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Construction of BAC and BIBAC libraries and their applications for generation of SSR markers for genome analysis of chickpea, *Cicer arietinum* L.

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Abstract Large-insert bacterial artificial chromosome (BAC) libraries, plant-transformation-competent binary BAC (BIBAC) libraries, and simple sequence repeat (SSR) markers are essential for many aspects of genomics research. We constructed a BAC library and a BIBAC library from the nuclear DNA of chickpea, *Cicer arietinum* L., cv. Hadas, partially digested with *Hin*dIII and *Bam*HI, respectively. The BAC library has 14,976 clones, with an average insert size of 121 kb, and the BIBAC library consists of 23,040 clones, with an average insert size of 145 kb. The combined libraries collectively cover ca. 7.0× genomes of chickpea. We screened the BAC library with eight synthetic SSR oligos, $(GA)_{10}$, $(GAA)_7$, $(AT)_{10}$, $(TAA)_7$, $(TGA)_7$, $(CA)_{10}$, $(CAA)_7$, and $(CCA)_7$. Positive BACs were selected, subcloned, and sequenced for SSR marker development. Two hundred and thirty-three new chickpea SSR markers were developed and characterized by PCR, using chickpea DNA as template. These results have demonstrated that BACs are an excellent source for SSR marker development in chickpea. We also estimated the distribution of the SSR loci in the chickpea genome. The SSR motifs $(TAA)_n$ and $(GA)_n$ were much more abundant than the others, and the distribution of the SSR loci appeared non-random. The BAC and BIBAC libraries and new SSR markers will provide valuable resources for chickpea genomics research and breeding (the libraries and their filters are available to the public at http://hbz.tamu.edu).

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Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world (FAO 2003, http://www.fao.org; Ladizinsky 1995) and a good source of plant protein (12.4–31.5%) and carbohydrates (52.4–70.9%) (Williams and Singh 1987). Nevertheless, relatively limited genetic and genomic tools are available for the crop. Chickpea is a self-pollinating diploid, 2n = 16, and has a genome size of 740 Mb/1C. While this size is slightly larger than that of the model legume, *Medicago truncacula* (530 Mb/1C), it is much smaller than other major legume crops such as soybean, peanut, garden pea, alfalfa, and lentil (Arumuganathan and Earle 1991). The first genetic map of chickpea was developed by combining the mapping results from three interspecific mapping populations (Simon and Muehlbauer 1997). However, due to the low polymorphism levels of isozymes (Ahmad et al. 1992; Kazan et al. 1993; Labdi et al. 1996), RFLPs, and RAPD markers (Udupa et al. 1993), this first map had a low marker density (Simon and Muehlbauer 1997).

Simple sequence repeat (SSR), or microsatellite markers, have been documented in several crop species to overcome the low polymorphism of other marker

types (Cregan et al. 1999a; Bhattramakki et al. 2000). SSRs are tandem repeats of di- to tetra-nucleotide sequence motifs flanked by conserved sequences (Tautz and Renz 1984). Because different alleles vary in length, depending upon the number of repeats, PCR with primers complementary to the flanking sequences of the SSR locus can detect these length polymorphisms (Cregan et al. 1999a). Because they are highly polymorphic, PCR-based, and readily portable within a species (Edwards et al. 1996), SSR markers have been the DNA markers of choice in many crop species. Weising et al. (1992) demonstrated that the simple sequence motifs, $(GACT)_4$, $(GATA)_4$, $(GTG)_5$, $(CA)_8$, and $(GGAT)_4$, are present as polymorphic repeats in the chickpea genome. Sharma et al. (1995) found that SSRs are abundant in the chickpea genome and have a high level of intraspecific polymorphism, suggesting that SSR markers are well suited for chickpea genome mapping and gene tagging. Winter et al. (1999) reported the first chickpea genetic map based on SSR markers and a list of 174 primer pairs flanking such loci. These SSR markers have been extensively used to study the genetic relationships among *Cicer* species (Udupa et al. 1999; Choumane et al. 2000), construct genetic maps (Tekeoglu et al. 2002; Flandez-Galvez et al. 2003), and map genes of agronomic importance (Winter et al. 2000; Cho et al. 2002; Rajesh et al. 2002, 2004; Udupa and Baum 2003). Despite the high demand for SSR markers in chickpea, only 174 have been reported to date. In contrast, more than 2,000 SSR loci have been mapped in soybean (Cregan et al. 1999a; http://129.186.26.94/). Thus, a large number of additional SSR markers are needed for comprehensive chickpea genome research and marker-assisted breeding.

Large-insert arrayed DNA libraries have been documented to be essential resources for advanced genome research. Because of their high stability, low chimerism, and easy DNA purification, large-insert bacterial artificial chromosome (BAC) libraries, especially plant-transformation-competent binary BAC (BIBAC) libraries, have emerged as the large-insert arrayed libraries of choice for plant genome research (Ren et al. 2004). BAC and BIBAC libraries have been developed for all major model and crop plants such as soybean, maize, rice, sorghum, cotton, and wheat (e.g., see http://hbz.tamu.edu) and are widely used in many areas of genomics and genetics research, including high-resolution gene mapping, positional cloning, integrative physical and genetic mapping (e.g., Chang et al. 2001; Tao et al. 2001; Chen et al. 2002; Wu et al. 2004a), region-targeted marker development (Cregan et al. 1999b), and comparative genome analysis. To facilitate chickpea genome research, Rajesh et al. (2004) recently prepared a BIBAC library of 23,780 clones, with an average insert size of 100 kb and a coverage of 3.8× genome equivalents. However, it has been demonstrated in other species that BAC libraries constructed with multiple enzymes and having higher genome coverage are needed for comprehensive genome research (Ren et al. 2004; Wu et al. 2004b, c).

Several methods have been developed for SSR isolation, including small-insert (~500 bp) genomic DNA libraries (Hüttel et al. 1999), small-insert SSR-enriched DNA libraries (Edwards et al. 1996), and large-insert BAC libraries (Bhattramakki et al. 2000). Utilizing BAC clones as a source for SSR isolation has several advantages over the other methods. First, since most BAC clones have inserts of 100 kb or larger, a significant proportion of the chickpea BACs would be expected to contain at least one SSR. The SSR loci contained in the BACs can be identified and cloned by simply screening a BAC library with SSR oligos, followed by subcloning the SSR-containing BACs (Bhattramakki et al. 2000). Second, although it is not difficult to isolate non-targeted SSR markers from many different sources, it is more problematic to isolate an SSR marker for a specific gene of interest from small-insert libraries than from BAC libraries. Cregan et al. (1999b) generated SSR markers from soybean BAC libraries for two regions involved in resistance to the soybean cyst nematode. Rajesh et al. (2004) isolated two BAC clones from the existing chickpea BAC library that hybridized to the SSR marker Ta96, which is tightly linked to a Fusarium wilt resistance gene (*FOC3*). Third, using a BAC as an intermediary facilitates the development of additional markers for fine mapping of genes and QTLs located in a particular genomic region (Cregan et al. 1999b). Finally, the BAC library utilized for the isolation of the SSR markers could also be used for the development of a chickpea physical map, with the simultaneous anchoring of the SSR markers on the physical map (Wu et al. 2004a).

In this study, we developed two new BAC and BIBAC libraries for chickpea and generated 233 new SSR markers from the BAC library. These new BAC and BIBAC libraries, combined with the recently developed chickpea BIBAC library (Rajesh et al. 2004), will provide sufficient clone resources for comprehensive genome research of the species. The 233 new SSR markers developed in this study together with the 174 SSRs developed by Winter et al. (1999) will significantly facilitate high-density genetic map development, gene mapping, and positional gene cloning in chickpea.

Materials and methods

Construction and characterization of BAC and BIBAC libraries

BAC and BIBAC libraries were constructed from the nuclear DNA of cv. Hadas according to a procedure previously developed in our laboratory (Zhang 2000; Ren et al. 2004; Wu et al. 2004b). Young leaves were used for megabase-sized DNA preparation (Zhang et al. 1995; Zhang 2000). In order to minimize plastid DNA contamination, the nuclei were purified by several washes and centrifugations. The nuclear DNA was prepared at 5 μg DNA per 100-μl plug. The plugs were

partially digested with *Hin*dIII or *Bam*HI, under conditions producing the largest number of fragments in the size range of 100–300 kb. The fragments were subjected to two rounds of size selection by pulsed-field gel electrophoresis. The fragments were ligated into either *Hin*dIII-digested and dephosphorylated pIndigoBAC-5 (Epicentre Technologies, USA) or *Bam*HI-digested and dephosphorylated binary vector pCLD04541 (Tao and Zhang 1998). The ligated DNA was transformed into electrocompetent DH10B cells (Invitrogen, USA). Recombinant colonies were selected on LB agar plates containing IPTG and Xgal with chloramphenicol for the pIndigoBAC-5 clones or tetracycline for the pCLD04541 clones (Zhang 2000; Wu et al. 2004b). White colonies were arrayed into 384-well plates containing freezing media (Zhang et al. 1996; Zhang 2000; Ren et al. 2004) with appropriate antibiotics.

To estimate the insert sizes of the clones, random clone samples from the BAC and BIBAC libraries were analyzed. DNA was isolated, digested with *Not*I, and subjected to pulsed-field gel electrophoresis (Zhang 2000; Ren et al. 2004; Wu et al. 2004b), and the genome coverages of the libraries were estimated (Wu et al. 2004b). For library screening with different probes, the libraries were double gridded onto Hybond-N$^+$ membrane (Amersham, USA) in a format of 3×3, using the GeneTAC G3 Robotic Workstation (Genomic Solutions, USA), and the high-density clone filters of the libraries were processed as described by Zhang (2000). To estimate the percentage of clones that were derived from the chloroplast genome, two of the BIBAC library high-density clone filters were screened with the probes derived from three chloroplast gene clones, *ndhA*, *rbcL*, and *psbA*.

