

Sensitive detection of pre-integration intermediates of long terminal repeat retrotransposons in crop plants

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1 **Title**

2 Sensitive detection of pre-integration intermediates of LTR retrotransposons in crop plants

3

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24 **Abstract**

25 **Retrotransposons have played an important role in the evolution of host**
26 **genomes^{1,2}. Their impact is mainly deduced from the composition of DNA sequences that**
27 **have been fixed over evolutionary time². Such studies provide important “snapshots”**
28 **reflecting the historical activities of transposons but do not predict current transposition**
29 **potential. We previously reported Sequence-Independent Retrotransposon Trapping (SIRT)**
30 **as a method that, by identification of extrachromosomal linear DNA (eclDNA), revealed**
31 **the presence of active LTR retrotransposons in *Arabidopsis*³. However, SIRT cannot be**
32 **applied to large and transposon-rich genomes, as found in crop plants. We have**
33 **developed an alternative approach named ALE-seq (amplification of LTR of eclDNAs**
34 **followed by sequencing) for such situations. ALE-seq reveals sequences of 5' LTRs of**
35 **eclDNAs after two-step amplification: *in vitro* transcription and subsequent reverse**
36 **transcription. Using ALE-seq in rice, we detected eclDNAs for a novel *Copia* family LTR**
37 **retrotransposon, *Go-on*, which is activated by heat stress. Sequencing of rice accessions**
38 **revealed that *Go-on* has preferentially accumulated in *indica* rice grown at higher**
39 **temperatures. Furthermore, ALE-seq applied to tomato fruits identified a developmentally**
40 **regulated *Gypsy* family of retrotransposons. A bioinformatic pipeline adapted for ALE-seq**
41 **data analyses is used for the direct and reference-free annotation of new, active**
42 **retroelements. This pipeline allows assessment of LTR retrotransposon activities in**
43 **organisms for which genomic sequences and/or reference genomes are either unavailable**
44 **or of low quality.**

45

46 **Main**

47 **Chromosomal copies of activated retrotransposons containing long terminal repeats**
48 **(LTRs) are transcribed by RNA polymerase II, followed by reverse transcription of transcripts**
49 **to extrachromosomal linear DNAs (eclDNA); these integrate back into host chromosomes³.**
50 **Because of the two obligatory template switches during reverse transcription, the newly**
51 **synthesized eclDNA is flanked by LTRs of identical sequence. Their subsequent divergence**
52 **due to the accumulation of mutations correlates well with length of time since the last**
53 **transposition, and thus transposon age⁴. However, the age of LTR retrotransposons cannot**

54 be used to predict their current transpositional potential. Moreover, predictions are further
55 complicated by recombination events that occur with high frequency between young and
56 old members of a retrotransposon family⁵; thus old family members also contribute to the
57 formation of novel recombinant elements that insert into new chromosomal positions⁵.
58 Although, retrotransposon activities can be relatively easily measured at the transcriptional
59 level⁶, the presence of transcripts is a poor predictor of transpositional potential due to
60 posttranscriptional control of this process^{7,8}. In addition, direct detection of transposition by
61 genome-wide sequencing to identify new insertions is too expensive and time-consuming to
62 be applied as a screening method. Clearly, the development of an expeditious approach to
63 identify active retrotransposons that predict their transposition potential would be
64 welcomed. We previously described the SIRT strategy for *Arabidopsis* that led to the
65 identification of eclDNA of a novel retroelement and subsequent detection of new
66 insertions³. Thus, the presence of eclDNAs, the last pre-integration intermediate, was shown
67 to be a good predictor of retrotransposition potential.

68

69 **Results**

70 Development of ALE-seq

71 Retrotransposons include a conserved sequence known as the primer binding site
72 (PBS), where binding of the 3' end of cognate tRNA initiates the reverse transcription
73 reaction³. Met-iCAT (Methionine tRNA-CAT anticodon) PBS was chosen for SIRT as it is the
74 site present in the majority of annotated *Arabidopsis* retrotransposons³. To examine
75 whether Met-iCAT PBS sequences are also predominant in LTR retrotransposons of other
76 plants, we used the custom-made software *LTRpred* for *de novo* annotation of LTR
77 retrotransposons in rice and tomato genomes (see Materials and methods). Young
78 retroelements were selected by filtering for at least 95% identity between the two LTRs and
79 subsequently examined for their cognate tRNAs (Supplementary Figure 1). As in *Arabidopsis*,
80 around 80% of LTR retrotransposons in the tomato genome contained Met-iCAT PBS
81 (Supplementary Figure 1). In contrast, only 30% harboured Met-iCAT PBS in rice, and Arg-
82 CCT (Arginine tRNA-CCT anticodon) PBS was found in 60% of young LTR retrotransposons
83 (Supplementary Figure 1). Nonetheless, we used Met-iCAT PBS in our initial experiments

84 because most retrotransposons known to be active in rice callus (e.g. *Tos17* and *Tos19*)
85 contain Met-iCAT PBS. Initially, SIRT was performed on DNA extracted from rice leaves and
86 calli; however, we did not detect eclDNAs for *Tos17* and *Tos19* in rice tissues by this method
87 (Supplementary Figure 2). We reasoned that the short stretch of PBS used for primer design
88 in SIRT may have impaired PCR efficiency due to the many PBS-related sequences present in
89 larger genomes containing a high number of retroelements, as is the case in rice.

90 To counter this problem, we developed an alternative method, named ALE-seq, with
91 significantly improved selectivity and sensitivity of eclDNA detection. A crucial difference to
92 SIRT is that ALE-seq amplification of eclDNA is separated into two reactions: *in vitro*
93 transcription and reverse transcription (Figure 1a). This decoupling of the use of the two
94 priming sequences followed by the digestion of non-templated DNA and RNA is significantly
95 more selective and efficient than the single PCR amplification in SIRT.

96 ALE-seq starts with ligation to the ends of eclDNA of an adapter containing a T7
97 promoter sequence at its 5' end and subsequent *in vitro* transcription with T7 RNA
98 polymerase. The synthesized RNA is then reverse transcribed using the primer that binds
99 the transcripts at the PBS site. The adapter and the oligonucleotides priming reverse
100 transcription are anchored with partial Illumina adapter sequences (Supplementary Table 1),
101 which allows the amplified products to be directly deep-sequenced in a strand-specific
102 manner. The ALE-seq-sequences derived from retrotransposon eclDNAs are predicted to
103 contain the intact 5' LTR up to the PBS site, flanked by Illumina paired-end sequencing
104 adapters. We used the Illumina MiSeq platform for sequencing because its long reads of 300
105 bp from both ends cover the entire LTR lengths of most potentially active elements. It is
106 worth noting that the Illumina adapters were tagged to the intact LTR DNA without
107 fragmentation of the amplicons. This together with the long reads of MiSeq allowed us to
108 reconstitute the complete LTR sequences, even in the absence of the reference genome
109 sequence. The reconstituted LTRs were analysed using the alignment-based approach that
110 complements the mapping-based approach when the reference genome is incomplete
111 (Figure 1b).

112 First, we tested ALE-seq on *Arabidopsis* by examining heat-stressed Col-0
113 *Arabidopsis* plants⁹, *met1-1* mutant³ and epi12⁸, a *met1*-derived epigenetic recombinant

114 inbred line. ALE-seq cleanly and precisely recovered sequences of complete LTRs for *Onsen*,
115 *Copia21* and *Evade* in samples containing their respective ecDNA (Supplementary Figure
116 3)^{3,8,9}. Due to priming of the reverse transcription reaction at PBS, the reads were explicitly
117 mapped to the 5' but not to the 3' LTR, although the two LTRs have identical sequences. The
118 ALE-seq reads have well-defined extremities, starting at the position marking the start of
119 LTRs and finishing at the PBS, which is consistent with their ecDNA origin. The ends of LTRs
120 can also be inspected for conserved sequences that would further confirm their ecDNA
121 origin (Supplementary Figure 4). This reduced ambiguity of read mapping in ALE-seq analysis,
122 combined with the clear-cut detection of LTR ends, allows for explicit and precise
123 assignment of ALE-seq results to active LTR retrotransposons.

124 Since SIRT failed to detect ecDNAs of rice retrotransposons known to be activated in
125 rice callus, we examined whether ALE-seq would identify their ecDNAs. As shown in Figure
126 1c to f, ALE-seq unambiguously detected ecDNAs of *Tos17* and *Tos19* in rice callus, but not
127 in leaf samples. To test whether detection of 5' LTR sequences requires the entire ALE-seq
128 procedure, we performed control experiments with depleted ALE-seq reactions, for example,
129 in the absence of enzymes for either ligation, *in vitro* transcription, or reverse transcription.
130 All incomplete procedures failed to produce sequences containing 5' LTRs derived from
131 ecDNAs (Figure 1e and f). Taken together, the data show that ALE-seq can detect ecDNAs
132 of LTR retrotransposons in *Arabidopsis* as well as in rice with considerably greater efficiency
133 than the SIRT method.

134 To examine the suitability of ALE-seq for quantitative determination of ecDNA levels,
135 we carried out a reconstruction experiment spiking 100 ng of genomic DNA from rice callus
136 with differing amounts of PCR-amplified full-length *Onsen* DNA from 1 ng to 100 fg (Figure
137 2a to d). The results in Figure 2a and b show that the readouts of ALE-seq for *Onsen*
138 correlate well with the input amounts ($R^2=0.99$). The initial ALE-seq steps of ligation and *in*
139 *vitro* transcription impinged proportionally on the input DNA, resulting in unbiased
140 quantification of the ecDNA and minimal quantitative distortion of the final ALE-seq data.
141 Noticeably, the levels of *Tos17* were similar in all the spiked samples, indicating that
142 addition of *Onsen* DNA did not influence the detection sensitivity of *Tos17*, at least for the
143 amounts tested (Figure 2c and d). Thus, ALE-seq can be used to accurately determine
144 ecDNA levels.

145 Most rice retrotransposons harbour Arg-CCT PBS (Supplementary Figure 1). We
146 tested whether the reverse transcription reaction can be multiplexed to capture both types
147 of retrotransposons (containing Arg-CCT or Met-iCAT PBS) and whether multiplexing of the
148 reverse transcription primers compromises the sensitivity of the procedure. ALE-seq was
149 performed on DNA from rice callus, testing each of the reverse transcription primers
150 separately or as a mixture of both primers in a single reaction. As shown in Figure 2e and
151 Supplementary Figure 5, the levels of *Tos17* recorded in the samples with both primers were
152 similar to the Met-iCAT primer alone. Importantly, we also detected the eclDNAs of the
153 *RIRE2* element containing Arg-CCT PBS (Figure 2f), which was known to be transpositionally
154 active in rice callus⁷.

155

156 Identification of *Go-on* retrotransposon using ALE-seq

157 We next used ALE-seq to search for novel active rice retrotransposons. Since many
158 plant retrotransposons are transcriptionally activated by abiotic stresses^{9,10}, we subjected
159 rice plants to heat stress before subjecting them to ALE-seq. In this way we identified a
160 *Copia*-type retrotransposon able to synthesize eclDNA in the heat-stressed plants (Figure 3a
161 to c) and named this element *Go-on* (the Korean for 'high temperature'). The three
162 retrotransposons with the highest eclDNA levels in heat-stress conditions all belong to the
163 *Go-on* family (Figure 3b and Supplementary Figure 6). Although, eclDNAs were detected for
164 all three copies, *Go-on3* seems to be the youngest and, thus, possibly the most active family
165 member, containing identical LTRs and a complete ORF (Supplementary Figure 6). As
166 depicted in Supplementary Figure 6, the 5' LTR sequences of the three *Go-on* copies are
167 identical; thus the ALE-seq reads derived from *Go-on3* LTR were also cross-mapped to other
168 copies that are possibly inactive or have reduced activities. To further determine whether
169 sequences of *Go-on* LTRs recovered by ALE-seq are indeed derived from *Go-on3* or also from
170 other family members, we performed an ALE-seq experiment using RT primers located
171 further downstream of the PBS, including sequences specific for each *Go-on* family member
172 (Supplementary Figure 6). The amplified ALE-seq products revealed that the eclDNAs
173 produced in heat-stressed rice originated only from *Go-on3* (Supplementary Figure 6). We

174 validated the production of ecdDNAs of *Go-on3* by sequencing the junction of the adapter
175 and the 5' end of LTR (Supplementary Figure 6) and by qPCR (Supplementary Figure 7).

