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Characterization of an *Aedes aegypti* bacterial artificial chromosome (BAC) library and chromosomal assignment of BAC clones for physical mapping quantitative trait loci that influence *Plasmodium* susceptibility

L. V. Jiménez[1], B.-K. Kang, B. deBruyn, D. D. Lovin and D. W. Severson

Center for Tropical Disease Research and Training, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA

Abstract

Previous studies have confirmed a genetic basis for susceptibility of mosquitoes to *Plasmodium* parasites. Here we describe our efforts to characterize a bacterial artificial chromosome genomic library for the yellow fever mosquito, *Aedes aegypti*, and to identify BAC clones containing genetic markers that define quantitative trait loci (QTL) for *Plasmodium gallinaceum* susceptibility. This library (NDL) was prepared from the *Ae. aegypti* Liverpool strain and consists of 50 304 clones arrayed in 384-well microplates. We used PCR analysis with oligonucleotide primer pairs specific to 106 genetic markers (as sequence-tagged sites or STS) to screen the NDL library. Each STS identified between one and thirteen independent clones with an average of 3.3 clones. The average insert size was 122 kb and therefore the NDL library provides approximately 7.87-fold genome coverage. The availability of the NDL library should greatly facilitate physical mapping efforts, including positional cloning of QTL for traits of interest such as *Plasmodium* susceptibility and for whole genome sequence determination and assembly.

Keywords: genomics, positional cloning, Diptera, Culicidae, vector competence.

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Correspondence: Dr David W. Severson, University of Notre Dame, Department of Biological Sciences, Notre Dame, IN 46556–5645, USA. Tel.: +1 574 631 3826; fax: +1 574 631 7413; e-mail: David.W.Severson.1@nd.edu

[1]Present address: University of Puerto Rico-Aguadilla, Department of Natural Sciences, Aguadilla, Puerto Rico, 00603, USA.

Introduction

Many mosquito species serve as obligate intermediate hosts and vectors for a wide variety of parasitic and viral diseases that represent significant sources of human morbidity and mortality worldwide. For example, the World Health Organization estimates that malaria affects 300–500 million people with over one million deaths annually, largely among young children in sub-Saharan Africa (WHO, 1998). The emergence of drug-resistant parasites and insecticide-resistant mosquitoes, coupled with the lack of success in vaccine development, is driving efforts to identify novel strategies to control the transmission of vector-borne diseases (Blair *et al*., 2000; Adelman *et al*., 2002; Moreira *et al*., 2002), particularly malaria. Elucidation of the molecular mechanisms underlying the susceptibility of mosquitoes to the parasite is therefore of great importance to developing control strategies for malaria and other arthropod-borne diseases.

The yellow fever mosquito, *Aedes aegypti*, is highly susceptible to the avian malaria parasite *Plasmodium gallinaceum*, is genetically one of the best-characterized mosquito species and in contrast to many mosquito species is readily tractable in laboratory culture. Although *Ae. aegypti* is not a vector for the human malarial parasites, including *Plasmodium falciparum*, its vector–pathogen interaction with *P. gallinaceum* provides an excellent model to understand a genetic basis of vector competence for malaria transmission (Kilama & Craig, 1969; Thathy *et al*., 1994). A genetic linkage map based on restriction fragment length polymorphism (RFLP) markers developed mainly from random cDNA clones was previously reported for *Ae. aegypti* (Severson *et al*., 1993), and was recently expanded to include PCR-based marker loci identified as single strand conformational polymorphisms (SSCPs) or single nucleotide polymorphisms (SNPs) (Severson *et al*., 2002). These markers have been used to identify and characterize specific genome regions that influence *P. gallinaceum* susceptibility (PGS). Two quantitative trait loci (QTL) were initially linked to PGS: a major effect QTL (*pgs1*) located on

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chromosome two and a minor effect QTL (*pgs2*) on chromosome three (Severson *et al*., 1995). Detailed analyses with additional mapping populations have identified four new QTL regions associated with PGS, designated as *pgs3*, *pgs4*, *pgs5* and *pgs6*, with each located on chromosome two (Meece, 2002). The identification of six QTL regions, five of which are physically linked, illustrates the complexity in the genetic mechanisms involved in PGS. The construction of a physical map of these QTL regions and its correlation with the genetic map would provide an important framework for efforts directed toward map-based cloning of the associated genes.

