GMG MOUSE GENOTYPING SERVICE Q&A

1 WHAT KIND OF SAMPLES CAN I SUBMIT?

The analysis method of our Mouse Genotyping protocol is sensitive to the quality of the DNA that we work with. Ideally you provide us with tissue samples:

- ear clips at least 3mm^2 large (you can submit more than one earclip of the same mouse in one tube).
- tail tips 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 form (no sample confusion).

- DNA samples (at least 35ul of 15ng/ul 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)

We have observed that purified DNA gives the best results. Following is a list of DNA preparation methods ordered from worst to best:

-Tissue cooked in NaOH + salt to adjust pH

-Tissue Proteinase K digested

-Tissue Proteinase K digested and Ethanol precipitated

-Tissue Proteinase K digested and spin column or magnetic bead cleaned.

We recommend to take the effort to clean the proteinase K digestion via columns or magnetic beads. Any Qiagen or similar cleanup kit will do a great job (for example Qiagen Blood /Tissue

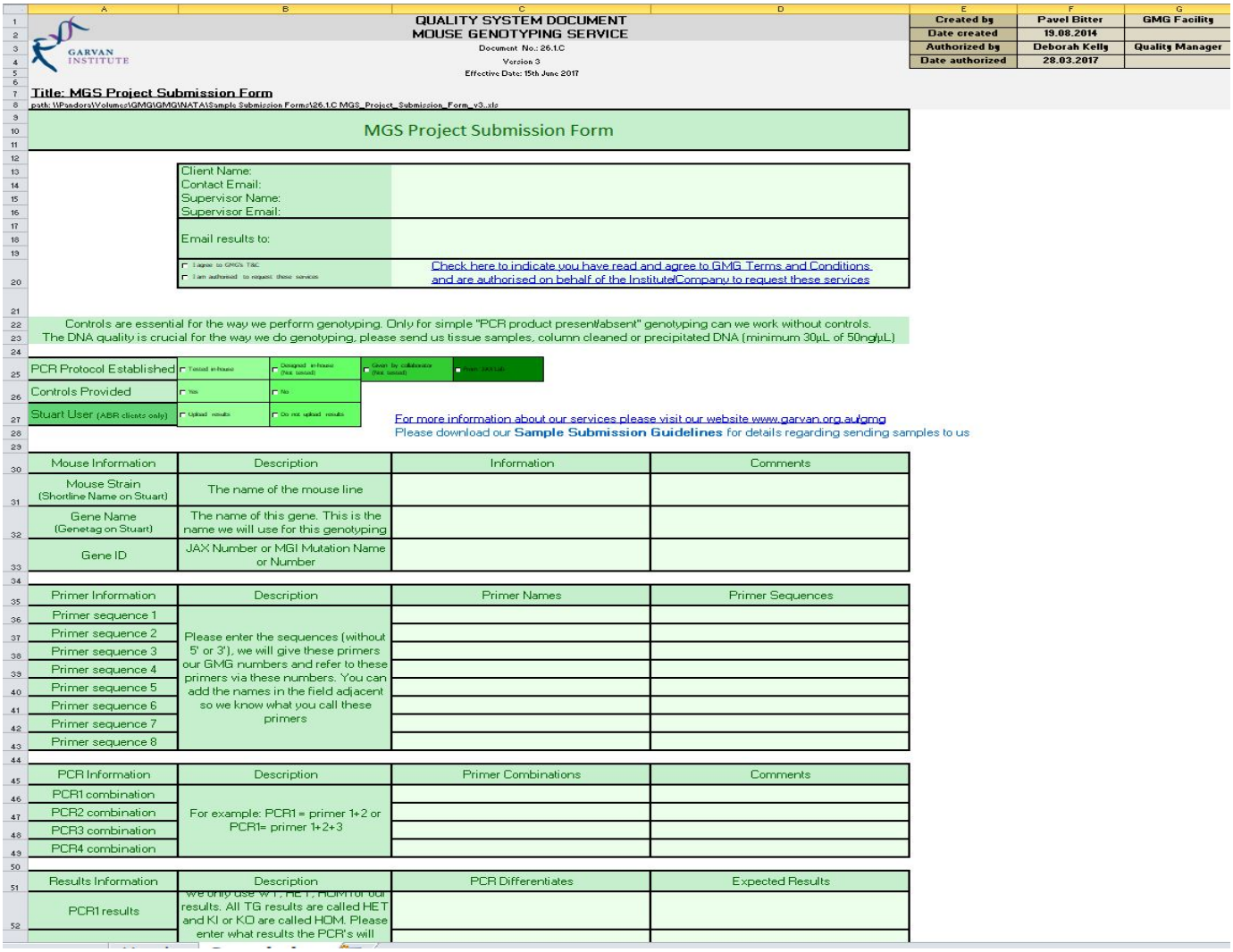
extraction kit). We will accept DNA that has not been treated in the recommended way but we must make you aware that we observe a dropout rate of ~20% of samples. Also, there is an increased risk of genotyping errors due to higher salt concentrations or inhibitors. The melt-curve analysis is highly sensitive to the salt concentration and variation of the salt concentration shifts the peak of the genotype relative to the controls.

2 HOW DO I SUBMIT MY PROJECT INFORMATION?

Please use our “New Project Submission Form” for new genetags or mousseline crosses that you want to establish with us. This template can be downloaded from our webpage:

https://www.garvan.org.au/research/capabilities/molecular-genetics/mouse-genotyping>New Project Submission Form

Please fill in whom to report to, the gene name, the primer sequences, the primer combinations & product sizes, and expected genotypes (eg wildtype, heterozygous, homozygous). We will need some controls (HET, WT, HOM) to get us started. These controls can be samples that have been genotyped and confirmed for their genotype before.



