Supplemental Methods and Supplemental Tables 1 – 9

An accurate and efficient method for large-scale SSR genotyping and applications

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ABSTRACT

Accurate and efficient genotyping of Simple Sequence Repeats (SSRs) constitutes the basis of SSRs as an effective genetic marker with various applications. However, the existing methods for SSR genotyping suffer from low sensitivity, low accuracy, low efficiency and high cost. In order to fully exploit the potential of SSRs as genetic marker, we developed a novel method for SSR genotyping, named as AmpSeq-SSR, which combines multiplexing PCR, targeted deep sequencing and comprehensive analysis. AmpSeq-SSR is able to genotype potentially more than a million SSRs at once using the current sequencing techniques. In the current study, we simultaneously genotyped 3,105 SSRs in 8 rice varieties, which were further validated experimentally. The results showed that the accuracies of AmpSeq-SSR were nearly 100% and 94% with a single base resolution for homozygous and heterozygous samples, respectively. To demonstrate the power of AmpSeq-SSR, we adopted it in two applications. The first was to construct discriminative fingerprints of the rice varieties using 3,105 SSRs, which offer much greater discriminative power than the 48 SSRs commonly used for rice. The second was to map *Xa21*, a gene that confers persistent resistance to rice bacterial blight. We demonstrated that genome-scale fingerprints of an organism can be efficiently constructed and candidate genes, such as *Xa21* in rice, can be accurately and efficiently mapped using an innovative strategy consisting of multiplexing PCR, targeted sequencing and computational analysis. While the work we present focused on rice, AmpSeq-SSR can be readily extended to animals and microorganisms.

Supplemental Methods – Construction and sequencing of Ampli-Seq libraries

Supplementary Table 1. The rice varieties used in this study.

Supplementary Table 2. The genotypes, coverages, stutter ratios and amplicon lengths of the 3,105 target SSRs in varieties A to H.

Supplementary Table 3. The accuracy of AmpSeq-SSR on pseudo-heterozygous variety ij

Supplementary Table 4. The decrease of SSR stutter ratios in varieties B-H when using only the target SSRs whose stutter ratios were no greater than 0.2 in variety A.

Supplementary Table 5. Some SSRs tend to be incorrectly genotyped.

Supplementary Table 6. Many inconsistent SSRs in the eighth variety can be filtered by the other seven varieties.

Supplementary Table 7. Comparable SSR pairs and length differences between their amplicons.

Supplementary Table 8. The genotypes, coverages and stutter ratios and amplicon lengths of the differential SSRs between each of the three pairs of NILs.

Supplementary Table 9. AmpSeq-SSR possesses high reproducibility.

Supplemental methods – Construction and sequencing of Ampli-Seq libraries

This protocol has been used on eight rice varieties in the current study. The genomic DNAs of varieties A-N in Supplemental Table 1 were extracted. Ampli-Seq libraries of varieties A-N were then constructed and sequenced using the S5 sequencing platform. Ampli-Seq libraries of varieties A-H were also constructed separately and sequenced using the Miseq sequencing platform. The main reagents for Ampli-Seq library constructions were included in Ion AmpliSeq™ Library Kit 2.0 (Cat No. 4475345, Thermo, USA), and methods for Ampli-Seq library constructions followed the main principles in its manual (Publication Part Number MAN0006735, Revision B.0). The Ion AmpliSeq™ Library Kit 2.0 includes the components of 5× Ion AmpliSeq™ HiFi Mix, FuPa Reagent, Switch Solution, DNA Ligase, Ion AmpliSeq™ Adapters, Platinum® PCR SuperMix HiFi, Library Amplification Primer Mix and Low TE.

Step 1. DNA extraction and quantitation

Whole plants at the first leaf stage were harvested for extraction of genomic DNA, following the protocol of E-Z 96® Mag-Bind® Plant DNA Kit (Cat. No. M1027, Omega bio-tek, USA). The Qubit® dsDNA HS Assay Kit (Cat. no. Q32851 or Q32854) was used for DNA quantification.

Step 2. Preparation of master mix for multiplex PCR reactions

Add the following components to each PCR tube: 4 µl 5× Ion AmpliSeq™ HiFi Mix, 10 µl the synthesized multiplex primers and 10 ng extracted genomic DNA of one of varieties A-N. Vortex the master mix thoroughly and spin down to collect droplets.

Step 3. Amplify SSR targets

Load each PCR tube from step 2 to the thermal cycler (2720, ABI, USA), and run the following program to amplify SSR targets: 99℃/2 min; 16 or 30 cycles of 99℃/15 sec and 60℃/8 min. For the S5 and MiSeq Ampli-Seq libraries, the PCR cycles were 16 and 30, respectively.

Step 4. Partially digest the primer sequences on both ends of SSR amplicons

During the synthesis of SSR multiplex primers, base T in the primers was substituted by base U. Therefore, the primers on both ends of SSR amplicons could be digested by FuPa reagent. Add 2 µl FuPa Reagent to each PCR tube from step 3. Vortex thoroughly and spin down to collect droplets. Load the PCR tube to the thermal cycler (2720, ABI, USA) and run the following program: 50℃/10 min; 55℃/10 min and 60℃/20 min.

Step 5. Ligate sequencing adapters to SSR amplicons

Add the following components to each PCR tube from step 4: 4µl Switch Solution, 2µl DNA ligase and 2µl barcode adapter for S5 Ampli-Seq libraries (Cat. No. 44712150, ABI, USA) or for MiSeq Ampli-Seq libraries (Cat. No. FC-131-2001, Illumina, USA). Vortex the mixture thoroughly and spin down to collect droplets. Load the PCR tube to the thermal cycler (2720, ABI, USA), and run the following program: 22℃/30 min and 72℃/10 min.

Step 6. Purify the ligation products of SSR amplicons

Add 45µl Agencourt® AMPure® XP Reagent (Cat. No. A63881, Beckman Counter, USA) to each PCR tube from step 5. The following purify steps follows the recommended procedure in the manual book of Agencourt® AMPure® XP Reagent. The purified SSR amplicons were eluted in 50µl Low TE. The purified ligation products were the SSR Ampli-Seq libraries and ready for high-throughput sequencing.

Step 7. High-throughput sequencing SSR libraries

The S5 SSR Ampli-Seq libraries from step 6 were sequenced on the S5 system in our lab. Thirty days later, the MiSeq SSR Ampli-Seq libraries were sequenced on the MiSeq system in BestNovo Co., Ltd, China, using a 2×300 bp paired-end sequencing mode.

