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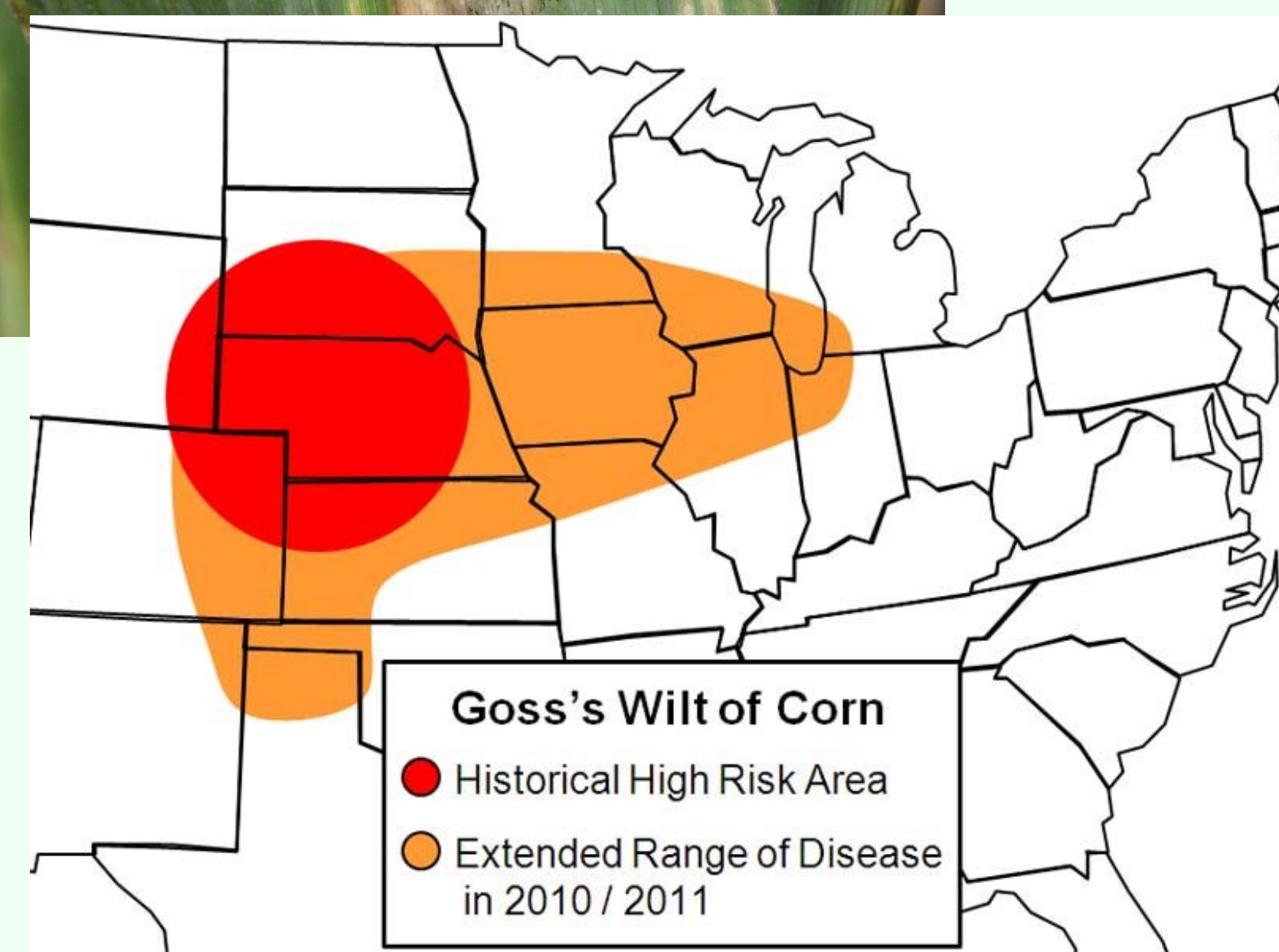
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Molecular Mechanisms of Goss's Wilt

