



MASSEY GENOME SERVICE JULY 2011 NEWSLETTER

Welcome to another issue of the Massey Genome Service (MGS) Newsletter. Over the past few months there have been some changes to the service as we prepare for the re-launch of Next Generation Sequencing with some exciting new technology and improve our overall operations.

UPDATE ON STAFF

The Massey Genome Service (MGS) currently employs the following staff:

Lorraine Berry – Laboratory/QA Manager: Has been with MGS for 13 years in a technical role and has recently taken up this new role. Lorraine is responsible for the overall operations of the laboratory and Quality Assurance Management.

Richard Fong – Senior Laboratory Technician: Has worked at MGS for 4 years, mostly in ABI Sanger Sequencing and also some Next Generation Sequencing. Richard is mostly involved in the technical operation of the laboratory and troubleshooting.



Left to Right: Lorraine Berry, Olga Kardailsky, Richard Fong

Olga Kardailsky – Laboratory Technician: Olga has been working with the service for 4 years and mainly undertakes the routine processing and testing of the samples submitted for ABI Sanger sequencing and well as assisting with customer enquiries.

Please direct all customer service enquiries to Lorraine Berry and all technical enquires regarding template quality, concentrations and preparation, and troubleshooting of data, can be directed to Richard Fong. Our e-mail and phone contacts are provided on the MGS website at <http://genome.massey.ac.nz>

INTRODUCTION OF ILLUMINA MiSEQ INSTRUMENTS FOR NEXT GENERATION SEQUENCING

Massey University in collaboration with other research organisations, are in the process of building a partnership with New Zealand Genomics Ltd (NZGL) to create a collaborative approach to extending New Zealand's expertise in Next Generation Sequencing and Bioinformatics. Under NZGL's collaborative model, there will be a range of Next Generation Sequencing services available later in 2011. NZGL will be investing in bioinformatics personnel and state-of-the-art Illumina HiSeq 2000 and MiSeq instrumentation to be implemented at various sites around NZ. These services will cater for researchers undertaking very large sequencing projects (using the Illumina HiSeq 2000) through to the smaller sequencing projects (using the Illumina MiSeq).



Massey Genome Service (MGS) will keep you informed of progress in this exciting new development and we look forward to continuing to service your Next Generation Sequencing needs in the near future.

MGS has been working with Illumina to assist in providing you with a convenient solution for Next Generation Sequencing services during the interim period when such services are not available at Massey University or elsewhere in NZ. Illumina has kindly arranged through its Fast Track Service Laboratory, based in the USA, to provide preferential pricing to NZ customers for Next Generation Sequencing in the meantime. The Service utilises the Illumina HiSeq 2000 based on the same proven GAllx Illumina SBS sequencing technology. Karl Sluis (NZ Territory Account Manager) from Illumina is the primary contact for you and you may discuss your project needs directly with him. Karl will coordinate access to the Fast Track Services, including quotation arrangements of the preferential pricing. Karl's contact details are as follows: Mobile 021 455 864 or ksluis@illumina.com. This arrangement with the Illumina Fast Track Service will remain in place until Next Generation Sequencing services have been re-established in NZ.

ABI SERVICE CAPILLARY SEPARATION AND PLATE SERVICES WITH REACTION CLEANUP

On the 1st April 2011 MGS introduced two new sequencing services:

- Capillary Separation Service with Reaction Cleanup
- Plate Service with Reaction Cleanup

These two services allow customers to do set up their own sequencing reactions, run the PCR amplification and provide the MGS with the fluorescently labelled sequence products for reaction cleanup. The MGS uses the commercial X-Terminator™ Kit from Applied Biosystems Inc. to remove the unincorporated fluorescently labelled ddNTPs and salts from the sequencing reactions. Our service has found that this method is very effective at removal of the dye fronts which are problematic with all fluorescent Sanger sequencing based methods. For more information regarding Big Dye® X-terminator™ protocol please refer to life technologies website.

The uptake of this service has been very popular and the customers who have used this service to date have been very satisfied with their sequencing results.

To submit sequencing for either of these two services, when you submit your online request select either "Capillary Separation Service with Reaction Cleanup" or "Plate Service with Reaction Cleanup" from the 'Procedures' pull-down menu.

Further information on these services is provided on the MGS website at <http://genome.massey.ac.nz>, under the section called "ABI Sequencing and Genotyping Services", or contact Lorraine Berry, e-mail: l.berry@massey.ac.nz.

BIOANALYSER SERVICES

In May 2011, MGS starting offering the "Bioanalyser Service" for the quality and quantification assessment of total RNA and mRNA. This service is run using the Agilent 2100 Bioanalyzer and the RNA 6000 Nano & Pico LabChips. The MGS is not providing a service for the quality checking of DNA.

The Agilent 2100 Bioanalyzer is a microfluidic-based electrophoresis platform for the quality and quantification analysis of RNA, DNA and protein. It is designed to deliver high quality digital data from very small amounts of sample. It is a very valuable tool for assessing the quality of your RNA samples before proceeding with expensive Next Generation sequencing, Microarray and Gene Expression experiments.

MGS is trialling this service for a three month period, to assess the uptake of the service, and at which time pricing may be reviewed.

