SERVICE GUIDE

Plasmid Express and Amplicon Access Service

CustomerCare@agrf.org.au
1300 247 301
www.agrf.org.au
AGRF Ltd.
SVG2509WPLASAMP

Service Guide

Plasmid Express and Amplicon Access

Service Guide

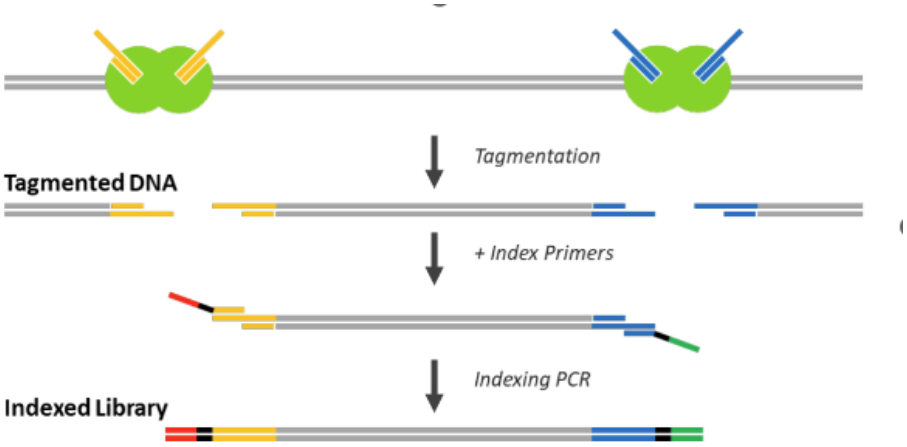
Plasmid Express and Amplicon Access

1.0 Overview

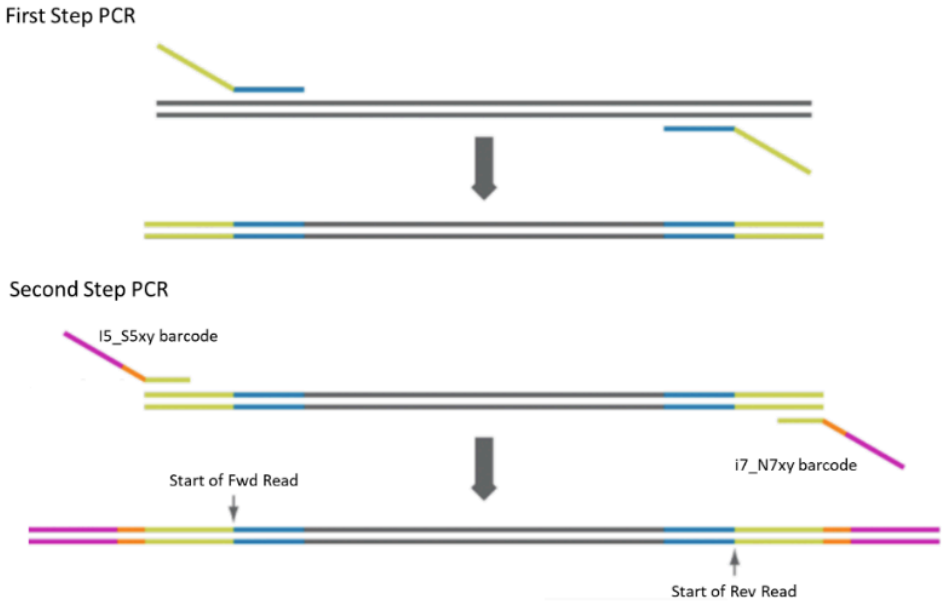
AGRF's Whole Plasmid and Amplicon Access service is a small-scale next generation sequencing (NGS) service designed to make the Illumina short read platform accessible to researchers for small projects, or genomes. The service is ideally suited to the sequencing of whole plasmids and long PCR products (> 1 kb), but also caters for short PCR amplicons (150 - 250 bp) that have been prepared using Illumina Nextera overhangs. Please note Amplicon Access Indexing is only available in our Brisbane Laboratory.

Figure 1 Schematic of the two available workflows for the preparation of DNA libraries for Plasmid Express and Amplicon Access.

