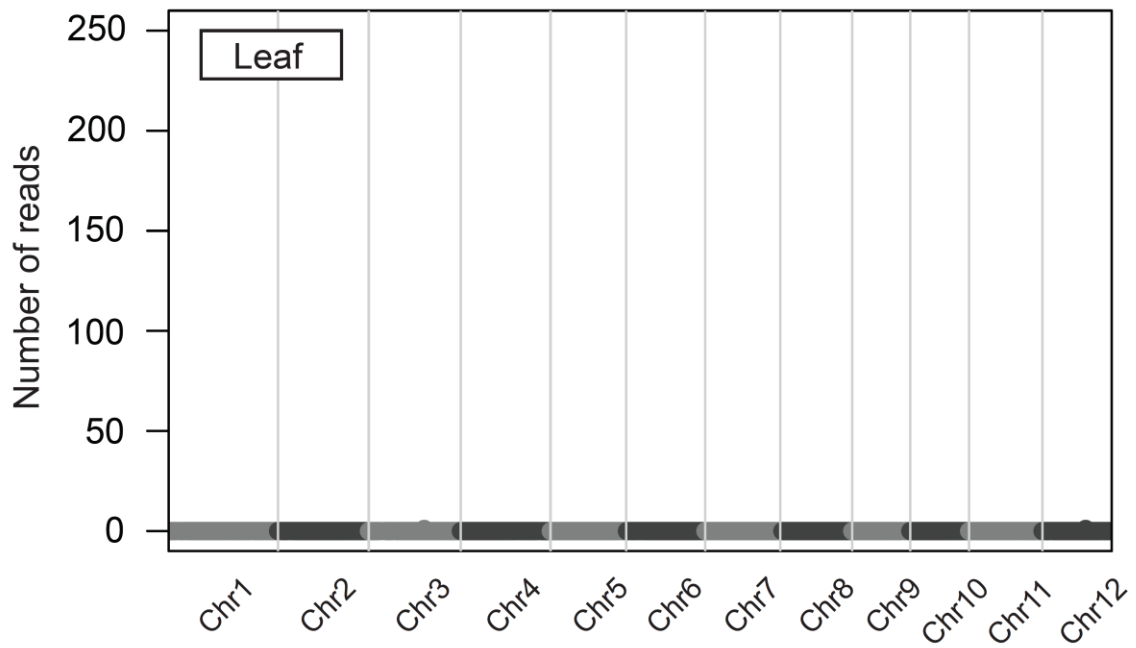1

Supplementary Figure 1. PBS sequences of LTR retrotransposons in *Arabidopsis*, rice and

tomato

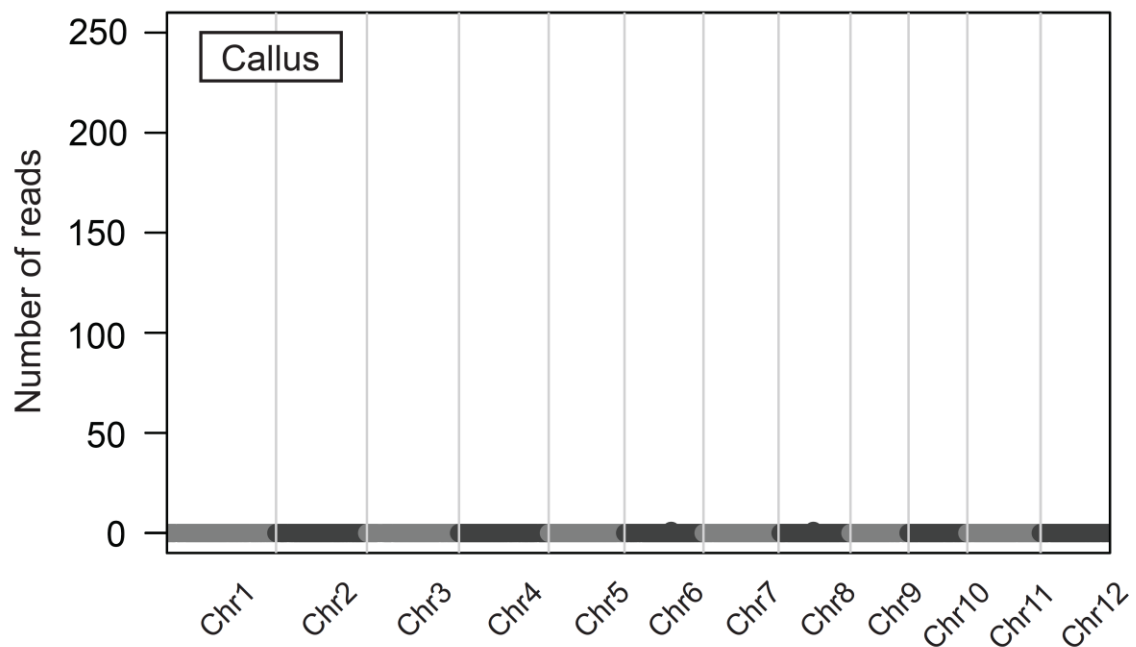The frequency of tRNAs used for targeting PBS. LTR retrotransposons were annotated by

LTRpred (http://github.com/HajkD/ALE) and selected for young elements by filtering LTR

similarities higher than 95%. The total numbers of retrotransposons analysed in each

species are shown below the plots.

