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*Genetic Resources*

# Constancy of RAPD Primer Amplification Strength among Distantly Related Taxa of Flowering Plants

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**Abstract:** A survey of 480 10-mer primers for RAPD markers revealed general consistency in primer amplification strength among the flowering plant genera *Datisca, Helianthus* and *Yucca*. Six characteristics of primer base sequences were analyzed: total (G+C) content; the amounts of G, A, C, and T taken separately; and the (G+C) content in the last four bases of the 3' end. Of these, total (G+C) content showed the most value in predicting primer amplification strength. Since the consistency of amplification strength is true only globally, there are still many primers showing high variation in amplification strength among genera, most probably due to DNA sequence differences, but perhaps also resulting from experimental artifact. Nevertheless, we suggest that this survey be used as a rough guide for prioritizing primer deployment in RAPD studies involving plants in the hope of improving efficiency during the search for adequate levels of polymorphism, with the understanding that taxon-specific differences in primer amplification strength are bound to occur.

The use of the polymerase chain reaction in generating random amplified polymorphic DNA (RAPD) has already proven valuable in the construction of genetic maps (Quiros et al., 1991; Klein-Lankhorst et al., 1991; Giovannoni et al., 1991; Reiter et al., 1992; Rieseberg et al., in press), the production of genetic markers linked to specific

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**Abbreviations:** RAPD, random amplified polymorphic DNA; CTAB, hexadecyltrimethylammonium bromide.

phenotypic traits (Mulcahy et al., 1992; Paran et al., 1991; Martin et al., 1991; Michelmore et al., 1991), parentage determination (Welsh et al., 1991), clone identification (Wilde et al., 1992; Smith et al., 1992), and population dynamics (Arnold et al., 1990; Fritsch and Rieseberg, 1992). In our laboratory we have used RAPDs in plants to construct a genomic map of a diploid hybrid species (Rieseberg et al., 1992), to help document the occurrence of natural introgression, and to estimate outcrossing rates in natural populations (Fritsch and Rieseberg, 1992). In order to develop arrays of genetic polymorphisms for the several plant species under study, we conducted extensive primer surveys using a small number of DNA samples. During initial stages of these surveys we noticed that some primers showed excellent amplification no matter which species was tested, whereas others consistently produced poor or no amplification. This general trend remained apparent as more and more primers were surveyed, and we began to suspect that rough predictions could be made as to the amplification strength of a primer for a new taxon being surveyed based on previous results from other taxa. Here we provide a table of amplification strength for primers used in polymorphism surveys of three distantly related genera of flowering plants and test the general concordance of primer amplification strength among them. Several characteristics of primer sequence base composition are also tested as predictors of amplification strength.

## Materials and Methods

Four-hundred-eighty oligodeoxynucleotide primers, prepared as arbitrary 10-mers, were obtained from the University of British Columbia Biotechnology Laboratory (primers 101-500) and Operon Technologies (primer kits A-D; Table I), and tested for amplification products from genomic DNA of *Datisca glomerata* (Dicotyledonae, Dilleniidae, Datiscaceae), two species in the genus *Yucca* (*Y. baccata* and *Y. schidigera*, Monocotyledonae, Liliidae, Agavaceae), and *Helianthus annuus* (Dicotyledonae, Asteridae, Asteraceae). A minimum of two DNAs for *D. glomerata*, a single DNA for each *Yucca* species, and a minimum of five DNAs for *H. annuus* were surveyed with each primer. DNAs were isolated using a CTAB extraction technique (Doyle and Doyle, 1987) modified by a reduction in reagent volumes in order to allow isolations to be conducted in 1.5-mL microcentrifuge tubes. Additional modifications were the inclusion of sodium metabisulfite (1% w/v) in the DNA isolation buffer and a double rather than single chloroform extraction. Amplification reactions and cycle parameters followed Williams et al.

(1990). Amplification products were separated by electrophoresis on 1.5% agarose gels and detected by staining with ethidium bromide.

Primer amplification strength was scored for each genus using a scale from *0* to *3*, where *0* indicates that no product or, at most, one or two faint bands were detected in only some DNAs within a genus; *1* indicates that at least one faint product was detected more or less consistently among most DNAs; *2* indicates that at least one moderately strong product was detected among most DNAs; and *3* indicates that at least one very strong product was detected among all DNAs (Fig. 1).

In order to evaluate the degree of agreement in overall primer amplification strength among the three genera, we used Kendall's coefficient of concordance, *W*. *W* equals 1 when the data is completely concordant (i.e., when all genera possess the same degree of amplification for each primer) and *0* when the data is completely discordant. Also, several characteristics of primer base composition (the "predictor variables" in Table II) were analyzed with a Model I linear regression to see if any of them were useful in predicting amplification strength.

## Results

The degree of amplification of each of the 480 primers (Table I) was strongly similar among the three genera ($W = 0.694$) and was highly

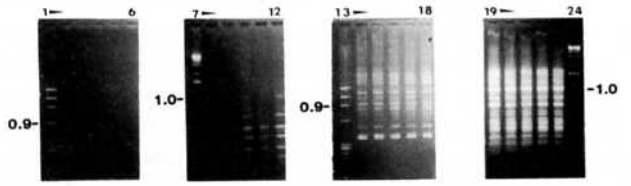


**Fig. 1 Examples of primer amplification strength measurements as scored in *Helianthus annuus*.** The far-left profile (primer 207) was scored as *0*, the second from left (primer 120) as *1*, the third from left (primer 177) as *2*, and far right (primer 153) as *3*. Each primer was surveyed with the same five DNAs. Molecular markers are shown in lanes 1 and 13 (ΦX174 DNA-*Hae* III digest, New England Biolabs, Inc.) and lanes 7 and 24 (1-kb ladder, Gibco BRL). Numbers adjacent to each marker indicate fragment size in kbp. Primers are from the University of British Columbia Biotechnology Laboratory.

significant ($X^2 = 997$, $df = 479$, $p < 0.001$). Thus, there is strong agreement among these three genera as to the similarity of primer amplification strength. This suggests that the ability of a particular primer to produce strongly, poorly, or no amplified products is largely independent of the source of DNA, at least among these three genera and perhaps generally among other plant groups as well.