Large-insert genomic libraries are critical resources for the development of physical maps of the chromosomal regions containing QTL and also for genetic marker development aimed at increasing the statistical resolution of individual QTL positions (Fahrenkrug *et al*., 2001). The improvement in bacterial artificial chromosome (BAC) cloning technology facilitates applications requiring large-fragment genomic clones (Rogel-Gaillard *et al*., 2001). BAC clones have the advantage of being bacterial-based, stable, rarely chimeric, capable of carrying inserts larger than 300 kb and can be isolated relatively easily (Shizuya *et al*., 1992). BAC end-sequence data can be employed for the targeted construction of physical contigs (Ripoll *et al*., 2000) and as sequence-tagged connectors to assist in the assembly of whole genome shotgun sequence data (Mahairas *et al*., 1999). In this study, we describe the construction and general characterization of an *Ae. aegypti* BAC library (NDL) and the identification of BAC clones containing genetic markers that define QTL for *P. gallinaceum* susceptibility.

Results

The Notre Dame Liverpool (NDL) *Ae. aegypti* BAC library was constructed with partially *Hin*dIII-digested high-molecular-weight DNA prepared from L1-stage larvae and consists of 50 304 clones arrayed in 131 384-well microplates. To estimate genome coverage, the average insert size of sixty-one randomly selected BAC clones was determined by pulsed-field gel electrophoresis. The average insert size was 122 kb, with a range between 30 kb and 280 kb (Fig. 1). Seventy-nine percent of the clones are 100 kb or larger. Based on a genome size of approximately 780 Mb (Rao & Rai, 1987; Warren & Crampton, 1991), the NDL library is estimated to contain about 7.87 haploid genome equivalents (i.e. 122 kb × 50 304 clones/780 Mb = ~7.87). For the sixty-one clones for which size was determined, forty-seven (77%) did not contain endogenous *Not*I sites, eleven (18%) contained one *Not*I site and three (5%) contained two *Not*I sites, as evidenced by the observation of one, two or three restriction fragments, respectively.

We used a combination of PCR-based plate-pool DNA screening and radiolabelled oligonucleotide probe hybridization to nylon plate replicates to assess the complexity of the NDL library. A total of 106 sequence-tagged site (STS) oligonucleotide primer pairs corresponding to genetic markers from each of the three *Ae. aegypti* chromosomes (Fig. 2) were used to screen the library. Twenty-eight of these markers are within five genome regions containing QTL that influence PGS. Each STS primer pair detected between one and thirteen clones, with an average of 3.3 clones. The 106 STS oligonucleotide primer pairs identified a total of 346 putative independent clones including 102, 154 and ninety clones containing genetic markers located on chromosome one, two and three, respectively. Of the 154 clones identified on chromosome two, ninety-four contain genetic markers within the PGS QTL regions.

Discussion

The availability of high-quality large-fragment genomic clones is essential for mosquito genomic studies, including

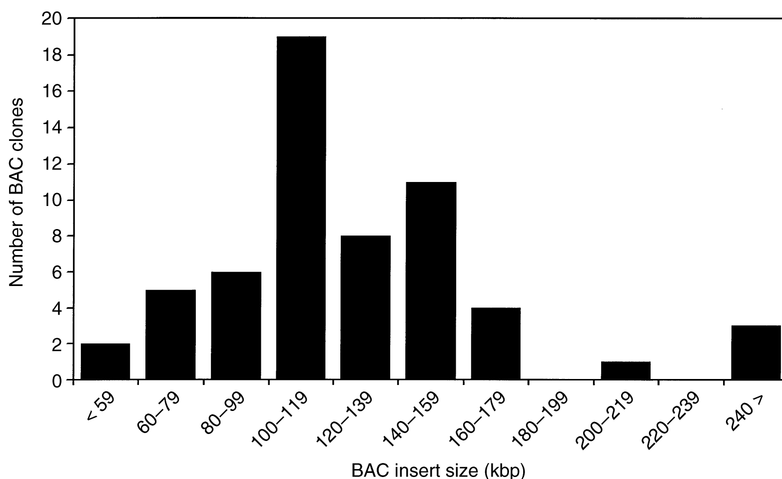

Figure 1. Insert size distribution of BAC clones in the NDL library based on size estimates of sixty-one random clones by pulsed-field gel electrophoresis.

