

Introduction to



SKILODGE

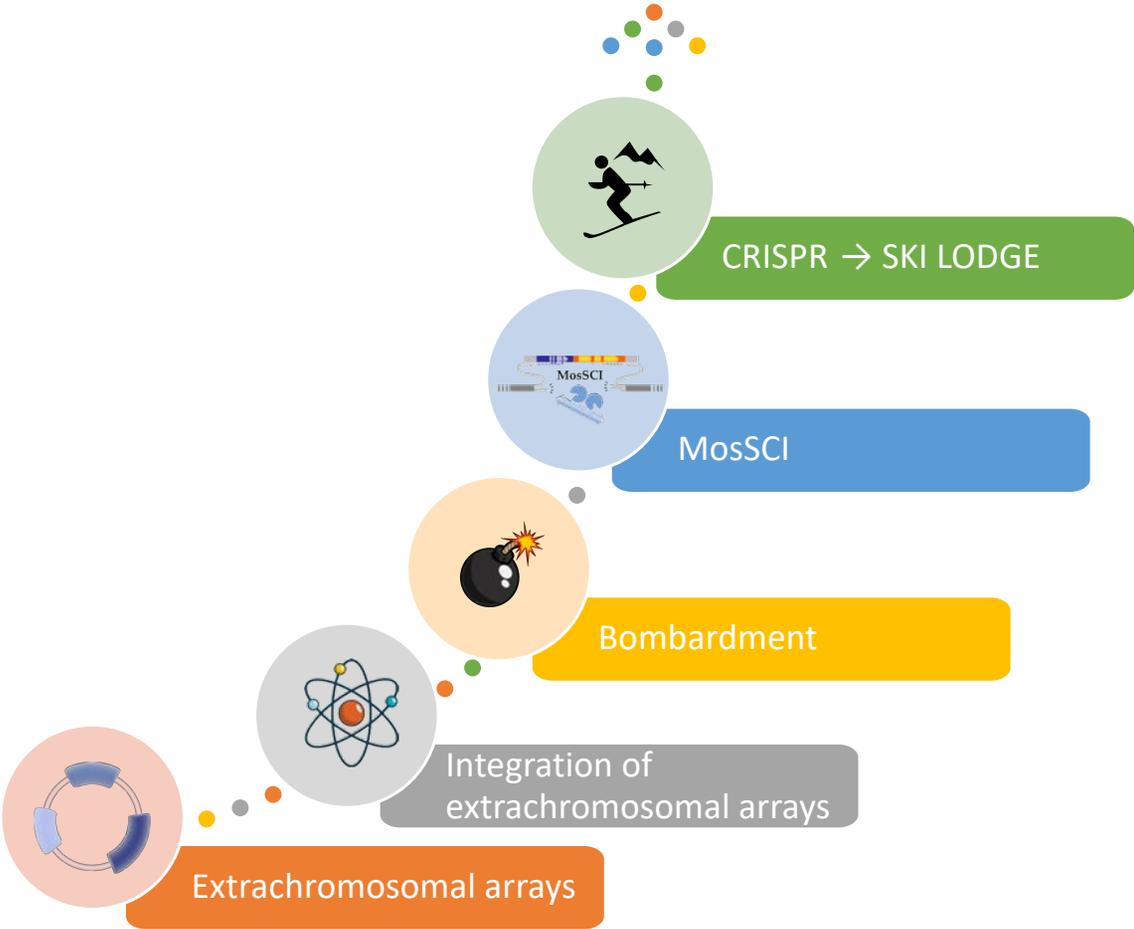
a single-copy knock-in system for defined gene expression

Carlos Giovanni Silva-García
The Mair Lab

Harvard T.H. Chan School of Public Health
Department of Genetics and Complex Diseases

