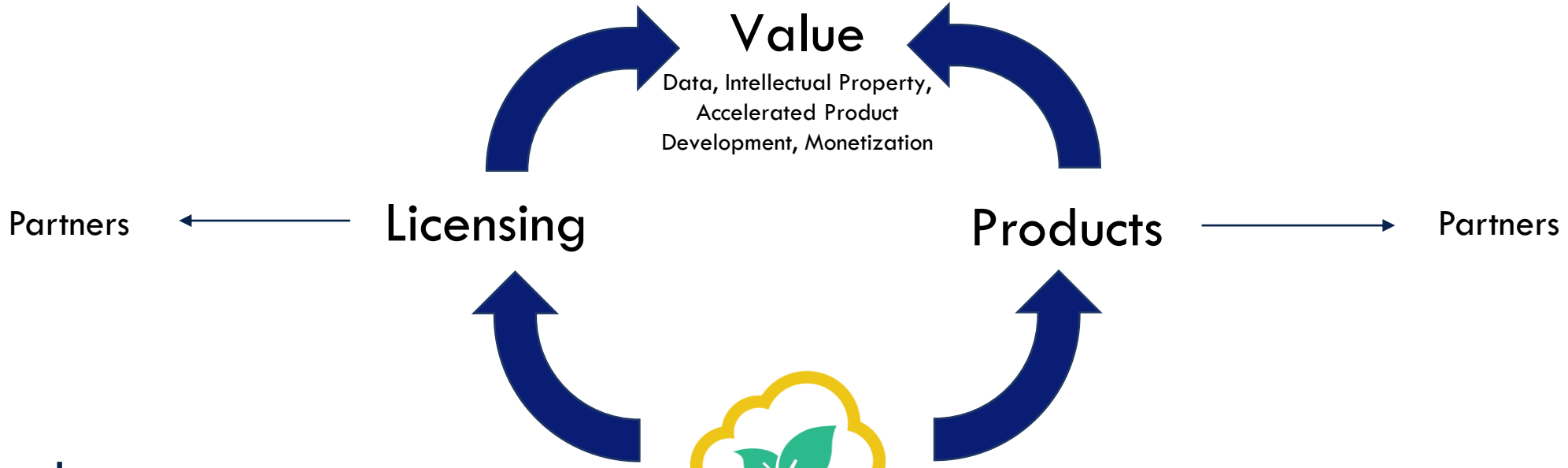
BENSON HILL

Enabling crop innovation with CropOS across breeding, genome editing, and trait development platforms

Benson Hill partners with companies to license one or more pieces of the platform with varying levels of support



