

# iMITS User Manual

## Table of Contents

- A. Overview
- B. Workflow
- C. How to add donor vectors prior to entering CRISPR injection information in iMITS
- D. Glossary
- E. Statuses in iMITS

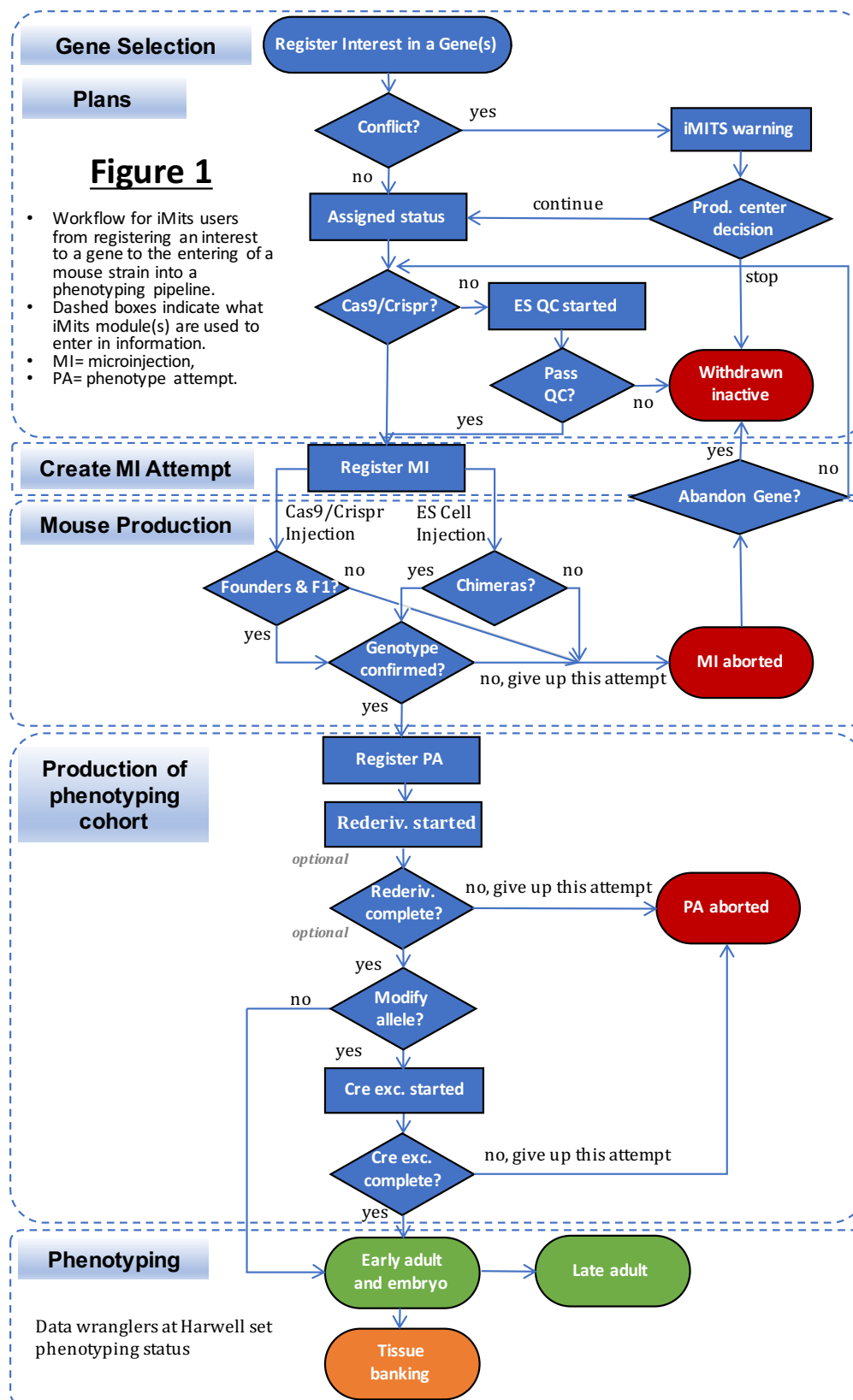
## A. iMITS Overview

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.

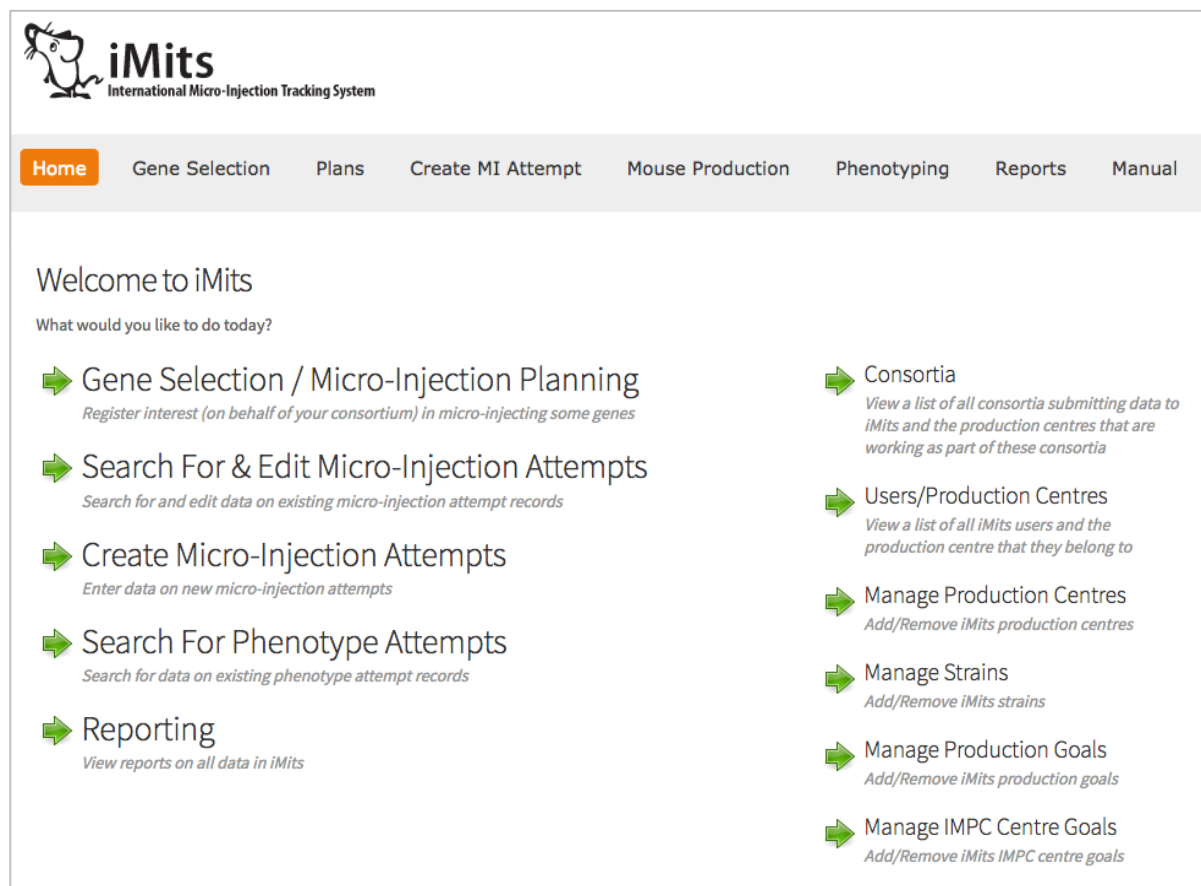
iMITS serves mouse production facilities from around the world as part of its funded mission to support the KOMP2 project – a NIH Common fund project to generate and phenotype 5,000 knockout mouse strains in the next five years (1U54HG006370). Mouse production and phenotype centers in the KOMP2 project 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



