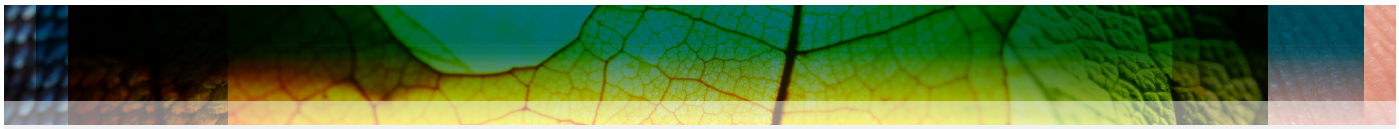


RNA Sample Reference Card

Preparing Samples for RNA-sequencing

For most RNA-seq experiments, please submit total RNA extracted from your organism of interest. Cofactor is able to provide RNA extraction services from certain sample types, contact your Project Scientist to find out if this service is available for your samples. For certain library types we may be able to accept cDNA, contact us for more details on submission and QC requirements for these samples.

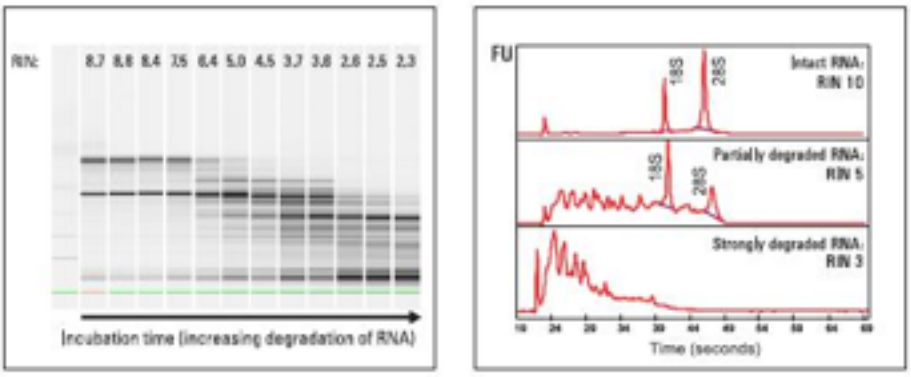
The key to a successful RNA-seq experiment is starting with a sufficient quantity of high quality material. For most RNA extractions, we recommend the mirVana extraction kit from Ambion or the RNeasy kit from Qiagen, however, you may use your own lab protocols or equivalent kits if they have yielded high quality RNA in the past. Small RNA sequencing also requires total RNA as starting material, however, the extractions must be modified to allow retention of small RNAs.
All RNA extractions must also be accompanied by a DNase treatment. Qiagen protocols allow this step to be performed on the column. For guidance with DNase treatment using other kits and protocols please contact us at sample@cofactorgenomics.com. Finally, all RNA samples must be resuspended in nuclease-free water and shipped frozen on dry ice. For international shipments we can receive RNA precipitated in ethanol or other buffers to allow for increased stability over longer shipping times. This option can be pre-arranged by contacting us at sample@cofactorgenomics.com.

Assessing Sample Quantity and Quality

Please measure RNA concentration with a fluorescence based measurement such as a Qubit or Picogreen assay. These measurements tend to be specific for RNA and are preferable to nanodrop or other UV based measurements which also measure contaminating genomic DNA. If your lab does not have a way of making a fluorescence based measurement, please use whatever method is available and indicate this information when submitting your sample. For nanodrop measurements, samples must fulfill the requirements below:

UV absorption: 260/280 >1.9 and 260/230 >2.0

RNA sample quality is best assessed on an Agilent Bioanalyzer or equivalent instrument (see sample traces below). Alternatively, if a Bioanalyzer is not available, please run the RNA samples on a denaturing agarose gel to confirm the integrity of rRNA peaks. The Bioanalyzer will produce an RNA Integrity Number (RIN) which is an objective measure of RNA quality.



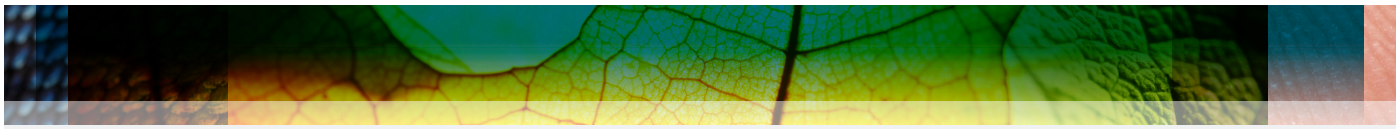
RNA analysis results obtained with the Agilent 2100 Bioanalyzer and the Agilent 2200 TapeStation are displayed in form of a gel image, electropherograms and in a tabular format. RNA quality assessment is based on the RNA Integrity Number (RIN). Image source: Agilent Technologies.

RIN scores vary from 1-10 with 10 being the highest quality samples showing the least degradation. RINs should be >7. For some sample types such as RNA from insects, the Bioanalyzer RIN may not be a reliable measure of RNA quality if the organism does not show conventional eukaryotic or prokaryotic ribosomal RNA peaks. Please contact us at sample@cofactorgenomics.com for assistance in assessing the quality of these samples. FFPE samples are another exception to the sample quality requirement since the RNA from these is heavily fragmented. Please contact us for FFPE RNA-seq input submission requirements prior to initiating your project.

How much RNA do we need for sequencing?

The following are minimum starting RNA requirements for library construction and QC. You are always encouraged to submit more than the recommended amount.

Sequencing Platform	Library type	Minimum RNA amount
Illumina	Whole Transcriptome/ rRNA depleted	>1 ug
Illumina	polyA / polyA +DSN	>1 ug
Illumina	DSN	>200 ng
Illumina	Clontech SMARTer RNA amplification	>100 pg
Illumina	NuGen Ovation RNA-seq V2 amplification	>1 ng
Illumina	small RNA	>1 ug



RNA Sample Reference Card

Sample Submission Guidelines

At the time of sample submission, please provide the genomic or transcriptomic reference DNA sequence to which your sequence data will be aligned (Reference sequences are not needed for No Analysis projects). Reference sequences can be submitted by email to sample@cofactorgenomics.com as a FASTA file or a URL where the FASTA can be downloaded.

Please use the following checklist for sample submission.

If the sample type is not purified RNA, please contact us for shipping recommendations.

- ☐ RNA submitted in 1.5 ml micro-centrifuge tubes clearly labeled with sample names, preferably sealed with parafilm. The RNA must be sent frozen on dry ice.
- ☐ Sample submission form completed online at http://cofactorgenomics.com/sample-submission/. Please attach the sample list spreadsheet to the online submission form electronically.
- ☐ Printed copy of the sample list spreadsheet in the package.
- ☐ Printed copy of your project quote in the package.
- ☐ Genomic or transcriptomic reference to sample@cofactorgenomics.com.
- ☐ International customers please refer to the “Guidelines for International Shipments” document for additional shipping instructions.
- ☐ Ship packages to:

Cofactor Genomics
Attn: Sample Submission
4044 Clayton Ave
St Louis, MO 63110

Still have questions? Send us an email at sample@cofactorgenomics.com or give us a call at 314-531-4647 and we’ll be happy to help you out!