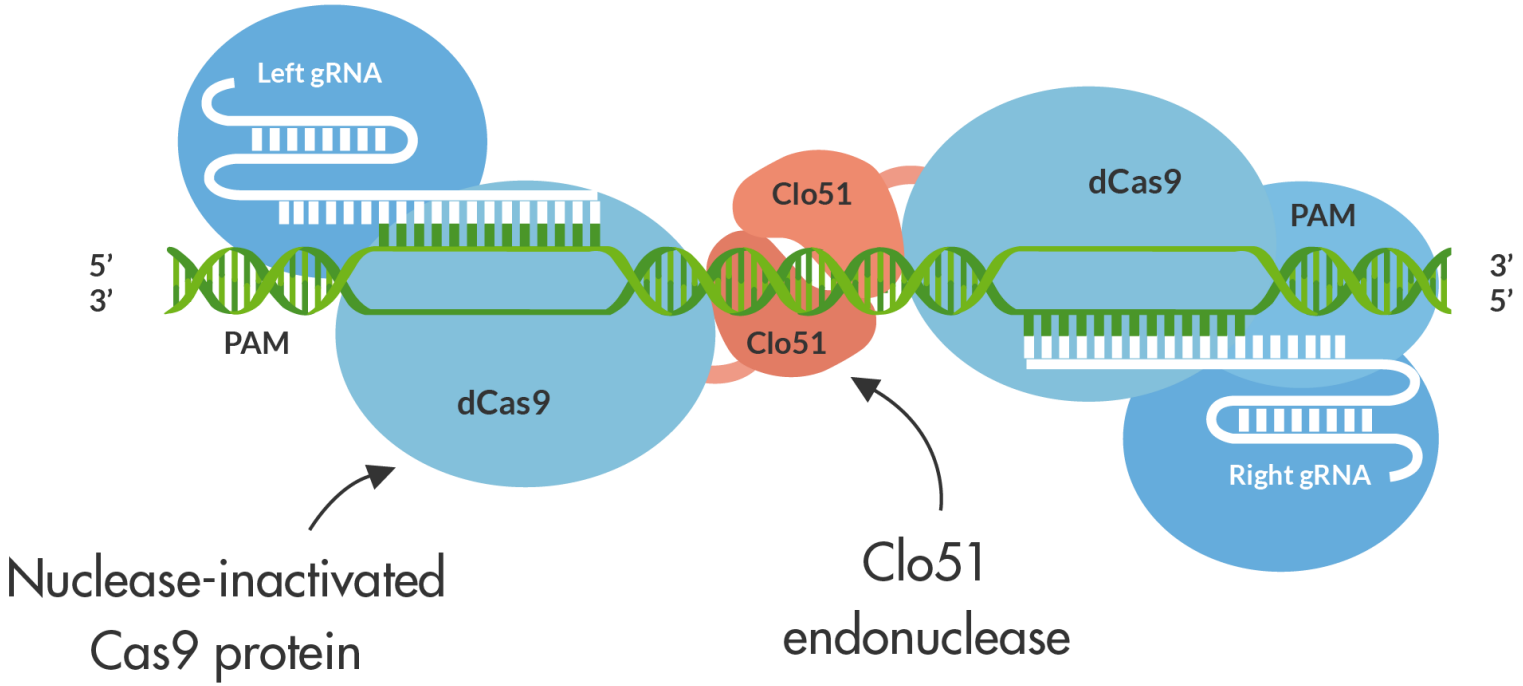
Plant Gene Editing with Cas-CLOVER™
"the clean alternative to CRISPR/Cas9"

What is Cas-CLOVER?

Hybrid gene editing system using nuclease-inactivated Cas9 protein fused to Clo51 endonuclease.

dCas9 serves as a fusion protein only. It is mutated and unable to cut DNA.

Cleavage activity depends on the dimerization of an "obligate dimer" of Clo51.



