

# NEB expressions

a scientific update

## in this issue

- 2 Development of a high-throughput data analysis method for quantitative real-time PCR (qPCR)
- 6 Introducing Luna<sup>®</sup> Universal Products for qPCR & RT-qPCR
- 8 NEBuilder<sup>®</sup> HiFi DNA Assembly – How does it compare to In-Fusion<sup>®</sup> HD?
- 10 Learn about the benefits of NEB<sup>®</sup> Golden Gate Assembly Mix
- 11 Meet the 2016 Passion in Science Awards<sup>®</sup> winners

Winter sunset on the  
New England Biolabs  
campus, Ipswich, MA



be **INSPIRED**  
drive **DISCOVERY**  
stay **GENUINE**

# Development of a high-throughput data analysis method for quantitative real-time PCR (qPCR)

by Gregory C. Patton, Ph.D., Andrew N. Gray, Ph.D., Nathan A. Tanner, Ph.D., Janine G. Borgaro, Ph.D., Yan Xu, Ph.D., Julie F. Menin, M.S. & Nicole M. Nichols, Ph.D., New England Biolabs, Inc.

Over the last 20 years, quantitative real-time PCR (qPCR) has become an essential technique in molecular biology for detecting and quantifying nucleic acids. Workflow simplicity and advances in instrumentation now permit sizeable quantities of data to be generated rapidly, with 96, 384, or even 1536 reactions in one qPCR experiment. The challenge lies in the details: qPCR experiments require thoughtful design and analysis to capture all relevant information, such that accurate and appropriate conclusions can be drawn.

Development of NEB's [Luna<sup>®</sup> qPCR](#) product line required repeated data collection on a series of test panels, each containing multiple targets. It became clear during early development that a more scalable approach to data analysis and visualization was required to better understand how changes in reagent composition impacted performance. In order to compare various amplicon panels over multiple qPCR runs, instruments, reagents and conditions, a high-throughput data analysis method termed "dots in boxes" was developed. The output of this analysis captures key assay characteristics, highlighted in MIQE guidelines, as a single data point for each qPCR target. This method of analysis permits multiple targets and conditions to be compared in one graph, allowing concise visualization and rapid evaluation of overall experimental success.

## INTRODUCTION TO qPCR

qPCR is a powerful fluorescence-based technique that detects and quantifies nucleic acids in a variety of samples. In 1992, Higuchi et al. showcased the first example of real-time PCR by using a camera during the amplification reaction to continuously monitor the incorporation of ethidium bromide, an intercalating dye that fluoresces in the presence of double-stranded DNA under ultraviolet light (1). Currently, most qPCR experiments commonly employ the dsDNA intercalating dye SYBR<sup>®</sup> Green I or hydrolysis probes (e.g., TaqMan<sup>®</sup>) to monitor amplification (2). Plotting the measured fluorescence signal versus PCR cycle number results in a graphical representation of amplification. The point at which the fluorescence signal exceeds the background fluorescence level is known as the quantification cycle ( $C_q$ ). Comparing  $C_q$  values permits evaluation of relative target abundance between two or more samples. Alternatively,  $C_q$  values can be used to calculate absolute target quantities via reference to an appropriate standard curve, derived from a series of known DNA

or RNA dilutions. This technique can be more powerful than traditional PCR, allowing both qualitative information (presence or absence of a target sequence), as well as the quantitative data (nucleic acid quantity) to be determined without opening the reaction tube. Greater sensitivity and lower risk of carryover contamination has resulted in qPCR replacing end-point PCR in many applications. Today, the technique is used in a variety of fields, from molecular diagnostics to agricultural research, and in applications including mutation detection, genotyping, copy number variation and gene expression analysis.

## MIQE GUIDELINES

Rapid adoption of qPCR and its relatively straightforward execution (mixing amplification reagents, primers and template) has led to the generation of an enormous amount of data, as evidenced by the numerous publications containing qPCR experiments. However, the ease of generating qPCR data has also proven to be the technique's greatest challenge (3). A diverse set of protocols, instruments, reagents and analysis methods can be found in the scientific literature, with many publications reporting invalid or conflicting data. The lack of consensus on best experimental practices for qPCR resulted in the establishment of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines by Bustin et al. (4).

The MIQE guidelines established a set of qPCR performance metrics that should be determined and reported in peer-reviewed publications to ensure robust assay performance and reproducibility. These assay characteristics include:

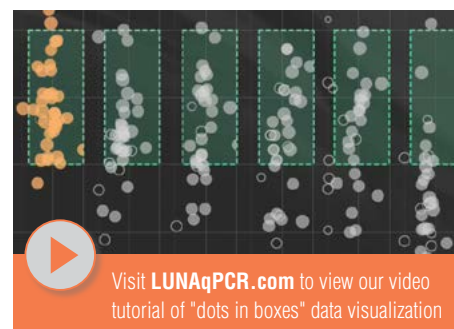
- PCR efficiency
- Dynamic range
- Limit of detection (LOD)
- Target specificity
- Precision

One of the most important assay characteristics is PCR efficiency, which is a measure of product duplication at every amplification cycle. PCR efficiency is measured by amplifying multiple known concentrations of nucleic acid to obtain  $C_q$  values for each concentration. A standard curve is created by plotting the observed  $C_q$  values on the y-axis and the  $\log_{10}$  of the template concentration on the x-axis. Efficiency is calculated using the equation: PCR efficiency =  $10^{-1/\text{slope}} - 1$ . A slope of -3.32 represents 100% PCR efficiency and indicates doubling of the target amplicon at each PCR cycle.

The dynamic range establishes the upper and lower limits for quantification and should be linear for at least three  $\log_{10}$  concentrations of template. Preferably, the dynamic range encompasses five to six orders of magnitude. Linearity over a dynamic range is reported by the  $R^2$  coefficient of determination for the  $C_q$  values linear fit to the standard curve.

The limit of detection is often defined as the lowest concentration at which 95% of target sequences are detected in positive samples. An ideal Poisson distribution and single copy detection dictate the lowest theoretical LOD is 3 molecules per PCR. Its determination establishes the lower boundary for target detection with 95% confidence (5).

Target specificity should be confirmed by product size, sequencing or melt curve analysis, since primers may unexpectedly amplify off-target regions. In addition, some primer sets have a propensity to form primer dimers during amplification, resulting in inaccurate quantification or false positive results. In order to identify spurious amplification products, no-template controls (NTC) should be included in every qPCR run. As NTCs can identify both unintended amplification products as well as contamination, criteria should be established for using these controls to determine when data should be accepted or rejected.



