

Functional studies of candidate genes associated with hybrid vigor

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USDA-ARS

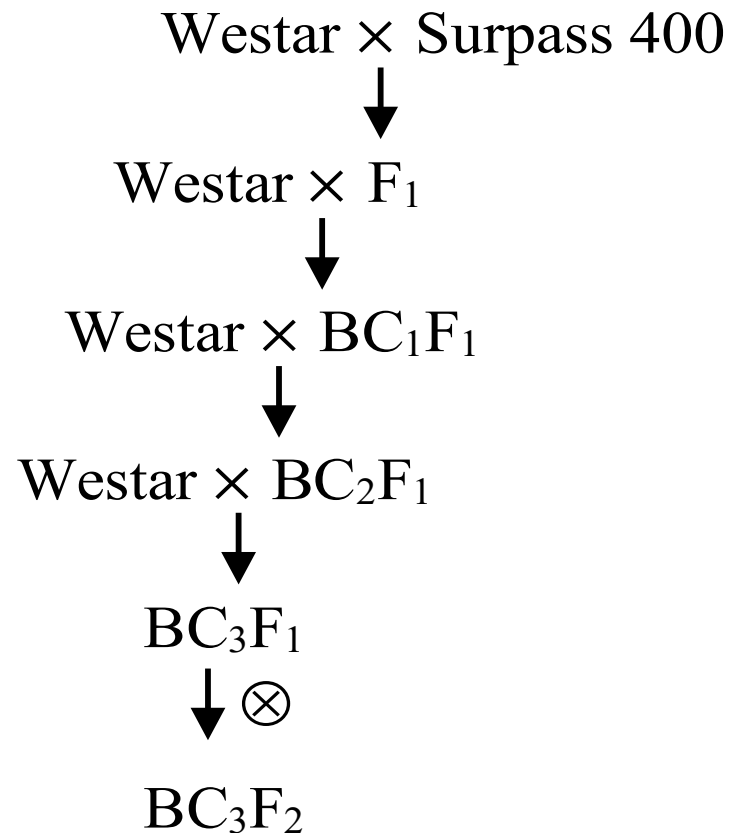
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Functional studies of candidate genes associated with hybrid vigor

- ❖ Molecular mapping of heterotic locus and statistical analysis of field data.**
- ❖ Genes reside in the 26-Kb mapped region.**
- ❖ CRISPR technology.**

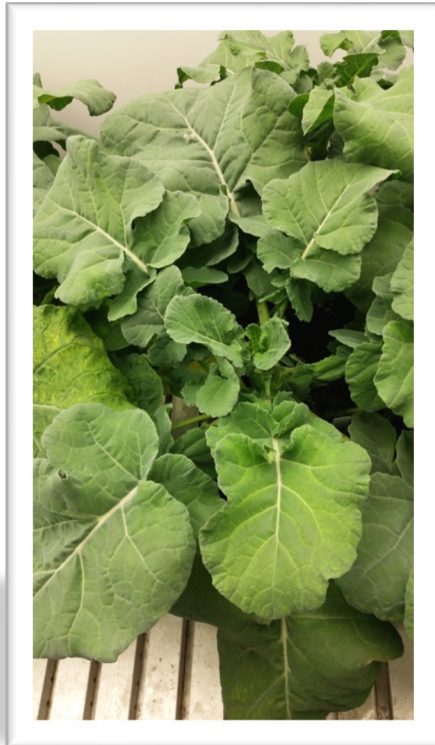
Molecular mapping of heterotic locus and statistical analysis of field data



Chromosome segment substitution line was obtained from BC₃F₂ population based on marker-assisted selection.

