# Cre Portal (<u>www.creportal.org</u>) Tutorial

<u>This tutorial contains two parts</u>: first, a step-by-step example that you can use to follow along on your computer; and second, a short set of questions (with answers) for you to test your skills.

#### **Aims**

- 1. Find your way to the Cre Portal and search for a cre transgene or knock-in that uses a particular driver/promoter or is known to have activity in a particular anatomical/tissue system.
- 2. Explore the contents of the Results Page returned and learn about customizing it for your use.
- 3. Explore detailed information about the cre activity of a specific cre transgene or knock-in and learn about customizing the view and linking to other data.
  - Cre construct information
  - Availability of mouse resources from repositories
  - Specifics of cre activity/specificity for a transgene in an anatomical location (here reproductive system)
  - Images
  - Publications involving this cre transgene/knock-in
  - Phenotypes observed when mice carrying this cre transgene/knock-in were mated to different gene knock-out heterozygotes
- 4. Return to the Cre Portal main page and view the data report for 'all cre transgenes/knock-ins'.

#### Introduction

Cre is currently the most commonly used recombinase. It catalyzes site-specific recombination of DNA between loxP sites, thus making possible conditional mutagenesis, where specific genes can be knocked-out in particular tissues and at particular developmental time points. Other non-cre recombinases (such as Flp, Dre, phiC31, etc.) and various inducible forms of recombinases have been and are being developed. The Cre Portal contains curated data about all recombinase-containing transgenes and knock-ins developed in mice to provide a comprehensive resource delineating known specificity patterns and allowing users to find relevant mouse resources for their studies.

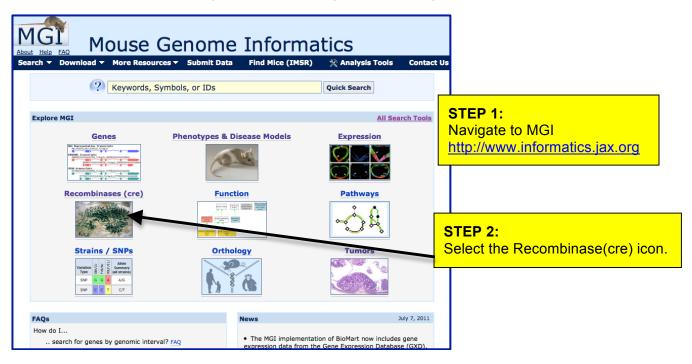
At present, researchers can search for "cre" data using the driver/promoter gene symbol or using an anatomical system in which the "cre" is expressed. In addition, data are available for all cre's in a html viewable table, or as downloadable tab-delimited text.

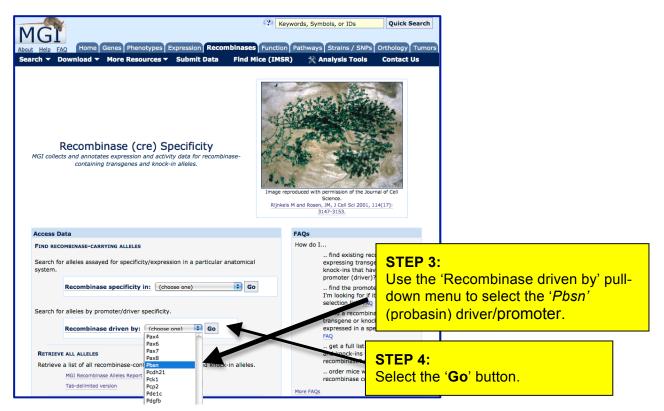
Help with using the Cre Portal is available on the <u>www.creportal.org</u> site. See the list of FAQs in the right-hand column or the 'Help Documents' tab in the bottom section of this page. You can also use the 'Contact Us' link in the navy blue navigation bar near the top of any of our web pages.

## Worked examples:

# 1. Find the Cre Portal and search for a cre transgene or knock-in with a particular driver/promoter [or with cre activity in a particular anatomical/tissue system].

Type the URL: <a href="http://www.creportal.org">http://www.creportal.org</a> into your web browser; OR go to the Mouse Genome Informatics (MGI) homepage (<a href="http://www.informatics.jax.org">http://www.informatics.jax.org</a>) and select the Recombinase(cre) icon. The following illustrates the latter route; visiting the MGI homepage and selecting the icon.

