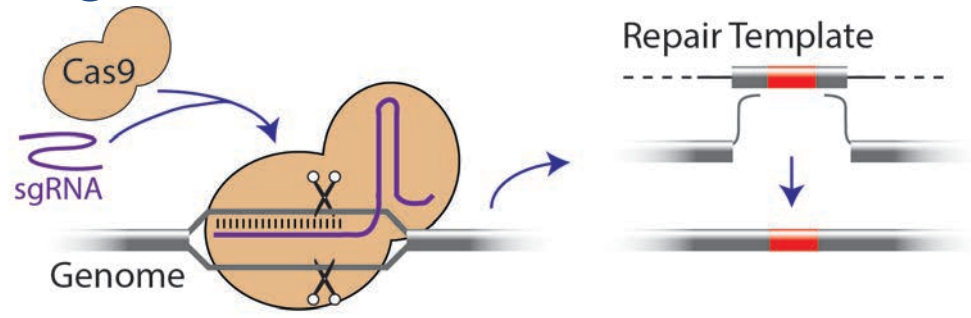


# New tools for high-efficiency CRISPR/Cas9 gene tagging

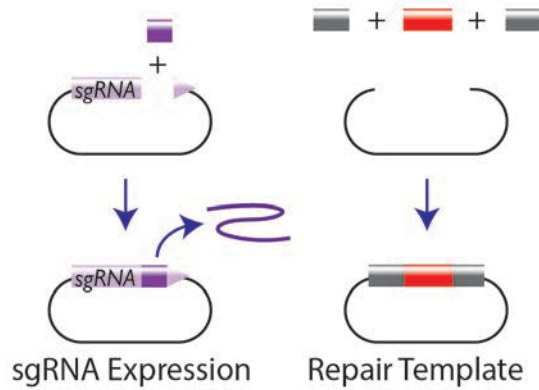
Matthew Schwartz  
Jorgensen Laboratory  
University of Utah