## 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).

The screenshot shows the iMits Gene Selection interface. The top navigation bar includes Home, Gene Selection (highlighted), Plans, Create MI Attempt, Mouse Production, Phenotyping, Reports, and Manual. The Gene Selection form has a search bar with '1110002L01Rik' entered. Below the search bar are dropdown menus for Consortium (BaSH), Production Centre (Harwell), and Priority (High). There are checkboxes for Phenotype Only, Mutagenesis Via Crispr/Cas9, and Register Interest. A table below shows the results for the selected gene, including columns for View In IMPC, Production History, Tree, # IKMC Projects, # Clones, Non-Assigned Plans, Assigned Plans, Aborted MIs, MIs in Progress, Genotype Confirmed MIs, and Phenotype Attempts. The table shows 1 IKMC Project and 4 clones for the selected gene.

**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).

▼ Allele Structure

*Bespoke Allele?*  
☐

*Knockout First Tm1a Allele?*  
☒

*Deletion Allele?*  
☐

*Cre Knock In Allele?*  
☐

*Cre Bac Allele?*  
☐

*Conditional Tm1c Allele?*  
☐

*Point Mutation Allele?*  
☐

*Conditional Point Mutation Allele?*  
☐

*Mutagenesis Via Crispr/Cas9?*  
☐

*Allele Symbol Override*

*Allele Comment*

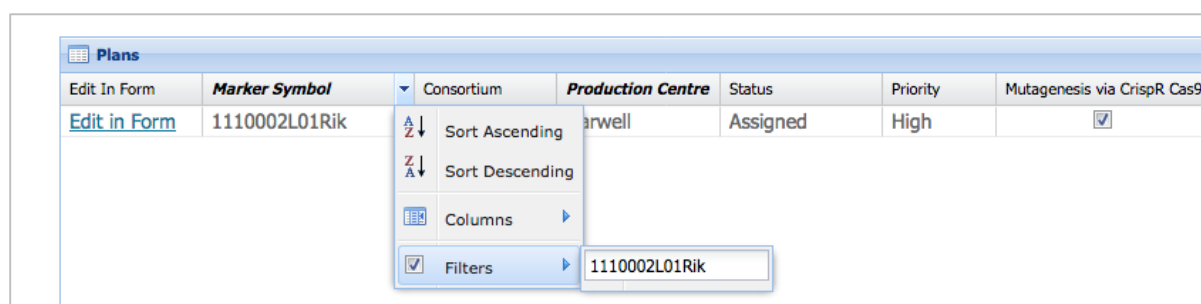
**Figure 3** Dialogue for changing details for an ES cell clone.

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.**

**ES Cell QC Form (Top):**

- ▼ ES Cell QC
- Number Of Es Cells Starting Qc: 2
- Number Of Es Cells Passing Qc:
- Es QC Comment:

**Summary Box (Top):**

- Marker Symbol: Cbx1
- Consortium: MGP
- Production Centre: WTSI
- Status: Assigned - ES Cell QC In Progress
- Phenotype Only: ☐
- Mutagenesis Via Crispr/Cas9?: ☐

**ES Cell QC Form (Bottom):**

- ▼ ES Cell QC
- Number Of Es Cells Starting Qc: 2
- Number Of Es Cells Passing Qc: 2
- Es QC Comment:

**Summary Box (Bottom):**

- Marker Symbol: Cbx1
- Consortium: MGP
- Production Centre: WTSI
- Status: Assigned - ES Cell QC Complete
- Phenotype Only: ☐
- Mutagenesis Via Crispr/Cas9?: ☐

**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.

## Edit Plan

[History](#)

▼ Details

*Marker Symbol*  
1110002L01Rik

*Consortium*  
BaSH

*Production Centre*  

Harwell

*Status*  
Aborted - ES Cell QC Failed

*Phenotype Only*  
☐

▼ Allele Structure

*Bespoke Allele?*  
☐

*Knockout First Tm1a Allele?*  
☐

*Deletion Allele?*  
☐

*Cre Knock In Allele?*  
☐

*Cre Bac Allele?*  
☐

*Conditional Tm1c Allele?*  
☐

*Point Mutation Allele?*  
☐

*Conditional Point Mutation Allele?*  
☐

*Mutagenesis Via Crispr/Cas9?*  
☒

**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).

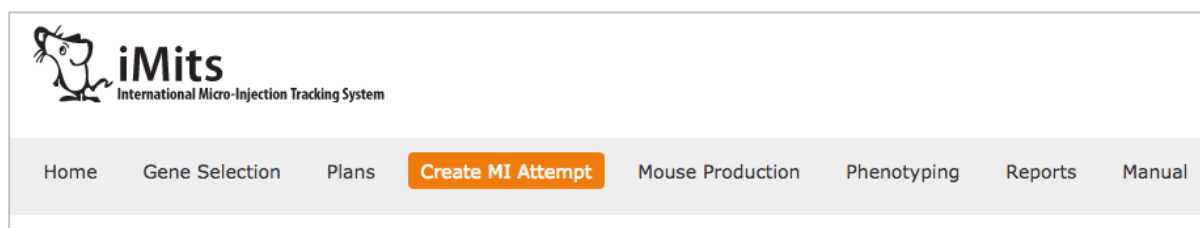
All of these changes will mean the plan does not 'compete' with other consortia looking to make plans on that gene.

The screenshot shows a web form titled 'Other' with a dropdown arrow. It contains several fields and checkboxes:

- Completion Note:** A dropdown menu currently showing '[none]' with a blue arrow icon to its right.
- Completion Comment:** A large, empty text area with a diagonal line in the bottom right corner.
- Report To Public:** A checkbox that is checked, indicated by a blue checkmark.
- Ignore Available Mice:** An unchecked checkbox.
- Recovery:** An unchecked checkbox.
- Is Active:** A checked checkbox, indicated by a blue checkmark.
- Withdrawn:** An unchecked checkbox.

**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).

**Search for ES cells**

Search by marker symbol | Search by ES cell name

Enter marker symbol

Atp6v0b Search

Choose an ES cell clone to micro-inject

ES Cell	Marker Symbol	Pipeline	Mutation Subtype	LoxP Screen
EPD0738_6_B05	Atp6v0b	EUCOMM	conditional_ready	pass
EPD0738_6_C05	Atp6v0b	EUCOMM	targeted_non_conditional	not confirmed
EPD0738_6_C06	Atp6v0b	EUCOMM	targeted_non_conditional	not confirmed
EPD0738_6_D05	Atp6v0b	EUCOMM	targeted_non_conditional	not confirmed
EPD0738_6_D06	Atp6v0b	EUCOMM	targeted_non_conditional	no reads dete...
EPD0738_6_D08	Atp6v0b	EUCOMM	conditional_ready	pass