<https://www.themairlab.com/skilodge>

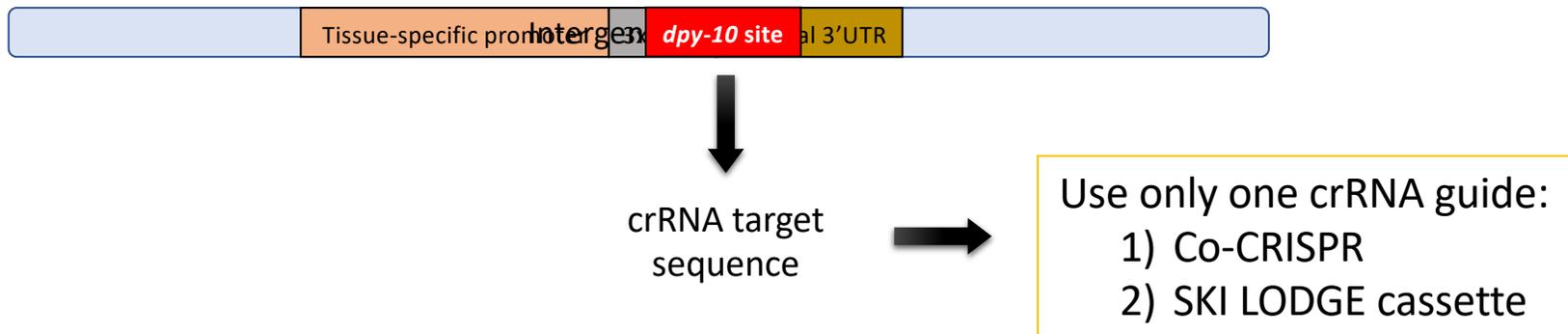
Single-copy transgene expression for defined gene expression



Single-copy Knock-In LOci for Defined Gene Expression

SKI LODGE

“The idea”



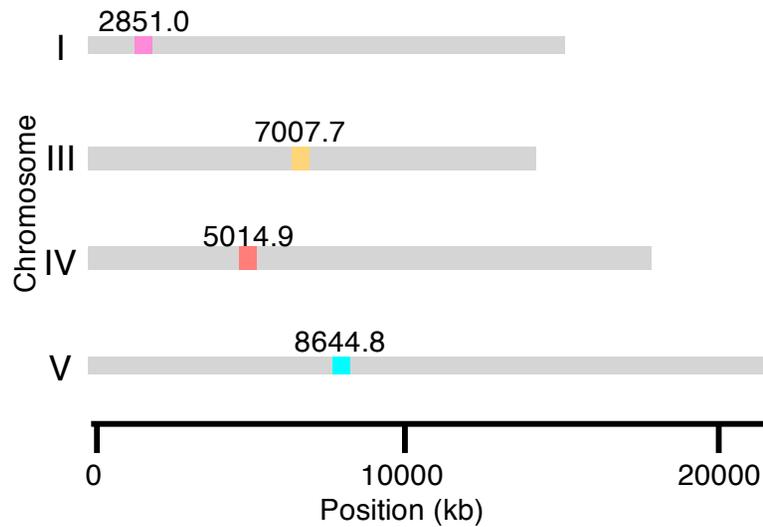
1] How we made the SKI LODGE strains.

2] How to use the SKI LODGE strains.

[1]

