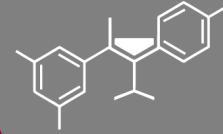


April 2024

Statistical Genomics Workshop



BRAGATO
RESEARCH INSTITUTE
RANGAHU KAREPE, WĀINA O AOTEAROA



**GRAPEVINE
IMPROVEMENT**

New genomics tools for
managing climate
resilience in grapevine &
other clonally-propagated
crops
Darrell Lizamore, Ellie Bradley, Amy
Hill, Cen Liau, Bhanupratap Vanga,
Solomon Wanté, Annabel Whibley

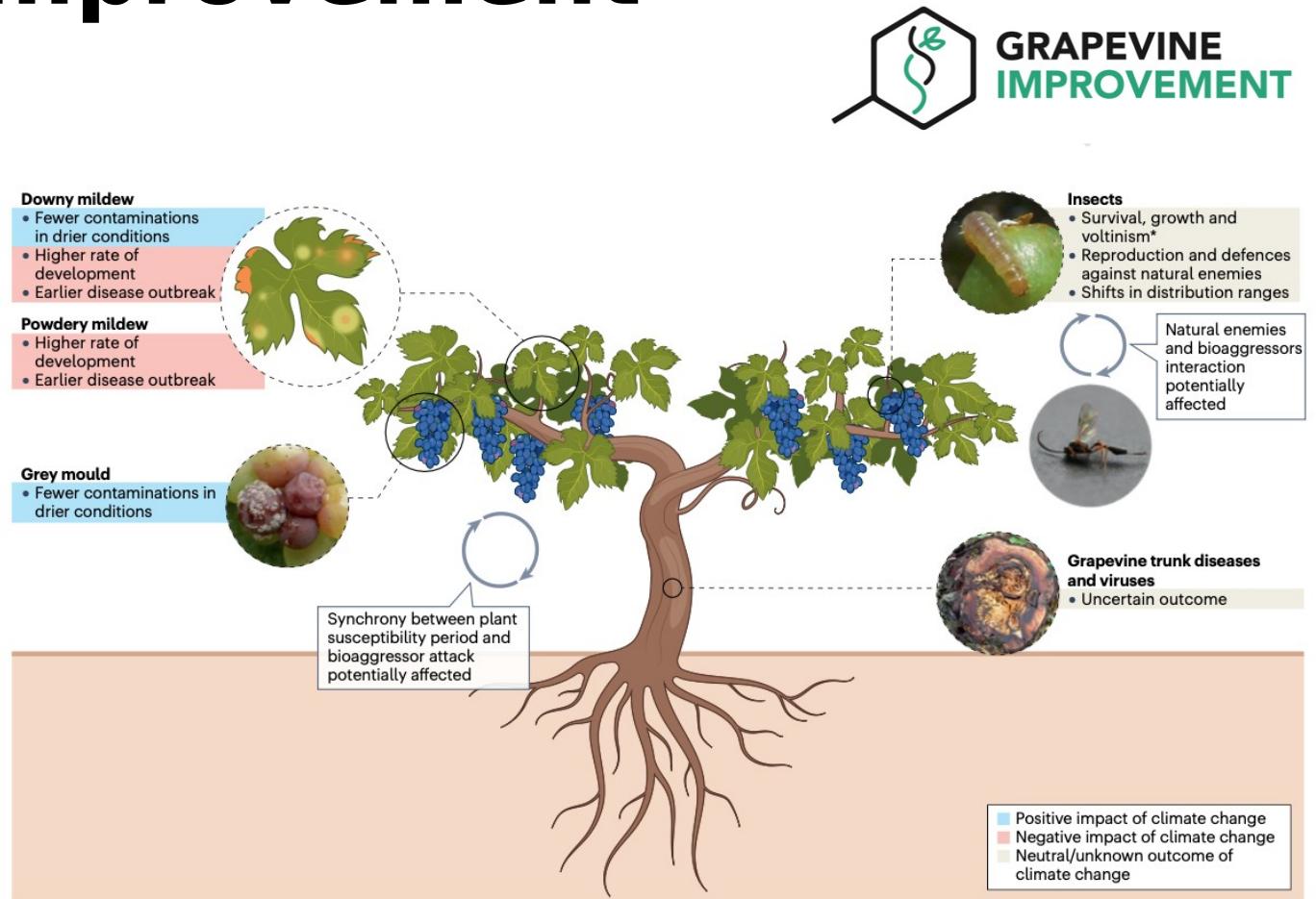
Global grapevine improvement research

Key focus areas:

- **Reducing inputs** (sprays, water, diesel, labour).
- **Pest & disease pressures.**
- **Climate adaptation.**

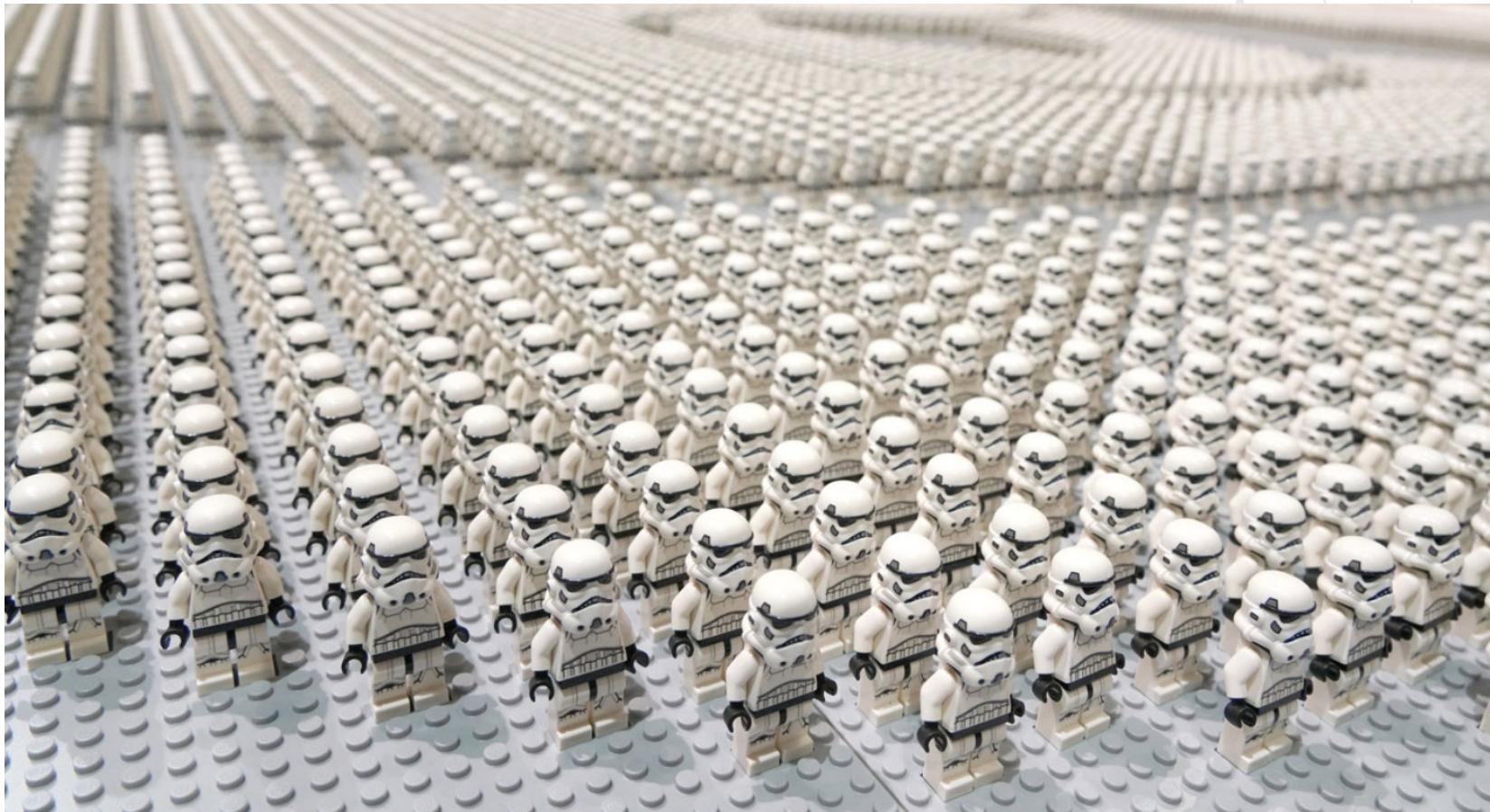
Which translate to investments in:

- The **genetics of resilient plants**.
- **Large datasets** about vines and their environments.
- Improving the **efficiency and speed of breeding**.

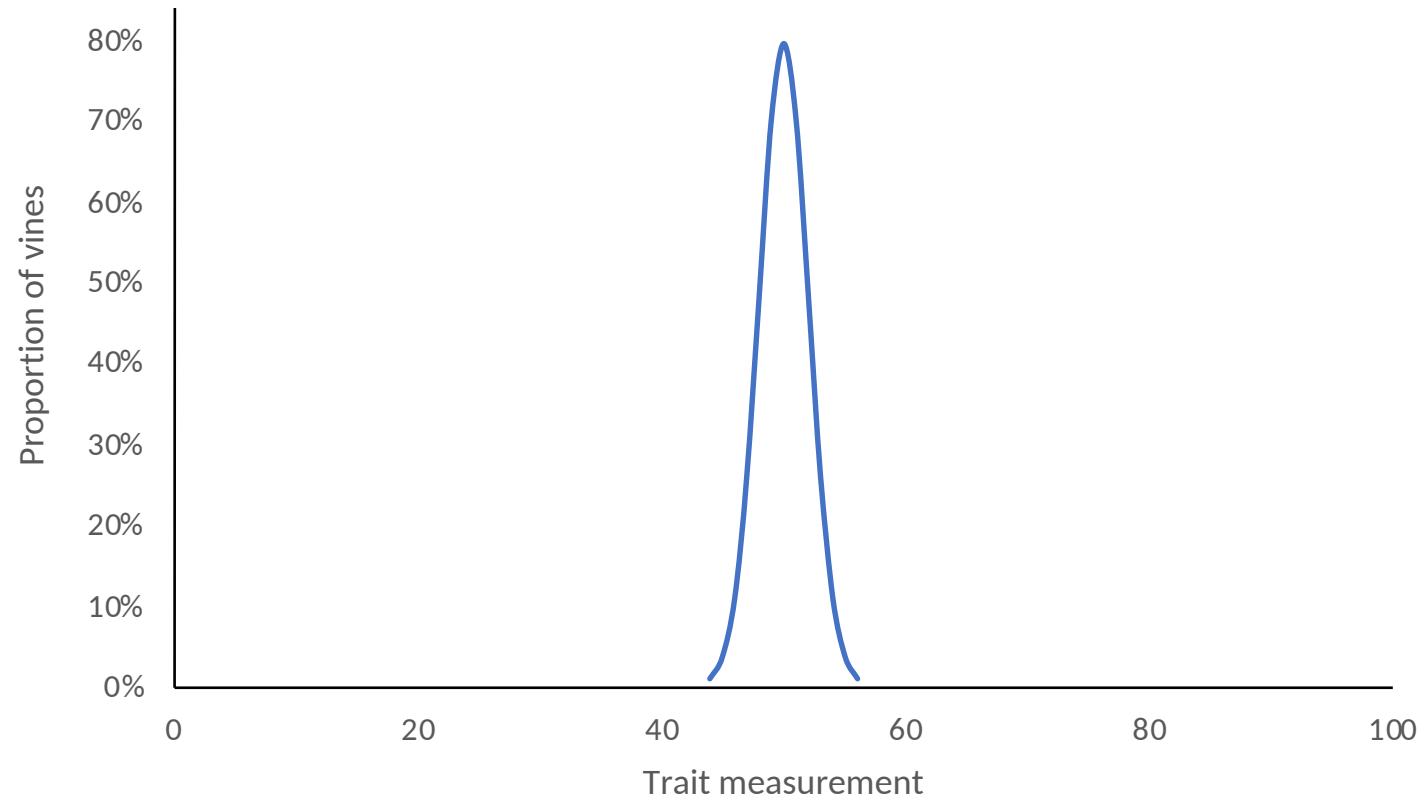


Likely impacts of climate change on grapevine pests & diseases. From van Leeuwen, C., et al. (2024) *Nat Rev Earth Environ.*