Genotype and phenotype of CSSL

Chromosome segment substitution line (CSSL)

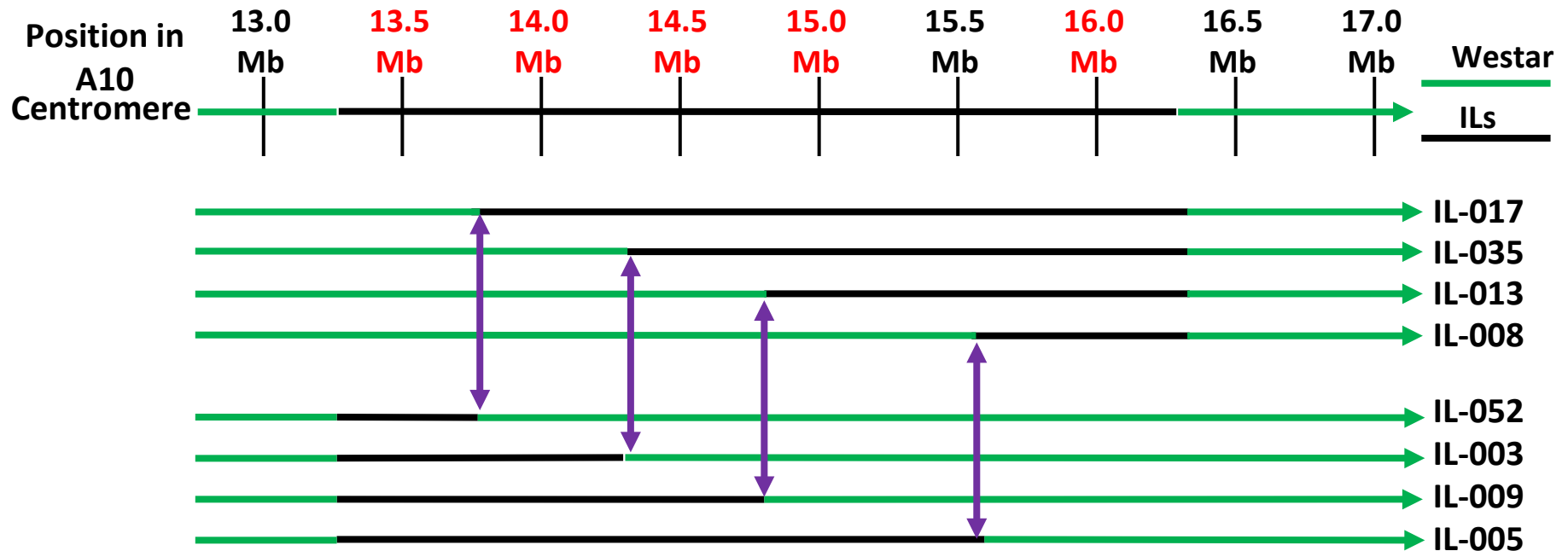


CSSL



Westar

Eight introgression lines (ILs)



