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DNA Extraction Protocols from Dormant Buds of Twelve Woody Plant Genera

Barbara Gilmore[1], Nahla Bassil[2] and Kim Hummer[1,2]

Abstract

Standard plant DNA extraction protocols call for samples of newly expanding leaves and shoots yet analysis is sometimes needed when plants are dormant. We evaluated three DNA extraction protocols using dormant buds from 40 species and four hybrids of 12 genera. Two protocols were from ready-to-use kits (the Omega E-Z 96 Plant DNA Kit and the Fast ID 96-Well Genomic DNA Extraction Kit) and the third included commercial lysis and protein precipitation reagents (Qiagen). The genera included: *Actinidia* (Hardy Kiwi), *Rubus* (red raspberry), *Ribes* (gooseberry and currant), *Cydonia* (quince), *Sorbus* (mountain ash), *Juglans* (butternut), *Amelanchier* (service berry), *Pyrus* (pear), *Mespilus* (medlar), *Corylus* (hazelnut), *Paeonia* (peony), and *Vaccinium* (blueberry). In each of the genera tested, except for *Juglans,* both the Qiagen and Omega protocols generated large amounts of DNA (averaging 40 and 14.8 µg, respectively, from 30 to 36 mg of tissue) from dormant buds. For *Juglans*, none of these procedures provided satisfactory amounts of DNA from dormant buds. The positive result for 11 genera expanded the options for the sources of tissue as well as time of tissue collection for DNA extraction. The highest DNA yield was obtained with the Qiagen protocol, which was the least expensive of the three. However, in this protocol the bud scales must be removed to obtain a clear DNA extract. The Omega protocol may be more efficient if DNA is to be extracted from a large number of samples. In each of these 11 genera, DNA produced by at least one of the three protocols was of sufficient quality to apply in downstream molecular techniques, such as sequencing.

The US Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (NCGR) in Corvallis, OR, was dedicated in 1981 to conserve fruit, nut, and specialty crop genetic resources. This genebank manages more than 26 genera of horticultural crops (13). The NCGR is located at the Lewis Brown Horticultural Research Farm of Oregon State University (OSU). The USDA ARS Arctic and Subarctic Plant Gene Bank (ASPGB), located at the University of Alaska Fairbanks, Agricultural and Forestry Experiment Station, Matanuska Experiment Farm, Palmer, AK, was established in 1999 (12). The ASPGB maintains collections of 32 genera, including *Ribes, Mentha, Paeonia* and *Rheum* (11, 16). Both of these genebanks conserve and manage horticultural genetic resources, establishing backup collections for each other, for germplasm security. The genetics program at each location has examined molecular markers of their collections using newly-expanded leaves for DNA extraction (2, 4, 5) in Corvallis and for *Rheum* in Alaska (12).

Having DNA extraction protocols that work across a wide number of species at different times of the year would be advantageous. Sometimes plant identity is questioned during fall and winter. At this time plants are dormant and vigorously growing leaves are unavailable. In some cases nursery growers may wish to determine the identity of dormant trees or roots, such as those of *Paeonia suffructicosa* or intersectional hybrids. Woody species have a higher percent of phenolics and polysaccharides than do non-woody annuals and biennials. These compounds can contaminate DNA and interfere with downstream analysis (10). During dormancy, these secondary compounds tend to accumulate (8). We sought to determine if adequate DNA can be extracted from dormant tissue for further molecular analyses.