**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/>).

**Create mutation Factor**

Search for crisors  
Enter marker symbol

Nxn

**Select by Exon** **Search by gRNA Sequ**

Exon

All

ENSMUSE00000392903

ENSMUSE00000295885

ENSMUSE00000110352

ENSMUSE00000110354

ENSMUSE00000110358

ENSMUSE00000110357

ENSMUSE00000110355

ENSMUSE00000342546

**Crisprs** **Crispr Pairs**

Sequence	Chr Start	Chr End
AACACGACAGGATCCTGGATGGG	76398547	76398569
AAGACACGAAGACGATCTCCAGG	76398784	76398806
ACACGAAGACGATCTCCAGGCGG	76398787	76398809
ACACGACAGGATCCTGGATGGG	76398548	76398570
ACCGTAGAAGGCGGCCAGGCTGG	76398887	76398909
ACGATCTCCAGGCGGTGCCGGG	76398795	76398817
AGAACGTGGACCGCGCTGCAGG	76398647	76398669
AGACGATCTCCAGGCGGTGCCGG	76398793	76398815
AGAGGGGAGCCCCGCCACCGGG	76399167	76399189
AGATAGAGATGCAGGCCTATGG	76398591	76398613
AGATTGCCTGGACTTAAGGCAGG	76399335	76399357

**Mutagenesis Factor: Crispr Selection**

Sequence:  Chr:  Chr Start:  Chr End:  Truncated: ☐

Sequence	Chr	Chr Start	Chr End	Truncated	gRNA Concentration
AACACGACAGGATCCTGGATGGG	11	76398547	76398569		
ACACGAAGACGATCTCCAGGCGG	11	76398787	76398809		

**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.

**Create mutation Factor**

Search for crisors  
Enter marker symbol

Nxn

**Select by Exon** **Search by gRNA Sequ**

Exon

All

ENSMUSE00000392903

ENSMUSE00000295885

ENSMUSE00000110352

ENSMUSE00000110354

ENSMUSE00000110358

ENSMUSE00000110357

ENSMUSE00000110355

ENSMUSE00000342546

**Crisprs** **Crispr Pairs**

Sequence	Chr Start	Chr End
AACACGACAGGATCCTGGATGGG	76398547	76398569
AAGACACGAAGACGATCTCCAGG	76398784	76398806
ACACGAAGACGATCTCCAGGCGG	76398787	76398809
ACACGACAGGATCCTGGATGGG	76398548	76398570
ACCGTAGAAGGCGGCCAGGCTGG	76398887	76398909
ACGATCTCCAGGCGGTGCCGGG	76398795	76398817
AGAACGTGGACCGCGCTGCAGG	76398647	76398669
AGACGATCTCCAGGCGGTGCCGG	76398793	76398815
AGAGGGGAGCCCCGCCACCGGG	76399167	76399189
AGATAGAGATGCAGGCCTATGG	76398591	76398613
AGATTGCCTGGACTTAAGGCAGG	76399335	76399357

**Mutagenesis Factor: Crispr Selection**

Sequence:  Chr:  Chr Start:  Chr End:  Truncated: ☐

Sequence	Chr	Chr Start	Chr End	Truncated	gRNA Concentration
AACACGACAGGATCCTGGATGGG	11	76398547	76398569		10
ACACGAAGACGATCTCCAGGCGG	11	76398787	76398809		15

**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).

▼ Mi-Plan Details

Select a Plan

Consortium	Production Centre	Sub Project	Knockout First Tm1a	Conditional tm1c	Deletion	Cre Knock In	Cre Bac	Point Mutation	Conditional Point Mutation	Active
BaSH	BCM		false	false	false	false	false	false	false	true
DTCC	UCD		false	false	false	false	false	false	false	true
JAX	JAX		false	false	false	false	false	false	false	true

Create new miplan

▼ Universal Details

Micro-Injection Date

Mi Attempt External Reference

Report Micro Injection Progress To Public

☒

Experimental?

☐

Is Active?

☒

Comments

**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.

▼ Mutagenesis Factor Details

Marker Symbol

Nxn

Delivery Method

Cytoplasmic Injection

0. Electroporation Details

Voltage

Number Of Pulses

1. Strain Injected

Zygote / Blast Strain Name

C57BL/6N:MGI:2159965

Parent Colony Name of Mutant Allele

2. Nucleases

mRNA Nuclease

CAS9

Concentration

ng/uL

Protein Nuclease

Concentration

ng/uL

**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.**

### 3. Crisprs

gRNA Concentration  ng/uL

☐ Individually Set Concentrations

Sequence	Chr	Chr Start	Chr End	Truncated Guide
AACACGACAGGATCCTGGATGGG	11	76398547	76398569	
ACACGAAGACGATCTCCAGGCGG	11	76398787	76398809	

[Edit Crispr Selection](#)

### 4. Vectors/Oligos

Donor	Concentration	Preparation	Action
Vector Name <input type="text"/>			
OR			
Oligos Sequence (FASTA format) <input type="text"/>	<input type="text"/> ng/uL	<input type="text"/>	<a href="#">remove</a>

[Add Vector/Oligo](#)

### 5. Reagents (inhibitors etc.)

Name	Concentration	Action
<input type="text"/>	<input type="text"/> ng/uL	<a href="#">remove</a>

[Add Reagent](#)

**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).

Genotype Primers

Name	Sequence	Coordinate Start	Coordinate End	Action
				remove
				remove

Add Genotype Primer

Crispr Injection Details

Number of Embryos Injected

Number of Embryos Survived

Embryo Transfer Day

Same Day

Number of Embryos Survived to 2 cell stage (Leave blank if transferred on same day)

Number Transferred

Founder/G0 Litter Details

Number of G0 Pups Born

Assay Type Used

Number of G0s Assayed

G0 Assay Results (Leave fields blank if not tested in your G0 screen)

Assay results for allele

Number of G0 with a Detected Mutation

G0 Allele Screen Details

Number of G0 with NHEJ Mutation

Number of G0 with Deletion Mutation

Number of G0 with HR Mutation

Number of G0 with HDR Mutation

G0 Donor Insertion Details

Number of G0 with All Donors Inserted

Number of G0 with Subset of Donors Inserted

Number of G0s Selected For Breeding

F1 Colonies

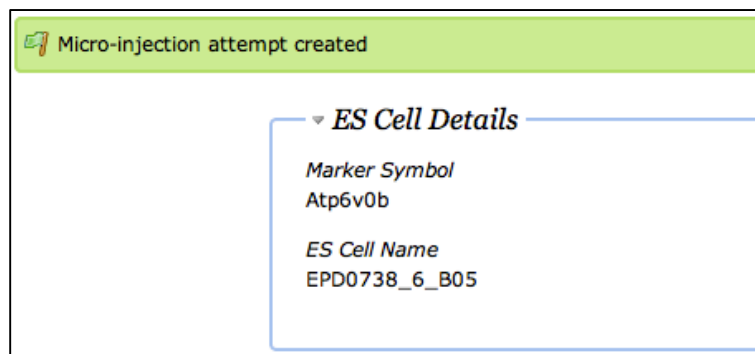
F1 Colonies can be entered after the Micro-Injection has been created.

Create

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

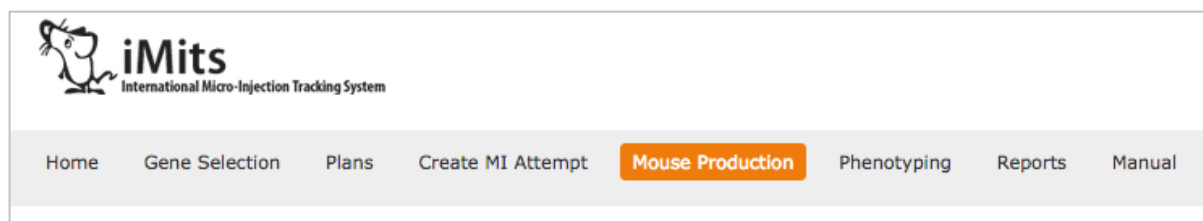


Once your ES Cell or Mutagenesis Factor details are setup, this successfully creates the MI Attempt with status 'Microinjection in progress' (Figure 15).