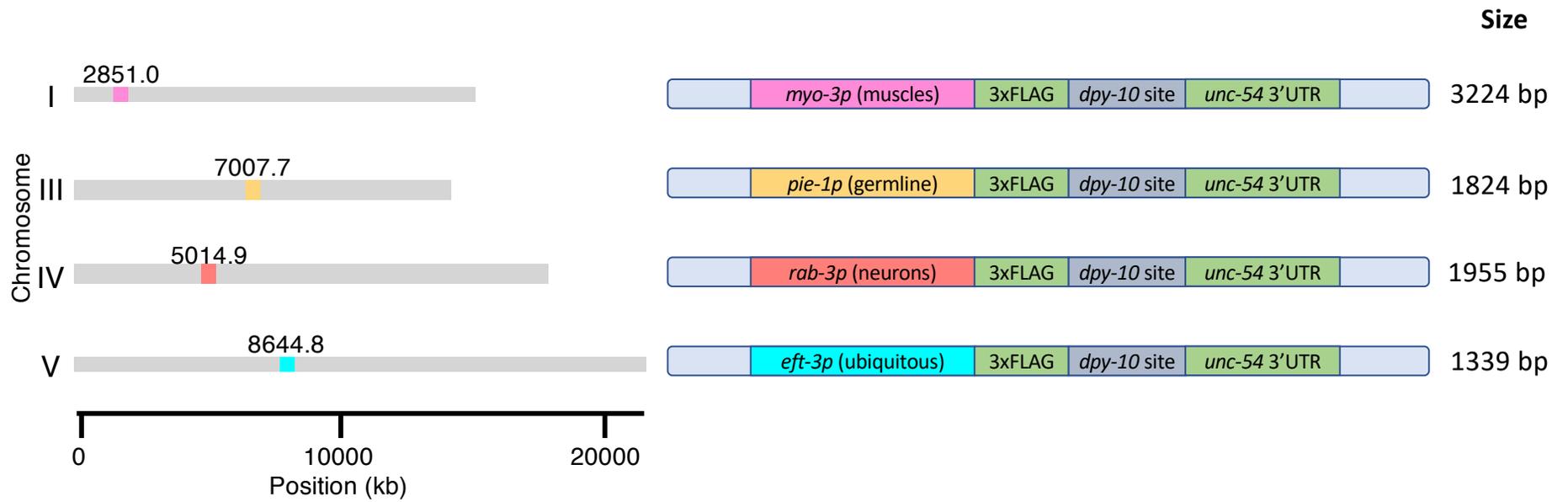
How we made the SKI LODGE strains

Making the strains



- **CRISPR protocol:**
Paix *et al.*, Genetics, 2015.
- **Intergenic regions:**
Frøkjær-Jensen *et al.*, Nature Genetics, 2008. MosSCI

Strains

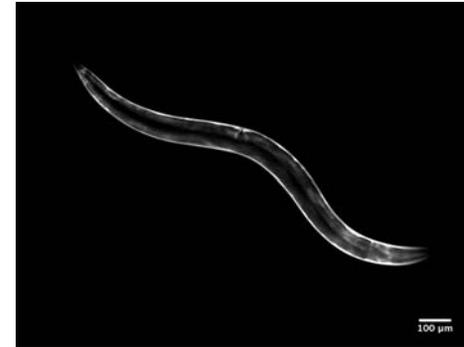


Testing the strains

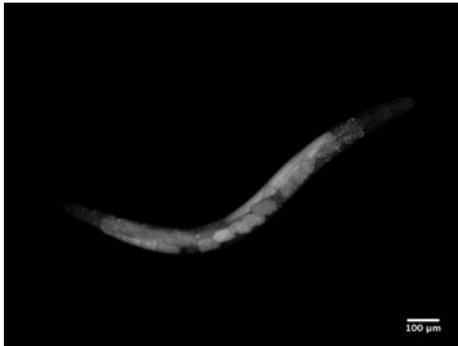
eft-3p (ubiquitous) 3xFLAG wrmScarlet *unc-54* 3'UTR



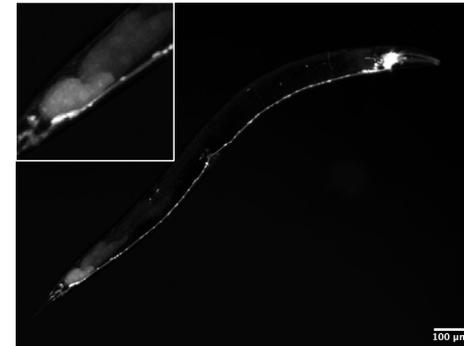
myo-3p (muscles) 3xFLAG wrmScarlet *unc-54* 3'UTR