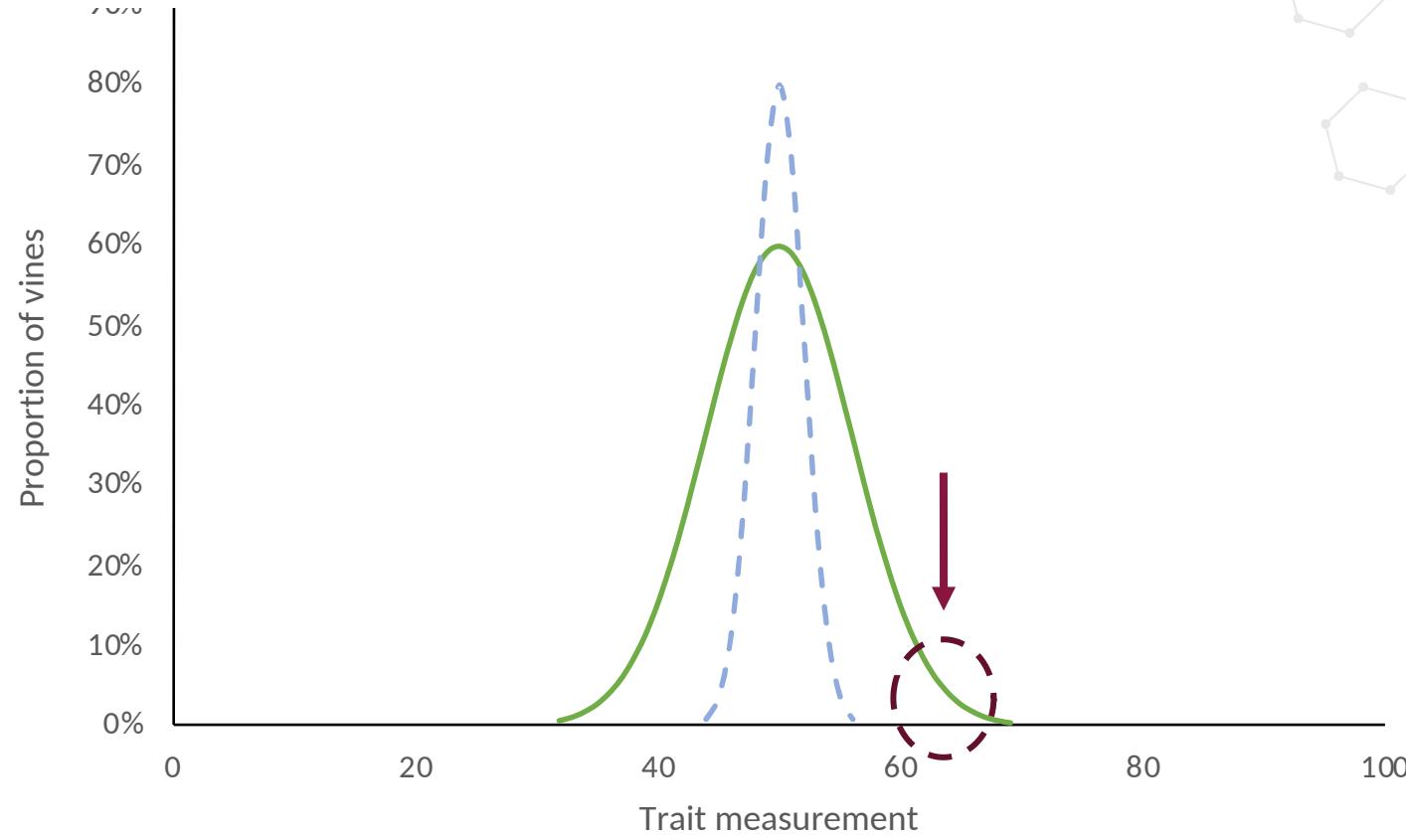
"We're just clones, sir. We're meant to be expendable"



Distribution “spike”



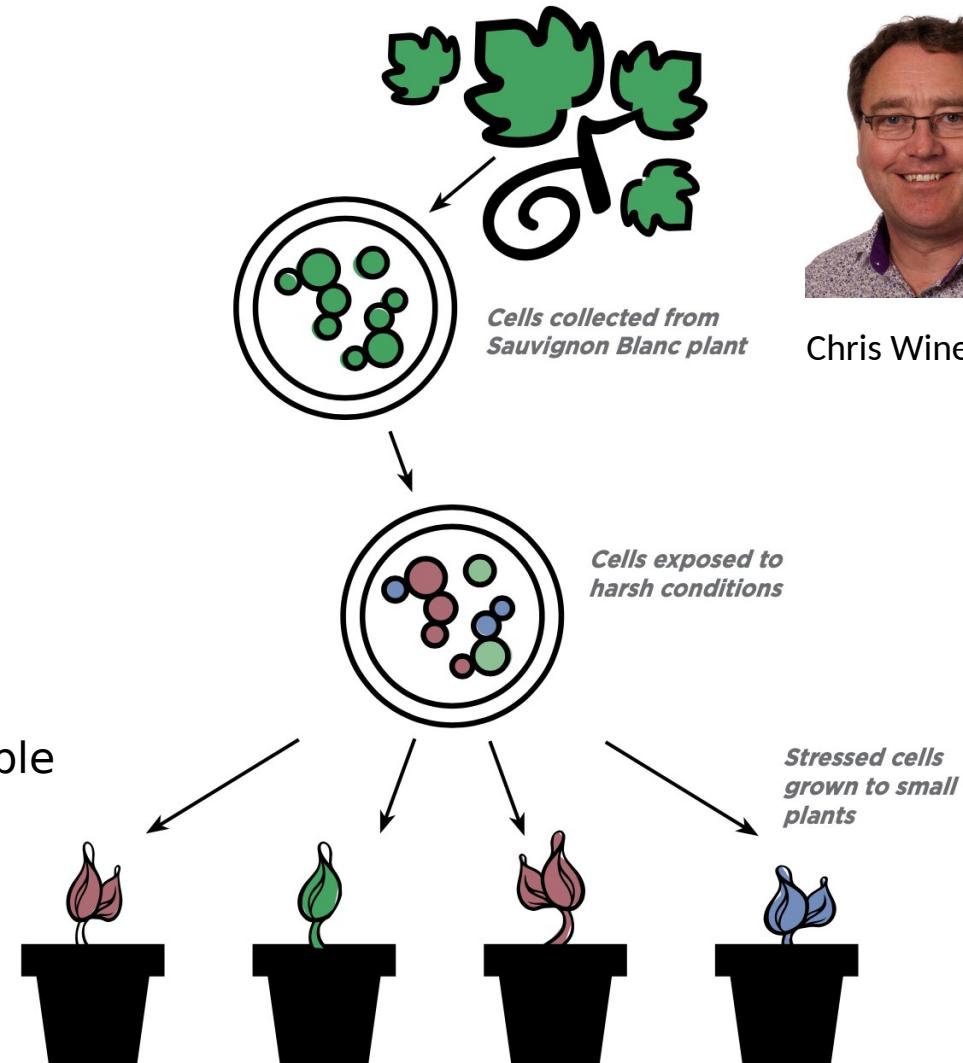
Flattening the curve



Accelerated somatic variation

X-ray source for grapevine improvement

- Varietal ID is an important market signal
- Intravarietal diversity is very limited in NZ
- Clonal selections typically from 'bud sports'
- Knowledge and resources to produce domestic clones by stimulating transposable elements to produce somatic variation
- Advantages over alternatives:
 - Breeding - new variety, directional, multiple backcrosses required
 - NBT - directional, ambivalence, needs functional genomic information



Chris Winefield



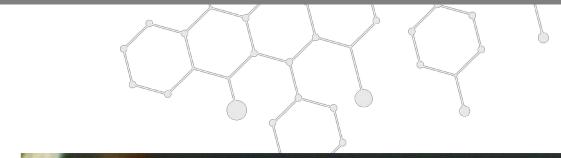
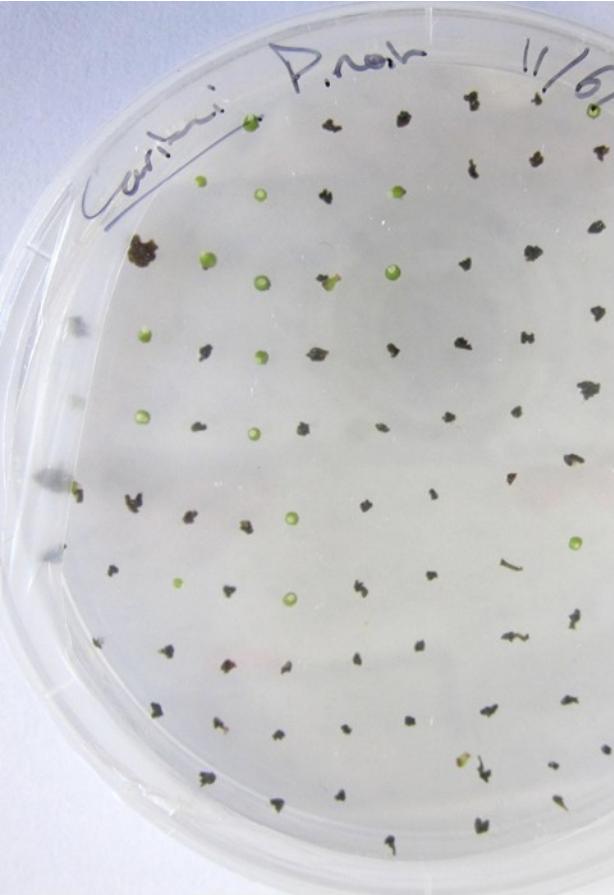
Ross Bicknell

Each plant has changed slightly, and randomly, following its exposure to the stress

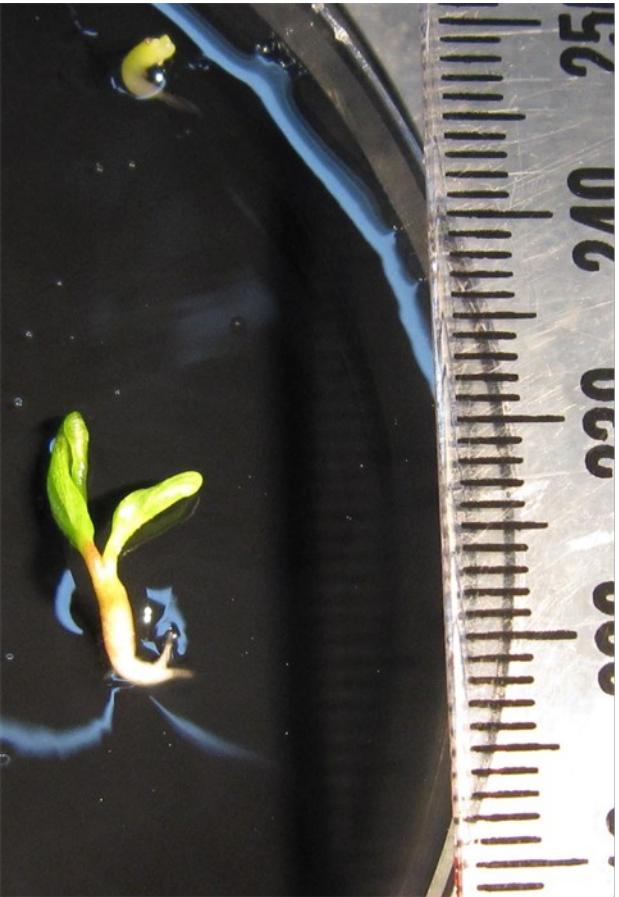
Somatic embryogenesis



Somatic embryogenesis



Somatic embryogenesis

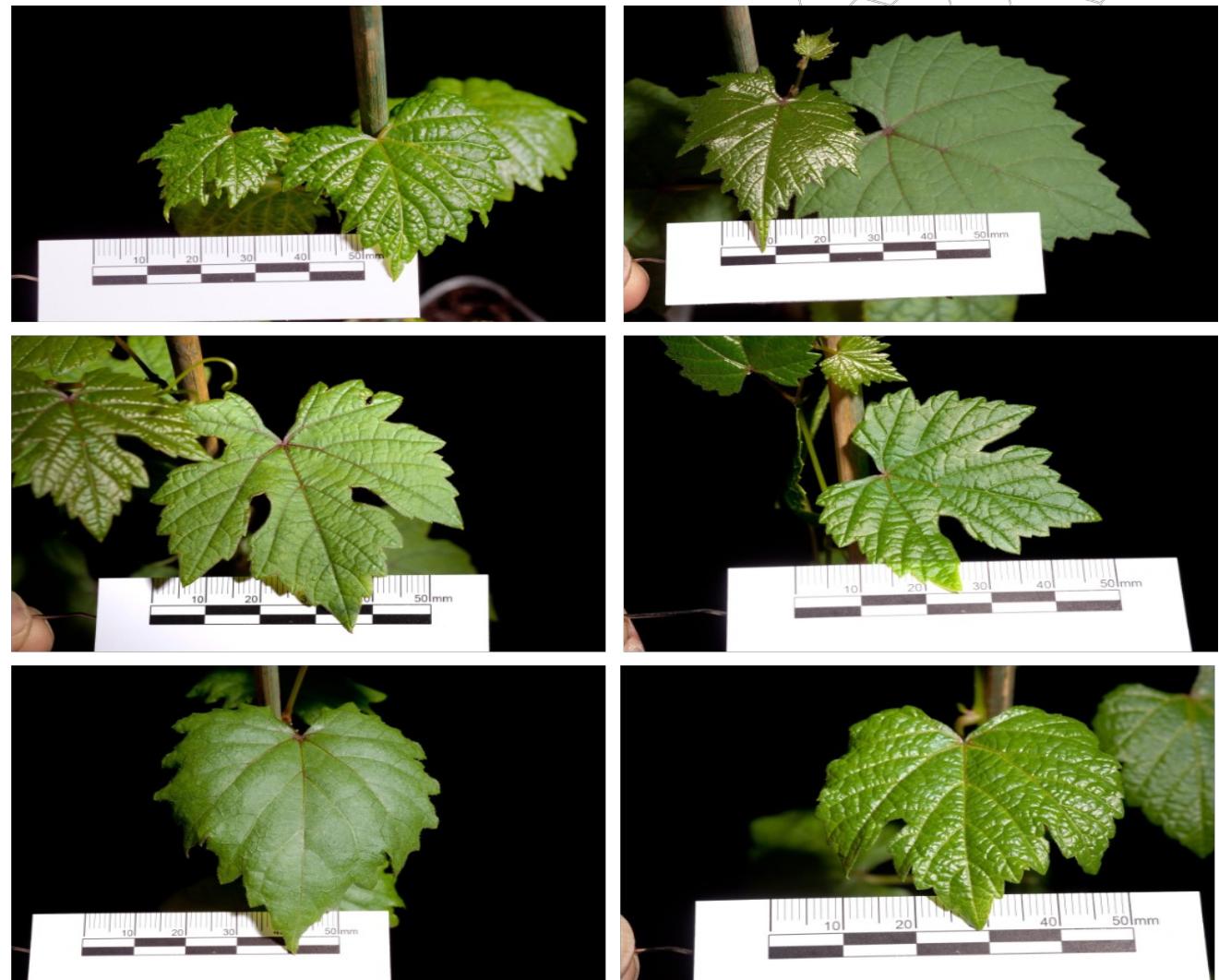


New clones



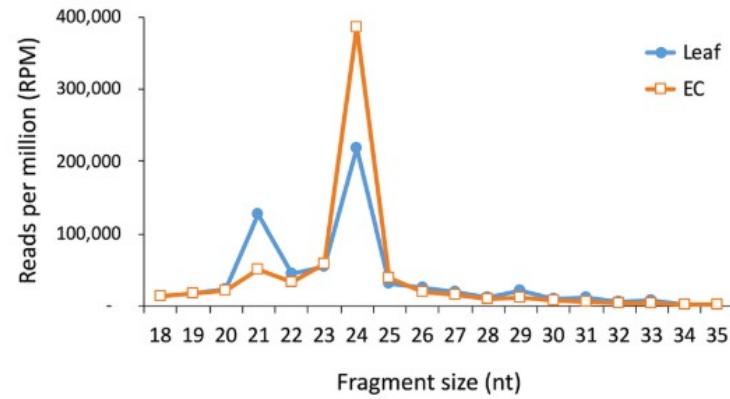
Phenotypes

- Atypical leaf morphology
- Anthocyanin production
- Reversed (summer) dormancy
- Altered susceptibility to powdery mildew
- Increased branching (reduced apical dominance)
- Internode distance