Please refer to the MGS website at <http://genome.massey.ac.nz>, under the section “Bioanalyzer Service” for information on the assays provided, pricing, sample requirements, and sample submission guidelines.

QUALITY ASSURANCE SYSTEM

MGS is current working on the implementation of a Quality Management System for the laboratory, in order to improve operating procedures, implement increased quality control and overall improve the quality of data you receive. This will be an ongoing and evolving process to better improve all aspects of our service from customer relations to data quality. MGS values and appreciates your feedback on the services we are providing.

WEBSITE UPDATE

The MGS website was updated last year but we are continuing to receive feedback from customers indicating they have a problem logging into their account to download results or submit requests. Please note the MGS website is no longer hosted by the Allan Wilson Centre and the service is now hosted by Massey University. Please use the website link <http://genome.massey.ac.nz> to access your account, by clicking on “Customer Login”. If you continue to experience problems logging into your account, please contact us.

JANUS[®] LIQUID HANDLING ROBOT

Recently, we updated our Janus[®] Liquid handling instrument to accept 15µl of sample for the Full Sequencing Services. Prior to this, we required the samples in minimum volume of 30µl. We have been working with SCIMED[™] to adjust the conditions to accept the smaller volume.

Thank you for your feedback regarding sample volume requirements as a number of customers suggested they have difficulty with providing higher amounts of DNA template.

PLEASE NOTE: The minimum premix volume for our “Full Sequencing Service” is now 15µl or 14µl if you require primer from us.

RECOMMENDATIONS ON PCR TEMPLATE PREPARATION FOR ABI SEQUENCING SERVICES

The Massey Genome Service (MGS) has talked to a few of the very experienced users of the service and have asked them to share their protocols and experience regarding template purification and preparation. We have taken this information and summarised it below to help other customers who are new to sequencing or are having difficulty with obtaining good quality sequencing from their templates.

PLEASE NOTE: The protocols listed below are not complete protocols, they are recommendations only. You will need to follow the protocol provided with the commercial purification kit you plan to use and the instructions provided with instrumentation and apparatus being used. The information below are recommendations to be used in conjunction with those provided with the commercial kits and the relevant protocols. The information below was kindly provided by Trish McLenachan, Adrian Cookson and Susanna Leung.

CLEAN DNA

The cleanliness of the DNA is the most important factor in the success of automated DNA sequencing. The DNA should be free of proteins, RNA, polysaccharides and genomic DNA. This can best be achieved by using either a commercial plasmid mini-preparation kit, or by sequencing a PCR amplified fragment.

In both cases if using a column based purification method:

- It is advisable to follow the protocol for the kit you are using in the preparation of the template
- Be sure to dry off all ethanol residues after the last recommended spin and leave the column to dry for an extra 5-10 minutes before adding the elution buffer

CUSTOMER RECOMMENDED PROTOCOLS FOR TEMPLATE PREPARATION

The majority of problems are caused by sequencing from PCR products, and in particular gel purified PCR products. Hence, the recommendations below relate to the preparation of PCR products.

Protocol 1

- PCR reactions are set up in a total volume of 50 µl
- Cleanup PCR reactions using a Qiagen QIAquick PCR purification kit. Refer to the supplier for ordering and pricing details
- Elute the product from the Qiagen column using 30 µl filtered water
- Quantify using the Nanodrop
- **NOTE:** The customer who uses this method prepares PCR products which are approximately 1500 bp in size. Approximately, 30 ng is used per sequencing reaction which equates to approximately 2ng per 100 bp.

Protocol 2

- PCR reactions are set up in a total volume of 20 µl.
- 2 µl of the PCR reaction is analyzed by gel electrophoresis to determine the size and concentration of the PCR product. The PCR products are run against a size standard containing bands of a similar size to the products.
NOTE: It is important to get a clean strong band, which indicates that the PCR product represents a unique sequence and not a mixture of products.
- The PCR products are treated with SAP and EXO1 to remove primer sequences.
- To determine the amount of PCR product to use per sequencing reaction, the following formula is used:

Length of DNA fragment (bp) ÷ 20 = Amount of DNA to Use (ng)

Example:

Length of PCR product = 1000 bp. Using the formula above as follows: 1000bp/20 = 50ng
(Uses 5ng of PCR product per 100 bp for each sequencing reaction.)

NOTE: The SAP + EXO1 method of purification will not remove double-stranded DNA contamination, such as primer-dimers or non-specific amplicons. Spin columns or gel purification methods can be effective in the removal of these types of contaminants.

- It is not recommended to sequence samples that have been cut and purified from a gel. If you are unable to get a clean sequence band from PCR it is recommended that you clone.
- Salt contamination can be a major problem with capillary sequencing. If you are concerned with salt contamination, you can dilute your samples in Milli-Q grade water instead of TE or elution buffer. In most situations, this will greatly reduce the presence of salt.

NEXT ISSUE OF MGS NEWSLETTER

The next issue of the MGS Newsletter will be in October 2011. If you have any concerns and issues with sequencing and genotyping with the MGS please feel free to contact our friendly staff who will be happy to assist you with your concerns and provide you with helpful advice.