pie-1p (germline) 3xFLAG GFP *unc-54* 3'UTR



rab-3p (neurons) 3xFLAG wrmScarlet *unc-54* 3'UTR



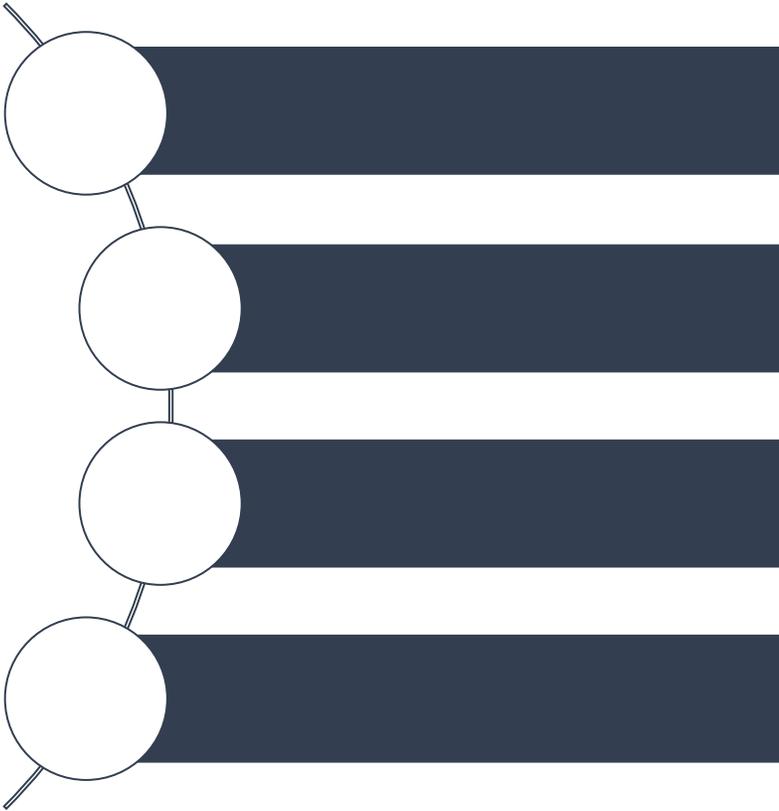
The SKI LODGE strains are available at the CGC

Strain	Short description	Expression	Chromosome position	Verified expression with	Genotype
WBM1119	pie-1 promoter	Germline	III: 7.00 MB	GFP	N2, wbmIs60[pie-1p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR, III:7007600]
WBM1126	myo-3 promoter	Muscles	I: 2.85 MB	wrmScarlet	N2, wbmIs61[myo-3p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR, I:2851000]
WBM1140	eft-3 promoter	Ubiquitous	V: 8.64 MB	wrmScarlet	N2, wbmIs65[eft-3p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR, V:8645000]
WBM1141	rab-3 promoter	Neurons	IV: 5.01 MB	wrmScarlet	N2, wbmIs66[rab-3p::3XFLAG::dpy-10 crRNA::rab-3 3'UTR, IV:5015000]
WBM1179	eft-3 promoter	Ubiquitous	IV: 5.01 MB	GFP	N2, wbmIs76[eft-3p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR, IV:5015000]
WBM1214	eft-3 promoter + SL2::wrmScarlet	Ubiquitous	V: 8.64 MB	N/A	N2, wbmIs88[eft-3p::3XFLAG::dpy-10 crRNA::SL2::wrmScarlet::unc-54 3'UTR, *wbmIs67]
WBM1215	rab-3 promoter + SL2::wrmScarlet	Neurons	IV: 5.01 MB	N/A	N2, wbmIs89[rab-3p::3XFLAG::dpy-10 crRNA::SL2::wrmScarlet::rab-3 3'UTR, *wbmIs68]
WBM1216	ges-1 promoter + SL2::wrmScarlet	Intestine	V: 8.64 MB	N/A	N2, wbmIs96[ges-1p::3XFLAG::dpy-10 crRNA::SL2::wrmScarlet::unc-54 3'UTR, *wbmIs88]