QUALITY SYSTEM DOCUMENT
MOUSE GENOTYPING SERVICE
Document No.: 26.1.C
Version 3
Effective Date: 15th June 2017

Created by	Pavel Bitter	GMG Facility
Date created	19.08.2014	
Authorized by	Deborah Kelly	Quality Manager
Date authorized	28.03.2017	

Title: MGS Project Submission Form

path: \\Pandora\Volumes\GMG\GMG\NATA\Sample Submission Forms\26.1.C MGS_Project_Submission_Form_v3..xls

MGS Project Submission Form

Client Name: Contact Email: Supervisor Name: Supervisor Email:	
Email results to:	
I agree to GMG's T&C I am authorised to request these services	Check here to indicate you have read and agree to GMG Terms and Conditions and are authorised on behalf of the Institute/Company to request these services

Controls are essential for the way we perform genotyping. Only for simple "PCR product present/absent" genotyping can we work without controls.
The DNA quality is crucial for the way we do genotyping, please send us tissue samples, column cleaned or precipitated DNA (minimum 30µL of 50ng/µL)

PCR Protocol Established	Tested in-house	Designed in-house (Not tested)	Given by collaborator (Not tested)	From JAX Lab
Controls Provided	Yes	No		
Stuart User (ABR clients only)	Upload results	Do not upload results		

For more information about our services please visit our website www.garvan.org.au/gmg
Please download our **Sample Submission Guidelines** for details regarding sending samples to us

Mouse Information	Description	Information	Comments
Mouse Strain (Shortline Name on Stuart)	The name of the mouse line		
Gene Name (Genetag on Stuart)	The name of this gene. This is the name we will use for this genotyping		
Gene ID	JAX Number or MGI Mutation Name or Number		

Primer Information	Description	Primer Names	Primer Sequences
Primer sequence 1	Please enter the sequences (without 5' or 3'), we will give these primers our GMG numbers and refer to these primers via these numbers. You can add the names in the field adjacent so we know what you call these primers		
Primer sequence 2			
Primer sequence 3			
Primer sequence 4			
Primer sequence 5			
Primer sequence 6			
Primer sequence 7			
Primer sequence 8			

PCR Information	Description	Primer Combinations	Comments
PCR1 combination	For example: PCR1 = primer 1+2 or PCR1= primer 1+2+3		
PCR2 combination			
PCR3 combination			
PCR4 combination			

Results Information	Description	PCR Differentiates	Expected Results
PCR1 results	We only use WT, HET, HOM for our results. All TG results are called HET and KI or KO are called HOM. Please enter what results the PCR's will		

We will order the primers and once they have arrived we will establish the project. In case you only have a gene name or sequence we can design primers too. Once primers have arrived (usually 4 days) we will establish the project with our genotyping protocol. We then will be in contact with you and inform you about the progress.

In case time matters and you need us to establish your project very quickly, you can provide us with primer aliquots and we will start immediately to establish the project.

3 HOW DO I SUBMIT MY SAMPLES?

For routine genotyping we prefer samples to be in a 96-well format as these plates fit to our robot liquid handlers.

- For DNA samples please use fully skirted 96 well PCR plates (Biorad plates #HSP-9901 & Microseals #PCR MSB-1001).
- For tissue samples (Corning Microcentrifuge tubes 1.2mL, Individual, Racked, #CLS4410-960EA)

- Less than 20 samples can be submitted in 1.5ml Eppendorf tubes (3810X PCR clean 1.5ml, Eppendorf #0030125.215).

Additional positive controls

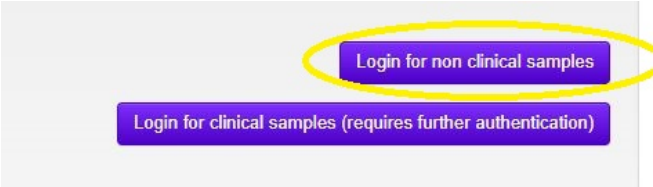
Some researchers prefer to add positive controls to each plate they are sending to us and for each test they want us to perform. We greatly appreciate this as it adds additional safety to the tests. If you can, please include positive and negative controls in your submission.

Sample Submission Portal:

Sample manifests are submitted through the Sample Submission Portal. These manifests will indicate what each sample is and what genetag to use on it. For details on how to submit manifests through the portal, please see the document: QS_15_GMG Sample Submission Portal Client Instructions_v1. It is also available on the login screen for the portal, at: https://www.garvan.org.au/research/capabilities/molecular-genetics/documents/qs_15_v1.pdf

The portal can be accessed at: https://gmg-submit.gimr.garvan.org.au/#/login

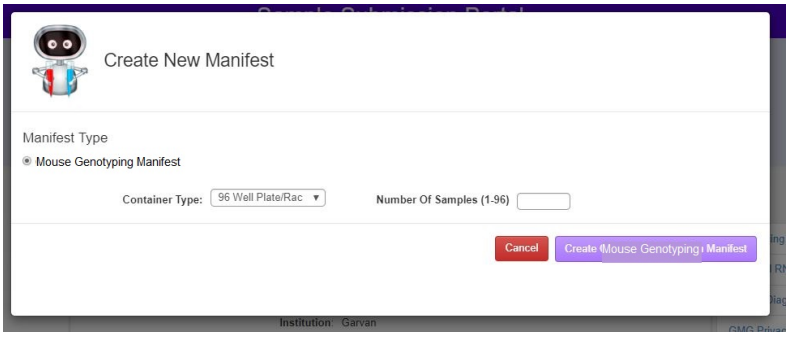
New clients need to create an account (please follow instructions after clicking on the "Create Account" button on the landing page), existing clients log into their account by selecting the button "Login for non clinical samples":



After logging in you the next screen will show your account. Here you can submit new samples and view previous submissions. To submit a new manifest for mouse genotyping, select "New Mouse Genotyping Manifest" from these options:

In the following popup window, select the container type and the amount of samples that will be submitted. We strongly prefer that samples are submitted in barcoded tubes in 96-well racks. We