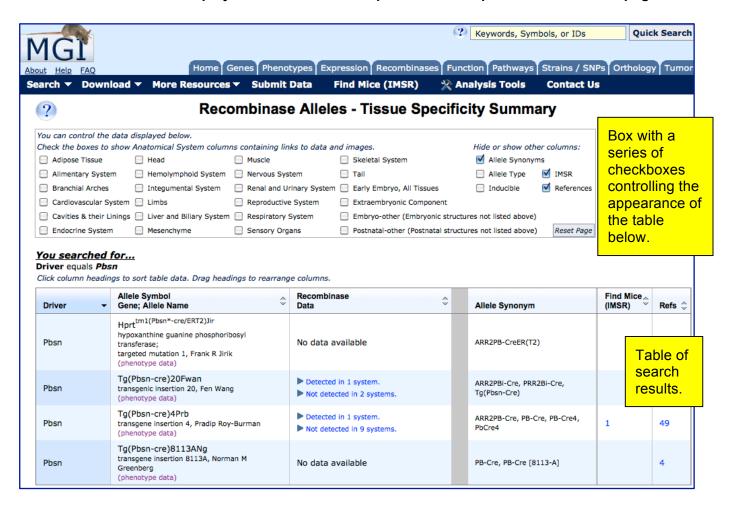




# 2. Explore the contents of the Results Page returned from your search and learn about customizing it for your use.

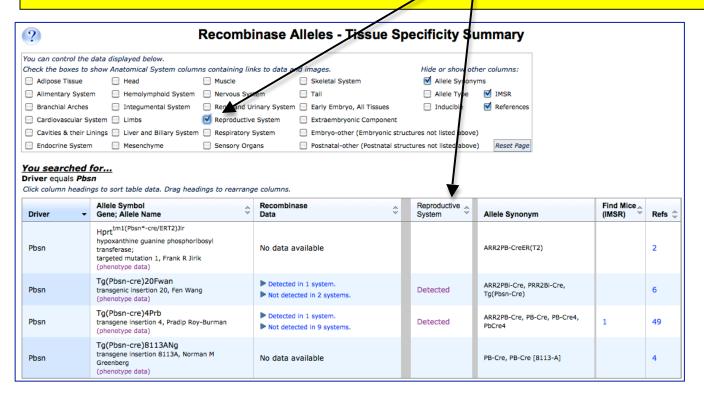
This is the default Results Page from the search illustrated on the previous page. Now let's explore the features of this page. First, we will examine the **top box** that controls the data displayed in the table. Then we will look at the contents of the **table** itself.

You can control the data displayed in the table with options in the top box section of the page.

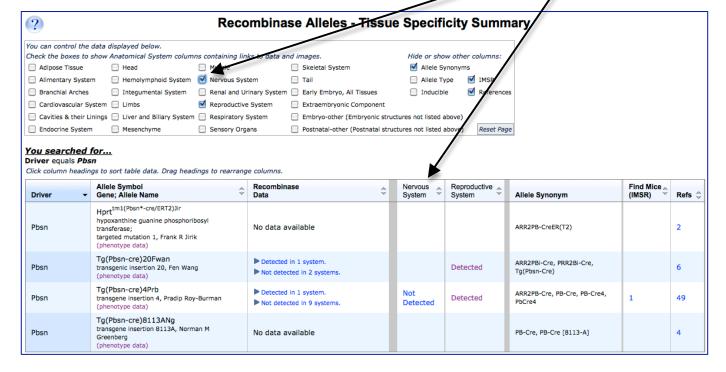


**STEP 1:** Sequentially check the box '<u>Reproductive System'</u>, check the box '<u>Nervous System'</u>, and un-check the box '<u>Allele Synonym'</u> in the box at the top to see how, with each of these actions, the columns of the table display change. See screenshots labeled (a)(b)(c) below.

(a) Screenshot showing the consequences of 'checking' the box for 'Reproductive System'. A new column is added to the table, displaying, for each cre allele in your search result, (here those with a Pbsn driver), whether cre activity has been 'Detected' or 'Not detected' in the Reproductive System.



(b) Screenshot showing the consequences of adding another 'check' in the box for 'Nervous System'. A new column is added to the table, displaying for each cre allele in your search result (those with a Pbsn driver), whether cre activity has been 'Detected' or 'Not detected' in the Nervous System.



(c) Screenshot showing the consequences of 'un-checking' the checkbox for 'Allele Synonym'. This column has now been deleted from the table.