Supplemental Table 1: The rice varieties used in this study

Variety code	Variety name	The types of the variety
A	t2092	*Indica* CMS line
B	ShengDao 808	*Japanica* inbred rice
C	JiNongDa 858	*Japanica* inbred rice
D	AoFuB	*Indica* maintainer line
E	HuaZhan	*Indica* inbred rice
F	Jiangyu F8412A	*dica* CMS (Cytoplasm Male Sterile) lines
G	YueGuang No. H3	*Japanica* inbred rice
H	Ha 04-29	*Japanica* inbred rice
I	9311	NILs of R11
J	R11	NILs of 9311
K	IR24	NILs of R21
L	R21	NILs of IR24
M	D62B	NILs of R62
N	R62	NILs of D62B

Supplemental Table 2: The genotypes, coverages, stutter ratios and amplicon lengths of partial target SSRs in varieties A-H

SSR	Amplicon	Primer sequence	
		forward	Reverse
1	AMPL1119350	AAATCAGGGTGCATGAACGACATA	TGTGAGGAGAAGAGACGAACAATTG
2	AMPL1118764	AGAAGAAGGAGAGATCTGAGCCAT	AACTTTTATCAAGAACCTGTACTACATGCT
3	AMPL1122080	CCAGTTCACCTTGTCATAAGCTTTC	GGATTCTATGTTTGCCATAAGTTTGATGAT
4	AMPL1118986	CAAGCTCATGGAGGAAAGTGTG	CGGATTAGCACATGACTACTTAAACTTGAA
5	AMPL1122956	CCACCTTAGGATTTAAGCGAGGAGTA	GCCTCCTTAGATAGCAGGAGAGATTC
6	AMPL1122956	CCACCTTAGGATTTAAGCGAGGAGTA	GCCTCCTTAGATAGCAGGAGAGATTC
7	AMPL1119030	ACCATTGTACATGCCCTAACATCAG	GGTCATATTTCAGTTCTCATCACAGGATC
8	AMPL1119030	ACCATTGTACATGCCCTAACATCAG	GGTCATATTTCAGTTCTCATCACAGGATC
9	AMPL1119030	ACCATTGTACATGCCCTAACATCAG	GGTCATATTTCAGTTCTCATCACAGGATC
10	AMPL1120471	AATCTCACCAGTATATGCTCTTCGATTTTT	GCCAAAACAGAAAACCAAATATTTGTCTTC
11	AMPL1121792	AAAACCAACATATCAACTACTGTCAGCT	CCCATGTCACTGGAGATCAATGC
12	AMPL1122521	CTGTCGTGAGCTTCACCATTCT	CTAGCCCAGCAAAACACATGGAT
13	AMPL1119455	TCTGACTTCTGAGAGGTGCTCAT	CCTTGATTTTCTTCGGTACTACTTCCTC
14	AMPL1122277	GCGGTCTTGCGTGATCAAAG	CGCCGTGTGTGTACAAATAATATGC
15	AMPL1122277	GCGGTCTTGCGTGATCAAAG	CGCCGTGTGTGTACAAATAATATGC
16	AMPL1118548	CCACCACCTCCCATGTTTCAAG	GCCGATACATTTGACTGTCCGAAA
17	AMPL1118548	CCACCACCTCCCATGTTTCAAG	GCCGATACATTTGACTGTCCGAAA
18	AMPL1142246	GTCCAGCTACAGCAGCTTCA	CGGCTCTTCTTCTTCTTCTTCTTCG
19	AMPL1142246	GTCCAGCTACAGCAGCTTCA	CGGCTCTTCTTCTTCTTCTTCTTCG
20	AMPL1122643	TTGATTGTTTGTTCCTTATGCAAAGGTTT	GGGATAAATCTGAAAAAGAGAAGTGACAGA
21	AMPL1118456	GATCATACGACACCATTTGATTCTGTG	ACTTACTGAGATGATTTAAGTCGGCAATTA
22	AMPL1118841	CGACGCGTCCTTTCGTTT	CCCAAACCGAATCCTGACTAACC
23	AMPL1121609	CAAATAACAGTCAAATTGGACCAAAATGTT	TATTGTTTGCATAGCTCTTTCACATTCATG
24	AMPL1142057	GGCATGTACGTTTTGTTCGGAT	CCAATAGGTACAAATGAACAGCATTTTGAA
25	AMPL1142057	GGCATGTACGTTTTGTTCGGAT	CCAATAGGTACAAATGAACAGCATTTTGAA
26	AMPL1123212	AAACCAAAATGACAGTCCTGGACTTA	TTTGGATCGTCTGACATCATCTTAGC

Supplemental Table 2: The genotypes, coverages, stutter ratios and amplicon lengths of partial target SSRs in varieties A-H

SSR	A_MiSeq				B_MiSeq				C_MiSeq				
	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype
1	AT32	2	0.50	245	AT6	896	0.01	193	NA	NA	NA	NA	AT6
2	CAGG5	87	0.02	275	CAGG4	125	0.01	271	CAGG5	212	0.00	275	CAGG4
3	TAGA5	1771	0.02	275	TAGA4	2553	0.01	265	TAGA5	2275	0.02	275	TAGA4
4	AACA5	218	0.01	275	AACA5	167	0.02	288	AACA5	293	0.01	275	AACA5
5	GC6	1569	0.05	273	GC5	893	0.08	273	GC5	1492	0.51	273	GC6
6	GCGT5	1345	0.19	273	GCGT4	895	0.01	273	GCGT3	1480	0.01	273	GCGT1
7	AT15	1	0.00	NA	AT14	476	0.65	NA	NA	NA	NA	NA	AT12
8	TTAT1	1	0.00	NA	TTAT1	1063	0.01	NA	NA	NA	NA	NA	TTAT1
9	AT0	1	0.00	NA	AT0	1067	0.00	NA	NA	NA	NA	NA	AT0
10	TA24	22	0.59	279	NA	NA	NA	NA	TA24	15	0.80	273	NA
11	CTC8	69	0.01	275	CTC7	120	0.04	272	CTC8	128	0.05	275	CTC7
12	TCC7	945	0.05	NA	TCC8	738	0.07	NA	TCC7	997	0.05	NA	TCC8
13	AT25	2	1.00	290	AT3	1	0.00	NA	AT17	9	1.00	280	AT27
14	CGG4	49	0.02	#N/A	CGG4	77	0.00	#N/A	CGG4	56	0.02	#N/A	CGG4
15	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA
16	TA42	9	0.67	297	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	GA11	20	0.15	297	NA	NA	NA	NA	NA	NA	NA	NA	NA
18	CGC7	1200	0.04	#N/A	CGC5	2611	0.02	#N/A	CGC7	3506	0.02	#N/A	CGC5
19	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA
20	CA21	8	0.63	274	CA19	2	1.00	274	CA21	9	0.67	275	CA22
21	TA11	41	0.39	NA	TA8	47	0.11	NA	TA10	49	0.49	NA	TA11
22	GCC7	124	0.02	256	GCC7	152	0.07	256	GCC7	273	0.04	256	GCC7
23	AAAT6	413	0.02	275	AAAT6	9	0.22	260	AAAT6	492	0.02	275	AAAT1
24	TA5	2	0.50	285	TA5	75	0.00	NA	TA5	19	0.05	275	TA5
25	TA13	1	1.00	285	TA5	74	0.01	NA	TA17	12	0.33	275	TA5
26	NA	NA	NA	NA	AC7	15	0.13	256	NA	NA	NA	NA	AC9

Supplemental Table 2: The genotypes, coverages, stutter ratios and amplicon lengths of partial target SSRs in varieties A-H

