Genomics-led radiation mutagenesis in rapeseed

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IRC | 2019 | Berlin
INTERNATIONAL RAPESEED CONGRESS

19th June, 2019

The evolution and relationships between members of the plant genus Brassica

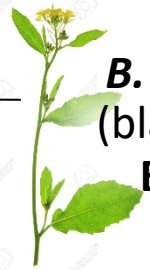
B. napus **(2n=38)**
(oilseed rape/canola, swede)
AACC 1200 Mb
5-10 kya

„triangle of U“
Woo Jang-choon, 1935
(Nagaharu U)

B. oleracea **(2n=18)**
(broccoli, cabbage, etc.)
CC 650 Mb

B. rapa **(2n=20)**
(turnip, Chinese cabbage, etc.)
AA 550 Mb

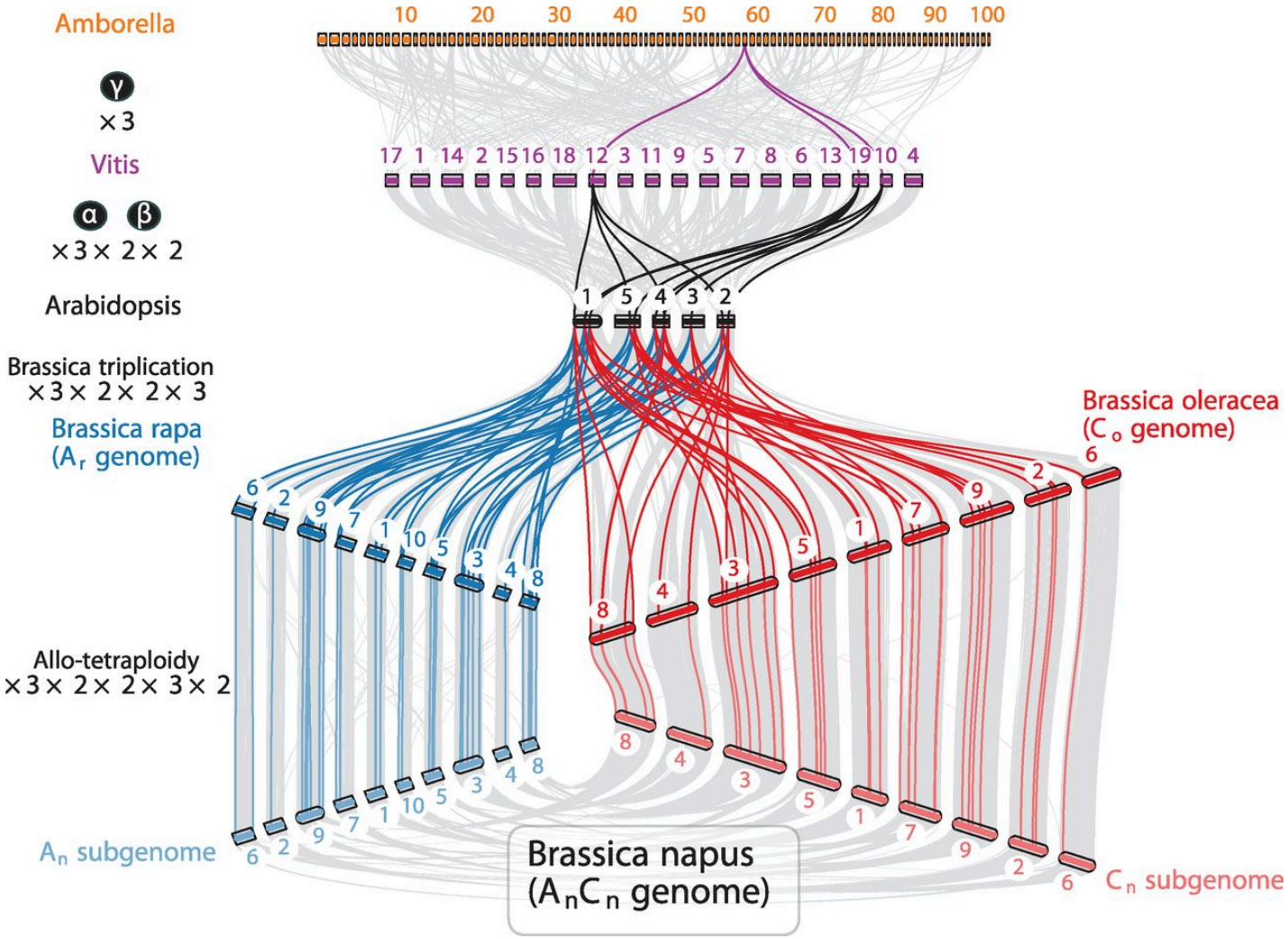
B. carinata **(2n=34)**
(Ethiopian mustard)
BBCC 1250 Mb