a

Leaf

Number of reads

250
200
150
100
50
0

Chr1 Chr2 Chr3 Chr4 Chr5 Chr6 Chr7 Chr8 Chr9 Chr10 Chr11 Chr12

b

Callus

Number of reads

250
200
150
100
50
0

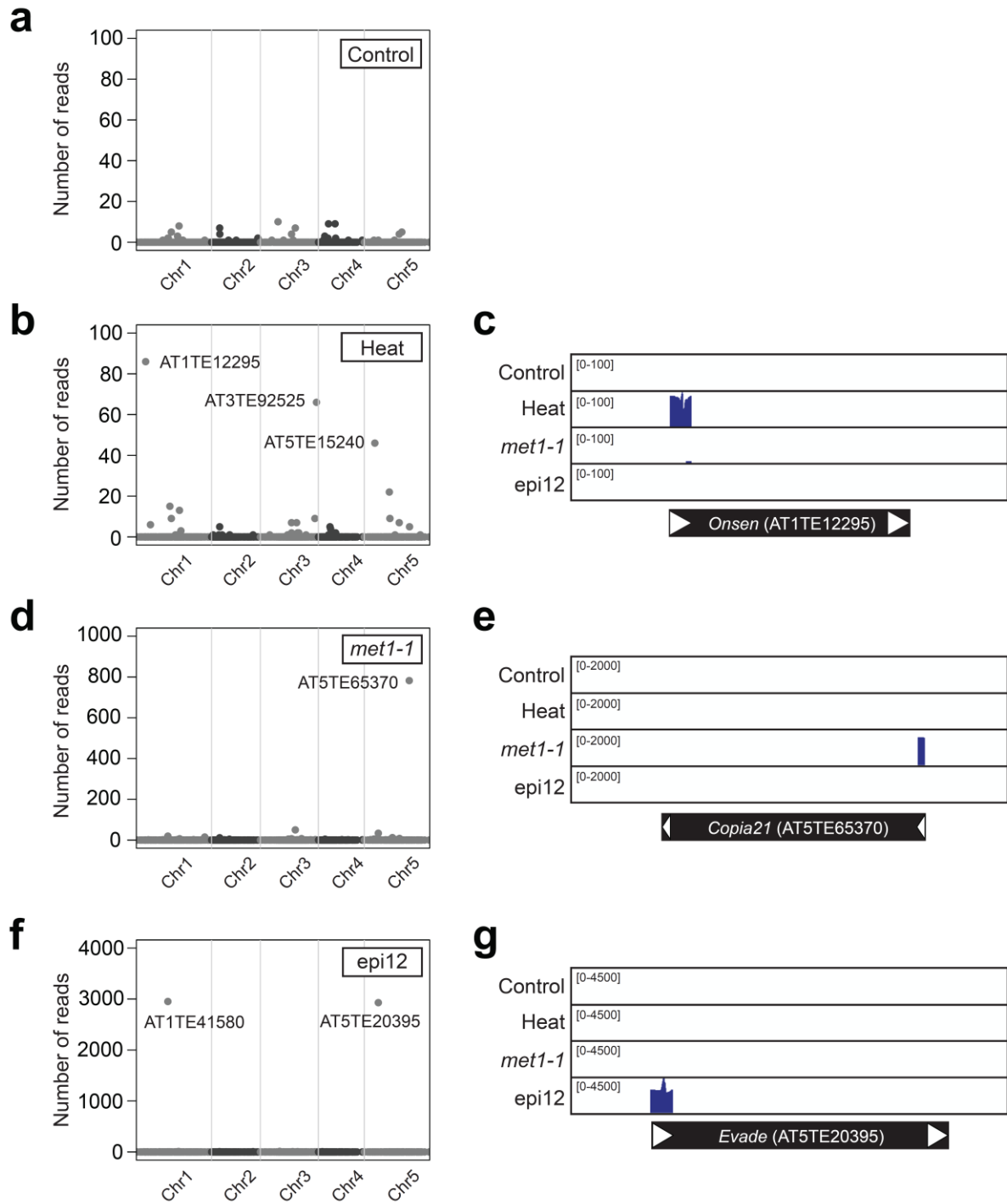Chr1 Chr2 Chr3 Chr4 Chr5 Chr6 Chr7 Chr8 Chr9 Chr10 Chr11 Chr12

9

10   **Supplementary Figure 2.** SIRT results from leaves and calli of rice

11   **a** and **b,** Genome-wide plots for SIRT performed in leaves (**a**) and in calli (**b**) of rice.
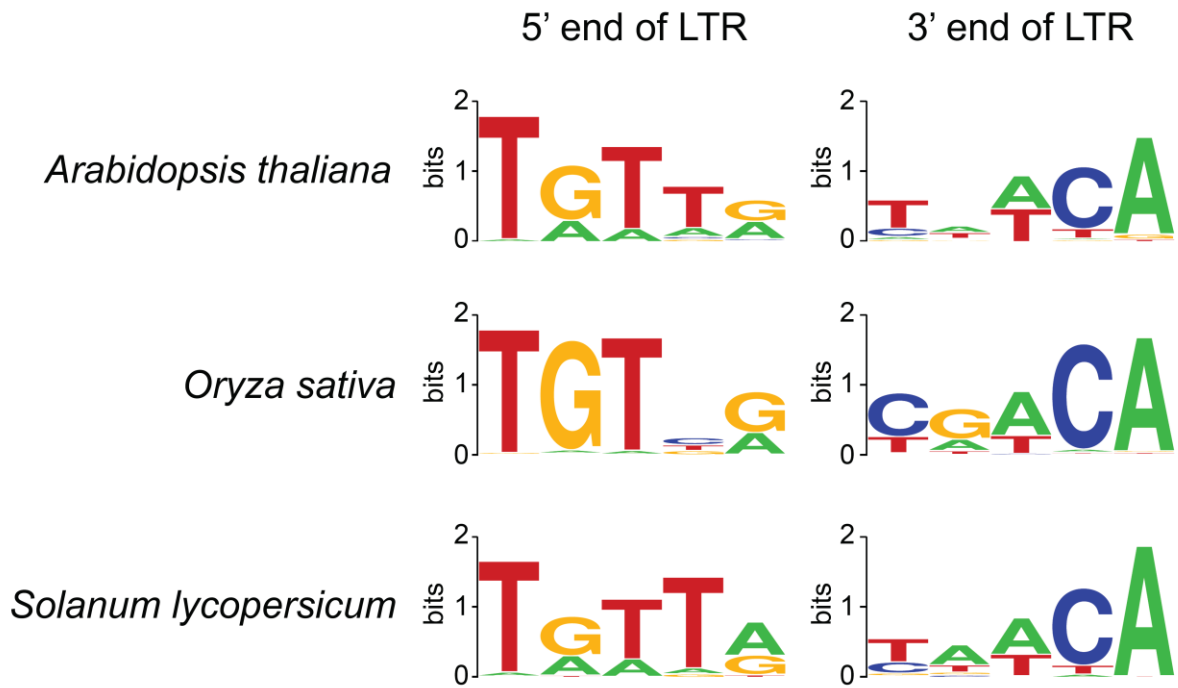
12

Supplementary Figure 3. ALE-seq detection of eclDNAs of *Arabidopsis* retrotransposons

Genome-wide plots (**a**, **b**, **d** and **f**) and read coverage plots (**c**, **e** and **g**) for ALE-seq profiles of *Arabidopsis* Col-0 wt (**a**), heat-stressed Col-0 (**b** and **c**), *met1-1* (**d** and **e**), and epi12 (**f** and **g**).