176 Next, we examined whether *Go-on3* is transcriptionally activated in rice subjected to
177 heat stress. RNA-seq and the RT-qPCR data clearly showed that *Go-on* is strongly activated
178 in heat-stress conditions (Figure 3d and Supplementary Figure 7). Similar to many other
179 retrotransposons including *ONSEN* of *Arabidopsis*^{9,11,12}, the LTR sequence of *Go-on3*
180 contains *cis*-acting regulatory element such as the heat shock transcription factor HSFC1-
181 binding sequence motif (Supplementary Figure 7), which is suggestive to its heat stress-
182 mediated transcriptional activation (Figure 3d). To determine whether *Go-on* is also
183 activated in *indica* rice, we heat-stressed plants of *IR64* for three days and examined *Go-on*
184 RNA and DNA levels. Similar to *japonica* rice, *Go-on* RNA and DNA accumulated markedly
185 under heat stress (Supplementary Figure 8), suggesting that the trigger for *Go-on* activation
186 is conserved in both of these evolutionarily distant rice genotypes. Analysis of the RNA-seq
187 data from the heat-stressed rice plants revealed a poor correlation between the mRNA and
188 ecdDNA levels of retrotransposons (Supplementary Figure 9). Given that ecdDNAs captured
189 by ALE-seq in *Arabidopsis* and rice (Figure 1c to f and Supplementary Figure 3) are all known
190 well for their transposition competence, this possibly agrees with the notion that the
191 ecdDNA level is a better predictor of retrotransposition than the RNA level.

192 To possibly relate accumulation of *Go-on* copies in plant populations grown in
193 different temperatures, we analysed the historical retrotransposition of *Go-on* using the
194 genome resequencing data of rice accessions from the 3,000 Rice Genome Project¹³. First,
195 we retrieved the raw sequencing data for all 388 *japonica* rice accessions and the same
196 number of randomly selected sequences of *indica* rice accessions. Using the Transposon
197 Insertion Finder (TIF) tool¹⁴, *japonica* and *indica* sequences were analysed for the number of
198 *Go-on* copies and their genome-wide distribution. Only non-reference insertions that were
199 absent in the reference genome were scored and the cumulative number of new insertions
200 was plotted (Figure 3e to g). Figure 3e shows that the *indica* rice population grown in a
201 warmer climate¹⁵ accumulated significantly more *Go-on* copies than the *japonica* population.
202 As controls, we also examined the accumulation of *Tos17* and *Tos19*, which were not
203 activated by heat stress in our ALE-seq profile (Figure 3a and b). Both retrotransposons
204 showed more transposition events in *japonica* than in *indica* rice (Figure 3f, g and

205 Supplementary Figure 10). Therefore, the copy number of *Go-on* in rice accessions
206 correlated with their growth temperatures, which could possibly be related to occasional
207 *Go-on* activation in elevated ambient temperatures.

208

209 Identification of *FIRE* retrotransposon using ALE-seq

210 It was reported previously that the tomato genome (*Solanum lycopersicum*)
211 experiences a significant loss of DNA methylation in fruits during their maturation, which
212 leads to transcriptional activation of retrotransposons¹⁶. However, it was not known
213 whether these transcriptionally activated tomato transposons synthesise ecDNA. It was
214 questionable whether the ALE-seq strategy is sensitive enough to detect ecDNA in the ~950
215 Mb tomato genome, which is almost three times as large as ~400 Mb of rice¹⁷. To address
216 these questions, ALE-seq was carried out on DNA samples from fruits at 52 days post
217 anthesis (DPA), when the loss of DNA methylation is most pronounced¹⁶, and from leaves as
218 a control. It is important to note that we used tomato cultivar (cv.) M82 for these
219 experiments, as it is commonly used for genetic studies^{18,19}, and that the sequence of the
220 current tomato reference genome is based on cv. Heinz 1706¹⁷. Since retrotransposon
221 sequences and their chromosomal distributions differ largely between genomes of different
222 varieties within the same plant species²⁰⁻²², we could not use the standard mapping-based
223 annotation of the ALE-seq results. As a consequence, we developed a reference-free and
224 alignment-based approach that adopts the clustering of reads based on their sequence
225 similarities (Figure 1b). Briefly, the reads from both samples were pooled and then clustered
226 by sequence homology (See Materials and methods). The consensus of each cluster was
227 determined and used as the reference in paired-end mapping. Subsequently, the consensus
228 sequences were used for a BLAST search against the reference genome for the closest
229 homologues. In this way, the BLAST search was able to map the clustered ALE-seq output to
230 reference genome annotated retrotransposons, which are most similar to the ALE-seq
231 recovered sequences. Applying this strategy, we identified a retroelement belonging to a
232 *Gypsy* family (*FIRE*, *Fruit-Induced RetroElement*) that produces significant amounts of
233 ecDNA at 52 DPA during fruit ripening (Figure 4a and b). We also determined the transcript
234 levels of the *FIRE* element in leaves and 52 DPA fruit samples. As shown in Figure 4c, fruit

235 RNA levels were enhanced twofold compared to leaves, where *FIRE* ecDNA was barely
236 detectable (Figure 4a). Finally, we found that the DNA methylation status of the *FIRE*
237 element was lower in fruits than leaves in all three sequence contexts (Figure 4d and f). In
238 contrast, the DNA methylation levels of sequences directly flanking *FIRE* were similar in
239 leaves and fruits (Figure 4e to g).

240

241 **Discussion**

242 Recently, a novel active retrotransposon was identified in rice by sequencing
243 extrachromosomal circular DNA (eccDNA) produced as a by-product of retrotransposition or
244 by nuclear recombination reactions of ecDNAs^{23,24}. Although the method of eccDNA
245 sequencing has certain advantages over SIRT, such as increased sensitivity and the recovery
246 of sequences of the entire element, it also has certain limitations. For example, the method
247 requires relatively large amounts of starting material but still shows serious limits in
248 efficiency and indicative power for retrotransposition. The method did not detect the
249 eccDNA of *Tos19* in rice callus, where this transposon is known to move²³, however, direct
250 comparison of both methods on the same biological samples was not performed. More
251 importantly, eccDNAs may also be the result of genomic DNA recombination²⁵ and these
252 background products may be misleading when extrapolating to the transpositional potential
253 of a previously unknown element. In this respect, ALE-seq is a significantly improved tool
254 that largely overcomes the above-mentioned limitations of previous methods and requires
255 only 100 ng of plant DNA.

256 The heat-responsiveness of *Go-on*, the novel heat-activated *Copia* family
257 retrotransposon of rice detected using ALE-seq, seems to be conferred by *cis*-acting DNA
258 elements embedded in the LTR, which are similar to the heat-activated *Onsen*
259 retrotransposon in *Arabidopsis*^{11,12}. Although heat stress can induce production of mRNA
260 and ecDNA of *Onsen*, its retrotransposition is tightly controlled by the small interfering RNA
261 pathway⁹. Given that real-time transposition of rice retrotransposons has only been
262 detected in epigenetic mutants^{26,27} and triggered by tissue culture conditions causing vast
263 alterations in the epigenome⁷, or as a result of interspecific hybridization²⁸, an altered
264 epigenomic status seems to be an important prerequisite for retrotransposition. In fact, we

265 failed to detect transposed copies of *Go-on* in the progeny of heat-stressed rice plants. Thus,
266 although *Go-on* produces eLDNAs after heat stress, it may be mobilized only at low
267 frequency in wild type rice due to epigenetic restriction of retrotransposition. Nevertheless,
268 on an evolutionary scale, the higher number of new insertions of *Go-on* in *indica* rice
269 populations grown at elevated temperatures might suggest its potential mobility.

270 Many retrotransposons are transcriptionally reactivated during specific
271 developmental stages or in particular cell types^{29,30}. In tomato, fruit pericarp exhibits a
272 reduction in DNA methylation during ripening¹⁶. This is largely attributed to higher
273 transcription of the *DEMETER-LIKE2* DNA glycosylase gene³¹. Despite massive transcriptional
274 reactivation of retrotransposons in tomato fruits, it has been difficult to determine whether
275 further steps toward transposition also take place. Using ALE-seq, we identified eLDNA that
276 we annotated using a reference-free and alignment-based approach to a novel *FIRE* element.
277 *FIRE* has 164 copies in the reference tomato genome and in a conventional mapping-based
278 approach the ALE-seq reads of *FIRE* cross-mapped to multiple copies, making it difficult to
279 assign eLDNA levels to particular family members (Supplementary Figure 11). Therefore,
280 our strategy can be used in situations where sequence of the reference genome is
281 unavailable or the mapping of reads is hindered by the high complexity and multiplicity of
282 the retrotransposon population.

283 ALE-seq could also be applied to non-plant systems. For example, numerous studies
284 in various eukaryotes, including mammals, found that retrotransposons are transcriptionally
285 activated by certain diseases or at particular stages during embryo development^{32,33}. It was
286 also suggested that retrotransposition might be an important component of disease
287 progression³⁴. Given that the direct detection of retrotransposition is challenging, it would
288 be interesting to use ALE-seq to determine whether such temporal relaxations of epigenetic
289 transposon silencing also result in the production of the eLDNAs, as the direct precursor of
290 the chromosomal integration of a retrotransposon.

291

292

293 **Materials and methods**

294 Plant materials

295 Seeds of *Oryza sativa ssp. japonica* cv. *Nipponbare* and *Oryza sativa ssp. indica* cv. *IR64* were
296 surface-sterilized in 20% bleach for 15 min, rinsed three times with sterile water and
297 germinated on ½-MS media. Rice plants were grown in 10 h light / 14 h dark at 28°C and
298 26°C, respectively. For heat-stress experiments, 1-week-old rice plants were transferred to a
299 growth chamber at 44°C and 28°C in light and dark, respectively. Rice callus was induced by
300 the method used for rice transformation as previously described³⁵.

301 Tomato plants (*Solanum lycopersicum* cv. *M82*) were grown under standard greenhouse
302 conditions (16 h supplemental lighting of 88 w/m² at 25°C and 8 h at 15°C). Tomato leaf
303 tissue samples were taken from 2-month-old plants. Tomato fruit pericarp tissues were
304 harvested at 52 days post anthesis (DPA).

305

306 Annotation of LTR retrotransposons

307 Functional *de novo* annotation of LTR retrotransposons for the genomes of TAIR10
308 (*Arabidopsis*), MSU7 (rice) and SL2.50 (tomato) was achieved by the *LTRpred* pipeline
309 (<https://github.com/HajkD/LTRpred>) using the parameter configuration: minlenltr = 100,
310 maxlenltr = 5000, mindistltr = 4000, maxdisltr = 30000, mintsd = 3, maxtsd = 20, vic = 80,
311 overlaps = "no", xdrop = 7, motifmis = 1, pbsradius = 60, pbsalilen = c(8,40), pbsoffset =
312 c(0,10), quality.filter = TRUE, n.orf = 0. The plant-specific tRNAs used to screen for primer
313 binding sites (PBS) were retrieved from GtRNAdb³⁶ and plantRNA³⁷ and combined in a
314 custom fasta file. The hidden Markov model files for gag and pol protein conservation
315 screening were retrieved from Pfam³⁸ using the protein domains RdRP_1 (PF00680), RdRP_2
316 (PF00978), RdRP_3 (PF00998), RdRP_4 (PF02123), RVT_1 (PF00078), RVT_2 (PF07727),
317 Integrase DNA binding domain (PF00552), Integrase zinc binding domain (PF02022),
318 Retrotrans_gag (PF03732), RNase H (PF00075) and Integrase core domain (PF00665).
319 Computationally reproducible scripts for generating annotations can be found at
320 <http://github.com/HajkD/ALE>.