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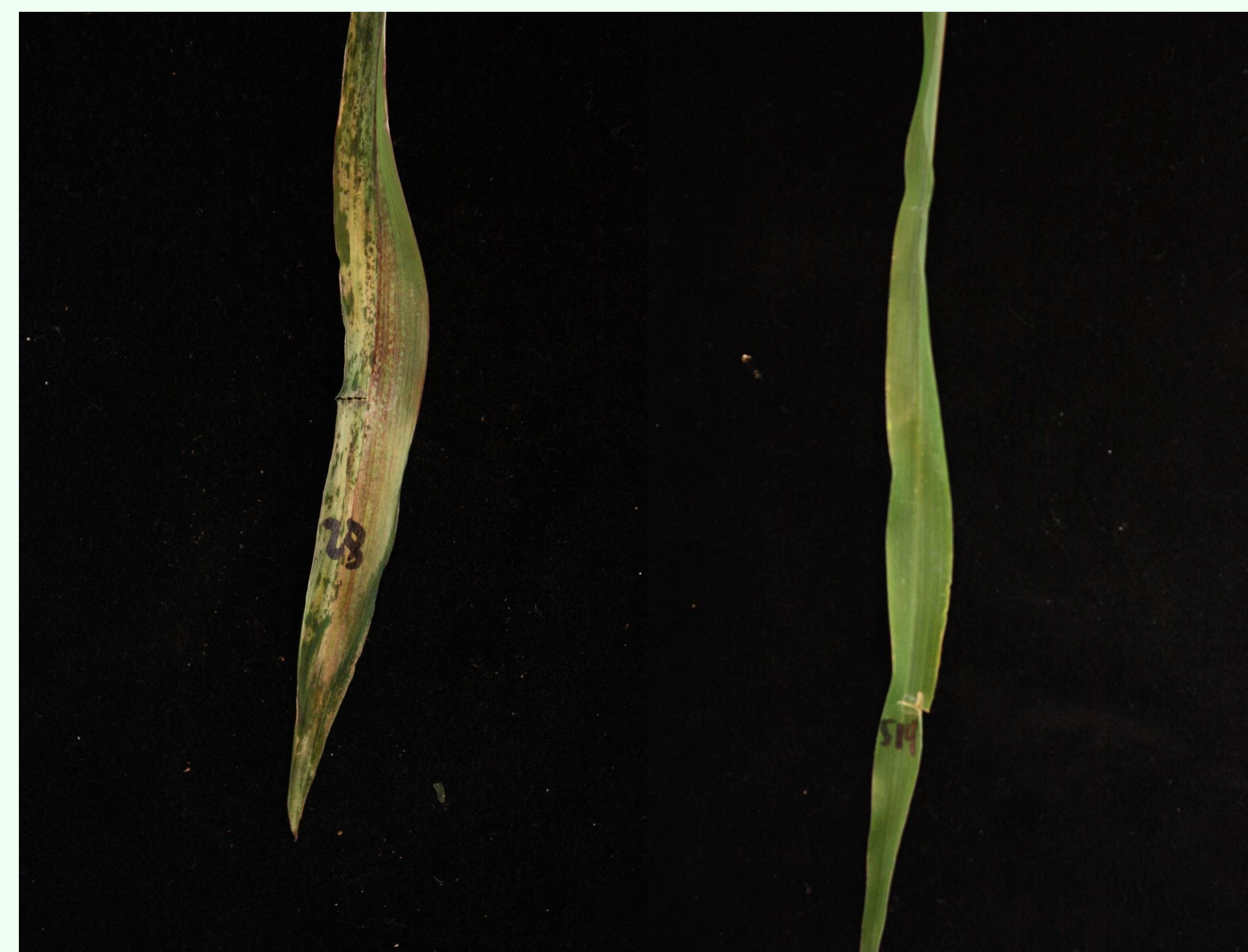
Abstract

Clavibacter michiganensis subsp. *Nebraskensis* (*Cmn*) is a pathogen responsible for Goss's Wilt in maize in the high plains. Strains of *Clavibacter michiganensis* subsp. *Nebraskensis* that are virulent and non-virulent in maize have been isolated, but the mechanism of this differentiation is not understood. Investigation of the genetic differences between virulent and non-virulent strains is providing an explanation as to how *Cmn* causes disease. *Cmn* mutants with reduced or removed virulence have been created using Tn5 transposon transformation to randomly knockout virulence factors. Potential effectors have also been identified in a genomic "virulence island" region by using bioinformatics.



