

# Ecotilling: High-throughput discovery of variation in DNA

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# Outline

- TILLING
  - principle
  - method
- Ecotilling
  - principle
  - method
  - examples

# TILLING

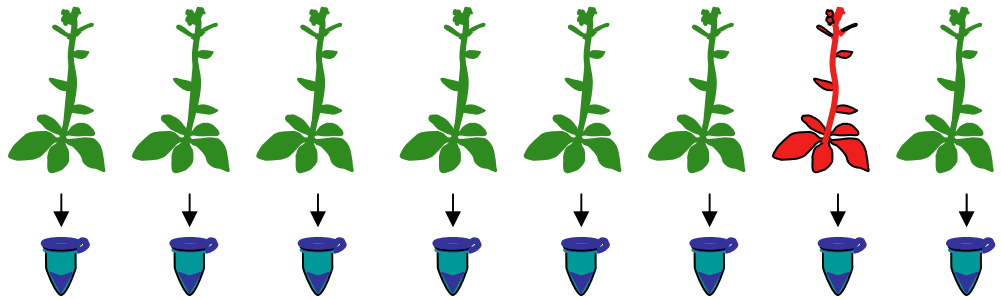
## Targeting Induced Local Lesions IN Genomes

1. Make EMS-mutagenized population
2. Target gene of interest with PCR primers
3. Search for rare mutants among many individuals

Bentley, 2000, *Genetics* **156**, 1169

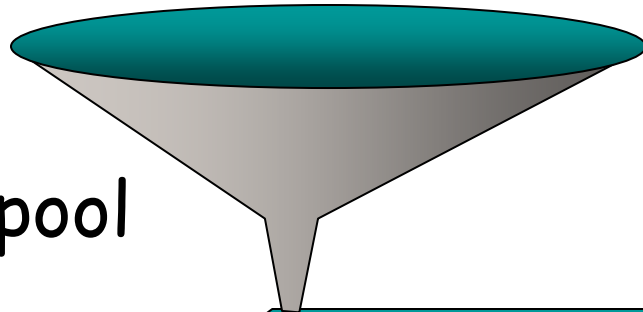
McCallum, 2000, *Nat.Biotech*, **18**, 455