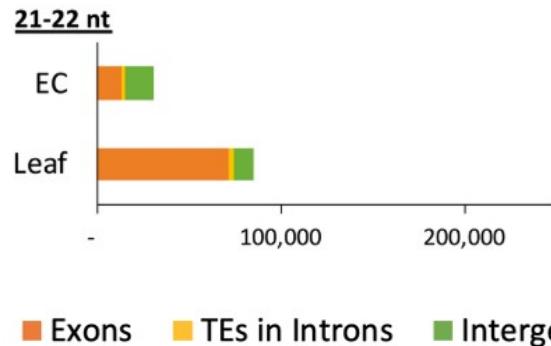
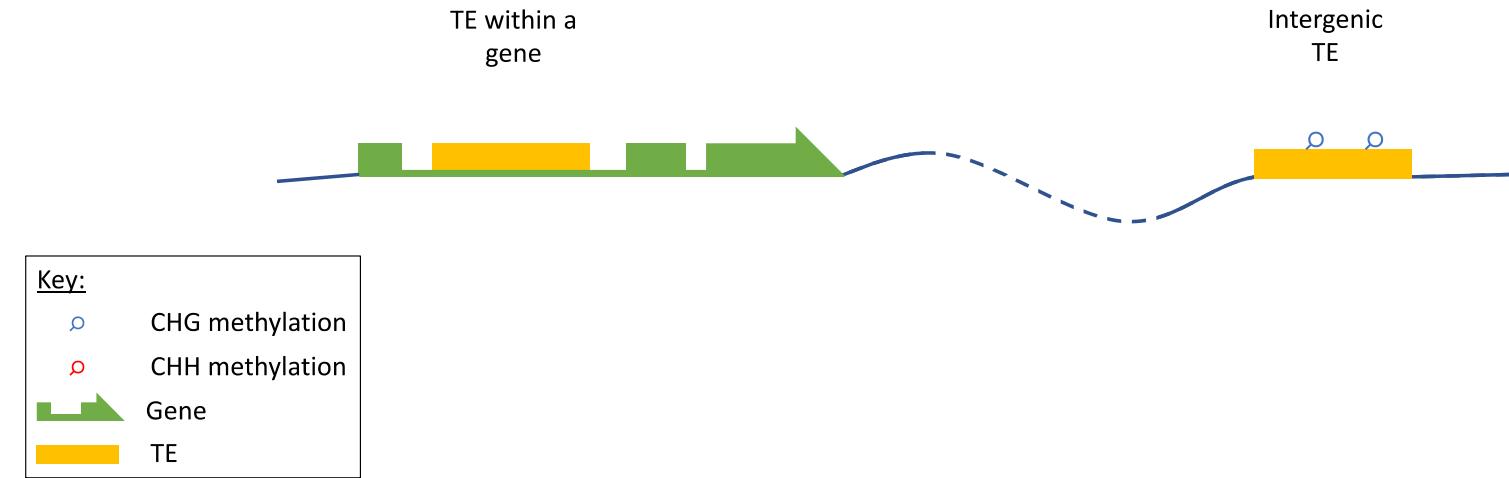


Stimulating TE bursts

- ↓ 21 nt siRNAs

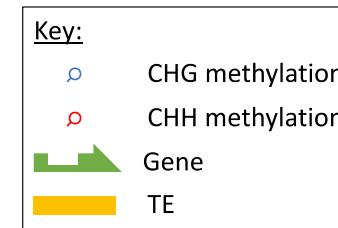
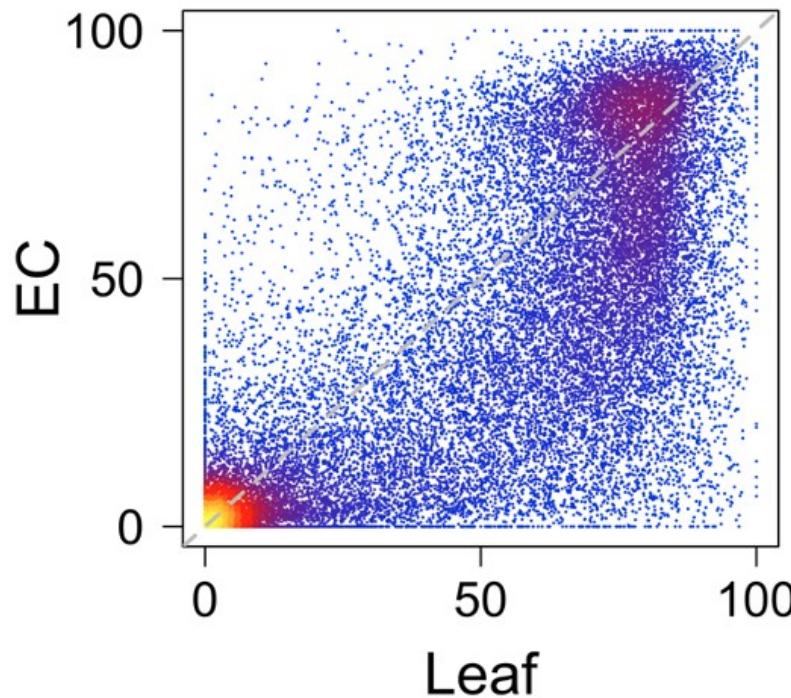


Embryogenic callus:

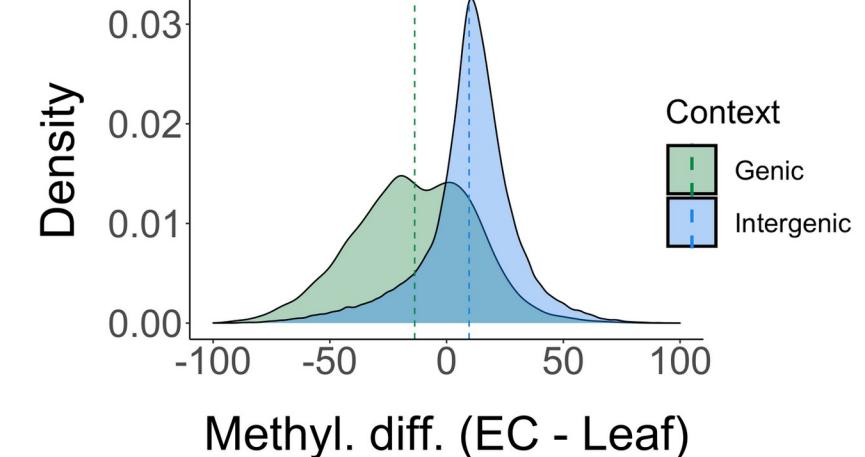
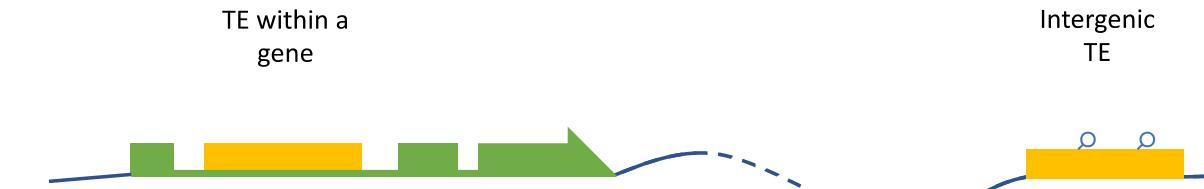


Stimulating TE bursts

- ↓ 21 nt siRNAs
- ↓ CHG methylation of **TEs within genes**

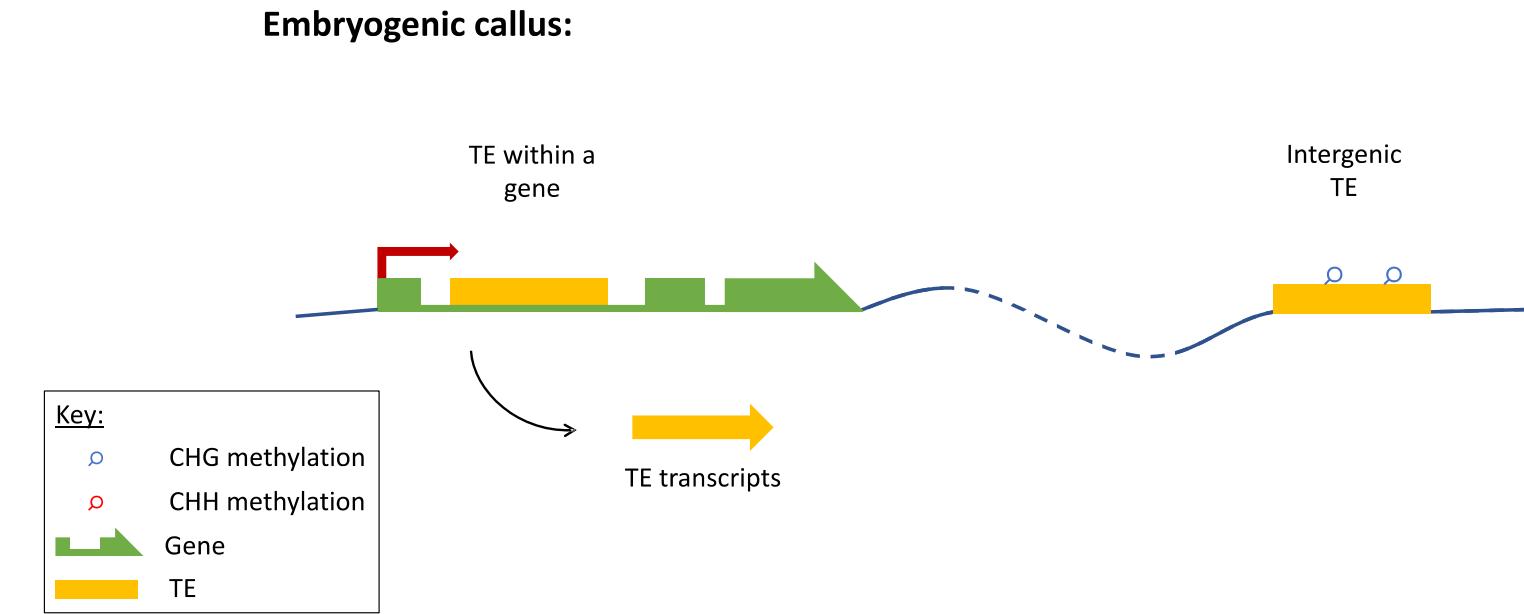
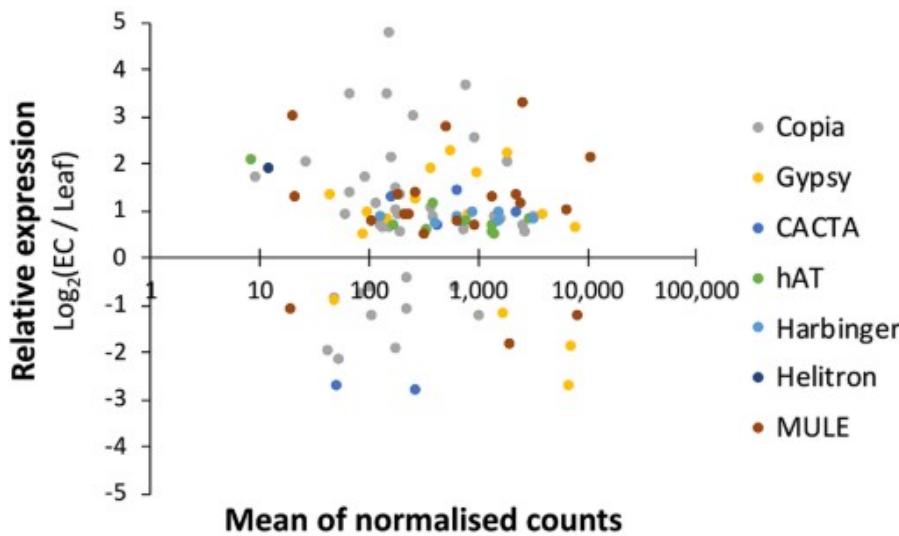


Embryogenic callus:



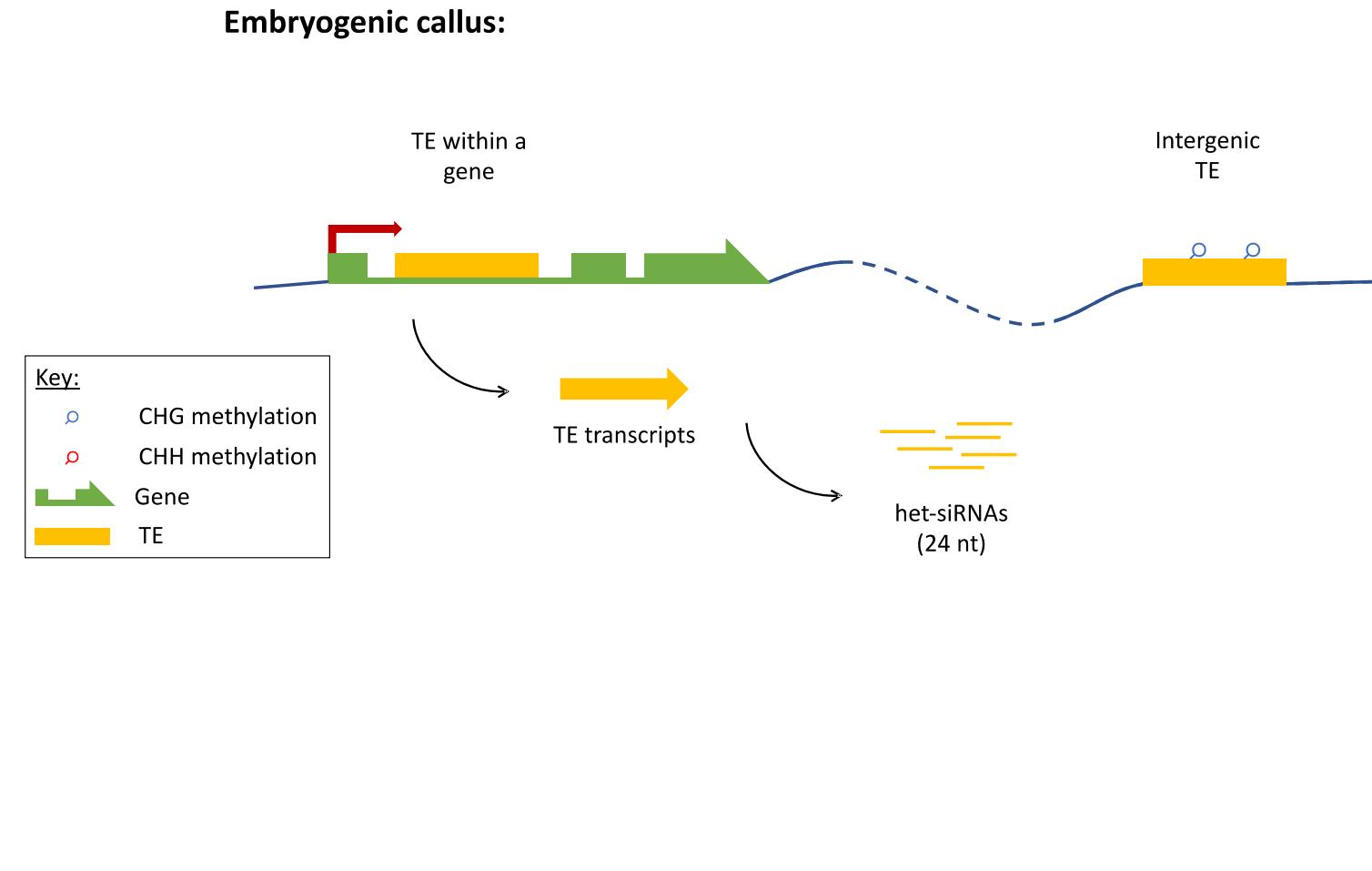
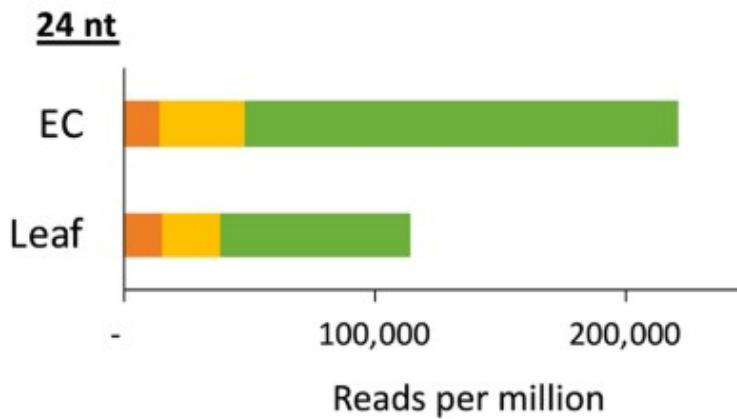
Stimulating TE bursts

- ↓ 21 nt siRNAs
- ↓ CHG methylation of TEs within genes
- ↑ TE expression



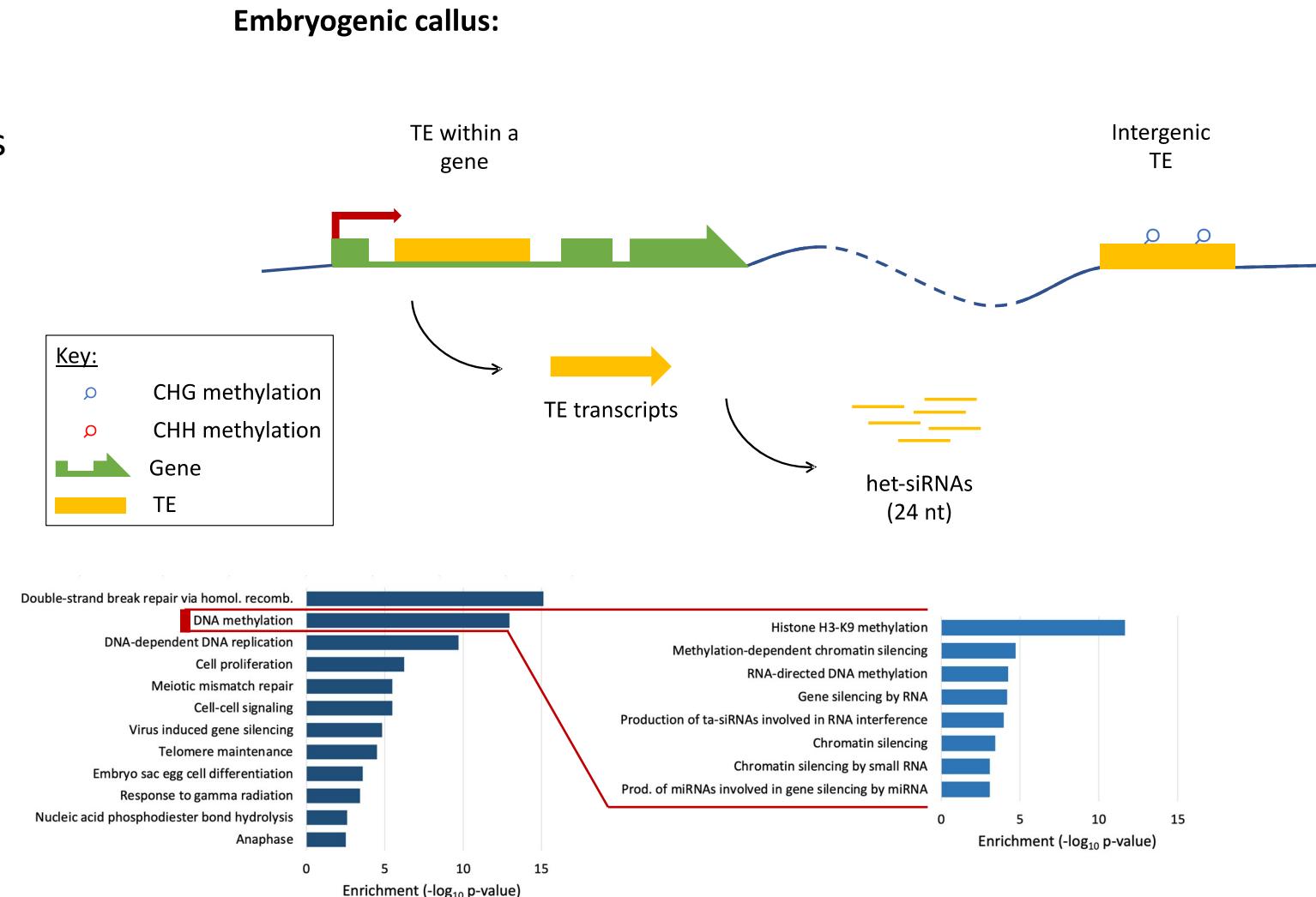
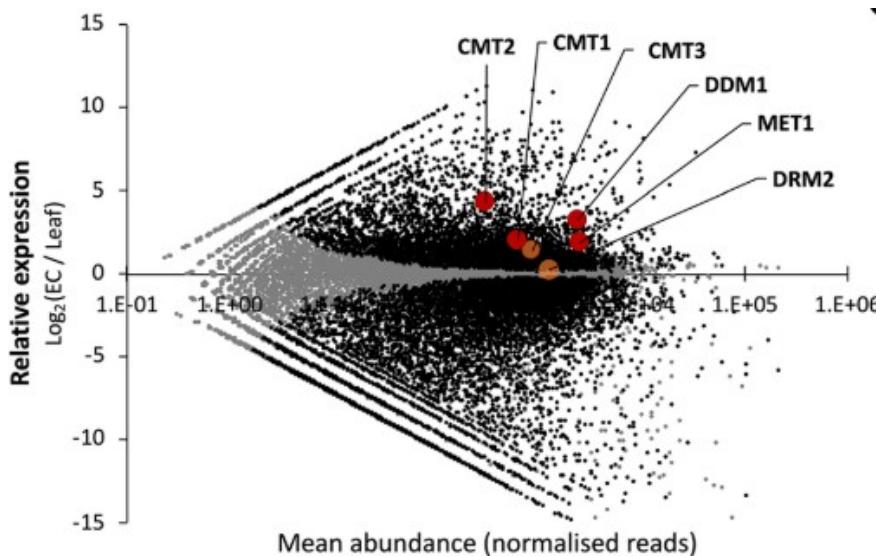
Stimulating TE bursts

- ↓ 21 nt siRNAs
- ↓ CHG methylation of TEs within genes
- ↑ TE expression
- ↑ TE-specific 24 nt siRNAs



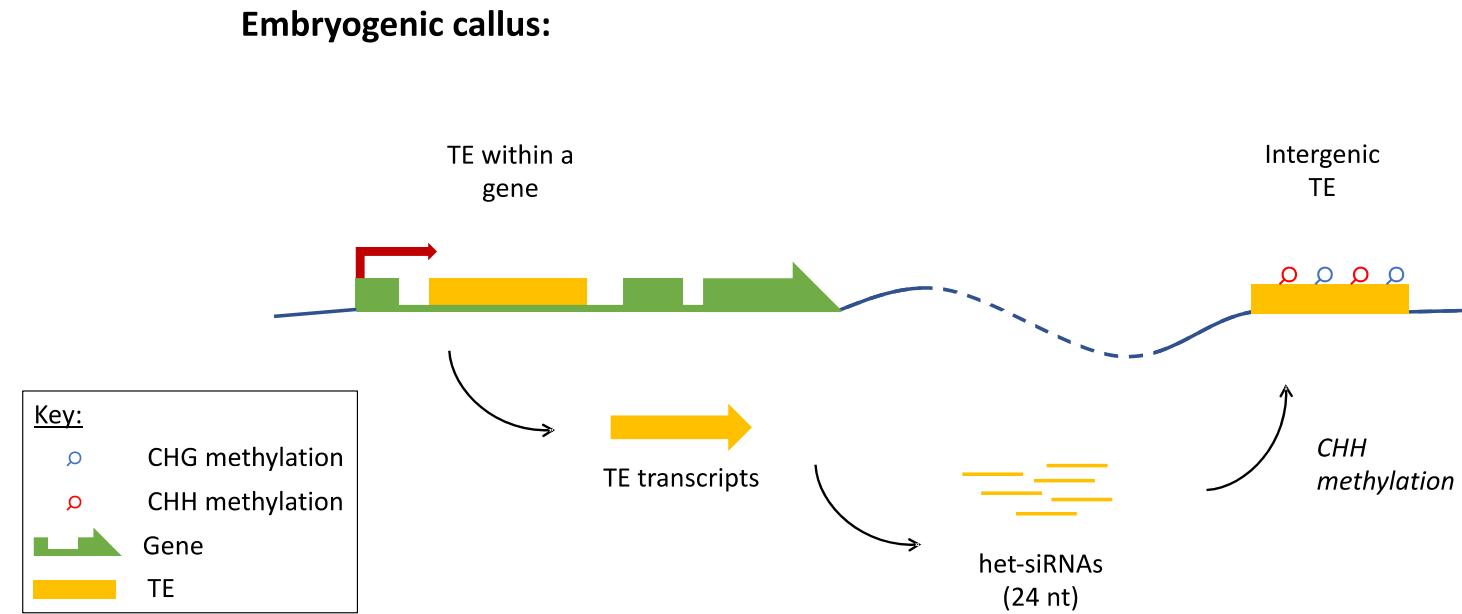
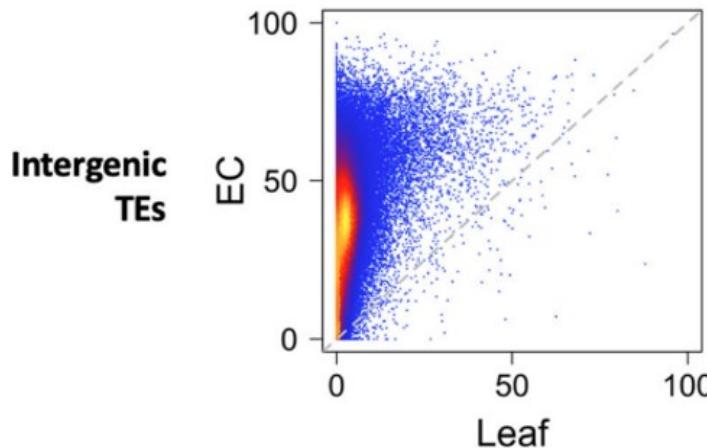
Stimulating TE bursts

- ↓ 21 nt siRNAs
- ↓ CHG methylation of TEs within genes
- ↑ TE expression
- ↑ TE-specific 24 nt siRNAs
- ↑ DNA methyltransferase expression



Stimulating TE bursts

- ↓ 21 nt siRNAs
- ↓ CHG methylation of TEs within genes
- ↑ TE expression
- ↑ TE-specific 24 nt siRNAs
- ↑ DNA methyltransferase expression
- ↑ genome-wide CHH methylation of TEs