B. nigra **(2n=16)**
(black mustard)
BB 600 Mb

B. Juncea **(2n=36)**
(brown mustard)
AABB 1150 Mb

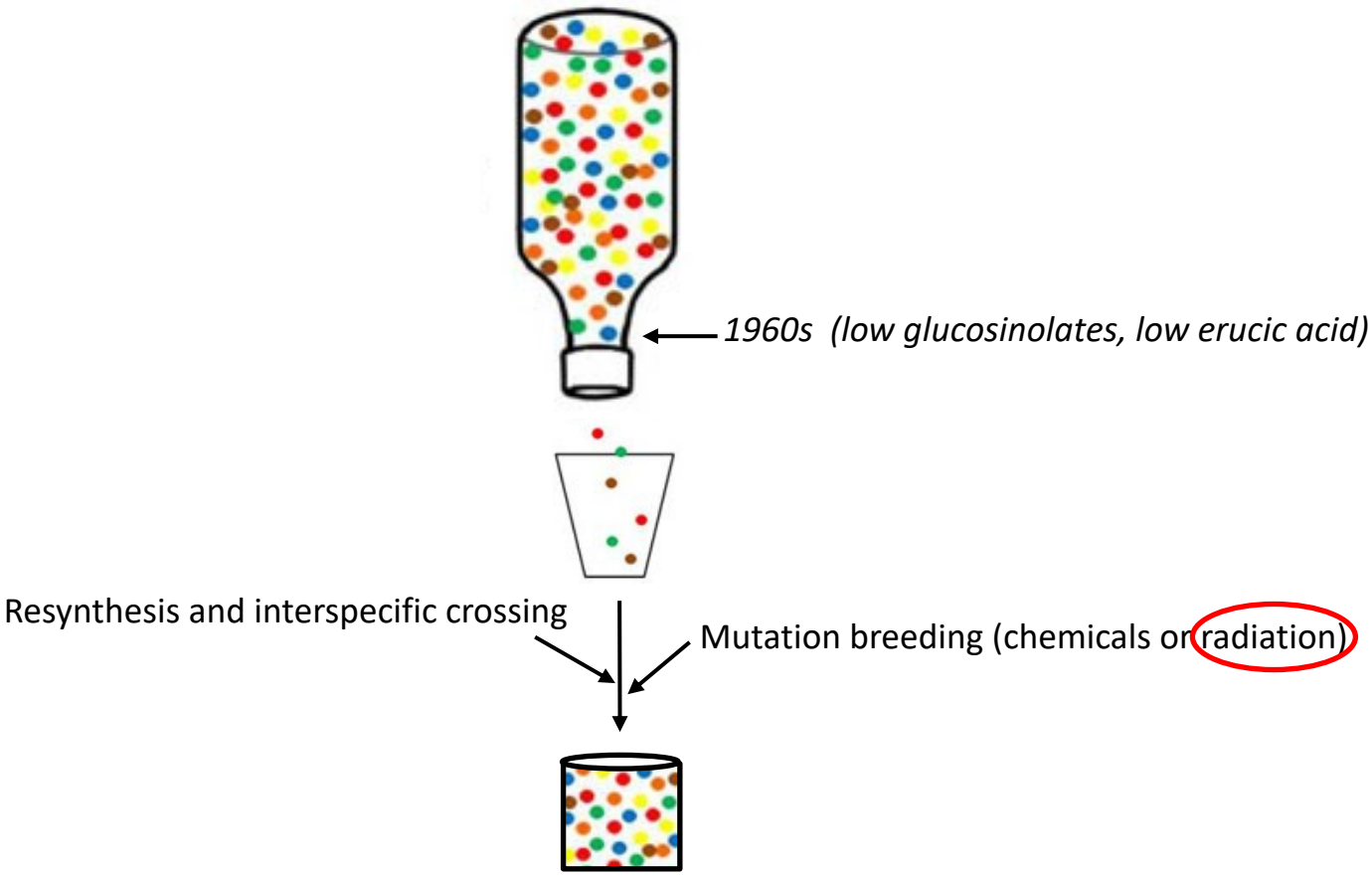
Highly duplicated genome



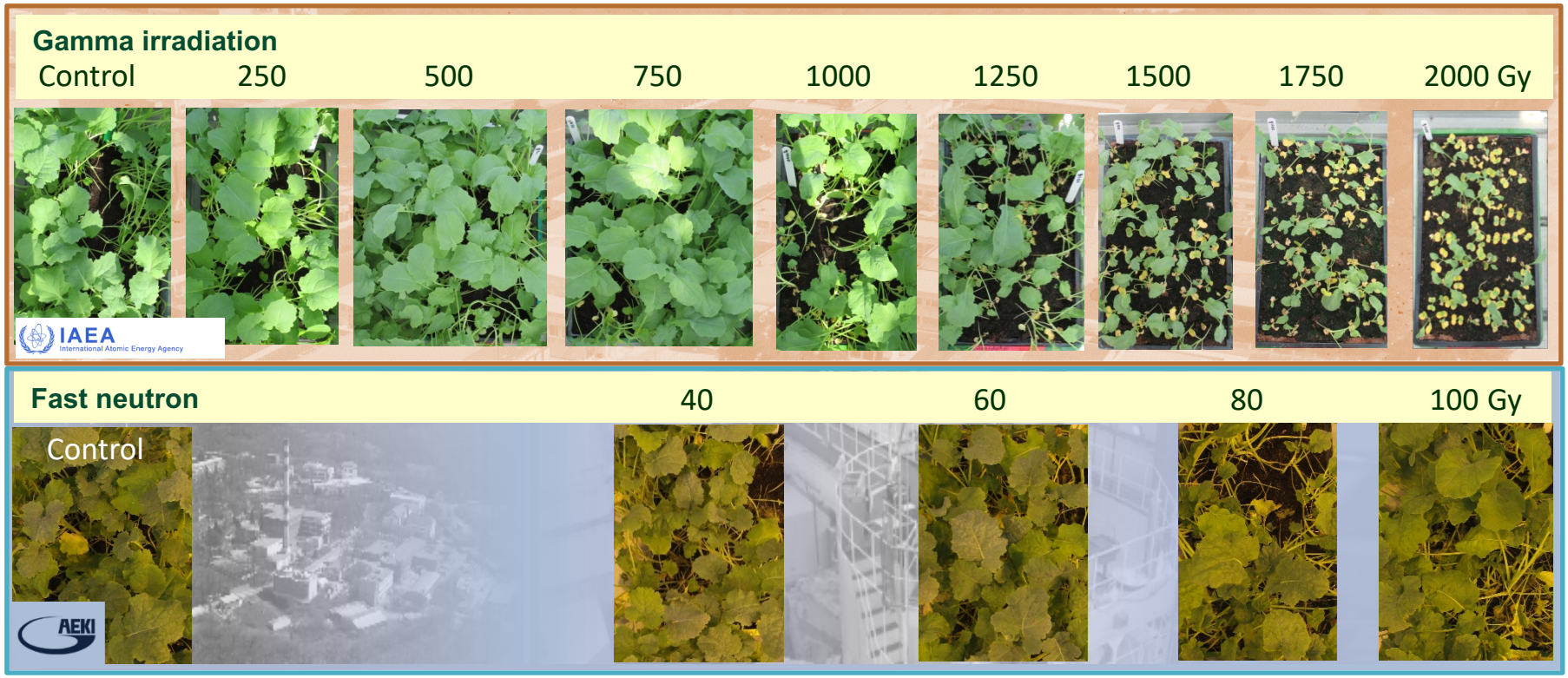
Science AAAS

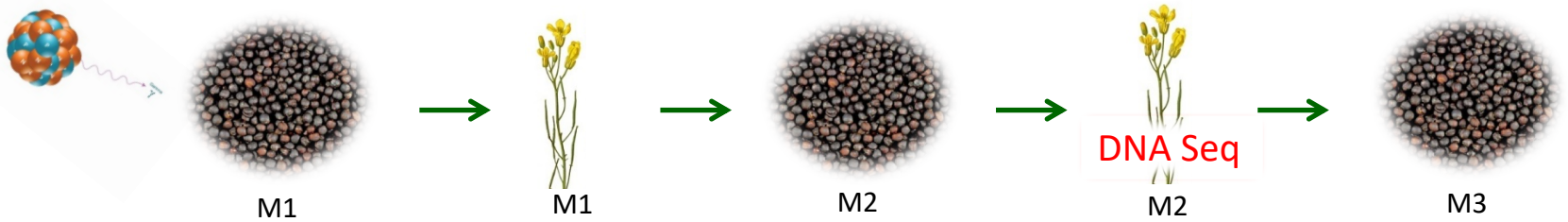
Chalhoub et al. (2015) Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* 345:950-953.

Genetic bottleneck during intensive selection



RIPR radiation mutagenesis panel





FN/Gamma irradiation

Selfed plants

~1K selfed M_2 plants per one M_1 individual

Seed stock

Type of mutations

- **Structural variation** (duplications/deletions)
- **Single base mutation** (introduction of premature stop codons and nonsynonymous base substitutions)
- **InDels** (frame-shift mutations)

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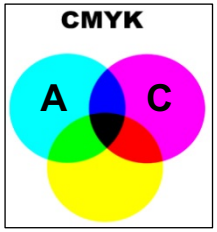
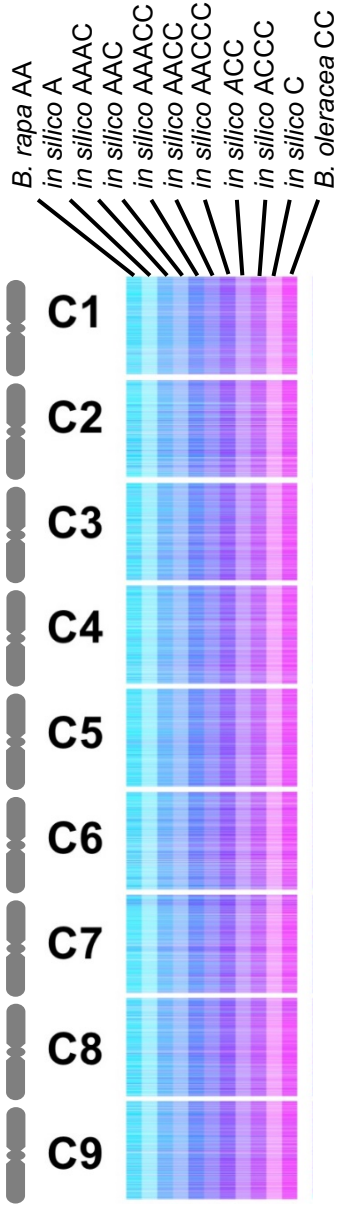
Genome copy number variation for larger deletions and duplications



- GDTP visualization based on DNAseq data



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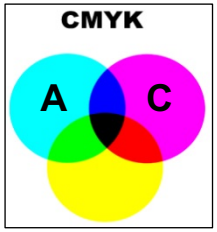

