iMits
International Micro-Injection Tracking System

iMITS User Manual

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A. iMITS Overview

iMITS coordinates the production of mutant mice for high-throughput production pipelines to minimize overlap and maximize efficiencies. iMITS captures the intention of a particular consortium to produce mutant mice on a particular gene, the progress of mouse production and modified allele creation on the original mouse, and the capture of phenotype data on the mouse.

iMITs serves mouse production facilities from around the world as part of its funded mission to support the KOMP2 project – a NIH Common fund project to generate and phenotype 5,000 knockout mouse strains in the next five years (1U54HG006370). Mouse production and phenotype centers in the KOMP2 project are required to track mouse production through iMITS to minimize overlap and maximize efficiencies. KOMP2 production is coordinated with other mouse production centers through participation in the International Mouse Phenotyping Consortium (IMPC) whose goal is to generate a knockout mouse strain for every protein-coding gene in the mouse genome in the next 5 years. To ensure a common genetic background, IMPC partners use the mouse lines generated by the International Knockout Mouse consortium (IKMC). The majority of IMPC production centers use iMITs to track production to help prevent duplicate strain production and identify how their progress compares to other centers.

The information in iMITS is provided by each individual production center/consortia through the iMITS graphical interface. This is a trust system – iMITS does not have access to a center's laboratory management system and thus cannot double check entries. Therefore, it's crucial that personnel entering in the information understand how the interfaces work. In this document, we will provide a step-by-step guide. A glossary explains unique terms and a Status Table explains what is meant when iMITS assigns a status to a particular field.

B. WORKFLOW

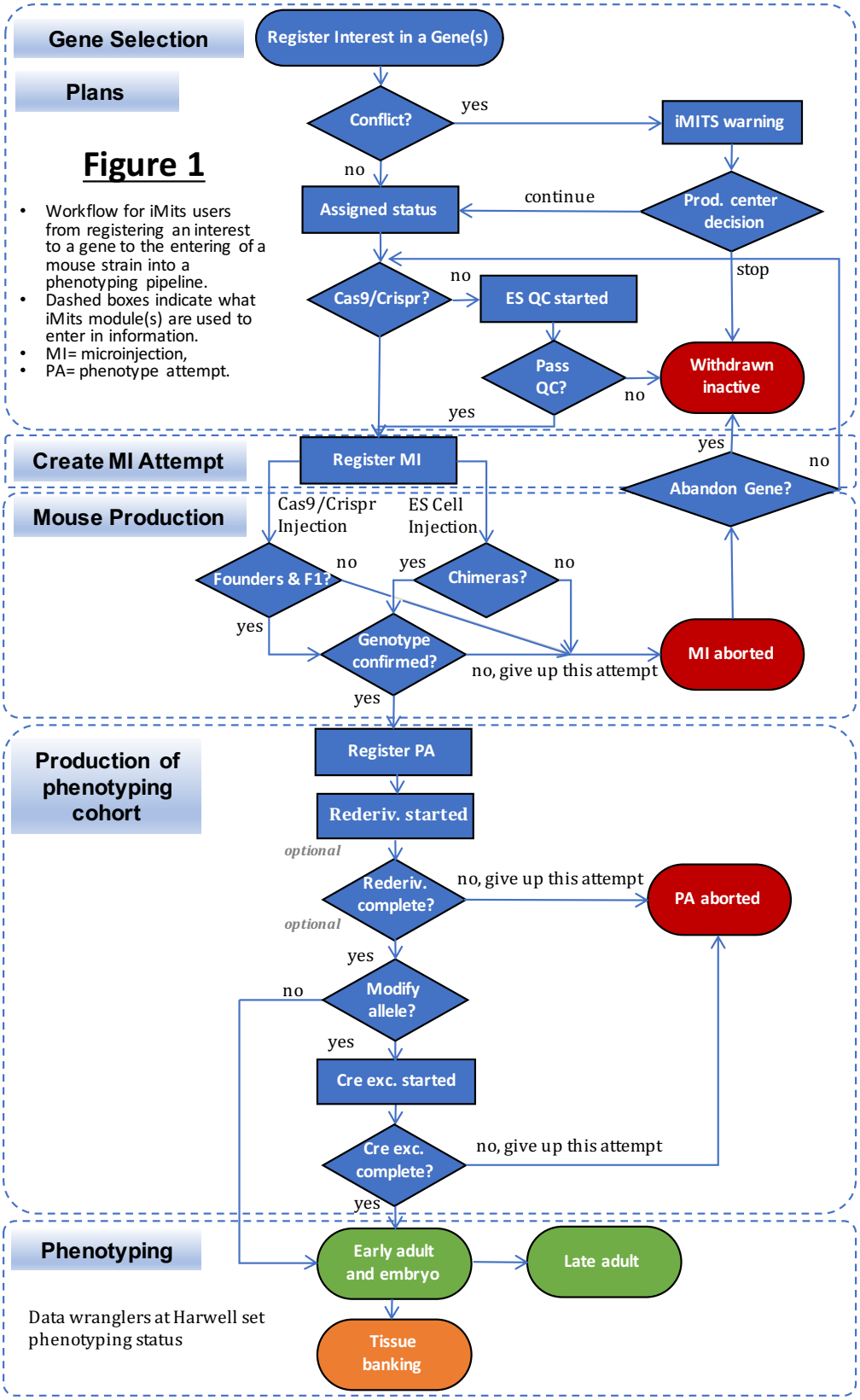
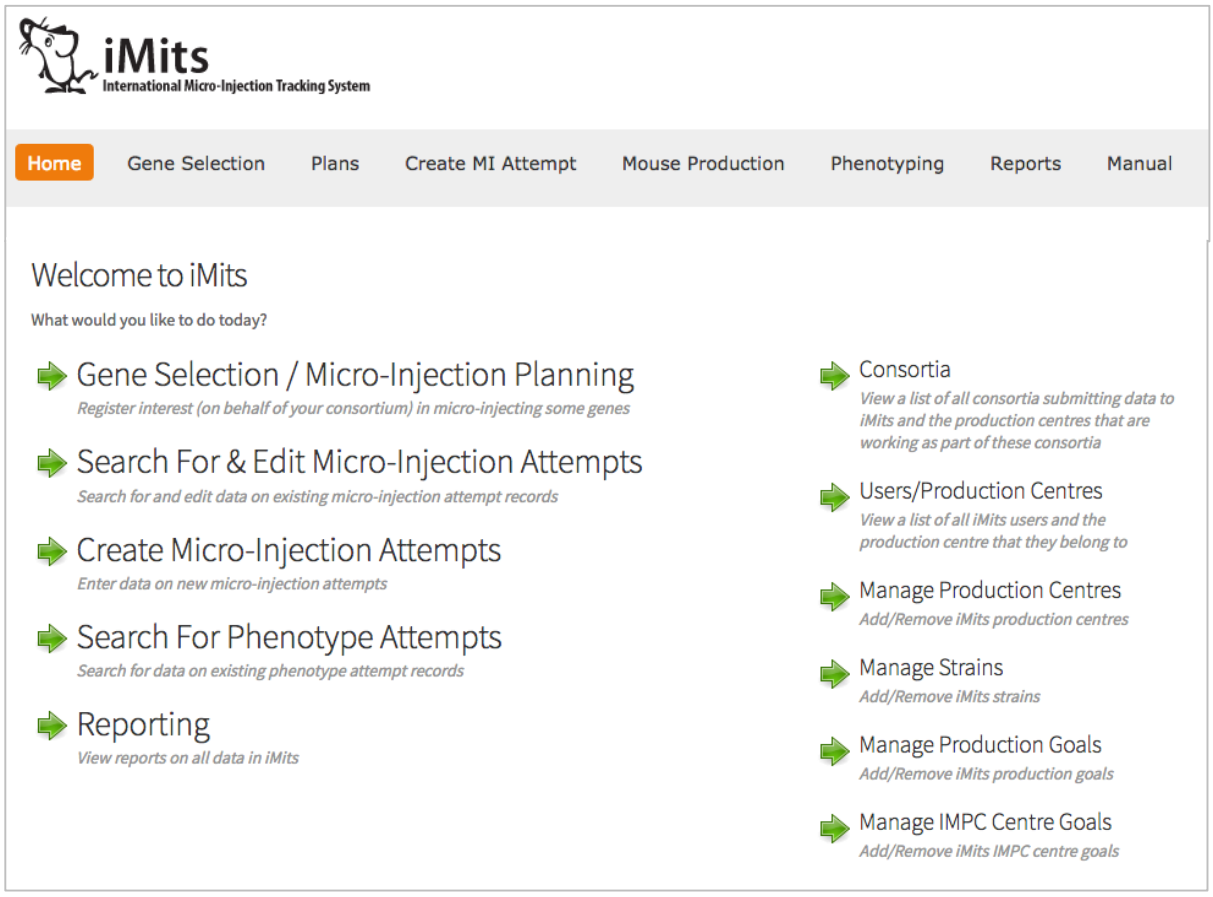


Figure 1

- Workflow for iMits users from registering an interest to a gene to the entering of a mouse strain into a phenotyping pipeline.
- Dashed boxes indicate what iMits module(s) are used to enter in information.
- MI= microinjection,
- PA= phenotype attempt.

1. Gene Selection: selecting a gene for Mouse Production, creating a Plan.



Users acting on behalf of consortia select genes for mouse production. Users will be alerted if mouse strains with targeted mutations of this gene are in production or have been produced/phenotyped by other IMPC partners. The process works in the following manner:

Users create a plan for the gene(s) of interest. When a plan is created, iMITS will check whether any other consortia have plans (or mice) for the gene. If not, the plan will be immediately put in status "Assigned". If there are other existing plans or mice, iMITS will put the plan in status "Inspect – Conflict" or "Inspect – MI Attempt" or "Inspect – GLT Mice" depending on the known products.

Conflict statuses do not preclude the centre from actually acting (starting ES-QC or starting mouse production – see below). If the centre does start activity, the plan will be changed by iMITS to have status "Assigned – ES QC in progress", etc.

NOTE. – iMITS' role is not to stop production centres from creating duplicate mouse strains, but rather to inform production centres of similar efforts so they may make informed decisions. Duplicate strain production is to be minimized under the KOMP2 funding mechanism.

1. Select Gene(s) of interest. Click on the "Gene Selection" tab from the top menu (see screen capture below) or from the list on the homepage (see screen capture above). Note you can adjust the width of the columns as well as use the filters next to the column heading.

Here I have selected **1110002L01Rik** and filled in the Consortium, Production Centre and Priority using the drop-down menus. Priority reflects centre's own priority for that gene. If the Crispr/Cas9 box is ticked, then it is assumed that the Centre will conduct Crispr/Cas9 mutagenesis for mouse production. If the Crispr/Cas9 box is not ticked, production needs ES cells; in such a case, the Priority field is used to help prioritize ES cells distribution from the ES cells repository. Tick the "Phenotype only" box to reflect interest in phenotyping an existing colony produced by another production centre (sections 2b and 5c provides more information on this latter aspect).

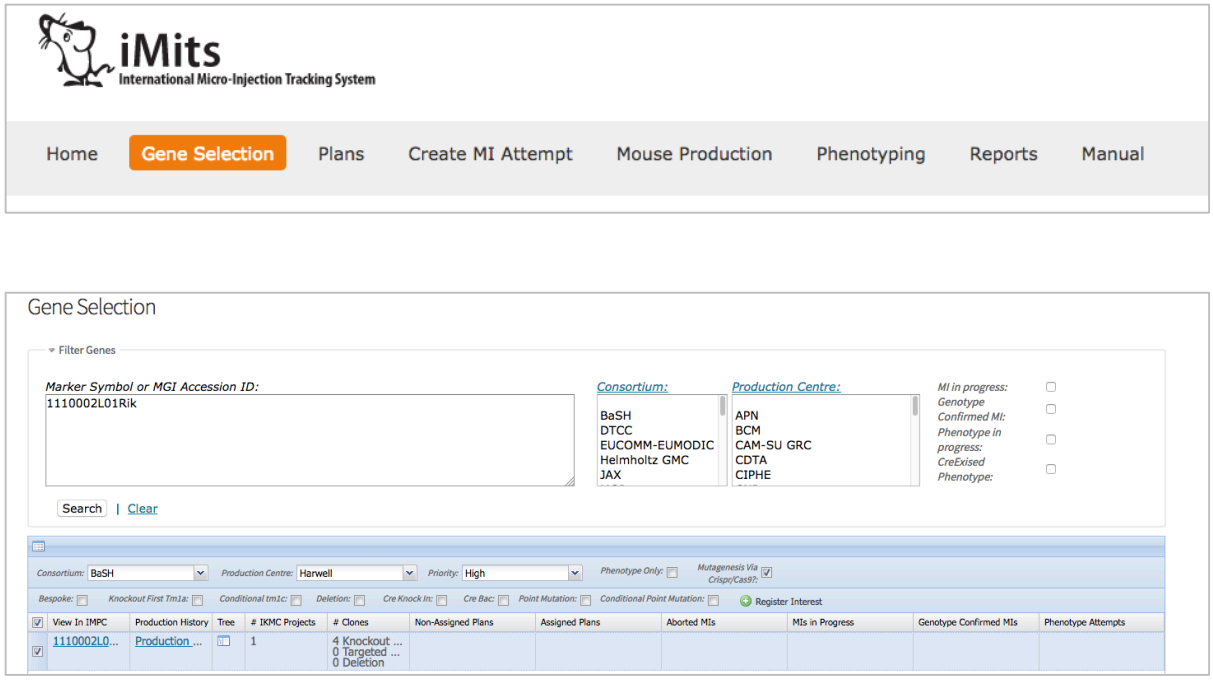


Figure 2 Register interest in microinjecting genes or phenotyping only a mouse model.

2. Push the "Register Interest" button to create the Plan. In this case, the Plan has been *Assigned*, because there were no other competing plans or production.

3. Edit the Plan – if necessary – to specify what kind of allele you are intending to make. Clicking on the plan will bring up the plan edit page. This page has a section that allows the user to select an allele type (Figure 3). This is very useful if you want to distinguish intent – for instance, if you want to make it clear to other users that you intend to make a Knockout First Tm1a allele (or a Cre-Knockin). In the following, I've checked the 'Knockout First Tm1a' box to make it clear for reporting purposes that I am going to make a Knockout First Tm1a (in case there are any deletion clones around).

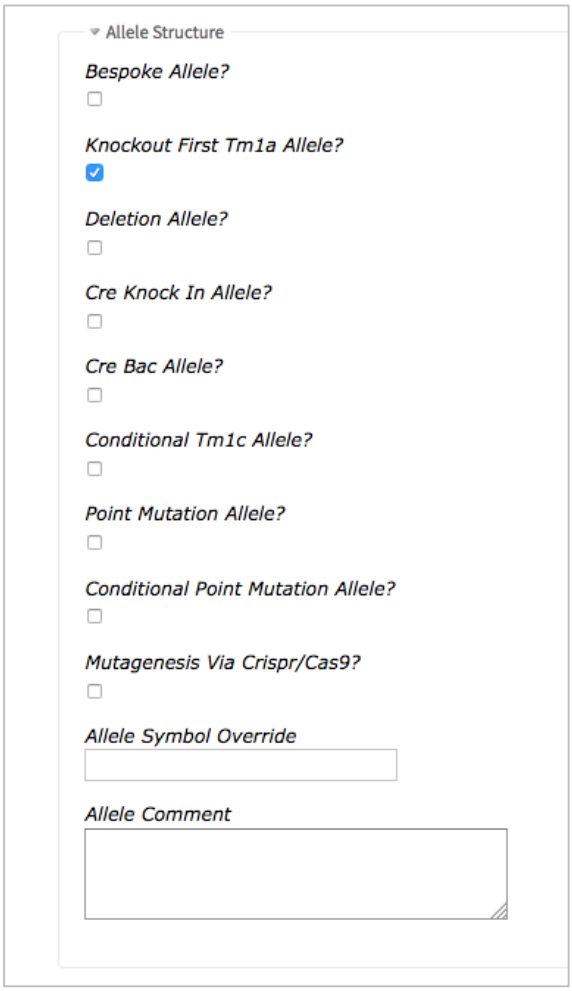
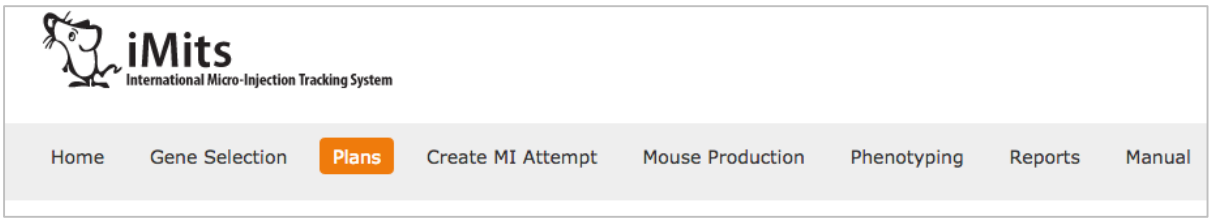


Figure 3 Dialogue for changing details for an ES cell clone.

