



MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

MASSEY GENOME SERVICE

**Sanger Sequencing and Genotyping
using Applied Biosystems™
3730 and 3500xL Genetic Analyzer**

**SEQUENCING TECHNICAL BULLETIN
August 2024**



BULLETIN INCLUDES

**Step by step online submission guide for submitting
sequencing and genotyping requests for the ABI service**

**Enquiries regarding online request submissions contact:
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Massey Genome Service

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<https://www.massey.ac.nz/about/clinics-and-services-for-the-public/massey-genome-service/>



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MASSEY GENOME SERVICE (MGS)

Sanger Sequencing and Genotyping using Applied Biosystems™ 3730 and 3500xL Genetic Analyzer ONLINE SUBMISSION & DOWNLOAD OF RESULTS BULLETIN

This bulletin contains a step-by-step guide for customers who are submitting online requests to the Massey Genome Service (MGS).

1. Go to the MGS website at <https://www.massey.ac.nz/about/clinics-and-services-for-the-public/massey-genome-service/>.
2. Go to 'Customer Login'.
3. If you are a new customer and have not yet "registered" with the MGS, please register by clicking on 'register' in the customer login. To register you will be asked to create your own 'username' and 'password', which you will use each time you submit an online request for sequencing or genotyping with the ABI service. You will also be asked to supply other information, such as your name, results address, invoicing address, and other contact details.
4. Once you have registered with the MGS you can then submit an online request. To submit the online request, go back to 'Customer Login', and click on 'login'.
5. Enter in your 'Username' and 'Password', then click 'submit'.
6. This will take you to the 'Massey Genome Service Main Menu'.
 - If you are submitting an online request for sequencing, select '1. Request Sequencing Reaction'.
 - If you are submitting an online request for genotyping, select '2. Request Genotyping Reaction'.

The online request form will come up on the screen.

Refer below to the specific requirement for filling in the online request for both sequencing and genotyping.

Submission of online sequencing form

1. Fill in the 'Account/order number', with the account or order number this request is to be charged to.
For internal Massey University customers please supply an account number
For external customers please supply an order number. You will need to obtain a purchase order (PO) from your accounts department.
2. Fill in the 'Customer Notes', if you have any additional information the MGS needs to know regarding this request submission. If you do not have any additional information that the MGS needs for this request submission, please do not fill this in.
3. Proceed with filling in the columns in the 'Table':
 - **Template Tube No./Well No:** If you are submitting this request for the 'Full Sequencing Service' or 'Capillary Separation Service', enter in the 'Template Tube No.', and for the 'Plate Service' enter in the 'Well No.'.
 - **Template Name:** The name for identifying the template/sample used.
 - **Primer Name:** The name for identifying the primer used.
 - **Template Type:** This is the type of sample used, and can be either a PCR product, plasmid, cosmid, flosmid, lambda clone or bacterial genomic DNA.
 - **Product size (Kb):** This is the total size of your product including insert and vector. Enter this in kilobases (Kb).
 - **Template (ng/μl):** The concentration of template in ng/ul in the sequencing reaction premix. Please note the concentration should be calculated from dividing the total amount of template (in ng) by the volume of the premix (20ul if using your own primer, 19ul if using MGS primer)