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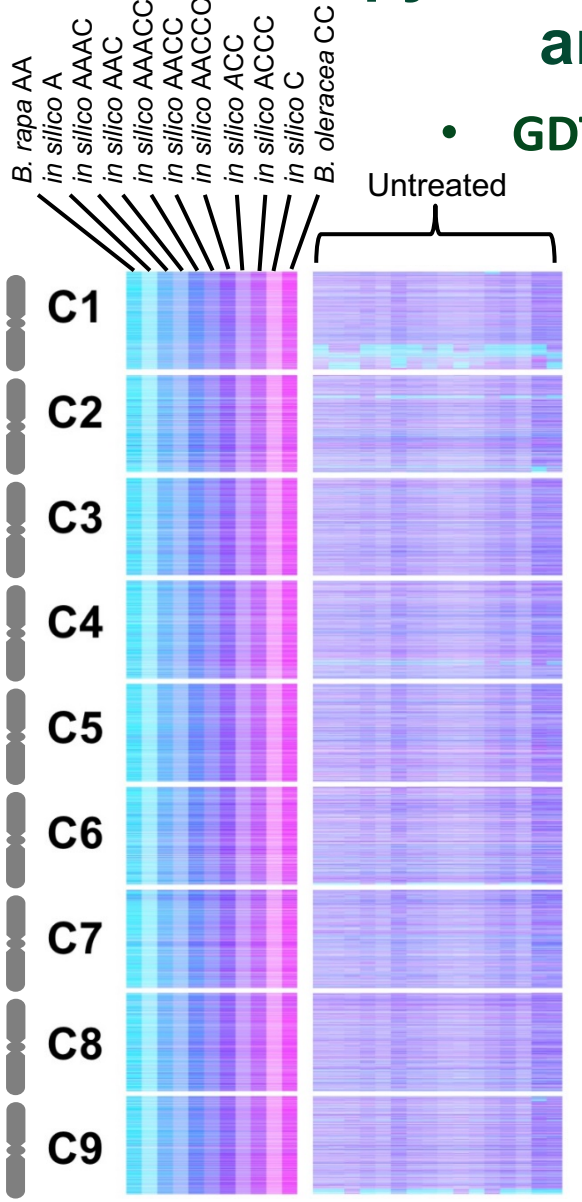
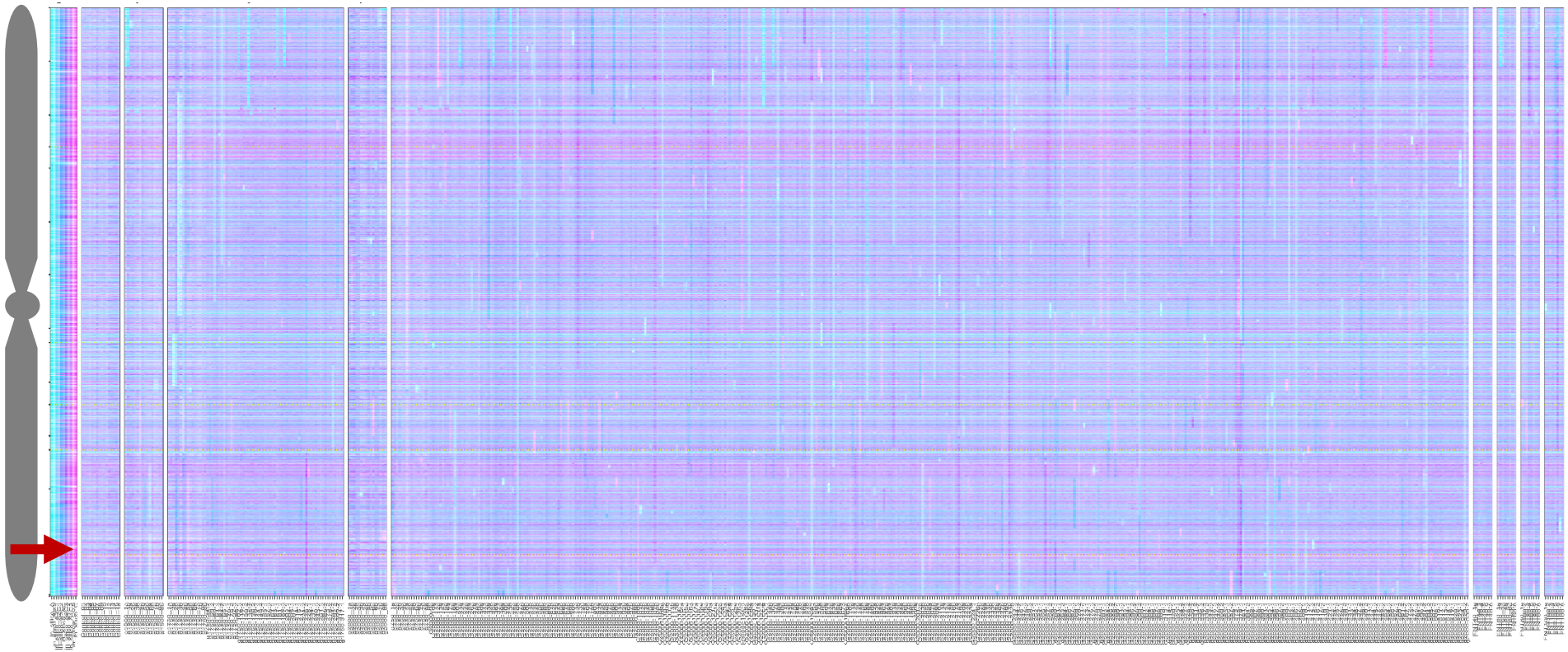


Genomics-led radiation mutagenesis

Identifying gene dosage variants using the RIPR radiation mutagenesis panel

e.g. FAE1.C3 deletion

Chromosome C3



All 600 GDTP visualization (C03) based on 12x Illumina genome re-sequencing

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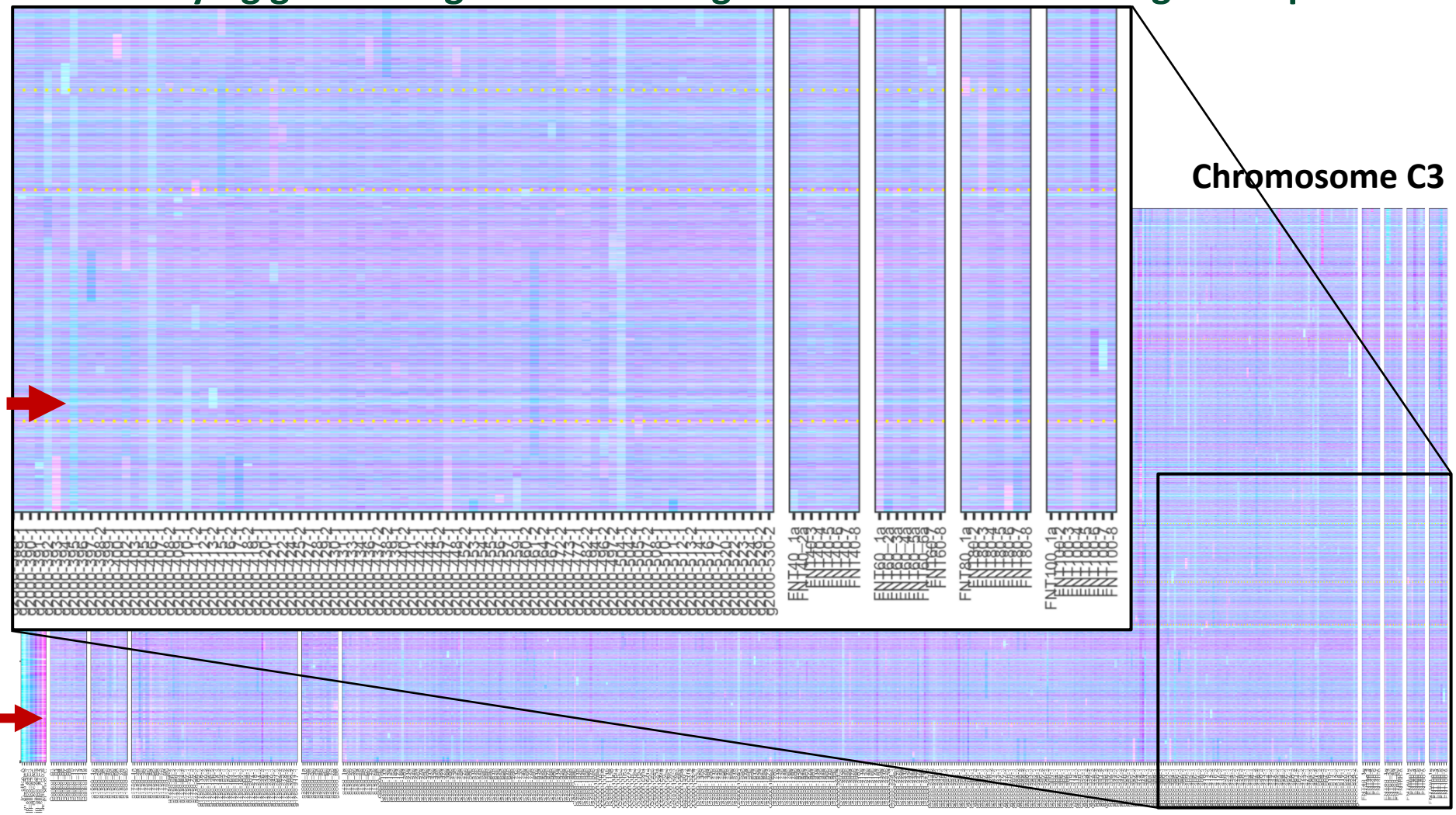

