bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Title: DeLTa-Seq: direct-lysate targeted RNA-Seq from crude tissue lysate. (8/15 words)

Running Title: Development of direct-lysate targeted RNA-Seq method

Authors: Makoto Kashima[1,2], Mari Kamitani[1,3], Yasuyuki Nomura[1], Hiromi Hirata[2], Atsushi J. Nagano[4,*]

Author's Addresses:

1. Research Institute for Food and Agriculture, Ryukoku University, Yokotani, Seta Oe-cho, Otsu, Shiga, Japan
2. Department of Chemistry and Biological Science, College of Science and Engineering, Aoyama Gakuin University, Sagamihara, Japan
3. Center for Ecological Research, Kyoto University, Hirano, Otsu, Shiga, Japan
4. Faculty of Agriculture, Ryukoku University, Yokotani, Seta Oe-cho Otsu, Shiga, Japan

Corresponding Author: Atsushi J. Nagano, Faculty of Agriculture, Ryukoku University, Yokotani, Seta Oe-cho Otsu, Shiga, 520-2194, Japan,

Tel: 077-599-5656(2056), Mail: anagano@agr.ryukoku.ac.jp

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Abstract:

Using current mRNA quantification methods such as RT-qPCR and RNA-Seq, it is very difficult to examine thousands of tissue samples due to cost and labor of RNA extraction and quantification steps. Here, we developed Direct-RT buffer in which homogenization of tissue samples and direct-lysate reverse transcription can be conducted without RNA purification. We showed that appreciate concentration of DTT prevented RNA degradation but not RT in the lysates of several plants' tissues, yeast, and zebrafish larvae. Using the buffer, direct reverse transcription on the lysates could produce comparable amount of cDNA with that synthesized from purified RNA. Furthermore, we established DeLTa-Seq (**D**irect-**L**ysate reverse transcription and **Ta**rgeted RNA-**Seq**) method. DeLTa-Seq is a cost-effective, high-throughput and highly-precise quantification method for the expressions of hundreds of genes. It enables us to conduct large-scale studies using thousands of samples such as chemical screening, field experiments and studies focusing on individual variability. (145/150 words)

Keywords: Direct-lysate reverse transcription, targeted RNA-Seq, reducing regent, gene expression analysis

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Introduction

Quantification of gene expression is a popular approach to study various biological phenomena[1–8]. Recently, large-scale transcriptome analysis by RNA-Seq of hundreds of samples has been emerged in plant biology[9–12]. Although sequencing cost has been reduced drastically in the last decade[13], the requirements for labor and cost at library preparation step obstruct routine use of RNA-Seq. To overcome these difficulties, several cost-effective RNA-Seq library preparation methods were developed[14–19]. Particularly, the two methods developed based on the 3' RNA-Seq protocols for single cell RNA-Seq, Lasy-Seq[19] and BRB-Seq[18], enables us to early-pooling of samples resulting in reducing labor and cost into about two dollar per sample. In this situation, RNA extraction is remained as the most laborious and costly step in RNA-Seq. Especially, because plants tend to contain large amount of polysaccharide and high RNase activity, it costs more expensive to purify RNA from plant samples than animal cultured cells[20]. To obtain high quality RNA from plant samples, we homogenize and lyse the sample in the buffer containing guanidine hydrochloride to denature RNase, following by RNA purification with phenol chloroform extraction, silica column or magnetic beads that selectively bind to nucleic acid. If these RNA extraction steps can be skipped, the required cost and labor for RNA-Seq will be reduced more and make it easier to use RNA-Seq in large-scale

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

53 studies with thousands of samples. Although several studies showed that RT can be
54 possible directly from lysate of cultured animal cells in cell-lysis buffers[21–23], in our
55 knowledge, no study showed direct-lysate RT from crude tissue lysates. A previous study
56 showed that reducing regents such as dithiothreitol (DTT) irreversibly deactivate intrinsic
57 RNase in wheat[24]. Yet, whether DTT can portect RNA in the lysate of other speiseses and
58 whether DTT does not inhibit reverse transcription has not been examined.

59 There is the tread-off between comprehensive gene quantification and
60 sequencing cost[25]. For example, in the case of human transcriptome, it is estimated that
61 ~40 million reads are required for the reliable quantification of moderately-abundant
62 transcripts, and as many as 500 million reads are required to comprehensively quantify
63 rare transcripts such as transcription factors[26,27]. In some cases, it is enough to focus on
64 the limited number of genes of interest. When we focus on the limited number of genes,
65 we can measure gene expression more accurately with small number of reads. This will
66 enable us to examine biological phenomena with higher resolution of time, space,
67 perturbation and individual variability. Thus, cost-effective methods of gene
68 quantification such as targeted RNA-Seq and its relevant techniques to quantify hundreds
69 of transcripts[29–32] are required to understand complex behaviors of biological systems.

70 Here, we showed that high concentration of DTT can inhibit intrinsic RNase in

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

plant and animal crude tissue lysates but not a reverse transcriptase. Using Direct-RT buffer containing DTT, we succeed in direct-lysate RT from plant, yeast and animal samples and RNA-Seq using the lysate. Finally, we combined direct-lysate RT and targeted RNA-Seq (DeLTa-Seq). DeLTa-Seq can drastically reduce cost and labor of RNA-Seq, and keep accuracy in quantification. DeLTa-Seq will pave the way for a large-scale research.

Results

Reducing regents inhibit RNA degradation in plant, yeast and animal lysates

For direct-lysate RT from plant tissues, we tried to apply the three regents [CL buffer[23], CellAmp (TaKaRa, Kusatsu, Japan) and SuperPrep (TOYOBO, Osaka, Japan)] designed for direct-lysate RT from cultured mammalian cells. *Arabidopsis thaliana* seedlings were homogenized in these regents followed by an hour incubation at 22 °C. Then, RNA was purified from the lysates and checked with electrophoresis. Frozen rice (*Oryza sativa*) leaves were also homogenized with zirconia beads and dissolved in the regents. Incubation and RNA purification were conducted same as above. In both cases, any peaks of 18S and 28S rRNA were not observed, but short fragments of degraded RNAs were observed (Supplemental Fig. 1). These electropherograms indicated that

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

89 these regents for direct-lysate RT from cultured mammalian cells cannot inhibit RNA
90 degradation in plant lysates.

91 In the previous research[24], inactivation of RNase in an extract prepared from dark
92 grown wheat required 0.5 mM DTT or more than 0.014% 2-mercaptoethanol (2ME)[24].
93 Thus, we examined the ability of reducing reagents to protect RNA in plants' lysates from
94 RNase. We prepared 90 mM Tris-HCl (pH7.6) containing 10 mM, 50 mM, and 100 mM
95 DTT and 2.5% 2ME, and homogenized *A. thaliana* seedlings in these buffers. After an
96 hour incubation at 22 °C, RNA was purified from the lysates, followed by quality check
97 with electrophoresis. In the electropherogram of the purified RNA from the lysate in
98 negative control buffer (no reducing regents), no peaks of rRNAs were observed (Fig. 1),
99 indicating that RNA was degraded in the lysate. In contrast, the clear peaks of rRNAs
100 were observed in the electropherograms of the purified RNA from lysates containing DTT
101 or 2ME. In the 10 mM DTT buffer, slight degradation of rRNA was observed in the region
102 shorter than the 18S rRNA (Fig. 1). In the higher concentration of DTT or 2.5% 2ME
103 buffer, degradation of RNA was inhibited (Fig. 1). In addition to *A. thaliana* seedling, we
104 examined the effect of adding reducing regents to the lysates of *O. sativa* leaf and root
105 and *Triticum aestivum* coleoptile with first leaf. Frozen rice leaves and wheat coleoptile
106 were homogenized with zirconia beads, and Tris-HCl buffers containing the reducing

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

107 regents were added. Like the results of *A. thaliana,* after an hour incubation at 22 °C,
108 degradation of rRNA was observed in rRNA purified from the lysates in the buffer without
109 any reducing regents and 10 mM DTT but not 50 mM and 100 mM DTT and 2.5% 2ME
110 (Fig. 1). Furthermore, we examined whether 100 mM DTT can protect RNA form
111 degradation in the lysates of yeast and animal tissue (zebrafish larva). A pellet of liquid-
112 cultured *S. cerevisiae* was homogenized with zirconia beads, and 2 days post fertilization
113 larvae of zebrafish were homogenized with a pestle in Tris-HCl buffers with or without
114 100 mM DTT, followed by an hour incubation at 22 °C and purification of total RNA. As
115 expected, clear rRNA peaks could be observed in the RNA from the lysates with DTT but
116 not without DTT (Fig. 1). It is noted that the commercial regents for cultured animal cells
117 could not prevent RNA degradation in the lysate of zebrafish (Supplemental Fig.1). We
118 therefore concluded that high concentration of reducing regents can protect RNA form
119 intrinsic RNase.
120
121 **Moderate concentration of reducing regents do not inhibit reverse transcription**
122 Next, we evaluated the efficiency of RT in the presence of the reducing regents.
123 We added several concentrations of DTT or 2ME to RT reaction mixes and performed RT
124 of purified total RNA from *O. sativa*. Then, qPCR for *Os03g0836000* transcript coding

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

125 for an Actin protein was conducted. Because, in manufacturer's protocol of RTase,
126 addition of 5 mM DTT is recommended for RT reaction to protect RT enzyme from
127 oxidization, 5 mM DTT is positive control in this experiment. RT products of 5 mM DTT
128 showed 4.4 lower Ct value than the negative control (Fig. 2a). As the concentration of
129 DTT increased, Ct value slightly increased (Fig. 2a). Same tendency was observed for
130 2ME, but 10 % 2ME drastically diminished the efficiency of RT reaction (Fig. 2a). These
131 results indicate that high concentration of reducing regent (ex. 10% 2ME) inhibits RT
132 reaction but moderate concentration of reducing regent dose not.