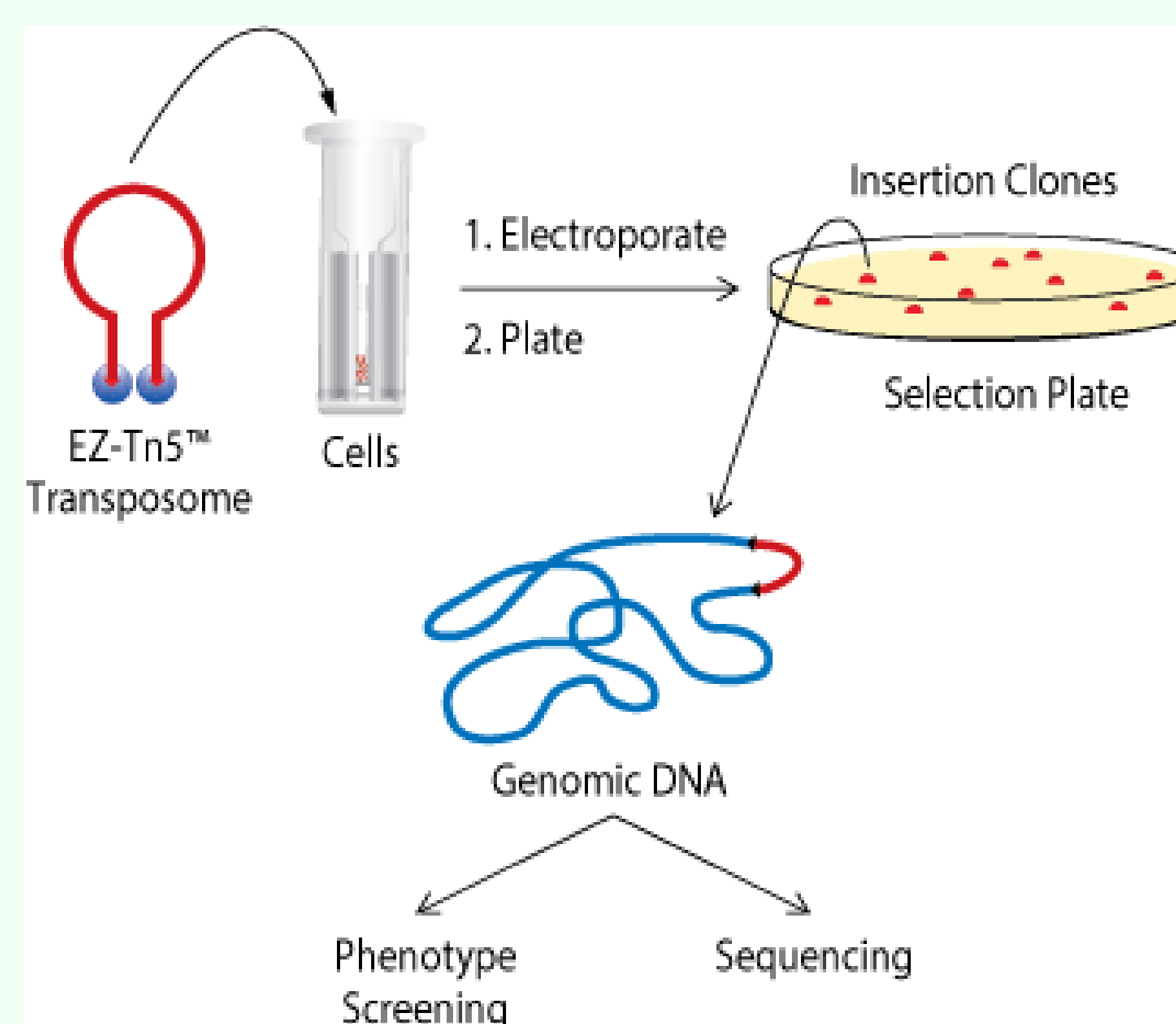
Goss' Wilt Symptoms

Goss's Wilt is a vascular pathogen that causes tissue necrosis parallel to leaf veins, with characteristic spots of bacterial exudate. Yield loss due to Goss's Wilt can reach 50% in severely infected fields. Goss's Wilt is historically a Nebraska pathogen but the range of disease increases during hot, dry years.