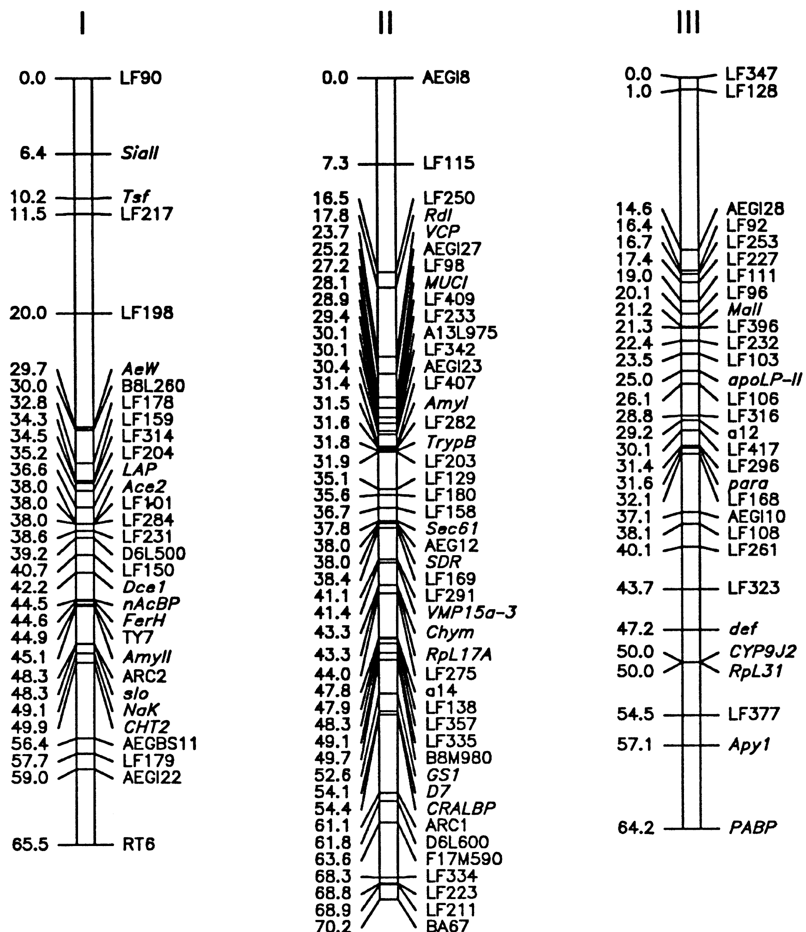

Figure 2. Chromosomal locations of the 106 *Aedes aegypti* genetic markers used as STS to identify BAC clones (after Severson *et al.*, 2002). Map distances are listed in Kosambi centiMorgans (cM).

the physical mapping of genome regions of interest and the establishment of the relationship between genetic and physical map distances (Brown *et al.*, 2001; Severson *et al.*, 2001). We have produced and characterized a 7.87-genome equivalent BAC library (NDL) for the Liverpool strain of the mosquito *Ae. aegypti* composed of 50 304 clones with an average insert size of 122 kb. Results from PCR screening of plate-pool DNAs indicated that known STS primer pairs identified between one and thirteen positive clones, with an average of 3.3 positive clones per screen. Considering that the library is estimated to represent 7.87 haploid genome equivalents, this average number of positive hits per STS marker is considerably less than expected. Similar inconsistencies were also observed by Budiman *et al.* (2000) and Hong *et al.* (2003) with their tomato BAC library and *Anopheles gambiae* BAC library, respectively. The specific reason for the observed inconsistency is unclear, but could reflect errors in the estimation of genome size or the under-representation of some genome regions in the NDL library. Regardless, the *Ae. aegypti* genome is clearly well represented and therefore our NDL library provides a valuable resource for physical mapping.