# Overview: three stages of CRISPR

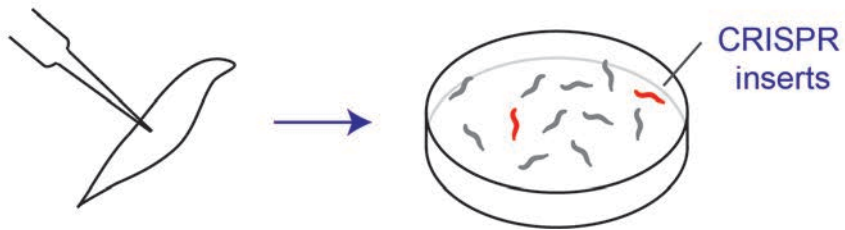
1. Design



2. Build

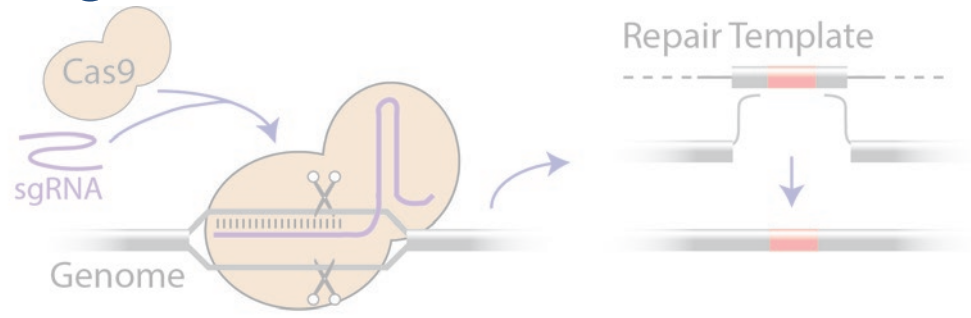


3. Edit

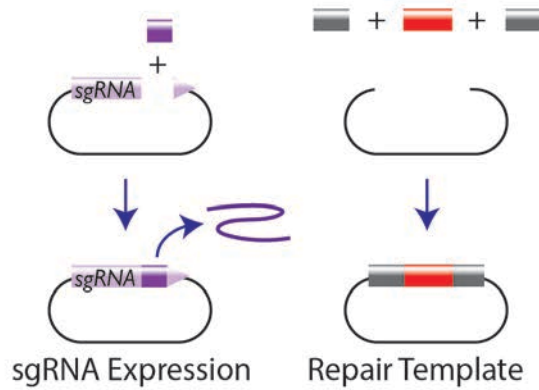


# Overview: three stages of CRISPR

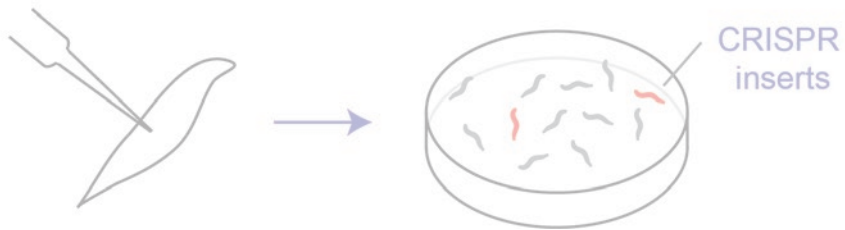
1. Design



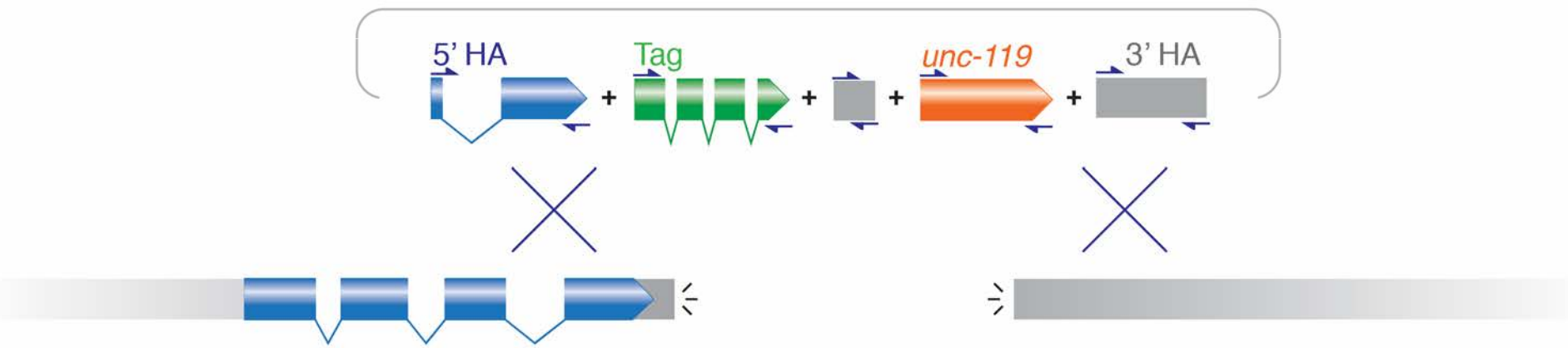
2. Build



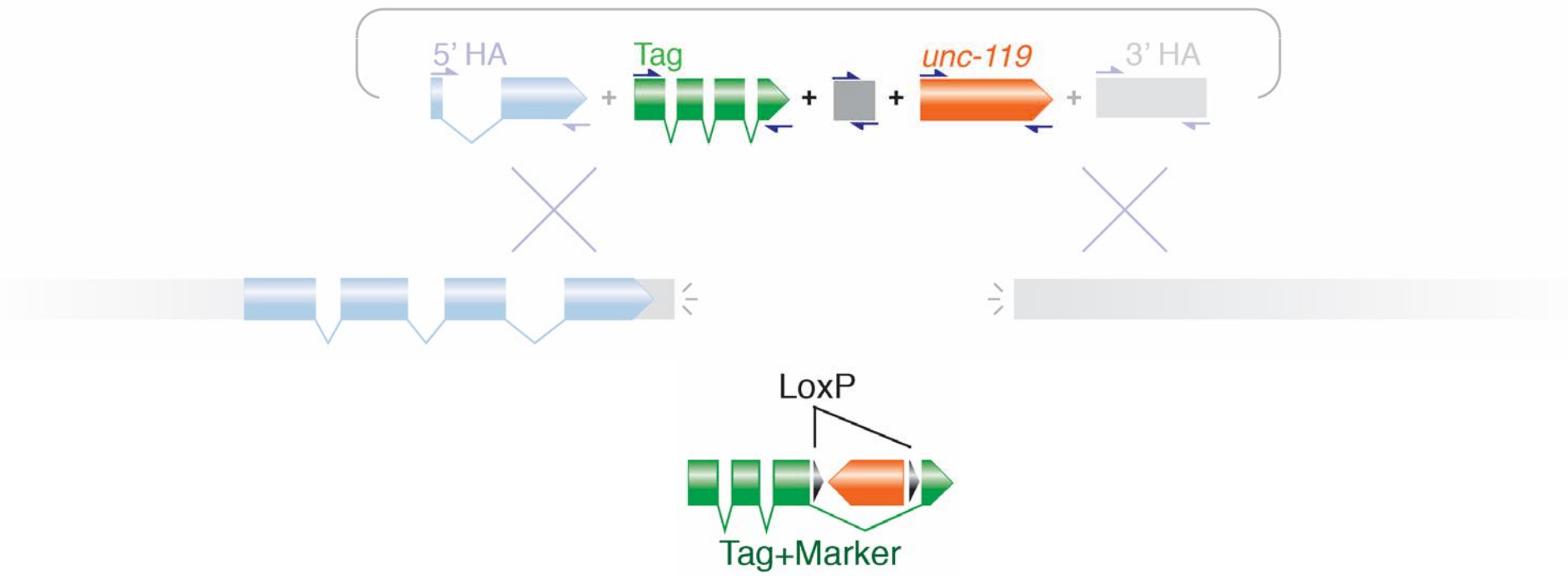
3. Edit



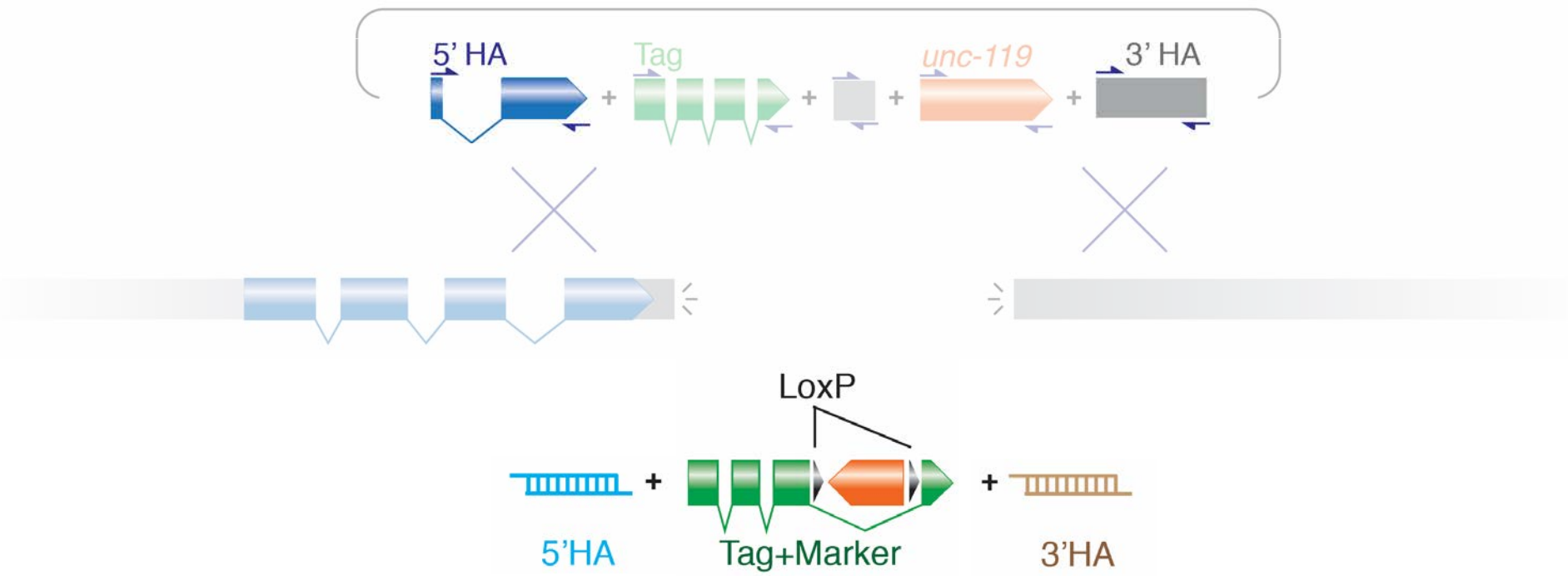
# SapTrap: a Golden Gate toolkit for CRISPR



