Cryopreserved stocks of 8 parasite lines

8 parasite lines in culture (grow to ~2% at 10 ml)

8 synchronized parasite lines in culture (10 ml)

Thaw after ~2 life cycles, triple sorbitol sync 0, 48, and 56 hrs

After 2 life cycles, set up competitions in plate: biological replicate 2

Every life cycle, count and cut parasitemia to 1% and collect genotyping samples

Cuts from plate to dilute parasitemia (genotyping samples stored at -80°C until ready for analysis)

Repeat for new thaws for biological replicates

After 1 life cycle, set up competitions in plate: biological replicate 1

Genotype PCR with fluorescently labeled primers

Set up CEQ run plate

Fragment analysis on CEQ 8000

Analysis: determine relative amounts of each parasite

NF54 (WT, F)

NHP4076 (E252Q, S)

Day 0 - 4 hours

Day 4 - 8 hours

Day 7 - 12

~48-96 hours

Day 10-12

~2 hours

~1 hour

~2-8 hours

~2 hours

Day 14-16

Continue plate maintenance for 20-60 days

Day 30 - 73

~1 hour

~2-8 hours

~2 hours