Xpress Genomics AB
Cancer Centrum Karolinska
Visionsgatan 56
171 64 Solna, Sweden

Revision: 3.1

Xpress-seq bulk RNA-seq sample submission guidelines

Please read and follow the sample submission guidelines carefully to ensure optimal results and smooth handling of your sample submission.

1. **Extract total RNA**. RNA can be extracted according to your preferred method or kit (e.g. Qiagen RNeasy, RNeasy/miRNeasy micro, Thermo Fisher MagMAX, Zymo Direct-zol, etc)

2. **Quantify the RNA**. Quantification is best performed using fluorimetric methods, e.g. using tube-based Qubit RNA HS assay (Thermo Fisher, #Q32852) or equivalent plate-based assays Quant-it RiboGreen RNA Assay Kit (Thermo Fisher, #R11490)

3. **Optional QC**. We recommend to check RNA quality, for example by Bioanalyzer or TapeStation (RNA integrity) as well as purity by Nanodrop (260/280 and 260/230 ratios). It may not be necessary to QC all samples if the processing was done similarly. See Table a) at the end of this document for optimal RIN-values for the two different Xpress-seq bulk RNA-seq protocols we offer.

4. **Normalize the RNA**. During this essential step, please accurately normalize RNA concentration across all samples. We recommend a range of 5-25 ng/µl, however we support concentrations as low as 0.25 ng/µl. See Table a) at the end of this document for input requirements for the two different Xpress-seq bulk RNA-seq protocols we offer. Contact us in case you have any questions about RNA input. In case samples are submitted without normalization, a fee of 750 SEK per plate is charged for normalization.

5. **Plate the RNA**. Please plate at least 20 µl of RNA (recommended: >25 µl volume) in a full-skirted 96-well PCR plate (we recommend BioRad Hard-Shell PCR Plate #HSP9601, Thermo Fisher Armadillo 96-well PCR Plate #AB2396 or Eppendorf twin.tec PCR Plate #0030128605). Plate the samples row-wise starting from well 1A, see Figure 1 at the end of this document for clarification. Seal well with cold-storage seal (eg.

Axygen #PCR-AS-600). Please follow the instructions carefully. If re-plating is required, a fee of 750 SEK will be applied.

6. **Ship plates**. Please ship the plates to our lab on dry ice (make sure that plates are completely in the ice). Please notify us when the shipment is underway and share the tracking number if available.

   Xpress Genomics AB
   R8:00, Cancercentrum Karolinska
   Visionsgatan 56
   171 64 Solna
   Sweden

   Delivery Contact Phone: +46 760079794
   Delivery Contact Email: lab@xpress-genomics.com

Protocol	Optimal RIN-value	Lowest RNA input	Desired RNA input
Total RNA-seq by rRNA depletion	>7.0	20 ng	200 ng
Oligo-dT primed RNA-seq	>6.0	1 ng	20 ng

Table a). Optimal RIN-values and input requirements for the two different Xpress-seq bulk RNA-seq protocols we offer.

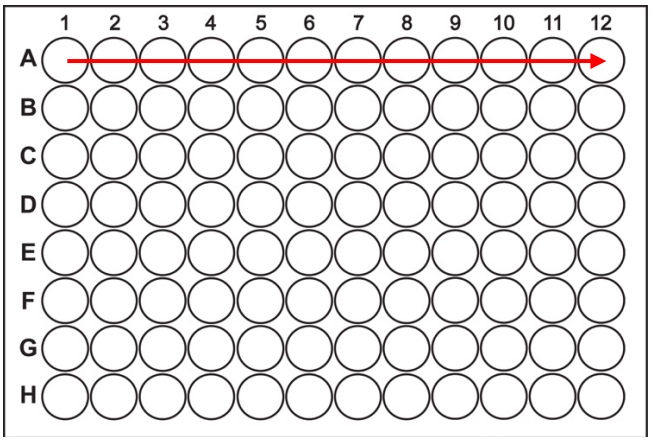

Figure 1. How to plate RNA row-wise in a 96-well plate.