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PRIMER NOTE

Microsatellite markers for northern red oak (Fagaceae: *Quercus rubra*)

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Abstract

We provide primer sequences for 14 $(GA)_n$ microsatellite loci developed from northern red oak, an important timber species. We screened loci using two sets of samples. A parent–offspring set included DNA from seven acorns collected from one mother tree along with maternal DNA, to determine that all progeny carried a maternal allele at each locus. The other set was comprised of 10 adult trees sampled from Indiana old-growth forest, providing a measure of diversity revealed by each locus.

Keywords: capillary, CEQ, enriched, Indiana, old-growth, SSRs

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Quercus rubra L. (northern red oak) is an important timber species in the United States yet many aspects of the gene pool remain undescribed. Only a few isozyme studies exist covering a small portion of the geographical range (Manos & Fairbrothers 1987; Guttman & Weigt 1989; Schwarzmann & Gerhold 1991; Hokanson *et al.* 1993). Hybridization among closely related taxa is widely reported based on morphology (Jensen 1995), and isozyme data generally show limited interspecific differentiation. Here, we describe a panel of novel northern red oak microsatellites and present preliminary data on their genetic variability and mode of inheritance.

Newly emerged leaves were harvested from greenhouse grown *Q. rubra* seedlings. DNA was extracted using the Murray & Pitas (1996) nuclei isolation method and were sent to Genetic Information Services for construction of microsatellite libraries (Peacock *et al.*, in press) enriched for $(GA)_n$ $(CA)_n$ $(AAG)_n$ and $(GAC)_n$ repeats. Clones ($N = 1061$) of the enriched $(GA)_n$ library were sequenced in a single direction (Purdue Agricultural Genomics Core Facility), and those containing simple sequence repeat (SSR) loci with repeats > 10 were sequenced ($N = 96$) in the reverse direction to obtain quality flanking sequences. Consensus sequences were generated through alignment with the CONTIGEXPRESS subroutine of VECTORNTI (Suite 6, InforMax, Inc.) and used for designing primers with OLIGO 6.4 (Molecular Biology Insights, Inc.). Sample tissues from leaves, acorns and bark cambium were broken up using a metal bead in an agitating rotor (FastPrep Bio101, Savant) prior to DNA extraction with the DNeasy Plant Minikit (Qiagen, Inc.), modified as follows: twice the recommended volume of lysis buffer (AP1), precipitation buffer (AP2), and binding buffer (AP3), and an extra wash step with 500 µL AW1 wash buffer. DNAs were quantified on an FL600 Microplate Fluorescence Reader (Bio-Tek Instruments, Inc.) using Hoescht Dye 33258, and concentrations were adjusted to 5–10 ng/µL prior to amplification.

We used a four-tier primer optimization process. First, we tested for polymerase chain reaction (PCR) products of the expected size in 1.5% agarose gel (1×TAE). Reactions (25 µL) included: [1× Ex*Taq* Buffer (Panvera, proprietary except 2.0 mM $MgCl_2$), 100 µM dNTP each, 72 nm each upper and lower primer, 0.01 U/µL Takara Ex Taq Polymerase (Panvera), and 0.2–0.4 ng/µL DNA]. The PCR protocol was: 94 °C for 1 min; 50 cycles of 94 °C for 30 s; T_a, for 45 s; 72 °C for 1.5 min; anf finally 72 °C for 10 min. Second we optimized amplifications with a temperature gradient (36–60 °C, 12 steps) on a PTC-225 Peltier Thermal Cycler (MJ Research). Third, microsatellites were re-amplified with

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Table 1 Characteristics of 14 $(GA)_n$ microsatellite loci isolated from *Quercus rubra*

