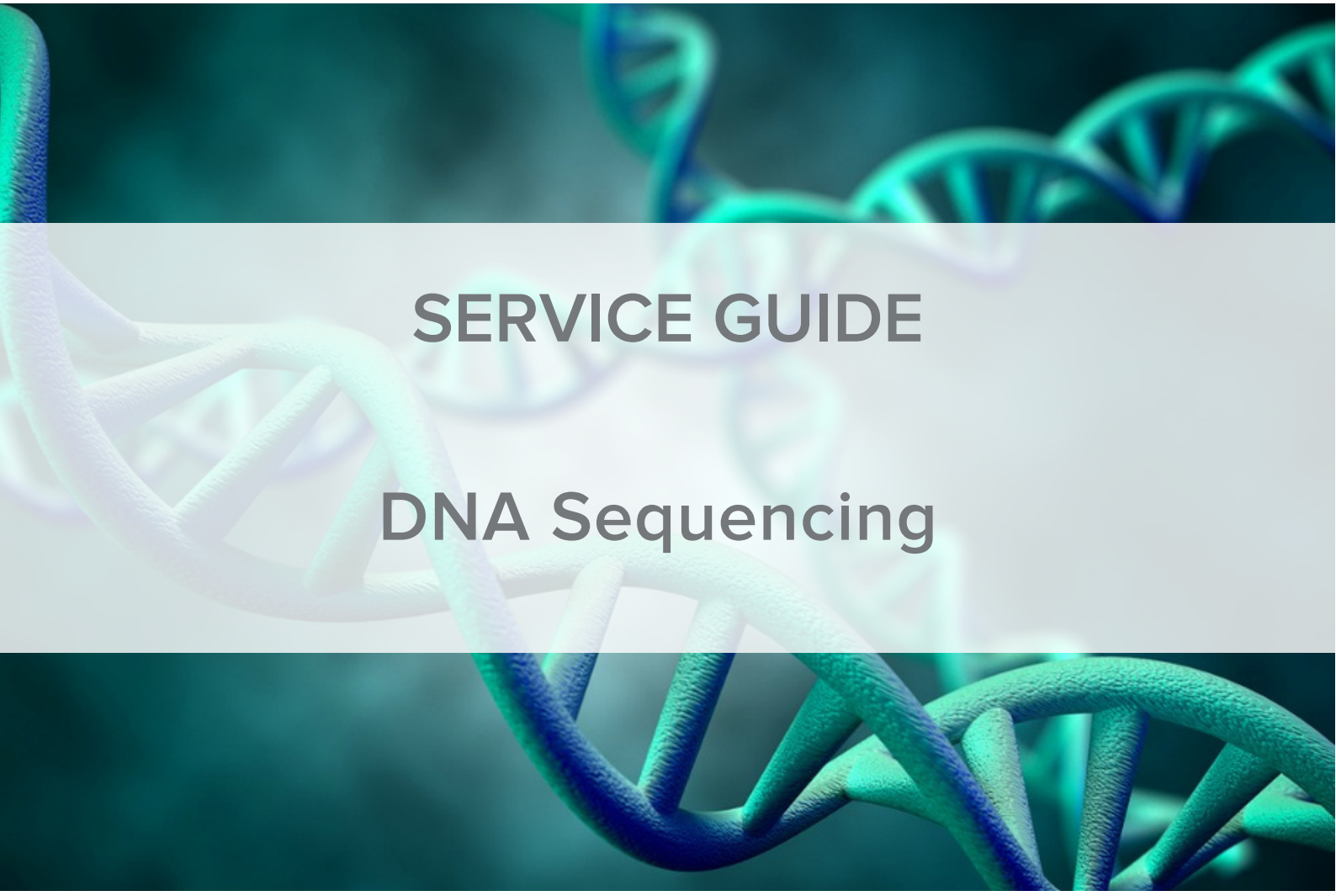

SERVICE GUIDE

DNA Sequencing

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

SVG2402DNA

Service Guide
DNA Sequencing

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Service Guide
DNA Sequencing Service

1.0 Overview

Next-generation sequencing technologies, such as Illumina sequencing, employ clonal amplification of DNA templates on solid supports to enable high-throughput sequencing. Prior to sequencing, DNA samples undergo library preparation, a process that involves fragmenting the DNA and attaching specific oligonucleotide adapters to facilitate the sequencing process. Whole genome sequencing (WGS) is employed for a comprehensive analysis of genome variance and structure. Additionally, DNA sequencing can be applied to samples that are enriched for specific loci using techniques such as PCR, hybrid capture, or immunoprecipitation. AGRF provides a range of DNA library preparation services suitable for various sample types and applications, including de novo sequencing, re-sequencing, and ChIP-Seq.

