รายงานวิจัยฉบับสมบูรณ์

โครงการ

การพัฒนาเครื่องหมายพันธุกรรมชนิด Microsatellite และการสร้างแผนที่พันธุกรรม ของ มันสำปะหลัง (*Manihot esculenta* Crantz)

Development of microsatellite markers and construction of genetic linkage maps of cassava (*Manihot esculenta* Crantz)

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา
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Abstract

Simple sequence repeat (SSR) provide powerful tool for genetic linkage map construction that can be applied for identification of quantitative trait loci (QTL). In this study, a total of 640 new SSR markers were developed from an enriched-genomic DNA library of the cassava variety 'Huay Bong 60' and 1500 novel expressed sequence tag-simple sequence repeat (EST-SSR) loci were developed from the Genbank database. To construct a genetic linkage map of cassava, a 100 F_1 line mapping population was developed from the cross Huay Bong 60 by 'Hanatee'. Polymorphism screening between the parental lines revealed that 199 SSRs and 168 EST-SSRs were identified as novel polymorphic markers. Combining with previously developed SSRs, we report a linkage map consisted of 510 markers encompassing 1,420.3 cM, distributed on 23 linkage groups with a mean distance between markers of 4.54 cM. Comparison analysis of the SSR order on the cassava linkage map and the cassava genome sequences allowed us to locate 284 scaffolds on the genetic map. Although the number of linkage groups reported here revealed that this F_1 genetic linkage map is not yet a saturated map, but it encompassed around 88% of the cassava genome indicating that the map was almost complete. Therefore, sufficient markers now exist to encompass most of the genomes and efficiently map traits in cassava. In addition, 34 QTL underlying fresh root yield and starch content were identified. Most of the QTL identified here are considered as a major QTL due to their %PVE of grater than 10%. Some of the QTL were found at the same locus or within the same region, indicating that they are reliable and potential QTL. The information found in this study will be useful for future study in order to pin point the position of tightly linked markers and identify gene controlling the traits as well as applicable to marker assisted selection of cassava.

Keywords: Cassava; Expressed sequence tag-simple sequence repeat (EST-SSR); Genetic linkage map; Quantitative trait loci (QTL); Simple sequence repeat (SSR

บทคัดย่อ

การสร้างแผนที่พันธุกรรมนั้นสามารถนำมาใช้เพื่อศึกษาหาตำแหน่งยีน (Quantitative trait loci: QTL) ที่ควบคุมลักษณะที่สนใจ โดยเครื่องหมายพันธุกรรมที่นิยมนำมาสร้างแผนที่พันธุกรรมมากที่สุด คือ เครื่องหมายพันธุกรรมชนิดไมโครแซทเทลไลท์ (simple sequence repeat: SSR) ในการศึกษาครั้งนี้จึงได้พัฒนาเครื่องหมายพันธุกรรมชนิดไมโครแซทเทลไลท์จากห้องสมุดจีโนม (enriched-genomic DNA library) ของมันสำปะหลังสายพันธุ์ห้วยบง 60 จำนวน 640 คู่ และพัฒนาเครื่องหมายพันธุกรรมชนิดไมโครแซทเทลไลท์จากฐานข้อมูล EST (expressed sequence tag SSR: EST-SSR) ของมันสำปะหลัง จำนวน 1,500 คู่ จากนั้นนำเครื่องหมายพันธุกรรมทั้งหมดมาวิเคราะห์ความแตกต่างทางพันธุกรรม (polymorphism) ระหว่างมันสำปะหลังสายพันธุ์ห้วยบง 60 และห้านาที พบเครื่องหมายพันธุกรรม SSR จำนวน 199 คู่และเครื่องหมายพันธุกรรม EST-SSR จำนวน 168 คู่ แสดงความแตกต่างทางพันธุกรรมระหว่างมันสำปะหลังสองสายพันธุ์ โดยเครื่องหมายพันธุกรรมดังกล่าวถูกมาศึกษาหารูปแบบพันธุกรรมในตัวอย่างมันสำปะหลังลูกผสมรุ่นที่ 1 จำนวน 100 ต้น เพื่อนำมาใช้ในการสร้างแผนที่พันธุกรรม โดยข้อมูลพันธุกรรมที่วิเคราะห์ได้นี้ถูกนำมารวมกับข้อมูลทางพันธุกรรมที่ได้จากการศึกษาก่อนหน้านี้ พบว่าแผนที่พันธุกรรมประกอบด้วย 23 กลุ่ม มีเครื่องหมายพันธุกรรม 510 ตำแหน่ง (loci) ความยาวรวม 1,420.3 cM และมีค่าเฉลี่ยระหว่างเครื่องหมายเท่ากับ 4.54 cM โดยแผนที่พันธุกรรมจากการศึกษาครั้งนี้ครอบคลุมจีโนมของมันสำปะหลังถึง 88% เมื่อเปรียบเทียบลำดับเครื่องหมายพันธุกรรมบนแผนที่พันธุกรรมกับลำดับเบสของดีเอ็นเอเส้นยาว (scaffold) จากฐานข้อมูลมันสำปะหลัง พบว่าสามารถระบุตำแหน่งลำดับเบสของดีเอ็นเอเส้นยาวจำนวน 248 scaffold บนแผนที่พันธุกรรม และผลการวิเคราะห์หาตำแหน่งยีนที่ควบคุมลักษณะปริมาณผลผลิตและปริมาณแป้ง พบ 34 ตำแหน่งที่สัมพันธ์กับลักษณะดังกล่าว โดยตำแหน่งของยีนส่วนใหญ่อธิบายความแปรผันทางลักษณะ (phenotypic variance explained: PVE) มากกว่า 10 % นอกจากนี้พบตำแหน่งของยีนอยู่บริเวณเดียวกัน ซึ่งถือได้ว่าเป็นบริเวณที่มีความน่าเชื่อถือว่าเป็นตำแหน่งยีนที่ควบคุมปริมาณผลผลิตและปริมาณแป้งในมันสำปะหลัง ผลการศึกษาในครั้งนี้เป็นประโยชน์อย่างยิ่งต่อการศึกษาหาเครื่องหมายพันธุกรรมที่สัมพันธ์และอยู่ใกล้กับตำแหน่งของยีนซึ่งควบคุมลักษณะที่แสดงออกมากที่สุด และสามารถนำเครื่องหมายพันธุกรรมดังกล่าวมาประยุกต์ใช้ในการคัดเลือกสายพันธุ์ (marker assisted selection: MAS) ในมันสำปะหลังให้มีลักษณะที่สนใจต่อไป

คำสำคัญ: มันสำปะหลัง; เครื่องหมายพันธุกรรมชนิดไมโครแซทเทลไลน์จากฐานข้อมูล EST; แผนที่พันธุกรรม; ตำแหน่งยีนที่ควบคุมลักษณะเชิงปริมาณ; เครื่องหมายพันธุกรรมชนิดไมโครแซทเทลไลน์; ปริมาณแป้ง; ปริมาณผลผลิต

Executive Summary

Introduction

Cassava (*Manihot esculenta* Crantz) is a major food crop that constitutes the most important sources of energy in the diet of most tropical countries of the world. Thailand is the world largest exporter cassava products. Every part of cassava can be utilized, but the important part is starchy roots which are used in starch industrial applications. In the past, improvement of cassava line relies on the conventional breeding that faces several limitations such as expensive, labor intensive, long cropping cycle and a number of years to develop new varieties that lead to application of Marker Assisted Selection (MAS) to identify those progenies that have desirable traits using genetic markers that linked to the traits.

Most of agronomically important traits are quantitative traits, which are typically measured in a continuous scale and resulted from the effect of multiple genes and environmental factors. The Quantitative Trait Locus (QTL) is a region on the chromosome that determines the variation of the quantitative trait. In order to use MAS to locate the position of the QTL, it is necessary to develop and identify markers that linked to the QTL, and have sufficiently mapped its genome.

There are several types of DNA markers that can be applied into MAS such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Microsatellite or Single Sequence Repeat (SSR), and Expressed Sequence Tag (EST). In this project, however we aimed to develop SSR markers from cassava variety Huay Bong 60, construct genetic linkage map and identify the QTL underlying yield and starch content of cassava.

Objectives

1. To develop new SSR markers for cassava
2. To construct genetic linkage map of cassava using F_1 progenies
3. To identify QTL of yield and starch content traits

Materials and Methods

An enriched SSR library and development of SSR marker

An SSR enriched-genomic DNA libraries were constructed from genomic DNA of cassava varieties "Huay Bong 60" (HB60) and "Hanatee" (HT) described by Sato et al. (Sato et al. 2005) with some modifications. Genomic DNA was extracted using QIAGEN kit and measured by NanoDrop. Five ug of DNA was digested with 3 different enzymes; *Alu*I, *Hae*III and *Afa*I. Each digestion was purified using Wizard SV gel and PCR clean-up system (Promega) and ligated with double strand linker. For the first four enriched-genomic DNA libraries from HB60, 2 µl of each digested-ligated DNA was pooled together and hybridized with $(AC)_{12}/(CT)_{12}$, $(AT)_{12}/(CTT)_8$, $(ACC)_8/(AGC)_8$ and $(GC)_{12}/(GGC)_8$ biotinylated-oligo probes, respectively for overnight. Whereas libraries 5 to 7 were developed from HT and hybridized with $(GAT)_8$, $(GGA)_8$ and $(AAAC)_6$ probes, respectively.

Hybridized-biotinylated oligo DNA was bound with Dynabeads-streptavidin for obtaining DNA fragments that contained SSR motifs. Then linker-ligated and oligo-probe hybridized DNA were amplified with linker to increase the number of DNA fragments and PCR products were run on 1% Seakem GTG. The expected size about 700-2,500 bp. was excised from agarose gel and purified using QIAquick gel extraction kit (Promega). Purified DNA was ligated using TOPO TA cloning Kit for sequencing according to the manufacture's instructions. The ligation product was transformed

into ElectroTen-Blue competent cells (Stratagene) by electroporation. The transformants were selected on LB-amp containing X-gal and IPTG. The insert size of white colonies was checked by PCR using T7 and T3 primers and PCR product was run on 1% agarose gel. Finally, amplified product was sequenced and sequencing results were used to find SSR motif and design SSR primer.

EST SSR analysis

A total of 76,566 ESTs obtained from the Genbank EST database was assembled to 28,940 unique sequences. Of these, 1,500 unique sequences were subjected to prime design using the Primer 3 program. The PCR product size was designed to be in range of 90-300 bp.

Construction of QTL map for yield and starch content

1. Plant materials and phenotypic measurement

In 2006, the F_1 mapping population comprising of 100 F_1 plants from a cross between Huay Bong 60 (female parent) and Hanatee (male parent) were developed. In 2007, stem cutting of each progeny of 100 F_1 were planted at Rayong Research Center. Phenotypes such as fresh root yield, fresh starch and extracted starch were evaluated at 12 months after planting base on 10 plants per line. In 2008 each sample was cut and planted at Lopburi and Rayong Research Centers. Fresh root yield (kg/rai) and fresh starch content (%) were measured at 12 months after planting. Finally, in 2009 each sample was cut and planted at Rayong Research Centers and fresh root yield (kg/rai) and fresh starch content (%) were measured.

2. SSR and EST-SSR analysis and genetic linkage construction

Genomic DNA of parental lines and 100 F_1 samples was isolated from frozen leaf using CTAB method (Sambrook and Russell, 2001). A total of 1,339 SSR primer pairs, 667 developed by CIAT (The International Center for Tropical Agriculture) and Mba et al (2001) and 672 in this study, were used to screen with the parents and the informative primers were genotyped in the F_1 population. In addition, 1,500 EST-SSR primer pairs that were developed with collaboration with Kazusa DNA Research Institute (KDRI) were also used in SSR analysis.

For SSR and EST-SSR analysis, PCR reactions were performed in 15 μl total volume containing 25 ng of DNA template, 0.06 μM each of reverse and forward primers, 0.2mM of each dNTP, 1X PCR buffer, 2.5 mM MgCl2 and 0.5 U *Taq* DNA polymerase. PCR cycle was set up at 95 ºC, 2 min then 35 cycles of denaturing at 95 ºC for 2 min, annealing at 55 ºC for 45 sec and extension at 72 ºC for 1 min following with final extension at 72 ºC for 5 min. PCR products were separated on 5% denaturing polyacrylamide gel and visualized by silver staining method (Benbouza et al., 2006). Genotypic data were scored as CP code as described by Ooijen and Voorrips (2001).

For linkage map construction, all informative SSR markers were used to construct linkage map by JoinMap version 3.0 (Van Ooijen and Voorrips, 2001). The threshold value of LOD score was set at 3.0 with recombination fraction of 0.5. Recombination fractions were converted to map distance (cM) using Kosambi mapping function.

3. QTL analysis

MapQTL 4.0 software (Van Ooijen et al., 2002) was used to perform the quantitative trait loci analysis of all traits. Permutation tests were performed to estimate appropriate chromosome wide and genome wild significant thresholds corresponding to an error level α =0.05 for declaring a significant QTL.

First QTL analysis was performed using interval mapping (IM) and the LOD score exceeded the chromosome wide significant thresholds were included in automatic cofactor selection analysis. Only markers significantly associated with each trait at $p < 0.02$ were used in multiple QTL method (MQM). If the LOD value for QTL linked with cofactor dropped below the chromosome wide threshold, it was removed from the cofactor list and MQM was run again to obtain the best set of QTL. The identified QTLs were mapped with Mapchart 2.1 software (Voorrips 2002)

Results

In this study, 712 (58.3%) useful SSR primer pairs were designed from those sequences. The predominant microsatellite motifs were di-nucleotide repeats (73.03%), while 18.68 % and 8.29 % were tri- nucleotide repeats and tetra- nucleotide repeats, respectively. The majority of di-nucleotide motifs were AC/TG repeats (56.15%); the majority of tri- nucleotide repeats were AAT/TTA (21.05%) or AAG/TTC (21.05%); and the predominant tetra- nucleotide repeat was AAAT/TTTA (28.81%). A total of 640 of the 712 (89.89%) useful SSR primer pairs were suitable for detection of polymorphisms between the DNA of the population parents. The analysis of the population showed 439 (68.59%) SSR primer pairs that were successfully amplified in all DNA samples. Of these, 199 (31.09%) markers were found to be informative markers that showed heterozygous pattern in either or both parental lines, whereas 240 (37.50%) pairs were non-informative. Additional 1,500 new primer pairs amplifying microsatellites in the cassava EST database were screened. These primers were designed to amplify microsatellites with di- (1,012; 67.47%), tri- (438; 29.20%) and tetra- (50; 3.33%) nucleotide repeats. Genomic DNA of parental lines were tested with all new EST-SSR primers, the results showed that 1,222 (81.47%) primer pairs successfully amplified genomic DNA. Of these, 168 (13.75%) primer pairs showed informative polymorphism.

In addition to these newly synthesized primers (199 SSRs and 168 EST-SSRs), 277 of informative SSRs that were developed by CIAT and 81 informative EST-SSR markers from Kunkeaw et al. (2010b) were also included in this genetic linkage map construction. In total, 725 informative markers were successfully genotyped within the 100 F_1 individuals of the mapping population and subjected to linkage map analysis. All of 725 markers were used for linkage map analysis with LOD score set at 4.0. The linkage map of the F_1 population consisted of 510 SSR markers distributed on 23 linkage groups (Fig 1). The map encompassed 1,420.3 cM. Linkage groups ranged in length from the 2.0 cM of LG23 to the 120.42 cM of LG2. The total number of markers in each linkage group varied from 2 to 71. The mean size of linkage groups was 61.8 cM containing 22.2 loci. The mean distance between linked markers was 4.54 cM but ranged from 0.1 to 26.1 cM.

To search for the location of SSR loci on the cassava genome sequence, primer sequences were searched for sequence homology against the scaffolds of the cassava genome (ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v5.0/Mesculenta) using BlastN. We were able to identify the locations of 481 (94.3%) SSR loci on the linkage map scattering on 284 scaffolds of the cassava genome.

QTL analysis was performed using MQM method of MapQTL 4.0. Seventeen QTL were identified in fresh root yield trait from different locations, Rayong and Lopburi Field Crop Research Stations. Percentage of phenotypic variance explained (PVE) ranged from 9.1% to 23.3%. For fresh starch content, seventeen QTL were identified with the PVE value ranged from 9.8% to 47.6%. For starch content, 17 QTL were identified consisting of 14 and three QTL found at Rayong and Lopburi locations, respectively with %PVE ranged from 9.8-47.6. Two QTL, fstr_Ry08_3 and fstr_Ry09I_4 were found in the same region (ESSRY48-EME303) on linkage group 12 as well as QTL fstr_Ry09I_1 and fstr_Ry09II_2 were found at the region between NS248-NS978 on linkage group 7. The QTL that were found within the same region indicated that they are potential QTL. More over,

those QTL that have %PVE of grater than 10% especially the QTL fstr_Ry09I_5 which has %PVE of 47.6 are also considered as major QTL.

Conclusion and discussion

Microsatellites or SSRs are the most widely used DNA markers for genetic linkage map analysis. SSRs provide powerful tool for genetic linkage map construction that can be applied for identification of QTL. Importantly, the marker linked to the QTL can be further applied to marker-assisted selection (MAS) in cassava breeding program for selecting cassava plant that contains desirable phenotype. This study, a total of 640 SSR and 1500 EST-SSR primer pairs were developed. All of these primers were tested with the parental lines, Huay Bong 60 by Hanatee, resulting of 181 SSR and 118 EST-SSR polymorphic loci. These loci were then genotyped with 100 sample of F_1 population. The genotypic data was then combined with previous one which was generated using 277 SSRs and 81 EST-SSR markers. In total, 725 informative markers were used to construct a genetic linkage map of cassava. The results showed that our map encompassed 1420.3 cM or around 88% of the genome indicating that the map was almost complete. The map was then used for QTL analysis. Phenotypic data of fresh root yield and starch content was obtained and analyzed together with the genotypic data. The results showed that 17 QTL specific to fresh root yield and other 17 QTL for starch content were identified. Most of the QTL identified here are considered as a major QTL due to their %PVE of grater than 10%. Some of the QTL were found at the same locus or within the same region, indicating that they are reliable and potential QTL. All of the information will be useful and applicable for future study in order to pin point the position of tightly linked markers and identify gene controlling the traits. However, additional markers within the QTL regions will be required before applying the linked markers to marker assisted selection of cassava.

เนื้อหางานวิจัย

Introduction

Cassava (*Manihot esculenta* Crantz) is an important crop that is commonly grown in tropical and subtropical areas. It is used as a major source of carbohydrate for over 500 million people (El-Sharkawy 2004). Cassava crop is also used to produce chip/pellets for starch production, animal feed and used as a raw material for ethanol fermentation. Cassava has an allopolyploid genome, with 2n=36 chromosomes with the estimated size of the haploid genome of about 772 mega-base pairs (Bennett et al. 1982). It has DNA content of 1.67 pg per cell nucleus (Awoleye et al. 1994).

Molecular markers are powerful tools for marker-assisted selection (MAS) in plant breeding (Collard and Mackill 2008; Ribaut and Hoisington 1998). MAS is more efficient, effective, reliable and cost-effective than conventional selection for many traits during plant breeding (Collard et al. 2005). Microsatellite or SSR markers are widely used to construct genetic maps, associate traits with underlying genomic regions and for MAS (Varshney et al. 2005). Microsatellites are found in all eukaryotic genomes. They consist of 1-6 bp. of nucleotide motifs repeated in 5-20 copies (Field and Wills 1996) distributed throughout the genome both in coding and non-coding regions (Kashi et al. 1997). Moreover, SSRs are co-dominant in inheritance, reproducible, highly polymorphic, simple and cheap to use (Gupta and Varshney 2000; Song et al. 2004). The use of genomic DNA enriched for satellites to produce-libraries for DNA sequencing was a common and reliable technique to develop markers in many plant species, including wheat (Song et al. 2005), maize (Sharopova et al. 2002), peanut (He et al. 2003), onion (Tsukazaki et al. 2007) and red clover (Sato et al. 2005). Alternatively, SSRs have been developed from published sequence databases of ESTs (Feng et al. 2009; Raji et al. 2009; Tangphatsornruang et al. 2008; Yu et al. 2004a; Yu et al. 2004b) and/or BAC end sequence databases (La Rota et al. 2005; McCouch et al. 2002; Shultz et al. 2007; Temnykh et

al. 2001). Although EST derived SSRs showed less polymorphism than genomic SSRs, they are directly linked to expressed genes (Cho et al. 2000; La Rota et al. 2005; Shultz et al. 2007). Therefore, direct association can be made between genotype and phenotype leading to identification of QTL underlying the traits of interest using EST linkage map (Rudd 2003).

For cassava, SSRs have been developed and used in genetic linkage map construction (Fregene et al. 1997). The first genetic linkage map of cassava was constructed from F_1 intra-specific cross using SSR, RFLPs, RAPDs, and isoenzymes. Later more SSRs (Chavarriaga-Aguirre et al. 1998) and EST-SSRs (Raji et al. 2009; Tangphatsornruang et al. 2008) were developed for germplasm evaluation in cassava and its related species. In addition, 172 SSR markers were developed from genomic DNA derive satellite enriched library and mapped in an F_1 population (Mba et al. 2001). In 2006, a genetic map of an F_2 population was developed using SSR markers (Okogbenin et al. 2006). Kunkeaw et al. (2010a; 2010b) presented a composite map of an F_1 population that consisted of AFLP, SSR and EST markers. However, none of these maps could completely encompass the genome of cassava. A recent genetic map of cassava was constructed using F_1 population (Chen et al. 2010). The map consisted of 18 linkage groups with a total length of 1,707.9 cM., however the map was based on AFLP markers.

Here the aim was to develop additional SSR markers from genomic DNA enriched for SSRs and the Genbank EST database from cassava and to construct genetic map using a F_1 mapping population.

Methods

Plant materials

Huay Bong 60 is a commercial cassava variety for Thailand that was developed by a cross between 'Rayong 5' and 'Kasetsart 50'. Huay Bong 60 is widely grown due to its high starch content and biomass yield. However, it is a bitter type with high cyanide content. 'Hanatee' is a local variety with low levels of starch content, biomass and yield. It is classified as a sweet type due to low cyanide content. An F_1 population consisting of 100 individuals, derived from crosses of Huay Bong 60 by Hanatee was developed in 2006 and used for linkage map analysis. All F_1 plant samples were propagated by stem cutting and grown at the Rayong Field Crops Research Center, Thailand. The distance between planting row was 1.5 m and 1 m between individual plants. Fertilizer (15:15:15), 312.5 kg/Hectare, and chicken manure, 3,100 kg/Hectare was be applied at one month after planting. Pest management was applied as necessary.

Phenotypic evaluation

Phenotype, fresh root yield, starch content was collected in years 2007, 2008 and 2009. In 2007 and 2008, plants were grown at Rayong Field Crop Research Center and the phenotypic data was evaluated at 10-12 months after planting. In 2009, all phenotype will be evaluated in comparison between two locations, Rayong Field Crop Research Center and Lop Buri Research Station. For starch content, a total of five kg of fresh root of cassava plant will be randomly selected and starch content will be measured using Reimann scale. Each line of the cassava that planted at the same location will be evaluated using four replications of the samples. Fresh root yield value of individual plant was recorded as fresh root weight. The recorded values were averaged in order to obtain the candidate value of each plant line.

SSR-enriched genomic libraries construction

An enriched-genomic DNA library was constructed from Huay Bong 60 as described by Nunome et al. (2006) with some modifications. Leaf tissue was collected for DNA extraction using DNeasy Plant Mini Kit (QIAGEN, Hilden Germany). Four libraries were constructed. For the first library, 20 µg of genomic DNA was digested with two different restriction enzymes; *Alu*I (Fermentas, Hanover, MD U.S.A.), *Hae*III (TaKaRa Bio Inc., Ohtsu-shi, Japan). The other 3 libraies were each made from DNA digested with three different restriction enzymes; *Alu*I (Fermentas), *Hae*III (TaKaRa Bio Inc.) and *Afa*I (TaKaRa Bio Inc.). Each digested DNA was purified using Wizard SV Gel and PCR Clean-Up System (Promega, Madsison WI, U.S.A.) and ligated with double stranded DNA linkers (linker 1: 5'-GTTTAGCCTTGTAGCAGAAGC-3' and linker 2: 5'-pGCTTCTGCTACAAGGCTAAACAAAA-3'). Ligation reactions were performed in 60 µl reactions containing 10 µM DNA linkers, 1x Rapid ligation buffer, 24 U of T4 DNA ligase (Promega), 20 U *Xmn*I (Promega), 10 U of each restriction enzyme in individual reaction: *Alu*I, *Hae*III and *Afa*I. Ligation reactions were accomplished by 25 cycles of 30 min at 16 ºC, and 10 min at 37 ºC, and incubated at 16 ºC overnight.

For the first library, 5 µl of linker-ligated DNA of *Alu*I and *Hae*III were pooled and hybridized with 1.5 µM of a biotinylated oligo probe of $(AC)_{12}/(CT)_{12}$. Libraries 2, 3 and 4, three different linker-ligated DNAs of *Alu*I, *Hae*III and *Afa*I, were pooled and hybridized with $(AT)_{12}/(CTT)_8$, $(ACC)_8/(AGC)_8$ and $(GC)_{12}/(GGC)_8$ probes, respectively.

All hybridized reactions were incubated at 95 ºC for 15 min followed by incubating overnight at different temperatures; 42 ºC for libraries 1 and 2, 50 ºC for library 3 and 58 ºC for library 4. One hundred and fifty microliters (1,500 µg) of Dynabeads (Dynabeads M-280 Streptavidine-DYNAL; Invitrogen Dynal AS. Smestad, Norway) were washed with B&W buffer (10mM Tris-HCl pH 7.5, 1mM EDTA, 2M NaCl) following by centrifugation and collection of magnetic beads (repeated 3 times). After adding hybridized-biotinylated oligo DNA into Dynabeads, the mixture was incubated at

43 ºC for 2 h, and the supernatant was removed by placing on a magnetic separation stand. The captured complexes were washed with 400 µl of 2× SSC/0.1% (w/v) SDS, twice for 5 min at room temperature followed by twice at 42 ºC for 5 min with 1× SSC/0.1%. After the supernatant was discarded, 120 µl of pre-heated TE buffer (at 95 ºC) was added and incubated for 10 min at 95 ºC to elute the captured DNA.

The eluted DNA was used for PCR amplifications with linker 1 primer. The PCR reactions were set up using 1x PCR Buffer (Mg^{2+} plus), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.8 µM linker, and 5U Ex-*Taq* Polymerase (TaKaRa Bio Inc.). The PCR profile was 94 ºC for 30 s, followed by 30 cycles of 94 ºC for 30 s, 60ºC for 1 min, 68 ºC for 1 min, then 68 ºC for 7 min. PCR products derived from the captured DNA were separated by electrophoresis on 1% Seakem GTG agarose gel (FMC, Bioproducts, Rockland, U.S.A.) at 20 V overnight and stained with ethidium bromide. The expected DNA bands of sizes between 750 bp and -2.5 kbp was excised in a block and embedded by converting the large size of DNA fragment on the top of 0.8% Sea Plaque GTG agarose gel (FMC Bioproduct). After that, the agarose was cut into 50 ml tube and weighed to determine the appropriate volume of 1x agarase buffer (1g of gel equal to 10 ml 1x agarase buffer). The gel was melted at 70 ºC for 15 min and cooled down to 45 ºC before β agarase (2 U/200 mg of gel) was added and incubated at 45 ºC for 1 h The DNA fragments were ethanol precipitated and the pellet washed in 70% (v/v) ethanol. The PCR products A-tailed by the following reaction mixture: 150-200 ng of PCR product, 1x Buffer, 0.2 mM dATP, 1.5 mM of $MgCl_2$, 2.5 U r*Taq* polymerase (TaKaRa Bio Inc.) and incubated at 70 ºC for 30 min.

The A-tailed PCR products were cloned into pGEM-T Easy vector (LigaFast Rapid DNA ligation system, Promega) according to the manufacturer's instructions. The enriched library was transformed into the ElectroTen-Blue Electroporation competent cells (Stratagene, La Jolla, CA, U.S.A.) by electroporation. The transformants were selected on LB-agar plates containing ampicillin,

X-gal and IPTG (TaKaRa Bio Inc.). White colonies were used to find the insert size by PCR using T7 and SP6 primers after growth in 96-well plates. Inserts were amplified using TempliPhi 100 Amplification Kit (GE Healthcare, Piscataway, NJ) according to the manufacture's instructions. Finally, Amplified DNA was sequenced.

EST SSR analysis

A total of 76,566 ESTs obtained from the Genbank EST database was assembled to 28,940 unique sequences (Kunkeaw et al. 2010b). The identification and localization of microsatellites were conducted as described in Kunkeaw et al. (2010b).

Primer designs

DNA sequences of clones were trimmed and analyzed to identify repeat regions. SSR primers were designed from flanking regions of SSR containing sequences using the Primer 3 program. The PCR product size was designed to be in range of 90-300 bp (Sato et al. 2005).

Analysis of SSR markers

SSR primers were analyzed with genomic DNA of cassava varieties Huay Bong 60 and Hanatee. Informative SSR markers that showed heterozygous pattern in either female or male parent were used to genotype 100 F_1 progenies of the cross. Polymerase chain reaction (PCR) was performed as described in Kunkeaw et al. (2010a) in 15 µl reaction volumes containing 25 ng of genomic DNA, 0.2 µM of each primer, 200 µM dNTPs (Promega), 1x PCR Buffer, 1.5 mM $MgCl_2$, and 1 U *Taq* DNA polymerase (Promega). PCR was accomplished by 2 min at 94 ºC, followed by 30 cycles 45 sec at primer annealing temperature, and 1 min at 72 ºC for 30 cycles. The PCR amplification products were visualized on 5% (w/v) denaturing polyacrylamide gel and visualized by silver staining (Benbouza et al. 2006).

Genetic linkage analysis

Genotypic data of SSR markers were scored as CP codes as described by (Van Ooijen and Voorrips 2001). All informative SSR markers were used to construct a genetic linkage map using JoinMap® version 3.0 (Van Ooijen and Voorrips 2001). The genotype data was scored as CP codes (eg. <abxcd>, <efxeg>, <lmxll>, <nnxnp> and <hkxhk>). Linkage groups were determined using a LOD threshold of 4.0. Map construction was performed, using the Kosambi mapping function with JoinMap parameter settings as follows: Rec = 0.5, LOD = 4.0 and Jump = 5 (Kosambi 1944). To compare the order of SSR loci with the cassava genome sequence, primer sequences were searched for sequence homology against the scaffolds of the cassava genome (ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v5.0/Mesculenta) using BlastN.

QTL analysis

The genetic linkage map was used for QTL analysis, based on the segregation of DNA markers in the population from a cross between heterozygous parents. By the use of computer package program mapQTL®/version 4.0 (Van Ooijen et al., 2002), quantitative trait loci of starch content, yield, cyanide content, amylose content and other agronomically important traits were identified using a composite interval mapping (Zeng, 1994) or multiple-QTL models (MQM) mapping (Jansen and Stam, 1994). The MQM was done using a hybrid of simple interval mapping and multiple regressions (Visscher et al., 2000) on marker genotypes. Markers on same chromosome and on different chromosomes were used as cofactors to absorb the effects of nearby QTL, thereby increasing the power for mapping. Therefore there should be greater power in detecting a QTL, and the effects of the QTL should be estimated more precisely (Broman and Speed, 1999). By this method, a two-stage MQM analysis was performed. In the first stage, map QTL permutation tests was carried out on all the mapped markers to determine appropriate threshold value for declaring a significant QTL effect with interval mapping. The LOD values of $P = 0.05$ and $P = 0.01$ were taken as the estimated critical values in which to declare the presence of a QTL. The LOD profiles from

interval mapping were inspected and the closest marker to each LOD peak was selected as a cofactor and used in MQM mapping analysis to search for other peaks in the LOD profiles. This suggested further co-factors to be included in the analysis. These co-factor markers were implemented in the MapQTL® 4.0 to select the best model for the second stage MQM analysis. Kruskal-Wallis analysis is the nonparametric analogue of a one-way ANOVA supported in mapQTL version 4.0. It was most commonly used when the measurement variable does not meet the normality assumption of an analysis of variance. In this method, single marker analysis was used to test a marker associated with the segregation of a QTL between individual markers and the trait at significant $0.0001 \leq P \leq 0.01$. QTL mapping on genetic linkage map was drawn by software MAPCHART Version 2.2 (Voorrips, 2001).

Results

Ninety-six hybridizing clones from each library were selected for sequencing. The results showed that library 1 contained most SSRs that were useful for design primers. Additional clones (2,016) from the library 1 were then selected and sequenced. From 2,400 clones, 2,269 (94.5%) were successfully sequenced. Of these, 1,576 (69.4%) clones contained microsatellite regions with 1,221 (77.4%) in non-redundant sequences. This indicated that our genomic libraries are highly enriched with microsatellite regions.

In this study, 712 (58.3%) useful SSR primer pairs were designed from those sequences. The predominant microsatellite motifs were di-nucleotide repeats (73.03%), while 18.68 % and 8.29 % were tri- nucleotide repeats and tetra- nucleotide repeats, respectively. The majority of di-nucleotide motifs were AC/TG repeats (56.15%); the majority of tri- nucleotide repeats were AAT/TTA (21.05%) or AAG/TTC (21.05%); and the predominant tetra- nucleotide repeat was AAAT/TTTA (28.81%) as shown in Table 1.

A total of 640 of the 712 (89.89%) useful SSR primer pairs was suitable for detection of polymorphisms between the DNA of the population parents. The analysis of the population showed 439 (68.59%) SSR primer pairs that were successfully amplified in all DNA samples. Of these, 199 (31.09%) markers were found to be informative markers that showed heterozygous pattern in either or both parental lines, whereas 240 (37.50%) pairs were non-informative.

In this study, we screened 1,500 new primer pairs amplifying microsatellites in the cassava EST database. These primers were designed to amplify microsatellites with di- (1012; 67.47%), tri- (438; 29.20%) and tetra- (50; 3.33%) nucleotide repeats, as present in Table 2. Genomic DNA of parental lines were tested with all new EST-SSR primers, the results showed that 1,222 (81.47%) primer pairs were successfully amplified genomic DNA. Of these, 168 (13.75%) primer pairs showed informative polymorphism. The sequences of SSR and EST-SSR primers developed in this project are available upon request.

In addition to these newly synthesized primers (199 SSRs and 168 EST-SSRs), 277 of informative SSRs that were developed by CIAT and 81 informative EST-SSR markers from Kunkeaw et al. (2010b) were also included in this genetic linkage map construction. In total, 725 informative markers were successfully genotyped within the 100 F_1 individuals of the mapping population and subjected to linkage map analysis. All of 725 markers were used for linkage map analysis with LOD score set at 4.0. The linkage map of the F_1 population consisted of 510 SSR markers distributed on 23 linkage groups (Fig 1). The map encompassed 1,420.3 cM. Linkage groups ranged in length from the 2.0 cM of LG23 to the 120.42 cM of LG2. The total number of markers in each linkage group varied from 2 to 71. The mean size of linkage groups was 61.8 cM containing 22.2 loci. The mean distance between linked markers was 4.54 cM but ranged from 0.1 to 26.1 cM.

To search for the location of SSR loci on the cassava genome sequence, primer sequences were searched for sequence homology against the scaffolds of the cassava genome (ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v5.0/Mesculenta) using BlastN. We were able to identify the locations of 481 (94.3%) SSR loci on the linkage map scattering on 284 scaffolds of the cassava genome (Table 3).

QTL analysis was performed using MQM method of MapQTL 4.0. Chromosome wide significant threshold (αc) was used for accepting QTL. For fresh root yield, fourteen QTL were detected on nine linkage groups (LG 1, 4, 6, 7, 9, 10, 11, 12, and 14) from Rayong in 2007 (three QTL), 2008 (three QTL), 2009 (five QTL) and 2010 (three QTL) as shown in Table 4 and Figure 2. Percentage of phenotypic variance explained (PVE) or %PVE was ranged from 9.1-23.3. For Lopburi location, three QTL were found in 2009 and mapped on three linkage groups (LG1, 13 and 15) with %PVE value of 13.0-18.3%. Interestingly, some QTL were found at the same position, such as yld_Ry10I and yld_Ry10I_2 found on LG 9 at locus MeES249, yld_Ry08_3 and yld_Ry09II_2 at locus EME195 on LG 11, and QTL yld_Ry07_2 and yld_Ry09II_3 were found on the same region, ESSRY48-EME303 on LG 12. These QTL that were located at the same position indicated that they are potential QTL. More over, the QTL that have %PVE of grater than 10% are also considered as major QTL.

Total of 17 QTL for fresh starch content were identified consisting of 14 and three QTL identified from Rayong and Lopburi locations, respectively with %PVE ranged from 9.8-47.6. These 14 QTL from Rayong were located on linkage group 2, 4, 6, 7, 10, 11, 12, and 13. Four QTL were identified in year 2008, while 7 and 3 were found in year 2009 and 2010, respectively. For Lopburi location, three QTL were found in year 2009. These QTL were located on linkage group 11 and 13 and had %PVE ranged from 15.5-18.6. In addition, two QTL, fstr_Ry08_3 and fstr_Ry09I_4 were found in the same region (ESSRY48-EME303) on linkage group 12 as well as QTL fstr_Ry09I_1 and

fstr_Ry09II_2 were found at the region between NS248-NS978 on linkage group 7. The QTL that were found within the same region indicated that they are potential QTL. More over, those QTL that have %PVE of grater than 10% especially the QTL fstr_Ry09I_5 which has %PVE of 47.6 are also considered as major QTL. The details of identified QTL underlying starch content are shown in Table 5 and Figure 3.

Discussion

Microsatellites are the most widely used DNA markers for genetic linkage map analysis (Ritschel et al. 2004). The satellite enriched-genomic library was a simple procedure used to characterize SSR markers in many plant species, including cassava (Chavarriaga-Aguirre et al. 1998; Mba et al. 2001). To characterize and develop additional SSR markers of cassava, 4 enriched-genomic DNA libraries were constructed from cassava and each library was enriched with a different biotinylated DNA oligomer. The most abundant microsatellite motif in plant genomes was the $(GA/CT)_n$ repeat (Li et al. 2002; Morgante et al. 2002; Saha et al. 2006). The (GA/CT) SSR motif was used to construct enriched-libraries in cassava (Chavarriaga-Aguirre et al. 1998; Mba et al. 2001). Therefore, this study choose different SSR motifs, $(AC)_{12}/(CT)_{12,}$ $(AT)_{12}/(CTT)_8$, $(ACC)_8/(AGC)_8$ and $(GC)_{12}/(GGC)_8$ to construct enriched-libraries. The results showed that AC/TG (56%) repeats were common and could be used to design primers useful for mapping. There was no evidence for clustering of repeat motifs seen in other paleopolyploid genomes (Shultz et al. 2007). In the previous study, Mba et al. (2001) constructed GA-enriched libraries with 45-60% enrichment efficiency. In this study, the highest efficiency of enriched library, 74% was found in the library using $(AC)_{12}/(CT)_{12}$. The average efficiency of other plants that ranges from 50% to 90% (Butcher et al. 2000). The enriched libraries increased the rate of SSR discovery compared to the sequencing of genomic DNA in BAC end sequences (13-15%) (La Rota et al. 2005; McCouch et al. 2002; Shultz et al. 2007; Temnykh et al. 2001). However, the problem of SSR enriched library for marker development is partly caused by

small insert size that lead to short or redundant flanking regions not suitable for primer design (Sharopova et al. 2002).

There was concern that the F_1 population may not be ideal for map development (Okogbenin et al. 2006). However, the process of population development for cassava was limited by the long growing cycle and low seed number per pollination that results in limits to developing F_2 or derived populations for classical genetic studies (Kunkeaw et al. 2010a). Conversely, several genetic linkage maps of cassava and other perennials have been constructed using F_1 populations. Therefore, genetic mapping populations in cassava were usually derived from crosses between heterozygous parents, F_1 crosses (Fregene et al. 1997; Mba et al. 2001; Kunkeaw et al. 2010b). In the previous work on the construction of the cassava linkage map of cassava using EST markers by Kunkeaw et al. (2010b), there were more than 7,000 SSR loci identified in the Genbank ESTdb. The authors screened 425 primer pairs for polymorphism between the parental lines and found that 81 primer pairs were informative and 56 EST-SSR loci were mapped in the F_1 population from crosses of Huay Bong 60 by Hanatee (Kunkeaw et al. 2010b). Based on the same mapping population as Kunkeaw et al. (2010b), 168 primer pairs were informative and used in the genetic linkage map analysis.

The linkage map in this study added 299 new microsatellite markers (181 SSR and 118 EST-SSR loci) into the previous map constructed by Kunkeaw et al. (2010b). Based on the genome size estimation method from linkage data (Hulbert et al. 1988), the size of the cassava genome was estimated to be around 1,610 cM (Fregene et al. 1997). Here, we reported the map which encompassed 1420.3 cM or around 88% of the genome indicating that the map was almost complete. SSRs provide powerful tool for genetic linkage map construction that can be applied for identification of QTL. Importantly, the marker linked to the QTL can be further applied to marker-assisted selection (MAS) in cassava breeding program for selecting cassava plant that contains desirable phenotype.

In general, SSR loci in the same scaffold of the cassava genome sequence located in the same linkage group. For example, on the LG5, there were 39 loci which can be located on 18 scaffolds of the cassava genome sequences (Fig. 1). Fifteen SSR loci could be located on the scaffold03614. However, 2 out of 15 loci from the scaffold03614 were separated by markers from other scaffolds on the LG5. This information could help bringing scaffolds in the incomplete cassava genome sequences. However, the incorresponding order of markers between genetic linkage map and the cassava genome sequence is probably due to different genetic backgrounds used in the linkage map construction and the cassava genome project. In some cases, the comparison analysis may help bridging linkage groups. For example, the scaffold02960 contained 3 loci (CA23, CA241 and CA258) on the LG14 and 1 locus (SSRY337) on the LG19. It is possible that the LG14 and LG19 are not linked on the linkage map due to a lack of bridging markers. However, more experiments are required to evaluate these hypotheses.

For QTL analysis, this study have identified 17 QTL specific to fresh root yield and other 17 QTL for starch content. Most of the QTL identified here are considered as a major TL due to their %PVE of grater than 10%. Some of the QTL were found at the same locus or within the same region, indicating that they are reliable and potential QTL. All of these information will be useful and applicable for future study in order to pin point the position of tightly linked markers and identify gene controlling the traits. However, additional markers within the QTL regions will be required before applying the linked markers to marker assisted selection of cassava.

Table 1: Type and number of microsatellite motifs from SSR enriched genomic libraries that were used to screen for linkage map construction.

Type of SSR motif	No. of clones	%	Repeat motif	No. of clones	%
di- nucleotide repeats	520	73.03	AC/TG	292	56.15
			AG/TC	200	38.46
			AT/TA	28	5.38
tri- nucleotide repeats	133	18.68	AAT/TTA	28	21.05
			AAG/TTC	28	21.05
			ATC/TAG	13	9.77
			AAC/TTG	16	12.03
			AGC/TCG	23	17.29
			ACT/TGA	4	3.01
			GGT/CCA	8	6.02
			GGA/CCT	7	5.26
			GGC/CCG	6	4.51
tetra- nucleotide repeats	59	8.29	AAAT/TTTA	17	28.81
			GGGT/CCCA	2	3.39
			GGGA/CCCT	5	8.47
			AATT/TTAA	3	5.08
			AAAG/TTTC	16	27.12
			AAAC/TTTG	6	10.17
			AATG/TTAC	2	3.39
			GACT/CTGA	3	5.08
			GGCT/CCGA	1	1.69
			AATC/TTAG	1	1.69
			AAGC/TTCG	1	1.69
			GGAT/CCTA	1	1.69
			GAGC/CTCG	1	1.69

Table 2: Type and number of EST microsatellite motifs that were used to screen for linkage map construction.

