Strawberry GenBank-derived and Genomic Simple Sequence Repeat (SSR) Markers and Their Utility with Strawberry, Blackberry, and Red and Black Raspberry

K.S. Lewers^{1,2}, S.M.N. Styan³, and S.C. Hokanson⁴

USDA–ARS Fruit Laboratory, 10300 Baltimore Avenue, BARC-West Building 010A, Beltsville, MD 20705-2350

N.V. Bassil²

USDA–ARS NCGR, 33447 Peoria Road, Corvallis, OR 97333

ADDITIONAL INDEX WORDS. *Fragaria*, *Fragaria ×ananassa*, *Fragaria chiloensis*, *Fragaria iinumae*, *Fragaria vesca*, *Fragaria virginiana*, *Rubus*, *Rubus idaeus*, *Rubus occidentalis*

ABSTRACT. Although simple sequence repeat (SSR) markers have been developed for species in the closely related genera *Fragaria* L. (strawberry) and *Rubus* L. (raspberry and blackberry), the number of SSRs available is insufficient for genetic mapping. Our objective was to use and compare multiple approaches for developing additional SSRs for *Fragaria* and *Rubus*. The approaches included: the development of SSRs from GenBank sequences from species of varied relatedness to *Fragaria* and *Rubus* and identified with two different data-mining methods (BLAST and SSRIT); the evaluation of some previously published SSRs designed from related species; and the development of SSRs from a genomic library made from *F. ×ananassa* Duschene ex Rozier ‘Earliglow’. When an SSR was developed from a known gene sequence, the location of the repeat in the gene was determined to evaluate the effect on amplification and polymorphism detection. Cross-generic amplification between closely related *Fragaria* and *Rubus* as well as transference from species of varied relatedness to *Fragaria* and *Rubus* also was evaluated and indicated limited transference within the subfamily Rosoideae. However, development of SSRs for *Fragaria* and *Rubus* from *Rosa* L. (rose) and Rosaceae genera outside Rosoideae was not efficient enough to be practical for new map development. SSRIT was superior to BLAST for identifying GenBank sequences containing repeats. SSRs developed from repeats found in either the 5’UTR (80% polymorphic) or 3’UTR (85% polymorphic) were most likely to detect polymorphisms, compared with those developed from coding regions (30%). SSRs developed from the genomic library were only slightly superior to GenBank-derived SSRs in their ability to detect polymorphisms.

Single sequence repeat (SSR) molecular markers have become valuable genetic markers in plant studies, with applications that include linkage mapping (Akkaya et al., 1995), cultivar identification (Rongwen et al., 1995), marker-assisted selection (Fazio et al., 2003), and gene flow characterization (Aldrich and Hamrick, 1998; Chase et al., 1996). SSRs are especially advantageous in genetic mapping research because they are easy to use, they map to similar locations in multiple populations (Cregan et al., 1999),

and are associated with gene clusters, and potentially with traits, in plant genomes (Morgante et al., 2002). Some researchers have tried to reduce the time and expense of SSR development by using SSRs from one species for genetic research on related species (Cipriani et al., 1999; Cordeiro et al., 2001; Dayanandan et al., 1997; Decroocq et al., 2003; Dirlewanger et al., 2002; Downey and Iezzoni, 2000; Echt et al., 1999; Isagi and Suhandono, 1997; Peakall et al., 1998; Rajora et al., 2001; Scott et al., 2000; Steinkellner et al., 1997; Whitton et al., 1997). Other researchers designed primers from SSR-containing genomic and EST sequences archived in GenBank (Broun and Tanksley, 1996; Cho et al., 2000; da Silva, 2001; Holland et al., 2001; Kantety et al., 2002; Smulders et al., 1997).

Although SSR markers have been developed for species in the closely related genera *Fragaria* and *Rubus*, the number of SSRs available is insufficient for genetic mapping. Ten SSRs were developed for the diploid *F. vesca* L. (James et al., 2003); 22 were developed from the diploid *F. viridis* Weston (Sargent et al., 2003); four were developed for the paternal octoploid progenitor, *F. virginiana* Mill., of cultivated strawberry (Ashley et al., 2003); and four were developed from the cultivated octoploid strawberry *F. ×ananassa* (Ashley et al., 2003). Twenty-one SSRs developed from *Prunus persica* (L.) Batsch (peach) were reported to amplify products from *F. ×ananassa* DNA (Dirlewanger et al., 2002). Ten SSRs were developed from *R. idaeus* L. (red raspberry) (Graham et al., 2002) and eight SSRs were developed from *R. alceifolius* Poir. (Amsellem et al., 2001). Our objective was to use and

Received for publication 2 Mar. 2004. Accepted for publication 6 July 2004. We thank Isabella Mackey and Kate Rappaport for managing the PCR work and DNA extractions, and Peter Boches, Sony Thomas, and Michael Henry for helping with the DNA extractions and/or completing PCR amplifications. Thanks also to Sam Garrett for plant maintenance. We also thank Dr. Ben Matthews and Margaret McDonald for soybean and peach DNA, Dr. Tom Davis for DNA from parents of the *F. vesca* mapping population, and Sam Garrett, Dr. Courtney Weber, Dr. John Clark, and Eric Stafne for raspberry and blackberry leaf tissue. In addition, we would like to thank Dr. Tad Sonstegard and Tina Sphon for genotyping, and the Beltsville Agricultural Research Center for supporting the genotyping effort. Finally, we would like to thank Dr. Freddi Hammerschlag, Dr. Kim Hummer, Dr. Jeannie Rowland, Dr. Perry Cregan, Dr. Hilde Nybom, and the anonymous reviewers for helpful criticism of this work. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Dept. of Agriculture.

¹To whom reprint requests should be addressed. E-mail: lewersk@ba.ars.usda.gov

²To be considered co-first authors.

³Current address: Pioneer Hi-Bred International, Inc. A DuPont Co., Waimea Research Center, P.O. Box 609, Waimea, HI 96796.

⁴Current address: Dept. of Horticulture and Landscape Architecture, Univ. of Minnesota, St. Paul, MN 55108.

compare multiple approaches for developing additional SSRs for *Fragaria* and *Rubus*. The approaches included the development of new SSRs from GenBank sequences from species of varied relatedness to *Fragaria* and *Rubus* and identified with two different data-mining methods (BLAST and SSRIT); the evaluation of some previously published SSRs designed from related species; and the development of new SSRs from a genomic library made from *F. xananassa*. When an SSR was developed from a known gene sequence, the location of the repeat in the gene was determined to evaluate the effect on amplification and polymorphism detection. Cross-generic amplification between closely related *Fragaria* and *Rubus* as well as transference from species of varied relatedness to *Fragaria* and *Rubus* also was evaluated.

Materials and Methods

DETERMINING FUNCTIONAL TAXONOMIC DISTANCE FOR SSR DEVELOPMENT. An initial study was conducted to determine how taxonomically distant the source of the repeat sequence could be and still supply primers that would amplify a product from the two target species. We tested six (CT)_n-containing primer pairs from each of nine genera with varied relatedness to *Fragaria* and *Rubus*. We searched GenBank using the National Center for Biotechnology Information's (NCBI) BLAST program (Altschul et al., 1990; National Center for Biotechnology Information, 2004) for genomic and EST sequences containing CT repeats in nine genera, using the sequence (CT)₃₀ as the query sequence. A total of 54 sequences containing (CT)_n repeats were selected for primer design. The taxonomically farthest genus from which primers were designed was within Rosidae, which includes the model species, *Arabidopsis thaliana* (L.) Hehnh; six repeat-containing *A. thaliana* sequences were selected for primer design. From the next taxonomic level closer to *Fragaria* and *Rubus*, the eurosids I, 12 primers were designed from the Fabaceae Lindl., nom. cons. family [six from *Glycine max* (L.) Merr. (soybean); and six from *Medicago truncatula* Gaertn. (a model legume)], and six from the Malvaceae family [*Gossypium* L. (cotton)]. Within the Rosaceae family, six primer pairs were designed from *Prunus persica* representing the subfamily Prunoideae, and 12 primer pairs were designed from species representing the subfamily Maloideae (six from *Pyrus communis* L. and six from *Malus xdomestica* Borkh). In the subfamily Rosoideae, to which the two target genera, *Fragaria* and *Rubus*, belong, six primer pairs were obtained from *F. xananassa* and six from *Rosa* (rose). Primers were developed with the on-line version of Primer3 software (Whitehead Institute for Biomedical Research, 2004) using the default settings (Rozen and Skaletsky, 2000). The recommended primers were synthesized without modification by Operon Technologies (Qiagen, Valencia, Calif.). In addition, several published SSR primers were tested and included 21 primer pairs designed from *Prunus persica* and reported to amplify a product from strawberry (Dirlewanger et al., 2002); eight primer pairs developed from *Rubus alceifolius* (Amsellem et al., 2001); four SSR primer pairs designed from *F. virginiana* genomic sequences (Ashley et al., 2003); and 10 designed from *F. vesca* (diploid) genomic sequences (James et al., 2003).