**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:

Search for Mouse Production

ES Cell Name, Gene Symbol, or Colony Name:

Production Centre:

Status:

[Search](#) | [Clear](#)

Micro-Injection Attempts - Summary									
Everything	ES Cells	Consortium	Production Centre	MI External Ref/ Colony Name	Status	Genotype Confirmed Colonies	# Active Phenotypes	Mouse Allele Symbol	Distribution Centres
<a href="#">Edit in Form</a>	Vhl	BaSH	Harwell		Micro-injection in progress				
<a href="#">Edit in Form</a>	Vhl	BaSH	Harwell		Micro-injection aborted				

**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:

Search for Mouse Production

ES Cell Name, Gene Symbol, or Colony Name:

Production Centre:

Status:

[Search](#) | [Clear](#)

Micro-Injection Attempts - Summary									
Everything	ES Cells	Consortium	Production Centre	MI External Ref/ Colony Name	Status	Genotype Confirmed Colonies	# Active Phenotypes	Mouse Allele Symbol	Distribution Centres
<a href="#">Edit in Form</a>	Vhl	BaSH	Harwell		Micro-injection in progress	1			
<a href="#">Edit in Form</a>	Vhl	BaSH	Harwell		Micro-injection aborted				

[Update](#) [Cancel](#)

**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”.

The screenshot shows a web form titled "Chimera Mating Details". It contains the following fields:

- Test Cross Strain Name: C57BL/6N:MGI:2159965
- Date Chimeras Mated: 11/06/2017
- Number Of Chimera Matings Attempted: 5
- Number Of Chimera Matings Successful: 5
- Number Of Chimeras With GLT From CCT: 5
- Number Of Chimeras With GLT From Genotyping: 3
- Number of Chimeras with 0-9% GLT: (empty)
- Number of Chimeras with 10-49% GLT: (empty)
- Number of Chimeras with 50-99% GLT: 5
- Number of Chimeras with 100% GLT: (empty)
- Total F1 Mice From Matings: (empty)
- Number Of CCT Offspring: (empty)
- Cassette Transmission Verified: (empty)
- Number Of HET Offspring: 7
- Number Of Live GLT Offspring: 5

**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.

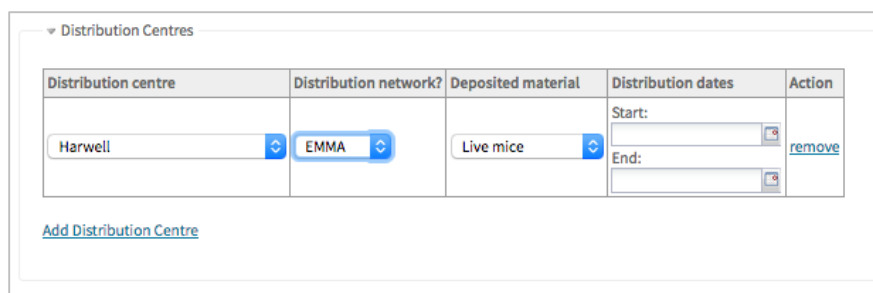
The screenshot shows the 'F1 Colonies' section of the iMITS portal. The 'Colony Details' form for colony 'j25475' is displayed. The 'Genotype Confirmed?' checkbox is checked. The 'Background Strain Name' is 'C57BL/6J-MGI:3056279'. The 'Allele' section shows 'Allele Type' as 'NHJ - CRISPR injection; Mutation resulted from Non Homology End Joining'. The 'MGI Allele Symbol Superscript' is 'em22'. The 'Report Mouse Strain To Public' checkbox is also checked.

**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.



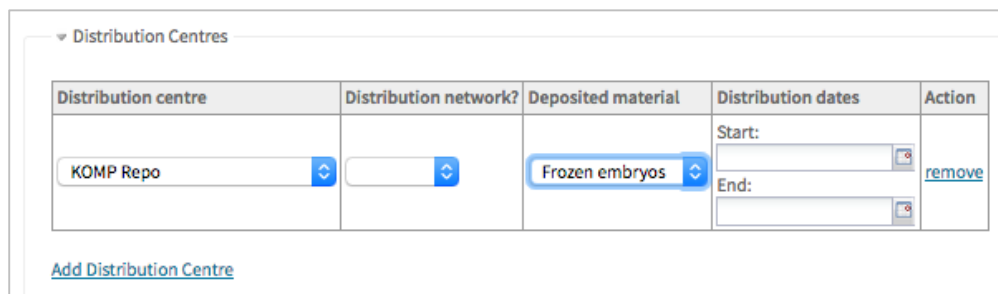
The screenshot shows a web form titled "Distribution Centres". It contains a table with the following columns: "Distribution centre", "Distribution network?", "Deposited material", "Distribution dates", and "Action". The "Distribution centre" dropdown is set to "Harwell". The "Distribution network?" dropdown is set to "EMMA". The "Deposited material" dropdown is set to "Live mice". The "Distribution dates" section has "Start:" and "End:" input fields, both of which are empty. The "Action" column has a "remove" link. Below the table is a link that says "Add Distribution Centre".

Distribution centre	Distribution network?	Deposited material	Distribution dates	Action
Harwell	EMMA	Live mice	Start: End:	<a href="#">remove</a>

[Add Distribution Centre](#)

**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):



The screenshot shows a web form titled "Distribution Centres". It contains a table with the following columns: "Distribution centre", "Distribution network?", "Deposited material", "Distribution dates", and "Action". The "Distribution centre" dropdown is set to "KOMP Repo". The "Distribution network?" dropdown is empty. The "Deposited material" dropdown is set to "Frozen embryos". The "Distribution dates" section has "Start:" and "End:" input fields, both of which are empty. The "Action" column has a "remove" link. Below the table is a link that says "Add Distribution Centre".

Distribution centre	Distribution network?	Deposited material	Distribution dates	Action
KOMP Repo		Frozen embryos	Start: End:	<a href="#">remove</a>

[Add Distribution Centre](#)

**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:

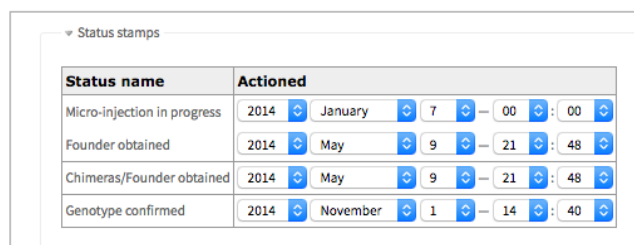


The screenshot shows a single button labeled "Update".

Update

## 4e. Managing status progress dates (Status stamps)

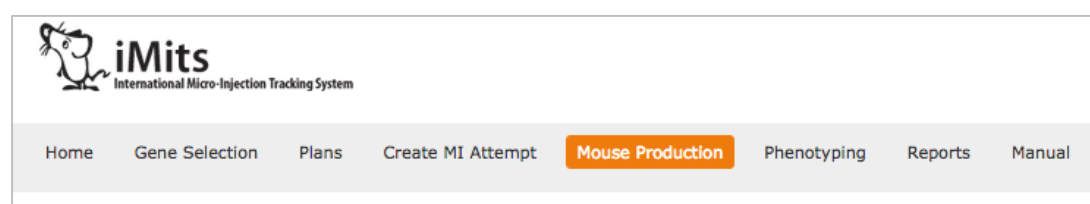
Note the “Status stamps” section at the bottom of the page. Status progress dates will be automatically allocated, but can be manually modified, too.



