Requirements for QC and Shipment of Premade Pooled Libraries

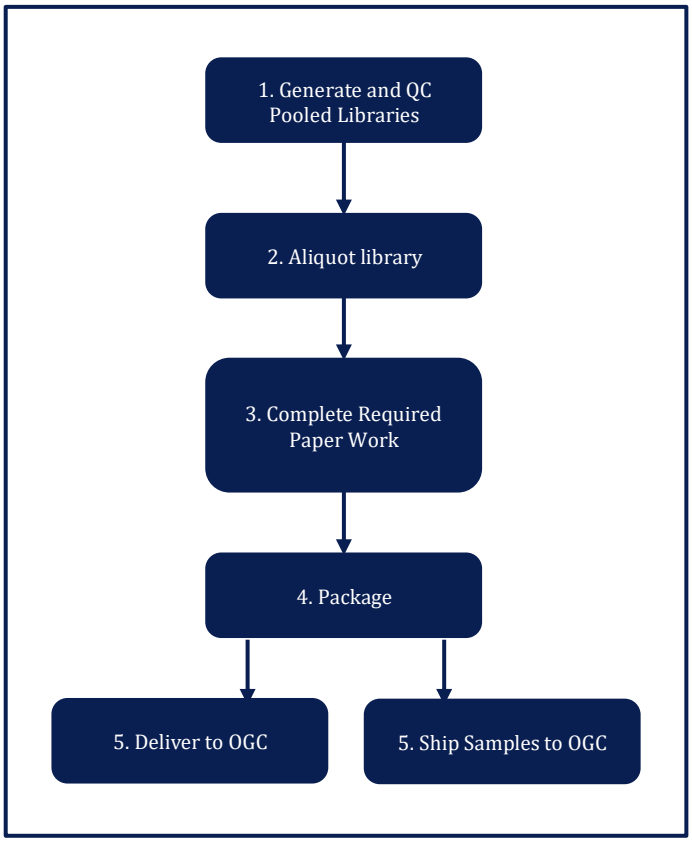
OGC receives thousands of samples every month. Help us to keep your libraries safe and to return good quality data in a timely manner by taking the time to read and follow these instructions. Failure to follow these guidelines and incorrect submission of libraries could:

1. Delay the initiation of your project
2. Put your libraries 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 with 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 sample extraction and collection.



OGC offers a number of different service types and this document is designed to help you if you have made your own pooled libraries. Your quote should state Library QC, if not then this is not the document for you, please return to the main page or contact your project manager if you are unsure.

Prepare and QC Libraries

How should libraries be QC'd?

Quantification should be done by Qubit or Picogreen. Nanodrop greatly overestimates the amount of material present and reliance on this method of quantification will risk the library failing. 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.

Libraries should be run on an appropriate bioanalyzer or tapestation to determine that the profile is correct.

We perform an in-house QC, in the event that your library does not meet our QC criteria your project manager will contact you to inform you regarding the problems. 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 library, additional costs for QC will be levied for each replacement library.

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

When submitting prepared libraries, these must be at least 10nM. When quantifing using fluorescence, the table below gives a guide to the concentrations required for different library sizes, for example a library with an average size of 250bp will need to be at a concentration of at least 1.7ng/µl whereas a library of 500bp will need a minimum concentration of 3.3ng/µl. If diluting libraries, please use EB.

Average Size of Library (bp)	Minimum Concentration of DNA (ng/µL)
250	1.7
300	2
400	2.6
500	3.3

For MiSeq and NextSeq we require at least 25µL of each prepared pooled library.

For NovaSeq 6000, which requires a larger loading volume, we require 50µL of each prepared pooled library.

Type of Sample	Amount required	Concentration	Volume
Prepared Libraries for MiSeq or NextSeq	~45ng	~3ng/µl	25µl
Prepared Libraries for NovaSeq	~150ng	~3ng/µl	50 µl

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.