BAC library screening with SSR oligos

To identify the BACs containing SSR loci, the filters of the chickpea BAC library were screened with the synthetic SSR oligos $(GA)_{10}$, $(GAA)_7$, $(AT)_{10}$, $(TAA)_7$, $(TGA)_7$, $(CA)_{10}$, $(CAA)_7$, and $(CCA)_7$. The filters were prehybridized for at least 2 h at 37°C in the hybridization solution containing 5× SSC, 0.5% SDS, 0.025 M potassium phosphate buffer, pH 6.5, and 5× Denhardt's solution. The oligos were end-labeled in a reaction containing 100 ng oligo mix, 1× Kinase forward buffer, 5 U T4 polynucleotide kinase (Invitrogen), and 200 μCi of γ[^{32}P]-ATP in a total volume of 10 µl. The reactions were incubated at 37°C for 30 min. Approximately 1 ng random primer-labeled pIndigoBAC-5 DNA was included in the probe to provide background hybridization to aid positive clone identification. The filters were hybridized overnight at 37–42°C, depending on oligo sequences, in a hybridization oven. The filters were washed twice for 15 min each in 2× SSC, 0.2% SDS, and 0.05% Na-pyrophosphate, twice for 1 h each in 1× SSC, 0.1% SDS, and 0.05% Na-pyrophosphate at 37–42°C, depending upon the oligounucleotides used as a probe, and then were exposed to XAR-5 film.

Subcloning of SSR-positive BACs

Positive BAC clones were selected and re-arrayed into 384-well plates, using the GeneTAC G3 Robotic Workstation. Clones were grown overnight in LB medium containing appropriate antibiotics. DNA was isolated and digested with *Sau*3AI, which has 4-bp recognition site and thus cuts the DNA frequently. Because the restriction sites of *Sau*3AI are complementary to the internal four nucleotides of the *Bam*HI sites, its restricted fragments can be cloned into the *Bam*HI site. The digests were electrophoresed on agarose gels, and DNA fragments in the size range of 400–1,500 bp were excised from the gel and purified by electroelution and dialysis. The size-selected DNA was ligated into the *Bam*HI-digested and dephosphorylated pGEM11 (Promega, USA). The ligated DNA was transformed into DH10B cells by electroporation (Invitrogen). Recombinant clones were selected on the LB agar with 50 mg/l ampicillin, IPTG, and Xgal. White colonies were picked into 384-well plates containing freezing media (Zhang 2000) with ampicillin.

Screening of SSR-positive subclones

High-density clone filters of the subclone library were prepared and screened with the above SSR oligos, along with a small amount of random primer-labeled pGEM11 to provide background hybridization for clone orientation. Positive subclones were re-arrayed into 384-well plates, using the GeneTAC G3 Robotic Workstation. Subclone DNA was isolated with the Qiaprep Spin Mini Prep Kit (Qiagen, USA), using the manufacturer's protocol and sequenced with 3.2 pmol of primer (SP030 or SP010, Operon Technologies, USA) in 10-µl reaction, using ABI PRISM BigDye Terminator (version 3.0) Ready Reaction Cycle Sequencing Kits (Applied Biosystems, USA). Sequencing reactions were run on an ABI PRISM 3100 DNA Analyzer (Applied Biosystems). The complementary reaction was run, whenever necessary, to aid in sequencing the complete SSR locus and its flanking regions. The sequence data were analyzed using the Sequencher, version 2.1 software (Gene Codes, USA). Sequences found to contain SSR motifs were subjected to BLAST search.

Primer design and optimization of amplification reactions

Primers complementary to the flanking regions of the selected SSR loci were designed using the Primer3 software (http://www-genome.wi.mit.edu/genome_software-other-primer3.html). The following criteria were applied: (1) PCR product size = 100–500 bp, with an optimal size of 200 bp; (2) primer size = 18–25 nucleotides with an optimal size of 20 nucleotides;

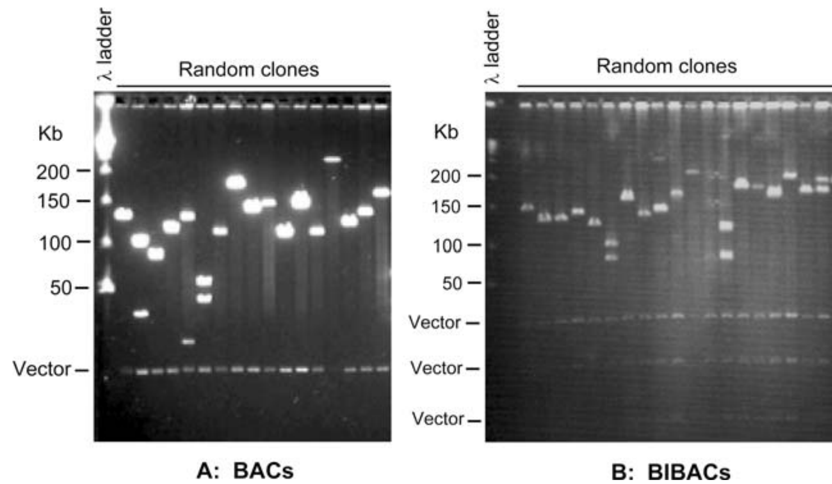

Fig. 1 Bacterial artificial chromosomes (*BACs*) and plant-transformation-competent binary BACs (*BIBACs*) randomly selected from the chickpea BAC and BIBAC libraries. The clones were grown in LB medium with appropriate antibiotics overnight. DNA was isolated, digested with *Not*I to release the inserts from the cloning vector, and subjected to pulsed-field gel electrophoresis with lambda ladder markers. The gels were stained with ethidium bromide and photographed. The insert size of each clone was estimated by the sum of the sizes of all insert bands of the clone

however, when SSRs consisted of more than 20 repeats of TA or TAA, the optimal primer size was 25 nucleotides with a maximum of 28; and (3) primer T_m = 50–65°C, with an optimal T_m of 60°C. For each primer pair, an optimal combination of annealing and elongation temperatures was determined using genomic DNA of cv. Hadas. Each PCR reaction was conducted in a volume of 15 µl containing 15 ng template DNA, 1 µM of each primer, 0.2 mM each dNTP (Biological Industries, Israel), 0.7 U of *Taq* DNA polymerase (Taq-Zol of Tal-Ron, Israel), and 1.5 µl 10× PCR buffer containing 3.5 mM $MgCl_2$. The reaction was carried out in a Biometra TGradient thermocycler (Biometra, Germany). The PCR temperature regime comprised an initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturating at 94°C, 20 s, annealing at a range of 50–62°C, 30 s, and elongation at 60, 65, or 72°C for 50 s, and a final elongation at 60, 65, or 72°C for 5 min. The reduction in elongation temperature relative to the standard 72°C was necessary to stabilize the long (TA/TAA) SSRs (Su et al. 1996). PCR products were initially evaluated on 1.8% agarose gels stained with ethidium bromide. Once the optimal PCR conditions were established, the amplicon number and size were determined by amplifying the SSR loci, using fluorescently labeled dUTP and analyzing the PCR products with ABI PRISM 377-XL DNA Sequencer (Applied Biosystems). The amplification reaction (8 µl) consisted of 7.5 ng DNA, 1 µM each primer, 0.2 mM each dNTP (Biological Industries), 0.35 U of *Taq* DNA polymerase (Taq-Zol of Tal-Ron, Israel), 0.8 µl of respective 10× PCR buffer (containing 3.5 mM $MgCl_2$), and 62 nM of ABI PRISM [R110]dUTP (Applied Biosystems). Electrophoresis was performed on 4.25% polyacrylamide gels following the standard procedures for the ABI PRISM 377-XL DNA Sequencer. Fragment sizes were calculated using the ABI PRISM GeneScan Analysis Software, version 2.1 (Applied Biosystems), by comparison with the internal size standard (ABI PRISM GeneScan-500 ROX, Applied Biosystems).

Results

BAC and BIBAC library construction and characterization

We constructed a BAC library and a BIBAC library for chickpea cv. Hadas. The BAC library was constructed in the *Hin*dIII site of the pIndigoBAC vector and consisted of 14,976 clones arrayed in 39 384-well microplates. Analyzing a random sample of 50 clones showed that the library had an average insert size of 121 kb, with a range from 40 kb to 170 kb, and 72% having insert sizes of greater than 100 kb, thus providing a genome coverage of 2.5× for chickpea (Fig. 1a). The BIBAC library was constructed in the *Bam*HI site of the binary BAC vector pCLD04541 and consisted of 23,040 clones arrayed in 60 384-well microplates. Analyzing a random sample of 79 clones showed that the library had an average insert size of 145 kb, with a range from 45 kb to 295 kb, and 84% having insert sizes of greater than 100 kb, resulting in 4.5× genome coverage (Fig. 1b). Therefore, the combination of the two libraries resulted in a total coverage of 7.0× genome equivalents, providing a probability of greater than 99.9% obtaining a single-copy clone. Analysis of the BIBAC library with chloroplast DNA probes showed that less than 0.3% of the clones were derived from ctDNA, an extremely low percentage of ctDNA-derived clones.

Development of SSR markers from BACs

To identify the SSR loci in the chickpea genome, the BAC library was double gridded onto membranes and

hybridized with radioactively labeled synthetic SSR oligonucleotides in two phases. In the first phase, 4,608 random clones were selected and screened with $(CA)_{10}$, $(GA)_{10}$, $(GAA)_7$, and $(TAA)_7$. Three hundred eighty-four of the positive BAC clones were randomly selected, subcloned, blotted, and hybridized with each of the labeled SSR oligos, respectively. A total of 293 positive subclones were selected and sequenced. The insert sizes of such subclones ranged from 200 bp to 1,500 bp, with an average size of 761 bp. Sequence comparative analysis of the subclones resulted in 117 singletons and 53 contigs containing 176 subclones. In other words, the 293 subclones represented a total of 170 independent SSR loci. The 53 contigs each consisted of two to seven subclones, with an average of 2.2 subclones per contig, with one exception for the contig identified with the $(CA)_{10}$ oligo consisting of 41 subclones. The sequence of the contig was found to be identical to the *Cicer arietinum* satellite DNA CaSat2 at the nucleotide level (BLAST code no. AJ006005.1/CAA006005 or AJ006006.1/CAA006006).

