For each line of interest culture was struck from frozen onto YPD plates and grown for 72 hours at 30°C, at which point colonies were inoculated into 10mL YPD and grown for 48 hours. To initiate the competition experiment, 100μL of the competitor and 100μL of the strain of interest were inoculated into 10mL of YPD. Four replicates were initiated for each line of interest. We performed transfers that exactly mimicked the initial evolution experiment (100 μL transferred from each tube after 24 hours into fresh medium) for three days. Each day (including the initial day of the experiment), exactly two hours after transfer, 1mL of culture from each tube was aliquoted into an eppendorf, pelleted, and resuspended in sodium citrate. 150μL from each eppendorf was aliquoted into one well of a 96 well plate and immediately run on an LSRII flow cytometer with the High Throughput Sampler attachment. 10000 cells were measured from each well.

Data was analyzed in FlowJo version 8.7 (Tree Star, Inc.). Small debris was excluded with an initial gate then gates were drawn around the two clusters of non-fluorescing and fluorescing cells, by examining plots of FITC-A and AmCyan-A. Clusters were always distinct and easily distinguished. The absolute number of cells in each gate for each day of the experiment was determined. The competitive fitness (m) was determined for each line using the formula for evolutionary change:

$$NonFluor = \frac{p_0 e^{mT}}{1 - p_0 + p_0 e^{mT}} \tag{4}$$

where *NonFluor* is the fraction of non-fluroescing cells, p_0 is the initial fraction of non-fluorescing cells at the start of the experiment, T is the generation number (measurements were done on days 0,1,2, and 3 which corresponds to 0, 6.7, 13.6, and 20.1 generations) and m is the Malthusian parameter of the experimental strain minus that for the YFP-marked competitor (relative growth rate). We use the nls function in the R programming language [**?**] to determine the best fitting p_0 and m for each competition assay.