RNA Sample Submission & QC Guidelines for Array

We expect that your samples will be supplied in the correct containers and at the required concentration and quality (requirements are stated below). Timely completion of your project depends on you following these guidelines.

⚠️ In the event that one or several samples does not meet our QC criteria:
Your project manager will contact you to inform you of the problems with sample QC. It will then be at your discretion whether we carry on with the experiment. If you choose not to proceed further and instead provide us with a replacement sample(s), drop that sample or cancel your project you will still be charged for sample QC and for reagent purchased for the project if applicable. Additional costs for QC will be levied for each replacement sample. Your project will be added to the end of the queue for the next batch of samples to be processed.

The following sample submission guidelines are broken down into:

1. General Considerations
2. Suitable QC Methods and Criteria
3. Volume and concentration of RNA to send:
   a. Affymetrix Whole Transcript (WT) or 3’ IVT Amplification
      i. Standard input, low input or FFPE samples
   b. RNA input for Affymetrix miRNA arrays
4. Submitting Samples

Please read the relevant sections and ensure that your samples meet our QC criteria. If you are unsure please contact your project manager prior to sending the samples. A sample of poor quality is likely to produce a poor, or biased data.

**General Considerations**

Ensure that RNase-free tubes, plates, lids and tips are used throughout. Take all additional precautions suitable for RNA work. All RNA samples should be DNase treated with a PCR-grade DNase. The enzyme should be inactivated with EDTA and heat after treatment and then cleaned up. All RNA samples must be in ultrapure water or RNase free buffer from extraction kit when submitted.

- Plate RNAs into a 96 well 0.2ml skirted plate (e.g. 4titude Frame Star 96 well ref: 4ti-0960 or Thermo-Fast 96 well ref: 10039522, but most of hard shell 0.2ml skirted plate with clear wells should be ok, but please check with us).
- The samples need to be layout in columns (A1, B1, etc) Please see info in sample submission template.
- The plate sealed using strong adhesive seals those capable of withstanding extremely low temperatures(e.g. ABgene adhesive PCR film, #AB-0558).
- Two separate aliquots should be provided (one for processing and one for QC) on a
different plates, see Volume and Concentration section for project type specific details.

For further details on packing and submitting your samples, please see the following document ‘Guidelines for Preparing and Submitting Samples’ and the video: sample submission best practice, in the Guides area of our website.

**Suitable QC Methods and Criteria**

Quantification should be done using the Qubit RNA Broad-Range Assay kit for standard amount. Nanodrop is a viable alternative to give a rough quantification of RNA samples but please be aware that any reading will likely be an overestimate. **For low starting RNA amount** quantification should be done using an RNA Pico kit on the bioanalyser or tapestation. If samples will be 5ng/µl or above then the Qubit RNA High-Sensitivity Assay kit can be used for quantification.

Ideally samples should be run on a RNA bioanalyzer or tapestation chip to determine the quality, **RIN values should be 7 or above**. If that is not possible we will perform this as part of your project but it is best practice to obtain an estimate of integrity using a 1% RNA gel, below is an example of good quality RNA (Figure 1).

![Figure 1: Lanes 1-3 are nuclear RNA, lanes 4-6 are cytoplasmic RNA (figure taken from http://www.norgenbiotek.com)](http://www.norgenbiotek.com)

Please check the OD ratios by nanodrop. The 260/280 ratio should be 1.8-2.0 and the 260/230 ratio should be close to 2.0

It is known that some extraction kits routinely produce samples with low 260/230 ratios and this could have a negative impact on the amplification of your samples. If you see low 260/230 ratios, we recommend that you perform an ethanol precipitation. If this is not possible or you have concerns then please contact your project manager.

**Volume and concentration of RNA to send**

Please note samples should be normalized, if the amounts of RNA are variable across a project we will have to consider input options and will normalise as we think is appropriate. If you have specific normalization requirements please talk to your project manager.

When optimal sample concentrations cannot be obtained it may still be possible to proceed but the success of the assay/quality of the data cannot be guaranteed, please discuss this with your project manager.
To avoid multiple freeze thaw cycles we require **two aliquots** of each sample **on separate plates**; one plate for processing and one plate for QC. The samples should be normalised prior to plating out so that the QC plate is an identical representation (in terms of concentration) of the processing samples.

**WT or 3’ IVT Amplification**

The type of array chosen will determine if the samples will be processed with either WT or 3’ IVT Amplification. However, for both methods there is a standard input and a low input (pico) version of the kit, which require different starting amounts of total RNA. It is preferable to use the standard input kit where possible. For FFPE samples the pico version of the kit will be used. The table below outlines the required concentration and volumes of total RNA.

**WT and 3’ IVT Amplification Requirements:**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Conc. Range</th>
<th>Min vol. for Process</th>
<th>Min vol. for QC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard input</td>
<td>17-167 ng/µl</td>
<td>5µl</td>
<td>6µl</td>
</tr>
<tr>
<td>Low input - Pico</td>
<td>33 pg/µl -3.3 ng/µl</td>
<td>5µl</td>
<td>6µl*</td>
</tr>
<tr>
<td>FFPE- Pico</td>
<td>167 pg/µl -16.7 ng/µl</td>
<td>5µl</td>
<td>6µl*</td>
</tr>
</tbody>
</table>

* We might not be able to QC low input samples in-house so please make sure you have performed your own QC prior to sending the samples

**FlashTag Biotin HSR for miRNA**

For the miRNA assay, the starting amount will depend on whether the sample is total RNA or if it is enriched Low Molecular Weight RNA.

**FlashTag Biotin HSR for miRNA:**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Conc. Range</th>
<th>Min vol. for Process</th>
<th>Min vol. for QC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RNA</td>
<td>16.5-125 ng/µl</td>
<td>10µl</td>
<td>6µl</td>
</tr>
<tr>
<td>Enriched LMW RNA</td>
<td>16.5-40 ng/µl</td>
<td>10µl</td>
<td>6µl</td>
</tr>
</tbody>
</table>

**Submitting Samples**

1. Sign your quotation and return via email along with a grant code (if within the WTCHG) or a PO number (if external). If outside of the University of Oxford, please provide a PDF copy of the PO
2. Complete the sample submission form, ensuring that the label on your plate(s) exactly matches the entry in the submission form and return via email.
3. With all shipment methods, please contact Christine Blancher (project manager) or the person responsible for your project to prior to shipping or drop off.
4. RNA should always be received on dry ice.
Internal / local to the WTCHG

1. To ensure that the RNA samples are transferred quickly to the -80°C storage freezer and not allowed to thaw, please arrange with your project manager a suitable time to drop off your samples with a member of the array team, either in Lab 3 or at WTCHG reception.
2. If necessary, it is possible to leave samples in the sample submission cupboard in the WTCHG reception between 10am and 12pm on any working day. This area is not secure and will become warm, please put all samples on dry ice and make sure your project manager is aware that they are being dropped off.
3. After 12pm, nobody will return to check for samples until the following working day unless special arrangements have been agreed beforehand with your project manager.

Shipping Samples

1. Samples should only be shipped between Monday and Wednesday (or 2 working days before the start of the weekend in the case of bank holidays).
2. All samples should be on dry ice. Some couriers have specific guidelines for shipping samples on dry ice and should be contacted for details prior to packaging up your samples.
3. Forward any courier details to the project manager so that the samples are expected and can be tracked.
4. Please send to:

FAO: Dr Christine Blancher
Wellcome Centre For Human Genetics,
University of Oxford,
Department of High-Throughput Genomics,
Roosevelt Drive,
Headington
Oxford, OX3 7BN