Standard RNA-seq:

For projects with >1 ug of RNA, concentrations >50 ng/uL, and RIN >6.3, we will help the investigator to outsource the RNA for RNA-seq.
For projects with 10 ng-1 ug of RNA, we will perform RNA-seq in-house.

- 100 ng-1 ug of RNA in 1-9uL is used to generate dual indexed RNA-seq libraries. The SMART-Seq Stranded Total RNA Hi Mammalian library prep kit (Takara, 634873) uses random hexamer cDNA priming and ribosomal depletion to generate strand specific cDNA libraries.
- 10 pg-10 ng of RNA in 1-9uL is used to generate dual indexed RNA-seq libraries. The SMART-Seq Stranded library prep kit (Takara, 634444) uses random hexamer cDNA priming and ribosomal depletion to generate strand specific cDNA libraries.
- 250 pg-10 ng of RNA in 1-9uL is used to generate dual indexed RNA-seq libraries. The SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian, library prep kit (Takara, 634413) uses random hexamer cDNA priming and ribosomal depletion to generate strand specific cDNA libraries.
- 250 pg-10 ng of RNA in 1-9uL is used to generate dual indexed RNA-seq libraries. The SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian, library prep kit (Takara 634411) uses random hexamer cDNA priming and ribosomal depletion to generate strand specific cDNA libraries.

Low input RNA-Seq:

81-1000 cells sorted directly into RLT + BME buffer or 10 pg-10 ng of RNA is used to generate dual-indexed RNA-seq libraries. RNA is isolated using the Quick-RNA MicroPrep kit (Zymo, 11-328M). 9.5 uL of RNA input is used to generate cDNA using the SMART-seq v4 Ultra Low Input cDNA Synthesis kit (Takara, 634888). 200 pg of cDNA is tagmented and adapter ligated (NexteraXT kit-Illumina, FC-121-10300) to generate oligo dT cDNA primed libraries. The library is quantified using the KAPA qPCR Library Quantification Kit (KAPA, KK4844). Libraries are pooled at equimolar ratios and sequenced using 75 bp paired-end Illumina chemistry at 20M reads per sample.

A couple of other important notes regarding sample type and RNA-seq:

Globin reduction is required for RNA derived from **blood** and will add to the cost of the library. RNA from **FFPE** samples typically has low quality integrity. Some FFPE sample are ineligible for RNAseq. Those samples which do qualify for RNAseq will require additional fees for processing.

Coverage:

20M PE reads is adequate for detection of up/down regulation. 50M-100M PE reads can detect alt splicing events and lower expressed transcripts. Check with your analysis team for what is most appropriate for your experimental aims.