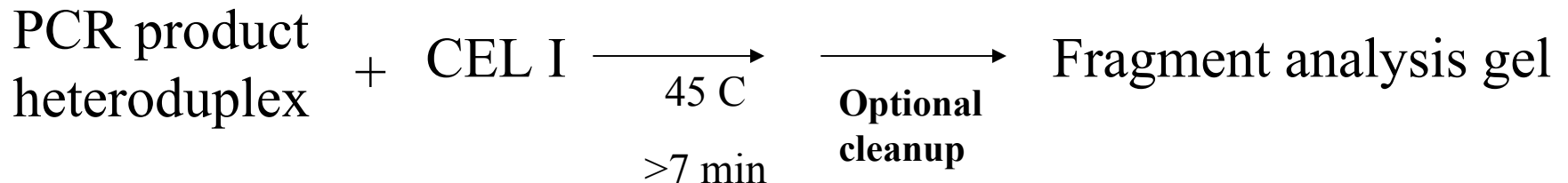




FOX CHASE  
CANCER CENTER

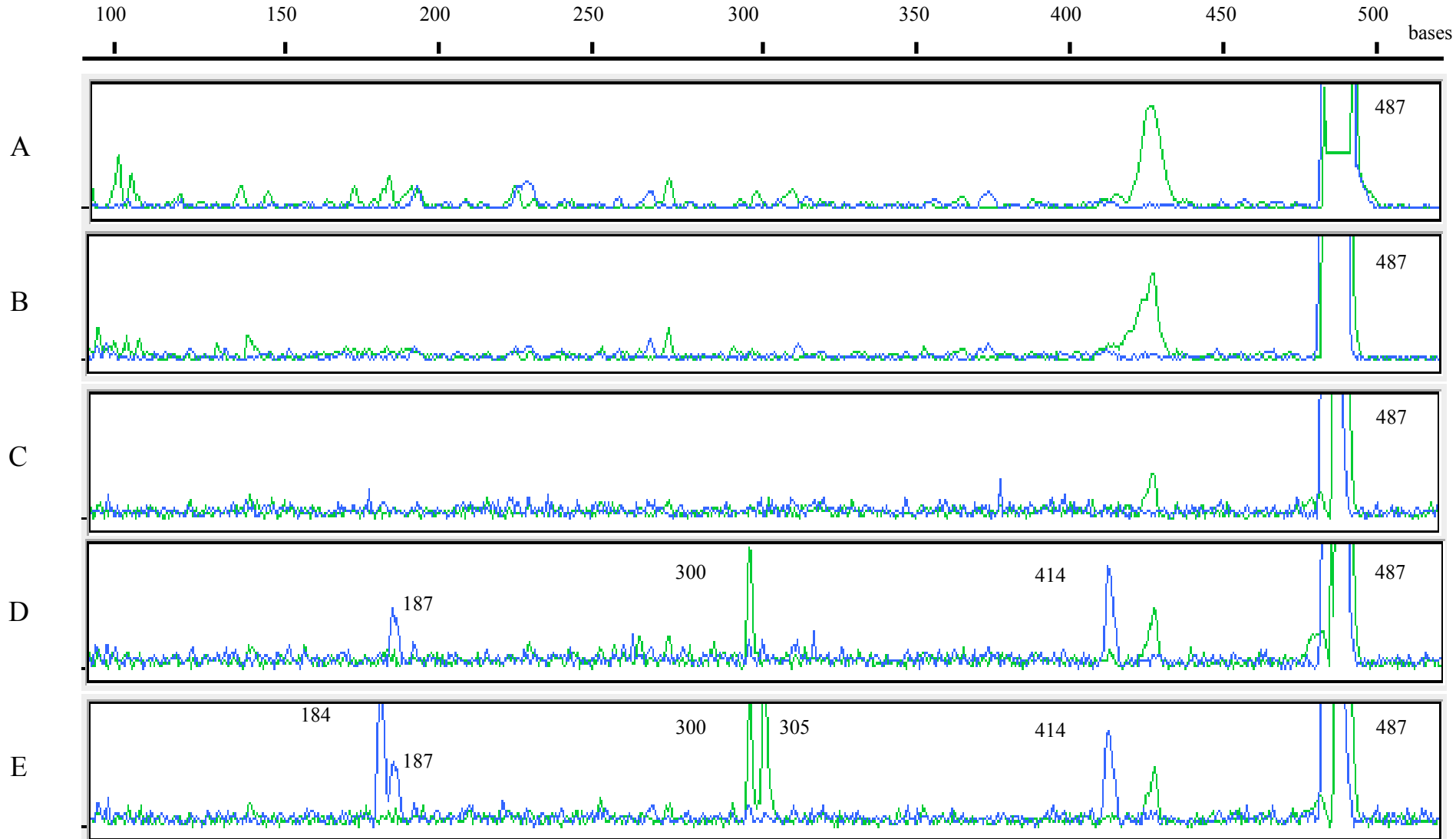
# CEL I nuclease for mutation detection

- CEL I, from celery, is representative of a family of plant nucleases that cut at DNA helix distortions, probably for programmed cell death.



