Frequently Asked Questions (FAQs) for MMA

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- **How do I log on to MMA?**
- **How long is each session after I logged on?**
- **Can I save changes for my projects?**
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General Questions

- **How do I get access or input my projects on MMA?**

  There are two ways of accessing projects on the Mouse Model Archive:
  1. You can register and choose your own username and password.
  2. We can assign you a username and password.

- **How do I log on to MMA?**

  You would need a username and password to log on to MMA. Without a username or password, only published projects will be accessible.

- **How long is each session after I logged on?**

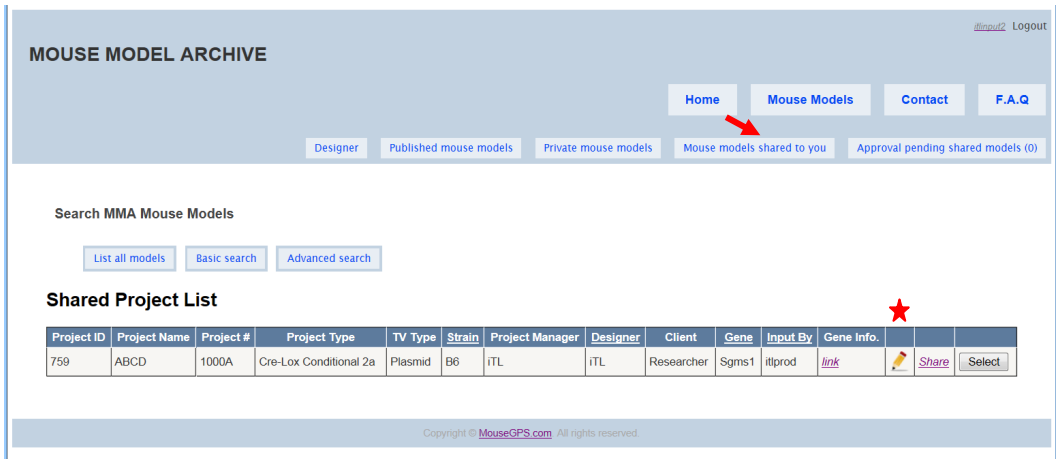
  Each session is 20 minutes of continuous keystroke activity. If your computer is idle, a prompt will be flashed up on your screen when the last 5 minutes is to be expired. By hitting any key, it will renew you for another 20 minutes. After 20 minutes of continuous inactivity, you will be automatically logged off.

- **Can I save changes for my projects?**

  If someone shared the project to you as View Only, you will be able to make any changes but once you are logged off, the changes will not be saved. However, if you copy the project to your own private list, you will be able to save all your changes. If someone shared the project to you as collaboration, you can make and save any changes including the targeting vector sequences and design. If you input your own project, you will be able to make and save any changes.

- **How do I know if someone shared a model or project to me as view only or as a collaborator?**

  You will find a list of mouse models and projects shared to you when click on **Mouse models shared to you** tab. You will see an icon next to the project. View only () is that you could view the project but cannot save any changes. Collaborator () allows you to make and save any changes including the targeting vector sequences and design.



- **What modifications can I made on the MMA as View Only?**

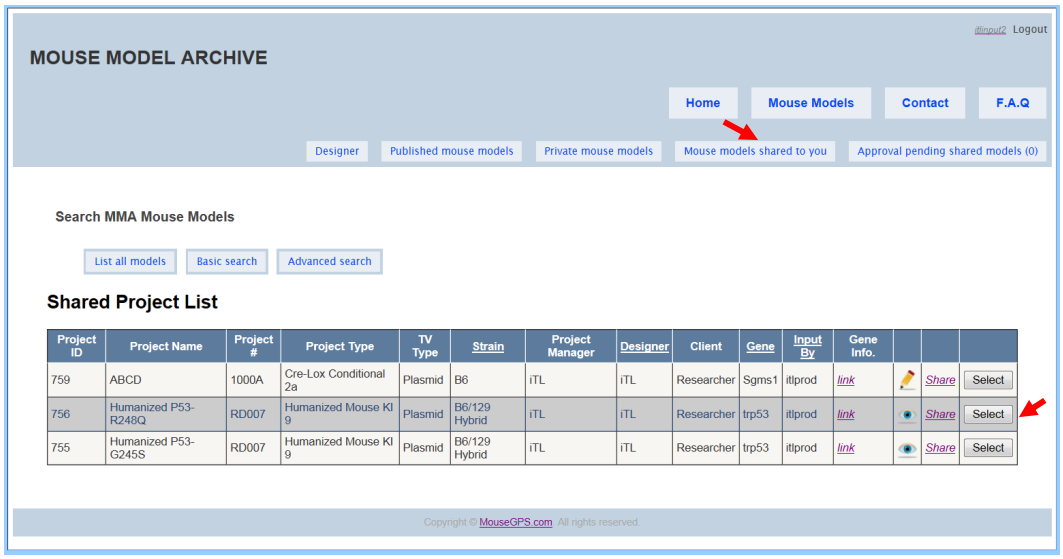
  If the project is shared to you as **View Only**, you have the option to make changes but your changes will not be saved after you logged off.

  Below are some modifications that you could make on the MMA:

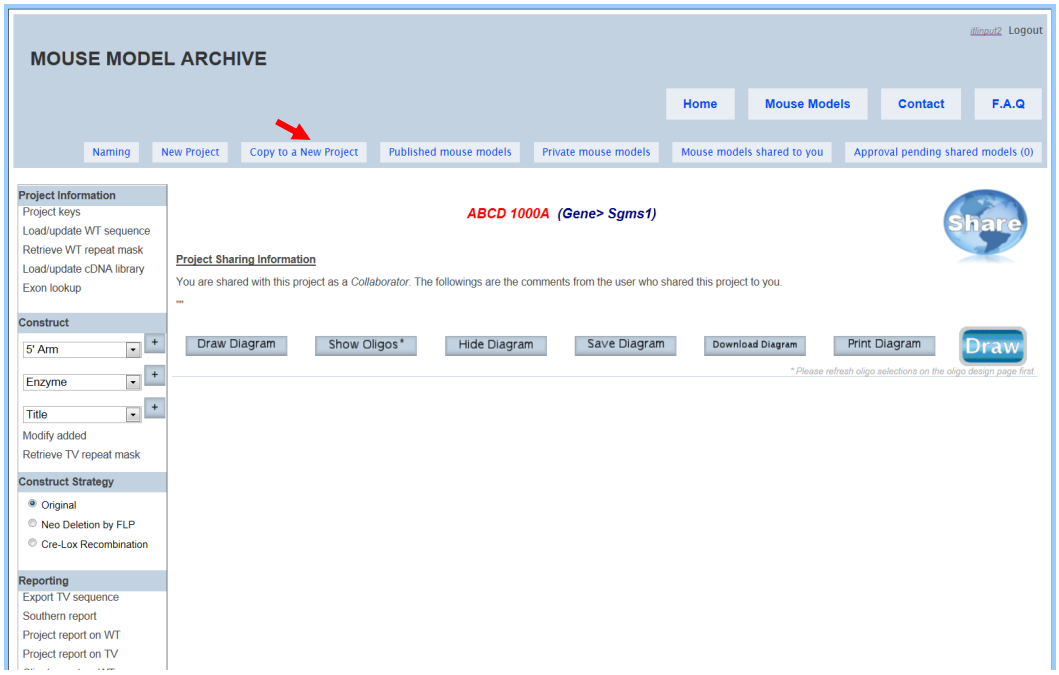
  * Design your own probes and display Southern strategy with selected enzyme(s)
  * Display all possible cutting sites for selected enzyme(s)
  * Visualize different mating schemes (FLP/Cre) on schematic diagram
  * Select oligos display on schematic diagram but can't design new oligos
  * Generate reports for project/client WT or TV
  * Generate repeat mask reports but can't show repeat regions on schematic diagram
  * Change schematic title and description
  * Change flanking region outside 5' and 3' arms
  * Replace a different sequence for selection cassette or any vector components
  * And many more options

- **How do I copy a project from a project that was shared to me?**

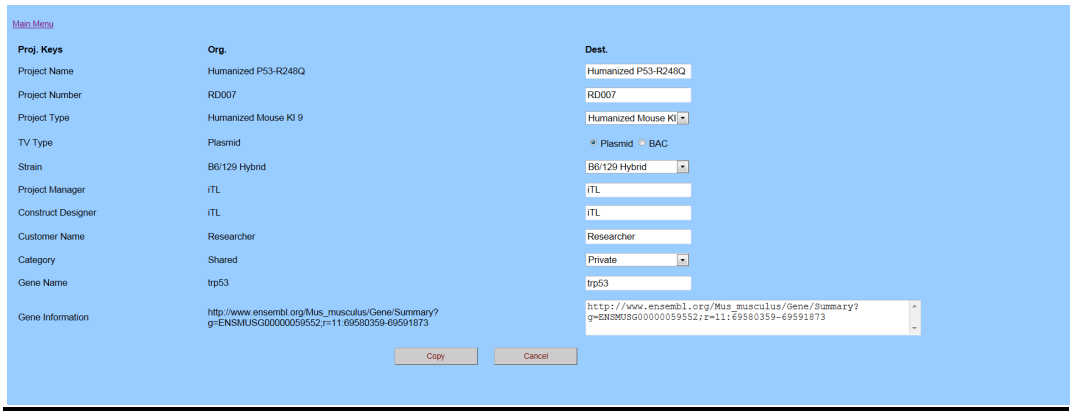
  After you logged on, select the **Mouse models shared to you** tab and a list of projects shared to you will be displayed. Select the project that you would like to make a copy.



Select **Copy to a New Project**.



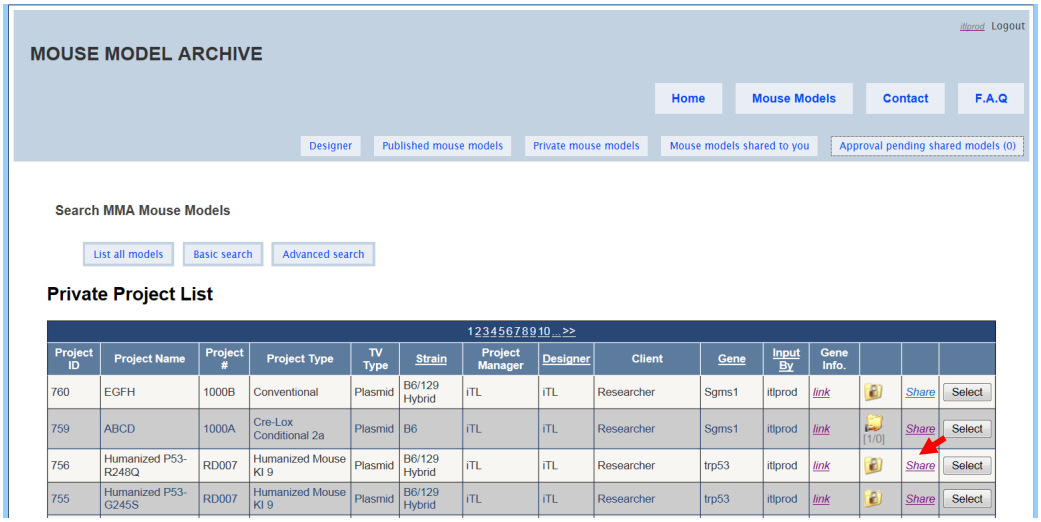
The original and a copy of the project are displayed. You have the option to change any fields on the project information page except the gene name and information. Then press the **Copy** button. Your copied project will be displayed in the **Private mouse models** and you will be able to make and save any changes.



- **How do I share my project with a colleague?**

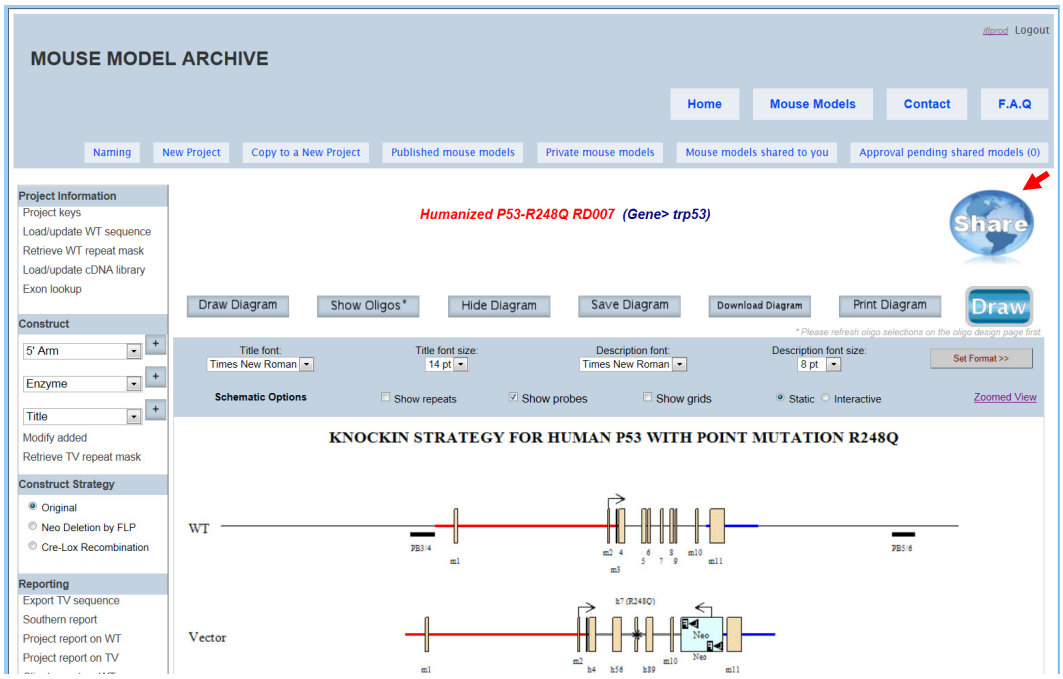
  There are two options that you can share your project to your colleague:

  1) You can use select **Share** in your **Private Project List**



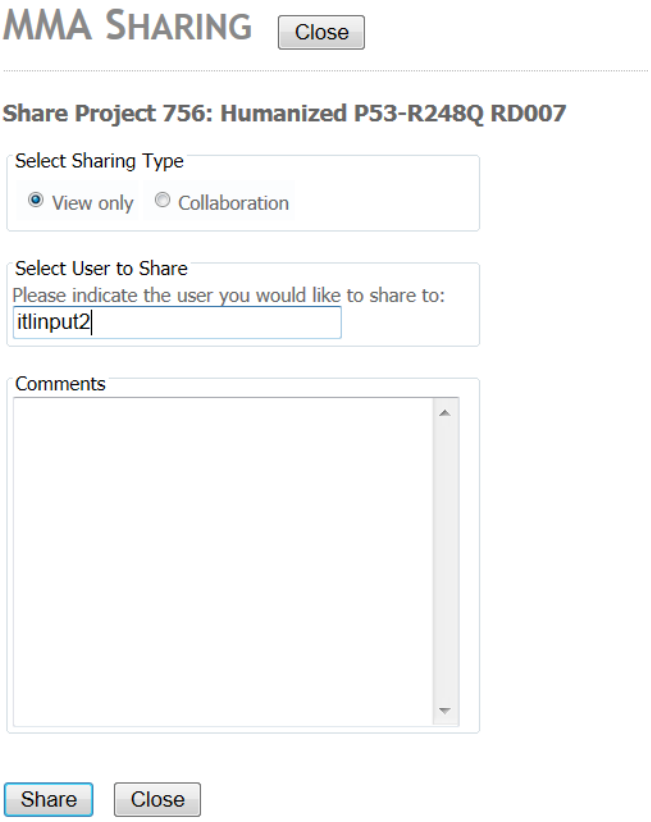
  or

  2) **Share** button on top of the schematic diagram.

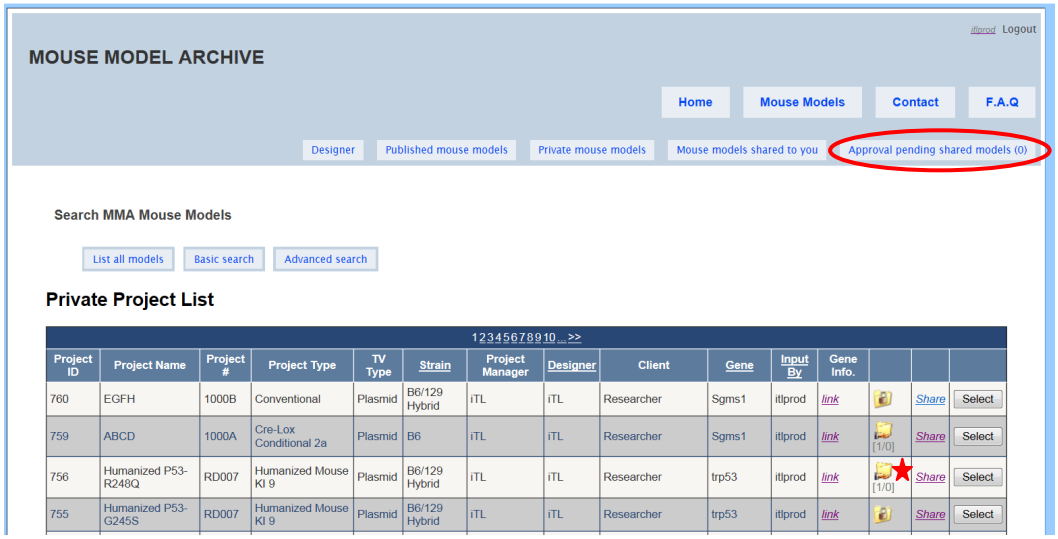


After clicking on one of the **Share** buttons, MMA Sharing page will be displayed. You can select Sharing Type either as View only or Collaboration. If you shared your project as **View only**, your colleague can not save any changes. If you shared your project as **Collaboration**, your colleague can make and save all changes including the targeting vector design.

Then enter your colleague's registered username and add any comments in the box below. Click **Share** button to share your project to your colleague.



Your Private Project List will display that the project has been shared.

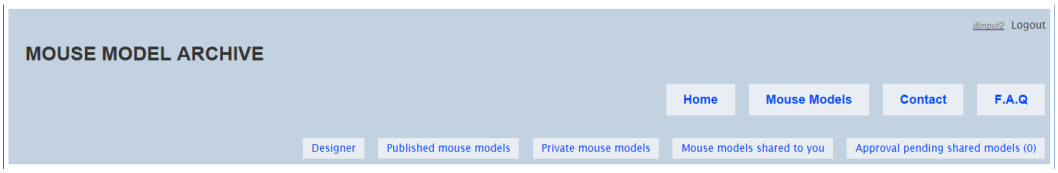


Also, if you go under **Approval pending shared model** tab on the Navigation bar as circled in red, it will display all projects and usernames that you have shared the project(s) to.

Navigation Bar

What are the tabs on the Navigation bar?

Below is a description of each tab on the Navigation bar.



Designer – new project can be input through here.

Published mouse models – a list of public projects that is available and accessible to registered and non-registered users. Changes cannot be made for these projects.

Private mouse models – a list of private projects input by you as a registered user.

Mouse models shared to you – a list of projects that shared to you by another registered user. You will have the privilege of view only or collaboration.

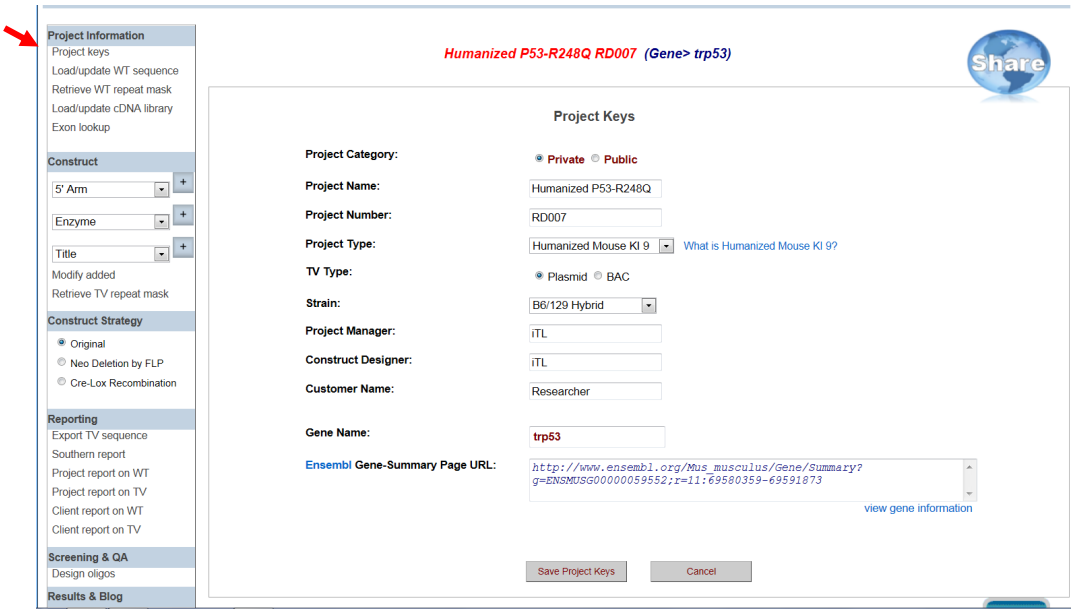
Approval pending shared models – a list of projects that you have shared with other registered user(s). Also a list of projects that is pending for your approval to be shared from one user to another user for your shared project.