SSR	D_MiSeq			E_MiSeq				F_MiSeq				G_MiSeq		
	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio
1	720	0.01	193	AT6	699	0.02	193	NA	NA	NA	NA	AT6	963	0.02
2	119	0.03	271	CAGG4	118	0.03	271	CAGG5	121	0.02	275	CAGG4	138	0.06
3	2446	0.01	265	TAGA5	1965	0.01	275	TAGA5	1059	0.01	275	TAGA5	2668	0.09
4	189	0.02	288	AACA5	183	0.01	275	AACA5	152	0.01	275	AACA5	258	0.01
5	95	0.75	NA	GC6	72	0.51	NA	GC6	2032	0.04	273	GC4	542	0.47
6	1	0.00	NA	GCGT1	1	0.00	NA	GCGT5	1822	0.15	273	GCGT5	198	0.24
7	668	0.48	NA	AT12	639	0.51	NA	NA	NA	NA	NA	AT12	844	0.48
8	1250	0.01	NA	TTAT1	1214	0.00	NA	NA	NA	NA	NA	TTAT1	1639	0.00
9	1252	0.00	NA	AT0	1218	0.00	NA	NA	NA	NA	NA	AT0	1644	0.00
10	NA	NA	NA	TA27	3	0.33	NA	TA24	11	0.91	277	TA23	2	0.50
11	120	0.08	272	CTC7	82	0.05	272	CTC8	96	0.03	275	CTC7	112	0.10
12	679	0.09	NA	TCC8	744	0.07	NA	TCC7	659	0.06	NA	TCC7	765	0.50
13	1	1.00	308	AT4	2	0.00	NA	AT4	3	0.67	290	AT4	5	0.40
14	77	0.00	#N/A	CGG4	66	0.00	#N/A	CGG4	22	0.00	#N/A	CGG4	58	0.00
15	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA
16	NA	NA	NA	NA	NA	NA	NA	TA30	15	0.67	273	TA30	5	0.80
17	NA	NA	NA	NA	NA	NA	NA	GA11	38	0.16	273	GA11	12	0.08
18	2856	0.02	#N/A	CGC5	2016	0.49	#N/A	CGC7	4505	0.02	#N/A	CGC7	4894	0.10
19	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA
20	4	0.50	277	CA21	5	1.00	275	CA21	5	0.40	275	CA21	8	0.13
21	31	0.39	NA	TA11	1	0.00	NA	TA10	50	0.34	NA	TA10	2	0.00
22	197	0.03	256	GCC7	159	0.06	256	GCC7	221	0.05	256	GCC7	194	0.05
23	4	0.75	246	AAAT6	27	0.04	275	AAAT6	305	0.02	275	AAAT6	368	0.02
24	54	0.02	NA	TA5	65	0.00	258	TA5	95	0.03	NA	TA5	31	0.00
25	55	0.00	NA	TA6	62	0.03	258	TA5	95	0.02	NA	TA6	30	0.03
26	2	0.00	271	AC17	3	1.00	277	AC9	1	0.00	270	AC8	2	0.50

Supplemental Table 2: The genotypes, coverages, stutter ratios and amplicon lengths of partial target SSRs in varieties A-H

SSR	H_MiSeq				A_S5				B_S5				
	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length
1	193	AT30_AT4	1	0.00	251	AT6	1	0.00	147	AT6	168	0.02	161
2	271	CAGG5	132	0.02	275	CAGG5	31	0.06	231	CAGG4	48	0.04	227
3	275	TAGA5	2101	0.01	275	TAGA5	380	0.02	223	TAGA4	373	0.04	214
4	275	AACA5	208	0.01	275	AACA5	82	0.07	227	AACA5	85	0.12	240
5	NA	GC6	2002	0.04	273	GC6	186	0.18	223	GC5	184	0.10	227
6	NA	GCGT5	1935	0.07	273	GCGT5	157	0.45	223	GCGT4	226	0.01	227
7	NA	AT12	1	0.00	NA	NA	NA	NA	NA	AT14	71	0.27	158
8	NA	TTAT1	1	0.00	NA	NA	NA	NA	NA	TTAT1	105	0.00	158
9	NA	AT0	1	0.00	NA	NA	NA	NA	NA	AT0	102	0.02	158
10	NA	TA23	5	0.80	NA	NA	NA	NA	NA	NA	NA	NA	NA
11	272	CTC8	102	0.05	275	CTC8	49	0.12	231	CTC7	54	0.07	228
12	NA	TCC7	948	0.05	NA	TCC7	272	0.04	229	TCC8	103	0.07	232
13	282	AT3	23	0.26	NA	AT1	2	0.50	195	AT2	1	1.00	155
14	#N/A	CGG4	38	0.03	#N/A	CGG4	19	0.00	#N/A	CGG4	25	0.08	#N/A
15	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A
16	273	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	273	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
18	#N/A	CGC7	3273	0.02	#N/A	CGC7	105	0.07	#N/A	CGC5	190	0.02	#N/A
19	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A
20	275	CA21	8	0.25	275	CA21	1	1.00	221	CA22	1	1.00	188
21	NA	TA11	30	0.23	NA	TA11	33	0.45	236	TA8	78	0.08	230
22	256	GCC7	192	0.02	256	GCC7	9	0.11	220	GCC7	10	0.10	221
23	275	AAAT6	420	0.03	275	AAAT6	88	0.26	217	AAAT6	47	0.26	114
24	NA	NA	NA	NA	NA	NA	NA	NA	NA	TA5	77	0.01	221
25	NA	NA	NA	NA	NA	NA	NA	NA	NA	TA5	76	0.01	221
26	269	AC15	1	0.00	NA	AC14	1	0.00	228	AC7	46	0.17	208

Supplemental Table 2: The genotypes, coverages, stutter ratios and amplicon lengths of partial target SSRs in varieties A-H

SSR	C_S5				D_S5				E_S5			
	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length
1	NA	NA	NA	NA	AT6	115	0.03	148	AT6	168	0.02	161
2	CAGG5	22	0.09	231	CAGG4	55	0.02	227	CAGG4	41	0.05	227
3	TAGA5	193	0.01	224	TAGA4	247	0.02	214	TAGA5	286	0.02	224
4	AACA5	20	0.15	227	AACA5	85	0.05	240	AACA5	82	0.05	227
5	GC7	86	0.74	NA	GC6	22	0.18	NA	GC6	11	0.27	NA
6	GCGT3	61	0.08	NA	GCGT0	1	0.00	NA	NA	NA	NA	NA
7	NA	NA	NA	NA	AT12	76	0.22	155	AT12	87	0.22	155
8	NA	NA	NA	NA	TTAT1	115	0.01	155	TTAT1	132	0.00	155
9	NA	NA	NA	NA	AT0	109	0.05	155	AT0	131	0.01	155
10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
11	CTC8	40	0.05	231	CTC7	45	0.02	228	CTC7	87	0.08	228
12	TCC7	248	0.05	229	TCC8	74	0.14	232	TCC8	102	0.10	232
13	AT3	11	0.27	153	AT4	2	1.00	210	AT2	27	0.00	232
14	CGG4	28	0.00	#N/A	CGG4	22	0.09	#N/A	CGG4	32	0.03	#N/A
15	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A
16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
18	CGC7	127	0.02	#N/A	CGC5	184	0.03	#N/A	CGC5	117	0.36	#N/A
19	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A
20	NA	NA	NA	NA	CA21	1	1.00	239	CA21	1	1.00	177
21	TA10	19	0.16	234	TA11	34	0.29	236	NA	NA	NA	NA
22	GCC7	11	0.18	219	GCC7	2	0.50	206	GCC7	10	0.30	222
23	AAAT6	24	0.21	229	AAAT6	24	0.33	232	AAAT6	43	0.23	218
24	TA5	2	0.00	239	TA5	49	0.00	221	TA5	35	0.00	224
25	TA19	1	1.00	239	TA5	48	0.02	221	TA6	33	0.06	224
26	NA	NA	NA	NA	AC9	4	0.00	231	NA	NA	NA	NA