Visit [LUNAqPCR.com](http://LUNAqPCR.com) to view our video tutorial of "dots in boxes" data visualization

The last factor that should be evaluated is assay precision. Multiple replicates of the same sample should typically have high concordance. Variation inherently increases as the copy number decreases, but also can be attributed to factors such as pipetting errors and instrumentation.

## DOTS IN BOXES ANALYSIS OF qPCR DATA

The MIQE-highlighted metrics described above served as a guide for evaluating reagent performance during development of NEB's new Luna qPCR and RT-qPCR product line. To ensure strong performance across a range of amplicons, multiple test panels were created, with each panel containing a minimum of five targets that could be run in 96 or 384-well formats. Panels comprised of gDNA and cDNA targets were used to evaluate DNA-based qPCR master mixes, whereas RNA targets of varying abundance were used to assess RT-qPCR reagents. In general, targets spanned typical qPCR amplicon lengths (~70 to



TABLE 1: Criteria for developing quality score metrics for dots in boxes analysis

	Intercalating Dye Chemistry	Hydrolysis Probe Chemistry
<b>Linearity</b>	$R^2 \geq 0.98$	$R^2 \geq 0.98$
<b>Reproducibility</b>	Replicate curves shall not vary by more than 1 $C_q$ .*	Replicate curves shall not vary by more than 1 $C_q$ .*
<b>RFU Consistency</b>	Maximum plateau fluorescence signal for all curves shall be within 20% of the mean. Fluorescence signal shall not be jagged.	Increase of fluorescence signal shall be consistent for all curves, exhibiting parallel slopes. Fluorescence signal shall not be jagged.
<b>Curve Steepness</b>	Curves shall rise from baseline to plateau within 10 $C_q$ values or less.	Curves shall rise from baseline to 50% maximum RFU within 10 $C_q$ values or less.
<b>Curve Shape</b>	Curves shall exhibit a sigmoidal shape, resulting in a plateau of fluorescence signal.	Curves need not be sigmoidal, but shall appear to be reaching a horizontal asymptote by the last PCR cycle.

\* At extremely low input (e.g., single copy), the lack of amplification due to the Poisson distribution is taken into consideration.

200 bp), as well as GC content (~40 to 60%). Given the large data set that was created during development, data mining to decipher what changes impacted performance became challenging, and it was clear that a better, more scalable approach to data visualization was needed.

The fundamental performance criteria outlined in the MIQE publication therefore served as a basis for the development of a high-throughput data analysis method termed "dots in boxes" (Figure 1). For each amplicon, PCR efficiency, dynamic range, target specificity and precision was captured as a single data point plotted in two dimensions, with the PCR efficiency plotted on the y-axis and the delta  $C_q$  ( $\Delta C_q$ ) as the x-axis.  $\Delta C_q$  is the difference between the  $C_q$  values of the NTC and the lowest template dilution. Setting guidelines around the typical accepted values for these two plotted parameters (PCR efficiency of 90 to 110% and  $\Delta C_q$  of 3 or greater) created a graphical box, highlighting where successful qPCR experiments (dots) should fall.

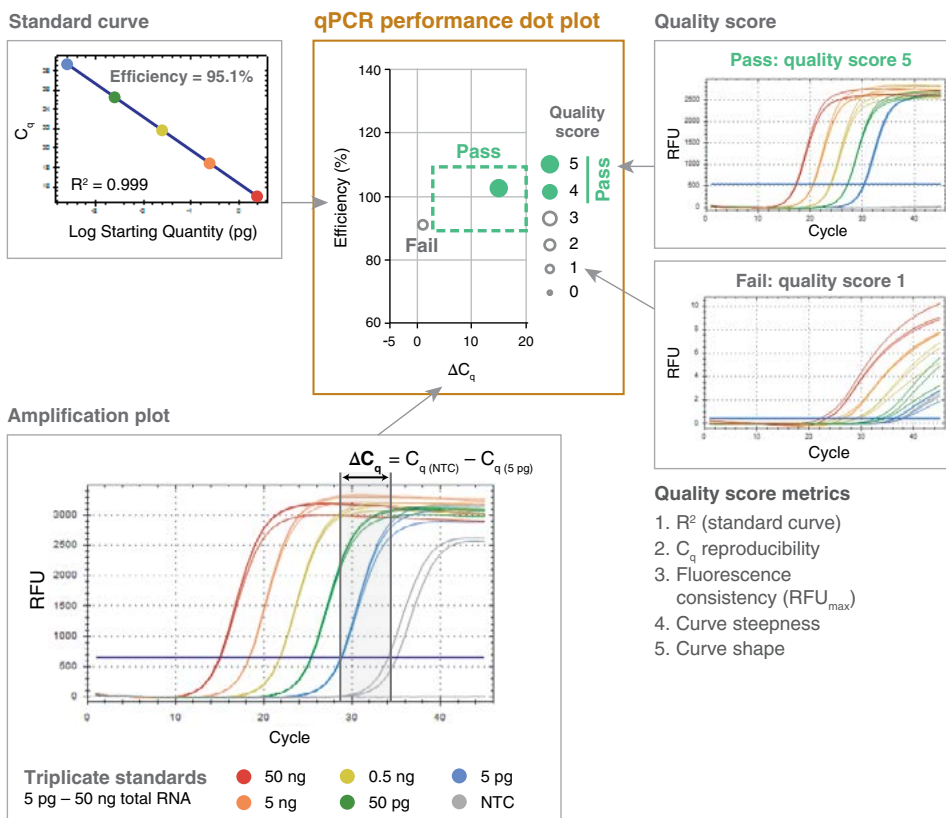
While this simple dot plot was informative on its own, it wasn't sufficient to capture all of the relevant details of each qPCR experiment. In order to represent additional information, such as the linearity of the dynamic range ( $R^2$ ), the overall quality of the qPCR data was scored on a scale of 1 to 5, with 5 representing the highest quality. This scoring method was built upon previous work by Hall et al. (6). Additional performance criteria captured using the 5-point quality score included precision (reproducibility), fluorescence signal consistency, curve steepness and sigmoidal curve shape. Parameters for these five criteria were established to identify when the quality score should be penalized. Scoring criteria differed slightly for probe-based chemistry compared to intercalating dye-based detection (Table 1) due to differences in typical curve shape.

