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MOLECULAR BIOLOGY

New Dinucleotide and Trinucleotide Microsatellite Marker Resources for Cotton Genome Research

O. Umesh K. Reddy,† Alan E. Pepper,†* Ibrokhim Abdurakhmonov, Sukumar Saha,†
Johnie N. Jenkins, Thomas Brooks, Yuksel Bolek, and Kamal M. El-Zik

INTERPRETIVE SUMMARY

The use of microsatellite markers has greatly accelerated the mapping of important traits and the characterization of genome structure in several plant and animal species. Microsatellites (also known as simple sequence repeats) are small, repetitive DNA structures, typically distributed throughout the genome, which are highly mutable and show substantial variation in size (polymorphism). To exploit microsatellites as molecular markers, they are amplified by polymerase chain reaction (PCR); gel electrophoresis or other analytical methods determine the size of the resulting DNA product. In organisms with genomes as large and complex as those of tetraploid cottons, several thousand microsatellite markers will be required for genetic mapping and genome analysis. To date, microsatellites have not been used extensively in cotton, in part because of the complex and labor-intensive methods for identifying microsatellites from large genomes.

Here we address the problem of microsatellite marker discovery, and present the initial results from a low-cost, easy-to-use, efficient method for microsatellite capture. Using an optimized protocol, more than 10,000 microsatellite-containing fragments were obtained from *Gossypium hirsutum* L. genomic DNA. Sequences from 588 of these DNA fragments were determined, and oligonucleotide primers for PCR amplification of 307 microsatellite markers were designed. A subset of markers was tested in a set of G. *hirsutum* L. and *Gossypium barbadense* L. varieties. Approximately 49% showed length polymorphism. Intraspecific polymorphism was observed in some cases. A lack of redundancy with two independently derived microsatellite marker sets implies that there is a large resource of unique microsatellites that have yet to be developed as molecular markers.

ABSTRACT

A collaborative multi-institutional program was initiated to streamline the process of microsatellite capture and characterization, development of microsatellites into informative molecular markers, and dissemination of marker information to the cotton research community. A simple and efficient biotin capture method was optimized and used to capture more than 10,000 **fragments. Out of 588 fragments sequenced, nearly all contained a microsatellite repeat structure. Several repeat types were represented, including AGA, GA, CA, and ACA. Primers were designed to amplify 307 unique microsatellite loci (305 nuclear and two chloroplast-encoded). One hundred fifty-two microsatellite loci were amplified from *G. hirsutum* L. cv. TM-1 and Tamcot SP37, and *G. barbadense* L. cv. Pima 3-79 and Pima S-7. In this comparison, 74 of the primers (~49% of the subset) showed detectable polymorphism. In a comparison of upland *G. hirsutum* cultivars, ~26% of the primers exhibited intraspecific polymorphism. Polymorphism was widely distributed among the various repeat types and structures (e.g., imperfect and compound repeats). Redundancy with two other previously derived microsatellite marker sets (BNL, CM) was low, implying that the total pool of microsatellites present in the cotton genome is large enough to satisfy the requirements of extensive genome mapping and marker-assisted selection projects.**

O.U.K. Reddy, A.E. Pepper, and I. Abdurakhmonov, Dep. of Biology, and T. Brooks, Y. Bolek, and K.M. El-Zik, Dep. of Soil and Crop Sciences, Texas A & M Univ., College Station, TX 77843; S. Saha and J. Jenkins, Crop Sci. Res. Lab., USDA, ARS, P.O. Box 5367, Mississippi State, MS 39762. Received 28 Nov. 2000. †Contributed equally to work. *Corresponding author (apepper@bio.tamu.edu).

Abbreviations: AFLP, amplified fragment length polymorphism; BNL, Brookhaven National Laboratory; CM, cotton microsatellite; EDTA, ethylene diamine tetraacetic acid; PCR, polymerase chain reaction; PVDF, polyvinylidene fluoride; RFLP, restriction fragment length polymorphism; SDS, sodium dodecyl sulfate; SSLP, simple sequence length polymorphism; SSR, simple sequence repeat.

Use of molecular markers in genome analysis, the systematic mapping of agriculturally important traits, and marker-assisted selection have been greatly advanced by the development of reliable PCR-based markers. These include amplified fragment length polymorphisms (AFLPs) (Zabeau and Vos, 1993; Vos et al., 1995); PCR-RFLP, also known as cleaved amplified polymorphic sequences (Konieczny and Ausubel, 1993); and microsatellites, also known as simple sequence repeats (SSRs) (Akkaya et al., 1992) or simple sequence length polymorphisms (SSLPs) (Bell and Ecker, 1994). Of these PCR-based markers, microsatellites are of particular utility because they are typically both co-dominant and multiallelic in nature. Furthermore, a given pair of microsatellite primers often will amplify a specific microsatellite locus from a diversity of species within a particular genus, sometimes out to the family level (Fredholm and Wintero, 1995; Plaschke et al., 1995; Zardoya et al., 1996; Steinkellner et al., 1997; Westman and Kresovich, 1998). Microsatellite markers are, therefore, readily "portable" among mapping populations; they are potentially useful for studies of genome evolution and comparative genomics and for the efficient utilization of wild and primitive germplasm resources in marker-assisted selection (Tanksley and McCouch, 1997).

McCouch and co-workers (1997) estimated that 5700 to 10,000 microsatellite repeat loci are present in the relatively small rice genome. Many of these loci have been incorporated into a high-density microsatellite marker map for rice (Akagi et al., 1996; Chen et al., 1997). High-density microsatellite marker maps containing 5264 and 7377 loci have been developed for the human (Dib et al., 1996) and mouse (Dietrich et al., 1996) genomes, respectively. Knapik et al. (1998) constructed a genetic linkage map of zebrafish (*Danio rerio*) consisting of 705 microsatellite markers with an average resolution of 3.3 cM. The zebrafish map has been expanded recently to include more than 2000 microsatellite loci (Shimoda et al., 1999).

Microsatellite identification and mapping in cotton lags far behind these milestones. More than 500 microsatellite-containing clones, containing mostly $(GA)_n$ repeats, have been identified at the Brookhaven National Laboratory. Several of these sequences are redundant. Primer-pairs for the amplification of ~240 of these loci (designated BNL for Brookhaven National Laboratory) have been made available to the cotton research community through purchase from Research Genetics, Huntsville, AL. An additional 150 $(GA)_n$ repeat loci (designated CM for cotton microsatellite) have been isolated at Texas A & M University (Connell et al., 1998; Reddy and Pepper, unpublished data, 1999). Liu et al. (2000) reported on the chromosomal assignment of several BNL and CM microsatellite markers using cytogenetic stocks. Despite these advances, a large number of additional microsatellite markers will be needed to achieve the goals of the International Cotton Genome Initiative and to meet the needs of marker-assisted selection applications.

In the past, microsatellite loci were identified by surveys of genomic and cDNA sequence databases, and by systematic screening of DNA libraries using colony hybridization with radioactively labeled repeat oligonucleotides (Condit and Hubbell, 1991; Akkaya et al., 1992; Morgante and Oliver, 1993; Wu and Tanksley, 1993; Bell and Ecker, 1994). These methods are typically costly, time-consuming, and labor-intensive. More recently, various approaches have been employed to enrich libraries in microsatellite-containing clones (Karagyozov et al., 1993; Edward et al., 1996; Lench et al., 1996), including methods employing the hybridization of adapter-ligated genomic DNA fragments to biotinylated oligonucleotides and their subsequent capture using streptavidin-coated magnetic beads (Kijas et al., 1994; Prochazka, 1996; Connell et al., 1998).

In the present study, a large number of new microsatellite sequences, comprising a diversity of dinucleotide and trinucleotide repeat structures, were retrieved from the *G. hirsutum* L. genome using an optimized and highly simplified biotin capture protocol. On the basis of sequence analysis of these captured fragments, appropriate primers were designed for amplification of these microsatellite loci in cotton genetic and genomic research. In addition, a subset of these markers was tested for polymorphism in *G. hirsutum* L. and *G. barbadense* L. to evaluate their informativeness in interspecific and intraspecific mapping populations. The potential value of these microsatellites in marker-assisted selection, comparative genomics, and efficient germplasm utilization is discussed.

MATERIALS AND METHODS

Microsatellite Isolation

A microsatellite-enriched library was prepared by a highly modified and simplified protocol based on the biotinylated-oligonucleotide capture methods of Kijas et al. (1994) and Prochazka (1996). In our protocol, no size fractionation steps or radioactive hybridizations were employed. Genomic DNA was isolated from *G. hirsutum* L. cv. Tamcot Sphinx by the method of Iqbal et al. (1997). A 2-µg sample of genomic DNA was digested for 3 h in a single reaction mixture containing restriction endonucleases *Hae*III, *Rsa*I, and *Dra*I (20 units of each), as well as 50 ng of RNaseA. This digestion resulted in a diverse population of blunt-ended restriction fragments with an average size of ~550 bp. Digested DNAs were purified using a QIA-quick PCR purification column (Qiagen, Valencia, CA), eluted with 50 µL of 5 m*M* Tris-pH 8.0, then dried completely under vacuum. The double-stranded adaptor molecule AP11/12 was prepared by mixing equal molar amounts of oligonucleotides AP11 (5´CTCTTGCTTAGATCTGGACTA3´) and AP12 (5´pTAGTCCAGATCTAAGCA-AGAGCACA3´, where p = 5´ phosphate), heating to 94°C, then cooling to 25°C over a period of 5 h. Digested genomic DNA fragments were resuspended in a 30-µL ligation reaction containing 100 ng of AP11/12 double-stranded adaptor and 30 Weiss units of T4 DNA ligase. Ligation was carried out at 14°C for 16 h.

Preamplification of adaptor-ligated products was performed using 2 µL of the ligation reaction as a template for 10 cycles of PCR in a 50-µL reaction volume using the single primer AP11. An annealing temperature of 55°C was employed in all PCR reactions. Approximately 100 ng of the preamplified product was then added to a single-reaction mixture containing 6X SSC (0.9 M NaCl, 90 mM sodium citrate, pH 7), 0.1% SDS (sodium dodecyl sulfate), and 200 ng each of biotinylated oligos b$(TA)_{30}$, b$(CA)_{20}$, b$(GA)_{20}$, b$(AGA)_{15}$, b$(TGA)_{15}$, and b$(ACA)_{15}$ (b = 5´ biotinylation). After denaturation at 95°C for 5 min, preamplified genomic DNA fragments were annealed in the presence of biotinylated oligonucleotides for 1 h at 60°C, then added to 200 µg of fresh streptavidin-coated paramagnetic beads (Promega, Madison, WI) previously equilibrated with 6X SSC. Beads were incubated at 60°C with gentle agitation for 15 min, then the liquid was removed by separation using a magnetic stand (Stratagene, San Diego, CA). Beads were washed twice in 300 µL of 6X SSC, 0.1% SDS for 15 min at room temperature with gentle agitation. Beads were further washed twice in 300 µL 6X SSC, 0.1% SDS for 15 min at 60°C with gentle agitation. Finally, beads were briefly washed twice with 6X SSC at room temperature. After removing the final wash, captured DNAs were eluted from the beads with the addition of 100 µL of 60°C 0.1 *M* NaOH. After neutralization with 100 µL of 1 *M* Tris-pH 7.5, captured DNAs were desalted and equilibrated with 10 m*M* Tris-pH 8.0, 1 m*M* EDTA-pH 8.0 (to a final volume of ~50 µL) using a 100-kDa MW cutoff size filtration column (Millipore, Bedford, MA). Five µL of desalted DNA sample were used as a template for 30 cycles of PCR in a 50-µL reaction volume using primer AP11. Six microliters of the resulting PCR reaction (~60 ng) was cloned into the TA-cloning vector pCR4-TOPO through topoisomerase-mediated ligation (Invitrogen, San Diego, CA) and transformed into chemically competent *Escherichia coli* TOP10. Recombinant colonies were identified by positive selection through insertional inactivation of the *ccd*B (control of cell death) open reading frame. Colonies were transferred to 96-well microtiter plates for archival storage. Additional experimental details on the microsatellite capture method can be found at our cotton microsatellite resources Web site (http://plantbiol.tamu.edu/cottonSSRs/htm).