Supplemental Table 2: The genotypes, coverages, stutter ratios and amplicon lengths of partial target SSRs in varieties A-H

SSR	F_S5				G_S5				H_S5			
	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length	Genotype	Coverage	Stutter ratio	Amplicon length
1	AT6	1	0.00	161	AT6	181	0.02	161	AT6	1	0.00	148
2	CAGG5	45	0.07	231	CAGG4	31	0.10	227	CAGG5	48	0.04	231
3	TAGA5	116	0.03	224	TAGA5	128	0.12	223	TAGA5	265	0.01	223
4	AACA5	68	0.12	227	AACA5	32	0.16	227	AACA5	138	0.06	227
5	GC6	223	0.17	223	GC4	14	0.71	NA	GC6	222	0.17	223
6	GCGT5	194	0.39	223	GCGT5	11	0.18	NA	GCGT5	203	0.34	223
7	AT12	1	0.00	155	AT12	106	0.26	155	NA	NA	NA	NA
8	TTAT1	1	0.00	155	TTAT1	159	0.00	155	NA	NA	NA	NA
9	AT0	1	0.00	155	AT0	154	0.03	155	NA	NA	NA	NA
10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
11	CTC8	37	0.24	230	CTC7	64	0.03	228	CTC8	56	0.11	230
12	TCC7	235	0.03	229	TCC7	260	0.20	229	TCC7	303	0.05	229
13	AT1	10	0.10	177	AT2	35	0.09	232	AT1	6	0.17	163
14	CGG4	23	0.04	#N/A	CGG4	27	0.00	#N/A	CGG4	22	0.00	#N/A
15	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A
16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
18	CGC7	175	0.06	#N/A	CGC7	168	0.17	#N/A	CGC7	120	0.05	#N/A
19	NA	NA	NA	#N/A	NA	NA	NA	#N/A	NA	NA	NA	#N/A
20	CA20	2	0.50	224	CA19	1	1.00	213	NA	NA	NA	NA
21	TA10	30	0.13	234	TA10	4	0.25	234	TA11	61	0.18	236
22	GCC7	5	0.20	220	GCC7	3	1.00	223	GCC7	5	0.20	219
23	AAAT6	34	0.15	218	AAAT6	15	0.33	218	AAAT6	52	0.38	217
24	TA5	28	0.00	221	TA5	21	0.00	224	NA	NA	NA	NA
25	TA5	27	0.04	221	TA6	20	0.05	224	NA	NA	NA	NA
26	AC9	4	0.25	203	AC9	5	0.20	186	AC14	2	0.50	226

Supplemental Table S3: the accuracy of AmpSeq-SSR in pseudo-heterozygous

i^{th} varieties	j^{th} varieties	Correct genotypes	Referece genotypes	Accuracy A_{ij}
A	F	1396	1446	96.54%
A	B	1084	1173	92.41%
A	E	1087	1166	93.22%
A	H	1409	1463	96.31%
A	C	1388	1447	95.92%
A	D	1078	1166	92.45%
B	H	1098	1179	93.13%
B	D	1240	1294	95.83%
C	F	1412	1458	96.84%
C	B	1102	1183	93.15%
C	H	1403	1458	96.23%
C	D	1092	1178	92.70%
E	F	1105	1183	93.41%
E	B	1210	1267	95.50%
E	H	1085	1158	93.70%
E	C	1101	1175	93.70%
E	D	1240	1285	96.50%
F	B	1112	1196	92.98%
F	H	1424	1467	97.07%
F	D	1110	1186	93.59%
H	D	1071	1155	92.73%
Average		1202.24	1270.62	94.47%
Standard deviation		140.41	126.51	1.69%

Supplemental Table 4: The decrease of SSR stutter ratios in variety B-H when using only the target SSRs whose stutter ratios were no greater than 0.2 in variety A.

Variety	B	C	D	E	F	G	H	Average
Before selecting	12.83%	15.96%	14.31%	14.95%	16.18%	17.27%	14.42%	15.13%
After selecting	7.19%	6.80%	8.27%	9.12%	7.39%	10.57%	6.25%	7.94%
The decrease ratio	43.97%	57.38%	42.23%	39.01%	54.31%	38.82%	56.66%	47.48%

Supplemental Table 5: Some SSRs tend to be incorrectly genotyped

Variety 1	Variety 2	Comparable SSRs (Nij)	Obervered error-prone SSRs	Inconsistant SSRs only in variety 1	Inconsistant SSRs only in variety 2
B	C	921	0	3	4
B	G	1036	1	3	3
B	H	1062	1	2	4
A	B	1030	1	4	2
E	H	1047	1	4	2
A	G	944	0	2	4
B	F	1030	0	3	3
B	E	1137	2	1	4
D	H	1057	1	3	2
G	H	969	0	3	2
D	F	1023	0	3	2
C	G	891	0	1	4
F	G	954	0	2	3
C	E	913	0	2	3
C	D	915	0	3	2
C	F	1107	1	2	2
A	E	1007	1	1	3
E	F	1010	1	3	1
D	G	1045	1	3	1
A	D	1019	0	2	2
D	E	1164	2	1	2
C	H	1152	2	2	1
A	C	1118	2	1	2
B	D	1197	1	1	2
F	H	1286	3	2	0
A	F	1218	2	1	1
A	H	1309	5	1	0
E	G	1077	3	0	0

Supplemental Table 6: Many inconsistent SSRs in the eighth variety can be filtered by the other seven varieties

The eighth variety	A	B	C	D	E	F	G	H	Average
Consistent SSR (Mi)	1383	1314	1216	1334	1309	1367	1201	1460	2646
Inconsistent SSRs (mi)	8	8	5	6	8	6	6	10	7.125
Error-prone SSRs (m_{ei})	6	6	2	4	7	3	4	8	5
The filtering rate*	75.00%	75.00%	40.00%	66.67%	87.50%	50.00%	66.67%	80.00%	70.18%

*The filtering rate = Error-prone SSRs / Inconsistent SSRs.