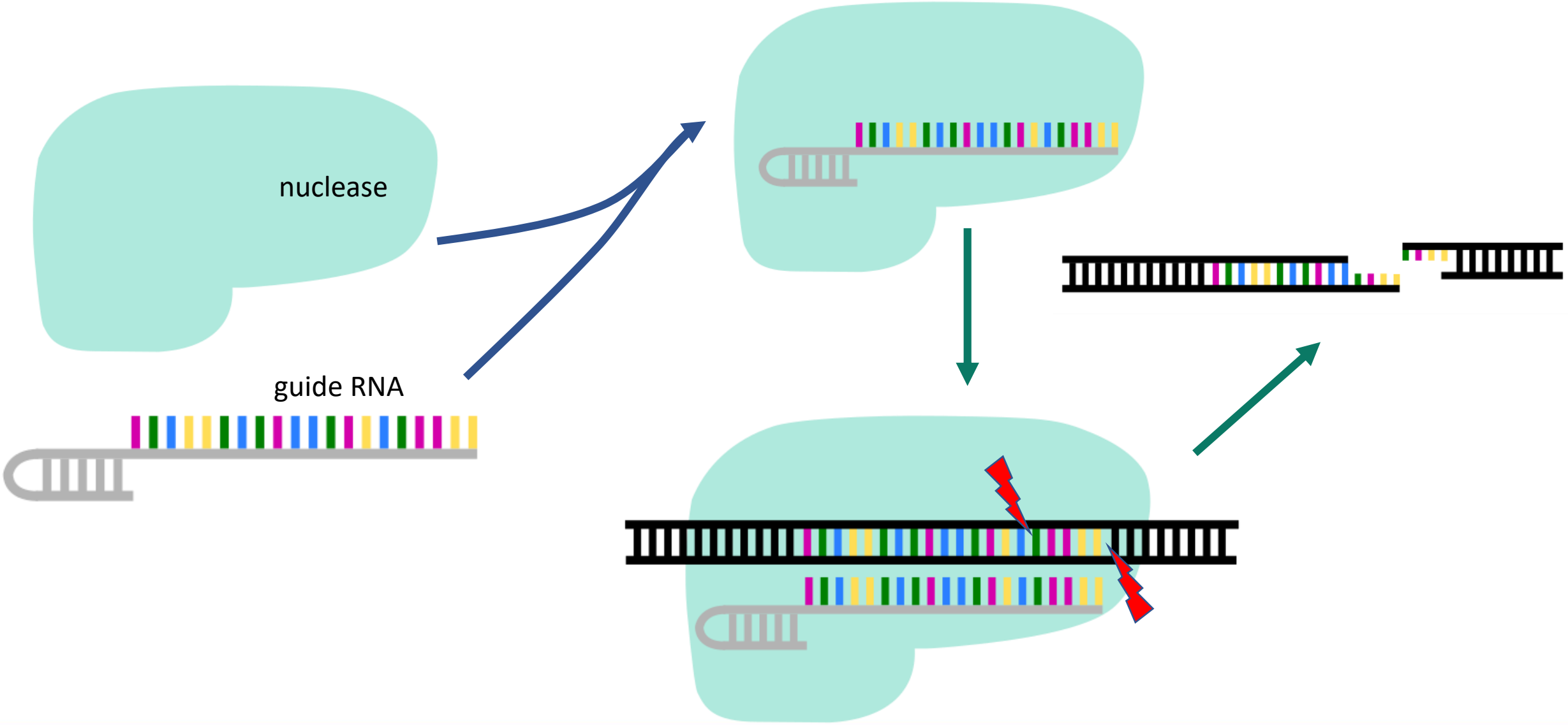
Participants

Platform

CRISPR Portfolio

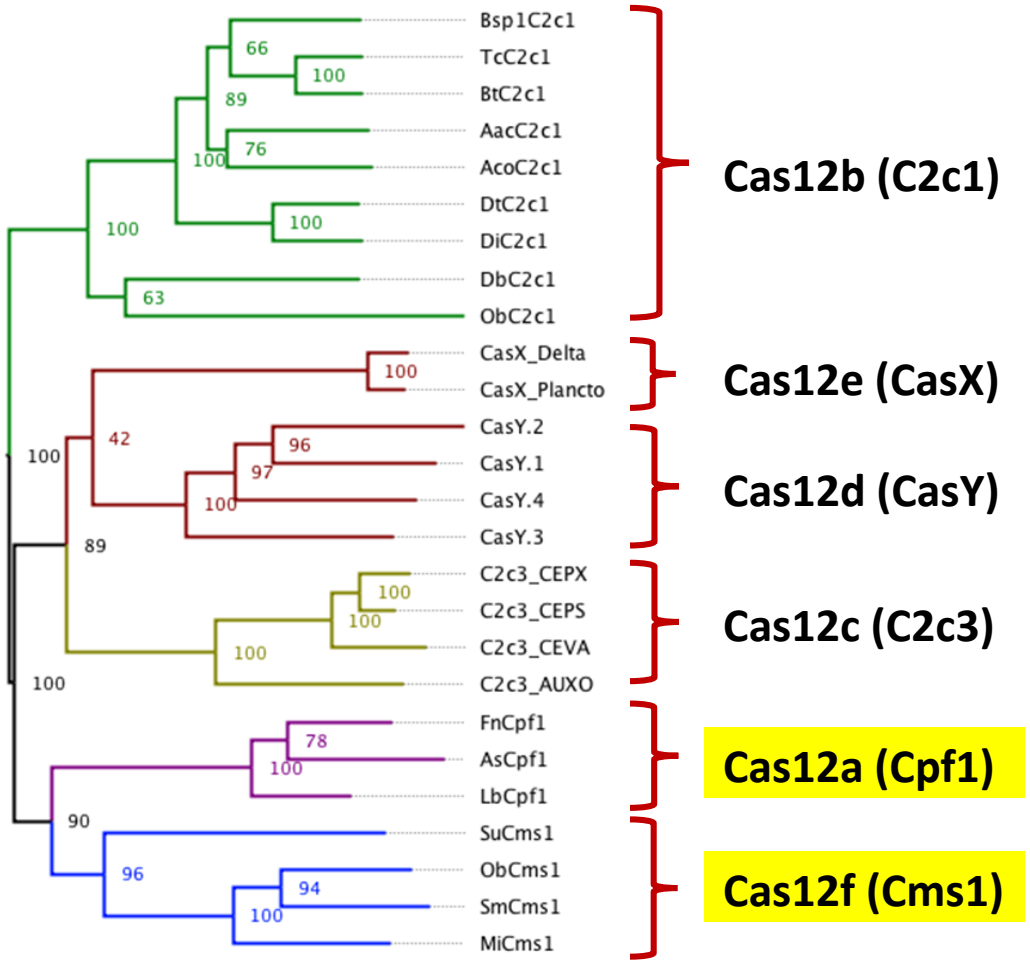
BENSON HILL

CRISPR is a highly programable gene editing technology



BHB Genome Editing Toolbox – CRISPR 2.0 and 3.0

Enabling Scientists Across the World to Meet Genome Editing Goals



Cas12b (C2c1)

Cas12e (CasX)

Cas12d (CasY)

Cas12c (C2c3)

Cas12a (Cpf1)

Cas12f (Cms1)

Existing Plant Editing Toolbox

Non-CRISPR Nucleases	CRISPR Nucleases
• TALENs	• Cas9
• Meganucleases	• Cpf1
• Zinc Fingers	

Benson Hill Biosystems' Expanded Toolbox

CRISPR 3.0 Nucleases

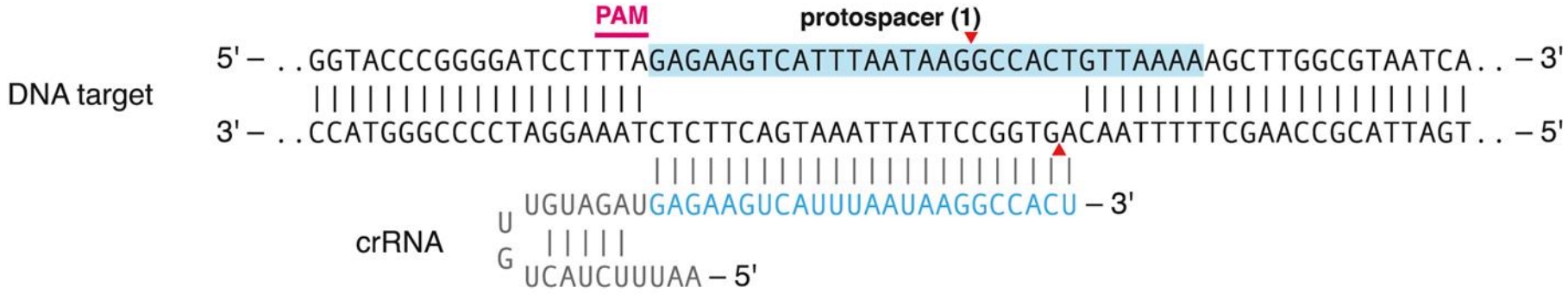
- **SmCms1**
- **ObCms1**
- **MiCms1**
- **SuCms1**
- **AuxCms1**
- **Adurb**
- **LahsCms1**
- **Unk38Cms1**
- **Unk1Cms1**

CRISPR 2.0 Nucleases

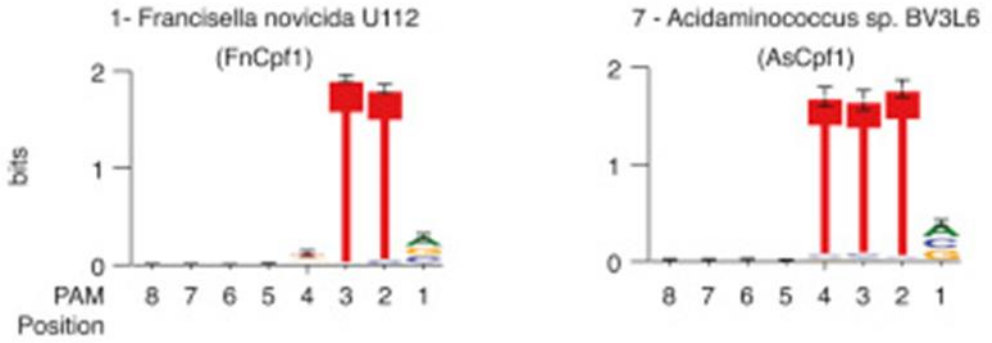
- **Pb2Cpf1**
- **Lb6Cpf1**
- **EcCpf1**
- **PsCpf1**
- **McCpf1**

“Sticky End” nuclease activity distal from PAM site; associated with high efficient homology-directed repair

Type V CRISPR nucleases have several advantages over Cas9



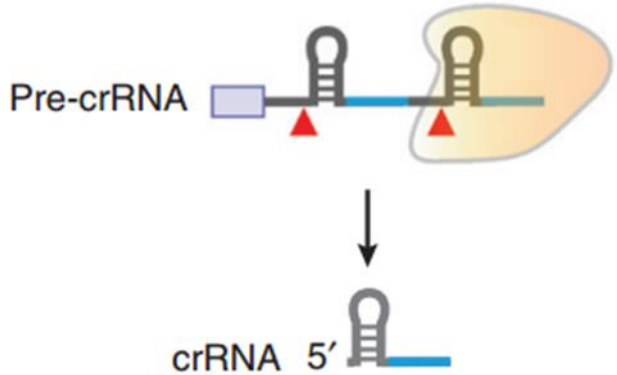
AT Rich PAM Sites



Zetsche et al 2015. Cell DOI: http://dx.doi.org/10.1016/j.cell.2015.09.038

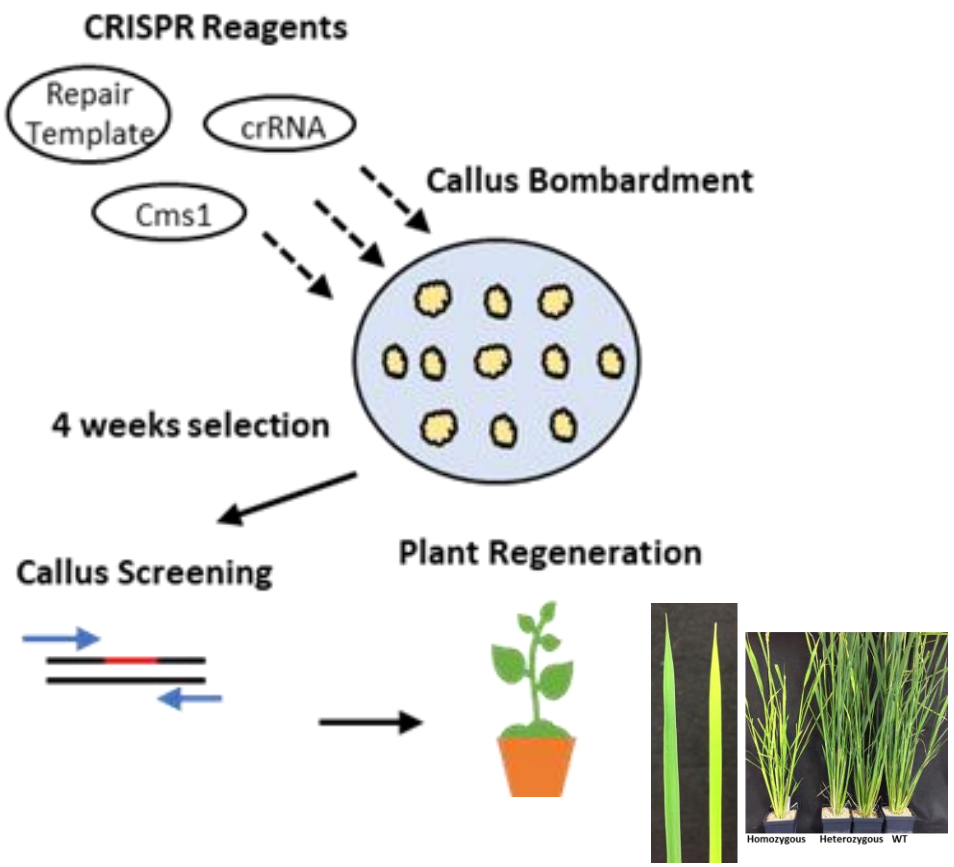
Self processing multiplex arrays enables easier multiplexing

