Sharpening the tools: genomics-assisted radiation mutagenesis in winter oilseed rape

Ian Bancroft

16th October, 2023

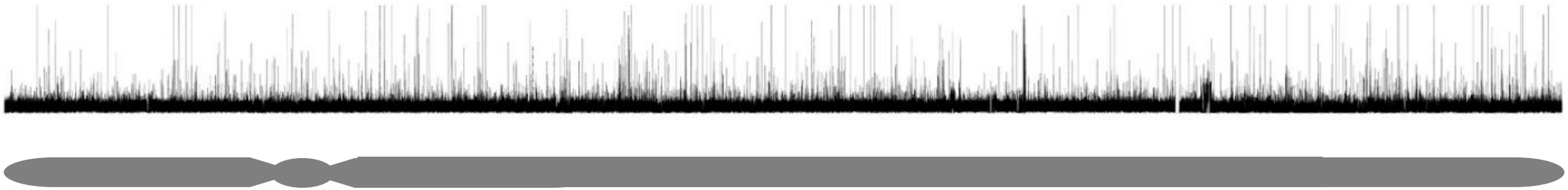
- **Visualizing genome structural variation in oilseed rape**
- **Trimming the Rfo introgression**
- **A prototype WOSR genome re-sequenced radiation panel**

Visualizing genome dosage variation using genome re-sequencing data

Quantify read depth (RPKM) mapped to AC genome reference sequence

↓

Display height of histogram bars based on RPKM values



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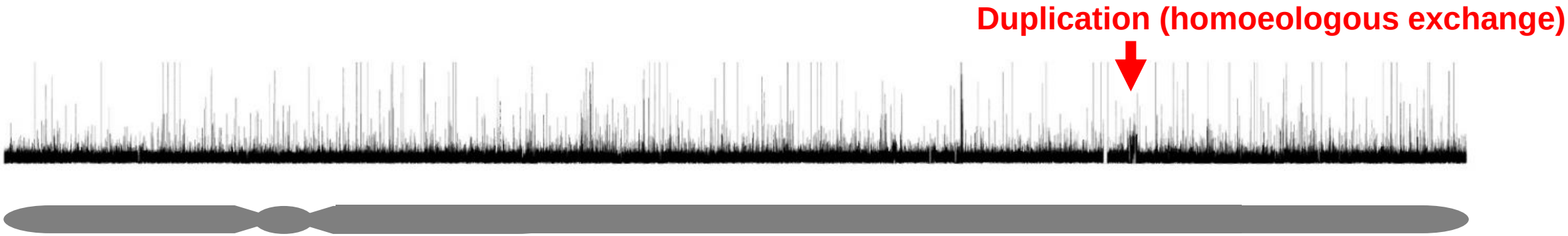

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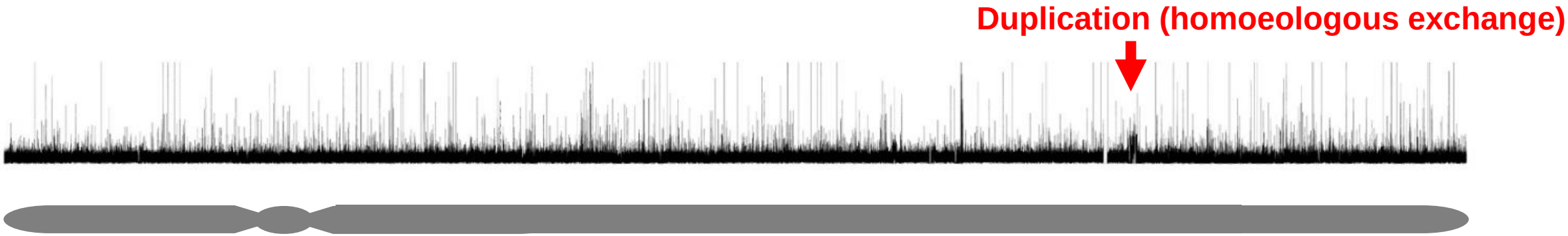


- **Preferable to have a more compact display to compare many genomes**

Visualizing genome dosage variation using genome re-sequencing data

Quantify read depth (RPKM) mapped to AC genome reference sequence

↓

Code intensity of shading based on RPKM values

↓

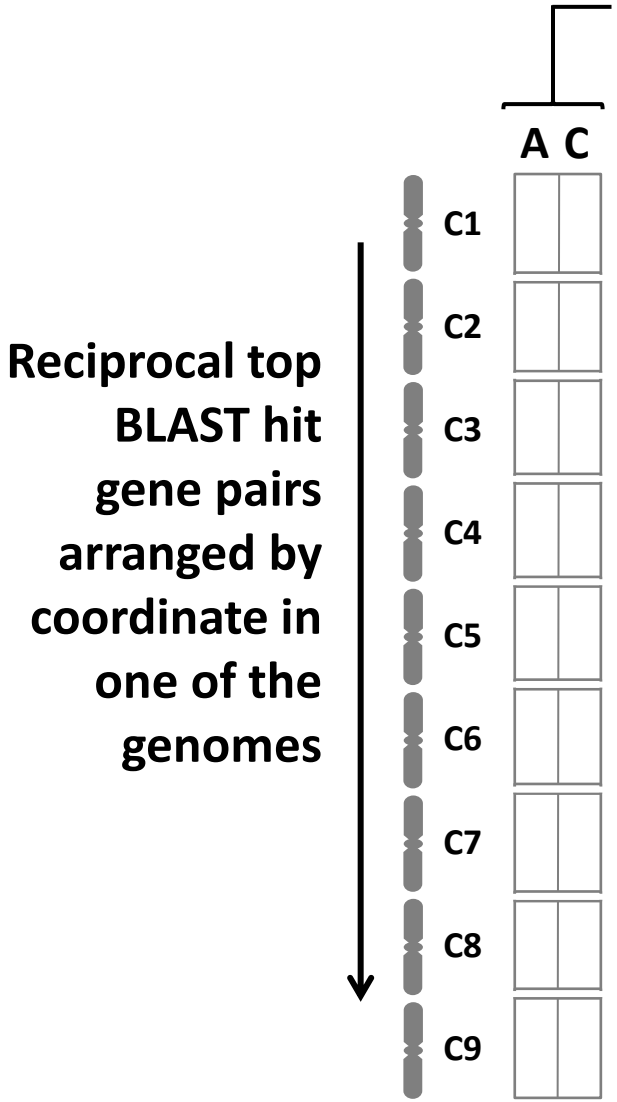
Filter data for reciprocal top BLAST hit gene pairs

↓

Visualise using tile plots with gene pairs ordered by one genome

Visualizing genome dosage variation using genome re-sequencing data

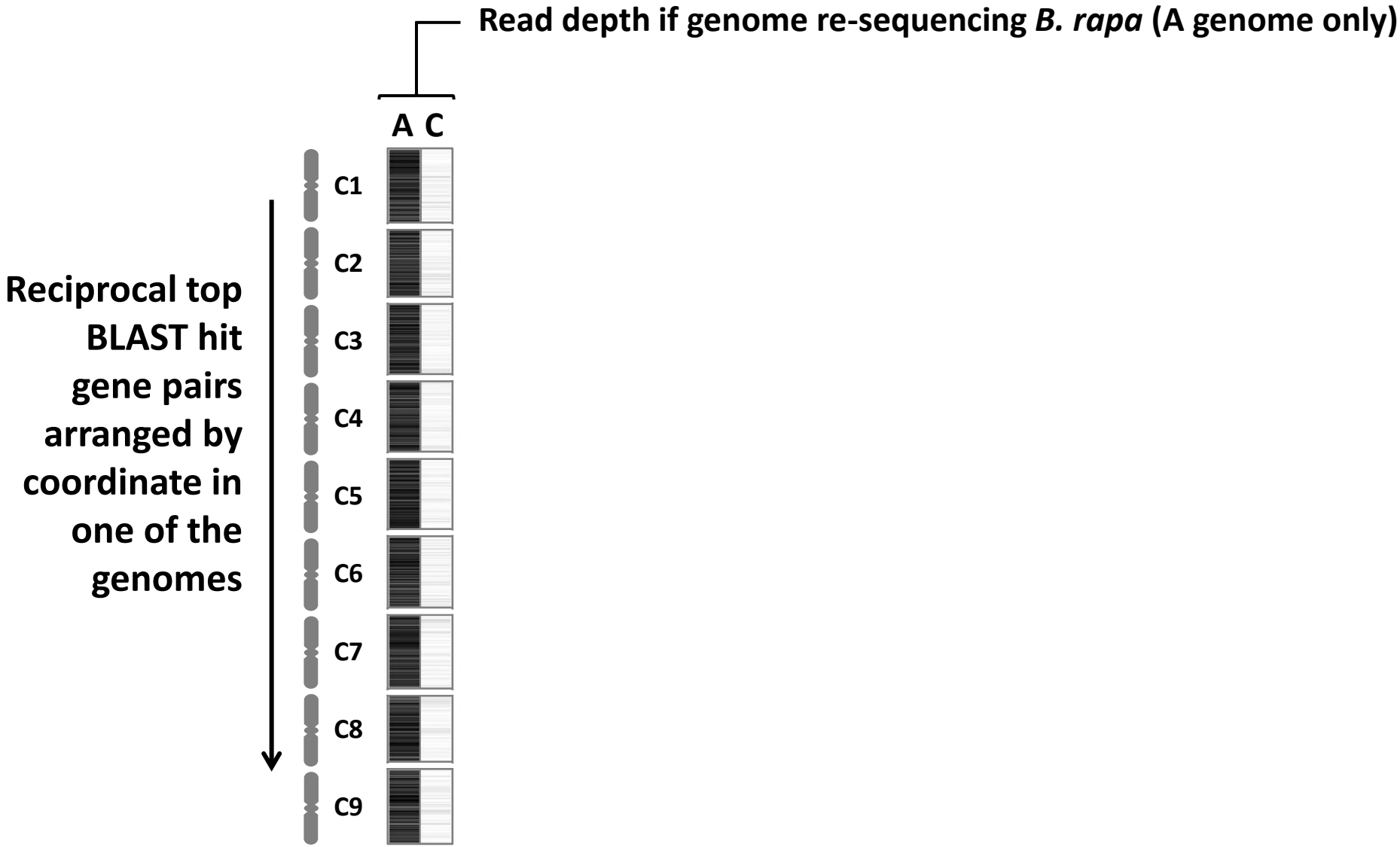
Positions of 31,591 homoeologous gene pairs, arranged by the order in the C genome of the C genome homoeologoue



Reciprocal top BLAST hit gene pairs arranged by coordinate in one of the genomes

	A genome homoeologue	Position in A genome	Order in A	Order in C	Position in C genome	C genome homoeologue
2	Cab020597.1	A01_000007596_000004363	1	1	C01_000004096_000006192	Bo1g001030.1
3	Cab020596.1	A01_000009147_000008191	2	25377	C07_048354208_048355539	Bo7g120870.1
4	Cab020595.2	A01_000011403_000009778	3	2	C01_000007519_000009142	Bo1g001040.1
5	Cab020594.1	A01_000012765_000011917	4	3	C01_000009720_000010577	Bo1g001050.1
6	Cab020592.1	A01_000021944_000019956	5	5	C01_000037620_000038611	Bo1g001080.1
7	Cab020591.1	A01_000024271_000022281	6	6	C01_000039858_000040949	Bo1g001090.1
8	Cab020590.1	A01_000032446_000028836	7	7	C01_000046852_000050484	Bo1g001100.1
9	Cab020589.1	A01_000033293_000032595	8	30597	C09_021661539_021662053	Bo9g071600.1
10	Cab020588.1	A01_000035983_000033952	9	9	C01_000068965_000070059	Bo1g001140.1
11	Cab020581.1	A01_000063572_000063039	10	10	C01_000103201_000103782	Bo1g002150.1
12	Cab020579.1	A01_000079019_000078723	11	11	C01_000112982_000113278	Bo1g002190.1
13	Cab020577.1	A01_000088358_000088041	12	12	C01_000124351_000124668	Bo1g002200.1
14	Cab020576.1	A01_000094861_000093230	13	13	C01_000128563_000130200	Bo1g002210.1
15	Cab020575.1	A01_000097996_000095110	14	14	C01_000130440_000133386	Bo1g002220.1
16	Cab020574.1	A01_000100108_000098057	15	15	C01_000134318_000135251	Bo1g002230.1
17	Cab020573.1	A01_000105326_000101322	16	16	C01_000137577_000141372	Bo1g002250.1
18	Cab020571.1	A01_000115025_000112704	17	17	C01_000148961_000151258	Bo1g002280.1
19	Cab020570.1	A01_000119528_000116614	18	18	C01_000153250_000156261	Bo1g002300.1
20	Cab020569.1	A01_000129775_000126831	19	19	C01_000164958_000168351	Bo1g002310.1
21	Cab020568.1	A01_000135472_000131405	20	20	C01_000172779_000174206	Bo1g002330.1
22	Cab020567.1	A01_000143020_000139039	21	21	C01_000175196_000180272	Bo1g002340.1
23	BnaA01g05330D	A01_000146772_000145212.00	22	23	C01_000183323_000183774	Bo1g002360.1
24	BnaA01g05340D	A01_000146772_000145212.00	23	24	C01_000184847_000187483	Bo1g002370.1
25	Cab020565.1	A01_000146829_000145011	24	22	C01_000181177_000182520	Bo1g002350.1
26	Cab020563.1	A01_000155768_000155126	25	25	C01_000191317_000191852	Bo1g002400.1
27	Cab020562.1	A01_000158109_000156564	26	26	C01_000192637_000194204	Bo1g002410.1
28	Cab020560.1	A01_000162233_000159755	27	27	C01_000195435_000197330	Bo1g002420.1
29	Cab020558.1	A01_000164388_000163736	28	29	C01_000202463_000202892	Bo1g002450.1
30	Cab020557.1	A01_000167670_000166905	29	30	C01_000205351_000206116	Bo1g002460.1
31	Cab020556.1	A01_000168725_000168246	30	31	C01_000206881_000208172	Bo1g002470.1
32	Cab020555.1	A01_000170941_000170440	31	32	C01_000209076_000210429	Bo1g002480.1
33	Cab020554.1	A01_000171955_000171563	32	33	C01_000210647_000211042	Bo1g002490.1
34	Cab020553.1	A01_000180461_000175822	33	34	C01_000216852_000221622	Bo1g002500.1
35	Cab020552.1	A01_000184155_000183811	34	35	C01_000223558_000223902	Bo1g002510.1
36	Cab020550.1	A01_000190655_000188381	35	37	C01_000228012_000229293	Bo1g002520.1
37	Cab020549.1	A01_000199980_000195942	36	38	C01_000237167_000240760	Bo1g002530.1
38	Cab020547.1	A01_000210518_000207773	37	39	C01_000268432_000270656	Bo1g002540.1
39	Cab020546.2	A01_000233705_000220053	38	40	C01_000278884_000293749	Bo1g002550.1
40	Cab020545.1	A01_000236573_000235062	39	41	C01_000294476_000298099	Bo1g002560.1
41	Cab020544.1	A01_000240300_000237136	40	42	C01_000298888_000301646	Bo1g002570.1
42	Cab020543.1	A01_000244118_000242423	41	43	C01_000305351_000307099	Bo1g002590.1
43	Cab020540.1	A01_000247210_000246059	42	44	C01_000312999_000314150	Bo1g002620.1
44	Cab020539.1	A01_000249298_000248129	43	45	C01_000315809_000316990	Bo1g002630.1

Visualizing genome dosage variation using genome re-sequencing data



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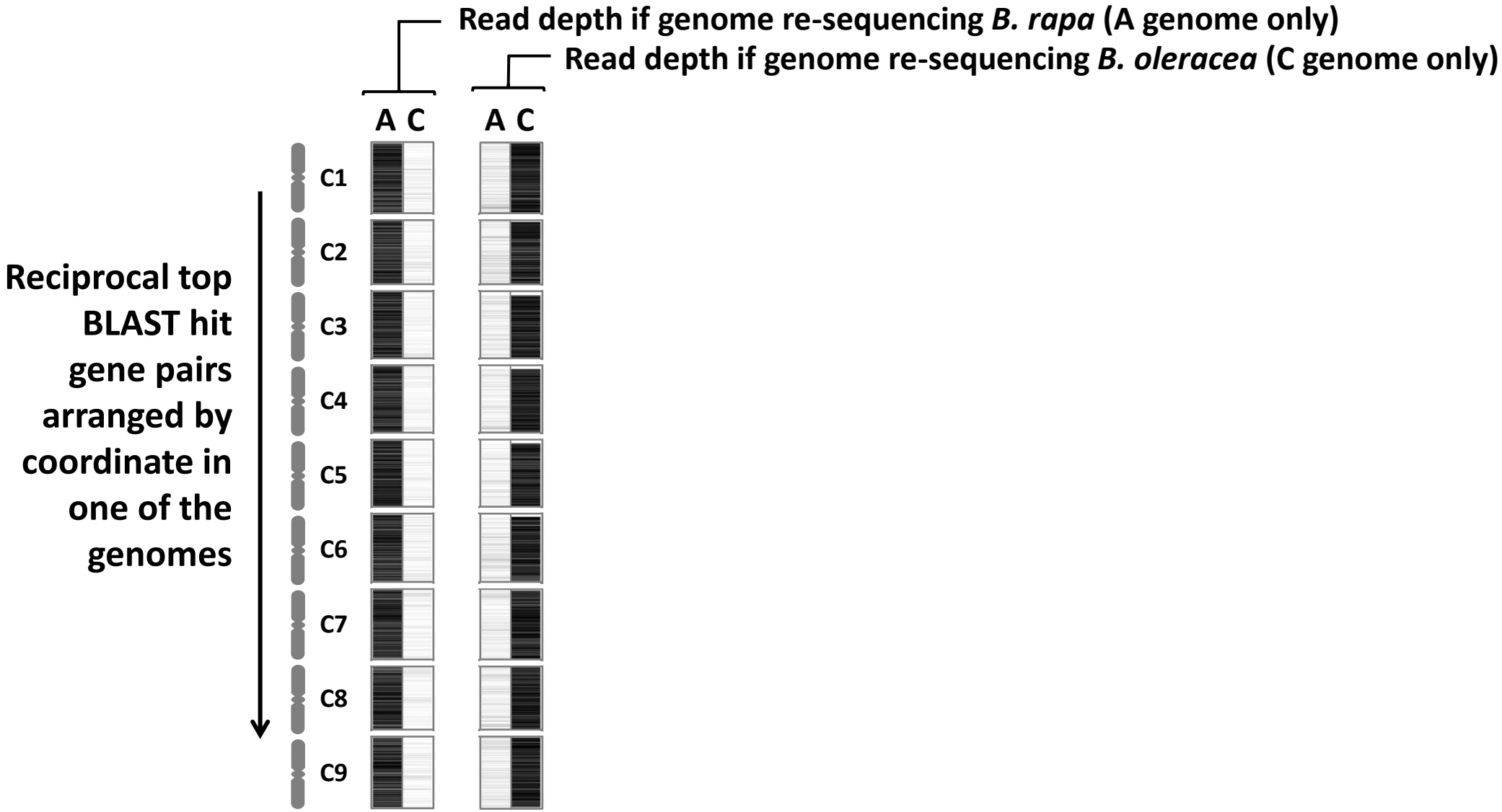

