

Supplemental Table 7: Overview of the segregation analysis on kanamycin and hygromycin of T4 seeds, collected from T3 plants resistant to 5-Fluorocytosine. The T3 plants originated from T2 plants containing a recombined and a non-recombined allele, indicating that the T3 plants resistant to 5-Fluorocytosine were homozygous for the GOI and marker-free. Several T4 plants, selected on kanamycin, were transferred to the greenhouse; DNA was purified from leaf material for Southern blot analysis (Fig. 5).

T4 seeds	Selection on Kanamycin			Selection on Hygromycin		
	R	S	$K_{S/R}^1$	R	S	$H_{R/S}^2$
SDS-1-2-H3/FC1	56	0	0	0	96	0
SDS-1-2-H3/FC2	49	0	0	0	113	0
SDS-1-2-H3/FC4	54	0	0	0	100	0
SDS-1-2-H6/FC1	52	0	0	0	47	0
SDS-1-2-H6/FC2	30	0	0	0	30	0
SDS-1-2-H6/FC6	55	0	0	0	102	0
SDS-1-14-H5/FC2	46	0	0	0	57	0
SDS-1-14-H5/FC4	43	0	0	0	52	0
SDS-1-14-H5/FC5	47	0	0	0	45	0
SDS-1-14-H7/FC1	49	0	0	0	58	0
SDS-1-14-H7/FC3	52	0	0	0	111	0
SDS-1-14-H7/FC4	51	0	0	0	102	0
SDS-1-21-H5/FC1	46	0	0	0	79	0
SDS-1-21-H5/FC2	33	0	0	0	97	0
SDS-1-21-H5/FC3	51	0	0	N.D. ³	N.D.	N.D.
SDS-1-21-H6/FC2	43	0	0	0	81	0
SDS-1-21-H6/FC3	44	0	0	0	83	0
SDS-1-21-H6/FC5	37	0	0	0	81	0
SDS-1-23-H5/FC3	38	0	0	N.D.	N.D.	N.D.
SDS-1-23-H5/FC6	53	0	0	0	116	0

SDS-1-23-H5/FC9	49	0	0	0	110	0
SDS-1-23-H10/FC1	53	0	0	0	107	0
SDS-1-23-H10/FC2	33	0	0	0	94	0
SDS-1-23-H10/FC5	48	0	0	0	100	0
SDS-11-2-H6/FC2	52	0	0	0	36	0
SDS-11-2-H6/FC3	55	0	0	0	54	0
SDS-11-2-H6/FC6	48	0	0	N.D.	N.D.	N.D.
SDS-11-2-H11/FC1	44	0	0	N.D.	N.D.	N.D.
SDS-11-2-H11/FC2	35	0	0	0	61	0
SDS-11-2-H11/FC3	25	0	0	0	64	0

¹ $K_{R/S}$: ratio resistant to sensitive plants on kanamycin.

² $H_{R/S}$: ratio resistant to sensitive plants on hygromycin.

³ N.D.: Not determined.