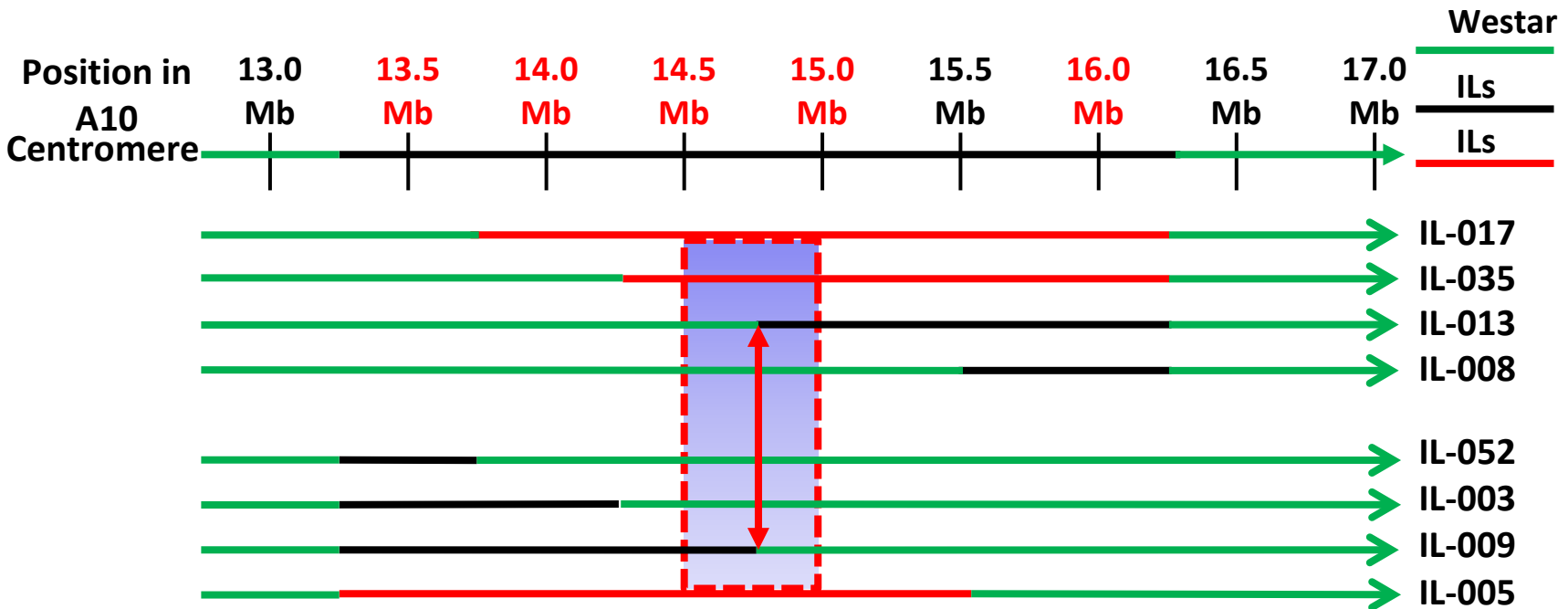
- Five co-dominant polymorphic markers are shown in red color.
- We crossed 8 IL lines to Westar reciprocally and generated 16 hybrids.

Three years grain yield results

Pedigree	2017		2018		2019		Avg (2017, 2018, &2019)	
	Avg (kg/ha)	±BP (%)	Avg (kg/ha)	±BP (%)	Avg (kg/ha)	±BP (%)	3 Yrs (Avg)	±BP (%)
Westar	3078		420		2402		1966	
IL052	1858		320		1729		1302	
IL052 × Westar	2575	-16	639	52	2265	-6	1826	-7
Westar × IL052	2334	-24	561	34	906	-62	1267	-36
Westar	2698		541		2193		1811	
IL003	2231		319		1413		1321	
IL003 × Westar	2199	-18	234	-57	1764	-20	1399	-23
Westar × IL003	2526	-6	300	-45	1343	-39	1390	-23
Westar	3035		301		2340		1892	
IL009	3088		349		2268		1902	
IL009 × Westar	2941	-3	290	-17	2410	3	1880	-1
Westar × IL009	3045	0	390	12	2101	-10	1845	-3
Westar	3076		328		1014		1472	
IL05	2924		687		2304		1972	
IL05 × Westar	3258	6	1307	90	2461	7	2342	19
Westar × IL005	3195	4	963	40	2729	18	2296	16
Westar	3076		184		1073		1444	
IL08	2786		129		933		1282	
IL08 × Westar	3033	-1	262	43	1424	33	1573	9
Westar × IL008	2712	-12	231	26	1118	4	1354	-6
Westar	2898		370		2110		1793	
IL013	984		543		726		751	
IL013 × Westar	3097	7	240	-56	1681	-20	1673	-7
Westar × IL013	2179	-25	226	-58	1345	-36	1250	-30
westar	2581		323		1831		1578	
IL035	2268		649		1961		1626	
IL035 × Westar	2868	11	884	36	3300	68	2351	45
Westar × IL035	2784	8	961	48	2507	28	2084	28
Westar	2989		397		1130		1505	
IL017	2260		803		1546		1536	
IL017 × Westar	3153	5	661	-18	1698	10	1838	20
Westar × IL017	3021	1	826	3	2764	79	2204	43

BP: better-parent value; yield values for better parents are shown in bold.
Green highlight: both reciprocal hybrids had increased grain yield compared to their better-parent value.