Sequence Analysis

Recombinant bacterial colonies were inoculated into 300 µL of 2X YT broth in a 96-well 0.6-mL-deep plate (Marsh Bioproducts, Rochester, NY). Cultures were agitated at 500 rpm in a HiGro high-density shaking-incubator (GeneMachines, San Carlos, CA) for 16 to 18 h at 37°C. The bacteria were pelleted by centrifugation and resuspended in 50 µL of plasmid mini-prep Solution I (25 m*M* Tris-pH 7.5, 10 m*M* EDTA, 50 m*M* glucose) containing 0.2 µg mL^{-1} RNaseA and mixed in a microplate shaker for 10 min. Cells were lysed with 100 µL of Solution II (0.2 *M* NaOH, 1% SDS); then 75 µL of Solution III (3 *M* K acetate-pH 4.8) and 25 µL of Procipitate reagent (LigoChem, Fairfield, NJ) were added to precipitate proteins and

chromosomal DNA. The supernatent was filtered through a 0.45-µm polyvinylidene fluoride (PVDF) membrane in 96-well format (Whatman Unifilter 350, Whatman, Clifton, NJ) and nucleic acids were precipitated with an equal volume of isopropanol. After centrifugation, the pellet was washed in 70% ethanol, air-dried, and resuspended in 10 µL of ultrapure distilled, deionized water. From 1 to 2 µL of each template preparation were sequenced using BigDye terminator cycle sequencing (Perkin-Elmer Applied Biosystems, Foster City, CA) using 8 pmol of M13-forward or M13-reverse primer in a 10-µL reaction. Standard cycle sequencing conditions were employed. Sequencing products were precipitated with an equal volume of isopropanol, washed with 70% ethanol, air-dried, and resuspended in 10 µL of ultrapure water. Electrophoretic separation of sequencing products was performed on an ABI PRISM 3700 DNA Analyzer 96-capillary automated sequencer (Perkin-Elmer Applied Biosystems).

Base calling was performed using Sequencing Analysis ver. 3.6 software (Perkin-Elmer Applied Biosystems). Assembly of double-stranded DNA sequence contigs from each clone, and identification of redundancy and overlaps between clones were performed using Sequencher 3.0 (Gene Codes, Ann Arbor, MI). Primers for the amplification of microsatellite loci were designed manually using the following criteria, if possible: (i) amplified product should be less than 200 bp in length for optimum resolution of polymorphic alleles; (ii) primers ideally should have a base composition of greater than 40% G+C; (iii) primers should not contain repetitive DNA. Primers selected by these criteria were evaluated further for melting temperature, internal structure, and propensity for primer-dimer formation using publicly accessible Worldwide Web resources (Sigma-Genosys, The Woodlands, TX).

Microsatellite Analysis

Genomic DNA was obtained from *G. hirsutum* L. cv. TM-1 and Tamcot SP37, and *G. barbadense* L. cv. Pima 3-79 and Pima S-7 by the method of Iqbal et al. (1997). Microsatellite loci were amplified by standard PCR methods (Bell and Ecker, 1994). A horizontal agarose gel electrophoresis system, yielding resolution of two base-pair polymorphisms, and a vertical acrylamide gel electrophoresis system, resolving single base-pair polymorphisms, were used to evaluate polymorphism of microsatellite amplification products. In the agarose system, samples were electophoresed on a 20-cm-long horizontal gel (Owl Separation Systems, Portsmouth, NH) containing 2% agarose plus 2% Metaphor agarose (Cambrex, North Brunswick, NJ) at 5.3 V cm^{-1} in 0.5X TBE buffer (45 m*M* Tris-Borate, 1 m*M* EDTA, pH 8) with buffer-chilling to 4°C. Gels were stained briefly with ethidium bromide prior to photodocumentation. In the acrylamide system, samples were electrophoresed (20 V cm^{-1} in a 10-cm-high by 33-cm-wide by 1-mm-thick vertical gel rig (CBS Scientific, Del Mar, CA) containing 6% polyacrylamide with 10% Vol./Vol. Spreadex NAB polymer (Elchrom Scientific, Cham, Switzerland) in 1X TAE buffer (45 m*M* Tris-Acetate, 1 m*M* EDTA, pH 8)), stained with ethidium bromide, then visualized. Additional experimental details on the agarose and acrylamide gel systems can be found at our cotton microsatellite resources Web site (http://plantbiol.tamu.edu/cottonSSRs/htm).

RESULTS

Several steps in the biotin capture protocol were modified to optimize the frequency of microsatellite repeats among captured genomic DNA fragments. Perhaps the most important single factor was the age of the streptavidin-coated paramagnetic beads. With beads only six months past the date of manufacture, stored at 4°C as per the manufacturer's recommendation (Promega), the yield of PCR product after the second amplification diminished greatly, and the final frequency of microsatellite-containing clones fell from close to 100% to between 20 and 30%. The relatively high quantity of T4 DNA ligase used in the adapter-ligation step (30 Weiss units) was also an important factor affecting the overall efficiency of the method.

A single capture reaction transformed into *E. coli* TOP10 cells (Invitrogen) yielded a total of $>1 \times 10^4$ colony-forming units, equivalent to $>2 \times 10^5$ colony forming units μg^{-1} of pCR4-TOPO vector. A random survey of primary colonies by PCR (using M13-forward and M13-reverse primers) indicated >98% of the transformants contained plasmids with detectable DNA inserts. The typical insert size was 500 ± 100 bp—a range well suited for complete sequencing using vector-specific

primers. A collection of 588 colonies, picked at random from primary transformation plates, was inoculated into 96-well culture plates for high-throughput sequencing. Sequence analysis showed that virtually all of the inserts contained microsatellite repeat motifs matching one or more of the biotinylated oligonucleotides used in the selection process—although some motif lengths were quite small [e.g., $(AGA)_2$ or $(GA)_3$]. Due to the judicious selection of the restriction enzymes used in the initial fragmentation (*Hae*III, *Rsa*I, and *Dra*I), none of the insert fragments were truncated within, or at the end of, a microsatellite repeat. In all cases, the unique sequences flanking both ends of the microsatellite repeat were obtained. This result is in contrast to that of the CM collection (Connell et al., 1998), in which genomic DNAs were mechanically fragmented using a high-pressure nebulizer, and a substantial portion of the clones were truncated within the microsatellite.

Of the 588 clones sequenced, 50 (8.5%) were redundant with other clones in the same collection. Internal redundancy was expected, since two PCR amplification steps were employed in the isolation process. Of the remaining 526 nonredundant clones, 309 were selected for further analysis on the basis of the following criteria: (i) the length of the microsatellite was five repeat units or greater; (ii) the unique 5′ and 3′ flanking sequences were both of suitable structure and composition for the design of efficient primers. Microsatellites with flanking sequences that were highly repetitive in nature and/or had a very high content of A+T nucleotides were eliminated.

These 309 new microsatellite loci (described in Table 1, appendix) were designated "JESPR" (after the names of the principle investigators, to reflect their collaborative development). Microsatellite loci containing a single repeat type made up 75% of the JESPR collection, including 98 (32%) with dinucleotide repeat types, 131 (42%) with trinucleotide repeat types, and 4 (1.3%) with hepta- and hexanucleotide repeat types. Of the microsatellites containing a single repeat type, 92% had "perfect" repeats, uninterrupted by nonrepeat nucleotides (Fig. 1). A further 25% of the collection was composed of "compound repeats" consisting of more than one repeat type at a single locus. Microsatellites with the AGA repeat type were the most abundant in our collection, followed by the GA type, with CA and ACA types making up the bulk of the remaining collection (Fig. 2).

Only two clones (both GA-repeats) were redundant with the more than 500 clones of the BNL microsatellites, and none were redundant with the 150 GA-repeat microsatellites of the CM collection. Thus, the overwhelming majority of sequences in our collection represented new microsatellite loci, and the total number of microsatellites present in the cotton genome appears to be quite large. Because two PCR amplification steps were employed in our biotin selection procedure, the level of internal redundancy within the JESPR collection is uninformative with regard to abundance of various microsatellite repeats in the genome.

To explore the potential utility of these new microsatellite loci in sequence-based genomic comparisons with the well-studied model plant *Arabidopsis thaliana* (L.) Hyenh, the JESPR sequences were subjected to basic local alignment search tool searches (Altschul et al., 1990) against *Arabidopsis* genomic sequence databases (http://www.Arabidopsis.org/blast/). A significant fraction of the loci (~13%) could be aligned with sequences in the *Arabidopsis* genome with basic-local-alignment-search-tool probability scores of $1 \times e^{-20}$ or less - a level at which we confidently

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JESPR219 (Perfect repeat):    GAGAGAGAGAGAGAGAGAGAGAGA
JESPR126 (Imperfect repeat): GAGAGAGAGAGAGAGAAAGAGAGAGA

JESPR210 (Simple repeat):     CTCTCTCTCTCTCTCTCTCTCTCTCTCT
JESPR211 (Compound repeat):   CTCTCTCTCTCTCTCTCTCTCACACACACA
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Fig. 1. Examples of categories of microsatellite repeat structures identified in this study. Underlined nucleotides indicate the distinguishing features of imperfect and compound repeats, respectively.

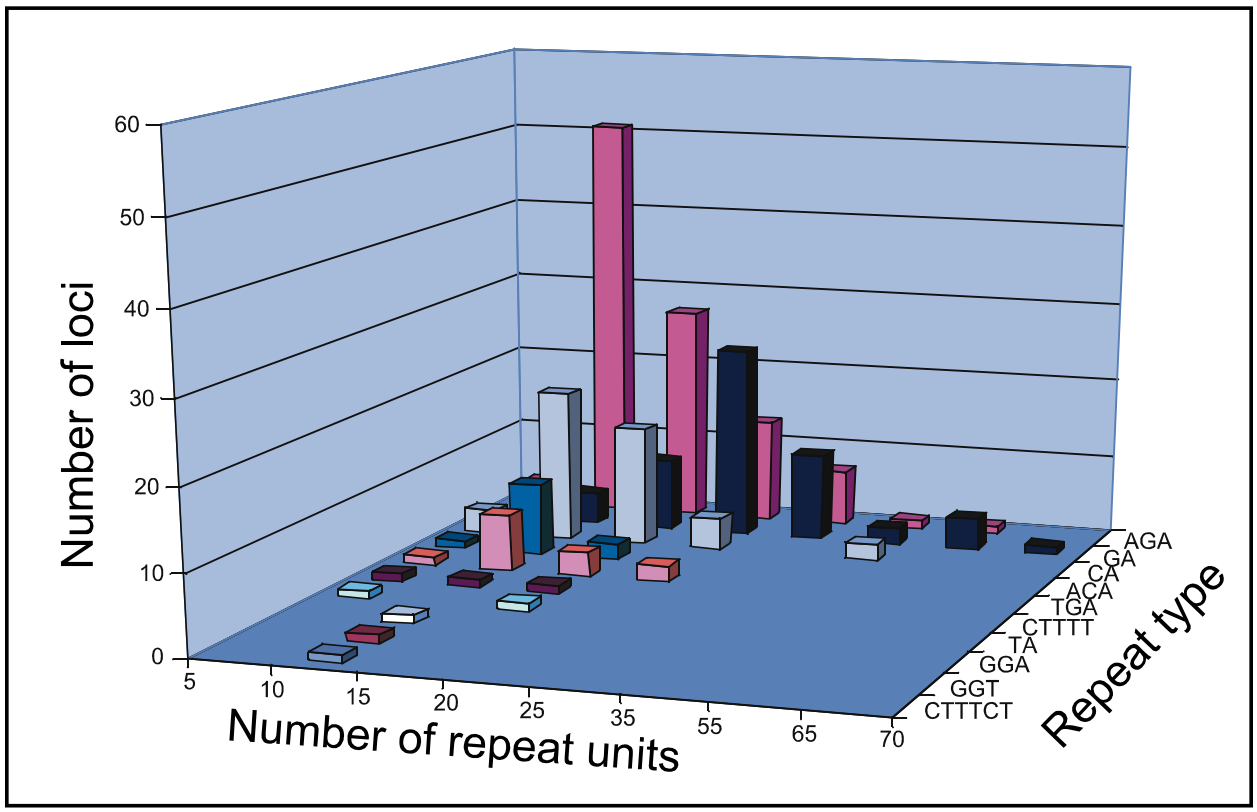

Fig. 2. Distribution of repeat lengths and types among 307 loci in the JESPR collection. Loci with repeat lengths of less than five units were not included in this collection.

could assign each *G. hirsutum* L. clone a single "most similar" sequence in the *Arabidopsis* genome (Fig. 3A). A representative sequence alignment between *Arabidopsis* and cotton, showing the location of the microsatellite repeat, is shown in Fig. 3B. On the basis of basic local alignment search tool results, the annotation of the *Arabidopsis* sequence database, and the presence of open reading frames, nearly all of the sequences with this level of similarity were predicted to be either protein-encoding genes or pseudogenes in both cotton and *Arabidopsis*. Sequences adjacent to the JESPR53 microsatellite showed high similarity to the trnI and 16S ribosomal rRNA-encoding genes from several higher plant chloroplast genomes. Similarly, sequences flanking the JESPR74 microsatellite showed high similarity to the chloroplast-encoded cytochrome-b 550 alpha subunit gene. None of these sequences were present in current publicly accessible cotton expressed sequence tag databases (http://www.cugisearch.clemson.edu).