Key Functions on Left Column

Project Information

- **What is Project Keys?**

  In the Project Information section on the left-hand column, click **Project Keys**. The Project Keys provide pertinent information relating to your project. Project Information page contains project category (private/public), project name, project number, project type (a list of detailed project types available), targeting vector (TV) type (plasmid/BAC), ES strain used, project manager, construct designer, customer name, gene name, and link for ensemble gene summary page.



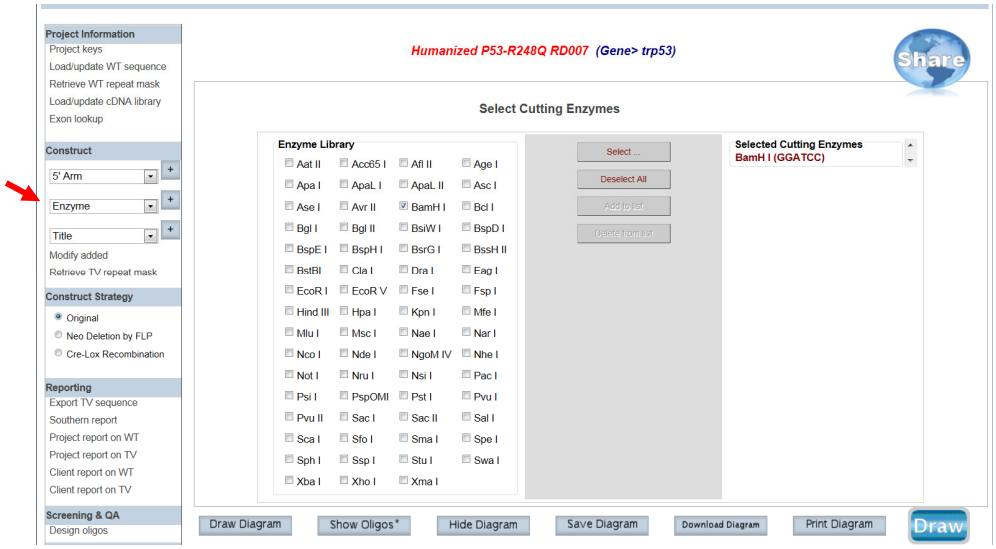
- **What is private vs. public?**

  If you select private on the Project Keys page, your project will be viewed by you when using your username and password unless you share your project with another registered user. If you select public, your project can be viewed by anyone with or without a registered username.

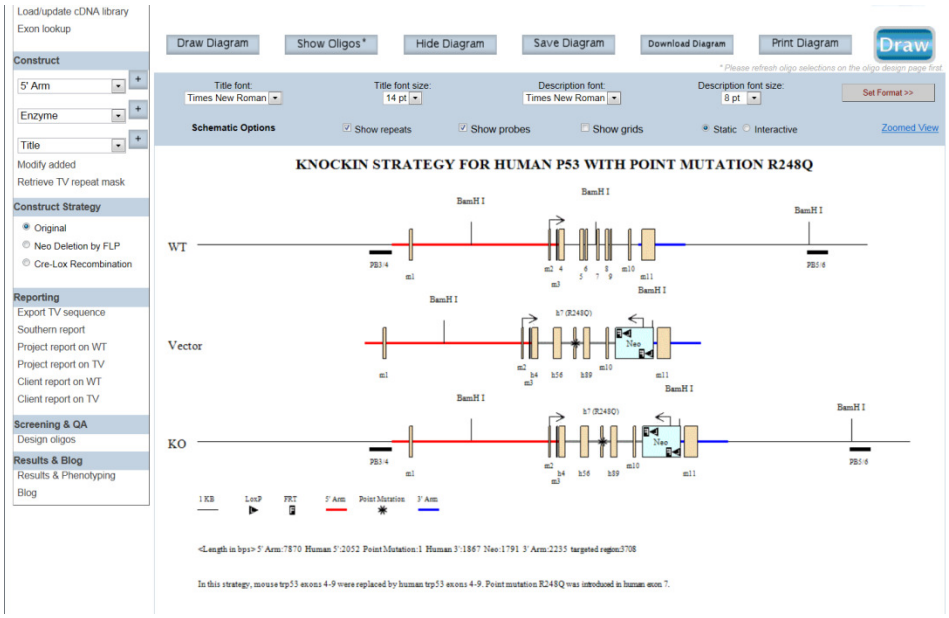
Construct

- **How do I select an enzyme to display all possible cutting sites on the schematic diagram?**

  In the Construct section on the left-hand column, select Enzyme and click the **[+]** button. It will take you to the Select Cutting Enzymes page. The Enzyme library has 63 unique enzymes to choose from. Select the enzyme of your choice by checking off the box next to your enzyme. Then click **Select** button. Your selected enzyme will be listed on the right side of the screen. You can select multiple enzymes by checking off the boxes and then click **Select**. If you want to clear all of your selected enzymes, click **Deselect All** button or remove the check from the box next to the enzyme.

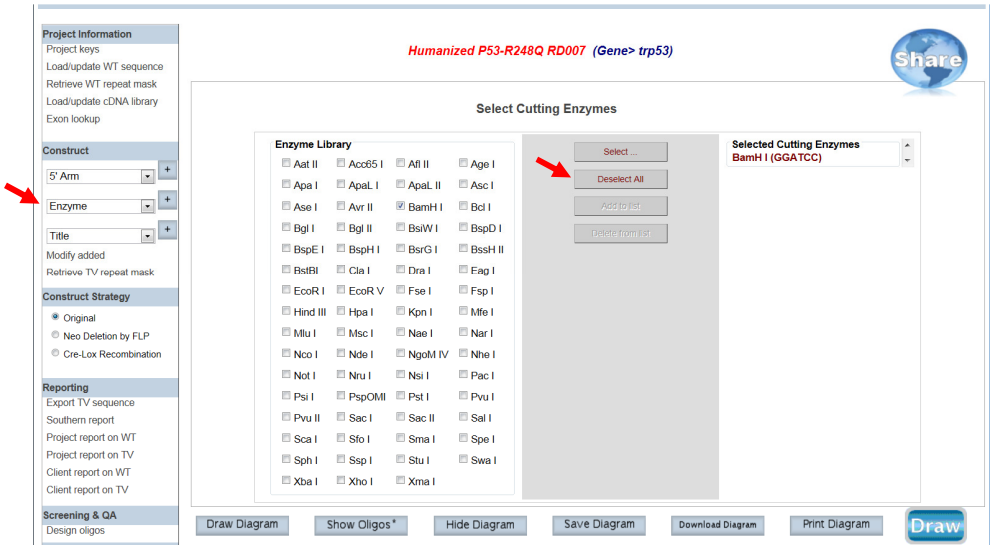


Click **Draw** or **Draw Diagram** and all possible cutting sites for your selected enzyme will be displayed on the schematic diagram. Below is an example of all possible cutting sites for the selected enzyme Bam HI.



- **How do I remove the selected enzyme(s) from displaying on the schematic diagram?**

  In the Construct section on the left-hand column, select Enzyme from the drop-down menu and click the **[+]** button. It will take you to the Select Cutting Enzymes page. Click **Deselect All** button to clear all selected enzymes or remove the check from the box next to the enzyme to deselect a specific enzyme. Enzymes will not be displayed on the schematic diagram when you click **Draw** or **Draw Diagram**.



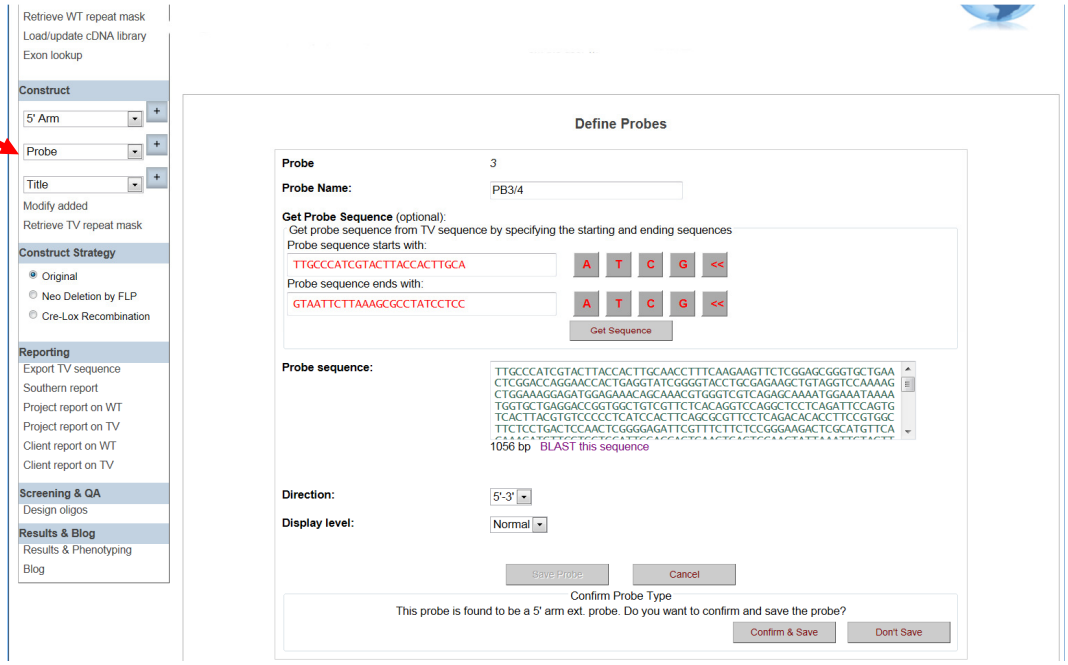
- **How do I design a probe for Southern blot?**

  In the Construct section on the left-hand column, select Probe from the drop-down menu and click the **[+]** button. It will take you to the Define Probes page.

  Enter your Probe Name. Enter the start and end sequences (5'->3') of your probe (a minimum of 10 bases) per entry. To enter the sequences, you have the option to type in the sequences using your keyboard or select the nucleotide boxes on the left panel. The box **<<** is for backspace or deletion. Then press **Get Sequence** button. The entire sequence of your probe will be displayed in the Probe Sequence box. If any base was incorrectly entered, sequences will not be displayed in the Probe Sequence box and an error message will be prompted.

  You also have the option to paste the entire probe sequence directly into the Probe Sequence box.

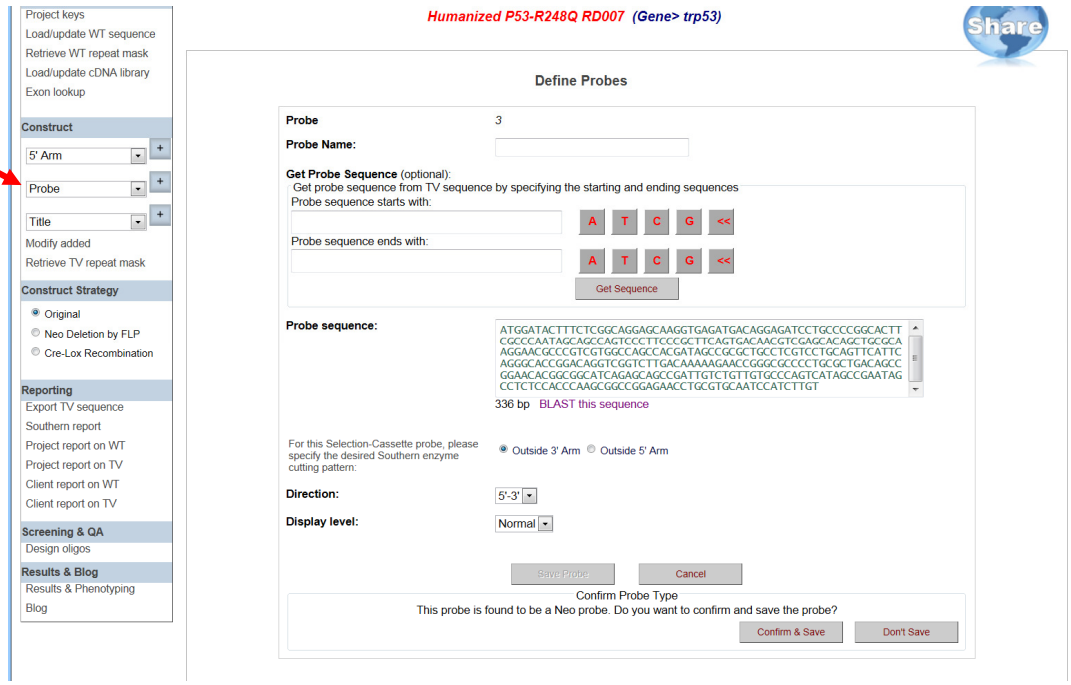
  Click **Save Probe** button to add your designed probe. A message for Confirm Probe Type will be prompted. It will indicate if the probe that you created is located 5' or 3' and external or internal. Click **Confirm & Save** button to save your probe.



- **How do I design a Neo probe for Southern blot?**

In the Construct section on the left-hand column, select Probe from the drop-down menu and click the **[+]** button. It will take you to the Define Probes page. Enter your Probe Name. Enter the start and end sequences for your Neo probe and press **Get Sequence** button or paste entire Neo probe sequence into the Probe Sequence box. A prompt will ask you to select the strategy for outside 5' or 3' arm or Long Arm (LA) or Short Arm (SA). A Southern report will be provided based on which arm you selected.

Click **Save Probe** button to add your Neo probe. A message will be prompted that it recognizes the probe as a Neo probe. Press **Confirm & Save** to save your probe.

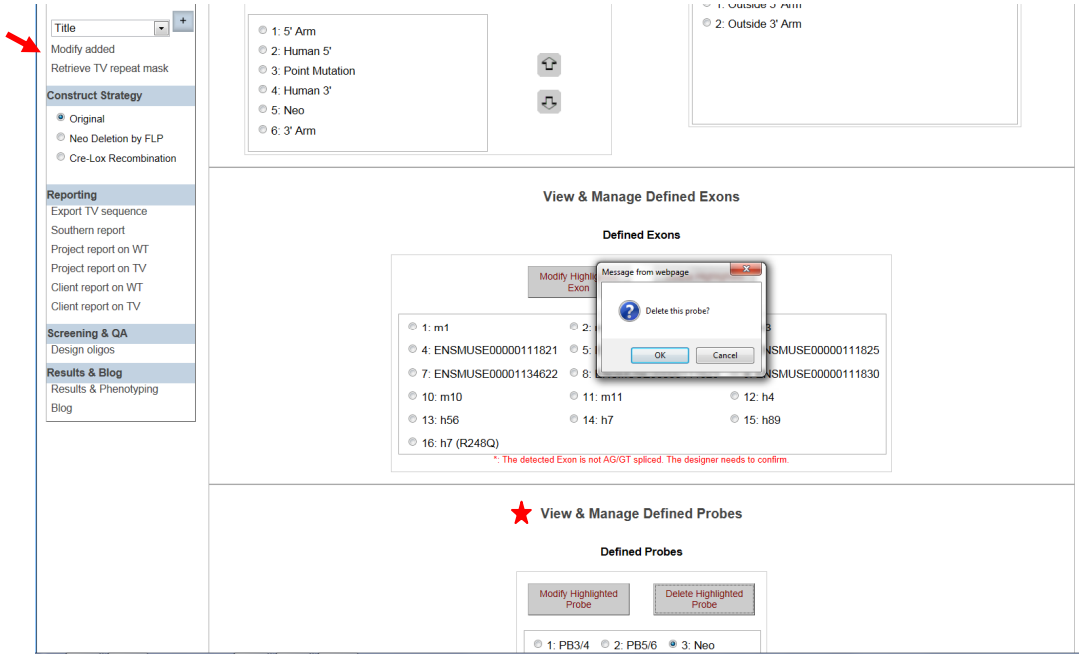


- **How do I modify my probe after I have designed it?**

  In the Construct section on the left-hand column, click **Modify Added**. It will take you to the View & Defined Probes section. Highlight the probe name that you'd like to modify then click **Modify Highlighted Probe** button. It will take you to the Define Probes page. After making changes, click **Save Probe** to update your new probe and **Confirm & Save**.

- **How do I delete my probe after I have entered it?**

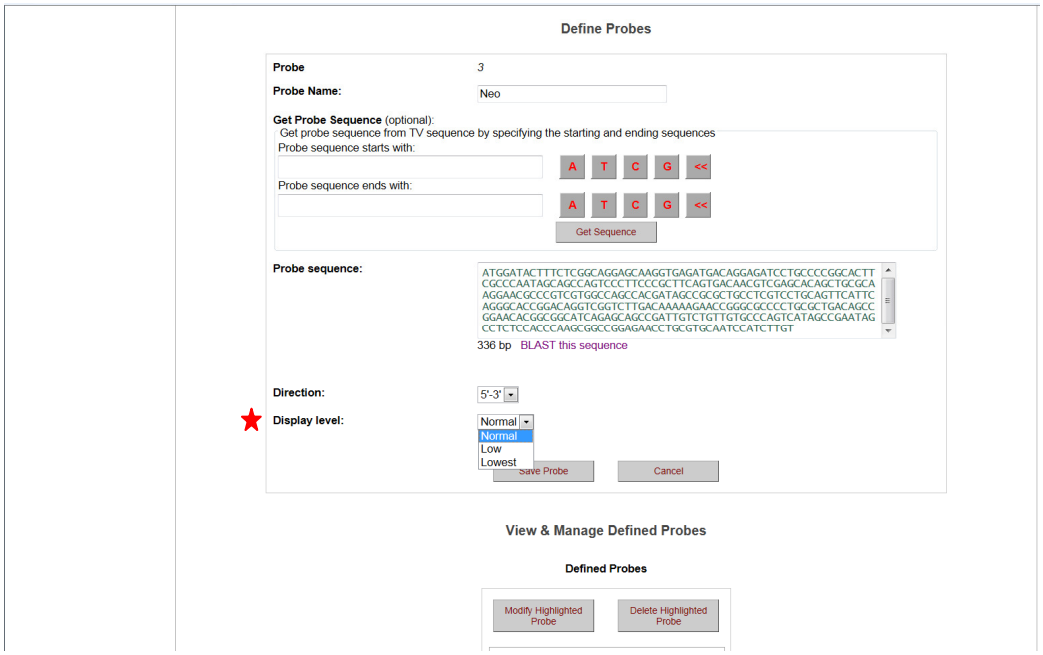
  In the Construct section on the left-hand column, click **Modify Added**. It will take you to the View & Manage Defined Probes section. Click the probe name that you'd like to delete then click **Delete Highlighted Probe** button. A prompt will ask you to confirm. If you click **OK**, your probe will be deleted.



- **How do I change the way my probe displayed on the schematic diagram?**

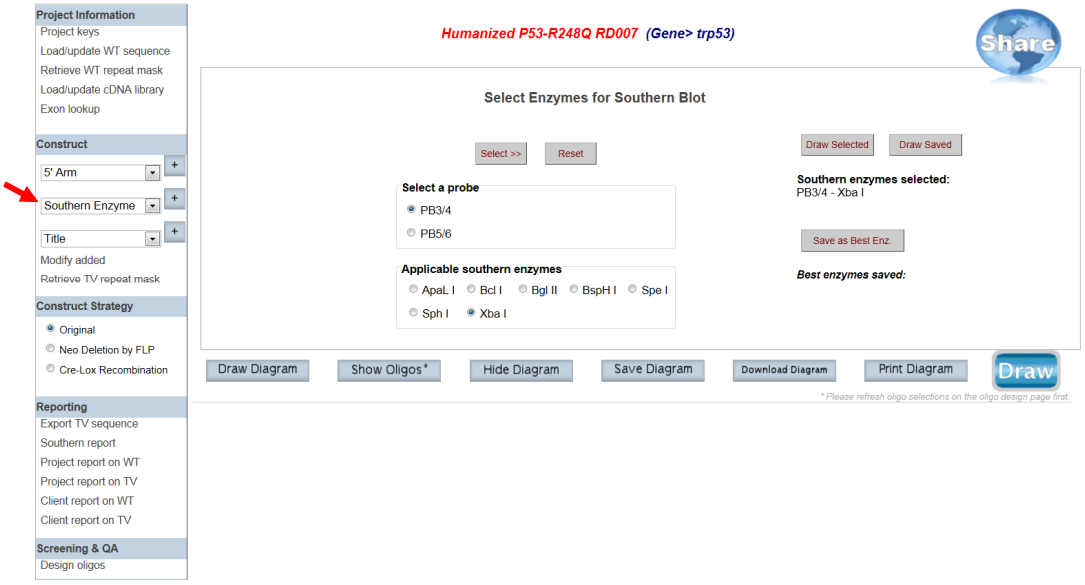
  In the Construct section on the left-hand column, click **Modify Added**. It will take you to the View & Manage Defined Probes section. Click the probe name that you'd like to modify then click **Modify Highlighted Probe** button.