16

5' end of LTR   3' end of LTR

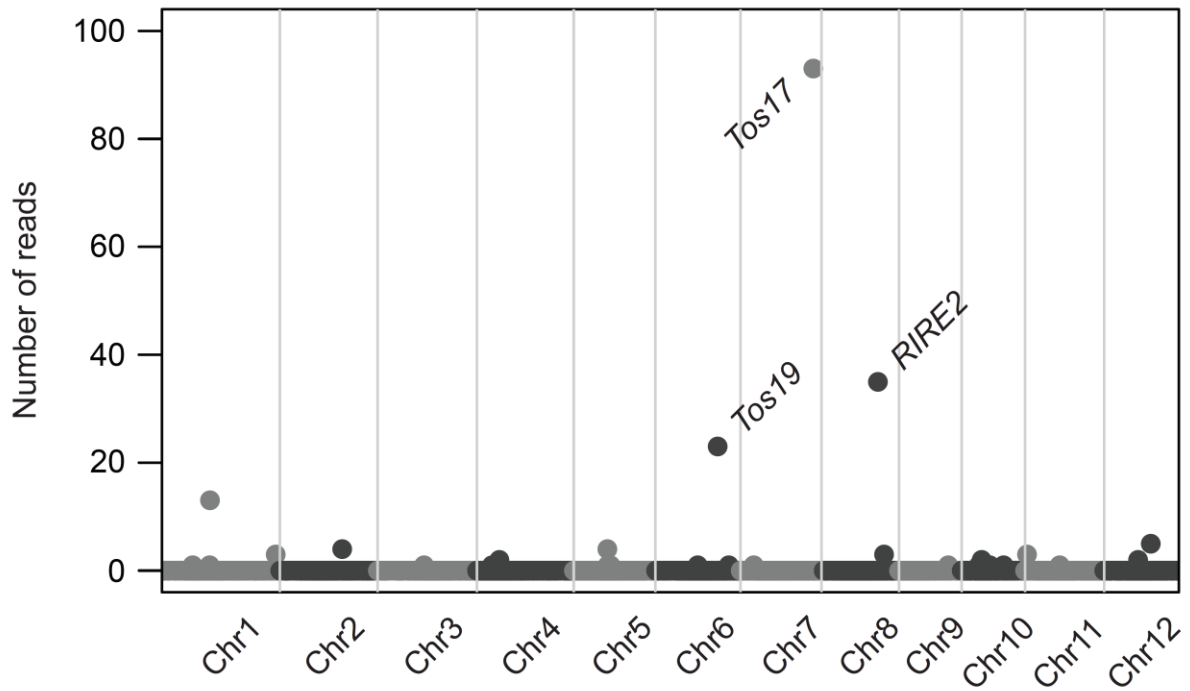*Arabidopsis thaliana*

Oryza sativa

Solanum lycopersicum

17

Supplementary Figure 4. Conservation of end sequences of LTR

The conserved sequences of 5' and 3' ends of LTR. The first and last five nucleotides of LTRs

are displayed. The images were generated by the WebLogo tool

(http://weblogo.berkeley.edu/logo.cgi).

22
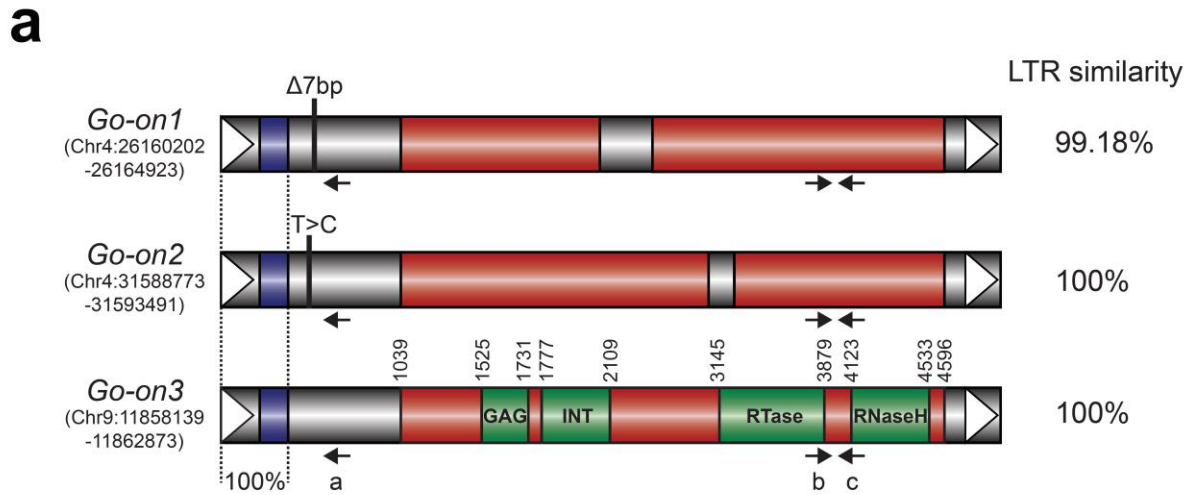
23

Supplementary Figure 5. ALE-seq detection of eclDNAs of rice retrotransposons using multiplexed PBS primers

Genome-wide plot for ALE-seq profiles of rice callus using pooled PBS primers of Met-iCAT and Arg-CCT.

a

Go-on1
(Chr4:26160202
-26164923)

Δ7bp

LTR similarity

99.18%

Go-on2
(Chr4:31588773
-31593491)

T>C

100%

Go-on3
(Chr9:11858139
-11862873)

1039 1525 1731 1777 2109 3145 3879 4123 4533 4596

GAG INT RTase RNaseH

100%

100% a b c

b

```
Go-on1  123  TGGTATCAGAGCCAATCGGCTGGTGGCTGGCGACGG-------CTAAACCCTAGCCTCGCCGGAG
Go-on2  123  TGGTATCAGAGCCAATCGGCTGGCGGCTGGCGACGGGCGACGGCTAAACCCTAGCCTCGCCGGAG
Go-on3  123  TGGTATCAGAGCCAATCGGCTGGTGGCTGGCGACGGGCGACGGCTAAACCCTAGCCTCGCCGGAG
clone1  123  TGGTATCAGAGCCAATCGGCTGGTGGCTGGCGACGGGCGACGGCTAAACCCTAGCCTCGCCGGAG
clone2  123  TGGTATCAGAGCCAATCGGCTGGTGGCTGGCGACGGGCGACGGCTAAACCCTAGCCTCGCCGGAG
clone3  123  TGGTATCAGAGCCAATCGGCTGGTGGCTGGCGACGGGCGACGGCTAAACCCTAGCCTCGCCGGAG
clone4  123  TGGTATCAGAGCCAATCGGCTGGTGGCTGGCGACGGGCGACGGCTAAACCCTAGCCTCGCCGGAG
clone5  123  TGGTATCAGAGCCAATCGGCTGGTGGCTGGCGACGGGCGACGGCTAAACCCTAGCCTCGCCGGAG
```

c

ACACGACGCTCTTCCGATCT TGTTGAGTTATGTATGTGTT
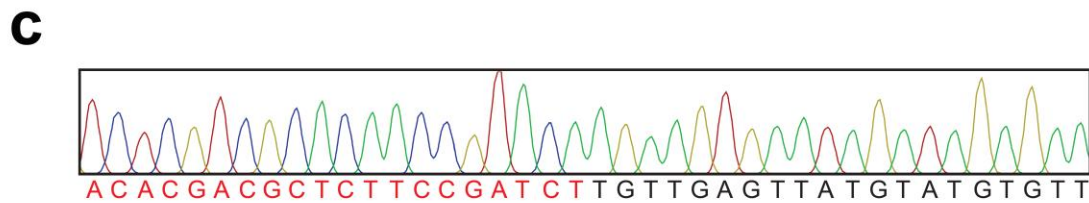
29

30   **Supplementary Figure 6.** *Go-on* retrotransposon family

31   **a**, Schematic structure of *Go-on* retrotransposons. The genomic coordinates and LTR

32   similarities of each copy are shown at the left and right, respectively. Red boxes, ORFs;

33   green boxes, regions encoding protein domains; blue boxes, PBS; white arrowheads, LTRs.

