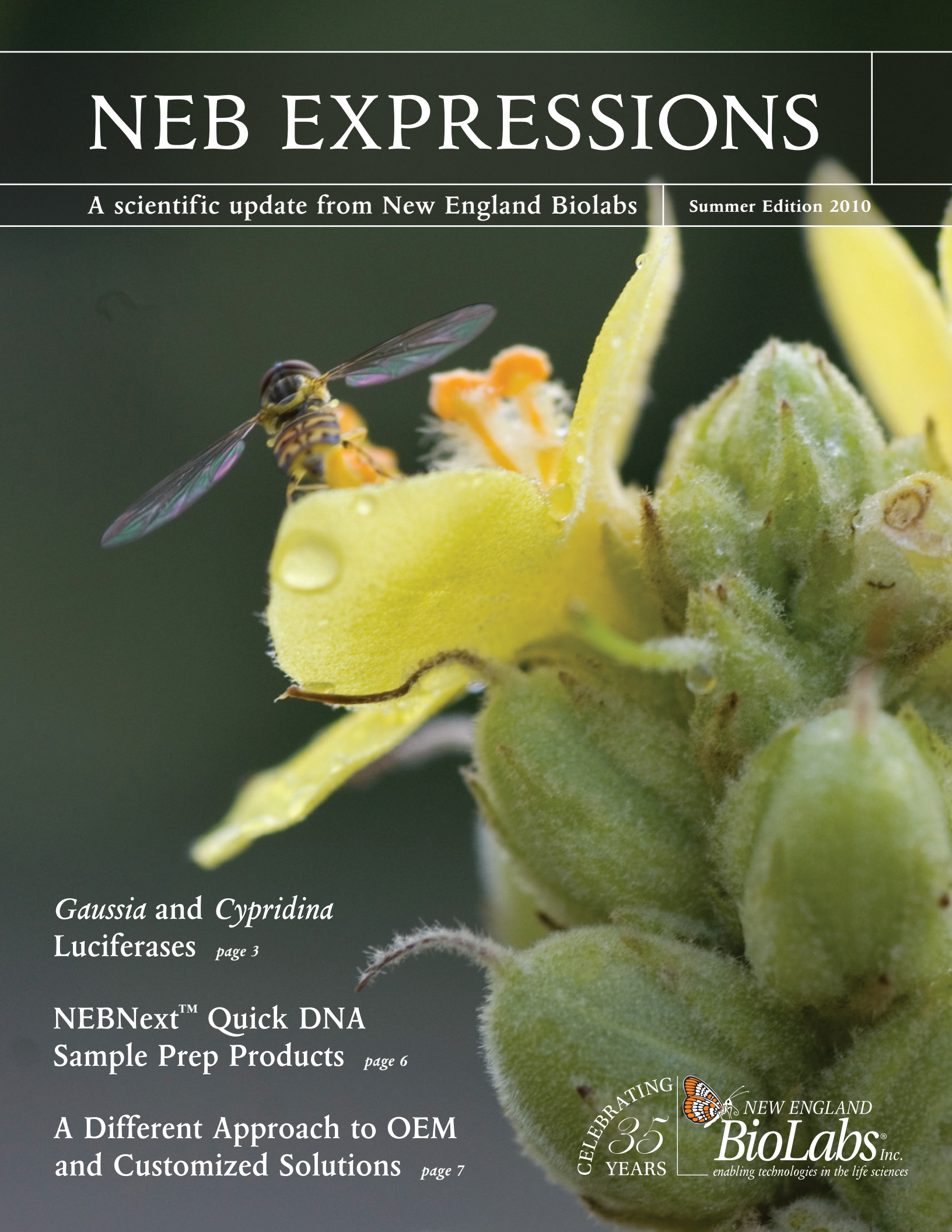
NEB EXPRESSIONS

A scientific update from New England Biolabs | Summer Edition 2010



CELEBRATING 35 YEARS

NEW ENGLAND BioLabs® Inc.

enabling technologies in the life sciences

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Upcoming Tradeshows

Visit the NEB booth at the following meetings:

- **American Association for Clinical Chemistry** (AACC) – July 25-29, 2010 – Anaheim, CA www.aacc.org
- CHI: **Evolution of Next-Generation Sequencing** (NGx) – September 27-29, 2010 – Providence, RI www.healthtech.com

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A Letter from NEB

Dear Researcher,

For 35 years, New England Biolabs has been committed to meeting the needs of our customers by providing exceptional quality products and technical support. Our focus on the advancement of science has helped to build relationships with our customers so that we may better understand their needs and develop the appropriate reagents to facilitate their research. This commitment applies both to the individual scientist and the OEM customer.