Total (G+C) content had the most predictive value in estimating the quality of the amplification product of any characteristic of primer base composition analyzed (Table II). The coefficient of determination of the least squares linear regression ($r^2$) for this predictor variable was 0.129 ($t = 14.57$, $df = 1438$, $p < 0.001$). Thus, nearly 13% of the variation in primer amplification quality can be predicted by total (G+C) content, and high total (G+C) content is a relatively good estimator for good amplification quality. Note that since 100 minus total % (G+C) content equals % (A+T) content, (A+T) content is just as equally good an estimator for poor amplification quality as total (G+C) content is for good amplification quality. Similarly, since T content varies inversely with total (G+C) content and estimated nearly 9.2% of the variation in amplification strength, T content is a good estimator for poor amplification quality. Other percentages of bases (G, A, and C) or combinations of bases ([G+C] in the last four bases of the 3' end of the primer) were poor estimators of the variation in amplification strength (Table II). It is important to note that base percentages are separate and nonadditive when used to predict amplification strength, since the base percentage variables are highly dependent.

## Discussion

There are probably many factors accounting for both variation and uniformity in primer amplification strength among the three genera surveyed. The most likely source of variation is the presence of genomic differences resulting in primer-specific variation among genera. This is supported by the typically high variation in primer banding profiles observed among the genera surveyed (data not shown). Variation in DNA purity also may lead to variation in amplification strength. Although weak amplification due to impurities did not appear to be a problem for the DNAs used for the present survey, some primers may be more highly sensitive to the presence and amount of impurities than others. Thus, if our DNA samples varied at all among genera in terms of purity, which is likely, some variation in amplification strength might be

predicted. We have obtained significantly improved amplification product yield after purifying poorly amplifying DNA with the Elu-Quik DNA Purification Kit (Schleicher & Schuell). Minor sources of variation could have come from primer degradation during long- term storage (primer surveys within each genus were not performed simultaneously), as well as human error (most primer-genus combinations were replicated only if results were satisfactory).

There two likely explanations for similarities seen in primer amplification strength across genera. The first is that it is known that primer base sequence composition influences general amplification ability and strength (Williams et al., 1990). In this regard, our surveys confirm the existence of a positive correlation between primer (G+C) content and ability and strength of amplification (Williams et al., 1990). The second is that DNA sequence conservation among genomes will tend to equalize amplification strength values (as well as result in primer profile similarities).

The finding that the ability of a particular primer to produce strongly amplified products is to a large extent independent of the DNA source suggests that our data can be used in future studies as a rough guide for prioritizing primer deployment during the screening process for polymorphisms. We recommend an itinerary of first sampling all primers that show an amplification strength of 3 for all genera included in the present survey, then sampling those with a 3 for two genera and a 2 for the third, then either 3-2-2 or 3-3-1, and so on. Although we expect that the general trend in primer amplification strength consistency will be maintained additional extensive surveys will, undoubtedly show that some of the primers listed here are weaker than currently believed, while others are stronger. Preliminary primer surveys of other plant genera both from our laboratory (*Salvia, Ipomoea, Pogogyne* [Rieseberg, unpublished] and *Lipochaeta* [S. Keeley, unpublished]) and the laboratory of A. Liston (personal communication) indicate agreement with the values of many of the primers surveyed here. To improve the efficiency of polymorphism surveys for all biologists using RAPDs in their research, we think it important to publish primer surveys that include not only a list of primers showing polymorphism, but also a measure of primer success for all primers tested.

Although our data on primer amplification strength should be of practical value to workers employing RAPD markers, additional types of information would be helpful. For example, it would be useful to know whether certain primer sequences are more likely to produce polymor-

**Table I. Primer Amplification Strength Values for *Datisca glomerata*, *Yucca baccata* plus *Y. schidigera*, and *Helianthus annuus*.** See text for explanation of amplification strength values.