Early experiences with short reads

Genome mapping for insert localisation

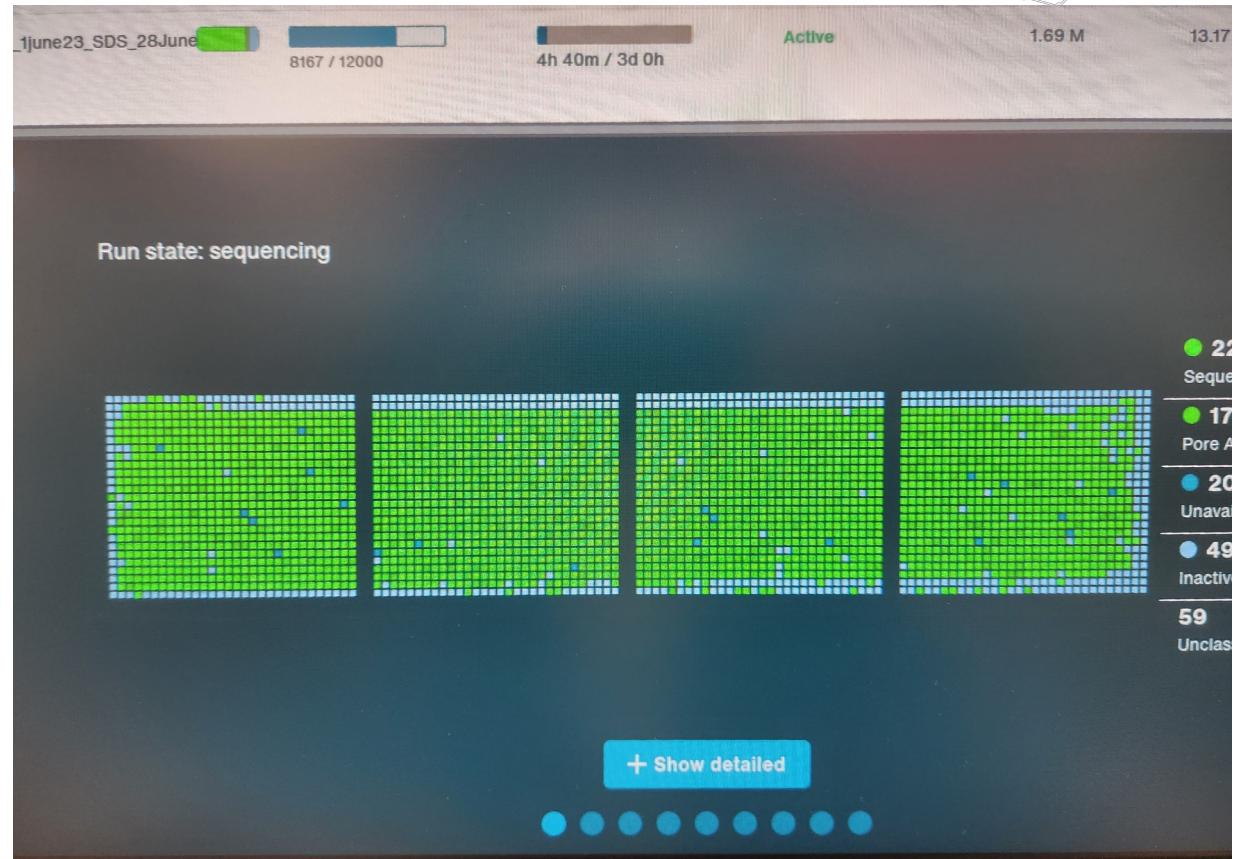
- Probe-based enrichment for mobile elements
- Insertion sites difficult to localise & compare
- Need high depth or longer reads
- Need a good (diploid) reference
- No methylation data
 - Epigenetic variability important for characterising phenotype
 - New somaclones demonstrate epigenetic changes