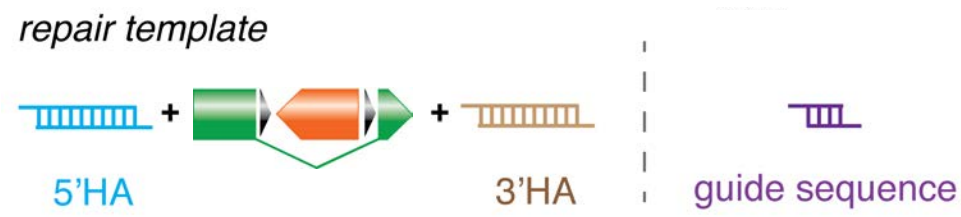
# SapTrap: a Golden Gate toolkit for CRISPR



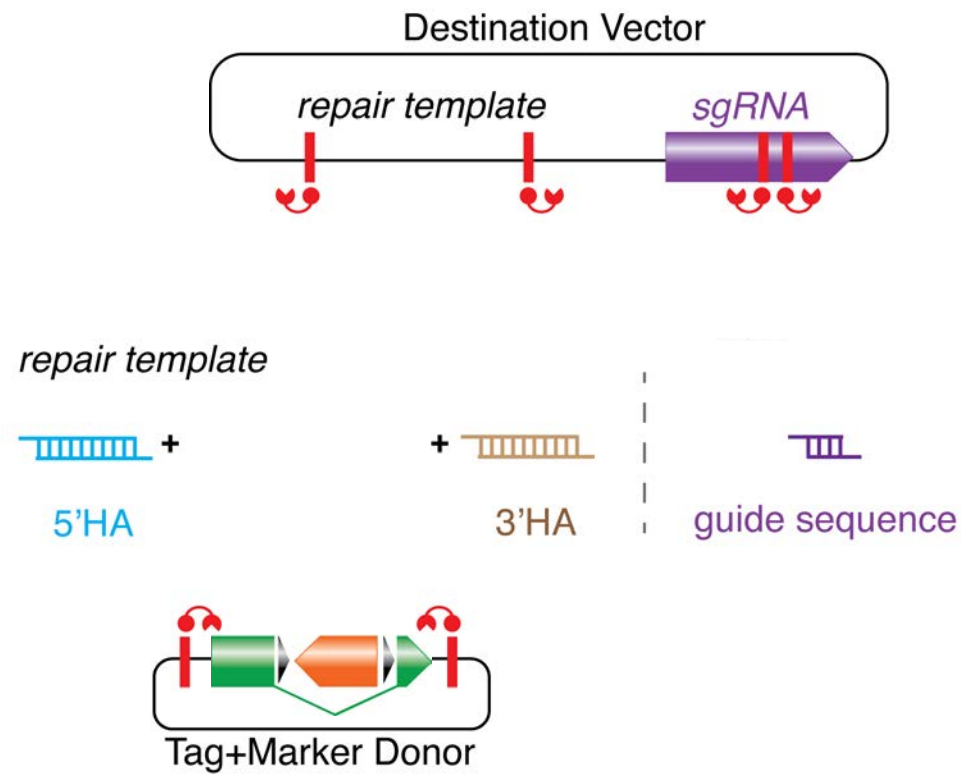
# SapTrap: a Golden Gate toolkit for CRISPR