34   Note that the sequences of the upstream LTRs through the PBS are identical in all three

35   copies. The sequence variation specific for each element is indicated. Protein domains were

36   predicted by NCBI BLASTP tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Nucleotide

37   positions indicating the start and end of ORF and protein domains are provided. Primers

38   used for sequencing and qPCR analyses are shown as arrows. **b**, Multiple sequence

39   alignment of the genomic sequences of three *Go-on* copies and the sequenced ALE clones.

40   ALE-seq was performed using the RT primer specific to *Go-on3* indicated as "a" in **a**. The

41   resulting single-stranded first strand cDNA was PCR-amplified, cloned to the pGEM T-easy

42   vector, and sequenced. Multiple sequence alignment was performed by ClustalW

43   (http://www.genome.jp/tools-bin/clustalw) and visualized by boxshade tools

44   (https://www.ch.embnet.org/software/BOX_form.html). **c**, Sequencing of the ALE-seq

45   product of *Go-on3* showing the junction region of the adapter and LTR. Sequences in red

46   and black are the adapter and *Go-on* LTR, respectively.

47

a
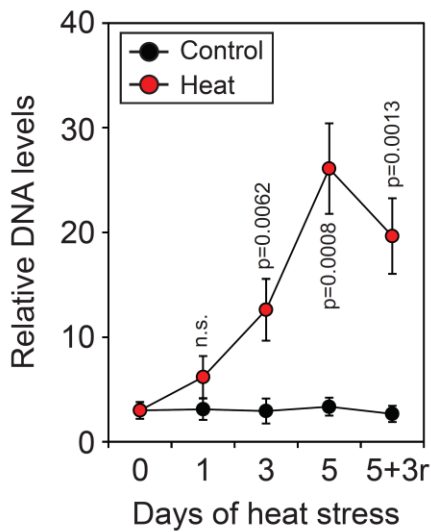
b
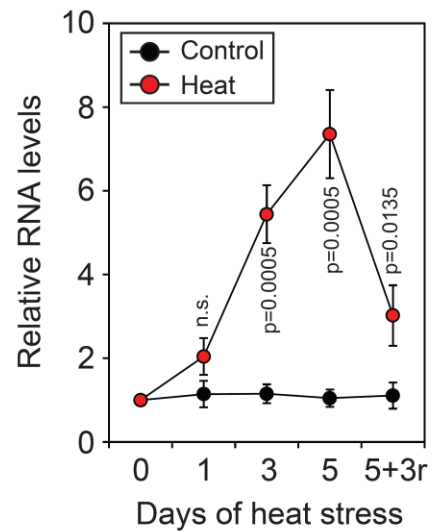
c

5′-TGTTGAGTTATGTATGTGTTGGCCCATGAGGCCCATATACTAC
1   10   20   30   40

TCATATGTACATGTATATAGCAGAGTTAGAGAAATGAAAAAAGTAG
         50   60   70   80

TGAAGCTTCTAGAGAAAAATTCCCAAAACTTCATGGTATCAGAGC-3′
90   100   110   120   130   134

Supplementary Figure 7. Heat stress-triggered transcriptional activation of *Go-on*

a and **b**, The relative levels of DNA (**a**) and RNA (**b**) of *Go-on3* determined by qPCR. Heat treatment (44°C) was applied to 1-week-old rice seedlings for the periods indicated; +3r means 3 days of recovery in normal growth conditions after heat stress. The levels are means ± sd of three biological replicates. For DNA analysis, Day 0 levels are set to 3, reflecting three genomic copies of *Go-on* in *japonica* rice. Normalization was done against *eEF1α*. P-values were calculated by a two-tailed Student's t-test; n.s., not significant. **c**, The sequence of the left LTR and PBS of *Go-on3*. The sequence in red is the heat-related HSFC1-binding sequence motif predicted by PlantPan 2.0 tool (http://plantpan2.itps.ncku.edu.tw/index.html) with statistically significant enrichment of P= 4.28e^{-10} as determined by Fisher's exact test. The enrichment of the sequence motif was

60 calculated by comparing the ten most similar sequences of *Go-on* found in rice genome with

61 1,000 random genomic loci of 150bp. The PBS is shown in blue.

62

63
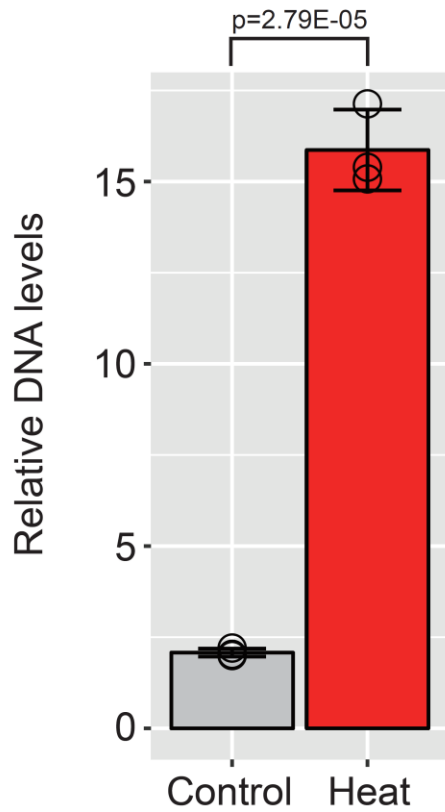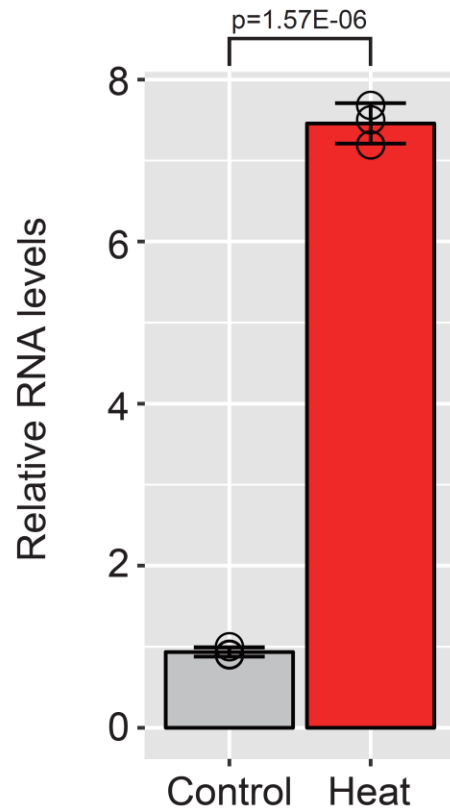
Supplementary Figure 8. Heat stress-triggered activation of *Go-on* in *indica* rice