When optimal library concentrations cannot be obtained, it may still be possible to sequence the library, but the amount of data cannot be guaranteed. Please discuss this with your project manager.

Additional Considerations if using NovaSeq 6000

There are some small changes in the way clustering and sequencing is carried out on the NovaSeq 6000, which restricts the type of libraries that can be run. Here are some factors to consider:

- Insert sizes- clustering favours material at the lower end of the size range. This means that material at the upper end of a broad peak may be underrepresented in the data. It also means that if there are multiple peaks, the first peak will be favoured, this is also the case if the first peak corresponds to adapters.
- Complexity- these platforms do not cope well with low complexity libraries, this is true even for a short stretch of low complexity. Depending on the library in question, a spike of a control library or a switch to another sequencing platform may be required.

OK, I have my library, what happens next?

Once you have your library, it is necessary to aliquot them correctly.


We will **only** accept samples in Eppendorf LoBind 1.5ml microcentrifuge tubes. If the libraries are in the wrong containers, we reserve the right to return at your cost or to charge a processing fee.

1. **Correct tubes**: Tubes are of varying qualities. Please use Eppendorf LoBind 1.5ml microcentrifuge tubes (catalogue number 0030108051) to ensure that your library does not reduce in concentration over time.
2. **Label appropriately**: The sealed tubes must be uniquely labeled with a clear library identifier, we suggest labelling with your initials and a number, that match those filled in on the submission form.
3. **Protect from extreme cold**: Tubes should be packaged together in a sealed container (e.g. small bag or box) before putting onto dry ice, this will protect them and ensure that we can find them. The sealed container must be labeled with your name and project number, which we'll provide at a later stage, see below.

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

Before delivering libraries, complete the paperwork

These steps should be carried out at least two days before you wish to ship your libraries, 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 premade library 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 tube(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 for each individual sample is:

- sample name
- sample type
- reference genome
- index used
- pooling details (e.g. the library name)

For the pooled libraries we need both provided pool concentration and provided pool volume. Please extend the table as appropriate and ensure that there are no duplicate sample names. Please select your reference genome of choice from the dropdown list in column C. Further details can be found on the reference genomes tab. If your genome is not in our current list, please enter "other" and provide a link to a fasta file for the reference genome that you wish to use. The index category has to be selected from cell H32. Details of each index category and the index sequences can be seen on the indexes tab. Please note that companies often re-order their index sequences in different kits so it is essential that you check that the index sequences are correct. Once you have selected the correct category in D21, you will then be able to select the correct index number from the dropdown list in column H. If the index category is not in the current list, please select the "Other" category and contact your project manager.

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


When submitting pre-made libraries for sequencing, it is your responsibility to provide the relevant instructions, if the sequencing does not follow the standard Illumina protocol, either by email or on the submission form,. This is relevant, for instance if the libraries require custom primers or non-standard read lengths. In the absence of any specific instructions, the libraries will be run according to Illumina standard protocol.

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 tubes are already labelled but need to have this project number added. This can be done without defrosting, by wiping the tube 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 libraries?

All libraries should be on dry ice, with care taken to protect the tubes 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 Pooled Libraries

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.

Generation & QC for Pooled Libraries		
No.	Processes	Tick
1.	Quantification (Qubit/ Picogreen)	
2.	> 10nM	
3.	Suitable volume	
Tubing		
4.	Eppendorf LoBind 1.5ml microcentrifuge tubes (NB we will only accept samples in tubes detailed above)	
5.	Correct label on the tube (initials, number, project number)	
6.	Placed in a labelled plastic bag	
Paperwork PRIOR sending		
7.	Quotation signed and returned	
8.	Emailed purchase order to project manager	
9.	Completed sample submission form?	
10.	Emailed sample submission form to project manager?	
11.	Received ‘go- ahead’ from project manager?	
Packaging & Shipping		
12.	Suitable box with dry ice?	
13.	Delivery	