Once assigned, the quality score for each amplicon was represented by the dot size and opacity. The higher the quality score, the larger the dot. Additionally, quality scores of 4 and 5 were represented as solid dots while a score of 3 or less was captured as an open circle for simple visual screening of performance. Amplicons falling in the box and receiving a quality score of 4 or 5 represented high quality, reliable qPCR data. The dots in boxes method allowed multiple targets and conditions to be plotted on



FIGURE 1: Breaking it down: how we translate qPCR data into dots in boxes

NEB has developed a method to better evaluate the large amount of qPCR data generated in an experiment. The output of this analysis is known as a dot plot, and captures the key features of a successful, high-quality qPCR experiment as a single point. This method of analysis allows many targets and conditions to be compared in a single graph. For each experiment, triplicate reactions are set up across a five-log range of input template concentrations (Amplification plot, bottom-left). Three non-template control (NTC) reactions are also included, for a total of 18 reactions per condition/target. Efficiency (%) is calculated (Standard curve, top-left) and is plotted against  $\Delta C_q$  (dot plot, center), which is the difference between the average  $C_q$  of the NTC and lowest template dilution. This parameter captures both detection of the lowest input and non-template amplification. Acceptable performance criteria are defined as an Efficiency of 90-110% and a  $\Delta C_q$  of  $\geq 3$  (green box). Other performance criteria are captured using a 5-point Quality Score (top-right). Quality Score is represented by the size and fill of the plotted dot, with experiments that pass all performance criteria represented by a solid dot within the box.



a single graph and compared quickly, creating an efficient, high-throughput visual method for data analysis.

To rigorously test qPCR performance, experiments were designed to simultaneously evaluate efficiency over a broad dynamic range of input concentrations; sensitivity by assessing low-input detection; and specificity by assessing off-target amplification. To accomplish this, qPCR efficiency was measured over a five-log dilution of template with data collected in triplicate for each dilution and a NTC. For genomic targets, an average of ~2 copies per reaction was routinely tested to assess the limits of low input detection. Since the  $\Delta C_q$  incorporates both the  $C_q$  of the lowest input and that of the NTC ( $\Delta C_q = C_{q(\text{NTC})} - C_{q(\text{lowest input})}$ ), it allows sensitivity and specificity to be captured in a single variable. Inability to amplify the lowest template dilution results in a  $\Delta C_q$  of 0 in most cases, since curves failing to cross the threshold are automatically given a  $C_q$  value corresponding to the total number of amplification cycles. The presence of non-specific or contaminating amplification in NTC reactions also reduces the  $\Delta C_q$ , such that either lack of low-input amplification or excessive off-tar-

get amplification can push the  $\Delta C_q$  below the passing ( $\geq 3$ ) threshold. Target specificity was also evaluated using denaturation or melt curves for all intercalating dye-based qPCR assays, although this information was not captured in the dot plot.

Pairing dots in boxes with an existing custom laboratory information management system (LIMS) permitted the performance of reagents to be screened and tracked on all amplicon panels. The LIMS, previously established for the development of NEB's Q5<sup>®</sup> High-Fidelity DNA Polymerase products, was modified to capture all relevant experimental details. The database connected results from each qPCR experiment (e.g.,  $C_q$  values, PCR efficiency, and linearity) to the contents of each well in that experiment (e.g., target, template concentration, primer concentration, qPCR master mix, additives, etc.) such that performance could be linked to reaction variables and conditions. Additional details including the operator, real-time PCR instrument ID, and cycling conditions were also recorded. Tableau<sup>®</sup>, an analytics software package, was used to analyze the data and to create graphical displays of the dot plots. An example outcome is

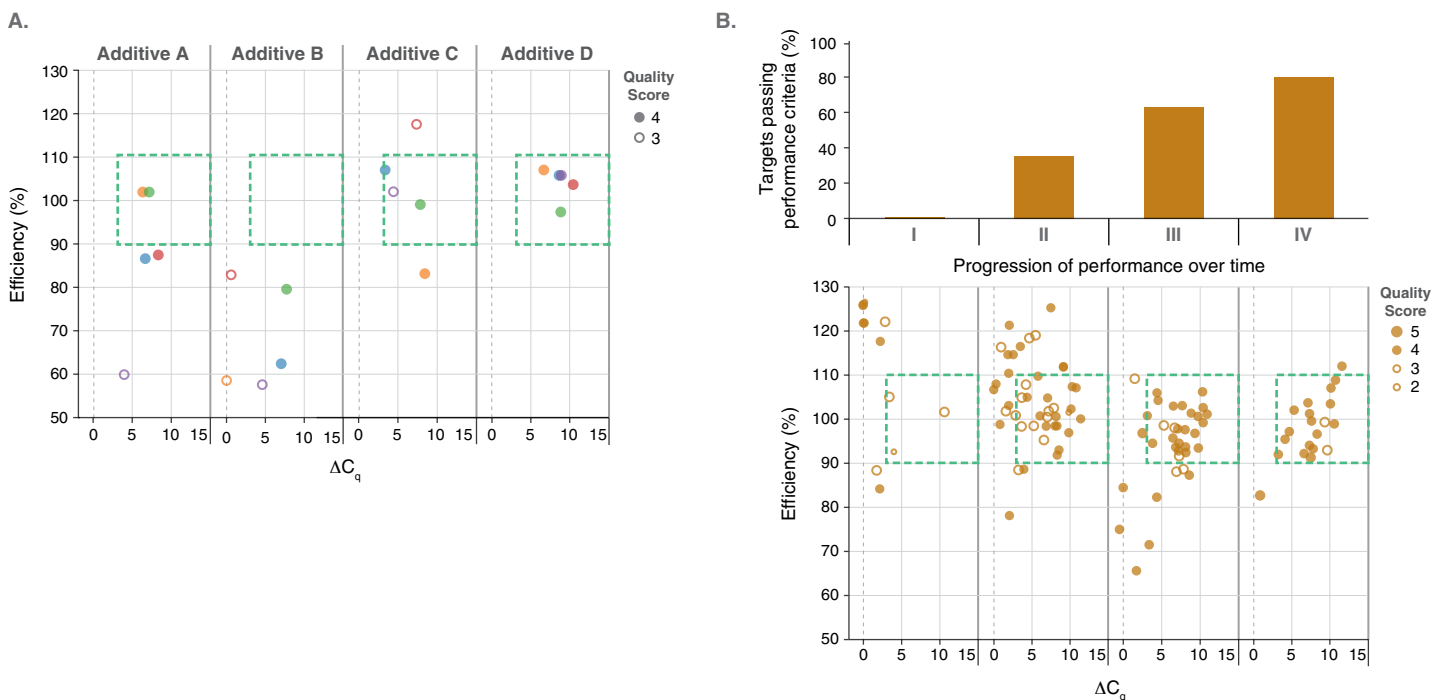
shown in Figure 2A. Here, the impact of known PCR additives and the concentration ranges that were beneficial to performance were quickly assessed on a development lot of the DNA dye-based master mix. Additive D resulted in the best performance on this particular panel of five amplicons. Unfortunately, improved performance on one particular qPCR panel did not necessarily translate to positive performance across all panels evaluated. Thus, the development process was by necessity methodical and iterative. This made the ability to analyze and visualize large sets of results, covering multiple test panels, formulations and experimental conditions, all the more crucial. Dots in boxes thus played a critical role in the development of NEB's Luna products, driving reagent optimization by quickly identifying compositions with increased performance across all test panels. Successful compositions were built upon and fine-tuned, progressively improving the percentage of amplicons that fell in the box with high quality scores (Figure 2B), and thus overall performance. As a result, the final Luna qPCR formulations exhibit robust performance on diverse targets from a wide range sample types and sources.



