Welcome to the June 2014 issue of the Massey Genome Service (MGS) Newsletter.

ILLUMINA MiSEQ NEXT GENERATION SEQUENCING

A reminder, all enquiries regarding Illumina MiSeq projects are to be directed to NZGL via their website enquiry at http://nzgenomics.co.nz. Please provide a detailed description of your project and experimental plan, so that NZGL genomics and bioinformatics personnel involved with scoping of projects are provided with a good description of your project requirements.

Please send enquiries regarding quotations to Jenny Shackelford, Project Manager, NZGL. Contact details are:
E-mail: jenny.shackelford@nzgenomics.co.nz
Phone: +64 3 470 3543

We have information on our website regarding the Illumina MiSeq service at http://genome.massey.ac.nz, under “Next Generation Sequencing Services”. The information includes:

- Process to follow for submission of work to our service
- Applications currently being provided
- Sample preparation, quantification and quality requirements
- Sample and library QC checks preformed by our service
- Sample delivery information
- Storage and retention of samples by the service
- Sequencing Run report delivered with the data upon completion of the work
- NZ Genomics Ltd Contact

As mentioned in the previous newsletter, Illumina has recently released a new library preparation kit for the preparation of libraries from total RNA called TruSeq RNA Stranded Total RNA sample preparation kit. The “TruSeq Stranded Total RNA sample preparation” method will convert total RNA in a library containing molecules of known strand origin, and will provide you with strand information on transcripts as well as multiple forms of non-coding RNA.

Illumina has also released a couple of new DNA library preparation kits for the preparation of genomic DNA and PCR amplicon libraries:

- Illumina TruSeq DNA PCR-Free Library Preparation Kit – PCR enrichment of the adapter ligated library can lead to under representation of library fragments in genomic regions which have a high CG or AT content. Library preparation without PCR enrichment will eliminate this artefact. This method eliminates the need for gel size selection of library fragments and instead utilises a magnetic bead size selection. The minimum input
requirement for this library preparation method is 1µg of purified genomic DNA for a 350bp insert library and 2µg for a 550bp insert library.

- **Illumina TruSeq DNA Nano Library Preparation Kit.** This method also incorporates a magnetic bead size selection of library fragments, eliminating the need for gel size selection. The input requirement for this library preparation method is 100-200ng of purified genomic DNA.

- **MGS** is also offering 16S rRNA amplicon sequencing, which targets the V3-V4 hyper-variable region of 16S. The libraries are prepared using a “Two Step PCR Approach”, using Illumina tailed primers for the first PCR step, and the Illumina Nextera XT PCR primers for the second PCR step.

MGS will keep you informed of future developments with these services and we look forward to servicing your Next Generation Sequencing needs in the near future.

**NZGL BIOINFORMATICS SERVICES**

NZGL offers bioinformatics services for NGS work, which is provided through each of the three collaborators, Massey University, University of Otago and University of Auckland, and is charged at a hourly rate. If you want NZGL bioinformatics assistance please include this in your NZGL online enquiry.

**NZGL Q&A Sessions: Talk to a Bioinformatician**

NZGL is still providing weekly Q&A Sessions where you can spend some time talking to one of the NZGL bioinformatics staff about your proposed project before you proceed with an NZGL enquiry. Locations and times are listed below.

**Massey University - Palmerston North**

**Day of week:** Friday  
**Time:** 11am - 12 midday  
**Location:** Massey University, Turitea campus, Science tower D rooms 5.30-5.33  
**Virtual location:** Skype patrick.j.biggs or dave.wheeler75  
**Staff available:** Dave Wheeler and Patrick Biggs  
**Other contact information:** Dave Wheeler, +64-6-3569099 ext 84598

**Massey University - Albany**

**Day of week:** Friday  
**Time:** 10:30 - 11:30am  
**Location:** Massey University Albany, Oteha Rohe campus, Building 14, Office 14.10  
**Staff available:** Sebastien Schmeier and Austen Ganley  
**Other contact information:** +64 9 414 0800 (ext: 41541)

**The University of Auckland**

**Day of week:** Friday  
**Time:** 1:30 - 2:30 pm  
**Location:** The University of Auckland - exact location to be advised (please check website)  
**Virtual location:** Skype dan_nzgl
ABI SERVICE ONLINE SAMPLE SUBMISSION
A reminder to all clients, all Sanger sequencing and genotyping work for the ABI Service must be submitted via our online submission system. Clients who have not used our services before, you will need to set up a new account by registering with our service. This can be done on line via the Massey Genome Service website at http://genome.massey.ac.nz. To register click on “Customer Login”, then click on “register”. You will be asked to create your own username and password, fill in your name and contact details. Once you have registered you will need to use the username and password each time you submit a new online request for sequencing or genotyping.
In the “Customer Login” section of the MGS website there is a PDF download which provides detailed instructions on the submission of samples and the downloading of results for the ABI service.

