PCR Protocol

*Platinum® PCR SuperMix High Fidelity may be stored at 4°C
*Reactions may be assembled either at room temperature or on ice.

Protocol

1. Dilute the Primers to 20uM (arrive at 200uM)
    a. Add 10ul primer to 90 ul dH_20

2. Prepare Each Reaction Tube (50uL)
    a. Add 45 µl Platinum PCR SuperMix High Fidelity
    b. Add 1.25ul of both Forward and Reverse Primer Solutions
    c. Template DNA solution
        ~Approximately 1ul (between 30-50ng) (see chart below)
    *A standard 50-µl PCR reaction includes a combined primer and template volume of approx. 5 µl.

3. Cap tubes and load in thermal cycler.

4. Incubate tubes at 94°C for 2 min to completely denature the template and activate the enzyme.

5. Perform 25-35 cycles of PCR amplification as follows:
    a. Denature 94°C for 30 s
    b. Anneal 55°C for 30 s
    c. Extend 68°C for 1 min per kb – (see chart below)

Data for Individual PCR Reactions					
	Amount per50 uL tube				
	Forward Primer	Reverse Primer	DNA Solution	Extension Time	PCR Supermix
SoxR-SoxS	1.25ul	1.25ul	~1ul	60 sec	45uL
SoxR gene	1.25ul	1.25ul	~1ul	60 sec	45uL
SoxS Promoter	1.25ul	1.25ul	~1ul	60 sec	45uL
RBS+Blh	1.25ul	1.25ul	~1ul	60 sec	45uL
RBS+RaldhII	1.25ul	1.25ul	~1ul	120 sec	45uL
hIl-6 HlyAs	1.25ul	1.25ul	~1ul	60 sec	45uL

*Everything 1 min extension except RaldhII- 2 min extension
**Note: Only 2 different rounds of PCR need to be ran.