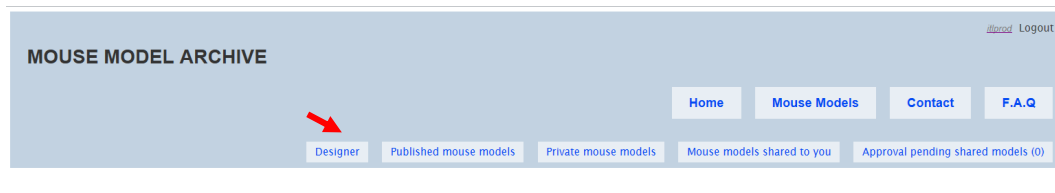


How do I input my targeting vector for a conditional (cre-lox) knockout?

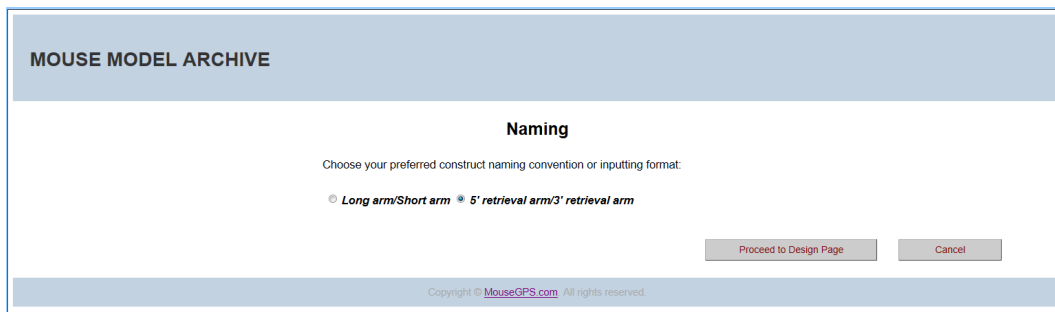
1. Click on **Designer** tab on the first page



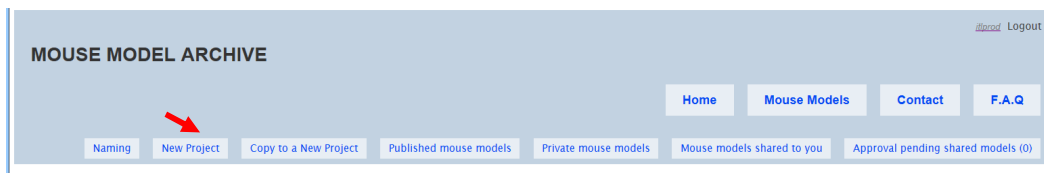
2. Click on **Naming** tab.



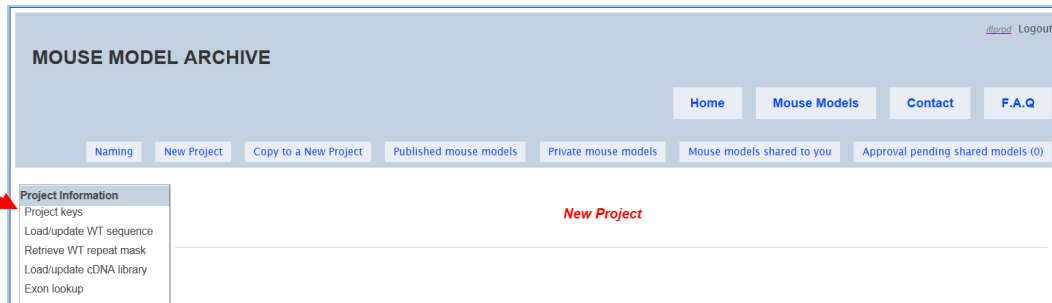
3. Choose which naming convention for your targeting vector and click **Proceed to Design Page** button.