An ES Cell distribution centre (or a mouse clinic doing data entry on behalf of an ES distribution centre) can indicate the progress of ES QC for a plan by editing the data on the plan. This edit page is also accessible from the Plans module (next section).

2a. Editing plans for Mouse Production.



The "Plans" module is selected by clicking the "Plans" tab. By default, the grid is filtered on the Users' Production Centre. This can be overridden by unticking the corresponding filter. You can search the grid by opening the drop-down menus next to the column headers. Here we search for **1110002L01Rik** we selected for production above (Figure 4).

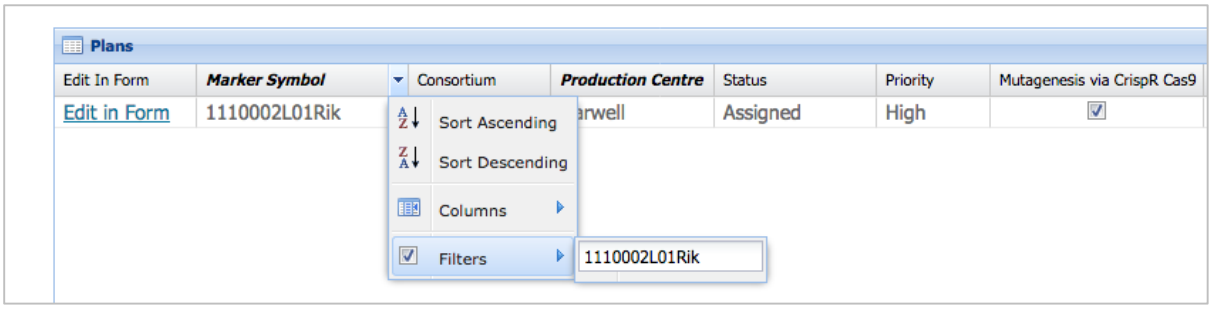


Figure 4 Filtering by marker of interest.

Clicking 'Edit in Form' will allow you to edit the plan.

Entering a non-zero value in "Number of ES Cells starting QC" will cause the plan to change state from its previous status ("Assigned" or "Inspect …", etc.) to "Assigned – ES Cell QC in Progress" (Figure 5).

Entering a non-zero value in "Number of ES Cells passing QC" will cause the plan to change status to "Assigned – ES Cell QC Complete" (Figure 5). If the "Number of ES Cells passing QC" is set to zero, then the status of the Plan will change to "Aborted – ES Cell QC Failed". This indicates that attempts to thaw / pass clones prior to Mouse production have failed for this consortium.

Note. – Do not forget to click "Update" to see changes to the plan implemented.

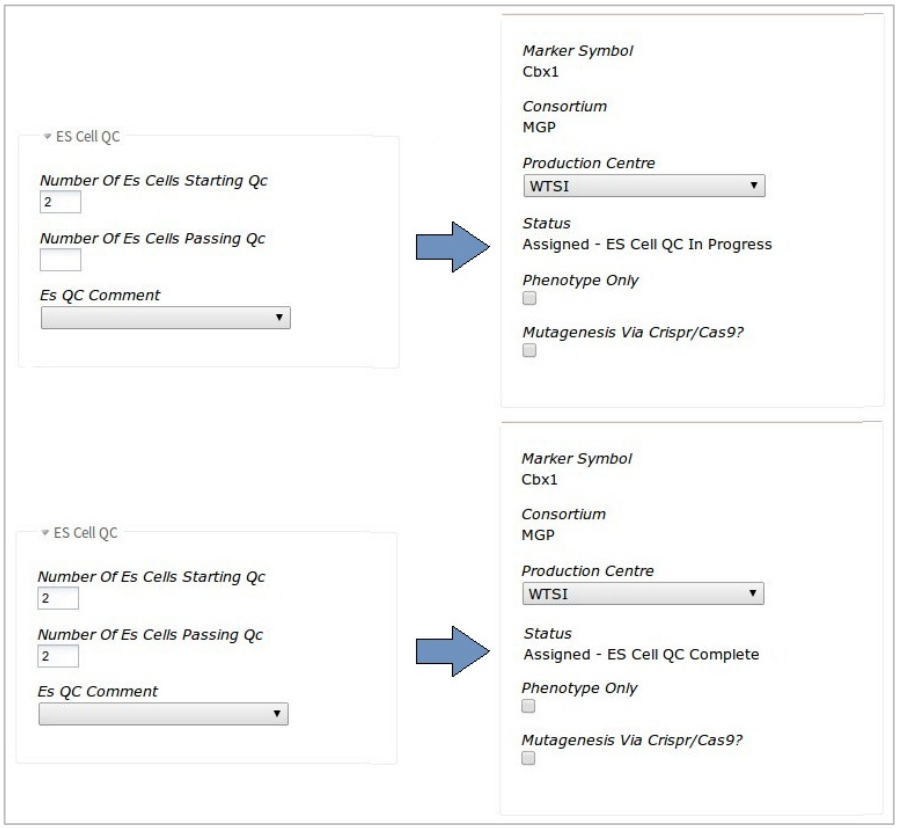


Figure 5 Status change of ES clone to "ES Cell QC in Progress" (top) or to "ES Cell QC Complete" (bottom).

2b. Editing a plan to indicate CRISPR injection or phenotyping.

The Plans are not only used to show intent to produce mice from clones. They can also be used to show intent to:

- Create a mouse colony through mutagenesis via CRISPR injection (with or without a vector/ oligos).
- Phenotype an existing colony produced by another production centre.

Note this could have been already selected during the Gene Selection step, in section 1 above, but changes are possible at this stage (Figure 6).

1. To show intent to create a mouse colony through mutagenesis via Crispr injection tick the 'Mutagenesis Via Crispr/Cas9' box.

2. To show intent to phenotype an existing colony produced by another production centre tick the 'phenotype_only' tick box.

Note. – Do not forget to click "Update" to see changes to the plan implemented.

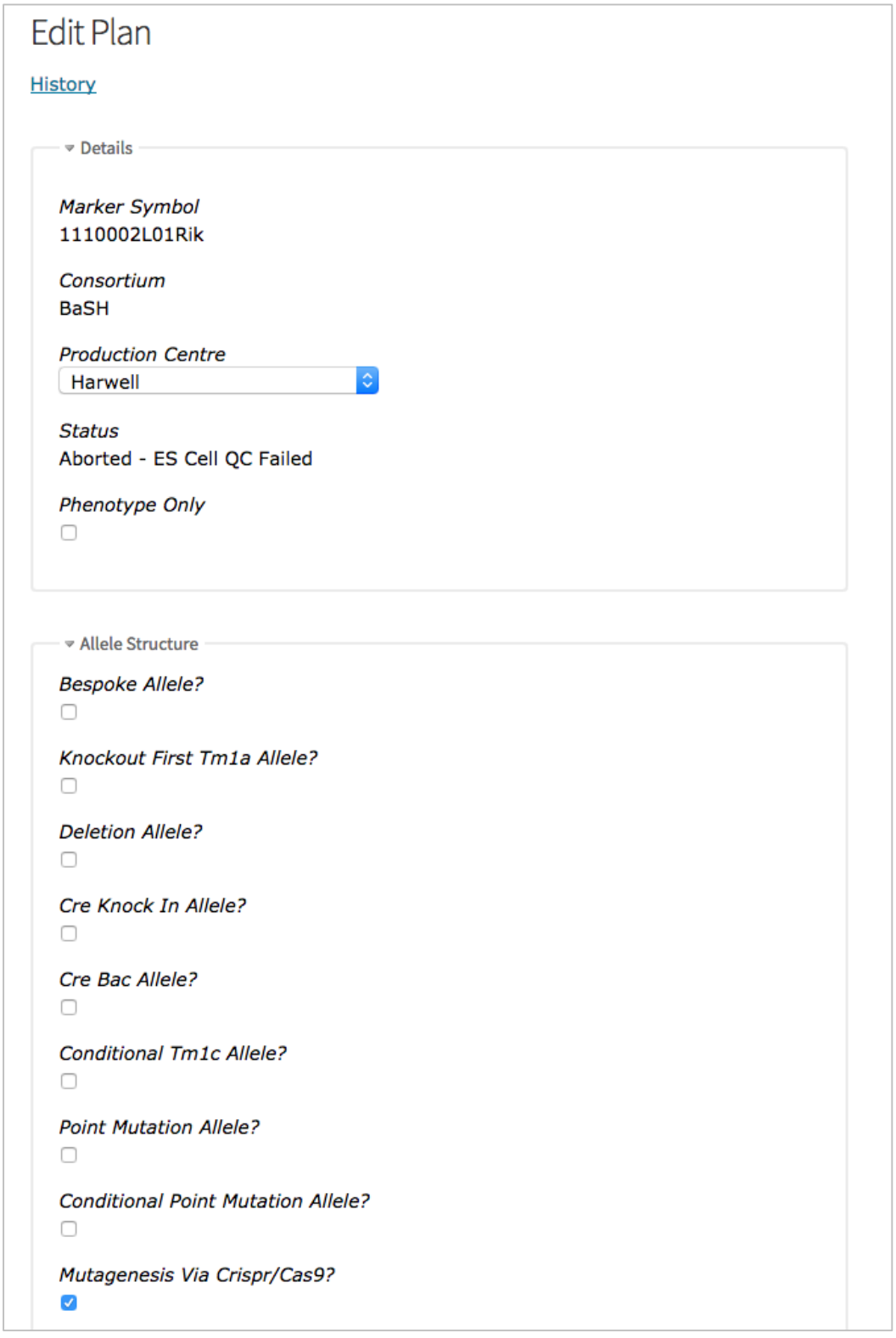


Figure 6 Updating a plan.

2c. Plans module: withdrawing interest in a gene, inactivating a plan, deleting a plan.

A user can:

- Withdraw interest in a plan prior to the start of any mouse production;
- Inactivate a plan with no *active* mouse production;
- Ignore Available Mice, which hides mice produced by this plan from the public;
- Delete an existing plan

by clicking on the corresponding buttons on the plan's edit page (Figure 7).

All of these changes will mean the plan does not 'compete' with other consortia looking to make plans on that gene.

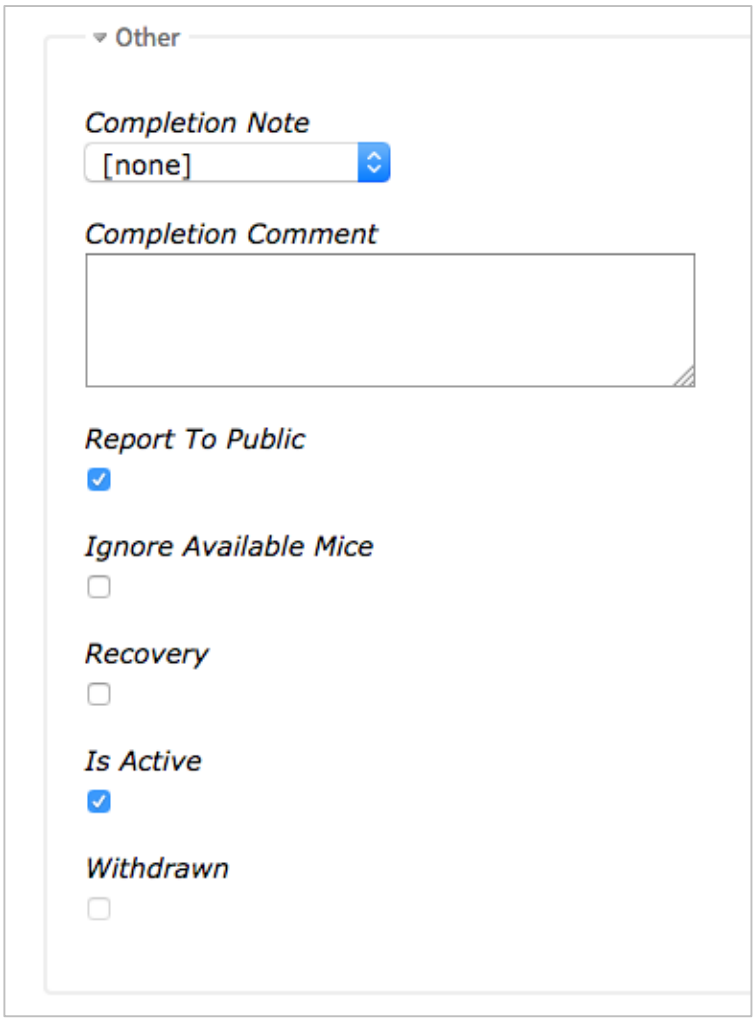
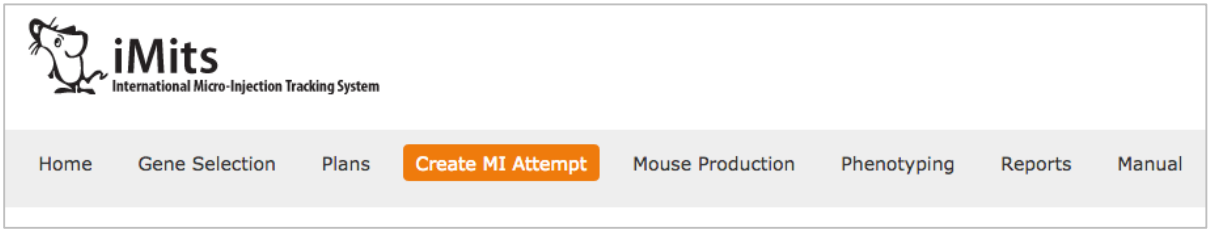


Figure 7 Withdrawing interest in a gene, aborting, deleting and hiding produced mice from the public.

3a. MI Attempts: Initiating Mouse Production for a gene.



iMITS tracks mouse production through microinjections of ES cells or mutagenesis factors (via Crispr/Cas9).

Note. – Before starting, check the appropriate Plans and that Targeting/HDR vectors have been created (for mutagenesis factor, see section C on adding donor vectors prior to entering CRISPR injection information in iMITS).

To record the start of a microinjection step, click the "Create MI Attempt" tab. This will give you two options:

i. Create ES Cell MI;

or

ii. Create Crispr MI.

(i) Mouse Production using ES Cell.

ES Cells will be sent from the ES distribution centre to the Mouse Clinic, where they will be prepared for microinjection.

Click the 'Select ES Cell' button and search for clones that are visible for that gene, by either gene symbol or clone name (Figure 8).

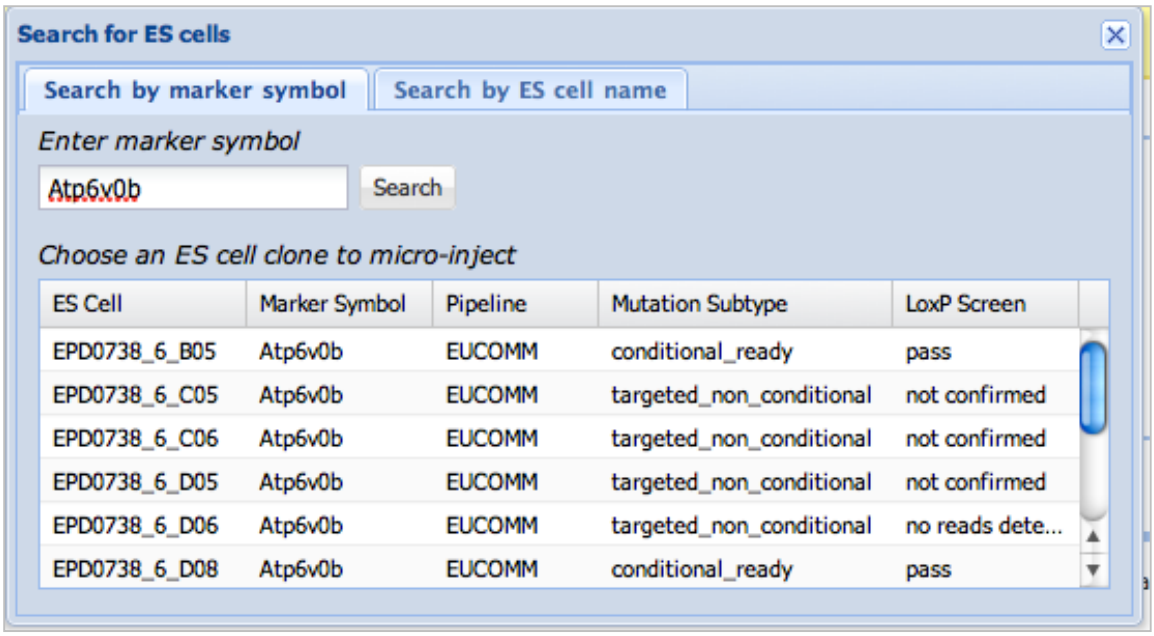


Figure 8 Searching for ES cell clones to be used in microinjection attempts.