Primers were designed for the 307 selected microsatellite loci using the criteria described in materials and methods. These primers (Table 1) had an average length of 20.4 nucleotides, with an average G+C content of 48% and melting temperature of 62°C. Predicted products ranged in size from 100 to 250 bp. An initial set of 152 primer-pairs was synthesized and tested in PCR using *G. hirsutum* L. cv. TM-1 genomic DNA as template. Of these, 123 (81%) yielded amplification products of approximately the expected size, on the basis of the sequence of the cloned microsatellite fragment. However, a minority of these amplified more than one locus, as we have observed previously in tetraploid cotton with several of the BNL and CM primers (Reddy and Pepper, unpublished data, 1999–2001). To determine the informativeness of the newly identified JESPR microsatellites in a *G. hirsutum* L. × *G. barbadense* L., interspecific mapping population amplification products from *G. hirsutum* L. cv. TM-1 and *G. barbadense* L. cv. Pima 3-79 were compared. In these analyses, 69 (45.4%) of 152 primer-pairs tested yielded polymorphic amplified products. Seventeen of the 152 primer-pairs tested (11.2%) showed

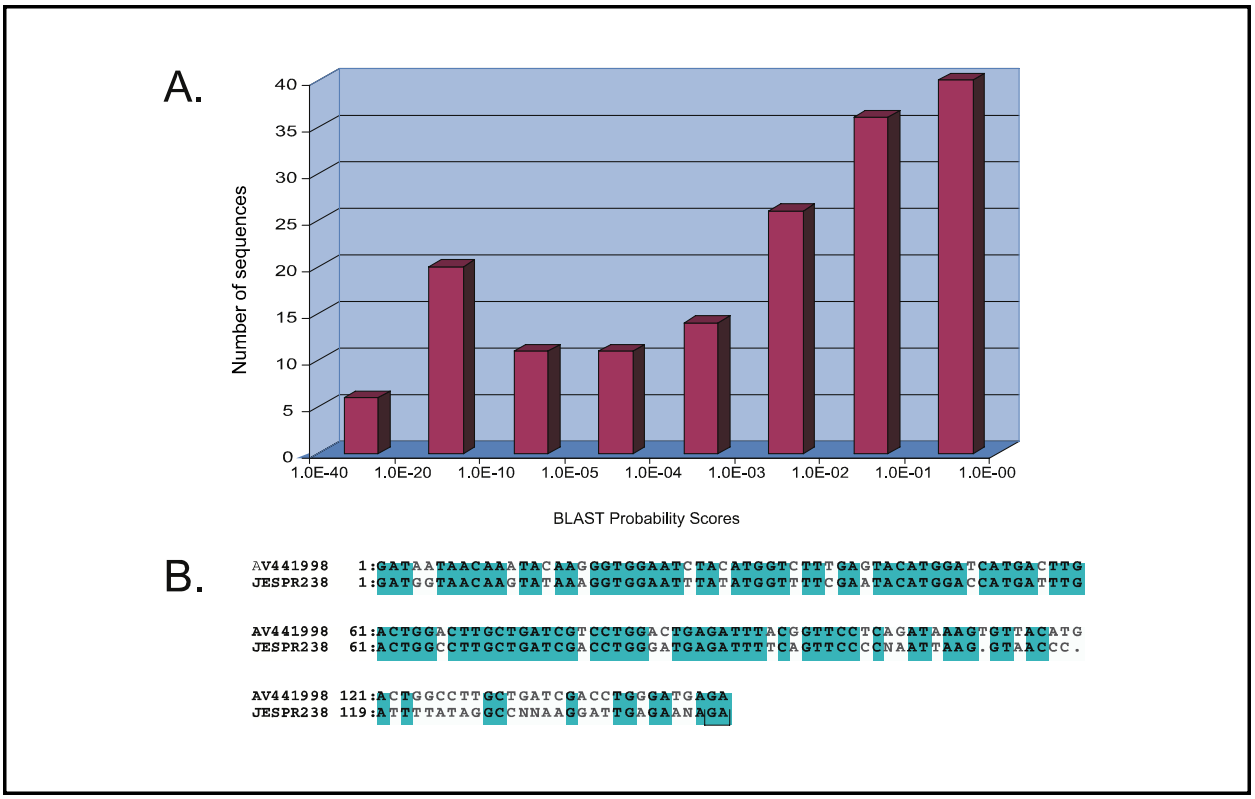

Fig. 3. Sequence-based comparison of unique sequences flanking JESPR microsatellite loci with *Arabidopsis thaliana*. (A) Distribution of basic local alignment search tool probability scores from 307 JESPR sequences searched against the *Arabidopsis* genome database. (B) An alignment of *Gossypium hirsutum* L. clone JESPR238 with *Arabidopsis* EST clone AV441998, a putative cCDC2-like kinase (p = 5.6 ¥ e-21, S = 370). The boxed GA dinucleotide corresponds to the location of the (GA)n microsatellite repeat in cotton.

intraspecific polymorphism between the *G. hirsutum* L. cv. TM^{-1} and Tamcot SP37. However, 25.7% of the primers amplified loci that were polymorphic between upland *G. hirsutum* L. cv. HS46 and MARCABUCAG8US-1-88, which are not closely related on the basis of pedigree records (Calhoun et al., 1997) and have been used to produce intraspecific genetic maps in upland cotton (Shappley et al., 1998). Three of the 152 primers (2%) amplified loci with intraspecific polymorphism between *G. barbadense* L. cv. Pima 3-79 and Pima S-7, which are very closely related (Calhoun et al., 1997).

In total, 74 primer sets (48.7%) showed polymorphism among the tetraploid cotton cultivars tested (Table 1). A minority of polymorphisms (21%) was dominant in nature, with the recessive allele not producing an amplification product (Table 1, Fig. 4). Dominant and co-dominant polymorphisms were widely distributed among the repeat types (AGA, GA, CA, etc.) as shown in Fig. 4. Approximately 34% of the compound repeat loci were polymorphic, while ~54% of the simple repeat loci were polymorphic. Furthermore, 38% of the imperfect repeats were polymorphic, while 54% of the perfect repeats were polymorphic.

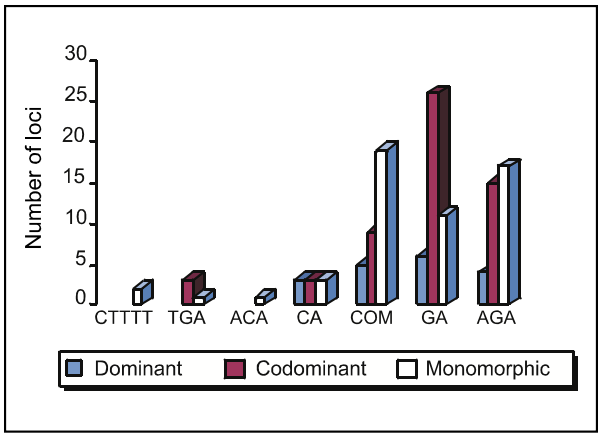

Fig. 4. Distribution of microsatellite polymorphism among categories and repeat types. COM indicates the values for compound repeats. Simple repeats identified by repeat type (GA, AGA, CA, etc.).

DISCUSSION

Microsatellite markers possess several important positive qualities for cotton genomic research. Among these is the broad spectrum of analytical detection methods that can be employed to detect polymorphisms. These methods include simple low-cost "low-technology" methods such as the Metaphor-agarose and Spreadex NAB-acrylamide gel systems described here, which utilize equipment and technical expertise that are ubiquitous in typical molecular biology laboratories. Such low-tech methods are particularly appropriate to researchers in developing countries where "hard currency" for major equipment and expensive reagents may be limiting, but labor costs are low. These methods can be performed at field stations and satellite experiment stations, without the need for major capital equipment or the logistical complications involved in shipping large numbers of samples for analysis. Consequently they are also appropriate for some marker-assisted selection projects involving analysis of only one or a few markers. However, the same microsatellite resources are also well-suited to more costly high-tech solutions, including detection of fluorescently labeled PCR products using automated and semiautomated DNA-sequencing instruments (Mitchell et al., 1997; Ponce et al., 1999), including high-throughput capillary electrophoresis devices (Baba, 1996). Future microsatellite detection methods probably will include matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (Taranenko et al., 1999) and microarrays (Cheung and Nelson, 1998).

An improved biotin selection method, along with the declining cost of DNA sequencing, has enabled us to identify more than 307 new microsatellite loci for cotton. The method was optimized using *A. thaliana* (where microsatellite composition and abundance are largely known from genome sequencing efforts) and recently has been used in our laboratory to isolate microsatellite-containing clones from species in the genera *Arabis* (Brassicaceae), *Caulanthus* (Brassicaceae), *Populus* (Salicaceae), *Silphium* (Asteraceae), and *Compsonurea* (Myisticaceae) (A. Pepper, T. Mitchell-Olds, L. Norwood, C. Loopstra, J. Manhart, and J. Janovec, unpublished data, 2001). In each case, the results were similar – nearly all clones contained at least a small repeat motif, and from 50 to 100% of the clones picked at random contained microsatellite repeats of a sufficient length (>5 repeat units) that they might be polymorphic in natural populations (Innan et al., 1997).

One of the key elements of our method was the use of a complex mixture of biotinylated oligonucleotides in order to cast a wide net for capturing several repeat types. Each of the repeat oligonucleotides used in our procedure captured several microsatellite-containing fragments (Fig. 2). The distribution of repeat types in our collection does not strictly represent their relative abundance in the cotton genome, but reflects the relative efficiency with which they were captured. For example, the lowest frequency of isolation was observed with the b$(TA)_{30}$ oligo, which identified only two repeat loci. This finding was probably due to the low melting temperature of the $(TA)_{30}$ repeat structure (T_{melt} = 51°C) relative to the 60°C hybridization temperature employed during the capture. Interestingly, a few $(CTTTCT)_n$ and $(CTTTT)_n$ repeat units also were captured in our isolation procedure. The $(CTTTCT)_n$ repeat was isolated in ~5% of the genomic DNA fragments captured from *Caulanthus amplexicaulis* (Brassicaceae) using a protocol that employed only the b$(CA)_{20}$, b$(GA)_{20}$, and b$(AGA)_{15}$ primers (L. Norwood and A. Pepper, unpublished data, 2001). We surmise that capture of this particular repeat motif probably was due to hybridization with the b$(GA)_{20}$ oligo. Similar mechanisms can be postulated for the capture of the GGA and GGT repeats, which also were not among our collection of biotinylated oligonucleotides. The mechanism of capture of $(CTTTT)_n$ repeats remains obscure.

A large percentage (~49%) of the microsatellite loci in the JESPR collection were polymorphic in tetraploid cottons. This value was highest in simple, perfect repeats (~54%), but other repeat structures also yielded informative polymorphisms at a significant frequency. Furthermore, ~46% of the microsatellite loci in the JESPR collection were informative for mapping in a *G. hirsutum* L. cv. TM-1 × *G. barbadense* L. cv. Pima 3-79 interspecific mapping population targeted by the International Cotton Genome Initiative. A wide distribution of product sizes (~100–250 bp) will facilitate the assignment to appropriate "bins" for multiplex analysis (Mitchell et al., 1997; Ponce et al., 1999). A substantial fraction of loci (~11–26%) were

polymorphic within upland cotton, and may be useful for intraspecific marker-assisted selection applications. Furthermore, in a set of 79 JESPR-primer-pairs tested on the diploid species *Gossypium raimondii* Ulbrich and *G. arboreum* L., all amplified a product of similar size to the expected product from tetraploid cotton, indicating that the primer binding sites may have been conserved in genomic DNA over several million years of evolution. These results imply that microsatellites will be useful—along with other DNA-based marker systems—in the comparative genetic mapping of tetraploid and diploid *Gossypium* species (Brubaker et al., 1999) and in tracking the introgression of agriculturally important traits from exotic diploid and tetraploid germplasm sources.