We identified BAC clones containing *Ae. aegypti* genetic marker loci at an average of 1.9 cM intervals across the entire genome. Furthermore, within the genome regions encompassing four QTL for *P. gallinaceum* susceptibility, e.g. *psg1*, *pgs4*, *pgs5* and *pgs6* (Meece, 2002), we have identified BAC clones at about 0.9 cM intervals. These clones provide physical anchors as starting points for contig identification and assembly across QTL for *Plasmodium* susceptibility in *Ae. aegypti*. Better understanding of the genetic basis for vector–parasite interactions in the *Ae. aegypti*/*P. gallinceum* system may provide significant insight regarding the genetic relationship between human malaria parasites and their *Anopheles* spp. vectors (Severson *et al.*, 1995), as well as other important phenotypes of interest.

In summary, our results indicate that the NDL library will be a valuable tool for genome analysis including: (i) construction of physical contigs across chromosome regions containing QTL, thereby facilitating gene discovery; (ii) alignment of the *Ae. aegypti* genetic and physical maps; (iii) establishment of a physical framework for comparative genome analysis with other mosquito species; and (iv) assembly of the complete genome sequence. Indeed,

the NDL library has been included with other existing libraries in an ongoing BAC end-sequencing effort that will provide critical preliminary tools for an anticipated annotated complete genome sequencing effort for *Ae. aegypti* (Knudson *et al*., 2002).

Experimental procedures

BAC library construction

Newly hatched larvae (L1) from the *Ae. aegypti* Liverpool strain were used as the source of genomic DNA for BAC library construction. The library was prepared at the Texas A & M BAC Center using their standard protocols (http://hbz.tamu.edu/bacindex.html). Briefly, high-molecular-weight DNA was extracted from L1 larvae and subjected to partial digestion with *Hin*dIII. Large *Hin*dIII fragments were selected by two rounds of size fractionization using pulsed-field gel electrophoresis and ligated into the *Hin*dIII-digested and alkaline-phosphotase-treated pECBAC1 vector. Ligation products were transformed into *E. coli* DH10B cells by electroporation. White recombinant colonies were arrayed robotically into 384-well microplates and stored at −80 °C.

Insert size analysis

BAC plasmid was isolated and purified using the QIAGEN-Large Construct kit following the manufacturer's protocol and then resuspended in 100 µl of TE (pH 8.0). To estimate insert size, 5 µl of BAC plasmid was incubated at 37 °C overnight with 10 U of *Not*I enzyme in a 50 µl digest volume containing 1× REact 3 (Invitrogen Life Technologies), 0.1 mg/ml BSA, 67 µg/ml RNAse and 4 mM spermidine. The *Not*I digests were separated by pulsed-field gel electrophoresis on a 1% agarose gel in 0.5× TBE buffer using an HG 100 Hula Gel™ apparatus (Hoefer Scientific Instruments). Electrophoresis was performed with a pulse time of 45 s, a field of 5 V/cm and a temperature of 11 °C for 45 h. The gel was stained with SYBR gold nucleic acid gel stain (Molecular Probes) for 1 h, and photographed.

BAC library screening

For screening by PCR, pools of DNA representing all clones within individual 384-well microplates were prepared. To make the pools, individual plates were first replicated on to LB agar plates containing 12.5 µg/ml chloramphenicol and incubated overnight at 37 °C. The plates were then flooded with LB broth containing 12.5 µg/ml chloramphenicol and agitated at 37 °C for 4 h, and thereafter used to prepare a 9.5 ml overnight culture. The individual plate cultures were subsequently used to prepare large-scale (75 ml) cultures that were used for DNA extractions. DNA extractions were performed following the large-scale alkaline lysis method (Birnboim & Doly, 1979).

