Step by step guide to insert your favorite gene into the SKI LODGE strains

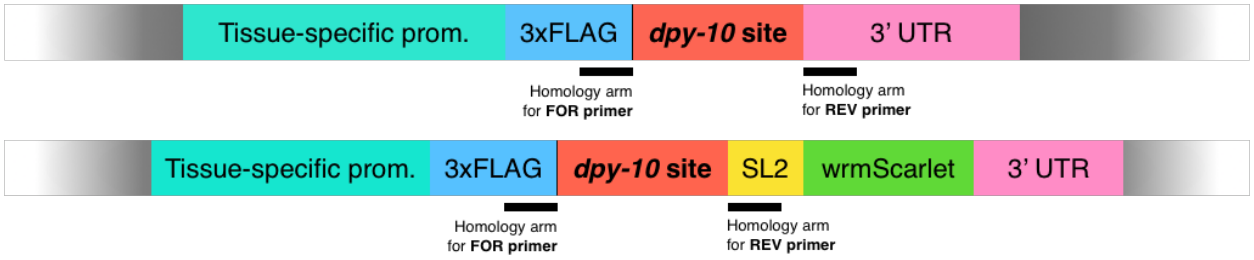
Step 1

Select the SKI LODGE strain to express your gene of interest

Step 2

Design primers with homologous recombination arms that contain at least 35 bp of SKI LODGE 3xFLAG sequence at the 5' end of the Forward primer, and at least 35 bp of the SKI LODGE 3'UTR sequences or SL2 sequence at the 5' end of the Reverse primer. Use the following models and table as a reference for primer designing.

Note: In your primer design, make sure the transcript to be inserted is in frame with the SKI LODGE epitope tag. The final correct edit should have your gene of interest in frame with the N-terminal epitope tag, followed immediately by SKI LODGE 3' UTR sequence or SL2 sequence. The entire *dpy-10* site will be removed.

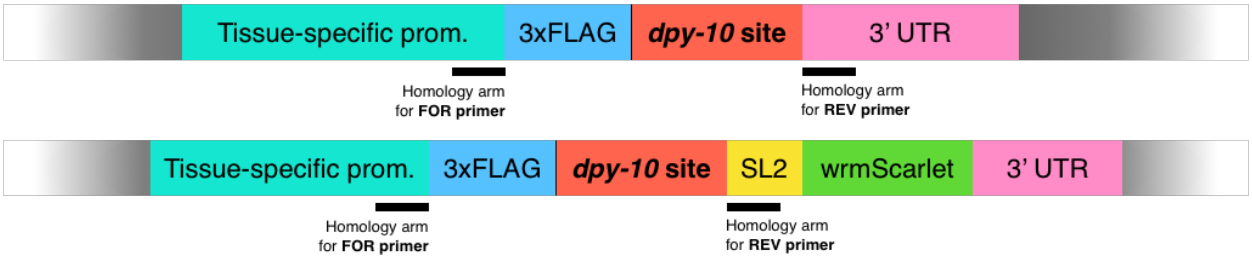


Sequences flanking *dpy-10* site in each SKI LODGE strain:

SKI LODGE strain	Left homology arm for **FOR primer** (3xFLAG) (60 bases)	Right homology arm for **REV primer** (3'UTR/SL2) (60 bases)	
myo-3p Chr. I:2851000 (WBM1126)	5'aaagaccatgacggtgattataaagatcatgac atcgattacaaggatgacgatgacaag	5'agcatgtagggatgttgaagagtaattgga cttagaagtcagaggcacgggcgcgagatg	*unc-54 3'UTR*
pie-1p Chr. III:7007700 (WBM1119)	5'aaagaccatgacggtgattataaagatcatgac atcgattacaaggatgacgatgacaag	5'agcatgtagggatgttgaagagtaattgga cttagaagtcagaggcacgggcgcgagatg	*unc-54 3'UTR*