| Primer | Sequence | *Datisca* | *Yucca* | *Helianthus* | Primer | Sequence | *Datisca* | *Yucca* | *Helianthus* |
|---|---|---|---|---|---|---|---|---|---|
| *Operon Technologies:* | | | | | | | | | |
| A-1 | CAGGCCCTTC | 3 | 3 | 3 | C-18 | TGAGTGGGTG | 3 | 2 | 3 |
| A-2 | TGCCGAGCTG | 0 | 3 | 3 | C-19 | GTTGCCAGCC | 3 | 2 | 3 |
| A-3 | AGTCAGCCAC | 3 | 3 | 3 | C-20 | ACTTCGCCAC | 3 | 3 | 3 |
| A-4 | AATCGGGCTG | 2 | 3 | 3 | D-1 | ACCGCGAAGG | 1 | 2 | 2 |
| A-5 | AGGGGTCTTG | 3 | 2 | 3 | D-2 | GGACCCAACC | 3 | 3 | 3 |
| A-6 | GGTCCCTGAC | 0 | 0 | 0 | D-3 | GTCGCCGTCA | 3 | 3 | 3 |
| A-7 | GAAACGGGTG | 2 | 2 | 2 | D-4 | TCTGGTGAGG | 2 | 2 | 2 |
| A-8 | GTGACGTAGG | 3 | 3 | 3 | D-5 | TGAGCGGACA | 3 | 3 | 3 |
| A-9 | GGGTAACGCC | 2 | 1 | 3 | D-6 | ACCTGAACGG | 0 | 3 | 1 |
| A-10 | GTGATCGCAG | 0 | 0 | 0 | D-7 | TTGGCACGGG | 3 | 3 | 2 |
| A-11 | CAATCGCCGT | 3 | 3 | 1 | D-8 | GTGTGCCCCA | 3 | 3 | 3 |
| A-12 | TCGGCGATAG | 2 | 2 | 1 | D-9 | CTCTGGAGAC | 1 | 3 | 1 |
| A-13 | CAGCACCCAC | 3 | 3 | 3 | D-10 | GGTCTACACC | 2 | 3 | 3 |
| A-14 | TCTGTGCTGG | 3 | 1 | 1 | D-11 | AGCGCCATTG | 3 | 3 | 2 |
| A-15 | TTCCGAACCC | 3 | 1 | 1 | D-12 | CACCGTATCC | 1 | 2 | 2 |
| A-16 | AGCCAGCGAA | 1 | 1 | 1 | D-13 | GGGGTGACGA | 3 | 3 | 3 |
| A-17 | GACCGCTTGT | 3 | 0 | 3 | D-14 | CTTCCCCAAG | 0 | 1 | 1 |
| A-18 | AGGTGACCGT | 3 | 3 | 2 | D-15 | CATCCGTGCT | 3 | 2 | 3 |
| A-19 | CAAACGTCGG | 2 | 0 | 3 | D-16 | AGGGCGTAAG | 3 | 2 | 3 |
| A-20 | GTTGCGATCC | 3 | 3 | 3 | D-17 | TTTCCCACGG | 0 | 0 | 1 |
| B-1 | GTTTCGCTCC | 3 | 1 | 3 | D-18 | GAGAGCCAAC | 3 | 3 | 3 |
| B-2 | TGATCCCTGG | 1 | 0 | 1 | D-19 | CTGGGGACTT | 0 | 1 | 2 |
| B-3 | CATCCCCCTG | 1 | 0 | 1 | D-20 | ACCCGGTCAC | 3 | 3 | 3 |
| B-4 | GGACTGGAGT | 3 | 3 | 3 | | | | | |
| B-5 | TGCGCCCTTC | 3 | 0 | 3 | *University of British Columbia:* | | | | |
| B-6 | TGCTCTGCCC | 2 | 2 | 2 | | | | | |
| B-7 | GGTGACGCAG | 3 | 3 | 3 | 101 | GCGGCTGGAG | 3 | 3 | 3 |
| B-8 | GTCCACACGG | 3 | 3 | 3 | 102 | GGTGGGGACT | 2 | 3 | 3 |
| B-9 | TGGGGGACTC | 3 | 0 | 1 | 103 | GTGACGCCGC | 3 | 3 | 3 |
| B-10 | CTGCTGGGAC | 0 | 0 | 1 | 104 | GGGCAATGAT | 3 | 3 | 3 |
| B-11 | GTAGACCCGT | 3 | 3 | 2 | 105 | CTCGGGTGGG | 3 | 3 | 3 |
| B-12 | CCTTGACGCA | 3 | 3 | 2 | 106 | CGTCTGCCCG | 3 | 3 | 3 |
| B-13 | TTCCCCCGCT | 3 | 3 | 3 | 107 | CTGTCCCTTT | 0 | 0 | 1 |
| B-14 | TCCGCTCTGG | 1 | 1 | 1 | 108 | GTATTGCCCT | 3 | 3 | 3 |
| B-15 | GGAGGGTGTT | 1 | 1 | 2 | 109 | TGTACGTGAC | 0 | 2 | 3 |
| B-16 | TTTGCCCGGA | 0 | 0 | 2 | 110 | TAGCCCGCTT | 0 | 2 | 3 |
| B-17 | AGGGAACGAG | 3 | 3 | 3 | 111 | AGTAGACGGG | 3 | 3 | 3 |
| B-18 | CCACAGCAGT | 3 | 3 | 3 | 112 | GCTTGTGAAC | 3 | 3 | 3 |
| B-19 | ACCCCCGAAG | 0 | 1 | 2 | 113 | ATCCCAAGAG | 0 | 0 | 0 |
| B-20 | GGACCCTTAC | 3 | 3 | 3 | 114 | TGACCGAGAC | 3 | 3 | 3 |
| C-1 | TTCGAGCCAG | 2 | 3 | 3 | 115 | TTCCGCGGGC | 3 | 3 | 3 |
| C-2 | GTGAGGCGTC | 3 | 3 | 3 | 116 | TACGATGACG | 2 | 3 | 3 |
| C-3 | GGGGGTCTTT | 0 | 0 | 0 | 117 | TTAGCGGTCT | 0 | 2 | 0 |
| C-4 | CCGCATCTAC | 2 | 2 | 3 | 118 | CCCGTTTTGT | 0 | 1 | 2 |
| C-5 | GATGACCGCC | 3 | 1 | 2 | 119 | ATTGGGCGAT | 2 | 3 | 3 |
| C-6 | GAACGGACTC | 3 | 3 | 3 | 120 | GAATTTCCCC | 2 | 3 | 3 |
| C-7 | GTCCCGACGA | 3 | 3 | 2 | 121 | ATACAGGGAG | 0 | 2 | 2 |
| C-8 | TGGACCGGTG | 3 | 3 | 3 | 122 | GTAGACGAGC | 3 | 3 | 3 |
| C-9 | CTCACCGTCC | 3 | 3 | 2 | 123 | GTCTTTCAGG | 0 | 2 | 3 |
| C-10 | TGTCTGGGTG | 3 | 3 | 3 | 124 | ACTCGAAGTC | 0 | 2 | 2 |
| C-11 | AAAGCTGCGG | 2 | 3 | 3 | 125 | GCGGTTGAGG | 3 | 2 | 3 |
| C-12 | TGTCATCCCC | 2 | 3 | 3 | 126 | CTTTCGTGCT | 1 | 0 | 1 |
| C-13 | AAGCCTCGTC | 3 | 3 | 1 | 127 | ATCTGGCAGC | 3 | 3 | 3 |
| C-14 | TGCGTGCTTG | 2 | 3 | 3 | 128 | GCATATTCCG | 0 | 0 | 3 |
| C-15 | GACGGATCAG | 3 | 3 | 3 | 129 | GCGGTATAGT | 0 | 0 | 3 |
| C-16 | CACACTCCAG | 3 | 3 | 3 | 130 | GGTTATCCTC | 0 | 0 | 1 |
| C-17 | TTCCCCCCAG | 0 | 1 | 1 | 131 | GAAACAGCGT | 1 | 0 | 2 |