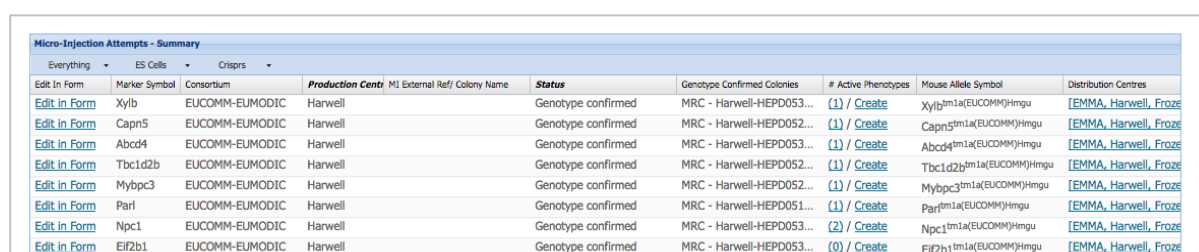
Status name	Actioned
Micro-injection in progress	2014 January 7 00:00
Founder obtained	2014 May 9 21:48
Chimeras/Founder obtained	2014 May 9 21:48
Genotype confirmed	2014 November 1 14:40

**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:



Everything	ES Cells	Crisprs							
Edit In Form	Marker Symbol	Consortium	Production Centre	MI External Ref/ Colony Name	Status	Genotype Confirmed Colonies	# Active Phenotypes	Mouse Allele Symbol	Distribution Centres
<a href="#">Edit In Form</a>	Xylb	EUCOMM-EUMODIC	Harwell		Genotype confirmed	MRC - Harwell-HEPD053...	(1) / <a href="#">Create</a>	Xylb <sup>tm1a</sup> (EUCOMM)Hmgu	<a href="#">EMMA, Harwell, Froze</a>
<a href="#">Edit In Form</a>	Capn5	EUCOMM-EUMODIC	Harwell		Genotype confirmed	MRC - Harwell-HEPD052...	(1) / <a href="#">Create</a>	Capn5 <sup>tm1a</sup> (EUCOMM)Hmgu	<a href="#">EMMA, Harwell, Froze</a>
<a href="#">Edit In Form</a>	Abcd4	EUCOMM-EUMODIC	Harwell		Genotype confirmed	MRC - Harwell-HEPD053...	(1) / <a href="#">Create</a>	Abcd4 <sup>tm1a</sup> (EUCOMM)Hmgu	<a href="#">EMMA, Harwell, Froze</a>
<a href="#">Edit In Form</a>	Tbc1d2b	EUCOMM-EUMODIC	Harwell		Genotype confirmed	MRC - Harwell-HEPD052...	(1) / <a href="#">Create</a>	Tbc1d2b <sup>tm1a</sup> (EUCOMM)Hmgu	<a href="#">EMMA, Harwell, Froze</a>
<a href="#">Edit In Form</a>	Mybp3	EUCOMM-EUMODIC	Harwell		Genotype confirmed	MRC - Harwell-HEPD052...	(1) / <a href="#">Create</a>	Mybp3 <sup>tm1a</sup> (EUCOMM)Hmgu	<a href="#">EMMA, Harwell, Froze</a>
<a href="#">Edit In Form</a>	Parl	EUCOMM-EUMODIC	Harwell		Genotype confirmed	MRC - Harwell-HEPD051...	(1) / <a href="#">Create</a>	Parl <sup>tm1a</sup> (EUCOMM)Hmgu	<a href="#">EMMA, Harwell, Froze</a>
<a href="#">Edit In Form</a>	Npc1	EUCOMM-EUMODIC	Harwell		Genotype confirmed	MRC - Harwell-HEPD053...	(2) / <a href="#">Create</a>	Npc1 <sup>tm1a</sup> (EUCOMM)Hmgu	<a href="#">EMMA, Harwell, Froze</a>
<a href="#">Edit In Form</a>	Elf2b1	EUCOMM-EUMODIC	Harwell		Genotype confirmed	MRC - Harwell-HEPD053...	(0) / <a href="#">Create</a>	Elf2b1 <sup>tm1a</sup> (EUCOMM)Hmgu	<a href="#">EMMA, Harwell, Froze</a>

**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.

Gene

Marker Symbol  
Snip1

MI-Plan Details

Select a Plan

Consortium	Production Centre	Sub Project	Knockout Pmt Tm1a	Conditional Impc	Deletion	Cre Knock In	Cre Bac	Pmt Mutation	Conditional Pmt Mutation	Active
EUCOMM-EU...	HMGU		false	false	false	false	false	false	false	true
EUCOMM-EU...	Harwell		false	false	false	false	false	false	false	true

Create new mipan

Original Allele Details

MI Attempt Colony Name

MRC - Harwell-EPD0039\_1\_B01-1

ES Cell Name

EPD0039\_1\_B01

Allele Name

tm1a(EUCOMM)Wtsi

Is Excision Required?

☒

Rederivation

Rederivation Started

☐

Rederivation Complete

☐

Allele Modification Details

Cre Excision via TAT-Cre?

☐

Deleter Strain Name

Number Of Cre Matings Successful

0

Allele Type

Is Active

☒

Report To Public

☒

Colony Details

Colony Name

Colony Background Strain Name

Phenotyping Experiments Started

QC Details

Create

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

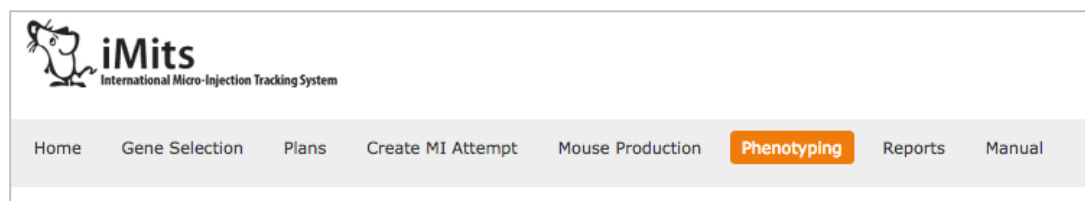
You select a Production Centre at the top of the page, next you tick the box for “Excision Required” if you are planning to modify the allele prior to phenotyping and, finally, you fill in the “Colony Name” information.

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.

