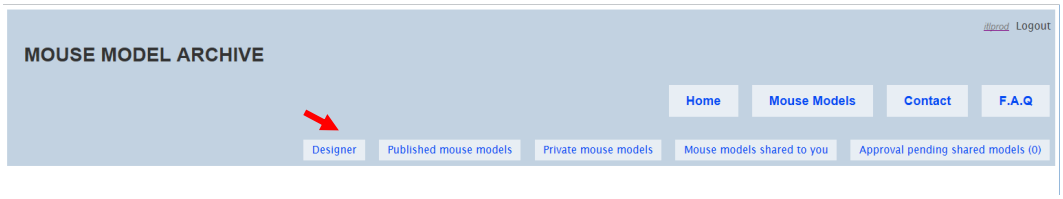
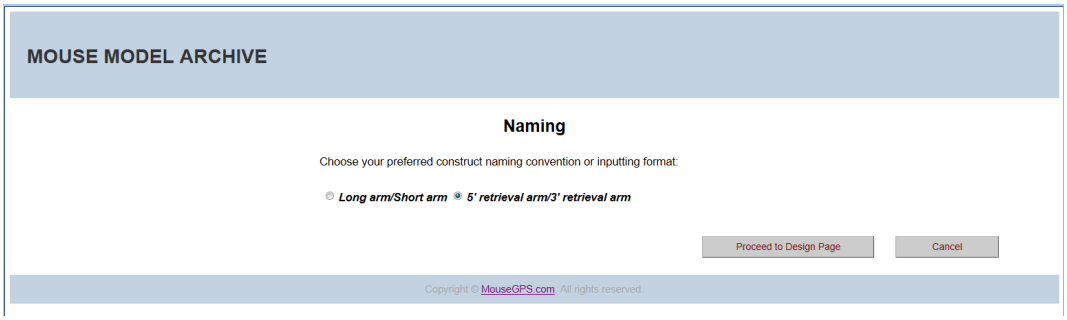
How do I input my targeting vector for a conditional (cre-lox) knockout?

1. Click on **Designer** tab on the first page

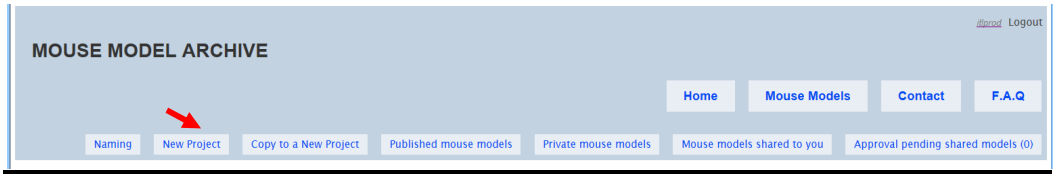


2. Click on **Naming** tab.

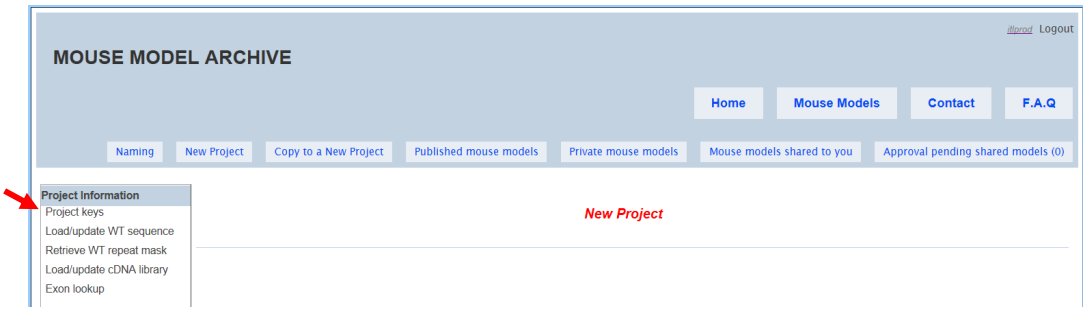
3. Choose which naming convention for your targeting vector and click **Proceed to Design Page** button.



4. Click on **New Project** tab.



5. Click on **Project Keys** under Project Information. Fill out all fields and click **Save Project Keys** button.

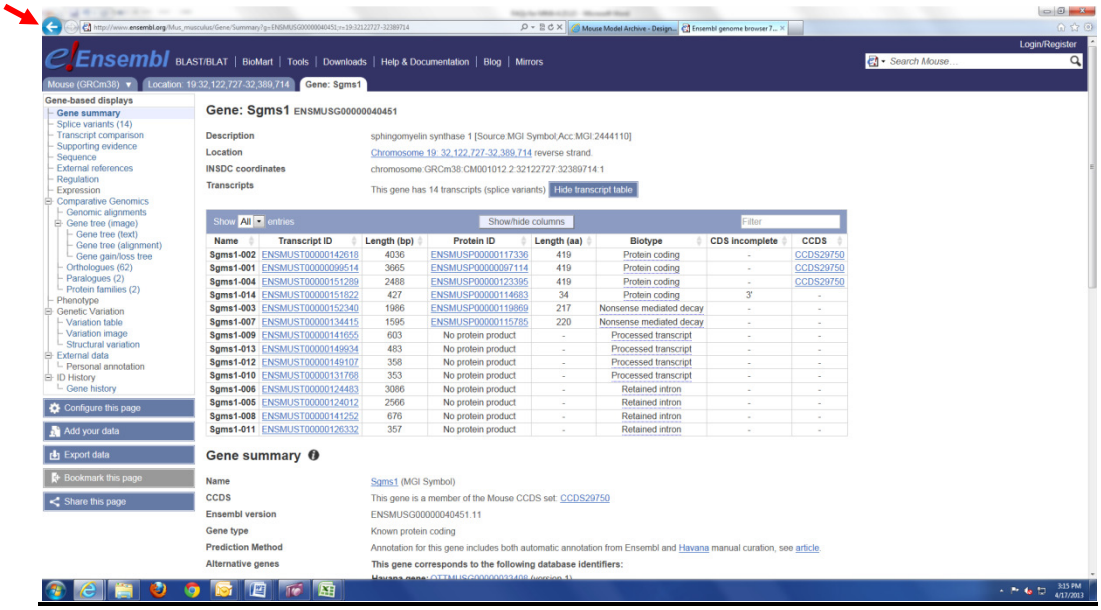


Project Category: Private – project can be seen only by you
Public – project can be seen by all users

