NEW ZEALAND WINE
PURE DISCOVERY

Lincoln University
Te Whare Wānaka o Aoraki
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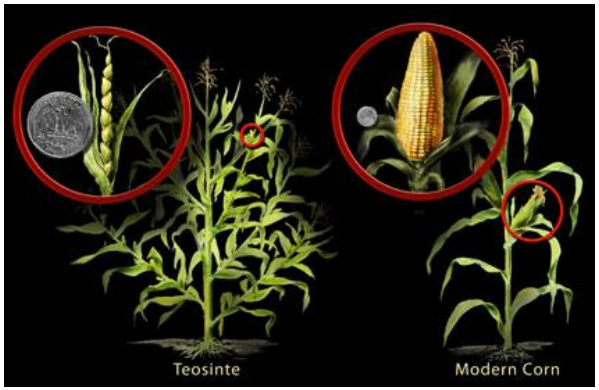
Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

Exploring the use of transposon mobilisation to produce a gene-tagged population for grapevine

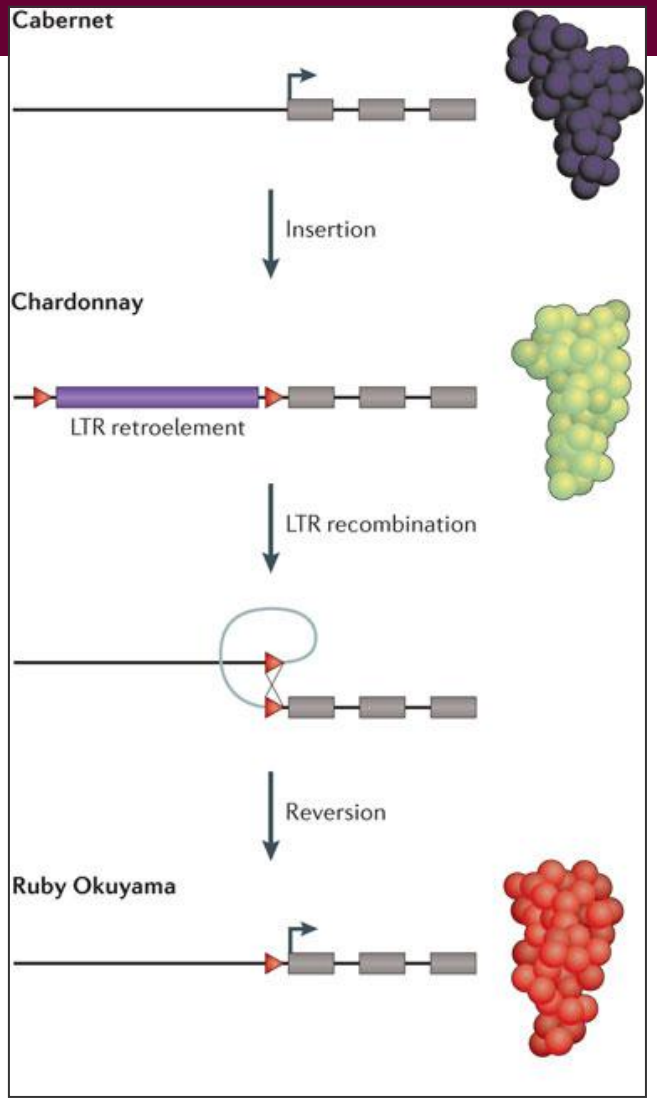
Ross Bicknell, Chris Winefield, Darrell Lizamore, Ting-Hsuan Chan, Tim Millar, Susan Thomson, Justine Larrouy, Philippa Barrell, Michelle Thompson, Sylvia Erasmuson and Dominik Merkert

The Role of Transposons (TE's) in Crop Improvement

- **Maize** (*Zea mays*): A Mite TE insertion near the *tb1* gene was a key event in the domestication of maize (Studer et al. 2011) .
- **Nectarines** (*Prunus persica*) are hairless peaches. Vendramin et al. (2014) found that the fuzzy-less nectarine phenotype is caused by a TE in exon 3 of the *PpeMYB25* gene.
- **Cauliflower** (*Brassica oleracea* var botrytis):
  - The purple mutant was found to be caused by a TE upregulating a DNA regulatory region controlling anthocyanin production (Chiu, Zhou et al. 2010).
  - The orange coloured mutant accumulates high levels of b-carotene in the curd, was found to be caused by a TE insertion into the *Or* gene (Lu, Van Eck et al. 2006).



