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著者	Shiokai Sachiko, Shirasawa Kenta, Sato Yutaka,
	Nishio Takeshi
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Improvement of the dot-blot-SNP technique for efficient and cost-effective genotyping

Sachiko Shiokai, Kenta Shirasawa ^a, Yutaka Sato ^b, Takeshi Nishio*

Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai 981-8555, Japan

* Corresponding author

a. Kazusa DNA Research Institute, 2-6-7 Kazusa-kamatari, Kisarazu, Chiba 292-0818, Japan

b. National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

Abstract

Although the dot-blot-SNP technique is a laborsaving, cost-effective method for SNP genotyping of a large number of plants, the synthesis of 5'-digoxigenin(DIG)-labeled oligonucleotides for use as probes is still costly. We developed two probe-labeling methods for this technique, one being digoxigenin labeling of oligonucleotides by PCR (PCR-DIG labeling) and the other being hybridization using a bridge probe and a 5'-DIG-labeled oligonucleotide (bridge hybridization). Bridge hybridization detected allele-specific signals under hybridization conditions similar to those for the 5'-DIG-labeled oligonucleotides and biotin-labeled oligonucleotides, while signals were detected only under a lower stringency condition by PCR-DIG labeling. As a method for genotyping using many markers at one time, two methods, i.e., PCR using mixed primer pairs and hybridization using mixed probes, were examined with successful results. Eighty-five SNP markers designed for genotyping of rice cultivars detected allele-specific signals, the genotyping results corresponding to the previously reported ones.

Keywords: SNP analysis, Dot-blotting, Large-scale genotyping, Cultivar identification

Introduction

Two methods, i.e., PCR-DIG-labeling and bridge hybridization (Fig. 1), were examined for detecting SNPs. 5'-biotin-labeled oligonucleotides were also used for comparison with 5'-DIG-labeled For preparation of the PCR-DIG-labeled oligonucleotides. oligonucleotide probe, a template oligonucleotide of 49 nt consisting of a 17-nt probe sequence inserted between 4-nt sequences (GGCC) recognized by HaeIII, and 12-nt sequences for annealing of primers both ends (5'-GATACAGTGTAC-3' at and 5'-TGTCACAGTGAC-3') was synthesized. The 49-bp oligonucleotide was amplified and labeled by PCR with primers (5'-GATACAGTGTACGG-3' and 5'-GTCACTGTGACAGG-3') using PCR-DIG-labeling mix (Roche Diagnostics, Switzerland). The PCR product was cleaved by Hae III. After denaturation in boiling water, equal volumes of the cleaved products of the DIG-labeled oligonucleotide and the unlabeled oligonucleotide as a competitive probe without removal of both end sequences were added to hybridization buffer. For bridge hybridization, a 48-nt oligonucleotide consisting of a 17-nt sequence of one allele of an SNP marker, a 6-nt spacer sequence (TATATT), and a 25-nt bridge arbitrary sequence, which is an sequence (5'-ACGAAGCCTCTTAATTGCGAATGTA -3'), was prepared as a bridge probe. A 48-nt oligonucleotide having a 17-nt sequence of the other allele of the SNP marker, the 6-nt spacer sequence, and 25-nt bridge sequence а (5'-TGAAAAGAAGCTTCATCCTTCTCGT-3') was also prepared as a competitive probe. A 5'-DIG-labeled oligonucleotide having a sequence complementary to the 25-nt bridge sequence of the bridge probe (5'-TACATTCGCAATTAAGAGGCTTCGT-3' or 5'-ACGAGAAGGATGAAGCTTCTTTTCA-3') was added to hybridization buffer. Signals were detected as previously described (Shirasawa et al. 2006).

SNP marker S10844, whose 'Nipponbare'-type allele is contained in 'Nipponbare', 'Koshihikari', 'Akihikari', and

'Kasalath' and whose variant-type allele is contained in 'Hatsuboshi', 'Kihou', 'Hitomebore', and 'Kirara397' (Shirasawa et al. 2006), was used for evaluation of the labeling methods. Under the highest stringency condition tested, i.e., hybridization and washing with 0.1 × SSC/0.1% SDS at 50°C, allele-specific signals were detected by 5'-DIG labeled oligonuceotides, but PCR-DIG-labeled oligonucleotides, bridge hybridization, and 5'-biotin-labeled oligonucleotides showed no signal (Fig. 2). On the other hand, non-specific signals were high in the analysis of the variant-type allele under the lowest stringency condition, i.e., hybridization and $1 \times SSC/0.1\%$ SDS washing at 40°C, by 5'-DIG-labeled oligonucleotides and bridge Allele-specific signals were detected by hybridization. PCR-DIG-labeled oligonucleotides and 5'-biotin-labeled oligonucleotides under this hybridization condition. Under intermediate stringency conditions, i.e., hybridization and 0.1 \times SSC/0.1% SDS washing at 40°C or hybridization and 1 \times SSC/0.1% SDS washing at 50°C, allele-specific signals for both alleles were clearly detected by bridge hybridization and 5'-DIG-labeled oligonucleotides, and weakly detected by 5'-biotin-labeled oligonucleotides, but not detected by PCR-DIG-labeled oligonucleotides. Seven SNP markers, which are necessary for distinguishing all the 17 Japonica cultivars used in this study (Table 1), were prepared with these two labeling methods and biotin labeling. Although all the labeling methods detected allele-specific signals, the optimum hybridization conditions were different not only between the SNP markers but also between the labeling methods (Table 2). higher Bridge hybridization vielded signals than PCR-DIG-labeled oliginucleotides.

The dot-blot-SNP technique is suitable for genotyping of many plant individuals, but not for analyzing a small number of plants. For identification of cultivars using a few plants, analysis with multiple SNP markers is desirable. Using bridge



Fig. 1. Scheme of PCR-DIG labeling and bridge hybridization. a: PCR-DIG-labeling. Probe sequences were amplified and labeled by PCR with DIG-dUTP using an oligonucleotide as a template. After cleavage with *Hae*III, the DIG-labeled probe was hybridized with dot-blotted PCR products together with an unlabeled competitive probe. Black boxes indicate sequences used as probes, and white boxes and gray boxes show recognition sites of *Hae*III and sequences for primer annealing in PCR-DIG labeling, respectively. b: Bridge hybridization. A bridge probe having an allele-specific sequence (black box), a spacer sequence (white box) and a bridge sequence (gray box) was hybridized with dot-blotted PCR products together with a competitive bridge probe and a 5'-DIG-labeled oligonucleotide having a sequence complementary to the bridge sequence.

hybridization probes and 5'-biotin-labeled oligonucleotides, we tested two methods of dot-blot hybridization with multiple SNP markers, one being multiplex PCR followed by dot-blot-SNP analysis with different membranes for each probe and the other being dot-blotting of PCR products amplified with each primer pair onto two membranes followed by hybridization with mixtures of multiple probes. After multiplex PCR using the primer pairs to amplify 300-bp DNA fragments for the seven SNP markers shown in Table 2, bridge hybridization detected allele-specific signals in both alleles of S0651, R1744, E61310, C53863, and E2439, but no signal in E1919. Analysis of variant-type alleles in R2702 showed many false signals (Fig. 3). 5'-biotin-labeled oligonucleotide probes yielded similar results, but slightly lower signal intensities.

Using the other method, i.e., separate PCR and hybridization with multiple probes, allele-specific signals without non-specific signals were detected in four of the seven SNP markers in bridge hybridization (Fig. 4). Non-specific signals were high in analyses of the both alleles of S0651 and E2439 and the 'Nipponbare' allele of R2702. Raising the hybridization temperature enabled detection of allele-specific signals of these SNP markers, but such a hybridization condition reduced the allele-specific signals of R1744 and E61310. In hybridization with 5'-biotin-labeled oligonucleotide probes, intensities of allele-specific signals were lower than those in bridge hybridization, but non-specific signals were also low, enabling easier genotyping.

Oligonucleotides for 45 loci having SNPs between Japonica rice cultivars (Shirasawa et al. 2007) were prepared and labeled by the PCR-DIG-labeling method. For detection of allele-specific signals, hybridization and washing conditions were optimized (Supplementary Table 1). 5'-biotin-labeled oligonucleotides for 20 loci were also designed (Supplementary Table 2). Genotypes of 17 Japonica rice cultivars were analyzed by dot-blot-SNP using the 45 PCR-DIG-labeled oligonucleotides and the 20 5'-biotin-labeled oligonucleotides, the results corresponding to the genotyping by PCR-RF-SSCP reported by Shirasawa et al. (2007). Bridge probes for 20 SNP markers were also prepared for graphical genotyping of low-temperature tolerant lines developed by a cross between 'Hitomebore' and 'Lijiangxintuanheigu' (Supplementary Table 3), and all of them yielded allele-specific signals.

Although the most powerful tools for large-scale SNP analysis are considered to be the microarray techniques based on allele-specific hybridization (Hacia et al. 1998) or primer extension (Syvänen 2001, Shen et al. 2005), these techniques require costly

							0.1	X	SSC	C/(0.1 ^o	%S	DS										1)	(SS	SC /	0.	1%5	SDS	5				
					5)°(C						40	°C							50	°C							40	°C			
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
PCR-DIG-	A							4								1	1	*							10	•		0	0	•	. 0	1.0	0
labeled probe	В																			-0	1					24	4	•	•		•		04
Bridge probe	A					. 0		-		•	•	0	0	•	9.0	0	•	•	•	0	0	•	0	0	•	•	•	•	•	•	•	•	•
Bridge probe	в									0	0	•	٠	0	•	•			÷.,	•	•		•	•		•	•	•	•	•	•	•	•
5'-biotin-	A	1	1								0			0	1		٥	•	0		6	0	•			•			13	•			
labeled probe	в					<i>a</i> .						0	0		0	0		1	5	a second	a		.0	20			100	0	0	3	0	0	
5'-DIG-	A	•	٠						٠	•	•	0	6	•	0	0	•	•	•	0	0	•	0	0	•	•	•	۰	٠	•	•	0	٠
labeled probe	в			۰	•		٠	٠	ø	0	0	•	•	0	•	•	0	•	0	•	٠	.0	•	•	0	•	•	•	•	٠	•	•	٠

Fig. 2. Dot-blot-SNP analysis using the three types of oligonucleotide probes under different hybridization conditions. Used SNP marker was S10844 (probe sequences, CAGTAGAGCTGTGGGAGA and CAGTAGAGTTGTGGAGA; primer sequences CTCTTCTACGCCAGGTTCCAA and GCACAGTAACCAGATCAACAG, Shirasawa et al. 2006). The probes were hybridized with PCR products of eight cultivars. A, 'Nipponbare'-type allele; B, variant-type allele. 1, 'Nipponbare'; 2, 'Koshihikari'; 3, 'Hatsuboshi'; 4, 'Kihou'; 5, 'Akihikari'; 6, 'Hitomebore'; 7, 'Kirara397'; 8, 'Kasalath'. Hybridization and washing were performed at 50°C or 40°C, and second washing was carried out with $0.1 \times SSC/0.1\%$ SDS or $1 \times SSC/0.1\%$ SDS. 5'-DIG-labeled oligonucleotides were used for control experiment.