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



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

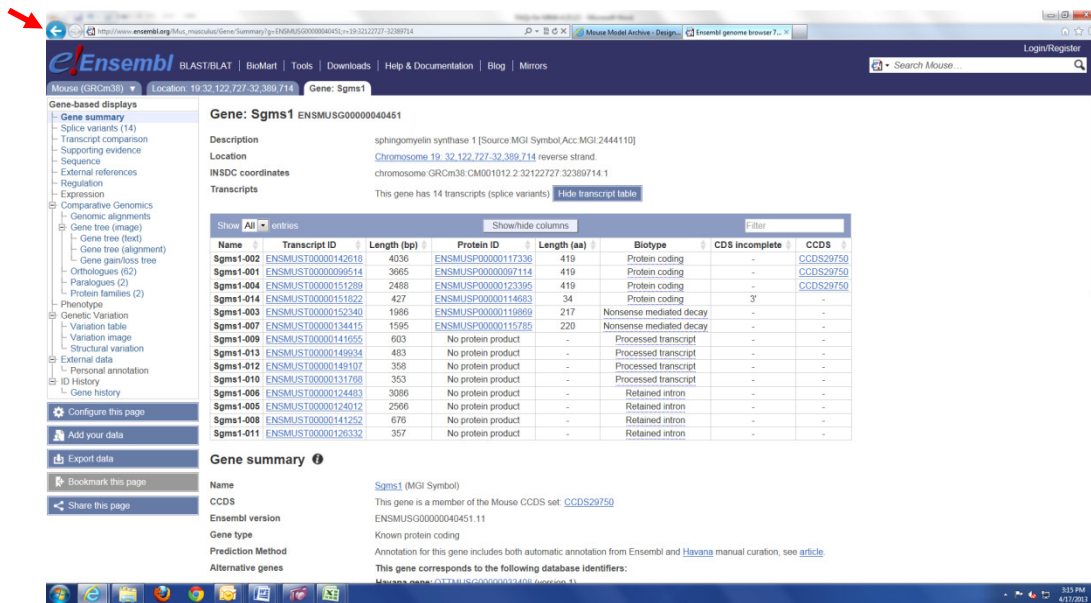


Project Category: Private – project can be seen only by you
Public – project can be seen by all users

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.

New Project

Project Keys

Project Category: ☒ Private ☐ Public

Project Name:

Project Number:

Project Type: [What is Cre-Lox Conditional 2a?](#)

TV Type: ☒ Plasmid ☐ BAC

Strain:

Project Manager:

Construct Designer:

Customer Name:

Gene Name:

Ensembl Gene-Summary Page URL: [view gene information](#)

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

MOUSE MODEL ARCHIVE

ABCD 1000A (Gene> Sgms1)