It will take you to the Define Probes page. Click on the drop-down menu for the Display Level and select how your probe will be displayed on the schematic diagram. Then click **Save Probe** to update your change.

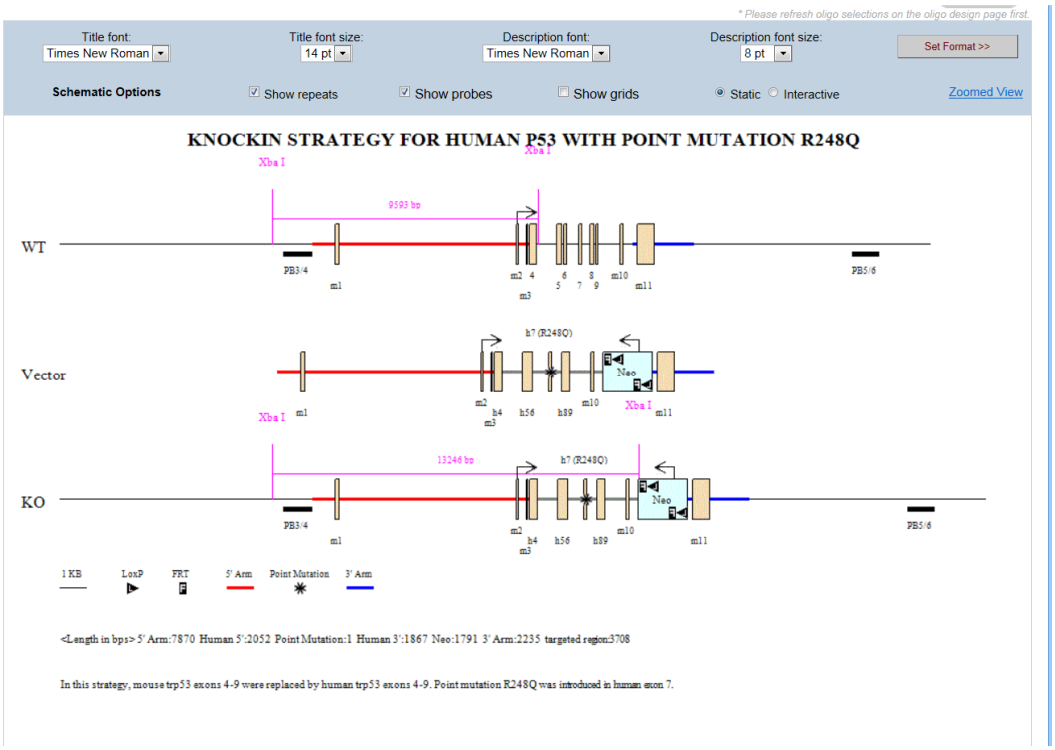


- **How do I display a Southern strategy of a specified probe and enzyme on the schematic diagram?**

  In the Construct section on the left-hand column, select **Southern Enzyme** from the drop-down menu and click the **[+]** button. It will take you to the Select Enzymes for Southern Blot page. All probes that you have designed and added are displayed. Select your probe of choice. A list of applicable enzymes for your specified probe is displayed below. Click your enzyme of choice and press the **Select** button. Your selected enzyme is displayed on the right side.



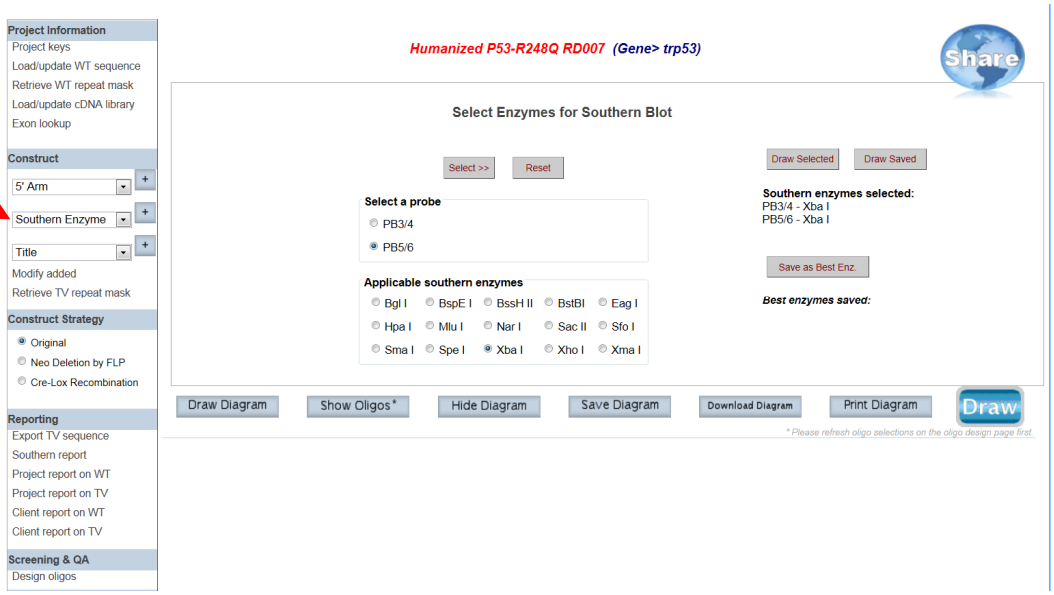
Click **Draw** or **Draw Diagram**. A schematic diagram is displayed with the specified probe, the selected enzyme, and the expected sizes for the WT and KO bands.



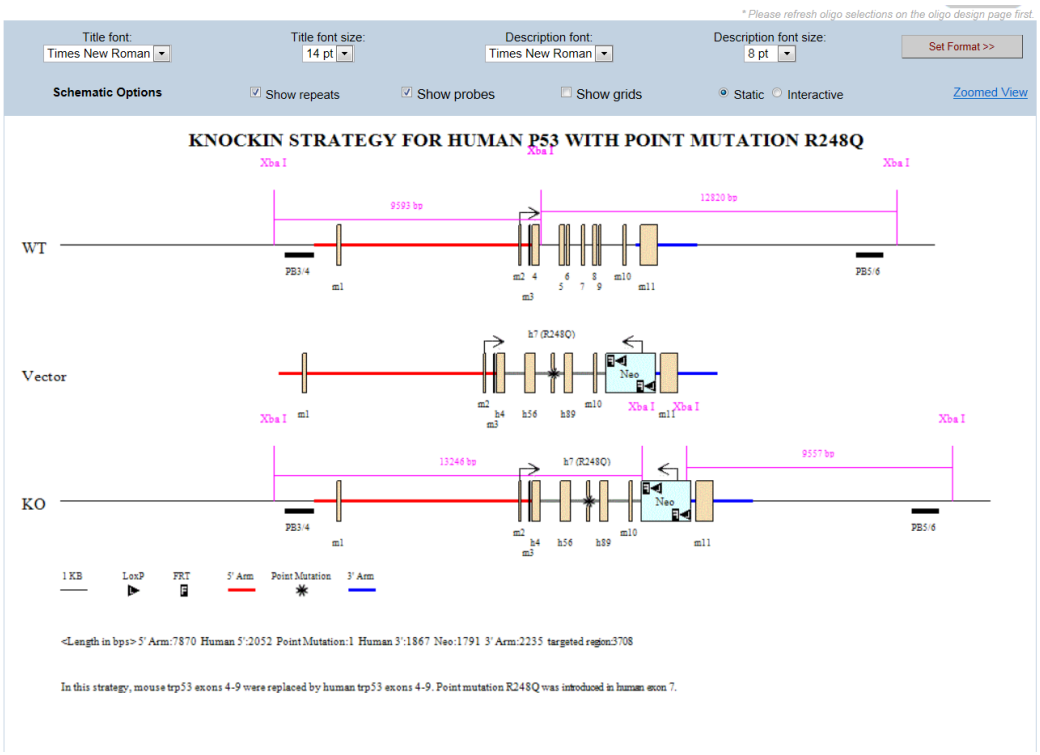
- **How do I display a Southern strategy with two probes and enzymes on the schematic diagram?**

  In the Construct section on the left-hand column, select Southern Enzyme from the drop-down menu and click the **[+]** button. It will take you to the Select Enzymes for Southern Blot page. All probes that you have designed and added are displayed. Select your probe of choice. A list of applicable enzymes for your specified probe is displayed below. Click your enzyme of choice and press the **Select** button. Your selected enzyme is displayed on the right side.

  Select another probe and a list of enzymes is displayed below. Click your enzyme of choice and press the **Select** button. Your selected enzyme is displayed on the right side with the probe name.

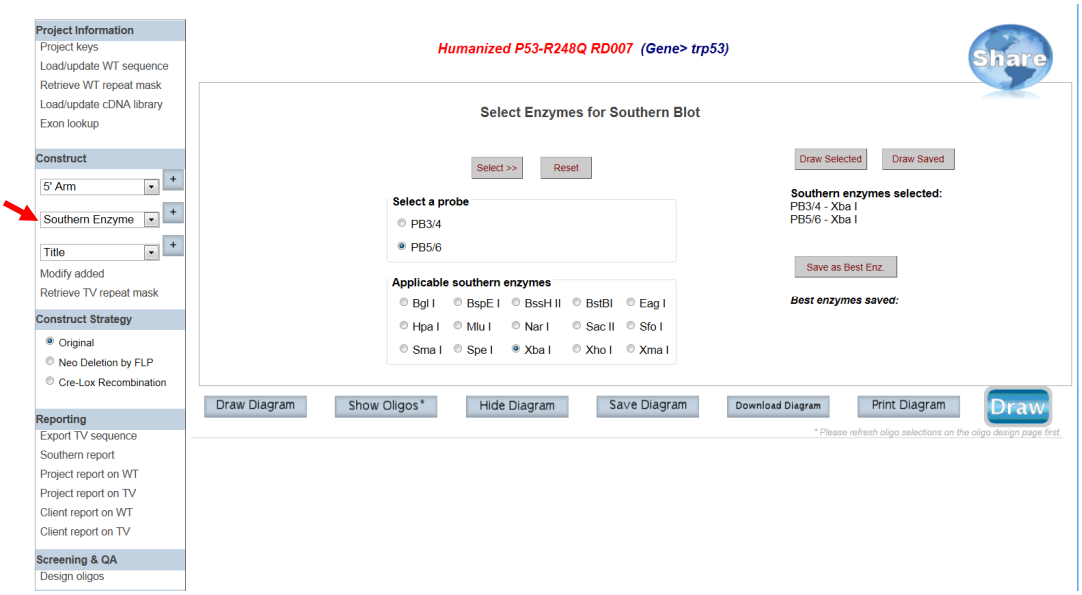


Click **Draw** or **Draw Diagram**. A schematic diagram is displayed with the specified probes, the selected enzymes, and the expected sizes for the WT and KO bands.



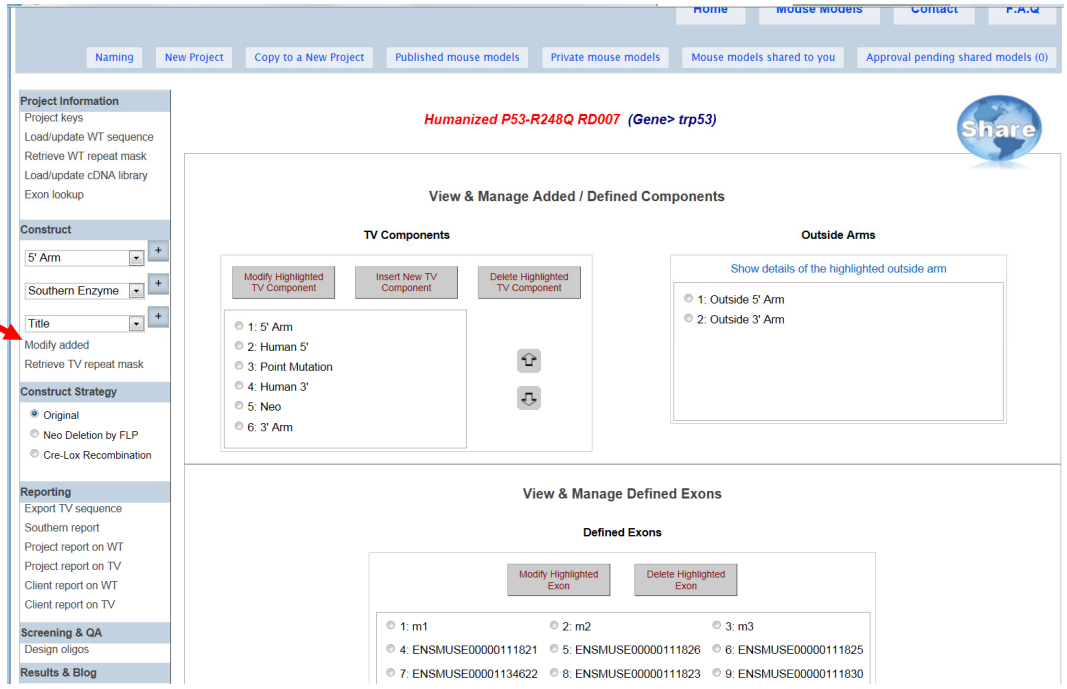
- **How do I remove the selected Southern enzyme from displaying on the schematic diagram?**

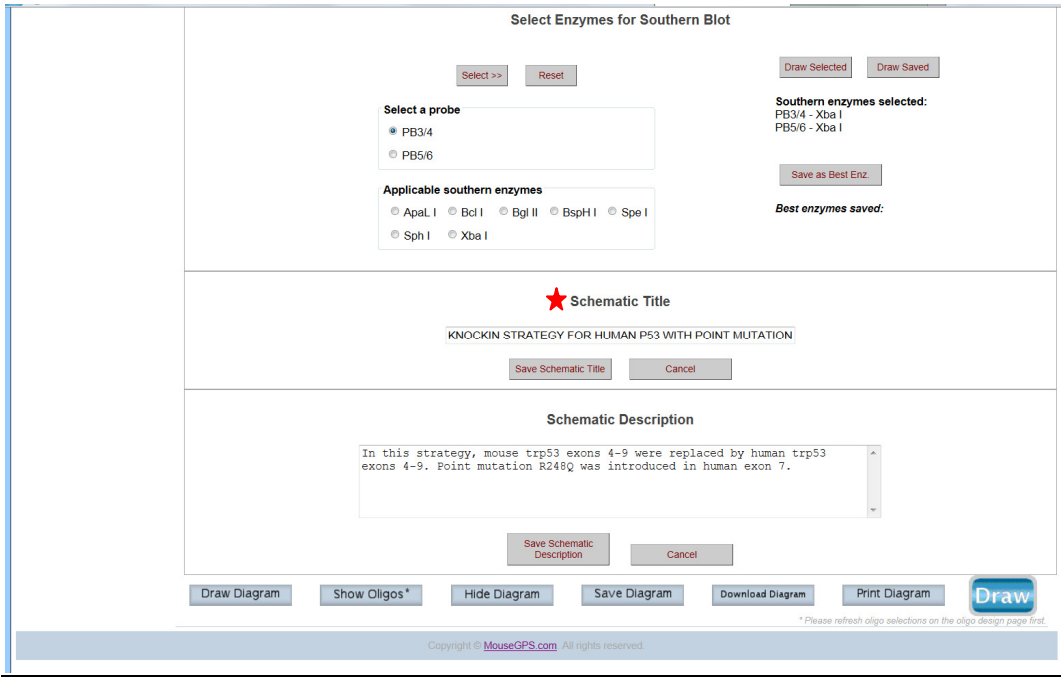
  In the Construct section on the left-hand column, select Southern Enzyme from the drop-down menu and click the **[+]** button. It will take you to the Select Enzymes for Southern Blot page. Click the **Reset** button. The selected enzyme(s) will be removed from the schematic diagram.



- **How do I modify the title on the schematic diagram?**

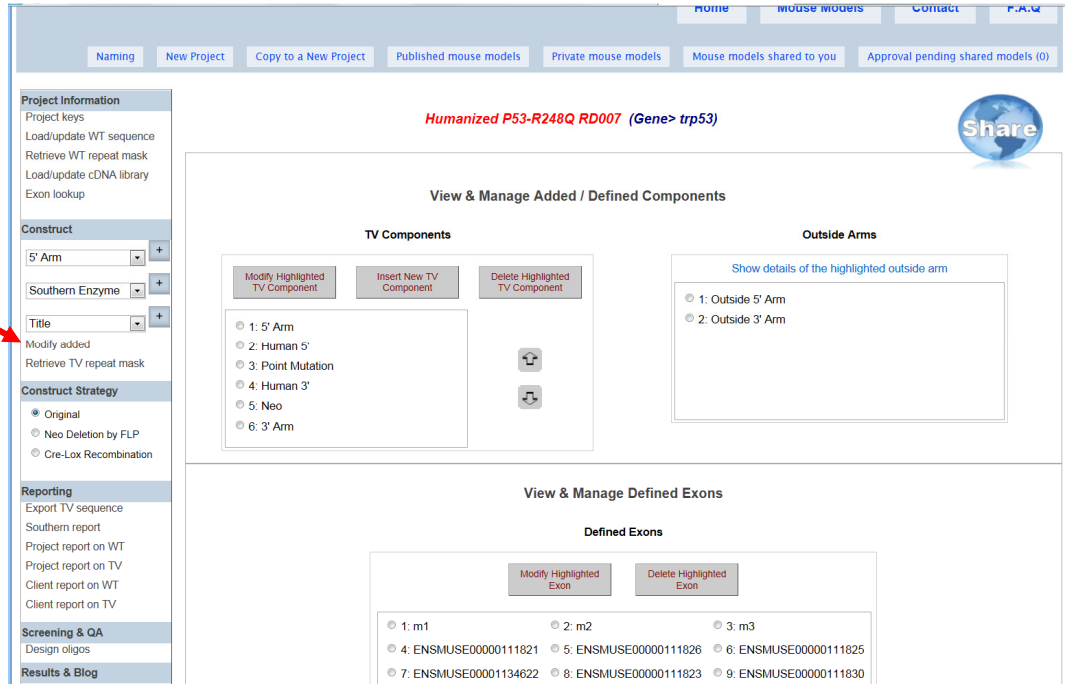
  In the Construct section on the left-hand column, click **Modify Added**. In the box under Schematic Title, type in a new title and then click **Save Schematic Title** button.

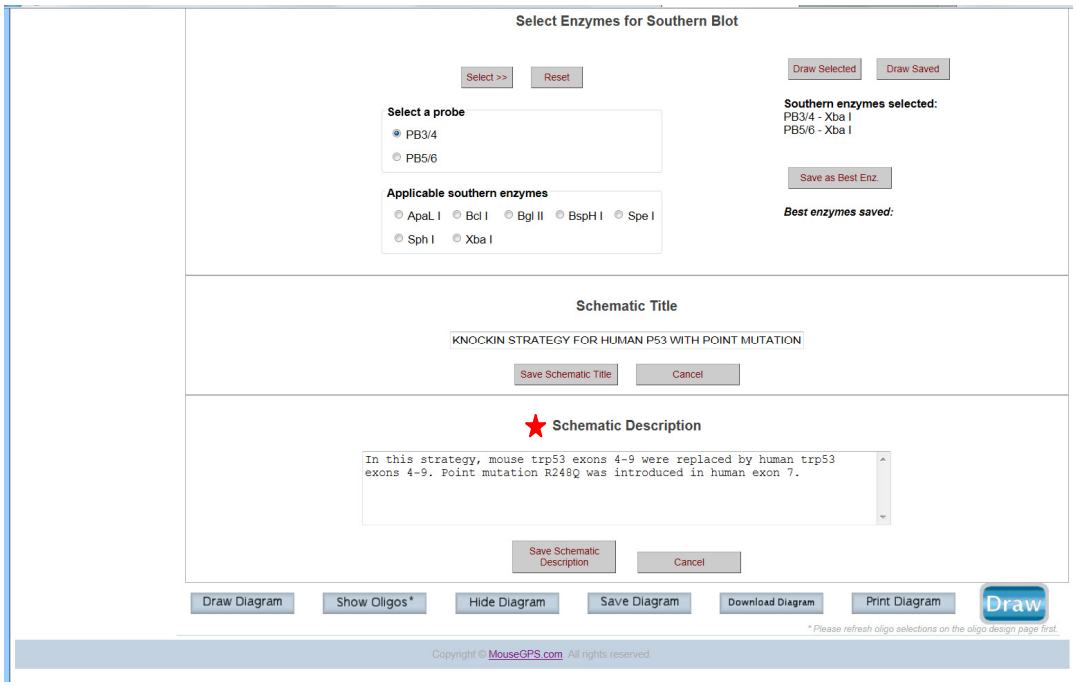




- **How do I modify the description on the schematic diagram?**

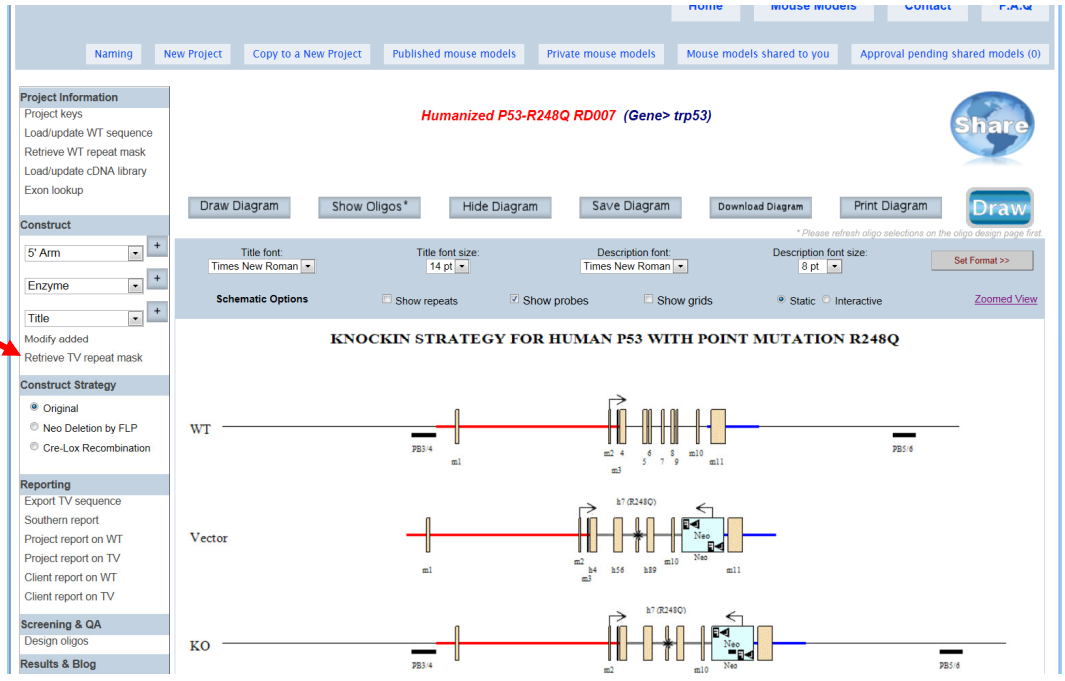
  In the Construct section on the left-hand column, click **Modify Added**. It will take you to the View & Manage Added/Define Components page. In the box under Schematic Description, type in a new description and then click **Save Schematic Description** button.