In the second phase, the entire BAC library was screened with $(GA)_{10}$, $(TA)_{10}$, $(GAA)_7$, $(TAA)_7$, $(CAA)_7$, and $(TGA)_7$. As before, selected positive clones were subcloned, re-screened, and sequenced. In this phase, a total of 359 SSR-positive subclones were sequenced, of which 145 were assembled into 60 contigs, with two or more subclones for each contig, and the remaining 214 subclones were singletons. Therefore, 274 independent loci were isolated from this second phase.

In summary of both phases, a total of 14,976 BAC clones were screened with the SSR oligos, and 1,720 (11.5%) were identified to strongly hybridize to at least one of the synthetic SSR probes. The two phases of SSR isolation resulted in a total of 444 independent loci.

Of the 444 subclones, 325 subclones contained SSR loci with four or more repeat units, while the rest (27%) had no SSR or had SSRs with three or fewer repeat units and therefore, were not considered for further analysis. BLAST analysis showed that 10 of the 325 SSR-containing subclones had high similarity to the chickpea Ty3-gypsy-like retrotransposon at the nucleotide level (BLAST code no. AJ411814.1/CAR411814), and 15 had high similarity to other repetitive sequences such as transposons and retrotransposons from various plant species. For eight subclones, the SSR loci were located too close to the cloning site, impeding the possibility to design primers. To limit potential redundancy of the chickpea SSRs, the loci isolated in the present study were compared to the published chickpea SSR markers (Hüttel et al. 1999; Winter et al. 1999). Since the sequence data of the primer pairs flanking the SSRs were the only sequence data available for such loci, the presence of both primers flanking the respective SSR motif was used to detect the redundancy. Only two subclones, H4D12 and H1P01, were found to match the detection criterion: H4D12 containing the primers and the SSR motif of marker TAA137 and H1P01 corresponding to those of marker GAA51 (Winter et al. 1999; Table 1). As a result, a total of 290 new subclones containing independent SSRs were found suitable for primer design.

Primer design and optimization of amplification reactions

Of the 290 SSR subclones, 25 contained two SSR loci and one had three loci, separated by at least 30 bp of non-repetitive DNA. Since neither of the markers previously isolated by Winter et al. (1999), TAA137 and GAA51, were mapped yet, independent primer pairs were also designed for these two loci. Therefore, primer pairs were designed for a total of 319 SSR loci. We evaluated 291 of the SSR primer pairs by PCR, using the genomic DNA of Hadas as template. Of the 291 primer pairs tested, 48 (16.5%) resulted in no PCR products under a number of annealing/elongation temperature combinations; ten (3.4%) resulted in smeared products, indicating that these primer pairs may be complementary to repetitive sequences; and 233 primer pairs resulted in clear amplicons. The sequences of the 233 primer pairs and respective SSR loci, the annealing/elongation temperatures employed for their amplification, as well as the expected and observed length of their PCR products in the cv. Hadas are presented in Table 1.

In general, the observed locus size matched the expected size, but there were a few exceptions: in three cases, the observed loci were significantly larger than expected (e.g., H1H07), and in 24 cases, the opposite was evident (e.g., H1A10). The stutter patterns that are commonly observed for long SSRs were in accordance with the SSR dominant motif: for dinucleotides-SSR motifs, the stutters appeared every 2 bp (e.g., H1H15, Fig. 2), while for trinucleotide-SSRs, the stutters appeared every 3 bp (e.g., H4G07 and H5H06, Fig. 2). Under the optimal PCR conditions (Table 1), most of the primer pairs (76.8%) amplified a unique locus (e.g., HIH15, Fig. 2); however, in 54 (23.2%) cases more than one locus was amplified. For instance, H4G07 and H5H06 primer pairs amplified two and four loci, respectively (Table 1; Fig. 2). We evaluated the allelic segregation of the multiple-fragment amplification products by using a recombinant inbred line population derived from the cross between the cv. Hadas and the desi Indian accession ICC5810. The independent segregation of at least one of the amplicons was established for 14 out of 21 primer pairs amplifying polymorphic patterns. Of these 14 primer pairs, 11 (H1D24, H1H08, H1J07, H1O06, H2B02, H2B061, H3C041, H3H122, H4D11, H5A04, and H5G12) produced only one polymorphic amplicon between Hadas and ICC5810—a direct indication of the independent segregation of such amplicons. For the other three primer pairs, H1B13 and H1P092 produced two polymorphic amplicons, while H3C11 produced three polymorphic amplicons. The polymorphic amplicons were confirmed to segregate

Table 1 Simple sequence repeats (*SSR*s) isolated from the chickpea genome by use of bacterial artificial chromosomes

Primer pair name	Primer pairs (5′–3′)	SSR loci [a]	Annealing/elongation Tem (°C) [b]	Fragment size [a] (bp)	
				Expected	Observed [c]
H1A06	TGGATAATTGTAGGGTAAGAAATGC TGTGTAATTTAAGTGTGGGGGTATT	$(TAA)_{23}$	60/72	181	181
H1A10	TTGGAAGTTTAAGTGTTTGCTTC TTCATAAAGAGAAACACTTGTTCAAT	$(TGA)_3$ $(TAA)_{54}$	57/60	289	112
H1A12	CGAGCCTCACTTAACCATTG CGATGTATAACTCGATTTTTCTTTT	$(TTA)_{29}$	50/60	308	302
H1A17	TGAGTGTTCTTTTCTTAGAGTGTGC CAAGCCAATTCTTCTCATAACAAC	$(TTA)_{31}$	58/65	235	155
H1A18	CTTTCCTTGGTCTTTGTCTTCAT TTGTAATTAATGAACATGAGTTAAGAGT	$(TTA)_{31}$ TTTTA T TAAAA $(TTA)_7$	58/65	244	244
H1A19	AGTGGAACCCACCAAATTTTA AACGAAACCCTTATATTTCTTCTCT	$(TTA)_4$	60/72	149	149
H1B02	GATGCCCTTACATAATTCAAATAGC ATCCCTATTCAACCTTCCTTCTAGT	$(TTA)_{43}$	57/65	425	425
H1B04	TAGTTGAAACACACGGGTTA AAAGTGAAATATGTCATCCTTATTA	$(TTA)_{35}$	50/60	260	204
H1B06	GACTCACTCTCCAAATGGAACC AAGCCCATGAAAACCATATATTC	$(TTA)_{28}$	60/72	197	155/197
H1B081	GGCCAACTATAACATCACCA GTAAACCCACCCAACAATTT	$(\mathit{TTA})_5$ 790 bp $(TTA)_{36}$ GTA $(TTA)_4$	54/72	140	140
H1B082	AAATTGTTGGGTGGGTTTACTC GGGTCTAGCCCAATAATACCTTT	$(TTA)_5$ 790 bp $(\mathit{TTA})_{36}$ GTA $(\mathit{TTA})_4$	58/65	225	110/125
H1B09	GGTTTCATGACCTGCACCTA AAGAACCGAAAACACTTGTGA	$(TAA)_{14}$ $(AT)_3$	58/65	210	210
H1B11	GCAGCTGTTGACATCTAATTTTG ACCGAAAACACTTGTGATTGTTA	$(TAA)_{20}$	62/72	203	156
H1B13	CGGTCTTCCTTTTCCTTTTTATTAT AAAATGTGTTTTATGGGTTAAGTTCA	$(TTA)_{40}$	58/65	204	148/197
H1B17	ATTCGAGGTGGTACCTCTAGTGA GAGGAACCGACGATGTATCTATT	$(TAA)_{38}$	57/65	199	199
H1C091	ACCACCGGAAGTCAATTTTT TTGGGTTTGACCCATATGAA	$(\mathit{GAA})_5$ 119 bp $(TAA)_{29}$	60/72	119	119
H1C092	CAATAAAACACTTTGTTCCTTTTT TGTAGAAAGAAAGCTAGCATGG	$(GAA)_5$ 119 bp $(\mathit{TAA})_{29}$	55/65	242	242
H1C19	AGAAAAGTTAAGATAAAAATCTCAGCA TCCTCTTCTCATAATTGAAATGAA	$(TAA)_5$ TGA $(TAA)_6$ 13 bp $(TAA)_8$	54/72	152	152
H1C22	ATTTATACAAAGTTTTTGAAGTCG CTTGTAAGTAGATAGTTTCACCAAA	$(CAA)_5$ $(TAA)_{47}$ AA $(TAA)_2$ TAAAA $(TAA)_{22}$	54/65	316	316
H1D02	TCCCTAGGTGCAATAGGAAAA CGTTCACATACCGCCATATT	$(TAA)_2$ $(TAG)_2$ TAA $(TGA)_3$ $(TAA)_{19}$	50/60	296	261
H1D221	TTCTAGAAACTGTCGACTGATAG ACTTAATCCATGAAATTTGTTTT	$(\mathit{TAA})_{25}$ CAA $(\mathit{TAA})_{23}$ 537 bp $(TAA)_4$	54/65	238	238
H1D222	AACAATCGTAATTAAAGGAGAAT TAACCGCTACCTTTTATTAATTT	$(TAA)_{25}$ CAA $(TAA)_{23}$ 537 bp $(\mathit{TAA})_4$	54/72	158	158/172
H1D24	TTTCGGTGAACAAAAACTAACTA ACGGTTAAATAGATGAGTCAAAA	$(TTA)_{14}$ TTG $(TTA)_6$	55/65	189	173/189/264/269
H1E06	GCAAATGTAACATCCTAAAATTAAAA	$(TTA)_{13}$ TAA $(TTA)_{14}$	57/72	201	201

Table 1 (Contd.)