A significant proportion (>13%) of our microsatellite loci could be directly linked, via substantial sequence similarity, to sequences in the fully sequenced *Arabidopsis* genome. Both traditional taxonomic treatments and modern molecular phylogenetics (Soltis et al., 1999) indicate a close evolutionary proximity of *Gossypium* (Malvaceae) to *A. thaliana* (Brassicaceae). When these microsatellites are mapped in cotton, they will contribute to the detection and delineation of regions of chromosomal colinearity ("synteny") between *Arabidopsis* and the A and D genomes of tetraploid *Gossypium* species and, in so doing, potentially accelerate gene discovery efforts based on positional cloning and candidate gene approaches.

Given the low level of redundancy between the JESPR, CM, and BNL marker sets, and the comparatively large 1C value of *G. hirsutum* L. (~2×10^9 bp [Arumuganathan and Earle, 1991]), the number of microsatellite repeat structures present in the cotton genomes is probably vast. The development of a high-density molecular marker map for tetraploid cotton, similar to those used for genetic mapping in humans, mouse, and zebrafish, probably will require the identification of more than 1000 polymorphic loci. A map developed from such a marker set would have an average density of markers of less than 5 cM, and probably give coverage of all chromosome arms. In our present collection, we have more than 1×10^4 additional, uncharacterized clones. Although some of these clones will be redundant with those already in hand, further development of this resource probably will satisfy the needs of the cotton research community for a sizable collection of polymorphic microsatellite markers. If required, additional biotin selections could be performed at a lower hybridization temperature to identify $(T)_n$, $(AT)_n$, and other A+T-rich repeat loci that are also abundant in many plant species. As new markers in our collection are developed, this information will be disseminated rapidly via the Worldwide Web through CottonDB (http://algodon.tamu.edu/cottondb.html) and through Web resources under development at Mississippi State University and at Texas A & M University (http://plantbiol.tamu.edu/cottonSSRs/htm).

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Table 1. Summary of JESPR microsatellite loci.

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-1-F	M	CCCTCTTCCTCTCACCCACC	$(GAA)_{18}$	AF351243
JESPR-1-R		GCTTGTGGTTTCTAGACACACC		
JESPR-2-F		ATCACCGGCATCATCATCAT	$(GTT)_5$	AF351244
JESPR-2-R		GCTCATCAAATATCTCACAATTTATC		
JESPR-3-F		CCGCATTTAAGAACCCTAAC	$(GAA)_9$	AF351245
JESPR-3-R		GGATTGGACAACCATTCTTC		
JESPR-4-F		GACATGTGGCATAAATGACG	$(CTT)_6$	AF351246
JESPR-4-R		GGCAGAGACACTTTTAACTAGAG		
JESPR-5-F		GTCTCCTTCCCCTTCCTCTTCTTC	$(GTT)_8$	AF351247
JESPR-5-R		CAACAACCCATGACGACGAC		
JESPR-6-F	NA	CTAAACCCTAAACACAATATCTCC	$(CTT)_{15}$	AF351248
JESPR-6-R		CATTATAAGGTCCCCAATGTC		
JESPR-7-F	P	GCTGACGGAAGTGACAGGACCCT	$(GAA)_{14}$	AF351249
JESPR-7-R		GTCTCCTTCCCCTTCCTCTTCTTC		
JESPR-8-F		GGCATCTTACGGTGGAAATGAC	$(GAA)_{12}$	AF351250
JESPR-8-R		GGTTAGGTTTGGGGTGTTACATAC		
JESPR-9-F	NA	GTTGACGAAGAGGCAAAAGGGTC	$(GAA)_{13}$	AF351251
JESPR-9-R		GGCTTCTCTTGCTTAGATCTGGAC		
JESPR-10-F	P	GAGGCAATGTCGGATGTGGGC	$(GAA)_{20}$	AF351252
JESPR-10-R		GCAAGTAGGTGGTGGCCGGAG		
JESPR-11-F		CTGGACTAACCTAAACTTGACAC	$(GAA)_{10}$	AF351253
JESPR-11-R		CCTATGTACATATGCTCTTC		
JESPR-12-F		CCTAGACATCTGATTTAGCCAC	$(CTT)_7$	AF351254
JESPR-12-R		GAAGAAGAAGAATCCGACAG		
JESPR-13-F	M	GCTCTCAAATTGGCCTGTGT	$(CTT)_{19}$	AF351255
JESPR-13-R		GGTGGAGGCATTCCTGCTAAC		
JESPR-14-F	P	GGGAGGGGGTGAATAAACGGTG	$(CTT)_{17}$	AF351256
JESPR-14-R		GGTCAGGTAAACTTGCCATAGTGGG		
JESPR-15-F		CTTCTCTTGCTTAGATCTGGAC	$(GAA)_7$	AF351257
JESPR-15-R		GCGAGTACTAGTAATGACTGTC		
JESPR-16-F		GATGTGAGTATTTGGCACTTTGAC	$(AT)_4TGA(AT)_3$	AF351258
JESPR-16-R		GCTATCTATATCCGACTCAGCCCG		
JESPR-17-F		CATGTCGTAGTGGTAACTGC	$(GAT)_6$	AF351259
JESPR-17-R		GCCTTGTTACTTAACTAATAGTCC		
JESPR-18-F		CGGCTTCTCTTGCTTAGATCTGG	$(GTT)_{10}$	AF351260
JESPR-18-R		CCTCTAGATTGCCCTCCTTGTGC		
JESPR-19-F		GAATTCTAATGTTTATAAAAAAATA	$(GAA)_{20}$	AF351261
JESPR-19-R		CTATATAAAGTATCAGAGAAATTC		
JESPR-20-F		GGCTTCTCTTGCTTAGATCTGG	$(CTT)_9$	AF351262
JESPR-20-R		CGGTACATGGCTCGAGAGAG		
JESPR-21-F	P	GAGGGGGTGAATAAACGGTGAGG	$(GAA)_{15}$	AF351263
JESPR-21-R		CGGCTTCTCTTGCTTAGATCTGGAC		
JESPR-22-F		GGCTAATGGTGGTTGTGGATGC	$(GAA)_{12}$	AF351264
JESPR-22-R		CCCATGAAGATTTTTCCAGGGGAG		
JESPR-23-F		GACTATGGCTTAAGGTTCAG	$(CTT)_9$	AF351265
JESPR-23-R		CCCATTAATGTTAATGGCAAC		
JESPR-24-F		CGGCTTCTCTTGCTTAGATCTGGAC	$(CTT)_2(CTCTT)_2(CTT)_2$	AF351266
JESPR-24-R		GCTGACGGAAGTGACAGGACC		
JESPR-25-F	M	GCCCTATCCACGTTGCTGTCG	$(GAA)_{17}$	AF351267
JESPR-25-R		CCCTCTCGGTCAAAATCAACAC		
JESPR-26-F		GGTGACATCAGTGTTGTTC	$(CAA)_9$	AF351268
JESPR-26-R		GTCGTTCACAGCAGCCCTATCCACG		
JESPR-27-F	NA	GATCTGGACTAACATATATTCCG	$(CT)_8(CA)_8(CGCAGA)_4$	AF351269
JESPR-27-R		GGAACTTACCTGGAATTTAGTG		
JESPR-28-F		CAACATCAACCTCGTGAACCAAT	$(CAA)_6$	AF351270
JESPR-28-R		GTGAGGCCCCTTCTATTATTAGAC		

†Polymorphism observed in tetraploid cottons. P, polymorphic; M, monomorphic; NA, not amplified.
‡GenBank submissions were trimmed at the first ambiguous nucleotide (N), and may not contain both primer binding sites.

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-29-F		CACCGTTTCCAAGTAAGATT	$(CTT)_9$	AF351271
JESPR-29-R		GGTTAATCTTAGTTGAGGTC		
JESPR-30-F		CAAGCTAAGCCACTTGTATTACC	$(GAA)_5A(GAA)_3$	AF351272
JESPR-30-R		GAAGAGAATAAGAATAGCCC		
JESPR-31-F		CCAGTTAACTTGCCACGGTG	$(GGA)_5$	AF351273
JESPR-31R		CCCCACCAATATAGTTGTTTCAAAT		
JESPR-33-F	M	GGATTTGCTTCCTAGTTCCCTTC	$(GTT)_9$	AF351274
JESPR-33-R		GACAGATTGTAATCAATCCAACACG		
JESPR-34-F		TGGTACCGGGGATTCAGTGTGCCAC	$(CTT)_{11}$	AF351275
JESPR-34-R		ATGTGGCGCATCAGATCCGGTC		
JESPR-35-F		GGTTCAGTTTCCTTTCAGCTCT	$(CTT)_9$	AF351276
JESPR-35-R		TTGGGAGGAAGGAAGGAAGGAA		
JESPR-36-F	NA	GGACTAAACTTGCCACGTTGG	$(CTT)_{20}$	AF351277
JESPR-36-R		GATGTTGGGTTAAGAAGTGGAGTG		
JESPR-37-F		GATCTGGACTAACTGTAGGGTC	$(CTT)_{14}$	AF351278
JESPR-37-R		GTCAGCGTAATTGATTGCTTC		
JESPR-38-F		GAATCTTACAGTGGAGCAAGG	$(GAA)_{12}$	AF351279
JESPR-38-R		GGG AAA GGC TAA TGT GGT TG		
JESPR-39-F		GGTTCAGTTTCCTTTCAGCTCT	$(CTT)_9(CCTT)_3$	AF351280
JESPR-39-R		TTGGGAGGAAGGAAGGAAGGAAG		
JESPR-40-F		CTTCTCTTGCTTAGATCTGGACT	$(CTT)_9$	AF351281
JESPR-40-R		GTGTGCTTATTATATGTGAATGTAG		
JESPR-41-F		GACCATTGGGAATTGCTGATACG	$(CTT)_8$	AF351282
JESPR-41-R		GGGTAATCGACATAGGTAACGGG		
JESPR-42-F	M	CGTTGCCGTCTTCGACTCCTT	$(CTT)_{12}$	AF352073
JESPR-42-R		GTGGGTGGCTAATATGTAGTAGTCG		
JESPR-43-F	M	CGGCTTACAACAACAACAAC	$(CAA)_{15}$	AF351283
JESPR-43-R		GCTTCTCTTGCTTAGATCTGGAC		
JESPR-44-F		CTCTTGCTTAGATCTGGACTAAC	$(GTT)_9$	AF351284
JESPR-44-R		CCTGTACCAGTAGTAATAGTAGC		
JESPR-45-F		GAAACTCGATCCCTCAAGATATG	$(GTT)_6$	AF351285
JESPR-45-R		ATGAAATGAAAGAAAAGAAGGGAGG		
JESPR-46-F	M	GCTGTTGACTAACACATAAATAC	$(GAA)_9$	AF351286
JESPR-46-R		ATTGTAAATGTTACTGTATGATGCC		
JESPR-47-F	M	GGG GTG AGG GAT TGG ACA ACA	$(CTT)_{10}$	AF351287
JESPR-47-R		CCC CCT AAC TGG CCA GGT AG		
JESPR-49-F		GCT GGA GAA GGA AAA GGT GG	$(GA)_5$	AF351288
JESPR-49-R		CCTTGCTCTCACTGTACCTCTG		
JESPR-50-F		GTA GTC TTC TCA ACT CCA CTG TT	$(CAA)_5$	AF351289
JESPR-50-R		GGTGACATCAGTGTTGTTC		
JESPR-51-F	M	CTTGGTACCGGGGATTCAGTGTGCC	$(GAA)_{11}$	AF351290
JESPR-51-R		CATAACATCTAGGTCAGGTTTGGGG		
JESPR-52-F		GCCGTACAATCACAGATTGGGAC	$(GAA)_{12}$	AF351291
JESPR-52-R		GCGCTTCTCATTGAGTCATCCTG		
JESPR-53-F		GCCAATGGGACTATATACCGGTG	$(GAA)_9$	AF351292
JESPR-53-R		CCATGTCCCACGCCAGATTG	(Chloroplast)	
JESPR-54-F		CTCACCTGAATCGCCCCATCTATC	$(GAA)_6(Y)_9GAAGA(GAA)_4$	AF351293
JESPR-54-R		GCTTAATTTGGCTGGGTCTCCAC		
JESPR-55-F	P	GTTCGAGGAGGATTGAGGTAGAGGA	$(CT)_6A(CA)_{11}$	AF351294
JESPR-55-R		CCCCTTCTCTTGCTTAGATCTGGAC		
JESPR-56-F	P	CCAGTTAGCACCAATTTAGG	$(GAA)_{23}$	AF351295
JESPR-56-R		CCACAATAACACACTGGAATC		
JESPR-57-F		CGCCCTTCTCTTGCTTAGATCTGG	$(TA)_6$	AF351296
JESPR-57-R		CTCAAGAGCAAAAGGAACTTAACTCG		
JESPR-58-F	P	CCGCCCTTCTCTTGCTTAGATCTGG	$(CTT)_{10}$	AF351297
JESPR-58-R		GGAGCCAATTGAGAAGTGAATCCAA		
JESPR-59-F	M	CGCCCTTCTCTTGCTTAGATCTGGA	$(CTTTT)_6$	AF351298
JESPR-59-R		TAGTAGAAGGATTCGGCTATGGGGG		
JESPR-60-F	M	CGCCCTTCTCTTGCTTAGATCTGG	$(AT)_4(GT)_4GA(GT)_{14}$	AF351299
JESPR-60-R		TAGATCTGGACTACCTACGAGACCC		
JESPR-61-F		CGCCCTTCTCTTGCTTAGATCTGGA	$(CA)_{10}$	AF351300

