

DELETION TILLING

De-TILLING

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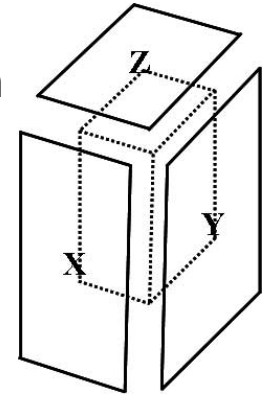
The contribution of De-TILLING to RevGenUK

Lower in cost

Sensitive PCR detection

High pooling Depth

3D pooling strategy



De-TILLING

Useful mutants

100% knockout mutants

Lower background mutations

Small deletions

Unique Applications

Small target genes

Tandem arrays of gene

Sequence Independent

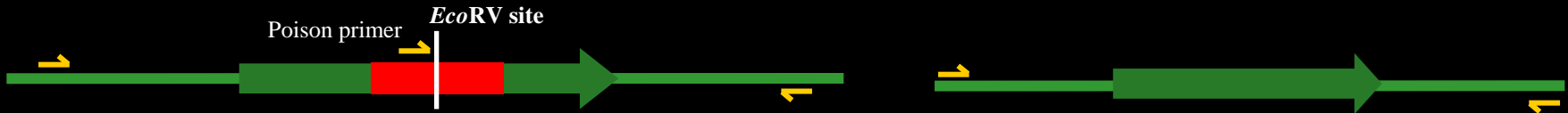
De-TILLING detection

Combines Restriction and Poison Primer Suppression

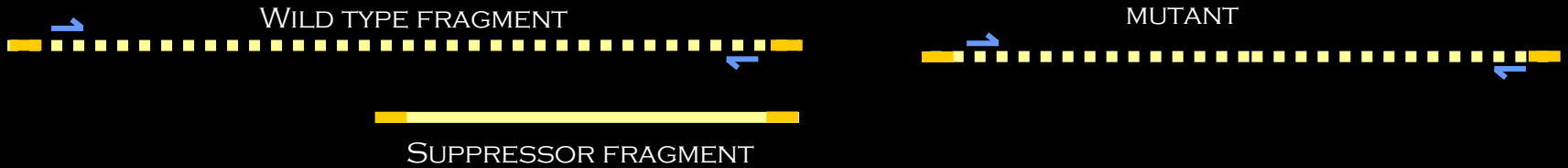
PREDIGESTED TEMPLATES

20,000 x WILD TYPE TEMPLATE

1 x MUTANT TEMPLATE



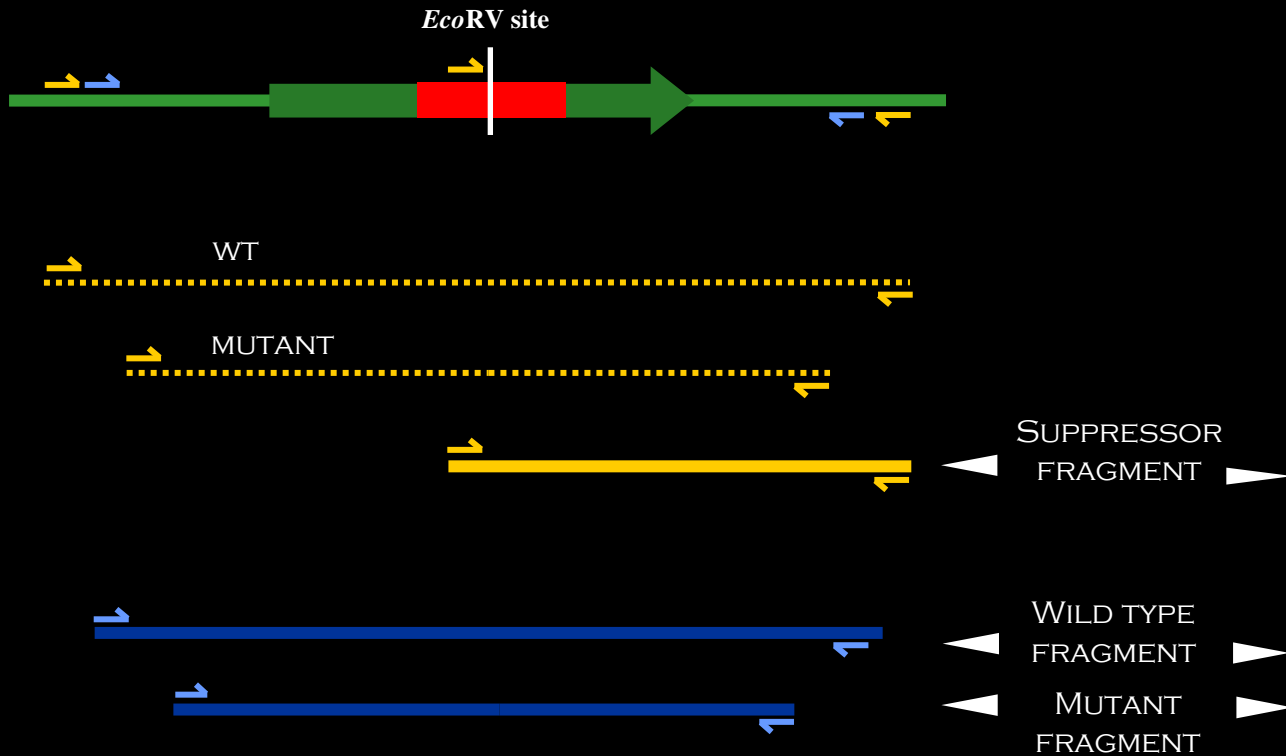
PRIMARY PCR



NESTED PCR

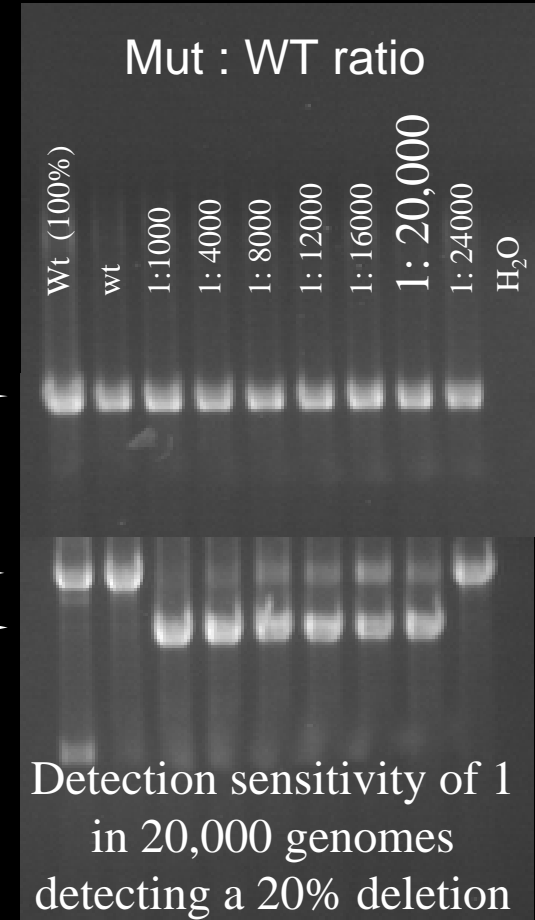


PCR suppression of 20,000 wild type alleles allows a single deletion allele to be amplified