HAND DELIVERY OF SAMPLES TO MGS
Laboratories within the Institute of fundamental Science (IFS) are required to meet PC1/PC2 compliance to function as a “Containment Facility”. In order to continue to meet compliance IFS has installed security doors throughout Science Towers C and D to restrict and control access to laboratories. A security door has been installed on level 3, right next to the Science Tower D lift, which restricts free access into the Massey Genome Service laboratory.
For all customers outside of IFS who require access to the Massey Genome Service laboratory to either hand deliver samples, or to talk to our staff, FIRST go to the IFS admin office where you will be instructed to sign for a temporary access card. The access card needs to be returned to the IFS admin office before you leave the building.
Activation of the security doors will take effect as of the 27th May 2014. The IFS admin office located on the 4th floor of Science Tower B, room ScB4.11.
IFS admin office contacts are:
- Cynthia Cresswell: ext 84706
- Colleen Blair: ext 84612
- Ann Truter: ext 84703

Natisha Magan and Andy Trow will also be holding a supply of temporary access cards, which you can use:
Natisha Magan: location – ScB3.33, ext 84588
Andy Trow: location – ScA3.18, ext 84601

Please contact Natisha Magan or Andy Trow if you have any concerns.

If you are only delivering samples and you do not have to talk to any of the MGS staff, we do have a sample drop off point, which is located outside the Inwards Good Office, ScD3.01, on the 3rd floor of science tower D, next to the photocopiers and printers. There is a grey rack containing PCR racks which you can put your samples into. This will eliminate the requirement to get an access card to enter the MGS facility.
We are waiting delivery of a small fridge which have been purchased for the MGS facility, which clients can put their samples in. This fridge will be located in the same place, on the 3rd floor of science tower D outside the Inwards Goods office, ScD3.01. This fridge will be installed an available for sample delivery soon. You will be notified when this fridge has been installed.

We are also looking into the possibility of having a fridge located on the 3rd floor of Science Tower D, next to the printers and photocopier, that customers can put their samples. I will update you on this when I have more information.

**BIOANALYSER SERVICE**

MGS is still providing the “Bioanalyser Service” for the quality and quantification assessment of total RNA and mRNA only, using the Agilent RNA 6000 Nano Labchip. The service is no longer providing the Agilent RNA 6000 Pico Labchip due to the lack of demand.

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.

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

**PRICING REVIEW FOR ABI SERVICE**

The Massey Genome Service carried out a review of pricing for the ABI sequencing and genotyping service back in December 2013 and as of 1 February 2014 the pricing for the following 3 services changed as follows:

- ABI Sequencing – Full Sequencing Service (plasmids and PCR products) – decrease from $12.50 to $12.00 per sequence
- ABI Sequencing – 96 well plate Service – increase from $420.00 to $440.00 per plate
- ABI Sequencing – 96 well plate service with reaction cleanup – increase from $470.00 to $490.00 per plate

**PURCHASE OF BIGDYE TERMINATOR MIX**

Clients who are purchasing BigDye Terminator Mix from MGS for the ABI Sequencing Service “Capillary Separation Services” and “Plate Services”, the price per aliquot increased as of 1 February 2014, due to increases in consumable costs.

The costs are as follows:

- Purchase 80 µL aliquot: Increase from $130.00 to $160.00 excl. G.S.T
- Purchase 800 µL aliquot: Increase from $1300.00 to $1600.00 excl. G.S.T

**LIQUID HANDLING AUTOMATION**

MGS has upgraded our PerkinElmer Janus liquid handling instrument, which will cater for the automation of library preparations for the Illumina MiSeq services. The service has also purchased the PerkinElmer Victor Fluorescent Plate Reader for the QC checking of samples and libraries. This instrument is in the process of being installed and the protocols validated.

Reminder: The processing of samples for the ABI “Full Sequencing Services” will still be carried out using our PerkinElmer Janus liquid handling instrument. However, due to the software and
hardware upgrades some changes to the volume of sample/primer mix you supply have had to be made.