Search for Phenotype Attempts

Gene Symbol, or Colony Name, or Mi Attempt Colony Name:

Production Centre:

Status:

Phenotype attempts	Colony Name	Consortium	Production Centre	Distribution Centres	Marker Symbol	Active?	Status	Rederivation started	Rederivation complete	Cre-deleter strain	# Cre Matings succ
<a href="#">Edit in Form</a>	null	EUCOMM-EUM...	Harwell		Secisbp2	<input checked="" type="checkbox"/>	Phenotype ...	<input type="checkbox"/>	<input type="checkbox"/>		
<a href="#">Edit in Form</a>	H-LAT2-F10...	MRC	Harwell		Lat2	<input type="checkbox"/>	Phenotype ...	<input type="checkbox"/>	<input type="checkbox"/>		
<a href="#">Edit in Form</a>	H-TREM2-A...	MRC	Harwell		Trem2	<input type="checkbox"/>	Phenotype ...	<input type="checkbox"/>	<input type="checkbox"/>		
<a href="#">Edit in Form</a>	EPD0327_3...	EUCOMM-EUM...	Harwell		Nxpe5	<input checked="" type="checkbox"/>	Phenotypin...	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
<a href="#">Edit in Form</a>	HEPD0549...	EUCOMM-EUM...	Harwell		Ric1	<input checked="" type="checkbox"/>	Phenotypin...	<input type="checkbox"/>	<input type="checkbox"/>		
<a href="#">Edit in Form</a>	HEPD0554...	EUCOMM-EUM...	Harwell		Epb4115	<input checked="" type="checkbox"/>	Phenotypin...	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
<a href="#">Edit in Form</a>	HEPD0636...	EUCOMM-EUM...	Harwell		Tmem255b	<input checked="" type="checkbox"/>	Phenotypin...	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		

**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”:

Edit Phenotype Attempt

[History](#)

MI-Plan Details

Marker Symbol

Inx3

Consortium

MRC

Production Centre

Harwell

Original Allele Details

MJ Attempt Colony Name

H-1rx3-DELS-EM2-B6N

Mutagenesis Factor

[MF-1333](#)

Allele Name

Is Excision Required?

☐

phenotyping

Phenotyping em2H Allele

Status

Phenotype Attempt Registered

Cohort Colony Name	Cohort Preparation	Phenotyping Details	Tissue Embedding and Block Banking
<div><div>H-1RX3-DELS-EM2-B6N</div><div><a href="#">remove</a></div></div>	<div><div>Phenotype Cohort Breeding Details</div><div><div>Cohort Production Centre Name</div><div>Harwell</div></div><div><div>Colony Background Strain</div><div>C57BL/6NTac:MG12164831</div></div><div><div>Rederivation Started</div><div><input type="checkbox"/></div></div><div><div>Rederivation Complete</div><div><input type="checkbox"/></div></div></div>	<div><div>Phenotyping Assignment</div><div><div>Consortium Name</div><div>MRC</div></div><div><div>Phenotype Center</div><div>Harwell</div></div></div> <div><div>Early Adult &amp; Embryo Phenotyping</div><div><div>Status</div><div>Phenotype Attempt Registered</div></div><div><div>Phenotyping Experiments Started</div><div>05/12/2016</div></div><div><div>Is Active</div><div><input checked="" type="checkbox"/></div></div><div><div>Report Phenotype Data to Public</div><div><input type="checkbox"/></div></div></div> <div><div>Late Adult Phenotyping</div><div><div>Status</div><div>Not Registered For Late Adult Phenotyping</div></div><div><div>Selected for Late Adult Phenotyping</div><div><input type="checkbox"/></div></div><div><div>Phenotyping Experiments Started</div><div></div></div><div><div>Is Active</div><div><input checked="" type="checkbox"/></div></div><div><div>Report Phenotype Data to Public</div><div><input checked="" type="checkbox"/></div></div></div>	<div><div>Tissue Distribution</div><div><div>Tissue Deposited</div><div>Distribution Details</div></div><div><div><a href="#">Add Tissue for Distribution</a></div></div></div>

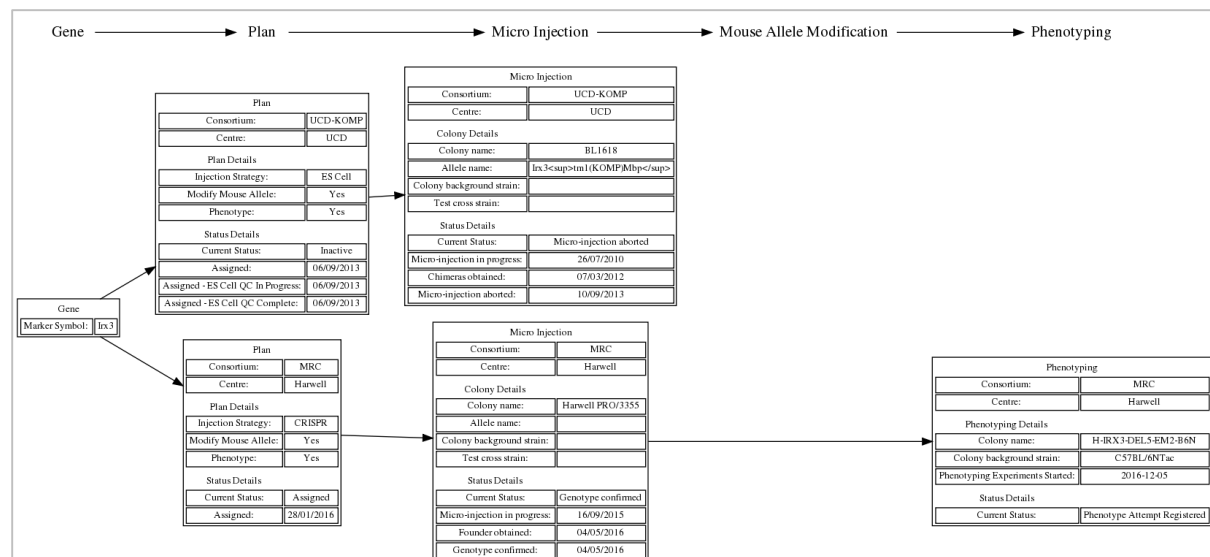
[Add Phenotyping Centre](#)

Status stamps

Update

**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 existing GLT mouse and the Phenotype Attempt already registered at Harwell.

Gene Selection

Filter Genes

Marker Symbol or MGI Accession ID:  
Irx3

Search | Clear

Consortium:  
BaSH  
DTCC  
EUCOMM-EUMODIC  
Helmholtz GMC  
JAX

Production Centre:  
APN  
BCM  
CAM-SU GRC  
CDTA  
CIPHE

MI in progress: ☐  
Genotype Confirmed MI: ☐  
Phenotype in progress: ☐  
Cre/Exel: ☐  
Phenotype: ☐

Consortium: BaSH Production Centre: BCM Priority: Medium Phenotype Only: ☒ Mutagenesis Via CRISPR/Cas9: ☐

Respoke: ☐ Knockout First Time: ☐ Conditional Im: ☐ Deletion: ☐ Cre Knock In: ☐ Cre BAC: ☐ Point Mutation: ☐ Conditional Point Mutation: ☐ Register Interest: ☒

View in IMPC	Production History	Tree	# DKHC Projects	# Clones	Non-Assigned Plans	Assigned Plans	Aborted MIs	MIs in Progress	Genotype Confirmed MIs	Phenotype Attempts
<input checked="" type="checkbox"/>	<a href="#">Irx3</a>	<a href="#">Production ...</a>	<input type="checkbox"/>	1	0 Knockout ... 0 Targeted ... 12 Deletion	<a href="#">[BaSH-BCM:Inspect - GLT Mouse]</a>	<a href="#">[UCD-KOMP:UCD:1]</a>		<a href="#">[MRC:Harwell:1]</a>	<a href="#">[MRC:Harwell:1]</a>

**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).

Search for Mouse Production

ES Cell Name, Gene Symbol, or Colony Name:  
Irx3

Production Centre: Harwell

Status:

Search | Clear

Micro-Injection Attempts - Summary

Everything	ES Cells	CRISPRs							
Edit in Form	Marker Symbol	Consortium	Production Centre	MI External Ref/ Colony Name	Status	Genotype Confirmed Colonies	# Active Phenotypes	Mouse Allele Symbol	Distribution Centres
<a href="#">Edit in Form</a>	Irx3	MRC	Harwell		Genotype confirmed	H-Irx3-DELS-EM2-B6N	<a href="#">(1) / Create</a>	Irx3 <sup>em2H</sup>	<a href="#">[Harwell, Live mice]</a>
						H-Irx3-INS2-EM1-B6N	<a href="#">(0) / Create</a>	em1H	<a href="#">[Harwell, Live mice]</a>

**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):

Gene

Marker Symbol

Irx3

Mi-Plan Details

Select a Plan

Consortium	Production Centre	Sub Project	Knockout First Tm1a	Conditional tm1c	Deletion	Cre Knock In	Cre Sac	Point Mutation	Conditional Point Mutation	Active
BaSH	BCM		false	false	false	false	false	false	false	true
MRC	Harwell		false	false	false	false	false	false	false	true

Create new miplan

Original Allele Details

MI Attempt Colony Name

H-Irx3-DEL5-EM2-B6N

Mutagensis Factor

MF-1333

Allele Name

Is Excision Required?

