

DNA MARKERS TO IDENTIFY SOUTHERN PINES AND THEIR HYBRIDS

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Why do it?

Pine hybrids are a concern for National Forest System (NFS) seed orchards that provide seed for reforestation on NFS lands in the Southern Region. The Forest Service has an obligation to make sure that its seed supply is genetically pure. Hybridization of longleaf and shortleaf pines with loblolly pine is of special concern for restoration initiatives. Mature hybrids and hybrid seedlings or seeds are not always possible to visually identify, but the right DNA markers can genetically identify hybrid material.

What was done?

By comparing pine chloroplast DNA (cpDNA) sequences from a public database, we designed DNA markers to provide species-specific DNA fingerprints (haplotypes) for longleaf, shortleaf and loblolly pines. cpDNA is paternally inherited, so these markers can identify the species of the pollen parent of an inter-species cross. We developed two variable cpDNA markers and one to serve as a PCR positive control. Together these provide diagnostic cpDNA haplotypes for longleaf, shortleaf, and loblolly pines. Slash, pitch, and Virginia pines were also tested.

To identify hybrid trees, the NFS Genetics program, with the National Forest Genetics Laboratory (NFGEL), haplotyped two ramets from each longleaf and shortleaf clonal family in every NFS Region 8 seed orchard. This included shortleaf 2nd generation material and scions for establishment of new 2nd generation seed orchards.

In the NFS seed orchards, we find that 2.5% of shortleaf clones, and no longleaf clones, are loblolly hybrids. A sample of a 2014 longleaf seed lot from Mississippi shows no excessive hybridization.

What's next?

- NF seed orchard managers will remove verified hybrid trees from all orchards
- Continue screening seed lots for hybrids and seed contaminants
- DNA fingerprint all families and verify ramet identity
- Cross check haplotyping results with published shortleaf hybrid analyses

Hybrid analyses based on chloroplast haplotypes

	source	# tested	# hybrid	% hybrid	notes
EXPCETED HYBRID					
longleaf x loblolly	nursery	20	20	100%	Sondereggers visually selected from longleaf seedling trays
longleaf x loblolly	Kisatchee NF	28	21	75%	Sondereggers visually selected from a longleaf plantation
NFSO SEED LOT					
longleaf	MS 2014	82	1	1.2%	Mississippi Forestry Commission seed orchard
shortleaf	range-wide	--	--	--	<i>In progress</i>
loblolly	range-wide	280	9	3%	Most "hybrids" from one lot, may be seed contamination
NFSO CLONES					
longleaf	range-wide	541	0	0%	Ramet count for 259 families, plus 28 from MFC orchard
shortleaf	range-wide	1144	29	2.5%	Ramet count for 677 families

Other pines tested: Pitch pine and shortleaf pine share their three haplotypes, but at very different frequencies, which may indicate reciprocal hybrids instead of shared haplotypes; slash pine shares its three haplotypes with loblolly pine, but only rarely, and these exceptions may be hybrids or seed contaminants. Virginia pine has an unusual haplotype (below).

PCR primer information for pine chloroplast markers

Marker name	Lab primer name	Primer sequence, 5' – 3'	Primer length, bp	RefSeq product len, bp	RefSeq Start pos	RefSeq Stop pos	T _m , °C
trnL-sifg1	PpaCPnuL_B3.f	CTTGAATCTGGCATCTATCTTACTT	25	260	69050	69074	56.7
	PpaCPnuL_B3.r	TGAATGGGTAATCCTGAGCCA	21		69309	69289	58.8
trnK-sifg1	PpaCP_D5.f	TGGATGATCTGCTCCTTCAGTATC	24	240	1627	1650	59.7
	PpaCP_D5.r	ATCCCTAGCTAATTCCTGGC	21		1866	1846	59.0
ycf1-sifg1	PecCPnuL_B1.f	AAAAGATGGTCAAGATCAAAAGG	23	215	100627	100649	55.4
	PecCPnuL_B1.r	CTTCACTATCACCATCGTCTTG	22		100841	100820	56.6

Nucleotide positions of start and stop sites are for the complete loblolly chloroplast reference sequence, RefSeq NC_021440.1 from NCBI GenBank. Marker trnL-sifg1 is a single-sized fragment seen only in loblolly pine. Its positive control marker, trnK-sifg1, is a single-sized fragment found in all species tested. Marker ycf1-sifg1 provides a single fragment that is variable in size among individuals with alleles largely specific to species. In Virginia pine (*Pinus* Subsection: Contortae), ycf1-sifg1 amplifies a complex pattern of fragments, indicating a very different ycf1 sequence structure from the southern pines (Subsection: Australes).



Nursery personnel cull any suspicious looking seedlings at the grading table, but visual grading doesn't always catch all the potential hybrids. Diagnostic DNA markers let us measure the hybrid frequency in seed lots before planting to ensure acceptable standards of genetic purity.