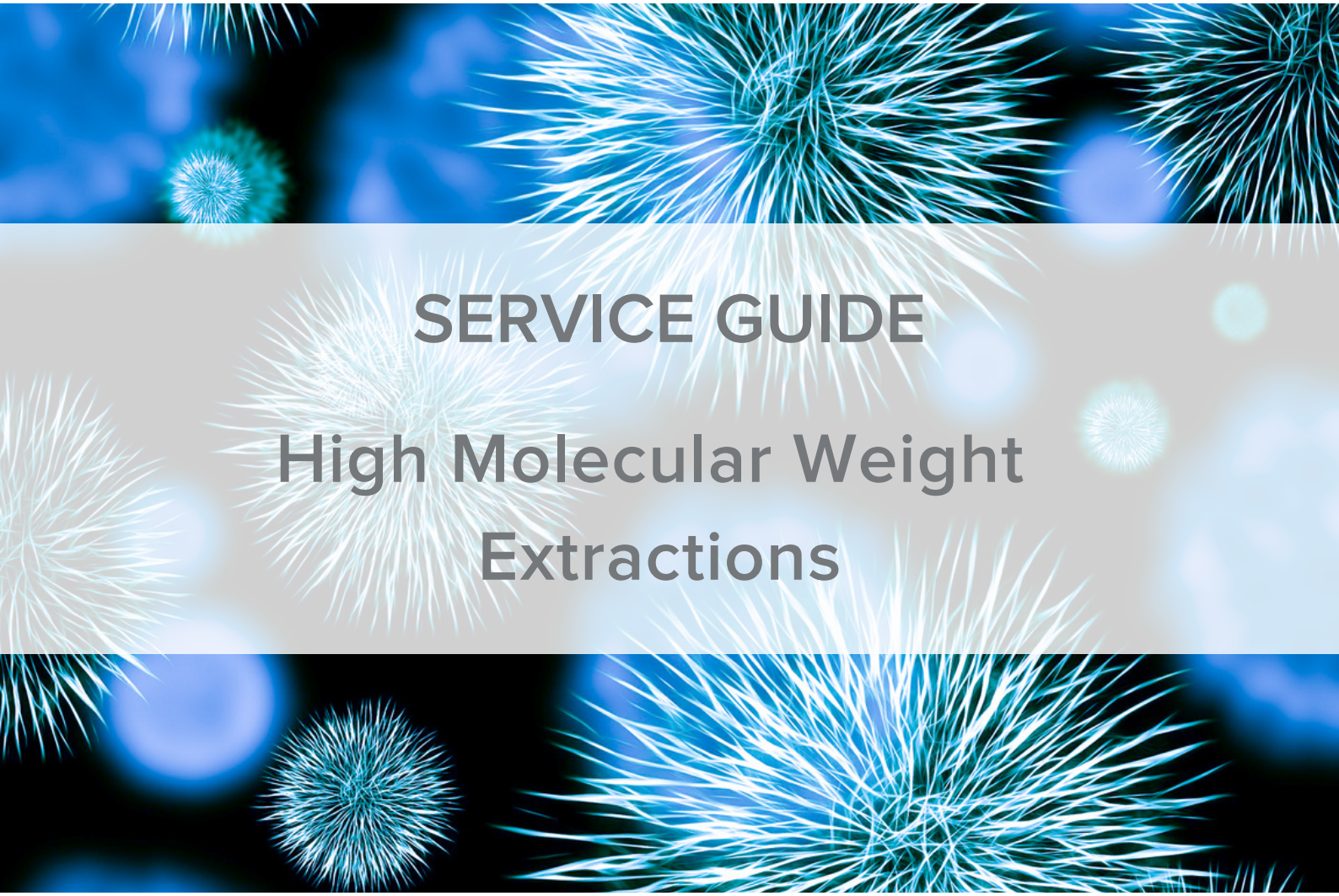

SERVICE GUIDE

High Molecular Weight Extractions

CustomerCare@agrf.org.au
1300 247 301
www.agrf.org.au

SVG2402HMWE

Service Guide

High Molecular Weight Extractions

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1.0 Overview

AGRF's third generation long read sequencing service requires the input of HMW genomic DNA for the best performance during sequencing. Standard DNA extraction protocols do not produce the required 10-15Kb for long read sequencing.

2.0 Submission

AGRF offers an HMW DNA extraction service from blood, tissue and cells. NOT sperm or plants.