Table 1 A set of SNP markers required for identification of the 17 cultivars

Marker name	Chr	bp	1. Nipponbare	2. Akihikari	3. Hatsuboshi	4. Hatsunishiki	5. Hinohikari	6. Hitomebore	7. Itadaki	8. Kihou	9. Kirara397	10. Koganebare	11. Koshihikari	12. Nourin 1	13. Nourin 6	14. Nourin 8	15. Nourin 22	16. Rikuu 132	17. Sasanishiki
E1919	1	4905189	Α	В	В	В	Α	В	В	Α	В	Α	В	В	Α	Α	Α	В	В
S0651	4	24229543	Α	Α	в	Α	Α	Α	в	В	Α	в	Α	Α	Α	Α	Α	Α	Α
R1744	7	259886	Α	в	Α	Α	Α	Α	Α	Α	Α	Α	Α	Α	В	В	в	В	Α
E61310	7	24283242	Α	в	в	в	в	В	Α	Α	Α	Α	В	в	В	Α	в	В	Α
C53863	7	27391821	Α	Α	Α	Α	В	Α	Α	Α	Α	Α	В	В	Α	Α	Α	Α	Α
E2439	10	14680293	Α	Α	Α	В	Α	Α	В	Α	В	Α	Α	В	Α	Α	Α	В	в
R2702	11	30616183	Α	в	в	Α	в	в	в	в	Α	Α	в	в	в	Α	Α	в	в

A, 'Nipponbare'-type allele; B, variant-type allele.

special equipment, which is not available in most laboratories or small research institutes. Furthermore, these techniques are not suitable for genotyping of a small number of loci, less than 50, of a large number of plants, more than 1,000, which are used in conventional crossbreeding programs. Despite being a classical technique, the dot-blot-SNP technique (Shirasawa et al. 2006) may be the most laborsaving, cost-effective technique among various SNP assays for analysis of a large number of individuals. Although hybridization and signal detection in the dot-blot-SNP technique is somewhat laborious, the labor and time for each sample are small because more than 1,000 samples can be analyzed at one time. This technique requires no special equipment and is usable in any small laboratory. One problem with this technique, however, is the high cost for synthesizing the 5'-DIG-labeled oligonucleotides.

Although PCR-DIG-labeled oligonucleotides detected no signal under the same hybridization condition as the 5'-DIG-labeled oligonucleotides, optimizing the hybridization condition enabled

detection of allele-specific signals. Oligonucleotides for PCR-DIG-labeled probes can be prepared at low cost, but the DIG-labeling process entails some labor. Bridge hybridization was found to be nearly as sensitive as direct hybridization of 5'-DIG-labeled oligonucleotides. Bridge hybridization is a complicated technique using three oligonucleotides for hybridization, i.e., a bridge probe having the sequence of an allele to be detected, a bridge probe having the sequence of the other allele as a competitive probe, and the 5'-DIG-labeled oligonucleotide that hybridizes only with the former bridge probe. However, one pair of 5'-DIG-labeled oligonucleotides can be applied to any SNP markers. Preparation of only a pair of unlabeled 48-nt oligonucleotides is required for producing a new SNP marker. Bridge hybridization is considered to be the most cost-effective and laborsaving method among the four labeling methods tested when analyzing SNPs at many loci.

Both multiplex PCR followed by hybridization with different oligonucleotide probes and separate PCR followed by hybridization

Table 2 N	ucleotide sequences and optimum hyb	oridization conditions of the seven SNP market	rs used for	identification	of the 17	rice cultiv	vars				
Marbar	Forward primer sequence (5'-3')	Probe sequence of 'Nipponbare' type (5'-3')	5.	biotin-labeling		PC	R-DIG-labeling		Bri	dge hybridizat	ion
name	Reverse primer sequence (5'-3')	Probe sequence of variant type (5'-3')	Temp."	Washing buffer	Ratio ^b	Temp.	Washing buffer	Ratio	Temp.	Washing buffer	Ratio
01010	AGCTGCTCGAAGTGAAGGAG	GTCATCCCaATTCCAGT	50	1 x SSC/ 0.1%SDS	1:5	40	1 x SSC/ 0.1%SDS	1:5	50	0.5 x SSC/ 0.1%SDS	1:10
51613	GCTTCAAACTTTATCGGAAC	GTCATCCCgATTCCAGT	50	1 x SSC/ 0.1%SDS	1:5	40	1 x SSC/ 0.1%SDS	1:5	50	0.5 x SSC/ 0.1%SDS	1:10
13200	GCCTGGGGGAATCAAGAAAAT	TATTGTGCcGGTGTTGA	50	1 x SSC/ 0.1%SDS	1:5	40	1 x SSC/ 0.1%SDS	1:5	50	0.5 x SSC/ 0.1%SDS	1:10
10000	AAACAATGACCAGGTACTGG	TATTGTGCtGGTGTTGA	50	1 x SSC/ 0.1%SDS	1:5	40	1 x SSC/ 0.1%SDS	1:5	50	0.5 x SSC/ 0.1%SDS	1:10
77210	CGCGTGGAAAATGAGAGAGT	GTACATACaTTGTGTAT	40	1 x SSC/ 0.1%SDS	1:5	40	1 x SSC/ 0.1%SDS	1:1	40	0.5 x SSC/ 0.1%SDS	1:10
KI / ##	TGAGATAAATAGGATCGACC	GTACATACgTTGTGTAT	40	1 x SSC/ 0.1%SDS	1:5	40	1 x SSC/ 0.1%SDS	1:1	40	0.5 x SSC/ 0.1%SDS	1:10
016170	CTGTAGCGTCGTTAAGCACG	TAAATGATaGAAATTCT	30	0.1 x SSC/ 0.1%SDS	1:1	30	1 x SSC/ 0.1%SDS	1:5	30	0.1 x SSC/ 0.1%SDS	1:1
E016103	ACCAGAAAAGCGCACAAAGT	TAAATGATgGAAATTCT	30	0.1 x SSC/ 0.1%SDS	1:1	30	1 x SSC/ 0.1%SDS	E	40	0.1 x SSC/ 0.1%SDS	1:10
0,0000	GAGCTGTTCACGCTCACGTA	GGTGTATTaCAGCGATT	40	1 x SSC/ 0.1%SDS	1:5	40	1 x SSC/ 0.1%SDS	1:5	40	0.5 x SSC/ 0.1%SDS	1:5
2085227	TGTCGCAGTGTAAAATCTGC	GGTGTATTICAGCGATT	40	1 x SSC/ 0.1%SDS	1:5	40	1 x SSC/ 0.1%SDS	1:1	50	0.5 x SSC/ 0.1%SDS	1:10
E3430	TCGGAGCTCCAGTTCCGAGC	AGGCTTTeGCCTCGGC	50	0.1 x SSC/ 0.1%SDS	1:5	40	0.1 x SSC/ 0.1%SDS	1:1	50	0.1 x SSC/ 0.1%SDS	1:5c
12439	GCCATGGTGTGTTTGCTCTA	AGGGCTTTgGCCTCGGC	50	0.1 x SSC/ 0.1%SDS	1:5	50	1 x SSC/ 0.1%SDS	E	50	0.1 x SSC/ 0.1%SDS	1:5c
00260	AGGATTTCTTCAACGGCA	CTGCTGGGgAAGTTTGA	50	1 x SSC/ 0.1%SDS	1:10	50	1 x SSC/ 0.1%SDS	1:1	50	0.5 x SSC/ 0.1%SDS	1:5
70/7V	TTCAGGATACCATTGGCATC	CTGCTGGG¢AAGTTTGA	50	1 x SSC/ 0.1%SDS	1:10	50	1 x SSC/ 0.1%SDS	1:5	50	0.5x SSC/ 0.1%SDS	1:5
a: Hybridi:	zation and washing temperature, b: Ra	tio of a labeled probe to an unlabeled competi-	itive probe	, c: Long brid	ge sequen	ces (25 nl	() were used for	r this ma	rker		

		Bridge	e probes	5'-biot	in labeled probes
		'Nipponbare'-type probe	Variant-type probe	'Nipponbare'-type probe	Variant-type probe
		1 2 3 4 5 6 7 8 91011121314151617	1 2 3 4 5 6 7 8 91011121314151617	1 2 3 4 5 6 7 8 91011121314151617	1 2 3 4 5 6 7 8 9 1011121314151617
E1919	S	• • • • • • • • • • • • • • • • • • • •	••• •• • •• ••		
S0651	SM	•••••		••••••••••••••••••••••••••••••••••••••	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
R1744	SM	• • • • • • • • • • • • • • • •	: :::	• • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • •
E61310	S M	• • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • •	· · · · · · · ·	• •
C53863	S M	••••••••••••••••••••••••••••••••••••••		••••••••••••••••••••••••••••••••••••••	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
E2439	S M	•••• •• • • •• •••			
R2702	S M	• • • • • • • • • • •		• • • • • • • • • • • • • • • • • • •	•••••••

Fig. 3. Dot-blot-SNP analysis using multiplex PCR. DNA fragments of the 17 cultivars (1 to 17, Table 1) were amplified by PCR with a single primer pair (S) or by multiplex PCR with a mixture of the seven primer pairs (M) for E1919, S0651, R1744, E61310, C53863, E2439, and R2702. Both bridge hybridization markers and 5'-biotin-labeled oligonucleotides were used.

	Bridge	probes	5'-biotin lat	peled probes
	'Nipponbare'-type probe	Variant-type probe	'Nipponbare'-type probe	Variant-type probe
	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
E1919 S0651	• • • • • • • • • • • • • • • • • • •	· · · · · · · · · · · · · · · · · · ·		
R1744		• • • • • • • • • • • • • • • • • • • •		
C53863		· · · · · · · · · · · · · · · · · · ·		
E2439 R2702	• • • • • • • • • • • • • • • • • • •	••••••••••	• • • • • • • • • • • • • • • • • • •	

Fig. 4. Genotyping of the 17 cultivars using mixed probes of dot-blot-SNP markers. 'Nipponbare'-type probes of seven SNP markers, i.e., E1919, S0651, R1744, E61310, C53863, E2439, and R2702, were mixed and hybridized separately with variant-type probe mixture. The 17 cultivars are shown as '1' to '17' (Table 1).

with multiple probes detected allele-specific signals and were found to be useful techniques for analysis of a small number of samples with many SNP markers. In multiplex PCR using seven primer pairs, five markers detected allele-specific signals by both bridge hybridization and 5'-biotin labeling. The inability to detect allele-specific signals is considered to be due to the low ability of some primer pairs to amplify the target sequences under the thermal cycling condition used for multiplex PCR. Hybridization with mixed probes also yielded allele-specific signals with four SNP markers, while three markers detected non-specific background signals. Further addition of the competitive oligonucleotides may reduce the background signals. Since optimum hybridization conditions are different between probes, choosing SNP markers having similar hybridization conditions would yield better results in hybridization with multiple probes.

The remaining laborious step of dot-blot-SNP analysis that should be improved is preparation of genomic DNAs from a large number of plants for PCR templates. DNAs prepared by the CTAB method (Murray and Thompson 1980) or the method of Edwards et al. (1991) are reliable PCR templates, but these methods require much time. In the method of Wang et al. (1993), only maceration of a plant tissue in alkaline solution and dilution of the solution are required. Although this method is highly simple, but success of DNA amplification has been found to be inconsistent (Shirasawa et al. 2006, our unpublished results). Blotting DNA from a plant tissue onto FTA[®] cards (Whatman, UK) can be used as a simple method for preparation of the PCR templates (Roy and Nassuth 2005), although it is costly. Recently we found a 1-mm leaf disk to be directly usable as a PCR template for dot-blot-SNP analysis (Shiokai et al. 2009).

The SNP markers developed in the present study can be used for genotyping of plants and identification of Japonica rice cultivars. Since DNA polymorphisms are infrequent between the genomes of Japonica rice cultivars (Nasu et al. 2002; Monna et al. 2006; Shirasawa et al. 2004, 2007), these markers may also be useful for QTL analysis and marker-assisted selection using progeny of a hybrid between Japonica rice cultivars. These dot-blot-SNP markers together with the 100 markers reported in our previous paper (Shirasawa et al. 2006) are good additional DNA markers for Japonica rice.