# Pool and array



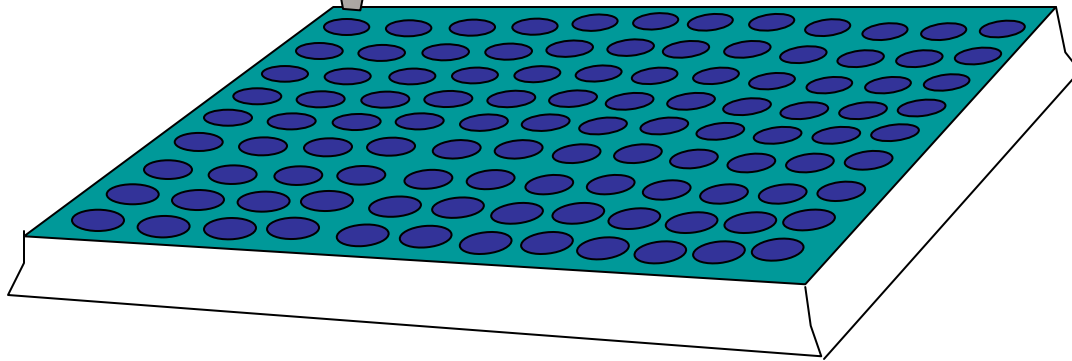
M2 plants

DNAs



8X pool

384x8 individuals/ plate  
2-D pooling provides  
address of unique individual

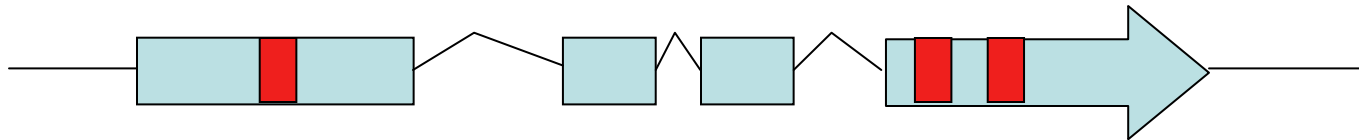


# Choosing the window to till

Exons vs introns  
Codon type (AAA vs TGG)  
Conserved motifs



gene model



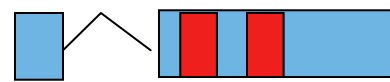
primers



PCR

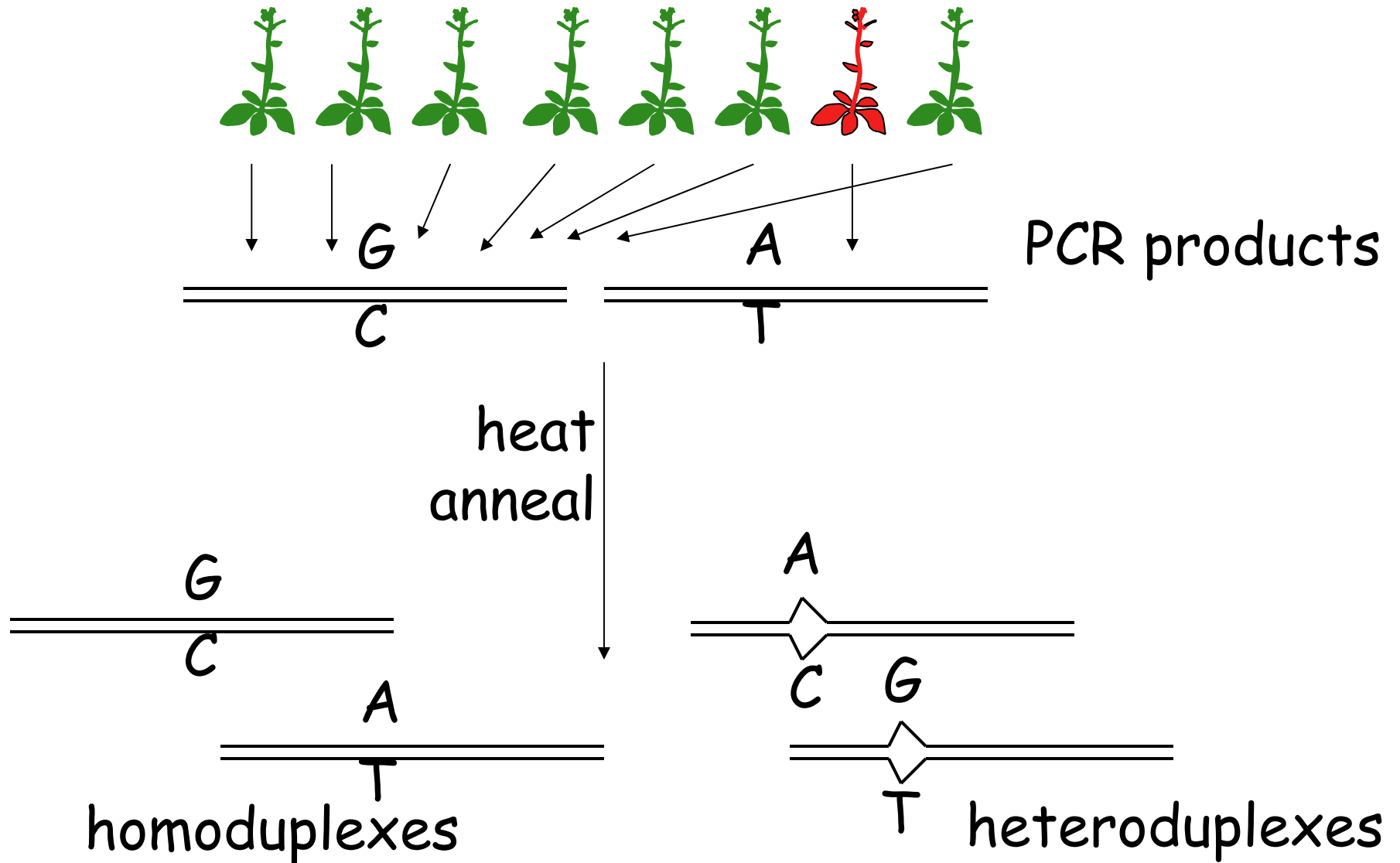


tiled fragment



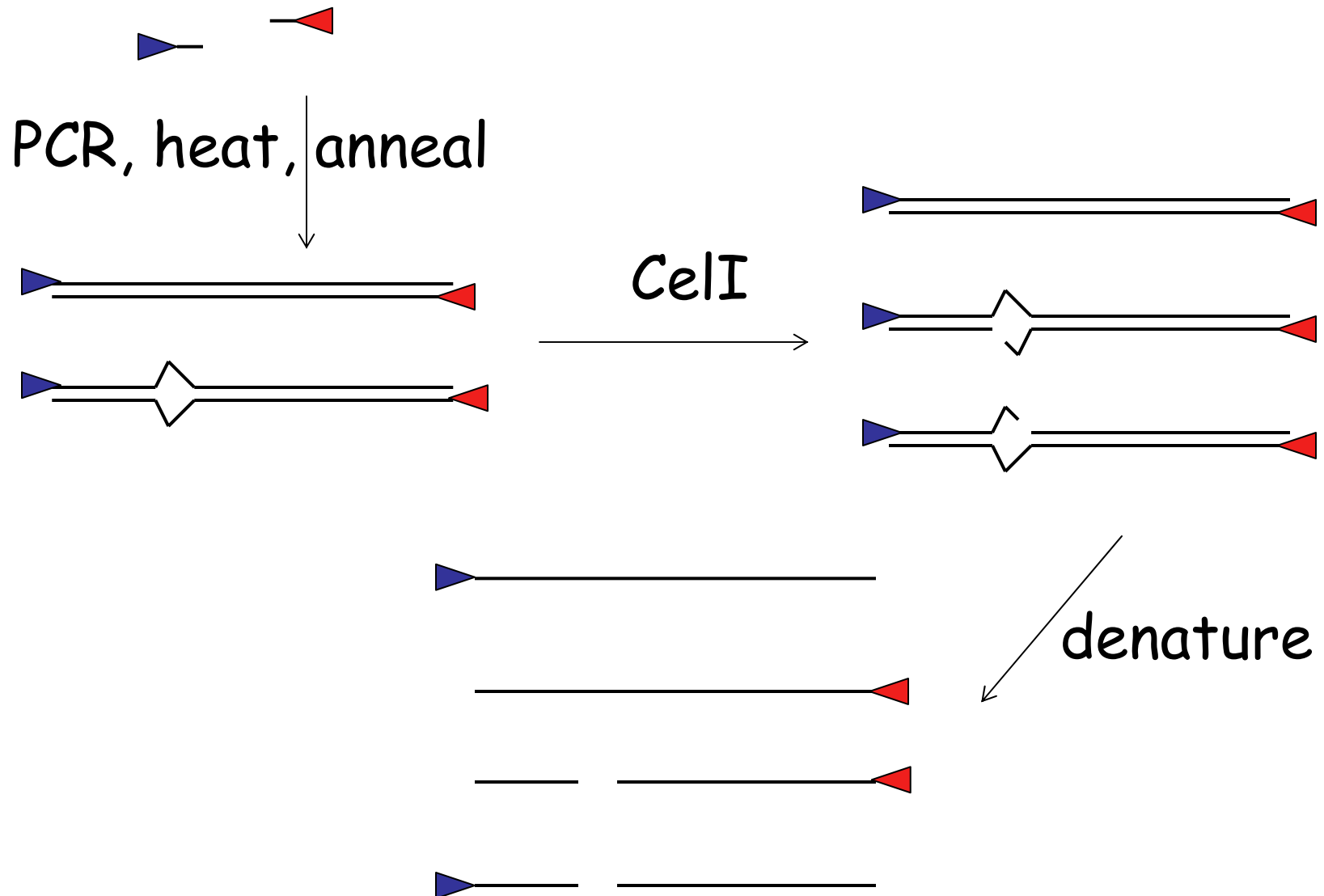
0.5 to 1.5 kb

# Formation of heteroduplexes in pooled DNAs

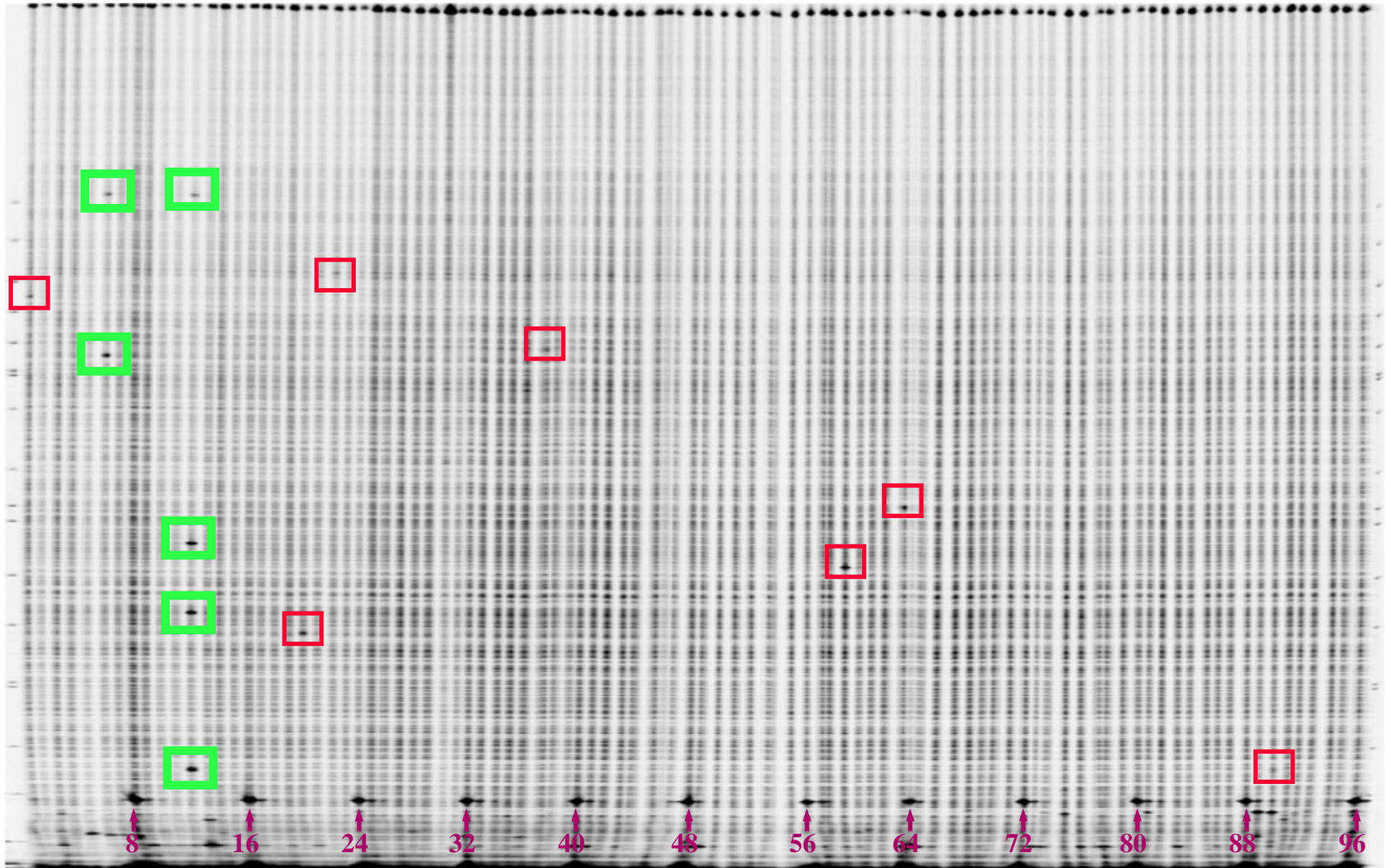


# CellI detection of mutations

fluorescent primers