Benefits

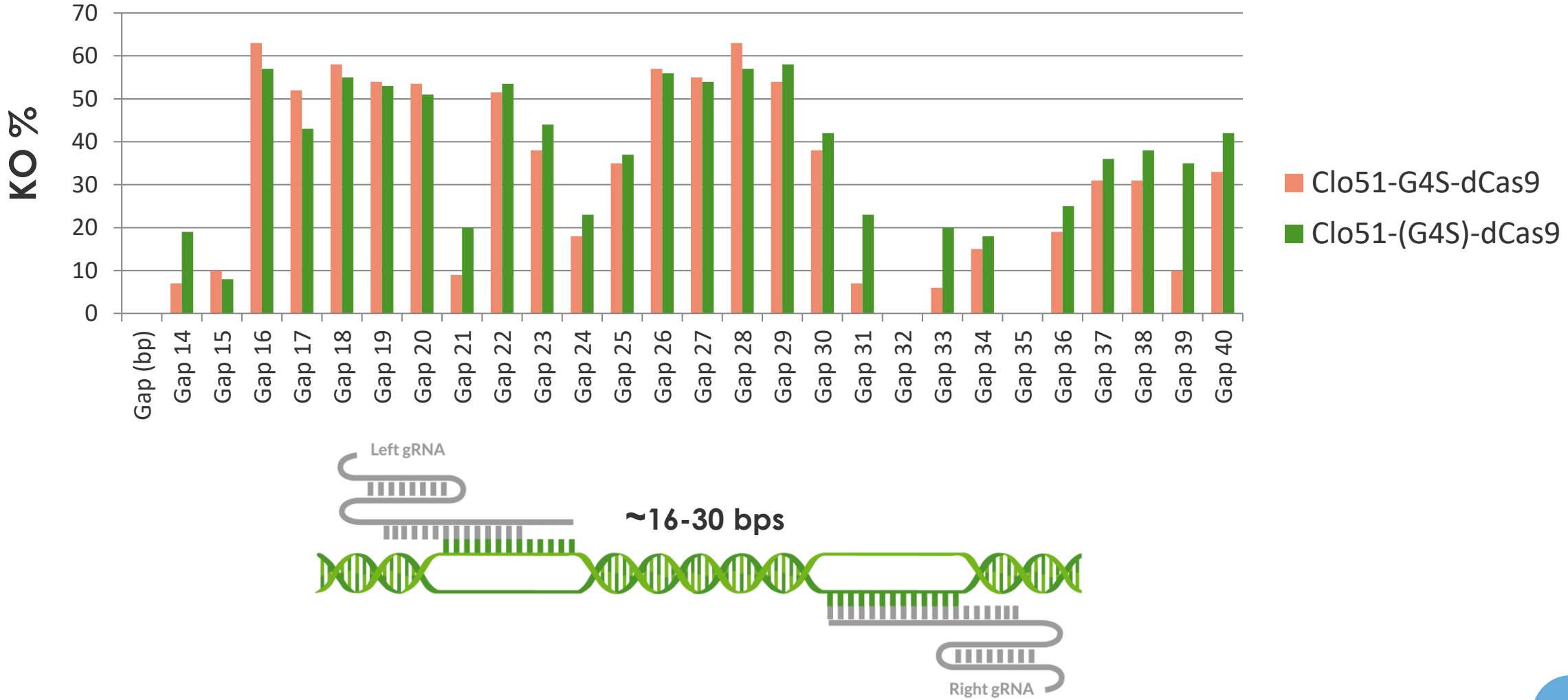
Ease of use: double gRNA guided

High efficiency: dCas9-guided RNA recognition

High fidelity: cuts only when Clo51 nuclease dimerizes

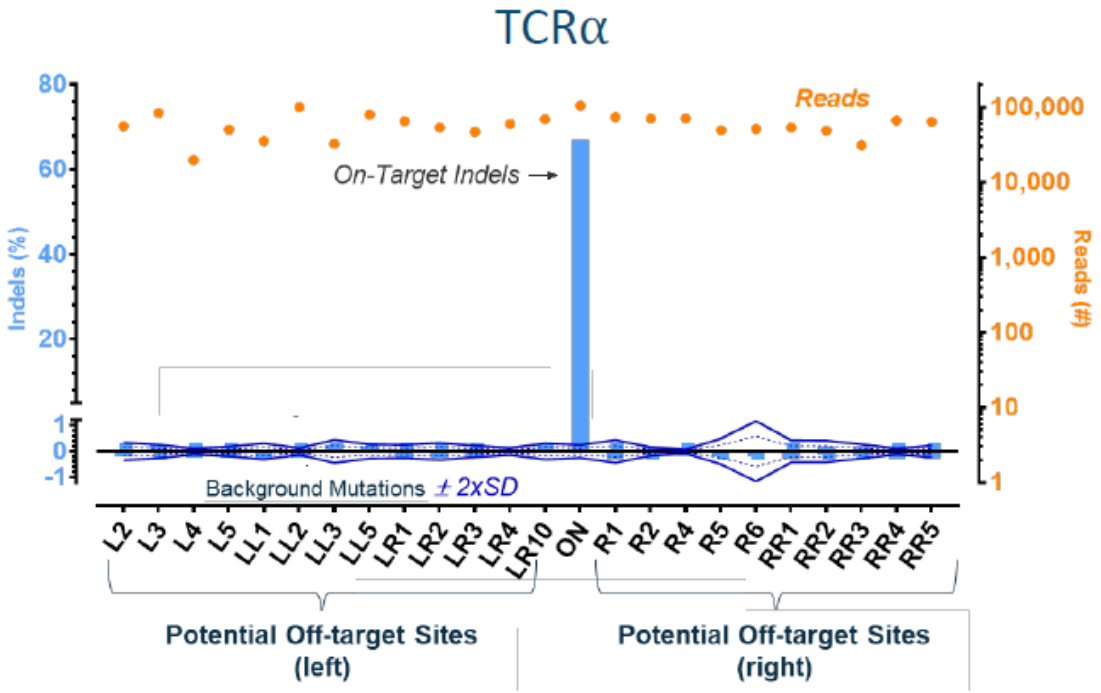
Optimal Activity Reported in ~16-30 Base Pair Range