**Table I.** Continued

| Primer | Sequence | *Datisca* | *Yucca* | *Helianthus* | Primer | Sequence | *Datisca* | *Yucca* | *Helianthus* |
|---|---|---|---|---|---|---|---|---|---|
| 132 | AGGGATCTCC | 2 | 0 | 3 | 194 | AGGACGTGCC | 3 | 3 | 3 |
| 133 | GGAAACCTCT | 0 | 0 | 2 | 195 | GATCTCAGCG | 0 | 0 | 0 |
| 134 | AACACACGAG | 0 | 0 | 2 | 196 | CTCCTCCCCC | 3 | 1 | 3 |
| 135 | AAGCTGCGAG | 3 | 2 | 3 | 197 | TCCCCGTTCC | 0 | 0 | 1 |
| 136 | TACGTCTTGC | 0 | 0 | 1 | 198 | GCAGGACTGC | 3 | 2 | 3 |
| 137 | GGTCTCTCCC | 3 | 0 | 3 | 199 | GCTCCCCCAC | 3 | 3 | 3 |
| 138 | GCTTCCCCTT | 2 | 0 | 2 | 200 | TCGGGATATG | 0 | 0 | 3 |
| 139 | CCCAATCTTC | 0 | 0 | 0 | 201 | CTGGGGATTT | 0 | 0 | 0 |
| 140 | GTCGCATTTC | 0 | 0 | 0 | 202 | GAGCACTTAC | 0 | 0 | 2 |
| 141 | ATCCTGTTCG | 0 | 0 | 1 | 203 | CACGGCGAGT | 3 | 0 | 3 |
| 142 | ATCTGTTCGG | 0 | 0 | 1 | 204 | TTCGGGCCGT | 2 | 2 | 3 |
| 143 | TCGCAGAACG | 0 | 0 | 2 | 205 | CGGTTTGGAA | 0 | 0 | 2 |
| 144 | AGAGGGTTCT | 0 | 0 | 0 | 206 | GAGGACGTCC | 0 | 0 | 0 |
| 145 | TGTCGGTTGC | 2 | 2 | 2 | 207 | CATATCAGGG | 0 | 0 | 0 |
| 146 | ATGTGTTGCG | 2 | 2 | 3 | 208 | ACGGCCGACC | 3 | 3 | 3 |
| 147 | GTGCGTCCTC | 3 | 3 | 3 | 209 | TGCACTGGAG | 1 | 0 | 1 |
| 148 | TGTCCACCAG | 1 | 1 | 2 | 210 | GCACCGAGAG | 2 | 0 | 3 |
| 149 | AGCAGCGTGG | 3 | 3 | 3 | 211 | GAAGCGCGAT | 2 | 2 | 3 |
| 150 | GAAGGCTCTG | 2 | 2 | 2 | 212 | GCTGCGTGAC | 2 | 2 | 3 |
| 151 | GCTGTAGTGT | 1 | 0 | 1 | 213 | CAGCGAACTA | 2 | 3 | 3 |
| 152 | CGCACCGCAC | 1 | 1 | 3 | 214 | CATGTGCTTG | 0 | 0 | 2 |
| 153 | GAGTCACGAG | 3 | 1 | 3 | 215 | TCACACGTGC | 3 | 0 | 3 |
| 154 | TCCATGCCGT | 1 | 1 | 2 | 216 | CATAGACTCC | 0 | 1 | 1 |
| 155 | CTGGCGGCTG | 3 | 3 | 3 | 217 | ACAGGTAGAC | 0 | 1 | 1 |
| 156 | GCCTGGTTGC | 3 | 3 | 3 | 218 | CTCAGCCCAG | 3 | 3 | 3 |
| 157 | CGTGGGCAGG | 3 | 3 | 3 | 219 | GTGACCTCAG | 3 | 3 | 3 |
| 158 | TAGCCGTGGC | 0 | 3 | 1 | 220 | GTCGATGTCG | 2 | 3 | 3 |
| 159 | GAGCCCGTAG | 2 | 3 | 3 | 221 | CCCGTCAATA | 1 | 1 | 2 |
| 160 | CGATTCAGAG | 0 | 2 | 1 | 222 | AAGCCTCCCC | 3 | 3 | 3 |
| 161 | CGTTATCTCG | 0 | 1 | 0 | 223 | GATCCATTGC | 0 | 0 | 2 |
| 162 | AACTTACCGC | 0 | 1 | 3 | 224 | TCTCCGGTAT | 0 | 0 | 0 |
| 163 | CCCCCCAGAT | 0 | 2 | 1 | 225 | CGACTCACAG | 2 | 1 | 2 |
| 164 | CCAAGATGCT | 2 | 3 | 3 | 226 | GGGCCTCTAT | 2 | 2 | 3 |
| 165 | GAAGGCACTG | 2 | 3 | 3 | 227 | CTAGAGGTCC | 1 | 1 | 1 |
| 166 | ACTGCTACAG | 0 | 3 | 0 | 228 | GCTGGGCCGA | 3 | 1 | 2 |
| 167 | CCAATTCACG | 0 | 2 | 2 | 229 | CCACCCAGAG | 2 | 1 | 3 |
| 168 | CTAGATGTGC | 1 | 2 | 3 | 230 | CGTCGCCCAT | 3 | 3 | 3 |
| 169 | ACGACGTAGG | 3 | 3 | 3 | 231 | AGGGAGTTCC | 1 | 1 | 2 |
| 170 | ATCTCTCCTG | 0 | 1 | 1 | 232 | CGGTGACATC | 2 | 2 | 2 |
| 171 | TGACCCCTCC | 3 | 3 | 3 | 233 | CTATGCGCGC | 0 | 0 | 0 |
| 172 | ACCGTCGTAG | 0 | 1 | 0 | 234 | TCCACGGACG | 3 | 2 | 3 |
| 173 | CAGGCGGCGT | 3 | 3 | 3 | 235 | CTGAGGCAAA | 0 | 1 | 3 |
| 174 | AACGGGCAGC | 3 | 3 | 3 | 236 | ATCGTACGTG | 2 | 1 | 2 |
| 175 | TGGTGCTGAT | 2 | 3 | 2 | 237 | CGACCAGAGC | 3 | 3 | 3 |
| 176 | CAAGGGAGGT | 1 | 3 | 2 | 238 | CTGTCCAGCA | 1 | 3 | 3 |
| 177 | TCAGGCAGTC | 3 | 3 | 2 | 239 | CTGAAGCGGA | 3 | 2 | 3 |
| 178 | CCGTCATTGG | 3 | 3 | 3 | 240 | ATGTTCCAGG | 0 | 3 | 1 |
| 179 | TCACTGTACG | 0 | 1 | 2 | 241 | GCCCGACGCG | 3 | 3 | 3 |
| 180 | GGGCCACGCT | 3 | 3 | 3 | 242 | CACTCTTTGC | 0 | 1 | 0 |
| 181 | ATGACGACGG | 3 | 3 | 3 | 243 | GGGTGAACCG | 3 | 2 | 0 |
| 182 | GTTCTCGTGT | 2 | 2 | 2 | 244 | CAGCCAACCG | 3 | 3 | 3 |
| 183 | CGTGATTGCT | 0 | 3 | 2 | 245 | CGCGTGCCAG | 1 | 2 | 3 |
| 184 | CAAACGGCAC | 3 | 3 | 3 | 246 | TATGGTCCGG | 0 | 2 | 2 |
| 185 | GTGTCTTCAC | 1 | 3 | 1 | 247 | TACCGACGGA | 0 | 0 | 1 |
| 186 | GTGCGTCGCT | 2 | 0 | 3 | 248 | GAGTAAGCGG | 3 | 3 | 2 |
| 187 | AACGGGGGAG | 0 | 0 | 0 | 249 | GCATCTACCG | 3 | 2 | 3 |
| 188 | GCTGGACATC | 3 | 3 | 3 | 250 | CGACAGTCCC | 1 | 3 | 3 |
| 189 | TGCTAGCCTC | 3 | 2 | 3 | 251 | CTTGACGGGG | 1 | 2 | 2 |
| 190 | AGAATCCGCC | 3 | 3 | 3 | 252 | CTGGTGATGT | 0 | 0 | 2 |
| 191 | CGATGGCTTT | 1 | 0 | 2 | 253 | CCGTGCAGTA | 0 | 3 | 3 |
| 192 | GCAAGTCACT | 3 | 2 | 3 | 254 | CGCCCCCATT | 3 | 2 | 3 |
| 193 | TGCTGGCTTT | 0 | 0 | 1 | 255 | TTCCTCCGGA | 0 | 0 | 0 |

