Speeding Up Life Science Research

High-Throughput Biology: Combining Chemistry and Technology for Faster Results

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Introduction

Life science research is undergoing a gradual transition in the way technology is being used to generate biological data. Much as physicists and chemists have moved away from the lab bench and into big science collaborations, biologists are increasingly turning to higher throughput instrumentation to stay at the boundaries of discovery.

As life science research evolves toward high-throughput biology, investigators, instrumentation companies and biological reagent companies must partner for success.

The move toward getting more data faster is driven, in part, by the realization that the complexity of biology can best be understood by measuring a number of parameters in a single experiment. Examples include measuring a large number of different cytokines in a single 96-well plate or using a microarray to measure 40,000 mRNA transcripts on a single slide. A fundamental driving force for this type of experimentation is that a number of variables are internally controlled when data are generated in a single experiment. Variability due to differences in environment, lot numbers and technicians are eliminated if all the data are generated at the same time. Such consistency is crucial when trying to generate information through a systems biology perspective. Another consideration is the ability to do pattern matching, which is only visible when looking at large data sets. Together, the data from the whole plate yield more information than the data from the individual wells. These advantages helped to create a new technology area that is generally referred to as "High-Throughput Biology".

The effort to sequence the human genome was an early example of industrialized biology. In this case the practical necessity of sequencing three billion nucleotides led to the development of specialized high-throughput instrumentation. Around the same time, pharmaceutical companies were developing instrumentation to screen compound libraries containing millions of compounds. In fact, much of what has become high-throughput biology was developed for high-throughput screening. An example of where instrumentation was crucial to the basic research discovery process was the use of 384-well technology and a yeast two-hybrid system to help understand the protein:protein binding matrix in a mammalian cell model (1). Elucidating this type of large protein interaction network would not have been feasible without advanced instrumentation.

In this issue of *Promega Notes* we provide three examples in which instrumentation is used to simplify the generation of large data sets. In the first example, described below, we used a reporter gene as a readout in an RNAi experiment using the new GloMax™ 96 Microplate Luminometer. This system provides a single source of instrumentation, software, reagents and support for laboratories that want turnkey solutions for luminescent assays. In a second article (page 4), we use a Tecan liquid handling platform to rapidly screen compounds for their development as biological probes for new drug candidates. Finally, in the third article (page 7), we describe an example of ultrahigh-throughput biology using nanoliter dispensing technology from Aurora Discovery. Together these examples provide an overview of biology as it transitions from single-tip pipettors to high-density plates.

The GloMax™ Luminometer Offers Sensitive Detection for Multiple Reporter Assays

The GloMax™ Luminometer was used to measure multiple reporter assays in the context of an RNAi experiment. Cells were transfected with a psiCHECK™-2 Vector that expresses *Renilla* luciferase mRNA fused to sequences from the human p53 gene. This vector allowed us to monitor the RNAi effect with a *Renilla* luciferase reporter assay. In addition, this vector also expresses the firefly luciferase gene for the normalization for transfection efficiency. Cells were also transfected with psiSTRIKE™ U6 vector constructs that express hairpin RNAs (shRNA) directed against *Renilla* luciferase, p53, or a nonspecific target. The shRNAs to *Renilla* luciferase and p53 should target the fusion *Renilla*-p53 mRNA expressed from the psiCHECK™-2/p53 Vector, reducing the mRNA levels and thus proteins levels, for *Renilla* luciferase. Two days following transfection, the cells were assayed for *Renilla* luciferase activity using EnduRen™ Live Cell Substrate or the Dual-Glo™ Luciferase Assay System, which measures firefly luciferase. Cell number was monitored using the CellTiter-Glo® Luminescent Cell Viability Assay.

The firefly luciferase and CellTiter-Glo® signals were similar for the nonspecific shRNA, *Renilla* shRNA, and the p53 shRNA as expected (Figure 1). However, the *Renilla* luciferase signal was dramatically reduced in those cells that expressed a shRNA directed against either *Renilla* luciferase or p53 as compared to the nonspecific shRNA. The *Renilla* signal measured by the EnduRen™ Substrate showed the same result (data not shown).

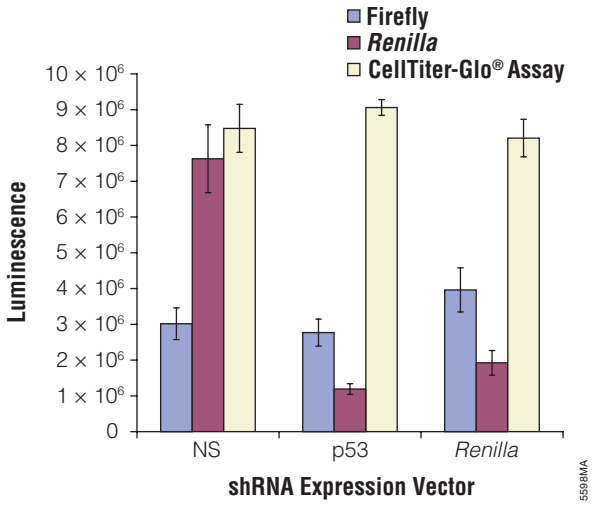

Figure 1. Detection of various luminescent reporter assays. The indicated luminescent reporter assays were used in 293T cells expressing psiCHECK™-2/p53 Vector and shRNAs to a nonspecific target (NS), p53, and *Renilla* luciferase. The results are the average of quadruplicate samples for each assay.

Cell-Based and Biochemical Assays for High-Throughput Compound Profiling

Using a panel of different assay types, it is possible to obtain a broad view of compound activity. Determining IC_{50} and EC_{50} dose responses offers more accurate drug potency data than single-point screenings.

In the article beginning on page 4, we describe the combination of Promega homogeneous screening assays and the Tecan Freedom EVO® system to create a high-throughput profiling system.

Miniaturized, High-Density Assays Meet the Demands of Ultrahigh-Throughput Screening

Many research groups are turning to high-density, 1,536-well plate formats as a way to generate larger, more relevant data sets. However, not all assays currently being used are amenable to such s low-volume format.

In the article beginning on page 7 of this issue, we use the BioRAPTR FRD™ Aurora Discovery workstation to demonstrate the easy miniaturization of Promega bioluminescent cell-based and biochemical assays. This combination of low-volume, non-contact dispensing instrumentation and Promega chemistries offers an excellent solution to the challenges and demands of ultrahigh-throughput drug discovery.

Discussion

This issue of *Promega Notes* includes examples of instrumentation that greatly facilitate high-throughput biology applications. In this article we show example systems that can help the traditional life science research laboratory begin the transition to automated biology. As life science research evolves from small-scale experimentation into a highly competitive big science endeavor, investigators will progress toward higher throughput instrumentation. This will require that investigators, instrumentation companies and biological reagent companies partner for success.

Reference

1. Stelzl, U. *et al.* (2005) *Cell* **122**, 957–68.