Requirements for QC and Shipment of RNA

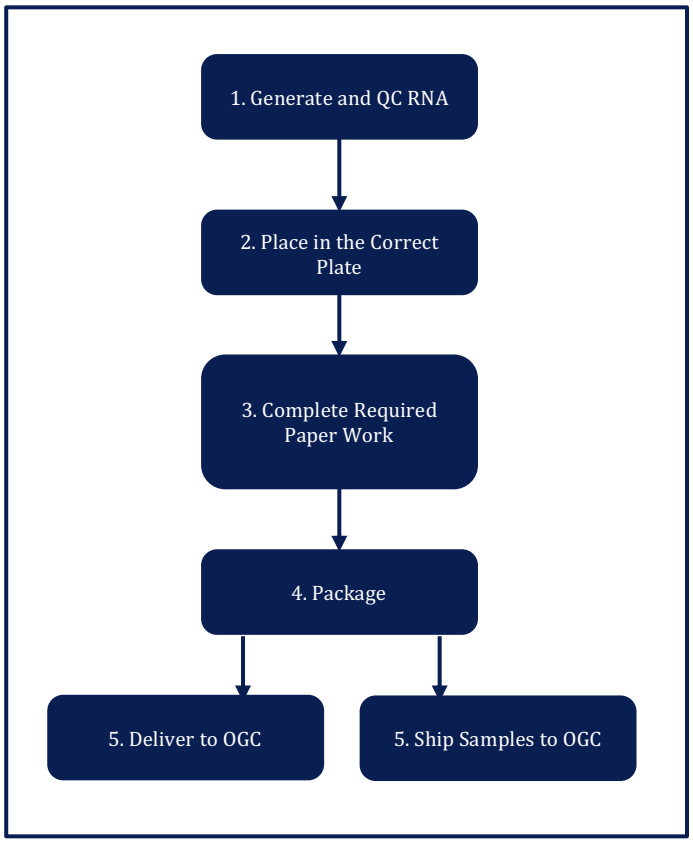
OGC receives thousands of samples every month. Help us to keep your samples safe and to return good quality data in a timely manner by taking the time to read and follow these instructions. Failure to follow these guidelines and incorrect submission of samples could:

1. Delay the initiation of your project
2. Put your samples at risk
3. Lead to poorer quality data
4. Result in additional charges being applied to your project

Obviously, as we've written this document for you, we think it is all important but there are some really key points, which are highlighted with the symbol below. If using a screen reader, these will be marked up as the "key points" style.

If in doubt at any point then please consult your project manager.

The schematic below is designed to give you an overview of the steps that you are required to take, to ensure the necessary amount of material is provided in the correct container, at the right temperature and with all the required paperwork. Further details are provided below the schematic. We have outlined the steps to take in order. However, we recommend reading the whole document through prior to starting sample extraction and collection.



As technologies improve, the boundaries between low input and high input RNA start to blur. This document is written for any library type that requires 10ng or more of input RNA, the only prep in our portfolio that this excludes is the RNA-Seq ultra low prep. If your quote states RNA-Seq ultra low, then this is not the document for you, please return to the main page or contact your project manager if you are unsure.

This requirements document is only suitable for library types including 3' mRNA, RNA-Seq No Isolation, RNA-Seq PolyA, RNA-Seq Ribozero, Small RNA and RNA-Seq TCR.

RNA is susceptible to degradation: things to do

- **Take all precautions suitable for RNA work**, including using RNase-free plasticware throughout.
- **DNase treatment with a PCR-grade DNase is essential**. Preferably, this would be done as part of the extraction process (e.g. on-column DNAse step); but if samples must be DNase treated subsequently, then the enzyme should be inactivated afterwards if necessary for the kit being used and purification should be carried out using columns or phenol/chloroform.

How should RNA be QC'd?

Quantification should be done using the Qubit RNA Broad-Range Assay or Quant-iT RiboGreen. 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. There is also likely to be some instrument to instrument variation, even with fluorescence-based methods. Because of these factors, you should give us more than the minimum requirement.