Transposon-induced Colour Change in Grape



Lisch, D. (2013) Nature Reviews Genetics; **14**, 49–61.

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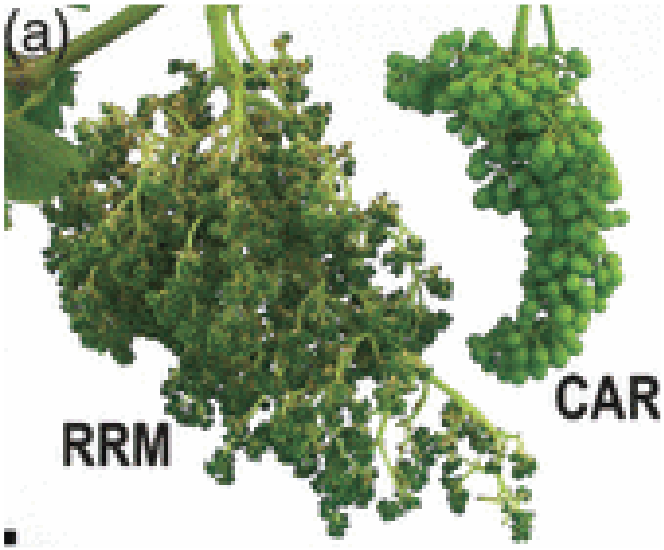
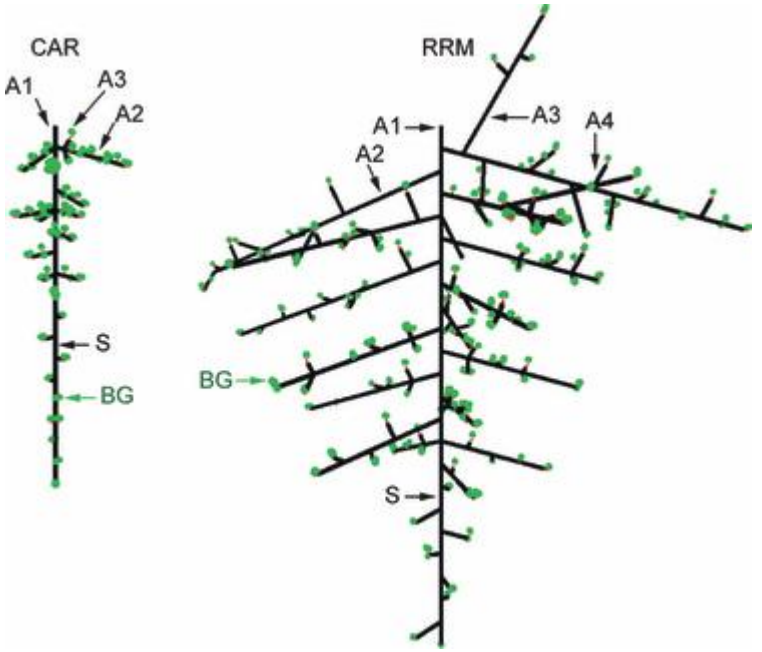
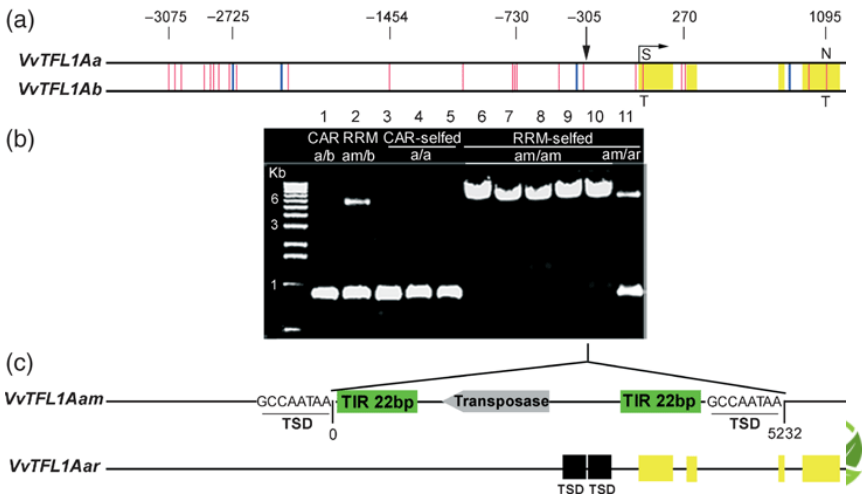
The *Reiterated Reproductive Meristem* 'RRM' Mutant