Screening by PCR was performed with 106 STS oligonucleotide primer pairs (Severson & Zhang, 1996) designed using Primer (Lincoln *et al*., 1991) for individual clones previously mapped genetically (Severson *et al*., 2002). PCR reactions were performed in a total volume of 25 µl containing 50 mM KCl, 10 mM Tris (pH 9.0), 0.1% Triton X, 1.5 mM $MgCl_2$, 200 µM dNTPs, 2.5 pmol of each primer, 4 ng of plate pool DNA and 1 unit of *Taq* polymerase. PCR conditions were 5 min at 94 °C, followed by thirty cycles of 1 min at 94 °C, 1 min at the appropriate primer annealing temperature, and 1 min at 72 °C, and then 10 min at 72 °C for a final extension. The STS oligonucleotide primer sequences and anneal temperatures are listed in Table 1. STS-positive plate pools were identified using a gel-free melting curve analysis (DASH™, Hybaid) system. Assays were performed in ninety-six-well microplates containing 5 µl PCR reaction, 5–7.5 µl formamide, 5 µl 10× SYBR green (Molecular Probes) in a 50 µl volume. Samples were heated at 0.04 °C/s from 40 °C to 90 °C. Denaturing profiles were analysed using the DASH system software. Putative STS-positive plate pools were verified by electrophoresis on 2% agarose gels.

The individual well position within positive microplates was determined by DNA–DNA hybridization. Positive microplates were replicated to Colony/Plaque screen hybridization membranes (NEN™, Life Science Products) and incubated overnight at 37 °C. Thereafter, membranes were soaked in 10% SDS for 3 min, 0.5 M NaOH for 5 min and 1.5 M NaCl/0.5 M Tris (pH 8.0) for 5 min, air dried for 1 h, and prehybridized in 10% SDS, 5 mg/ml BSA, 0.5 M EDTA (pH 8.0) and 1 M orthophenanthroline for 3 h (shaking at room temperature). Hybridizations and radiolabelling of the STS clones were conducted following our standard probing procedures (Severson, 1997). Positive hybridization signals were confirmed by direct PCR on the positive well within the 384-well microplate glycerol stock.

Table 1. STS primer sequences

Clone	Linkage group	Primer sequence (forward/reverse)	Product size (bp)*	Annealing temperature (°C)	Accession no.
LF90	1	TTTAAGCTAGCAGAATGGCTCC ATCCGTGACATGGACGAAG	152	60	T58320
*Sia*II	1	GCGTTTCCATCACTGTCTCA CAGACAAAACCTCCCCTCAA	246	55	AF108099
Tsf	1	CAACGTGGACTACACCATGC CATTCTTCTCTCCCGCTTTG	173	60	AF019117
LF217	1	ACCTGGACGAGAAATGATCG CGCCCAGCTCCTTAGTGTTA	179	53	BM005473
LF198	1	TGAAAAAGGAATGGTGCGAT GAGCGGTTACAGGTCATCGT	144	60	T58319
AeW	1	GAACAAGGAAGCTGAGTGCC ACGTTCTTTCCGGAATTGG	108	60	U73826
B8L260	1	GTATATTATCCTTTTTCG CGGTATACACATGAAAAA	134	60	BH214532
LF178	1	GTATCTTCGATCTCGGTCGC AATACGTCCGGTTTCCAGG	161	60	T58309