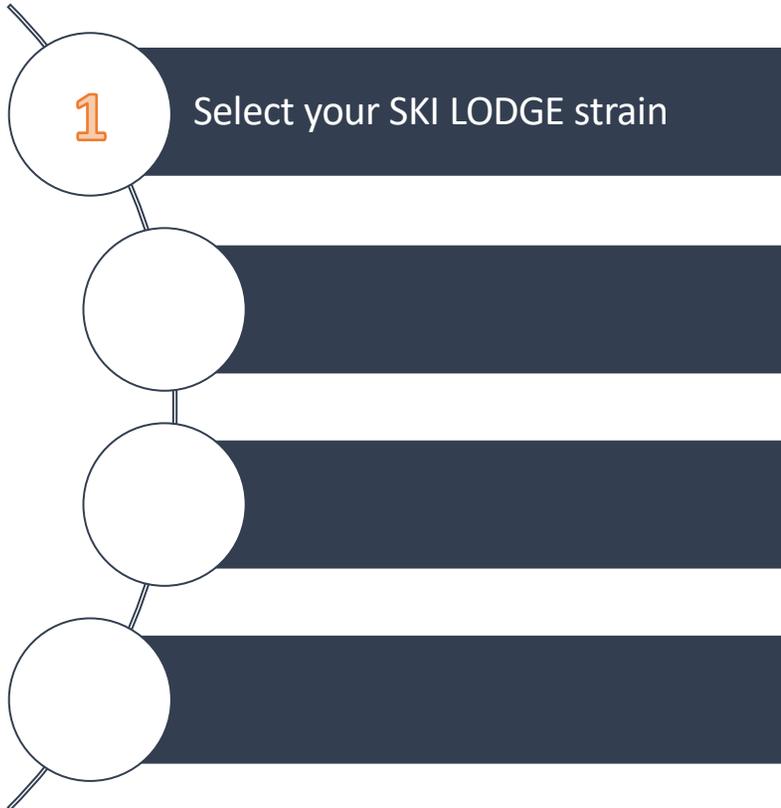
[2]

How to use the SKI LODGE strains

Using the SKI LODGE strains

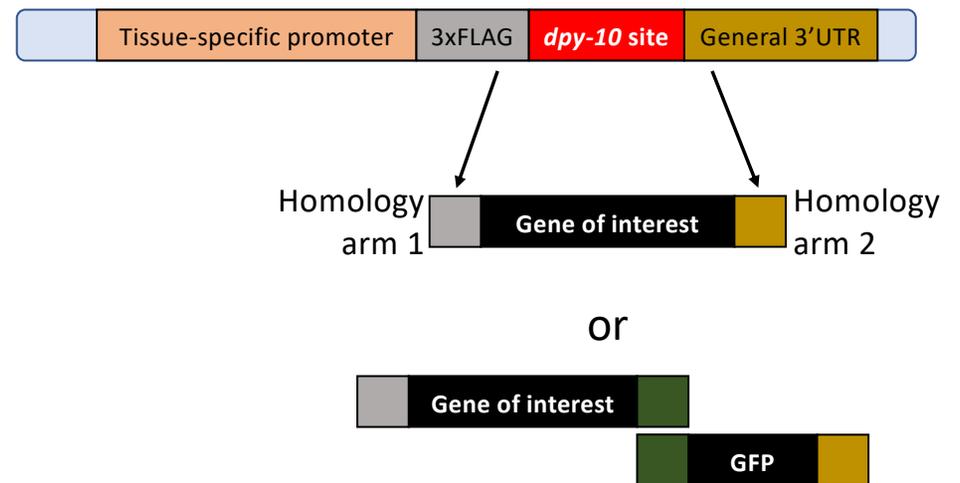
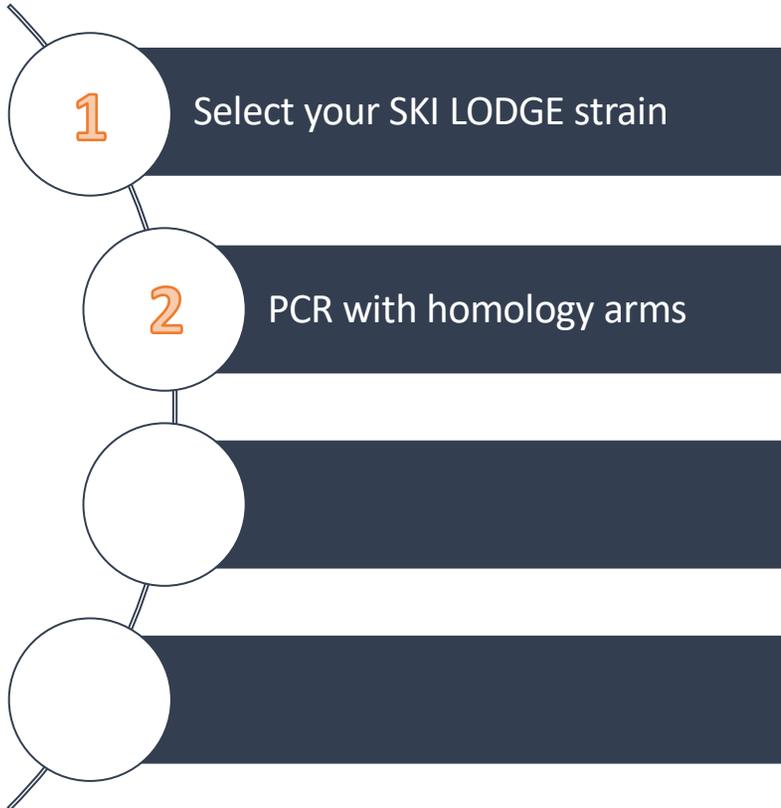