1.1 Illumina Libraries

Illumina sequencing systems are compatible with two basic library types: Ligation and Transposase.

1.1.1 Ligation Libraries

Ligation libraries (e.g. IDT xGen cfDNA & FFPE library prep etc) are created by ligation of double- stranded oligonucleotide adapters to fragmented DNA and cDNA.TruSeq adapters contain multiple sequence elements required to bind to Illumina flow cells, sequencing primers and indexes (or barcodes) to allow multiplexed sequencing.

1.1.2 Transposase Libraries

Transposase libraries are created using a hyperactive variant of the Tn5 transposase to fragment DNA and simultaneously ligate double stranded oligo nucleotide to the ends of the DNA (Figure 3). Transposase adapters consist of a 19bp Mosaic End sequence required for transposition and a linker sequence for PCR amplification (figure 4). The primers used for amplification contain the sequence elements required to bind to Illumina flow cells and sample indexes. The Illumina DNA PCR-Free (tagmentation) library prep does not require PCR amplification and generates a highly complex library without PCR errors and with a lower input.

Figure 1: Diagram of dual indexed ligation adapters .

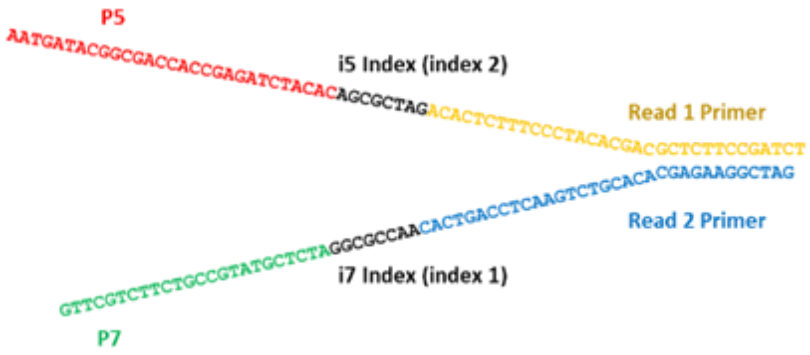

Figure 2: Diagram of the final product ready for sequencing.

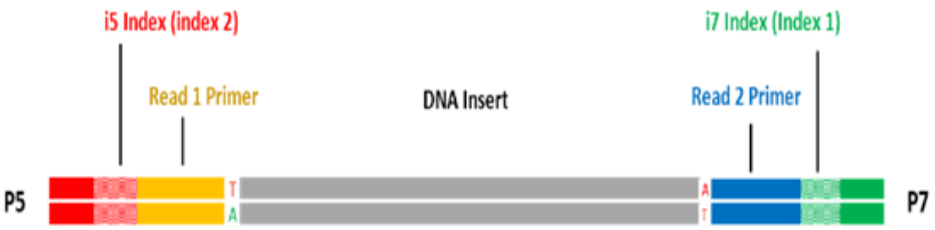

Figure 3: Workflow for DNA library preparation via adapter ligation.

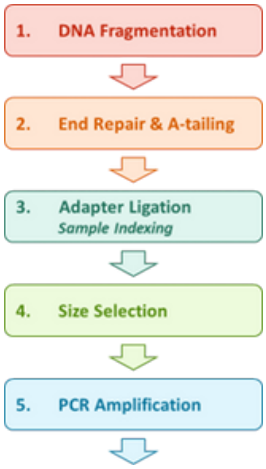

Figure 4: Incorporated UMI's into library preparation for quantitative data analysis.

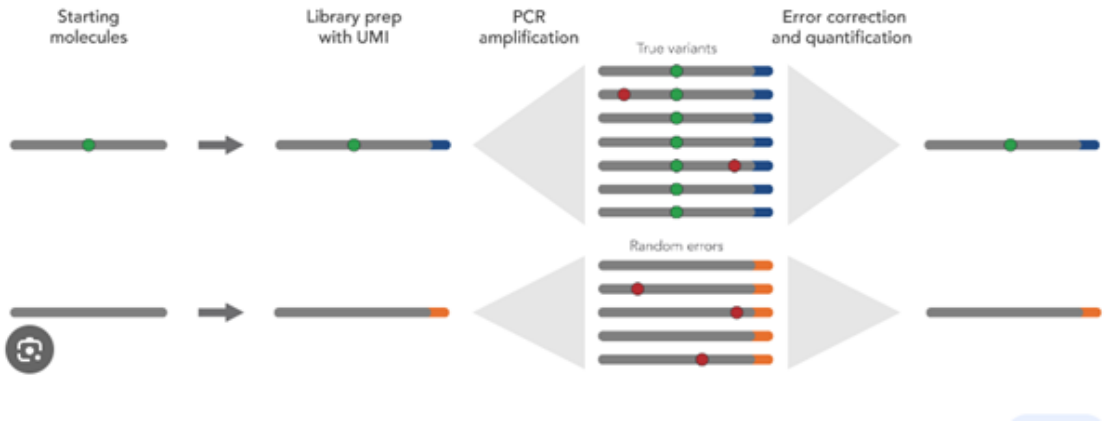

Figure 5: Diagram of Tn5 mediated transposition of linker sequences for transposase-based library preparation.