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Supplementary Data

Supplementary Table 1 Nucleotide sequences and hybridization conditions of PCR-DIG-labeled dot-blot-SNP markers

Supplementary Table 2 Nucleotide sequences and hybridization conditions of 5'-biotin-labeled dot-blot-SNP markers

Supplementary Table 3 Nucleotide sequences and hybridization conditions of bridge hybridization markers

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Supplementary	table	1 Nucleotide	sequences and hybridization condition	s of PCR-DIG-labeled dot-blot-SNP markers	lemperature of	
Marker name	Chr.	SNP position	Forward primer sequence (5'-3') Reverse primer sequence (5'-3')	Probe sequence of 'Nipponbare' type (5'-3') Probe sequence of variant type (5'-3')	hybridization and second	Second washing buffer
S0063	1	1017259	AGTTAACCGCCAGGCTTACA	TTGAAACCaTATACATC	40	1xSSC/0.1%xSDS
C0122	1	28629759	GGTCATCTATGCTAGCACAC	GACTACAGCACAATTGC	40	1x55C/0 1%x5D5
50122		20020700	ATCCTCTGGAACTTGCTTGC CCCTGTAACAAGGAGTTGAT	GACTACAGtACAATTGC TGAAAAATaCTATCAAC	40	
R0655	1	34355286	ACCTCCTTGAAGTTCTGCTGTCT	TGAAAAATgCTATCAAC	40	1xSSC/0.1%xSDS
C63989	1	34611539	ACCTTCTACTGCTCCATTAG	TGGATACAgCATTGGCT	40	1xSSC/0.1%xSDS
S4284	1	39470663	GTGTGCAGCTATTGTGTAGA GTACAATTGCTGACATGTGG	TATTAAGCgTATGCTAT TATTAAGCcTATGCTAT	40	1xSSC/0.1%xSDS
C30021	1	40211213	CTAAGTTCCTTGGGCGACAA	TGAGTTACtGCCATTTG	40	1xSSC/0.1%xSDS
\$13049	2	2924042	ATCTGTGAAATGTCCGACCT	CTAGTTCAtGAAATTAG	40	1xSSC/0 1%xSDS
010040	-	2024042	TAGGACAAAGCCAGGAAGGA ACACACAAACGGTTGGTTCA	CTAGTTCAgGAAATTAG AGCATACAITGTAACCA	40	
C60715	2	2882443	CGGCTCTAGCGGTCAACGAG	AGCATACACTGTAACCA	40	1x55C/0.1%x5D5
R0910	2	3142223	GCACAGCATGAAAGCAATGG	GTCAATGGaGTCTGCGG	40	1xSSC/0.1%xSDS
E10780	2	17696138	GGACTGAGGTGAGAGATCTTGAG TCATCCGTATTTGAGTCGGG	AAAATCATgAGCTGCAA AAAATCATaAGCTGCAA	40	1xSSC/0.1%xSDS
E31585	2	23267087	CTACCACTAACATCTGAACC	ATTGATTTAATAAGCTA	40	1xSSC/0.1%xSDS
\$20768	2	25028486	TTGTAATGCCAACTGCTGAC	ATCAGTTATACAACTG	40	1xSSC/0 1%xSDS
040040	-	20020100	CCTGCAAAGCAGAACTGTTGTA ACTGGCTTGCAATGCTTTCT	ATCAGTTACTACAACTG AATTAACAtGATAAGAA	10	1:000/0.11/::000
513818	2	33206902	ACTAAGCTCGACAGGACCAC	AATTAACAcGATAAGAA	40	1x55C/0.1%x5D5
C12409	2	36572369	TCTGCAGCTATTGACCACTG	TATGGATCAAAGTAGAA	40	1xSSC/0.1%xSDS
E11192	2	31563824	CTGTGTGCTGATTTATTCGG GGAGGAGGGTGTACTCTTGCTC	TTACACCTgAAGTGATT TTACACCTcAAGTGATT	40	1xSSC/0.1%xSDS
E60742	3	33298583	GGCCCAGTATTGACTTTGTTTC	TCGGATTCaTCAGTCTT	50	0.1xSSC/0.1%xSDS
E610/6	4	1030701	AAGGTAATGGTGCAGTAGCTCAG	ATAGTTTCcGACATCTA	40	1×550/0 1%×505
201340		1000701	TTGCTGTAGCGCTGTTGCCG GGTCCCTCAAGGTCATCAAA	ATAGTTTCaGACATCTA TCCAGTTGaCAAGCAAC	40	1,000,0.17,0000
E1294	4	14861640	ACTCAAGAGTTCCTGTAAGC	TCCAGTTGgCAAGCAAC	40	1xSSC/0.1%xSDS
S0651	4	24229551	AAACAATGACCAGGTACTGG	TATTGTGCCGGTGTTGA	40	1xSSC/0.1%xSDS
S3010	4	32432915	TGATGCATGCGTGTTCTCTT CCTTGCCTTGGAGGTGATTC	GATGCCTCcGGAGAAAT GATGCCTCcGGAGAAAT	40	1xSSC/0.1%xSDS
S14881	6	17611605	GATGACCGGTCCTGTTGCTT	CTCCTCCGaCCAGGAGC	40	1xSSC/0.1%xSDS
C30223	6	20107088	GAAGCACTAGAAGCCCCCTATTA	TCATGGGACCCTTCCTG	40	1×550/0 1%×505
030223	0	2019/000	AGACAGTTGGAACTATTGCC		40	18330/0.1/08303
E61502	6	22378340	CTATAGTGCACATGGATGCTAAGG	CTATTACTaACTTCGAA	30	1xSSC/0.1%xSDS
C12560	6	24422125	GCATCTAACCTTGCGGATAG	ACACTAGGETCAGTACT	40	1xSSC/0.1%xSDS
E60075	7	5490161	GTCGTATGTTGCAGGAACCA GTATTGGAGCACAACATGCC	AATGTTGTgAAGCACAT AATGTTGTcAAGCACAT	50	0.1xSSC/0.1%xSDS
E50426	7	9023318	CGCACCATTTAGCAGTTTGA	GTTGCATAgAACCTATT	40	1xSSC/0.1%xSDS
E 1 2 E E	-	12579070	GGTGTACTAGTGTCCAGAAG	CATTGAACaAACCTATT	10	1/200/0 10/ 2000
E01200	'	13576072	CTACAGGTTCCCATCTGCTC	CATTGAACgAACCCATG	40	1x330/0.1%x3D3
C11630	7	28357612	TTGCTGCAGTGTCTGGTGTA	GCTTCTGCcAACATTAT	50	1xSSC/0.1%xSDS
C10985	7	28362096	ATGTTTCCATATTCTTCAGG	GGATGGTAtGGATGAAC GGATGGTAcGGATGAAC	40	1xSSC/0.1%xSDS
E30622	7	29014196	TAGGACATTGGGTGAGCTTG	ATTCAGTGCTGATACGA	40	1xSSC/0.1%xSDS
R3089	7	18997374	ATCGCCTCCGCTTCTGCTTT	ATCAACTAcGCTATTTA	40	1xSSC/0 1%xSDS
015651		5265002	CCAACAGGTATGGCAATATG	ATAATTGCgTGATCCCT	10	1/200/0 10/ 2000
515051	8	5365093	CTATTTACACGAGGCATTGTCG	ATAATTGCCTGATCCCT	40	1x55C/0.1%x5D5
S2092	8	5221699	GCACGAGGATGCACATGTGG	AATTTCCCaGGCCTCAA	40	1xSSC/0.1%xSDS
S4853	8	5226393	AACAGTTTGTGCCTGCACCTA	IAGGAG IAtATAATTTC TAGGAGTAcATAATTTC	40	1xSSC/0.1%xSDS
E20920	8	6037745	ATCAGCGACCGCCAATGTCG	TCGATCTCCTTCTGCCT	40	1xSSC/0.1%xSDS
C52909	8	20314028	GGCTGGTGCGCTGTACATAA	CCCTGCATgATTGAGAT	N: 50, V: 40	1xSSC/0.1%xSDS
E64000		6040500	I GAI GCAGCAGTCAAACACA CTCACTCGGTGCATGGAATG	CCCTGCATaATTGAGAT ACCGTCACtAGCTGAAA	40	1/200/0 10/000
E01980	8	6240593	CCTGATAGTGCCAGATTAAT		40	1X330/0.1%X8D8
E4156	8	28163769	ACCATGTACATTAGCGTACC	CAATCCACtGTATTTGA	40	1xSSC/0.1%xSDS
S13017	9	12729050	GGACCTGTTCACTTTGGTGC ATTCTACCGGTCCAAGTCGTC	ATCGTAGCcAAAATAAA ATCGTAGCtAAAATAAA	40	1xSSC/0.1%xSDS
S0065	10	8699830	GTGGGATGTGGGACTCAAAC	AAAATATCaTTAGTCCA	40	1xSSC/0.1%xSDS
S14938	11	256540	GGTAGATTATTATGAGGACTGAC	GCTGTCTGgTTCCGGGG	40	1xSSC/0.1%xSDS
E 44005		0050110	TGGCAATAGACATAGGCTAG AAGCCTGTTCTACTACCACC	GCTGTCTGaTTCCGGGG AAAATTTTgGTAAGGTT		4.000/0.4% 000
E11325	11	2256140	GCCAATAGACGATCGGGCGC	AAAATTTTaGTAAGGTT	30	1x55C/0.1%xSDS
E0935	11	9119726	CTAAGCCAGATTGAGGACAT	ATCATTGACCATACAAT	30	1xSSC/0.1%xSDS
E3876	11	25197076	AGCATTTGGCTCGTAGCAAT CCTCCAATATGCTTGCAGGC	TCAAATGCaTCGACGAG TCAAATGCgTCGACGAG	50	0.1xSSC/0.1%xSDS
R2664	11	25335264	ACTGGGACACACGCTACAAGATA	GGTAGCTAaCAGCGATC	40	1xSSC/0.1%xSDS

a: 'N' represents a 'Nipponbare' type and 'V' does a variant type.