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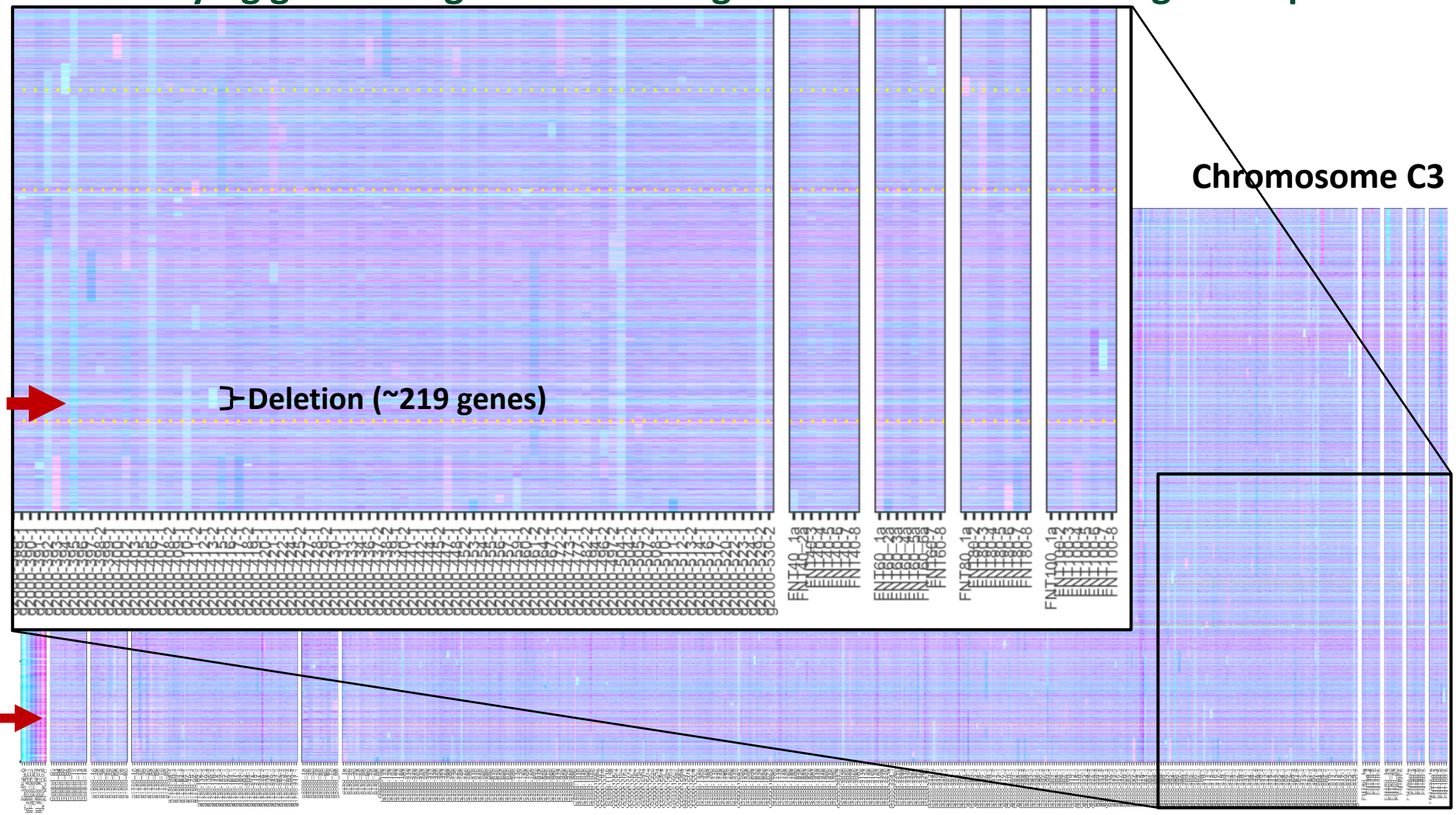

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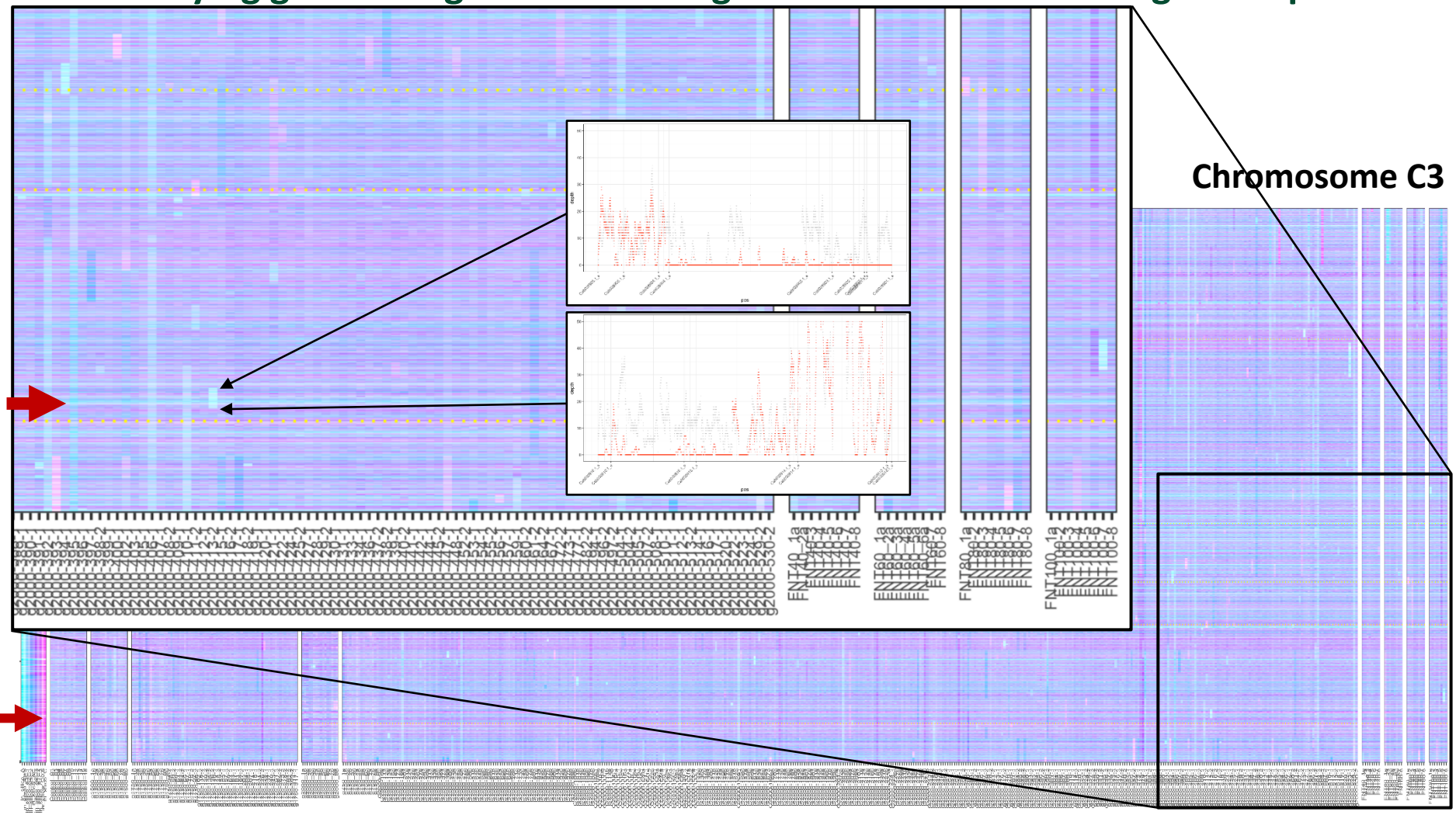

