

Table S1. Primers used to construct *GRA* knockouts and complementations.

Primer	Primer Sequence	Primer Use	KO Construct	Corresponding TGME49 locus
GRA12AF1	TTGGGTAACGCCAGGGTTTTCCAGTCACGACGCTCTAGACACGATACGCTGTGGTGGGTC	Pru GRA12A KO 5' target FP	pRS416.GRA12A	TGME49_220890 chrII 37,902 to 41,699 (-)
GRA12AR1	GCGGGTTTGAATGCAAGGTTTCGTGCTGATCAAGTTTAAACGCTCGCTAGAGCGTTTACCGTAACC	Pru GRA12A KO 5' target RP		
GRA12AF2	TTCTGGCAGGCTACAGTGACACCGCGGTGGAGGGTTTAAACGGAAGCCGGTGTGGATTGGTC	Pru GRA12A KO 3' target FP		
GRA12AR2	GTGAGCGGATAACAATTTACACACAGGAAACAGCGCGGCCGCGCACACCACTTCTTAAGTCCAC	Pru GRA12A KO 3' target RP		
GRA12BF1	TTGGGTAACGCCAGGGTTTTCCAGTCACGACGACTAGTGGCTCAGCGTCACGTGAGAAG	Pru GRA12B KO 5' target FP	pRS416.GRA12B	TGME49_275860 chrIII 180,806 to 184,525 (+)
GRA12BR1	GCGGGTTTGAATGCAAGGTTTCGTGCTGATCAAGTTTAAACCTGCGAGCGCACTAAGCTCTG	Pru GRA12B KO 5' target RP		
GRA12BF2	TTCTGGCAGGCTACAGTGACACCGCGGTGGAGGGTTTAAACCTCACAGATCCTGTAATACTGCGCTCC	Pru GRA12B KO 3' target FP		
GRA12BR2	GTGAGCGGATAACAATTTACACACAGGAAACAGCAITTTAAATGTCCTCCACTGGTGAGCACGTTTC	Pru GRA12B KO 3' target RP		
GRA12CF1	TTGGGTAACGCCAGGGTTTTCCAGTCACGACGACTAGTGAACGCTGCTCACCAGTGGGAC	Pru GRA12C KO 5' target FP	pRS416.GRA12C	TGME49_275850 chrIII 185,445 to 189,438 (+)
GRA12CR1	GCGGGTTTGAATGCAAGGTTTCGTGCTGATCAAGTTTAAACCATGCTTGCACCTCTTAGGCGAG	Pru GRA12C KO 5' target RP		
GRA12CF2	TTCTGGCAGGCTACAGTGACACCGCGGTGGAGGGTTTAAACAGCATGTTCCAGTGGGTGAAGC	Pru GRA12C KO 3' target FP		
GRA12CR2	GTGAGCGGATAACAATTTACACACAGGAAACAGCAITTTAAATCTTCCGTTTCGATGCCGGTCTC	Pru GRA12C KO 3' target RP		
GRA12DF1	TTGGGTAACGCCAGGGTTTTCCAGTCACGACGATTTTAAATGATCTTGGCCGGTACACAC	Pru GRA12D KO 5' target FP	pRS416.GRA12D	TGME49_308970 chrXI 408,907 to 412,526 (+)
GRA12DR1	GCGGGTTTGAATGCAAGGTTTCGTGCTGATCAAGTTTAAACCGGTCAACGGATGCGTGACC	Pru GRA12D KO 5' target RP		
GRA12DF2	TTCTGGCAGGCTACAGTGACACCGCGGTGGAGGGTTTAAACCATGACTATGTCATTGCGAGGTACCG	Pru GRA12D KO 3' target FP		
GRA12DR2	GTGAGCGGATAACAATTTACACACAGGAAACAGCGCGGCCGCGCAGTCATCATGGGGAATTGTGATGGC	Pru GRA12D KO 3' target RP		
GRA12AF1	TTGGGTAACGCCAGGGTTTTCCAGTCACGACGCTCTAGACACGATACGCTGTGGTGGGTC	Pru GRA12A 5'target FP	pRS416.GRA12A	TGME49_220890 chrII 37,902 to 41,699 (-)
GRA12AR4	TCACTTGTGCTGCTGCTCCTTGTAGTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGG AACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGAACCTTTTCTGGATTCAAAT GCCCT	Pru GRA12A coding+tag RP		
GRA12AF4	CGCTGACTACAAGGACGACGACGACAAGTGACGCTGCATCGTAGATGGCAC	Pru GRA12A coding+tag FP		
GRA12AR2	GTGAGCGGATAACAATTTACACACAGGAAACAGCGCGGCCGCGCACACCACTTCTTAAGTCCAC	Pru GRA12A 3'target RP		
GRA12BF1	TTGGGTAACGCCAGGGTTTTCCAGTCACGACGACTAGTGGCTCAGCGTCACGTGAGAAG	Pru GRA12B 5'target FP	pRS416.GRA12B	TGME49_275860 chrIII 180,806 to 184,525 (+)
GRA12BR4	TTACTTGTGCTGCTGCTCCTTGTAGTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGGA ACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTAAGCTTCTAGCAGCTTCAACGTAGG	Pru GRA12B coding+tag RP		
GRA12BF4	CGCTGACTACAAGGACGACGACGACAAGTAAGGAGGCGCCTAGGGGTTG	Pru GRA12B coding+tag FP		
GRA12BR2	GTGAGCGGATAACAATTTACACACAGGAAACAGCAITTTAAATGTCCTCCACTGGTGAGCACGTTTC	Pru GRA12B 3'target RP		

