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Highly Efficient Selection System For Directed Evolution of Novel Endonuclease

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Designing a Selection System for Directed Evolution of a Novel Endonuclease

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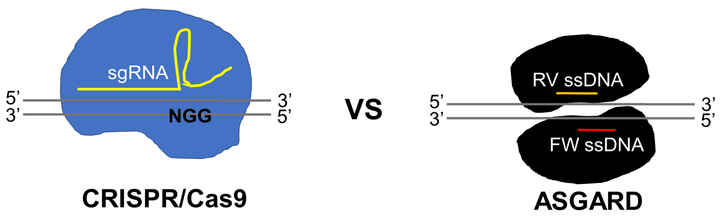
Binding constraints restrict the use of popular gene-editing tools

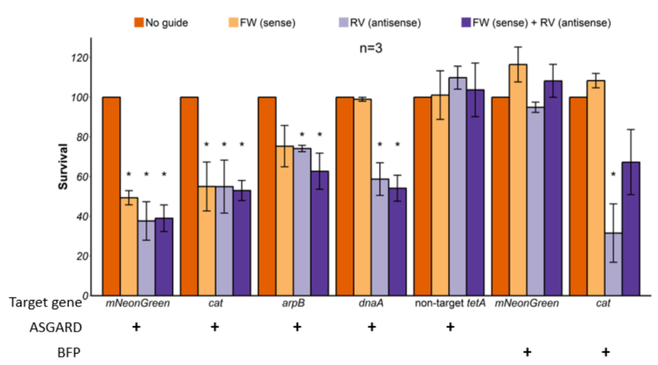


Endonucleases edit the genetic code by cutting DNA and allowing for edits to be made at a specific location in the genome. CRISPR/Cas9[1] is currently the most popular method of genetic manipulation, which is a method that can be used to cure diseases and can improve crop resistance and adaptation to different environments.

Some disadvantages of CRISPR are its "off" target activity and the requirement of a specific binding site to bind DNA.

Previously, we showed that our novel endonuclease ASGARD can be programmed to cut DNA. ASGARD has the potential to be more flexible than CRISPR as it does not require a motif.

ASGARD can be programmed to cleave DNA



Limitations of ASGARD can affect its efficiency

-off-target activity: Unprogrammed cutting of other parts of the genome

-Insolubility: The highest degree of solubility is desired in order to increase ASGARD functionality

