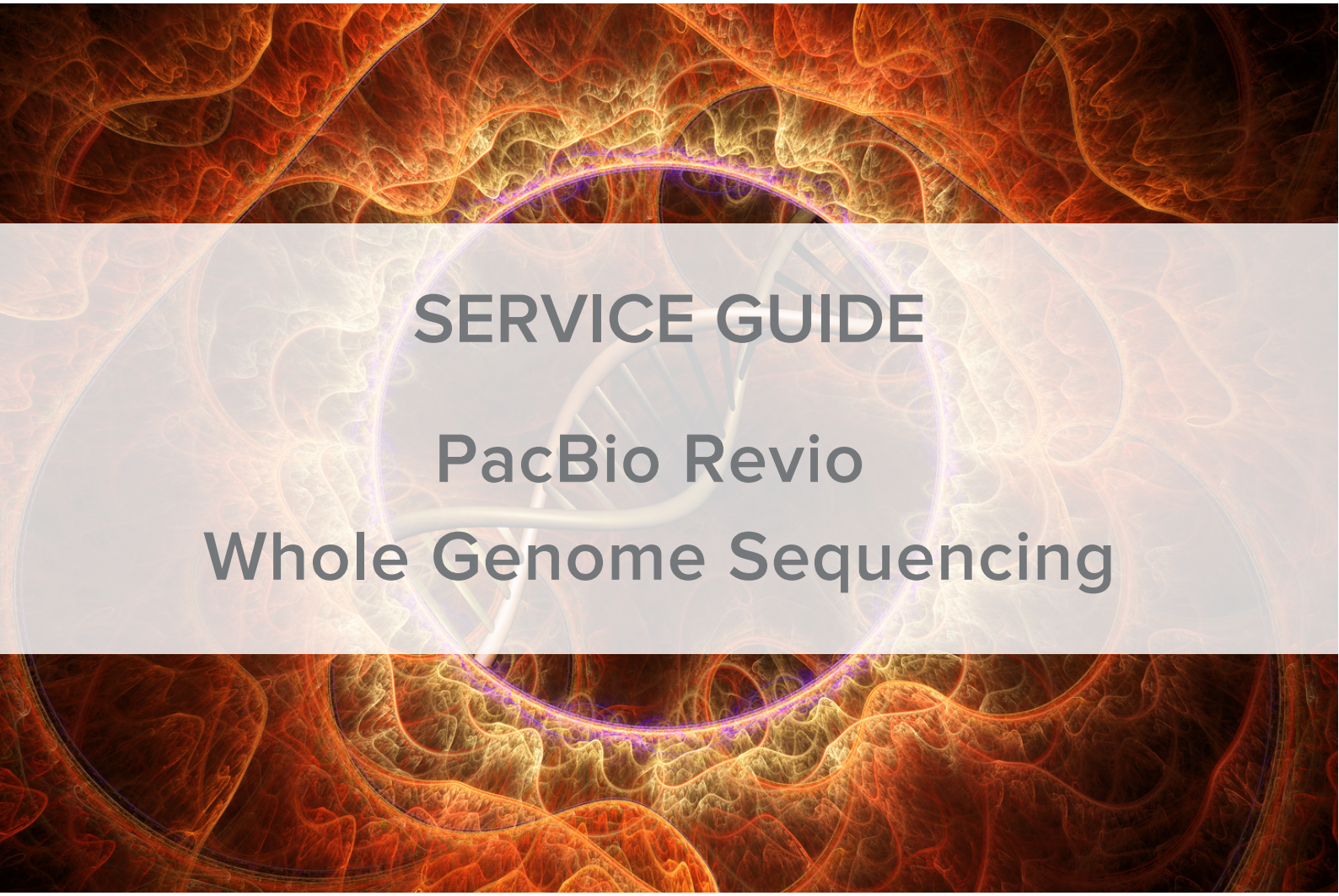


SERVICE GUIDE

PacBio Revio
Whole Genome Sequencing

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

SVG2404PCBWGS

Service Guide

PacBio Revio WGS

1.0 Overview	3
2.0 HiFi reads for Whole Genome Sequencing (WGS)	3
3.0 Sequencing Recommendations and sample requirements	4
4.0 Sample requirements	5
5.0 Size Selection	6
6.0 Data Output	7
7.0 Sample Submission	7
8.0 Sample returns and discards	8
9.0 Quality Statement	8