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Table 1. *(Continued)*

Clone	Linkage group	Primer sequence (forward/reverse)	Product size (bp)*	Annealing temperature (°C)	Accession no.
LF159	1	AATTGCTGGATCGTTTGTCC GCCTTGTCCTCATCTTCGTC	250	60	T58315
LF314	1	GCTTTGGCGCCACTACTTC TGGTTCAGGTGCAACTTGTC	213	62	BM005509
LF204	1	ACCGTTTTACGTCGAGTTGG CGGTAAGGTCAAGGGTCAA	163	60	BM378050
LAP	1	GAAGCAATTGCCGATACCG TGAAAACGTCTCCCAAAATCC	105	58	M95187
Ace2	1	GCTCCATCTTGGCTTCTCTATC TCTAGGTGTGTAGATCTTTGGC	147	58	AAB35001
LF101	1	AGAAGCTGAGCGGAATCAAG GCCTTCAGCACCTAGACGAC	172	60	BM005475
LF284	1	AGGTCATCTAGGCCAAGGAC GTTCAGGACCTTACGGATTTC	300	60	BM005502
LF231	1	CGGTATGGAATTGCTGAAGG CAAACAGAGCGAACGGTGTA	198	60	BM005478
D6L500	1	GCAGTTGACCCCATGAACTT TAACCTGATTCCACCCGAAG	202	60	BH214541
LF150	1	ACACATCGCAATATACTGGACG ACCTTTGACACATGCCATGG	209	60	BM005476
Dce1	1	GACCAGGAAGGAAACGTGTG GCCCTTGATGATTTCCTTGA	123	60	AF288384
nAcBP	1	AATTGTACGGTGCCGGA GGATTTCCTTGTTTCTCTGCTG	226	53	AY040341
FerH	1	GTTCCTGTTCGACAAGAACC GCATTTTCCGGTGGTCTTC	150	60	AF326341
TY7	1	CCTAAATTGGAAGTTCCGTCC GTTCAAAAATCTCGACTCCTGG	151	60	R19560
*Amy*II	1	GAACGCTATCAGCCTGCTTC GTATGGCACTGCTGGGAAAT	198	63	AF000568
ARC2	1	GCAAGATCAAGCGACAGGAC CTAACTTCGGGTGGATTTGG	156	62	BH214538
slo	1	CGGAAATGGTGGTGTTTTCT GGTAGTGGTTTGACCCGAGA	239	60	AF443282
NaK	1	CGGATTTCACCCATGAGAAC GAAGGCAATCACGAAGAAGG	64	58	AF393727
CHT2	1	CCACTACTATGGCACCAGCA GGTCGTTGCACAGGTTTCTT	172	60	AF026492
AEGBS11	1	AGAGTGCAGCCTGTTCAAC AAAGCAACATCACAACACTTCC	154	60	AY033622
LF179	1	GGCTTCACCCAACAACAAGT CCAGCTTCAAGTCTCCAACC	188	60	BM005479
AEG122	1	GGTGGTTTCATCGCTAGCTC GAAAGCAGCAAAACCGTCTC	168	52	BI099650
RT6	1	CAACGTTAGCATGGCATCAG CATGTTGCTGGTTTTGCTGT	225	60	BH214544
AEGI8	2	CGAGAGTGGAAAACCAGAGC GCATAGACTGCGGACAGACA	250	60	AF326340
LF115	2	CCTATGTCCAATCGAAGGGA GTTGTGTTTCCTTGAGAACCG	124	58	R67978
LF250	2	AACCCCATCCCGAAGAAG AACACCCCCTTCCACTCAG	102	62	T58310
Rdl	2	TTAGCGAACACCGAGAGGTT TGTTTGCTGCTGCTACTGCT	221	60	U28803
VCP	2	AATCGCCGGGTACAAGAAG AGGTGATCATGTCGAAAGCC	110	65	L46594
AEGI27	2	CGATTCGAGCTATACGGAGG CTGGGGGTGAAATTTTGTTG	177	60	BG937399
LF98	2	CACGTCCAGCTTCAGAAACA GTATAGCTGGACTCTGGCCG	137	60	T58313
MUCI	2	ATATTTGGAGCAGTCCGACG ATCGATCCCACCTAAGGACC	103	60	AF308862
LF409	2	CTTCGTCTGCGGAGTGGT CCATTACCGTTGACTAAGGTCC	220	58	BM005511
LF233	2	TGGAGTCCTCTTCCGCTG CACCGAAAGAACATCCGG	152	58	T58327