Choose the ES cell you have used for microinjection (pay attention to the difference between conditional ready and targeted non-conditional clones, which is read from the IKMC targeting repository classification of these clones). I clicked on the row "EPD0738_6_B05", which opens the Create form (continue in section 3b).

(ii) Mouse Production using a Mutagenesis Factor.

Click the 'Create Crispr MI' button and type in the gene marker symbol (I entered Nxn) and click search. This will show you a list of exons which can be used to search for Crisprs/Crispr Pairs (Figure 9). You can also search by gRNA sequence, and enter more than one gRNA sequence.

The underlining tool that is used here is WGE (Whole-Genome Editing), which was developed to aid with genome editing of human and mouse genomes by the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/htgt/wge/).

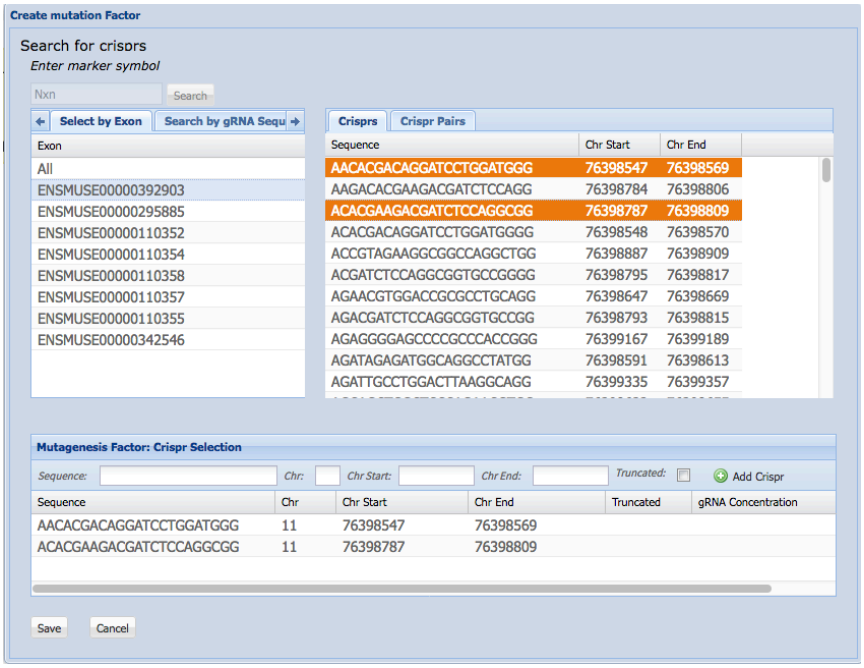


Figure 9 Crispr selection using exon search. Sequences highlighted in orange have been selected.

Alternatively, you can manually enter the 23-base sequence (this includes the PAM sites) and positional information (at least chromosome) of the Crispr (Figure 10). By clicking the box "Truncated", the system allows you to enter sequences shorter than 23 bases. The last column in the Crispr selection pane (scroll right, not in Figure) contains a red button that allows you to delete a sequence.

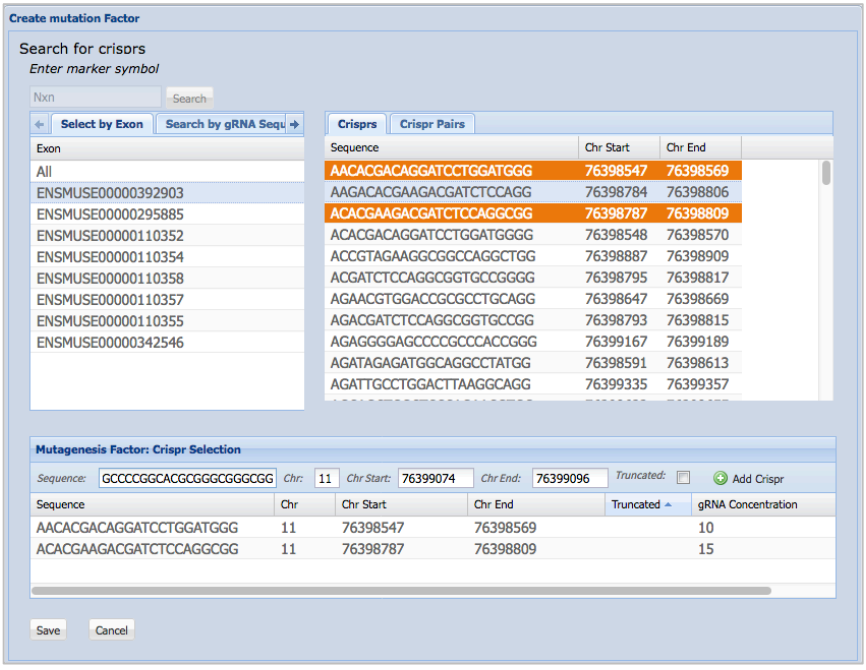


Figure 10 Manually entered Crisprs.

I did my selection of Crisprs for this microinjection and clicked 'Save', which opened up the microinjection Create form to provide further details.

3b. MI Attempts: Mouse Production for a gene.

The first step is to select your Gene Selection plan from the top table, that you created earlier (section 1), and provide the microinjection date (Figure 11).

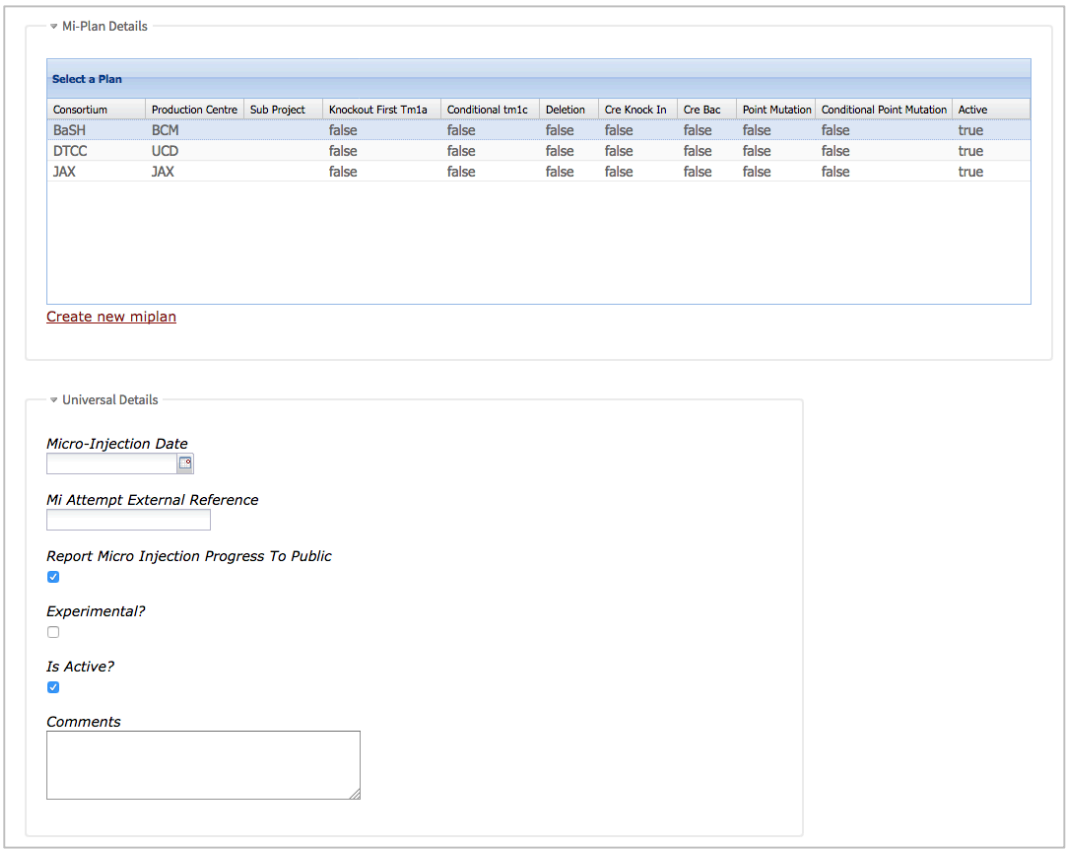


Figure 11 Plan selection and microinjection date.

Next, about the mutagenesis factor details, I have provided Delivery Method, Strain Injected and Nucleases (at least one) using the drop-down menus (Figure 12). If you are microinjecting in an existing mutant strain, you can provide the Parent Colony Name.

NOTE. – The Colony Name is a UNIQUE name CHOSEN BY YOU for the group of mice (the "mouse line") that will arise from this microinjection. WTSI have colony names which are four-letter words such as "MECD". UCD use colony names like "BL1253". Typically, this name is an "external" reference, determined by the name of the colony in the "real" mouse-tracking system available on the mouse clinic campus. This name will be transmitted to the outside world via portals, marts, etc.

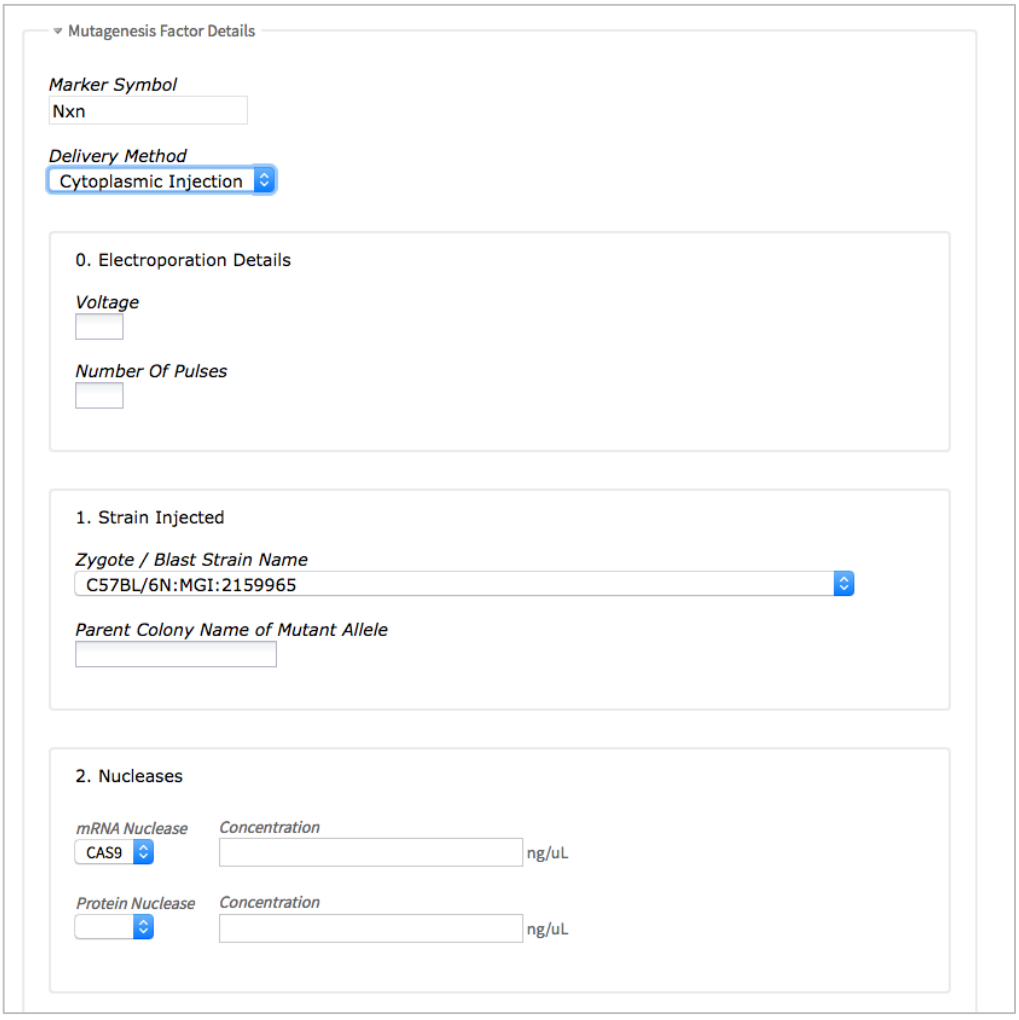


Figure 12 Mutagenesis factor details.

The Crispr sequences I selected in the previous step appear in the Crispr section. Concentrations can be provided individually when different. Multiple vectors can be added by clicking on "Add Vector/Oligo", as well as multiple reagents to increase efficiency by clicking on "Add Reagent" (Figure 13).

Note. – At this point you can still edit your Crispr selection if incorrect.

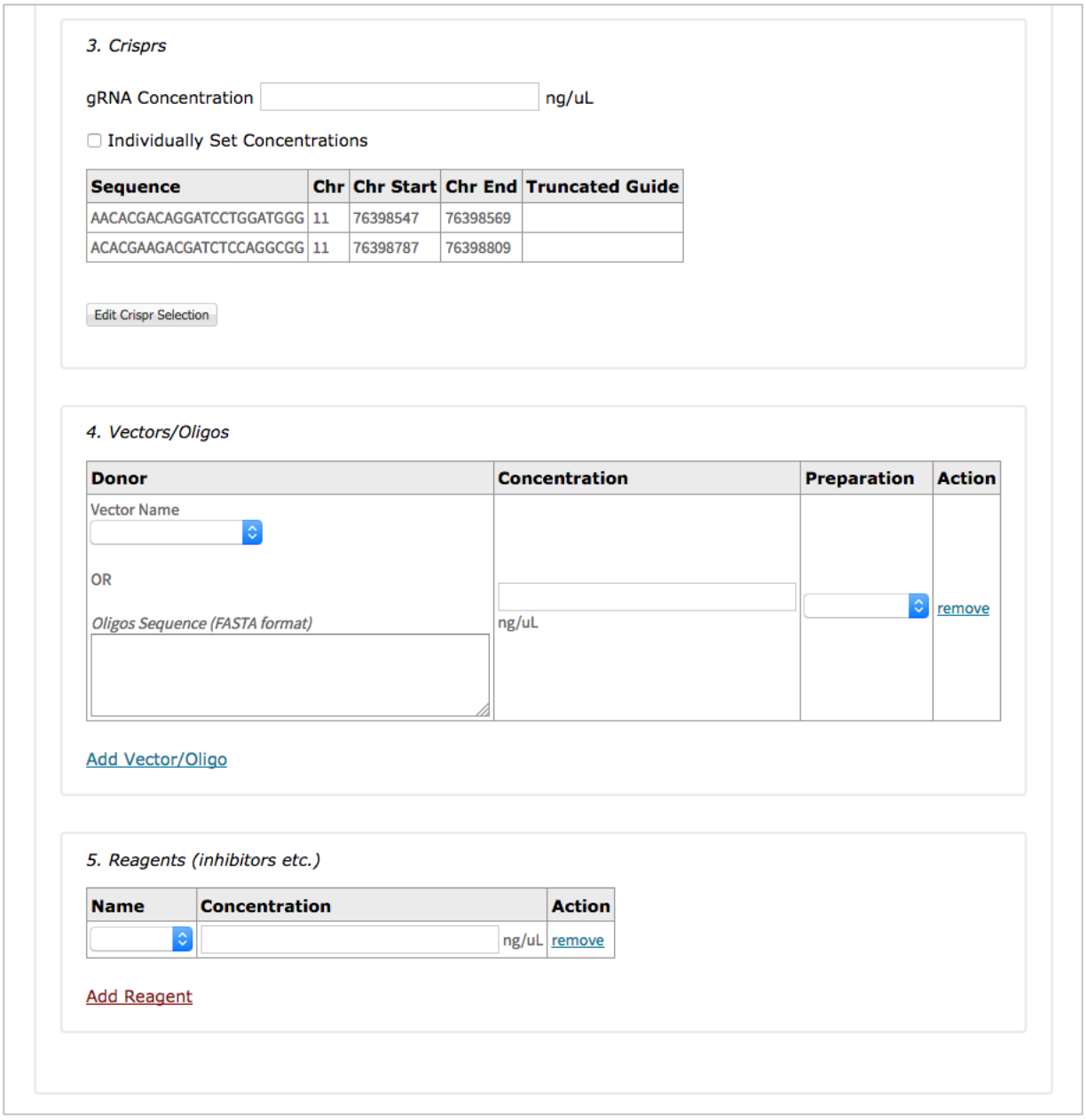


Figure 13 Crispr, Vectors/Oligos and Reagents to increase efficiency.

