

New Tools for the AID System

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Ward Lab

UCSC



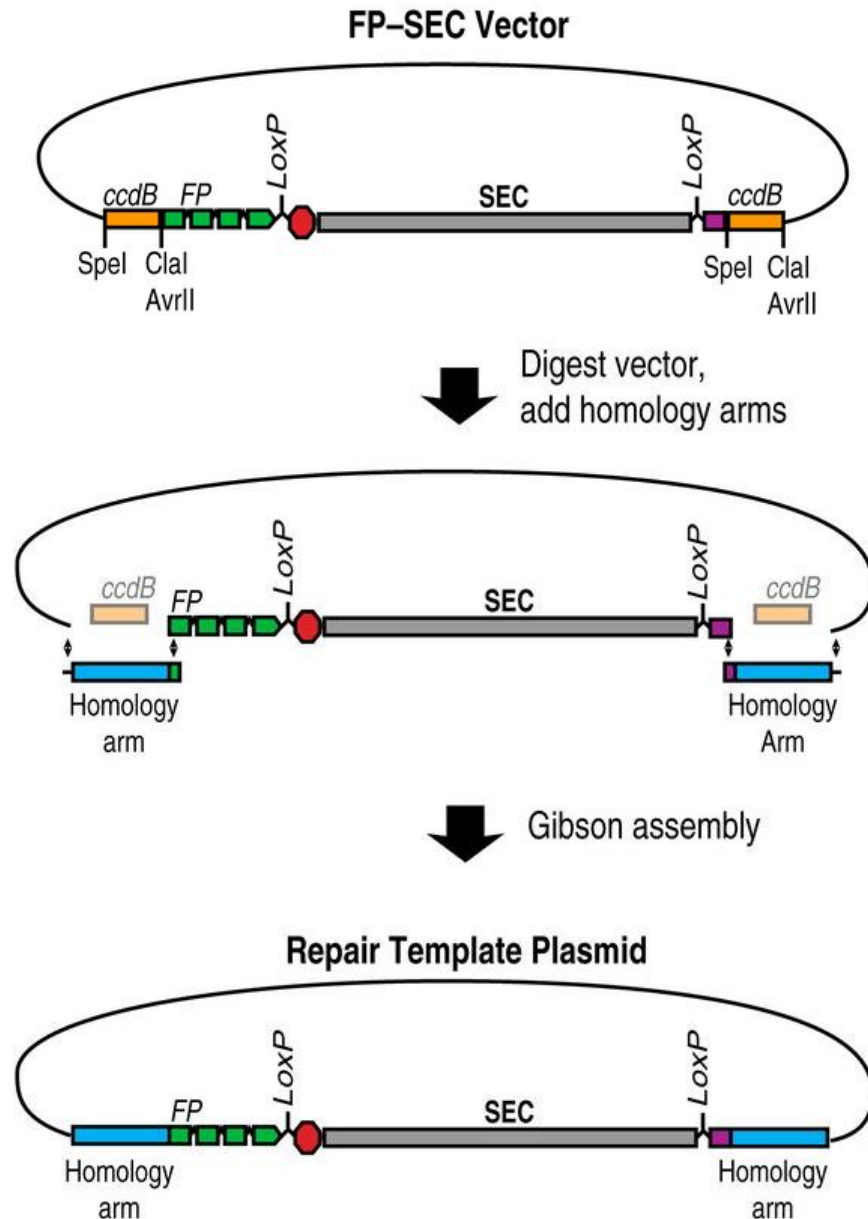
A 3D-rendered illustration of a dark grey asphalt road with white dashed lane markings, curving from the bottom left towards the top right. Three blue rectangular signposts with rounded corners and white text are positioned along the road. The first signpost is on the left side of the road, the second is on the right side, and the third is further up the road on the right side. Each signpost is supported by two thin grey metal poles. The background is plain white.