☐

Colony Details

Colony Name

NewColonyName

Colony Background Strain Name

Phenotyping Experiments Started

Create

**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):

## Edit Phenotype Attempt

[History](#)

MI-Plan Details

Marker Symbol  
Irx3  
Consortium  
BaSH  
Production Centre  
BCM

Original Allele Details

MI Attempt Colony Name  
H-Irx3-DELS-EM2-B6N  
Mutagenesis Factor  
[MF-1333](#)  
Allele Name  
Is Exclusion Required?  
☐

phenotyping

Phenotyping emZH Allele  
Status  
Phenotype Attempt Registered

Cohort Colony Name	Cohort Preparation	Phenotyping Details	Tissue Embedding and Block Banking
<div> <input type="text" value="NewColonyName"/> <a href="#">remove</a> </div>	<div>             Phenotype Cohort Breeding Details           </div> <div>             Cohort Production Centre Name Harwell           </div> <div>             Colony Background Strain           </div> <div>             Rederivation Started <input type="checkbox"/> Rederivation Complete <input type="checkbox"/> </div>	<div>             Phenotyping Assignment           </div> <div>             Consortium Name BaSH Phenotype Center BCM           </div> <div>             Early Adult &amp; Embryo Phenotyping           </div> <div>             Status Phenotype Attempt Registered Phenotyping Experiments Started <input type="text" value=""/> Is Active <input checked="" type="checkbox"/> Report Phenotype Data to Public <input checked="" type="checkbox"/> </div> <div>             Late Adult Phenotyping           </div> <div>             Status Not Registered For Late Adult Phenotyping Selected for Late Adult Phenotyping <input type="checkbox"/> Phenotyping Experiments Started <input type="text" value=""/> Is Active <input checked="" type="checkbox"/> Report Phenotype Data to Public <input checked="" type="checkbox"/> </div>	<div>             Tissue Distribution           </div> <div> <div>Tissue Deposited</div> <div>Distribution Details</div> </div> <div> <a href="#">Add Tissue for Distribution</a> </div>

[Add Phenotyping Centre](#)

Status stamps

**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 Allele Modification Details” (Figure 32):

Phenotype attempt created

Edit Phenotype Attempt

Wt Panel Details

Marker Symbol  
Jax  
Consortium  
B6H  
Production Centre  
BCR

Original Allele Details

MO Attempt Colony Name  
m3x3-0015-EMO-005  
Multiplexed Factor  
M3x3x3  
Allele Name  
Is Excision Required?  
☒

Rederivation

Rederivation Started  
☐  
Rederivation Complete  
☐

Allele Modification Details

One Excision via TAD-CAP?  
☐  
Donor Strain Name  
Number of One Mating Successful  
0  
Allele Type  
Is Active  
Request To Public

Colony Details

Colony Name  
New Colony Name  
Colony Background Strain Name

QC Details

phenotyping

Phenotyping attempt Allele  
Status  
Phenotype Attempt Registered

Colony Name	Colony Preparation	Phenotyping Details	Tissue Embedding and Block Marking
<div>Phenotyping attempt New Colony Name</div>	<div>Phenotype Consent Breeding Details Colony Production Centre Name Name Colony Background Strain Rederivation Started Rederivation Complete</div>	<div>Phenotyping Assigned Consortium Name B6H Phenotype Center BCR Early Adult &amp; Embryo Phenotyping Status Phenotype Attempt Registered Phenotyping Experiment Started Is Active Request Phenotype Data to Public Late Adult Phenotyping Status Not Registered For Late Adult Phenotyping Selected for Late Adult Phenotyping Phenotyping Experiment Started Is Active Request Phenotype Data to Public</div>	<div>Tissue Distribution Tissue Distribution Details Add Tissue for Distribution</div>

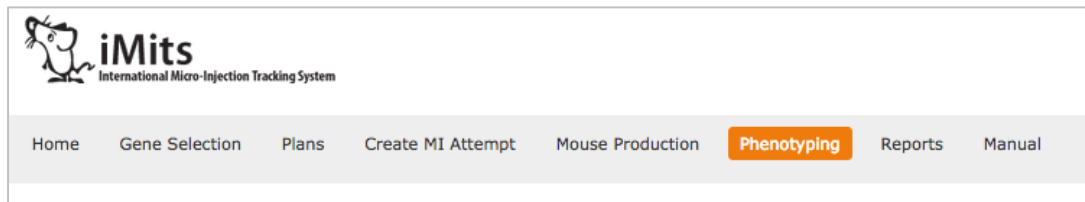
Add Phenotyping Details

Status details

update

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):

The screenshot shows the 'Search for Phenotype Attempts' form. The search criteria are 'Irx3' and 'Production Centre' is set to 'BCM'. The results table shows two entries: one at Harwell and one at BCM.

Gene Symbol, or Colony Name, or Mi Attempt Colony Name:	Production Centre:	Status:
Irx3	BCM	

Search | Clear

Phenotype attempts							
Edit In Form	Colony Name	Consortium	Production Centre	Distribution Centres	Marker Symbol	Active?	Status
<a href="#">Edit in Form</a>	H-IRX3-DEL...	MRC	Harwell		Irx3	<input checked="" type="checkbox"/>	Phenotype ...
<a href="#">Edit in Form</a>	NewColony...	BaSH	BCM		Irx3	<input checked="" type="checkbox"/>	Phenotype ...

**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):

Original Allele Details

MI Attempt Colony Name  
H-Irx3-DEL5-EM2-B6N  
  
Mutagenesis Factor  
[MF-1333](#)  
  
Allele Name  
  
Is Excision Required?  
☒

Rederivation

Rederivation Started  
☐  
  
Rederivation Complete  
☐

Allele Modification Details

Cre Excision via TAT-Cre?  
☐  
  
Deleter Strain Name  
  
  
Number Of Cre Matings Successful  
  
  
Allele Type  
  
  
Is Active  
☒  
  
Report To Public  
☒

**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.

Status stamps

Status name	Actioned
Phenotype Attempt Registered	2017 June 22 15 : 49
Rederivation Started	2017 June 22 15 : 49
Rederivation Complete	2017 June 22 15 : 49

**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.

The screenshot shows the 'Allele Modification Details' section of the iMITS Phenotyping Attempt edit form. It includes the following fields and options:

- Excision Status:** A dropdown menu currently set to 'Rederivation Complete'.
- Cre Excision via TAT-Cre?** A checkbox that is currently unchecked.
- Deleter Strain Name:** A text input field containing 'MGI:3046308: Hprt<tm1(CMV-cre)Brd>'.
- Number Of Cre Matings Successful:** A text input field containing '0'.
- Allele Type:** A dropdown menu.
- Is Active:** A checkbox that is currently checked.
- Report To Public:** A checkbox that is currently checked.
- Colony Details:** A section containing:
  - Colony Name:** A text input field containing 'NewColonyName'.
  - Colony Background Strain Name:** A dropdown menu.