The NCGR laboratory has been routinely extracting DNA from actively growing leaves in most of these genera using commercial cell lysis and protein precipitation solutions (Qiagen, Inc., Valencia, CA), hereby referred to as

[1] USDA ARS Arctic and Subarctic Plant Gene Bank, 1509 S. Georgeson Drive, Palmer, Alaska

[2] USDA ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, Oregon 97333-2521

the Qiagen protocol. Recently we switched to using the Omega E-Z 96 Plant DNA Kit (Omega) (Omega Bio-Tek, Inc., Norcross, GA) and routinely recover adequate DNA from leaves using that method (unpublished). The Fast ID Genomic DNA Extraction Kit (Fast ID) (Fast ID NA, Inc., Fairfield, IA) was reported to produce good quality DNA from commercially processed blackberry purée (3). The objectives of this study were to determine if DNA could be successfully extracted from dormant buds and to compare these three extraction protocols using dormant buds from a broad selection of woody genera to identify economical methods that produce quality DNA in sufficient quantities for marker or sequence analyses.

Materials and Methods

Plant materials. Eight accessions per genus were examined for 40 species and four hybrids from 12 genera (Table 1). The sample collection started in early February 2011. In most of the genera minor bud swelling was beginning. Either floral or leaf buds were used, based on availability. For example, the *Corylus* trees were blooming, so leaf buds were the only available buds. Buds were removed from branches, placed in a collection storage box, without coolant, and transported to the laboratory. The collection storage box was kept at 4°C until buds were processed, either the same day or the following day. Scales on the buds were removed; the buds were then weighed, crushed with forceps, placed into cluster tubes,

Table 1. Eight species per genus, name and USDA Plant Introduction (PI) number.

Taxon	Plant Name	NCGR acc. no.[z]	Taxon	Plant Name	NCGR acc. no.[z]
Actinidia arguta	Ken's Red	PI617109	*Amelanchier lamarckii*	Autumn Brilliance	PI559442
Actinidia arguta	A. arguta 74-32	PI617113	*Amelanchier x grandiflora*	Robin Hill	PI559443
Actinidia sp.	Red Princess	PI617118	*Amelanchier canadensis*	Prince William	PI559439
Actinidia kolomikta	Aromatnaya	PI617124	*Amelanchier lamarckii*	Princess Diana	PI559445
Actinidia kolomikta	Pautske	CACT98	*Amelanchier alnifolia*	Thiessen	PI652535
Actinidia kolomikta	Sentyabraskaya	PI617149	*Amelanchier hybrid*	Success	PI652537
Actinidia callosa	A. callosa female	PI641094	*Amelanchier alnifolia*	Forestburg	PI652538
Actinidia hybrid	A. hybrid #211	PI637809	*Amelanchier arborea*	*A. arborea* Stumphouse	PI652540
Rubus idaeus	Scepter	PI553370	*Pyrus communis*	Baronne Leroy	PI215321
Rubus idaeus	Thames	PI553439	*Pyrus pyrifolia*	Shu Li	PI132103
Rubus idaeus	Marcy	PI553446	*Pyrus spinosa*	*P. spinosa* (amygdaliformis)	PI349021
Rubus idaeus	Hilton	PI553447	*Pyrus communis subsp. caucasica*	*P. communis* subsp. caucasica - Gofitskoye	PI440632
Rubus idaeus	Chief	PI553508	*Pyrus cordata*	*P. cordata* - Turkey	PI541571
Rubus idaeus	Pocahontas	PI553516	*Pyrus cossonii*	*P. cossonii* - Russia	PI541592
Rubus idaeus	Norfolk Giant	PI618401	*Pyrus salicifolia*	*P. salicifolia* [*P. orientalis*]	PI541950
Rubus idaeus	Glen Ample	CRUB2331	*Pyrus ussuriensis*	*P. ussuriensis*	PI542020
Ribes roezlii var. cruentum	*R. roezlii var.* cruentum BLJ-14-2	PI555806	*Mespilus germanica*	*Medlar OSU 9-20*	*PI660782*
Ribes niveum	*R. niveum* NF 400 B	PI556250	*Mespilus germanica*	Medlar OSU 9-18	PI660783
Ribes alpinum	*R. alpinum*	PI555751	*Mespilus germanica*	Medlar OSU	PI660784
Ribes komarovii	R. komarovii female	PI556114	*Mespilus germanica*	Medlar - Corbett, OR	PI660785

