Submission Guidelines for Production-Grade Sequencing Projects

Preparing Samples for RNA isolation and sequencing

Cofactor's standard pipeline accepts total RNA extracted from your organism of interest. If you would prefer to ship biological materials for RNA extraction, and this has not already been added to your project quote, please contact sales@cofactorgenomics.com.

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. The RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE tissues is also an excellent option for these samples.

Note that small or microRNA 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 re-suspended in nuclease-free water and shipped frozen on dry ice.

Shipping Samples for Extraction - Ensuring Sample Quantity and Quality

Fresh and frozen tissue should be snap-frozen on dry ice/ethanol or in liquid nitrogen as soon as possible after harvest or excision, in the quantity recommended in the table below.

FFPE samples are an exception to the minimum tissue requirement. We suggest removing as much excess block material as possible prior shipping.

How much tissue or how many cells do you need for RNA extraction?

The following are minimum starting tissue requirements for RNA isolation, library construction, and QC.
If you don't see your project listed below, please contact samples@cofactorgenomics.com.

If your samples do not meet the minimum requirements described below, please contact samples@cofactorgenomics.com ahead of sample submission to confirm samples can be accommodated.

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How many cells do we need for sequencing? Continued…

Product Name	Catalog Numbers	Library type	Minimum Amount of Tissue
mRNAble	CFG001-CUSTOM	Standard mRNA Sequencing (poly-A enrichment)	10-30 mg tissue, 5 mL bacterial culture pellet, 10^6 mammalian cell pellet
Total RNAble*	CFG002-CUSTOM	Total RNA Sequencing (whole transcriptome/rRNA depleted)	10-30 mg tissue, 10^6 mammalian cell pellet
FFPExact*	CFG004-CUSTOM	Fragmented RNA Sequencing (whole transcriptome/rRNA depleted)	4 each 5-10 µm FFPE sections‡
miRNAble	CFG005-CUSTOM	miRNA enrichment	10-30 mg tissue, 5 mL bacterial culture pellet, 10^6 mammalian cell pellet
RNAccess*	CFG006-CUSTOM	Low-input/Low-quality (targeted) RNA Sequencing	1 each 5-10 µm FFPE sections‡ or 5-10 mg tissue

**Total RNAble, FFPExact, and RNAccess are specifically for RNA derived from human, mouse, and rat samples. All other products/catalog numbers are amenable to any organism. If a description for your sample type is not listed, please contact samples@cofactorgenomics.com for guidance on the amount of material to ship.*

‡*Some tissues such as epidermal tissue may require larger amounts. Contact your Project Scientist for guidance.*

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:

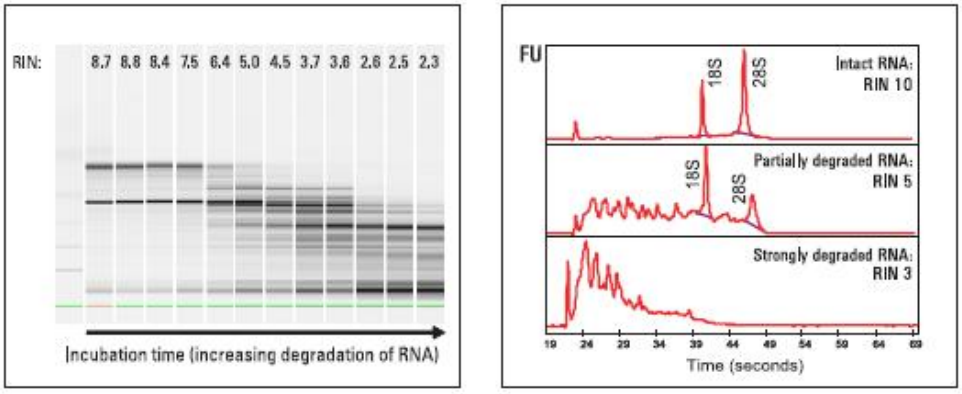
UV absorption: 260/280 >1.9 and 260/230 >2.0

RNA sample quality is best assessed on an Agilent Bioanalyzer or equivalent instrument (see example traces on next page). 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. RIN scores vary from 1-10 with 10 being the highest quality samples showing the least degradation. RINs for all samples (except from FFPE, as described below) should ideally 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 requirements since the RNA from these is heavily fragmented. Often RIN scores for fragmented RNA are misleading. Do not be concerned if the RIN scores for these samples are significantly below the threshold. We will assess quality upon arrival at Cofactor.

Samples for Total RNAble, RNAccess and RNAmplify may also be lower RIN values. Ideally, you have measured this quality information prior to deciding upon a final experimental design with your Project Scientist.



