A coincidence reporter-gene system for high-throughput screening

To the Editor: Originally developed as sentinels of transcriptional activity to map the regulatory function of genetic elements, reporter-gene assays have been extensively used in high-throughput screening (HTS) to identify chemical modulators of cellular pathways[1]. However, HTS chemical libraries consist of structurally diverse small molecules that frequently interact directly with the reporter, thus skewing data interpretation and complicating candidate selection. For example, a recent study indicated that >80% of the apparently active compounds from a 500,000-compound HTS were assay artifacts[2]. For reporter-gene assays, this poses a formidable challenge. To distinguish compounds that target a biological pathway from those that interfere with a reporter, we designed a coincidence 'biocircuit' based on the principle that it is easier to tell signal from noise when the signal is reported by two or more detectors. This concept is implemented here using co-translational expression of nonhomologous reporters: for example, proteins having different catalytic, light-emitting or fluorescence properties (**Fig. 1a**).

We confirmed the function of a preliminary biocircuit design by stoichiometric coexpression of the unrelated bioluminescent reporters firefly luciferase (FLuc) and *Renilla* luciferase (RLuc), using 'ribosome skipping' facilitated by the short P2A peptide[3] in a HEK293 cell. (**Supplementary Fig. 1** and **Supplementary Methods**). We demonstrated the accurate discrimination of forskolin-activated adenylyl-cyclase signaling through the cAMP-response element from signals mediated by the known FLuc and RLuc stabilizers, PTC124 and BTS, respectively (**Fig. 1b**,**c** and **Supplementary Fig. 2**). Using the LOPAC1280 chemical library, we conducted a quantitative HTS experiment, in which full titrations of each compound are tested[4] to identify potentiators of the CREB pathway (**Supplementary Figs. 3** and **4** and **Supplementary Table 1**). The screen revealed, for example, coincident FLuc and RLuc signal outputs for 17 adenosine analog agonists of endogenous purinergic 2Y known to signal through G proteins in this cell type[5] (**Supplementary Table 2a**). We observed excellent correlation between the half-maximal effective concentration (EC_{50}) values calculated from the orthogonal reporter outputs (**Fig. 1d**). Our experiments also illustrated the phenomenon of reporter-dependent artifacts: we identified five aryl sulfonamides that showed selective agonist activity for RLuc only (**Supplementary Table 2b**, compounds 20–24). These compounds share a similar core scaffold with two known RLuc inhibitors and selectively inhibit the enzymatic activity of RLuc over that of FLuc (**Supplementary Fig. 5**), thus tying these particular artifacts to the phenomenon of reporter stabilization[4]. Cross-section data analysis of the screen (**Supplementary Fig. 6**) also demonstrates how coincidence detection enhances the testing of compound libraries in single-concentration format.

We conclude that coincidence reporter strategies rapidly discriminate compounds of relevant biological activity from those interfering with reporter function and stability, using a single assay platform. This study outlines an approach to improved use of reporter genes in HTS with numerous coincidence combination types and stoichiometries possible.

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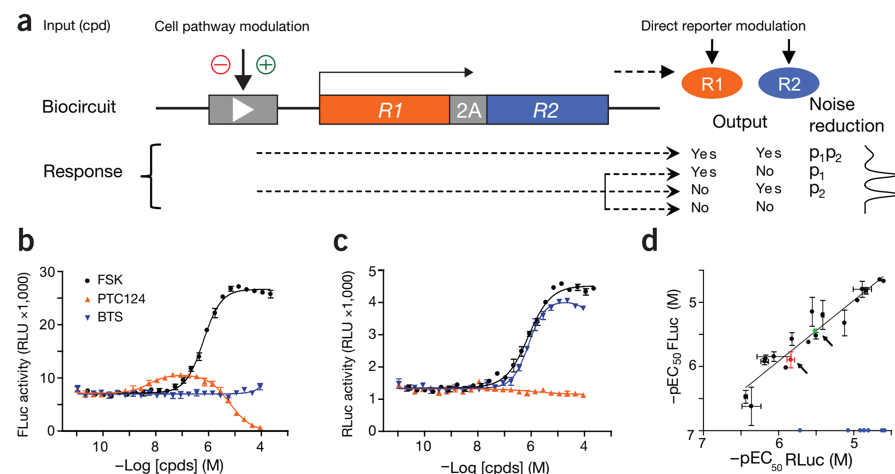

Figure 1 | Coincidence reporter biocircuit. (**a**) General scheme, where *R1* and *R2* are nonhomologous reporter genes giving rise to proteins R1 and R2. Cpd, compound; p, noise probability for respective reporter. (**b**,**c**) Response of FLuc (**b**) and RLuc (**c**) to treatment with FSK and inhibitors of FLuc (PTC124) and RLuc (BTS). RLU, relative luminescence units. (**d**) EC_{50} correlation plot for compounds activating FLuc and RLuc expression equally; $r^2 = 0.87$. Three classes of compounds were identified: purinergic Y2 receptor agonists (black circles); compound 18, a muscarinic receptor agonist (green); and the adenylyl-cyclase activator FSK (red). EC_{50} values of compounds that selectively increased RLuc (blue) are plotted along the *x* axis. Data plotted are averages of replicate ($n = 2$) determinations; error bars, s.d.

Note: Supplementary information is available at http://www.nature.com/doifinder/10.1038/nmeth.2170.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Ken C-C Cheng[1] & James Inglese[1,2]

[1]National Center of Advancing Translational Sciences, NIH, Bethesda, Maryland, USA. [2]National Human Genome Research Institute, NIH, Bethesda, Maryland, USA.

e-mail: jinglese@mail.nih.gov

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