Supporting Information

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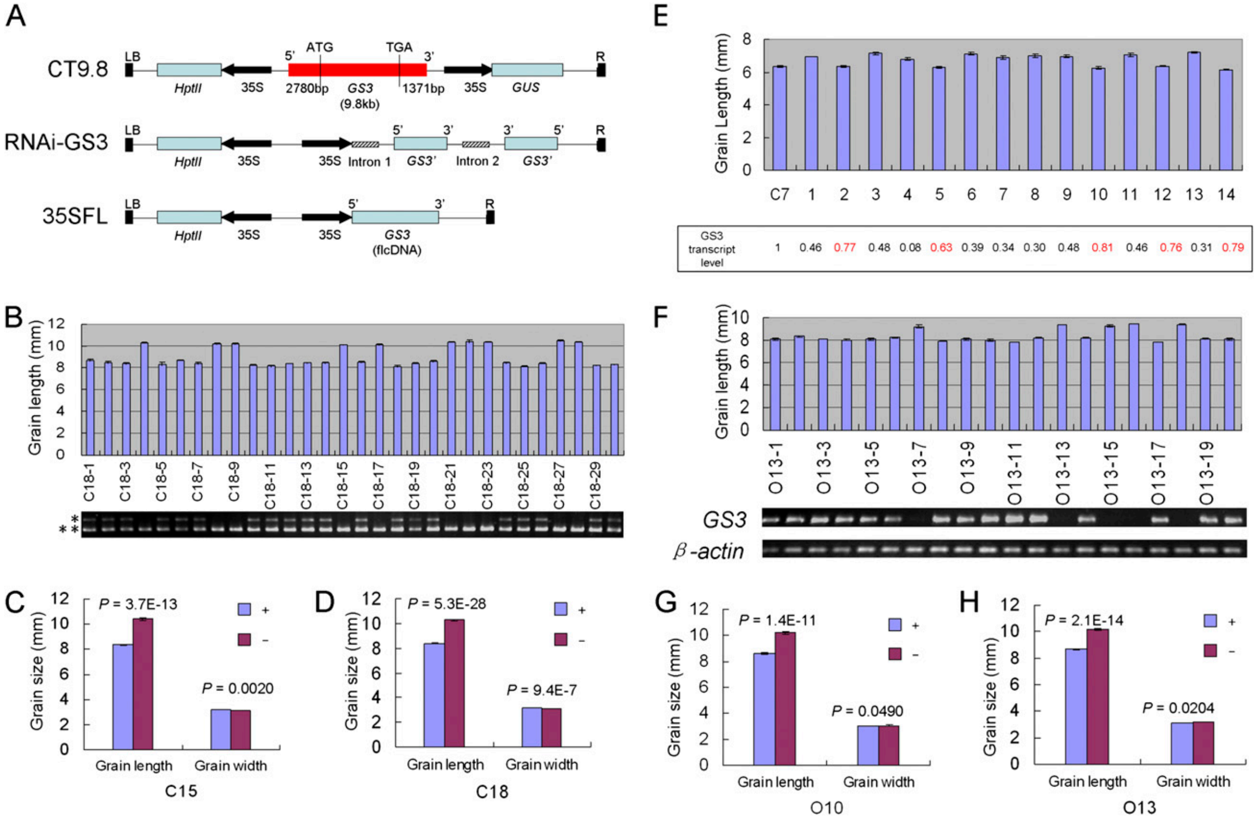

Fig. S1. Effects of transformation on grain size using three strategies. (*A*) Transformation constructs. CT9.8: the complementation construct in which *GS3* fragment was amplified from Chuan 7 genome DNA and cloned into pCAMIA1301. RNAi-GS3: RNAi construct in which intron 1 is *Adh1* intron and intron 2 is rice *Waxy* gene intron. 35SFL: the overexpression construct, in which *GS3* is under the control of CaMV 35S promoter. (*B–D*) Analysis of cosegregation between grain length and the transgene (CT.9.8). (*B*) Cosegregation between grain length and the transgene. *Bottom:* Genotype of T_1 segregants revealed by PCR amplification of the genomic DNA using a pair of primers, SF28 and SR17. The PCR product of the negative segregants is 1,065 bp (*), and transgene has another band of 1,403 bp (**). (*C* and *D*) Grain length and width of transgene-positive (+) and transgene-negative (–) plants in two T_1 families. All data are given as mean ± SEM. *P* values are generated using *t* tests. (*E*) Relationship of grain length and expression level of *GS3* in the *GS3*-suppression (RNAi-GS3) plants of T_1 generation. *Bottom: GS3* transcript level in T_1 segregants. The numbers in red represent the transcript level of negative segregants. C7 is the parent as a control. (*F–H*) Relationship of grain length and expression level of *GS3* in the *GS3*-overexpression (35SFL) plants of T_1 generation. (*F*) Cosegregation between the transgene and grain length using a T_1 family as an example. Overexpression was detected in leaves using primers GS3F and GS4R, by which nonoverexpression segregants do not show clear bands because of low expression of the native gene. *β-Actin* was used as an internal control. (*G* and *H*) Grain length and width of transgene-positive (+) and transgene-negative (–) plants in two T_1 families. All data are given as mean ± SEM. *P* values are generated using *t* tests.