Type of SSR motif	No. of clones	%	Repeat motif	No. of clones	%
di- nucleotide repeats	1012	67.47	AC/TG	68	6.72
			AG/TC	709	70.06
			AT/TA	233	23.02
			GC/CG	2	0.20
tri- nucleotide repeats	438	29.20	AAC/TTG	12	2.74
			AAG/TTC	168	38.36
			AAT/TTA	55	12.56
			ACG/TGC	4	0.91
			ACT/TGA	1	0.23
			AGC/TCG	61	13.93
			ATC/TAG	60	13.70
			GGA/CCT	38	8.68
			GGC/CCG	10	2.28
			GGT/CCA	29	6.62
tetra- nucleotide repeats	50	3.33	AAAC/TTTG	2	4.00
			AAAG/TTTC	22	44.00
			AAAT/TTTA	12	24.00
			AAGC/TTCG	1	2.00
			AATC/TTAG	2	4.00
			AATG/TTAC	1	2.00
			AATT/TTAA	2	4.00
			AGGT/TCCA	1	2.00
			GAGC/CTCG	2	4.00
			GATC/CTAG	2	4.00
			GGAT/CCTA	2	4.00
			GGGA/CCCT	1	2.00

Table 3: A list of number and scaffold name from the cassava genome project present on each linkage group. Italic scaffolds represent scaffolds with markers on more than one linkage group

Linkage group	No. of scaffold	Scaffold name
LG1	33	scaffold00069, scaffold00109, scaffold01520, scaffold02658, scaffold03623, scaffold03648, scaffold04043, scaffold04450, scaffold04457, scaffold04587, scaffold05280, scaffold05434, scaffold05938, scaffold06446, scaffold06512, scaffold06609, scaffold06748, scaffold06871, scaffold06916, scaffold07035, scaffold07520, scaffold07571, scaffold07591, scaffold07991, scaffold08473, *scaffold08477*, scaffold08860, scaffold08910, scaffold09856, scaffold11341, scaffold11581, scaffold11998, *scaffold12946*
LG2	30	scaffold00368, scaffold00708, scaffold01743, scaffold01782, scaffold01945, scaffold02040, scaffold02421, scaffold02610, scaffold02912, scaffold02993, scaffold03049, scaffold03277, scaffold03342, scaffold03692, scaffold03812, scaffold03975, scaffold04300, scaffold04465, scaffold04486, scaffold05019, scaffold05206, scaffold05772, scaffold06265, scaffold06278, scaffold07896, scaffold08828, scaffold10563, scaffold11882, scaffold12147, scaffold12753
LG3	26	scaffold00010, scaffold00053, scaffold00363, scaffold00749, scaffold01481, scaffold02581, scaffold02853, scaffold03040, scaffold03136, scaffold03750, scaffold04324, scaffold04450, scaffold05859, scaffold06700, scaffold06754, scaffold07351, scaffold07378, scaffold07902, scaffold08446, *scaffold08477*, scaffold08801, scaffold08873, scaffold10045, scaffold10504, scaffold12498, scaffold12525
LG4	26	*scaffold01072*, scaffold02307, scaffold02559, scaffold02630, scaffold03055, scaffold03168, scaffold03219, scaffold03402, scaffold03667, scaffold03741, scaffold06484, scaffold06868, scaffold07007, scaffold07036, scaffold07528, *scaffold07543*, scaffold08261, *scaffold08265*, scaffold08691, scaffold09260, scaffold09274, scaffold09880, scaffold10673, scaffold11661, scaffold11689, scaffold12725
LG5	18	scaffold00621, scaffold02264, scaffold02892, scaffold03564, scaffold03602, scaffold03614, scaffold03651, scaffold03718, scaffold05421, scaffold06043, scaffold06591, scaffold08000, scaffold08368, scaffold09426, scaffold10758, scaffold10919, scaffold11928, scaffold12086
LG6	16	scaffold00520, scaffold00876, scaffold03115, scaffold03604, scaffold06089, scaffold06631, scaffold06656, scaffold07845, scaffold09410, scaffold10292, scaffold10963, scaffold11179, scaffold11232, scaffold11425, scaffold11970, scaffold12794
LG7	21	scaffold00480, scaffold00506, scaffold00746, scaffold02915, scaffold02967, scaffold02973, scaffold02994, scaffold03569, *scaffold03572*, scaffold04151, scaffold05432, scaffold05693, scaffold05875, scaffold05958, scaffold06327, scaffold08151, scaffold08316, scaffold10797, scaffold11494, scaffold12262, scaffold12327

Table 3: A list of number and scaffold name from the cassava genome project present on each linkage group. Italic scaffolds represent scaffolds with markers on more than one linkage group (cont.)

Linkage group	No. of scaffold	Scaffold name
LG8	20	scaffold00224, scaffold00271, scaffold00999, scaffold02538, scaffold02998, scaffold03735, scaffold03883, scaffold04155, *scaffold04165*, scaffold06145, scaffold06314, scaffold06407, scaffold07891, scaffold08380, scaffold09809, scaffold10689, scaffold11378, scaffold11495, scaffold12023, scaffold12439
LG9	16	scaffold00631, scaffold00821, scaffold02811, scaffold02949, scaffold04175, scaffold04881, scaffold05214, scaffold06711, scaffold06825, scaffold06906, *scaffold07543*, scaffold07827, scaffold07933, scaffold10960, scaffold11042, *scaffold12946*
LG10	10	scaffold00889, scaffold01305, scaffold03581, scaffold03942, scaffold04209, scaffold05898, scaffold06334, *scaffold08265,* scaffold10136, scaffold11998
LG11	14	scaffold00318, scaffold00560, scaffold01195, scaffold01493, scaffold02717, scaffold05335, scaffold06028, scaffold08498, scaffold08715, scaffold08847, scaffold09120, scaffold09180, scaffold09372, scaffold12091
LG12	10	scaffold00325, *scaffold01072*, scaffold01551, scaffold03741, scaffold06582, scaffold07069, scaffold07296, scaffold07660, scaffold10173, scaffold10746
LG13	7	scaffold01624, scaffold03299, scaffold03484, scaffold04106, scaffold07012, scaffold10217, *scaffold12946*
LG14	6	scaffold00847, *scaffold02960,* scaffold04651, scaffold08542, scaffold10587, scaffold11069
LG15	9	scaffold00341, scaffold03264, scaffold03834, scaffold04083, scaffold04681, scaffold08359, scaffold09434, scaffold09876, scaffold10899
LG16	11	scaffold00570, scaffold01584, *scaffold03572*, scaffold03802, scaffold04308, scaffold04653, scaffold05157, scaffold10305, scaffold10761, scaffold11110, scaffold11297
LG17	7	*scaffold04165*, scaffold04450, scaffold04622, scaffold07070, scaffold11606, scaffold12004, scaffold12272
LG18	6	scaffold01718, scaffold04489, scaffold05269, scaffold07859, scaffold10114, scaffold10493
LG19	5	scaffold02895, *scaffold02960*, scaffold04024, scaffold04260, scaffold09870
LG20	3	scaffold06914, scaffold09151, scaffold11106
LG21	1	scaffold00081
LG22	2	*scaffold08265*, scaffold03283
LG23	1	scaffold09761

Table 4: Identified QTL specific to fresh root yield in mapping population of a cross between Huay Bong 60 (F) X Hanatee (M)

Trait	Location	Year	Rep.	Abbreviation in picture	IM				MQM						
					LG	LOD	% exp	Locus	LG	LOD	% exp	Locus	KW	α_c	α_g
Fresh root yield	Rayong	2007	-	yld_Ry07	12	3.6	28.3	ESSRY48-EME303	7	3.46	16.3	EME222	*	3.1	4.4
					14	2.75	11.9	CA258	12	3.89	20.3	ESSRY48-EME303	***,-	2.9	
									14	5.21	15.2	CA258	***	2.5	
		2008	-	yld_Ry08	4	4.23	17.7	SSRY165	4	4.77	14.8	SSRY165	*****	2.9	4.3
					5	3.35	15	ESSRY5	10	3.41	10.5	SSRY81	**	3	
					7	3.49	14.8	MeES548	11	3.60	11.1	EME195	-	2.9	
					10	3.63	16.1	SSRY81							
		2009	I	yld_Ry09I	1	4.3	19.2	CA343	1	4.30	19.2	CA343	******	3.4	4.5
			II	yld_Ry09II	6	4.52	21.6	CA185	6	5.86	18.6	CA185	**	3	4.4
					14	2.79	12.9	CA241	11	3.05	9.1	EME195	-	3	
									12	3.33	15.8	ESSRY48-EME303	*,-	3	
									14	5.54	16	CA241	**	2.5	
		2010	I	yld_Ry10I	9	3.63	17	MeES249	9	3.63	17	MeES249	****	3.0	4.7
			II	yld_Ry10II	9	2.73	13	SSRY249	6	3.4	23.3	SSRY242	-	3.1	4.5
									9	4.43	21.6	MeES249	-	2.7	
	Lopburi	2009	I	yld_Ry09I	1	3.34	16.9	MeES1019	1	3.39	13	MeES1019	***	3.3	4.4
					13	2.72	22.3	MeES482	13	2.77	18.3	MeES482	-	2.6	
									15	2.90	14.4	MeES1372	-	2.7	
			II		No QTL										

12 Table 5: Identified QTL specific to fresh starch content in mapping population of a cross between Huay Bong 60 (F) X Hanatee (M)

Trait	Location	Year	Rep.	Abbreviation in picture	IM				MQM						
					LG	LOD	% exp	Locus	LG	LOD	% exp	Locus	KW	α_c	α_g
Fresh starch content	Rayong	2008	-	fstr_Ry08	2	3.7	15.8	SSRY152	2	5.02	15.7	SSRY152	****	3.2	4.4
					3	3.28	18	MeES1437-EME54	7	3.22	10	CA172	-	3.1	
					7	3.42	14.9	CA172	12	2.98	12.2	ESSRY48-EME303	***,-	2.9	
					13	3.35	14.3	MeES1405	13	3.23	9.8	MeES161	****	2.7	
		2009	I	fstr_Ry09I	13	3.05	55	CA695-CA204	7	3.65	14.3	NS248-NS978	*,-	3.2	4.6
									10	5.23	16.1	SSRY231	-	2.8	
									11	3.44	23.1	MeES1125	-	3	
									12	4.68	13.3	ESSR48-EME303	**,-	3.1	
									13	4.77	47.6	CA204	-	3	
			II	fstr_Ry09II	4	3.36	17.1	MeES666	4	3.24	13.7	MeES666	**	3.2	4.6
					7	4.38	28.7	NS248-NS978	7	4.05	20.8	NS248-NS978	****,-	3.3	
		2010	I		No QTL										
			II	fstr_Ry10II	2	3.35	36.5	EME373-3	2	3.31	14.1	EME332-EME373-3	***,**	3.2	4.5
					6	4.79	21.3	CA526	6	5.5	17.3	CA526	*******	3.1	
					9	3.35	15.5	NS162	11	3.37	10.9	NS1021	**	2.9	
	Lopburi	2009	I	fstr_Lp09I	13	2.85	15.5	MeES161	13	2.85	15.5	MeES161	*	2.7	4.5
					20	2.41	12.6	SSRY229							
			II	fstr_Lp09II	11	3.02	16.3	SSRY200	11	4.01	18.2	SSRY200	**	2.9	4.3
					13	3.29	16.7	MeES396	13	4.29	18.6	MeES396	***	2.6	
					15	3.27	17.9	MeES1372							

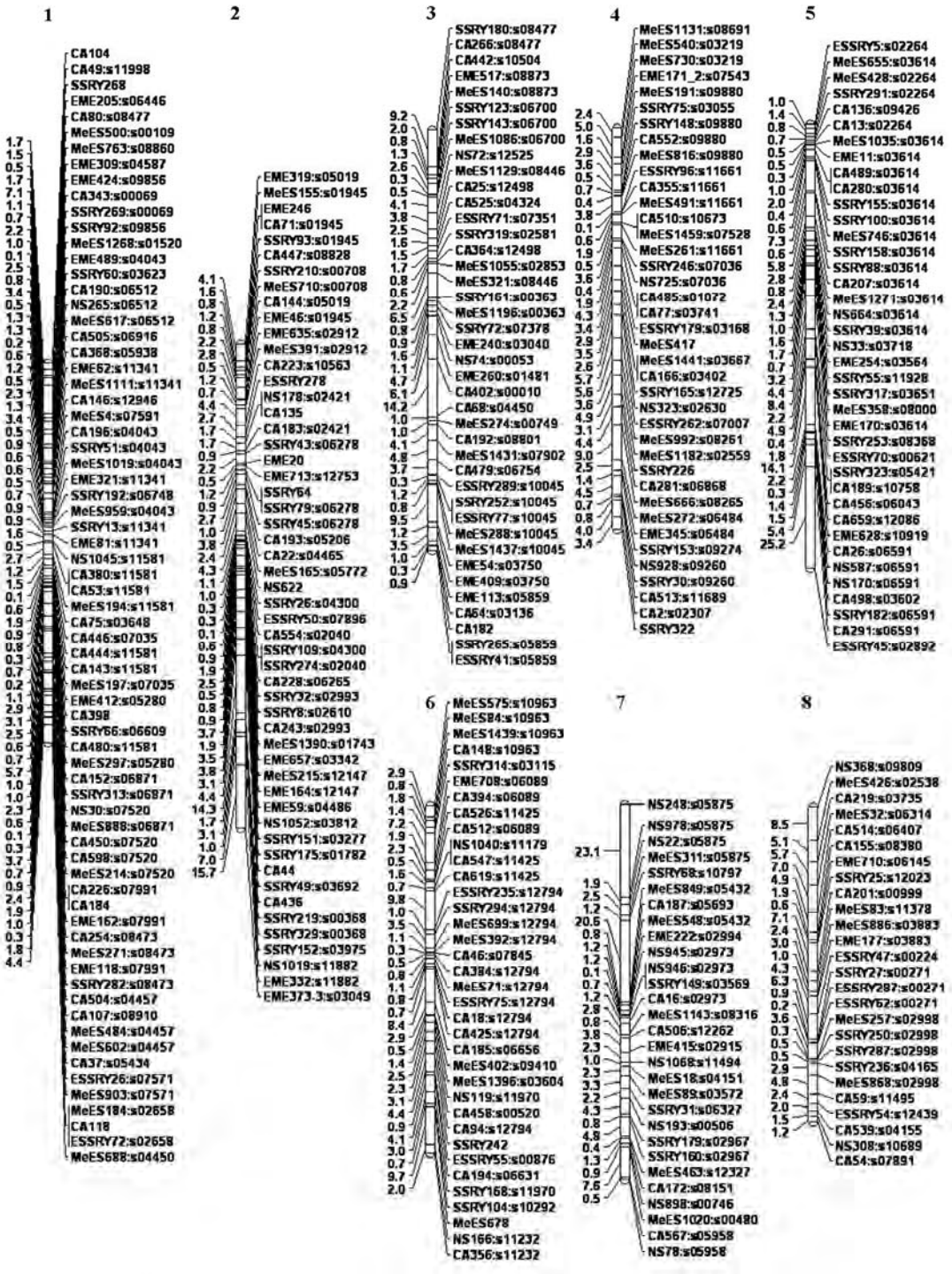

Fig. 1 The genetic linkage map of cassava F_1 population (*Manihot esculenta* Crantz) developed from SSR and EST-SSR markers. The map is composed of 510 SSR loci, covering 1420.3 cM on 23 linkage groups. On the map, the SSR loci name is followed by the scaffold name (s) that each locus is located in the cassava genome project

(ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v5.0/Mesculenta)

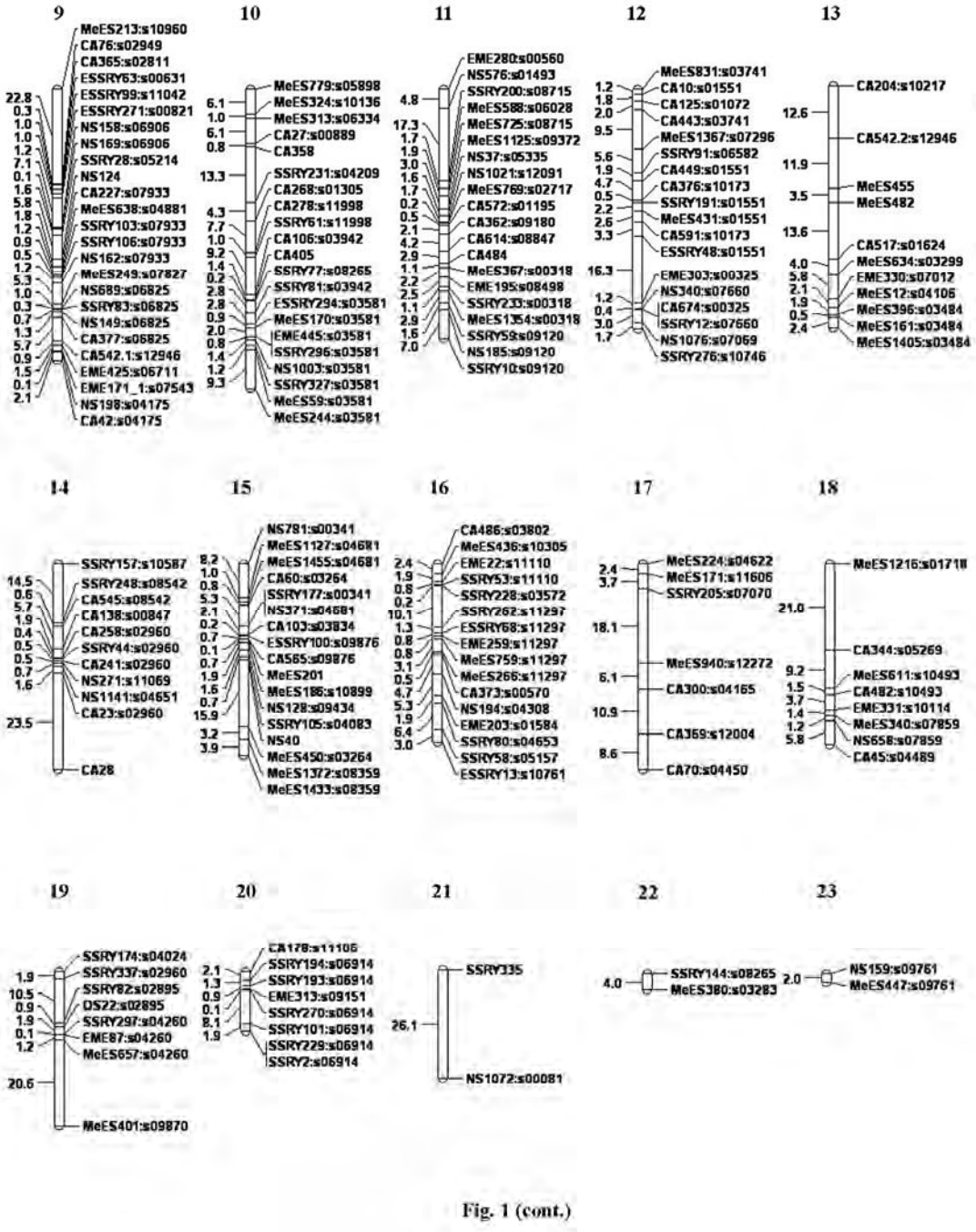

Fig. 1 (cont.)

Fig. 1 The genetic linkage map of cassava F_1 population (*Manihot esculenta* Crantz) developed from SSR and EST-SSR markers. The map is composed of 510 SSR loci, covering 1420.3 cM on 23 linkage groups. On the map, the SSR loci name is followed by the scaffold name (s) that each locus is located in the cassava genome project (ftp://ftp.jgi-psf.org/pub/JGI_data/phytozome/v5.0/Mesculenta)

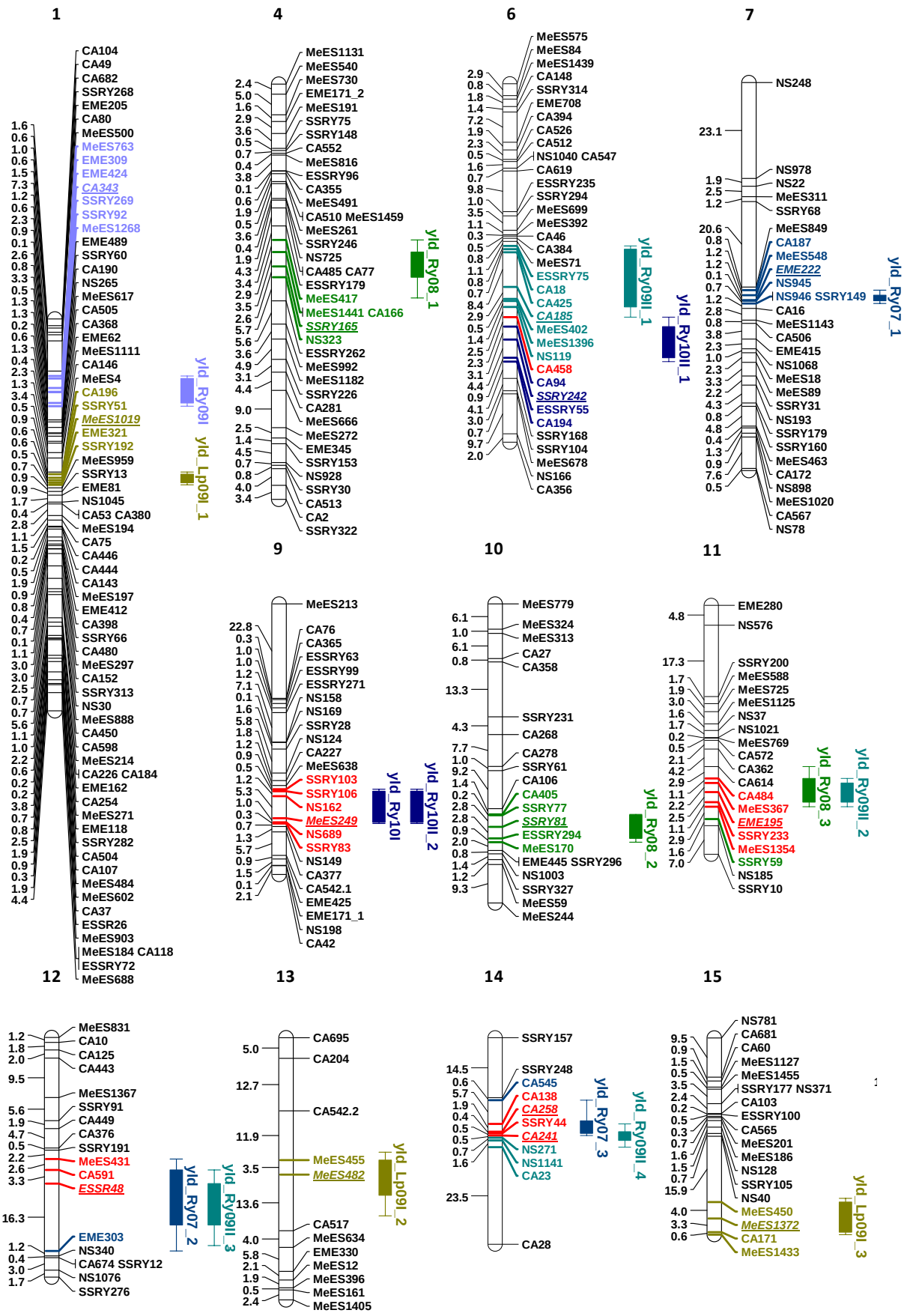

Figure 2. QTL specific to fresh root yield (red color indicates the markers that are overlap between linked QTL; underline indicates highly linked marker with the trait)

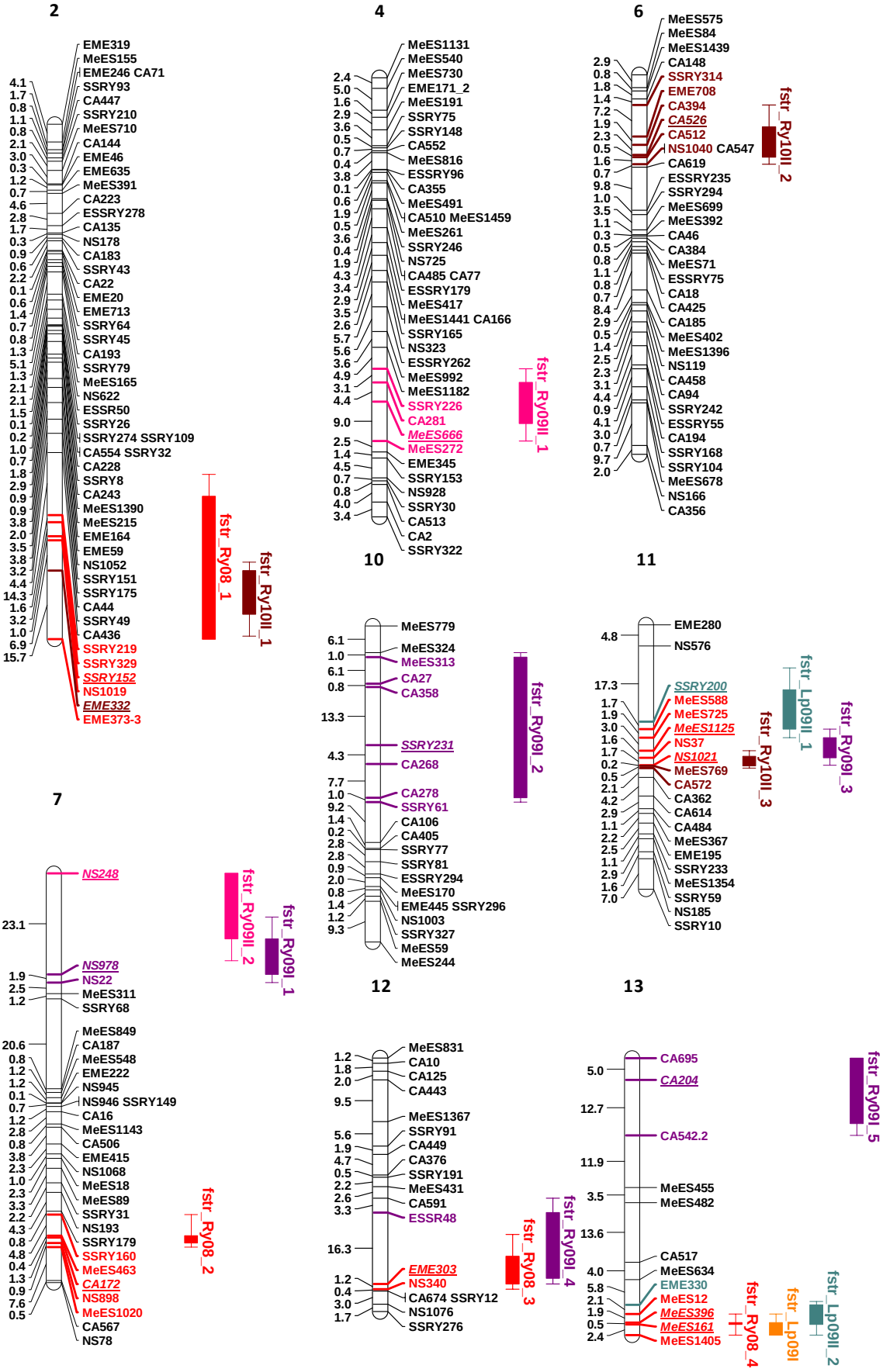

Figure 3. QTL specific to fresh starch content (red color indicates the markers that are overlap between linked QTL; underline indicates highly linked marker with the trait)

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Output ที่ได้จากโครงการ

1. ผลงานตีพิมพ์

2.1 Supajit Sraphet, Athipong Boonchanawiwat, Sithichoke Tangphatsornruang, Opas Boonseng, Satoshi Tabata, Shigemi Sasamoto, Kenta Shirasawa, Sachiko Isobe, David A. Lightfoot and **Kanokporn Triwitayakorn** (2010) Development of simple sequence repeat markers and construction of genetic linkage map of cassava (*Manihot esculent*a Crantz). (revised)

2.2 Sukhuman Whankaew, Supajit Sraphet, Ratchadaporn Thaikert, Duncan R. Smith and **Kanokporn Triwitayakorn** (2010) M-AFLP based development of microsatellite markers for the characterization of cassava (*Manihot esculenta* Crantz). (submitted)

2.3 Sukhuman Whankaew, Supannee Poopear, Supanath Kanjanawattanawong, Sithichoke Tangphatsornruang, Opas Boonseng, David A Lightfoot and **Kanokporn Triwitayakorn** (2010) A genome scan for quantitative trait loci affecting cyanogenic potential of cassava root in an outbred population. (submitted)

2.4 Athipong Boonchanawiwat, Supajit Sraphet, Opas Boonseng, David A Lightfoot and **Kanokporn Triwitayakorn** (2010) QTL underlying plant height and first branching height in cassava (*Manihot esculenta* Crantz). (submitted)

2.5 Suparat Kunkeaw, Jeerapun Worapong, Duncan R Smith and **Kanokporn Triwitayakorn** (2010) An *in vitro* detached leaf assay for pre-screening resistance to anthracnose disease in cassava (*Manihot esculenta* Crantz). Australasian Plant Pathology. (in press)

2.6 S. Kunkaew, T. Yoocha, S. Sraphet, A. Boonchanawiwat, O. Boonseng, D.A. Lightfoot, **K. Triwitayakorn**, and S. Tangphatsornruang (2010) Construction of a genetic linkage map using simple sequence repeat markers from expressed sequence tags for cassava (*Manihot esculenta* Crantz). Molecular Breeding. (in press)

2.7 Kunkeaw S, Tangphatsornruang S, Smith DR and **Triwitayakorn K** (2010) Genetic linkage map of cassava (*Manihot esculenta* Crantz) based on AFLP and SSR markers. Plant Breeding. 129:112115

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2. การผลิตนักศึกษา

2.1 ระดับปริญาเอก

2.1.1 นส. สุภารัตน์ ขันเขียว

(สอบวิทยานิพนธ์วันที่ 14 กันยายน 2553)

2.1.2 นส. ศุภจิต สระเพชร

(อยู่ระหว่างรอการตอบรับผลงานตีพิมพ์ คาดว่าจะจบการศึกษาภายในปี 2553)

2.1.3 นส. สุขุมาล หวานแก้ว

(อยู่ระหว่างรอการตอบรับผลงานตีพิมพ์ คาดว่าจะจบการศึกษาภายในปี 2553)

2.1.4 นายธัญญ์วนิช ธัญสิริวรรธน์

(คาดว่าจะจบการศึกษาภายในปี 2554)

2.2 ระดับปริญาโท

2.2.1 นส. สุภารัตน์ ขันเขียว (จบการศึกษาปี 2550)

2.2.2 นส. สุพรรณี ภู่เปีย (จบการศึกษาปี 2551)

2.2.3 นายอธิพงษ์ บุญชนะวิวัฒน์ (จบการศึกษาปี 2552)

ภาคผนวก A

- Sequences of SSR primers

- Sequences of EST-SSR primers

Table A1: SSR sequences

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0001	GCCGACTCCGAGACTATCAG	CCTGCCAAaGAACAAACCAt
CA0002	GGATAGGTGGTCAAAGTGCAA	GAGGACCTGGGACAAGTTGA
CA0003	TGGTTCTATTTGAACGGTGC	TGAGAAAAGCACCATATGCC
CA0004	TTTCAGACACCCAAGAACCC	AGAAGCGCCTTGTAGCAGAA
CA0005	CCTTGTAGCAGAAGCTGCCT	GTAGCAGAAGCCTGGAATGC
CA0006	CTTCTCAATTCCAAGCTGGC	CCAATCAGAAAGTTTGGGGA
CA0007	CCCGCTAGTGTGCAACATAA	GAAGTGAAATGAGGATTGCTCC
CA0008	AAGCCTTCCATTTCCCAACT	ACGTGTAGCGAAGGGTCTGT
CA0009	AATGAGGCTAGGGGTGTGTG	CCCCCAGGTCTACTTCTGTG
CA0010	AATTCTGCTCCAGGTTTCCA	CCCACATGTAAATCTCATCCC
CA0011	TCATGCAGAGATTCTTCCCC	GGTAGCCTGCCTGACTATGC
CA0012	AGCAGAAGCCTATGTGCAGG	CTTCATCAATGGACCCATcc
CA0013	TTATGTGTGGGCAAGTGAGC	CATATTGTTCACCACTGCGG
CA0014	CAGCAACACATTGGCATAGG	TGCTTAGGCTGAAGGCTTTG
CA0015	ACCCTTGGGTTCTTCCAACT	CTTCCTCAAACCAGTGAGGG
CA0016	TGGACGAGTGCACTGCTACTAT	AATTTGCCAAAACAATTGCC
CA0017	TTCGGATGTCACTGGCAATA	CCTTTGTCGACTTTGGTGGT
CA0018	AATTGTAGCTGTTGCCCCAC	TATGGCAATGGGAGGTCATT
CA0019	TCCTTGGTGTTCTTTGACCC	CCTCCCCAAACCTACTCCTC
CA0020	AGCAGAAGCCCTTgtTtTCA	CAAGAGCCCTcTCCAcTTTg
CA0021	CCAGTGCCAAGAACCAATTT	TCGAATTGCAAGTTGTGGAA
CA0022	GGCTTAAAGGCAAAAAGGACA	CCCTTTTCGGTTCTTTTAGGT
CA0023	ATCGACGTAATGGCACAACA	TGCCTGAAGCAGAGAAGTTG
CA0024	TGGAGCCCCAGAACTATGAG	TTTCACGACAACACCCGTAA
CA0025	CAATGGCTTCTCAAATCGGT	ACTTGCCTCTCCCAGACCTT
CA0026	ACACACACACACACACACGC	TTCCTTTACAATTCTGGACGC
CA0027	CTTACCAGCCTGCATTGTCA	ATTCCTCTGGAGAGATGGCA
CA0028	GAAGCCTGCACAGACAAACA	ATTACCCGTTCCCAATCACA
CA0029	GATACTCTTTCCGGCAGCAC	ATTTGCATCCTAAATGCCCA
CA0030	GAATCACCAAAGGAAGCCAA	TAAGCGAAAGAGCTGGTGCT
CA0031	GTGTGGGGTCCGAAATTATG	AAATTAATGCCCAAAACCGA
CA0032	TTCCAGTTTTCACCAGTCCA	ATTCTGCCTATGTCACGCAC
CA0033	CATGTGTGTGAAGATTGTGGC	AACAGACTGGGCAAACAACC
CA0034	GCAATTGCGACTTGCTCATA	AGAAAGAtAGCcAGCCAGtCA

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0035	CACAGAGGACATCACATGGC	CCCTGTCACAAGGGAGTTTT
CA0036	TAGCAGAAGCCTGAGCAACA	AATGACCAGGTTAAGCCACAA
CA0037	GCATGCATGTGTGTGTGTGT	CAGAGATTGATTGGGCGTTT
CA0038	AAAGGCAAGCCAACTCAGAA	GAGGACACCAAAGGCAATGT
CA0039	GGAGTCCAAATGCCAATCAT	TGCAATTACATTGGAAGCCA
CA0040	GCGGATCCAAGTAAGGATGA	TTTTGAGCCCTACCATGTCC
CA0041	TTAGCAATGGGGAAGGTGAG	CCTTGTAGCAGAAGCCTTGG
CA0042	GAATGTAGCACTTGCTCCCC	GGTGTTCTTCTGTGGCAGGT
CA0043	GGTTTGTCCTTTGCCTGTGT	GGAAGAGAAAATTCCAGCCC
CA0044	CCCGAGTTCTTTTGCTCTTG	TTTTTcgGGgGAGACACAC
CA0045	GTCTGTGGACCAAGAGGAGG	GGAGGTGAAAAGGGGGATTA
CA0046	AACTCTCTGCCGCTACCAAA	GCCGAATAACAATCGGAGAC
CA0047	AATCAACAGGTTGCTTCAGGA	GGGTCTCAACAGAAAACCCA
CA0048	CTTTGTCCTTCCTATCGCCA	CAATACCAAGAGCGCTGTGA
CA0049	CTCCCCTACTCCCTTTTTGC	TCTGCCCCAATTTACACCAT
CA0050	CGTGCATTTGTGTGTGTGTG	AGGCGTTGCAAATCATTCTT
CA0051	ATTGCATAAGCCCTGGACTG	CAGTATAAGAAATCTCCCCCAGA
CA0052	TTCCAATAAGAAAATCCCAATCA	TCCAAACCTCCCTCTTCCTT
CA0053	CTGTGTGAGGACTGGGATTG	TGCCTTATCACTGATGCTCTCT
CA0054	CCATTAAACGACAATCCGCT	AAATGCCAAAGCGAGAGAGA
CA0055	TTCATGCGATAGTTGGTGGA	GGCGTCTCCAATCTAAGTGC
CA0056	TGTGCAAACCGCAATGTACT	GTGCTGTTTCTCCTGCACAA
CA0057	GGAAATTGGTTATGTCCTTTCC	TGTAGCAGAAGCCTACCCAGA
CA0058	GAGACCACAGGAAGCAGGAG	GCAACAATCTGGGCGTTTAT
CA0059	GCCAGGCTGCAAAAGTACA	ATAGCACTAAGCGGAGCGAA
CA0060	TGGGTTCTCATTTCTGGAGG	AACATCAGGTCATCAACGCA
CA0061	AGCCTGGGGAACTTTTGTTT	AGCAGAAGCCCACGAATAGA
CA0062	CCGCAGATACAGCAAGAAAG	TCACCTAGAAGAAGGGCCAA
CA0063	TTGTAGCAGAAGCCCAGGAT	CAAGAGCCCTCTCCAGTTTG
CA0064	GACAGACTTGTGGGGGAAAA	GTTAGTGCGCCCACTCTCTC
CA0065	AGCAGAAGCGGTTCGATAAG	CGCTGATGCAAACAGACATT
CA0066	GTGAAGTGTGTGCGAAGTGC	TGGCTTAGCACAAGGGATTT
CA0067	ATTTGGGGGATCTAGGGTTG	TTTTCAATAAGCCTGGCTGG
CA0068	GGGTTGCATGTTTGCTTTCT	TGATGCAAGTGTTTGTGAAAA

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0069	TGCCTTTCTCTGTCAAGCCT	GGCCTCTTTTCTTCTCTCCC
CA0070	AGCAGAAGCCTTGTCCACAT	GGGTTGCATGTTTGCTTTCT
CA0071	AAACTAAAACCCACCCCAGG	TTGTAGCAGAAGCCCCAGTT
CA0072	CTTGTAGCAGAAGCCCAACC	AAGGAAAGGGAAAGAGCAGC
CA0073	GCAGTTTCTTGCACAGGTGA	ACCAAGATACCCCTTCTCGC
CA0074	CCACAAAATGACCAATGGTTT	TGGGATTTCCGTCTTTGATT
CA0075	CCTCCCTCCTTGGCTAATCT	CCAAACTCAACTCTGGAAGCA
CA0076	TTGTCCCGCTCTCTCCTTTA	ACTGTAACCATGCCTGAGGG
CA0077	TAGACAAAGAACCGCCAAGC	CACCAGACGAATCATCAACG
CA0078	CCTTCAATATCGGAGACGGA	CTTTCCAGCCCATCAAACAT
CA0079	TAGTGCTGCCTCACGTTTTG	CGGGATTTGATCATAGGCAT
CA0080	TTGATCCAATTCCCACCTTC	GAAATGGCTCTGCATTGGTT
CA0081	AGCCTGGTCCGGATTCTATT	ACCAAGTCAACAGTTTGGGC
CA0082	GCCTCTACCCGCTTTCTTTT	CTGAAGGGGAAGAACCAACA
CA0083	GAAGCCTGCACAGACAAACA	GCCGAATAACAATCGGAGAC
CA0084	CATTAGGCATCCATGCACAG	GATCATGTCAAACGGGTTCC
CA0085	ATGGAAACACCGAGTTCAGG	GGAGAAAAAGGAGGAGTTTCC
CA0086	CTAACGCTTCAAATCCCCAA	TTGCAAAGGAAGTTGTGCTG
CA0087	TCCCGGTTTCTGTTTGTTTC	GGATTTTGTGTGGTTCCGAG
CA0088	CAGAAGCCCTTTTGGTTTGA	AATCGAGCAATCATCCTTCG
CA0089	GAGAGTTCACCAGCCGCTAC	GTGGGATCCCTGGGTAAATC
CA0090	TCAACAACTACAGTGCCCCA	TTTCAGTGAGATCTGAGATGGTG
CA0091	CCTTTTCTTTTGCAAGCCAG	AACTGCTGGGTATGCCAGTC
CA0092	ACAAAAAGCTACCAACCCCA	TGATTTTTGTTTCTAGATGGGTGA
CA0093	TCTGCGTTGCTTTCTCCTTT	TCTTGCTTTCTGGCTCTGGT
CA0094	AGTCGCAATTCTAACCGTGG	GCGCACACACACACACAC
CA0095	TTATGGAGGTTGGGTTGCTC	CCGTCATATGCCTTTTGGAA
CA0096	AGGGACGAGAAAAAGGAGGA	GGAAAAGCTGTTATGGCACG
CA0097	CCACATAATATCGTGCGTGC	TGGTGGAATCCATGAAATGA
CA0098	GCTCATCCCCACCACACTAT	ATGGAGTTGGAGCTGTGTCC
CA0099	CCACTTCATTTTCGCAACCT	CTTGGAAGTAGGGTGGTGGA
CA0100	GCAGAAGCCTGTGTGTGTGT	GACCGAATTCGCTTCAAATC
CA0101	GTGGTCTTCCTCCCTTCTCC	AGGCCCCTACCACCATCTAC
CA0102	AGCAGAAGCCTCATTTGCAT	GCCCATTTGCTTAAAGTGGA

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0103	AGAAGCCCATTGTTTGCTTG	CTTCTGTCCCTCTCCCCTCT
CA0104	CCTGCTGAATGGACCATCTT	TCCAATTCCCATTTCTTTGC
CA0105	AGTTCAAGTGGGTGGTCAGG	TGGCAACCTCAATGACTCAA
CA0106	GAACTGTGCCACCCAAGATT	TTGTCTGGCCATATCAACCA
CA0107	TCTTGTCACTGGTGGCACTC	CTCCACCGTTGCATTCCTAT
CA0108	TCAAGACCCTTGCTTTGGTT	ATCAAGGCGCAAAAGTCAAT
CA0109	AGAAGCCTTCAGGTGGATCA	CCTTTTGGTTGCTCGTTTGT
CA0110	TCTGGTCTTTCAGTTCGTGTTG	AATTCTGCTCCAGGTTTCCA
CA0111	TGGAGCAGCACTTTCACATC	CAGCTGTGATCCGTGAAAGA
CA0112	AATTTGCCACCTACTCCCCT	GCAGAAGCCTTCCCTCTCTT
CA0113	CGCGGGAATTCGATTAGTTA	AAGCTACCAACCCTTAGCCC
CA0114	TTCAAGGATGTGACGTTGGA	TGGCTGCTGGAAACAAATAA
CA0115	AATGTCTGCTTGCACCATCA	TTGCCAATTCCCCTTTCTAA
CA0116	ATGCTGCTACACCCACACTT	CCGTCATTTGCTTTATGCCT
CA0117	TCATGACAGCAACATGCAGA	ACTTGCCTCTCCCAGACCTT
CA0118	AAGCCTTCAGCATCTTTCCA	TCAATTTCTGCAGGGAAACC
CA0119	GGTGCGTGAGCGTTAACATA	CACTGGTTCCCATGACACAA
CA0120	TTTGGTGTCCTAAGTCCGCT	TCGCCATGTGGAATATGGTA
CA0121	TCTTGCATTGGCTTTCTCCT	CCTGAATCTGGCGTACCTGT
CA0122	CCCCAGCATCTTTACAATAGC	GCATACGAAACCAATAGCAATG
CA0123	CCTCCTTTCTCCCCAAATCT	CCGTCTTGATGTCTCTGTGTG
CA0124	AGGAGAGGGAAGGGGTACAA	TGGCAGTTAAGGAAGACTTGG
CA0125	GCTCGTCTGTCCTTTTGTCC	CACAGGTGAACCCACTTCCT
CA0126	GCCCATCTTTGTTTCAATTTC	AGTGAAATGGACACCCTTTTT
CA0127	AATTCTCCCCGAACTCCACT	CCTTATTAGCCTTGCTTGCG
CA0128	AGTGAGAATTTTGCCTGCGT	ATCCATTGAGGGAGCAACAG
CA0129	AAGCCTGATAGGGCTAAGGC	GAAGCCTAAAAATAGCGCCC
CA0130	CCTCCCTCCTTGGCTAATCT	TTGACTCGCAGCAATTTGAG
CA0131	TGGCTTTGGTTGTATGTGGA	AAAGGGAGATTTGCAGAGCA
CA0132	TGAAAGCATGCGTACCAATAA	ATGTTGATTGGGCCTTCATC
CA0133	CCTGAACACGGGATTTTGTC	TTTATCCCTCCCTCCAAGGT
CA0134	AACCATCACATGCAAACGAA	CTTGTGTCGCAGTTTCCAGA
CA0135	TATGATTTGCTTTTCGGGGA	GCTACTTGGAAGGCTGCACT
CA0136	CGTCAGTGTACTCCATCACCA	GGGATTTGCTGAATATAATGGG

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0137	AACCAAGGCTTCTCAGGACA	TTGCCAAGTTTACTGCTGGA
CA0138	CCAAATCAAAGAAGGGTCCA	TCTTTACCTTTTTGGCCAGG
CA0139	CAGAATTCCTGCATTTTTGGA	TGAATGCAATCGCATCCTTA
CA0140	TTCGAGTTTGGATCCTGTCC	GGATGCCTTGGCTAAAACAA
CA0141	GTTGTCGTTGAGGCCTTGTT	TGACAAAATCACCCATCCCT
CA0142	CCAACCCCTTCTCTAGCTCC	TCCATGTGATGTGTGTGCAT
CA0143	GGAAAGCTCCAGGATTGTCA	TCTTCTTTGAGAGCACTTGCAG
CA0144	GACATATGCAACCAAATGCG	AACTCCAACTTGTTCCCACG
CA0145	TTTGCAATCGATACCCACCT	TCTTGTATAAAAGGGGGTTGAAA
CA0146	CTTTGCAGTGCCAACAAAAA	AGGTTGTGGCAAGAGCAAGT
CA0147	AGAAGCGCCTTGTAGCAGAA	ATCAACACCAAAGACTGGGG
CA0148	AGTTCAAGTGGGTGGTCAGG	ACCCCAGACATGTTGCATTT
CA0149	AGGAGAACGAGCCTGTCCTT	CAAGCCATAATGCCCTCAAT
CA0150	GAAAATTGCACTTCGGGAAA	TTTCCTCCATTTCTTGGTGG
CA0151	ATTTTTGAGATGGGGCAGTG	CGGATTTGACAAGAACAGCA
CA0152	TTGCCAATTCCCCTTTCTAA	ACCCTGATCATCACATGGAA
CA0153	CAGAAGCCTGGGAGATGAAG	TGTGGATAAACACAGGGCAG
CA0154	TTGTGTGCTTGCAGGTTAGG	TGTACTCCACCTTTCACCCC
CA0155	ACATTTGTTTGTTCCCCTCG	GGGGCATGAATTATCCAACA
CA0156	AGCATTGGATTGCTTCATCC	ACAAGCAAGCCATCTCCTGT
CA0157	TGAAGCATGAAGAGCCAATG	TCCTTCGCCATTTCACTTTC
CA0158	AAGGCGGAGTAACACCATGT	AAAGGAATTAAACATCCAACGG
CA0159	TCTTGCATTGGCTTTCTCCT	AACAATCATGCTCCCATTGA
CA0160	TTTATAGGACGGGGAAAGGG	GGTCTGGTTCGGTTTCACTC
CA0161	AAACTTCCACACAGATCGCA	CACTTTGATTTTGATGGTTTGTTC
CA0162	AGAAGCGCCTTGTAGCAGAA	CTGCAAAAACCAGCTGTACG
CA0163	AACTTGCAATCGGAGAATGG	CGTTGACAAAACGATGAACG
CA0164	ACAAAATGTCATTCCGGCTC	AATCAGGTTCATTCCCCAAC
CA0165	AGGATGACCTAGCCATGCAC	AGTGTTTGCGAATGGCTCTT
CA0166	TTGAAGCTTGCATGTCCATC	GCCAAGTGTTCTTCTGGTCC
CA0167	GCCAAACAGCACACCTGTAT	ATGATGTCCTTGTTGGGCAT
CA0168	TATGGGCCTCCTTACGAGTG	GGCTTTTGATGAGAACCCAG
CA0169	CCACACCTGAGAGCAATCAA	CCCAACTCCTCCAGGAAAAT
CA0170	GCAACCAGCAGTCTTCATCA	GGTTGCTGCAAGATGTCAAA

