

New SSR markers for barley derived from the EST database

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Introduction

There are currently 1,196 microsatellite [or simple sequence repeat (SSR)] marker primer sets that have been developed for barley (from both genomic libraries and EST databases) of which 504 have been mapped (Saghai Maroof et al., 1994; Becker and Heun, 1995; Liu et al., 1996; Struss and Plieske, 1998; Ramsay et al., 2000; Pillen et al., 2000; Holton et al., 2002; Thiel et al., 2003; Li et al., 2003; Yu et al., 2005). Unfortunately, the available SSR markers provide uneven coverage of the barley genome and are concentrated near the centromeres. We have compared the available mapped SSRs and identified 62 BINs out of the total of 99 barley BINs (<http://barleygenomics.wsu.edu/>; <http://rye.pw.usda.gov/cgi-bin/gbrowse/BarleyBinMaps>) that have poor coverage. Although this represents, 63% of the barley genome, only 31% of the available SSRs map to these BINs. Additional SSR markers are needed to increase coverage in these BINs. Moreover, some of the SSR markers recently published are restricted from being used to develop new barley varieties, thus there is still a need for additional publicly available SSR markers that can be used without restrictions.

Materials and Methods

Barley ESTs used for primer development were selected by using either rice BAC or wheat EST sequences in a BLASTn search for publicly available barley ESTs with the low complexity filter turned off (BLASTn searches were completed between October, 2002 and October, 2004) (<http://www.ncbi.nih.gov/BLAST/>). Matches with e-values between 0 and 1e-2 were used. The resulting barley ESTs were processed through the Tandem repeats finder which measures the rate at which the actual EST sequence matches a perfect repeat sequence (Benson, 1999; <http://tandem.bu.edu/trf/trf.submit.options.html>). Primers were designed for SSRs with 85-100% matches to the perfect repeat sequence. Primer pairs were designed to flank SSR motifs using Primer3 software (Rozen and Skaletsky, 2000; http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and screened on a set of mapping parents to identify which population(s) were appropriate for mapping. Primer pairs were tested using two to four PCR protocols to identify a protocol that resulted in clear products (Tables 1 and 2). Products were separated on 6% polyacrylamide gels and visualized using silver staining.

Newly developed SSR markers were mapped on the appropriate mapping population(s) including: Steptoe x Morex (Kleinhofs et al., 1993), Chevron x M69 (Canci et al., 2003), Frederickson x Stander (Mesfin et al., 2003), or Atahualpa x M81 (unpublished). We used JoinMap 3.0 for map construction (Van Ooijen and Voorrips, 2001). Assignment to BINs was based on adjacent mapped markers that have been previously assigned to BINs (<http://barleygenomics.wsu.edu/>; <http://rye.pw.usda.gov/cgi-bin/gbrowse/BarleyBinMaps>). Wheat STS markers were mapped on the barley populations to increase the coverage of chromosome 3 (3H) (Liu and Anderson, 2003).

Results and Discussion

A total of 76 new markers were developed that produce between two to six alleles per locus among the twelve mapping parents: Atahualpa, M81, Chevron, M69, Frederickson, Stander, Harrington, OUH602, Hor211, Lacey, Steptoe and Morex (Table 3). Sixty of the markers were mapped using the Steptoe x Morex, Chevron x M69, Frederickson x Stander, and Atahualpa x M81 populations (Figure 1 and Table 3). Of the 60 mapped markers, 41 (68%) have mapped to BIN positions that were previously identified as being poorly covered with the currently available SSR markers. These markers should provide additional tools for barley genetic mapping and marker-assisted selection.

Acknowledgements

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Table 1. PCR recipes for UMB SSR markers

PCR recipe	A	B	C
dNTPs (1.25 mM each)	1.0	1.0	1.0
MgCl ₂ ² (25 mM)	1.0	1.0	0.5
10 X buffer *	1.0	1.0	1.0
Forward Primer (5μM)	0.6	0.6	1.0
Reverse Primer (5μM)	0.6	0.6	1.0
Betaine (5 M)	0	2.0	0
DMSO (100%)	0	0.5	0
Taq polymerase*	0.075	0.075	0.075
H ₂ O	2.725	0.225	2.425
DNA (10 ng/μL)	3.0	3.0	3.0
Total	10.0	10.0	10.0