# CEL I mutation detection in BRCA1 gene of breast cancer patients

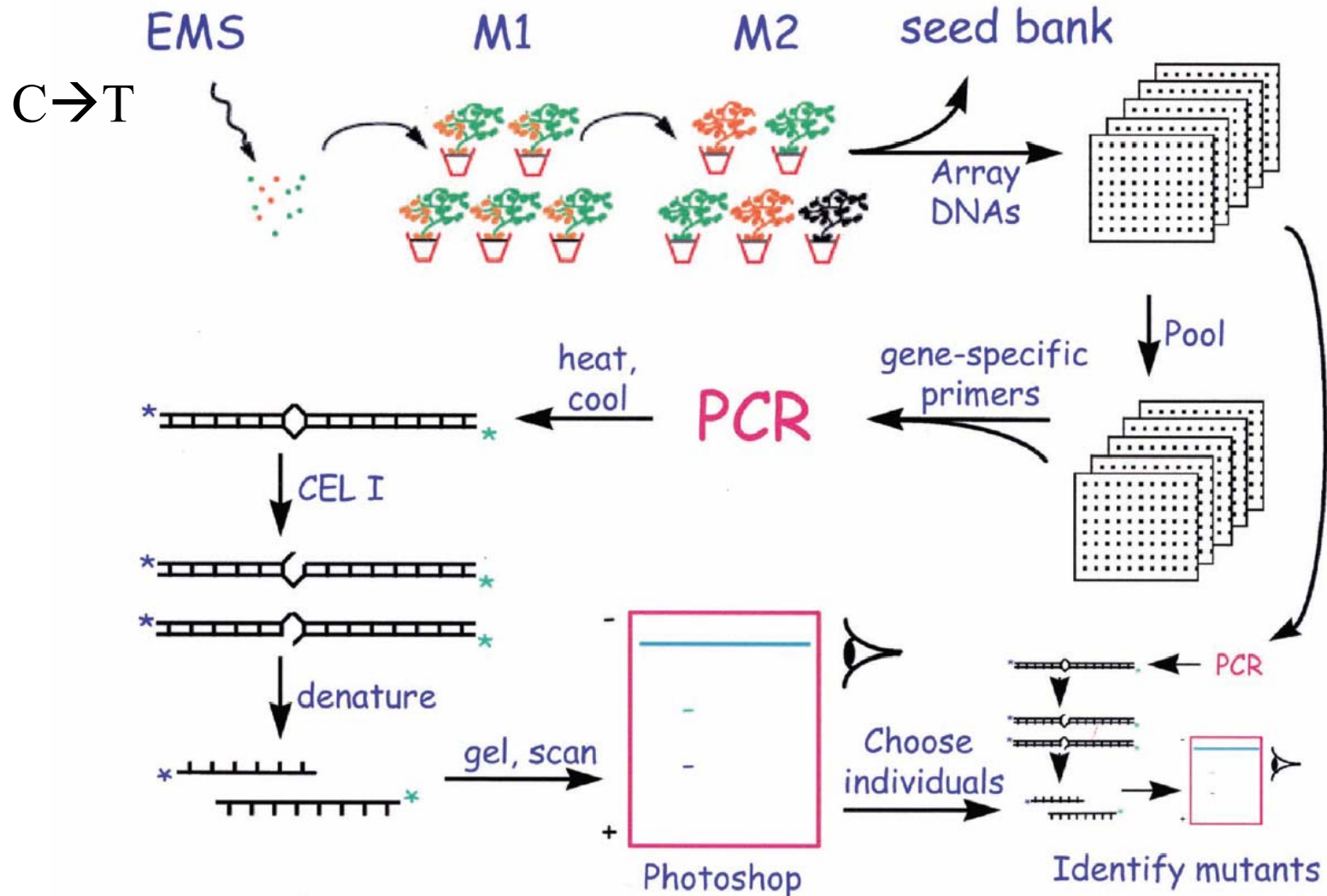
ABI 377 slab gel sequencer Genescan analysis. Three polymorphisms in in E and two in D, in a 500 basepair region are detected in patients, versus controls in A-C. Blue top strand, green bottom strand.



# CEL I is a useful mutation detection tool:

## Example in plant genetics: TILLING

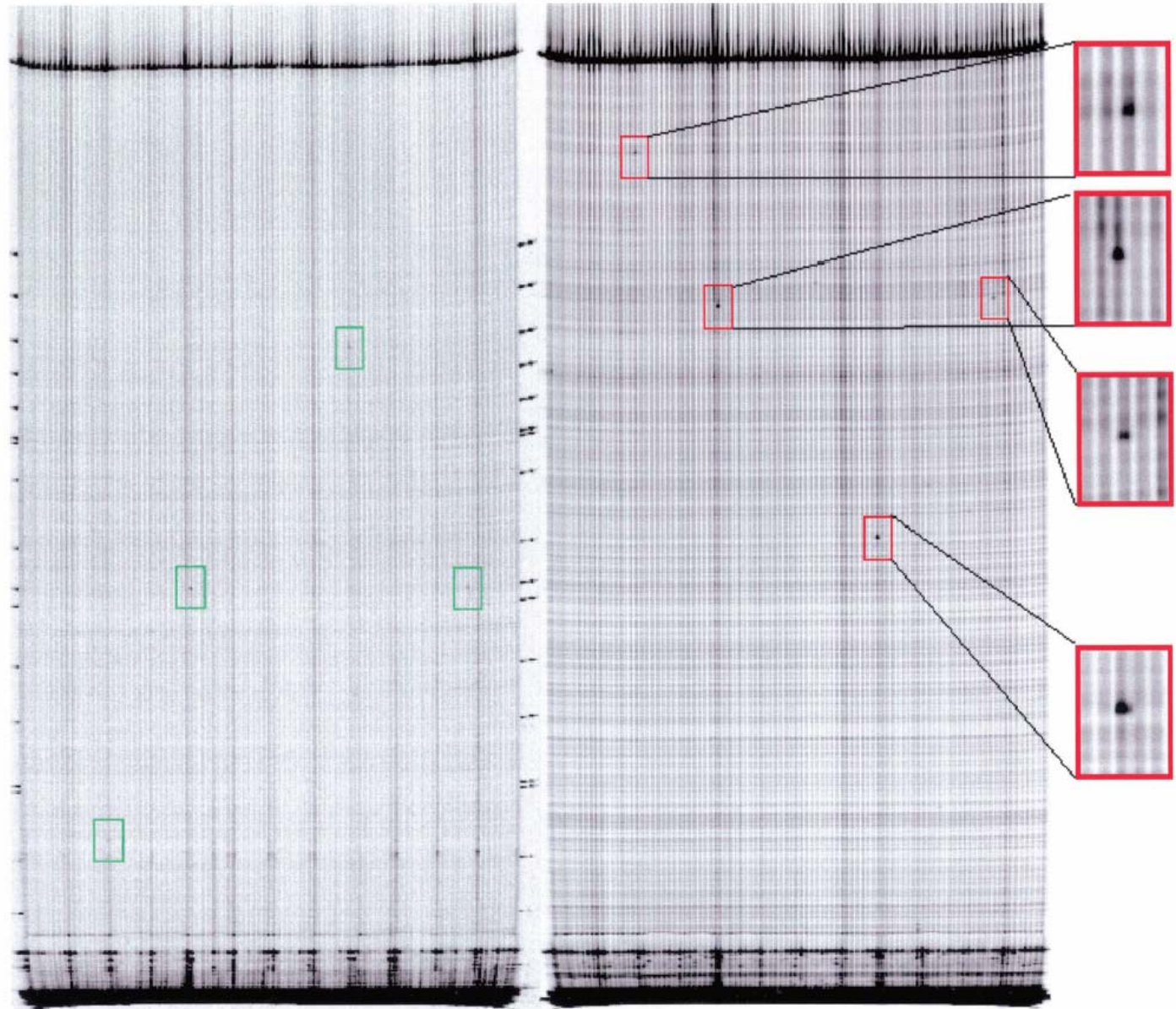
### (Targeting Induced Local Lesions IN Genomes)