Cms1

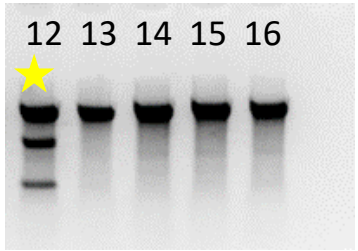


Zetsche et al 2017. Nature Biotech DOI:doi:10.1038/nbt.3737

BHB developed a robust high throughput screening for CRISPR activity in rice



Typical T7 Endonuclease Assay result



Cms1 nucleases generate indels distal from the PAM site

Rice CAO1 Target Site #2

Sample	PAM	Target Sequence	Indel
WT	TTGCTTTC	TCCAGTGACCTAAAAGACGATACA	ATGGTA
#38	TTGCTTTC	TCCAGTGACCTAAAA---------	ATGGTA -9
#38	TTGCTTTC	TCCAGTGACCTAAAA--------	CAATGGTA -7
#77	TTGCTTTC	TCCAGTGACCTAAAA---GATACA	ATGGTA -3
#77	TTGCTTTC	TCCAGTGACCTAAAA--------	CAATGGTA -7
#86	TTGCTTTC	TCCAGTGACC----------	TGGTA -15
#88	TTGCTTTC	TCCAGTGACCTAA---------	CAATGGTA -9
#90	TTGCTTTC	TCCAGTGACCTAAA--------	CAATGGTA -8

Deletions observed at the predicted cut site

- Predicted cut site is ~ 19bp distal to the PAM site
- Deletions range from 3-75 base pairs in length

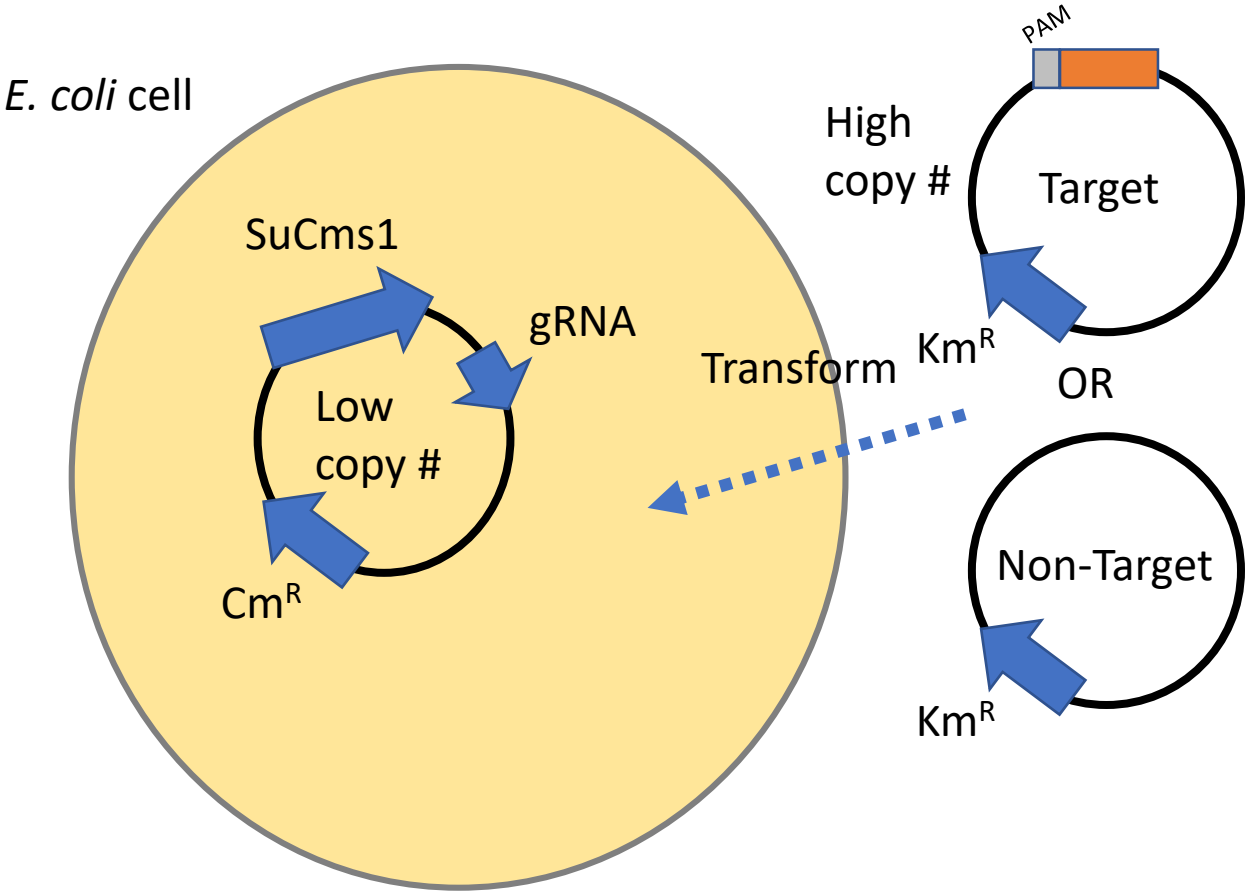
Precise insertion and guided editing of higher plant genomes using Cpf1 CRISPR nucleases

Matthew B. Begemann[1], Benjamin N. Gray[1], Emma January[1], Gina C. Gordon[1], Yonghua He[1], Haijun Liu[1], Xingrong Wu[1], Thomas P. Brutnell[1,2], Todd C. Mockler[2] & Mohammed Oufattole[1]

