Gene Expression Center
University of Wisconsin-Madison Biotechnology Center

Illumina Bulk RNA-Seq Service Policy

Illumina sequencing library preparation services are available for a variety of RNA sample types- total RNA, reduced rRNA, small RNA, Ip RNA, and mRNA. We use a variety of commercial kits to prepare RNA libraries that have been tested in-house on multiple sample types.

Projects are processed in the order they are received. Estimated turnaround time from point of QC approval to library preparation completion is 7-10 business days.

RNA QC typically occurs within 1-3 business days of sample submission. Each sample is assayed on the NanoDrop One Spectrophotometer or Agilent BioTek Synergy H1 Microplate reader to confirm sample concentration and purity as well as the Agilent 4200Tapestation or Bioanalyzer to assess the integrity (RIN^e). Results are emailed directly to the client. It is the client's responsibility to decide to continue with services following QC or to resubmit sample(s). Staff are available to discuss options upon request.

It must be noted, there are contaminants (polysaccharides, polyphenols, humic acid, collagen, etc.) that may be present in samples that neither the NanoDrop nor Agilent assay will detect. It is the client's responsibility to determine the appropriate RNA extraction protocol to remove the known contaminants in their sample type.

Samples that meet the recommended QC metrics and have been approved for library preparation by the client, will be prepared for sequencing using the library prep option chosen by the client. Libraries that do not meet final QC metrics (yield, adapter/dimer contamination >1%) will be shared with the client along with a recommendation.

1) Adapter/dimer (AD) contamination >1%: If library concentration > 2 ng/µl, library will be recommended for an additional bead cleanup at a cost of $35/sample. We are unable to guarantee re-purification will be successful. If library concentration < 2 ng/µl and client has their own 10B lane for sequencing, library may proceed as is. Disclaimer- smaller template such AD may be preferentially sequenced over the longer library template.
2) Insufficient yield: options will be discussed with client. Re-prep option is available at cost of rgts/consumables per sample.

Every effort will be made to provide a successful and satisfactory experience but unfortunately, we cannot guarantee the results. Within reason, we will do our best to assist with troubleshooting if needed on a per project basis at the discretion of the core director.

RNA Quality Recommendations:

1) RNA samples should be DNase treated.
2) A_{260}/A_{280} ratio range 1.8 – 2.1; A_{260}/A_{230} ratio should be similar indicating pure nucleic acids. Inaccurate ratios may be encountered at very low concentrations (<10 ng/µl).
3) The absorbance peak of each RNA sample should be directly over 260nm. Residual chemical contamination from RNA extraction procedures may result in an overestimation of the nucleic acid concentration and/or negatively influence downstream analysis.
4) RNA concentration should be 30-300 ng/µl, if >300ng/µl please dilute.
5) RNA samples with concentrations <10 ng/µl should be quantified on a fluorometer for a more accurate concentration. If you would like RNA to be quantified on a fluorometer by the GEC, please be aware 2 µl will be used for the assay.
6) Agilent generated RIN^e >7 is recommended. Samples with a RIN^e <7 may not attain full gene body coverage.

Sample Submission Guidelines

- RNA-Seq projects with ≥8 samples, samples must be submitted in a -80C thermostable sealed clear full or semi skirted v-bottom 96-well plate. Plate must be filled consecutively by column (A1-H1, etc.). Plates incorrectly filled will be returned to the client at a charge of $150. Submission Number, PI name and date must be on the side of the plate. A $75 service charge will be charged for incompletely labeled plate(s).
- RNA-Seq projects with <8 samples, samples may be submitted in a 1.5mL microfuge tube with the sample ID on the lid and PI name, date, and submission number on the side. A $75 service charge will be charged for incompletely labeled tubes. The sample ID on the lid must match the Sample Submission sheet.
- We request 15uL per sample as 3-4uL is used for RNA QC. If the RNA is suspended in something other than nuclease-free water, please include an aliquot when submitting your samples.
- Samples may be returned to the client upon request.
- Samples will be discarded six months post project completion.

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