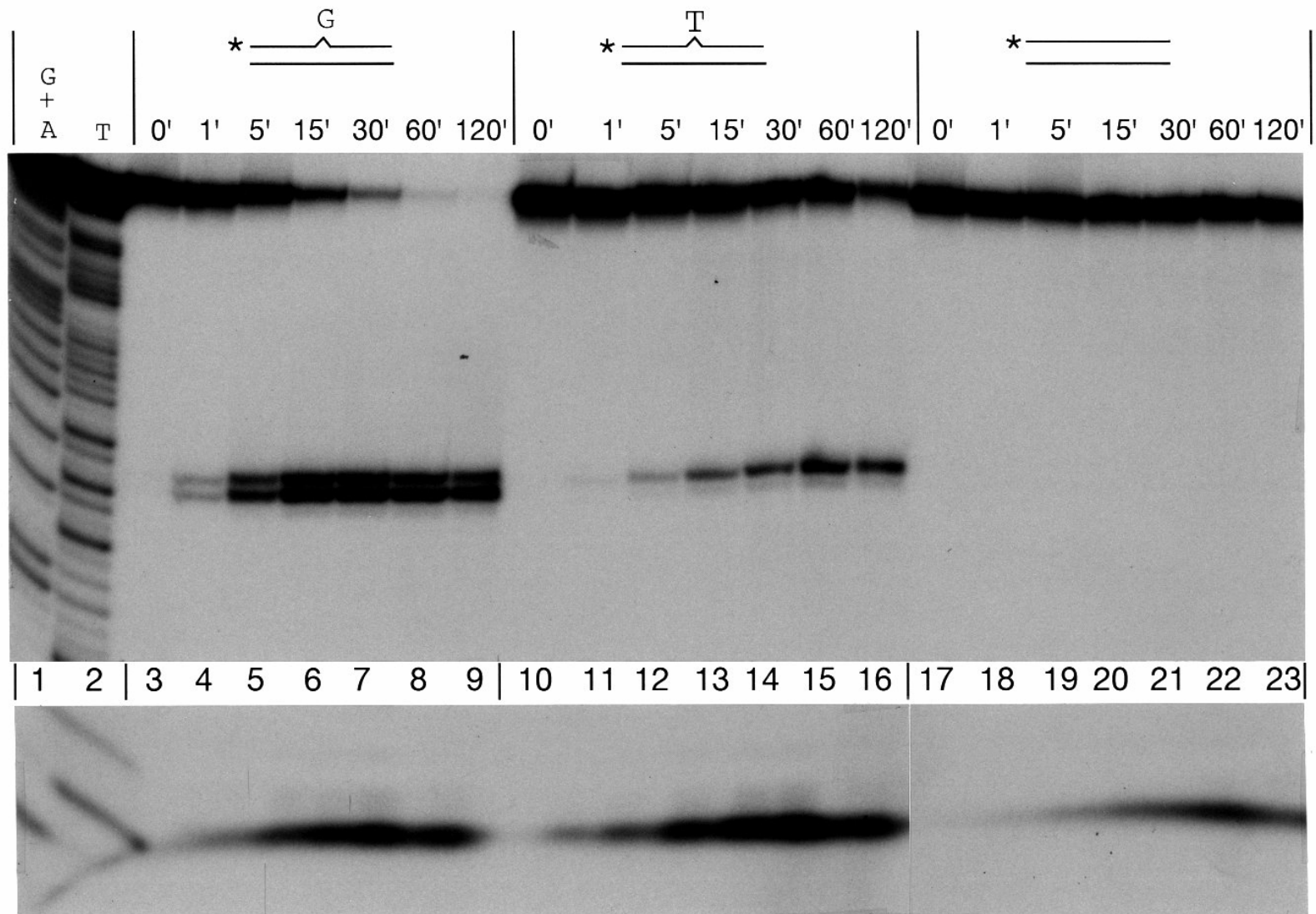
# Tilling

## Screening for EMS

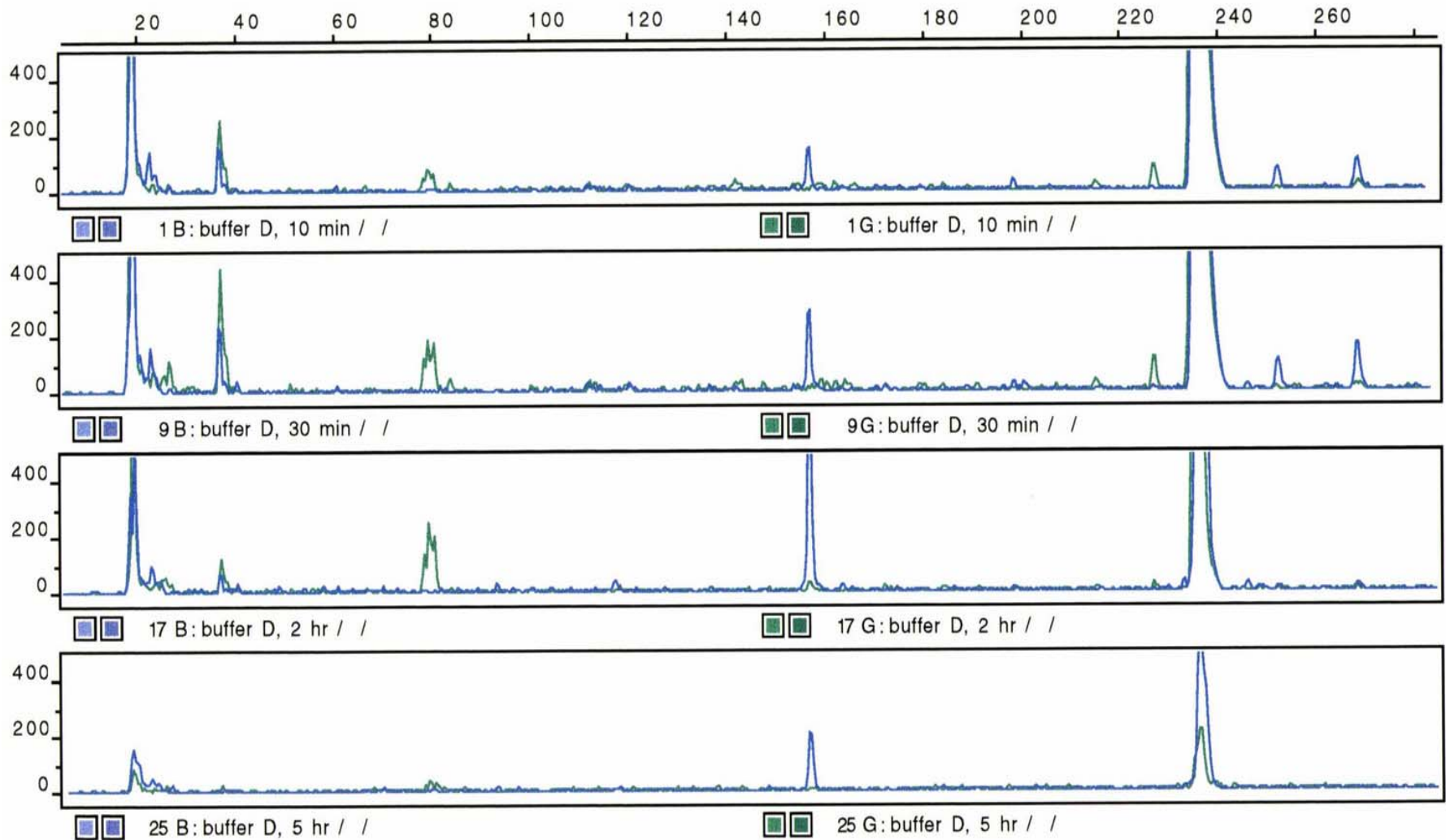
**C → T mutants** This is a 96 lane Li-Cor DNA sequencer examining the top strand (left) and bottom strand (right) of a PCR product heteroduplex by CEL I mutation detection. Each lane has the DNA of five plants, total 480 plants. Signals on left have