Designing multiple Cas-CLOVER sites within target genes is straightforward.

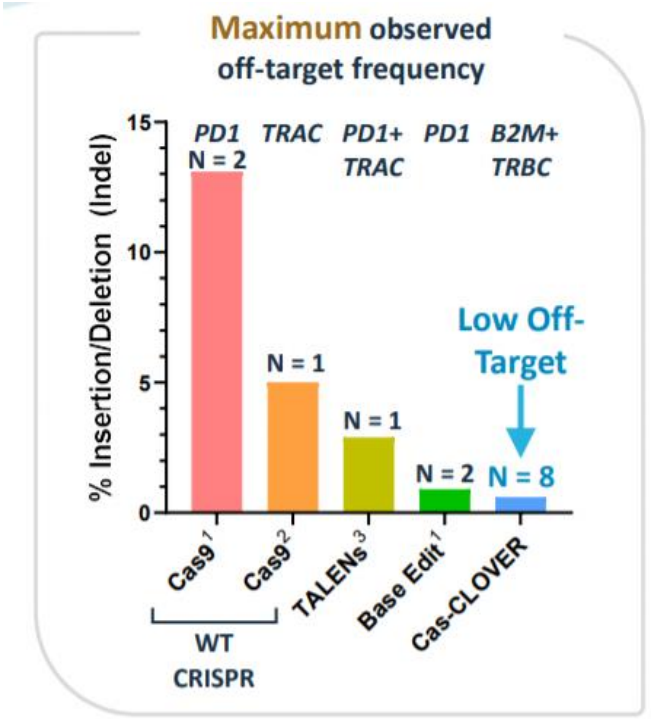


Li et al (2018) Cas-CLOVER™: A High-Fidelity Genome Editing System for Safe and Efficient Modification of Cells for Immunotherapy. Precision CRISPR Congress Poster.

Extensive studies reveal minimal off-target activity



- Left y-axis = percent knockout (indels) for the given gRNA compbination
- Right y-axis = number of next-generation sequencing (NGS) reads. Comprehensive coverage from around 100,000 reads
- X-axis bar graphs = percent knockout (indels) the different gRNA combinations for predicted off-target and on target "ON"
- X-axis lines = dotted blue line is 1 standard deviation (SD) from background mutation rate. Solid blue line is 2 SD from background mutation rate



- Other studies examine few (10 to 25) candidate off-target sites[1-3].
- Our Cas-CLOVER off-target study is ~10x broader and includes 8 donor lots.

- "unbiased" off-target analysis iGuide-seq followed by Ampli-seq

Li et al (2018) Cas-CLOVER™: A High-Fidelity Genome Editing System for Safe and Efficient Modification of Cells for Immunotherapy. Precision CRISPR Congress Poster. Posieda Therapeutics Virtual R&D Day 2021

Cas-CLOVER plant genome engineering

Robust genetic tools for trait development

Clean editing for possible non-GMO crop designation

Optimization of the gene editing system: targeting the PDS gene in tobacco for visual phenotype read-out

Utilized visual PDS read-out to optimize cutting efficiencies to 100%, demonstrating editing of all 4 alleles

