SERVICE GUIDE

Sequencing Ready Libraries

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

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Service Guide

Sequencing Ready Libraries

1.0 Overview

Our Sequencing Ready Library Service is designed for clients who prefer to prepare their own libraries and have greater control during the library preparation process. The process starts with clients preparing a library, then submitting it to AGRF for sequencing on our Illumina MiSeq, NextSeq 2000, or NovaSeq X Plus platforms. This service is also suitable if you have prepared a library using workflows that we do not offer.

2.0 Workflow

This service is split into two workflows:

- Pooled libraries ready for sequencing.
- Individual libraries requiring QC, normlisation, pooling and then sequencing.

We strongly recommend submitting dimer-free libraries. If your library contains a dimer, an additional clean-up fee will be applied. Please note that we do not guarantee the performance or success of the clean-up. It is important that clean-ups are completed prior to submission as additional clean-ups can bias your sequencing profiles.

Libraries provided for both workflows will also undergo vigorous quality control prior to sequencing to ensure the best sequencing outcome is produced and confirm libraries are intact during transportation. This includes:

- Library sizing assessment: Agilent Tape Station
- Library quantitation: qPCR

The sequencing configurations we offer as part of this service are listed in Table 1. However, if you require custom read lengths such as 50 bp paired-end (PE) or 200 bp single-read (SR), or custom sequencing primers on the NovaSeq X Plus, you will need to purchase the entire flow cell as we are unable to apply custom configurations to individual lanes (see section Technical Considerations below for more details). Further, custom sequencing primers on the NextSeq 2000 require an additional ancillary kit to be purchased due to limitations of the system.

3.0 Technical Consideration

We cannot guarantee sequencing outputs or Q30 for libraries we have not prepared, however, as per Table 1, we can provide minimum data outputs and Q30 estimates as a rule for most workflows. To ensure we can provide you with the best outcomes, we recommend the following:

1. Please provide us with the protocol you have used to generate your libraries. This allows us to ensure the sequencing requirements of your protocol will be met.

Please check your libraries prior to submission and ensure you complete a bead-cleanup to remove excess dimers. If we find that your libraries contain dimers, you can either resubmit them after cleanup or we can clean them for you at a cost. AGRF will perform quality control prior to commencing a project, however, we recommend the client also checks libraries before submission. We are happy to review QC data prior to submitting samples. Please forward to techsupport@agrf.org.au

2. If you are providing custom sequencing primers, please ensure that each primer must be 100 µM in concentration.

3. Amplicon libraries have the following requirements to ensure the highest quality data is generated with reasonable data outputs. If you request an alternative to this, we are happy to facilitate it, but we cannot guarantee the outcome. It is important that we are notified if you're submitting an amplicon library:

- MiSeq – all amplicon runs are clustered at 7 pM with a minimum 25% PhiX.
- NextSeq 2000 – all amplicon runs are clustered at 650 pM with a minimum 35% PhiX.
- NovaSeq X – all amplicon runs are clustered at 150 pM with a minimum 35% PhiX.

4. If you are completing a 10X library preparation or Nanostring workflow you must:

- Provide us with the exact 10X Nanostring workflow utilized as each workflow has different PhiX requirements.
- If you require the custom 10X sequencing configuration, you must purchase a flow cell (not a lane), as we cannot apply the configuration to individual lanes.
- Alternatively, if you have the capability to mask bases or trim reads to your desired length and you'd like to purchase a lane, we can offer 150 PE lanes. Please note: All Nanostring libraries require the purchase of an entire flow cell.

5. It is important that clients refer to the manufacture's guidelines for low-plex sequencing pooling guidelines before assigning indexes to your libraries. Illumina have recently updated there pooling strategies to exclude using Illumina UD indexes Set C and Set D from low-plexity pooling for sequencing on the NovaSeq X plus. Please refer to link.

Table 1: AGRF's standard Illumina sequencing configurations. The minimum read outputs and quality scores are based on a high diversity library prepared using a standard library preparation workflow such as Illumina DNA PCR-Free Prep. Minimum reads do not factor PhiX spike-in or non-standard workflows.