133 To assess whether addition of the reducing regents into homogenization buffer
134 makes it possible to perform RT from plant lysates, we conducted direct-lysate RT of *A.*
135 *thaliana* seedling and *O. sativa* leaf, root and *T. aestivum* coleoptile, and quantified their
136 actin cDNAs (*AT3G18780*, *Os03g0836000* and *HP620998.1*, respectively) (Fig. 2b-e).
137 Because the genomic DNA remains in the lysates, we conducted RT with dT primers
138 harboring an adapter sequence at the 5' end (Supplemental Table 1), followed by RT-
139 qPCR with a gene-specific and the adapter-specific primers to detect exclusively cDNA
140 synthesized by the direct-lysate RT (Fig. 2f). Compared to the RT(-) negative controls,
141 amplification of the target cDNAs were observed in the lysates with the reducing
142 regents (Fig. 2b-e). Consistent with RNA degradation levels in the lysates (Fig. 2a), the

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

143 lysate in the 10 mM DTT buffer showed less cDNA production than the others (Fig.
144 2b,c). For all plant lysates, the 100 mM DTT buffer showed high production of the actin
145 cDNAs (Fig. 2b-e). In addition, to check the linearity of cDNA synthesis by direct-
146 lysate RT, we conducted direct-lysate RT of varied amounts of the *O. sativa* leaf lysate
147 and quantifies the cDNA with qPCR. The series of direct-lysate RT showed a good
148 linearity between the input amount of the lysate and the cDNA production ($R^2 = 0.991$,
149 Fig. 2g). Thus, Direct-RT buffer (100 mM DTT/90 mM Tris-HCl (pH7.6)) was used for
150 the following experiments of direct-lysate RT.
151
152 **Direct-lysate RT can produce cDNA comparable to RT from purified RNA.**
153 We examined bias in direct-lysate RT comparing with RT of purified total RNA.
154 We compared the results of 3' RNA-Seq of purified RNA and lysate. The lysate of *O.*
155 *sativa* leaves was divided into two aliquots. One was purified and used for 3' RNA-Seq
156 library preparation, whereas the other was directly used for 3' RNA-Seq library
157 preparation. 29 and 30 technical replicates for cDNA from the purified RNA and lysate
158 were sequenced, respectively. To remove noise derived from total read-number variation,
159 we analyzed 100000 reads subsampled from each technical replicate. The mapping rate
160 onto the transcriptome reference in RNA-Seq of the lysate is approximately 1% higher

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

than that of the purified RNA (Fig. 3a). Also, ratio of rRNA reads of the lysate is approximately 1.7% higher than the purified RNA (Fig. 3b). The numbers of detected genes in the purified RNA and lysate were almost same (Fig. 3c). Comparison of each gene expression level showed that almost all gene were quantified equally in both methods, although 396 of 38194 genes were differentially quantified (Fig. 3d). Among them, 329 genes were quantified as higher amount in the lysate than the purified RNA (Fig. 3d). The transcript length of the highly-quantified genes tended to be short; the average length of the highly-quantified transcripts is 799 nt, while the average length of all transcripts in the reference is 1537 nt (Fig. 3e). There is possibilities that direct-lysate RT could efficiently produce cDNA of short transcripts in the lysate of *A. thaliana* seedlings and/or that some of short transcripts might be diminished through RNA purification process. Also, we conducted same comparison of RNA-Seq of lysate and purified RNA from budding yeast and zebrafish larva. In the case of the yeast, among 6713 genes, 37 and 48 genes were highly and lowly quantified in RNA-Seq of the lysate (Supplemental Fig. 2a). On the other hand, in the case of the zebrafish larva, among 57775 genes, 6913 and 1048 genes were highly and lowly quantified in RNA-Seq of the lysate (Supplemental Fig. 2b). Unlike *A. thaliana* lysate, any bias in the transcript length was not observed in both yeast and zebrafish lysates (Supplemental fig. 2c and d). We

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

179 concluded that direct-lysate RT method can produce comparable, or even higher, amount
180 of cDNA to that synthesized from purified RNA, although RT efficiencies of some
181 transcripts could be specifically altered by the composition of lysates.
182
183 **Direct-lysate targeted RNA-Seq (DeLTa-Seq)**
184 To develop a cost-effective and accurate gene quantification method applicable for using
185 direct-lysate RT, we tried to take advantage of targeted amplicon RNA-Seq[29]. We
186 designed the gene-specific primers for all *A. thaliana* genes (googlesite URL). Here, we
187 focused on the 100 marker genes focused on in the previous study[33]. We integrated the
188 PCR-based targeted RNA-Seq with direct-lysate RT, named "**D**ire**c**t-**L**ysate **Ta**rgeted
189 RNA-Seq (DeLTa-Seq) (Fig. 4a); First, 1st index was added by the direct-lysate RT with
190 oligo-dT primer harboring 1st indexes, following by sample pooling (up to 384 samples).
191 The RT primer contains unique molecular identifier (UMI). Second, a part of the adapter
192 sequence was added to the cDNA of target genes using gene-specific primers harboring a
193 part of the adapter sequence. Third, 2nd indexing and target amplification were conducted
194 with PCR. If needed, up to 384 libraries can be pooled, which enables us to sequence up
195 to 147456 samples at once. To evaluate target enrichment by DeLTa-Seq, we prepared
196 two libraries, non-targeted RNA-seq and targeted RNA-Seq (DeLTa-Seq) from the same

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

197 lysate of *A. thaliana* seedling, followed by sequencing with MiSeq, resulting in 6694751
198 reads of the non-targeted RNA-Seq and 203450 reads of DeLTa-Seq. All target genes are
199 quantified more highly in targeted RNA-Seq than in non-targeted RNA-Seq (Fig. 4b).
200 The ratio of reads corresponding to the target genes increased from 0.45% in the non-
201 targeted RNA-Seq to 94.9% in the targeted RNA-Seq; more than 200-fold-enrichment for
202 the target cDNA was achieved even for direct-lysate RT (Fig. 4c).
203
204 **Targeted RNA-Seq works with low amount of input RNA.**
205 Next, we examined the required amount of input RNA for the targeted RNA-Seq.
206 We prepared targeted RNA-Seq libraries from 0.01 ng, 0.1 ng, 1 ng and 10 ng of a same
207 purified *A. thaliana* total RNA with 96, 96, 10 and 5 technical replicates, respectively.
208 After sequencing with MiSeq, 10,000 reads were subsampled from each replicate. The
209 mean ratios of the target reads to the subsampled reads were 89.5%, 87.1% and 87.3% in
210 the libraries of 0.1 ,1 ng and 10 ng inputs, respectively, whereas 1.31% in the libraries of
211 0.01 ng input (Fig. 5a). While 86 genes were totally detected, the number of detected
212 target genes was saturated in the libraries of more than 0.1 ng input (69.6 and 74.4 genes
213 on average in 1 ng and 10 ng), whereas very few target genes (1.26 genes on avarage)
214 were detected in the libraries of 0.01 ng input (Fig. 5b). Pearson's correlation coefficient

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

of log2 RPM+1 of the target genes in each library to each 10-ng-input library was also saturated in the libraries of more than 0.1 ng input (Fig. 5c). Based on these results, targeted RNA-Seq roughly requires 1 ng input of total RNA, and would be applicable for small tissues.

Targeted RNA-Seq improved reproducibility and cost of gene quantification.

To examine the reproducibility and performance of mRNA quantification by targeted RNA-Seq, we prepared 96 technical replicates of targeted and non-targeted RNA-Seq libraries, respectively, using single tube of RNA of *A. thaliana* seedlings and sequenced them with HiSeq. In our targeted RNA-Seq, we used 10-bases unique molecular identifiers (UMI), which can theoretically distinguish a million of RNA molecules of each target transcript[31,34]. All reads were used after UMI preprocessing, resulting in 96 technical replicates of non-targeted RNA-Seq of 1786180 UMI counts on average and of targeted RNA-Seq of 344215 UMI counts on average. UMI conversion efficiencies were 0.805 on average in non-targeted RNA-Seq, and 0.434 on average in targeted RNA-Seq, respectively (Supplemental fig. 3). We merged all UMI counts of targeted and non-targeted RNA-Seq (33044648 and 171473284 UMI counts, respectively) to be used as 'full data'. First, to evaluate reproducibility of our targeted RNA-Seq protocol, we

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

233 subsampled from 10000 to 150000 UMI counts from each technical replicate of targeted
234 RNA-Seq. Then, Pearson's correlation coefficients of log2 RPM+1 of the target genes in
235 each subsampled data set for the full data were calculated. With 150000 UMI counts, 96
236 replicates showed high reproducibility (the average of Pearson`s correlation coefficients
237 for the full data: 0.977 ± 0.0039 [mean ± S.D.]) (Fig. 6a). Next, to examine quantification
238 performance, we merged every eight technical replicates of non-targeted and targeted
239 RNA-Seq into one, resulting in 12 pseudo-technical replicates, respectively. Then, we
240 subsampled from 1000 to 2000000 UMI counts of targeted RNA-Seq and from 10000 to
241 10000000 UMI counts of non-targeted RNA-Seq from each pseudo-technical replicate.
242 Reproducibility of quantification of each target gene was almost saturated at 250000 UMI
243 counts in the targeted RNA-Seq, while 5000000 UMI counts in the non-targeted RNA-
244 Seq (Fig. 6b). Similarly, Pearson's correlation coefficients of log2 RPM+1 of the target
245 genes in each subsampled data set for the full data was saturated at 250000 and 5000000
246 UMI counts in the targeted and non-targeted RNA-Seq, respectively (Fig. 6c). Thus,
247 targeted RNA-Seq (DeLTa-Seq) would enable us to conduct cost-effective and accurate
248 quantification of target gene expression by reducing sequencing cost.
249

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Discussion

Direct-lysate RT are utilized in analyses of cultured animal cells to reduce cost and labor for RNA purification, followed by gene expression analysis such as RT-qPCR and RNA-Seq[21,35]. In these direct-lysate RT methods, RNase inhibitors are usually added to the lysis buffers to inhibit RNA degradation by intrinsic RNase. Because commercial RNase inhibitors are expensive, it is not realistic to use them in preparation of lysates from large size and/or large number of crude plant and animal tissues. Notably, we demonstrated that these methods cannot be applicable to plant and animal tissues (Supplemental fig. 1). In this study, we developed Direct-RT buffer that realizes cost-effective direct-lysate RT of crude plant, yeast and animal samples. DTT could reduce disulfide bonds required for the activity of RNase in lysates[24]. Because no loss of RNA in purification occurs through direct-lysate RT, this technique is beneficial also for gene expression analysis in small tissues from which it is difficult to purify enough amount of RNA for the experiments. Furthermore, DTT may contribute long-term maintenance of endogenous RNase inhibitor activity under storage at -20 °C[23,36]. Thus, our Direct-lysate RT technique will be useful not only for gene expression analysis but also RNA storage.

Comparison of the non-targeted RNA-Seq results of the purified RNA and the lysate revealed that some transcripts were differentially reverse transcribed in the direct-

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

lysate RT than in RT of the purified RNA (Fig. 3d and Supplemental fig. 2). For example, in the case of *A. thaliana* seedlings, the amount of cDNA of rRNA was twice in the direct-lysate RT as much as in RT with the purified RNA, but less than 4% (Fig. 3b). Previous studies showed that unspecific priming from A-rich region of rRNA with dT primer occurs in RT step[19,37]. Considering that lysate will contain higher concentration of salt than purified RNA solution and that salt increases the stability of base-paring of nucleic acid[38], unspecific priming by dT primer against non-polyA RNA such as rRNA would occur in direct-lysate RT more than in RT with purified RNA. Also, direct-lysate RT in the lysate of *A. thaliana* seedlings might produce more cDNA of relatively short transcripts (Fig. 3e). Given that numerous transcripts were differentially quantified in the lysate of zebrafish (Fig. S2), there is another possibility that the composition of the lysates affected the binding between transcripts and the magnetic beads, causing biases in RNA purification step. It is recommended to compare the RNA-Seq of a lysate with the RNA-Seq of purified RNA as a pilot experiment.