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

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

Export data		Exons ⓘ					
Bookmark this page		Show All entries					
Share this page		Show/hide columns					
Download view as RTF		Filter					
No.	Exon / Intron	Start	End	Start Phase	End Phase	Length	Sequence
5' upstream sequence							
1	ENSMUSE00000789664	32,349,316	32,348,693	-	-	624cttcctggatcaaggtctatcatgattattcatgttttataggagtcgtg AGACAGCCCTCCAGGAGCCACAAAGCAATGCTTTTATAGCTCTCTAAGAAATATCATTC ATTTTAACTAGTAGTAGTATTTTATAAATGCTTTGTGTGTGTTTATGGGTGGACACTTG GGCTTTCAATGCTGTGCCCTCTCACACCCCTGACTATCTCAGACAGCTAGGATTATGT CAGTGGGCGACAGACACACAGCTACACTTCTCTTCAGCCCAAGAGGCTTTT TAGGAAGCAAGCTGGGTATTTACTGGGATCTTCAGTTGTAAATATTATAGTCTGTGCT AAATTCAGGGGACAGTAAAGCTTAAAGCTCTCAGTGATGCTTCCGTGCTCAGAGGC CTCTGACCAAGGTGGAAATCTGTTTCCCACTTGGAGGAGGGGCTAGGTGGACAA CAGTCTTCCCTCTGCAATCTCAGACTTTCGAAGGAACCCGGGAAGGACCATTTGTG AATCTCGAGTCACTGTGTGAGGTGAAGTCCACATAGTTTGTCTCTCTCAGACTTGA GTCTGTTTATCTTTTAGAGATTACATTTCCCAAGAGGCTCAATCTCGGGATTC GTGAAGTCTCTCTCAGAGGAG
Intron 1-2		32,348,692	32,289,632			59,061	gtaggcttaccagatagagaggcg.....ttatttcctgaatttgttattctag GGAAAGCTTCAGTGGTGTATGAGCTGTGTACCTTTTACAGAGGTATATTGGAGCACAG ACCGGGGATATATATACAGACACACACAG
2	ENSMUSE00000513323	32,289,631	32,289,541	-	-	91	gtaaagtaaaccttgatctccac.....ttacttgtgtgttaattccacag gtaaagtaaaccttgatctccac.....ttacttgtgtgttaattccacag
Intron 2-3		32,289,540	32,260,098			29,443	gtaaagtaaaccttgatctccac.....ttacttgtgtgttaattccacag
3	ENSMUSE00000820259	32,260,097	32,259,976	-	-	122	GGGCACTGGATATATATGTTTGATGAAATGGCTTCTCAGGAATGTCACATAGAGAG GTGTGGCTTCTCTGAAGGAAGTCTTGTCTACAGAGGTGGGTTTGAAGTTTCCAGTCTC AG
Intron 3-4		32,259,975	32,248,037			11,939	gtgaggtccagtgctcactcttctt.....tttatatgttctaatttctcttag ACCGAGAAAGAGCTGATGAAGAAAGTAAACGATGATTGAG
4	ENSMUSE00000508860	32,248,036	32,247,994	-	-	43	gtaaatggagctttaattcttttat.....atgagattcttataccoggttaacag gtaaatggagctttaattcttttat.....atgagattcttataccoggttaacag
Intron 4-5		32,247,993	32,223,591			24,403	gtaaatggagctttaattcttttat.....atgagattcttataccoggttaacag
5	ENSMUSE00000509651	32,223,590	32,223,509	-	-	82	GTATATGGAGCTACTTGGACACCGCGGAGAGCTAGTATCTTGCAGGAAGCGAAGATGAG GAGGCTCTTTTAAATATCTC
Intron 5-6		32,223,508	32,160,364			63,145	gtaaatgaattttgtgagttctt.....ctgacattttctttgtccacag gtaaatgaattttgtgagttctt.....ctgacattttctttgtccacag
6	ENSMUSE00000518710	32,160,363	32,159,524	-	2	840	AAGGAAGAAATCTGTTGAGAAATGAGGCGAAGCAATGTTTGGACACCTTGTGATGAAGTG TTTTAAATCTGCCCTGACATCTTGGCAATGAGTCTCTGCTCATGAGGCCCAACAAGAT GAACACGACAGGAGGATATGTTCTGCTCTGACAGACAGTGTGAGCTGAGCTGTGAG AGCAAGCTGGGGTACTGAATGTTCTGCGAGACATGAGGAAGTGTTTACTGTGTC ACCAAGAAAGGTGGCAGACTGGCTCTGCGAGATGCTATGTCAGATATCTGTGAGCTCT GGACACTCTCAGAGCCAGAGCTTATCTACCTAACCAACAGAGATTCTAAAAACCCCTC ACTGTCCAGCTCTCTTCTCAGATGGGAGCACTCTTAAGATGATATAGAACTCTGAA GATGGAGCACAATATGGAACACACAGATAGGACCCACCAACAGACACTCAGCATTTGG CTTGTGATCTCAGACCCGATGGAGCTTCAAGCTGAGACTAACTCAGAGATGCTC AAATGGGTTTAGGAAGAGATGATCAGATCCCATGCGAAGACCGAGGCGTCCAGTA TCCATGGAGTGGGCGAGAGCTTCTGAGCTTTTATGCACTTCTGTTTGTGTTCT CACTACAGTGTGATCTGCTGCTCTCTCAAGACATATCTCTTAAGAGGTGAGCTCTC ACTACCGGACAGCTTTTTGACCAATTTAAACGGGTGCGATGGGGTTTTCTATTGCGA AATTACGGGATGATCTTTTAGGAGCTCTGGCTATTTCAAGTGTGCTCTTAAATACAA

- 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.