a and **b**, The qPCR analyses for DNA (**a**) and RNA (**b**) levels of *Go-on* in *indica* rice. The levels are means ± sd of three biological replications. The levels of control sample are set to 2 (**a**) reflecting 2 genomic copies of *Go-on* in *indica* rice. P-values are determined by two-sided Student's t-test.
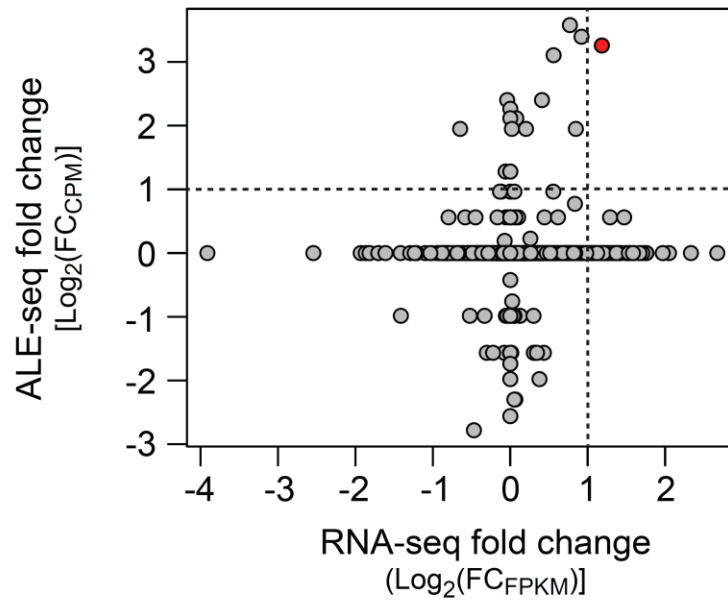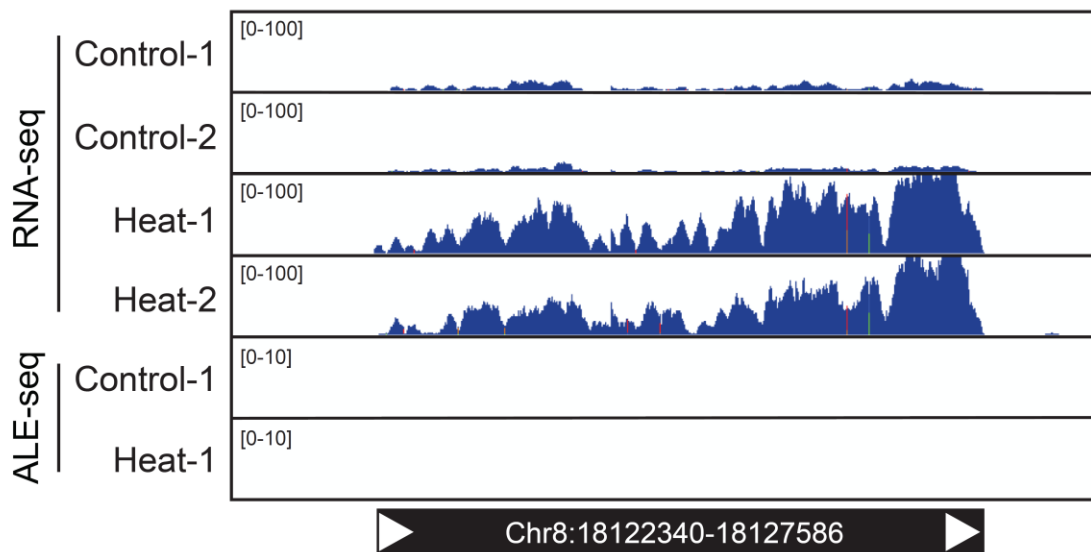
a, Scatter plot for log2-fold changes (FCs) in RNA-seq and ALE-seq profiles in the control and

heat-stressed rice plants used in Figure 3. FCs were calculated by dividing heat samples

values by control samples values of CPM (counts per million reads) and FPKM (fragments

per kb per million reads) for ALE-seq and RNA-seq data, respectively. Each dot represents an

76   individual retroelement and the dashed lines mark log2-FC one. Th retrotransposon in red

77   has log2-FC higher than one in both ALE-seq and RNA-seq. **b**, Read coverage plot for a

78   selected retrotransposon showing evidence of transcriptional activation upon heat stress

79   not followed by synthesis of eclDNAs.

80

81
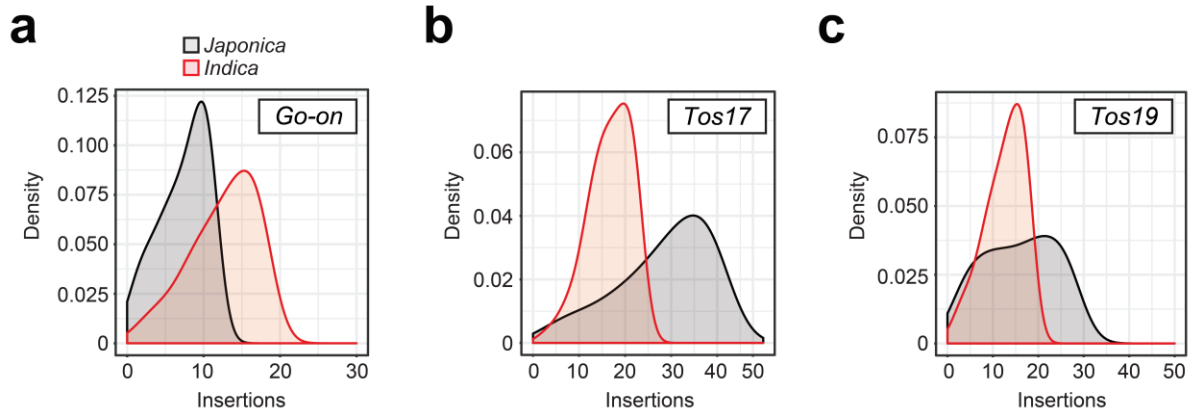
Supplementary Figure 10. Retrotransposon insertions in *japonica* and *indica* rice

a-**c**, Density plots for number of non-reference insertions in randomly selected 200 accessions out of 388 iterated by 1,000 times.