Supplemental Table 7: Comparable SSR pairs and length differences between their amplicons

Variety pairs	Comparable SSR pairs										
	Total (No.1)	With different SSR genotypes									
		Total		Lengh difference between SSR genotypes							
				> 5 bp		⩽ 5bp		Between 1 bp and 5		= 0 bp	
		No.2	Ratio (No.2/No.1)	No.3	Ratio (No.3/No.2)	No.4	Ratio (No.4/No.2)	No.5	Ratio (No.5/No.2)	No.6	Ratio (No.6/No.2)
A.vs.H	1774	119	6.71%	51	42.86%	68	57.14%	62	52.10%	6	5.04%
F.vs.H	1711	130	7.60%	46	35.38%	84	64.62%	79	60.77%	5	3.85%
G.vs.E	1426	137	9.61%	62	45.26%	75	54.74%	60	43.80%	15	10.95%
A.vs.F	1750	152	8.69%	59	38.82%	93	61.18%	84	55.26%	9	5.92%
H.vs.C	1712	162	9.46%	58	35.80%	104	64.20%	99	61.11%	5	3.09%
F.vs.C	1693	190	11.22%	76	40.00%	114	60.00%	108	56.84%	6	3.16%
A.vs.C	1757	190	10.81%	71	37.37%	119	62.63%	109	57.37%	10	5.26%
B.vs.D	1418	228	16.08%	107	46.93%	121	53.07%	103	45.18%	18	7.89%
E.vs.D	1400	265	18.93%	131	49.43%	134	50.57%	119	44.91%	15	5.66%
B.vs.E	1375	277	20.15%	136	49.10%	141	50.90%	126	45.49%	15	5.42%
G.vs.D	1440	305	21.18%	145	47.54%	160	52.46%	133	43.61%	27	8.85%
G.vs.B	1412	326	23.09%	137	42.02%	189	57.98%	160	49.08%	29	8.90%
F.vs.E	1411	597	42.31%	330	55.28%	267	44.72%	221	37.02%	46	7.71%
F.vs.B	1433	597	41.66%	316	52.93%	281	47.07%	241	40.37%	40	6.70%
F.vs.G	1584	603	38.07%	295	48.92%	308	51.08%	242	40.13%	66	10.95%
F.vs.D	1427	611	42.82%	327	53.52%	284	46.48%	237	38.79%	47	7.69%
B.vs.C	1424	611	42.91%	324	53.03%	287	46.97%	254	41.57%	33	5.40%
E.vs.C	1413	622	44.02%	342	54.98%	280	45.02%	242	38.91%	38	6.11%
G.vs.C	1581	627	39.66%	310	49.44%	317	50.56%	257	40.99%	60	9.57%
B.vs.H	1441	634	44.00%	333	52.52%	301	47.48%	261	41.17%	40	6.31%
C.vs.D	1430	634	44.34%	349	55.05%	285	44.95%	240	37.85%	45	7.10%
G.vs.H	1596	635	39.79%	304	47.87%	331	52.13%	263	41.42%	68	10.71%
E.vs.H	1419	635	44.75%	346	54.49%	289	45.51%	244	38.43%	45	7.09%

H.vs.D	1435	654	45.57%	351	53.67%	303	46.33%	250	38.23%	53	8.10%
A.vs.E	1458	655	44.92%	361	55.11%	294	44.89%	248	37.86%	46	7.02%
A.vs.B	1475	658	44.61%	340	51.67%	318	48.33%	278	42.25%	40	6.08%
A.vs.G	1646	660	40.10%	327	49.55%	333	50.45%	266	40.30%	67	10.15%
A.vs.D	1480	678	45.81%	361	53.24%	317	46.76%	268	39.53%	49	7.23%
Average	**1518.61**	**449.71**	**29.61%**	**228.39**	**48.28%**	**221.32**	**51.72%**	**187.64**	**44.65%**	**33.68**	**7.07%**
Standard Deviation	**131.52**	**219.87**	**15.29%**	**125.39**	**6.28%**	**96.01**	**6.28%**	**77.27**	**7.37%**	**20.38**	**2.18%**
Maxium	**1774**	**678**	**45.81%**	**361**	**55.28%**	**333**	**64.62%**	**278**	**61.11%**	**68**	**10.95%**
Minium	**1375**	**119**	**6.71%**	**46**	**35.38%**	**68**	**44.72%**	**60**	**37.02%**	**5**	**3.09%**

Supplemental Table 8:The genotypes, coverages and stutter ratios and amplicon lengths of the differential SSRs between each of the three pairs of NILs

		Primer sequence	
Amplicon	Variety	forward	Reverse
AMPL1562757	9311 vs. R11	GCTTCTCCTGCAGGTTCATCTC	CATATGGTCAGCGACAGGTACA
AMPL1562757	IRBB24 vs. R24	GCTTCTCCTGCAGGTTCATCTC	CATATGGTCAGCGACAGGTACA
AMPL1562757	D62B vs. R62	GCTTCTCCTGCAGGTTCATCTC	CATATGGTCAGCGACAGGTACA
AMPL1562999	9311 vs. R11	GTGAGACCTTGTAGTGATGTGCTATTTA	CGGAGAGGAAGAACAGAGAGTTTC
AMPL1563098	D62B vs. R62	GGACGATCGCCCTGTACAAG	CTCAAGGCTCACCTTATTATACTAATCTGC
AMPL1563104	D62B vs. R62	CTCAATGCCAACTTGATATATATCCGGT	GTGACGGAGTAATTGTGTTGCA
AMPL1563170	D62B vs. R62	ACCTGTCACCTTTACTGTGATCTATTTG	CGGGCAGGTATTGGGATTATTTATTAATCC
AMPL1563183	D62B vs. R62	CTATATGCTTTCTGTAATGCAATGCCA	ACCTTCTTCATATAATGTCAAAGGTTGGAA
AMPL1563209	D62B vs. R62	AAGAGGCACTGAAAAATACCTCAAGATT	TCAGATTTGACAGTTTCACAAAGAGGT
AMPL1563216	D62B vs. R62	CAAACTTGCTGCATACACACCTTAAT	ACCCTAACCCAACCTAACCAAC
AMPL1563217	D62B vs. R62	CATGCACACTATTGTTATAGATGCCATTG	CCGTATCTTCATGCATCAACATATCATGA
AMPL1563226	D62B vs. R62	TGAGCAGAAGCACTCTAGTATATTTAGCT	GCACATGTTCGTCGACCAAGAA
AMPL1563228	D62B vs. R62	GAAGAGGTGGAGGCCATGT	GAGACGACGAAGGCAGATAAGTC
AMPL1563230	D62B vs. R62	GCTTCTCCTTGTCTCGAAGCAA	TCCATCCACCCAGAGTAAAAGGA
AMPL1563277	IRBB24 vs. R24	TCATGTTCAAACGAGCCCAAGAA	GAGGCTGTGGGTCATTCTTCTC
AMPL1563300	D62B vs. R62	CTATCTTGGCGTCCATCTTGAGT	GTTGAATCAAATACAGTTCGTTTCAGGTT
AMPL1563309	D62B vs. R62	CCACACGACACGAGCTAGTA	GCCATCGCCCTTTTCGTCTT
AMPL1563321	D62B vs. R62	CTCCCATCTTTTTGCTGCAATCTTT	GCGATCGATCGACCTAGCTATTG
AMPL1563350	D62B vs. R62	CATGCCTGCTATACCTTCCTGA	CATCTCTAGCTACTAGTTGTTCTCTTCTCT
AMPL1563354	D62B vs. R62	CCACAGAGTAGCATCAAACGCA	GCAAAGTTTATGCTCACTTTGCCA
AMPL1563355	D62B vs. R62	CACCTCCTCGAAGAACTGCT	CCGCCATTAAAATTGCTCCAAG
AMPL1563368	IRBB24 vs. R24	CGCCAGGATGATGCTCGA	ATGCCAACACGGTTCATTTCAC
AMPL1563368	D62B vs. R62	CGCCAGGATGATGCTCGA	ATGCCAACACGGTTCATTTCAC
AMPL1563370	IRBB24 vs. R24	GACTTCGTTTCAAGTGGTAAACCTG	AGCCTATTCTTGTTTTCCTTCAACATCT
AMPL1563370	D62B vs. R62	GACTTCGTTTCAAGTGGTAAACCTG	AGCCTATTCTTGTTTTCCTTCAACATCT
AMPL1563371	IRBB24 vs. R24	GCGCATTCATGTCATCGTCTCA	ATCTTTCCAGTTTTTATTGCTCAAAGCTT
AMPL1563371	D62B vs. R62	GCGCATTCATGTCATCGTCTCA	ATCTTTCCAGTTTTTATTGCTCAAAGCTT
AMPL1563372	IRBB24 vs. R24	CCGAGGGTTAGCTTGTTAGTGC	GAACAAGACAAGAGAAGCAATATTTGGTG
AMPL1563372	D62B vs. R62	CCGAGGGTTAGCTTGTTAGTGC	GAACAAGACAAGAGAAGCAATATTTGGTG
AMPL1563384	D62B vs. R62	TGTTGTTGTTGCGTCCCTCTA	CCCGAGCTGGAATGGACTAC

