LEXOGEN
The RNA Experts

Quick Checklist for RNA-Seq Wet Lab Experiments

Get your samples ready for RNA-Seq by following our Checklist! Next to every step, we have given some best practice advice and a few tips & tricks. Before you start, **ready all your samples and controls**, and please follow the manufacturer's instructions for individual steps.

☐ **RNA Extraction**	Ensure you are familiar with the protocol, have all the (RNAse-free) reagents and consumables you need, wear your PPE, and create an RNAse-free environment. To prevent wasting your valuable samples, run a pilot experiment with less valuable samples so that you can spot any pitfalls of your RNA extraction protocol.
☐ **Quality Control for Extracted RNA**	Check the RNA quality, e.g., by using a microfluidics device such as Bioanalyzer or Fragment Analyzer, and quantify the RNA amount for sample preparation and for adding spike-in RNA controls if they were not added in the previous step.
☐ **DNase Treatment (optional)**	If unsure if "to treat or not to treat" check out our RNA LEXICON Chapter on DNAse Treatment.
☐ **Poly(A) Enrichment / rRNA Depletion (optional)**	Deciding if any enrichment or depletion of RNA is needed heavily depends on your experimental needs, library prep, and sample constraints. E.g., poly(A) enrichment makes sense when you want to focus on protein-coding poly(A)-mRNAs, and rRNA-depletion enables a view of the entire transcriptome, both coding and non-coding transcripts. Only high-quality RNA (RIN, RQN >8) is suitable for poly(A) enrichment, for low quality RNA and FFPE samples use rRNA depletion or 3' mRNA-Seq library preps, such as QuantSeq, to assess mRNAs.
☐ **Library Preparation**	Prepare your RNA-Seq library preps by following the manufacturer's instructions. Make sure to use the optimal indexing strategy for your multiplexing needs. Optimizing the PCR cycle number used for library amplification is recommended for high quality results. This avoids under- and over-cycling of RNA-Seq libraries which may lead to accumulation of PCR duplicates and can distort sequencing data. For more information, see Amplification of RNA-Seq libraries: the correct PCR cycle number.
☐ **Library Quality Control & Quantification**	Library QC and quantification are essential. Depending on the preparation, each library is assessed individually or as a combination of many samples when sample-barcoding and early pooling are chosen. If you identify shorter by-products, such as adapter dimers, adding a clean-up step to remove these by-products and thus save sequencing space is beneficial. To reduce library loss and save hands-on time, prepare the lane mix first and re-purify your lane pool.
☐ **Lane Mixes Prepared**	Combine all samples into one pooled lane mix for loading the sequencer. Apply the desired mixing ratios required for your experimental needs. Mix all samples equally or according to their molarity. Use our Library Quantification Calculation File to prepare equimolar lane mixes. If you need to sequence some samples deeper than others, apply the desired sequencing depth ratio to the samples based on their molarity. Repeat the QC procedure for the pooled lane mix and re-purify if you observe shorter by-products, such as adapter dimers. Shorter fragments can be amplified more efficiently on the flow cell, therefore, always mix samples according to molarity (i.e., taking into account their respective length distribution) and avoid mixing very long and very short library types, e.g., avoid sequencing whole transcriptome libraries together with small RNA libraries.

Congrats! You are ready for Sequencing!

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