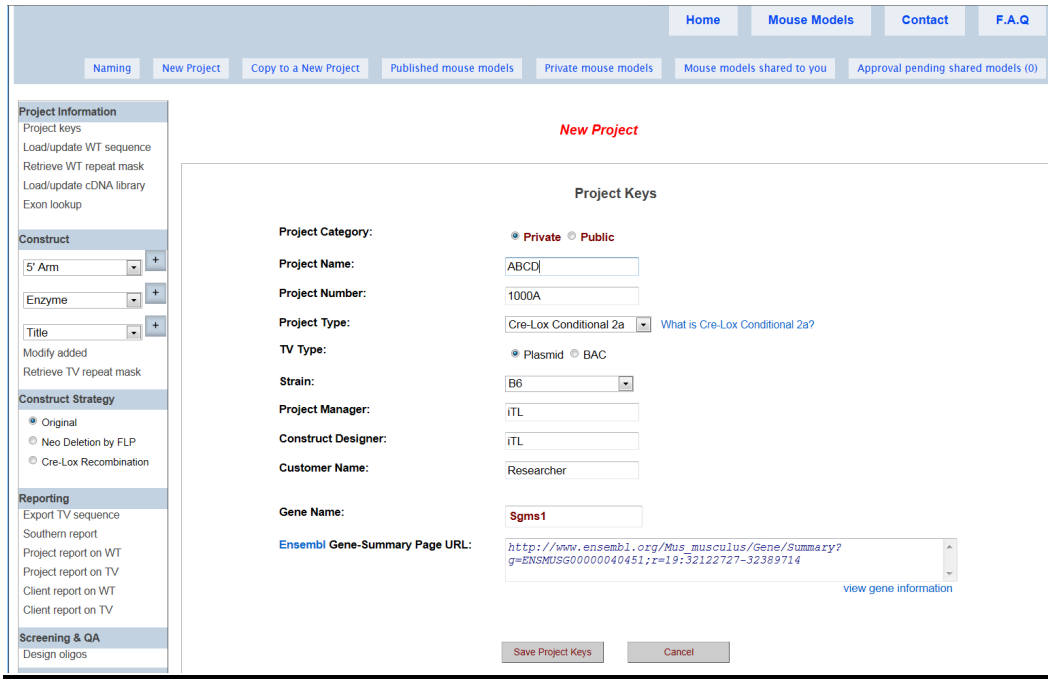
Project Type: Choose an appropriate project type for your targeting design. Click on "***What is . . . ?***" to see details on classification of various project types.

TV (Targeting Vector) Type: Plasmid or BAC

Ensembl Gene-Summary Page URL: Copy the link from the Ensembl Gene Summary page (see red arrow below) and paste it on Project Keys page.



Below is an example of a new project completed for a conditional knockout of the mouse Sgms1 gene.



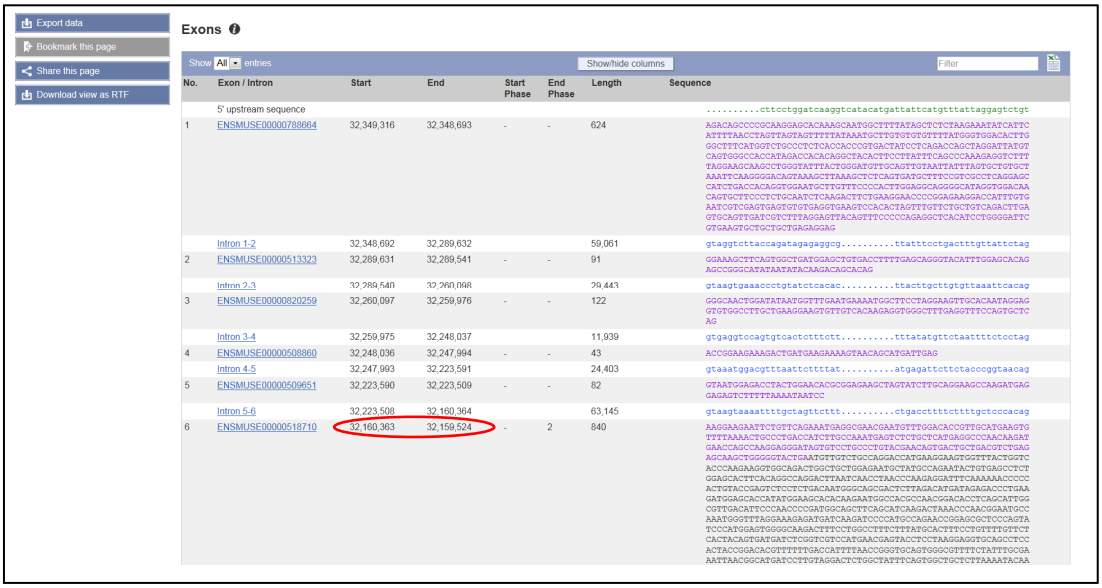
6. Click on **Load/Update WT Sequence** under Project Information to retrieve your genomic sequences from Ensembl.

You would need to keep in mind which exon(s) to be targeted for your targeting vector.