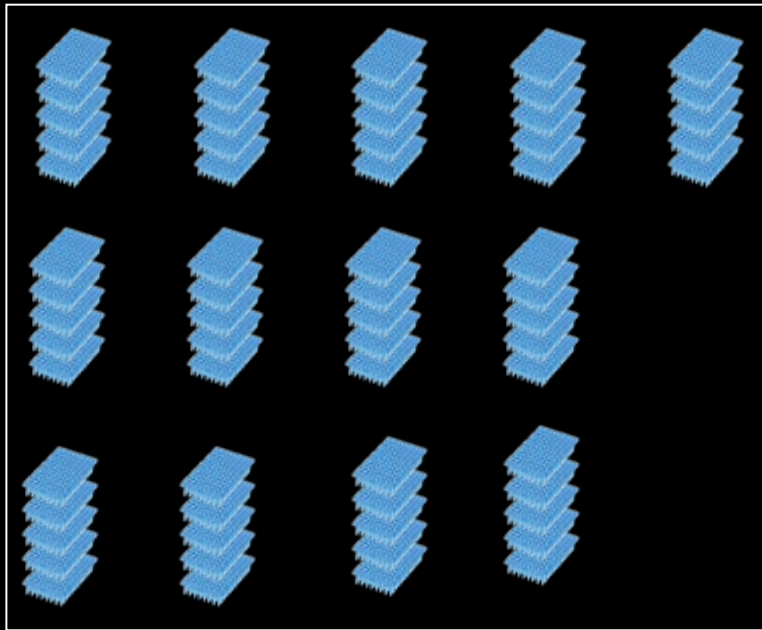


MtMutDetect.pl

The 435bp (20%) *nsp2* deletion is detected within a 2168bp target using restriction suppression and the poison primer technique.



The JIC Medicago De-TILLING Population



13 TOWERS

31,200 M1 lines

156,000 M2

Medicago truncatula A17
Fast Neutron Mutagenesis
Deletions

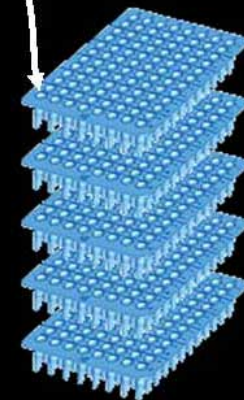
1 pool



25 M2 plants



5 mutagenised M1s



480 pools

50ng ul

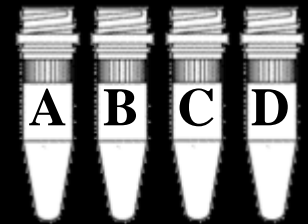
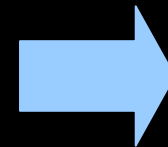
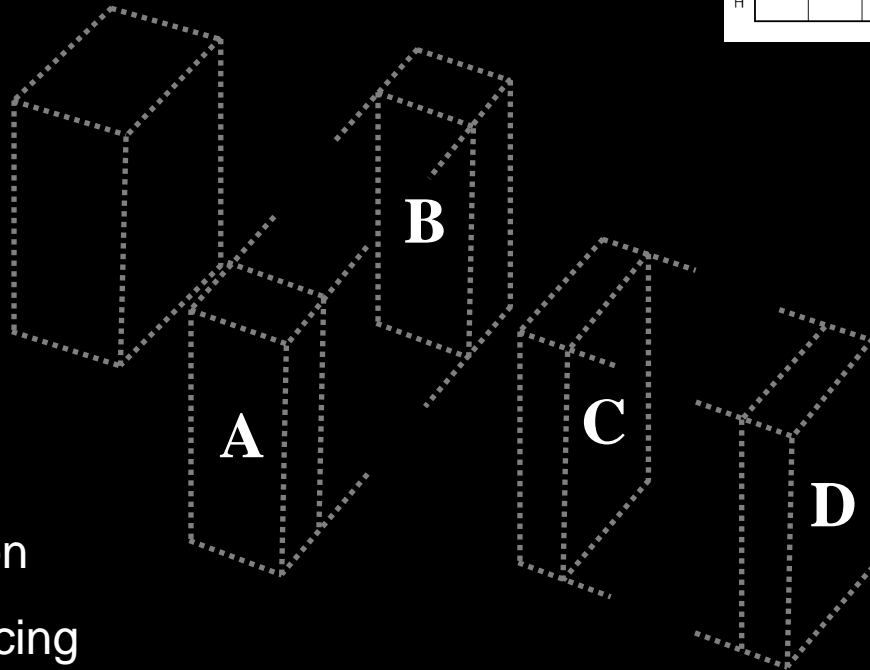
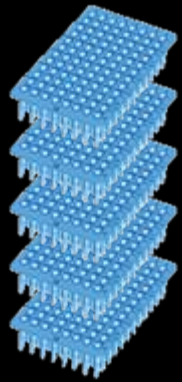
12,000 M2
plants/tower

Towers are screened as half tower pools (HTP)

HTP stock plate (52 templates)

