Tips, Tricks, and Strong Suggestions for Sample Submissions

Sample prep has been performed here with whole transcriptome RNA-seq, coding mRNA-seq, 3'-RNA-seq, 10x single cell seq, ChIP, FAIRE, VDJ, Amplicon, and much more. We specialize in difficult samples, so contact us if you want to try something unconventional, or have a low input/degraded sample. This page has general RNA prep info, as those requirements are more strict than most DNA applications. Beyond that, there are an ever growing number of sequencing methods which are up for discussion and trial. A list and description of potential sequencing applications can be found if you click here.

Further info on Sequencing and selecting the appropriate prep can be found at this link.

Yield and Quality

Submitting high yield and high quality samples is the best thing that can be done to ensure great data. Lower input will produce lower coverage and a need for more PCR cycles which increases bias. We have worked with less than 1ng but greater than 50ng total RNA is prefered for ideal cDNA conversion and RNA library prep on the neoprep system. When sorting cells for Single Cell applications or otherwise, check cell viability using trypan exclusion post sort.

For NGS sequencing on the illumina platform there are some basic sample requirements and recommendations to obtain the best possible results. In general, clean and undegraded samples are crucial, but when working in special circumstances degraded or fragmented sample may be used in a 3'-sequencing protocol as long as most of the sample doesn't fragment below 200bp. Samples should be or will be cleaned of anything that can contaminate upstream processes. Be sure to clarify that the sample is properly isolated for its specific type of sequencing.

RNA Isolation

RNA extraction and isolation may be slightly different depending on the type of sequencing (e.g. whole transcriptome, coding mRNA, miRNA, or fixed tissues and cells). For any whole transcriptome application gDNA needs to be eliminated by DNase treatment, column removal, or other procedure. For mRNA-seq, Poly A selection is performed, so non-degraded and relatively clean total or mRNA is all that is required. There are a multitude of kits, trizol type extractions, and other methods which can also be used and may be valuable for various challenges. Please talk to me about planning the best way to isolate RNA for your experiment.

Keep the workplace, reagents, and all samples nuclease and RNase free. Keep samples cold unless otherwise noted, complete experiments in a timely manner, and store samples in the proper manner. It's highly recommended to use Ambion's additive, Superase In, at 1:20 in all eluted RNA in order to prevent post isolation degradation. Low yield samples can be improved by using column free techniques such as trizol or RNAzol reagents and LPA carriers. If samples need DNase treatment the DNase will need to be inactivated or removed after the step is complete.

DNA Isolation

DNA isolation may be dependent on the type of experiment being run. In general clean nuclease free supplies and reagents should be used, samples should be treated with RNase A and Proteinase K, and isolation can best be performed using standard PCR purification techniques such as column extraction and/or Ethanol precipitation. If using a fragmentation protocol such as ChIP it is necessary that average sizes fall between 100-800bp and ideal 200-500bp.

Storage

Isolated RNA in nuclease free water stored at -80°C is stable for months, while samples stored in strong lysis buffers at -80°C can be stable for years. If need be RNA will be most stable in an NH_4OAc/ethanol precipitation mixture at -80°C. Clean DNA is much more stable and can be stored for weeks at room temperature, but is best in any of the above conditions and at -80°C long term.

Sequencing Strategy

Biological duplicates are highly recommended, biological triplicates are more helpful still, and replicates beyond that may be called for depending on the experiment. It is suggested to sequence the first pair to check for any biological contaminations or other abnormalities before starting further replicates. The same thinking may be applied to starting with a smaller sequencing run at first to make sure the sample is pure and proper before going on to deeper sequencing.

When possible, submitted samples will be run through QC to ensure that they are of the best quality for sequencing. Best practices and sample test runs may be needed to optimize successful results when quality is blinded due to low yield or other experimental limits that prevent standard QC checks.

Submissions for Sequencing

When submitting we need as much information from you as possible. For RNA samples please provide us with a Unique Sample ID, Sample label on the tube, Organism, cell type, sample buffer type, Total Volume, Total concentration, quantification method, and the type of sequencing you would like to do. Submission form can be accessed here:
https://docs.google.com/spreadsheets/d/1GQZflKCmjk8xECCKSynvOSIbx4oegWAKh-IjMoVG4LQ/edit?pref=2&pli=1#gid=1299211430

For RNA include RIN score and trace if available, answer has it been Dnase treated? answer does it have a RNase inhibitor added?
For DNA include average fragment size and picture, A260/280, and A260/230 if available.
If any other assistance is needed please do not hesitate to contact us.

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