中国科学技术大学
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Genome manipulation using CRISPR/Cas9 technology in *C. elegans*

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2021-10-17

THE GENETICS OF *CAENORHABDITIS ELEGANS*

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Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England

Manuscript received December 10, 1973

Methods are described for the isolation, complementation and mapping of mutants of *Caenorhabditis elegans*, a small free-living nematode worm. About 300 EMS-induced mutants affecting behavior and morphology have been characterized and about one hundred genes have been defined. Mutations in 77 of these alter the movement of the animal. Estimates of the induced mutation frequency of both the visible mutants and X chromosome lethals suggests that, just as in Drosophila, the genetic units in *C. elegans* are large.

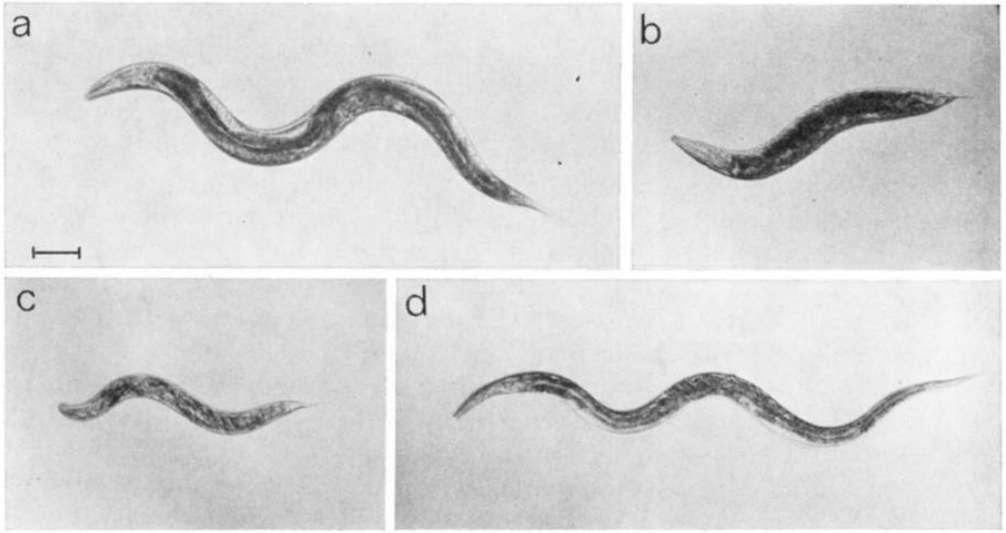


FIGURE 1.—Photomicrographs of *C. elegans* and some of its mutants. a: wild type, b: dumpy (*dyp-1*), c: small (*sma-2*), d: long (*lon-1*). The scale is 0.1 mm.

Phenotypes of mutants:

Uncoordinated mutants

Roller mutants`

Dumpy and small mutants

Long mutants

Blistered mutants

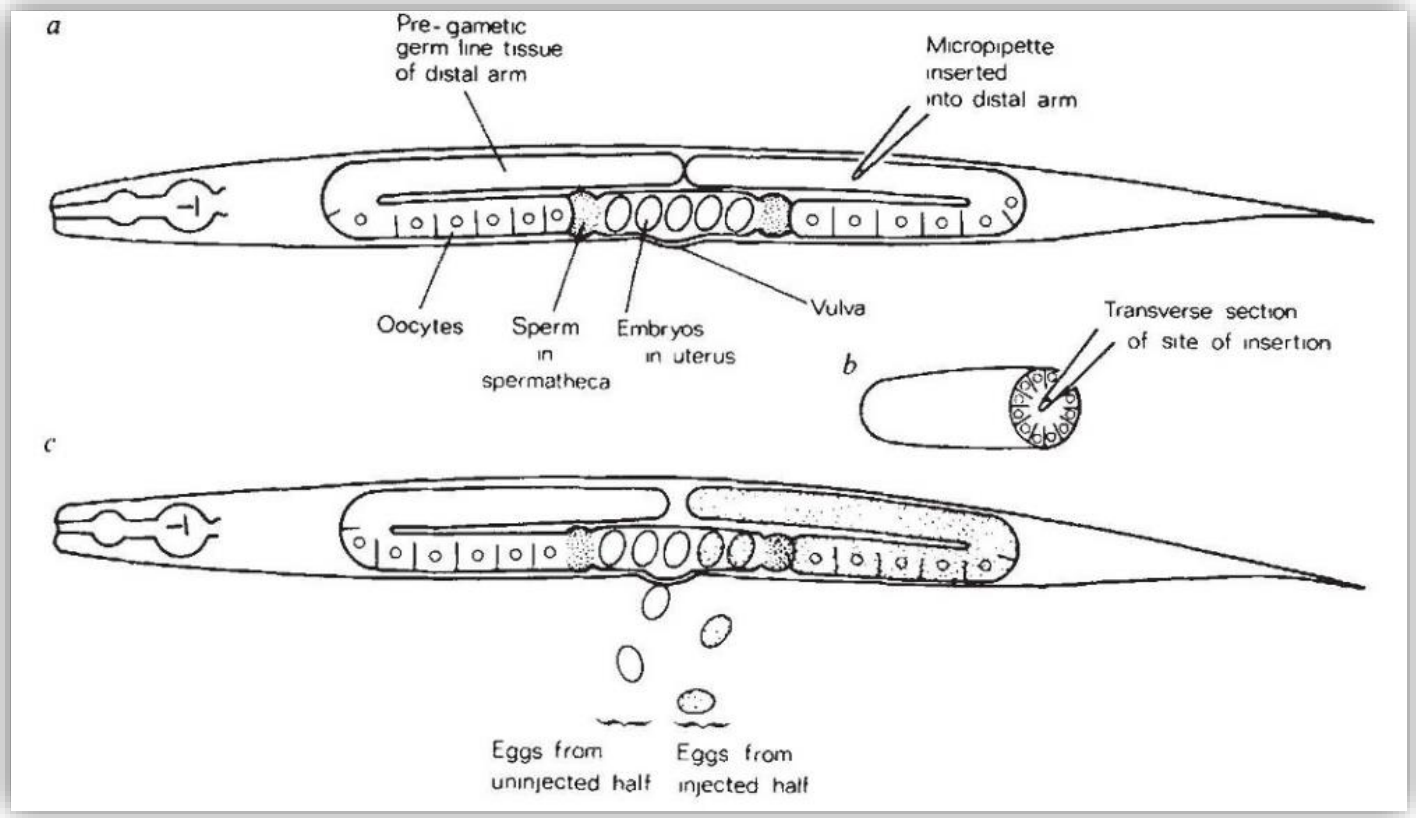
.....

Methods for genome manipulation in *C. elegans*

Method	Types (references)	Purposes
Genome-wide mutagenesis		
Chemical mutagenesis	EMS (Brenner, 1974) TMP/UV (Barstead and Moerman, 2006) ENU (De Stasio and Dorman, 2001) formaldehyde (Johnsen and Baillie, 1988) NTG (Greenwald and Horvitz, 1980) DES (Greenwald and Horvitz, 1980) acetaldehyde (Greenwald and Horvitz, 1980) DEO (Anderson and Brenner, 1984) DEB (Trent et al., 1991)	Forward genetic screens, target-selected mutagenesis
Radiation mutagenesis	Short wave UV (Stewart et al., 1991) IR (Rosenbluth et al., 1985) ^{32}P decay (Babu and Brenner, 1981)	Forward genetic screens, generate deficiencies
Transposon insertional mutagenesis	Tc1 (Martin et al., 2002) *Mos1* (Boulin and Bessereau 2007)	Forward genetic screens, generate insertion mutants for use in gene-targeted mutagenesis
Target-selected mutagenesis		
PCR-based methods	restricted extension time (Jansen et al., 1997) poison primer (Edgley et al., 2002) thermostable restriction enzymes (Huang et al., 2006; Wei et al., 2002)	Recover deletion in specific gene after whole genome mutagenesis
TILLING	(Gilchrist et al., 2006)	Isolate point mutations/allelic series in a gene of interest
G4 DNA-induced mutations	(Pontier et al., 2009)	Isolate deletion alleles for genes near G4 genomic site
Gene-targeted mutagenesis		
Transposon-based methods	Tc1 (Zwaal et al., 1993; Barrett et al., 2004) MosTIC (Robert, 2012) MosDEL (Frøkjaer-Jensen et al., 2010; Frøkjaer-Jensen et al., 2012)	Make targeted changes or deletions in gene of interest without mutagenizing entire genome
Enzyme-based methods	ZFNs (Wood et al., 2011) TALENs (Wood et al., 2011) CRISPR/Cas (Friedland et al., 2013; Dickinson et al., 2013)	Create small indels or repair off a transgene to create mutations

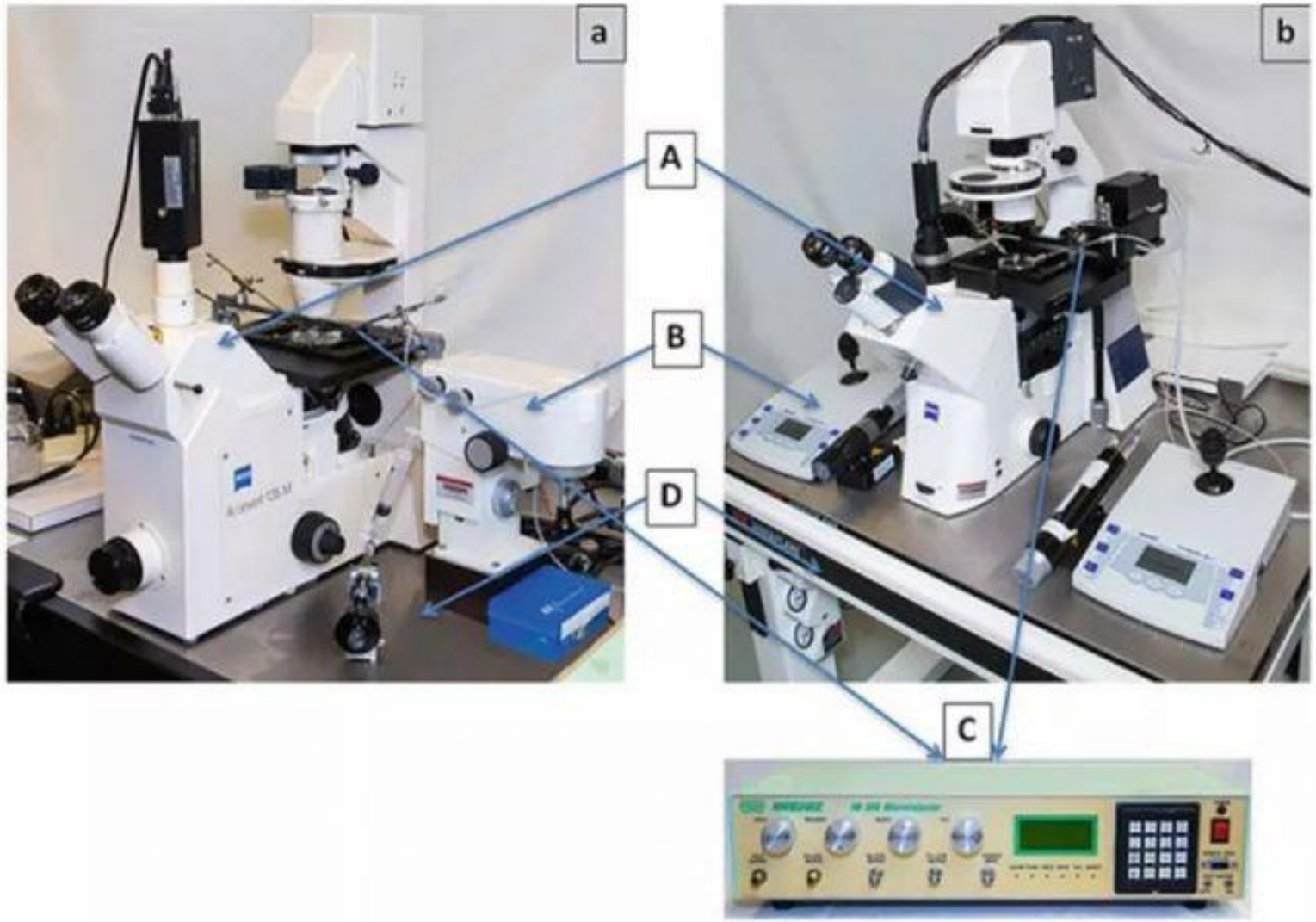
- UV/TMP genome manipulation
- Microparticle bombardment
- Cre/LoxP and FLP/FRT systems
- MosI system
- ZFNs and TALEN technologies
- CRISPR/Cas9 technology

Introduction of tRNA into embryos by microinjection into the parental gonad to in vivo suppress an amber mutant

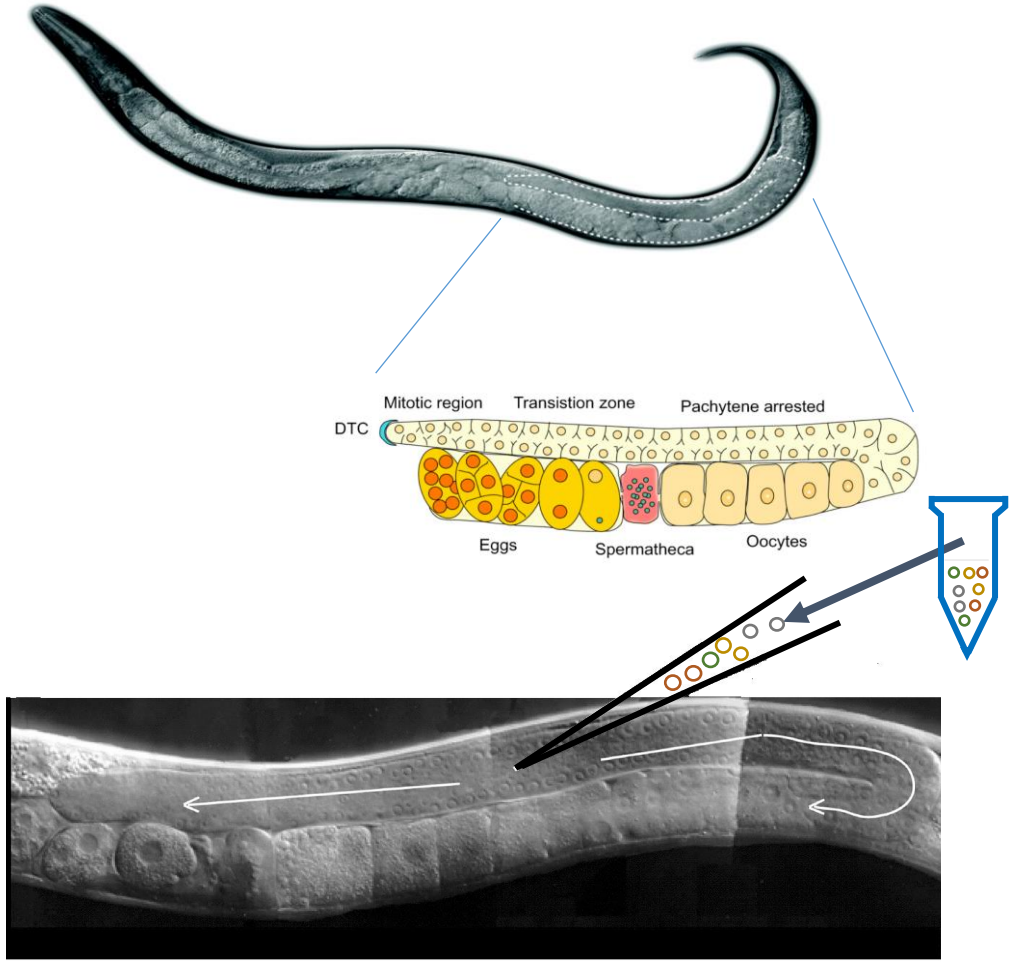


Judith Kimble, et al. Nature, 1982

John Smith Lab


a
b
A
B
D
C

The microinjection platform in *C. elegans*

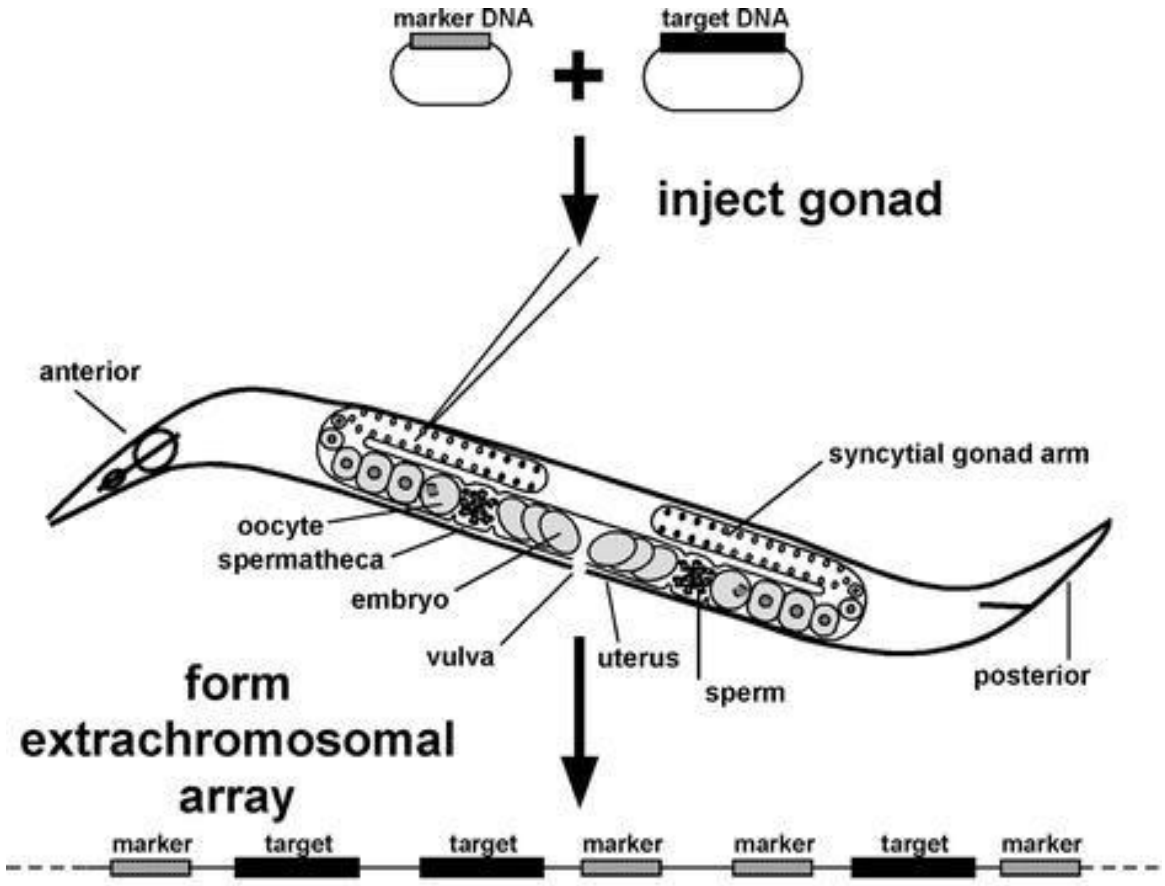


P0 : Injection of plasmids mix into the germline

F1 : Single worms with the marker to NGM plates.

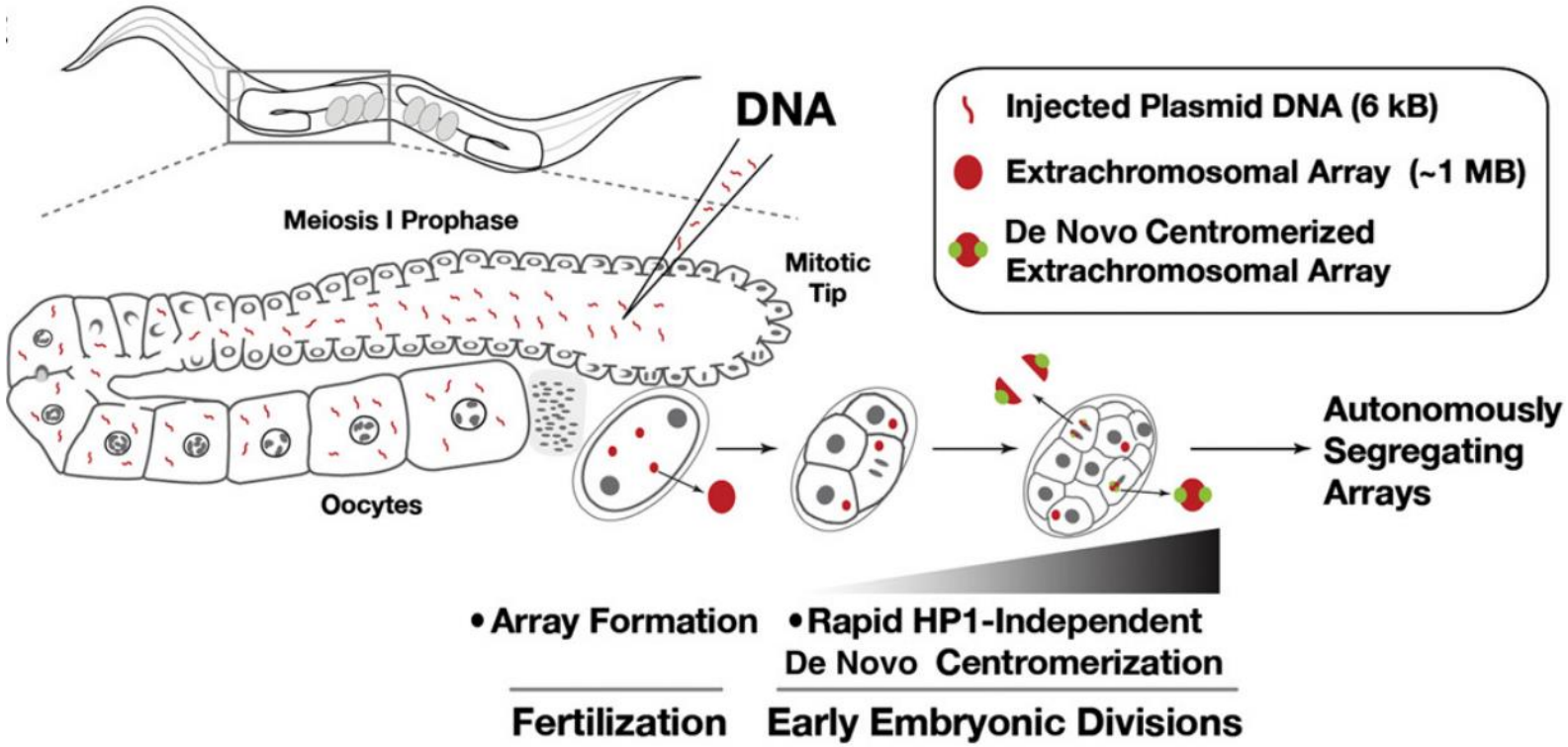
F2 : PCR amplification and DNA sequencing

DNA injected into the *Caenorhabditis elegans* germline forms extrachromosomal arrays



Pavan Kadandale, et al. MIMB, 2008

DNA injected into the *Caenorhabditis elegans* germline forms extrachromosomal arrays



