal Genome. For the community of IFG. Placerville, CA. January 29, 2015.

- Hipkins, VD. Linking science to management: using DNA to support resource decisions. National Advanced Silviculture Program (NASP) –Region 5/6 Module. Placerville, CA. June 12, 2015.

- Bower, AD and VH Hipkins. Genetic diversity and population structure in the rare, endemic Baker cypress (*Cupressus bakeri*). Joint Western Forest Genetics Association (WFGA)/Northwest Seed Orchard Managers Association (NWSOMA) Meeting. Seattle, WA. June 23 – 24, 2015.

- Potter, KM, BS Crane, and VD Hipkins. Project CAPTURE: a national prioritization assessment of tree species for conservation, management and restoration. Joint Western Forest Genetics Association (WFGA)/Northwest Seed Orchard Managers Association (NWSOMA) Meeting. Seattle, WA. June 23 – 24, 2015.

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## Professional Activities

- Continued technology transfer to use allozyme electrophoresis to identify species of *Botrychium* (moonwort ferns), working with Donald Farrar from Iowa State University. (DeWoody)

- DeWoody, J., H. Trewin, and G. Taylor. 2015. Genetic and morphological differentiation in *Populus nigra* L.: Isolation by colonization or isolation by adaptation? Molecular Ecology 24(11): 2641-2655.

## Attendance

- Expert Group Meeting on Forensic Analysis in Support of Law Enforcement Operations Related to Forest Crime. United Nations, Global Program for Combating Wildlife and Forest Crime. Vienna, Austria. December 10 – 12, 2014. (Hipkins)
- Plant and Animal Genome Conference XXIII. San Diego, CA. January 10-14, 2015. (DeWoody)
- US Forest Service, Washington Office, Forest Management Staff Retreat. Washington DC. January 20 – 23, 2015. (Hipkins and DeWoody)
- Reforestation Summit. US Forest Service, Washington Office. Washington DC. April 12 – April 16, 2015. (Hipkins)
- Joint Western Forest Genetics Association (WFGA)/Northwest Seed Orchard Managers Association (NWSOMA) Meeting. Seattle, WA. June 23 – 24, 2015. (Hipkins)

## Technical Review

- Advisory Committee, American River College Biotechnology Program. (DeWoody)
- Advisory Committee, Folsom Lake College Work Experience Program. (DeWoody)

## Team Participation

- Member of USDA Forest Service National Safety Committee (January 2001 - current). (Meyer)
- Member of the PSW Placerville Safety Committee. (2013 –current, DeWoody; 2001 – current, Meyer)
- Interim Site Collateral Duty Safety Officer. July 2015 – current. (DeWoody)
- Member of the PSW-RS Community Enhancement and Diversity (civil rights) Team. (Meyer)
- Co-Lead (with David Gwaze) of organizing committee for "Gene Conservation of Forest Trees – Banking on the Future" workshop being planned for spring/summer 2016. (Hipkins)
- Member of the Forest Management Complexity Team. US Forest Service, Washington Office. (DeWoody)
- Member of the Forest Management Communications Team. US Forest Service, Washington Office. (Meyer)
- Member of the Forest Management Career Development and Mentoring Team. US Forest Service, Washington Office. (Hipkins)

## Outreach

- Hosted Work Experience students from the Los Rios Community College District (WEXP program), Fall 2014, spring 2015, spring 2015. (DeWoody)
- Featured in an article about the large aspen clone "Pando" on Science Friday Picture of the Week, April 28, 2015. NFGEL Project #213. (DeWoody)
- Featured on a radio podcast ("Theoretically Speaking", CJSW-FM) discussing extremely large organisms, discussing Pando in particular (Episode 3). NFGEL Project #213. (DeWoody)