Supplymentar	y table	2 Nucleotide	sequences and hybridization condition	ons of 5'-biotin-labeled dot-blot-SNP markers		
Marker name	Chr	SNP position	Forward primer sequence (5'-3')	Probe sequence of 'Nipponbare' type (5'-3')	Temperature of hybridization and second	Second washing buffera
Marker Harrie	0111.	ora position	Reverse primer sequence (5'-3')	Probe sequence of variant type (5'-3')	washing	
C0101	1	4851683	CTCCATCTCCCATCCAGGTA	CTTGCTGTcGTTAGTGT	40	1xSSC/0.1%xSDS
			GGAACTACATATTCAGATCC	CTTGCTGTtGTTAGTGT		
C10106	1	8793184	CAGTGCTGTACTAGGCTTAG	AGCAGTAAcCAGTGCTG	40	1xSSC/0 1%xSDS
010100		0.00101	AACAGAGTCTGGCTTCTGCTG	AGCAGTAAaCAGTGCTG	10	110001011101020
C1456	1	26469268	GGATGTGTTAAAGGAGCCAG	AGCGTGCAtGGCGGAAC	50	0 1xSSC/0 1%xSDS
01100	•	20100200	AGCACTCTTGGTGCAACCAG	AGCGTGCAcGGCGGAAC		0.110000.0.1700000
S10045	1	30926011	GGACGTTGCCATGGAAGTCT	TTCATCTTgTTAGTGTT	40	1xSSC/0 1%xSDS
010040	•	00020011	CTGTGCAGGTACACAACAGAAAG	TTCATCTTaTTAGTGTT		110001011101020
C53722	2	17816703	CAGCATGGAGGTGGCATGAT	GAGGGCAAaGAGCTGGA	40	1xSSC/0 1%xSDS
000722	-		TAATGGGCAGATCACGACAG	GAGGGCAAgGAGCTGGA		incool of this back
E60475	2	18828120	GACAGIGACACAGIGACACC	GACAACTITICTAATAGC	40	1xSSC/0.1%xSDS
200110	-		CCACTGAAATGGGTAACTCTGTC	GACAACTTaGCTAATAGC		
C30024	3	5901203	GATGCTAGTGGTGCTGTCCG	ATAAACTGtATTTTTT	40	1xSSC/0 1%xSDS
000021	0	0001200	CACGCGTTTATCACTAGTGA	ATAAACTGaATTTTTTT	10	110001011101020
C0820	4	6896396	AAGGTTCATCAGTAACCATC	AAATGCTCaTTGAAGGA	40	1xSSC/0 1%xSDS
00020	-	0000000	TGTTCCTGGGCTCAATTAAC	AAATGCTCtTTGAAGGA	40	12000/011/02020
C0106	4	16667671	AACAGCATGAGAATAAAAACGC	CATACATAaACAAAAT	40	1xSSC/0 1%xSDS
00100	-		TATGCGGTTCTGCTGTATTG	CATACATAtcaACAAAAT	10	110001011101020
S21013	4	20120616	CACTCAGCTGGTTGAGGATG	CCATAATGgTGGACACT	40	1xSSC/0 1%xSDS
021010	-	20120010	GGATGCGTATTGGACCATCT	CCATAATGaTGGACACT	40	12000/011/02020
F20943	5	16554327	GGGTTCCTCCCTCGTGTATT	TCTGTTGGcCAATTGAT	40	N:1xSSC-V:0 1xSSC/0 1%xSDS
220010	0	10001021	TTTGCTACTTGAAGAACCAC	TCTGTTGGgCAATTGAT	10	
\$5407	6	4430027	GGATAGTAAGTGCTAGCAAA	TACACGAAGAAGAATAACA	40	1xSSC/0 1%xSDS
00407	v	4400021	TTGGAGTAGGGCATTGTTCA	TACACGAAGAAgaaGAATAACA	40	12000/011/02020
E61502	6	22377949	CGCTAAACAGCTCCACATTG	GTGGAGTGgAGTGGAGG	40	1xSSC/0 1%xSDS
201002	Ũ	22011010	GTACACAAGCGCTGACAAAATG	GTGGAGTGaAGTGGAGG	10	110001011101020
F0211	6	22841785	GAAGGCTGCGGCAGATAATA	gtcacttACCATCATTA	40	1xSSC/0 1%xSDS
20211	Ũ	22011100	ACCGTTGACCAAAATGGCTA	cagtcACCATCATTAGG	10	110001011101020
R1804	7	3324222	CGCAAGTACAGCATGCATTC	GTGTATTTgACTATCTG	50	1xSSC/0 1%xSDS
	•	0021222	GACCCCCTATAGCTTTGATTTCTC	GTGTATTTtACTATCTG		110001011101020
R1488	7	5290923	AGATAAGCAGAGGAAGGTCC	AAGATGGCtGAAGTATC	50	1xSSC/0 1%xSDS
	•	0200020	CTAGAGCATGAGAACTGACC	AAGATGGCcGAAGTATC		110001011101020
C61928	7	19182659	ATATTAGTCGTCCTAACAACTG	GATAAGAGagTGGTTAGG	40	1xSSC/0 1%xSDS
001020		10102000	CACGGTCCAAGTACCAAGGC	GATAAGAGTGGTTAGG	10	110001011101020
S4853	8	5227221	AAAACCGGAAGGAGTAATAGGG	ATAATAATCATATTGTT	30	1xSSC/0.1%xSDS
0.000	Ũ	0227221	GAATACTGCTTCCATCCCAG	ATAATAATtATATTGTT		110001011101020
\$3680	8	18344367	TGACCAGTTCAGATAACCTG	AAAACTACaTTTTAGCA	40	1xSSC/0 1%xSDS
23000	5	.00.4007	GGAGGTATGTCTTCCACATGCTA	AAAACTACgTTTTAGCA	.0	
F0349	9	17110873	CGAAGTGCTAATCACATCTG	ATGCAGGCcTCTCATAT	40	1xSSC/0.1%xSDS
20040			CCATGGATATCATGGATCTC	ATGCAGGCtTCTCATAT		1,000,01,1,0,020

a: 'N' represents a 'Nipponbare' type and 'V' does a variant type.