Primer pair name	Primer pairs (5′–3′)	SSR loci [a]	Annealing/ elongation Tem (°C) [b]	Fragment size [a] (bp)	
				Expected	Observed [c]
	TCTTATAATAAAATTAAAAACACGTCAA				
H1E12	TGACATTTGACGTTTGTGCT ACCCCAATAGCGAATTTGAC	$(TA)_7$ $(CA)_4$	60/72	131	131
H1E192	AAAACCCTTGCCACCTCAT GGAGAATGGAAGAAAAGAAGGA	$(TA)_7$ T $(TAA)_{26}$ 61 bp $(\mathit{CA})_7$	57/65	125	119
H1E20	CTGAATCTGTGTTGGCCATT TCAACCACCTCCTAAGACCA	$(GAA)_4$	57/72	119	119
H1E22	TCATCTTAGAGTTCAACGAGAGA TGAGTAGTGGCTTCTAACAAAGA	$(TTA)_{14}$	57/65	139	113
H1F021	GAGGCGAGAAGAAGGAAGAG GGAAGAAAAATAGTAACAAGAAGACCA	$(\mathit{GAA})_5$ 39 bp $(TTA)_{29}$ [CTA $(TTA)_5]_2$	60/72	100	100
H1F022	GGTTCTGGTCTTCTTGTTACTATTT TGAAATATGTCATCCTTATTACTAACT	$(GAA)_5$ 39 bp $(\mathit{TTA})_{29}$ [CTA $(\mathit{TTA})_5]_2$	55/60	226	187
H1F05	ATAACTCAAATCGTTTCACAAGA AAACCCCTTTTTATTTTCAATTT	$(TAA)_{36}$	57/65	173	173
H1F14	GAGAGAGAGGAAGGGAAACG TCCTAACTTGCTCCTTAACCTTG	$(TTTA)_4$ $(TTA)_{11}$	60/72	204	204
H1F17	GGGGAGGAAGAAGATGGAA GCGTTATGGGTGAAATGGTA	$(TA)_{27}$	56/72	239	233
H1F21	GTTTCGCTCACATACCATCG GGGAAAGTCTTGCTCCTACG	$(TAA)_{20}$	58/65	316	316
H1F22	TAATGTAATTTTGTCTTTAACGTTTCC ATTGTGTTGTGTTATTTTAACTTTTGG	TTACTA$(TTA)_{24}$TTG$(TTA)_{11}$CTA TTACTA $(TTA)_9$TTG$(TTA)_8$CTA [TTACTA$(TTA)_{18}$	57/65	306	171/306
H1F24	AACGGAGAGTTGATTTCACA TGCTAGCTCTACACATGATATACT	$(TAA)_5$ TGA $(TAA)_6$ 13 bp $(TAA)_8$	50/60	268	268
H1G11	GCAAACAAGAACCGAAAACA GCTTGACATGCAAATTGTTG	$(TTA)_{11}$	60/72	196	195
H1G16	GTTTGCTTTCAACACCGAGA CCCATGAAGGCCTGAATTAT	$(TAA)_2$ TAT $(TAA)_{17}$	58/65	265	265
H1G20	TCAACACTTGTTTGAGATTGTT GGTTCTCTAATGGCTTTATTCA	$(GA)_7$	56/72	271	271
H1G22	AACAGACGAGACTGGGGTTC CTTCATCATCACGCCTCATC	(GA)n, $n=3$–8. In total, 8 SSRs interrupted by 9–23 bp	60/72	303	301/307
H1G24	CTTTCCCCCTTTTTCATTCA ACGCTCGCGTTTATTCTCAG	$(GA)_5$ 17 bp $(GA)_5$ 17 bp $(GA)_5$ - Except for 1 base the 17 bp are identical	57/72	235	192/207/235
H1H011	CATGTGCCCAAATGCTATTA CAAGTTTGAAATGCCAATTTTT	$(\mathit{GA})_{16}$ 139 bp $(TA)_5$	60/72	157	157
H1H06	TTCATGGGTAAAGCCCAAGT TTTTGACGAAAACGGAGAAA	$(GA)_{10}$	56/72	190	170/190
H1H07	CATCAAATAATGATGTGCTTGC AAATTGTTGATTTTAACTAACCAAGA	$(GA)_{27}$	56/72	165	192
H1H08	GTGAGACACATGAGTGCAAAAA GTGAGACACATGAGTGCAAAAA	$(GA)_{24}$ AA $(GA)_6$	60/72	167	120/167
H1H11	TGATTTTTGCTGGAATCAAT	$(GA)_{15}$	57/72	152	152

	TCAACCAGACAAAATCTACCTGA				
H1H13	TTCCTTTATCGCACCCTTCT CCAGAGAAAGAGCAGCTACG	$(GA)_{17}$	56/72	198	198
H1H14	AGGAATGATTTTCCGGTGAG TCGAGTGAACAAACCACACA	$(GA)_7$ GCA $(GA)_{11}$ 14 bp $(GA)_8$ CA $(GA)_5$	56/72	200	200
H1H15	GGCGAGAAGGAAATTAAACG TTGGCACGTGTTGGATACTT	$(GA)_9$ 23 bp $(GA)_{11}$	56/72	239	239
H1H18	TCTCGCGAAAAACAGTAACC ACACATGCTTTACCCCATGA	$(GA)_{13}$ 22 bp $(GA)_5$	57/72	169	169
H1H20	ACGCAACAACCTTTCAAAAA GCTAGACCGTCTGTCATGGA	$(CA)_7$ $(TA)_{17}$ AA $(TA)_{17}$	58/65	375	375
H1H22	GGGAGAAAGACAGATGAGTGC TGTCCAAGATACAAGAAATCAAGA	$(GA)_3$ AA $(GA)_{10}$ GT $(GA)_2$ 14 bp $(GA)_4$ T $(GA)_4$	53/65	253	253
H1H24	CTCGCTCCTCAGTTGCCTAC CCAAATTGCTCAACCCTACA	$(CT)_2$ TT $(CT)_7$	56/72	193	193
H1J24	GCGTAGGGAGGAAGAAGATG TTAATGCGTTACGGGTGAAA	$(CA)_{15}$ $(TA)_7$	60/72	224	242
H1I01	TGGGAAGTTGAAAACGTGAG ATCATTACGCCTCATCAACG	$(GA)_{10}$	57/65	153	139/145/153/172
H1I05	TGCAAAACTTGCATGTCATTA TCGTAGAGTTTGGCCTTTCA	$(GA)_8$	56/72	203	195
H1I08	CTTCCTTCACCAACACTTG AAAAATCGTTCTCATAGAGTC	$(GA)_5$ 17 bp $(GA)_5$ 17 bp $(GA)_5$ - Except for 1 base the 17 bp are identical	57/72	429	197
H1I09	AGGGATTTGAAAAGATGGTT TTTCCACACACAATACAAATAC	$(GA)_8$ AA $(GA)_7$ AA $(GA)_5$	53/65	173	173
H1I16	GACATGAAATTCGGTGCATT AACGCCCTAAACCTCTTGGT	$(GA)_{20}$	56/72	169	169
H1I17	AGTTTATTATCTCTCATGTCTTTT GAGAGCTAAATTGAAGAAAGG	$(CTT)_2$ $(CT)_{12}$ CATAT $(CT)_3$ CCC $(CT)_3$	53/72	204	204
H1I18	GCCATGCCAGAAAGATGTAA GTGGCTAGAGATTGCCATGA	$(AG)_{14}$ AC $(AG)_2$	56/72	205	205
H1I20	CCGTCAACAATGGCATACAT TCAACCATTTCTTCCTTCCTTT	$(CT)_8$	56/72	150	150
H1I24	CGCCACTTCAAAGTCTCATT ACGAGATAACGACACGAACG	$(GA)_9$	60/72	124	124/129/160
H1J04	TTAGTTGGGCACAACAGGAC AGGGGGAGGATGTCCTTAAC	$(GA)_{16}$	57/72	157	157
H1J07	GGATTATAGGTGCCCTTTTGG AGCAATACCAAAACTGTTGGAC	$(CT)_4$ GT $(CT)_6$	60/72	135	105/135
H1J12	GCGTCGCTTCCAAAGTTAG ATGGGAGGTGATGTGGAAAT	$(CA)_{94}$ $(TA)_4$	57/72	295	295
H1J15	CACAGCGTGCACTCTCACTA CGAACAATCAAACAAACACAA	$(CA)_7$ $(TA)_2$ CA $(TA)_5$	56/72	205	205
H1J16	TGAAGAAGGAGTGTCGCAAG TCAAAATGATTTGAGAAGTGATGA	$(TA)_{19}$ TTAC $(TAA)_4$ $(TA)_2$ TAA $(TA)_4$ $(TAA)_2$	58/65	222	178/189/205
H1K18	GTATGAGCCACAAGCTCCAA CAAACAAAACAGAGGATGTTGA	$(TA)_{12}$ $(TG)_8$	56/72	232	226
H1K23	GACCCTTCTTAATTTGTTTTATGC CACTGCTTCCAATGCAATCT	$(CA)_6$	57/72	163	163
H1L161	ACTATTCTAAGTGTTTGATGTTGAA TGCTCGCAAATTTTTATTTC	$(TA)_{21}$ 48 bp $(CA)_4$ $(CT)_3$	54/65	206	206

Table 1 (Contd.)