Next are some details about the verification of the success of the Crispr experiment, including genotyping primers to characterize the nucleotide changes induced, embryos injected and survived, and G0 details; F1 colony details can be entered at a later stage (Figure 14).

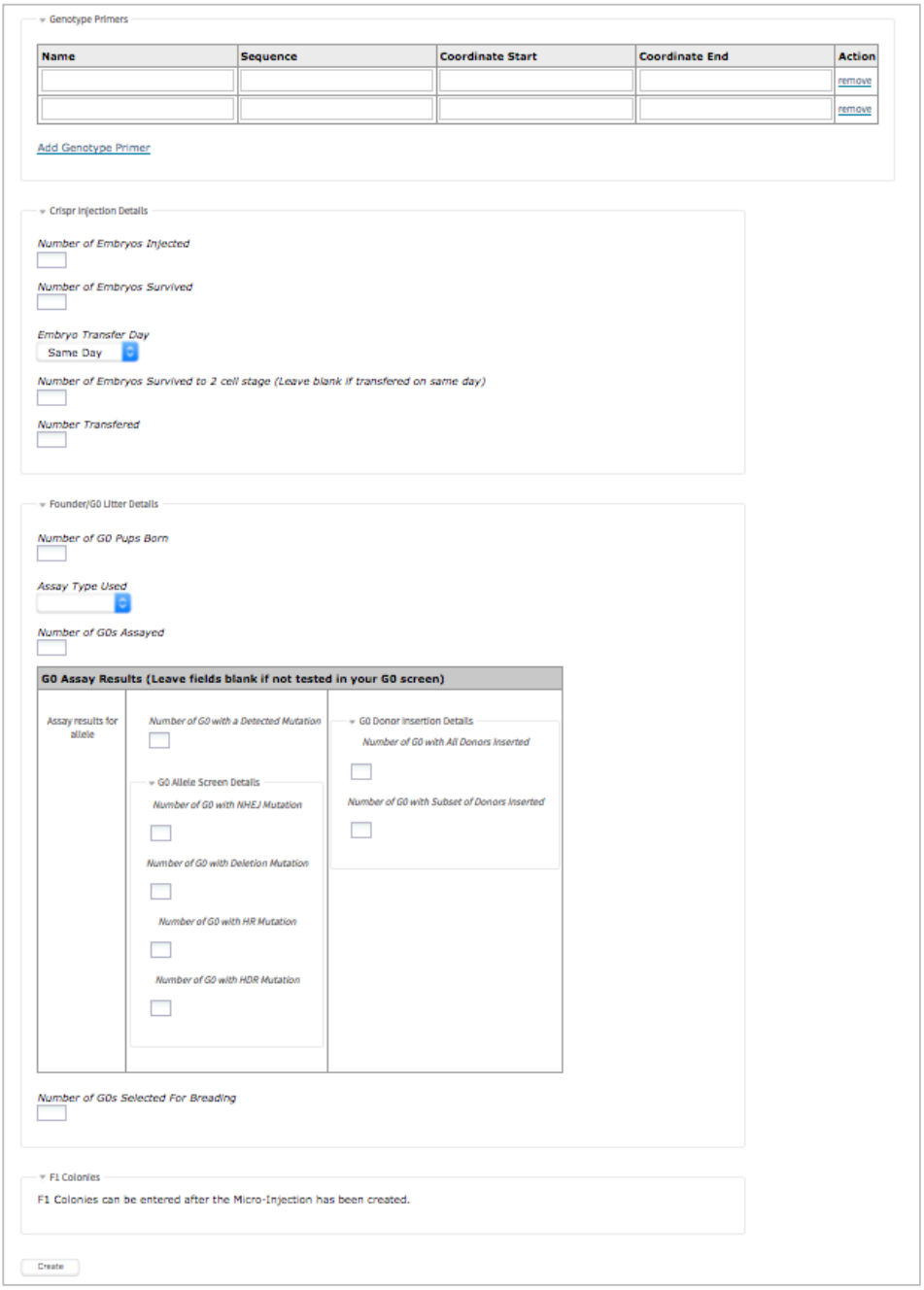


Figure 14 Verification details regarding the success of the Crispr experiment.

Once your ES Cell or Mutagenesis Factor details are setup, this successfully creates the MI Attempt with status 'Microinjection in progress' (Figure 15).

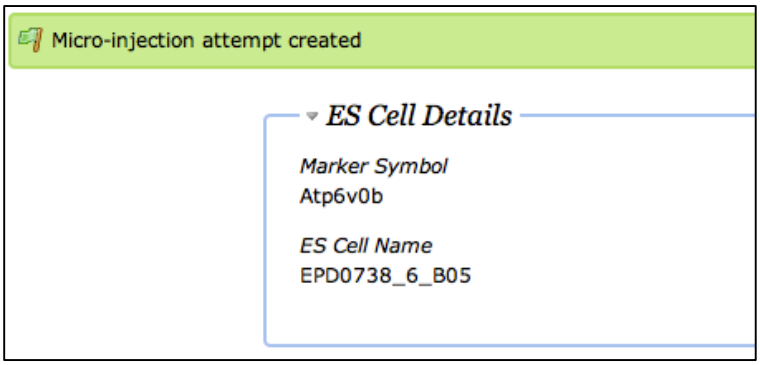
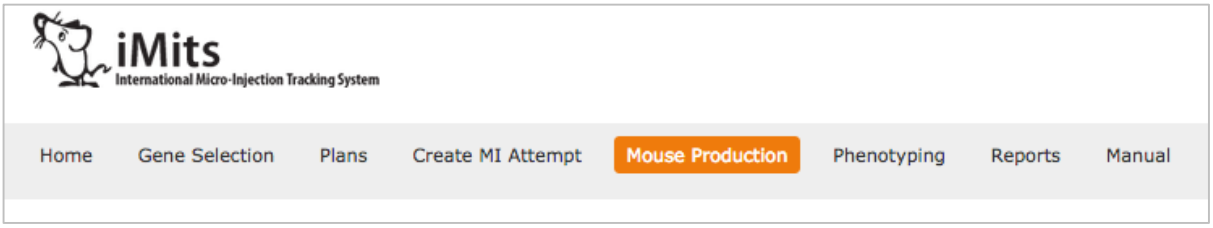


Figure 15 Green flag showing a microinjection attempt has been successfully entered.

4a. Mouse production attempts: Indicating the production of chimeras/founders for a mouse line.



Mouse production attempts can be found and edited via the "Mouse Production" tab. The grid can be searched by any column header. In Figure 16, I used gene symbol. Note also you can filter by production centre or by consortium; by default, the grid is filtered for your Centre (by clicking on the "Production Centre" column header and unclicking "Filter" you can undo this).

Here I've found my microinjection project by filtering by gene symbol Vhl:

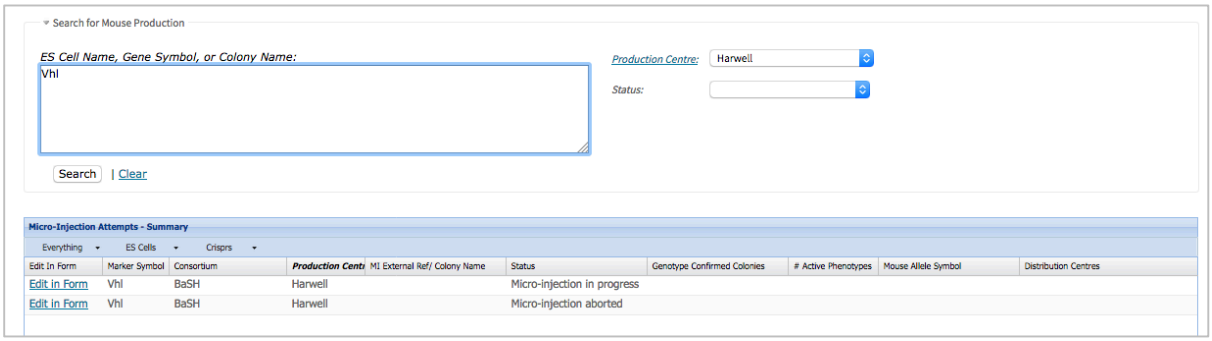


Figure 16 Searching through microinjection attempts.

You can edit this Mouse Production attempt in two ways:

1. By clicking on the row, which will make certain fields in the row editable. Here I've clicked on the row, showing the field with "Genotype Confirmed Colonies" ready to type in:

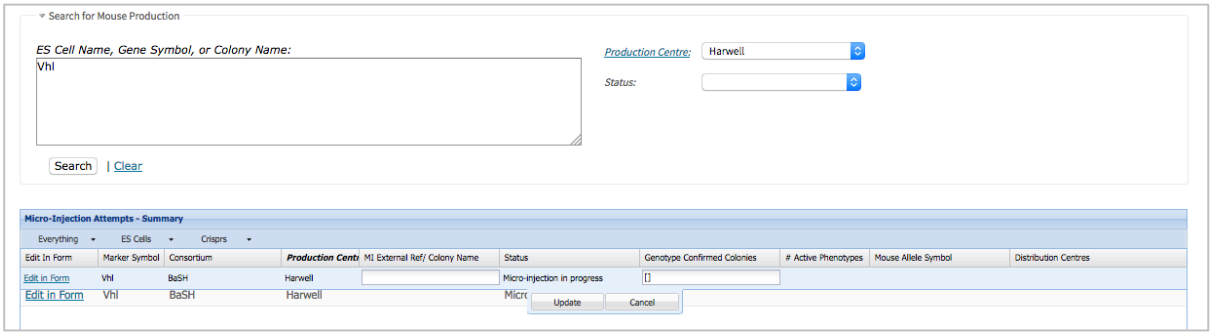


Figure 17 Editing within the mouse production grid.

2. You can also click on the "Edit in Form" link to the left, which will show the MI Attempt in a traditional HTML page.

'Chimeras Obtained' (ES Cell micro-injection) and 'Founders Obtained' (Mutagenesis Factor) statuses are equivalent for the different microinjections (Figure 1). Clicking on the "Edit in Form" link will display slightly different forms depending on the different microinjections.

4b. Mouse production attempts: Indicating Genotype Confirmed mice for a mouse line.

(i) Indicating genotype confirmed for ES Cells

A mouse line changes status to Genotype Confirmed when either of two fields is set to > 0: “Number of Chimeras with GLT from Genotyping” OR “Number of HET offspring” in the “Chimera Mating Details” section (Figure 18).

The following fields must also be filled in, so that details of the mouse line can be used to yield a correct strain name:

1. Test Cross Strain Name: the name of the initial test cross strain used for breeding to establish chimerism.
2. Number of Chimeras with GLT from genotyping. The number of chimeras (i.e., parents) which had germline-transmitting pups, where the GLT was established by genotyping the pups. A non-zero number here will set the MI Attempt status to “Genotype Confirmed”.
3. Number of chimeras with x – y % chimerism. These bins are for information purposes only (they can be reported on).
4. Number of HET offspring. A non-zero number here will set the MI Attempt status to “Genotype Confirmed”.

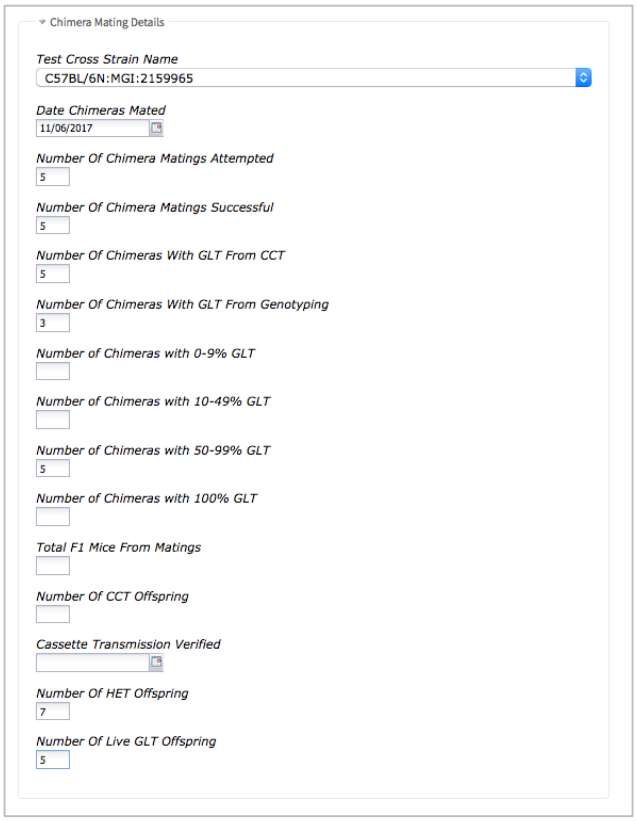


Figure 18 Changing status of mouse production to Genotype Confirmed for ES Cells.

(ii) Indicating genotype confirmed for Crispr mutagenesis

A mouse line changes status to Genotype Confirmed when at least one F1 colony gets the "Genotype Confirmed?" ticked and "Background Strain Name", "Mutant Nucleotide Sequence" and "Mgi Allele Symbol Superscript" reported.

1. Colony Name: as provided in the microinjection project set up details.
2. Background Strain Name: the name of the backcross strain (eventually the colony background) for the genotype-confirmed mouse colony.

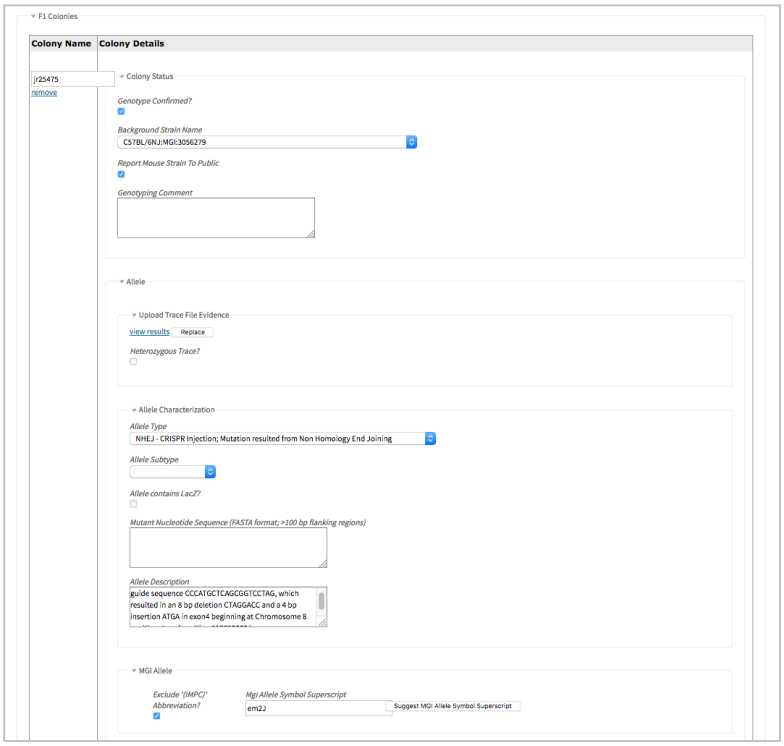


Figure 19 Changing status of mouse production to Genotype Confirmed for Crispr mutagenesis.

NOTE. – Clicking on Report Mouse Strain to Public indicates that the mouse strain is made public in the IMPC portal.

4c. Mouse production attempts: Distribution Centres for Genotype Confirmed Mice.