Karen W.Y. Yuen, et al. Current biology, 2011

Arshad Desai Lab

Integrative transformation of *Caenorhabditis elegans*

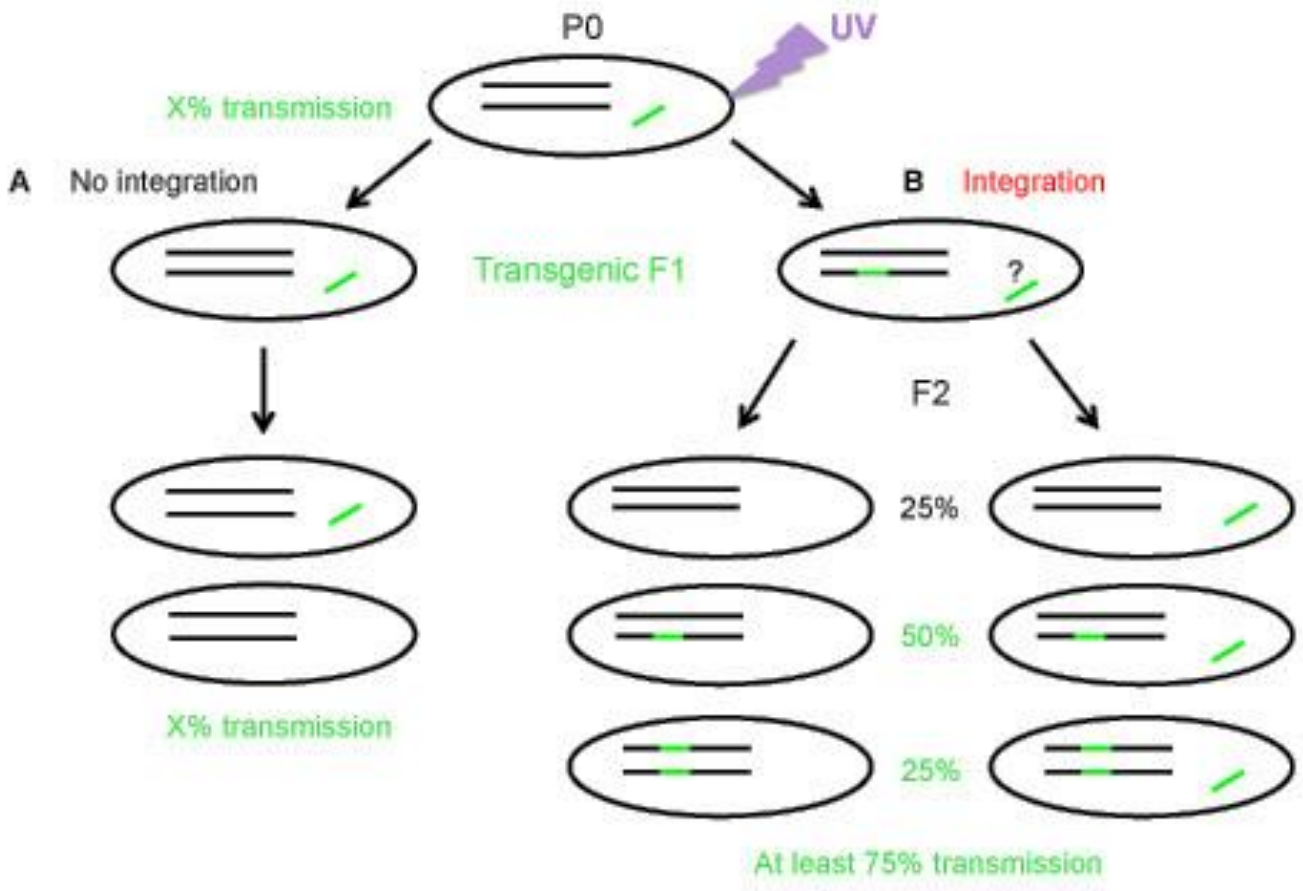
No heat shock

Heat shock

HSP-βGAL

Andrew Fire, The EMBO Journal, 1986

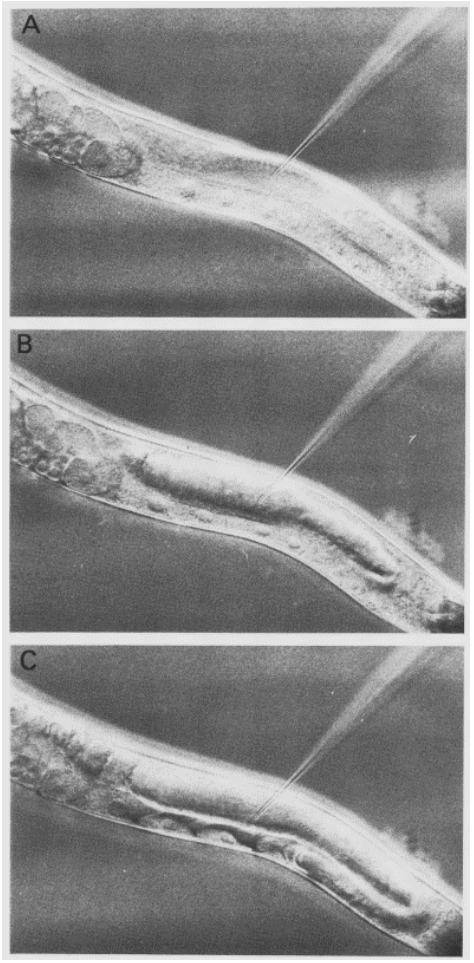
Integration of extrachromosomal DNA arrays into a chromosome by UV- irradiation



Expressed in the soma
Silenced in the germline

Marie-Christine Mariol, et al. JoVE, 2013
Kathrin Gieseler Lab

Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences

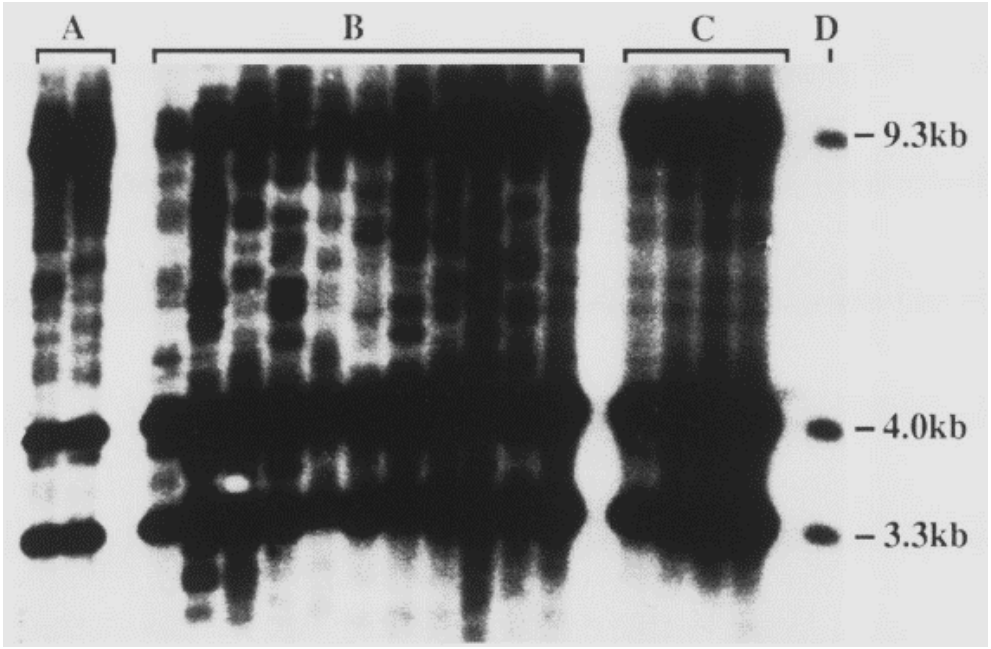


Plasmid: pRF4

A: F1 rollers from independent injected animals;

B: Independent F1 rollers from one injected animal;

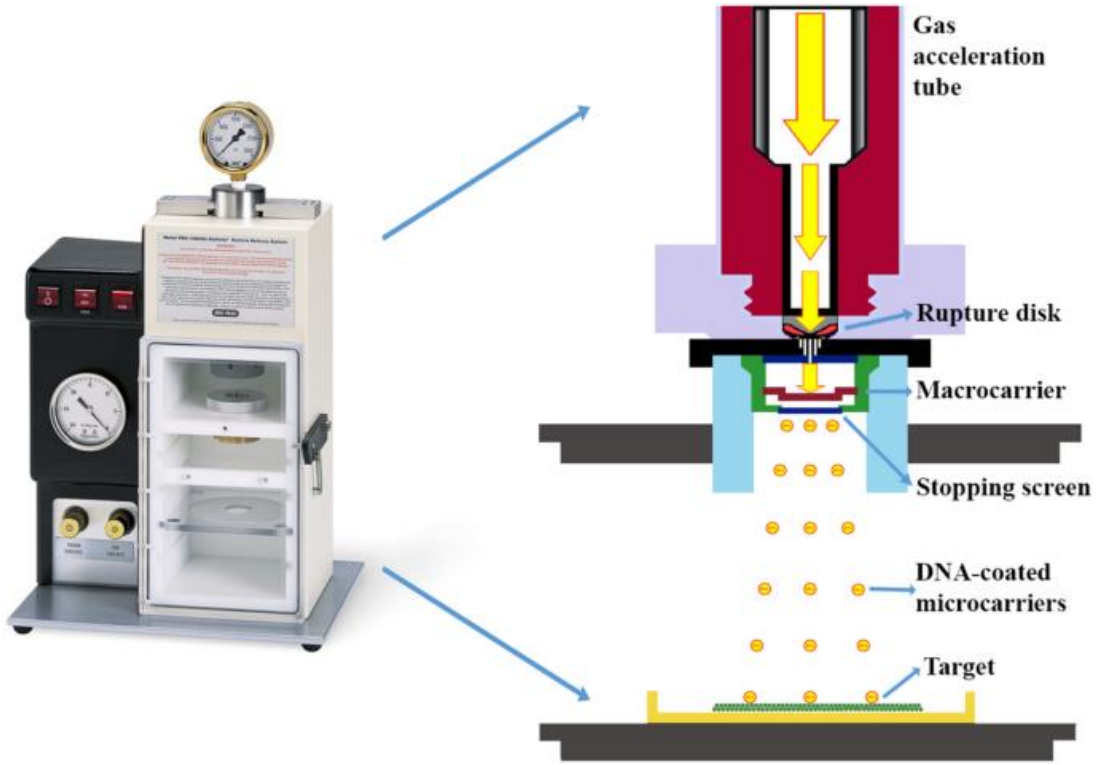
C: Independent F2 progeny derived from the same F1 roller.

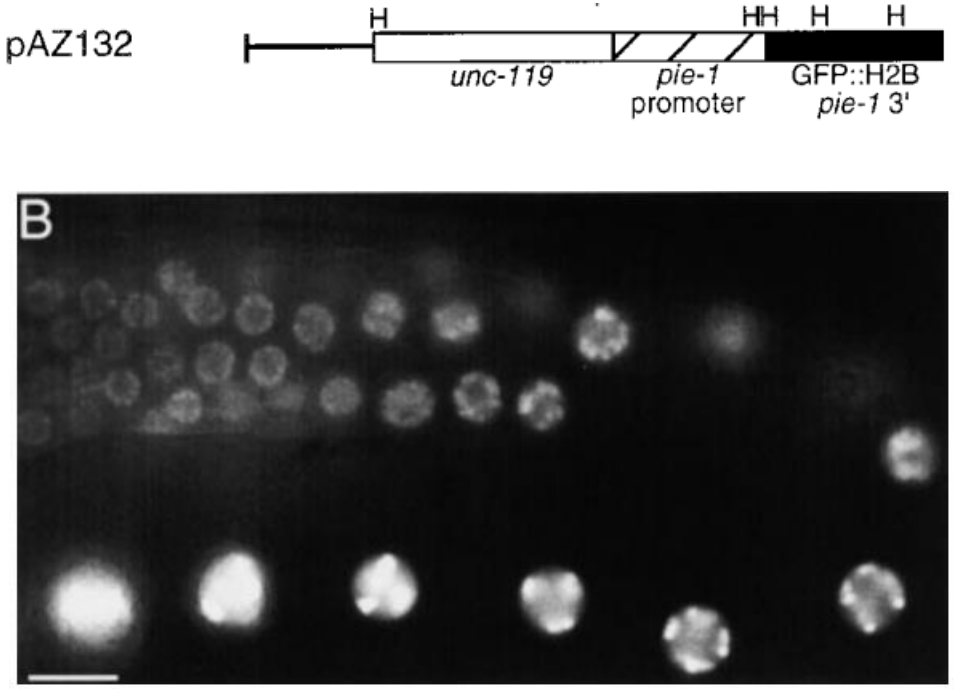


Craig Mello, et al. The EMBO Journal, 1991

Victor Ambros Lab

Creation of **Low-Copy** Integrated Transgenic Lines via microparticle bombardment



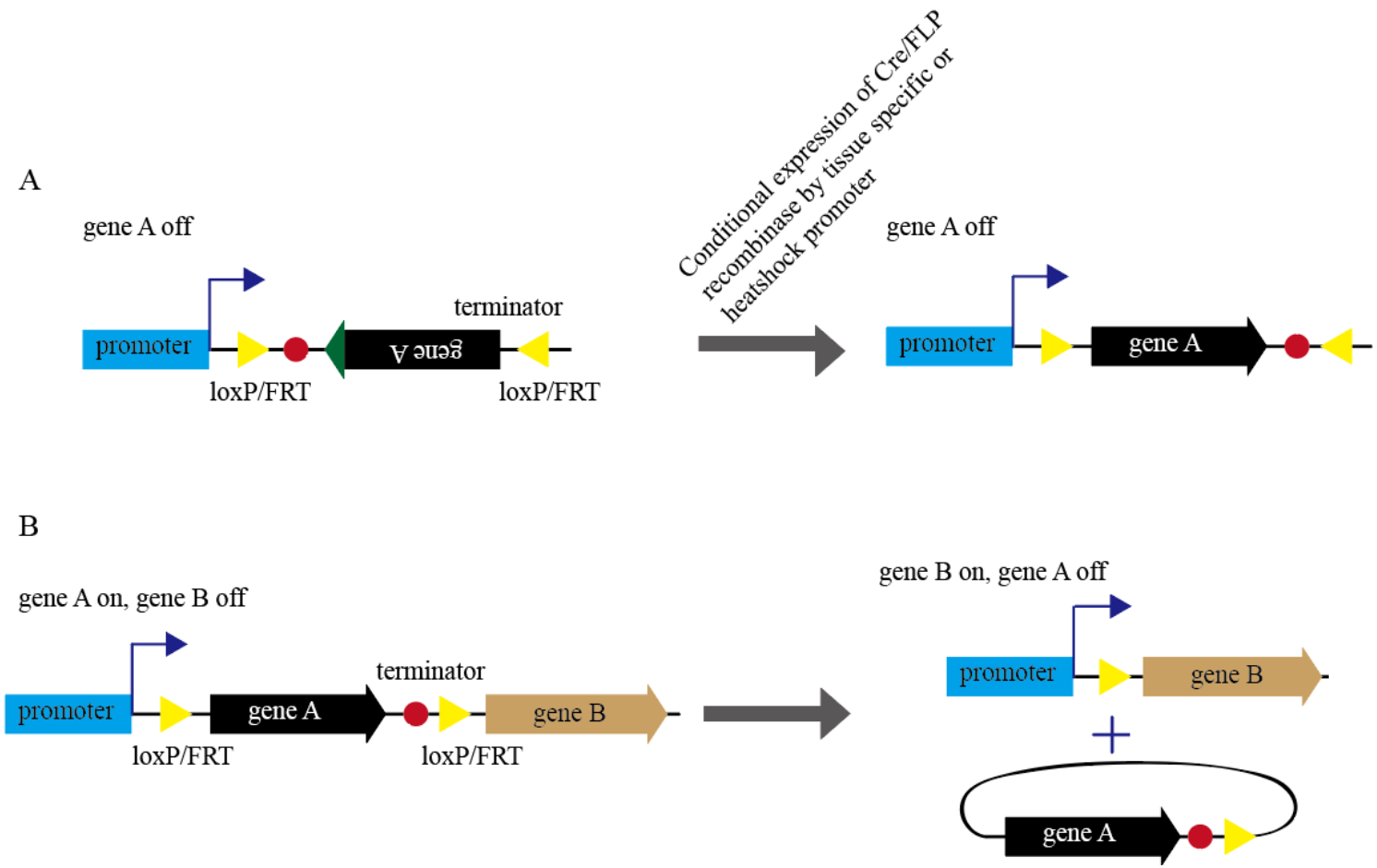


Vida Praitis, et al. GENETICS, 2001

Judith Austin Lab

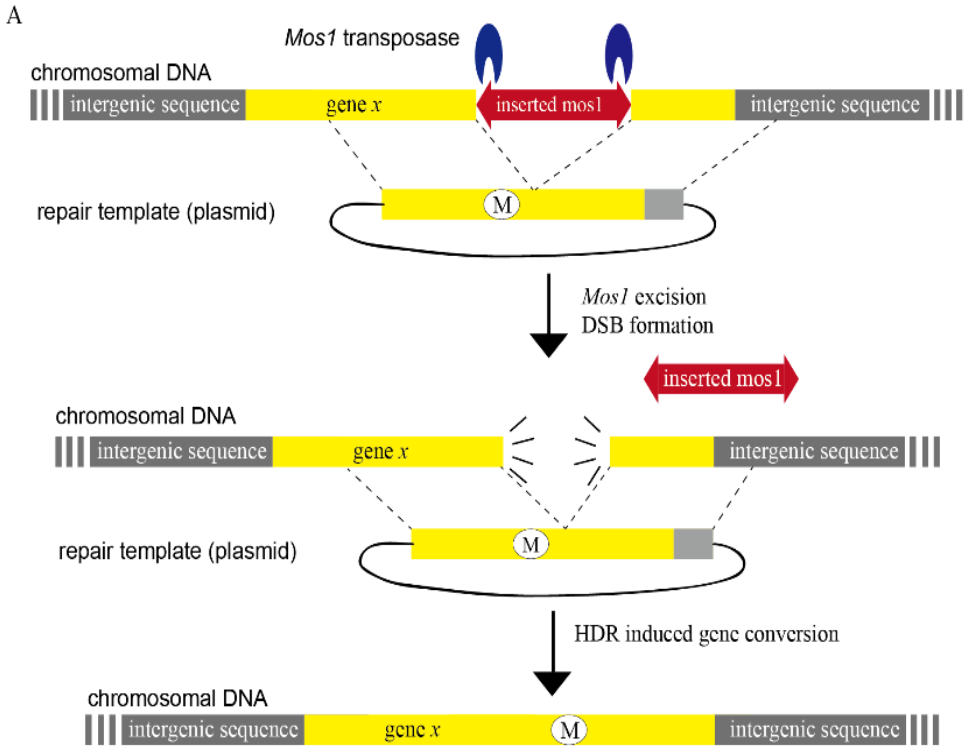
FLP/FRT and Cre/LoxP recombination technologies

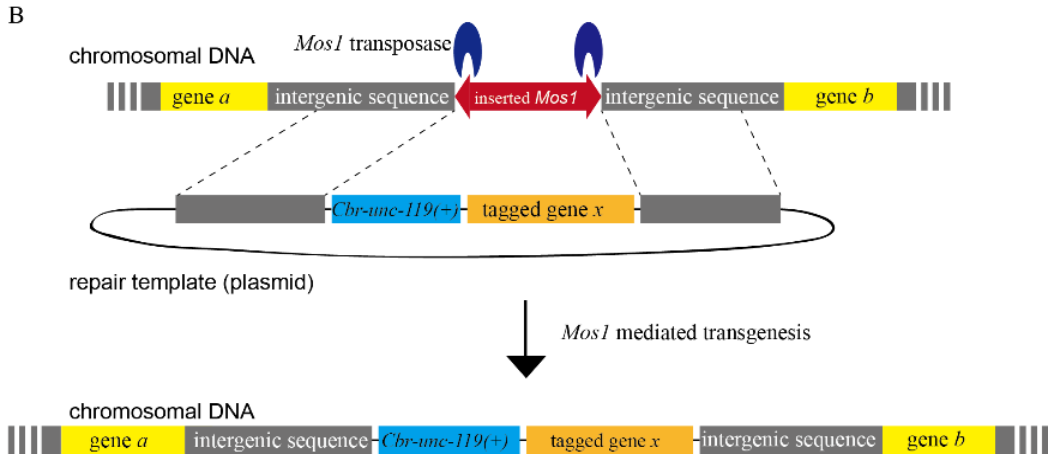
Conditionally activate or inactivate gene expression

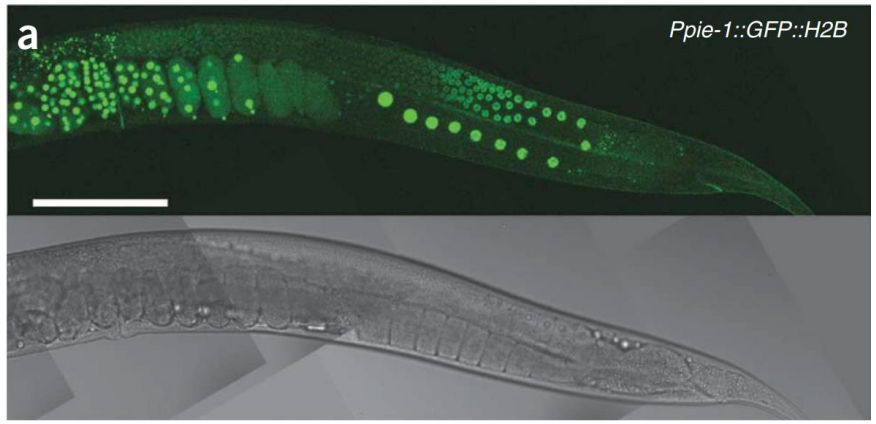


MosTIC-induced targeted gene conversion

Mos1-mediated Single Copy Insertion (mosSCI)



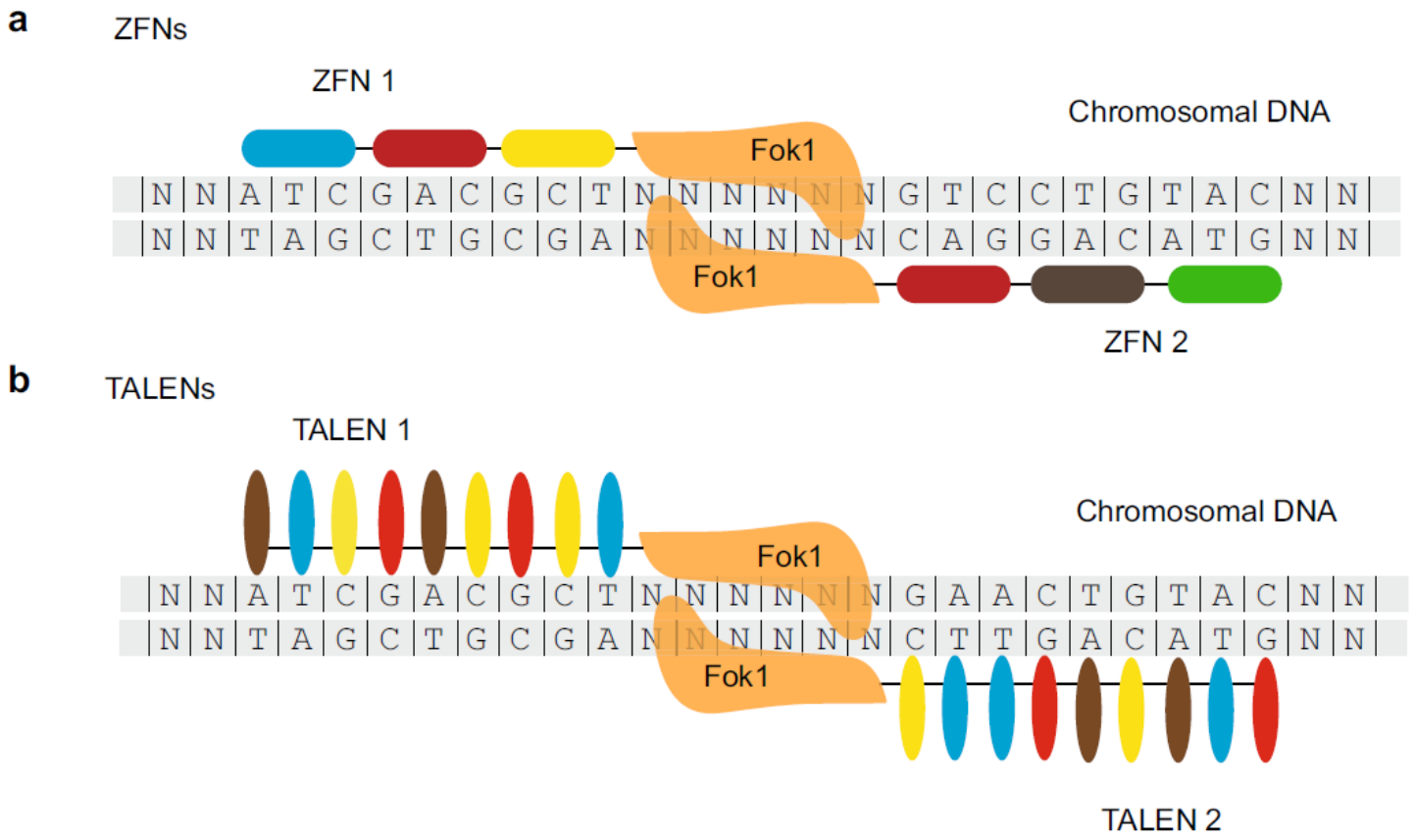




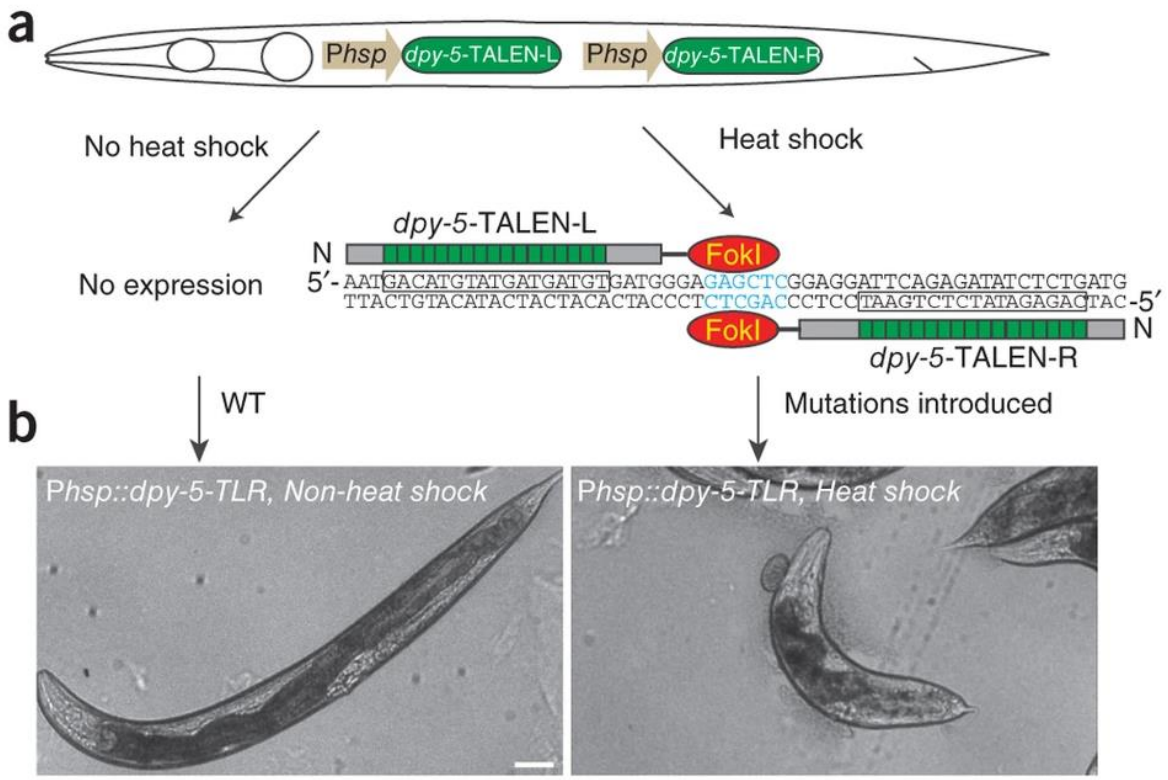
Christian Frøkjær-Jensen, et al, Nature Genetics, 2008