**FIGURE 2:**  
**Dots in boxes enables visual screening of reagent optimization**

Data was collected for qPCR targets varying in length and GC content, using Jurkat genomic DNA as input. Results were evaluated for efficiency, low input detection and lack of non-template amplification (where  $\Delta C_q = \text{average } C_q \text{ of non-template control} - \text{average } C_q \text{ of lowest input}$ ). In addition, consistency, reproducibility and overall curve quality were assessed (Quality Score, Table 1).

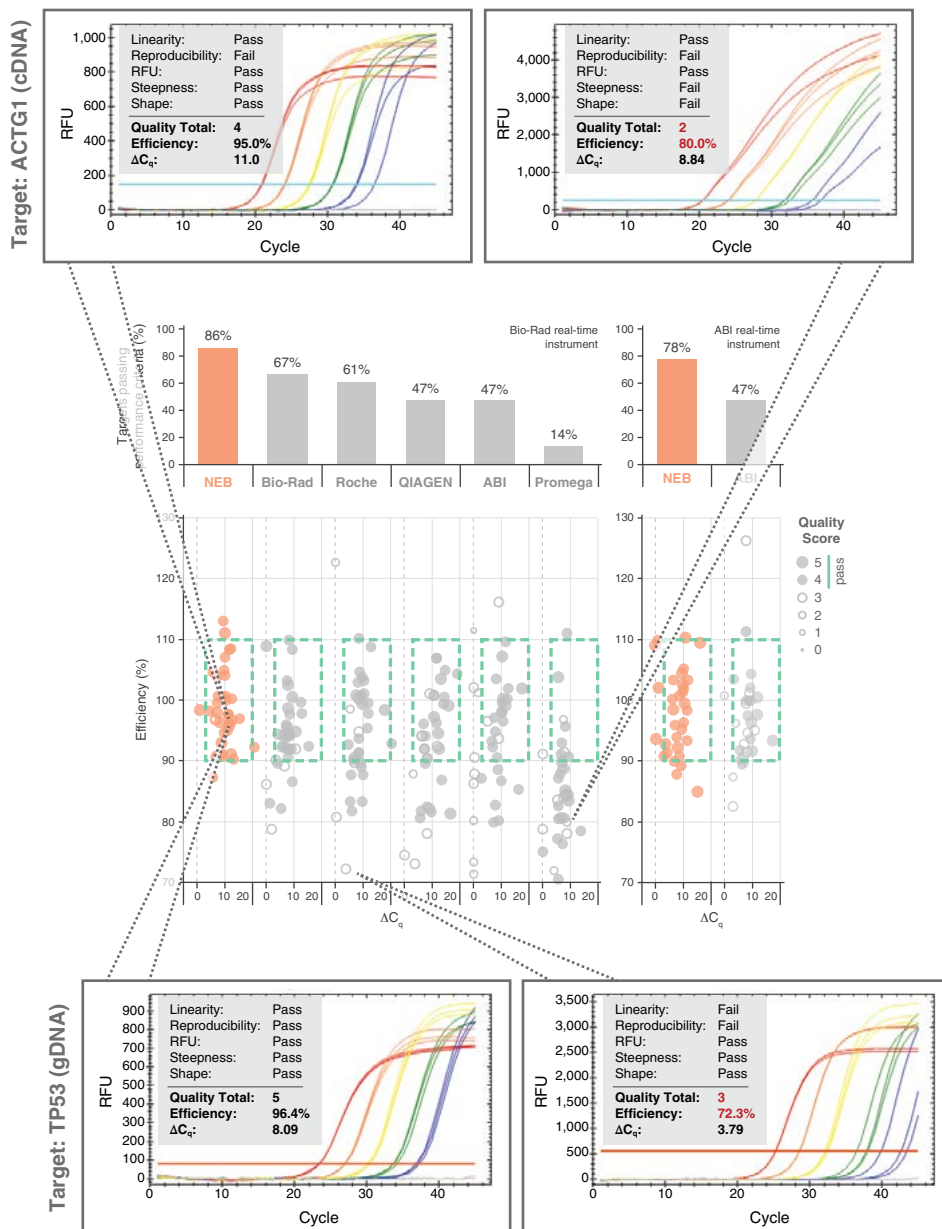
A) In this example, additives A through D were screened on five amplicons, each represented by a colored dot, to examine their effect on qPCR performance. Additive D resulted in successful amplification of all targets while Additive B was detrimental to amplification, resulting in low PCR efficiencies. B) Dots in boxes permitted large volumes of data to be compared over multiple master mix compositions, ultimately driving reagent optimization. Progression of performance is displayed for several predecessors of the Luna Universal qPCR Master Mix (NEB #M3003). Mixes with successful qPCR performance were built upon to establish the final composition of the Luna products.





**FIGURE 3:**  
**Luna qPCR products outperform other commercially-available reagents**

qPCR reagents from NEB and other manufacturers were tested across 16–18 qPCR targets varying in length and GC content, using either Jurkat genomic DNA or Jurkat-derived cDNA as input (10 genomic DNA targets and 8 cDNA targets on Bio-Rad® real-time instrument, 9 genomic and 7 cDNA targets on ABI instrument). For each testing condition, data was collected by 2 users and according to manufacturers' specifications. Results were evaluated as described in Figure 2. Representative curves are shown for two targets, ACTG1 (top) and TP53 (bottom), to demonstrate the correlation of dots in boxes with typical qPCR data. Results for NEB and other major manufacturers are shown: Bio-Rad, SsoAdvanced™ Universal SYBR Green Supermix; Roche®, FastStart™ SYBR Green Master; QIAGEN®, QuantiTect® SYBR Green PCR Kit; ABI, PowerUP™ SYBR Green Master Mix; Promega®, GoTaq® qPCR Master Mix. [Luna Universal qPCR Master Mix](#) from NEB outperformed all other reagents tested.