A Genotype Confirmed line does not automatically inherit a distribution centre**. These must be added manually by the mouse production centre** at the bottom of the "F1 colonies" section (Figure 20). The process is the same whether it is an ES Cells or Crispr mutagenesis project In Figure 20, I have clicked the "Add Distribution Centre" link, and chosen Harwell to act as an EMMA "node", serving live mice. This tells the IMPC portal to refer users to the EMMA repository. It also tells the EMMA repository to expect to advertise this mouse line as orderable.

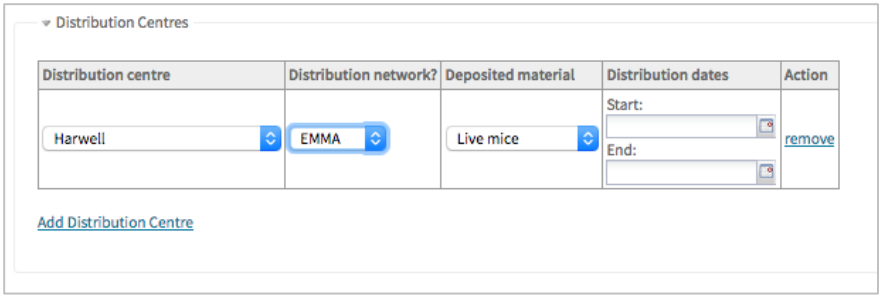


Figure 20 Adding distribution centre to genotype confirmed mice. In this example, Harwell as distributor under the EMMA framework.

In the case of the KOMP Repository, you do not need to select a distribution network (see Figure 21):

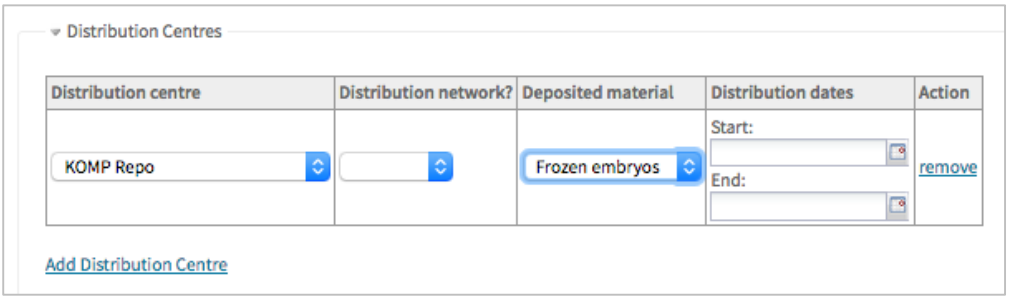


Figure 21 Selecting KOMP repository for archiving of frozen embryos.

Note. – To actually add the centre, you have to push the "Update" button at the bottom of the page:

4e. Managing status progress dates (Status stamps)

Note the "Status stamps" section at the bottom of the page. Status progress dates will be automatically allocated, but can be manually modified, too.

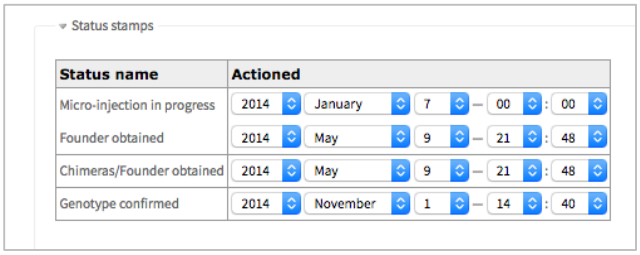
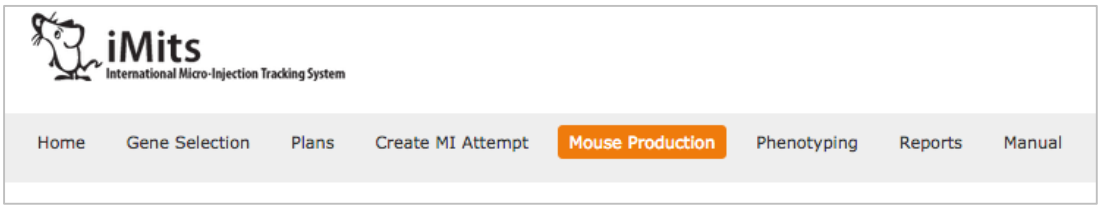


Figure 22 Relevant dates since the microinjection was attempted until the genotype was confirmed.

5a. Phenotyping Attempts: Registering a Genotype Confirmed Mouse for Modified Allele creation and Phenotyping.



A Genotype Confirmed mouse line can be registered for phenotyping by the same centre that created it, or a different one. To register it for phenotyping by the same centre that created it requires no further planning. Otherwise, you need to create a phenotyping plan at the Gene Selection tab and choose option "Phenotype only" (as described in section 1; see section 5c for more information). Find the mouse line in the "Mouse Production" grid (see Figure 23) and click on the "Create" link in the "Phenotype" column. Here I have filtered for "Genotype confirmed" in the "Status" column header:

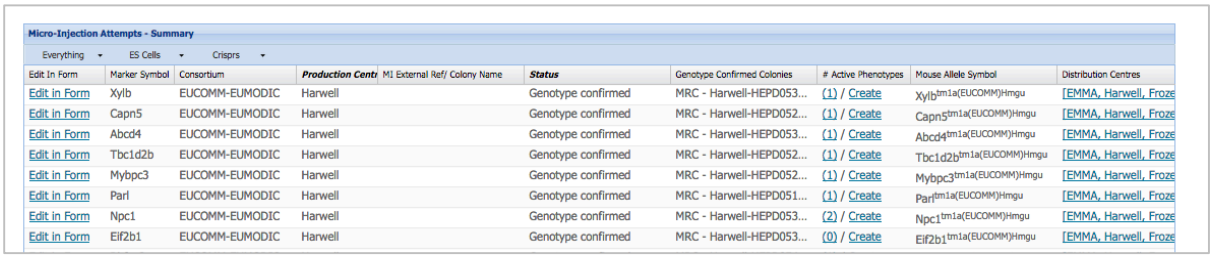


Figure 23 Selecting "Create" in the Mouse Production grid to start a Phenotyping Attempt.

When a 0 is indicated, phenotyping has not been carried out by any centre. You can always click on “Create” to be offered a Phenotype Attempt edit page (Figure 24), which allows you to Register a Phenotype Attempt for this mouse line.

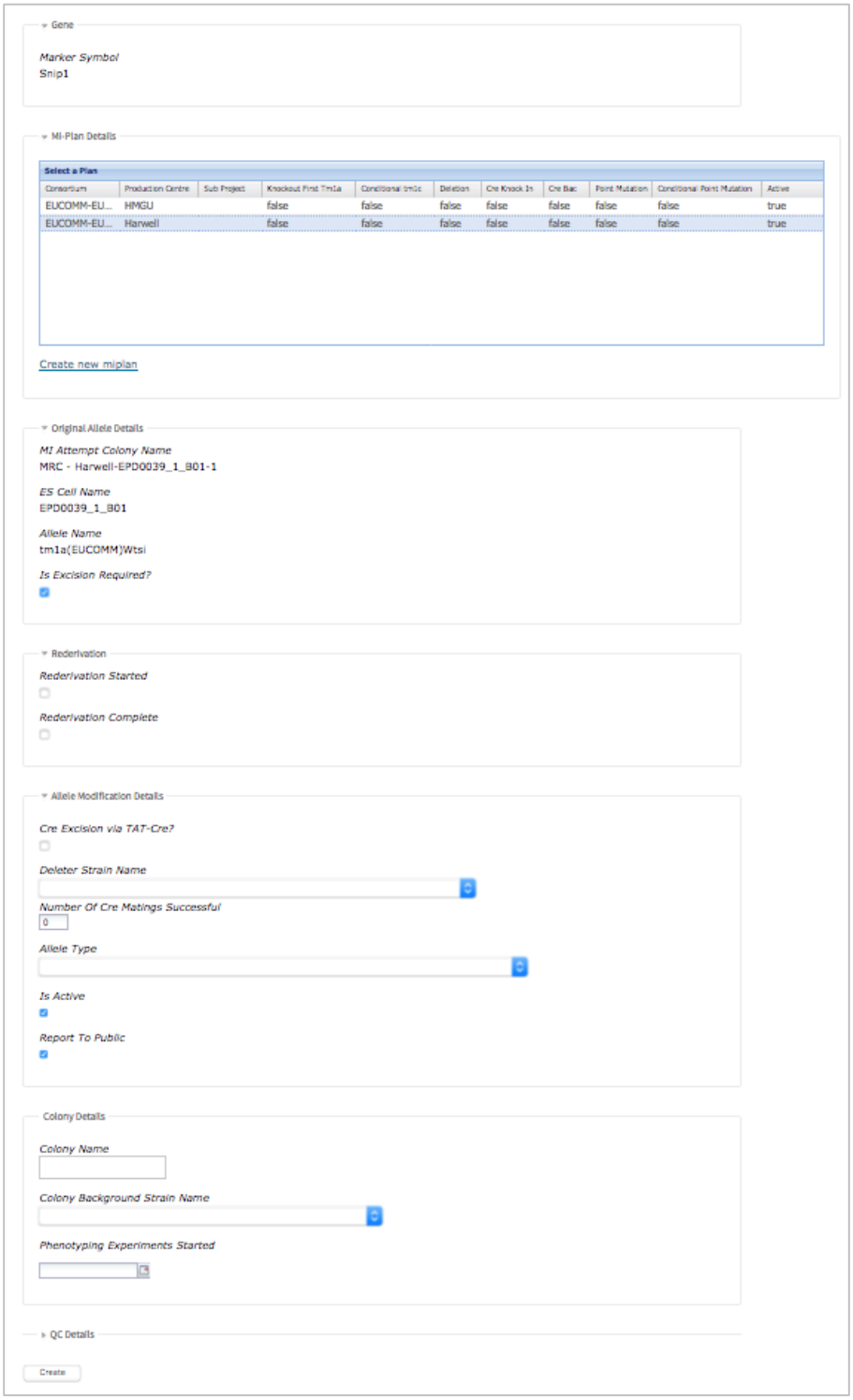


Figure 24 Creating a Phenotyping attempt, for the same Consortium and Production Centre as the generated mouse.

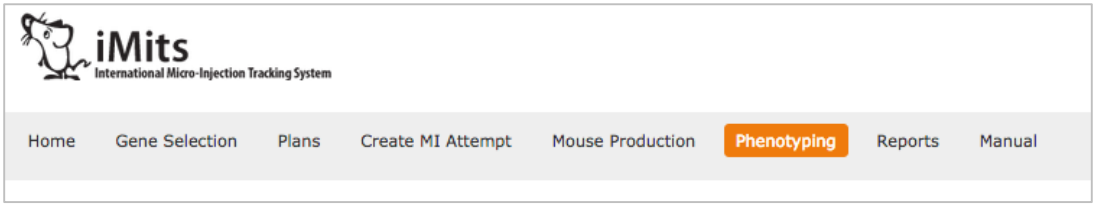
You select a Production Centre at the top of the page, next you tick the box for "Excision Required" if you are planning to modify the allele prior to phenotyping and, finally, you fill in the "Colony Name" information.

The COLONY NAME for the Phenotype Attempt is set BY YOU. **This name must match the colony name sent with your mouse clinic's phenotyping data to the DCC.**

If you ticked "Excision Required", a section in the form opens with the "Allele Modification Details" that you can fill in at this stage or later (see section below for details). The "Allele modification details" describes the new strain that you are creating for phenotyping.

After you push "Create" for this Phenotype Attempt, it is in status "Registered". You can find the Phenotype Attempt for update later by going to the "Phenotyping" tab (next section).

5b. Phenotyping Attempts: Editing your Phenotyping Attempt.



Under the Phenotyping tab, you can find and edit your individual Phenotyping Attempts, for example by entering the Gene Symbol in the search box and/or selecting a specific Production Centre or Status, and then click "Search" (Figure 25). Alternatively, the columns of the grid allow you to filter by Consortium, Production Centre, Colony Name, Status, etc.

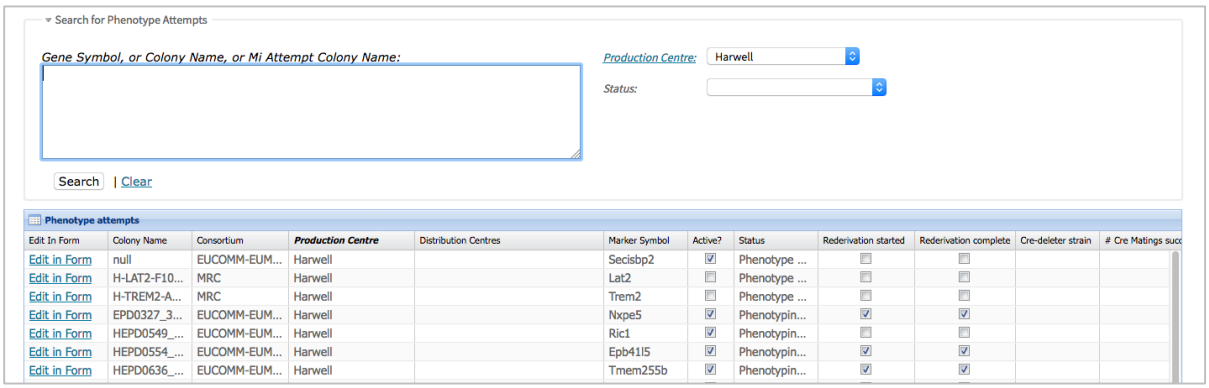


Figure 25 Searching for Phenotyping Attempts already created in the Mouse Production grid.

Clicking on “Edit in Form” brings up the Phenotype Attempt edit form, which allows updates to the status of the Phenotype Attempt. Here I selected one of the forms searching for Status “Phenotype Attempt Registered”:

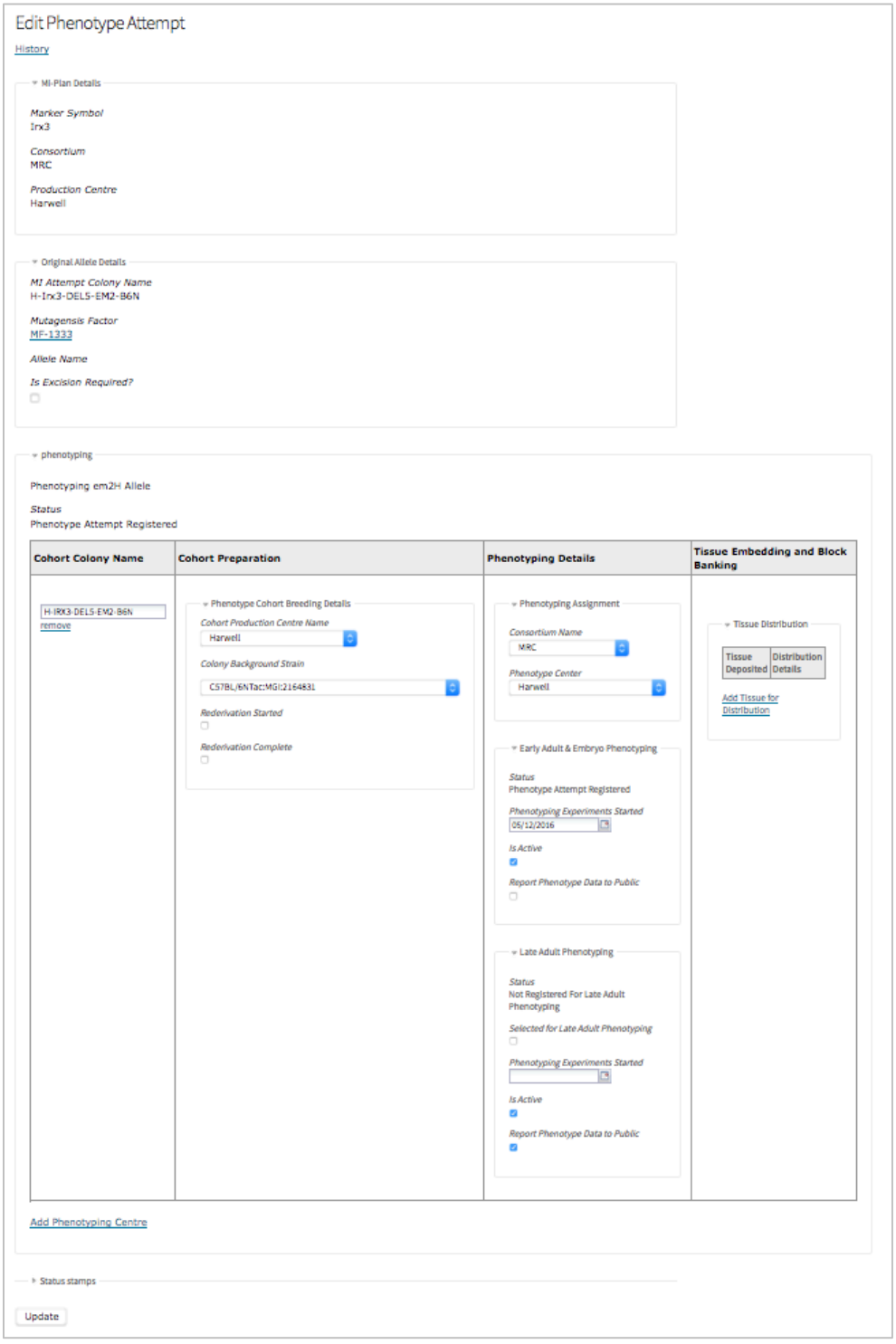


Figure 26 Editing a Phenotyping Attempt form.

