Genus-Wide Microsatellite Primers for the Goldenrods (Solidago; Asteraceae)

Authors: Beck, James B., Semple, John C., Brull, Justin M., Lance, Stacey L., Phillips, Mai M., et al.

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Primer Note

Genus-wide microsatellite primers for the goldenrods (*Solidago*; Asteraceae)[1]

James B. Beck[2,8], John C. Semple[3], Justin M. Brull[2], Stacey L. Lance[4], Mai M. Phillips[5], Sara B. Hoot[6], and Gretchen A. Meyer[7]

[2]Department of Biological Sciences, Wichita State University, 537 Hubbard Hall, Wichita, Kansas 67260 USA; [3]Department of Biology, University of Waterloo, Waterloo, Ontario NL2 3G1, Canada; [4]Savannah River Ecology Laboratory, University of Georgia, Aiken, South Carolina 29802 USA; [5]Conservation and Environmental Science Program, University of Wisconsin–Milwaukee, 3209 N. Maryland Ave., Milwaukee, Wisconsin 53201 USA; [6]Department of Biological Sciences, University of Wisconsin–Milwaukee, 3209 N. Maryland Ave., Milwaukee, Wisconsin 53201 USA; and [7]Field Station, University of Wisconsin–Milwaukee, 3095 Blue Goose Road, Saukville, Wisconsin 53080 USA

- *Premise of the study:* Microsatellite primers were developed for studies of polyploid evolution, ecological genetics, conservation genetics, and species delimitation in the genus *Solidago*.
- *Methods and Results:* Illumina sequencing of a shotgun library from *S. gigantea* identified ca. 1900 putative single-copy loci. Fourteen loci were subsequently shown to be amplifiable, single-copy, and variable in a broad range of *Solidago* species.
- *Conclusions:* The utility of these markers both across the genus and in herbarium specimens of a wide age range will facilitate numerous inter- and intraspecific studies in the ca. 120 *Solidago* species.

Key words: Asteraceae; Illumina sequencing; polyploidy; simple sequence repeat (SSR) markers; *Solidago.*

The ca. 120 species of goldenrod (*Solidago* L.; Asteraceae) are largely confined to North America and occupy an impressive array of habitats, including tundra, rock outcrops, bogs, sand dunes, prairies, barrens, rockhouses, and a variety of woodlands (Semple and Cook, 2006). This taxonomic and ecological diversity has led to *Solidago*'s popularity as a study system in evolution and ecology. Microsatellite, or simple sequence repeat (SSR), markers could represent a valuable tool in many of these instances, for example, allowing for the estimation of kinship, the identification of invasive genotypes, and the estimation of gene flow among populations.

Microsatellite data could also help clarify *Solidago* species boundaries. The taxonomic complexity of the genus is widely recognized, a problem stemming from sheer species richness, low overall levels of genetic differentiation, occasional interspecific hybridization, and frequent polyploidy (Semple and Cook, 2006). An accurate delimitation of *Solidago* species would provide a robust account of biodiversity in the genus and enhance the evolutionary and ecological studies noted above. Given the low overall genetic divergence among *Solidago* species (Schilling et al., 2008), it should be possible to identify SSR loci that amplify in most species, providing a standard comparative genetic toolkit for the genus.

METHODS AND RESULTS

Silica-dried tissue from a diploid individual of *S. gigantea* Aiton (confirmed by a meiotic chromosome count) was collected in Chester County, Tennessee, USA. A voucher specimen for this collection (*Beck 1258*) has been deposited at the Wichita State University Herbarium (WICH). Total DNA was extracted with a DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA). An Illumina paired-end shotgun library was prepared by shearing 1 μg of DNA using a Covaris S220 ultrasonicator (Covaris, Woburn, Massachusetts, USA) and following the standard Illumina TruSeq DNA Library Kit protocol (Illumina, San Diego, California, USA) using a multiplex identifier adapter index. Sequencing was conducted on the Illumina HiSeq 2000 with 100-bp paired-end reads. Five million of the resulting reads were analyzed with the program PAL_FINDER_v0.02.03 (Castoe et al., 2012) to extract those reads that contained di-, tri-, tetra-, penta-, and hexanucleotide SSRs. Once positive reads were identified in PAL_FINDER_v0.02.03, they were batched to a local installation of Primer3 version 2.0.0 (Rozen and Skaletsky, 2000) for primer design. To avoid targeting multiple-copy loci, only those for which either primer sequence occurred one or two times in the 5 million reads were selected. A total of 1888 loci met this criterion.

