RNA submission guidelines for RNA-seq

IN SHORT: Prepare your RNAs (gDNA-free) according to these guidelines and hand them over to us directly, on dry ice, during working hours, in minimum 7ul (more is better). In parallel, send the RNA submission excel form to gecf@epfl.ch . No info should be transmitted only orally.

How to submit your RNA samples?

- Fill in the **RNA submission excel file** found on our website and email it to us. This must contain all relevant information. No information can be transmitted only orally.

- If you submit several **independent projects**, use different submission forms.

- Provide the **RNA profile analysis** if available, ideally with the excel report of the instrument. If you haven't done one, we will do it before starting the prep. Since this can induce delays if RNA quality is poor, we suggest you perform it before submitting your RNAs. We can train you on our user-friendly TapeStation.

- If you need to **modify** or comment on your submission in any way, do it by email only.

- In parallel to submitting the form, bring RNAs on **dry ice** during regular office hours and hand them over to us (do not put them in our -20° drawer). RNAs should be in RNase-free **PCR-grade 0.5ml-2ml microtubes**, but not in PCR tubes/strips/PCR plates.

- Prefer **short sample codes** to label tubes, especially when samples names are long. Alternatively, write short sample codes to tubes in addition to the sample names, and indicate them in the submission sheet.

- Indicated tube **labels** in the submission form must match exactly the ones on your physical tubes. If available, names of profiles must also match perfectly either tube labels or sample names.

- Do not use tape to label tubes as it detaches at -80°.

- Avoid using "S1", "S2"… or "R1", R2"… in your samples names, as these characters are already appended to fastq files by the sequencer.

- The GECF is an open-access facility, therefore we cannot guarantee the privacy of data. If this is an issue, give codes to your samples. Upon request we can create access-controlled folders for data delivery.

- With good quality RNA, there is no lower limit to the **RNA quantity**, but more RNA will give more sensitivity and accuracy. In more details:

- The dream world quantity to submit is >2 ug RNA.
- There are no strong constraints on volumes, but a reasonable amount is between 7ul and 50 ul.
- Above 10-20ng (in max 50ul) we can still use our gold standard stranded method. This is clearly what you should aim for as a minimum, samples allowing.
- Above 2ng total (in 20ul max) we can use with good results the smart-seq v4 method, even though it yields non-stranded data and is more expensive.
- Below that, we use the smart-seq v4 as well but with decreasing results quality.

- Concentration or total ng amount submitted don't need to be normalized/identical across samples.

Recommendations for RNA preparation

- **RNA quality and quantity** are crucial factors for the outcome of the RNA-seq. There are RNA-seq methods starting from tiny amounts of poor-quality RNA, but these will give lower quality data. Therefore, we recommend that you optimize RNA extraction in a pilot when working with tricky samples. For these reasons as well, follow **good RNA-specific lab procedures** when preparing/handling RNAs.

- Some tissues such as **lungs, spleen or pancreas** are very RNases-rich, so look for validated tissue-specific protocol when working with these tissues.

- For a differential expression analysis, we recommend at least **triplicate biological replicates**.

- Avoid **batch effects** when designing your experiment: distinct biological groups should not be processed in distinct batches during samples preparation/RNA extraction.

- If you are interested in **small RNAs**, use a suitable RNA extraction method (not all are).

- Avoid **bacterial contamination**, for instance during dissection or FACS sorting (e.g. using old buffers).

- **Extraction methods** finishing with an affinity column and washes (such as RNeasy…) yield RNA of better quality than methods finishing with precipitation/resuspension (such as Trizol…). If you still prefer using a Trizol/Trizol-like method, consider coupling it at its final steps with a column-based method, for instance the Zymo "RNA Clean and Concentrator-5 kit with DNase" kit, following manufacturer's instructions.