# SapTrap: a Golden Gate toolkit for CRISPR

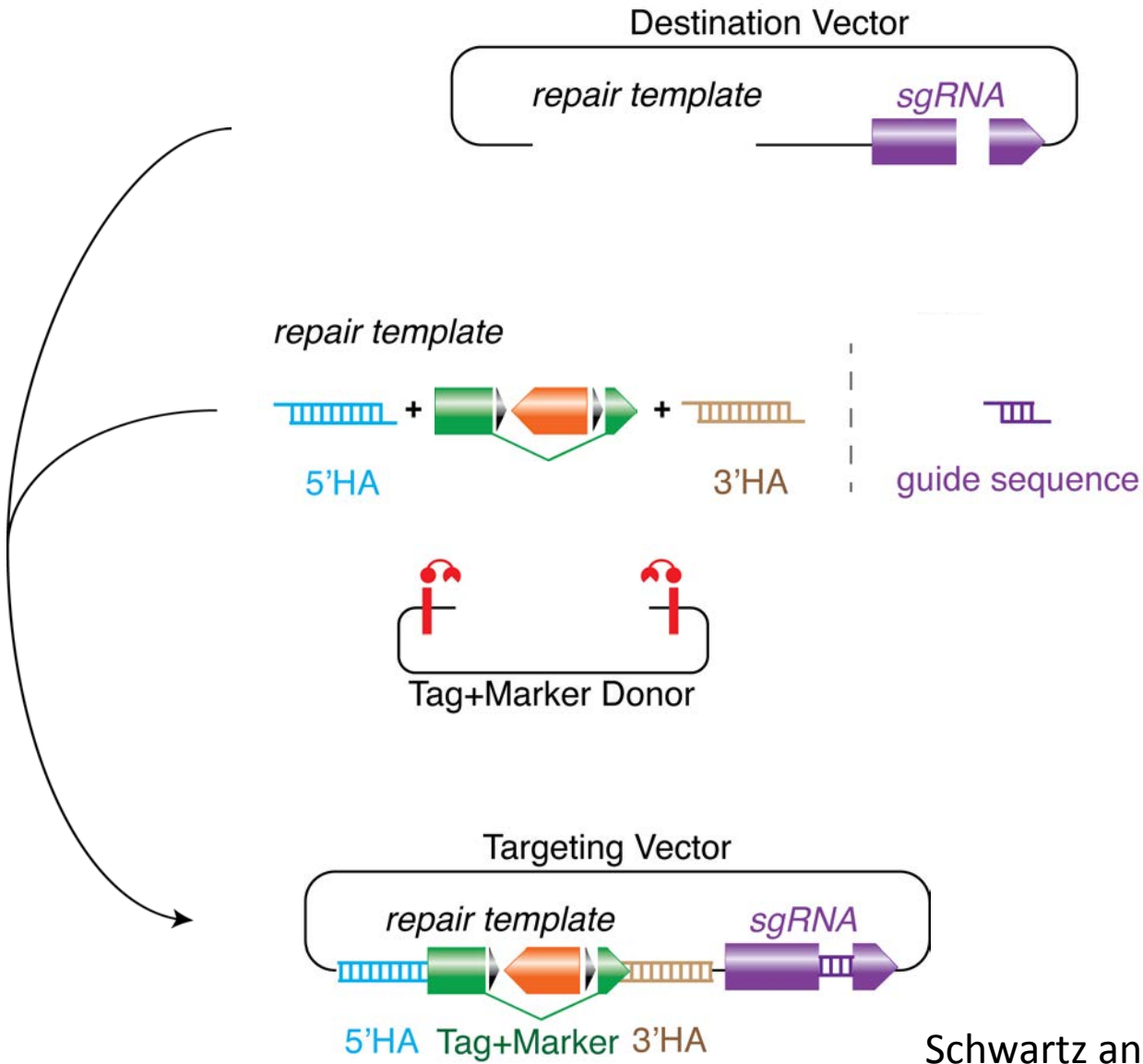


# SapTrap: a Golden Gate toolkit for CRISPR



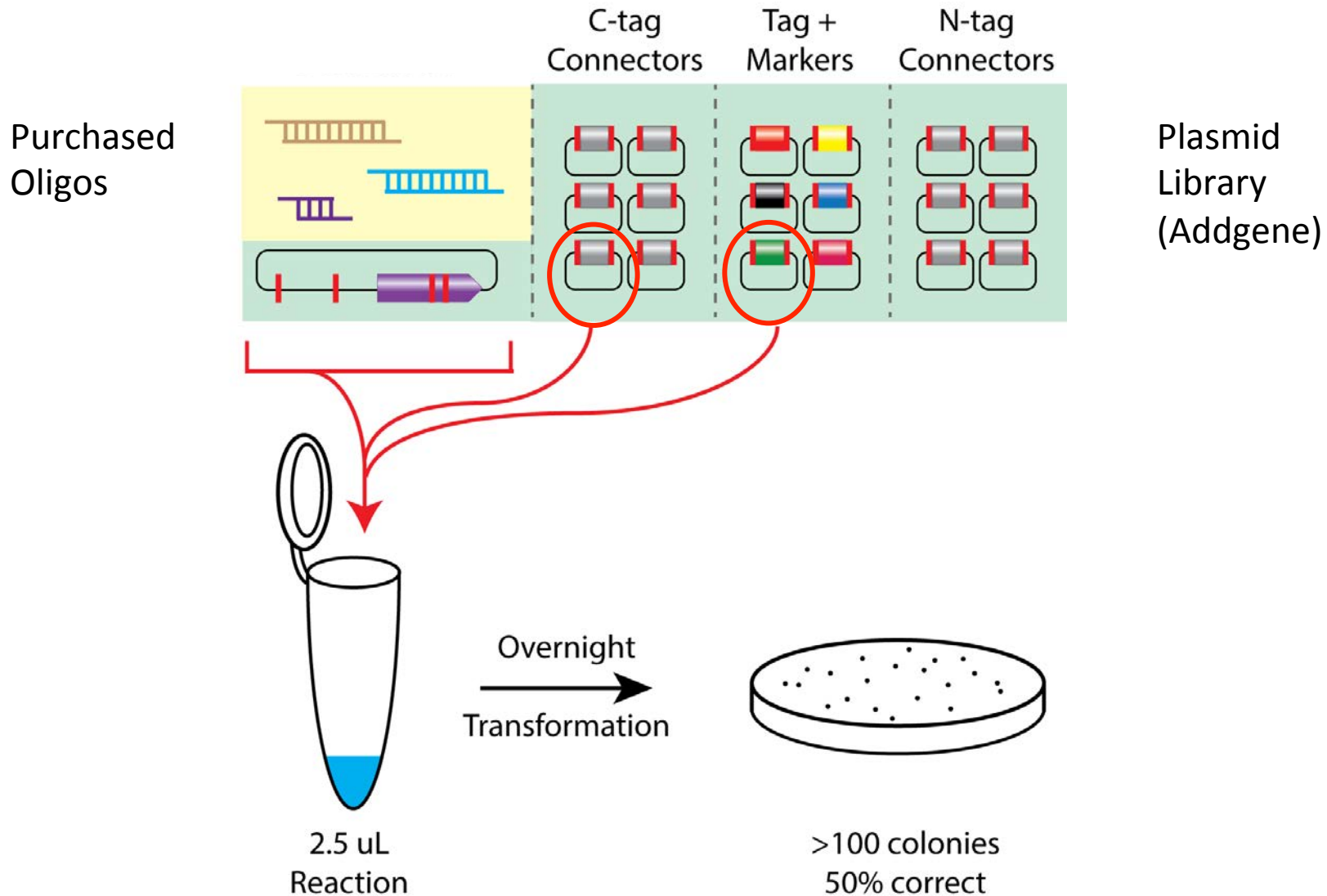
Schwartz and Jorgensen, Genetics. 2016.  
Plasmids available from Addgene.

# SapTrap: a Golden Gate toolkit for CRISPR



Schwartz and Jorgensen, Genetics. 2016.  
Plasmids available from Addgene.

# A simple procedure to generate CRISPR plasmids

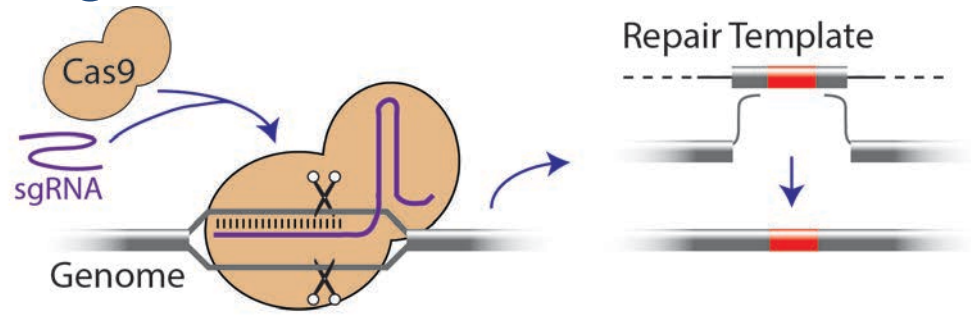


Dickinson et al., uPub Biology. 2018.  
Plasmids available from Addgene.