Workflow

TIR Drivers

AID knock-in

Gibson Assembly Method

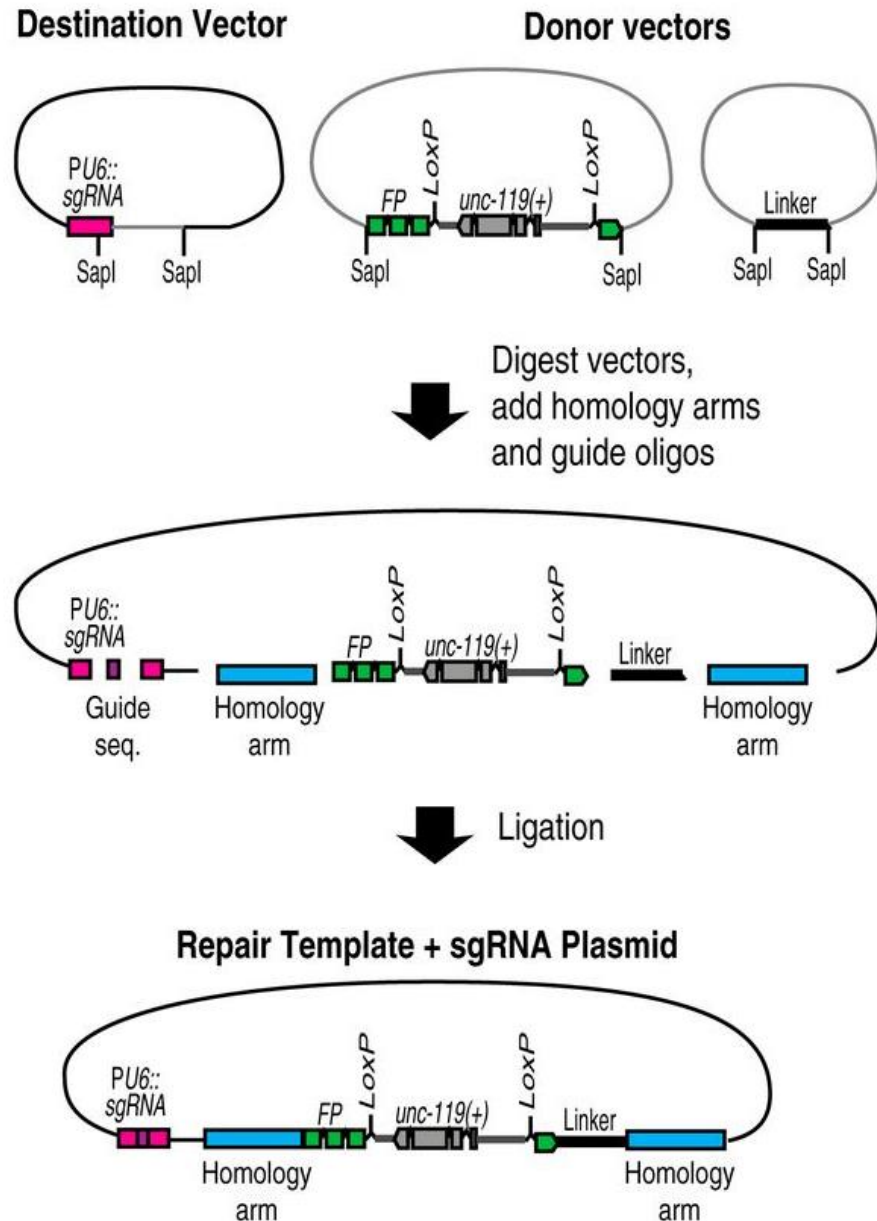


- Ward lab initially used Gibson cloning to generate knock-in repair templates
- Cloned AID cassette downstream of Dickinson FP[^]SEC[^]3xFLAG plasmids

AID versions available

- GFP (pJW1583)
- YPET (pJW1584) [\[in AddGene\]](#)
- mKate2 (pJW1986)
- mNG (pJW1582) [\[by direct request\]](#)

Golden Gate Assembly Method



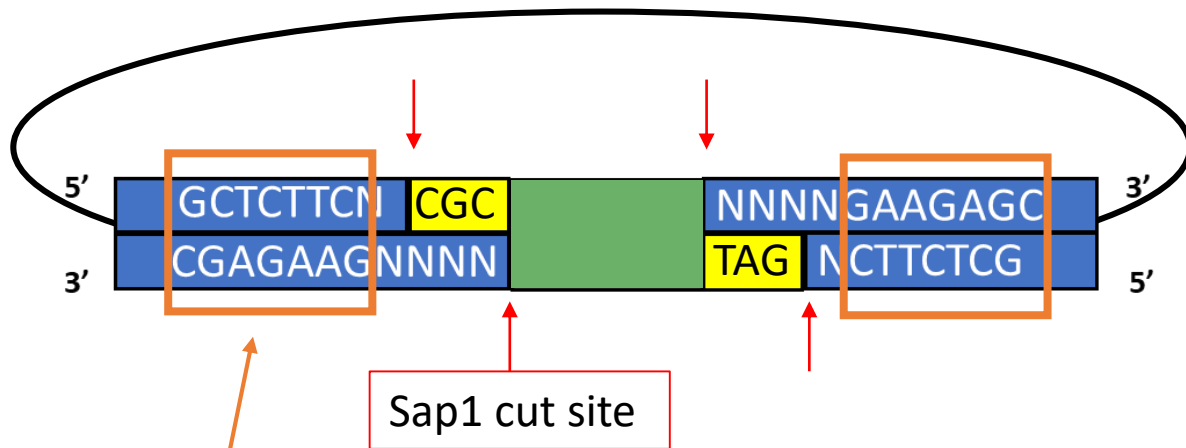
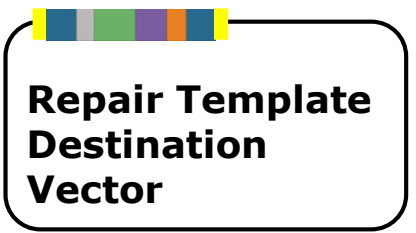
- Moved towards SapTrap assembly to generate new vectors
- Modular, type II restriction enzyme based assembly
- Initially described with *unc-119(+)* selection
- Dan Dickinson developed SapTrap compatible SEC cassettes:

<https://www.micropublication.org/journals/biology/w2kt0n/>

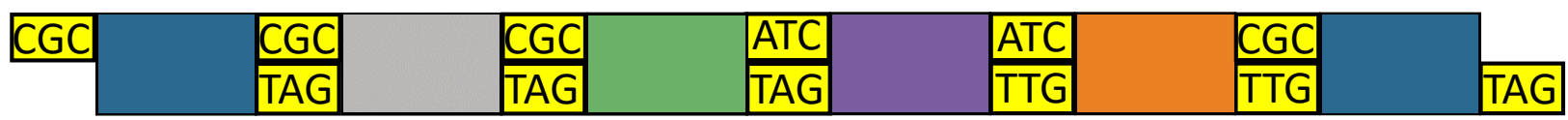
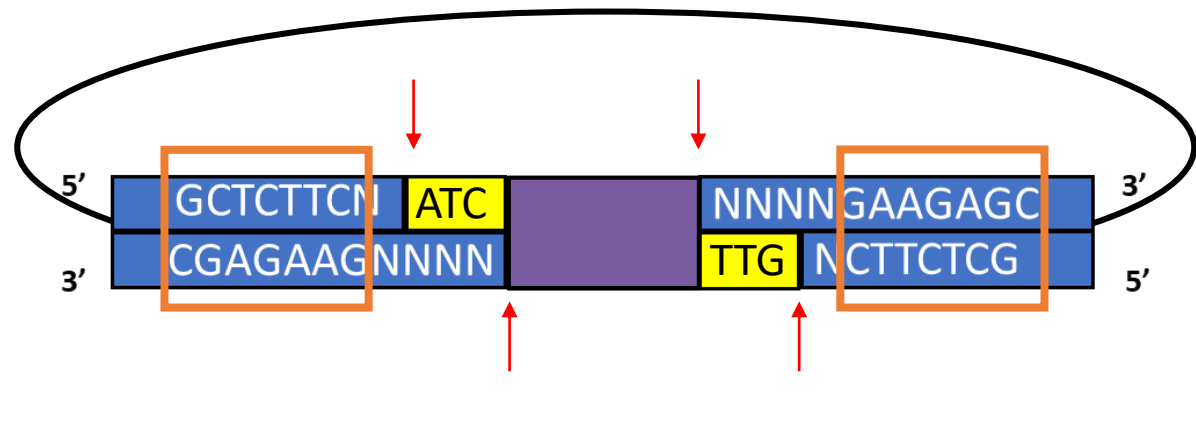
A Pipeline for Genome Editing Using CRISPR