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0171	GCACAAGAAGAGCAAGAGACC	GGAAGTCGCACTTCTAACCG
CA0172	TTTTGTGACTGCGTTTATTGC	GAACCAAATATCCCTGCCCT
CA0173	AAGATCCGAGGGAGGAAAAA	TTGGAGGACCTGGTAAGTGG
CA0174	CACTCACACGCATCTCCATT	AGCTCACATCAGCTGCCATA
CA0175	AGAAGCCTTTCAAGCCAACA	AGGGTTTTCACACCACAACC
CA0176	CCTATCGGGACCTGCTACAA	GCCTCCCAAGTCAATATCCA
CA0177	GTTACCACACACAAACCCCC	TGCAGCATGTTATCATTTCCC
CA0178	TGCTCGCATGTACTACCACC	CATGTGTGGAATCTCCCCTT
CA0179	GGGAGAGGAACAGTTGCTTG	CATTCCTCCACCACAACGAT
CA0180	AAGCATTCACCTCAGCGTTT	CATGGCTTCTGGTAGGTGGT
CA0181	ATGCTGGTCATTGCCCTAAC	ACTGTGACCCAATCATGCAA
CA0182	TGATCGAGAAGAAAATGAGTGAA	CAATGCTTTGGCTAAGACCC
CA0183	TCTTTGATATAGCGTGGGGC	CAAGAGAAGCACAAGGGGAA
CA0184	GCAGAAGCCTTCAGAAATGC	AAGCAGTTCCAGCCAGAAAG
CA0185	ACTTTCACGTGGGTACAGGC	TTCTCATCTCCACCTCCACC
CA0186	AAACCGAAGGCTTGAAAACA	TGATCATGGGACAGGACAAA
CA0187	AAGTCCCCACATCTCACAGG	ACAGAGCCCTCTGGAACTGA
CA0188	AGCCTTGTAGCAGAAGCAGC	AAGGCCTATCCGATCATGG
CA0189	TACATGTGCGTTTTGGTGGT	GCTGTTTAATCAAGTGGTGGTG
CA0190	CCTGAATCTGGCGTACCTGT	TTCATGTTGCAAAATCCCAA
CA0191	GCAAACGTGCACGAAAAGTA	TGTGCCACTACAACACATTGAA
CA0192	AGTGTGCAATGCTGACCAAC	CCAAATTGCCTGTTTGGTG
CA0193	TGCAAACTGGAATCTTTACCC	ACATGGGAGTTGGAAGATGC
CA0194	TCGAGAGCTCATCACTGGAA	TCACCATCGAATGAACAATACC
CA0195	GGTGGTTCTAGCAAAGACGC	AGTGCCTGAGGGAAGTTTGA
CA0196	GAAGCCTGATGCCAAATCAT	AAAGTTGTTCCTTGTGGGGA
CA0197	CCTTGTAGCAGAAGCCCAAG	TCCTCATGAAGGTTTCACCA
CA0198	TCAATTTGCACAAACAAGCC	TTGCGGTTTAGGCTGATCTT
CA0199	GCATGGAGGTGAGTCCTGTT	GCATCATCTACAATTCATCTGTGC
CA0200	TGGAGTAGAGTCCACGAGCA	GCTTATGCATGTCAAATGGG
CA0201	ATGGACCGGGGAAAATTAAA	CAAAGTAAGGGCACTAAAAAGACA
CA0202	TGATCTGGTGCAGAATGGAA	CACTTGCCACTTCCTGGTTT
CA0203	TGTAGCAGAAGCCTGAAGCA	TATAACCGAACCGAACCGAA
CA0204	TTATGCTGTGTCTGGCCTTG	GCCCATCTTTGTTTCAATTTC

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0205	CGGGAATTCGATTGTAGCCT	CACCTCATTTTCCTTCAAACAA
CA0206	AATAATGCACAGCCGCACTT	TGGATGTCTGGGATTTGAGC
CA0207	AGCGCTTTAAAATCGAGCAC	TCAGGAACCAGAAGTCCAGG
CA0208	TTTGCTGAAACAAAAATGCG	ACAGTTCACCATGGCAATCA
CA0209	TGTGGTTCTCAACAAGGCAG	GCAGAAGCCCAAAAGAAGTG
CA0210	GAAGATTCCGCTACGTGCTC	CAATGCACCAGCATTCATTT
CA0211	TTCGCCTCTTTGTCCCTTTA	GCAGCGTTCAAAAACCAAAT
CA0212	CTCATCGAAGTGGCTGAACA	CCCAGCCATGACGTAAAAGT
CA0213	AGATTGCATCAGGCAAAAGG	CAGGTAGGCTGTGGGTTCAT
CA0214	GCAACCAGCAGTCTTCATCA	GGTTGCTGCAAGATGTCAAA
CA0215	TGCATGAATTAGGTGTGGGA	TGGGATCATGGAAAGAAAGC
CA0216	ACCTGTCAGGGATTGCTCAG	GTAGCAGAAGCCTGATCCCA
CA0217	TCCAGGCCAGTTACCTCCTA	TTCTTGCCCCTTCTAAGCAA
CA0218	GCCAAGAAATTGATAGGGCA	TTGAGGAGATCACATGGCAA
CA0219	TGAGCTTGTGGGTGAAAATG	ATCATATGCCAGGTCAGGCT
CA0220	TGTATGCATGTGGGTGTCGT	GCAGAAGCCTAGTTGGATGG
CA0221	TTCCATCCAGCTTTTTCCAG	TGTAGCAGAAGCCTCAAGCA
CA0222	TCAGGGATGTCCAAAGAAGC	ATGTTGGTCCCAGTCCCTCT
CA0223	TGGGATTTCCGTCTTTGATT	GCATGTGCAAAAGAACTCCA
CA0224	TTCCTCACTTGTGCCACGTA	TCTTTGGTGGCATATTGCTG
CA0225	GCATCACCAAACAAAACCCT	AGATTGTCCAATTGTGGCCT
CA0226	CTGTGAGGCAAAGAAAAGGG	ATCCGTGGAGGGCTTAAGTT
CA0227	GCTGGAAAATTTGGATTGGA	TGCAACCCAACCACTTATGA
CA0228	TATACTCCCTCCGTCCCACA	CAATGTAGGTGTGGGGTTCC
CA0229	AGGATTTGGCCACATTGAGA	CACCATTTCCAACTCCAACC
CA0230	GTTGGGGAAGATGCAGGATA	ACACACTGCAGCACCTGTTC
CA0231	TCGCAGTCATGCACTAATTGA	GCAAATGAACTAGCATGGGG
CA0232	CGCAACTTTTTAAGGCAAGC	TTCCCTATCCTTCATCAGCG
CA0233	GTAGCAGAAGCCTCCACAGC	AGGCTAAAACCCACACCTCC
CA0234	AAGGCCATCAGGGAAAAGTT	CTGTTGCCAGGTCACCCTAT
CA0235	GGTGTTGCCTCCAGAGAGAG	CTTGTAGCAGAAGCCCAACC
CA0236	TCCAAGATCCCCTCACAAGT	CCTGTGTCCCTGAACTTGAA
CA0237	TACAGCAAACCCTACCCCAG	ACTCAAATGGGTATCGCCTG
CA0238	TTACACTGGACGCAAACCAA	CCAGAAATGTTGTCAAGGTGA

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0239	GCAATCTCGGGTGTGTTTCT	CCATGTGCAACTGGAACTGT
CA0240	GCCTGTGGGTTTCTCTTGAT	GCATGCTAGTCCTTGTAATCCC
CA0241	ATCGACGTAATGGCACAACA	TGCCTGAAGCAGAGAAGTTG
CA0242	CAGCAACAGAAGCACCAAAA	CCCTCTACAGGAACTTCCCC
CA0243	AATTGGTTCGGTGTCTACGC	TCATCCCATCTCAACAATCAA
CA0244	TCTCTCCCTCCTTTGCCTTT	ATTTTTGCAGTTCCAGGCAG
CA0245	TCCTTGCCTTTGAAAAGTTGA	AGGGCAACTGATAGAGACGC
CA0246	TCCTTAGCAGCCAATTTTTCA	TGGAAAATCAATGGGGTCAT
CA0247	TAGGCATCCATGCTGATTTG	TGGTCATGGCAGTCACTTGT
CA0248	ACTCGAGTGCCAAATAACGG	CCGTGTAAAGGAGGTTTCCA
CA0249	CACACACCCACACACTCACA	CCAGTGGTTAATGATGCACG
CA0250	AAAATTGGAAACCCATGCAC	CCTTTTCCCCTCTCTGTGTG
CA0251	CCATATCCACCCTACATGCC	ATCTCCCGTGTTGAATTTCG
CA0252	GATGCCTAAAACACCAGGGA	CATGCTTGATGCTGTCACCT
CA0253	TGTGTGTGTGTGTGCATGTG	ATGATGAGGTTTGCCCTTTG
CA0254	TAACCACGCACAGACACACA	TCCAGAGAGGGAGGAGTGAG
CA0255	CCCACCTTGAGCATTAGCAT	TCCTTAAGGCTTTGGAAGCA
CA0256	GGGTCAGAACAAACAGGGAA	TTCCATGGCAAACACACATT
CA0257	TAAACACCATGCGCCATAGA	GAAGCCTAGATGCAAAGCCA
CA0258	TGCCTGAAGCAGAGAAGTTG	ATCGACGTAATGGCACAACA
CA0259	AGCAGAAGCCCTGCAATAAA	GACATGGGAGCAAGGAAACT
CA0260	CCTTCGTCTTCGTCTTCGTC	TAAGCAATTCCACGGGAAAC
CA0261	GGCATCCAAAAGGTTAAAGAGA	GTCAGTTAACATTGGTCCGC
CA0262	CACGCGCAAATAGCAATAGA	GAATGGCGAAAAAGGATTCA
CA0263	TCATGGTTGAAACAAGGGGT	GCCAGTCGCCAGTTTTAGTC
CA0264	AATCGAGAATCGAAGCGAAA	TGAAATTTAGATGAGTGTTTCAGCA
CA0265	CAACACATGTTATGCCACCC	TACGCCAAACTCAACCTGTG
CA0266	CACAGGTCCATTTTGTCTGC	CATCCCAATTAGGGGGATCT
CA0267	TCTACCCAGGTCCAGCAAAC	AACCATTCCCTTGCCTTCTT
CA0268	TAGAGCAAGGGGCTAAAGCA	AACAGGCCAGAAAGTCCCTT
CA0269	GCAGCCAGACTTGTGGTTCT	AAAATCTGGAGCACCCATTG
CA0270	CACTGCTTTCTTCTCCCCTG	AGGGCTTCATAATGCCTCCT
CA0271	AGCCAAAGCGCTAAAACTCA	TGCTTAATTGCTGTGCAGATG
CA0272	CTAACAGGACCTGAGGGCAG	CACTTGCCCAAGAAGGTCAT

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0273	GTGTGATCCATCAGTGGCAG	TCCATCCTGACCTCTGAACC
CA0274	CAGAAGCCtGTGCTCATCAA	ACAGAAATGGGCAGAGTTGG
CA0275	CTGTGATGTTGGTGGACTGG	ATTCAATGCCCAACAAGGAC
CA0276	TAGCCCTCTGTTCCTGCATC	TCTTTCTGGGCCTTATCTCG
CA0277	TGATCAATTTGTTGGCCTCA	TGGTTATCAGGACAGGGCAT
CA0278	TGTCCACTTCCACCACTCAA	TATGATTGACCTCCGCATCA
CA0279	AACATCCCGTCTTCTTGTGC	ATGATGCATCCAACAAAGCA
CA0280	TCTTGTAGCGTTTGTTTGCG	AAACTGATTTGCGAAGGTCG
CA0281	TGCAAGCTCCAGGTTTCTTT	GGCATAGCCAAGTCACAGGT
CA0282	TTATTAGAGTGTGGGGCAGGA	AAGCACACACTTTAACACTTCACA
CA0283	TTGTCTACAATTCCGAAGTTCA	CAATCAAATCGTAAGGTTCGC
CA0284	ATCGCGTCAGAGCTGTCTTT	TCTTCAATGGTTATTTGATTGGT
CA0285	CCAGTATTGACAGCCCCACT	TGTAGCAGAAGCCCATTGTG
CA0286	CTTGTGGATGTTCGGATGTG	GAACCATAAGGTCAGCTGGAT
CA0287	ACCCTTGCTCACATTTCACA	TTGCCATGACCAATAGATTTT
CA0288	AATCATTTTGCCAGGTCCAG	CTCTTGCTCTCCTAGCCAGC
CA0289	AACCCTGCTCTGTTTCATGC	TGTGGTGTTCCAGCATGTTT
CA0290	CCGAACATGAAGGTGGTTCT	AATGCCTGTGAAATTGAGGG
CA0291	TGCTGGTCTTCTTCCTCCAT	ATTTCCCCGCCCATATAAAA
CA0292	AGCGTCTCCGATGACTCTGT	GGAGACCCAAAATCCAGTGA
CA0293	CAGATGAGCAGGTTGAACACA	CTGCCTGCAACAAGCATCT
CA0294	CGGTGAGTAGGGAACTCTGC	AGGGCATGCTTCAGTTATGG
CA0295	CCCATGCATGAGGAAGGTAT	CTACCTGCAGTGTTCACCCA
CA0296	TCGATTACTCGTGCGACAAC	TGAATACGTTTACGCTGCCA
CA0297	GCGAGATTAACTCAAAGCCG	CGGTATCGGCTGTCTTATCC
CA0298	TGTGAATTGTGCCATTGTGA	GGCCTCACCAGAAATGTTGT
CA0299	CCTTGTAGCAGAAGCCCAAG	TGAGACATTGTCCAAACCGA
CA0300	TTTTGGGAAGGGAATAAGGG	AGCCTCACTGAAGCAATGGT
CA0301	GCAACTGCACCAGAACTGAA	TTCCCCAACAGATCAAGGAG
CA0302	GCTGGCATACGTGTTGAAGA	ACCTAGCGAAAACGCCAGTA
CA0303	AACCCCAATAGCCACACTTG	ATGCCCACCACCCAATATAA
CA0304	CGAACAAGGTAAGCATAGCG	AGCAGCTGCATTGTTGAAGA
CA0340	GACCAGGAAGCAAGACAAGG	ATATaGTCGACCcgCAGGC
CA0341	CCTGGCAGAGAACTGGAGAC	GCAGTGGAACTCGATGGTTT

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0342	CCTTCCCTGAAGACCAAACA	TCCATAAAACCCAAATCCCA
CA0343	CAGGAAAACATGAAAGGGGA	AATCTGGCTGAAGTGAGCAGT
CA0344	GTGCAGATAACATGGCCAGA	TTGGTGAAATGGAGATGCAA
CA0345	TGCTTTCTCTGCCTTGGTTT	GCCTGGATATCTATCTGCCG
CA0346	TGCAGATCCGAACAAGAAGA	CATGATCTAATGCACTGCCG
CA0347	CCTGCAGTGGCTGTCAGTAA	GCAGAAGCCTAAACCCATTG
CA0348	CCAAGTCTGCATCACAAAGG	GAACCAACATGCTTCACCAA
CA0349	GCAGAAGCCTTAGACTCCCA	TATAAAGTCCGATCGTCGCC
CA0350	AATGCATGAATTTGCACGAG	GCAGAAGCCTGTACTCAGCC
CA0351	AATGCGTTCTGGGTCTTCAG	TATGGCAATGGGAGGTCATT
CA0352	AGACGATGCCATTTCAACAA	GGCTACGAGTTCAATTCCCTC
CA0353	GTAGCAGAAGCCTTCGTTGG	CGTGCTTTTGAAGCATTTGT
CA0354	CGAGTTGTCACATGAATCGG	GCTGCTGGTCAAAAGAGTCC
CA0355	AGGGATTGATTGGGGGTTAG	TGCTTGGAGAATGTAAAGCG
CA0356	CCAATGCTTGCCAATTTTCT	AAAATGGCACTTCATCTGGC
CA0357	AGGCTGCAAGAACAAGGAAA	ATTAGGGCGAGGGGTTTATG
CA0358	AGCAGAAGCCTCGAtGAAGA	TaGACTGTCCTTGCTCGGCT
CA0359	AAGGAACACAAGTCTTGATTGG	AACAGTCCTGCACAGAGGGT
CA0360	GCAGCTATGCACCTGGAGA	TCACTTGTGTTGGGGTCTTG
CA0361	AGGATATGGAGGAGGGGCTA	CGATTTTGCGAATTACCACC
CA0362	AAGTATCGCCTGCCTCTGAA	GTGCACTTTTATGTGCGAGC
CA0363	TCATGACAGCAACATGCAGA	TGATCAATTTGTTGGCCTCA
CA0364	AATGGAGCCAAAATCACCAC	CAAATGCTATCAAGCACAAGG
CA0365	GGTGGTAACCGCCAACTCTA	ACTCGCACATAGACGCACAC
CA0366	GCCATGAAAATCTAGAAATGGAA	GCATTAACAACAAGGTGAAGCA
CA0367	GCCTCAAGTACCCATCCTCA	GCAGAAGCCTAATCTGTTGGA
CA0368	CCTCCCTGCAAATGACAAAT	GCGACTTTCTGGATGGATTC
CA0369	CTAATCGTCGGTCCACTGGT	AGGATTTGGCATAGGGGAAC
CA0370	GCTCCATATTCCCAGCAATC	TGAATGAAATCCGGGAAAAA
CA0371	CGAATCCTCCATTGTCGTCT	GGCCCAAAGAACACGTAGAG
CA0372	TTGTAGCAGAAGCCTCGTGA	AGCAGAAGCCTCCAACAAAG
CA0373	TCAGGAGTGTTGCTGGTCTG	TGTGCAGAGATTAGCTCCCA
CA0374	CAGCACCATTGTCAGCAAGT	GCAGAAGCCTGATGTGTTGA
CA0375	GCCTGTTCCCAAATTAACGA	ATTCCCTGTTCTCTTCACGC

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0376	CCAAGACTGCCAACGAAAGT	CGTTGAGGTTGTCTGAACGA
CA0377	TCGGTTCTGGTTTTCTGCTT	GCCCTGGATCACATTCATCT
CA0378	AATTGCAAGAGCGGAGCTTA	TCCTTCTGGTGTTCAGGTCTC
CA0379	TAGCAGAAGCCTAGGCGAGA	GCCTCATCTTGTTGGGTGTT
CA0380	CATTCGTGCCTCAGTGGTAA	TGAAGCCCAAGAGCAAATCT
CA0381	AAATGGCAAAAACCCTTGTG	CCTCGAAGCAATGTCTGTGA
CA0382	TAATTTTGGCAATTGGGACC	ACGAAGCTTGCCTGTGGATA
CA0383	AGTCCTCGGATGTTTTCACG	AGAAGCCTTTTCAGACACGC
CA0384	TCATTTCCCTCCATTTCTCG	AGCCTCTCCTCCGATAAAGC
CA0385	GTACCAAGATTTTGGGGGCT	AACCTCAAGCAGTACAGGGG
CA0386	GGAGGTAGTGGCAAGGTTGA	GGATTCAATTACCTCTGGGC
CA0387	CCTCGTCACATTTGTGTAGCA	GAGGCATACCAGACCCAAAA
CA0388	GAGAGAACACCCAATCCCAA	TTTCAAGCCCATGACAAATG
CA0389	CATGGCTGATCCATTTTCCT	AAAAACACGTGGCATTAGCC
CA0390	AGTGCCAGCGAACGTAGAAT	CGCATCATTAATGCCATGAG
CA0391	AGTTTTCCAGGGATTTGGCT	TCCTGCAACTTCTGCAACTG
CA0392	ATCAAGGCGCAAAAGTCAAT	TTTCCCAGTGATGAGAACACA
CA0393	AATGATGAGGTTGCCCTTTG	TGACAATCAGGACTGGAGCA
CA0394	CCGTCACTTTCTGGTTTCGT	GCGTAAAGCAGACCCTGAAA
CA0395	TACAAGAGGAGAGCCACCCT	GAAAGAAACGCACAAGGCTC
CA0396	GCGTCTATTTGCACCCCTAA	GGCTGATGTTGTTGCTGCTA
CA0397	TCCAAATCGAATGGGCATA	TGTAGCAGAAGCCTTTTCCC
CA0398	TCCTATGTCCCCTGTGAACC	TTTTCATCGCTGAGAAGGCT
CA0399	CGTTTGTGCAGCAATCAATC	CGTTTCTGCGATTTTTGAGC
CA0400	GCGACTTTCTGGATGGATTC	GAACACTGTCCCAATTGCCT
CA0401	AAACAATCACGAGGGGACAA	TTGCAGTTCCTGAGGAGGAT
CA0402	GAAACCGGCACAAGACAAAC	ACATCCACAGCCCAACTTTC
CA0403	CAGCACATTCTCACCATGAAG	TTTTTGTCGCAGAGAGGAAG
CA0404	TGGTTGGCTAGGAAATCTGG	ACACATTTGTGGGAGGTGTG
CA0405	GAGACTGCTCGGTTCTGACC	GGTGGGATCTAATGGTGGTG
CA0406	TTGTAGCAGAAGCCCCATTT	TTTGCCAGAATGAAACCCTC
CA0407	CCACTGGCTTCCCCTAAAAT	GCCTTCCCAAAATATCCTCC
CA0408	ACTTCAACTTCCCATGTGGC	GGTAACGATAACCGGCTGAA
CA0409	GCTCAAGTGGCTTTGTAGGG	CCTCTACGAACAAACCCCAA

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0410	ATTCTGTGGGAGGTGGTCAG	TAGCAGAAGCCTTGTCAGCA
CA0411	GAGTTTTGCCTCTTTTCCCC	CTTGTCGCGGTTGGTTAAAT
CA0412	AAGCCCCATaaAAGGCTCTC	TTGTAGCAGAAGCCCAGGTT
CA0413	CTGATCAGCAGGATGCATGT	ATTCCAAAATCACAACCGGA
CA0414	TACCGTTGCTTCACGCTATG	CCCTTGTGAAGGCACACAC
CA0415	TTGGGATTGAGCCTGTTTTC	AAGCAGATGAAACCCACGAC
CA0416	ACGCCTACGACAAACAAACC	ACGCTTCGTGCATTCTTTCT
CA0417	ACCTCCACCTCCTTCGCTAT	AAAGCACACATGCAAAACCA
CA0418	TAATCCTCTTGGCTGGATGG	TGCCTTTGGAAGAAGCAAAT
CA0419	GCAACTCATGGACATTGGTG	TTCATCAAAGTCTCGCAACG
CA0420	TGCCAAGAAAACATCATGGA	ACTATGATGCCACAGCCACA
CA0421	GTCAACAAAGGGAACTGGGA	TACATCAAAGCAAATGGGCA
CA0422	TGACTCTCCTTCTCGCAACA	TGTGGGGGAGTGATGGTATT
CA0423	TGAACCAGCAAGGTTCTTCC	CGCTGATAGTCGTTCCCTTC
CA0424	GGTGGGATCTAATGGTGGTG	TCAAGATGAGTTTTTGCCCC
CA0425	CCCGTGATGCCAAAGAGTAT	TCCCACAAACCATAATTCGG
CA0426	CACCGTCAAGAGGATTTGGT	AATGACCATGCCAACACAAG
CA0427	GTTGTCACGTAATGCAACCG	TGTCATGGACAAAGGACTTGA
CA0428	TCCCAGAACAGAGTTGCTCC	TTGTAGCAGAAGCCCTCCAT
CA0429	TGTAGCAGAAGCCTTCACCC	TCGCACACTCTTTTGCTTTG
CA0430	CATTTGCCCTATCGGACACT	CTGAGGGTTTTGACGCTCTC
CA0431	ACCCCTAGAGTGAAGGTCCC	ATCAGCATACCCAGGGACAG
CA0432	TCTGCTCCGACAACTTCTCTT	TCGCTGTATGCAATACTTCGTT
CA0433	AATGTGCCATTTCAGGCTTC	TTGGGGGCTTTAAGTTGTTG
CA0434	TTGCATTGTGGTTGTGCTTT	TTGGAGCTTCCATTGCCTAT
CA0435	TGCTGTAGTGGGTATGCAGC	CTCATCCCAACTCGGTTCAT
CA0436	GGCTTTGTGGATGCTTCAAT	CCTCTGTACTGGCTTGGCTC
CA0437	TGAAGGGTAAAAAGGGAGGG	GTCTGGCTTACAAGGTCCCA
CA0438	AAAGGGGGAAAGGACAAAAA	TTCAGGTTGCAGTCCATCTG
CA0439	AGAAAGGgTAGGGCAATGGT	CCAATCAAATGCAGCAAAAA
CA0440	TGTGACTGAGGTTGGATGGA	TGCGGTTTTTAGGTTGGTTC
CA0441	TTCATTCTTTGCTTGCTCCA	ATGTGCACACAGGCATCG
CA0442	GAGGGAGCACTTTCCCTTTT	AAGATCATGGGAATGGCTTG
CA0443	GCTTCAGACAATGCAACAGG	GTTCCTACAATGCACTGCCA

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0444	AGTTCTTTGGGCCACTTCCT	CTTTTGCCACCTTCCTCATC
CA0445	tgGGTGGAATCATTTTTGGT	CATTTTATGCCAAGGGGTTG
CA0446	ACCCCAGTGCAAGAGAGAAA	CTTTACCTGCATGCCATTGA
CA0447	CTTTCACATGGGTTCCGTCT	TGATTCCTGCCTTTTTGCTT
CA0448	CAGAAGCCTAGGTTTGCAGC	GTTGCAGATGGTGGTTGTTG
CA0449	TGCAGATCCGAACAAGAAGA	CGGTACAACCGCTCTCTCTC
CA0450	AGGATTGTGGTTGACAGGCT	GAGACAACGGGGACAAAAGA
CA0451	AAGACAGCAAGCCAATCCAT	TGATAATCATGGGAATCCGC
CA0452	ATTAGGGCGAGGGGTTTATG	CGCTATTAACATGCACAAGCA
CA0453	CGCGGGAATTCGATTTGT	TATTGGTTTTCCAATGCCGT
CA0454	TGAGACAAAAAGTGGGCAAA	AACTTGTGTGGCATGTCCTG
CA0455	GGGTGCCAAACTCTCATTGT	GGTGAGAGCCTAACCTGTGC
CA0456	CTTAGTGCAATCCTGGCACA	ATTGCACGTCTCAATTGGTG
CA0457	GTAGCAGAAGCCCAGGTCAG	GCATGTTTTGAACCCAAGGT
CA0458	GAAGGATGCTGAGCAATGTG	ACACTAGCATCAGATGGGGG
CA0459	GTCGTGGAGTTGGGGATAGA	TGTTGCTTGGCTGACTTGAG
CA0460	AGCCTCCAGAAAATCGGAGT	GGATGACTTTTCTTGCCTGC
CA0461	GGCCATTGAGAATGCAACTT	GCCTTGTAGCAGAAGCATCC
CA0462	TTGCTTCCATTTTGTGTAGCC	CAGTTGTCCCTTCGGTATTG
CA0463	TCATTTGCCCCTCTGTTCTC	AATGCAGCTCGCTTTCTCTC
CA0464	ATTCCCTTTCAAGCTCCCAT	CGTTCTGTTTTTCCCCTCAG
CA0465	CACCCCACGAGTTCAGACTT	GAAGCCTCCCCAGTTCTCTT
CA0466	TCGCCATGTGGAATATGGTA	TTTGGTGTCCTAAGTCCGCT
CA0467	CACCGCCTTCCCATCTATTA	TCATCGCTGTGTTGTTTTCC
CA0468	ATTGCTTTTCGCAGTGCTTT	GTTCCAGCTGTGCTCATCAA
CA0469	GCTCACATAAATGCGCAAAA	AGTGCTAAACCCGCTCGTTA
CA0470	CCCATTCTGCAGTTCTGGAT	GCCTTGCTTGTGTTTGATGA
CA0471	GGAGACGCTCTGTAGGATGC	CATGAAGTCGAGCTTGTCCA
CA0472	GGAAAGCCTTGATTGTGGAA	GTGGACCGTGAGAACAACCT
CA0473	TGTTGGCCATCTTGCTACAG	CCAAATTAATGGCAGCCAAC
CA0474	GCGGGAATTCGATTAAAAGG	CAAGTTTGAGCCCACAACAA
CA0475	GACTTCCAGACGGGATGTGT	AGCACATGGCGTGAATGTAA
CA0476	TCACGGACCAGTTTTTAGGG	ATGGTAAGCCAACACCAACA
CA0477	CAACAGCAGCAACTTTTCCA	TTGCAATTCTGGCTTGTCTG

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0478	CACCGGACTCACCCTAAGAA	TTTGGAGACCTTTTGACGCT
CA0479	CGTCACCACCTGATCCAATA	AAAAAGTTTCTCTCCGCTTGG
CA0480	TTCAAAATTCAAACCGGTCC	TGAGCCATGACTGCAGAAAC
CA0481	GGAGAGGATGCTTTTGGTTG	CTCCCTCCTTCCTCTGCAAT
CA0482	GGACTGCTTGGATCCATTAAA	CACCATTCCCTCTGGAGAAA
CA0483	GCTGGAGCGTAACCTTGGTA	AGCCTTGTAGCAGAAGCCAG
CA0484	AACGAGTTCCTGTTGCCAAG	GGGGACTCCCATGTAATCCT
CA0485	GGAAAAATTTTGATGCCCAA	AGCTGCAGGAGTTTCTCCAA
CA0486	AACTTGGCTGAGAGTGTGCAT	ATTGGCTTCTGGAAAACACG
CA0487	TTTCCCACTTGGGGAATTTT	GCCTTGTAGCAGAAGCATACG
CA0488	AAGGAAATGCACCATGCTCT	TGGCAAATATGAAAGCAATCTG
CA0489	GCAGAAGCCTCCAACCAATA	TCTTGTAGCGTTTGTTTGCG
CA0499	GCATGTTTTGAACCCAAGGT	GTAGCAGAAGCCCAGGTCAG
CA0490	GCAGAAGCCTGCTAGCGTAT	TCCATTTAATCATGCCCACC
CA0491	GAAGCCTCCATGTTTCTTCG	ATGGACACCTCCGTGACTTC
CA0492	CAGAAGCCTTTTCTGGCTTG	CCGACACAGAAACCTTTGGT
CA0493	ACTGTAACCATGCCTGAGGG	CATCAGTGCTTTTTCAGCCA
CA0494	GAAGCCCCTTTTGAAATCCT	CCGTCATATGCCTTTTGGAA
CA0495	CATGCACCAACCCAGTACAC	GGTGTTGATTTGGTTCCAGG
CA0496	TTCCTCTACAGGATGGCACC	TGTGTGAGTTTCCAGGGTGA
CA0497	ACCAATGGGATTACCCACAA	TGCGTTGTGTTTCAGTGGTT
CA0498	GCTTGAAGTTTTGGCTGCTT	AAAAACCCTTAATGCCCCTG
CA0500	CGCTAAAATCCACATGCAAA	GTGCGAGACTTTCTGTGCAA
CA0501	TTAGCCTCGTAGCAGAAGCC	GCTCACAGCATTACGGTTGA
CA0502	CATCATCCGTGCATAGTTGG	AAGGGCTTTTCTTTGTTGGG
CA0503	TTGCCCCTGTTTACAAGTCC	TCCGCTCCTTTCAACAATCT
CA0504	GGGTCTGGCATAAAGTGGAA	CAGAAGCTGTTCGTGGCATA
CA0505	CTGTGTGAGGACTGGGATTG	GCTCTTGCAACCTTGAGACC
CA0506	TCTATCCCTCCTCCGGTCTT	TGCAAGAGGAAACTTCAGCA
CA0507	CTCCAAACCGAAAAGCAAAA	TTTCCATTCCTGTTTCCTCG
CA0508	CAGCAATGTCTGTGAATGGG	GAGATCCCCATCTCTTTCCC
CA0509	TGAGCCTCCTCATGTAAGCC	TGCTCACTATCATTGAGTGCG
CA0510	CCAGCACCATAAACCAACCT	GAAGGCTTGAAGAAATCGGA
CA0511	CCTAAGCCACCATCCACCTA	TGGATAGCCATTTGAGGAGG

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0512	AGGCAGGTGTTCATTGGTTC	TATGATTTCATGCATCCCCC
CA0513	GGCACTTTTGGAAATTGGAA	CAAAGGATCCAACAGGGAGT
CA0514	AGCCAATGGCCATGAATTAG	GCTGCTGAAATGTTGACATGA
CA0515	TTGCTAAAAATCCCTGGACC	CCCAGCAAGGTTTGCTACAT
CA0516	GCGAAAGTTTCTTGAGTGCC	AATGGTTATTCCCGGAGGTC
CA0517	AAAATCTCGGAATTGCCCTT	GTCCGAAAAGATTGCTCCAA
CA0518	CCTAAGCCACCATCCACCTA	GCTTGCTGCTTCTGGTTCTT
CA0519	GCTCCTCGAAGAGAAGCAGA	TGGAGAGAAATGCGTAAGCTC
CA0520	AATGGTCCCATTCTGGATGA	TATGATGCAAAACCAAGCCA
CA0521	TATAAAGTCCGATCGTCGCC	TGCAATCAGGAGGAATAGGG
CA0522	AGGGGCACATTACCCCTATC	CAGGCCAGAATTCATCCACT
CA0523	GTTGCGGTTTGGTTTTGGTA	CACATCCAACCTCTGCGTTA
CA0524	TGCACAACCTTTTGCAGGTA	TGTATTGCACTTGTGGGGTG
CA0525	GCAGCTGGCTGTGACATTAG	TCAGCTCAGCTCAACGAAGA
CA0526	AGCTCCATCATCAAGCCATT	GGTGCAAAGTGGCAGTTTCT
CA0527	CGGCCAGAATAACTCCAAAC	TAGCAGAAGCTTTTCCTCCG
CA0528	TGGTTGGCTCATCACTTTTG	TCTGCAAGGCAGTGTGATTC
CA0529	AAGGATGTCCATTCAGACGC	TGGCAACTAAGTCTCCCATGT
CA0530	TGAGAAAACAGAGGCTTTTGG	CATTGTCCTTAAATTCAGGTTGTG
CA0531	CAACAATTGATGAGACCATGAGA	AGCTGATTCCAACAGGACTGA
CA0532	TTAAACAGCAGGAACATGCG	GCCAAGGACTGAGAATAGCG
CA0533	TTTGTGTTGACGAAATGGGA	GAGGCTGATATTTCATGGATCA
CA0534	GTCGGCTCCAAATACTGGAA	TCTATTTGCACCCCTAACGC
CA0535	CAAAGCTTCCAGTACTGCCC	TGCACGAGGATTTAGTTAAGCA
CA0536	CATTCTCTTGGTCCCTTCCA	TTTTTGAGGTTGGGAGCCTA
CA0537	GCCTTGTAGCAGAAGCCaac	AGTGCGAAACATAACCCAGC
CA0538	CTCCGTTGCAGGCATTATTT	AGCCTAAGAATTTCCCGCAT
CA0539	ACAGCCGTGTGTATTCTCCC	GGAGATGGGTACTCACCGAA
CA0540	GGAATTAACCACCTCGCAAA	CTGCCAAGTCGTTTTGTTGA
CA0541	TTAGCCTTGAAGCAgAAgCC	TAgcCTTGTAGCACGAAGCA
CA0542	CTTTTTATTTTGGCGACCCA	AGCACGAGAGAAAGGGACAA
CA0543	GTGGCACACTTGGGTTCTTT	GGTCCTTCCTCAAACACGAA
CA0544	CGCACCAAGTCGTAATATCG	TGACACTAGTCCCTCCCGTT
CA0545	ATGTCAAGAAACCCCACAGC	AGCCTGTTTCGGTACAATGG

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0546	CTTGGCTTCCACACACACAC	AAAACCAAATGCTTCCCAGA
CA0547	ATTTTCAGGAAGCATGGCAG	TTGCTGCAAATCTCGAACC
CA0548	ATGCAGCAGCTAAATGGTCA	TGAACCAAGCGTTGCAATAA
CA0549	GCCTTTCACATTTGAACACCT	TGTGTGAGTGTGTTTGTGCG
CA0550	GAAGTCCCTGATCCTGCTGA	TTTCAGGTCCATGTAGAAGGG
CA0551	GCTCCAGAGTTTTCAGAGCC	GTCAGGGCATAGTCAAAGGC
CA0552	AACCAGATGCAAGGTCATCC	ACTCTCAAACGACCACACCC
CA0553	AGGCTTCAAGAGCCATGAGA	AGCAGAAGCCTCACTTGACC
CA0554	CAGCGAGAGACACACACACA	CCACCAATGGTCGGATAAGT
CA0555	CGTGGGGTTGCTTATGAAGT	GCATTGGAAAGGCCTATTGT
CA0556	AGCCAAATTAGGCACTGTGG	TTTGTGGTCCTCACGATTCA
CA0557	TTTTAATGTGCAGGGCTGTG	CATCCCATCATGGACACAAA
CA0558	ATCTGGTCCAAACCCAACAG	CCAACACTGCAAAGAGCAAA
CA0559	CGGAAGAACAGCACAATTCA	AAAGATCCAGAAGGGAGGGA
CA0560	GGCTCGTGACTGTCAAAGTG	TGTGCATGAGAGGTCAGGTT
CA0561	TCTGCGCATATTCTTCTGGTC	ACAACAGTTTGGACTTGGGG
CA0562	AGGTGGGGAAGATTGCTTTT	GCCCCCAAAAGTCATGTAGA
CA0563	CTTGTCATGGACCGAGGACT	GGTCTGTGGAAAGACCAAGC
CA0564	CTCTCTCAATCAGTCAATCTCACC	TGCATAGCAGGTGCATTAGG
CA0565	TTCCTCCCTCTTTCTGCTTG	GGGAGGTGGTTTGTGAAACT
CA0566	GGAGATGATGTCCGTCCAAA	TTTCCCAGTGATGAGAACACA
CA0567	ACACGCACATGCATACACAA	GGCTGATGTTGTTGCTGCTA
CA0568	TTGTTTGGGGTGCAATGTTA	CCCCTGAATTGTGTGGAATC
CA0569	TGCAGCATGTTATCATTTCCC	GGCTCCATTCTTTCTTTCTGC
CA0570	TACAAATTGGGTCATGCTGG	TCCCAAACGAGGTGTTAAGG
CA0571	ACTACCTCGACAGAGCGCAT	ACCTGTGGGTCCTCTGCTTA
CA0572	TAGGCAAGCCATACCCTTTG	CGGATTTTTGGTCAATTGCT
CA0573	GGGCAAGCCTAATTGTCAGA	TGCAACTTTTTGCTCCACAC
CA0574	AAGGCATACACACCGCTTTC	ACGAGACCACCAAAAACCTG
CA0575	CGGTAGCATTAATTTGCTGG	TGTACACTGCAAATCTGGGC
CA0576	AAACGTTTGATTAATTGCCCA	AGTGTTGCACGTGAGATTGC
CA0577	TCCTTCCATGGGATTACCAA	TGAGCTCTTTGGATTCAGGG
CA0578	TCACAAACCCAAAACCCATT	GTCCCTCTTTCTCTCCGTCC
CA0579	GAAGCCTCAACTGTCCCTTG	CTCCACGCACAAGAGAACC

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0580	CACAGGTGAACCCACTTCCT	TTACAAAATCATGCCCACACA
CA0581	GTTCTGTGGGTTGGTGTGTG	CTTGGCAACCAAACCATCTT
CA0582	ATGAACACCCATTTCCCAAA	TATCAGCAGTGATCTCCCGA
CA0583	GCAGTAGCAGCAGCAATACG	CCTTGATACCCAACGCCTAA
CA0584	TGCCATTTTCTTCCTCAACC	AATGCCTCTGGATCTTGGTG
CA0585	AGAGACGAGAGGAACCGTGA	CTCAAGCCTTCCATCTCCAG
CA0586	AGGACTATCTCGCTGACGGA	CCTGCCATCCTATCTTGATG
CA0587	TCCGTTAAACGACACAACCA	TCGCAGAAGCTTTCAAAACA
CA0588	TCCATTTGAAAAAGGGCATAA	ATGGCCCTGCAACAATAGTC
CA0589	CATCCCCAACAATGTCACAA	GGGATTGCACTGTTGACCAT
CA0590	ACATGGGAGTTGGAAGATGC	TGCAAACTGGAATCTTTACCC
CA0591	GGACGAGTCCCTGTCCATAA	GGGACCATGTCGCTAGAAAA
CA0592	AAGGTTTCAGCCACTCATGG	TGGTCTTTACACTCCCCCTG
CA0593	ATTGCAGGACTTTGAAGGGA	CCATGAGCAAGCCACCTTAT
CA0594	GGATGGCATGAAAGAGGAAA	TTGGGTGAATGCATTGTTGT
CA0595	CCCCAGAATTTGTTGCTGTT	CCTGTTCCCAATTACCCCTT
CA0596	GCTGATTGGCAATGCTTTTT	TTTCAGATTAATTCACGCTTGG
CA0597	TGCGTTTTAGGAATTAGCTGC	GCACTTAGGAACAGCCAAGG
CA0598	AACCTTGGCAAAAATGCAAC	GGAACAATGGGAAAGCTTCA
CA0599	GCCTTTGTACCCAATGATGG	ATCATTACAGTTTTGCGGGC
CA0600	AACGCTCCTCTCCGATGTAA	CGCAGAACTTGAAAAGGAGG
CA0601	GGGCGCCTATTACTGTGAAA	CaGAGGGCTCTcTTACTcGC
CA0602	GATTCCCAGGAGTGCACAGT	TGTAGAAACAGCAACAGCGG
CA0603	GAAGGGGAGGGAAAGAGTTG	TTTGAAGGTAGCCCCTCTCAT
CA0604	GTCTCTCCATTGGGAGGTCA	TAGTTCTGTTCCCGCGTTCT
CA0605	AATCCAACGAGCCACAAAAC	GCCTAATCATCCAACCCAAA
CA0606	ACCGAGTCTGCCTTACCCTT	CTCAAGAAATTCCAGGTGCC
CA0607	CTTCCCGACAAGTTTCAGGT	GTCTCGCCAATTTAAGGCAA
CA0608	TTTGAATTGAGAAATGGGGG	CTGTTGCCATTCCTACGGTT
CA0609	TTAGGAGGTGTGTCCCATCC	ACAGCATCAACAGCAACAGC
CA0610	AAGCCAAACAACGGAATCAC	CGGTTATgGCATAGcTGGTT
CA0611	TAAGCAACGGATGCATTGAA	ATTCGTTTGGTTATCCGGTG
CA0612	ACAAAAGCAGAACCACCACC	CAGCACAGATTCCAGGAACA
CA0613	AATTGGTTCGGTGTCTACGC	TCATCCCATCTCAACAATCAA

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0614	CGCTGTAAGAGAAAGGCCAG	GACGAGCAAGTGTGCTTTTG
CA0615	AGGCACATAACGTAAACGGC	ACACCGAACCAGGAAGACTG
CA0616	CCCCCATGGGTTCTAAACTT	GATTGATGCATCCCTGCTTT
CA0617	TTTGTACTTTCGGCAACGTG	TTCTTTCACAAGGGTTTTTGC
CA0618	TGGTTGTGGGTACGAATGAA	TGCATTTGATACCTTGCATTG
CA0619	CTTCAAGCTTTCCACTTGGG	GGGAGGTACCGATCAAAGGT
CA0620	TAGAGTGCGGGGCATGATA	GCACACACTTTAGCACTTCACA
CA0621	AGCACACACTGACCTCTCCC	TCTACTGTCTGGTTTGGGGC
CA0622	ATCTGATGCTCCTGCCAAAT	TGAGCATGGATTCTGCTGTT
CA0623	CAATGATGGCTGAAGAAGCA	TTCTTCACCAATGGCTACCG
CA0624	GGATTTATGATGCAATGGGG	ACCTGTCAAAGCACGTGTGA
CA0625	TTGTGAAGTTTGAAGTGCGAA	CAAACCCCTTCACACCACTT
CA0626	CGGAATCTAGCCTGGCATTA	AGCCAGTGGAGTTCAGGAGA
CA0627	AGCTTGTAGCAGAAGCCTCG	GTCAGGTGCATGCTGTCACT
CA0628	ACCAGAATGGCTCCTTGTTG	CTGGACCTGCAGACAAAACA
CA0629	TaGGtTGCGTCTCATCCTCC	CGAAGTTCGACCATGACAGA
CA0630	CAGTGGGCCCTATGTTGAGT	ATGGACTCTTCCACCACGTC
CA0631	CCACCTATTGAGTCCTTGCC	GGTGTCTTCCGTTGCATTTT
CA0632	GGTTAGTGTGCCAGAGAGGG	GAGGGATAAGAGGCTGACCC
CA0633	ATTTGGTACTTGGGGGAAGG	CCCAACACAGTGCAAAAGAA
CA0634	AGTCAAAGTGGGTGCAGCTT	TGTAACAGCATTACACCCCC
CA0635	GGCAGAATTGAGGGAGATGA	TAGCAGAAGCCCACAGGATT
CA0636	CAACGGTTGTTGTTTTGTGC	AAGCCTCAAAGTCGGGAAAT
CA0637	TTCTGCCCTTTCATTCCATC	GGTAAGCCTTCCTCCTCGTC
CA0638	TCTGGACACGCAGATCAGAC	ACCAGACAACAGAAATCCCG
CA0639	GGAAATTAGAAAGTCCCGCA	TAAGGCCTGGTCTTCGTGTT
CA0640	TCCCAACCGAATCAAGTCTC	CTGCGATTGAGACAAATGGA
CA0641	AAGCCTCACATGGCAAAAAT	TGTCAGGGTGCAACGAATTA
CA0642	TAGCCACAGATGAGTTGGCA	ATCAGAGAGAGGCAACTGGC
CA0643	TCACAGATACACACGCCCAT	GCAAGACTCCTGCATCCTTC
CA0644	TTGTTCAGATGTACTGCCGC	TGGAAGAATCCACAGGTTCC
CA0645	ACCTGGTCTTCACGTTGTCC	CAGATCCCGTAAGATAGGCG
CA0646	AGAAAGATGATTGTGCCGGT	TTCGTCGTCATCAATCCAAC
CA0647	CCGCTGTTGCTGTTTCTACA	AGCAGAAGCCTCAAAAACCA