In this study, we examined how much input are required for accurate gene quantification. At least, 0.1 ng total RNA per sample was required for stable quantification of the target genes (Fig. 5b,c), but targeted-RNA-Seq with 0.1 ng total RNA resulted in abundant useless reads (Fig. 5a). The amount of the 100 target RNAs in 0.01 ng total

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

286 RNA could be less than 0.1 pg (1% of total RNA) equal to mRNA amount of one cell[39],
287 resulting in few detected targets (Fig. 5b) as observed in single cell RNA-Seq. Dropouts
288 is known as the phenomenon in which single-cell RNA-Seq can capture only small
289 fraction of the transcriptome of each cell and the count of most genes are zero[40].
290 Considering that one cell contains 10 pg of total RNA[39], the requirement of DeLTa-Seq
291 will be acceptable for small tissue sample consisting of roughly 100 cells.

292 A high resolution of time, space, perturbation, and stochastic variation should be
293 required to understand complex biological systems via transcriptome analysis. From the
294 viewpoint of sequencing cost, it is difficult to apply non-targeted RNA-Seq strategy to
295 thousands of samples. Although the required read counts for robust quantification by
296 targeted RNA-Seq depends on expression levels of target transcripts, our study indicated
297 that 150000 UMI counts could be enough for targeted RNA-Seq to quantify target
298 transcripts (Pearson's correlation coefficient > 0.98) (Fig. 6). Given the high throughput
299 of recent illumina DNA sequencers and our UMI conversion efficiencies (Supplemental
300 Fig. 3), we can sequence thousands of samples per lane, resulting in cost-effective
301 quantification of genes of interest. For example, with HiSeq 4000, which can generate
302 300 million reads per lane, two thousand samples can be sequenced at a time. Coupled
303 with skipping RNA purification step, DeLTa-Seq can drastically reduce cost and labor in

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

304 mRNA quantification. DeLTa-Seq is useful not only for large scale studies handling
305 thousands of samples such as field transcriptome and chemical screening but also in-depth
306 studies of specific biological systems. For example, a researcher will conduct limited
307 number of comprehensive RNA-Seq, and find genes of interest. DeLTa-Seq can measure
308 expressions of the genes of interest in many samples required for in-depth analysis.
309 Notably, in our protocol, same cDNA can be shared for both non-targeted and targeted
310 RNA-Seq. This advantage enables us to select non-targeted and targeted RNA-Seq
311 according to the aim of each round. DeLTa-Seq will realize cost-effective and accurate
312 quantification of these genes in numerous samples. DeLTa-Seq can be applicable for
313 animals as well as plants. This technique will open up a novel field of both plant and
314 animal biology. (3522/5000 words)
315

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

316 Materials and Methods

317 **Plant materials**.

318 Seeds of *Oryza sativa* L. *japonica* 'Koshihikari' were sown in nursery trays followed by

319 cultivation for two weeks under 16-h light/8-h dark and 30°C/ 20°C cycles.

320 Seeds of *Arabidopsis thaliana* L. (Col-0, CS70000) were sterilized in 90% EtOH.

321 Then, they were sown on 1/2× Murashige and Skoog medium with 0.25% gellan gum

322 (Wako, Osaka, Japan), 0.05% (v/v) PPM-100 (Plant Cell Technology, Washington, D.C.,

323 USA), 1.25 mM MES-KOH (pH 5.7) and 0.5% sucrose following by cultivation for 21

324 days at 20°C under 16-h light/8-h dark cycles.

325 Seeds of *Triticum aestivum* L. 'Chinese Spring' were sown on 200 µL zirconia

326 beads YTZ-1 (AS-ONE, Osaka, Japan) with 110 µL nuclease-free water in 2 mL

327 microtubes LT-0200 (INA OPTICA, Tokyo, Japan) followed by cultivation for 3 days

328 under 24-h dark and 4°C and 8 days under 24-h light and 20°C.

329

330 **Budding yeast material**

331 *S. cerevisiae* BY4742 strain was cultured in Synthetic Complete (SC) medium at 28 °C.

332 The yeast in the exponential phase was used in this study.

333

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

334 **Animal material**

335 Fertilized eggs of zebrafish (*Danio rerio*) were obtained by cross of lab stock AB provided
336 by Zebrafish International Resource Center (ZIRC) located at the University of Oregon.
337 They were maintained at 28 °C under dark condition till sampling. Larvae were sacrificed
338 at 48 hours post fertilization.

339

340 **Preparation of purified *O. sativa* and *A. thaliana* RNA**

341 At two weeks after seeding, the youngest fully expanded leaves of *O. sativa* were frozen
342 in liquid nitrogen, and stored at -80 °C until RNA isolation for RNA-Seq. Frozen samples
343 were homogenized with zirconia beads YTZ-4 (AS-ONE, Osaka, Japan) using
344 TissueLyser II (Qiagen, Hilden, Germany), and total RNA was then extracted using the
345 Maxwell 16 LEV Plant RNA Kit (Promega, Madison, WI, USA) and Maxwell 16
346 Automated Purification System (Promega). The concentration of RNA was measured
347 using QuantiFluor RNA System (Promega) and Quantus Fluorometer (Promega).

348 Seven days after seeding, bulked seedlings of *A. thaliana* were homogenised
349 with zirconia beads YTZ-4 and TissueLyser II (Qiagen, Hilden, Germany), and total RNA
350 was then extracted using the Maxwell 16 LEV Plant RNA Kit (Promega, Madison, WI,
351 USA) and Maxwell 16 Automated Purification System (Promega, Madison, WI, USA).

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

352 The concentration of RNA was measured using Quantus Fluorometer (Promega, Madison,
353 WI, USA).
354
355 **Preparation of lysate**
356 Seedlings of *A. thaliana* (Max 9 seedlings) were homogenised with zirconia beads YTZ-
357 4 and TissueLyser II in 400 µl of 90 mM Tris-HCl (pH 7.6) containing reducing regents
358 (10, 50 or 100 mM DTT or 2.5% 2ME), CL buffer [50 mM Tris-HCl pH 7.6, 0.1% Igepal
359 CA-630, 150 mM NaCl and 1 mM DTT][23] or CellAmp Processing Buffer (TaKaRa,
360 Kusatsu, Japan) or Lysis Solution of SuperPrep (TOYOBO, Osaka, Japan). DTT solution
361 should be freshly prepared or stored at -20 °C. According to the manufacture's manual,
362 for SuperPrep, 76 µl Stop solution (TOYOBO) and 4 µl RNase Inhibitor (TOYOBO)
363 were added after incubation for 5 min at 22 °C.
364 Each youngest fully expanded leaf of *O. sativa* was frozen in liquid nitrogen, and
365 stored at -80 °C until homogenization. Each frozen sample was homogenized with
366 zirconia beads YTZ-4 using TissueLyser II. Then, 400 µl of the same buffers used for *A.*
367 *thaliana* seedlings were added, and, immediately, the samples were mixed well by
368 vortexing. According to the manufacture's manual, for SuperPrep, 76 µl Stop solution
369 and 4 µl RNase Inhibitor were added 5 min after incubation at 22 °C.

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

370 Each coleoptile with first leaf of *T. aestivum* was frozen in liquid nitrogen, and
371 stored at -80 °C until homogenization. Each frozen sample was homogenized with the
372 zirconia beads YTZ-4 using TissueLyser II. Then, 400 µl of the Direct-RT buffer was
373 added, and, immediately, the samples were mixed well by vortexing.

374 *S. cerevisiae* in the exponential phase was homogenised with zirconia beads
375 YTZ-1 and MS-100R (TOMY, Tokyo, Japan) in 350 µl of 90 mM Tris-HCl (pH 7.6)
376 containing 100 mM DTT.

377 Three *D. rerio* larvae were homogenized in 50 µl of the same buffers used for *A.*
378 *thaliana* seedlings with Biomasher II (Nippi, Tokyo, Japan). According to the
379 manufacture's manual, for SuperPrep, 9.5 µl Stop solution and 0.5 µl RNase Inhibitor
380 were added after incubation for 5 min at 22 °C.

381 The concentration of RNA in the lysates were measured using the Quant-iT RNA
382 Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and Infinite M1000 PRO
383 (TECAN, Zurich, Switzerland).

384

385 **Purification of RNA from lysates**

386 Lysate was incubated for an hour at 22 °C. Then, 2.5× volume of AMpure XP beads
387 (Beckman Coulter, Brea, CA, USA) was added to a lysate. The mixture was purified

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

388 following the manufacturer's instructions. The RNA was then eluted with the equal
389 volume of RNase-free water to the lysate. One microliter of the purified RNA solution
390 was used for electrophoresis using a Bioanalyzer 2100 with Agilent RNA nano or pico
391 kit (Agilent Technologies, Santa Clara, CA, USA) to check for the quality.
392
393 **Direct-lysate reverse transcription**
394 RT reactions were conducted with 1 μL of 2 μM RT primer
395 (CAGAAGACGGCATACGAGATGCGTCTACGTGACTGGAGTTCAGACGTGTGC
396 TCTTCCGATCNNNNNNTTTTTTTTTTTTTTTTV), 0.4 μL of 25 mM dNTP
397 (Advantage UltraPure dNTP Combination Kit) (TaKaRa), 4.0 μL of 5× SSIV Buffer
398 (Thermo Fisher Scientific), 0.1 μL of SuperScript IV reverse transcriptase (200 U/μL,
399 Thermo Fisher Scientific), lysates and nuclease-free water to make a volume of 20 μL.
400 Reverse transcription was carried out at 65°C for 10 min, following by incubation at 80°C
401 for 15 min to inactivate the enzyme.
402
403 **Quantitative PCR analysis of cDNA (RT-qPCR) for figure 2**
404 250 ng (*A. thaliana* and *O. sativa*) or 140 ng (*T. aestivum*) of total RNA in lysate was used
405 for RT. Then, the reaction mix of *A. thaliana* and *O. sativa* was diluted with nucleases-

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

406 free water by 20 times. The reaction mix of *T. aestivum* was used for the following
407 experiments without dilution. The cDNA amount of *AT3G18780*, *Os03g0836000* or
408 *HP620998.1* was measured by qPCR analysis. The composition of the qPCR mixture is
409 described below; diluted cDNA solutions 2 µl, gene-specific primer
410 (CTTGCACCAAGCAGCATGAA for *AT3G18780* or GTGTGTCGGTACTTTCGTCG
411 for *Os03g0836000*, TGACCGTATGAGCAAGGAG for *HP620998.1*[41], P7 primer for the
412 adapter sequence added in the RT step (CAAGCAGAAGACGGCATACGAGAT) and 5
413 µl of KAPA SYBR FAST qPCR Master Mix (2×) (KAPA BIOSYSTEMS, Wilmington,
414 MA, USA). qPCR was conducted using LightCycler 480 System II (Roche Diagnostics,
415 Basel, Switzerland) with the following program: enzyme activation at 95 °C for 3 min,
416 40 cycles at 95 °C for 3 s and 60 °C for 30 s for amplification.
417
418 **Non-targeted RNA-Seq for figure 3**
419 Non targeted RNA-Seq were conducted according to Lasy-Seq ver. 1.1 protocol[19]
420 (https://sites.google.com/view/lasy-seq/). RT reactions were conducted with 1 µL of 2 µM
421 of a RT primer
422 (CAGAAGACGGCATACGAGATxxxxxxxxGTGACTGGAGTTCAGACGTGTGCTC
423 TTCCGATCNNNNNNTTTTTTTTTTTTTTTTVT, xxxxxxxx indicate index

