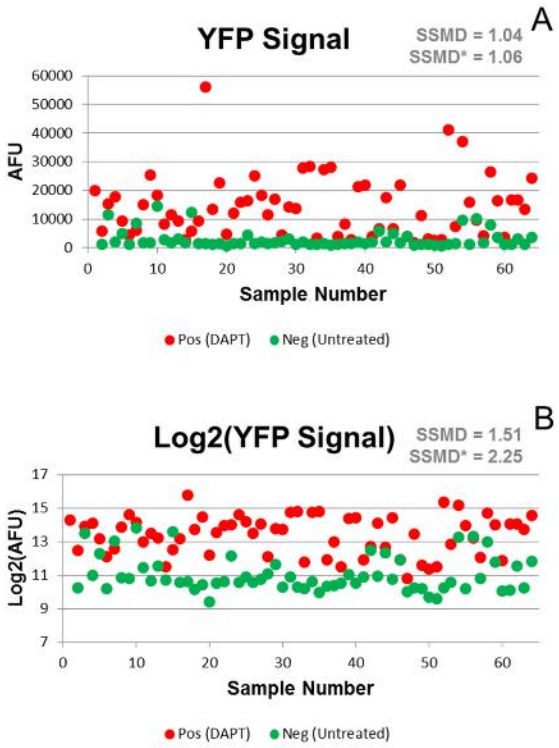
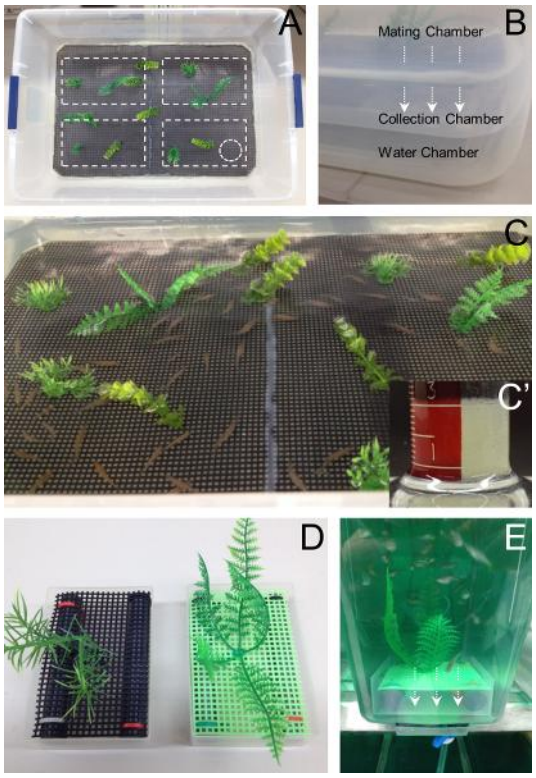


Supplementary Figure 1

Data transformation.

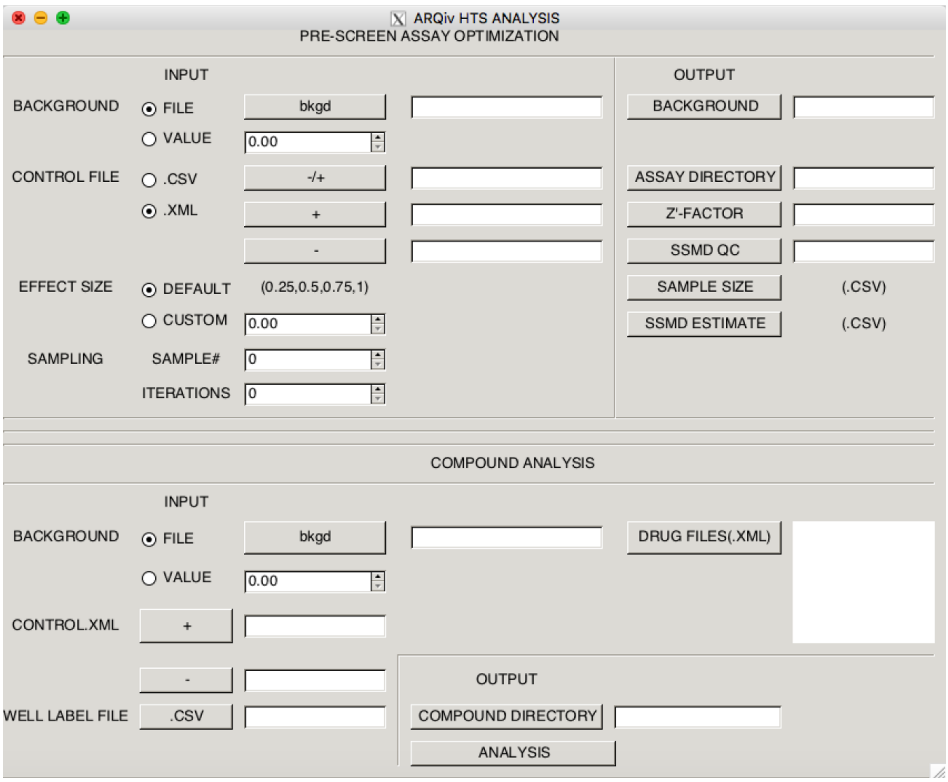
A: Representative data from an ARQiv-HTS assay quality test with arbitrary fluorescent units (AFU) expressed on an arithmetic scale. The data show unequal variance between the negative (green) and positive (red) controls. B: Base 2 log transformation of the data shown in A. Log transformation achieves more symmetrically distributed data around the respective means, as in a normal distribution, and can also facilitate a larger dynamic range of the effect size metrics used to evaluate tested compounds (e.g., increased SSMD scores, compare upper right in A and B).