Service Guide

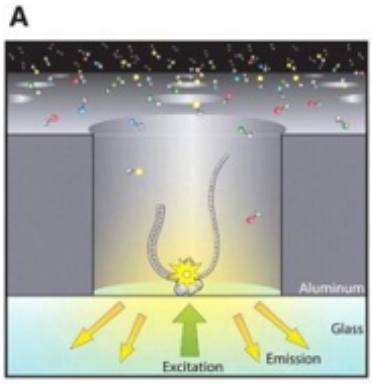
PacBio Revio WGS

1.0 Overview

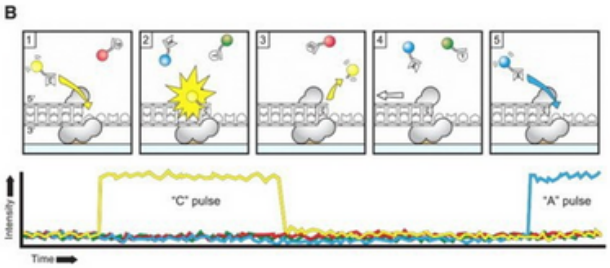
The Pacific Biosciences (PacBio) long-read sequencing platform utilizes single-molecule, Real-Time (SMRT) sequencing technology, incorporating circular consensus sequencing to generate Q20+ High-Fidelity (HiFi) reads and automatically provide simultaneous epigenetic information along with your sequencing output. At AGRF, we proudly feature the cutting-edge PacBio Revio system, offering significantly enhanced capabilities in long-read sequencing and producing substantially more high-quality data than its predecessors.

Figure 1: Visual representation of SMRT sequencing in action.

A) Template material is bound within the ZMW with a DNA polymerase incorporating a fluorescently labelled nucleotide complimentary to the template material.



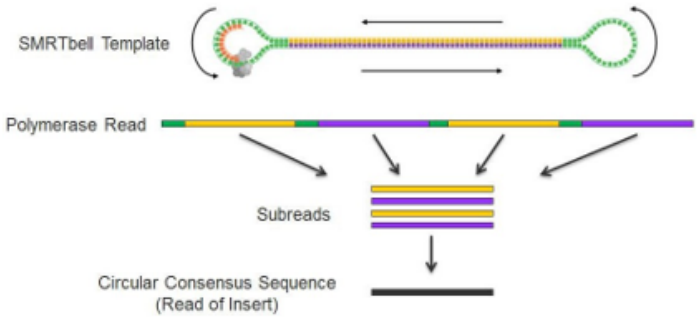
B) As the polymerase moves along the template material, the fluorophore fluoresces as it's incorporated, and the emission is detected using a nano-sized camera with each colour representing a different nucleotide.



2.0 HiFi Reads for Whole Genome Sequencing

Before sequencing, the target material must undergo library preparation which is the process of preparing your samples for sequencing. This process typically involves fragmentation and attaching of oligo- nucleotide adapters to facilitate sequencing. PacBio libraries require circularisation which is the process of attaching SMRTbells to the template, allowing the polymerase to repeatedly sequence the same template for improved accuracy (Figure 2). This allows the generation of circular consensus (CCS) reads ranging from Q20 up to Q50 in quality. In a typical WGS library, an average Q30 score is observed.

Figure 2: Graphical representation of the SMRTBell adapters as part of the template.This allows the polymerase to perform multiple passes of the same template molecule to generate high quality long read data. The multiple passes are polymerase reads and then the consensus of the polymerase reads are the CCS reads.



Samples submitted for High-Fidelity (HiFi) library construction are derived from genomic DNA (gDNA) and undergo a thorough quality control (QC) screening process. The preparation involves mechanical shearing, targeting a length range of 15-18 kb, followed by SMRTbell® prep kit 3.0 library construction. Subsequently, the library undergoes a BluePippin size selection, eliminating all fragments below 10 kb. The final step involves sequencing on the PacBio Revio instrument (refer to Figure 3). Detailed DNA sample requirements for both workflows are outlined in Table 1.