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

424 sequences for multiplex sequencing. See supplemental table 1), 0.4 μL of 25 mM dNTP,
425 4.0 μL of 5× SSIV Buffer, 2.0 μL of 100 mM DTT, 0.1 μL of SuperScript IV reverse
426 transcriptase, template RNA (lysate or purified RNA solution) and nuclease-free water to
427 make a volume of 20 μL. Reverse transcription was carried out at 65°C for 10 min,
428 following by incubation at 80°C for 15 min to inactivate the enzyme. Then, all RT mixture
429 of samples were pooled and purified with Wizard SV Gel and PCR Clean-Up System
430 (Promega, Madison, WI, USA) according to the manufacture's manual except for using
431 80% EtOH instead of Membrane Wash Solution. The purified cDNA was eluted with 30
432 μL of nuclease-free water. Second strand synthesis was conducted on the pooled samples
433 (30 μL) with 4 μL of 10× blue buffer (Enzymatics, Beverly, MA, USA), 2 μL of 2.5 mM
434 dNTP (Advantage UltraPure dNTP Combination Kit), 1 μL of 100 mM DTT, 1 μL of
435 RNaseH (5 U/μL, Enzymatics), 2 μL of DNA polymerase I (10 U/μL, Enzymatics). The
436 reaction was conducted at 16°C for 2 h and kept at 4°C until the next reaction. To avoid
437 the carryover of large amount of rRNAs, RNase treatment was conducted on the mixture
438 with 1 μL of RNase T1 (1 U/μL, Thermo Fisher Scientific) and 1 μL of RNase A (10
439 ng/μL, NIPPON GENE, Tokyo, Japan). The reaction was conducted at 37°C for 5 min
440 and 4°C until the next reaction. Then, purification was conducted with 0.8× volume of
441 AMpure XP beads according to the manufacturer's manual, following by elution with 36

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

μL nuclease-free water. Fragmentation, end-repair and A-tailing were conducted in the mixture of 17.5 μL of the dsDNA, 2.5 μL of 10× Fragmentation Buffer (Enzymatics, Beverly, MA, USA) and 5 μL of 5× WGS Fragmentation Mix (Enzymatics, Beverly, MA, USA). The mixture was kept at 4°C before the following reaction. The reaction was conducted at 4°C for 1 min, 32°C for 7 min, 65°C for 30 min and 4°C until the next reaction. Adapter for the next ligation step was prepared by annealing 100 mM of A*C*C*GAGATCTACACACTCTTTCCCTACACGACGCTCTTCCGA*T*C*T and /5Phos/G*A*T*CGGAAGAGCGTCGTGTTAAATGTA*T*A*T (* signifies a phosphorothioate bond. /5Phos/ signifies a phosphorylation.) using a thermal cycler with the following program: 95°C for 2 min, slow-cooled to 45°C (0.1°C/s), followed by 45°C for 5 min. 10 μL of 5× Ligation buffer (Enzymatics), 8 μL of nuclease-free water, 2 μL of 4 mM annealed adapter were added to the above reaction mix, following by adding 5 μL of T4 DNA ligase (Enzymatics). The ligation was conducted at 20°C for 15 min. The adapter-ligated DNA was purified with 0.8× volume of AMpure XP beads, twice, followed by elution with 17 μL of nuclease-free water. To optimise PCR cycle for library amplification, qPCR was conducted with 3.5 μL of the adapter-ligated DNA, 5 μL of KAPA HiFi HotStart ReadyMix (KAPA BIOSYSTEMS), 0.5 μL of Evagreen, 20× in water (Biotium, Fremont, CA, USA), 0.5 μL of 10 μM 5× WGS_Pr_R1-5' primer

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

(AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTC) and 0.5 µL of 10 µM PCR_P7 primer (CAAGCAGAAGACGGCATACGAGAT). Reaction was carried out using LightCycler 480 II at 95°C for 5 min, 30 cycles of 98°C for 20 s, 60°C for 15 s, 72°C for 40 s, followed by 72°C for 3 min, then held at 4°C. The optimal number of the PCR cycle was the cycle corresponding to the middle of exponential phase. Then, library was amplified with 12 µL of the adapter-ligated DNA, 15 µL of KAPA HiFi HotStart ReadyMix, 1.5 µL of 10 µM 5× WGS_Pr_R1-5' primer and 1.5 µL of 10 µM PCR_P7 primer. Reaction was carried out at 95°C for 5 min, the optimized cycles of 98°C for 20 s, 60°C for 15 s, 72°C for 40 s, followed by 72°C for 3 min, then held at 4°C. The amplified library was purified with equal volume of AMpure XP beads and eluted with 15 µL of nuclease-free water. One microliter of the library was used for electrophoresis using a Bioanalyzer 2100 with Agilent High Sensitivity DNA kit to check for the quality. Sequencing of 50-bp single-ends using HiSeq (Illumina, San Diego, CA, USA) was carried out.

The design of gene-specific primers for *A. thaliana*

We designed gene-specific primers for *A. thaliana* cDNA sequences using primer3[42]. The parameters were as below; Concentration of Monovalent Cations : 40 mM,

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Concentration of Divalent Cations：6mM, Annealing Oligo Concentration：25nM, Primer Size Min/Opt/Max : 20/23/25, Primer Tm Min/Opt/Max : 60/65.5/70, Primer GC% Min/Opt/Max : 30/50/55, Max Tm Difference : 5, Product Size Ranges : 289-889, Number To Return : 5,PRIMER_RIGHT_INPUT : CAAGCAGAAGACGGCATACGAGAT, PRIMER_MAX_END_STABILITY. Then, candidates of gene-specific primers were concatenated as below; TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG+gene-specif-perimer. All candidates of gene-specific primers for all *A. thaliana* genes are available in google site URL.

Direct-lysate targeted RNA-Seq (DeLTa-Seq) for figure 4

RT reactions and sample-pooling were conducted according to the above protocol for the non-targeted RNA-Seq, following by elution of cDNA with 40 µl of nuclease-free water. 20 µL of the pooled cDNA was used. RNase treatment was conducted on the mixture with 1 µL of RNase T1 and 1 µL of RNase A. The reaction was conducted at 37°C for 5 min and 4°C until the next reaction. Then, purification was conducted with 0.8× volume of AMpure XP beads according to the manufacturer's manual, following by elution with 10 µL nuclease-free water. The adapter sequence was added to the target

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

transcripts in the library amplification step. The reaction was conducted as below; 4 µL of the RNase-treated cDNA solution, 5 µL of KAPA HiFi HotStart ReadyMix and 1 µL of gene-specific primer mix (total 100 µM, See Supplemental Table 2). The reaction was conducted at 95°C for 5 min, 98°C for 20 sec, 60°C for 15 sec, 72°C for 40 sec 5 min and 4°C until the next reaction. Then, purification was conducted with 0.8× volume of AMpure XP beads according to the manufacturer's manual, following by elution with 12 µL nuclease-free water. To optimise PCR cycle for library amplification, qPCR was conducted with 3.5 µL of the adapter-added DNA, 5 µL of KAPA HiFi HotStart ReadyMix, 0.5 µl of Evagreen, 20× in water, 2nd-index primer (AATGATACGGCGACCACCGAGATCTACACxxxxxxxxACACTCTTTCCCTACAC GA, xxxxxxxx indicates index sequences for multiplex sequencing. See supplemental table 3) and 0.5 µL of 10 µM PCR_P7 primer (CAAGCAGAAGACGGCATACGAGAT). Reaction was carried out using LightCycler 480 II at 95°C for 5 min, 30 cycles of 98°C for 20 s, 60°C for 15 s, 72°C for 40 s, followed by 72°C for 3 min, then held at 4°C. The optimal number of the PCR cycle was the cycle corresponding to the middle of exponential phase. Then, library was amplified with 7 µL of the adapter-added DNA, 10 µl of KAPA HiFi HotStart ReadyMix, 1 µL of 10 µM 2nd index primer (Supplemental Table 2) and 1 µL of 10 µM PCR_P7 primer. Reaction was

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

514 carried out at 95°C for 5 min, the optimized cycles of 98°C for 20 s, 60°C for 15 s, 72°C
515 for 40 s, followed by 72°C for 3 min, then held at 4°C. The amplified library was purified
516 with equal volume of AMpure XP beads and eluted with 15 μL of 10 mM Tris-HCl (pH
517 7.6). One microliter of the library was used for electrophoresis using a Bioanalyzer 2100
518 with Agilent High Sensitivity DNA kit to check for the quality. Sequencing of 140-bp
519 single-ends was carried out with MiSeq (Illumina, San Diego, CA, USA). The sequences
520 of the first 50-bp of 5'-end were used in the following analysis.
521 An update of DeLTa-Seq protocol is available in https://xxxxxx.
522

523 **Targeted RNA-Seq for figure 5**

524 RNA was extracted from bulked homogenate of approximate 10 seedlings of *A. thaliana*
525 in 14 days after sowing. Then, 0.01 ng, 0.1 ng, 1 ng and 10 ng of the purified RNA were
526 used for the reverse transcription. The number of technical replicates were 96, 96, 10 and
527 5, respectively. The following experiments were conducted as described in the section of
528 'Direct-lysate targeted RNA-Seq (DeLTa-Seq) for figure 4'.
529

530 **Preparation and sequencing of 96 technical replicates of non-targeted and targeted**
531 **RNA-Seq for Figure 6**

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

RNA was extracted from bulked homogenate of approximate 250 seedlings of *A. thaliana* in 7 days after sowing. RT reactions, pooling and purification were conducted as described in the section of 'Non-targeted RNA-Seq for figure 3' except for the amount of template RNA (250 ng) for each technical replicate. The pooled cDNA was eluted with 50 μL of nuclease-free water in the purification step.

For non-targeted RNA-Seq, 20 μL of the pooled cDNA was used. The following steps were conducted as described in the section of 'Non-targeted RNA-Seq for figure 3' with some changes; dsDNA after RNase treatment was eluted with 10 μL nuclease-free water. The optimization of PCR amplification cycle were conducted with 4 μL of the adapter-ligated dsDNA. Then, library was amplified using 7 μL of the adapter-ligated DNA. The amplified library was purified and eluted with 15 μL of 10 mM Tris-HCl (pH 7.6).

For targeted RNA-Seq, the following experiments were conducted as described in the section of 'Direct-lysate targeted RNA-Seq (DeLTa-Seq) for figure 4'.

Equal amount by mole of non-targeted and targeted RNA-Seq library were mixed based on qPCR-based quantification with Kapa Library Quantification Kit (KAPA BIOSYSTEMS, Wilmington, MA, USA). Sequencing of 150-bp paired-ends using HiSeq was carried out.

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Subsampling of RNA-Seq read for figure 5 and 6.

