Supplementary material 1

RNA sequencing protocol

RNA extraction. TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) was directly added to the cells (1 ml/1x10^6 cells), followed by vortex mixing at 3,000 rpm for 15 sec and standing for 5 min. The mixture was centrifuged at 4˚C at 12,000 x g for 5 min and the supernatant was transferred to a new EP tube for extraction. A total of 300 μl chloroform/isoamyl alcohol (24:1) was added and the mixture was vigorously vortexed at 3,000 rpm for 15 sec to ensure thorough mixing. The mixture was centrifuged at 4˚C and 12,000 x g for 8 min. The clear upper aqueous layer was transferred to a new tube. To purify the sample, another 300 μl of chloroform/isoamyl alcohol (24:1) was added, followed by vortexing at 3,000 rpm for 15 sec and centrifugation at 4˚C and 12,000 x g for 8 min. The upper aqueous layer was collected. Following centrifugation at 12,000 x g for 15 min at 4˚C, the upper aqueous layer was transferred to a new 1.5 ml EP tube with a 2/3 supernatant volume of isopropanol, inverted to mix and placed in a -20˚C refrigerator for 2 h. The precipitation mixture was centrifuged at 17,500 x g at 4˚C for 25 min. The supernatant was discarded, and the precipitation was washed with 0.9 ml 75% ethanol. The precipitation was suspended by inverting the tube and collected by centrifuging at 17,500 x g for 3 min at 4˚C. Supernatant was discarded. Centrifugation at 12,000 x g for 15 min at 4˚C was performed again and the precipitation was dried for 3-5 min. Finally, 20-200 μl DEPC-treated or RNase-free water was added to dissolve the RNA. Total RNA was qualified and quantified using a Fragment Analyzer or Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) or Qseq-400 (BiOptic, Inc.).

Library preparation. Library preparation was performed using Optimal Dual-mode mRNA Library Prep kit (cat. no. LR00R96; BGI Group). A total of 1 μg of RNA was denatured at 65˚C for 5 min to open the secondary structure and mRNA was enriched by oligo (dT) attached magnetic beads. After reacting at 94˚C for 8 min, RNAs were fragmented with fragmentation reagents. First-strand cDNA was generated using random hexamer-primed reverse transcription, followed by second-strand cDNA synthesis. The double-stranded cDNA underwent end repairment. A single A nucleotide was added to the 3' ends of the blunt fragments through A tailing reaction. Sequencing adaptors were ligated to the cDNA fragments. The resulting library products were amplified through PCR using the library preparation kit. The thermocycling conditions were as follows: initial denaturation at 95˚C for 3 min; 12 cycles of denaturation at 98˚C for 20 sec, annealing at 60˚C for 15 sec, and extension at 72˚C for 30 sec; followed by a final extension at 72˚C for 10 min.

Next, the single-stranded library products were produced via denaturation. Circularization reagents provided in the library preparation kit were used to obtain the single-stranded cyclized DNA products. Any uncyclized single stranded linear DNA molecules were digested using the digestion enzymes provided in the kit at 37˚C for 30 min. The concentration of the final single-stranded circularized library was measured using a Qubit ssDNA Assay Kit (Thermo Fisher Scientific, Inc.). Subsequently, 60 fmol circularized library was amplified using Phi29 DNA polymerase at 30˚C for 20 min to make DNA nanoballs (DNBs), which carry >300 copies of the initial single stranded circularized library molecule. The DNBs were loaded into the patterned nanoarray and sequenced using the DNBSEQ-T10x4RS High-throughput Sequencing Set (FCL PE150; cat. no. 940-000100-00; MGI Tech Co., Ltd.). Prior to loading, the final library concentration was measured using a Qubit 4.0 Fluorometer (Thermo Fisher Scientific, Inc.). Paired end 100/150 base pair reads were generated on G400/T7/T10 platform (BGI Group).

Read filter and quality control. The filtering steps were as follows: Raw sequencing reads were filtered and subjected to quality control using SOAPnuke software (version 2.2.6; github.com/BGI-flexlab/SOAPnuke). The filtering process was performed using the specific parameters of -n 0.001 -l 10 -q 0.5 -Q 2. Specifically, the filtering steps were as follows: i) reads exhibiting >50% overlap with adapter sequences were removed; ii) reads containing an excessive number of unknown bases ('N' ratio >0.1%) were discarded; and iii) low-quality reads were excluded if the proportion of low-quality bases (quality score <10) exceeded 50% of the entire read length. The resulting high-quality clean reads were subsequently used for all downstream analyses.

Gene differential expression analysis. Expression of genes was calculated by RNA-Seq by Expectation-Maximization (v1.3.1; github.com/deweylab/RSEM). The heatmap was drawn by pheatmap (v1.0.12; cran.r-project.org/web/packages/pheatmap/) according to the gene expression difference. Differential expression analysis was performed using the DESeq2 (v1.34.0; https://bioconductor.org/packages/DESeq2/), DEGseq (v1.48.0; https://bioconductor.org/packages/DEGseq/) or Poisson distribution method with Q-value ≤0.05 or false discovery rate ≤0.001.