You can view the Production Graph (Figure 27) of this whole gene (in this example, Irx3) by selecting it in the "Gene Selection" tab, and clicking on the "Production Graph" link under Production History. You will see the gene, the plan, the mouse and the phenotype attempt, all linked up:

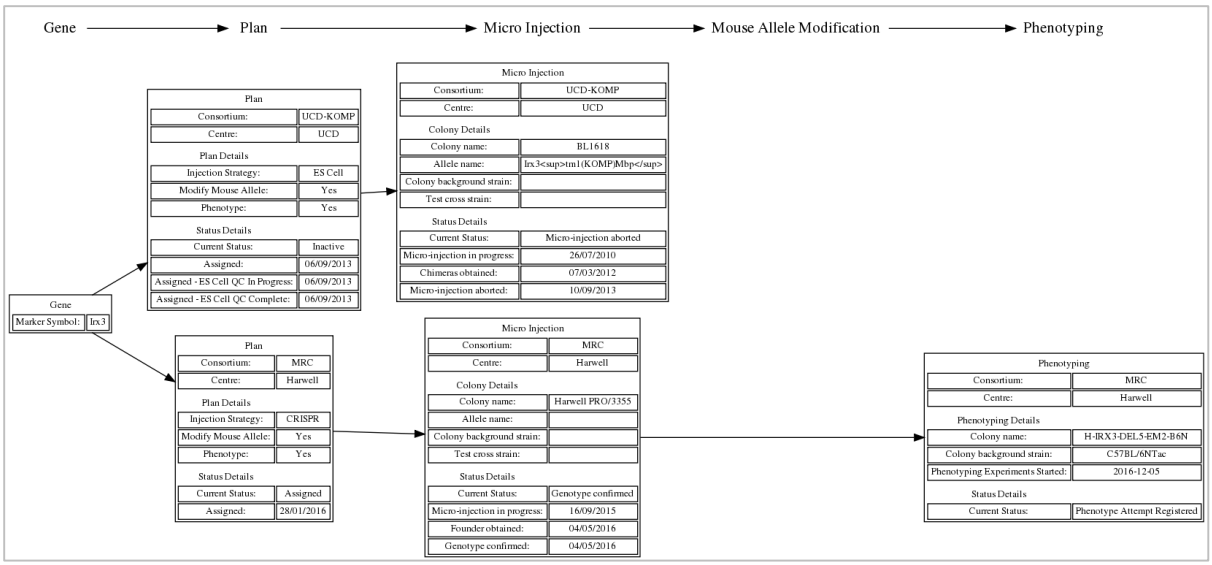


Figure 27 The Production Graph for the mouse and phenotype attempt, visible via the Gene Selection tab under column header Production History.

Out of curiosity, you may want to have a look at the Production Graph for Nxn.

5c. Phenotype Attempts: Having multiple Phenotype Attempts for a single mouse line (from different consortia or phenotyping centres)

What if another Production Centre also wanted to Phenotype this same mouse line? Well, they could! But it would require the creation of a new PLAN.

As we saw in the first section of this manual, it is possible to create a plan for phenotyping only for a centre different for the one creating the mouse (Figure 2). Here, I have created a new PLAN for Irx3 for BaSH / BCM / Priority Medium and clicking on Register Interest using the Gene Selection tab (see Figure 28).

Notice that it is in status "Inspect – GLT Mouse" because iMITS has observed the existing GLT mouse at Harwell, and is questioning BCM's intention to produce. Note also that iMITS is showing the exising GLT mouse and the Phenotype Attempt already registered at Harwell.

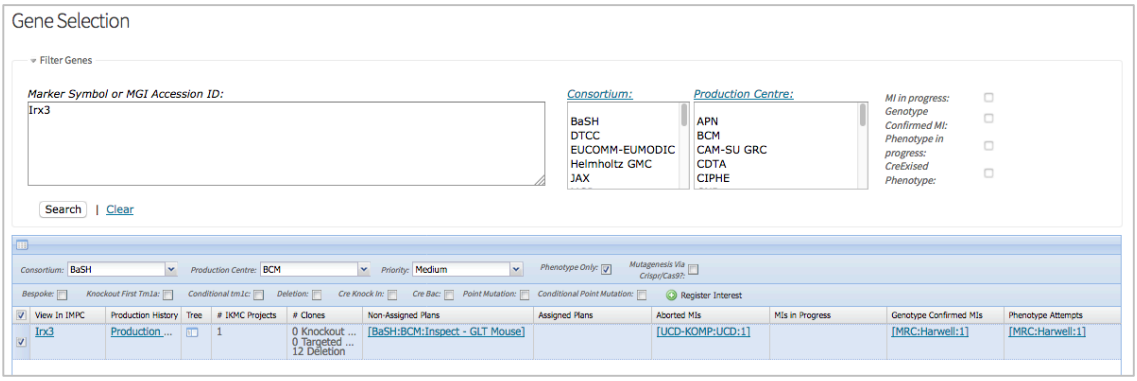


Figure 28 Creating a new plan for BaSH / BCM for the Irx3 gene.

Now that we have made a plan for BaSH / BCM, the NEXT thing to do is to actually FIND the mouse and make a Phenotype Attempt attached to the BaSH / BCM plan. Go to the Mouse Production tab, search for Irx3, and click on the 'Create' link in the Phenotype column (Figure 29).

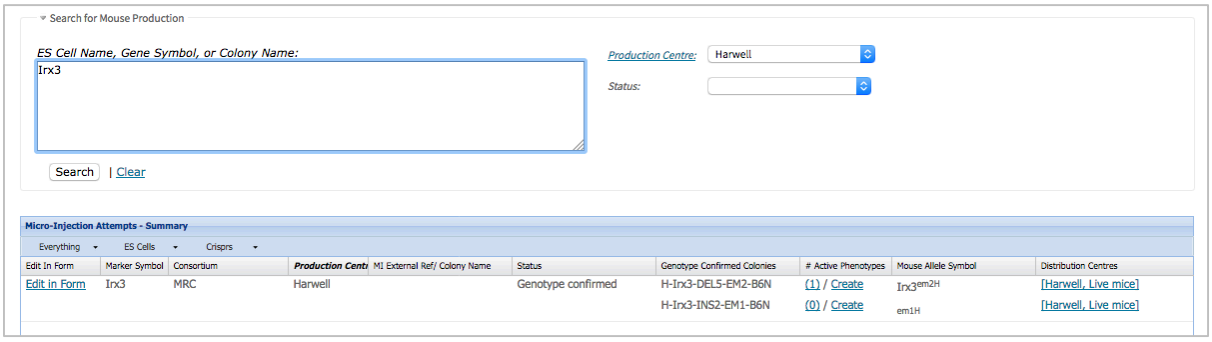


Figure 29 Finding the original Irx3 mouse in order to create a BaSH / BCM phenotyping attempt.

THIS time you want to create a Phenotype Attempt for consortium BaSH and production centre BCM (Figure 30):

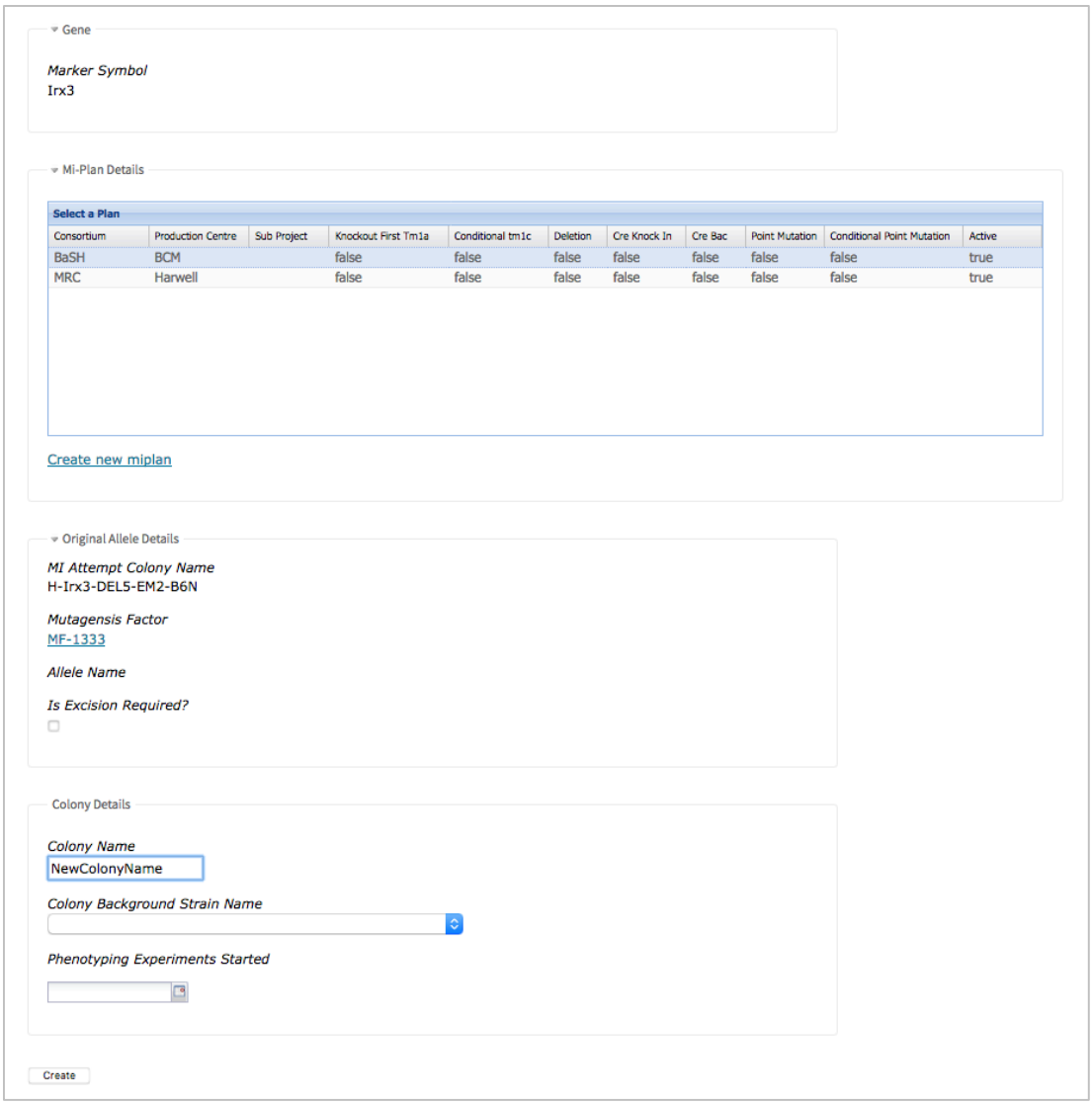


Figure 30 Selecting BaSH/BCM when creating a Phenotyping Attempt for a MRC/Harwell mouse. You need to provide a Colony Name before you push Create.

After you push "Create" at the bottom of the screen, you will see a green flag and a message to indicate that the Phenotype Attempt was successfully created (Figure 31):

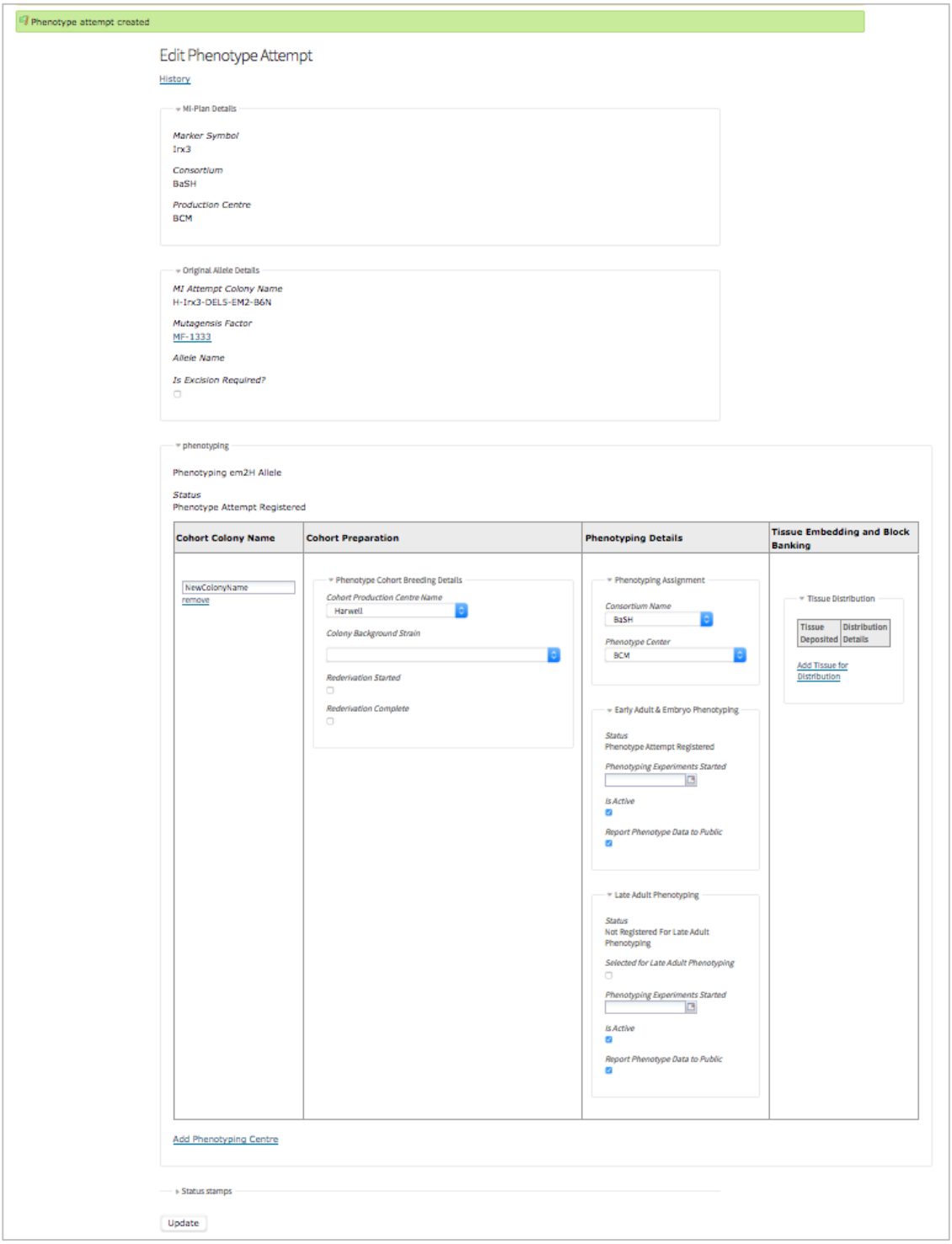


Figure 31 Phenotype attempt successfully created.

Next, you can check your work by looking at the Production Graph for this gene (like you did before for Figure 27) via the row for the gene in the Gene Selection tab. You will see that a new phenotype attempt is registered), that is, there is a *separate* BaSH/BCM Phenotype Attempt for the *same* mouse.

5d. Phenotyping Attempts: Indicating the start / finish of rederivation.