You must now to submit a total volume of 20µl of sample/primer mix (increased from 15µl). You need to modify the template and primer amounts as tabled below:

**NEW TEMPLATE AND PRIMER REQUIREMENTS**

<table>
<thead>
<tr>
<th>Requirements</th>
<th>PCR Template</th>
<th>Plasmid Template</th>
<th>Cosmid/Fosmid Template</th>
<th>Bacterial Genomic Template</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Template Amount</strong></td>
<td>2.5ng/100bp</td>
<td>250-625ng/20µl</td>
<td>0.625-1.25µg/20µl</td>
<td>2.5-3.75µg/20µl</td>
</tr>
<tr>
<td><strong>Primer Amount</strong></td>
<td>4pmol</td>
<td>4pmol</td>
<td>6.4pmol</td>
<td>6.4pmol</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td>20µl</td>
<td>20µl</td>
<td>20µl</td>
<td>20µl</td>
</tr>
</tbody>
</table>

These sample and primer requirements for the “Full Sequencing Services” have been updated on our website. To find these requirements please click on “ABI Sequencing & Genotyping Services”, then click on “ABI Sequencing Service Technical Information”.

An example of how to make up the template/primer mix is provided on our website.

MGS requires all “Full Sequencing Service” samples to be supplied in a 20µL volume. You can start transitioning over to supplying your samples at the new volume as of now.

**MGS DNA SEQUENCING QC SOFTWARE**

MGS purchased QualTrace™ QC software back in 2010 for the ABI Sanger Sequencing service. The QualTrace™ software enables client to detect sequencing problems with the data received from the MGS. A QualTrace™ Report is generated for each sequencing request and provided as a file that can be downloaded along with the result. The QualTrace™ software can automatically identify nine problems either with the preparation of the DNA sequencing reactions or with the DNA instrument itself. Below are the lists of sequencing issues that can be detected by the QualTrace™:

1. Sequence traces that contain no signal data due to either a failed reaction or a blockage of the DNA sequencer capillary.
2. Mixed trace signal resulting from multiple DNA templates in the sequencing reaction.
3. Noisy or very low signal data traces that provide only short reads.
4. Very weak signal strength at the end of the DNA sequencing trace resulting in short reads.
5. Early mixed trace signal due to template contamination by PCR products.
6. Delays in the starts of the DNA sequencing trace signal that indicate significant overloading of sequencer capillary with template DNA and/or other contaminants.
7. Problems with the DNA sequencer’s spectral calibration resulting in major channel cross-talk signal.
8. The presence of significant leftover BigDye sequencing mix in the loaded DNA sequencing reactions (dye blobs).
9. Traces which show a rapid decline in peak signal.
10. Indels (traces with insertions and deletions).
11. Total number of high quality bases.
12. Peak signal start.
13. Instrument diagnostics such as machine name, run module, capillary length, run date, tray name, tray well, polymer lot, sample, sample id and user.
14. The point in the traces where the trace signal reaches the noise threshold.
15. The level of peak blur (poor resolution).

Please have a look at this information when you download your result as the QualTrace™ software provides invaluable information on your DNA sequencing result.
MGS SUPPLIED PRIMERS
The MGS offers the following primers:
- M13 forward
- M13 reverse
- T7
- SP6

The primer sequence for each of these primers is available on our website at http://genome.massey.ac.nz > ABI Sequencing and Genotyping Services > ABI Sequencing Service Technical Information.

A reminder that MGS is no longer be supplying the T3 primer as a part of the “Full Sequencing Service”. The “Tick Box” on the sequencing online request form has been removed for this primer.

However, MGS still has a small amount of the T3 primer left that needs to be used up. Should you want to use this primer please note this in the “Customer Notes” section of the sequencing online request form. Once the T3 primer has been used up you will be notified.

We would also like to emphasize that we have a re-run policy. MGS does provide a re-run (under strict criteria) and you will have to speak to us and report your problems accordingly. You are welcome to use the report fault ‘function’ on your MGS account under the ‘results page’. Please refer to the “Submission of Online Problem Report” section in the downloadable “Submission of Online Request Bulletin”, which is located on our website in the “Customer Login” section. If you fall under one of our re-run policy criteria, we will provide an additional run for you. Please do not submit a re-run without informing us, or else we assume it is a new sequencing run.

COURIER BAG SERVICE
MGS provides a free courier service for both our ABI sequencing and genotyping services. Prepaid courier bags can be mailed to New Zealand customers free of charge.

If you wish to use our free courier service, please send an email to Pani Vijayan (e-mail: p.vijayan@massey.ac.nz) stating the number of courier bags to be sent to you, your courier address, postal address and contact phone number.

NEXT ISSUE OF MGS NEWSLETTER
The next issue of the MGS Newsletter will be in September 2014. 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.