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0648	TGTTGAGGAACATCCAACCA	CTGCACGGGAGAAAAGAATC
CA0649	TAAAGGGCCATTTTCAACCA	GCCGTATCTCGTCATCAAGAA
CA0650	CCACGCTCTGTTCGTTGATA	AGACTTATCGCATCGTTGGC
CA0651	TGCTTCCTCGTCACATCAAG	ATGCACCAGATCTGAAACCC
CA0652	TGGGTAGCAGTGTTGCTCAG	GAAAGGCATCATTCATTGGG
CA0653	ATAGCAATTCCAGCGTCAGG	TTCTCCATGTTTTCCCCTTG
CA0654	TCTTTTGGAGGCAATGGTTC	TGCCCTAGGCCATTATCTTG
CA0655	CGCACTTTGGTTGTTGAAAA	GCAATTGGGAAAACTCCTGA
CA0656	GCCTTGTAGCAGAAGCGACT	TCTCTTTTCCATCTCCACCG
CA0657	GCACGACTTGAGGTTCAACA	GGGAACTCCTGCAACATCTC
CA0658	GCCATTTTCATCGTTCTCGT	CAGGGTTATCAGAGGAGCCA
CA0659	TCTTGCTGGAAGGCTTCTGT	GGCCAGTTGCCTAAAGAACA
CA0660	ACGGGGAGAGAGAGATGGAT	GCGTCGATATTGGAAGGAAA
CA0661	CTAGCTTCACTGGACCGAGG	AAGCCATTCTGGAAGCTCAA
CA0662	GTTTATGGCATGTTTTGGGG	CAGAATCCGAGCACAACTCA
CA0663	GACATAGCAAAATATGTGTCATTGC	AGCCTGGAGGAATTTGCTTT
CA0664	TCATCATAGTTCAACGGCGA	CCTTTCAAATTGCAGGATGG
CA0665	GCCTTGTAGCAGAAGCAtcc	GAAGCCCGTCCTTCTCTTTT
CA0666	ATCCCATTTCTCCCAAAACC	TGATTCTCATGCTGCCACTC
CA0667	GTTAATCCCACCCAGAGCAA	GCTCCTGGTAGAGACCCTCC
CA0668	GCAGAAGCCTCTATAGCCCA	CAGACAGGAAGTCCAGGAGC
CA0669	CATCAGAACACCCAATGACG	CAGCTTCTGGTAGTGTGGCA
CA0670	CTCTCCCTGCATCTTTCCAG	TTCCAATGGGTTCTTCTTGC
CA0671	TCCATCCTGTCCTCTGAACC	AGTTGCTCTAGGAGCCGTCA
CA0672	AAAGGGGAGTGCTTACTTTATGG	GCAGTCGTTGTCGTTTTCCT
CA0673	CAAGGGATGTTGATTTGGCT	CCACATCAGCTAGACTCGCA
CA0674	TCTTCTTCACATGTTTGCCG	CATCTGCCCTACTCTTCCCA
CA0675	CCTTGTTTCAGGAGCCAGAG	CTAGGCTGCTGTGTTTGTGC
CA0676	GAAATGCTGGGTTAAGGGGT	GGTGGGGGTATTTTGTGTTG
CA0677	GCGTCTAGCCGGGTATTACA	GGAGACCCGGGAGTTCTTAC
CA0678	AGCAATAGCCACAACATCCC	CGTGTGTGATCGTCGATTCT
CA0679	GATTGGATTGCAGAGGGAAA	AAAGGGGGAAAAACCTGAGA
CA0680	ACTACTGATCGCGCCATTCT	TGAAAAGGGGCTGAAACTATG
CA0681	ACGGACAAAGAATCTGGACG	CCTGTTTCTACCTCTCCCCC

Table A1: SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
CA0682	TCTTGGAGGCAATGCAAAAT	GTGCAAACCGCAATGTACTG
CA0683	TTCCGTGCTGTCACATTTTT	TGCCTGGACCATTACAAAATC
CA0684	CCCACTATCACAATCTCGCA	TTGTTGGACTGGGCCTATTG
CA0685	TGAAGCAGTTTCCTTCTGAGG	CGGTTTCCGAGCTGTTACAT
CA0686	ATGGATGGGATGTCACAGGT	TTCCTATGGTGCTTGACCAAC
CA0687	GAGGTGATGCGGATATTGCT	TCCCAACAAACCCACCTAAA
CA0688	GCTGTTGCTCCACAAAATGA	CCTCCACCCAATcgtAAGAA
CA0689	TTGGATAACTGGGGCTTCTG	ATCCCATGTATCCACCCTCA
CA0690	TACCATCTGCACCCAACTCA	ATCCACCCTCCCCTATTCAC
CA0691	TTTCACAGATCGACGAGCAC	TTGCAGGTTCATGTGTTTTCA
CA0692	ATCCTTCCCGTCCTTGTCTT	GGCATTCTCATCCGTGAACT
CA0693	GTCTTCGCGAGACTTGAACC	GAAACTTCTTTTTCGTATTCGATTG
CA0694	TGGTTACAGGGGTCCAAAAC	AAGGGTTTATGGGAGTTGGG
CA0695	TTATGCGACAAAAACCCCTC	CTGGTTGGTCGCTGGTTATT
CA0696	TGAAACCCAACGGTCTATCA	AAGCCATGGCCTCTTCACTA
CA0697	TTGGAAGTCGTCTGACATGC	CTGCTGCTTCTCCTTCCCTA
CA0698	TGCCCTAGGCCATTATCTTG	CGGGTCCATCCAATACTTTG
CA0699	AGTATAGCCAACCCGGGAGT	TGTCTGTGGATGGAAAGCAG
CA0700	CATTTCTGCATATGTCACACACA	TGGATTGATGTCCCTCTTCC
CA0701	CCTCGACCTGGACTTCCATA	CAAGGACCCCAGCAGTAAGA
CA0702	GAACTAATGCATCCTGCCCT	GCATGGTTTAGGCGGTTCTA
CA0703	TGAAGGATTTTCGTCTGTTTATGA	TTCGCAAGAATGAAATCCAA
CA0704	ACCTTGCTCCCACTAGCTGA	AGCAAACCCTACCCCAGG
CA0705	ACAAGAGCCCAGTGAGGAGA	CCCTGGCACAGTTGGACTAT
CA0706	AGGTGCTTGAGTTTCTGGGA	CATCCTTCTACCCAGGTCCA
CA0707	TCGCTATCAAGGGCTAAGGA	GTAGCCCAGAGTGAGCCAAG

Table A2: EST-SSR sequences

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0001	CTCATGCTCATCTTCGCTGA	TTCTTCCTCGTCTTCCAGTGA
MeES0002	CCTATAAACGGCATAAGCGTG	TCCACTGTCACGCCATAAAA
MeES0003	AAGAGCTTTAGGCGGTCACA	CCACAGCAAACGAGAACTCA
MeES0004	CTCCTCCTCTCCCTCAGCTT	CCTCAAACTTTCCGTGCAAT
MeES0005	GAAAAGATTGGAGGAAGCCC	TTGTTGAGCTTCATATTTGATTCC
MeES0006	AGCTAGACAAAGCAGCTCGT	TGTCGGTGCCCTACATACAA
MeES0007	GCACTTCCCTGACTCACTTCA	GAGCCGGTGTAGAGTTGAGG
MeES0008	CACTATAGAACCAGAATCCTAGCG	GCAGCTACTTGGGTGTGGTT
MeES0009	CCATAATAAATGTTTCAACCTCG	ATTGGACTTTTGTGGGCAAC
MeES0010	AAAACCATGACTGCCGAGAC	CCTCAGCTTCATCTTCGTCC
MeES0011	AGCACTGCGTTTGTCATCTG	TGCACCAAATATTCCCCATT
MeES0012	ACCTCCTCCTCCTCTTCTGC	TCCGGAGAAGTTCGAAGAAA
MeES0013	TCCTTCGAAAACAGCAAACA	TGCAAGTCTGCAACCCATAG
MeES0014	CGTGGATCTATCTCCTCCCA	AGAAGGGCAGATCTCAAGCA
MeES0015	TCTTCCCCTTTGGATCCTCT	TGACTTCAGAACCGAAAGCC
MeES0016	CAGTTCATTTCCCTCCGAAA	AATTCAGAGCCCCTAGCCTC
MeES0017	TCTGCTTCTGCCTATGTCCA	CGAATTCACGGAAACTTGAAA
MeES0018	AAATTGCTGTAAACGACGGC	TGCATGGAACAACGTTAAAAA
MeES0019	ACGCCACACCAGTTCTCTCT	TTCATTTTGGACCGTTGGAT
MeES0020	CAGTCGCAACAAAGAGCAAA	GCTTTCTCTCCAAATCCCCT
MeES0021	ACTCGCCTAATCAGATCCCC	CCAGAGGACAGTGATGAGCA
MeES0022	AGGACGGTCTGAGGCTTTTT	TTTCTCATCATGCACAACACTG
MeES0023	GTAGGACGGAAGAGGGGAAC	TTCCACGGAAGATCTGGTTT
MeES0024	GAGATCATACCTCTCATTCTCTCTCA	ATGTGGTCACAAAGGAAGCC
MeES0025	GGGTAAACTTTGCTCCTCAGC	CTCGAGCTGTTTCCCAAAAG
MeES0026	TTAAACTTCGAACCCGAACG	AGCTAATGTGCGAGTTCCGT
MeES0027	CCTTACCAATCCTGCGAAAA	AGCACAATGAGTGCGATGAG
MeES0028	CATCCGTCCAAATGACACAG	AACCAACAACAACCACAGCA
MeES0029	GGTTTGTTCTGGGCACAAGT	ACACCGTTGTACAGCAACCA
MeES0030	CGTCTCAGTCGATCCTTTCC	TCCCGGGTCTTCTCTGTATG
MeES0031	TTCCATGACTCTCGATGCTG	CCCAACAAGGGCTGTACAAT
MeES0032	TTGGCAGAGGTTTATGGGTC	TGAAATTCACCTTCATGTGAGAA
MeES0033	TTTGGTGAGAGGAGGAGACTTT	TGATTCAAGCAAATGGATGC
MeES0034	ACACAGCACAACTGAGCAGC	TGCCCTCAACTTCCATTTTC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0035	CTGCTTCCCTCTGCCAAA	AATGGCAAAAACTCCCCTCT
MeES0036	TCGAGGAAGCTCGGTACACT	GGTAGGCATATCGTGCGAGT
MeES0037	CGGAGTTTCCGTCTCTCTTG	GCCCAAGTAGCACCCATAGA
MeES0038	TGGTTTCTAAGATCAGGGCG	TAAACATTGGCCCTTCTTGG
MeES0039	CGTGGATCTATCTCCTCCCA	AGAAGGGCAGATCTCAAGCA
MeES0040	ACGTGATGCAGGTGAGGTTA	TTCACTCATGCATCCTCAAAA
MeES0041	TTTTTCTGTCTTTCCCTCCTTTT	GGCCCTTGAAATCCCTAAAG
MeES0042	CAGGAATTGCGGTTTCCTTA	CCAAACTCTCATATCGCCGT
MeES0043	TATTTAGCGGGCAACTTTGG	GATAGGCAGAGCTCACCTGC
MeES0044	TGTTGCCAAGTGTTTGTTCC	CGGAATATATCCTTCAAGACCC
MeES0045	AGCTCTTGTTGCTTTGTGCC	ATTGCTCTGACCCTCCTCCT
MeES0046	CTAGCCCCATCTTTCACCTG	GAGCAACAGATGTGCTTCCA
MeES0047	CACCCTATTTCCCGCTCATA	GGGGGAAAAACTCACTTTCC
MeES0048	TTGTTCACACCACCCACTTC	GAAAATTCCAAGCAAGTCGC
MeES0049	GACAACTCCTCTCGCCGTAG	GTCCACATAGCCATCCCATC
MeES0050	GGGAATCGTCTGGTTTCTTG	CCTCGAGCACCATGGTTAGT
MeES0051	AACTCGCGCCAAATACAAAC	CCTGGACGGTATCCTTAGCA
MeES0052	CCTGCTTCCACACAGCATAA	AAACAACAGACGGCAAATCC
MeES0053	TAGGGTTTTCCGATTTGCAC	GGAGCAGAGGATCAGAGTCG
MeES0054	ATAGCACCCCACCACCTGTA	ACCTCCGGAATGACTAGCCT
MeES0055	ACAGAGCCTACTGATGATGTGA	GGAGCCATCCACTTAGTCCA
MeES0056	GGCTTCATTTTAAGGGGGAA	GCTCTTGATTTCGTGAGATGG
MeES0057	CTTCCTCACCACTCCTCGC	AGTTGGCTATGGCAGCTTGT
MeES0058	TTTGTTAAGTGTTGCTGCCG	CCCACCTCCGAAATCACTAA
MeES0059	GTCAGCGCCTTCTTTTTGTC	TTCAAATGGATCCGGAAGAG
MeES0060	CCCGAATCCATCTCAGAAAA	CGAGACTCTTGGTTTCGAGG
MeES0061	TCAGGGGCTTCTACTCTCCA	CCCTAGAAGGGAGAGGATGC
MeES0062	AAAATCTCCATCTCCCTCCC	TTCTCCTTCTCCTTCCCCAT
MeES0063	CAACCGTCCAACCATCTTCT	GAAAAGTTGAGGCAAGCAGG
MeES0064	AGGTCTCCCTCACTTTGGCT	TGGCCTCTCTTGACTCCCTA
MeES0065	GAGCGTGGATTCCCTTAAAA	GATTGGGCAACTATCGCTGT
MeES0066	TCTCGTTTCCCAGCTTCACT	ACATCTTCACCCAATCGAGC
MeES0067	ACCGAAGAGAAACCAGCTCA	GATTGGGGTTGGCTTTGTAA
MeES0068	GGCAGAAGGGAATACAAACG	AACACCGTCAATTCTCCGTC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0069	ATGGGAAGGCTTTTGTTCCT	CATTGTTTGGACATGGCAAG
MeES0070	TTTATATCTTCGGCGTTCCG	CGACATCACACGGAATGAAC
MeES0071	TACGTCTCCCTGCTGCTTTT	TTGATTCCCCGATTGTTTGT
MeES0072	GGACGTAGCCCTTCAAACAA	TGAAAGCACAAGAATGCCAA
MeES0073	TCAGCACCAGTCAACATTCC	TTTGAGTTTCCTGCCAATCC
MeES0074	GCCTTTGAATCCTCCATCAA	ACAGCATGCGAAAAAGTCCT
MeES0075	GCAATGAGAGGCCACTTAGC	TTTGCATTGACGGTGAAGAG
MeES0076	GTGGCAATAACACGCAGACC	GATTGGTGGCGACTGTTTCT
MeES0077	GATTCACATCTCCGCTCCA	ACGAGGGTTATACGACGCAC
MeES0078	ATCGACACGTCGTCTCCTCT	TCCGCTCTTCCTCTCTCTCA
MeES0079	GGGGGAGGAAAGAAGAGAGA	AATTGAAACTCGCCCTGAGA
MeES0080	AGCAGAAGAAGAGCGTCTGTG	AGCTCCACAACTTCTTGACCA
MeES0081	CAAAACTGAACAACCAGCGA	TCTCCACCTCCCCTTTCTTT
MeES0082	GACCATCAAAGAGAAAGAAAGAGAA	TGGGTAGTGCAGTGAAGCAG
MeES0083	CTAAACAACGGCCTCTCACG	AAAATCAACGTTCGGGTCAC
MeES0084	GTAAGGGCAGTCACCAAACG	GAACCTTATTTGCAACCGGA
MeES0085	CCACCTCCTTTAATGAGCCC	AGATGCGAAGAAACCAGCAT
MeES0086	TTTCATTTCCATCTCCAGGC	ATTCCAAGAGCGAAGCAAAA
MeES0087	GTCGAGAAAGCTCCTCCCTC	CAAAAGGGGAATAGATGGCA
MeES0088	ACTCCCATGTTTGTCTTCCG	GCTTCGTAAAGGTAAGCCCC
MeES0089	TCCATGGATGGGATTTCTTA	TGGATGGCAAATGAAGTGAA
MeES0090	GGCTTCAAGAAAAGCTTCGTT	GACATGAAAAAGGCTCCGAA
MeES0091	GAGACATTTTGGTGGGTGCT	TTTCATGGGGCCATACACTT
MeES0092	GCGCTTTACAGGCGTTTTTA	GTCTTTGCTCCGTCGTTACC
MeES0093	GCTTGGGTTACTTGCAGCTC	GCACAGTTGATGCTGTGGAT
MeES0094	TTTGTATTCTCATCTCCCTCCC	GGAGGAATGGATTCTCCCAT
MeES0095	TCAGACCCGTCCTCTTGATT	TGAAGCTTAGTTGTTGTTCGAGA
MeES0096	GCCGAAGAATGGGTATAGCA	CAGAAGAGGGGAAAACACGA
MeES0097	ACCGATTGACAATGGAAAGC	AGTGATACGCGTTCTTCGCT
MeES0098	CTTCAGCTCTCTGGCCAACT	AACTGGGAGCTTCCAAAACA
MeES0099	AAACTCAAGAAACTGCCCCA	GATCAAGTCCCATTTTTCTTCA
MeES0100	CAGCTATTGCGTGCTTCGTA	CAGAGAACACCGCACTCAAA
MeES0101	GCTTTGCTGTGTGTCACCTC	AGCTCCACGAAATGCTCTGT
MeES0102	TCCATCCTCATCCTCTTCCA	GGGTGACGTTTTCATGCTTT

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0103	TGAATAAAGGGACAGAGGGAAA	CATCACTGCAGCCAGAGAAA
MeES0104	AAGTTGAGATTGTTTATCGCCT	TCATTCGAGTTTAATTCACACACA
MeES0105	ATTAAACCTTGGGGTCAGGG	AGGTGACAGTTTCTGGTGGG
MeES0106	TAGATCTGACCGCGAAAGGT	GGCCAATAAGTGCCAAAAGA
MeES0107	AACTCGCGCCAAATACAAAC	CCTGGACGGTATCCTTAGCA
MeES0108	TCAAACAAAATTACCCCACCA	CTTTCTTGGGATGGGAGGAT
MeES0109	TGCTACTTCTGGCTTCCTCAA	GCTGATGTTCCCCTCCATAA
MeES0110	AGAATCAGAGCCCAAAACCC	GCTCTTTTTCCTTGGCTGTG
MeES0111	GCCTCTTTTTCTCCCCATTC	GGGTTATGAAGCGCAGTGAT
MeES0112	TAATTGCAACTGGTCGGACA	CCCTACGTCCTTGCATTCAT
MeES0113	GATGGACGCCACCATTCA	GGCGTTTCAAAATCACCATT
MeES0114	GAATTCATCGTTGCGAAGC	GTTACAGCTCTCTCGACGGC
MeES0115	CCTCAAGCAAAGCAAAGGAC	TCTGCAGTAGAAGTGCGTGG
MeES0116	CAAAAAGTGAAGGCTGAGGG	TTATCCATTGGGAATAAGCG
MeES0117	ACAGGCAGAGGAGATGAGGA	GAAGGCCAGACCCAACAATA
MeES0118	CGAAATATTCTGCCTGGGAA	CCCCCATCTTATCTTGCTGA
MeES0119	TTGTTTCATGTGGGGAGAGA	CAGCTCTCTGCCTCTGTGTG
MeES0120	GTCACCGTCGTCATCATCAG	GCTCTCTCAAGCGCAGATTT
MeES0121	GCTGTAGAAAGGCAGCTTGG	CCATTGAATTGTTGTGCAGG
MeES0122	GAAAAGCCATACAAACAAAGAAAA	ATCTTGAACGCCGAAATGAG
MeES0123	CCACCGAGAGATCTTCTTCCT	CAACCCTCCAAGGATTTGAA
MeES0124	TTTTCCATTAAAGAAAATATGAGACA	GTTTGTCTTGGTTTGGTGGG
MeES0125	TGTTTTCTTTCCCTTGCTCG	TGAAGGAAGAATTTGCCCAC
MeES0126	AAGACTGCTGACCTTTCCCA	ACTATCCTGCAACCACAGGG
MeES0127	GGATTTATGCTGTTGTTTATTTTCC	TCATCGTCTGAACAAGCGAC
MeES0128	TCGTTAAACGGTGAGTTCTGA	GTGGTTCGATGCTGTTTTCA
MeES0129	TTTCAGAGATGGGAATTGGG	TACGATGGAAGCAAATCACG
MeES0130	TCCTCGAATTCATCTTTGGC	AAAAGCGCTACCGAGTGAAA
MeES0131	CCAATCCATCCTTCCTCTCA	AGGAAGCAAAGACGACCTGA
MeES0132	ACCCGGGACTTCATACTTCC	TTTCAAGTGGTCCCCTTTGT
MeES0133	CCTTTCCAGAGCAAAATTCG	CCTCCGTAAACTCAAAGCAA
MeES0134	TTCTCGTCGGCTCCTTTCTA	CCCCACTTGATCTGCCTTTA
MeES0135	TAGGAGATGGTGCCTCCAAG	GGTTTCACTTTCCTGAAATCCT
MeES0136	TCAAGCGAAGAGCATCAGAG	GATCTGGGAGGATGTCAGGA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0137	TGCCGAAGAGACTGAGGAAT	TTCGCAAACTGTACTGTGCC
MeES0138	TGATTACAGGGCAACCATCA	AGAGCATCCAAGATTCCCCT
MeES0139	TGACAGGCCTAGAGATTCCG	TGACTCGTACCTGCTCAACG
MeES0140	CCGACCACAACCCTTGTACT	AAGCCAAACGAGGTGAAATG
MeES0141	TGAACCAACAGTGGCAACAT	TCAGGGCCTCCCTATCTATG
MeES0142	TTTTTCGGAGATGGATTTGG	CCTCCATCTCTATCCGCATC
MeES0143	CAGCCTCATTTCTGGGTTGT	TCTGGGTGAATCTCTCTTTGG
MeES0144	TCGTTTGCAGCATTCATCTC	CCAGGAAGTTACGAGCTTGG
MeES0145	TGGTTTTTGTTTCTGTTCTTGG	AGGGTGTGAAAATTGATGGG
MeES0146	TTCATCAAAGTCTCGCAACG	GCAACTCATGGACATTGGTG
MeES0147	ATTACCAGCAGCAGCACTCC	TGATCCGAGGAACGAATAGG
MeES0148	GGAGGTTGTGGCAGAAACA	GTGGTAGCGGTGACGATTTT
MeES0149	AGGAAAGAAAGGAAAGGGGA	TTTATGGCGCGTGTTTCATA
MeES0150	GCAAAAGATCCAATCAGGGA	GATTGGCTGAAGAGCCTTTG
MeES0151	CCTGCTGCCATTCTCACTTT	AGCAGTCAAAACAGTCGCAA
MeES0152	CATCTGAAACAGTTACCAAGCC	AAGGACCGACATTGAGATGG
MeES0153	ACTCCACCATTCCAGCAATC	CAGCAAGCTTTTTCCTTTCG
MeES0154	ATCAATCCACTGCAACTCCC	GTGGGCGCTGAATTCATACT
MeES0155	CTTCATATGCCACCTGCCTT	GCGCTAAACCTAGCAACCAC
MeES0156	AAAGAACAACCAAACAGGCG	AGCTAAAATGCGAAGCCAAA
MeES0157	CGCGTCCTCTCGAATAAAAA	GTTCCTTCAAGATCCGCTTG
MeES0158	CGAATCGTTTCAACCACAAA	GGTGCCATAAAAGAATTGCC
MeES0159	TCAGCCAGACAGAGAGATGC	ACTGCAAACCGCTCTCCTTA
MeES0160	CATGCCAAAACCTCTCCATT	CCTCCTCCAAAAACAGCAAA
MeES0161	TCCCTAAGTATGCAATGGGC	TCAATTGTCAAACAAGGGGG
MeES0162	TCTTGAAAGGGTCAATAAGTTGC	TCAAGGCCTAGAGCTTCCAA
MeES0163	TCCTTGAGACAGTATACCAGCG	TGCTCGACAAAGGAACACTG
MeES0164	TTCTTTTGCATCCCCTGTTC	GCAAATTTACGCTTGTCTTGC
MeES0165	GGAAGCATGTTTCTTTGATAATTC	ATCTGGTTCTCGTGGGAGTG
MeES0166	GCTTCTTGACCTTCCATTGC	AACAAGACAAACCCACGAGC
MeES0167	TGGTACTTCTGTGTCGCATGT	ACTACAATGCGGGAGGTCAG
MeES0168	GCGTTAAGAGTGGCAAAAGC	CCCCTCCGATATTTTGGTTT
MeES0169	TCAGGAACAAGTGCCAGGTA	GGATAGAAACCCAAAGCGAA
MeES0170	TCTTCCTCCTTTTCGAACCC	TCATTTCTGGCTTGTCCCTT

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0171	GATACATAGATCGCTTCCTTGAA	TCAGTCGAAGAGGAAAGGGA
MeES0172	GATACAAAGCCCCAAGCTCA	ATGCATTTCTCAGCGGAGTT
MeES0173	CGGTTTCTTCTATCTCCGGG	AAAAACCCACGCAAATCATC
MeES0174	GACAAGTGATAATTGCCTTACCTT	GAGAGCTGTTTTGATTCCGC
MeES0175	ACTTTGCCCCTTCTCCAAGT	TGCTAACAACCCCACTGTCA
MeES0176	TGCTTTCTCTGCCTTGGTTT	CGCTTCCAGGAGCTAGAGAG
MeES0177	ATTCGGCCAGAAATGCAGTA	CGCTCATATTGGGTATGGCT
MeES0178	GCCTTTCTATTTCGCTGCTC	CAAAACCATGGGGATGATTC
MeES0179	GCCCAATTACCAAAAGCTGA	CTGAAAACCAACCCACAACC
MeES0180	GCGGAGCTAAAACTCCCAA	CTGCATAAGATGGATCGGCT
MeES0181	GGGTTCTCACAGTGACGGTT	AATCCCAAAGGCACACAAAC
MeES0182	TCTTGCCAGTCCACTTTCCT	GGTCCACTCCAATTCCTTGA
MeES0183	TCAGTGGATCAGTTTAGGGAAG	AGTTGCGAACCACAAAATCC
MeES0184	ACAGGGACCCGAGAGAAAAA	CGGAGCATTTGGAAAATCAT
MeES0185	GCCATTTGCAGACCTTGATT	TTCATTCCCATTTTGTGCAG
MeES0186	TCCTAGTGTCCTGTTTGCCC	TGAGACGACCATCCAAATCA
MeES0187	CGTTCAAAGAAAAGGCTCAA	TGGCCTTCAGTTCCAAAAAC
MeES0188	ATTACCCCGGTTTCTCGTCT	CCATCGCCTTGAGATTTGTT
MeES0189	CAGAGGAAATTGGTGGAGGA	AGAAGACCCAGAGGCCAGAC
MeES0190	AGCGCAGCATTACGAACAC	CAGGAGACTTGTGCTCTCCC
MeES0191	CATCTGATTCCGGTTGTCCT	TGGGACGTTGATCCCATATT
MeES0192	GAACCTGTGGTATGGGCATC	AATTTGTTCATGGCCAATCAT
MeES0193	TGGTTGATCCCTTTTCAGTTTT	AGCAGAGCCCACCAAAATTA
MeES0194	AACGAAACAGACGCTCAACC	GCAGGGAGGTATGAGTGGAA
MeES0195	GCAGAGAGCATCGTTGATGA	TCATCGTCACCGTCCAATAA
MeES0196	CTAGGGCTCCTCCACCGT	GTAGGCTTCTGAGCCGTCAC
MeES0197	AAATGGGCAAAATTCACCCT	AGAGGACAGTGCCCTTGAGA
MeES0198	TGGGGAAACGAGAGAAGAAA	AATCATTATGCTTGCCGTCC
MeES0199	GAAGACGACCCATTAGTGCC	AAAGGGATGCTAACGGGAAC
MeES0200	CATACATAAACACAAGCTATCTCAGC	GAGATGATGGCTTTCTGGGA
MeES0201	ATGCTCTCTGCCAAAAGGAA	CTGCCATGGAAGAAGCTACC
MeES0202	ACCGATTTCTGTCGGTGATT	GTAACAGAGGAAGATGCGGC
MeES0203	TGTTCCTTCTCTCTCGTTTCC	ATGAATGGCATAGGATTGGC
MeES0204	AGAGAGAGAGAGAGTGAGAGAGGG	GATTGAAGTCACGCGCAGTA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0205	TGGATGGCTGGCTAAAAATC	ATACGCCTTTTGGAGCAATG
MeES0206	GGTGTAGCAGGGAATGGCTA	TCCGGTTCTCTTGATTGGAC
MeES0207	AACCAACTCAATAGGCGGC	TTGATTTGTGGGTTCCCAAT
MeES0208	AACATGGATAAGCGTCCCAG	TTGCGAGGAACTGGAAAATC
MeES0209	ACAACAGGGTTCTACACGCC	TTTGTCTTCCAAGACCGTCC
MeES0210	CTGCCCCACTTGTCAGAAAT	TCATTGCCTTGAACATATCAGTG
MeES0211	TCTCCAATCCATCTCCTTCG	GAATTCACGGCTAGACCCAA
MeES0212	AAGCATCCCCTCTCTCCAAT	GCAACATCCTGGCTTTAGGA
MeES0213	CCACCTCGTAACCCTCATTG	ACAAGGCTTCTGCTCCGTAA
MeES0214	ACGCAGCAGCTCTTAGTGGT	GCAACATGTCGAGCAGAAAA
MeES0215	CACAAAACTAGCGGCACTCA	GGAAGCCACAATTGACAGGT
MeES0216	TTCCTAAAGTATGGAGACAGGG	GCATGAGTTTGTGGGATGTG
MeES0217	TCGTCTCCCATTTTTACAATGTT	GGGTTTGCAACAAATATGGG
MeES0218	AAAAGGACCAAAGCCCACTT	TTGACCCACCAAGTGTTTGA
MeES0219	AATTTCAATTGCCCCAAACA	TTGGATGAAAATGTGCTTGC
MeES0220	GATGAAAGAGATTGACGCCC	GCGCGAATGATTAGGAGAAA
MeES0221	TCCTTCCGTGGCTACTTCAC	AGAGGTCCAGCAACTCCAGA
MeES0222	TCCCGTTTCTTTGGTGAATC	TAATGAAGGGACCGTGAAGG
MeES0223	TTTTTCTTCGCACATGTTGTTT	CGCTTCTTCTGCTTGAGACC
MeES0224	TTTCACCTTAATTACGCCCG	CCGGTCCAAATTAGCATCAT
MeES0225	CCAATTTCAGAGGTCTGTGCT	GCACTCGTTTCCTGCTTAGG
MeES0226	GGAGGTTTGGCGTTGTTCTA	AGGGATTCATGGCTCTTCCT
MeES0227	GCCCTCCTAAGGATTTCTGG	CGTTCTTCTGCAGCCTTCTT
MeES0228	TACCCACCTCACTTCCCATC	TGCAACCTTCTTGTTGGCTA
MeES0229	CAAGCAATTCCCCAGTCTTC	ACCAAAGTTTCCAGACCCAA
MeES0230	TCCCCATCTTCCCTCTACCT	TCTGGCCTCTTTCTTCCTCA
MeES0231	CTCCTTCTCCTTCTCCTTCTCA	AAAGGGACTTTTAGCCCCAA
MeES0232	CCCAGGCTAATTATTGGGGT	CTGAGGAGCAGAGCCGTTAC
MeES0233	GTGGATAAAGCAGCGGACAT	TGCAGAGAACTGCCACTGTT
MeES0234	AGGAGGATGTGAAGGTGTGG	AATATTCTCCGGCAATGCAA
MeES0235	TTCAATGATGGCTGAGCAAG	ACACCTTCTGAGAAAGCCGA
MeES0236	GTTGAGAGTGCCAAGAAGGC	TGAAAATGCGAGTTCTGCAC
MeES0237	TCCAAAGCCAGAGGAGTTGT	ACAAAGGTGCAAACCTGTCC
MeES0238	GTCTGCTGATTCTGAGCGTG	GGCAAACTCAAATATGTTAACTTCAG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0239	TGAGAGACGAGCGTTCATTG	GAATGCCTCCCAAACATCTC
MeES0240	AGAGGGAGTCTGCTTCTGCTT	GGCGCTCGAAAAGATAGACA
MeES0241	CCTTCGCCTTTCTTCCGTAT	CTGCAGTGGCTTGATTTTGA
MeES0242	TACTGTTGTTGTGGCCGTGT	TCTCTCTCTCCCTCTGCCTCT
MeES0243	TTTTATACAACACGAACTCGATCA	TGTCAAATCTGAGGATGGCA
MeES0244	GCTCAAGTGGCTTTGTAGGG	TTTCCACAAGCATTCCAACA
MeES0245	GTCAACGAAGCACAAGAACG	TGTACTGCGAGGCGTGTTAC
MeES0246	TTGTGACTGAGGTTCGATGG	CCACTGCCTTTCAAGGGATA
MeES0247	TCGATCAGGGGTTTTGAGAC	GTTGCAACTGTGAGAGCCAA
MeES0248	TTCCTGCTGATGAAGCATTG	AGATGAGCTCGGAGACGGTA
MeES0249	ATGGTGGAGAAAGTGATGGC	TGGTCCTTAACAGCAATCAAGA
MeES0250	TCAAAAAGTTTTGGTTGTTGC	TAGCCAGAGTTGCTTCCACA
MeES0251	AGAGGGAGAGAACGAAAGGG	TCAACCGAGTGAGATCATGC
MeES0252	ACGATCGCTCATGCTTCTCT	TCAAAAATGTAATTGGCGCA
MeES0253	TGATTTTTAAAGTGAAATTTGTTTAGG	TTGATGACTGACCATCACGG
MeES0254	GCTTCAAGAGGTTCAATCGC	AGTGATGCTCTTCCGGTTGT
MeES0255	AAGGTTTGATTCAAGGCGTTT	CAAATTGCGTTAAAACACAAAATCT
MeES0256	CGGAGCGTGACTGTCAAAT	GGGATTCCCGAGAAAAAGAG
MeES0257	GATCGGATGTCTGAGGAGGA	TGCTAGTCCTTTTTGGCAGG
MeES0258	AGCACCCTTCATCATCTCCA	GTGACAGCGAAAGCATGAAA
MeES0259	CGCCATTTACAAGCCAAAAT	GGCTGCAGTTTTCATCCTTC
MeES0260	TGGTGGGAACTAGGAAGACG	TGAAGAATGAAGAACCCGAAA
MeES0261	CGATTTTCCCATCTCTGGAA	GCAGACACAAAGTCCAGCAA
MeES0262	CATTCCCCATATAACGGCAT	TGAAAACGAAAGCGAAAACA
MeES0263	TTTTTCATACAATTAATGCGACTTT	TTCGTAAGGCCCTCTTTTGA
MeES0264	CATTGCCTCAACATCCTTCA	AAAAATGTTGGCATTGGAGC
MeES0265	TTGGGTTTCTTCGTTTCTTTTC	AAGAGCCAGCAAATCAATGG
MeES0266	GAGGCACCAAAACAAGGAAA	CGAAGGGAGCTTGATTTCAC
MeES0267	TAATCCGGTGATCCTGAAGC	AATTGTCGAGGACAAGTGGG
MeES0268	GCCTTGAAGCCATTGAAGAG	GACCATGCAATCTAGCCGAT
MeES0269	CCAATCAGAAAGTTTGGGGA	TTATGCTGAGAACTGTGGCG
MeES0270	ACCACTGCTGCACTCCTCTT	CAAATTTTCAACACAAGCAAGC
MeES0271	ATTCTCGCATTCACAATCCC	AAAGCTGCTCAAACCCAAAA
MeES0272	CGATCATTTGATGCTGTGCT	TTTTTGCGCCAAGACTTTCT

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0273	TAGGCAGCCAAAGCAAGATT	CCCTTTGAAAGCAATACCCA
MeES0274	TTGTCTCTGCCAGCTTGACT	CTATGGTGGCCTTGGAGTGT
MeES0275	TTTTCTTCATCAACTGCGGA	TCCAAGAATCGAGAACCACC
MeES0276	TTGAGGACTTTACGGATGCC	TCAACCTTTTTCAGCTATTTGAACT
MeES0277	ATGGTCGCTGCCTACCCT	TGTAGAGGGGTGGCGATTAG
MeES0278	GGGTTTTCATCTTCCCATCT	TTCCCTTCCTTTCCTTGTGA
MeES0279	CAAGCTTTCTCCTTCATGCC	AGCTGCGATGGTGAAAATCT
MeES0280	GAACGCGAAGAACAGACACA	CGATAATCGTCCTCAGAGCC
MeES0281	TGGGTATGGCTTTCGGTTTA	TGTTCTGCTCTTGCATTTGG
MeES0282	ATGACCATCCAACCATCACC	CCATGCAGATTGCTTGAAGA
MeES0283	ATTCGCAATAGTTGGGAGGA	GCTGGCATCAAATGGAAACT
MeES0284	GGAGCTAAACTCCAAGCTCA	GCGACTTCCACAAAAGGAAA
MeES0285	TTCCTTTCATCCTTTGTGCTC	CAATCGCTAAAAAGATGCCC
MeES0286	AAGAGAGAGCCAGAGAGGGG	GTCAACAGAGCAAGGGCTTC
MeES0287	TGAAAACTCGGTTTGCTGTG	GCTTCCACATTCATTATAGAAACAAG
MeES0288	AAGTAATGGATGTCGGTGCC	AACCCAAACCTGCAACTCAC
MeES0289	GCAGAGGCGCATAAAAAGAG	ATGCTTGCAAAGCCCTAAAA
MeES0290	CCAGCCACTCTGCTGTGTTA	CACCTGAACCAACATCAACG
MeES0291	GCTTTGGGGTGGTGTCTATG	GCTGAAACCCCCAACTGTAA
MeES0292	CCAAATGGGCAATAGTGTCA	GGCCTGTATGGAACAAGGAA
MeES0293	AGCTTGTAATGTCGCCAACC	TTGATAAAAGGCCAAGGGAA
MeES0294	GGCCAGCTCTGTATTCTTGG	TCGGTTTTATGCGAGAGCTT
MeES0295	TGGACCTGTTTTCTTTTCCTG	GCACCCACCACTGAAGTACA
MeES0296	TGTTTCAAACCTGCCACAAA	TCCACAGAGAAAAGGTTGCC
MeES0297	GAGTTCTTGCCGAAAGATGG	ATCAATCACTGCCCAATTCC
MeES0298	TGCAAAATCCACTTGGTGAA	TGCAGAGAAACAGGCAAAAA
MeES0299	GCCTAGGGCTCTGTGTGTGT	TCAAAAACCTAAGGGGGAGG
MeES0300	TGCAACTCCTTCGGGATAAC	AGCGATACACGGAATCACCT
MeES0301	AGTCTGAAGGCGGACTGAAA	TACGCTCCCAATCACCGTAT
MeES0302	TTCCAGGACATGGACTCCTC	TTCCATGCCGACATAGATCA
MeES0303	TCGACATCGCTAAACGACAG	CAGCATCATCTTGAAAGCGA
MeES0304	TCTACAACCCCTTTTGCCTC	GGAGATCACACCCGTAATCG
MeES0305	CAATGGGTAGGATTGTGGCT	GCTGTCATGGTCACTCCCTT
MeES0306	ACACCAAACGCTCCATCTTC	CCTTGGAGCCAAAAAGTTCA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0307	TTCGTCTGAGAGGGAGAGGA	TTGGAGATGAGATGGGAAGG
MeES0308	AAGGTGCCCTAAAGGTTGGT	CCATAATCAAGGCCCAAAGA
MeES0309	TAATGCCTGTGGTCCTCTCC	CCACCTTCTTTGCTTTAGCG
MeES0310	TTTTGCAGCTTGATGACTGG	GGCATCCAGTAGGTTGGAGA
MeES0311	TGAGAATGCTGAGACGGATG	AAAAGGGCAAGAAAACAAGAAA
MeES0312	TAAGATGTTCCCGTTCCCTG	ACCGTCGATTGATCACTTCC
MeES0313	TGGGAATTAGGAGGAAAAAGG	CCATCGTCATCTCGTGTTTG
MeES0314	GGTGAGTTTGATTTTGATGGC	CCCTTAGCTGATCAAGTGCC
MeES0315	TGCAGACCAGCAGTAGCAGT	TGAGTTGCCTACGGATGATG
MeES0316	CGGCCAGACAGAAAATTGAT	GGAATACGCACGTCTGGAAT
MeES0317	GTCCTTTTCTCCGACTGTGC	CCCGTAGAGAGGACTGACCA
MeES0318	CATCGCTGTACCACACTGCT	TCGCTCACGTTCAAGATCAC
MeES0319	TGGTTGCATGTGTGGAATCT	TGCTCGCATGTACTACCACC
MeES0320	CAACCACATTGATGCACTCC	CGTCCCATGGTTTAACATCC
MeES0321	TTCTTACCTGGCTCGCTGAT	AAACGCAAGGAAGACGAAGA
MeES0322	TTGAGAACTACCACCACCCC	TCGAGTTGGAACGAGATGTG
MeES0323	GAGGGTTTTGTCGCTCAAAG	GGTAGCGGACGTCTTCTCAG
MeES0324	GATTGAGCGGTTGGATTTGT	CCTGCACCTTGTGGAGAGAT
MeES0325	TGGAAGGATGAAACATGCAA	TAATATTCCCCGTCCCACAA
MeES0326	TCTAACGACCTGTCCCAACC	TGAAATCGATGGGAGAAAGC
MeES0327	TTCTCTGATCGCAGCCTTCT	TCATGTTTTGATGGGCAAGA
MeES0328	TTTCCCCACTCTCTGTCCAC	ACTTGCCCACTAACTGGCAC
MeES0329	TTTCCTCATTCCCAATGTCC	TACCAATTGCCCCTTGTAGC
MeES0330	TGTCGTTTTGCCAGTCAGAG	TGCTTGTCTCAACAAGTGCAG
MeES0331	GGAACCACCTGGGTTTCTTT	GGCGCTAAAATTCCCATACA
MeES0332	CTCCTCTTCTACCGCCACTG	CGGAGTTGAGATTCGAGGAG
MeES0333	GATGATAAAAGGGATGCGGA	ATTCACGAGCAAAATGACCC
MeES0334	TTAAGCGCCGCAGAAATACT	CAAACACAACATCTCCATTGC
MeES0335	TGACGATTTTCAAGGGAAGG	TTCTGCCACTTTTGTTGCTG
MeES0336	TCCACTCTTCTCTCTCCTCCC	GAACGCCATTTCAGTGGTTT
MeES0337	ATAACAGAACACGAACCCCG	AGAATTTCAGCTCCAACTGTGA
MeES0338	ATTCCCTCCTCTTTCTCCCA	ACAGCACAAACAGGAAACCC
MeES0339	AGGCGTTTTCACTTCCTCAG	CTAGCAATACCCCAGCCAAA
MeES0340	CAACTTCAAGCAGTATGTTCTCTC	TTAATAACGAGGCTTGCGCT