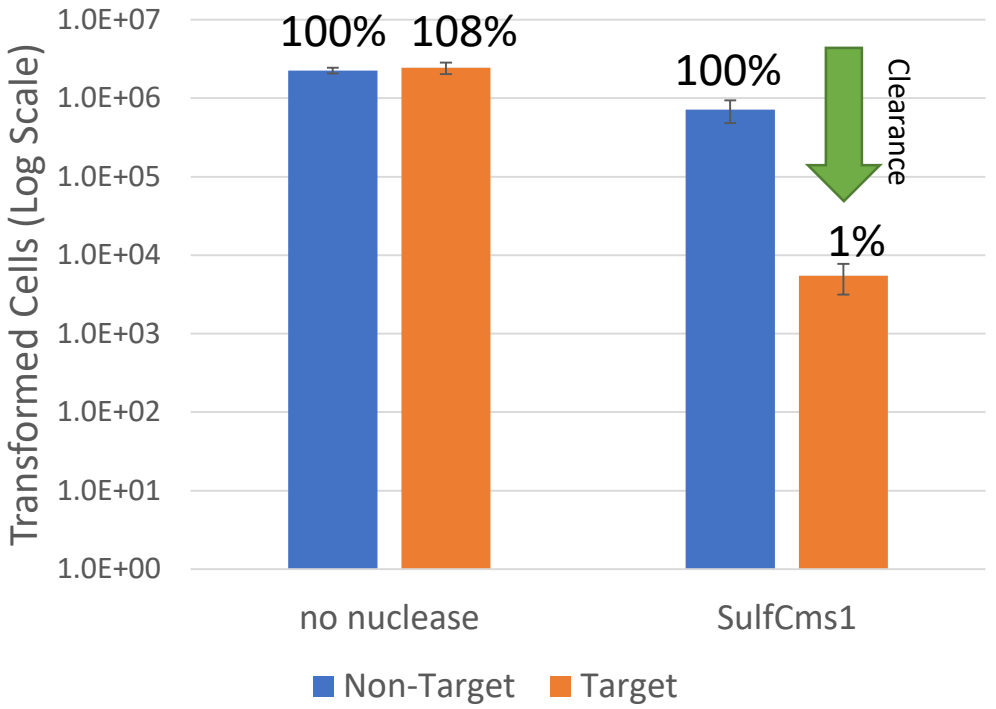
SCIENTIFIC REPORTS

A microbial assay was developed to characterize activity of 3.0

E. coli with nuclease:RNA complex transformed with target or non-target plasmids

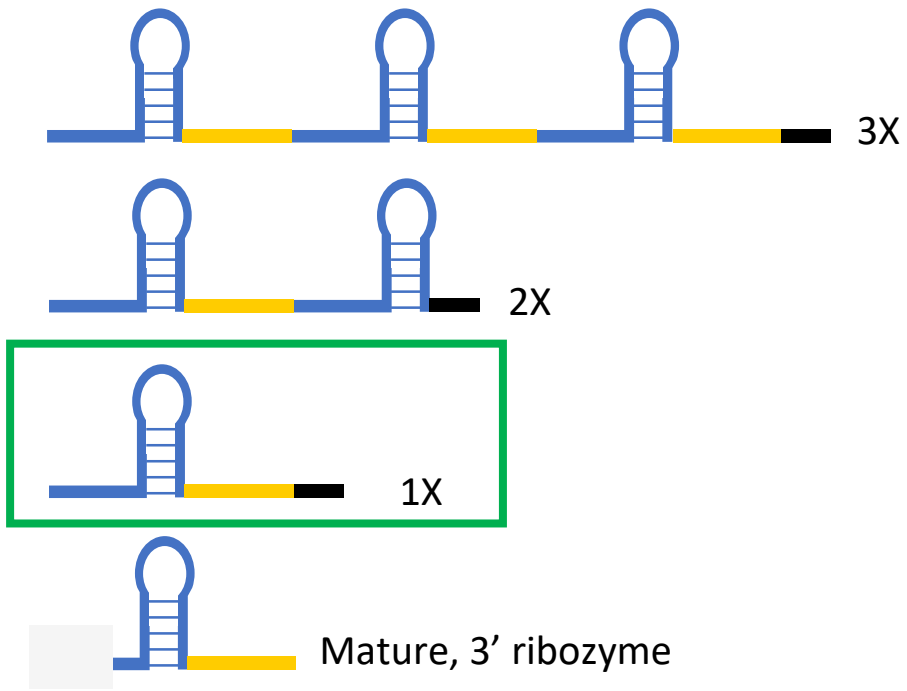


Clearance of the target plasmid observed with SuCms1

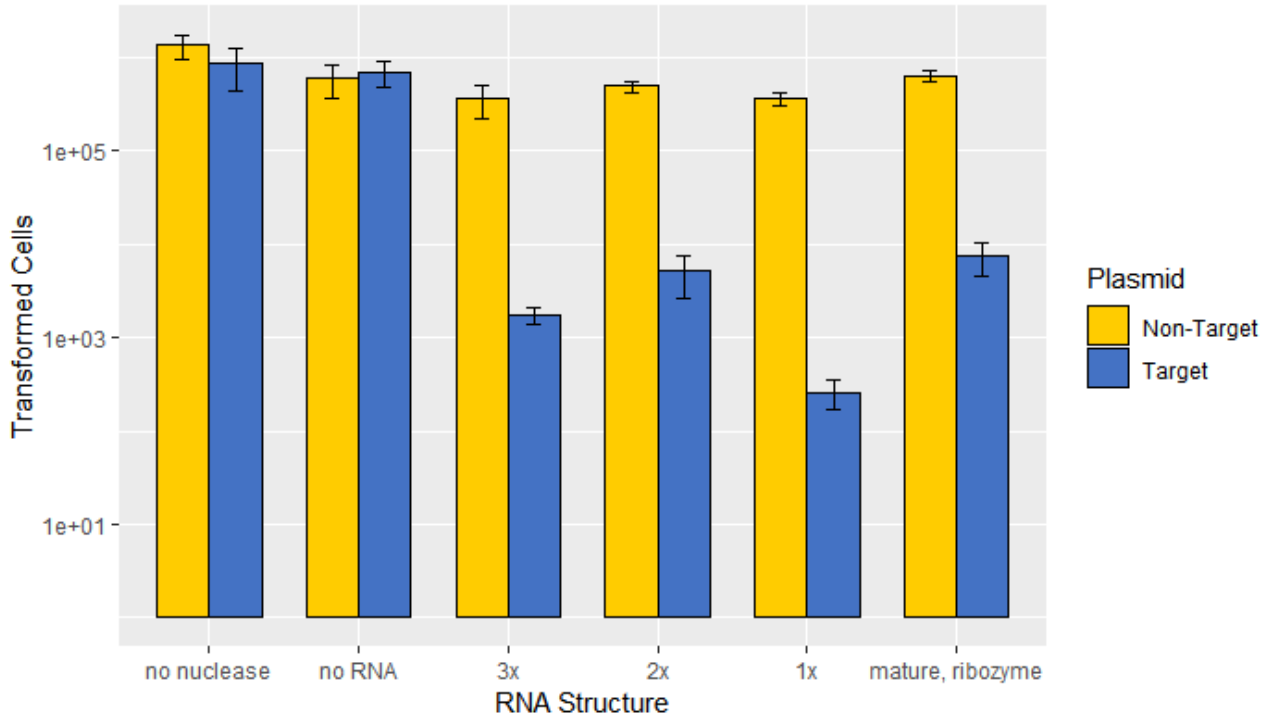


SuCms1 can process and cut efficiently with multiple gRNA structures

