SERVICE GUIDE

Tissue Extraction Service

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SVG2402TEX

Service Guide

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1.0 Overview

AGRF's Tissue DNA Extraction Service provides DNA for entry into a wide range of services.

Please note DNA yield, average molecular weight and quality will vary depending on genome size, age of sample, sample collection, storage and shipping conditions. Recovery of a DNA yield and molecular weight suitable for intended downstream purposes are not guaranteed.

We request that you retain a working aliquot of all samples to mitigate the unlikely risk of sample loss during transport or processing.

Tissue Samples

Successful genomic DNA extraction from tissue requires proper handling and storage before purification.
Improper handling and storage of tissue samples is one of the most common reasons for extraction failure.

Immediately after collection, tissue samples should be preserved to prevent DNA degradation by cellular nucleases. This can be done by using a preservation reagent or flash freezing the samples in liquid nitrogen.

If the DNA extraction involves FFPE (Formalin-Fixed Paraffin-Embedded) samples, we recommend utilizing freshly cut tissue sections for optimal results. We do not accept slides or whole blocks.

Use of poor-quality starting material, (FFPE), will lead to reduced quality and yield of purified DNA. Repeated freeze/thaw cycles should be avoided as this can lead to reduced quality and quantity of DNA. It is not advisable to put frozen tissue samples in ethanol or any other preservation reagent after freezing as this will cause the samples to degrade. Frozen samples will need to stay frozen until extraction has been performed.

Samples that are stored for long periods of time at room temperature, 4°C or -20°C will show degradation over time. To prevent this, immediately freeze tissue samples with liquid nitrogen and store them at -80°C. Alternatively, you can use stabilising reagents to protect the sample and enable storage for longer periods of time at 4°C or -20°C. Always read the manufacturer's instructions for the stabilising reagent you are using and store the tissue as recommended by the manufacturer.

Ethanol as a Preservation Reagent

Ethanol can be used for tissue preservation; however, it is not a very good preservation reagent. It should not be used for High Molecular Weight extractions for PacBio Sequencing. Samples should be fully immersed in the ethanol and kept under the level of the solution. Samples should only be left at room temperature in ethanol for a very short period. The samples should be frozen at -80°C for longer term storage.
A common method to produce ethanol with a higher concentration than 95% is to use additives that disrupt the azeotrope composition and allow further distillation. For this reason, absolute ethanol sometimes contains trace amounts of additives such as benzene. Denatured ethanol, either 95% or absolute, contains additives such as methanol and isopropanol. Methylated Spirit is a denatured alcohol with additives. We strongly advise that you do not use denatured ethanol or ethanol with any additives for tissue preservation.

Tissue Preservation Reagents

Preservative reagents need time to diffuse into animal tissue, the smaller the piece of animal tissue the quicker the diffusion time and less degradation is seen. It is best to store samples under cool or cold conditions while in the field and under refrigeration in the laboratory, when using preservation reagents. It is also best to make sure you have the maximum amount of surface area and smaller amount of tissue for faster absorption of the preservative reagent. It is therefore recommended you cut your 20mg piece of tissue into smaller pieces before placing it in the preservation reagent.
You should always read the manufacturer's instructions for the preservation reagent you are using to make sure that you are storing the sample appropriately for that reagent. It is always best to store samples for long-term storage at -80°C.

High Molecular Weight Extraction

Samples for HMW extraction should be collected and flash frozen in liquid nitrogen, the sample should be stored in a -80C freezer and should always be kept frozen until extraction, the sample should be transported on dry ice.
You cannot use ethanol for samples that are to be extracted for HMW DNA for the PacBio, other preservation reagents can be used if needed. Best practice is new collected samples flash frozen transported on dry ice and extracted as soon as possible after collection. Please refer to our HMW service guide for further information.

DNA Degradation

DNA degradation can result from:

- Using very old tissue samples, not stored correctly.
- Freezing and then thawing your samples to place in a preservation reagent.
- Freezing and thawing your samples repeatedly.
- Using samples stored at room temperature for extended periods.
- Using samples stored at 4°C for extended periods.
- Using samples stored at -20°C for extended periods.
- Using absolute denatured ethanol with an additive to preserve your samples.
- Using FFPE tissue.

IMPORTANT: If your samples are frozen, then they should not be allowed to thaw. Please send them to AGRF on dry ice to ensure they stay frozen. Letting the samples thaw will increase degradation and influence the quantity and quality of the DNA.

2.0 Sample Submission Requirements

Tissue samples should be pre-sized to 20mg and sent in a clearly labelled Eppendorf tube. To obtain optimum DNA yield and quality, it is important not to overload the extraction, as this can lead to significantly lower yields than expected. The amount of tissue should not exceed 20mg. In large tissue pieces, nucleases will degrade the DNA before the Proteinase K can lyse the tissue and release the DNA.

FFPE material needs to be freshly cut sections, each with a thickness of up to 10 µm. Up to 8 sections maximum, each with a thickness of up to 10 µm and a surface area of up to 250 mm2, can be combined in one preparation. Place sections in a clearly labelled Eppendorf tube. Do not send slides or blocks.

Please note exceeding the recommended amount of starting material will reduce quality and quantity of DNA.

Some types of samples are not suitable for all downstream services. We suggest you talk to your Account Manager to determine if your samples are suitable for your intended downstream service.

3.0 Turnaround Time

Turn around time is sample number dependant and should be discussed with your account manager at the time of submission.

4.0 Sample Storage

Your DNA will not be stored at AGRF Adelaide as all DNA will be sent for downstream processing. If you require your DNA after downstream processing, please indicate this to your account manager at the time of quoting. Raw sample material will also not be stored at AGRF Adelaide as all sample material will be extracted and discarded after quantification.

5.0 Shipping Your Samples for Extraction

IMPORTANT: if you need your DNA returned to you, please let your Account Manager know as this will incur a cost. Please ask your Account Manager to add this to your original quote to save doubling up on paperwork and delaying sample delivery. All Samples should be shipped on dry ice.

Please send your samples with a printed submission sheet.

Submission sheets are generated when you login via the AGRF portal and submit your samples online.

AGRF can organise dry ice shipment for your samples as part of your quoted services or you can use our free shipping between nodes once a week service. For information on this service go to Free Shipping.

6.0 Online Sample Submission

Online Submission:

- Log in to MY AGRF HUB
- From the drop-down menu, select the Service Type:
- Select 'Extraction and (ongoing service)' if the DNA is going to be moved into another AGRF service, or
  Select 'Extraction' if the DNA is not going to be moved to an ongoing service.
- Please complete and upload the 'Template File' excel template.
- Send your samples to the address below.

Physical address (courier) and postal address (mail):

AGRF Adelaide
PLANT GENOMICS CENTRE
HARTLEY GROVE
URRBRAE, SA 5064

7.0 Quality Statement

Non-clinical works are performed following the strict requirements of ISO17025: 2005. AGRF Ltd is accredited in the field of Biological Testing (Scope: DNA Analysis) according to the ISO17025: 2005 standard by the National Association of Testing Authorities (NATA). Staff and analysis processes follow Standard Operating Procedures, which define responsibilities and quality checks to achieve reported standards. Compliance is monitored at regular reviews and during internal audits. All work is supervised by a person with relevant qualifications and is checked while in progress and upon completion to ensure that it meets the necessary ISO17025: 2005 standards.