rab-3p Chr. IV:5014900 (WBM1141)	5'aaagaccatgacggtgattataaagatcatgac atcgattacaaggatgacgatgacaag	5'gcgtttggaatttgggaaaattttgagttttta tagatagtataatagaacgtagaattt	*rab-3 3'UTR*
eft-3p Chr. V:8644800 (WBM1140)	5'aaagaccatgacggtgattataaagatcatgac atcgattacaaggatgacgatgacaag	5'agcatgtagggatgttgaagagtaattgga cttagaagtcagaggcacgggcgcgagatg	*unc-54 3'UTR*
eft-3p Chr. IV:5014900 (WBM1179)	5'aaagaccatgacggtgattataaagatcatgac atcgattacaaggatgacgatgacaag	5'agcatgtagggatgttgaagagtaattgga cttagaagtcagaggcacgggcgcgagatg	*unc-54 3'UTR*
rab-3p SL2 Chr. IV:5014900 (WBM1215)	5'aaagaccatgacggtgattataaagatcatgac atcgattacaaggatgacgacgataag	5'taaagagaagcatagattttaagacaagca gttaactaggtgaaagtaggatgagacagc	SL2
eft-3p SL2 Chr. V:8644800 (WBM1214)	5'aaagaccatgacggtgattataaagatcatgac atcgattacaaggatgacgacgataag	5'taaagagaagcatagattttaagacaagca gttaactaggtgaaagtaggatgagacagc	SL2
ges-1p SL2 Chr. V:8644800 (WBM1216)	5'aaggaccatgacggtgattataaagatcatga catcgattacaaggatgacgacgataag	5'taaagagaagcatagattttaagacaagc agttaactaggtgaaagtaggatgagacagc	SL2

Removing N-terminal epitope tag. To remove 3xFLAG tag, the Forward primer needs to contain an arm with at least 35 bp of SKI LODGE promoter sequences at the 5' end instead of the 3xFLAG sequence. The final correct edit should have the specific SKI LODGE promoter, then your gene of interest, followed immediately by SKI LODGE 3' UTR sequence or SL2 sequence. The entire 3xFLAG sequence and *dpy-10* site will be removed. Use the following models and table as a reference for primer designing when the 3xFLAG is not desired.



Sequences flanking 3xFLAG::*dpy-10* site in each SKI LODGE strain:

SKI LODGE strain	Left homology arm for **FOR primer** (end of promoter) (60 bases)	Right homology arm for **REV primer** (3'UTR/SL2) (60 bases)	
myo-3p (WBM1126)	5'cacttttaccgtctaattttcagggcagggagc catcaaacccacgaccactagatccat	5'agcatgtagggatgttgaagagtaattgga cttagaagtcagaggcacgggcgcgagatg	*unc-54 3'UTR*
pie-1p (WBM1119)	5'aggtttctttttgcagtatttcgtttcccaaacaa ttaaaaatcaaattttcttttccag	5'agcatgtagggatgttgaagagtaattgga cttagaagtcagaggcacgggcgcgagatg	*unc-54 3'UTR*
rab-3p (WBM1141)	5'tgctcttttaaaataaatctacagtagccctattt tcagatgacaagtttgtaccccggg	5'gcgtttggaatttgggaaaattttgagtttttat agatagtataatagaacgtagaattt	*rab-3 3'UTR*
eft-3p (WBM1140)	5'cgcactcttcttacttttaaattaaattgttttttttt cagttgggaaacactttgctca	5'agcatgtagggatgttgaagagtaattgga cttagaagtcagaggcacgggcgcgagatg	*unc-54 3'UTR*
eft-3p (WBM1179)	5'cgcactcttcttacttttaaattaaattgttttttttt cagttgggaaacactttgctca	5'agcatgtagggatgttgaagagtaattgga cttagaagtcagaggcacgggcgcgagatg	*unc-54 3'UTR*
rab-3p SL2 (WBM1215)	5'tgctcttttaaaataaatctacagtagccctattt tcagatgacaagtttgtaccccggg	5'taaagagaagcatagattttaagacaagca gttaactaggtgaaagtaggatgagacagc	SL2
eft-3p SL2 (WBM1214)	5'cgcactcttcttacttttaaattaaattgttttttttt cagttgggaaacactttgctca	5'taaagagaagcatagattttaagacaagca gttaactaggtgaaagtaggatgagacagc	SL2
ges-1p SL2 (WBM1216)	5'tcgtctgcgtcttacagtttcaggcttcaaaaa tctattacatatcttatctttgaattc	5'taaagagaagcatagattttaagacaagca gttaactaggtgaaagtaggatgagacagc	SL2

Step 3

Make a mastermix with your newly designed primers and amplify your homology repair template by PCR using the following conditions:

Reagent	**Volume (µl)**
H_2O	Up to 300
FOR primer (100 µM)	1.5
REV primer (100 µM)	1.5
DNA template	400-600 ng
Phusion 2x Master Mix	150

Divide mastermix between 6 PCR tubes, 50 µl per tube. Use the following gradient and PCR cycle conditions:

Tube	Tm	PCR cycle steps		
1	60 ºC	**1**	98° 2'	
2	62 ºC	**2**	98° 30"	35x cycles
3	64 ºC	**3**	**Gradient 30"**	
4	66 ºC	**4**	72° *	
5	68 ºC	**5**	72° 10'	
6	70 ºC	**6**	**4**° ∞	

*Extension time will depend of the length of your gene of interest. The typical recommendation is to allow 1 min of extension time for every 1 kb to be amplified.

Step 4

Check PCR products on a gel to verify that the Homologous Recombination (HR) template has amplified as a clean single band. Add 5 µl of 10x orange loading buffer to each sample and run 5 µl on a 1% agarose gel.

Step 5

Purify and concentrate your homology repair template by pooling all reactions that amplified as a strong single band into a single sample, and pass it through a Qiagen minelute column (#28006). Follow kit instructions, and elute with 10 µl of water. Determine the concentration of your purified template.

Step 6

Assemble of CRISPR/Cas9 complex *in vitro*:

Reagent	Volume (µl)
H_2O	Up to 10
Hepes pH7.4 (200 mM)	0.375
KCl (1 M)	0.25
tracrRNA (4 µg/µl)	2.5
dpy-10 crRNA (2.6 µg/µl)	0.6
dpy-10 ssODN (500 ng/µl)	0.25
PCR Template (ng/µl)	*
Purified Cas9 (12 µg/µl)	2.0
Total:	10 µl

*Note that we have had great success getting CRISPR edits using a final concentration of HR template of 400-600 ng/µl in the CRISPR mix.

Injection mixes can be prepared in advance without Cas9 protein, separated into 2 tubes of 4 µl, and stored at -80ºC. We have found that frozen mixes stored up to one year at -80ºC are still effective at generating CRISPR edits. Before injecting, thaw one 4 µl mix, add 1 µl purified Cas9 (12 µg/µl), mix by pipetting, spin for 2 min at 13000 rpm, and incubate at 37ºC for 10 min. Inject into day 1 adults of the relevant SKI LODGE line.

Step 7

3-4 days after injection, screen for plates that have produced many dumpy and/or roller progenies. From these plates, individually plate single *dpy* animals and allow them to lay eggs before screening them for your desired edit, either by looking for fluorescent expression and/or genotyping them by PCR. We have annotated forward primers close to the end of every tissue-specific promoter, and reverse primers at the beginning of 3'UTR or wrmScarlet that can be used for screening, genotyping, and sequencing your desired edit. Use the next models and table as a reference for primer designing.

Note: if you design new primers for genotyping, choose them outside of the homology arms to look for successful insertion.

Primers flanking the insertion in each SKI LODGE cassette:

SKI LODGE strain	**FOR primer** (end of promoter)	**REV primer** (beginning of 3'UTR/wrmScarlet)	**SKI LODGE band without insertion (bp)**
myo-3p (WBM1126)	CGSG 123 5' ggattctttgcttgtcaaccagc	CGSG 117 5' aatagggggtgggagcacag	279
pie-1p (WBM1119)	CGSG 130 5' cggcggcaaaaatggatttctc	CGSG 117 5' aatagggggtgggagcacag	299
rab-3p (WBM1141)	CGSG 105 5' ctacagtagccctattttcagatgac	CGSG 168 5' ggaaaactgagagctacgcgc	243

eft-3p (WBM1140)	eft-3p seq fwd_2 5' ctaccgtccgcactcttctta	CGSG 117 5' aatagggggtgggagcacag	255
eft-3p (WBM1179)	eft-3p seq fwd_2 5' ctaccgtccgcactcttctta	CGSG 117 5' aatagggggtgggagcacag	255
rab-3p SL2 (WBM1215)	CGSG 105 5' ctacagtagccctattttcagatgac	CGSG 285 5' ttgagcttggcggtttggg	598
eft-3p SL2 (WBM1214)	eft-3p seq fwd_2 5' ctaccgtccgcactcttctta	CGSG 285 5' ttgagcttggcggtttggg	555
ges-1p SL2 (WBM1216)	ges-1p INT SEQ 5' cgtctgcgtcttacagtttcaggc	CGSG 285 5' ttgagcttggcggtttggg	546