corresponding signals on right. One technician in one afternoon can use the CEL I mutation detection method to find five mutants in a gene of interest in *Arabidopsis*.



# 5' exonuclease activity of CEL I



37°C



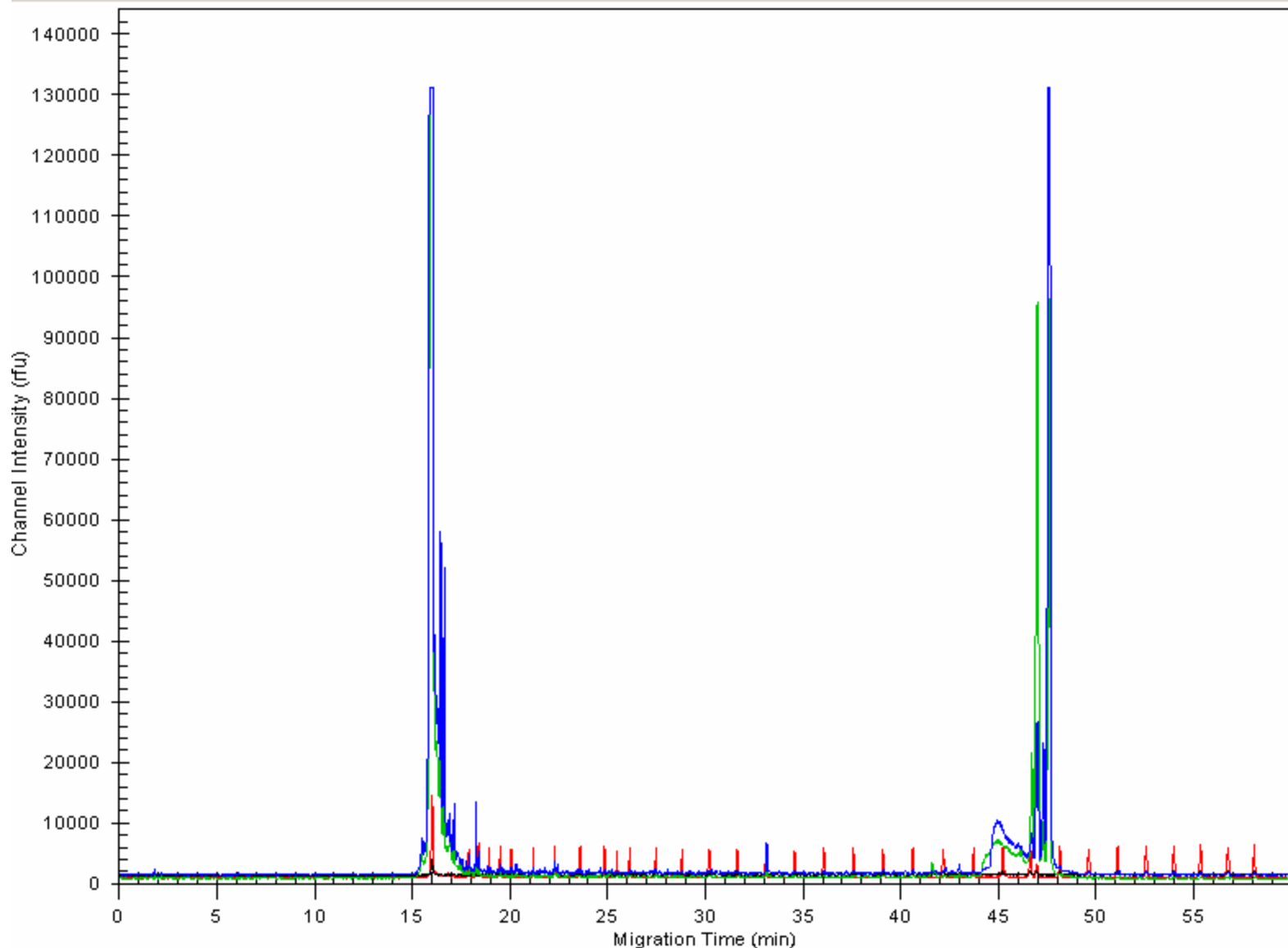
Time course of CEL I converting PCR product to the signals of mutation detection to degraded products