Primer pair name	Primer pairs (5′–3′)	SSR loci [a]	Annealing/elongation Tem (°C) [b]	Fragment size [a] (bp)	
				Expected	Observed [c]
H1M07	GAAAAACACTTTTGACAGAAACG ACGTTTAGATCGCCACCAGT	$(CA)_8$	56/72	192	192
H1N12	AAAAATTGGTTCTCAAGAGTAAA ATGAGGATTGGACGTAATCA	$(CA)_5$ 46 bp $(CA)_5$	57/65	187	187
H1O01	GCCGTTCTGCCATAAGATT AGCCCGTAAGTGGTATCCAA	$(TG)_{16}$ $(TA)_5$	56/72	194	184/194
H1O06	CGTTCTCGAATCAAAGAGGA AGGAAGGCAGAAAGGAAACA	$(GAA)_{21}$ 69 bp $(AT)_7$ 14 bp $(AT)_{12}$	60/72	226	183/188/226
H1O09	CATGGGATGATTCAGAGGAA TCATTTCCCATTTGTTGACAT	$(GAA)_{132}$	60/72	478	133
H1O10	TGGTTTTTCCAAGAATGCAA TTTTGGATGATGAATAAAGGAA	$(GAA)_{10}$	57/72	197	175/197
H1O12	GAAAATGGAAATGCAGCAGA GCCACTTTCACTTGAAACCA	$(CTT)_2$ 9 bp $(CTT)_{11}$	60/72	224	224
H1O14	AGCAGAAAAGCAACCAGACA TGTCACTGATACTGCCTCCTG	$(GAA)_5$	56/72	148	148
H1P01	CAAAGCAGAATGTCCATCAAA CTGTTTTTGCCATCAAGCAT	$(GAA)_6$	56/72	146	146
H1P181	ATGGCAACATGGGATGATT TTTTGGTTTTGCCATTAAGC	$(GAA)_{90}$ 30 bp $(GAA)_4$	57/65	321	81
H1P182	TATGCTTAATGGCAAAACCA TTTTGGTCATTTTTGTCCTT	$(GAA)_{90}$ 30 bp $(GAA)_4$	54/72	100	100/198
H1P02	AAGAGGAAAGCTCCTCCAAC TCTTCAACAGGTTTGCCTTT	$(GAA)_5$	60/72	128	128
H1P091	GTCTCGGTTTTGCATGACAC GGAACACCGGTTCCCATC	$(GAA)_6$ 61 bp $(TTA)_{16}$ TCA$(TTA)_{22}$ TTG$(TTA)_5$	60/72	146	126/146
H1P092	GAACCGGTGTTCCCTTTTT GAAAGGTATAGAATGTGTTTAATGGA	$(GAA)_6$ 61 bp $(TTA)_{16}$ TCA $(TTA)_{22}$ TTG $(TTA)_5$	57/65	229	163/229
H1P17	TGCCTCCCACTTACATTAGG TTGCACGAAGACCATTAGAA	$(CAA)_{12}$ $(GAA)_8$	60/72	205	205
H1P23	ACCAAAGGCTCAAGGAGAAA TGGAACTTGAGGTGTTGCTT	$(TAA)_5$	60/72	193	193
H2A02	TGATATTATTGTGATATTGGATGTCG TCCTCGGAATCACTAGGTTTG	$(TGA)_4$	60/72	199	196/347/372/396/406
H2A04	GATTTTCTTGAAAACAACATATAGTCA VTGTGTTGGAGGTTTGTTGA	$(TA)_7$ $(TGA)_5$	60/72	134	134
H2A08	AGGCAATTATTAGGGGTTAGTG TTGGTTTATGCTGCACGTTT	$(GA)_9$	60/72	136	136/139
H2A11	GCATGTTCGATGGAAGAGAA TAGAACCAGAGGCAGAGCAA	$(CAA)_5$	60/72	133	133
H2B02	GCCATGAAATTACACAAAATGG GGTGGTTTACGGGTTGTTCT	$(GAA)_6$ 24 bp $(GAA)_{10}$	62/72	200	140/192/200
H2B061	TCTTGAAGCAAAAGAAGTCAAAAG CAAGTGATAAGTAGGAAGGCAGAA	$(GAA)_2$ G $(GAA)_{19}$ 70 bp $(TA)_7$ 14 bp $(TA)_{11}$	60/72	163	120/128/163
H2B18	CAATTTAATCGGAACATTGTCTTC TTCACAAACATCACAACCATAAAC	$(TTG)_7$	60/72	170	130/137/160/203/221

H2B19	CTACTTGGAAAAATGCTTCCTTCT AGTTGCGACGAGAGCTAGATATT	$(TA)_6$ 22 bp $(TA)_6$	60/72	144	144
H2B201	AATTTTCTTGGTGTTTGTTTGTGT GCAATACGCATGTTTATTTCTTGT	$(TA)_7$ AA $(TA)_8$ 49 bp $(TGA)_6$ 89 bp $(CTA)_5$ GTG $(TA)_7$	60/72	159	159
H2B202	GAAATAAACATGCGTATTGCTACA ATTTATCGGTATCTCCAACGGTAG	$(TA)_7$ AA $(TA)_8$ 49 bp $(TGA)_6$ 89 bp$(CTA)_5$ GTG $(TA)_7$	60/72	117	117
H2B203	ACAAACGAACTTATTTGACTACCG ATAATTTTTGAGAAGGCATGACAG	$(TA)_7$ AA $(TA)_8$ 49 bp $(TGA)_6$ 89 bp $(CTA)_5$ GTG $(TA)_7$	60/72	148	148
H2C03	ATTTGTTTGGTTTCTGTCATGATT AATCGTAGAAGTAGGTGGTGAGTG	$(CAA)_4$	60/72	130	130
H2C10	AATGAAGGATAAGGTTTGGGTTT GATTAGTTAAGGAAACGGGAAAAA	$(TAG)_5$	60/72	149	149/205
H2E13	TGGGGGTATACATGAATTGAATAA AATCCCATCAATGTTGTACTTTTC	$(GA)_9$	60/72	154	154
H2E23	TTTTACCTTATTAGGAGTTGAGATAA AACCTAAGACGTAAAATTTGTTTTT	$(TAA)_{27}$	58/65	186	186
H2F24	ACTTCAACTTTATTAGGAGGTTTGT TCTCTGTTTTATGTTTAATTACTTTCA	$(GA)_{15}$	54/65	170	170
H2I01	AACATTCTGAACAGACACTTTTCTCTA TTTTCTTCTTTTAACACATAGCCTTTT	$(GA)_{12}$	60/72	144	144
H2I06	GAGGTGAGACACATTACTTCCTTCA TGTAATTCAAACACATCAAAAATCA	$(GTT)_3$ GT $(GTT)_4$	58/65	186	186
H2I10	CATTAATTTGGGATTTTGTTTCAA GCATCACATTATTTTGTTCTTGTG	$(TAA)_{35}$	60/72	225	225
H2I12	AGACACTTACTCCCTCCTCCTTTT TCATTACGTAACTCAAACACATCAA	$(TTG)_2$ TGG $(TTG)_4$ TTA $(TTG)_2$	60/72	177	177/155/165/197
H2I20	TGTTTTGCTCATCTGTTAAATCAA AGCATGCCTCTGATGAATAGTAAC	$(TTA)_{13}$ CTA $(TTA)_{19}$	60/72	190	190
H2J04	GGTGATGTGATAAATTTGTGATGA GCCTATGGTACTCCATTAAGACCT	$(TGA)_4$ $(TG)_3$	60/72	190	190
H2J09	AACGAAAAACAAGGGAGAAAAA TATTTCTTTGACTCCCCCTAACTT	$(GA)_{18}$	58/65	183	183
H2J10	CAGGAAGAACACACAAACAATCTT ATGTGATATTGAATGTCGAATGAA	$(TGA)_4$	60/72	193	193
H2J11	TCCCTCACATATAACATTTCCCTA ACAACACAAATGCAAAAGTTTCTT	$(TGA)_4$	60/72	169	169
H2J19	TTTGAGTTCTTAACAAATCCTTGA TGTGTGTAAAATTTCAATTTCAGTTC	$(TTG)_4$	60/72	151	151
H2J20	TCAAAGAGATAAACACTTGTTCAAAA GGAACTTTAAGTGTTTGCTTCTAGC	$(TTA)_{30}$ CTA $(TTA)_5$	58/65	207	207
H2L101	AGGGTTTATGGGTTCAATTTATGT ACTCAAATCTCATCCACATTTCAT	$(GA)_6$ 58 bp $(TTTA)_8$ $(TTA)_{29}$	60/72	151	151
H2L102	GAAGAGAAAATGTTTAATGGGTAAAT GAATAACTTCACACCGTTTCATAA	$(GA)_6$ 58 bp $(TTTA)_8$ $(TTA)_{29}$	60/72	204	204
H3A03	ACGGTAGAAACTTGCGAGAAAAT TTATGAAAGCTTCAGGTGGGTAA	$(TA)_4$ 165 bp $(TG)_3$	60/72	253	253
H3A052	ATACAAAGGAAGGATACGTTGGA ATCACTTAAGGTGACGGATTTGT	$(CA)_3$ $(ATC)_3$ 253 bp $(TC)_6$ 5 bp $(TC)_{17}$	60/72	252	252
H3A07	GCGACACCTATTCCTCTTTTCTA TCATTTTTGGAATATTTTAGTGACAA	$(TTA)_{20}$	60/72	246	246

Table 1 (Contd.)