T0 and T1 generations were screened for stable gene editing

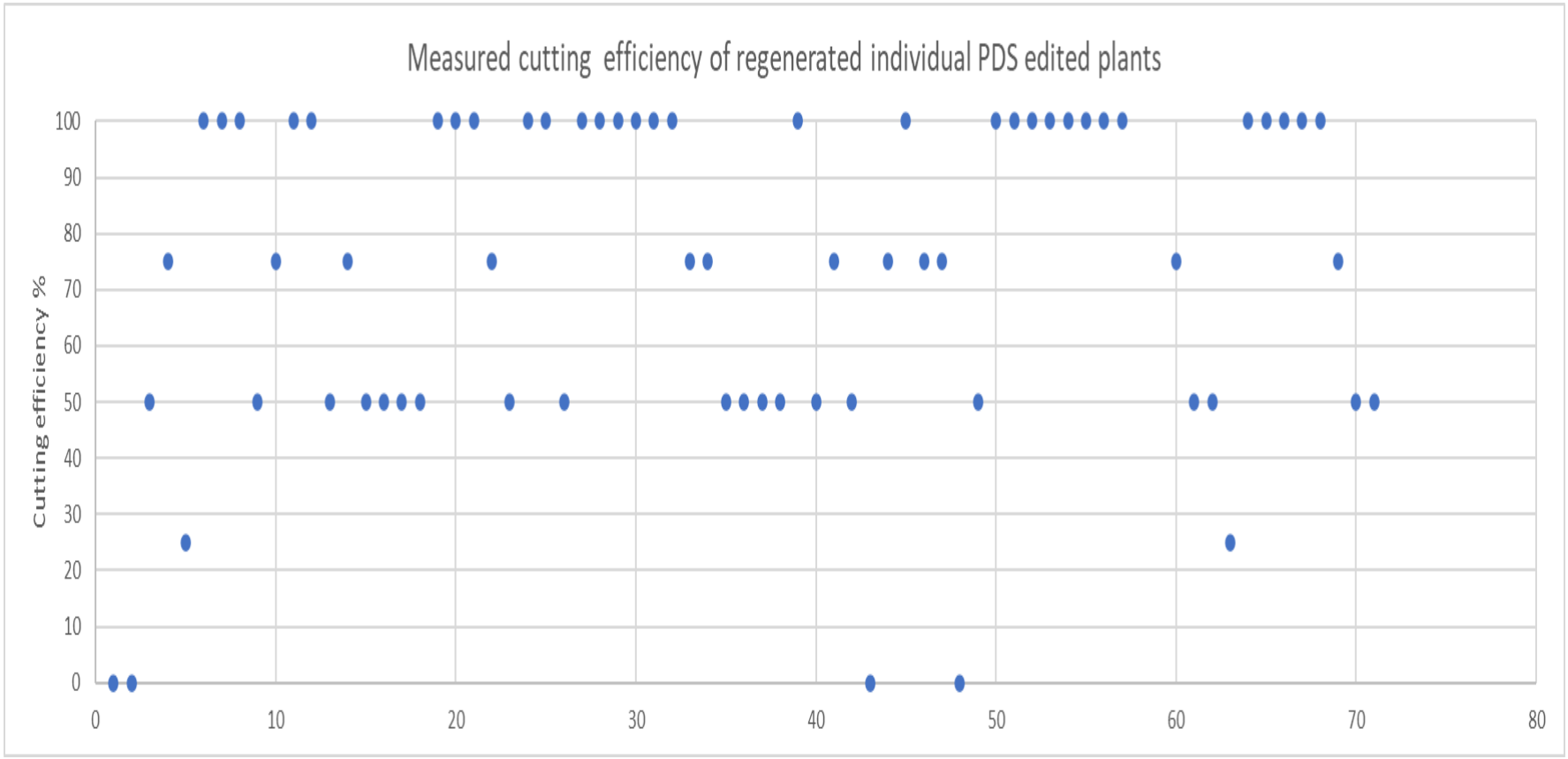
First proof-of-concept: PDS Knockout

Knockout mutation of the phytoene desaturase (PDS) gene results in a visually pale and white phenotype

Green, pale mixed, and white shoots were all screened to determine cutting efficiencies. Afterwards, we verified that only white and pale material was edited at an overall average of around **50%+ editing efficiency (white shoots/total shoots from plates).**