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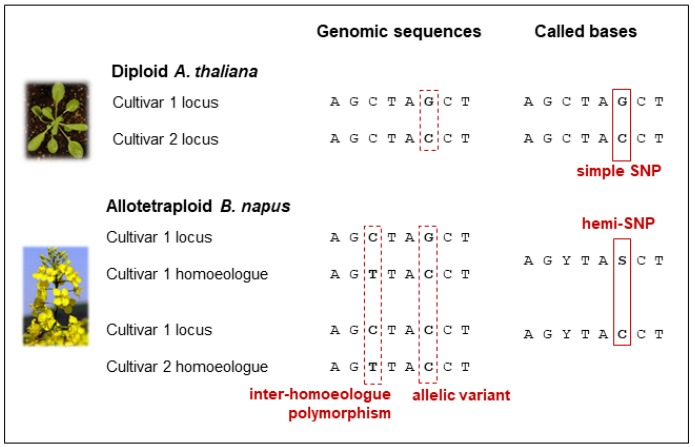
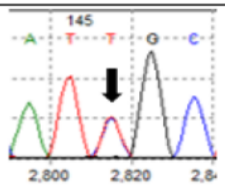
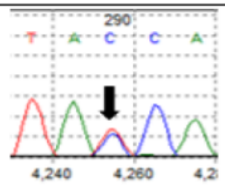
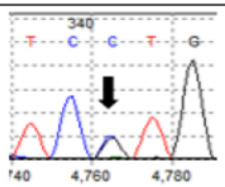
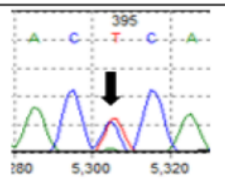
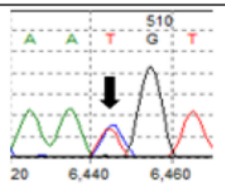
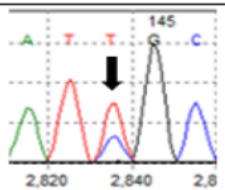
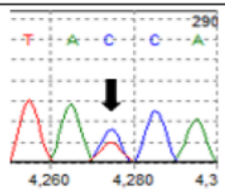
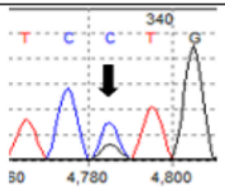
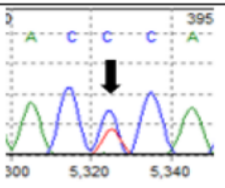
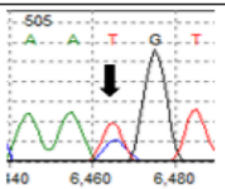
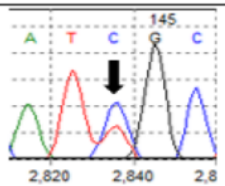
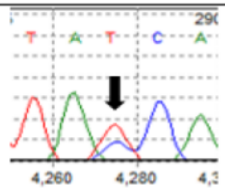
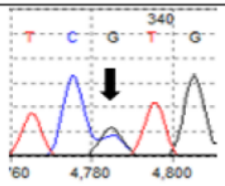
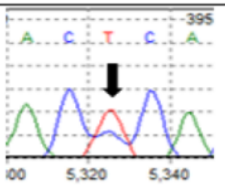
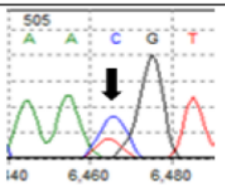
Inter-Homoeologue Polymorphism (IHPs)

Using IHPs to confirm structural variation (deletion/duplication) in the radiation mutagenesis panel



	IHP 168	IHP 312	IHP 363	IHP 417	IHP 531
Bna.FAE1.A8	C	T	G	T	C
Bna.FAE1.C3	T	C	C	C	T
WT Predicted AACC					
G1750_5a_10 Predicted ACC					
G1750_2b_1 Predicted AAACC					

Acknowledgement: Yen Peng Chew

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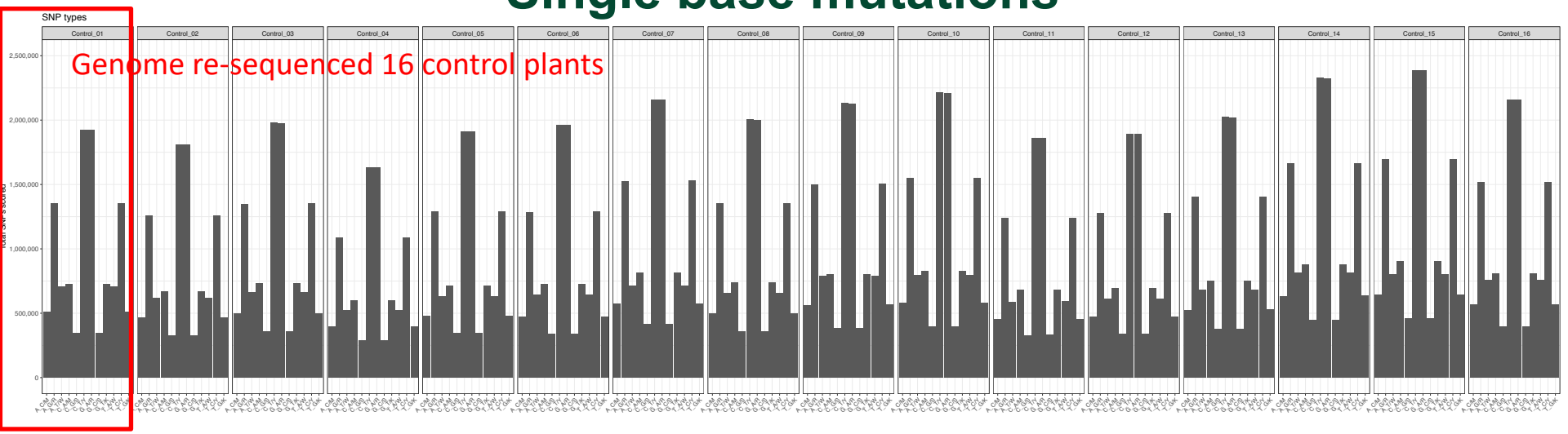
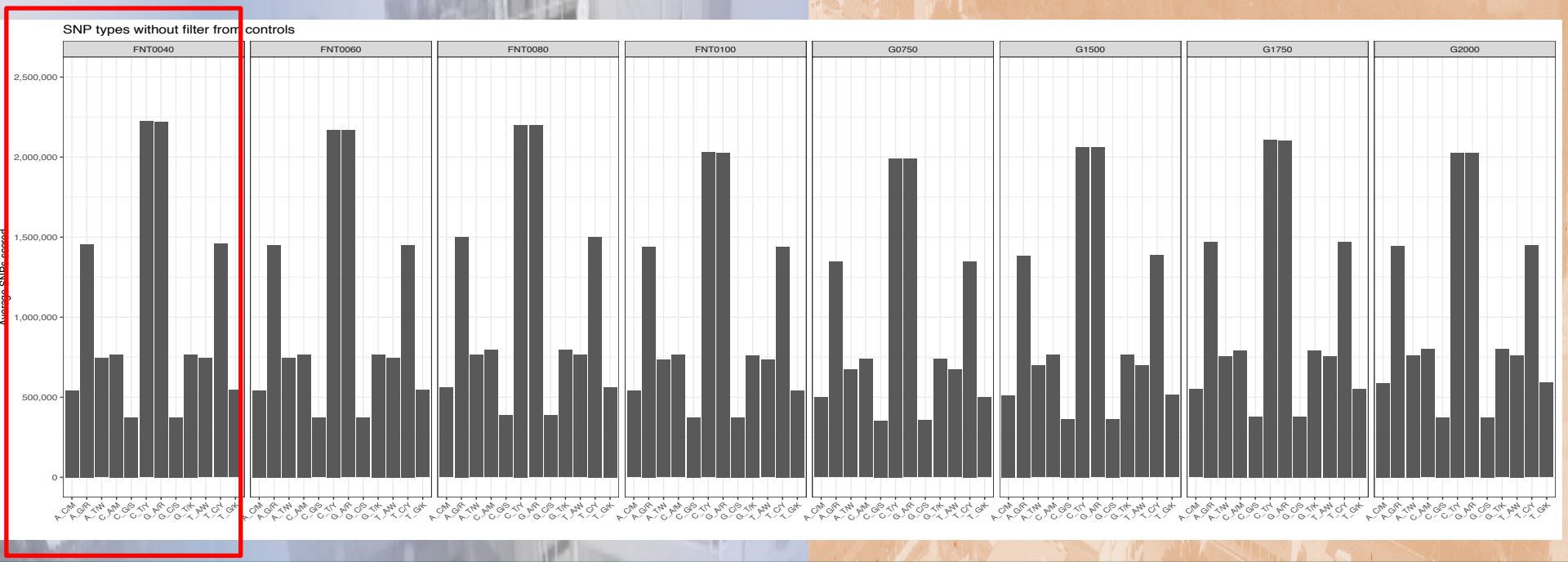
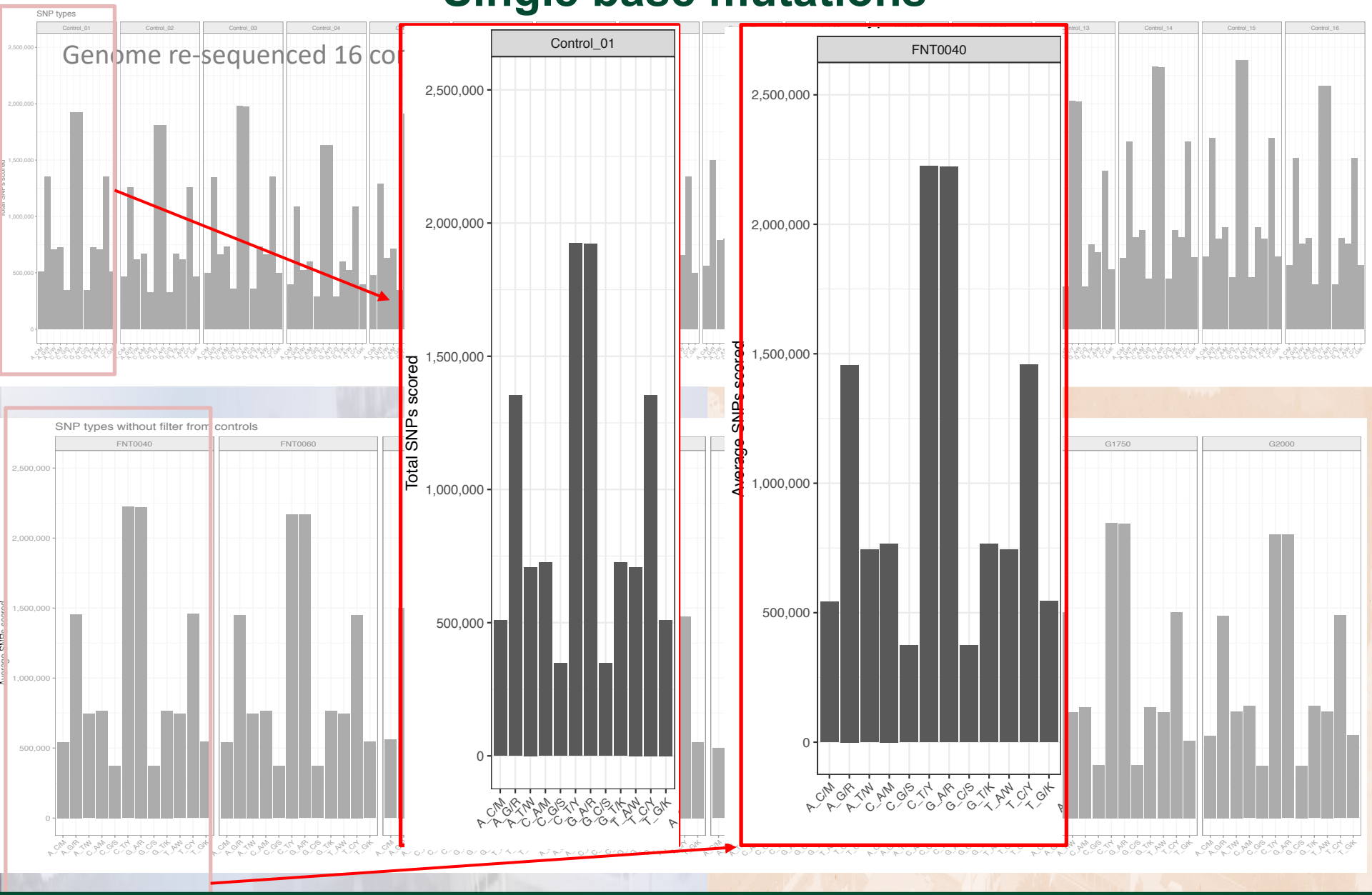
Single base mutations