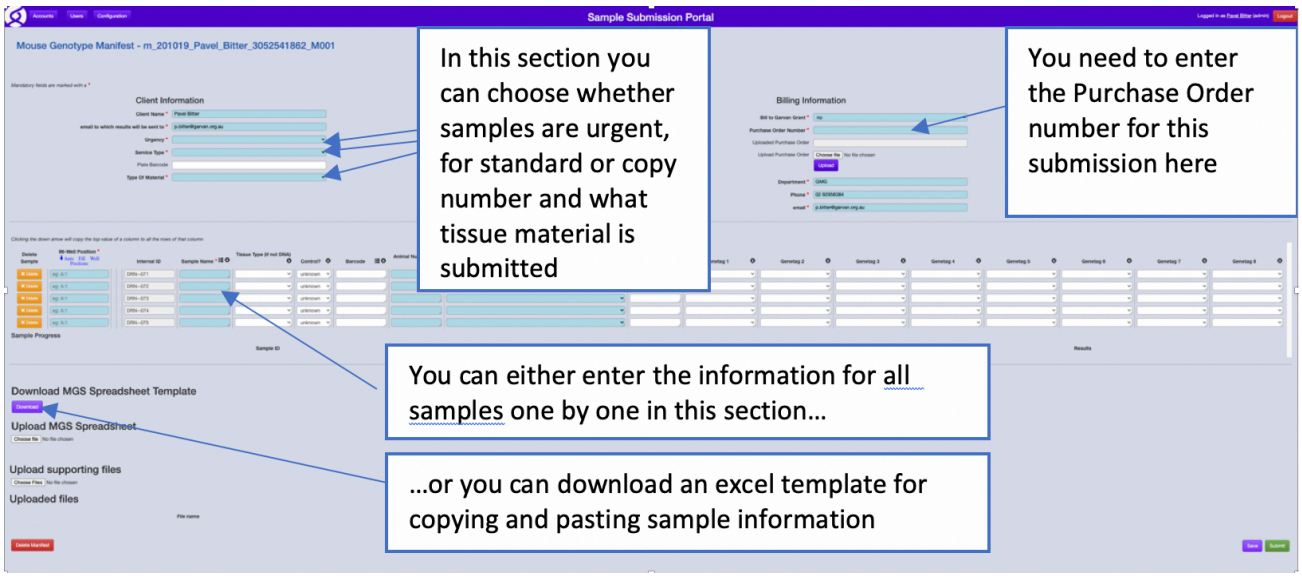
can supply these (racks, lids, tubes, barcodes) to you at no additional cost if you email us at mgs@garvan.org.au to request them.



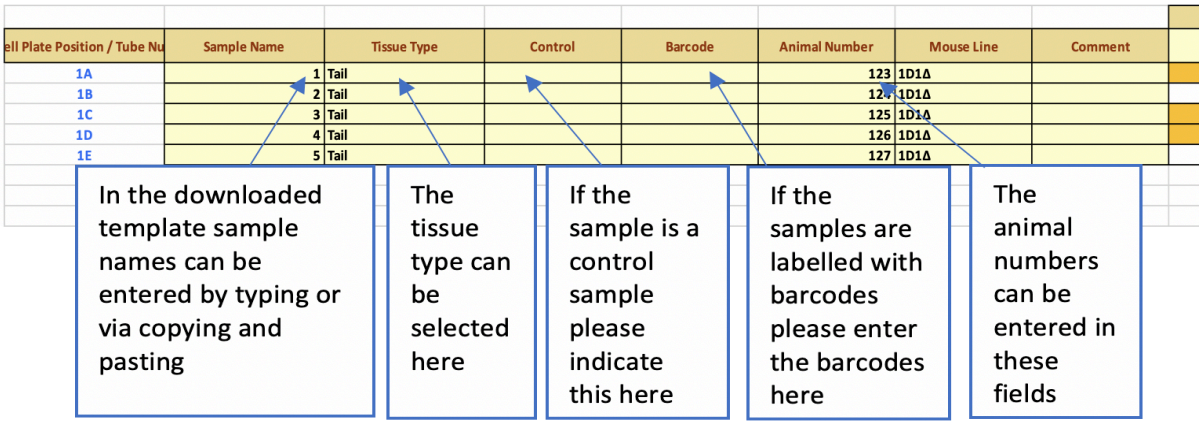
After clicking “Create Mouse Genotyping Manifest”, the new Manifest will open (see below). Please fill out the client and billing information fully and include a Purchase Order (PO) if your institution requires one to process invoices. If you can confirm that your institution does not need a PO in order 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 sheet form under “Download MGS Spreadsheet Template”.

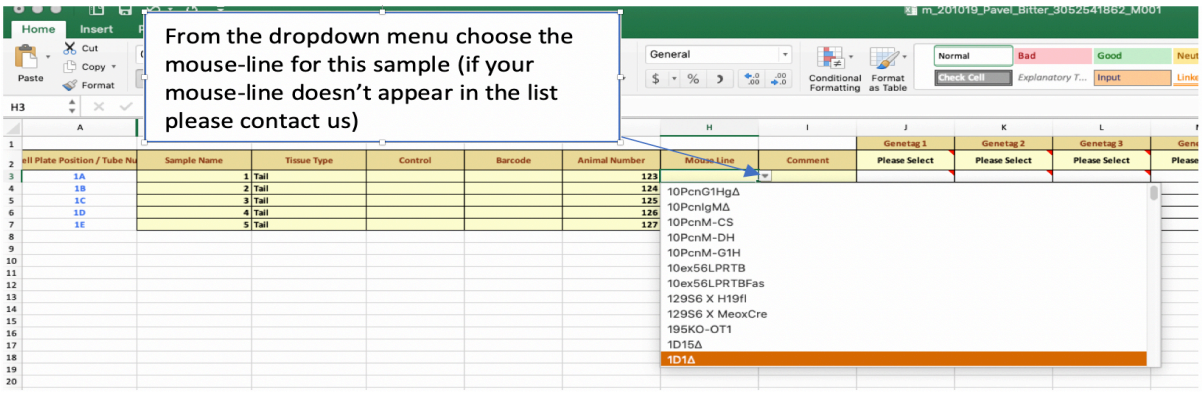
When filling out the webpage form, once a mouse line is selected, the genetag dropdown menu will show only genetags that are associated with that line. Please let us know if any of your lines are missing, or if there are any genetags that you would like added to a line.



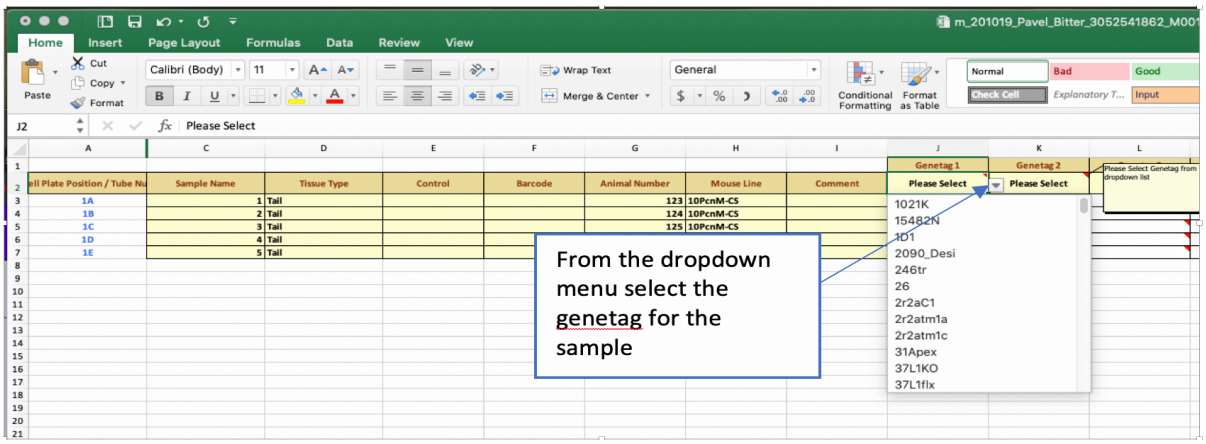
The downloaded template can be filled out as follows (see below). Please provide unique sample names (you can use the sample barcode as the sample name as well, to ensure uniqueness).