## DOTS IN BOXES AS A COMPARISON TOOL

Dots in boxes also permitted large-scale performance comparisons between the Luna Universal qPCR and RT-qPCR reagents to various other commercial product offerings. Each commercial mix was challenged against test panels containing a range of targets. Amplicon panels used during the development were tested with a commercial primer/probe sets and a variety of commercial mixes. Data was collected by two separate users and experiments were performed according to each manufacturer's specific product recommendations. The results for the Luna Universal qPCR Master Mix (NEB #M3003) are shown in Figure 3. Luna generates the highest quality qPCR data of all reagents tested, with 86% of all amplicons tested falling in the box with high quality scores. Strong performance was selected for each Luna product; dots in boxes performance comparisons for each Luna product can be found at [LUNAqPCR.com](#).

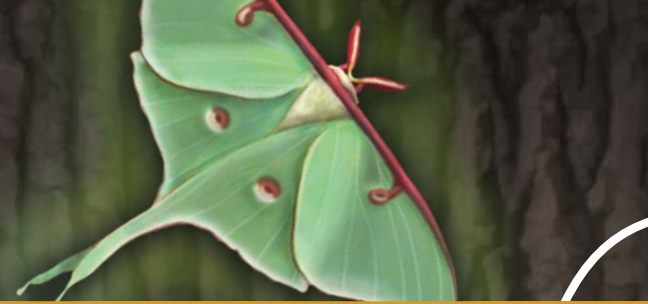
## CONCLUSION

Dots in boxes is a powerful, high-throughput data analysis method based on the MIQE guidelines. It enables rapid, concise comparison of qPCR performance across many targets and for multiple reagents, conditions and/or protocols, permitting an overview of qPCR performance over thousands of reactions where such visualization was not previously possible. Combining the dots in boxes analysis method, a range of target test panels, and a custom LIMS enabled us to create and mine large data sets for information, identify critical variables that affect amplification in qPCR, and harness this information to optimize qPCR reagents. The dots in boxes analysis tool was thus invaluable in development of the Luna qPCR and RT-qPCR reagents, and will continue to benefit future qPCR evaluation and development efforts.

### References

- Higuchi, R., et al. (1992) *Biotechnology*. 10, 413-417.
- Navarro, E., et al. (2015) *Clin. Chim. Acta*. 439, 231-250.
- Bustin, S.A., and Nolan, T. (2004) *J. Biomol. Tech.* 3, 155-156.
- Bustin, S.A., et al. (2009) *Clin. Chem.* 55, 611-622.
- Wittwer, C.T., et al. (2004) *Washington: ASM Press*. 71-84.
- Hall, A.T., et al. (2013) *PLOS One* 8: e73845.

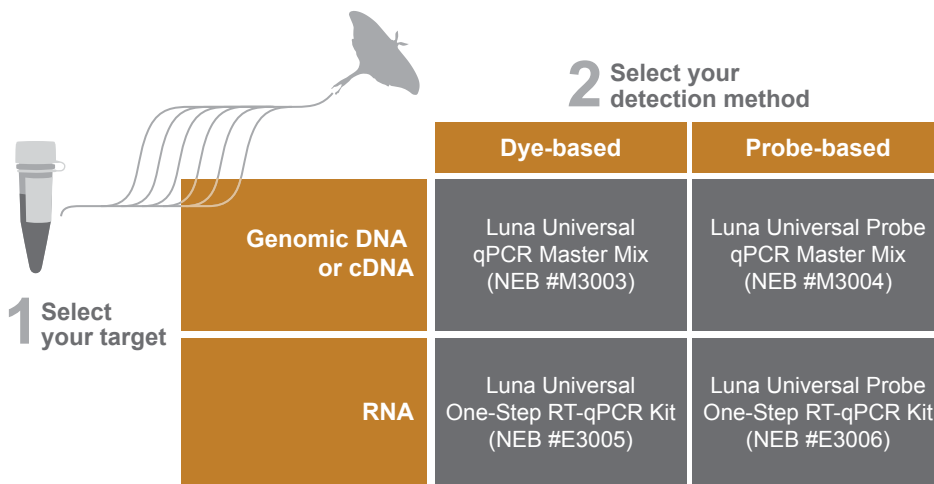
# Lighting the way.™



## Introducing Luna® Universal Products for qPCR & RT-qPCR

Fluorescence-based quantitative real-time PCR (qPCR) allows sensitive and specific detection and quantitation of nucleic acids. [Luna products](#) from NEB are optimized for qPCR or RT-qPCR, and are available for either intercalating dye or probe-based detection methods. All Luna products provide robust performance on diverse sample sources and target types, and have been formulated with a unique passive reference dye that is compatible across a wide variety of instrument platforms, including those that require a ROX reference signal. The mixes also contain dUTP, enabling carryover prevention when reactions are treated with NEB's [Antarctic Thermolabile UDG](#) (NEB #M0372). The reverse transcriptase featured in the Luna RT-qPCR products is a novel, engineered WarmStart® enzyme, developed for robust performance and increased thermostability. These features, combined with rapid, sensitive and precise real-time qPCR performance, make Luna the universal choice for all your qPCR and RT-qPCR experiments.

Finding the right Luna product for your application is simple!



### Make a simpler choice

- One product per application simplifies selection
- Convenient master mix formats and user-friendly protocols simplify reaction setup
- Non-interfering, visible tracking dye helps to eliminate pipetting errors

### Experience best-in-class performance

- All Luna products have undergone rigorous testing to optimize specificity, sensitivity, accuracy and reproducibility
- Products perform consistently across a wide variety of sample sources
- A comprehensive evaluation of commercially-available qPCR and RT-qPCR reagents demonstrates superior performance of Luna products

### Optimize your RT-qPCR

- Novel, thermostable reverse transcriptase (RT) improves performance
- WarmStart RT paired with Hot Start Taq increases reaction specificity and robustness

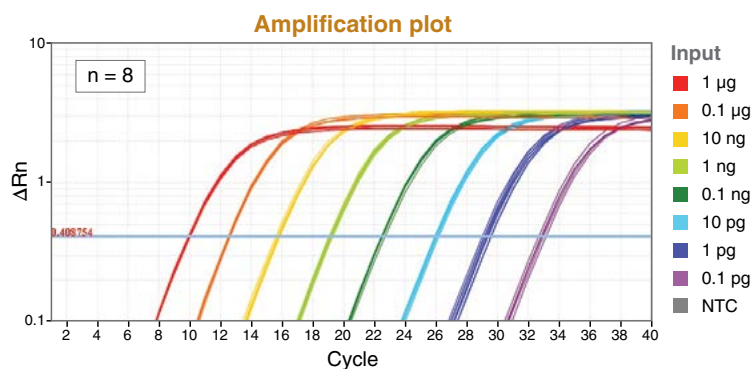


How will Luna perform in your hands? Find out by requesting a sample at [LUNAqPCR.com](http://LUNAqPCR.com).