This issue highlights the utility of luciferases as reporter systems, and introduces two secreted luciferases with several unique advantages over existing systems. Also, we are pleased to introduce two new products to our growing line of NEBNext™ sample preparation reagents for next generation sequencing that will help to reduce both sample loss and prep time. NEBNext reagents are highly pure and undergo stringent quality controls, ensuring maximum yield, convenience and value.

Wishing you continued success in your research,

New England Biolabs

View of the NEB laboratory facility

35th Anniversary Offers

NEB would like to thank its customers for 35 years of support. Join us in celebrating our 35th anniversary by visiting **www.neb.com** to find 12 months of exciting offers, including significant product discounts and giveaways.

There are only three months left to take advantage of these offers! Thus far, customers have received significant discounts on HF restriction enzymes, T4 DNA Ligase, DNA ladders and competent cells, as well as prize drawings for iPOD Touches® and ice buckets filled with valuable NEB products.

Look for the 35th Anniversary Offers icon on our website to learn about the next monthly special offer.

As we celebrate our 35th year, share your thoughts or comments about your experience with NEB through our **online guestbook** by visiting http://nebiolabs.wordpress.com/

Gaussia and *Cypridina* Luciferases – Ultrasensitive secreted reporters and their use in dual assays

Scientists have long been interested in the biology of bioluminescent (i.e. light-emitting) organisms. Bioluminescence has evolved independently in a number of different phyla and is used by a wide range of marine and terrestrial organisms in behaviors as diverse as predator avoidance and sexual selection (1). Studies in this area led to the identification of multiple bioluminescent proteins and enzyme systems, some of which have found direct applications in biological research (2).

George Tzertzinis, Ph.D., Ana Egaña, Ph.D. and Cathy Shea, Ph.D., New England Biolabs, Inc.

Luciferases are enzymes that produce light as a by-product of the oxidation of specific substrates called "luciferins". This light emission can be easily detected and measured, making luciferases ideal for the development of biological reporter systems. The advantages of luminescence, over fluorescence, include the absence of background, the amplification of signal and a high dynamic range that spans many orders of magnitude. Since light emission depends strictly on the chemical reaction between the substrate and the luciferase, there is no background light originating from the sample. Futhermore, the turnover of the light reaction significantly amplifies the reporter signal. Luciferases have become indispensable for the study of fundamental cellular processes including the regulation of transcription and translation, mRNA and protein stability, and nucleic acid/protein interactions. They also serve as markers of cellular physiology and responses (2).

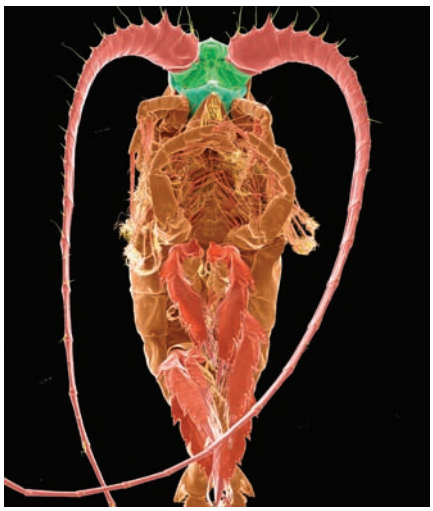
The first generation of luciferase reporters developed were based on Firefly Luciferase (FLuc). Insect luciferases, including *Photinus pyralis* (firefly), catalyze the oxidation of a benzothiazol luciferin requiring ATP as a cofactor. Later, a marine luciferase from the sea pansy *Renilla reniformis* was isolated. *Renilla* Luciferase (RLuc), like most marine luciferases, uses a different luciferin, the benzylimidazo-pyrazinone coelenterazine. This feature allows measurement of RLuc activity from the same samples expressing FLuc.

