# Modulation of Tomato Fruit Texture by Silencing Cell Wall Structure-Related Genes

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## Introduction

**The challenge is to breed tomato varieties that have a reasonable postharvest shelf life while maintaining excellent eating quality. Shelf life depends on the extent of softening during the ripening process. Cell wall remodelling including solubilisation and depolymerisation of pectin polysaccharides, and depolymerisation of hemicelluloses normally occurs during the process of tomato fruit softening and is a consequence of the combined action of multiple gene products. More than 50 cell wall modifying genes are expressed during fruit development and ripening (Tomato Genome Consortium, 2012). In this project we are focusing on the six most highly ripening-related cell wall genes to test if knocking them out by DNA editing can influence the progress of tomato fruit softening and extend shelf life.**

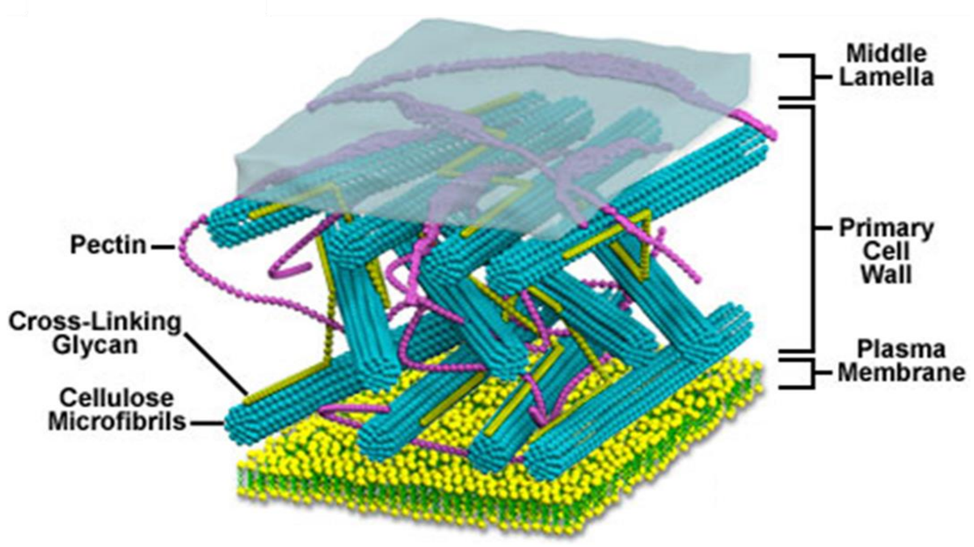



**Figure 1 Cartoon of the structure of plant cell wall**

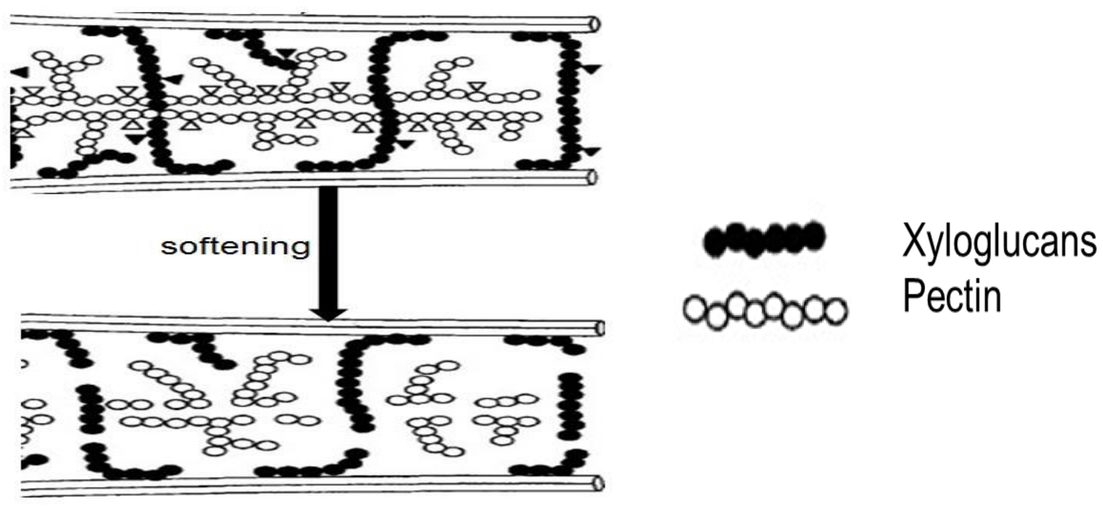
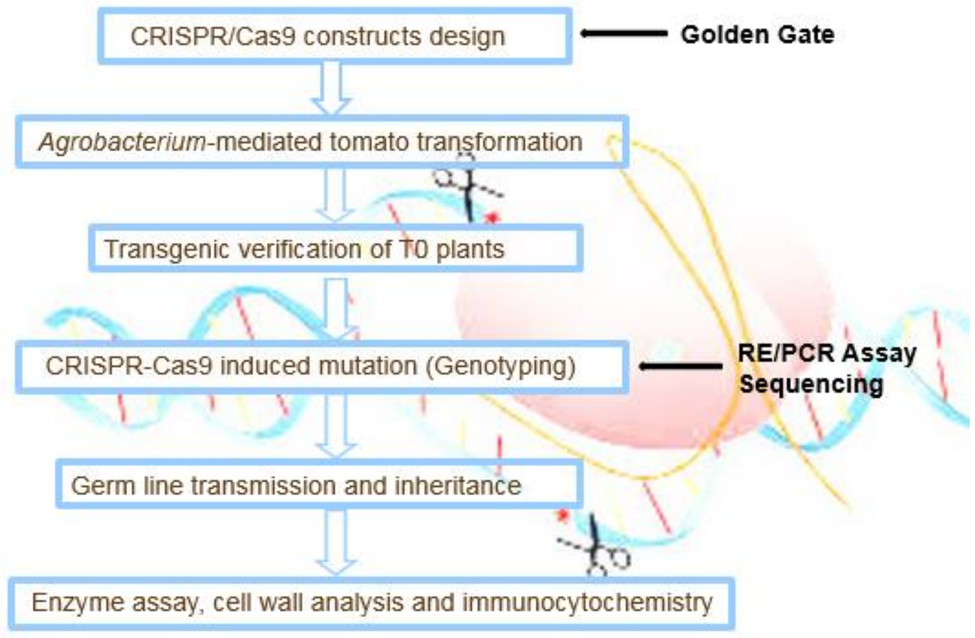