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Naming New Project Copy to a New Project Published mouse models Private mouse models Mouse models shared to you Approval pending shared models (0)

Project Information
Project keys
Load/update WT sequence
Retrieve WT repeat mask
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

ABCD 1000A (Gene> Sgms1)

Share

WT Sequence

Input Genomic Information of WT Sequence:

Chromosome#	Start	End	Strand	Sgms1 Transcript ID
19	32139554	Swap 32180363	Reverse na Forward Reverse	na

Or enter WT sequence here:

View Area
Retrieve Sequence

BLAST WT sequence

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

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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

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Share

WT Sequence

Input Genomic Information of WT Sequence:

Chromosome#	Start	End	Strand	Sgms1 Transcript ID
19	32139554	Swap 32180363	Reverse	na

Or enter WT sequence here:

View Area
Retrieve Sequence

ENSMUST00000142618
na
ENSMUST00000142618
ENSMUST00000099514
ENSMUST00000151289
ENSMUST00000151822
ENSMUST00000152340
ENSMUST00000134415
ENSMUST00000141655
ENSMUST00000149934
ENSMUST00000149107
ENSMUST00000131768
ENSMUST00000124483
ENSMUST00000124012
ENSMUST00000141252
ENSMUST00000126332

BLAST WT sequence

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.

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Project Information
Project keys
Load/update WT sequence
Retrieve WT repeat mask
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

ABCD 1000A (Gene> Sgms1)

WT Sequence

Input Genomic Information of WT Sequence:

Chromosome#	Start	End	Strand	Sgms1 Transcript ID
19	32139554	32180363	Reverse	ENSMUST00000142618

Swap

View Area
Retrieve Sequence

Sequence retrieval options
☒ WT, cDNA and Exons (replace existing exons) ☐ WT, cDNA and Exons (append to existing exons) ☐ WT only
Retrieve

Or enter WT sequence here:

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.

Project keys

- Load/update WT sequence
- Retrieve WT repeat mask
- 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
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- Client report on TV

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- Results & Phenotyping
- Blog

ABCD 1000A (Gene> Sgms1)

Component Sequence/Specifications

Component#:

1

Name:

5' Arm

Get Sequence (optional):

Method 1: Get component sequence from WT by specifying locations on the chromosome

Start Location:

End Location:

[Get Sequence](#)

Method 2: Get component sequence from WT by specifying starting and ending sequences

Sequence starts with:

A
T
C
G
<<

Sequence ends with:

A
T
C
G
<<

[Get Sequence](#)

Actual Sequence:

(Note: For Point Mutation, specify the region from the 1st PM to the last PM)

CAGACCATTATTTATAATTCTGTTATCCTGAGACAACAGGAAGTTACAAATAATGC
CTGGACTTTCATCCCATTTGATTTATCATGCATTATATTAAGCAAAATGATGTTTA
TAGCTGATGTCCTCATCAAAACATCAATATCACTATAAATATAGAAATGGGATAC
AATCTTGACTAGGACTTAGTCAGC-TGTTTAATGTTTGGTTTGGTTTGGTTTGGGG
TTTTTTTTTTTTTTTTTTTTTTTGGTTTTCGAGACAGGGTTTCTCTGTATCGCCCT
GGCTGTTCTGGAACCTCACTCTGTAGACCAGACTGGCCTTGAACTCAGAAATCCCTTG

[Show Uploaded](#) 6189 bps [BLAST this sequence](#)

Direction (only):

5'-3' v

Symbol:

Notation:

- 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.