**Table I.** Continued

| Primer | Sequence | *Datisca* | *Yucca* | *Helianthus* | Primer | Sequence | *Datisca* | *Yucca* | *Helianthus* |
|---|---|---|---|---|---|---|---|---|---|
| 256 | TGCAGTCGAA | 0 | 2 | 3 | 318 | CGGAGAGCGA | 3 | 3 | 3 |
| 257 | CGTCACCGTT | 0 | 0 | 3 | 319 | GTGGCCGCGC | 3 | 3 | 3 |
| 258 | CAGGATACCA | 0 | 2 | 1 | 320 | CCGGCATAGA | 3 | 1 | 2 |
| 259 | GGTACGTACT | 0 | 1 | 2 | 321 | ATCTAGGGAC | 0 | 0 | 0 |
| 260 | TCTCAGCTAC | 0 | 0 | 0 | 322 | GCCGCTACTA | 3 | 3 | 3 |
| 261 | CTGGCGTGAC | 3 | 3 | 2 | 323 | GACATCTCGC | 1 | 2 | 2 |
| 262 | CGCCCCCAGT | 3 | 3 | 3 | 324 | ACAGGGAACG | 2 | 1 | 2 |
| 263 | TTAGAGACGG | 0 | 0 | 1 | 325 | TCTAAGCTCG | 1 | 1 | 0 |
| 264 | TCCACCGAGC | 3 | 3 | 3 | 326 | CGGATCTCTA | 1 | 0 | 0 |
| 265 | CAGCTGTTCA | 1 | 2 | 2 | 327 | ATACGGCGTC | 2 | 1 | 2 |
| 266 | CCACTCACCG | 3 | 3 | 3 | 328 | ATGGCCTTAC | 1 | 1 | 0 |
| 267 | CCATCTTGTG | 0 | 1 | 1 | 329 | GCGAACCTCC | 3 | 3 | 3 |
| 268 | AGGCCGCTTA | 3 | 2 | 3 | 330 | GGTGGTTTCC | 0 | 0 | 0 |
| 269 | CCAGTTCGCC | 3 | 3 | 2 | 331 | GCCTAGTCAC | 0 | 1 | 1 |
| 270 | TGCGCGCGGG | 3 | 3 | 2 | 332 | AACGCGTAGA | 0 | 0 | 0 |
| 271 | GCCATCAAGA | 0 | 2 | 2 | 333 | GAATGCGACG | 1 | 1 | 0 |
| 272 | AGCGGGCCAA | 3 | 3 | 3 | 334 | ATGGCAAAGC | 0 | 0 | 0 |
| 273 | AATGTCGCCA | 3 | 3 | 2 | 335 | TGGACCACCC | 0 | 3 | 2 |
| 274 | GTTCCCGAGT | 1 | 2 | 3 | 336 | GCCACGGAGA | 0 | 1 | 1 |
| 275 | CCGGGCAAGC | 3 | 3 | 3 | 337 | TCCCGAACCG | 1 | 2 | 3 |
| 276 | AGGATCAAGC | 3 | 2 | 3 | 338 | CTGTGGCGGT | 1 | 1 | 3 |
| 277 | AGGAAGGTGC | 3 | 3 | 3 | 339 | CTCACTTGGG | 0 | 0 | 3 |
| 278 | GGTTCCAGCT | 3 | 3 | 2 | 340 | GAGAGGCACC | 2 | 2 | 2 |
| 279 | AGACATTAGA | 0 | 0 | 0 | 341 | CTGGGGCCGT | 3 | 3 | 2 |
| 280 | CTGGGAGTGG | 3 | 3 | 3 | 342 | GAGATCCCTC | 0 | 0 | 1 |
| 281 | GAGAGTGGAA | 0 | 2 | 2 | 343 | TGTTAGGCTC | 0 | 0 | 1 |
| 282 | GGGAAAGCAG | 3 | 3 | 2 | 344 | TGTTAGGCAC | 1 | 0 | 1 |
| 283 | CGGCCACCGT | 3 | 3 | 3 | 345 | GCGTGACCCG | 3 | 3 | 3 |
| 284 | CAGGCGCACA | 3 | 3 | 3 | 346 | TAGGCGAACG | 3 | 1 | 1 |
| 285 | GGGCGCCTAG | 3 | 2 | 2 | 347 | TTGCTTGGCG | 1 | 0 | 1 |