Supplemental Table 8:The genotypes, coverages and stutter ratios and amplicon lengths of the differential SSRs between each of the three pairs of NILs

		Primer sequence	
Amplicon	Variety	forward	Reverse
AMPL1563405	9311 vs. R11	GCGTGGAACTCCAAGATCTTCC	AAGCCGATCAGGACCGTAAAG
AMPL1563406	9311 vs. R11	GTGGCGAAATCCAGGCAGAT	GTCGTCGGTTATAAACTTATAATGGTCTGA
AMPL1563411	D62B vs. R62	CTGCCCTGCTCTCAAGTTTCTA	GGGAGAAAAACTAACCAAGTGTACTCTTAT
AMPL1563413	D62B vs. R62	CAGAAAAAGCCATGCGTGAAGT	CCGTATCAAAAATTCGATGTGACATATCTC
AMPL1563426	9311 vs. R11	GTAGTGATTGAGAGTAGTACTGTTCACTTG	ATCTCCTGTGGTCTGTTGAATCTATTG
AMPL1563494	IRBB24 vs. R24	ACAAACTATCTGGAAGCTTCTGGAAG	GATATTAGCAGTGGATTCATGGAGCT
AMPL1563540	D62B vs. R62	GGATCAGGACAGCACAAGGAATG	GCGGATGAGATGAGAATTTTCAACTAAAAT
AMPL1563601	D62B vs. R62	GGTGTATGAGGGTGGTGACAAG	TTGTTAGCTGAAACATTACAAAACAAACCT
AMPL1563618	D62B vs. R62	CCGTTATAACTGTGAACTGTGATATTTACG	GGCAGAGAGACCTAATTCCTAGTTGAT
AMPL1563638	9311 vs. R11	GCCTTCTTGCACCGTTGA	AAGGCAGTTTCACTGACGTGA
AMPL1563638	D62B vs. R62	GCCTTCTTGCACCGTTGA	AAGGCAGTTTCACTGACGTGA
AMPL1563640	9311 vs. R11	TGACCGTACAATCCTACTACTCGT	GGAAAAATATACTACCTCGAAAAGGTTGGA
AMPL1563640	D62B vs. R62	TGACCGTACAATCCTACTACTCGT	GGAAAAATATACTACCTCGAAAAGGTTGGA
AMPL1563642	9311 vs. R11	TGAGCTTCTCCATCACTCCCAT	TCAAATCGTTCGACCACGTGAT
AMPL1563671	D62B vs. R62	CCCTAGTGGCTGTATCAAGTTACG	GGCTCTGCTGCTATCGTCATTT
AMPL1563688	D62B vs. R62	CGGCCATGTACAGCTCATAC	GGTACCCGTCGAGCATGAAG
AMPL1563697	D62B vs. R62	GATAATATCATATGGCGGTTTAGGAGCAT	GCCCAGCAGGCCTAATGTAC
AMPL1563717	D62B vs. R62	GCGCACATGATCATGGTTCAAC	AGGGATGATTTTACAAGGATTTCATCAGAA
AMPL1563739	D62B vs. R62	ACTATTTGAGGGTGTCCAATTGGAC	GTCAGCCGTGAGTACACCAT
AMPL1563778	9311 vs. R11	GTTCGCTGAATATAATAACGATAGCAATGC	GACGATCGAGTGAAGACGATGAG
AMPL1563795	D62B vs. R62	GCAACGCACTCTCTATCTCGTA	GATGAGGAATCGAGGGCAGAAAT
AMPL1563807	IRBB24 vs. R24	AAACTGACATACAAGGCGATAAAACAATG	TGAGGTCGGTCTAGGGTTTTAGTATAATT
AMPL1563810	IRBB24 vs. R24	GTGGGCCAAGCTGCACAAG	TGGTGGTCGCTAGCTGAC
AMPL1563810	D62B vs. R62	GTGGGCCAAGCTGCACAAG	TGGTGGTCGCTAGCTGAC
AMPL1563812	IRBB24 vs. R24	GGAGCTAAGTCCTTGCCATCG	GAAAATTGCGAAATAGTACTGTACTGGG
AMPL1563812	D62B vs. R62	GGAGCTAAGTCCTTGCCATCG	GAAAATTGCGAAATAGTACTGTACTGGG
AMPL1563820	IRBB24 vs. R24	AACAGTGGTCGCAATTGTTTCAC	CGGAGGTTGTGGGAGATGATTTC
AMPL1563820	D62B vs. R62	AACAGTGGTCGCAATTGTTTCAC	CGGAGGTTGTGGGAGATGATTTC
AMPL1563821	IRBB24 vs. R24	ATATACGTCTTGGTCAGCCAAGTTTATC	GATTCCACTGAACATTCAAACACATAACAT
AMPL1563821	D62B vs. R62	ATATACGTCTTGGTCAGCCAAGTTTATC	GATTCCACTGAACATTCAAACACATAACAT

Supplemental Table 8:The genotypes, coverages and stutter ratios and amplicon lengths of the differential SSRs between each of the three pairs of NILs