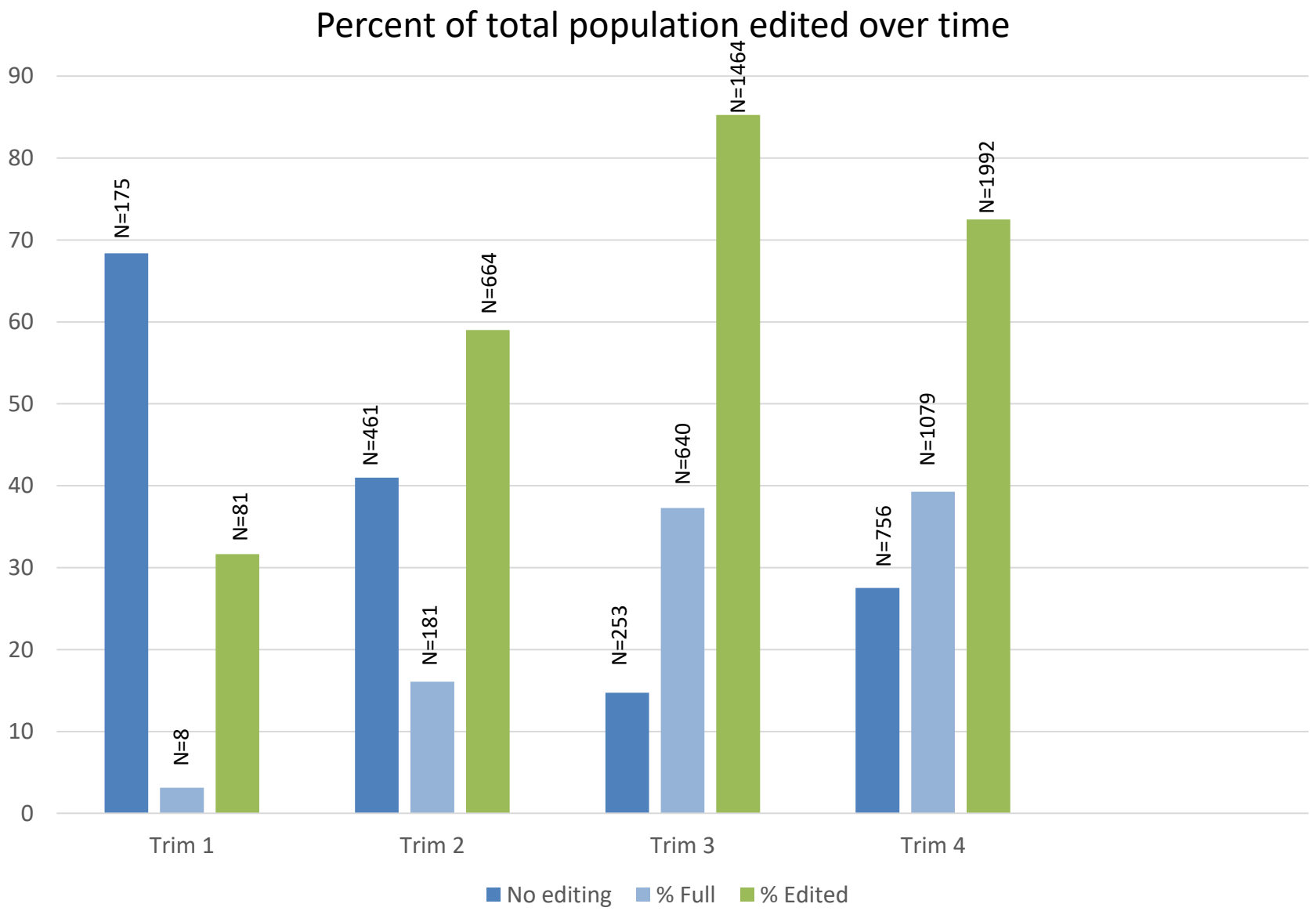
Optimizing cutting efficiencies of Cas-CLOVER in plants

Demeetra developed an optimized protocol which is transferred to our licensees and partners



Improved system consistently yields 100% editing efficiency, modifying all four alleles in tobacco