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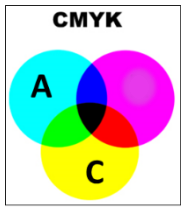
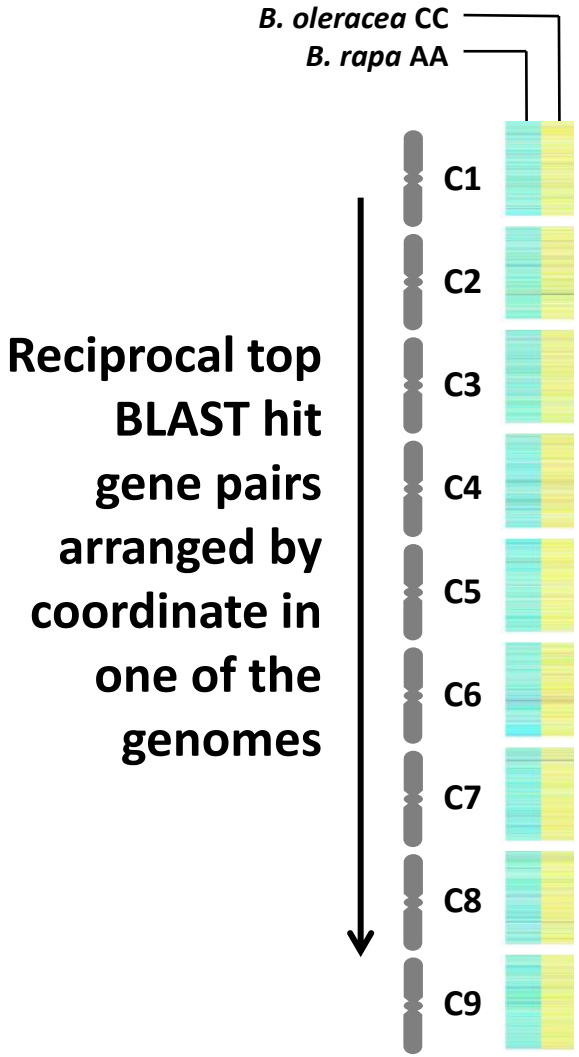


Display top BLAST pairs



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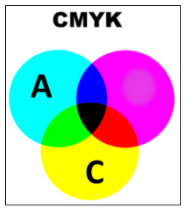

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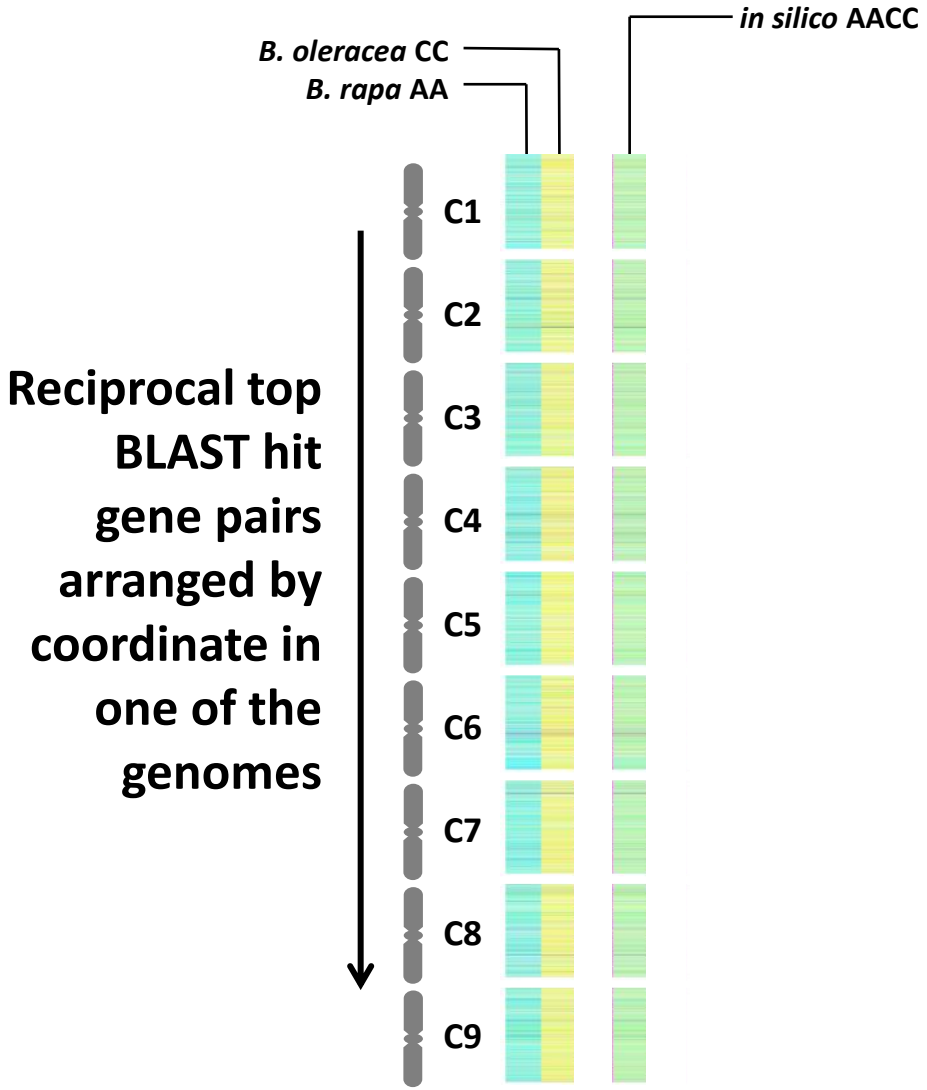

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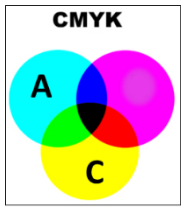

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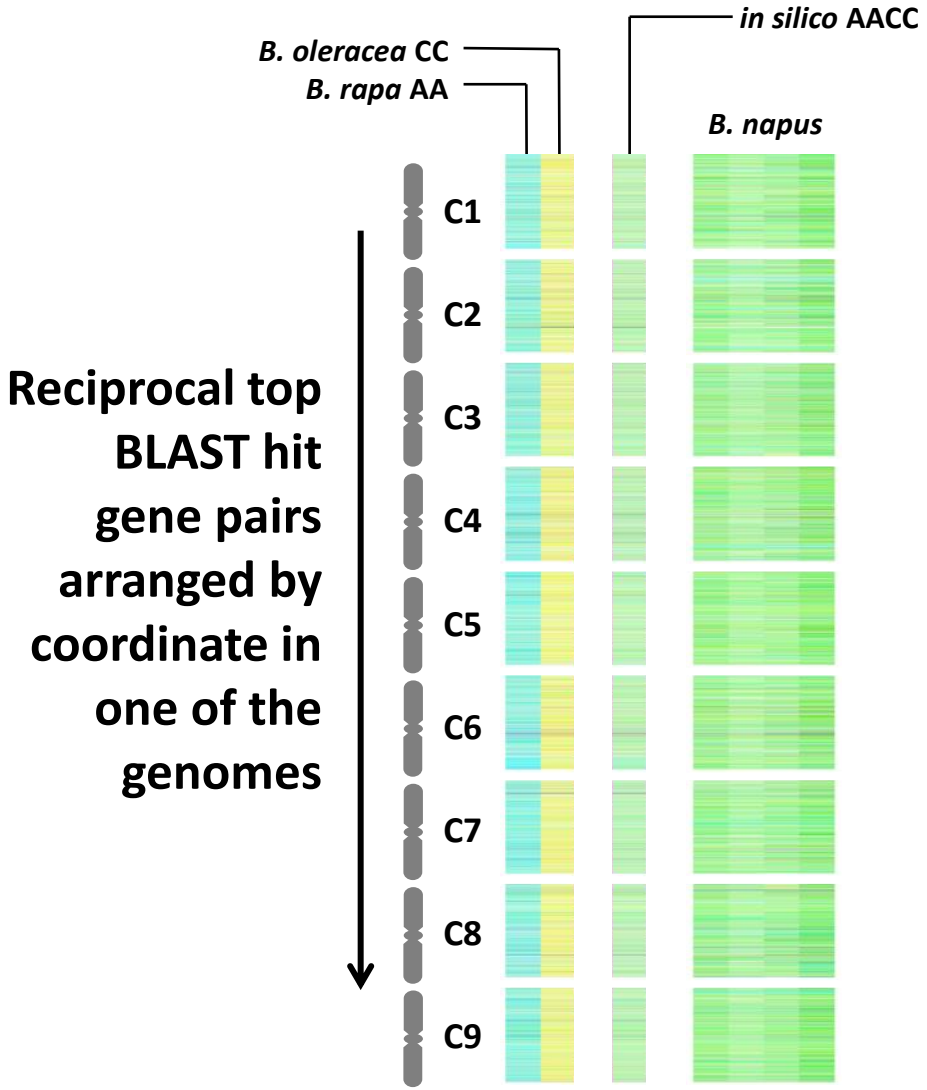

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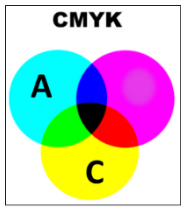

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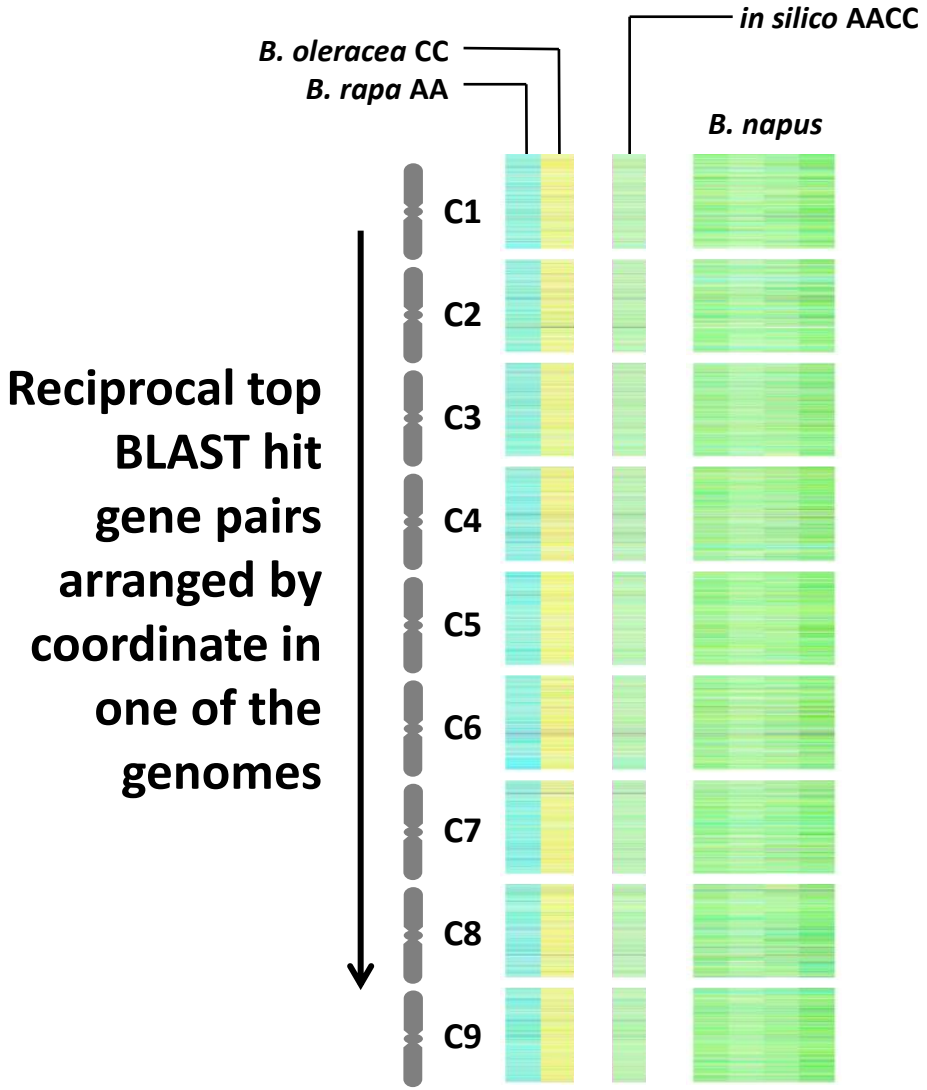

- **Method for compact display of relative genome dosage in oilseed rape**

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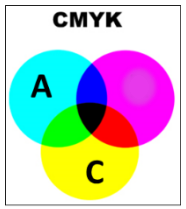

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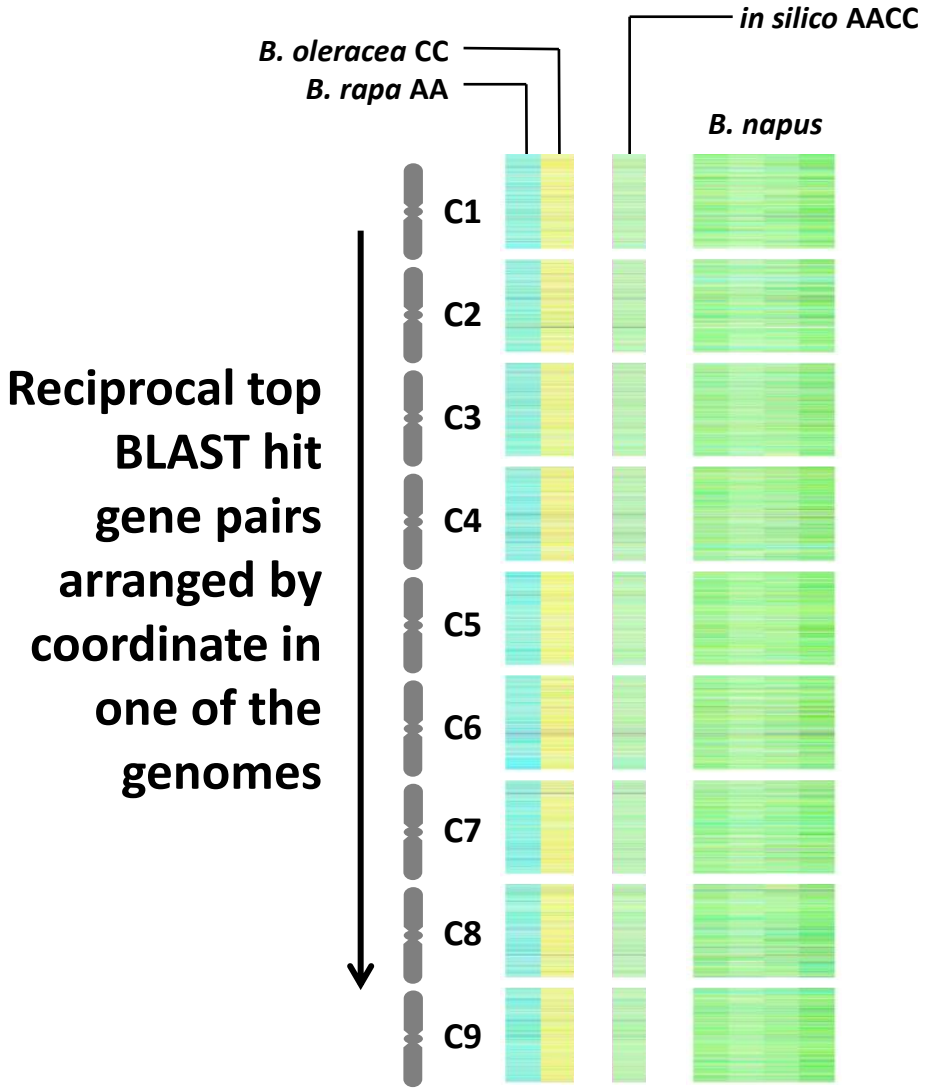
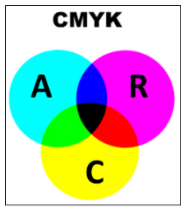
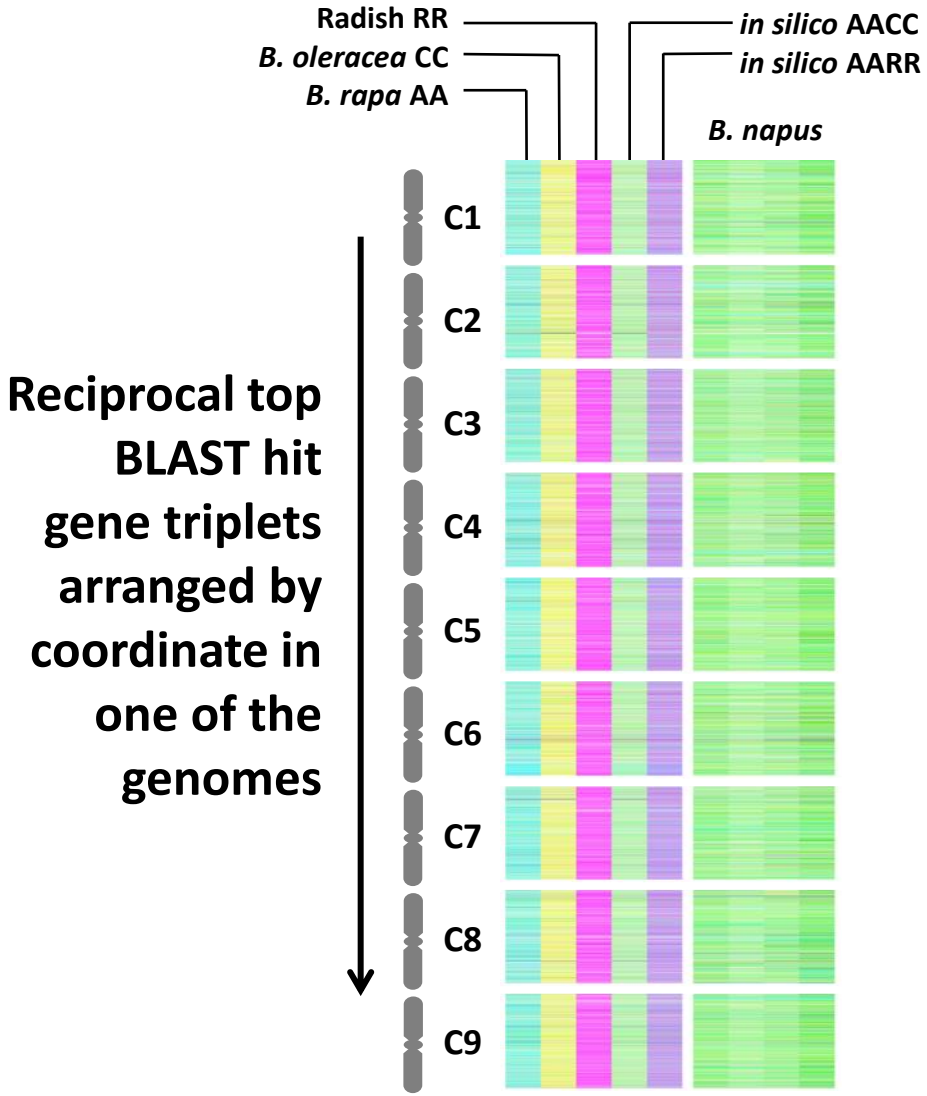