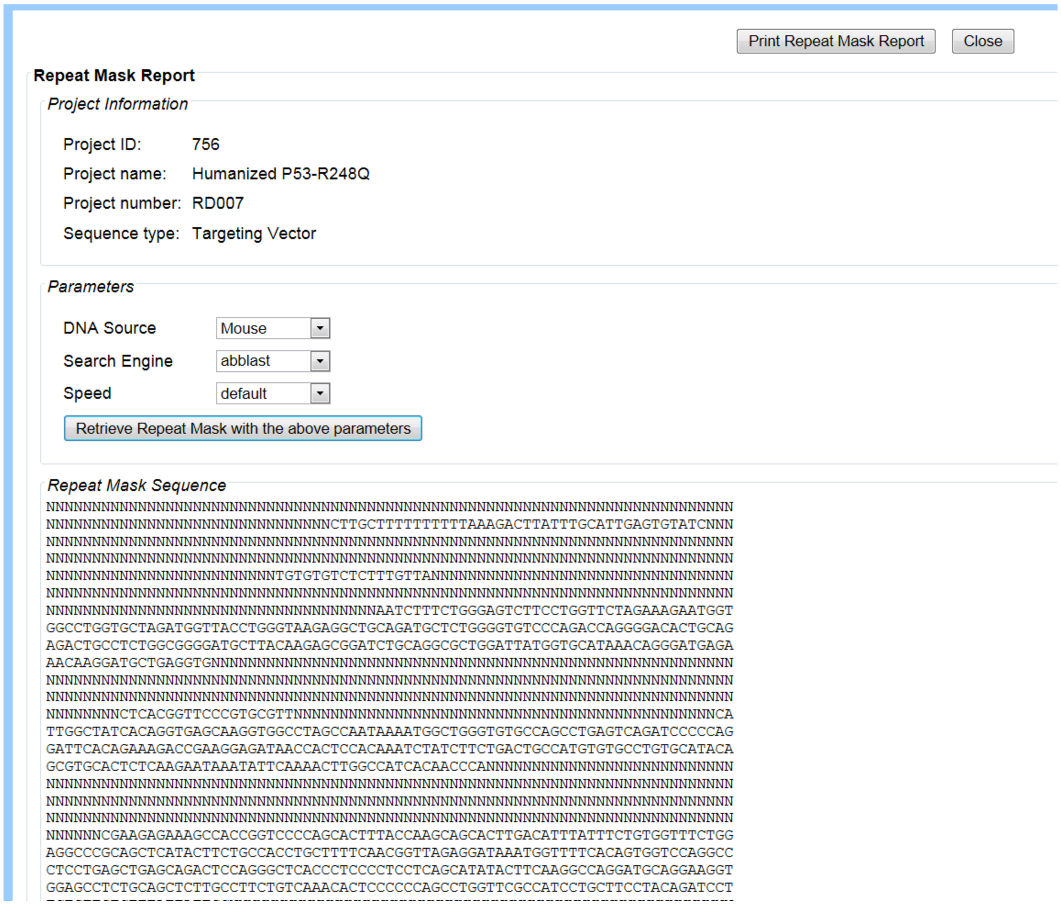
- **How do I retrieve repeat mask for my sequences?**

In the Construct section on the left-hand column, click **Retrieve TV repeat mask**.



Your entire input sequences including the targeting vector and the flanking sequences were sent out to RepeatMasker ((http://repeatmasker.org/), which is a program that

screens DNA sequences for interspersed repeats and low complexity DNA sequences. The Repeat Mask Report shows a detailed annotation repeats, where Ns are representing repeats.


Print Repeat Mask Report | Close

Repeat Mask Report

Project Information

Project ID: 756
Project name: Humanized P53-R248Q
Project number: RD007
Sequence type: Targeting Vector

Parameters

DNA Source: Mouse
Search Engine: abblast
Speed: default

Retrieve Repeat Mask with the above parameters

Repeat Mask Sequence

```
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCTTGCTTTTTTTTTTAAAGACTTATTTGCATTGAGTGTATCNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNTGTGTGTCTCTTTGTTANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAATCTTTCTGGGAGTCTTCCTGGTTCTAGAAAGAATGGT
GGCCTGGTGCTAGATGGTTACCTGGGTAAGAGGCTGCAGATGCTCTGGGGTGTCCCAGACCAGGGGACACTGCAG
AGACTGCCTCTGGCGGGGATGCTTACAAGAGCGGATCTGCAGGCGCTGGATTATGGTGCATAAACAGGGATGAGA
AACAAGGATGCTGAGGTGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNCTCACGGTTCCCGTGCGTTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCA
TTGGCTATCACAGGTGAGCAAGGTGGCCTAGCCAATAAAATGGCTGGGTGTGCCAGCCTGAGTCAGATCCCCCAG
GATTCACAGAAAGACCGAAGGAGATAACCACTCCACAAATCTATCTTCTGACTGCCATGTGTGCCTGTGCATACA
GCGTGCACTCTCAAGAATAAATATTCAAAACTTGGCCATCACAACCCANNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNCGAAGAGAAAGCCACCGGTCCCCAGCACTTTACCAAGCAGCACTTGACATTTATTTCTGTGGTTTCTGG
AGGCCCGCAGCTCATACTTCTGCCACCTGCTTTTCAACGGTTAGAGGATAAATGGTTTTCACAGTGGTCCAGGCC
CTCCTGAGCTGAGCAGACTCCAGGGCTCACCCTCCCCTCCTCAGCATATACTTCAAGGCCAGGATGCAGGAAGGT
GGAGCCTCTGCAGCTCTTGCCTTCTGTCAAACACTCCCCCCAGCCTGGTTCGCCATCCTGCTTCCTACAGATCCT
```

Load/update cDNA library
Exon lookup

Construct

5' Arm
Enzyme
Title
Modify added
Retrieve TV repeat mask

Construct Strategy

- Original
- Neo Deletion by FLP
- Cre-Lox Recombination

Reporting

Export TV sequence
Southern report
Project report on WT
Project report on TV
Client report on WT
Client report on TV

Screening & QA

Design oligos

Results & Blog

Results & Phenotyping
Blog

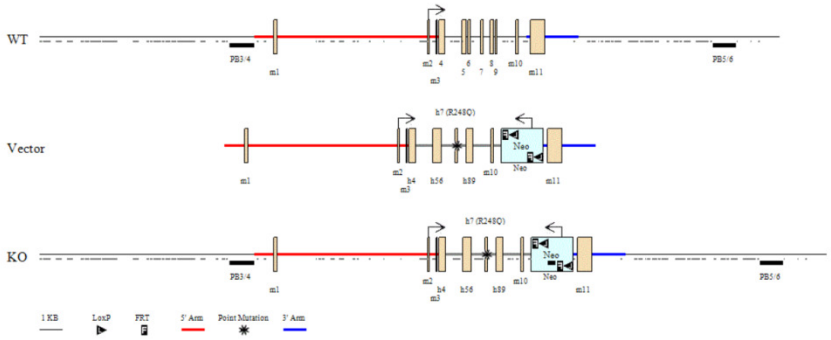
Draw Diagram | Show Oligos* | Hide Diagram | Save Diagram | Download Diagram | Print Diagram | Draw

*Please refresh oligo selections on the oligo design page first.

Title font: Times New Roman | Title font size: 14 pt | Description font: Times New Roman | Description font size: 8 pt | Set Format >>

Schematic Options | Show repeats | Show probes | Show grids | Static | Interactive | Zoomed View

KNOCKIN STRATEGY FOR HUMAN P53 WITH POINT MUTATION R248Q



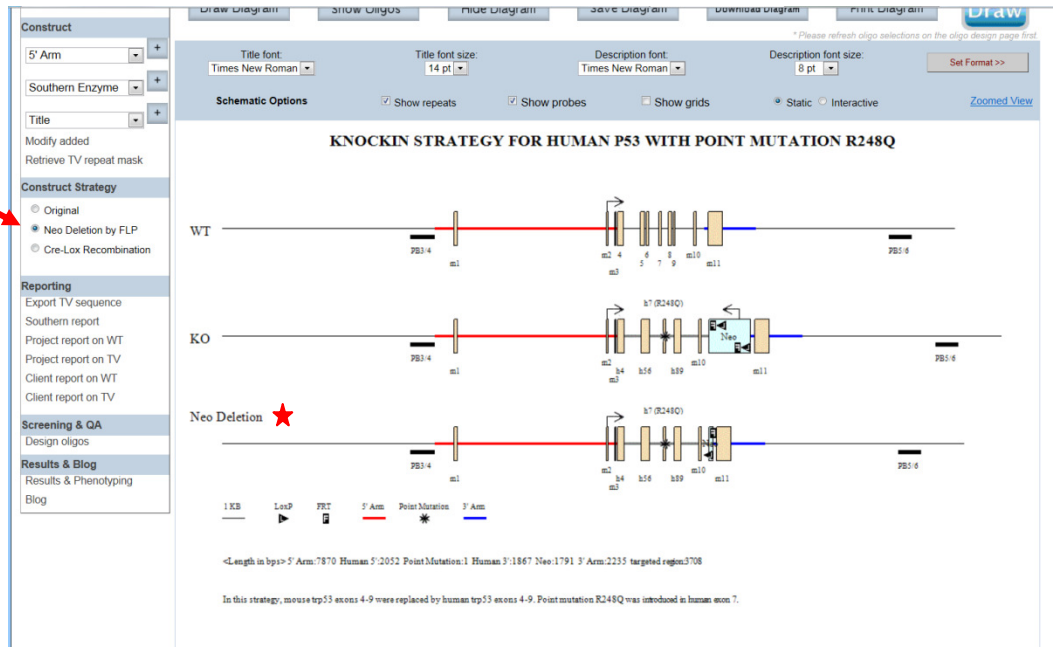
<Length in bps> 5' Arm:7870 Human 5':2052 Point Mutation:1 Human 3':1867 Neo:1791 3' Arm:2235 targeted region:3708

In this strategy, mouse trp53 exons 4-9 were replaced by human trp53 exons 4-9. Point mutation R248Q was introduced in human exon 7.

Construct Strategies

- **How do I display the schematic diagram after FLP recombinase has been introduced?**

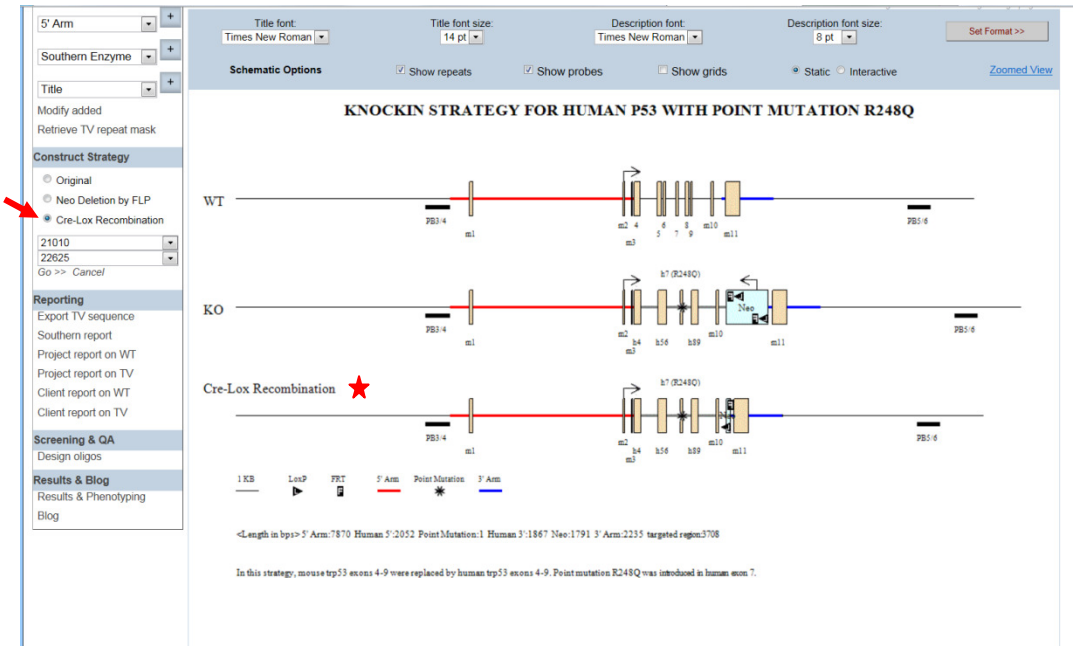
  In the Construct Strategy section on the left-hand column, click on the circle next to **Neo deletion by FLP**. Click **Draw** or **Draw Diagram**. The third line on the schematic diagram (Neo Deletion) displays the remaining components after introducing FLP recombinase.



- **How do I display the schematic diagram after Cre recombinase has been introduced?**

  In the Construct Strategy section on the left-hand column, click on the circle next to **Cre-Lox Recombination**. Select 1st LoxP Site with a drop-down menu of location for all loxP sites is shown. Select the location of the 1st loxP site of your choice. Go to Select 2nd LoxP Site and select the location of the 2nd loxP site of your choice.

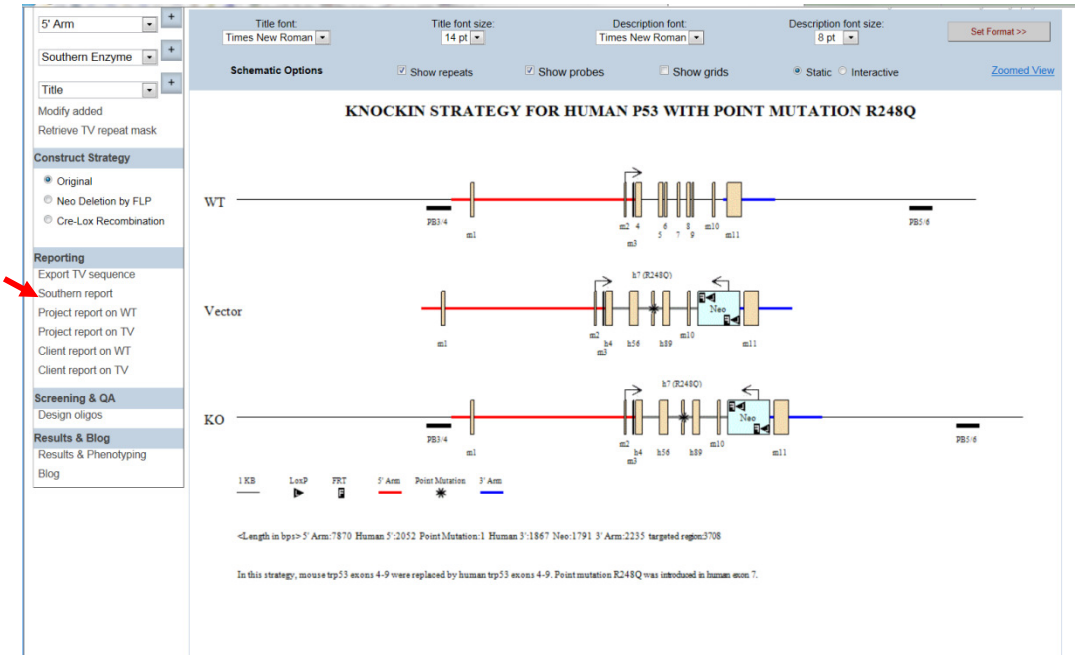
  Click **Go >>** button**.** A schematic diagram is displayed. The third line on the schematic diagram shows the remaining components after introducing Cre recombinase for the selected loxP sites.


5' Arm
Southern Enzyme
Title
Modify added
Retrieve TV repeat mask
Construct Strategy
Original
Neo Deletion by FLP
Cre-Lox Recombination
21010
22625
Go >> Cancel
Reporting
Export TV sequence
Southern report
Project report on WT
Project report on TV
Client report on WT
Client report on TV
Screening & QA
Design oligos
Results & Blog
Results & Phenotyping
Blog
Title font:
Times New Roman
Title font size:
14 pt
Description font:
Times New Roman
Description font size:
8 pt
Set Format >>
Schematic Options
Show repeats
Show probes
Show grids
Static
Interactive
Zoomed View
KNOCKIN STRATEGY FOR HUMAN P53 WITH POINT MUTATION R248Q
WT
KO
Cre-Lox Recombination
h7 (R248Q)
Neo
PB3/4
PB5/6
m1
m2
m3
m4
m5
m6
m7
m8
m9
m10
m11
h4
h5/6
h8/9
1 KB
LoxP
FRT
5' Arm
Point Mutation
3' Arm
<Length in bps> 5' Arm:7870 Human 5':2052 Point Mutation:1 Human 3':1867 Neo:1791 3' Arm:2235 targeted region:3708
In this strategy, mouse trp53 exons 4-9 were replaced by human trp53 exons 4-9. Point mutation R248Q was introduced in human exon 7.

Reporting

- **How do I view possible Southern strategies including enzymes and expected sizes for a specific probe?**

  In the Reporting section on the left-hand column, select **Southern report**. A list of all probes that you have designed is displayed in the report including Probe Name, possible enzymes, and expected sizes for the WT and KO. The enzymes that are highlighted in red are not recommended to use.



- **What is the difference between Project Report and Client Report?**

  Project Report displays sequences for the forward and reverse strands for your project of interest. Client Report displays only sequences for the forward strand (5'->3') for your project of interest. Sites of restriction enzymes are indicated only in the Project Report. 5' and 3' retrieved homology arms, insertion sites of vector components, locations of oligos, probes, exons, repeats, etc. are annotated in each report.

  Note if the sequence is >50kb, the report will not be displayed especially for BAC targeting constructs.

- **What is the difference between WT and TV on Project Report and Client Report?**

  WT displays wild-type genomic sequences before modifications. 5' and 3' retrieved homology arms and locations of probes, exons, and repeats are indicated. TV displays sequences after modifications including annotated sequences of 5' and 3' retrieved homology arms, vector components, selection cassette, locations of oligos, probes, exons, repeats, etc.

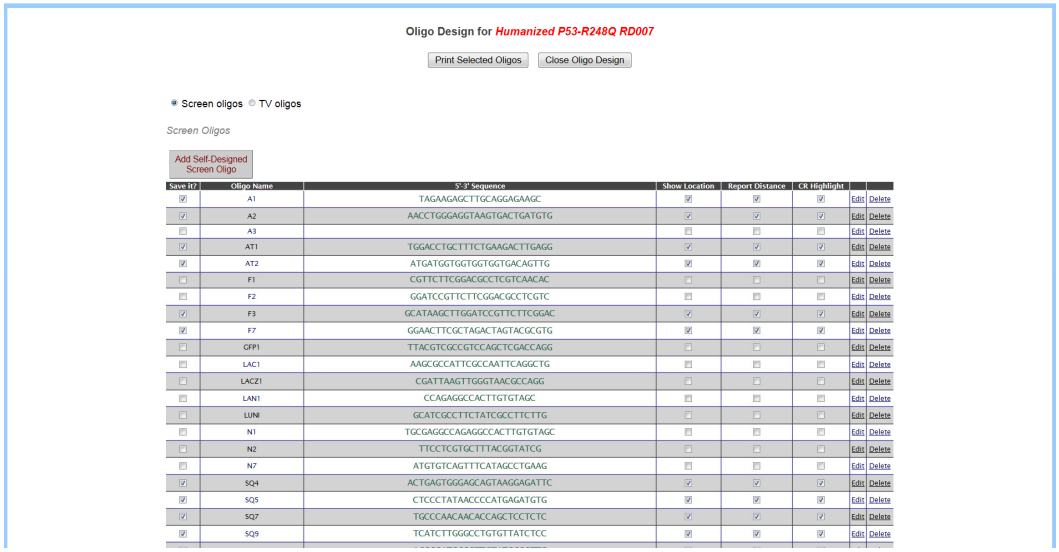
  Note if the sequence is >50kb, the report will not be displayed especially for BAC targeting constructs.

