

Homoeologous non-reciprocal translocation explains a major QTL for seed lignin content in oilseed rape (*Brassica napus* L.)

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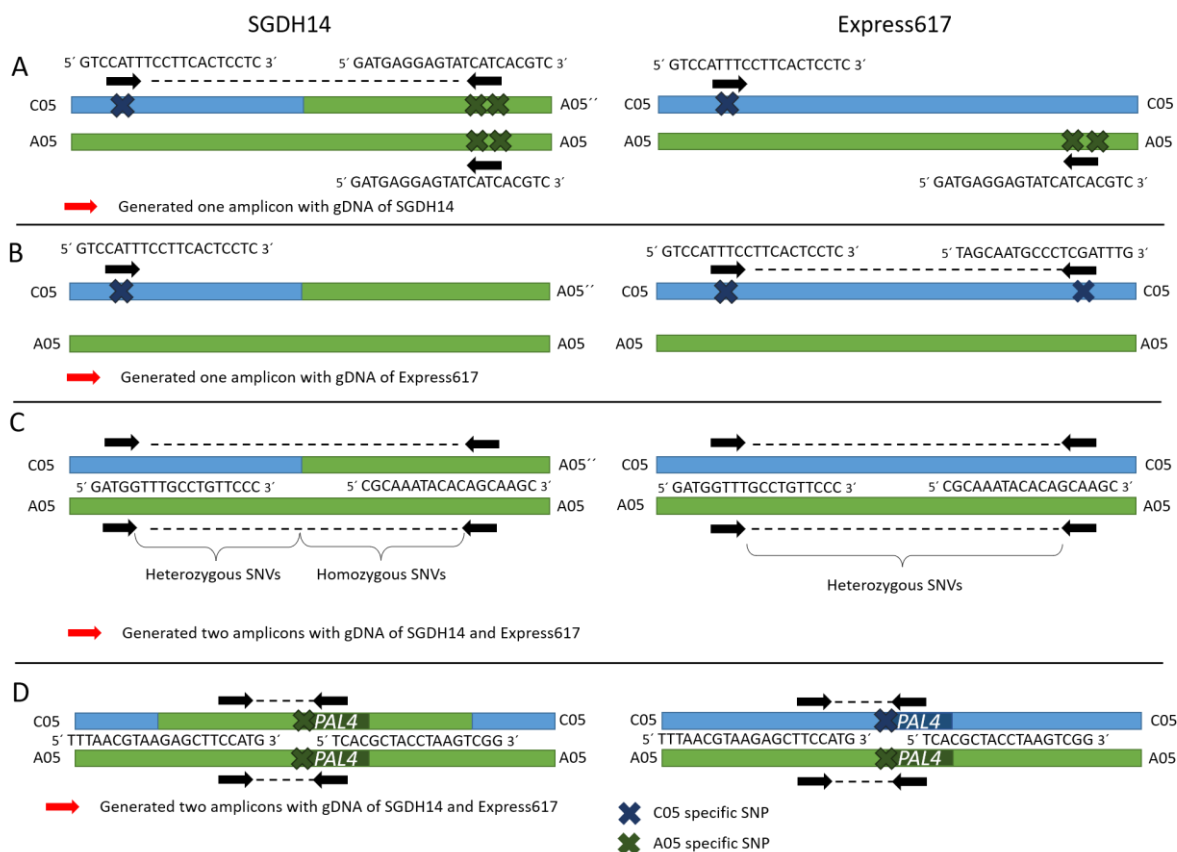
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Supplementary Figure 1: Oligonucleotide design strategies for validation of the HNRT border sequences.

(A) The subgenome-specific for SGD14, (B) the subgenomes-specific for Express 617 and (C) not subgenome-specific oligonucleotide design strategies used for the validation of the border sequences are shown, (D) subgenome-specific oligonucleotide design to determine if the *PAL4* gene of the A and/or the C chromosome is present. Oligonucleotides are marked as black arrows. Each strategy was applied for the left and right border, respectively.

Supplementary Table 1: Oligonucleotides used in this study for the three different strategies and for the amplification of *PAL4*. PCR products were sequenced to confirm the correct gene was amplified. The oligonucleotides written in bold and italics indicate the positions which are different between the subgenome-specific primers on the A and C chromosome.

Strategy	Oligonucleotide name	Strand	Sequence (5' to 3')	Annealing Temp. [°C]
A – left border	SGDH14_spec_fw_L	forward	GTCC <i>C</i> ATTTCCCTTCACTCCTC	60
A – left border	SGDH14_spec_rev_L	reverse	GATGAGGAGTATC <i>A</i> TACAGTC	
A – right border	SGDH14_spec_fw_R	forward	TCAGAC <i>G</i> GC <i>A</i> GCGTTTAC	60
A – right border	SGDH14_spec_rev_R	reverse	TTGCCACCACCACC <i>T</i> AC	
B – left border	Exp_spec_fw_L	forward	GTCC <i>C</i> ATTTCCCTTCACTCCTC	60
B – left border	Exp_spec_rev_L	reverse	TAGCAATGCCCTC <i>G</i> AT <i>T</i> TG	
B – right border	Exp_spec_fw_R	forward	TGGTCAGATG <i>G</i> C <i>T</i> CCGTTTAC	60
B – right border	Exp_spec_rev_R	reverse	TTGCCACCACCACC <i>T</i> AC	
C – left border	Not_spec_fw_L	forward	GATGGTTTGCCTGTTCCC	56
C – left border	Not_spec_rev_L	reverse	TCGCTGAATAGTCGCAAG	
C – right border	Not_spec_fw_R	forward	AACCAAATCCGTTGATGC	56
C – right border	Not_spec_rev_R	reverse	TTGCGTGACTGCTCCAAG	
D – PAL4	PAL_nspec_fw_1	forward	TTTAACGTAAGAGCTTCCATG	55
D – PAL4	PAL_nspec_rev_2	reverse	TCACGCTACCTAAGTCGG	

Supplementary Table 2: PCR program for the different subgenomes-specific strategies (A, B) and not subgenome-specific strategy (C), as well as for the amplification of the *PAL4* gene (D).

Steps	Temp. [°C]	Time [sec]	No. of Cycles
PCR program for strategy A and C; PAL4			
initial denaturation	95	60	1x
denaturation	95	15	35x
annealing	54, 56, 58, 60	15	
elongation	72	30	
final elongation	72	180	1x
hold	8	infinite	
PCR program for strategy B			
Steps	Temp. [°C]	Time [sec]	No. of Cycles
initial denaturation	95	180	1x
denaturation	95	30	15x
annealing	70 _ 0.7°C decrease each cycle	45	
elongation	72	45	
denaturation	95	30	25x
annealing	60 or 61	45	
elongation	72	45	
elongate	72	300	1x
halt reaction	4	300	1x
hold	20	infinite	1x