Ribes aureum var. aureum	*R. aureum* var. aureum	PI555764	*Mespilus germanica*	Puciu Super Mol	PI660789
Ribes niveum	*R. niveum* WM 224.1	PI556015	*Mespilus germanica*	Nefle Precoce	PI660799
Ribes rubrum	O-399	PI617830	*Mespilus germanica*	Nefle d'October	PI660800
Ribes nigrum	*R. nigrum* 26 C 18	PI653028	*Mespilus germanica*	Nefle Tardive	PI660801
Cydonia oblonga	Pillnitz 1	PI194160	*Corylus avellana*	Rote Zellernuss	PI271280
Cydonia oblonga	Pillnitz 2	PI194161	*Corylus avellana*	Badem	PI304630
Cydonia oblonga	BA-29 (Provence)	PI559884	*Corylus sieboldiana*	*C. sieboldiana* - Japan-73	PI557402
Cydonia oblonga	Quince - Angers, France	PI559886	*Corylus hybrid*	Bountiful Ridge 3-6	PI557347
Cydonia oblonga	W-4	PI162494	*Corylus avellana*	Bergeri	PI557114
Cydonia oblonga	Quince - OSU Medford	PI559887	*Corylus colurna*	*C. colurna* N550 - Geisenheim, German	PI557256
Cydonia oblonga	Tashkent AR-232	PI502332	*Corylus avellana*	Grande	PI617189
Cydonia oblonga	Quince S (= Pigwa S-1)	PI655043	*Corylus avellana*	L. Smith Pioneer Hazelnut	PI617279
Sorbus lanata	*S. lanata*	PI635895	*Paeonia lactiflora*	Yan li (hua zhan xiao)	PPAE47
Sorbus aucuparia x Pyrus communis?	Krasavitsa	PI635898	*Paeonia lactiflora*	Kong que luo fen chi	PPAE49
Sorbus aucuparia x *Pyrus communis?*	Rubin	PI635902	*Paeonia lactiflora*	Tie gan zi	PPAE51
Sorbus decora	*S. decora*	PI635910	*Paeonia lactiflora*	Zhu sha pan	PPAE55
Sorbus forrestii	*S. forrestii*	PI635943	*Paeonia suffruticosa* subsp. *suffruticosa*	Fen zhong guan	PPAE92
Sorbus intermedia	Swedish Whitebeam	PI635947	*Paeonia suffruticosa* subsp. *suffruticosa*	Zhao fen	PPAE94
Sorbus alnifolia	*S. alnifolia*	PI635959	*Paeonia suffruticosa* subsp. *suffruticosa*	Juan ye hong	PPAE96
Sorbus torminalis	*S. torminalis* (L.) 2.1	PI635973	*Paeonia suffruticosa* subsp. *suffruticosa*	Xian chi zheng chun	PPAE100
Juglans cinerea	#850 Argos, IN	CJUG35	*Vaccinium corymbosum*	Ivanhoe	PI554807
Juglans cinerea	Collier #2	CJUG42	*Vaccinium corymbosum*	Jersey	PI554808
Juglans cinerea	Heron Rookery	CJUG45			
Juglans cinerea	Maxwell #1	CJUG49	*Vaccinium elliottii*	V. elliottii NC 84-15-3	PI554924
Juglans cinerea	Planton #1	CJUG56	*Vaccinium corymbosum*	Duke	PI554892
Juglans cinerea	Sheets / Ransburg	CJUG64	*Vaccinium corymbosum*	Herbert	PI554895
Juglans cinerea	Snyder	CJUG66	Vaccinium virgatum	Beckyblue	PI554960
Juglans cinerea PI618127	Clay Hill #2	CJUG72	Vaccinium arctostaphylos	V. arctostaphylos - Turkey	

NCGR accession No.[z] =Plant introduction (PI) and then number assigned by ARS Germplasm Resources Information Network (GRIN). If the number is not a PI number then it refers to a local number, which is assigned to the plant before the PI number is awarded. For example CJUG72 refers to C = Corvallis, Jug = Juglans, and 72 is the 72nd butternut tree that the repository has acquired.

frozen in liquid nitrogen, and stored at -80°C. The weight of each sample ranged from 30 to 36 mg with an average of 33 mg of dormant bud tissue.

