Values from activity assays are in “Tloop\_fluor\_and\_FI.xlsx”. Each sheet includes all assay data for a given library. Within each sheet, columns denote unique sequences with names assigned based on coordinates of gridded plate in each round. Each value is a ratio of the fluorescence measurement and the optical density at 600 nm. Column 1 denotes whether the value is in absence of ligand (-), presence of ligand (+), or the fold induction (FI) ration. Each unique sequence has at least 9 values for 9 unique measurements, since many have more, those cells that were left empty contain “-2” to be removed during analysis. Analysis of these data completed by converting each sheet into a .csv file to run in Tloop\_project\_analysis.Rmd.

Results from analysis via Tloop\_project\_analysis.Rmd are compiled in “Tloop\_data\_table.xlsx”. Each library has two sheets, one for all data values “X\_all” one with only colonies with FI >= 2.0. Each sheet includes the colony name (rows), median OD corrected fluorescence in presence of CNCbl (col B) absence of CNCbl (col D), the standard error of each (col C and E) and the median fold induction (col F). Additional data was added to output files in col F-L. Those media values are also reported as env8 corrected values (col G-I) and important sequence elements and the full sequence was reported in the neighboring columns.

FASTA files were created by converting the sheets from “Tloop\_data\_table.xlsx” into .csv and using:

awk -F , '{print ">"$1"\_"$4"\n"$9}' {file}.csv > {file}.fas

The information indexed can vary. The above example creates a fasta file with the fluorescence in the presence of cobalamin ($4) but that information can be varied or removed.