**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”.

The screenshot shows a web form titled "Allele Modification Details". It contains two main sections: "Excision Status" and "Colony Details".

**Excision Status**

- Excision Status**: Cre Excision Started
- Cre Excision via TAT-Cre?**: ☐
- Deleter Strain Name**: MGI:3046308: Hprt<tm1(CMV-cre)Brd> (dropdown menu)
- Number Of Cre Matings Successful**: 5 (text input)
- Allele Type**: b - Knockout-First, Post-Cre - Reporter Tagged Deletion (dropdown menu)
- Is Active**: ☒
- Report To Public**: ☒

**Colony Details**

- Colony Name**: NewColonyName (text input)
- Colony Background Strain Name**: C57BL/6N:MGI:2159965 (dropdown menu)

**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).

Colony Details

Colony Name

NewColonyName

Colony Background Strain Name

C57BL/6N:MGI:2159965

Distribution Centres

Distribution centre	Distribution network?	Deposited material	Distribution dates	Action
BCM		Live mice	<div>Start:</div> <div></div> <div>End:</div> <div></div>	<a href="#">remove</a>

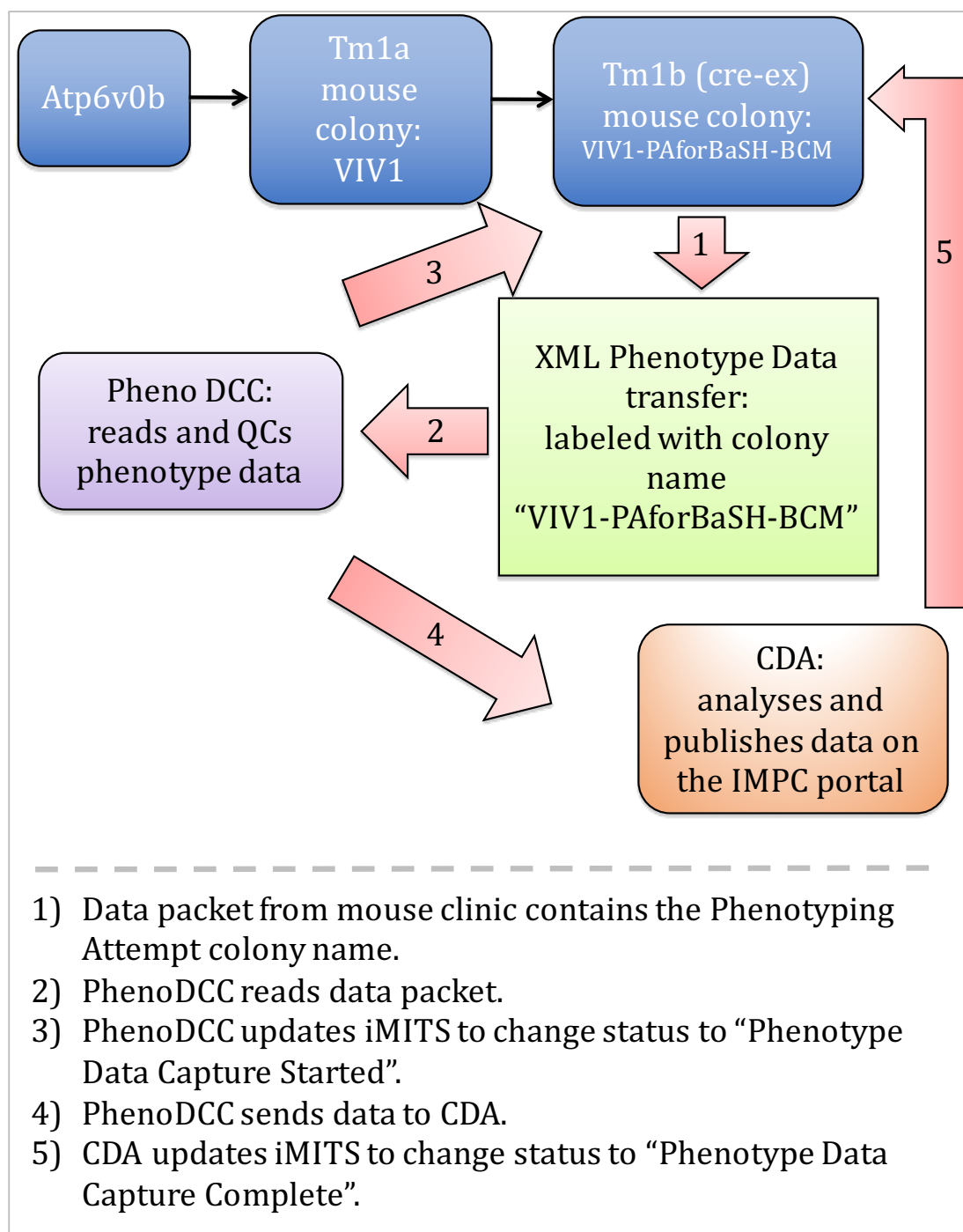
[Add Distribution Centre](#)

**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:



**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.

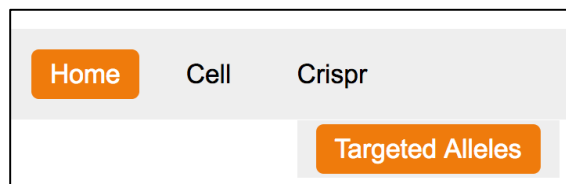
## C. How to add donor vectors prior to entering CRISPR injection information in iMITS

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.**

### Create an allele

Gene Filter

Genomic Position

Chromosome  
11 ▼

Strand  
+ ▼

Assembly  
GRCm38

Design Details

Mutation Method  
Targeted Mutation ▼

Mutation Type  
Conditional Ready ▼

Mutation Subtype  
Domain Disruption ▼

Backbone  
L3L4\_pD223\_DTA\_T\_spec

Cassette  
pL1L2\_GT2\_LF2A\_nEGFPO\_T2A\_CreERT\_puro\_dr

Cassette type  
Promotorless ▼

Genbank files Details

Targeting Vectors Genbank File

```

LOCUS      K0-
first_condi...ready_114372_MGI:2677836
21996 bp    dna            circular UNK
DEFINITION Mus musculus targeted K0-first,
conditional ready, lacZ-tagged
mutant vector Dolk targeting
project(s): 114372
ACCESSION   unknown
SOURCE      Mus musculus
ORGANISM    Mus musculus
Eukaryota; Metazoa; Chordata;
Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria;
Euarchontoglires; Glires; Rodentia;
Sciurognathi; Muroidea; Muridae;
Murinae; Mus.
COMMENT     cassette:

```

### Targeting Vectors

Targeting Vector	Intermediate Vector	
nxn_tv		<a href="#">Remove</a>

[Add a targeting vector](#)

**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](http://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
<b>Plan</b>	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 <i>not yet</i> 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).

<b>MI Attempt</b>	MI In Progress	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	
<b>Phenotype Attempt</b>	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 settable by the DCC and the CDA – that is, they cannot be set by the Mouse Clinic (Figure 35)