Extraction protocols. Prior to DNA extraction using any of the three protocols, samples were ground while frozen in liquid nitrogen, with 4 mm stainless steel beads (McGuire Bearing Company, Salem, OR) in the Retsch MM301 Mixer Mill, (Retsch, Inc., Hann, Germany) rapidly at a frequency of 30 cycles•sec^{-1} using three 30 second bursts. Grinding was performed the day before the extraction and samples were stored at -80°C until the DNA extraction.

The NCGR laboratory has been routinely using the Qiagen protocol for DNA extraction from young actively growing leaves of many genera (2, 4, 5, 6). For this protocol, the cell lysis solution (Qiagen, Inc., Valencia, CA, Cat. No. 158908) is a sodium-dodecyl sulfate (SDS) based solution (9) and was heated to 65°C before addition of 500 µl to the ground tissue immediately after removal from the -80°C freezer.

The manufacturer's protocol was modified in our routine use to include proteinase K (60 µg per sample) (BioExpress, Kaysville, UT, Cat. No.C-5011-100) and RNase A (15 µg per sample) (Thermo Fisher Scientific, Inc. Waltham, MA, Cat. No. BP2539-100) treatments in addition to repeating the protein precipitation (Cat. No.158912) step as recommended by the manufacturer for DNA extraction from tissue containing high polysaccharides.

We followed the Omega E-Z 96 Plant DNA Kit (Cat. No.D1086-02) protocol with changes that were suggested by the Omega technical staff. These modifications included: heating the SP1 solution to ~80°C to prevent the buffer from freezing and to allow our -80°C stored samples to mix with the buffer; adding 500 µl (instead of 400 µl) of SP1 to each sample; adding proteinase K (60 µg per sample) (BioExpress, Kaysville, UT, Cat. No.C-5011-100) before incubation at 65°C in a water bath; and removing the supernatant in two steps. The first step involved taking as much supernatant as possible, and then centrifuging the sample again. The second step involved transferring 400 µl to a new tube that contained 2.5 µl RNase A stock solution provided by the kit (this second transfer allowed us to avoid contaminating the sample with any of the soft pellet material). The final suggested change was letting the binding plate dry for an additional 15 minutes at 37°C after the second SPW Wash buffer (personal communication with Omega technical staff).

We followed the Fast ID 96-well Genomic DNA Extraction Kit protocol with some changes that were suggested by the technical staff at Fast ID (personal communication, Pradheep Chhalliyil). These modifications included: heating the genomic lyse buffer to ~65°C to prevent the buffer from freezing and allow the -80°C stored samples to mix with the buffer; adding 600 µl of genomic lyse buffer (the protocol allows the researcher to choose the amount) to each sample and then incubating the plate for 30 minutes; transferring the supernatant twice instead of the recommended one step (this extra step allowed for less chance of plugging the DNA binding plate with sediment); and finally after the ethanol washes, thoroughly drying the binding plate by incubating at 37°C in an incubator to prevent residual ethanol from hindering elution of the DNA.

DNA was quantified with the Perkin Elmer, Wallac Victor 3 V, 1420 Multilabel Counter using two different methods: the UV absorbance 260/280 method; and fluorometry at 485nm/535nm for 1.0 second with Quant-iT™ PicoGreen® dsDNA Kit reagents (Invitrogen Corp., Carlsbad CA). The Quant-iT PicoGreen was used according to manufacturer's recommendations. It is a more stringent method of quantifying DNA due to its sensitivity and results are not skewed by proteins, ssDNA, RNA or phenols (1**).**

One DNA sample per extraction method from each genus was electrophoresed on a 1.25% agarose gel to estimate DNA quality and band size. An OD260/280 ratio of 1.8

was considered to indicate excellent quality and lack of proteins and polysaccharides in the extract. Because the quality for each genus (Table 2) was an average from the eight accessions, an OD260/280 of 1.4-1.6 was considered to be adequate for molecular analyses.