Supplementary Figure 2

Economic large-scale egg production units.

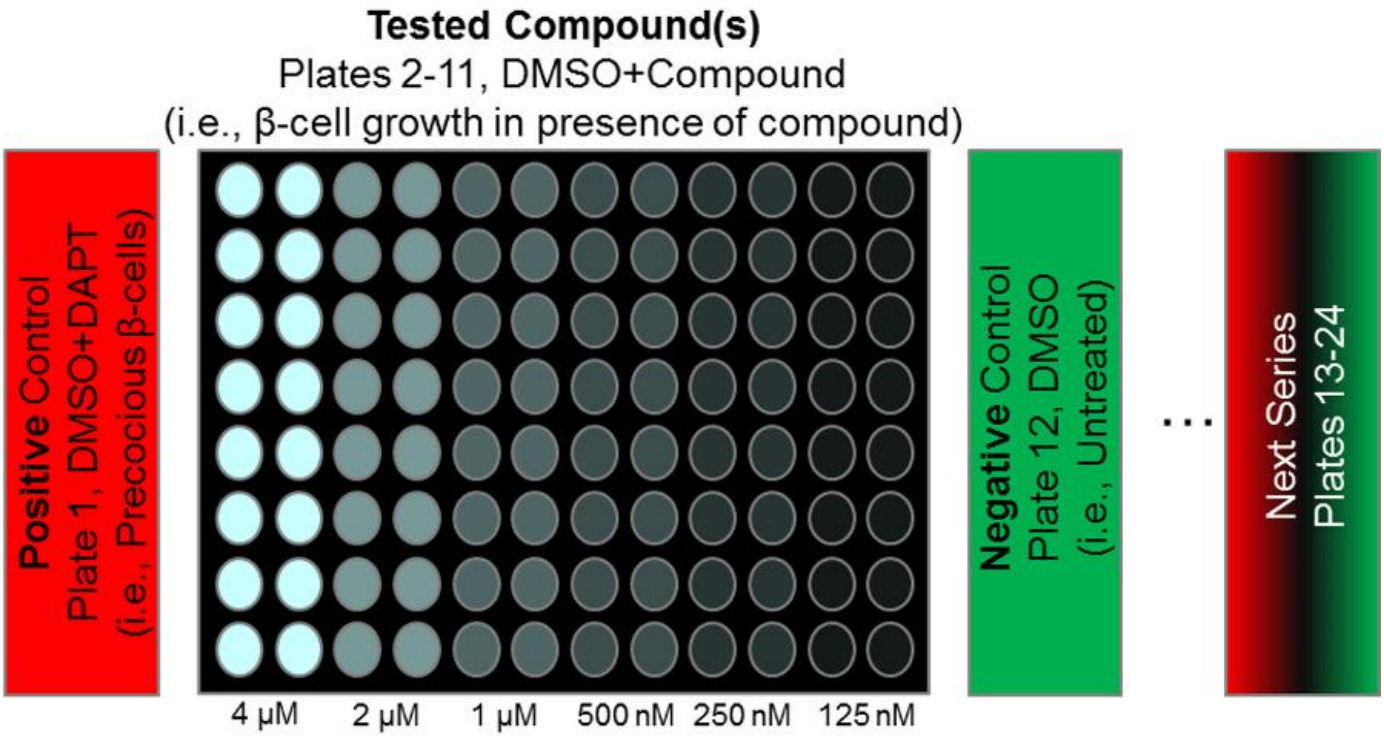
A: Top view of simple grouped breeding system assembled from plastic storage units showing the uppermost mating chamber (empty). Dashed boxes represent areas where inserted mesh screen allows eggs to pass through to a middle collection chamber; dashed circle shows area where micron mesh is inserted in the collection chamber to allow water drainage. B: Close-up side view of all three chambers, mating, collection, and water, seated one inside the other. Arrows indicate egg movement from mating to collection chamber. C: Grouped breeding unit in use, egg production is correlated to density of breeders in mating chamber, plastic 'plants' can be dropped in to further stimulate breeding. C': Measurement of eggs with graduated cylinder (once settled, 500-600eggs/mL). D: Top view of empty drop-in mating chambers. E: Side view of drop-in mating chamber (in use). Arrows indicate egg movement from tank to collection chamber.