Marker name Chr. SNP position Reverse primer sequence (5:3) Probe sequence of variant type (5:3) "briddication and becond washing General washing Reconstruction and building NLSNP1 1 1971498 GTAGGTCATCCATAGTCCCATTC CGTGGATGGGATGGGAGGG 50 0.1xSSC0.1%SDS 1.5 1.5 NLSNP4 2 37753 CGGGACTGGATCGACCATC TGCTGCACGGGGGAGGGACGACCATC TGCTGCACGGGGGAAGGACTAAT TGCTGGATGGTGAGGCGAC 50 0.1xSSC0.1%SDS 1.5 1.5 NLSNP4 2 1706838 GGTAGCCAGACGAACTAA TGCTGGATGGTGAGGCGACGAC 50 0.1xSSC0.1%SDS 1.5 1.5 NLSNP3 3 2192296 GGAACTGGAACGAACAA ACTIGCTCAATCTTAT 40 1xSSC0.1%SDS 1.5 1.5 NLSNP1 3 3338220 GGTGGATCCATAAGGTGAACAA ACGTGCACTGAAGACCA TAGGTCACTAAGAGACA ACGTGCACTGAAACAACA ACGTGCACACAACAACAAGA ACTIGCTCAACTAAGGTAA 50 1xSSC0.1%SDS 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1				Forward primer sequence (5'-3')	Probe sequence of 'Nipponbare' type (5'-3')	Temperature of		Rati	O ^a
NLSNP1 1 1914468 CACCTCATCCCATATICCCATTC CCTGCATGGTAGGCGG 50 0.14SSC0.1%SDS 1.5 1.5 NLSNP4 2 197753 CGGGACTGGATGAGACCTAC TGCTGCCCGGCGAACCGGC 50 0.14SSC0.1%SDS 1.5 1.5 NLSNP4 2 1705538 GGTGGCGTGACGAGCTAC TGCTGCCCGGCGAACCGGC 50 0.14SSC0.1%SDS 1.5 1.5 NLSNP4 2 1705538 GGTGGGTGAACGAGCTAC TGCTGCCGGCGAACCGGC 50 0.14SSC0.1%SDS 1.5 1.5 NLSNP7 3 984000 GCTGGGTGAAAGGCTACCTTC TCTTGAAGGGGAATATCATCAGAGAAT ACTTGCTCAATTT 40 1XSSC0.1%SDS 1.5 1.5 NLSNP8 3 21928296 AGGTGCATGAAAAGGTAACATT TTGAAGGGGAATATCATTAGGGAATATCATTAGGTCAAAT 50 1XSSC0.1%SDS 1.5 1.5 NLSNP3 3 3338920 GGTGCTGGTGGAAGAACATTAGGTGAAAAC ATCGATAGGGGATATCAT TGCGGGTGATGAGAGATATCATTAGGGGATATCAT TGCGGGCTGCAA 1 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5	Marker name	Chr.	SNP position	Reverse primer sequence (5'-3')	Probe sequence of variant type (5'-3')	hybridization and second washing	Second washing buffer	Nipponbare' type	Variant type
Inclumination Image: Construct of the second s	NI SNP1	1	1071/068	GTACGTCATCCATAGTCCGATTC	CGTGCATGtGTGAGGCG	50	0.1×550/0.1%505	1.5	1.5
NLSNP45 2 97753 CTGGGATGGATGGAGCATT TGCTGCCGGCGAACCGC 50 0.1x8SC0.1%SDS 1.5 1.5 NLSNP4 2 1705938 GGATGCGATGAGCACTAT TCTTGCAGGCGAACCGC 50 0.1x8SC0.1%SDS 1.5 1.5 NLSNP4 2 1705938 GGATGCATGAGGAACTAT TCTTGCAGGCGATGATGAGGCTATT TCTTGCAGGCGATGATGAGGCTACTAT 50 1x8SC0.1%SDS 1.5 1.5 NLSNP7 3 984009 CTGGGTATGAGGCACCAGAGGAACTAC ACTGCTCATGTAGGCTAATT 50 1x8SC0.1%SDS 1.5 1.5 NLSNP7 3 984008 CGAGGGAATTGAGGCACAGGACGACTACA TAGAGTCACTGATGCATGGAGGAATT 50 1x8SC0.1%SDS 1.5 1.5 NLSNP11 3 3398929 CCAGATTTGCTGGGCTAA ATCGATGCACACCATG 50 1x8SC0.1%SDS 1.5 1.5 NLSNP13 6 648665 GGTCCACCACGCATTC CCAGTTACATGGACTACC ATGCAAGCACACCTCT 50 1x8SC0.1%SDS 1.5 1.5 NLSNP15 6 1899200 GGATGCATGGACGACCACCT TCGCACCAGGAGTACCACGCAGTTAC TC	NEONE I	1	19714908	AAAAATGCGTGGACGTTAGC	CGTGCATGcGTGAGGCG	50	0.12330/0.176303	1.5	1.5
LINN CGGACTGACTAGACCTAC TCCTGACGCGACACAT TCCTTGACGCGCACAT TCTTGACGCTGATT D INSCR INS INS NLSNP4 2 17058398 GTTACCAGACATCAT TCTTGACGCTGATT D 1xSSCR 1	NI SNP45	2	977553	CTGGGACTGGATGAGACGTT	TGCTGCCGgCGAACCGC	50	0.1xSSC/0.1%SDS	1:5	1:5
NLSNP4 2 1798339 GFTACCETEGGTACAGCACTAT TCTTTGAAGCTTAGTT 50 1xSSC0.1%SDS 1.5 1.5 NLSNP7 3 964009 CTGGCAGACAAGCAACAA ACTTGCTCATTTT 40 1xSSC0.1%SDS 1.5 1.5 NLSNP8 3 2192226 GAACGGAGAAGTACTAACAACAA ACTTGCTCATTTTT 40 1xSSC0.1%SDS 1.5 1.5 NLSNP8 3 2192226 GAACGGAGAAGTACTAA TAGAGTCACTGCATAGT 50 1xSSC0.1%SDS 1.5 1.5 NLSNP1 3 338920 CCACGTAGTATGTCACACAC ATGCATGCAGTGAGAGTAGT 50 1xSSC0.1%SDS 1.5 1.5 NLSNP67 6 579699 CCGCGTATATGTGGCTA ATGCAAGGCAACTTCT 50 1xSSC0.1%SDS 1.5 1.5 NLSNP13 6 6458683 GGCCATCTACAGTAGTACACAC ATGCAAGGCAAGCACTT 1xSSC0.1%SDS 1.5 1.5 NLSNP14 6 1899203 GAATGCTTACAGAGT TTGCATGCAGGAGAGAGCA 11 1xSSC0.1%SDS 1.5 1.5 NLSNP14 8		-	077000	CGGAGCTGGATCAGACCTAC	TGCTGCCGaCGAACCGC		0.1.1.000.0.1.1.000.0		
Instruction Instruction Generation Gener	NI SNP4	2	17056398	GTTACCGTGGGTACCAGCACTAT	TCTTTGAAtGCTTGATT	50	1xSSC/0 1%SDS	1:5	1.5
NLSNP739840009CCTGGCTGATAGAGCTACCTTCACTTGCTCATCTATTT401xSSC/0.1%SDS1.51.5NLSNP832182298GACGAGGAAGAACAAGAACTTGCTCATCTATTT401xSSC/0.1%SDS1.51.5NLSNP832182298GAGGATGCATAGAGGAACAAGAACTAGCTAGTGAGAGAT501xSSC/0.1%SDS1.51.5NLSNP11333898200GCAGATTTGCTGCACAACATCAGTACATGCTAGT501xSSC/0.1%SDS1.51.5NLSNP3642817142GTGCATTGGATAGGGGACTGACTGGTACTGGCTAGT501xSSC/0.1%SDS1.51.5NLSNP3665478999GCGGTATAGTGGACGGACTGGTACTGGCTAGT501xSSC/0.1%SDS1.51.5NLSNP1366458953GCCCCATTACCAGAGTATCCTCGACTTCGCTTGTTCCCAAGC401xSSC/0.1%SDS1.51.5NLSNP15619892003GAATCCTACGAGATTACCCAGCCATCGGTAAAGTGGACGAGC501xSSC/0.1%SDS1.51.5NLSNP1882084144TGCAAGGCCAGGTATTACCAGCCATCGGTAAGATGCACGAGC501xSSC/0.1%SDS1.51.5NLSNP1882084144CAGGCCCTGTCAAGGTATACCCATCGGTAAGATGAAGC501xSSC/0.1%SDS1.51.5NLSNP3898418891TTGCGTAACACTGGCAGGAATTGCAAGATGAAGCC501xSSC/0.1%SDS1.51.5NLSNP39911444861CTGCGCATGACTAGCCAGCGTAAGCTAGCCC501xSSC/0.1%SDS1.51.5NLSNP39911444861CTGCGCATGTAACACGCAGGAGGCAAGCCC <td></td> <td>2</td> <td>17000000</td> <td>GGCTTGCAGAAGAGTGAAGAAT</td> <td>TCTTTGAAgGCTTGATT</td> <td>00</td> <td>1,000,0.1,10000</td> <td>1.0</td> <td>1.0</td>		2	17000000	GGCTTGCAGAAGAGTGAAGAAT	TCTTTGAAgGCTTGATT	00	1,000,0.1,10000	1.0	1.0
INSUMP Instruction Instrestruction Instruction	NI SNP7	3	9640009	GCTGGGTGATAGAGCTACCTTC	ACTTGCTCcATCTATTT	40	1xSSC/0 1%SDS	1:5	1.5
NLSNP832192828GAACCGAGGAAGTACATGAGAACAC TAGACTACATGAGGAATTAGAGTACATGAGAAT TAGAGTACATGAGAAT501xSSC0.1%SDS1.51.5NLSNP1133338280CCAGATTTTGTCTTGGCCTAA GGTGATCCTAATTAGTAGACAC GGTGATCCTAGTTGACTGGCTAAGT CCAGATTTGCTTGGCCTAA GGTGATCCTGGTTGTTGCGCTAA CCCGGTATGATGGGGATACTAC CCGGTATGATGGGGATACTAC CCGGTATGATGGGGATACTACTGGGCTAG CCGGTATGATGGGGATAGT501xSSC0.1%SDS1.51.5NLSNP36428171432CCCCCTCTCTCTCTGGGGGTA CTCCTCCTCTCTCTGGGGGAT CCGGTATGATGGGGATACTGGTGACTGGGCTAG 		5	3040003	CTTGGCACACAGAACAAAGA	ACTTGCTCtATCTATTT	40	1x000/0.170000	1.0	1.0
INCSING32120229AGGATGCATGAGGGGATATCTATAGAGTCAGTGAGAGT30INSCIENTINGED1.31.3NLSNP11333389280GGTGATGCAAATAGGTACATG501xSSCI0.1%SDS1.51.5NLSNP36428171432GGTACCTTGGTTACTTGGGCTACTGGTACTGGGCTAG500.1xSSCI0.1%SDS1:01.5NLSNP36428171432GGGGATGCAAATAGTGGGACTGACTGGTACTGGGCTAG500.1xSSCI0.1%SDS1:51:5NLSNP1366458663GGCGCATATAGTGGACTCAATGCAAGGCAACCTCT501xSSCI0.1%SDS1:51:5NLSNP15618992003GGCGCATCTACATGAGGATTCCCCATCGCTGTGCTCCAGAC401xSSCI0.1%SDS1:51:5NLSNP16621979144TCGAGGCACTACGTGATCCCATCGGTAGATGAGC501xSSCI0.1%SDS1:51:5NLSNP70621979144CGAGGCCATGCTCAGCTAGCTCATGGTAACAGGAGC501xSSCI0.1%SDS1:51:5NLSNP8898418891TCGCAGCCACTCGCAGAGGAATAGATACCACAGCC501xSSCI0.1%SDS1:51:5NLSNP8991146481CGGGGGGGATTACAAATAGATACCACAGCC501xSSCI0.1%SDS1:51:5NLSNP2091375405CCGCGTATGCGTACAGGAAGAAGCTAACAGGACGACAAGAAGCTAACAGGAGCGACC501xSSCI0.1%SDS1:51:5NLSNP21101210820GGGGGGGGAAAATACACGGCACCTCGACTTCAACAAGGGGCACCACCTACAAGAAGCTCGCCGCC501xSSCI0.1%SDS1:51:5NLSNP3410 <td></td> <td>2</td> <td>21026206</td> <td>GAACGGAGGAAGTACATGAGAAAC</td> <td>TAGAGTCAcGTAGAGAT</td> <td>50</td> <td>1×550/0 1%505</td> <td>1.5</td> <td>1.5</td>		2	21026206	GAACGGAGGAAGTACATGAGAAAC	TAGAGTCAcGTAGAGAT	50	1×550/0 1%505	1.5	1.5
NLSNP1133339828CCAGATTTTGTTTGCCCCTAAATCAGTACATGCTAAGT ATGATACGTACAGT501xSC/0.1%SDS1:51:5NLSNP36428171432GGTGCATTGGTAGTGCAAAGGTACAACATCAGTACGTGGCTAG CCGGTATGATGGGTA500.1xSSC/0.1%SDS1:101:5NLSNP5765478999GGCGGTATTAGTGGGCTACTGGTACGTGGGCTAG CCGGTATGATGGGGGTAC500.1xSSC/0.1%SDS1:51:5NLSNP5766495853GGCCGATTATAGTGGACTGCTCGCTGGTTCCAGAC401xSSC/0.1%SDS1:51:5NLSNP15618992003GAATGCTTACCAGAGTAGCACCCATCGGTAGAGGCAGACC1xSSC/0.1%SDS1:51:5NLSNP70621979144GGCCGATTACAGTATACAGCACCATCGGTAGAGCAGAGC GAATGCTTACCAGTAGACACCTGGGCTATACAGTGAATTAAGTAACCAGTGAACC1xSSC/0.1%SDS1:51:5NLSNP70621979144GGGGGGGATTGCAAGAGGTGAATTAACTATTTACATAGATACCAGTGAACC1xSSC/0.1%SDS1:51:5NLSNP70621979144CGGGGTCCTGGCAGTAACAGTGAATTAACTATTTACATAGATACCAGTGAACC1xSSC/0.1%SDS1:51:5NLSNP8898418891CGGGGTGGTGAAGAGGATAGATACCAACTGG501xSSC/0.1%SDS1:51:5NLSNP89911464961CTGGCACTGCAGGAGGATAGATACCAACTGG501xSSC/0.1%SDS1:51:5NLSNP2091374905CGTGGTGGAGGGAAATTACCAAGACCTGCACTG0.1xSSC/0.1%SDS1:51:51:5NLSNP9310866031AGGACTGCACTGCAGAGGCACGAGACCAACCGGCACA	NEGNEO	3	21920290	AGGATGCATGAGGGGATATCTA	TAGAGTCAtGTAGAGAT	50	1x330/0.176303	1.5	1.5