The result of a transposed hAT element into the promoter of *VvTFL1A*, a meristem identity factor in the variety Carignan.

Fernandez et al. (2010). *Plant Journal 61*, ***545-557.***



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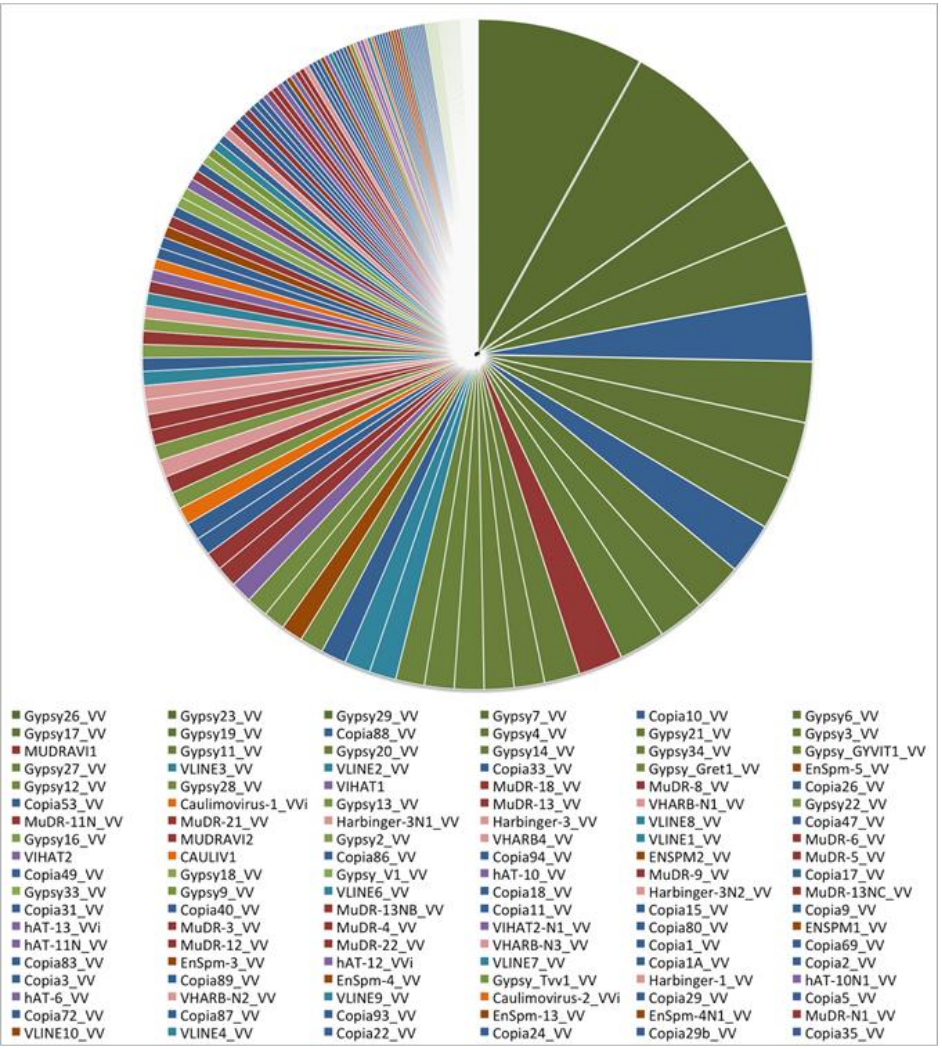
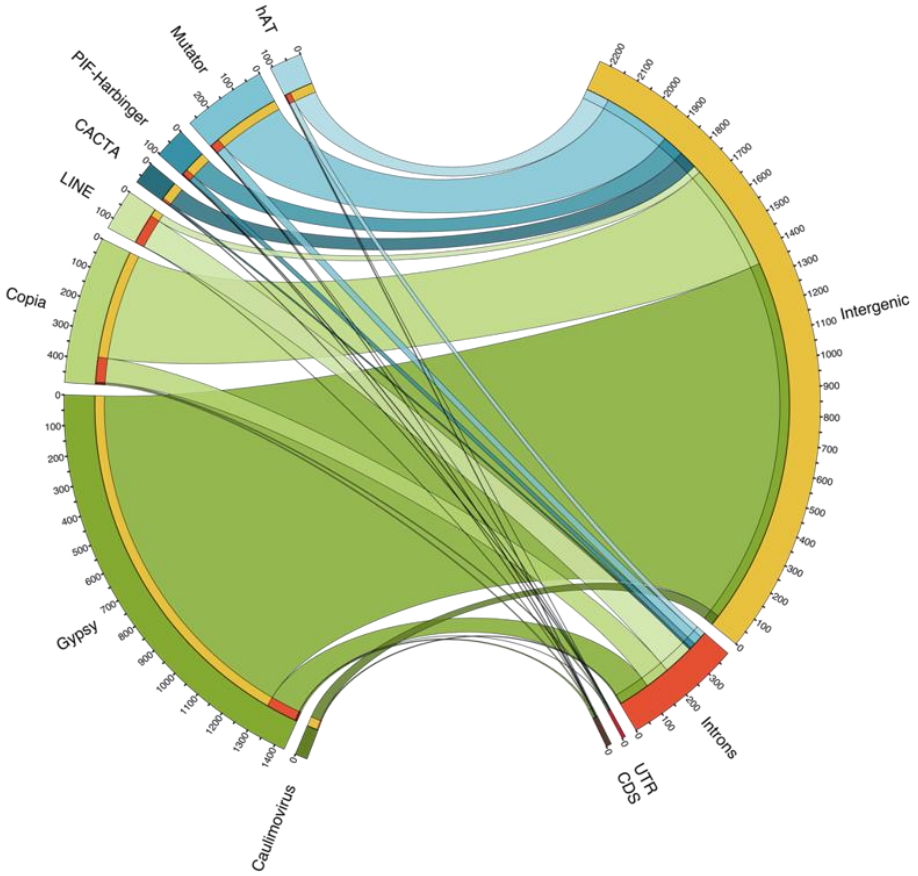
Grape TE Programme Goals