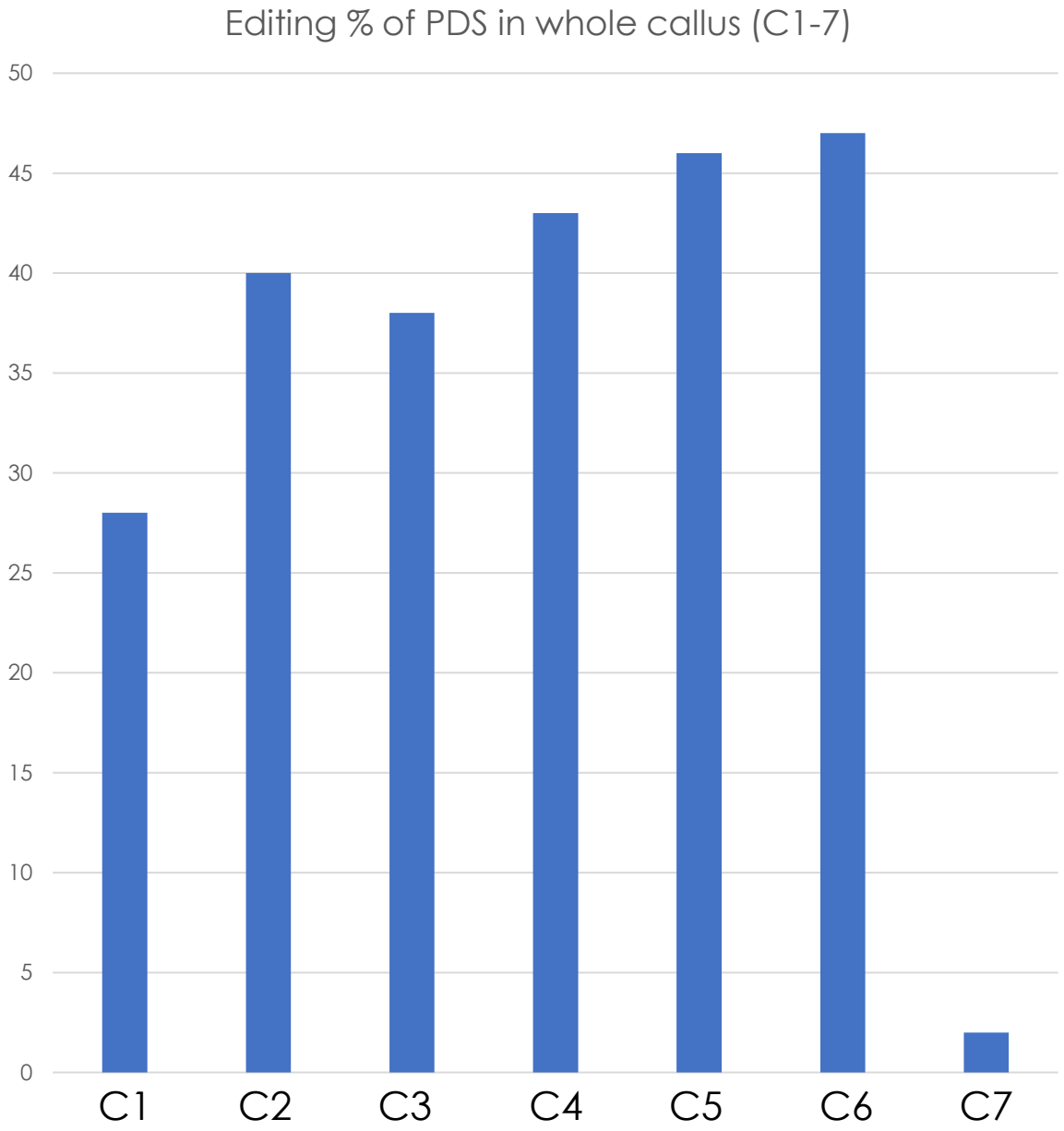
Editing Over Time



- Correctly targeted plants can be identified in a relatively short period of time
- Editing efficiencies plateaued at the Trim 3 timepoint

Green = no editing
Yellow = 1-2 alleles edited
White = 3-4 alleles edited
Note: Yellow plants can be crossed to produce fully edited plants. Therefore, the data represent white phenotype as "% fully edited" and white + yellow "% edited"

Whole callus editing identification



Next, to speed up and simplify our process, we aimed to test whole callus from plates for cutting efficiency with Cas-CLOVER

-Whole callus were homogenized, DNA was extracted, PCR amplified, sequenced, then analyzed by editing software