Using the SKI LODGE strains

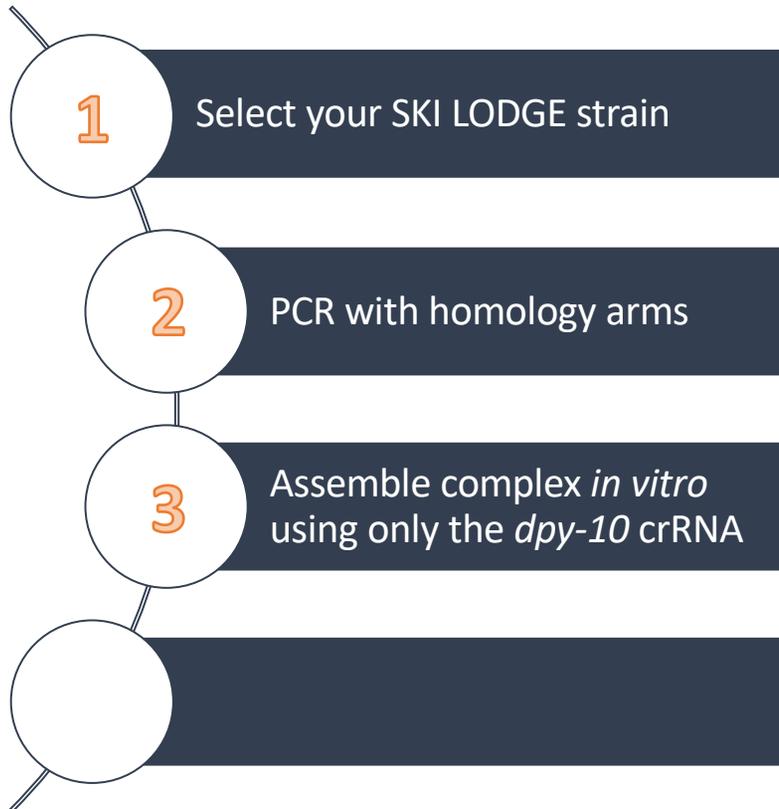


Strain	Short description
WBM1119	pie-1 promoter
WBM1126	myo-3 promoter
WBM1140	eft-3 promoter
WBM1141	rab-3 promoter
WBM1179	eft-3 promoter
WBM1214	eft-3 promoter + SL2::wrmScarlet
WBM1215	rab-3 promoter + SL2::wrmScarlet
WBM1216	ges-1 promoter + SL2::wrmScarlet