Instrument/mode	Chemistry	Read length	% bases ≥30	Min # Read/lane
MiSeq	V2	50 cycles (50SR)	≥85	9 M
		300 cycles (150 PE)	≥80	
		500 cycles (250 PE)	≥75	
	V3	150 cycles (75 PE)	≥80	12 M
		600 cycles (300 PE)	≥70	
	V2 Nano	300 cycles (150 PE)	≥ 80	750 K
		500 cycles (250 PE)	≥ 75	
	V2 Micro	300 cycles (150 PE)	≥ 80	3M
NovaSeq X Plus	10B	300 cycles (150PE)	≥ 85	1 B
NextSeq 2000	P1	100 cycles (100 SR)	≥ 85	Up to 100 M
	P1	300 cycles (150 PE)	≥ 85	
	P1	600 cycles (300 PE)	≥ 80	Up to 100 M (Amplicons typically yield 50 – 70M usable reads)
	P2	100 cycles (100 SR)	≥ 85	Up to 400 M
	P2	300 cycles (150 PE)	≥ 85	
	P2	600 cycles (300 PE)	≥ 80	Up to 300 M (Illumina limit outputs for P2 600 kits)
	P3	100 cycles (100 SR)	≥ 85	Up to 1.2 B
	P3	300 cycles (150 PE)	≥ 85	

6. We can demultiplex libraries based on i5/i7 indexes. Libraries containing in-read indexes cannot be demultiplexed using our standard pipelines. This will require either custom demultiplexing with our bioinformatics team or we can supply you bcls or non-demultiplexed fastq files.

7. We do not complete adapter trimming as part of this service. If you require this, please let us know and our Bioinformatics Team will be able to assist you.

Please feel free to email AGRF NextGenTeam at techsupport@agrf.org.au if you have any concerns or require additional technical information.

4.0 Submitting Libraries to AGRF

Indexes must be supplied with your submission details. Failure to fill in these details will delay the processing of your samples.

4.1 MiSeq Submissions.

- ≥ 20 µL of each pool/library containing no dimers and ≥ 10 nM in concentration.
- Pools/libraries should be supplied in either Qiagen Buffer EB or nuclease-free water or 10 mM Tris-HCl pH 8.5.
- A Bioanalyzer/GX/TapeStation etc report must be provided during submission for us to compare against.
- If your libraries are found to contain dimers and require cleaning, a charge will be applied per cleanup. AGRF takes no responsibility if cleanup does not perform as intended.
- Indexes must be supplied as per the standard MiSeq workflow (not reverse complimented i5).

4.2 NovaSeq X Plus / NextSeq 2000 submissions.

- ≥ 30 µL of each pool/library containing no dimers and ≥ 10 nM in concentration.
- Pools/libraries should be supplied in either Qiagen Buffer EB or nuclease-free water or 10 mM Tris-HCl pH 8.5.
- A Bioanalyzer/GX/TapeStation/etc report must be provided during submission for us to compare against.
- If your libraries are found to contain dimers and require cleaning, a charge will be applied per clean-up. AGRF takes no responsibility if cleanup does not perform as intended.

Indexes must be supplied as per the standard NovaSeq X Plus /NextSeq 2000 workflow, (NOT reverse complimented i5).

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 How to Submit Samples

Online Submission:

- Submit your sample details online.
- Select: “Next-Generation Sequencing” as the Service Type.
- 0≤23 pools/libraries:
  - Please complete tube submissions.
- ≥24 pools/libraries:
  - Please complete plate submissions, (an additional handling charge of $1.50 per sample will occur if tubes are used).
- We recommend shipping plates that are heat-sealed, or strip-cap sealed on 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.

- Submission Format – by selecting tube or plate, the “SampleFile” template link will appear. Click “Download Template” and enter your sample details:
- Sample Name can be up to 40 characters in length.
- You can use numbers, letters, underscores and hyphens.
- The first character must be a letter or number. For example: Sample-1_b.
- An error will occur if a name is duplicated.
- Save and close completed Template File locally, select “Browse” to upload file.
- Submit and print a paper copy of your sample submission receipt that will be generated as a PDF file.

This receipt must be included with your sample package.

Post/send/deliver samples to the addresses below:

Melbourne MiSeq/NextSeq2000/NovaSeqX service:
MELBOURNE CLIENT LIBRARY SERVICE
AUSTRALIAN GENOME RESEARCH FACILITY
LEVEL 13,
VICTORIAN COMPREHENSIVE CANCER CENTRE
305 GRATTAN STREET
MELBOURNE, VIC, 3000

Sydney MiSeq/NextSeq2000 service:
AUSTRALIAN GENOME RESEARCH FACILITY
THE WESTMEAD INSTITUTE FOR MEDICAL RESEARCH
176 HAWKESBURY ROAD
WESTMEAD, NSW 2145

Perth MiSeq service:
AUSTRALIAN GENOME RESEARCH FACILITY
LEVEL 6, MEDICAL RESEARCH FOUNDATION BUILDING
ROYAL PERTH HOSPITAL
REAR 50 MURRAY STREET
PERTH, WA 6000

7.0 Results and Data Outputs

All client library sequencing projects will undergo quality control to assess the quality of the sequencing run. AGRF will provide the following results and data;

- The raw FASTQ outputs for your individual samples.
- We do not complete adapter trimming.

Please contact us if you require bioinformatics or adapter trimming.

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.