Figure 1: A guide to outputs from various blood, tissue and cell types. AGRF recommends submitting at least double the maximum input amount, in case a re-extraction is required. Please note that if submitting tissue not as a powder, then ensure that each piece is no greater than the maximum amount indicated in "maximum input amount".

SAMPLE TYPE	RECOMMENDED INPUT AMOUNT	TYPICAL YIELD (µg)	DIN	MAXIMUM INPUT AMOUNT
TISSUE				
Tail (mouse)	10 mg	12-20	8.5-9.5	25 mg
Ear (mouse)	10 mg	18-21	8.5-9.5	10 mg
Liver (mouse and rat)	10 mg	15-30	8.5-9.5	15 mg
Kidney (mouse)	10 mg	10-25	8.5-9.5	10 mg
Spleen (mouse)	10 mg	30-70	8.5-9.5	10 mg
Heart (mouse)	10 mg	9-10	8.5-9.5	25 mg
Lung (mouse)	10 mg	14-20	8.5-9.5	15 mg
Brain (mouse and rat)	10 mg	4-10	8.5-9.5	12 mg
Muscle (mouse and rat)	10 mg	4-7	8.5-9.5	25 mg
Muscle (deer)	10 mg	5	8.5-9.5	25 mg
BLOOD**				
Human (whole)	100 µl	2.5-4	8.5-9.5	100 µl
Mouse	100 µl	1-3	8.5-9.5	100 µl
Rabbit	100 µl	3-4	8.5-9.5	100 µl
Pig	100 µl	3.5-5	8.5-9.5	100 µl
Guinea pig	100 µl	3-8	8.5-9.5	100 µl
Cow	100 µl	2-3	8.5-9.5	100 µl
Horse	100 µl	4-7	8.5-9.5	100 µl
Dog	100 µl	2-4	8.5-9.5	100 µl
Chicken (nucleated)	10 µl	30-45	8.5-9.5	10 µl
CELLS				
HeLa	1 x 10^6 cells	7-9	9.0-9.5	5 x 10^6 cells

2.1 Tissue Submissions

Figure 1 shows the expected outputs from frozen tissue powder. For the best outcome submit tissue samples in very small pieces frozen at -80°C. We recommend snap freezing in liquid nitrogen and pulverising to tissue powder and storing at -80°C. If submitting insects avoid including gut tissue, (high bacterial content) and eyes, (pigment will affect sequencing). Soft organ tissues (e.g., liver, kidney, pancreas and intestine), have high nuclease content. Isolating high quality gDNA from such tissues tends to be more challenging than with other samples. However, if samples are stabilised and cut to small pieces (or are processed as frozen tissue powder), good yields can be obtained. Tissues can be submitted in stabilising agents. AGRF will not except fresh specimens. Ethanol should never be used as a preservation reagent.

2.2 Blood Submissions

- Blood stored in anticoagulants, EDTA, citrate and heparin, fresh or frozen, can all be processed. Fresh blood samples should not be stored at 4–8°C for longer than a week, and therefore we recommend submitting° all blood samples frozen.

For archiving samples of whole blood, storage at -80°C is recommended. Frozen blood samples will give excellent quality gDNA but it is essential that samples are not thawed before the purification procedure.

2.3 Cell Submissions

Submit cell pellet frozen or fresh.

3.0 Turnaround Time

Turnaround time is dependent on sample numbers. Please discuss with your account manager prior to submission.

4.0 Quality

DNA degradation can result from:
• Using tissue samples not stored at -80°C.
• Freezing and then thawing your samples.
• Using samples stored at room temperature for extended periods without a preservation reagent.
• Using samples stored at 4°C for extended periods without a preservation reagent.
• Using samples stored at -20°C for extended periods without a preservation reagent.
• Using ethanol

DNA yields vary depending on genome size and age of sample. Sample collection, storage and shipping will also influence DNA quality and quantity. DNA yields and average molecular weights are highly sample dependent. Recovery of a DNA yield and molecular weight suitable for intended downstream purposes are not guaranteed by AGRF.

AGRF requests that you retain a working aliquot of all samples to mitigate the unlikely risk of sample loss during transport or processing. In the event of accidental sample loss during processing, AGRF's liability will be limited to a full discount of the price of sample processing.

5.0 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.

6.0 Online Sample Submission

Online Submission:
• Log in to MY AGRF HUB
• From the drop-down menu, select the Service Type:
• Select ‘HMW 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.

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.

Physical address (courier):
AGRF Adelaide
PLANT GENOMICS CENTRE UNIVERSITY OF ADELAIDE
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.