



SERVICE GUIDE

Plasmid and Amplicon Express Service

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SVG2403PLASAMP

Service Guide

Plasmid and Amplicon Express



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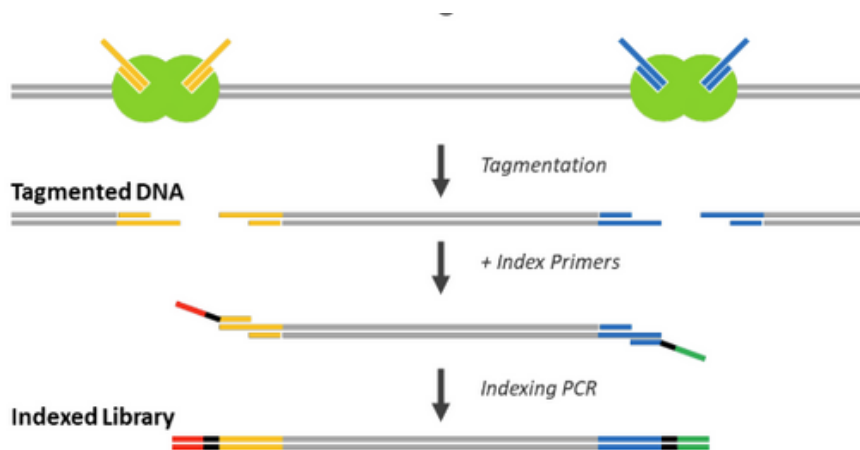
Plasmid and Amplicon Express

1.0 Overview

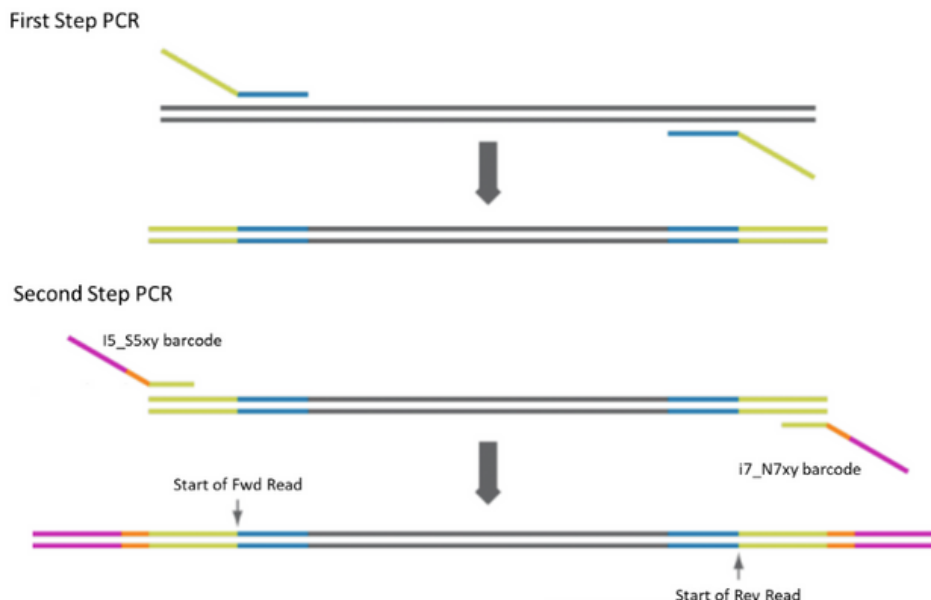
AGRF's Plasmid and Amplicon Express service is a small-scale next generation sequencing (NGS) service designed to make the Illumina short read platform accessible to researchers for small projects, or genomes. The service is ideally suited to the sequencing of whole plasmids and long PCR products (>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

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

1a) Transposase Libraries – Whole Plasmids and Long PCR Products (> 1kb)



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



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.

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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 Transposase, or Amplicon Indexing Libraries. Sample entry points highlighted (2.1, 2.2).



2.1 Transposase Libraries – Plasmids and Long PCR Amplicons (> 1kb).

This is a method of creating a small-scale library using Tn5 transposase, which inserts a custom DNA linker sequence through a "cut and paste" process called tagmentation. This linker sequence enables subsequent PCR-based indexing with dual 10bp indexes. Following this samples are purified and selected for size by magnetic beads. After passing QC, the libraries are pooled, normalized, and sequenced.

2.2 Amplicon Indexing – Short PCR Amplicons (150 - 250bp amplicons, not including tags/barcodes). Perth 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 10bp 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 Indexing is only available in our Perth Laboratory.

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3.0 Technical Considerations

- Sequencing is performed on a MiSeq, therefore the amount of data is only appropriate for samples such as plasmids or large PCR amplicons. Larger bacterial, or fungal genomes can be sequenced with our larger-scale WGS service.
- For amplicons less than 1kb in size: the transposase will not fragment DNA evenly, and as a result amplicons <1kb 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.

Indexed Amplicons:

- The sequencing configuration for Plasmid and Amplicon Express is 150bp paired ends. Therefore, the indexed amplicons should be between 150 - 250bp 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: 150bp and 250bp, 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 Perth.

4.0 Sample Submission Requirements

Transposase Library Requirements

- Purified Plasmid: 400ng resuspended in 20µL water or 10mM Tris.HCl.

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

Amplicon Indexing Requirements (Amplicon Indexing is only available in our Perth Laboratory).

- 150-250bp PCR product: 20uL 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 Returns/Discards

Samples are stored with AGRF for 1 months 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.

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.
- \leq 23 pools/libraries:
 - Please complete tube submissions.
- \geq 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.
- 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: Transposase 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	25	20			Tran	3.5kb	
	A02	Plasmid_2	25	20			Tran	10kb	
	A03	Long Amplicon_1	25	20			Tran	5.2kb	
	A04	Long Amplicon_2	25	20			Tran	5.2Kb	

Table 2. Plate Format: Amplicon Indexing 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: Transposase 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	25	20			Tran	3.5kb	
2	Plasmid_2	LP_2	25	20			Tran	10kb	
3	LongAmplicon_1	LA_1	25	20			Tran	5.2kb	
4	Long Amplicon_2	LA_2	25	20			Tran	5.2Kb	

Table 4. Tube Format: Amplicon Indexing 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:

ATTENTION: PLASMID AND AMPLICON EXPRESS SERVICE
 AGRF PERTH
 LEVEL 6, MEDICAL RESEARCH FOUNDATION BUILDING
 ROYAL PERTH HOSPITAL
 REAR 50 MURRAY STREET
 PERTH, WA 6000

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Postal Address (mail)

ATTENTION: PLASMID EXPRESS SERVICE
AGRF SYDNEY
AUSTRALIAN GENOME RESEARCH FACILITY
PO BOX 285
WESTMEAD, NSW 2145

Physical address(courier)

ATTENTION: PLASMID EXPRESS SERVICE
AGRF SYDNEY
AUSTRALIAN GENOME RESEARCH FACILITY
THE WESTMEAD INSTITUTE FOR MEDICAL RESEARCH
176 HAWKESBURY ROAD
WESTMEAD, NSW 2145

7.0 Results and Data Outputs

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

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

For 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.