? Recombinase Alleles - Tissue Specificity Summary									
You can control the	data displayed below.								
Check the boxes to show Anatomical System columns containing links to data and images.									
Adipose Tissue	☐ Head ☐ Muscle	Skeletal System	Allele S	ynonyms					
Alimentary System	m 🔲 Hemolymphold System 🗹 Nervous Sy	stem 🔲 Tail	Allele T	ype 🗹 IMSR					
Branchial Arches	☐ Integumental System ☐ Renal and U	Irinary System 🔲 Early Embryo, All Tissues	Inducib	le 🗹 Reference	s				
Cardiovascular Sy	ystem 🗌 Limbs 🗹 Reproductiv	e System Extraembryonic Component							
Cavities & their Li	☐ Cavities & their Linings ☐ Liver and Billary System ☐ Respiratory System ☐ Embryo-other (Embryonic structures not listed above)								
Endocrine System	Mesenchyme Sensory Org	gans Postnatal-other (Postnatal st	ructures not listed	above) Reset Pag	e				
You searched for  Driver equals Pbsn  Click column headings to sort table data. Drag headings to rearrange columns.									
Driver <b>▼</b>	Allele Symbol Gene; Allele Name	Recombinase	Nervous System	Reproductive System	Find Mice	Refs \$			
Pbsn	Hprt <sup>tm1(Pbsn*-cre/ERT2)Jir</sup> hypoxanthine guanine phosphoribosyl transferase; targeted mutation 1, Frank R Jirik (phenotype data)	No data available				2			
Pbsn	Tg(Pbsn-cre)20Fwan transgenic insertion 20, Fen Wang (phenotype data)	<ul><li>Detected in 1 system.</li><li>Not detected in 2 systems.</li></ul>		Detected		6			
Pbsn	Tg(Pbsn-cre)4Prb transgene insertion 4, Pradip Roy-Burman (phenotype data)	<ul><li>Detected in 1 system.</li><li>Not detected in 9 systems.</li></ul>	Not Detected	Detected	1	49			
Pbsn	Tg(Pbsn-cre)8113ANg transgene insertion 8113A, Norman M Greenberg (phenotype data)	No data available				4			

### Step 2. Now let's look at the contents of the table (lower) portion of this page.

This section of the page provides data summaries and links to additional information. Let's review a single data line representing one cre transgene. Outlined in red is the row of the table describing transgene, **Tg(Pbsn-cre)4Prb**, a transgene with cre driven by the *Pbsn* promoter.

Driver	Allele Symbol  Gene; Allele Name	Recombinase Data	\$ Nervous System	Reproductive System	Find Mice (IMSR)	Re s
Pbsn	Hprt <sup>tm1</sup> (Pbsn*-cre/ERT2)Jir hypoxanthine guanine phosphoribosyl transferase; targeted mutation 1, Frank R Jirik (phenotype data)	No data available				2
Pbsn	Tg(Pbsn-cre)20Fwan transgenic insertion 20, Fen Wang (pnenotype data)	➤ Detected in 1 system.  ➤ Not detected in 2 systems		Detected		6
Pbsn	Tg(Pbsn-cre)4Prb transgene insertion 4, Pradip Roy-Burman (phenotype data)	▼ Detected in 1 system. reproductive system  ▼ Not detected in 9 systems. alimentary system, cardiovascular system, embryo-other, hemolymphoid system, integumental system, liver & biliary system, nervous system, renal & urinary system, respiratory system	Not Detected	Detected	1	49
Pbsn	Tg(Pbsn-cre)8113ANg transgene insertion 8113A, Norman M Greenberg (phenotype data)	No data available				4

The columns of the table can be sorted (as indicated by the up/down arrows in the column headers). For example, perhaps you want an alphabetic sort of the Drivers in your Results Page; or to sort by the number of references (an indicator of how widely this cre line is used); or to sort by the Find Mice column to bring all those cre lines that are in public repositories to the top of your Results Page.

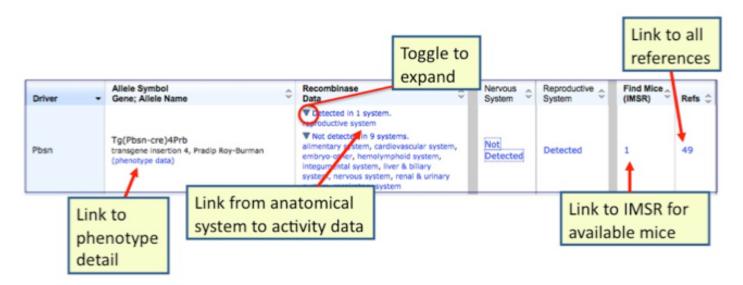
Each line of the table provides basic information about the cre transgene or knock-in, its driver, official symbol/name, a high level summary of location of cre activity and links to additional information.