Directed evolution can target limitations

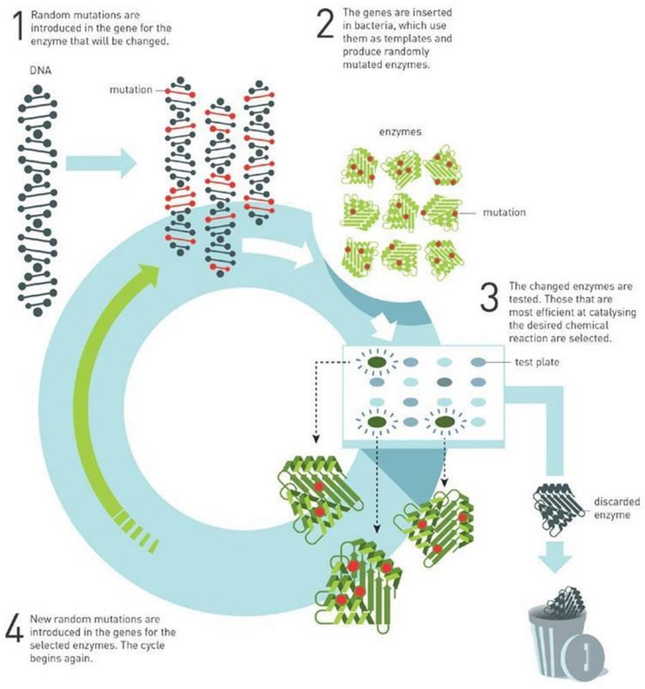


Photo credit: Johan Jarnestad/The Royal Swedish Academy of Sciences

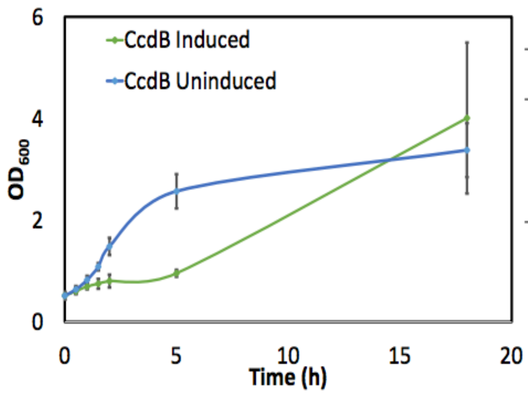
Increase the efficiency of ASGARD by performing directed evolution and selecting mutants with higher on-target activity and higher solubility.

A viable selection system is required for efficient screening of mutants

There is a critical need to develop a positive screening system with forgiving, selective, and tunable characteristics.

We tested the following three systems:

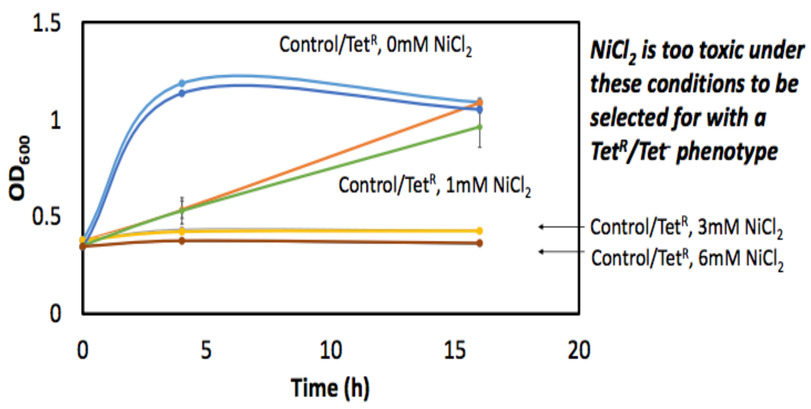
CcdB



CcdB colonies after induction	-ve control	+ NgAgo
Replicate 1	0	1
Replicate 2	0	4
Replicate 3	0	2

CcdB is overly stringent with inconsistent growth inhibition

$Tet^R/NiCl_2$



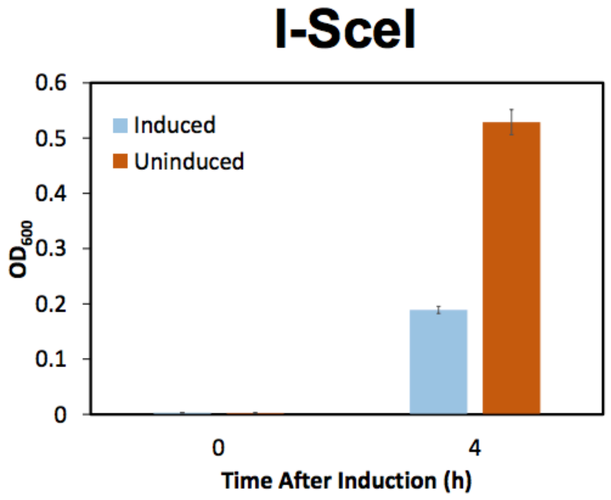
$NiCl_2$ is too toxic under these conditions to be selected for with a Tet^R/Tet^- phenotype

Conclusions and future directions

CcdB and $Tet^R/NiCl_2$ potential selection systems proved to be inefficient while I-SceI had good results with consistency. Future steps will be to introduce our novel endonuclease through subsequent rounds of directed evolution with the I-SceI selection system

	I-SCEI	TET/NICL	CCDB
Forgiving	X	X	
Selective	X		X
Tunable	X	X	X

I-SceI



I-SceI shows clear growth phenotype differentiation

Reference

Jinek et al., A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, Science. 2012 Aug 17;337(6096):816-21.

Acknowledgement

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