Primer pair name	Primer pairs (5′–3′)	SSR loci [a]	Annealing/elongation Tem (°C) [b]	Fragment size [a] (bp)	
				Expected	Observed [c]
H3A09	CTGTGATAGGTCTGAAACTCGAA TTCGGTATTAAATTCTTCGCAAC	$(TTA)_{23}$ TTG $(TTA)_2$ TTT $(TTA)_2$ $(TTG)_3$ $(TTA)_{12}$ TTT $(TTA)_{15}$	58/65	391	391
H3A10	TTTAAGGCTTCAGGTATTGATTTCT TCACACATGCCAACTTAAAATAAAA	$(TTA)_{24}$	58/65	246	246
H3A12	AACCTTAGACTGTGTTCGCTGA TCAATCTTTTGTTGTTACTATGAATCTG	$(GA)_{11}$	60/72	179	179
H3B01	AGTTGCGACGAGAGTAGTTATTTTT AATGTTTTTCTTTCACTCACACTTG	$(TA)_5$ T $(TA)_3$	60/72	254	254
H3B04	TGTTTCCTGATGTTGAGAAACTC TATTTTATGATATCCGCGGTGAC	$(TC)_9$ $(AC)_7$	60/72	197	197
H3B08	TGTAATAAAACAAAATCCTCACACC AGATTAAGCCTGAATTGGTTGAA	$(ATC)_5$	58/65	199	199
H3C041	GGTGAAGAGAGAGAGAAAGTGAAAC CTCTCTCTCATCAATCAATCAGTTTT	$(GA)_5$ *11 bp* $(GA)_5$ *35 bp* $(GA)_4$ *13 bp* $(GA)_9$ *23 bp* $(GA)_4$ 21 bp $(GA)_4$ 13 bp $(GA)_4$ 20 bp $(GA)_4$ 31 bp $(ATG)_4$	60/72	199	153/188/199
H3C042	AAACGTGAGAGAGATAGAAGAAACG CTTTTAGGTGAAACTTCCTCTTGAC	$(GA)_5$ 11 bp $(GA)_5$ 35 bp $(GA)_4$ 13 bp $(GA)_9$ 23 bp $(GA)_4$ 21 bp $(GA)_4$ 13 bp $(GA)_4$ 20 bp $(GA)_4$ 31 bp $(ATG)_4$	60/72	206	206
H3C06	AATTTCGTGAATCATTAAAAATAGAGG CACATGACTATCTAGACATTTTATTTATC	$(TAA)_{23}$	58/65	171	171
H3C08	TTGTTTGAGAAGAAGATGGGTTT ATGCACAGACTGCATTAAATGAT	$(GA)_4$ 15 bp $(GA)_4$ 22 bp $(TGA)_4$	60/72	183	183
H3C10	TTTTGTCTATTGTGTTGAATTACTTTTT AGGTTGATATCCTAAACAAGGACTCT	$(TAA)_{33}$ 40 bp $(TAA)_4$	60/72	224	165/196/224
H3C11	GCCCATATTCAATTCTTACCATTATTA ACCTTTAACGCTAATAGAGTGAGTTTA	$(TTA)_{40}$	60/72	205	142/205/240
H3D05	AGACGTGTTCCCTTTCTTTTAACTA GCCGACACAAAGTTTATGATTTT	$(TAA)_{41}$	58/65	307	307
H3D09	GGCAAAATCTCTCCATAAGAGG CACACTTTAGCACAATGCAGAA	$(TC)_{10}$ $(TA)_{12}$	55/65	224	224
H3E04	GATTTAACGTGTCGCGTCTTC GCCTTATGTGTTTTCCTTAGTGATT	$(TTA)_{36}$ $(CTA)_5$	58/65	313	313
H3E052	TAGACCCTTGCTTCTTGTTCCT AATCTTGTTGGTTCTTTGGTCA	$(TTC)_3$ $(TAC)_2$ 22 bp $(TC)_{22}$ $(AC)_{11}$ 251 bp $(TTC)_3$ *20 bp* $(TC)_7$ *5 bp* $(CA)_5$ *CC* $(CA)_4$	60/72	184	184
H3E08	CGTCGTTAAATGTGTGGAATTT GCAGGAAGAACACACAAACAAT	$(GAT)_4$	60/72	270	270
H3F08	AAACACCCGTGATTCTCTAAAGTT TGACACCTAATTTTATTCGGTTTTT	$(TTA)_{34}$	58/65	246	246
H3F09	AGCATGTAGTAGGAGGCAAGTATG GTAGGTTCCCGCTACATTACTTTTA	$(TTA)_{44}$	60/72	241	241
H3G031	CCCAAGAACTTGGAAAAATATG AACCATCTTTCCCTTTTCTTTTT	$(GA)_3$ *AA* $(GAA)_5$ $(GAAA)_3$ *25 bp* $(GA)_5$ 50 bp $(TTA)_{31}$ $[TTTAA\ (TAA)_3]_2$ T $(TA)_4$	60/72	201	201

H3G032	AAAAAGAAAAGGGAAAGATGGTT TTTTTATAAGGGGAGGAGATTAACA	$(GA)_3$ AA $(GAA)_5$ $(GAAA)_3$ 25 bp $(GA)_5$ 50 bp $(TTA)_{31}$ $[TTTAA\ (TAA)_3]_2$ $T\ (TA)_4$	58/65	203	203
H3G06	AATTCAAGGACGAATTTTTATAACG GGAAGGAAAAATGAATTAAAAATGA	$(TA)_8$ $(TAA)_{19}$	58/65	197	130/197
H3G09	AATTGTGCTTTGCCATTTCTAA AAAATTTCTCTATAAGGGGAGAGGA	$(GA)_{10}$	60/72	190	190
H3G11	TTAGAGGTATGCGTTTTGGGTA CTCCACTCTTCCTCATCTCAAA	$(GAT)_3$ 9 bp $(GAT)_3$	60/72	199	199
H3H021	GGGGTAAAAACTGTCCCTTTTA TCCTTTCCTGTCTTCATCTCTG	$(GA)_8$ 235 bp $(CAAA)_3$ 25 bp $(TC)_{11}$ 25 bp $(TTTC)_3$ $(TC)_4$	60/72	182	182
H3H022	TTGAGTTAAGGAAGCAGACAGC CACCCTAAAGTTGTCCAATCAA	$(GA)_8$ 235 bp $(CAAA)_3$ 25 bp $(TC)_{11}$ 25 bp $(TTTC)_3$ $(TC)_4$	60/72	197	197
H3H04	CACACAAATCAATATATAACGCAAT GAAGATATTTATGTGTTGACCGATT	$(TTA)_{33}$ 27 bp $(TA)_9$ 9 bp $(TAA)_3$	58/65	216	216
H3H07	GAGGCATAGTACCTCAATTTTATTCA AAGAAAGACAGGTTATCTGTGTGGT	$(TAA)_{16}$	58/65	289	286/289
H3H11	CTCCTTCCTTCTCTCTCTCAACA TCCATAGAACACAAACCCTAACC	$(TTC)_5$	60/72	196	196/210/213
H3H121	TCTTCACCGTAACTTGATTCACATA ATGAGAGGAATGAATTGAAAAGAAG	$(TTTA)_{21}$ 8 bp $(TTGAA)_3$ $(TTC)_4$ 228 bp $(TC)_{10}$ 44 bp $(TTC)_3$	60/72	214	141/154/214/231
H3H122	CTTCCTAGTGCTGCTGGTAAAG CGAAAATGTGGGTGAAAGTAAG	$(TTTA)_{21}$ 8 bp $(TTGAA)_3$ $(TTC)_4$ 228 bp $(TC)_{10}$ 44 bp $(TTC)_3$	58/65	187	187/205
H4A03	ACAAGAATTAGAGGCAAACAAGAAC TGACACCTAATTTTGACCAATTTTT	$(TTA)_{17}$	60/72	194	168/179/194/200
H4A04	GCAAATTCTCACCATTTCTTTTT TGTTTTGACGAATGAGAAGTAAAGA	$(TTA)_{32}$	58/72	240	240
H4A07	ACCCTCATGGATTTTCCTTTTA GCACCTGCTGTTATTCAAGAGA	$(TTC)_4$	60/72	204	204
H4A09	ATTACAGAGCAAATGACCCTCA TAATACTCCTCCCATTCCCAAA	$(TC)_{10}$	60/72	203	203
H4B041	ACAAACTACCATGGCTCGAGTA GCAACATCTTTGCCTTTATCCT	$(ATC)_4$ 226 bp $(GAA)_4$	60/72	199	199
H4B042	TTAATGGGTGATGCTATGAAGG ATGTTCTGGCTTCTTGGATTTT	$(ATC)_4$ 226 bp $(GAA)_4$	60/72	151	151
H4B06	CCATTAATACCGTCATCTCAGG TGTGTTGTTGTATGACTAATGATAGTG	$(GAT)_4$	60/72	148	90/148
H4B09	GATACAAAAGATGGGAACTTGAGAT GAGAGAAAAGCTTGTTCAAGTTAGA	$(TAA)_{37}$	57/60	218	218
H4C04	AAGAGAGAAATACTTGTTCAAGTTAGAAA AACTTAAAAAGTTGCTTCTAACAAAGC	$(TTA)_{29}TTT(TTA)_{16}(CTA)_3CTG(CTA)_2(TTA)_8T(TTA)_{11}TTTT(TTA)_4TTT(TTA)_3$	58/65	334	104
H4D011	ATCCTCCTACTCCCGACACTAA TTGTAATGTGAAATATGAACGACA	$(TA)_7$ 14 bp $(TA)_4$ $(AT)_4$ $(GT)_3$ 70 bp $(TA)_4$ AAT $(TA)_9$ 37 bp $(TA)_8$ $(CA)_8$	58/65	207	207
H4D012	TGTCGTTCATATTTCACATTACAA AGAGAATGATGAAGGCAGTGAC	$(TA)_7$ 14 bp $(TA)_4$ $(AT)_4$ $(GT)_3$ 70 bp $(TA)_4$ AAT $(TA)_9$ 37 bp $(TA)_8$ $(CA)_8$	58/65	158	158

Table 1 (Contd.)

Primer pair name	Primer pairs (5′–3′)	SSR loci [a]	Annealing/ elongation Tem (°C) [b]	Fragment size [a] (bp)	
				Expected	Observed [c]
H4D02	CAAATCCCTTTTATTTTTCCTTCATA CTCCCTAAGTAGAACTCACCAATTGTA	$(TAA)_5$ CAA $(TAA)_4$ TAG $(TAA)_5$ TAG $(TAA)_{43}$	58/65	288	288
H4D07	AGAGTGAGTTTTGCGAAGTCTG CCACTGATTCTCCGTAGGTAAA	$(GA)_9$	60/72	205	200/205/213
H4D08	TGTCCTTTATTTCTTAAGCACACAT GAGATGGATGTTATTGGACTCATC	$(TAA)_4$ 9 bp $(TAA)_{25}$	58/65	189	189
H4D11	TCTTATGCACACATTTATTCTGAAATC CATCTGTTAAAATATGGTTGCCTATAA	$(TAA)_{48}$ 14 bp $(TAA)_3$	58/65	276	141/173/276
H4D12	GTGGCAGCCATAATAATCAATGT TTTGTTTCATATTTCTTGTTTCGTT	$(TAA)_{23}$	57/60	200	200
H4E04	ACTTTATGATGTAAAATATGCATGGTC TCTTTTGTTTTACTAATGCTCATGTCT	$(TTA)_{56}$ $(TTTA)_3$	58/65	346	346
H4E09	TGCTATTTGTACTAGGACTTAAGGAAA TGTTTAAAGTACCCATTAAAAACGTAA	$(TTA)_{35}$	58/65	253	253
H4F01	ATTCACTTTGCTTTGGTGTGAT ATTGAGCATTGACAACAAAAGG	$(TC)_9$	60/72	217	206/217
H4F02	ATAAATAGCTAATGGCCTATTGTGTTC TCTTTTGCACAAGTAAAATAATGATAA	$(TTA)_{35}$	58/65	181	151/181
H4F03	CCTTTAACAAACAAAACAAACTTCC GGTCGTCTGTAACATCCTATATCAA	$(TAA)_{25}$	58/65	284	284
H4F07	AACGCCTGCATTTTATTTTTGT GGATTCTATTCAAAGCCCAATC	$(GAT)_5$	60/72	170	170
H4F09	TCATCGACTGTATTGAGGAAAAA GCACCTTCAGTTTGAATTGTGT	$(TC)_{11}$	60/72	266	266
H4G01	ATTGACTTTATGTTGTGATTTTCTTCA TATCATGCATATTTTCAACTTGTCCTA	$(TTA)_{29}$ TTG $(TTA)_{19}$	58/65	255	255
H4G02	AAATAACTCATTACGTACCCCGAAC TGGGTGAAATTTTTGAGTTCTTAAC	$(ATC)_7$ $(AAC)_5$ $A(CAA)_5$ $(CACAA)_2CAAT(CAA)_4$ 6 $bp(CAA)_3$	60/72	188	188
H4G04	TGCATAAAATTAATTCAACAACCA GTTTGAATGTGTGGCATTTGATA	$(CA)_3$ C $(CA)_3$	60/72	200	94/105/153
H4G05	TGCTAAACTATCTTCTGACCTTTTTG AAAAATGCTATTACTGGATAAACACAA	$(TAA)_{17}$ $(CAA)_3$	57/60	202	202
H4G07	ATTAGAGGCAAACAAGAACTTGAAAC TGACACCTAATTTTATTCGGTTTTTAT	$(TAA)_{33}$	57/60	265	197/265
H4G08	AAATGAAAAAATGGGGTTAGGAA CGTTCTTTGACTTGAAGGATTT	$(GAT)_6$	58/65	239	239
H4G09	GGGGATGAGTGAAATTTTTGAG TTTCAACTTCAGTGCAAACTCAT	$(TTG)_3$ T $(GTT)_6$	60/72	176	152/162
H4G10	CACAAATCAGTATACAACACATCACTC AACTCACGTAGTTCTTGGATAGAAAA	$(CTA)_2$ $(TTA)_{29}$ ATA $(TTA)_2$ TCA $(TTA)_9$ $[TCA\ (TTA)_2]_2$ TCA 25 bp $(TA)_5$ 9 bp $(TAA)_3$	58/65	279	167
H4G11	ATCTAAGTGAGCGGCTACTAAATCA GTAGTCATGCAGCCTATAAAAACAA	$(TAA)_{18}$	58/65	195	195
H4H01	TAGTATTTTTCTTTCATTTCCTTCGTT TTGCTTTAATTCATTATGGTATATTTATG	$(TTA)_{43}$ CTA $(TTA)_{30}$	58/65	299	299