Three genomes can be visualized using genome re-sequencing data

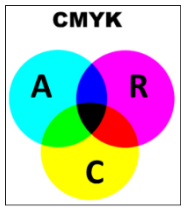


Display top BLAST triplets



- **Read mapping to 3 genomes can be visualized, based on homoeologous gene triplets**

Fertility restorer introgression from radish can be identified and delineated



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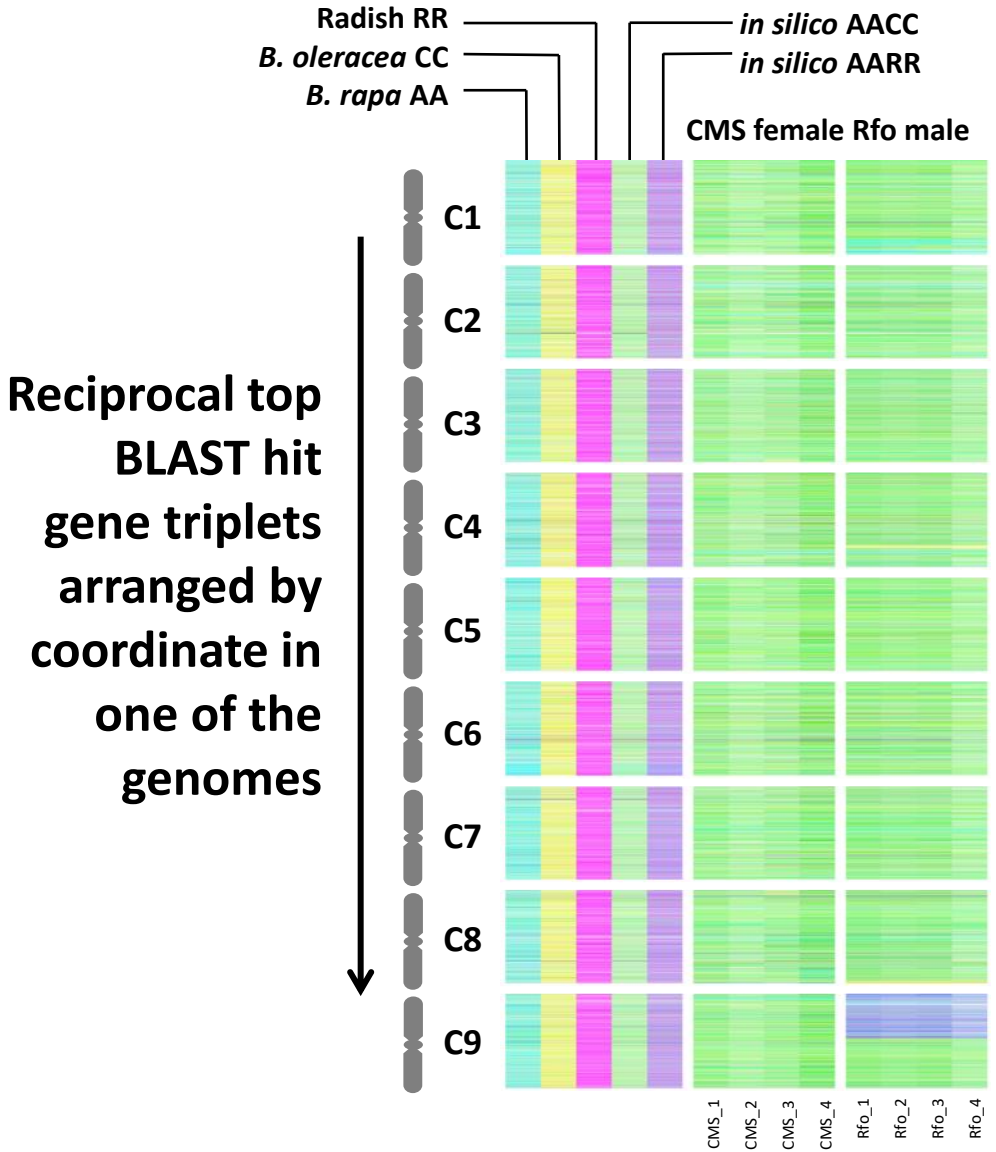

Reciprocal top BLAST hit gene triplets arranged by coordinate in one of the genomes

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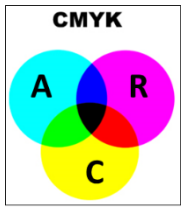

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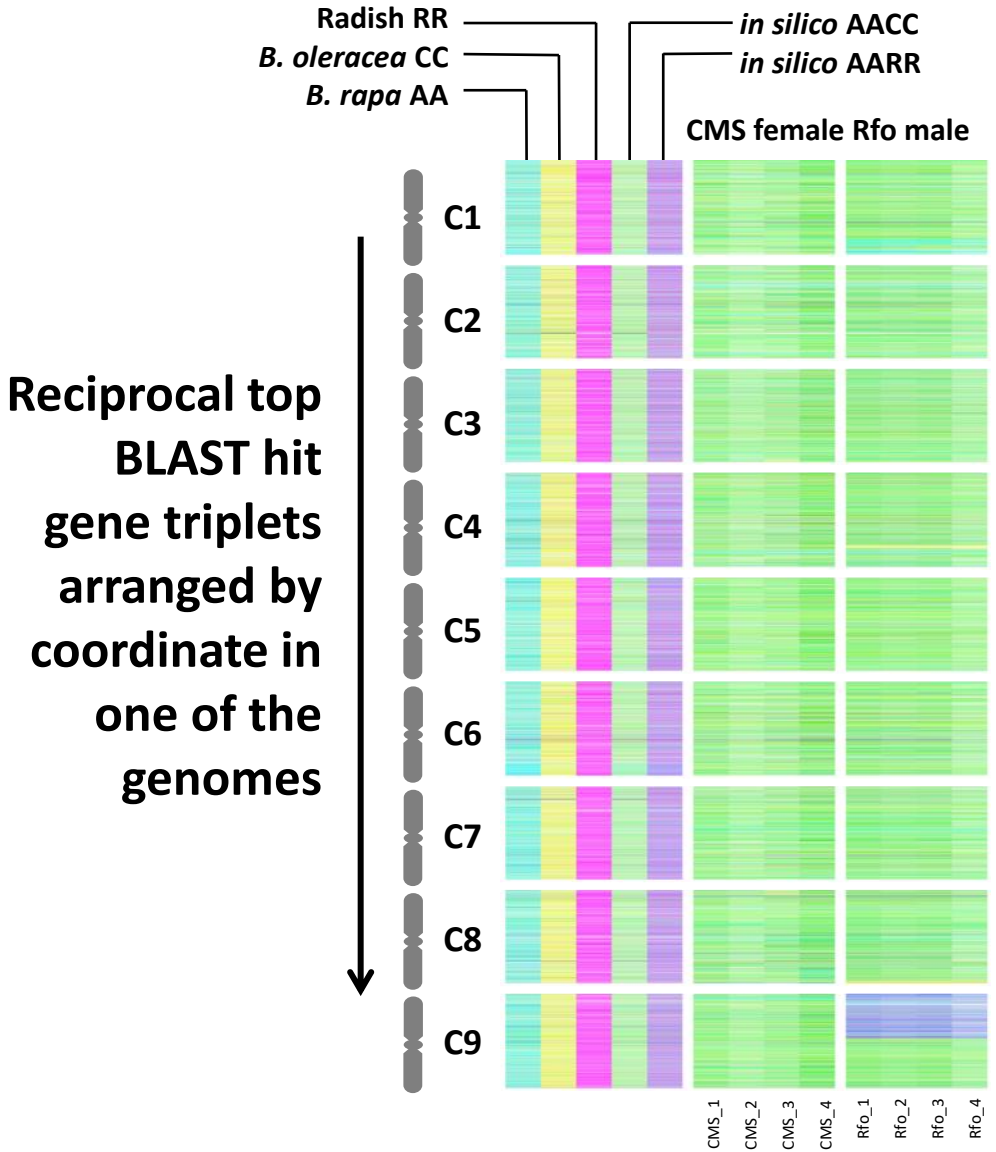
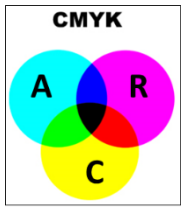


- **Generalized method for visualization of wild relative introgressions**

Visualizations can be ordered by any genome



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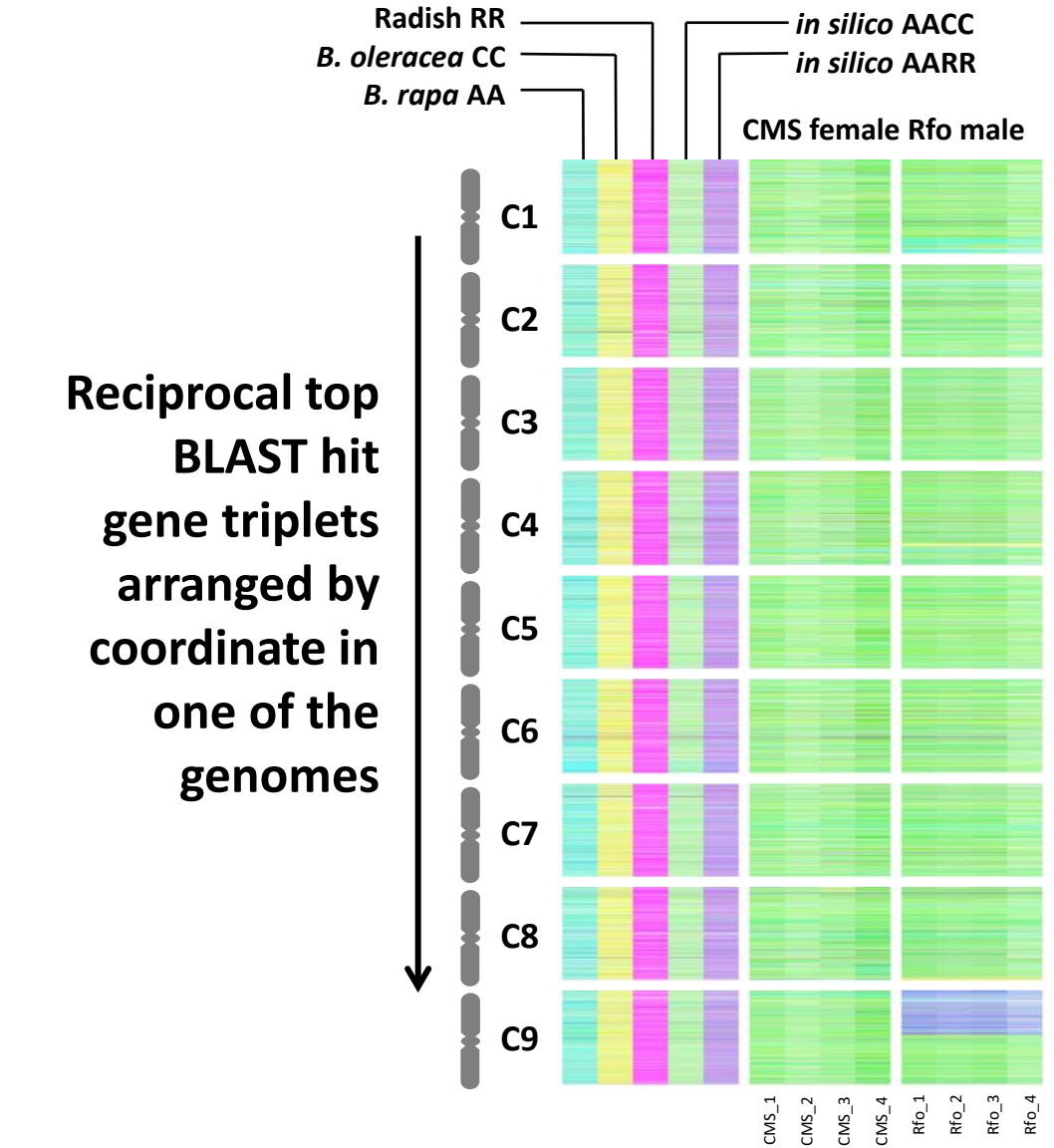
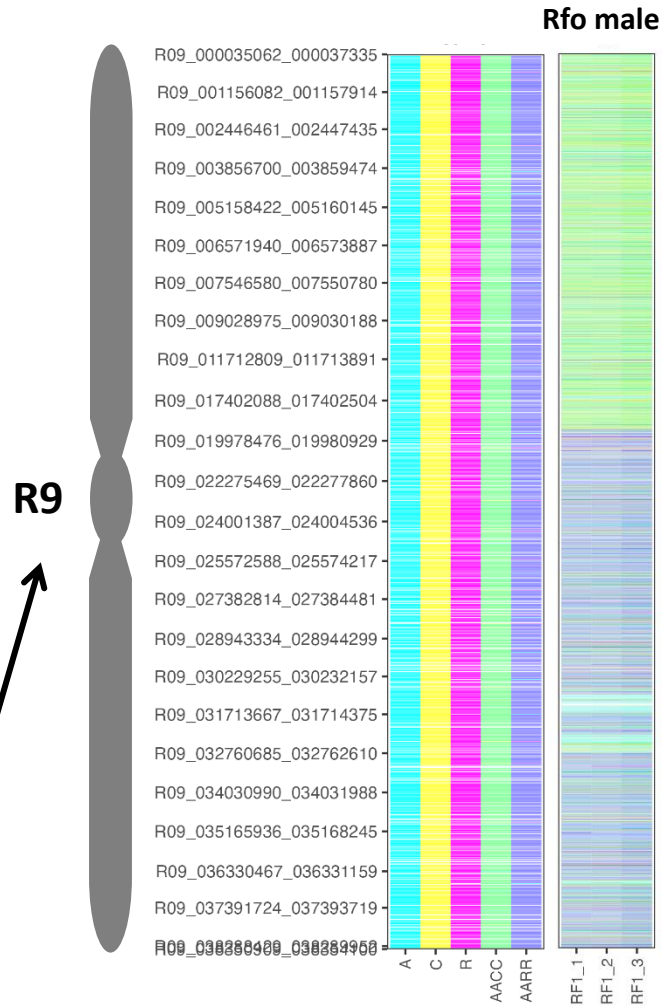
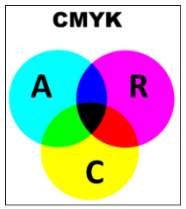