Samples should be run on a RNA bioanalyzer or tapestation to determine the quality, where RIN values should be 8 or above. A sample of poor quality is likely to produce a poor, or biased library and although there are some library preparation techniques that can overcome this issue, it will still be a factor.

Please check the OD ratios by nanodrop. It is known that some extraction kits routinely produce samples with low 260/230 ratios and this could have a negative impact on the library preparation. 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. OGC will not measure 260/280 or 260/230 ratios.

If the 260/230 or 260/280 ratios you record on the submission form are out-of-range, we will still proceed with library prep.

To prevent delays to projects that are batch-processed using our automated systems, **in the event that a sample does not meet our QC criteria, we will still proceed with the library preparation.** Your project manager will contact you to inform you regarding the problems with sample QC. It will then be at your discretion whether you sequence the library. If you choose not to sequence the library and instead provide us with a replacement sample(s), drop that sample or cancel your project you will still be charged for library prep and sample QC. Additional costs for QC and library preparation will be levied for each replacement sample. All new samples will be added to the end of the queue for the next batch of samples to be processed.

How much RNA do I need to provide and in what?

The concentrations and volumes required for each library preparation type are given in the table below, along with some specific library-type notes in numbered bullets underneath the table. These concentrations are based on measurement by ribogreen.

All RNA samples must be in **ultrapure water** when submitted.

Samples for the **PolyA** workflow should be provided in 30 µl at a concentration of 3.3-33ng/ µl for a total of 100ng-1 µg.

For the **Ribodepletion** library type, we need 100ng-1µg to be provided in 15µl, with a concentration of 10-100ng/µl.

The **No selection** library type requires 5µl of samples to be submitted at 2-20ng/µl, for a total amount of 10-100ng.

Samples for the **Small RNA** library type should be provided at 40-220ng/µl in a total volume of 9µl and a total amount of 0.36-2µg.

If submitting the samples for a combination of the **Small RNA** and **PolyA** workflows, we will need a total of 0.46-3µg of total RNA to be submitted in 10-14µl at a concentration of 40-200ng/µl.

For the **3'mRNA** library type, we need 1.2-1.5µg to be provided in 12µl, with a concentration of 100-125ng/µl.

Samples for the **TCR** workflow should be provided in 10 µl at a concentration of 40-200ng/ µl for a total of 0.4-2 µg.

Please note **samples should be normalized** to the same concentration across all samples within a project where appropriate. If a subset of samples within your project fall below the recommended concentration range, please leave them undiluted but normalise the rest to the required concentration range e.g. 30ng/ul. If you have specific normalization requirements, please talk to your project manager.

As standard, we will normalize input amounts to 100ng of total RNA for both polyA and ribodepletion protocols. If the in-house QC indicates low RIN values, then input requirements may be increased. Please provide sufficient material to accommodate for this and also to repeat a library preparation if considered necessary. For samples <100ng, we will use the appropriate maximum input. The absolute minimum is 10ng. In all cases where we have 20ng or more, we will aim to keep 10ng back in case a repeat is needed.

It is easier, cheaper and faster for you to arrange for leftover material to be retrieved than it is to resend top-up material for additional rounds of QC, which will also result in project delays and additional cost.

Type of Library Preparation	Amount of total RNA required	Concentration	Volume	OD 260/280 and 260/230
PolyA[1]	100ng-1µg	3.3-33ng/µl	30 µl	~2
Ribodepletion[1,2]	100ng-1 µg	10ng- 100ng/ µl	15 µl	~2
No selection[3]	10-100ng	2-20ng/ µl	5 µl	~2
Small RNA[4]	0.36-2µg	40-220ng/µl	9 µl	~2
PolyA and smallRNA	0.46-3 µg	40-200ng/µl	10-14 µl	~2
3' mRNA[5]	1.2-1.5 µg	100-125ng/µl	12 µl	~2
TCR[6]	0.4-2 µg	40-200 ng/µl	10 µl	~2