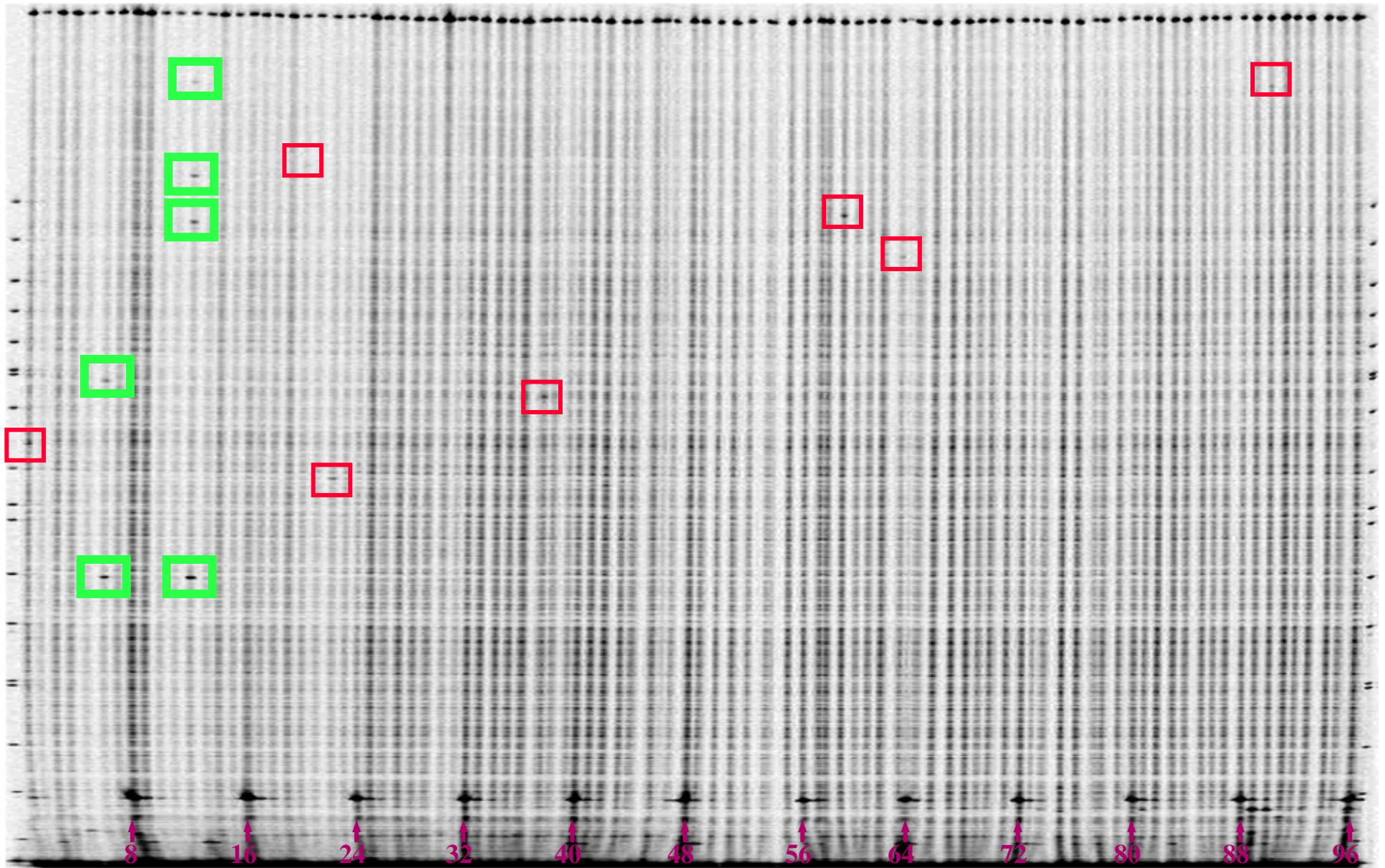


Red boxes mark mutations in pools





# Second fluorescence channel detects bottom strand

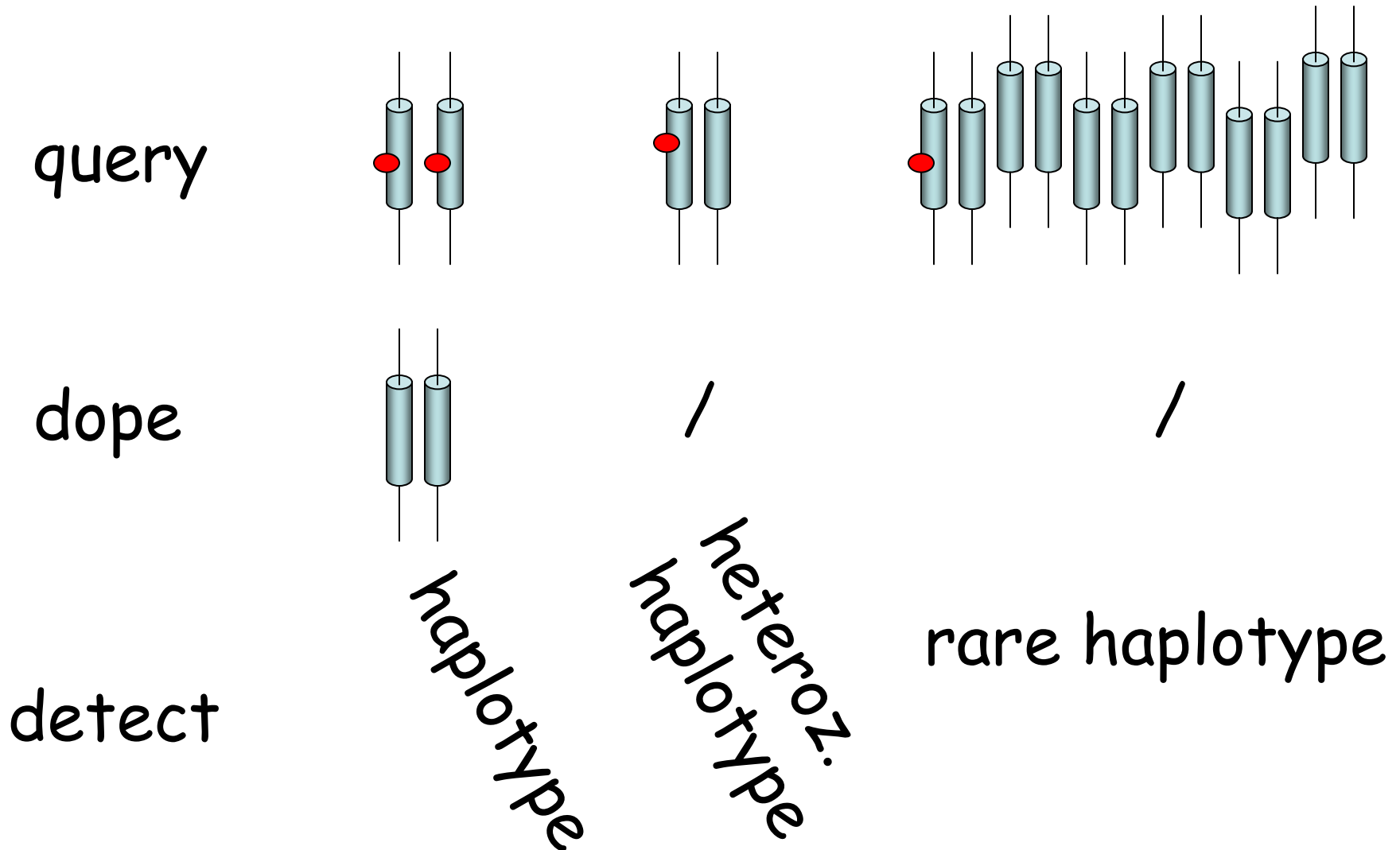


# TILLING SERVICES

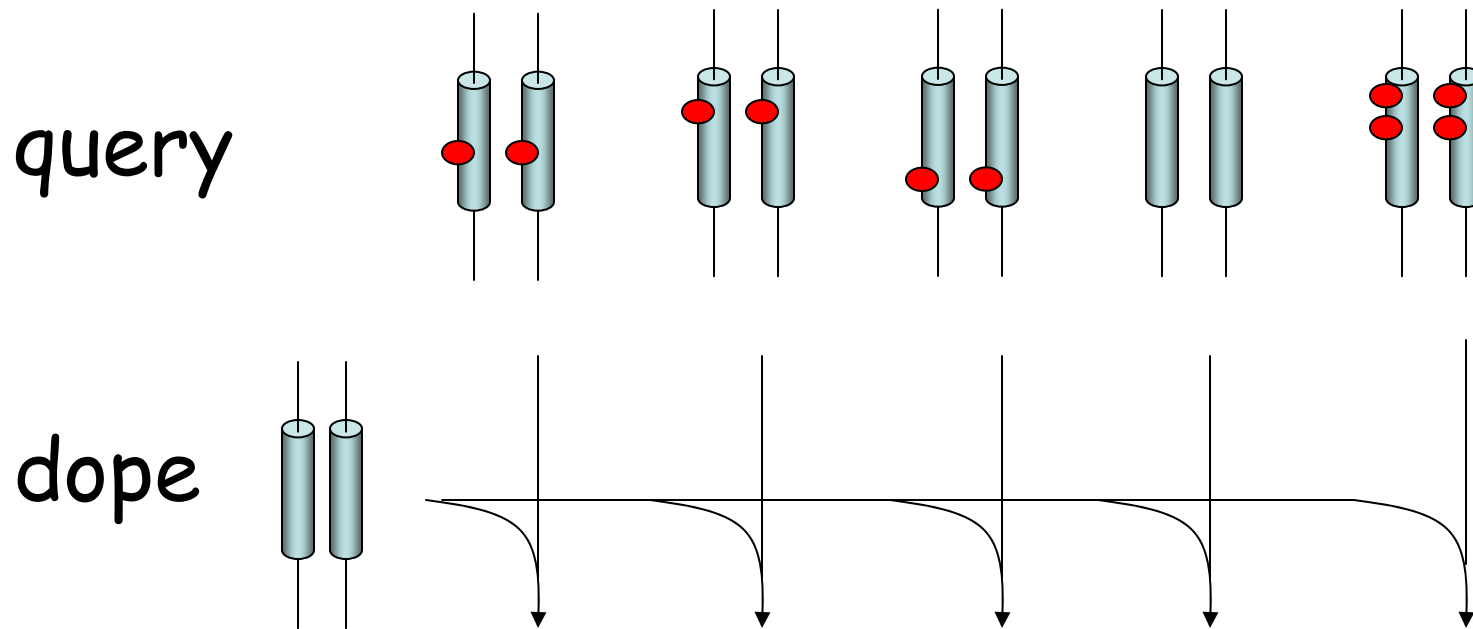
- Arabidopsis:
  - opened 2002
  - 600 genes, 6,300 mutations, 2.4 Gigabases DNA
  - 1 gene, 4 Mb: \$1,500
  - <http://tilling.fhcrc.org:9366/>
- Maize: opened Dec 2004,  
<http://genome.purdue.edu/maizetilling/main.htm>
- Fly: opened March 2005,  
<http://tilling.fhcrc.org:9366/fly/>

Can the technology used for TILLING  
be used to discover natural variation?