20kb upstream and 20kb downstream sequences of the targeted exon(s) will be retrieved from Ensembl. If your gene is located on the forward strand, subtract 20kb from the start location of the targeted exon and add 20kb to the end location. If the gene is on the reverse strand, make sure to add 20kb to the start location of the targeted exon and subtract 20kb from the end location.

For example, exon 6 will be conditionally targeted for the mouse Sgms1 gene. Look up the start and end location of exon 6 on the Transcript Page in Ensembl.

Transcript page of Sgms1-002



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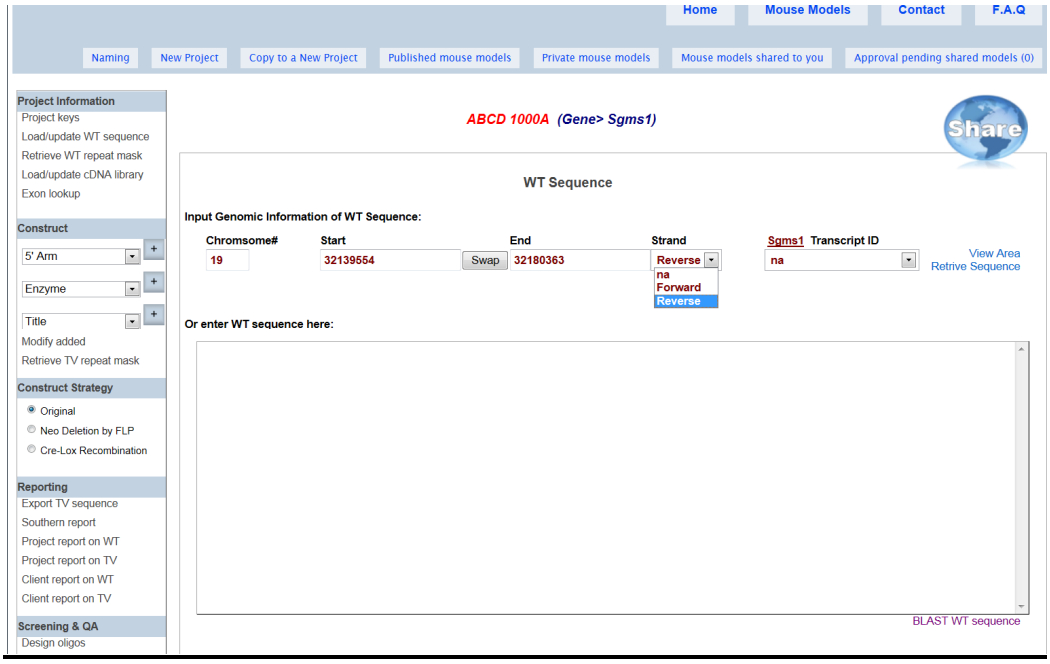
Exons

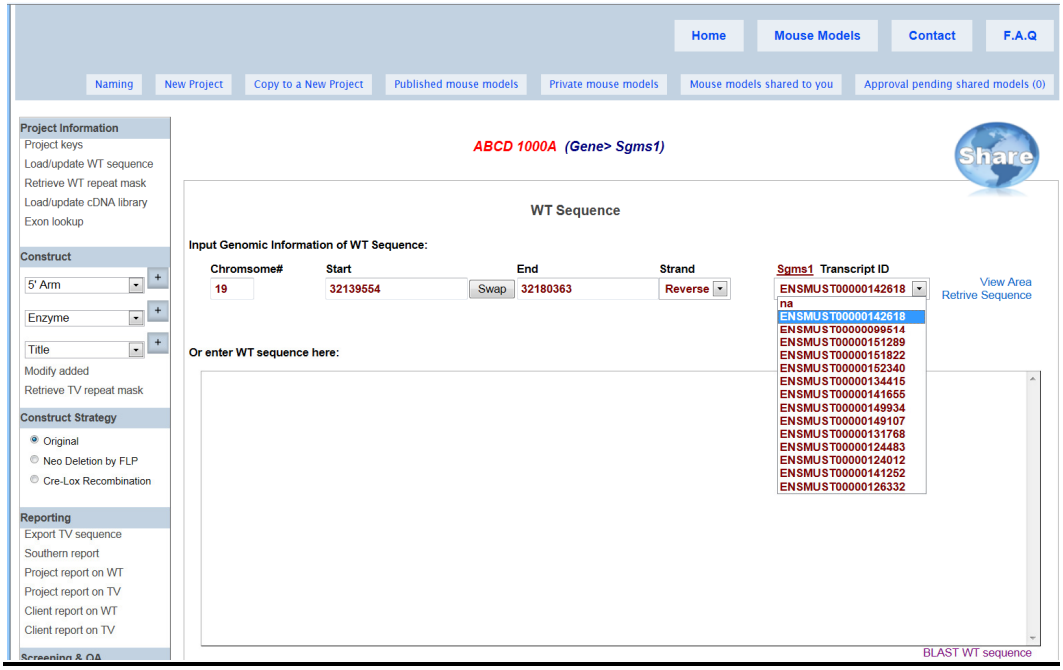
Show All entries | Show/hide columns | Filter