SapTrap: High Throughput Cloning

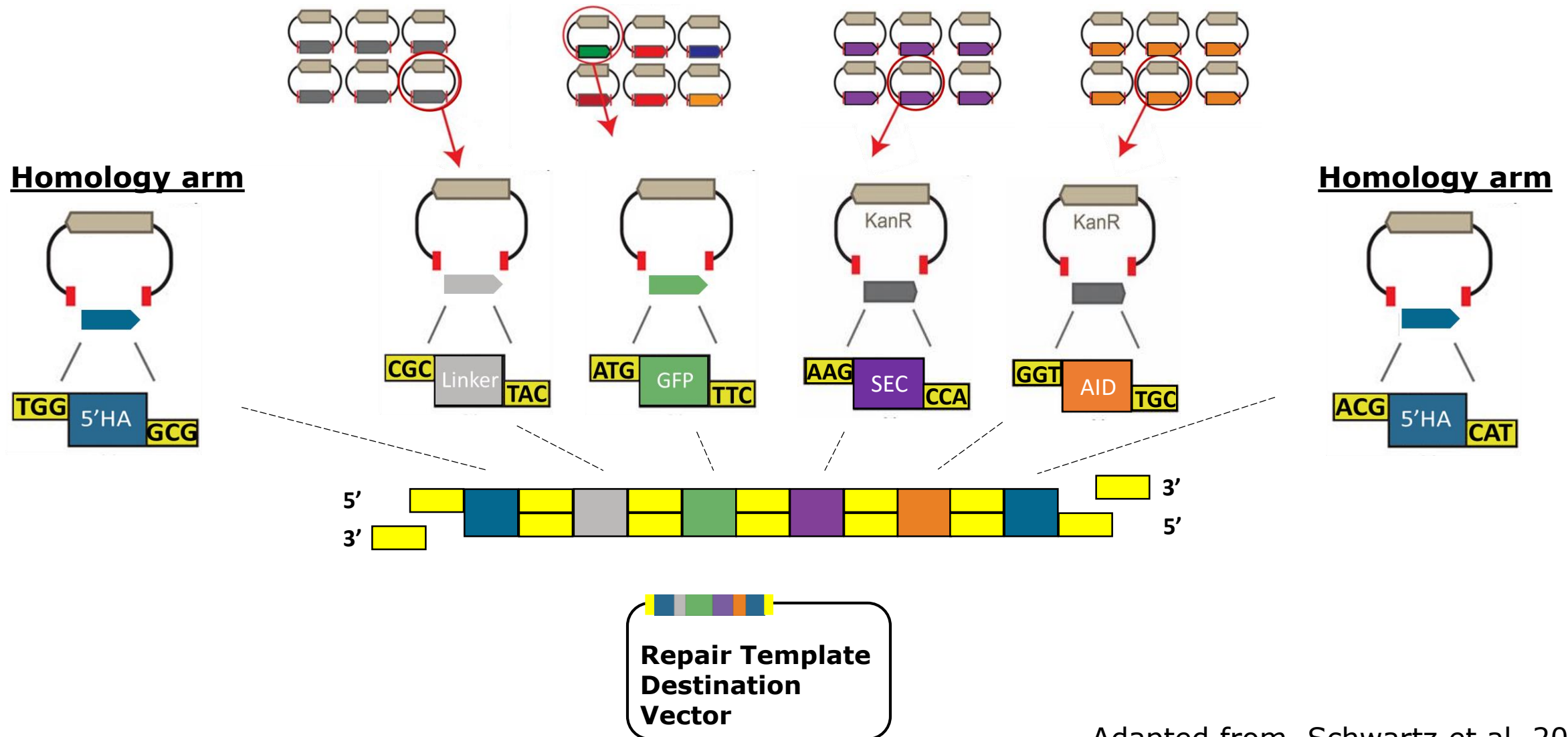


Sap1 restriction site

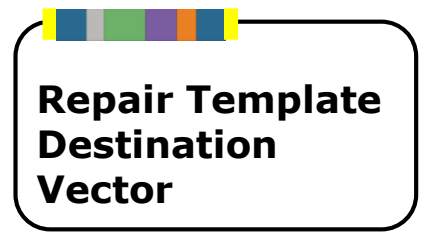


SapTrap: High Throughput Cloning

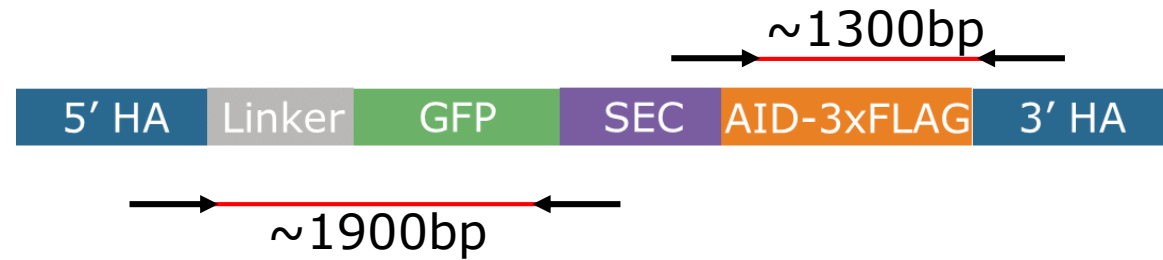
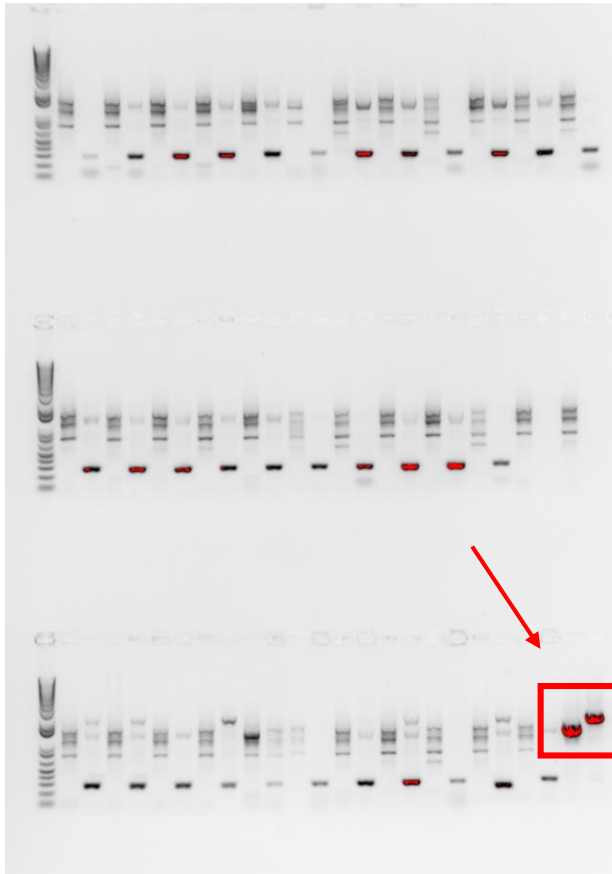
Repair Template
Destination
Vector