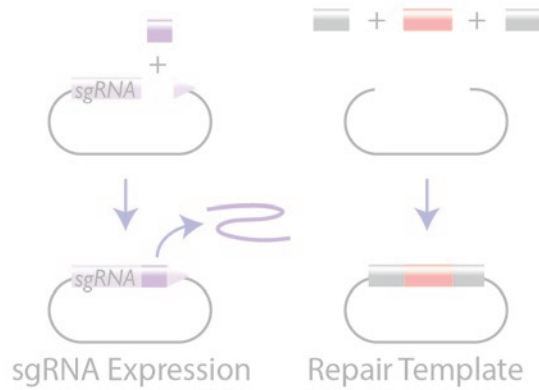
Schwartz and Jorgensen, Genetics. 2016.  
Plasmids available from Addgene.

# Overview: three stages of CRISPR

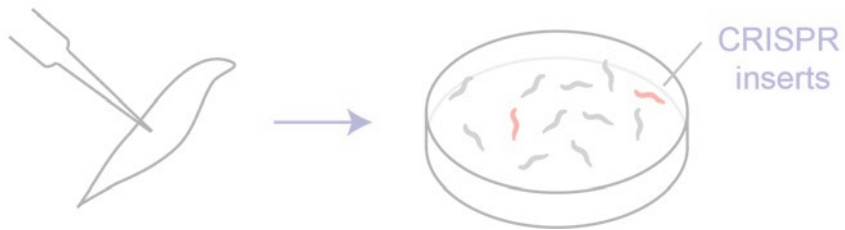
1. Design



2. Build



3. Edit



# SapTrap Builder: CRISPR design software



SapTrap Builder

Job Information: Job Name:  Type:  Output:

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

Sequence Entry:  Select gRNA:

Select Translation Frame:  Mutate Repair Template:

C-tag:  Review Design:

Set Insert Site:  Get Reagent Sequences:

Wild Type Translation: F S F E K I L A E E R E A E E N L \*

Repair Template Translation: F S F E K I L A E E R E A E E N L \*

Repair Template DNA: CATCTCATTCGAAAAATCCTTGCTGAAGACCGTGAAGCTGAGGAGAATCTC| -INSERT-| TAAgatcacctcggccacttcaaacagtgtgacatcgacgttcgacaaatctttaat

Wild Type DNA: CATCTCATTCGAAAAATCCTTGCTGAAGACCGTGAAGCTGAGGAGAATCTC| -INSERT-| TAAgatcacctcggccacttcaaacagtgtgacatcgacgttcgacaaatctttaattatta

Guide RNA binding site: GAGAATCTC| -INSERT-| TAAgatcacctcgg

PAM: (8) /\ cgg

Restriction Sites: SapI

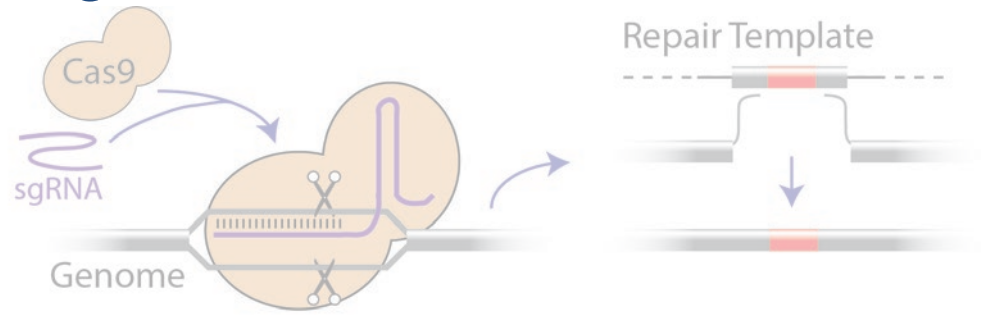
SapI site in repair template. Mutate SapI site for SapTrap assembly.

Review Design  
Repair Template trimmed to specified length.

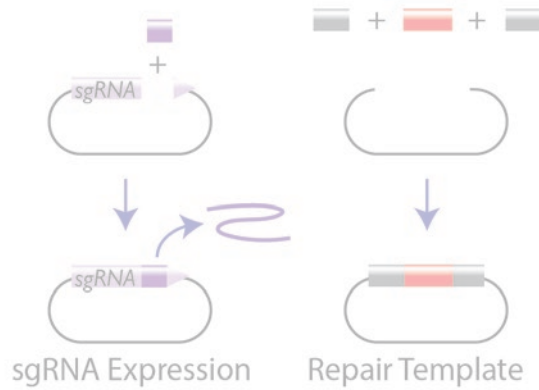
- Works on Windows, Mac, Linux
- User guide and Youtube tutorials