INCSNP17335388280GGTGATGCAAATAGGTACAACATACATACATGATGCTAAGT50INSSCID: 1,45031.31.3NLSNP36428171432GGTACCTTGGTACTTGGGCTACTGGTacACTGGGCTAGG500.1xSSC/0.1%SDS1:101:5NLSNP676547899GGCGGTATAGTGGACTGAATGCAAGGCAACCTCT501xSSC/0.1%SDS1:51:5NLSNP1366458563GGCCCATCTACAGTATACACACCTTGCATGGTAGCTAGAC401xSSC/0.1%SDS1:51:5NLSNP15618992003GAATGCTTACCGAAGTACACCCACCTCATCGGTAGCTAAGC601xSSC/0.1%SDS1:51:5NLSNP70621979144ACAAGCCATGGTGCTTTTGTTGAATTACACGCAAC501xSSC/0.1%SDS1:51:5NLSNP1882084104GGGAGGCACTGCACGTAGCGGGAGGCCACTGCACGTAACCCAGTGGTAGCGAAGC501xSSC/0.1%SDS1:51:5NLSNP8898418691TGCAGGCTAGCTAACCCAAGTACCACAGGGAAGGAATAGATACCACAGC501xSSC/0.1%SDS1:51:5NLSNP89911464681CTGGCTGTGTCTGGCCCAACTACAGGGAAGC501xSSC/0.1%SDS1:51:5NLSNP9310965031AGAAGGGAGGGAAAATAACGGAAGGGAAGACAAAGAAGCTGAGGGAGCG501xSSC/0.1%SDS1:51:5NLSNP941016148652ATATTTCACACATGAGGTGGGTGGCACTCGAGGGCGC500.1xSSC/0.1%SDS1:51:5NLSNP941016148652ATATTTCACACATGAGGTGGGGGTGGAGGAGAAATAACGACAAGAGCGAGGCGC501xSSC/0.1%SDS <td></td> <td>2</td> <td>22260200</td> <td>CCAGATTTTGTCTTGGCCCTAA</td> <td>ATCAGTACaTGCTAAGT</td> <td>50</td> <td></td> <td>1.5</td> <td>1.5</td>		2	22260200	CCAGATTTTGTCTTGGCCCTAA	ATCAGTACaTGCTAAGT	50		1.5	1.5
NLSNP36428171432GGTACCTTGGTCATTGGGCTA GCCCTCATCTCTTGGGGTAT GCCCGTATAGTGGGGCTAG GCCGGTATAGTGGGGCTAG GCCGGTATAGTGGGGACTGA CCGGTATAGTGGGACTGA GCCGGTATAGTGGGACCTCA GCCGGTATAGTGGGACCTCA GCCCGTATAGTGGGACCTCACAGTATACCACC GCCCGTATAGTGGTACCGCGCATGC GCCCCATCAGAGTATACCACC GCCCCATCGCAGAGTATCCCCACC GCCCCATCGTAGAGTACACCCCT GCCCGTCACAGTATACCACC GCCCCATCGCAGAGTCCCACC GCCCCATCGCAGAGTCCCCACC GCCCCATCGCGAGAGCCCCCC GCCCACTGCGTAGAGTCACCC GCCCACTGCGTAGAGTCACCC GCCCACTGCGTAGAGTCACCC GCCCACTGCGTAGAGTCACCC GCCCACTGCGTAGAGTCACCC GCCCACTGCGTAGAGCTAGC GCCCACTGCGTAGAGCTCCC GCCCACTGCGTAGAGCTCCC GCCCACTGCGTCCCCCCCCCCCCCCCCCCCCCCCCCCCC	INLOINF I I	3	33309280	GGTGGATGCAAATAGGTACAAC	ATCAGTACgTGCTAAGT	50	1x330/0.1/03D3	1.5	1.5
NLSNP3428171422CTCCTCTCTCTCTTGGGGTATCTLGGATACTGGGGCTAG500.1XSSC/0.1%SDS1.101.3NLSNP6765476999GGCGGTATAGTGGAGCTGAATGCAAGGCAACCTCT501xSSC/0.1%SDS1.51.5NLSNP136645553ACTCCACGAGGTATGATGGATTCCTTCGCTTGCTTCCAGAC401xSSC/0.1%SDS1.51.5NLSNP15618992003GACTACATGAGGTCCAGCTATGCCATCGTGATGACGACCTTCGCTTGGTCCAGAC501xSSC/0.1%SDS1.51.5NLSNP70621979144TGCAGGCACCACTCGGCAGAGGGAATAGATACCACATGG501xSSC/0.1%SDS1.51.5NLSNP18820864104CAGTTACCACGAGAGGAATAGATACCACATGG501xSSC/0.1%SDS1.51.5NLSNP8898418691TTCGCAGCACTCGCAACTACAAGGAAGGAATAGATACCACATGG501xSSC/0.1%SDS1.51.5NLSNP89911464861CTCGCCACTGCTTCTGGCTCTCCAACTACAAGACACTGG501xSSC/0.1%SDS1.51.5NLSNP89913754905GATAAAGCAGAGAGGAAGATGTGGCACTCCGAGGCTC500.1xSSC/0.1%SDS1.51.5NLSNP93109659031AGGACTCCTCGGTCTCTGGGGGAGGAAGATTGGAGAGAGATTGGACAAAAGCCAAGCGCA501.5SC/0.1%SDS1.51.5NLSNP941016148652ATAGAGAGGGGAAGGTGTGGCACTGCGGGGTGCCT500.1xSSC/0.1%SDS1.51.51.5NLSNP941016148652AATGGCAGGCAACTACAACGACAAACCCTGCG500.1xSSC/0.1%SDS<			00171400	GGTACCTTGGTTACTTGGGCTA	CTGGTacACTGGGCTAG	50	0.1,0000/0.10/000	1.10	1.5
NLSNP6765476999GGCGGTATAGTGGGACTGA CGGTATGATGGGAGTGTTC CGGTATGATGGAGGGTGTCC CGGGTATGATGGAGGGTGTCC CGGGTATGATGGAGGGTAGTCCTCGAC GGCCCATCTACAGGTATACCCACCATGCAAGGCAACCTCT ATGCAAGGCAACCCC1:SSC/0.1%SDS1:51:5NLSNP1366458863ACTCCACGAGGTATCCCCGAC GACTACTAGGGCCAGCTTACCAGGTATACCCACCTTCGCTTGTTCCAGAC CATCGGTAAGCTAGAC01xSSC/0.1%SDS1:51:5NLSNP15618992003GAATGCTTACCGAGATTCCC GATCACTGAGGCCACGCATTGCCATCGGTAAGCTAGACC CATCGTAAGGTCACACC01xSSC/0.1%SDS1:51:5NLSNP70621979144ACAAGCCCTTGGCTTTTT TGCAGGCACTGGTTCTCGTCGTTGAATTAATTTTTA CATGGAGCACCAGTAGCA01xSSC/0.1%SDS1:51:5NLSNP78820861104CAGGTTACACGTGGGCACCTC CGGCTGTCTGGTCTCTGGTCCCTCCAACTACAAGCAACTTGG CAGGCTACCTGGTCCCATC01xSSC/0.1%SDS1:51:5NLSNP8898418891TTCGCCACTGGCTCCACTC CGGCTGTGGAGAGACAACAAATAGATACCACAAGCAGC AAGAAGCTAAGCAGCG01xSSC/0.1%SDS1:51:5NLSNP80911464861CTGGGGGGAAAATAA CGGCAGGGAAAATAA CGGCACTGAGGGAACGGACGGCACGAACCCCATATAT CGACAAACCCATATAT CGGCGGGGAAAATAA CGACAAAACCCATAAAGCCTATAAT CGACAAACCCATATAT CGACAGAGCGACCAACA CGGCGTGTGGGGTGGGCTGCC01xSSC/0.1%SDS1:51:5NLSNP93109665031 CGGCGTGTGGGGGAAAATAA CGACAAACCCATCGAAACCCATATAT CGGCGTGGGGAAAATAA CGACAAACCCATAGAAAACCCATATAT CGACAGAGCAGCACCACA CGGCGTGTGGGGTGGGCTGCC01xSSC/0.1%SDS1:51:5NLSNP941016148652TT	INLSINP30	4	281/1432	CTCCTCCTCTCTCTGGGGTAT	CTtGGTtACTGGGCTAG	50	0.1x330/0.1%3D3	1.10	1.5
NLSNP67b54/6999CCGGTATGATGGATGGATGTCTATCCAAGGG(CACCTCT50IXSC/0.1%SDS1.51.5NLSNP1366458563GGCCCACAGGGTAGTCCTCGACTTCGCTTGCTTCCAGAC401xSSC/0.1%SDS1:501:5NLSNP15618992003GAATGCTTACGGATATCCACCCATCGGTAGACTAGAC501xSSC/0.1%SDS1:51:5NLSNP70621979144ACAGGCCTGGGTGCACGTATGCCATCGGTAGACTAGAC501xSSC/0.1%SDS1:51:5NLSNP70621979144CAGGGCCACTGACTTACAGTTGAATTACAATTTTTA401xSSC/0.1%SDS1:51:5NLSNP78820864104CAGGGCACTGACTTCCACCACAAGGATAGATACCACACTGG501xSSC/0.1%SDS1:51:5NLSNP8898418691CAGGCTTCCTGTTCTCGTCCAACTACAAGCAAGCTGG501xSSC/0.1%SDS1:51:5NLSNP8898418691CAGGCTCCTGTTCTCGTCCAACTACAAGCAAGCTGG501xSSC/0.1%SDS1:51:5NLSNP89911464861CTGGCTATGGGGCACCCCAACTACAAGCAAGCTGG501xSSC/0.1%SDS1:51:5NLSNP20913754905GATAAACCAGAGGGGAAAATAACGACAAAAGCTAAGCCACCC500.1xSSC/0.1%SDS1:51:5NLSNP33109665031AGGACTCCCGGTCGAAAAAACCCTATAAT501xSSC/0.1%SDS1:51:5NLSNP941016148652TTGGACGAGGGTGTCTACAGAGCAGGCTCTGC500.1xSSC/0.1%SDS1:51:5NLSNP941016148652TTGGCACGAGGGTGTCCTAC		0	F 470000	GGCGGTAATAGTGGGACTGA	ATGCAAGGcCAACCTCT	50	4.000/0.40/ 000	4.5	4.5
NLSNP1366458563ACTCCACGAGGTAGTCCTCGAC GGCCCATCTACAGATATACACACCTTCGCTTGCTTCCAGAC TCGCTTGCTTCCCAGAC401xSSC/0.1%SDS1:501:50NLSNP15618992003GAAGGCTCTACCGAGATTCCATC GACTACATGAGGTCAGGCTATGCCATCGTAGAGACTAGAC GACTACATGAGGTCCAGCTATGC501xSSC/0.1%SDS1:51:51:5NLSNP70621979144ACAAGCCTCTGGTGCTTTT TGCAGGCCACTGGACTAACAGTTGAATTCAATTTTTA GTTGAATTACAAGCAC401xSSC/0.1%SDS1:51:51:5NLSNP18820864104CAGTTAACCACTTCGACGAAGG GGGAGGGATTGCACTAACAGTTGAATACCACAGC GGGAGGGATTGCACTTAACA501xSSC/0.1%SDS1:51:5NLSNP8898418691CTCGCCACTGTTCCTCC CAGCTCATGGGTCCACTCAACTACAAGAAGCTAGCCAAGGC AAGAAGCTAACCACAGCA0.1xSSC/0.1%SDS1:51:5NLSNP89911464861CTCGCCATGTTCAAGAAGC CGGTGTGAGAGACGAACAAAGAAGCTAACCACAAGCGC AAGAAGCTAACCAAGCAGCC0.1xSSC/0.1%SDS1:51:5NLSNP93109665031CGTGTGGGAGGAAAATAA CGACGAAGAGGGGAAGAATAACGACAAAACCCTATAAT CGACAAAAGCCATACAA01xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGCAGCAGTGTGTGG CGACGTGGTGACTACCACAGGTGTCGAGCCATCTGC CCAGTCCACACA0.1xSSC/0.1%SDS1:51:51:5NLSNP941016148652AAATTGCAGCAGTGTCT CGCGCGGTGTACTACACACCAGGCCAGCACTGCC CCAGGCCACCTCCC01xSSC/0.1%SDS1:51:51:5NLSNP941016148652AAATTGCAGCAGTGCTCC CGAGGCAGGCACCTACCACCAGGCAGG	NLSNP07	0	5476999	CCGGTATGATGGATGGTTTC	ATGCAAGGtCAACCTCT	50	1x330/0.1%3D3	1.5	1.5
NLSNP1366498963GGCCATCTACAGTATACACACCTTCGCTTGITTCCAGAC401x8SC/0.1%SDS1:501:5NLSNP15618992003GAATGCTTACCAGTATGCCATCGTAGAGCTAGAC501xSSC/0.1%SDS1:51:5NLSNP70621979144ACAAGCCTCGGTGCTTTTGTTGAATTAATTTATTA401xSSC/0.1%SDS1:51:5NLSNP70621979144TGCAGGCCACTGACTAACAGTTGAATTAATTTATTA401xSSC/0.1%SDS1:51:5NLSNP18820864104GGCAGGATTCGACAGAAGGAATAGATALCCACAGAAGC501xSSC/0.1%SDS1:51:5NLSNP8898418691TTCGCTGCTGTCTCGTCCAACTACAGCAACCTTGG501xSSC/0.1%SDS1:101:5NLSNP89911464861CTGGGTGTGAGAGACGAACAAAGAAGCTAGGCACGC500.1xSSC/0.1%SDS1:51:5NLSNP20913754905GATAAGCAGAGAGGGAAGATGTGGCACTCCGAGGCTCC500.1xSSC/0.1%SDS1:51:5NLSNP93109665031CGTGTGGGAGGAAAATAACGACAAAACCTATAAT501xSSC/0.1%SDS1:51:51:5NLSNP941012100820CAGTGTTGCTCCATCTCCAGGTGCGAGGCGCTCC500.1xSSC/0.1%SDS1:51:51:5NLSNP941016180907AATTGCAAGCGAGGTGTCTACCAAGCAAGCCGAGCTGTGCT500.1xSSC/0.1%SDS1:51:51:5NLSNP96116180907ACTGATGACAGTGGGGGAACAATACGCAAGCAAGCCAGCTGCC500.1xSSC/0.1%SDS1:51:51:5NLS				ACTCCACGAGGTAGTCCTCGAC	TTCGCTTGcTTCCAGAC	10	1.000/0.40/0000	1.50	4.5