## Union Activities

- Union President – Pacific Southwest Research Station (PSW-RS), and Chief Steward Eldorado National Forest (ENF) – Region 5. (Meyer)
- NFFE Forest Service Council, Safety. (Meyer)
- NFFE Forest Service Council, Safety Committee Chair (2006 – Current). (Meyer)
- NFFE Forest Service Council, Union Representative on USDA Strengthening Services Administrative Solutions (SSAS) Homeland Security Workgroup. (Meyer)
- NFFE Forest Service Council, Union Representative, Work Group, ATV/UTV Handbook update. (Meyer)
- NFFE Forest Service Council, Union Representative, Work Group, Comprehensive Safety Policy Review. (Meyer)
- NFFE Forest Service Council, Union Representative on Accident Investigations. (Meyer)
- Sierra Fire LTBMU Fatality, Team Member. (Meyer)
- NFFE, Union Representative, Federal Advisory Council on Occupational Safety and Health (FACOSH) on Field Federal Safety and Health Council (FFSHC) Improvements. (Meyer)
- NFFE Forest Service Council, Union Representative on USDA Strengthening Services Administrative Solutions (SSAS) Homeland Security Workgroup. (Meyer)
- NFFE Forest Service Council, Union Representative, Coordinated Response Protocol (CRP). (Meyer)
- NFFE Forest Service Council, Union Representative, Emergency Medical Services (EMS). (Meyer)
- NFFE Council Vice President – Region 5, Union Representative, Region 5 Self Contained Breathing Apparatus (SCBA) Working Group (Meyer)
- NFFE Forest Service Council, Union Representative, eSafety – OWCP Project working with Kirk Powel (Meyer)
- NFFE Council Vice President – Region 6, Union Representative, Supervisor Training and Team Education Program (STTEP) Pilot (Meyer)
- NFFE Forest Service Council, Pre-Assignment Drug Testing Of Seasonal Employees Negotiation Team (Meyer)
- NFFE Forest Service Council, Union Representative, Facilitated Learning Analysis Continuous Improvement Team (FLACIT) (Meyer)
- NFFE Forest Service Council, Union Representative, USDA USFS Avian Flu Support Working with APHIS (Meyer)
- NFFE Council Vice President – Region 5, Union Representative, Growth Respect Opportunity Workshop (GROW) PTSD Sub-Group (Meyer)

# Hosted Groups

NFGEL is pleased to host a variety of visitors throughout the year. Tours of the facility and operation are provided that range from simple walk-through visits of the laboratory to more extensive experiences where visitors obtain hands-on experience that demonstrate how laboratory genetics contributes to restoration and native plant species management.

| TOUR DATE | TOUR GROUP | NFGEL GUIDES |
|---|---|---|
| December 1, 2014 | Local Public (2 elementary aged children and grandparents) | Hipkins |
| December 17, 2014 | Eldorado NF Placerville RD Meeting – Host | Meyer |
| December 19, 2014 | USDA-FS Homeland Security – Host (NFFE) | Meyer |
| January 8, 2015 | CL Raffety (Treasurer and Tax Collector, Eldorado County) and Joe Hairns. Satellite UC Davis Branch exploration | Hipkins |
| January 13, 2015 | USFS employees from Camino and Sacramento | Hipkins |
| February 6, 2015 | Safety Engagement III | All NFGEL Staff |
| February 10, 2015 | Safety Engagement 3 VTC, PSW Station, NFFE Follow-up | Meyer |
| February 27, 2015 | Girl Scout Troop (12 girls + 3 chaperones) | Hipkins |
| March 16/17, 2015 | Eldorado NF Fire Training – Host | Meyer |
| April 9, 2015 | CALFIRE and El Dorado NF Annual Fire Meeting – Host | Meyer |
| April 17, 2015 | Master Gardeners | DeWoody |
| April 24, 2015 | USFS Middle Leader Training, 45 participants | Hipkins |
| April 30, 2015 | Eldorado NF Forest Protection Officer Training – Host | Meyer |
| May 11, 2015 | Region 5 Tree Climbing Meeting – Host | Meyer |
| June 2, 2015 | King's Beach Academy (3rd grade students, teachers, and chaperones) | DeWoody |
| June 12, 2015 | National Advanced Silviculture Program (NASP) –Region 5/6 Module (15 participants) | Hipkins |
| June 18, 2015 | Eldorado NF Fire Protection Officer Refresher – Host | Meyer |
| July 24, 2015 | IFG 90th Celebration; 100 participants | All NFGEL Staff |
| September 21, 2015 | Eldorado NF Law Enforcement Squad Meeting – Host | Meyer |