Subsampling of RNA-Seq reads was conducted with 'seqtk' (https://github.com/lh3/seqtk) to normalize total read. The seed parameter, which is specified as -s, was differently specified in each subsampling.

Calculation and normalization of RNA-Seq count data for figure 3, 4, 5 and 6.

All obtained reads were processed with Trimmomatic (version 0.3.3) (Bolger, Lohse and Usadel, 2014) using the following parameters: TOPHRED33 ILLUMINACLIP: TruSeq3-SE.fa:2:30:10 LEADING:19 TRAILING:19 SLIDINGWINDOW:30:20 AVGQUAL:20 MINLEN:40. This procedure removed adapter sequences (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10), leading and trailing low quality or N bases (below quality Phred score 19) (LEADING:19 TRAILING:19). Also, this trimmed the reads when the average quality per base drops below 20 with a 30-base wide sliding window (SLIDINGWINDOW:30:20). Finally, trimmed reads with length > 39 nucleotides and average quality score > 19 were output. The trimmed reads were then mapped to the *O. sativa* reference sequences of IRGSP-1.0_transcript[43,44], rRNAs, and transcripts coded in the mitochondria and chloroplast genomes (GenBank; NC_001320.1

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

568 and NC_011033.1), the *A. thaliana* reference sequence of Araport11 representative
569 transcripts[45], the *S. cerevisiae* cDNA R64-1-1 reference sequence of ensembl[46] or the *D.*
570 *rerio* GRCz11.cdna.all reference sequence of ensembl[46] with RSEM (version 1.3.0)[47],
571 using Bowtie (version 1.1.2)[48] with default parameters.

572 In the case of using UMI in the analysis, all obtained paired reads were processed
573 by using dynacomkobe/biodocker_rnaseq_pipeline:ver.0.2.0 with the default parameters,
574 with which quality trimming with Trimmomatic, quantification with RSEM and bowtie
575 were conducted.

576 Expected counts of each gene in the RSEM outputs were used with R (version
577 3.4.2)[49] in the following analysis. The R script used in this study is deposited in xxxxxxx.
578 Briefly, normalization of read counts were conducted by calculating read per million
579 (RPM) by using total read count of all transcripts as denominators. Differentially
580 quantified genes (FDR = 0.05) were detected as described by Sun et al[50]. (2947/3000
581 words)

582

583

584 Funding

585 This work was supported by the JST CREST JPMJCR15O2 to A. N..

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

586

587 Disclosures

588 The authors declare no competing interests

589

590 Data availability

591 All RNA-Seq sequence data is deposited in PRJNA643885 of SRA.

592

593

594 Acknowledgements

595 We thank Yoichi Hashida, Yuko Kurita, Yuma Wakamatsu, and Takahiro Mochizuki and

596 Fumiyoshi Abe for their advices on of *A. thaliana* and *O. sativa*, breeding of *D. rerio* and

597 a kind gift of *S. cerevisiae* respectively. We also thank all our laboratory members for

598 their help and encouragement.

599

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

600 References

601 1. Choudhury, F. K., Devireddy, A. R., Azad, R. K., Shulaev, V. & Mittler, R.
602 Rapid Accumulation of Glutathione During Light Stress in Arabidopsis. *Plant*
603 *Cell Physiol.* **59**, 1817–1826 (2018).

604 2. Cui, P. *et al.* Tung Tree (Vernicia fordii, Hemsl.) Genome and Transcriptome
605 Sequencing Reveals Co-Ordinate Up-Regulation of Fatty Acid β-Oxidation and
606 Triacylglycerol Biosynthesis Pathways During Eleostearic Acid Accumulation in
607 Seeds. *Plant Cell Physiol.* **59**, 1990–2003 (2018).

608 3. Lin, Y. *et al.* Comparative Transcriptome Profiling Analysis of Red- and White-
609 Fleshed Strawberry (Fragaria×ananassa) Provides New Insight into the
610 Regulation of the Anthocyanin Pathway. *Plant Cell Physiol.* **59**, 1844–1859
611 (2018).

612 4. Yoshida, T., Kawanabe, T., Bo, Y., Fujimoto, R. & Kawabe, A. Genome-Wide
613 Analysis of Parent-of-Origin Allelic Expression in Endosperms of Brassicaceae
614 Species, Brassica rapa. *Plant Cell Physiol.* **59**, 2590–2601 (2018).

615 5. Yuan, S. *et al.* Transcriptomic and Metabolic Profiling Reveals 'Green Ring' and
616 'Red Ring' on Jujube Fruit upon Postharvest Alternaria alternata Infection. *Plant*
617 *Cell Physiol.* **60**, 844–861 (2018).

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

618 6. Yuan, S. *et al.* Transcriptomic and Metabolic Profiling Reveals 'Green Ring' and
619 'Red Ring' on Jujube Fruit upon Postharvest Alternaria alternata Infection. *Plant*
620 *Cell Physiol.* **60**, 844–861 (2019).
621 7. Huang, R. *et al.* Heat Stress Suppresses Brassica napus Seed Oil Accumulation
622 by Inhibition of Photosynthesis and BnWRI1 Pathway. *Plant Cell Physiol.* **60**,
623 1457–1470 (2019).
624 8. Ishimaru, T. *et al.* Laser Microdissection-Based Tissue-Specific Transcriptome
625 Analysis Reveals a Novel Regulatory Network of Genes Involved in Heat-
626 Induced Grain Chalk in Rice Endosperm. *Plant Cell Physiol.* **60**, 626–642
627 (2019).
628 9. Nagano, A. J. *et al.* Deciphering and prediction of transcriptome dynamics under
629 fluctuating field conditions. *Cell* **151**, 1358–1369 (2012).
630 10. Nagano, A. J. *et al.* Annual transcriptome dynamics in natural environments
631 reveals plant seasonal adaptation. *Nat. Plants* **5**, 74–83 (2019).
632 11. Anacleto, R. *et al.* Integrating a genome-wide association study with a large-scale
633 transcriptome analysis to predict genetic regions influencing the glycaemic index
634 and texture in rice. *Plant Biotechnol. J.* pbi.13051 (2018) doi:10.1111/pbi.13051.
635 12. Kremling, K. A. G. G. *et al.* Dysregulation of expression correlates with rare-

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

allele burden and fitness loss in maize. *Nature* **555**, 520–523 (2018).

13. Muir, P. *et al.* The real cost of sequencing: scaling computation to keep pace with data generation. *Genome Biol.* **17**, 53 (2016).

14. Kumar, R. *et al.* A high-throughput method for Illumina RNA-Seq library preparation. *Front. Plant Sci.* **3**, 1–10 (2012).

15. Nagano, A. J., Honjo, M. N., Mihara, M., Sato, M. & Kudoh, H. Detection of plant viruses in natural environments by using RNA-Seq. *Plant Virol. Protoc. Methods Mol. Biol.* **1236**, 89–98 (2015).

16. Townsley, B. T., Covington, M. F., Ichihashi, Y. & Zumstein, K. BrAD-seq : Breath Adapter Directional sequencing : a streamlined , ultra-simple and fast library preparation protocol for strand specific mRNA library construction. *Front. Plant Sci.* **6**, 1–11 (2015).

17. Ishikawa, T. *et al.* Unfolded protein response transducer IRE1-mediated signaling independent of XBP1 mRNA splicing is not required for growth and development of medaka fish. *Elife* **6**, 1–29 (2017).

18. Alpern, D. *et al.* BRB-seq: ultra-affordable high-throughput transcriptomics enabled by bulk RNA barcoding and sequencing. *Genome Biol.* **20**, 71 (2019).

19. Kamitani, M., Kashima, M., Tezuka, A. & Nagano, A. J. Lasy-Seq: a high-

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

654 throughput library preparation method for RNA-Seq and its application in the
655 analysis of plant responses to fluctuating temperatures. *Sci. Rep.* **9**, 7091 (2019).
656 20. Yockteng, R. *et al.* A Method for Extracting High-Quality RNA from Diverse
657 Plants for Next-Generation Sequencing and Gene Expression Analyses. *Appl.*
658 *Plant Sci.* **1**, 1300070 (2013).
659 21. Shatzkes, K., Teferedegne, B. & Murata, H. A simple, inexpensive method for
660 preparing cell lysates suitable for downstream reverse transcription quantitative
661 PCR. *Sci. Rep.* **4**, (2014).
662 22. Huang, D. *et al.* An optimised direct lysis method for gene expression studies on
663 low cell numbers. *Sci. Rep.* **5**, 1–10 (2015).
664 23. Wang, X., Teferedegne, B., Shatzkes, K., Tu, W. & Murata, H. Endogenous
665 RNase inhibitor contributes to stability of RNA in crude cell lysates:
666 Applicability to RT-qPCR. *Anal. Biochem.* **513**, 21–27 (2016).
667 24. Chen, Z., Ling, J. & Gallie, D. R. RNase activity requires formation of disulfide
668 bonds and is regulated by the redox state. *Plant Mol. Biol.* **55**, 83–96 (2004).
669 25. Clark, M. B. *et al.* The Reality of Pervasive Transcription.
670 doi:10.1371/journal.pbio.1000625.
671 26. Blencowe, B. J., Ahmad, S. & Lee, L. J. Current-generation high-throughput

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

672 sequencing: Deepening insights into mammalian transcriptomes. *Genes and*
673 *Development* vol. 23 1379–1386 (2009).
674 27. Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L. & Wold, B. Mapping
675 and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods* **5**, 621–
676 628 (2008).
677 28. Katagiri, F. Attacking complex problems with the power of systems biology.
678 *Plant Physiology* vol. 132 417–419 (2003).
679 29. Blomquist, T. M. *et al.* Targeted RNA-Sequencing with Competitive Multiplex-
680 PCR Amplicon Libraries. *PLoS One* **8**, (2013).
681 30. Subramanian, A. *et al.* A Next Generation Connectivity Map: L1000 Platform
682 and the First 1,000,000 Profiles. *Cell* **171**, 1437-1452.e17 (2017).
683 31. Fu, G. K. *et al.* Molecular indexing enables quantitative targeted RNA
684 sequencing and reveals poor efficiencies in standard library preparations. *Proc.*
685 *Natl. Acad. Sci. U. S. A.* **111**, 1891–1896 (2014).
686 32. Sato, M. *et al.* A high-performance, small-scale microarray for expression
687 profiling of many samples in Arabidopsis-pathogen studies. *Plant J.* **49**, 565–577
688 (2007).
689 33. Biswas, S. *et al.* Tradict enables accurate prediction of eukaryotic transcriptional

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

states from 100 marker genes. *Nat. Commun.* **8**, (2017).

34. Kivioja, T. *et al.* Counting absolute numbers of molecules using unique molecular identifiers. *Nat. Methods* **9**, 72–74 (2012).

35. Sasagawa, Y. *et al.* Quartz-Seq2: A high-throughput single-cell RNA-sequencing method that effectively uses limited sequence reads. *Genome Biol.* **19**, 1–24 (2018).

36. Dickson, K. A., Haigis, M. C. & Raines, R. T. Ribonuclease Inhibitor: Structure and Function. *Prog. Nucleic Acid Res. Mol. Biol.* **80**, 349–374 (2005).

