Mouse Genotyping & Health Screen Service Sample Submission Guidelines

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Online Sample Submission Portal

Please use our Sample Submission Portal (MSSS) available at https://gmg-submit.gimr.garvan.org.au/#/login to submit samples. Find instructions on how to use the sample submission portal here, https://www.garvan.org.au/research/capabilities/molecular-genetics/documents/qs_15_v1.pdf, and also a short video here, https://www.youtube.com/watch?v=8eL-JJ8SIGU&feature=youtu.be. Further information on our services can be found at our website www.garvan.org.au/gmg. New clients need to create an account (please follow instructions after clicking on the 'Create Account' button on the landing page), existing clients log into their account by entering their login details and selecting the button 'Login', see Figure 1

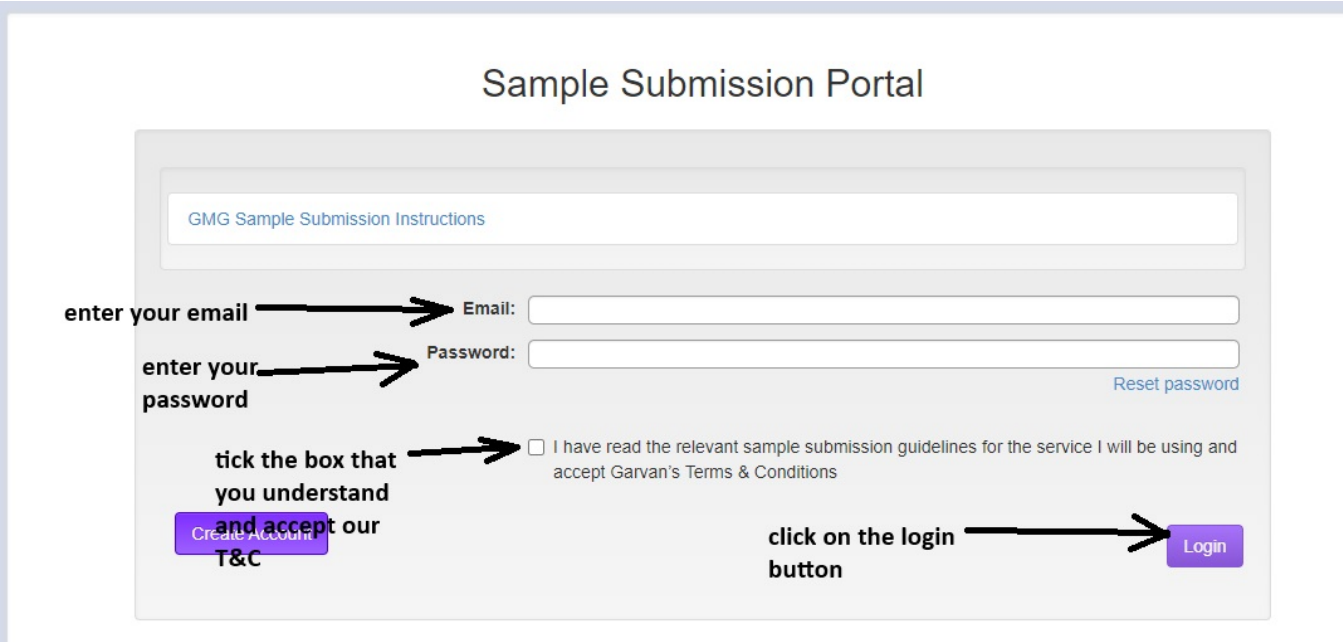

Figure 1: MSSS login screen

After logging in the next window will show your account where you can submit new samples and view previous submissions, To submit a new manifest select 'New Mouse Genotyping Manifest' from the options available, see Figure 2

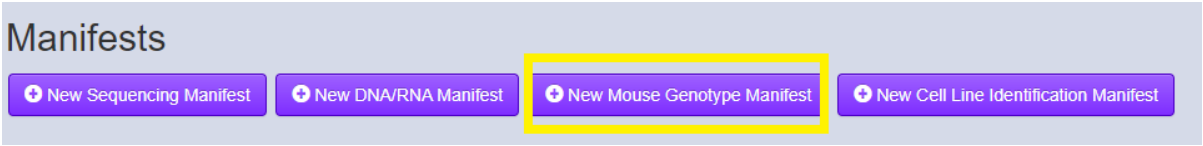

Figure 2: Manifest choice

In the pop-up window select 'Container Type'; '96 Well Plate/Rack' or 'Single tubes' and enter the number of samples that will be submitted, see Figure 3. Click on the 'Create Mouse Genotype Manifest'. We recommend samples are submitted in barcoded tubes placed in a 96well rack, we provide these supplies at no extra cost to you please contact the lab at mgs@garvan.org.au to request supplies.

Figure 3: Create new manifest

After clicking 'Create Mouse Genotyping Manifest', the new Manifest page will open, see Figure 5. Please ensure the 'Client Information' and 'Billing Information' sections are fully complete, including a Purchase Order number (PO). If your financial dept need a quotation for generating a PO please contact us and we will generate a quotation for you. If you can confirm that your institution does not need a PO in order for invoices to be paid, you can fill this field with a "0". The PO can also be updated after the manifest has been submitted (but must be before results are released).