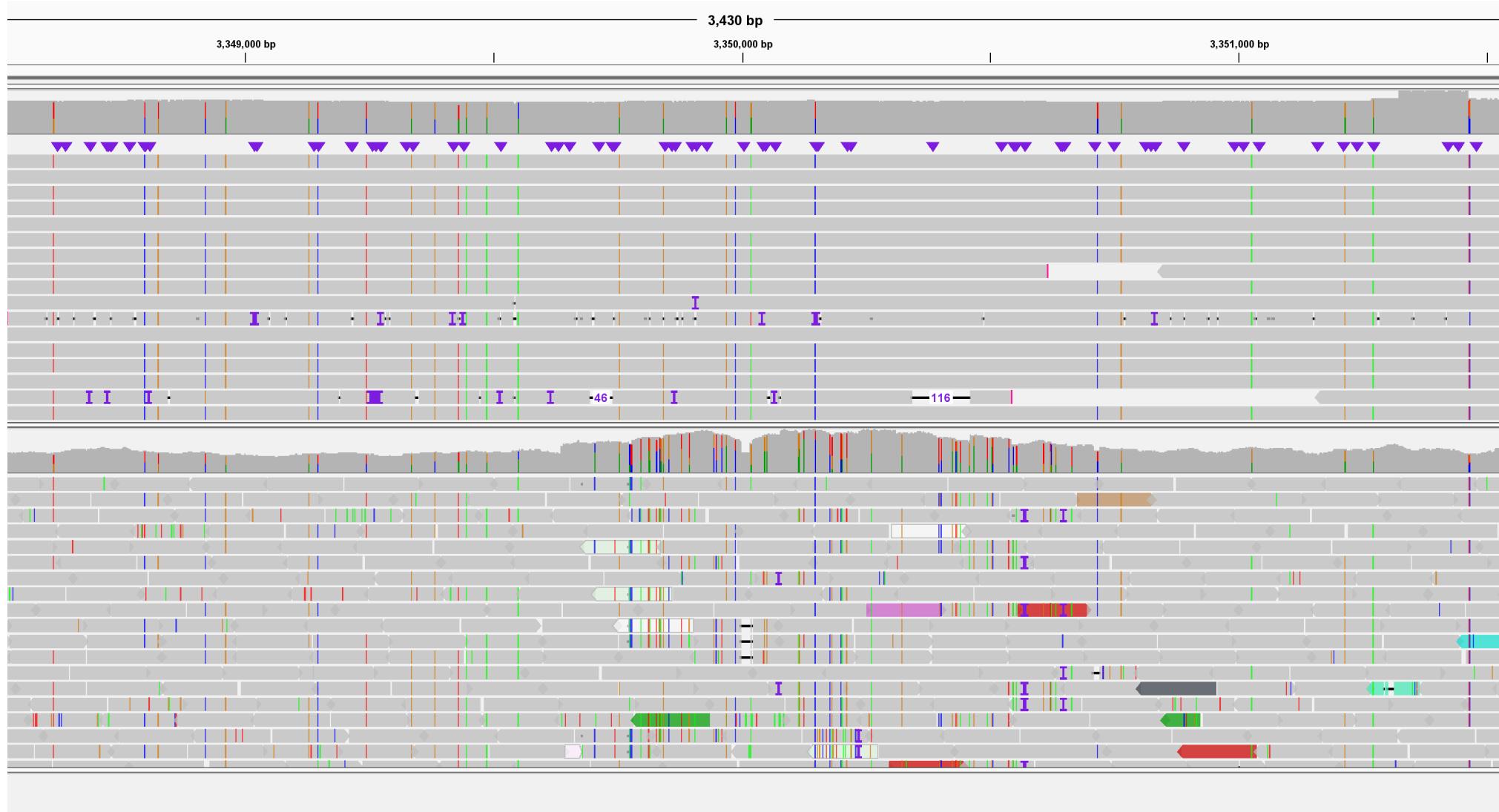
Chris Winefield Susan Thomson

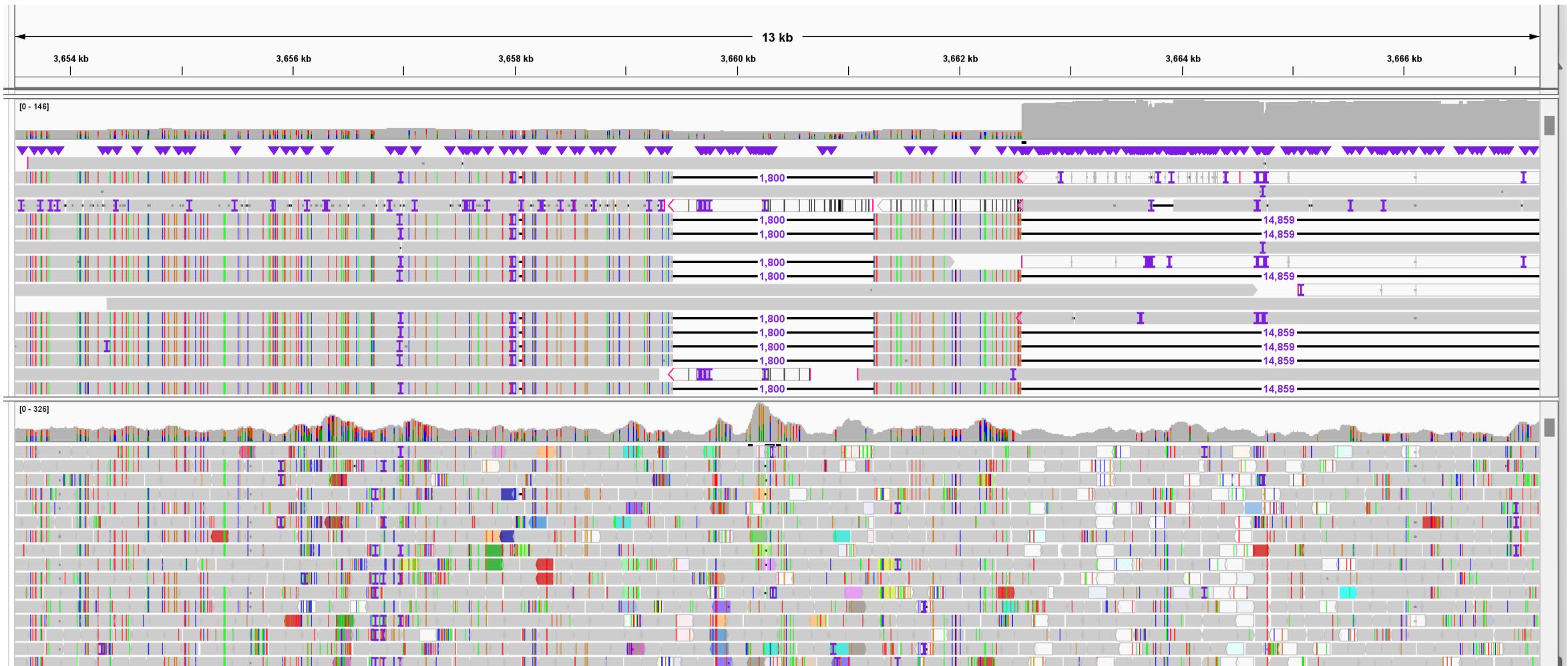
Tim Millar

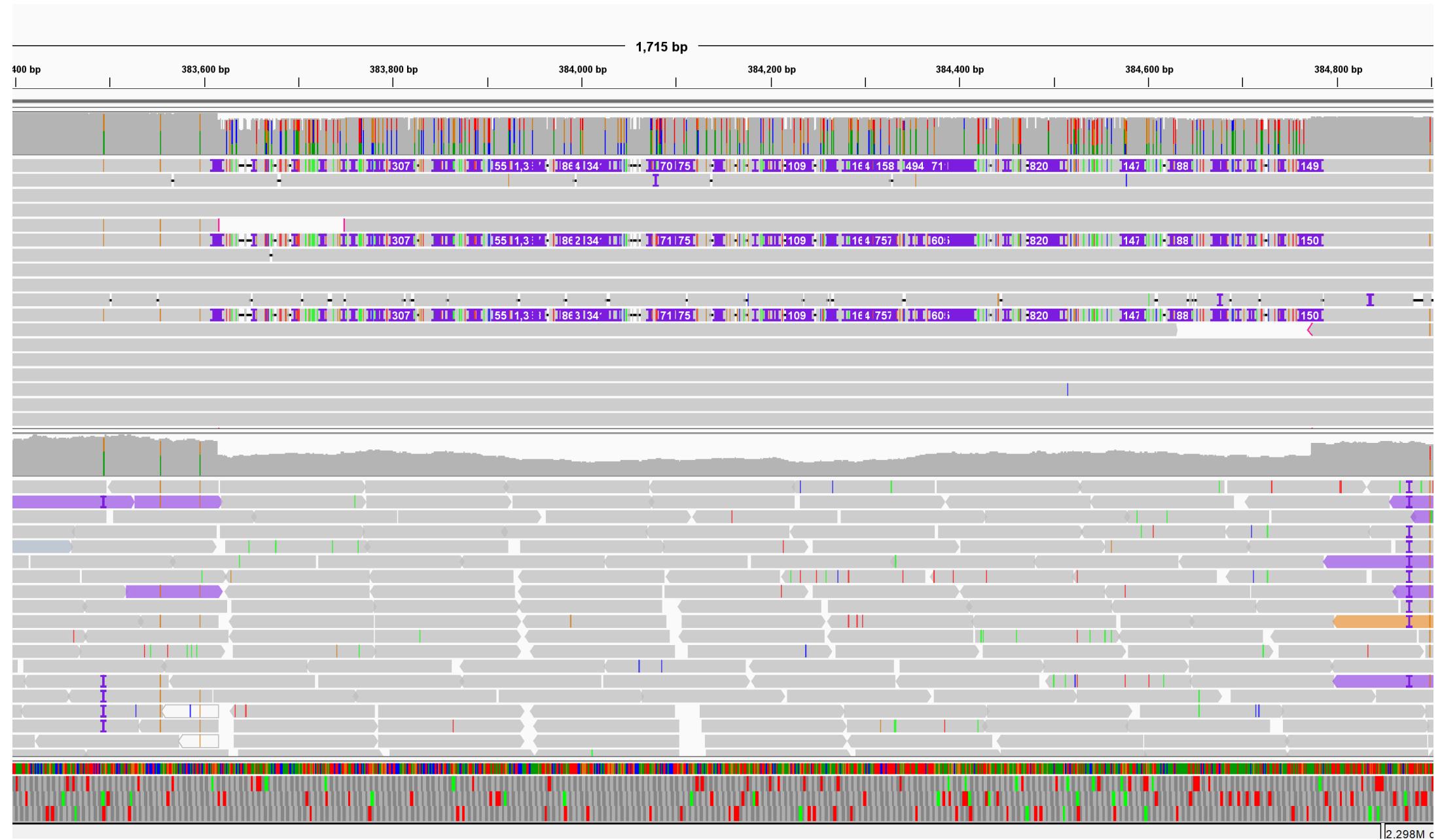


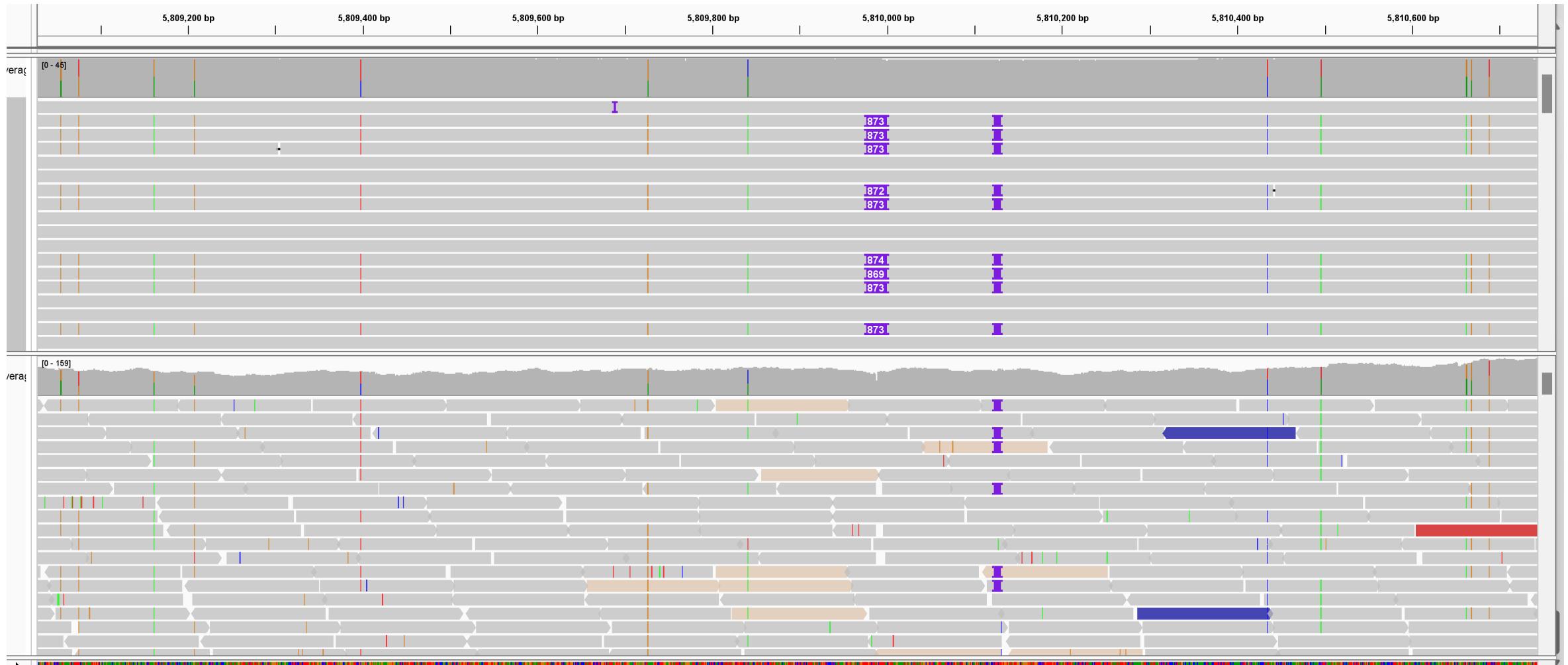


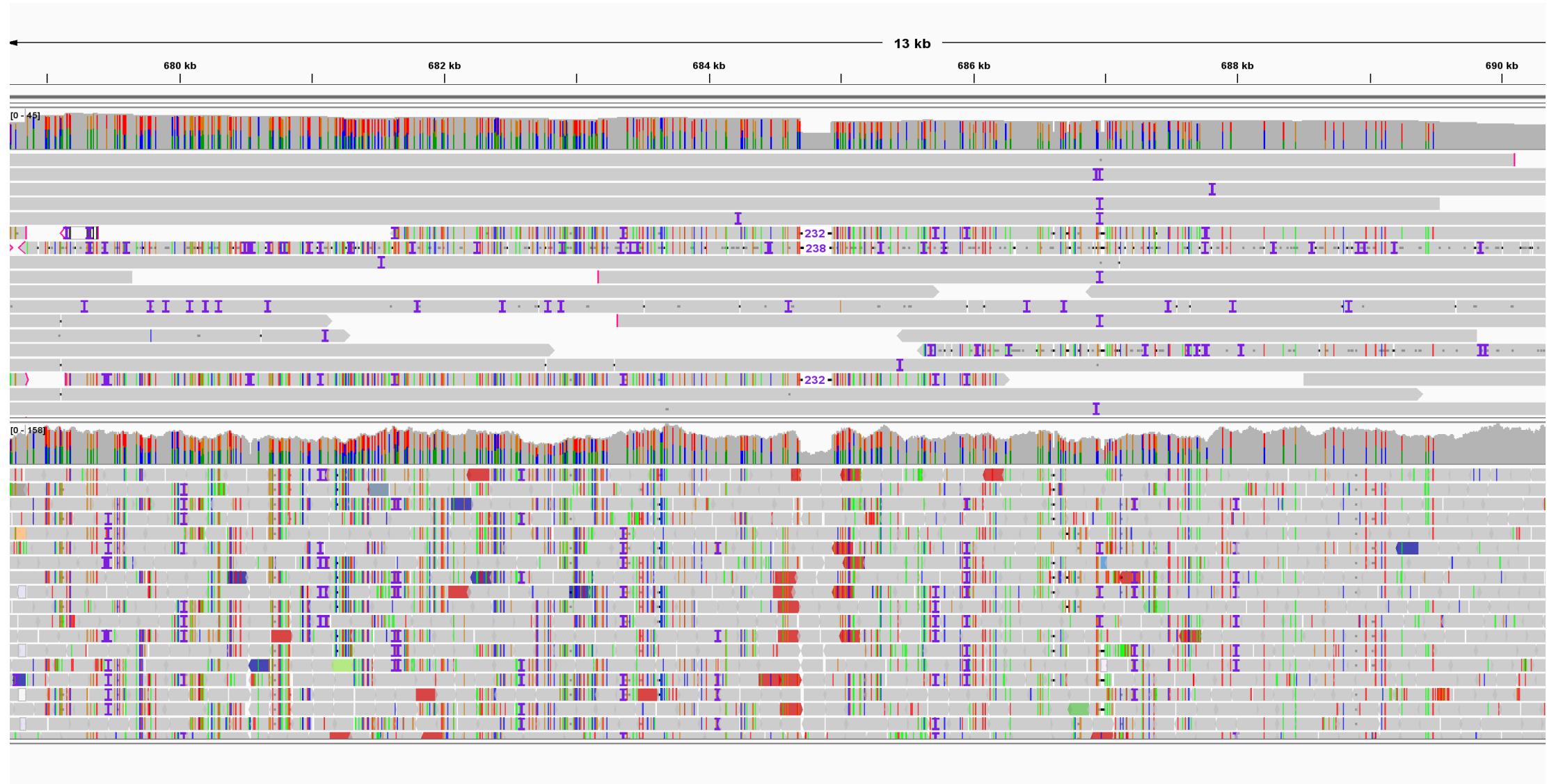
Nanopore vs short reads











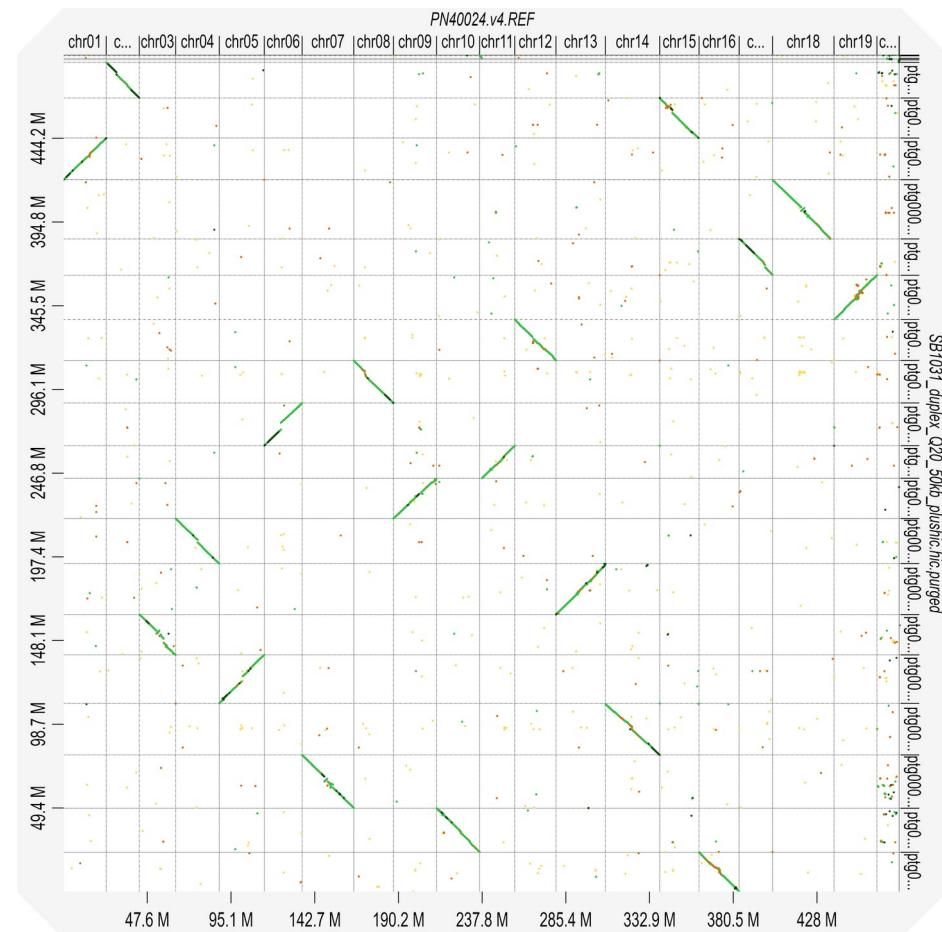
A reference genome

High-accuracy duplex makes all the difference



Annabel Whibley

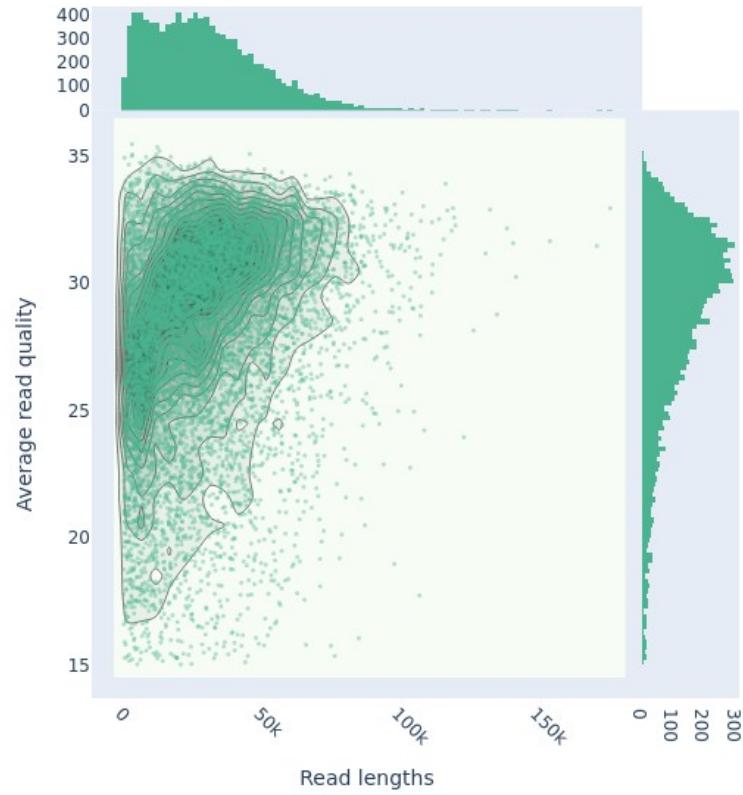
- 2 PromethION flow cells (10.4.1):
 - HMW DNA (not nuclei isolation)
 - Ligation libraries (LSK114)
 - 5kHz
- Filtering: Duplex + >50kb (~30% retained)
- Hifiasm



A reference genome

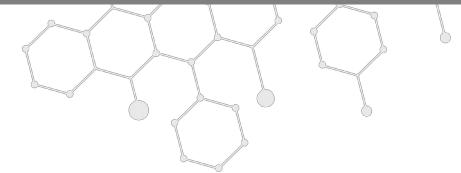
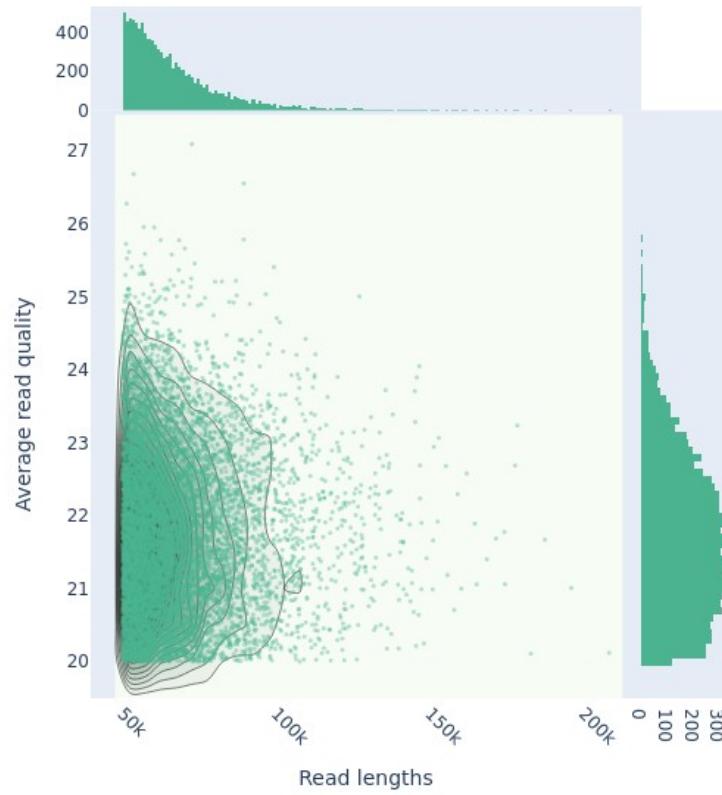
Duplex: 22GB,