37. Nam, D. K. *et al.* Oligo(dT) primer generates a high frequency of truncated cDNAs through internal poly(A) priming during reverse transcription. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 6152–6156 (2002).

38. Nakano, S., Fujimoto, M., Hara, H. & Sugimoto, N. Nucleic acid duplex stability: influence of base composition on cation effects. *Nucleic Acids Res.* **27**, 2957–2965 (1999).

39. Sasagawa, Y. *et al.* Quartz-Seq: a highly reproducible and sensitive single-cell RNA-Seq reveals non-genetic gene expression heterogeneity. *Genome Biol.* **14**, R31 (2013).

40. Zappia, L., Phipson, B. & Oshlack, A. Splatter: simulation of single-cell RNA

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

sequencing data. *Genome Biol.* **18**, 174 (2017).

41. Paolacci, A. R., Tanzarella, O. A., Porceddu, E. & Ciaffi, M. Identification and validation of reference genes for quantitative RT-PCR normalization in wheat. *BMC Mol. Biol.* **10**, 11 (2009).

42. Koressaar, T. & Remm, M. Enhancements and modifications of primer design program Primer3. **23**, 1289–1291 (2007).

43. Kawahara, Y. *et al.* Improvement of the Oryza sativa Nipponbare reference genome using next generation sequence and optical map data. *Rice* **6**, 4 (2013).

44. Sakai, H. *et al.* Rice annotation project database (RAP-DB): an integrative and interactive database for rice genomics. *Plant Cell Physiol.* **54**, 1–11 (2018).

45. Cheng, C.-Y. *et al.* Araport11: a complete reannotation of the *Arabidopsis thaliana* reference genome. *Plant J.* **89**, 789–804 (2017).

46. Yates, A. D. *et al.* Ensembl 2020. *Nucleic Acids Res.* **48**, (2020).

47. Li, B. & Dewey, C. N. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12**, 323 (2011).

48. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10**, R25 (2009).

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

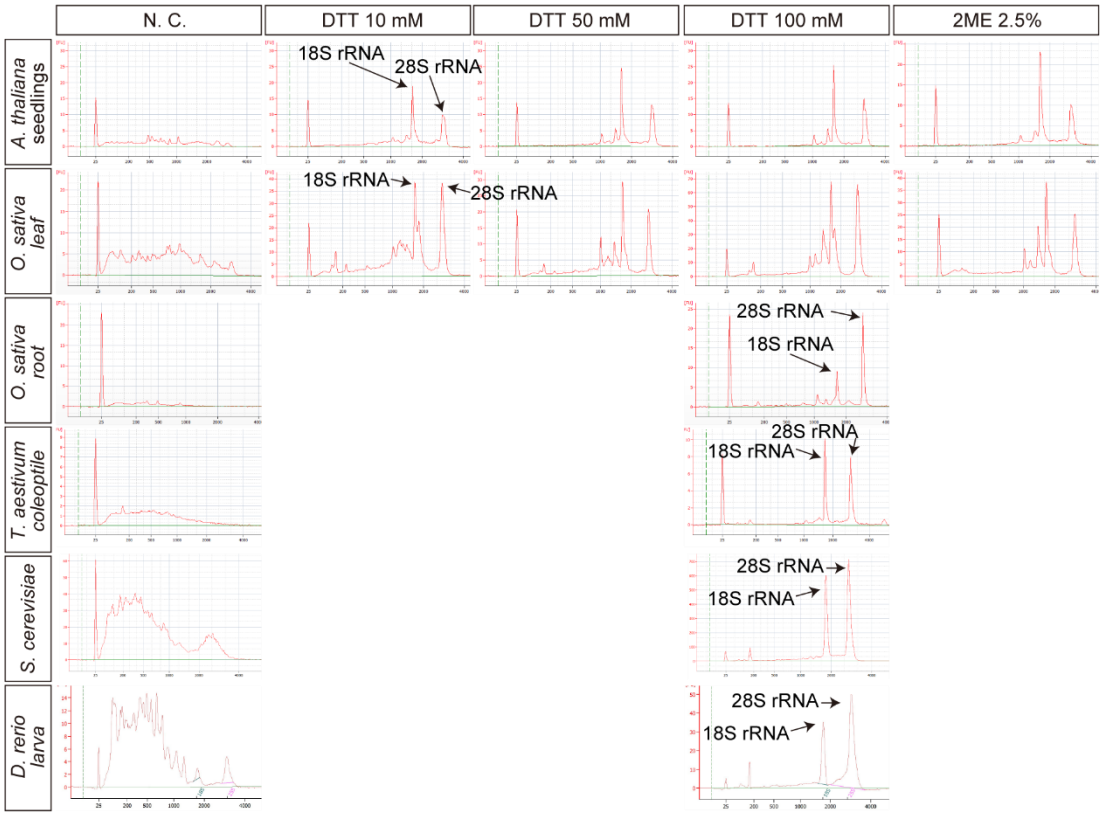
726 49. R Core Team. R: A language and environment for statistical computing. R
727 Foundation for Statis-tical Computing, Vienna, Austria. (2017).
728 50. Sun, J., Nishiyama, T., Shimizu, K. & Kadota, K. TCC: An R package for
729 comparing tag count data with robust normalization strategies. *BMC*
730 *Bioinformatics* **14**, 1 (2013).

731

732

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

733 Figures



734

735 **Figure 1 Reducing regents inhibited RNA degradation in plant and animal tissues'**

736 **lysate.**

737 Bioanalyzer electropherograms of purified RNA from tissue lysates (*A. thaliana* seedlings,

738 *O. sativa* leaves, *O. sativa* root, *T. aestivum* coleoptile, *S. cerevisiae* and *D. rerio*)

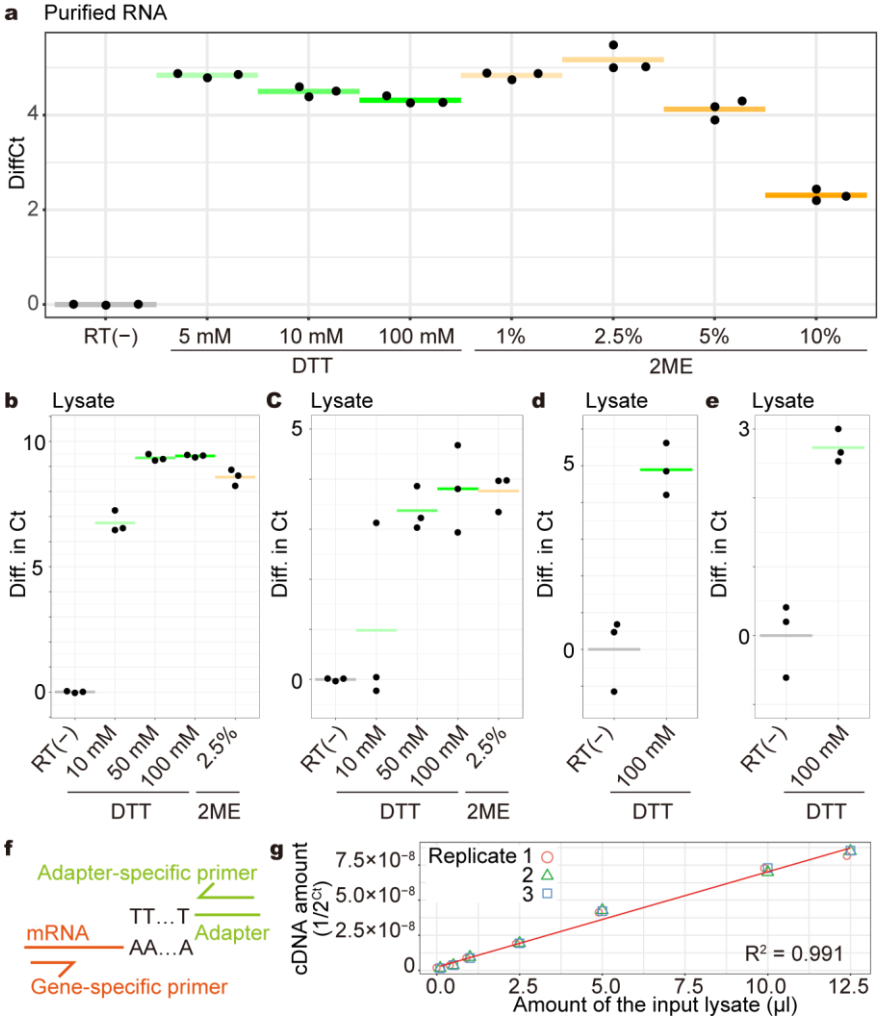
739 containing the reducing regents. The columns indicate that the concentrations of the

740 reducing regents in the homogenization buffers. N.C. represents no reducing reagents in

741 buffers (negative control). (65/350 words)

742

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



743

744 **Figure 2 Quantification of cDNA synthesized by direct-lysate reverse transcription.**

745 (a) qPCR results for *Os03g0836000* transcript in the cDNA synthesized from mixtures of

746 purified *O. sativa* total RNA and reducing regents. The horizontal axis indicates the

747 concentrations of the reducing regents in the RT reaction mixes. The vertical axis

748 indicates the differences in Ct value compared to the RT(-) negative control. Each point

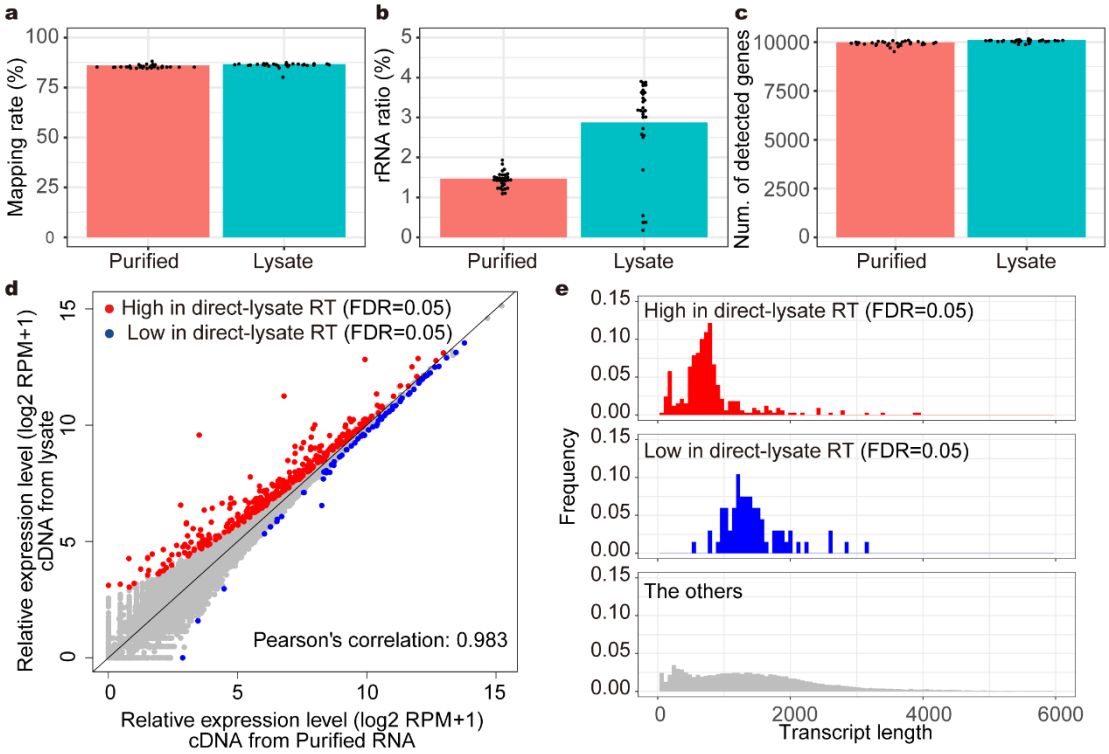
749 indicates a value of each replicate. Each horizontal line indicates the average of each