DEVELOPMENT OF ADDITIONAL SSRs FROM FRAGARIA SEQUENCES IN GENBANK. Two methods were used to identify GenBank *Fragaria* sequences containing repeats. The first was the direct BLAST search method used above with (CT)-containing SSRs to determine the taxonomic distance for SSR transference to *Fragaria* and *Rubus*. Genomic and EST sequences of *Fragaria*

GenBank sequences were searched using all possible (dinucleotide)₃₀ and (trinucleotide)₂₀ repeats as a query sequence. The second method of finding GenBank sequences containing repeats used the Perl script SSRIT (simple sequence repeat identification tool) (Gramene, 2004; Temnykh et al., 2001). A total of 306 genomic and EST *Fragaria* sequences present in the nucleotide database of the NCBI were screened for repeat sequences. The maximum motif length group selected was pentamer, and the minimum number of tandem repeats was set at five (and then also later at four to identify additional repeat-containing sequences).

The location of the simple sequence repeat in the gene was determined to evaluate the effect on amplification and polymorphism detection. If available, the genomic as well as the coding sequence of the repeat-containing GenBank sequences were aligned using the CLUSTALW algorithm (Institute for Chemical Research, 2000; Thompson et al., 1994), enabling the identification of the repeat location with respect to the gene. Alternatively, the repeat-containing sequence was compared against known gene sequences archived in GenBank using the BLASTN algorithm. For each sequence, the location of the repeat region [5' untranslated region (5'UTR), intron, exon, or 3' untranslated region (3'UTR)] was determined. Whenever possible, primers were designed in the coding regions of the gene. After eliminating duplicated sequences and sequences that contained repeats at their ends, primer pairs were designed from 25 sequences using Primer3 (Table 1).

SSR DEVELOPMENT FROM A GENOMIC LIBRARY. *Fragaria xananassa* 'Earliglow' genomic DNA was digested with restriction enzymes *Sma* I, *Msc* I, and *Rsa* I. The resulting DNA fragments were phosphorylated and electrophoretically separated through a 0.8% agarose, 1× TBE gel. The DNA fragments between 400–800 base pairs (bp) were extracted and purified from the gel according to the QIAquick Gel Extraction Kit protocol (Qiagen). Purified DNA fragments were ligated into the *Sma* I site of the pUC 19 vector with T4 ligase (New England BioLabs, Beverly, Mass.), and transformed into Epicurian Coli Ultracompetent Cells (Stratagene, La Jolla, Calif.). Transformed cells were grown on LB-agar plates containing 50 mg·mL⁻¹ of ampicillin, 40 mg·mL⁻¹ of X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), and 40 mg·mL⁻¹ IPTG (isopropyl-1-thio-β-D-galactopyranoside). White colonies were transferred to Optitran BA-S85 membranes (Schleicher & Schuell, Keene, N.H.), and hybridized with ³²P-labeled (CT)₁₅ probe. After three rounds of screening, single bacterial colonies were grown and selected for plasmid DNA isolation using the QIAprep Miniprep Kit (Qiagen).

Plasmid DNA from the selected positive clones was diluted to 100 ng·μL⁻¹ for sequencing reactions. Clone inserts were sequenced from both ends using M13 17-mer forward and 24-mer reverse sequencing primers (New England Biolabs) in the BigDye Terminator Cycle sequence ready reaction mixture (Applied Biosystems, Foster City, Calif.) according to the supplied protocol. The reaction products were analyzed with an ABI 310 Automated DNA Sequencer (Applied Biosystems). Repeat-containing sequences were compared against known gene sequences contained in GenBank using the BLASTN and BLASTX algorithms.

Oligo Lite, Version 6 (Molecular Biology Insights, Cascade, Colo.) was used for primer design. Primers were chosen under the highest stringency conditions possible with criterion including primer efficiency (PE) values >400, duplex values >4, and a melting temperature difference of ≤20 °C between primer pairs. Twenty-eight primer pairs were synthesized by BioServe Biotechnologies, Ltd. (Laurel, Md.) (Table 1).

GENOTYPES USED FOR PRIMER TESTING IN *FRAGARIA* AND *RUBUS*. SSR primers were tested in PCR amplification with genomic DNA from 31 genotypes (Table 2): 12 *F. xananassa* (octoploid) genotypes (cultivated octoploid strawberry), five *F. chiloensis* (L.) Mill. genotypes (octoploid maternal progenitor species of cultivated strawberry), three *F. virginiana* genotypes (octoploid paternal progenitor species of cultivated strawberry), one *F. virginiana* × *F. xananassa* hybrid (octoploid), four *F. vesca* (diploid) genotypes, one *F. iinumae* Makino (diploid), and six *Rubus* genotypes (four tetraploid blackberry, one diploid red raspberry, and one diploid black raspberry). Many of these genotypes are parents of existing mapping populations and were selected so that we could obtain polymorphism data for multiple mapping populations (Tables 2 and 3). Plants were grown at multiple locations: the USDA–ARS National Clonal Germplasm Repository at Corvallis, Ore.; the USDA–ARS Beltsville Agricultural Research facility at Beltsville, Md.; the Univ. of Arkansas station near Clarksville, Ark.; and Cornell Univ. near Geneva, N.Y. Not all of the primers were tested on every genotype.

DNA EXTRACTION. DNA was obtained from plants by harvesting young leaves from greenhouse- or field-grown plants. At Corvallis,

DNA was extracted from four leaf discs using the PureGene kit (Gentra Systems, Minneapolis) according to the recommended plant protocol. DNA concentrations were estimated spectrophotometrically using a Biophotometer (Brinkmann Instruments, Westbury, N.Y.) and diluted to 2.5 ng·μL⁻¹ with 1× TE buffer. At Beltsville, DNA was extracted using Qiagen's DNeasy Plant Maxi Kit or DNeasy Plant Mini Kit. DNA was quantified using the Spectramax 190 spectrophotometer (Molecular Devices, Sunnyvale, Calif.), and diluted with 1× TE buffer to 30 ng·μL⁻¹. Dilutions to 0.03 ng·μL⁻¹ were made with ddH₂O.

PCR CONDITIONS. At Corvallis, PCR amplifications were carried out in 10 μL total volume of 1× Biolase reaction buffer, 2 mM MgCl₂, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.3 μM each of forward and reverse primers, 0.25 U of Biolase *Taq* DNA polymerase (Bioline USA, Randolph, Mass.), and 2.5 ng of template DNA. DNA was amplified for 35 cycles in an Eppendorf Gradient thermocycler (Brinkmann Instruments, Westbury, N.Y.) or an MJ Research Tetrad thermocycler (MJ Research, Waltham, Mass.) programmed for a 40-s denaturation step at 94 °C, a 40-s annealing step at the optimum annealing temperature, and a 40-s extension step at 72 °C. The optimum annealing temperature

Table 1. Primer pairs tested in polymerase chain reactions (PCRs) with template DNA from several *Fragaria* and *Rubus* genotypes. The primer pairs were either published previously or developed from nucleotide sequences containing simple sequence repeats (SSRs). The SSR-containing sequences were identified from among *Fragaria* or *Rubus* sequences archived in the GenBank database or from a genomic library made from *F. xananassa* 'Earliglow' DNA. The source sequence GenBank accession number, clone number, and/or reference (if published) for each primer pair are given. Location of the SSR with respect to the gene is indicated when known. If a primer pair amplified a product in PCR reactions with any tested genotype in the listed species, a "+" sign is indicated. If the primer pair also detected polymorphisms among the tested genotypes in the listed species, a "+/+ " sign is indicated. If the primer pair did not amplify a product from any of the genotypes tested in the listed species, a "-" sign is indicated. If the primer pair was not tested on any of the genotypes in the species, no sign is given.

Marker ^z	GenBank or library accession, source genome, sequence similarity ^{y,x}	Primer sequences 5' to 3', or source reference if published	T _m °C	Size (bp) ^w	Repeat ^v	Location	<i>F. iinumae</i> (diploid)	<i>F. vesca</i> (diploid)	<i>F. chiloensis</i> (octoploid)	<i>F. virginiana</i> (octoploid)	<i>F. xananassa</i> (octoploid)	Blackberry (tetraploid)	Raspberry (diploid)
Previously published SSRs													
Fvi6b	None, <i>F. virginiana</i> , NA	Ashley et al., 2003	56	282	(ga) ₁₅	NP	-	+	+/+	+/+		-	
Fvi9	None, <i>F. virginiana</i> , NA	Ashley et al., 2003	60	175	(ca) ₁₁	NP	+	+	+/+	+/+		-	
Fvi11	None, <i>F. virginiana</i> , NA	Ashley et al., 2003	60	137	(ga) ₁₆	NP	+	+	+/+	+/+		-	
Fvi20	None, <i>F. virginiana</i> , NA	Ashley et al., 2003	60	162	(ga) ₂₀	NP	+/+	+	+/+	+/+	+	+	
EMFv1	AJ508244, <i>F. vesca</i> , NA	James et al., 2003	55	230	(cgg) ₇	NP	+	+	+	+/+	-	-	
EMFv2	AJ508245, <i>F. vesca</i> , NA	James et al., 2003	60	278	(agc) ₁₀	NP	+	+	+	+	-	-	
EMFv3	AJ508246, <i>F. vesca</i> , NA	James et al., 2003	50	241	(ag) ₂₂₋₁	NP	+/+	+	+	+/+	-	-	
EMFv4	AJ508247, <i>F. vesca</i> , NA	James et al., 2003	50	277	(ag) ₁₅	NP	+/+	+	+	+/+	-	+/+	
EMFv5	AJ508248, <i>F. vesca</i> , NA	James et al., 2003	60	194	(cgg) ₇	NP	+	+	+	+/+	-	-	
EMFv6	AJ508249, <i>F. vesca</i> , NA	James et al., 2003	60	228	(ag) ₃₁	NP	+/+	+	+	+/+	-	-	

Table 1 continued next page

Table 1. Continued.