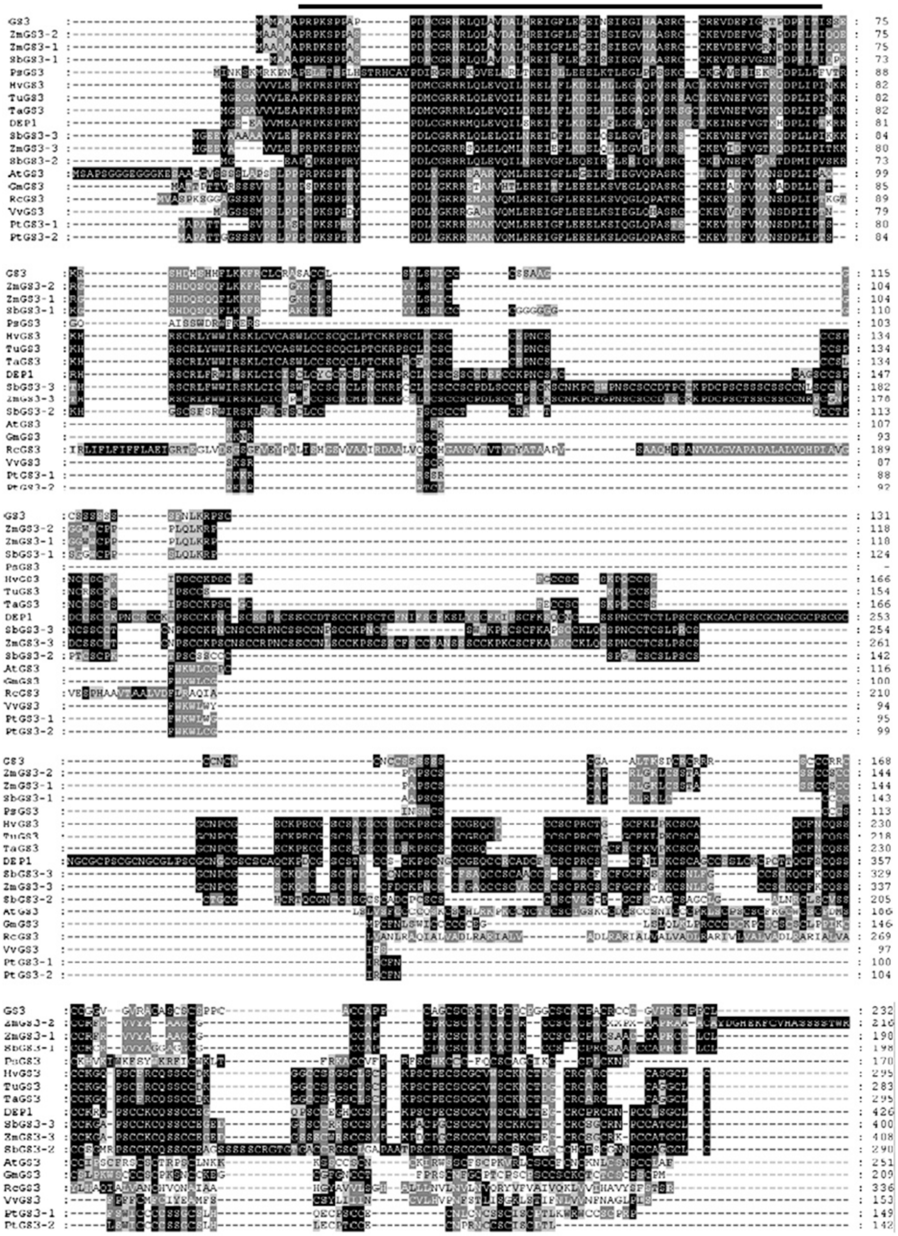

Fig. S2. Sequence alignment of GS3 and homologous proteins from different species. Black bar on the top indicates the highly conserved N-terminal region, which we named the OSR (organ size regulator) domain. Accession numbers are as follows: *ZmGS3-1*, *Zea mays*, ACZ02400; *ZmGS3-2*, NP_001144472; *ZmGS3-3*, NP_001152197; *SbGS3-1*, *Sorghum bicolor*, XP_002465152; *SbGS3-2*, XP_002444469; *SbGS3-3*, XP_002460275; *DEP1*, *Oryza sativa*, BAD38070; *TuGS3*, *Triticum urartu*, ACT78691; *AtGS3*, *Arabidopsis thaliana*, NP_680175; *TaGS3*, *Triticum aestivum*, ACI25444; *HvGS3*, *Hordeum vulgare*, ACI25445; *PtGS3-1*, *Populus trichocarpa*, XP_002324141; *PtGS3-1*,XP_002326287; *VvGS3*, *Vitis vinifera*, CBI27799; *RcGS3*, *Ricinus communis*, XP_002326287; *GmGS3*, *Glycine max*, ACU19287; *PsGS3*, *Picea sitchensis*, ABK26455.

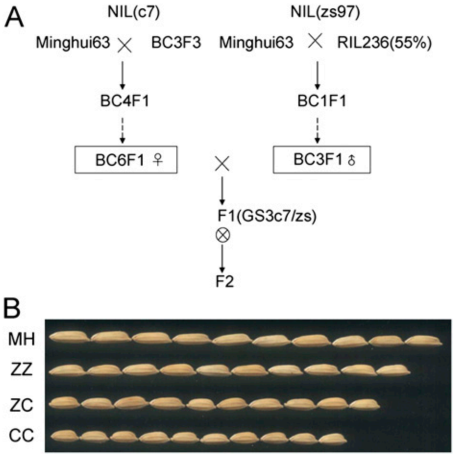

Fig. S3. Construction of NILs and their grains. (*A*) Process of near isogenic line (NIL) constructions. For developing NIL(c7), BC_3F_3 was further backcrossed three times to Minghui 63 to get BC_6F_1. For making NIL(zs97), RIL236, a recombinant inbred line containing the chromosome segment RZ403-C1087 from Zhenshan 97 and 55% of the genetic background from Minghui 63, from a population of 240 RILs, was backcrossed three times to Minghui 63. The two resulting lines were crossed to produce F_1 (GS3c7/zs), which was self-pollinated to produce F_2. (*B*) Grains of the NILs. CC, NIL(c7); ZZ, NIL(zs97); ZC, NIL(het); MH, Minghui63.