Genescan fluorogram from ABI 377 DNA sequencer



## 2 SNPs of T to C base substitutions

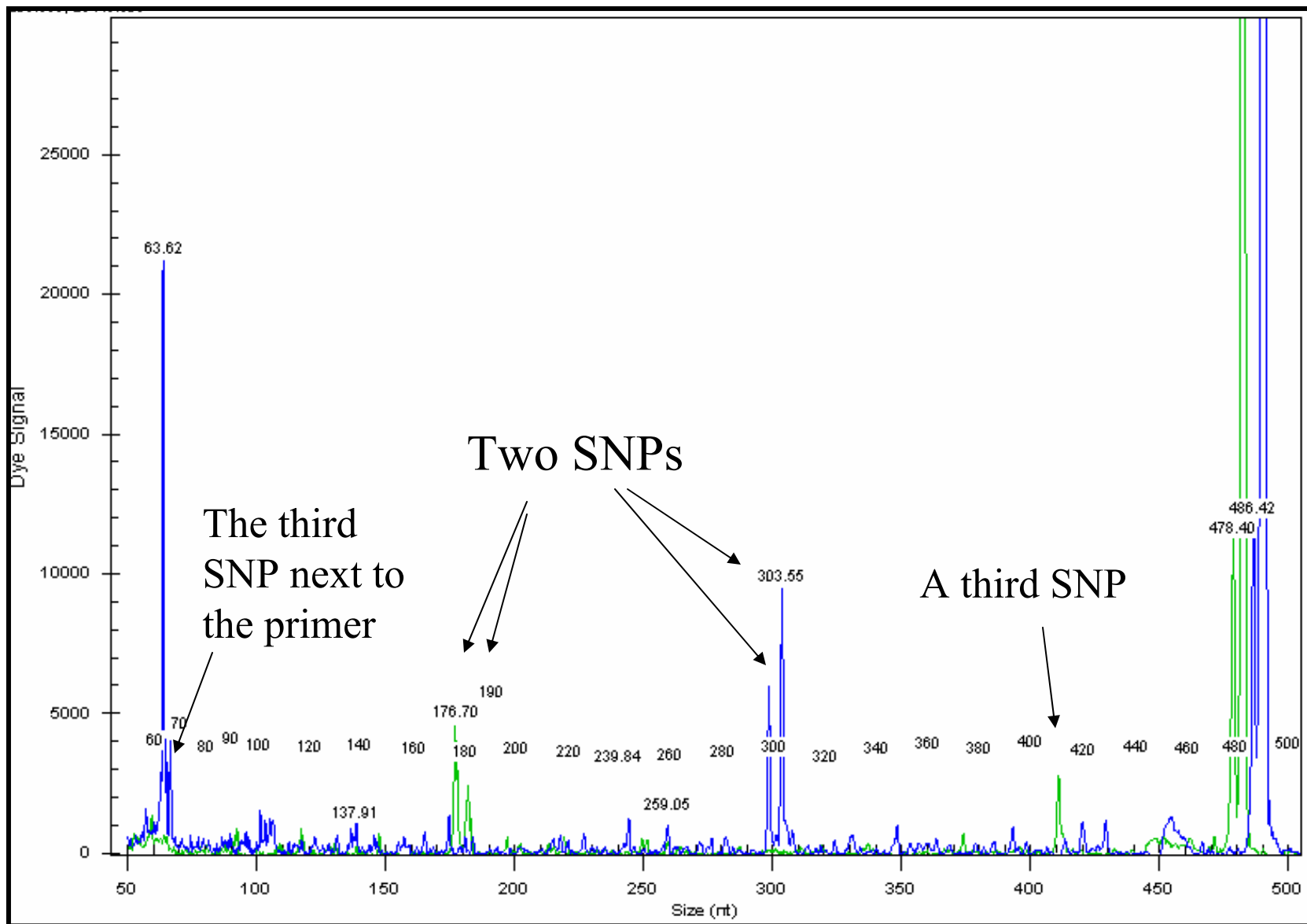
Cy5 and Cy5.5 primers were used. 2.5 nL\* of CEL I for 5 min at 45 C.