Read lengths vs Average read quality kde plot



Simplex: 24GB, Q20+,
50kb+

Read lengths vs Average read quality kde plot

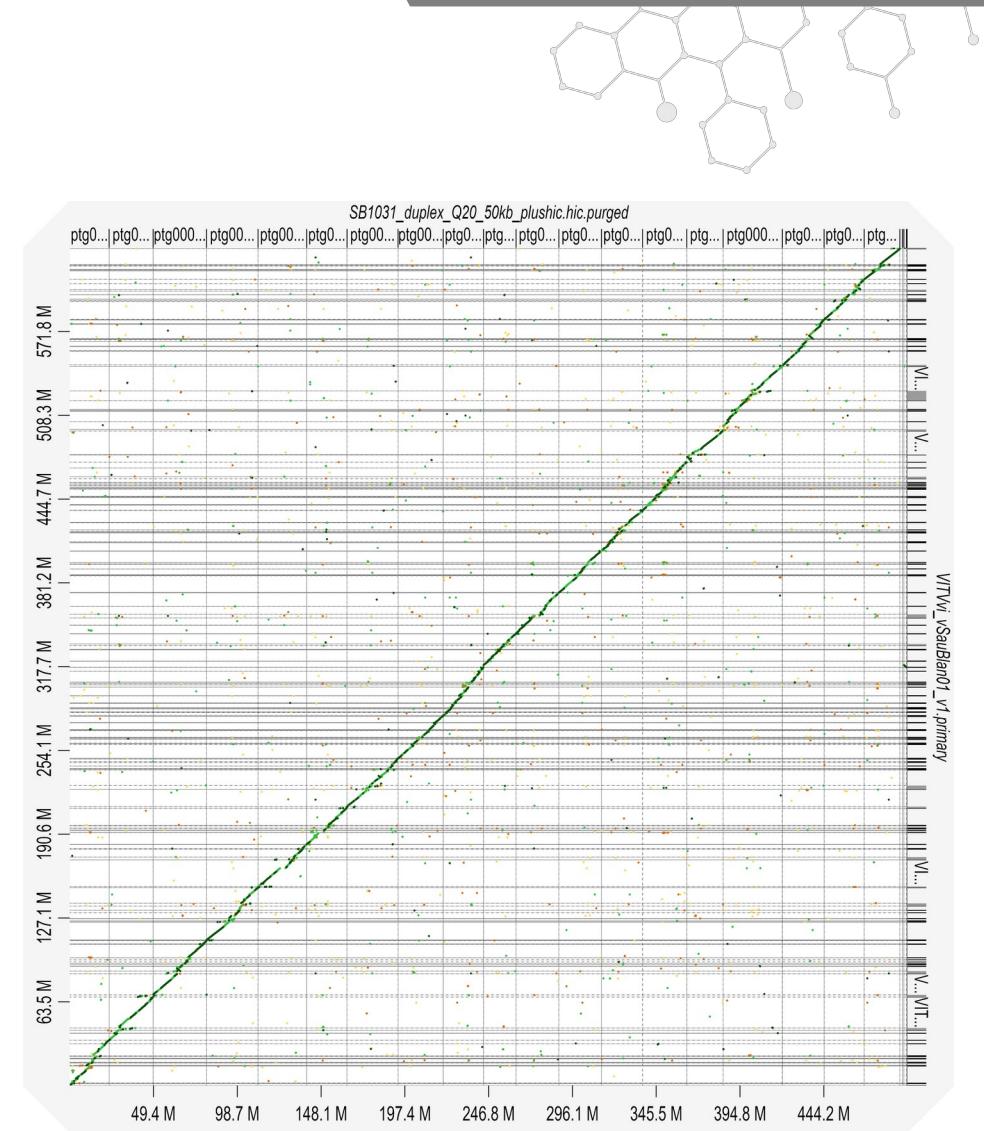


A reference genome

QC metrics

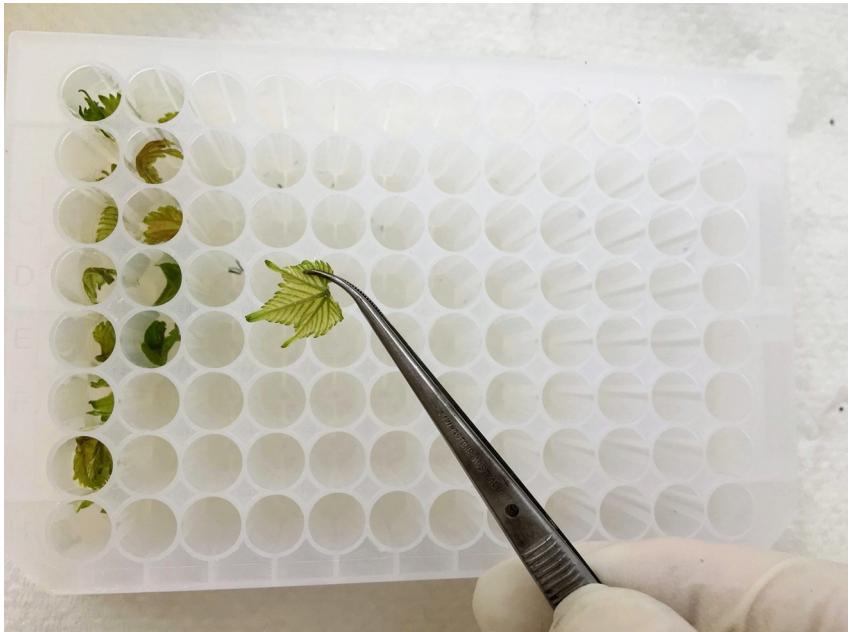
	Primary	Hap1	Hap2
Assembly length (Mb)	493.6	491.4	508.2
Number of contigs	26	41	38
Largest contig (Mb)	34.9	37.8	31.2
Contig N50 (Mb)	25.2	20.6	23.8
Contig N90 (Mb)	21.4	8.6	13.3
Contig L50	9	10	10
Contig L90	17	25	20
Compleasm Completeness (%)	99.18	99.36	99.44
Duplicated (%)	1.29	1.55	1.16
Fragmented(%)	0.43	0.47	0.43
Missing (%)	0.39	0.17	0.13

Of the eudicot BUSCO gene set (n=2326), there are 4 missing from hap1, 3 from hap2 (two are absent from both)



Genetic testing

- Varietal ID
- Or ~50 SNPs in mapped WGS data
- Rapid clonal ID

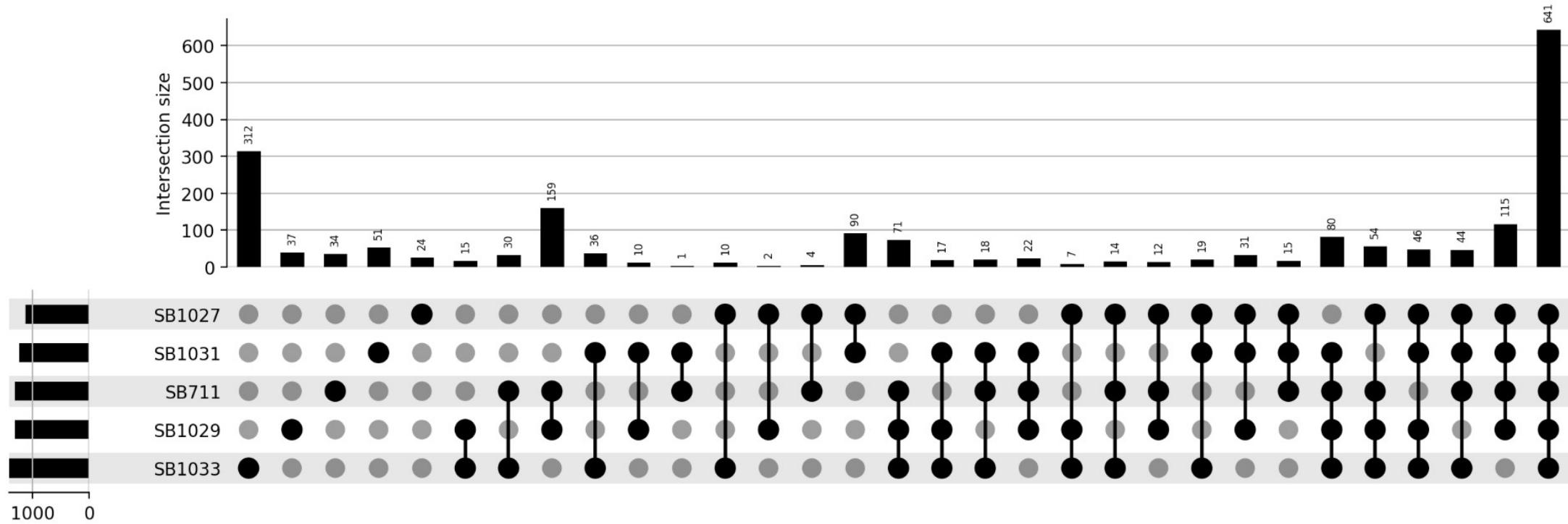


		REF	ALT	1031 calls	1031_GT	Expected	Expected PN	Expected Chardonnay
chr01	20954936	T	C	0/1	CT	CT	CC	CC
chr02	4766274	C	T	0/1	CT	CT	CT	CC
chr02	6963808	C	T	0/1	CT	CT	CT	CT
chr04	2969982	C	A	0/1	AC	AC	AA	AA
chr04	18743245	C	T	0/1	CT	CT	CT	CT
chr05	4539511	T	C	1/1	CC	CC	CT	CC
chr05	24789326	G	A	1/1	AA	AA	GG	AG
chr06	2487136	A	C	0/1	AC	AC	AA	AC
chr06	17789284	C	T	0/1	CT	CT	CC	CT
chr07	11426014	C	T	0/1	CT	CT	TT	TT
chr07	25754639	A	G	0/1	AG	AG	AG	AA
chr08	12922556	T	C	0/1	CT	CT	CT	CT
chr09	5749389	C	T	0/1	CT	CT	CT	CT
chr09	10799024	A	G	0/1	AG	AG	GG	GG
chr10	13771162	T	G	1/1	GG	GG	GG	GG
chr11	3453240	C	T	1/1	TT	TT	CT	CC
chr11	5398715	C	A	1/1	AA	AA	AC	CC
chr12	6693128	G	A	0/1	AG	AG	AG	AG
chr12	21989175	T	G	1/1	GG	GG	GT	GT
chr13	276322	C	T	0/1	CT	CT	CT	CT
chr14	4665397	G	T	0/1	GT	GT	GG	GG
chr15	20344092	G	T	0/1	GT	GT	GT	GG
chr16	16781162	A	G	0/1	AG	AG	AG	AG
chr16	18545342	G	A	0/1	AG	AG	AG	AA
chr17	17989459	T	C	0/1	CT	CT	CT	CT
chr18	11502220	T	C	0/1	CT	CT	CT	CC
chr18	34524656	G	T	0/1	GT	GT	GT	GT
chr19	24135821	A	G	0/1	AG	AG	AA	AA

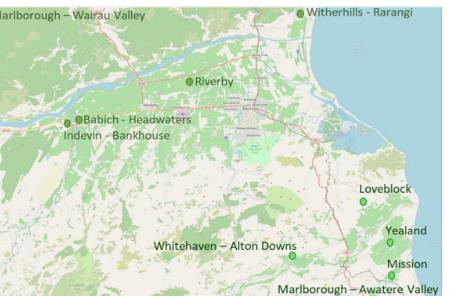
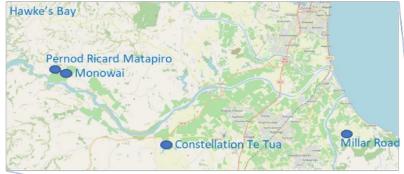


Amy Hill

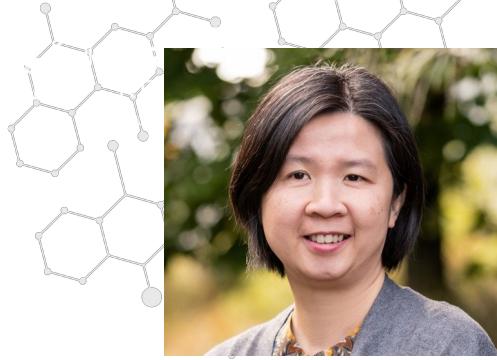
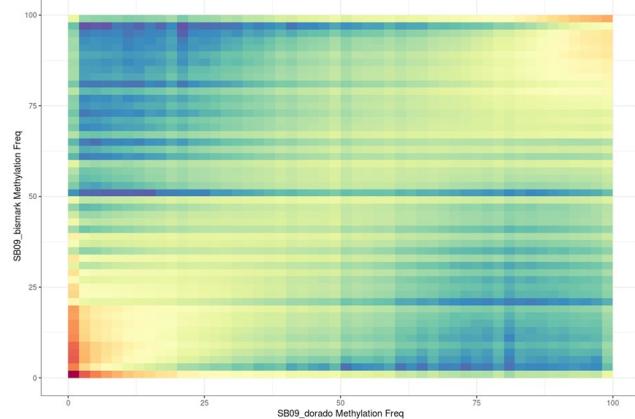
Clonal discrimination