Results and Discussion

Removing the bud scales was very time intensive and this must be taken into consideration when preparing sample tissue for DNA extraction. Some of the genera required as much as two hours to remove scales and weigh tissue for the four replicates of each of the eight samples, one per DNA extraction protocol. One of the four replicates was held in reserve. In preliminary work (not reported), the color of the final DNA extract was dark when using the Qiagen protocol and clear with the Omega protocol. However, when the bud scales were removed, both protocols yielded a clear DNA extract. This indicated that the Qiagen protocol is inefficient at removing polyphenols when extracting DNA from buds that contained scales and the latter must be removed prior to DNA extraction. However, it is not necessary to remove bud scales when using the Omega protocol to obtain a clear DNA extract, free of polyphenols.

Each of the three protocols were straightforward, with easy to follow directions. Neither of the two Qiagen solutions is hazardous but both Omega and Fast ID kits contained reagents that carried health warnings.

The Qiagen DNA extraction protocol that our laboratory has been routinely using takes more than a day and a half to complete; the two other extraction kits took less than a day each. Cost and time were prime considerations. Consumables and reagents were calculated, but extraction time was not included due to lack of practice with Fast ID. When calculating the cost per sample, the total was divided by 192, which would be the number of samples in a two 96-well box extraction. The cost of each sample varied by extraction method used; the least expensive extraction method was the Qiagen protocol, at US$1.95 per sample, followed by Omega at US$2.46 per sample and finally Fast ID at US$2.97 per sample.

When DNA was run on a gel, the sizes of all bands were approximately the size of the lambda bands, 48.5 Kb (not shown). A light smear indicating possible degradation was observed in some of the DNA samples isolated using the Fast ID kit. RNA was not seen in any of samples that were run on the gels.

SNP platforms like the Infinium and GoldenGate assays (Illumina, Inc., CA) use high concentrations of DNA, averaging 50 $ng \bullet \mu l^{-1}$. Our laboratory has been preparing samples for these platforms where an accurate DNA measurement is essential. When comparing DNA quantities using UV absorbance to DNA quantities measured with Quant-iT™ PicoGreen®, the average amount of DNA across samples was 2.6 (OD260 vs. fluorometry reading) times higher for Omega, 3.9 times higher for Qiagen and 4.6 times higher for Fast ID. We therefore recommend using Quant-iT™ PicoGreen® for estimating DNA quantity in samples submitted for SNP detection using these platforms. The quantity of DNA isolated in all except for *Juglans* exceeded 100 ng $DNA \bullet mg^{-1}$ of tissue (Table 2) and therefore is adequate for SNP detection.

The three protocols performed poorly with *Juglans* where DNA quantity and quality were poorest (40 $ng \bullet mg^{-1}$ of tissue, OD260/280 = 1.2, respectively) with the Fast ID. Other researchers have used the hexadecyltrimethylammonium bromide (CTAB) buffer method with *Juglans* (7, 14, 15, 17, 18), but that method requires working in an extraction hood. Therefore with *Juglans*, we recommend either using the CTAB protocol or developing a better DNA extraction protocol, if needed. In the remaining genera, the average total amount of DNA extracted across all samples was 40 µg for the Qiagen protocol, 14.8 µg for Omega and 5.2 µg for Fast ID. The highest yield was consistently obtained with the Qiagen kit and ranged from a total of 18 µg or 0.55 $\mu g \bullet mg^{-1}$ of tissue in *Vaccinium* to a total of 116 µg or 3.5 $\mu g \bullet mg^{-1}$ of tissue in *Paeonia* (Table 2). Total DNA yield observed with the Fast ID in all ex-

Table 2. Amount of DNA recovered from each genus using each of the three methods [Qiagen, Omega E-Z 96 Plant DNA Kit (Omega), and Fast ID 96-Well Genomic DNA Extraction Kit (Fast ID)].