**Figure 2 Schematic representation of remodelling of the cell wall during fruit softening**

## Methods




## Results

**Identification of sgRNA:Cas9-induced mutations in T0 transgenic tomato plants using RE/PCR assay and sequencing**

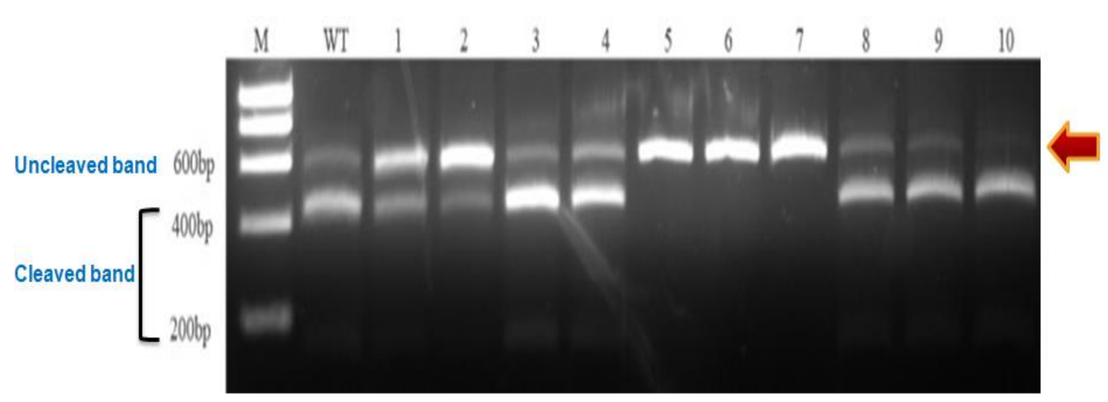



**Figure 3 Schematic of the PCR/RE assay used to detect indels**
Genomic DNA from sgRNA- and Cas9-transformed transgenic plants was amplified by PCR. The amplicons were then digested with restriction enzymes that recognize the wild-type target sequences. Mutations introduced by CRISPR were resistant to restriction enzyme digestion because of the loss of the restriction sites, and they resulted in an uncleaved band (indicated by red arrowhead) in agarose gels. WT: Control digested; 1-10: Transgenic plants

**Table 1 Identification of CRISPR/Cas9-induced target mutations in T0 generations by sequencing.**

Homozygous, biallelic and heterozygous mutations were all detected.

| Zygosity | Mutation type and length (bp) |
|---|---|
| Homozygote | d3 |
| | i1 |
| Bi-allele | d1d4 |
| | d1d2 |
| | d4i1 |
| | d6i1 |
| | d7i1 |
| Heterozygote | wt:d4 |

**CRISPR mutations in tomato pectate lyase (PL) resulted in reduced PL mRNA levels, removed ripening-related PL activity and resulted in firmer and longer lasting fruits (published Nature Biotechnology, September 2016).**

## Conclusions and Future Work

**Results indicated that the CRISPR/Cas9 system was efficient in tomato for generating Double Strand Break-induced target gene editing. Germ line transmission and heritability analysis of CRISPR/Cas9-generated mutations indicated that gene mutations could be passed to the next generation. Enzyme activity was significantly reduced in CRISPR transgenic lines compared with the control azygous wild-type line. One of these gene, known as pectate lyase (PL) was found to be responsible for a substantial portion of tomato fruit softening. Immunocytochemistry is underway on a range of transgenic plants with altered cell wall enzyme levels.**

## References

Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*. 485: 635–641.

Uluisik et al.,2016. Genetic improvement of tomato by targeted control of fruit softening. *Nature Biotechnology*. 34(9), doi:10.1038/nbt.3602