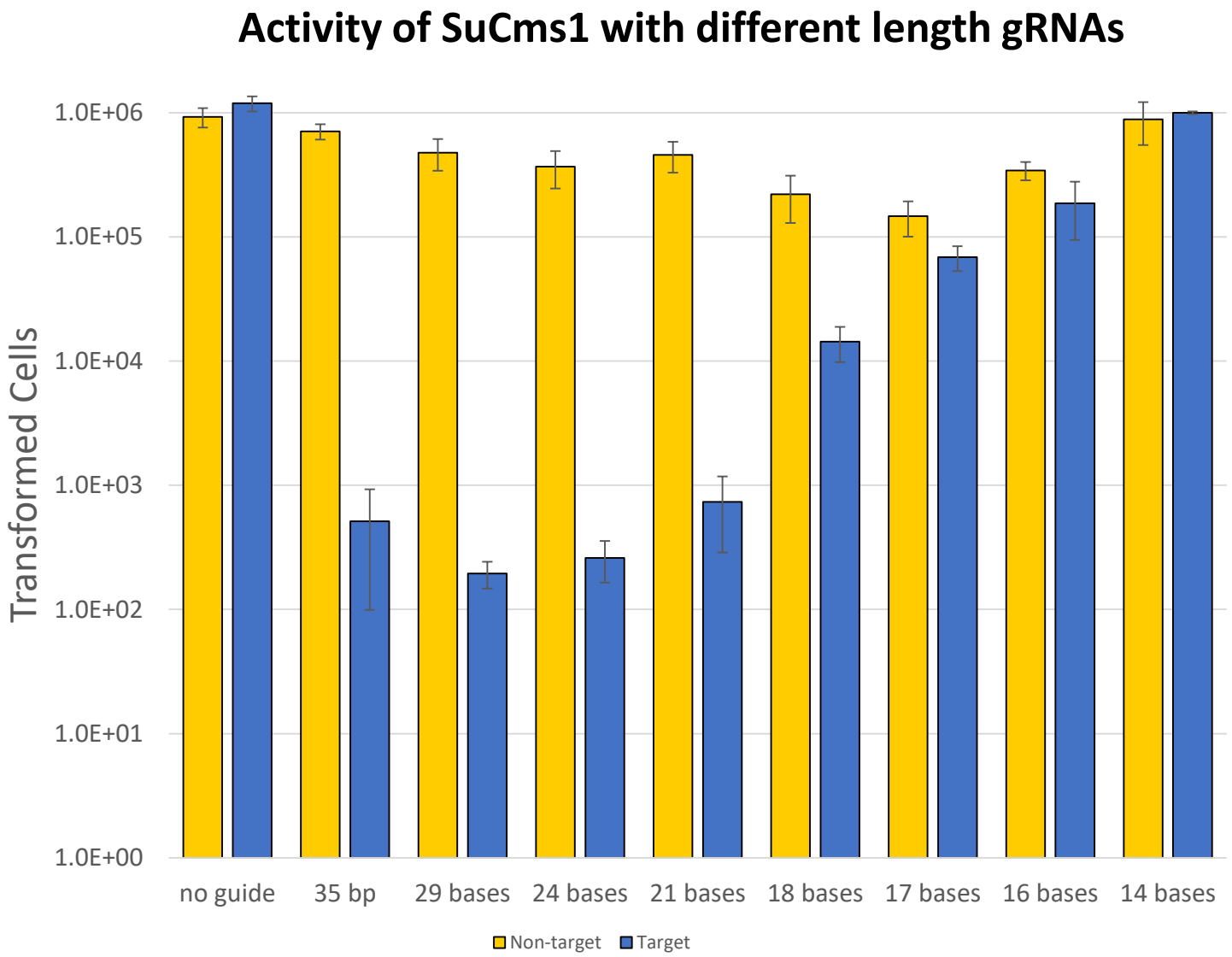
Diagram of RNA Structures



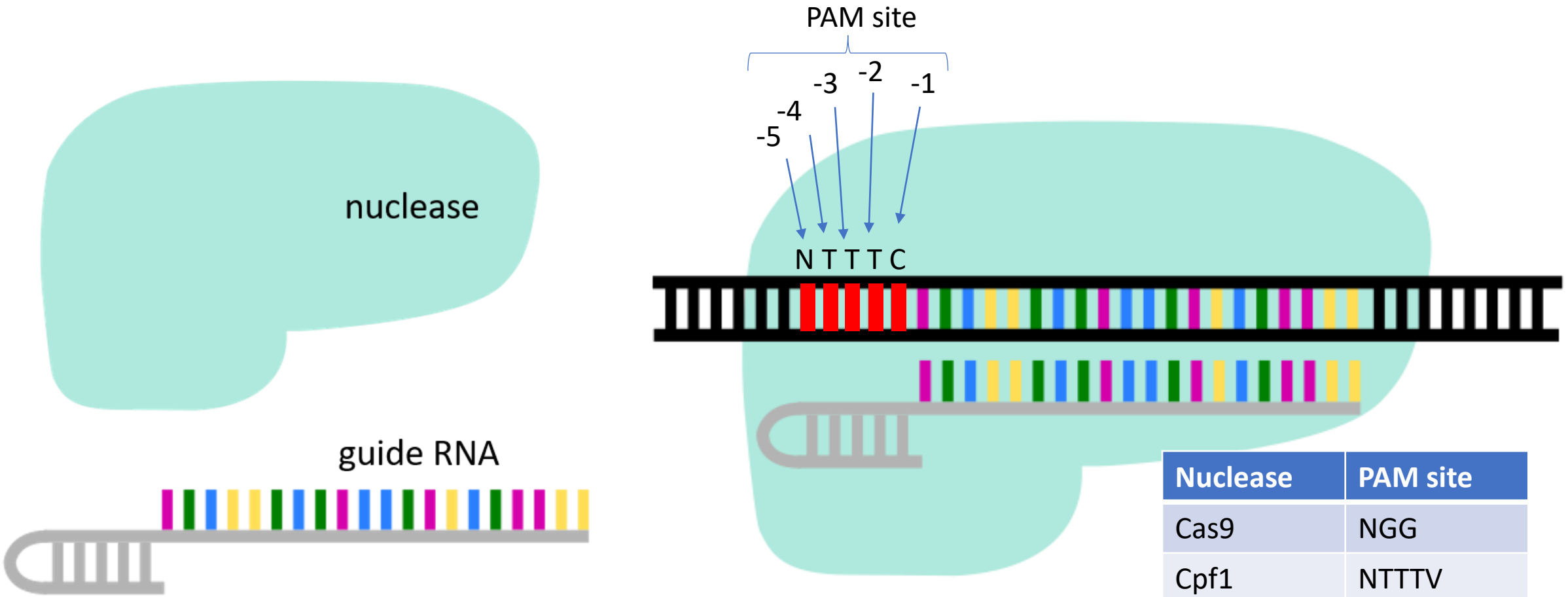
SuCms1 Activity with Diverse RNA Structures



SuCms1 has an optimal gRNA length similar to Cpf1 – 24 nucleotides



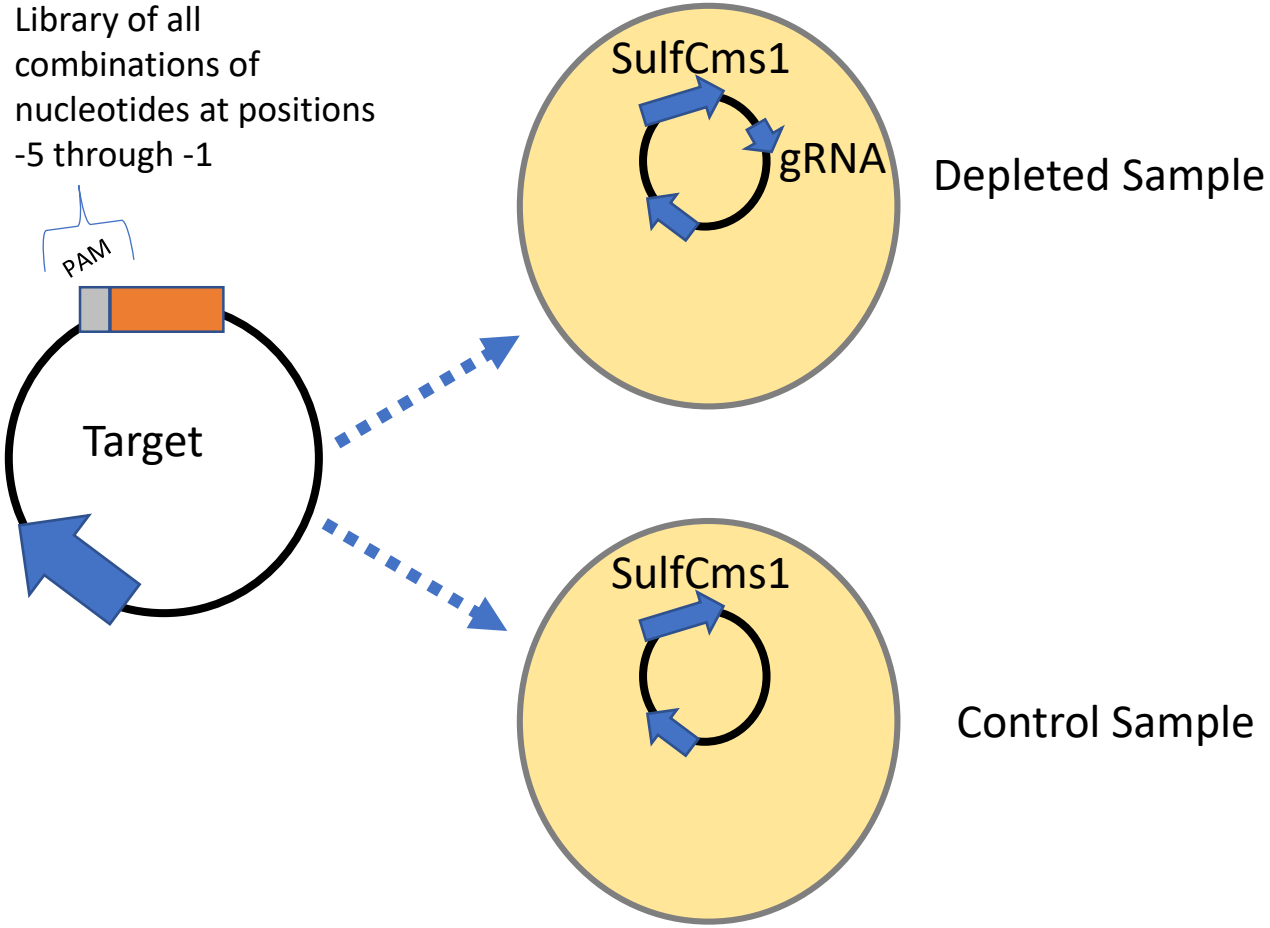
CRISPR Review: Protospacer adjacent motif (PAM) sites



Nuclease	PAM site
Cas9	NGG
Cpf1	NTTTV
Cms1	?