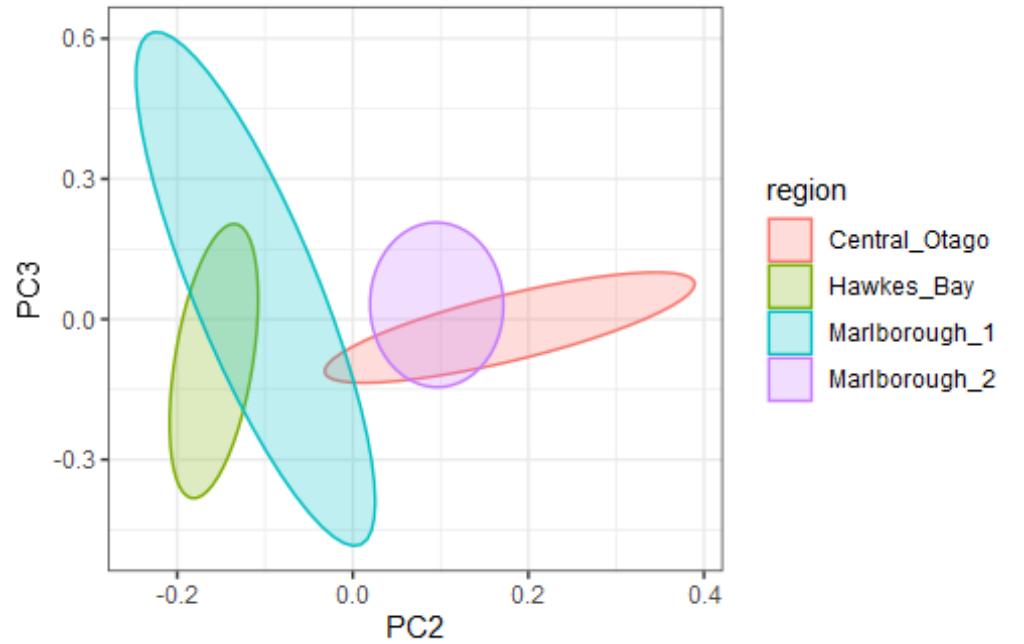
Methylation Calling



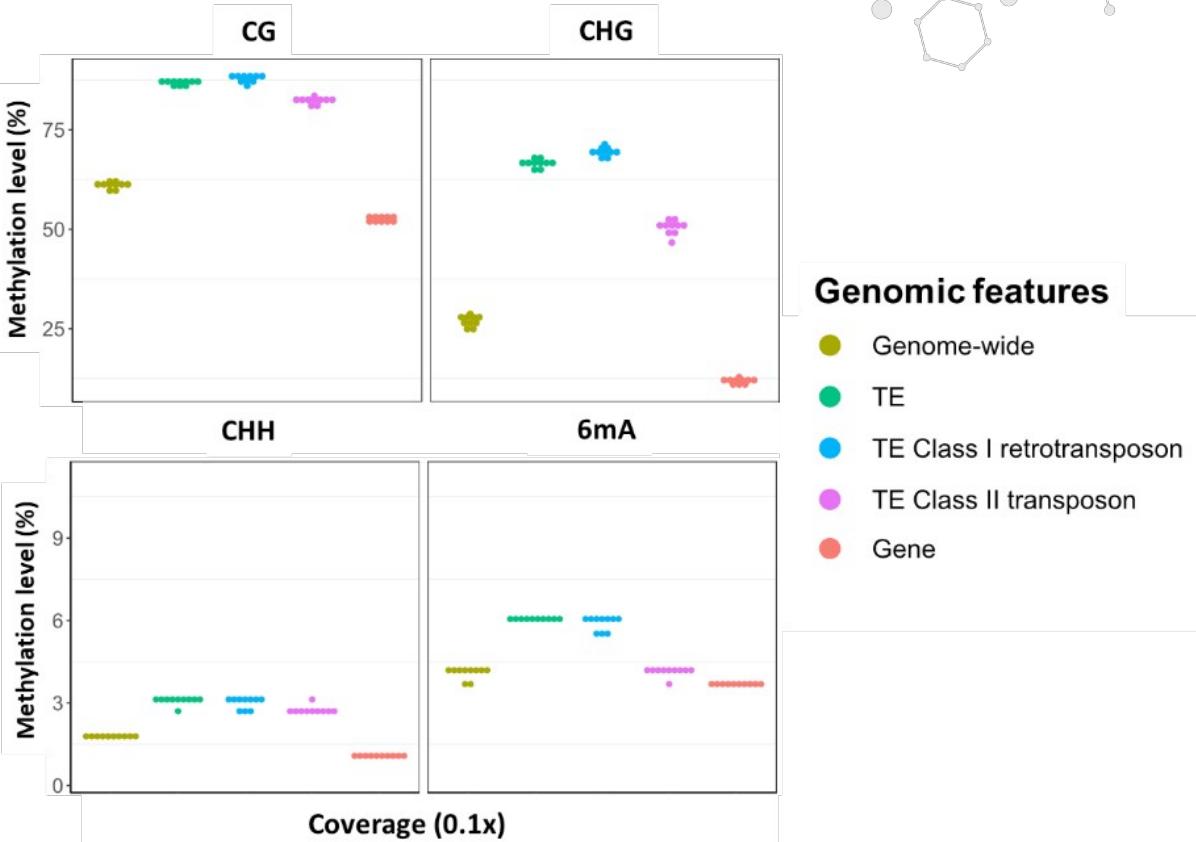
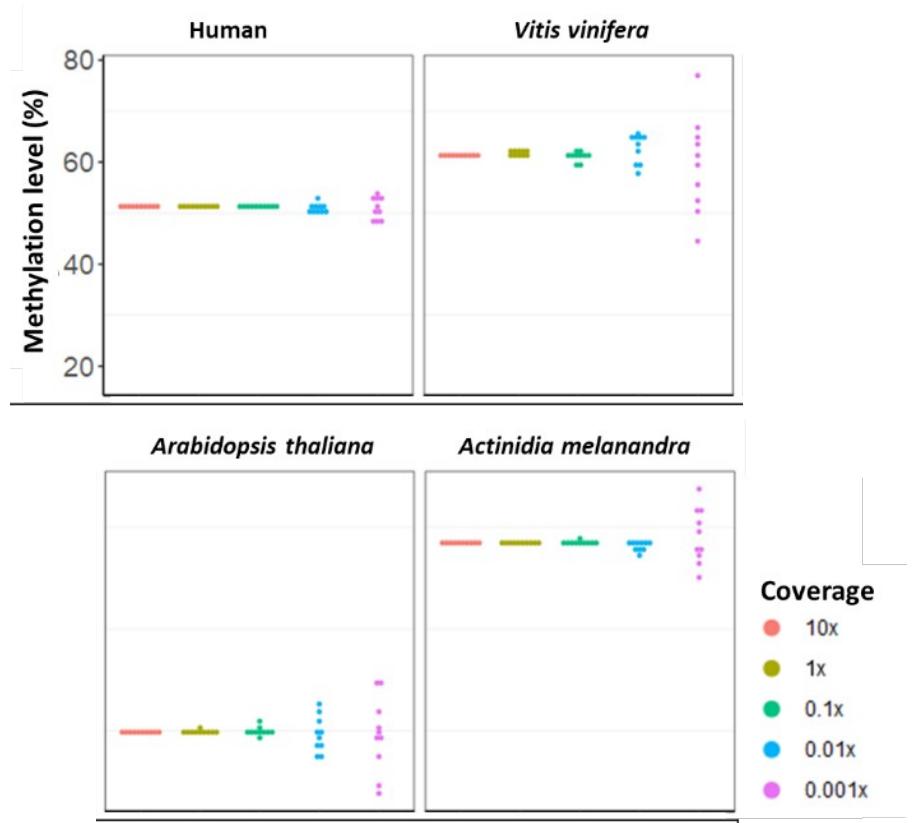
N = 112082432 r = 0.936
Correlation Dorado-5khz Bismark all coverage >=5



Cen Liau

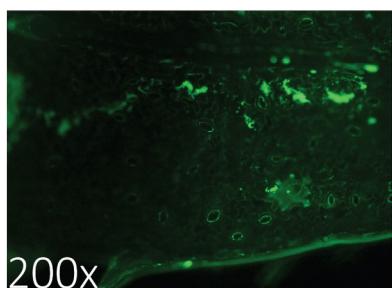
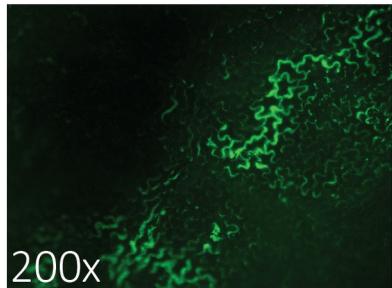
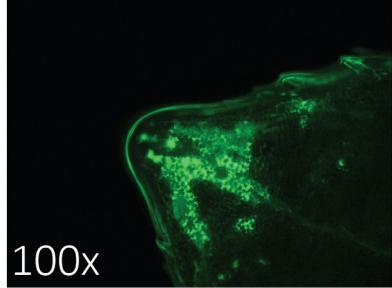


Methyl Skim-seq

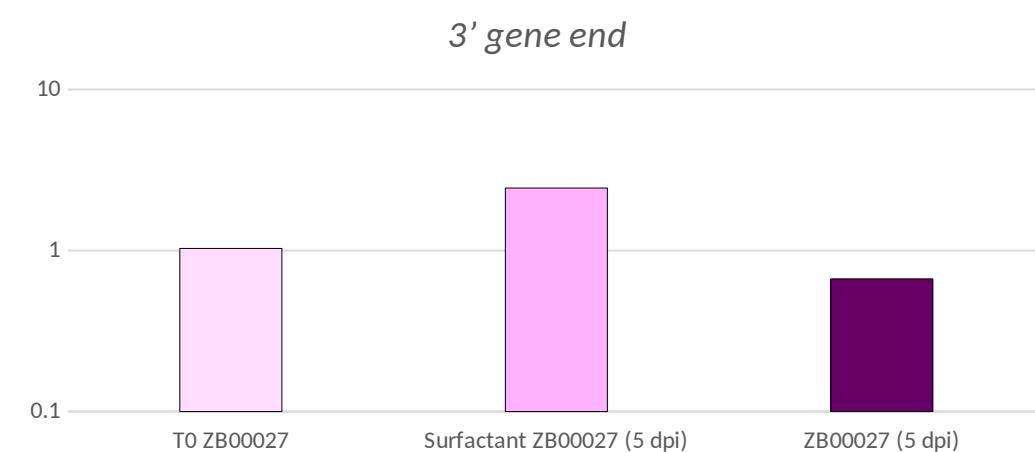
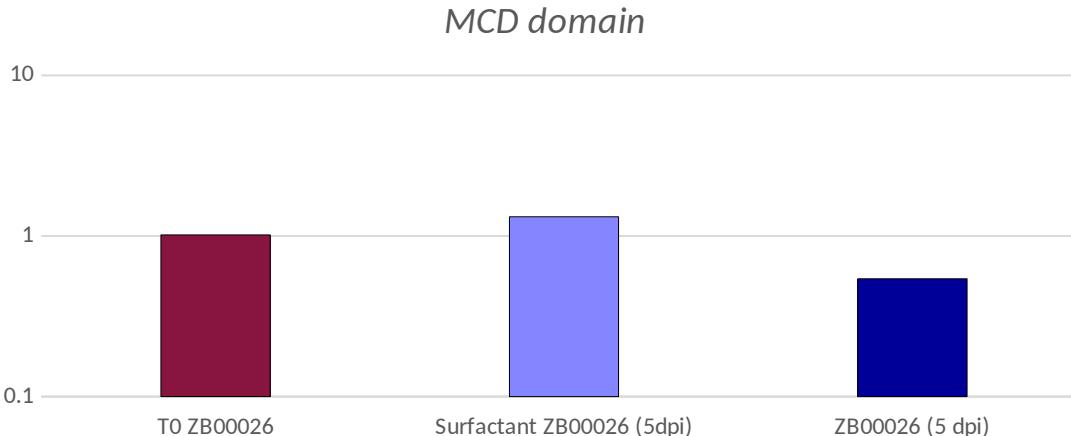
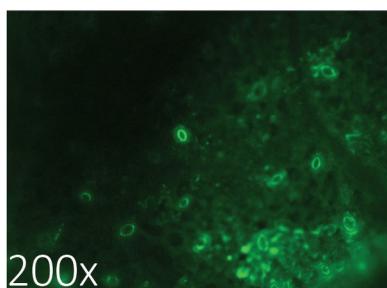
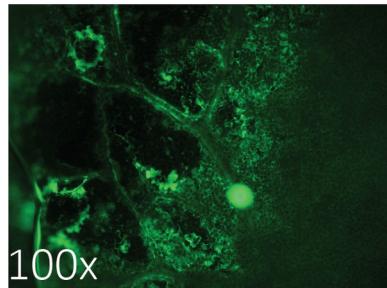
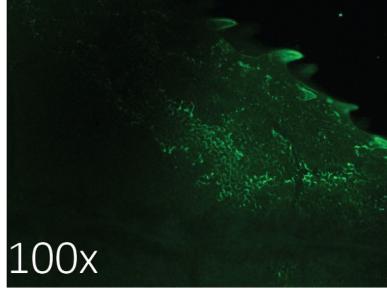


RNA for gene expression management

Wash at 10 min



Wash at 90 min



Ellie Bradley

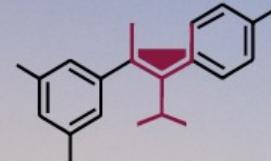


BRI Grapevine
Improvement Team
Ellie Bradley
Amy Hill
Cen Liau
Bhanupratap Vanga
Solomon Wante
Annabel Whibley
Darrell Lizamore

Collaborators
World-leading research from grape to glass



New Zealand Wine
Altogether Unique.



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NEW ZEALAND GRAPE AND WINE RESEARCH
RANGAHAU KAREPE, WĀINA O AOTEAROA

 Plant & Food
Research
Rangahau Ahumāra Kai


LINCOLN
UNIVERSITY
TE WHARE WĀNAKA O AORAKI