Erik M. Jorgensen Lab

ZFNs and TALENs create DNA lesions through the utility of sequence-specific DNA-binding modules



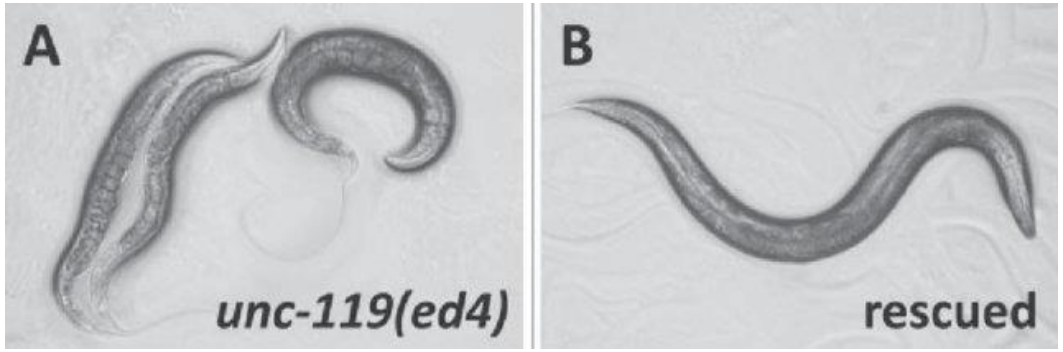
Conditional targeted genome editing using somatically expressed TALENs in *C. elegans*



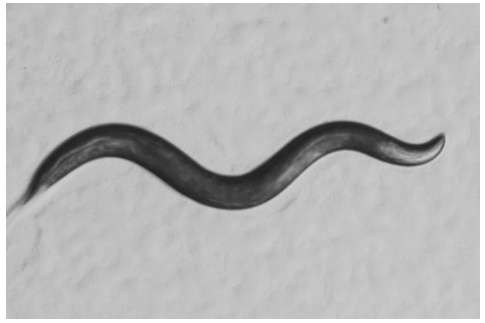
Ze Cheng, at al. Nature Biotechnology. 2013

Guangshuo Ou Lab

Multiple observable physical properties of *C. elegans*



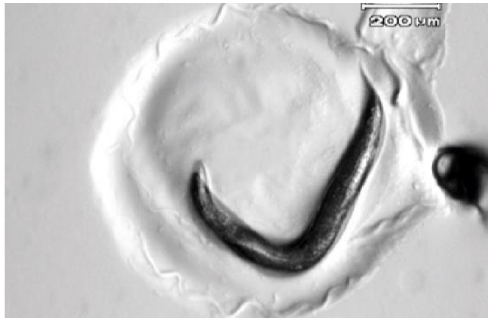
WT



sqt-1(e1350)

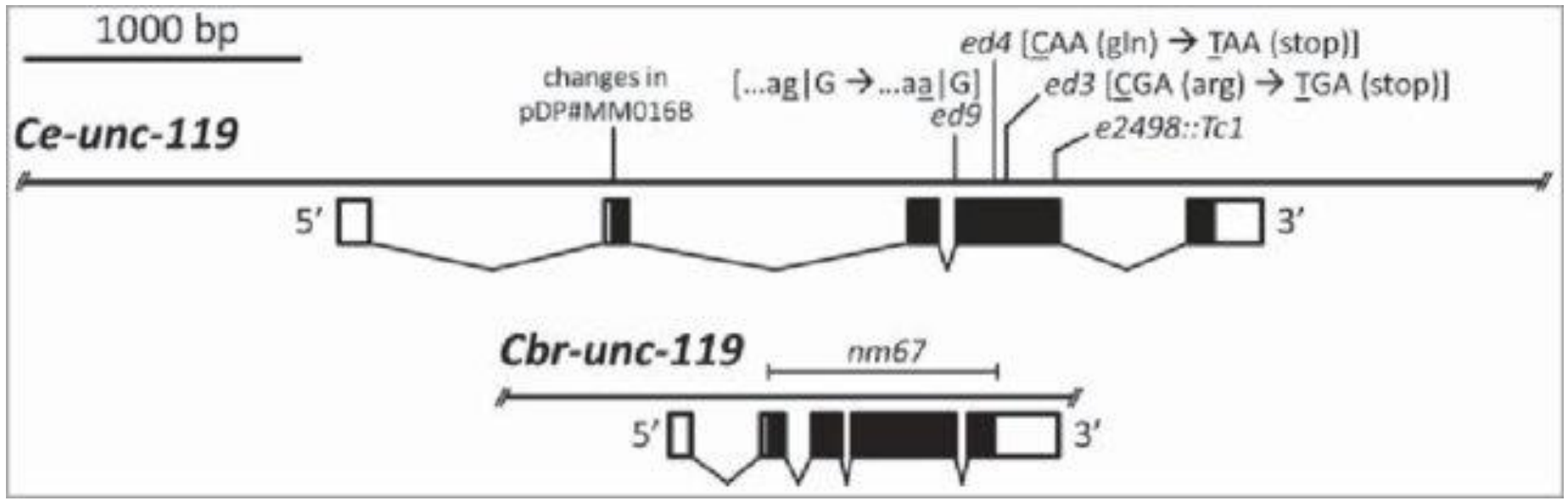
left handed roller

rol-6(su1006)

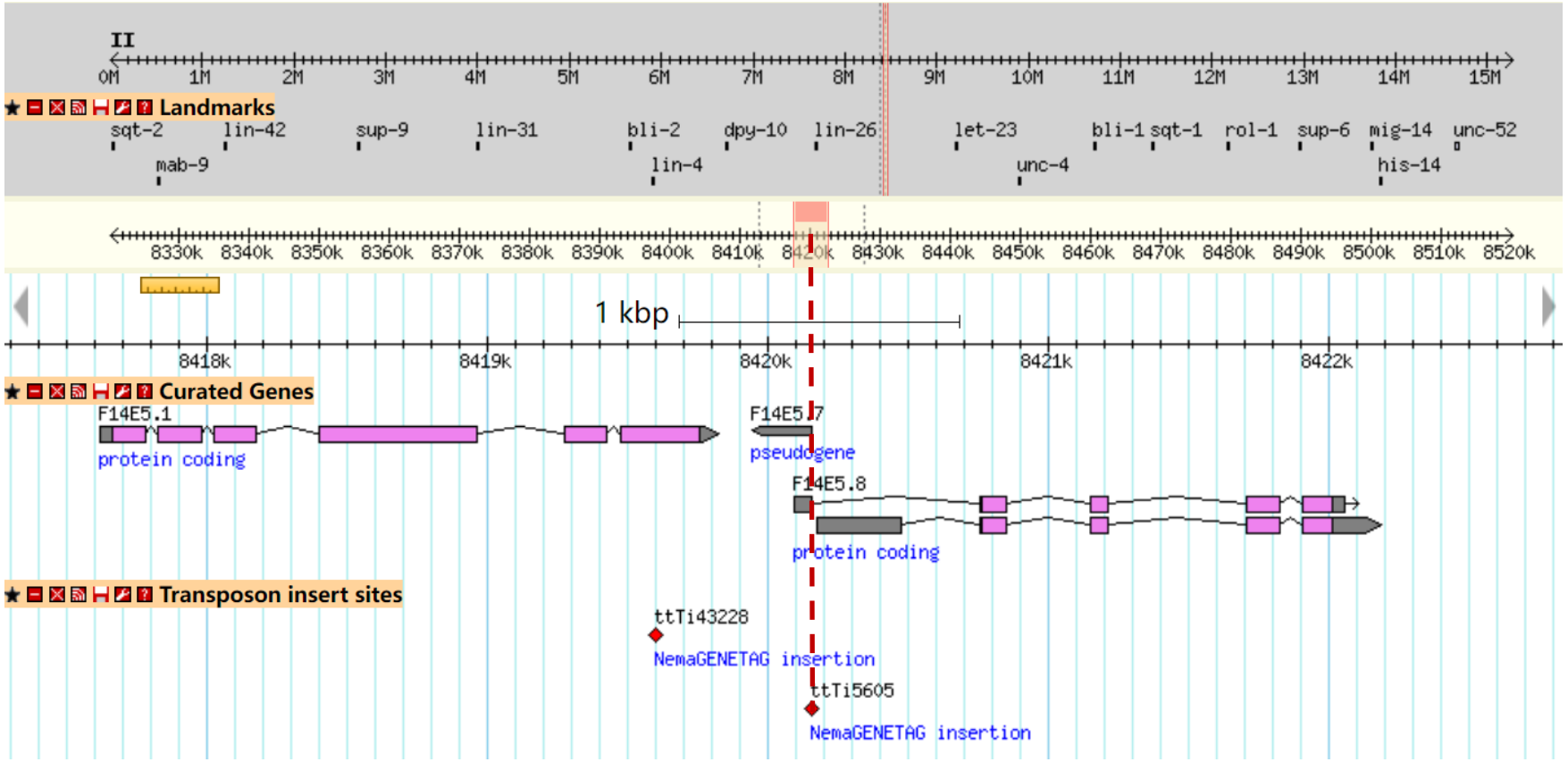


right handed roller

The C. elegans and C. briggsae unc-119 loci

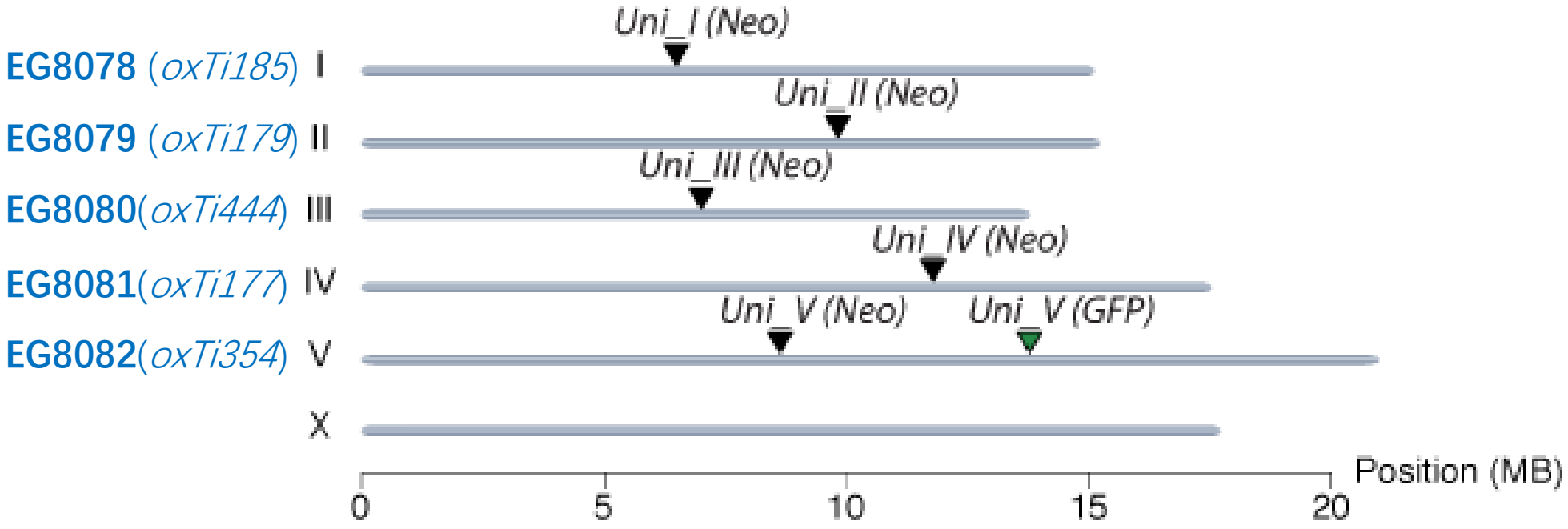


EG4322: strain widely used for MosSCI



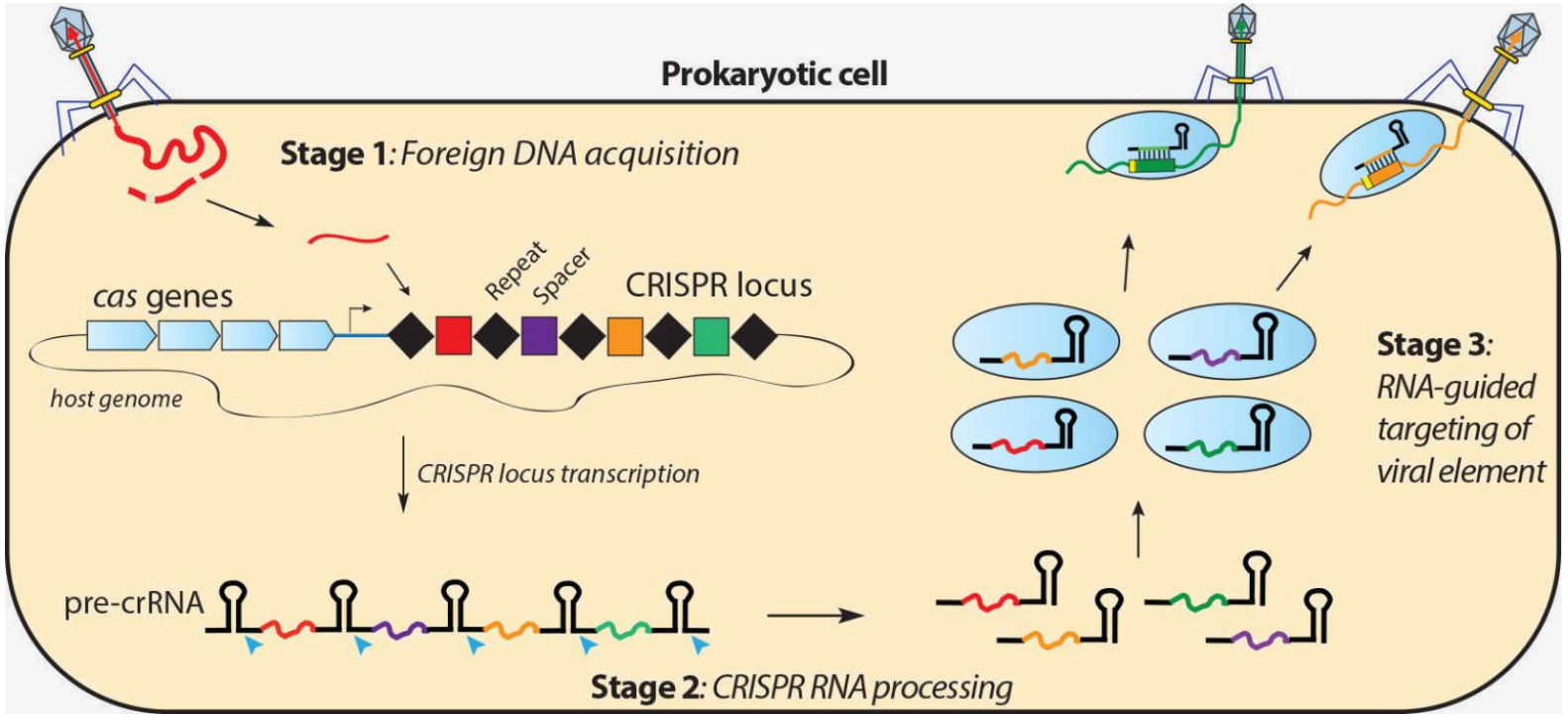
***ttTi5605* (LGII: 0.77)**

Modified universal MosSCI insertion strains

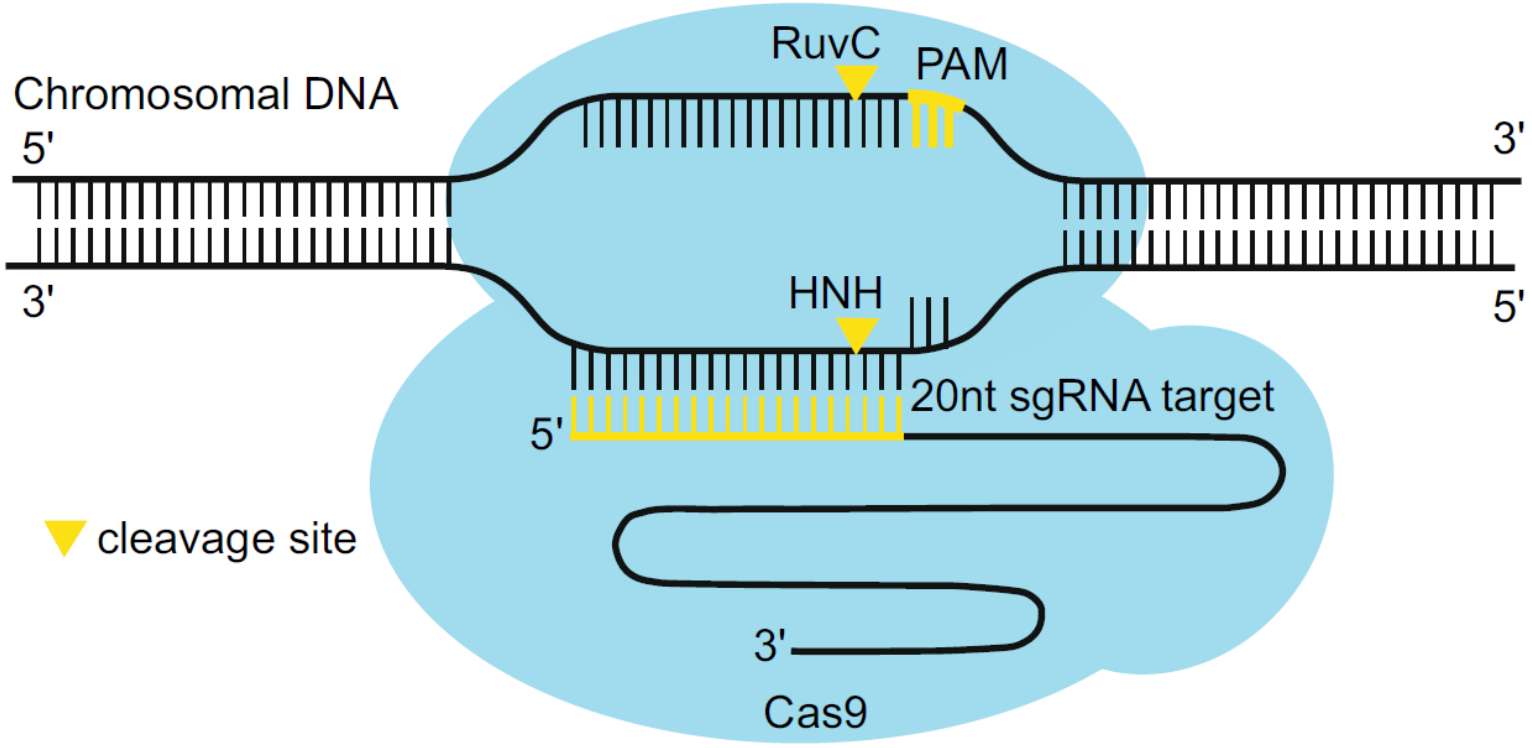


CRISPR/Cas9 technology

CRISPR : Clustered Regularly Interspaced Short Palindromic Repeats

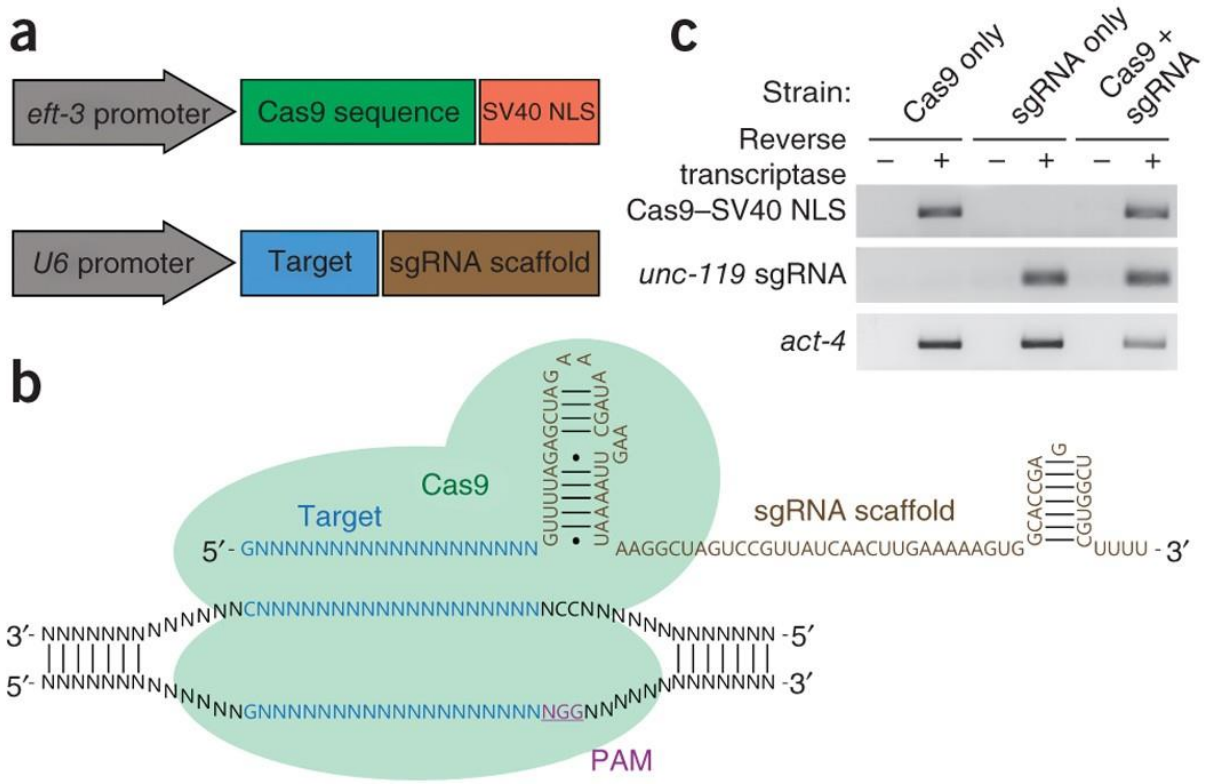


Schematic of the CRISPR/Cas9 system



CRISPR/Cas9 technology in *C. elegans*

Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system



Ari E Friedland, et al. nature methods. 2013

John A Calarco Lab

GENETICS
Vol. 195 No. 3 November 2013

COMMENTARY

635–642 **Exciting Prospects for Precise Engineering of *Caenorhabditis elegans* Genomes with CRISPR/Cas9**
Frøkjær-Jensen, Christian

NOTES

METHODS, TECHNOLOGY, AND RESOURCES

1167–1171 **Transgene-Free Genome Editing in *Caenorhabditis elegans* Using CRISPR-Cas**
Chiu, Hui, Hillel T. Schwartz, Igor Antoshechkin, and Paul W. Sternberg

1173–1176 **Targeted Heritable Mutation and Gene Conversion by Cas9-CRISPR in *Caenorhabditis elegans***
Katic, Iskra and Helge Großhans

1177–1180 **Heritable Gene Knockout in *Caenorhabditis elegans* by Direct Injection of Cas9–sgRNA Ribonucleoproteins**
Cho, Seung Woo, Jihyun Lee, Dana Carroll, Jin-Soo Kim, and Junho Lee

1181–1185 **Heritable Custom Genomic Modifications in *Caenorhabditis elegans* via a CRISPR–Cas9 System**
Tzur, Yonatan B., Ari E. Friedland, Saravanapriah Nadarajan, George M. Church, John A. Calarco, and Monica P. Colaiácovo