Screening & QA

- **What is on the Design Oligos page?**

  Note that projects that are shared as View Only will not be able to design new oligos.

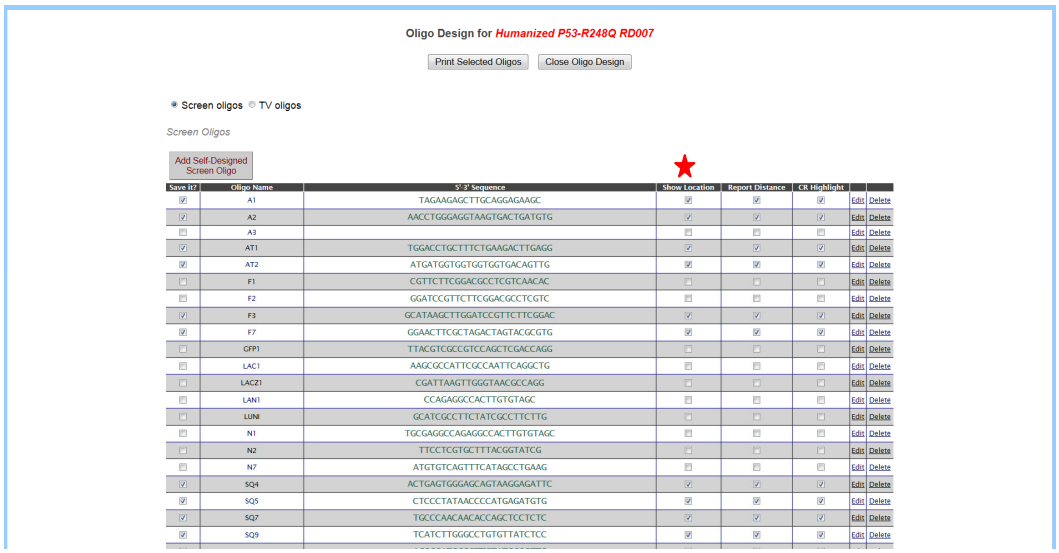
  In the Screening & QA section on the left-hand column, click **Design Oligos**. It will bring you to the Oligo Design page for Screen Oligos. All PCR screening oligos specifically designed for your project are displayed. The Oligo Design page displays the oligo name, its sequences (5'->3'), location of oligo on the forward/reverse strand, and expected sizes of oligo pairings.



- **How do I display oligos on the schematic diagram?**

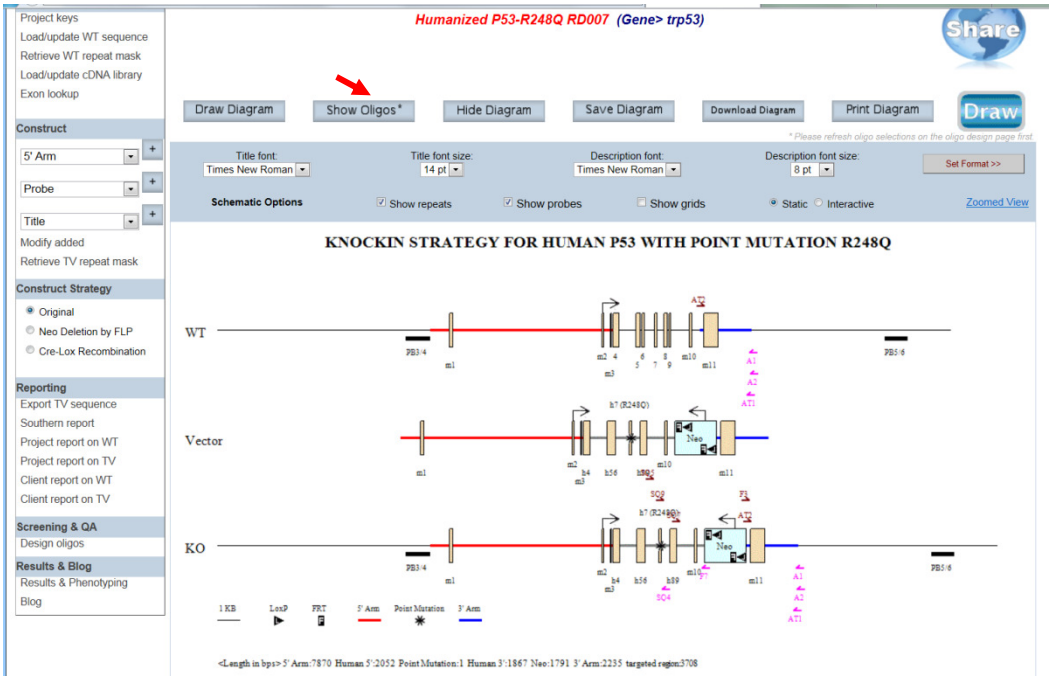
  In the Screening & QC section on the left-hand column, click **Design Oligos**. It will bring you to the Oligo Design page for Screen Oligos. PCR screening oligos designed specifically for your project and generic oligos are displayed.

  To view oligos on the schematic diagram, select the oligo of your choice and check off the box in the column **Show Location** as shown below in red asterisk.



Press **Select Oligos** button to save your changes. **Close Oligo Design** page.

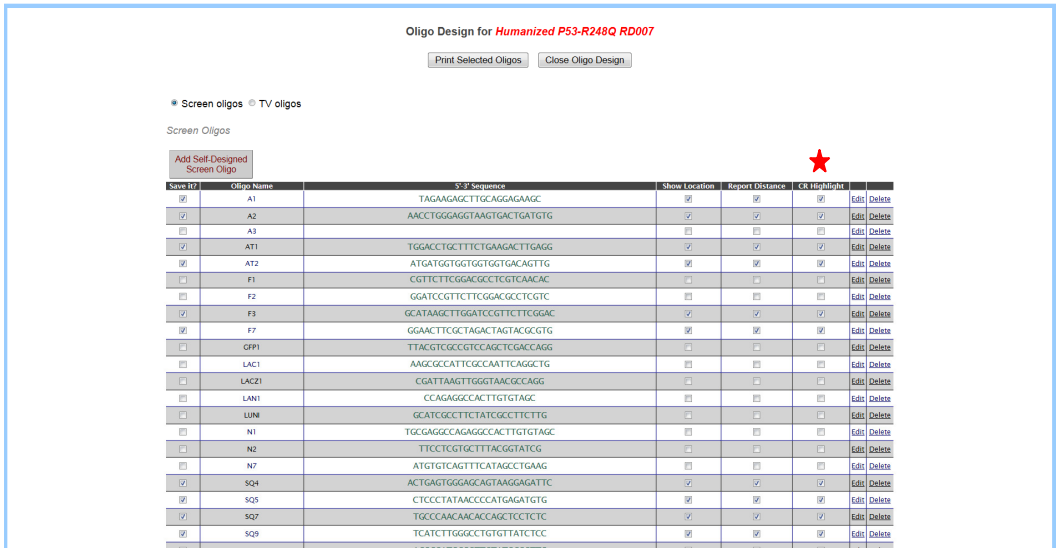
To view oligos on the schematic diagram, click **Show Oligos** button in the top panel.



- **How do I display oligos on the Client Report?**

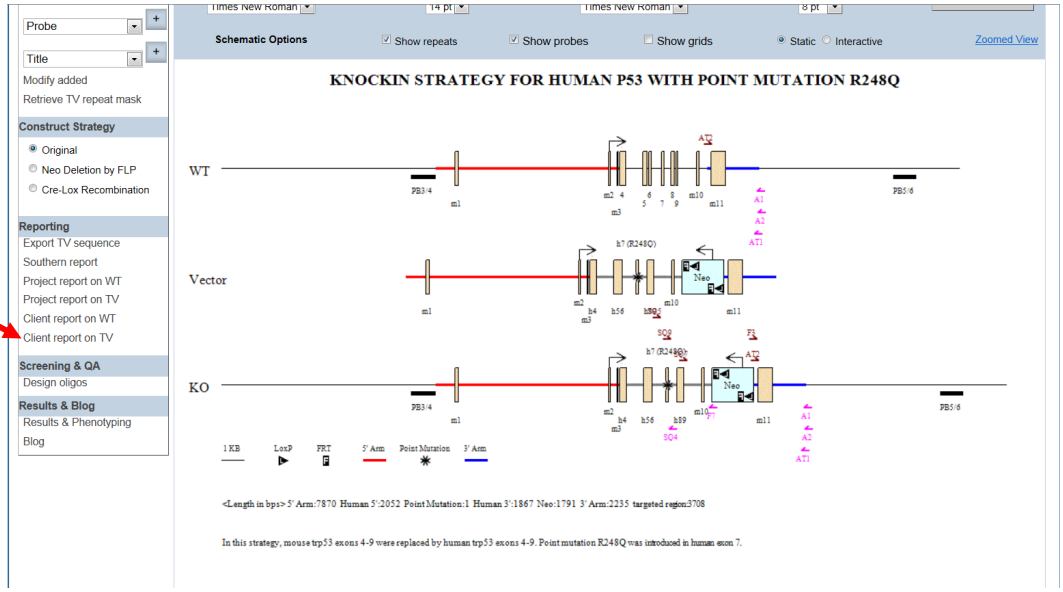
In the Screening & QC section on the left-hand column, click **Design Oligos**. It will bring you to the Oligo Design page for Screen Oligos. PCR screening oligos designed specifically for your project and generic oligos are displayed.

To view oligos on the client report, select the oligo of your choice and check off the box in the column **CR Highlight** as shown below in red asterisk.



Press **Select Oligos** button to save your changes. **Close Oligo Design** page.

To view oligos on the Client Report on TV, click **Client Report on TV** button under Reporting section.



Below is a display of Client report on TV. Forward oligos (5'-3') are highlighted in orange and reverse oligos (3'-5') are highlighted in magenta.

Formatting convention:

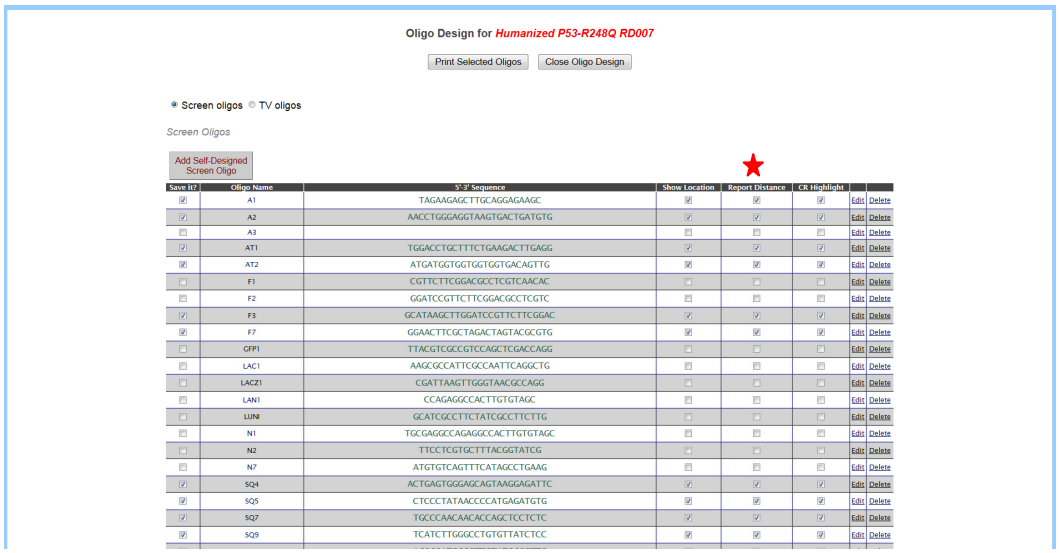
1. Outside 5' Arm (9142 bp): Plain text (e.g., ATCG)
2. Outside 3' Arm (8559 bp): Plain text (e.g., ATCG)
3. 5' Arm (7870 bp): Bold and italic text (e.g., ***ATCG***)
4. Self-Designed (2052 bp): Blue text, (e.g., ATCG)
5. Point Mutation (1 bp): Red, bold and italic text (e.g., ***ATCG***)
6. Self-Designed (1867 bp): Blue text, (e.g., ATCG)
7. Neo (1791 bp): Red text (e.g., ATCG)
8. 3' Arm (2235 bp): Underlined text (e.g., ATCG)
9. LoxP: Red text, highlighted in yellow (e.g., ATCG)
10. FRT: Red and underlined text, highlighted in gray (e.g., ATCG)
11. Exon: Highlighted in pink (e.g., ATCG)
12. Probe: Highlighted in sandy brown (e.g., ATCG)
13. Oligo (5\'-3\'): Highlighted in orange (e.g., ATCG)
14. Oligo (3\'-5\'): Highlighted in magenta (e.g., ATCG)

CCTGGAACTCGATTTATAGAGCAGGCTGACCTCAAACTCAAAGAGATCCACCTGCCTCTGCCTCCCAAG
TGCTGGGGCTAAAGGTGTGCACCACCACCGCCCAGCTCTTGCTTTTTTTTTTAAAGACTTATTTGCATT
GAGTGTATCTGTGTCTGTACGTCTGTACATACAAGTGTGGGTGCCTACAGAGGCCAGAAGAGTTGAATC
CCTAGGAGCTGGACTGACAGGGAACCATGAGCTGCCTGACACGGGCACAGGAATGGAGCTTAGGTCTTC
TGCAGGAGAACAGTCCATGCTCTGAACCGCTGAGCCATCTCTCCAGCCCTGTGTGTCTCTTTGTTATTG
TTGTTGTTGAGGCAGTCTCGGTATCCCTGGCTGGCGTGGGACTCACTATGTACCAGATCACAGACAGGT
GTCAGTCCTATGGTGGCTCTGTGTTGTCTCACACATACCGAGATTACAATTCGAGTGCCATCTTGACTG
GCTAATCTTTCTGGGAGTCTTCCTGGTTCTAGAAAGAATGGTGGCCTGGTGCTAGATGGTTACCTGGGT
AAGAGGCTGCAGATGCTCTGGGGTGTCCCAGACCAGGGGACACTGCAGAGACTGCCTCTGGCGGGGATG
CTTACAAGAGCGGATCTGCAGGCGCTGGATTATGGTGCATAAACAGGGATGAGAAACAAGGATGCTGAG
GTGTGGCTGCTTTCCTCACTGCTGTGATAAGTGTCCTACACAGCGACTGAAGGGAGCGTTACTTTTGGT
CACAGTTCGAGGGTGTAGTCCACCACTGCAGGGAAGTCAGAGCAGAAGTACTTTATCCTCAGGCCAGAA
GTCAGAGGTGTGGGAAGCTCATACTCTCCCCACTTTTATCTTTTTATCCAGCTCAGAACCCACACCCGT
AGAGAGGGAATCTCACGGTTCCCGTGCGTTCCCTGACAGACACAACAGGTTTATCTCCTGGGTGATTCT
AGACCAGCATTGGCTATCACAGGTGAGCAAGGTGGCCTAGCCAATAAAATGGCTGGGTGTGCCAGCCTG
AGTCAGATCCCCCAGGATTCACAGAAAGACCGAAGGAGATAACCACTCCACAAATCTATCTTCTGACTG
CCATGTGTGCCTGTGCATACAGCGTGCACTCTCAAGAATAAATATTCAAAACTTGGCCATCACAACCCA
CTGGGAATGTGGCTCCAGTGCCGTGCTTGTCTAGCATGCATGAAGCCTTATGTTCACACACATACACAC
ACACACACACACACACACACACACACAGAGGCAAAAAGAGGAGAAAAGAATGTAAGAGAATACGATTCT
AGTGTGGTGGCTCGTACCTAAAATCTCAGTATTTGGGAGTTCAAGGCCAGTCTCGGGTATGTAGTGAGC
TTCAGGCCACTCTGAGCTGCAGAATGAGACCTTGTCTAACTAACAAGCAAACGAAGAGAAAGCCACCGG

- **How do I find the expected size for PCR amplification for an oligo or primer pair?**

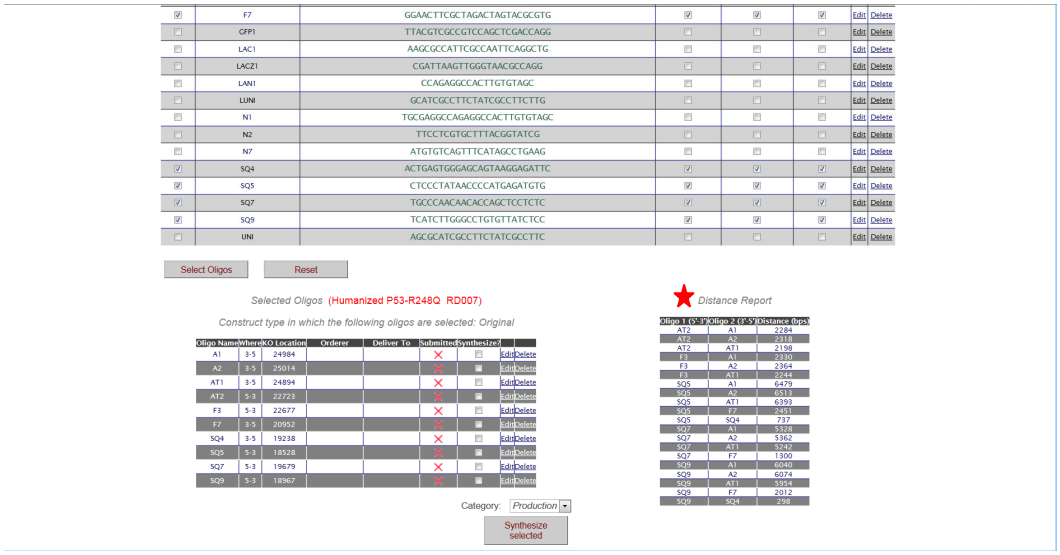
  In the Screening & QC section on the left-hand column, click **Design Oligos**. It will bring you to the Oligo Design page for Screen Oligos.

  To find the expected size of the oligo or primer pair, select the oligo of your choice and check off the box in the column **Report Distance** as shown below in red asterisk.



Press **Select Oligos** button to save your changes.

In the lower right-hand corner, a Distance Report section displays all possible pairings of oligos that were checked off for Report Distance. Oligo 1 column is for all forward oligos (5'->3') and Oligo 2 column is for all reverse oligos (3'->5'). The Distance column displays an expected size in base pairs from Oligo 1 to Oligo 2. This information will provide you the expected size if Oligo 1 and Oligo 2 were used for pairing in PCR amplification.

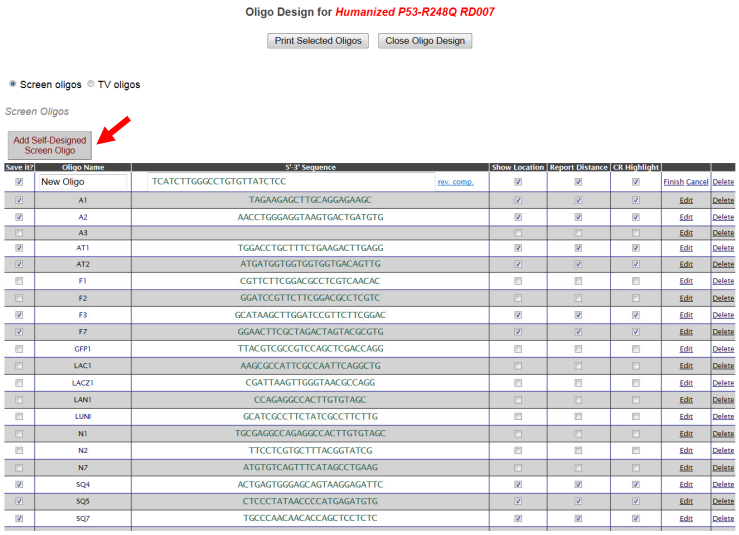


- **How do I design my own PCR oligo or primer?**

In the Screening & QC section on the left-hand column, click **Design Oligos**. An Oligo Design page for Screen Oligos will be displayed. Click **Add Self-Designed Screen**

Oligo. A blank entry will be created on top of the box. Click **Edit** to enter your Oligo Name and its sequence (5'->3'). Check off the boxes for **Show Location**, **Report Distance**, and **CR Highlight**. When done, click **Finish** or **Cancel** if you decide to cancel the entry. Click **Delete** if you would like to delete the entry. Click **Select Oligos** button below the box to select your new entry.

