

## Requirements for QC and Shipment of Amplicons

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:

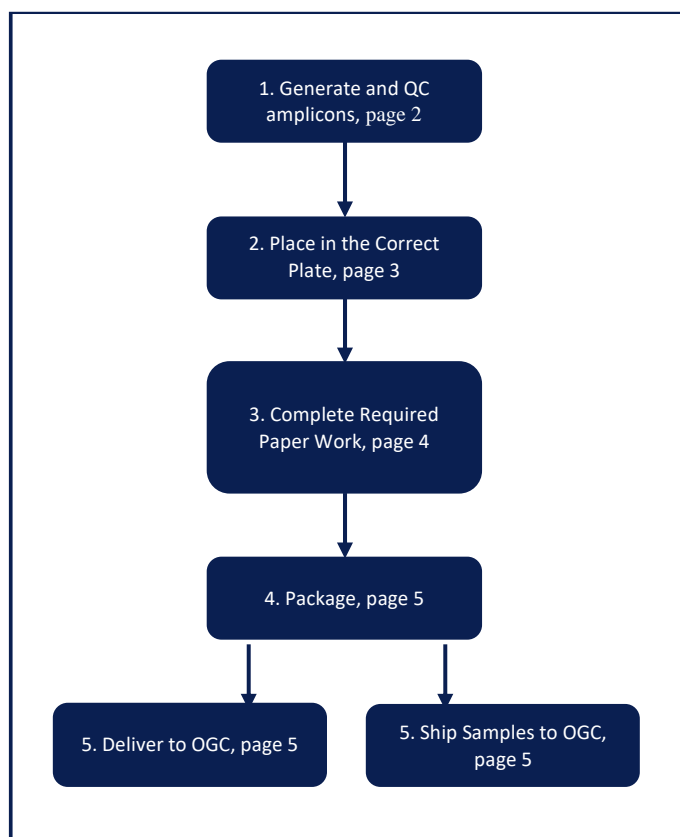
1. Delay the initiation of your project
2. Put your samples at risk
3. Lead to poorer quality data
4. Result in additional charges being applied to your project

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 sample extraction and collection.



The library type discussed here is Amplicon, if you have any other starting material other than PCR amplicons, and if your quote states any other library type- then this is not the document for you, please return to the main page or contact your project manager if you are unsure.

## Generate and QC amplicons

### What should my amplicons look like?

PCR amplicons should either be

1. longer than 1.5Kb
2. at least 50bp shorter than the combined read length of the run (e.g. for a 150bp PE run, your amplicon should be no longer than 250bp)
3. short enough to produce a library and with the area of interest within the regions that will be sequenced.

Your project manager will be able to talk through the options with you.

**Each PCR product must be cleaned up using a PCR purification kit** (e.g. the Qiaquick kit from Qiagen or Ampure XP beads from Beckman Coulter Genomics).

When amplicons are being pooled prior to delivery to OGC for library preparation, this must be done so that each amplicon is represented in equimolar concentrations within the pool. The size of each amplicon within the pool should be stated in the “notes/description” column of the submission form.

### How should amplicons 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.

Nanodrop should however be used to confirm that the 260/280 ratio is between 1.8 and 2 and that the 260/230 ratio is between 2-2.2, this will not be done by OGC.



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.

Amplicons should also be run on a 0.7% agarose gel or a bioanalyzer/ tapestation chip to check the integrity. Only a single, clean band should be obtained.



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 DNA do I need to provide and in what?

We need 0.66-1µg of amplicons to be **normalised** and provided at 20-30ng/µl in 33ul of 10mM Tris-Cl, pH 8.5. These details are summarised in the table below.

Type of Library Prep	Amount required	Concentration	Volume	OD 260/280	Agarose gel
Amplicons	0.66-1µg	20-30ng/µl	33µl	1.8 -2.0	0.7%



**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.

If you are unable to obtain the required amount of DNA, contact your project manager to discuss alternative options.

## Place in the Correct Plate

OK, I have my DNA, what happens next?

Once you have your DNA, 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.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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, 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.

## **Complete the Required 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 into 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, we ask for you to add the project number 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. As these are amplicons, please also include the size of the expected products.

**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.

## **Package and Ship Samples**

### 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 Amplicons

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