	1	2	3	4	5	6	7	8	9	10	11	12
A		TOWER 2				TOWER 9						
B		TOWER 3				TOWER 10						
C		TOWER 4				TOWER 11						
D		TOWER 6				TOWER 12						
E		TOWER 7				TOWER 13						
F		TOWER 8				TOWER 14						
G		TOWER 1										
H												

pre-digested templates

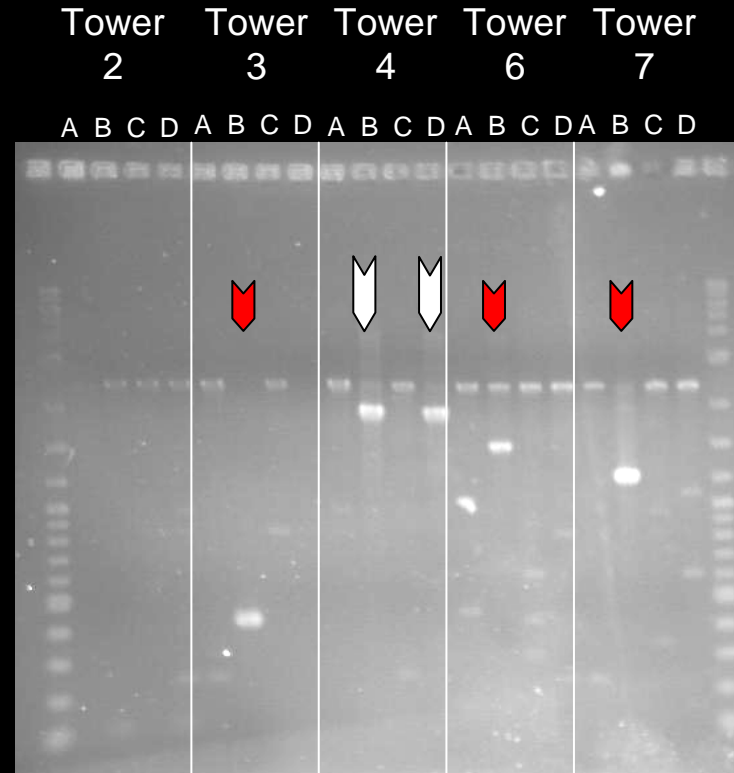
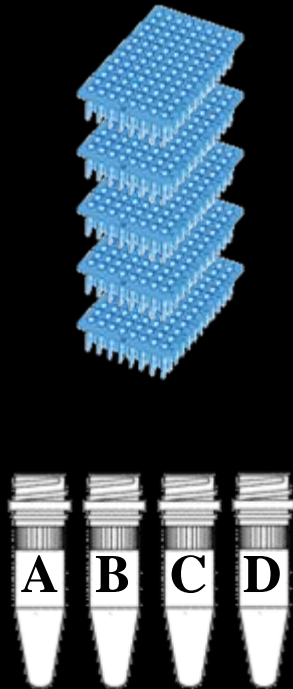


dual detection
dual sequencing

$\frac{1}{2}$ Tower pool
= 12000 haploid
genomes

A Medicago De-TILLING assay

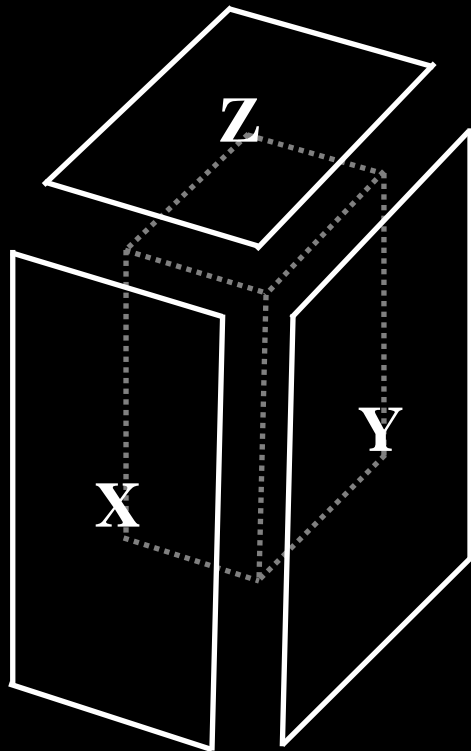
dual detection
dual sequencing



Real detection events (↯)
spurious PCR products (↯)

Locating the mutant

Aim: To identify a single mutant containing family



3-dimensional pools

X pools

12 templates

Y pools

8 templates

Z pools

5 templates

25 PCRs to identify
single pool

