**PCR Program for Use with Primer3-Designed Primers**

The program (WI-S):

1. 96°C 5:00min
2. 96°C 0:30min
3. 57°C 1:00min
4. 72°C 1:00min
5. Goto 2 34 times
6. 72°C 5:00min
7. 4°C hold

Note that the original program had annealing and extension times of 2 minutes each.

The 10X WI Buffer:

15mM MgCl2
0.5M KCl
0.1M Tris-HCl pH 9.3(!)

Protocol:

10ng Template
5pmole each primer
4nmoles each dNTP
0.025 until/ul Taq
20 ul Total reaction volume

Notes:

We used our primers at 5pmol each, diluting the stock to 2.5pmol/ul and using 2ul for each 20ul reaction
We used our dNTPs at 5mM each (2.5mM each per ul)

A typical reaction would look like this:

2ul (c)DNA
2ul 10X WI Buffer
2ul dNTP mix
2ul primer mix
11.8ul PCR H2O
0.2ul Taq (or try just 0.1ul)
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20ul