* 10 X buffer is provided with purchase of Taq polymerase

Table 2. PCR programs for UMB SSR markers

Stage	Temp. (°C)	Time (min.)	Cycles	Stage	Temp. (°C)	Time (min.)	Cycles
Program A*				Program F*			
Initial Hold	95	9:00	1	Initial Hold	-	-	-
Thermal Cycles-1	94	1:00	18	Pre-PCR	95	9:00	1
	64	0:30			58	1:00	
	72	1:00			72	1:00	
Thermal Cycles-2	94	1:00	30	Thermal Cycles	94	0:30	30
	55	1:00			58	0:30	
	72	1:00			72	0:30	
Ending Hold	72	5:00	1	Ending Hold	72	5:00	1
Final Hold	4	∞	1	Final Hold	4	∞	1
Program J*				Program L58			
Initial Hold	95	8:00	1	Initial Hold	95	9:00	1
Thermal Cycles	94	1:00	35	Thermal Cycles	94	0:10	30
	60	1:00			58	0:20	
	72	2:00			72	0:45	
Ending Hold	72	5:00	1	Ending Hold	72	2:30	1
Final Hold	4	∞	1	Final Hold	4	∞	1

* Programs A, F and J are as in Ramsay et al., 2000.

Table 3. PCR and mapping information for UMB markers.

Name	Chr.	BIN ¹	Population ²		Quality Score ³	Alleles ⁴	PCR Program	PCR Recipe
			Polymorphism	Mapped				
UMB101	1 (7H)	11-12	A/M; F/S; H/O; H/L; S/M	A/M; F/S; S/M	2	3	L58	A
UMB102	1 (7H)	11-12	A/M; H/O; H/L; S/M	S/M	2.5	2	L58	A
UMB103	1 (7H)	13	A/M; F/S; H/O; H/L; S/M	A/M; F/S; S/M	2	4	L58	A
UMB104	1 (7H)	7-8	C/M; F/S; H/O; H/L; S/M	F/S; S/M	1.5	2	F	A
UMB105	1 (7H)	10-11	A/M; C/M; H/O	A/M; C/M	3	2	L58	A
UMB106	1 (7H)	4-5	C/M; H/L; S/M	C/M; S/M	2	2	L58	A
UMB107	1 (7H)	4-5	F/S; H/O	F/S	3	4	J	C
UMB108	1 (7H)	2	A/M; F/S; H/O; H/L; S/M	F/S; S/M	1	3	A	B
UMB201	2 (2H)	6-7	C/M; F/S; H/O; H/L; S/M	C/M; F/S; S/M	2.5	4	L58	A
UMB202	2 (2H)	6-7	A/M; C/M; F/S; H/O; S/M	A/M; C/M; F/S; S/M	2	2	F	A
UMB203	2 (2H)	4-5	C/M; H/L	C/M	2	2	J	C
UMB204	2 (2H)	7-8	F/S; H/O	F/S	2.5	2	L58	A
UMB205a	2 (2H)	4-5	C/M	C/M	1	2	J	C
UMB205b	2 (2H)	4-5	A/M; C/M; H/O	C/M	1	3	J	C
UMB206	2 (2H)	2-3	C/M; F/S; H/O; H/L; S/M	F/S; S/M	2	3	L58	B
UMB301	3 (3H)	1-2	C/M; F/S; H/L; S/M	C/M; F/S; S/M	3	3	F	A
UMB302	3 (3H)	4-6	A/M; C/M; F/S; H/O; S/M	A/M; C/M; F/S; S/M	1	4	L58	A
UMB303	3 (3H)	4-6	A/M; C/M; F/S; H/O; S/M	A/M; C/M; F/S; S/M	2	6	L58	A
UMB304	3 (3H)	4-6	A/M; C/M; F/S; H/L; S/M	A/M; C/M; S/M	1	4	J	C
UMB305	3 (3H)	2-4	A/M; H/O; H/L	A/M	1	2	F	A
UMB306	3 (3H)	2-4	A/M; H/O; H/L	A/M	1	3	F	A
UMB307	3 (3H)	2-4	A/M; H/O; H/L	A/M	1	3	L58	A
UMB308	3 (3H)	1-2	A/M; C/M; F/S; H/O; S/M	F/S; S/M	2.5	2	F	B
UMB309	3 (3H)	4-6	A/M; C/M; F/S; S/M	S/M	3	4	J	B
UMB310	3 (3H)	4-6	A/M; C/M; F/S; H/L; S/M	C/M; S/M	2	3	L58	B
UMB311	3 (3H)	16	CM; F/S; H/L	C/M	3	3	J	B
UMB401	4 (4H)	4-5	H/O; H/L; S/M	S/M	1	2	J	B
UMB402	4 (4H)	2-3	H/O; H/L; S/M	S/M	1	2	L58	A

Table 3 cont. PCR and mapping information for UMB markers.

