UNSW
Ramaciotti Centre
for Genomics

Genomic Sample Requirements:

Long Read Sequencing Services

UNSW | Australia's Global University

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1 INTRODUCTION

Obtaining long sequencing reads requires long undamaged ultra-pure DNA fragments, as the quality of the DNA starting material will be directly reflected in the sequencing results.

Any irreversible DNA damage present in the input material (e.g. inter-strand crosslinks, etc.) will result in impaired performance and lower read lengths. Contamination present in the sample can not only affect enzymatic reactions in library preparation but also affect efficiency of pore occupation in sequencing, decreasing data yield.

High quality, high molecular weight genomic DNA is imperative for obtaining long read lengths and ensuring optimal sequencing performance.

2 GENERAL GUIDELINES FOR HANDLING HIGH MOLECULAR WEIGHT DNA

In general, the following precautions should be taken when handling genomic DNA:

- Avoid over drying of genomic DNA. Allow DNA to air dry and do not use heat.
- When resuspending DNA be gentle. Either carefully invert the tube several times after adding buffer or tap the tube gently. Alternatively, allow the DNA to stand in buffer overnight at room temperature (20-22°C).
- Avoid vortexing or harsh pipetting as it can shear genomic DNA. Mix by inversion and use wide-bore pipette tips.
- DNA should be eluted in a neutral, buffered solution. TE is recommended for long term storage of DNA.
- To prevent enzymatic inhibition during downstream sample library preparation, avoid buffers containing high concentration of EDTA.
- If gel purification is required avoid using ethidium/UV based methods. Alternative methods are SYBR® Safe (Thermo/Invitrogen) and visualization with blue light or the Sage Blue Pippin.
- If RNase treatment is required, ONT recommends ThermoFisher RNase Cocktail Enzyme Mix (AM2286).
- DNA should be stored at 4°C (short-term) or –20°C / –80°C (long-term).
- Repeated freeze-thawing of gDNA should be avoided as this will lead to DNA shearing.
- PCR products should be a clean single band product, i.e. no non- specific products visible on the agarose gel.

3 FACTORS THAT IMPACT DNA QUALITY

To maximize read length and quality, it is ***essential*** that the DNA sample:

- Is double-stranded.
- Has not undergone multiple freeze-thaw cycles as this can lead to DNA damage.
- Has not been exposed to high temperatures (e.g. > 65°C for 15 min) or pH extremes (< 6 or >9).
- Has an OD260/OD280 ratio of ~1.8-1.9 and an OD260/OD230 ratio of 2.0-2.2.
- Does not contain insoluble material.
- Does not contain RNA contamination.
- Has not been exposed to intercalating fluorescent dyes or ultraviolet radiation. SYBR dyes are not DNA damaging. Avoid ethidium bromide.
- Does not contain denaturants (e.g., guanidinium salts or phenol), detergents (e.g. SDS or Triton X100) or chelating agents.
- Does not contain carryover contamination from the original organism/tissue (haem, humic acid, polyphenols, etc.)
- DNA should run above 50kb in size as assessed on a pulse field gel or equivalent.

For a guide on the effects of common contaminates in DNA see:
https://community.nanoporetech.com/contaminants#etoh_nanodrop_readings&

4 QUALITY ASSESSMENT

The following checks are required steps to ascertain DNA integrity, purity and concentration before submitting samples for sequencing:

1) **Integrity:** Genomic DNA integrity should be assessed by pulse field gel electrophoresis or equivalent. Regular agarose gels do not have sufficient resolution to determine integrity of high molecular weight DNA. If pulse field is not available, Tapestation genomic tapes offer a overview of the DNA size range up to 60kb. Tapestation analysis is included in the Centre's sample QC workflow.

Recommended methods

1. Agilent Femto Pulse (0-150Kb size range)
2. Biorad Chef Mapper (0-10Mb size range)
3. Sage Pippin Pulse (0-100kb size range)
4. Tapestation (0-60kb size range)

2) **Purity:** DNA purity should be determined by using the NanoDrop® instrument or equivalent spectrophotometer. Readings of both A260:A280 and A260:A230 ratios need to be obtained. Low ratios indicate the presence of contaminants. Additional sample purification may be required.

Please note that although the Nanodrop system is highly useful in determining purity of nucleic acids, it cannot assess all types of contamination. The concentration obtained from the Nanodrop and Qubit (see below) should be compared. If the Nanodrop reading is more than twice the reading obtained from Qubit, this indicates that there is too higher level of contamination present in that sample.

3) **Concentration:** Qubit® or Picogreen® must be used to specifically measure double stranded DNA. Please note that NanoDrop® is not specific for dsDNA. A large discrepancy between Nanodrop and Qubit concentrations indicates the presence of contaminants. Additional sample purification may be required.

5 QUANTITY OF DNA REQUIRED

The table below summarises the quantity and concentration of DNA required for ONT library preparation and sequencing. For other ONT library prep methods and RNA, please contact the Ramaciotti Centre for more information:

Library Type	Minimum Quantity*	Minimum Concentration**
ONT DNA by ligation (No shearing)	7.0ug	80ng/ul
ONT Rapid library prep (No shearing)	1.5ug	80ng/ul
ONT 16s library prep	150ng	5ng/ul

***** Recommended amounts for submission represent DNA quantities required for one library prep and additional QC.

****** Please note that purely spectrophotometric based methods will overestimate concentration.

6 DNA EXTRACTION GUIDELINES

6.1 Before DNA extraction

a. Avoid incubation in complex or rich media.

b. Harvesting from several replicate cultures rather than a single, high-density culture during early to mid-logarithmic growth phase is preferred.

c. Extraction of small volumes is preferred over large volumes to avoid accumulating high concentrations of potentially inhibiting secondary components.

6.2 Options for DNA extraction

When choosing an extraction kit, be aware that some kits will shear the DNA to below 50kb, making it unsuitable for long read sequencing.

The Oxford Nanopore website contains suggested DNA extraction protocols for some species and tissue types. Please see the "Protocol Builder" section:
https://community.nanoporetech.com/knowledge/protocol_builder/

The list below contains some examples of HMW extraction kits available. Protocol/kit performance will vary across different species and tissues. Optimisation of the DNA extraction process will need to be performed.

- Circulomics® Nanobind Big extraction kit (50-300kb)
- QIAGEN® Genomic-tip kit (50-100 kb)
- QIAGEN® Gentra® Puregene® kit (100-200 kb)
- QIAGEN® MagAttract® HMW DNA kit (100-200 kb)

6.3 Additional DNA Clean Up

If additional sample clean-up is required, we recommend the following methods.

a. Purification of DNA with the AMPure® XP kit.

b. Some researchers have found that running DNA samples through a Blue Pippin gel helps to remove contaminates (particularly in plant samples).

Please note that listed third party products are not officially endorsed by the Ramaciotti Centre and are only provided as possible options.

7 SHIPPING GUIDELINES

- Please ship via the fastest method available (same day/overnight if available).
- Ensure all our contact details are correct and that you have forwarded on the tracking details to the contact person at the Ramaciotti Centre.
- For interstate and overseas shipping, schedule your shipments to go out at the start of the week (Monday or Tuesday).
- If you are shipping from overseas the Ramaciotti Centre can provide an import permit.
- DNA stored at 4°C, should be shipped on enough 4°C cold packs to maintain temperature throughout the journey.
- DNA that has been stored at -20°C should be shipped on enough frozen ice packs to maintain temperature throughout the journey.