There are two ways to fill out the sample form; either on the webpage (this may be more convenient for you if you are only submitting a few samples) or via the excel template form under "Download MGS Spreadsheet Template".

In the manifest view under "Service Type" select whether you are submitting mouse health screen samples or standard genotyping samples, see Figure 4.

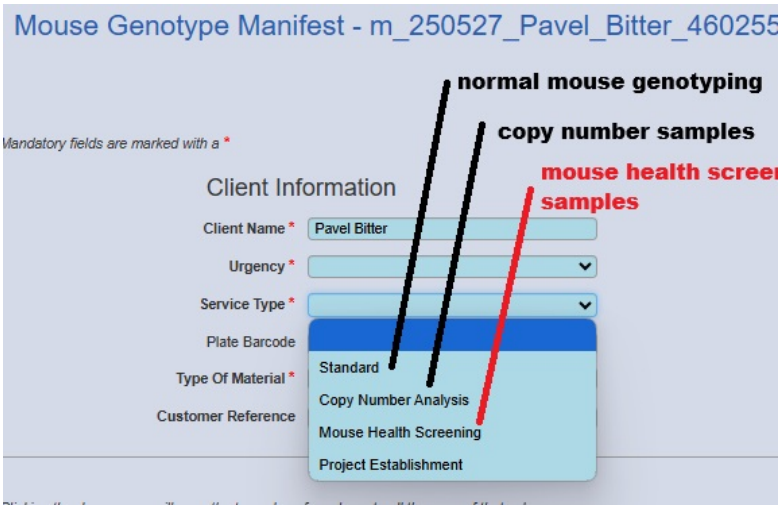

Figure 4: Service Type Selection

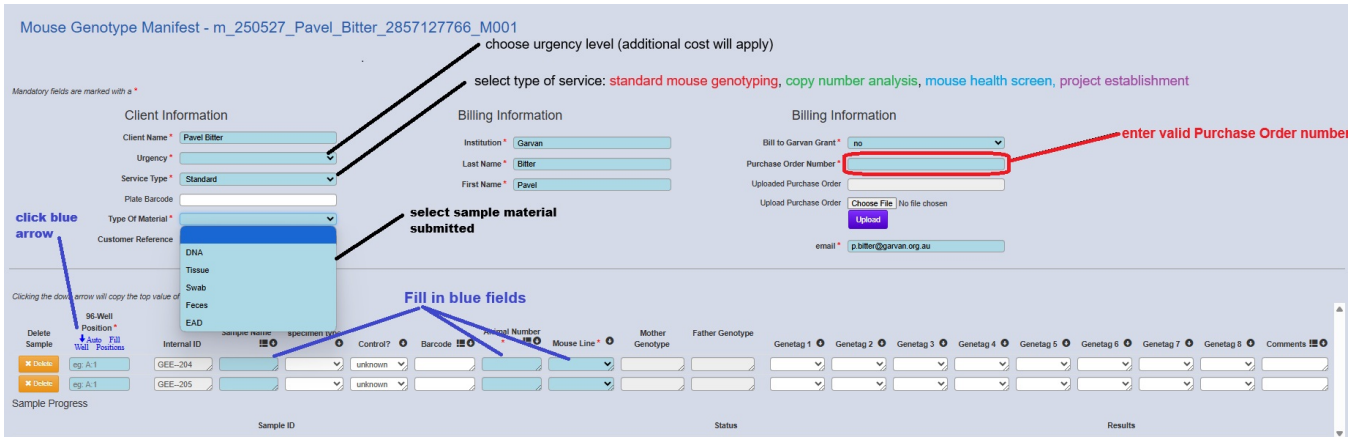

Figure 5: MGS manifest page

Figure 6: MHS manifest page

When completing the form (online or the download), once a mouse line is selected, the genetag dropdown menu will show only genetags that are associated with that line. Please let us know if any of your lines are missing, or if there are any genetags that you would like added to a line.

All the fields marked with the red asterisks need to be entered. You can automatically fill the sample locations by clicking on the **blue arrow down button**. Alternatively, you can give other locations for your samples manually in the format A:1 etc.

Standard Genotyping

Our standard mouse genotyping is based on high resolution meltcurve analysis. We require **controls with known genotypes** (tissue or DNA) to perform this analysis. Exceptionally we can deduct the genotype without controls if you take responsibility for the results. To start a new genotyping assay please fill in the **New Project Submission Form** which you can download from our webpage https://www.garvan.org.au/research/services/molecular-genetics/mouse-genotyping under "Accessing our services"

Submitting DNA samples

Mouse genotyping results greatly depend on the DNA quality of the submitted samples. The meltcurve analysis that is used by us is very sensitive to low quality DNA or the salt concentration.