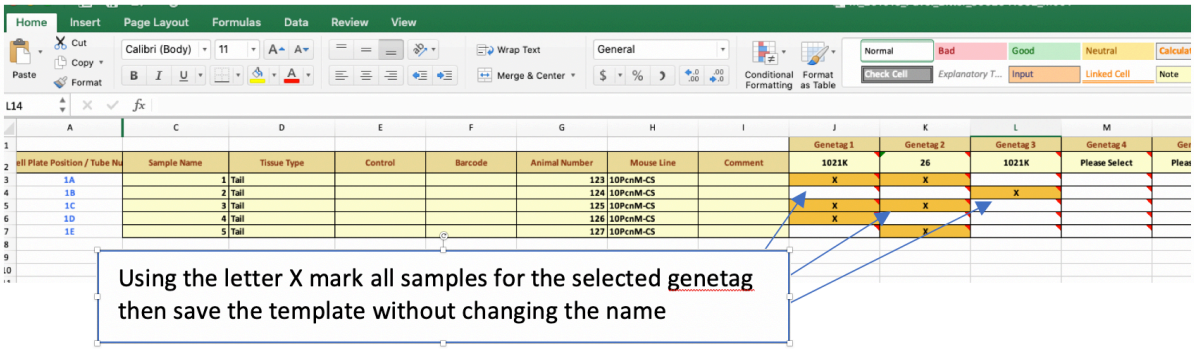


Similarly to the webpage form, you will be able to select your mouse lines from the dropdown menu.



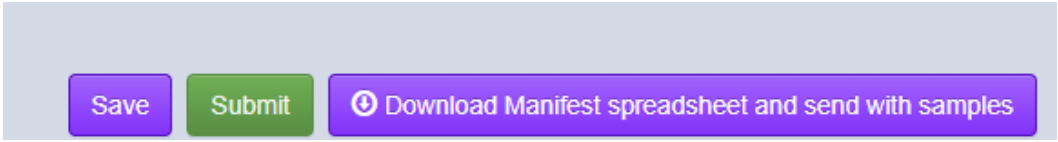
After your mouse line has been selected, please select what genetags you would like run for your samples. This is achieved by selecting the relevant genetag from the dropdown menu and then typing "x" in the row for what samples you want run with it (see the images below). You will see every genetag in this view, but please ensure you only select genetags that are associated with the line you have selected.





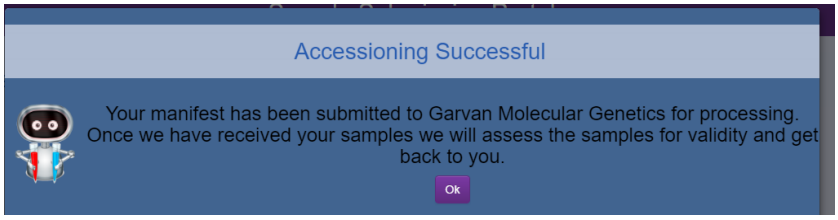
Once you have completed this form, save it (do not change the file name) and upload it by selecting “Choose File” under the “Upload MGS Spreadsheet” header. If there are any issues with the excel sheet, the system will tell you in an error message. If anything if unclear in this message or you are unable to rectify it, please let us know. Once the sheet is successfully filled out and selected, click the “Upload” button that appears and select “ok” once the upload has completed. After this, you will see the webpage form will be updated with the information you have provided in the spreadsheet.

When you are ready, scroll to the bottom of the page, and select the green “Submit” button.



If there are any issues with your submission, the manifest will not be submitted. Any fields with missing information or that have been filled out incorrectly will be highlighted with a red outline. Hovering over this will supply further context on what needs to be fixed.

Once the form has been submitted successfully, you will see the following popup:



Samples

Labeling of samples

Samples need to be positioned and labeled according to the electronically submitted sample submission form. Ideally a unique identifying number is written onto the tube with a permanent

marker, or the 96well plate is labeled with the name of the submitter and the date of submission. If you are submitting DNA in a 96-well plate, please enter the plate's barcode in the field "Plate Barcode" on the Sample Submission Form.

Shipping of samples

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:

If possible, please use express post envelopes and avoid sending over the weekend. Please send your samples to:

Garvan Institute
Loading Dock
West Street (off Burton Street)
Darlinghurst, NSW, 2010

Dock times: 8am to 4pm

Phone: 02 9295 8640

Personal Sample Delivery

Samples for genotyping can be placed into the "Sample reception fridge" which is located on level8 in the corridor in front of room 8.05. There is a reception tray in the fridge clearly marked. Please place your samples here and send an accompanying email with the sample submission form to gmg@garvan.org.au

4 HOW DO YOU GENOTYPE MY SAMPLES?

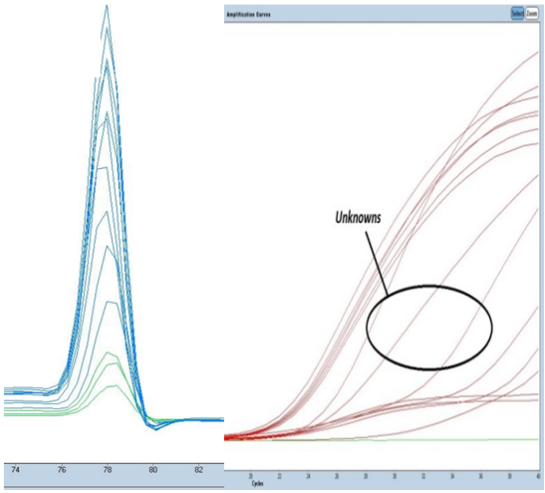
We perform a touchdown realtime PCR in a 384well plate scale. Samples are processed via an *in-house* developed PCR programming software with robotic liquid handlers that work fully automated from worklists. After PCR amplification on our LightCycler 480 we perform a Meltcurve analysis. The control meltcurve peaks are compared to the unknown sample peaks. The test format is also used by the Jackson Laboratories in the USA.