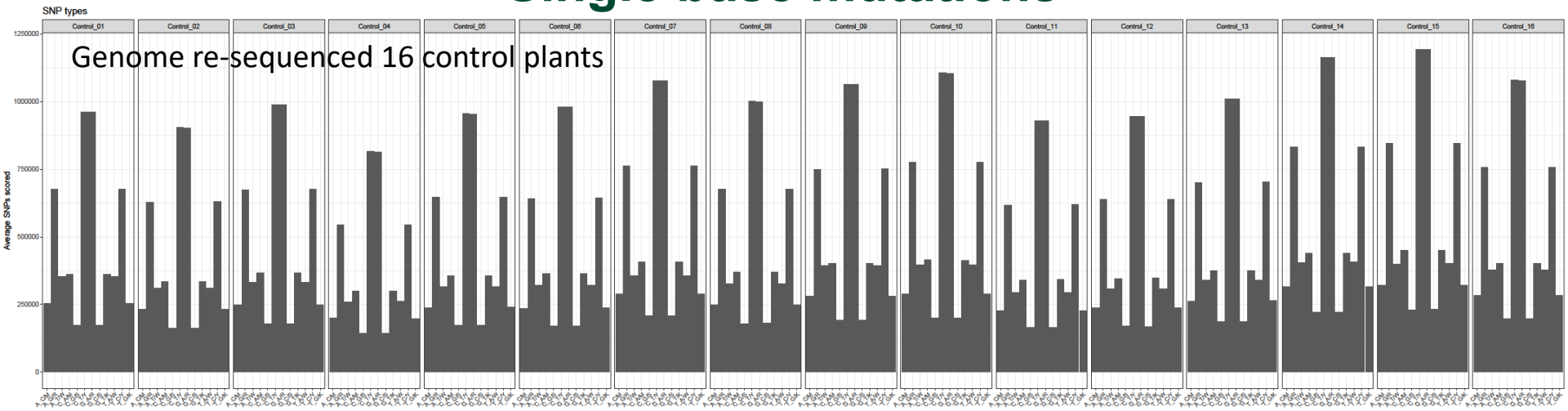


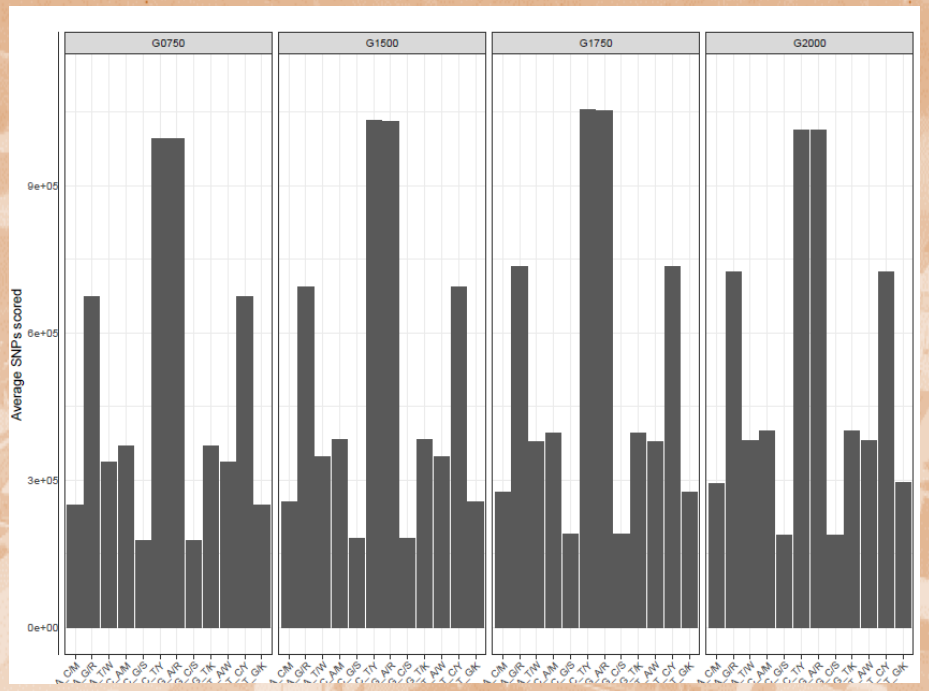
Single base mutations



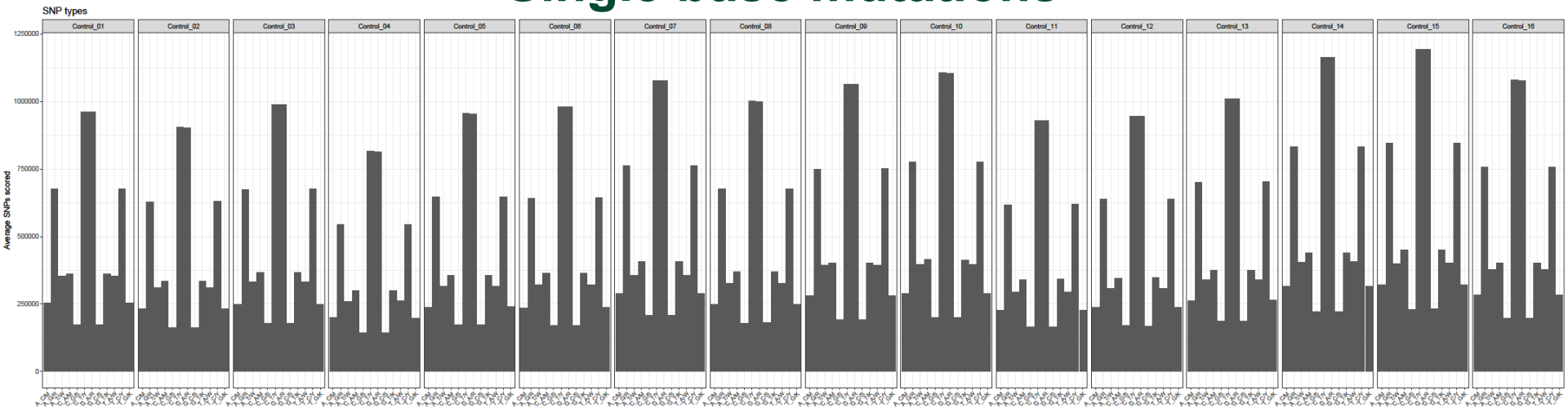
Single base mutations

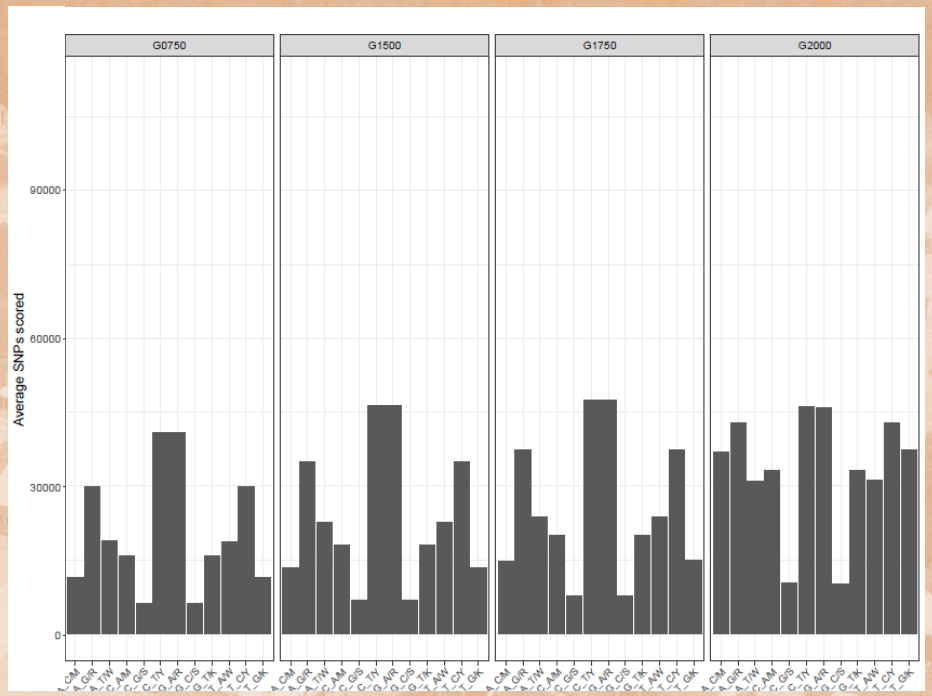
Genome re-sequenced 16 control plants