Name	Chr.	BIN ¹	Population ²		Quality Score ³	Alleles ⁴	PCR Program	PCR Recipe
			Polymorphism	Mapped				
UMB403	4 (4H)	4-5	A/M; C/M; H/O; H/L; S/M	C/M; S/M	2	4	L58	B
UMB404	4 (4H)	5-6	C/M; H/O; S/M	C/M; S/M	1	2	J	B
UMB501a	5 (1H)	6	C/M; F/S	C/M; F/S	2	2	L58	B
UMB501b	5 (1H)	6	C/M; F/S	C/M; F/S	3	2	L58	A
UMB502	5 (1H)	12-13	A/M; F/S; H/O	A/M; F/S	1	3	L58	A
UMB503	5 (1H)	2	A/M; C/M; F/S; H/O; H/L; S/M	C/M; S/M	2	6	L58	A
UMB504	5 (1H)	11-12	A/M; F/S; H/O; H/L; S/M	F/S; S/M	2	4	L58	B
UMB505	5 (1H)	2	A/M; C/M; H/O; H/L; S/M	S/M	3	3	J	B
UMB506	5 (1H)	6	C/M; F/S; H/O; H/L	C/M; F/S	2	2	J	B
UMB507	5 (1H)	6	C/M; F/S; H/O; H/L	C/M; F/S	1	3	L58	B
UMB508	5 (1H)	14	A/M; C/M; F/S; H/O; H/L	C/M	2.5	3	L58	B
UMB601	6 (6H)	13-14	A/M; F/S; S/M	A/M; F/S; S/M	1	2	F	B
UMB602	6 (6H)	11-13	A/M; C/M; F/S; H/O; H/L	A/M; C/M; F/S	2	4	F	A
UMB603	6 (6H)	13-14	A/M; C/M; F/S; H/O; H/L; S/M	A/M; C/M; F/S; S/M	2	4	L58	A
UMB604	6 (6H)	7-9	A/M; C/M; F/S; H/O; H/L; S/M	C/M; F/S; S/M	1	5	L58	A
UMB605	6 (6H)	14	A/M; C/M; F/S; H/O; H/L	C/M; F/S	1	2	L58	B
UMB606	6(6H)	1	A/M; C/M; F/S; H/O; H/L; S/M	S/M	3	3	F	B
UMB701	7 (5H)	9	A/M; F/S; H/O	F/S	2	2	J	B
UMB702	7 (5H)	10-11	C/M; H/O; H/L	C/M	1	3	F	A
UMB703	7 (5H)	7-8	A/M; S/M	S/M	1.5	2	L58	A
UMB704	7 (5H)	2-4	A/M; F/S; H/O; H/L	A/M; F/S	1	4	L58	A
UMB705	7 (5H)	5-6	F/S; H/O	F/S	1.5	3	L58	A
UMB706	7 (5H)	9	A/M; C/M; H/O; H/L	A/M; C/M	1	2	L58	A
UMB707	7 (5H)	6-7	A/M; F/S; H/O; S/M	A/M; S/M	1	3	L58	A
UMB708	7 (5H)	10-11	A/M; H/O	A/M	1	2	F	A
UMB709	7 (5H)	10-11		A/M; H/O; S/M	S/M	2	2	F
UMB710	7 (5H)	11-12		S/M	S/M	2	2	L58
UMB711	7 (5H)	11-12		A/M; F/S; H/O;	F/S; S/M	2	3	L58

Table 3 cont. PCR and mapping information for UMB markers.

Name	Chr.	BIN ¹	Population ² Polymorphism	Mapped	Quality Score ³	Alleles ⁴	PCR Program	PCR Recipe
UMB712	7 (5H)	13-14	A/M; F/S; H/O; H/L; S/M	F/S; S/M	2	3	F	B
UMB713	7 (5H)	13-14	A/M; F/S; H/O; S/M	S/M	2	4	L58	B
UMB714	7 (5H)	13-14	A/M; F/S; H/O; H/L; S/M	F/S	3	2	F	B
UMB715	7 (5H)	13	A/M; F/S; H/O	F/S	2	5	J	B
UMB001			H/L		2	3	L58	B
UMB002			H/O		3	2	F	A
UMB003			H/O		2	4	F	A
UMB004			H/O; H/L		3	2	J	C
UMB005			H/O		1.5	2	L58	A
UMB006			H/O		3	2	L58	A
UMB007			H/O; H/L		2	3	L58	A
UMB008			H/O; H/L		1	3	A	C
UMB009			H/O		2	3	L58	A
UMB010			A/M; H/O; H/L; S/M		2.5	3	F	A
UMB011			A/M; H/O		1	3	L58	B
UMB012			H/O		1	2	L58	B
UMB013			H/L		2	2	L58	B
UMB014			A/M; C/M; H/O; H/L		2	3	J	B
UMB015			C/M; H/O; H/L; S/M		3	5	F	B
UMB016			H/O		1	2	A	B

¹ Bold type indicates BINs with poor SSR marker coverage

² Mapping populations: A/M=Atahualpa x M81; C/M=Chevron x M69; F/S=Frederickson x Stander;
H/O=Harrington x OUH602; H/L=Hor211 x Lacey; S/M=Steptoe x Morex

³ Scale from 1-5 where 1=Very easy to score and 5=Very hard to score

⁴ Number of Alleles based on the twelve mapping parents

Table 4. Design information for UMB primers.