85

a

b

86
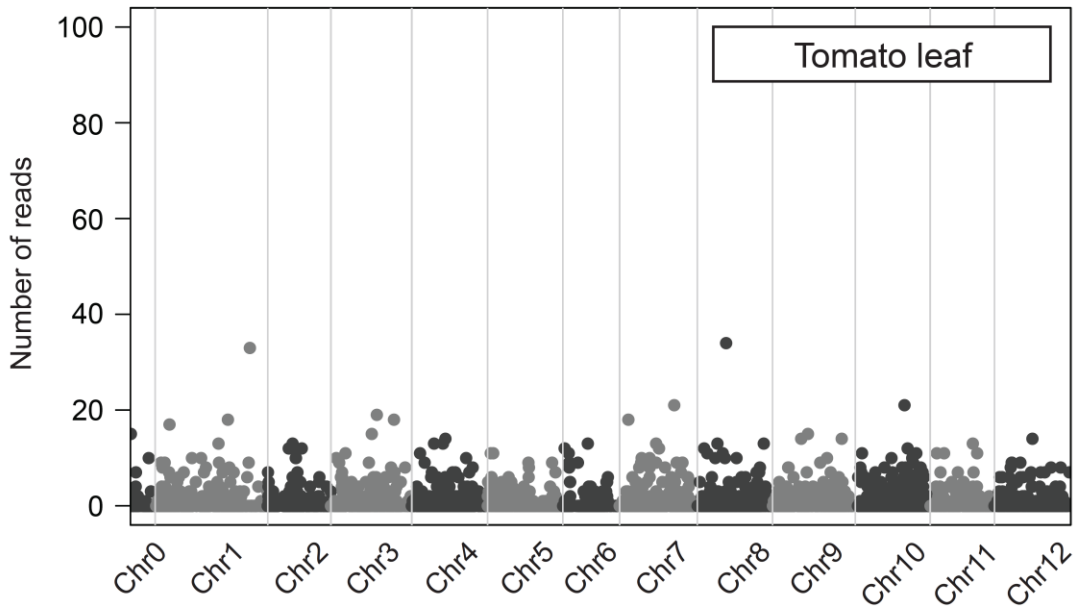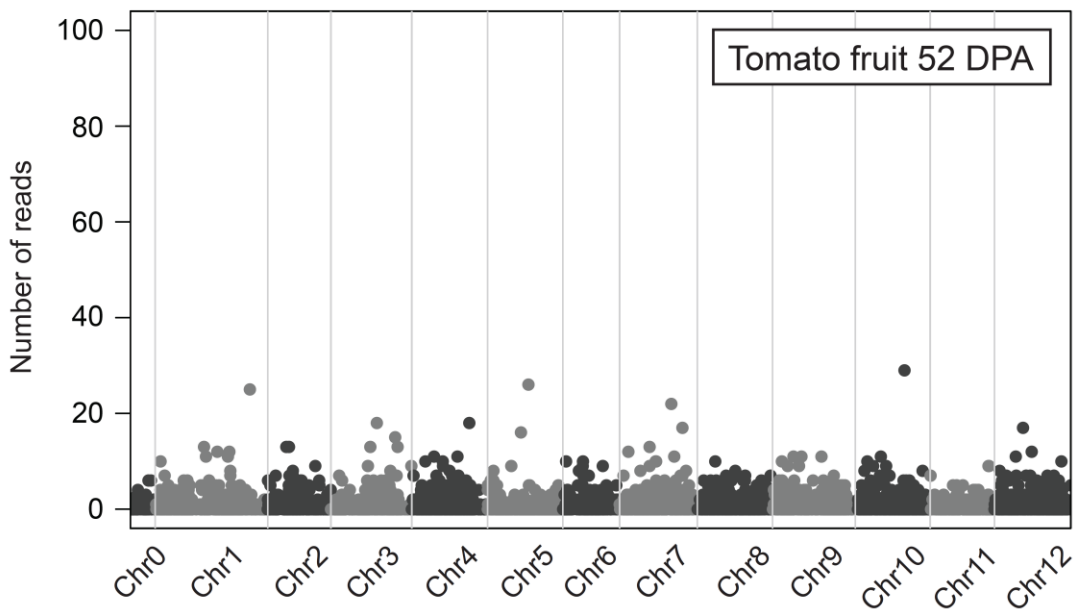
Supplementary Figure 11. ALE-seq profile of tomato leaves and fruits

a and **b**, Genome-wide plots for ALE-seq profiles performed in tomato leaves (**a**) and fruits 52 DPA (**b**). Each dot represents an individual retrotransposon.

90

91　**Supplementary Table 1.** Sequences of oligonucleotides used in this study

92　T7 promoter sequence is underlined and in bold is partial Illumina adapter sequence.

Primer	Sequence (5' → 3')
ALE adapter top strand	AGAGAG<u>TAATACGACTCACTATAGGG</u>**ACACGACGCTCTTCCGATCT**
ALE adapter bottom strand	**AGATCGGAAGAGCGTCGTGT**<u>CCCTATAGTGAGTCGTATTA</u>CTCTCT
ALE RT Met-iCAT-R	AGACGTGTGCTCTTCCGATCTGCTCTGATACCA
ALE RT Arg-CCT-R	AGACGTGTGCTCTTCCGATCTCCTGGCGCGCCA
ONSEN full length-F	TGTTGAAAGTTAAACTTGATTTTG
ONSEN full length-R	TGTTAGAGTAAAATTCTTTTAG
Go-on-F (b of Figure S5)	GGCAGAATACAGGGCAATGTC
Go-on-R (c of Figure S5)	GCCGACTTATTGTCACACCAC
Go-on RT-R (a of Figure S5)	TCTCTGCACGCCTCGACAAG
eEF1α-F	GCACGCTCTTCTTGCTTTCACTCT
eEF1α-R	AAAGGTCACCACCATACCAGGCTT
FIRE RT-F	GAGTTGGCTACGTATCGTTTGC
FIRE RT-R	AGCCTCCACAAATTCATCCCAT
FIRE copy number-F	GGTGTTCTCGTTGTGGTAAGT
FIRE copy number-R	TAAGGTGACACTCCCTCATAGT
SlCAC-F	CCTCCGTTGTGATGTAACTGG
SlCAC-R	ATTGGTGGAAAGTAACATCATCG
SlGAPDH-F	ATGCTCCCATGTTTGTTGTGGGTG
SlGAPDH-R	TTAGCCAAAGGTGCAAGGCAGTTC

93

94 **Supplementary Table 2.** Summary of ALE-seq libraries

95 Numbers of reads sequenced and mapped are summarised. Both unique-mappers and
96 multi-mappers are considered. "% mapped to LTRs" refers to all reads mapped throughout
97 retrotransposon.