### NEB's Luna Universal One-Step RT-qPCR Kit offers exceptional sensitivity, reproducibility and RT-qPCR performance

RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit over an 8-log range of input template concentrations (1  $\mu\text{g}$  – 0.1 pg Jurkat total RNA) with 8 replicates at each concentration. Reaction setup and cycling conditions followed recommended protocols, including a 10-minute RT step at 55°C for the thermostable Luna WarmStart Reverse Transcriptase. NTC = non-template control



#### ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
Luna Universal qPCR Master Mix	<a href="#">M3003S/L</a>	200/500 rxns
Luna Universal Probe qPCR Master Mix	<a href="#">M3004S/L</a>	200/500 rxns
Luna Universal One-Step RT-qPCR Kit	<a href="#">E3005S/L</a>	200/500 rxns
Luna Universal Probe One-Step RT-qPCR Kit	<a href="#">E3006S/L</a>	200/500 rxns

Learn more at  
[LUNAqPCR.com](http://LUNAqPCR.com)

- View [performance data](#) for all Luna products
- Learn more about qPCR in our [overview tutorial](#)
- Download our [Luna product brochure](#)
- [Request your Luna sample](#) and share your feedback

## Learn molecular biology in two weeks!

### New England Biolabs Molecular Biology Summer Workshop

**Date:** July 23 – August 5, 2017 (apply by July 1st)

**Location:** Smith College, Northampton, MA, USA

**Tuition:** \$4,300 per participant (includes lab manual, use of equipment & supplies, and room & board).

*No molecular biology experience required!*

Learn more and apply at [www.neb.com/summerworkshop](http://www.neb.com/summerworkshop)



# NEBuilder<sup>®</sup> HiFi DNA Assembly

[NEBuilder HiFi DNA Assembly](#) enables virtually error-free joining of DNA fragments, even those with 5'- and 3'-end mismatches. This flexible kit enables simple and fast seamless cloning utilizing a proprietary high-fidelity polymerase. Find out why NEBuilder HiFi is the next generation of DNA assembly and cloning.

NEBuilder HiFi DNA assembly offers unique features over other DNA assembly methods, including:

- [3'- and 5'-end mismatch removal](#)
- [Bridging of two ds-fragments with a ssDNA oligo](#)
- [Improved methods for site-directed mutagenesis](#)

## Not your average DNA assembly reagent

NEBuilder HiFi DNA Assembly can be used for a variety of DNA assembly methods.

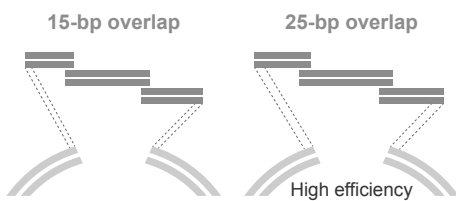
### 2-fragment assembly



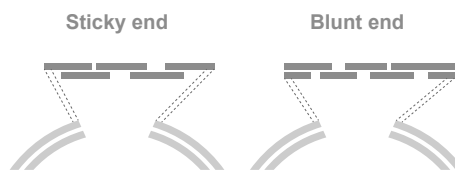
### ssOligo & dsDNA assembly



### 4-fragment assembly



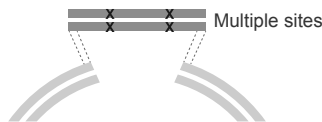
### Annealed-oligo assembly



### 3'- and 5'-end mismatch assembly



### Site-directed mutagenesis



## 4 reasons to choose NEBuilder HiFi

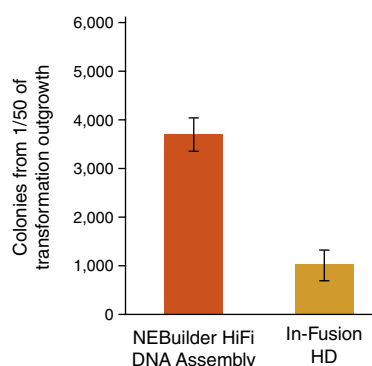
- 1 Save time** – Enjoy simple and fast seamless cloning in as little as 15 minutes
- 2 Flexibility** – Use one system for both "standard-size" cloning and larger gene assembly products, up to 12 fragments
- 3 Compatible with downstream applications** – DNA can be used immediately for transformation or as template for PCR or RCA
- 4 Adaptable** – Adapts easily for multiple DNA manipulations, including site-directed mutagenesis



## How does NEBuilder HiFi compare to In-Fusion® HD?

### NEBuilder HiFi delivers higher colony yield than In-Fusion HD

Two-fragment reactions were set up using the positive control from the In-Fusion HD Cloning Kit (ClonTech® Takara Bio USA, Inc), according to recommended protocols. Two microliters of assembly reaction was transformed into supplied competent cells. 1/50 of outgrowth was spread on an Ap<sup>R</sup> plate.



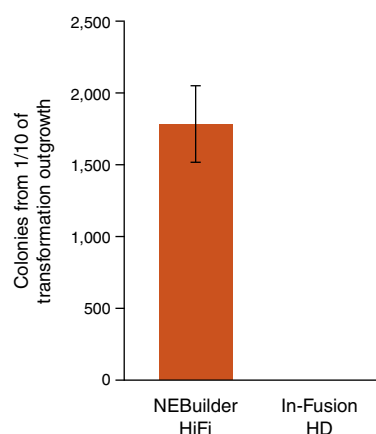
### The NEBuilder HiFi DNA Assembly Cloning Kit can assemble annealed ssDNA oligos with 3' and 5' overhangs or blunt ends

A. Seven annealed oligos, which yield a dsDNA with nicks and 3' and 5' overhangs, were assembled with a pUC-based vector to restore a fragment of *lacZ* gene.