Map of heterotic locus



- Green lines represents Westar genome. Red and black lines represents introgressed Surpass 400 chromosome segment. Red color indicates both reciprocal crosses showed increased yield compared to the best-parent value. Black color indicates one of the reciprocal crosses or both did not show increased yield compared to the best-parent value.
- Field test determined that IL groups (IL017, IL035, and IL005) increased grain yield in both reciprocal crosses compared to the best-parent value.

Fine map of heterotic locus

Centromere A10

IL-013



IL-009



The width of each column represents the distance between two adjacent SNP markers

Westar	a	a	a	aa	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a		
CSSL	b	b	b	bb	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	
IL-013	a	a	a	aa	a	a	a	a	a	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	
IL-009	b	b	b	bb	b	b	b	b	b	u	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a

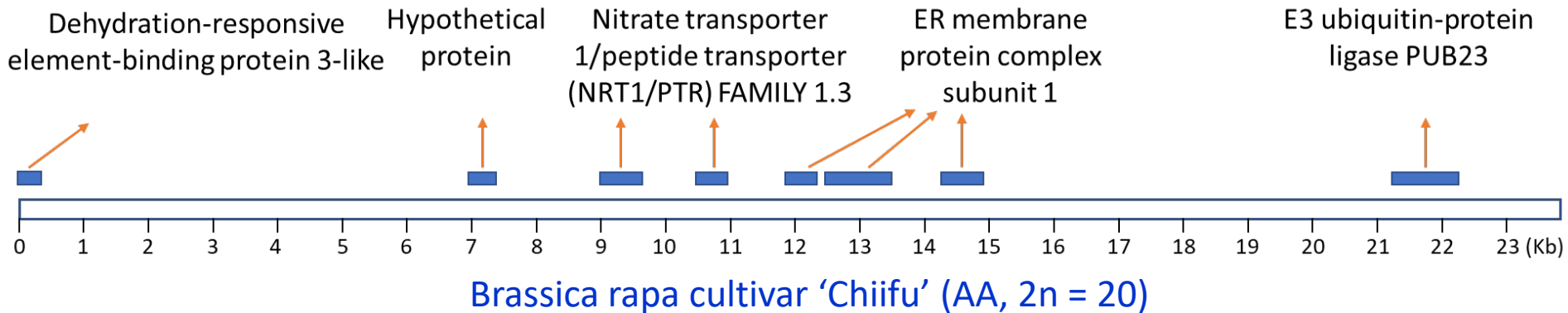
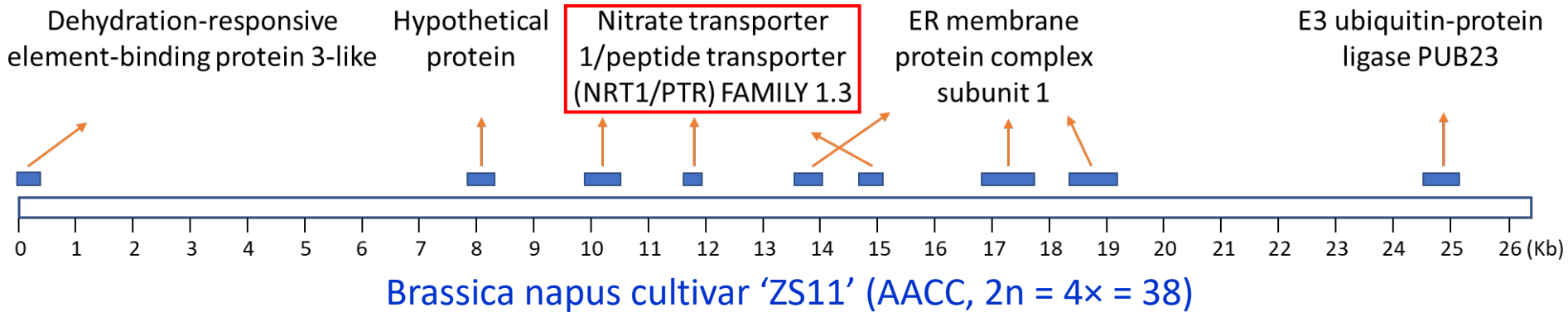
26-Kb

a and b: markers represented Westar and CSSL lines, respectively.
 u: markers were ambiguous in this region.