1 - For our standard **PolyA and Ribodepletion** prep, we prefer 100ng-1µg in the volumes detailed, but we can start from as little as **10ng** if required, please discuss with your project manager if you expect low amounts of RNA. In both cases, if you request that a previous kit is used, we will need more material from you.
2 - Please note that the input volume that can be used in the **Ribodepletion** prep is limited, so we will only add 10µl to the prep.
3 - **We will not QC the samples in-house,** so please make sure you have performed your own QC prior to sending the samples.
4 - For **small RNA** library preps, we need additional material for QC, but please note that we can only put a maximum of 5µl into the prep. Although you do not need to enrich for small RNA, please make sure that you use an RNA extraction kit that isolates large and small RNA molecules.
5 - For **3' mRNA** preps, we prefer to have enough for a repeat if needed. We use 500ng in 5µl, but we can start with varying quantities (we always require 5 µl for the prep itself) down to as little as 500pg if required. Please discuss with your project manager.
6 – The optimal input will vary with **TCR** diversity and the clonotype abundance. In the case of a first experiment, manufacturer's recommendations are to start with 200ng input.

N.B. When the optimal sample concentrations cannot be obtained it may still be possible to proceed, please discuss this with your project manager.

OK, I have my RNA, what happens next?

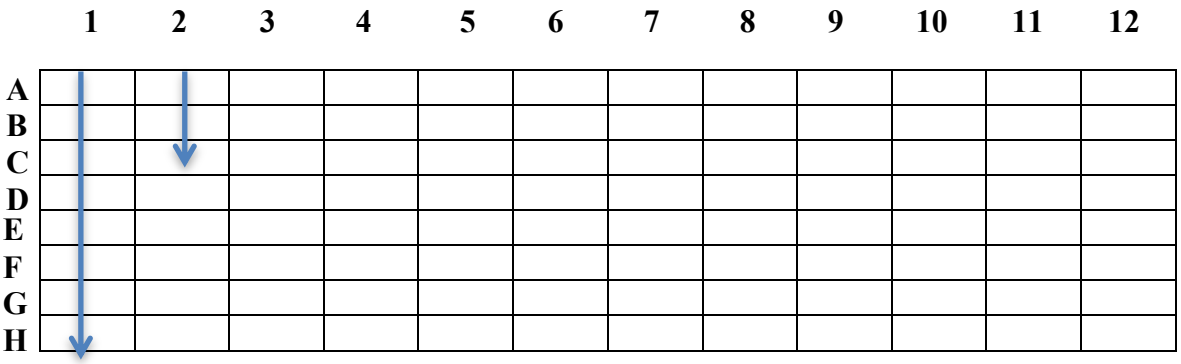
Once you have your RNA, it is necessary to plate them correctly.

We will **only** accept samples in plates. If the samples are in the wrong containers, we reserve the right to return at your cost or to charge a processing fee.

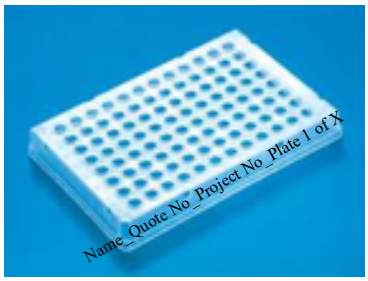
Please use this checklist to ensure that samples and plates are correctly packaged. Many of these details can also be found in our handy video guides for sample submission.

1. **Correct plates and seals**: Plates and seals are of variable quality, some seals do not stick to plates properly and can allow contamination of samples. To avoid the seal from lifting and resulting contamination, please use:
    a. Fully skirted, clear plastic, 96-well plates (ThermoFisher Thermo-Fast 96 Skirted plates, catalogue #AB-0800 or 4Titude FrameStar® 96 Well Skirted PCR Plates # 4ti-0960)
    b. Sealed with an adhesive seal (Thermo Scientific Adhesive PCR Seals #AB-0558).
2. **Plate layout**: The overall plate layout should be in columns A1-H1, A2-H2 etc. with **no gaps** between samples. Samples that are to be multiplexed should be grouped and assembled on a plate so that samples in a given multiplex are in consecutive wells. If you are multiplexing different sample types, please discuss this with your project manager.

