

	1	60
S _{inbred} [S _{1a}]	TTCCGCGAGGAGTATCCGGATAGAATCATGGCTTCAT TTC TCCGTTGTTCCATCACCAAAGG	
S _{inbred} [S _{1b}]	----- TTC -----	
RS ³ [R ₂]	----- TTC -----	
RS ³ [R _{1c}]	----- TTC -----	
RS ³ [R _{1b}]	----- TTC -----G	
RS ³ [R _{1a}]	----- TTC -----G	
	61	120
S _{inbred} [S _{1a}]	TAATGTATCCC·ACAGCAGTCCTTTTT·GAGATCGTATTTACAGGTTCTCGAGGTGCAA	
S _{inbred} [S _{1b}]	-----TA---T-----G	
RS ³ [R ₂]	-----TA---T-----G	
RS ³ [R _{1c}]	-----TA---T-----G	
RS ³ [R _{1b}]	---T---T-TG---TC-----C-----G-----G-----G-----G	
RS ³ [R _{1a}]	---T---T-TG---TC-----C-----G-----G-----G-----G	
	121	180
S _{inbred} [S _{1a}]	TCCATTTTACGAACTCTTCACATTGATGCGCAACCGTGAAATGTGCGAAGAAGTTATGTT	
S _{inbred} [S _{1b}]	-----G-----	
RS ³ [R ₂]	-----G-----T-----	
RS ³ [R _{1c}]	-----G-----T-----	
RS ³ [R _{1b}]	C-----CG---T---T---CA---TTT---A---G-----C---T-----	
RS ³ [R _{1a}]	C-----CG---T---T---CA---TTT---A---G-----C---T-----	
	181	240
S _{inbred} [S _{1a}]	TATAGGTTTCCGATACCGTTGTGGAACCTTACAATGCCACTCTTTCTGTACACCAGTTGG	
S _{inbred} [S _{1b}]	-----	
RS ³ [R ₂]	-----	
RS ³ [R _{1c}]	-----	
RS ³ [R _{1b}]	-G---A-----	
RS ³ [R _{1a}]	-G---A---C-----T---A---	
	241	300
S _{inbred} [S _{1a}]	TTGAAAATACCGAT GAAACATTC TGCATCGATAATGAAGCTCTGTACGATATCTGCTTCC	
S _{inbred} [S _{1b}]	----- GAA --- TTC -----	
RS ³ [R ₂]	----- TTA --- TTC -----	
RS ³ [R _{1c}]	----- GAA --- TAC -----	
RS ³ [R _{1b}]	----- GAA --- TAC -----	
RS ³ [R _{1a}]	-A---C---G AAC - TAC -T-----G-----	
	301	360
S _{inbred} [S _{1a}]	GCACCTTAAAACACAAATCCAACCTTATGGCGATCTCAATCACTTAGGTGAGGTTTAAAT	
S _{inbred} [S _{1b}]	-----T-----	
RS ³ [R ₂]	-----T-----	
RS ³ [R _{1c}]	-----T-----	
RS ³ [R _{1b}]	-----T-----	
RS ³ [R _{1a}]	-----G-----C-TAC-T-	
	361	420
S _{inbred} [S _{1a}]	ATAGGTTCTATGAATATTAGCTCAGCCATCTTTT·TAAATGATATGTATTGTGGTTTT	
S _{inbred} [S _{1b}]	-----	
RS ³ [R ₂]	-----T---G---T-----A-----	
RS ³ [R _{1c}]	-----T---G---T-----	
RS ³ [R _{1b}]	-----T---G---T-----C-----	
RS ³ [R _{1a}]	---A-CCT-·-A-G-----T-----C-C---C-C-----C-----	

S3 Fig. ClustalW multiple alignment of selected gDNA sequence variants from the central region of the β -tubulin *isotype-1* gene from S_{inbred} and RS³ worms showing amino acid codon positions 167, 198 and 200 (bold), SNPs and positions of introns (shaded areas).

For the purposes of genotyping individual male worms from S_{inbred} and RS^3 populations, three “alleles” were recognized (S_1 , R_1 and R_2) and allele-specific PCR strategies designed to distinguish between these. In both S_1 and R_2 alleles, codon 200 encoded Phe, but in the case of the R_2 allele a mutation at codon 198 resulted in a Glu/Leu substitution. In all R_1 variants amino acid codon 200 encoded Tyr. Note that in order to accommodate the presence of SNPs in sequence flanking codon 200 in variants of the BZ resistant R_1 allele, the allele-specific primer TubRASFWd was designed to be degenerate in three nucleotide positions (see Methods).