# Overview: three stages of CRISPR

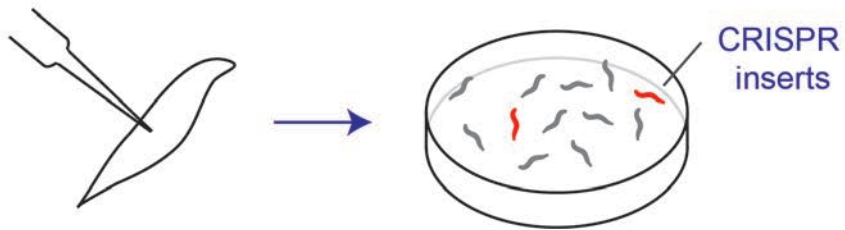
1. Design



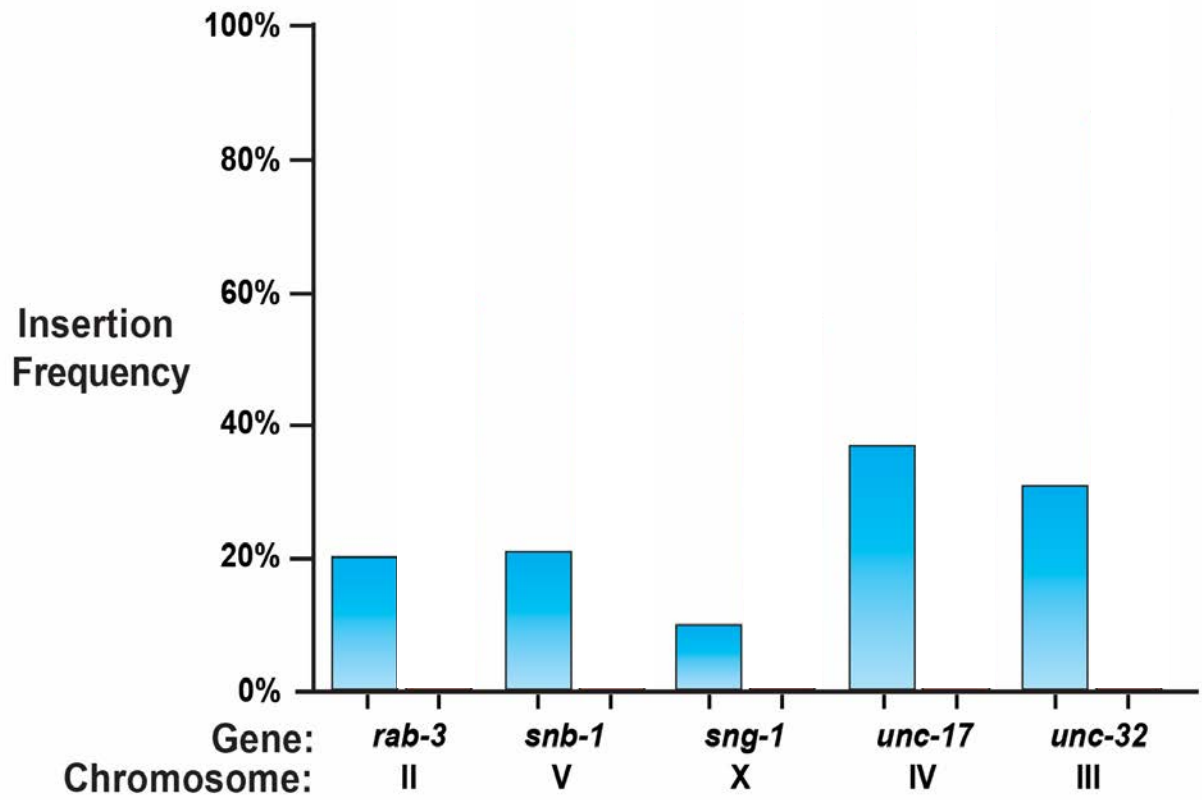
2. Build



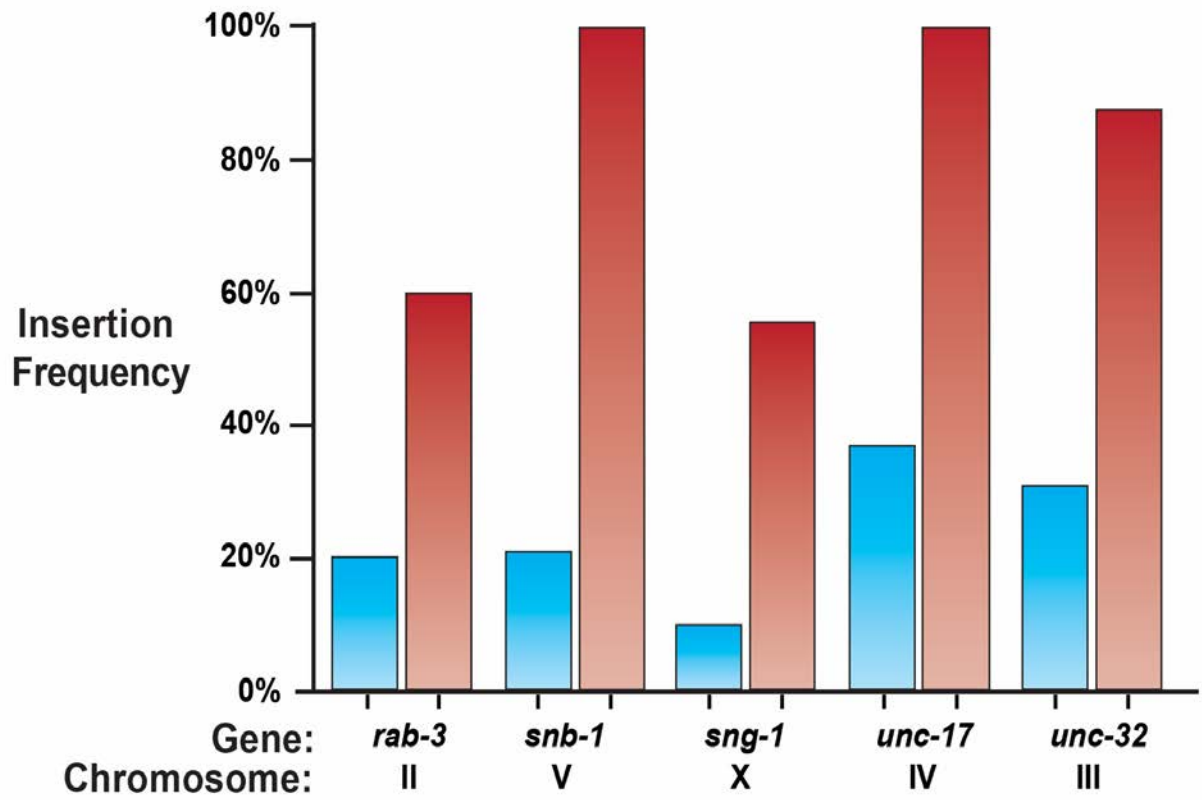
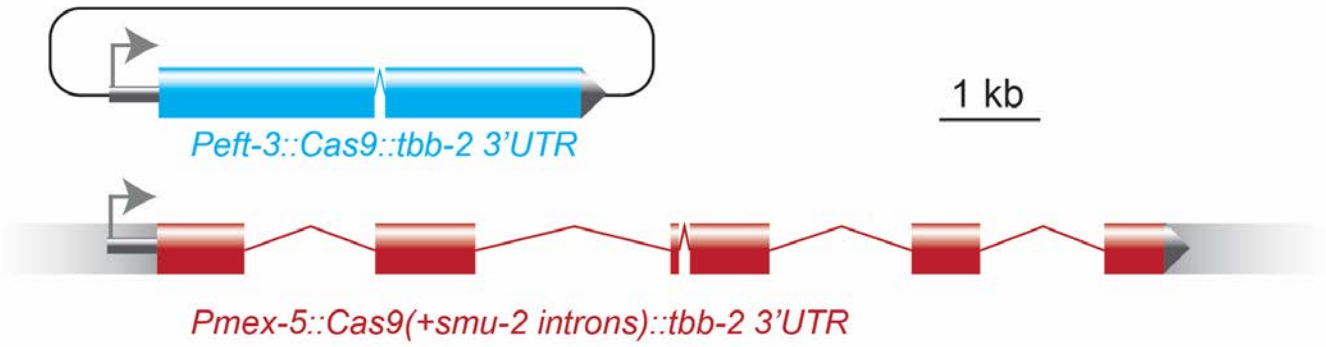
3. Edit