Driver	Allele Symbol ▼ Gene; Allele Name	A V	Recombinase \$\hfrac{1}{2}\$	Nervous System	Reproductive \$\hfigsetarrow\$	Find Mice (IMSR)	Refs 💠
Pbsn	Tg(Pbsn-cre)4Prb transgene insertion 4, Pradip Roy-Burman (phenotype data)		➤ Detected in 1 system. ➤ Not detected in 9 systems.	Not Detected	Detected	1	49

Within a row of data, each of which represents a single cre line transgene or knock-in, there are a number of links and actions possible that lead to further data details (shown below).

The link to **phenotype data in the Allele/Symbol column** directs you to a page showing phenotypes reported in offspring of mice bearing this cre transgene and mice carrying floxed alleles of various genes (see page 11 of this tutorial).

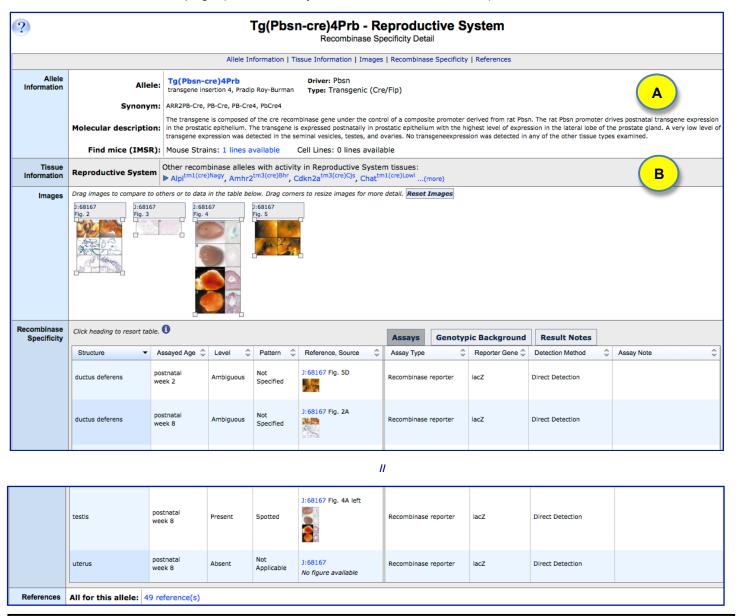
The Recombinase Data column contains toggles ( ) that will expand to show the anatomical systems where cre activity has been "Dectected in..." or "Not detected in..." In turn, each anatomical system term is a link directing you to a page showing the data for cre activity in that system (next page of this tutorial). The number displayed in the Find Mice (IMSR) column is a link to the International Mouse Strain Resource (IMSR) page with information on what repository holds relevant cre lines and how to obtain them. The number in the Refs column shows the number of publications about this cre and links to the full list of these publications (this number gives an idea of how widely this particular cre transgene has been used experimentally).



**Step 3**. Click on the term 'reproductive system' in the expanded **Recombinase Data column** in the row for **Tg(Pbsn-cre)4Prb** to go to the detail page for cre activity/specificity in the reproductive system for this cre transgene.

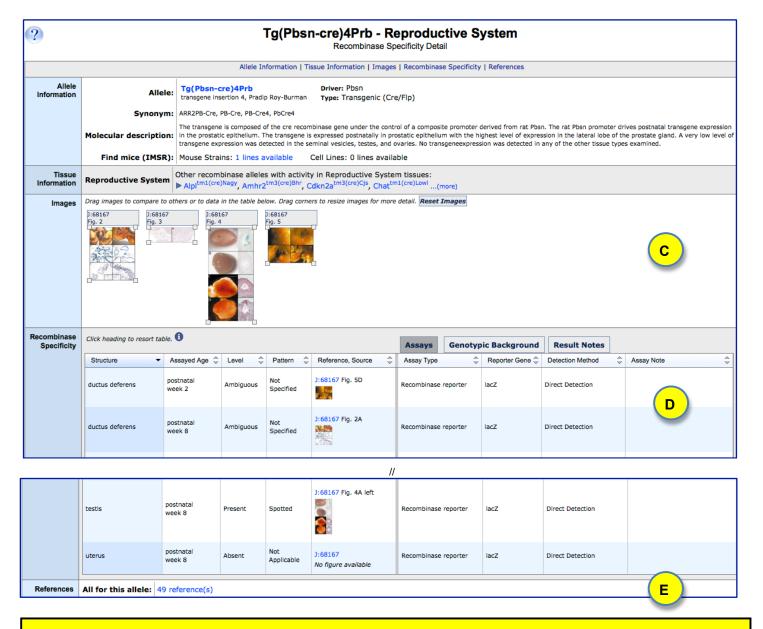
# 3. Explore detailed information about the cre activity of a specific cre transgene or knock-in and learn about customizing the view.