1a) ExpressPlex Library – Whole Plasmids and Long PCR Products (> 1 kb) Service only available in Sydney



1b) Amplicon Access – Short PCR products (between 150 – 250 bp). Service only available in Brisbane



This service is based on the Illumina 2-Stage (or 2-Step) library preparation protocol designed to partner with our Illumina MiSeq platform and provides you with the flexibility to reduce the cost of amplicon-based NGS by having you perform the first-stage PCR (see Section 2.2). This is helpful for people working with CRISPR or challenging templates because it allows you to adjust and perform the initial PCR step using customised protocols, which may not be compatible with our full 2-Stage library preparation kit.

2.0 Workflow

There are two workflows available for this service, which are dependent on the type and size of the submitted DNA sample (see Figure 2 for Workflow Entry Points).

Figure 2. Workflow outline for the creation and sequencing of Plasmid Express, or Amplicon Indexing Libraries. Sample entry points highlighted (2.1, 2.2).

2.1 seqWell ExpressPlex – Whole Plasmids and Long PCR Amplicons (> 1kb).

We employ the seqWell ExpressPlex™ 2.0 Library Preparation Kit, which utilizes a streamlined, one-step tagmentation process powered by the engineered TnX transposase. This method simultaneously fragments DNA and inserts custom adapter sequences, facilitating efficient library construction.

Please note Plasmid Express is only available in our Sydney Laboratory.

2.2 Amplicon Indexing – Short PCR Amplicons (150 - 250 bp amplicons, not including tags/barcodes). Brisbane only.

This service requires you to complete the first-stage PCR using the Nextera-tagged, target specific primers:

- Forward primer: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[Target Specific Primer]-'3
- Reverse primer: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[Target Specific Primer]-'3

After the first-stage PCR is complete, and successful amplification has been confirmed on a gel (which must be provided to AGRF at the time of submission), clients can send the uncleaned amplicons to AGRF for further processing.

Submitted amplicons will be cleaned using magnetic beads, and dual unique 10 bp indexes added by PCR amplification. Following indexing, the samples will again be cleaned and submitted for QC. Libraries passing QC will be pooled, normalized, and sequenced.

Please note Amplicon Access is only available in our Brisbane Laboratory.

3.0 Technical Considerations

- Sequencing is performed on a MiSeq, therefore the amount of data is only appropriate for samples such as whole plasmids or large PCR amplicons. Larger bacterial, or fungal genomes can be sequenced with our larger-scale WGS service.
- For amplicons and whole plasmids less than 1 kb in size: the transposase will not fragment DNA evenly, and as a result samples <1 kb are not recommended for this service.
- Large amplicon libraries will have a lower coverage of sequence at the ends of the amplicon.
- Large PCR amplicons should not contain any internal modifications, fluorophores or dark quenchers.
- Please note that our Plasmid Express service is unable to detect plasmids that contain concatamers, dimers, trimers, or tetramers. This limitation arises because short-read assembly algorithms cannot distinguish between these structures; they interpret them as increased coverage of the same sequence rather than as extensions of the plasmid.
- Important: If you observe double bands on your agarose gel, this is an indication that your plasmid may contain these structures. To accurately identify and resolve these complex plasmid forms, we recommend using Sanger sequencing or long-read sequencing technologies.

Indexed Amplicons:

- The sequencing configuration for Plasmid Express and Amplicon Access is 150 bp paired ends. Therefore, the indexed amplicons should be between 150 - 250 bp in length (this is the length of the targeted amplicon minus the Nextera tags).
- Amplicons of different sizes in the same sequencing run, for instance the two extremes: 150 bp and 250 bp, may yield different numbers of sequencing reads. This is due to amplicons of smaller size clustering more efficiently on the flow cell, leading to their higher representation (and thus, generate more data) within the sequencing pool. AGRF will employ a pooling offset to account for the differences in size and to minimize variability in sequencing reads.
- Short PCR amplicons should not contain any internal modifications, fluorophores or dark quenchers.
- Service only available in Brisbane.

4.0 Sample Submission Requirements

Plasmid Express Library Requirements

- Purified Whole Plasmid: 400 ng resuspended in 20 µL water or 10 mM Tris.HCl.
- Purified Long (≥ 1 kb) PCR amplicon: 400 ng resuspended in 20 µL nuclease-free water or 10 mM Tris.HCl.