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0341	CAACCCAAACCACCACTTCT	ACCAGCAGCTATAGAGGCCA
MeES0342	ATCTCTCGGCATCCAACAAG	GGAAACTATGAAGCCGTGGA
MeES0343	GTTGGGCAGAACCATCAAGT	CTCCTCTGGTGCTCAATCCT
MeES0344	TTCCAGTAATGCTTCCGAGC	CAGAGCACGATTGGAAGTGA
MeES0345	GCCACGAGCATAACCATTTT	TCCTGTGCTTCTTCGGAGTT
MeES0346	TGATCTTCTTGGAGCTGCCT	CACCTTCAGAATGTCCAGCA
MeES0347	CCTCATCTGGGATTCTCCAA	GAAGAGTAGGGGCTTGCTCA
MeES0348	ACAACAGTCCAAATCCAGCC	CCATCTTCACGAACTGGGTT
MeES0349	CATGGGAGGAATGAAGGAAA	GTTGAGCCAGAAATGGCTTT
MeES0350	TCTCTCTCCATTTCAAAAACCA	GCCATGTAGACGCTCTCCTC
MeES0351	TCCTCGCCACTTGAAAAATC	TGAAGGGAACAGGAGCATCT
MeES0352	GTCCTAAATGACGGCTTCCA	TCAACACCCACATGTACAGC
MeES0353	CCATACCACTGGAACAGCCT	TTGCAAGTTCTTGGCATCAG
MeES0354	CTCATCTCTCGCAACCCTTC	GGGCTTGCTCAAAGTAGTGC
MeES0355	TCATGGCTCTTTTATTGGGC	TTTGAGTGCTCCTCGTGATG
MeES0356	GGCCCCCATTATCAAGTTTT	TGGAACCCAGCCTCATAATC
MeES0357	GGGCAGTGGATCCTCAAATA	CAGGAACCAGACACAACCCT
MeES0358	CAGGTTCTGGTTCTGGGTGT	TTGGTATCCGGCAGAGAATC
MeES0359	AATGGCTCAAATGCAGCAG	GCAGTTCCAGAGCTATTGGC
MeES0360	CCCATTGATTTCCTCCCTCT	GAGTGGGGCTGAGTGAGAAG
MeES0361	CTTATTCCAGCTCACCCCAA	AATCAAAGAGACGGCGAAGA
MeES0362	GTCGACGGCTAAAATTGCAT	ATTTTTCATCATTGCCTCGG
MeES0363	TCCTCTCCTCCTCCAAAACC	CAAGGGCTTTGGACCTGTTA
MeES0364	AAGCTTCAATGGTGATAATTTCAAG	GCATTACACGAACAGAAAATTCA
MeES0365	TTTGCAACAGCAGGAGTTTG	TTGCTTCAGGCTTGGAAGAT
MeES0366	TTCACCATAATGAGCACCCA	TTGCATTTGTTGTGGCAACT
MeES0367	CACCCCTTCCCTTTCAAGAC	AAGGCGGTTTCTTTGGAGAT
MeES0368	CAGAAGCATGGGGCAATAGT	CGTTGCTTTAGAGATCATCTTTTT
MeES0369	CTACAACGAGAGAGGTGGGC	TCTCACAGCAAATGGCAGTC
MeES0370	GGGTGAATTACTTGAGGCCA	CTGCTGCTGTTGCTGTTGTT
MeES0371	TCCAGCTTCCAGTCTCGATT	TCGACTCTAGCATCAGCCAA
MeES0372	GGACGAACCGACAATGAAGT	TGGGCTATCCTCCAAACAAC
MeES0373	TAAAGAGGAAGGGCAGTGGA	AACGGAGTCGCAGAGAATGT
MeES0374	AAGGTGGCTGTTCGATGAAA	CGAGGAAAATGAGAGGTCCA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0375	GGAGCGAGCGAAACTAATTG	GCTTGGCAGGTTTTGTAGGA
MeES0376	GCGTTTCATGGAAGCTGTCT	AATAGCTCTGATGCGCTCGT
MeES0377	TGCCAAACAAGTAAATAACAAAGT	CCAACAACATTCCAGAGCCT
MeES0378	GCTCTGCAACTGCTGTGTTC	TCCTTCCTCTCTCCTCCCTC
MeES0379	GCGCTCACTCTTCAGTTCAC	CTTATCCGCCATTCCTTCAA
MeES0380	CAGGATTCTGCGTCGTTTCT	TCACCTTCTCCTCGTCGTCT
MeES0381	TTCGCTAACCCTCTCTTCCA	TCCCCCAAAAATCCCTTAAC
MeES0382	TACATGCGATGCGTCTATGG	CCCACCTCACAGGCAATTAT
MeES0383	TGCGACTTTATAGGAACTTCCAG	GGATTGGTTTCTCCCAGGAT
MeES0384	CTCTGCTGTTGCATCCTTCA	GTATTTCCCGAGTCCGATCA
MeES0385	TCTCATGCTCACAATGCACA	CTGTCGATGAAAGGGGAAAA
MeES0386	AACTACAAATTCCCCATGAAAAA	GAGACTGCTGGGGATGTGAT
MeES0387	CCATGGGAAATGGCAAATAC	GATCAGCTGACAAACCAGCA
MeES0388	GCTGCTTCTTTTTGCTCCAC	CAGCGGGAATAGGCATACAT
MeES0389	CGCTCATATTGGGTATGGCT	GCAAGAACTCCACCGTTTTC
MeES0390	TCACATTCCTTTCACACCCA	CCGTGTAAAGGAGGTTTCCA
MeES0391	CCTTGGGAAACTGAACCTCA	GCAGCAAGACAATGCAAGAA
MeES0392	TTGCCATATACAAATCTAACGCA	AGGTGAGGCACACGAACTCT
MeES0393	TCTAGAAGAAACCCACCCGA	AGCACTCCACAAAACGATCA
MeES0394	CACACCAATTTCCACAGTCG	GAATCCATTGCCAGATTCGT
MeES0395	TCCTCACAAGTTTGCTGTGC	AACAACATGCATACAACGCC
MeES0396	GCCATAAGAAATGCCGTTGT	ATCGTTTTCCCCTTCCAGAT
MeES0397	TCTGGTCCGAGGTCCACTAC	AAGAAACAATTTACCCCCGC
MeES0398	CATGGAAGGATGGTCCAGTT	CAACATTCTCAGCTCCGTGA
MeES0399	TGGCCTCCAAGAGACAAACT	CAATTAATGCGACTTGAACTTCC
MeES0400	ATGAGGCGAATGGCATAAAG	ACGCGCAGAATTAACGAAAC
MeES0401	AGTGTCGCTTTCCATTTCAGA	GGATCTTGGTTGCCGTAGAA
MeES0402	GCAGCCTGATGAAATGGAAC	ATGCATCTGCATGCCTATTT
MeES0403	CCCCAGGTCCACTTCTCTTT	CCTCAGCTGCAGTTACTCCC
MeES0404	TGCACATGCACCATGTATCTT	TCGATGAATAACTTTCGGTGA
MeES0405	GTGTCTCTGACTCTGCGAGAA	TAAACCAGCTCTCTTGGGGA
MeES0406	TTTTCCCCGAGGATGTAGTG	TGTTTCTCCTTCCCTTCTTCA
MeES0407	GGGTCCTAACTGCACATGCT	AAGGCCAGAGCCACAGAATA
MeES0408	ACCACAGTCTCCTGTGGGAC	TCACTTTGCTGCTGTTGAGC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0409	AGCTTGCTGAAGCCATTGAT	AGAAGACAAGCAACATGGGG
MeES0410	GGAGCCCTTTTTCCAATTTT	AATGAAAGAAAAGTTGTTTAATACCC
MeES0411	CACGCACACACTCCTAGTGC	ATTGCTTGGTGTCAGGAAGG
MeES0412	CTCTTCTTCAGGCGGAAATG	CCATGATCAGATCCAAACCC
MeES0413	CTTTTGCGATTGATCGCTTC	AAACCCACTTGCCATCTCAC
MeES0414	TATCATCGCCACCTCCTTTT	GTTGGCTCACCAAGCTTCTC
MeES0415	GCCAAATCCTTACGAGCATC	GCCATGATGGGGTGATTAAG
MeES0416	CAATCAGAGGAGGTTTGGGA	GTAGAGGAGGGTGGCTGTGA
MeES0417	TTCTCATCTCCCTCTTTCGC	CTTCACCGGAGACGAAACTC
MeES0418	AAAGAGGGTGACGAATGGTG	CAAAAAGGGCACTTGAGCAT
MeES0419	AACTTAGCTTTGGGGGAGGA	CATTTACCCTCAGCAGCCAT
MeES0420	CTCACAAGGTCCCTAACCCA	GCAGCTACAATGGCCTTAGC
MeES0421	TGGATGTAAATGTGAGGGCA	AAGTCACCCCTCATGTCCTG
MeES0422	CTTCACAGCTCCTCAAAGGG	TGTTTCTCCATGTTTTCCCC
MeES0423	TGTTTGTGGAAACCATGGAA	AGCCAACAAAACCAAACAGC
MeES0424	AGATAAACTGGGCTGGGGAT	GGCTCTTCTGGGCTTAGGTC
MeES0425	CAAAACCAGACTACAAGATGCAA	GGGAAGCTAGTGAACAAGGG
MeES0426	GGGGAAACGAGGAGAGTTT	CAGAAGCTGGTGAAACCCAT
MeES0427	GGGGAAAAAGGAATAACCCA	TGCTGCTGCATATGAGGTTC
MeES0428	TAACCTTTCGCCGTCTTCTG	GAAGGAAGGGGGAATTTGAG
MeES0429	ATTCATCGCCATACTCCAGC	TGTATGGGAATTTTAGCGCC
MeES0430	AACAAACTCATCTTGGTCTCACAA	AAGGCCATTCCACACTTCAG
MeES0431	CATCGTTTTTGTCGGGTTCT	ACCAATGATCCCTGCTTCTG
MeES0432	GGTGCCCTCTCCTCTTCTCT	CCAAAGCAGCTTAAACGACC
MeES0433	ACCTAACACGCCACAACCAC	TCGGTTGAGCTCCAAATACC
MeES0434	CCTCGTCCGAGAAGACTCAC	ACCAGCGGTCTCATTTTCAG
MeES0435	CACCATTTCCTTCCCTCAAA	TGCCCCTCTACCAAAATCAC
MeES0436	TGGAGCTGTCAAATGTAATTGTTT	ACCATATTTGCCTGCAGAGG
MeES0437	AGGTTACGGTCAGGGTTGTG	CTCCTGGAGCTTCTCGATTG
MeES0438	AGGGCTGAGAGGTTTCAACA	TCATCAAGGGTTTCTGGAGG
MeES0439	AGGCATCTTTGATCCTCCCT	AAAAATACAGCTTCTGATGCAAA
MeES0440	AAGACTAGCCCAAGCAACGA	ATTTTTGGAATGCCTGATGG
MeES0441	AGCCAGCAAAGTCGTTTCTC	CGGCGAGAGATCCGTAATAG
MeES0442	CAGCGACTAGGAGGAGGCTA	TTTCGACGGGTCAATTTTTC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0443	AACCTCAATCAAACGCCAAC	GCTCAGCTGATGATCTGCAA
MeES0444	TGTGCAGATTATGGGGTTCA	CAAAGCTCAACCCTGTGTAGC
MeES0445	CCATCCTTTGGGAAACACAT	TCCCTTCACGAAGAAAAGTGA
MeES0446	CAACGTTGATTTCGCCATAA	TTATTTTCCAATGTTCCGGC
MeES0447	GCGCTTCTTCTAAAACACCG	GACTTGAAGGGAAGAAGGGG
MeES0448	TGAAAGAAAAACCGACCCAA	CGTGCCCTATTTGCAGGTAT
MeES0449	GCTTTCTCCATCCAAATTTCA	GATCAAAGCTGGTGATGGGT
MeES0450	TGGCCCGCTACTAAAGCTAA	CTCCTGGATGACGAAGAAGC
MeES0451	TGAATCAGACGGGAAACACA	AGGAACCCTTGGAGAAGGAA
MeES0452	GCTTCTCACAGCAATCCACA	GAGTCGAACCCAATGAAGGA
MeES0453	GGTTGACATGTTTGGCCTCT	TCTGGATATCCCTTCAACCG
MeES0454	TCTCAAATAAAATCACTCGCTTT	GAAAGCTCGATTGAGTTCCG
MeES0455	GGGAGTCTGTATTGAGTCAGGAA	ACCCAAGCAGCCTTGAGATA
MeES0456	CCAAACTCTCATATCGCCGT	TATTGGGGTTTCCAAACGAA
MeES0457	CGTTCTGTCGGGTTTTTCAT	GCAGCCTTAACCAAAAGTGG
MeES0458	TGCTAGCGGCAACTTTAGGT	TGAACGAAAACAACAAAGAGGTT
MeES0459	TCTGCCCTGTCTGACCTTCT	TGCTGGGCAGATCTTTTTCT
MeES0460	CAAGCAAAGACCCCACAAAT	GTGGTCCTCAGAATCACCGT
MeES0461	TCATCTGGCCATTTCCTTTC	GGGAAAGATCTTCTCAGGGG
MeES0462	TACAACTTCTGGGACTCGCC	TCTTCCTGCTCCAAGCGTAT
MeES0463	ATCCATGGCTGCACTCTTTT	CAATGGCAAGAGCAGTTTGA
MeES0464	ATCTCTCGCCTTCCCTTTGT	GATGCTGAGGGTGGAACAAT
MeES0465	AGCCTCTGTGCTTGTGGTTT	TTTCCTGTTCCGAGCAAAGT
MeES0466	CAACCCGACAAAAGTTCTTCA	ATAGATGCTTCGCATGAGGG
MeES0467	TACGGAATCCCAGAAGCAAC	TGACTAGTGTCCGGAAGGCT
MeES0468	AAAGGGCAGCAACAAGAAGA	ATTGTGGGATTTGTTCAGGC
MeES0469	GGAAGATTGAAACTCTGATGGAA	AGCTCCAGTCACGTTTGCTT
MeES0470	GCAAGGGCAACCAAAGTAAA	CAAAGCAGCTCCTTATGTGTTG
MeES0471	TTAGGTGCATAGGGCATGGT	GCAGTTCGTACTGTCAACGC
MeES0472	TCACAAACAGACACCTCCCC	GCAAAGAAAATGGCCAAAAA
MeES0473	ATTTGCACTCCCATGGCTAC	TCCTCAAACCACCGAATCTC
MeES0474	GGCCAGAACCCTAGACGAAC	CTTCAGGCTTCACGTCCTTC
MeES0475	TGCAGGGCTCTCTCTCTCTC	CTTGTACGAGCATCCCCATT
MeES0476	TGGCCTCACAATTTAGGTTACA	TGTGTCAGCCACCATGTTTT