Name	EST Accession ¹	Forward Primer	Reverse Primer
UMB101	BE421034	CGGGTTCCATTGAGAAGAAC	CACAAATACAGATGCCGCAC
UMB102	CB881209	TTGTGTTTGAGATATCCTGTACTTTTC	ACCTTTTGCCGGCTTTTATT
UMB103	BQ762328	TGCCCATGAAGCCTCTTTAC	GGAACGGAGGGAGTATTAAGC
UMB104	CB881555	GGAAAAATAAACTATTCAACATCCTG	CAGCGCATGTGTTCTCAGAT
UMB105	BJ485220	GCCCCTGGTAAGAACTCCAT	CTGGGAACCGTACAGTGTTG
UMB106	AL502019	AGCATAAAGCCGCAAAAGAA	GCGTCCTGATGAAGAGGTGT
UMB107	CB873957	ACGCACGGGCATTTGTA	GCCTGCATCATTGTGTTGTG
UMB108	CV055381	TCAAGCTGCTGCATTGCT	AGCCCAAACCCTTTTGTTTT
UMB201	BJ480735	GCTCCTGAAAAGGACCTCAG	TCTCCGCCACCTACACATAG
UMB202	BG299528	GGTCGGCTCCCTCTTCTACT	CGAGCGACATGAGGAACAT
UMB203	CB873608	TTTCATTGCTGTGACGGATG	AGCCTCACCCGGACTACC
UMB204	BM371159	GAATCCTCGGCCTTCTCAAC	GCGGAGCTTGACCTCGAC
UMB205a	CB881957	CGGTCGTAGAACGGAATCAG	GCACTTCCACCACAAGAAGC
UMB205b	CB881957	CGGTCGTAGAACGGAATCAG	GCACTTCCACCACAAGAAGC
UMB206	CB863325	GCGCTAGCTATCCACACAAA	AACATTAAGGGCGACAAGGA
UMB301	AV944239	CTTCACATGTCTGGGAAAACA	GACATGTTGGAAGGTGGCTT
UMB302	CD663662	ACCACAGGTAACCTCGCAAC	AAAGTGCTGGGAGCTTGAAA
UMB303	BU979287	CACGAGGGATGCTCTTGAGT	TGTATATTTCAAGCTCCCAGCA
UMB304	BM443659	CTTCGCTTACCGCTTTCG	TTTCAAGCTCCCAGCACTTT
UMB305	BJ484842	CAGAGCGGGCTCAACGTA	ACTTGCTGTCATCCTTGCTG
UMB306	BJ467519	GCAGAGCTGGCTCAACGTA	TTCACTGATCGACCACTTGC
UMB307	BJ461914	CTGCAGAGCTGGCTAACGTA	TTCACTGATCGACCACTTGC
UMB308	CB859861	CCCCTCAGGTTGTTTCATCAT	AGCAGCAGCAACAACAACAG
UMB309	BF265771	GCTCGACTTCGAGGACACC	ATTCTTGCGGAACGACCTC
UMB310	CB879994	CTCCAGCACTTTTACCATC	CCGATGCTCTTGAGTCGTG
UMB311	CA592691	ATCCAGTTTCAGCCACCAAC	ACCGCAGTGATCAGTGACAA
UMB401	AL507067	CGTCTTCGTA	ATCGAGATGCACTCCCTCAT
UMB402	BQ466542	TCGATCCATCCAAACATGAG	CGTGTCACGTGTGTGTGTGT

Table 4 cont. Ordering and design information for UMB primers.

