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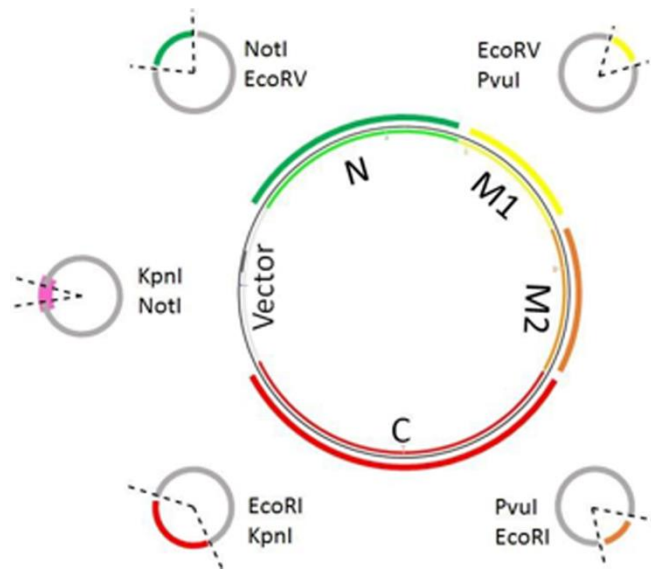
Supplementary Data

Supplementary Methods M1

To facilitate fragment reassembly, a hybrid plasmid vector was used, composed of the multiple-cloning-site (MCS) from pcDNA3.1(-) (Invitrogen, Carlsbad, CA, USA), plus the addition of two extra restriction enzyme cut sites (HindIII and MluI cut sites) at the 5' and 3' ends of the MCS respectively, spliced into a pIZ/V5-His plasmid vector (Thermo-Fisher Scientific, Waltham, MA, USA) in place of its original MCS. The four fragments (N, M1, M2 and a modified C) to be re-incorporated into the linearised hybrid pIZ/V5-His plasmid vector where then re-assembled into a whole intact full-length cDNA corresponding to the PxRyR ORF by ligation in the presence of the cut hybrid pIZ/V5-His plasmid vector. The total DNA concentration in the ligation reaction was maintained at ~10ng/ul. The addition of HindIII and MluI cut sites at the 5' and 3' ends of the pcDNA3.1(-) MCS, allowed the amplified sequence reassembled in the hybrid vector to be easily transferred across into pIZ/V5-His.

Supplementary Table S1: Primers used to generate amino acid substitutions in the *P. xylostella* RyR.

Mutation	Oligonucleotide 1	Oligonucleotide 2
K4700R	GTTCTACACCTTgCGTACGTGGCGCTGG	CCAGCGCCACGTACcgCAAGGTGTAGAAC
Y4701F	GTTCTACACCTTGAAGTTCGTGGCGCTGG	CCAGCGCCACGgACTTCAAGGTGTAGAAC
I4790C	GTATCGCTGGCTgtCTGATCGGGTACTAGGATTGAAGG	CCTTCAAATGGTAGTACCCGATCAGacaAGCCAGCGATAC
S4919L	CTCTTCTGTACTtaCTGTGGTACTTCTCGTTCTCTGTATGGGC	GCCCATCACAGAGAACGAGAAGTACCACAGtaAGTACAGGAAAGAG
V4945M	CGCTCATCTGTTGGACGTGGCTaTgGGGTTCAAGACGTTGAGG	CCTCAACGTCTTGAACCCcAtAGCCACGTCCAACAGATGAGCG
	CCTCGCCAGGAAGTTCTACACCTTGAAGTACGTGGCGCTGGTGTGGCC	
RFCLM (MULTI)	GCACTCTATAGTATCGCTGGCTATACTGATCGGGTACTACCATTGAAGG TCCGCG CGATCACAGACAACCTTTCTGTACTTCTGTGGTACTTCTCGTTCTCTGT	No reverse primer required in Lightning Multi Reaction



Supplementary Figure S1: *PxRyR* construct fragment assembly and restriction sites used for cutting out and re-ligating. The WT-*PxRyR* sequence was digested into four fragments, as labelled in the diagram. N ('N-terminus', green) - 4061bp M1 (yellow) - 2350bp M2 (orange) - 2684bp C ('C-terminus', red) - 6326bp Vector (pIZ/V5-His) - 2900bp