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-61-R		CACATCCTCCTCCCTACTCCCTCC		
JESPR-62-F	P	GAATTGAGTGGAAAAGGGGGG	$(GA)_{14}(Y)_4(GT)_{11}$	AF351301
JESPR-62-R		CCTTCTCTTGCTTAGATCTGG		
JESPR-63-F		CATCTTGGGTATTTTTTGAGTG	$GAAA(GAA)_5AAA(GAA)_5$	AF351302
JESPR-63-R		GACTACCAAATGCACCATCTC		
JESPR-64-F	P	CGCCCTTCTCTTGCTTAGATCTGGA	$(CCCACA)_5$	AF351303
JESPR-64-R		GCAATTGAGGGGTGGGGTTGTCTG		
JESPR-65-F	P	CCACCCAATTTAAGAAGAAATTG	$(GAA)_{25}$	AF351304
JESPR-65-R		GGTTAGTTGTATTAGGGTCGTTG		
JESPR-66-F	P	CTGGACTAACTATTTGGTATCCCTC	$(GA)_{20}$	AF351305
JESPR-66-R		GATCTGGACTACCGCTAATCAC		
JESPR-67-F	P	GTAAAGAGCAACCTACACCTACCT	$(GAA)_{31}$	AF351306
JESPR-67-R		CCAAGATAGTTCATACTTCCCTC		
JESPR-68-F		GATATTTATTGTGTTTAACAGCAG	$GAAA(GAA)_3AAAGAA$	AF351307
JESPR-68-R		TACTCTTATCGATGTCCTTTTCA		
JESPR-69-F	M	CGCCCTTCTCTTGCTTAGATCTGG	$(CA)_{15}$	AF351308
JESPR-69-R		AAACTTTGCCGTTGATGGAGACCC		
JESPR-70-F	P	CTGGACTAAAAGGAAGATGAGAG	$(GA)_{17}$	AF351309
JESPR-70-R		GAATACAGGTTCAAAGTTGATA		
JESPR-71-F		CGCCCTTCTCTTGCTTAGATCTGG	$(GA)_{11}$	AF351310
JESPR-71-R		GCACCCTGCTCCAATCCTCTTTC		
JESPR-72-F	P	CGCCCTTCTCTTGCTTAGATCTGG	$(CTT)_{10}$	AF351311
JESPR-72-R		GGGCAAGCTGACGATGAGGAATG		
JESPR-73-F	P	CCACCGAAATCGATAGAGAGCAAT	$(GA)_5G(GA)_2$	AF351312
JESPR-73-R		CACTGTCCGACTAGGCCAATAC		
JESPR-74-F		CCCTTCTCTTGCTTAGATCTGGAC	$(CA)_{12}$	AF351313
JESPR-74-R		GCATTATGCTTGCTAGTTCCCTGC	(Chloroplast)	
JESPR-75-F	P	CTTCTCACGTTACCATTGATTCTTC	$(CT)_9$	AF351314
JESPR-75-R		GGCTGTTCACGGACTAGCTGTA		
JESPR-76-F	NA	CCCTTCTCTTGCTTAGATCTGGAC	$(GT)_5(Y)_7(GA)_{20}$	AF351315
JESPR-76-R		CAGTTGCTTCCAATGCAGCTACAG		
JESPR-77-F		TCTCTTGCTTAGATCTGGACT	$(GA)_{11}$	AF351316
JESPR-77-R		CTTATCCTTACTTTGTGGCG		
JESPR-78-F		GAAGTGCTCATAGTCCATCATAG	$(GA)_{14}$	AF351317
JESPR-78-R		GTCTGGCAGGACATAGAGAAG		
JESPR-79-F		GGGACAAATGTAATCTTGCATCCAG	$(GGT)_7$	AF351318
JESPR-79-R		CGCAGTGTCTGAATCGCCTTC		
JESPR-80-F		CTCTTGCTTAGATCTGGACT	$(CTT)_7$	AF351319
JESPR-80-R		ATGACTATGATTTAGCAGCG		
JESPR-81-F		GTGGAATGGTTGATAAGCATGTTG	$(AT)_5$	AF351320
JESPR-81-R		GGATATAACACCAGGCACAAATAC		
JESPR-82-F		GCAAAACATGGAATTTAAGTC	$(CTCTT)CTA(CTCTT)_3$	AF351321
JESPR-82-R		CTAGATATTAGTTCCCGAATCAC		
JESPR-83-F		CATAGGCAAGCCTTGTAGCAATC	$(CT)_6$	AF351322
JESPR-83-R		CCTCTTCTTTCACTACCACCTGC		
JESPR-84-F	P	GACTCCCGGAGGCAATCAGAG	$(CTT)_{20}$	AF351323
JESPR-84-R		CCAGGGCTCATACTATCGCTGC		
JESPR-85-F	P	CCACCCAAATTTTTCATGGAGAG	$(GA)_{14}$	AF351324
JESPR-85-R		CCTTCCTCATGTATGACATTGATGG		
JESPR-86-F	M	GGAGGAAGTTAGGAGCATGTCTCAG	$(GT)_2TGG(GT)_4$	AF351325
JESPR-86-R		ACAGGGTAGTCACGTAACAACTGC		
JESPR-87-F	P	GCCCTTCTCTTGCTTAGATCTGGA	$(GAA)_{22}$	AF351326
JESPR-87-R		CAAAACGGTCGTAGCTAGGGTATG		
JESPR-88-F		GTCAGCACAGTGAGGGTAAGAG	$(GA)_4GG(GA)_2(GAA)_2(GA)_2$	AF351327
JESPR-88-R		GAATACTCCCTCTTCCCTCG		
JESPR-89-F	P	CCCCAACCCACGAACATTCCA	$(GAA)_{10}(GAAAAA)_2(GAA)_3$	AF351328
JESPR-89-R		GGTGTTAACTGGATTGCTGACGTGG		
JESPR-90-F		CATGGAGTTTCAATGGCGAAGAATC	$(CTT)_7$	AF351329
JESPR-90-R		GGAACCGCTGATGTGGCTAGTTAAC		
JESPR-91-F		GGGGTGTTGAGTAGAATGGTAG	$(GAA)_8$	AF351330
JESPR-91-R		CGACATTTGCGATAAGTTGTG		

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-92-F	P	GGGACCTCTATTGAATAGCTGGAG	$(GAA)_{23}$	AF351331
JESPR-92-R		CTCTTGGCATCATTAGTTCCTGG		
JESPR-93-F		GCTTAGATCTGGACTACCCGTTG	$(GAA)_5$	AF351332
JESPR-93-R		GGTCGTGGTGGTGGTCTTGC		
JESPR-94-F		GCAAGACCACCACCACGACC	$(GAA)_5$	AF351333
JESPR-94-R		GTCTGAATCGCCCTTCTCTTGC		
JESPR-95-F		GCTTTTCTCGTAGACGTATG	$(CTT)_9$	AF351334
JESPR-95-R		GCATATTTATATACCAAGTCCCTC		
JESPR-96-F		CATCAGGTTCGAGATTGTCCCTCTG	$(GT)_5$	AF351335
JESPR-96-R		CGTCCTGCTGCACACTCTACTCTC		
JESPR-97-F	P	CTTCTCTTGCTTAGATCTGGAC	$(CT)_{11}$	AF351336
JESPR-97-R		GAAGAGGCTTTTCTTTATGATTC		
JESPR-98-F		CTTGATGAGGACCTATTTCCC	$(GA)_3G(GA)2A(GA)_3$	AF351337
JESPR-98-R		CACATCTCATCTTCACACTCTC		
JESPR-99-F		GCTATGCAGGCTCTGGGAAGGCTC	$(GT)_{10}$	AF351338
JESPR-99-R		CCCCTTCTCTTGCTTAGATCTGGAC		
JESPR-100-F		CACATGGTTGACCGTACCGCCTCG	$(CA)_8$	AF351339
JESPR-100-R		GCTAGGTCCTGGAGTGCTCGGTG		
JESPR-101-F	P	CCAAGTCAAGGTGAGTTATATG	$(TA)_3(GT)_{15}$	AF351340
JESPR-101-R		GCTCTTTGTTACTGAAATGGG		
JESPR-102-F	P	CTTGTGAAGTCCTTTAGGGC	$(CTT)_5TT(CTT)_5$	AF351341
JESPR-102-R		GTTATCCATCATGGTCAAATGC		
JESPR-103-F		CTATGAAACTCAAAGCCAAACTC	$(GAA)_7$	AF351342
JESPR-103-R		CCAAGATTCGTTCGATCGACC		
JESPR-104-F		GATGTTTAAGAATAACTATG	$(GA)_{10}$	AF351343
JESPR-104-R		GGAAATTTTGATACACATCCAC		
JESPR-105-F	M	GGAAGACCAACCAAGTCAAG	$(TA)_6(GT)_{19}$	AF351344
JESPR-105-R		GGATATGATATTCCAAAGCCC		
JESPR-106-F	M	CTAACAACTCTAACCTCTAACTG	$(CAA)_{10}$	AF351345
JESPR-106-R		GGACTAAAAGTTGTTATTTG		
JESPR-107-F		GACAATCCAGGCAGTCAGAG	$(CA)_{11}$	AF351346
JESPR-107-R		CATACTAATTAGCCATTCTCACCC		
JESPR-108-F		CGATAGTCTCTAGCCTCAAATTC	$(CTT)_3(CT)_3$	AF351347
JESPR-108-R		CGTAACACTAGTCGAACGAGC		
JESPR-109-F		GATTACAGTATGTTCTCTGAGG	$(GAA)_7$	AF352074
JESPR-109-R		CACACAATATCTCTCTTCTC		
JESPR-110-F	P	GGCGAAGAGCTACCTGTGAATGGC	$(GA)_{16}$	AF351348
JESPR-110-R		CCAATGGGGACTCTACATGTGGC		
JESPR-111-F		CTCTTGCTTAGATCTGGACTACC	$(GAA)_2GA(Y)_4GAAGAAA$	AF351349
JESPR-111-R		CTCCTTCCTCATCATCCTCTC		
JESPR-112-F	NA	CTTCTCTTGCTTAGATCTGGAC	$(CA)_9(TA)_5$	AF351350
JESPR-112-R		CAATGGCTCTCTAGCTTACTTG		
JESPR-113-F	M	CCCCCGAAGCCTTCAAGTAAGTTAC	$(GAA)_{11}$	AF351351
JESPR-113-R		CCCTTCTCCTTGCTTAGATCTGGAC		
JESPR-114-F	P	GATTTAAGGTCTTTGATCCG	$(GT)_{12}$	AF351352
JESPR-114-R		CAAGGGTTAGTAGGTGTGTATAC		
JESPR-115-F	M	GAAACATGATTGTATGGTAATG	$(TA)_5T(GT)_{10}$	AF351353
JESPR-115-R		CAGGAATCACTATTTTGGAC		
JESPR-116-F		GGTCACATTCAAACTAAATGTTCC	$(GAA)_2GAG(GAA)_2GAG$	AF351354
JESPR-116-R		CAAATAGCCTCTTTAGCAGTGG		
JESPR-117-F		CAAACATCTGGCTTTTTAACTC	$(GA)_2CA(GA)_2CAGA$	AF351355
JESPR-117-R		CTGTCTTCCAGTTTCAGAGC		
JESPR-118-F		CTTTTTCTCTTTTCAACACGTG	$(CT)_{12}$	AF351356
JESPR-118-R		GTTGAAAGGAAGACTCCAAAC		
JESPR-119-F		CTCAGGGAACTATTTGTAGTAGC	$(CA)_{10}$	AF351357
JESPR-119-R		GATCCACAAGAAACTGAAACTAG		
JESPR-120-F		GTAACCGAATACCCCTCAACTTAAG	$(CT)_8$	AF351358
JESPR-120-R		ACAGCCGCTTTGTCGAAGAT		
JESPR-121-F		CCTCAGATCAATTAACTATGATTC	$(CA)_{12}$	AF351359
JESPR-121-R		CGGTTGTAAAACTATACTATTTGTC		
JESPR-122-F		GCTGCTGGTTTTACTTGTTGG	$(CAT)_5$	AF351360
JESPR-122-R		CTATGGTGGAGGAGCAACAAC		