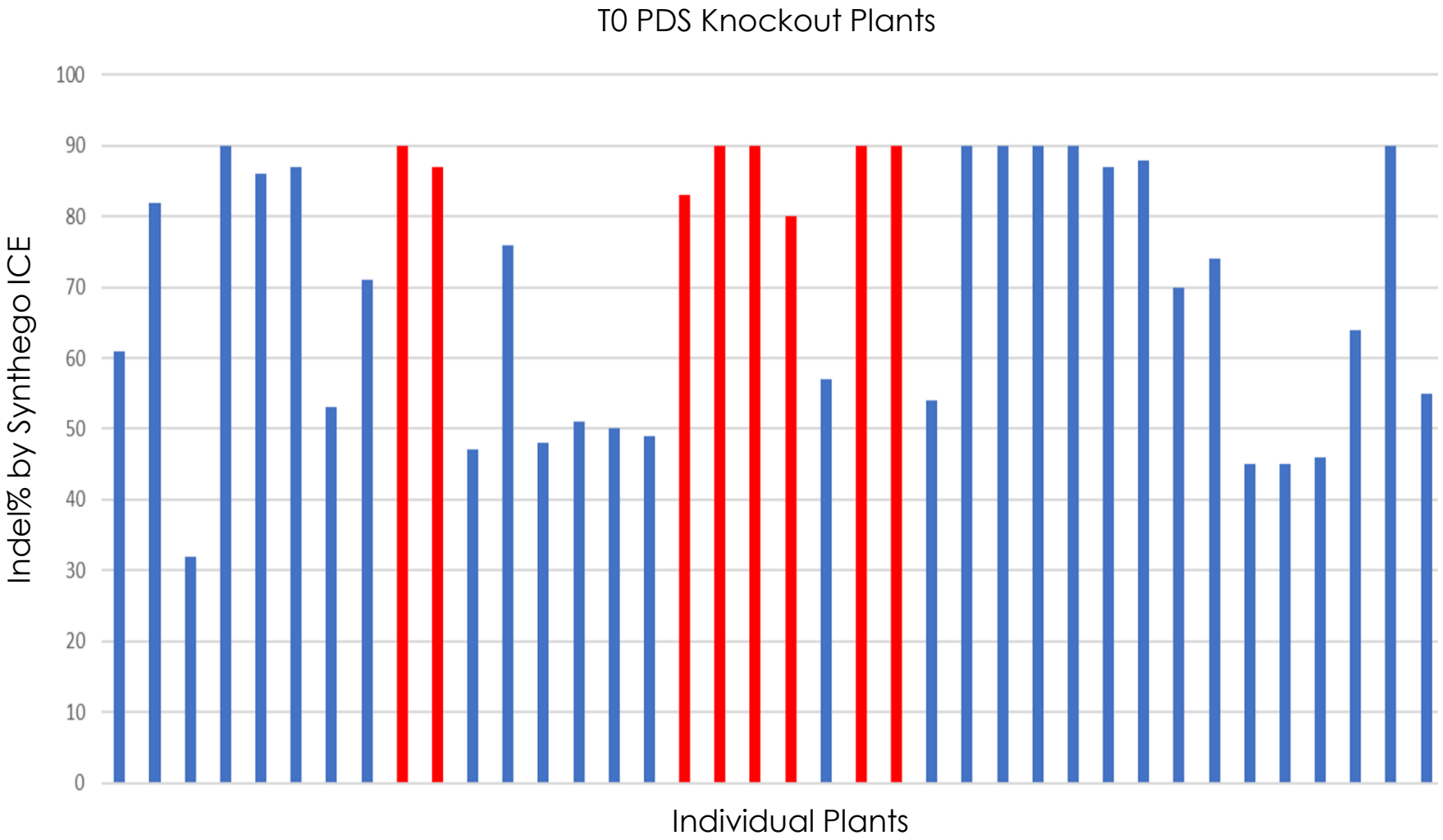
-These data are from a single promoter expressing Cas-CLOVER, Demeetra has tested multiple promoters with varying efficiencies.

Examples of edited sequences

Relatively large deletions (in comparison, CRISPR/Cas9 generates 1-3 bp deletions) is consistently observed with Cas-CLOVER

This results in simpler, faster and more cost-effective screening of plants for editing, see our post on this.

Stability of plant edits over time

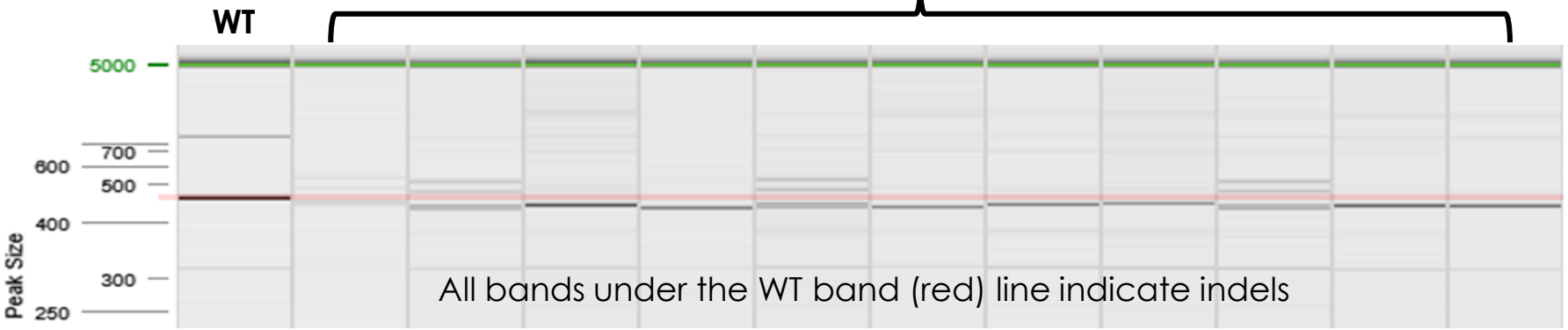


- Red annotated plants were propagated to maturity
- Mature plants in soil demonstrated stable editing levels at flowering stage