An example of a newly designed oligo is shown below.



Your New Oligo is displayed in the Selected Oligos box. It will show your oligo name, the direction of your oligo (5'->3' for forward; 3'->5' for reverse), and the location on the KO alelle. Note that if your oligo sequence is incorrectly entered, instead of displaying the direction of the oligo, NA will be displayed in the Where column next to your Oligo Name. Ordering oligos is not functional for this version.

Below is a correct oligo sequence that was entered.

Selected Oligos (Humanized P53-R248Q RD007)

Construct type in which the following oligos are selected: Original

Oligo Name	Where	KO Location	Orderer	Deliver To	Submitted	Synthesize?		
A1	3-5	24984			✗	☐	Edit	Delete
A2	3-5	25014			✗	☐	Edit	Delete
AT1	3-5	24894			✗	☐	Edit	Delete
AT2	5-3	22723			✗	☐	Edit	Delete
F3	5-3	22677			✗	☐	Edit	Delete
F7	3-5	20952			✗	☐	Edit	Delete
★ New Oligo	5-3	18967			✗	☐	Edit	Delete
SQ4	3-5	19238			✗	☐	Edit	Delete
SQ5	5-3	18528			✗	☐	Edit	Delete
SQ7	5-3	19679			✗	☐	Edit	Delete
SQ9	5-3	18967			✗	☐	Edit	Delete

Category: *Production*

Synthesize selected

Below is an incorrect oligo sequence that was entered. The incorrect oligo will not be displayed on the schematic diagram, highlighted on the client report, or provided distance report.

Selected Oligos (Humanized P53-R248Q RD007)

Construct type in which the following oligos are selected: Original

Oligo Name	Where	KO Location	Orderer	Deliver To	Submitted	Synthesize?		
A1	3-5	24984			✗	☐	Edit	Delete
A2	3-5	25014			✗	☐	Edit	Delete
AT1	3-5	24894			✗	☐	Edit	Delete
AT2	5-3	22723			✗	☐	Edit	Delete
F3	5-3	22677			✗	☐	Edit	Delete
F7	3-5	20952			✗	☐	Edit	Delete
★ New Oligo	NA	-1			✗	☐	Edit	Delete
SQ4	3-5	19238			✗	☐	Edit	Delete
SQ5	5-3	18528			✗	☐	Edit	Delete
SQ7	5-3	19679			✗	☐	Edit	Delete
SQ9	5-3	18967			✗	☐	Edit	Delete

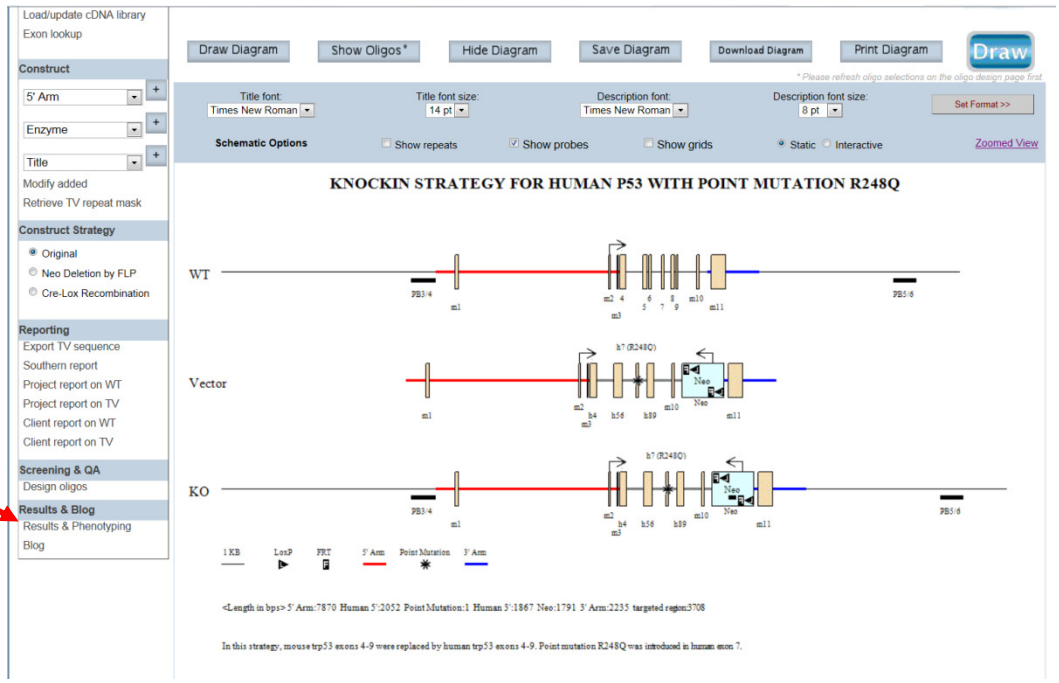
Category: *Production*

Synthesize selected

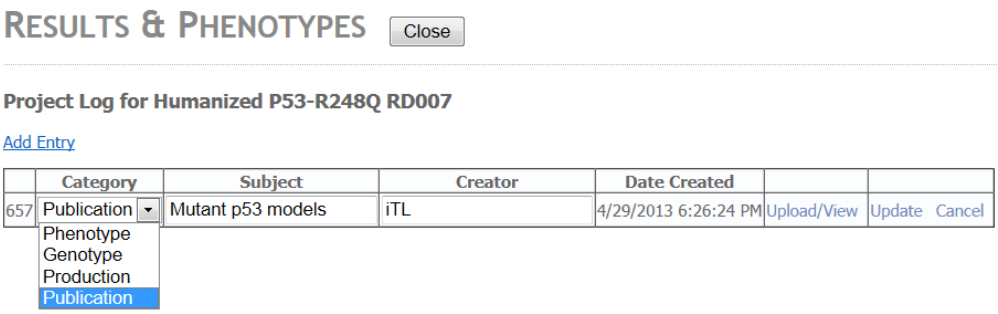
Results & Blog

- **How do I upload a file (i.e. photo, document, publication, etc.) for my project?**

  In the Results & Blog section on the left-hand corner, click **Results & Phenotyping**.



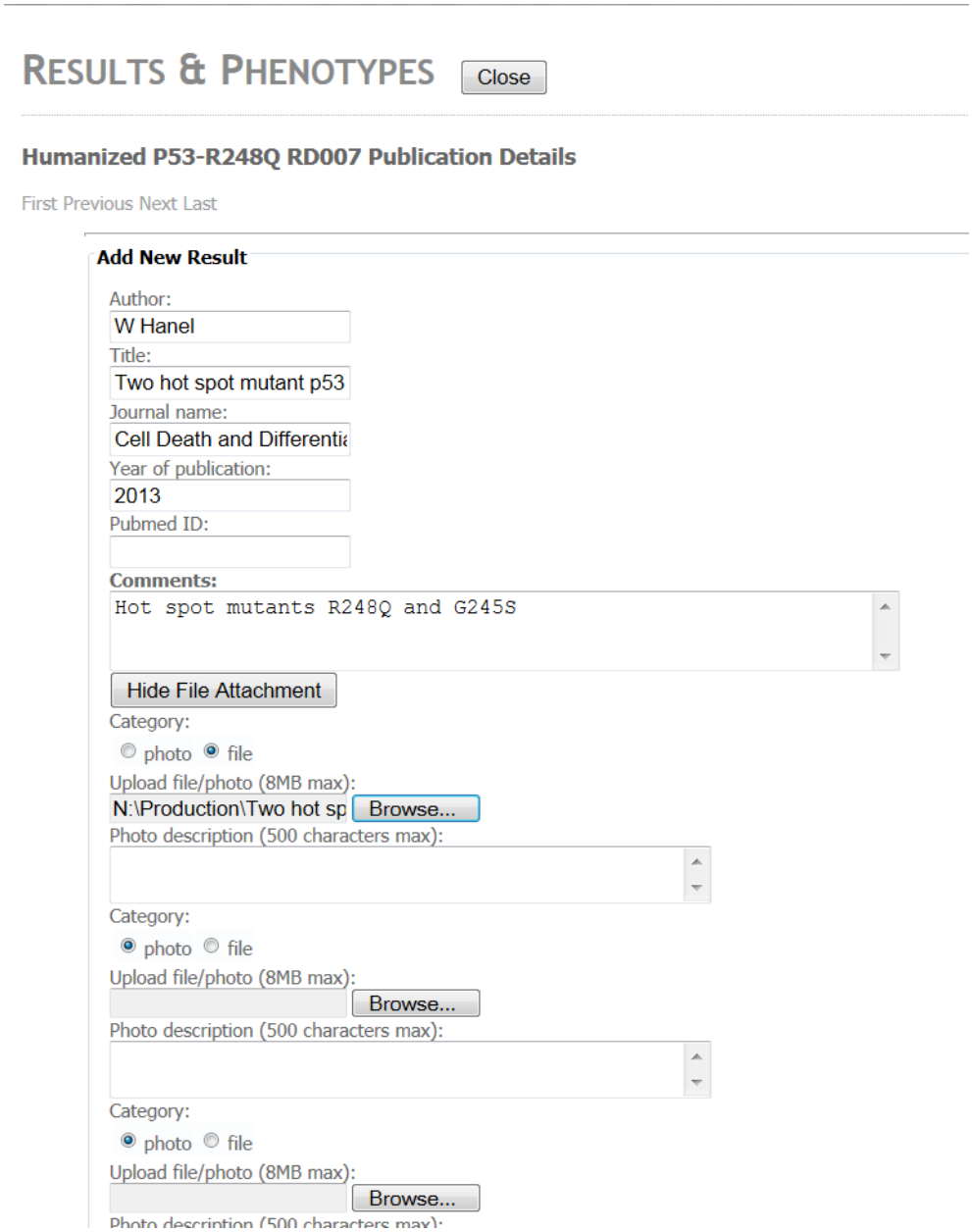
A Project Log for the project will be displayed. Click on **Add Entry**. A new entry is added below. Click on **Edit** to update your entry. Under Category, a drop-down menu of the file type can be selected. Enter the Subject and the name of the Creator for this entry. Click on **Update** and then **Upload/View** to upload your file.



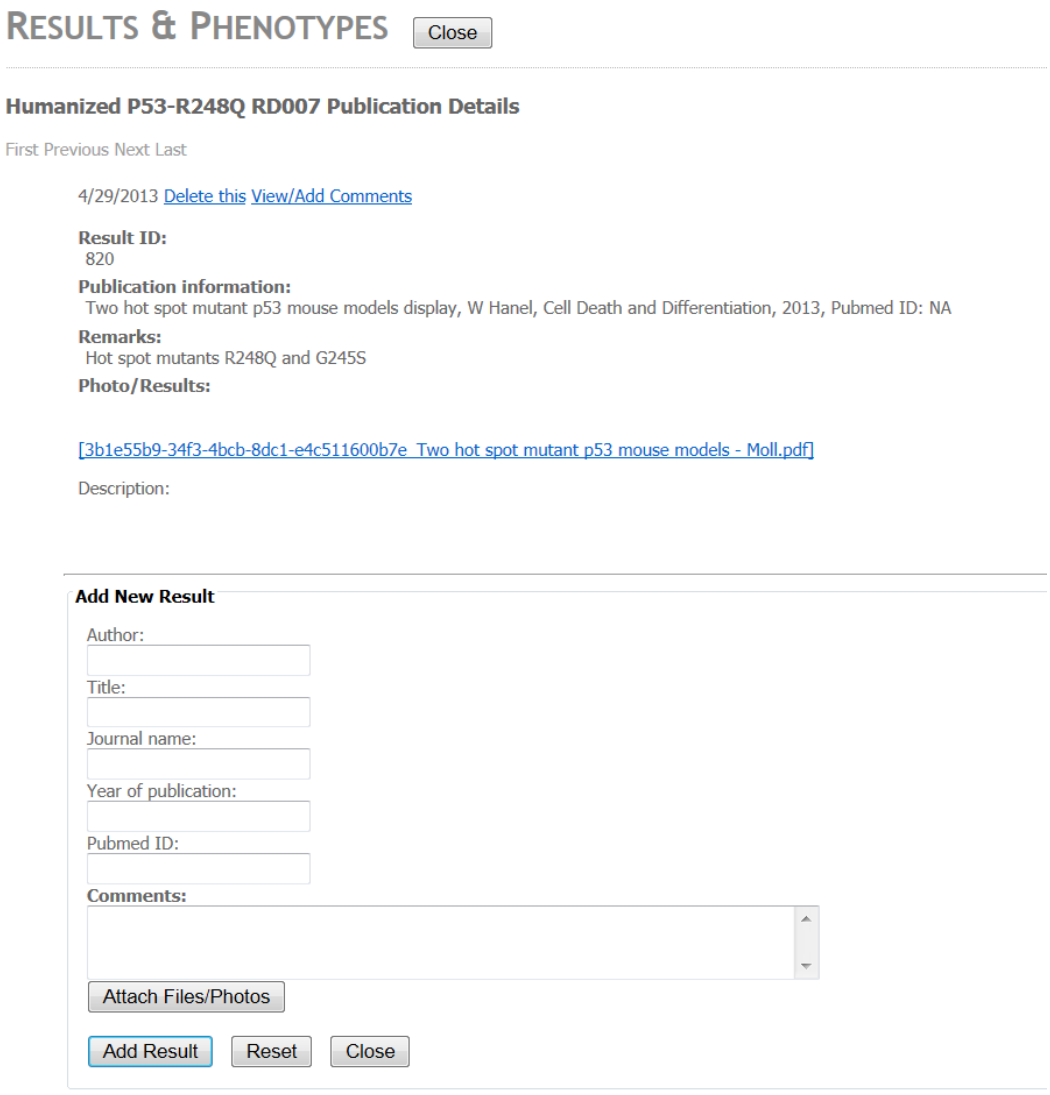
If Publication is selected:

If you selected Publication for Category, the following details for the publication and comments can be entered. To upload the file or photo, click on **Attach Files/Photos**

button. Select the file or photo option. Find your file or photo through **Browse**. Then click on **Add Result** button.

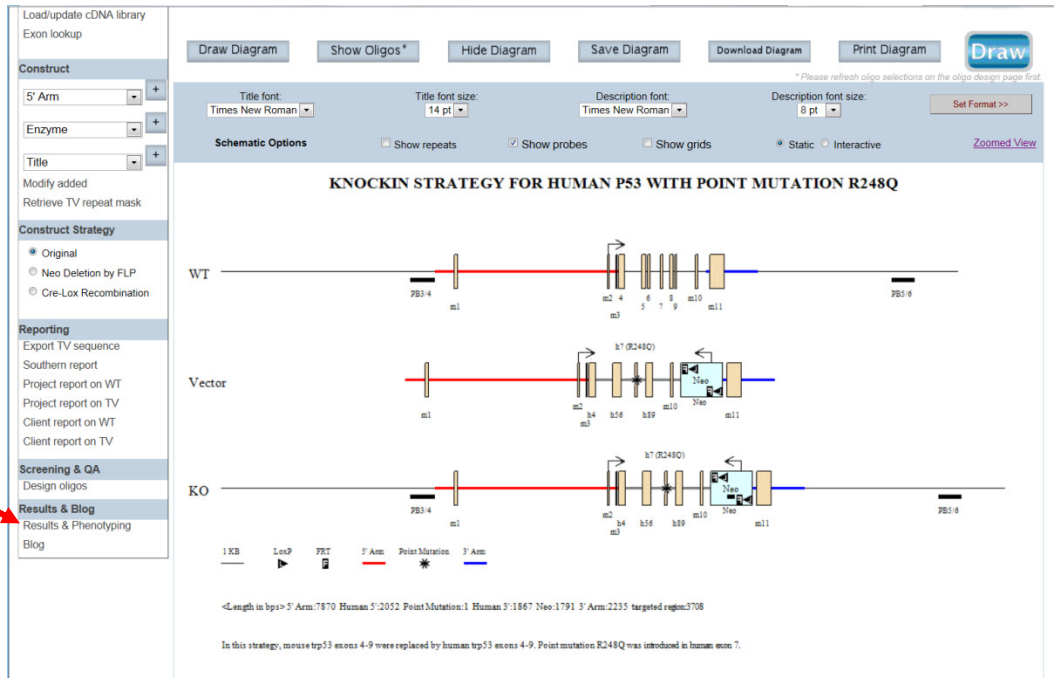


Below is a display of a pdf file uploaded for Publication.

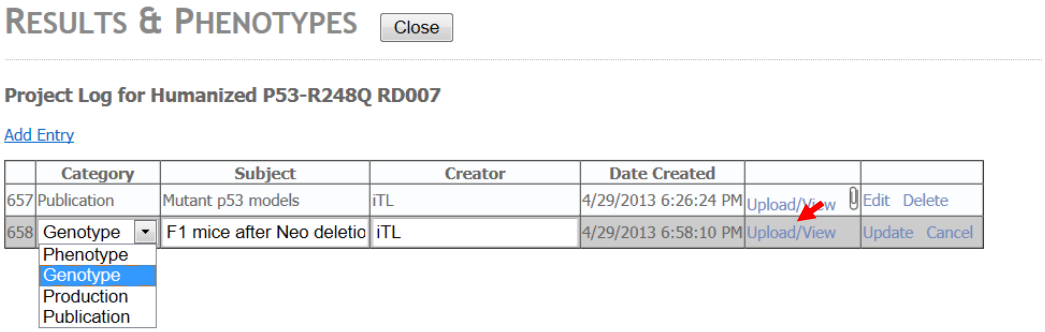


- **How do I add or view comment to my uploaded files?**

  In the Results & Blog section on the left-hand corner, click **Results & Phenotyping**.



A Project Log for the project will be displayed. A project log for project is displayed. Click on **Upload/View** to view your file for a specific entry.



Click on **View/Add Comments** on the entry page for the uploaded file(s). A list of comments will be displayed for that specific entry.

RESULTS & PHENOTYPES Close

Humanized P53-R248Q RD007 Genotype Details

First Previous Next Last

5/13/2013 Delete this View/Add Comments

Result ID:
890

Remarks:
Please review genotyping data.