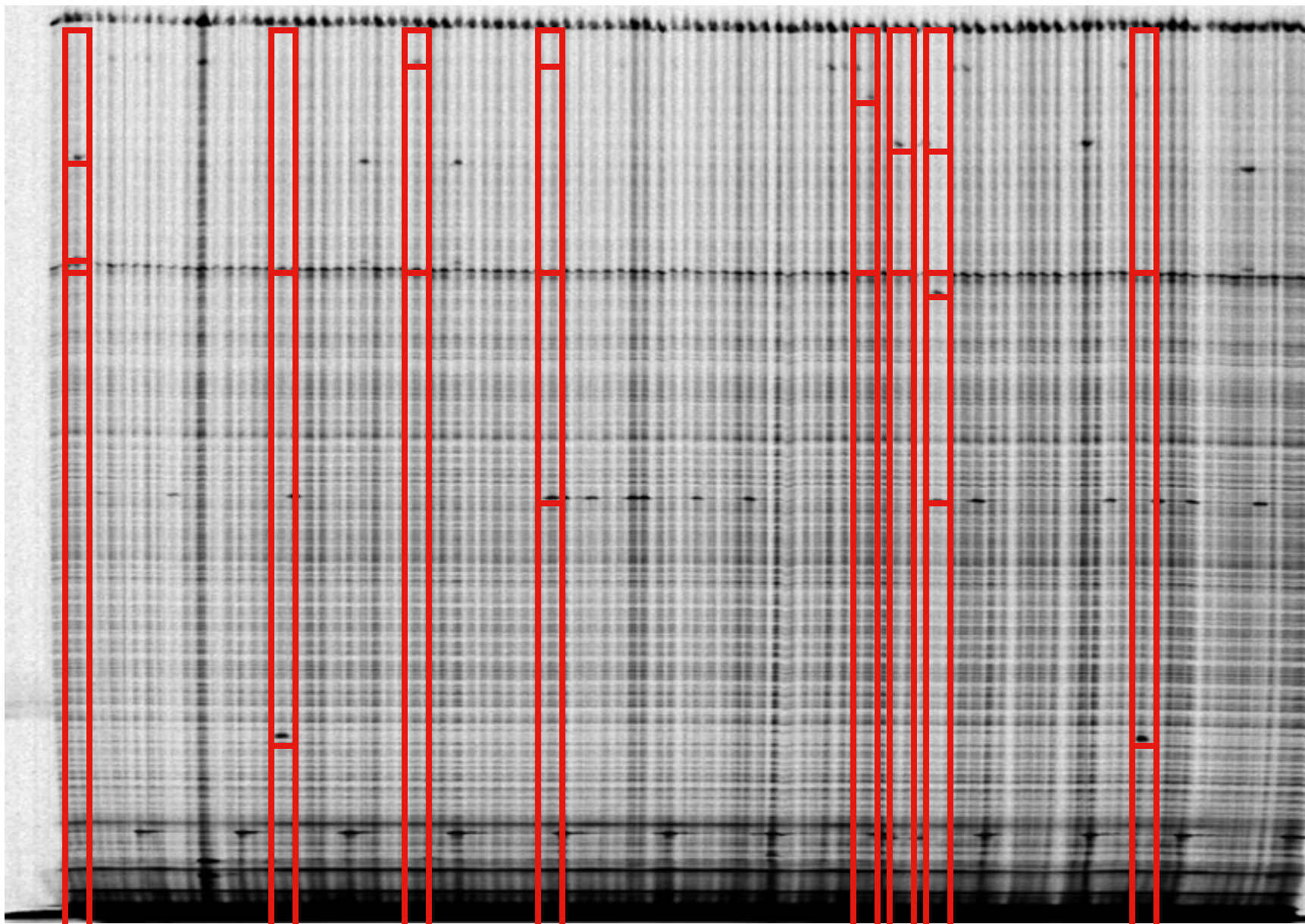
# Versions of Ecotilling



# Ecotilling of inbred arabidopsis and rice



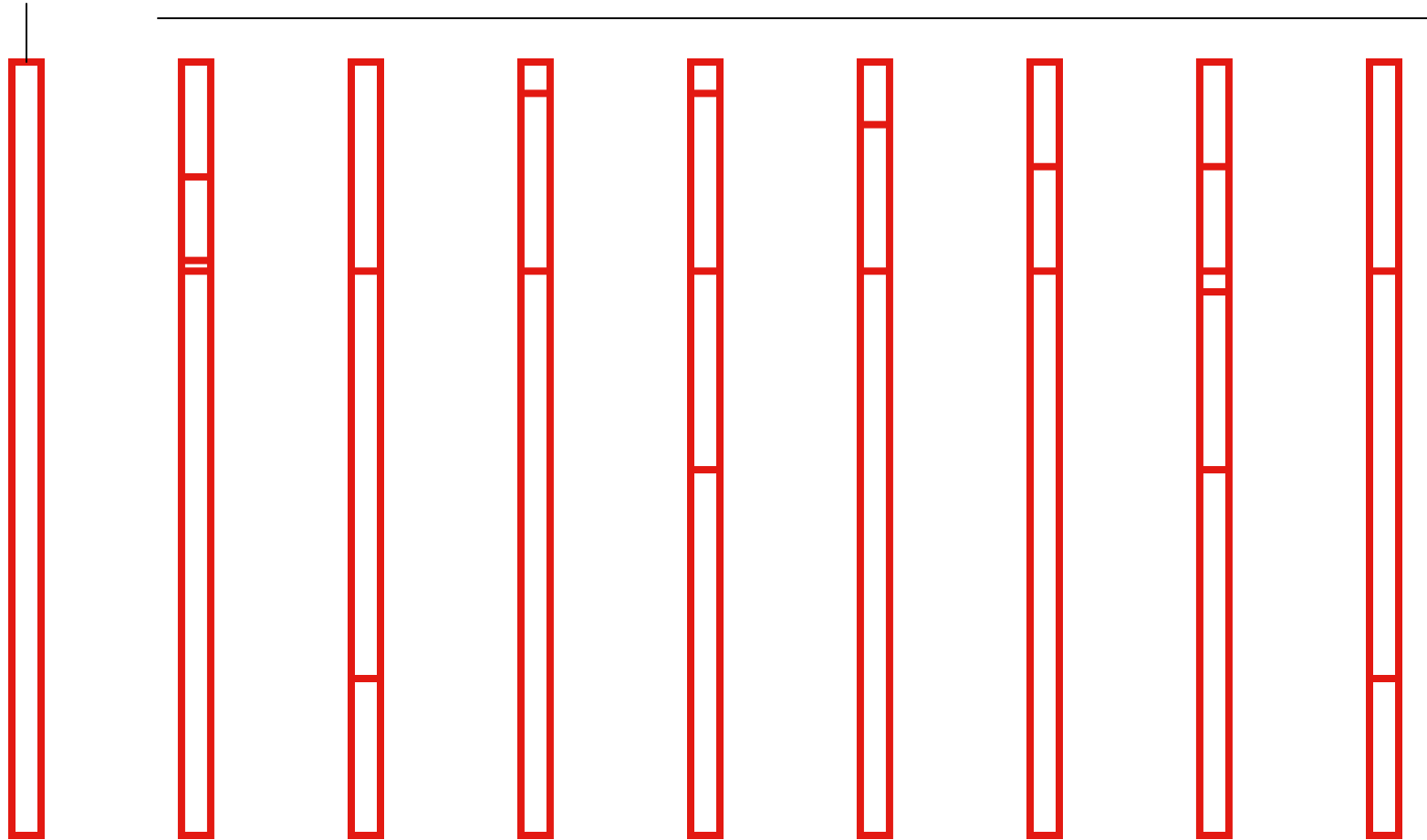
Dope each accession DNA with standard DNA