Table S1. Primers used to construct *GRA* knockouts and complementations.

Primer	Sequence	Primer Use	KO Construct	TGME49 Replacement Locus
GRA12CF1	<i>TTGGGTAACGCCAGGGTTTTCCAGTCACGACGACTAGTGAACCGTGCTCACCAGTGGGAC</i>	Pru GRA12C 5'target FP	pRS416.GRA12C	TGME49_275850 chrIII 185,445 to 189,438 (+)
GRA12CR3	GAGA ACTTGGGGCTGTCTGCC	Pru GRA12C coding RP		
GRA12CDF	GTGTTCCGGTACATATGGCAGACAGC	Pru GRA12C coding FP		
GRA12CR4	CTACTTGTCGTCGTCGTCCTTGTAGTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATCTGG AACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGTA CTTCTTTTGTGAAGGTTTCATGAA CGTCG	Pru GRA12C coding+tag RP		
GRA12CF4	CGCTGACTACAAGGACGACGACGACAAGT AGGAAGCTGTGCACGCGG	Pru GRA12C coding+tag FP		
GRA12CR2	<i>GTGAGCGGATAACAATTTACACAGGAAACAGCA</i> <u>TTTTAAATCTTCCGTTTCGATGCCGGTCTC</u>	Pru GRA12C 3'target RP		
GRA12DF1	<i>TTGGGTAACGCCAGGGTTTTCCAGTCACGACGCA</i> <u>TTTTAAATGATCTTGCGGCCGGTACACAC</u>	Pru GRA12D 5'target FP	pRS416.GRA12D	TGME49_308970 chrXI 408,907 to 412,526 (+)
GRA12DR4	TCACTTGTCGTCGTCGTCCTTGTAGTCAGCGTAATCTGGAACATCGTATGGGTAAGCGTAATC TGGAACATCGTATGGGTAAGCGTAATCTGGAACATCGTATGGGT ACAATTCACGGTACCTGC GAATGACATAG	Pru GRA12D coding+tag RP		
GRA12DF4	CGCTGACTACAAGGACGACGACGACAAGT GATTCGGTTTTGCAAGTCGAAAGTACC	Pru GRA12D coding+tag FP		
GRA12DR2	<i>GTGAGCGGATAACAATTTACACAGGAAACAGCGCGGCCAGTCATCATGGGGAATTGTGATGGC</i>	Pru GRA12D 3'target RP		

*Italicized nucleotides indicate regions of crossover in yeast recombination cloning, underlined nucleotides indicate restriction enzyme sites, and bold nucleotides indicate specific genomic priming target regions corresponding to type II ME49 in the *Toxoplasma gondii* database (ToxoDB version 30). FP indicates forward primer and RP indicates reverse primer. GRA12C replacement loci was pulled out in three parts.