- **Primer Conc (pmol/μl):** The concentration of primer in pmol/ul used in the sequencing reaction premix sent to the MGS. Please note the concentration should be calculated from dividing the total amount of primer (in pmol) by the volume of the premix (20ul if using your own primer). If you are using one of the MGS primers with the corresponding template, please enter in 'NA'.
- 4. Fill in the 'Template Purification Method', used to purify your template products, by selecting one option for the 'Pull-down Menu'. There is also an 'Other' option which you can fill in if your method of template purification is not listed in the 'Pull-down Menu'.
- 5. Fill in the 'Template Quantification Method', used to quantify your template products, by ticking either 'Spectrophotometry', 'Fluorometry', or 'Agarose Gel Electrophoresis'. You can tick more than one.
- 6. Fill in the 'Reaction Type', by selecting either 'BigDye Terminator v3.1' from the 'Pull-down Menu'.
- 7. Fill in the 'Procedure', by selecting 'Full Sequencing Service for Plasmids or PCR Products', 'Full Sequencing Service for BAC Templates or Cosmids', 'Full Sequencing Service for Bacterial Genomic DNA', 'Capillary Separation Service', 'Capillary Separation 96-well Plate Service' 'Capillary Separation Service with reaction cleanup' or 'Capillary Separation 96-well Plate Service with reaction cleanup', from the 'Pull-down Menu'.
- 8. If you are using any of the four 'Capillary Separation Services' or 'Capillary Separation 96-well Plate Services', you can purchase 80μl tubes of 'BigDye Terminator v3.1' reaction mix from the MGS. This 80μl tube of reaction mix is enough to set up 80 sequencing reaction if you are doing a 1/8 dilution of your reaction mix. Included will be a tube of 5X sequencing buffer, if you are diluting your reaction mix.
Please select the number of tubes of reaction mix you want to purchase from the 'Pull-down Menus'.
- 9. Please indicate how you want your 'Electropherogram' file sent to you. Please tick either 'Website', 'E-mail', 'Printout' or 'Postscript'. You can select more than one.
- 10. Please indicate how you want your 'Text file' of the sequencing read sent to you. Please tick either 'Website', or 'E-mail'. You can select more than one.
- 11. Please indicate how you want your 'Analysis Report' of your sequencing data sent to you. Please tick either 'Website', 'Printout' or 'Postscript'. You can select more than one.
- 12. If you tick 'Automatic Blast Search', you will get information back with your data which will tell you what organism your sequence read is from.
- 13. Click on 'Submit', to submit your online request for sequencing. This online request will come through to the MGS server.
- 14. Once you have clicked on 'Submit' the submitted online request form will come up on the screen. Please print this submitted request form in 'Landscape' format, and send to the MGS with your samples. The printout of the submitted request form is needed for sample identification.

Submission of online genotyping form

1. Select either 1. Microsatellite Analysis, for any microsatellite genotyping you want to have done with the MGS, or 2. Genotyping, for any other fragment analysis you want to have done with the MGS.
2. Fill in the 'Account/order number', with the account or order number this request is to be charged to.
For internal Massey University customers please supply an account number.
For external customers please supply an order number. You will need to obtain a purchase order (PO) from your accounts department.
3. Fill in the 'Customer Notes', if you have any additional information the MGS needs to know regarding this request submission. If you do not have any additional information that the MGS needs for this request submission, please do not fill this in.
4. Proceed with filling in the columns in the 'Table':
 - **Tube No./Well No:** If you are submitting this request for the 'Capillary Separation Service' enter in the 'Tube No.', and for the '96-well Plate Service' enter in the 'Well No.'.
 - **Sample Name:** The name for identifying the sample being run.
 - **Dye Set:** Select which dyes have been used for each sample.
5. Fill in the 'Procedure', by selecting either 'Capillary Separation Service', if you are sending your samples to the MGS in tubes, or '96-well Plate Service' if you are sending your samples in a 96 well plate to the MGS, from the 'Pull-down Menu'.
6. Please indicate how you want your 'Genotyping (.fsa) file' sent to you. Please tick either 'Website' or 'E-mail'. You can select more than one. **The 'Website' download is the default**

setting. If you do not want your genotyping (.fsa) file to be uploaded to the MGS server, you must de-select the 'Website' option.

7. Please indicate how you want your 'fragment sizing table file' sent to you. Please tick either 'Website' or 'E-mail'. You can select more than one. **The 'Website' download is the default setting. If you do not want your text file data to be uploaded to the MGS server, you must de-select the 'Website' option.**
8. You can receive printouts of the chromatograms. There is a cost for this. Please refer to 'ABI Pricing' on our website at <http://genome.massey.ac.nz> for details. If you want a printout of the chromatograms, please tick the 'Printout' box.
9. Please note that the MGS will not allele call genotyping data for customers. There is software which can do this for you. Please contact the MGS if you need any help with this.
10. Click on 'Submit', to submit your online request for sequencing. This online request will come through to the MGS server.
11. Once you have clicked on 'Submit' the submitted online request form will come up on the screen. Please print this submitted request form in 'Landscape' format, and send to the MGS with your samples. The printout of the submitted request form is needed for sample identification.

Downloading Results

Instructions for downloading results from the MGS server once you have received the automatic e-mail notification that the results have been uploaded:

- Login to your account using your username and password, from the "Customer Login" tab.
- From the "Massey Genome Service" main menu, click on "Results".
- From the "Download Results As" column in the table, click on either "WinZip" or "tar.gz" for the request you want to download.

Submission of online problem report

If you have any problems and concerns with the quality of your sequencing or genotyping data, please submit an online 'Problem Report' to the MGS.

Instructions for submitting an online "Problem Report":

- Login to your account using your username and password, from the "Customer Login" tab.
- From the "Massey Genome Service" main menu, click on "Results".
- Click on "Report Problem" for the request in question and fill in your online report submission.

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