Genes reside in the 26-Kb mapped region

- ❖ Identify predicted genes according to the reference genome sequence of *Brassica napus* cultivar 'ZS11' (AACC, $2n = 4x = 38$) and *Brassica rapa* cultivar 'Chiifu' (AA, $2n = 20$).
- ❖ Sequence the 26-Kb region in Westar, CSSL, IL009, and IL013 using a primer walking method.

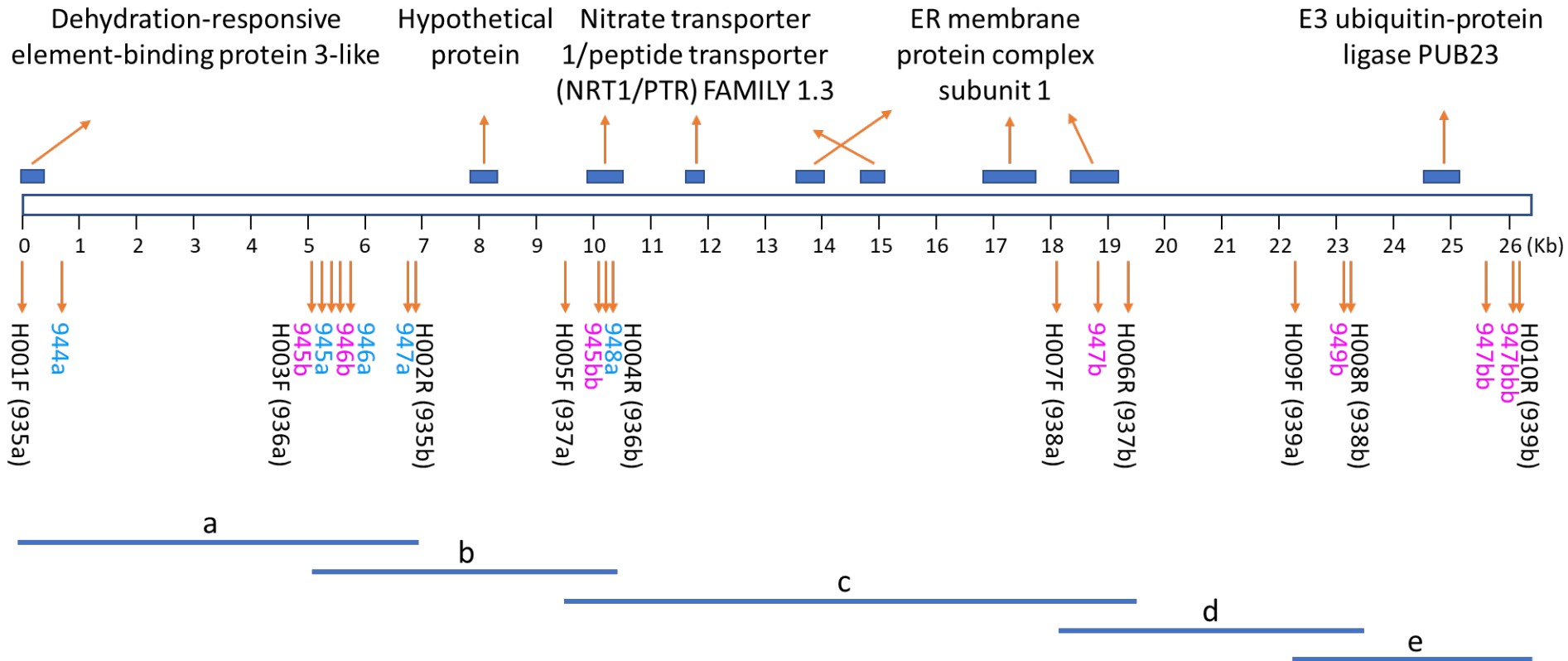
Five genes reside in the 26-Kb mapped region of chromosome A10



Sequence the 26-Kb region

- ❖ In Westar, Chromosome Segment Substitution Line (CSSL), IL009, and IL013
- ❖ Using primer walking method