\* Transgenomic CEL nuclease concentration is different

# Close-up of CEL I cut at 3 SNPs

## Two T to C and one G to A base substitutions

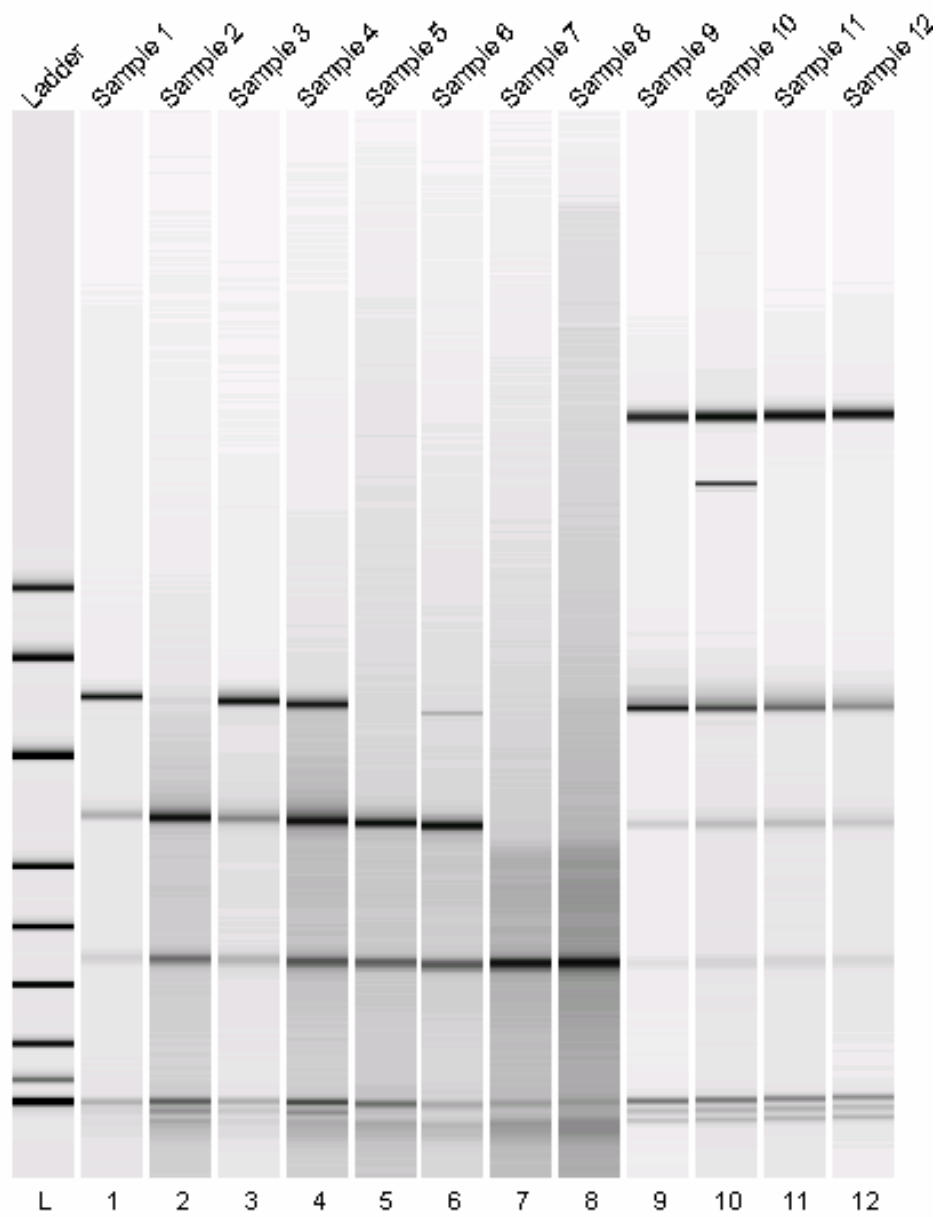


2:1 cold to  
labeled  
primers,  
30' 2.5nL  
CEL I

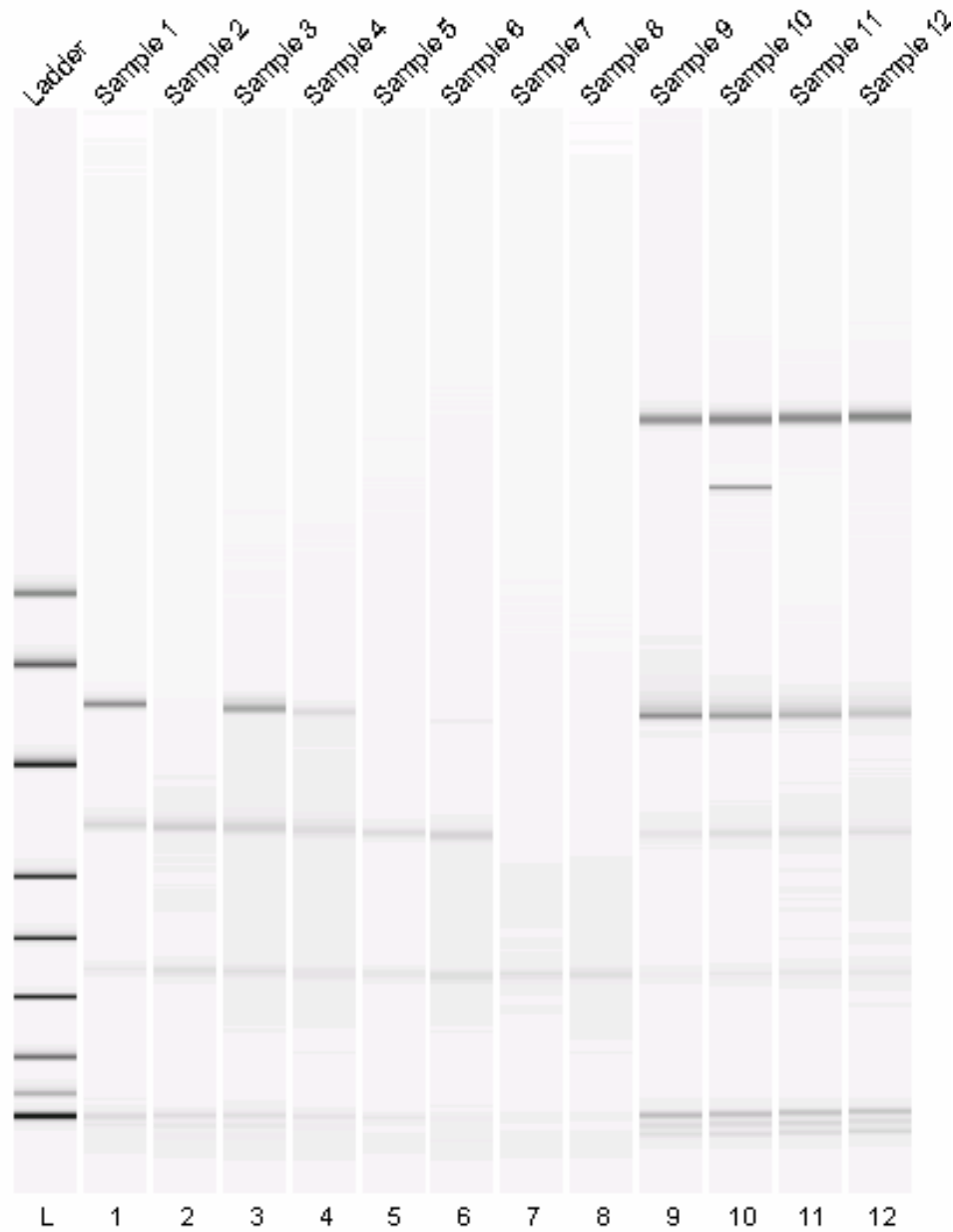