Table 1. *(Continued)*

Clone	Linkage group	Primer sequence (forward/reverse)	Product size (bp)*	Annealing temperature (°C)	Accession no.
A13L975	2	CAACAGTGAGCCTTGTTTGG AGAAACGCCTATTTAGCAGTGC	209	60	BH214533
LF342	2	GATTATCGCCGAAAAGTGGA TAAACGAGGGCCTCTTCTAGC	216	65	BM005512
AEGI23	2	CCGAAGCGGACATAAAAGAG TTTTACACTTCCACGGCTCA	190	58	AY033624
LF407	2	AATTGTACGGTGCCGGA CCTAGCTCATTATTTGTTGTCG	163	53	BM005510
*Amy*I	2	CCATTCTTCTTCCCTGGACA TGTTGAACGGTACACCTGGA	382	60	AF000569
LF282	2	TACGTTCCAAATGCATACGG AGGATTTCCTTCCTTCGCAT	113	56	T58328
TrypB	2	ATTCAACGACCGAGTCCAAC CTGTAGGCCTTCGAGCATTC	174	60	M77814
LF203	2	GTCGTCCAGGACAAGGAGAA CAGCACTTCCATTAGACCA	170	53	BM005503
LF129	2	GCACCAGGAAAGTCGGAATC GCAGGAACGCTTCATAGCAT	200	60	BM005504
LF180	2	AAGGTGTACGGAATTGTGCC AAATGATCGCTGGTCCTTTG	153	65	BM005486
LF158	2	GGAAGGGTCTGAAGATCACC AGCACTTCCTTGCAGGTACC	239	54	BM005485
Sec61	2	TCGTGTGGAAGGCTTTCTCT GGGCAGATTTGATTGGAAGA	240	60	AF326338
AEGI2	2	ATTCTTCGACGAAACGGTTG GGCAACGAGATTGAAGAAGC	226	60	AY038041
SDR	2	AAGCACAGCGAAAAAACCCA TCCACTAAATCAAAACGCACT	200	53	AY0033621
LF169	2	GGACACGACTACACCCGAAG GCTGCTCGAACGGTTTAAGCG	84	60	BM378049
LF291	2	GAACACCTTGGCCGAGTG GTGCACAGGGAACCATCG	250	60	BM005482
VMP15a-3	2	TGAGCGACGATAGAACTAA TACGCCAGCTAGGAAATGTA	351	59	U91682
Chym	2	GGTGAAACAGTCCGGTGAAG AAATAACATCACATCCGAGTCC	174	63	AY038039
RpL17A	2	CGTAAGAAGGTCATGCCAG CCTTATCCAATCCAATCCGTC	227	60	AF315597
LF275	2	AGCGAAGAATGTGGCAGAAC TCTGGGACCTGTTCCACTTC	153	60	BM005500
a14	2	CGACAACTGCCTAGTTTTTGC GCTACGGATTCATGGTGTTG	222	58	BH214531
LF138	2	ATCGTGTTCAACTACGACATGC CGAGACAAATGTGATGGCC	104	63	T58332
LF357	2	AGTCAGCAATCGCTCGAAAT CCAAGCAACCCAAGAGAAAG	197	60	BM005495
LF335	2	CGTACTCGACACCGAAGACA GGATGACGGATTTGGTGTTC	179	60	BM005505
B8M980	2	TGAAGATGACTCTTACGGGATTC CGTAAATCGAAAAACAGTGAGC	320	60	BH214534
GS1	2	TATTGATGGAAGGCCTTTGG GATATCGTCAGCGCACTTCA	232	60	AF004351
D7	2	TCAAGGAGCACACTGATTGC TCAAAACCTTTTCGAGTGCC	187/132	56	M33156
CRALBP	2	CCGTGTGCTGCTGCTCAA CCCACACCAGCTTGAAGATGTA	300	60	AF329893
ARC1	2	GCCACATCAAGGGACAAATC GGAACGGCGATTTTTTCC	107	58	R19561
D6L600	2	GGTCGGATTGGAGCAGTAGA ATTTCGCAACAAGTCGTCCT	300	60	BH214535
F17M590	2	ATAACAGCGATGCCACATCA AAAGATTAACCCGGGAATGG	172	53	BH214537
LF334	2	GTGCTGGTTTTGGAGCTAGG GCTCAACCTTGGCGTACTTC	156/350	63	BM005506
LF223	2	TACGCGCATCTCCTTATTCC TTTCCCCATCAACTGTGTCA	300	60	BM005515