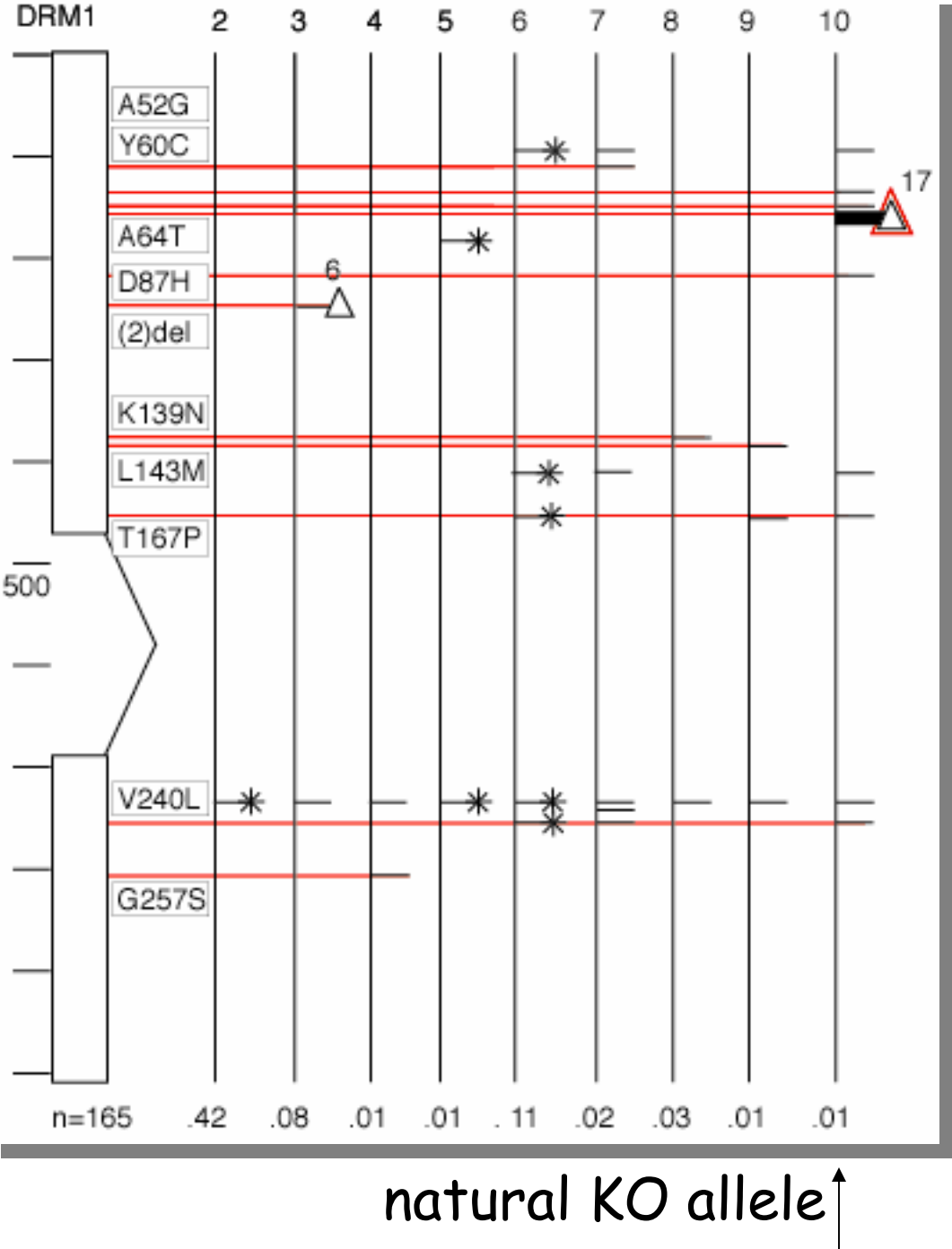
# Catalog alleles, sequence if desired

standard

variants

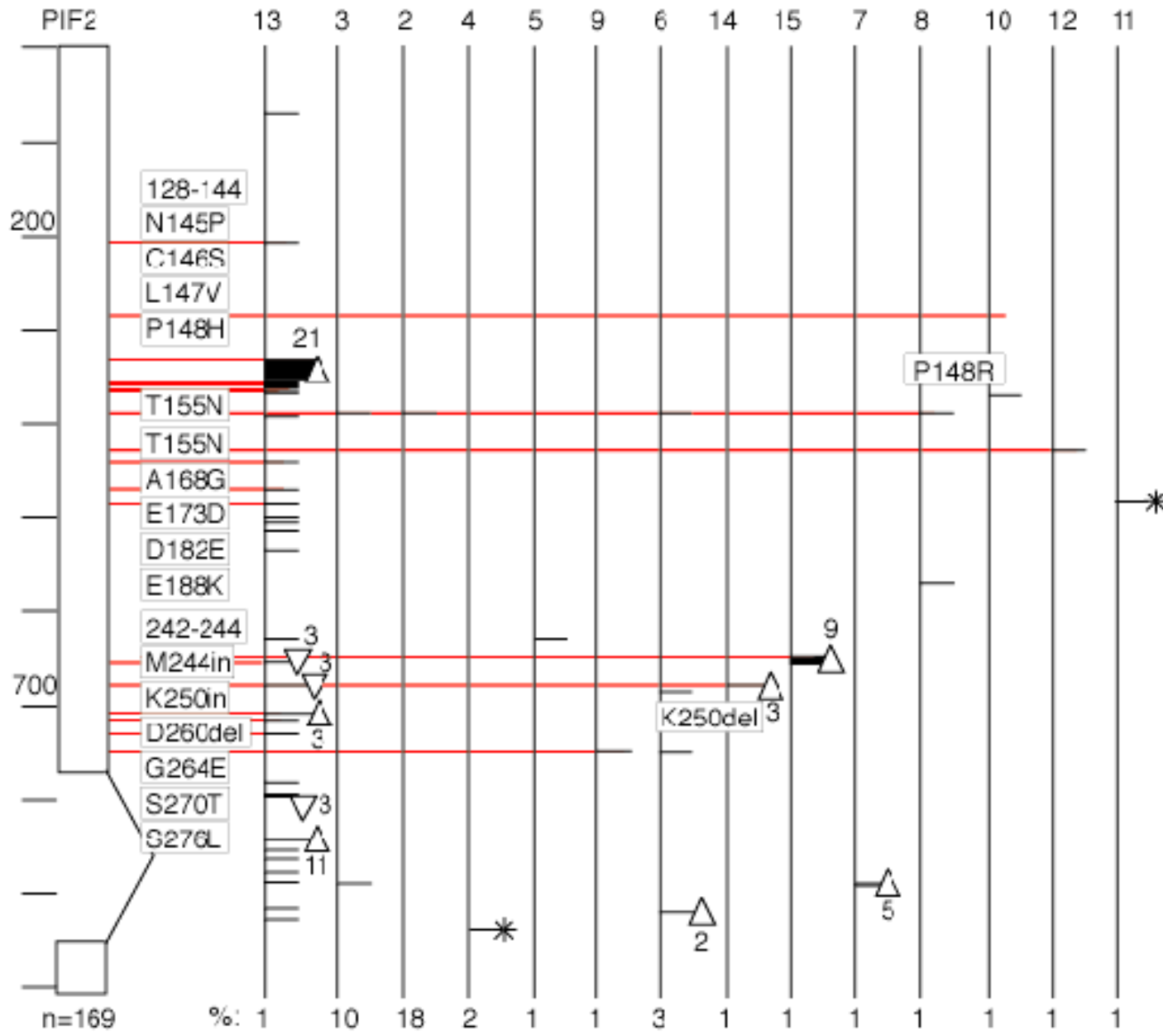


# Ecotilling DNA methylase DRM1



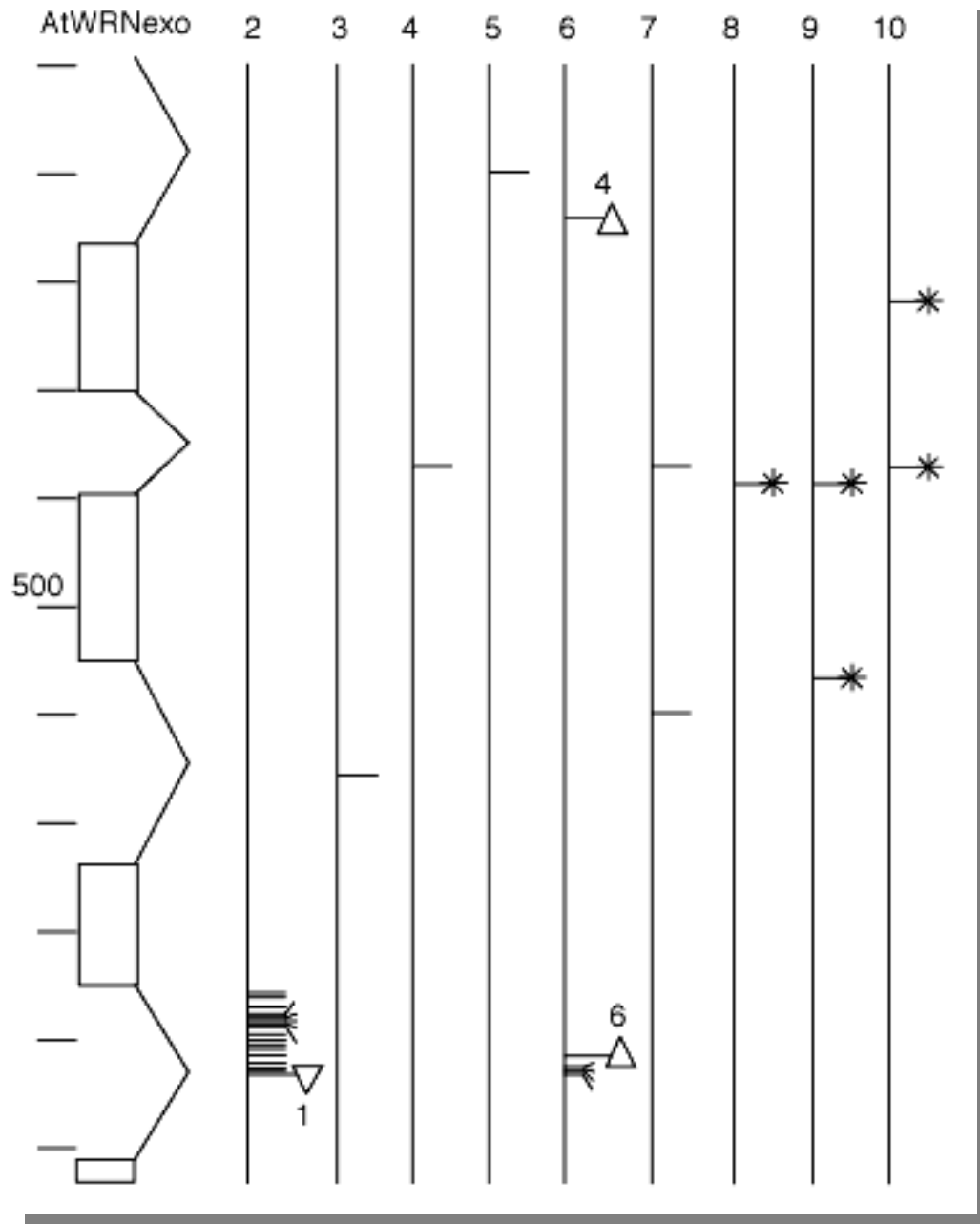


# Ecotilling PIF2



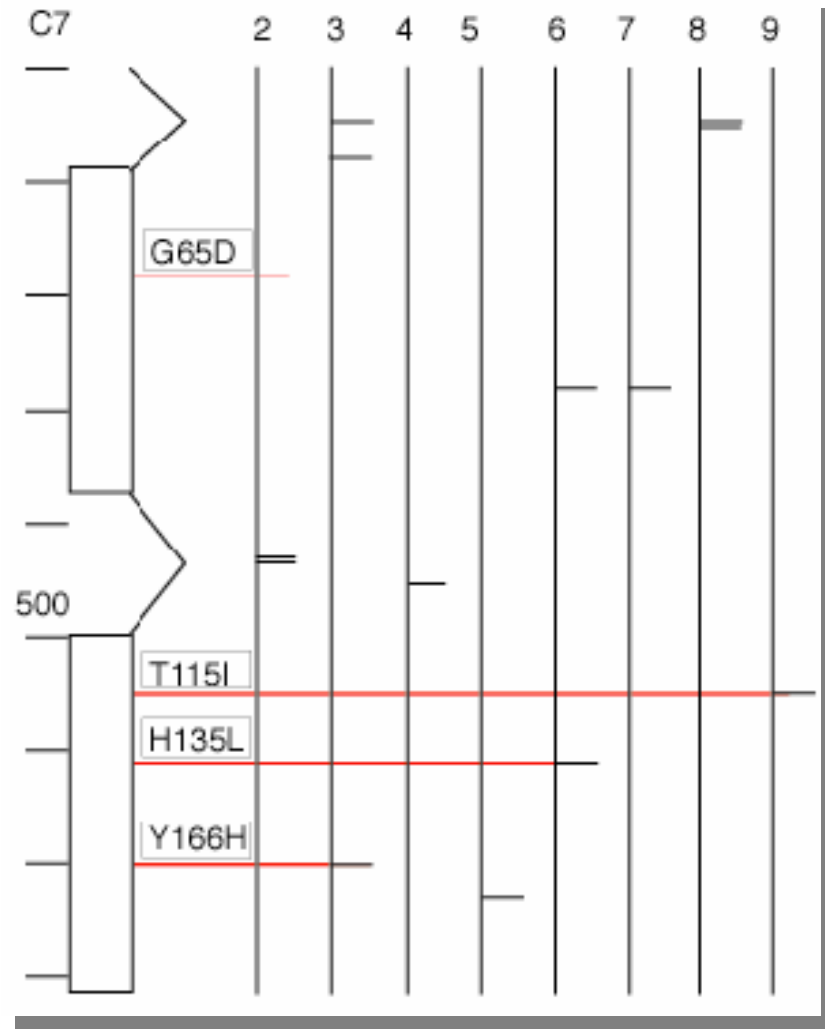
- PIF2-13
- 96% ID to PIF2-1
- variable coding regions
- conservation for function