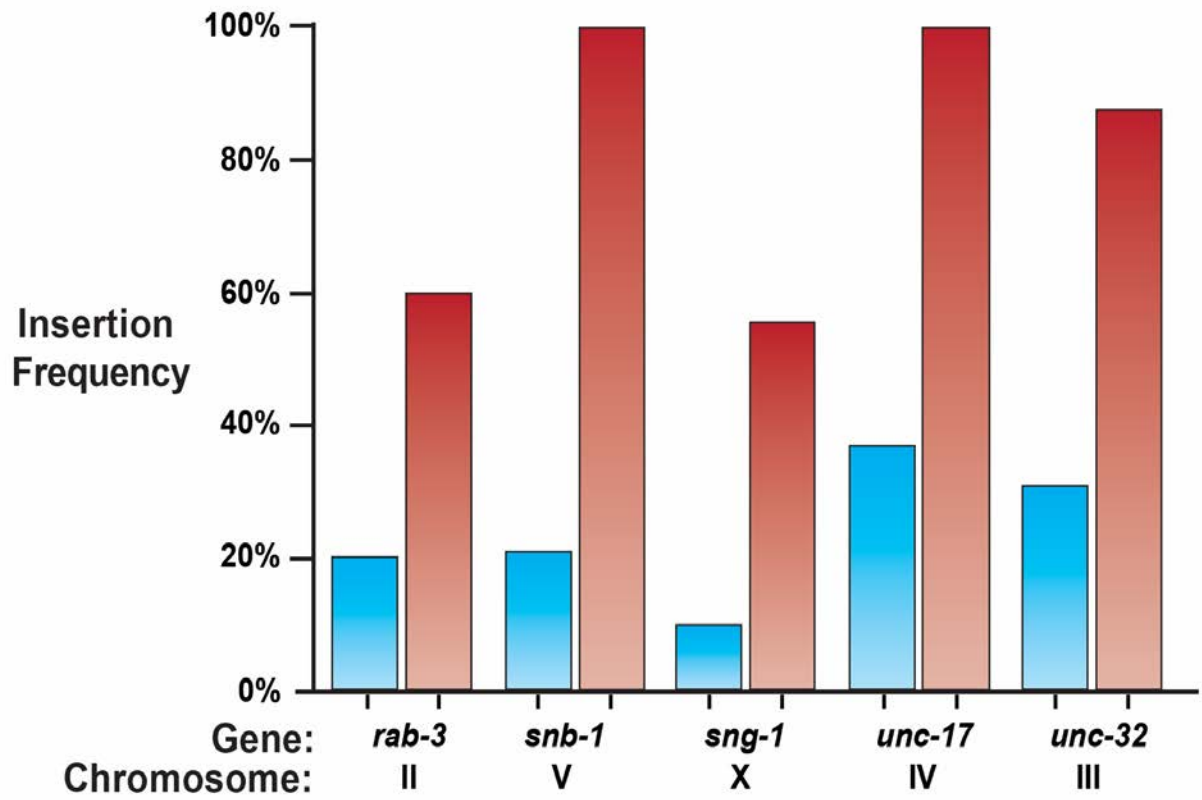
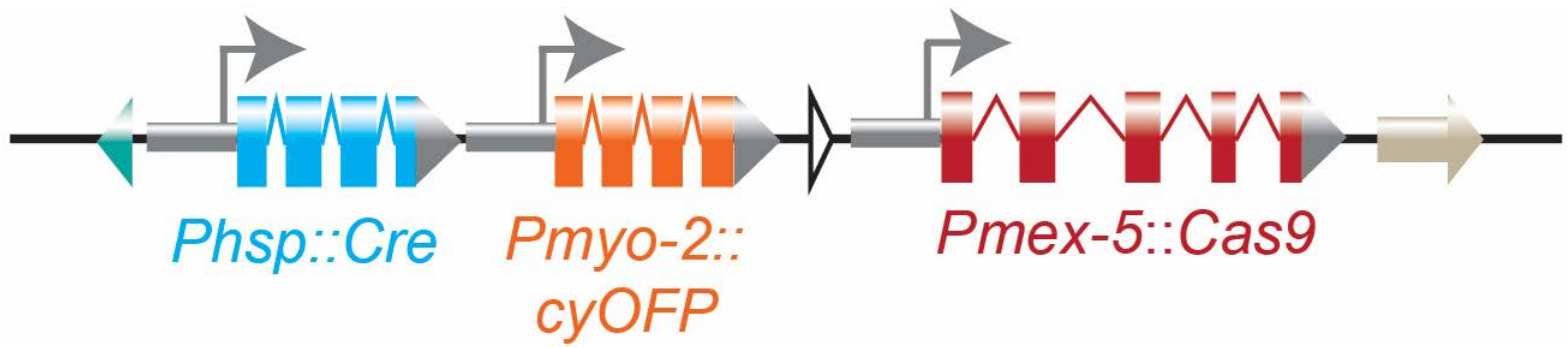
# Integrated Cas9: High efficiency CRISPR