High-throughput experiment for PAM site characterization

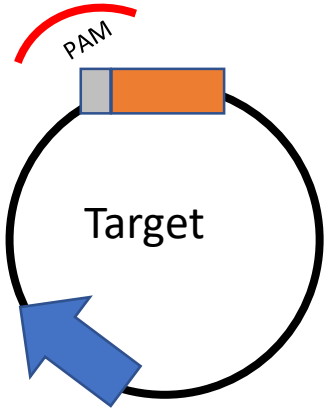
Library of all combinations of nucleotides at positions -5 through -1

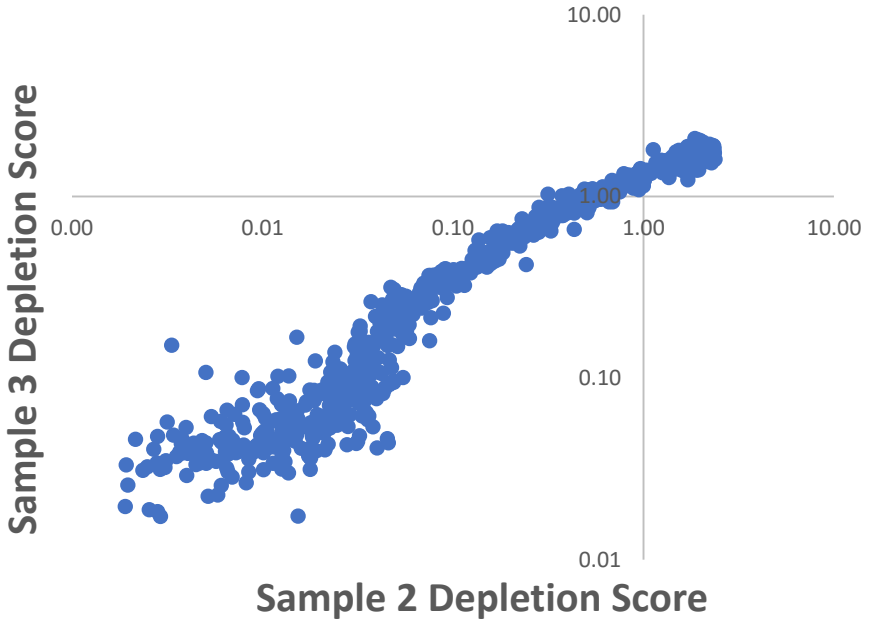


Depleted Sample

Control Sample

- Replicates 1, 2, & 3
- Submitted Replicates 2 & 3 for NextGen sequencing
- Calculated Log2 depletion compared to WT for each of the 1,024 PAM sites





Top 30 Depleted PAM sites for SuCms1 – Diversity in PAM Sites!

1	ATTGA	7	GTTCG	13	ATTCT	19	TGTCC	25	ATTAC
2	ATTCG	8	ATTCA	14	ATATC	20	GTTAC	26	ATCTC
3	GTTTC	9	TCTTA	15	GTCTG	21	ATTAA	27	ATTAG
4	ATCTG	10	ATATA	16	CTTTG	22	GTTTG	28	ATTTA
5	ACTTA	11	GTTCA	17	ACTTG	23	TGTTC	29	ATATG
6	GTCTA	12	GTTGA	18	ATTTC	24	GCTTA	30	GCTTG

AsCpf1

Rank of depleted PAMs	PAM	Depletion score	Rank of depleted PAMs	PAM	Depletion score
1	GTTTG	12.9	11	ATTTC	7.2
2	GTTTA	12.3	12	TTTTC	6.8
3	CTTTC	11.0	13	ACTTA	5.3
4	TTTTG	10.7	14	GCTTA	5.0
5	CTTTA	10.6	15	GGTTA	4.6
6	ATTTG	10.0	16	TCTTA	4.5
7	TTTTA	9.9	17	ACTTC	4.4
8	GTTTC	8.4	18	TCTTG	3.6
9	CTTTG	8.3	19	AGTTA	3.6
10	ATTTA	7.9	20	TCTTC	3.2

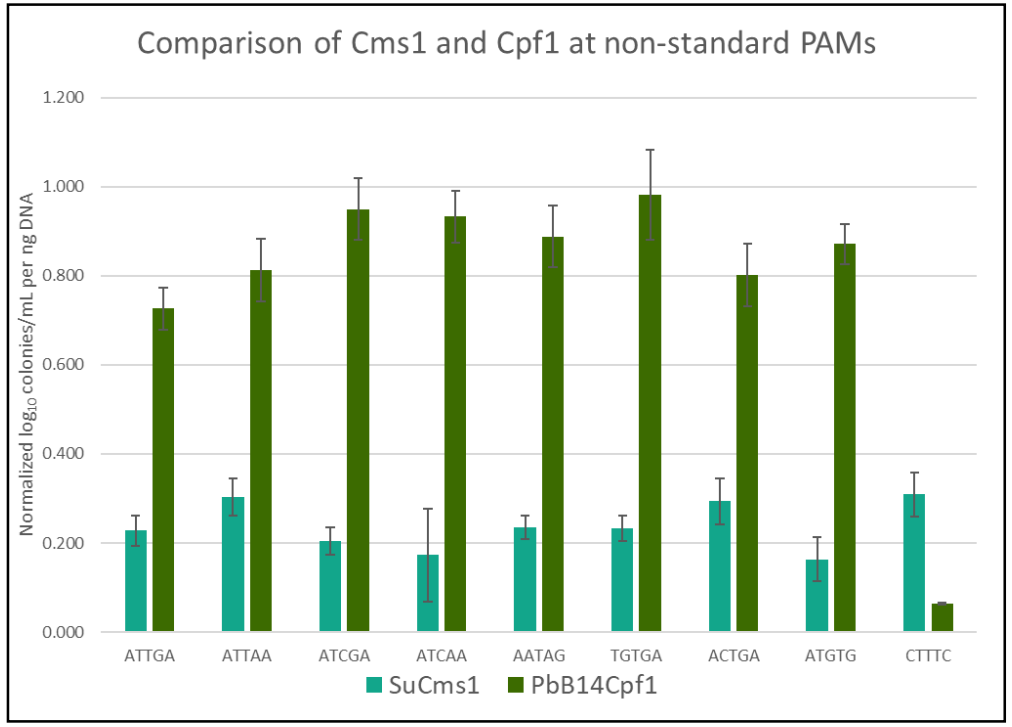
NTTTV

Other

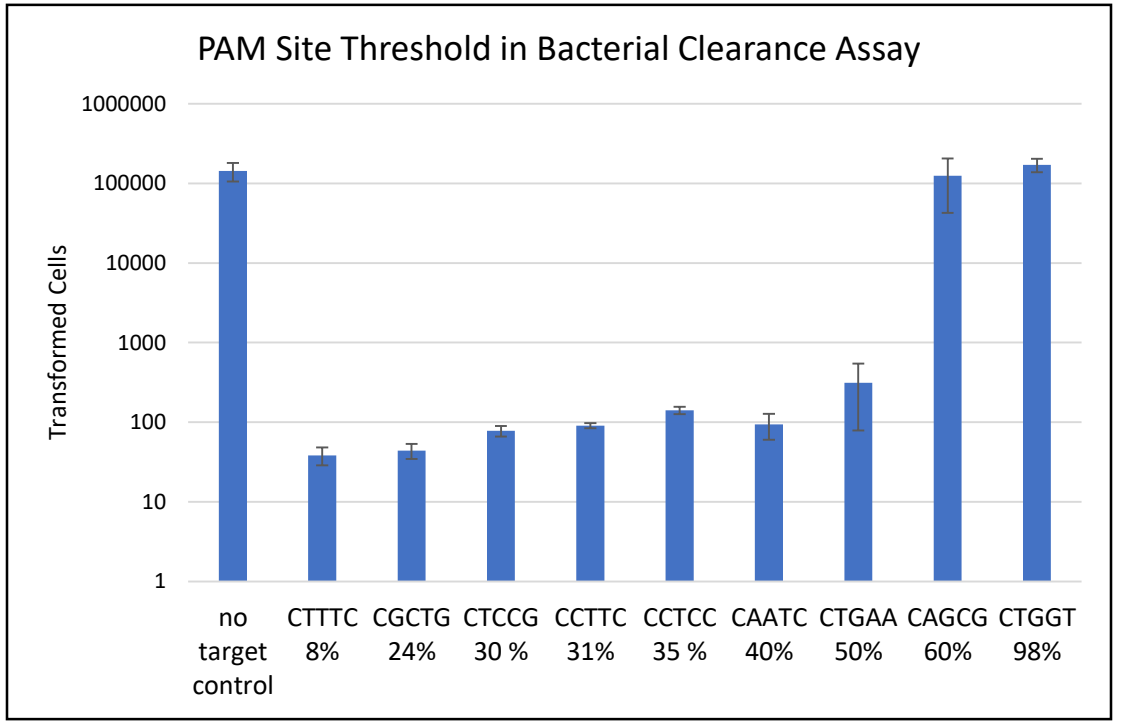
Jacobsen, T., Liao, C., & Beisel, C. L. (2019). *FEMS Microbiology Letters*