321

322 ALE-seq library preparation

323 Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen) following the
324 manufacturer's instruction. Genomic DNA (100 ng) was used for adapter ligation with 4 µl of
325 50 µM adapter DNA. After an overnight ligation reaction at 4°C, the adapter-ligated DNA
326 was purified by AMPure XP beads (Beckman Coulter) at a 1:0.5 ratio. *In vitro* transcription
327 reactions were performed using a MEGAscript RNAi kit (Thermo Fisher) with minor
328 modifications. Briefly, the reaction was carried out for 4 h at 37°C and the template DNA
329 was digested prior to RNA purification. Purified RNA (3 µg) was subjected to reverse
330 transcription (RT) using a Transcriptor First Strand cDNA Synthesis Kit (Roche). Transcriptor
331 First Strand cDNA Synthesis Kit was chosen because the RTase of the kit is thermostable.
332 This allowed the RT reaction at higher temperature (55°C) that reduces the RT-inhibiting
333 RNA secondary structure formation. The custom RT primers were added as indicated for
334 each experiment. After the RT reaction, 1 µl of RNase A/T1 (Thermo Fisher) was added to
335 digest non-templated RNA and the reaction mixture was incubated at 37°C for at least 30
336 min. Single-stranded first strand cDNA was PCR-amplified by 25 cycles using Illumina TruSeq
337 HT dual adapter primers and the PCR product was purified by AMPure XP beads (Beckman
338 Coulter) at a 1:1 ratio. After purification, the eluted DNA was quantified using a KAPA
339 Library Quantification Kit (KAPA Biosystems) and run on the MiSeq v3 2 X 300 bp platform in
340 the Department of Pathology of the University of Cambridge. Due to the nature of ALE-seq
341 that specifically amplifies ecDNAs, some ecDNA-free samples did not produce enough
342 library DNAs which, although suboptimal loading, were nevertheless sequenced. It is
343 advisable to spike in PCR-amplified retrotransposon DNA as described below. The
344 oligonucleotide sequences are provided in Supplementary Table 1.

345

346 Preparation of full-length *Onsen* DNA

347 The full-length *Onsen* copy (AT1TE12295) was amplified using Phusion High-Fidelity DNA
348 polymerase (New England Biolabs). PCR products were run on 1% agarose gels. The full-
349 length fragment was then purified by QIAquick Gel Extraction (Qiagen) and its concentration

350 was measured using the Qubit Fluorometric Quantitation system (Thermo Fisher). Primers
351 used for amplification are listed in Supplementary Table 1.

352

353 RT-qPCR analyses

354 Samples were ground in liquid nitrogen using mortar and pestle. An RNeasy Plant Mini Kit
355 (Qiagen) was used to extract total RNA following the manufacturer's instructions. The
356 amount of extracted RNA was estimated using the Qubit Fluorometric Quantitation system
357 (Thermo Fisher). cDNAs were synthesized using a SuperScript VILO cDNA Synthesis Kit
358 (Invitrogen). Real-time quantitative PCR was performed in the LightCycler 480 system
359 (Roche) using primers listed in Supplementary Table 1. LightCycler 480 SYBR green I master
360 premix (Roche) was used to prepare the reaction mixture in a volume of 10 μ l. The results
361 were analysed by the $\Delta\Delta$ Ct method.

362

363 RNA-seq library construction

364 Total RNA was prepared as described above. An Illumina TruSeq Stranded mRNA Library
365 Prep kit (Illumina) was used according to the manufacturer's instructions. The resulting
366 library was run on an Illumina NextSeq 500 machine (Illumina) in the Sainsbury Laboratory
367 at the University of Cambridge.

368

369 Analysis of next-generation sequencing data

370 For RNA-seq data analysis, the adapter and the low-quality sequences were removed by
371 Trimmomatic software³⁹. The cleaned reads were mapped to the MSU7 version of the rice
372 reference genome (<http://rice.plantbiology.msu.edu>) using TopHat2⁴⁰. The resulting
373 mapping files were processed to the Cufflinks/Cuffquant/Cuffnorm pipeline⁴¹ guided by the
374 annotation file which includes the MSU7 reference gene annotation
375 ([http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/
376 pseudomolecules/version_7.0/all.dir/](http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_7.0/all.dir/)) and our custom retrotransposon annotation.

377 Visualization of sequencing data was performed using an Integrative Genomics Viewer
378 (IGV)⁴².

379 For ALE-seq data analysis, the adapter sequence was removed from the raw reads using
380 Trimmomatic software. For the mapping-based approach, paired-end reads were mapped to
381 the reference genomes (Arabidopsis, TAIR10; rice, MSU7; tomato, SL2.50) using Bowtie2⁴³
382 with minor optimization. In most short-read sequencing platforms, it is often difficult to
383 assign the multi-mapped reads of TEs to precise genomic location. However, as MiSeq
384 outputs relatively longer reads, we presumed that ALE-seq reads have less ambiguity than
385 other sequencing platforms and set the parameters dealing with multi-mappers to default.
386 It is only the maximum fragment length option which is set to 500 by default that was
387 manipulated to 3000 (-X 3000). The numbers of reads mapped throughout each
388 retrotransposon were counted by the featureCounts tool of the SubRead package⁴⁴ using
389 the custom annotation file created by *LTRpred*. Since featureCounts recognizes multi-
390 mappers by SAM file's NH tag that bowtie2 does not generate, multi-mapped reads are
391 counted as one read aligned to a single genomic location, which reduces quantitation bias
392 that often happens to multi-mappers. IGV was used to visualize the sequencing data. For the
393 alignment-based approach, the forward and reverse reads were merged to yield the full-
394 length fragment sequences and converted to fasta files using the BBTools
395 (<https://jgi.doe.gov/data-and-tools/bbtools/>). The fasta files created for all the samples
396 were concatenated to get a master fasta file that is later inputted to CD-HIT software⁴⁵ to
397 cluster the reads by sequence similarity with the following options: -c 0.95, -ap 1, -g 1. CD-
398 HIT outputs a fasta file of representative reads for each cluster. The resulting fasta file was
399 used as reference for paired-end mapping of initial fastq files. The mapped reads were
400 counted with the featureCounts tool. Those clusters that significantly differed in the number
401 of mapped reads in different samples were further analysed for their identities using BLAST
402 search.

403 For Bisulfite sequencing analysis, raw sequenced reads derived from tomato fruits (52 DPA)
404 and leaves were downloaded from the public repository (SRP008329)¹⁶ and re-analysed as
405 previously described⁴⁶, with minor modifications. Briefly, high-quality sequenced reads were
406 mapped with Bismark⁴⁷ on the cv. Heinz 1706 reference genome (<https://solgenomics.net>),
407 including a chloroplast sequence obtained from GenBank database (NC_007898.3) to

408 estimate the conversion rate. After methylation call and correction for unconverted
409 cytosines, the methylation proportions at each cytosine position with a coverage of at least
410 3 reads were used to generate a bedGraph file for each cytosine context, using the R
411 Bioconductor packages DMRCaller⁴⁸ and Rtracklayer⁴⁹. The IGV browser was used to
412 visualize the methylation profiles.

413

414 Detection of retrotransposon insertions

415 The insertions of selected retrotransposons were detected from the genome resequencing
416 data of *japonica* and *indica* rice accessions downloaded from the 3,000 rice genome project
417 (PRJEB6180). The Transposon Insertion Finder (TIF) program¹⁴ was used to identify the split
418 reads in the fastq files and detect newly integrated copies. We used MSU7
419 (<http://rice.plantbiology.msu.edu>) and ShuHui498 (<http://www.mbkbase.org>) for the
420 reference of *japonica* and *indica* rice, respectively. Only non-reference insertions were
421 considered and common insertions found in multiple accessions were counted as a single
422 retrotransposition event.

423

424 Data availability

425 The next generation sequencing data that support the findings of this study are available in
426 the Sequence Read Archive (SRA) repository with the identifier SRP155920.

427

428 Code availability

429 The custom scripts used in this study are available in <http://github.com/HajkD/ALE>.

430

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- 538

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542

543 **Author Contributions**

544 J.C. and J.P. conceived the research. J.C., M.B., M.C., H.-G.D., A.B. and M.O. performed
545 experiment. J.C., M.B., M.C. and H.-G.D. analysed data. J.C. and J.P. wrote and revised the
546 manuscript.

547

548 **Competing Interests**

549 The authors declare that no competing interests exist.

550

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554

555

556 **Figure Legends**

557 Figure 1. Detection of ecDNA by ALE-seq

558 **a**, The workflow of ALE-seq. The colour code is indicated in a box. **b**, Analysis pipeline of ALE-
559 seq results. The sequenced reads can be mapped to the reference genome or aligned to
560 each other to obtain a cluster consensus. **c** and **d**, Genome-wide plots of rice ALE-seq results
561 from leaf (**c**) and callus (**d**). The levels are shown as number of reads mapped to each
562 retrotransposon. Dots represent annotated retrotransposons; those corresponding to *Tos17*
563 and *Tos19* are indicated. **e** and **f**, Read coverage plots mapped to *Tos17* (**e**) and *Tos19* (**f**).
564 The black bars represent retrotransposons and white arrowheads indicate LTRs.

565

566 Figure 2. Sensitivity and specificity of ecDNA detection by ALE-seq

567 **a-d**, ALE-seq reconstruction experiment with varying amounts of PCR-amplified *Onsen* DNA
568 added to rice callus DNA. Genome browser image with the read coverage (**a** and **c**) and
569 quantitated read counts (**b** and **d**) for *Onsen* (**a** and **b**) and *Tos17* (**c** and **d**) loci. The amounts
570 of *Onsen* DNA added were 1 ng, 100 pg, 10 pg, 1 pg or 100 fg; 100 ng of rice callus DNA was
571 used. Note that read coverage values are log₁₀-converted in **a**. For **b** and **d**, values are shown
572 as log₁₀-converted counts per million sequenced reads. **e** and **f**, Read coverage plots for the
573 ALE-seq of rice callus using different RT primers. *Tos17* and *RIRE2* transposons are depicted
574 below the plots as in Figure 1.

575

576 Figure 3. Identification of a novel heat-activated retrotransposon in rice

577 **a** and **b**, Genome-wide plots of rice ALE-seq results as in Figure 1. Control (**a**) and heat-
578 stressed (**b**) rice plants were used. One-week-old seedlings were subjected to heat stress
579 (44°C) for 3 days. Met-iCAT PBS primer was used in RT. The levels are shown as the number
580 of reads mapped to retroelements. Three *Go-on* copies are indicated in **b**. **c**, Read coverage
581 plot for *Go-on3*. **d**, RNA-seq data showing *Go-on3* and a neighbouring gene. RNA-seq data
582 were generated using the same plant materials as in **a** and **b**. The experiment was repeated
583 independently two times with similar results. **e-g**, Cumulative plots for the number of non-

584 reference insertions of *Go-on* (**e**), *Tos17* (**f**), and *Tos19* (**g**) in the genomes of 388 *japonica*
585 and *indica* rice accessions. The statistical difference was determined by iterating random
586 selection of 200 accessions out of 388 and performing the two-tailed Wilcoxon test. ** P
587 $=2.2e^{-16}$.