To select a set of loci for initial screening, we focused on loci with tetra- and trinucleotide repeat motifs and with primer melting temperatures between 55°C and 65°C. Furthermore, loci were targeted for which only one of the paired-end reads sequenced into the repeat motif to avoid relatively small fragment sizes. Using these criteria, 80 loci were chosen for initial screening using a "CAG-tag" strategy similar to the M13 approach in Schuelke (2000). The forward primer from each locus was 5′ modified with an engineered "CAG-tag" sequence (5′-CAGTCGGGCGTCATCA-3′) to enable use of a third, fluorescently labeled primer (identical to the CAG-tag) in PCR. In addition, the "PIG-tail"

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[8]Author for correspondence: james.beck@wichita.edu

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sequence GTTT was added to the 5′ end of the reverse primer to reduce double peaks. Reactions (10 µL) included 1× Promega GoTaq Buffer (Promega Corporation, Fitchburg, Wisconsin, USA); 0.2 mM each dNTP; 2.5 mM $MgCl_2$; 0.025 µg bovine serum albumin (BSA); 0.5 U Promega GoTaq; 0.4 µM unlabeled primer; 0.04 µM CAG-labeled primer; 0.4 µM labeled CAG-tag, and ca. 30 ng DNA template. PCR amplification involved the touchdown cycling protocol outlined in Lance et al. (2010). CAG-tag screening included DNA extracted from eight herbarium specimens representing species from four subsections of *Solidago* sect. *Solidago* (*Solidago* subsect. *Triplinerviae* (Torrey & A. Gray) G. L. Nesom, *Solidago* subsect. *Glomeruliflorae* (Torrey & A. Gray) A. Gray, *Solidago* subsect. *Squarrosae* A. Gray, and *Solidago* subsect. *Junceae* (Rydb.) G. L. Nesom) and a sample of *Brintonia discoidea* (Elliott) Greene, representing a monotypic genus potentially sister to *Solidago* (Schilling et al., 2008). Full details for these eight specimens are provided in Appendix 1.

Fourteen loci (Table 1) were identified as variable, interpretable, and broadly amplifiable across the four tested *Solidago* subsections and outgroup *Brintonia* Greene. These loci were then further evaluated in a larger set of diploid individuals from *Solidago* subsect. *Triplinerviae* (47 samples representing 10 species), *Solidago* subsect. *Squarrosae* (47 samples representing 10 species), and *Solidago* subsect. *Junceae* (32 samples representing seven species). Full specimen details are provided in Appendix 1. All 126 samples were extracted from herbarium specimens archived at the University of Waterloo Herbarium (WAT), the University of Tennessee Herbarium (TENN), the Duke University Herbarium (DUKE), or the Missouri Botanical Garden Herbarium (MO) using the modified cetyltrimethylammonium bromide (CTAB) protocol detailed in Beck et al. (2012). Forward primers (minus the CAG-tag) were dye labeled with either 6-FAM or HEX, while reverse primers retained the PIG-tail for all but two loci (Table 1). Sets of two or three loci were simultaneously amplified using the multiplex PCR protocol described in Beck et al. (2012). Amplicons were sized using the GeneScan 500 LIZ Size Standard on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, California, USA) at the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility (Chicago, Illinois, USA). Alleles were determined using GeneMarker 1.9 (SoftGenetics, State College, Pennsylvania, USA).

The 14 loci were variable and generally transferable across the 27 species representing three *Solidago* subsections (Table 2). The number of alleles per locus ranged from seven to 51, and all loci (if amplifiable) were polymorphic in all three subsections. A null allele was inferred if no amplification was observed in all individuals of a given species, and seven of the 14 loci exhibited no evidence for null alleles in any of the 27 species (Table 2). Not surprisingly, the fewest null alleles were observed in subsect. *Triplinerviae* (11 of 140 locus/species combinations), the subsection to which *S. gigantea* belongs. In only one case did all species in a subsection exhibit a null allele for a given locus (Sg_7 in subsect. *Squarrosae*). Lineage-specific locus duplication was inferred in two cases based on the observation of more than two alleles per individual in multiple confirmed diploid samples (Sg_4 in subsect. *Junceae* and Sg_5 in subsect. *Squarrosae*).

CONCLUSIONS

The general transferability, single-copy status, and variability of these loci suggest that primers designed for a single *Solidago* species should be applicable across the genus. Screening of the 14 SSR loci described here and those previously reported for *S. sempervirens* L. (Wieczorek and Geber, 2002), *S. canadensis* L. (Zhao et al., 2012), and *S. altissima* L. (Sakata et al., 2013) should therefore provide a set of >20 informative SSR loci for any goldenrod species. These loci were also readily amplifiable from herbarium specimens of a wide age range (1932–2007, Appendix 1), creating opportunities for the broad inclusion of archived museum material in future studies.