Radiation treatment can be used to disrupt introgressions



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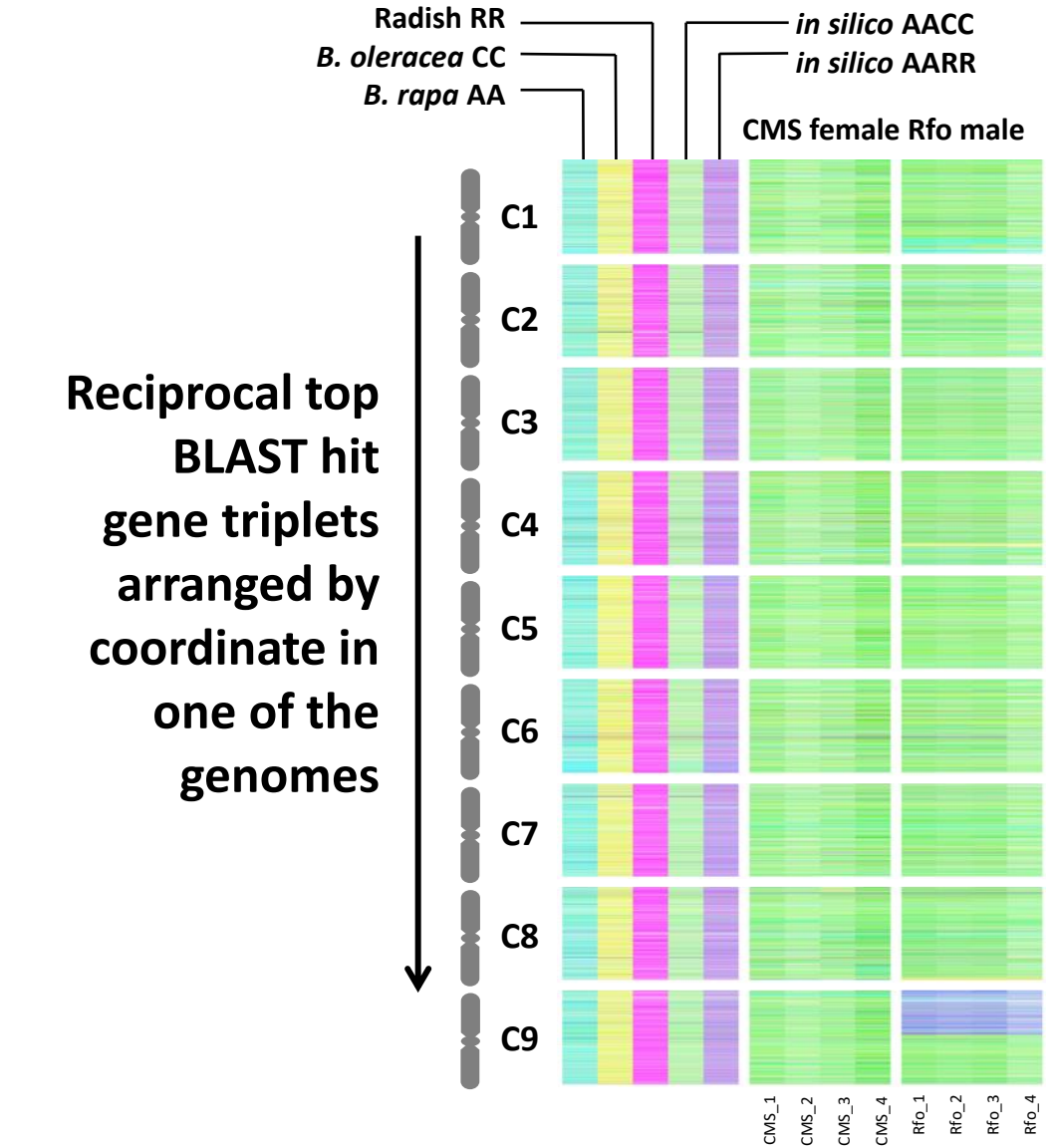
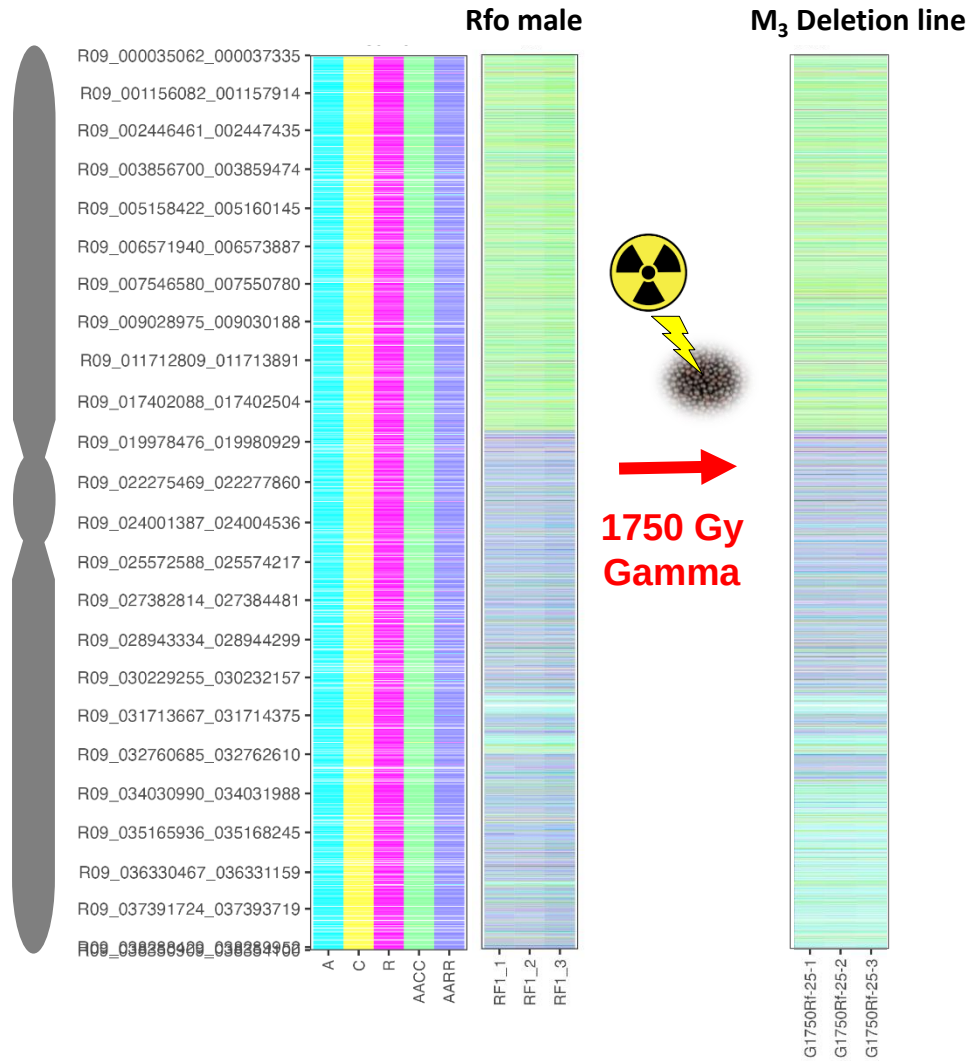
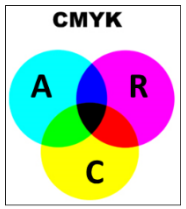




Visualizing genome dosage to assess radiation-induced variation genome-wide



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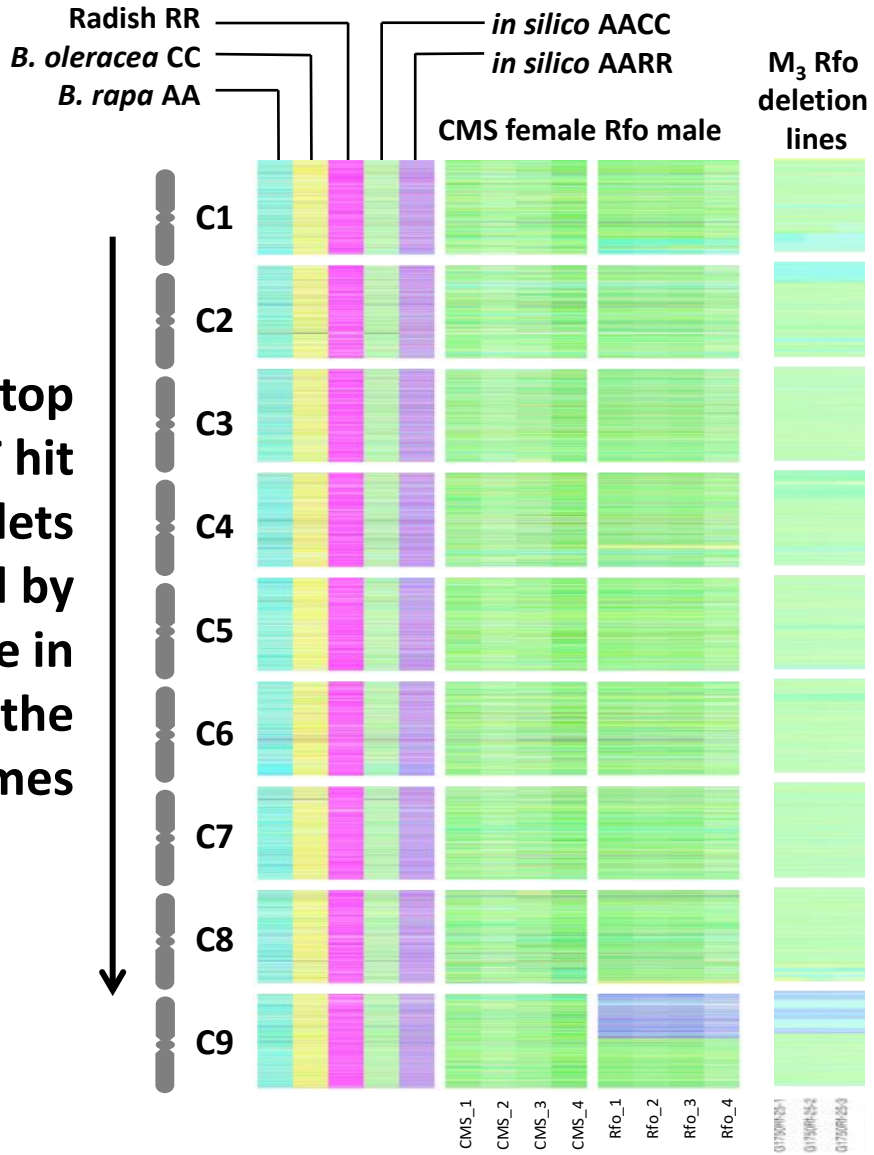

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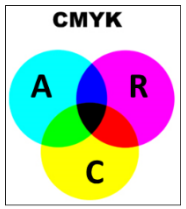

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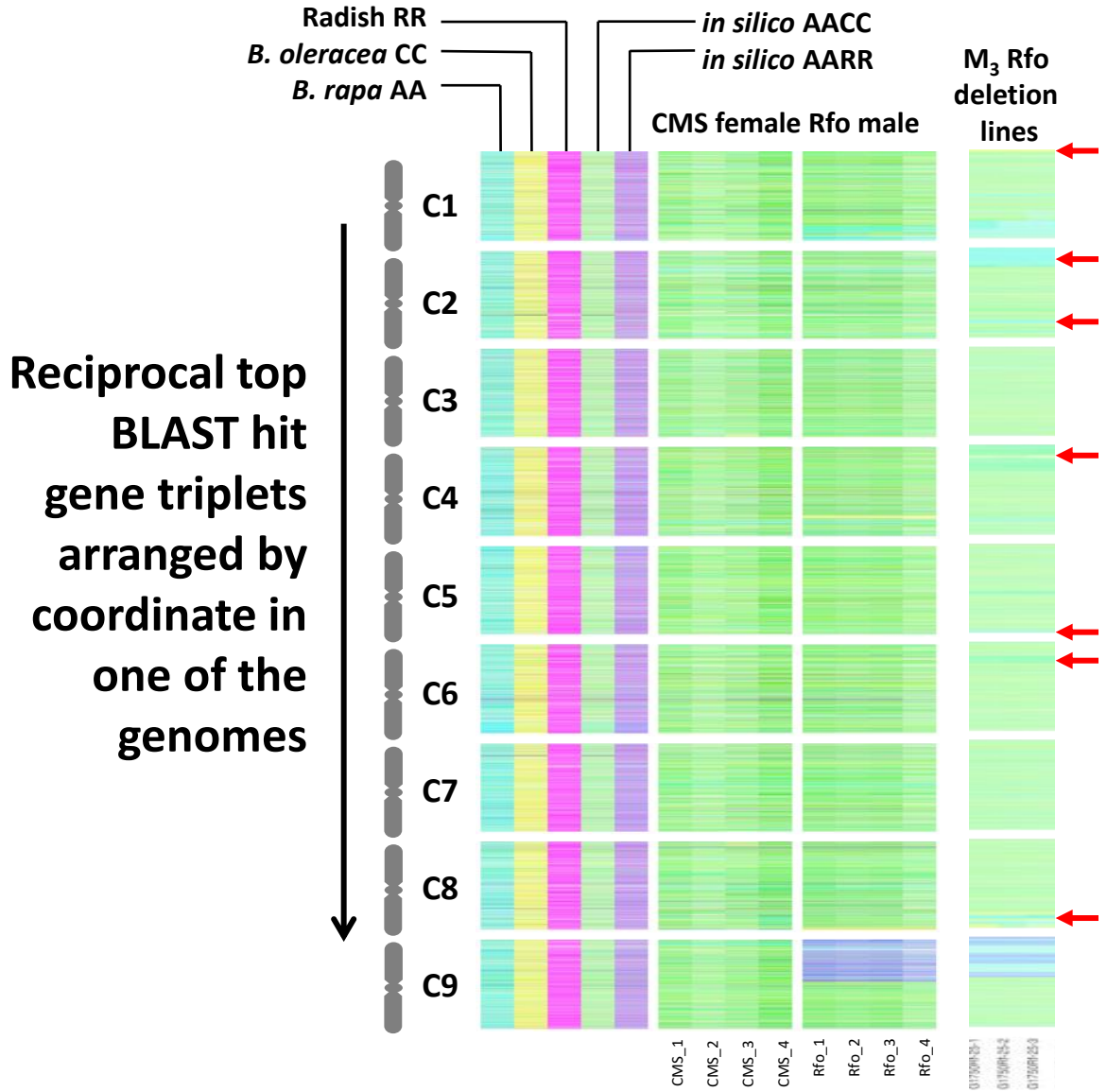

Reciprocal top BLAST hit gene triplets arranged by coordinate in one of the genomes

Radiation-induced lesions elsewhere in the genome

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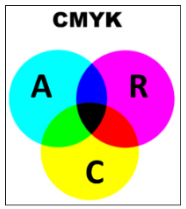

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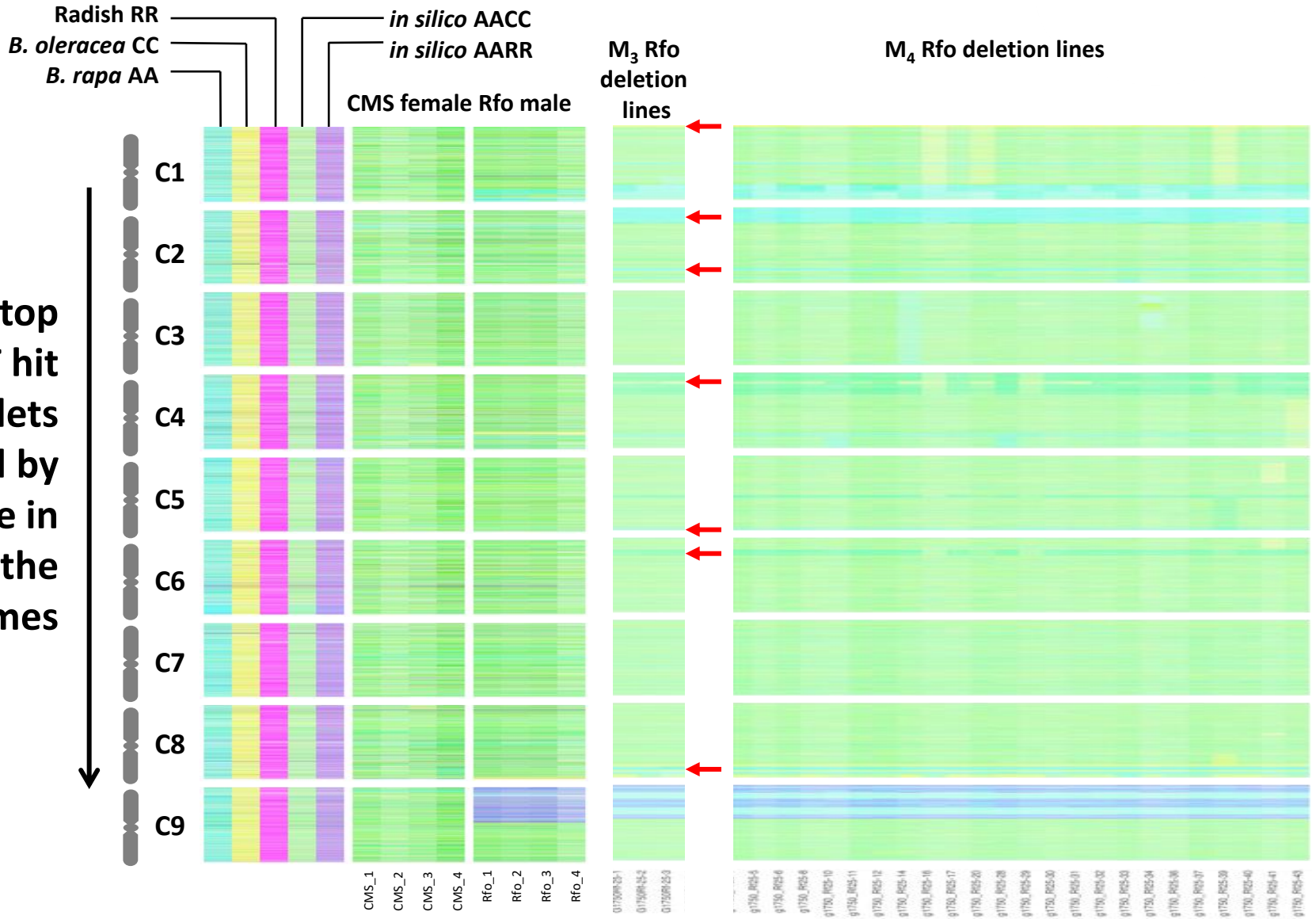
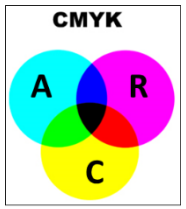