Figure 3: HiFi and Microbial Multiplexing workflow summary.

3.0 Sequencing recommendations and Sample Requirements.

The sequencing and input requirements for all PacBio Revio services provided can vary significantly based on the sample type and the intended type of analysis.

Please note that the standard WGS metrices are based on a Human genome - The multiplexing level can vary based on the size of the genome you are sequencing.

Table 1: Summary of input requirements for AGRF-offered PacBio Whole Genome Sequencing (WGS) library preparation. *Expected output can vary based on many factors, including provided sample quality and sample type.

	Standard WGS	Microbial WGS
Required input	> 5µg per sample	> 1µg per sample
Multiplexing per SMRT Cell	Up to 3 (Human)	Up to 96
Target Insert Size	15-18kb	7-10kb
Fragmentation	Mechanical	Mechanical
Expected output	60-90Gb* HiFi data	Minimum 150Mb per microbe

Please note: Standard whole-genome sequencing (WGS) protocols can multiplex over 10 samples for small genomes on a single SMRT cell.

Table 2: General recommendations for sequence depth per SMRT Cell.

Application	Coverage requirement
De novo genome assembly	>15x **per haplotype**
Microbial Whole Genome Assembly	>15x per microbe
Variant Detection	>10x for structural variants >30x for all variant classes

4.0 Sample Requirements.

The quality of the sample is a critical factor for the success of PacBio experiments. While AGRF conducts quality control (QC) before initiating a project, we strongly recommend that you verify your material before submission.
For DNA samples, the assessment of DNA integrity can be carried out through agarose gel electrophoresis or microfluidic assays, such as the Agilent gDNA TapeStation assay, Agilent FemtoPulse, Sage Science PippinPulse, or PerkinElmer GX systems (see Figure 4). High-quality DNA should appear as a clear, high molecular weight band without any indication of RNA contamination, as evidenced by faint bands or smears toward the bottom of the gel. Additionally, quantification of genomic DNA (gDNA) using a double-stranded DNA (dsDNA) assay with fluorescence, such as Quantifluor or PicoGreen, or Qubit, is highly recommended (refer to Table 3).

Figure 4: Example of DIN assessment using an Agilent 2200 gDNA TapeStation assay

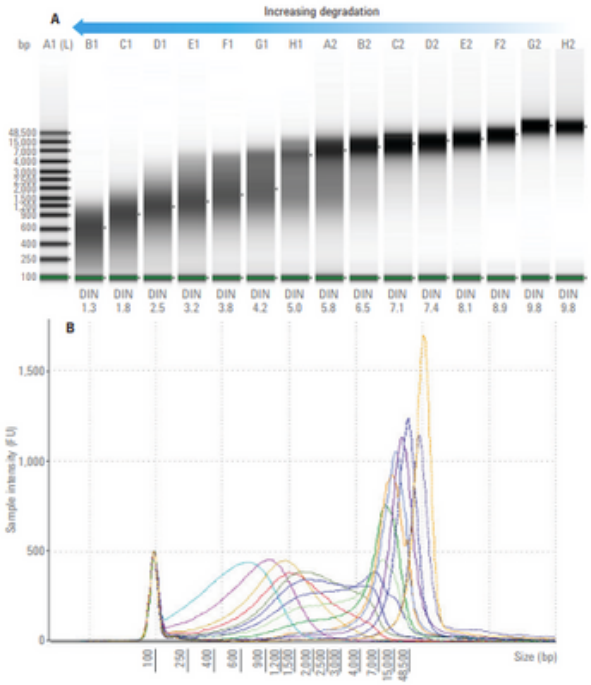


Table 3: Recommendations for sample purity as assessed by absorbance spectra.

Ratio	Target (RNA)	Target (DNA)	Low Ratio (<1.6) indications
A260/280	2.0	1.8-2.0	Residual phenol from extraction, proteins, or very low conc. of nucleic acids (<1ng/µl)
A260/A230	≥2.0		Residual guanidine from the extraction protocol Carryover of carbohydrates (e.g. plant polysaccharides)

Table 4: Sample Requirements of DNA library preparation services.