- Determine the TE diversity in grape
- Establish reference genomes for our key varietals
- Establish tissue culture systems for TE mutagenesis
- Determine treatments that mobilise TE's in grape
  - Induction of transcription
  - Achievement of integration
  - Tools required to measure these events accurately
- Genotyping and Phenotyping
- Build a web-based browser for data visualisation
- Make the data available for forward and reverse genetic approaches

Transposable Elements in Grape

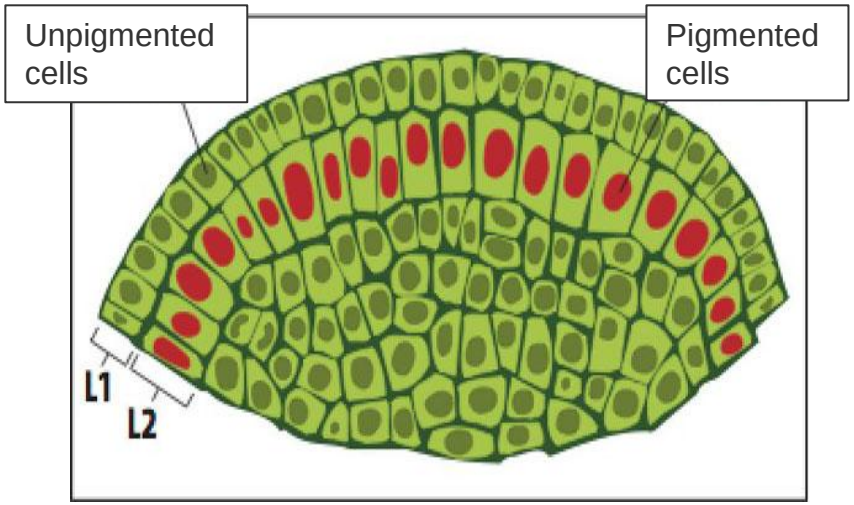




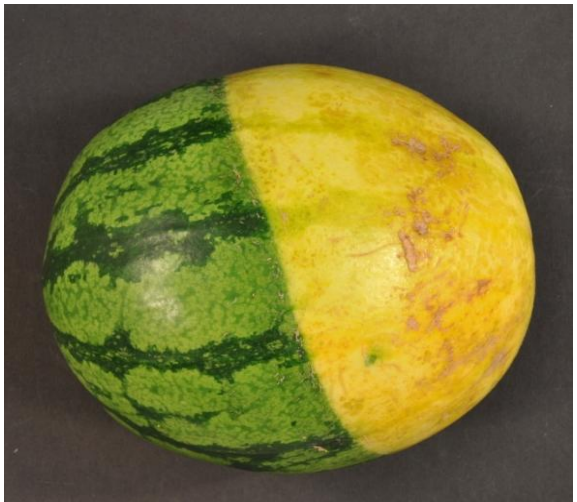
TE's make up 40-50% of the grape genome, 32,500 different types identified, over 220,000 copies per cell