Samples	Reads sequenced	% mapped to genome	% mapped to LTRs	% not mapped to LTRs	Accession number
Arabidopsis Col-0	56,057	93.20	17.77	75.43	SAMN09748167
Arabidopsis heat-stressed	45,554	90.33	15.85	74.48	SAMN09748168
Arabidopsis *met1-1*	58,029	94.79	16.03	78.76	SAMN09748169
Arabidopsis epi12	45,545	96.19	31.18	65.01	SAMN09748170
Rice leaf	27,063	97.54	12.33	85.21	SAMN09748171
Rice callus	25,183	95.67	13.58	82.09	SAMN09748172
Rice callus -Lig	37,610	83.48	11.75	71.73	SAMN09748173
Rice callus -T7	870	2.82	0.12	2.70	SAMN09748174
Rice callus -RT	516	1.26	0	1.26	SAMN09748175
Rice callus pooled PBS	22,939	90.06	14.16	75.90	SAMN09748176
Rice non-stressed	31,819	90.39	12.29	78.10	SAMN09748177
Rice heat-stressed	31,525	97.63	13.54	84.09	SAMN09748178
Tomato leaf	46,421	96.65	13.24	83.41	SAMN09748179
Tomato fruit 52 DPA	73,067	96.97	28.53	68.44	SAMN09748180

98

Supplementary Table 3. Non-reference insertions of *Go-on*.

Neo-insertions of *Go-on* detected by TIF. The positions are provided as coordinates of target
site duplication.

Chromosome	Start	End	Accession	Category
Chr4	31968290	31968294	ERS467756	*Indica*
Chr4	31968290	31968294	ERS467757	*Indica*
Chr1	43029985	43029989	ERS467791	*Indica*
Chr4	31968290	31968294	ERS467794	*Indica*
Chr4	31968290	31968294	ERS467830	*indica*
Chr1	43029985	43029989	ERS467831	*indica*
Chr4	31968290	31968294	ERS467831	*indica*
Chr7	29937784	29937788	ERS467843	*indica*
Chr1	43029985	43029989	ERS467872	*indica*
Chr4	31968290	31968294	ERS467876	*indica*
Chr1	43029985	43029989	ERS467877	*indica*
Chr1	43029985	43029989	ERS467878	*indica*
Chr8	5518300	5518306	ERS467878	*indica*
Chr7	29937784	29937788	ERS467880	*indica*
Chr4	31968290	31968294	ERS467883	*indica*
Chr11	1485949	1485953	ERS467910	*indica*
Chr1	43029985	43029989	ERS467915	*indica*
Chr4	31968290	31968294	ERS467924	*indica*
Chr7	29937784	29937788	ERS467925	*indica*
Chr4	31968290	31968294	ERS467926	*indica*
Chr1	20049396	20049400	ERS467927	*indica*
Chr8	5518300	5518306	ERS467934	*indica*
Chr1	43029985	43029989	ERS467938	*indica*
Chr1	20049396	20049400	ERS467943	*indica*
Chr8	3790507	3790511	ERS467943	*indica*
Chr1	20049396	20049400	ERS467944	*indica*
Chr4	31960603	31960607	ERS467952	*indica*
Chr8	5518300	5518306	ERS467952	*indica*
Chr8	5518300	5518306	ERS467959	*indica*
Chr1	20049396	20049400	ERS467960	*indica*
Chr1	43029985	43029989	ERS467961	*indica*
Chr1	20049396	20049400	ERS467962	*indica*
Chr4	13737528	13737532	ERS467962	*indica*
Chr7	29937784	29937788	ERS467962	*indica*
Chr1	43029985	43029989	ERS467966	*indica*
Chr1	43029985	43029989	ERS467969	*indica*
Chr4	31968290	31968294	ERS467969	*indica*
Chr1	43029985	43029989	ERS467979	*indica*
Chr8	5518300	5518306	ERS467980	*indica*
Chr1	43029985	43029989	ERS467986	*indica*
Chr4	31968290	31968294	ERS467995	*indica*
Chr7	29937784	29937788	ERS467995	*indica*
Chr12	16846383	16846387	ERS467996	*indica*
Chr4	31968290	31968294	ERS467996	*indica*
Chr8	5518300	5518306	ERS467996	*indica*
Chr5	287377	287381	ERS467998	*indica*
Chr1	43029985	43029989	ERS467999	*indica*
Chr8	3790507	3790511	ERS468001	*indica*
Chr1	20049396	20049400	ERS468004	*indica*
Chr8	5518300	5518306	ERS468004	*indica*
Chr1	20049396	20049400	ERS468006	*indica*
Chr8	5518300	5518306	ERS468006	*indica*
Chr4	31960603	31960607	ERS468008	*indica*
Chr7	29937784	29937788	ERS468011	*indica*

Chr8	5518300	5518306	ERS468011	*indica*
Chr1	43029985	43029989	ERS468014	*indica*
Chr12	16846383	16846387	ERS468016	*indica*
Chr4	31960603	31960607	ERS468018	*indica*
Chr1	43029985	43029989	ERS468023	*indica*
Chr1	43029985	43029989	ERS468025	*indica*
Chr8	5518299	5518306	ERS468028	*indica*
Chr1	43029985	43029989	ERS468029	*indica*
Chr4	31968290	31968294	ERS468042	*indica*
Chr7	29937784	29937788	ERS468048	*indica*
Chr1	43029985	43029989	ERS468049	*indica*
Chr8	5518300	5518306	ERS468050	*indica*
Chr1	43029985	43029989	ERS468052	*indica*
Chr8	5518300	5518306	ERS468053	*indica*
Chr4	13737528	13737532	ERS468055	*indica*
Chr8	5518300	5518306	ERS468055	*indica*
Chr7	29937784	29937788	ERS468059	*indica*
Chr7	29937784	29937788	ERS468060	*indica*
Chr6	31319589	31319593	ERS468065	*indica*
Chr1	43029985	43029989	ERS468066	*indica*
Chr4	13737528	13737532	ERS468068	*indica*
Chr8	5518300	5518306	ERS468071	*indica*
Chr1	20049396	20049400	ERS468072	*indica*
Chr7	29937784	29937788	ERS468073	*indica*
Chr4	31968290	31968294	ERS468074	*indica*
Chr4	31960603	31960607	ERS468075	*indica*
Chr12	16846383	16846387	ERS468077	*indica*
Chr1	20049396	20049400	ERS468078	*indica*
Chr8	5518300	5518306	ERS468084	*indica*
Chr11	29783248	29783252	ERS468086	*indica*
Chr1	20049396	20049400	ERS468087	*indica*
Chr1	20049396	20049400	ERS468088	*indica*
Chr8	5518300	5518306	ERS468088	*indica*
Chr8	5518300	5518306	ERS468089	*indica*
Chr12	22672020	22672024	ERS468095	*indica*
Chr4	31960603	31960607	ERS468101	*indica*
Chr1	43029985	43029989	ERS468102	*indica*
Chr1	43029985	43029989	ERS468104	*indica*
Chr4	31968290	31968294	ERS468106	*indica*
Chr12	16846383	16846387	ERS468111	*indica*
Chr1	43029985	43029989	ERS468112	*indica*
Chr1	43029985	43029989	ERS468115	*indica*
Chr8	5518300	5518306	ERS468121	*indica*
Chr4	31968290	31968294	ERS468126	*indica*
Chr8	5518300	5518306	ERS468131	*indica*
Chr8	5518300	5518306	ERS468133	*indica*
Chr1	20049396	20049400	ERS468134	*indica*
Chr4	31968290	31968294	ERS468136	*indica*
Chr1	43029985	43029989	ERS468138	*indica*
Chr4	31968290	31968294	ERS468139	*indica*
Chr8	5518300	5518306	ERS468142	*indica*
Chr4	31968290	31968294	ERS468154	*indica*
Chr4	31968290	31968294	ERS468157	*indica*
Chr1	43029985	43029989	ERS468160	*indica*
Chr1	43029985	43029989	ERS468161	*indica*
Chr4	31968290	31968294	ERS468163	*indica*
Chr1	20049396	20049400	ERS468166	*indica*
Chr4	31968290	31968294	ERS468169	*indica*
Chr5	19524953	19524957	ERS468170	*indica*
Chr4	31968290	31968294	ERS468174	*indica*
Chr8	5518300	5518306	ERS468184	*indica*

