Wheat Inf. Serv. 124: eWIS124.2, 2017. www.shigen.nig.ac.jp/ewis

Research Information

High-quality RNA isolation from wheat immature grains

Shigeo Takumi[1]*, Moeko Okada[1], Asami Michikawa[1], Yuka Miki[1], Ryoko Ohno[2] and Kentaro Yoshida[1]

[1] Graduate School of Agricultural Science, Kobe University, Nada-ku, Kobe, Hyogo 657-8501, Japan

[2] Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe, Hyogo 657-8501, Japan

*Corresponding author: Shigeo Takumi (E-mail: takumi@kobe-u.ac.jp)

Grain quality is one of the most important targets in wheat breeding. Transcriptome analyses of wheat developing grains and endosperm have been performed using the microarray and RNA sequencing (RNA-seq) approaches (Wan et al. 2008, 2009; Nemeth et al. 2010; Pellny et al. 2012; Dong et al. 2015). For the RNA-seq analysis of the grain transcriptome and precise quantification of each transcript in developing grain and endosperm, the high-quality RNA is essential. For the microarray analysis, ≥7.3 RIN (RNA integrity number) value for the RNA sample quality is required according to the Agilent microarray protocol. In the previous report for the transcriptome of wheat developing grains, the total RNA samples with ≥8.0 RIN values were used for the RNA-seq analysis based on the PacBio and Illumina platforms (Dong et al. 2015). Some RNA extraction buffers containing SDS, CTAB, or TRIzol® reagent (Thermo Fisher Scientific, Waltham, Massachusetts) and several commercial kits for RNA isolation have been used to isolate total RNA from wheat grain and endosperm (Kawakami et al. 1992; Wan et al. 2008; Kang et al. 2013). However, total RNA samples from the wheat developing and immature grains are often damaged due to high content of polysaccharides and high stickiness of the solution homogenized with the RNA extraction buffer, and thus extraction of the high-quality RNA with high RIN value is quite difficult. Here, we report a protocol for the wheat grain RNA extraction using Maxwell RSC Plant RNA Kit (Promega, Madison, Wisconsin).

Two tetraploid wheat lines, *Triticum turgidum* ssp. *durum* cv. Langdon and *T. timopheevi* KU-107-1, a wild Einkorn wheat line, *T. boeoticum* KU-3620, and a wild tetraploid relative, *Aegilops cylindrica* KU-6953, were used in this study. The seeds were supplied by the National BioResource Project (NBRP)-Wheat, Japan (https://www.nbrp.jp), and plants were grown in a field at Kobe University (34˚43'N, 135˚13'E). Selfed seeds with 7 to 30 days after pollination (DPA) were ground in liquid nitrogen, and then the ground tissues were mixed with the 600 µL Homogenization buffer attached in the Promega kit or RNase-free phosphate-buffered saline (PBS) buffer (pH7.4, Thermo Fisher Scientific) including 20 µL/mL 1-thioglycerol. 200 µL Lysis buffer of the Promega kit was added to the 400 µL sample solution and mixed, and then the mixed solution was centrifuged at 14,000 g for 2 min. According to the protocol of the Maxwell RSC Plant RNA Kit, the samples were set to the Maxwell RSC Instrument (Promega) to start RNA purification. When the first seed sample amount was excessed, the magnetic beads were sometimes contaminated to the eluted RNA solution. After removing the magnetic beads, the RNA solution was purified using Plant Total RNA Extraction Miniprep System (VIOGEN, Taipei Hsien, Taiwan) as the occasion demands. The extracted total RNA was finally dissolved in 50 µL DEPC-treated water. The extracted RNA quality was estimated by NanoDrop 2000 (Thermo Fisher Scientific) and BioAnalyzer 2100 (Agilent Technology, Santa Clara, CA).

Excess seed samples were related to the high stickiness of the solution homogenized with the

RNA extraction buffer. Use of the PBS buffer instead of the Homogenization buffer greatly alleviated the stickiness. However, higher RIN values were obtained in the extracted RNAs using the Homogenization buffer than in those using the PBS buffer (Table 1). Presumably, use of the Homogenization buffer tended to induce the contamination of magnetic beads in the eluted RNA solution due to the high stickiness. The beads-contaminated RNAs could be more purified using the other RNA purification kit after removing the magnetic beads. Quality checking using BioAnalyzer 2100 showed that no damage in the RNA quality was observed by the additive purification step (Fig. 1). The RNA isolation protocol enables us to extract easily the high-quality RNA with ≥8.0 RIN value from developing grains of diploid and polyploid wheat and their relatives.