H4H02	TAGGAGACACTCAAGAAACTCTCAA GTAAAAGTTTTGTTTCCCTCAAAAA	$(TA)_8$ A $(TA)_2$ $(TAA)_5$ TAT $(TAA)_8$	60/72	174	174
H4H05	ATGCGTTGTTGTATGATTGATG TATTTTCTCCCCTCGTTGGTAT	$(CA)_3$ C $(CA)_3$	60/72	182	182
H4H06	CACAACAGCTCCAACAGATTG ATGTGCAACTTCAACCACCTAT	$(TTTA)_6$	60/72	213	213
H4H07	TCTTTACGTATTTTTACTTTGATTCTCA CTGAAAACACTTGTGATTGTTAAAAG	$(TAA)_{16}$ $(AT)_3$	60/72	235	204
H4H08	GTGTAACTACAACACATATACCAACCA TATTACTTTCTCACTTGAATCGTGAAG	$(TAA)_{29}$ GAA $(CAA)_6$ $(TAA)_{17}$ $(CAA)_4$	58/65	329	329
H4H10	GGCATTGCATTCATAAACAGAA TTCGTTCAACACCTTTTTCATC	$(GAT)_3$ 23 bp $(GAT)_3$ T $(GAT)_3$	60/72	200	200
H4H11	ATATTCCCCCTCCCTATAAACG TCTCATTGCAATCCTTTCAAGT	$(ATC)_7$	58/65	212	204/212
H4H12	TCAAACAGAAATCCACCAAAAC CAAATTCATTTCCCATTTGTTG	$(GAA)_7$	60/72	193	193
H5A04	CTTCTTCCTTTTTGTTGACACCT ACCGAAAACACTTGTGATTGTTA	$(TAA)_{16}$	58/65	201	201 (+smear: 163–173)
H5A08	AGGAGAGAAAATGTAACATCCTAAATC CAAATTGGTTATTGATTACAATTAGGT	$(TTA)_{18}$ CCA $(TTA)_8$ TT $(TTA)_3$	58/65	218	218
H5B04	CATAATTTTAAAAGAGGCACGTTAAAT ATATAAGCAAAAATAAAGATGAGTTGC	$(TAA)_{11}$ ATAAAA $(TAA)_{25}$	57/60	211	211
H5B06	TGTCCAATTACATGGTAATGCTT CACAAAAACAAATTCTTCGACTG	$(TC)_6$ TT $(TC)_5$	60/72	266	266
H5B09	AATCACAAGACTCAACAATATAAGCAG TTCTCTCTAAAGGTGGTATGTTTGTAG	$(TTA)_{50}$	58/65	267	123
H5C121	GATTTGGATGAAACATGAAAACA TTGTTGCTAGGAAAAAGAGGAAT	$(\mathit{GA})_7$ 280 bp $(CAA)_5$	60/72	185	185
H5C122	ATTCCTACCACCAAGCTATTCAA CTGTGAAAAATTGAAGATTGCAG	$(GA)_7$ 280 bp $(\mathit{CAA})_5$	60/72	199	199
H5D02	TCAATTTAACTTGGCCCACTAAC AAGGTTTGTTGGTGTGAAGAACT	$(CAT)_6$	60/72	142	142
H5E02	AAGGAACTTAAACAGCGGCTACT GAGAATGAGCTAAAGATGACCTG	$(CAA)_5$	57/60	183	183
H5E05	AAAAAGCTCCTAGAGAGGGAGAG AATGATATCTTTTTAAAACCAGAAGG	$(GA)_4$ GG $(GA)_4$	60/72	192	192
H5E08	GAGAAATTTTATTTGTGGGGATG ACTCCCTCATTTTTCTCCTGTTT	$(GA)_5$	58.65	178	178
H5E11	ATTTATTTAGTGGCCTCACTCTCC GATTTCAAAATCCCTTTGTTCTAGTTA	$(TTA)_{34}$	58/65	218	218
H5F011	CATTGTTGTATCAAGGGATATGG CTATGGCAACTAGAGCCATGAAT	$(\mathit{TTA})_5$ 238 bp $(GAT)_6$ 22 bp $(TTG)_4$	60/72	173	173
H5F021	CAAATTTTGTAATTTGGTAAGTAGC GAAAATATATTAAAAATACAATTGGACGA	$(\mathit{TTA})_{31}$ 167 bp $(TTA)_4$ T $(TTA)_4$ 18 bp $(TTA)_9$ T $(TTA)_2$ $(AT)_3$ TAT $(TTA)_2$ TAT $(TTA)_7$	58/65	234	234
H5F11	CGTGACAACATCTTCCACTTATG GGTGATGTGATAAATTTGTGATGA	$(ATC)_5$	60/72	198	190/198
H5G01	ACCACTAAGCAATGTCTTCCTTC GACCTTTTCCTCCAGTCTTGTTT	$(TC)_4$ 7 bp $(TCA)_8$	60/72	192	192
H5G12	AAAGAGAGAAACACTTGTTCATGATAG CTTGAGTAGTTGCTTCTAACAAAGATG	$(TAA)_{36}$	57/60	201	104/201

Table 1 (Contd.)

Primer pair name	Primer pairs (5′–3′)	SSR loci [a]	Annealing/ elongation Tem (°C) [b]	Fragment size [a] (bp)	
				Expected	Observed [c]
H5H02	CATATTGTTGTACTGTCCTTTTGAG TATAATAAATACCTTCGGGGTTGAA	$(TTG)_3$ TTT $(TTG)_5$ TTT $(TTG)_2$ TTT $(TTC)_3$	60/72	148	148/190/197
H5H032	AATACCCCAATTTTGTCTGATACAT TGAAGCAAACCGAATAAAAGTTATC	$(TC)_{32}$ $(TA)_{12}$ 241 bp $(TA)_{13}$	58/65	213	213
H5H06	ACTTATTTTGCTCAACATCAAGACAC CACCGAAAATAATTGATTTAAGTAACA	$(TTA)_{33}$	58/65	221	187/196/208/221
H5H12	TTGTTACAATGCATATTTTTTTAGC AACTTATCCTTGTGTTTTTATAGGG	$(GA)_7$ GC $(GA)_4$ AAGG $(GA)_4$ CA $(GA)_9$ GG $(GA)_5$ GG $(GA)_7$	60/72	183	183
H6A03	GGGAGCTTAATTATTTCTTCATTACA ATGAATTCACTCAAAGACACAGATT	$(ATC)_3$ 27 bp $(TC)_4$	60/72	181	181
H6B11	AGCTCTCCATATCTGAGGCTTT TCACTGTATCGGAGTTCTACTGC	$(TTC)_6$	60/72	193	170/193/202
H6B12	TTTCTCACCTCGTTGGTATATGA CGTTTGATTGATGATAGTGATGC	$(ATC)_4$	60/72	183	183
H6C06	ACAATGCAATACTAATATGCAAAATTC AACCAGATTAAATAGGTGATTTTATGA	$(TAA)_3$ TGTA $(TAA)_{33}$	60/72	226	184
H6C07	CAAAAGTGCAATTAAGCCTACATAATA CCATTTTGTTTTAACACATATTTAACG	$(GAT)_3$ $(TAA)_{17}$ TCA $(TAA)_2$ TCA $(TAA)_{13}$TCA $(TAA)_5$	58/65	201	201
H6C09	TATGTGCGGTGAGACACTTATTT TTTGAGAAAGAGAGTGGAGGAAC	$(GTT)_3$	60/72	186	186
H6C11	TTGGGTTATCCAAAGAATAAAATATAA TTATATGGACTATGTATGTCAATTAAATC	$(TAA)_{23}$ $(CAA)_2$ $(TAA)_2$	58/65	188	188/191/212/218
H6D02	CTTCCGAATATGGACTTGGTTT CATAAATCTAAGTTACGGGTCTGGT	$(TA)_7$ AC $(TA)_7$	58/65	185	170/185
H6D05	TCTTTATCAAATCATTCAACTTACGA GACTAAACGTAGGAATAGTCTACTATGAAC	$(GA)_4$ AC$(GA)_3$ GG$(GA)_5$ TA$(GA)_3$[CA$(GA)_3]_3$	60/72	210	210
H6D11	AAAGATGGGAACTTGAGATGTTG AATAGCTACTCAAGGCTGAAGAAA	$(TAA)_{11}$	58/65	200	278
H6E07	GTTTAAATGGGTCTAAACAGTCGATTT GTGTGTGCATGCATATGTGTATAAT	$(TTA)_{41}$	58/65	240	240
H6F01	AAAGCAGAATGTCCATCAAATAAA TTGTTGACATAAATGACATGGTTC	$(GAA)_{10}$	60/72	204	192
H6F09	GCTGATTCAGAGTATGAGAAATTCA AGAAATGTGATTCCAACAATTCAA	$(TC)_8$ $(TA)_8$ $(TG)_9$	60/72	222	222
H6F10	TCATCACAAATTTATAACATCACTGTC TTTGATTAGATGAGTTTACGTGAGG	$(ATC)_4$ 46 bp $(ATC)_4$ 45 bp $(ATC)_4$	60/72	232	232/245
H6G01	ATTGAAGAAGGAACTAAACAACTATCG TCATCTTAGAGTTCAAAGAAAGAAACA	$(TAA)_{10}$ CAA $(TAA)_{14}$ [TTA $(TAA)_3]_2$ TTA $(TAA)_6$ TAT $(TAA)_{14}$ $(CT)_2$ $(CA)_2$ $(CT)_2$	60/72	321	168
H6G06	AAACCTATCAGCCCTAATTCAAA GACGAAGAATAAGAACCCAGATG	$(ATC)_4$	60/72	193	193
H6G07	TCTATCAGAGATATTAAGTTGAACG CGTGACAGAATTAGCCTCTTGT	$(TAA)_{23}$	60/72	189	189