AtWRN-exo

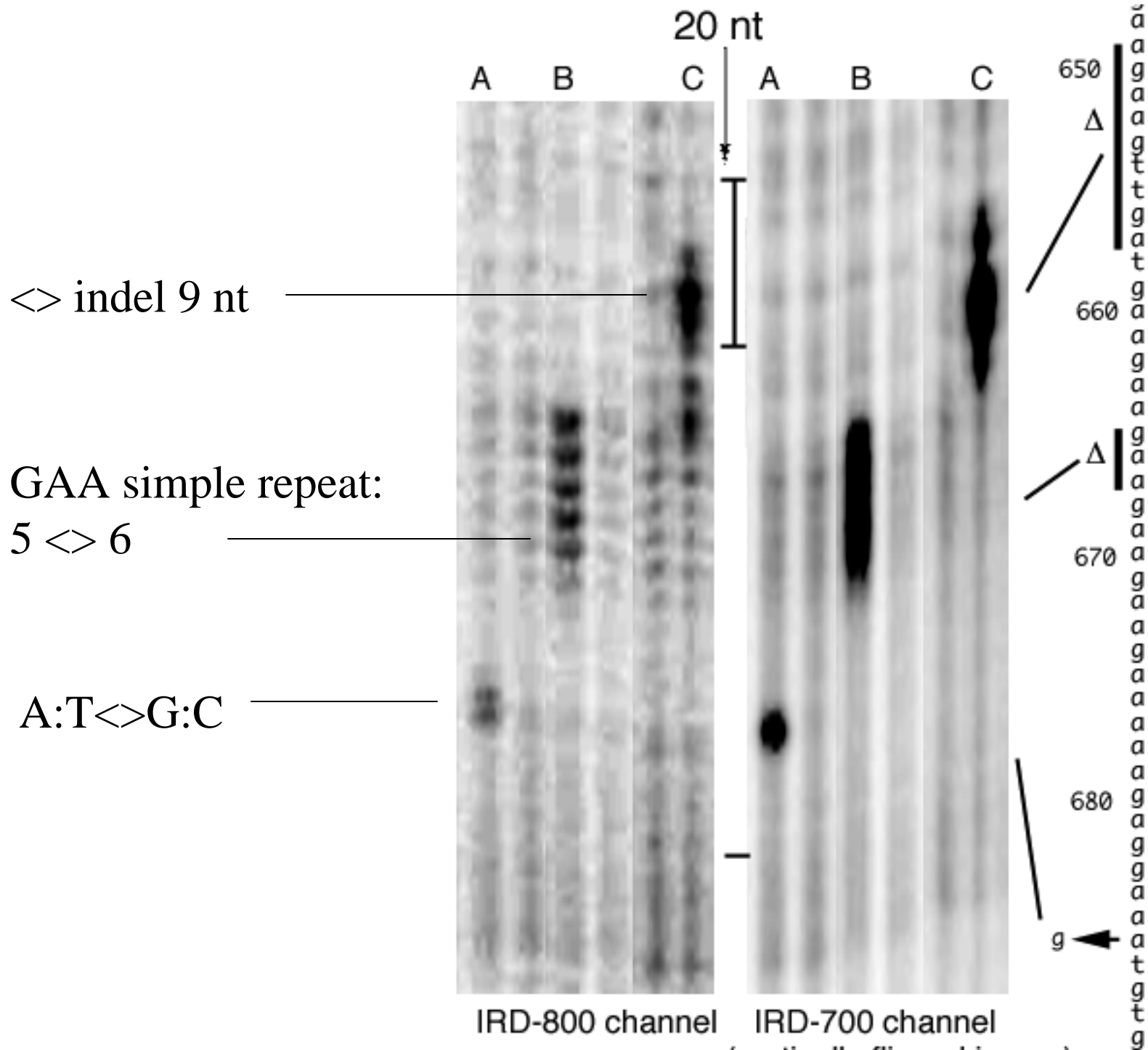


No (?) protein variants

# Ecotilling GATA- TF "C7"

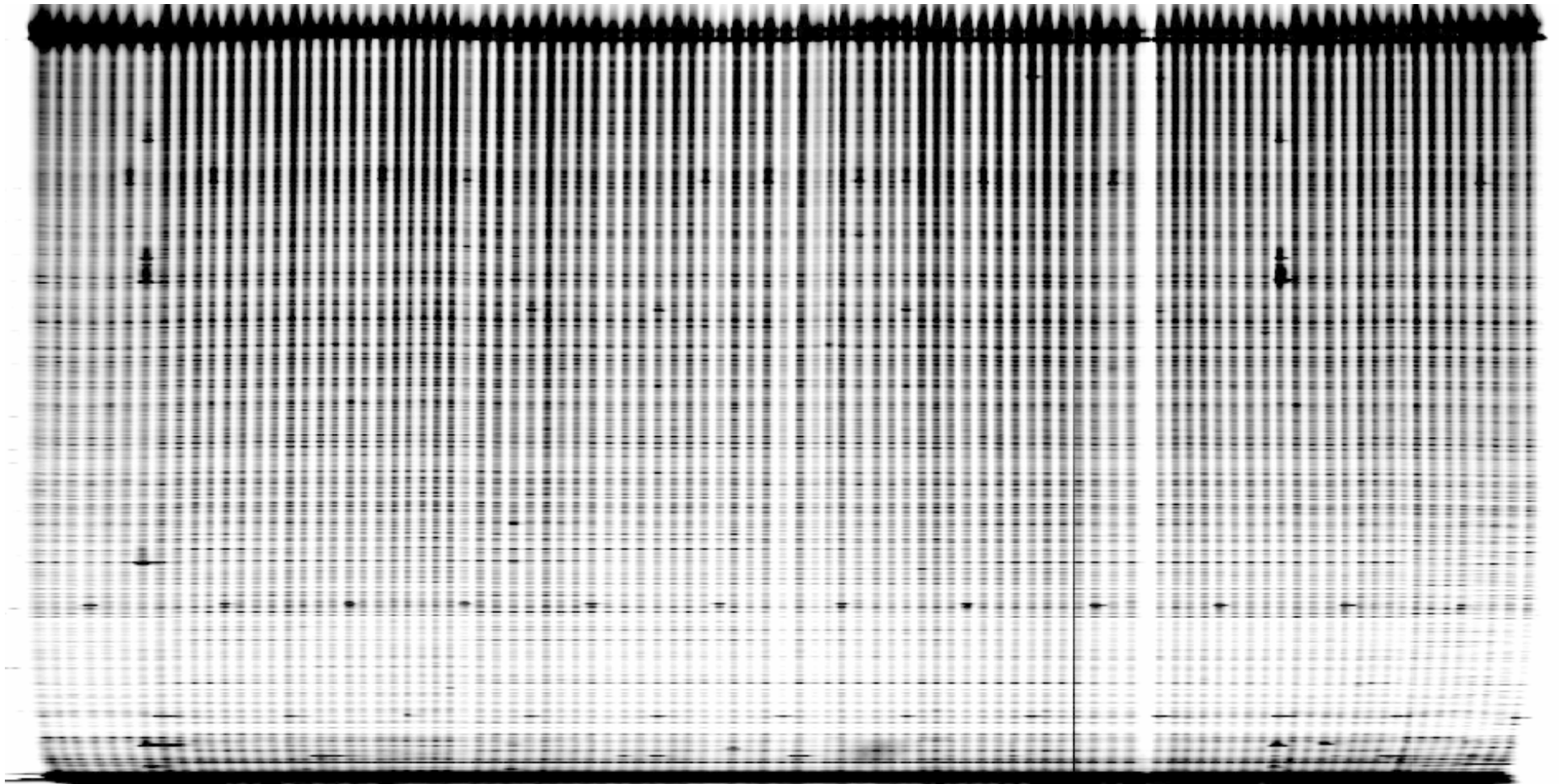


Protein variants

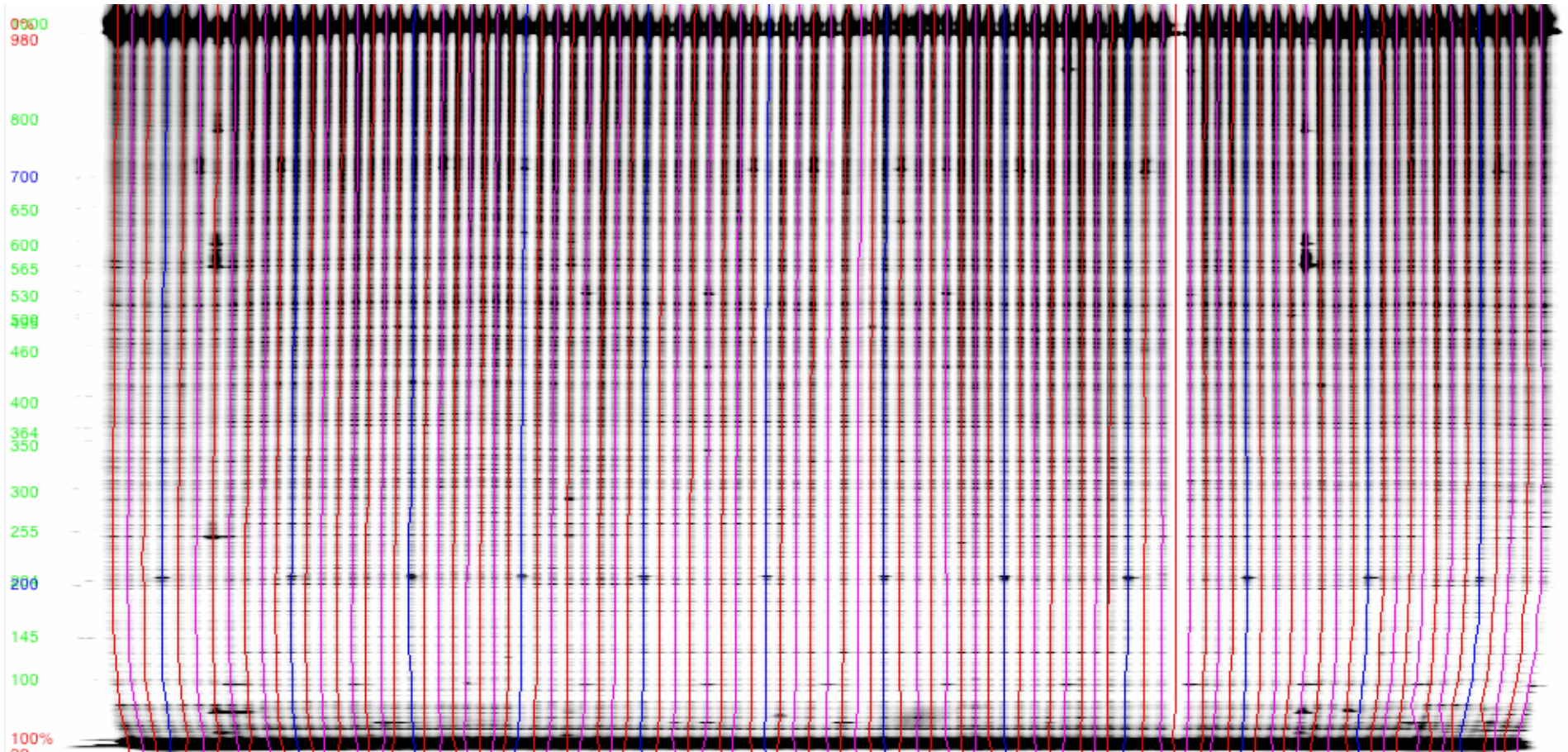


# Rice Ecotilling

96 accessions: spike each with Nipponbare DNA and assay

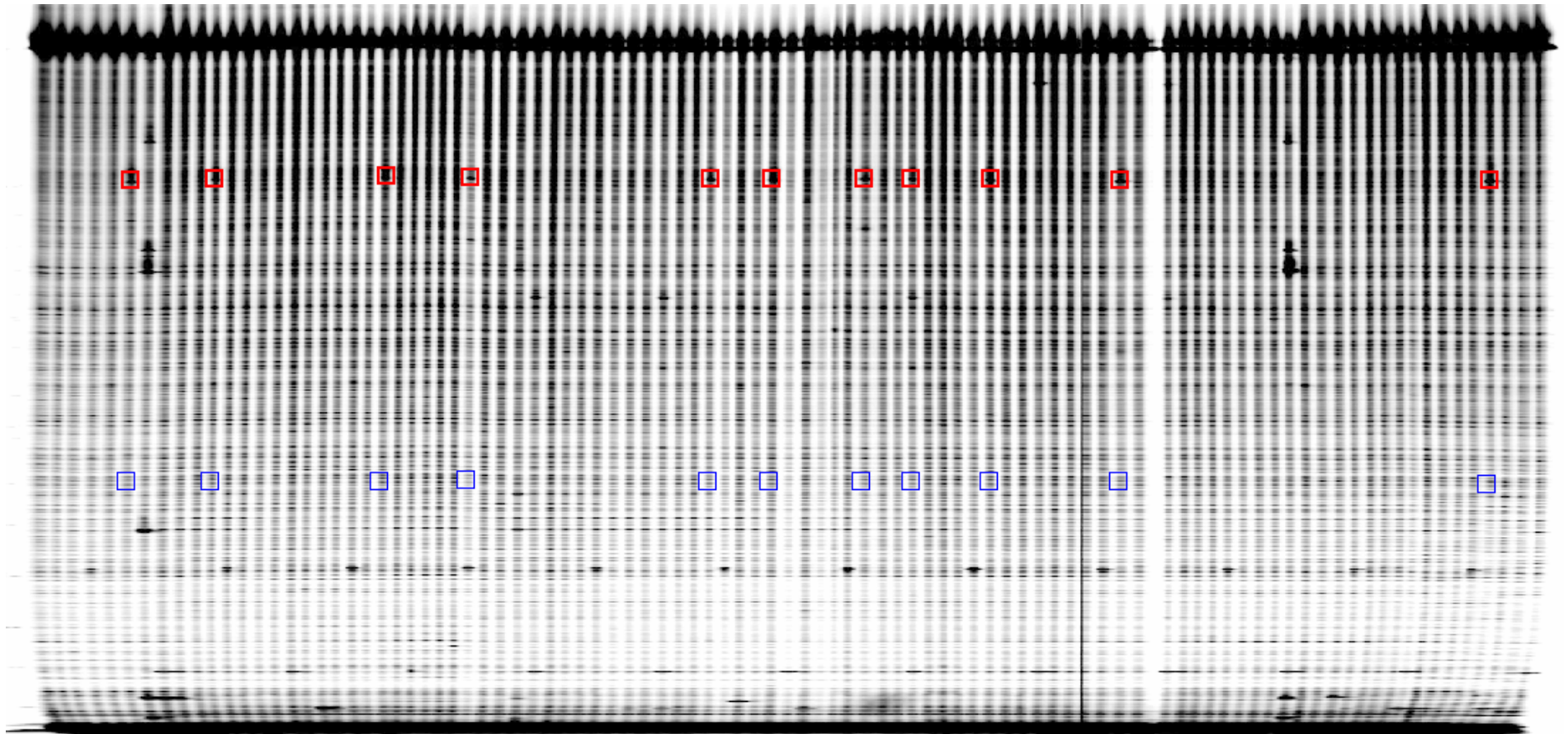


# Lane calling in GelBuddy

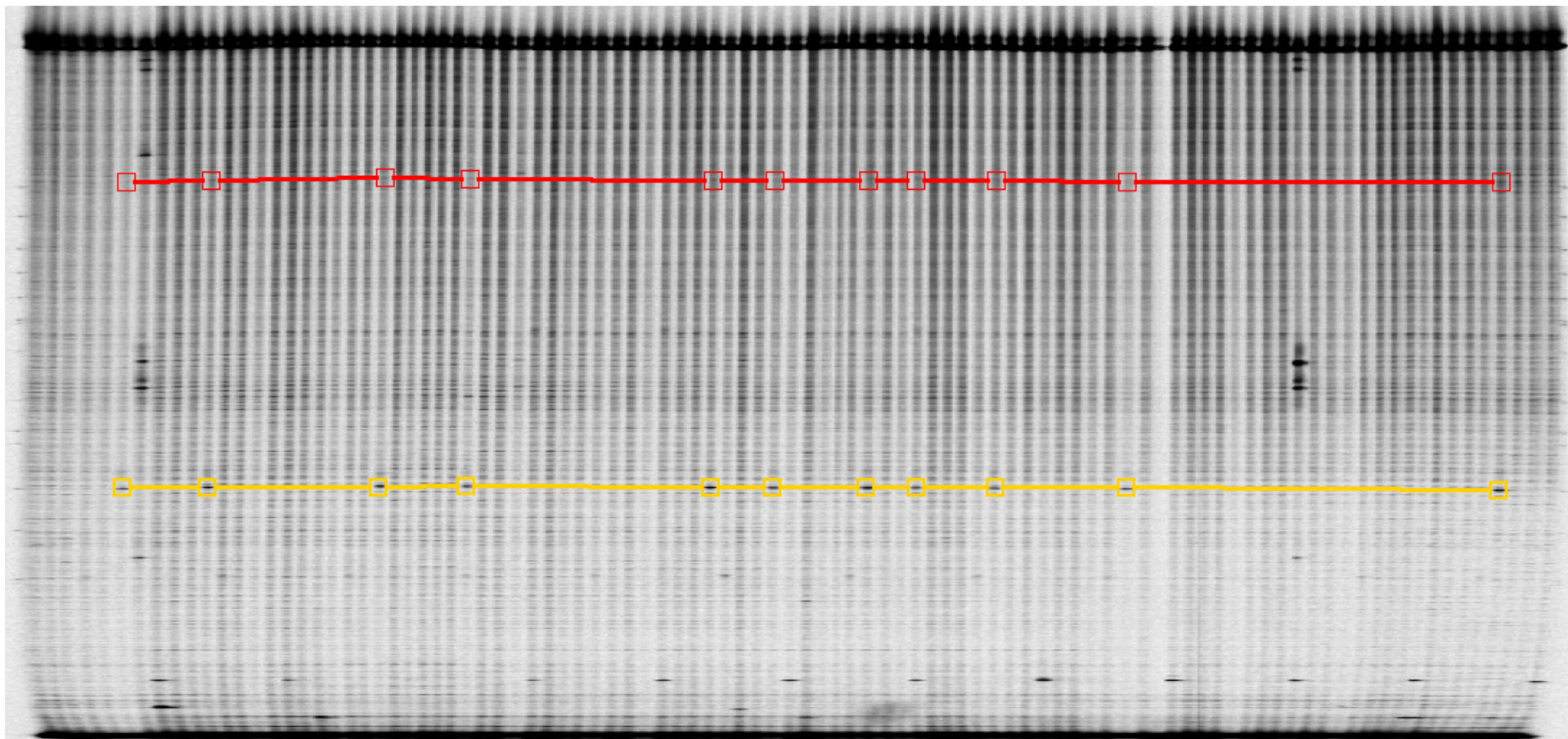


GelBuddy: Zerr and Henikoff, 2005, NAR 83, 2806

# Identifying polymorphisms



# Grouping polymorphisms





# GelBuddy Output

Lane	700% ( MW)	800% ( MW)	(Total)
6	19.5 ( 710)	63.2 ( 304)	( 1014)
11	19.4 ( 710)	63.1 ( 305)	( 1015)
22	19.5 ( 710)	63.2 ( 304)	( 1014)
28	19.5 ( 709)	63.0 ( 306)	( 1015)
43	19.6 ( 708)	63.1 ( 305)	( 1013)
47	19.4 ( 710)	63.3 ( 304)	( 1014)
53	19.5 ( 709)	63.3 ( 304)	( 1013)
56	19.6 ( 709)	63.3 ( 304)	( 1012)
61	19.6 ( 708)	63.3 ( 304)	( 1012)
69	19.6 ( 709)	63.2 ( 304)	( 1013)
93	19.5 ( 709)	63.5 ( 302)	( 1011)

## Similar Lanes

-----

