

Transcription Analyses

DOI

<https://dx.doi.org/10.17504/protocols.io.75uhq6w>

Fu-Hao Lu¹, Neil McKenzie¹, Laura-Jayne Gardiner², Ming-Cheng Luo³, Anthony Hall², Michael W Bevan¹

¹Department Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK;

²Earlham Institute, Norwich Research Park, Norwich, NR4 7UZ, UK;

³Department of Plant Sciences, University of California, One Shields Avenue, Davis, CA 95616, USA



Fuhao Lu

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account



DOI: <https://dx.doi.org/10.17504/protocols.io.75uhq6w>

Protocol Citation: Fu-Hao Lu, Neil McKenzie, Laura-Jayne Gardiner, Ming-Cheng Luo, Anthony Hall, Michael W Bevan .
Transcription Analyses. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.75uhq6w>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Created: October 12, 2019

Last Modified: October 12, 2019

Protocol Integer ID: 28564

Keywords: Paragon; AL8/78, RNAseq, rnaseq data of wheat, rnaseq data, transcription analysis, transcription analyses this protocol, wheat

Abstract

This protocol describes how we preprocessed the RNAseq data of wheat and *Aegilops tauschii*.

Materials

Triticum aestivum Paragon

Aegilops tauschii AL8/78

RNA extraction

- 1 RNA samples from wheat and *Ae. tauschii* lines AL8/78, Clae23 and ENT336 were extracted as described by Oñate-Sánchez and Vicente-Carbajosa
- 2 RNA quality was assessed using a Qubit fluorometer (Invitrogen) and 2100 Bioanalyzer (Agilent Technology) in the Earlham Institute (Norwich, UK).

RNAseq

- 3 Illumina TruSeq mRNA libraries were constructed using the Illumina TruSeq RNA Sample preparation guide v2 (Illumina Inc.) in accordance with the manufacturer's protocol.
 - 3.1 One µg of total RNA used to purified mRNA using two rounds of poly-T oligonucleotide purification attached magnetic beads. During the second elution of poly-A RNA, the RNA was fragmented and primed for cDNA synthesis.
 - 3.2 cDNA synthesis was carried out using SuperScript II Reverse Transcriptase (Invitrogen) and random primers. Second strand cDNA synthesis was carried out and the DNA was subjected to end repair, "A" tailing and ligation.
 - 3.3 cDNA templates were enriched by 15 cycles of PCR as per manufacturer's instructions. The amplified library was quantified using a Bioanalyzer DNA 100 Chip.
 - 3.4 The library was normalised to 10 nM for generation of sequence clusters on a sequencing flow-cell on the Illumina c-Bot instrument.
 - 3.5 Sequencing library cluster generation was carried out on a paired-end flow cell on the Illumina cBot according to the manufacturer's instructions.
 - 3.6 All sequencing was carried out on an Illumina HiSeq 2500, with 100 bp paired-end read metric, TruSeq SBS V3 Sequencing kit and version 1.12.4.2 RTA.
 - 3.7 FASTQ files were generated and demultiplexed according to library-specific indices by CASAVA (v. 1.8.2, Illumina)

Quality Control

- 4 Adaptor sequences were trimmed using CutAdapt v 1.6 and low quality sequences were removed by Trimmomatic v0.30 with parameter ILLUMINACLIP:2:30:10 HEADCROP:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36

Read mapping

- 5 To estimate gene expression differences between Paragon and *Ae. tauschii*, the HISAT2-StringTie pipeline was used to compute Transcripts Per Million (TPM) values.
- 5.1 HISAT2 (v2.1.0) was used to create genome indices and map the trimmed RNAseq reads to each genome.
- 5.2 Transcripts were mapped to the Triticum3.1 wheat assembly with the 2,703 PacBio-based 3DL scaffolds replaced by the 3DL pseudomolecule, and to the *Ae. tauschii* AL8/78 assembly.
- 6 A two-step StringTie (v1.3.3b) [\[30\]](#) strategy was used to measure transcript abundance. For each RNA-Seq sample,
 - 6.1 StringTie was used to assemble the read alignments;
 - 6.2 A non-redundant set of transcripts observed in all the RNA-Seq samples assembled previously was generated using the stringtie --merge mode;
 - 6.3 finally the second run was performed with the merged transcript models, for each RNA-Seq sample, using the -B/-b and -e options in order to estimate transcript abundances and generate read coverage tables.