Marker ^z	GenBank or library accession, source genome, sequence similarity ^{y, x}	Primer sequences 5' to 3', or source reference if published	T _m °C	Size (bp) ^w	Repeat ^v	Location	<i>F. inumae</i> (diploid)	<i>F. vesca</i> (diploid)	<i>F. chiloensis</i> (octoploid)	<i>F. virginiana</i> (octoploid)	<i>F. ×ananassa</i> (octoploid)	Blackberry (tetraploid)	Raspberry (diploid)
Previously published SSRs													
EMFv7	AJ508250, <i>F. vesca</i> , NA	James et al., 2003	60	214	(ag) ₂₂	NP	+/+	+	+	+/+	-	-	
EMFv8	AJ508251, <i>F. vesca</i> , NA	James et al., 2003	60	206	(ag) ₁₈	NP	+/+	+	+	+/+		-	
EMFv9	AJ508252, <i>F. vesca</i> , NA	James et al., 2003	60	206	(agc) ₈	NP		+	+	+	+/+	+	+/+
EMFv10	AJ508253, <i>F. vesca</i> , NA	James et al., 2003	55	243	(cgg) ₇	NP	+/+	+	+	+		-	-
mRaCIRRI1G3	AF205116, <i>Rubus alceifolius</i> , NA	Amsellem et al., 2001	58	195-265	(ga) ₂₈	NP		+	+	+	-	+	+/+
mRaCIRRIIV2A8	AF261693, <i>R. alceifolius</i> , NA	Amsellem et al., 2001	59	191-237	(ca) ₁₂ (ct) ₁₁	NP		+/+	+	+	-	+/+	+/+
Derived from GenBank sequences													
FAC-013	AF041394, <i>F. ×ananassa</i> , FANR40, cDNA	F:tggttgaaaagtgtggac R:gatatcaatatcaatactagataacag	58	192	(aag) ₁₀		-	+/+	+/+	+/+	+/+		
ARSFL_97	AF041401, 'Brighton', UDP glucose glucosyl-transferase	F:caagcaatccaacagctcaa R:acgcctctaagcacttctcg	59	185	(ca) ₅	Coding		+/+	+	+	+/+	+	-
ARSFL_34	AF041403, 'Brighton', flavonoid-3-hydroxylase(4)	F:tctcaacgatcccaaggag R:ccaatctcctcaccttga	59	155	(ag) ₁₀₋₃	Coding		+	+	+	+	-	-
ARSFL_35	AF054615, 'Selva', cellulase (Cel2) mRNA	F:tggtgatctgcttaggctttt R:aagccacttttaccctcaa	59	206	(ag) ₈	3'UTR		+	+/+	+	+/+	-	-
ARSFL_134	AF158652, <i>F. ×ananassa</i> , cytosolic ascorbate peroxidase (ApxSC)	F:ttctttatggttgctgtct R:ctccatccgcttgaactca	59	184	(tgg) ₂ (tgg) ₄	Intron2		+	+	+	+	+	+
ARSFL_99	AF159563, 'Chandler', expansin auxin-independent	F:gattagggaaggcaacgtg R:gcttcaagcaaatgcatca	59	178	(at) ₁₃₋₁	3'UTR		+/+	+	+	+/+	-	-
FAC-011	AF159563, 'Chandler', expansin auxin-independent	F:gttttcaggcgggtcaattcta R:gcttcaagcaaatgcatcatc	64	298	(ta) ₇ a(at) ₆	3'UTR	+	+/+	+	+/+	+/+		
FAC-010	AF220491, <i>F. ×ananassa</i> , O-methyltransferase (omt1)	F:gctgtgatgctttcaacaccta R:ttgctgacccatacttagaaa	48	278	(ctt) ₅	3'UTR	-	-	-	-	-		

Table 1 continued next page

Table 1. Continued.

Marker ^z	GenBank or library accession, source genome, sequence similarity ^{y, x}	Primer sequences 5' to 3', or source reference if published	T _m °C	Size (bp) ^w	Repeat ^v	Location	<i>F. inumae</i> (diploid)	<i>F. vesca</i> (diploid)	<i>F. chiloensis</i> (octoploid)	<i>F. virginiana</i> (octoploid)	<i>F. × ananassa</i> (octoploid)	Blackberry (tetraploid)	Raspberry (diploid)
ARSFL_33	AF226700, <i>F. × ananassa</i> , expansin 3 (Exp3) mRNA	F:ttcaacaatggcttgagctg R:tgaaccttatgcctcctgct	59	250	(ag) ₄	Coding		+	+	+	+	+	+/+
FAC-001	AF320110, <i>F. × ananassa</i> , Cinnamyl alcohol dehydrogenase	F:aaatcctgttctgccagtg R:tgtgtgacgtattgggtgatg	64	212	(aaat) ₇	5'UTR	+	+/+	+/+	+	+/+		
ARSFL_31	AF339024, <i>F. × ananassa</i> , Pectate lyase B	F:cgaccagcgactacattg R:actttaaccgccaccaactg	59	166	(ag) ₁₀	5'UTR		+	+/+	+	+/+	-	-
FAC-007	AF339024, <i>F. × ananassa</i> , Pectate lyase B	F:gacggaccgacactaaacttg R:ctagctgacctcattgctctgt	62	350	(ag) ₁₀	5'UTR	+	+	+/+	+/+	+/+		
ARSFL_32	AF339025, <i>F. × ananassa</i> , Pectate lyase	F:gggtgattgtgggagctcatt R:caggccgtaatccatttctt	59	229	(ag) ₁₀₋₁	5'UTR		+	+	+	+/+	-	-
FAC-006a	AF339025, <i>F. × ananassa</i> , Pectate lyase	F:actggtggaggagaggactgta R:tgtggagcagaggaattgaag	65	213	(gt) ₅ (tc) ₇	5'UTR	+	-	+/+	+/+	+/+		
FAC-006b	AF339025, <i>F. × ananassa</i> , Pectate lyase	F:acactgcgtttgtgtcctta R:caggccgtaatccatttcttat	60	136	(ag) ₄ aa(ag) ₅	5'UTR	+	-	-	-	-		
FAC-006c	AF339025, <i>F. × ananassa</i> , Pectate lyase	F:ttgacatgtgcgttctctt R:agaaatgagccagcttttccct	58	299	(ta) ₁₀	5'UTR	-	-	-	+/+	+/+		
FAC-006d	AF339025, <i>F. × ananassa</i> , Pectate lyase	F:atttaggagcaggtgcagaag R:ccc aaat gtatgca gat gaaaa	58	188	(cca) ₅	3'UTR	+	+	+	+	+		
ARSFL_100	AF380299, 'Chandler', endopolygalacturonase	F:tgatgtattgcatttcgtgct R:ctatctccgggtgctttgac	59	243	(at) ₇	Exon3		+/+	+	+	+/+	-	-
FAC-005a	AF380299, 'Chandler', endopolygalacturonase	F:gatgtattgcatttcgtgctaa R:gcacataatgagaaagcagcac	65	103	(at) ₇	Exon3	-	+/+	+/+	+/+	+/+		
FAC-005b	AF380299, 'Chandler', endopolygalacturonase	F:actgtggagggtctagcattgt R:gcacataatgagaaagcagcac	65	171	(at) ₇ (tta) ₄	Exon3	+	+/+	+/+	+/+	+/+		
FAC-003a	AF401220, <i>F. × ananassa</i> , Transcription factor MYB1	F:agctaattctcaggccacaag R:ttcattgccagttgaattacg	64	166	(ta) ₇	3'UTR	+	-	+	+/+	+/+		
FAC-003b	AF401220, <i>F. × ananassa</i> , Transcription factor MYB1	F:tcgacctcactctaa gcatcaa R:agataagcttcttggcctga	64	310	(gaa) ₅	3'UTR	+	-	+	+	+/+		

Table 1 continued next page

Table 1. Continued.

