UNIVERSITY OF LIVERPOOL | CENTRE FOR GENOMIC RESEARCH

Sample requirements for SureSelect RNA sequence capture libraries

- The Agilent SureSelect RNA target enrichment protocol includes an initial polyA RNA selection step. For certain sample types it may, however, be required to use rRNA-depleted RNA in order to remove bacterial rRNA or simultaneously remove prokaryotic and eukaryotic rRNA.
- Unfortunately, for submission of already depleted or enriched RNA samples, the CGR can take no responsibility for the level of data mapping to rRNA.
- The RNA sample should be free from DNA contamination. Thus, we recommend treating the RNA sample with DNase and remove the enzyme prior to library preparation.
- Accurate quantification of nucleic acids in the sample(s) is necessary. Use a dye based method such as Qubit (Life Technologies).
- Sample purity should be confirmed by values of ≥1.80 for both NanoDrop 260:230 and 260:280 ratios. If the samples need further purification after submission, the additional expenses will be added to the formal quote.
- Please provide a gel image of all samples to confirm sample integrity. The protocol recommends working with intact total RNA with RIN values ≥8 as starting material for depletion or enrichment. This value is calculated by the Agilent Bioanalyser software for most types of RNA. However, for some species the software cannot compute the RIN value. In those cases, RNA integrity can be estimated by the sharpness of the rRNA bands and a baseline value close to zero in the 200-1000 bp range.

Required quantities of purified RNA (including RNA for initial QC) in nuclease- and RNase-free water (free of DNA, contaminating salts, metal ions, ethanol, and phenol):

Submitted material	RNA quantity and sample volume for each sample
Total RNA for polyA selection	300-4000 ng in ≤25 µl
Total RNA for low input RiboZero treatment	≥300 ng in ≤15 ul
Total RNA for standard input RiboZero treatment	1000-5000 ng in ≤30 µl

- **Sample labelling:** If possible, please label the samples and tubes 1, 2, 3 etc. in order to ease our sample identification. Please remember to underscore numbers that can be read upside-down such as 6, 9, 16, 91, 69, 96 etc.