Table 1. Characteristics of 14 loci broadly amplifiable in *Solidago*.[a]

Locus[b]	Primer sequences (5′–3′)[c]	Repeat motif[d]	Allele size range (bp)[e]
Sg_1	F: GCGTACTTATTAAATTGATTTCTATAACCG R: ACAGATGGCTTCCATGATCG	(TTGG)	116–153
Sg_2	F: TCTAAACTGTAAGTCTTTGATGAAACC R: GCCGTCAATCCTTACAATCC	(AATG)	167–248
Sg_3	F: TTGAAGATCAAATGCTTCCACC R: **GTTT**AACCAATTTGTCACTTCAGATCG	(AAC)	92–182
Sg_4	F: CAATCTTGTCAGTTTAATCATTTCTTCC R: **GT**TTCATAAGGAGTGGCATGTTCC	(TTCC)	104–201
Sg_5	F: TTGTCCTGATACAAATTTCCTACTCG R: **GTTT**AACAATGAGAATAAGTGGACAACCC	(TTC)	256–296
Sg_6	F: TTTACCTTTGAATTGCGGC R: **GTTT**AGTACCAATCAACCATGGGC	(AAAT)	200–244
Sg_7	F: TTTGTATGCAAGTCAAAGGCG R: **GTTT**CACAGCTGCCAATAAATCCC	(AAAG)	360–378
Sg_8	F: TCCCTCTTTATTCTTTCAACAAACC R: **GTTT**AACACCAACATTGCAATCCC	(AAAG)	126–172
Sg_9	F: GACGTGGCTAAATTAAGGTGTACG R: **GT**TTGCAACGTAATCCACCTCC	(AATG)	170–190
Sg_10	F: CGTTTGTTCTTTGTCCCTTTCC R: **GTTT**CTATACCTCGTGCGTGTCGG	(ATCT)	276–330
Sg_11	F: GAGTCTCTTCAGTATAAGTTTATCTTGGC R: **GTTT**AAGACTGTCTACATTTCACCTCTCC	(AAC)	119–155
Sg_12	F: CTAGAAGATGTGGATTGACCAGC R: **GTTT**CAAATGAGTCAGTCGGTGCC	(AAAT)	182–208
Sg_13	F: TTGAAATGTTTGTATCATTAGGGTATGG R: **GTTT**CATATCCCGTTTCGGCAGG	(AAC)	153–172
Sg_14	F: AACCTTTGTTTGGTATGTAAATTAGG R: **GTTT**ATGTTTCTACGTTGGGAGGG	(AAC)	317–355

[a] Paired-end sequence data are deposited in the Dryad Digital Repository: http://doi.org/10.5061/dryad.72p7k (Beck et al., 2014).
[b] A multiplex amplification protocol incorporating a single annealing temperature (see text) was used for all loci.
[c] Nucleotides added to create PIG-tail are noted in boldface for relevant primers.
[d] Total repeat motif number is not reported because it could not be determined whether paired-end reads sequenced through the entire repeat region.
[e] Full size range across the three *Solidago* subsections evaluated in the broad analysis.

Table 2. Number of alleles, size range, and amplification success in three *Solidago* subsections. Loci successfully amplified in all taxa are shown in bold.