1187–1191 **CRISPR/Cas9-Targeted Mutagenesis in *Caenorhabditis elegans***
Waaijers, Selma, Vincent Portegijs, Jana Kerver, Bennie B. L. G. Lemmens, Marcel Tijsterman, Sander van den Heuvel, and Mike Boxem

Injection mix

Cas9 mRNA and sgRNA

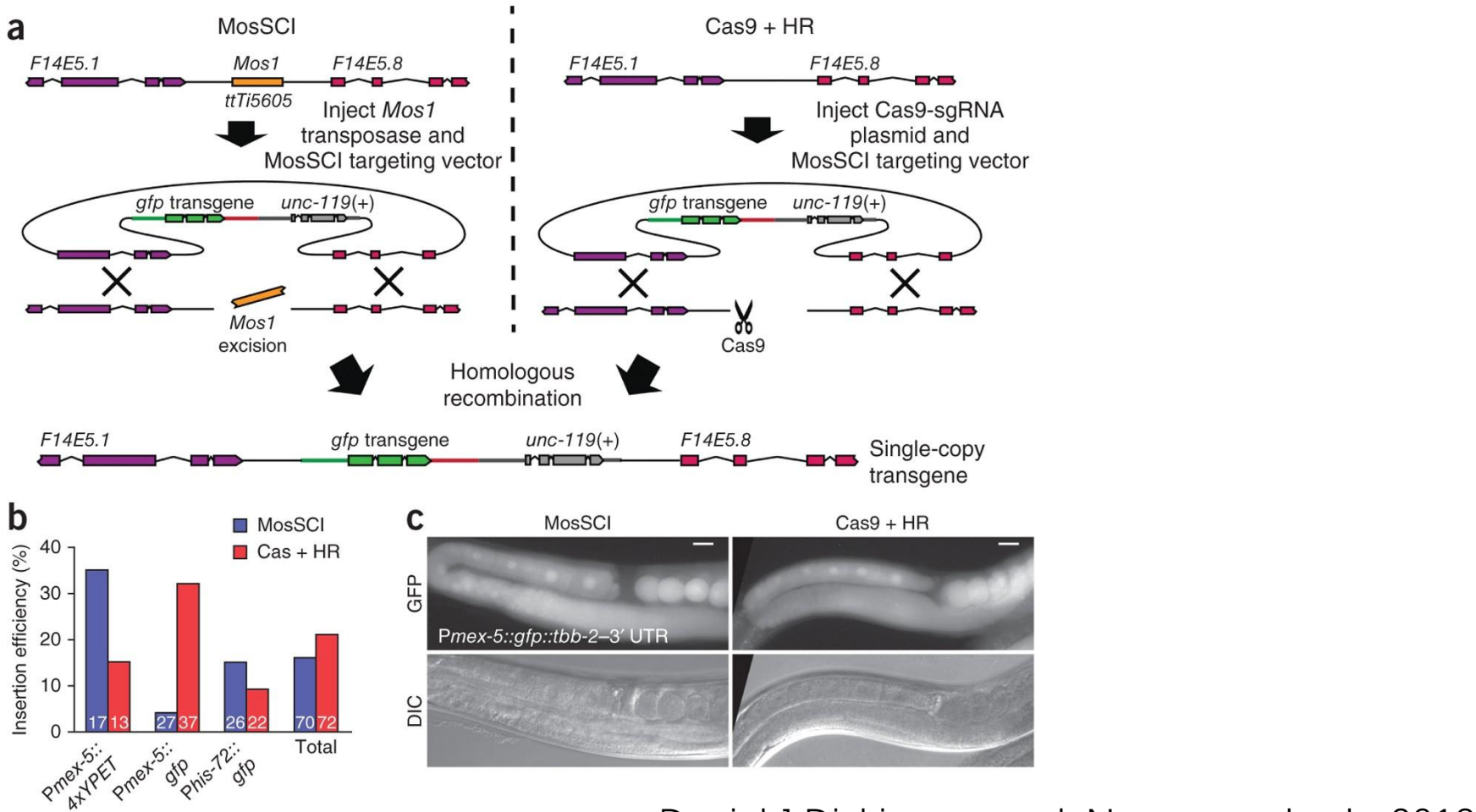
Cas9 mRNA and sgRNA

Cas9/sgRNA complex

Cas9 and sgRNA plasmids

Cas9 and sgRNA plasmids

Engineering the genome using Cas9-triggered homologous recombination



Daniel J Dickinson, et al. Nature methods. 2013

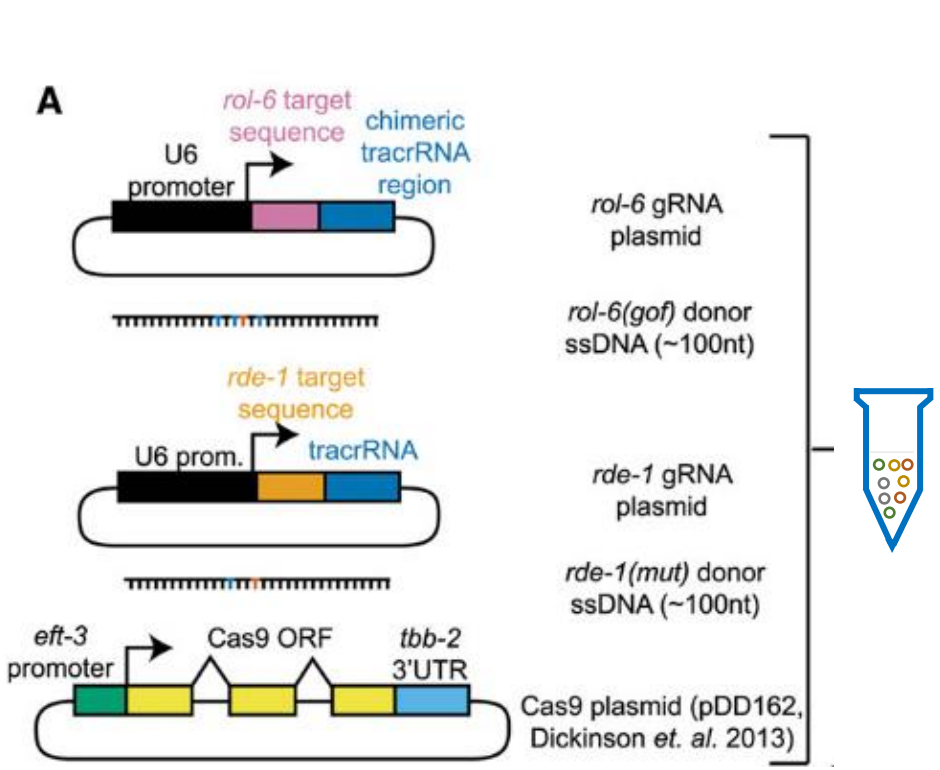
Bob Goldstein Lab

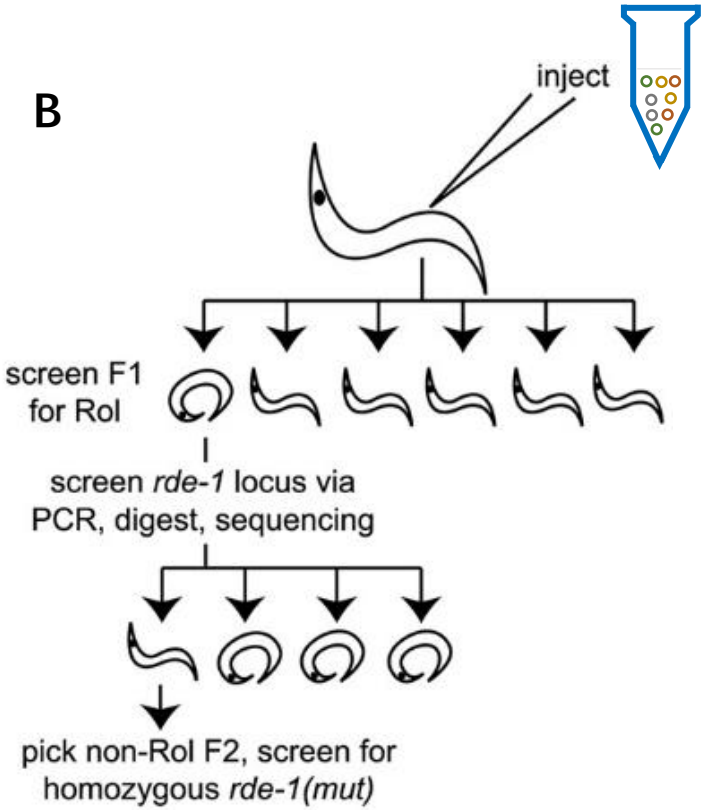
How to be timesaving and laborsaving?

- 1. Efficient identification of genome-modified *C. elegans* strains
- 2. Increase the efficiency via the optimization of sgRNA and Cas9 protein
- 3. Simplification of the construction of repair template

Efficient identification of genome-modified *C. elegans* strains

Efficient Marker-Free Recovery of Custom Genetic Modifications Via **Co-conversion**

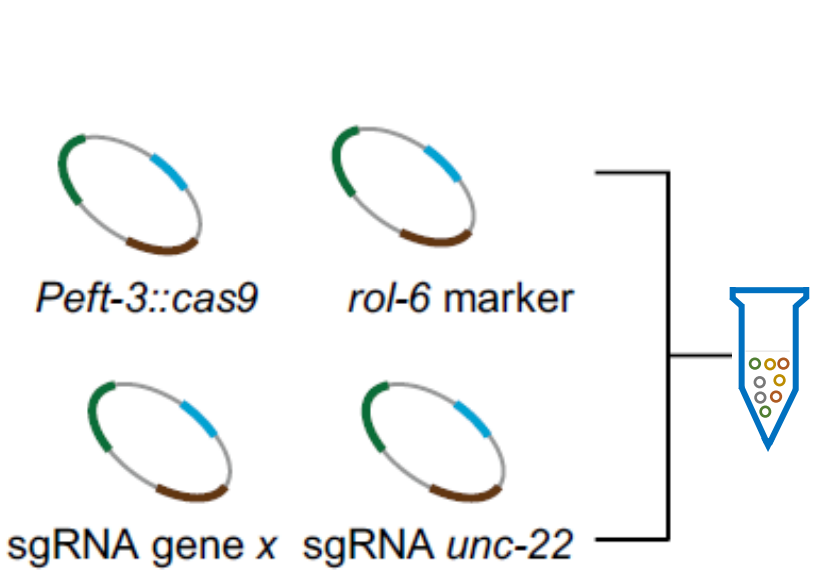


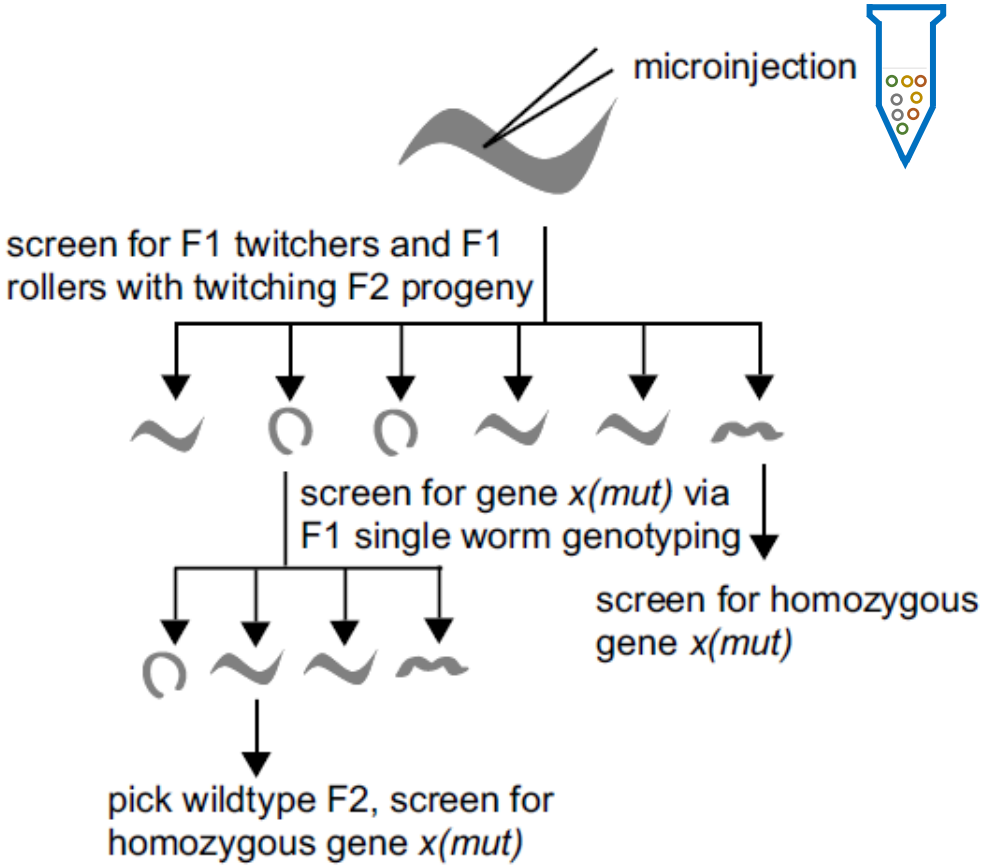


Joshua A. Arribere, et al. GENETICS. 2014

Andrew Z. Fire Lab

A **Co-CRISPR** Strategy for Efficient Genome Editing

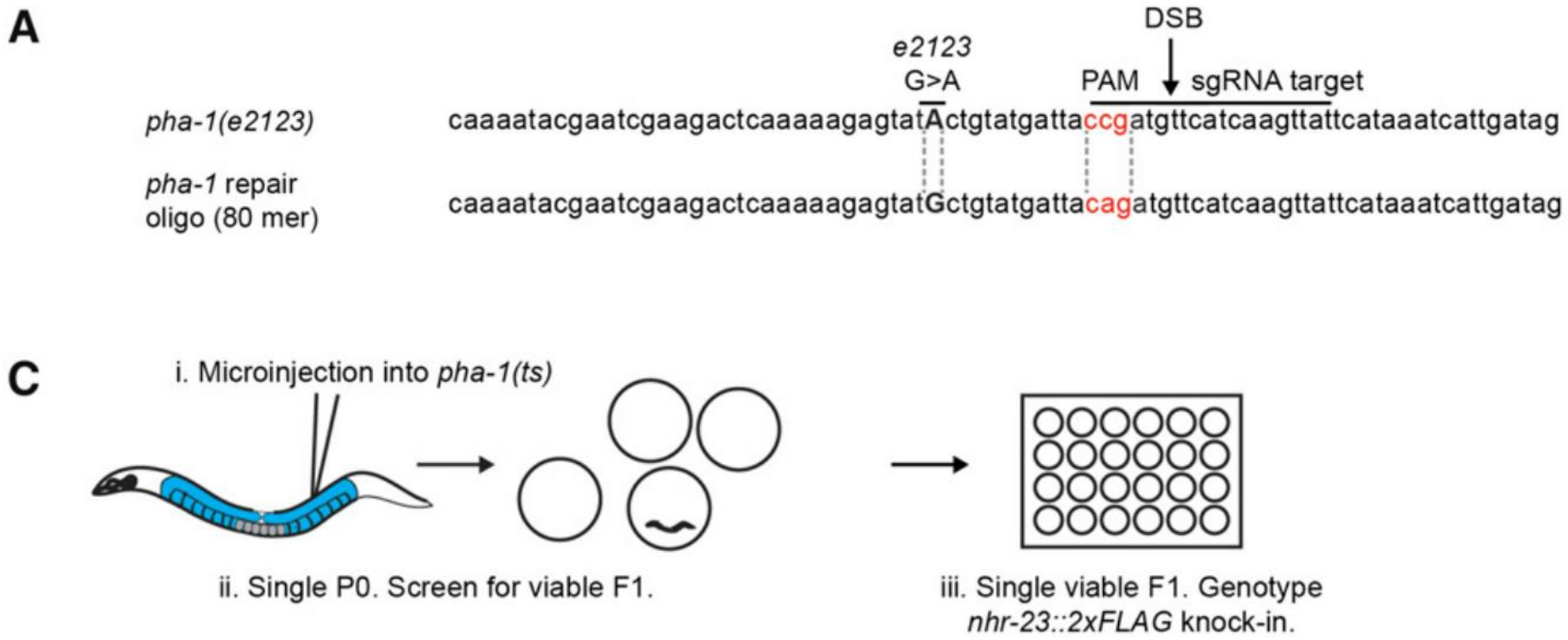




Heesun Kim, et al. GENETICS, 2014

Craig C. Mello Lab

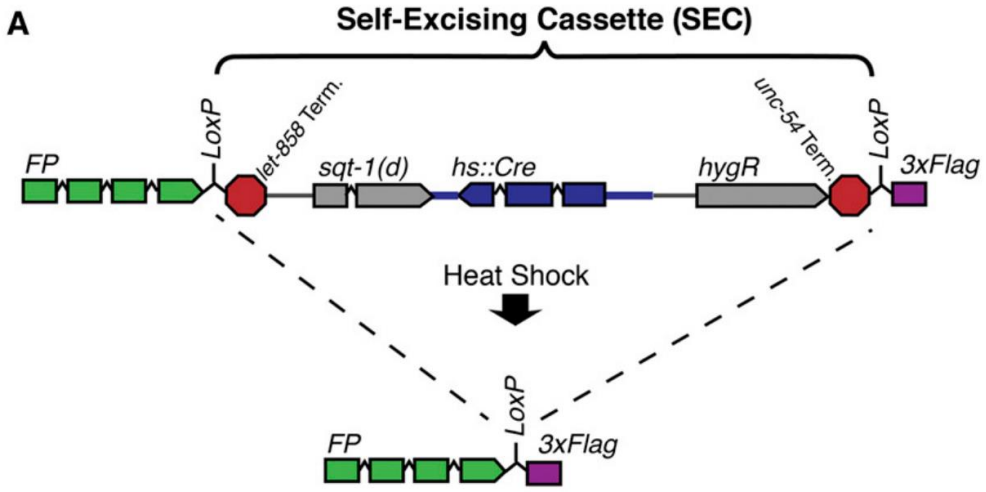
Lethal Mutation Co-Conversion and Inactivation of NHEJ Repair



Jordan D. Ward. GENETICS, 2014

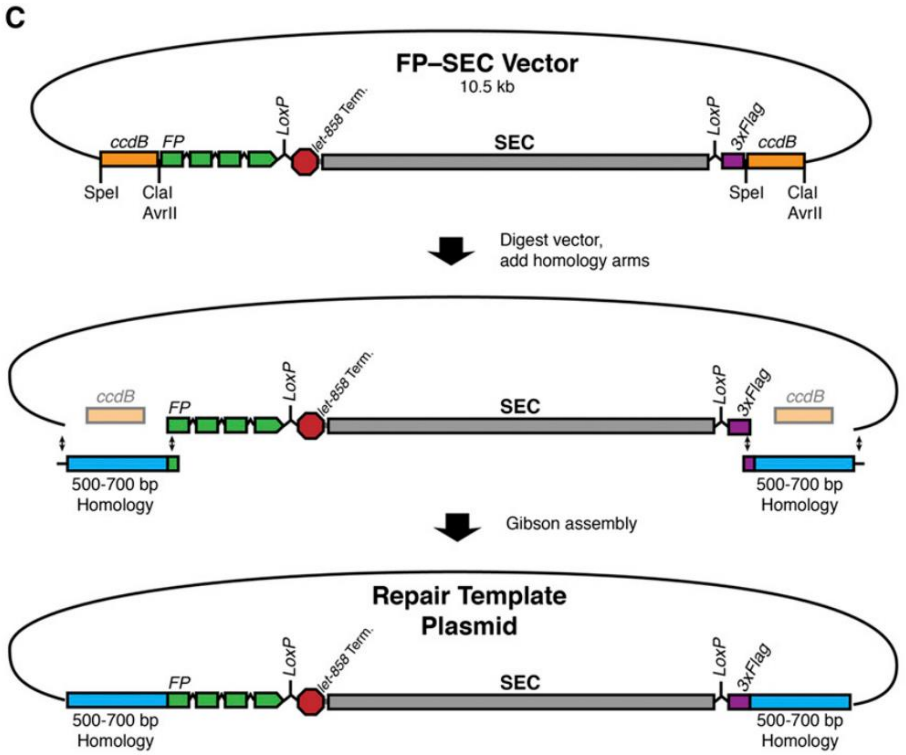
Jordan D. Ward Lab

Streamlined Genome Engineering with a Self-Excising Drug Selection Cassette



SEC elements:

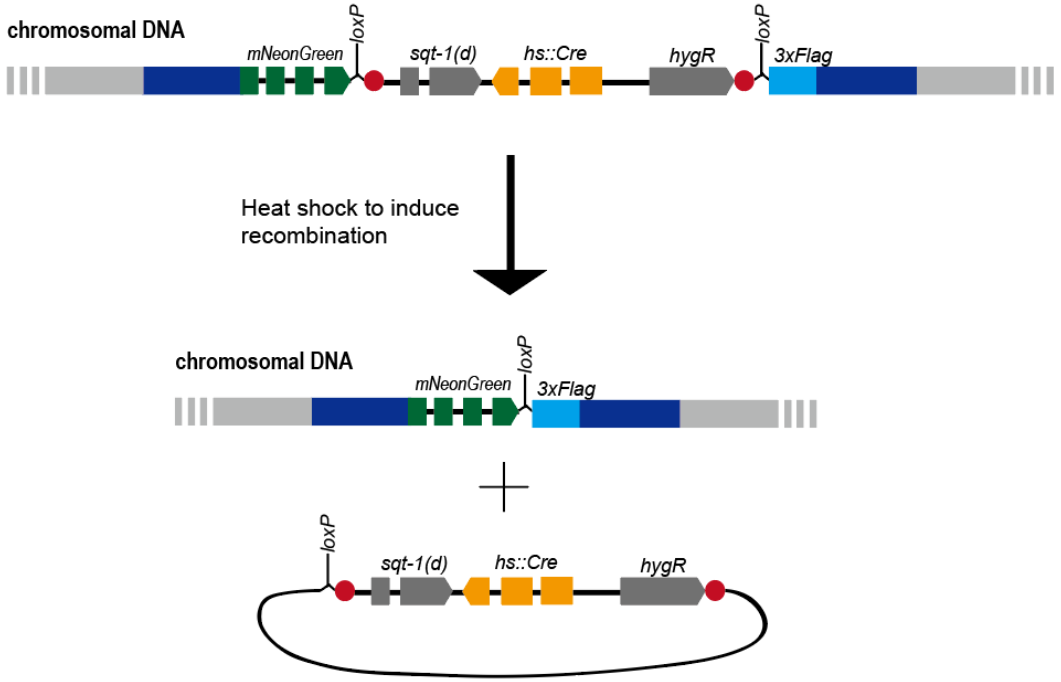
1. *sqt-1(e1350)*
2. *Hs::Cre*
3. HygR

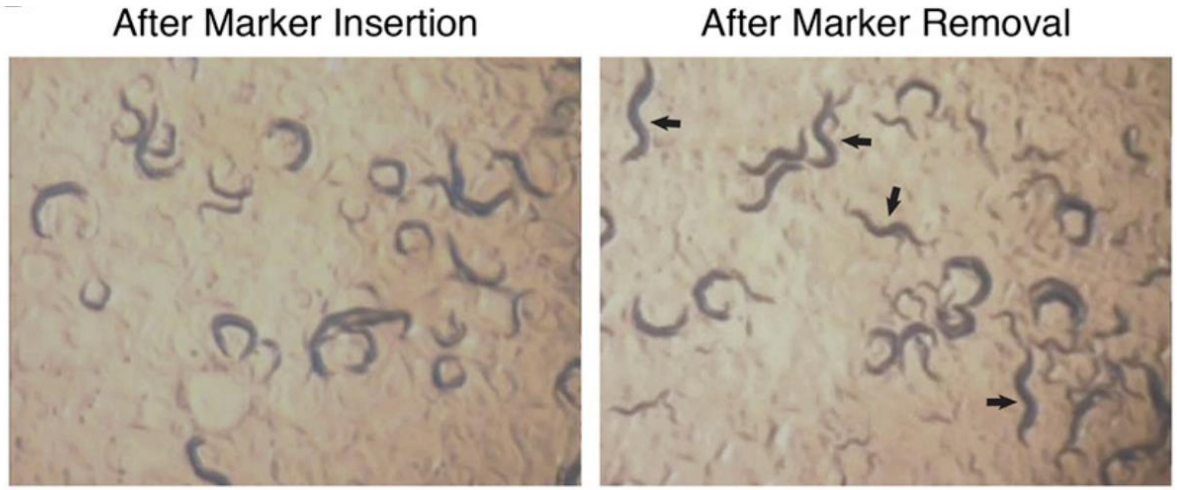


Daniel J. Dickinson, et al. GENETICS. 2015

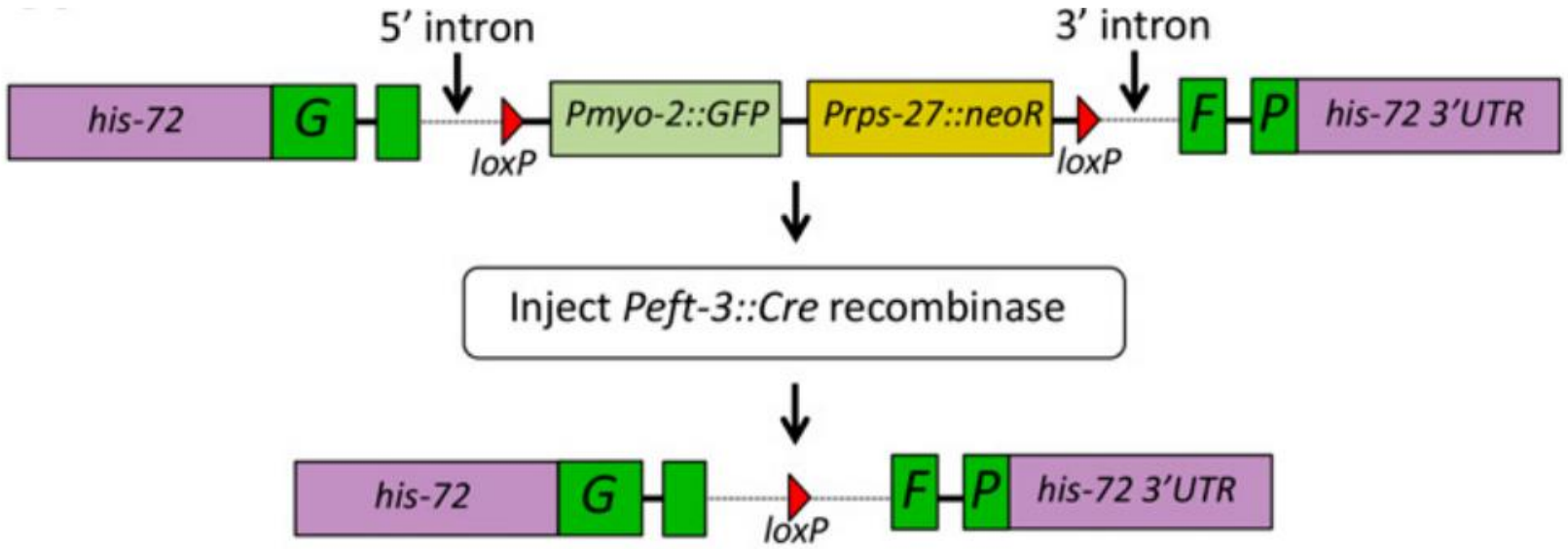
Bob Goldstein Lab

Streamlined Genome Engineering with a Self-Excising Drug Selection Cassette





Efficient Genome Editing in *Caenorhabditis elegans* with a Toolkit of Dual-Marker **Selection Cassettes**