Using the SKI LODGE strains



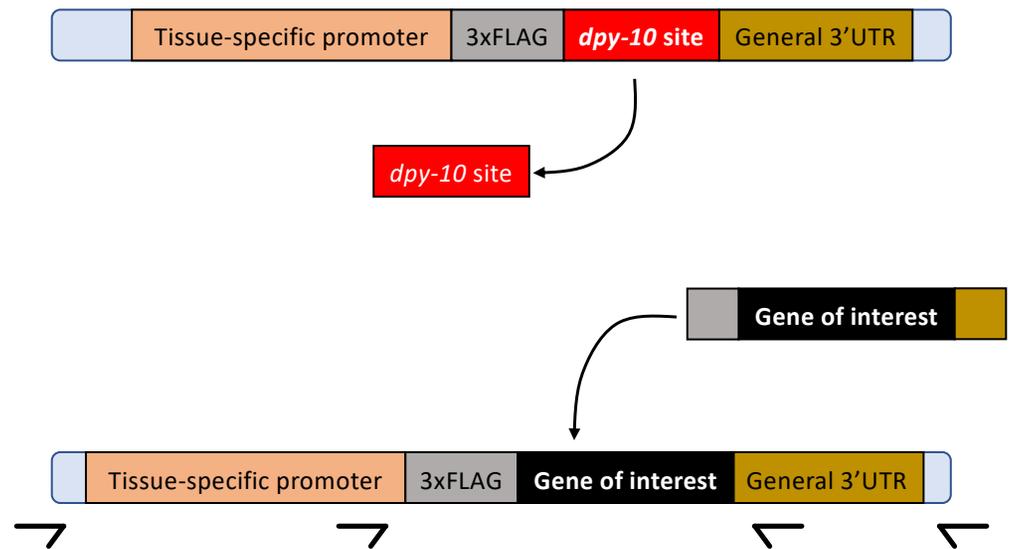
Using the SKI LODGE strains



- a) PCR template.
- b) *dpy-10* crRNA:
 - Co-CRISPR marker
 - Target SKI LODGE cassette
- c) Purified Cas9 + other reagents following the Paix protocol (2015), & Silva-García (2019).

Using the SKI LODGE strains

- 1 Select your SKI LODGE strain
- 2 PCR with homology arms
- 3 Assemble complex *in vitro* using only the *dpy-10* crRNA
- 4 Inject into SKI LODGE strain and screening



Tips!



PCR template length (~2000 bp).

High template concentration (500-600 ng/ul).

Large homology arms are better (>50 bp).

Well-honed microinjection technique
(10 injected worms = >200 dpys).

Good PCR genotyping strategy.

Conclusions

The SKI LODGE system will give you defined gene expression from single copy transgenes.

Use your favorite CRISPR protocol for the SKI LODGE lines.

Use the *dpy-10* crRNA.



William Mair (PI): wmair@hsph.harvard.edu

My email: cgsilva@hsph.harvard.edu

Single-Copy Knock-In Loci for Defined Gene Expression in *Caenorhabditis elegans*

Carlos G. Silva-García, Anne Lanjuin, Caroline Heintz, Sneha Dutta, Nicole M. Clark, and William B. Mair¹
Department of Genetics and Complex Diseases, Harvard T. H. Chan School of Public Health, Harvard University, Boston, Massachusetts 02115
ORCID ID: 0000-0002-0661-1342 (W.B.M.)



SKI LODGE

A B O U T

We have generated a single-copy knock-in loci for defined gene expression (SKI LODGE) system to insert any DNA by CRISPR/Cas9 at defined safe harbors in the *C. elegans* genome. Utilizing a single crRNA guide, which also acts as a Co-CRISPR enrichment marker, any DNA sequence can be introduced as a single copy, regulated by different tissue-specific promoters. The SKI LODGE system provides a fast, economical and effective approach for generating single-copy ectopic transgenes in *C. elegans*.

(Silva-García *et al.* G3 2019)

[OVERVIEW](#)[STEP BY STEP GUIDE](#)[STRAINS](#)

Thanks!



[@TheMairLab](#)

William Mair, Anne Lanjuin, Caroline Heintz, Sneha Dutta, Nicole M. Clark.

Pallas Yao, Porsha Howell, Arpit Sharma, Rohan Sehgal, Hannah Smith, Malini Bhadra.

- *Yerby Fellowship Program*
- *Aramont Fellowship Program*



**HARVARD
T.H. CHAN**

SCHOOL OF PUBLIC HEALTH
Powerful ideas for a healthier world



DEPARTMENT OF
**GENETICS AND
COMPLEX DISEASES**

POSTERS:
1063C (Hannah)
1065B (Arpit)
1066C (Porsha)