Table S1. Grain traits of segregants in a T_1 family of *GS3*-suppressed plants

Genotype	No. plants	Grain length (mm)	Grain width (mm)	1,000-grain weight (g)
Positive	35	6.97 ± 0.02	2.49 ± 0.01	12.80 ± 0.08
Negative	13	6.28 ± 0.02	2.50 ± 0.01	11.65 ± 0.13
P		1.48×10^{-25}	0.3566	1.01×10^{-9}

All data are given as mean ± SEM. *P* values are the results of *t* tests.

Table S2. Comparison of yield traits of two independent transgenic lines (O10, O13) overexpressing *GS3* with Minghui 63

Traits	Minghui 63	O10	O13
Grain length (mm)	9.90 ± 0.01	8.73 ± 0.02	8.75 ± 0.02
P		4.88×10^{-46}	4.63×10^{-44}
Grain width (mm)	2.95 ± 0.01	2.98 ± 0.08	2.97 ± 0.01
P		0.0349	0.0812
1,000-grain weight (g)	27.95 ± 0.22	19.27 ± 0.19	20.25 ± 0.23
P		2.66×10^{-32}	3.49×10^{-28}
No. grains per panicle	104.15 ± 2.47	45.56 ± 1.64	35.67 ± 2.39
P		2.13×10^{-24}	1.58×10^{-24}
No. tillers per plant	10.29 ± 0.73	14.21 ± 0.91	13.42 ± 0.60
P		0.0008	0.0009
No. days to heading	91.92 ± 0.43	91.21 ± 0.51	91.38 ± 0.42
P		0.1450	0.1837

All data are given as mean ± SEM. Each *P* value is obtained from a *t* test between the transgenic line and Minghui 63 (*n* = 24 for each genotype).