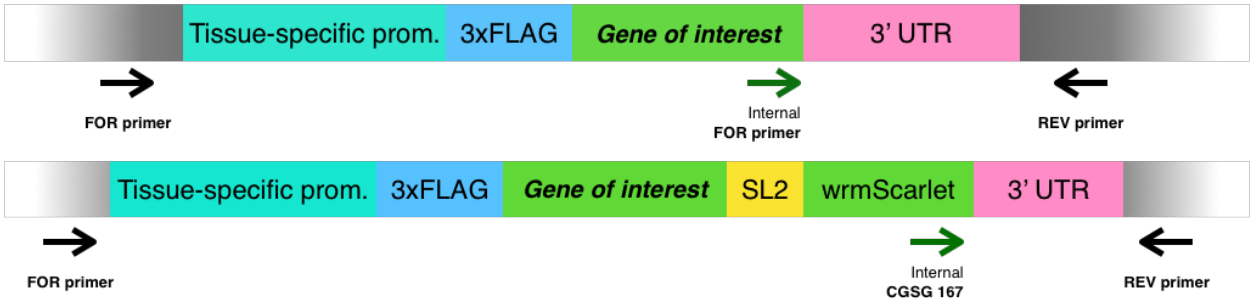
Step 8

From those F1 positives, individually plate single F2 *dpy* animals again, allow them to lay eggs and then screen them. Continue doing this until getting homozygous animals for your desired edit. Confirm that the knock-in CRISPR edit is correct by sequencing.

Step 9

Outcross your newly made SKI LODGE line to get rid of the *dpy-10* Co-CRISPR edit, as well as any other off-site mutations. Note that a good outcrossing genotyping strategy is necessary since outcrossing will be done using the N2 strain and not SKI LODGE strains. The following models and table show primers flanking the SKI LODGE cassette that can be used to outcross the new SKI LODGE line to N2. We also recommend designing an internal Forward primer close to the 3' end of your knocked in gene or use CGSG 167 primer localized at the end of wrmScarlet for SL2 lines. Using this 3 primer PCR strategy, you will obtain a SKI LODGE knock-in band around 1000 bp.

Figure of final SKI LODGE strains after knock in, and positions for primers:



Primers flanking each SKI LODGE cassette for outcrossing:

SKI LODGE strain	FOR primer	REV primer	Wild-type band (bp)
myo-3p (WBM1126)	CGSG 143 5’ taagcttctcacacctttctctcg	CGSG 124 5’ cctcccctcatctcaattatcccg	245
pie-1p (WBM1119)	CGSG 129 5’ cctgggaacaataagtcggtgaag	CGSG 131 5’ atgtctggcggtccaaagtg	441
rab-3p (WBM1141)	CGSG 104 5’ gaactttgcagtttggttgtagtg	CGSG 106 5’ tcaatccgttcatttgagccc	252
eft-3p (WBM1140)	CGSG 205 5’ accttcgacctcactttccctc	CGSG 206 5’ ccgtcctgaagtatacccagatcc	357
eft-3p (WBM1179)	CGSG 104 5’ gaactttgcagtttggttgtagtg	CGSG 106 5’ tcaatccgttcatttgagccc	252
rab-3p SL2 (WBM1215)	CGSG 104 5’ gaactttgcagtttggttgtagtg	CGSG 106 5’ tcaatccgttcatttgagccc	252
eft-3p SL2 (WBM1214)	CGSG 205 5’ accttcgacctcactttccctc	CGSG 206 5’ ccgtcctgaagtatacccagatcc	357
ges-1p SL2 (WBM1216)	CGSG 205 5’ accttcgacctcactttccctc	CGSG 206 5’ ccgtcctgaagtatacccagatcc	357

Step 10 (optional)

The major predicted off-target site of the *dpy-10* crRNA is in an exon of R12E2.15. After outcrossing, to check potential off-target events from the *dpy-10* crRNA, we recommend using primers CGSG 273 and 274. These primers amplify a fragment of 873 bp from the R12E2.15 gene flanking the potential off-target sequence. Use this PCR product for sequencing with the same primers.