No.	Exon / Intron	Start	End	Start Phase	End Phase	Length	Sequence
	5' upstream sequence						..........cttcctggatcaaggtcatacatgattattcatgtttattaggagtctgt
1	ENSMUSE00000788664	32,349,316	32,348,693	-	-	624	AGACAGCCCGCAAGGAGCACAAAGCAATGGCTTTTATAGCTCTCTAAGAAATATCATTC ATTTTAACCTAGTTAGTAGTTTTTATAAATGCTTGTGTGTGTTTTATGGGTGGACACTTG GGCTTTCATGGTCTGCCCTCTCACCACCCGTGACTATCCTCAGACCAGCTAGGATTATGT CAGTGGGCCACCATAGACCACACAGGCTACACTTCCTTATTTCAGCCCAAAGAGGTCTTT TAGGAAGCAAGCCTGGGTATTTACTGGGATGTTGCAGTTGTAATTATTTAGTGCTGTGCT AAATTCAAGGGGACAGTAAAGCTTAAAGCTCTCAGTGATGCTTTCCGTCGCCTCAGGAGC CATCTGACCACAGGTGGAATGCTTGTTTCCCCACTTGGAGGCAGGGGCATAGGTGGACAA CAGTGCTTCCCTCTGCAATCTCAAGACTTCTGAAGGAACCCCGGAGAAGGACCATTGTG AATCGTCGAGTGAGTGTGTGAGGTGAAGTCCACACTAGTTTGTTCTGCTGTCAGACTTGA GTGCAGTTGATCGTCTTTAGGAGTTACAGTTTCCCCCAGAGGCTCACATCCTGGGGATTC GTGAAGTGCTGCTGCTGAGAGGAG
	Intron 1-2	32,348,692	32,289,632			59,061	gtaggtcttaccagatagagaggcg.........ttatttcctgacttttgttattctag
2	ENSMUSE00000513323	32,289,631	32,289,541	-	-	91	GGAAAGCTTCAGTGGCTGATGGAGCTGTGACCTTTTGAGCAGGGTACATTTGGAGCACAG AGCCGGGCATATAATATACAAGACAGCACAG
	Intron 2-3	32,289,540	32,260,098			29,443	gtaagtgaaaccctgtatctcacac.........ttacttgcttgtgttaaattcacag
3	ENSMUSE00000820259	32,260,097	32,259,976	-	-	122	GGGCAACTGGATATAATGGTTTGAATGAAAATGGCTTCCTAGGAAGTTGCACAATAGGAG GTGTGGCCTTGCTGAAGGAAGTGTTGTCACAAGAGGTGGGCTTTGAGGTTTCCAGTGCTC AG
	Intron 3-4	32,259,975	32,248,037			11,939	gtgaggtccagtgtcactcttttctt.........tttatatgttctaattttctcctag
4	ENSMUSE00000508860	32,248,036	32,247,994	-	-	43	ACCGGAAGAAAAGACTGATGAAGAAAAGTAACAGCATGATTGAG
	Intron 4-5	32,247,993	32,223,591			24,403	gtaaatggacgtttaattcttttat.........atgagattcttctaccggtaacag
5	ENSMUSE00000509651	32,223,590	32,223,509	-	-	82	GTAATGGAGACCTACTGGAACACGCGGAGAAGCTAGTATCTTGCAGGAAGCCAAGATGAG GAGAGTCTTTTTAAAATAATCC
	Intron 5-6	32,223,508	32,160,364			63,145	gtaagtaaaattttgctagttcttt.........ctgacctttttttgtcccacag
6	ENSMUSE00000518710	32,160,363	32,159,524	-	2	840	AAGGAAGAATTCTGTTCAGAAATGAGGCGAACGAATGTTGGACACCGTTGCATGAAGTG TTTTAAAACTGCCCTGACCATCTTGCCAAATGAGTCTCTGCTCATGAGGCCCAACAAGAT GAACCAGCCAAGGAGGGATAGTGTCCTGCCCTGTACGAACAGTGACTGCTGACGTCTGAG AGCAAGCTGGGGGTACTGAATGTTGTCTGCCAGGACCATGAAGGAAGTGGTTTACTGGTC ACCCAAGAAGGTGGCAGACTGGCTGCTGGAGAATGCTATGCCAGAATACTGTGAGCCTCT GGAGCACTTCACAGCCCAGGACTTAATCAACCTAACCCAAGAGGATTTCAAAAAACCCCC ACTGTACCAGTCTCCTCTGACAATGGGCAGCGACTCTAGACATGATAGAGACCCTGAA GATGGAGCACCATATGGAAGCACACAAGAATGGCCACGCCAACGGACACCTCAGCATTGG CGTTGACATTCCCAACCCGATGGCAGCTTCAGCATCAAGACTAAACCCAACGGAATGCC AAATGGGTTTAGGAAAGAGATGATCAAGATCCCCATGCCAGAACCGGAGCGCTCCCAGTA TCCCATGGAGTGGGGCAAGACTTTCCTGGCCTTTCTTTATGCACTTTCCTGTTTTGTTCT CACTACAGTGATGATCTCGGTCGTCCATGAACGAGTACCTCCTAAGGAGGTGCAGCCTCC ACTACCGGACACGTTTTTTGACCATTTTAACCGGGTGCAGTGGGCGTTTTCTATTTGCGA AATTAACGGCATGATCCTTGTAGGACTCTGGCTATTTCAGTGGCTGCTCTTAAAATACAA

a) Enter the start and end location number including 20kb upstream and downstream sequences of your targeted exon. Indicate the direction of your gene under Strand. Make sure the strand direction is entered correctly otherwise an incorrect sequence will be retrieved for your project.



b) Choose the transcript ID for your targeting vector using the drop down menu.