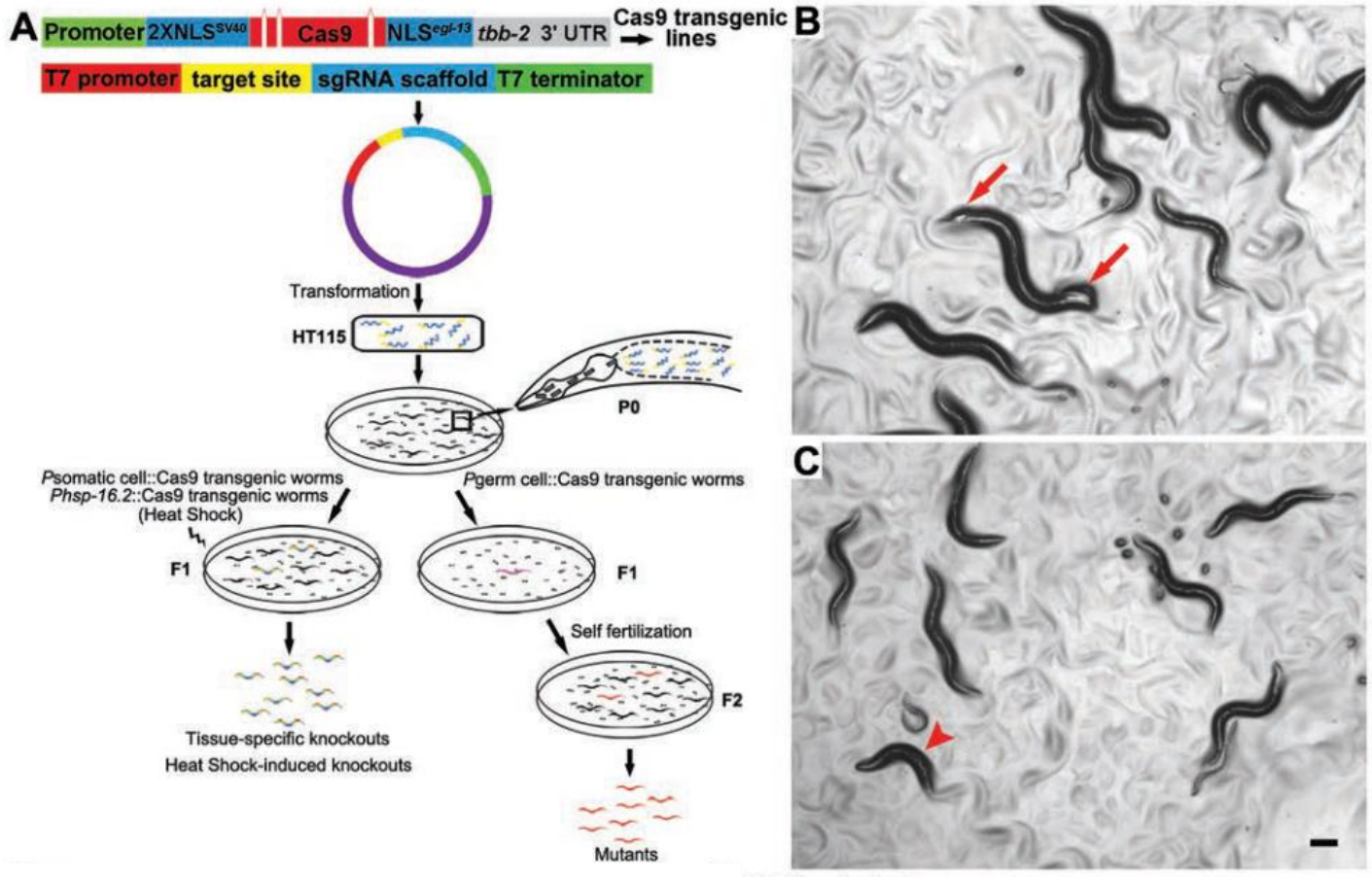
Cassette elements:

1. *Pmyo-2::GFP*
2. *NeoR*

Adam D. Norris, et al. GENETICS. 2015

John A. Calarco Lab

Heritable/conditional genome editing in *C. elegans* using a CRISPR-Cas9 feeding system



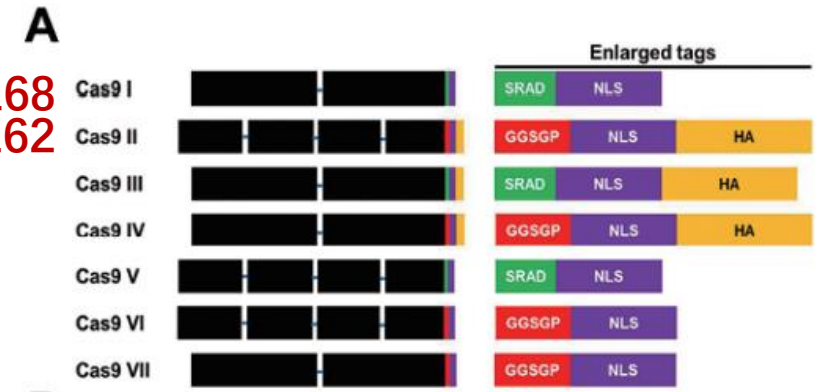
Pengpeng Liu, et al. Cell research. 2014

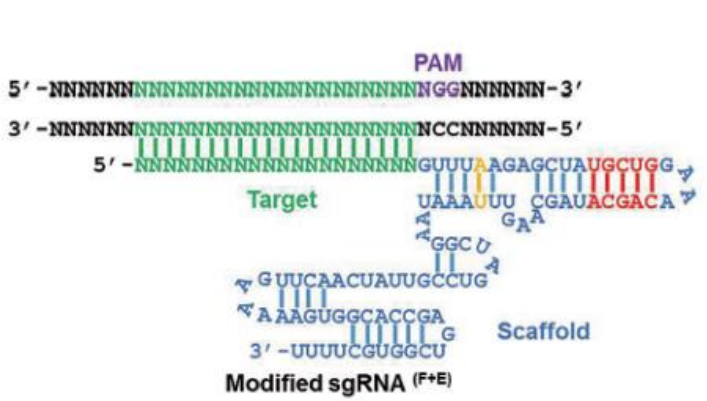
Dong Liu Lab

Increase the efficiency via the optimization of sgRNA and Cas9 protein

One-step homozygosity in precise gene editing by an improved CRISPR/Cas9 system

#46168
pDD162





sgRNA(F+E)

B

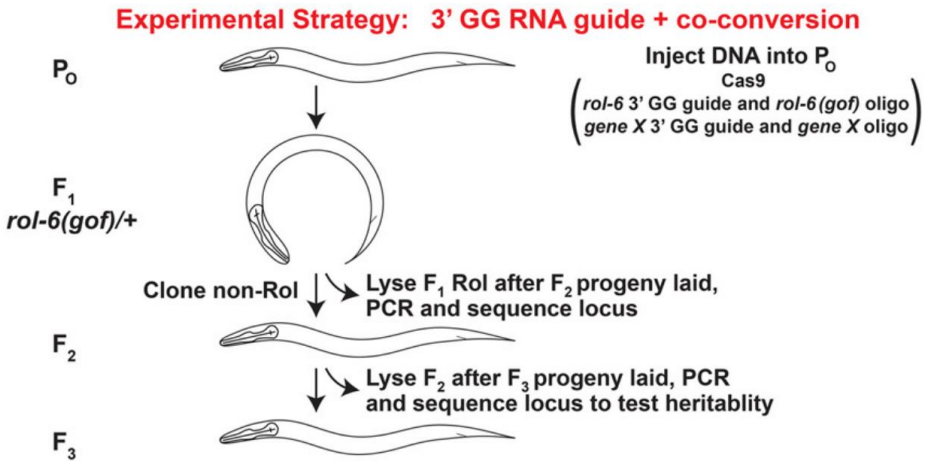
editing	Cas9	Benomyl^{R}/F1	Knock-ins/Sequenced	Precise knock-ins/Sequenced	Normalized precise knock-ins/F1	fold
	I	15% (128/848)	30% (16/54)	20% (11/54)	3% (26/848)	1
	II	66% (498/759)	96% (48/50)	90% (45/50)	59% (448/759)	19.7
	III	2% (19/950)	33% (4/12)	33% (4/12)	0.6% (6/950)	0.2
ben-1^{153}	IV	76% (621/822)	79% (15/19)	79% (15/19)	60% (490/822)	20
	V	12% (114/939)	40% (8/20)	35% (7/20)	4% (40/939)	1.3
	VI	41% (425/1031)	88%(23/26)	81%(21/26)	33% (343/1031)	11
	VII	54% (512/957)	83%(24/29)	83%(24/29)	44% (423/957)	14.7

Pei Zhao, et al. Cell research. 2016

Ding Xue Lab

Dramatic Enhancement of Genome Editing by CRISPR/Cas9 Through Improved Guide RNA Design

$GN_{17}GGNGG$



GG Guides

Target Gene	Guide RNA	Protospacer Sequence (PAM)	sgRNA Bases 19,20	Mutagenesis Rate (%)
lir-2	3' GG	GGCTGATTTTCGCAGTTCGG (GGG)	GG	72
Y62E10A.17	3' GG	CGCACCGATGCTCTCCGAGG (AGG)	GG	57
sex-1	3' GG (1)	GGATGAGAATCTGACAAAGG (TGG)	GG	54
cpsf-2	3' GG	CACTTTCAATTTGATAATGG (AGG)	GG	52
sex-1	3' GG (2)	AACATTTCCACAACGAGAGG (AGG)	GG	51
fox-1	3' GG (1)	ATATGAGGGGAGTGAGGCGG (TGG)	GG	29
fox-1	3' GG (3)	ATTACAGTGAAGTACAGCGG (AGG)	GG	21
fox-1	3' GG (2)	AATATCGTTTACCAAAACGG (GGG)	GG	13
xol-1	3' GG	AGCGATTTCTGGCGATTGGG (GGG)	GG	10
			median:	**51**

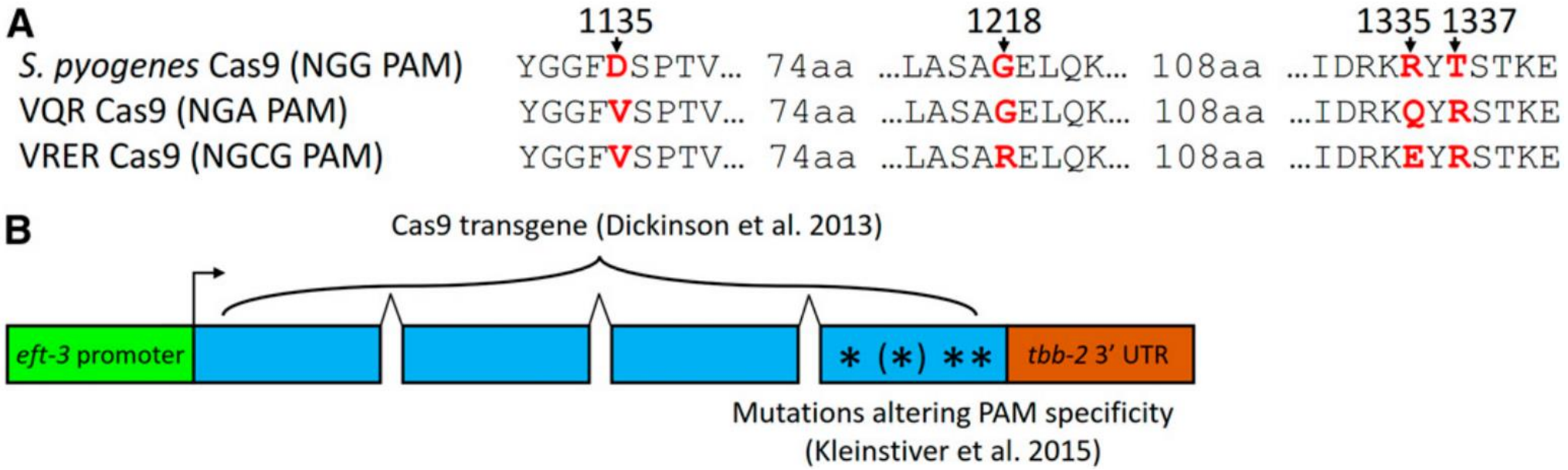
Non-GG Guides

sex-1	3' GG-shift (1)	AACGGATGAGAATCTGACAA (AGG)	AA	21
fox-1	3' GG-shift (1)	CATTTGATATGAGGGGAGTG (AGG)	TG	20
Y62E10A.17	3' GG-shift	ATACGCACCGATGCTCTCCG (AGG)	CG	14
sex-1	3' GG-shift (2)	TGGAACATTTCCACAACGAG (AGG)	AG	8
lir-2	3' GG-shift	CTCGGCTGATTTTCGCAGTT (CGG)	TT	1
cpsf-2	3' GG-shift	AAACACTTTCAATTTGATAA (TGG)	AA	0
fox-1	3' GG-shift (2)	TTGAATATCGTTTACCAAAA (CGG)	AA	0
fox-1	3' GG-shift (3)	ACAATTACAGTGAAGTACAG (CGG)	AG	0
xol-1	3' GG-shift	TCTAGCGATTTCTGGCGATT (GGG)	TT	0
cpsf-2	3' non-GG (1)	GTGGTTGGGATGAGCGATTC (GGG)	TC	0
lir-2	3' non-GG (1)	AATCAGCCGAGATGTAAGTT (TGG)	TT	0
lir-2	3' non-GG (2)	TTGACTCGTTCCATTTCAGC (TGG)	GC	0
sex-1	3' non-GG (1)	AAACCTGCCTCCTCTCGTTG (TGG)	TG	0

Behnom Farboud, et al. GENETICS, 2015

Barbara J. Meyer Lab

Cas9 Variants Expand the Target Repertoire

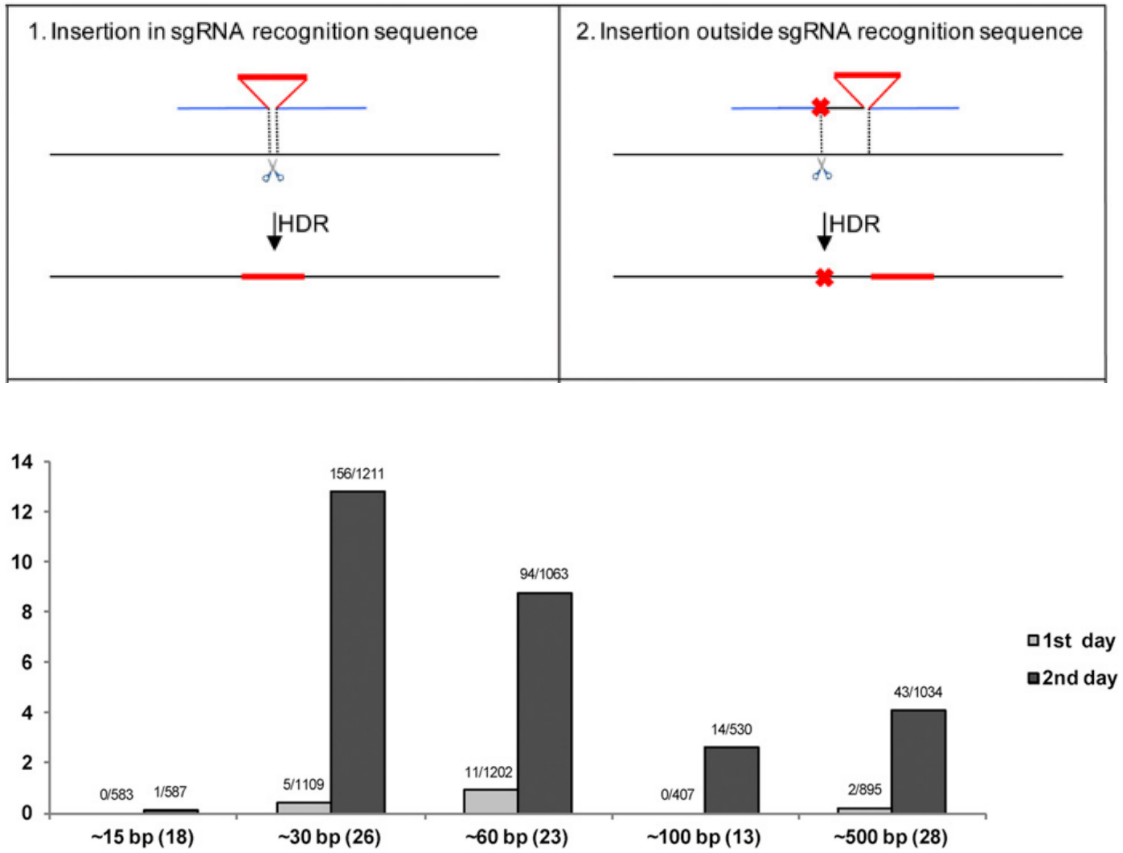


Ryan T. Bell, et al. GENETICS, 2015.

Andrew Z. Fire Lab

Simplification of the repair templates construction

Scalable and Versatile Genome Editing Using Linear DNAs with Microhomology to Cas9 Sites

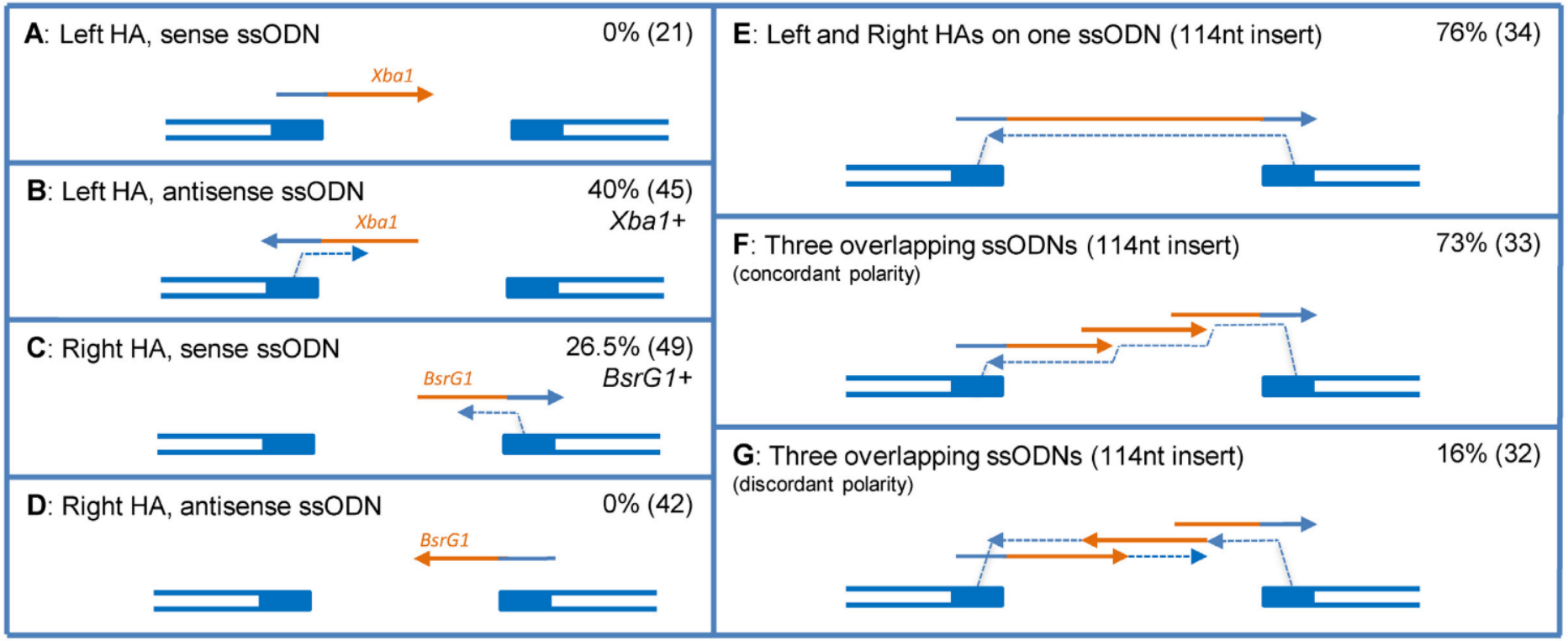


Alexandre Paix, et al. GENETICS. 2014

Geraldine Seydoux Lab

Genome editing using in vivo assembly of linear DNAs

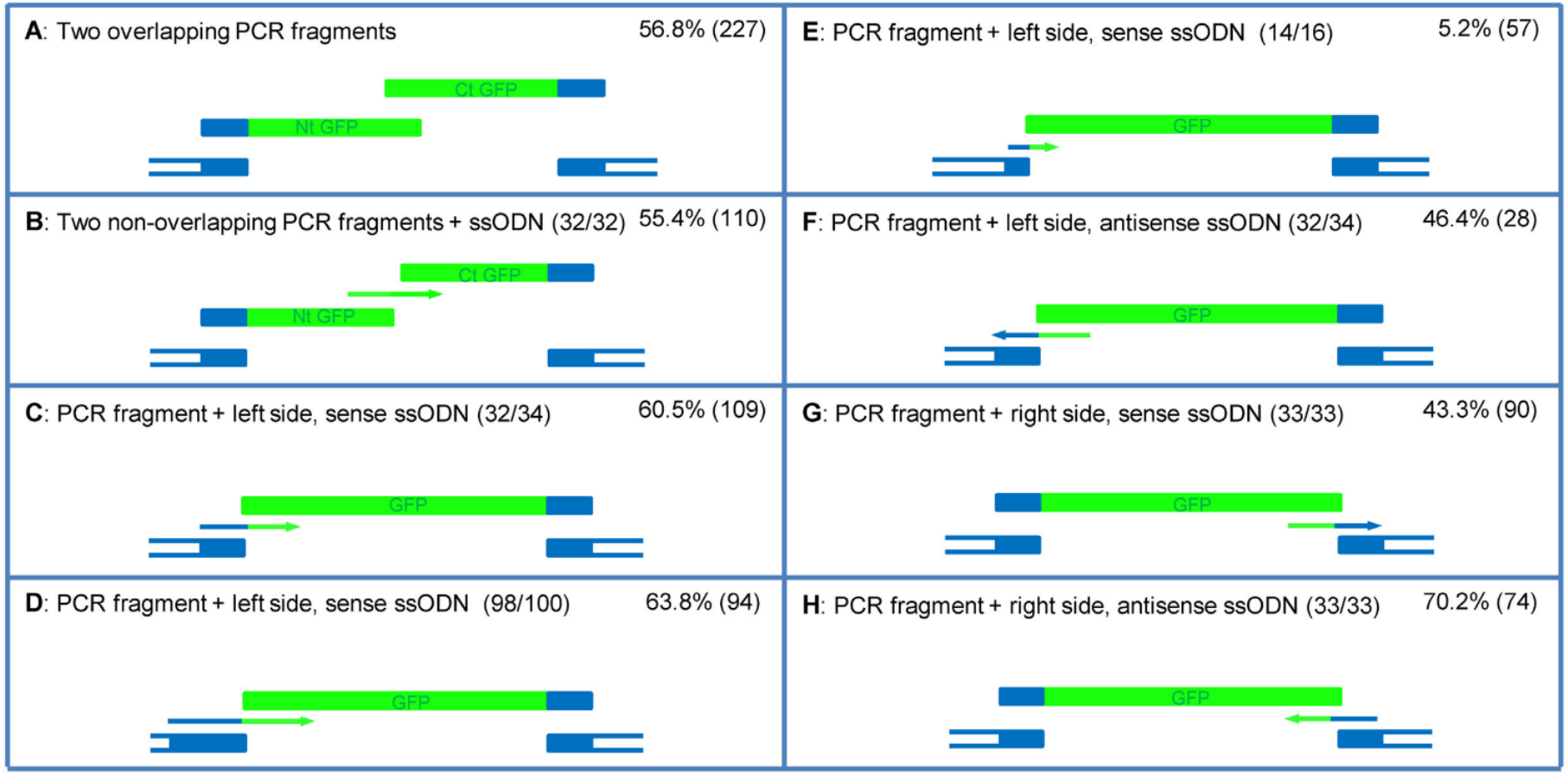
Template switching



Alexandre Paix, et al. NAR, 2016.