| 286 | CGGAGCCGGC | 0 | 1 | 0 | 348 | CACGGCTGCG | 2 | 0 | 2 |
| 287 | CGAACGGCGG | 3 | 3 | 3 | 349 | GGAGCCCCCT | 3 | 3 | 3 |
| 288 | CCTCCTTGAC | 0 | 2 | 1 | 350 | TGACGCGCTC | 3 | 3 | 3 |
| 289 | ATCAAGCTGC | 3 | 3 | 3 | 351 | CTCCCGGTGG | 2 | 0 | 2 |
| 290 | CCGCGAGCAC | 3 | 3 | 3 | 352 | CACAACGGGT | 2 | 0 | 2 |
| 291 | AGCTGAAGAG | 0 | 2 | 1 | 353 | TGGGCTCGCT | 2 | 1 | 2 |
| 292 | AAACAGCCCG | 3 | 3 | 3 | 354 | CTAGAGGCCG | 3 | 3 | 2 |
| 293 | TCGTGTTGCT | 2 | 3 | 2 | 355 | GTATGGGGCT | 2 | 2 | 2 |
| 294 | TGATTGGCCA | 0 | 0 | 0 | 356 | GCGGCCCTCT | 3 | 3 | 3 |
| 295 | CGCGTTCCTG | 3 | 3 | 3 | 357 | ACGCCAAATG | 2 | 3 | 3 |
| 296 | CCGCTGGGAG | 3 | 1 | 3 | 358 | GGTCAGGCCC | 3 | 3 | 3 |
| 297 | GCGCATTAGA | 3 | 3 | 3 | 359 | AGGCAGACCT | 3 | 3 | 2 |
| 298 | CCGTACGGAC | 1 | 1 | 0 | 360 | CTCTCCAGGC | 0 | 0 | 0 |
| 299 | TGTCAGCGGT | 3 | 2 | 3 | 361 | GCGAGGTGCT | 2 | 2 | 2 |
| 300 | GGCTAGGGCG | 3 | 1 | 3 | 362 | CCGCCTTACA | 1 | 1 | 1 |
| 301 | CGGTGGCGAA | 3 | 3 | 3 | 363 | ATGACGTTGA | 1 | 1 | 2 |
| 302 | CGGCCCACGT | 3 | 3 | 3 | 364 | GGCTCTCGCG | 1 | 0 | 1 |
| 303 | GCGGGAGACC | 3 | 3 | 3 | 365 | TAGACAGAGG | 2 | 0 | 1 |
| 304 | AGTCCTCGCC | 0 | 0 | 1 | 366 | CCTGATTGCC | 1 | 0 | 0 |
| 305 | GCTGGTACCC | 3 | 1 | 2 | 367 | ACCTTTGGCT | 1 | 0 | 0 |
| 306 | GTCCTCGTAG | 1 | 1 | 1 | 368 | ACTTGTGCGG | 3 | 3 | 1 |
| 307 | CGCATTTGCA | 1 | 1 | 1 | 369 | GCGCATAGCA | 0 | 0 | 0 |
| 308 | AGCGGCTAGG | 3 | 3 | 3 | 370 | TCAGCCAGCG | 0 | 0 | 0 |
| 309 | ACATCCTGCG | 1 | 1 | 1 | 371 | TCTCGATTGC | 0 | 0 | 1 |
| 310 | GAGCCAGAAG | 3 | 1 | 2 | 372 | CCCACTGACG | 2 | 2 | 1 |
| 311 | GGTAACCGTA | 0 | 0 | 0 | 373 | CTGAGGAGTG | 1 | 1 | 2 |
| 312 | ACGGCGTCAC | 3 | 3 | 3 | 374 | GGTCAACCCT | 2 | 1 | 1 |
| 313 | ACGGCAGTGG | 3 | 3 | 3 | 375 | CCGGACACGA | 3 | 3 | 3 |
| 314 | ACTTCCTCCA | 2 | 1 | 1 | 376 | CAGGACATCG | 3 | 3 | 2 |
| 315 | GGTCTCCTAG | 2 | 1 | 1 | 377 | GACGGAAGAG | 3 | 0 | 0 |
| 316 | CCTCACCTGT | 2 | 1 | 0 | 378 | GACAACAGGA | 0 | 0 | 0 |
| 317 | CTAGGGGCTG | 3 | 1 | 3 | 379 | GGGCTAGGGT | 3 | 3 | 3 |