Transposon Movements are Cell-autonomous Events. They Result in Chimerism

Periclinal chimeras



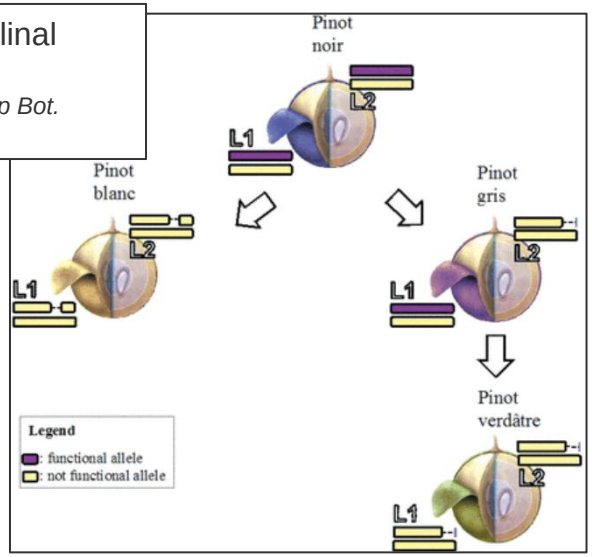
Anticlinal chimeras



Chimerism – Genetic Variation Within a Plant

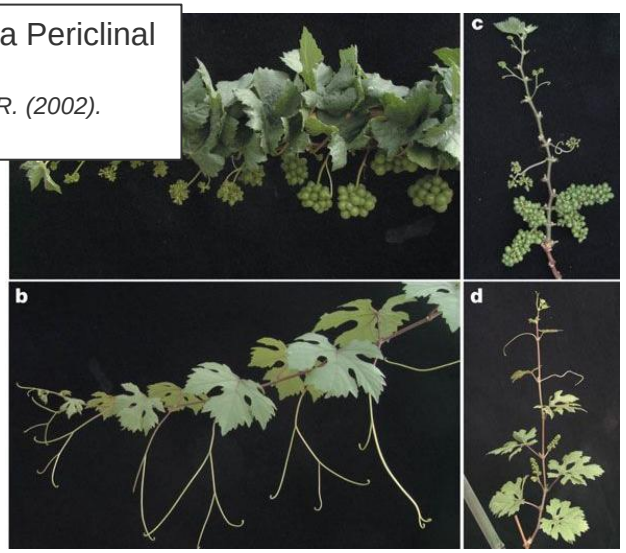
Pinot gris is a Periclinal chimera

Vezzulli et al. (2012) J Exp Bot. 63:6359-6369