Table S3. Accessions of rice germplasm used for comparative sequencing

Accession ID*	Variety name	Subspecies	Country of origin	Grain length (mm)	Genotype
	Chuan 7	*indica*	China	6.4	GS3-4
W5-008	Dandongludao	*japonica*	China	7.6	GS3-1
W5-019	Liushizao	*indica*	China	7.45	GS3-1
W5-023	Jinxibai	*indica*	China	8.1	GS3-1
W5-024	Jiefangxian	*indica*	China	8.6	GS3-1
W5-025	Sanbaili	*indica*	China	8.3	GS3-1
W5-026	Taisannuo	*indica*	China	7.65	GS3-1
W5-027	Aihechi	*indica*	China	8.25	GS3-1
W5-028	Aimi	*indica*	China	8.05	GS3-1
W5-030	Jinbaoyin	*japonica*	China	8.75	GS3-1
W5-031	Minbeiwanxian	*indica*	China	8.25	GS3-1
W5-032	Lucaihao	*indica*	China	7.05	GS3-1
W5-033	Wukezhan	*indica*	China	7.85	GS3-1
W5-035	Yanshuichi	*indica*	China	7.15	GS3-1
W5-037	Simiao	*indica*	China	7.25	GS3-1
W5-038	Esiniu	*indica*	China	7.95	GS3-1
W5-039	Qimei	*indica*	China	7.9	GS3-1
W5-040	Nanxiongzaoyou	*indica*	China	7.9	GS3-1
W5-041	Baikehuoluo	*indica*	China	8.25	GS3-1
W5-042	Heidu 4	*indica*	China	7.8	GS3-1
W5-046	Hengxianliangchun	*indica*	China	8.05	GS3-1
W5-047	Aizaizhan	*indica*	China	7.7	GS3-1
W5-050	Qiyuexian	*indica*	China	6.85	GS3-1
W5-053	Dongtingwanxian	*indica*	China	8.5	GS3-1
W5-055	Xuanenchangtan	*indica*	China	7.95	GS3-1
W5-056	Bawangbian 1	*japonica*	China	7.5	GS3-1
W5-057	Baikezaohe	*indica*	China	8.3	GS3-1
W5-058	Xugunuo	*japonica*	China	7.5	GS3-1
W5-061	Wanlixian	*indica*	China	8.1	GS3-1
W5-062	Xiangdao	*indica*	China	7.95	GS3-1
W5-063	Hanmadao	*indica*	China	8.05	GS3-1
W5-065	Mamamgu	*indica*	China	7.95	GS3-1
W5-067	Meihuanuo	*indica*	China	8.45	GS3-1
W5-068	Zhongnong 4 hao	*indica*	China	7.75	GS3-1
W5-076	Qitoubaigu	*japonica*	China	8.2	GS3-1
W5-080	Xianggu	*japonica*	China	8.1	GS3-1
W5-081	Xiaohonggu	*japonica*	China	8	GS3-1
W5-082	Qingke	*indica*	China	7.8	GS3-1
W5-083	Wuzidui	*japonica*	China	7.05	GS3-1
W5-086	Gongju 73	*japonica*	China	8.15	GS3-1
W5-090	Qitougu	*indica*	China	8.75	GS3-1
W5-094	Mowanggunei	*japonica*	China	7.35	GS3-1
	Zhenshan 97	*indica*	China	8.2	GS3-1
W5-001	Funingzipi	*japonica*	China	7.75	GS3-2
W5-002	Longhuamaohu	*japonica*	China	8.45	GS3-2
W5-003	Gaoyangdiandao	*japonica*	China	8.25	GS3-2
W5-005	Yelicanghua	*japonica*	China	8.1	GS3-2
W5-010	Laoguangtou 83	*japonica*	China	6.75	GS3-2
W5-011	Baimaodao	*japonica*	China	8.2	GS3-2
W5-014	Youmangzaojing	*japonica*	China	6.85	GS3-2
W5-015	Huangkezaogan	*japonica*	China	7.2	GS3-2
W5-016	Baigedao	*japonica*	China	6.9	GS3-2
W5-018	Tieganwu	*japonica*	China	7.25	GS3-2
W5-022	Feidongtangdao	*japonica*	China	7.25	GS3-2
W5-029	Hongmisandan	*japonica*	China	6.6	GS3-2
W5-043	Chikenuo	*indica*	China	8.25	GS3-2
W5-049	Hongainuo	*indica*	China	7.85	GS3-2
W5-052	Dongtingwanxian	*indica*	China	8.3	GS3-2
W5-059	Muguanuo	*japonica*	China	7.25	GS3-2
W5-060	Hongqi 5 hao	*japonica*	China	7.8	GS3-2
W5-064	Xibaizhan	*japonica*	China	7.9	GS3-2
W5-066	Nantiangangjiu	*japonica*	China	7	GS3-2

Table S3. Cont.