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-123-F		CTCTTGCTTAGATCTGGACTACC	$(GT)_9$	AF351361
JESPR-123-R		CTAAAACTACAGTCGAAAGGGG		
JESPR-124-F		GGATATCGCTCTCCCTCT	$(GT)_9$	AF351362
JESPR-124-R		CTCTTGCTTAGATCTGGACTAAC		
JESPR-125-F		CATTGTGATCAACCCCACCAAC	$(CA)_{11}$	AF351363
JESPR-125-R		GTTGGTGGGGATGGTCACATGC		
JESPR-126-F		GCATGTGACCATCCCCACCAAC	$(CA)_6$	AF351364
JESPR-126-R		GCCAGTGTGCTGAATCGCCCTTC		
JESPR-127-F	P	GATTTGGGTAACATTGGCTC	$(GA)_9AA(GA)_5$	AF351365
JESPR-127-R		CTGCAGTGTTGTGTTGGGTAGA		
JESPR-128-F		CTGAAAAATCTGCATTTCCG	$(GT)_{10}$	AF351366
JESPR-128-R		CTCCTAGATTTTGCTCTCCTGTC		
JESPR-129-F	M	CCTAACCTTTATCATCATGATC	$(CA)26(TA)_5$	AF351367
JESPR-129-R		CAGTATTGAAAAGCATTTGATC		
JESPR-130-F		GCCCAATTACAACATTTCCAAC	$(CA)_5$	AF351368
JESPR-130-R		GGTAGAGCCCACTTTTATGTCTC		
JESPR-131-F	M	GGCACTACCGGTTTGTCTTTC	$(CT)_3CC(CT)_7(CA)_4$	AF351369
JESPR-131-R		GAGGTGAATGGATATCATGAATG		
JESPR-132-F		GATCGAACAGATGGGTTAGTG	CTCTTCTTTCTTTTT	AF351370
JESPR-132-R		GCTTAGATTGGACTAACATCTTG		
JESPR-133-F		CAAGGATAAGGTTGAAGCTTC	$(GAA)_6$	AF351371
JESPR-133-R		CCTCATCACCTACGGCTCTAC		
JESPR-134-F		GTCAGAGTCTTCGGGTTGTC	$(CTTTT)(CTT)_6(CTTTT)$	AF351372
JESPR-134-R		GTAACAGCAGAGAAGTCGGTG		
JESPR-135-F	P	CAAAACCATCATCACTCTCAAG	$(CT)_{11}$	AF351373
JESPR-135-R		CGAGAGCCCACTAACAGAAAAG		
JESPR-136-F		GCAGGAAGCATTGGTATCTC	$(TAC)_6$	AF351374
JESPR-136-R		CTCTGATGTAAGAGTTGAATCAAG		
JESPR-137-F	M	CTTCTCTTGCTTAGATCTGGAC	$(CA)_9$	AF351375
JESPR-137-R		GCAAGTGGTGATGTAATAAGTTG		
JESPR-138-F		GATCAACTATCAGTCCAATTGG	$(GT)_{10}$	AF351376
JESPR-138-R		GTACTGCAATGAACATATATTCC		
JESPR-139-F	M	CCTTCTCTTGCTTAGATCTGG	$(CTT)_{10}$	AF351377
JESPR-139-R		CTTGCATGTCCTGAGAATACC		
JESPR-140-F		CTTCTCTTGCTTAGATCTGGAC	$(CA)_4T(CA)_3$	AF351378
JESPR-140-R		GGAGCAACTGTGTATGTGTG		
JESPR-141-F	M	CTCAAGCTCTTCCCCCTTC	$(CTTTT)_6$	AF351379
JESPR-141-R		ACTACCATGACAAGGAAGGTGG		
JESPR-142-F	M	CTCTTGCTTAGATCTGGACTAAC	$(CTT)_{10}$	AF351380
JESPR-142-R		GAGCAATAATGCCTTTCTTG		
JESPR-143-F		CCTTCTCTTGCTTAGATCTGG	$(GAAAA)_4$	AF351381
JESPR-143-R		GCCTCTGATAATGAAGATAACTG		
JESPR-144-F	NA	CTCTTATTTGTGTAACTACTGTTCC	$(GTGTAT)_{14}$	AF351382
JESPR-144-R		CATACACATACACATACACATACAC		
JESPR-145-F	M	CGCCCTTCTCTTGCTTAGATCTGG	$(CTT)_9$	AF351383
JESPR-145-R		GCGAGCAACAATCAATTTCACCTC		
JESPR-146-F		CGCCCTTCTCTTGCTTAGATCTGGA	$(CA)_7$	AF351384
JESPR-146-R		CGTTTCAGCCATCAGAATAGCTCC		
JESPR-147-F		GCTTAGATCTGGACTACCGAATCCT	$(GAA)_7$	AF351385
JESPR-147-R		CCAAATCAACATCCTCCATTACCC		
JESPR-148-F	P	GCTTCTCTTGCTTAGATCTGG	$(CTT)_{11}$	AF351386
JESPR-148-R		GTCGCTTTGTAAGTGAATGAG		
JESPR-149-F		GTTCTTAAGTGAGGATTGGACG	$CTTCT(CTT)_6CTTTTTCT$	AF351387
JESPR-149-R		CTCATTAAGACCCTAGGTAGGC		
JESPR-150-F	M	GCTTAGATCTGGACTAACATACG	$(GAA)_9$	AF351388
JESPR-150-R		GATAATTTCATGTAAAATCCCTG		
JESPR-151-F	P	CTGGACTAAAAACCTTAACTGG	$(GAA)_9(Y)4(GAA)_{10}$	AF351389
JESPR-151-R		CTCGATTCTAACTCAATCACG		
JESPR-152-F	P	GATGCACCAGATCCTTTTATTAG	$(GAA)_{50}$	AF351390
JESPR-152-R		GGTACATCGGAATCACAGTG		
JESPR-153-F	P	GATTACCTTCATAGGCCACTG	$(CTA)_{18}$	AF351391
JESPR-153-R		GAAAACATGAGCATCCTGTG		

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-154-F		GTTCCCTCAGTTGCTCAGAAG	$(CTT)_9$	AF351392
JESPR-154-R		GGAGGAGTTGGCAGAAAATAGC		
JESPR-155-F	M	GCTTAGATCTGGACTAAAATAGCC	$(GAA)_{23}$	AF351393
JESPR-155-R		GATTTACAGAGGAGGGAACATG		
JESPR-156-F		GCCTTCAATCAATTCATACG	$(CTT)_6CCTT$	AF351394
JESPR-156-R		GAAGGAGAAAGCAACGAATTAG		
JESPR-157-F	P	CAAGTTCCCACCATCTTTAC	$(CTT)_9(Y)_{10}(CTT)_4(Y)_3(CTT)_4$	AF351395
JESPR-157-R		CTTCTTTGACTGAAATTGCTC		
JESPR-158-F		CACCATTCGGCAGCTATTTC	$(CTT)_{12}$	AF351396
JESPR-158-R		CTGCAAACCCTAGCCTAGACG		
JESPR-159-F		CAGCTGACTGCATTGGTTCAATCTC	$(CTT)_9$	AF351397
JESPR-159-R		AACGAGTTGAAGAGAGTGAGGATCC		
JESPR-160-F	P	CTTGCTTAGATCTGGACTAACC	$(GAA)_4(Y)_{27}(GAA)_4$	AF351398
JESPR-160-R		CACCGAGACATTCATATCAC		
JESPR-161-F		CGGAAGGGCTGCTGATGGAG	$(GAA)_5$	AF351399
JESPR-161-R		CTACCCCCATTTTTTGGATTCACC		
JESPR-162-F		CGGCTTCTCTTGCTTAGATCTGG	$(CTT)_9$	AF351400
JESPR-162-R		CATGTTGATCGTCAATCTGGGG		
JESPR-163-F		CTCCAGTTCACTCCAAATTATC	$(CTT)_6$	AF351401
JESPR-163-R		GGCACTACTACTGAGAAACAAG		
JESPR-164-F	M	GCGCCTATTAGCCATGAACTCAAGG	$(CTT)_8(CTTTT)_5$	AF351402
JESPR-164-R		GACGTTGGCTCGAGTTGTTAAAGG		
JESPR-165-F		CAAAACTCACCATGGGGAAAC	$(CTT)_{10}$	AF351403
JESPR-165-R		GAATCAATGGCAGAAGTGTTGAAG		
JESPR-166-F	M	GGCTTCTCTTGCTTAGATCTG	$(CTT)_3CTC(CTTTTT)_4$	AF351404
JESPR-166-R		CAAGCTTGAGTTTCGGGAAC		
JESPR-167-F		CTCCCCTCTTCTCTTGTTGTC	$(CTCTT)_5(CT)_2G(CTCTT)_2$	AF351405
JESPR-167-R		GTCAACAACACTTGAAGCAC		
JESPR-168-F		GGCTTCTCTTGCTTAGATCTG	$(TAG)2(Y)_3(TAG)_5(Y)_3(TAG)_5$	AF351406
JESPR-168-R		GTGCTAATAGAGACCAGCTG		
JESPR-169-F	P	CTCAGATCTAATGATTGGGTTGG	$(GA)_5(CTT)_{10}$	AF351407
JESPR-169-R		GAGTAAATTGACCACTTGTTCGC		
JESPR-170-F		CCCATATTTCAACGTTGACAC	$(CTT)_{10}$	AF351408
JESPR-170-R		CTTATCCTCCAGGTTTCACC		
JESPR-171-F	P	CTGCAAGGTGGAAACTGAACCTG	$(CTT)_{15}$	AF351409
JESPR-171-R		CCATGCATGTATTAAATTGTGAG		
JESPR-172-F		CAATCTGAAAAACCATAAGACC	$(GAA)_5$	AF351410
JESPR-172-R		ACCATTGAAACACCATGTC		
JESPR-173-F		GACCATTTTAGTCCCTTCATC	$(CTT)_6$	AF351411
JESPR-173-R		GGAGAAACAAATAGATGTCGAAG		
JESPR-174-F	M	CAACAGGTTCCTACAGGTCTG	$(CTT)_9$	AF351412
JESPR-174-R		GATTGCTGAAATCACAGAGG		
JESPR-175-F		CCCCTATTGGCTGCTGAAAG	$(CTT)_{12}$	AF351413
JESPR-175-R		GTTTCTTTTTTTTTCCCCTGTA		
JESPR-176-F	P	CGGCTTCTCTTGCTTAGATCTGGAC	$(GAA)_{15}$	AF351414
JESPR-176-R		GGGCATGATAAATGACAATCCTCC		
JESPR-177-F		GCTTAATCTGGACTAACATATGC	$(CTT)_9$	AF351415
JESPR-177-R		CGGTACATACAGCAAAATGC		
JESPR-178-F	M	CCGCTGATGTGGCCAGTTAACTTGCC	$(GAA)_{21}$	AF351416
JESPR-178-R		GATGCTTGTCCAACATGGCTTTC		
JESPR-179-F		CTGACACTGTATGCTTGCAG	$(CTT)_{12}$	AF351417
JESPR-179-R		CATATTTGGCATATCACATAGAG		
JESPR-180-F	P	GCGTAGTACATATAGATGCCC	$(GA)_5(GA)_{20}$	AF351418
JESPR-180-R		CTTGGAGTATGTATGCTCTATTC		
JESPR-181-F		CAACTTTTAGATTTGGAAATGG	$(GAA)_8$	AF351419
JESPR-181-R		GAGTTGAAGCTTGACCTGTC		
JESPR-182-F	M	CTTAGATCTGGACTAGGAGCC	$(CTT)_6CTC(CTT)_2$	AF351420
JESPR-182-R		GGAAGTGGATTGATAATGAGG		
JESPR-183-F		CATTTGTTTCACTTCAGGTCC	$(CTT)_6$	AF351421
JESPR-183-R		CATGCTACCATTGCTTCTCATC		
JESPR-184-F	M	CTTAGATCTGGACTAAACTCTTGC	$(CTT)_{16}$	AF351422
JESPR-184-R		GTTGATGGGGATAGTTCAAGTG		