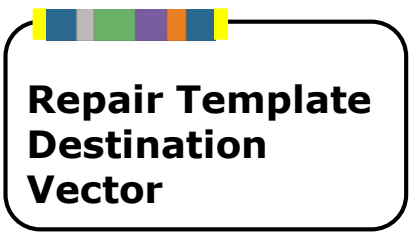
SapTrap: High Throughput Cloning



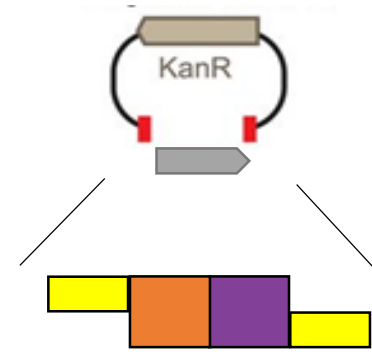
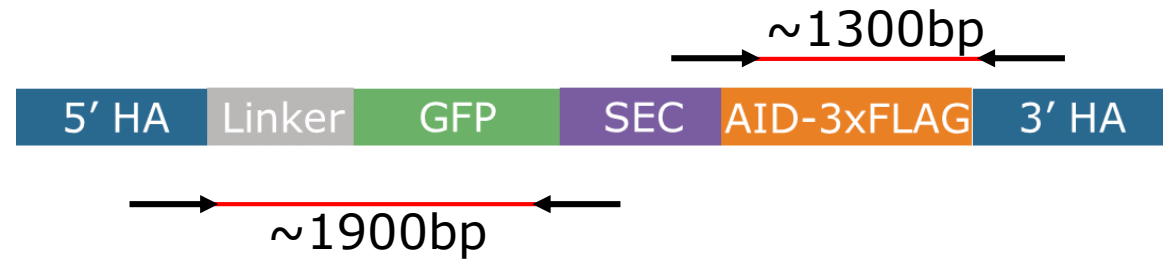
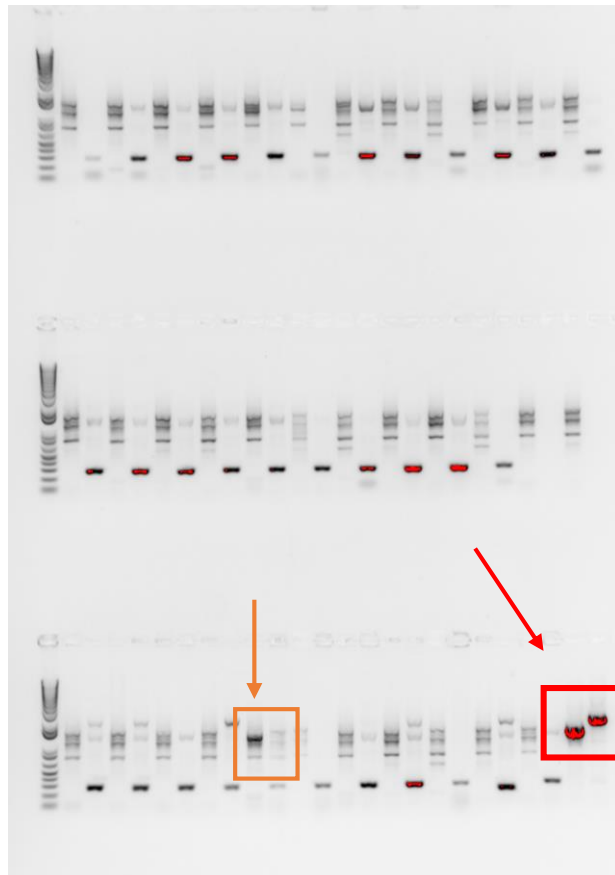
3% efficiency