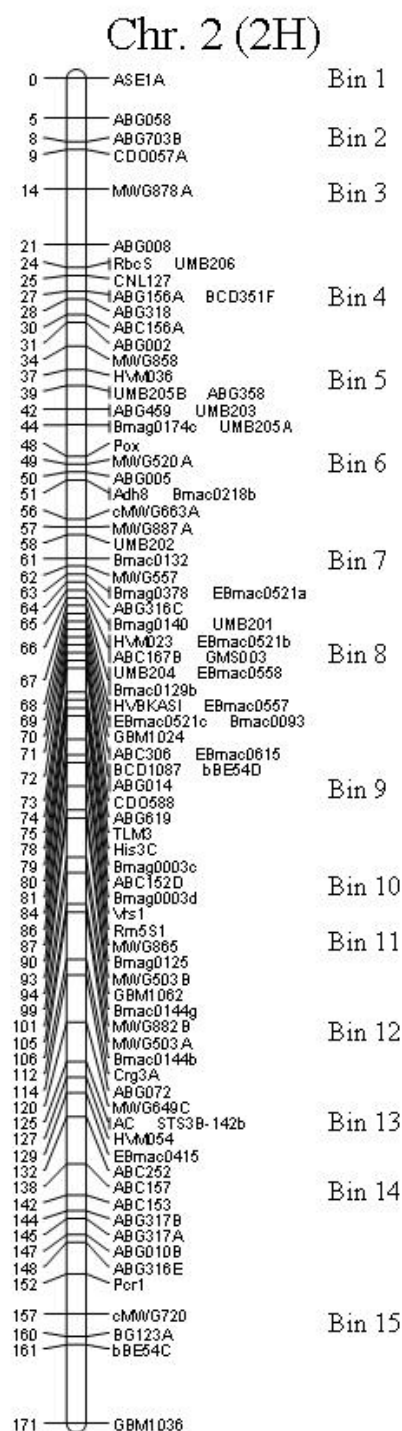
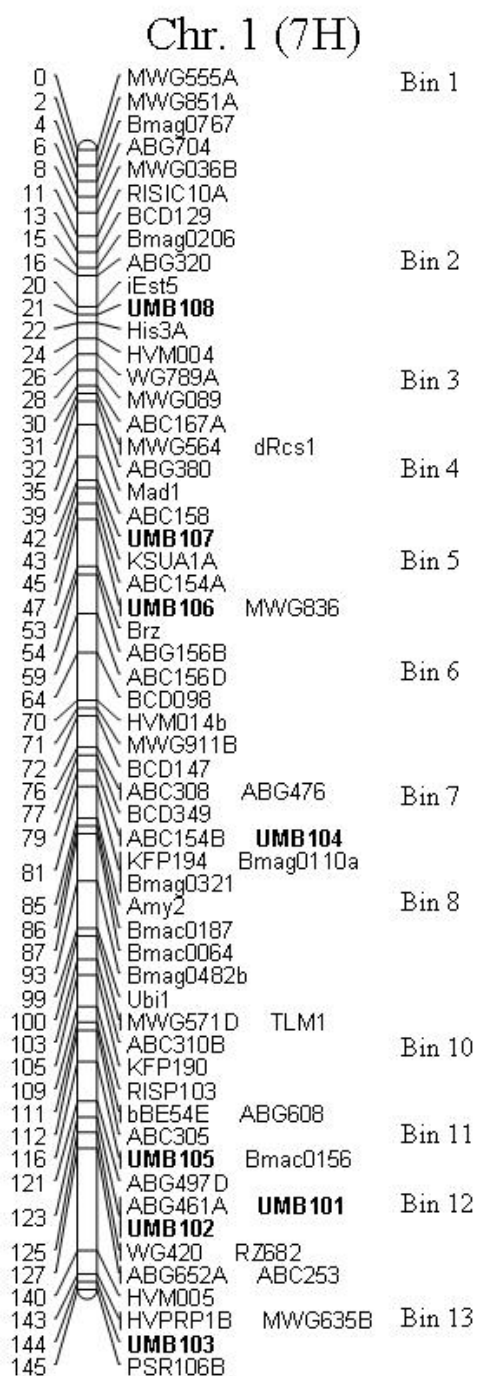
Name	EST Accession ¹	Forward Primer	Reverse Primer
UMB403	BM372825	TTCCGCAGATTCATTTCCAC	GCTGGACAGGCGTTAAAAAG
UMB404	AJ475924	GGAGGCAAGAACACTTGACAG	GCTCGATCTCCTCCTTGTTG
UMB501a	BE421033	CACACAGGCGACCATTTTC	CAGCTAGACGCTATGAGCCA
UMB501b	BE421033	CACACAGGCGACCATTTTC	CAGCTAGACGCTATGAGCCA
UMB502	BI953342	ATCCCATCTCCCTCCTCCTA	TGGAGTGCTCCTCCCAGTAG
UMB503	BQ766039	TCCCGGTGCCATATACAAAT	TTTGATGAAACGAAGGGAAA
UMB504	CV054443	CAAAGTGCGCGTGAGAAATA	AATCACCACCAGCTTCTTGG
UMB505	BE195848	ATGTTGCAGCAGAGCAGTTG	ATTGTTGGGGTTGTTCTTGC
UMB506	CD663377	CTCTTCCGTGAAACGAAACC	CGAGCAAGGACGTGGTAGAT
UMB507	CD663377	ATGTTTCAACAGGCCATTCC	CATGAAAACAGATGACGATGC
UMB508	BF621983	GATTAAGGCGTCCAATTCCA	TCGGGATGTGAAGAAGGAAC
UMB601	BJ486149	AAATACCGTATGGAGGGTGCT	CTACCCCTACGTCCGAGATG
UMB602	CB877685	AGGAGTGGGTCTCAGGTTTG	CAAGCAGATGCAACTACACCA