Marker ^z	GenBank or library accession, source genome, sequence similarity ^{y,x}	Primer sequences 5' to 3', or source reference if published	T _m °C	Size (bp) ^w	Repeat ^v	Location	<i>F. inumae</i> (diploid)	<i>F. vesca</i> (diploid)	<i>F. chiloensis</i> (octoploid)	<i>F. virginiana</i> (octoploid)	<i>F. × ananassa</i> (octoploid)	Blackberry (tetraploid)	Raspberry (diploid)
FAC-008	AF421492, <i>F. × ananassa</i> , Glyceraldehyde 3-phosphate dehydrogenase 1	F: tactgtgcacgcaacaacag R: ctctccaatccttcattgat	50	138	(tc) ₅ a(tc) ₄	Intron9	+	+/+	+/+	+/+	+/+		
ARSFL_98	AI795160, 'Elsanta', Metallothionein-like protein	F: cccctattcgacaaccaatg R: tggctaccaaaagaacacgaa	59	217	(ac) ₆	3'UTR		+	+	+	+/+	-	-
FAC-009	AI795160, 'Elsanta', Metallothionein-like protein	F: catcgactgcaagtgtggac R: tggctaccaaaagaacacgaa	64	244	(tg) ₆	3'UTR	+	+	+	+	+		
ARSFL_105	AJ001448, <i>F. vesca</i> , 'Reine des Valles', ripening-induced protein, clone 2.5.R1	F: cagctccagctcgtcaagt R: gacctcagtgctcgtcgt	59	295	(gaa) ₇₋₁			+	+	+	-	-	-
FAC-014	AJ001448, <i>F. vesca</i> , 'Reine des Valles', ripening-induced protein, clone 2.5.R1	F: gactgaaggagaagctcaaggaa R: gacctcagtgctcgtcgtag	62	224	(gaa) ₇₋₁		-	+/+	+/+	+/+	+/+		
ARSFL_101	AJ001451, <i>F. vesca</i> , 'Reine des Valles', putative cystathionine gamma synthase	F: cagctaaaaccctgctctcg R: gtgacgataggccgtgaaac	59	218	(agg) ₆	Exon1		+	+	+	+	-	-
ARSFL_107	AJ001451, <i>F. vesca</i> , 'Reine des Valles', putative cystathionine gamma synthase	F: tccaacaagactccgacct R: gtccttcaaatcaggcaagc	59	183	(ggc) ₆₋₁	Exon1					-	-	-
ARSFL_30	AJ006349, 'Chandler', faEG3 gene for endo-beta-1,4-glucanase	F: ttcgaagattggagaaagagg R: aagccactttttaccctcaa	59	161	(ag) ₁₂	3'UTR		+	+	+	+/+	+/+	-
FAC-002	AJ006349, 'Chandler', faEG3 gene for endo-beta-1,4-glucanase	F: tcactctcttcacccactt R: tcaaaagacttggaaatgttgc	64	236	(ga) ₇ a(ag) ₅	3'UTR	+	+	+/+	+	+/+		
ARSFL_106	AJ315844, 'Chandler', lpt46 gene for lipid transfer protein	F: ttcatctctatgtcggaagaa R: ttgttgatgaaatgccaca	59	221	(gaa) ₆	5'UTR	-	+	-	+/+	-	-	

Table 1 continued next page

Table 1. Continued.

Marker ^z	GenBank or library accession, source genome, sequence similarity ^{y,x}	Primer sequences 5' to 3', or source reference if published	Tm °C	Size (bp) ^w	Repeat ^v	Location	<i>F. iinumae</i> (diploid)	<i>F. vesca</i> (diploid)	<i>F. chiloensis</i> (octoploid)	<i>F. virginiana</i> (octoploid)	<i>F. ×ananassa</i> (octoploid)	Blackberry (tetraploid)	Raspberry (diploid)
FAC-004a	AJ315844, 'Chandler', lpt46 gene for lipid transfer protein	F:atgggagatgctcttagtgga R:acagattcgggtcggttattga	48	230	(tg) ₆	5'UTR	+	-	+/+	+/+	+/+		
FAC-004b	AJ315844, 'Chandler', lpt46 gene for lipid transfer protein	F:atcaataaacgaaccgaatctg R:gtcctgaagggtggaagaatcag	48	254	(ta) ₆	5'UTR	-	-	+/+	+/+	+/+		
FAC-004c	AJ315844, 'Chandler', lpt46 gene for lipid transfer protein	F:gtccatactttaagccgaatgc R:acgtcccttcacataaaatgg	48	334	(ta) ₈ (tg) ₅	5'UTR	-	-	+	-	+		
FAC-004d	AJ315844, 'Chandler', lpt46 gene for lipid transfer protein	F:gccaatgtcgatgttcacta R:tccttgggtcgatcacataaat	58	350	(gaa) ₆	5'UTR	+	+/+	+/+	+	+/+		
FAC-016	AX025481, <i>F. ×ananassa</i> , 3-ketoacyl-CoA thiolase	F:tttcaagacaggagcaagatca R:agtggtgggtcccatctttdta	64	233	(gct) ₆		+	+	+	+	+		
ARSFL_133	AY158836, <i>F. ×ananassa</i> , quinone oxidoreductase (QR) gene	F:aaacttgattggcggagaga R:ttctgtttgaggcccaagac	59	203	(gaa) ₃	5'UTR		+	+	+	+	+	+
FAC-012	FVY17185, <i>F. vesca</i> 'Reine des Valles', putative cystathionine gamma synthase	F:tacacgtgtcctagggtttca R:agcggagaatgagtgacgatag	62	169	(cct) ₆	Exon1	-	-	+	+	+		
ARSFL_103	FXU19944, 'Pajaro', clone RJ39 unknown protein	F:ggcagttgatgcttatgatga R:attggatcttcaggcacacac	59	171	(agg) ₉₋₄	Coding		+	+	+	+	-	-
ARSFL_104	FXU63631, 'Chandler', LMW heat shock protein	F:ctcgaatcgactggaaggag R:gaacctcctcacgaacttgc	59	200	(agg) ₄₋₁	Coding		+	+	+	+	+	+
FAC-015a	X91839, <i>F. ×ananassa</i> , auxin-binding protein	F:gaatttgaccgttgattgat R:attgca gaa gaa gaa gcaagc	60	161	(ctt) ₅	Exon1	+	+	+	+	+		
FAC-015b	X91839, <i>F. ×ananassa</i> , auxin-binding protein	F:gaacacgaggatttacaagtgc R:agtggtgtccagttcaagacat	64	292	(ttta) ₃	Intron 4	+	-	+	+	+		

Table 1 continued next page

Table 1. Continued.

Marker ^z	GenBank or library accession, source genome, sequence similarity ^{y, x}	Primer sequences 5' to 3', or source reference if published	Tm °C	Size (bp) ^w	Repeat ^v	Location	<i>F. iinumae</i> (diploid)	<i>F. vesca</i> (diploid)	<i>F. chiloensis</i> (octoploid)	<i>F. virginiana</i> (octoploid)	<i>F. ×ananassa</i> (octoploid)	Blackberry (tetraploid)	Raspberry (diploid)
Derived from a genomic library													
ARSFL_1	10C9, 'Earliglow', NS	F:gcggaccatagcacactgttgac R:gcgccttccttgatacaacttac	52	259	(ga) ₁₇		+/+	+	+	+/+	-	-	
ARSFL_2	10H6, 'Earliglow', NS	F:gcgaagcgaagcggatg R:gcgaacgtcgaaggacattctcat	52	237	(ct) ₂₀		+/+	+/+	+	+/+	-	-	
ARSFL_3	11A4, 'Earliglow', NS	F:gcgggtgcttaggtttcacaact R:gcgcaagtggtatttaagggttag	52	291	(gt) ₆ (ga) ₁₂		+/+	+/+	+	+/+	-	-	
ARSFL_4	11B7, 'Earliglow', NS	F:gcggtcgcattgagttggaaggata R:gcgtagccaacaccgatctacc	52	166	(ga) ₁₃		+/+	+	+	+/+	+	-	
ARSFL_7	11H7, 'Earliglow', NS	F:gcgcgcataaggcacaag R:gcgaatggcaatgacatcttctct	52	256	(ct) ₁₆ (ga) ₁₃		+	+/+	+	+/+	+	+	
ARSFL_8	12G6, 'Earliglow', NS	F:gcggaccgaagatgacctcaccc R:gcgttagccgaagatgttctactg	52	297	(ga) ₁₆₋₁		+/+	+	+	+/+	-	-	
ARSFL_9	12H3, 'Earliglow', NS	F:gcgagcgatcatggagaga R:gcgttctacgtcccaataaatc	52	222	(ga) ₁₀		+/+	+/+	+	+	-	+/+	
ARSFL_10	13A11, 'Earliglow', NS	F:gcgtcagccgtatgtagcag R:gcgcaagccctcaaatatc	52	252	(ga) ₂₀		+/+	+/+	+	+/+	+/+	-	
ARSFL_11	13C2, 'Earliglow', NS	F:gcgaagcataactggcagatctcg R:gcggcctaggtgatcttggga	52	279	(ga) ₂₆		+/+	+/+	+	+/+	+	-	
ARSFL_12	13F7, 'Earliglow', NS	F:gcggaaccaaagcaataagatg R:gcgaccacgacagtttctcactct	52	264	(ct) ₁₁		+/+	+	+	+/+	-	-	
ARSFL_13	13H3, 'Earliglow', NS	F:gcgggcagcctccagatctcctta R:gcgccctatcttcgaccga	52	259	(ga) ₁₆₋₁		+/+	-	+	+/+	-	+/+	
ARSFL_14	14B2, 'Earliglow', NS	F:gcgttaacggaaacttagagaga R:gcggaaagggtcaaacatc	52	233	(ga) ₁₃		+/+	+	+	+/+	-	-	
ARSFL_15	14C5GA, 'Earliglow', NS	F:gcgggctgtccacactcctttct R:gcgatgcgtaagctcttcaaata	52	203	(ga) ₁₇		+/+	+	+	+/+	-	-	
ARSFL_16	14C5TA, 'Earliglow', NS	F:gcgtatggcgtttctctcac R:gcgagcggtcagccaaagagaact	52	177	(ta) ₁₀		+	+	+	+/+	-	-	
ARSFL_17	14D6, 'Earliglow', NS	F:gcgcatacaatcgccatagaaac R:gcgaacacgccttcaacaaccac	52	218	(ct) ₁₀		+/+	+/+	+	+/+	-	-	
ARSFL_18	14F5, 'Earliglow', NS	F:gcgggaccactggtaaaaactact R:gcgcccaactcattattactt	52	291	(ct) ₂₃₋₁		-	-	-	-	-	-	
ARSFL_19	14G5, 'Earliglow', NS	F:gcgaaaccgaagaaagaacaaatgc R:gcggcccaaagggaaga	52	251	(ga) ₁₂		+	+/+	+	-	-	+	
ARSFL_20	15A4, 'Earliglow', NS	F:ggcgagcaatgttggtgcact R:gcgctaccattcttctcccgtgat	52	190	(ct) ₁₉₋₁		-	-	-	-	-	-	
ARSFL_22	15E7, 'Earliglow', NS	F:gcgaaccccattaacagcttca R:gcgatcaaatccctctaacaat	52	158	(ga) ₁₁		+/+	+/+	+	+/+	+/+	+/+	
ARSFL_23	16D6, 'Earliglow', NS	F:gcggccgcttgaaaggag R:gcgtcccactgtcaaggtaaa	52	237	(ga) ₁₃		-	+	+	+/+	+/+	-	
ARSFL_24	16F8, 'Earliglow', NS	F:gcgtggatctatgatcgttgcc R:gcggggttcttcttgggaatg	52	195	(ct) ₂₀		+	-	+	+/+	-	-	
ARSFL_26	17A1, 'Earliglow', NS	F:gcgtgaggtcccttaagcactaaa R:gcgcagggtaacgaaacctaaa	52	282	(ga) ₁₂		+	+	+	+/+	-	-	
ARSFL_27	17C4, 'Earliglow', NS	F:gcgaagccagactcaattacc R:gcgtaccgccattgttac	52	164	(ct) ₄₅₋₁		+	+	+	+/+	-	-	