Inoculated corn symptom comparison

The severity of Goss's Wilt symptoms was recorded in all inoculated plants. Transformed strains that caused significantly reduced or absent symptoms were flagged for analysis and further testing.



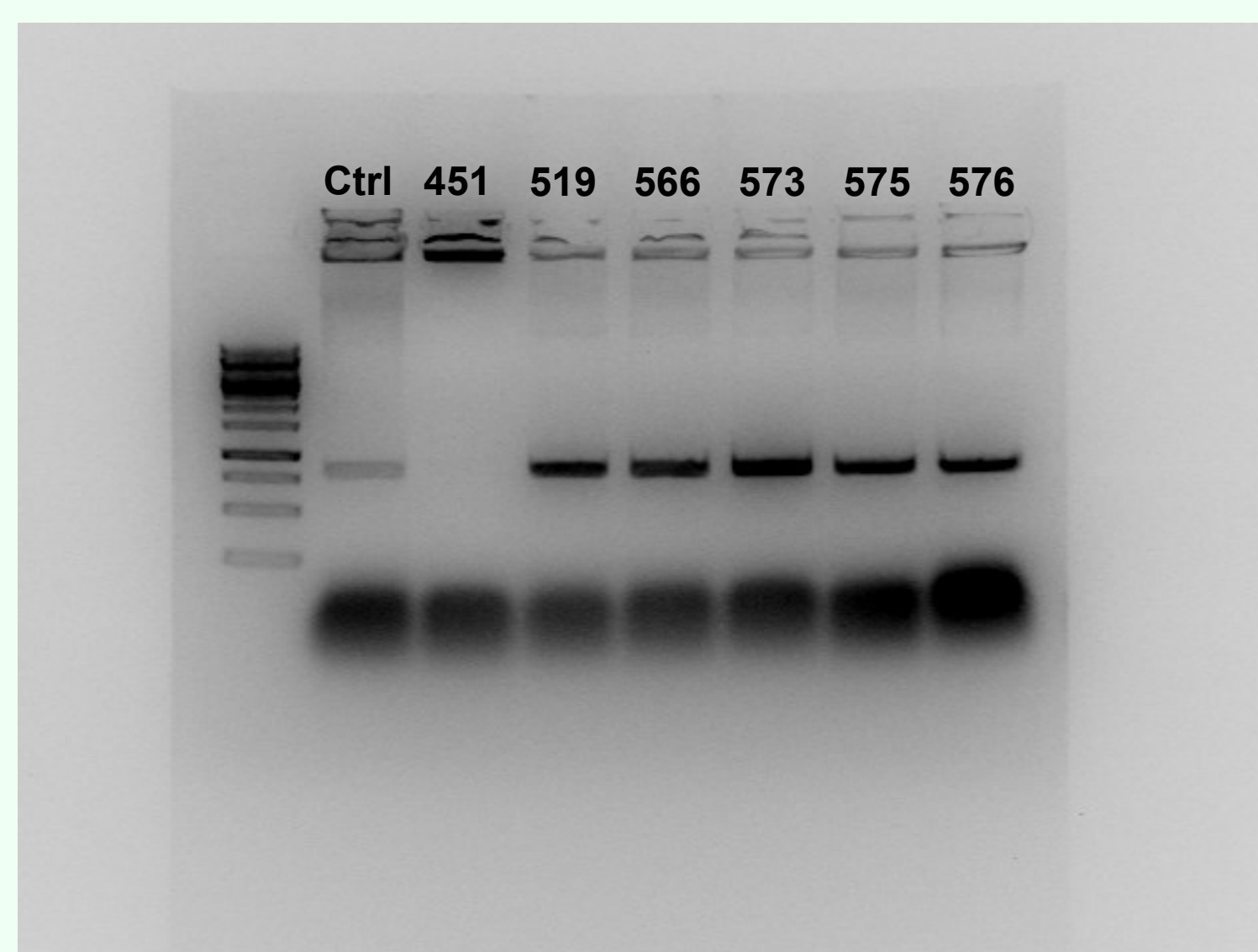
Tn5 Transposome Random Mutagenesis

By transforming *Cmn* with a transposon carrying a selectable antibiotic-resistance gene, over a thousand random mutants of *Cmn* were created. The Tn5 transposon inserts randomly into the genome, breaking any gene in which it lands.