Reverse transcription (RT)-PCR analysis of the wheat *Cell Division Control Protein* (*CDCP*) gene, identified as the most stably expressed gene in different tissues (Paolacci et al. 2009), was performed using the RNA samples without adjustment of their concentrations, and first-strand cDNA was synthesized from DNase I-treated RNA samples with oligo-dT primers using the high fidelity ReverTra Ace reverse

Table 1. Quality check of the RNA isolated from wheat grains

Sample Name	DAP	Sample weight (mg)	Buffer*	A260/A280 ratio**	RNA conc. (ng/μL)***	RIN value***
Langdon	20	58	PBS	2.18	435.0	7.6
Langdon	20	116	PBS	2.17	708.0	7.4
Langdon	15	48	PBS	2.16	402.0	7.4
Langdon	15	96	PBS	2.19	1,543.0	7.6
KU-107-1	30	72	PBS	2.19	690.0	7.4
KU-107-1	30	144	PBS	2.17	5,991.0	7.3
KU-107-1	20	69	PBS	2.18	330.0	7.5
KU-107-1	20	138	PBS	2.17	190.8	7.0
Langdon	20	58	HB	2.10	130.6	7.8
Langdon[a]	20	116	HB	2.22	121.0	8.7
Langdon[a]	15	48	HB	2.23	76.1	8.5
Langdon[a]	15	96	HB	2.25	87.5	8.7
KU-107-1[a]	30	72	HB	2.23	103.6	8.7
KU-107-1[a]	30	144	HB	2.27	89.1	8.5
KU-107-1[a]	20	69	HB	2.22	63.1	8.9
KU-107-1[a]	20	138	HB	2.19	61.4	7.7
KU-3620	20	51	PBS	2.17	102.0	6.9
KU-3620	20	102	PBS	2.13	307.5	6.7
KU-3620	15	33	PBS	2.19	346.0	6.6
KU-3620	15	66	PBS	2.17	156.0	6.5
KU-3620	7	17	PBS	2.19	26.0	6.7
KU-3620	7	34	PBS	2.18	127.0	6.3
KU-6953	20	51	PBS	2.17	125.0	3.9
KU-6953	20	102	PBS	2.17	1,150.0	6.8
KU-3620	20	51	HB	1.99	406.0	9.9
KU-3620[a]	20	102	HB	2.19	79.8	8.8
KU-3620	15	33	HB	2.16	259.0	9.8
KU-3620	15	66	HB	2.06	432.0	9.7
KU-3620	7	17	HB	2.17	262.0	9.4
KU-3620	7	34	HB	2.18	302.0	9.4
KU-6953	20	51	HB	2.02	100.0	9.4
KU-6953[a]	20	102	HB	2.15	47.2	9.2

*PBS, 1x PBS buffer; HB, Homogenization buffer in the Promega kit

**Estimated by NanoDrop 2000

***Estimated by BioAnalyzer 2100

[a]The total RNA was purified with the VIOGEN kit after removing the magnet beads.

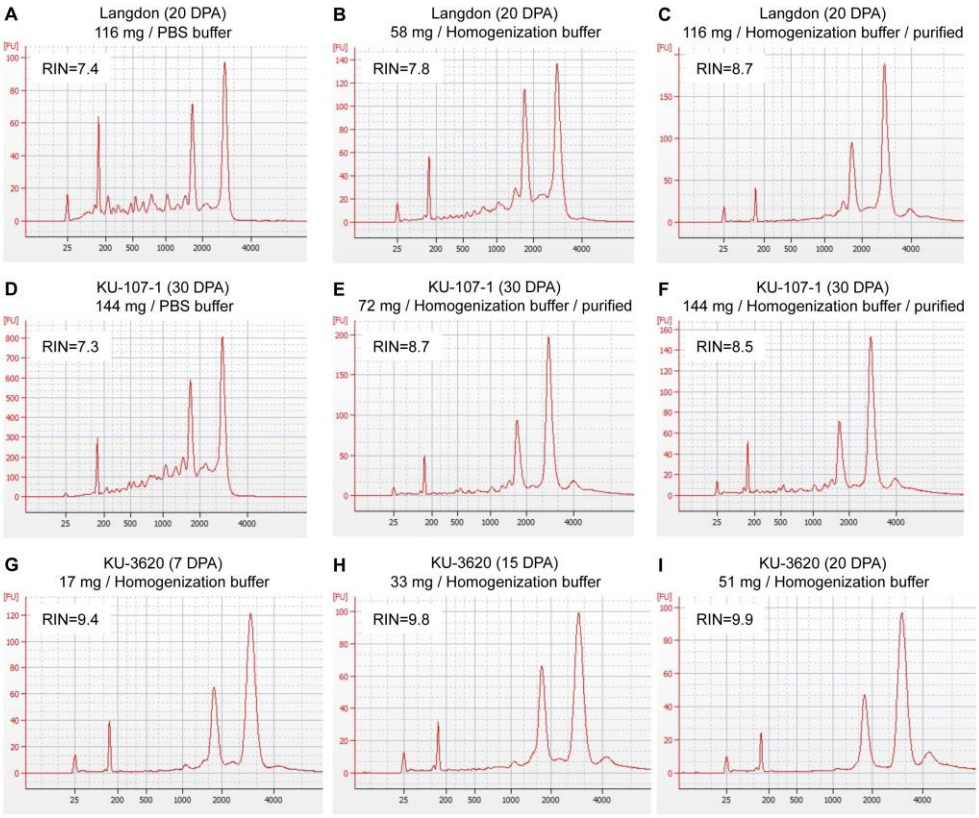

Figure 1. Quality check data of the isolated RNA samples by BioAnalyzer 2100.