	subsect. *Triplinerviae*			subsect. *Squarrosae*			subsect. *Junceae*			All samples		
Locus	*A*	Allele size range (bp)	Amplification success[a]	*A*	Allele size range (bp)	Amplification success[a]	*A*	Allele size range (bp)	Amplification success[a]	*A*	Allele size range (bp)	Amplification success[a]
Sg_1	**5**	**121–144**	**10/10**	**7**	**129–153**	**10/10**	**9**	**116–153**	**7/7**	**13**	**116–153**	**27/27**
Sg_2	**32**	**167–226**	**10/10**	**37**	**175–248**	**10/10**	**24**	**171–209**	**7/7**	**48**	**167–248**	**27/27**
Sg_3	39	92–182	8/10	11	99–141	7/10	23	94–139	7/7	51	92–182	22/27
Sg_4	29	127–201	10/10	17	104–171	10/10	—	—	—	38	104–201	20/27
Sg_5	30	256–296	10/10	—	—	—	13	266–290	7/7	31	256–296	17/27
Sg_6	**11**	**214–244**	**10/10**	**13**	**200–239**	**10/10**	**6**	**214–226**	**7/7**	**21**	**200–244**	**27/27**
Sg_7	7	360–378	3/10	0	0	0/10	2	368–372	4/7	7	360–378	7/27
Sg_8	**17**	**126–172**	**10/10**	**14**	**138–172**	**10/10**	**13**	**134–172**	**7/7**	**22**	**126–172**	**27/27**
Sg_9	**4**	**178–190**	**10/10**	**6**	**170–185**	**10/10**	**7**	**173–186**	**7/7**	**10**	**170–190**	**27/27**
Sg_10	**13**	**268–330**	**10/10**	**8**	**274–283**	**10/10**	**7**	**276–290**	**7/7**	**17**	**276–330**	**27/27**
Sg_11	10	119–143	10/10	3	143–149	6/10	11	122–155	7/7	13	119–155	23/27
Sg_12	**6**	**190–208**	**10/10**	**6**	**182–200**	**10/10**	**4**	**182–200**	**7/7**	**10**	**182–208**	**27/27**
Sg_13	10	153–171	10/10	4	156–165	4/10	10	155–172	7/7	16	153–172	21/27
Sg_14	15	322–355	8/10	16	317–352	9/10	6	328–340	4/7	23	317–355	21/27

Note: — = duplicated; *A* = number of alleles.
[a] Number of taxa with successful amplification/number of taxa attempted.

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Appendix 1. Sampling information for the eight individuals used in CAG-tag screening followed by the 126 individuals analyzed in the broader survey of locus transferability. Information presented: taxon, sample number, collector and number, herbarium, country: state/province/region, year collected.

***Solidago altissima* L.**, S206, *Semple 11415*, WAT, USA: Nebraska, 2006.

***Solidago caesia* L.**, S351, *Semple 10778*, WAT, USA: Kentucky, 1999.

***Solidago gigantea* Aiton**, S208, *Cook C-456*, WAT, USA: Iowa, 2001. S215, *Semple 10165*, WAT, USA: Mississippi, 1991. S217, *Semple 9620*, WAT, USA: Kentucky, 1991.

***Solidago pinetorum* Small**, S536, *Semple 11625*, WAT, USA: North Carolina, 2006.

***Solidago squarrosa* Muhl.**, S384, *Semple 11529*, WAT, Canada: New Brunswick, 2006.

***Brintonia discoidea* (Elliott) Greene**, S298, *Semple 11194*, WAT, USA: Alabama, 2003.

***Solidago* subsect. *Junceae* (Rydb.) G. L. Nesom**

***Solidago confinis* A. Gray**, S506, *Semple 8984*, WAT, USA: California, 1987. S507, *Semple 9632*, WAT, USA: California, 1990. S508, *Semple 9347*, WAT, USA: California, 1990. S510, *Semple 8970*, WAT, USA: California, 1987. ***Solidago gattingeri* Chapm. ex A. Gray**, S521, *Semple 5288*, WAT, USA: Missouri, 1980. S522, *Dietrich 49*, MO, USA: Missouri, 1994. S524, *McNeilus 93-1443*, TENN, USA: Tennessee, 1993. S525, *Nordman s.n.*, TENN, USA: Tennessee, 2000. S526, *Baily s.n.*, TENN, USA: Tennessee, 2000. ***Solidago guiradonis* A. Gray**, S502, *Semple 9356*, WAT, USA: California, 1990. S503, *Semple 9351*, WAT, USA: California, 1990. S504, *Semple 9355*, WAT, USA: California, 1990. S505, *Semple 9352*, WAT, USA: California, 1990. ***Solidago juncea* Aiton**, S540, *Semple 10677*, WAT, USA: Pennsylvania, 1999. S542, *Semple 4897*, WAT, Canada: Nova Scotia, 1980. S543, *Semple 2757*, WAT, USA: Missouri, 1977. S544, *Semple 2759*, WAT, USA: Michigan, 1977. ***Solidago missouriensis* Nutt.**, S527, *Semple 7699*, WAT, USA: Colorado, 1985. S528, *Semple 9195*, WAT, USA: Nebraska, 1990. S530, *Semple 9263*, WAT, USA: Utah, 1990. S531, *Semple 8844*, WAT, USA: Wisconsin, 1987. S532, *Semple 9381*, WAT, USA: New Mexico, 1990. S534, *Semple 2669*, WAT, Canada: Manitoba, 1977. ***Solidago pinetorum* Small**, S535, *Semple 11223*, WAT, USA: North Carolina, 2003. S536, *Semple 11625*, WAT, USA: North Carolina, 2006. S537, *Semple 11599*, WAT, USA: North Carolina, 2006. S538, *Semple 9734*, WAT, USA: North Carolina, 1991. ***Solidago spectabilis* (D. C. Eaton) A. Gray**, S511, *Semple 8717*, WAT, USA: California, 1986. S512, *Semple 9301*, WAT, USA: California, 1990. S513, *Semple 9299*, WAT, USA: California, 1990. S514, *Semple 9310*, WAT, USA: California, 1990. S516, *Semple 8401*, WAT, USA: California, 1986.