Strain	Virulence	Date Inoculated	Mutant Type	Method of Inoculation	Type of Corn	Location
511	+	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
512	+	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
513	+	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
514	+	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
515	+	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
516	+	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
517	+	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
518	+	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
519	-	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC

Clavibacter Virulence Data

Virulence readings on over 1000 randomly generated mutants were taken and recorded. Potentially avirulent strains are shown in green to show reduced or absent virulence in the plant.



Strain-Specific Colony PCR

By including strain-specific primers in the PCR reaction, potentially avirulent strains were either identified as arising from the original transformed strain, or identified as a contaminant.

Strain	Virulence	Date Inoculated	Mutant Type	Method of Inoculation	Type of Corn	Location
519	-	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
562	-	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
573	-	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
575	-	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
576	-	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
586	-	2/12/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
623	-	2/19/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
628	-	2/19/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
658	-	2/19/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
681	-	2/19/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
720	-	2/26/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC
721	-	2/26/16	EzTn5 Mutant (2)	Nick-Dab	Heirloom GB	GC

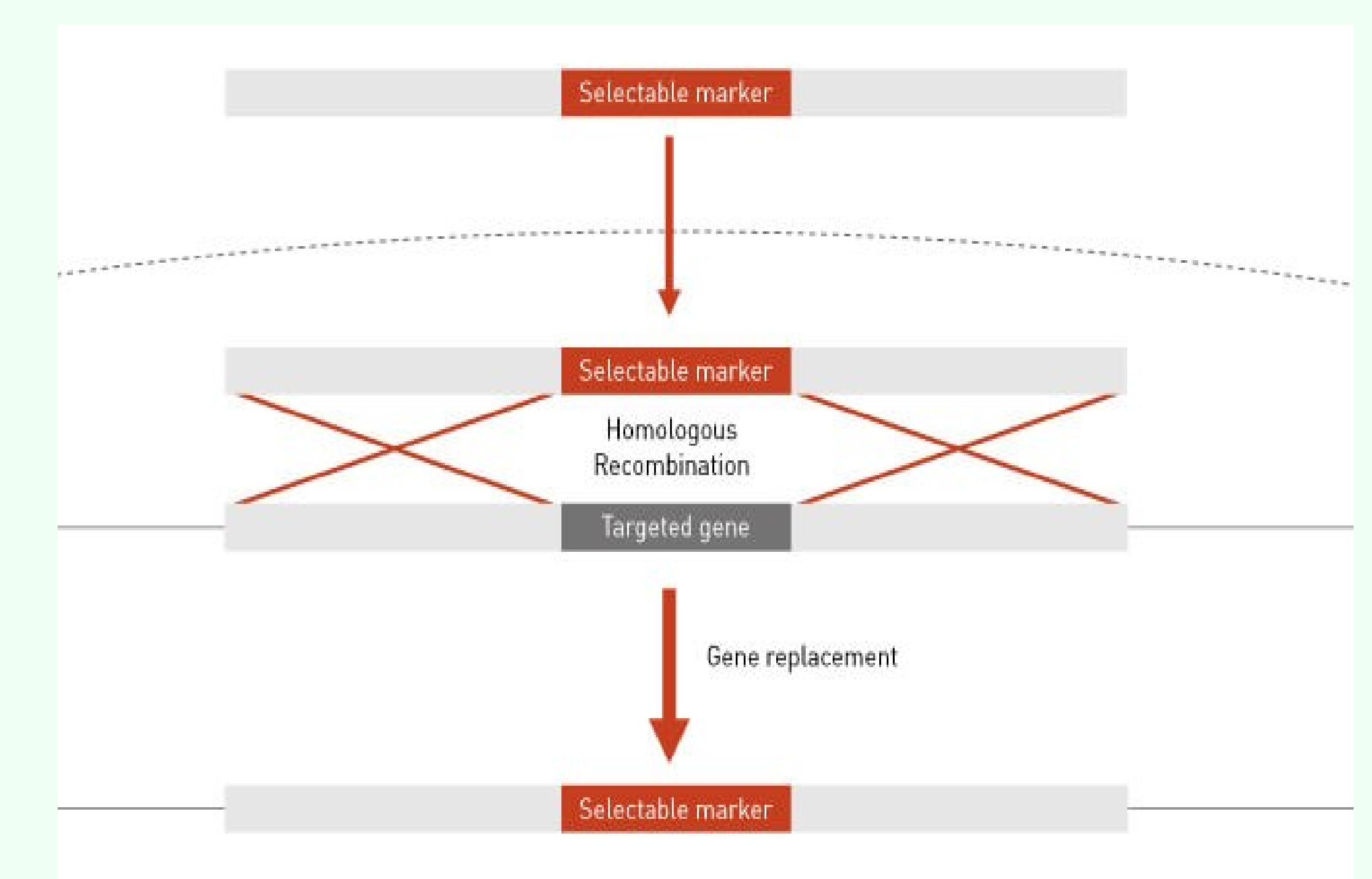
Avirulent Mutants

Out of almost 1200 Tn5 mutants, 12 showed no virulences on maize

Pro. ID	Gene Name	Character	Size(bp)	GC%	GC Diff. (%)
PROKKA_00090	hypothetical protein	unknown	93	73.1	0.1%
PROKKA_00133	hypothetical protein	unknown	210	79	6.0%
PROKKA_00432	Phage_integrase	Phage_integrase	1116	63.9	-9.1%