Amplicon Access Requirements.

- 150-250 bp PCR product: 20 uL of unpurified PCR product.

Please include a Gel QC image of isolated plasmid, or successful amplicon generation with your sample submission.Note: DNA concentration is best determined by fluorometry (i.e. QUBIT). Amplicon concentrations are best estimated from gel QC using a DNA molecular weight ladder with bands of known mass.

5.0 Sample and Data Storage

Samples are stored with AGRF for 1 month after you receive your data. 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:

- Return your samples by courier at ambient (please ask your account manager for a quote).
- 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.

Files will remain on the AGRF server for 1 month before being removed. A backup copy will be archived at AGRF for a further 2 months. Charges will apply for restoring archived files to the server.

6.0 How to Submit Samples

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 samples:
  - Please complete tube submissions.
- ≥24 samples:
  - 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.
- Submit the form and print the submission receipt to be included with your sample package.

Packaging of Samples

- DNA samples can be shipped at room temperature via courier or express post.
- To prevent leakage in transit use parafilm to seal your tubes. Plates should be heat sealed or sealed with strip caps.

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.

Table 1. Plate Format: Plasmid Express Library submissions.

Plate	Well	Sample name	Vol (µl)	[DNA] (ng/µl)	Index (i7)	Index (i5)	Pooling % / Phix / Comments	Sample Group	Gender
Plate 1	A01	Plasmid_1	20	20			Tran	3.5kb	
	A02	Plasmid_2	20	20			Tran	10kb	
	A03	Long Amplicon_1	20	20			Tran	5.2kb	
	A04	Long Amplicon_2	20	20			Tran	5.2Kb	

Table 2. Plate Format: Amplicon Access library submissions.

Plate	Well	Sample name	Vol (µl)	[DNA] (ng/µl)	Index (i7)	Index (i5)	Pooling % / Phix / Comments	Sample Group	Gender
Plate 1	A01	Short amplicon_1	20	10			Index	200bp	
	A02	Short amplicon_2	20	10			Index	250bp	
	A03	Short amplicon_3	20	10			Index	200bp	
	A04	Short amplicon_4	20	10			Index	250bp	

Table 3. Tube Format: Plasmid Express library submissions.

Plate	Well	Sample name	Vol (µl)	[DNA] (ng/µl)	Index (i7)	Index (i5)	Pooling % / Phix / Comments	Sample Group	Gender
1	Plasmid_1	LP_1	20	20			Tran	3.5kb	
2	Plasmid_2	LP_2	20	20			Tran	10kb	
3	LongAmplicon_1	LA_1	20	20			Tran	5.2kb	
4	Long Ampliocn_2	LA_2	20	20			Tran	5.2Kb	

Table 4. Tube Format: Amplicon Access library submissions.

Plate	Well	Sample name	Vol (µl)	[DNA] (ng/µl)	Index (i7)	Index (i5)	Pooling % / Phix / Comments	Sample Group	Gender
1	Short amplicon_1	SA_1	20	10			Index	200bp	
2	Short amplicon_2	SA_2	20	10			Index	250bp	
3	Short amplicon_3	SA_3	20	10			Index	200bp	

Post/send/deliver samples to:

Plasmid Express Service

Sydney

Attention: Plasmid Express Service AGRF Ltd.
THE WESTMEAD INSTITUTE FOR MEDICAL RESEARCH
176 HAWKESBURY ROAD
WESTMEAD, NSW 2145

Amplicon Access Service

Brisbane

Attention: Amplicon Access AGRF Ltd.
Level 5 Gehrmann Laboratories (Building 60)
Research Road
University of Queensland
St Lucia, QLD 4072

7.0 Results and Data Outputs

All Amplicon Access projects will undergo quality control to assess sequencing and indexing quality. AGRF will provide the following results and data:

- > 5000 reads (at 150 bp paired end) per sample.
- The FASTQ outputs for your individual samples.

For Whole Plasmid Express submissions:

- Fasta formatted text file of assemblies generated by an automated Shovill Pipeline

8.0 Downstream Data Analysis

Our knowledgeable Bioinformatics team is well-experienced to handle your data analysis and visualizations. Please contact your Account Manager if you require bioinformatics.