Supplementary Figure 3

ARQiv package graphical user interface (GUI).

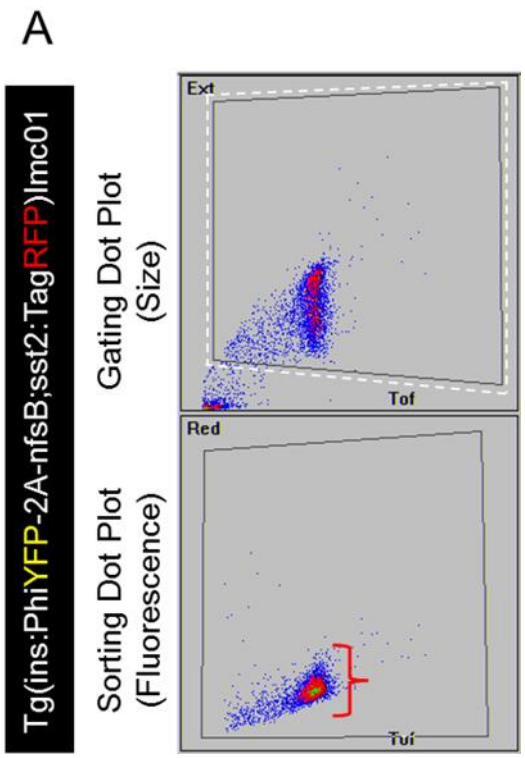
Upon installing the ARQiv R-based package, the GUI above is available for simplifying ARQiv data processing. The use of this GUI is detailed within relevant sections of the protocol. Briefly, the ARQiv R package includes functions that fall into two categories - those applied to ‘Pre-screening Assay Optimization' (upper panel) and 'Compound Analysis' (lower panel). The functions allow the user to calculate background signal, determine sample size, run quality control tests, perform virtual experiments to simulate compound efficacy - and finally, to perform compound analysis during iterative drug screen cycles.



Supplementary Figure 4

Titration-based ARQiv-HTS assay diagram.

Compounds are tested at a total six concentrations at a sample number of 16 per compound concentration, thus one 96 well plate per compound (center 96 well plate). To account for the possibility of signal changes over time, positive and negative control plates (red and green, respectively) 'bookend' every set of 10 tested compound plates. This process is reiterated for each series of tested compounds.



Supplementary Figure 5

COPAS-based larval fish sorting and microtiter plate dispensing.

Sorting and dispensing of transgenic larvae into microtiter plates can be automated using the COPAS-XL system. Fish are illuminated with a 561 solid-state nm laser as they pass through a flow cell (analysis chamber) wherein they are gated/sorted based on extinction (i.e., size and internal structure of object), time of flight (i.e., length of object), and fluorescence (emission at 610 nm +/-10). Upper panels are 'gating dot plots' denoting extinction (Ext, y-axis) and time of flight (Tof, x-axis) parameters used for size-based sorting of fish/non-fish objects as determined by user-defined 'gate region' (e.g., interior to dashed line in upper panel). Lower panel is fluorescence-based sorting of transgenic fish via user-defined 'gate region' in 'sorting dot plot' denoting 'RFP' signal (Red, y-axis) and time of flight (Tof, x-axis). Red bracket represents transgenic fish sorted into wells.