	Qiagen				Omega				Fast ID					
	DNA				DNA				DNA					
	conc. ng•µl^{-1}	total µg	µg•mg^{-1} of tissue	260/280	conc. ng•µl^{-1}	total µg	µg•mg^{-1} of tissues	260/280	conc. ng•µl^{-1}	total µg	µg•mg^{-1} of tissue	260/280	Highest yield of DNA	Best quality of DNA
Actinidia	183	37	1.12	1.5	70.6	14.1	0.43	1.8	57.5	5.8	0.18	1.8	Q^{y}	O^{x} ,F^{w}
Amelanchier	215	48	1.51	1.7	66.9	13.4	0.39	1.6	43.9	4.4	0.13	1.6	Q	O
Corylus	123	25	0.74	1.6	73.8	14.8	0.44	1.8	50.7	5.1	0.16	1.6	Q	O
Cydonia	271	54	1.69	1.8	89.3	17.9	0.55	1.8	67.7	6.8	0.21	1.8	Q	Comparable
Juglans	47	9	0.29	1.3	45.6	9.1	0.27	1.3	12.1	1.2	0.04	1.2	Q /0	Poor quality-all three
Mespilus	152	30	0.91	1.7	61.0	12.2	0.37	1.6	42.1	4.2	0.12	1.7	Q	Q ,F
Paeonia	386	116	3.50	1.8	140.4	28.1	0.85	1.8	111.3	11.1	0.34	1.7	Q	Comparable-excellent
Pyrus	109	22	0.66	1.6	56.6	11.3	0.34	1.5	48.8	4.9	0.15	1.5	Q	Comparable
Ribes	158	32	1.00	1.6	67.5	13.5	0.44	1.4	37.4	3.7	0.12	1.4	Q	Comparable
Rubus	106	21	0.67	1.7	61.8	12.4	0.40	1.5	56.5	5.6	0.18	1.7	Q	Q ,F
Sorbus	166	42	1.29	1.6	68.4	13.7	0.41	1.5	34.3	3.4	0.11	1.4	Q	Comparable
Vaccinium	90	18	0.55	1.6	61.1	12.2	0.37	1.6	48.1	4.8	0.14	1.4	Q	Comparable

Q^{y} = Qiagen, O^{x} = Omega, F^{w} =Fast ID

cept for *Juglans* (1.2 µg) and *Paeonia* (11.1 µg) ranged from 3.4 to 6.8 µg which is comparable to that reported from 25 to 50 mg of fresh walnut leaf tissue where 2.3 to 5.2 µg were obtained (7).

The quality of the DNA with the three extraction methods was comparable in six genera (*Cydonia, Paeonia, Pyrus*, *Ribes, Sorbus,* and *Vaccinium*). Depending on the genus, either the Qiagen or the Omega kit produced the best quality DNA, as estimated by OD260/280 ratios (Table 2).

Conclusion

In 11 of the genera sampled, both the Qiagen and Omega protocols generated large amounts of DNA from dormant buds, thus expanding the sources of tissue as well as time of tissue collection for DNA extraction in these plants. For the genus *Juglans*, DNA extraction from dormant buds could not be recommended with any of these three extraction methods and a different DNA extraction protocol should be used. The highest DNA yield for most genera was obtained with the Qiagen protocol, although it required the extra step of bud scale removal. In each of the genera studied, DNA from at

least one of the three protocols was of sufficient quality for use in molecular techniques such as DNA sequencing or SNP detection that require pure DNA. Even though these three DNA isolation methods did not perform extractions on dormant buds equally well, this in no way reflects the effectiveness of these techniques for other tissue and in other genera. Quality and quantity of DNA, time, cost, ease, and use of chemicals that don't require an extraction hood are major considerations for choosing one of these extraction methods.