PROKKA_00434	hypothetical protein	unknown	372	65.05	-8.0%
PROKKA_00435	hypothetical protein	unknown	243	59.25	-13.8%
PROKKA_00441	hypothetical protein	unknown	408	65.2	-7.8%
PROKKA_00442	hypothetical protein	unknown	420	65.48	-7.5%
PROKKA_01195	hypothetical protein	unknown	138	73.2	0.2%
PROKKA_01305	hypothetical protein	unknown	990	51	-22.0%
PROKKA_01416	BsuMI modification methylase subunit YdG0	putative BsuMI modification methylase subunit YdG0	1140	57.63	-15.4%
PROKKA_01495	hypothetical protein	unknown	120	79.2	6.2%
PROKKA_01504	hypothetical protein	unknown	183	75.41	2.4%
PROKKA_01554	hypothetical protein	unknown	156	78.2	5.2%
PROKKA_02089	hypothetical protein	unknown	246	73.2	0.2%
PROKKA_02139	hypothetical protein	unknown	117	75.21	2.2%
PROKKA_02246	hypothetical protein	unknown	312	81.93	8.9%
PROKKA_02310	hypothetical protein	unknown	135	77.8	4.8%
PROKKA_02646	hypothetical protein	unknown	294	65	-8.0%
PROKKA_02648	hypothetical protein	unknown	174	67.8	-5.2%
PROKKA_02652	hypothetical protein	unknown	246	65.9	-7.1%
PROKKA_02653	hypothetical protein	unknown	402	68.7	-4.3%
PROKKA_02654	hypothetical protein	unknown	513	67.3	-5.7%
PROKKA_02655	hypothetical protein	unknown	180	65	-8.0%
PROKKA_02681	hypothetical protein	unknown	99	57.6	-15.4%

Reverse Genetics Approach

By comparing the sequence of the virulent strain of *Cmn* to other, avirulent strains, candidate genes for potential effectors were identified. The genes in green are located clustered together with a gene for a Phage integrase protein, suggesting a region of virulence genes (a "virulence island"), responsible for the acquisition of pathogenicity and Goss's Wilt symptoms.



Gene Knockout using Homologous Recombination

Using Homologous Recombination the virulence region described above and all the genes contained within will be knocked out all at once. A specially-prepared version of DNA with identical sequences from up- and down-stream of the virulence region will bind to the genomic DNA and replace the region with a selectable antibiotic marker

Acknowledgements

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Laboratory Setup

Plants were inoculated by dabbing bacterial solution on a leaf cut. They were then kept for 1 week in a growth chamber at 24 degrees C.

