# The Establishment of the TILLING Experimental Techniques Based on Capillary Electrophoresis

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## Abstract

The CEL I enzyme that specifically cleaves the mismatch in DNA double strands is an important component of the TILLING experiment platform. Due to the expensive price of commercial CEL I, it was not conducive to large-scale the TILLING experiments. Refered to published literature, the active crude extract of CEL I enzyme was obtained from the celery growning in Guiyang, Guizhou province. In this study, the effective CEL I digestion system was established: The CEL I enzyme effectively cut the mismatch DNA in the 15μL digestion reaction solution including 8μL PCR product, 4.5μL ddH2O, 1.5μL 10×cleavage buffer (pH 7.5, 500mmol/ L KCL, 100mmol / L Tris-Cl, 15mmol / L MgCl2 ) and 1μL the 20 times dilution of CEL I crude extraction after 60-min incubation under 42℃.

## Introduction

Targeting Induced Local Lesions IN Genomes(TILLING) offers a powerful way to create novel mutant alleles at a selected locus. This approach makes it possible to directly identify plants that carry a specific modified gene from the nucleotide sequence data.

As TILLING helps locate an allelic series of induced point mutations, including missense and truncation lesions, it is useful both in organisms, such as tobacco, with sophisticated gene knockout methods, and in organisms lacking practical reverse-genetics tools, where a knockout would be highly desirable. This makes TILLING an attractive strategy for a wide range of applications from basic functional genomic study to practical crop breeding.

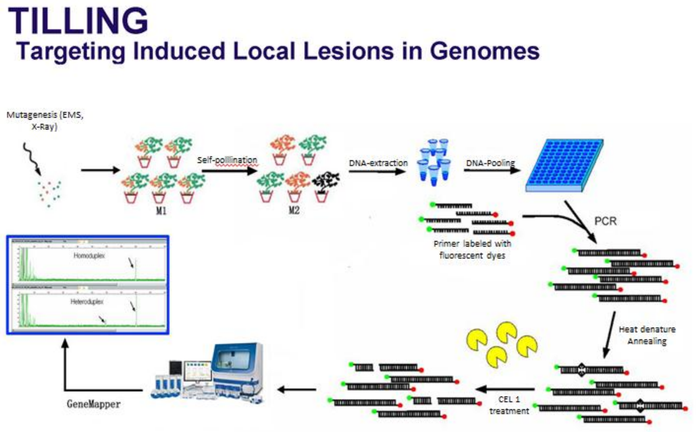


Fig 1. Outline of the development of a tobacco mutant population and the process of mutant screening employing the TILLING approach.

> ZYD

TCCTGTTCCTCAAGTCAAGCTTTGTAGTGAGCAGATATAATGGCTGTTGAAAGGTCCGGAATTGCCAAAGAT
GTTACGGAATTGATTGGTAAAACCCCATTAGTATATCTAAATAAACTTGCGGATGGTTGTGTTGCCCGGGTTG
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AGAAGAATCATTCTATTAGCTTTTGGAGCTGAGTTGGTTCTGA

> BG

TCCTGTTCCTCAAGTCAAGCTTTGTAGTGAGCAGATATAATGGCTGTTGAAAGGTCCGGAATTGCCAAAGAT
GTTACGGAATTGATTGGTAAAACCCCATTAGTATATCTAAATAAACTTGCGGATGGTTGTGTTGCCCGGGTTG
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CAGAAGAGAAGGGACTTATCACACCTGGAAAGAGTGTCCTCATTGAGCCAACAAGTGGTAATACTGGCATT
GGATTAGCCTTCATGGCAGCAGCCAGGGGTTACAAGCTCATAATTACAATGCCTGCTTCTATGAGTCTTGAG
AGAAGAATCATTCTATTAGCTTTTGGAGCTGAGTTGGTTCTGA

Fig 2. The double-stranded DNA containing base mismatches

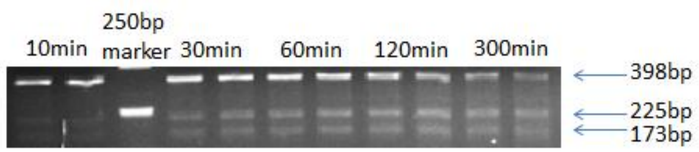


Fig 3. The identification of CEL I activity and the effect of incubation time on digestion results

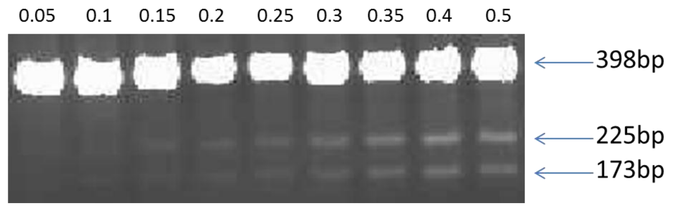


Fig 4: Effect on mismatch cleavage of different concentration of CEL I crude extraction

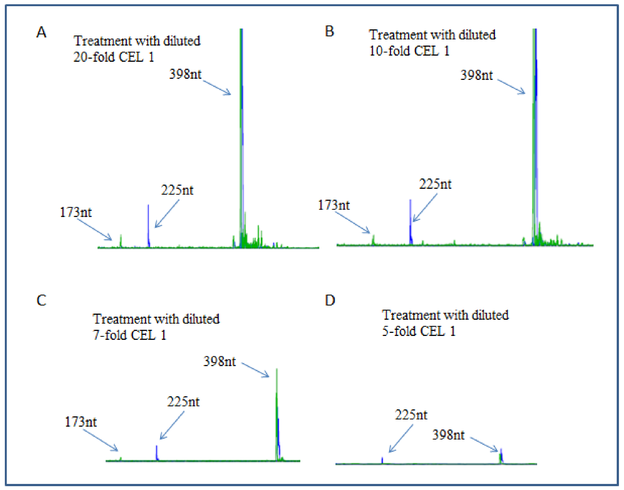


Fig 5: Effect on mismatch cleavage of different concentration of CEL I crude extraction by ABI 3500 genetic analyzer

## Conclusions

The CEL I enzyme effectively cut the mismatch DNA in the 15μL digestion reaction solution including 8μL PCR product, 4.5μL dd$H_2O$, 1.5μL 10×cleavage buffer (pH 7.5, 500mmol/ L KCL, 100mmol / L Tris-Cl, 15mmol / L MgCl2 ) and 1μL the 20-fold dilution of CEL I crude extraction after 60-min incubation under 42℃.