H6G10	AAGACCTTCAATGGTAAAATTCG AGAGGATAAATCACCCATTTTGA	$(GA)_{12}$	60/72	200	200
H6H04	TTCAACCCTCAATTCTCTTTGAT GAGACCTCAATTGGGTACAAGAG	$(TC)_4$ TTCCC $(TC)_4$ 15 bp $(CAT)_4$ CACC $(CAA)_3$ 39 bp $(TC)_4$ 23 bp $(TC)_4$ 10 bp (TC)4	60/72	241	241
H6H111	AGTCATCAGAAAGAAAAGGCAAG TGTCAAAAGAAGGAAACATTCCA	$(\mathit{GAT})_5$ 99 bp $(GA)_4$	60/72	182	182
H6H112	TCTCACTTTCTTGTGTTTTCCAG TTACCCTAATAGATGGGTGTGGA	$(GAT)_5$ 99 bp $(\mathit{GA})_4$	60/72	171	171

[a]When more than one SSR was detected for a given locus, the SSR flanked by the specified primer pair is shown in *italics*
[b]Based on data of genomic DNA from the kabuli chickpea cv. Hadas
[c]Determined on 4.25% polyacrylamide gels, using the ABI PRISM 377-XL DNA Sequencer system. Amplicon size was established according to the highest amplicon peak in the GeneScan histogram

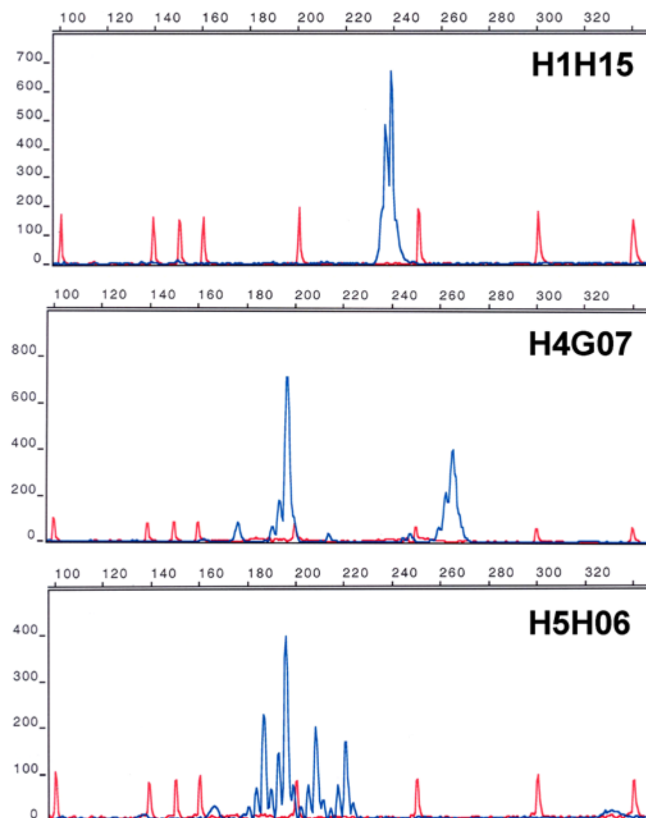

Fig. 2 Amplification products of the primer pairs H1H15, H4G07, and H5H06, using genomic DNA of cv. Hadas as template. The amplicons are depicted as *blue-lined peaks* in the histogram of readings of fluorescently labeled dUTP detected with the ABI PRISM 377-XL DNA Sequencer and analyzed with the GeneScan software. *Red lines* correspond to the internal size standard

independently among the recombinant inbred lines generating two and three markers, respectively.

Discussion

We constructed a BAC library and a BIBAC library from chickpea, *C. arietinum*. The two libraries contain a total of 38,016 clones and are equivalent to ca 7.0× genomes of chickpea, providing a greater than 99.9% probability of obtaining a single-copy sequence from the libraries. These two new libraries along with the recently published BIBAC library that has 23,780 clones equivalent to 3.8× genomes of chickpea (Rajesh et al. 2004) total 61,796 clones, equivalent to 10.8× genomes. This number of genome equivalents should be sufficient for various aspects of chickpea genomics research. Since the three libraries were developed from two different restriction enzymes with complementary recognition sites in which *Hin*dIII is AT-rich and *Bam*HI is GC-rich, it is expected that the genome coverage of these libraries would be more representative of the chickpea genome than the coverage based on a library constructed with a single enzyme. This is especially critical in the development of physical maps using the libraries (Chang et al.

2001; Tao et al. 2001; Ren et al. 2003, 2004; Wu et al. 2004a, c, d; Xu et al. 2004). Moreover, since different chickpea genotypes were employed for the construction of the libraries (Rajesh et al. (2004) used cv. FLIP 84–92C vs cv. Hadas used in the present study), the new BAC and BIBAC libraries represent different set of alleles that might be useful for future gene discovery studies. Additionally, the libraries were prepared using two different vectors, the F plasmid-based pIndigoBAC-5 that was used in the chickpea BAC library and the P2 plasmid-based pCLD04541 that was used in the BIBAC libraries developed in the present study and by Rajesh et al. (2004). The use of two different vector systems further enhances the true genome coverage of the libraries (Ren et al. 2004; Wu et al. 2004b). Finally, the transformability of the BIBAC libraries via *Agrobacterium* in plants would further enhance the utility of the libraries for chickpea genome research, especially positional cloning and functional analysis of the chickpea genome. It has been reported in several species that DNA fragments of larger than 100 kb could be transformed into plants via BIBACs by use of the *Agrobacterium*-mediated transformation (Hamilton et al. 1996, 1999; Liu et al. 1999, 2002; He et al. 2003). Application of the chickpea transformation protocols (Kar et al. 1996; Krishnamurthy et al. 2000) in combination with these plant-transformation ready BIBACs may allow the transformation of large regions containing desirable traits into cultivated chickpea lines for genetic improvement.

In the present study, we generated and characterized 233 new SSR markers for chickpea. These SSR markers significantly increase the available arsenal of 174 SSR markers previously reported by Winter et al. (1999). For 27 of the markers (11.6%), discrepancies were observed between the expected locus and the PCR products in terms of amplicon size and/or amplicon number. Presumably, the discrepancies in fragment size might be a result of artificial deletions or insertions during the DNA duplication in the host bacterium often observed due to the repetitive nature of the loci, but further studies will be needed to answer this question. Röder et al. (1998) found that the primer pairs that amplified fragments with unexpected sizes were non-functional since they were usually monomorphic. We tested the primer pairs, using two *C. arietinum* cultivars, Hadas and ICC5810; 39% of them resulted in clear polymorphic patterns (data not shown). Eleven percent of the fragments whose sizes deviated from the expected (Table 1) were found to be polymorphic among the chickpea lines, but about three to four times less than the general estimate of 39%. Of the primer pairs that produced more than one amplicon, 40% were found to be polymorphic among the lines. The SSR markers developed herein have resulted in useful tools for chickpea molecular genetic map construction and gene mapping (J. Lichtenzveig et al., in preparation). The SSR markers reported here were generated from a large-insert BAC library, whereas those of Winter et al. (1999) from a small-insert DNA library. The development of SSR markers from large-insert BACs has added several advantages to the markers (see "Introduction").

Analysis of the SSR motifs $(GA)_{10}$, $(TA)_{10}$, $(GAA)_7$, $(TAA)_7$, $(CAA)_7$, and $(TGA)_7$ (prefixed with H3-, H4-, H5-, or H6- in Table 1) showed that the SSR abundances were significantly different among different motifs in the chickpea genome, with the $(TAA)_n$ and $(GA)_n$ being the most abundant and the $(TGA)_n$ being extremely rare. The percentage (50.6%) of the SSR loci containing perfect repeats is much lower than that identified from a small-insert DNA library by using $(TAA)_n$, $(GA)_n$, and $(GAA)_n$ as probes (Winter et al. 1999). This discrepancy may be due to the different SSR oligo probe combinations, the source libraries, and/or preferential selection of strongly hybridizing clones for SSR isolation. The high percentage (49.4%) of the SSR loci containing interrupted and compound repeat motifs within a genomic span of 1,500 bp (the longest sequence) might suggest that the SSR loci tend to cluster in the genome. The clustering distribution of SSR loci was previously observed by genetic mapping of SSRs (Winter et al. 1999; J. Lichtenzveig et al., in preparation).

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