

Alternative RNA-Seq Sample Submission Workflows

Sample Submission for mRNA-Seq

Samples may be prepared and submitted for mRNA-seq by using one of the following alternative workflows:

- A. Investigator performs all quality assessments and quantifications: \$0.00
- B. Investigator performs quantification, Genomics Division performs quality assessments: \$9.00/sample
- C. Genomics Division performs the quality and quantity assessments: \$15.00/sample

I. mRNA-Seq Sample Submission

- A. Investigator performs all quality and assessments and quantification workflow:
 - 1. DNase I treatment and removal is required
 - 2. Measure the samples on a spectrophotometer (Nanodrop) to determine concentrations (ng/ul) and purity (260/280, 260/230). The 260/280 values should be ~1.8-2.0 and the 260/230 values should be above 2.0 (or at the very least above 1.0). If the concentrations are above 100ng/ul, dilute at least 1ug to around 60 ng/ul for measuring on the Qubit.
 - 3. Perform Bioanalysis to assess RNA quality. RIN should be greater than 8.0 to continue with this workflow.
 - 4. Measure samples (under 100 ng/ul based on Nanodrop and/or Bioanalyzer) on the Qubit with the RNA High Sensitivity kit. Use these results to aliquot 600 ng, then add water to a total volume of 60 ul.
 - 5. Prior to submitting the samples please provide all the QA/QC results that includes:
 - a. Bioanalyzer results
 - b. Excel file that includes: Nanodrop (ng/ul, 260/280, 260/230, Qubit HS (ng/ul); The volume that was aliquoted and the volume of water that was added).
 - 6. **Submit 600 ng Total RNA** as measured with a Qubit RNA HS kit diluted in 60 uL nuclease free water. If you are able please double this amount (1.2 ug in 120 ul). Samples should be submitted in 1.5 ml low-bind tubes labelled with simple numbers.
- B. Investigator performs quantifications, division performs the quality assessments workflow:
 - 1. DNase I treatment and removal is required
 - 2. Measure the samples on a spectrophotometer (Nanodrop) to determine sample concentration (ng/ul).
 - 3. Submit 7 ul of 100-400 ng/ul for quality assessment in a 0.5ml tube.
 - 4. Once you receive the quality assessment, use the Trinean concentration OR measure on the Qubit with the RNA High Sensitivity kit. Use these results to create an aliquot containing 600 ng in a total volume of 60 ul in nuclease-free water. If you are able please double this amount (1.2 ug in 120 ul). Samples should be submitted in 1.5 ml low-bind tubes labelled with simple numbers

5. Prior to submitting the samples please provide all the QA/QC results that includes:
 - a. Bioanalyzer results
 - b. Qubit HS (ng/ul)
6. **Submit 600 ng Total RNA** as measured with a Qubit RNA HS kit diluted in 60 uL nuclease free water. If you are able please double this amount (1.2 ug in 120 ul). Samples should be submitted in 1.5 ml low-bind tubes labelled with simple numbers.

II. Total RNA-Seq Sample Submission

- A. Investigator performs all quality and assessments and quantification workflow:
 1. DNase I treatment and removal is required
 2. Measure the samples on a spectrophotometer (Nanodrop) to determine concentrations (ng/ul) and purity (260/280, 260/230). The 260/280 values should be ~1.8-2.0 and the 260/230 values should be above 2.0 (or at the very least above 1.0). If the concentrations are above 100ng/ul, dilute at least 1ug to around 60 ng/ul for measuring on the Qubit.
 3. Perform Bioanalysis to assess RNA quality. RIN should ideally be greater than 6.0. *If not, please contact us.*
 4. Measure samples (under 100 ng/ul based on Nanodrop and/or Bioanalyzer) on the Qubit with the RNA High Sensitivity kit. Use these results to aliquot 600 ng, then add water to a total volume of 12 ul.
 5. Prior to submitting the samples please provide all the QA/QC results that includes:
 - a. Bioanalyzer results
 - b. Excel file that includes: Nanodrop (ng/ul, 260/280, 260/230); Qubit HS (ng/ul); The volume that was aliquoted and the volume of water that was added).
 6. **Submit 600 ng Total RNA** as measured with a Qubit RNA HS kit diluted in 12 ul nuclease free water. If you are able please double this amount (1.2 ug in 24 ul). Samples should be submitted in 1.5 ml low-bind tubes labelled with simple numbers.
- B. Investigator performs quantifications, core performs the quality assessments workflow:
 1. **DNase I treatment and removal is required**
 2. Measure the samples on a spectrophotometer (Nanodrop) to determine sample concentration (ng/ul).
 3. Submit 7 ul of 100-400 ng/ul for quality assessment in 0.5 ml microfuge tube.
 4. Once you receive the quality assessment, use the Trinean concentration OR measure samples (under 100 ng/ul based on Nanodrop) on the Qubit with the RNA High Sensitivity kit. Use these results to aliquot 600 ng, then add water to a total volume of 12 ul.
 - a. Prior to submitting the samples please provide all the QA/QC results in an Excel file that includes: Nanodrop (ng/ul, 260/280, 260/230); Qubit HS (ng/ul) or Trinean (ng/ul);
The volume that was aliquoted and the volume of water that was added.
 5. **Submit 600 ng Total RNA** as measured with a Qubit RNA HS kit diluted in 12 ul nuclease free water. If you are able please double this amount (1.2 ug in 24 ul). Samples should be submitted in 1.5ml low-bind tubes labelled with simple numbers.