Table 1 continued next page

Table 1. Continued.

Marker ^z	GenBank or library accession, source genome, sequence similarity ^{y, x}	Primer sequences 5' to 3', or source reference if published	T _m °C	Size (bp) ^w	Repeat ^v	Location	<i>F. iinumae</i> (diploid)	<i>F. vesca</i> (diploid)	<i>F. chiloensis</i> (octoploid)	<i>F. virginiana</i> (octoploid)	<i>F. ×ananassa</i> (octoploid)	Blackberry (tetraploid)	Raspberry (diploid)
ARSFL_28	17H3, 'Earliglow', NS	F:gcgccggtgctgattcttctgt R:gcgccttctcttactgcgacaaa	52	287	(ga) ₁₈		+	+	+	+/+	-	-	
ARSFL_29	16H6#2, 'Earliglow', NS	F:gcggggggatattggtggtgatg R:gcgcgggtttcacgtaatttctt	52	284	(ga) ₁₃₋₁		+	+	-	+/+	-	-	
ARSFL_92	16H6, 'Earliglow', NS	F:tcgggtgacgaatctaaagg R:gaaagaacaagcaccaccaca	59	179	(ct) ₈ (ga) ₁₃₋₁		+	+	+	+	-	-	
ARSFL_96	21B6, 'Earliglow', NS	F:agtctaggctgcttgggttg R:ccaagggaagaacagacatga	59	208	(ct) ₂₄		+	+	+	+/+	-	-	

^zARSFL primer pairs were designed in Beltsville, Md., and FAC primer pairs were developed in Corvallis, Ore.

^ySimilarity of the source sequence to other sequences archived in the GenBank database are provided if possible; if the similarity is not available, "NA" is indicated, and, if no similarity was found, "NS" is indicated.

^xSometimes the cultivar name was provided for *F. ×ananassa*-derived sequences; if a cultivar name is provided, it is *F. ×ananassa* unless otherwise stated.

^wThe expected PCR product size in base pairs (bp) is based on the sequence from which each primer pair is derived.

^vThe simple sequence motifs identified in the source sequences are indicated as the simple sequence repeats, enclosed in parentheses, and the number of repeats found in the source sequence in subscript. For example, (ct)₄ is interpreted as "ctctctct". When the subscript number is indicated as, for example, n-1, this means the repeat was not perfect and either a base pair was missing or a base pair substitution was present in the repeat, compared with a perfect repeat sequence.

Table 2. These *Fragaria* (strawberry) and *Rubus* (raspberry and blackberry) genotypes were used to evaluate published and newly developed simple sequence repeat (SSR) primers in polymerase chain reactions (PCRs).

Accession or plant patent no ^z	Plant species	Cultivar or other designator	Location tested ^y	Comments
PI 616505	<i>F. iinumae</i>	CFRA 1008	C	Another diploid species
PI 551507	<i>F. vesca</i>	'Baron Solemacher'	B	Diploid strawberry mapping population
None	<i>F. vesca</i>	WC6	B	Diploid strawberry mapping population
PI 9000	<i>F. vesca</i>	UC5	B	Diploid virus indicator
PI 551646	<i>F. vesca</i> subsp. <i>bracteata</i>	CFRA 389	C	Diploid core accession, day-neutral
PI 551908	<i>F. vesca</i> subsp. <i>vesca</i>	'Snow King'	C	White-fruited diploid strawberry
PI 612490	<i>F. chiloensis</i>	'Scott's Creek'	B	Large-fruited, super core octoploid
PI 612489	<i>F. chiloensis</i>	HM 1	B	Highly productive, super core octoploid
PI 552038	<i>F. chiloensis</i>	Island of Lemuy	C	White-fruited octoploid
PI 551736	<i>F. chiloensis</i> f. <i>chiloensis</i>	CFRA 372	C	Super core octoploid
CFRA 1806	<i>F. virginiana</i>	US 4808	B	Octoploid resistant to <i>Xanthomonas fragariae</i>
CFRA 1807	<i>F. virginiana</i> × (<i>F. ×ananassa</i>)	US 4809	B	Octoploid resistant to <i>Xanthomonas fragariae</i>
PI 616561	<i>F. virginiana</i> subsp. <i>grayana</i>	CFRA 1170	C	Octoploid core accession
PI 552250	<i>F. virginiana</i> subsp. <i>virginiana</i>	WC 26	C	Octoploid day-neutral
PP 9,866	<i>F. ×ananassa</i>	'Rosa Linda'	B	Florida-bred octoploid cultivar
PP 5,266	<i>F. ×ananassa</i>	'Selva'	B	Day-neutral octoploid parent in mapping population with 'Delmarvel'
PI 616589	<i>F. ×ananassa</i>	'Delmarvel'	B	<i>Phytophthora Fragariae</i> -resistant octoploid parent in mapping population with 'Selva'

Table 2 continued next page

Table 2. Continued.

Accession or plant patent no ^z	Plant species	Cultivar or other designator	Location tested ^j	Comments
None	<i>F. ×ananassa</i>	‘Pelican’	B	<i>Colletotrichum</i> species crown and fruit rot-resistant octoploid parent in mapping population with ‘Chandler’
PP 5,262	<i>F. ×ananassa</i>	‘Chandler’	B	Widely adapted octoploid parent in mapping population with ‘Pelican’
PP 8,729	<i>F. ×ananassa</i>	‘Sweet Charlie’	B	Susceptible Florida-bred octoploid parent cultivar in mapping populations with US 4808 and US 4809
PI 551394	<i>F. ×ananassa</i>	‘Earliglow’	B,C	Octoploid genotype from which a genomic library for SSR development was derived
PI 551398	<i>F. ×ananassa</i>	‘Titan’	C	Octoploid cultivar
PI 551416	<i>F. ×ananassa</i>	‘Scott’	C	Red stele-resistant octoploid cultivar
PI 551406	<i>F. ×ananassa</i>	‘Allstar’	C	Widely adapted mid-Atlantic octoploid cultivar
PI 551490	<i>F. ×ananassa</i>	‘Hecker’	C	First day-neutral octoploid cultivar released in U.S.
None	<i>R. hybrid</i> (blackberry)	APF12	B	Primocane-fruiting tetraploid blackberry in mapping population with ‘Arapaho’
PP 8,510	<i>R. hybrid</i> (blackberry)	‘Arapaho’	B	Summer-fruiting tetraploid blackberry in mapping population with APF12
PI 553276	<i>R. hybrid</i> (blackberry)	‘Merton’	B	Tetraploid source of thornless trait in U.S. blackberry genotypes
PI 553348	<i>R. hybrid</i> (blackberry)	‘Illini Hardy’	B	Tetraploid blackberry that produces secondary floral initiation buds to replace those damaged by cold
None	<i>R. idaeus</i> (red raspberry)	NY322	B	Diploid red raspberry in mapping population with ‘Jewel’
PI 553742	<i>R. occidentalis</i> (black raspberry)	‘Jewel’	B	Diploid black raspberry in mapping population with NY322

^zPI numbers refer to accessions in the USDA–ARS germplasm collection. CFRA numbers refer to accessions in the USDA–ARS germplasm collection at Corvallis that have not yet been given a PI number. PP numbers refer to US Plant Patent numbers.

^jSome accessions were tested at Beltsville, Md. (B), and some were tested at Corvallis, Ore. (C).

Table 3. Simple sequence repeat (SSR) primers derived from *Fragaria* (strawberry) and *Rubus* (bramble) sequences were tested in polymerase chain reactions (PCRs) with strawberry, blackberry, and raspberry genotypes. For each group of tested genotypes, the number of primers that were tested, the number that amplified a product, and the number that detected polymorphisms are reported.

