

**Table S4** Diagnostic PCR-based screening of breakpoints associated with the *Rose-comb* alleles.

A. Primer sequences for breakpoint analysis PCR

Primer	Sequence	Strand in WT
Rose-16.5-out	CAGAGCCCCCAGCGAA	+
Rose-16.5-in	GAAGTGAAACGGCCGTGAG	-
Rose-23.79	GCTGTTGATGCTGCTGGTG	-
Rose-23.88-in	CTGTCTGTGAGGCATTTCCAGT	+
Rose-23.88-out	CTAAATACCTGCCCTTCCTGA	-

B. PCR mix for breakpoint analysis

Reagent	Volume ( $\mu$ l)
5X Kapa2G GC Buffer w/ 1.5mM MgCl <sub>2</sub>	2
dNTPs (20 mM)	0.1
Rose-16-out (10 $\mu$ M)	0.2
Rose-16-in (10 $\mu$ M)	0.3
Rose-23.79 (10 $\mu$ M)	0.1
Rose-23-in (10 $\mu$ M)	0.4
Rose-23-out (10 $\mu$ M)	0.4
Kapa2G Robust HotStart DNA Polymerase (5U/ $\mu$ l)	0.08
DNA (10 ng/ $\mu$ l)	1
dd H <sub>2</sub> O	5.42
Total	10

C. PCR cycle conditions for breakpoint analysis

Temp	Time
95°C	5:00
95°C	0:30

26x	68°C	0:30	-0.5°C/cycle
	72°C	1:00	
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9x	95°C	0:30	
	55°C	0:30	
	72°C	1:00	
	72°C	7:00	
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	8°C	∞	

#### D. Result breakdown for breakpoint analysis

Genotype:	<i>R1R2</i>	<i>R1R1</i>	<i>R2R2</i>	<i>rr</i>	<i>R2r</i>	<i>R1r</i>		Primer1	Primer2
	155	155				155	23.89 <i>R1</i> inversion band	Rose-23-out	Rose-16-in
	240		240	240	240	240	23.89 <i>r/R2</i> band	Rose-23-in	Rose-23-out
	350		350		350		23.79 <i>R2</i> duplication band	Rose-23.79	Rose-16-in
				482	482	482	16.5 <i>r</i> band	Rose-16-out	Rose-16-in
	561	561	561		561	561	16.5 <i>R1/R2</i> inversion band	Rose-16-out	Rose-23-in