Requirements for QC and Shipment of DNA for PacBio long-read sequencing

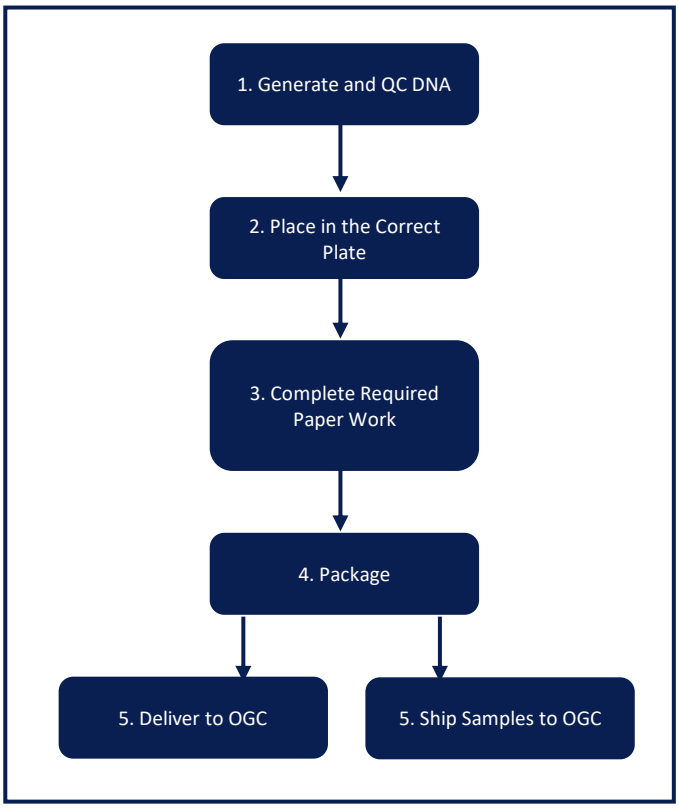
OGC receives thousands of samples every month. Help us to keep your samples safe and to return good quality data in a timely manner by taking the time to read and follow these instructions. Failure to follow these guidelines and incorrect submission of samples could:

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 list 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. We have outlined the steps to take in order. However, we recommend reading the whole document through prior to samples extraction and collection.



OGC offers a number of different library types from genomic DNA and it's important to get the right set of guidelines. The library type discussed here is for PacBio long-read HiFi sequencing of genomic DNA (gDNA) only.

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 DNA

In order to sequence long fragments of DNA using single molecule real-time (SMRT) technology it is important to start with high-quality, high molecular weight (HMW) gDNA.

There are a number of commercial kits available designed to purify HMW gDNA from a variety of materials. PacBio recommendations can be found via their website (www.extractdnaforpacbio.com).

Quantification of gDNA

Quantification should be done by Qubit with either the Broad Range (BR) or High Sensitivity (HS) dsDNA assay kits. Nanodrop can overestimates the amount of material present and reliance on this method alone should be avoided. 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 **$A_{260/230}$ ratio is 1.8** and that the **$A_{260/280}$ ratio is greater or equal to 2.0**, this will not be done by OGC.


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

DNA should also be run on a 0.7% agarose gel to check the integrity and to ensure that no RNA is present. On the gel, samples should give distinct bands with no smearing.


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?

The concentrations and volumes required for each library preparation type, as detailed in your quote, are given in the table below with the majority gDNA size indicated. For all of these preparation types, DNA should be **normalized** in 10mM Tris-HCl, pH 8.5-9.0.


Pure gDNA suspended in an appropriate buffer can be safely stored at 4°C for several weeks to months. For long-term storage (months) DNA can be frozen at -20°C or below. Freezing will induce some low-level of shearing from the ice-crystals.

Type of Library Prep		Amount required	Concentration	Volume
High complexity, high DNA input libraries (>40Kb)	Minimum	15 µg	83.3 ng/ µl	180 µl
	Preferred	>30 µg	167 ng/ µl	185 µl
High complexity, low input DNA input libraries[1] (>30Kb)	Minimum	400 ng	83.3 ng/µl	4.8 µl
	Preferred	>800 ng	167 ng/µl	15 µl
High complexity, ultra-low DNA input libraries[2] (>20Kb)	Minimum	5 ng	100 pg/µl	50 µl
	Preferred	>20 ng	200 pg/µl	105 µl
Low complexity (microbial) gDNA libraries[3] (>20Kb)	Minimum	1µg	10 ng/µl	100 µl
	Preferred	>2 µg	20 ng/µl	105 µl
Full length 16s gene amplificon libraries[4] (>20Kb)	Minimum	2 ng	0.2 ng/µl	10 µl
	Preferred	4 ng	0.4 ng/µl	10 µl

