**Rhodopsin Amplification PCR Protocol**

**PCR Reagents: 1X(µl):**

**Qiagen Multiplex MM (2X) 5**

**Primer Mix (2µM)\* 1**

**Molecular Grade H2O 2.5**

 **8.5 µL + 1.5 µL of DNA**

\*Dilute brand new lyophilized primers to get a 100µM master stock.

100µM = X nmoles lyophilized primer + ( X \* 10µL molecular grade H2O)

Dilute the 100µM master stock by 10 to get a 10µM stock.

To get 100µL of 2µM primer mix working stock: 20µL of each 10µM primer + 60µL of PCR grade H2O.

**PCR Cycling Conditions:**

**Step Temperature Time**

**Initial Heat Activation 95˚C 15 min**

**Denaturation\*\* 94˚C 30 sec**

**Annealing\*\* 57˚C 1 min + 30 sec**

**Extension\*\* 72˚C 1 min**

**\*\*3-step cycling number of cycles: 40**

**Final Extension 60˚C 30 min**

**Hold 4˚C ∞**

**Materials:**

**Qiagen Multiplex PCR Kit, cat. no. 206143**

**Primers were ordered from IDT**

**Primer Sequences:**

Avian RHO E1 F: CTACGTGCCCATGTCCAACAAG

Avian RHO E1 R: GTGACGTACAGCGTGAGGAAG