Amplification of five overlapping DNA fragments in Westar, CSSL, IL009, and IL013 for sequencing

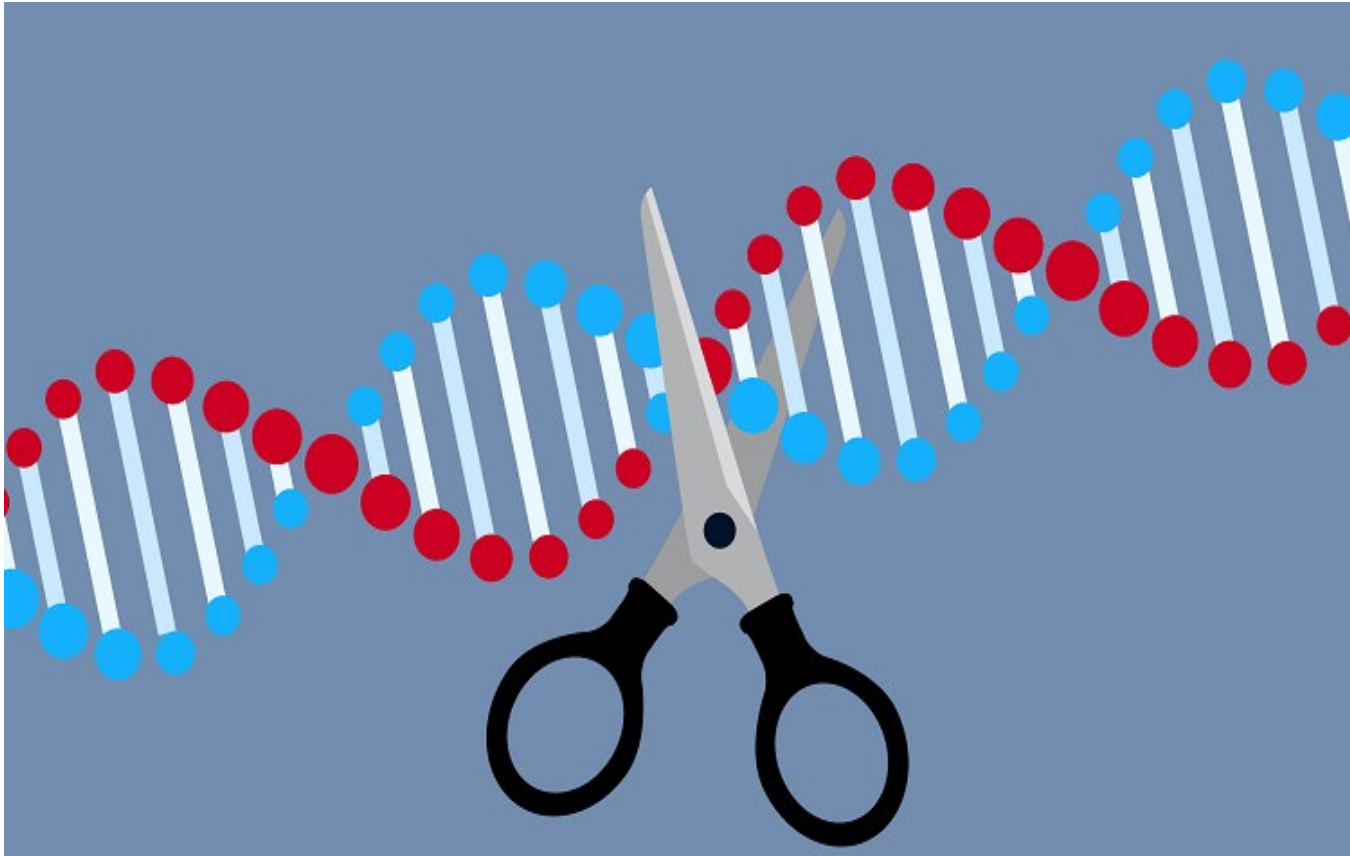


The locations of five PCR primer pairs (in black color) and forward (light blue) and reverse (pink) sequencing primers are determined based on the reference genome of *Brassica napus* cultivar 'ZS11' .