**Table I.** Continued

| Primer | Sequence | *Datisca* | *Yucca* | *Helianthus* | Primer | Sequence | *Datisca* | *Yucca* | *Helianthus* |
|---|---|---|---|---|---|---|---|---|---|
| 380 | AGGAGTGAGA | 3 | 3 | 2 | 440 | CTGTCGAACC | 3 | 3 | 3 |
| 381 | ATGAGTCCTG | 3 | 1 | 1 | 441 | CTGCGTTCTT | 1 | 0 | 1 |
| 382 | ATACACCAGC | 3 | 3 | 3 | 442 | CTACTCGGTT | 0 | 0 | 0 |
| 383 | GAGGCGCTGC | 3 | 2 | 1 | 443 | TGATTGCTCG | 3 | 2 | 3 |
| 384 | TGCGCCGCTA | 3 | 0 | 0 | 444 | GCAGCCCCAT | 3 | 3 | 3 |
| 385 | ACCGGGAACG | 0 | 0 | 2 | 445 | TAGCAGCTTG | 3 | 3 | 3 |
| 386 | TGTAAGCTCG | 1 | 1 | 0 | 446 | GCCAGCGTTC | 3 | 3 | 3 |
| 387 | CGCTGTCGCC | 1 | 1 | 0 | 447 | CAGGCTCTAG | 2 | 2 | 2 |
| 388 | CGGTCGCGTC | 3 | 3 | 1 | 448 | GTTGTGCCTG | 2 | 3 | 3 |
| 389 | CGCCCGCAGT | 3 | 3 | 1 | 449 | GAGGTTCAAC | 3 | 3 | 3 |
| 390 | TCACTCAGAG | 0 | 0 | 0 | 450 | CGGAGAGCCC | 3 | 3 | 3 |
| 391 | GCGAACCTCG | 3 | 3 | 0 | 451 | CTAATCTCGC | 1 | 2 | 3 |
| 392 | CCTGGTGGTT | 1 | 1 | 1 | 452 | CTAATCACGG | 1 | 0 | 2 |
| 393 | TTCCATGCCT | 1 | 0 | 0 | 453 | AGTACAAGGG | 0 | 0 | 1 |
| 394 | TCACGCAGTT | 0 | 0 | 0 | 454 | GCTTACGGCA | 0 | 0 | 0 |
| 395 | TCACTTGAGG | 0 | 0 | 0 | 455 | AGCAAGCCGG | 3 | 3 | 3 |
| 396 | GAATGCGAGG | 1 | 2 | 1 | 456 | GCGGAGGTCC | 3 | 3 | 3 |
| 397 | GGGCTGTGCC | 0 | 0 | 0 | 457 | CGACGCCCTG | 3 | 3 | 3 |
| 398 | CAGTGCTCTT | 0 | 0 | 0 | 458 | CTCACATGCC | 2 | 2 | 3 |
| 399 | TTGCTGGGCG | 1 | 1 | 1 | 459 | GCGTCGAGGG | 3 | 3 | 3 |
| 400 | GCCCTGATAT | 0 | 0 | 1 | 460 | ACTGACCGGC | 3 | 3 | 3 |
| 401 | TAGGACAGTC | 0 | 1 | 0 | 461 | CCCGTATGTC | 0 | 0 | 0 |
| 402 | CCCGCCGTTG | 0 | 3 | 1 | 462 | CATAGCGGCA | 3 | 2 | 3 |
| 403 | GGAAGGCTGT | 3 | 3 | 3 | 463 | AGGCGGAAGC | 0 | 0 | 1 |
| 404 | TCTCTACGAC | 0 | 0 | 0 | 464 | CACAAGCCTG | 3 | 3 | 3 |
| 405 | CTCTCGTGCG | 0 | 1 | 1 | 465 | GGTCAGGGCT | 3 | 3 | 3 |
| 406 | GCCACCTCCT | 3 | 1 | 2 | 466 | TTCTTAGCGG | 0 | 0 | 1 |
| 407 | TGGTCCTGGC | 3 | 3 | 1 | 467 | AGCACGGGCA | 3 | 3 | 3 |
| 408 | CCGTCTCTTT | 0 | 0 | 0 | 468 | ACGGAAGCGC | 3 | 3 | 3 |
| 409 | TAGGCGGCGG | 3 | 3 | 3 | 469 | CTCCAGCAAA | 3 | 3 | 3 |
| 410 | CGTCACAGAG | 1 | 2 | 1 | 470 | AGGAGCTGGG | 3 | 3 | 3 |
| 411 | GAGGCCCGTT | 1 | 2 | 1 | 471 | CCGACCGGAA | 3 | 2 | 3 |
| 412 | TGCGCCGGTG | 2 | 3 | 2 | 472 | AGGCGTGCAA | 3 | 3 | 3 |
| 413 | GAGGCGGCGA | 3 | 3 | 3 | 473 | ATCCCCAAGA | 0 | 0 | 0 |
| 414 | AAGGCACCAG | 3 | 3 | 3 | 474 | AGGCGGGAAC | 3 | 3 | 3 |
| 415 | GTTCCAGCAG | 3 | 3 | 3 | 475 | CCAGCGTATT | 1 | 2 | 3 |
| 416 | GTGTTTCCGG | 3 | 2 | 3 | 476 | TTGAGGCCCT | 2 | 3 | 3 |
| 417 | GACAGGCCAA | 3 | 3 | 3 | 477 | TGTTGTGCCC | 3 | 3 | 3 |
| 418 | GAGGAAGCTT | 3 | 3 | 3 | 478 | CGAGCTGGTC | 3 | 3 | 3 |
| 419 | TACGTGCCCG | 3 | 3 | 3 | 479 | CTCATACGCG | 1 | 2 | 3 |
| 420 | GCAGGGTTCG | 3 | 3 | 3 | 480 | GGAGGGGGGA | 0 | 0 | 1 |
| 421 | ACGGCCCACC | 3 | 3 | 3 | 481 | GTAATTGCGC | 0 | 0 | 0 |
| 422 | CACCTGCGGG | 0 | 0 | 0 | 482 | CTATAGGCCG | 3 | 3 | 3 |
| 423 | GGGTCTCGAA | 2 | 2 | 2 | 483 | GCACTAAGAC | 0 | 0 | 0 |
| 424 | ACGGAGGTTC | 0 | 0 | 1 | 484 | CTGGCAAGGA | 0 | 0 | 0 |
| 425 | CGTCGGGCCT | 3 | 3 | 3 | 485 | AGAATAGGGC | 0 | 0 | 0 |
| 426 | TCTCCCGGTG | 0 | 0 | 1 | 486 | CCAGCATCAG | 3 | 3 | 3 |
| 427 | GTAATCGACG | 3 | 3 | 2 | 487 | GTGGCTAGGT | 3 | 3 | 3 |
| 428 | GGCTGCGGTA | 2 | 2 | 3 | 488 | TTCGCTTCTC | 0 | 0 | 0 |
| 429 | AAACCTGGAC | 0 | 1 | 2 | 489 | CGCACGCACA | 3 | 3 | 3 |
| 430 | AGTCGGCACC | 3 | 3 | 3 | 490 | AGTCGACCTT | 3 | 3 | 3 |
| 431 | CTGCGGGTCA | 3 | 3 | 3 | 491 | TCCTGTCAAG | 0 | 0 | 0 |
| 432 | AGCGTCGACT | 3 | 2 | 3 | 492 | GTGACTGCTC | 3 | 3 | 3 |
| 433 | TCACGTGCCT | 2 | 2 | 2 | 493 | CCGAATCACT | 3 | 2 | 3 |
| 434 | TCGCTAGTCC | 0 | 0 | 0 | 494 | TGATGCTGTC | 1 | 2 | 3 |
| 435 | CTAGTAGGGG | 0 | 0 | 0 | 495 | CTTTCCTTCC | 0 | 0 | 1 |
| 436 | GAGGGGGCCA | 0 | 0 | 1 | 496 | CCTTTCAAGG | 0 | 0 | 0 |
| 437 | AGTCCGCTGC | 3 | 3 | 3 | 497 | GCATAGTGCG | 2 | 0 | 2 |
| 438 | AGACGGCCGG | 3 | 3 | 3 | 498 | GACAGTCCTG | 3 | 3 | 3 |
| 439 | GCCCCTTGAC | 3 | 2 | 3 | 499 | GGCCGATGAT | 3 | 3 | 3 |
| | | | | | 500 | TTGCGTCATG | 3 | 3 | 3 |