UMB603	CA006980	ATGAAACATCGCGAACTGTG	ACTGCAGTGAGGGAAGCTGT
UMB604	CA004840	GAGCAATCCCCTCATCCAAT	TCTTTGGTTTCCTCGTGTCC
UMB605	CD053629	GAGGCTTGTTCTCAGACCA	ATGAGGAAGAGCGGGATCAG
UMB606	CV056304	CGAGCAGCAGCAGATCGT	CTCCTGCGCTTGGAGAAG
UMB701	BE421177	ACGTCGTGGATCAACGTGTA	TTACATTGCGCACAGCTAGG
UMB702	BF265777	CAGCATCCATCAGCAATGAA	CATGTTTGGCTTCTTCGTCC
UMB703	CA025623	GCCGCCTCTTACTCTTTGC	GGAGATGCCGAGGGACTT
UMB704	AV942720	ATCCTCCAACGAGGCACATA	GAGTCCATTTACGGAGACC
UMB705	CB876579	TGCTGAGACACACACACACC	CGATGCACGAAAAGCTGTAG
UMB706	CB875298	TCAACAGATGACGTGCATGA	TCACACATTGAGGGAGGACA
UMB707	CB876579	TGCTGAGACACACACACACC	CGATGCACGAAAAGCTGTAG
UMB708	BE421505	CTCCTCAGCTCTGGAATGGA	GCGCATATACAAGCCAAACA
UMB709	CV063649	ACGACAAGACTATGGCAGCA	AAGGTTTCCTCAGCCTGTGA
UMB710	CV063745	ACAGTTCCCAACTTCCAAGC	CAGCACATCAGCCCGTACT
UMB711	BJ553047	GCAACGACACGTCTAAACCA	GTGGTGTGGTGTCTTCCTC

