GeneLab Standard Operating Procedure:

Use of ERCC spike-in mixes and UMRR/UHRR controls for Total RNA-Sequencing

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Document Revisions

Document Number	Revision Number	Date	Description of Changes
GL-SOP-5.2	1.1	03_30_2020	Original document

Scope and Purpose

As described in detail on GeneLab.nasa.gov webpage titled “GeneLab Sequencing Standards and Services” we encourage the researchers to use two levels of control in the Total RNA sequencing workflow. This SOP describes the suggested protocol.

Reagents

1. Universal Mouse Reference RNA (Agilent, Cat#740100)
2. Universal Human Reference RNA (Agilent, Cat#740000)
3. ERCC ExFold RNA Spike-In Mixes (Thermo Fisher Scientific, Cat#4456739)
4. UltraPure DNase/RNase-free Distilled Water (Thermo Fisher Scientific, Cat#10977023 OR similar)

Procedure

ERCC Spike-In Use

1. For long term storage, keep unopen, non aliquoted ERCC kits in -80C storage.
2. ERCC Spike In reagent prep:
    a. On the day of the experiment, thaw one kit (ERCC Spike-In Mix 1 and ERCC Spike-In Mix 2) on ice.
    b. Make ten 1uL aliquots of ERCC Spike-In Mix 1.
    c. Make ten 1uL aliquots of ERCC Spike-In Mix 2.
    d. Store aliquots in -20C until use.
3. Retrieve one tube of Mix 1 and one tube of Mix 2.
4. Dilute each mix 1:100 by adding 99uL of DNase/RNase-free distilled water into the ERCC mix tube, briefly vortex and spin down the tube.
5. Keep 1:100 ERCC Spike In mixes on ice until use.
6. On the day of the library preparation follow one of the following options:
    a. Normalize an RNA aliquot to 1.5ug of RNA in 17uL of RNase/DNase free water.
    b. If working with Pre-normalized RNA aliquots, take it out of the storage and fully thaw before adding the ERCC mixes. Quantify RNA using Qubit BR RNA reagent to make sure the concentration range is acceptable.

Note: Follow the ERCC RNA Spike-In User Guide if using a different RNA concentration/Spike in dilution.

Table 4 Guidelines for adding Spike-In Mixes to sample RNA

Amount of sample RNA	Volume of Spike-In Mix 1 or Mix 2 (dilution)†	
	Total RNA	Poly(A) RNA
20 ng	4 µL (1:10000)	2 µL (1:100)
50 ng	1 µL (1:1000)	5 µL (1:100)
100 ng	2 µL (1:1000)	1 µL (1:10)
500 ng	1 µL (1:100)	5 µL (1:10)
1000 ng	2 µL (1:100)	–
5000 ng	1 µL (1:10)	–

† ERCC RNA Spike-In Mix 1, ExFold Spike-In Mix 1, or ExFold Spike-In Mix 2.

7. Add 3uL of either Mix 1 or Mix 2 at 1:100 dilution into all of the thawed RNA aliquots of 1.5ug.
8. Mix by vortexing.
9. Centrifuge the RNA+Spike-in tubes.
10. Record the Lot number of the spike in used.
11. Record what Mix was added to each sample, mix dilution and volume added.
12. Proceed with library prep procedure as described in GL-SOP-5.1.

UMRR/UHRR Use

13. Add a minimum of 3 UMRR/UHRR samples to each library prep run.
14. If using automation, maintain the same location on the plate (A1, D6, H4 for example) if several plated are required to prep all libraries for the pool.
15. Make aliquots of UMRR/UHRR following the User Guide and store them at -80C until use
16. On the day of the library preparation, thaw sufficient amount of reference RNA.
17. Add ERCC Spike-in mix into UMRR/UHRR aliquots, maintain the same Spike-RNA ratio as used for test RNA samples.
18. Proceed with library prep procedure along with test RNA samples as described in GL-SOP-5.1.