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0477	TAGCAGAGGCATTAGGCGTT	AGAGTAATCTGCGGGAAGCA
MeES0478	TTCTCAAAGAAGCAGCAGCA	AAGCAGCGTCATGTTCCTCT
MeES0479	GGTGGGTTGAAGAATAATAGAGAA	TTCCCATTGTTGAAAGCCTC
MeES0480	TCCGAGGAGGATTTGTCATT	CAACTGACAACCGGTGAAGA
MeES0481	ATCTTTCTACCCCCACCACC	GCTTCGCGATTCTCAAATTC
MeES0482	GCGTTTACTTCATCCTCCCA	TGACCATTTCTGCAAACCAA
MeES0483	TTTGCTCACTGGTTCTGCAC	AACCCCCAGCTTACACACAG
MeES0484	TCCAACGTTTCCCTCTATCG	AGTTCAATTTTGGGTGTCGG
MeES0485	GCAGAAGAAGCATTCTCCGTA	GGTGCGATTAAAGCCGTAAA
MeES0486	GCAACTCACTCTCTTCCCCA	ACGACGTCCATAATAACGCC
MeES0487	TGCAGAGCATACTGAAACCG	CCTTCTTCTTCACTCGGTCG
MeES0488	AAACAGGGCCAAAGGAGAAT	CTATAAGTGGCCCTGCAAGC
MeES0489	TGCTCAGTTGCTTCCAGATG	TGGTACGTGGAGAGCTGCTA
MeES0490	ACCGTTGACAAAATCCCAAA	ATCCCAATTCCCCTAGCAAC
MeES0491	GAGGCGATTTTAGGCCTTTT	CTCTGTTCCAGGAATTGGGA
MeES0492	AAAACCCGAATTGATAAAACCA	GGAGGAAAGAGGGAGGTGTT
MeES0493	ACCCCTCAAATCTCTGCCTT	ACATCATCAGGGAGACCAGG
MeES0494	TGTCTGGGATGTACGGTCAA	TCTCCTCCTTGATACCCACG
MeES0495	CAGGATTTCATTAGTTTGCACG	AACGAAGGAATTCCCGAAAG
MeES0496	CAGCTTCCAGCTCTTCACCT	GGGTTCTTTCAAGACCACGA
MeES0497	CCTTCAGCTTCCTGTCAAGG	CCCACATGTAAAGGGTGGTC
MeES0498	CAACAAATAAATGAAGAGCCTCC	CAGGCTACCTCCATTGAAGC
MeES0499	ATTGTTCACACGATGGGGAT	TTTTCATCCTCCAAGCCATC
MeES0500	TCAGGCTCAATCACAAGCAC	TGCATGCTCTGTTCTGCTTT
MeES0501	CATTTGCTTTTGGCATGTTG	CACTCCTGTCACTGAAGCCA
MeES0502	AAGAAGGGAACAGCAAGCAA	GAGAGTAGGAAGCAAGTGGCA
MeES0503	CCTTGGTGCACAGATGCTTA	TCATCCAAGCTCCACAAACA
MeES0504	CCCAACTGAGCTCTTGAATG	TACCCTGAGGATGTCAAGCC
MeES0505	GAGAGAGCTCTTGGAGCGAA	CGATTTCAGGCCAAAACAAA
MeES0506	TGCCGTTTTATCCTCTTGCT	CTGAGAGACCCGAAAGTTGG
MeES0507	AGAGTAACCCTTTTGCCGGT	TCATCATCCCCTTTGCTAGG
MeES0508	ACTCGTTGCCGGAGAGTAGA	CTCCTCACAAATCTCGCCAT
MeES0509	GAAGAGGTGAGGAGACTGCG	CATTGGCAAATGCATGCTAC
MeES0510	ACCTCATTGATGATTTCGGG	CAAAACTTATGGGCATGCAA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0511	TTCGTGGACTCGGAAGAGTT	CGGGGTCAAATGACAGAAGT
MeES0512	AAGCACAGAGCATAAAGCATCA	TCCATGCGACATTCCAGATA
MeES0513	TCTCTTTTCACCGCTCTGGT	TTCAACACCAGGCAGCATAC
MeES0514	TTGTTAGGTTGTGGTGGTGG	TGAGAGTAGCTCATCAACCCA
MeES0515	TTGGGTAAATCAACTGGGCT	GAACAATGCCCTTTTCTCCA
MeES0516	CCTCATCCTCTCTGCCTTTTT	TGAACTTGTTCTGGCTGTGC
MeES0517	CATAAACGCGGTCCAAAAAT	ATCGACGCTGATAATCCCTG
MeES0518	TCTTCTTCTTCTTCTTCCTGTTTCA	TGGTGACAGTGCACCTTGTT
MeES0519	CTTTGATTGCTGGTCGGATT	TCATCTCAACGAAACATTCTCC
MeES0520	CCTTCTGTTGGGAAATCGAA	CAAAGTAACGTTGCTGCCAA
MeES0521	CCTCCTTGGTGAAATTGTGTG	CCAACACAGCAAATCTGAAA
MeES0522	ACCAACTATTGGATGTGGGC	ACAAACCTAGCCACTCGCAT
MeES0523	AAAGCAATGGCTGAAGCTGT	TGGCCATTTAAGTCACCCTC
MeES0524	ATGCTGACCAACATGTTCCA	GAATTTATTTCGGCGGTTGA
MeES0525	TTGAAGCTGGAACCCTGAAG	CTGATGCCAAGATCGAAACA
MeES0526	GTGGCTAACATCCCTGCAAT	GCAGCAGTGGCATTTGTTTA
MeES0527	GGTGGGATGATCAAATGGAG	AGCCCGTGTCATAGCAGAAC
MeES0528	GGAATCCGCTAAGCGATAAA	ATCCTCTACGCTGTCGCAAT
MeES0529	TGATTTTATTCTTGAGGGCCA	TCTCCCACTCCAAAACAACC
MeES0530	TAGAGGCATGGAAGTTGGCT	CGATGACTGCTTGTTGGATG
MeES0531	CATGAACTGAGCCTTCGACA	CTTTAGCCTCTGCCTCCTCA
MeES0532	GGTGACGACGCTCTTAGCTC	ACCGATCCCATGACAATGAT
MeES0533	CTGAATGATGATCCGTGGAA	TCAGCAATCCCAAAACAACA
MeES0534	TCGATTCTGTTTGGCTCTCG	GCTGGTATACCGCTACTCGC
MeES0535	TCTTTTTGCACACTCCGTCA	TGAGAGAACATCGCCAAGTG
MeES0536	ATCTCTCATCACTCGCCACG	GAACCCGGATGCAGATAAGA
MeES0537	CCTCGCTACTTTACCAACCG	GCAACCGTAGAGGACTCCAG
MeES0538	GGATTCCGGAGTTAAAATGATTC	GCTTGAGTTTGGCTTCTTGC
MeES0539	CCCCACAGCAGTTCTCTTCT	CATGGTGAGAGTGGTGGTTG
MeES0540	AGGCCCTGCTTCTTCTGTTT	TGCTCATCACTGCCATCTTC
MeES0541	GTAATACCGGCAAGTCACCC	ATGAAGGCGAAAGTAGCGAA
MeES0542	GTCCACCAGTGTTTCCGACT	TTCATTTCTCGTTGCTGCAC
MeES0543	AGGGGAAAGGGAAAGGAAAA	TGAGATGCCAAATGATGCTT
MeES0544	TACACGTGCGACAAAAGAGC	TTTTGAAGGGCTGCAAGAAG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0545	GCTAGTCTTCTTTTAGTGCAGTTGG	CGGAAAGAGAGCGAGAATTG
MeES0546	TCGCGAAGTTGATGAAAGTG	GCCGGATAATTCAAAACCAA
MeES0547	AGCCATTTGCTTTCTGCTTT	CAGAACAAACCCACAAACCC
MeES0548	CGGCTCTTGACTTCGTTAGG	ACGGCTAAACAGGCTCAGAA
MeES0549	CGTACGTTAGGTCCCGCTTA	TCCTTCATCCATGGGTCTGT
MeES0550	GGGGAGACGTCAAAGCCT	AAATCCAACGCCTCTCCTCT
MeES0551	GGGGTTTTTGTCGCATAAGA	GTCAAACCCGCTATTTTCCA
MeES0552	GAAATCGGATGGACTCCAAA	ATGCATAGCAAAACCAAGGG
MeES0553	GCTTTCAACTTCTTCTCCCC	TGAGGAGGTATTCCTGGCAC
MeES0554	TCGAGTCCGTCTCTACACTTTTC	ATGCAGGAAAATGGAAACCA
MeES0555	TCGCGTGTAAATGACTCGAC	AGCTACAAGCGGGAGTGAAA
MeES0556	CTTCTCAATTCCAAGCTGGC	AGAGTGAAATCCCCCTGTCC
MeES0557	GAGATCACTCGCTCGCTGTC	TCTCCAGAAAGAGAGCCCAA
MeES0558	ATCAGCATACCCAGGGACAG	ACCCCTAGAGTGAAGGTCCC
MeES0559	AACCTGGTGCTTCATTTGGT	CATTCAAACTTCGAGTGCCC
MeES0560	TGCAAGCCATTTGAAAAACA	GGTCACAACCGGCAATTAG
MeES0561	CGGCCAGTTCTGCATTTATT	ACGCTCAAGTCAAATCTGGG
MeES0562	CACTCTCTTGCAAGCCCTTC	GCACCCATGATTCTCCTGTT
MeES0563	AAACTTCGCCATGATGGAAC	AACAAACTGTTGAACTGAAACACA
MeES0564	ATGCGCCTAGTGCTGTTTCT	GCATGGTGACAGCTGAGAAG
MeES0565	TTTCGCAGGTCTCTCTCTCC	GAAAATAATGGCCAAAGCGA
MeES0566	ATTTCCATTGCTGCCAAAAC	ATCGAGTTCAGCTTCCCAGA
MeES0567	GCTCACACTTGGTGTTGGTTT	TCCAAATGGGGACTGTTCAT
MeES0568	AAAGCCTGGTCCTCACAAGA	CAATCAGCTTCCCTGCATTT
MeES0569	TGTTAGGAAGAGCAATCGGG	TTTGCCTCTGATTTTGGGTC
MeES0570	AGAAGCTAGAAGCAGCACGC	CTTGGAGAAAGTCACCTGCC
MeES0571	GCCTGCAACAAGAAAGTTCC	CCCAAAGATCACTTTGATAGCC
MeES0572	ACTTATTGCCGGAGCCTCTT	CGGACACTGGTAGAGGCAGT
MeES0573	ACACCTCTCCTCTGGAAGCA	TGTCGAACATCAAGAGCGTC
MeES0574	GACCTGTTTCTCCAAAGCTAGA	AGTCCTTGCTTTGCTCCTCA
MeES0575	AGCAGAAACCAAATCCAACG	TAGGTGAGCCGCTCTCTGAT
MeES0576	CCCATTCTCTTCTCCACCAA	ATCAAGAACCCAAGGCAAGA
MeES0577	AATAATGGCTCAACAAGCCG	ATGAAAGTCCAGCAATTGGG
MeES0578	CCTAATCGTCGCCTATCGAA	AGCTTCTGGTTGATCTCGGA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0579	TCCAAGCACAAAAACCATAAAA	TTCCATCCAATCCAAACACA
MeES0580	ACCCTCTAACACATCTGCGG	TTCTCAAAATTGGGGGAAGTT
MeES0581	CCTCTTCAACCTCTGCGTTC	TTGGGATAAGAAATGCCTGC
MeES0582	ACCACCTTCACGTCGATCTC	TACTGTGAATGACCGCCTCC
MeES0583	ACATCCAGAACGCCAATCTC	CATGGGCGATATCAGCATTA
MeES0584	AAATGGTGTTCGGAGATTGG	AACATTGGCTGCAGAAACCT
MeES0585	TCCTTGGCCTCCCTAACTTT	CAACCTCGTCTCGGAATCTC
MeES0586	AACGGTGATTCCTCGTCAAC	CAAAGAAGGGACCAACCAAA
MeES0587	AGGCGTGAATTGAAGAGGAA	TGCCTGCTCTCTGTATCCGT
MeES0588	CTCGGTTGCTGTGGATTTTT	TCCTCCGTTTCACTCAATCC
MeES0589	GCAGGCTTGTACAGGGAAAA	ACTTTGAGGATGGGTGTTGC
MeES0590	CGTAGCATCCTTTAAGCCCA	TTGGTCATGGATCTGAGTGG
MeES0591	GGGTTTGGAGGAAAAACCAT	AGGCATCGTTCATTGGGTAG
MeES0592	GCTTGGTCTCGATTGTGGAT	GGGATTGGCGTTCTACAGTG
MeES0593	TTCCAGACGATTAGGCCTTG	AAACCCTTGGTTTCCCATTC
MeES0594	GCCGATGTGGAGAAGATGAT	GCGGTGAGACAATCTGTGAG
MeES0595	ACCCACAAATCTCTTTCCCC	ATCACAGACGCTCATGTTGC
MeES0596	TGGTTTCCCCTTTTCTTGTG	AACAAAAAGAAACCCTCCCC
MeES0597	CTCTACTCTGCCCACCAACC	GTGGCTGACTGATTCGGATT
MeES0598	CGCTTCTCAAATCTCCGTTC	CCAAACTCGCCATTCTGTTT
MeES0599	TCAACGCTACCACTAGCACG	GGAAGCAGATCTCTCACAGGA
MeES0600	TCAAAGTTAAACGCTGTGAGTGA	GGGAAGCCCTTTTAATCTGC
MeES0601	CTCAGTCCTTGCCTGTCTCC	TTTCCCAATCTGGCAATCTC
MeES0602	TTGCTGCTATTGTTTTCCCC	CCCATCAACCGAATCAAACT
MeES0603	TGCTTCTGGTGAAACTGGTG	CCCTTCTCTTTGCTCCTCCT
MeES0604	CACTCCATCACCACCAACTG	TCCTTAGCGCTACTGCCAAT
MeES0605	GATACGTGCATCGAAGCTGA	TGTCCATCAAAGCCACAGAG
MeES0606	CAACAAATGACAAATCTGCTGAA	TTCCACCAGAGACTCCAAGG
MeES0607	CTTCTCTGCCTTTCCCTTCA	TACCGGTAGCAACCCAAGAC
MeES0608	GACCGAACCTGAAAACCTCA	TAGCTGAGCCGAGCTAGGAG
MeES0609	GCTCTTCCACCCAAACAAAA	CATCTTGAGAAGCGAGGGAG
MeES0610	GGTTTCACAGAGACCGCAAT	ATGGGGATGTGAGAGCAAAG
MeES0611	TTTCCAATCACTTCTTCGCA	GATTTCTGGGTTTGAAGGCA
MeES0612	AGGCCAGGACAGTCAAAGAA	CAAATATCTTTCGGGTGGGA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0613	CTTCTTGTGCCCAACTCCAT	GGCTAAGGTCCTCGACTGTG
MeES0614	GGCACTTTCTCCTGCAACAT	CCAACAAGAACAAGCCCATT
MeES0615	CTCTGATGGGCTTGAGGGTA	CCCAGCAAGTTCTGGATTGT
MeES0616	GGGTGAAACCATCAGTTGCT	TTGGTACAGCCACCTAACACA
MeES0617	GCGAAATTATTGCCCTTCAG	CCAAATTGACCTGCAAGGAT
MeES0618	AAACCCACAAGCAGAGGAAA	AAACCAATCCATGGGTGAAA
MeES0619	AAAGGATTCGTTTCCCTTGC	ATCGACAGCGTACTCATCCC
MeES0620	TGCAGTCGTTCGTGCTATTC	TTCGTTCAAGCTCCAGTGTG
MeES0621	TCAAAAGCAACCCCATTACC	TCCAATGCTGAGACGAACTG
MeES0622	TTCTGAAGGCCACAATTTTCTT	CAAGAAGATCGCCGGTAGAG
MeES0623	TTGTTTTTCTTGCTGTTGCG	CTGGGCCTTCAATTTGGATA
MeES0624	TAGCTACGGGGGATTACACG	TGGACTCAACACATTGGCAT
MeES0625	GGGATGCTCGTCTTGTTCAT	AAGCAGAAGTAGCCGTTCCA
MeES0626	ATGGGTTTTGCGTACTCAGG	CATTGCTGGGAACTTGTCCT
MeES0627	ATGAAACCAGCCACCATAGC	TGGTCATGGATCCTGCAATA
MeES0628	GAGGAGGAGGAGGAGGAAGA	CATATCAGCTGGACAAGGCA
MeES0629	TCTTCCCATTCGAAAGAAGG	CTAGAATCCGCCATGGAAAA
MeES0630	AGACTGCGGCTCTAAGCAAG	TCAGAGTCATCAGAATCCTCCTC
MeES0631	GCCTCTTTTACCTGGGGTTC	TACAGCCAGAACAAGGGGTC
MeES0632	GGTGTTGGAGGAACTTTGGA	AGCCATCACAGTCCATCCTC
MeES0633	CTGGTTGCAAAGCTTGTTGA	AATCCTGCTTTTTCTGCCAA
MeES0634	CTCGAAGGCTATTGGAGCAC	GTCTCCCTCTCGCTTCCTTT
MeES0635	GCAAGAGGAAACACTGGAGC	AATTGTCGATCAGACGGGTT
MeES0636	GATCACGTGCAAGAGAACCA	CGTTATCTCCTCCCCACTCA
MeES0637	TTTCCATCAAACGGGTTCTC	GGAACTTCATGAGCCTCTGC
MeES0638	CTGATGCTTGTGGCTGAGAA	CAGGTGTTTGAGCAGCAAAA
MeES0639	CTGGCAGTTGGCTTTGAAAT	GTTGAGATTTCCCCATCTGC
MeES0640	CGTCTCTTCCCAGCTGTTTT	TTCCCTTGAATTTGTGGCTC
MeES0641	TGCTATGCTTGGTTTTGCTG	TCCATGATTTTCTCCTTTTTGG
MeES0642	AGAGATTCGGATTCCACCCT	TGGAAAATCATGGACTGAGAA
MeES0643	TCCCCAGAACACCAACATTT	GGAAGCTGGTGGTTTTTGAA
MeES0644	GCGATTATTGAAAGGGCTCA	ATGCTTTTGCAGCGTTTGTA
MeES0645	GATGTCCTTTGCCCCATAAC	TGATGGGTTTTGATGCTTGA
MeES0646	TTCAAAGCTTCTCGCTGGAT	AAACCCACTTGCCATCTCAC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0647	GGCCTTACAAGCAAAGCGT	AGGAGGCATGGAGTATGGTG
MeES0648	TTTCCTTCCTCGTTCTCGG	GAGCTGAGGAAGCCAAAATG
MeES0649	TGTGTCCATAAACCCCCTGT	AGCCACTACCGAGAAACAGC
MeES0650	GCAGTGACCATTGTCAGGAA	ATGGGCTTATTACAGCAGCG
MeES0651	TGGACCCGTATGAGAACTGA	GGGCAAATCGTAGAGGACAA
MeES0652	CCCACATAATTTTGTTTATAGTGCC	ACAGGGGAAGGAAAATGAGG
MeES0653	CCGGAGAGTAACGGAAACTG	CGCAGAGAGAGGGAGAGAGA
MeES0654	AATCGTAAGCAAACGGAAGC	CCATTTGCCTTTGCTTCTTC
MeES0655	GGGATTAGATTACAATTCCAACCA	TGGAGTTGTACTGGGCCTTC
MeES0656	ATCCAACCCCATGAACACAT	GCCCCCATGATCCTAACTTT
MeES0657	GCACAAGCCCACTAGAGACC	GAGCTGCAGATCCCAGAAAA
MeES0658	ATGCAAATAAAACATCCGCC	ATGGTTGGAGCTGTTTCAGG
MeES0659	CCTTCGTGCTGCTTCTTCTT	CTACGAAGCAAAGAATCGCC
MeES0660	GAGACACACCCCCTTCTTCA	TCCTCCACCTTCTGACAAGC
MeES0661	AAGCCTCTGCTACTACTCTCTCG	AATCGGGCAAAAGAACAGTG
MeES0662	TTCCCTAAAATTCTCCCCAAA	GGGGTTTTCGACTTTTCACA
MeES0663	CCAAAGTACGCCACCAACTT	TAAGCAACTCATGGGCAGTG
MeES0664	TGGTGAAACACCAAGCACAT	CCTCTGGTACTTGCACGGAT
MeES0665	AGCATGAGAAAGCAGAAGCC	ACAAGATTCTCCATGGCTGG
MeES0666	CTTGCTGGAACTTTCTTGGC	ACATCCCTCATGCCCAAATA
MeES0667	AATTCAAGTTCAGGCGTGGT	GCCTATGGCCTACCCAAAAT
MeES0668	CCAATCCAAGCTTGCAGAAT	TGAAGTGATCGACATCTAATTGC
MeES0669	GCTTGGGCACTCTCATCTTC	GACAAGCTTGAATGTCGCAA
MeES0670	TTCCTGCCGATGATATTCCT	TTTTGGAAACCGAAGAACATT
MeES0671	TCAGCTCCACATTCTGGTTC	TGTCAATGATTCAAAAAGCCA
MeES0672	GGTATGAGACCGCGATGATT	AATGTGTATGGCTTAGCGGC
MeES0673	TTGGCAAATTGAGATGGTGA	ACTTGCTGCCGCATATGTTA
MeES0674	ATGCAGATCAAATCAAGCCC	CAAGTGCCACATTGCTTCAT
MeES0675	TGTTGCAACTCCTGCTCTTG	TAAGAACCACAGCCTGCCTT
MeES0676	TCCCTTTGCTTGAACTAGGC	AAGGCTGCATGTTTTATGGG
MeES0677	GTTCAACATCTGGGGCAGTT	ATGGGAACTTGGGTTGATGA
MeES0678	CAAATCTTCCTCCCTCCTCC	GCATTCGAGACGCAGTACAA
MeES0679	CACACAAGCCAATGTTCCTG	TCTGCTCTTCTTGGGAAGGA
MeES0680	ACGACGAATCTTTGGAGGC	TTACCGGGAAGCTTATGCAG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0681	CAAAAAGGAAGCCATTGCATA	TTAAGCAAGACCAAGCCGTT
MeES0682	GGAGCAGGTGGGGGTATTAT	CCAGCCTTCGGTTTTAATGA
MeES0683	CAACCCCTTCCATCAAAAGA	CCTGCAAGTGAGCAAATGAA
MeES0684	TGTGTTTCTCAAGCAGCAGG	TCAAGATGCACAAATCGCTC
MeES0685	TAATTTGGGCAACTGGGTGT	GGGGAGAAAAGCCCTAACTG
MeES0686	CTTCCTGAACTATCCGAGCG	GAACGAGGGAGTCATCGGTA
MeES0687	ATGTCCTCGTAAGCACACCC	TGCTTCCTGTACCTCTGCAA
MeES0688	CCACCCCCTCATCTCAAGTA	TCTTCCAACCAAACCAGAGG
MeES0689	CAAATGAAACACGCAAATGG	AACTTAGCTTTGGGGGAGGA
MeES0690	CCAAAACCATGTTTTCCTCG	TGTGCTGCCATGGATAAAAG
MeES0691	TGCCATACAAAGAAGAAAAGCA	AGATGGTGATGGTAACCCCA
MeES0692	AGAAGGAACAGCCTCAGGAA	TGCCCATGAAGGAAAGAAGT
MeES0693	AGTGCGATACAAAGGGCAAC	TTCCCACAGGGTTTTACAGC
MeES0694	AGGGTTCCTTCCTTGCTTTC	AAATGCTGAACGCCACTTCT
MeES0695	GGACAATTGGCAATGAGGAA	AGAATTCCTGCTCCAAGCAA
MeES0696	ACAGAGCGATTTGCAGTTCA	GGACGCCATTTTTGTTCAGT
MeES0697	TTCATTCGCTCGTTCATTCA	GAGTGTGCTGCGTAGTGGAA
MeES0698	CCTTTTCACTCCCTCAGCAG	ATAGGCAGAAGCGAGAACCA
MeES0699	TTCCTGGGTTCCTTGAAGTG	GCAGGCACAATCTTCAGTCA
MeES0700	TTCCAACAACAAAAGCAGCA	TCGTGTCTTTGATCTCAGCG
MeES0701	CTTGCTATCGACACGGCATA	CGAATTCCACGGTCTGATTT
MeES0702	GAGAAGTGGTTGGGGATTCA	TCAAGAGCTGGACTTGAGGAA
MeES0703	CTCTTTACGCCGCTCAAATC	TGCTCGCTCCACCTTAGACT
MeES0704	ATCTCAGGTTTCGACGCATT	TGCAACACAAGAAGGGAGACT
MeES0705	AACCTGAACCCACGATTCTG	TGAGGTACCTGCAAGAGGCT
MeES0706	GGCTGTTCCAAATGCAAGAT	TCGAAAAGCGGAAATATTGG
MeES0707	AGCTCAGATCACTCCGTCGT	GTGCTAAAACAAAGGCACCG
MeES0708	TTTAAGGAAAAAGCGAGCCA	CGGAATGGTCAATACCCTTG
MeES0709	CAGTGGTGGAGCTAGTGGGT	TTCTCCGGTGGCAAACTATC
MeES0710	AATCACAGAGGATTGCCCAC	CTCCATCTCACCCTCACCAT
MeES0711	CAACTGTGATTGTCAACCCG	GAGTAGGCTGGCATTTCGAG
MeES0712	CGGAAGGGTTACCTCAGCTT	GAGAGTCCCACGAAGACAGC
MeES0713	CCTTCATGTTTGGGAGGATG	AGAAACCACCACTCGTCCAC
MeES0714	CACTGTGCCTTCTTGCGTTA	TGCTTGGCTACAACATAATTCC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0715	GACTTGACGAAGCCAAAAGC	TTGCGACTCATTCGTGACAT
MeES0716	GCAAGCTGTGATCAAATTCAGA	GCTTCAACCCTGAGAAGCAC
MeES0717	ACCAATCACACACGTTTCCA	TGATCACTTGGATTGGCTCA
MeES0718	TTCTTCCAAGCAAAGAAGAAGAA	CCAAAACCCAAAACTCCCTT
MeES0719	CAGGGTGTTCTCCAATGCTT	TCAGTGTGTGGTCTTTTGTCA
MeES0720	TTCCTGGAGACGGTTTCATC	CTCACCTAGAAAGCCCTCCC
MeES0721	TGCTCTTCCTCCTCTCTCCTT	CGGTGGTGGGTACGTAAGAT
MeES0722	GGGCTGCTTTTCTGAAACAA	TGGTGCCTTCCATTTCTATTG
MeES0723	AAATGGGATGTGGCTCAAAG	AATGAAGTTGCCACCACCTC
MeES0724	TTTAATGGCCTCTCTCCCCT	CCGATATCGAAAGAAGACGG
MeES0725	GGTTCCATCGAGGAAGATGA	ACAAAGCGACCGAGAAAAGA
MeES0726	AGAAATGCCTTCAGCAGAGC	CAACAATTCATCCGCATCAG
MeES0727	TAGCACCAGCCCTTCACTCT	TCTTGGACCGAATTCTCCTG
MeES0728	GCTGACGTGAACGAGAATGA	TGATTAGTTGCAGCAGCAGG
MeES0729	TTTCTGACCAGGAAGAGCGT	CCAAAGCCAAACAAACACAA
MeES0730	ATTCTCCCTGTTGCTGCTTT	ACCGCTGCAGGTTGAAGTAT
MeES0731	CTGATAAGCCTGTGGCCG	TTGCCCTTCAGCTTCATCTT
MeES0732	AGAAAAGCAAAAGCAGCCCT	AAACCCTAGGTGCAAACACG
MeES0733	TGTTCTCCTTCCCAGAATGG	GATTCGACGGGAAACAAAGA
MeES0734	TCTGAGTTTGGTGATGCGAG	CGTAATACCACACAAGCCGA
MeES0735	TTGCGAAATCAAGCAACAAG	GAACCCTTGGGAGAAGAAGG
MeES0736	TCCCATCCTGCTAATCATCC	CTTCCAATTCCCAGTTTCCA
MeES0737	CATCATGAATGTCGGGTCTG	TGAAGCTTTGACGCAATCAG
MeES0738	TTCAGTTCCGGAGCAGCTAT	AAAGCCGAACTCCTTCTTCC
MeES0739	CAATTCAGAAGAAGCAGGGG	CACAGGGGTACCAATTGCC
MeES0740	CTCTCGATTTTGCTGCAGTG	AGCCAATAGCAAGCACATCC
MeES0741	TTGATTGTATGTCACCCACCA	CCAGTACCCTCCCTCCTCTC
MeES0742	CTCTCTCTTTCCATCGCCTG	GGATCATTCCCATCCACAAC
MeES0743	TTGCATGCATCTTCTCATCC	GTGCATCCAATGATCGTGAC
MeES0744	ATTCCAAACAGCCAATTTCG	CTGCAACGAAAGAACACTCG
MeES0745	GAAGCATAGGAACCTGCGTC	TCCAGCTGTAGCTGTTGTGG
MeES0746	TTCCGATTCTCCAATTCACC	GCAATGGGTGTCGAGCTAAT
MeES0747	TTTAGGGGGAGGGAAGAAGA	ATATCTCCAACCGCATGACC
MeES0748	TCTCCAAGGAGAAGCGAAAA	GAAGACGCTTTGAAGTTCGG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0749	ACGATGATGGAGGGAGTGAG	TCCCAAACGACTTGGACTTC
MeES0750	CACCAACATGAATGGCAAAG	TGCAATATTTGCAATAGAAAGTTAAAA
MeES0751	CCGGCATCAAAGCAAAGTAA	ACGGTCACGATGTCAAACAA
MeES0752	CATCAATGCTGAGCTGCTTC	ATCACCCGCTTCACATTCTC
MeES0753	GACAGATAGCCCCCTCCTTC	TGAGGTTCAGGTTTGGGTTC
MeES0754	CACGACATCCTCTCCGCC	CGCCATCTAATGCTCCCTTA
MeES0755	GCAACAAGTAGCATTTCTTCTCC	ATCAAAAGGGTGTTGCTTGG
MeES0756	TTGAAGGACCAAGGGTTTTG	GTGCTCATGGACCCAAATCT
MeES0757	CGAATTGGAAAGCCATCTGT	AAAATTGCTGCATCCGTCA
MeES0758	GCGGAAGATAGTGAAGACGG	CAAAAAGCCTCTCCAACTCG
MeES0759	AAATGGCCCCCATAAGAGAT	GAGCTTCCTTCCAGGTGATG
MeES0760	GGAAGCCTGTACCACTACTGC	TCGTCTGCGTCTTCATCATC
MeES0761	ACTTCGGGTCCAAGTTTTCC	GGCAGTTGTTTGAGGTGGTT
MeES0762	TCGTTACCGGAAACCACTTC	AAAGCTTTTGCACGGACAAC
MeES0763	TCCCTCCACTCAACTGAGGA	CAATGGCGCTGCATAGTAGA
MeES0764	GTCTCTTACGCCTTTCGTCG	GGTGATTCTACGGGAAACGA
MeES0765	CAACAGCCAAAAACCAACCT	GGCGGATTGAGCTCTGATAG
MeES0766	AGTGCCAGTGGCTGAGAAAT	CGGGCCTGAGAAGTAGTTTG
MeES0767	ACAACCATGTGAACCCACCT	TTTGACTCACAGATGCTGGC
MeES0768	GCAAAAGGAGGGTTCTAGGG	TGCGTGCTCAAGGACTGTAG
MeES0769	GTTCTCTCTTCCCCCACCTC	TCAGAGGAGCGCGAATAACT
MeES0770	TTCAGAAATGTGTTCAAGACCC	ATATAACGGACAAGGCAGGC
MeES0771	CATTTCGTCCAATTTCGCTT	GGTTTGAGATGGAAGGACGA
MeES0772	GCAACTTCACTTAATCGCCC	TCACAAACCAGCAACTCCAG
MeES0773	TGGAATTTTGAAGCACACCA	CGCAAGTCACATTCTCAGGA
MeES0774	AAGAAACGGAAGCATGATGG	CTCAGCTCATTCCACGCATA
MeES0775	CAATCCAGCTCACAAAACCC	CCACGCTTTGCAGACTGATA
MeES0776	TTCAGATTCATCACCTCCTTCTC	CCTTTTCCGCCATGATTAGA
MeES0777	GATTGGAGCTCCGGATGATA	AGGGAGGATTTTCTAGCCCA
MeES0778	GATTCCCTCGGGTTCTTCTC	CATATCCATTCTCAACCGCC
MeES0779	GGGCTCTCTTTGTAGCATCG	TCATTACCACCGACCCTTTC
MeES0780	TGATGCTGAGGCATATGAGG	GGCTCCTGAATTTGGATTGA
MeES0781	AAAGAAATGAATGTTTTTAGCTCCA	AGCTTCTGCCCAAACAAAGA
MeES0782	TGGTCTCGCTCTATCTCGCT	TAATCAGTCCAGTCGGGAGG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0783	CCCGCTTTCGCTTCTTTT	GGACCCTCAACCAAATCTGA
MeES0784	CCGCCAGTACACATCTACCA	CCACGCTCATACTGTTGGTG
MeES0785	TTTTGCGTTTGATTCTTTTGG	CAACTGTTTGAGAGCTGGGTC
MeES0786	TTCTCTGTCATAATTCTTCCATTTC	GGTTGCAGAGGTGGTGTGAT
MeES0787	TTCCGTGGATTTCCTTCTTG	TCTTGGTCACCACCATCTTG
MeES0788	TTTTGTCACTTTTGCTTGCG	CTCCCTCTACCACCACTCCA
MeES0789	ATGGCCTGTCACTTTTCCAG	TCAGACAACCTGCATAACCG
MeES0790	AGCCAAAAACCATACCCACA	CTGCTATTGCTGTGTGGTCC
MeES0791	CCCAAAACAAACCTCCATTG	TTTTAAAGAAAGCGCCCTCA
MeES0792	CCCCTCTGCGAGAGAAAA	CCTCACGCTCTCACAAAACA
MeES0793	ACAACACAAACCACAAGCCA	CTTTCACCCCTCTTCAACCA
MeES0794	GGGAGAGCCTTTGCTAATCC	GAGAGGCCCTAAGGGAATTG
MeES0795	TACTCGAATCCCTTTTCCCC	CTCTTCAAGCCAAAAGCGTC
MeES0796	TGCAAAACTATTCCAGCTATGG	TACAGAAATTTGATGGGGGC
MeES0797	CAGTGGGTAGCTTAGCCTGC	GAAAGATTTGCACTTTCGGC
MeES0798	TTGAAAAACCATTTGAGGGC	TCCACCCAAAACGAGAGAAC
MeES0799	ATTCTCTGCGCATTTTGCTT	ACCCATAAACAAGGCCATCA
MeES0800	CGATTTTTAGATAACGAAGAAGCAC	ATCACTCGCTCACTTTCGCT
MeES0801	AGGGCCACATCCAAACACT	ACAGCATCGTCTTCTGCCTT
MeES0802	CATTCCCTTCATCATCTCTTCA	GAGTCCTCGAGCCAGACAAC
MeES0803	TATTTTCCACACATCAGCGG	CTTGCATCCACCACTACCCT
MeES0804	TTGCCTGCAACAAGAATGAG	GGAGAGAAACTGGAGGGAGG
MeES0805	CATCTGCCTGCTAGGGTTTC	GGTATTTGCTGCGAAAGAGG
MeES0806	TGCCCTCAAATTTTCTCCAT	AAACATCCAGTGCAAATGTGA
MeES0807	GAATTGACTGGGTCCTGAGC	TATGATGATTTGCTCCCGGT
MeES0808	GGTTTGTTCAGTCCGTTCGT	CAACACCTCTCTCTCTCTCTCTC
MeES0809	CAGGTTTCTCTCTCCTCTCTCC	CAAATGCAAATAGATCCGGG
MeES0810	TGTAGAGTGTGGCGAAGTGG	CATTGCAGCAAAGCAAAAAG
MeES0811	GGAAAACACCCTGTACTGTATCG	CTGGGCACCATTCTTCCTAA
MeES0812	TCACCACCTACCCCTTTGTC	CCAGTATCCCAAACAATGCC
MeES0813	AAAGTGCAACCCAGCAATTC	TGTGAGAGACATAATCAAACCG
MeES0814	TTTGAAAATCAGTGTAAAGGAACA	TGGTTTGTCTTCGCTGACTG
MeES0815	TTTCCGCTTTCCTGGTACTG	GGGATGGTGGAGAGAACTGA
MeES0816	GACTCGTTCTTTCTCAGCGT	ATGTCGCGGAAAATCAAAAG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0817	ATGGTCCACAGACAAGGAGG	GCTTCTTGAAGGCTTGCACT
MeES0818	GGGATTCAATCTCCTTAACGC	CCCAGCCATCATGAGAGAAT
MeES0819	TCAACCACCAGTATCCATGC	TCCATGGAGATTTCAATGACTG
MeES0820	TTCATCCAAAATCCGACTCC	GCCTAACGTTCTCCTTGCTG
MeES0821	TCATTTTTCCTCCTCCACAAA	GCCCTTTTGAACGTCAATGT
MeES0822	AAACCCTAATTCAAAGAAAAGGC	GGAATCACCAGGCAGTGAAT
MeES0823	GAATGAGGCTTTGTTCTCGG	CGTCTGATTTTGGGTTCACA
MeES0824	GGGTTACTTTCCCTCTTTGACA	AGGGTCTCTCTCGCTTCCTC
MeES0825	TCTCCAGATCCAAACATCCTG	CCTTGGCACCATTCTTCCTA
MeES0826	TTTCCAAAATTAAGCGTCGTC	TGCCTTGGTTATCGTCCTTT
MeES0827	CCGTCTCCCACTCTCTTCAA	CACAAGCATTCTCCACGAAA
MeES0828	GTTCTTGTGCTGGTCGCTG	TAAGTTGCATGCTTTGCTGG
MeES0829	CTTTCAAAAGCTCCAGACCG	TCTCCACAGACAAGTGCCAG
MeES0830	ACCAAATCAGAACCAGCCAC	TCGACGTAGAAGTGGTGTGC
MeES0831	GCTTCAGACAATGCAACAGG	GTTCCTACAATGCACTGCCA
MeES0832	CCTTCTCATTTCACAACTCTCTCTC	TTCTTCTACTGGGGAAGCCA
MeES0833	TCTCACCTCAGCTGATCCCT	GGGTGCTTTGATTGCTCATT
MeES0834	AAAACCCATCACCAACCCTT	CCAGACATCTCCCCACAAAT
MeES0835	TCAAATTAGCGCACGATGAC	GATTCTGAATATCGCCGGAA
MeES0836	TCCTTTTCCTGGATGCTCTC	ATCCAATCCAATCCAATCCA
MeES0837	CAAGCTTGCAATTACCATTTATT	CTGTGGCTGCAACTCATCAT
MeES0838	GGGCAACAGTGCTCTCTCTC	CCCTTTTTCACTCCTATTCAGC
MeES0839	CCCATGAACTCTTGAAATCTCC	GATGATGTGGTTCAATCCCC
MeES0840	GCTACGAGATCGAGGGACTG	CCTCTTCTTCTCTTGCCCCT
MeES0841	TCAAGCAATTCAAGCTCTCA	TGATTGTTGGCCAGGTTGTA
MeES0842	TTGACCATAACTCTCTAATCTCTCC	TAATGTTTGCCCATGCTTTG
MeES0843	TTTTGTAGCGTGGAGTGCAG	CAAAATTATTGGAACCTAAAAAGGAA
MeES0844	TTTTGTTTTGGTTTAATTTGCTCTT	GAATTGTTTGGTTGAGGGGA
MeES0845	GATGGACTCTCTCTAAAAACTTGC	CTGCCATTTCTCTCTCCACC
MeES0846	TGACCTCCTAACTTCACCACC	TTGGGAAGATGAAGGAGTGG
MeES0847	GTTTGTGCCAAAACCCTTCT	GATTCCGGTGAGAATGAGGA
MeES0848	AAAGGCGAGGAAAGAGGAAG	GGATCGTGGGGTTTCTGTTA
MeES0849	CAGACAAGGCCATTTTCATTT	AAAGCTTGGAGAGAGAGGGG
MeES0850	GGGTTTGGATGGTTGGTTTT	GAAAGCTGAAACCCCCTCTC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0851	AGAGAGAGAAAGCGCACTGG	GCACAGACGCAGATCCATAA
MeES0852	TGTTTGGTAGAAAAGAAAAAGAAAGA	CCTGAAGAAGATTCCACCCA
MeES0853	TGCCTCAAACACTCTCCCAT	TGGCCAACATCAGCATTAAA
MeES0854	CCTTCTCTCACCGTCTCTGC	AAAAACGCAGCAAGACAAGAA
MeES0855	TCTTTGCTTTCCTTCTCGGA	ATTGACGGATAGACCTTGCG
MeES0856	TGACATCTTCCGCCTTCTCT	CCGTAAAATGGTGAGACGGT
MeES0857	GATCATCTGGGTTTTGGCTG	TTTCAGTATGAAGACAAAAGGGG
MeES0858	TGGGTCCTGCGTTTAAGGTA	ACGTCCTCTTCAGAGCCAGA
MeES0859	GATCCCATCTCCCACTCCTT	CAGTTGATCCGCTGGGTTAT
MeES0860	AGAAACACGCGGAATGCTAC	CCAGTGGAACCCCATGATTA
MeES0861	GGAGAGGAAGAGGGAGAGGA	AAGCCCATGACTTCTTCCTG
MeES0862	GCGAAGGTTGCAGATTTTTC	ATTTTTCATCAAGAATTCACAATTT
MeES0863	ATTACCTCCTCCCAACTGCC	TCGAAGATCTGAGCGTTCCT
MeES0864	TTCAGCAAAGCTGCAGAAGA	TTCAGCAAAGGTTTTGGAGG
MeES0865	TTGATTTGGTGCATTCAGGA	TGCCTATAAAAGCATGTGCG
MeES0866	AAAATTGCAGGATGATTGCC	ACCTCCTTGTTCGATCTTCC
MeES0867	ACGCTCCCTTCAACTGTCAT	TGCTCCCCGGATACTACTTG
MeES0868	GATCGGATGTCTGAGGAGGA	TGCTAGTCCTTTTTGGCAGG
MeES0869	GCGTCTTGTGGCTCTCTGTA	ACAGCTGATATCCGAGCGAT
MeES0870	CTCACCCCTCCTATTGGTCA	TGCAAGTCGAATGTCCAGAG
MeES0871	CAGCCCAAAAGGAGTTCAAA	CACCCAATCTTTCTGTCCGT
MeES0872	TCTCCTCCCACCTCCTTTTT	GGGAAGTGTTTACGTTGCGT
MeES0873	CAGAGCAGAAGGCAATCACA	ATTTACGAGGGGAGGGAATG
MeES0874	TCCTCCCTCGCAGATTATTG	GCTTGGGCCTGATTCTAGTG
MeES0875	TCCATATCTCCTAGGTCCCAA	AGTTCTTGATCCGCTGCACT
MeES0876	GTGGAGTGGACACCACAATG	GAGGAGGAGCTGCAGAATTG
MeES0877	GGATTTTCATTCCACGCCTA	GGCCGAATTTAAGGACATGA
MeES0878	AAGAGTCCAACTTTGGAAAATCA	TTTGAAGGAAAGCAACATGG
MeES0879	CAATCATTTTCATGGTGGATG	GCCTCTGCTTGCGGTATTAG
MeES0880	AGGGAGTCCTAGTGCAAGACC	TGGGATTCTTGGGAGTTTTG
MeES0881	TCCTTCACCACCACAAATCA	ATGCTTAAATGCAAATCCGC
MeES0882	CCACGACGTCTCCTTTCATT	GGAAGCGTGCAGAAGAGAGT
MeES0883	CAGCCGAAGAGAGATATGGC	GCTCAAGCAACCTCCCATAA
MeES0884	GAGACCCTTTCTTGGTGCTG	GGTTATCCCCCTAGAGCTGC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0885	GCAGTCCCATTATTTCGACTTT	CCACCGCATACTGTTCCTTT
MeES0886	TCATTCAAGCACGATCAGATG	GAAGGCGACAAGAGAGATGG
MeES0887	AAGCCAGCTCGCTCTGTTAG	TGCTGAGAATCAAATCGAAAAA
MeES0888	CATGTCTTTAGAAAATCACATGTCAAG	GTCTTTTGCATTCCCCACAT
MeES0889	GCTATCCATTCCATCTTGATTACC	CTTCCCCAACAGCTCCACTA
MeES0890	GGCTGAGACCAAAGGAACAA	GCCCATCAAAGACACCTGTT
MeES0891	TGGCTATTGTAGCGGAGCTT	TAAGAACAAAAGCCCCCAAA
MeES0892	TGCTCAAGGCTGAACCTTTC	GAGCAAACACTTCTCGCACA
MeES0893	CAAGGCAAAGCATCTCCTCT	AGCTAGCAAAGCAAACCTGC
MeES0894	CGTGCATGAATTCCGTTACA	CGGGAACTGATTTGGAAAGT
MeES0895	AAGCGAGAACCTTCCCATCT	AGCAACCCCTTTCATCCTCT
MeES0896	GGGTTTTGATGGGGAGTTTT	GCCCTCCCAACTAAACCTGT
MeES0897	TAGCTTGGTTATTGGACGGC	TTCATCCTCGAGCCATATCC
MeES0898	GGGATCTCAACATCTAAAGGAA	GGTCATAGCATCAGCCACCT
MeES0899	TCATACATAAAATCTTTAGGCCTTG	TATGGATCAACCAGCAACCA
MeES0900	AGCCACCTCTGCTGTAAGGA	CGAATGTCAAGGCCAGATTT
MeES0901	CGGTTCCAAGAAGATAGAGAGAA	CGGAGTCGTAGAAGGAGACG
MeES0902	CCAAAGAAACCTGGAAAGCA	TGCATGATGCTCTCTTCTTTTT
MeES0903	AAATCTCAAAACGCCACCAC	AGTCAACGAACAGCGGAAAC
MeES0904	CAAACTGAAAACCCTCCGAA	CCTTTTCTTGAACTGGGCCT
MeES0905	TGCTTGCTGGTTTCAGATTG	TCTGTCTCTCTCCGACTCCC
MeES0906	TGAATAAAAACATAATTCAAAGTGGC	CCTGAATTCCTCCTTCCACA
MeES0907	AGGGATTGTGGGAGGAGTTC	TCCCTTCACCAATCCTTCTG
MeES0908	CGGCCTTCTTTCTGTCACAT	CCAGTGTGCTTTTCCCTGAT
MeES0909	CTCTCCAGGATGCTCTCCAG	AGCCCTTAGGTCCCATGTTT
MeES0910	TGGCATAAAACCAACGTGAA	GTGCGAATGGTGAATGAATG
MeES0911	AGGCATGCCAATATGTCCTC	GATCAACTACATCCAACTCTGCC
MeES0912	TGTTGAGCTGTATGCGAAGG	TCATGGGCTGTGAAAATGAA
MeES0913	CGGCCAAGATTCTCTCTCAC	GCATGATGAGGATCTCCGAT
MeES0914	CCTATTGCCTTACCTGCTGC	AAAATCCCGCACAAGAAAAA
MeES0915	CCACCAAGCCAACTTTCAAC	TGCTTGGAAGTAGCTAAGCGAT
MeES0916	GGTGCCTGAATCAAAAGGAA	AAGAAAGAGCAGGCAAGAAAAA
MeES0917	CCTCTCCAAGAACGCTCAAG	GTGCGATGGATGTGGAGTC
MeES0918	CCATCGTCTTTGCAGCATTA	GAGAAAAACAACCACCCGAA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0919	AGGAGAGCAAAAGAGAGGGG	AACCCCCACACCGTACAATA
MeES0920	GCTACGGTGGCGTAAATGAT	TTTTATTCAGAGAGAGGGTAGGC
MeES0921	TAATAATGGGAAGGGCATGG	CTCACCAAATTTCCTTCTTTGT
MeES0922	GCTTTCATTCCATCTCTGGC	TGACCATGGGGATTAAGCAT
MeES0923	CCAAGGACATCACTCCACCT	GCACTCAAGTGCAAAATCACTC
MeES0924	TGCCCCTGTTTTATTTCCTG	CATGTTCAATCACTCCAAAGAGA
MeES0925	ACAAGAATGCAAACGTGCAG	TTAGCACACCAACAGCTTGC
MeES0926	GACTCGTGCATCAAGCAAAA	GAGTTAAGCCGAGTGCCAGT
MeES0927	GGCAAAAATTCATCATACCCA	TTCTGTTGCTGTGGTTGCTC
MeES0928	AGCTTGTTCACGAGCTGACA	CCCGGTGAAGACCATAAAGA
MeES0929	TTTATCGTGGAGCAGAACCC	CGATGTTAAAAGAGCATAGGCA
MeES0930	ACATTCAAAATGCAAAGGGG	CGAACCATAGCAGCAAGACA
MeES0931	TCATCTTAACGTGGTGCTGC	CTATGGCGATTGTCTGGGTT
MeES0932	GGACTAGGAGCCTGCCTTTT	AAGACCTCCTCCAAAGCCAT
MeES0933	AAGTTCCAAAAGCCTCAGCA	CAGCAGCAAAGCAGAAAGAG
MeES0934	TGTTGCACTCAGCTTCCACT	TGCCACAGCTAGCATCAATC
MeES0935	TTGCGACAGTGTGCTTTTTC	ATTTCGGTGAAGCAATGGAC
MeES0936	ATAGGCATCTTTTCCCCTCC	AACGAGCAAACGCTCACTCT
MeES0937	GAATTCGCTGATTTTCCCTC	GCGAAAAAGATCCGTAGCTG
MeES0938	ATTCTGAGATGCCAAGTCGG	ATTCGGAGGCTGTCTAGCAA
MeES0939	TGCACCTTCTCCCTTTTCTC	GGTTTTGTTGTGGAGGCACT
MeES0940	CATCTCTCTGCAGTCCGTCA	GGTCGATGAGAGGGAAATCA
MeES0941	TGCCTACAGGTATGCGTCGT	TAAGCGTGCCTGTGTAGTGG
MeES0942	GCCAGGTCAGGCTAAGATCA	TGAGCTTGTGGGTGAAAATG
MeES0943	GAGGGGCCTCTCTCTTCAAC	GTGGTTGCCCTTCTTGATGT
MeES0944	TTACCTCGCAAACCCAACTC	TGCCTCTGCTAGTTGGTCCT
MeES0945	GGCTGCTTGAGCTTATCCAC	TTTTCTCTGCAGGTTGGCTT
MeES0946	CGCTCAGAAATTACAAAACCC	GCCCTCTTGATCTGTTCGTC
MeES0947	CCCATAATTCCCAATCAACG	TGAGCGTTCTCTCTCTGCAA
MeES0948	CAAACTTCAAAGCACCAGCA	GGAAAAAGCAAGAACCAGCA
MeES0949	ATCACTGGAATTGGGCTTTG	TTTGCGTAGCCCTTCTGAAT
MeES0950	ATACACGCGCGCACTCAC	AGTGTTTCCATGCATCACCA
MeES0951	TCTTCTCGTACCAAAGTTCACAA	ATCAATCCCACCAAACCTCA
MeES0952	GCTTGAAGGAAGTGAGTGCC	ACTTAGAGGCGGCTTTCCTC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0953	GGAGAGTTGGCTAAACACGC	TGGATTGAGAAAATCCAAGC
MeES0954	GCAATGGCGTTTATGGAGAT	CTTCAGAAGCTACATGGGCA
MeES0955	TCCGAACATCCTCTCCATTC	GAGCACACCAAGATCTGCAA
MeES0956	TTTTGCTTTGCTCGAAGGAT	TATCCAAAATCCACTTGCCC
MeES0957	CTGCAGCAGCAAACATTGAT	GCGGAAAACAAGATCCATTC
MeES0958	GGATCTGTTGGGGAGTTGAA	CAGCCTTGCAAGATCCACC
MeES0959	GATTGTGTGATCATGGCTGG	GAATCCATCGCGTGATTTG
MeES0960	AATGCACGGTGGTGATCC	TTTGACGAAGTCCCATCACA
MeES0961	GCTGAGGCCATTTCCATTTA	TGCCTCAAAAACACACAAATAA
MeES0962	CGGTGAGCCAAATCTTTCAT	GGTGAACATATTTCGATGTTATTGA
MeES0963	CAAAGAGGCGAAGGAAGATG	CTGCCCCAAGCTAACTGAAC
MeES0964	TGAGATGGAGCAGAAAAGCA	TTGCCTCAGTCTCCGAAGTT
MeES0965	CCATCCCATAAAACTTGCAGA	ATAGGGAAGGATGTGGAGCA
MeES0966	ACCAACCTCCACTGTCCAAG	AAGGAAATGGAAGCAGCAGA
MeES0967	TCGCAAGCAGAAACTAGCAG	AGTTTCCTGGCTCCTTGGAT
MeES0968	AGTTACAGGACGCGCAGTG	TCACCTGACTTAACCCCCTG
MeES0969	GCAGAGAGACGGAGAGTGCT	GCCTCTCTCTAAAAGGGCAAA
MeES0970	CCTGCCTTCTCTGTGCTCTC	ACAGATCAATCCTTGCGCTT
MeES0971	TTCCGGTCACTCCAGATAGG	CAAAGGAACACATAAGACGTCAA
MeES0972	AAAACCCTCTCCCTCCTCAC	AAACAAAGGAAGAAGGCGGT
MeES0973	TTAGGAGGGCATCTCTGGAA	AGGGAAGAGAATCGGAGAGG
MeES0974	CAGTCCCCTCTTACAACCCA	TTTCAAGGTGGTTGGTAGCC
MeES0975	TGGTCAACACAGTGTGAAATTG	ATCAAATCGTGTCCGAGAGC
MeES0976	CCAACTTCACCATTTCCACC	ACGAGACAGAGGATGGGAGA
MeES0977	CATCACCACTGGCCCTTACT	ATTCGGGGTTCCTTTTCATC
MeES0978	CTTTTATGTGGTGTTCCTACCG	GACTTAACAATGCCGAGGGA
MeES0979	GGGTTGCGTACTCTTCTCGT	AGAGGGAGCAACTGTAGCCA
MeES0980	GAGAGACCGGAGGGGAGA	ACGCTTCAAAGTCGAGGAAA
MeES0981	GGCTTTAAATGCTTCTTAATATAGGG	GTTTTTGCCTTTAGTGCCCA
MeES0982	TGCTCTTTTTCCCAATTTGC	CGCCATTGATTTTGGTTCTT
MeES0983	CTGCTTTTCTTTCCTTTGGC	TTTCCAACAGCTCCATCTCC
MeES0984	GAAGTTGGCGTAGTTGGGAG	TGCAGCCAAAATCATCTCAG
MeES0985	GTTATCATGCGCCCTTTTTC	ACAAGATGACGGAAACCCTG
MeES0986	GCATCTGAACGGATCGTTTT	CAAGAACGTCAAACACCGAA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES0987	CAGCCTTATCAAGGCGAGAG	AAAATATGGGTGTTCATTGAAGTG
MeES0988	CATCCTTGTTTTGGGCTTGT	AGCTCGCACCATTTTCTGAG
MeES0989	GGTATGAGAATTGCTGGAATCTG	CGAGTCTCTTACGCCCTTACA
MeES0990	AGCCATCGTCCAGCGATTA	TCATCAGCATCGTGAGGAAG
MeES0991	CTGATTCCGATCCGGTTTTA	CTCCTGACAGCGACAAATGA
MeES0992	GATGAACAGCCATAGCCCAT	TACCGCCACTGCTCTCTCTT
MeES0993	CCAGGCTGTCAAAACCCTAA	AGAGCAAATTCCGCTCTTCA
MeES0994	CATCGCCATGTGATCTTCTG	GCAGGTGAAATGGGTCTTTC
MeES0995	CCGTTTTCTCCACCATAGGA	TAAGAGCACCAGGTGGAACC
MeES0996	CAATGAATTGAGGGGGTTTG	AGCCGCAAAGAAAAACTGAA
MeES0997	ACAGCCTCTTCTTCTTCCGA	CATATCTTCCCCTGCCTGAA
MeES0998	AGCAAAATCCCGCTCTTTTT	ATGGGGGATTTAAAGGATGC
MeES0999	GGAAGAAAAAGAAACCCTAGCTC	GGGTCGGACTGAATACCCTT
MeES1000	ATGGAGGCTATGTGGTGAGG	CAATCCCTCGAGTGCTCTTC
MeES1001	TTGCACTTGTGTGGCATGTA	AATTTCACTGGCCACATCCT
MeES1002	AAACGGGAAGAGAGGCAAAT	GGCAAAGTAGATGCAGAGGC
MeES1003	TCTGCTCTGCTTCTTCTCTTCA	GCAACAGGAACAACCCAAGT
MeES1004	CATCCAGCGTTACCAAACCT	TAAAGCGTCGGAGTCTCGTT
MeES1005	AAAATCTCCTTTGTTCTCTTATAATCA	GTTTGTGGTTGCAACAATGG
MeES1006	GAAGGATGGGATCTTGACAGA	TTTCCCGAATTGGAAACACT
MeES1007	TCAAGTTCCAAGCCAAGGAG	GAGAGCCAAACCAAACATCG
MeES1008	TGGGCAAGAAAGATTGGAAC	TGATTATAGTTCGTAAGCATCAACAG
MeES1009	CTTTCTTCCCTTGCGGCTA	TCGCCATTTCCGATAAAATC
MeES1010	GTTGGAAATTGCGAAAGCAT	GATCTGGCACGATTTGGAGT
MeES1011	AGATCTTTATGCAGAAGAAGCTG	GCTTCAACACCCAAGGAAAA
MeES1012	CGGCTGACATGTTGAATGAG	TTGCAACAGCATAAGAACCG
MeES1013	ATCAGCAACAGCATCTGCAA	TAAGAGGGAAGGTGCTGACG
MeES1014	TGAAGTTGCAGGTTTTTGGA	CCTACCCAAATCTCTCTTGGC
MeES1015	TCCGGATTCGTTTCAAGTCT	TGGACAAATGTTTGGAGCTTT
MeES1016	CAGAGGAAAACAAGGCCAAA	TTTGTGGGTCTTGAGCCAAT
MeES1017	GATTCTGCCAAAAACAAGAAA	CAATGCAGAGGCTTGTCAAA
MeES1018	AGCTGAAGGACATGGCAGTT	AGGTGGAGCAAAGAACAAAAA
MeES1019	AGAATGGATGCAGGAGTGCT	AAGTTGGATGCTTGATGGAA
MeES1020	GCTCATTCGGTTGGTTCAGT	AAGAGAGGATATGAGAGTTTAAGTGTG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1021	AAAGTTGTGTGGCAGTGTGC	GCTCAGACACCAATTGCAGA
MeES1022	CACGAAACCATTTCGGTCTT	TGACCTGGTTAGGGACCAAG
MeES1023	CCAAAAATTTCATTTTGCACAT	GGCCTGATATCCCTGATTGA
MeES1024	GACCGCTCACTACTCACTCTGA	ATTCGATCTCCACTTGCCAC
MeES1025	GCATTCTCCTTCCTTTCGTG	AGGGTTAGCGGTGAAGGTTT
MeES1026	ACAACCCAATCTTCCCAAAT	GCGGCACTGAGAATGAGAAC
MeES1027	ACTCTAAAGACAGCCGCCAA	CCACATGCTGAATCCCTTCT
MeES1028	ACGCGGCAATAATTAAGTGG	TGAAAGAGAGCCTGGATTGC
MeES1029	CGCTTATCAAGCCCCTTTCT	CAATGCTTCAAAACCGACCT
MeES1030	GATATGATTGCGGTCTCCGT	GACACCAGGCTCGAAGAAAA
MeES1031	GCGGGACTTCTGTGAATCTT	AGTTTGGCAGTGGGTTTCAC
MeES1032	GGACCACCCATATAATTGCG	TGGGGGTGTGTTCATCTTTT
MeES1033	CAGCTCATCATGACATTTCCA	AGATTTCGGGTCTCCCTTGT
MeES1034	TGCGGGAAAATAAGAGGATG	TGCCCTTGCTTAGCACTCTT
MeES1035	CATAAAGCTGGAGCAAAGGC	GCCCCTCGTGAAAACAATTA
MeES1036	ACTTTTGAGGGTTTTTGGGG	CCCTGTGAAGGGTACAAGGA
MeES1037	ACAGAGGGAACATGGAGCTG	AATTCGCTGATCCAACATGG
MeES1038	GGATCCTCTGGTCCCTTCTG	CCGGGACAGTCAAGAAATGT
MeES1039	CATCATATAACATCGGGGCA	AAGCTCCAAGGCTGCTGTAA
MeES1040	TGCTCTGTGTTGCAGAGGTT	CGACCTCTTCCTCGTAGTCG
MeES1041	GGGTTCGTGAACTGGATTTA	CTCTCGACACATACTCGCCA
MeES1042	TCAGCTGTCTCACAGTCGCT	AATCCTTTGGAGGAAATGGG
MeES1043	GACGCGTGACGAGGACAG	CAGTGGACAATGTGGTGGAG
MeES1044	AAAGCTCGTGCCAATCTTTG	TTTCACCCATACCCAAAAACA
MeES1045	TCATCATACATAGTTGGGAAGCA	TTGGTGGATTCTTTTCCCTG
MeES1046	AAGTCTTGGCTAACGCGAAA	ACAGATCACCCATGCTCCTC
MeES1047	ATATAACGTGGGGGCATCAA	GGCTGCTGCTTCTAGGAAAA
MeES1048	GCTGTGGTTGCAGAAATTCA	AGCTGTAGCAAAGAAATGCAG
MeES1049	TCTCTCTCCCAGTCAGCCAT	GATGGTCGGCCACTAGACAT
MeES1050	CAGTTGCTTTGGCTGCATTA	TAGCTCTAGCGGCAGAAACC
MeES1051	CTCTCCCCGCATAATCACAT	CCCATGTTTACCCAATTTGC
MeES1052	TCCTCCTCCGTCTCTTCTCA	GTAGCCTTCTCTTGCGGTTG
MeES1053	AGTGGAGGGGAGAAAGAAGG	TAGGCATATTGGGGATTTGG
MeES1054	TCCTTGCCGCTAGTTCTTGT	CAGTTTCGACCCTTCTCTGG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1055	AGCGAAGCGAAGAAAGATCA	CTTGAAACCCTAGCTGACCG
MeES1056	GGACATGGGATCCTAAAGCTC	GAAGCCAAAGCCAAACAGAC
MeES1057	ATCACGATCCCACCATCAAT	TATTGAAACCATGAACGGCA
MeES1058	GCCGCTCTTCTTCTTCTCCT	ATGCTAAGAAAACGCCTGGA
MeES1059	CTAAGCCGTTCAACACGTCC	TACTTTCTGAGCCGGTTGCT
MeES1060	ATCGTGTTCCGTGTTCTTCC	TTACGGGAACTCTGTCTGGG
MeES1061	AACAGCCAAAACAACCAAGC	AGCGGAAGGGAAAAGTTCAT
MeES1062	AGCGGAAGAAGATGATGGTG	GATCCTTTCCTCTATCCCGC
MeES1063	GCAAAAGTTGGCCAAATACG	TGAAATCCCACATTGCAGAA
MeES1064	GTCTTCGACACGGTTTCGTT	ATAATTGCCAGCACTAGCCG
MeES1065	TTCCTCTCATTTTTCCCACG	CTTCGCTTCTTCCTGTGCTT
MeES1066	GGAAAATTCTCAACCAACGG	AAGGCCTAAGAATTTGGGGA
MeES1067	CACTTGCTGTAGCACCTGGA	TTCGCATATCTGTTGTCCCA
MeES1068	ATTAGTGGCCCTTCTCCCAG	TCCCTCAGTGCTTCCCATAC
MeES1069	ATCAGATACCCCTCGTGTGC	AAGAAACGCCATGAGAAGGA
MeES1070	GGCCCTTGCCTTTAGATTTC	GTGAAGCACTTGTTGTTGCC
MeES1071	TTCCGTCTCCCATCTTCATC	TCCCGTTTGTTGATCTTCATC
MeES1072	TTCAGCCCCTCGTTTTTATG	CAAGTATGCCGCGAGAAAAT
MeES1073	GAGCTTCCCTTACCGTCTCC	CCTTCTCTGCTCCAGCAATC
MeES1074	AACGCCTACACTCAAATGGG	TGCACAGCCCTTATACACCA
MeES1075	TGTTGGGCCTGTCTCTCTCT	TCTCTGGGCACAGTCATCAG
MeES1076	AAAAGGCGCTTCCTTCATTT	GAGAACAGGGTCAAGCCATT
MeES1077	TCTGCAAACCTTTCATCACG	GGAGAAAGGGACCAGAGACC
MeES1078	GCTTGAACCTTGTTGACAGGA	AGAGGAGGGAGCTTGAAAGG
MeES1079	GACGGTGATTCCCCTGATAC	GTATTCCCGCCATCGAAGTA
MeES1080	CAACTTCCCAGAGCAAAAGG	CATGTTGAGAGGTGCTTGGA
MeES1081	TGAAGATGAGGAGAATGATAAAGG	CTTGAAGCGATGGGGTTTAC
MeES1082	GAAAATGGAGAGGGAGAGGC	CCTTCGGTTTTCTTGTCAGC
MeES1083	TTTGATTCCAGAACACAAAATCA	TTGAAAATGAAAGTATTTTTCTCACA
MeES1084	TGCCTTTCATGCTTGCTAGA	TGAGATTCCCACCCACCTTA
MeES1085	ACCCAATTATTTTGGCGCTT	CTTGAGGGATGAGACGGGTA
MeES1086	GCGCTTGCAGTTGCTCTATC	GCCACAACACACGCATATCT
MeES1087	GGGGATTGAAGAGGGGATAA	CACATGACAATGGCGAAAAG
MeES1088	GAAGCACGTGTCCACTCAGA	AAGGGAGTAGGATATGAGAGAAGGA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1089	ATCCCAACAACGAAAGCAGT	AGTGTCTCTGAGAGCTCGGC
MeES1090	ATCTCGCTCCTTCTCTTCCC	CTCCAAAACCACAAGCCAAT
MeES1091	CACTTCAAAGCCCAAGGAAA	AGGGGAAAACAAATGGGATT
MeES1092	TTGATCCCATCCTAAAGCCA	CAATGGCTGGTTTTTGTTGA
MeES1093	AACTGCATTCAAGTTAGATTTTGC	TCCTTTCACCCTGAAACCTT
MeES1094	CTTGGGTTCTCTCTGTTCCG	CGAATTCGAGAATCTGGTCTG
MeES1095	GCAGCCACTTCGTTCAACTC	GCAACCCAACACAAGGAACT
MeES1096	TTTTCCACTTCTGCAAGCTG	GAGTGGGGTTAGAGCGAGTG
MeES1097	GGGACTGATTCATCTCTCTCTCTC	CCTTTGGAAAATCCTCCCAT
MeES1098	TTAAAGCACTCTCATTGGCA	AGCGTTGAGAGCTGGGATTA
MeES1099	GCTTTGGAGAATTTGTTGCC	ACATTGCACATAGCCAGCAG
MeES1100	AGGGAGGAGAGAAATGGGAA	CTGCTTTCTCTGTCCCGTTC
MeES1101	GGTAGCTTCCCAACAATTTTCA	TCCGTTTGTTCTTGATGCTG
MeES1102	GCATTCGCTTCTCTGTTTCTC	ACGCCGTTTTTGGATTTATG
MeES1103	CAAGGCTTATGTTTTCTTACACGA	GCAGCAGTAGAATGAAGGGG
MeES1104	TCCAAGAAAAACAAGCCAGTC	GTGCCATGTGATGTTTGGAG
MeES1105	GAAAGGAGAAGAGCAGATACGA	GAAGATGTTCATGCCCTGGT
MeES1106	ATTTCCTGGTGGTTCAAACG	AGCATTGCTTCCCCAATATG
MeES1107	TGCCTCTTGTCTCCTCTGCT	AACGGGAGAACTCGTACCCT
MeES1108	TGGTGAAAGGGAATGAGGAG	CCTCCAACACCAGCATCTTT
MeES1109	CCAAAACGGCATTTCTCATT	AAAGCTGGGAGAGAAGGAGG
MeES1110	GGCGTTGATGGCTCTTTTT	AGCTGAGCAGCAAGAGGAAA
MeES1111	CGTGGGAAGGCTGCTATTAC	CGCCTTTCCTGTTTCATCTC
MeES1112	TGAAGTATGGCAGCATCTACATT	CCTGGGTTTAAGGGCTTTTC
MeES1113	TCCTCGAACACCCACTCTCT	CAAGCTATTCATCGCAGCAA
MeES1114	CAGATCTGTCTCCCTCGTCA	GTTTTCGACCGGTCTTGTGT
MeES1115	CTCTTTGGAAGCCGAGTTTG	TTCAGAAATCCCTCACCCTG
MeES1116	TCAAGCAATTCCTCAAACACC	CATGTCGTGGAGGTGACAAG
MeES1117	CATTTCCTTTAATTTTCTATTGCG	ATCATGGTTGCCTTCTACGG
MeES1118	CCATGGAAATGTTGAGACCC	ACAAATGTGGAAAACGAGGG
MeES1119	TCATCCGATTAAGCTCCCAC	TTGCGATCTTGAACATGAAAG
MeES1120	GAAGGAGAGCCATCAACAGC	TTGCAAATGTCTAATGCCAAA
MeES1121	TGGTAGAAGGCCTTGCAGAT	CAACAGCCTGTGCAAAACAT
MeES1122	TCGCACACTCTTCCATACCTC	TCCTTGCAAACACACCATGT