Lane 6, 11, 22, 28, 43, 47, 53, 56, 61, 69, 93

19.5 ( 708- 710) 63.2 ( 302- 306)

# Eco-Squint output

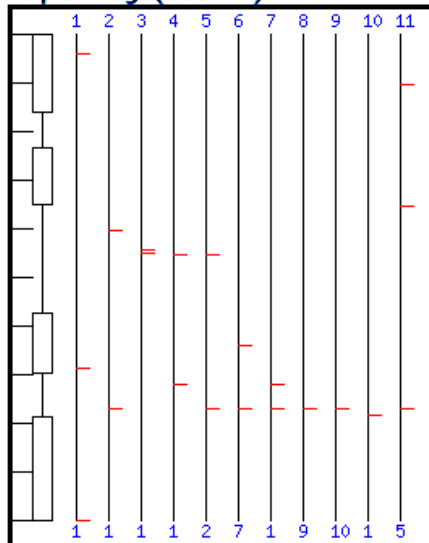
Views:  ⌵

[Download Tab-Separated Table](#)

Licor Run Name: hira3\_ei6xa\_eg Full-Length MW=1026 50 marker=99 700 marker= 23

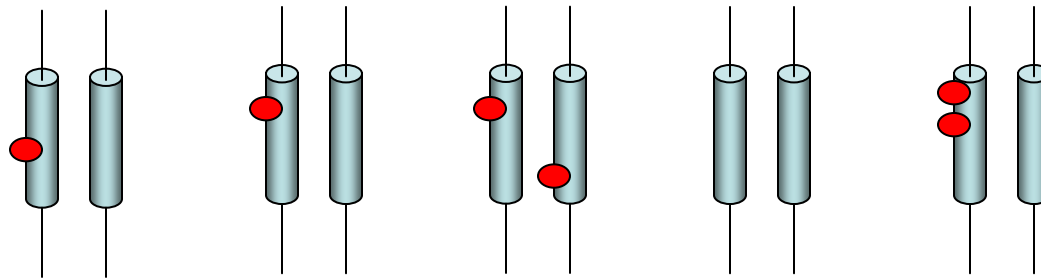
haplo	Lane	P700	mw700	P800	mw800	Sum	Confidence
1	91	0,4,65.5	0,958,311	83.5,83,25	174,178,678	174,1136,989	2
2	29	33,76	594,230	54,18	404,759	998,989	3.5
3	71	37,37.5	555,550	51,50.5	429,434	984,984	1.5
4	59	38,69.5	545,280	49,22	446,712	991,992	2.5
5	83,85	38,76	545,230	49,18	446,759	991,989	2.5
6	7,17,25,27,61,67,73	59.5,76	359,230	29.5,18	629,759	988,989	3
7	57	69.5,76	280,230	22,18	712,759	992,989	3
8	3,5,49,53,63,65,69,81,93	76	230	18	759	989	3
9	31,33,35,37,39,45,47,51,75,89	76.1	230	18.1	758	988	2
10	41	78	215	16.5	778	993	3
11	21,23,77,79,87	28,76,8	645,230,895	61,18,81	347,759,193	992,989,1088	3.333333

HaploImage(700MW)



