



Supplemental Figure 1: Assay optimization using varying concentrations of MgSO₄ and betaine

The amplification time difference between the DNA positive and negative samples of 33 minutes, 30 minutes and 32 minutes respectively in reactions containing 2mM MgSO₄ and 800mM, 400mM and 200mM of betaine respectively. In reactions containing 800mM, 400mM and 200mM of betaine and no MgSO₄, the reaction time difference between the negatives and positives were 36minutes, 33minutes and 28minutes respectively. There was no amplification when 6mM of MgSO₄ was added to the reaction containing 800mM, 400mM and 200mM of betaine.

ID:31		dimer(minimum)dG=-2.46						
label	5'pos	3'pos	len	Tm	5'dG	3'dG	GCrate	Sequence
F3	761	778	18	59.1	-4.2	-6.3	0.56	AGAAGTCCTGCTGCAACG
B3	933	952	20	60.2	-5.1	-5.3	0.5	AGGTCTGCACATTCATGCTG
FIP			41					TTGTCTTGGCAGTCCTGACAGG-GTAGTACGTGCTGCACGAA
BIP			42					GTTTCGGTGTACGCGACAGACAC-GCACCAAGGCTAAAGAGGAA
F2	779	797	19	59.2	-3.3	-5.4	0.53	GTAGTACGTGCTGCACGAA
F1c	821	842	22	64.3	-4.4	-5.4	0.55	TTGTCTTGGCAGTCCTGACAGG
B2	907	926	20	59.6	-6.4	-4.7	0.5	GCACCAAGGCTAAAGAGGAA
B1c	845	866	22	65.2	-5.8	-4.9	0.59	GTTTCGGTGTACGCGACAGACAC

Supplemental Table 1: Primers used in the development of the assay

GTGCTCCGCTTGCCAACTGCGCGCGCGTGCATTACAATGGGCACCATGATCAGAC
ATACATTTACGCATAGGTGCGGAGCGCTCCTGTGCGCGCTGGCGTTGGGAAGCTC
CACTATGGCTGCGACCGCCGCTGCAAAGCCAAGACGACGGGGCAAATGCAGAA
ATTGCGGCGGGCGGCCGGTGTGGGCGCCCACCGGGCGGGCGGTATGCGTCTTTGG
ACGGTGCCTTTACCGCGCTGGCAAATGATGCAAGTTTCTTTGAGGCAAATCCGGC
AGGAAGTGCGAACATGACGCACGGGGAGCTGGCTTTCTTCCATACCACTGGCTTT
GGCTCGTTTTACGCCGAAACGCTTTTCTACGTTGGCCAGCGGGGCAAATGGGGAT
ACGGCGCGTTCGATGCGTATGTTTTTCCCCGAATCCGGGTTTAACTTTTCTACCACC
ACGGAGACCGTGTGCACACCTGCTTCGGCCCCATTAAGCAGCGCGGGGCAATT
GGAATCATCAACTTTGCCCGGCGTATCGGGGGTCTCTCCCTGGGAGCCAACCTGA
AGGCGGGGTTCCGCGACGCGCAGGGCCTGCAGCACACCTCTGTCTCCAGTGACA
TCGGTTTGCAGTGGGTGGGGAACGTTGCCAAGTCCTTTACCTCTGAGGAGCCCAA
CCTGTACATCGGGCTTGCGGCCACCAACTTGGGATTGACCGTAAAGGTCTCGGAC
AAGATAGAGAACTGCACGAGTACCTGTGAAAAGTGTGGTTGCTGCAAGGAGAAGT
CCTGCTGCAACGGTAGTACGTGCTGCACGAAGAAGTGCGAAGGCTGTAAGTCCCC
CTGTCAGGACTGCCAAGACAAAGGTTTCGGTGTACGCGACAGACACCATGCTGCGT
GCAGGGTTTTCGTACCGGCCCTTCAGCTGGTTCCTCTTTAGCCTTGGTGCCACCA
CCAGCATGAATGTGCAGACCTTGGCTAGTAACGCCAAGTCGCTGTACGAGAATCT
GGCTTACAGCATAGGCGCCATGTTTGATCCCTTCAGCTTCCTGAGCTTGAGTTCGA
GCTTCCGCATCAACCACAAGGCTAACATGCGAGTGAGAGTGGGTGCACAGGCGC
GAAGGCTTCGCACTACCTTTCTTGGGTGGCGCGATACTGCTCGGCCGAAATTAAT
TCATAA

Supplemental Figure 2: The 200 bp sequence of tp0967 with the location of primers used in the assay