This is the cre specificity detail page for **Tg(Pnsn-cre)4Prb** in the **Reproductive System**. Many rows of the **Recombinase Specificity** table have been omitted to save space in this tutorial document. We will explore each of the sections of this page (delineated by the blue title column at left).



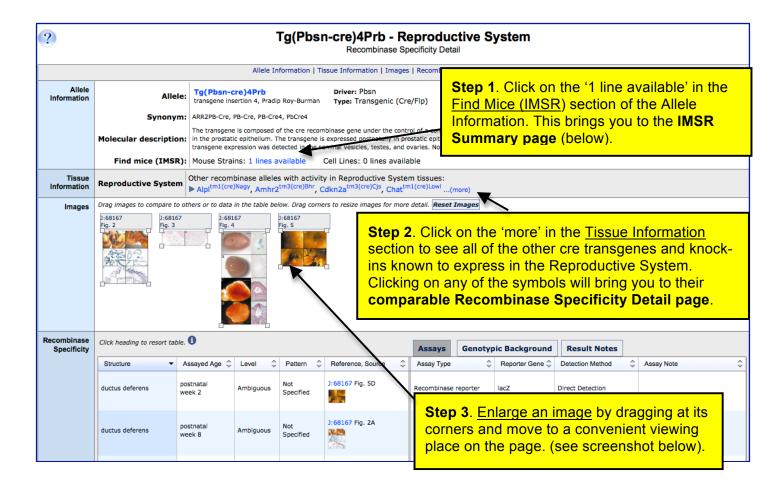
**A. Allele Information**. This section contains essentials about the cre transgene itself; its correct nomenclature (symbol and name), the <u>driver</u> involved, other <u>synonyms</u> that have been used in publications, a <u>molecular description</u> of the what the transgene is carrying (in this case, the cre recombinase gene under the control of a composite promoter derived from the rat *Pbsn* gene) and a <u>Find mice</u> IMSR summary indicating whether mice or cell lines carrying this transgene are available from pubic repositories.

**B. Tissue Information**. This section lists all other cre transgenes and knock-ins that have reported activity in the Reproductive System. Each symbol is a link to the cre specificity page for that transgene or knock-in for the Reproductive System. The ...(more) indicates there are others and clicking on (more) will bring back the entire list.

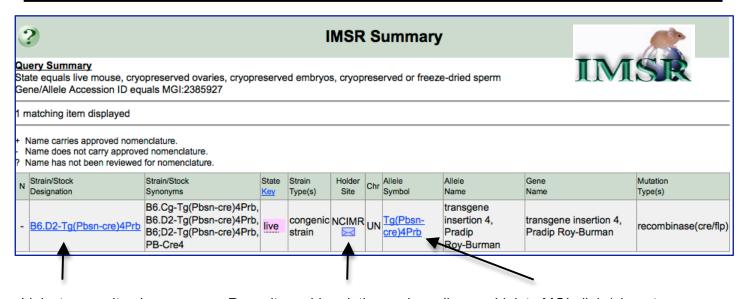


- **C. Images**. This section presents a gallery of thumbnail images for cre activity for the tissue being viewed. Images can be enlarged by dragging at the corners and may be moved around the window for convenient viewing. Enlarging an image also activates a pop-up with caption and attribution information.
- **D. Recombinase Specificity**. This tabular section provides the annotation detail for the cre specificity, including anatomical structure, age, activity level & pattern, and data source. The last columns of the table (separated by a gray bar) are controlled by tabs at the top of the table and allow this section of the table to show variably 'Assay' or 'Genotypic Background' information or 'Result Notes'. The view shown here is 'Assay' information, consisting of Assay Type, Reporter Gene, Detection Method, and Assay Notes. Observe that all columns in this table are sortable, as indicated by the up/down arrowheads after the column heading title. For example, one might want to sort by age to group all data together for a certain age; or by structure; or by level to place all the 'present' vs. 'absent' together. Finally, clicking on the small image in the table highlights the corresponding image in the image gallery so it is easier to identify the image you may want to enlarge.
- E. References. This section lists the number of publications for this cre transgene and links to the full list.

# Now we will look at some of the links and actions on this page....



**Below, Result of Step 1**. View of IMSR (International Mouse Strain Resource) results and access to a Tg(Pbsn-cre)4Prb bearing strain.