# Ecotilling humans: one by one, no dope

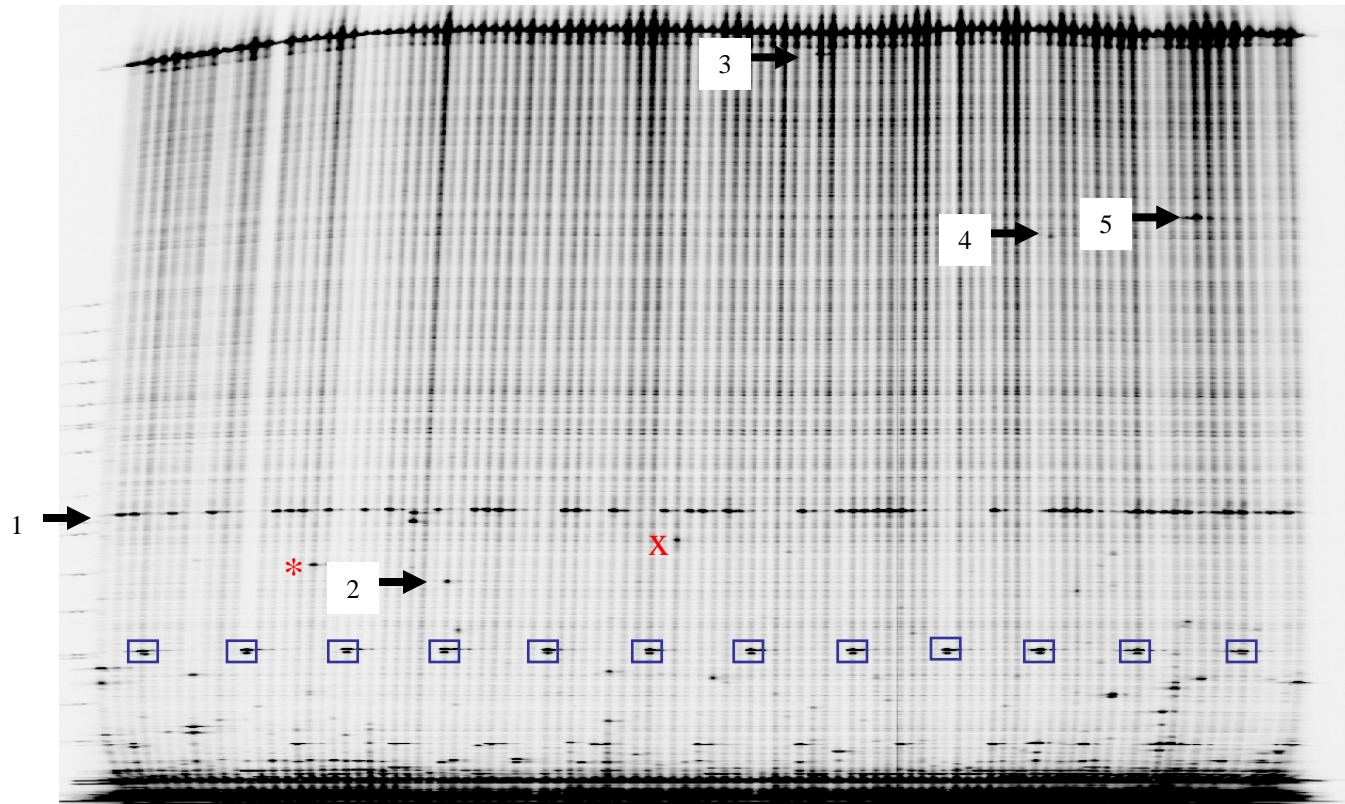
query



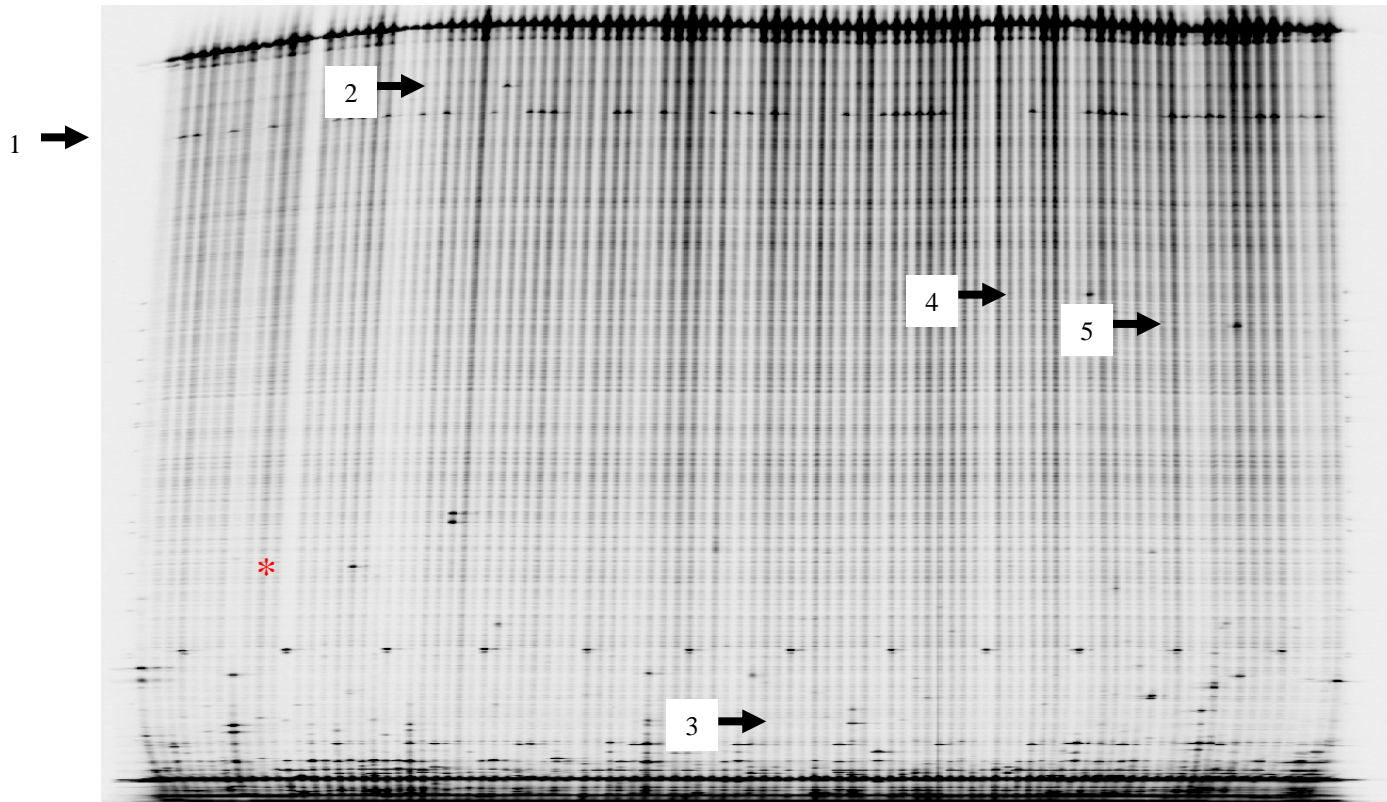
throughput:  $288/\text{Licor} \times \text{day}$

# Human DCLRE1A (cross-linked DNA repair protein)

A

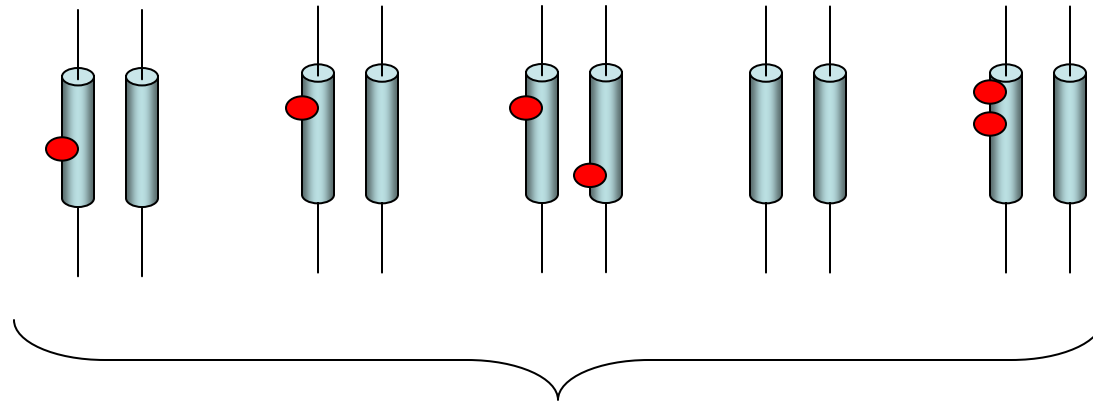


# DCLRE1A (cross-linked DNA repair protein)



# Ecotilling pools for rare haplotypes

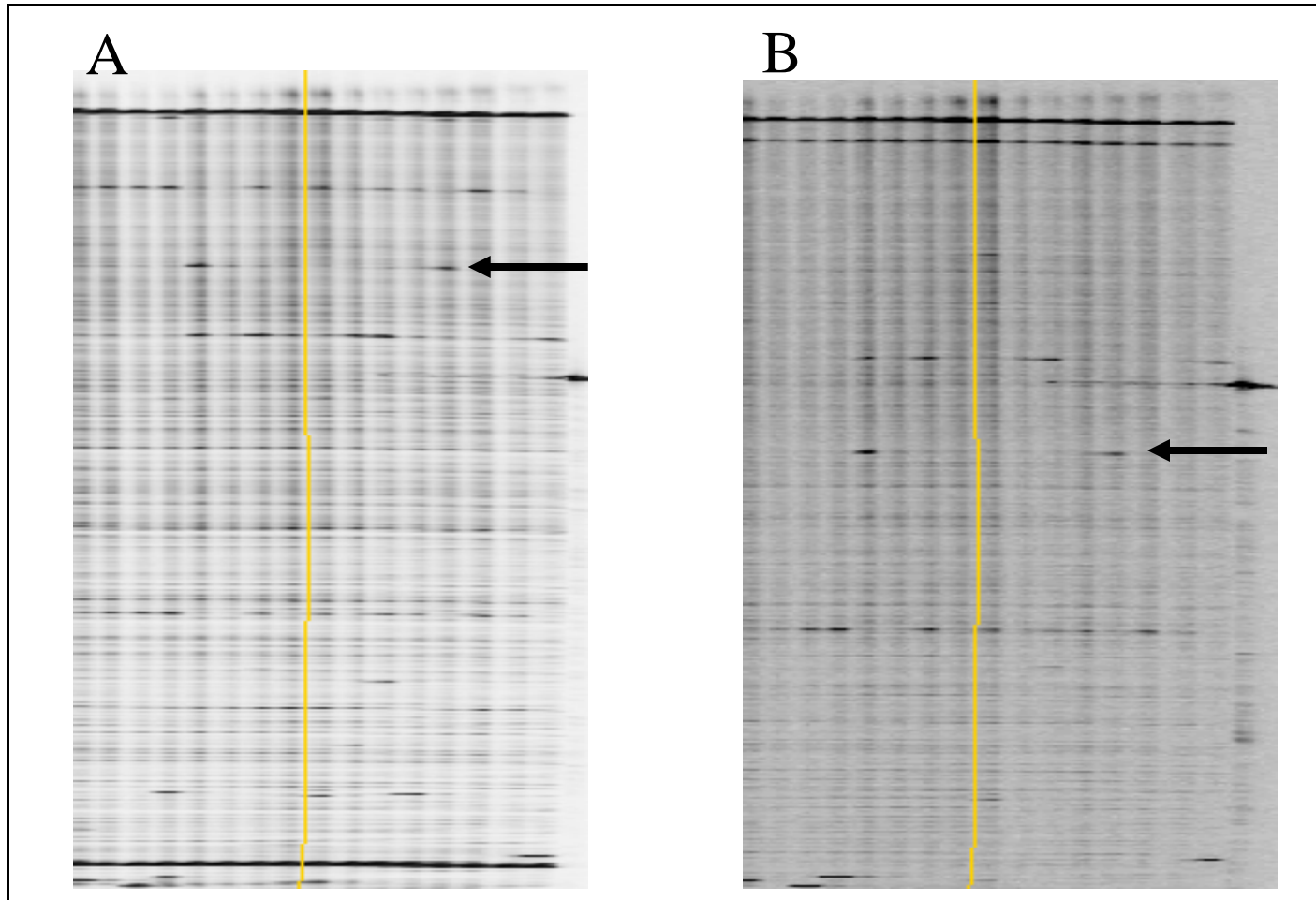
query



pools of 8

throughput: 1152/Licor x day

# Discovery of rare polymorphism by pooling



# Uses of ecotilling

1. Screen many individuals at one locus
2. Not good for few individuals at many loci
3. Discover rare haplotypes
4. Good in heterozygous populations



# Credits

## FHCRC/UW TILLING lab

Brad Till  
Jenny Cooper  
Kim Young  
Rob Laport  
Christine Codomo  
Chris Burtner  
Elisabeth Bowers  
Aaron Holm  
Nina Miller  
Steve Reynolds

## FHCRC Bioinformatics

Elizabeth Greene  
Jorja Henikoff  
Troy Zerr

## PIs

Steve Henikoff  
Luca Comai

## Collaborator

Debbie Nickerson  
UW, Seattle

## Support