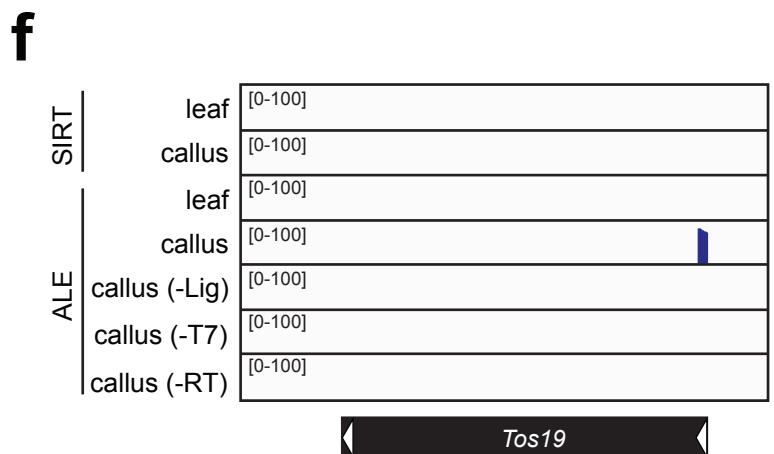
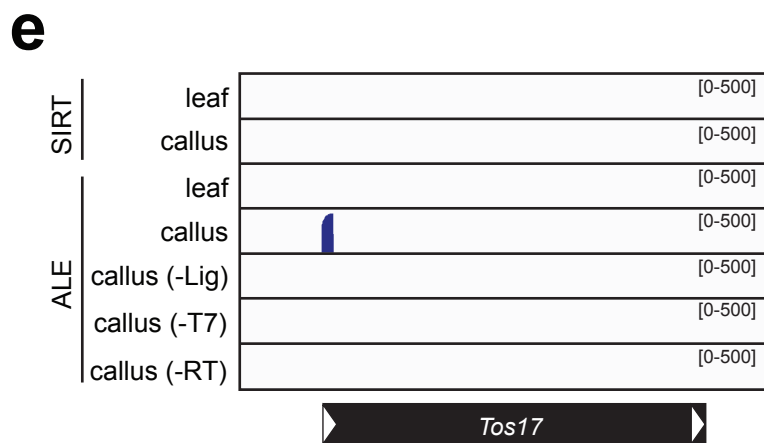
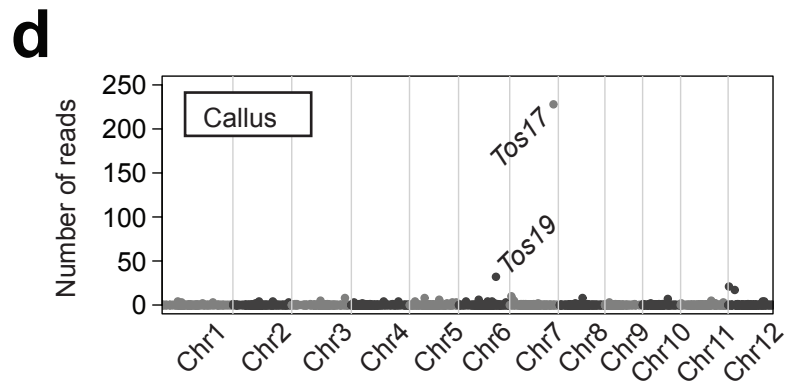
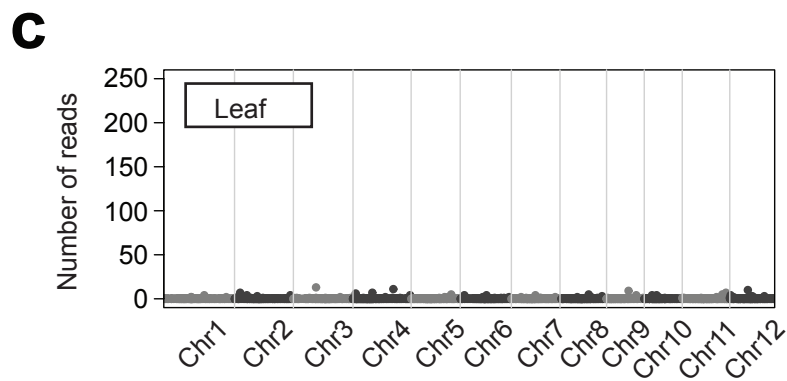
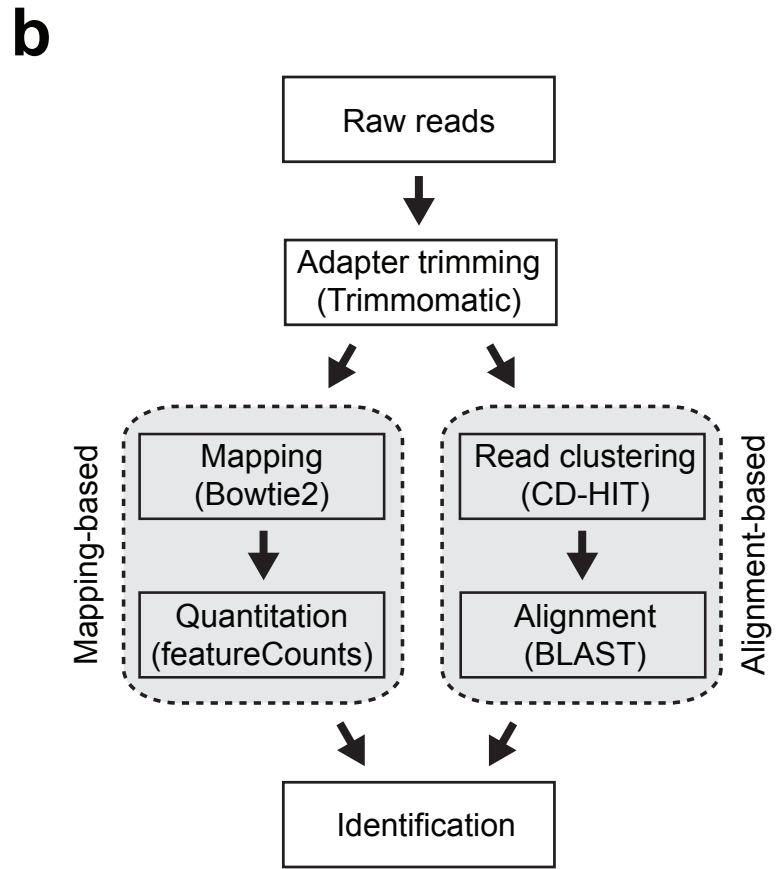
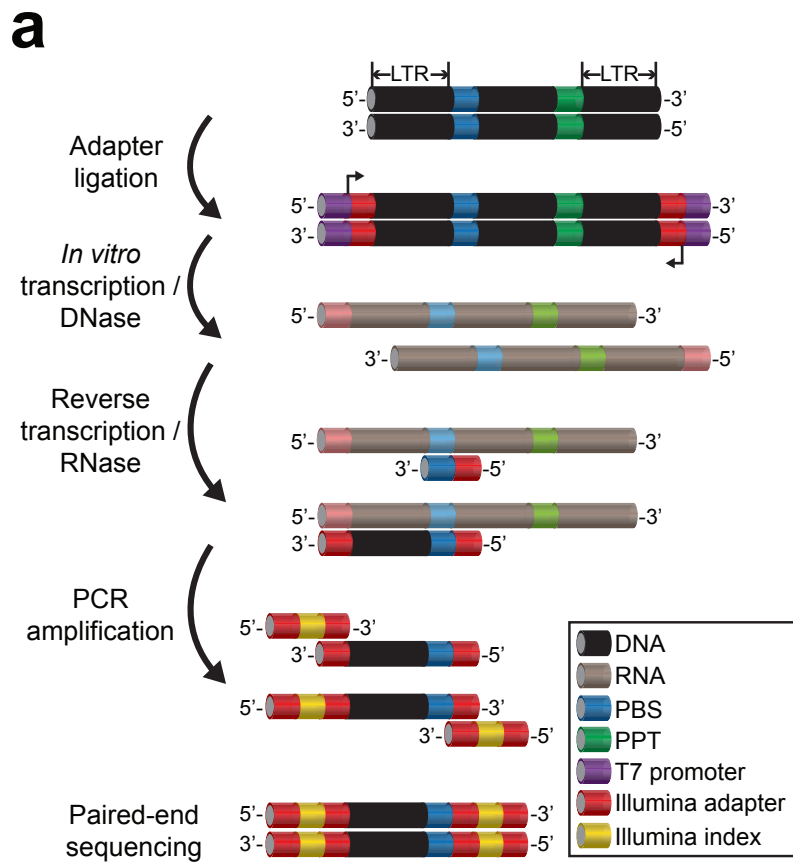
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589 Figure 4. Identification of a tomato retrotransposon activated in fruit pericarp

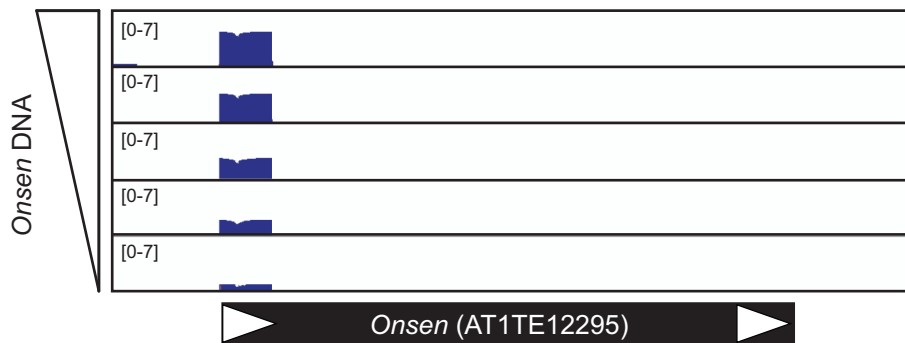
590 **a**, Read coverage plot for the *FIRE* retrotransposon identified in tomato fruit pericarp by
591 ALE-seq. Met-iCAT PBS primer was used in RT. **b** and **c**, The DNA (**b**) and RNA (**c**) levels of
592 *FIRE* in leaves and fruits determined by qPCR. The levels are means of two biological
593 replicates. Normalization was done against *SIGAPDH* (Solyc03g111010) and *SICAC*
594 (Solyc08g006960) for DNA and RNA analyses, respectively. **d**, Genome browser image for
595 the DNA methylation levels at *FIRE* element in leaves and fruits of tomato. The levels are
596 shown as percent methylation of each cytosine. **e-g**, Violin plots for DNA methylation levels
597 at the upstream (**e**), *FIRE* (**f**) and downstream (**g**) regions. Only cytosines supported by at
598 least three reads in both samples were considered. In *FIRE* locus, for example, 4,032 out of
599 4,078 cytosines in both strands were analysed. The upstream and downstream regions are
600 immediate flanking sequences taken for the same length as *FIRE* of 9.362 kb. P-values were
601 determined by a two-sided Fisher's t-test using 558 CG and 717 CHG sites at *FIRE* locus.
602 Other samples with insignificant statistical difference are not shown for the p-values.

603

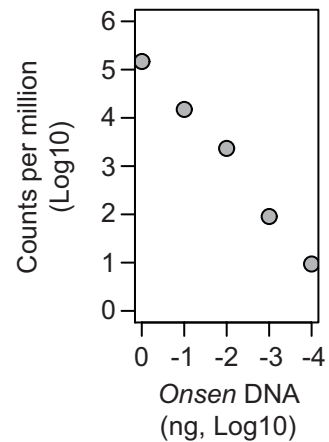
Figure 1.