Although both of these intracellular luciferases are widely used in life science research, they have the disadvantage of requiring lysis of the expressing cells in order to measure their activity. This becomes a disadvantage in many types of experiments. For example, measurement of activity at different time points involves experimental design with large numbers of wells and large amounts of reagents. Additionally, cell lysis precludes additional downstream procedures.

Recently, a new generation of bioluminescent reporters with unique characteristics that expand the flexibility and applications of luciferase reporter systems has become available. The secreted luciferases derived from *Gaussia princeps, Metridia longa* and *Cypridina noctiluca* do not require cell lysis to measure activity and are significantly "brighter" than firefly and *Renilla.* These features make these luciferases ideal for repeated assays, sensitive measurements in miniaturized assays and single cell applications.

Gaussia Luciferase Naturally secreted from the deep sea copepod *Gaussia princeps* (3) (Figure 1), *Gaussia* Luciferase (GLuc) was first used in a sensitive analytical assay in 2002 (4) and later commercialized by NEB for expression in mammalian cells. GLuc is a monomeric enzyme of only 185 amino acids (19 kD), and is the smallest known luciferase. It catalyzes the oxidative decarboxylation of coelenterazine to produce coelenteramide

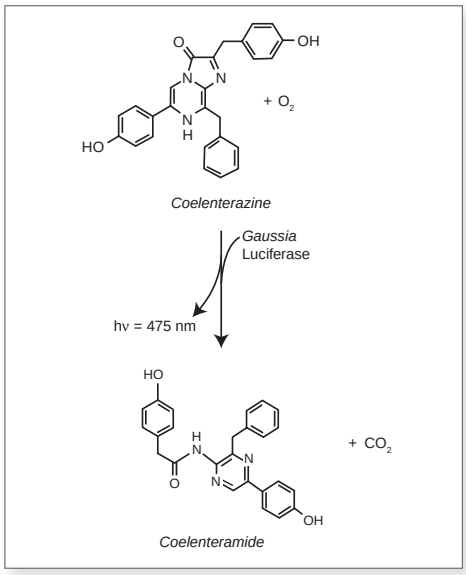
Figure 1: The copepod *Gaussia princeps*



Electron micrograph courtesy of Microangela

via an excited state intermediate, which upon relaxation to the ground state emits blue light (475 nm) (Figure 2). The catalytic properties of GLuc make it an extremely sensitive detection reporter. Recombinant GLuc has been shown to

Figure 2: The Photo-oxidation catalyzed by *Gaussia* Luciferase



Haddock, S.H.D., McDougall, C.M. and Case, J.F., The Bioluminescence Web Page, http://lifesci.ucsb.edu/~biolum/ (created 1997; updated 2005).

produce the highest number of photons per mole of any luciferase (5). The first report describing the utility of GLuc in *in vivo* imaging demonstrated superior sensitivity over *Renilla* and Firefly by several orders of magnitude. Despite the fact that secretion causes its diffusion in the locality of the tissue that express it, the secreting cells were readily detectable due to this high sensitivity (6).

The robust secretory signal, small size and sensitivity of GLuc, as compared to standard bioluminescent reporters, give GLuc some unique advantages in many assay systems. This is illustrated in the following examples.

The native secretion signal of GLuc has been shown to be functional in every eukaryotic system tested so far, from algae and nematodes to mice and mammalian cells (7,8). Functionality is dependent on the ER-Golgi pathway for proper folding and secretion. Thus the GLuc assay provides a fast, quantitative and sensitive technique to monitor the secretory pathway and ER stress (9,10).

The small size of *Gaussia* is favorable for achieving fusions with heterologous proteins without detrimental effects to either partner. Capul and de la Torre developed an elegant assay for viral infectivity using such a fusion. GLuc activity in the culture medium reflected the budding activity of the virus and the system was used for antiviral agent screening (11). In a different study, a chemokine-GLuc fusion was used to monitor interactions with its receptor by measuring cell-associated luciferase activity (12). Notably, the receptor was activated by the chemokine-GLuc chimera in a manner indistinguishable from the unfused chemokine. In both of these examples, the secretion signal was contributed by the fusion partner.