Geraldine Seydoux Lab

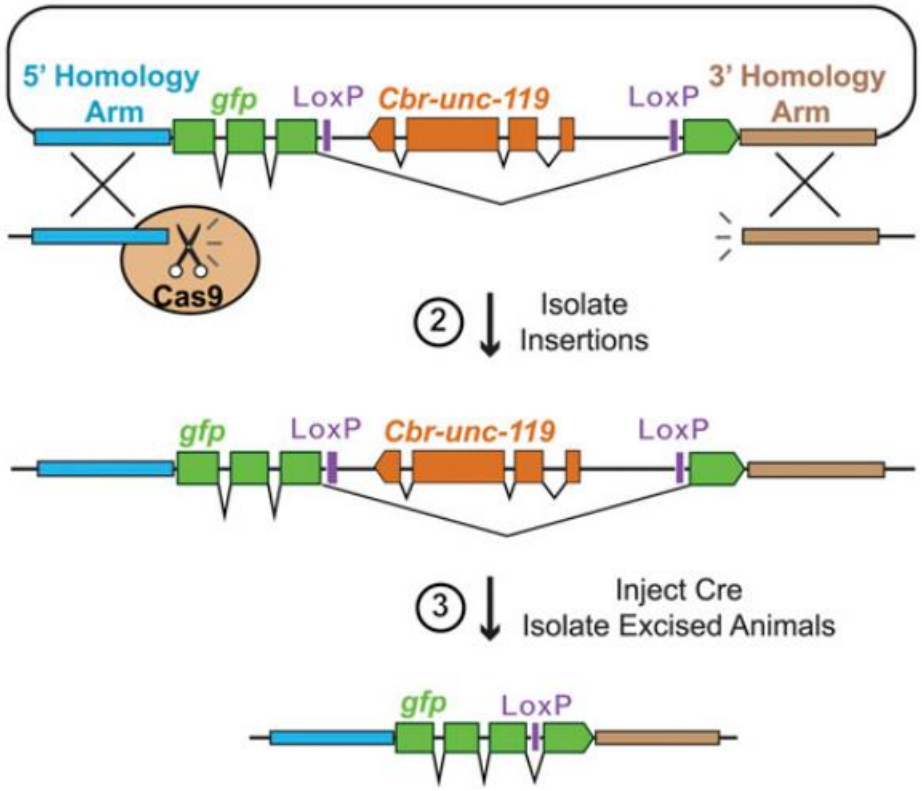
Genome editing using in vivo assembly of linear DNAs



Alexandre Paix, et al. NAR, 2016.

Geraldine Seydoux Lab

SapTrap, a Toolkit for High-Throughput CRISPR/Cas9 Gene Modification in *Caenorhabditis elegans*



SapI

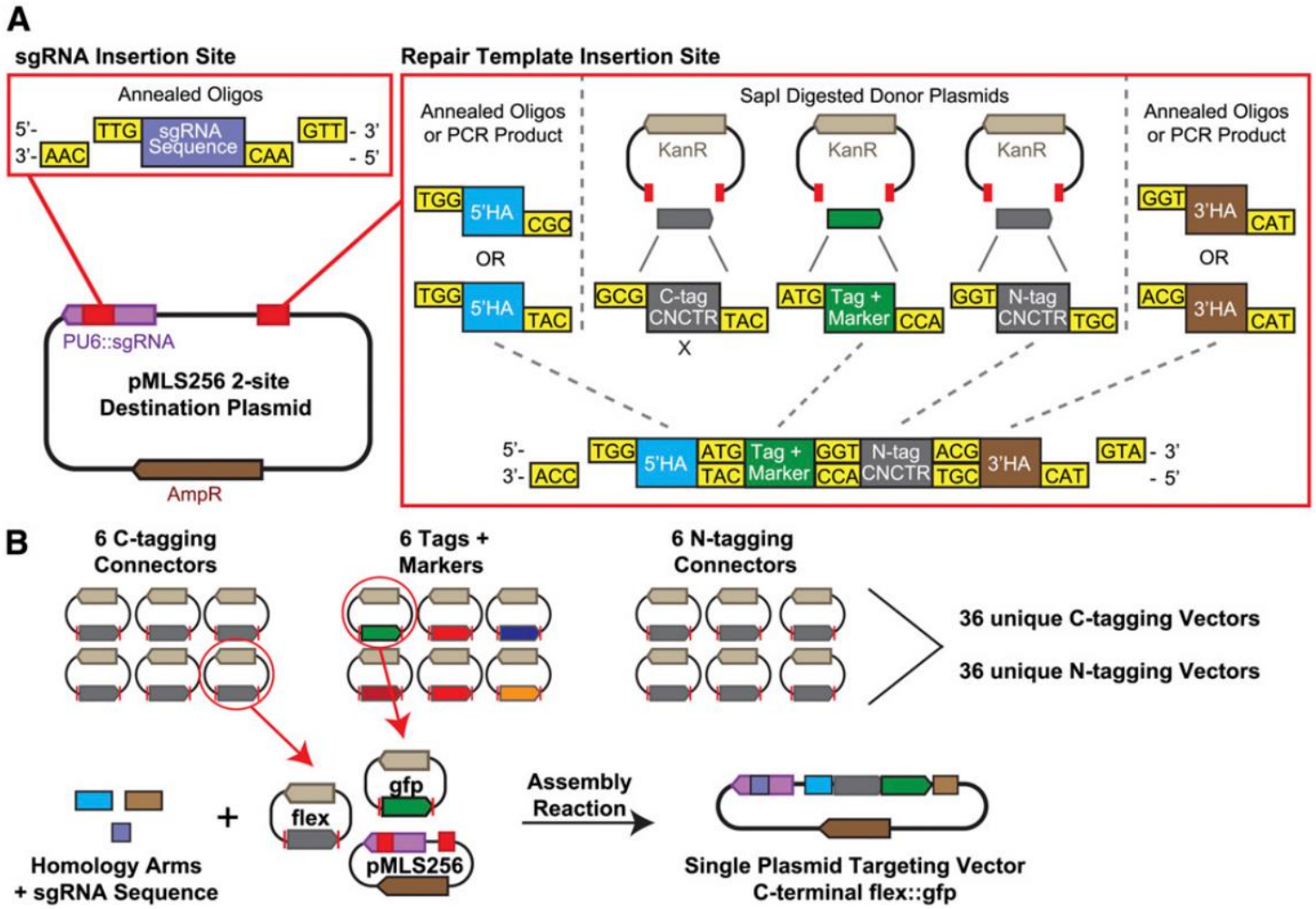
5' G C T C T T C N_1↓ 3'

3' C G A G A A G N_4↑ 5'

Matthew L. Schwartz, et al. GENETICS, 2016

Erik M. Jorgensen Lab

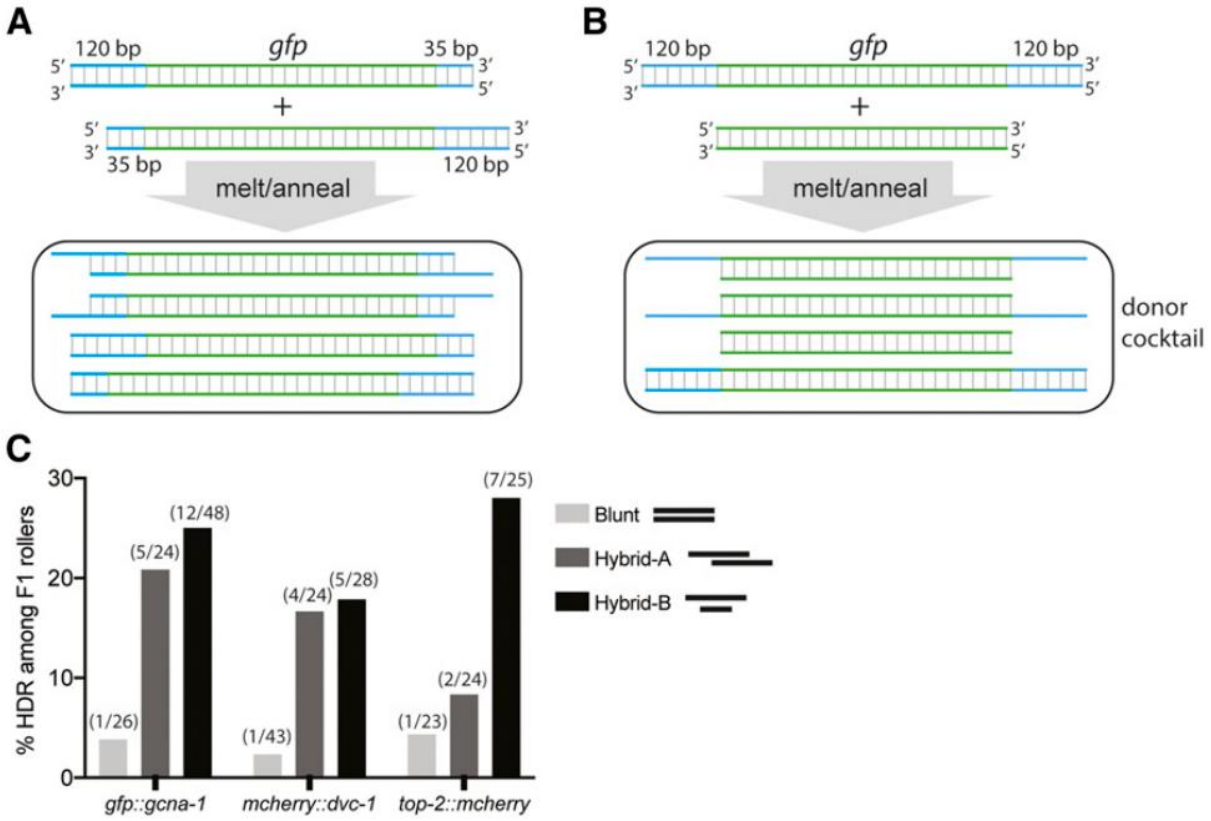
The SapTrap assembly method



Matthew L. Schwartz, et al. GENETICS, 2016

Erik M. Jorgensen Lab

Efficient editing with long, partially single stranded dsDNA donors

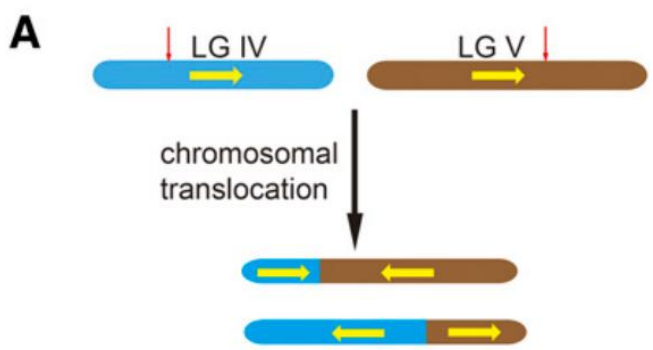


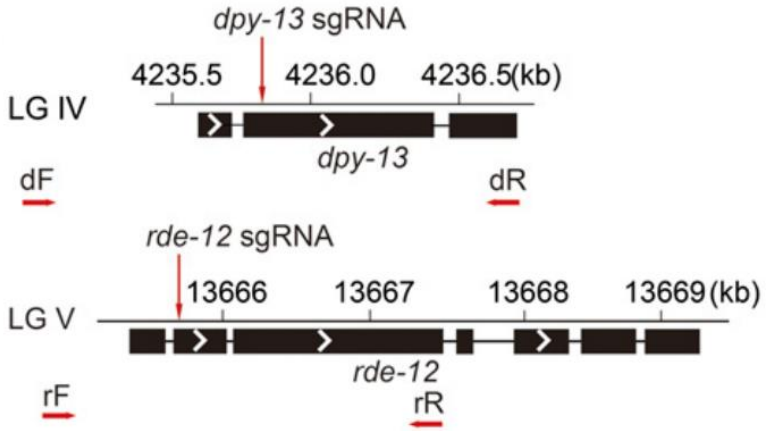
Gregoriy A. Dokshin, et al. GENETICS, 2018

Craig Mello Lab

Chromosome manipulation

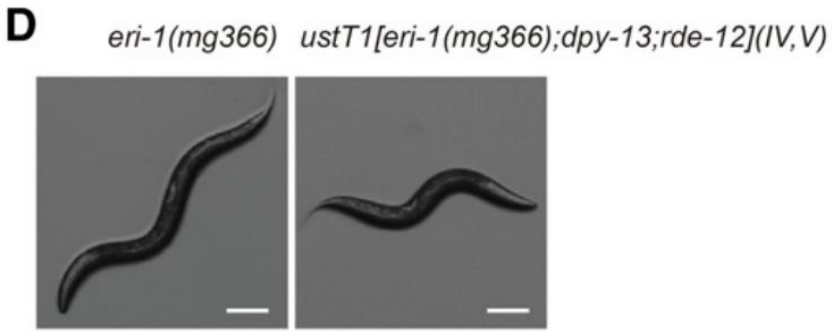
Targeted Chromosomal Translocations and Essential Gene Knockout Using CRISPR/Cas9 Technology in *C. elegans*





C

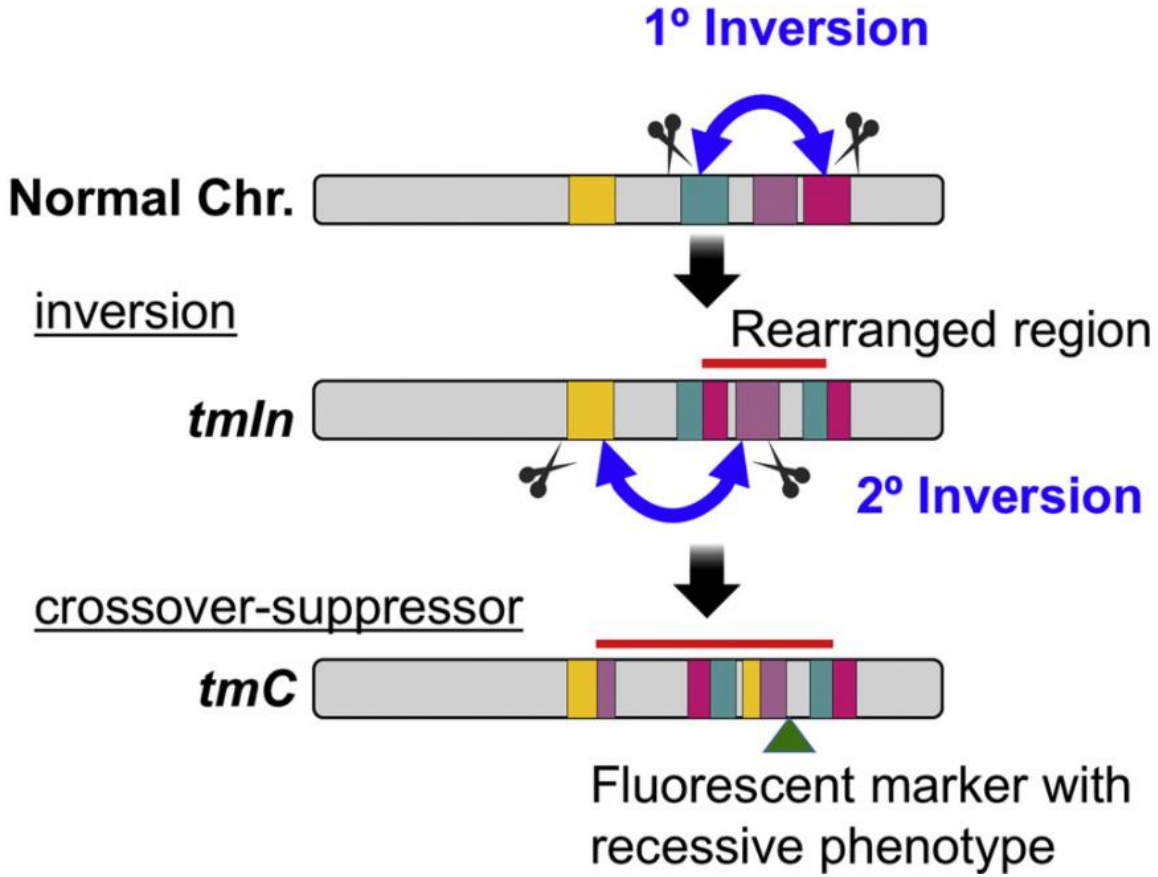
dpy-13 sgRNA + *rde-12* sgRNA	
P0 injected	150
F1 with mCherry	369
F2 with ≤25% dumpy	32
F2 with 100% dumpy	2
translocation strain	1



Xiangyang chen, et al. GENETICS, 2015

Shouhong Guang Lab

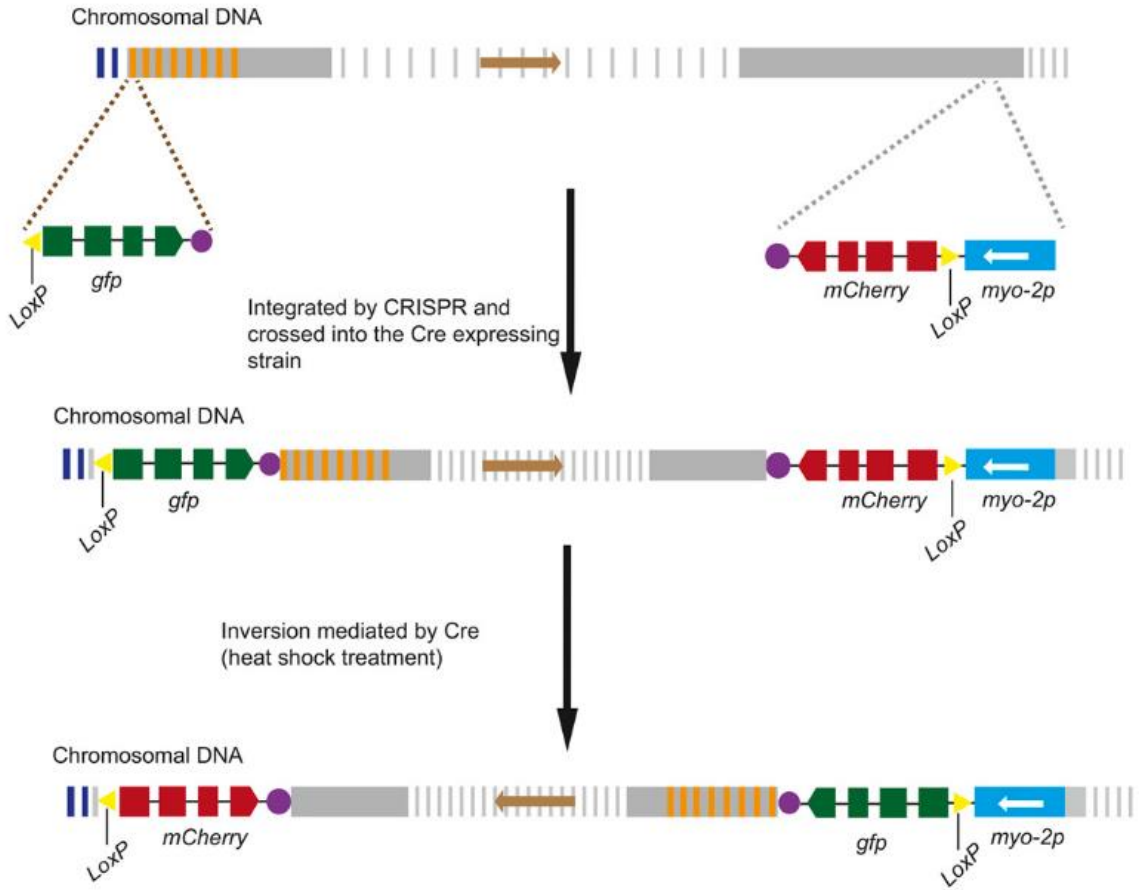
Generation of a set of structurally defined and aneuploidy-free balancer chromosomes via CRISPR/Cas9



Katsufumi Dejima, et al. Cell reports. 2018

Shohei Mitani Lab

Targeted Chromosomal Rearrangements via Combinatorial Use of CRISPR/Cas9 and Cre/LoxP Technologies in *C. elegans*