SSR sequence source	Strawberry					Blackberry			Raspberry		
	Number tested	Number amplified	Number polymorphic	Polymorphic in octoploid parents ^z	Polymorphic in diploid parents ^y	Number tested	Number amplified	Polymorphic in tetraploid parents ^x	Number tested	Number amplified	Polymorphic in diploid parents ^w
<i>Fragaria</i> genomic	28	24	24	19/24	9/18	23	6	3/6	28	5	3/5
<i>Fragaria</i> GenBank	34	26	15	10/17	-	19	6	1/5	20	4	1/4
<i>F. vesca</i>	10	10	8	7/10	-	8	1	0/1	10	2	2/2
<i>F. virginiana</i>	4	4	4	1/1	-	1	1	0/1	4	1	0/1
<i>Rubus alceifolius</i>	8	0	-	-	-	8	2	1/2	8	2	2/2

^zThe octoploid strawberry mapping parents are US 4808 and ‘Sweet Charlie’.

^yThe diploid strawberry mapping parents are ‘Baron Solemacher’ and WC6.

^xThe tetraploid blackberry mapping parents are ‘Arapaho’ and Univ. of Arkansas selection APF-12.

^wThe diploid raspberry mapping parents are Cornell Univ. red raspberry selection NY322 and black raspberry cultivar Jewel.

for a primer pair was initially determined by gradient PCR using template DNA from two cultivars of *F. xananassa* 'Rainier' and 'Earliglow' at 55 °C ± 10 °C.

At Beltsville, PCR components included 1×Qiagen PCR buffer (1.5 mM MgCl₂), 0.67 mM dNTPs (higher than usual to facilitate amplification through repeat regions), 0.5 μM each primer, and 0.4 U HotStar Taq polymerase enzyme, and template DNA at either 2 ng·μL⁻¹ (standard stringency) or 0.002 ng·μL⁻¹ (high stringency) in a total reaction volume of 15 μL. Reaction components were heated to 95 °C for 15 min to activate the polymerase. The heat-activation period was followed by 30 cycles of 40 s at 95 °C, 40 s at 52 °C (for primers designed from the genomic library) or 59 °C (for primers designed from GenBank sequences), and 40 s at 72 °C, followed by 10 min at 72 °C and storage at 4 °C. The thermal cycler was the MJ Research Engine Tetrad with 96-well blocks. For some reactions a PCR gradient was used from 52 °C through 62 °C using the gradient option.

PCR FRAGMENT ANALYSIS. At Corvallis, PCR products and a 100-bp-size standard (Promega, Madison Wis.) were separated on 3% GenePure LE agarose (ISC BioExpress, Kaysville, Utah) at 100 V for 180 min. The DNA fragments were visualized after ethidium bromide staining using the GelDoc 2000 gel documentation system (BioRad, Hercules, Calif.). To detect polymorphisms, fragment sizes were estimated by Quantity One software (BioRad).

To determine if the reactions resulted in a product, at Beltsville, PCR products were diluted in water and visualized after 12 min of electrophoresis through a 2% agarose gel using Invitrogen's E-gel 96 system. Gel images were captured in .tif files using the Alpha-Imager (Alpha Innotech Corp., San Leandro, Calif.), analyzed with the E-gel 96 Editor software (Alpha Innotech Corp.).

To size the PCR products and identify polymorphisms at Beltsville, fluorescently labeled primers were ordered from Applied Biosystems and PCR was repeated. Fluorescently labeled PCR products were separated by capillary gel electrophoresis and detected using the Applied Biosystems, 3700 DNA Analyzer. The size standard used was GENESCAN 400HD [ROX]. Sizing data were analyzed using ABIs Genotyper (Applied Biosystems) software.

Results

DETERMINING FUNCTIONAL TAXONOMIC DISTANCE FOR SSR DEVELOPMENT. GenBank sequences containing (CT)_n repeats were obtained from *F. xananassa*, *Rosa*, *Prunus persica*, *Malus xdomestica*, *Pyrus communis*, *Medicago truncatula*, *Glycine max*, *Gossypium*, and *Arabidopsis thaliana*, and primers designed from six of each species were initially tested to see if they would amplify products from *Fragaria*, *Rubus*, *P. persica*, or *G. max* template DNA in PCR amplifications. None of the *P. persica*, *M. xdomestica*, *P. communis*, *M. truncatula*, *G. max*, *Gossypium*, or *A. thaliana* primers amplified products from *Fragaria* or *Rubus* DNA. Of the previously published SSRs tested, the 21 primer pairs designed from *P. persica* genomic sequences and reported by Dirlewanger et al. (2002) to amplify products from *Fragaria* genomic DNA, successfully amplified a product from *P. persica* DNA but failed in our attempts with varying PCR conditions to amplify products from *Fragaria* or *Rubus* DNA. In addition, only *Prunus*-derived primers amplified products from *P. persica* DNA, and only *G. max*-derived primers amplified a product from *G. max* DNA. Therefore, transference of SSRs from distantly related genera was less successful than expected based on previous reports.

Transference of SSRs among Rosoideae genera was tested using previously published SSRs from *Fragaria* and *Rubus* species, including four primer pairs designed from *F. virginiana* genomic sequences (Ashley et al., 2003), 10 SSRs designed from genomic sequences of *F. vesca* (diploid strawberry) by James et al. (2003), and eight primer pairs developed from *R. alceifolius* (Amsellem et al., 2001), as well as six new GenBank-derived *F. xananassa* primers. One of the four primer pairs designed from *F. virginiana* genomic sequences (Ashley et al., 2003) amplified both blackberry and raspberry DNA. Three of the 10 SSRs designed from *F. vesca* (diploid strawberry) genomic sequences by James et al. (2003) amplified products from blackberry, and two of those amplified products from raspberry. One of the six *F. xananassa* primers amplified a product from blackberry, and one amplified a product from raspberry. In total, 27% and 19% of these *Fragaria*-derived primer pairs amplified a product in blackberry and/or raspberry, respectively. Interestingly, of the eight primer pairs developed from *R. alceifolius* (Amsellem et al., 2001), only two amplified a product from blackberry and raspberry, so that using the *R. alceifolius*-derived SSRs with blackberry and raspberry was no better than using the *Fragaria*-derived SSRs. None of the eight *R. alceifolius*-derived primer pairs amplified a product from *Fragaria* DNA. In addition, none of the *Rosa*-derived primers amplified a product from *Fragaria* or *Rubus*. To confirm this finding, 24 additional *Rosa*-derived SSR primer pairs (data not shown) were tested with *Fragaria* and *Rubus* DNA, and, again, none amplified a product. Therefore, some SSRs were transferable between some Rosoideae genera, but not others.

Transference of SSRs between *Fragaria* congeners was highly successful. The published *F. virginiana* and *F. vesca* SSRs (Ashley et al., 2003; James et al., 2003) were used in addition to six GenBank *F. xananassa*-derived primers to test for intragenomic amplification. These 20 primer pairs amplified products from all the *Fragaria* templates tested, including *F. xananassa*, *F. virginiana*, *F. chiloensis*, and *F. vesca* (Tables 1 and 3). These results support the conclusion made by Ashley et al. (2003) and Sargent et al. (2003) that primers designed from one *Fragaria* species generally can be used with other *Fragaria* species.

GENBANK-DERIVED SSRs AND CROSS-SPECIFIC AMPLIFICATION AND POLYMORPHISM IN FRAGARIA. Because transference of SSRs between *Fragaria* congeners was so successful, we developed new strawberry SSRs using *Fragaria* sequences archived in GenBank.

Of the two methods used to identify SSR-containing *Fragaria* sequences from GenBank, SSRIT was superior to BLAST in that it detected a larger number of repeats as well as a greater diversity of repeat motifs. From the 306 *Fragaria* sequences in GenBank, SSRIT identified 42 with a minimum of five tandem repeats and 227 sequences with a minimum of 4 tandem repeats. The motifs uniquely identified by SSRIT included AAAAT (FAC-001), CTT (FAC-010, FAC-010), AAG (FAC-003, FAC-013), and GCT (FAC-016). BLAST identified 57 *Fragaria* sequences containing repeats.

All primer pairs designed from GenBank sequences using both methods amplified a product in the source species *F. xananassa* except for FAC-010. SSRs FAC-012 and FAC-014, designed from *F. vesca*, amplified PCR fragments of the expected size in *F. chiloensis*, *F. virginiana*, and *F. xananassa*. It is of interest to note that FAC-012 failed to amplify in the source species, *F. vesca*. The amplification rate using GenBank-derived SSR primers was high within the *Fragaria* genus (73% in *F. vesca*; 79% in *F. iinumae*; 88% in the octoploid *F. chiloensis* and *F. virginiana*;

and 92% in the source species *F. xananassa*). Likelihood of polymorphism detection seemed to increase as the number of simple sequence repeats increased, though we were not able to determine if the apparent increase was statistically significant. Of GenBank-derived SSR primers that amplified a product, 68% with six or more repeats detected polymorphisms among tested accessions, and 43% with fewer than six repeats detected polymorphisms. Between the octoploid strawberry parents (US 4808 × 'Sweet Charlie') of a genetic mapping population, 59% of the GenBank-derived primers detected polymorphisms.