We run PCR specific and general positive & negative, contamination check, reference and DNA extraction controls for each test and only release results when all controls have worked.

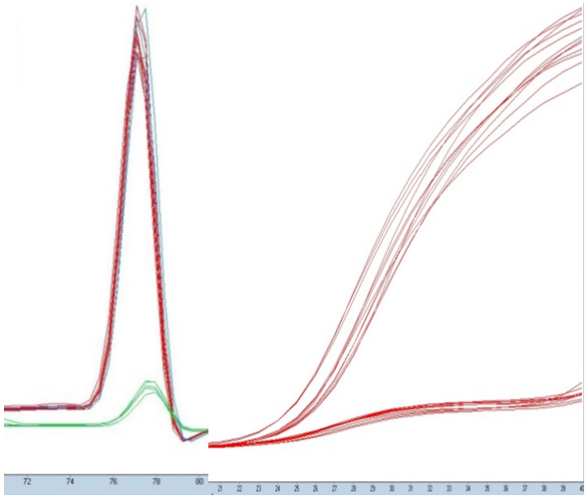
All our procedures are NATA ISO 17025 certified which means that we work in a quality controlled environment which is regularly internally and externally audited.

Here are some examples of typical Meltcurve results:

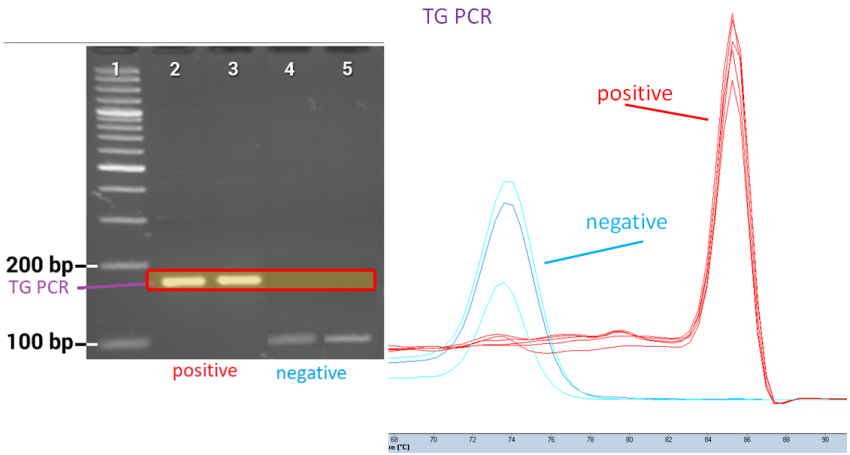
bad quality DNA

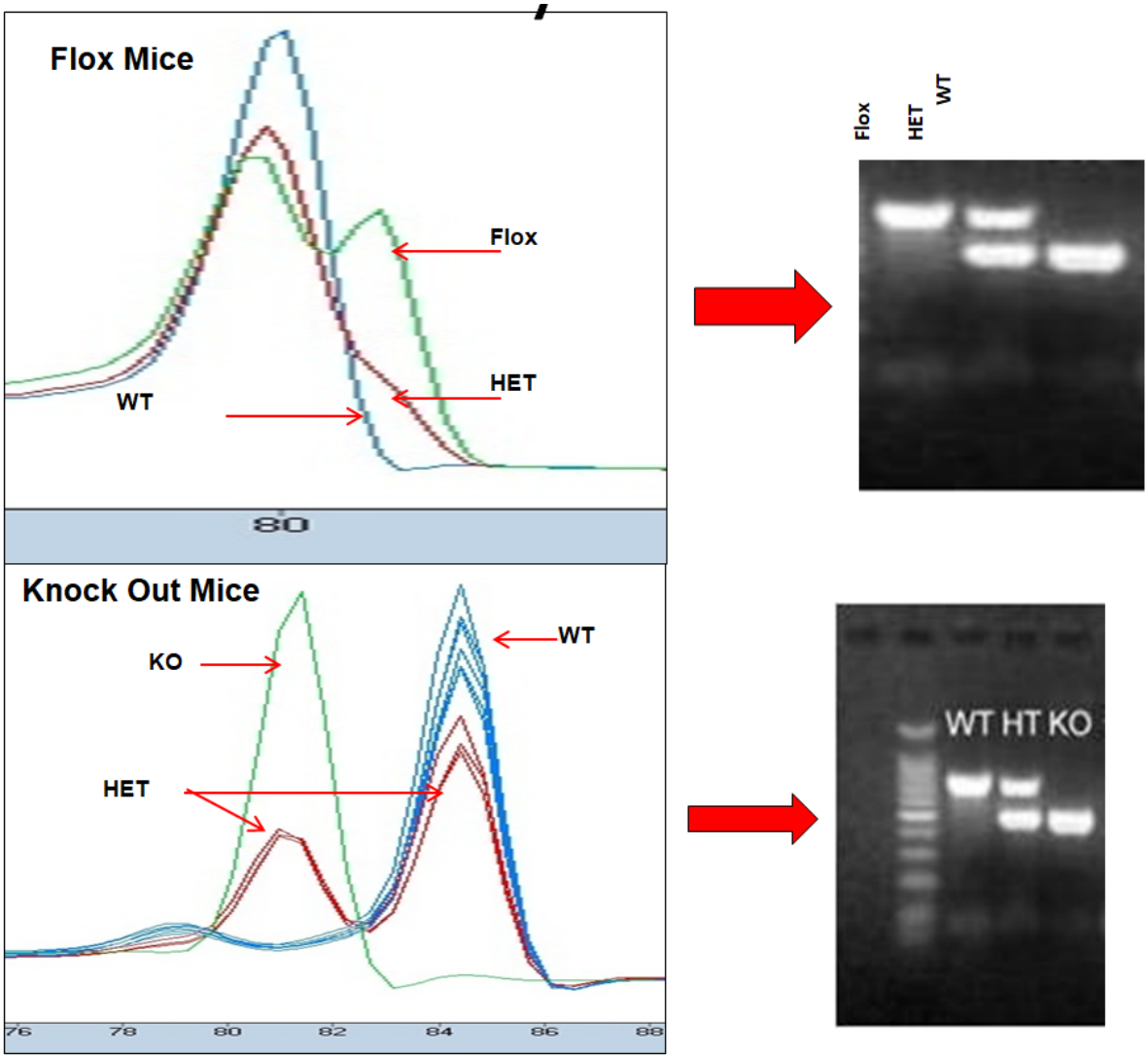


spin column DNA



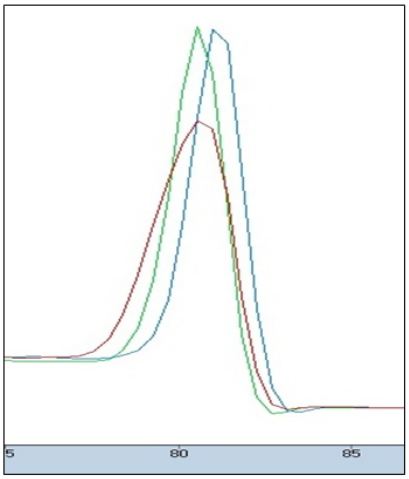
HRM Analysis of 1 PCR system



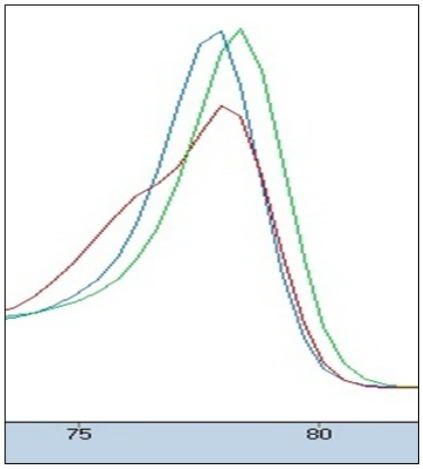

Flox Mice
Flox
HET
WT
80
Knock Out Mice
KO
WT
HET
76
78
80
82
84
86
88
Flox
HET
WT
WT HT KO

HRM Analysis of SNPs

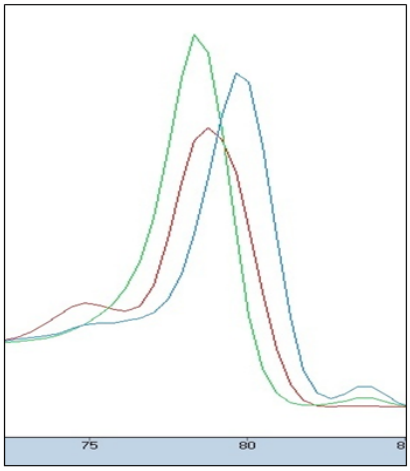
OB C→T



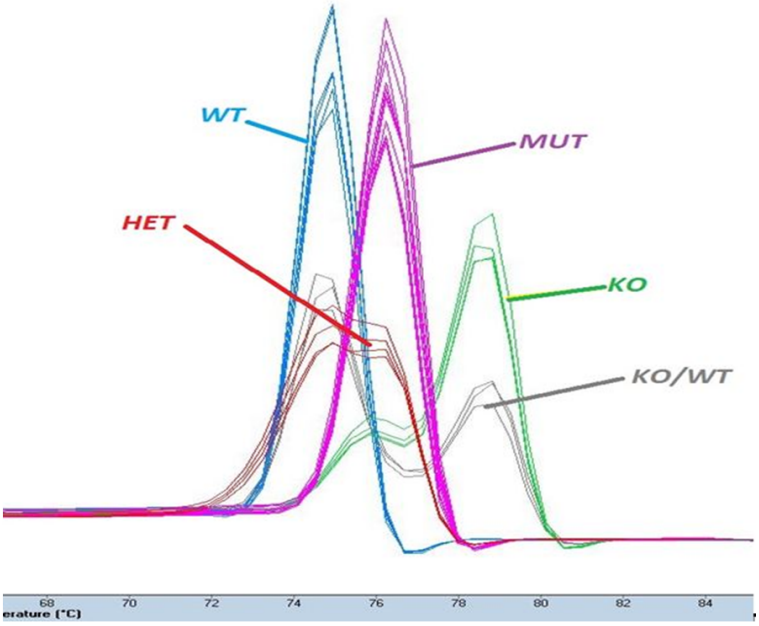
MC4R T → C



NEK8 G → T



HRM Analysis
multiple genotypes in one PCR



5 HOW DO I GET MY RESULTS?

Our online sample submission portal will send clients an email with a link to download results within an approximate turnaround time of 48-72h for standard genotyping (copy number results take several working days). Clients will also receive an email copy of the results (excel format). If the client is a Stuart software user, results will also be uploaded into Stuart or sent to “Draft Genotypes” where they can be accepted manually.

What is the turnaround time?

We usually complete standard genotyping within 72 hours after sample reception (faster for submitted DNA samples).

In case we have dropouts we will repeat them once. If they still do not come up we will inform you about the problem. In exceptional cases the genotyping might be delayed by a day or 2 if instruments are out of order or staff is sick, or if we are processing an unusually high number of samples.

You can also label your samples as urgent and we will make a special effort to process them ASAP. Samples can be analysed with highest priority within 36 hours after reception with an additional charge for urgency.

What are the prices?

Prices can be found on our webpage:

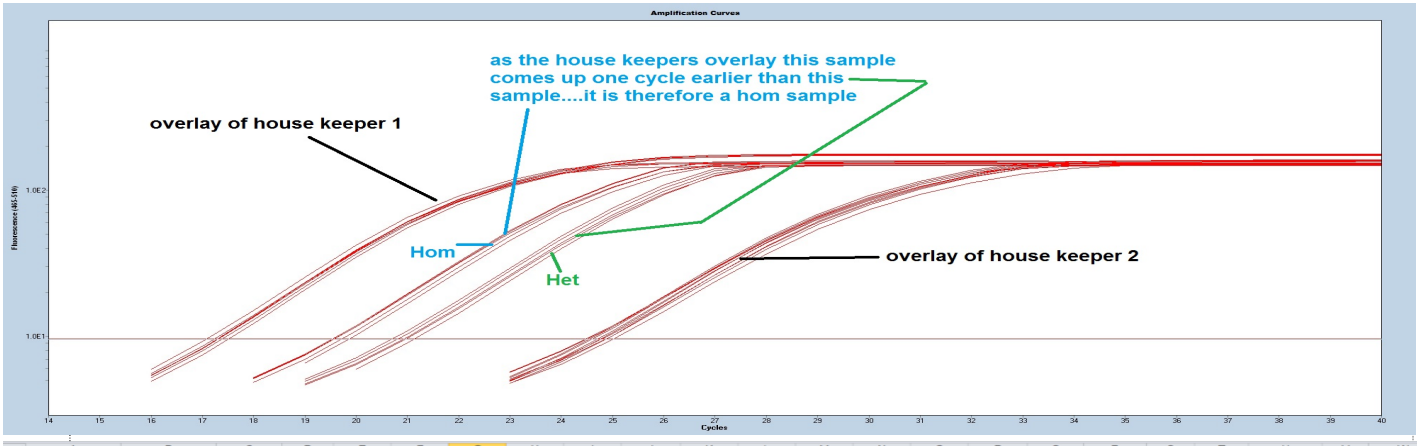
https://www.garvan.org.au/research/capabilities/molecular-genetics/shop

6 DO YOU DO COPY NUMBER VARIATION ANALYSIS?

Yes we do Copy Number Analysis to differentiate Het from Hom animals if there is no other way to differentiate TG animals other than Southern Blot. We use realtime PCR quantification to do the trick. The idea is that when exactly the same amount of DNA is added to a PCR reaction and the amplification process monitored via realtime PCR a homozygous sample will have a crossing point exactly one cycle earlier than a heterozygous sample.

Therefore we can distinguish heterozygous samples from homozygous samples for any given gene by this protocol. For the method to work we quantify the DNA exactly, dilute to the same concentration and set up 5 PCRs (repetitions) of each sample for the target gene and for two house keeper genes. We then amplify in realtime mode and form the average Ct value for each sample, subtract the house keeper and compare to controls. This test only works with very clean

DNA of a concentration >60ng/ul (min 50ul volume) and a het and a hom control.



Date: 06-02-17
Contact: Jason

Copy Number Analysis C-alpha

Original Plate Name	Sample ID	Shortline Name	Animal ID	37L1KO cT Values					37L1KO Average	C-alpha cT Values					C-alpha Average	ΔTarget	ΔControl	ΔΔ Cz	Fold Change	Calculated Result	Visual Result	Final Genotype
240117-1N	30165021	37L1null	92	24.23	24.31	24.26	24.3	24.29	24.28	24.75	24.86	24.8	24.78	24.76	24.79	-0.51	-0.30	-0.21	1.16	Hom	Hom	Hom
240117-1N	30165022	37L1null	93	24.51	24.48	24.48	24.51	24.47	24.49	24.86	24.77	24.8	24.75	24.75	24.79	-0.30	-0.30	0.00	1.00	Hom	Hom	Hom
240117-1N	30165023	37L1null	94	25.09	25.12	25.12	25.14	25.11	25.12	24.71	24.67	24.68	24.67	24.72	24.69	0.43	-0.30	0.73	0.60	Het	Het	Het
240117-1N	30165024	37L1null	95	25.18	25.24	25.21	25.19	25.2	25.20	24.75	24.69	24.68	24.72	24.77	24.72	0.48	-0.30	0.78	0.58	Het	Het	Het
240117-1N	30165025	37L1null	96	25.36	25.28	25.31	25.48	25.35	25.36	24.75	24.74	24.67	24.83	24.75	24.75	0.61	-0.30	0.91	0.53	Het	Het	Het
240117-1N	30165026	37L1null	97	25.51	25.51	25.56	25.56	25.56	25.54	24.7	24.73	24.86	24.72	24.79	24.76	0.78	-0.30	1.08	0.47	Het	Het	Het
240117-1N	30165027	37L1null	98	24.28	24.5	24.35	24.46	24.31	24.38	24.63	24.79	24.81	24.75	24.78	24.75	-0.37	-0.30	-0.07	1.05	Hom	Hom	Hom
240117-1N	30165028	37L1null	99	24.33	24.24	24.2	24.22	24.15	24.23	24.79	24.64	24.67	24.69	24.67	24.69	-0.46	-0.30	-0.16	1.12	Hom	Hom	Hom
240117-1N	30165029	37L1null	1	25.52	25.36	25.29	25.29	25.38	25.37	24.91	24.78	24.8	24.77	24.85	24.82	0.55	-0.30	0.85	0.56	Het	Het	Het
240117-1N	30165030	37L1null	2	24.33	24.47	24.35	24.18	24.23	24.31	24.85	24.82	24.79	24.77	24.77	24.80	-0.49	-0.30	-0.19	1.14	Hom	Hom	Hom
240117-1N	30165031	37L1null	3	24.19	24.28	24.17	24.23	24.29	24.23	24.98	24.76	24.83	24.77	24.83	24.83	-0.60	-0.30	-0.30	1.23	Hom	Hom	Hom
291216-5N	30162390	37L1null	63	25.05	25.15	25.09	25.17	25.08	25.11	25.17	25.12	25.12	25.28	25.13	25.16	-0.06	-0.30	0.24	0.84	Hom	Hom	Hom
291216-5N	30162392	37L1null	65	25.26	25.34	25.33	25.26	25.23	25.28	24.85	24.84	24.97	24.86	24.84	24.87	0.41	-0.30	0.71	0.61	Het	Het	Het Control

