bio-protocol

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Published: May 20, 2024

Update Notice: TGIRT-seq Protocol for the Comprehensive Profiling of Coding and Non-coding RNA Biotypes in Cellular, Extracellular Vesicle, and Plasma RNAs

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After official publication in Bio-protocol (https://bio-protocol.org/e4239), we noticed that the R1 sequencing adapter in this protocol, which was derived from the NEBNext small RNA library preparation kit, is not compatible with the i5 sequencing primer used for demultiplexing reads by unique dual indices (UDIs) in newer versions of Illumina sequencing kits, particularly for sequencing on the Illumina NovaSeq platform. The adapter sequences described in the published protocol still allow demultiplexing by using the i7 index and overall read quality is unaffected. We are adding this note to update the R1 adapter sequence for use with the Illumina NovaSeq platform, enabling UDI demultiplexing for high-depth sequencing on this instrument.

The following information was replaced in the updated published *Bio-Protocol* article:

For applications requiring Illumina Unique Dual Indices (UDI), the protocol for steps c, d, and e should be as follows:
c. R1R DNA 5'/5Phos/AGA TCG GAA GAG CGT CGT GTA GGG AAA GAG TGT/3SpC3/3'
Note: The Read 1 (R1) sequence corresponds to the adapter sequences used in the Illumina TruSeq RNA Library Prep Kit v2 for Illumina sequencing.
d. 6N unique molecular identifier (UMI) R1R DNA
5'/5Phos/NNN NNN AGA TCG GAA GAG CGT CGT GTA GGG AAA GAG TGT/3SpC3/3'
Note: UMI nucleotides (machine-mixed equimolar A, C, G, and T residues, denoted N) are added at the 5' end of the R1R sequence. The number of N nucleotides can be changed to suit the complexity of the samples being sequenced and the number of duplicates expected after PCR.
e. Illumina barcode PCR primer (P5)
5'-AAT GAT ACG GCG ACC ACC GAG ATC TAC AC BARCODE ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT
Note: All the sequences in (a-e) should be updated to correspond to any changes in Illumina sequencing kits in the future.

Cite as: Xu, H. et al. (2024). Update Notice: TGIRT-seq Protocol for the Comprehensive Profiling of Coding and Non-coding RNA Biotypes in Cellular, Extracellular Vesicle, and Plasma RNAs. Bio-protocol 14(10): e4999. DOI: 10.21769/BioProtoc.4999.