Accession ID*	Variety name	Subspecies	Country of origin	Grain length (mm)	Genotype
W5-069	Honggu	*indica*	China	8	GS3-2
W5-071	Sanjiugu	*japonica*	China	7.65	GS3-2
W5-072	Haomake (K)	*japonica*	China	8	GS3-2
W5-077	Benbanggu	*japonica*	China	8.3	GS3-2
W5-087	Lengshuigu 2	*japonica*	China	8.2	GS3-2
W5-089	Lamujia	*japonica*	China	7.8	GS3-2
W5-092	Yuyannuo	*japonica*	China	7.7	GS3-2
	Nipponbare	*japonica*	Japan	7.3	GS3-2
	Zhonghua 11	*japonica*	China	7.5	GS3-2
W5-017	Cunsanli	*japonica*	China	9.6	GS3-3
W5-044	Sanlicun	*japonica*	China	9.45	GS3-3
W5-075	Sanqishi	*japonica*	China	9.15	GS3-3
W5-078	Dawannuo	*indica*	China	9.5	GS3-3
W5-079	Zimi	*japonica*	China	9.35	GS3-3
W5-091	Zinuo	*indica*	China	9.3	GS3-3
	Minghui 63	*indica*	China	10.1	GS3-3
W5-021	Leihuozhan	*indica*	China	9.15	GS3-3
W5-054	Liuyezhan	*indica*	China	9	GS3-3
W5-070	Sankecun	*indica*	China	9.6	GS3-3
W5-074	Haobuka	*japonica*	China	9.65	GS3-3

*Rice accession ID from core collection of Chinese rice germplasm.

Table S4. Comparison of grain length between accessions with or without the TCC insertion

Group	TCC insertion	No. of accessions	Grain length (mm)	*t*	*P*
Group 1	With	2	7.33 ± 0.28	0.7191	0.2393
	Without	25	7.61 ± 0.11		
Group 2	With	40	7.94 ± 0.07	0.2932	0.3854
	Without	3	8.02 ± 0.14		

Data of grain length are given as mean ± SEM. *P* value is obtained from a *t* test.

Table S5. Measurements of yield component traits of the near isogenic lines in comparison with Minghui 63 from field trial

	NIL (c7)	NIL (het)	NIL(zs97)	Minghui 63
No. grains per panicle	113.08 ± 6.08 ab	123.60 ± 4.80 a	117.62 ± 5.31 ab	105.60 ± 4.20 b
No. tillers per plant	5.92 ± 0.36 Aa	7.58 ± 0.36 Ab	8.54 ± 0.51 Bb	8.90 ± 0.53 Bb
Yield per plant (g)	10.68 ± 0.95 A	16.05 ± 1.00 Ba	18.71 ± 1.54 Bab	22.09 ± 1.44 Cb
1,000-grain weight (g)	18.42 ± 0.19 A	20.82 ± 0.16 B	22.80 ± 0.41 C	26.78 ± 0.39 D
Grain length (mm)	7.57 ± 0.04 A	8.23 ± 0.02 B	9.02 ± 0.03 C	9.85 ± 0.03 D
Grain width (mm)	3.07 ± 0.01 bc	3.08 ± 0.01 cd	3.05 ± 0.01 ab	3.04 ± 0.01 a

All data are given as mean ± SEM. A, B, C, D: ranked by Duncan test at $P \leq 0.01$; a, b, c, d: ranked by Duncan test at $P \leq 0.05$.