EFFECT OF REPEAT LOCATION ON AMPLIFICATION AND POLYMORPHISM DETECTION. Simple sequence repeats were found in all regions of genes: ten were present in 5'UTRs, eight in 3'UTRs, eleven in coding regions and three were detected in introns (Table 1). The SSRs identified from the 5'UTRs contained mostly dinucleotide repeats (7 out of 10) and 80% detected polymorphisms among the *F. xananassa* accessions tested. All five of the dinucleotide-containing SSRs isolated from 3'UTRs detected polymorphisms among *F. xananassa*, while only one out of the three trinucleotide-containing SSRs detected polymorphisms. Trinucleotide repeats were the most abundant type found in the coding regions as previously reported in other plant species (Smulders et al., 1997). Seven of the eleven SSRs identified in exons contained trinucleotide repeats. Three of these trinucleotide-containing SSRs (42%) identified in *Fragaria* coding sequences detected polymorphisms. The number of SSRs detected in introns was small in this study. Out of 3 intron-located SSRs, only one, FAC-008, detected polymorphisms within *F. vesca*, *F. chiloensis*, *F. virginiana* and *F. xananassa*. In spite of these trends, ARSFL_33, derived from a *Fragaria* sequence and having only four tandem repeats in a coding region detected polymorphisms between raspberry genotypes.

SSRs DEVELOPED FROM A *FRAGARIA* GENOMIC LIBRARY. Both the amplification success rate and the percentage of polymorphism detected by SSRs derived from the genomic library were 85%. Seventy-seven percent of SSRs derived from the genomic library, averaged across *F. xananassa*, *F. vesca*, and *F. virginiana*, detected polymorphisms. Of the SSRs derived from the *F. xananassa* genomic library, 79% detected polymorphisms between parents of an octoploid population (US4840 × 'Sweet Charlie') and 50% between the parents of a diploid population ('Baron Solemacher' × WC6) (Davis and Yu, 1997). The SSR primers derived from the genomic library detected more polymorphisms than those derived from GenBank sequences (Table 3).

GENOMIC- AS OPPOSED TO GENBANK-DERIVED SSRs IN *FRAGARIA*. There were no noticeable differences in ability to amplify a product that could be associated with SSR derivation from the genomic library or GenBank sequences. Among SSRs derived from the genomic library, all but ARSFL_18 and ARSFL_20 amplified a product from at least one genotype, and among GenBank-derived SSRs, all but FAC-010, ARSFL_105, and ARSFL_107 amplified a product. ARSFL_19, derived from the genomic library, amplified a product from some genotypes but not its source genome *F. xananassa*. Similarly, GenBank-derived SSRs FAC-006b and FAC-012 did not amplify a product from their respective source genomes, *F. xananassa* and *F. vesca*, though they did amplify products from other genomes. However, SSRs developed from the genomic library rather than GenBank sequences detected more polymorphisms (Table 3).

CROSS-SPECIES AMPLIFICATION AND POLYMORPHISM OF SSRs WITHIN *FRAGARIA*. Within *Fragaria*, there were no significant differences in SSR ability to amplify a product associated with

SSR source species (Tables 1 and 3). SSRs developed from *F. xananassa*, *F. virginiana*, and *F. vesca* seemed equally capable of amplifying products from any *Fragaria* species and detecting polymorphisms between genotypes within species. Twenty-one of the twenty-two primer pairs obtained from *F. xananassa* were cross-transferable to the progenitor octoploids, *F. chiloensis* and *F. virginiana*. In the diploid species, *F. iinumae* and *F. vesca*, 19 and 17 of the 22 *F. xananassa* primers, respectively, amplified the expected size fragments. Furthermore, 66% of the *F. xananassa*-derived SSRs detected polymorphisms between parents of the octoploid strawberry genetic mapping population (US 4808 × 'Sweet Charlie') and 56% between parents of the diploid strawberry population ('Baron Solemacher' × WC6).

CROSS-GENERIC AMPLIFICATION AND POLYMORPHISM OF SSRs BETWEEN *FRAGARIA* AND *RUBUS*. Because some SSRs were transferable between some Rosoideae genera, but not others, cross-generic amplification between *Fragaria* and *Rubus* was evaluated further using new *Fragaria* BLAST-identified SSRs as well as 22 *F. xananassa* genomic SSR primer pairs. Between 26% to 31% of the new *Fragaria* genomic and GenBank-derived primer pairs resulted in amplification of product from blackberry and 17% to 20% from raspberry (Table 3). In total, ten *Fragaria*-derived SSR primers amplified products from both blackberry and raspberry, although only two detected polymorphisms. Five SSR primers amplified products from only blackberry (three detected polymorphisms), and three SSR primers amplified products from only raspberry (two detected polymorphisms). Of SSR primers that amplified a product, the percentage that detected polymorphisms ranged from 20% to 60% with 35% average for blackberry, and 43% average for raspberry.

Discussion

Transference of SSRs among distantly related genera was unsuccessful as sequences derived from other genera did not amplify a product from the *Fragaria* and *Rubus* genotypes we tested. Although rose is in the same subfamily, Rosoideae, the thirty primer pairs we designed from rose sequences did not amplify a product from any strawberry or bramble genotype. And, although SSRs derived from peach have been reported to amplify a product from strawberry, the 21 peach-derived SSRs reported by Dirlewanger et al. (2002) to amplify products from strawberry failed in our attempts with varying PCR conditions to amplify products from *Fragaria* or *Rubus* DNA. However, these and six primers we designed from peach sequences in GenBank amplified a product from peach DNA. This is especially significant, because peach recently has been proposed as a model species for the entire Rosaceae family. Our findings indicate that peach SSRs will not be as useful as initially hoped for *Fragaria* and *Rubus* species and support the findings of Peakall et al. (1998) and Decroocq et al. (2003) on limited cross-species transferability in the Rosaceae family. Decroocq and co-workers (2003) found that optimal utility of *Prunus armeniaca* L. (apricot) EST SSR markers was obtained for closely related species belonging to the same subgenus *Prunophora*, and that the threshold distance after which no amplification can be expected is short in the Rosaceae family. It is likely that a small percentage of primers designed from *Prunus* and other Rosaceae genera will reliably amplify a product from strawberry, raspberry, or blackberry, and these primers eventually will be very valuable for genome comparison. However, the percentage appears to be too small to be practical in quickly developing SSRs and maps for marker-assisted selection.

Therefore, genome comparisons among species from different Rosaceae subfamilies might be more efficient with hybridization techniques than with PCR-based techniques.

Sequences from any *Fragaria* species appear to be equally useful for designing SSRs for any other *Fragaria* species. Current research projects developing EST sequences from *F. vesca* as a model for studying gene expression in strawberry should yield many repeat-containing sequences from which SSRs can be designed and used with any of the *Fragaria* species. Other research projects developing ESTs from *F. xananassa*, likewise, should yield widely useful SSRs. The SSRs we tested have limited transference between *Fragaria* and *Rubus*, and these SSRs are particularly interesting for comparative mapping. For example, SSRs associated with resistance to a pathogen in strawberry may be associated with resistance to a similar pathogen in raspberry and blackberry.

Although 30% to 50% of strawberry-derived SSRs amplified a product in raspberry and blackberry, only about 30% to 50% of these detected a polymorphism between parents of the raspberry ('Jewel' × NY322) or blackberry (APF-12 × 'Arapaho') mapping populations we tested. Therefore, only about 10% of the strawberry-derived SSRs were useful in mapping in these two populations. It seems clear that dependence on strawberry-derived SSRs is insufficient for genetic mapping in *Rubus*, and *Rubus* sequences are required.

Transference among *Rubus* species was not as successful as among *Fragaria* species. SSRs from the Asian (Indonesia and south east China) native noxious weed *R. alceifolius*, a member of the subgenus *Malachobatus*, exhibited only limited cross-species transference to raspberry (subgenus *Idaeobatus*) or to blackberry (subgenus *Rubus*, formerly *Eubatus*). *Rubus* is one of the most diverse genera in the plant kingdom and considerable differentiation of species exists in the three subgenera used in this study (Jennings, 1988). It may be necessary to develop raspberry SSRs for use with raspberry and blackberry SSRs for use with blackberry.

Both BLAST and SSRIT can be used to screen in-house library sequences for SSRs without first having to deposit sequences in GenBank. Other SSR identification tools include two designed to work with the Linux operating system: TROLL (Castelo et al., 2002; Source Forge, 2001); and Sputnik available at (Espresso Software Development, 2003). A fourth tool, MISA, present at [Institute of Plant Genetics and Crop Plant Research (IPK) Plant Genome Resources Center, 2002; Thiel et al., 2003] is designed to interface with the Primer3 primer design software with the help of two PERL scripts available on the web site. We compared BLAST and SSRIT and found SSRIT was superior to BLAST, because it identified more GenBank sequences containing repeats and detected a greater diversity of repeat motifs.

SSR primers developed from the genomic library seemed better able to detect polymorphisms among accessions and between parents of mapping populations (Table 3). It is possible that the SSRs derived from the genomic library were more likely to detect polymorphisms because of the larger repeat regions found in the genomic library sequences (Table 1). Among SSRs derived from GenBank accessions, the ability to detect polymorphisms among accessions increased from 43% to 68% as the number of repeats increased from fewer than six repeats to six or more repeats. This suggests SSRs derived from GenBank sequences with six or more repeats would be as likely to detect polymorphisms as would SSRs derived from a genomic library. If a genomic library is not already available, development of SSRs from GenBank

sequences may be more efficient than from a genomic or cDNA library. On the other hand, if the number of sequences deposited in GenBank is insufficient for genetic mapping, development and sequencing of a library is warranted. Since Morgante et al. (2002) showed that SSRs are associated with gene clusters in plants, SSRs developed from cDNA sequences or genomic sequences may be equally likely to be associated with traits. The choice of whether to make a cDNA library or genomic library for SSR development could depend on several factors, including: additional research interests that could be better addressed with one library compared to the other; relative cost of making the two different library types; and technical skills of and services available to the researchers involved.