		Primer sequence	
Amplicon	Variety	forward	Reverse
AMPL1563823	IRBB24 vs. R24	CAGATCCGGCAATGGTGAAGAT	GGTATCCTCTAATCTCACCGATCAAATG
AMPL1563823	D62B vs. R62	CAGATCCGGCAATGGTGAAGAT	GGTATCCTCTAATCTCACCGATCAAATG
AMPL1563825	IRBB24 vs. R24	TCTTCCGAAGGCTTCGAACTTT	TCGTGGTCTCTTCTTCTTCATCCATA
AMPL1563825	D62B vs. R62	TCTTCCGAAGGCTTCGAACTTT	TCGTGGTCTCTTCTTCTTCATCCATA
AMPL1563826	D62B vs. R62	GGCGGTTTAGAAGCGTTTATATGAG	GTGGTTACAAGAGTCTAAGTATCACTTGAG
AMPL1563844	D62B vs. R62	CATTGGTTTTGCCTCGCTGAAT	GTGTGTATCCCACGAAGGAACAA
AMPL1563846	D62B vs. R62	GGGCTTCTTCTCCTTCTCTCCA	CACCAAATATGCAAATACCACCTTTTGTAT
AMPL1563850	D62B vs. R62	CACTGTGTGAACTGCTGAACAG	GTGTTGCAGGTAAAGCATGCAA
AMPL1563853	D62B vs. R62	TTCTTACATCTGACAGCTGTTTTGGT	TGATCATGTTTACCAAAAACAACAACTACA
AMPL1563866	D62B vs. R62	CTCCGATGCCTTCTTCCTCTTG	CCTGCGACCCTTCCACTACTATA
AMPL1563876	D62B vs. R62	CTAATAGCAATTGTAGGAGCGCCATA	CCTTGTGGTCATGCTTCCTCATC
AMPL1563877	D62B vs. R62	TGGCCCACGAAGTTAACTAATTAAACAT	GGAGCTCAGATATTTAGCACCTCATATTTT
AMPL1563890	D62B vs. R62	TTGGCCAATCAAATCCAAAGAACC	CCAAGAAGAGTGATCAAGAATTGGGTAAAA
AMPL1563893	D62B vs. R62	GAAAATGTATGGCGAGACCCTACA	TGTGTCACAAGGCTAATCCTTGTC
AMPL1563896	D62B vs. R62	TTGAAGTCGGTGGCGTGT	AGCGGATGCAGGAGCTCTC
AMPL1563897	D62B vs. R62	GATTCTCCTCGCTCATCTGAGTG	GGGATTCCAAGTCGATGACCAAT
AMPL1563903	D62B vs. R62	GCATCCATCCATCCATCATAATGC	GTACAAATCACTTCTCCAATTCCTTACCA
AMPL1563906	D62B vs. R62	CAAAGCAGTGCAAAGAACATTTCTCT	GTGTTTATCTACCTCCTTTCTAGACCGATA

Supplemental Table 8:The genotypes, coverages and stutter ratios and amplicon lengths of the differential SSRs between each of the three pairs of NILs

		Receiptor parent			NIL parent			SR location on reference genom		
Amplicon	Variety	Genotype	Coverage	Stutter rati	Genotype	overag	Stutter ratio	Chr.	Begin	End
AMPL1562757	9311 vs. R11	CGC4,	250	0.14	CGC7,	198	0.10	chr11	21463893	21463913
AMPL1562757	IRBB24 vs. R24	CGC4,	279	0.10	CGC7,	190	0.09	chr11	21463893	21463913
AMPL1562757	D62B vs. R62	CGC4,	218	0.11	CGC7,	142	0.11	chr11	21463893	21463913
AMPL1562999	9311 vs. R11	GA10,	90	0.06	GA8,	70	0.11	chr7	22587884	22587905
AMPL1563098	D62B vs. R62	CTT11,	219	0.09	CTT7,	234	0.05	chr1	2377852	2377899
AMPL1563104	D62B vs. R62	NA0,TC3,	22	0.09	AG13,TC4,	78	0.27	chr1	4636778	4636982
AMPL1563170	D62B vs. R62	TA13,	27	0.41	TA12,	24	0.38	chr1	30551523	30551546
AMPL1563183	D62B vs. R62	CT6,TG9,	180	0.09	CT7,TG10,	138	0.17	chr1	35240853	35240887
AMPL1563209	D62B vs. R62	TCC11,	23	0.13	TCC9,	45	0.18	chr10	4780097	4780126
AMPL1563216	D62B vs. R62	GA8,	31	0.10	GA7,	14	0.21	chr10	12483340	12483351
AMPL1563217	D62B vs. R62	AG9,	95	0.25	AG10,	52	0.35	chr10	12761302	12761321
AMPL1563226	D62B vs. R62	TCG5,	297	0.14	TCG9,	90	0.11	chr10	18888150	18888173
AMPL1563228	D62B vs. R62	AG11,TG4,	113	0.17	AG18,TG4,	27	0.19	chr10	19150841	19150906
AMPL1563230	D62B vs. R62	CT8,GCC7,	59	0.25	CT8,GCC8,	56	0.27	chr10	19512581	19512640
AMPL1563277	IRBB24 vs. R24	AG19,	35	0.26	AG7,	297	0.10	chr11	18274552	18274565
AMPL1563300	D62B vs. R62	TCA16,	45	0.18	TCA15,	20	0.15	chr12	2433282	2433308
AMPL1563309	D62B vs. R62	CG6,CCG8,	94	0.15	CG6,CCG5,	151	0.15	chr12	8834290	8834446
AMPL1563321	D62B vs. R62	CA4,GCC7,	103	0.13	CA4,GCC8,	84	0.12	chr12	14109142	14109175
AMPL1563350	D62B vs. R62	GAA15,	192	0.11	GAA8,	694	0.06	chr2	3836790	3836816
AMPL1563354	D62B vs. R62	5_TCT12,AG8,	10	0.60	A0,AG7,CT4	50	0.08	chr2	5453224	5453366
AMPL1563355	D62B vs. R62	CCG6,	141	0.10	CCG9,	109	0.09	chr2	6159658	6159681
AMPL1563368	IRBB24 vs. R24	AAT4_AAT7,	489	0.03	AAT4_AAT10	394	0.08	chr2	8760457	8760474
AMPL1563368	D62B vs. R62	AAT6,	481	0.07	AAT4_AAT10	283	0.08	chr2	8760457	8760474
AMPL1563370	IRBB24 vs. R24	AGA9,	186	0.13	AGA8,	539	0.07	chr2	11068833	11068862
AMPL1563370	D62B vs. R62	AGA9,	325	0.09	AGA8,	440	0.10	chr2	11068833	11068862
AMPL1563371	IRBB24 vs. R24	ATG22,	134	0.37	ATG15,	274	0.18	chr2	11389818	11389844
AMPL1563371	D62B vs. R62	ATG18,	184	0.33	ATG15,	188	0.20	chr2	11389818	11389844
AMPL1563372	IRBB24 vs. R24	GTCT6,GTAT4,	168	0.08	TCT4,GTAT	104	0.04	chr2	11466628	11466679
AMPL1563372	D62B vs. R62	GTCT6,GTAT4,	131	0.10	TCT4,GTAT	85	0.02	chr2	11466628	11466679
AMPL1563384	D62B vs. R62	TC10,TCG6,	45	0.09	TC9,TCG6,	18	0.33	chr2	18770047	18770137

Supplemental Table 8:The genotypes, coverages and stutter ratios and amplicon lengths of the differential SSRs between each of the three pairs of NILs