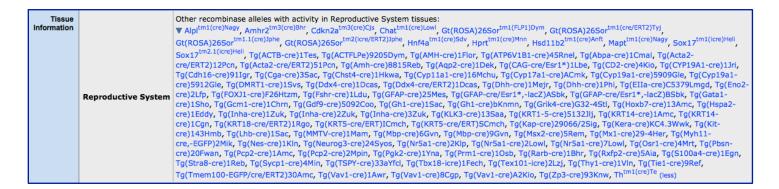


Links to repository's strain information page

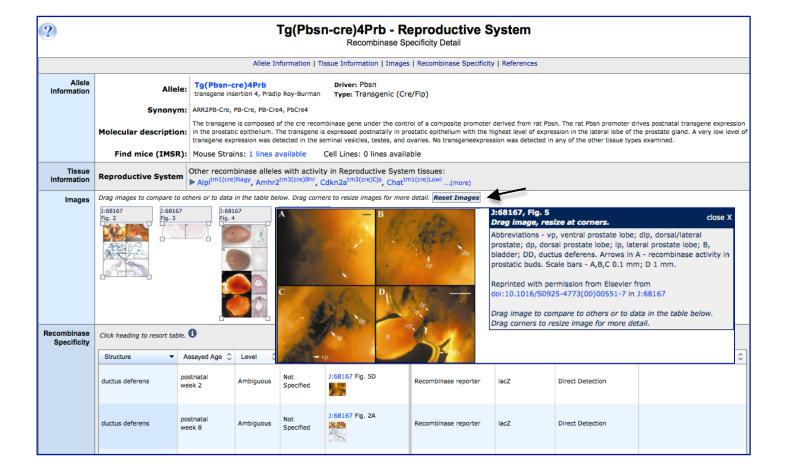
Repository abbreviation and email link for questions or to order mice. (NCIMR is the NCI Mutant Resource)

Link to MGI allele/phenotype page for this transgene (later in this tutorial, page 11)

**Below, Result of Step 2**. List of all other cre transgenes and knock-ins that show activity in the Reproductive System (there are 100 listed here). Each symbol is a link to the respective Reproductive System cre details for that transgene or knock-in. Note the (less) at the end of the list, which will collapse the list back to its original few.

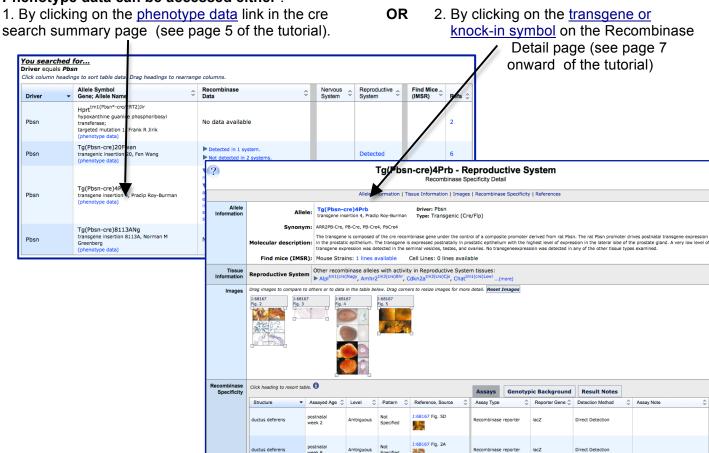


**Below, Result of Step 3**. The enlarged image was made by dragging the image corners. The image legend and attribution has popped-up to the right of the image. Multiple images may be enlarged and moved as you work. Note the 'Reset Images' Button (arrow) that can be used to bring all images enlarged or moved back to their original size and placement on this page.