Copy Number Analysis Internal Control

Original Plate Name	Sample ID	Shortline Name	Animal ID	37L1KO cT Values					37L1KO Average	IC cT Values					IC Average	ΔTarget	ΔControl	ΔΔ Cz	Fold Change	Calculated Result	Visual Result	Final Genotype
240117-1N	30165021	37L1null	92	24.23	24.31	24.26	24.3	24.29	24.28	18.23	18.37	18.36	18.31	18.24	18.30	5.98	5.98	0.00	1.00	Hom	Hom	Hom
240117-1N	30165022	37L1null	93	24.51	24.48	24.48	24.51	24.47	24.49	18.57	18.46	18.52	18.5	18.48	18.51	5.98	5.98	0.00	1.00	Hom	Hom	Hom
240117-1N	30165023	37L1null	94	25.09	25.12	25.12	25.14	25.11	25.12	18.14	18.07	18.05	18.05	18.09	18.08	7.04	5.98	1.06	0.48	Het	Het	Het
240117-1N	30165024	37L1null	95	25.18	25.24	25.21	25.19	25.2	25.20	18.22	18.25	18.25	18.12	18.29	18.23	6.98	5.98	1.00	0.50	Het	Het	Het
240117-1N	30165025	37L1null	96	25.36	25.28	25.31	25.48	25.35	25.36	18.16	18.27	18.21	18.3	19.5	18.49	6.87	5.98	0.89	0.54	Het	Het	Het
240117-1N	30165026	37L1null	97	25.51	25.51	25.56	25.56	25.56	25.54	18.32	18.28	18.5	18.28	18.37	18.35	7.19	5.98	1.21	0.43	Het	Het	Het
240117-1N	30165027	37L1null	98	24.28	24.5	24.35	24.46	24.31	24.38	18.48	18.53	18.43	18.46	18.45	18.47	5.91	5.98	-0.07	1.05	Hom	Hom	Hom
240117-1N	30165028	37L1null	99	24.33	24.24	24.2	24.22	24.15	24.23	18.24	18.24	18.23	18.25	18.17	18.23	6.00	5.98	0.02	0.98	Hom	Hom	Hom
240117-1N	30165029	37L1null	1	25.52	25.36	25.29	25.29	25.38	25.37	18.24	18.18	18.17	18.18	18.09	18.17	7.20	5.98	1.22	0.43	Het	Het	Het
240117-1N	30165030	37L1null	2	24.33	24.47	24.35	24.18	24.23	24.31	18.3	18.26	18.27	18.32	18.35	18.30	6.01	5.98	0.03	0.98	Hom	Hom	Hom
240117-1N	30165031	37L1null	3	24.19	24.28	24.17	24.23	24.29	24.23	18.01	17.99	18	17.94	18.05	18.00	6.23	5.98	0.25	0.84	Hom	Hom	Hom
291216-5N	30162390	37L1null	63	25.05	25.15	25.09	25.17	25.08	25.11	19.07	19.24	19.06	19.16	19.12	19.13	5.98	5.98	0.00	1.00	Hom	Hom	Hom
291216-5N	30162392	37L1null	65	25.26	25.34	25.33	25.26	25.23	25.28	18.76	18.77	18.88	18.71	18.81	18.79	6.50	5.98	0.52	0.70	Het	Het	Het Control

We also offer a Mouse SNP panel for genetic monitoring of inbred strains

We also offer a 28 SNP panel for quality controlling your inbred mouse lines routinely. The panel assesses genetic contamination in colonies containing a large number of genetically diverse mouse strains. This test is used by the Jackson Laboratory for their mouse lines and investigates 28 relevant loci which cover over 300 inbred, wild-derived, congenic, consomic and recombinant inbred strains.

7 CAN I GET THE DNA FOR FURTHER TESTING IN MY LAB?

After genotyping results have been sent you can pick up the remaining DNA from the sample reception fridge if you needed the samples for further testing or you can ask us to send the DNA with a courier to you (at your cost).

We will keep these samples for 6 months after the results have been sent to you. The DNA extraction we perform is a spin column extraction which gives you very good DNA quality and

usually there is at least 50ul of ~30ng/ul DNA concentration.

Screenshot of our Programming software

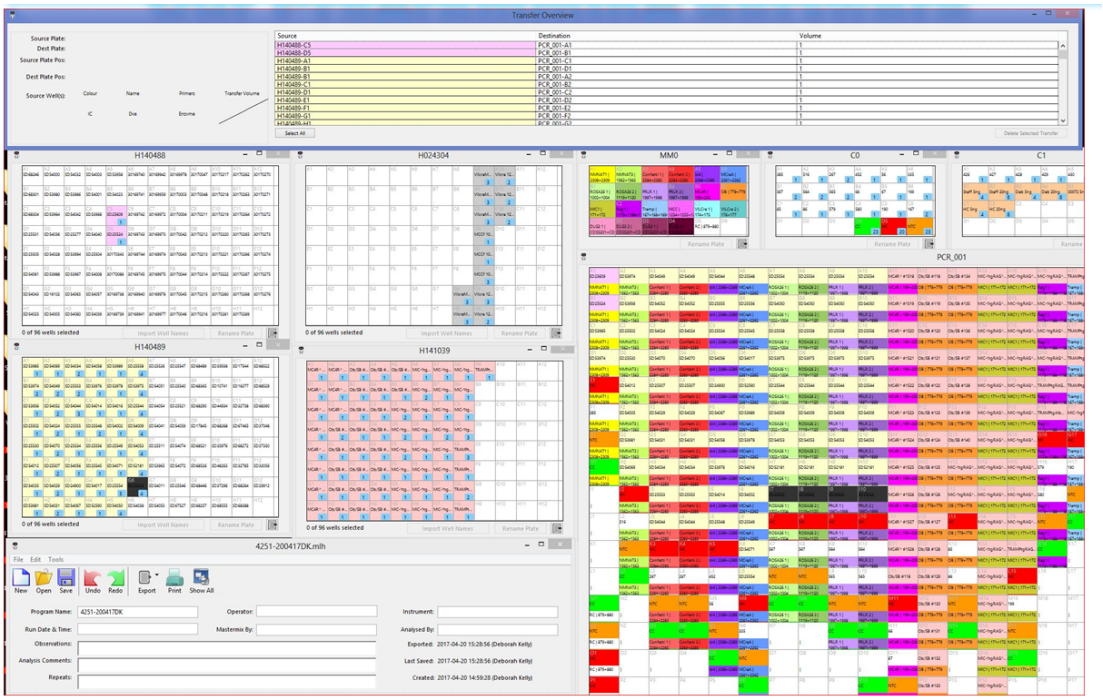


Photo of our PCR setup facility

Working under ISO 17025 means:

- **Procedures are detailed in SOPs**
- **Tests are validated (verified)**
- **Equipment is calibrated**
- **Staff is trained (and retrained)**
- **Complaints, errors and false results are followed up on**
- **Continuous Improvement, Quality goals/quality objectives**
- **Management review / user group meetings / surveys**

NATA

ISO 17025

NATA quality control system means for PCR setup:

- No template control NTC
- Contamination Check Control CC
- Standard WT control MC
- PCR specific Positive Controls (Numbers)
- Internal control IC
- Reference control RC
- DNA extraction control PC

ISO 17025