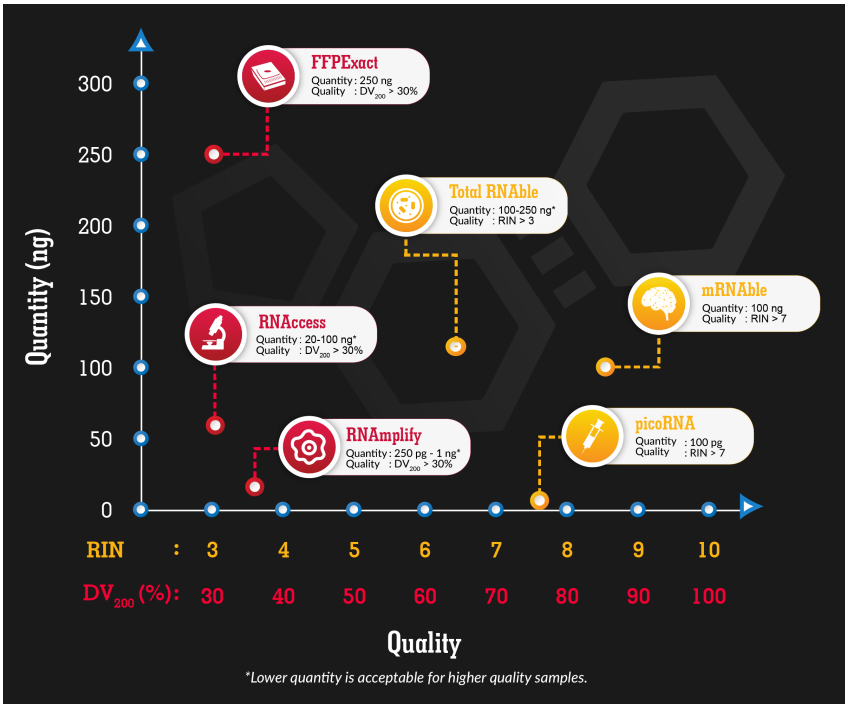
RNA analysis results obtained with the Agilent 2100 Bioanalyzer and the Agilent 2200 TapeStation are displayed in form of a gel image, electropherograms and in a tabular format. RNA quality assessment is based on the RNA Integrity Number (RIN).
Image source: Agilent Technologies.

How much RNA do we need for sequencing?

View a larger version of this image.

The values above represent the minimum inputs into an RNA library protocol.

View a summary table and concentration requirements on the following page.



How much RNA do we need for sequencing?

The following are recommended RNA requirements which allow sufficient material for QC and library construction. You are always encouraged to submit more than the recommended amount. If you don't see your project listed below, please visit the Sample Submission website (http://cofactorgenomics.com/sample-submission/) and download our Custom Projects Submission Guide.

If your samples do not meet the minimum requirements described below, please contact samples@cofactorgenomics.com ahead of sample submission to confirm samples can be accommodated.

Product Name	Catalog Numbers	Library type	Recommended RNA Quantity‡	Minimum Concentration‡
mRNAble	CFG001-CUSTOM	Standard mRNA Sequencing (poly-A enrichment)	≥500 ng	≥5 ng/µl
Total RNAble	CFG002-CUSTOM	Total RNA Sequencing (whole transcriptome/rRNA depleted)	≥500 ng	≥25 ng/µl
picoRNA	CFG003-CUSTOM	Low-input mRNA Sequencing (poly-A enrichment)	≥100 pg or equivalent cell number**	≥100 pg/µl
FFPExact	CFG004-CUSTOM	Fragmented RNA Sequencing (whole transcriptome/rRNA depleted)	≥500 ng or equivalent FFPE sections†	≥25 ng/µl
miRNAble	CFG005-CUSTOM	miRNA enrichment	≥1 µg	≥100 ng/µl
RNAccess	CFG006-CUSTOM	Low-input/Low-quality (targeted) RNA Sequencing	≥40 ng	≥20 ng/µl
RNAssemble	CFG010-CUSTOM	Normalized mRNA Sequencing (poly-A + DSN)	≥500 ng	≥5 ng/µl
RiboPRO	CFG011-CUSTOM	Ribosomal Profiling	Contact Us	
All_Splice	CFG012-CUSTOM	PacBio Long-Read RNA Sequencing	Contact Us	
CUSTOM	CFG000-CUSTOM	Custom Protocol	Contact Us	
R&D	CFG000-R&D	R&D Protocol	Contact Us	

‡Quantities listed above represent Qubit quantified material; RNA quantified using NanoDrop or other absorbance methods will require additional material.

*Total RNAble and FFPExact are specifically for RNA derived from human, mouse, and rat samples. All other products/catalog numbers are amenable to any organism.

**Equivalent cell number is required for sorted cells shipped to Cofactor in extraction buffer. Contact sample@cofactorgenomics.com for more information on this method.

Sample Submission Guidelines Checklist

At the time of sample submission, please provide the genomic or transcriptomic reference sequence to which your sequence data will be aligned. Reference sequences can be submitted by email to sample@cofactorgenomics.com as a FASTA file or a URL where the FASTA can be downloaded.

If your experimental design allows, we recommend shipping additional samples as "backup" in case of sample QC failure. Please note which samples are considered "backup" on the Sample Submission Form.

Please use the following checklist for sample submission:

- ❑ RNA is DNase treated and is re-suspended in nuclease-free water.
- ❑ Sample quality, quantity, and concentration pass above thresholds.
- ❑ RNA is submitted in 1.5 ml micro-centrifuge tubes clearly labeled with sample names matching the Sample Submission Form (below), preferably tubes are sealed with parafilm.
- ❑ Tissue, cell pellets, and FFPE sections are submitted in 1.5 ml micro-centrifuge tubes clearly labeled with sample names matching the Sample Submission Form (linked below), preferably tubes are sealed with parafilm.
- ❑ Fresh tissues and extracted RNA are frozen and will be shipped on plenty of dry ice. Avoid shipping samples on Fridays to keep samples from prolonged exposure to ambient temperature.
- ❑ 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 is placed in the package.
- ❑ Printed copy of your signed project quote is placed in the package.
- ❑ Genomic or transcriptomic reference sequence is sent to sample@cofactorgenomics.com.
- ❑ Ship packages to:

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

International customers please refer to the "Guidelines for International Shipments" document for additional shipping instructions.

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!