		Receptor parent			NIL parent			SSR location on reference genom		
Amplicon	Variety	Genotype	Coverage	Stutter ratic	Genotype	Coverag	Stutter ratio	Chr.	Begin	End
AMPL1563405	9311 vs. R11	TCG4,GCG5,	120	0.37	TCG4,GCG8,	44	0.30	chr2	25505108	25505143
AMPL1563406	9311 vs. R11	GCC5,	271	0.04	GCC7,	230	0.08	chr2	25672593	25672616
AMPL1563411	D62B vs. R62	TAA17,	15	0.27	TAA10,	14	0.14	chr2	28872633	28872680
AMPL1563413	D62B vs. R62	AG8,	208	0.08	AG15,	43	0.28	chr2	29585757	29585772
AMPL1563426	9311 vs. R11	GA16,	23	0.26	GA20,	29	0.41	chr3	2454209	2454248
AMPL1563494	IRBB24 vs. R24	GT8,GA13,	220	0.15	GT9,GA14,	117	0.50	chr3	33157739	33157790
AMPL1563540	D62B vs. R62	TTAA3,	202	0.02	TTAA5,	259	0.03	chr4	18599429	18599448
AMPL1563601	D62B vs. R62	AG8,	117	0.15	AG10,	87	0.22	chr5	4295803	4295826
AMPL1563618	D62B vs. R62	GCA8,	248	0.15	GCA9,	179	0.40	chr5	16811593	16811619
AMPL1563638	9311 vs. R11	AG6,TTTA5,	62	0.06	AG5,TTTA16	35	0.20	chr5	25016101	25016222
AMPL1563638	D62B vs. R62	AG6,TTTA5,	53	0.09	AG5,TTTA16	24	0.21	chr5	25016101	25016222
AMPL1563640	9311 vs. R11	CAC5,CGA7,CG	157	0.22	AC7,CGA5,CC	165	0.13	chr5	25771849	25772019
AMPL1563640	D62B vs. R62	CAC5,CGA7,CG	150	0.21	AC7,CGA5,CC	103	0.12	chr5	25771849	25772019
AMPL1563642	9311 vs. R11	C4,CCG5,AG15,N	87	0.54	4,CCG5,AG9,	43	0.47	chr5	26910831	26911031
AMPL1563671	D62B vs. R62	CTG5,	229	0.04	CTG6,	175	0.05	chr6	8815673	8815699
AMPL1563688	D62B vs. R62	CAC9,	36	0.11	CAC11,	10	0.30	chr6	16852149	16852175
AMPL1563697	D62B vs. R62	GATA6,	402	0.18	GATA13,	332	0.10	chr6	17297623	17297678
AMPL1563717	D62B vs. R62	TC16,	86	0.48	TC11,	159	0.23	chr6	23738108	23738133
AMPL1563739	D62B vs. R62	CCG6,	116	0.05	CCG11,	30	0.10	chr7	742939	742968
AMPL1563778	9311 vs. R11	CATC7,	220	0.03	CATC4,	185	0.02	chr7	18959633	18959668
AMPL1563795	D62B vs. R62	GTC5,TCC0,	420	0.14	GTC7,TCC0,	359	0.17	chr8	551775	551799
AMPL1563807	IRBB24 vs. R24	ATA5,ATA10,	40	0.13	ATA5,ATA13	35	0.11	chr8	6763734	6763817
AMPL1563810	IRBB24 vs. R24	GCG4,	273	0.04	GCG11,	88	0.07	chr8	9387098	9387121
AMPL1563810	D62B vs. R62	GCG4,	224	0.04	GCG7,	147	0.04	chr8	9387098	9387121
AMPL1563812	IRBB24 vs. R24	GAA7,AG4,	83	0.06	GAA9,AG4,	50	0.06	chr8	10524151	10524184
AMPL1563812	D62B vs. R62	GAA7,AG4,	126	0.05	GAA8,AG4,	54	0.09	chr8	10524151	10524184
AMPL1563820	IRBB24 vs. R24	CTAT5_CTAT4	243	0.05	CTAT5,	271	0.03	chr8	15835187	15835222
AMPL1563820	D62B vs. R62	CTAT5,	258	0.05	TAT5_CTAT	228	0.04	chr8	15835187	15835222
AMPL1563821	IRBB24 vs. R24	CT9,TA11,	37	0.14	CT11,TA9,	69	0.10	chr8	15972723	15972785
AMPL1563821	D62B vs. R62	CT10,TA9,	41	0.17	CT9,TA11,	31	0.29	chr8	15972723	15972785

Supplemental Table 8:The genotypes, coverages and stutter ratios and amplicon lengths of the differential SSRs between each of the three pairs of NILs

		Receiptor parent			NIL parent			SSR location on reference genome		
Amplicon	Variety	Genotype	Coverage	Stutter ratio	Genotype	Coverage	Stutter ratio	Chr.	Begin	End
AMPL1563823	IRBB24 vs. R24	CGAT5,CT9,	201	0.21	CGAT5,CT7,	320	0.16	chr8	17541009	17541050
AMPL1563823	D62B vs. R62	CGAT5,CT7,	226	0.15	CGAT5,CT9,	160	0.19	chr8	17541009	17541050
AMPL1563825	IRBB24 vs. R24	AAG11,AAG5,	129	0.12	AAG8,AAG5,	238	0.08	chr8	17945092	17945127
AMPL1563825	D62B vs. R62	AAG8,AAG5,	249	0.09	AAG11,AAG5	96	0.14	chr8	17945092	17945127
AMPL1563826	D62B vs. R62	AGAT7,	58	0.05	AGAT13,	90	0.14	chr8	18763626	18763685
AMPL1563844	D62B vs. R62	AGG5,	321	0.05	AGG7,	225	0.04	chr8	26322442	26322465
AMPL1563846	D62B vs. R62	AAG9,	278	0.13	AAG6,	232	0.08	chr8	26549759	26549782
AMPL1563850	D62B vs. R62	TG4,TGA11,	56	0.09	TG4,TGA4,	85	0.02	chr8	26728356	26728527
AMPL1563853	D62B vs. R62	CT8,	287	0.12	CT9,	209	0.10	chr8	27049866	27049887
AMPL1563866	D62B vs. R62	CCT4,CTT9,	83	0.10	CCT4,CTT8,	52	0.13	chr9	152507	152564
AMPL1563876	D62B vs. R62	GATA5,	205	0.04	GATA14,	117	0.08	chr9	6601053	6601088
AMPL1563877	D62B vs. R62	AGAT7,	216	0.13	AGAT5,	134	0.05	chr9	6780406	6780425
AMPL1563890	D62B vs. R62	TCT10,	28	0.18	TCT5,	49	0.08	chr9	10128853	10128885
AMPL1563893	D62B vs. R62	TTTA5,	58	0.03	TTTA3,	63	0.05	chr9	12313181	12313200
AMPL1563896	D62B vs. R62	CGG6,AGG3,	53	0.13	CGG3,AGG3,	151	0.02	chr9	14812719	14812798
AMPL1563897	D62B vs. R62	TCAA7,	145	0.06	TCAA5,	135	0.08	chr9	14977341	14977376
AMPL1563903	D62B vs. R62	CT16,	56	0.27	CT11,	46	0.57	chr9	19320231	19320428
AMPL1563906	D62B vs. R62	CT16,	17	0.53	CT10,	68	0.31	chr9	20174852	20174871

Supplemental Table 9: AmpSeq-SSR possesses high reproducibility

Stutter ratio (⩽ s) Coverage (⩾c)	≤0.1	≤0.2	≤0.3	≤0.4	≤0.5	≤0.6	≤1
≥1	97.50%	97.49%	97.21%	96.49%	95.22%	94.79%	89.89%
≥10	99.97%	99.88%	99.81%	99.65%	99.46%	99.26%	97.89%
≥20	99.98%	99.90%	99.84%	99.73%	99.62%	99.49%	98.42%
≥30	99.98%	99.92%	99.87%	99.78%	99.68%	99.54%	98.65%
≥40	99.98%	99.91%	99.89%	99.80%	99.68%	99.58%	98.78%
≥50	100.00%	99.97%	99.94%	99.85%	99.75%	99.66%	99.54%
≥100	100.00%	99.97%	99.95%	99.95%	99.91%	99.84%	99.37%
≥200	100.00%	100.00%	100.00%	100.00%	99.95%	99.95%	99.60%
≥500	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%