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
ESPR-185-F	P	CCCAAGCTACAGAGATAACC	$(GAA)_{13}$	AF351423
JESPR-185-R		CACACAAATTGGGTAAGAATAG		
JESPR-186-F		CCGTGTTGTGAGTGGTACAGGTC	$(GAA)_{13}$	AF351424
JESPR-186-R		AGGTTAGGTTTGGGGTGTTACATAC		
JESPR-187-F		CAGGTCATGAGGAGCAGAAG	$(CTT)_{11}$	AF351425
JESPR-187-R		GTGCTTAATTTGCAAAAAGGACC		
JESPR-188-F		GGCTTCTCTTGCTTAGATCTG	$(CTT)_{9}$	AF351426
JESPR-188-R		GGTTTATTTGATTGCTAAGTCC		
JESPR-189-F		CCATAGACTTGGTTCATGACC	$(CTT)_{8}$	AF351427
JESPR-189-R		GTTCCAGAGTCGTACAGTCG		
JESPR-190-F		GCCCGCCATCTTTGAGGATCCG	$(CTT)_{9}$	AF351428
JESPR-190-R		GGCAAAACTTGACAATTTTCTCGGC		
JESPR-191-F		GGTCTAGCCTTTCGGAATTTG	$(GAA)_{10}$	AF351429
JESPR-191-R		GAATCATTCTCGTTCTCGGAC		
JESPR-192-F		GGAACCTCTACTGAATAGTCGGAG	$(GAA)_{9}$	AF351430
JESPR-192-R		CAGGGATTTCAGCTGACTGC		
JESPR-193-F		GATCTGGACTAACTATCTTCTTG	$(CTTTCT)_{3}$	AF351431
JESPR-193-R		GTGGTATAAGTTAGTACTTGATGG		
JESPR-194-F		GAGTTTATTGAGAAAGGCTTTCC	$(GAA)_{11}$	AF351432
JESPR-194-R		CTCAAAGTGGCTGTGCTTTG		
JESPR-195-F	P	GATCTGGACTAAACTAGTTGATGTG	$(GAA)_{20}$	AF351433
JESPR-195-R		GCCAATAATGGATGAAGGTTAC		
JESPR-196-F		CCCTAACACCTCTCAGTTTCACAGC	$(CTT)_{6}$	AF351434
JESPR-196-R		GGGGGCTGCTGATGGAGTTAAGACC		
JESPR-197-F	P	CAATACCTGGAACATAGACAAATG	$(TAC)_{11}$	AF351435
JESPR-197-R		CTTGAGGCTTGCAAAAAATG		
JESPR-198-F		GCTTCTCTTGCTTAGATCTGG	$(GAA)_{12}$	AF351436
JESPR-198-R		GTTTGAATGGCTTCACAAAG		
JESPR-199-F		GGCAAAGTCCAAAGGCGGTGG	$(GAA)_{8}$	AF351437
JESPR-199-R		CCATCAAAACAGGTGATTGTATTTGG		
JESPR-200-F		CTTCTCTTGCTTAGATCTGGAC	$(CTT)_{13}$	AF351438
JESPR-200-R		GTGATGTGACCAGTTAACTAGC		
JESPR-201-F		TCGATCAGTTAGGGTTTTGG	$(GAA)_{6}$	AF351439
JESPR-201-R		CGAATCTCAACCAGATTTCC		
JESPR-202-F		CACCCGGGAAAAGCTAATGTGGTTG	$(GAAA)_{4}(GAA)_{10}$	AF351440
JESPR-202-R		GCCATGAACTCAAGGTACCCATTG		
JESPR-203-F	M	CTCTTGCTTAGATCTGGACTAAC	$(GAA)_{11}$	AF351441
JESPR-203-R		CTGCTGAAAGAAATTGTTACCCC		
JESPR-204-F	P	CTCCAGGTTCAATGGTCTG	$(CTT_{20}$	AF351442
JESPR-204-R		GCCATGTTGGACAAGTAGTC		
JESPR-205-F	P	CCCAACTCTTTCCAAACTTGAG	$(CTT)_{9}(CT)_{9}$	AF351443
JESPR-205-R		GTACATATAGATGCCCTCGTG		
JESPR-206-F	P	CACAGTCTCCCAGAAGCTCCC	$(GA)_{52}$	AF351444
JESPR-206-R		TAGTTGCGTGTGTCTCCTCTTCTC		
JESPR-207-F	M	CAGCAAAGGAACAAGAAACCAGA	$(GA)_{35}$	AF351445
JESPR-207-R		GTTAATGCACTAAGACTTGGAAG		
JESPR-208-F	P	CGCAACCAAACATATACTTCACAC	$(CT)_{15}$	AF351446
JESPR-208-R		CCCTTTCCATCCATAGAACG		
JESPR-209-F	M	ATTGAGAGGCATTTTGGTC	$(GA)_{41}$	AF351447
JESPR-209-R		AGATGACTAAAAATTGTGCC		
JESPR-210-F	P	GCATGTTCTACAATGGTAAGCATA	$(CT)_{26}$	AF351448
JESPR-210-R		GAATTCTGCTTCTCTTGCTTAGAT		
JESPR-211-F		CATCATTTTTCCAAGTTCCAATTTC	$(CT)_{25}$	AF351449
JESPR-211-R		CAAACCGTTAAGGCTCCAGC		
JESPR-212-F	M	CCAAAGGTTTTTGTTGTTGCTC	$(CT)_{21}(CA)_{7}$	AF351450
JESPR-212-R		CAGATTCTGCTTCTCTTGCTTAG		
JESPR-213-F	NA	TATGGAAACCCTAGGAGAG	$(GA)_{17}$	AF351451
JESPR-213-R		GACAAGAGAACTTACCCAATTAAGC		
JESPR-214-F	P	GTAACATTGACGCGATTTATCC	$(GA)_{62}$	AF351452
JESPR-214-R		CCCTCGACGGATACATATGG		
JESPR-215-F	P	CGAGAAGATGAGATTGGAGGAG	$(GA)_{22}$	AF351453
JESPR-215-R		CCCTTCTGAGTTTTCTTTGG		

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-216-F	M	CAAGAGAACTTACCCAATTAAGCC	$(CT)_{16}$	AF351454
JESPR-216-R		GGAAACCCTAGGAGAGAGAG		
JESPR-217-F	M	GCTCTTGCTTAGATCTGGAC	$(CT)_{18}$	AF351455
JESPR-217-R		GGTGATTCATCCCATGAAATG		
JESPR-218-F	P	GGGGCTAAACTTGAAAAATGACC	$(GA)_{21}$	AF351456
JESPR-218-R		CATGCAGCTTCCAGTTTTG		
JESPR-219-F	M	GCATAGTTATGAATGACTCTCTCT	$(CT)_{19}$	AF351457
JESPR-219-R		GGGGAGTTGAAAAGAAGTATC		
JESPR-220-F	P	CGAGGAAGAAATGAGGTTGG	$(GA)_{20}$	AF351458
JESPR-220-R		CTAAGAACCAACATGTGAGACC		
JESPR-221-F	P	CTTAGGTGCTTCAGGCATGATTC	$(GA)_{18}$	AF351459
JESPR-221-R		CCCAACCCCTTCCTTC		
JESPR-222-F		GGGCCAACATCTTGC	$(CT)_{10}$	AF351460
JESPR-222-R		GGGGGACATTAATGATTGG		
JESPR-223-F	P	TGGTCCAAAGCTCAAAG	$(CT)_{18}$	AF351461
JESPR-223-R		CGTTACGGATTATTGGACATG		
JESPR-224-F	P	GGGGAGCAACGAAAACTTAGC	$(GA)_{22}$	AF351462
JESPR-224-R		CCACCATTCTCTTTCATTTTCTCC		
JESPR-225-F	M	GTTATGGCGAGGAATATAAC	$(GA)_{44}$	AF351463
JESPR-225-R		ACTCAAGTGTCCCATCTC		
JESPR-226-F	P	GAGGCATGAATATTCAG	$(GA)_{24}$	AF351464
JESPR-226-R		GAGACATCAAAGTTTGCA		
JESPR-227-F	P	CGAGAAGATGAGATTGGAGGAG	$(GA)_{19}$	AF351465
JESPR-227-R		GGTTTTCCATTCTCTTTCATTTTC		
JESPR-228-F	P	CAGAACAACACCATCAACACTCTCAG	$(GA)_{21}$	AF351466
JESPR-228-R		GGCAAGCAAAGCAAAACTC		
JESPR-229-F	P	CCATTCTCTTTCATTTTCTCC	$(CT)_{22}$	AF351467
JESPR-229-R		GTTGAAACGAGAAGATGAG		
JESPR-230-F	P	GGGACTAAAGAAGTAATTATGCC	$(GA)_{38}$	AF351468
JESPR-230-R		GAAACCCTTGGCCATGAG		
JESPR-231-F	P	GCTGGTGGGATTCTCTG	$(GA)_{22}$	AF351469
JESPR-231-R		CTATGAACTGCTGGCTATGG		
JESPR-232-F	P	CAGACCACGCTATTTTTGCC	$(CT)_{18}$	AF351470
JESPR-232-R		CGTTGTATTATTTCCAGTGCTCG		
JESPR-233-F	M	GAGACATCAAAGTTTGCAGC	$(CT)_{18}$	AF351471
JESPR-233-R		CCCAAACTATTGAACCAAC		
JESPR-234-F	P	GCATAGTTATGAATGACTCTC	$(CT)_{18}$	AF351472
JESPR-234-R		CTAACTCGAATCCGTCAC		
JESPR-235-F	P	GAGCAAGGATGAGGAACGAG	$(GA)_{46}$	AF351473
JESPR-235-R		CAAATTACTCAAGTGTCCCATCTC		
JESPR-236-F	P	GACTGACATGCAGCTTCCAG	$(CT)_{22}$	AF351474
JESPR-236-R		GGGGCTAAACTTGAAAAATGAC		
JESPR-237-F	P	GGCATCTCCATGTAGAAATAG	$(GA)_{17}$	AF351475
JESPR-237-R		TGTCAGTCCCCCATCACC		
JESPR-238-F		CAGAGAGCTTAGTTAACCC	$(GA)_{20}$	AF351476
JESPR-238-R		GTTGATCCTTATTTTTCCCC		
JESPR-239-F	M	CGACCTGGGATGAGATTTTC	$(GA)_{18}$	AF351477
JESPR-239-R		CAATAGTGAAGCCCAGTAAG		
JESPR-240-F	P	CAGATCCCCTTTTCTTTC	$(CT)_{16}$	AF351478
JESPR-240-R		GAAGAAGCAAAGCGAGAG		
JESPR-241-F		CTTACCCAATTGACCTATG	$(GA)_{16}$	AF351479
JESPR-241-R		GATTCTTCTTATCATCCCC		
JESPR-242-F	P	CAATGCGATTTTCAAACCC	$(CT)_{17}$	AF351480
JESPR-242-R		GCCAGTGTGATGGATATCTGC		
JESPR-243-F	P	GTGTGTTCTTAGGTGCTTCAG	$(GA)_{16}$	AF351481
JESPR-243-R		CCAAACCAAAATAATAGACATCC		
JESPR-244-F		GAAGATCTTCATCATTTTTCCAAG	$(CT)_{18}$	AF351482
JESPR-244-R		CAGAGAGCTTAGTTAACCCA		
JESPR-245-F		GAGACACCAAAGTTTGCAGC	$(CT)_{18}$	AF351483
JESPR-245-R		GTTTGGAGGCTGAAGGATGTC		
JESPR-246-F		GGAGCTTTACGGAGAGAGTTG	$(CT)_{13}$	AF351484
JESPR-246-R		GAGCTCCACTCCAAAGCC		

