

Draft template for Primer Notes & Protocols in the Plant Sciences
Short Title for Running Head: AJB Primer Notes & Protocols – + 2 or 3 Descriptive Words
[e.g., Antennaria microsatellites]

**Microsatellite primers in the native perennial herb,
Antennaria plantaginifolia (Asteraceae)¹**

[no more than 125 characters; after a species name, include family name in parentheses]

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Number of words: YYYY *[1200 or fewer]*.

¹Manuscript received _____; revision accepted _____.

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Acknowledgments should be limited to no more than 75 words.

ABSTRACT

The abstract of no more than 150 words should capture the interest of the general botanical community as well as specialists.

The abstract is 150 words or less, written in a structured format:

- Premise of the study (why the work was done)
- Methods and Results
- Conclusions (In the case of primer development, briefly mention other related species in which the markers also amplify.)

Avoid references; if essential, cite parenthetically with journal name, volume number, pages, and year.

Here is a sample abstract:

- *Premise of the study:* Microsatellite primers were developed in the native perennial herb, *Antennaria plantaginifolia*, to investigate potential hybridization events with related taxa promoting the evolution of polyploidy within the genus.
- *Methods and Results:* Using a non-radioactive protocol, 16 primer sets were identified in North American populations of *A. plantaginifolia*. The primers amplified di-, tri- and pentanucleotide repeats with 1-11 alleles per locus. Most primers also amplified in *A. neglecta*, *A. solitaria*, *A. virginica*, *A. parlinii*, and *A. neodioica*
- *Conclusions:* These results indicate the utility of primers in *A. plantaginifolia* for future studies of polyploidy and hybridization as well as their applicability across the genus.

Key words: *Antennaria plantaginifolia*; hybridization; perennial herb; polyploidy.

[Please list 3 to 6 key words here in alphabetical order, separated by semicolons.]

INTRODUCTION

This section should consist of no more than two paragraphs outlining the reasons behind the study, a brief explanation of its importance, and any information regarding the species or the protocol that would be of interest to the general botanical community. Of particular appeal is the

potential for widespread applicability of the markers or techniques to other species or systems.

Formatting will follow that for the *American Journal of Botany* (available at:

<http://www.amjbot.org/misc/ifora.shtml#64Text>).

METHODS AND RESULTS

The combined **Methods and Results** section will consist of no more than five paragraphs. In the first one to three paragraphs of this section, the methods used to develop the genetic markers or conduct the protocols should be described. The description of methods must contain enough detail that other botanists can replicate the results. For primer development papers, these details will normally include: primer sequences (including GenBank accession numbers), specific quantities of chemicals used for amplification, temperature conditions for amplification, and source information for chemicals and supplies (if necessary). The number and geographic origin of samples analyzed must also be included in primer development papers. In protocol development papers, the new method must be described in sufficient detail to allow other botanists to apply it to their own materials.

In the final one to two paragraphs of this section, the authors must demonstrate the usefulness of their primers or their new protocol. For primer development papers, the authors must show that they have tested all primers on a reasonable number of individuals, e.g., more than five individuals per populations and two or more populations unless the species being studied is extremely rare. Data on the number and frequency of alleles detected for at least five primer pairs (including both monomorphic and polymorphic primers) should be reported. For protocol development papers, the authors must demonstrate that the new method produces reliable results and describe the advantages and limitations it has relative to existing methods.

CONCLUSIONS

In this section, the author(s) should clearly state in one paragraph the main conclusions that have been reached, focusing on the effectiveness and applicability of the markers or protocols being described. Protocol papers should also include a description of the advantages of the new method over current techniques.

LITERATURE CITED *[no more than 10]*

- GOUDET, J. 1995. FSTAT: a computer program to calculate *F* statistics, version 1.2. *Journal of Heredity* 86: 485–486.
- STEBBINS, G. L. 1974. Flowering plants: evolution above the species level. Belknap Press, Cambridge, Massachusetts, USA.
- STEVENS, P. F. 2001 onward. Angiosperm phylogeny website, version 8, June 2007 [more or less continuously updated]. Website <http://www.mobot.org/MOBOT/research/APweb/> [accessed 00 Month Year].
- TURNER, B. L., AND R. M. KING. 1977. Chromosome numbers in the Compositae. VIII. Mexican and Central American species. *Southwestern Naturalist* 9: 27–39.
- WHITE, T. J., T. D. BRUNS, S. B. LEE, AND J. W. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: A guide to methods and applications, 315–322. Academic Press, San Diego, California, USA.

Tables

For instructions on formatting tables, please see the Instructions for Authors at

<http://www.amjbot.org/misc/ifora.shtml#tables>.

For marker development papers, two tables should be presented. One should contain the names of the forward and reverse primers, their DNA sequences, repeat, fragment size range, annealing temperatures, and GenBank ID. The second should report the number of alleles and observed heterozygosity in each population surveyed. In species expected to be primarily outcrossing, results of tests for departures from single-locus Hardy-Weinberg expectations and for departures from gametic equilibrium may be included. See example below. For protocol development papers, up to two tables may be included if needed to explain the protocol or to document its reliability.

Table 1. Characteristics of 16 microsatellite primers developed in *Antennaria plantaginifolia*.

Shown for each primer pair are the forward and reverse sequence, repeat type, size of the original fragment (bp), annealing temperature when run individually (T_a) and the GenBank accession number. All values are based on 50 samples representing North American populations located in Florida, Tennessee, and Michigan (N = 14-20 for each).

Primer	Sequence	Repeat	Size	T_a	GenBank
Primer A1	F: CATGGGACACCATTTTAAGTG R: TCCATGTCATCCACAATACCA	(GT) ₁₆	164	53	AJ842064
Primer B5	F: GCTGGGTAGATTGAGCTGCTT R: TCAACGATGCAATAGTGGGTA	(TC) ₈	219	57	AJ842074
Primer X8	F: CATCCACCAACCCACACATA R: CTAGCAACACACAGGGCATC	(GAA) ₅	169	55	AJ842065

Table 2. Results of initial primer screening in populations of *Antennaria plantaginifolia*. Shown for each primer pair are the number of alleles (A), and mean values of observed (H_o) heterozygosity. The sample size for each population is shown in parentheses.

	No. of alleles	Observed heterozygosity
Population 1 (N = 14)		
<i>Primer A1</i>	5	0.45
<i>Primer B5</i>	7	0.68
<i>Primer X8</i>	4	0.38
Population 2 (N = 20)		
<i>Primer A1</i>	3	0.27
<i>Primer B5</i>	2	0.18
<i>Primer X8</i>	1	0.00

Figure and Legend

[limited to one optional figure, which should be uploaded as a separate file; the legend should be included in the text file]