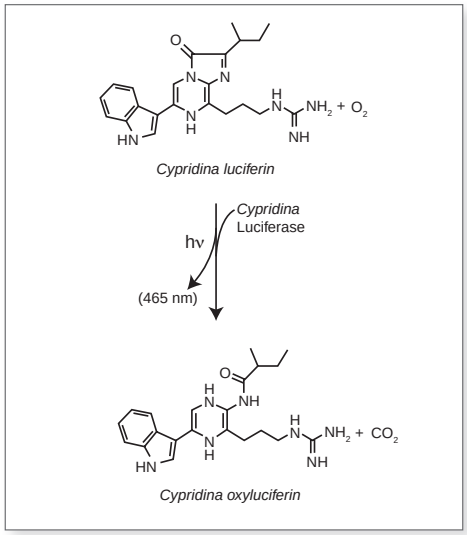
In research performed at NEB, scientists used *Gaussia* Luciferase activity secreted from cells subjected to RNAi, as a surrogate for cytoplasmic mRNA levels. The siRNA target sequence was introduced in the 3´-UTR of GLuc and the GLuc activity reflected the degree of silencing and the relative potency of the siRNAs tested (13). Despite the indirectness of the assay, reporter activity correlated well with RT-qPCR measurements of the mRNA levels, making the siRNA potency assay rapid, convenient and amenable to higher throughput formats.

Most reporter assays benefit by measuring a different reporter from the same experimental sample as a control. In the NEB study mentioned above, two additional reporters were measured from the cell lysates: beta-galactosidase and Firefly Luciferase. In a different study, GLuc was combined with Firefly Luciferase (FLuc) to study microRNA biogenesis (14). FLuc was used to monitor the activity of the miR-23 microRNA promoter, while GLuc was used to monitor the silencing activity of the mature microRNA. Although GLuc can also be measured in cell lysates, firefly, beta-galactosidase and *Renilla* activity can not be measured in the supernatant, making an additional secreted luciferase reporter desirable.

Cypridina Luciferase: a second secreted reporter The first luciferase from *Cypridinae* ostracods was isolated from *Vargula hilgendorfii* and characterized several decades ago (15). This luciferase oxidizes a luciferin known as Vargulin, which is another common substrate of marine luciferases. Problems with substrate stability and the cost of production, however, precluded its wider application in analytical screening and cell-based applications. In 2004, a luciferase from a related species, *Cypridina noctiluca,* was reported to offer some advantages as a secreted reporter in mammalian culture medium (16). Furthermore, the development of synthetic approaches to produce *Cypridina* substrates resistant to rapid oxidation, helped establish this system as optimal for bioluminescent assays including reporter gene studies, live cell monitoring and BRET applications *in vivo*.

Cypridina Luciferase (CLuc) shares many of the features of *Gaussia* Luciferase, including secretion, emission of large amounts of light and high protein stability. Both GLuc and CLuc are inherently stable due to the presence of multiple disulfide bonds (4 and 17, respectively). This feature confers significant stability at high temperatures, making them useful for applications where other luciferases are rendered inactive. For example,

Figure 3: The photochemical reaction catalyzed by *Cypridina* Luciferase

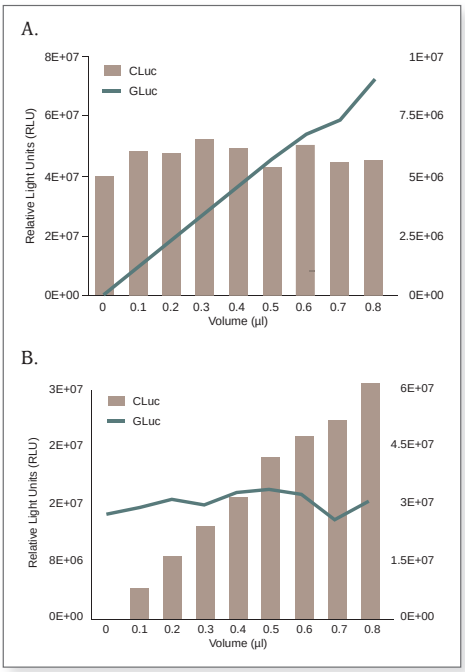


Adapted from Nakajima, Y. et al. (16)

both proteins maintain activity at 55°C, a treatment that inactivates most viruses, or in the presence of 0.1 mM β-mercaptoethanol, a component of mouse embryonic stem cell culture media. In practice, this high stability allows the safe storage of luciferase containing extracts for later (re)measuring without any loss of activity.