750 condition. (b-e) qPCR results for *AT3G18780* transcript (b), *Os03g0836000* transcript (c,

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

751 d) and *HP620998.1* transcript (e) in cDNA synthesized from lysate of *A. thaliana*
752 seedlings (b), *O. sativa* the youngest fully-expanded leaves (c), *O. sativa* roots (d) and *T.*
753 *aestivum* coleoptile (e). The horizontal axis indicates the concentrations of the reducing
754 regents in the homogenization buffers. The vertical axis indicates the differences in Ct
755 value compared to the RT(-) negative control. Each point indicates a value of each
756 replicate. Each horizontal line indicates the average of each condition. Same amount of
757 total RNA was used as cDNA template according to RNA concentrations in lysates (250
758 ng of *A. thaliana* and *O. sativa* or 140 ng of *T. aestivum*). (f) A schematic diagram of the
759 primer sets for cDNA detection. (g) qPCR results for *Os03g0836000* transcript in cDNA
760 synthesized from lysate of *O. sativa* leaves. In the direct-lysate reverse transcription, 0.1,
761 0.5, 1, 2.5, 5, 10 and 12.5 µL of the lysate were used. Three technical replicates were
762 prepared. The red line is a linear regression line. (253/350 words)
763

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



764

765 **Figure 3 Direct-lysate reverse transcription could generate cDNA comparable to**

766 **reverse transcription of purified RNA.**

767 The results of 3' RNA-Seq of purified RNA and lysate of *A. thaliana* seedlings. 100000

768 subsampled reads for each technical replicate were used for the following analysis. (a)

769 Boxplot of mapped read ratio to 100000 reads. (b) Boxplot of the rRNA read ratio to

770 100000 reads. (c) Boxplot of the number of detected genes. Each point indicates value of

771 each technical replicate. (d) Scatter plot of log2 RPM+1 of each gene. Average of

772 technical replicates was plotted. Differentially quantified genes (DQGs) were detected

773 between RNA-Seq for purified RNA and lysate (FDR = 0.05). (e) Transcript length

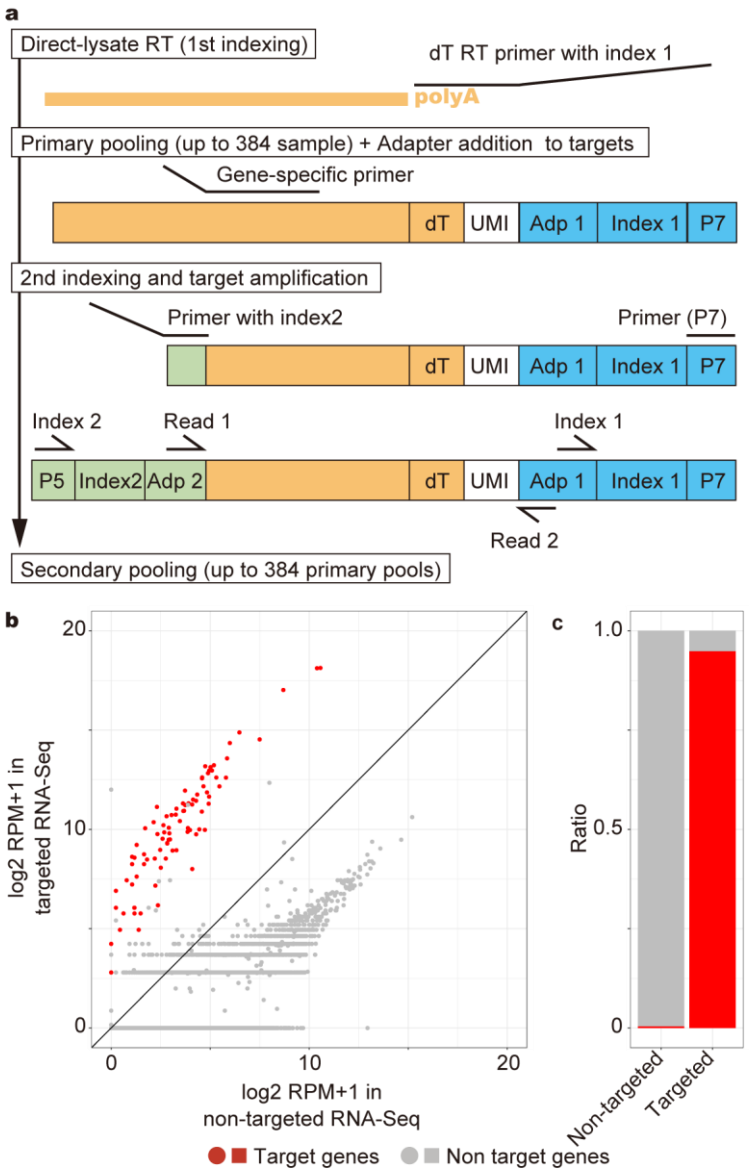
774 distribution of DQGs with higher rpm values in the method using lysate and the other

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

775 genes. The histograms from 0 nt to 6000 nt are shown. (137/350 words)

776

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



777

778 **Figure 4 DeLTa-Seq works for *A. thaliana* seedlings.**

779 (a) A schematic diagram of DeLTa-Seq library preparation. P7 and P5 are adapter

780 sequences binding with the oligonucleotides on the flow cells supplied by illumina Adp

781 1 and Adp 2 are adapter sequences for the sequencing. UMI indicates unique molecular

782 identifier. (b) Scatter plot of log2 RPM+1 values of each gene in targeted RNA-Seq and

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

783 non-targeted RNA-Seq of *A. thaliana* seedlings' lysate. (c) The bar graph represents the

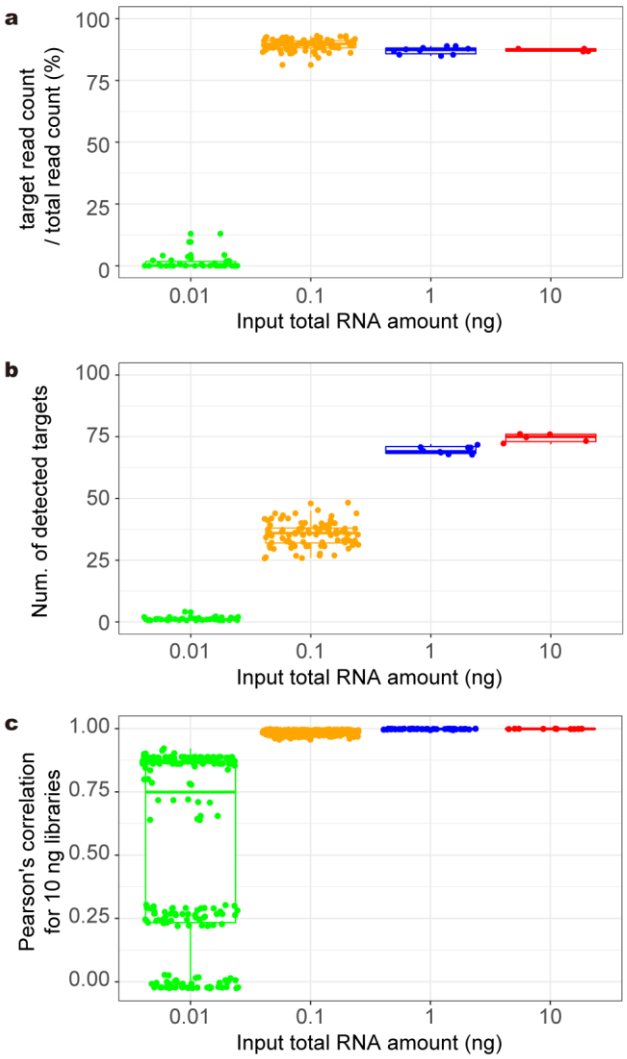
784 ratio of reads correspond to target and non-target genes in targeted RNA-Seq and non-

785 targeted RNA-Seq. (91/350 words)

786

787

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



788

789 **Figure 5 Required amount of input RNA for the targeted RNA-Seq**

790 (a-c) The result of targeted RNA-Seq using 0.01 ng (n = 96), 0.1 ng (n = 96), 1 ng (n =

791 10) and 10 ng (n = 5) of purified *A. thaliana* RNA. 10000 reads subsampled from each

792 library was used for the analyses. Boxplots of the ratio of target reads per total read (a),

793 the numbers of detected target genes (b) and Pearson's correlation coefficients to each

794 results of 10ng RNA. (c). Each point indicates value of each technical replicate. (92/350

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

795 words)