Sometimes a mouse clinic will have to rederive a colony after the reception of cryopreserved stock (embryos, sperm). iMITS allows the optional indication of that process by the checking of the "Rederivation Started" and "Rederivation Complete" checkboxes on the Phenotype Attempt edit page. If you tick the box for "Is Excision Required?" that you see in Figure 31, two new sections appear: "Rederivation" and Alle Modification Details" (Figure 32):

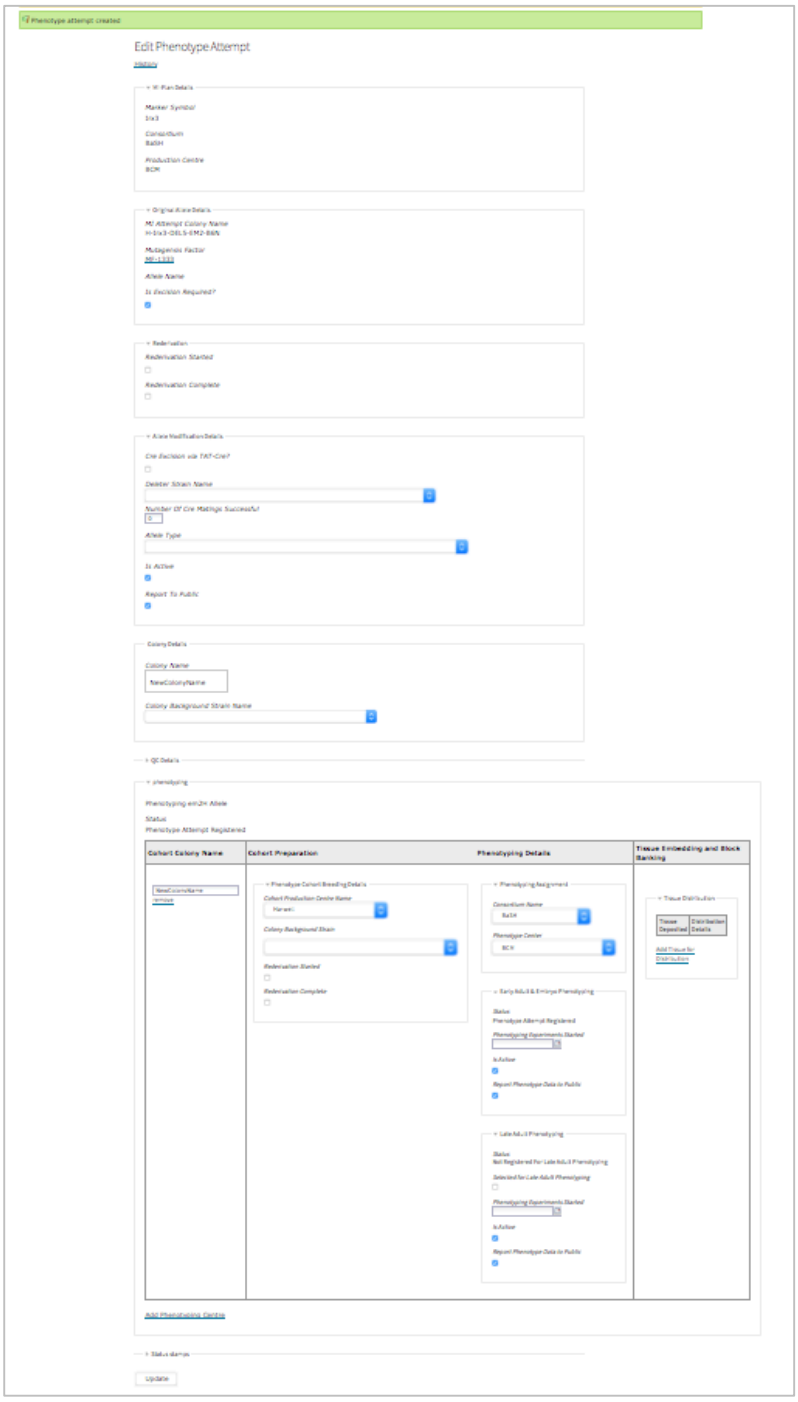
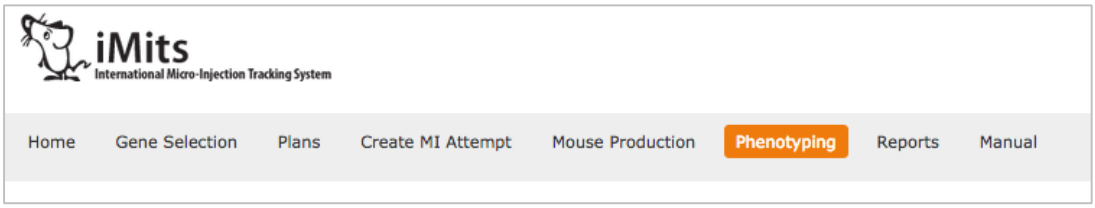


Figure 32 Phenotyping Attempt form allowing to indicate Rederivation.

You can also get to the Phenotyping Attempt form we just created via the Phenotyping tab:



and search by entering the gene name (remove the 'Production Centre' filter or select BCM from the dropdown menu before pushing Search; Figure 33):

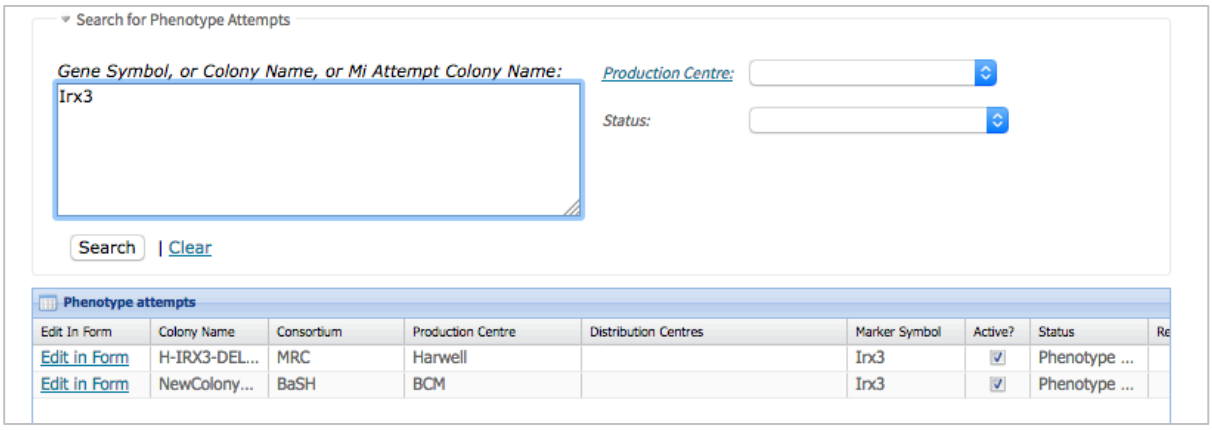


Figure 33 Finding the Phenotyping Attempts for Irx3 in the Phenotyping Attempt grid, in order to update the BaSH/BCM attempt.

Notice that we're now finding two Phenotype Attempts: one at Harwell and one at BCM. Click on the "*Edit in Form*" link for the BCM row. This will bring up the Phenotype Attempt Edit form. The middle of the form (Figure 32) has checkboxes for "Rederivation Started" and "Rederivation Complete". Here you can see a close-up (Figure 34):

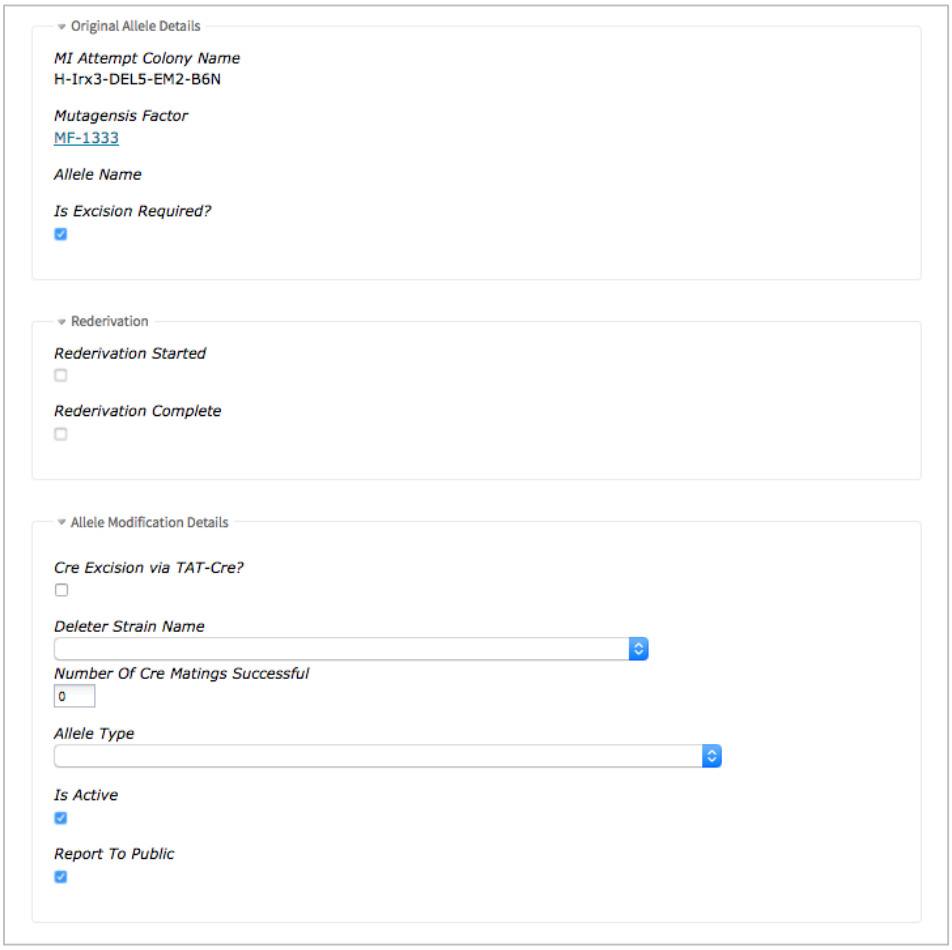


Figure 34 The Rederivation started / complete fields in the Phenotyping Attempt edit form.

Simply check these boxes on the dates when Rederivation of this mouse line has started / finished. **AFTER** you push Update at the bottom of the page, this will be shown in the Status Stamps at the bottom of the page (Figure 35), and also in the Production Graph.

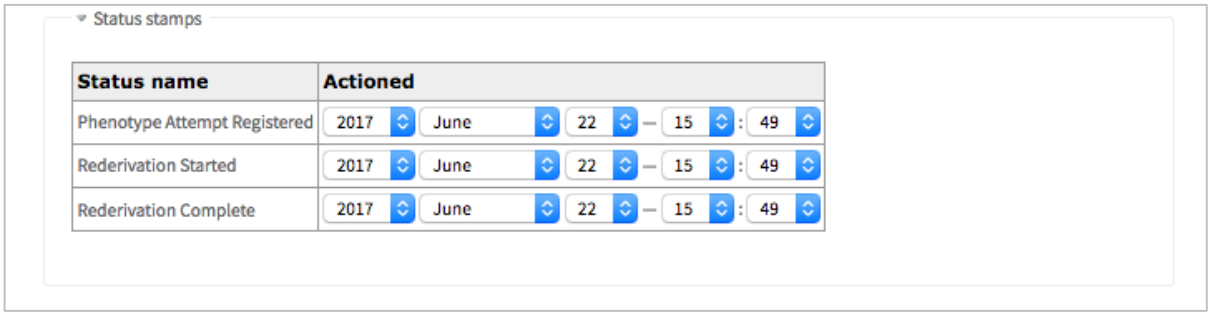


Figure 35 Status stamps for Irx3 after the BaSH/BCM Phenotyping Attempt has become "Rederivation Complete".

5e. Phenotyping Attempts: Cre Excision

(i) Indicating the start of Cre Excision

Cre-excision is the crossing of a "tm1a" or "tm1" mutant with a cre-expressing mouse strain in order to flox the critical exon (if this is applicable) and to remove any promoter from the trapping cassette. The *start* of cre-excision is indicated in iMITS by the *choice* of a cre-deleter strain. We have polled each mouse clinic and attempted to compile a list of strains currently in use. If your strain isn't in this list, we have to add it – please contact us. I have indicated the strain in the Phenotyping Attempt edit form below (Figure 36), and pushed "Update". After you push Update, a new entry is added to the Status stamps table at the bottom of the page. Cre excision via TAT-Cre is an alternative to using a deleter strain.

Figure 36 Specifying the cre-deleter strain to alter the status of the Phenotyping Attempt to "Cre Excision Started".

(ii) Phenotyping Attempts: Indicating the finish of Cre Excision

The *finish* of the Cre Excision happens when a mouse clinic has genotyped the offspring of the cre-mating and determined which matings have successfully removed the promoter / floxed the critical exon(s).

To change to this status, the mouse clinic must indicate *all* of:

- a positive number of "Cre Matings Successful";
- a Mouse Allele Type (this should be a "b" for a conditional allele and a ".1" for a deletion); and
- a Colony Background Strain. This is the colony background of the stain which will be phenotyped.

We have made choices in the form shown below (Figure 37). Pushing "Update" will change the status of the Phenotype Attempt to "Cre Excision Complete".

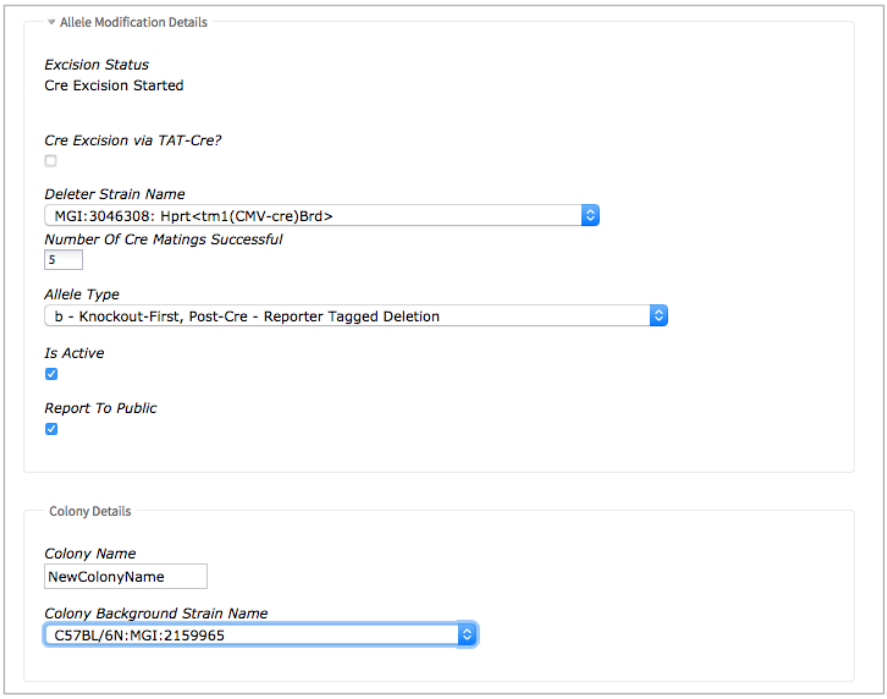


Figure 37 Specifying data to indicate that Cre Excision is Complete.

You can check the Production Graph again, with the BCM Phenotype Attempt now advanced to "Cre Excision Complete".

(iii) Phenotyping Attempts: Distribution Centres for Cre-Excised Mice.

A Cre-Excised mouse line can be distributed as a resource available to the scientific community, just like the original conditional – ready mouse line.

Check the "Distribution Centres" panel for your Phenotype Attempt: you can add a distribution centre there - and indicate if it is to be distributed via the EMMA network - in the same way as the original mouse lines (Figure 38).