Photo/Results:

[e61264d0-328d-4d6c-94ea-21b1c7915ff4_p53 Genotyping Document (04-27-2009).doc]

Description:

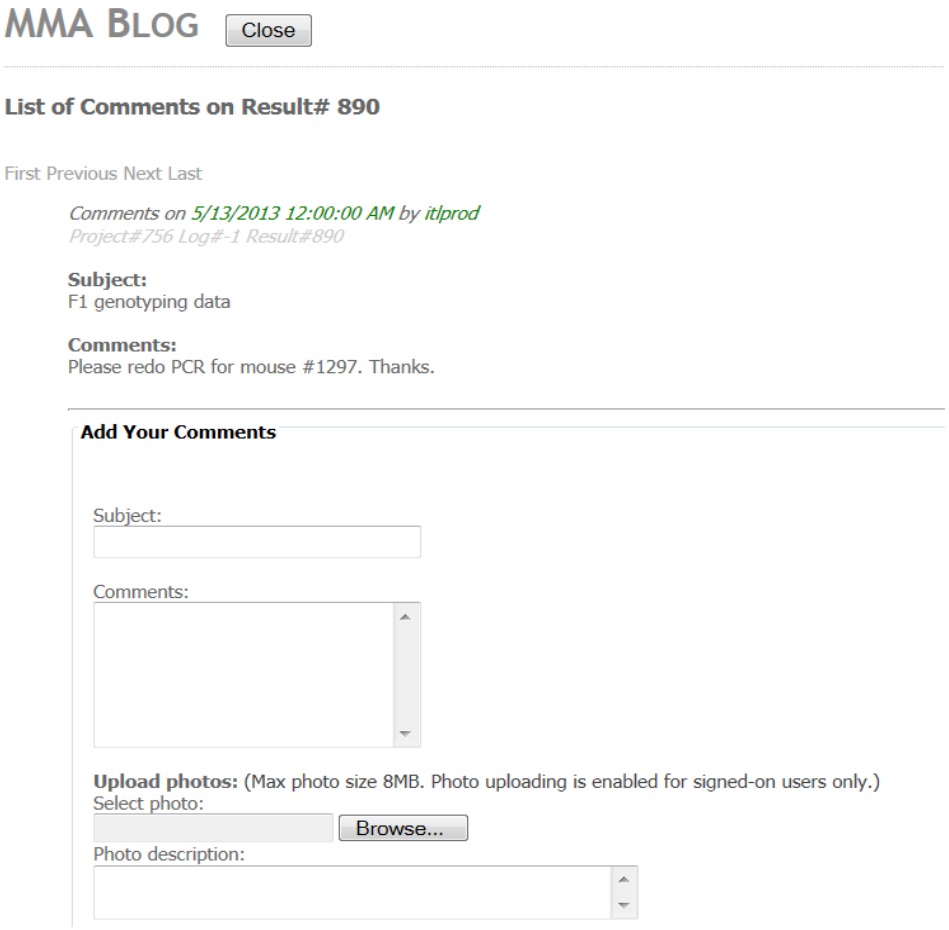
Add New Result

Category:
Genotype

Comments:

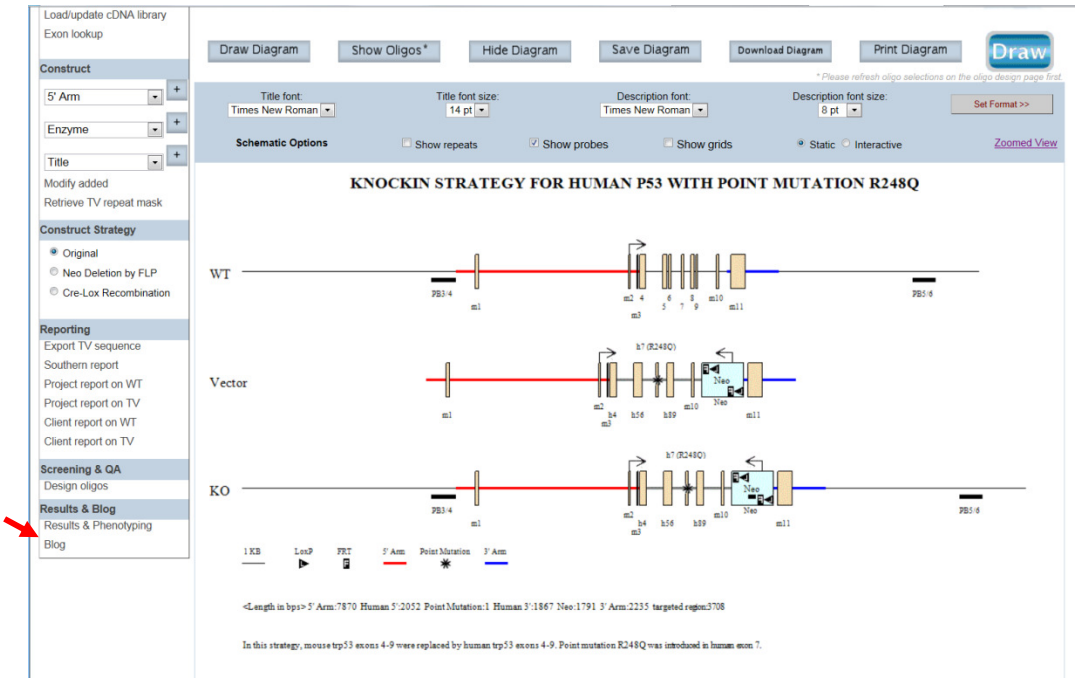
Attach Files/Photos

Add Result Reset Close

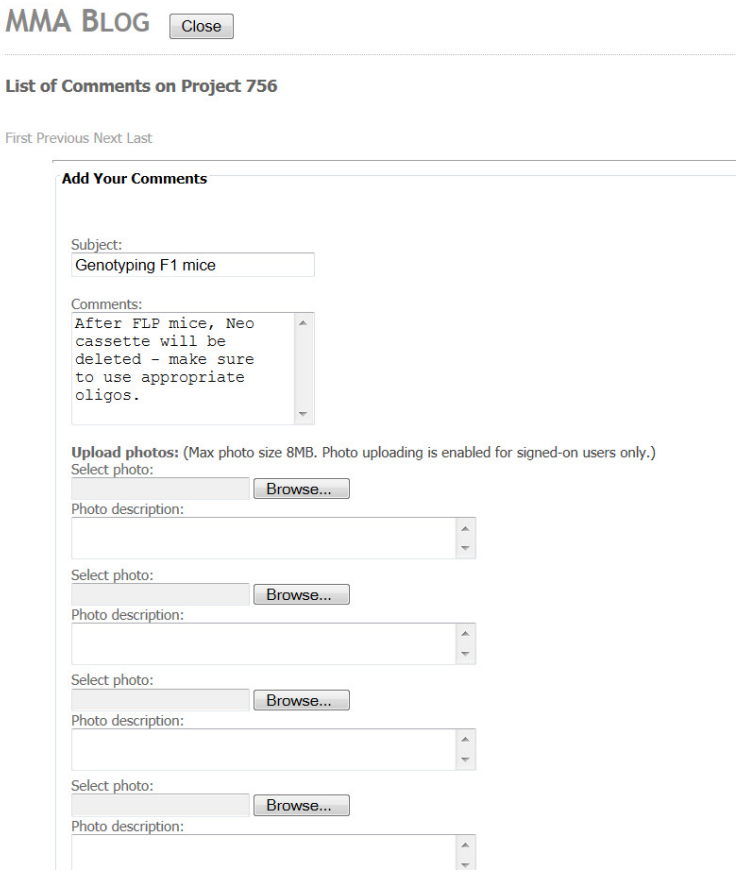


- **How do I blog about a specific project?**

  The blog is used for communicating to your lab members about your project. In Results & Blog section on the left-hand corner, click **Blog**.



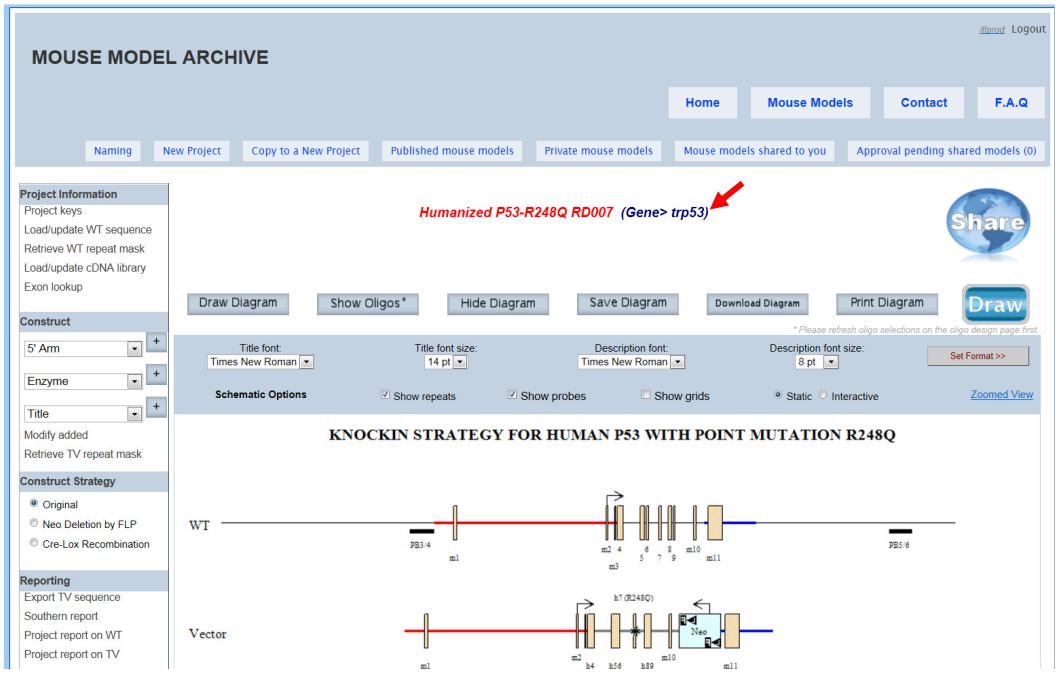
A blog page is displayed for the project. Add your comments or files to the blog. All comments for the project will be listed here.

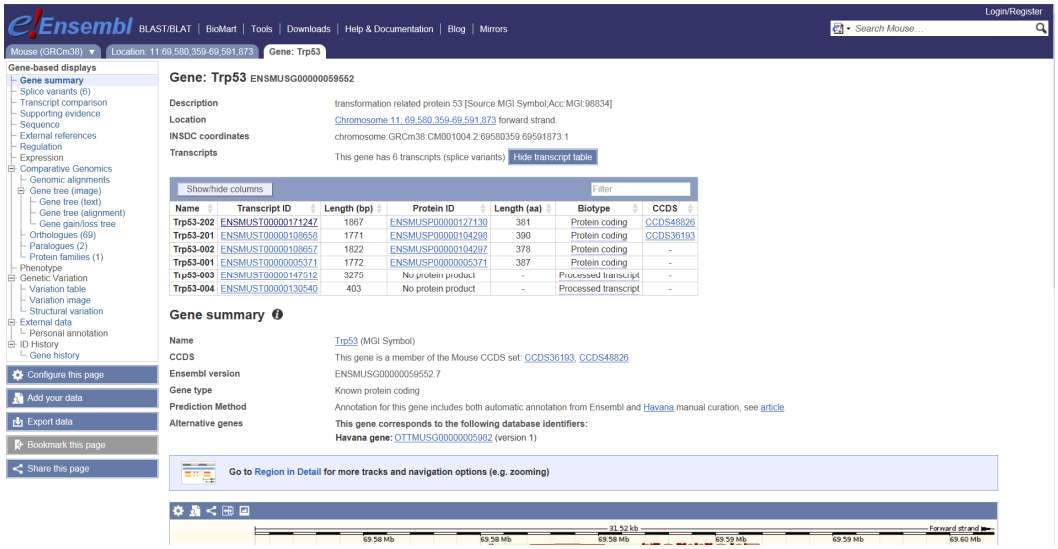


Key Functions on Top Panel

- **How do I get to the Ensembl website?**

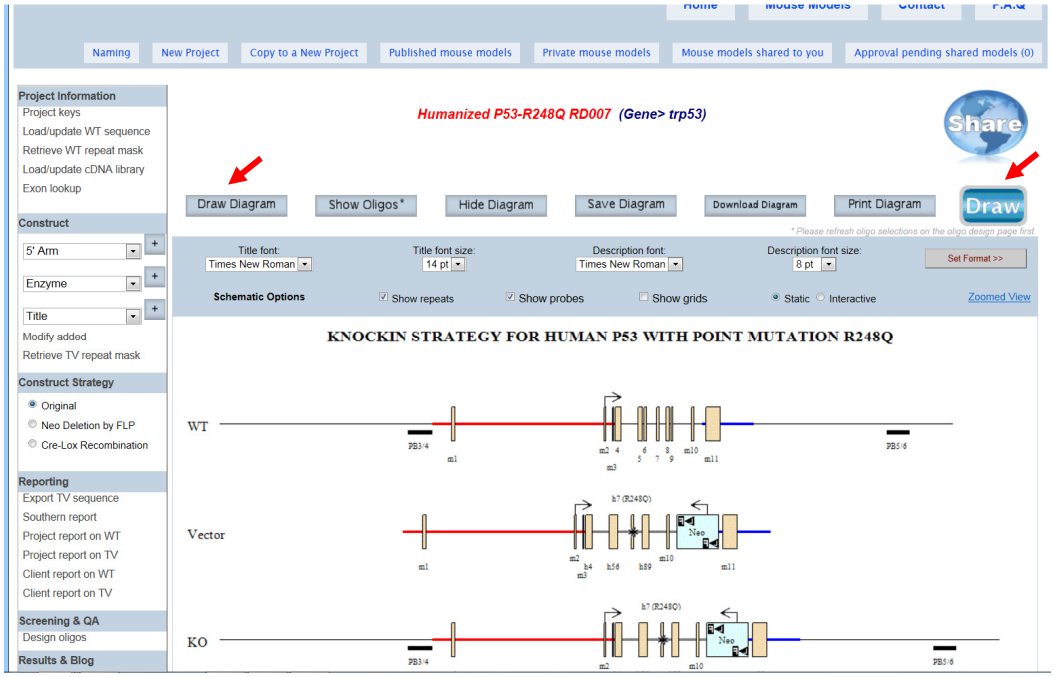
  Click on the Gene name and it will link you to the Ensembl gene summary page for your gene of interest.





- **How do I display the targeting vector on the schematic diagram?**

On the top panel, click **Draw** or **Draw Diagram** button and a schematic diagram of the targeting vector of your project will be displayed.

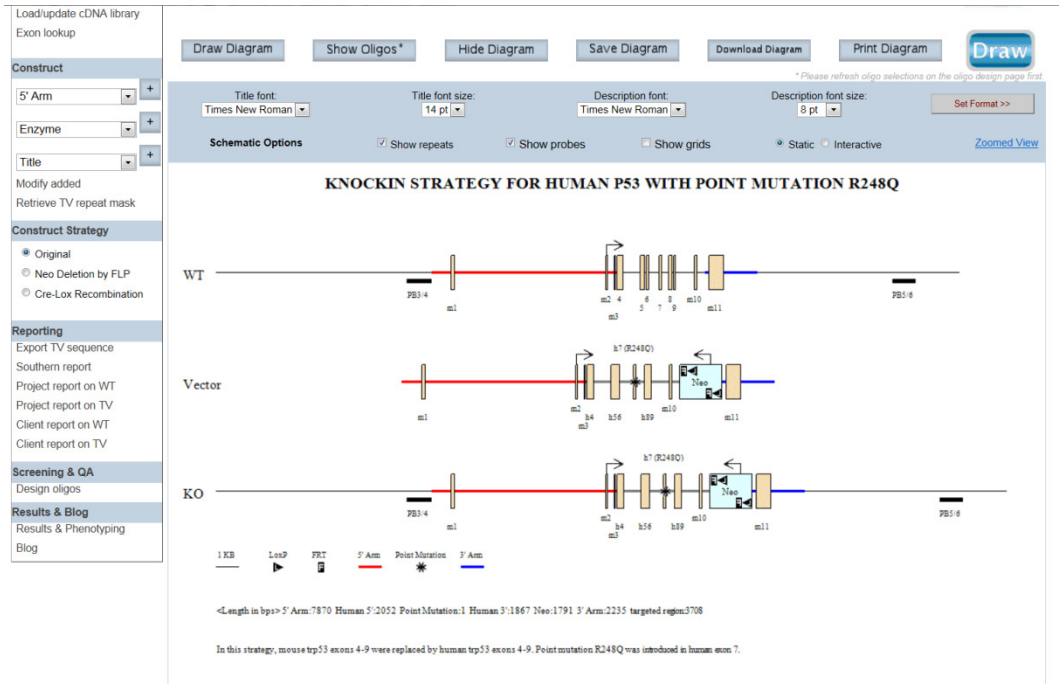


- **What is displayed on the schematic diagram?**

  The title of the schematic diagram is displayed on the top and a description of targeting strategy is indicated below the diagram.

  Three images are shown on the diagram. The first image (WT) displays the wild-type genomic sequences with boxes as exons and color-coded lines for 5' and 3' or long and short, and middle arms. The second image (Vector) displays the vector components without the vector backbone. Flags are used to indicate FRT or loxP sites and boxes are used for cassettes. The third image (KO) shows the targeting vector integrated into the genome. The WT sequence is replaced by the targeting vector.

  Legends of symbols and colors used in the diagram, lengths of the retrieval arms and vector components, and pertinent information about your targeting vector are provided below the diagram.

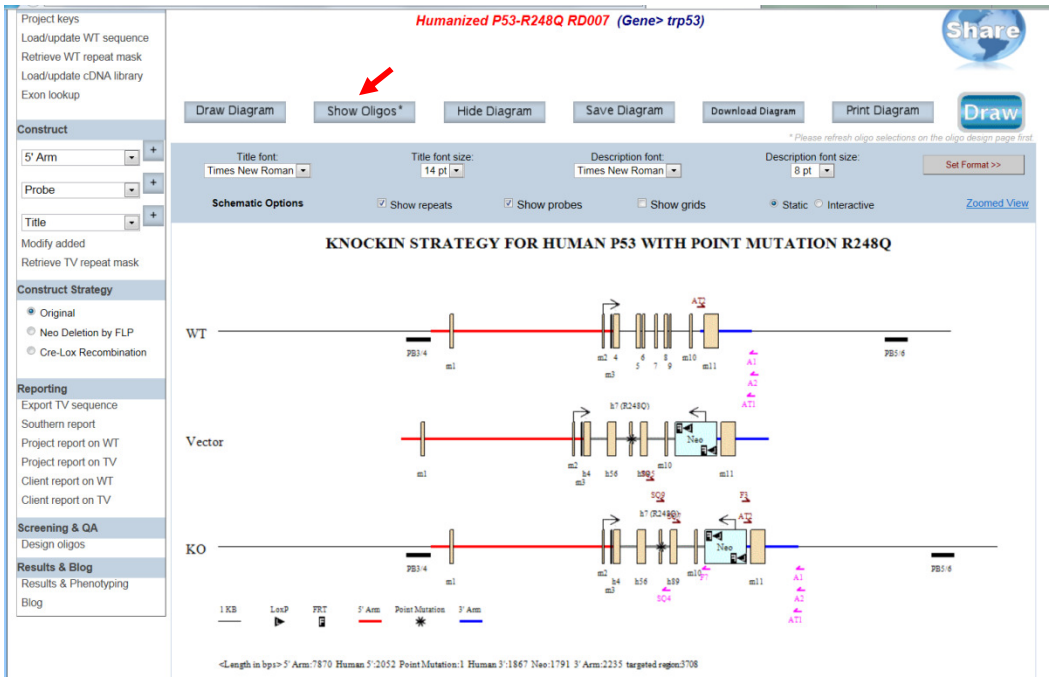


- **How do I hide the schematic diagram?**

  On the top panel, click **Hide Diagram** button and the schematic diagram will not be displayed.

- **How do I display my selected oligos on the schematic diagram?**

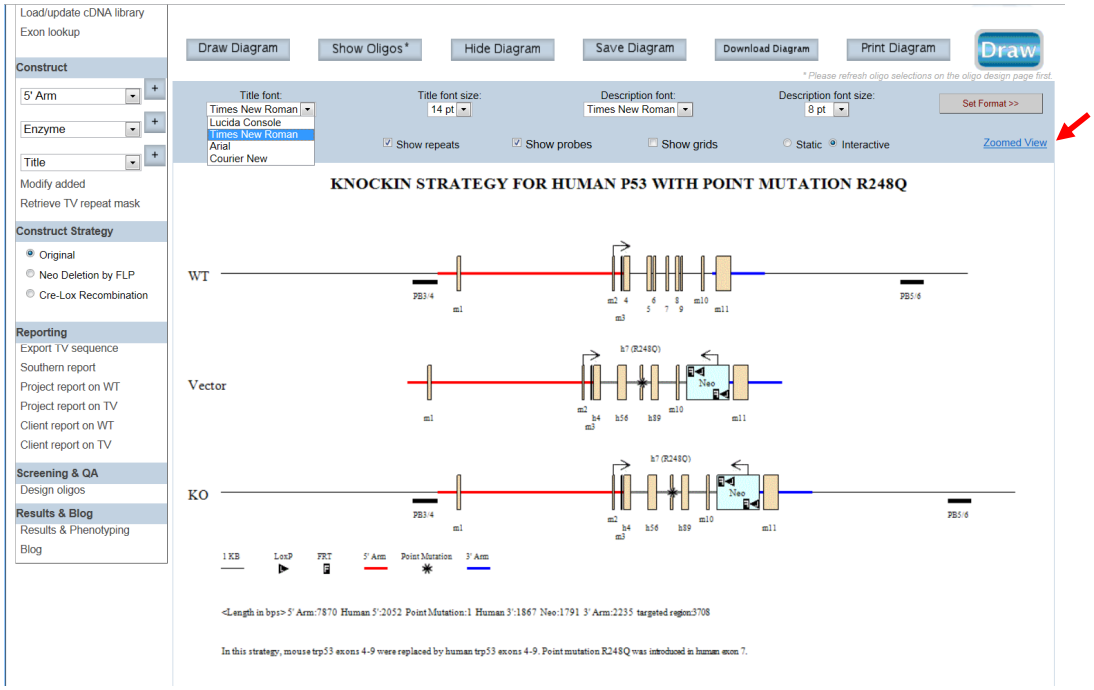
  On the top panel, click **Show Oligos** button and all oligos are displayed forward or reverse direction on the KO strand in the schematic diagram. Note: If you make any changes on the Oligo Design page, you must press **Select Oligos** first on the Oligo Design page. Then click **Show Oligos** to display changes.