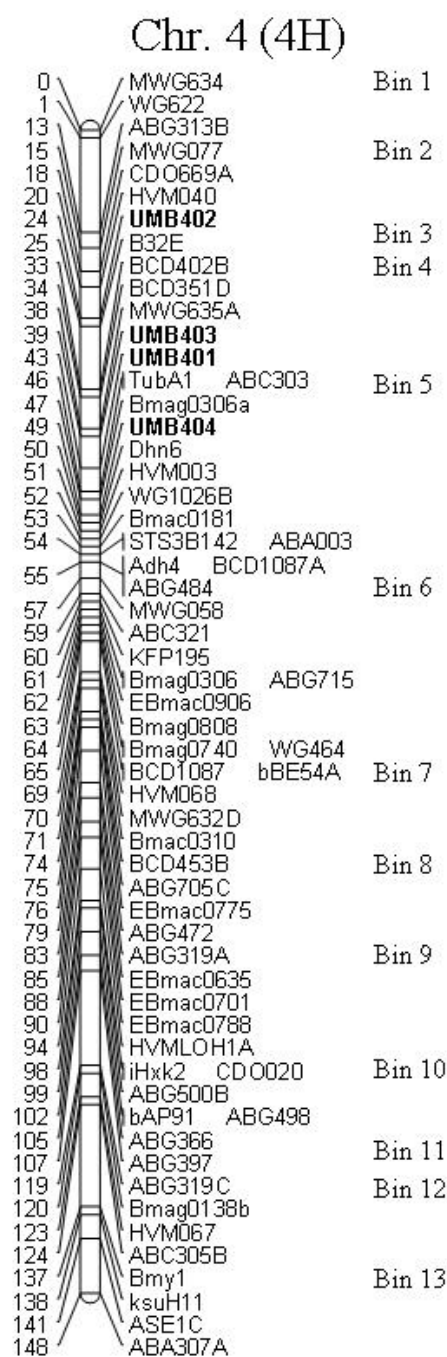
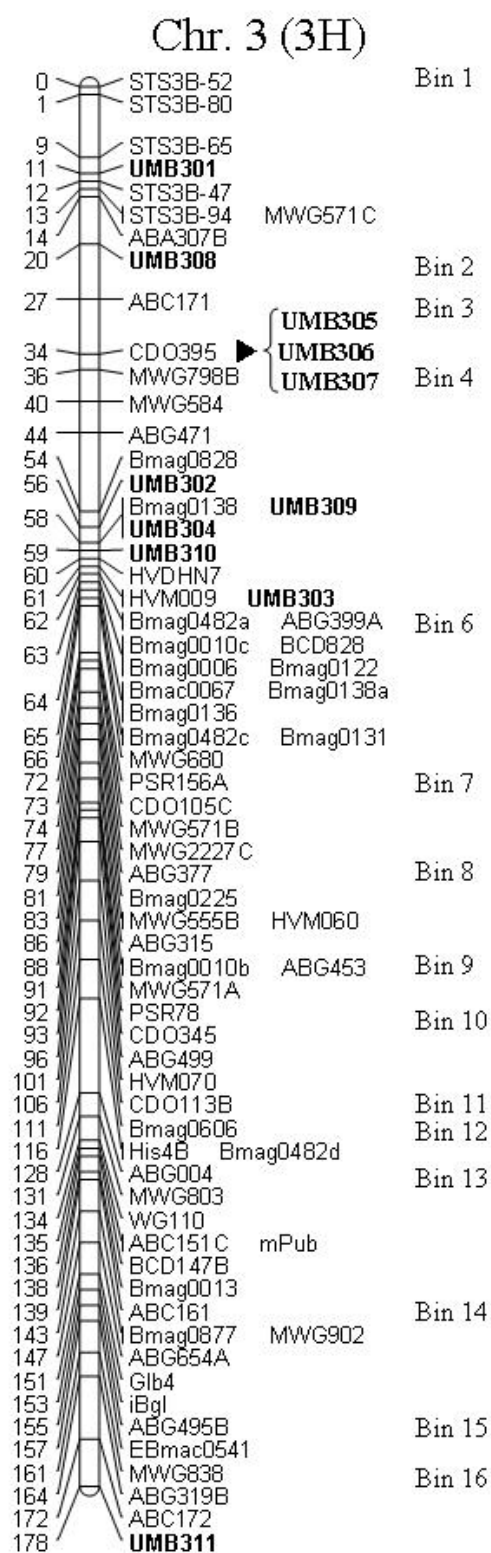
Table 4 cont. Design information for UMB primers.