Chr8	5518300	5518306	ERS468186	*indica*
Chr4	31968290	31968294	ERS468187	*indica*
Chr8	5518300	5518306	ERS468187	*indica*
Chr4	31968290	31968294	ERS468191	*indica*
Chr4	31968290	31968294	ERS468192	*indica*
Chr4	31968290	31968294	ERS468193	*indica*
Chr8	5518300	5518306	ERS468195	*indica*
Chr4	31968290	31968294	ERS468202	*indica*
Chr7	29937784	29937788	ERS468202	*indica*
Chr4	31968290	31968294	ERS468204	*indica*
Chr4	31968290	31968294	ERS468205	*indica*
Chr8	5518300	5518306	ERS468207	*indica*
Chr8	5518300	5518306	ERS468209	*indica*
Chr7	29937784	29937788	ERS468210	*indica*
Chr11	30168035	30168039	ERS468212	*indica*
Chr5	287377	287381	ERS468212	*indica*
Chr1	43029985	43029989	ERS468215	*indica*
Chr4	31968290	31968294	ERS468222	*indica*
Chr4	31968290	31968294	ERS468230	*indica*
Chr4	31968290	31968294	ERS468232	*indica*
Chr4	31968290	31968294	ERS468234	*indica*
Chr1	43029985	43029989	ERS468237	*indica*
Chr4	13737528	13737532	ERS468240	*indica*
Chr7	29937784	29937788	ERS468249	*indica*
Chr1	43029985	43029989	ERS468250	*indica*
Chr3	431859	431863	ERS468252	*indica*
Chr7	29937784	29937788	ERS468252	*indica*
Chr11	29783248	29783252	ERS468255	*indica*
Chr1	19257271	19257275	ERS467801	*japonica*
Chr5	23638163	23638167	ERS467889	*japonica*
Chr11	1514174	1514178	ERS467893	*japonica*
Chr8	5635769	5635774	ERS467904	*japonica*
Chr11	1514174	1514178	ERS468026	*japonica*
Chr5	258622	258626	ERS468308	*japonica*
Chr8	5635768	5635774	ERS468310	*japonica*
Chr1	41968356	41968360	ERS468380	*japonica*
Chr8	5635768	5635774	ERS468383	*japonica*
Chr8	5635768	5635774	ERS468384	*japonica*
Chr8	5635768	5635774	ERS468387	*japonica*
Chr8	5635768	5635774	ERS468402	*japonica*
Chr6	24954457	24954461	ERS468442	*japonica*
Chr6	22413483	22413487	ERS468446	*japonica*
Chr7	29379081	29379085	ERS468449	*japonica*
Chr8	5635769	5635774	ERS468449	*japonica*
Chr8	5635768	5635774	ERS468456	*japonica*
Chr8	5635768	5635774	ERS468458	*japonica*
Chr8	5635769	5635774	ERS468595	*japonica*
Chr8	5635768	5635774	ERS468596	*japonica*
Chr8	5635768	5635774	ERS468604	*japonica*
Chr8	5635768	5635774	ERS468613	*japonica*
Chr8	5635768	5635775	ERS468617	*japonica*
Chr8	5635768	5635774	ERS468620	*japonica*
Chr6	22413483	22413487	ERS468649	*japonica*
Chr5	258622	258626	ERS468684	*japonica*
Chr7	29379081	29379085	ERS468704	*japonica*
Chr8	5635768	5635774	ERS468705	*japonica*
Chr5	258622	258626	ERS468721	*japonica*
Chr5	23638163	23638167	ERS468734	*japonica*
Chr1	41968356	41968360	ERS468902	*japonica*
Chr1	41968356	41968360	ERS468917	*japonica*
Chr8	5635769	5635774	ERS468993	*japonica*

Chr2	1659944	1659948	ERS469049	*japonica*
Chr8	5635768	5635774	ERS469069	*japonica*
Chr8	5635768	5635774	ERS469132	*japonica*
Chr8	5635768	5635774	ERS469177	*japonica*
Chr8	5635768	5635774	ERS469199	*japonica*
Chr8	5635768	5635774	ERS469215	*japonica*
Chr8	5635768	5635774	ERS469302	*japonica*
Chr8	5635768	5635774	ERS469307	*japonica*
Chr8	5635768	5635774	ERS469556	*japonica*
Chr8	5635768	5635774	ERS469602	*japonica*
Chr8	5635768	5635774	ERS469604	*japonica*
Chr8	5635768	5635774	ERS469605	*japonica*
Chr8	5635768	5635775	ERS469637	*japonica*
Chr8	5635768	5635774	ERS469650	*japonica*
Chr8	5635768	5635774	ERS469668	*japonica*
Chr8	5635768	5635774	ERS469669	*japonica*
Chr8	5635768	5635774	ERS469689	*japonica*
Chr8	5635768	5635774	ERS469694	*japonica*
Chr8	5635768	5635774	ERS469696	*japonica*
Chr8	5635768	5635774	ERS469699	*japonica*
Chr8	5635768	5635774	ERS469746	*japonica*
Chr8	5635768	5635774	ERS469758	*japonica*
Chr8	5635768	5635774	ERS469845	*japonica*
Chr8	5635768	5635774	ERS469880	*japonica*
Chr8	5635768	5635774	ERS469978	*japonica*
Chr8	5635768	5635774	ERS469985	*japonica*
Chr8	5635768	5635774	ERS470129	*japonica*
Chr8	5635768	5635774	ERS470132	*japonica*
Chr8	5635768	5635774	ERS470188	*japonica*
Chr8	5635768	5635774	ERS470344	*japonica*
Chr8	5635768	5635774	ERS470439	*japonica*
Chr8	5635768	5635774	ERS470516	*japonica*