# BUDGET

NFGEL received an annual allocation from the Washington Office, National Forest System's Forest Management staff group of $473,500. In addition to these funds, NFGEL received $50,000 from State and Private Forestry, Forest Health Protection and also other individual partner program dollars collected for projects outside the prioritized program of work. These dollars were used for additional salary, chemical, supply, equipment, repair needs, and travel.

| | |
|---|---|
| **ALLOCATION** | |
| WO — NFS — Forest Management | $476,922 |
| WO — State and Private Forestry — Forest Health Protection | $50,000 |
| Private Company (NFGEL Projects 303 and 307) | $26,819 |
| NW Tree Improvement Cooperative (NFGEL Project 277) | $28,977 |
| WCF Special Project (NFGEL Project 315) | $5,349 |
| Center for Natural Lands Management (NFGEL Project 299) | $8,020 |
| Pacific Southwest Research Station (NFGEL Project 301) | $1,236 |
| Region 6 (NFGEL Project 319) | $7,500 |
| Private Company (NFGEL Project 311) | $690 |
| United Nations , Office on Drugs and Crime (combating timber theft) | $3,850 |
| *TOTAL* | $609,363 |
| **EXPENDITURES** | |
| Salary - Permanent Employees | $305,993 |
| Salary - Temporary Employees | $23,846 |
| Salary - Contracts | $51,247 |
| Site Utilities and Rents | $21,101 |
| Chemicals and Supplies | $78,934 |
| Equipment and Repair | $83,096 |
| Computer and Office Supplies | $2,323 |
| Postage | $254 |
| Administrative Costs (awards, uniforms, lync pass, hazardous waste removal) | $4,484 |
| Vehicle | $3,500 |
| Contract – USFS (Gene Conservation of Forest Trees Workshop) | $15,000 |
| Travel and Training | $13,690 |
| *TOTAL* | $603,468 |
| | |
| **BALANCE** | $5,895 |

US Forest Service
The National Forest Genetics Laboratory (NFGEL)
2480 Carson Road
Placerville, CA 95667

Valerie D. Hipkins (Director)
530-622-1609 (direct office phone)
nfgel@fs.fed.us

## STRATEGIC ALIGNMENT

NFGEL's work is consistent with the strategic direction outlined in the USDA Strategic Plan (2011 – 2015) and the Forest Service Strategic Plan (2015 – 2020). Our work aligns to the following Agency Strategic Plan measures:

Goal 1: Sustain our Nation's Forests and Grasslands, with the objective to foster resilient, adaptive ecosystems to mitigate climate change.

Goal 3: Apply Knowledge Globally, with the objectives to (a) advance knowledge, (b) transfer technology and applications, and (c.) exchange natural resource expertise.

Goal 4: Excel as a High-Performing Agency, with the objectives to (a) recruit a diverse workforce, (b) promote an inclusive culture, and (c.) attract and retain top employees.

## MISSION AND PURPOSE

The National Forest Genetics Laboratory (NFGEL) provides genetic testing and information for integrated solutions to on-the-ground problems faced by natural resource managers and policy makers. Solutions are provided for public agencies, non-government organizations, and private industries across the United States, often spanning geographical and organizational boundaries. NFGEL addresses conservation, restoration, and management of all plant species using molecular genetic techniques.

The purpose of NFGEL is to analyze molecular genetic markers (protein and DNA) in plant material submitted by Forest Service employees and those from other cooperating entities. NFGEL provides baseline genetic information, determines the effect of management on the genetic resource, supports genetic improvement program, and contributes information in the support of conservation and restoration programs, especially those involving native and TES (threatened, endangered, and sensitive) species. NFGEL serves the needs of the national forests and provides natural resource managers with the means for evaluating the genetic consequences of vegetation establishment actions.

**NFGEL**
http://www.fs.fed.us/NFGEL/