Genome-wide variation can be related to traits such as seed yield



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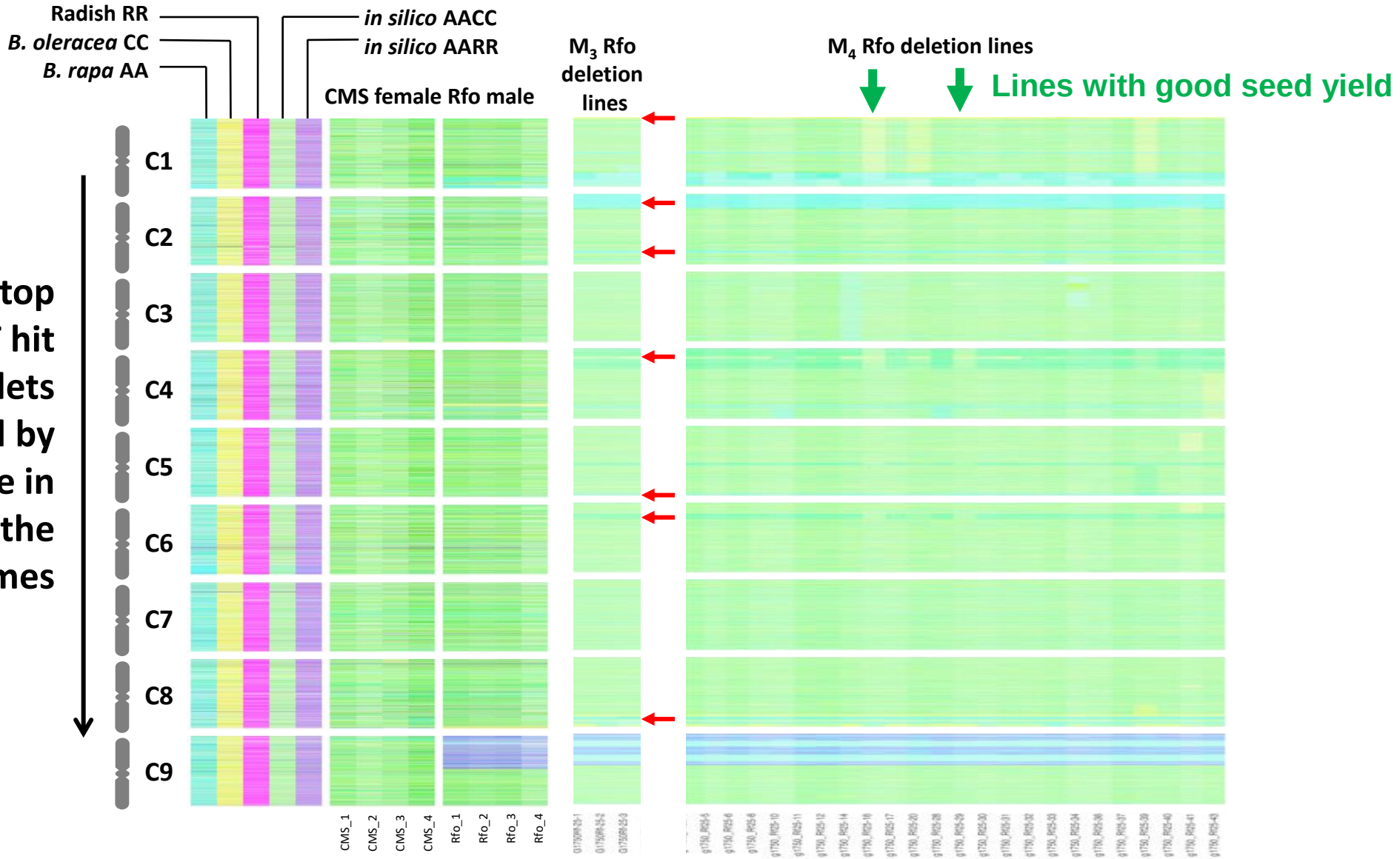
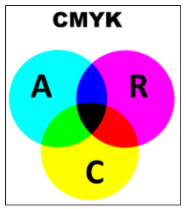
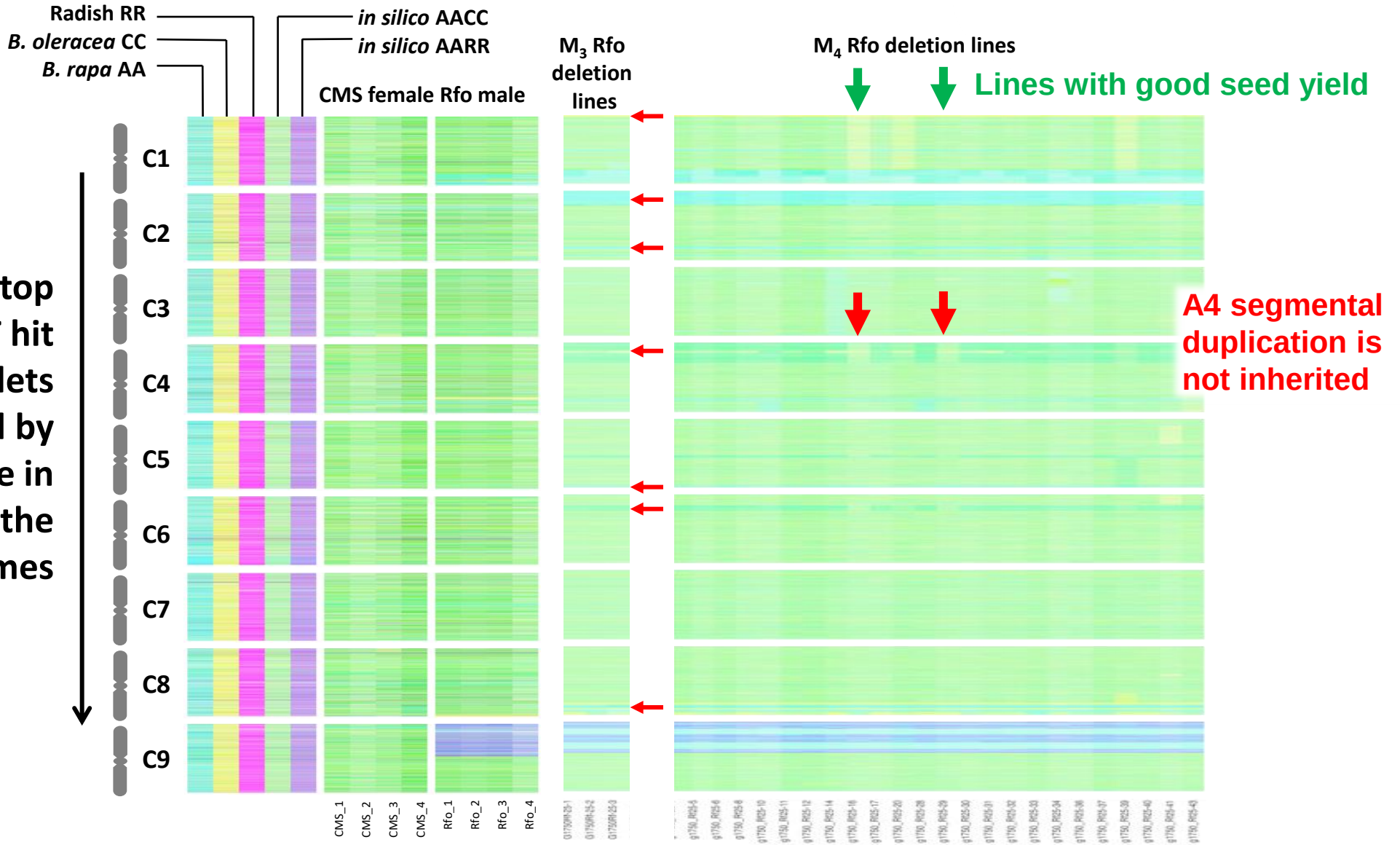


Genome-wide variation can be related to traits such as seed yield



Display top BLAST triplets

Reciprocal top BLAST hit gene triplets arranged by coordinate in one of the genomes



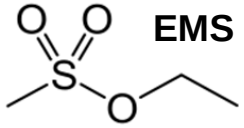
Sharpening the tools: genomics-assisted radiation mutagenesis in winter oilseed rape

Ian Bancroft

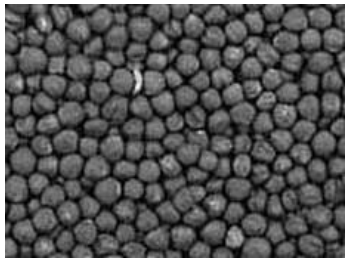
16th October, 2023

- Visualizing genome structural variation in oilseed rape
- Trimming the Rfo introgression
- **A prototype WOSR genome re-sequenced radiation panel**

EMS mutagenesis for “TILLING”



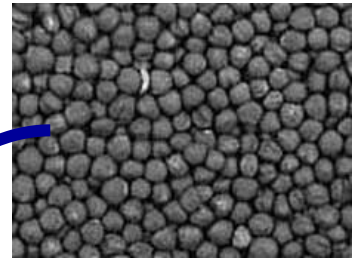
Treat seeds



Sow at high density

Grow and self plants from treatments with ~50% kill

Collect seed from M_1 plants



Grow

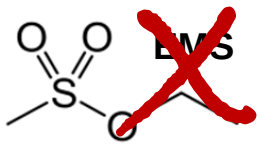
Make DNA from a leaf of each M_2 plant from which seeds will be collected

Collect selfed seed from one M_2 plant per M_1 individual

Panel of C/G to T/A mutations

Seed stocks

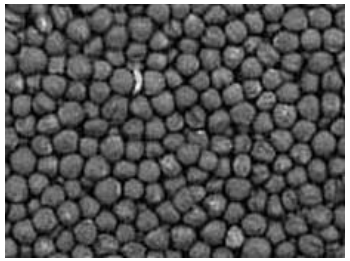
Radiation mutagenesis



γ Radiation

IAEA International Atomic Energy Agency

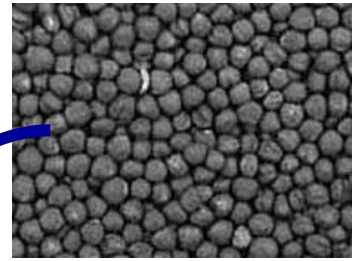
Treat seeds



Sow at high density

Grow and self plants from treatments with ~50% kill

Collect seed from M_1 plants



Grow

Make DNA from a leaf of each M_2 plant from which seeds will be collected

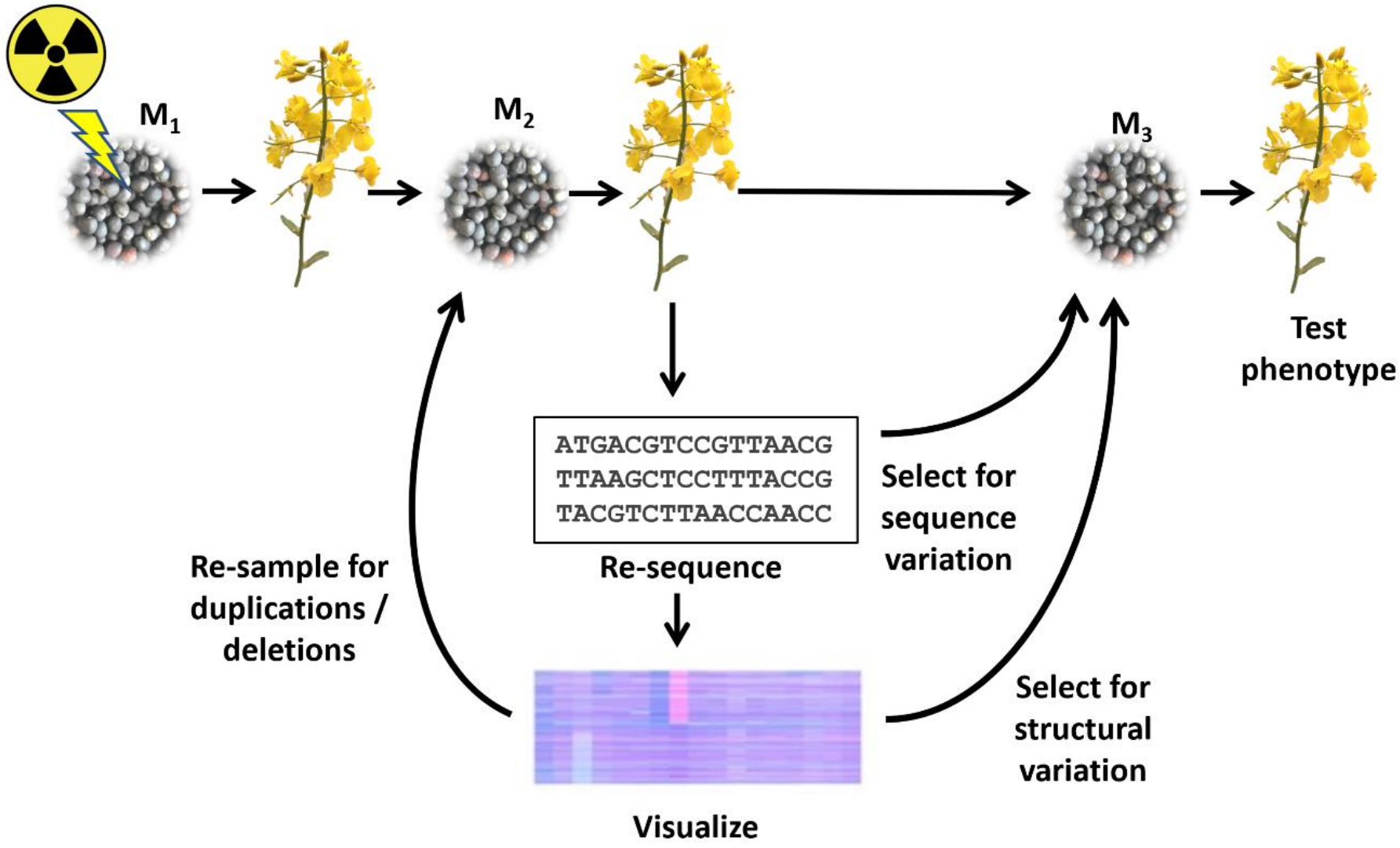
Collect selfed seed from one M_2 plant per M_1 individual

Point, frame-shift and larger deletion mutations

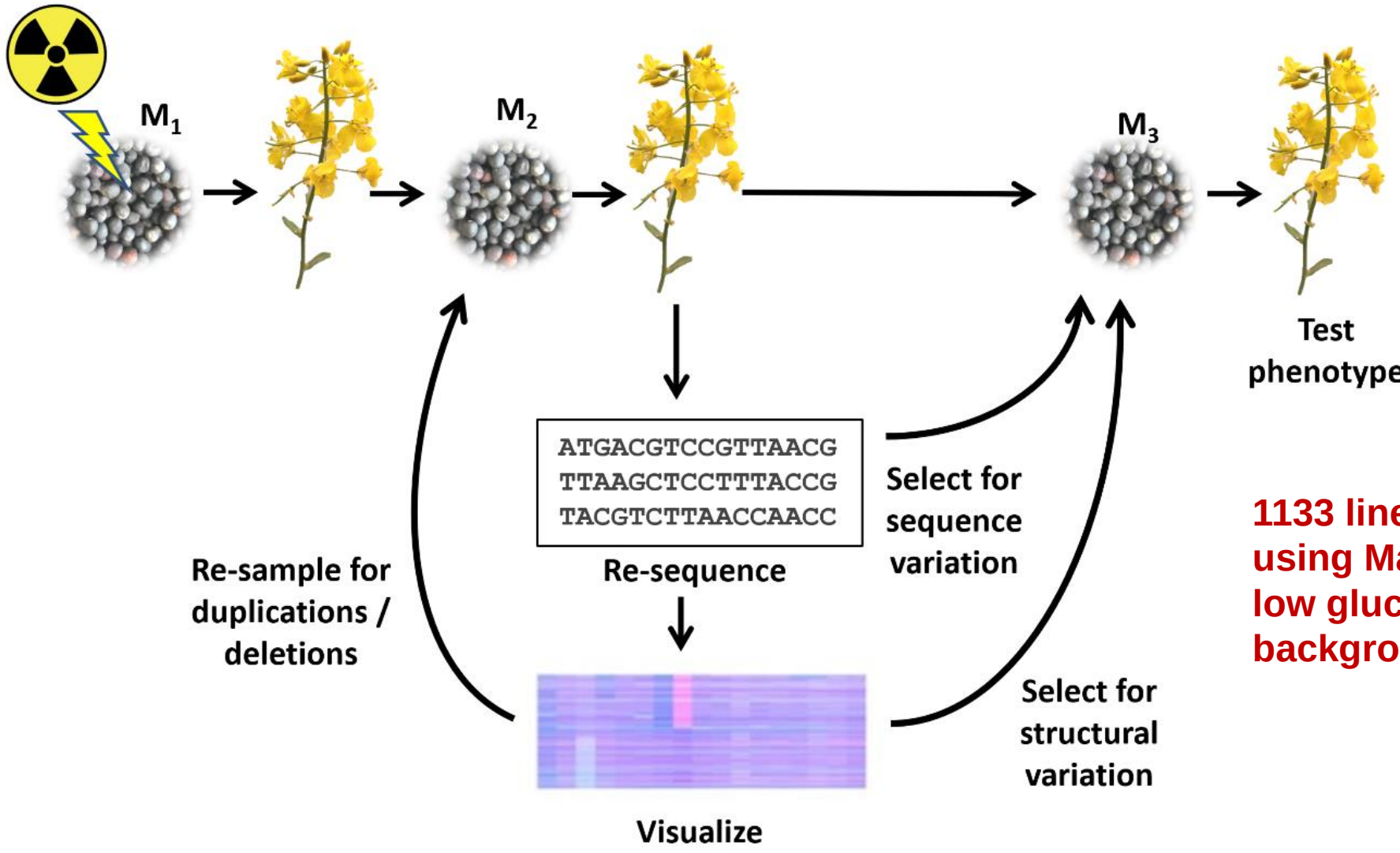
Panel of C/G to T/A mutations

Seed stocks

Development of a genome re-sequenced radiation panel for reverse genetics



Development of a genome re-sequenced radiation panel for reverse genetics

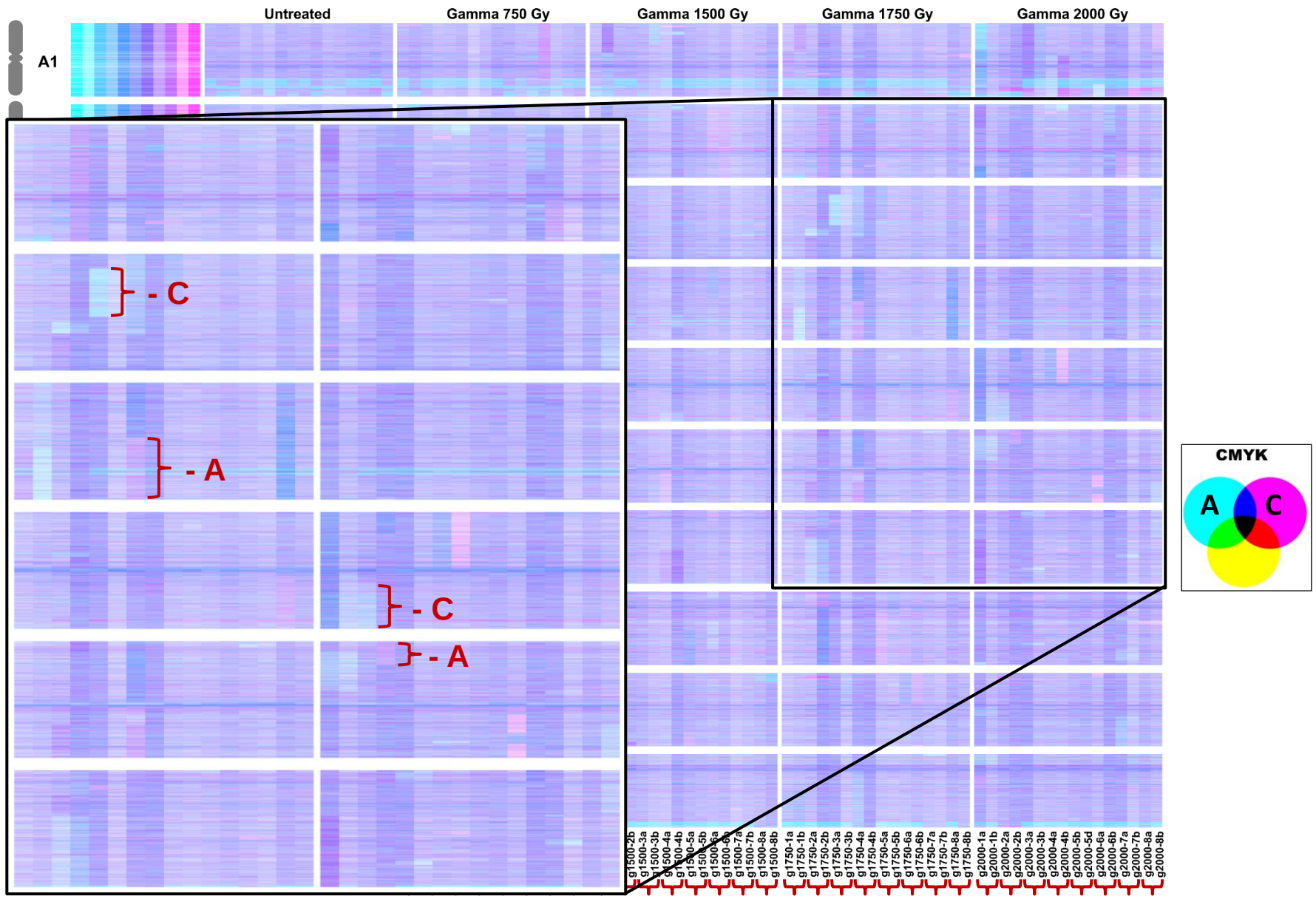


1133 lines in protype panel using Maplus (high erucic, low glucosinolate) WOSR background