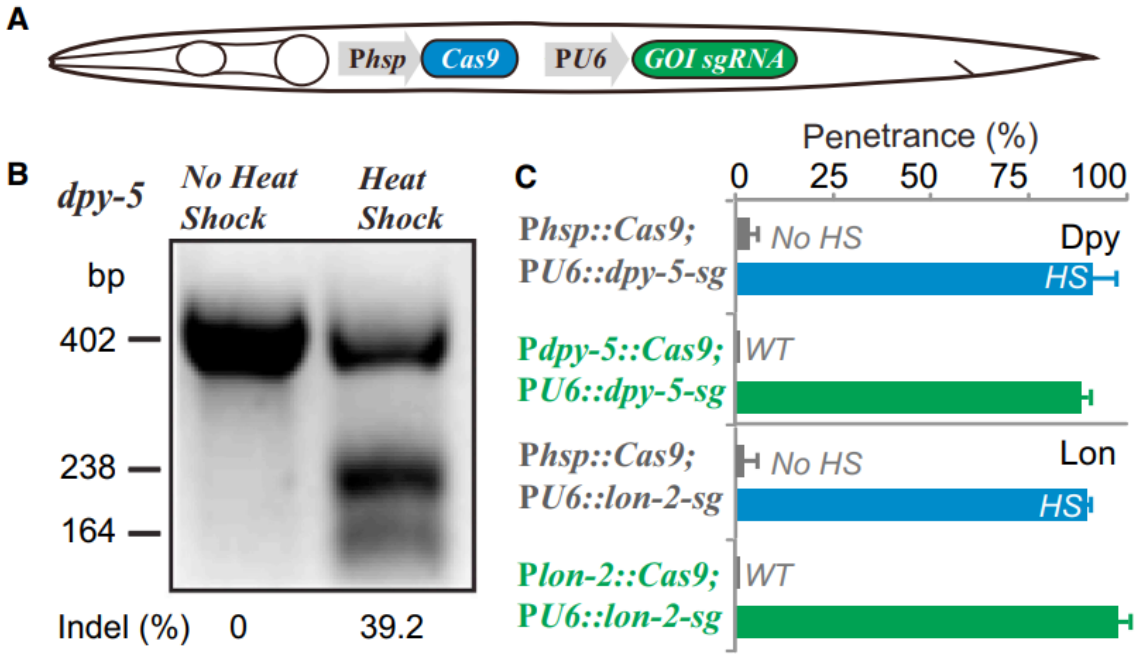


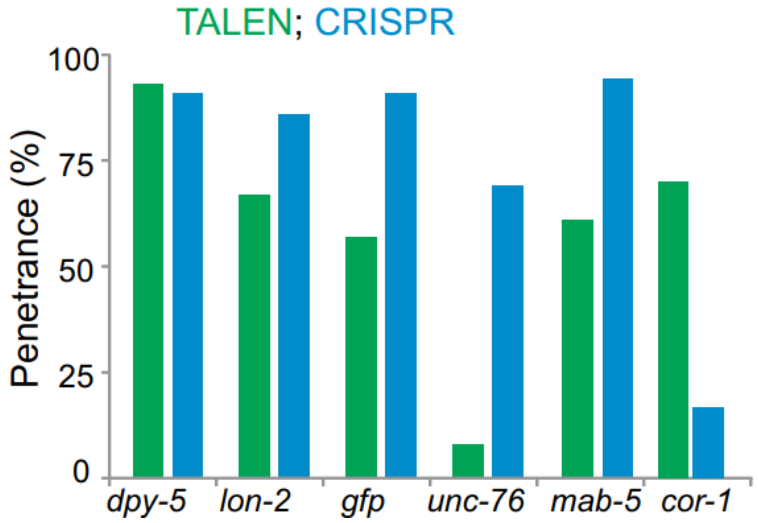
Xiangyang chen, et al. G3, 2018

Shouhong Guang Lab

Essential gene manipulation

Somatic expression of the CRISPR-Cas9 system induces conditional mutations

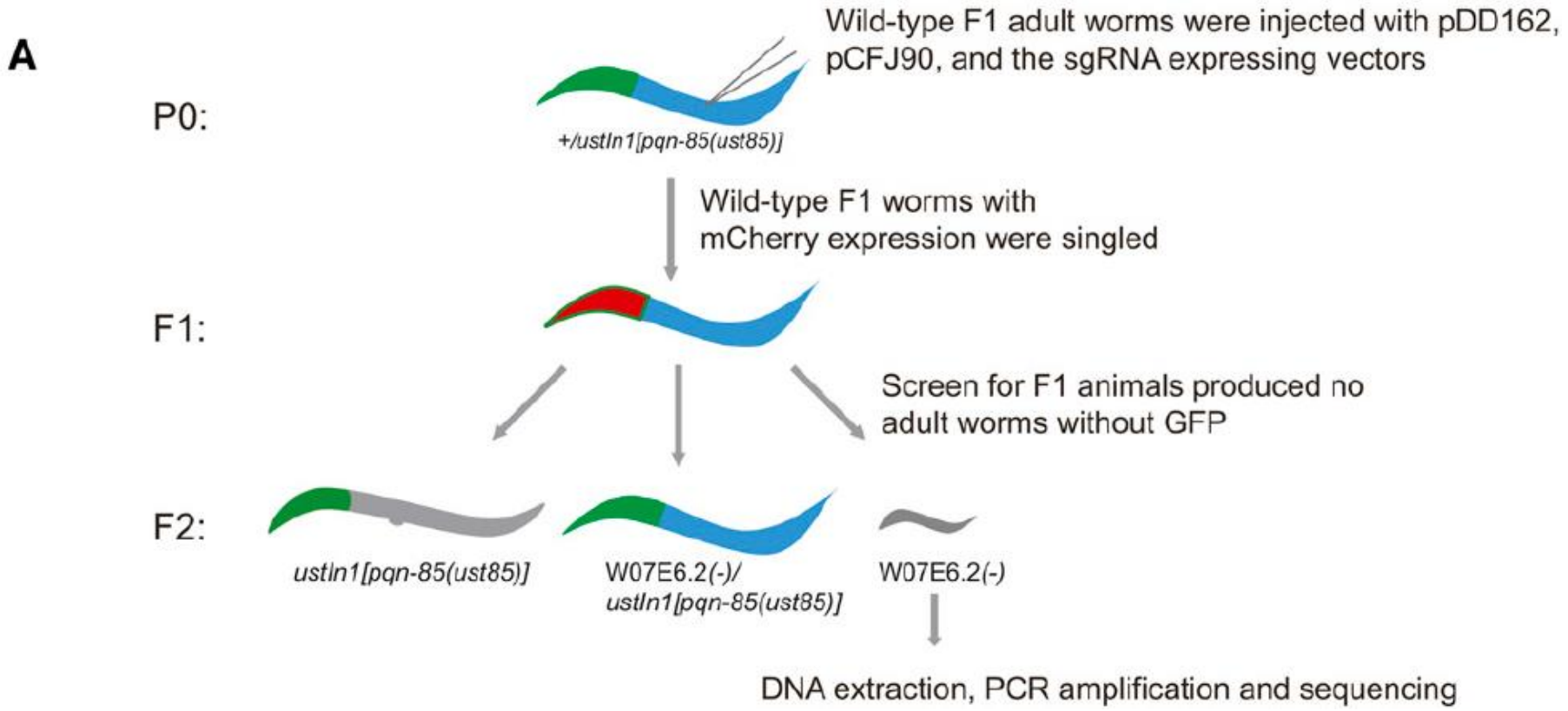




Zhongfu Shen, et al. Development cell. 2014

Guangshuo Ou Lab

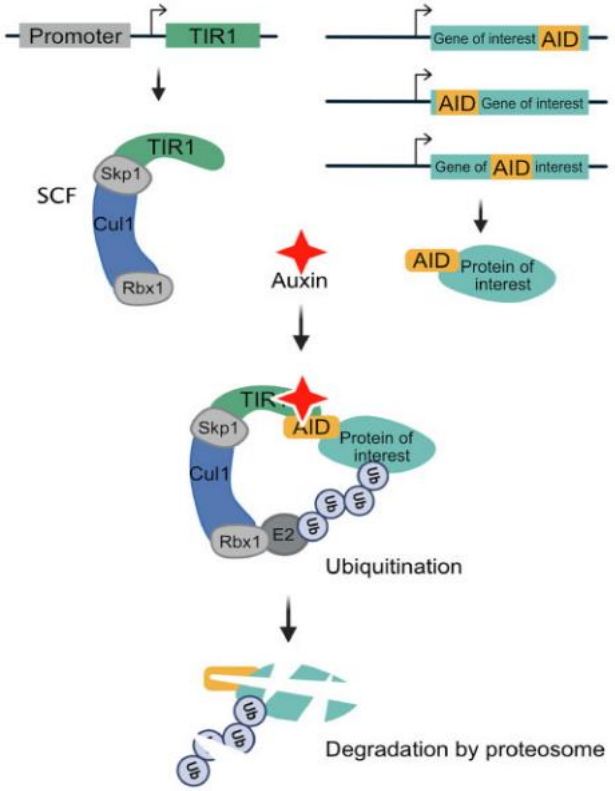
Construction of W07E6.2 balancer using the CRISPR/Cas9 system

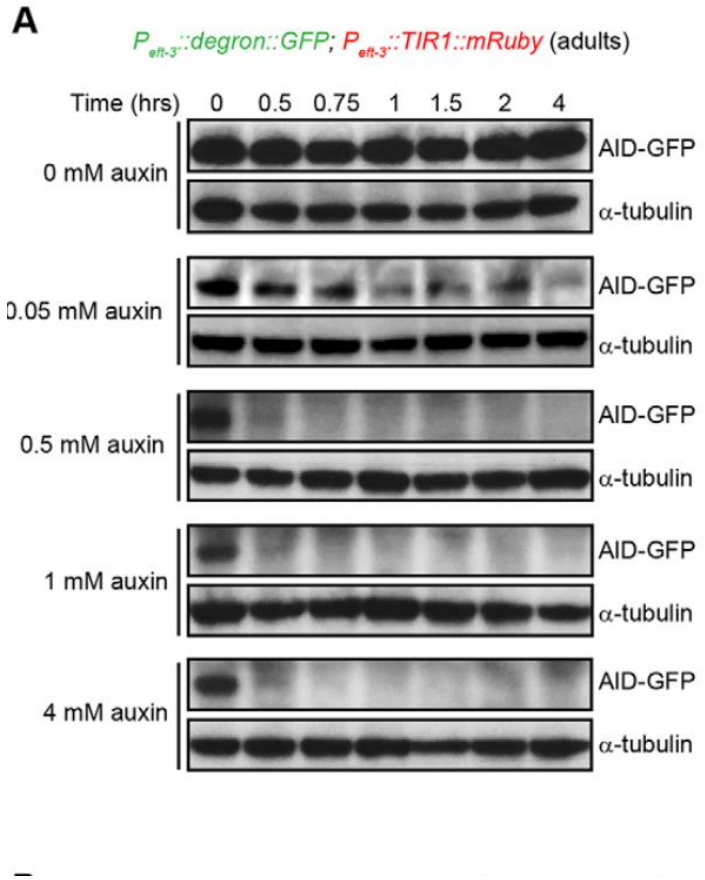


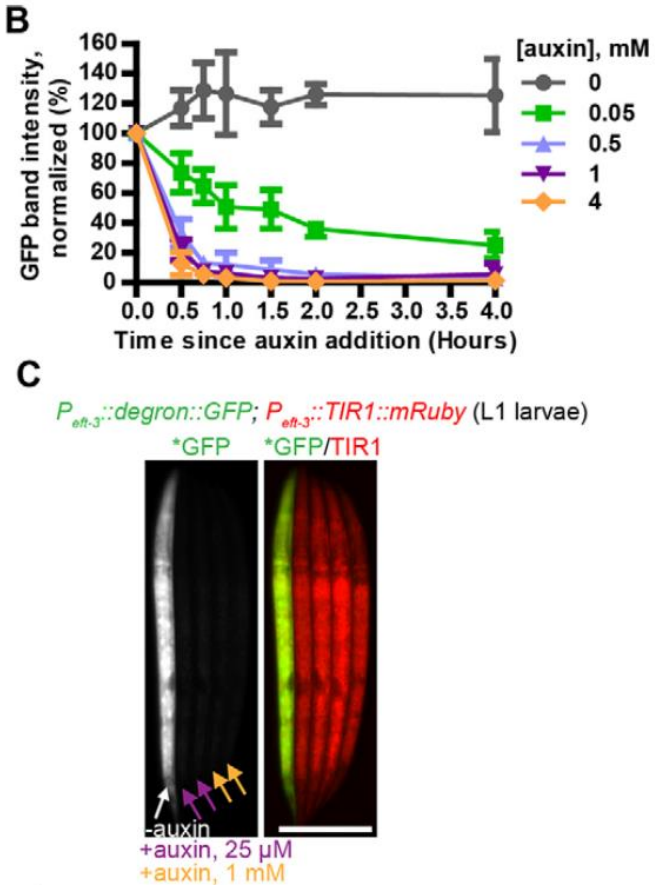
Xiangyang chen, et al. G3, 2018

Shouhong Guang Lab

The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in *C. elegans*





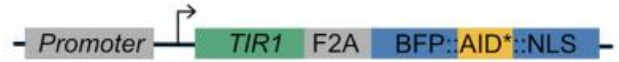


Liangyu Zhang, et al. 2015, Development

Abby F. Dernburg Lab

An expanded auxin-inducible degron toolkit for *Caenorhabditis elegans*

TIR1 driver strains



Strain	Promoter	Tissue	Genotype	Insertion site
LP869	*vha-8p*	Excretory cells, Hypodermis, Gut, unidentified head cells	*cpSi171[vha-8p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
JDW225	*eft-3p*	Soma	*wrdSi23[eft-3p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
DV3799	*col-10p*	Hypodermis	*reSi1[col-10p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
DV3800	*col-10p*		*reSi2[col-10p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	II:-0.77
JDW227	*dpy-7p*		*wrdSi45[dpy-7p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	II:-0.77
JDW229	*dpy-7p*		*wrdSi47[dpy-7p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
JDW231	*SCMp*‡	Seam cells and	*wrdSi44[SCMp*::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	II:-0.77
JDW233	*SCMp*‡	Hypodermis	*wrdSi46[SCMp*::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
JDW221	*mex-5p*	Germline	*wrdSi18[mex-5p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
JDW223	*mex-5p*		*wrdSi35[mex-5p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	II:-0.77
JDW10	*sun-1p*		*wrdSi3 [sun-1p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	II:-0.77
DV3801	*unc-54p*	Muscle	*reSi3[unc-54p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
DV3825	*unc-54p*		*reSi11[unc-54p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	II:-0.77
LP871	*myo-3p*		*cpSi174[myo-3p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
DV3803	*ges-1p*	Intestine	*reSi5[ges-1p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
DV3826	*ges-1p*		*reSi12[ges-1p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	II:-0.77
DV3805	*rgef-1p*	Neuron	*reSi7[rgef-1p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32
DQM526	*cdh-3p*	Anchor cells, Seam cells, L4 Vulval precursor cells	*bmd176[cdh-3p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	II:-0.77
LP870	*myo-2p*	Pharynx	*cpSi172 [myo-2p::TIR1::F2A::BFP::AID*::NLS::tbb-2 3'UTR]*	I:-5.32

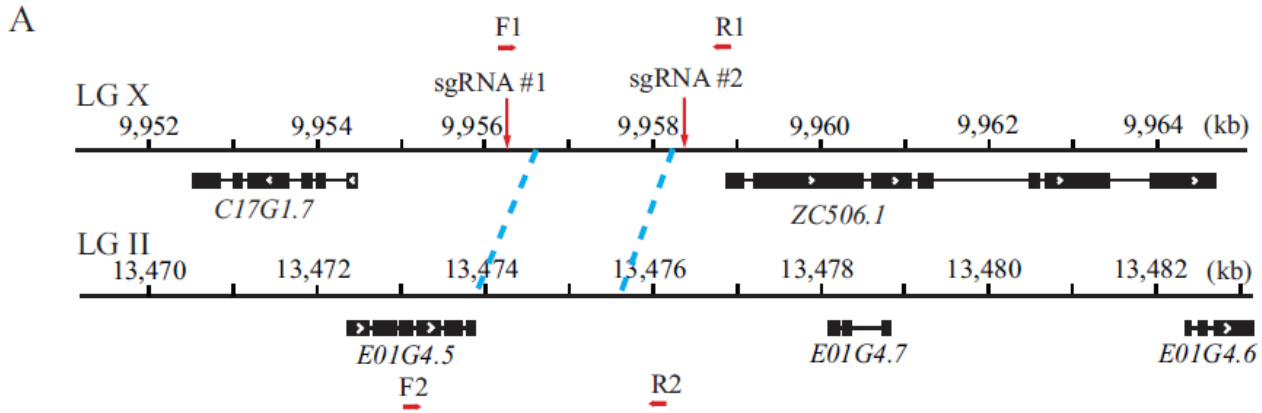
‡ SCM promotor is a 573bp enhancer from *arf-3* intronic sequence + *pes-10Δ*

Guinevere E Ashley, et al. GENETICS. 2021

Jordan D Ward Lab

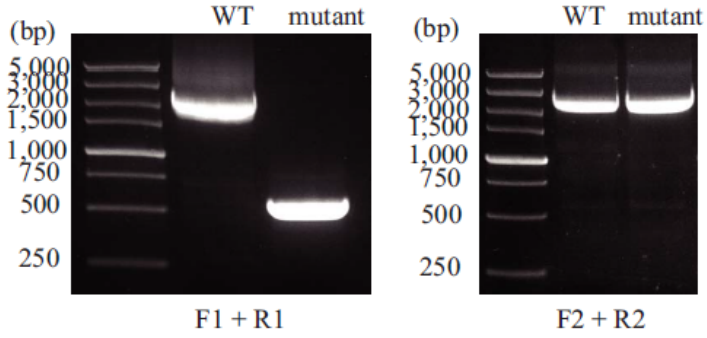
CRISPR in our Lab

Dual sgRNA-guided deletion of a repetitive sequence



B

	sgRNA #1 + #2
P0 injected	20
F1 with mCherry	30
F2 with large deletion	1



D

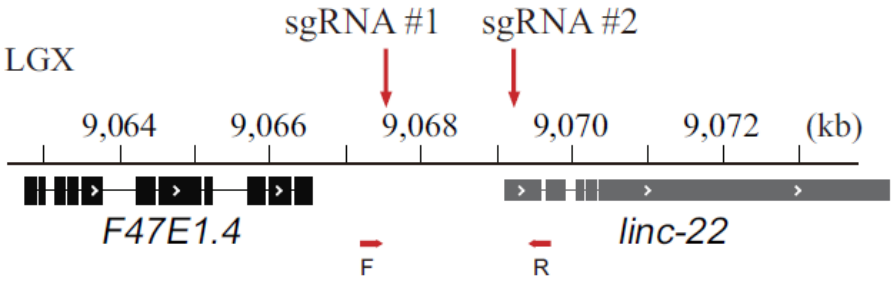
sgRNA #2 PAM

WT TGATGCCA(1994bp)CACCTATCACTATTCATTGACATTCAATT

mutant TGATGCCA----------CATTCAATT (-2014 bp)

Dual sgRNA-directed deletion of lincRNAs

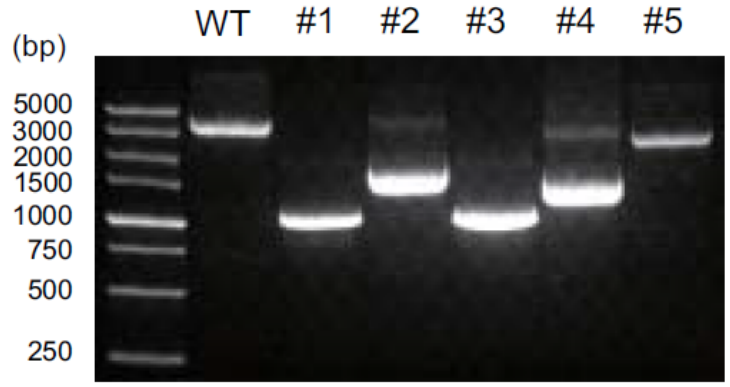
A



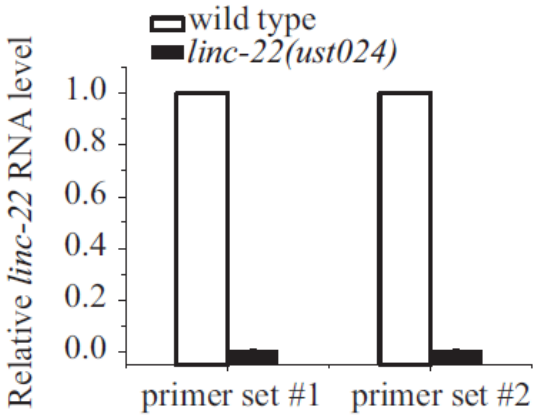
B

sgRNA #1 + #2	
P0 injected	100
F1 with mCherry	168
F2 with large deletion	5

C

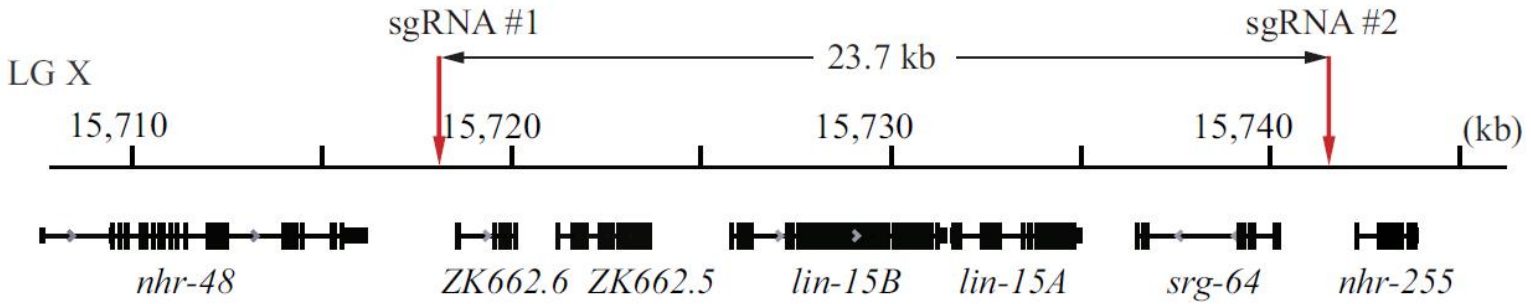


E

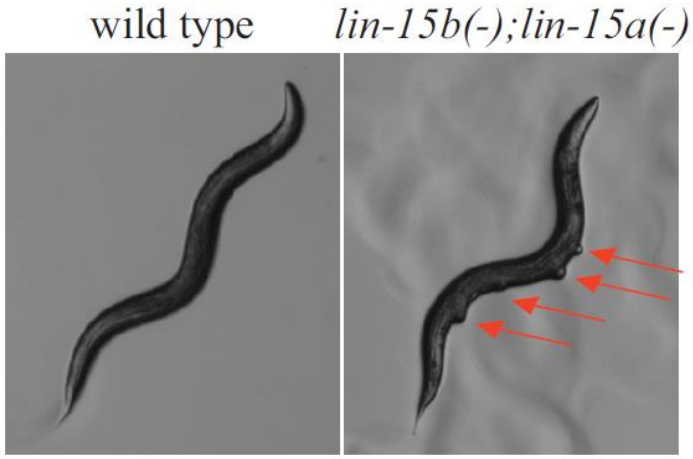


Dual sgRNAs can direct the deletion of large chromosome segments

D



E



F

	sgRNA #1 + #2
P0 injected	100
F1 with mCherry	136
F2 with Muv	6
F2 with large deletion	2
	(-23,717 bp)
	(-23,445 bp)

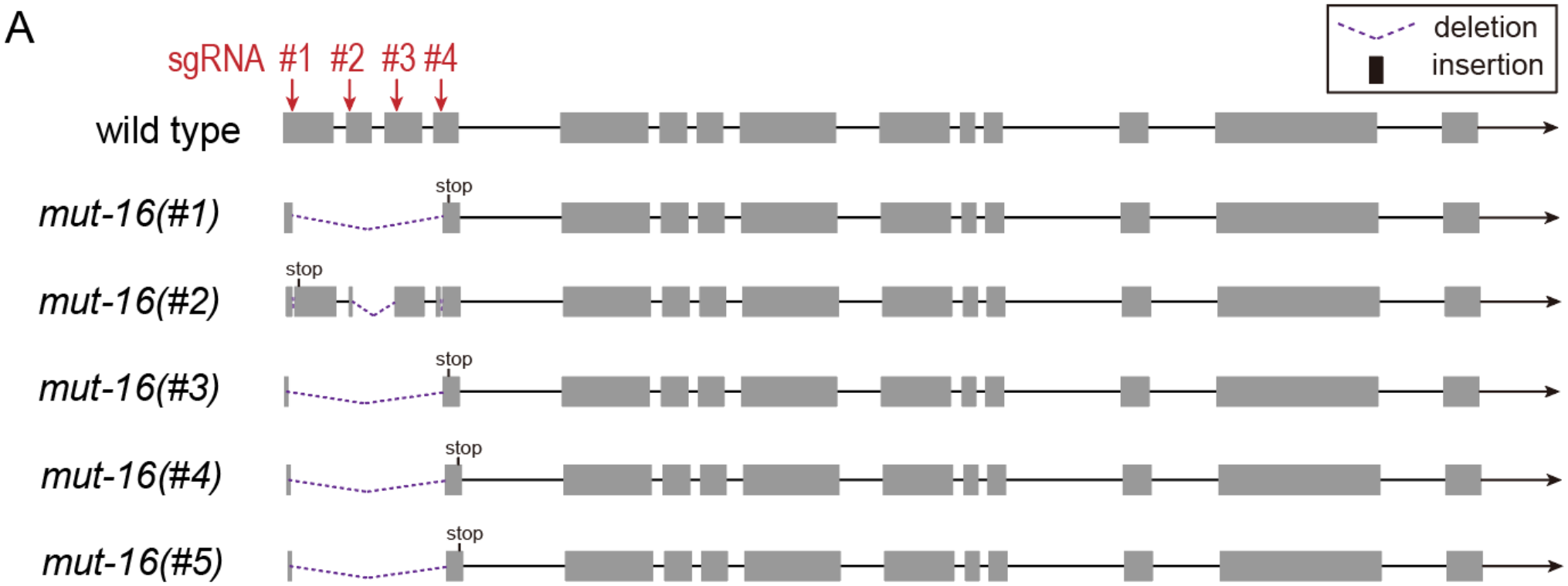
Xiangyang Chen, et al. Sci Reports. 2014

Shouhong Guang Lab

Summary of dual sgRNAs experiments

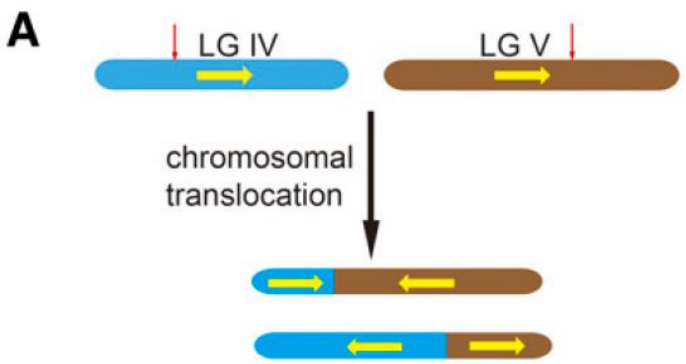
	Targeted region (kb)	F1 with mCherry	F2 with deletion	Ratio (%)
rde-12	1	46	1	2.2
linc-22 promoter	1.9	168	5	3.0
E01G4.5 repeat sequence	2.1	30	1	3.3
dpy-7 region	8.5	216	6	2.8
	16.5	126	1	0.8
lin-15b/a region	23.7	136	2	1.5
f39b2 region	100.2	143	2	1.4