796

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

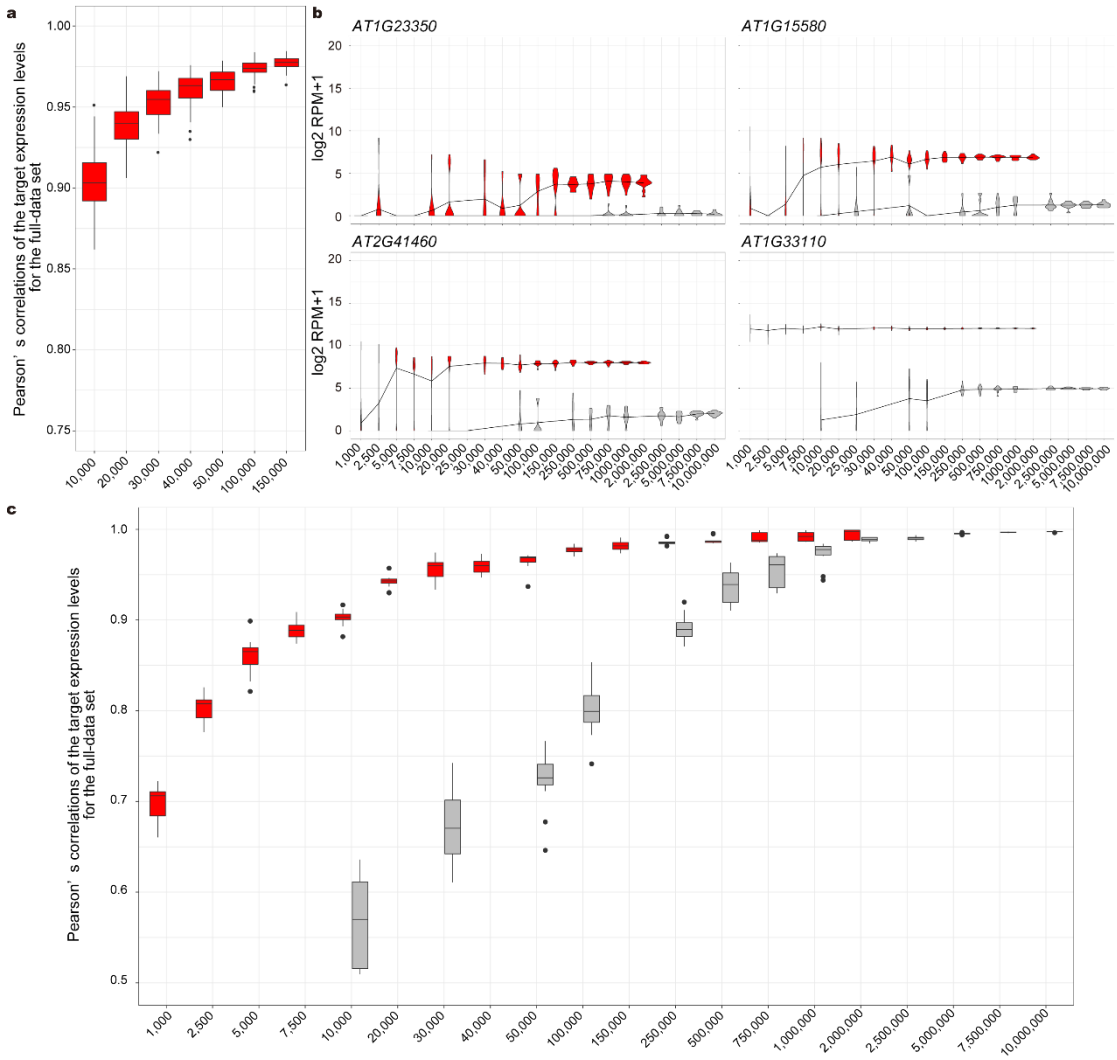


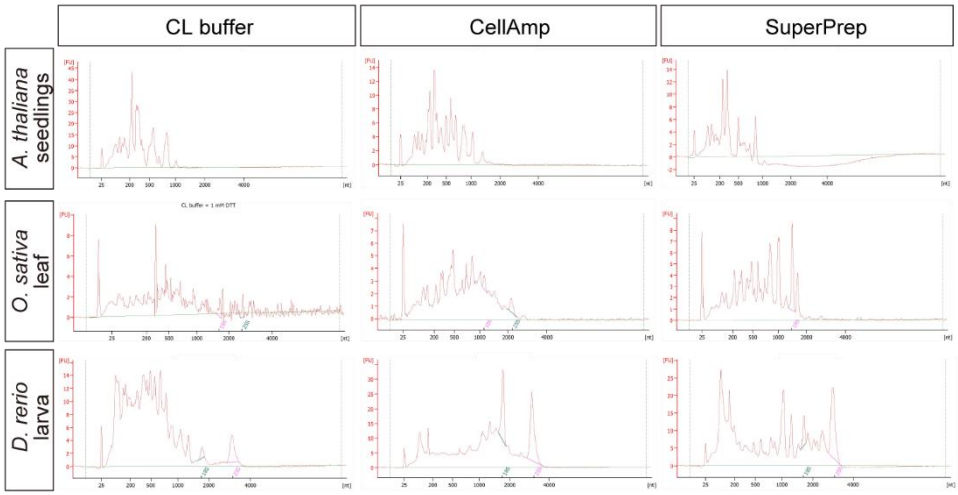
Figure 6 Reproducibility and required UMI counts for the targeted and non-targeted RNA-Seq

(a) A plot of Pearson's correlation coefficients of the target log2 RPM+1 between each subsampled 96 technical replicates and the full-dataset of targeted RNA-Seq. The replicates were prepared using single tube of purified RNA of *A. thaliana* seedlings. (b) Plots of examples of the target log2 RPM+1 in each size of subsampled sets from 12 pseudo-technical replicates of targeted and non-targeted RNA-Seq results. The same

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

805 purified RNA was used for the preparation of 96 technical replicates of non-targeted
806 RNA-Seq. Then, eight RNA-Seq results were merged, resulting in 12 pseudo-technical
807 replicates. (c) Plot of Pearson's correlation coefficients of the target log2 RPM+1 between
808 each subsampled set from the 12 pseudo-technical replicates and the full-dataset of
809 targeted and non-targeted RNA-Seq, respectively. (a-c) Red indicates targeted RNA-Seq.
810 Gray indicates non-targeted RNA-Seq. horizontal axes indicate the numbers of
811 subsampled UMI counts. (150/350 words)
812

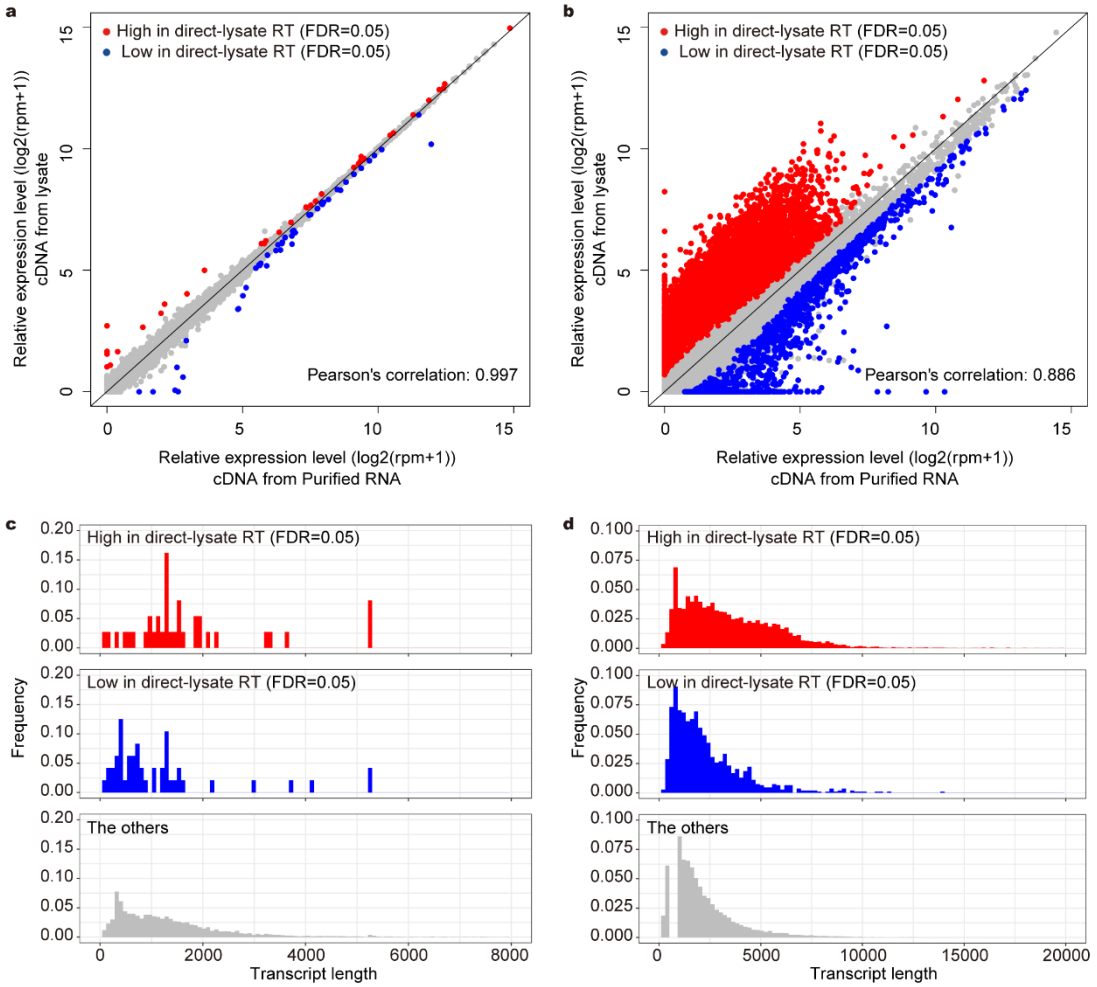
bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



Supplemental figure 1 The performance of previously reported buffers for mammalian cultured cells in direct-lysate reverse transcription of *A. thaliana*, *O. sativa* and *D. rerio*

Bioanalyzer electropherograms of purified RNA from plants' and zebrafish larvas' lysates homogenized in CL buffer and the buffers in CellAmp kit and in SuperPrep kit. (50/350 words)

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



Supplemental figure 2 Comparison of RNA-Seq results of purified RNA and lysate of *S. cerevisiae* and *D. rerio*.

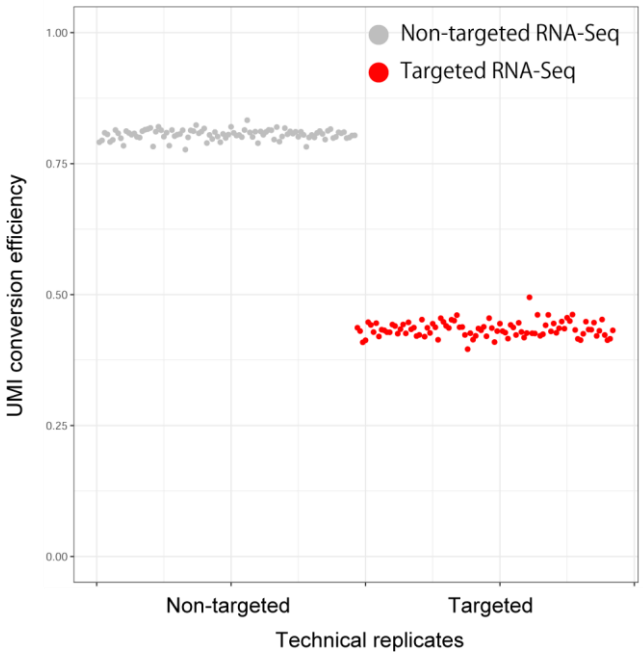
The results of 3' RNA-Seq of purified RNA and lysate of *S. cerevisiae* (a, c) and *D. rerio* (b, d) larva. (a, b) Scatter plot of log2 RPM+1 of each gene. Differentially quantified genes (DQGs) were detected between RNA-Seq for purified RNA and lysate (FDR = 0.05, n=6). (c, d) Transcript length distribution of DQGs with higher rpm values in the method using lysate and the other genes. The histograms from 0 nt to 8000 nt in (c) and from 0 nt

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

828 to 20000 nt in (d) and are shown. (108/350 words)

829

bioRxiv preprint doi: https://doi.org/10.1101/2020.09.15.299180; this version posted September 17, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



Supplemental figure 3 UMI conversion efficiencies of 96 technical replicates of non-targeted and targeted RNA-Seq.

A plot of UMI conversion efficiencies of each technical replicates of non-targeted and targeted RNA-Seq in Fig.4. Red indicates targeted RNA-Seq. Gray indicates non-targeted RNA-Seq. (40/350 words)