File S5

Supplemental Materials and Methods

Detailed protocol for sequencing library construction

1) 400ng of amplified mRNA was fragmented to 10-200nt using 10x RNA fragmentation buffer (Ambion) and was purified using regular ethanol precipitation method with 0.35µl of GlycoBlue (Ambion). 2) 3' end the RNA samples were dephosphorylated using 10x Antarctic Phosphatase Buffer and 0.5 µl Antarctic Phosphatase (NEB) at 37 °C for 20 minutes. The reaction was heat inactivated at 75°C for 10 minutes. 3) 5' end of RNA samples was phosphorylated using 10x T4 DNA ligase buffer (it has 1mM ATP final) and T4 PNK (NEB) at 37 °C for 30 minutes. The RNAs in the reactions were purified using ammonium acetate and ethanol precipitation with 2µl of GlycoBlue (Ambion). 4) The RNA samples are then ligated to 3' linker (5'-/5rApp/CTG TAG GCA CCA TCA AT/3ddC/-3') (synthesized by IDT) using T4 RNA ligase 1 (NEB), 5X ATP-free T4 RNA ligase buffer (16.5 mM DTT, 41.5% glycerol, 250 mM HEPES-KOH, pH8.3, 50 mM $MgCl_2$, 50 µg/ml acetylated BSA), and 10% DMSO at 37 °C for one hour. The RNAs in the reactions were purified using ammonium acetate and ethanol precipitation with 2µl of GlycoBlue (Ambion). The RNA samples were then run on 6% TBE-Urea PAGE Gel (Invitrogen). The 100-200nt bands were cut and elute overnight with 400µl stop solution (1M ammonium acetate and 10mM EDTA) at 4°C overnight. The RNAs in the supernatant was purified using regular ethanol precipitation method with 2µl of GlycoBlue (Ambion). 5) The RNA samples are ligated to 5' linker (with bar code) using T4 RNA ligase 1 (NEB), 10x T4 RNA ligase 1 buffer (NEB), and 10% DMSO at 37°C for 1 hour. The RNAs was purified by ammonium acetate and ethanol precipitation and gel purification as described in step 4. The 5' barcoded linkers are synthesized by IDT. IPC1: 5'-/5AmMC6/ ACG CTC TTC CGA TCT rCrUrGrG-3', IPC2: 5'-/5AmMC6/ ACG CTC TTC CGA TCT rCrGrUrC-3', Control 1: 5'-/5AmMC6/ ACG CTC TTC CGA TCT rArCrUrU-3', Control 2: 5'-/5AmMC6/ ACG CTC TTC CGA TCT rCrCrCrU-3'. 6) cDNA of the RNA samples were reverse transcribed using SuperScript III (Invitrogen) following manufacture's protocol. The primer sequence used for reverse transcription is 5'-ATT GAT GGT GCC TAC AG-3'. 7) The cDNA samples were amplified using Taq (NEB) following manufacture's protocol. Forward primer: 5'-GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T-3'. Reverse primer: 5'-CAA GCA GAA GAC GGC ATA CGA GCT CTT CCG ATC TAT TGA TGG TGC CTA CAG-3'. The PCR products (200-300nt) were purified using Qiagen PCR purification kit. The purified PCR samples were diluted to 10nM and were sequenced using Illumina GA II sequencing system.