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Literature Cited

1. Ahn, S.J., J. Costa and J. Rettig Emanuel. 1996. PicoGreen quantitation of DNA: effective evaluation of samples pre- or post-PCR. Nucleic Acids Res. 24(13): 2623–2625.
2. Bassil, N.V., B. Gilmore, J.M. Oliphant, K.E. Hummer and J.A. Henning. 2008. Genic SSRs for European and North American hop (*Humulus lupulus* L.). Genet. Resour. Crop Ev. 55:959-969.
3. Bassil, N.V., M. Muminova and W. Njuguna. 2010. Microsatellite-based fingerprinting of Western blackberries from plants, IQF berries and puree. Acta Hort. 859:73-80.
4. Bassil, N.V., A. Oda and K.E. Hummer. 2008. Blueberry microsatellite markers identify cranberries. Acta Hort. 810:181-187.
5. Bassil, N.V. and J. Postman. 2010. Identification of European and Asian pears using EST-SSRs from *Pyrus*. Genet. Resour. Crop Ev. 57:357-370.
6. Castillo, N., B. Reed, J. Graham, F. Fernandez-Fernandez and N.V. Bassil. 2010. Microsatellite markers for raspberry and blackberry. J. Amer. Soc. Hort. Sci. 135(3):271-278.
7. Chen, D.H. and P.C. Ronald. 1999. A rapid DNA minipreparation method suitable for AFLP and other PCR applications. Plant Mol. Biol. Rep. 17: 53-57.
8. Cheng, F.S., S.K, Brown and N.F. Weeden. 1997. A DNA extraction protocol from various tissues in woody species. HortScience 32(5):921-922.
9. Clewell, D.B. and D.R. Helinski. 1970**.** Properties of a super coiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry 9(22):4428–4440.
10. Couch, J.A. and P.J. Fritz. 1990. Isolation of DNA from plants high in polyphenolics. Plant Mol. Biol. Rptr. 8:8-12.
11. Furman, B., N. Robertson and K.E. Hummer. 2010. New clonal emphasis for the Arctic and Subarctic Plant Gene Bank in Palmer, AK. HortScience 45 (8):S182.
12. Pantoja, A. and J.C. Kuhl. 2009. Morphologic variation in the USDA/ARS rhubarb germplasm collection. Plant Genetic Resources: Characterization and Utilization 8(1): 35–41.
13. Postman, J., K.E. Hummer, E. Stover, R. Krueger, P. Forsline, L. Grauke, F. Zee, T. Ayala-Silva and B. Irish. 2006. Fruit and nut genebanks in the U.S. National Plant Germplasm System. HortScience 41(5):1188-1194.
14. Potter, D., F. Gao, S. Baggett, J.R. McKenna and G.H. McGranahan. 2002. Defining the sources of paradox: DNA sequence markers for North American walnut (*Juglans* L.) species and hybrids. Scientia Hort. 94:157-170.
15. Ross-Davis, A. and K.E. Woeste. 2008. Microsatellite markers for *Juglans cinerea* L. and their utility in other Juglandaceae species. Conserv. Genet. 9(2):465-469.
16. SARU. (2009). Subarctic Agricultural Research Unit: http://www.ars-grin.gov/cgi-bin/npgs/html/site.pl?PALM
17. Tai, T.H. and S.D. Tanksley. 1990. A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. Plant Mol. Biol. Rep. 8(4):297-303.
18. Victory, E.R., J.C. Glaubitz, O.E. Rhodes and K.E. Woeste. 2006. Genetic homogeneity in *Juglans nigra* (Juglandaceae) at nuclear microsatellites. Amer. J. Bot. 93(1):118–126.