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1123	ATGCCTGAACAACCCTTGTC	TGCCATGATCGTGAACAAAT
MeES1124	ACGGACATGGAAAATAAGCG	TCAAAGCAAAGCCTGCAGTA
MeES1125	GATCCATCTGCAACTTCGTG	TCAAATTAATGGAATCGCGTC
MeES1126	TTGGAAGTTGCAAGTCGTCA	AAAACTCAGTGCCATAACTCTTGA
MeES1127	CAATTGGAAGCCCAACACTT	GGAAAAACAGAGCACAAGCA
MeES1128	TACAGCAGTAACCAGCCACG	TGCTTCAACGTCTCATAAGCA
MeES1129	GCATCGAAGCTTCCTTTAGC	GCGAAAAAGATCCGTAGCTG
MeES1130	CCTTTTGAAGATGGTGGGAA	GTTTCAGCCGAAGAACCAGT
MeES1131	GATCGGGACTATGACAGGGA	CTCCTTGAATGACTCCTCCG
MeES1132	ATCATGGGTTTGCTGAAAGC	TGGGCATTCACGTGATCTTA
MeES1133	TTTCCCATTTTTGAGGAACG	CAAAGATCGAATCCCAAGGA
MeES1134	CTGGCCTAGCTTCCAACAAG	TCTGCCTTTCCAAGCAAACT
MeES1135	AACAATTGCCTCCCAAACAC	CATATTGGCTCCCAGGAGAA
MeES1136	TGCAGCGAGACTGAACTTTTT	CATTCTTGATTCAGCCAGCA
MeES1137	CTTCTTGGTGCGTCTTGTGA	AAACGATCTATCAGGCACGC
MeES1138	CTGGAGTTTATGAAGGCGCT	AATGCCCGACACTGGTATTC
MeES1139	GCTGAAGGAGTTGGCTTCTG	TTGACATCAACGTCACCGAT
MeES1140	CTAGCCATGGTGGAACTGCT	CCAACGAAATTCAGACACTCAA
MeES1141	GCTGATGAACGGAAAACACC	CATTGCTCGAAAACCTCTCC
MeES1142	CCTTCAAAGATGGAGAGGCA	ATGCTAGCTGCCAGATCCAC
MeES1143	TCTGTTTTCACTTTTTGAGCCA	AACGTGTCTTACCAGGTGCC
MeES1144	GGCTTTGTCCAATTCACGAT	GCCACATGAACAGGCAACTA
MeES1145	TATCTCCATCAACCCAAGCC	TGCAAACCTCATAAAAGCGA
MeES1146	CGAGGAACTCGTCCAAAATC	CCACGAGCACCCAGATCTAT
MeES1147	GATGGGAATGTTGTTTGGCT	TTGGAGGGGAAGGAGAGATT
MeES1148	AGGCATGTGAAACCATAGGC	GTGGAAAACCCTGCAGAAAT
MeES1149	GTCTTGCTTGCTAGTTGCCC	TTACAAGGTCCACACCACGA
MeES1150	CCATCAAAGGAACCGAGAAA	TTGGAGAGGGAAGCAGAAGA
MeES1151	ACAAAGAGCTTGGCGAGGTA	AGAGAAAACGCAATTCCGAG
MeES1152	TACCCCTTGGAAGGGTCTGT	TTTCTGATGGGAAGGTTTCG
MeES1153	CAGCACAGAGAAGAGGGAGG	CAGCTATGGCGGAAGTTCAT
MeES1154	AGGGTCGGGTTCTTCTTCAT	TCACAACTCACAATAGCCGC
MeES1155	AGTCGCTGGTTCTCTTCGTC	GTTCTTTCTGAAGGCGGTTG
MeES1156	AAGAAGCCAATCCAAGCTGA	TAAAAAGCTTGTTGGGGGTG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1157	TCACTGTTAATCTCAATACCTACCTTG	TCTGTCGGAGGGAGCTAAGA
MeES1158	TGATATGGAATCCTTGCAAAA	CTCTTCTGCGTGCTCCTCTT
MeES1159	GGCCCACAAAGAAAGAGTGA	CTGCTTTTGAACTTGTGGCA
MeES1160	CTTGAAACAGCGGAGAAAGG	CCACTAAAACGCCCACAACT
MeES1161	ACAACTTAAACCTGGGGGCT	TCATCACCAGCAAACTGCTC
MeES1162	ACGCGTGAATTCTTGCTTTT	AAAGCATGCGCAGAGGTAGT
MeES1163	CCAGGCCGTTTAATTCCTTT	GCAGTGACGTCTCGATCAAA
MeES1164	AGCATCGAAAACCGTGACTC	TGGTGAATACCCACATCGAA
MeES1165	CCATATTGGCGTACTTCGCT	CCTCCAATAGCAACTTCGGT
MeES1166	CCCACTGTCACTCCGTTTTT	TCGTCGCCATCAATTTCATA
MeES1167	GCTCCCATTGCTGACTCTTC	AGCTGGTAAGCTGAAGTGGG
MeES1168	AAGCAAACGTCCAAACAACC	ATGGTGGATACCTGCAGAGC
MeES1169	CAACGGACTCCTTCTGTGGT	GTCGCTATTGACCTCGGTTC
MeES1170	AAGGAAATCCTCAATCGCCT	AGGCGATCTAAAGCCGAAAT
MeES1171	ACAATCCCCAGTAGCAGCAG	CGAGGATTACCAGCAGAAGC
MeES1172	CCGTTTTCGTTTCCCTAGAA	TCGTTCCACAATAACCACGA
MeES1173	TGAGGAGGAAAAACAACGCT	ACCAGCAGCCTCTGTTAGGA
MeES1174	TTATCCAAAGCAAAGCTCGC	CATGATGTGATTCCAGCACC
MeES1175	TAAACGCGCTTCGCTACTTT	GGGAAGCTTAAGGGAGGTTG
MeES1176	GATCATTGCTGCACAACACC	CCTATTGGAGGATTGAGGCA
MeES1177	CCAACGTCCATTAACATCCC	CTGAGCTTTAGGGAGGGCTT
MeES1178	CAACAATCTTTGCTTCTGGC	GCACAAAGTGCGAAATCAGA
MeES1179	TTTGATGCGTTGAAGCTTTG	TCGATTAGGTCTTCGCGTTT
MeES1180	TTCCTAATATTATAACTGGGGTTTCAA	TGCTTCGTAAACTTTGCAGC
MeES1181	GCCCCAGTGTAGGAAGAAAA	TTTCTCGCAACTGCATCATC
MeES1182	GTTCGTGTACATGGGGAACC	GGATGTTTCCATCCAGGAAC
MeES1183	TGCGAACTCTCCCTCTCATT	CTGTTGTCGCTCATCATCGT
MeES1184	ATCCCCAAAATTTCACCCTC	CGTGCTAGTGGTAGCGTTGA
MeES1185	CGTACAATTCCCAACGGTTT	ACCGTTTCAACACTGGGAAG
MeES1186	AGGCCGGACTATCGGTACTT	ACCATCAGAAGCCAACGAAG
MeES1187	GCTTTCTCGGATAAGGGTCC	GCAAGAAAATCTTGGGCGTA
MeES1188	TTCACTCATTCATGGGCAGA	TCAGACTGCTTCCACCACAG
MeES1189	TCTGCCACCGAGTTTTCTCT	AATGAAACTCCGTCGTTTGG
MeES1190	CCATGCTCTCATCAGTGGAA	GAGCAGCCTTCGAAGAACAC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1191	CCTTTGCTGGTCGTCTTCTC	CTCTAAATCCAACACCGGGA
MeES1192	CTAATTTTGCCACCCCCTTC	TGGGTTATTCTCCAAGTCGC
MeES1193	CAGGGCCAAACGAAAGAATA	GAGATGTGGCCTCCTTTCTG
MeES1194	CCCTCAAGAATCCAGAGGCT	GAAATGGAAAGAATGGCGAA
MeES1195	ACAGGGTGGAATGGTTCAAG	ATAAGAAGGCAAGCAGGCAA
MeES1196	AGCAACTCGTCAAGGCTGTT	TGTTGCTTCATCCTTTTCCC
MeES1197	TGATTAATTTGGGTTATGGGAAA	TGAGGGAAAAGCAAACATCC
MeES1198	CCCGTCAATGGTGGATAAAC	CGACCCAGCAAGAAAAAGAG
MeES1199	GGCTTGCCAAATGCTACTTT	GCAGAGTCTCCAGGGATGAG
MeES1200	CGAGTACCAACAAACAGACGC	CTTGTCGTCCCATTTGGAGT
MeES1201	TCATCAACCCCCATCCTAAA	ACCCTACAAATCGCCATCAG
MeES1202	TTGGAAAGATCCACACCACA	TCTCTCGATCCCTCAAGCTC
MeES1203	GTAGAATCCGCCTTTTTCCC	CCCAGCAACGAAAATCAAAT
MeES1204	CAGTTTTTCCTTTTGCCTGC	ATGCTTCAGATTGGGCTTGT
MeES1205	TCATTGTTTTCGTTTTTGGG	TGAAGCCTCAAAGCCTTGTT
MeES1206	GCATTGAAGCAACCAACCAT	TTTTAGTATCAGTGGCGGGG
MeES1207	CTCTCCAAAAACCCCAACAA	GGAAAGGAGACTCAAAGGGG
MeES1208	TTCCCCTACTCCTCCTCTCC	CTACAGTGCGTGGACTCGAA
MeES1209	AACCTTGTGGGTCTGGTCTG	CGCTAGGACTGAGGAGTTGG
MeES1210	GCAAGTCATCGGTCCAACTT	CCACATTTCAGGAGGCAACT
MeES1211	ACCTCCCTGTTTTCTTCGCT	AAGCGACGATCATGACACAG
MeES1212	GGCCGAACATCTACCTTGTT	GACCAGCCCTCAAATGGTAA
MeES1213	TGTCCCGTAGGGTTTCGTAG	AGAAATTGAAAGGGCCTGGT
MeES1214	GAGCTCACCCAATAATCAAGC	CGAGTGTGCTGAAAGGATGA
MeES1215	TCCCAATCTTACGACTCGCT	GTTGTAGATGCGAAACCCGT
MeES1216	AGATACCCAAAAACCCCCAC	ATTCGAATCACGTCCAAAGC
MeES1217	GCTCTCTCCCTCCTCATCCT	CATCGAGTCCTCCGTGGTAT
MeES1218	ACGGTCACATTTTGCACAGA	TACGTTGCGCAGTACCACAT
MeES1219	TCAACGTTACTCGCTCCTCA	ATTGGGGGAATGAAGGAAAC
MeES1220	TTGCATCATTTTCCTTTCCA	ACCCACTTATACCCGCTCCT
MeES1221	GTTCTCCCTCTCGTCTTCCC	ACACAAATTGCACACCGAAA
MeES1222	TTTTCAATTTCACCCCTTTTC	GGTGGGGAAGGATTTAGGAA
MeES1223	TCTCTCCGAACTCTCCCAGT	CCATACCTGGAGAACGCAAT
MeES1224	TGCTACATATCCAGAGAGCCAA	ATGCTGGGGATGAACAGAAC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1225	GGGAGGGAGATTGTGAGAGA	GATGAACAGACGTGGCAATG
MeES1226	CTTGGTGACTGGGAGGTCAT	ATCTTTTACCGGGCAGTCCT
MeES1227	TTGACCTAATATATCTGCTCTGCTG	GTGACTTCAGCAGCTCCCTC
MeES1228	AATGCGACCATCATTTCTCC	GGATCCAGTTCGCAAGGTAA
MeES1229	TCTCTCCTCCCATCATCACC	GCTGCCATAGTGAAGGAAGG
MeES1230	CCATCTCTCTTTCCCTTTCTCA	AGCCCAGAACCCAGAAAACT
MeES1231	AAATTCAGCCAGCCAGACAC	CTGAGGCACACATGGTGAAG
MeES1232	CCTTCTGCAACACTGAGCTG	TTACACAACAAGCCCCAACA
MeES1233	GACCGGTCACCATTGCTACT	TCCAAGTACCCTGAGCTTCC
MeES1234	GCTAGCAAACTGCACCATAGG	GGACCACAGGTAAAGAAAGCA
MeES1235	TTAAAGCACTCTCATTGGCA	AGCGTTGAGAGCTGGGATTA
MeES1236	AGAGATAGAAAGAAAGAAAGAAACGA	AATCCACTCCTCGTTCTCCA
MeES1237	CCATCACTCAAACCGCATC	GGTGATCGAATTGGAGGAGA
MeES1238	CACAGACGAATTGTTCAAGGG	TGGTGTTGGGTCATGCTCTA
MeES1239	CACACTCGGAAACTCCAACA	ACTGTTCCCAAGGAGGAGGT
MeES1240	TTCCAGGTAGTAGGGGGAGG	AAGATCGCAAGCTTTTTCCA
MeES1241	TCTGCAAAACTGCAATAAGCC	GACGATGACTCCCCTTGTGT
MeES1242	ACGAAAACGTTTCCTTCCCT	TACCGGTTAGCGGTGATTTC
MeES1243	AGGAAACCCCTCCCCTTATT	GTTTCAGCGAGCCAGAAAAC
MeES1244	TTCCTTTTCCACTGGTCCAC	AGAATGCAAAACAGAGAGAAAAA
MeES1245	GAGCAGTTGGATGTTGGAAA	TGGTAAATGCCACAGCCATA
MeES1246	GGGCGGTCTCTTCGTTCA	GTCCCATGTTTCGCTTTGTT
MeES1247	TCCCTTAGCGCTTGAGTTGT	TCTTGAAAGGTGGACGAACC
MeES1248	GGAACGCAAAAGAAATGAAA	TCTTGCTGTGATGTTGGCTC
MeES1249	TTTGCCCTCTTAGCCTCAAA	AAGAGAGAGCACATGGAAAGAT
MeES1250	CACCACCATTTCATTCCAAA	CCTTGAAGAGACAAGGCAGG
MeES1251	ACCTATATTCCCGTCGGGTC	GAGAAACCCAGAAACGGAAA
MeES1252	GGCGCATTAAAAATCTTGGA	AAGCAATGTCCAAAACCTGG
MeES1253	TTCATTTAGCTACTCAACCTCTCC	AGGCATGATTGGAAGAGTGG
MeES1254	TTTCTCCTCCATTCCGTTTC	CTTGAAGCGTAGGGAGATCG
MeES1255	TCAACCTGACCCTCTTGTCC	AGATTTGCATGCAGCCTTCT
MeES1256	AACACGGAAACAAGGTCAGG	AAAGTAGCCCGACCCAACAT
MeES1257	TAATGGTGACGCTGACCAAA	CCATCTGGGTTTTACCCCTT
MeES1258	TCTGCCCATCATTTCATTCA	GCTTCATTATTTGGACCGGA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1259	ATTACACAAGGCAAGGGCAA	ATTCATGGGCCCTTATCACA
MeES1260	GAGGGAGAGAGAAACAAGGGA	GAACACAGCAGTTGCAGAGC
MeES1261	CAAGCGCAAAATCTTCACAG	GAGGGAGGAAAGGAGAGGAA
MeES1262	CCCCCTTGTCTCTCTCGTTT	GACCGTACATTGGCGAGTCT
MeES1263	TCAGTCAGAAAACTCGCCCT	AAAGTGGTTCTCCATGGCTG
MeES1264	CGTGATGGAAGTGAAGCTGA	ACAGCAACCATGAGGGAAAG
MeES1265	ACAGGGACGCTCACAAAGAC	GGCAGTTGGGTTCTTGTTGT
MeES1266	GCCTTATTGGCGTGATTGTT	AAGCGCAGCTAACATCCATT
MeES1267	CACCATCTTCCGTTACACTCC	TTAATTCGGACCTCACCAGC
MeES1268	TGAATCAATATTGCTGGATCTTT	AGACACTGCAGCACCCTCTT
MeES1269	GCAAGGGCTTGAGAAAGAGA	TGGAAGCAAAAGCTGTCTCA
MeES1270	ATTGCCAGCGAAGCTTTCTA	GCAAGTAATGATCCGCCATT
MeES1271	GGGAAAATCACTGATAAAACCC	CGACATGGAGAGTAATCGCA
MeES1272	TCTCTCTCACCATTGGACCC	GGAAGAGGAAGAGGGAGGAA
MeES1273	ACACAATTGTTTGTTCGGCA	TTGGTGAACTGTCTGGCTTG
MeES1274	TCCTTCCATGGGATTACCAA	TGAGCTCTTTGGATTCAGGG
MeES1275	ATTGCTTCATCTTTGCAGCC	AGGCTTGTTTTGAGGAGGGT
MeES1276	AAAAAGAATGGAGCTACCGGA	TTTTCTGGGCATCCAAACTC
MeES1277	AGACGCGAAACAATCCATTC	CCTTTGCTTCTGCTTTCCAC
MeES1278	TGTTGGTGTTTCATCAGGGA	AGAGCTGGGAAGGAGAGGAC
MeES1279	GATTCGCTCTCCTCGTCTGT	GGAACTCCTCTTCGAAACCC
MeES1280	GGAAGGACTTTGCTAATCCCA	GAGAGGCCCTAAGGGAATTG
MeES1281	CAGACCCCTCTCTCTTCCG	ATTCCTTTTCTTCCTGGCGT
MeES1282	CCAGCAATCCAGCTTTTGAT	GATTCCTTCTTCCGGTCCTC
MeES1283	CAGCGTTACTGATGGCAAGA	AATTGTTTCGTCAATCCCCA
MeES1284	ACTTGGCTCTCTCCTCCTCC	AAATCCCAAGACGACAGTGG
MeES1285	CAGAGCACCTGAAGATAATTTGG	GACAACATGGCTCAGGGTTT
MeES1286	AAAAGACATTTTAATAACAACCCAAAA	AATAGCCTTGGCAATTGGTG
MeES1287	ACAACTCAAGTGATACAAATCCTG	ATCCTCCAAAACTGGCACAC
MeES1288	GGATTGCTTTTCATGGCTGT	AATTTGGGTGCTGAGAGTGG
MeES1289	CATTCAAGTCGTCTTCTTTCCC	GAATCCTATGGCTGTCGGAA
MeES1290	AATTCACCCAATGGGAATCA	TCGATATGCCTCGTGATGG
MeES1291	CCTTACCACCTTCGTTTCCA	CCTGGGCCGTCAGATATAAA
MeES1292	TGTAAACTAAATCACACAGAGTCCA	CCTTGGCTAAGGACCAACAA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1293	AAACCCACCACTTCATCTGC	AGAATGATTTCCGGCATCTG
MeES1294	ACACCCCACAGTTTCTGCTT	AGCCATGCCTTCTGTCAACT
MeES1295	GAGGAAGGGGGAAAACAGTC	GTCGCTGATGGGATGAATCT
MeES1296	TTCCATGGCTTCTCCAAATC	GGCTTCTCCAGCAGAACAAC
MeES1297	TTCAGAAGCAACTGTTCCACA	TGAAAACCAAAAGTTCCTCCA
MeES1298	GATTGGACCCATTTGCATCT	TCCAACTCCCAAGAGAGGAA
MeES1299	ATTCTCTGTTTGGCGCGTAT	TTGGTTTTCTTGGTTTTGGC
MeES1300	CACCCAACCACTTCTCCAGT	GACACACTCCACGAACGAGA
MeES1301	TGAATTCTACATTTACAGGGGC	ATCCTTGCTGCCTCACAATC
MeES1302	GGCGTAGAGTACAAAATCCTCAA	ATGTGAACACAGCCATGGAA
MeES1303	GAGGTTAAAACAAACTCAGCCC	ACACCAAACAGGCTCAAACC
MeES1304	CGCACTTGGGATTCTTTCTC	CCGATTCCAGTCCAAGAAAA
MeES1305	TTGGCTTTGTCTTCATCCAA	TCTATGGCGAATCCAAGAATTT
MeES1306	CAAGCTAACCCAAGACCCAA	ACACCCAATTCTCATGCACA
MeES1307	TTTCCAGTCCTTCCATTTGC	TCCTCCAATAGCATTTTGCC
MeES1308	AAAACCCCTAATGCCCCTT	ATTGATGCTGGCTAGACGCT
MeES1309	CCAACATATGATTCAGGGGG	GGAAACAACATTTTGGGCAC
MeES1310	AAGTTGTTGCAACCTCCACC	GCTAGCAGAAGCAAACCCAC
MeES1311	GAGAAAAGGACGCCACTCTG	TGCTTTGTTCTTTGCCCTCT
MeES1312	AGGTGGTAAATGGAGCAACG	CAACTCCAAGTGCATAGCGA
MeES1313	CGGGGAGAGAAGAGAGACAA	AAAATCCGCACGACCACTAC
MeES1314	CCCACCGAAATTTCTCTCCT	GCTTCTTTGCAACACGATCA
MeES1315	TGGCTACAACCCTAAGGTGC	AATGAGCTTGACGAGGAGGA
MeES1316	TGCGAGCAAACACAAAAGAC	AGCCATACTGTCTCCTGACGA
MeES1317	TCCACTTATAGGCGCAGGAC	CACTGGAGGAAGCCATGAAT
MeES1318	TTCTCTCGCTGAGCGGTAAC	AATGGTTCTTTCACTGCGCT
MeES1319	AGAGTGCTCTGTGTGCGTGT	CCTTTGCGCTTCTCAGATTC
MeES1320	CGGACTTGAAGGTTAATTAGGG	GCGACACCTTTCCATCCTTA
MeES1321	GCTGCACAGTGTCTCCACAT	CATTCAACTGATAAACCGACCA
MeES1322	TCATGACAAAACTCGGTGGA	GCAGAGGAAAACGAAATGGA
MeES1323	CGACGCATTTTACGTTTTCC	CCCCCTCTCATGAATTGAAA
MeES1324	TCTCAGTTCACTGCCACCAG	AATTTAAAAGCGCATAAAAGAGAT
MeES1325	GAGGCTTGATGAGAGGTTGC	TATGCACACGAAACTGCTCC
MeES1326	GCACAAACTTAGTGGTCGCA	GTGATCATGTTGGAAACCCC

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1327	ACCCCATGAGTGGTTTCAAG	TTACATGAACAAGAGTGTAAGCCT
MeES1328	ACGAGGACCTGTCCCTCTCT	AACCATTTGGTTCCTCCCTC
MeES1329	CCCTTACGAGTGTGGGACAG	TGCATCAGCTTCATTCAACAG
MeES1330	AACTTCGAATTTCCAGGGGT	CAAAGAATTTCTAACTTCATTATGGTC
MeES1331	CGATGACGAAGAAGGTTTCC	AGGAAGAAGCAGGGGAAGAG
MeES1332	CACTCAAAGGAGTCCGAAGG	CAGCACCCAACACAAGAAGA
MeES1333	TCTCCATGGCCTTTCAATTC	AGAATCGCAAAAGAAGGCAA
MeES1334	GAACGCATTGAAAGGGAAAA	AATTCAGTGACCTCGCTGCT
MeES1335	CCATTTCCACTCCTTTGTCA	CAGGATCCTTTCCACTCCAA
MeES1336	CTGAGCTTGCCAAATTCCTC	CAGCCAAGCAAGATCATTCA
MeES1337	ATCACAAACCAAACCTTGGC	GGGGAGAATTGAAACTGTTCTCT
MeES1338	GCTCGATCTGGGAAGATTTG	ATGATGAACGTCAGTGCTGC
MeES1339	GCATCTGTCGCAGCAAAATA	GAAGGGAGCAACTAAGCCAA
MeES1340	CACAACAAAACACCTCACCG	GTGAAGAGGTCTTGCCGAAG
MeES1341	AACGCATTGCCCTTAATCAC	GCTCAAGATGAGTTGGCCTC
MeES1342	AAATTCCCACAAAGACACGC	AGCGGACGGGAATTTTAAGT
MeES1343	ATCCCCTCAAGCGAACTTTT	TCCTTCCCAAGGCCTTTACT
MeES1344	ATGGGTGTCCTTGTGCCTAC	CTGATCTGCTGCAATGGCTA
MeES1345	TCCATCAATGGCTCAATCAA	CTGAAGCATCCCCTCTCTTG
MeES1346	AAGGAGTTCTCGTGGGTCCT	TGCCCTGTCAGTAAATACAGGAT
MeES1347	TCCCAATAAGCTTTTGCCAC	GAATGACCAAAAGGAAGCCA
MeES1348	CCAAGCAGCCTGTCAATGTA	CTCTTAACCCACTGCCGAAG
MeES1349	TTTGCTGGCATAAACATGGA	TCAAACCCAACACCCTTCTC
MeES1350	CGAAGGCGAAGCATCTAAAA	CGGTCAAGCGAGAAAGATTC
MeES1351	CGAGAGAGAAAGAGATAGAGAGAGA	GAGGACAATATCCGCCAAGA
MeES1352	GACGTTTCTCGTCTACCCCA	TTCACTGTCGTTCTTGCATTG
MeES1353	TGGGTAACTGTGCATCACCT	CCTTGATGGGTTGCTTGAAT
MeES1354	TGGCCTAGACATTTTCAGGA	CCTCCTCTCCTTTCCTGCTT
MeES1355	AATAAGATTTTGTTATTTGCCACA	TCCCATTTGATTGACGAAAA
MeES1356	TCTGGTTTTGTTCCTGGGTT	CAGAAGGGGTTCCAATCTCA
MeES1357	ATTGGTTTTGGAGGCAGTTG	TTGCCAATAGTTTCGCCTTC
MeES1358	TTTGAGGCCGAGACCTAATG	CCTGTACATGGTGATGCTGC
MeES1359	ATGCATAGTAAGATGCGGGC	TCAGAAATTTTCGCATTAACTCC
MeES1360	GGAAGGAAAAAGCCATCACA	CAAATCCCTCGGTGTGAAAA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1361	TTTACAACAGTTAAGTCATTCTCCAA	GGCATCATACCAAAATGGCT
MeES1362	TTGTGCCAAAACCATCAAAA	CGGCAATCTTCTCTTTCCAG
MeES1363	GGACATGGATCTGTGGCTTT	TCATGCTTGATTCCATCCAA
MeES1364	AGCTGCCAAGGCATATGAAC	GACTATCGCCCAATTTGCAG
MeES1365	ATATTCCGGAAGGCCTGAGT	CCTCAGAAAGAAAATCCGCA
MeES1366	ATGGGTTCATGGTTTCCAGA	CAGTGAATTTGCCTCCCTGT
MeES1367	TTGCAGCTTCAAGATGGTTG	CGTTCCCAAACCTGAACTGT
MeES1368	AAGGTCTGAGGTTTTTGCCA	ACTTACATTCATGGCTGGGG
MeES1369	AGAGACGGGAAGAGGGAGAG	TTACAACACTTTCCTCACCACA
MeES1370	CCTCTCATTTTCCCGTTCAA	AAGATCGGTAGCCTCGGATT
MeES1371	TTGGGAAAGAATGGTTTTGG	TTCCTTCGATTTTGGCAATC
MeES1372	CAGGGCCTTCTTTGCTGTAG	TCCCAAGCAAGAGAGGAGAA
MeES1373	GCCGCTAGCAGAGTTGTACC	CTTGGCCCTGAGGTTATCAG
MeES1374	AGTTCCGCTATCAACGCTGT	TCATGTCGCTTTGCACTTTC
MeES1375	CGCCTACAATCACTGGTCCT	GAAGCGCAAAAATCTGAAGC
MeES1376	AGAGGGAATCAGCGAGTGAA	AGGGCGAGTTTAAGAGAGGC
MeES1377	GGCAGTCAAGCGGATATCAT	CCCGCCTAGATTCACAGAAA
MeES1378	AAGCTGGCTGATGGAATCAC	AATTTCCATTTCGGAAGCCT
MeES1379	CGGATGCTTTGTAGGGATGT	CACTCGGAATCCAATCCATC
MeES1380	CTCCTCGTCCTCCACCATAA	GGGTCATTGACATCCCATTC
MeES1381	TTTTGCATAGGATTGGGACC	AAGATTGTTGGCAATCTGGC
MeES1382	GAAAAGCAAAGAAAAGAAAGGAAA	GTCTGGCTTTTCTACTGCGG
MeES1383	GCACTAACTTGATGGGGGAG	TTGTGTGAGTTGCAGAGGAAA
MeES1384	CTGTTGACGGAGGAGCTAGG	GTGATCTCTCCGACCTCTGC
MeES1385	CCAAAATTATCTGCCATTGCT	TCATTTCAGATGCACTCAACTCT
MeES1386	AGATCGCTTGCTCAGCCTT	GGCGGTGATTCATCAAGATT
MeES1387	GCTCAAGTGGCTTTGTAGGG	TTGGTGAGGTTCGAAGCATT
MeES1388	ACAGAGCCTACTGATGATGTGA	GGAGCCATCCACTTAGTCCA
MeES1389	CAGCTTCCTCTTCGTCATCC	GCCTTTTGGCTTCATACCAG
MeES1390	GCTTCTGCTAGCCTCAAGGA	CTGGACGAGAGGGAGAGAAA
MeES1391	CAAAAAGTGAAGGCTGAGGG	TTATCCATTGGGAATAAGCG
MeES1392	GGGGACTCCATCCTGAGCTA	TTTCTTGCATGATTTGAGCG
MeES1393	TCATTTCACTCGGATCCCTC	TGGTTCGTAGCCTTTCCTTC
MeES1394	GGTTTCACTTTCCTGAAATCCT	TAGGAGATGGTGCCTCCAAG

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1395	GAGCCGGAAAAGTTCACAAA	GAGTTTCTGGGGGAGGAAAG
MeES1396	AATTCTAGGGTCGCCGATTT	AACCACCTTGGAAAACCCA
MeES1397	TTTTCTGCCAAGAAACAGGG	GACTAGGAGCTTTGCCATCG
MeES1398	CCATATTTGTGCCCATATGCT	GCAGTATCGCATACGCTCAA
MeES1399	CCAGCCACTCTGCTGTGTTA	CACCTGAACCAACATCAACG
MeES1400	ATGATGAGCTGCAACGTGAG	AATGCAAGAAATTGAAACAAGC
MeES1401	AAGTAATGGATGTCGGTGCC	AACCCAAACCTGCAACTCAC
MeES1402	ATAGCCCAAAACAGCCAAGA	AAGCTTGCAAGACCAGAAGC
MeES1403	CAGGGTACAACGCACTGAGA	AACACGAAAGGGCATGTAGC
MeES1404	CAAAAACAAATACACCCCAACA	GCATACAACGCCTGTGAAGA
MeES1405	GCCATAAGAAATGCCGTTGT	ATCGTTTTCCCCTTCCAGAT
MeES1406	AATCCACCCACTCCTTTTCC	AAGAGCTTCCCATTAGGCGT
MeES1407	TCGTGACGACATTGCTTCTC	AACAACAACGGCGGAGTAAC
MeES1408	GACGATGATGATGACGATGG	TTTCCCTTCAGGATTCATGC
MeES1409	CCTGAGAACTCCTTTCATGTCC	GGCGAAGGAAGAGTGTGAAG
MeES1410	TTCCAACCTAAGCAATCTCACA	TGGATCTAAGAATGGCAGGG
MeES1411	GACGATGATGATGACGATGG	TTTCCCTTCAGGATTCATGC
MeES1412	TGCAAGCCATTTGAAAAACA	TTTTGTTGGGTCACAACCG
MeES1413	AGCGGATTCTGGGGAGTTAT	TGAAACTCTACATCAACCCAGATG
MeES1414	CTTTGATTTCTCTCAGCCAGC	GACCCAAAAGCAACAAAAGC
MeES1415	AGAGAGTGGGGTTTTTGCCT	CCTCCCTTCCATCCTCCTAC
MeES1416	AGATACCAATCCGCTCCG	TGCTGAAGCTGAAACCATTG
MeES1417	ACAAGATTCTCCATGGCTGG	AAGACACGAAGACGGTTGCT
MeES1418	GCCTATGGCCTACCCAAAAT	AATTCAAGTTCAGGCGTGGT
MeES1419	TGGTGAAACACCAAGCACAT	CCTCTGGTACTTGCACGGAT
MeES1420	CCAAAGTACGCCACCAACTT	TAAGCAACTCATGGGCAGTG
MeES1421	GATTCCCTCGGGTTCTTCTC	CATATCCATTCTCAACCGCC
MeES1422	TTTGTTCTGTTCTGCGCAAT	TGGGGTCTTTCTGTTTTTCG
MeES1423	CCCAAAACAAACCTCCATTG	TTTTAAAGAAAGCGCCCTCA
MeES1424	AGCCAAAAACCATACCCACA	CTGCTATTGCTGTGTGGTCC
MeES1425	GGGTTTCTTTGTTAAAAAGGGG	GGTATTTGCTGCGAAAGAGG
MeES1426	TGCCCTCAAATTTTCTCCAT	AAACATCCAGTGCAAATGTGA
MeES1427	TCCGAACATCCTCTCCATTC	GAGCACACCAAGATCTGCAA
MeES1428	CCGTCTCCCACTCTCTTCAA	CACAAGCATTCTCCACGAAA

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1429	GTTGTGCCAAAACCCTTCTG	GATTCCGGTGAGAATGAGGA
MeES1430	TGGGTCCTGCGTTTAAGGTA	ACGTCCTCTTCAGAGCCAGA
MeES1431	GGAGGCTCAGAGGTTAGCAA	TTTCAGTATGAAGACAAAAGGGG
MeES1432	GATCCCATCTCCCACTCCTT	TTGCTATTGAGCACAGCCAC
MeES1433	TCCCAAGCAAGAGAGGAGAA	CAGGGCCTTCTTTGCTGTAG
MeES1434	GAAGCACCTAGCTCACCTCG	TTAACAGAACAGCCCAACCC
MeES1435	CAGGACCCATCTCGTTCAAT	TACCTTAAACGCAGGACCCA
MeES1436	CCATATTTGTGCCCATATGCT	GCAGTATCGCATACGCTCAA
MeES1437	AAGTAATGGATGTCGGTGCC	GCAAATGGACCTCTCTCTCTCT
MeES1438	CGACACAACCCAGACCTTCT	TCGAGAAAGCAAAGAAAGGG
MeES1439	GTAAGGGCAGTCACCAAACG	GAACCTTATTTGCAACCGGA
MeES1440	GGGACGCTTGTCTCTCAGTT	AAGGTGGTCTCCCCATATCC
MeES1441	GAAGAGGAAGATTTTGTTTTCCA	AGAGAAGCAGAGAGGAGGGG
MeES1442	GGTGGCTTTCAGCTTTTGAA	AAATTCAAAGTGGACAAAACAAAA
MeES1443	AAACAAACCGAATCTGCCAC	TGAAGAACAAATGTGGGCAA
MeES1444	AAAACCCTCTCCCTCCTCAC	AAACAAAGGAAGAAGGCGGT
MeES1445	TTAGGAGGGCATCTCTGGAA	AGGGAAGAGAATCGGAGAGG
MeES1446	TTCCGGTCACTCCAGATAGG	CAAAGGAACACATAAGACGTCAA
MeES1447	TCCTCTTCCTTCCGACTACTCTC	ACGAACTGGAAGCATGAACC
MeES1448	TTCTTGCAGCGAAAACAGTG	CCTCCACATATTGCTAGCCC
MeES1449	TCAAGTTCCAAGCCAAGGAG	AAGCTTTGGGCCAGCATAAT
MeES1450	TGGGCAAGAAAGATTGGAAC	TGATTATAGTTCGTAAGCATCAACAG
MeES1451	CAAGAGAAAAACTCCCGCAG	CACAGCTCCCATCTGTCCTT
MeES1452	GCAGGACATGGGATCCTAAA	GAAGCCAAAGCCAAACAGAC
MeES1453	CCATCTCACAAAACCCCCTA	CCACGCTTTGCAGACTGATA
MeES1454	GCTGTGGTTGCAGAAATTCA	AGCTGTAGCAAAGAAATGCAG
MeES1455	GCCCAACACTTGAGCAATTT	GGAAAAACAGAGCACAAGCA
MeES1456	GGTGGTGACGAGAGTGGAAT	AAGCCTCCCCCTTCAGTTAT
MeES1457	AAAGCAAAGCCTGCAGTACAA	ACGGACATGGAAAATAAGCG
MeES1458	GAAATGGGATCTTCTCAGAAACA	GGTGGGATCTTGCTACTCCA
MeES1459	TCTTTCCAAACAACTTTGAGCA	TACCAGGAGCCGAGAGAAAA
MeES1460	TGGGCATTCACGTGATCTTA	ATCATGGGTTTGCTGAAAGC
MeES1461	TTTGAGAGATGGGGTTCTTCA	TGAGATTGGAAGCAGAGGAGA
MeES1462	CCTTTTGAAGATGGTGGGAA	GTTTCAGCCGAAGAACCAGT

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1463	CCTTCTTCAACCTTCGCTTT	TTTTTAGCAACGCACCACAG
MeES1464	TCTGTTGCGACTCGAGAGAT	GACTTTCTGATTTGGCCGAG
MeES1465	GCTGCACAGTGTCTCCACAT	CATTCAACTGATAAACCGACCA
MeES1466	CTCCGAACAACAACGTTTCA	AAGATAAGCCTTCCCCCTCA
MeES1467	GTACTGGGTAGCAAGAGGCG	AATGGTTCTTTCACTGCGCT
MeES1468	GAGTGTAAGCCTATAAGCATCATTTG	GCTGTTATTTTGCTGCACGA
MeES1469	CAGCCAAGCAAGATCATTCA	CTGAGCTTGCCAAATTCCTC
MeES1470	GAAAAGCTTGAGCCTTTTAAGC	AAAATCCGCACGACCACTAC
MeES1471	CAAACAAGCTGATGAACGGA	ATTGCTCGAAAACCTCTCCA
MeES1472	GGGACAGACCGTAGCCATGT	GATTTGGCCGTGATGATTTT
MeES1473	GTGATCATGTTGGAAACCCC	GCACAAACTTAGTGGTCGCA
MeES1474	ATCCCCTCAAGCGAACTTTT	TCCTTCCCAAGGCCTTTACT
MeES1475	TCCATCAATGGCTCAATCAA	CTGAAGCATCCCCTCTCTTG
MeES1476	TGCATGAAGTCCTTGGTCAG	TAATGGGTATGGAACGGGAG
MeES1477	CACACTGTGGAGGATGGATG	CATCATTTGAACATAACGATCTCC
MeES1478	TCCACTTATAGGCGCAGGAC	GGGAGAGTTATAGCAGGACCC
MeES1479	GGGGAGAATTGAAACTGTTCTCT	AAATATGACCGCAGATTGCC
MeES1480	AACTTCGAATTTCCAGGGGT	TTTCAGCGACAAAGAATTTGTAA
MeES1481	GAATGACCAAAAGGAAGCCA	TCCCAATAAGCTTTTGCCAC
MeES1482	GAGGCTTGATGAGAGGTTGC	TATGCACACGAAACTGCTCC
MeES1483	TCTCCATGGCCTTTCAATTC	AGAATCGCAAAAGAAGGCAA
MeES1484	CTCTCCACCATGCCAAGAAT	GCTCAAGATGAGTTGGCCTC
MeES1485	TTCCTTCAGTGTGTGTGTGTGA	GCCCTGTAGAAGGACCATGA
MeES1486	TCCCATTTTTCTTTTAGGTCTGTC	TCATAACAACAACCCGCAAA
MeES1487	TCCATCAATGGCTCAATCAA	CTGAAGCATCCCCTCTCTTG
MeES1488	AAATTCCCACAAAGACACGC	CATAGCGTATCAGTTGCCGA
MeES1489	GCTCGATCTGGGAAGATTTG	ATGATGAACGTCAGTGCTGC
MeES1490	GTGAAGAGGTCTTGCCGAAG	CACAACAAAACACCTCACCG
MeES1491	AAGGAGTTCTCGTGGGTCCT	TGCCCTGTCAGTAAATACAGGAT
MeES1492	GAACGCATTGAAAGGGAAAA	AATTCAGTGACCTCGCTGCT
MeES1493	CGCACACTCCCATACAGAGA	GCGAGAAAGCAACCCAGATA
MeES1494	AAACCCAAAATTCCGAAACC	TTCTCCTTTTTCTGCTCCGA
MeES1495	CCATTTCCACTCCTTTGTCA	CAGGATCCTTTCCACTCCAA
MeES1496	GCGATCTGATATCAAAATCAAAATTA	CTCCTCGCGAATGAAATGAT

Table A2: EST-SSR sequences (cont.)

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')
MeES1497	GCATCTGTCGCAGCAAAATA	GAAGGGAGCAACTAAGCCAA
MeES1498	CGATGACGAAGAAGGTTTCC	AGGAAGAAGCAGGGGAAGAG
MeES1499	CTACGCTGTCCTATCGCCTC	GGCCTAAACTTTGCTTGTTGA
MeES1500	TTTCATGTTGTTGGATCCTTTTT	AAATAACACGCAGGAATCCG

ภาคผนวก B

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An *in vitro* detached leaf assay for pre-screening resistance to anthracnose disease in cassava (*Manihot esculenta* Crantz)

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Cassava anthracnose disease (CAD), caused by *Colletotrichum gloeosporioides* f. sp. *manihotis* is one of the most important fungal infections that affects cassava yield in many countries especially in Africa and including Thailand. In this study, a rapid screening method to identify cassava varieties that are resistant to CAD based on a detached leaf assay was developed. Three different varieties of cassava commonly grown in Thailand, Hanatee, Huay Bong 60 and Kasetsart 50 were used. Agar plugs (0.8 cm diameter) with mycelia of *C. gloeosporioides* f. sp. *manihotis* were applied to the centre of the middle lobe of the cassava leaves, while sterile potato dextrose agar plugs were used as controls. Lesions on the inoculated samples were measured on the fourth day after inoculation. The size of the lesions on each variety was compared using ANOVA and the results revealed that Huay Bong 60 showed the highest resistance to CAD whereas Hanatee was the most susceptible variety. The screening method developed in this study can be undertaken within a short time and without contamination of the pathogen into the environment. This methodology will be useful for identification of cassava varieties with resistance to CAD.

AP10024

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important crops utilised as a source of energy in the diet for people in many regions of the world (Ceballos *et al*. 2004). Moreover, cassava is also used as a raw material in many industrial applications. In Thailand, cassava is the third most important crop and generates a total farm value of 520 million USD annually (Sriroth *et al*. 2000).

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Cassava anthracnose disease (CAD) caused by the fungus *Colletotrichum gloeosporiodies* f. sp. *manihotis* (Fokunang *et al*. 2002b) has been reported to be one of the most important diseases of cassava in many countries especially in Africa (Owolade *et al.* 2005), and also in Thailand. CAD infection is characterised by cankers on stems, branches and fruits, leaf spots and tip die-back (Muimba 1982; Hahn *et al.* 1989*a*; IITA 1990). In susceptible varieties, CAD can cause wilt and death of plant shoots (Leuschner *et al.* 1982), while resistant genotypes can recover from CAD infection by sprouting new twigs from axillary buds at the lower edge of the necrosis (Hahn *et al*. 1989*a*). As the overall effects of the disease are a reduction in yield as well as the amount of healthy stems available to farmers (Owolade *et al*. 2005), resistance to CAD infection is an important target for cassava breeding programs. However, breeding programs for resistance can only be successful if there are varieties that show different levels of resistance to the disease, so that improved disease resistance can then be selected as a desirable trait. Disease resistant plants are especially useful for farmers in resource poor countries, where alternative forms of control, such as chemical control tend to be less used due to the high cost. Therefore, breeding for resistance to CAD appears to be the most efficient means of CAD control (Hahn *et al*. 1989*b*).

Previously, screening for *C. gloeosporioides* f. sp. *manihotis* susceptibility in cassava had been performed by either stem puncture and directly inoculating fungal spores into the cassava stem, or by field experimentation by planting cassava in high CAD incidence areas (Fokunang *et al*. 2002a). However, whole-plant testing is of limited application for large-scale screening because of cost and time, while field studies may be restricted only to quarantine facilities or suitable testing areas with an outbreak history. The detached leaf method has been used as a rapid screening tool in several studies investigating host defence responses to pathogens in various plants, such as wheat (Arraiano *et al*. 2001), rice (Jia *et al*. 2003), soybean (Surin *et al*. 1993), durian (Vawdrey *et al.* 2005) and alfalfa (Irwin *et al*. 2003). The detached leaf method has several advantages including increased replication, the ability to test and maintain susceptible lines (St Amand and Wehner 1995) as well as economy in labour, plant material, space and inoculum and control of environmental conditions such as humidity, light and contamination (Dhingra and Sinclair 1995). This study reports the development of an initial screening method based on the detached leaf assay for resistance to CAD infection in cassava, which will be a useful tool in pathogenic testing of cassava that is both simple and rapid.

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Materials and methods

Fungal isolation

Small pieces of cassava root that showed symptoms of anthracnose disease were collected from cassava fields. After cleaning with water and surface sterilisation for 3 min in 10% sodium hypochlorite solution followed by rinsing in sterile distilled water, the small pieces of cassava were placed on potato dextrose agar (PDA) plates containing 0.2 mg/mL Tetracycline to inhibit bacterial growth. Several fungal colonies with different characteristics such as colour and growth shape appeared after culture at room temperature for 5–7 days, and these were isolated individually and cultured further on PDA plates.

Fungal characterisation and identification

Fungal types were identified by colony characteristics and microscopic observation of spores. In addition, molecular identification of *C. gloeosporioides* f. sp. *manihotis* was performed by PCR using primers specific to fungi (ITS_F: 5′-GGAAGTAAAAGTAACAAGG-3′, ITS_R: 5′-GCTGCGTTCTTCATCGATGC-3′) (White *et al*. 1990) and to *Colletrotrichum* spp. (Col_F: 5′-AACCCTTTGTGAACATACCT-3′, Col_R: 5′- CCACTCAGAAGAAACGTCGTT-3′) (Cano *et al*. 2004). The PCR reactions were performed using DNA samples that were extracted according to the protocol of Shan *et al*. (2002) from fungal colonies of a pure culture isolated from infected cassava root and a culture from inoculated leaf samples. For ITS amplification, the primers are specific to the ITS region of rDNA of fungi, which can generate a PCR product from all fungi, although the sizes vary depending upon genus and species (White *et al*. 1990). The amplification reactions were performed in 20 µL consisting of 500 ng of DNA template, 1× buffer (15 mM $MgCl_2$, 500 mM KCl, 100 mM TRIS-HCl pH 8.3 and 0.1% gelatin), 2 mM dNTP, 1 µM each of primer, and 0.5 unit of *Taq* polymerase. PCR conditions were set at 94°C for 2 min, 20 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 30 s, and a final extension at 72°C for 10 min. The Col primers were designed from the conserved region of *C. gloeosporioides* f. sp. *manihotis* rDNA. PCR reactions were carried out in 50 µL with a final concentration of 0.1–10 ng of DNA template, 1× buffer containing 100 mM TRIS-HCl (pH 8.0 at 25°C), 500 mM KCl, 0.8% Nonidet P40, 15 mM $MgCl_2$ (Fermentas, Life Science, MD, USA), 2 mM dNTP, 1 µM each of primer, and 1.5 unit of *Taq* polymerase. PCR was performed at 94°C for 5 min, 20 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s with a final extension of 72°C for 7 min (Cano *et al*. 2004). The PCR products of each primer pair were analysed on 1.5% agarose gels by electrophoresis. The fungal samples identified as *C. gloeosporioides* f. sp. *manihotis* were maintained on PDA plates and used for plant inoculation.

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Plant inoculation

The detached leaf method was performed as described by Poopaibool *et al.* (2003) to screen for resistance to *C. gloeosporioides* f. sp. *manihotis* infection using three Thai cassava varieties, Hanatee (HT), Huay Bong 60 (HB60) and Kasetsart 50 (KU50). Healthy mature leaves of each variety were collected from cassava trees and surface sterility obtained by consecutive rinses in 70% ethanol and sterile distilled water. Agar plugs cut with a 0.8-cm-diameter cork-borer from the edge of a *C. gloeosporioides* f. sp. *manihotis* colony cultured on PDA were placed face down in the centre of the middle lobe of a cassava leaf that had been freshly wounded. Leaves were wounded by cutting the middle vein of the leaf with the cork-borer. The inoculated leaves were placed in a closed plastic bag with wet cottonwool to prevent evaporation and then incubated at room temperature. Each experiment was undertaken independently in triplicate and one experiment consisted of seven leaves of each of the three cassava varieties. One leaf in each experiment was used as a physical control, one leaf was used as a negative control and a further five leaves were subjected to inoculation. A physical control was set up using a cassava leaf without either PDA agar or fungal plugs in order to observe any changes in the leaf samples. A cassava leaf inoculated with a PDA agar plug was used as negative control in order to observe effects of the agar on the inoculated area.

Data analysis of infection size

The inoculated and control leaf samples were observed daily until the fourth day after inoculation for the development of lesions. Lesion length was measured and recorded. The lesion size was determined by measuring the longest length (A) and width (B) of the lesion and size calculated using the formula: (A + B)/2. One-way ANOVA was used to analyse the statistical differences between lesion sizes and a *P*-value of <0.05 was considered significant.

Results and discussion

Cassava root samples from plants showing symptoms of anthracnose disease yielded several different types of fungal colonies upon culture. These colonies were collected and cultured on PDA plates for morphological identification. A colony of *C. gloeosporioides* f. sp. *manihotis* on a PDA plate had white cotton-like mycelia with a greyish white to dark-grey colour (Fig. 1*a*). In addition, spores with rounded ends and hyphal morphology characteristic of *C. gloeosporioides* f. sp. *manihotis* were observed under the light microscope (Fig. 1*b*). The *C. gloeosporioides* f. sp. *manihotis* culture was maintained on PDA agar and used for a detached leaf assay. Molecular identification using DNA samples from mycelia of a pure culture, (which had been isolated directly from a cassava root with CAD symptoms), and the culture from a leaf lesion in the experimental cohort was performed along with a negative PCR control using sterilised

distilled water and DNA samples of *Curvularia* sp. and *Fusarium* sp. The ITS primer was initially used to identify different fungal types when similar fungal colonies were observed. The results showed that PCR products were obtained from the reactions using DNA samples of all fungal types and ITS primers (Fig. 2*a*). However, only two samples, *C. gloeosporioides* f. sp. *manihotis* from pure culture, and the culture isolated from a leaf lesion showed PCR products of ~150 bp (Fig. 2*b*) when analysed with the Col primer. The results indicated that the fungal type used in this assay that caused lesions on leaf samples was *C. gloeosporioides* f. sp. *manihotis*.

Differences in lesion size between the three cassava varieties, HT, HB60 and KU50 infected with *C. gloeosporioides* f. sp. *manihotis* in the detached leaf assay could be clearly observed (Fig. 3). The largest lesion (2.14 ± 0.56 cm) was found on HT leaf whereas the smallest (1.39 ± 0.44 cm) was found on HB60 leaf. Lesion area was calculated and a histogram of the results was generated (Fig. 4). Lesion sizes were significantly different when comparing HT with HB60 and HT with KU50 with *P*-values of 0.002 and 0.016, respectively. However, there was no statistically significant difference between HB60 and KU50 ($P = 0.411$). From the results of this study, it can be concluded that HB60 is the most resistant while HT is the most susceptible cassava variety to *C. gloeosporioides* f. sp. *manihotis* infection.

CAD caused by *C. gloeosporioides* f. sp. *manihotis* is one of the major economic diseases of cassava. Identification and development of new cassava varieties with resistance to the disease is the most effective alternative for disease control. Therefore, it is necessary to develop a simple and rapid method of screening for CAD resistance. This study developed a rapid and simple technique for screening of cassava resistant to CAD based on a detached leaf assay. This technique will be useful for large-scale screening, especially in cassava breeding programs.

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215 **Fig. 1.** (*a*) A colony of *Colletotrichum gloeosporioides* f. sp. *manihotis* isolated from cassava root with
216 Cassava anthracnose disease infection on potato dextrose agar; (*b*) spore and hyphal morphology of *C.*
217 *gloeosporioides* f. sp. *manihotis* staining with lactophenol cotton blue observed under the light
218 microscopes (40×).

219 **Fig. 2.** Molecular identification of *Colletotrichum gloeosporioides* f. sp. *manihotis* using internal
220 transcribed spacer (*a*) and Col (*b*) primers. (M: 100 bp + 1.5-kb marker; DS: sterilised distilled water; 1:
221 *C. gloeosporioides* f. sp. *manihotis* from pure culture; 2: *Curvularia* sp.; 3: *Fusarium* sp.; 4: *C.*
222 *gloeosporioides* f. sp. *manihotis* from inoculated leaf sample).

223 **Fig. 3.** Representative lesion areas caused by *Colletotrichum gloeosporioides* f. sp. *manihotis* on leaves
224 of three cassava varieties, Hanatee, Hauy Bong 60 and Kasetsart 50.

225 **Fig. 4.** Average lesion size caused by *Colletotrichum gloeosporioides* f. sp. *manihotis* on leaves of three
226 cassava varieties, Hanatee (HT), Hauy Bong 60 (HB60) and Kasetsart 50 (KU50). Bars indicate ± SD.
227 The results showed a statistically significant difference between HT and HB60 and between HT and
228 KU50 with *P*-values of 0.002 and 0.016, respectively.

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Construction of a genetic linkage map using simple sequence repeat markers from expressed sequence tags for cassava (*Manihot esculenta* Crantz)

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Abstract Cassava (*Manihot esculenta*) is an economically important crop that is grown in tropical and sub-tropical regions. Use of molecular technology for genetic improvement of cassava has been limited by the lack of a large set of DNA markers and a genetic map. Therefore, the aims here were to develop additional simple sequence repeat (SSR) markers from the public expressed sequence tags (ESTs), and to construct a genetic linkage map. In this study, we designed 425 EST-SSR markers from sequences obtained from the cassava EST database in GenBank, and integrated them with 667 SSR markers from a microsatellite-enriched genomic sequence received from the International Center for Tropical Agriculture (CIAT). Of these, 107 EST-SSR and 500 genomic SSR primer pairs showed polymorphic patterns when screened in two cassava varieties, Hauy Bong 60 and Hanatee, which were used as female and male parental lines, respectively. Within the 107 and 500 primer pairs, 81 and 226 EST-SSR and SSR primer pairs were successfully genotyped with 100 samples of F_1 progeny, respectively. The results showed 20 linkage groups consisting of 211 markers—56 EST-SSR and 155 SSR markers—spanning 1,178 cM, with an average distance between markers of 5.6 cM and about 11 markers per linkage group. These novel EST-SSR markers provided genic PCR-based co-dominant markers that were useful, reliable and economical. The EST-SSRs were used together with SSR markers to construct the cassava genetic linkage map which will be useful for the identification of quantitative trait loci controlling the traits of interest in cassava breeding programs.

Keywords Cassava · EST · EST-SSR · Genetic linkage map

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Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important food crops and is a source of calories for more than 500 million people in tropical and subtropical Africa, Asia and Latin America (El-Sharkawy 2004). Cassava can be used in various industrial processes including the production of starch and starch-derived products, alcohol and high fructose–glucose syrups. Due to increasing demands on both quality and quantity traits, it is necessary to continue genetic improvement of cassava with higher values.

Genetic markers are effective molecular tools for genomic study and development of new varieties in plant and animal breeding programs (Collard et al. 2005). One class of genetic markers known as expressed sequence tag (EST) markers have been useful for unraveling the complexities of eukaryotic organisms because they are directly linked to genic regions of the genome (Akagi et al. 1997). ESTs are short (300–800 bp) single read sequences randomly selected from cDNA clones or normalized libraries of clones (Akagi et al. 1997). EST approach has developed into an inexpensive and efficient method for gene discovery and marker development (Ohlrogge and Benning 2000). Once generated, ESTs are useful in cloning the encoding genes and for mapping the functional gene families in various related organisms (Akkaya et al. 1992). EST collections are often a prelude to genome sequencing. Genetic mapping programs based on ESTs identify the locations of active genes and identify diagnostic genic markers (Adams et al. 1991; Sasaki 1995).

For cassava and other members of the Euphorbiaceae family, several efforts have focused on generating EST sequence databases which were useful transcriptomic resources for the studies of genetic diversity and stress responses as well as growth and development (Akkaya et al. 1992; Lokko et al. 2007; Lopez et al. 2004; Anderson et al. 2004). Since ESTs represent coding regions of the genome, direct associations can be made between genotype and phenotype leading to identification of quantitative trait loci (QTL) underlying the traits of interest (Rudd 2003). Lopez et al. (2007) mapped 21 EST-SSR markers from sequences identified as genes putatively involved in plant disease resistance together with RFLP, CAPS, and SNP markers in order to identify the QTL underlying responses to bacterial blight disease resistance (Lopez et al. 2007). In addition, EST markers were useful in phylogenetic evolutionary studies due to their transferability across taxa.