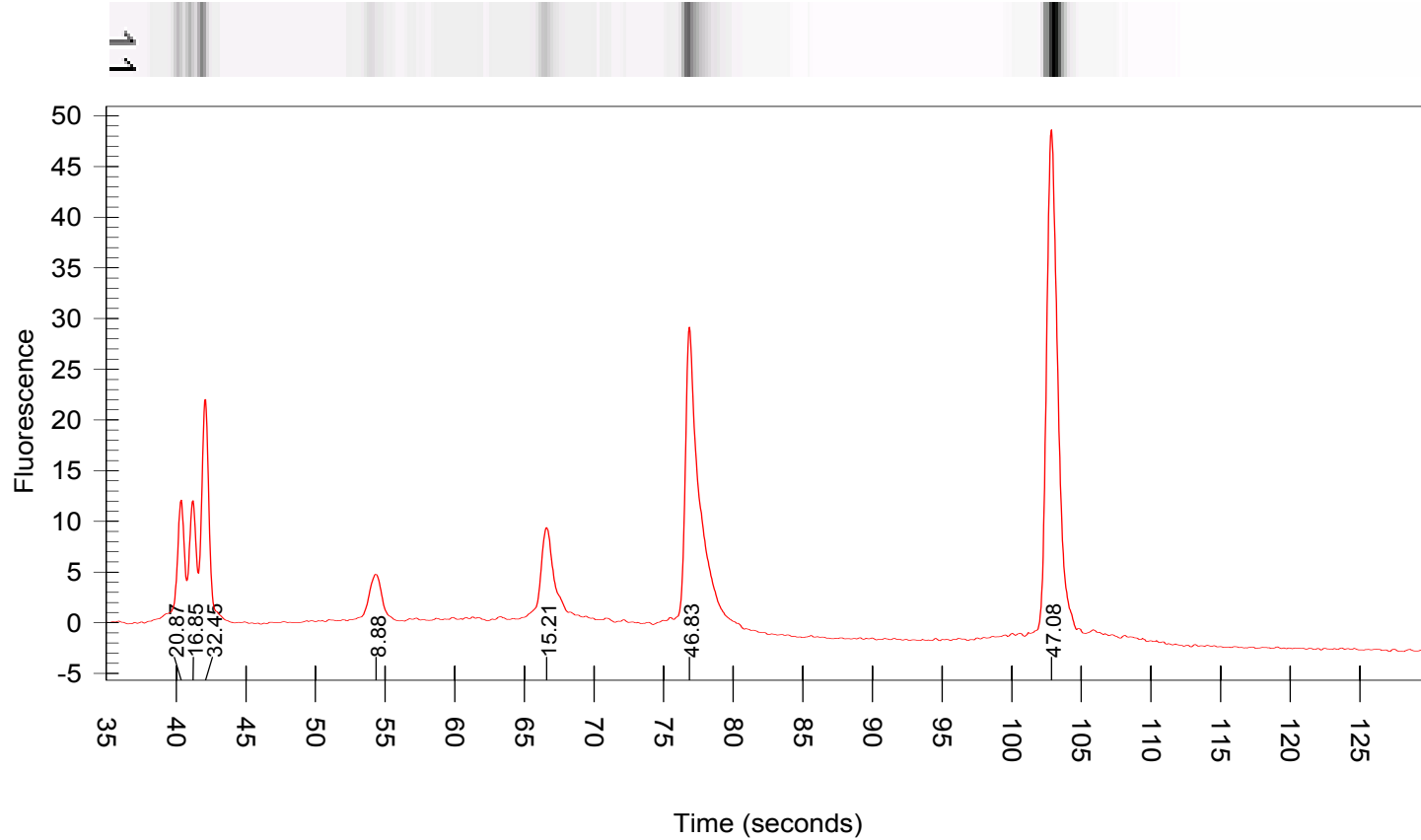
FOX CHASE  
CANCER CENTER





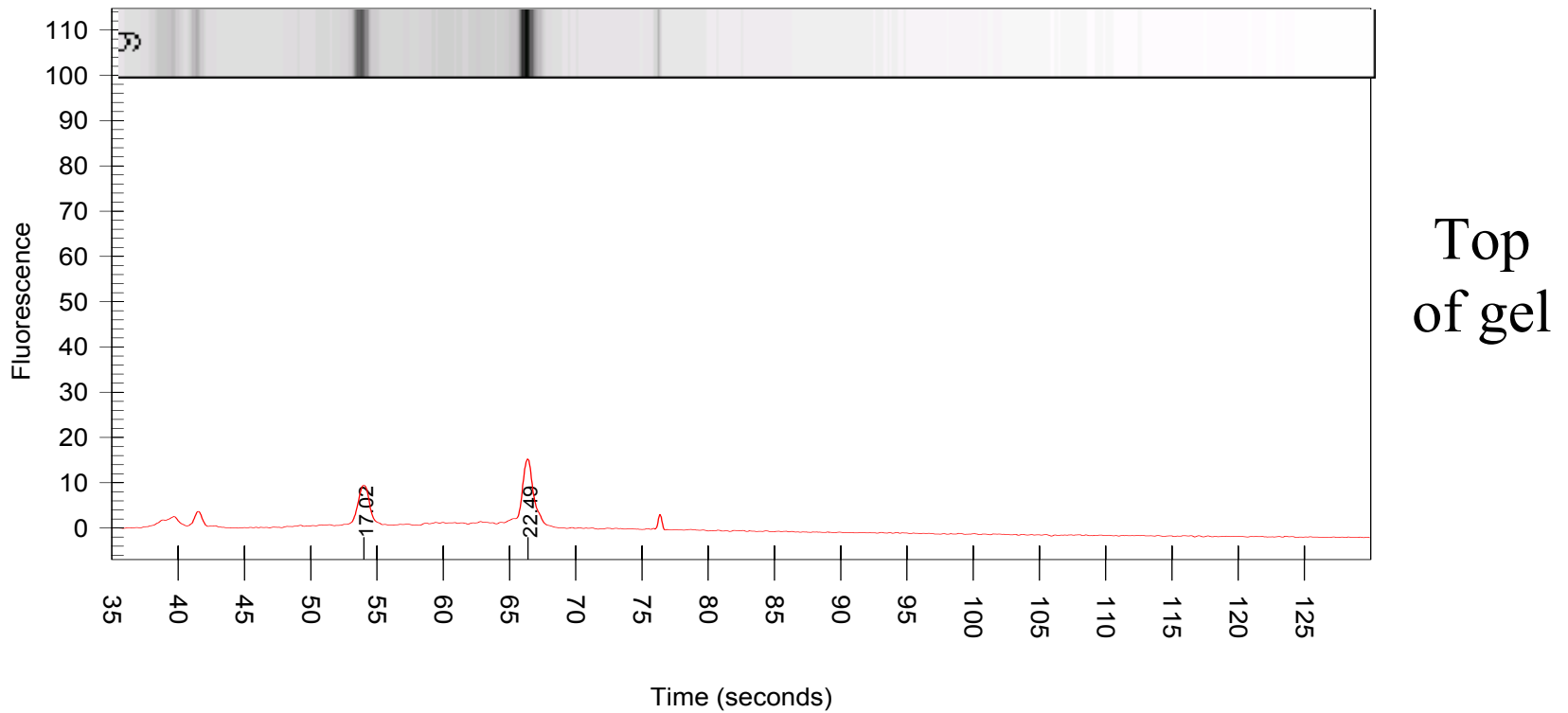
All lanes  
normalized to  
ladder lane  
on left





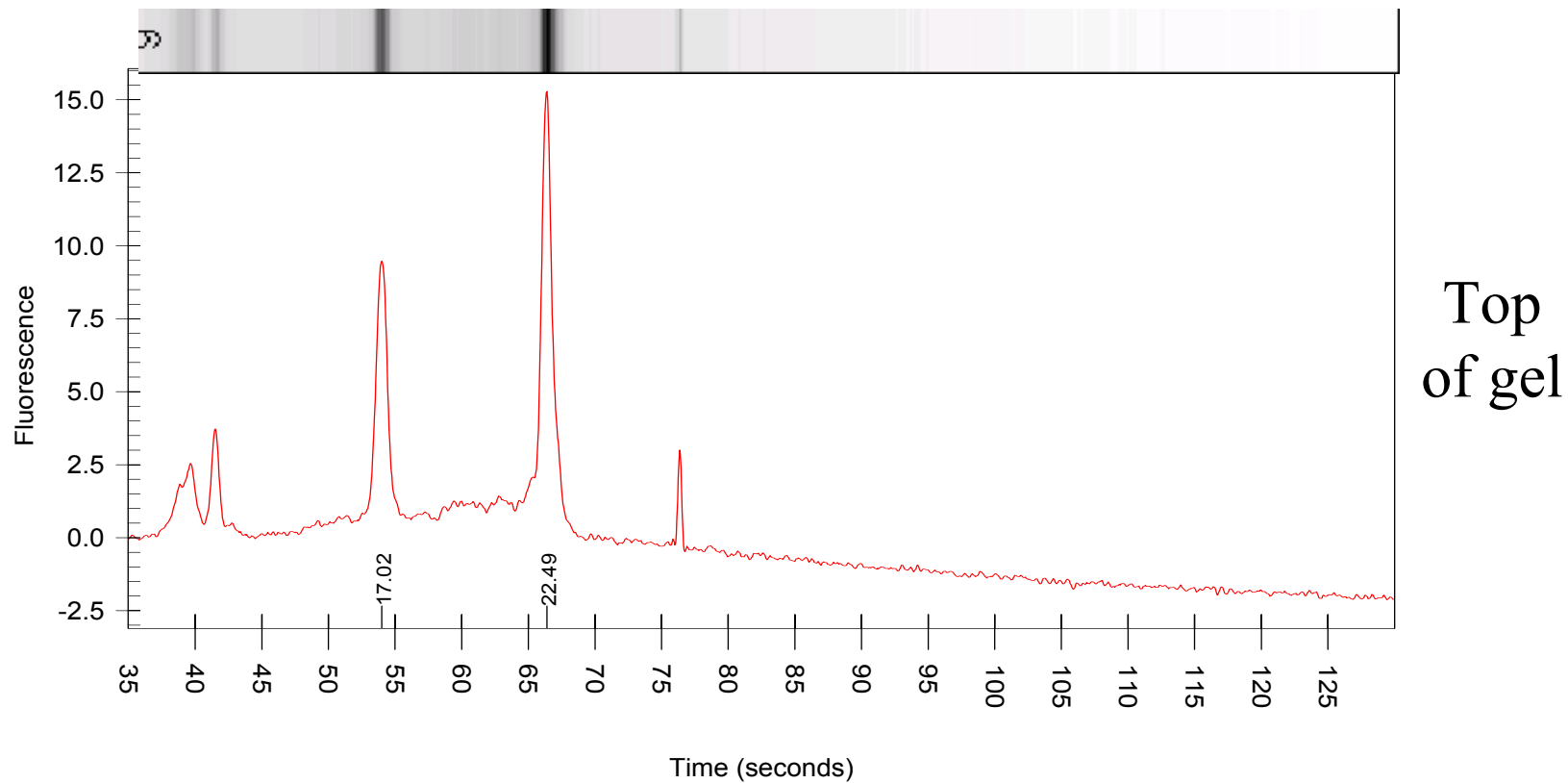
Top of  
gel

15' CEL I reaction. Lane loaded in presence of markers



No marker, 6 uLCEL I rxn 10 min.

Lane normalized to ladder lane.



No markers, 6 uLCEL I rxn 10 min.

Individual lane auto-scaled.

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Joanne Kulinski

Xiao Wen

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