[1] can also be used for multiplexing smaller genomes up to 1Gb (e.g. yeast)
[2] this protocol requires material amplification and a genome size limit of 500Mb (for de novo assembly). Larger genomes are possible (for variant detection), e.g. human if material is limited to 5-20ng
[3] multiplexing up to 48 samples per SMRTcell (8M)
[4] multiplexing up to 192 samples per SMRTcell (8M)

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 not able to obtain the required amount of DNA, please contact your project manager to discuss alternative options.

Here is a PacBio-compiled summary of best practice considerations:

- Use fresh or flash-frozen material
- Store flash-frozen material at -80°C and avoid freeze-thaw cycles
- Do not store blood samples longer than a week at 4-8°C before DNA extraction
- Grind tissue samples to a fine powder in liquid nitrogen
- Inactivate nucleases and DNA binding proteins with a protease, such as proteinase K
- Remove all RNA with RNase A
- Avoid guanidinium or guanidine thiocyanate (proteases are favoured over chaotropic agents)
- Avoid oxidative agents such as phenol and/or chloroform to minimise DNA damage
- Resuspend, or elute, DNA in low salt buffer, such as 10 mM Tris-HCL pH 8.5-9.0
- Check concentration on both the Qubit and NanoDrop systems for concordance
- Ensure DNA is pure with $A_{260/280}$ ration at 1.8, and $A_{260/230}$ ratio greater or equal to 2.0
- Library preparation should be completed with freshly isolated DNA

Place in the Correct Plate

OK, I have my DNA, what happens next?

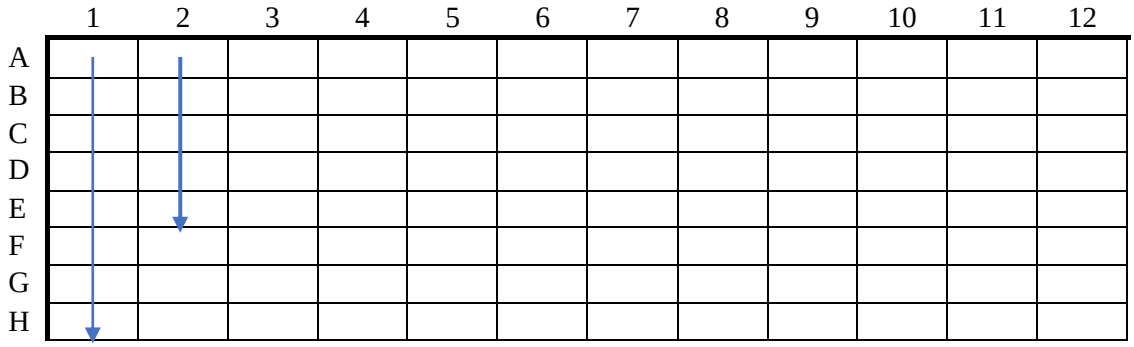
Once you have your DNA, it is necessary to plate them correctly.

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 http://www.well.ox.ac.uk/ogc/video-sample-submission-best-practice/

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.



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 labelled 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: If material is to be frozen for shipment, 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 Required Paperwork

Before sample delivery, please 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 www.well.ox.ac.uk/ogc/guides/. 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
- volume
- pooling (only 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, please also add the project number to the plate.

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 arrange delivery prior to receiving notification by email from your project manager that it is OK to do so. Samples received unexpectedly, poorly labelled or without correct paperwork will delay the initiation of your project, risk the safety of your samples and may incur additional charges.

Package and Ship Samples

How should I package and ship my samples?

Pure gDNA suspended in an appropriate buffer can be safely stored at 4°C for several weeks to months. For long-term storage (months) DNA can be frozen at -20°C or below. Freezing will induce some low-level of shearing from the ice-crystals. Depending on your particular situation, you may want to consider whether freezing your gDNA is necessary.

Where appropriate, DNA should be shipped frozen on dry ice. Keeping the DNA frozen helps prevent shearing that may occur as a result of bulk transport handling. Care should be 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 DNA (General)
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 DNA (General)		
1.	RNase Treatment	
2.	Quantification (Qubit)	
3.	260/230 or 260/280 ratio	
4.	0.7% Agarose Gel Check	
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 to 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 (if appropriate)	
17.	Delivery	