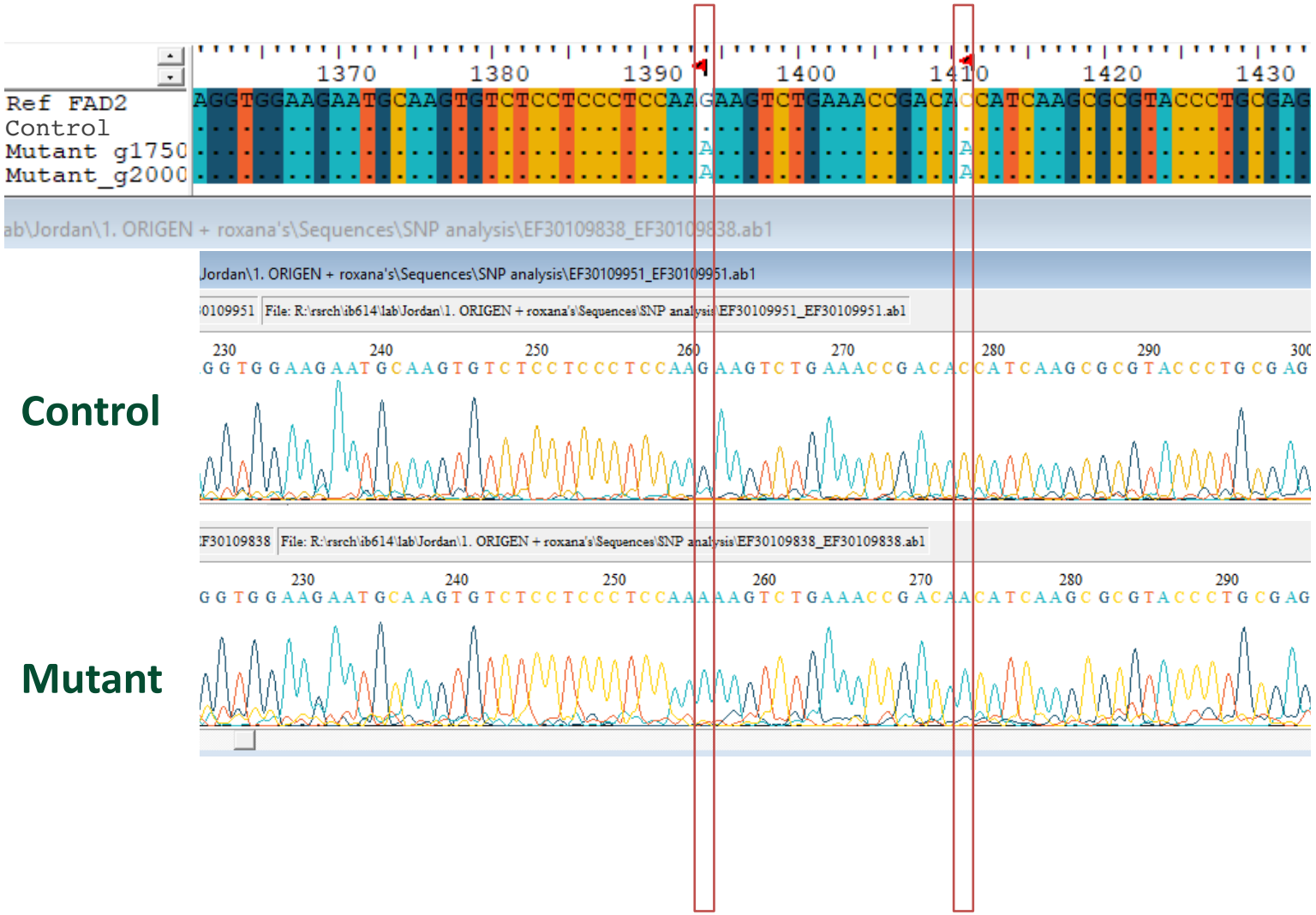


Single base mutations





Single base mutations
validation



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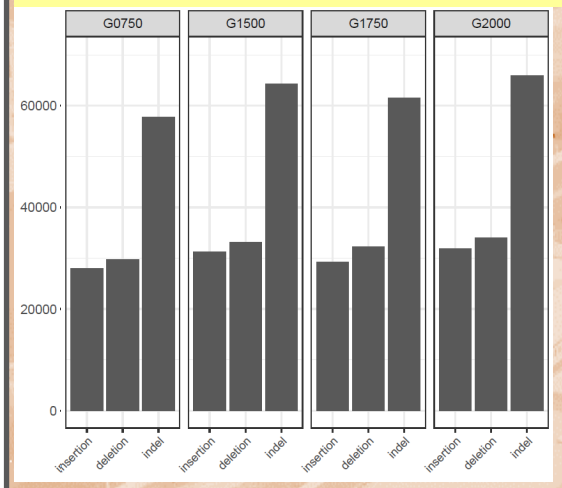
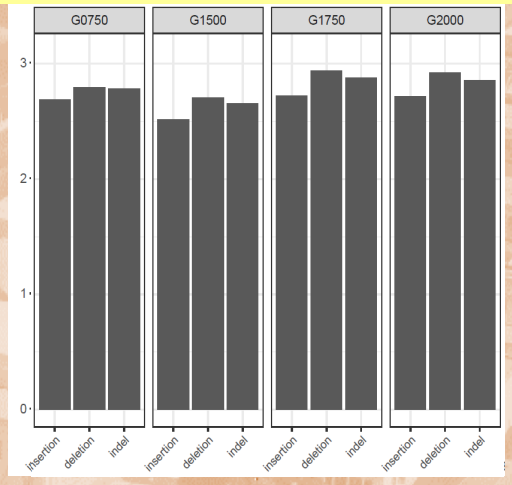
InDels