Locus	$(GA)_n$	Primer sequences (5′–3′)	Population diversity				Allele size (bp)	
			T_a	N_a	H_O	H_E	clone	population
quru-GA-0A01	$(GA)_{11}$	*CTCTCGCTCTGCACGTGACTCA TTTGATTGATATAATTGATCGCT	50 C	4	0.40	0.34	125	123–129
quru-GA-0C11	$(GA)_{15}$	*ATACCCAGCTCCCATGACCA TCCCCAAATTCAGGTAGTGT	53 C	8	0.70	0.78	214	204–222
quru-GA-0C19	$(GA)_{18}$	*TTAGCTTTTACGCAGTGTCG CGGCTTCGGTTTCGTC	50 C	8	0.80	0.84	239	218–242
quru-GA-0E09	$(GA)_{16}$	*TGCCATCCCTATACACAACCA CCTCCATCACAAAGTTGCC	53 C	13	1.00	0.91	192	186–230
quru-GA-0I01	$(GA)_{16}$	*GGGCTATCAAGTAAGTGCTTAAC ACGCCATCCCTATAACACA	53 C	7	0.70	0.83	200	196–218
quru-GA-0M05	$(GA)_{20}$	*CTACAAGTTACATGCCCAATCA CTTTGCGCAGGTCCATTAC	53 C	7	0.40	0.78	219	184–215
quru-GA-0M07	$(GA)_{19}$	*TTTAGCATCACATTTCCGTT TTTTGTGTCATCCGGTATTA	45 C	8	0.60	0.72	209	185–209
quru-GA-1C06	$(GA)_{29}$	*CAAATAAATATTGTGGGGTTCA GGAGGGGATCCGGAAAA	50 C	8	0.70	0.82	276	234–262
quru-GA-1C08	$(GA)_{29}$	*TCCCAATCGATGTTTGATAAGG GGGCTCTTGAGAGGATGTAGG	50 C	5	0.60	0.54	313	257–296
quru-GA-1F02	$(GA)_{15}$	*CCAATCCACCCTTCCAAGTTCC TGGTTGTTTTGCTTTATTCAGCC	50 C	8	0.80	0.76	181	166–184
quru-GA-1F07	$(GA)_{22}$	*CCGGTCAAAGAAGTTATCAGA GGGTGGATTGGGTTTCTACCTA	58 C	11	0.90	0.82	348	306–348
quru-GA-1G13	$(GA)_{14}$	*AAAACTCACACAGCCGATTACTA GATTCCATTGTCAACTGCGAAGA	50 C	4	0.60	0.68	184	177–193
quru-GA-2F05	$(GA)_{21}$	*CCGCTTCGTGACGATTATTC GAGGTTTGGAGGAGAGATCATTCT	53 C	6	0.60	0.64	300	294–322
quru-GA-2M04	$(GA)_{20}$	*GGAGAGGACGGGATGCC TACTATGTCAGCCGGATG	56 C	8	0.70	0.86	210	182–220

Locus name, repeat sequence in clone, primer sequences, labelled primer (*), annealing temperature (T_a), diversity for 10 adults (N_a, alleles per locus; H_O, observed heterozygosity and H_E, expected heterozygosity), and PCR product sizes.

Cy5-dCTP fluorescent label (Amersham Pharmacia Biotech, Inc.), 1 μM reaction concentration, allowing approximate sizing on an eight-capillary CEQ2000XL genotyper (Beckman Coulter). Loci with one or two easily interpreted peaks were accepted and the forward primer was 5′ end-labelled with phosphoramadite dyes (D2-PA, D3-PA or D4-PA, Research Genetics), permitting detection of ± 1 bp differences. (iv) Finally, we screened the end-labelled primers using DNA from a single tree on the Purdue campus and seven of its acorns. Loci carrying at least one maternal allele in each acorn were accepted.

We used the panel of 14 loci to screen for diversity in 10 adult *Q. rubra* from an old-growth stand at the Davis-Purdue Research Forest in east-central Indiana (Table 1). Species identifications were available from prior research at the site (Parker *et al.* 1985). Over all loci, 105 alleles were detected with a mean of 7.5 alleles per locus (range, 4–13 alleles). Expected and observed heterozygosities were close for nearly all loci; only quru-GA-OMO5 exhibited a notable deficit of heterozygotes that may indicate the presence of null alleles. PCR product sizes from the library did not always fall in the size ranges amplified from the Davis-Purdue Research Forest because we constructed the library from a single individual, geographically distant from the Davis-Purdue Research Forest. In summary, these primers should be widely applicable to genetic research on *Q. rubra*.

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