NLSNP15618992003GAATGCTTACCGAGATTCCATC GACTACATGAGGCCAGCTATGCCATCGGTAgAGCTAGAC CATCGTAGCC501xSSC/0.1%SDS1.51.5NLSNP70621979144ACAAGCCTCTGGTGCTTTTT TGCAGGCCATGGCTAGCTGTTGAATTAACATTTAAC GTTGAATTAATTTTA401xSSC/0.1%SDS1.51.5NLSNP18820864104CAGTTAACCACTTGGAGATAAC GGAGGATTTGCAGATTAAC TGCAGCTCTGTTCTTCGTCAATAGATACCACACGC CAACTACAGACACTTGG501xSSC/0.1%SDS1.51.5NLSNP8898418691CAGGCTTCCTGTCTCTGCC CTGCGCATGTGGACGAGAGAG CTGGGTGTGAAGAGAGAGAACA ACAGAGCTGACCTACAGCAACTTGG CCGCGTGTGAAGAAGCGAACAACT CCGGGTGTGAAGAAGCGAACAACT CGAGGCTTCCTGTTCAAGAGAAGC CGTGGGGGGAGAAAATAAAAGAAGCTaAGCGAAGCG CGGCACCAGAGCC CGGCTCCTCTAACACACACTGG CCCCCATTTGCACATTCAAGAAAATAA1.51.51.5NLSNP30109665031CGTGTGGGAGGAGAAAAATAA CGACGAAGAGCGGAGCGAAAAATAA CGACAAAACCCTATAAT CGACAAAAGCCTATCGG CGACTCCTCGGTCCTCCAAA CGACAAAAGCCTATCAGA501.xSSC/0.1%SDS1.51.5NLSNP311012100820CGTGTGGGAGGAAAAATAA CGACGAGGCGTGCT CGACCTTCTCTCCCGCTCTCAAA CGACAAAACCCTATAAT CGACAAAACCTATGCG500.1xSSC/0.1%SDS1.51.5NLSNP341016148652AAATTGGCAGGCGTGCTT CGACCTGTGACACACCTACA AAATTGGCAGGCGGTACCTACA CGACGCAGGGCTACCTACA CGACGCAGGGCTACCTACA CCAGCCCGTGGCT CGACCTCTTCTCTCCCCTCCTTACCC500.1xSSC/0.1%SDS1.51.5NLSNP36116180907AAATTGGCAGAGCAGGGCCACCTACA ACCAGCCGCGTGGTACCTACA ACCAGCCAGGCCTCCCTCCTACC CCAGCCCGGTGCTACCTACA CCAGCCCGGGTACCTACACCAGCC	NLSNP13	6	6458563	GGCCCATCTACAGTATACACACC	TTCGCTTGtTTCCAGAC	40	1x55C/0.1%5D5	1:50	1:5
NLSNP1561899203GACTACATGAGGTCCAGCTATGCCATCGGTACAGCTAGAC501XSSC/0.1%SDS1:51:5NLSNP70621979144ACAAGCCTCTGGTGCTTTTTGTTGAATTACAAGTTTTTA401xSSC/0.1%SDS1:51:5NLSNP18820864104CAGTTAACCACTCGACAGAAGGAATAGATAICCACAGC501xSSC/0.1%SDS1:51:5NLSNP8898418691CAGGCTTCCTGTTCTTGGTCCAACTACAAGAACACTTGG501xSSC/0.1%SDS1:51:5NLSNP8998418691CCGGCTTCCTGTTCTTGGTCCAACTACAAGAACCTTGG501xSSC/0.1%SDS1:51:5NLSNP89911464861CTGGCTGTGAGAGACGAACAAAGAAGCTAAGCGACGC500.1xSSC/0.1%SDS1:51:5NLSNP20913754905GATAAAGCAGAGAGGGGAAGATGTGGCACTCCGAGGCTCC CCCTCATTGCACATTCACAA700.1xSSC/0.1%SDS1:51:5NLSNP93109665031AGGACTTCCTCGTCTCTAACGACGACGAGAGGGGGAAAATAACGACAAAACCTATAAT CGACAGCGGCTGTCCT500.1xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTGTGGGGTGTCGAIGCTGTGCT CGACTCTTTCTCTCCCATCTTC6GTGTCGAIGCTGTGCT CGACTCTGTCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652TTGGACGAGCGTGTCCTT TGGCAGAGCACCACCTACAAACCAAGCAGCAGCTTCGC CCAGCCGTGGTACATACAACCAAGCAGCAGCTGCC CCAGCCGTGGTACATACACAGCGCGGGGTGACACCTACAACCAAGCAAGCATCTGC CCACGCCGTGGTACATACAACCAAGCCAACCTGC CCACGCCGTGGTACATACAACCAAGCAGCAACCTACA1:51:5NLSNP94				GAATGCTTACCGAGATTCCATC	CATCGGTAgAGCTAGAC	50	1.000/0.40/0000	4.5	4.5
NLSNP70621979144ACAAGCCTCTGGTGCTITTT TGCAGGCCACTGACTAACAGTTGAATTGAATTTTAA GTTGAATTAATTTTA401xSSC/0.1%SDS1:51:5NLSNP18820864104GGGAGGGATTGCAGAAGAG GGGAAGGAGTTGCAGATACAAATAGATACCACAAGC AATAGATACCACAAGC501xSSC/0.1%SDS1:51:5NLSNP8898418691CAGGCTCCTGTTCTTGGTC CGGCATGTCAATGGACACAC CTGGCGTGTGAGAGAAGAGCAATAGATACCAAAGCAACTTGG CAACTACAGCAACTTGG501xSSC/0.1%SDS1:101:5NLSNP89911464861CTGGGGTGTGAGAGACGA CTGGGTGTGAGAGACGAACAAAGAAGCTGAGCGC AAGAAGCTGAGCGC500.1xSSC/0.1%SDS1:51:5NLSNP20913754905GATAAAGCAGAGAGGGAAAATAA CGTGTGGGGGGAAAAATAACGACAAAACCTCGAGGCTCC CGCTGTGTGGGGGGAAAAATAA501xSSC/0.1%SDS1:51:5NLSNP21109665031AGGACTTCCTCGGTGTCATA CGACTCTTCTTCTTCTCTCTCCCATCTTC CGGTGTGGAGGCAAAATAAGGTGTGGAGGCAACATAA CGACAAAAGCCTATAAT CGACAAGACGCTGTGCT500.1xSSC/0.1%SDS1:51:5NLSNP241016148652TATTTCAGCAGGGTGTGCT TGGACAGGCGTGTCCTT AACTGGCAGCGGTGGCACCACC ATCCATCCCCTCCTTACCCCCAGTCCAAGCAGCATCTGC CCAGTCCAAGCCAGCATCTGC01xSSC/0.1%SDS1:51:5NLSNP96116180907ACTGATGACAGGTGAGCAAGC ACTGATGCACGTGGGTGGGAGGCAAGCT ATCTCATCCCTCCTTACCCCCAGTCCAAGTCGAGT CCAGTCGAGACCAAGCT CCAGTCCAAGCAAGCCAAGCT CCAGTCCAAGCAAGCCAAGCT CCAGTCCAAGCCAGCTAGCT1xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACGGTGGGGGAGCAAGT ACTGATGACGGGGGGAGCAAGT ACTGA	NLSNP15	6	18992003	GACTACATGAGGTCCAGCTATGC	CATCGGTAcAGCTAGAC	50	1xSSC/0.1%SDS	1:5	1:5
NLSNP706219/9144TGCAGGCCACTGACTTAACAGTTGAATTIAATTITTA401xSSC/0.1%SDS1:51:5NLSNP18820864104CAGTTAACCACTTCGACAGAAGG GGGAGGGATTGCAGATTAACAATAGATACCACAAGC CAAGGCTTCCTGTCTTCGTC501xSSC/0.1%SDS1:51:5NLSNP8898418691TTCCGTACTGGGTCCACTC CCGTCGTGTTCTTCGTCCAACTACAACAACAGC CAACTACAAGAACTTGG501xSSC/0.1%SDS1:101:5NLSNP89911464861CTCGCCATGTTCAAAGAAGC CTGGCTGTGAGAGAAGAGGGAACAAAAGAAGCTGAGCGACGC AGAAGCTAAGCGAACC500.1xSSC/0.1%SDS1:51:5NLSNP20913754905CCTCGTTTGCACATTCAGA CCTCATTTGCACATTCAGATGGCACTCCGAGGCTCC TGGCACTCCAAGGCTCC500.1xSSC/0.1%SDS1:51:5NLSNP33109665031GGTGTGGGGGGGAAAATAA AGGACTTCCTCCGTTCTCAAA CGACTAAAAGCAGAGGGGTGTGTGGGGTGTCGAGGCTGTGCT GGTGTCGAGGCTGTGCT500.1xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTTGTGG CGACTCTTCTCTCTCCATCTTC CGACGCGTGCTTGGTGTCGAGCTGTGCT ACCAAGCAAGCCAACTTGC500.1xSSC/0.1%SDS1:51:5NLSNP941016148652TTGGACAGAGCCAACCTACA ACCAGCCGCGCGCCACCTACAACCAAGCAGCAGCACTGC CCAGCGCGTGGTACTATGACCAGCGCCGCGGGTAACTATGAC CCAGCGCGTGGTACTATGACCAGCGCCCACCTACA CCAGCGCGTGGTAACTATGAC1:51:5NLSNP96116180907ACTGATGACAGGGACAAGT ACTGATGACAGGGGACCAAGTGGTTCACTATACTATTA GGTTCACTATACTATTA CCAGCGCGTGCGTACCTATACCAC1:51:51:5NLSNP2912				ACAAGCCTCTGGTGCTTTTT	GTTGAATTcAATTTTTA	10			
NLSNP188 20864104 CAGTTAACCACTTCGACAGAAGGAATAGATAICCACAAGC501xSSC/0.1%SDS1:51:5NLSNP889 8418691 CAGGCTTCCTGTTCTCGTCCAACTACAACAAGCAAGC501xSSC/0.1%SDS1:101:5NLSNP899 11464861 CTGGCACTGTGGAGACAACAAAGAAGCTGAGGCACGC50 $0.1xSSC/0.1\%SDS$ 1:51:5NLSNP899 11464861 CTGGCATGTGAAGAAGCAAGAAGCTGAGGCACGC50 $0.1xSSC/0.1\%SDS$ 1:51:5NLSNP209 13754905 GATAAAGCAGAGAGGGGAAAATAACGACAAAACCCTAGGCTCC50 $0.1xSSC/0.1\%SDS$ 1:51:5NLSNP93109665031CGTGTGGGAGGGAAAAATAACGACAAAACCCTATAAT50 $1xSSC/0.1\%SDS$ 1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTTGGGGGTGTCGAIGCTGGCT50 $0.1xSSC/0.1\%SDS$ 1:51:5NLSNP941016148652AAATGGCAGAGCAACCTACAACGAACAAGCAIGCAITCTGC50 $0.1xSSC/0.1\%SDS$ 1:51:5NLSNP96116180907ATCTCATCCCTCTCTTTACCAAGCAIGCAITCTGC50 $0.1xSSC/0.1\%SDS$ 1:51:5NLSNP991224186101ACGGGCGTGGAACCAACCTACAACCAAGCAIGCAITCTGGAT50 $1xSSC/0.1\%SDS$ 1:51:5NLSNP991224186101ACGTGGTGAACCAAGTGGTTCACTGGGT50 $0.1xSSC/0.1\%SDS$ 1:51:5NLSNP291224186101ACGTGGTGAACCAACGTGGTTCACTGGGT50 $1xSSC/0.1\%SDS$ 1:51:5NLSNP29	NLSNP70	6	21979144	TGCAGGCCACTGACTTAACA	GTTGAATTtAATTTTTA	40	1xSSC/0.1%SDS	1:5	1:5
NLSNP18820864104GGGAGGGATTTGCAGATTAACAATAGATACCCACAAGC501XSSC/0.1%SDS1:51:5NLSNP8898418691CAGGCTTCCTGTTCTTCGTCCAACTACAGCAACTTGG501xSSC/0.1%SDS1:101:5NLSNP88911464861CTCGCCATGTTCAAAGAAGCAAGAAGCTgAGCGACGC500.1xSSC/0.1%SDS1:51:5NLSNP89911464861CTGGGTGTGAGAGACGAAAGAAGCTgAGCGACGC500.1xSSC/0.1%SDS1:51:5NLSNP20913754905GATAAAGCAGAGAGGGAAAAATGGCACTCCGAGGCTCC500.1xSSC/0.1%SDS1:51:5NLSNP93109665031CGTGTGGGAGGGAAAAATAACGACAAAAgCCTATAAT501xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTGTGGGGTGTCGAIGCTGTGCT CGACTCTTCTCCCATCTTC6GTGTCGAIGCTGTGCT GGTGTCGAIGCTGTGCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGACAGAGCCAACCTACAACCAAGCAAGCAIGCATCTGC CAGCGCGTGGTAACTATGACCCAGCCCATCTCC500.1xSSC/0.1%SDS1:51:5NLSNP96116180907ACGGCGCTGGAACCTACAACCAAGCAAGCAAGCATCTGGAT ACCAGGAGCACCAACTGGTTCACTATACTATTA CGATCCACTCCTTTC501xSSC/0.1%SDS1:51:5NLSNP991224186101ACGGCGTGGAACCTACAACCAAGCAAGCAAGCATCTGGAT ACGAGGCAGGACCAAGT6GTTCACTATACTATTA CCAGCGCGTGGAACCTACA401xSSC/0.1%SDS1:51:5				CAGTTAACCACTTCGACAGAAGG	AATAGATAtCCACAAGC	50			
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	NLSNP18	8	20864104	GGGAGGGATTTGCAGATTAAC	AATAGATAcCCACAAGC	50	1xSSC/0.1%SDS	1:5	1:5