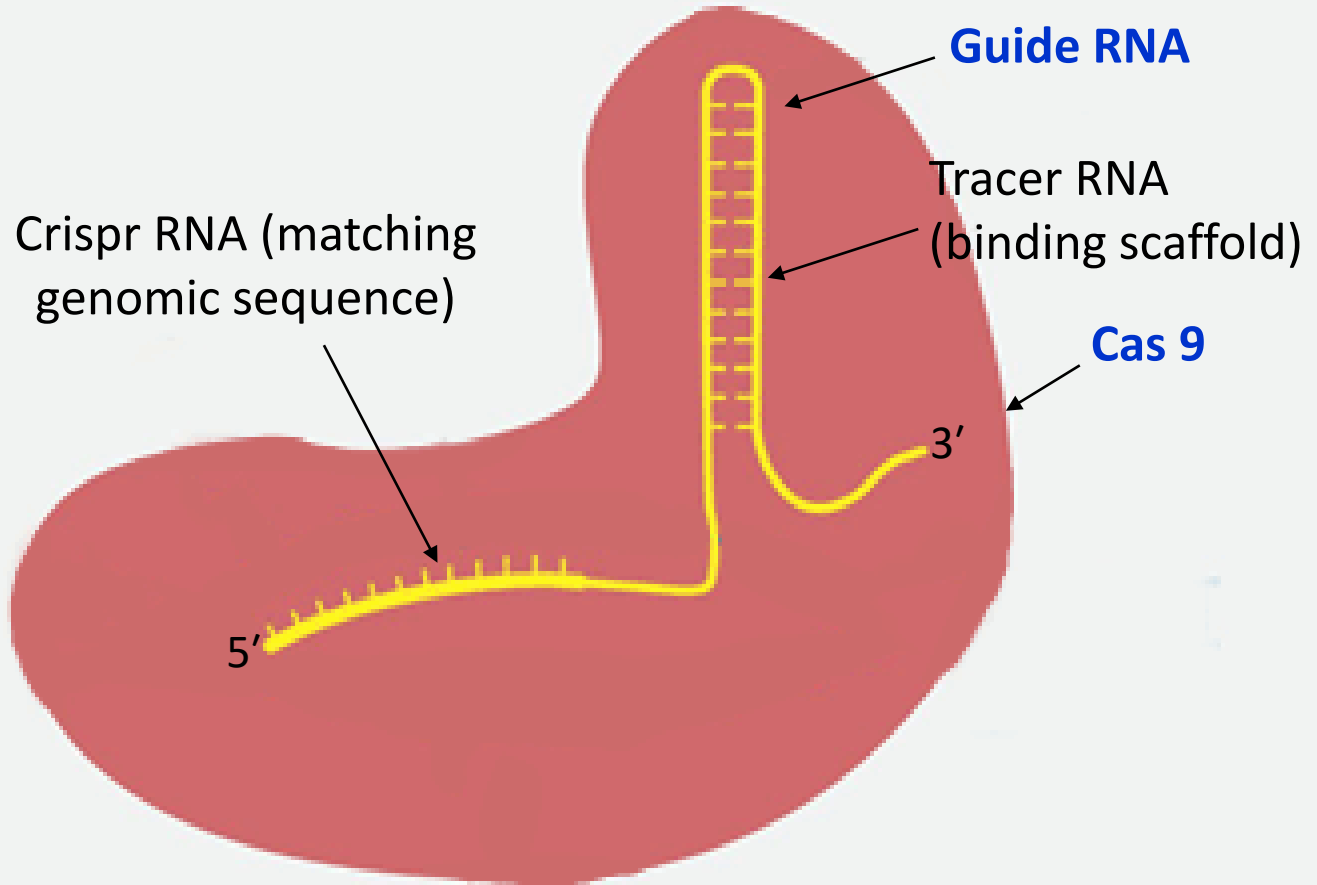
CRISPR technology

- ❖ **CRISPR/Cas9 genome editing: to develop gene knockout mutants for loss-of function phenotype.**