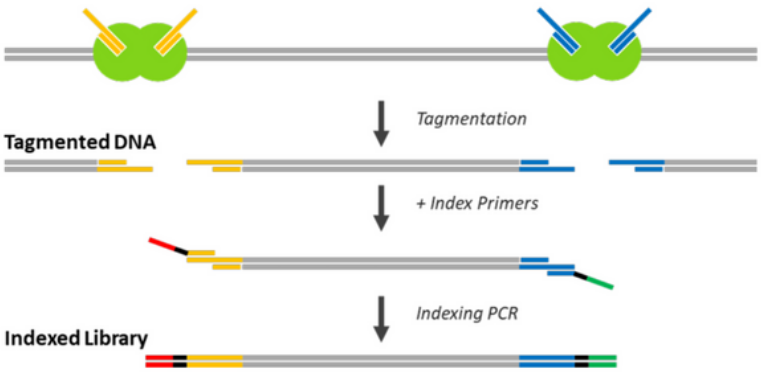

Figure 6: Workflow for tagmentation-based DNA library preparation with PCR.

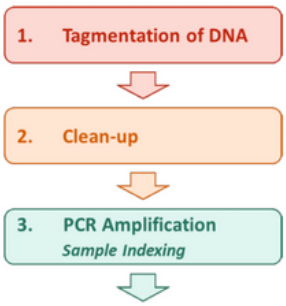

Table 1: Summary of AGRF's DNA library preparation kits.

	IDT xGen cfDNA & FFPE library prep	Illumina DNA PCR-Free	Illumina DNA Prep (M)
Input	1 - 250ng	>50ng	1-500ng
Multiplexing	384 UDI with UMI	384 UDI	384UDI
Target Insert Size	350bp	450bp +/- 75bp	350bp
Fragmentation	Enzymatic	Enzymatic	Enzymatic
Key features	Low inputs, Flexible insert sizes, FFPE-compatible, Contains in-read UMI	Flexible inserts, PCR-Free	Flexible Inputs
Sample Type	Complex Genomes, Small Genomes	Complex Genomes, Small Genomes	Complex Genomes, Small Genomes

2.0 Sequencing Recommendations for DNA Libraries

Sequencing requirements for DNA libraries can vary considerably depending on the type of sample and the intended analysis type. The following table provides general guidelines for genome sequencing applications.

Table 2: General recommendations for genome sequencing applications.

Sample Types	Application/Analysis	Coverage
Small Genomes <200Mb	Resequencing - SNV, MLSTs	30-50x
	de novo genome assembly	50-100x
Complex Genomes >200Mb	Resequencing - SNVs	30x
	Resequencing - Indels	60x
	Resequencing - CNVs	>5x
	de novo genome assembly*	>100x

**de novo assembly of large genomes using Illumina short reads will yield highly fragmented draft assemblies. High quality, highly contiguous assemblies may require a combination of sequencing technologies including long read technologies.*

3.0 Results and Data Outputs

All Illumina sequencing projects will undergo QC analysis to assess the quality of both the sequencing and library preparation. AGRF will provide untrimmed and unfiltered Illumina sequencing data in FASTQ format. AGRF also provides options for analysis of DNA sequencing including genome alignment and de novo assembly. If you have specific analysis requirements, please discuss these during the quotation process.

4.0 Sample Requirements

The standard input for any genome sequencing project is high quality genomic DNA. Our recommendations for isolation of DNA are listed below:

- Standard silica column-based extractions generally produce DNA of suitable quality for Illumina library preparations.
- Please ensure the extraction protocol includes an RNase treatment to ensure removal of RNA.
- Purified DNA should be eluted/resuspended in 10mM Tris-Cl, pH 8.5 or nuclease-free water.
- No AE buffer

We can also process other DNA sample types including Amplicons:

- Amplicon DNA requiring fragmentation must undergo PCR cleanup to prior to submission.
- Amplicon DNA should be eluted/resuspended in 10mM Tris-Cl, pH 8.5 or nuclease-free water.