Library Type	Sample Type	Quantity	DNA Concentration
HiFi	gDNA (≥8 DIN) >20-40Kbp peak size	≥5µg	≥50-100 ng/µl
Multiplexed Microbial	gDNA (≥8 DIN) >20-40Kbp peak size	≥1µg	≥50-100 ng/µl

The standard input for any genome sequencing project is high quality genomic DNA (DNA Integrity Number DIN ≥ 8). Some recommended protocols for the isolation of DNA for PacBio sequencing are listed below (alternatively, please contact us regarding HMW extraction at AGRF):

Column-based extraction protocols or equivalent, specific for high-molecular weight recovery (example kits below):

- PacBio Nanobind PanDNA kit.
- Qiagen Genomic-Tip 20/100/500/G kit.
- GeneJET Plant Genomic DNA Purification kit.
- Lucigen Master pure Kit.

Ensure the extraction protocol includes an RNase treatment to ensure removal of RNA. Purified DNA should be eluted/resuspended in 10 mM Tris-Cl (pH 8.5 – also known as buffer EB), or nuclease- free water. Do not use EDTA-containing buffers as this can inhibit PCR reactions.

For best results we recommend following the PacBio sample and extraction guides below.

- Technical Note - Preparing DNA for PacBio HiFi Sequencing - Extraction and Quality Control
- Technical Note - Preparing Samples for PacBio Whole Genome Sequencing for de novo Assembly - Collection and Storage

Organism specific protocols recommended by PacBio can also be found here.

5.0 Size selection

We aim to size select all samples using the Bluepippin system with 10kb cutoff, where DNA quantity allows. In circumstances where not enough DNA is provided, we will instead apply a bead-based size selection method. Smaller insert size averages, and potentially lower gigabase output is seen in some cases.

Figure 5. BluePippin size-selection library with a 10kb cutoff.

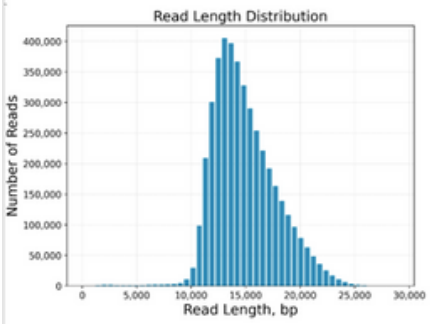
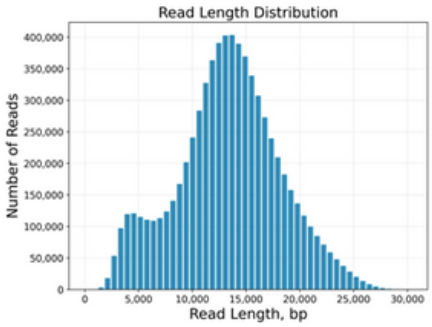


Figure 6. Bead-based size-selection library with a 3-5kb cutoff.



6.0 Data Output

All PacBio sequencing projects will undergo quality control (QC) analysis to assess the quality of both the sequencing and library preparation. AGRF will provide HiFi reads in the native PacBio BAM format.
If you are submitting for microbial WGS, we will also provide complimentary microbial genome assemblies, which will include base modification analysis.
AGRF also provides options for sequencing analysis including genome alignment, mapping as well as de novo assembly. If you have analysis requirements or wish to talk about your specific goals for your project, please discuss these during the quotation process.

7.0 Sample Submission

7.1 Online Submission

- In the client portal, select 'LRS' 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

7.2 Packaging 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.

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:

Postal Address (mail)

AGRF PACBIO REVIO SERVICE
GEHRMANN LABORATORIES RESEARCH RD
UNIVERSITY OF QUEENSLAND BRISBANE QLD 4072

Physical address (courier)

AGRF PACBIO REVIO SERVICE
AGRF LTD
LEVEL 5, GEHRMANN LABORATORIES RESEARCH RD
UNIVERSITY OF QUEENSLAND BRISBANE QLD 4072

8.0 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 let us 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.

9.0 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.