a



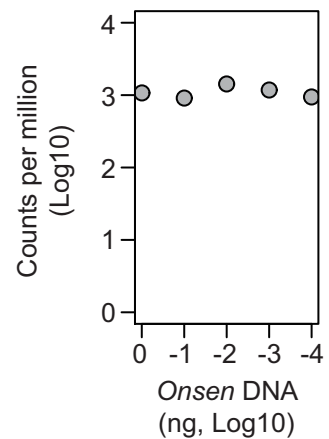
b



c



d



e



f

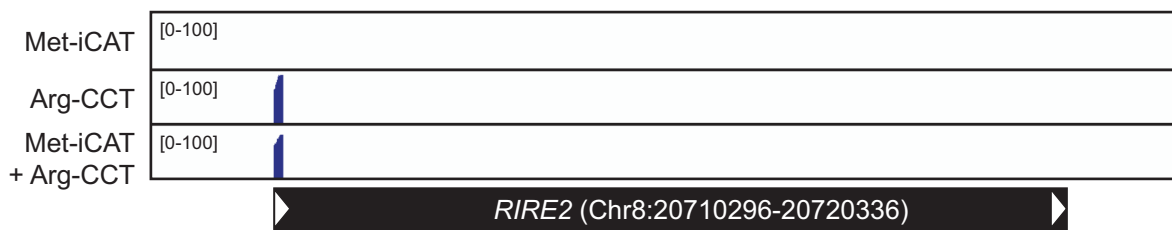
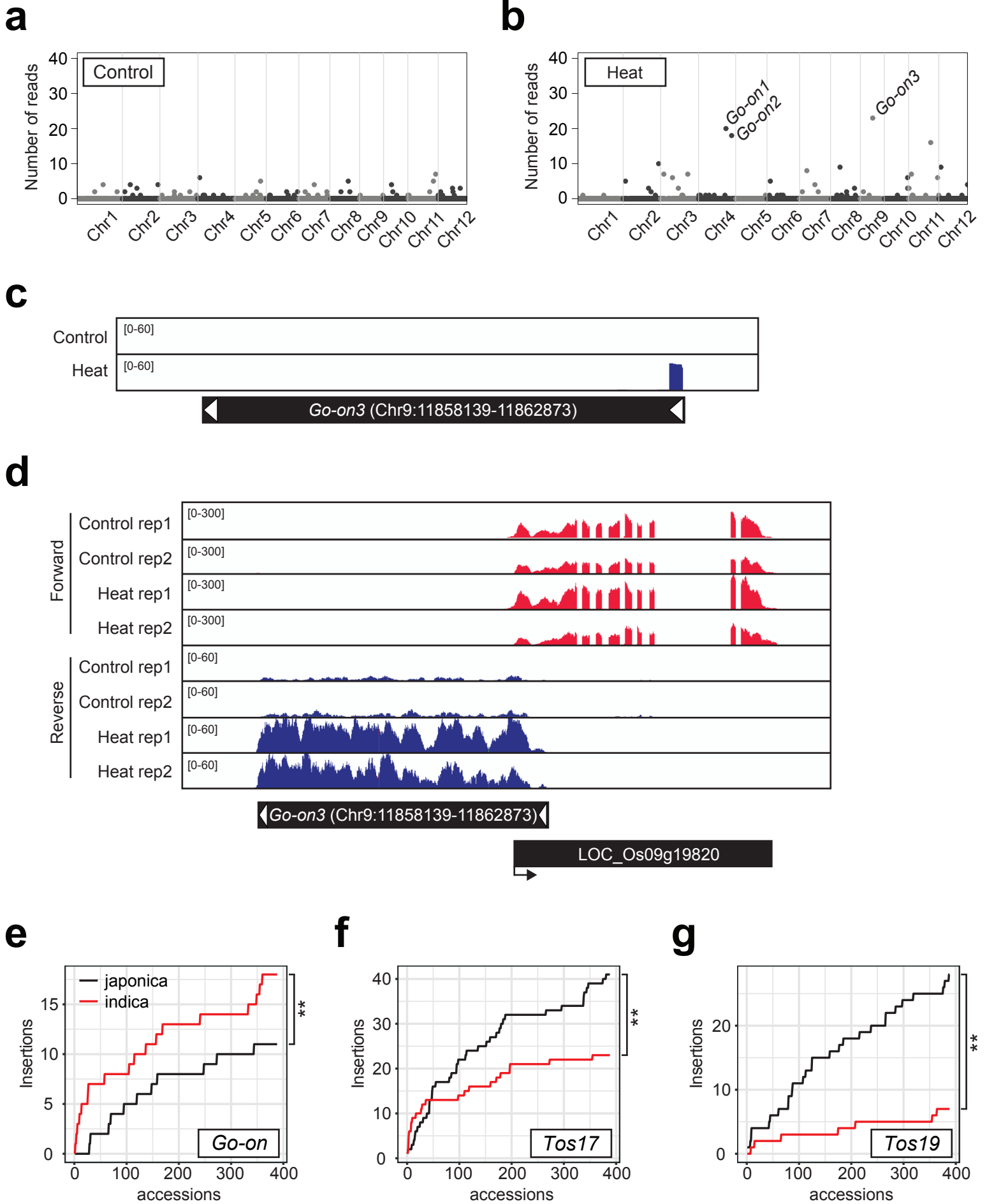
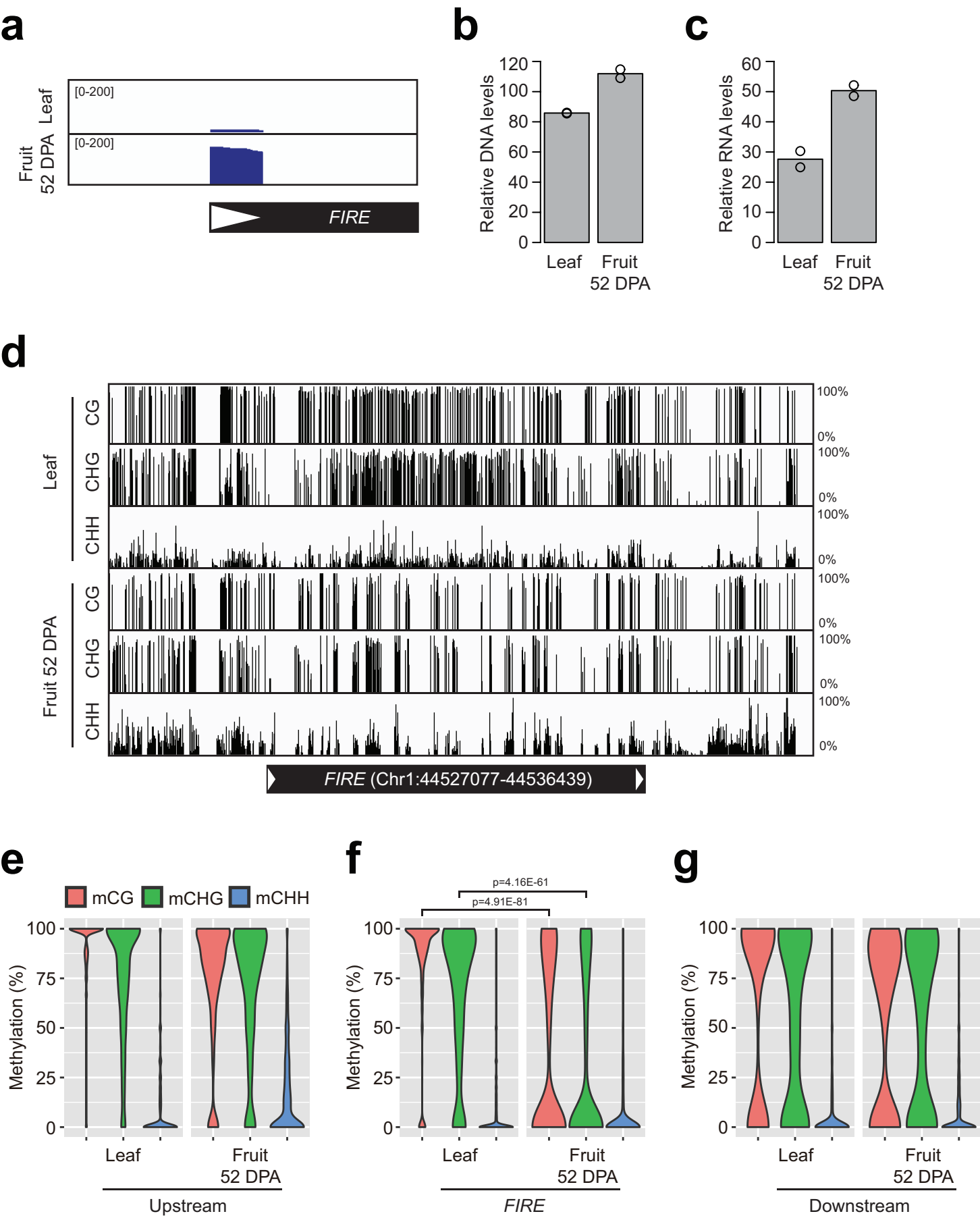
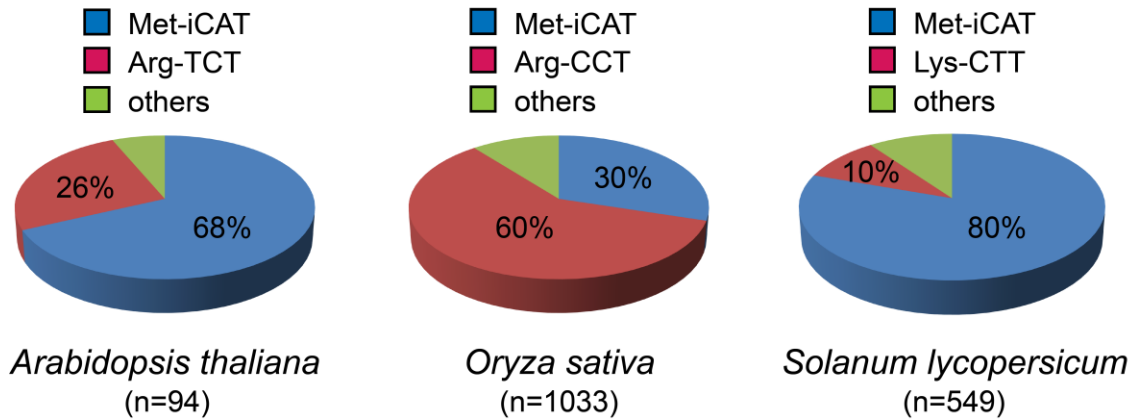


Figure 3.





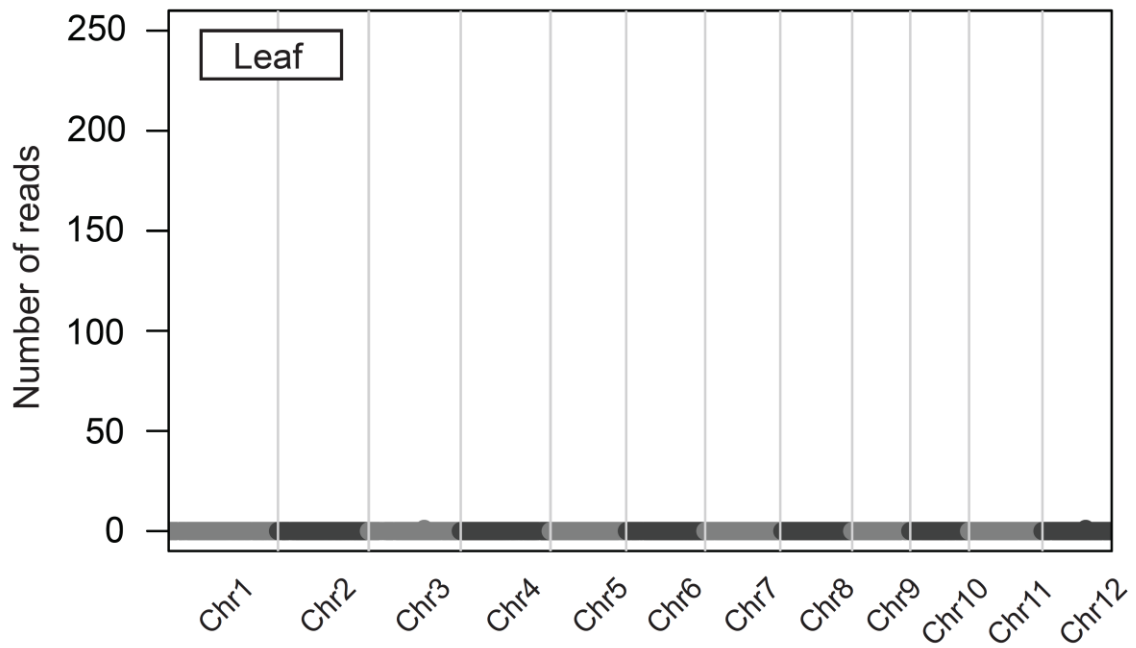
1

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

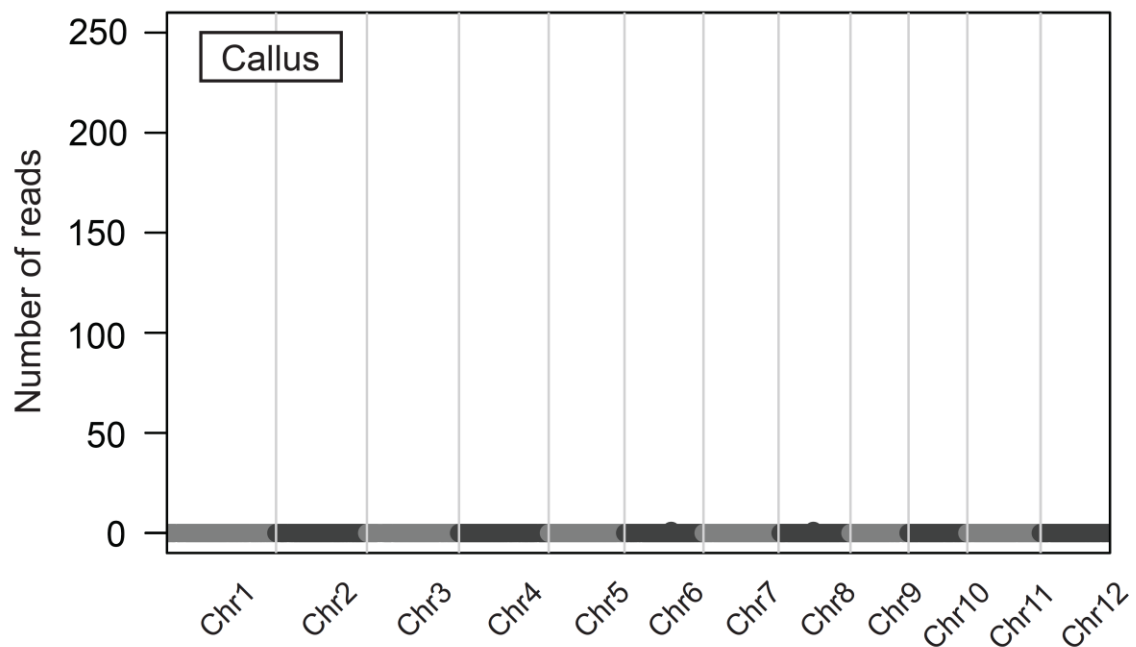
4 The frequency of tRNAs used for targeting PBS. LTR retrotransposons were annotated by
 5 *LTRpred* (<http://github.com/HajkD/ALE>) and selected for young elements by filtering LTR
 6 similarities higher than 95%. The total numbers of retrotransposons analysed in each
 7 species are shown below the plots.