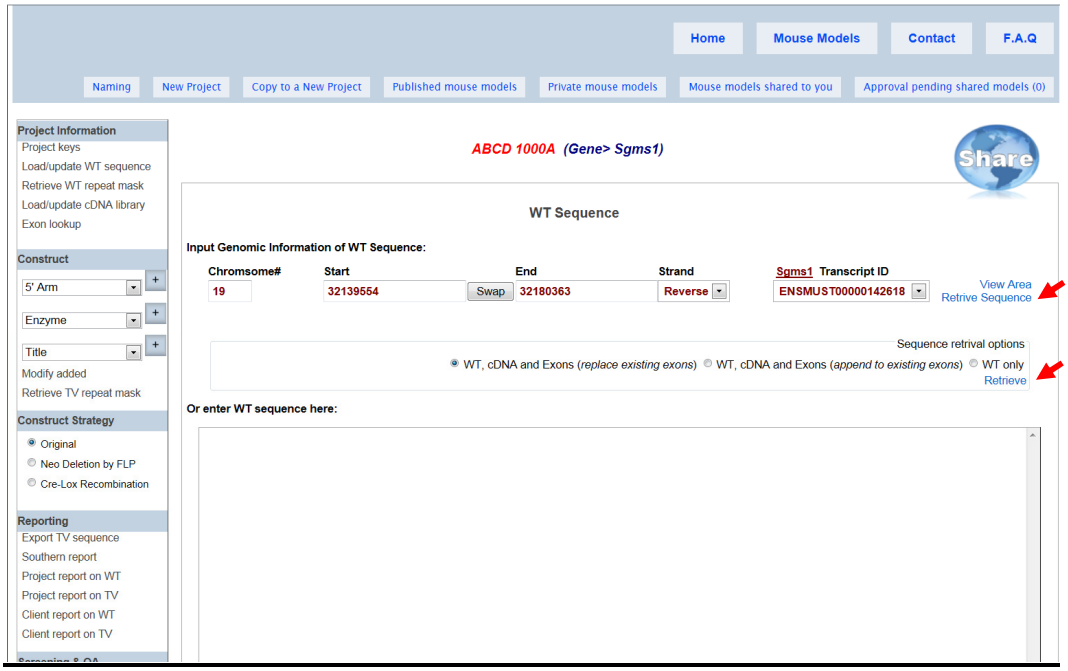


c) Click on **Retrieve Sequence**. Options for sequence retrieval will be provided.

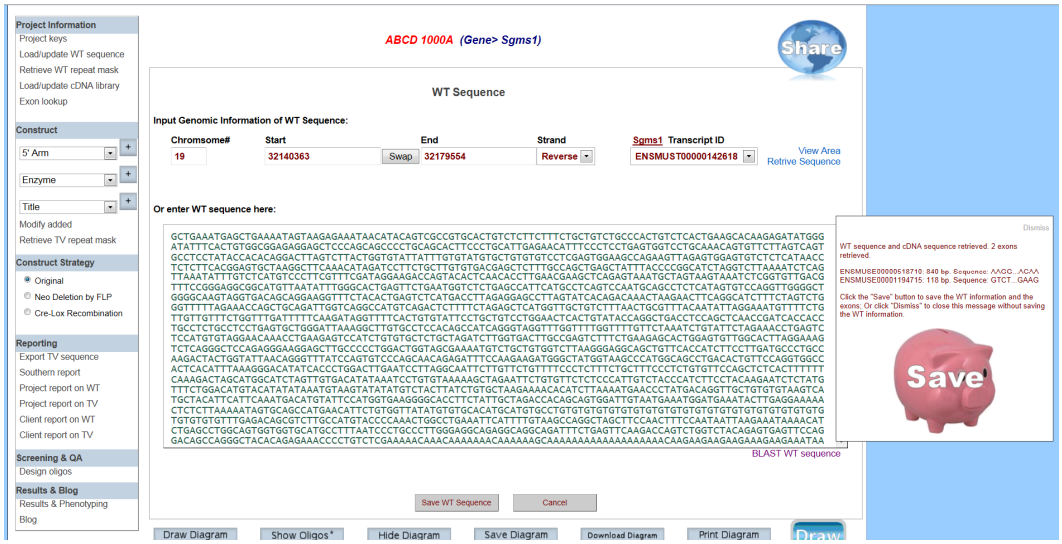
If your project is new, select **WT, cDNA and exons (replace existing exons)**.

Select **Retrieve** and the sequences will be retrieved from Ensembl using the indicated start and end location.

Note: If your sequence retrieval is slow or not working well, it would be likely that Ensembl website is slow or temporarily not working.



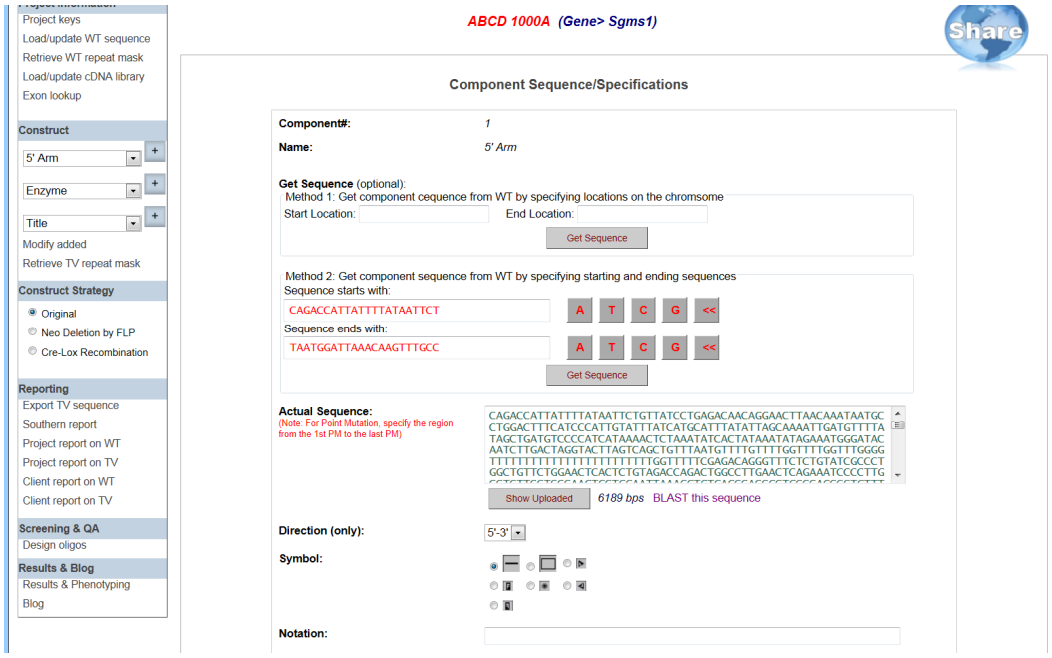
d) Genomic sequences will be retrieved. Number of exons will be noted. If no exons are indicated, check to make sure the start and end location numbers were entered correctly and that the direction of the strand was indicated correctly. Make sure to click **Save** on the piggy bank.