Non-standard PAMs were cross validated in E. coli - > 50% of PAM library variants have activity

Testing of non-standard PAM's (Beisel Lab)



PAM's across percentile tiers (1,024 total in library)



Nuclease	PAM Site	Total Sites Possible	Percent of Sites Available
No PAM Restriction	NNNNN	1,024	100%
SpCas9	NGGNN	64	6.25%
AsCpf1	NTTTV	12	1.17%
SuCms1	Complex	>300	~30%

Summary of published portfolio of BHB CRISPR Nucleases

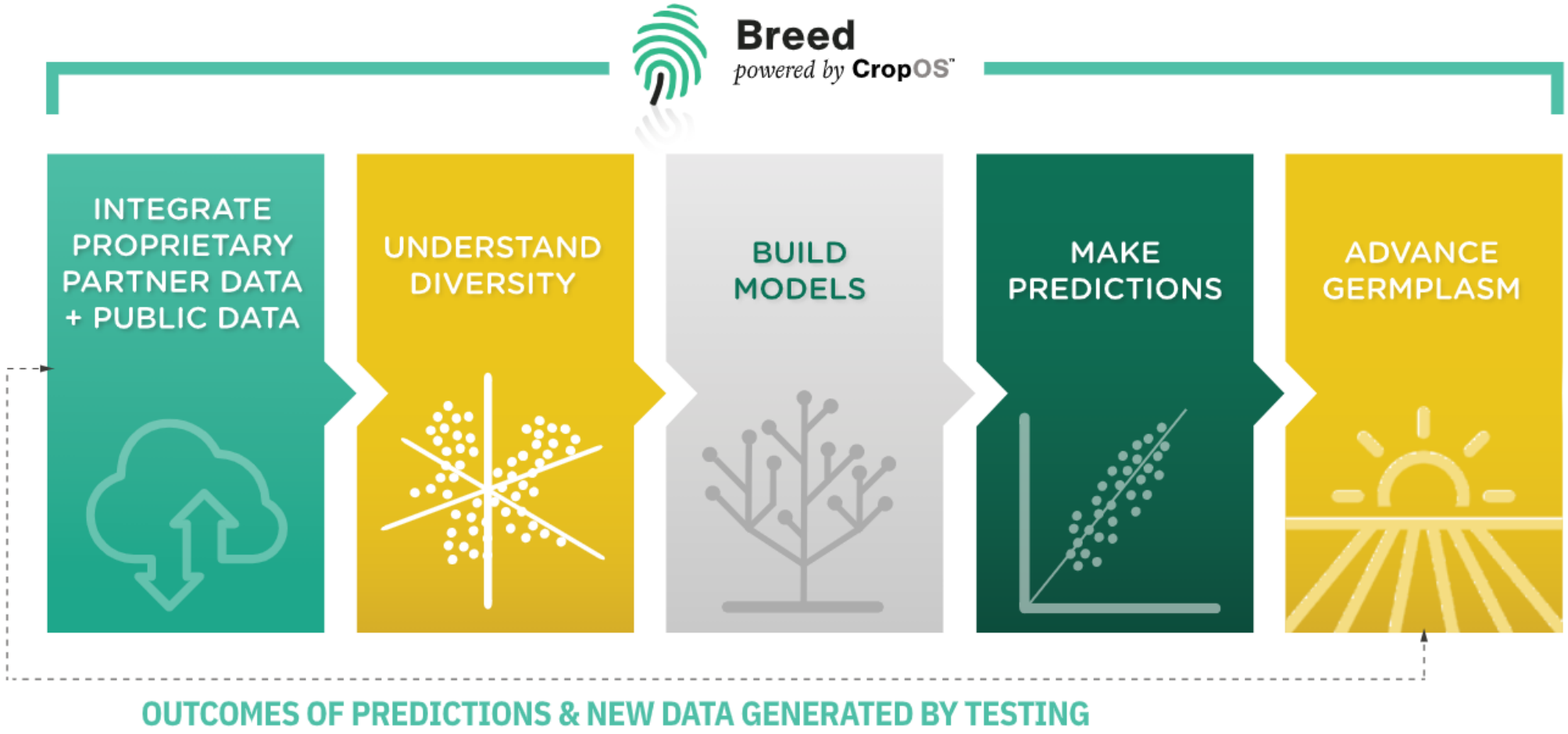
Nuclease	Type	CRISPR	Monocot	Dicot	*E. coli*	Mammalian	Yeast	Patent #
Pb2	Cpf1	2.0	O	O	O	O	Not tested	US 10,113,179
Lb6	Cpf1	2.0	O	O	O	O	Not tested	US 10,113,179
Ec	Cpf1	2.0	O	O	O	O	O	US 10,113,179
Mc	Cpf1	2.0	O	O	O	O	Not tested	US 10,113,179
Ps	Cpf1	2.0	O	O	O	O	Not tested	US 10,113,179
Su	Cms1	3.0	O	O	O	Testing with Partner in Progress	O	US 9,896,696
Sm	Cms1	3.0	O	O	O		O	US 9,896,696
Mi	Cms1	3.0	O	O	O		O	US 9,896,696
Ob	Cms1	3.0	O	O	O		O	US 9,896,696
Unk38	Cms1	3.0	O	O	O		Not tested	10,316,324
ADurb	Cms1	3.0	O	Not tested				10,316,324
Aux	Cms1	3.0	O	Not tested				10,316,324
LAHS	Cms1	3.0	O	Not tested				10,316,324
Unk1	Cms1	3.0	O	Not tested				10,316,324
Unk35	Cms1	3.0	O	Not tested				10,316,324