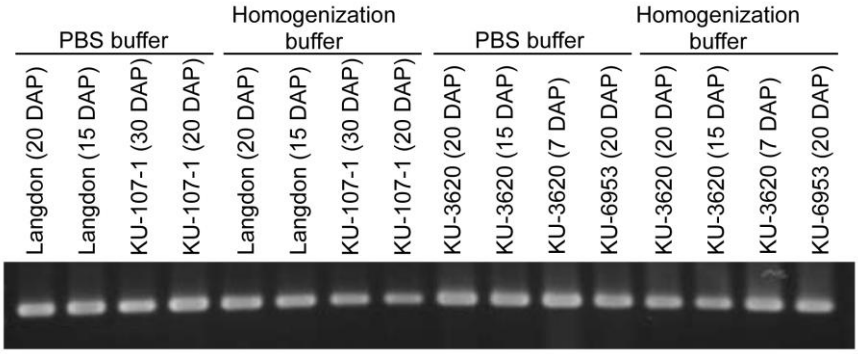

Figure 2. RT-PCR analysis of wheat *CDCP* gene expression using the RNA samples from grains.

transcriptase (Toyobo, Osaka, Japan). The gene-specific primer set for *CDCP*, which has been used as an internal control for wheat quantitative RT-PCR analyses (Paolacci et al. 2009; Rikiishi and Maekawa 2014; Iehisa and Takumi 2017), was 5'-CAAATACGCCATCAGGGAGAACATC-3' and 5'-CGCTGCCGAAACCACGAGAC-3'. The PCR condition was 1 cycle of 94°C for 2 min, 30 cycles of 94°C for 20 s, 58°C for 30 s, and 68°C for 45 s, and then 1 cycle of 68°C for 1 min. The RT-PCR products were separated by electrophoresis through a 1.5% agarose gel and stained with ethidium bromide. The RT-PCR fragments for *CDCP* were clearly amplified in all of the RNA samples (Fig. 2). The high-quality RNA isolated from immature grains using the protocol conducted here can be applied to any transcriptome analysis and precise quantification of each transcript.

Acknowledgments
This work was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Scientific Research (B) No. 16H04862).

References

Dong L, Liu H, Zhang J, Yang S, Kong G, Chu JSC, Chen N, Wang D (2015) Single-molecule real-time transcript sequencing facilitates common wheat genome annotation and grain transcriptome research. BMC Genomics 16: 1039.

Iehisa JCM, Takumi S (2017) Identification and evaluation of wheat reference genes for normalization of quantitative RT-PCR data during dehydration conditions. Wheat Inform Serv 124: eWIS124.1.

Kang GZ, Xu W, Liu GQ, Guo TC (2013) Comprehensive analysis of the transcription of starch synthesis genes and the transcription factor RSR1 in wheat (*Triticum aestivum*). Genome 56: 115-122.

Kawakami N, Miyake Y, Noda K (1992) Differential changes in levels of mRNAs during maturation of wheat seeds that are susceptible and resistant to preharvest-sprouting. Plant Cell Physiol 33: 511-517.

Nemeth C, Freeman J, Jones HD, Sparks C, Pellny TK, Wilkinson MD, Dunwell J, Andersson AAM, Aman P, Guillon F, Saulnier L, Mitchell RAC, Shewry PR (2010) Down-regulation of the *CSLF6* gene results in decreased (1,3;1,4)-ß-D-glucan in endosperm of wheat. Plant Physiol 152: 1209-1218.

Paolacci AR, Tanzarella OA, Porceddu E, Ciaffi M (2009) Identification and validation of reference genes for quantitative RT-PCR normalization in wheat. BMC Mol Biol 10: 11.

Pellny TK, Lovegrove A, Freeman J, Tosi P, Love CG, Paul Knox J, Shewry PR, Mitchell AC (2012) Cell walls of developing wheat starchy endosperm: comparison of composition and RNA-seq transcriptome. Plant Physiol 158: 612-627.

Rikiishi K, Maekawa M (2014) Seed maturation regulators are related to the control of seed dormancy in wheat (*Triticum aestivum* L.). PLoS ONE 9: e107618.

Wan Y, Poole RL, Huttly AK, Toscano-Underwood C, Feeney K, Welham S, Gooding MJ, Mills C, Edwards KJ, Shewry PR, Mitchell RAC (2008) Transcriptome analysis of grain development in hexaploid wheat. BMC Genomics 9: 121.

Wan Y, Underwood C, Toole G, Skeggs P, Zhu T, Leverington M, Griffiths S, Wheeler T, Gooding M, Poole RL, Edwards KJ, Gezan S, Welham S, Snape J, Clare Mills EN, Mitchell RAC, Shewry PR (2009) A novel transcriptomic approach to identify candidate genes for grain quality traits in wheat. Plant Biotechnol J 7: 401-410.