Table 1. *(Continued)*

Clone	Linkage group	Primer sequence (forward /reverse)	Product size (bp)*	Annealing temperature (°C)	Accession no.
LF211	2	TCGCAACAACGCTTACTGTC TGACTCCGTTTCCTGGTAGG	211	58	BM005514
BA67	2	GTATCTGTTCCTGCGGTTGC CCTGAAGTGCTGCTTCTGCT	600	60	AI561370
LF347	3	CACACTCGACTCGCGAAATA GACATCAAGCAGCAAATCCA	250	60	T58329
LF128	3	CTGGATGTGAAGCGTGTGTT TTTGGTACCGGTTTCTTGGA	226	55	BM005494
AEGI28	3	TCAGCAGAAGCGGTATGTTTC TGACACGGTACACTCGCAAT	178	58	BI096849
LF92	3	CCCAGATCAGCAGCGTTTG TTACTTCAGCTTCTTCTTCGGG	277	55	BM005493
LF253	3	AAGGCTTTGGAGAGCATCAA CCTGAGATGGGGAGTGTTGT	158	55	T58331
LF227	3	GCGAAAAAGGGCGAAAAA GCGCTCACCTCCATTCTTAG	210	55	T58323
LF111	3	CAACCGTAGCCCAAAACATC TAGCCTTCTTGATGCGGTTC	154	60	BM005492
LF96	3	ATCAAGCTCGCCAAGGTC GGGTTGTAGACGGCGTTG	164	60	BM005491
*Mal*I	3	ACGGAACCTGTAGCGCTG CGGGTTCTTTCCCCATAAAT	177/250	60	M30442
LF396	3	CGGCTGAAAGGAATCTTCTG GAGTTGATTGTCGCCAGGTT	250	60	BM005498
LF232	3	AAGCTATCCTGGCCCGTACT TGGCCTTCGTGTTGGTTT	246	51	BM005489
LF103	3	ATGCGCGATCTTCGGATC AGTGGACGGCAATCTTTTCG	124	50	BM005488
apoLP-II	3	GCTGGAATCGGTCAAACTCG CCGGCCTTAACTTGCTGGTA	350	60	AF038654
LF106	3	CAAAGCAGCCGCAAAAGA GGGTGATCAGATTGTAGG	175	54	BM005490
LF316	3	TCTCGAGCCAACCTAACACC CGCATGTCATCCAAGTTCTG	203	58	BM005516
a12	3	GGTGGCACGACGAAGTTATT TACACAGAAAGCGCAGCAAC	222	60	BH214530
LF417	3	GAAGACCAGGAAGCTACGTGG GACCAGCGACCACAGCTT	500	58	BM005499
LF296	3	CGGTCGGCAAAAATAAGGG GTTCAGTCCATTCGGTGGAAG	322	52	BM005501
para	3	AGTTTCGATACTTTCGGATGG GGTCATTAGACGAAAGGCAG	50	58	AF468968
LF168	3	AAATCGTCGTTATCGCTGCT GCGAGCGAAAGTTCAACATAAA	150	60	R47184
AEGI10	3	GCGAACAAGCGATTATCACA GGCAATGTCTTTGGGAGCTA	750	51	BI096854
LF108	3	TCATTAGCAAGCAGCGAGCT TACCATCCTTAAATCCTCTCGC	109	58	T58321
LF261	3	CTGATTCACCACTGGGACCT TGCTGAAGTGCTTGAATTGG	172	62	BM378052
LF323	3	GCTGAAGCAGTTCCTGTTCC TCGCGGACCAGTTTCTTACT	224	60	BM005507
def	3	TGTCATTTGTTTCCTGGCTCT AATTTCGACAGACGCAGACC	281/345	60	AF156088
CYP9J2	3	AAACGAAAAATGGTGCCTTG ATTTGCCTACGTGCGATTCT	90	55	AF329892
RpL31	3	GCCAGTGTCCACATTCAAG TTTTCCTCCTTCATTCCAACTC	227	60	AF324863
LF377	3	AGCGCGATTGATTGACTAGC CCCTTGACGTTGAAGAGTCC	240	56	BM005496
Apy1	3	GGAATGTGACGGCGGATTT TGGATCATGCGGCTGTTTG	625	60	L12389
PABP	3	ACCAACACTTCCACAGCCAC CTTGGCGGTATTGATTACCAGC	170	58	AY038043

*Both the cDNA and the genomic product sizes are listed for clones that represent genes containing an intron.

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