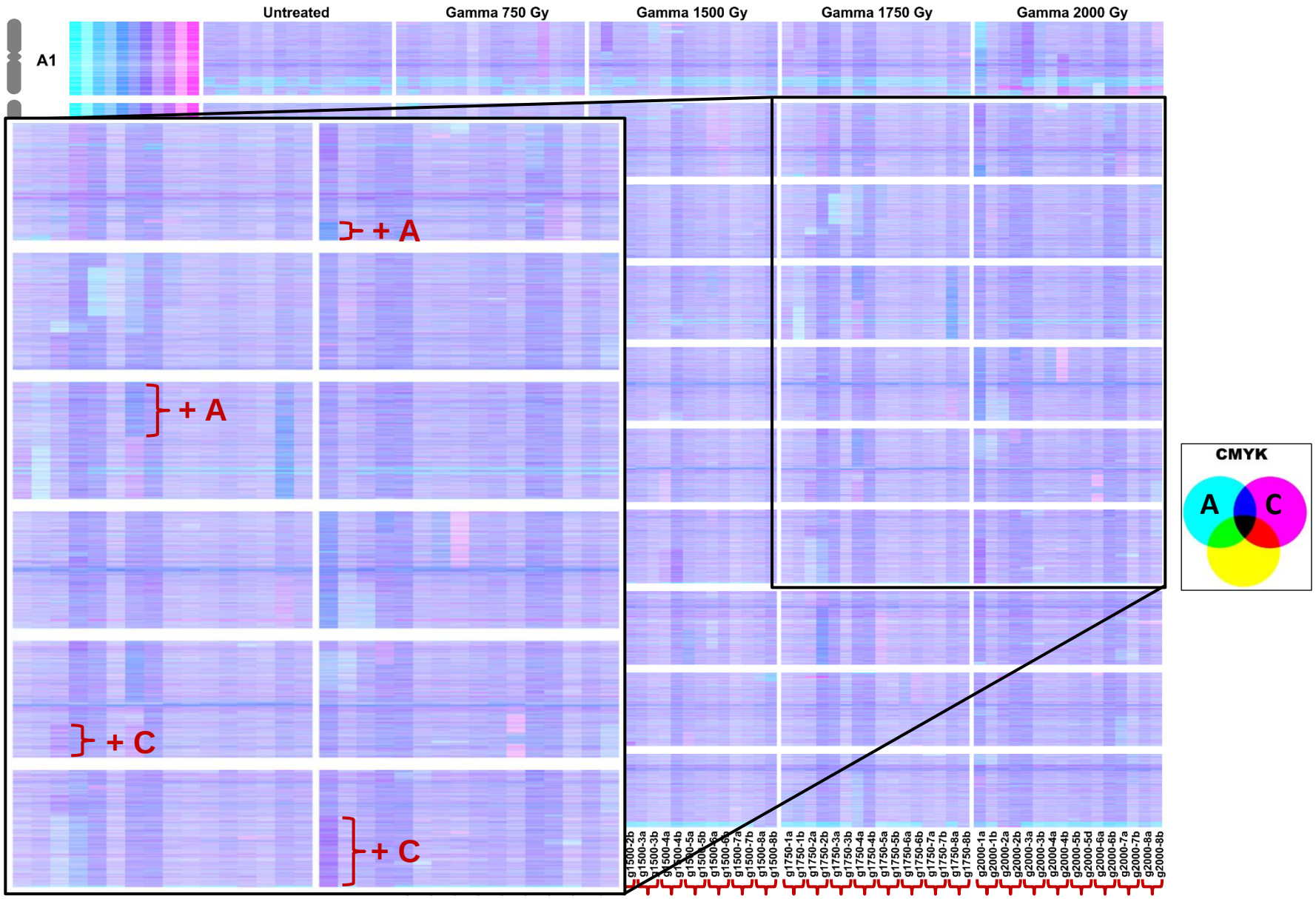
Visualizing gamma radiation-induced dosage variation

Pairs of siblings

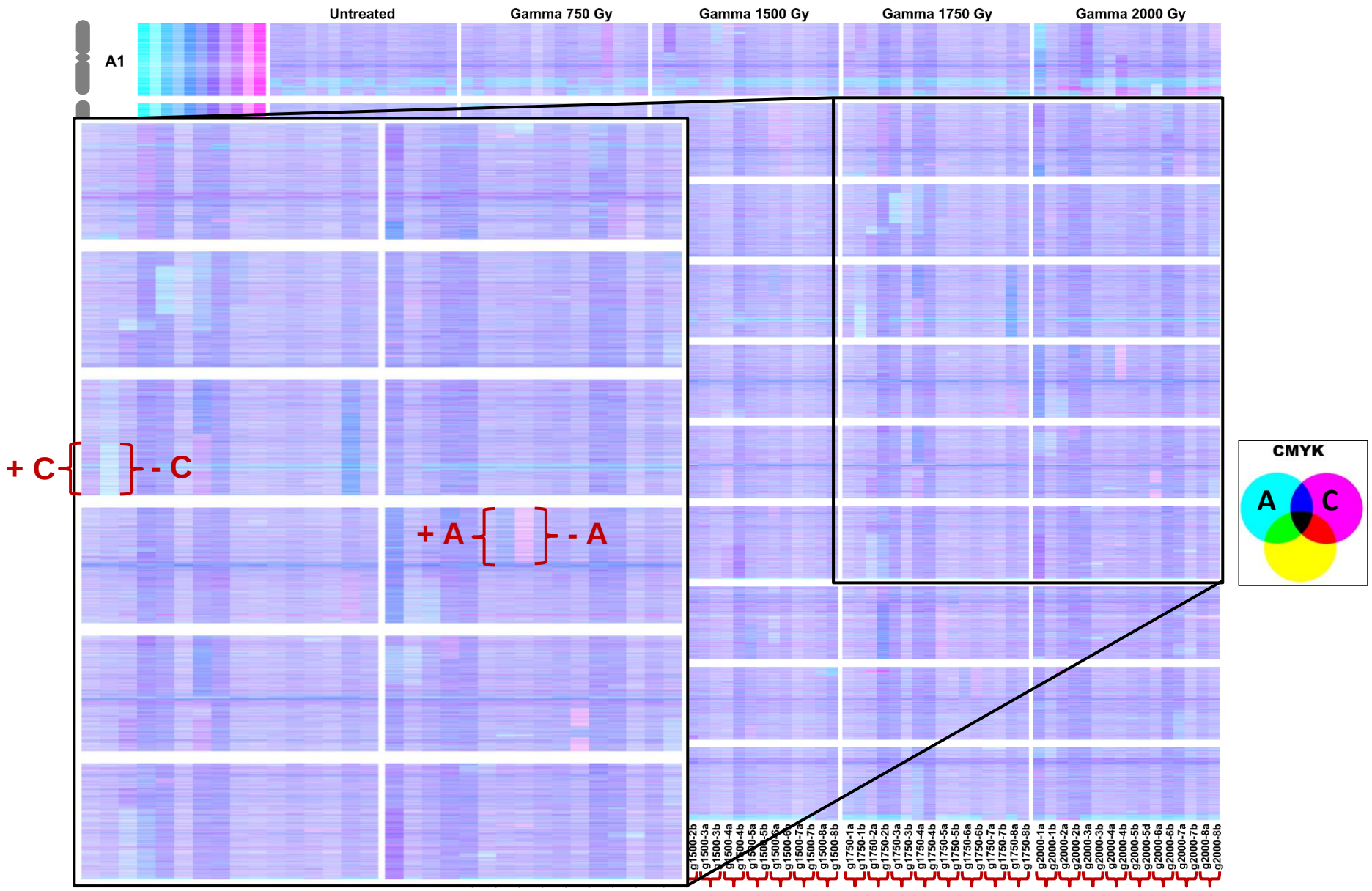
Visualizing gamma radiation-induced dosage variation



Visualizing gamma radiation-induced dosage variation



Visualizing gamma radiation-induced dosage variation



Frequency of large-scale lesions induced by radiation

Radiation dosage / Gy	A genome chromosome loss / plant	C genome chromosome loss / plant	A genome chromosome duplication / plant	C genome chromosome duplication / plant	A genome visible deletion / plant	C genome visible deletion / plant	A genome visible duplication / plant	C genome visible duplication / plant	Mean no. deletions and duplications / plant
NONE	0	0	0	0	0	0	0	0	0
Gamma									
750	0	0	0.06	0	0.5	0.88	0.06	0.20	0.41
1500	0.06	0.06	0	0	0.63	1.13	0.13	0.31	0.55
1750	0.06	0	0.06	0	1.31	1.69	0.56	0.31	0.97
2000	0	0.06	0	0	1.5	2.38	0.63	0.31	1.21
FNT									
40	0.13	0	0	0	0.38	0.25	0	0.25	0.22
60	0	0.13	0	0	0.75	0.63	0.13	0.50	0.50
80	0	0	0	0	1.25	1.38	0.38	0.63	0.91
100	0	0.13	0	0	0.75	2.38	1.25	0.75	1.28

- Gamma and Fast Neutrons induce similar types of variation
- Chromosome number variation detected but is independent of radiation dosage
- Lesion frequency increases with radiation dosage
- More lesions in the C genome than in the A genome
- ~4-fold more deletions than duplications

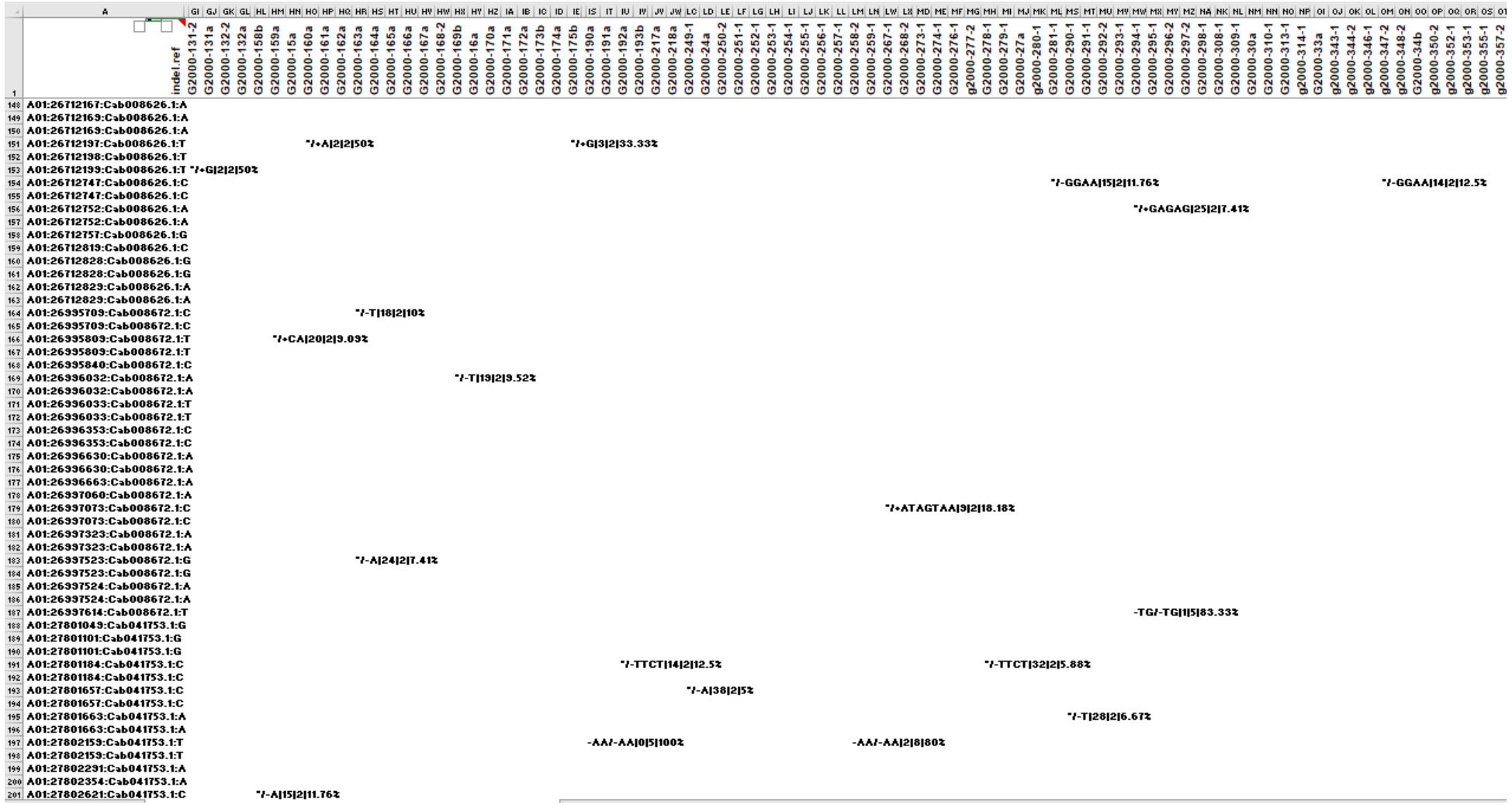
Identification of small-scale lesions induced by radiation