Table S6. Primers used in this study

Primer name	Sequence	Purpose
9247r	5′-GAGGTACCACGCACTTGCTCTGCACAAACAGCT-3′	Vector construction
1512f	5′-TGGGTACCCAAAACTGCCGAGATTTGTCACATT-3′	Vector construction
U9EcoRIF	5′-AGGGTTGACGAATTCATCGGAAGAACTCCTGAT-3′	Vector construction
RBP	5′-AGTTAAGCTTAGGTCCCTTTTCTCGTCCTCGA-3′	Vector construction
F1SacI	5′-CATCATgagctcTATCGGAACTTCGGAGTGACATGGCA-3′	Vector construction
VWFCKpnI	5′-TTTggatccTTAACACGGACTCTTCGTTAACGCCGCC-3′	Vector construction
DelPEcoRIR	5′-CCGAATTCCGCCGCCGCCATTGCCATGTCA-3′	Vector construction
DelVMluIR	5′-TATACGCGTGGAGCAGCTGCAGCTCGCGCACG-3′	Vector construction
DelVMluIF	5′-ACGCGTTAGTGATCGATCGATCGATTGA-3′	Vector construction
DelTMluIR	5′-TATAACGCGTTGAGCAGCCGCCGGCGGCGCT-3′	Vector construction
DelTMluIF	5′-ACGCGTTTAACGAAGAGTCCGTGTCG-3′	Vector construction
PVMluIF	5′-TGGACGCGTGCAGCACGCACTTGCTCTGCACA-3′	Vector construction
VMluIR	5′-TTACGCGTCGCCGCCGCCATTGCCATGTCA-3′	Vector construction
3UTRMluIR	5′-acgcgtAGTACGCGCGCAGCTCGATCGATCA-3′	Vector construction
3UTRMluIF	5′-acgcgtTAATTAGCTAGCTAGGTTCTGGTGT-3′	Vector construction
F-ExHindIIIR	5′-gaagcttTCATCGATCGATCGATCACAA-3′	Vector construction
CFPXbaIF	5′-GGGtctagaCGGAGTGACATGGCAATGGC-3′	Vector construction
GUS1.6R	5′-GAGTGAAGATCCCTTTCTTGTTACCG-3′	Positive test
GUS1.6F	5′-CCAGGCAGTTTTAACGATCAGTTCGC-3′	Positive test
PMCGR	5′-CTGAGCTACACATGCTCAGGTT-3′	Positive test
PMCGF	5′-GGCTCACCAAACCTTAAACAA-3′	Positive test
dsF1	5′-CCGCGAGATCGGATTCC-3′	Expression analysis
dsR1	5′-CGTGGATCCCTTCGATTGA-3′	Expression analysis
G53F	5′-GAACTCCTGATCCATTCATAACGATT-3′	Expression analysis
G53R	5′-CAAACAGCGAAACTTCTTCAAGAA-3′	Expression analysis
Actin1F	5′-TGGCATCTCTCAGCACATTCC-3′	Expression analysis
Actin1R	5′-TGCACAATGGATGGGTCAGA-3′	Expression analysis
SF18	5′-CCTTCAGTAAGAGAGATGTG-3′	Comparative sequencing
SR18	5′-AGTTGATGGTTTTGTGGGAT-3′	Comparative sequencing
GS64F	5′-CAACACCAGCAACGAACAAC-3′	Comparative sequencing
GS64R	5′-ACGAGGGATTATCAGCCATT-3′	Comparative sequencing
SF46	5′-ACTTTGACCCTTGACTACTCGT 3′	Comparative sequencing
SR46	5′-AGCATCTGGAGGCAGCGTGGA 3′	Comparative sequencing
SF47	5′-AGTCCGTGCTATAGCTGACT-3′	Comparative sequencing
GS3F	5′- TGACGAATTCATCGGAAGAACT-3′	Comparative sequencing
race3f	5′-TGGTCCACGCTAGTTTCTGAGTTCTG-3′	Comparative sequencing
SF16	5′-CGAATAGGAAGTCAATGGC-3′	Comparative sequencing
SR16	5′-GAGAGAGAAGAGCAGCGGA-3′	Comparative sequencing
SF17	5′-TGGCTTGATTTCCTGTGC-3′	Comparative sequencing
SR17	5′-TGTTCGTTGCTGGTGTTG-3′	Comparative sequencing
SF28	5′-TGCCCATCTCCCTCGTTTAC-3′	Comparative sequencing
SR28	5′-GTTGTTCGTTGCTGGTGTTG-3′	Comparative sequencing
SF29	5′-TCCCACAAAACCATCAACTT-3′	Comparative sequencing
SR29	5′-AACCACCATATCAGCCAAAA-3′	Comparative sequencing
SF45	5′-AACCTTCTCTTCCTACCCTT-3′	Comparative sequencing
SR45	5′-TCAGCAATCACGTACTCATC-3′	Comparative sequencing