**Table II. Coefficients of Determination ($r^2$), *t* Statistics and p Values for Seven Predictor Variables Involving Primer Base Composition.** $df = 1438$; (n.s.), not significant.

| Predictor Variable | $r^2$ | *t* | *p* |
|---|---|---|---|
| Total (G + C) Content | 0.129 | 14.57 | < 0.001 |
| (G + C) content (last four bases in 3'end) | 0.026 | 6.16 | < 0.001 |
| (A + T) Content | 0.129 | -14.57 | < 0.001 |
| G Content | 0.036 | 7.30 | < 0.001 |
| A Content | 0.000 | 0.80 | n.s. |
| T Content | 0.092 | -12.07 | < 0.001 |
| C Content | 0.008 | 3.30 | < 0.001 |

phic amplification products than other sequences and whether certain sequences produce more reliable amplification patterns. More work is needed to determine whether the 10-mer sequences used for most RAPDs to date is the optimal primer length (Williams et al., 1990; Welsh and McClelland, 1990). In this regard, preliminary data suggest that 15- to 20-mer primers actually produce stronger amplification products than 10-mer primers and may be less likely to produce spurious bands (Rieseberg, unpublished). Finally, it would be of interest to determine whether certain primer sequences are more likely to amplify co-dominant products than other sequences (*e.g.*, perhaps primers that amplify low-copy number sequences would be more likely to produce co-dominant products than those amplifying repetitive sequences). Answers to these questions will be necessary before an optimal approach to RAPD primer design can be achieved.

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