7. Now you will enter individual components of your targeting vector. Depending on your selected naming convention, Long Arm/Short Arm or 5' Retrieval Arm/3' Retrieval Arm will be indicated on the drop-down menu under Construct section on the left panel.

a) **Enter 5' arm sequence**: Select 5' Arm under Construct from the drop-down menu. Click **[+]** button. Component Sequence/Specifications page will be displayed. You have 3 options to enter your sequence on the 5' arm:

Below is an example of 5' arm sequence retrieved by using option 3.

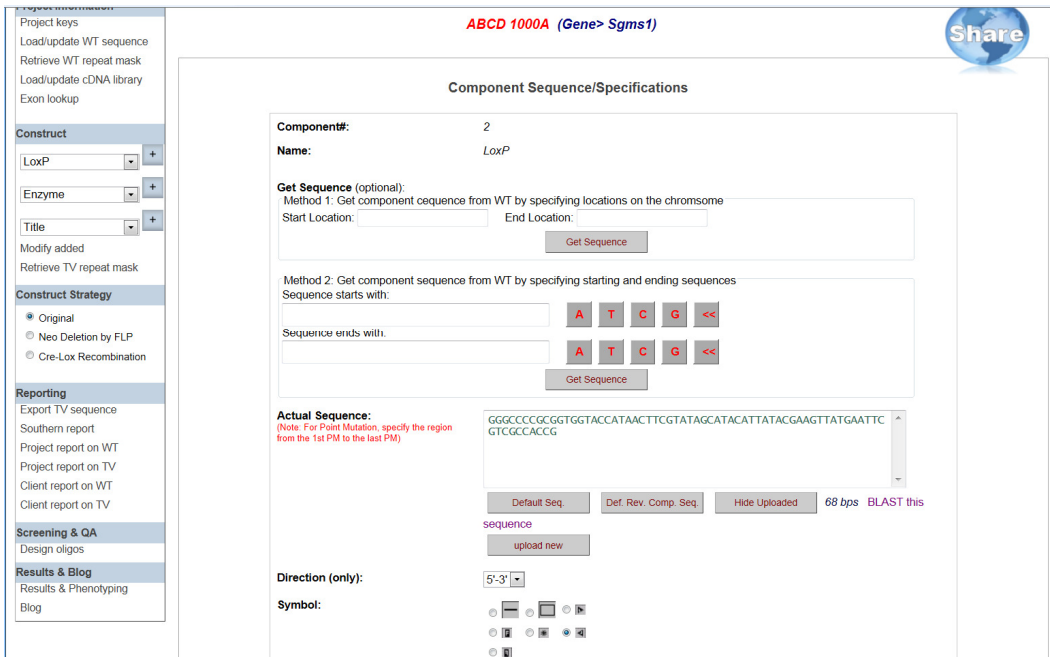


b) **Add LoxP site:** Select LoxP under Construct from the drop-down menu. Click **[+]** button. Component Sequence/Specifications page will be displayed.

Note: Sequences for LoxP site, selection cassette, reporter gene, etc. cannot be retrieved by **Get Sequence** button. The sequence will need to be pasted directly into the Actual Sequence box.

Paste your LoxP sequence into the Actual Sequence box.

Direction (only) is not applicable for LoxP site. Select a symbol for the LoxP site with the correct direction. Typically a triangle is used to indicate the LoxP site. When completed, press **Add/Update KO Component** button.



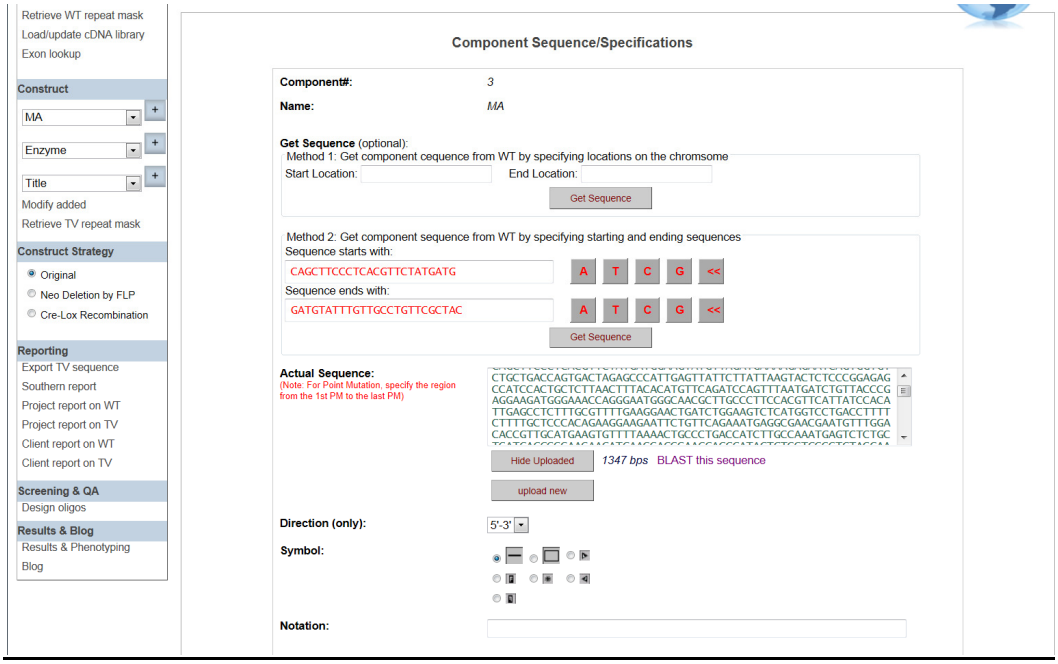
c) **Enter MA (middle arm) sequence:** Select MA under Construct from the drop-down menu. Click **[+]** button. Component Sequence/Specifications page will be displayed. You have 3 options to enter your sequence on the MA:

1) Enter start and end location. Press **Get Sequence** button.
2) Type in start and end sequences (5'->3') using your keyboard or the nucleotide boxes using your computer mouse. **<<** button is used for backspace and deletion. A minimum of 10 base pairs is required. Press **Get Sequence** button. If sequences were entered incorrectly, an error message will be prompted.
3) Paste your entire sequence of the 5' arm inside the Actual Sequence box.

Direction (only) is not applicable for homology arms. It is only applicable for selection/reporter cassettes, exons, cDNA, etc.

Select a symbol of your choice. Typically a line is used to indicate the homology arm.

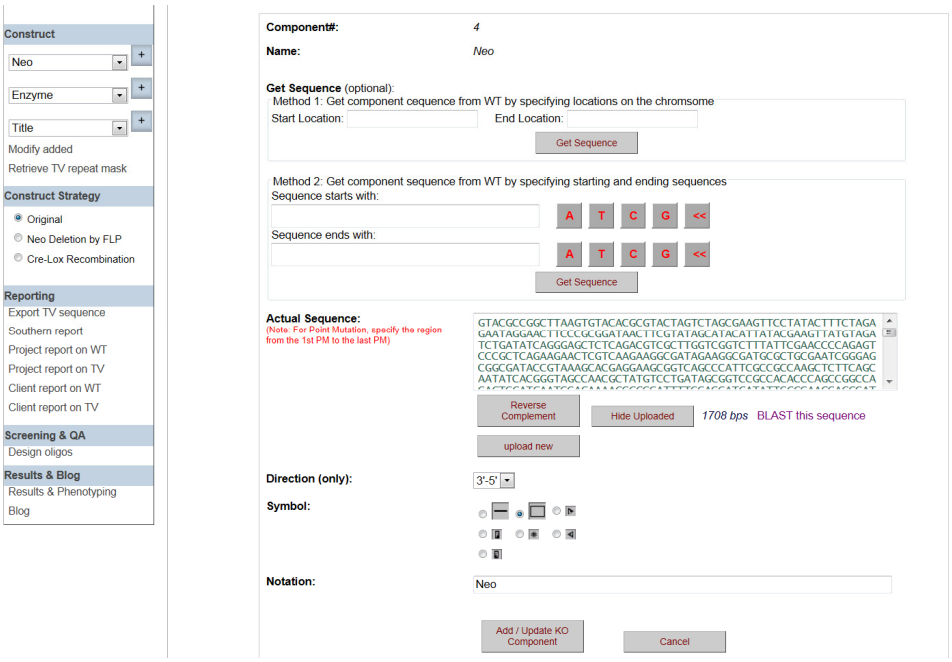
After you're done, press **Add/Update KO Component** button.



d) **Enter Neo selection cassette**: Select Neo under Construct from the drop-down menu. Click **[+]** button. Component Sequence/Specifications page will be displayed.

Note: Sequences for LoxP site, selection cassette, reporter gene, etc. cannot be retrieved by **Get Sequence** button. The sequence will need to be pasted directly into the Actual Sequence box.

Paste your Neo sequence into the Actual Sequence box. The program will recognize any wild-type LoxP or FRT sequences flanking the Neo cassette.



Select Direction (only) to show direction of the Neo cassette. Choose appropriate Symbol for the cassette. Typically a box is used to represent the Neo cassette. Add the Notation to indicate the cassette. When completed, press **Add/Update KO Component** button.

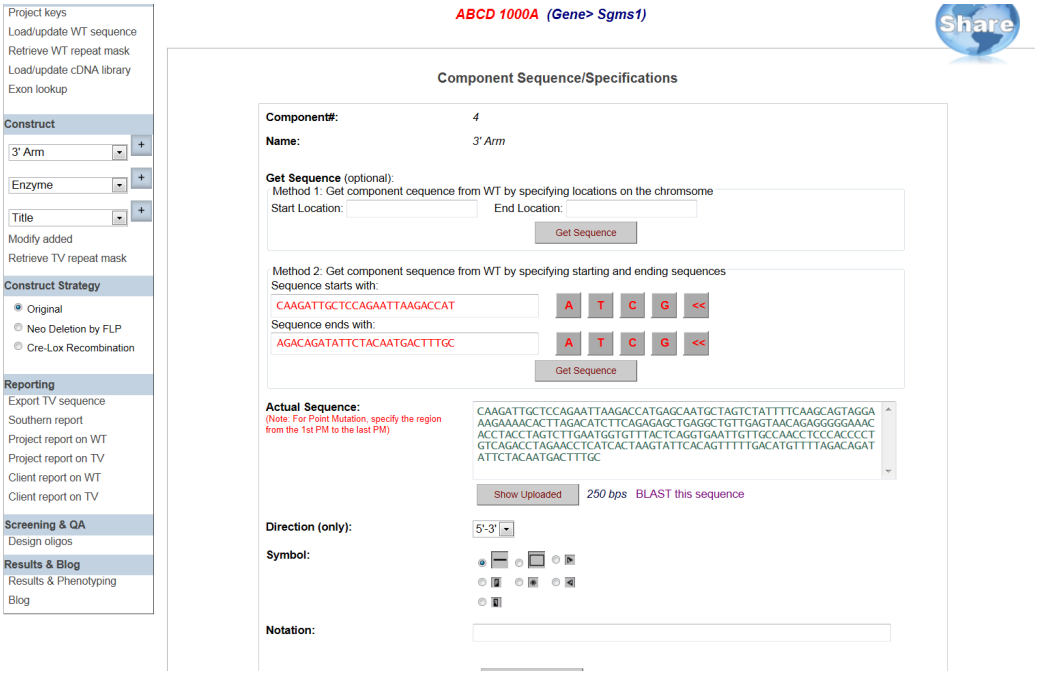
e) **Enter 3' arm sequence:** Select 3' Arm under Construct from the drop-down menu. Click **[+]** button. Component Sequence/Specifications page will be displayed. You have 3 options to enter your sequence on the 5' arm:

1) Enter start and end location. Press **Get Sequence** button.
2) Type in start and end sequences (5'->3') using your keyboard or the nucleotide boxes using your computer mouse. **<<** button is used for backspace and deletion. A minimum of 10 base pairs is required. Press **Get Sequence** button. If sequences were entered incorrectly, an error message will be prompted.
3) Paste your entire sequence of the 5' arm inside the Actual Sequence box.

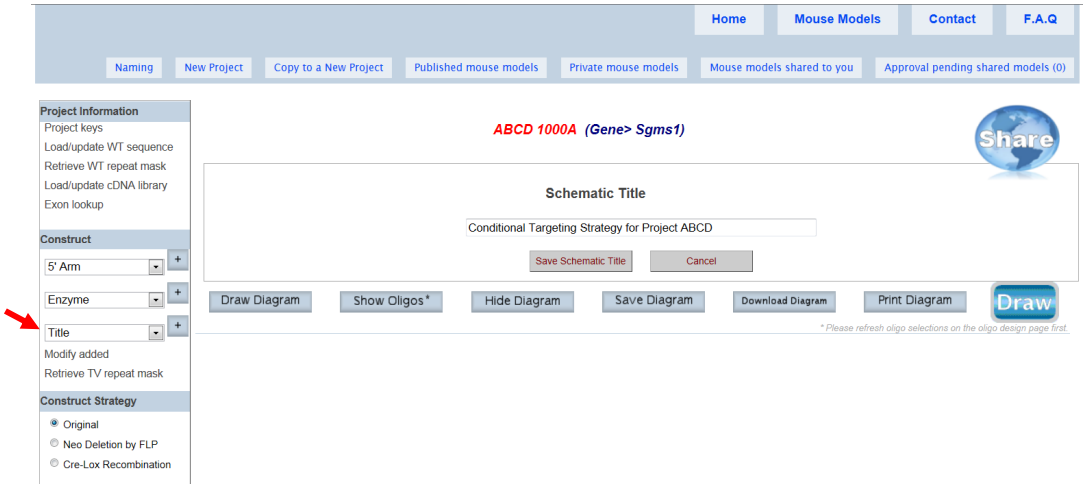
Direction (only) is not applicable for homology arms. It is only applicable for selection/reporter cassettes, exons, cDNA, etc.

Select a symbol of your choice. Typically a line is used to indicate the homology arm.

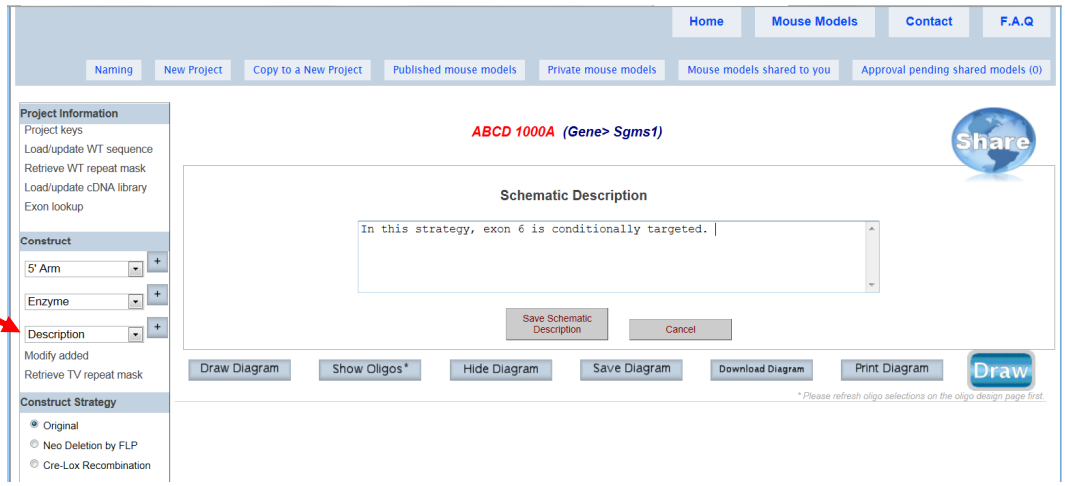
After you're done, press **Add/Update KO Component** button.



8. Title of the schematic diagram can be entered under Construct section on the left panel. Type in your title and click **Save Schematic Title**.



9. Description of the schematic diagram can be entered under Construct section on the left panel. Type in your description and click **Save Schematic Description**.



10. Click **Draw** or **Draw Diagram**. Your schematic diagram will be displayed with a title and a description. Your targeting vector is displayed with the WT (wild-type) and KO (knockout) allele.