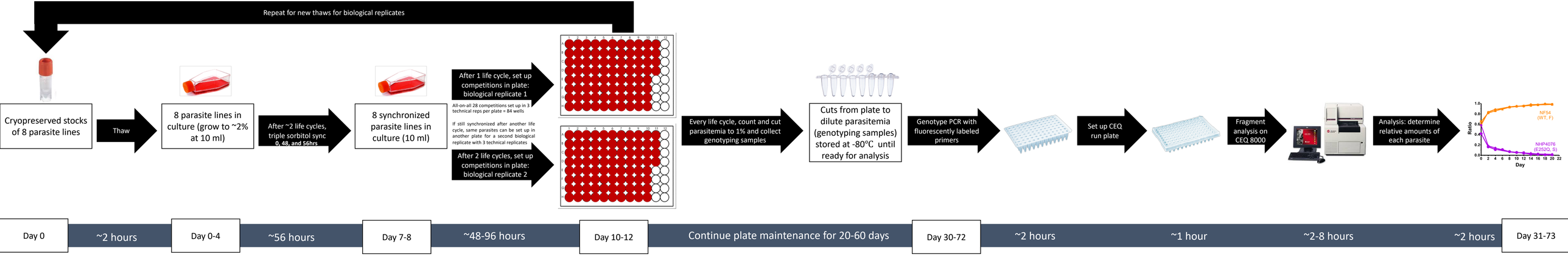
Repeat for new thaws for biological replicates
Cryopreserved stocks of 8 parasite lines
Thaw
8 parasite lines in culture (grow to ~2% at 10 ml)
After ~2 life cycles, triple sorbitol sync 0, 48, and 56hrs
8 synchronized parasite lines in culture (10 ml)
After 1 life cycle, set up competitions in plate: biological replicate 1
All-on-all 28 competitions set up in 3 technical reps per plate = 84 wells
If still synchronized after another life cycle, same parasites can be set up in another plate for a second biological replicate with 3 technical replicates
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
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)
Ratio
Day
Day 0
~2 hours
Day 0-4
~56 hours
Day 7-8
~48-96 hours
Day 10-12
Continue plate maintenance for 20-60 days
Day 30-72
~2 hours
~1 hour
~2-8 hours
~2 hours
Day 31-73