PCR Amplifications

For 50µL reactions:

Forward primer- 5'GGCTCAGAATTCGCGGCCGCTTCTAGAG3' (Prefix +6)
Reverse primer- 5'CTGGACCTGCAGCGGCCGCTACTAGTA3' (Suffix +6)

Table 1: Assemble reaction in a thin walled tube in the following order:

Component	50µL rxn	Final conc.
ddH_2O	40.5	
10x pfu buffer	5	1x
10mM dNTPs	1	200µM
Forward Primer	1	200µM
Reverse Primer	1	200µM
Template DNA*	0.5	50pg-1µg
pfu	1	0.25U/µL

*The concentration of the DNA template is 10ng/µL.

1. Run with the described thermocycler program

Table 2: Cycling instructions

Cycle Step	Temperature (°C)	Time (sec)	Cycles
Initial Denaturation	95	60	1
Denaturation	95	20	30
Annealing	58	30	
Extension	72	360	

Final Extension	72	600	1
Stop	4	∞	1