# Integrated Cas9: High efficiency CRISPR

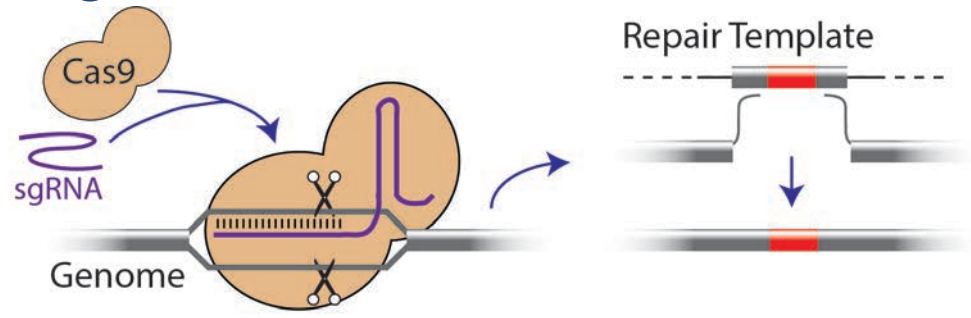


# Integrated Cas9: High efficiency CRISPR

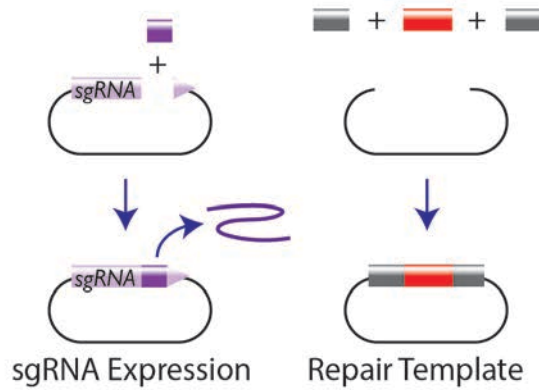


# Overview: three stages of CRISPR

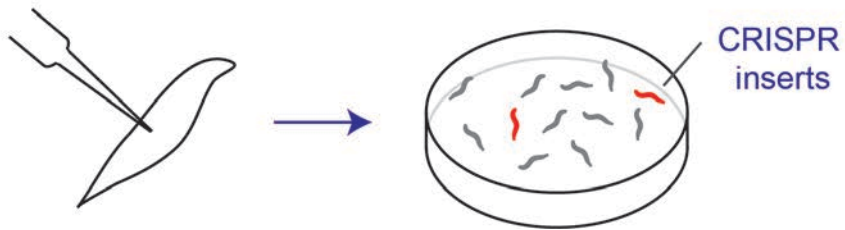
1. Design



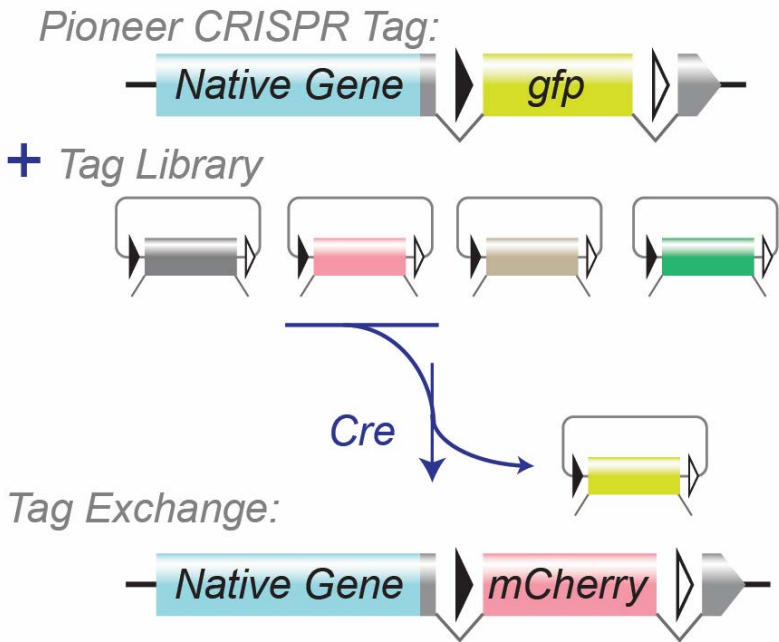
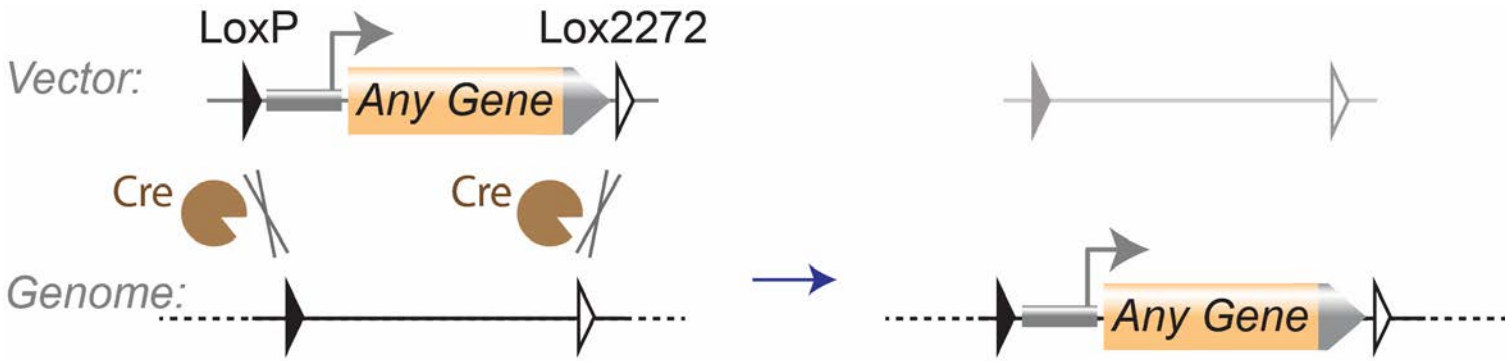
2. Build



3. Edit



# The Future: Cassette Exchange Tags



# Jorgensen Lab



**DEPARTMENT OF BIOLOGY**  
THE UNIVERSITY OF UTAH

PI: Erik Jorgensen

Research Associate Professor  
Wayne Davis

## Graduate Students

Siyu Chen

Eddie Hujber

Matt Labella

Patrick Mac

Tyler Page

Thien Vu

Christine Wnukowski

## Postdocs

**Matt Rich – Talk 116 Saturday**

**Matt Schwartz – Poster 614A Friday**

Nathan Okerlund

**Malan Silva – Poster 946C Sunday**

## Associates

Robert Hobson



**HHMI**  
HOWARD HUGHES MEDICAL INSTITUTE