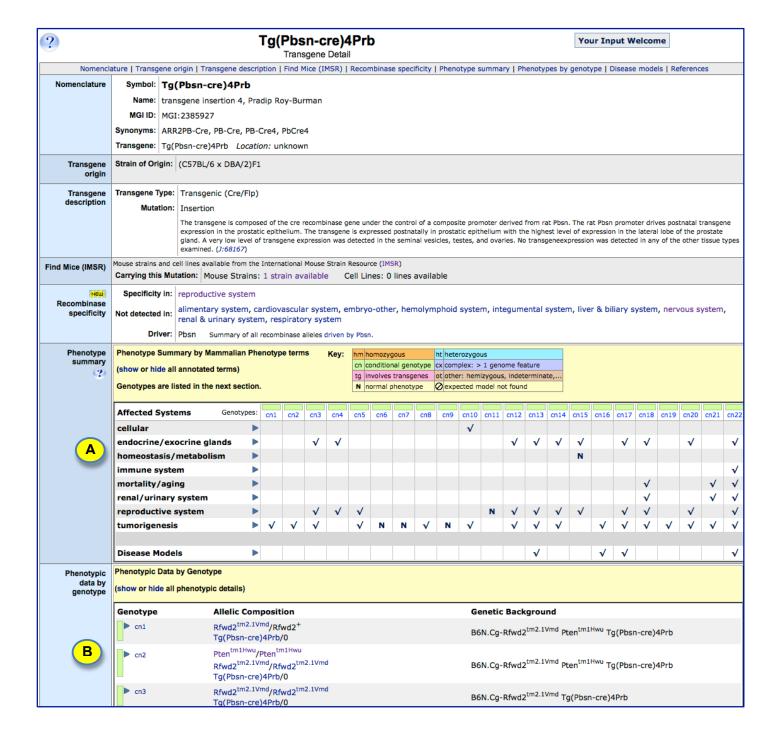


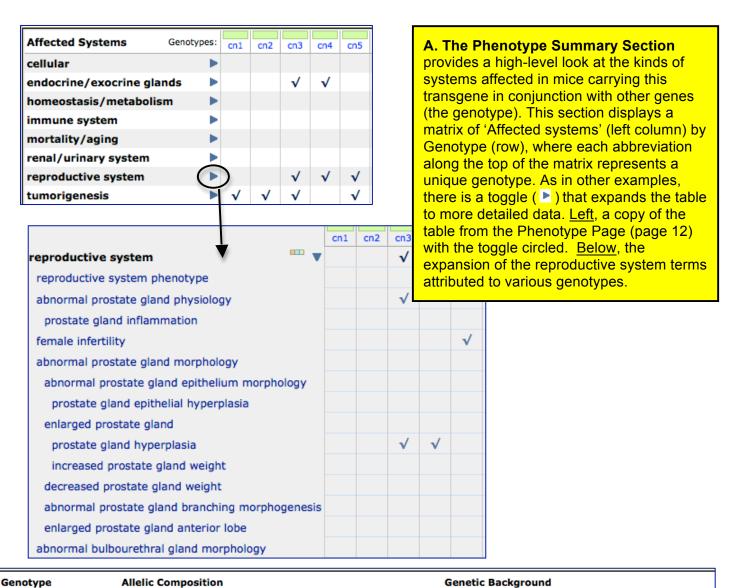
Finally, we learn about <u>phenotypes observed</u> when mice carrying a particular cre transgene/knock-in are mated to mice carrying different genes with loxP sites (genes that will be knocked-out when exposed to cre recombinase).

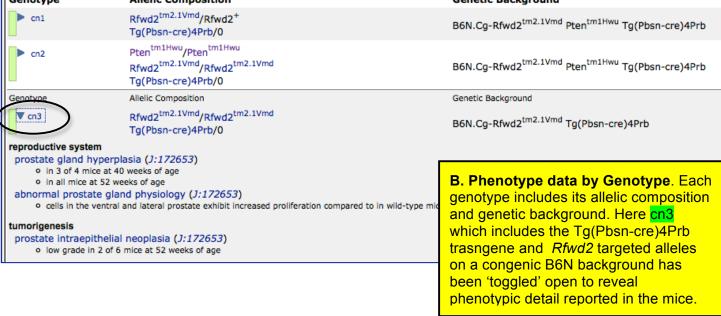
#### Phenotype data can be accessed either:



This is the **Transgene detail page for Tg(Pbsn-cre)4Prb, which contains the phenotype data**. Like the Recombinase Specificity page for the Reproductive System, the information at the top of the page is basic information on nomenclature, the description of the molecular construct and includes links to IMSR to obtain mice carrying this transgene. There is also a section summarizing recombinase specificity with links to the Recombinase Specificity pages as we have reviewed previously. Two phenotype sections on this page, labeled (A) Phenotype summary and (B) Phenotype data by genotype are described below.