Project keys
Load/update WT sequence
Retrieve WT repeat mask
Load/update cDNA library
Exon lookup
Construct
5' Arm
Probe
Title
Modify added
Retrieve TV repeat mask
Construct Strategy
Original
Neo Deletion by FLP
Cre-Lox Recombination
Reporting
Export TV sequence
Southern report
Project report on WT
Project report on TV
Client report on WT
Client report on TV
Screening & QA
Design oligos
Results & Blog
Results & Phenotyping
Blog
Humanized P53-R248Q RD007 (Gene> trp53)
Share
Draw Diagram
Show Oligos*
Hide Diagram
Save Diagram
Download Diagram
Print Diagram
Draw
* Please refresh oligo selections on the oligo design page first.
Title font:
Times New Roman
Title font size:
14 pt
Description font:
Times New Roman
Description font size:
8 pt
Set Format >>
Schematic Options
Show repeats
Show probes
Show grids
Static
Interactive
Zoomed View
KNOCKIN STRATEGY FOR HUMAN P53 WITH POINT MUTATION R248Q
WT
Vector
KO
Neo
1 KB
LoxP
FRT
5' Arm
Point Mutation
3' Arm
<Length in bps> 5' Arm:7870 Human 5':2052 Point Mutation:1 Human 3':1867 Neo:1791 3' Arm:2235 targeted region:3708

Schematic Options

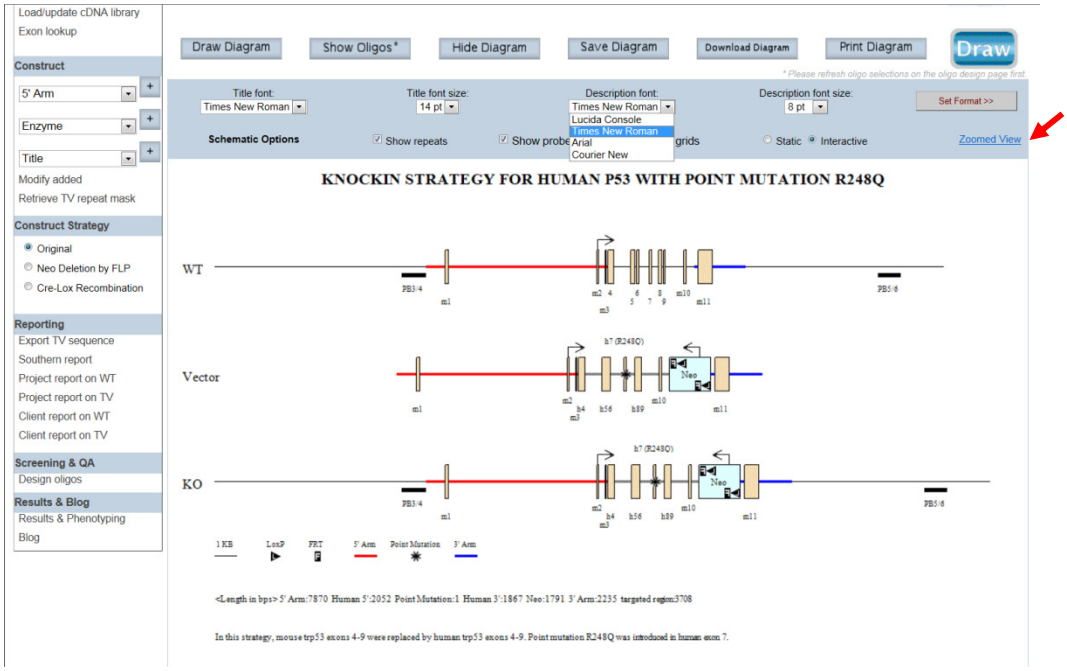
- **How do I change the font style and size for the title on the schematic diagram?**

  Click on the drop-down menu for Title font or Title size. Select the font style or size of your choice and press the **Set Format** button to display your change. Default is Times New Roman at size 14 pt.



- **How do I change the font style and size for the description on the schematic diagram?**

  Click on the drop-down menu for Title font or Title size. Select the font style or size of your choice and press the **Set Format** button to display your change. Default is Times New Roman at size 8 pt.

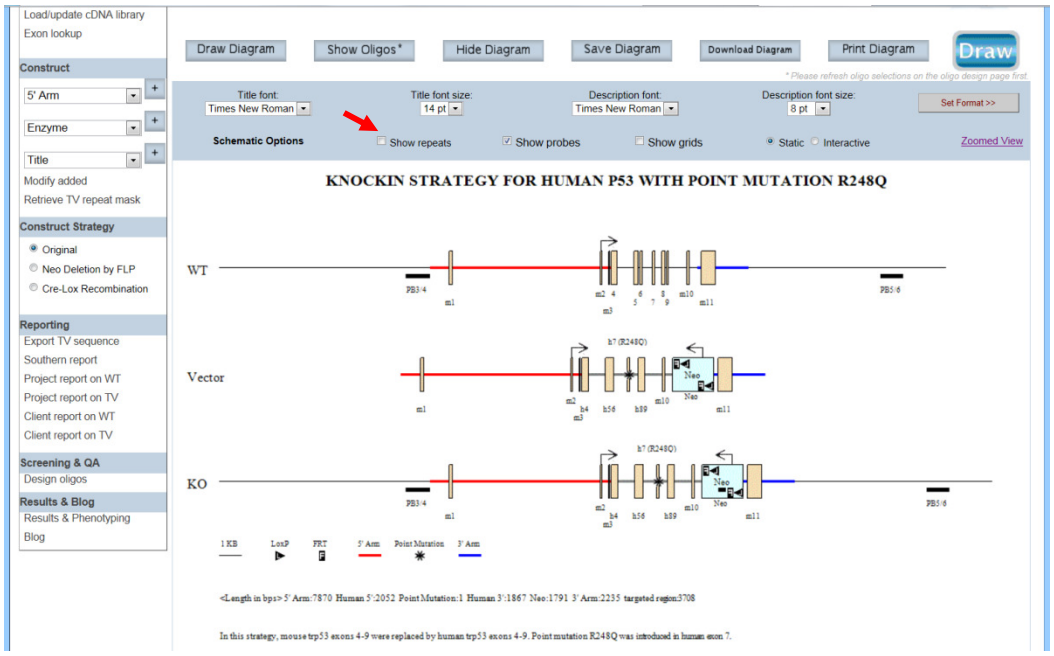


- **What are the small dashed lines drawn on the schematic diagram?**

  The small dashed lines are stretches of repeats that were retrieved from RepeatMasker (http://repeatmasker.org/) and displayed on the WT or KO allele. (See **How do I retrieve repeat mask for my sequences** for details.) The display of repeats can help you to avoid designing primers/probes around these repetitive sequences.

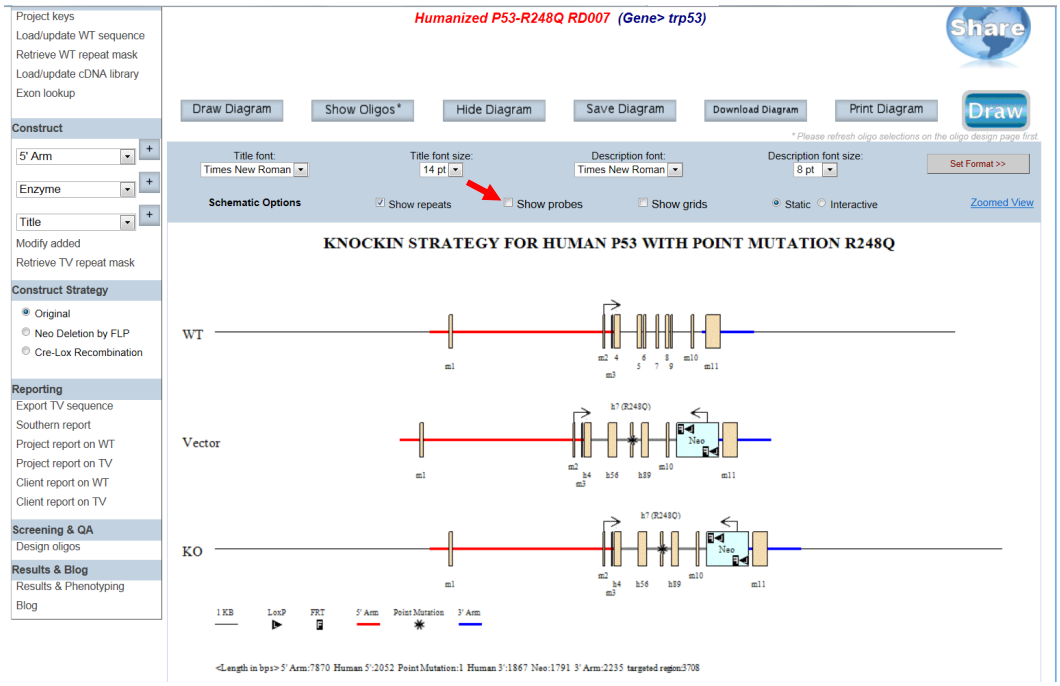
- **How do I remove the repeats from RepeatMasker displaying on the schematic diagram?**

  By default, the repeats are displayed on the schematic diagram. Deselect the box next to **Show Repeats**. The repeats will be hidden from the schematic diagram in the Schematic Options on the top panel.



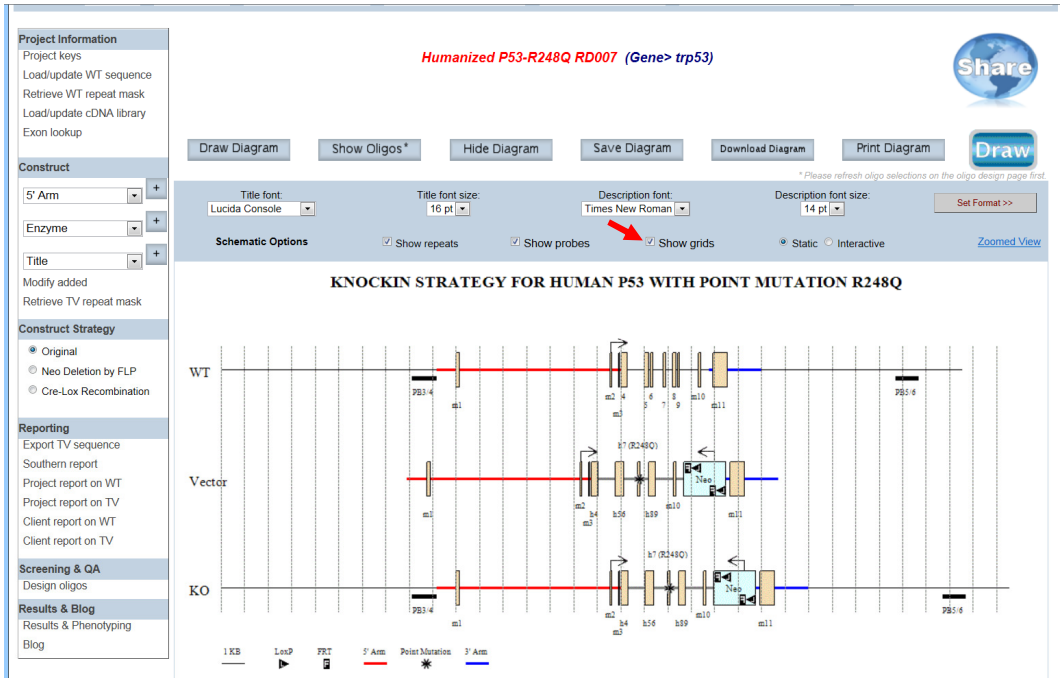
- **How do I hide my probe(s) on the schematic diagram?**

  By default, the probes are displayed on the schematic diagram. If you would like to hide the probes, deselect the box next to **Show Probes** in the Schematic Options on the top panel.



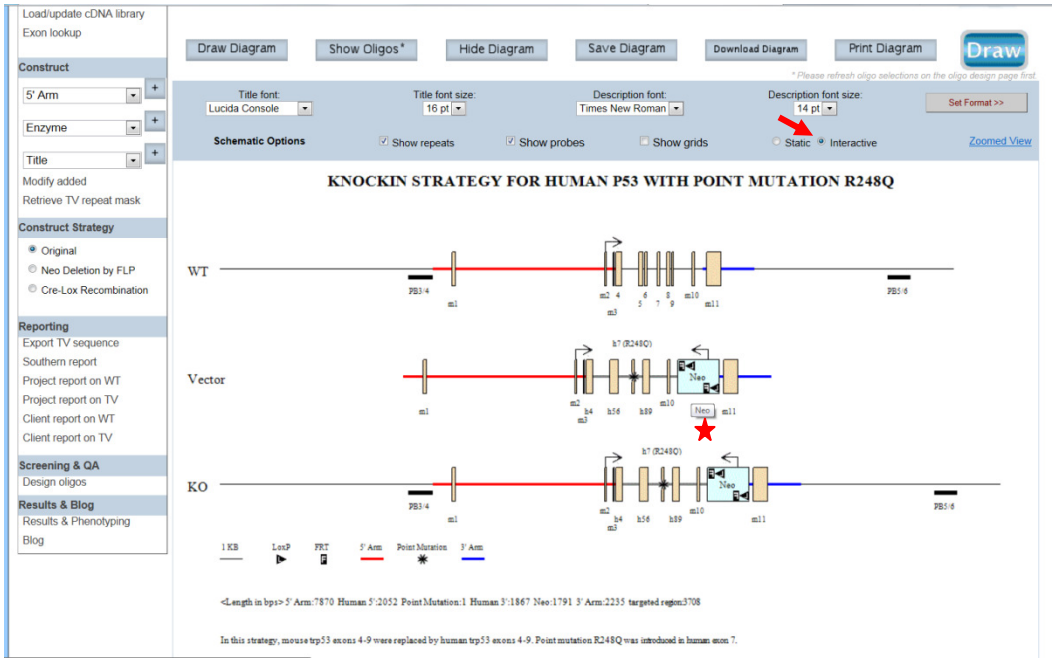
- **How do I display the relative distance or size on the schematic diagram?**

  By default, the grid is off. In the Schematic Options on the top panel, click the box next to **Show grids**. A grid of vertical lines will be overlaid on top of the schematic diagram. The space between two vertical lines is 1kb in length. This will allow you to estimate the distance or size on the schematic diagram.

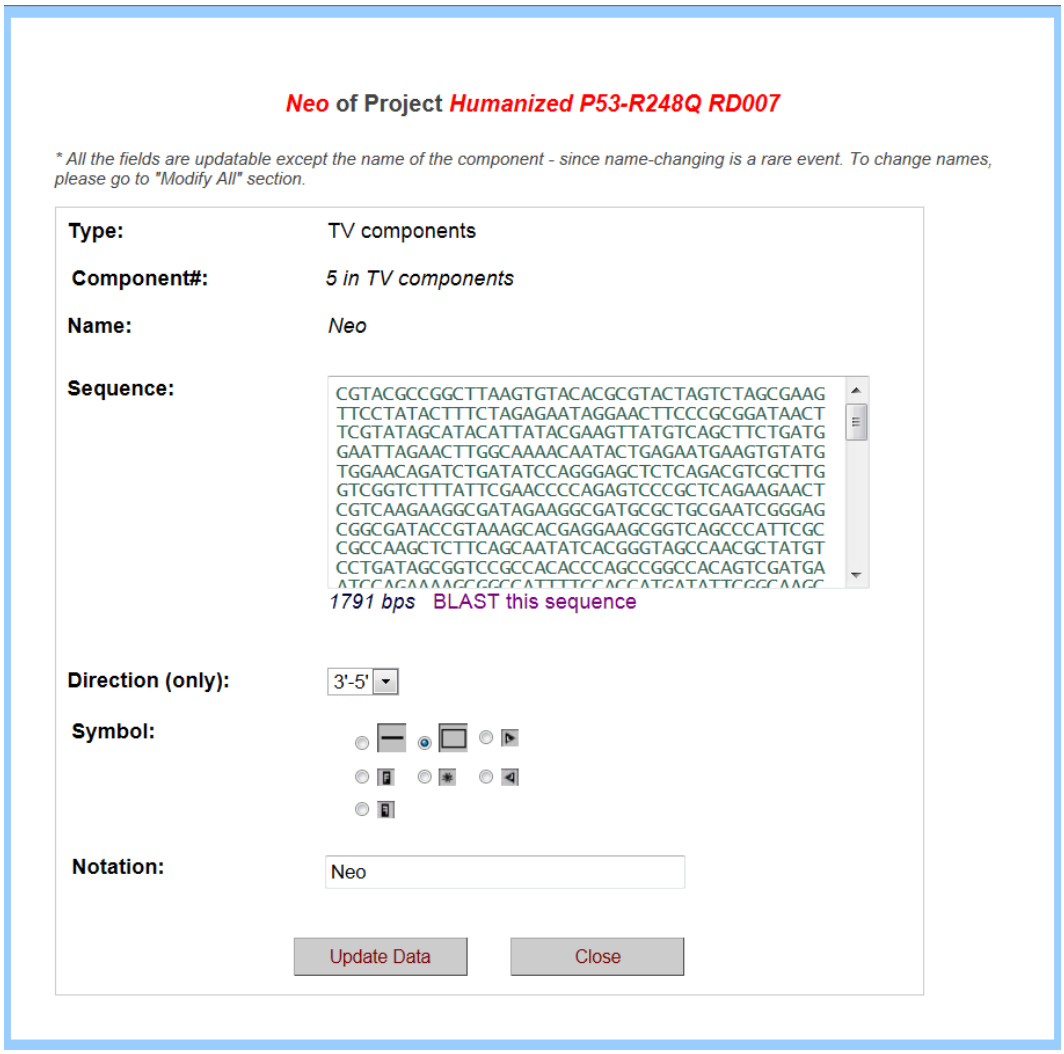


- **What is the static or interactive button on the schematic diagram?**

  By default, the schematic diagram is in a static mode. In the Schematic Options on the top panel, click the box next to **Interactive**. This will allow you to view the schematic diagram in the interactive mode. When moving your computer mouse on top of an image, a pop-up box will indicate the description of the component. The example below shows a pop-up box indicating the Neo cassette (in red asterisk).



When clicking on any image in the diagram, another window will be opened with detail about the vector or non-vector component. For example, below provides the sequence and size of the Neo cassette and other details.



- **Can I save the schematic diagram?**

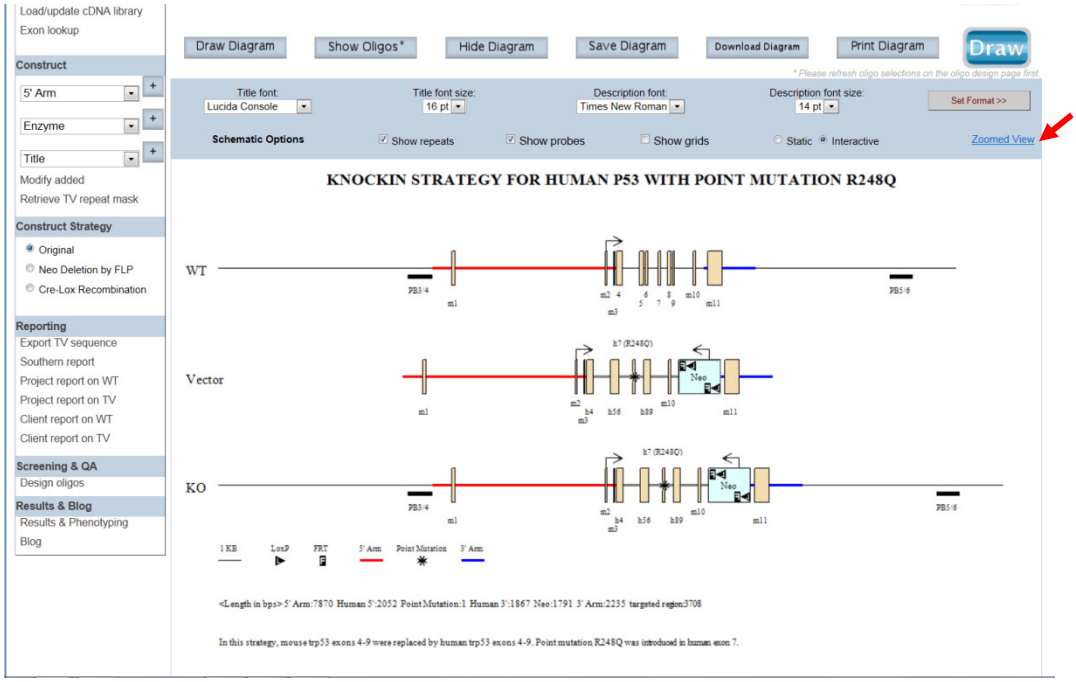
  If the project has been shared to you by View Only, the schematic diagram cannot be saved. Otherwise, you would be able to save the schematic diagram with the changes.

- **Can I download the schematic diagram?**

  If the project has been shared to you by View Only, the schematic diagram cannot be downloaded. Otherwise, you would be able to download the schematic diagram.

- **How do I zoom in to see details of the schematic diagram?**

  In the Schematic Options on the top panel, click **Zoomed View**.



Another window will be opened for the Diagram Zoomed View page. There are 2 ways to specify your region of interest (ROI). The first method is by entering the start and end sequences of your ROI outside the 5’ and 3’ arm. A minimum of 40bps is required per arm. The second method is by entering the length (in basepairs) flanking outside the 5’ and 3’ arm for your ROI. The maximum is 10,000bps. Then click **Draw Diagram** button. An enlarged and zoomed-in schematic diagram is displayed below. The **Save Diagram** and **Download Diagram** are not functional for this version. Select any changes in the Schematic Options and then click **Draw Diagram** to display changes in the schematic diagram.

Below is an example of an ROI that is specified by length outside of 5’ or 3’ arm. The length was entered for 1500 bps.

Diagram Zoomed View for **Humanized P53-R248Q RD007**

Specify ROI:

- By start/end sequences
- Length outside of long arm/short arm

Length outside of 5' Arm *(< 9142bps)*: 1500

Length outside of 3' Arm *(< 8559bps)*: 1500

Draw diagram | Save Diagram | Download diagram | Print diagram

with probes / without probes | with oligos / without oligos | static / interactive | Annotation Size: 7 pt | Increase Exon label size to: 100%