Knockout of CPL-3 gene in tomato using genome editing

Faisal Saeed[1], Ufuk Demirel[1] Allah Bakhsh[2]

[1]Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University, Nigde, Turkey.

[2]Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan.

*Correspondence Email: abthebest@gmail.com. faissalsaeed@hotmail.com.

Abstract

- Tomato (Solanum lycopersicum L.) is one of the important vegetables in the world due to its commercial and dietary value and its widespread production.
- All over reduction in crop production worldwide 20-40 % due to biotic stresses.
- Several genes of tomato plants have been identified related to a positive and negative regulator of the immunity gene.
- \In this study, we find out CPL-3 gene in tomato.
- Further, we are trying to knock out the CPL-3 gene using Crispr-Cas9 that is involved in downregulating immune responsive genes of crop plants under biotic stress conditions.

Introduction

- Turkey is the fourth-largest producer of tomatoes.
- Different bacterial diseases.
  - ❖ Bacterial Speck.
  - ❖ Bacterial Canker.
  - ❖ Bacterial Spot.
  - ❖ Bacterial Wilt.
- Many bacterial pathogens of plants and animals disarm and remodel host cells by injecting large repertoires of effectors.
- These bacterial pathogens released some proteins which effect metabolism of plants and suppress immunity of plant.
- They start necrosis of infected leaf or wilting process start and ultimately death of plant occurs.
- Once plant effected by these bacterial pathogens it's impossible to take effective measures against these pathogens
- Tomato as a dicot crop would be an idyllic candidate crop plant for the use of CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeat)-Cas9.
- By knocking out CPL-3, tomato plants will continue to express immune-related genes in response to biotic stresses, hence immunity against a pathogen will be achieved.

Methodology

PCR amplification and characterization

↓

TA Cloning of Eluted fragment

↓

Sequencing of Positive Clones

↓

Designing and Cloning of gRNA to vector (Crispr-P. 2.0)

↓

Agrobacterium mediated plant transformation

↓

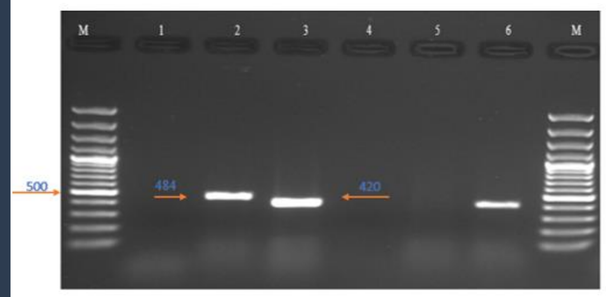
PCR Detection

↓

Screening of mutants

Results

Confirmation of Exon regions of CPL-3.



Lane: M 1 kb gene ruler. Lane:2 and Lane:4 shows Amplified exon regions

Conclusion

- **Cpl-3 gene identified and characterized using different set of primers.**
- **Designed gRNA against exon region of CPL-3 and add Bsa 1 restriction sites.**
- **Cloned gRNA into Crispr vector pKSE-401.**
- **Confirm cloning by restriction digestion analysis.**

Acknowledgements

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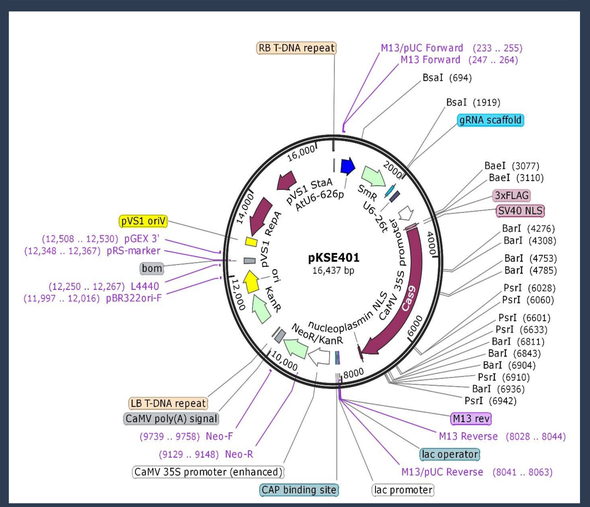
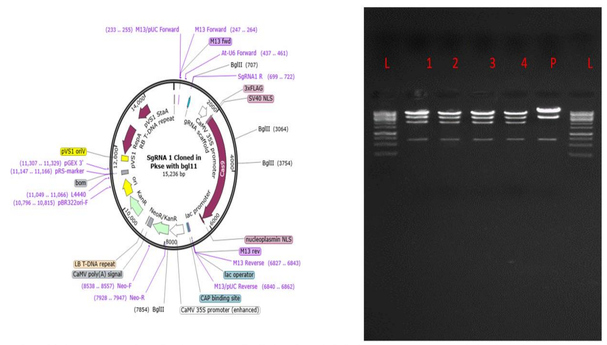
Crispr Vector pKSE-401



Cloning confirmation of gRNA in vector

Selected gRNA having a bgIII restriction site and confirm after ligation using restriction digestion