SapTrap: High Throughput Cloning




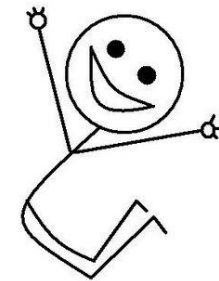
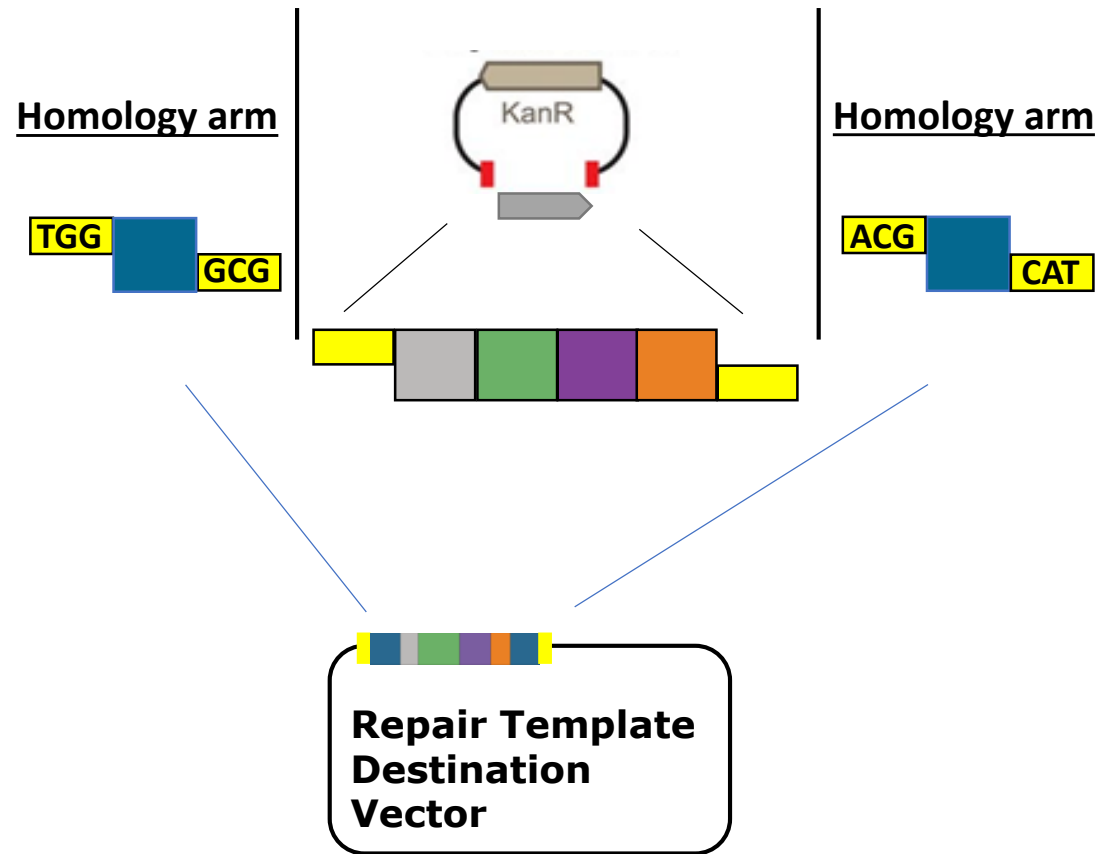
3% efficiency



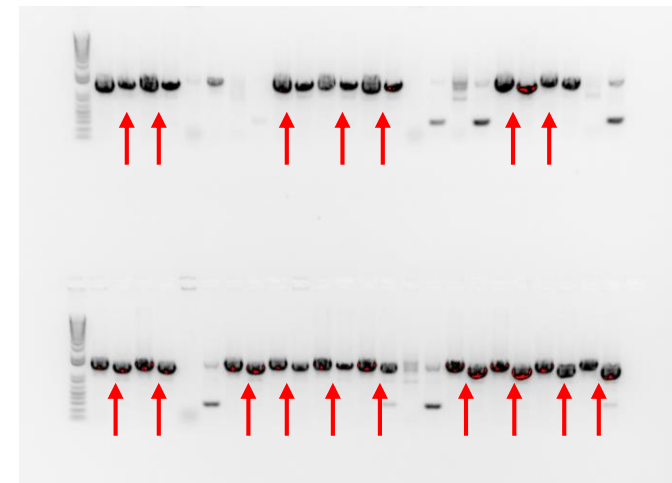
SEC-AID-3xFLAG assembly

SapTrap: High Throughput Cloning


Repair Template
Destination
Vector



71% efficiency



SapTrap builder: design tool

The screenshot displays the SapTrap Builder software interface. The window title is "SapTrap Builder".