Perhaps the most compelling aspect of the CLuc reaction is its compatibility with GLuc in

Figure 4: GLuc or CLuc activity can be measured without interference from the presence of the other luciferase

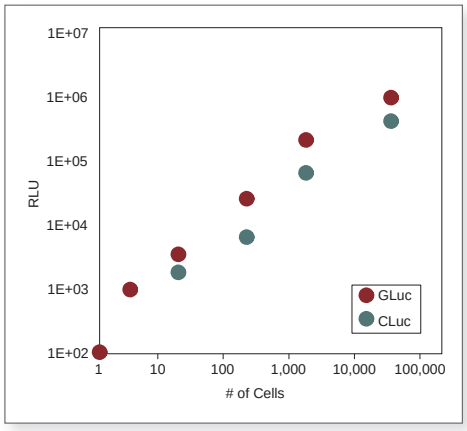


(A) Supernatant from CLuc-expressing cells was held constant while the volume of supernatant from GLuc cells was increased. (B) Supernatant from GLuc-expressing cells was held constant while the volume of supernatant from CLuc-expressing cells was increased. Activity was measured with the corresponding BioLux Assay System.

reporter assays, since GLuc and CLuc utilize two different substrates (Figure 2,3). The detection of one luciferase can be monitored without any cross-reactivity from the presence of the other in the same experimental sample (Figure 4). When expressed in mammalian cells, GLuc and CLuc are both secreted into the growth media, allowing the user to assay both luciferase activities from the same sample, multiple times over the course of an experiment. The use of these two secreted luciferase reporters in a dual secreted luciferase assay was first reported in 2007. This study demonstrated the activation of the promoter of the clock protein Bmal1, by the transcription factor RORa4 (17).

The ability to measure the activity of these ultra-sensitive secreted luciferases from the same cells at different timepoints was featured in a study by Watanabe, et al (18). These investigators offered an excellent example of this property by showing that the cycling activity of clock genes could be monitored by continuously secreted GLuc and CLuc reporters over several days (18).

Figure 5: Detection of low numbers of cells expressing GLuc and CLuc



The indicated number of cells expressing GLuc or CLuc was diluted with untransfected CHO cells and cultured in a 24-well plate format for 1 day. A 20 µl sample from each well was assayed for each luciferase.

Summary New England Biolabs now offers two compatible, secreted luciferases which can be used alone or together as a dual system. *Gaussia* Luciferase and *Cypridina* Luciferase are the brightest commercially available luciferases, enabling the user to detect activity in small samples or from very low expression (Figure 5). The non-destructive nature of these ultrasensitive assays allows the cells to remain intact so they can be used in further downstream assays including RT-PCR, Western blots, RNA expression analysis, live imaging, cell viability assays, etc. Their compatibility, secretion, sensitivity and stability, make GLuc and CLuc ideal for routine *in vitro* and *in vivo* reporter applications, as well as high throughput assays.

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Gaussia and *Cypridina* Luciferase products available from NEB