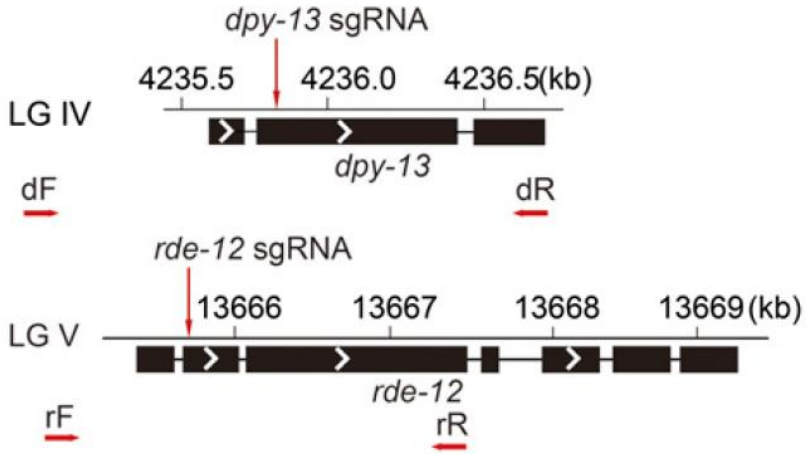
Gene knockout via multiple sgRNAs



Xiangyang Chen, et al. unpublished

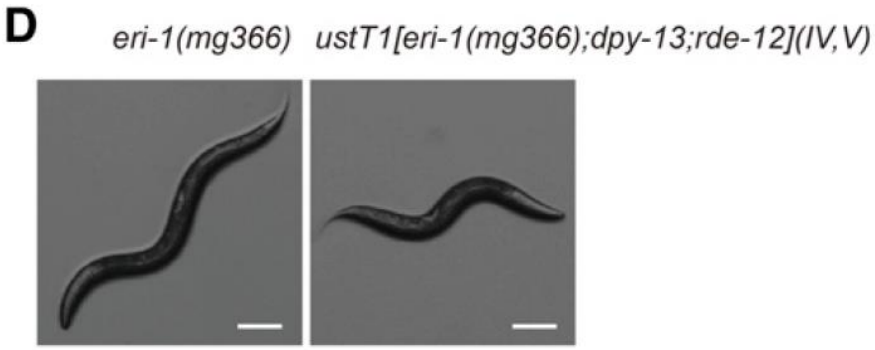
Cas9 directs chromosomal translocation between dpy-13 (LG IV) and rde-12 (LG V)



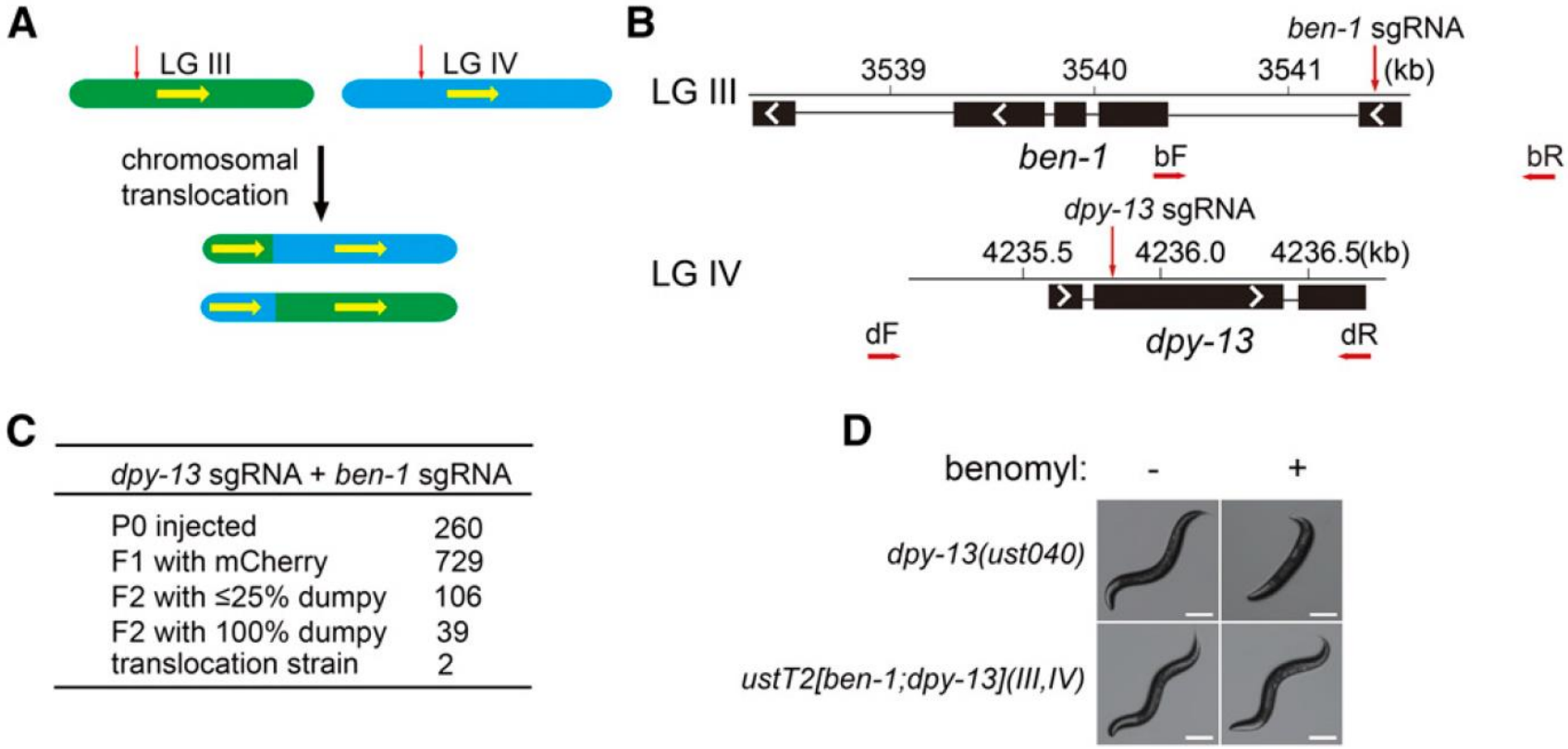


C

dpy-13 sgRNA + *rde-12* sgRNA	
P0 injected	150
F1 with mCherry	369
F2 with ≤25% dumpy	32
F2 with 100% dumpy	2
translocation strain	1



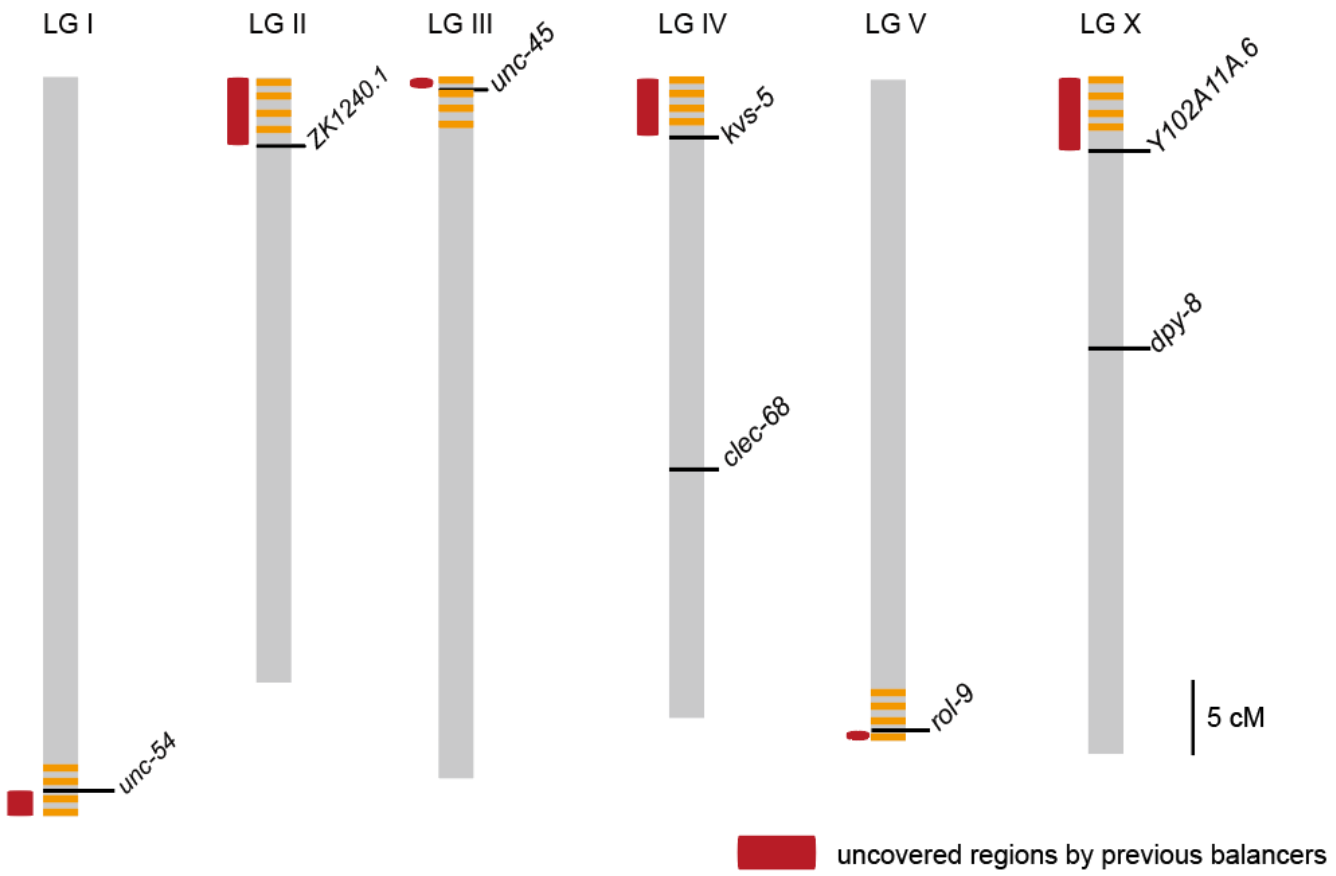
Cas9 directs chromosome translocation between ben-1 (LG III) and dpy-13 (LG IV)



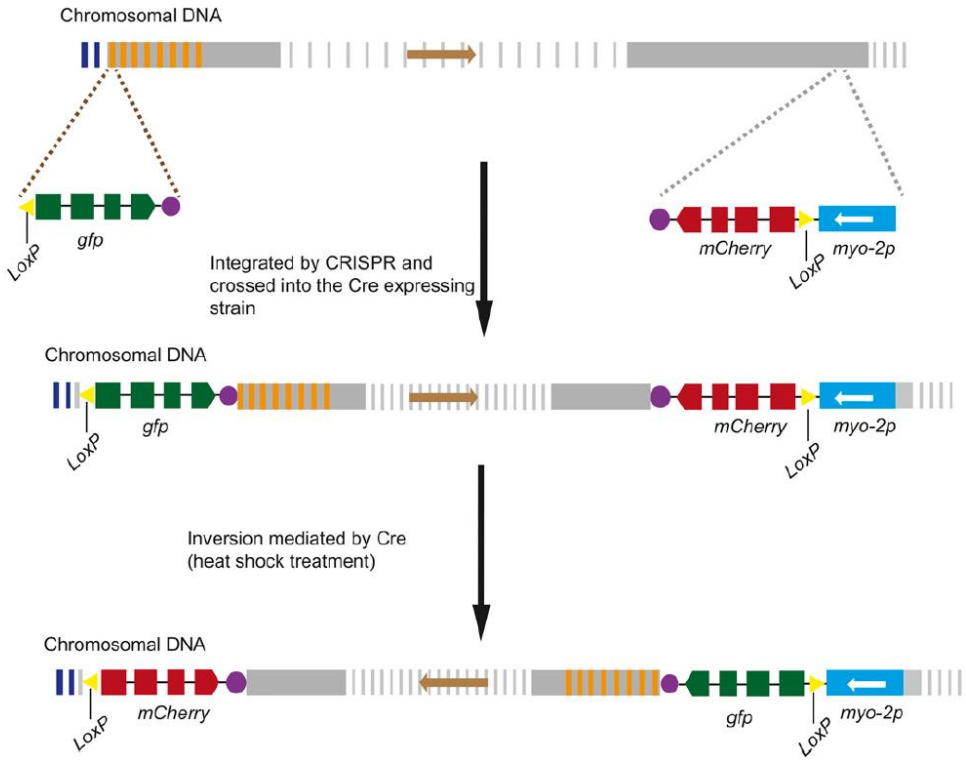
C

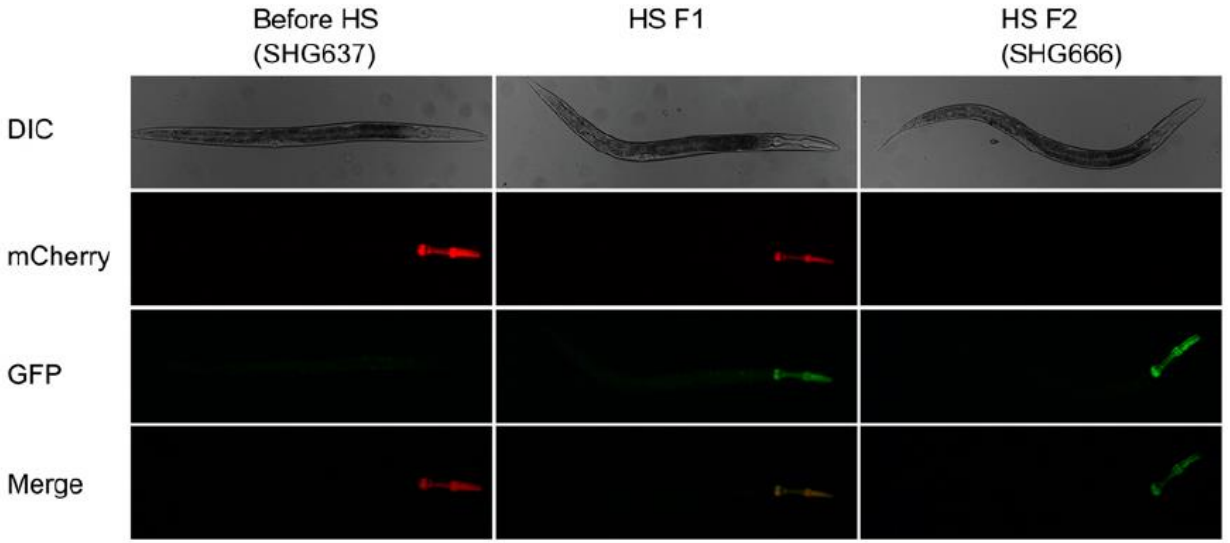
dpy-13 sgRNA + *ben-1* sgRNA	
P0 injected	260
F1 with mCherry	729
F2 with ≤25% dumpy	106
F2 with 100% dumpy	39
translocation strain	2

Schematic of the genomic regions uncovered by existing balancer systems in *C. elegans*



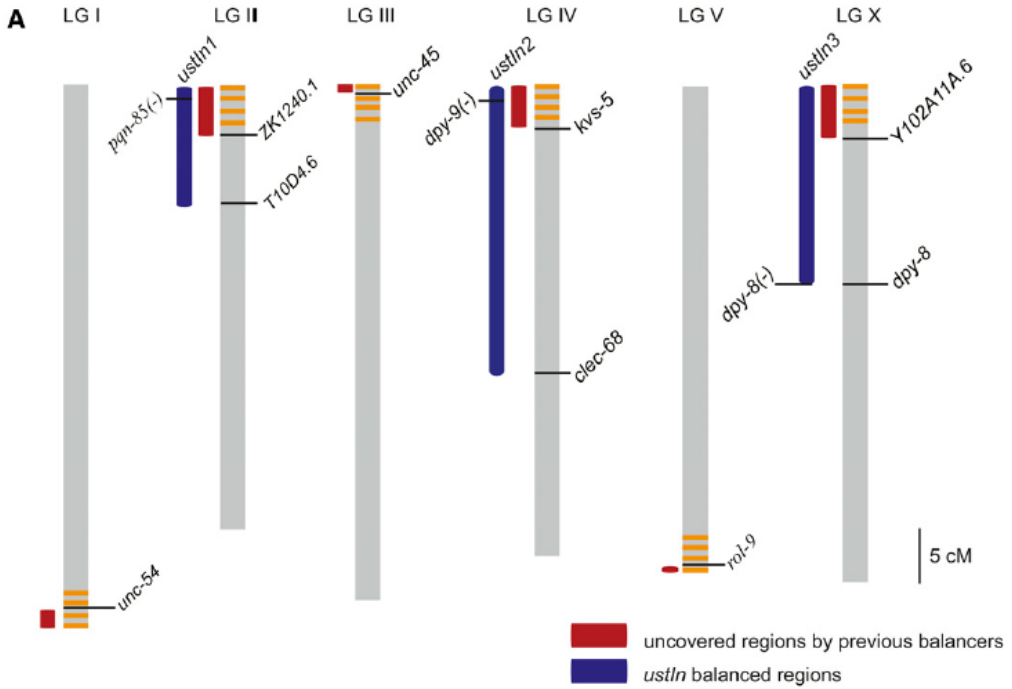
Strategy for chromosomal inversions through combinatorial use of the CRISPR/Cas9 and Cre/LoxP technologies





Summary of the chromosomal inversions

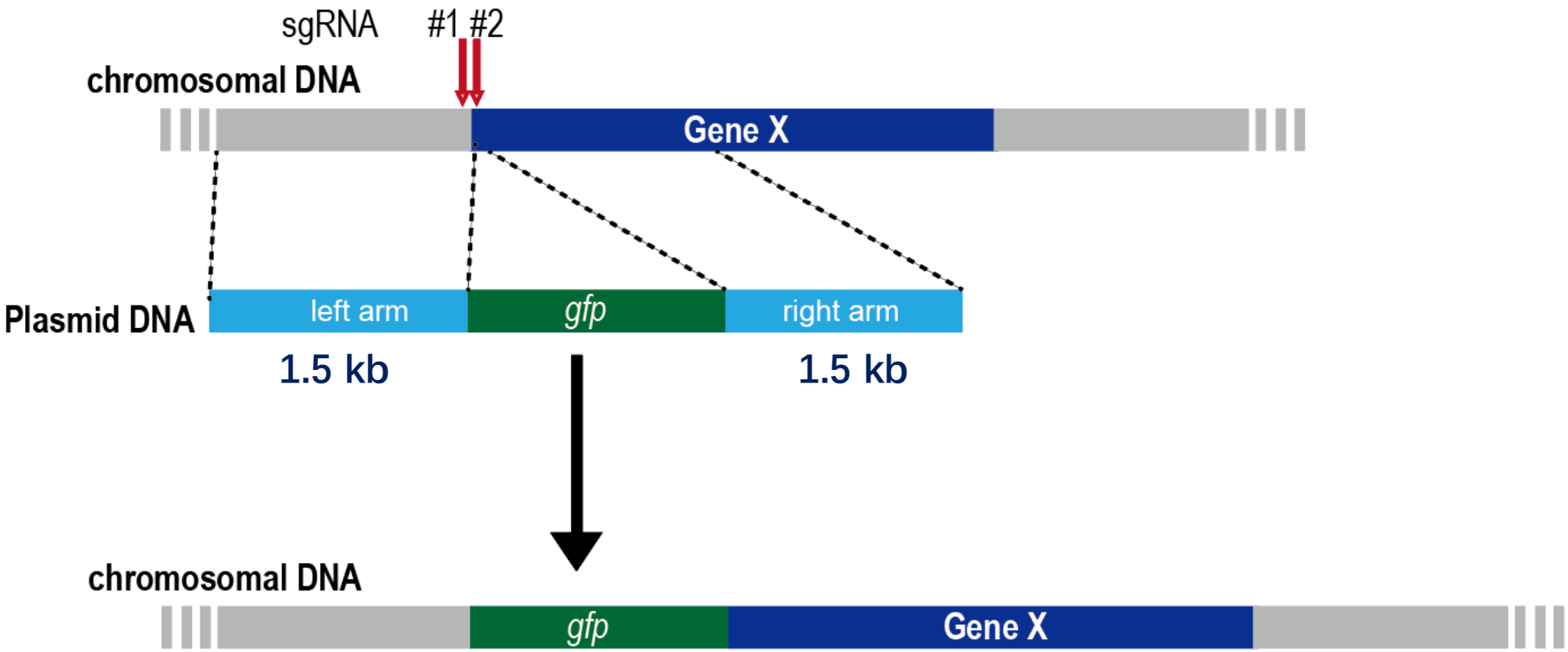
A



B

Strain	Genotype	Balanced region (cM)	Fluorescence marker	Morphological marker
SHG686	*+/ustIn1[pqn-85(ust85); best-4;t10d4.6]*	LG II, -18.01 ~ -8.47	*myo-2p::gfp*	*pqn-85(-)*
SHG687	*+/ustIn2[clec-68;dpy-9(ust86)]*	LG IV, -27.20 ~ -1.65	*myo-2p::gfp*	*dpy-9(-)*
SHG688	*+/ustIn3[dpy-8]*	LG X, -21.60 ~ -6.16	*myo-2p::gfp*	*dpy-8(-)*

Transgene construction via the CRISPR/Cas9



At least one **GN17GGNGG** sgRNA

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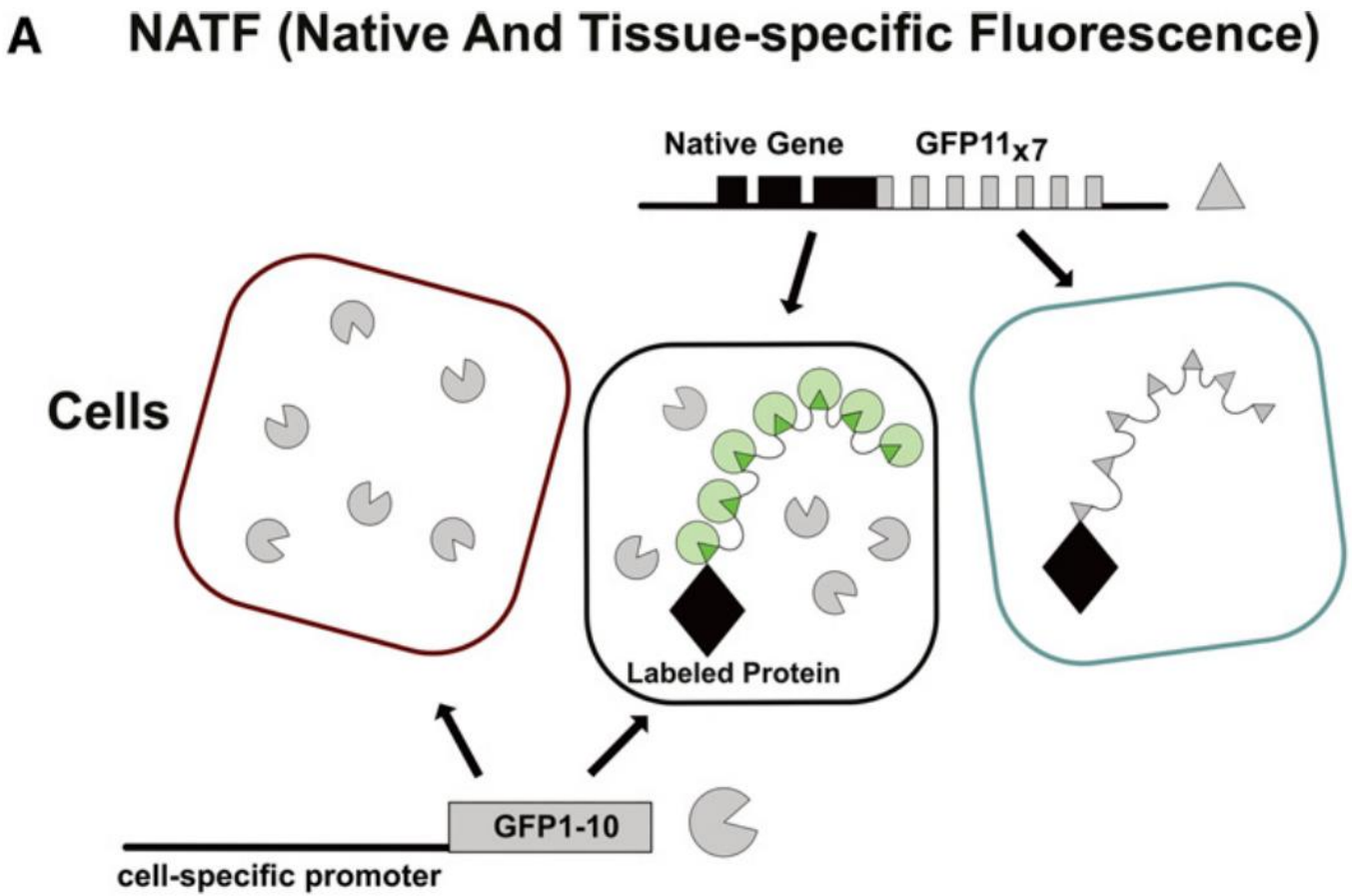
王小洋

NATF (Native and Tissue-Specific Fluorescence): A Strategy for Bright, Tissue-Specific GFP Labeling of Native Proteins



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