96-well plate layout



3. **Seal the plate thoroughly**: Please make sure the plates are sealed properly to minimize contamination, we recommend using a pen lid or credit card to apply pressure between each line of wells. Note that applying a seal to a cold surface will result in the seal lifting.
4. **Label appropriately**: The sealed sample plate must be labeled with your name and quote number, once you receive the project number, we ask for you to add that, like in the image below, on the skirt along the long edge of the plate. If submitting multiple plates, please include Plate 1 of X on the label.



5. **Protect from extreme cold**: Plates should be placed within a plastic bag and the bag sealed with tape (to prevent dislodging in transit) before putting on dry ice or into the freezer. This stops the plate seal from becoming brittle and lifting. Ideally, the bagged plate would be placed in a small box before putting into a larger polystyrene box with dry ice.

Only one aliquot of each sample should be submitted unless by prior arrangement.

Before delivering samples, complete the paperwork

These steps should be carried out at least two days before you wish to ship your samples, in order to give your project manager time to log the details in to our database.

Step 1
Sign your quotation and return via email along with a PO number. If outside the University of Oxford please provide a PDF copy of the PO.

Step 2
Download the latest submission form from our website. It is important to always use the most recent version because we make improvements and old versions will not work with our login process.

Complete the sample submission form ensuring that the label on your plate(s) exactly matches the entry in the submission form.

To ensure there are no delays in initiating your project, please confirm that all the requested information is provided on the submission excel form. There are more details on the form itself but the minimum required information is:

- sample name
- reference genome
- sample concentration (except for the occasional sample where quantification is impossible)
- volume
- pooling (if applicable, e.g. specify multiplex groupings by mp1 for all samples in the first multiplex, mp2 etc).

Please extend the table as appropriate and ensure that there are **no duplicate sample names**. The container ID should also be provided, this is what your plate is labelled with your name, quote number and plate number (e.g. plate 1 of 4). Once you have the project number from your project manager, below, we ask for you to add the project number to the plate also.

In 'additional comments', please note if you want your samples returned and if there is a priority order (1, 2, 3 etc) within the project, we will try to accommodate this where possible.

Email the completed form to your project manager.

Step 3
Wait for confirmation of receipt before shipping samples. Your project manager will assign you a project number. Your plates are already labelled but need to have this project number added. This can be done without defrosting, by wiping the edge with a tissue prior to labelling. Please also include the project number on the packaging and then ship accordingly following the instructions below.

To ensure the safety of your samples, please do not send them to us prior to receiving notification by email from your project manager that it is OK to do so. Samples received unexpectedly, poorly labeled or without correct paperwork will delay the initiation of your project, risk the safety of your samples and incur additional charges.

How should I package and ship my samples?

All samples should be on dry ice, with care taken to protect the plates and seals as detailed above. The amount of dry ice that you require will depend on the size and quality of your container as well as the number of days that the parcel will be in transit. Please include extra dry ice if your parcel needs to go through customs as there can be unexpected delays. If in doubt, please speak with your courier for advice.

For delivery details please see:
https://www.well.ox.ac.uk/ogc/sample-delivery/

The essential steps of this process are summarized as a checklist in Appendix 1.

Appendix 1

Checklist for RNA

After reading the full text, this checklist can be used to ensure all the steps are carried out prior sending any samples to OGC. These are only brief summaries of each step and this checklist should not be used as a standalone document.

No.	Processes	Tick
	Generation & QC for RNA	
1.	DNase Treatment	
2.	Quantification (Qubit/ Ribogreen)	
3.	RNA Bioanalyzer/ TapeStation	
4.	260/230 or 260/280 ratio	
5.	Suitable mass & volume	
	Plating	
6.	Fully skirted, clear plastic, 96 well plates (NB we will only accept samples in the plates detailed above)	
7.	Correct layout? (vertically and no gaps)	
8.	Sealed with our recommended adhesive seal	
9.	Correct label on the side of plate (name, quote, project number, plate number)	
10.	Placed in a labelled plastic bag	
	Paperwork PRIOR sending	
11.	Quotation signed and returned	
12.	Emailed purchase order to project manager	
13.	Completed sample submission form	
14.	Emailed sample submission form to project manager	
15.	Received ‘go- ahead’ from project manager	
	Packaging & Shipping	
16.	Suitable box with dry ice	
17.	Delivery	