Project keys
Load/update WT sequence
Retrieve WT repeat mask
Load/update cDNA library
Exon lookup

Construct
 LoxP
 Enzyme
 Title
 Modify added
 Retrieve TV repeat mask

Construct Strategy
☒ Original
☐ Neo Deletion by FLP
☐ Cre-Lox Recombination

Reporting
 Export TV sequence
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Component Sequence/Specifications

Component#: 2
Name: LoxP

Get Sequence (optional):
 Method 1: Get component sequence from WT by specifying locations on the chromosome
 Start Location: End Location:

Method 2: Get component sequence from WT by specifying starting and ending sequences
 Sequence starts with:
 Sequence ends with:

Actual Sequence:
 (Note: For Point Mutation, specify the region from the 1st PM to the last PM)

68 bps [BLAST this](#)

sequence

Direction (only): 5'-3'

Symbol:

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.

Retrieve WT repeat mask
Load/update cDNA library
Exon lookup

Construct
MA +
Enzyme +
Title +
Modify added
Retrieve TV repeat mask

Construct Strategy
☒ Original
☐ Neo Deletion by FLP
☐ Cre-Lox Recombination

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Component Sequence/Specifications

Component#: 3
Name: MA

Get Sequence (optional):
Method 1: Get component sequence from WT by specifying locations on the chromosome
Start Location: End Location:
Get Sequence

Method 2: Get component sequence from WT by specifying starting and ending sequences
Sequence starts with:
CAGCTTCCCTCACGTTCTATGATG A T C G <<
Sequence ends with:
GATGTATTTGTTGCCTGTTGCTAC A T C G <<
Get Sequence

Actual Sequence:
(Note: For Point Mutation, specify the region from the 1st PM to the last PM)

Hide Uploaded 1347 bps BLAST this sequence

upload new

Direction (only): 5'-3'
Symbol:
Notation:

- 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.

[illegible]

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.

Project keys
 Load/update WT sequence
 Retrieve WT repeat mask
 Load/update cDNA library
 Exon lookup

Construct
 3' Arm
 Enzyme
 Title
 Modify added
 Retrieve TV repeat mask

Construct Strategy
☒ Original
☐ Neo Deletion by FLP
☐ Cre-Lox Recombination

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Component Sequence/Specifications

Component#: 4
Name: 3' Arm

Get Sequence (optional):
 Method 1: Get component sequence from WT by specifying locations on the chromosome
 Start Location: End Location:

Method 2: Get component sequence from WT by specifying starting and ending sequences
 Sequence starts with:
 A T C G <<
 Sequence ends with:
 A T C G <<

Actual Sequence:
(Note: For Point Mutation, specify the region from the 1st PM to the last PM)

CAAGATTGCTCCAGAATTAAGACCATGAGCAATGCTAGTCTATTTTCAAGCAGTAGGA
 AAGAAACACTTAGACATCTTCAGAGAGCTGAGGCTGTTGAGTAACAGAGGGGGAAC
 ACCTACCTAGCTTGAATGGTGTCTACTCAGGTGAATGTTGCCAACCCTCCACCCCT
 GTCAGACCTAGAACCTCATCACTAAGTATTACAGTTTTTGACATGTTTAGACAGAT
 ATTCTACAATGACTTTGC

 250 bps [BLAST this sequence](#)

Direction (only): 5'-3'

Symbol:
☐ ☐ ☐ ☐

Notation:

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**.

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Project Information
 Project keys
 Load/update WT sequence
 Retrieve WT repeat mask
 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

ABCD 1000A (Gene> Sgms1)

Schematic Title

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

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**.

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Project Information
 Project keys
 Load/update WT sequence
 Retrieve WT repeat mask
 Load/update cDNA library
 Exon lookup

Construct
 5' Arm
 Enzyme
 Description
 Modify added
 Retrieve TV repeat mask

Construct Strategy
☒ Original
☐ Neo Deletion by FLP
☐ Cre-Lox Recombination

ABCD 1000A (Gene> Sgms1)

Schematic Description
 In this strategy, exon 6 is conditionally targeted.

Save Schematic Description Cancel

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

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

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.