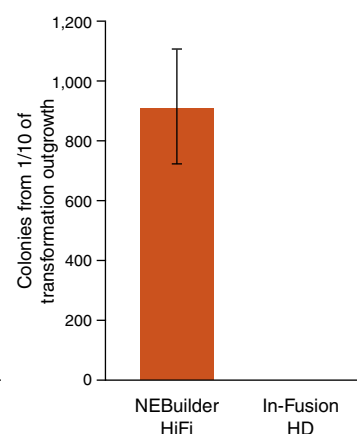
B. Nine annealed oligos, which yield blunt-end dsDNA with nicks, were assembled with a pUC-based vector to restore a fragment of *lacZ* gene.

Assembly reaction was set up according to recommended protocols. Two microliters of assembled products were transformed to NEB 5-alpha Competent *E. coli* (NEB #C2987). 1/10 of outgrowth was spread on Ap<sup>R</sup>, IPTG and X-Gal plates. Blue colonies indicated correct assembly. NEBuilder HiFi successfully assembled ssDNA oligo with 3' and 5' overhangs or blunt ends, while In-Fusion HD did not.

A. 7 oligos & vector



B. 9 oligos & vector




#### ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Cloning Kit	<a href="#">E5520S</a>	10 rxns
NEBuilder HiFi DNA Assembly Bundle for Large Fragments	<a href="#">E2623S</a>	20 rxns
NEBuilder HiFi DNA Assembly Master Mix	<a href="#">E2621S/L/X</a>	10/50/250 rxns*


\* Please inquire for bulk pricing

## Online resources at [NEBuilderHiFi.com](http://NEBuilderHiFi.com)

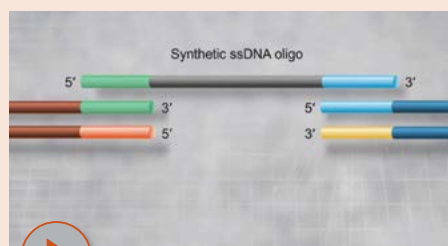
Need help designing primers for DNA assembly?


 Try **NEBuilder Assembly Tool** at [NEBuilder.neb.com](http://NEBuilder.neb.com)

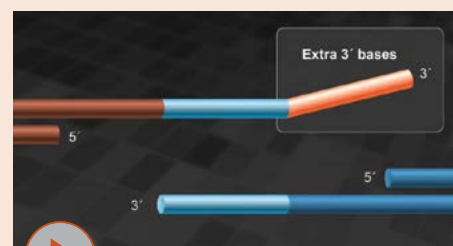
Did you know that NEBuilder HiFi can be used to generate an sgRNA-Cas9 expression vector?


 Download our **application note** and watch our **tutorial**

Want to learn more about the benefits of NEBuilder HiFi? Check out our tutorials.



 Learn how NEBuilder HiFi DNA Assembly bridges dsDNA with a ssDNA oligo



 See how NEBuilder HiFi removes 3' and 5' end mismatches prior to assembly

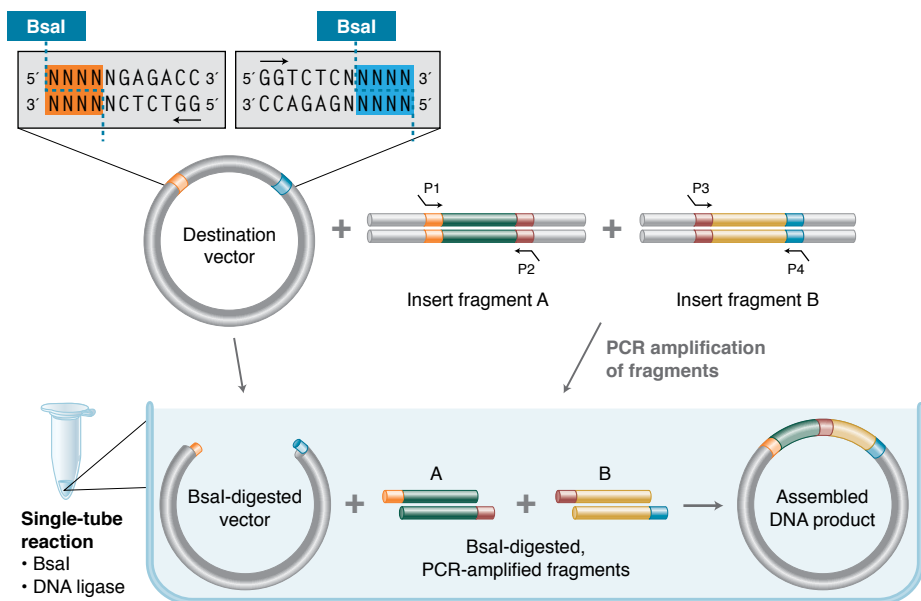
# NEB Golden Gate Assembly

The [NEB Golden Gate Assembly Mix](#) offers efficient and seamless assembly of DNA fragments, and can be used with a broad range of fragment sizes. It contains an optimized mix of [BsaI](#) and [T4 DNA Ligase](#), which work simultaneously in a single reaction. These enzymes, along with a highly optimized buffer, can direct the assembly of multiple inserts/modules using the [Golden Gate approach](#). Assembly is complete in as little as 5 minutes for single inserts, or one can utilize cycling steps for more complex assemblies with multiple inserts.



## NEB Golden Gate workflow

In its simplest form, Golden Gate Assembly requires a Type IIS recognition site, in this case, [BsaI](#) (GGTCTC), added to both ends of a dsDNA fragment. After digestion, these sites are left behind, with each fragment bearing the designed 4-base overhangs that direct the assembly.



New England Biolabs supplies a variety of reagents for use in Golden Gate Assembly, including the optimized NEB Golden Gate Assembly Mix, as well as individual restriction enzymes and ligases. For the full list, visit our Type IIS restriction enzyme table at [www.neb.com/TypeIISTable](http://www.neb.com/TypeIISTable).

### ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
NEB Golden Gate Assembly Mix	<a href="#">E1600S</a>	15 rxns
BsaI	<a href="#">R0535S/L</a>	1,000/5,000 units
BsaI-HF	<a href="#">R3535S/L</a>	1,000/5,000 units
BbsI	<a href="#">R0539S/L</a>	300/1,500 units
BsmBI	<a href="#">R0580S/L</a>	200/1,000 units
SapI	<a href="#">R0569S/L</a>	250/1,250 units
T4 DNA Ligase	<a href="#">M0202S/T/L/M</a>	20,000/100,000 units
T7 DNA Ligase	<a href="#">M0318S/L</a>	100,000/750,000 units

**— NOW AVAILABLE**  
at a lower price

## Advantages of NEB Golden Gate

- Seamless cloning – no scar remains following assembly
- Can be used to assemble areas of repeats
- Compatible with a broad range of fragment sizes (< 100 bp to > 3 kb)
- Efficient with regions with high GC content
- Speed up your experimental design with our online assembly tool at [NEBGoldenGate.neb.com](http://NEBGoldenGate.neb.com)



For the full selection of products available for DNA assembly, and for help in choosing which product would work best, visit [www.neb.com/DNAassemblychart](http://www.neb.com/DNAassemblychart)

# PASSION IN SCIENCE AWARDS®

## The 2016 Passion in Science Awards gathered 15 inspiring scientists from around the globe

New England Biolabs® celebrated its 40th anniversary with the introduction of [the Passion in Science Awards](#) in 2014 to recognize those within the scientific community working to solve many of today's challenges.

We were pleased to offer the Passion in Science Awards again in 2016, and welcomed the award winners to the NEB campus in Ipswich, Massachusetts for a two-day summit celebrating the dedication of these individuals, and exploring how scientists can create opportunities to explore their passions.



2016 Passion in Science Awards winners pictured outside the New England Biolabs facility in Ipswich, MA.

### Scientific Mentorship and Advocacy

Chris Martine – Bucknell University, Lewisburg, PA

Amanda Munson – Shenandoah University, Winchester, VA

David Ng – University of British Columbia, Vancouver, Canada

Sabah Ul-Hasan – University of California, Merced, CA

### Humanitarian Duty

Karishma Bhagani – Matone de Chiwit, Farmington, CT

Nina Dudnik – Seeding Labs, Boston, MA

Lucia Prieto Godino – TReND in Africa, University of Lausanne, Switzerland

Scott O'Neill – Monash University, Victoria, Australia

### Environmental Stewardship

Lisa Anderson – Massachusetts Institute of Technology, Cambridge, MA

Lucy Lahrita – Hokkaido University, Sapporo, Japan

Alexandra Polasko – University of California, Los Angeles, CA

### Arts and Creativity

Scott Chimileski – Harvard Medical School, Boston, MA

Christine Liu – University of California, Berkeley, CA

Will Ryan – Florida State University, Tallahassee, FL

Dana Simmons – University of Chicago, Chicago, IL



“ Nothing inspires me more than nature and the notion that we are still learning new things about the natural world everyday. I became an educator to share this with anyone I can reach, and my lab group comes in each day knowing they will be part of the discovery process. ”

– Chris Martine,  
Awardee for Scientific Mentorship and Advocacy  
Bucknell University, Lewisburg, PA



“ Science and art are sometimes thought of as opposing disciplines that attract opposite personalities. I use my project to demonstrate that people who view themselves as scientists can learn to appreciate and get involved with the arts, and people who view themselves as creative and artistic can appreciate the beautiful patterns created by nature and science. ”

– Dana Simmons,  
Awardee for Arts and Creativity  
University of Chicago, Chicago, IL

Learn more about the recipients and watch their inspiring presentations at [www.neb.com/PassionInScience](http://www.neb.com/PassionInScience)



New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723

Campus-Class™ Mail  
Intra-Mail Network

**USA**

New England Biolabs, Inc.  
Telephone: (978) 927-5054  
Toll Free: (U.S. Orders) 1-800-632-5227  
Toll Free: (U.S. Tech) 1-800-632-7799  
[info@neb.com](mailto:info@neb.com)

**Canada**

New England Biolabs, Ltd.  
Toll Free: 1-800-387-1095  
[info.ca@neb.com](mailto:info.ca@neb.com)

**China, People's Republic**

New England Biolabs (Beijing), Ltd.  
Telephone: 010-82378265/82378266  
[info@neb-china.com](mailto:info@neb-china.com)

**France**

New England Biolabs France  
Telephone : 0800 100 632  
[info.fr@neb.com](mailto:info.fr@neb.com)

**Germany & Austria**

New England Biolabs GmbH  
Free Call 0800/246 5227 (Germany)  
Free Call 00800/246 52277 (Austria)  
[info.de@neb.com](mailto:info.de@neb.com)

**Japan**

New England Biolabs Japan, Inc.  
Telephone: +81 (0)3 5669 6191  
[info.jp@neb.com](mailto:info.jp@neb.com)

**Singapore**

New England Biolabs Pte. Ltd.  
Telephone +65 6776 0903  
[sales.sg@neb.com](mailto:sales.sg@neb.com)

**United Kingdom**

New England Biolabs (UK), Ltd.  
Call Free 0800 318486  
[info.uk@neb.com](mailto:info.uk@neb.com)

For contacts in other countries, please visit  
[www.neb.com/international-support](http://www.neb.com/international-support)

For US customers only:

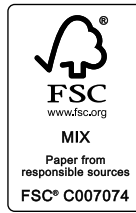


- ✓ Correct this address
- ✓ Unsubscribe from future mailings
- ✓ Remove name from organization

Simply go to [www.wastefreeemail.com](http://www.wastefreeemail.com) and enter Code below:



or fax this cover to 571-970-6072



One or more of these products are covered by one or more patents, trademarks and/or copyrights owned or controlled by New England Biolabs, Inc. However, its use may require you to obtain additional third party intellectual property rights for certain applications. For more information, please email us at [ghd@neb.com](mailto:ghd@neb.com).  
Your purchase, acceptance, and/or payment of and for NEB's products is pursuant to NEB's Terms of Sale at [neb.com/support/terms-of-sale](http://neb.com/support/terms-of-sale). NEB does not agree to and is not bound by any other terms or conditions, unless those terms and conditions have been expressly agreed to in writing by a duly authorized officer of NEB.  
BIO-RAD® is a registered trademark of Bio-Rad Laboratories, Inc. GOTAQ® and PROMEGA® are registered trademarks of Promega Corporation. IN-FUSION® and CLONTECH® are registered trademarks of ClonTech Laboratories, Inc. QIAGEN® and QUANTITECT® are registered trademarks of Qiagen, Inc. ROCHE® is a registered trademark of Roche. SYBR® is a registered trademark of Molecular Probes, Inc. TABLEAU® is a registered trademark of Tableau Software. TAQMAN® is a registered trademark of Thermo Fisher Scientific. FASTSTART™ is a trademark of Roche. POWERUP™ is a trademark of Thermo Fisher Scientific. SSOADVANCED™ is a trademark of Bio-Rad Laboratories

# Coming soon: The 2017-18 NEB Catalog & Technical Reference



[neb.com](http://neb.com)

