Table 1. Protocol for the p53RE qHTS Assay

Step parameter	Value	Description
Plate cells [a]	5 µL	4,000 p53RE-bla HCT-116 cells/well
Incubation time [b]	6 hr at 37°C	Cells adhere and acclimate
Tox21 compound library addition [c]	23 nL	92 µM to 0.59 nM titration series
Positive/vehicle control compound addition [d]	23 nL	Mitomycin C, 2 columns: 0.7 nM–23 µM; 11.5 µM Nutlin-3, 2 columns: 1.4 nM–46 µM; 23 µM Tetraoctylammonium bromide, partial column: 92 µM (cytotoxicity positive control) DMSO: 1 column
Incubation [b]	16 hr at 37°C	Induce p53 reporter
Reagent [e]	1 µL	Beta lactamase detection mix
Incubation	2 hr, room temp.	Cells load and cleave substrate
Assay readout [f]	Ex = 405/8 nm	EnVision™ plate reader

a 1536-well plates, single-tip dispensing of 4000 cells per well into all wells.

b Incubated at 37 ± 1°C under humidified atmosphere and 5% CO_2.

c Pintool transfer of library to columns 5–48.

d Pintool transfer of controls to columns 1–4; 2 columns with mitomycin C, 1 column with DMSO only, several wells in column 3 with tetraoctyl ammonium bromide (this column divided between MMC and TAB).

e LiveBLAzer™ B/G FRET substrate (Invitrogen, CA).

f Emission filters of 460/25 and 530/20 nm and an excitation filter of 405/8 nm.