- Include a **genomic DNA elimination step**, such as:

  - on-column DNase treatment during RNA extraction (e.g. Qiagen RNeasy with the optional DNase)
  - genomic DNA exclusion column during RNA extraction (e.g. Qiagen RNeasy PLUS kit)
  - on-column DNase treatment performed after RNA extraction (e.g. with Zymo RNA Clean and Concentrator-5 kit with DNase, #R1013 or R1014). *Useful for Trizol-like extraction methods.*

  Note: A simple post-extraction DNase treatment without subsequent column purification can cause major downstream problems so absolutely avoid doing that.

  Note: TapeStation/Fragment Analyzer runs do not detect contamination with intact genomic DNA.

  Note: This gDNA elimination step is especially crucial in these circumstances:

  - *RNA is expected to be partially degraded/low quality*
  - *A "total RNA" RNA-seq method is planned*
  - *A smart-seq or brb-seq method is planned*
  - *RNA amount is very low (<20ng total)*

- **RNA elution** should be done in RNase-free H2O or Tris, but not in a buffer containing EDTA such as TE.

- Nanodrop measurements of **RNA concentration** may be very inaccurate below 50 ng/ul where extraction buffer contaminations can be responsible for most of the absorbance. Therefore, report 260/280 and 260/230 ratios when nanodrop is used, and if possible use the "nanodrop One" instrument which corrects for most buffers contamination. Alternatively, use qubit RNA HS.

- In case nanodrop **260/280 and 260/230 ratios** deviate a lot from standard values, we cannot guarantee good results downstream in the library prep.

- **RNA storage** must be done at -80°C.

Determining RNA-seq library prep method

The choice of a library prep method depends directly on results from RNA quantification and QC and is a shared decision between the user and the GECF. This discussion will address the following topics:

- Are you interested only in mRNAs or also in **lncRNAs**?
- Is detection of **small RNAs** (miRNAs…) required?
- If many samples are to be submitted (such as >40), would a "**3' end method**" such as BRB-seq be suitable? These methods conserve only the 3'end of mRNAs and are cheaper.
- Do you need **long reads**, such as paired-end 150nt reads (2x 150nt)? If yes, libraries prep will be tweaked accordingly. Only certain projects benefit from such read length, notably for repetitive transcripts such as retrotransposons, or for quantifying splicing isoforms.
- Are your RNAs likely to contain **bacterial RNAs** (gut samples, infected samples…)? If yes, do they need to be sequenced?
- Is the total RNA content misleading in terms of the **actual mRNA content**? Typical cases are: 1) nuclear fraction was isolated (rRNA is lower, mRNA is higher); 2) fractionation/IP of lysates into polysomes and other ribosomal-related fractions (some fractions may not contain mRNA).

Sequencing settings

Defining sequencing settings is not strictly required at RNA submission stage, but can already be discussed with us or with a bioinformatician. A few questions you can already think about:

- Which reads length? (see above)
- How many reads per sample? Recommendations range from 15mio (low resolution broad transcriptional profiling), to 25-40mio (standard RNA-seq), and more if looking for SNPs.

- For commercial libraries prepared by us, we guarantee a reads quantity with a 25% margin.

Versions log

- v1.05 - 1.08: RNAs must be brought in parallel with form. Also send the excel report of the RNA profiling. Mentioned BRB-seq. Decreased minimum amount for stranded prep to 50ng. Do not put RNAs in our -20° drawer. Mentioned that lungs/pancreas/spleen are very RNases-rich. Avoid R1, R2 in samples names.

- v1.09: Indicated to favor short tube labels, or add tube labels codes if the sample names are long. Indicated that minimal volume is 7ul (contact us if an issue).

- v1.10 (29.10.2024): Mentioned cases where the gDNA elimination step is especially crucial. Added a disclaimer regarding RNAs with poor nanodrop ratios.

- v1.11 (04.09.2025): Upon request we can create access-controlled folders for data delivery. Gold standard method from 10-20ng instead of 50ng. Increased the guaranteed margin from 20 to 25% to accommodate the Aviti sequencer (this is largely compensated by reduced costs).