1. Manual inspection of Excel spreadsheets of RPKM data, aided by conditional formatting

1	cds_name	pos	G2000-38a	g2000-390-1	g2000-391-1	g2000-392-1	g2000-393-1	g2000-394-1	g2000-395-1	g2000-396-1	g2000-397-1	g2000-398-2	g2000-399-1	G2000-39a	g2000-3a	g2000-3b	g2000-400-1	g2000-402-1	g2000-403-1	g2000-405-1	g2000-406-1	g2000-407-1	g2000-408-2	g2000-409-1	G2000-40b	g2000-410-1	g2000-411-2	g2000-412-1	g2000-413-2	g2000-415-1	g2000-416-2	g2000-417-1	g2000-418-2	g2000-419-1
1263	A08p12620.1_BnaDAR	A08_013915393_013916338	9	4.5	7	6.8	4.7	5.5	6.9	5.7	6.2	7.3	4.6	7.9	5.8	6.2	5.7	4.1	6.2	5.3	5.8	6.8	7.2	5.6	6.3	5.4	6	6.5	5.2	5.3	5	6.8	6.2	5.1
1264	A08p12630.1_BnaDAR	A08_013917443_013920673	7	5.5	6	5.8	5	5.2	6.6	5.2	5.1	6.5	4.8	6.2	7	4.6	5.6	2.8	6.1	5	5.2	5	6.6	4.3	5.7	5	5	4.9	5.5	4.5	5	6.2	5.6	4.9
1265	A08p12640.1_BnaDAR	A08_013920747_013922022	7	4.4	6	6.2	4.9	4.2	6.3	4.2	5.1	5.4	5.5	5.4	5.8	4.9	4.9	2.9	5.3	4.3	5	5.3	5.8	4.4	5.1	3.5	6	5	4	4.2	6	7	5.8	4.4
1266	A08p12650.1_BnaDAR	A08_013924607_013925496	7	6.1	7	6	5.8	5	7	5.7	5.1	8	4.2	8.7	6.9	8	6	2.8	5.3	3.5	6.3	5.9	7	4.2	5.6	4.1	7	4.3	5.6	5.9	6	5.6	7.3	6.1
1267	A08p12660.1_BnaDAR	A08_013932792_013934931	4	3.2	3	3.7	2.7	3.3	3.3	2.7	2.4	2.8	2.9	3.9	3.7	2.7	2.9	1.4	2.4	2.3	2.1	2.3	3	2.6	2.2	2.8	3	2.4	2.7	3.3	3	3.3	3	2.5
1268	A08p12670.1_BnaDAR	A08_013948557_013952755	5	4	6	5.2	4.6	4.8	6.1	5.3	4.8	6.5	4.6	4.3	5.6	2.8	5.4	2.9	5.3	5.1	5.3	4.4	4.6	4.2	5.2	4.3	4	4.9	4.1	3.9	4	5.3	4.5	3.6
1269	A08p12680.1_BnaDAR	A08_013964720_013966814	9	7	9	7.2	6.5	7.1	7.9	6.4	7	9.6	6.9	8.2	8.9	4.7	6.8	4.4	7.8	5.8	7.7	6.7	6.8	5.3	7.6	7	7	6.9	5.8	5.9	7	8.1	8.6	7.3
1270	A08p12690.1_BnaDAR	A08_013972218_013972858	3	1.6	2	1.4	0.8	1.6	1	0.8	1.5	1.4	1.2	2.1	1.9	1.4	1.2	1.9	2.2	1.5	1.1	1.2	1.5	2	2.1	0.9	1	1.5	2.3	1.5	1	0.7	1.3	1.3
1271	A08p12700.1_BnaDAR	A08_013975872_013976939	3	1.9	8	2.3	2	2.3	2.6	2.2	2.8	2.4	2.2	8.7	2.4	1.8	1.9	1.7	4.1	2	2.5	1.5	2.1	7.1	3.2	2	2	2	1.7	1.4	2	2.8	2.8	2.4
1272	A08p12710.1_BnaDAR	A08_013979545_013980807	9	6.2	8	6.6	5.7	5.5	6.8	6.2	6.7	7.9	5.8	10	7.7	9.3	6	4.1	6.6	4.8	5.5	5.4	7	8.8	7.4	5.4	8	5.7	4.7	5.4	7	6.9	6.6	6.9
1273	A08p12720.1_BnaDAR	A08_013984556_013989227	6	4.4	6	5.7	4.7	4.4	6.5	5.3	5.6	6.1	4.8	6	6.1	3.5	5.3	4.8	5.3	4.6	4.8	4.2	5.3	4.7	5.5	4.5	5	5	4.4	4.4	5	5.7	5	4.1
1274	A08p12730.1_BnaDAR	A08_013991308_013991505	6	4.3	3	4.9	3.6	2.3	7.8	4.5	5.7	4.8	4.3	0	4.8	5.7	3.4	6.9	6.5	3.7	4.6	2.2	5	5.3	3.5	4.8	5	4.1	2.5	3.9	6	8.9	4.4	4
1275	A08p12740.1_BnaDAR	A08_013996917_013997440	5	2.9	4	2.8	3	3.4	4.8	4.2	4	2.8	3.6	0.1	4.3	1.9	3.3	3.8	2.2	2.4	2.8	2.8	3.9	2.4	3	2.3	4	2.3	2.8	4.3	4	4.1	3.2	2
1276	A08p12750.1_BnaDAR	A08_013998781_013999290	6	4.5	4	3	3.1	2.9	5.2	3.1	3.6	2.3	2.8	0.3	3.9	5.2	3.3	4.2	3.7	4.4	2.8	4.2	3.2	4.4	2.7	4.2	2	3.3	2.6	2.2	4	5.2	4.6	4
1277	A08p12760.1_BnaDAR	A08_014003084_014005936	7	5.4	7	6.4	4.6	5.7	6.7	5.1	5.6	6.8	4.8	6.6	6.1	3.3	5.2	4.8	5.3	4.4	4.9	5.1	5.6	5	5.8	4.9	5	4.8	4.3	5	5	6.1	5.3	5.9
1278	A08p12770.1_BnaDAR	A08_014006863_014008873	8	5.8	6	5.5	4.2	4.9	5.9	5.8	5.2	7.2	4.7	7.6	6.2	7.3	4.6	4.5	6.5	3.9	4	5.1	5.7	4.6	5.3	5.5	8	5	5	5.1	7	5.8	5.4	5.1
1279	A08p12780.1_BnaDAR	A08_014009852_014010472	8	4.1	7	6.4	6.3	7.4	7	5.8	6.4	6.7	4.2	9.7	7.6	5.7	6.2	6.6	5.6	5.4	6.2	5.4	6.1	6.9	7.5	6.6	6	5	4.4	5.3	6	6.9	6.3	4.9
1280	A08p12790.1_BnaDAR	A08_014012708_014015650	5	4	5	4.9	3.5	3.6	4.6	3.8	4.3	4.6	3.8	6.1	4.7	2.4	3.6	3.6	5.6	4	3.8	4.2	4.1	4.7	5.1	3.9	3	3.3	3.4	3.5	4	4.8	4.1	4.2
1281	A08p12800.1_BnaDAR	A08_014026164_014026915	9	5.7	7	6.8	5.1	6.1	7.6	4.9	4	8.5	5.3	6.9	6.6	7.9	6.7	7	5.9	4.7	5.7	6.1	6	6.1	6.5	5.9	9	6.3	6	5.7	10	6.9	7.4	5.2
1282	A08p12810.1_BnaDAR	A08_014028274_014029221	8	6.2	9	6.3	5.4	6.2	8.3	6.9	5.7	7.4	5.8	7.2	7.7	3.5	6.2	5.3	7.4	6.1	7.2	5.7	6.1	6.4	6.6	6.1	7	6	5.7	5.7	6	9.5	5	6
1283	A08p12820.1_BnaDAR	A08_014031218_014032245	6	3.7	5	5.3	4.3	4.3	5.4	5.3	5.6	6.1	4.5	5.9	6.3	3.1	5.1	6.4	5.9	4.7	6.3	4	5.4	4.6	5.6	4.6	5	3.5	3.3	4.2	5	5.7	4.2	4.9
1284	A08p12830.1_BnaDAR	A08_014034488_014035020	5	3.9	6	6.9	5.2	5.3	5.1	5.4	3.5	6	4	6.3	6	4	5	3.8	5.7	5.2	3.9	5	6.1	5.1	5.9	4.1	4	4.6	5.7	3.5	5	4.2	4.2	4.6
1285	A08p12840.1_BnaDAR	A08_014040254_014040662	3	2.2	4	4.3	4.3	2.6	5.9	3.5	3	3.3	3.1	2.8	3.8	2	4.6	4	4.9	2.5	2.5	2.1	3.5	3.5	2.4	3.1	4	5.2	2.5	4.1	1	5.5	4.3	2.1
1286	A08p12850.1_BnaDAR	A08_014040842_014043333	8	7.1	7	6.1	4.4	5.1	6.6	5.3	5.3	7.2	5.4	8.5	7.6	8.4	5.7	5	5.6	4.7	5.5	6.3	6	5.9	6.4	4.5	7	4.9	5.1	5.1	8	5.6	6.9	5.9
1287	A08p12860.1_BnaDAR	A08_014046623_014049044	7	5.7	6	5.5	4.5	4.7	6.9	5.6	5.4	7.1	4.6	6.6	6.7	5.8	5.5	5.2	5.4	4.7	4.8	4.7	5.4	5.3	5.7	5.4	7	5.1	4.9	5	6	6.2	6	6
1288	A08p12870.1_BnaDAR	A08_014049529_014050040	7	5	9	5.7	5.9	6.9	7	5.8	4.8	7.3	4.8	8.8	8.3	5.5	7.5	6.1	7.5	5.2	5.1	4.2	7	5.1	7.5	7.4	7	6.2	6.6	5	7	5.7	6.8	4.1
1289	A08p12880.1_BnaDAR	A08_014050130_014058487	2	1.7	2	2.1	1.9	2.1	2.4	2.8	1.9	2.1	3.1	3.5	2.4	1.2	2	3.1	2.2	2.6	2.6	2	3.2	3.3	3.1	1.8	2	3.5	2.1	1.8	2	2.9	2	2
1290	A08p12890.1_BnaDAR	A08_014060350_014066736	6	4.8	6	5.3	4.5	2.7	3.6	2.6	5.2	6.4	4.9	6	3.4	2	3	3.1	5.2	4.7	4.6	3.8	2.8	4.5	5.6	5.1	3	2.5	4.3	4.4	5	4.3	5	4.8
1291	A08p12900.1_BnaDAR	A08_014066665_014083352	6	4.8	6	5.7	4.5	4.9	5.9	5.1	5.2	6.3	4.5	6.1	6.3	3.3	5.1	5.1	5.6	4.7	4.8	5.2	5.1	5	5.1	4.8	5	4.9	4.5	4.6	5	5.7	5.4	5.1
1292	A08p12910.1_BnaDAR	A08_014095993_014099118	6	4.8	7	5.6	4.8	5.1	6.7	5.4	5.7	6.8	5.1	6	6.6	3.1	5.5	5	5.9	5	5.9	5.3	5.5	4.8	5.7	4.4	5	5.1	4.7	4.8	5	5.8	5.3	5.8
1293	A08p12920.1_BnaDAR	A08_014099000_014100521	8	6	7	6.2	5.3	6.1	6.6	6	6.1	6	4.9	7	6.5	5	5.8	5.1	6.5	5.4	4.8	5.4	5.8	4.9	7.2	5.9	6	5.8	5	5.5	7	6.9	6.5	7.1
1294	A08p12930.1_BnaDAR	A08_014104618_014106266	9	5.5	7	5.8	4.1	4.7	6.2	5.6	5.6	6.8	4.3	8.3	6.4	8.4	6.2	5.7	5.3	5.2	5	5	5	6.3	5.7	5.1	5	5	3.4	3.9	6	5.6	5.8	5.8

Identification of small-scale lesions induced by radiation

2. Computationally using VarScan, for both InDels and SNVs



| | A | indel.ref | G2000-131-2 | G2000-131a | G2000-132-2 | G2000-132a | G2000-158b | G2000-159a | G2000-15a | G2000-160a | G2000-161a | G2000-162a | G2000-163a | G2000-164a | G2000-165a | G2000-166a | G2000-167a | G2000-168-2 | G2000-169b | G2000-16a | G2000-170a | G2000-171a | G2000-172a | G2000-173b | G2000-174a | G2000-175b | G2000-190a | G2000-191a | G2000-192a | G2000-193b | G2000-217a | G2000-218a | G2000-249-1 | G2000-24a | G2000-250-2 | G2000-251-1 | G2000-252-1 | G2000-253-1 | G2000-254-1 | G2000-255-1 | G2000-256-1 | G2000-257-1 | G2000-258-2 | G2000-259-1 | G2000-267-1 | G2000-268-2 | G2000-273-1 | G2000-274-1 | G2000-276-1 | g2000-277-2 | G2000-278-1 | G2000-279-1 | G2000-27a | g2000-280-1 | G2000-281-1 | G2000-290-1 | G2000-291-1 | G2000-292-2 | G2000-293-1 | G2000-294-1 | G2000-295-1 | G2000-296-2 | G2000-297-2 | G2000-298-1 | G2000-308-1 | G2000-309-1 | G2000-30a | G2000-310-1 | G2000-313-1 | g2000-314-1 | G2000-33a | g2000-343-1 | g2000-344-2 | g2000-346-1 | g2000-347-2 | g2000-348-2 | G2000-34b | g2000-350-2 | g2000-352-1 | g2000-353-1 | g2000-355-1 | g2000-357-2 |
|---|---|---

Identification of small-scale lesions induced by radiation

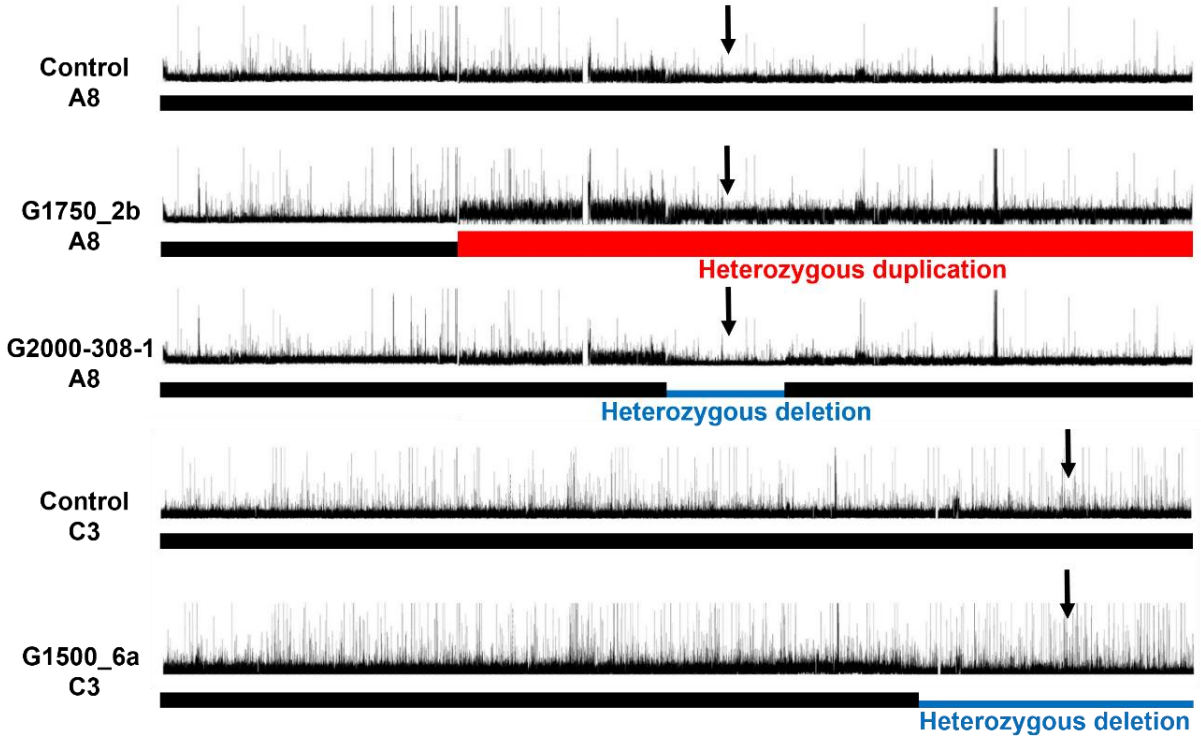
2. Computationally using VarScan, for both InDels and SNVs

Target gene	Line	Nucleotide position	Reference allele	Variant allele	Read depth reference allele	Read depth variant allele	Validated?
Bna.CytP450.A5	G2000-83b	A05:5736668	CT	C	0	13	Yes
Bna.eIF(iso)4E.A4	G2000-119-1	A04:7768451	GGAGAGGTAGAAGAT	G	0	3	Yes
Bna.FAD2.A5	G2000-137b	A05:36892995	CCACCACTTACTTCCCTCTCCT	C	11	2	Yes
Bna.FAD2.C5	G1750-356a	C05:51453809	T	A	5	4	Yes
Bna.FAD3.A4	G2000-145b	A04:16659808	CCTTT	C	6	6	Yes
Bna.FAE1.A8	G2000-173b	A08:16404035	GA	G	3	2	Yes
Bna.GTR1.A6a	g1750-153-1	A06:11068670	TA	T	0	10	Yes
Bna.GTR2.A6	G1750-327a	A06:34290460	GC	G	3	4	Yes
Bna.GTR2.A9a	G2000-396-1	A09:4453814	TGA	T	7	8	Yes
Bna.GTR2.C9	g2000-408-2	C09:5200267	A	ACAC	10	3	Yes
Bna.VTE4.C2a	g2000-403-1	C02:15001410	TA	T	0	7	Yes
Bna.VTE4.C2a	G1750-489b	C02:15002099	CCGT	C	0	7	Yes

- **High false discovery rate, especially for SNVs at low read depth**
- **Mutation load much lower than with EMS**
- **High proportion of mutants are deletions of a few bases that disrupt reading frames**

Testing predictive impacts of gene copy number variation: *FAE1* and oil erucic acid content

FAE1 orthologues control erucic acid content in an additive manner

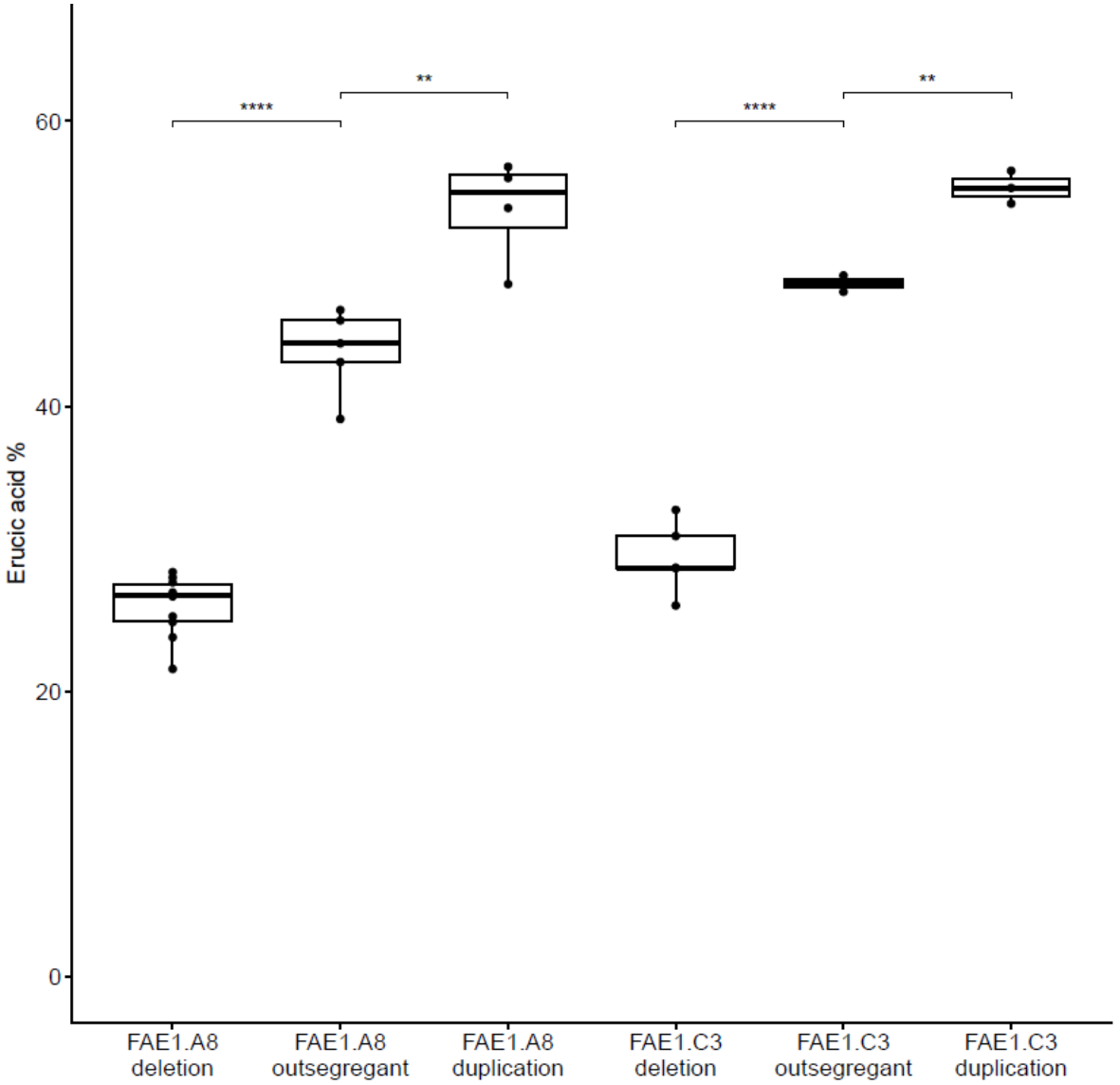


Identified radiation lines:

G2000-308-1 (*FAE1*.A8 deletion)
G1750_2b (*FAE1*.A8 duplication)
G1500_6a (*FAE1*.C3 deletion)
G2000-119a (*FAE1*.C3 duplication)

Gene model		G2000-108b	G2000-110-2	G2000-112a	G2000-113b	G2000-114a	G2000-115-1	G2000-117-1	G2000-118-1	**G2000-119a**	G2000-120-1	G2000-122-2	G2000-123-1	G2000-125-1	G2000-126-1	G2000-128a	G2000-132-2	G2000-133-1
Bo3g168710.1		1.17	1.29	1.22	1.41	1.11	1.00	0.97	1.37	**1.10**	1.26	1.18	1.02	0.99	1.23	0.85	1.01	0.79
Bo3g168720.1		0.90	1.20	1.21	1.08	1.29	1.23	1.31	1.25	**1.02**	1.26	1.04	1.29	0.85	0.95	0.92	1.11	1.06
Bo3g168740.1		0.78	1.02	0.63	0.49	0.79	1.05	0.74	0.74	**0.97**	0.66	1.05	0.49	0.65	1.28	0.57	0.57	0.99
Bo3g168750.1		1.04	1.25	0.90	1.27	1.00	1.25	1.11	0.62	**1.07**	0.51	1.14	1.40	1.45	1.40	1.32	0.97	0.82
BnaC03g65930D		0.83	0.20	1.01	1.50	1.48	0.88	1.59	1.33	**0.69**	0.62	1.38	0.56	0.59	1.05	0.84	0.59	1.43
Bo3g168770.1		1.05	1.16	0.89	1.02	1.04	1.04	1.21	1.28	**0.76**	1.04	1.26	1.08	0.71	0.96	1.22	1.07	1.18
Bo3g168780.1		0.75	1.05	0.66	1.23	1.32	1.10	0.92	0.88	**0.70**	0.53	0.88	0.97	0.79	0.75	0.92	0.61	0.57
Bo3g168790.1		0.97	0.87	1.00	0.91	0.92	0.77	0.99	1.16	**1.19**	1.07	0.89	0.76	0.98	0.88	1.04	1.10	1.26
Bo3g168800.1		1.16	1.24	0.97	1.04	1.17	0.84	0.88	1.35	**1.01**	0.66	0.88	1.34	1.41	1.22	0.85	0.80	0.92
Bo3g168810.1	*BnaFAE1.C3*	0.95	0.74	0.96	1.36	0.88	1.22	0.66	1.38	**2.21**	0.56	0.46	1.06	0.93	0.88	1.33	0.93	1.03
BnaC03g51250.1T		0.84	0.93	1.03	1.25	1.11	0.89	0.96	1.24	**0.71**	0.77	0.89	1.25	0.69	0.90	1.11	1.27	0.66
BnaC03g51270.1T		1.21	0.86	0.82	1.00	1.04	0.86	0.79	0.64	**1.18**	1.39	1.36	1.21	0.75	0.75	0.96	1.36	0.96
BnaC03g51310.1T		1.04	1.00	0.80	1.04	1.21	0.92	1.11	1.09	**1.08**	0.95	1.24	1.18	1.13	0.87	1.42	0.98	0.94
Bo3g168820.1		0.77	1.47	0.91	1.33	0.91	1.40	1.19	0.98	**1.05**	0.70	0.49	1.26	0.84	0.84	1.12	0.70	1.40
Bo3g168830.1		0.98	0.82	1.18	1.07	1.05	1.02	0.96	1.28	**1.06**	0.76	0.82	0.97	0.86	1.04	0.99	1.10	1.05
Bo3g168840.1		1.10	1.27	1.16	0.84	1.12	0.99	1.01	1.06	**0.87**	0.89	1.00	1.15	1.06	1.03	1.18	0.99	1.15
Bo3g168850.1		1.03	1.09	0.87	1.03	0.79	1.07	1.06	1.02	**0.82**	1.13	0.99	0.88	0.74	1.28	0.99	1.02	1.04
Bo3g168860.1		0.86	1.07	0.98	1.14	0.94	0.84	1.00	0.80	**1.01**	0.97	0.81	1.21	1.15	0.75	0.85	0.95	0.68
Bo3g168870.1		1.03	1.09	0.87	1.05	1.06	0.78	1.23	0.94	**0.87**	1.11	0.98	0.96	0.86	0.92	1.27	0.97	1.01

Testing predictive impacts of gene copy number variation: *FAE1* and oil erucic acid content



- **Deletions cause large drop in erucic acid content, as expected**
- **Duplications cause significant increase in erucic content**

Adjusted P-values for significance:
** <0.005; *** <0.0005; **** <0.00005

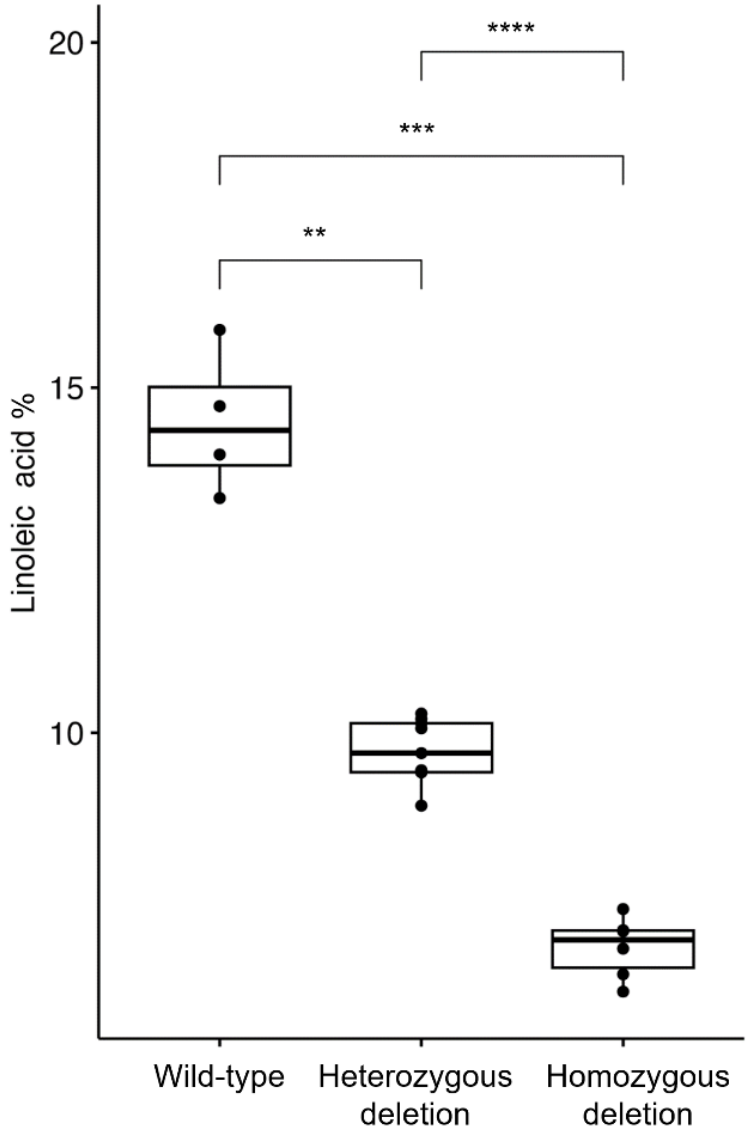
Testing predictive impacts of gene copy number variation: *FAD2* and oil linoleic acid content

FAD2 orthologues control PUFA (especially linolenic acid) content in an additive manner

Identified radiation line:
G2000-137b (*FAD2.*A5 21 base deletion)

Target gene	Line	Nucleotide position	Reference allele	Variant allele	Read depth reference allele	Read depth variant allele	Validated?
Bna.CytP450.A5	G2000-83b	A05:5736668	CT	C	0	13	Yes
Bna.eIF(iso)4E.A4	G2000-119-1	A04:7768451	GGAGAGGTAGAAGAT	G	0	3	Yes
Bna.FAD2.A5	G2000-137b	A05:36892995	CCACCACTTACTTCCCTCTCCT	C	11	2	Yes
Bna.FAD2.C5	G1750-356a	C05:51453809	T	A	5	4	Yes
Bna.FAD3.A4	G2000-145b	A04:16659808	CCTTT	C	6	6	Yes
Bna.FAE1.A8	G2000-173b	A08:16404035	GA	G	3	2	Yes
Bna.GTR1.A6a	g1750-153-1	A06:11068670	TA	T	0	10	Yes
Bna.GTR2.A6	G1750-327a	A06:34290460	GC	G	3	4	Yes
Bna.GTR2.A9a	G2000-396-1	A09:4453814	TGA	T	7	8	Yes
Bna.GTR2.C9	g2000-408-2	C09:5200267	A	ACAC	10	3	Yes
Bna.VTE4.C2a	g2000-403-1	C02:15001410	TA	T	0	7	Yes
Bna.VTE4.C2a	G1750-489b	C02:15002099	CCGT	C	0	7	Yes

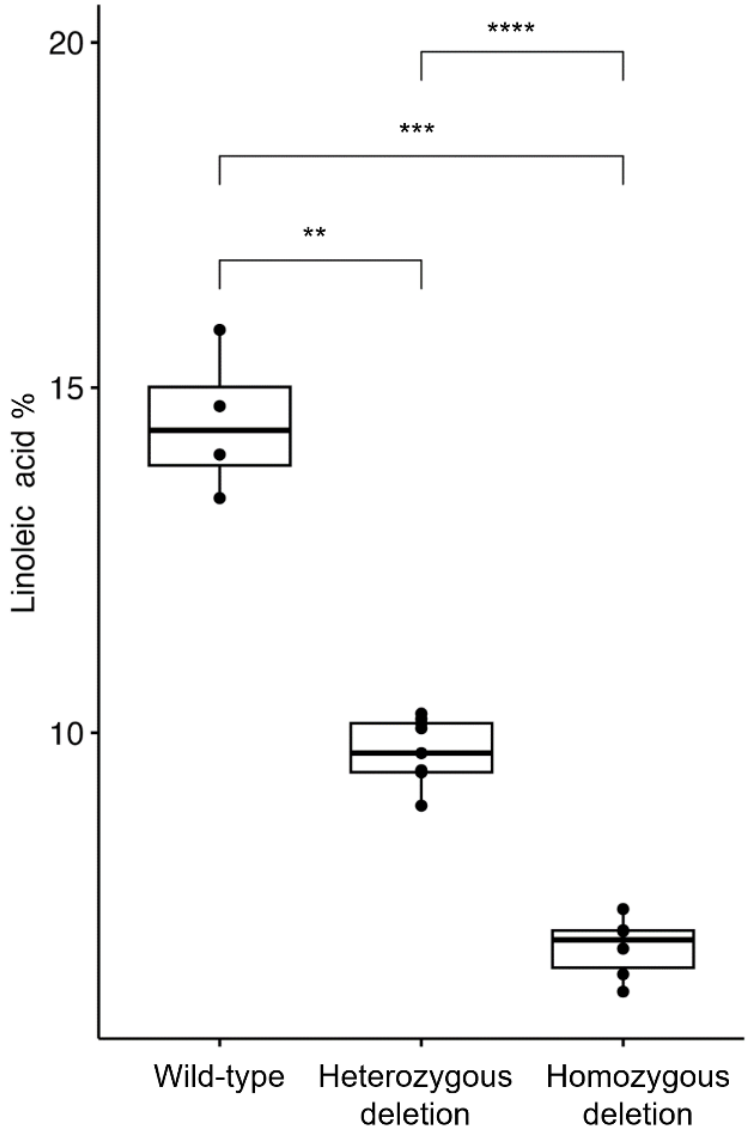
Testing predictive impacts of gene copy number variation: *FAD2* and oil linoleic acid content

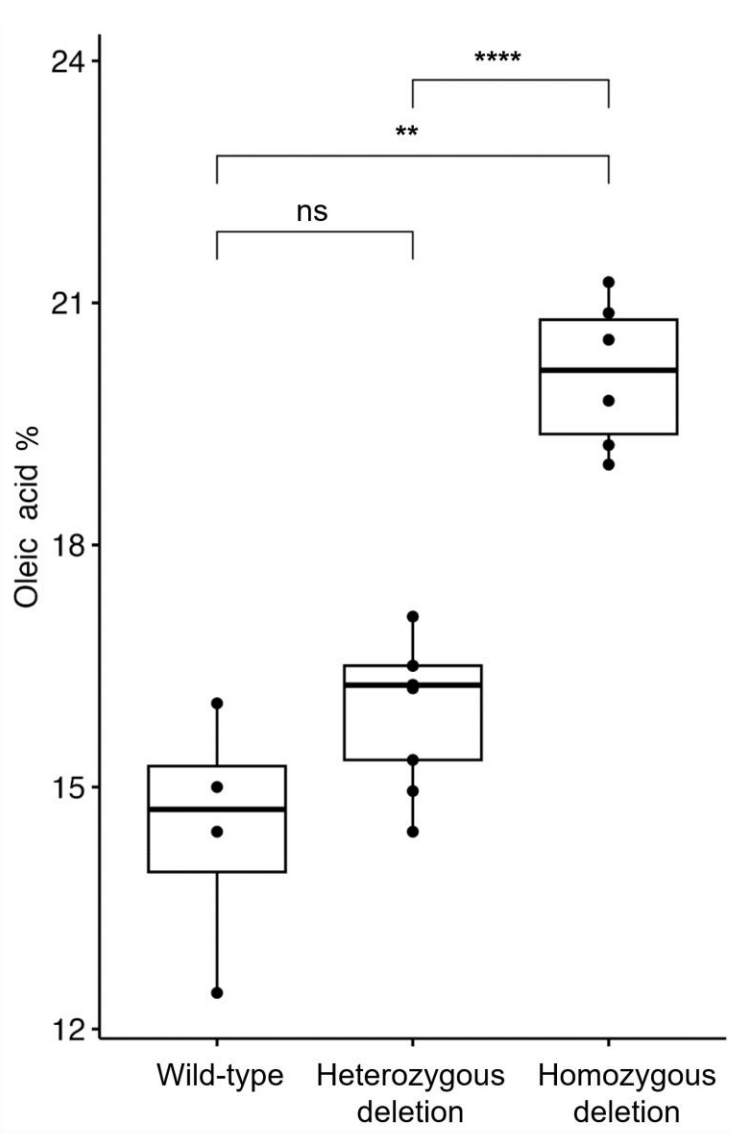


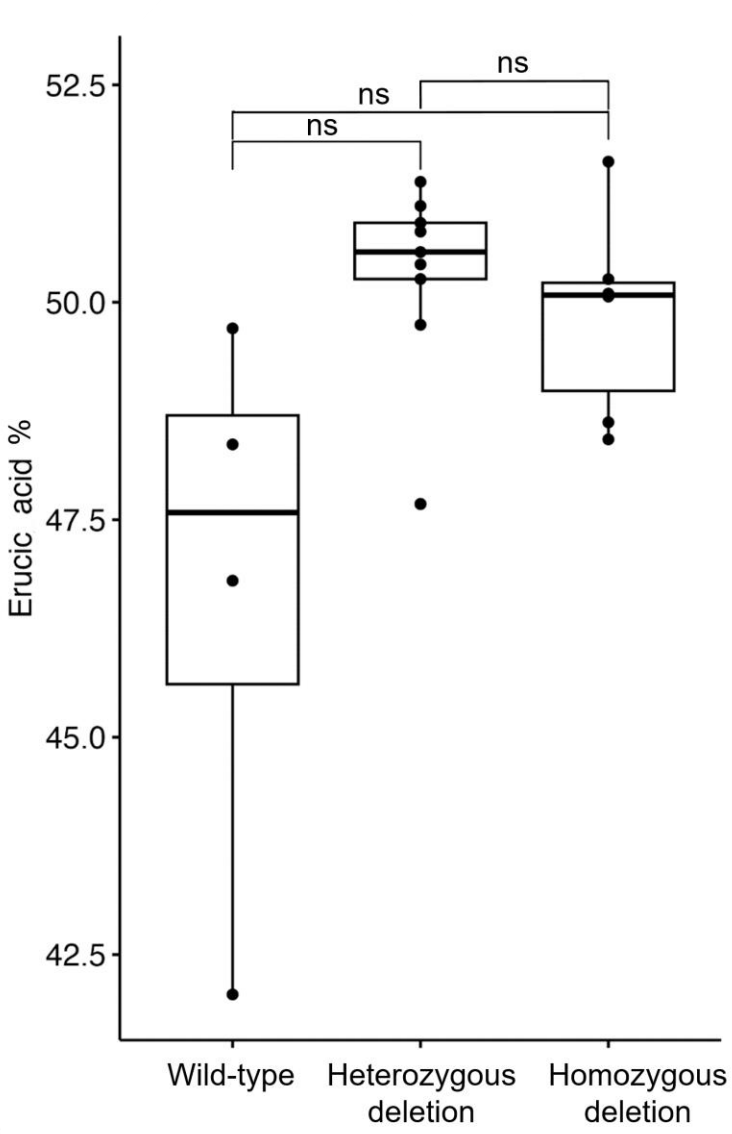
- ***FAD2*.A5 Knockout causes large drop in linoleic acid content, as expected**

Adjusted P-values for significance:
** <0.005; *** <0.0005; **** <0.00005

Testing predictive impacts of gene copy number variation: *FAD2* and oil linoleic acid content







Conclusions

- Radiation treatment is an effective tool for breaking up introgressions
- Visualization of whole genome enables identification of additional lesions
- Structural changes correlated with poor agronomic traits can be identified
- Genome re-sequencing enables identification of abundant large-scale deletions
- Panel of ~1100 lines sufficient to achieve saturation for large-scale deletions
- Deleted segments often inherited by siblings, so increasing copy number feasible
- High false discovery rate for single nucleotide variants
- Indels disrupting open reading frames are the most common small variant type
- Panel of ~1100 lines insufficient to achieve saturation for single gene knockout
- Predicted phenotypes confirmed for knockouts of two fatty acid synthesis genes
- Increased *FAE1* dosage results in novel increase in erucic acid content

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