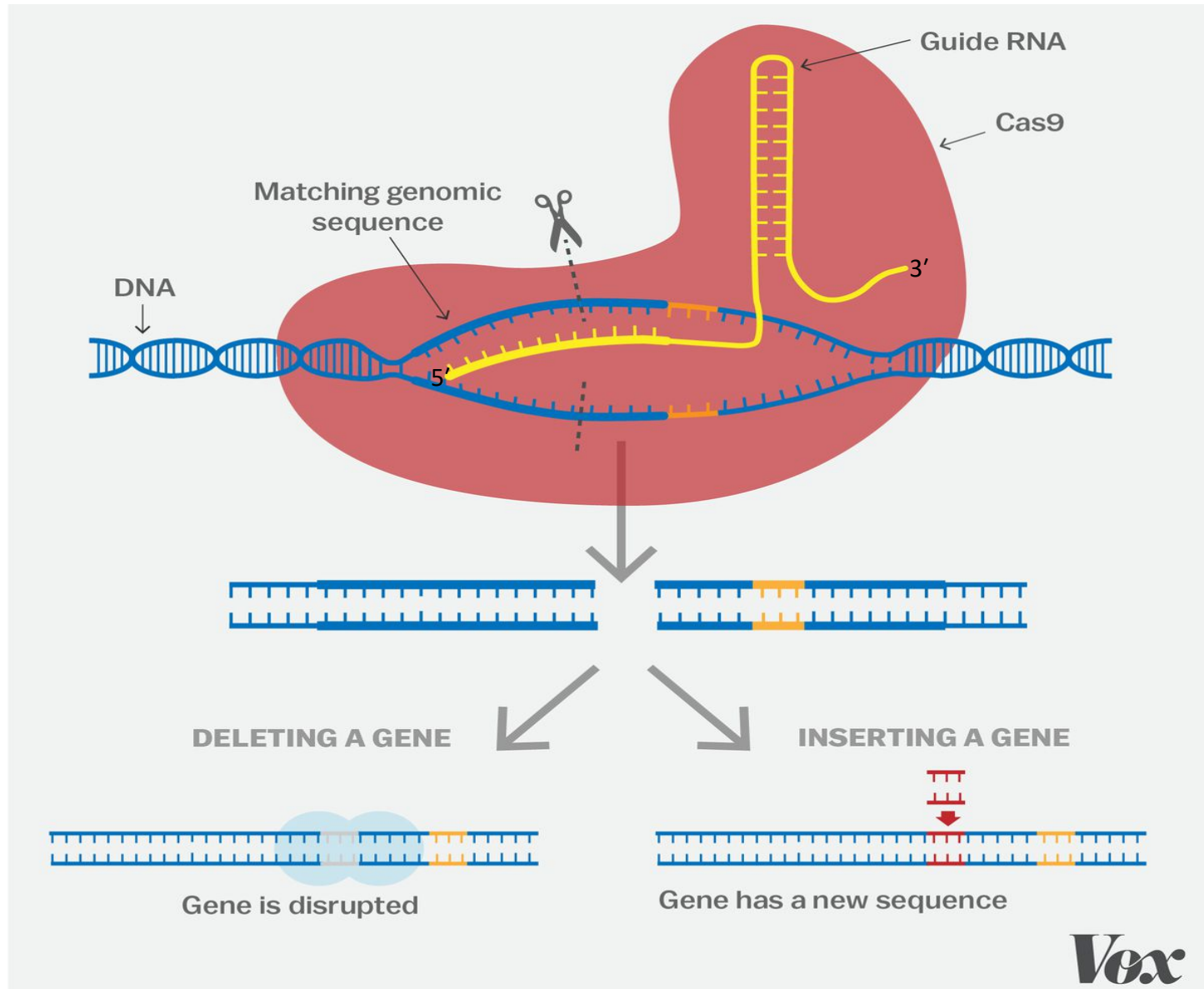
Clustered Regularly Interspaced Short Palindromic Repeat-Cas9 (CRISPR-Cas9)



CRISPR-Cas9



CRISPR-Cas9



Acknowledgements

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