O = Activity confirmed
O = Experiment in progress
O = Activity not detected

Breed, *powered by CropOS*

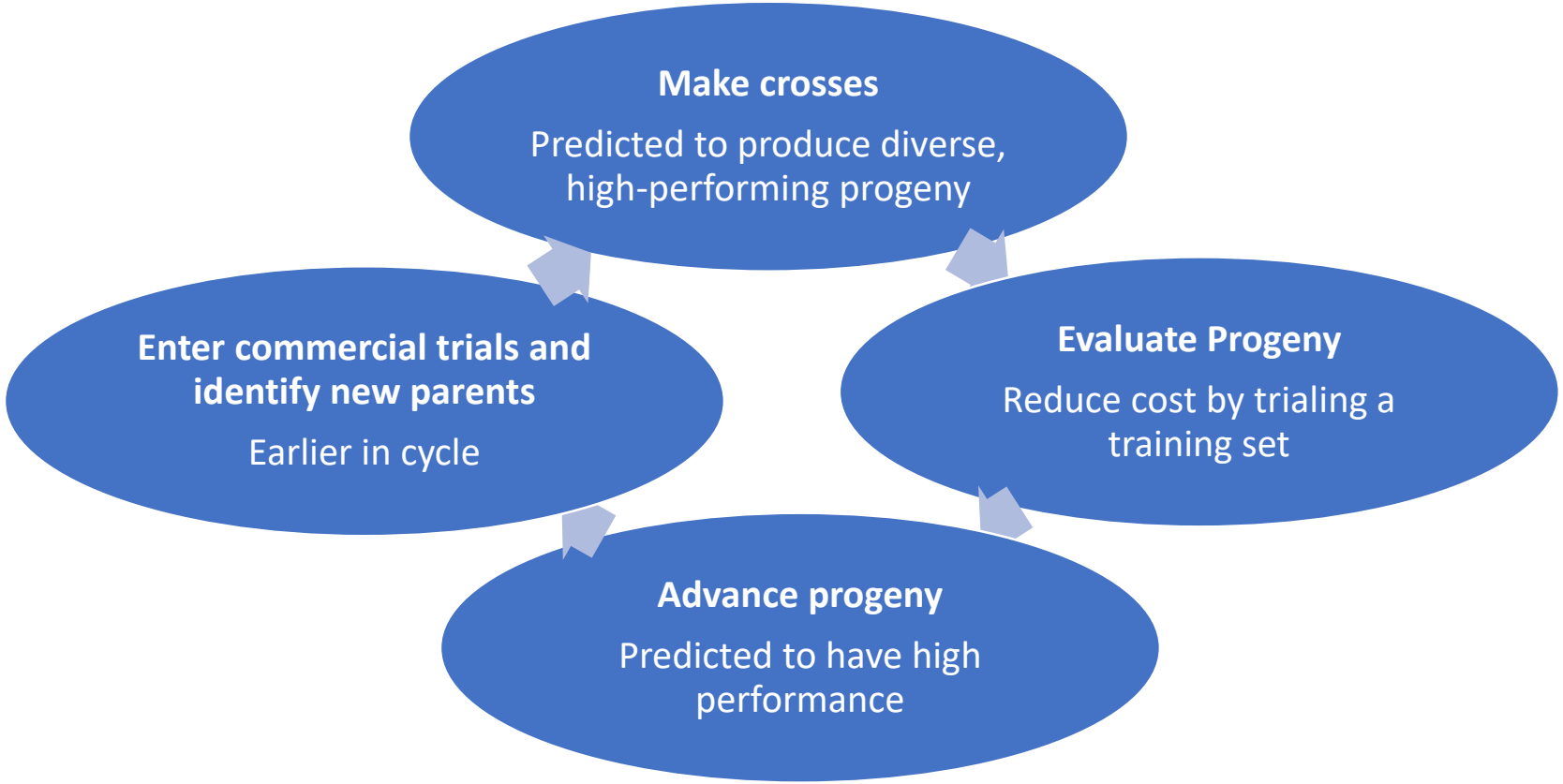
BENSON HILL

Breed empowers plant breeders by providing a user-friendly platform for genomic selection

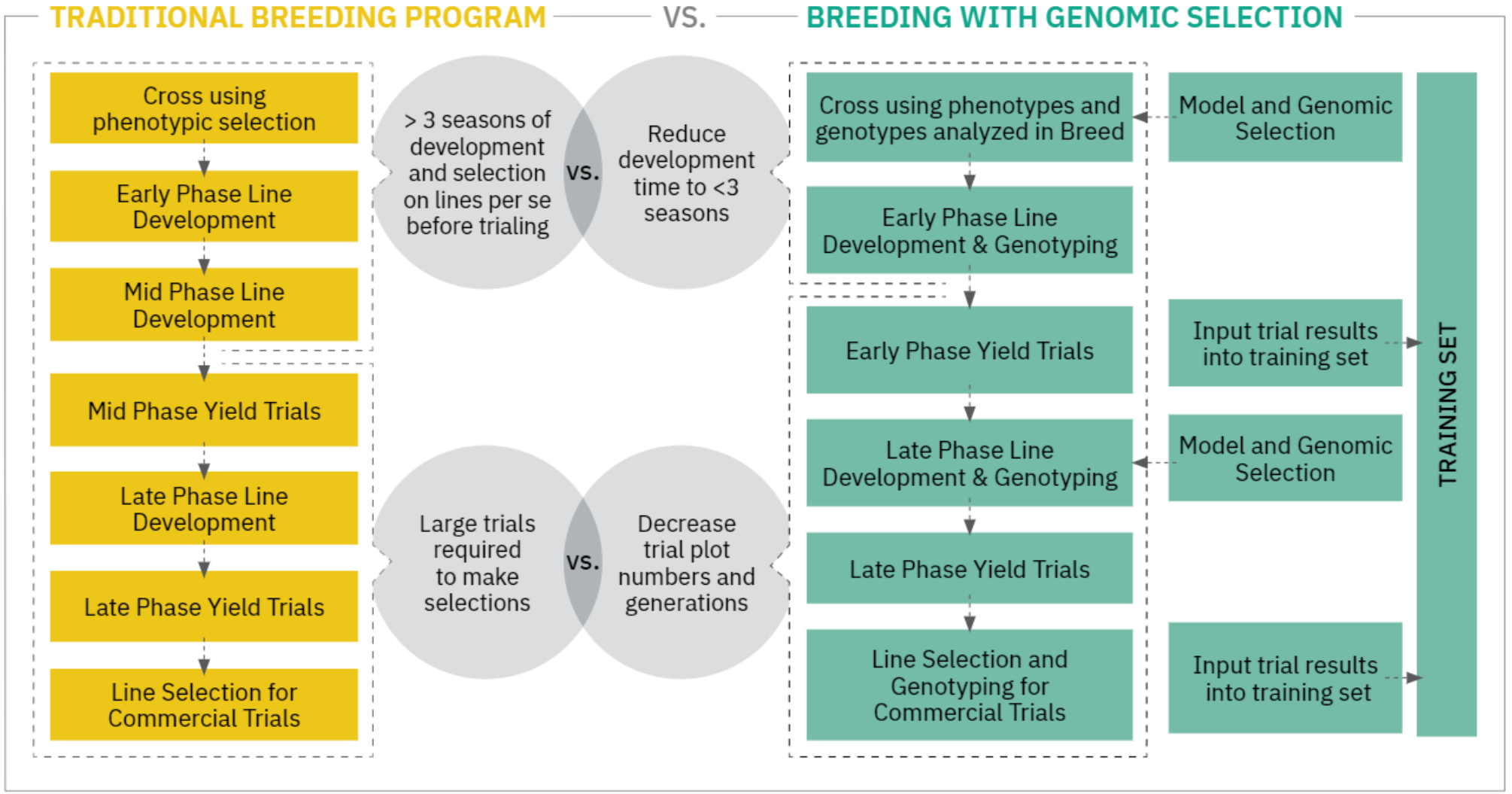


What is genomic selection?

Genomic selection is a breeding method that improves the speed and efficiency to identification of a new variety by utilizing genotypes, phenotypes, operations, and prediction.



Genomic Selection: Using genomics and predictive analytics to enhance decision making in the breeding process



Breed provides a user-friendly interface for each of the key steps in genomic selection

Key Step in Genomic Selection	Breed, *powered by CropOS*
1) Collect data and make it available	Data onboarding, phenotype analysis, and imputation available through online apps
2) Understand what you have	View, share, and asses genetic diversity and phenotype analysis results in one place
3) Use that information to create models	Select from different algorithms and apply to the training set of interest
4) Make predictions	Use trained models to calculate predicted breeding values for genotyped lines
5) Identify advancements and enter advanced trials	Lines to advance can be easily identified in interactive tables
6) Make crosses predicted to have high-performing progeny	*In silico* assessment of progeny performance
7) Enter next cycle of genomic selection	All results are stored and ready for review at the start of the next cycle

Benson Hill and partners work together to fully capture the power of predictive analytics in their breeding programs

Action	Responsible Party	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8
PROGRAM & GOAL ALIGNMENT									
Establish a working team and hold kick-off meeting	BHB/Partner								
Review current state and roadmap of Partner program	BHB/Partner								
Review current state and roadmap of CropOS	BHB/Partner								
DATA DISCOVERY									
Understand Partner's available phenotypic and genotypic data and if that data will support GS	BHB/Partner								
Determine additional data requirements if needed	BHB								
DATA AND USER ONBOARDING									
Train Partners on data formatting and onboarding workflow	BHB								
Onboard all available and relevant phenotypes and genotypes	Partner								
MODEL TRAINING									
Evaluate genetic diversity	BHB/Partner								
Define target populations	BHB/Partner								
Optimize training populations	BHB/Partner								
Create models for GS and dev cross prediction	BHB/Partner								
LINE AND CROSS SELECTION									
Generate predictions of phenotypes and the best crosses to advance in Breed	BHB/Partner								

Partnership examples

- Committed to developing and defining consumer acceptance guidelines around genome editing in the US
- Provide access to predictive analytics and molecular biology tools to accelerate product development
- Offer these tools for no cost to research organizations for research purposes

Benson Hill empowers innovators that are advancing food and agriculture

BENSON HILL