For our robotic PCR setup we need at least 40µL of 15ng/µL of DNA in each tube. The 260/280 ratio must be between 1.7 to 2.3 and the 260/230 ratio must be above 1.9. Only 1µL of the DNA will be used in each PCR reaction and the remaining DNA can be returned after the genotyping has been completed or is stored in our facility for up to 6 months.

Submitting tissue samples

If you submit ear clip samples please make sure they are **at least 3mm^2 large** (you can submit more than one ear clip of the same mouse in one tube, **we suggest a minimum of two ear clips)**. Please note, if the tissue size is too small we cannot extract sufficient DNA and will have to reject that sample. If you submit tail tips please submit them in a **range of 3 to 5mm**. Please make sure samples are not cross-contaminated by other tissue and that the tissue in the well corresponds with the sample submission manifest (no sample confusion).

Mouse Health Screen Samples

We offer single pathogen PCRs and PCR panels:

Individual PCRs

PPNUEM	Pasteurella pneumotropica	Oral swab, EAD
Cbovis	Corynebacterium bovis	Fur swab, EAD
SAUREUS	Staphylococcus aureus	Oral swab, EAD, Feces
HELICOSP	Helicobacter ssp.	Feces

Panels for mouse health screening

- Panel 1 MCL1 (Mouse Cell Line PCR panel 1)
  - MMV, MPV, MHV, MCMV, Myco, TMEV
- Panel 2 MCL2 (Mouse Cell Line PCR panel 2)
  - LDEV, LCMV, MMV, MNV, MPV, MCMV, Myco, ROTA, SEND, TMEV
- Panel 3 MFPCR1 (Mouse Fecal PCR panel 1)
  - HELICOPS, MMV, MHV, MPV, ASPIC, SYPH, ROTA
- Panel 4 MFPCR2 (Mouse Fecal PCR panel 2)
  - TYZ, HELICOPS, MMV, MHV, MPV, MNV, ASPIC, SYPH, ROTA, SALM TMEV
- Panel 5 EAD (Exhaust Air Dust Filter panel)
  - HELICOPS, MMV, MHV, MNV, MPV, MYOB, MYCOP, ASPIC, SYPH, ROTA, TMEV

Sample types

If you submit panel samples a **special tube** for your sample specimen must be used.

We will **provide this tube**, this tube contains a nucleic acid stabilizer to prevent the degradation of RNA.

Please contact gmg@garvan.org.au and ask for sending ***MHS Panel Sample Tubes.***

Place the specimen (feces, oral or fur swab, EAD) into the tube and keep and send cooled at 4C.

Copy Number Analysis via Real-Time PCR

If heterozygous and homozygous transgenic mice cannot be differentiated via standard PCR we can run a real-time PCR and separate the heterozygous from the homozygous samples via real-time Ct values, see *Shitara H. "Simple method of zygosity identification in transgenic mice by real-time quantitative PCR", Transgenic Res. 2004 Apr;13(2):191-194.*

Quality of submitted DNA samples

This is a very sensitive real-time PCR, we can only accept column-cleaned DNA for this service.

Sample volume and concentration

We will need at least **100µL of DNA** with a **concentration of >60ng/µL**. The concentration of the submitted samples must be adjusted to be the same concentration for all samples (+/- 5%) including the controls. We can prepare your samples for you for an added service fee. Please indicate in the sample submission form if you want us to perform the equilibration. The 260/280 ratio must be between 1.7 to 2.3 and the 260/230 ratio must be between 1.9 to 2.4. These are our acceptance criteria for DNA that will be accepted to the service. DNA that's fails these criteria can be sent to us and we can perform genotyping but we will not take responsibility for the results.

Controls

We will need at least one control which is either Het or Hom, ideally both, WT samples are of no use. The DNA of these control samples should be extracted at the same time as the samples that are submitted.

Turnaround time

The turnaround time for this analysis can take up to two weeks although usually and especially when requested results are available earlier.

Sending of Samples

We prefer sending you our tubes in racks with barcode labels. You can then use these tubes to send us your tissue samples.

Figure 7: tubes in rack

Alternatively submit your samples in 1.5mL Eppendorf tubes (*3810X PCR clean 1.5ml, Catalogue # 0030125.215 Eppendorf*). Please position these samples in the rack that we send you according to your online sample submission manifest.

We recommend to cool DNA or tissue samples when sending samples with mail or courier (cooling packs, ice or dry ice). However if the transport can be arranged overnight it is acceptable to send samples without cooling in express post envelopes or via courier.

Shipping Address

Our shipping address is:

Garvan Institute / GMG

Loading Dock

West Street (off Burton Street)

Darlinghurst, NSW 2010

Dock times: 8am to 4pm

Phone: 02 9295 8640

Pricing

Pricing information is available on our website at https://www.garvan.org.au/research/services/molecular-genetics/mouse-genotyping "Our Services" > "Pricing"

Results

Our online sample submission portal will send you an email with an attached excel file with your results within an approximate turnaround time of 48-72h for standard genotyping (copy number results take several working days). If you are a Stuart software customer, results will also be uploaded into Stuart for you.

Contact us

Figure 8: NATA logo

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