PRODUCT	NEB #	FEATURES/PROPERTIES
CONTROL PLASMID/VECTOR		
pGLuc-Basic	N8082S	Promoterless vector for promoter evaluation and screening
pGLuc Mini-TK	N8086S	Minimal promoter for promoter or enhancer screening
pCMV-GLuc	N8081S	Constitutive promoter, high expression vector
pTK-GLuc	N8084S	Constitutive promoter, medium expression vector
pCLuc-Basic 2	N0317S	Promoterless vector for promoter evaluation and screening
pSV40-CLuc	N0318S	Constitutive promoter, high expression vector
ASSAY KITS		
BioLux™ *Gaussia* Luciferase Assay Kit	E3300S/L	Contains reagents for assaying GLuc activity
BioLux™ *Gaussia* Luciferase Flex Assay Kit	E3308S/L	Reagents for GLuc activity include a more concentrated substrate and stabilizer; ideal for high throughput screening
BioLux™ *Cypridina* Luciferase Assay Kit	E3309S/L	Contains reagents for assaying CLuc activity
BioLux™ *Cypridina* Luciferase Starter Kit	E3314S	Contains the reagents for assaying CLuc activity, as well as cloning and control CLuc encoding plasmids

Advantages

- **Naturally secreted** – Amenable to live cell assays
- **Sensitivity** – Brightest luciferases available; enable single cell applications
- **Stability** – Samples can be stored for several days with no loss of activity
- **Easy-to-use** - Cell lysis not necessary
- **Non-destructive** - Living cells can be used in downstream assays
- **Flexible** - Activity can also be measured in cell lysates

NEBNext™ Quick DNA Sample Prep Reagent Set 2 & Master Mix Set 2

The NEBNext™ Quick DNA Sample Prep Reagent Set 2 provides all the enzymes and buffers required for fast fragment DNA library preparation appropriate for Titanium sequencing on the GS FLX™ and GS Junior™, as well as making expression libraries. Starting with only 0.5 µg of fragmented DNA, this protocol enables DNA end repair, dA-tailing, and adaptor ligation in a single tube, in less than two hours, with no column purifications between the steps. Elimination of these purification steps significantly reduces both sample loss and prep time. After adaptor ligation, the correct fragment size is isolated using the provided NEBNext Sizing Buffer with Agencourt® AMPure® beads (not included).

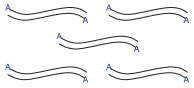
The NEBNext Quick DNA Sample Prep Master Mix Set 2 provides all the components included in the Quick DNA Sample Prep Reagent Set 2, but in a more convenient format. The reagents are condensed into one enzyme vial and one buffer vial per step, substantially reducing both the number of individual components and the required pipetting steps.

Advantages

- **Convenient formats** – All of the required enzymes, buffers and nucleotides are included, and are available in set or master mix format.
- **Functional validation** – Each reagent set or module is functionally validated by preparation of a genomic DNA library that is then sequenced using the Roche/454 GS FLX Titanium™ and by preparation of an expression library.
- **Stringent quality controls** – Additional QCs ensure maximum quality and purity.
- **Value pricing**

*Visit **nebnext.com** for more information and to learn about additional NEBNext products available.*

NEB Quick DNA Sample Preparation for 454

Fragmentation	End Repair/dA Tailing	Adapter Ligation	Size Selection
			
454 Rapid Genomic DNA Sample Prep Reagents			
• Nebulizer	• RL T4 DNA Polymerase • RL PNK • RL *Taq* DNA Polymerase • RL dNTP • RL ATP • RL 10X PNK Buffer	• RL Ligase • RL Adaptor	• Sizing Solution • TE Buffer
Available separately	**NEBNext Quick DNA Sample Prep Reagent Set 2**		
• dsDNA Fragmentase™	• T4 DNA Polymerase • T4 PNK • *Taq* DNA Polymerase • dNTPs • ATP • NEBuffer 2	• Quick T4 DNA Ligase	• NEBNext Sizing Buffer • TE Buffer
Available separately	**NEBNext Quick DNA Sample Prep Master Mix Set 2**		
• dsDNA Fragmentase™	• End Prep Enzyme Mix • End Repair Reaction Buffer	• Quick T4 DNA Ligase	• NEBNext Sizing Buffer • TE Buffer

Ordering Information

PRODUCT	NEB #	SIZE
NEBNext Quick DNA Sample Prep Reagent Set 2	E6080S/L	10/50 reactions
NEBNext Quick DNA Sample Prep Master Mix Set 2	E6090S/L	10/50 reactions

A Different Approach to OEM and Customized Solutions

John Pelletier, New England Biolabs, Inc.