8

a



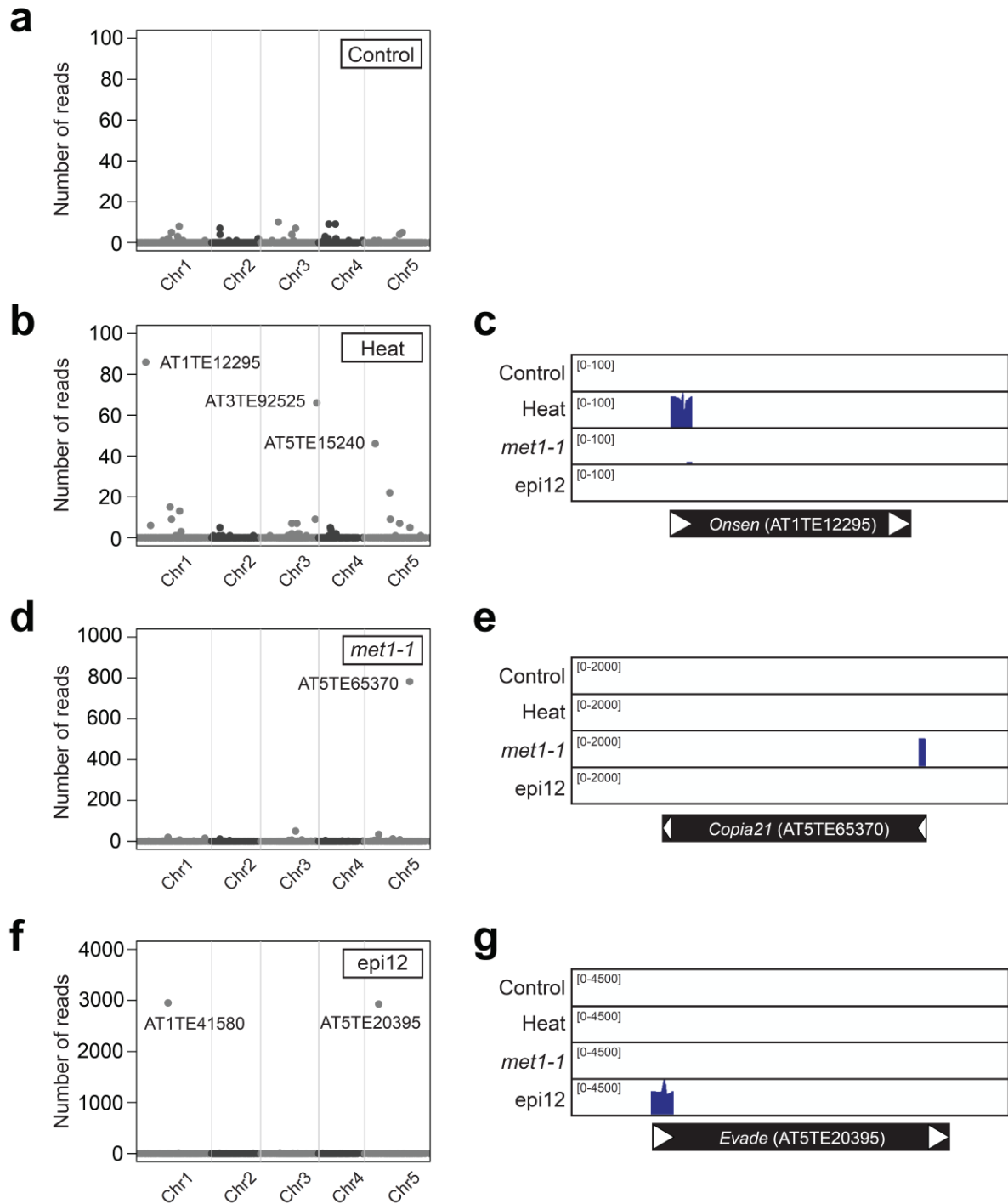
b



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.

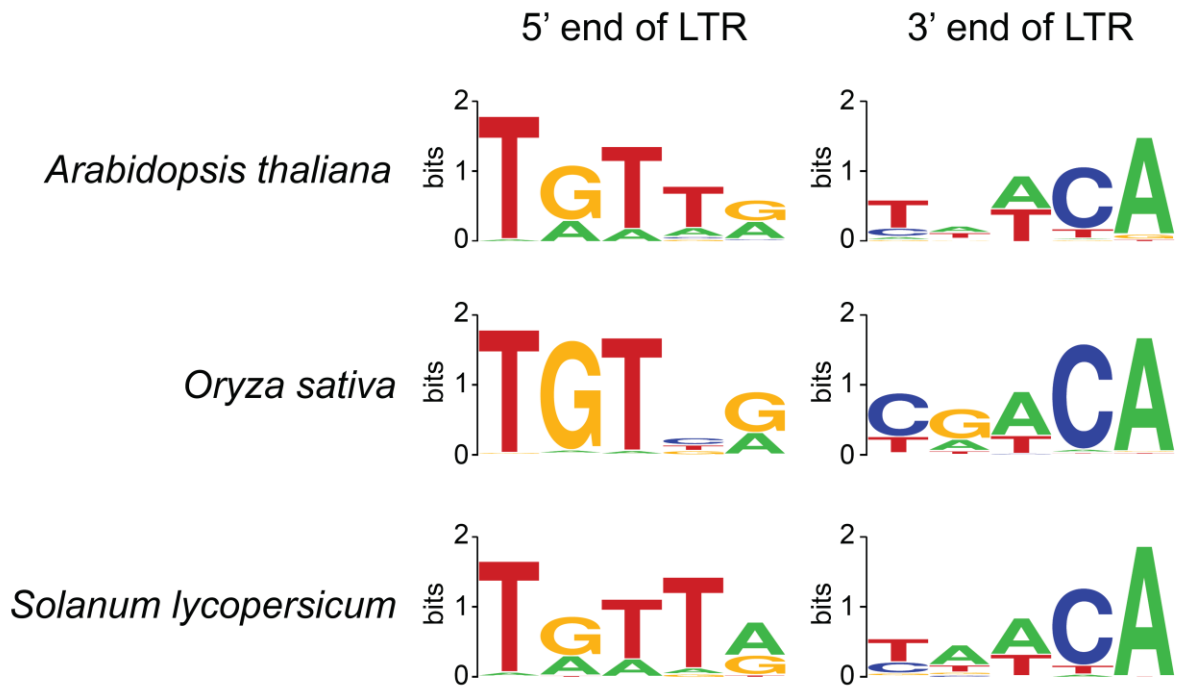


12

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

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

16

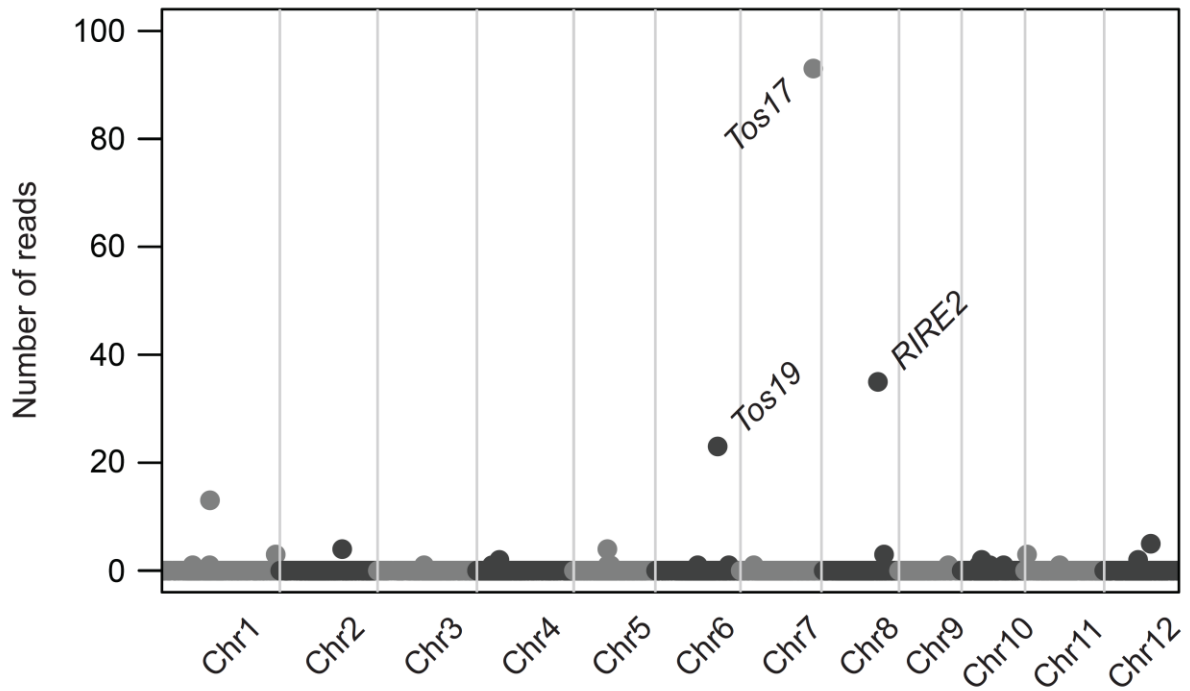


17

18 **Supplementary Figure 4.** Conservation of end sequences of LTR

19 The conserved sequences of 5' and 3' ends of LTR. The first and last five nucleotides of LTRs
 20 are displayed. The images were generated by the WebLogo tool
 21 (<http://weblogo.berkeley.edu/logo.cgi>).

22

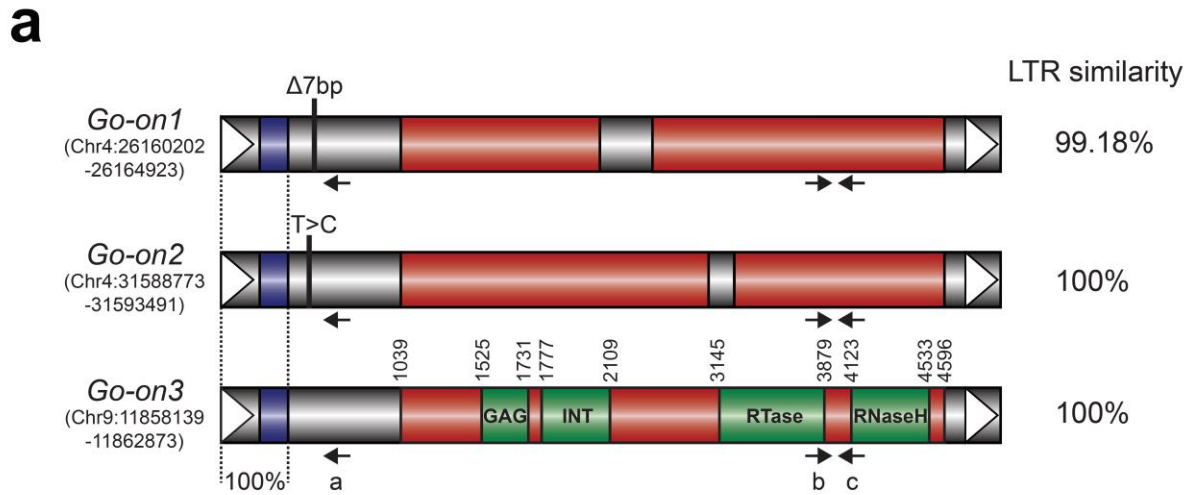


23

24 **Supplementary Figure 5.** ALE-seq detection of eLDNAs of rice retrotransposons using
 25 multiplexed PBS primers