***Solidago* subsect. *Squarrosae* A. Gray**

***Solidago bicolor* L.**, S385, *Semple 10681*, WAT, USA: West Virginia, 1999. S389, *Semple 5927*, WAT, USA: Virgina, 1981. S390, *Semple 3487*, WAT, USA: Vermont, 1978. S391, *Semple 9487*, WAT, USA: Pennsylvania, 1991. S393, *Semple 6002*, WAT, USA: North Carolina, 1981. S398, *Semple 3614*, WAT, USA: Connecticut, 1978. S400, *Semple 4708*, WAT, Canada: New Brunswick, 1980. S406, *Semple 11472*, WAT, Canada: Prince Edward Island, 2006. ***Solidago erecta* Banks ex Pursh**, S424, *Semple 5984*, WAT, USA: Virginia, 1981. S425, *Semple 11189*, WAT, USA: Tennessee, 2003. S428, *Semple 9501*, WAT, USA: New Jersey, 1991. S429, *Semple 9454*, WAT, USA: Kentucky, 1990. S433, *Semple 6098*, WAT, USA: South Carolina, 1981. S434, *Semple 10175*, WAT, USA: Mississippi, 1991. ***Solidago hispida* Muhl.**, S408, *Semple 3638*, WAT, USA: New York, 1978. S411, *Semple 4634*, WAT, USA: Maine, 1980. S418, *Semple 11065*, WAT, Canada: Ontario, 2001. S419, *Morton 12474*, WAT, Canada: Newfoundland, 1978. S420, *Semple 8298*, WAT, USA: Arkansas, 1985. ***Solidago pallida* (Porter) Rydb.**, S465, *Semple 8082*, WAT, USA: New Mexico, 1985. S462, *Semple 11304*, WAT, USA: South Dakota, 2004. S464, *Semple 11401*, WAT, USA: Wyoming, 2006. ***Solidago puberula* Nutt.**, S437, *Semple 11635*, WAT, USA: North Carolina, 2006. S440, *Kral 44276*, WAT, USA: Alabama, 1971. S441, *Semple 10137*, WAT, USA: Florida, 1991. S442, *Semple 9813*, WAT, USA: South Carolina, 1991. S445, *Cook C-118*, WAT, Canada: Quebec, 2000. S448, *Semple 7628*, WAT, USA: Maryland, 1984. S451, *Semple 6867*, WAT, USA: Massachusetts, 1982. S452, *Semple 10815*, WAT, USA: North Carolina, 1999. ***Solidago rigidiuscula* (Torr. & A. Gray) Porter**, S466, *Semple 10602*, WAT, Canada: Ontario, 1997. S467, *Semple 4532*, WAT, USA: Indiana, 1979. S468, *Semple 9121*, WAT, USA: Tennessee, 1986. S469, *Semple 5063*, WAT, USA: Wisconsin, 1980. ***Solidago roanensis* Porter**, S455, *Cook C-332*, WAT, USA: Tennessee, 2000. S457, *Cook C-557*, WAT, USA: North Carolina, 2001. S458, *Semple 9658*, WAT, USA: North Carolina, 1991. S459, *Poindexter 05-1580*, WAT, USA: North Carolina, 2005. ***Solidago speciosa* Nutt.**, S470, *Semple 6180*, WAT, USA: South Carolina, 1981. S471, *Semple 11613*, WAT, USA: Virginia, 2006. ***Solidago squarrosa* Muhl.**, S371, *Semple 2426*, WAT, Canada: Ontario, 1976. S375, *Semple 4660*, WAT, USA: Maine, 1980. S379, *Semple 3692*, WAT, Canada: Ontario, 1978. S383, *Cook C-125*, WAT, Canada: Quebec, 2000. S384, *Semple 11529*, WAT, Canada: New Brunswick, 2006. ***Solidago villosicarpa* LeBlond**, S460, *Semple 11645*, WAT, USA: North Carolina, 2006. S461, *Semple 11637*, WAT, USA: North Carolina, 2006.

***Solidago* subsect. *Triplinerviae* (Torrey & A. Gray) G. L. Nesom**

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