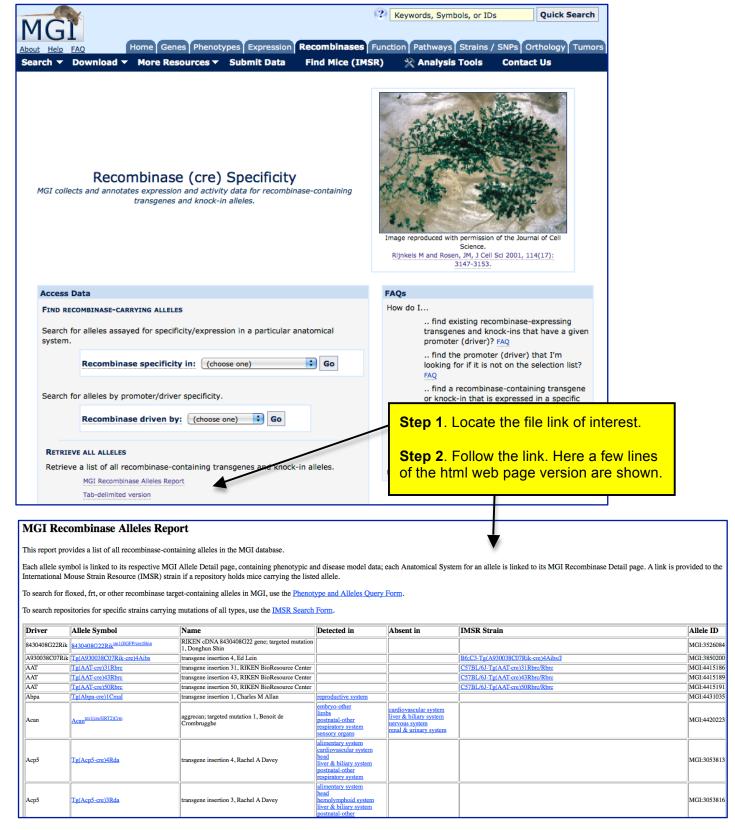






## 4. Return to the Cre Portal main page & view the data report for 'all cre transgenes/knock-ins'

Find the Cre Portal main page using http://www.creportal.org. Locate the "Retrieve All Alleles Section" and choose either the MGI Recombinase Alleles Report (an html page view format) or the Tab-delimited version if you wish to copy the file into Excel or some other analysis program.



### **Self Directed Tasks**

1. Using the Cre Portal, find cre carrying transgenes or knock-ins that are driven by *AMH*. How many are there?

Which one do you think has been used most experimentally?

Are any mice available with these cre transgenes from public repositories? If so, from where? For the *AMH* driven cre transgenes, what tissues are listed as "not detected" for cre recombinase activity? What ages were assayed?

(explanation note: You will note that the search is case in-sensitive. Do you know why there is a driver designated *AMH* and one designated *Amh*? This is because nomenclature follows that of the species where the gene originated. *AMH* is the human gene (all capital letters); *Amh* is the mouse gene (1<sup>st</sup> letter only capitalized).

2. For Tg(AMH-cre)1Flor, what tissues were reported with staining 'Present' (indicating cre activity) at postnatal day 35?

What reporter gene was used in the assays for Tg(AMH-cre)1Flor and Tg(Amh-cre)8815Reb?

3. For Tg(AMH-cre)1Flor, when mated to other targeted mutations, what other classes of phenotypes are observed in addition to "reproductive" related?

## Answers (These are likely to change due to database updates, etc.)

1. From <a href="www.creportal.org">www.creportal.org</a>, select the driver AMH in the 'search for alleles by promoter/driver specificity' and then click 'GO'.

There are 2 cre transgenes (**Tg(AMH-cre)1Flor** and **Tg(Amh-cre)8815Reb**) with an *AMH* driver. One is driven by the human *AMH* promoter; the other by the mouse *Amh* promoter. These symbols follow species' nomenclature convention. You can also learn this by reading the molecular description in the transgene detail pages.

Because there are 34 references for Tg(AMH-cre)1Flor and only 8 references for Tg(Amh-cre)8815Reb, the cre transgene Tg(AMH-cre)1Flor is likely to have been used more frequently.

Yes, both of these cre transgenes are available from public repositories.

Tq(AMH-cre)1Flor is available from the EMMA repository (EM) in Europe as frozen embryos.

Tg(Amh-cre)8815Reb is available from the Jackson Laboratory repository (JAX) as live mice.

From the summary page, if you expand the 'Not detected in 1 system' for the Tg(AMH-cre)1Flor transgene, the data indicate recombinase (cre) activity is not detected in the anatomical class "embryo-other". Following the link to this 'anatomical class' one sees that cre activity was not detected at embryonic day 12.5 and 13.5.

2. For Tg(AMH-cre)1Flor at day 35, staining was found in ovary antral follicle, ovary secondary follicle, ovary stratum granulosum.

The reporter gene for cre transgene Tg(AMH-cre)1Flor was lacZ; for cre transgene Tg(Amh-cre)8815Reb, the reporter gene was hemagglutinin.

3. Through the "phenotype data" link for Tg(AMH-cre)1Flor, you can see that there are endocrine/exocrine gland phenotypes and homeostasis/metabolism phenotypes observed.

We welcome your feedback in improving this resource. Contact us through the "Contact Us" link in the navy blue navigation bar at the far right; or email <a href="mailto:mgi-help@jax.org">mgi-help@jax.org</a>