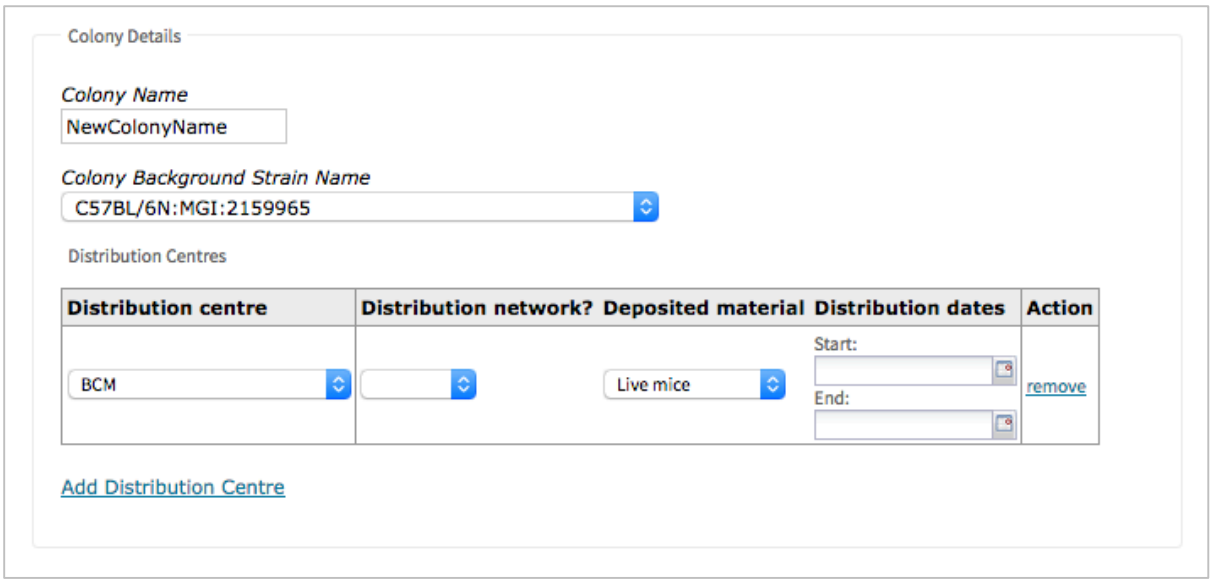
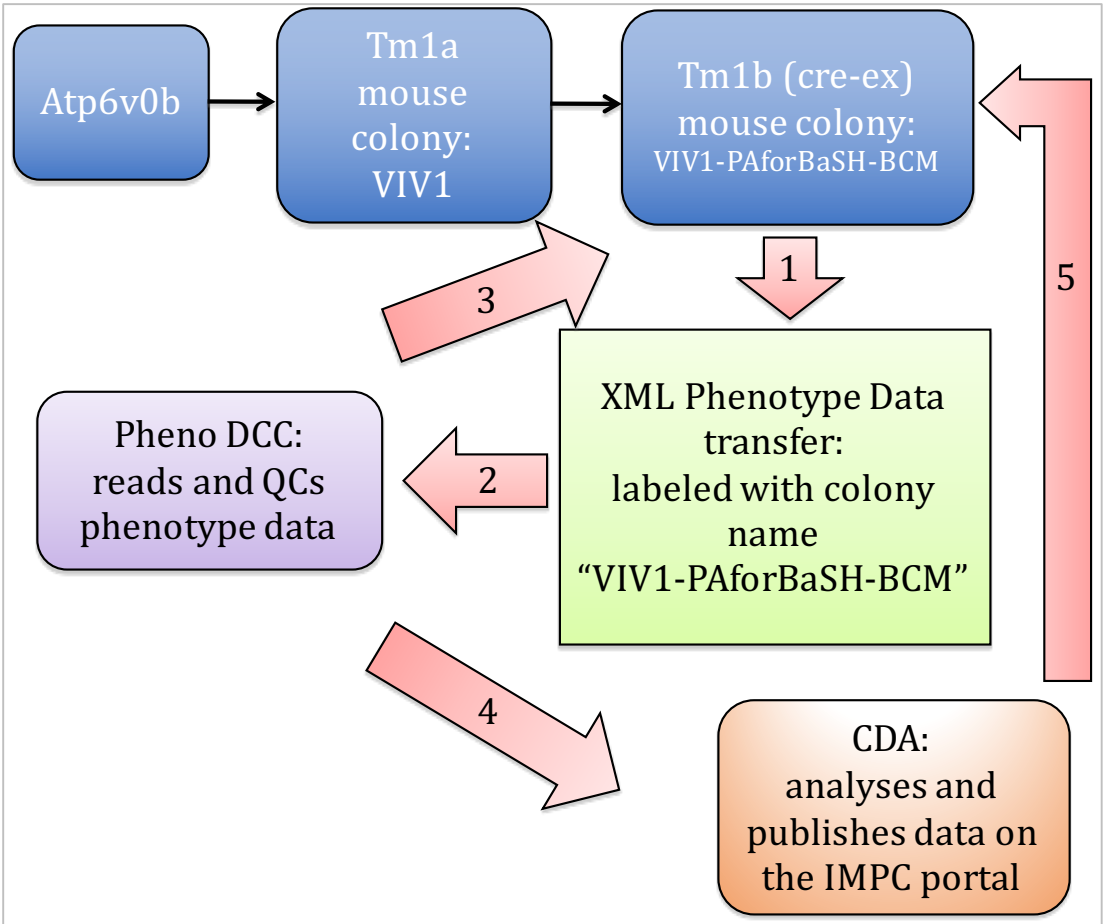


Figure 38 Specifying / adjusting the Distribution Centre for the Cre-Excised mouse on the Phenotype Attempt edit page.

5f. Phenotyping Attempts: Indicating the start and end of Phenotype Data Transfer

The mouse clinics need to indicate in the Phenotyping Attempt form the data in which the phenotyping experiments started. However, the iMITS status currently labeled "Phenotyping Started" and "Phenotyping Complete" represent the start and finish of phenotyping **data transfer** between the mouse clinic and the Pheno DCC - – the body responsible for the compilation and quality-control of phenotype data from the entire KOMP2 / IMPC effort.

Mouse clinics cannot directly indicate the start and end of data transfer inside iMITS: this signal must be sent to iMITS directly by the data wranglers at the Pheno DCC (Figure 35). Currently, this coordination requires that the Phenotype Attempt Colony name recorded in iMITS match the Colony Name attached to the XML containing the procedure / parameter results in the data transferred to the PhenoDCC:



1) Data packet from mouse clinic contains the Phenotyping Attempt colony name.
2) PhenoDCC reads data packet.
3) PhenoDCC updates iMITS to change status to "Phenotype Data Capture Started".
4) PhenoDCC sends data to CDA.
5) CDA updates iMITS to change status to "Phenotype Data Capture Complete".

Figure 35 Coordination between iMITS, data coming from the Mouse Clinic, the PhenoDCC and the CDA to indicate the flow of data via "Phenotyping Started / Complete" statuses.

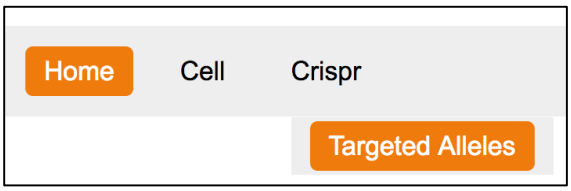
C. How to add donor vectors prior to entering CRISPR injection information in iMITS

The IKMC Targeting Repository (TargRep) stores all the mutant ES Cells and Targeting Vectors made by the pipelines in the International Knockout Mouse Consortium (IKMC). The TargRep has also been adapted to store the Targeting Vectors and Oligos created for Crispr/Cas9 microinjections.

Here we will cover how to add these new Targeting Vectors and Oligos.

Note. – TargRep will infer most of the alleles structure from the information provided when entering the Targeting Vectors and Oligos. Therefore, it is IMPORTANT that the information entered is correct to prevent an incorrect allele from being inferred.

On the top right corner of the iMITS web page, click on "Go to TargRep". TargRep's navigation bar has a Crispr and Cell section. Hover over "Crispr" and click on "Targeted Alleles".



Select 'New Allele'. The following form will pop up.

Here I have filled in the gene as Nxn, the genomic information, design details, the targeting vectors Genbank file and the name of the targeting vector (Figure 36).

Note. – You can enter more than one vector name if you have multiple copies of the vector. It is important that the Genbank file is accurately annotated as this will be used to infer the allele's structure.

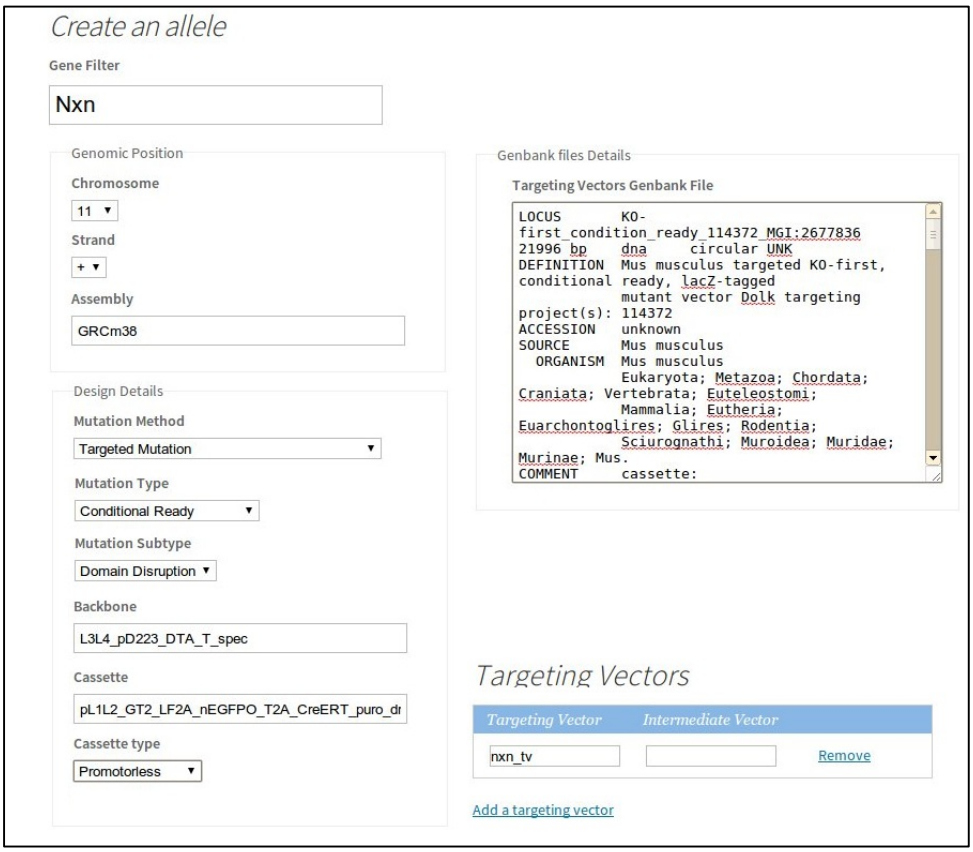


Figure 36 Creation of a targeting vector.

Click 'Create'. Now this vector will appear when recording the microinjection of a Mutation Factor (Crisprs and vector)

D. GLOSSARY

IMPC
The members of the International Mouse Phenotyping Consortium aim at creating 20,000 knockout mouse strains on a single background strain and at characterizing each through standardized phenotyping protocols. The IMPC also strives to integrate the data with human disease resources. The IMPC is currently composed of 19 research institutions and 5 national funders from 11 countries, and it is a global infrastructure recognised by the G7. See http://www.mousephenotype.org/ for more information.

IKMC
The members of the International Knockout Mouse Consortium (IKMC) have worked together to mutate all protein-coding genes in the mouse using a combination of gene trapping and gene targeting in C57BL/6 mouse embryonic stem (ES) cells. The IKMC includes the following programs: The Knockout Mouse Project, The European Conditional Mouse Mutagenesis Program, The North American Conditional Mouse Mutagenesis Project, The Texas A&M Institute for Genomic Medicine, and the EUCOMMTools Program. See www.knockoutmouse.org for details of IKMC products, alleles and contributors.

Genes
A mouse has (about) 20,000 *genes* inside every cell in its body, each encoded by a stretch of DNA. Each gene is labelled by an *MGI Accession Id*, which can be thought of as the logical key of the gene. The *Marker Symbol* is a human-readable string that labels the gene.

ES Cells
iMITS contains a list of all IKMC ES Cells for a particular gene. When a user production center wants to start mouse creation, they must first pick a gene, and then an ES Cell from a list of possible ES Cells for that gene.

Consortia
Mouse Production is funded by various Government and EU-related bodies known as Consortia. Each Consortium is granted money and held accountable for certain mouse-production goals. Examples of consortia include BaSH, the MGP, Phenomin, Helmholtz-GMC etc.

Production Centres
Mouse production occurs in physical locations called Production Centres. These correspond to actual labs that receive ES Cells (from the ES Cell distribution centres) and then proceed to check the ES Cells and breed mice derived from them. Examples of production centres include WTSI, BCM, MRC Harwell etc.

One Consortium can have work done by many mouse production centres, and one mouse production centre can do work on behalf of many different consortia. The connection between the two is held by the MI Plans – see below.

MI Plans (or just Plans, really)

The MI Plan encodes the *intention* of a consortium to produce a mouse for a particular gene at a particular production centre. The nature of the mutant mouse can be carefully specified by flags on the MI Plan.

MI Attempts

These records are linked to MI Plans and ES Cells, and represent the creation / progress of colonies (groups of genetically identical) mice based on a single mutant ES cells. The records are linked to a single ES Cell, and proceed through statuses:

Mutagenesis Factors

These are a combination of Crisprs/Cas9 and vectors that will be micro-injected into a mouse embryo to induce a mutation in the targeted gene.

Phenotype Attempts (better called "Modified Alleles")

These records are linked to MI Attempts (and independently to MI Plans if necessary) and represent the import of an existing mutant mouse, the modification (via cre-excision) of the allele in that mouse, and the start and end of phenotype data transfer to the DCC for that mouse.

E. Statuses in iMITS

The overall progress of mouse production from planning to end of phenotyping is captured by status on Plans, MI Attempts and Phenotype Attempts for a gene:

Entity	Status	Description
Plan	Assigned	There are no other assigned plans for this gene (no other Consortia and Centres have plans for this gene).
	Assigned – ES QC in Progress	ES Cells for this gene have started QC, either at the ES-cell-distribution centre or at the mouse clinic. Triggered by selecting non-zero ES Cells entering QC on this gene. Note – simply entering ES Cells for QC will cause the plan to enter this state, even if it was not assigned before.
	Assigned – ES QC Complete	ES Cells for this gene have (successfully) finished QC prior to mouse production. Triggered by selecting non-zero ES Cells finished QC on this gene.
	Assigned – ES QC Aborted	All ES Cells starting QC for this gene have failed to pass QC. The plan is *not yet* inactive or withdrawn.
	Inspect – Conflict	There are other plans from other consortia for this gene, but no visible mouse production. Note – the microinjection (MI) Plan can still be changed to status "Assigned" by starting ES QC, or by starting mouse production (MI).
	Inspect – MI Attempt	There are other visible (active) mouse production attempts for this gene. Note – the MI Plan can still be changed to status "Assigned" by starting ES QC, or by starting mouse production (MI)
	Inspect – MI Attempt / GLT Mouse	There are other visible Genotype Confirmed Mice for this gene. Note – the MI Plan can still be changed to status "Assigned" by starting ES QC, or by starting mouse production (MI).

MI Attempt	MI In Progess	Mouse production has commenced with Microinjection for this gene. There is a tracked mouse 'colony' – group of mice.
	Chimeras Obtained	The mouse colony has produced Chimeras. Triggered by entering > 0 male Chimeras for the MI Attempt.
	Founder Obtained	The mouse colony has produced founders from the micro-injection of the Mutagenesis Factor. Triggered by entering >0 Number of Mutant Founders
	Genotype Confirmed (ES Cell)	The mouse colony has produced F1 pups which have been assayed and found genotype confirmed. All centres except WTSI: Triggered by > 0 numbers in either of these fields: "Chimeras with glt offspring established by genotype confirmation" or "Number of het offspring".
	Genotype Confirmed (Mutagenesis Factor)	Genotype confirmation of the F1 (bread from the founders). CURRENTLY NOT POSSIBLE
	MI Aborted	
Phenotype Attempt	Phenotype Attempt Registered	This is the initial status on creation of a PhenotypeAttempt as a child of an MI Attempt
	Rederivation Stated / Finished	Triggered by the selection of a checkbox on the Phenotype Attempt.
	Cre Excision Started / Complete	Cre-started – triggered by selection of >0 for "Number of Cre Matings". Cre-complete: triggered by choice of "Mouse Allele Type" to "b".
	Phenotype Data Capture Started / Complete	These are only setable by the DCC and the CDA – that is, they cannot be set by the Mouse Clinic (Figure 35)