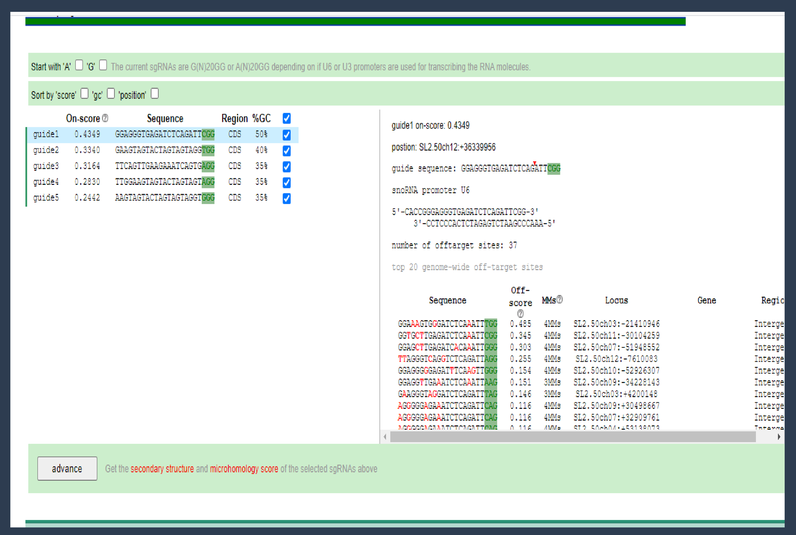


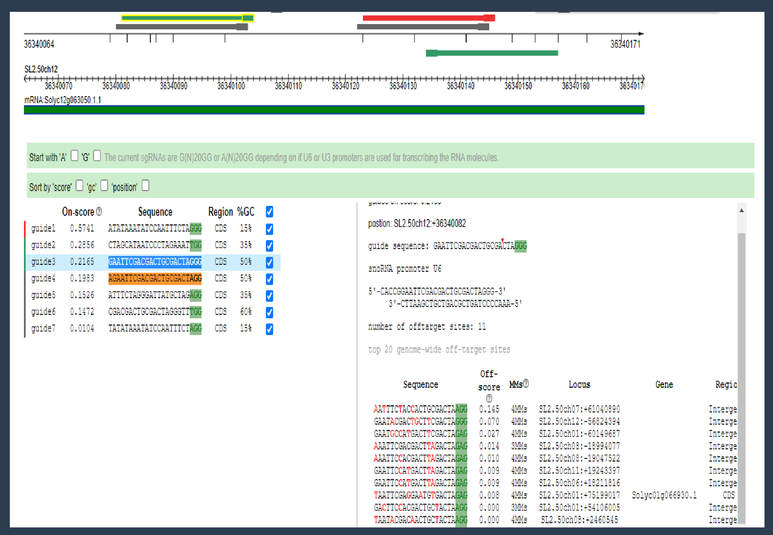
Lane M: 1 kb gene rule. Lane P: without sgrna, BglII sites. Lane 1, 2, 3, 4, cloned fragment restriction (showing one extra fragment)

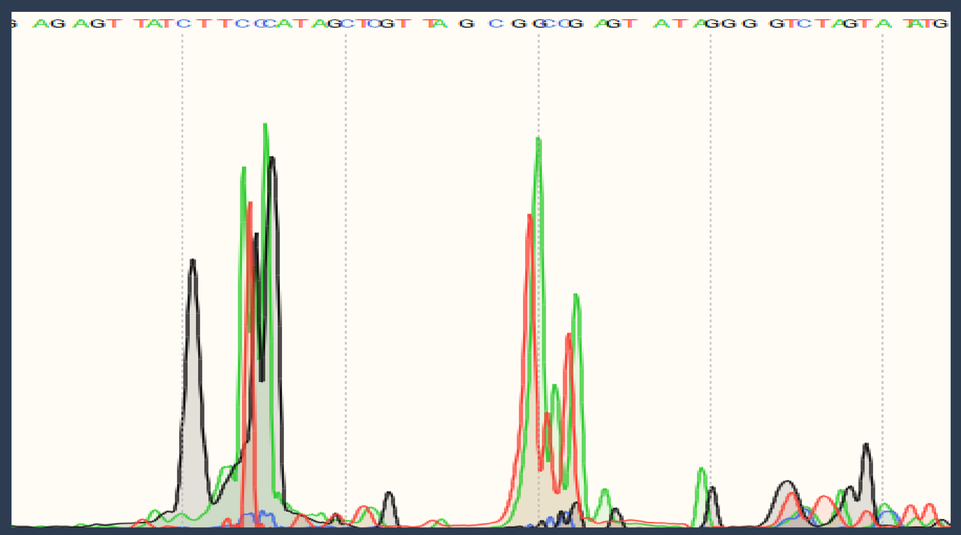
Annealing of gRNA

Reagents for annealing of gRNA

sgRNA forward (100 µM)	1 µL
sgRNA reverse (100 µM)	1 µL
10x T4 DNA ligation buffer	1 µL
T4 PNK	1 µL
ddH20	6 µL
Total	10 µL







Designing of gRNA using Crispr-p. V.2.0

@faissalsaeed