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

28

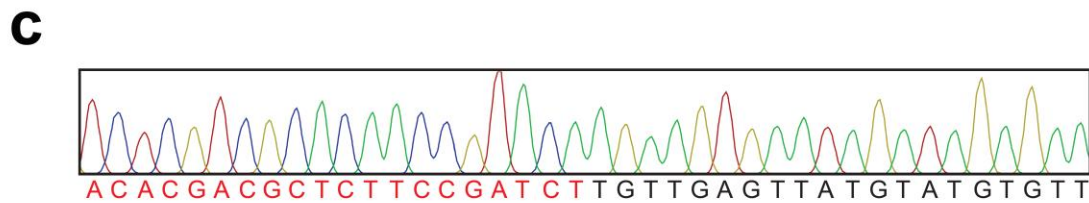


b

```

Go-on1 123 TGGT AT CAGAGCCAAT CGGCT GGT GGCT GGC GACGGG ----- CT AAACCCT AGCCT CGCCGGAG
Go-on2 123 TGGT AT CAGAGCCAAT CGGCT GG GGCT GGC GACGGGGC GACGGCT AAACCCT AGCCT CGCCGGAG
Go-on3 123 TGGT AT CAGAGCCAAT CGGCT GGT GGCT GGC GACGGGGC GACGGCT AAACCCT AGCCT CGCCGGAG
clone1 123 TGGT AT CAGAGCCAAT CGGCT GGT GGCT GGC GACGGGGC GACGGCT AAACCCT AGCCT CGCCGGAG
clone2 123 TGGT AT CAGAGCCAAT CGGCT GGT GGCT GGC GACGGGGC GACGGCT AAACCCT AGCCT CGCCGGAG
clone3 123 TGGT AT CAGAGCCAAT CGGCT GGT GGCT GGC GACGGGGC GACGGCT AAACCCT AGCCT CGCCGGAG
clone4 123 TGGT AT CAGAGCCAAT CGGCT GGT GGCT GGC GACGGGGC GACGGCT AAACCCT AGCCT CGCCGGAG
clone5 123 TGGT AT CAGAGCCAAT CGGCT GGT GGCT GGC GACGGGGC GACGGCT AAACCCT AGCCT CGCCGGAG

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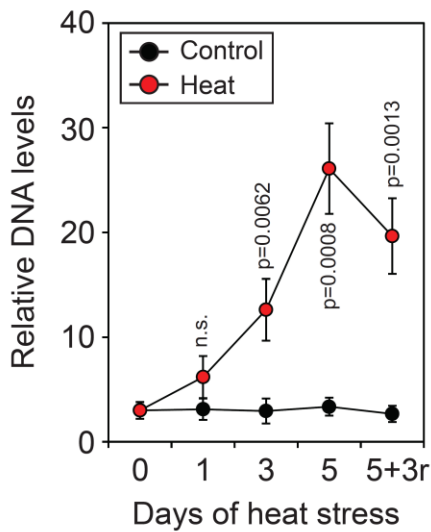
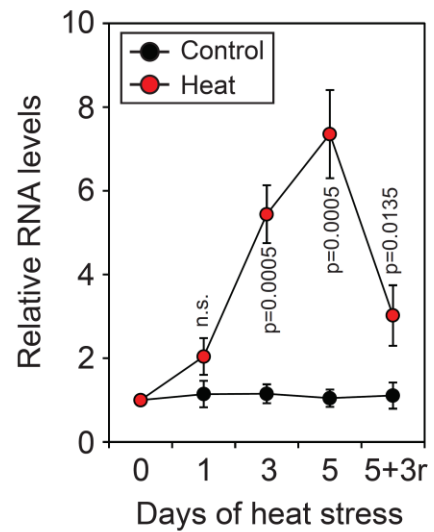
29

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
 TCATATGTACATGTATATAGCAGAGTTAGAGAAATGAAAAAGTAG
 50 60 70 80
 TGAAGCTTCTAGAGAAAAATTCCCAAAC TTCATGGTATCAGAGC - 3'
 90 100 110 120 130 134

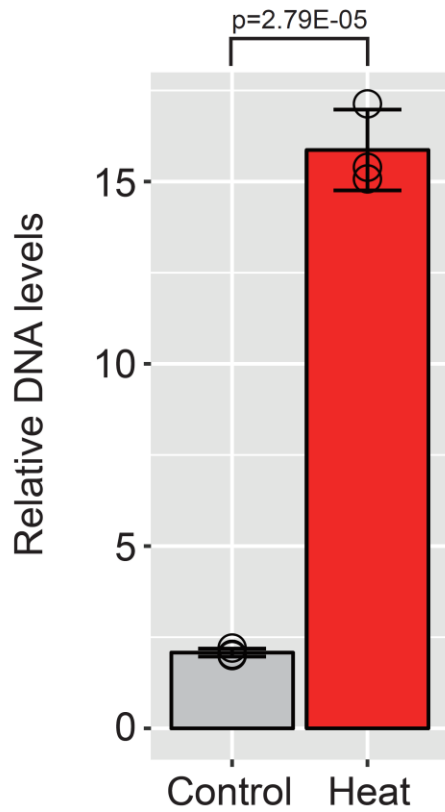
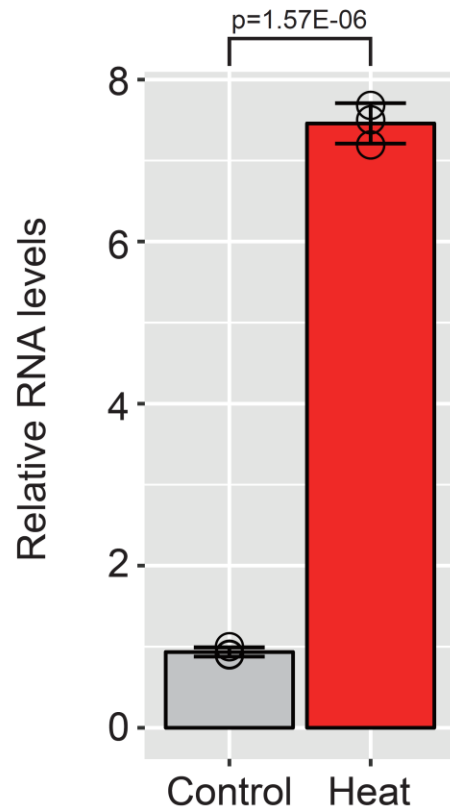
48

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

50 **a** and **b**, The relative levels of DNA (**a**) and RNA (**b**) of *Go-on3* determined by qPCR. Heat
 51 treatment (44°C) was applied to 1-week-old rice seedlings for the periods indicated; +3r
 52 means 3 days of recovery in normal growth conditions after heat stress. The levels are
 53 means \pm sd of three biological replicates. For DNA analysis, Day 0 levels are set to 3,
 54 reflecting three genomic copies of *Go-on* in *japonica* rice. Normalization was done against
 55 *eEF1 α* . P-values were calculated by a two-tailed Student's t-test; n.s., not significant. **c**, The
 56 sequence of the left LTR and PBS of *Go-on3*. The sequence in red is the heat-related HSFC1-
 57 binding sequence motif predicted by PlantPan 2.0 tool
 58 (<http://plantpan2.itps.ncku.edu.tw/index.html>) with statistically significant enrichment of
 59 $P= 4.28e^{-10}$ 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

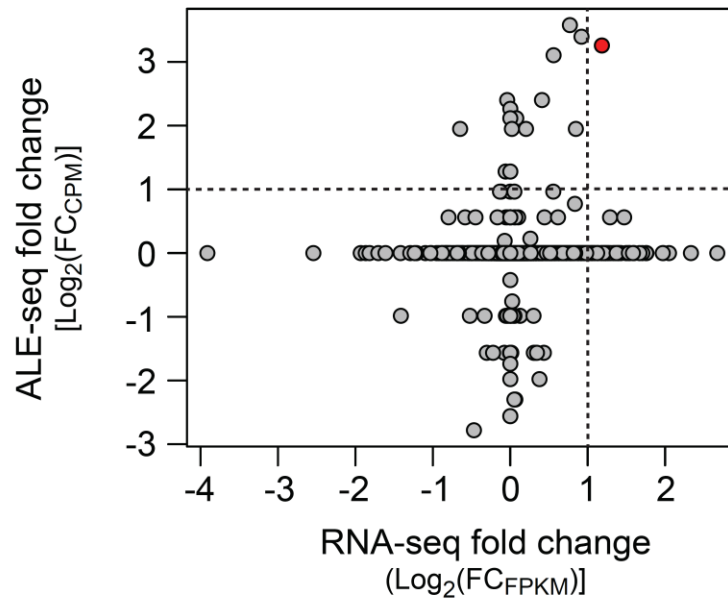
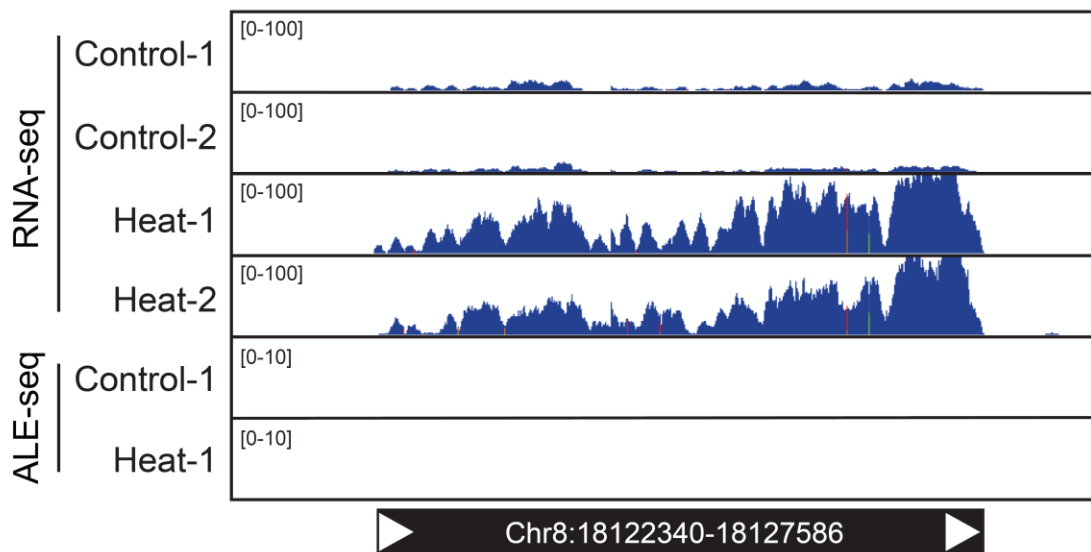
a**b**

63

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

65 **a** and **b**, The qPCR analyses for DNA (**a**) and RNA (**b**) levels of *Go-on* in *indica* rice. The levels
66 are means \pm sd of three biological replications. The levels of control sample are set to 2 (**a**)
67 reflecting 2 genomic copies of *Go-on* in *indica* rice. P-values are determined by two-sided
68 Student's t-test.

69

a**b**

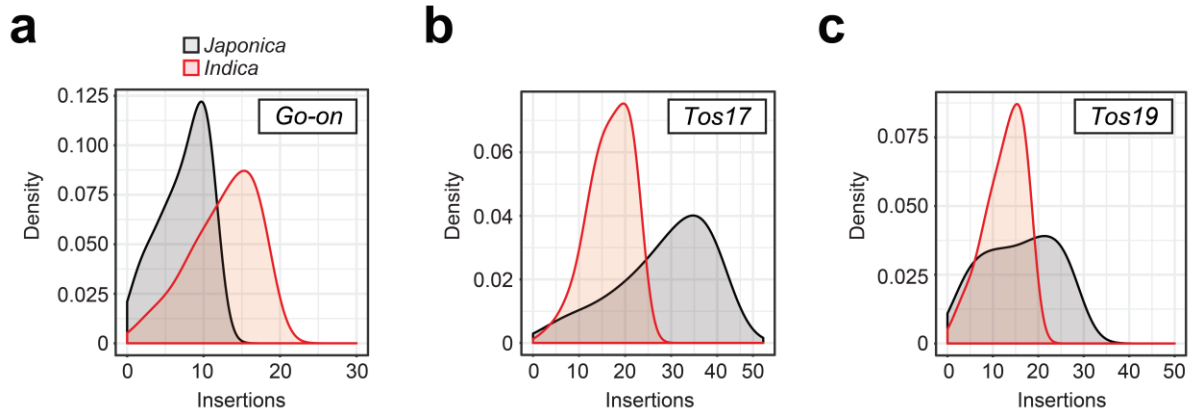
70

71 **Supplementary Figure 9.** Comparison of mRNA and ecdNA levels

72 **a**, Scatter plot for log₂-fold changes (FCs) in RNA-seq and ALE-seq profiles in the control and
 73 heat-stressed rice plants used in Figure 3. FCs were calculated by dividing heat samples
 74 values by control samples values of CPM (counts per million reads) and FPKM (fragments
 75 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. The 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 ecdNAs.

80



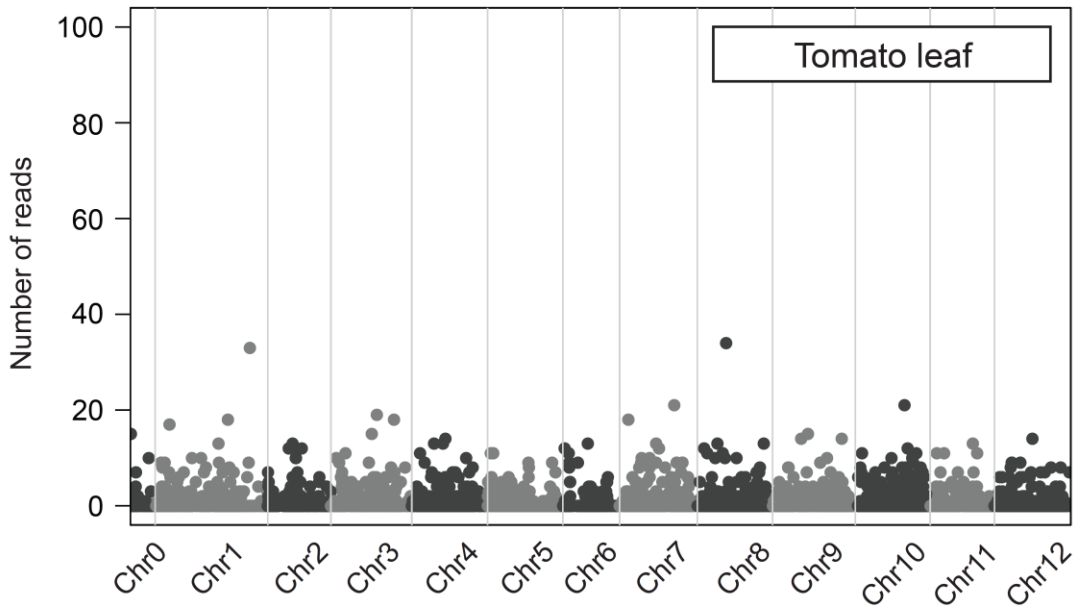
81

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

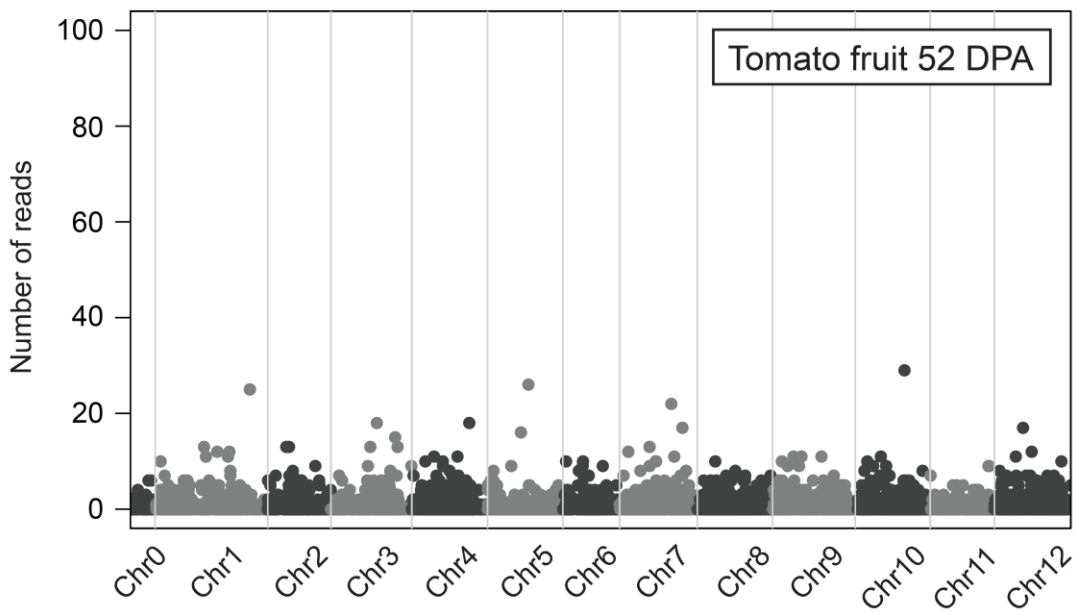
83 **a-c**, Density plots for number of non-reference insertions in randomly selected 200
 84 accessions out of 388 iterated by 1,000 times.

85

a



b



86

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

88 **a** and **b**, Genome-wide plots for ALE-seq profiles performed in tomato leaves (**a**) and fruits
89 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	AGAGAGT <u>AATACGACTCACTATAGGG</u> ACACGACGCTCTCCGATCT
ALE adapter bottom strand	AGATCGGAAGAGCGTCGTGT <u>CCCTATAGTGAGTCGTATTACTCTCT</u>
ALE RT Met-iCAT-R	AGACGTGTGCTCTCCGATCTGCTCTGATACCA
ALE RT Arg-CCT-R	AGACGTGTGCTCTCCGATCTCCTGGCGCGCCA
ONSEN full length-F	TGTTGAAAGTTAACTTGATTTTG
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
eEF1 α -F	GCACGCTCTTCTTGCTTTCACTCT
eEF1 α -R	AAAGGTCACCACCATAACCAGGCTT
FIRE RT-F	GAGTTGGCTACGTATCGTTTGC
FIRE RT-R	AGCTCCACAAATTCATCCCAT
FIRE copy number-F	GGTGTCTCGTTGTGGTAAGT
FIRE copy number-R	TAAGGTGACACTCCCTCATAGT
SICAC-F	CCTCCGTTGTGATGTAAGTGG
SICAC-R	ATTGGTGGAAAGTAACATCATCG
SIGAPDH-F	ATGCTCCCATGTTTGTGGGTG
SIGAPDH-R	TTAGCAAAGGTGCAAGGCAGTTC

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 <i>met1-1</i>	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

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

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

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

Chr8	5518300	5518306	ERS468011	<i>indica</i>
Chr1	43029985	43029989	ERS468014	<i>indica</i>
