**Primer Design:**

C term:

FWD: 5’ Last 80 bp of ORF w/o stop codon + GGTTCTGGTAGTGGTTCC 3’

REV: 5’ Rev comp of first 80 bp of 3’ UTR + CCAATTTGAGAGACCTGTGC 3’

N term:

FWD: 5’ Last 80 bp of 5’ UTR + GTATAATGCAGACCTGCTGC 3’

REV: 5’ Rev comp of first 80 bp of ORF + ACTACCCGATCCTGATCC 3’

**PCR Instructions:**

**200μl Reaction set up:**

ReagentAmount (μl)Final Concentration

H20: 118μl -

10μM FWD primer: 20μl 1μM

10μM REV primer: 20μl 1μM

2mM dNTPs: 20μl .2μM

Plasmid: 2μl 25 ng/μl

DMSO: 4μl 2%

Buffer: 20μl 1X

\* dNTPs must be 2mM, if 10mM dilute prior to adding to reaction mixture

\*Once the above mixture reaches 94°C in thermocycler add HiFi Polymerase

HiFi Polymerase: 2μl

**Thermocycler set up:**

TempTime

94°C 5:00’

94°C 0:15\

65°C 0:30 30X

72°C 2:00/

72°C 7:00

12°C ∞

**Transfection:**

After you confirm a singular strong band of about 1.5 kb on an agrose gel, purify the 200μl PCR reaction using phenol chloroform. Add 200μl of phenol chloroform (DNA) to PCR product and spin at RT for 10’ at max speed. Take the top layer, about 200μl, and place in fresh tube. To the tube with the top layer add 1mL 4°C 96% ethanol and 40μl of 3M sodium acetate, let precipitate at -20°C over night. Spin in centrifuge at max speed for 20 minutes at 4°C. Remove supernatant, wash with 70% ethanol, spin again and remove supernatant. Air dry for 2-5’ in the hood. Resuspend in 30μl of ddH20 in the hood. Quantify via nanodrop and use 10-12μg of the purified PCR product per transfection and transfect using normal transfection conditions. For transfection into procyclic form cells use 1x107 cells, or 2x107 cells for bloodstream form transfections .