total scored

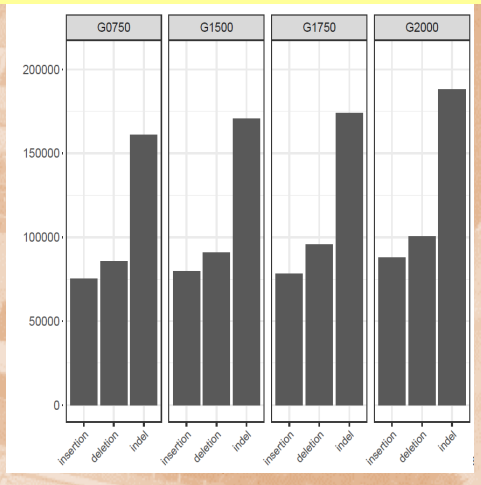


average size

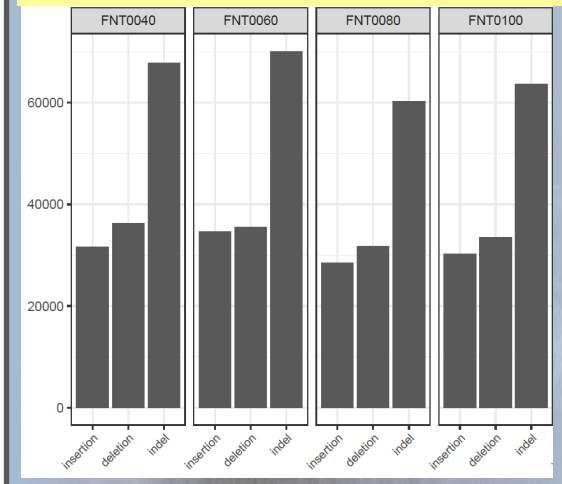
Gamma irradiation

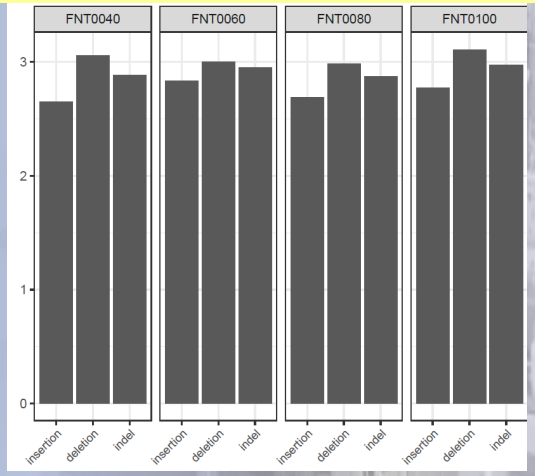


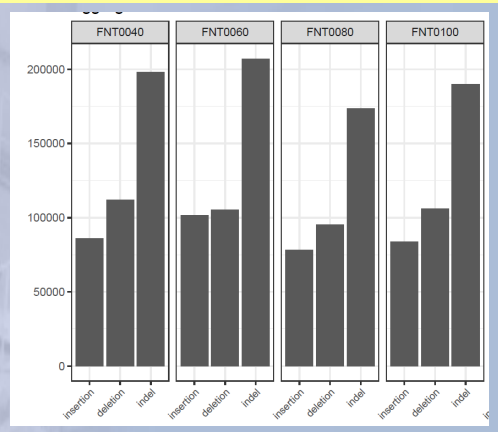
aggregate size



Fast neutron

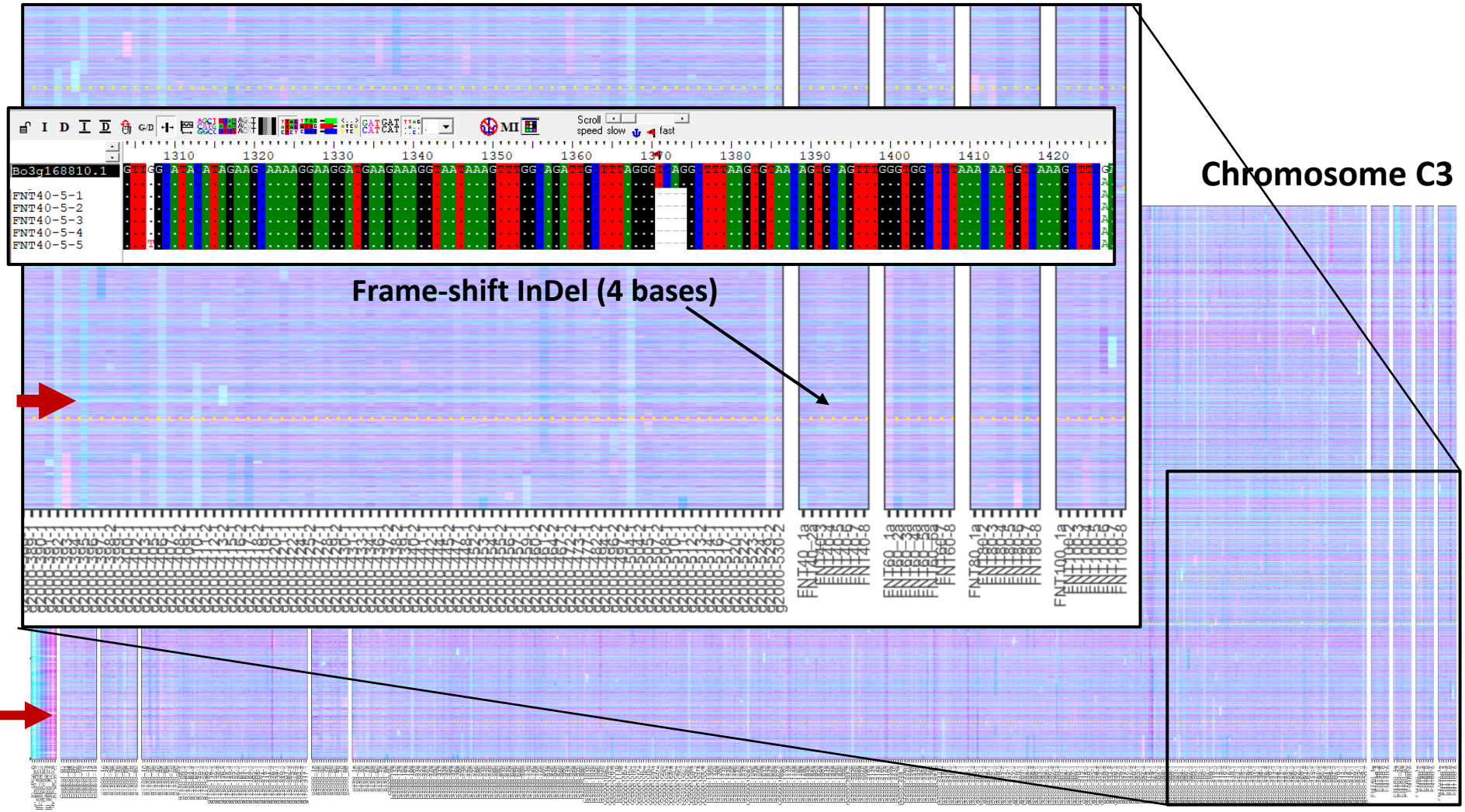






InDels

Identifying frame-shift mutations using the RIPR radiation mutagenesis panel



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Acknowledgement

BBSRC BB/L002124/1 RIPR