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-247-F	M	GCTTCTTCCATTTTATTCAAG	$(CT)_{15}$	AF351485
JESPR-247-R		CAGCGGCAACCAAAAAG		
JESPR-248-F		TCTCTCCCTTTCAAATCTC	$(CCT)_{8}$	AF351486
JESPR-248-R		GTGAATGAAAGGTGTGGTG		
JESPR-249-F	P	CCATTACTCTCCTCAAGTATG	$(GT)_{15}$	AF351487
JESPR-249-R		TCGTAGTCAATGTGGTAC		
JESPR-250-F		CCAAGAAATCCACCTCATAAG	$(GT)_{10}$	AF351488
JESPR-250-R		GAGTGCAAGGCTATGCTATTACC		
JESPR-251-F	P	CAACTAGAATGATAAGACAC	$(CA)_{15}$	AF351489
JESPR-251-R		CTTTAAGTACGTATGCATC		
JESPR-252-F	P	GCTATTGTTGATCTGATCCTG	$(GT)_{17}$	AF351490
JESPR-252-R		GATTGATCAATCCTGTAACTC		
JESPR-253-F		CAAACCACGTCTTCTCT	$(GT)_{11}$	AF351491
JESPR-253-R		CTTAACATCGTCGAATTTTC		
JESPR-254-F	P	GTATTGGTTTAATAAAAAGT	$(TA)_{5}(GT)_{10}$	AF351492
JESPR-254-R		GGTCCAGTTTGTTTACAGAG		
JESPR-255-F		GTATTGGTTTAATAAAAGGT	$(GT)_{8}$	AF351493
JESPR-255-R		GAACTGGTTAAACGAATG		
JESPR-256-F	M	GTCAATGAATGCAGAAC	$(CA)_{12}(TA)_{4}$	AF351494
JESPR-256-R		GTATATACAAGTATAAAGTATTGG		
JESPR-257-F		CAAATGATAATATAAAAGACTG	$(GT)_{9}$	AF351495
JESPR-257-R		GCATATACTCAATGGTATCAC		
JESPR-258-F	M	CAAAGTTGGGATTAGAGAC	$(CA)17(TA)_{5}$	AF351496
JESPR-258-R		GTATATACAAGTATAAAGTATTGG		
JESPR-259-F		CCCTTAAATCATAAGAAAACAC	$(CA)_{11}$	AF351497
JESPR-259-R		GAAGGAGGATCAACTATC		
JESPR-260-F	P	CTAGACTCATATGCCCATCTAC	$(CA)_{12}(TA)_{4}$	AF351498
JESPR-260-R		CAATAAATGCAAGAAGACAG		
JESPR-261-F	P	GGTCATCCTAGGTTCTC	$(CT)_{10}(CA)_{11}$	AF351499
JESPR-261-R		CAATAAATGCAAGAAGACAG		
JESPR-262-F		ACCATCTGTCTTTGGTTTTC	$(CA)_{13}$	AF351500
JESPR-262-R		CGTTTCTTTGCATCATC		
JESPR-263-F		CCTTTTTATCTCATGGAAACAC	$(CA)_{14}$	AF351501
JESPR-263-R		GGCGCGGTACTATGAAC		
JESPR-264-F		GCTTATTCCATTTTTCAAC	$(CA)_{12}$	AF351502
JESPR-264-R		CAACTGAAAGAGGATCAAC		
JESPR-265-F	M	GATCAACTACAACGCAC	$(CA)_{11}(TA)_{6}$	AF351503
JESPR-265-R		AATTCAGTGGTCAAAACA		
JESPR-266-F		GGTGACTCTAGCTCCG	$(CA)_{9}$	AF351504
JESPR-266-R		CCAGCAGTTTTGGTCTC		
JESPR-267-F	M	CCCTCTAACTTTCCCCA	$(CA)_{18}$	AF351505
JESPR-267-R		GAAAACCCAGCATTGC		
JESPR-268-F		GGTTGGAAAGGAAGGAC	$(CA)_{12}$	AF351506
JESPR-268-R		CATGTGTTGCCATGATATA		
JESPR-269-F		GCATCGGGATGGTGTG	$(GT)_{8}$	AF351507
JESPR-269-R		GATCAGCCACCCAAAATTAG		
JESPR-270-F	P	ACGCAACTCGCATATAAACAC	$(CA)_{15}(TA)_{3}$	AF351508
JESPR-270-R		GTAGCTTAGAATTTGAATGGC		
JESPR-271-F	M	GGAGCTGATGTCTTG	$(CA)_{12}(TA)_{5}$	AF351509
JESPR-271-R		GTATTGGTTTAATAAAAGG		
JESPR-272-F		GGGGCACAACAGAAGTCAG	$(CA)_{10}$	AF351510
JESPR-272-R		GGTCTCAACTGAAAGAGGATC		
JESPR-273-F	P	GGTGTGAGTTATCGCCAAAGG	$(TA)_{3}(CA)_{14}$	AF351511
JESPR-273-R		CTCGCATATAAACGCAACTCG		
JESPR-274-F	P	GCCCACTCTTTCTTCAACAC	$(CA)_{13}$	AF351512
JESPR-274-R		TGATGTCATGTGCCTTGC		
JESPR-275-F	M	CATTGTCTTATCCTCAAATACCGAATTC	$(CA)_{18}(TA)_{6}$	AF351513
JESPR-275-R		CTCATAGTGAGTTGATTTCAAAGTG		
JESPR-276-F	M	CACGAAAGACTGTACAC	$(CA)_{14}(TA)_{4}$	AF351514
JESPR-276-R		ATTGGTTTAATAAAAGGT		
JESPR-277-F	P	CGTAATGGGAAAAGGTCAGTG	$(GT)_{11}(TA)_{4}$	AF351515
JESPR-277-R		CGATAGAAGTCCTGATTTTTGG		

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-278-F	M	ACCCTTAAATCATAAGAGAAC	$(CA)_{10}(GA)_3$	AF351516
JESPR-278-R		CCGTAAGTTAAGGTACAAGG		
JESPR-279-F	M	GGAGTGAAAGCTAATGCCTG	$(CA)_{28}$	AF351517
JESPR-279-R		CGGGTCATTGGTTGTTTTTG		
JESPR-280-F		GGAGTACAAGGACCAGCAG	$(GT)_{10}$	AF351518
JESPR-280-R		GGATTAACTAATTAGTTTCCGC		
JESPR-281-F	M	TGATTGATCCTAGTTCTACG	$(TA)_4(GT)_{12}$	AF351519
JESPR-281-R		GTCTCCTTACTTCGCAAC		
JESPR-282-F		GGAGTACAAGGACCAGCAG	$(TG)_{10}$	AF351520
JESPR-282-R		CATAAGCCATGGTTGTAC		
JESPR-283-F	M	TCATCATGCTATTCATTGAACTAA	$(TA)_5(TG)_{14}$	AF351521
JESPR-283-R		GCAGCGAGAATTATCATGG		
JESPR-284-F	M	CAAGATCCATCTGCTGATTAG	$(CA)_{25}(TA)_5$	AF351522
JESPR-284-R		GTATATACAAGTATAAAGTATTGG		
JESPR-285-F		CCCGGATATAGTACTAAGGC	$(CA)_{10}$	AF351523
JESPR-285-R		ATGTATGGTGTTGAGTGC		
JESPR-286-F	P	GGAGGACATGGGTTTGAAC	$(CA)_{30}$	AF351524
JESPR-286-R		GCATGCATGTAAAATGTAATGG		
JESPR-287-F		TGATTATGTATTGAGGTTCTGG	$(GT)_{12}$	AF351525
JESPR-287-R		CGTTAAACTCATGGGAGC		
JESPR-288-F	M	CATGTATATACAAGTATAAAG	$(TA)_5(GT)_{11}$	AF351526
JESPR-288-R		CAATATAAGCACGTAAC		
JESPR-289-F		CATTGCATTTTGCCCC	$(GAA)_9$	AF351527
JESPR-289-R		AATCTAGCGCACAAGGGC		
JESPR-290-F		ACCGGTCAGTCCTCATAATC	$(CTT)_6$	AF351528
JESPR-290-R		GCCAAGGTCGTAGTCCAGG		
JESPR-291-F	P	CATTCCCCACTTTGCTCTTAC	$(CTT)_8$	AF351529
JESPR-291-R		CATGTTTCTTTGCCCATC		
JESPR-292-F		GCTTGCAATCTCCTACACC	$(CTT)_7$	AF351530
JESPR-292-R		GAATATGTTTCATAGAATGGC		
JESPR-293-F		CGAGATTTTAAGATTGTGC	$(GAA)_7$	AF351531
JESPR-293-R		TGATGGCAAAAGCACC		
JESPR-294-F	M	CTCCTCATTTTGCACGTCCTCTTC	$(AGA)_{10}$	AF351532
JESPR-294-R		AGGGCTTCATTCTTCTTCG		
JESPR-295-F		GCCTCGTTTAAGCCCATAAAC	$(CTT)_7$	AF351533
JESPR-295-R		GAGGGCCATAGTCACCGG		
JESPR-296-F	P	GGGTGTTACATAGAGTGTATAAAATTG	$(TCA)_8(CTT)_{13}$	AF351534
JESPR-296-R		TGACCTCAATTTAGAAACCC		
JESPR-297-F	P	GAGAACTCGTTAAAGCACAATG	$(GAA)_{12}$	AF351535
JESPR-297-R		GTTAATAGAGTTGGGTTTCTCATG		
JESPR-298-F	P	GATGCCCTCGTGTTAAAG	$(GAA)_{17}$	AF351536
JESPR-298-R		GGACCTTCGGAATAATTACC		
JESPR-299-F	P	CTGAACCTGCTCCTGAATC	$(CAT)_9$	AF351537
JESPR-299-R		GCCTAGGTGGAGTTCGTG		
JESPR-300-F	P	CGCATCACAAACCAAACAC	$(CTT)_5(CAT)_6$	AF351538
JESPR-300-R		CGGAAAATGATGATGATGAAGAAG		
JESPR-301-F		TGAGTTCCGAATTCCTTGG	$(CAT)_8$	AF351539
JESPR-301-R		CGGGCTAAGTGTTTTTCG		
JESPR-302-F		CACTCCTAGCTTCTTGGCATC	$(GAT)_5$	AF351540
JESPR-302-R		CTGCGATCTTGGCACAG		
JESPR-303-F		CATCGGAAAACTCTGAAC	$(CAT)_6$	AF351541
JESPR-303-R		GTAGCAGTACAGATGAAAGAG		
JESPR-304-F		GAAATGCATTCCCTCAAAAGC	$(GAT)_8$	AF351542
JESPR-304-R		AGACTCTATCGAATGACCCTG		
JESPR-305-F		CGATCCATCAAAGGCGAC	$(GAT)_6$	AF352075
JESPR-305-R		CCGCCTCAGCACCATTTAC		
JESPR-306-F	M	CCCCTTACATTATATTGACCTGC	$(CT)_{10}(CAT)_8$	AF351543
JESPR-306-R		CCATGTGAAAAGGGGATA		
JESPR-307-F		CTTGGCCATGTATTCCTTCA	(TGA)11	AF351544
JESPR-307-R		GAAAGACACTAAGCTGAGGC		
JESPR-308-F	P	GGCATCATCAGATTCTTTC	$(GAT)_7(GA)_4$	AF351545
JESPR-308-R		GCTGGTGGATATTTTATTC		

Name	Polym.†	Primer 5'–3'	Repeat motif	GenBank accession number‡
JESPR-309-F		CGAGACTCACANCGAGGACAC	$(TG)_5(GAT)_9$	AF351546
JESPR-309-R		GGGATTGAACAACACATGAAGC		
JESPR-310-F		GAGGCACATTGAGAAATGTTC	$(CAT)_8$	AF351547
JESPR-310-R		CAATGAGTGGGTTAGTATTGG		
JESPR-311-F	M	GGGGCTCGGTTAAAGGTAG	$(CT)_{20}$	AF351548
JESPR-311-R		GGATATCTGCAGAATACGGC		