Job Information:
Job Name: Unc-32 Ctag
Type: SapTrap
Output: Oligos + Annotated

Homology Arm Setup:
hUS target length: 57
hDS target length: 57
Min true length: 32

Buttons: Sequence Entry, Select gRNA, Select Translation Frame, Mutate Repair Template, C-tag, Review Design, Set Insert Site, Get Reagent Sequences.

Design Review:

```
Wild Type Translation: S F E K I L A E E R E A E E N L *
Repair Template Translation: S F E K I L A E E R E A E E N L *
Repair Template: TCTCATTGCGAAAAAATCCTTGCTGAAGAGCGTGAAGCTGAGGAGAATCTC | -INSERT- | TAAgatcacctcggccacttcaaacagtgatgacatcgacgttcgacaaatc
Wild Type DNA: TCTCATTGCGAAAAAATCCTTGCTGAAGAGCGTGAAGCTGAGGAGAATCTC | -INSERT- | TAAgatcacctcggccacttcaaacagtgatgacatcgacgttcgacaaatc
Guide RNA binding site: GAGAATCTC | -INSERT- | TAAgatcacctcgg
PAM: (8) /\ cgg
SapI Sites: gaagagc
```

Warning: SapI site in repair template. Incompatible with SapTrap assembly.

Review Design:
Repair Template trimmed to specified length.

Matthew L. Schwartz and Erik M. Jorgensen; 2018
http://jorgensen.biology.utah.edu/matts/saptrap_builder/

Coming Soon to AddGene

mScarlet multi-cassettes

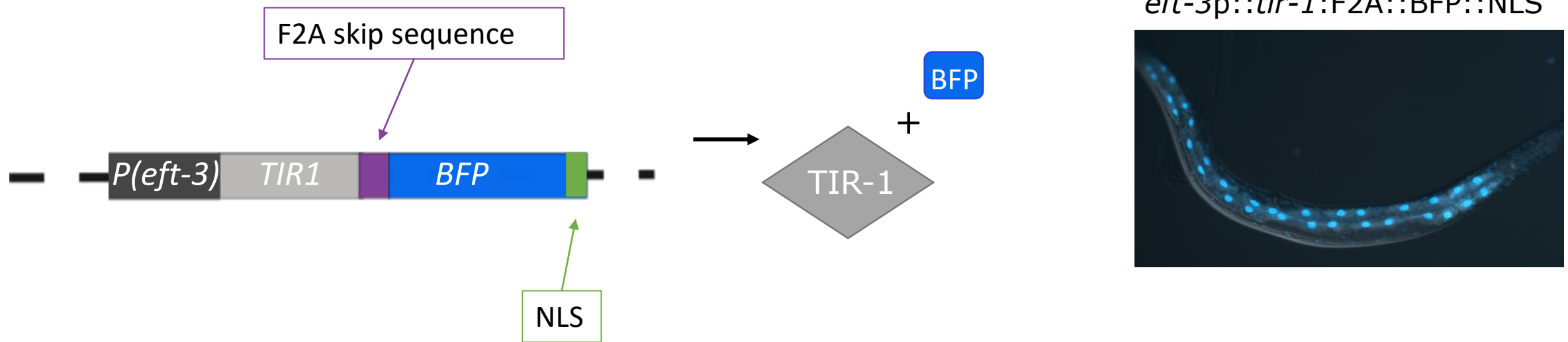
pJW1816	30xlinker::mScarlet::SEC+Lox511I::3xMyc
pJW1886	30xlinker::mScarlet::SEC+Lox511I::TEV-degtron-3xFLAG
pJW1821	mScarlet::SEC+Lox511I::3xMyc
pJW1927	mScarlet::SEC+Lox511I::TEV::degtron::3xFLAG