Pinot meunier is a Periclinal chimera

Boss and Thomas, M.R. (2002). Nature 416, 847-850

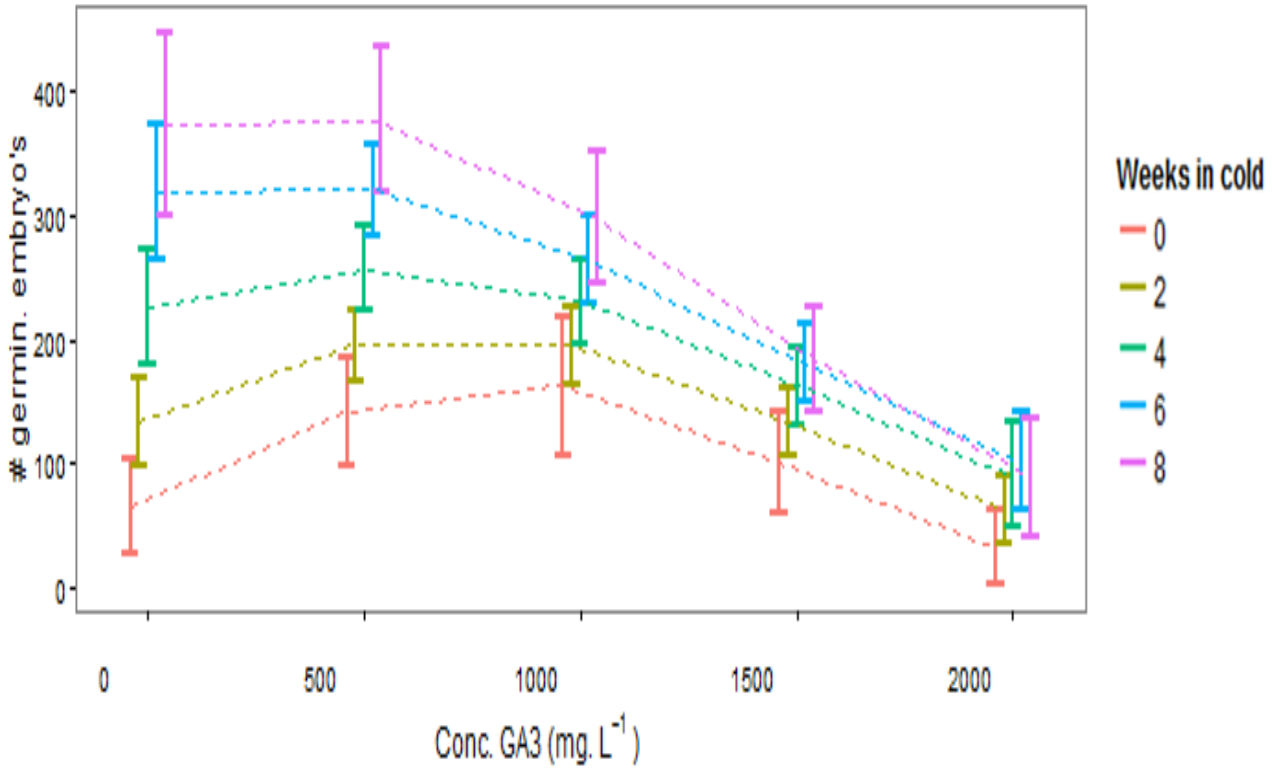


An anticlinal chimera of Sauvignon blanc

To minimise chimeras we use somatic embryogenesis

Optimising Somatic Embryogenesis in Grape

Embryo formation and germination can both be stimulated by cold and GA3 treatment