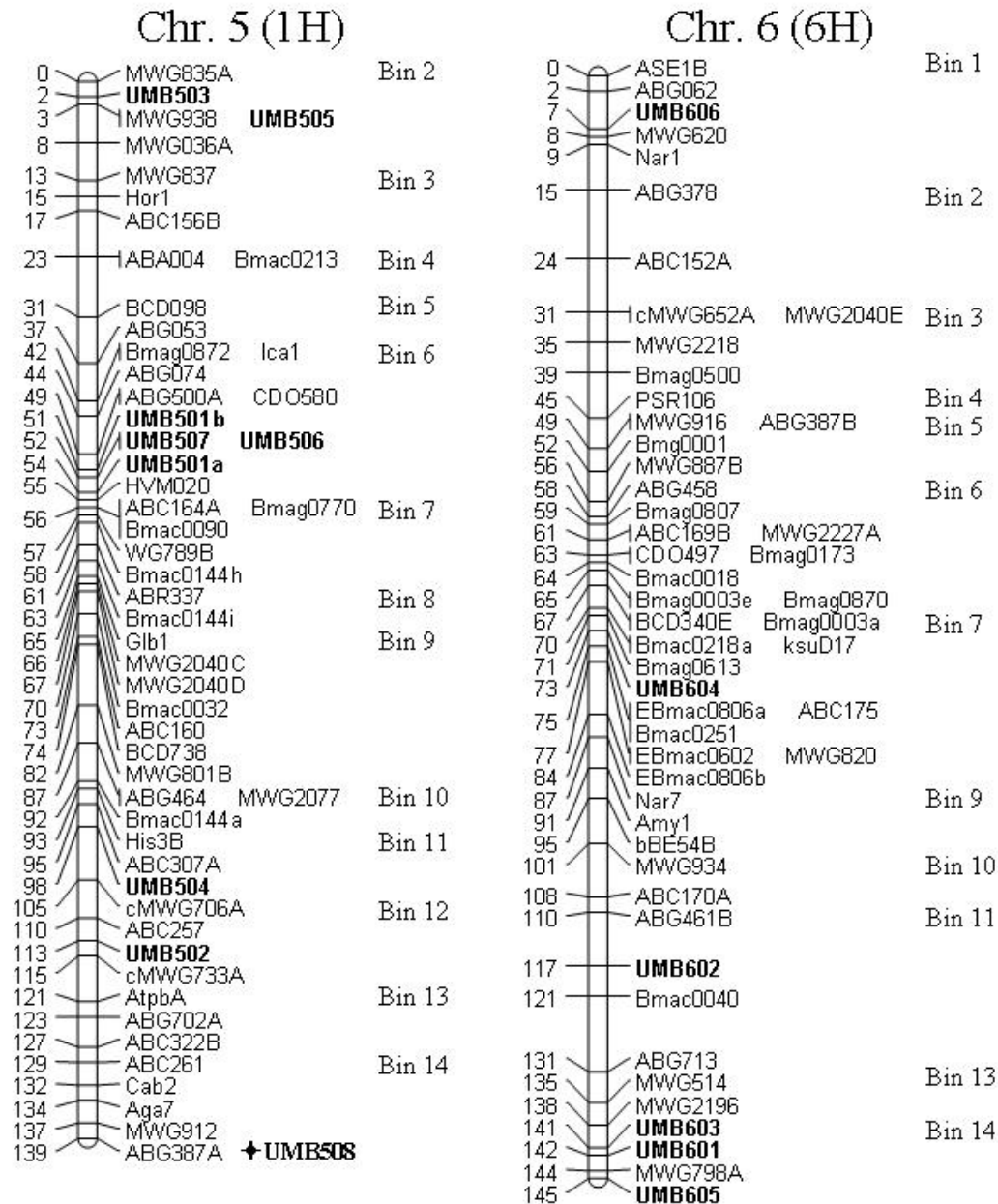
Name	EST Accession ¹	Forward Primer	Reverse Primer
UMB712	CB881537	CAAGAAGGAACGAAGCCAAG	TGCTAGTTTTTCCGGCTGAT
UMB713	CB881537	ATCAGCCGGAAAACTAGCA	CAAGCGAAGAGGAAGAGGAA
UMB714	CB881537	GCAAGCAAATCACACTCTGG	GCGTCATCTACGGCTGATTC
UMB715	CB879524	CCGCGCCTAATTAACAAAAA	AGCTGACTGCTGACCAACCT
UMB001	BI949870	TTCTCCATGTTTCGGCTTCTT	ATCAGCAATGAAGTTGTCCG
UMB002	BJ481660	GGCAAGTGAGCTCAACCTCT	GCCCTCACATGCAACATCTA
UMB003	CB881957	TCAGTGGTGACCGTGGTATC	GCACTTCCACCACAAGAAGC
UMB004	BG309433	ACCACGTCCACCACCATC	CTCTTTCTCCGCTCATCACC
UMB005	BQ763410	AAGAAAGCCCGGAAGAGAAG	ATGGTGCCATCCTGATTGAT
UMB006	BQ662872	CGGAGTCGCTTTCGAGATT	ACTGACAGCAACGGTGGTAG
UMB007	CB881851	CGCAATAAAATCGCAGGAAT	ACGGAAGACCGGGATAGTTT
UMB008	BU996005	CTGGAGCCTAGCTTGGAGTG	CTCCACCGTTCTTCACGTTT
UMB009	BI954579	CATCCCCATCCAGATCCA	GTCGAGAGGTGCGAGGATT
UMB010	BF621983	GCCACAGCCAAGAAAGCTAC	GATGGGATCTGCTTGGAGAG
UMB011	AV918630	TCTTCTCGCTAGCATCAGCA	CAAAGAAGGAGGTGGCTTCA
UMB012	BI960225	ATAGCGACGTGCTCCAGAGA	AGCAGCGACTTCCTTAGCAG
UMB013	CB873614	GAGCAAGCACGCACGTATTA	GGGACCTCGAGATGATCAAG
UMB014	CB882192	CAGGAGATCCGCGCTCTT	CGAGCAGTGAACGATGTACG
UMB015	CA030737	CGGACGAGGTTTACTCCAAA	AGCACAGGAGGATGAGGATG
UMB016	CA032410	CCATCACCCATTTCTTCCTC	GATGGATTTGTCCGTCCAAG

¹ EST accession from which the primers were designed (<http://www.ncbi.nih.gov/>)

Figure 1. Map locations of the UMB markers on consensus maps of the Steptoe x Morex (S/M), Frederickson x Stander (F/S) and Chevron x M69 (C/M) populations. Chromosome 7 (5H) is presented as a consensus map of the F/S and C/M populations with the S/M population separate. UMB508 (denoted with '✦') has had its location inferred from the C/M population (C/M is included in the consensus but the inferred marker did not map in the consensus). Markers denoted with '►' have had their location inferred from the Atahualpa x M81 (A/M) population. Marker "ABG497D" [chr. 1 (7H), BIN 11], was named "ABG497B" in Canci et al. (2003), but the "ABG497B" locus should map to chr. 1 (7H), BIN 4, therefore we have designated a new locus name.







Chr. 7 (5H)