As research in the life sciences industry continues to accelerate, so does the level of complexity and sophistication of the technologies driving it. In today's economic climate many companies face the challenge of keeping pace with product development for these advanced technologies. Often this involves focusing on core strengths while leveraging the capabilities of strategic partners.

With a 35-year commitment to basic research, New England Biolabs was founded on the principle of serving its customers "scientist to scientist." While NEB remains a world leader in the screening, design, development and production of molecular biology tools, it is also a valued and experienced collaborator and innovator. As ideas transition from benchtop to application, the depth of resource that NEB provides in furthering these technologies becomes increasingly relevant.

What is OEM? OEM, or "original equipment manufacturer", is a widely-applied term borrowed from the computer industry to define commercial relationships, whereby a supplier produces components to customer-defined specifications. Long recognized for its service to the scientific community, NEB also has a deep history of providing the life sciences industry with products and solutions on an OEM basis. NEB is not only a trusted source of key reagents, but also a source of ideas and solutions that contribute to the advancement of our customers' technologies. Examples of NEB's capabilities include protein engineering and optimization, protein fusion or modification, customized enzyme combinations, novel solutions for molecular manipulations, and custom formulations to meet specific reagent needs. Also, NEB has a large portfolio of non-commercialized enzymes, which can be screened for properties that meet particular customer needs.

A view of the tangential flow filtration unit situated in the purification process development laboratory.

Although other organizations often highlight similar OEM core capabilities, what differentiates NEB is its continuous focus on what is most important to the customer. Once our research and development teams have fully developed a reagent for integration into a platform, NEB then has the experience, capability and expertise to exceed customer requirements for scale, quality management and risk mitigation. Working with the OEM customer is a true collaboration with various departments within NEB, including Research, Production, Packaging, Business Development, Legal and Shipping/Logistics. Dedicated OEM staff facilitate relationships with each of these departments to address the critical needs of each customer, ensuring speed, flexibility and sound supply chain management.

A true collaboration. One example of NEB's approach to customized solutions and OEM began when NEB's Research team was approached by developers at a large diagnostics company with a novel DNA amplification technology to be applied in the detection of human diseases. NEB was chosen for its extensive collection of novel enzymes and its ability to optimize polymerases for unique applications. Initially, the platform utilized mesophilic enzymes; NEB provided two viable candidates from its existing portfolio, which were used in the company's initial development and proof-of-principle. However, a switch to thermophilic conditions was requested to enable greater specificity and robustness. NEB met the challenge, and through a close collaboration with the customer, quickly screened and offered a restriction enzyme that met the new demands of the platform. However, the system also required a polymerase with equally demanding specifications. An optimized polymerase was developed using one of NEB's proprietary expression systems, and this unique combination of restriction enzyme and polymerase allowed the customer to meet their development objectives. NEB then committed the resources necessary to accelerate improvements in expression and scale, assure the commercial viability of the platform, and meet critical timelines. As the reagents moved into production, additional quality assurance measures and stringent release criteria were implemented. NEB worked closely with the customer to produce multiple lots of product to assure stability and reproducibility of process and implement a series of stability studies. Later, to leverage the company's in-house cGMP manufacturing capabilities, a technology transfer was conducted, giving the customer direct control over manufacturing.

Throughout the process, NEB worked closely with the customer in the design and build-out of their manufacturing site, the training of staff, validation of the customer's processes and QA of lots manufactured in these facilities. This collaboration is just one example of NEB's commitment to its customer during all phases of product development.

For more information of customized solutions and OEM capabilities at NEB, contact ***oem@neb.com****.*

Our Custom Solutions experts are ready to work with customers in these areas:

Research & Development
- Access to accomplished R&D staff for collaborative product improvement
- Customer specific product formulations and specifications, including customer-defined quality control assays

Logistics & Risk Mitigation
- Custom packaging options including vial and label specifications, and private brand labeling for "drop-in" kit components
- Audits of NEB facility by regulatory staff
- Long-term supply agreements that include reserve stock level information
- Value-oriented supply and pricing terms
- Flexible delivery schedules
- Highly developed risk mitigation procedures
- Confidentiality agreements

Distribution
- Access to extensive global logistics through a well supported international network

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