Chr12	16846383	16846387	ERS468016	<i>indica</i>
Chr4	31960603	31960607	ERS468018	<i>indica</i>
Chr1	43029985	43029989	ERS468023	<i>indica</i>
Chr1	43029985	43029989	ERS468025	<i>indica</i>
Chr8	5518299	5518306	ERS468028	<i>indica</i>
Chr1	43029985	43029989	ERS468029	<i>indica</i>
Chr4	31968290	31968294	ERS468042	<i>indica</i>
Chr7	29937784	29937788	ERS468048	<i>indica</i>
Chr1	43029985	43029989	ERS468049	<i>indica</i>
Chr8	5518300	5518306	ERS468050	<i>indica</i>
Chr1	43029985	43029989	ERS468052	<i>indica</i>
Chr8	5518300	5518306	ERS468053	<i>indica</i>
Chr4	13737528	13737532	ERS468055	<i>indica</i>
Chr8	5518300	5518306	ERS468055	<i>indica</i>
Chr7	29937784	29937788	ERS468059	<i>indica</i>
Chr7	29937784	29937788	ERS468060	<i>indica</i>
Chr6	31319589	31319593	ERS468065	<i>indica</i>
Chr1	43029985	43029989	ERS468066	<i>indica</i>
Chr4	13737528	13737532	ERS468068	<i>indica</i>
Chr8	5518300	5518306	ERS468071	<i>indica</i>
Chr1	20049396	20049400	ERS468072	<i>indica</i>
Chr7	29937784	29937788	ERS468073	<i>indica</i>
Chr4	31968290	31968294	ERS468074	<i>indica</i>
Chr4	31960603	31960607	ERS468075	<i>indica</i>
Chr12	16846383	16846387	ERS468077	<i>indica</i>
Chr1	20049396	20049400	ERS468078	<i>indica</i>
Chr8	5518300	5518306	ERS468084	<i>indica</i>
Chr11	29783248	29783252	ERS468086	<i>indica</i>
Chr1	20049396	20049400	ERS468087	<i>indica</i>
Chr1	20049396	20049400	ERS468088	<i>indica</i>
Chr8	5518300	5518306	ERS468088	<i>indica</i>
Chr8	5518300	5518306	ERS468089	<i>indica</i>
Chr12	22672020	22672024	ERS468095	<i>indica</i>
Chr4	31960603	31960607	ERS468101	<i>indica</i>
Chr1	43029985	43029989	ERS468102	<i>indica</i>
Chr1	43029985	43029989	ERS468104	<i>indica</i>
Chr4	31968290	31968294	ERS468106	<i>indica</i>
Chr12	16846383	16846387	ERS468111	<i>indica</i>
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Chr8	5518300	5518306	ERS468121	<i>indica</i>
Chr4	31968290	31968294	ERS468126	<i>indica</i>
Chr8	5518300	5518306	ERS468131	<i>indica</i>
Chr8	5518300	5518306	ERS468133	<i>indica</i>
Chr1	20049396	20049400	ERS468134	<i>indica</i>
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Chr4	31968290	31968294	ERS468139	<i>indica</i>
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Chr4	31968290	31968294	ERS468157	<i>indica</i>
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Chr1	43029985	43029989	ERS468161	<i>indica</i>
Chr4	31968290	31968294	ERS468163	<i>indica</i>
Chr1	20049396	20049400	ERS468166	<i>indica</i>
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Chr4	31968290	31968294	ERS468174	<i>indica</i>
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Chr4	31968290	31968294	ERS468191	<i>indica</i>
Chr4	31968290	31968294	ERS468192	<i>indica</i>
Chr4	31968290	31968294	ERS468193	<i>indica</i>
Chr8	5518300	5518306	ERS468195	<i>indica</i>
Chr4	31968290	31968294	ERS468202	<i>indica</i>
Chr7	29937784	29937788	ERS468202	<i>indica</i>
Chr4	31968290	31968294	ERS468204	<i>indica</i>
Chr4	31968290	31968294	ERS468205	<i>indica</i>
Chr8	5518300	5518306	ERS468207	<i>indica</i>
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Chr1	43029985	43029989	ERS468215	<i>indica</i>
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Chr11	29783248	29783252	ERS468255	<i>indica</i>
Chr1	19257271	19257275	ERS467801	<i>japonica</i>
Chr5	23638163	23638167	ERS467889	<i>japonica</i>
Chr11	1514174	1514178	ERS467893	<i>japonica</i>
Chr8	5635769	5635774	ERS467904	<i>japonica</i>
Chr11	1514174	1514178	ERS468026	<i>japonica</i>
Chr5	258622	258626	ERS468308	<i>japonica</i>
Chr8	5635768	5635774	ERS468310	<i>japonica</i>
Chr1	41968356	41968360	ERS468380	<i>japonica</i>
Chr8	5635768	5635774	ERS468383	<i>japonica</i>
Chr8	5635768	5635774	ERS468384	<i>japonica</i>
Chr8	5635768	5635774	ERS468387	<i>japonica</i>
Chr8	5635768	5635774	ERS468402	<i>japonica</i>
Chr6	24954457	24954461	ERS468442	<i>japonica</i>
Chr6	22413483	22413487	ERS468446	<i>japonica</i>
Chr7	29379081	29379085	ERS468449	<i>japonica</i>
Chr8	5635769	5635774	ERS468449	<i>japonica</i>
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Chr8	5635768	5635774	ERS468458	<i>japonica</i>
Chr8	5635769	5635774	ERS468595	<i>japonica</i>
Chr8	5635768	5635774	ERS468596	<i>japonica</i>
Chr8	5635768	5635774	ERS468604	<i>japonica</i>
Chr8	5635768	5635774	ERS468613	<i>japonica</i>
Chr8	5635768	5635775	ERS468617	<i>japonica</i>
Chr8	5635768	5635774	ERS468620	<i>japonica</i>
Chr6	22413483	22413487	ERS468649	<i>japonica</i>
Chr5	258622	258626	ERS468684	<i>japonica</i>
Chr7	29379081	29379085	ERS468704	<i>japonica</i>
Chr8	5635768	5635774	ERS468705	<i>japonica</i>
Chr5	258622	258626	ERS468721	<i>japonica</i>
Chr5	23638163	23638167	ERS468734	<i>japonica</i>
Chr1	41968356	41968360	ERS468902	<i>japonica</i>
Chr1	41968356	41968360	ERS468917	<i>japonica</i>
Chr8	5635769	5635774	ERS468993	<i>japonica</i>

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