GFP multi-cassettes

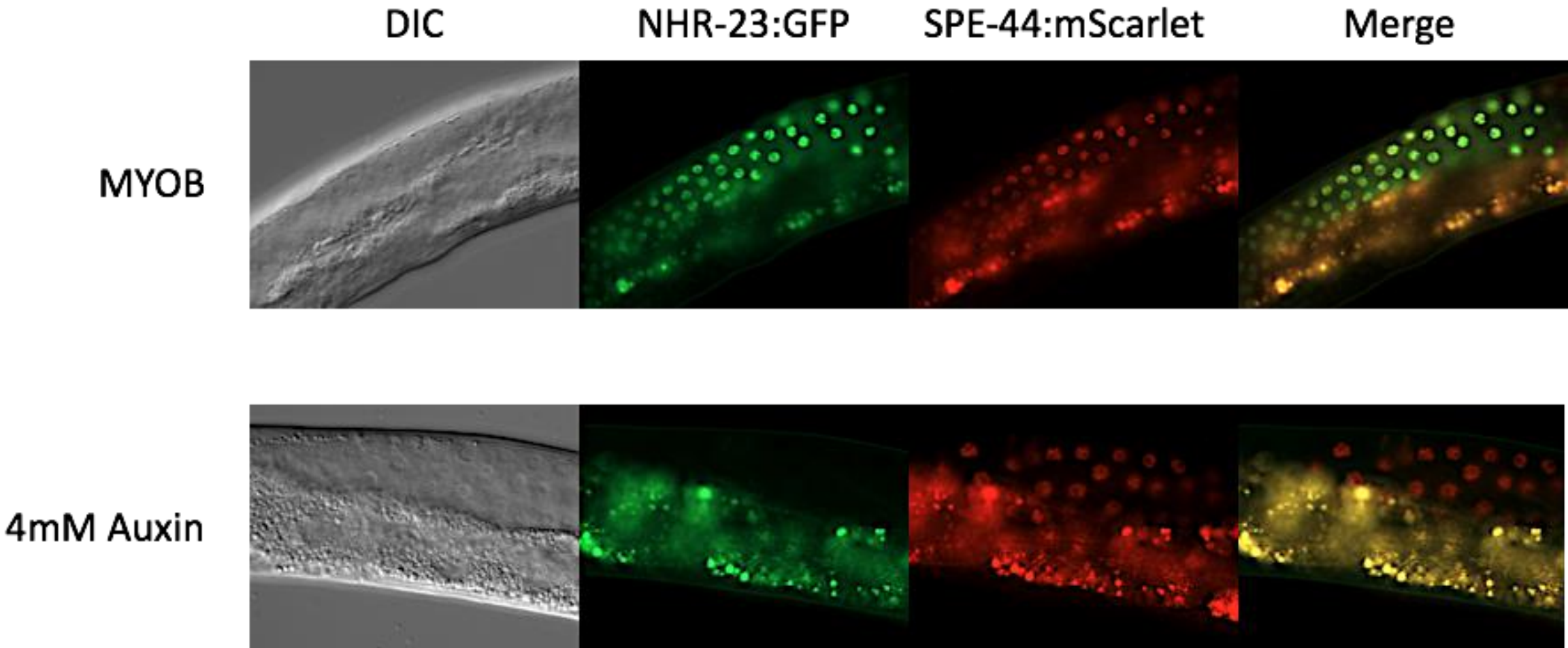
pJW1846	30xlinker::GFP::SEC-LoxP::degtron::3xFLAG
pJW1820	GFP::SEC+LoxP::degtron::3xFLAG

Developing a Tool-Kit: Improved *tir-1* Drivers

Original *tir-1::mRUBY* strains developed by Liangyu Zhang are available in Addgene
Zhang L, et al. 2015



Germline permissible expression and depletion of proteins using AID system



Developing a Tool-Kit: Improved *tir-1* Drivers



Dan Dickinson
Dave Reiner

Dickinson Lab	TIR-1 vector	
	Germline	<i>Psun-1</i>
Reiner Lab	BW Muscle	<i>Punc-54</i>
	Neurons	<i>Prgef-1</i>
	Hypodermis	<i>Pcol-10</i>
Ward Lab	Germline	<i>Pgld-1</i>
	Germline	<i>Pmex-5</i>
	Pan-soma	<i>Peft-3</i>
	Hypodermis	<i>Pgld-1</i>
	Seam cells	<i>Pscm-1</i>
	Gut	<i>Pges-1</i>

Thank you!

Ward Lab

Jordan Ward
Matt Ragle
Londen Johnson
Hannah Saeger
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Max Levenson
Diego Martinez
Joseph Aguilera
Brian Sangalang
Chiara Marley
Keanu Quilton
Brittany Davidson

Dan Dickinson

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Matt Schwarz

Erik Jorgensen

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UCLEADS

Always Be
Cloning



Photo Credit: Megan Durham



UNIVERSITY OF CALIFORNIA
SANTA CRUZ

NHR-23:GFP:degron:3XFLAG; *Pmex-5*::TIR1 adult male worms expressing and depleted of NHR-23 in the germline