4.1 Sample Quality

Sample quality is a key factor for successful NGS experiments. We will perform quality control prior to commencing a project, however, we recommend you check the DNA before submission. If you would like us to review your DNA QC prior to submission, please forword your files to AGRF.techsupport@agrf.org.au

4.2 Sample Purity

Purity of nucleic acid samples can be assessed by measuring the absorbance spectra via a spectrophotometer (e.g. Nanodrop). The ratio of absorbance values 260nm and 280nm or 230nm provide estimates of sample purity or the presence of common contaminants. Purified DNA is expected to have a A260/280 ratio of ~1.8.

Table 3: Recommendations for sample purity as assessed by absorbance spectra.

Ratio	Target	Low Ratio (<1.6) Indications
A260/280	1.8	Residual phenol from extraction or very low concentration of DNA (<10ng/µl)
A260/A230	>1.6	Residual guanidine from the extraction protocol Carryover of carbohydrates (e.g. plant polysaccharides)

4.3 DNA Integrity

DNA integrity can be assessed by agarose gel electrophoresis. DNA should appear as a clear, high molecular weight band. There should be no indication of RNA contamination (which will present as faint bands toward the bottom of the gel).

4.4 Sample Quantity

Quantification of gDNA by dsDNA assay such as PicoGreen of Qubit is highly recommended. Table 4, shows quantity and concentration of DNA requested for library preparation services.

Table 4: Sample requirements for DNA library preparation services*

Library Type	Sample Type	DNA Quantity	DNA Concentration	DNA Volume
Illumina DNA Prep PCR-Free (STD input)	gDNA, amplicons >500 bp	≥300ng	≥20ng/µl	≥30µl
Illumina DNA Prep-M (STD input)	gDNA, amplicons >500 bp	≥100ng	≥10ng/µl	≥25 µl
Illumina DNA Prep-M (STD input) larger genomes	gDNA, amplicons >500 bp	≥300ng	≥15ng/µl	≥25 µl
IDT xGen cfDNA &FFPE library prep (STD input)	gDNA, amplicons >100 bp	≥300ng	≥20ng/µl	≥25 µl

*please contact AGRF if your inputs are lower than listed. We can typically accommodate low inputs.

5.0 Sample Returns/Discards

Samples are stored with AGRF for 3 months after you receive your data except for our clinical WGS and clinical exome services which are stored for 1 year. If you wish for your samples to be returned, you must discuss this with your account manager during quoting or contact us after you receive your data. At the completion of your project, we can either:

1. Return your samples by courier at ambient (please ask your account manager for a quote).
2. Return samples by courier with dry ice (please ask your account manager for a quote).

If we are not notified within the specified time frame, samples will be automatically discarded.

6.0 Sample Submission

6.1 Online Submission

- In the client portal, select 'Next Generation Sequencing' from the service dropdown menu.
- Enter your species and submission format (tube or plate).
- Complete and upload the template file.
- ≤23 please complete tube submissions.
- ≥24 Please complete plate submissions, (an additional handling charge per sample will occur if tubes are used).
- We recommend shipping plates that are heat-sealed, or strip-cap sealed on dry ice.
- Submit the form and print the submission receipt to be included with your sample package.

6.2 Packing of Samples

- DNA samples can be sent at ambient temperature or on ice blocks.
- If you are sending your samples in plates, please use strip caps to seal the plates.
- Sample tubes or plates should be in a zip-lock bag or box to avoid direct contact with dry ice.

Samples must be shipped to AGRF in tubes or 96 well plates and be clearly labelled and sealed.

- Samples in tubes can be shipped at room temperature via express post.
- For samples in plate format, we recommend shipping on dry ice to avoid potential cross contamination of liquid between wells during transit due to air pressure changes in flight.

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.

Post/send/deliver samples to the addresses below:

Melbourne Service by post
ATTN Next Generation Sequencing AGRF
LEVEL 13, VCCC
305 GRATTAN STREET
MELBOURNE VIC 3000

If sending by courier::
ATTN Next Generation Sequencing AGRF
VCCC LOADING DOCK
14 FLEMINGTON ROAD
NORTH MELBOURNE VIC 3051
(note: our loading dock is open from 8am to 4pm weekdays)

7.0 Let AGRF Extract Your Samples for You

Avoid the hassle of extracting DNA yourself and let AGRF do this step for you. Our Extraction Service works with a wide range of DNA sources and prepares DNA to meet the requirements of our service. Please contact AGRF for a quote or assistance with your extraction.
Phone: 1300 247 301
Email: CustomerCare@agrf.org.au

8.0 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.