Literature Cited

- Akkaya, M.S., R. Shoemaker, J.E. Specht, A.A. Bhagwat, and P.B. Cregan. 1995. Integration of simple sequence repeat (SSR) DNA markers into a soybean linkage map. *Crop Sci.* 35:1439–1445.
- Aldrich, P.R. and J.L. Hamrick. 1998. Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. *Science* 281:103–105.
- Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.
- Amsellem, L., C. Dutech, and N. Billotte. 2001. Isolation and characterization of polymorphic microsatellite loci in *Rubus alceifolius* Poir (Rosaceae), an invasive weed in La Réunion island. *Mol. Ecol. Notes* 1:33–35.
- Ashley, M.V., J.A. Wilk, S.M.N. Styant, K.J. Craft, K.L. Jones, K.A. Feldheim, K.S. Lewers, and T.L. Ashman. 2003. High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill (Rosaceae). *Theor. Appl. Genet.* 107:1201–1207.
- Broun, P. and S.D. Tanksley. 1996. Characterization and genetic mapping of simple repeat sequences in the tomato genome. *Mol. Gen. Genet.* 250:39–49.
- Castelo, A.T., W. Martins, and G.R. Gao. 2002. TROLL—Tandem repeat occurrence locator. *Bioinformatics* 18:634–636.
- Chase, M.R., C. Moller, R. Kesseli, and K.S. Bawa. 1996. Distant gene flow in tropical trees. *Nature* 383:398–399.
- Cho, Y.G., T. Ishii, S. Temnykh, X. Chen, L. Lipovich, S.R. McCouch, W.D. Park, N. Ayres, and S. Cartinhou. 2000. Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 100:713–722.
- Cipriani, G., G. Lot, W.-G. Huang, M.T. Marrazzo, E. Peterlunger, and R. Testolin. 1999. AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L.) Batsch]: Isolation, characterization and cross-species amplification in *Prunus*. *Theor. Appl. Genet.* 99:65–72.
- Cordeiro, G.M., R. Casu, C.L. McIntyre, J.M. Manners, and R.J. Henry. 2001. Microsatellite markers from sugarcane (*Saccharum* spp) ESTs cross transferable to erianthus and sorghum. *Plant Sci.* 160:1115–1123.
- Cregan, P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler, N. Kaya, T.T. VanToai, D.G. Lohnes, J. Chung, and J.E. Specht. 1999. An integrated genetic map of the soybean genome. *Crop Sci.* 39:1464–1490.
- da Silva, J.A.G. 2001. Preliminary analysis of microsatellite markers derived from sugarcane expressed sequence tags (ESTs). *Genet. Mol. Biol.* 24:155–159.
- Davis, T.M. and H. Yu. 1997. A linkage map of the diploid strawberry *Fragaria vesca*. *J. Hered.* 88:215–221.
- Dayanandan, S., K.S. Bawa, and R. Kesseli. 1997. Conservation of microsatellites among tropical trees (Leguminosae). *Amer. J. Bot.* 84:1658–1663.
- Decroocq, V., M.G. Favé, L. Hagen, L. Bordenave, and S. Decroocq. 2003. Development and transferability of apricot and grape EST microsatellite markers across taxa. *Theor. Appl. Genet.* 106:912–922.
- Dirlewanger, E., P. Cosson, M. Tavaud, M. Aranza, C. Poizat, A. Zanetto, P. Arús, and F. Laigret. 2002. Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity

- analysis on peach and sweet cherry (*Prunus avium* L.). *Theor. Appl. Genet.* 105:127–138.
- Downey, S.L. and A.F. Iezzoni. 2000. Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. *J. Amer. Soc. Hort. Sci.* 125:76–80.
- Echt, C.S., G.G. Vendramin, C.D. Nelson, and P. Marquardt. 1999. Microsatellite DNA as shared genetic markers among conifer species. *Can. J. For. Res.* 29:365–371.
- Espresso Software Development. 2003. Sputnik. 6 June 2004. <<http://espressoftware.com/pages/sputnik.jsp>>.
- Fazio G., S.M. Chung, and J.E. Staub. 2003. Comparative analysis of response to phenotypic and marker-assisted selection for multiple lateral branching in cucumber (*Cucumis sativus* L.). *Theor. Appl. Genet.* 107:875–883.
- Graham, J., K. Smith, M. Woodhead, and J. Russell. 2002. Development and use of simple sequence repeat SSR markers in *Rubus* species. *Mol. Ecol. Notes* 2:250–252.
- Gramene. 2004. SSRIT—Simple sequence repeat identification tool. 6 June 2004. <<http://www.gramene.org/db/searches/ssritool>>.
- Holland, J.B., S.J. Helland, N. Sharopova, and D.C. Rhyne. 2001. Polymorphism of PCR-based markers targeting exons, introns, promoter regions, and SSRs in maize and introns and repeat sequences in oat. *Genome* 44:1065–1076.
- Institute for Chemical Research. 2000. Clustal W multiple sequence alignment. 6 June 2004. <<http://clustalw.genome.jp/>>.
- Institute of Plant Genetics and Crop Plant Research (IPK) Plant Genome Resources Center. 2002. MISA—MicroSatellite identification tool. 6 June 2004. <<http://pgrc.ipk-gatersleben.de/misa/>>.
- Isagi, Y. and S. Suhandono. 1997. PCR primers amplifying microsatellite loci of *Quercus myrsinifolia* Blume and their conservation between oak species. *Mol. Ecol.* 6:897–899.
- James, C.M., F. Wilson, A.M. Hadonou, and K.R. Tobutt. 2003. Isolation and characterization of polyploid microsatellites in diploid strawberry (*Fragaria vesca* L.) for mapping, diversity studies and clone identification. *Mol. Ecol. Notes* 3:171–173.
- Jennings, D.L.. 1988. Raspberries and blackberries: Their breeding, diseases and growth. Academic, San Diego.
- Kantety, R.V., M. La Rota, D.E. Matthews, and M.E. Sorrells. 2002. Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. *Plant Mol. Biol.* 48:501–510.
- Morgante, M., M. Hanafey, and W. Powell. 2002. Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature Genet.* 194–200.
- National Center for Biotechnology Information. 2004. BLAST. 6 June 2004. <<http://www.ncbi.nlm.nih.gov/BLAST/>>.
- Peakall, R., S. Gilmore, W. Keys, M. Morgante, and A. Rafalski. 1998. Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: Implications for the transferability of SSRs in plants. *Mol. Biol. Evol.* 15:1275–1287.
- Rajora, O.P., M.H. Rahman, S. Dayanandan, and A. Mosseler. 2001. Isolation, characterization, inheritance and linkage of microsatellite DNA markers in white spruce (*Picea glauca*) and their usefulness in other spruce species. *Mol. Gen. Genet.* 264:871–882.
- Rongwen, J., M.S. Akkaya, A.A. Bhagwat, U. Lavi, and P.B. Cregan. 1995. The use of microsatellite DNA markers for soybean genotypes identification. *Theor. Appl. Genet.* 90:43–48.
- Rozen, S. and H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers, p. 365–386. In: S. Krawetz, and S. Misener (eds.). *Bioinformatics methods and protocols: Methods in molecular biology*. Humana Press, Totowa, N.J.
- Sargent, D.J., A.M. Hadonou, and D.W. Simpson. 2003. Development and characterization of polymorphic microsatellite markers from *Fragaria viridis*, a wild diploid strawberry. *Mol. Ecol. Notes* 3:550–552.
- Scott, K.D., P. Eggler, G. Seaton, M. Rossetto, E.M. Ablett, L.S. Lee, and R.J. Henry. 2000. Analysis of SSRs derived from grape ESTs. *Theor. Appl. Genet.* 100:723–726.
- Smulders, M.J.M., G. Bredeijer, W. Rus-Kortekaas, P. Arens, and B. Vosman. 1997. Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. *Theor. Appl. Genet.* 97:264–272.
- SourceForge. 2001. TROLL—Tandem repeat occurrence locator. 6 June 2004. <<http://finder.sourceforge.net>>.
- Steinkellner, H., C. Lexer, E. Turetschek, and J. Glossl. 1997. Conservation of (GA)_n microsatellite loci between *Quercus* species. *Mol. Ecol.* 6:1189–1194.
- Temnykh, S., G. DeClerck, A. Lukashova, L. Lipovich, S. Cartinhour, and S. McCouch. 2001. Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, transposon associations, and genetic marker potential. *Genome Res.* 11:1441–1452.
- Thiel, T., W. Michalek, R.K. Varshney, and A. Graner. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* 106:411–422.
- Thompson, J.D., D.G. Higgins, and T.J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22:4673–4680.
- Whitehead Institute for Biomedical Research. 2004. Primer3. 6 June 2004.
- Whitton, J., L.H. Rieseberg, and M.C. Ungerer. 1997. Microsatellite loci are not conserved across the Asteraceae. *Mol. Biol. Evol.* 14:204–209.