NLSNP8898418691TTCGCTACTGGGTCCACTCCAACTACAGCAACTTGG501xSSC/0.1%SDS1:101:5NLSNP89911464861CTCGCCATGTTCAAAGAAGCAAGAAGCTGAGCGACGC500.1xSSC/0.1%SDS1:51:5NLSNP20913754905GATAAAGCAGAGAGGGAAGATGTGGCACTCCGAGGCTCC500.1xSSC/0.1%SDS1:51:5NLSNP93109665031GGTGTGGGAGGGAAAAATAACGACAAAACCTATAAT501xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCACCAGTGGGTTGTGGGGTGTCGA4GCAGCAGCATCTGC500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGCAGCAGTGTCCTTACCAAGCA4GCATCTGC500.1xSSC/0.1%SDS1:51:5NLSNP96116180907ACGCGCGTGGAAACTATGACCCAGCCAGTCCAATCTGGAT500.1xSSC/0.1%SDS1:51:5NLSNP291224186101ACGACGAGGGCAAAATGAACCAAGCAAGCATCTGCATTCGCAATCTGAAT501xSSC/0.1%SDS1:51:5NLSNP991224186101ACGACGCGTGGAACTATGACCCAGCCAGTCCAATCTGGAT501xSSC/0.1%SDS1:51:5				CAGGCTTCCTGTTCTTCGTC	CAACTACAaCAACTTGG				
NLSNP89911464861CTCGCCATGTTCAAAGAAGC CTGGGTGTGAGAGACGAACA CTGGGTGTGAGAGACGAACAAAGAAGCTAAGCGACGC AAGAAGCTAAGCGACGC500.1xSSC/0.1%SDS1:51:5NLSNP20913754905GATAAAGCAGAGAGAGGGGAAGATG CCTCATTTGCACATTCAGATGGCACTCcGAGGCTCC TGGCACTCCGAGGCTCC500.1xSSC/0.1%SDS1:51:5NLSNP93109665031CGTGTGGGAGGGAAAAATAA AGGACTTCCTCCGTCTCAAACGACAAAACCCTATAAT CGACTGCGAGGGTGTGGG500.1xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTTGTGG CGACTCTTCTCTCTCTCCATCTTCGGTGTCGAtGCTGTGCT GGTGTCGAdGCTGTGCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGCAGCAGTGTCCTT TTGGACAGAGCCAACCTACAACCAAGCAAGCATCTGC CCAGCCGTGGTAACTATGAC500.1xSSC/0.1%SDS1:51:5NLSNP96116180907ACGCGCGTGGTAACTATGAC ACTGATGACAGGTGAGACCAAGTCCAGTCCAATCTGGAT GGTTCACTATACTATTA501xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGGTGAGACCAAGT TACCTGATGCTACCAAGCTAGCAAGT TACGTCCTCTTATCCTGGTTCACTATATTA GGTTCACTATATTA401xSSC/0.1%SDS1:51:5	NLSNP88	9	8418691	TTCGCTACTGGGTCCACTC	CAACTACAgCAACTTGG	50	1xSSC/0.1%SDS	1:10	1:5
NLSNP89911464861CTGGGTGTGAGAGACGAACAAAGAAGCTAAGCGACGC500.1xSSC/0.1%SDS1:51:5NLSNP20913754905GATAAAGCAGAGAGAGGGGAAGATGTGGCACTCCGAGGCTCC500.1xSSC/0.1%SDS1:51:5NLSNP93109665031CGTGTGGGAGGGAAAAATAACGACAAAACCCTATAAT501xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTGTGGGGTGTCGAGGCTGTGCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGCAAGAGCAACATCAAACCAAGCAAGCATCTGC500.1xSSC/0.1%SDS1:51:5NLSNP96116180907CAGCGCGTGTAACTATGACCCAGTCCAGATCTGGAT500.1xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGGGGAGACCAAGTGGTTCCATATTAT501.xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGGTGAGACCAAGTGGTTCACTATATTAT501.xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGGTGAGACCAAGTGGTTCACTATATTATA501.xSSC/0.1%SDS1:51:5				CTCGCCATGTTCAAAGAAGC	AAGAAGCTgAGCGACGC				
NLSNP20913754905GATAAAGCAGAGAGGGGAAGATG CCCTCATTTGCACATTCAGATGGCACTCCGAGGCTCC TGGCACTC1GAGGCTCC500.1xSSC/0.1%SDS1:51:5NLSNP9310965031CGTGTGGGAGGGAAAAATAACGACAAAAcCCTATAAT CGACTCCTCCGTCTCAAA501xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTTGTGG CGACTCTTTCTCTCTCCTCCATCTTCGGTGTCGAtGCTGTGCT GGTGTCGAgGCTGTGCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGCAGAGCCAACCTACA AGGCCGCGTGGTACTATAGACACCAAGCAAGCATCTGC CCAGCCCTGTGCTACAA500.1xSSC/0.1%SDS1:51:5NLSNP96116180907CAGCGCGTGGTAACTATGAC ACTGATGACAGAGGCGAGCAACGTCCAGTCCAATCTGGAT GGTTCACTATCTATTA501xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGGTGAGACCAAGT TGCCTCCTTATGCTCCTCCTTATGCT TACCTATCCTTATGCCTATGCT CGTTCACTATACTATTA401xSSC/0.1%SDS1:51:5	NLSNP89	9	11464861	CTGGGTGTGAGAGACGAACA	AAGAAGCTaAGCGACGC	50	0.1xSSC/0.1%SDS	1:5	1:5
NLSNP20913754905CCCTCATTTGCACATTCAGATGGCACTCtGAGGCTCC500.1xSSC/0.1%SDS1:51:5NLSNP93109665031CGTGTGGGAGGGAGAAAATAACGACAAAAcCCTATAAT501xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTTGTGGGGTGTCGAtGCTGTGCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGCAGCAGTGTCTTACCAAGCAAGCATCTGC500.1xSSC/0.1%SDS1:51:5NLSNP96116180907CAGCGCGTGGTAACTATGACCCAGTCCAtTCTGGAT500.1xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGAGCAACGTGGGTTCACTaTACTATTA501xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGAGCCAACGTGGTTCACTaTACTATTA601xSSC/0.1%SDS1:51:5				GATAAAGCAGAGAGGGGAAGATG	TGGCACTCcGAGGCTCC				
NLSNP93109665031CGTGTGGGAGGGAGAGAAAATAA AGGACTTCCTCCGTCTCAAA AGGACTTCCTCCGTCTCAAACGACAAAACCCTATAAT CGACAAAAgCCTATAAT501xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTTGTGG CGACTCTTTCTTCTCTCCATCTTC CGACTCTTTCTTCTCTCCATCTTC TTGGACAGAGCCAACCTACAGGTGTCGAIGCTGTGCT GGTGTCGAIGCAGCAGCTGTGCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGCAGCAGCGAGTGTCCTT TTGGACAGAGCCAACCTACAACCAAGCAAGCAACCTACC ACCAAGCAAGCAACCTACA500.1xSSC/0.1%SDS1:51:5NLSNP96116180907CAGCGCGTGGTAACTATGAC ACTGATGACAGGTGAGACCAAGTCCAGTCCAJATCTGGAT GGTTCACTATCATTATA501xSSC/0.1%SDS1:51:5NLSNP291224186101TACGTGACGAGGCAACGT TACCTGATGCCTATGCTTATGCTGGTTCACTATACTATTA GGTTCACTATACTATTA401xSSC/0.1%SDS1:51:5	NLSNP20	9	13754905	CCCTCATTTGCACATTCAGA	TGGCACTCtGAGGCTCC	50	0.1xSSC/0.1%SDS	1:5	1:5
NLSNP93109665031AGGACTTCCTCCGTCTCAAACGACAAAAgCCTATAAT501xSSC/0.1%SDS1:51:5NLSNP211012100820ATATTTCAGCAGTGGGTTGTGGGGTGTCGAtGCTGTGCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGCAGCAGTGTCCTTACCAAGCAtGCATCTGC500.1xSSC/0.1%SDS1:51:5NLSNP96116180907CAGCGCGTGTAACTATGACCCAGCCCAGTCACAAACCAAGCAaGCATCTGGAT501xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGAGTGAGACCAAGTGGTTCACTaTACTATTAA501xSSC/0.1%SDS1:51:5NLSNP291224186101TACGATGACAGAGTGAGACCAAGTGGTTCACTATACTATTAA401xSSC/0.1%SDS1:51:5				CGTGTGGGGGGGGAAAAATAA	CGACAAAAcCCTATAAT				
NLSNP211012100820ATATTTCAGCAGTGGGTTGTGG CGACTCTTTCTTCTCTCCATCTTC GGTGTCGAGGGCTGTGGCT GGTGTCGAGGGCTGTGGCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGCAGGAGTGTCCTT TTGGACAGGAGCCAACCTACAACCAAGCAGGCATCTGC ACCAAGCAAGCATCTGC500.1xSSC/0.1%SDS1:51:5NLSNP96116180907CAGCGCGTGGTAACTATGAC ATCTCATCCCCTCCTTACCCCCAGTCCAAGCTCTGGAT CCAGTCCATGCTGGAT501xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGGTGAGACCAAGT TACCGACTGTACCTATGCACGGTTCACTATATA GGTTCACTATATA401xSSC/0.1%SDS1:51:5	NLSNP93	10	9665031	AGGACTTCCTCCGTCTCAAA	CGACAAAAgCCTATAAT	50	1xSSC/0.1%SDS	1:5	1:5
NLSNP211012100820CGACTCTTTCTTCTCTCCATCTTCGGTGTCGAGGGCTGTGGCT500.1xSSC/0.1%SDS1:51:5NLSNP941016148652AAATTGGCAGGCAGTGTCCTTACCAAGCAAGCATCTGC500.1xSSC/0.1%SDS1:51:5NLSNP96116180907CAGCGCGTGGTAACTATGACCCAGTCCAATCTGGAT501xSSC/0.1%SDS1:51:5NLSNP291224186101ACTGATGACAGGAGCAAGCTGGTTCACTATATA501xSSC/0.1%SDS1:51:5NLSNP291224186101TACCGATGCTCCTTACCCCCAGTCCATATCTATTA401xSSC/0.1%SDS1:51:5				ATATTTCAGCAGTGGGTTGTGG	GGTGTCGAtGCTGTGCT				
NLSNP94 10 16148652 AAATTGGCAGCAGTGTCCTT ACCAAGCAtGCATCTGC 50 0.1xSSC/0.1%SDS 1:5 1:5 NLSNP96 11 6180907 CAGCGCGTGGTAACTATGAC CCAGTCCAtATCTGGAT 50 1xSSC/0.1%SDS 1:5 1:5 NLSNP96 11 6180907 ACTCATCCCCTCCTTACCC CCAGTCCAtATCTGGAT 50 1xSSC/0.1%SDS 1:5 1:5 NLSNP29 12 24186101 TACTGATGCAGAGACCAAGT GGTTCACTATATA 40 1xSSC/0.1%SDS 1:5 1:5	NLSNP21	10	12100820	CGACTETTETTETETECATETTE	GGTGTCGAgGCTGTGCT	50	0.1xSSC/0.1%SDS	1:5	1:5
NLSNP94 10 16148652 TTGGACAGAGCCAACCTACA ACCAAGCAaGCATCTGC 50 0.1xSSC/0.1%SDS 1:5 1:5 NLSNP96 11 6180907 CAGCGCGTGGTAACTATGAC CCAGTCCAtATCTGGAT 50 1xSSC/0.1%SDS 1:5 1:5 NLSNP29 12 24186101 TACTCATCCCTTATCCT GGTTCACTATATA 40 1xSSC/0.1%SDS 1:5 1:5				AAATTGGCAGCAGTGTCCTT	ACCAAGCAtGCATCTGC				
NLSNP96 11 6180907 CAGCGCGTGGTAACTATGAC CCAGTCCAtATCTGGAT 50 1xSSC/0.1%SDS 1:5 1:5 NLSNP29 12 24186101 TACTGATGCTCCGTACCTATGCTAT GGTTCACTATATA 40 1xSSC/0.1%SDS 1:5 1:5	NLSNP94	10	16148652	TTGGACAGAGCCAACCTACA	ACCAAGCAaGCATCTGC	50	0.1xSSC/0.1%SDS	1:5	1:5
NLSNP96 11 6180907 ATCTCATCCCCTCCTTACCC CCAGTCCAgATCTGGAT 50 1xSSC/0.1%SDS 1:5 1:5 NLSNP29 12 24186101 TACTGATGACGAGACCAAGT GGTTCACTATACTATTA 40 1xSSC/0.1%SDS 1:5 1:5				CAGCGCGTGGTAACTATGAC	CCAGTCCAtATCTGGAT				
ACTGATGACGGGTGGAGACCAAGT GGTTCACTATACTAT	NLSNP96	11	6180907		CCAGTCCAGATCTGGAT	50	1xSSC/0.1%SDS	1:5	1:5
NLSNP29 12 24186101 Target ar ceta receta re					GGTTCACTATACTATTA				
	NLSNP29	12	24186101	TACGTGCTCCGTACGTATCCTAT	GGTTCACTATACTATTA	40	1xSSC/0.1%SDS	1:5	1:5

Supplymentary table 3 Sequences and hybridization conditions of bridge hybridization

a: Ratio of competitive probe of labeled probe,