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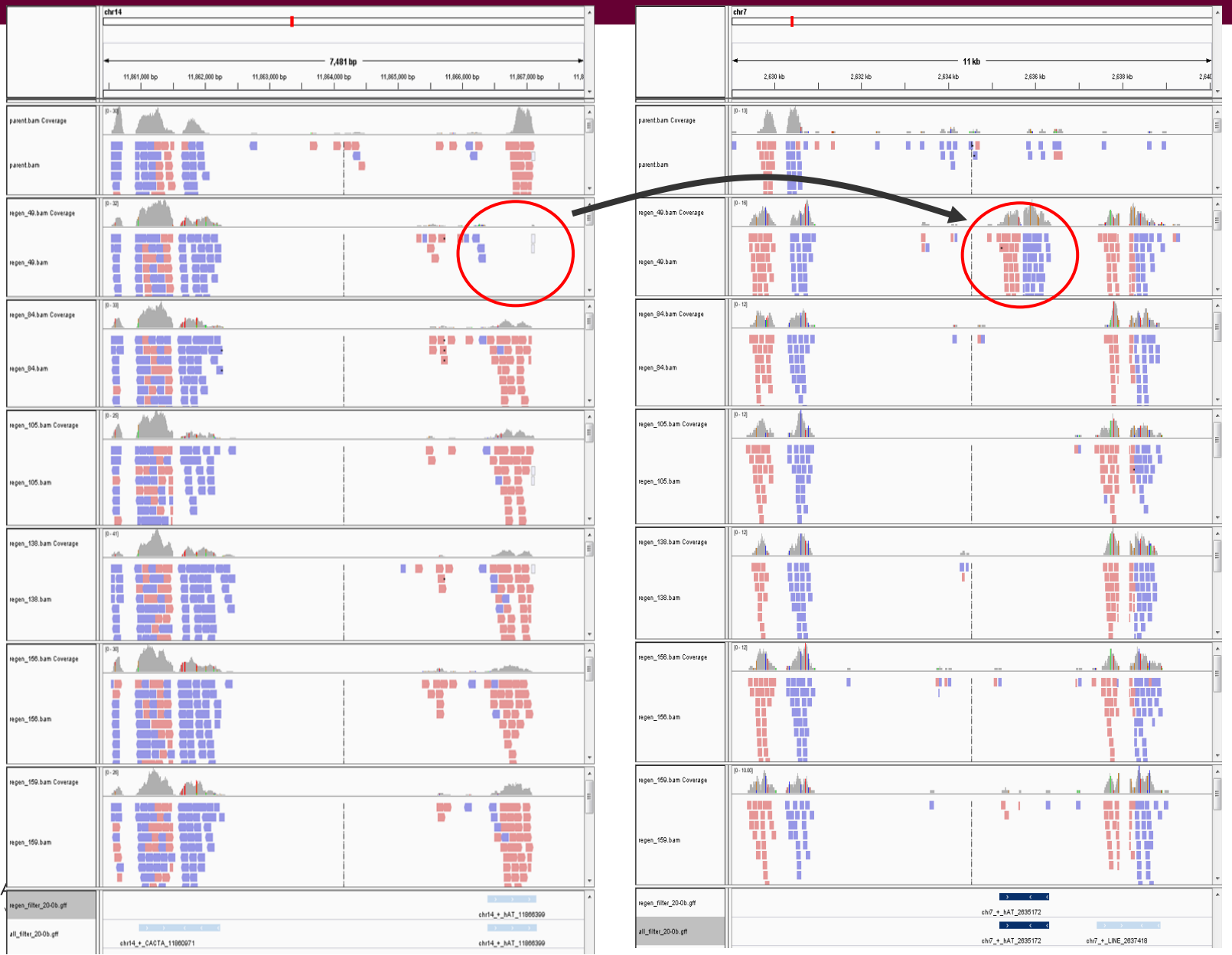
Quantification of TE Mobilisation ('TE Fingerprint')

- Aim: To identify new insertions in each individual
- **TE Fingerprint** (Tim Millar and Susan Thomson)
  - A computer programme written in Python
- Input data:
  - Paired end sequence data (Illumina)
  - Library of transposon sequences
  - Grape genome sequence
- Output
  - Identify paired reads at TE boundaries
  - Map those and identify differences between individuals

hAT Element Relocation (using ‘TE Fingerprint’)



New Gypsy Insertion (using ‘TE Fingerprint’)