A set of 49 EST primer pairs was developed using cassava EST sequences and deposited in published databases (Tangphatsornruang et al. 2008). These primers were also tested for cross-amplification with four different *Manihot* species. By 2009, the number of genetic markers of cassava was limited for applications such as the construction of a higher density genetic linkage map, QTL identification and the discovery of DNA markers tightly linked to the genes underlying traits of interest. Therefore, we report here the development of additional molecular markers from ESTs.

Materials and methods

Plant materials

Two cultivars of cassava in Thailand were used as parental lines to generate progeny populations. Huay Bong 60 (HB60) is a cassava cultivar widely used for industrial applications because of its high root yield, starch content, and cyanide content. The Hanatee (HT) variety has lower root starch and cyanide content. A total of 100 F_1 progenies from a cross of HB60 (female) and HT (male) was used as the mapping population. All the cassava samples were planted at Rayong Field Crop Research Center, Rayong Province, Thailand. Cassava leaves of parents and progenies were collected from the field and used for DNA extractions based on Sambrook and Russell (Sambrook and Russell 2001). Briefly, the young cassava leaves were powdered under liquid nitrogen and thawed into 600 µl of extraction buffer (2% (w/v) cetyltrimethylammonium bromide, 1.4 M NaCl, 1% (w/v) polyvinylpolypyrolidone, 20 mM EDTA, 100 mM TrisHCl, 0.1% (w/v) sodium metabiosulfite and freshly added 2% (v/v) 2-mercaptoethanol). The slurry was incubated at 65°C after 20 µl of 20% SDS were added for 20–30 min and occasionally mixed. One volume of chloroform:isoamyl alcohol (24:1) was added after incubation of the samples at room temperature (RT) for 1 min and mixed. After centrifugation at 10,000*g* for 10 min, the upper phase was transferred to new tubes. The DNA was precipitated with one volume of isopropanol, followed by centrifugation at 10,000*g* for 1 min. The

DNA pellet was washed with 70% (v/v) ethanol and centrifuged at 10,000*g* for 1 min, the supernatant was removed and the pellet dried at RT. The DNA pellet was suspended with sterilized distilled water or TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA).

Cassava EST-SSR analysis and primer design

EST sequences were obtained from the EST database at the NCBI (http://www.ncbi.nlm.nih.gov/dbest/). A total of 76,566 ESTs were obtained for this study (10 May 2008). All EST sequences were processed by trimming off the polyA/T and vector sequences, clustered and assembled using the TGICL software (http://www.tigr.org/tdb/tgi/software/) to remove redundant sequences. The unique sequences were used for searches to find SSR sequences using the Troll software (http://wsmartins.net/webtroll/troll.html, now http://wsmartins.net/websat/; Martins et al. 2009). The software was set to detect repeat motifs with more than four repeats (a mixture of type 1 and type 2 microsatellites; (Shultz et al. 2007). Both simple and complex microsatellite motifs under 8 bp were detected. Insertion or deletions of greater than 8 bp were also detected. EST primers were designed from conserved sequences flanking repeats where the main parameters were: GC content of 40–60; annealing temperature (T_m) of 53–57°C; and expected amplified products size of 100–300 bp. In some cases, candidate EST markers were prioritized based on presence of motif sizes or known polymorphisms in the EST database.

All the EST-SSR and SSR primer pairs were screened with the parental samples in order to identify the potentially polymorphic markers, used to genotype with 100 F_1 progenies. The PCR reactions for EST-SSR analysis were performed according to Tangphatsornruang et al. (2008). Briefly, the amplification reactions in 20 µl consisted of 50 ng of DNA template, 10 pmol of each forward and reverse primers, 200 µM dNTP, 1× PCR Buffer, 1.5 mM $MgCl_2$, and 1.5 U Taq DNA polymerase. The PCR conditions consisted of 1 cycle of 94°C for 1 min, followed by 30 cycles of 94°C for 45 s, annealing temperature for 1 min and 72°C for 1 min, and 1 cycle of 72°C for 5 min. The PCR reactions of SSR analysis were performed as described in Kunkeaw et al. (2010) in 15 µl reaction volumes containing 25 ng of genomic DNA, 0.06 µM of each primer, 0.2 mM dNTPs (Promega), 1× PCR Buffer, 1.5 mM $MgCl_2$, and 1 U Taq DNA polymerase (Promega). PCR was accomplished by 2 min at 94°C, followed by 30 cycles of 45 s at primer annealing temperature, and 1 min at 72°C for 30 cycles. The PCR products were analyzed by 5% (w/v) denaturing polyacrylamide gel electrophoresis and visualized by silver staining (Benbouza et al. 2006). Sequences of EST primers used in this study are shown in Table 1.

Data analysis

Data analysis was performed as described in Kunkeaw et al. (2010). The genotypic data was scored and collected manually according to Van Ooijen and Voorrips (2001). The linkage analysis was performed using the JoinMap®3.0 program (Akkaya et al. 1992). A logarithm of odd ratio (LOD) score was established for linkage and Kosambi's mapping function was used to determine map distance in centimorgans (cM) based on recombination frequencies of 40.

Results and discussion

EST-SSR identification and analysis

Out of 76,566 sequences with an average read length of 504 bp obtained from the GenBank EST database, the number of unique sequences was reduced to 28,940. Those sequences had an average read length of 609 bp, formed 10,902 tentative contigs and 18,038 singletons. The tentative contigs had an average read length of 790 bp whereas the singlets had an average read length of 500 bp. The Troll software identified 7,270 SSRs which represent an average frequency of EST-SSR of one SSR in every 2,424 bp in the cassava transcriptome. The frequency of EST-SSR found in cassava was similar to that found in *Hevea brasiliensis* (2.24 kb) (Altschul et al. 1990). Both cassava and rubber tree are closely related in the same Euphorbiaceae family where a large proportion of EST markers were cross-transferable (Tangphatsornruang et al. 2008). This frequency of EST-SSR was higher than that of wheat (1/15.6 kb) (Kantety et al. 2002), barley (1/6.3 kb) (Thiel et al. 2003), *Arabidopsis thaliana* (1/13.83 kb), tomato (1/11.1 kb), cotton (1/20.0 kb), soybean (1/7.4 kb), and poplar (1/14.0 kb) (Cardle

Table 1 The ESTs and homologous genes with primer sequences providing DNA markers, showing allele size and repeat motifs

Primer name	Sequence	Accession no.	Range size (bp)	Motif
EME11	F:CTAGGTTGCACGCACAG	gb|CK652737.1	250	TA6
	R:CCAGAATGGTAAAACTCATG			
EME20	F:CAGCACCAGTCAACATTCCTG	gb|CK649260.1	240	ATG9
	R:CCTTCTGGCAATGAGCTCATG			
EME22	F:CCAGGCTCCGTTGCAGG	gb|CK649470.1	190	In/Del
	R:AGGATATGCAGCTTGTCCCT			
EME46	F:CCGAAGACCAGTAAACGCAT	gb|DB935307.1	250	In/Del
	R:AGAGTTGCACCTCTCCGTC			
EME54	F:GTCGATGGCAAGGACCCTAA	gb|DV444255.1	190	In/Del
	R:GAAACATCCTTAAATATCCAAAAACC			
EME59	F:GGAGTGAGTGAGTGAGAGA	gb|DV449262.1	300	In/Del
	R:CACTGCTCCCAATCCCATTCC			
EME62	F:GAGGCAACATGCGTGATCC	gb|BM259702.1	250	In/Del
	R:TTACACCCAAAAGACTAGATGTTA			
EME81	F:GTGATGGAGACAGCTGAGG	gb|DV445454.1	500	In/Del
	R:CACATAATGCCCAAAACCTAACC			
EME87	F:GCTAGGGTGCTGGATTGG	gb|DV443734.1	180	In/Del
	R:CAGAGAGCAATTGTGACACTC			
EME113	F:CATGGGAGCACCACAACG	gb|DV450153.1	130	In/Del
	R:ACAGACAGCCATACAATGAG			
EME118	F:GCAGCATGGACATGGACC	gb|CK642382.1	250	In/Del
	R:GCCAAGTTAAGACCAGCAAAGC			
EME162	F:GGTCCTAACTGCACATGCTT	gb|DB948567.1	250	AGC7
	R:GGAAAAGAATCAAGGCCAGAG			
EME164	F:GAGCAACTAAGGAAGCCACA	gb|DB943756.1	180	GAT7
	R:AACTAGCGGCACTCAAATCAAT			
EME170	F:GTAGAGGTTGTGCAAGGTGA	gb|DV441144.1	1,000	TGA5
	R:TCCTTCTATGATTTCTGTTTCC			
EME171	F:CCAAGGAAGATGTGAAGGTG	gb|DB950271.1	160–190	GAA6
	R:GGCAACGCAATTCTACTGCT			
EME177	F:ATACAGAGGCATCCTTCCC	gb|DV441807.1	180	CAA4
	R:GCAGTCGTCATTGTTGTGTC			
EME189	F:CAGAGCACATCCAGAAATTGTT	gb|DB943802.1	170–200	CATGGT5
	R:GAAATAGATCAAGTGCCCCATC			
EME195	F:GAAACTCCAGCACCAACAGA	gb|DB924415.1	160–200	AAC8
	R:ACACGGCCTCTTCTTCTATC			
EME203	F:GGAACTCGTTCCGGAAGAAG	gb|DB925707.1	210	GAA12
	R:TTAACTGCTCTTTACTGAACGG			
EME205	F:CCAGAGCGTATAACTGGAAC	gb|DB941881.1	250	GAT4 + TAA6
	R:TGCAGGAGTGTGGATATGGTT			
EME212	F:GATATGGCTGCTCTTTTCATGG	gb|CK646517.1	130–210	AAT7
	R:CCCTTCAATCTCCTCTTCAC			
EME222	F:CCCACTCTCTGTCCACTTC	gb|DB923273.1	190	GAT6
	R:CTTCGACTCTTCTTTACGGG			

Table 1 continued

Primer name	Sequence	Accession no.	Range size (bp)	Motif
EME240	F:CCTCCTCCTAAAACCCTTCA	gb|DV455573.1	180	CAGCAC4
	R:GGTGCTAACTGCTTCTTTTGC			
EME246	F:GTGCTCTTCTGCGGAATATC	gb|DV445119.1	500	AT14
	R:ACCCTTCCCTACCGAACACT			
EME254	F:CAGACAGGGAGATGCTGCT	gb|DB926223.1	250	TTC12
	R:GCGATAGAAACTTGAGGAGC			
EME259	F:GCCTTATGGGGTTTGTGCTT	gb|DR083758.1	160	CTT6
	R:CAAGTTTAAGTGAAGGCAGAG			
EME260	F:GTTGGAGTTGTAGTTGCTGC	gb|DV442874.1	160	AT14
	R:CATGGGCTGTGAAAATGAACT			
EME280	F:AGGGGCTTTTGTCTACTGAGG	gb|DV451367.1	200	TTTTG5
	R:CTTAGTTCTCACTGTCCTTCG			
EME303	F:ATTGGGAAGCATTGGTGTAGAA	gb|DB949763.1	180	CT11
	R:CACAAACAAAACCCTGTGACCT			
EME309	F:GTAGTGATATTGGTGATCCCG	gb|CK643188.1	120–180	TGA9
	R:AACTGCACATCCGTTGACAC			
EME313	F:AGCAGGGATCTTCTGGTCAG	gb|DB945066.1	180	AAT5
	R:CGCATCATTCATACTCTTCATTC			
EME319	F:CGGGTCGCAGCTTCAATAAG	gb|DB927387.1	370	TG5 + TA5 + GA5
	R:TCTGGGTTGCTCTCATCTTG			
EME321	F:GCCTCATTTCTGGGTTGTTATT	gb|DB931037.1	150	TTC10
	R:TCTGGGTGAATCTCTCTTTGGT			
EME331	F:GAAGAGCATCAGGGCAAATC	gb|DB930842.1	160	GCA5
	R:GATTGTAGGGATTGACGGCT			
EME332	F:CGTGGCTACACTCTTCTCCAC	gb|DB931839.1	210	GA7
	R:GAGACGGCTAGGGTTGGATAC			
EME341	F:TGAGGTGTGTGGAACTTGTAAA	gb|DV452842.1	200	TTA6
	R:ATCTGGTCCTTAACAGCAATCA			
EME345	F:CTGTGGCTACTCCGTTCAGTAA	gb|CK646638.1	190	CCT6
	R:AGTCACCCCATTGTTCTTTGAC			
EME353	F:GATACTCCCCAAAACCAACAAG	gb|DV450189.1	170	TGGTGA4
	R:ACCTGCCTGAAACTCTTGCTAA			
EME373	F:GGTTGGAGAGTTGAGCTTTC	gb|DV455407.1	250	TGG7
	R:GTAGCTCCTTCGACCATAGC			
EME395	F:TCAAAGGTATCGGGGAGGTAG	gb|DV456045.1	200	TATGGC6
	R:GTTTACCCCACTAACATCGCAT			
EME409	F:AGCAGCAATTAAGGCTCTAGGA	gb|DB950509.1	210	TGG/A9
	R:ACCCTCAAAACCCTTGAAAGA			
EME412	F:GCATTACGAACACATACAGTG	gb|DB935045.1	300	AG12
	R:GATAAATCAACCGTCACCTCC			
EME424	F:TTAAAGACGAATCAAGGGAGGA	gb|DV448798.1	180–220	CAC5 + GAGCA4
	R:CTCTTGGCACTCAAAAGTGGTT			
EME425	F:AAATTGGACAGGAGAGGTTGG	gb|DV447765.1	110–150	TC7 + TGGAAT3
	R:ACGGAGGAGAGTTGGATTTACA			

Table 1 continued

Primer name	Sequence	Accession no.	Range size (bp)	Motif
EME445	F:CCTCCACAACCTTATCAATCA	gb|DV443092.1	300	TCT4
	R:CGGTAGCCATAGCCATAACA			
EME489	F:CAATTGCTACAATGAAGAACG	gb|DV448108.1	200	TTTC4 + AT5
	R:CATCGCGTGATTTGGTACAA			
EME512	F:CTCCCAATATCCACTCATCCAT	gb|DB922782.1	100–150	CTC5 + CGC7
	R:AATAGCTTGTGCCATCTGTGAA			
EME517	F:GTGAACGAGAATGAGGACACAA	gb|DR083949.1	100	GCA11
	R:GAGCCGCAGAAGAGTTATCAAT			
EME628	F:GTTGAGAGTTTTATCTGGTCC	gb|DV452468.1	100	TG10
	R:AACCAAGTGCCCAAGCAT			
EME635	F:CTCATAAGCAGCAAGACAATGC	gb|DB943826.1	180	AT9
	R:GACATAAACTCCATAGCCTGCC			
EME637	F:ATCTATCGCCTCCTGAAACCTT	gb|DB934835.1	200	AG14
	R:CAAACCAAACTCTCATATCGCC			
EME657	F:CTTGGTTGGCAGAGAGAACT	gb|DB930374.1	140	AAGAGG5
	R:CGACCCTTTTGACTTCTTGAA			
EME708	F:AAGACACAAGAAGGGCAATG	gb|DV445735.1	600	(TC)3(AT)3
	R:GATCACACTGAACTAAGGCA			
EME710	F:TGTGGAACGGGTCTGTGG	gb|DV451393.1	300	In/Del
	R:AGGCACAAACATTGAAGCAG			
EME713	F:TTTTTGTGAAGACCTTGACTG	gb|DV453603.1	200	In/Del
	R:GGTGAAGGGTTGATTCCTTC			

et al. 2000). However, it was lower than coffee (1/1.56 kb) (Aggarwal et al. 2007).

Of these SSRs, 2,596 (34.8%) were dinucleotide repeats or DNPs (Fig. 1). The TC/AG repeat was present at the highest proportion of ~72%, followed by TA/AT (20%) and AC/TG (7%), but GC/CG was very rare (0.15%). The GC/CG motif repeat is rare in most plant's ESTs, as previously reported in rice, corn, soybean (Gao et al. 2003), wheat (Nicot et al. 2004), *Arabidopsis thaliana*, apricot, peach (Jung et al. 2005), coffee (Aggarwal et al. 2007), and rubber tree (Feng et al. 2009). Trinucleotide repeats (TNPs) were found at 2,673 SSR loci (36.76%), which was similar to that of DNPs. The TTC (or AAG) repeat was found at 905 loci (33.86%), followed by AAT (9.95%) and GAG (9.84%). The common presence of the TTC motif repeat was also observed in *Arabidopsis thaliana* (Cardle et al. 2000), soybean (Gao et al. 2003), strawberrey (Folta et al. 2005), citrus (Chen et al. 2006), and rubber tree (Feng et al. 2009). Tetra- and pentanucleotide repeat motifs were found at 1,174 and 386 SSR loci, respectively. Repeat motifs with more than hexanucleotide repeats were found at 441 SSR loci.

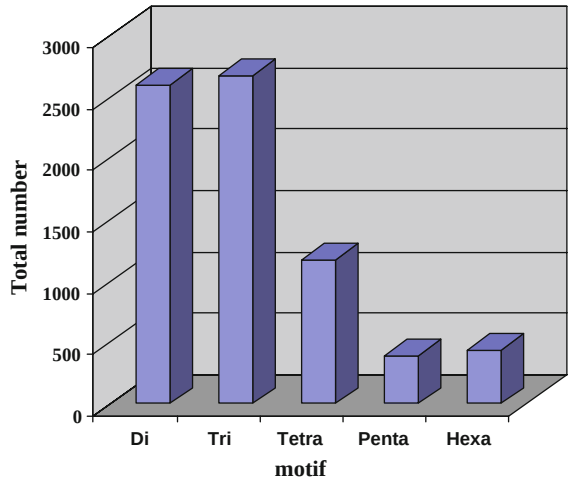

Fig. 1 Distribution of repeat motif size classes in the EST database of cassava *(Manihot esculenta* Crantz) using the Troll software

Construction of a genetic linkage map

A total of 425 EST-SSR and 667 genomic-SSR primer pairs were initially analyzed with the parental samples. The results of EST-SSR analysis showed 346 (81%) pairs were successfully amplified while 79 (19%) pairs gave no amplified PCR product. A total of 107 of the 346 amplifiable EST-SSR primer pairs showed polymorphic patterns between parental samples consisting of 81 and 26 informative and non-Mendelian markers, respectively. For SSR analysis, all 667 primer pairs were successfully amplified and 500 primer pairs showed polymorphic patterns between parental samples consisting of 226 and 274 informative and non-informative markers, respectively. The numbers of informative polymorphic EST-SSR (19%) markers were much lower than the informative genomic-SSR markers (33.88%). This may be because ESTs are generated from expressed regions which are more conserved than non-coding regions of the genome (Dreisigacker et al. 2004; Varshney et al. 2005). Mba et al. (2001) reported that a higher proportion of genomic SSR loci (66%) showed unique alleles in at least one of the parental lines (TMS 30572 and CM2177-2) (Mba et al. 2001).

All 81 EST-SSR and 226 SSR informative polymorphic markers were genotyped with 100 individuals of the F_1 progeny. Genotypic data was scored and recorded based on the study population model, which was a CP model as described by the JoinMap® 3.0 program (Altschul et al. 1990). This model is specific to a population created from a cross between two heterogeneous (heterozygous and homozygous) diploid parents (Altschul et al. 1990) that can divide the type of markers between homozygous and heterozygous alleles. The results showed that 56 EST-SSR and 155 SSR markers were mapped on 20 linkage groups spanning 1,177.57 cM with an average between markers of 5.6 cM and about 11 markers per linkage group (Fig. 2). The previously constructed genetic linkage map of cassava using 122 genomic-SSR markers consisted of 22 linkage groups

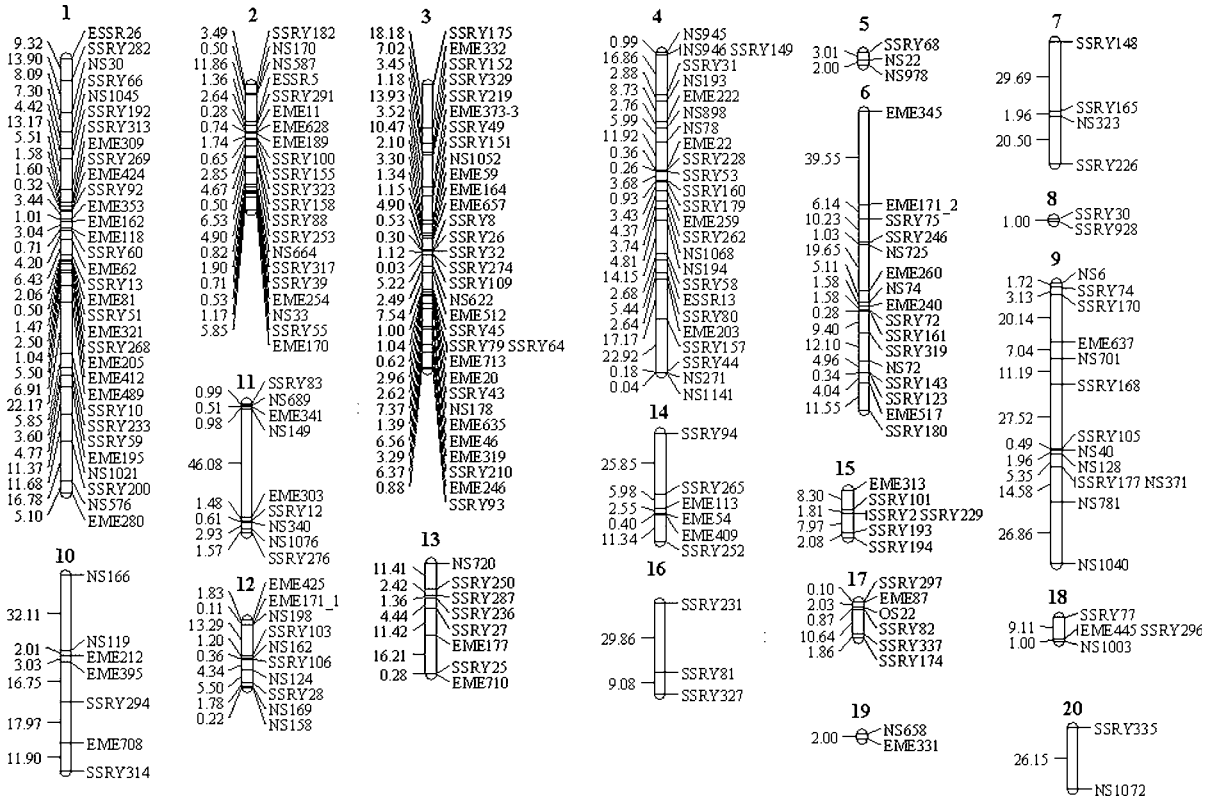

Fig. 2 An EST-SSR-based genetic linkage map of cassava derived from a cross between Huay Bong 60 and Hanatee cultivars. Map distances shown on the *left* are indicated in Kosambi map units (cM)

with an average marker distance of 18 cM (Okogbenin et al. 2006). Clearly, still more molecular markers will be needed to join unlinked markers and small linkage groups into a higher density map. Since ESTs are products from transcript abundances, EST markers that are associated with the traits might identify gene(s) or loci underlying traits. Therefore, the EST-based linkage map may be useful for the identification of both genes and QTL that can be further applied to marker-assisted selection of cassava.

Conclusion

Our study shows the utility of cassava EST-SSR for the construction of a genetic linkage map, with a remarkably high level of polymorphism within this species. It highlights a reliable and efficient method of obtaining microsatellite markers from the conserved genic regions of the cassava genome. The availability of additional sets of mapped EST-derived SSR markers for cassava will assist the development of molecular maps for *M. esculenta*, its integration into the genomic network and QTL mapping of agronomical traits.

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Short Communication

Genetic linkage map of cassava (*Manihot esculenta* Crantz) based on AFLP and SSR markers

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With 1 figure

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Abstract

To generate a genetic linkage map of cassava (*Manihot esculenta* Crantz), 58 F_1 progenies from a cross between Rayong 90 (female) and Rayong 5 (male) were examined in amplification fragment length polymorphism (AFLP) and simple sequence repeat (SSR) analyses. A total of 469 polymorphic markers consisting of 378 AFLPs generated from 76 primer combinations and 91 SSRs were identified. These markers were analyzed using the JOINMAP® 3.0 package program to construct a genetic linkage map. A total of 33 linkage groups of a common map were constructed from 119 AFLPs and 18 SSRs, spanning 1095 cM with an average of 7.99 cM between markers. The genetic linkage map generated in this study will be useful for genetic studies in cassava particularly for the identification of genetic markers linked to traits of interest, although the complex cassava genome suggests that maybe a long term objective.

Key words: AFLP — cassava — linkage map — SSR

Cassava (*Manihot esculenta* Crantz) is the sixth most important world food crop and is a source of calories for more than 500 million people in tropical and sub-tropical Africa, Asia and Latin America (EL-Sharkawy 2004). Cassava can be used in various industrial processes including the production of starch and starch-derived products, alcohol and high fructose–glucose syrups. In Thailand, cassava is the third most important crop after rice and sugarcane and is recognized as one of the most important crops to the Thai economy (Sriroth et al. 2000). Because of relatively few problems with pests and diseases of cassava in Thailand, the objectives of cassava breeding in Thailand mainly focus on the production of varieties with high root starch content and high yield.

Previous studies have used molecular markers as a developmental tool in cassava breeding programmes. The first genetic linkage map of cassava was constructed using RFLP markers on F_1 population of a full-sib intra-specific cross (Fregene et al. 1997). The map has so far provided an initial tool for the genetic analysis of important traits of cassava (Akano et al. 2002, Okogbenin and Fregene 2002, 2003). Moreover, the map was divided into male and female maps. A common genetic linkage map of cassava based on simple sequence repeat (SSRs) was then constructed by Okogbenin et al. (2006) using an F_2 population.

Because of the high heterozygosity in nature of the crop, long growing cycle and low seed yield per pollination that results in limits for the production of adequately sized F_2 populations and because of the complication arising from separate analyses of gametes segregating from male and female parents map development is complicated. This project aimed to use an F_1 population to develop a common genetic linkage map of cassava based on SSR markers that were characterized and identified by Mba et al. (2001) and AFLP markers using the JOINMAP® 3.0 program (Van Ooijen and Voorrips 2001).

Materials and Methods

Plant materials: A total of 58 F_1 progenies were developed from a cross between 'Rayong 5' (male) and 'Rayong 90' (female). All individual F_1 plants as well as the parents were planted at Khon-Kaen Filed Crop Research Center, Department of Agriculture, Thailand. Fresh leaf tissue of the parental lines and the F_1 samples were collected and used for DNA isolation by CTAB method (Sambrook and Russell 2001). DNA samples were kept at −20°C until used for AFLP and SSR analysis.

AFLP and SSR analysis: Amplification fragment length polymorphism analysis was performed according to the methodology developed by Vos et al. (1995). For SSR analysis, primer sequences used in this study and the reactions were preformed as described by Mba et al. (2001). The PCR products were analyzed by electrophoresis through 5% (w/v) denaturing polyacrylamide gels and the bands were visualized by silver staining (Sambrook and Russell 2001).

Genotyping and linkage analysis: All AFLP primer combinations and SSR primers were screened against the parental samples to identify polymorphic markers. The primers which showed informative results were then genotyped in the mapping population. The genotypic data was scored according to CP population type code as described in JOINMAP® 3.0 Manual (Van Ooijen and Voorrips 2001). The map was constructed using the computer package. The thresholds for declaring linkage was set at a LOD score of 3.0 and a recombination frequency 40 cM applying the Kosambi function.

Results and Discussion

A total of 58 F_1 progenies of a cross between Rayong 5 (male) and Rayong 90 (female) as well as the parental lines were screened with a total of 76 informative AFLP primer combinations and a total of 378 polymorphic AFLP markers were identified. In addition, 170 SSR primer pairs obtained from

Mba et al. (2001) were also analyzed with the parental samples and the results revealed that 91 primer pairs showed informative polymorphic patterns. These SSR primers were then genotyped with the F_1 progenies. The genotypic data from these two techniques were then integrated and used as target markers for construction of a genetic linkage map using the JOINMAP® 3.0 package program. A total of 469 markers consisting of 378 AFLP and 91 SSR markers were integrated to construct the common map. Of these, 295 AFLPs and 61 SSRs showed 1 : 1 segregation ratio and were subjected to

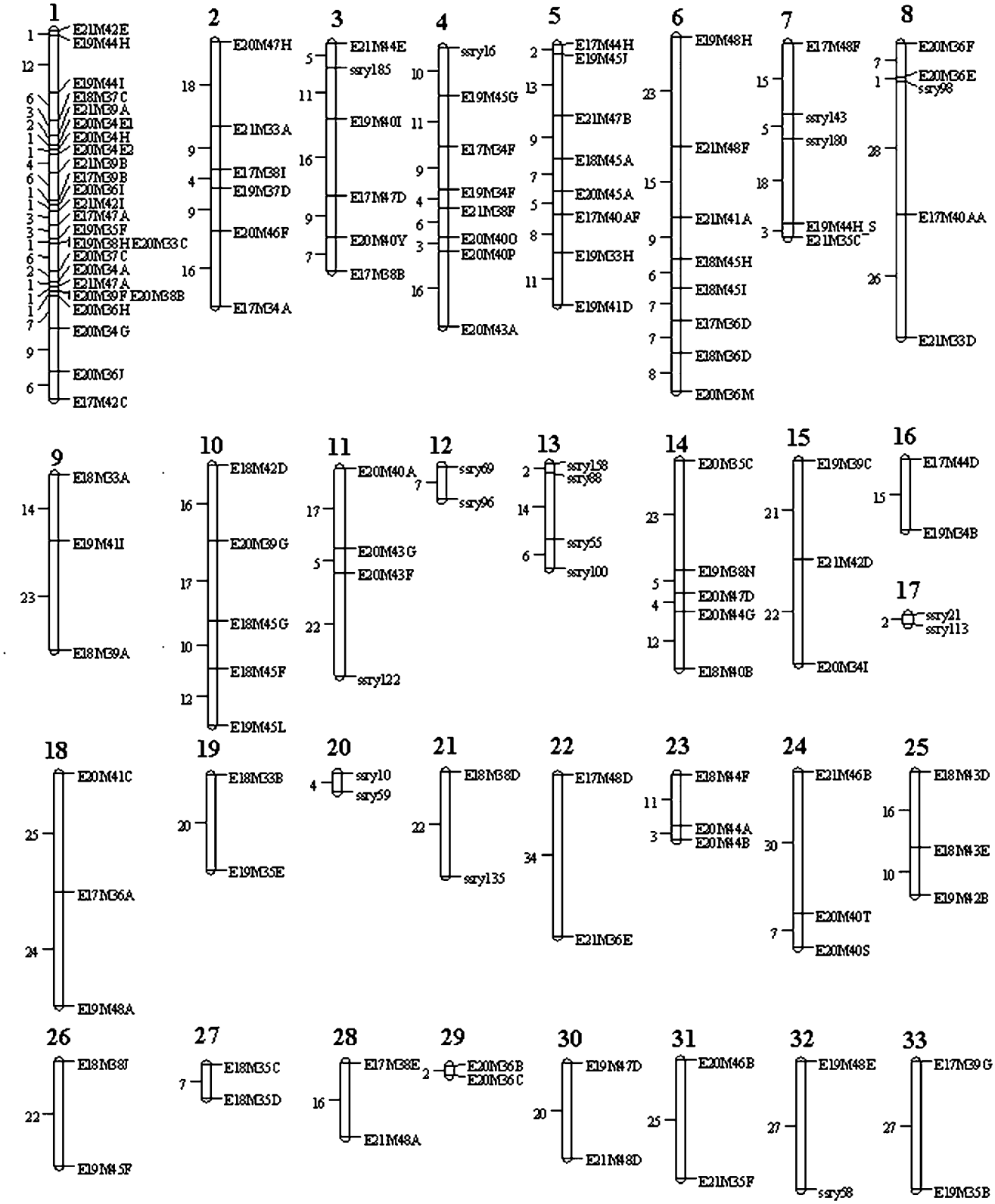

Fig. 1: The common map of Cassava derived from the cross of Rayong 90 and Rayong 5. Map distances that show on the left are indicated in Kosambi map unit (cM)

genetic linkage map construction. The result showed that 137 markers consisting of 119 AFLP and 18 SSR markers mapped into 33 genetic linkage groups spanning 1095 cM with an average of 7.99 cM between markers (Fig. 1), while others remained unlinked.

Cassava is an allopolyploid (2n = 36) in which the genome number is higher than the two sets of chromosome. Jos and Nair (1979) and De Carvalho and Guerra (2002) conducted the studies of cassava meiotic behaviour and observed 18 regular bivalent formations of the chromosomes. In this study, the number of linkage groups exceeds of the number of cassava's gamete chromosome and the low number of markers in some linkage group indicates that the map constructed in this project has an incomplete coverage of the genome.

The SSR markers resulting from in study can be compared with a previous study based upon the F_2 map of Okogbenin et al. (2006). The F_2 map consisted of 22 genetic linkage groups while 33 genetic linkage groups were generated in this study. Comparisons of linked markers of the common maps show that markers that are found to be linked markers in both maps are SSRY96 and SSRY69 on linkage group 5 of the F_2 map and are located in linkage group 12 of the F_1 map with distances between the markers of 34.1 and 7.0 cM, respectively. In addition, four other SSR markers, SSRY10, SSRY21, SSRY100 and SSR143 were found to be common markers that were placed on the two maps.

Discordant results between the genetic linkage maps generated in this study and the F_2 map are probably because of the small size of population used in this study, which decreases the power of the analysis and gives a lower accuracy in the statistical analysis. The positioning of markers which are located in the same linkage group of the F_2 map, but are found in different linkage groups as well as the number of unlinked markers in this study and the greater number of linkage groups than gametes are probably a consequence of missing bridge markers required to join some linkage groups together. Conversely, the fact that some markers are located in the same linkage group in this study, but which are found in different linkage groups in the F_2 map may arise as a result of the use of different molecular marker to construct the genetic maps. The numerous AFLP markers generated specifically for this study might possibly be able to link different linkage groups of the F_2 map together. Alternatively such a result could arise because of differences in the genotypes of the population used in this study and that of Okogbenin et al. (2006)'s study.

As genetic linkage maps are constructed and generated from specific cross populations, there is no guarantee that all of the markers will be polymorphic between different populations. Therefore, to correlate information from one map to another, common markers are required (Collard et al. 2005). Moreover, the integration of different DNA markers generated from different techniques on a study population has been used to produce a unified picture of the genome (Marcel et al. 2007) and such integration can be undertaken using the JOINMAP® program and such a map allows researchers to rapidly and accurately select SSR markers for chromosome regions of interest for cassava genetic and plant breeding studies (Gustafson et al. 2003).

Although a F_1 population is not an ideal population for genetic analysis (Okogbenin et al. 2006), but with time and cost effectiveness, F_1 populations can be applied to the construction of common genetic linkage maps using the JOINMAP® program. While this study was based on Thai germplasm, the information from the genetic linkage maps generated in this study are important basic knowledge for genetics studies in cassava worldwide. In particular, the identification of genetic markers that are tightly linked to traits of interest will be useful for the future identification of QTL. Although the genetic map constructed from a single cross will provide useful information, populations from multiple crosses should also be tested to confirm the position of the linked markers. Eventually, this will assist the improvement of the cassava line with high quality and quantity as well as to establish marker assisted selection programmes.

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PERMANENT GENETIC RESOURCES

Development of polymorphic markers from expressed sequence tags of *Manihot esculenta* Crantz

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Abstract

In this study, 49 primers were designed from sequences containing di-, tri-, tetra-, penta- and hexanucleotide motifs with a minimum of four repeats and presence of motif size polymorphisms (insertion/deletion) from cassava (*Manihot esculenta* Crantz) expressed sequence tags deposited in public sequence database. Each locus was subsequently screened on 29 *M. esculenta* Crantz obtained from 15 different countries. Cross-amplification was tested with *M. esculenta* Crantz (ssp. *flabellifolia*) and four different *Manihot* species, *M. chlorosticta*, *M. carthaginensis*, *M. filamentosa* and *M. tristis*. Of these, nine loci showed polymorphic profiles within *M. esculenta* Crantz, which revealed two to four alleles per locus. The average unbiased and direct count heterozygosities were 0.4901 and 0.5674, respectively.

Keywords: cassava, cross-species amplification, database, EST-SSR, *Manihot esculenta* Crantz, microsatellite

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Cassava (*Manihot esculenta* Crantz), an economically important crop in tropical areas, accumulates starch as its major storage product within its tuberous roots. It has been used as one of the most important sources of calories in the tropics, especially in Asia and Africa. Over 130 million tons of fresh cassava roots have been produced annually to be consumed by around 600 million people on a daily basis, used as animal feed and various industrial applications. Genetic diversity within germplasms provides a valuable source of undiscovered genetic materials for breeding programmes. In the past, several reports on assessing cassava genetic diversity and degree of relationship between cassava and its wild relatives using molecular markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers, yielded rather contradicting results (Fregene *et al*. 1994; Roa *et al*. 1997; Chavarriaga *et al*. 1998; Roa *et al*. 2000; Joaquim *et al*. 2001; Olsen & Schaal 2001; Fregene *et al*. 2003; Nassar 2003; Olsen 2004; Zacarias *et al*. 2004).

The codominant SSR markers are powerful tools for assessing genetic diversity, studying population genetics and helping in marker-assisted selection in many breeding programmes because of their simplicity, ubiquity, codominant behaviour, reproducibility and high level of polymorphism (Milbourne *et al*. 1997; Witsenboer *et al*. 1997). Polymorphisms can be easily detected by polymerase chain reaction (PCR) amplification with specific flanking primers. Early development of cassava SSR markers has employed a strategy to generate SSR markers from genomic DNA (gSSR) through construction of microsatellite-enriched genomic libraries (Chavarriaga *et al*. 1998 and Mba *et al*. 2001). The widespread use of microsatellites has been limited by the fact that PCR primers require a high degree of homology to work, implying that information on

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nucleotide sequences would have to be known. The cost of developing microsatellite markers is relatively high because of expenses in library construction and nucleotide sequencing. Recently, a large amount of cassava expressed sequence tags (EST) has been developed (Lopez *et al*. 2004; Lokko *et al*. 2007) and deposited in public database. Up to date, there are 38 413 cassava ESTs deposited in GenBank. With the available EST in public database, it is practical, economical and straightforward to search the available EST database for development of markers in transcribed regions, such as polymorphic EST-derived SSR, *in silico*. To date, the development of EST-derived SSR through mining EST database has become a fast, efficient and low-cost option for many plant species (Han *et al*. 2004; Feingold *et al*. 2005; Fu *et al*. 2006). Although, EST-derived SSRs are less polymorphic than those from intergenic regions, they are more easily transferred across taxa and more likely linked to genes for traits of interest (Jung *et al*. 2005). In this study, we reported the development of EST-SSR markers which are likely to be within the coding region of genes, and assessing the heterozygosity between *Manihot* species with those EST-SSR markers.

In order to identify EST-SSR markers, the cassava EST database in GenBank were clustered and assembled using TGICL software (www.tigr.org/tdb/tgi/software/) and screened for SSRs using TROLL software (http://wsmartins.net/webtroll/troll.html). The parameters were set for detection of di-, tri-, tetra-, penta- and hexanucleotide motifs with a minimum of four repeats. In some cases, candidate ESTs were prioritized based on presence of motif size polymorphisms in the EST database. Finally, 49 primer pairs were designed using PRIMER 3 (http://frodo.wi.mit.edu). The major parameters for primer design were set as follows: primer length from 18 to 22 nucleotides with 20 as the optimum, PCR product size ranges from 100 to 300 bp, optimum annealing temperature at 53 °C and 50% GC contents.

Young leaves were collected for DNA isolation using DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. The 49 primers were evaluated on 29 samples of *M. esculenta* Crantz cassava plants obtained from 15 countries (three samples from Thailand, two from each country: Argentina, Brazil, Peru, Venezuela, Columbia, Mexico, Cuba, Paraguay, Ecuador, Guatemala, Malasia, Nigeria, and one from China, and Fiji). The samples, except those from Thailand, are the samples from the collection provided by the International Center for Tropical Agriculture (CIAT) and maintained at Rayong Field Crops Research Center, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. Microsatellite survey across the species was also evaluated in three samples each of *M. esculenta* Crantz (ssp. *flabellifolia*), *M. chlorosticta*, *M. carthaginensis*, *M. filamentosa* and *M. tristis* (Table 1). PCR was performed in a total volume of 20 μL containing 50 ng of genomic DNA, 10 pmol each of forward and reverse primers, 200 μM dNTP (Promega), 1× PCR buffer, 1.5 mM $MgCl_2$, and 1.5 U *Taq* polymerase (Promega). PCR was accomplished by 1 min at 94 °C, 1 min at primer annealing temperature, and 1 min at 72 °C for 30 cycles. The PCR products were separated on 5% denaturing polyacrylamide gels and were visualized by silver staining according to Sambrook & Russell (2001). A 100-bp +1.5 Kb DNA ladder (SibEnzyme) was used to define allele sizes. The data were first analysed using TFPGA 1.3 (Miller 1997) for the unbiased and direct count heterozygosities. Hardy–Weinberg equilibrium and linkage disequilibrium were tested by GENEPOP 3.4 (Raymond & Rousset 1995).

Of the 49 primers tested with 29 individual *M. esculenta* Crantz, nine primers showed polymorphic loci with variations in genotypes. The number of alleles observed at a single locus ranged from two to four alleles per locus. The value of unbiased heterozygosity varied from 0.3522 to 0.7193 with the average of 0.4901, while direct count heterozygosity was from 0.2414 to 1.000 with the average of 0.5674 (Table 1). Analysis revealed significant ($P < 0.05$) deviations from Hardy–Weinberg equilibrium at two loci, ESSR26 and ESSR28 (Table 1). No evidence for linkage disequilibrium was detected for these loci ($P < 0.001$ for each pair of loci). Cross-species amplification was examined on other *Manihot* species and subspecies. All the nine primer pairs gave successful amplifications. Of these, polymorphisms were identified from *M. esculenta* Crantz (ssp. *flabellifolia*) when tested with ESSR4, ESSR10, ESSR9, ESSR11, ESSR18 and ESSR26 and *M. tristis* when tested with ESSR9 and ESSR26.

Sequence similarity search was conducted between the ESTs, from which these newly developed markers were derived, and the previously reported markers in cassava. The result showed no sequence similarity between the two sequence sets indicating that marker loci developed in this study are novel. These newly identified primer sets will be useful for genetic diversity studies, cultivar identification and genetic map construction in *Manihot* species. Additionally, six markers, ESSR4, ESSR11, ESSR26, ESSR28, ESSR33 and ESSR37 that showed polymorphism between Huay Bong 60 and Hanatee, two cassava varieties from Thailand used in this project will further be utilized to construct genetic linkage map of cassava using F_1 population from a cross between these two varieties. Furthermore, they can be used in assessing cassava genetic diversity, phylogenetic relationship as well as comparative genomic studies in related taxa.

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Table 1 Characteristics of nine polymorphic EST-SSRs derived from *Manihot esculenta* Crantz. The number of alleles and heterozygosities (unbiased and direct count) of each locus and significant deviations from Hardy–Weinberg equilibrium (P_{HW}) at the $P < 0.001$ level (*) were calculated for 29 *M. esculenta* Crantz (Mec) and three samples each of *M. esculenta* Crantz (ssp. *flabellifolia*) (MeF), *M. chlorosticta* (Mch), *M. carthaginensis* (Mca), *M. filamentosa* (Mfi) and *M. tristis* (Mtr) individuals

Locus	Accession no.	Primer sequences (5′–3′)	Size range (bp)	Motif	Het. (unbiased)	Het (direct count)	P_{HW}	No. of alleles					
								MeC	MeF	Mch	Mca	Mfi	Mtr
ESSR4	gb|DV443221.1|	F: CGTTCAGAACTTCCTCGT R: CGAGCCAATTTCTTATTAAC	280–282	$(TA)_6$	0.3902	0.2414	0.0330	2	2	2	1	1	1
ESSR10	gb|DV456590.1|	F: CTGTGAGTAGTGACTAGTG R: CAAATGGATATAGATGAAGC	240–245	In/Del	0.3522	0.3704	1.0000	2	2	2	1	1	1
ESSR9	gb|DV442443.1|	F: GAAGTGTCTGTTTGCAGAA R: GAACTTTGATGTCCCCAG	300–304	$(TTGG)_3$	0.5064	0.5862	0.7026	2	2	2	1	1	2
ESSR11	gb|DV456977.1|	F: CTAGGTTGCACGCACAG R: CCAGAATGGTAAAACTCATG	250–252	$(TA)_6$	0.4987	0.3571	0.2497	2	2	1	1	1	1
ESSR18	gb|DV445645.1|	F: GATTCATGATGTGGAGGCAA R: AGGTTCTTCAGTGCATTACC	320–323	$(CAT)_7$	0.6624	0.7241	0. 1788	3	2	2	1	2	1
ESSR26	gb|DV447683.1|	F: AGCGACTCAAGCGCCATC R: GGACAGTACCATTGAAGAGT	270–273	$(CAT)_7$	0.5064	0.9310	0.0000*	2	2	2	2	2	3
ESSR28	gb|CK652798.1|	F: CGGTGAGACAGCCACCG R: CAAGAATAGACCCATAAGT	190–192	In/Del	0.7193	1.0000	0.0000*	4	2	2	2	2	2
ESSR33	gb|DV448955.1|	F: GAGGCTATCCTGGATATGG R: AGGTGCCATATGCTATTAGC	230–236	$(CAG)_7$	0.3539	0.4483	0.2862	2	1	1	2	1	1
ESSR37	gb|DV445735.1|	F: AAGACACAAGAAGGGCAATG R: GATCACACTGAACTAAGGCA	600–602	$(TC)_3(AT)_3$	0.4217	0.4483	1.0000	2	2	2	1	1	2

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