DNA Sample QC Guidelines

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.

⚠️
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 of 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(s). All new samples will be added to the end of the queue for the next batch of samples to be processed.

For further details on packing and submitting your samples, please see the following document ‘Guidelines for Preparing and Submitting Samples’.

Sample QC

Please check the following guidelines to ensure that your samples meet our QC criteria. There are multiple library types prepared from DNA, some of which have different input requirements and these are broken down below into

1. DNA- gDNA, whole genome high complexity, exome and target capture
2. DNA- high multiplex whole genome
3. PCR Amplicons

In the sections below, there are examples of the product types that you’ll see on your quotes so that you know which requirements apply. If you are unsure please contact your project manager prior to sending the samples. If your sample(s) do not meet our QC criteria we will still proceed to make a library. A sample of poor quality is likely to produce a poor, or biased, library.

1. DNA - gDNA, whole genome high complexity, exome and target capture

If you are using this type of library prep, your quote will include one of the following products.
We require 1-5μg of DNA normalized to a concentration of 50ng/μl in 10mM Tris-Cl, pH 8.5 (please note, for Nimblegen captures, we require 3μg). If you are not able to obtain the required amount of DNA, please contact your project manager to discuss alternative options. For Nextera library prep we require at least 200ng at 10ng/μl. Quantification should be done by Qubit or Picogreen. Nanodrop overestimates the amount of material present and reliance on this method of quantification will risk the library failing. Nanodrop should 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. Material should be run on a 0.7% agarose gel. Samples should give distinct bands with no smearing, as shown in the figure below. Samples should be RNase treated.

![Figure 1: Good quality DNA, clear, crisp bands, no smearing](image)

2. **DNA- high multiplex whole genome**

This is a specific library preparation that is largely used for bacterial genome sequencing. In order to keep costs low and processing time to a minimum, for this ultra-high-throughput protocol, we require that samples are normalized to 20-30ng/μl and in a volume of 60μl. Material should be run on a 0.7% agarose gel. Samples should give distinct bands with no smearing, as shown in the figure below. Samples should be RNase treated.

3. **PCR Amplicons (final product for library prep)**

PCR amplicons should either be

1. longer than 1.5Kb
2. at least 50bp shorter than the combined read length of the run
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 should be cleaned up using a PCR purification kit (e.g. the Qiaquick kit from Qiagen) or Ampure XP beads (Beckman Coulter Genomics). All amplicons should be run individually on an agarose gel or on a bioanalyzer chip. Only a single, clean product should be obtained.

Where amplicons are being pooled prior to delivery to HTG 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.

We require 1-5μg of DNA normalized to a concentration of 10-50ng/μl in no more than 100μl of 10mM Tris-Cl, pH 8.5. Quantification should be done by Qubit or Picogreen. Nanodrop overestimates the amount of material present and reliance on this method of quantification will risk the library failing. Nanodrop can be used to confirm that the 260/280 ratio is between 1.8 and 2 and that the 260/230 ratio between 2-2.2.

Summary Table

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Amount required</th>
<th>Concentration</th>
<th>Volume</th>
<th>OD 260/280</th>
<th>Agarose gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. DNA for gDNA or whole genome high complexity</td>
<td>1- 5μg</td>
<td>10-50ng/μl</td>
<td>100μl</td>
<td>1.8 - 2.0</td>
<td>0.7%</td>
</tr>
<tr>
<td>1. DNA for exome or target capture*</td>
<td>3 μg</td>
<td>30-50ng/μl</td>
<td>100μl</td>
<td>1.8 - 2.0</td>
<td>0.7%</td>
</tr>
<tr>
<td>1. DNA for Nextera</td>
<td>100-200ng</td>
<td>10ng/μl</td>
<td>10μl</td>
<td>1.8 - 2.0</td>
<td>0.7%</td>
</tr>
<tr>
<td>2. DNA for high multiplex whole genome</td>
<td>1.2-1.8μg</td>
<td>20-30ng/μl</td>
<td>60μl</td>
<td>1.8 - 2.0</td>
<td>0.7%</td>
</tr>
<tr>
<td>3. Amplicons</td>
<td>1- 5μg</td>
<td>10-50ng/μl</td>
<td>100μl</td>
<td>1.8 - 2.0</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

*If DNA is limited and only 1 μg can be provided please do not exceed 55μl volume.