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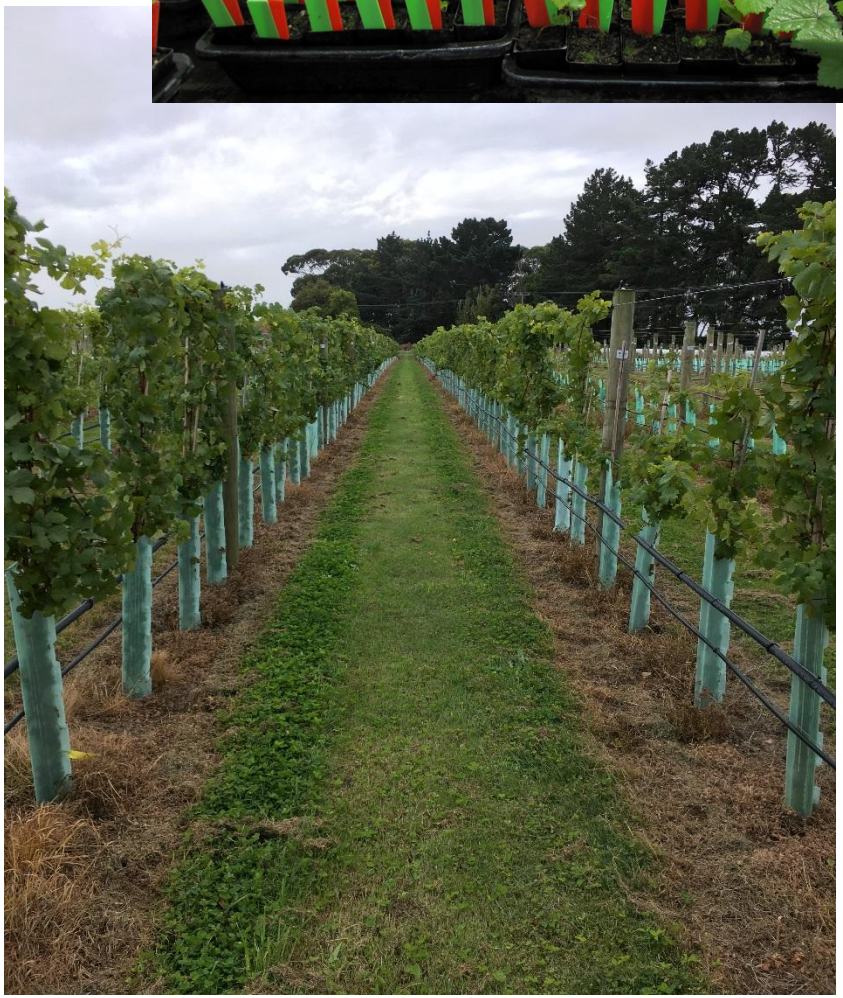
Field Planting

- 1,300 somaclones planted to-date
- Another 1,200 ready to plant next season
- The first 100 fully genotyped
- The first fruit was seen in the 2017/18 season



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Somaclonal Mutants

- One PN clone has altered dormancy. It breaks buds in mid winter and goes dormant in mid summer
- Three PN clones that are more susceptible to powdery mildew infection
- Two PN clones with a bushy habit (reduced apical dominance)
- Several plants with reduced vigour
- Fruit and bunch changes ? (too soon)

Thank You

UNCOVERING THE GENETIC AND EPIGENETIC IMPACT OF A TRANSPOSON BURST IN GRAPEVINE

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[2]Plant and food research, Lincoln, Canterbury, New Zealand.

Introduction

Endogenous transposable elements (TEs) can be stimulated, through a combination of tissue culture and stress treatments, to produce novel genetic diversity in plants. Using this approach, we are generating a **population of novel grapevine somaclonal mutants** as a resource for gene function studies and as novel germplasm for and the wine industry.

Figure 1: A sample of the variety in leaf shape phenotypes observed among vines regenerated from the same cell cultures

Novel molecular and bioinformatic tools enable semi-automated, **high throughput genotyping of genomic TE insertion sites** in each vine, so that a searchable **database of genetic variation** accompanies the population.

Whole genome bisulphite sequencing of a subset of these plants will also reveal the genome-wide epigenetic impact of a temporary loss of TE silencing and the consequent TE mutagenesis, a fundamental process in eukaryotic evolution.

Results & Discussion

Transposon-enriched sequencing requires **less than 1% of the genome** be sequenced to genotype greater than **85% of transposon insertion sites**.

Approximately **60,000 transposon insertion sites** are scored per genome.

TEFingerprint allows **rapid comparisons** of transposon loci among enriched sequence libraries.