T1 generation retain Cas-CLOVER edits

- Selected plants were self pollinated, and seed was harvested
- Unpurified PCR products screened on QIAxcel for rapid PCR detection

QIAxcel T1 Plant Knockout Gel



QIAxcel Mutagenesis Report

Individual Plant Samples			Deletion reads				WT reads			
	Pos	Sample Info	Found	Size [bp]	Conc. [ng/µl]	Rel. Abund. [%]	Found	Size [bp]	Conc. [ng/µl]	Rel. Abun [%]
	E2	21B2.2	Yes	440	0.49	13.55	No	n/a	n/a	n/a
	E4	21B2.5	Yes	438	0.97	26.83	Yes	468	0.37	7.46
	A2	21B2.9	Yes	448	0.18	4.83	Yes	456	0.17	3.45
	A3	21B2.10	Yes	437	0.46	12.73	No	n/a	n/a	n/a
	A4	21B2.11	Yes	446	2.66	73.35	No	n/a	n/a	n/a
	A5	21B2.12	Yes	439	1.65	45.53	No	n/a	n/a	n/a
	A6	21B2.13	Yes	441	0.56	15.59	No	n/a	n/a	n/a
	D11	21B2.14	Yes	443	0.18	4.89	Yes	463	0.39	7.80
	A7	21B2.15	Yes	442	1.31	36.18	No	n/a	n/a	n/a
	A8	21B2.16	Yes	448	1.51	41.64	No	n/a	n/a	n/a
	B5	21B3.10	No	n/a	n/a	n/a	No	n/a	n/a	n/a
	B9	21B4.1	Yes	438	0.54	14.83	No	n/a	n/a	n/a
	D8	21B4.8	No	n/a	n/a	n/a	Yes	469	0.08	1.67
	C10	21B8.10	Yes	445	2.11	58.27	No	n/a	n/a	n/a
	C11	21B8.11	Yes	444	1.80	49.58	No	n/a	n/a	n/a
	D1	21B8.13	No	n/a	n/a	n/a	Yes	473	4.98	100.0
	D3	21B8.15	Yes	444	2.05	56.63	No	n/a	n/a	n/a

- Cas-CLOVER produces larger indels than other technologies
- The large indels are rapidly and easily detected by peak size & translated into a gel image

→ Deletions double checked by Synthego ICE

T1 generation screened by ICE

- 14 of 16 samples sequenced and analyzed demonstrated 100% indel percentage
- the two noted samples below also showed detection of WT remaining by QIAxcel
- two other samples with noted WT peaks by QIAxcel revealed only very small deletions by sequence

SUCCESSFULLY ANALYZED SAMPLES

SAMPLE	GUIDE TARGET	PAM SEQUENCE	INDEL %	MODEL FIT (R^2)	KNOCKOUT-SCORE	
21B310_PDS9_Plate_CEUU47_B07	AAGGTAATTCAGCTTATCTT GCAGAAACAAGTCCAATTTG	TGG GGG	100	0.85	16	View Details
21B213_PDS9_Plate_CEUU47_G06	AAGGTAATTCAGCTTATCTT GCAGAAACAAGTCCAATTTG	TGG GGG	100	0.71	98	View Details
21B212_PDS9_Plate_CEUU47_F06	GCAGAAACAAGTCCAATTTG AAGGTAATTCAGCTTATCTT	GGG TGG	100	0.88	98	View Details

Licensing Options

Demeetra AgBio holds the exclusive license in Agriculture to an issued patent portfolio for Cas-CLOVER. We offer sublicenses and strategic development partnerships.

Evaluation

Technology transfer with reagents and protocols for a 6-month evaluation research license

Research

Economically attractive yearly fee. Freedom to switch technologies for commercialization to navigate gene editing regulatory landscape

Commercial

Flexibility of one-time fee-based license or more standard upfront/milestone/royalty structure

For More Information, Contact

Demeetra

Jack Crawford, M.S.

CEO, Demeetra AgBio, Inc.

Mobile: 315-351-9115

Email: jcrawford@demeetra.com