

# NEB expressions

a scientific update



## in this issue

- 2** Development of a high-throughput data analysis method for quantitative real-time PCR (qPCR)
- 6** Experience excellent Luna Universal qPCR & RT-qPCR performance in all your experiments!  
**Get a free Sample!**
- 8** Transform your DNA/RNA purification experience! Monarch Nucleic Acid Purification Kits.
- 9** Accelerate your research! Tips for Cloning in just one day!
- 10** Use NEB's competent cells for cloning
- 11** What is the difference between electrocompetent vs. chemically competent cells?
- 12** Let's grow wildflowers for butterflies and bees!

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

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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® 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® Green I or hydrolysis probes (e.g., TaqMan®) 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

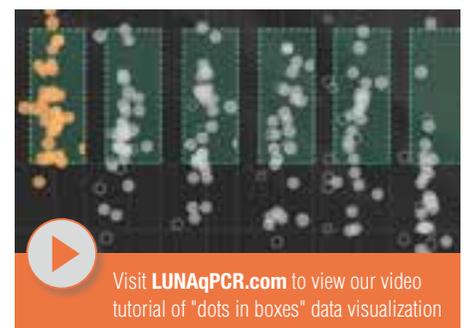
$C_q$  values for each concentration. A standard curve is created by plotting the observed  $C_q$  values on the y-axis and the  $\log_{10}$  of the template concentration on the x-axis. Efficiency is calculated using the equation:  $\text{PCR efficiency} = 10^{-1/\text{slope}} - 1$ . A slope of -3.32 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.

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.



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

## 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 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



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.

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.

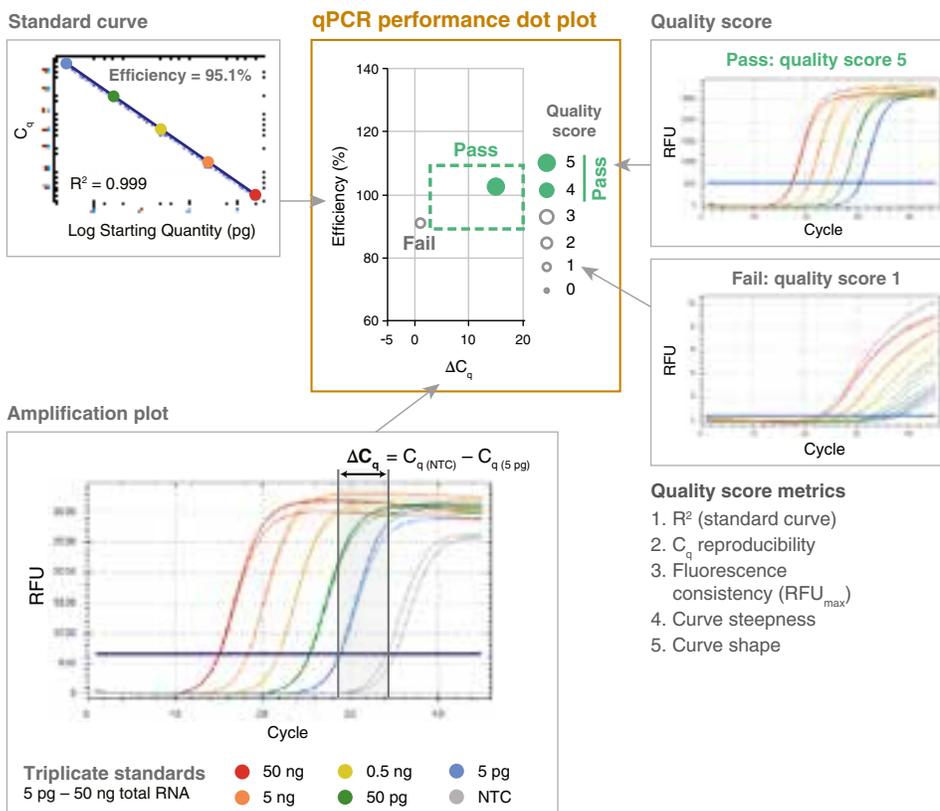
While this simple dot plot was informative on its own, it wasn't sufficient to capture all of the relevant details of each qPCR experiment. In order to represent additional information, such as the linearity of the dynamic range ( $R^2$ ), the overall quality of the qPCR data was scored on a scale of 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 a single graph and compared quickly, creating an efficient, high-throughput visual method for data analysis.



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.



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-target 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

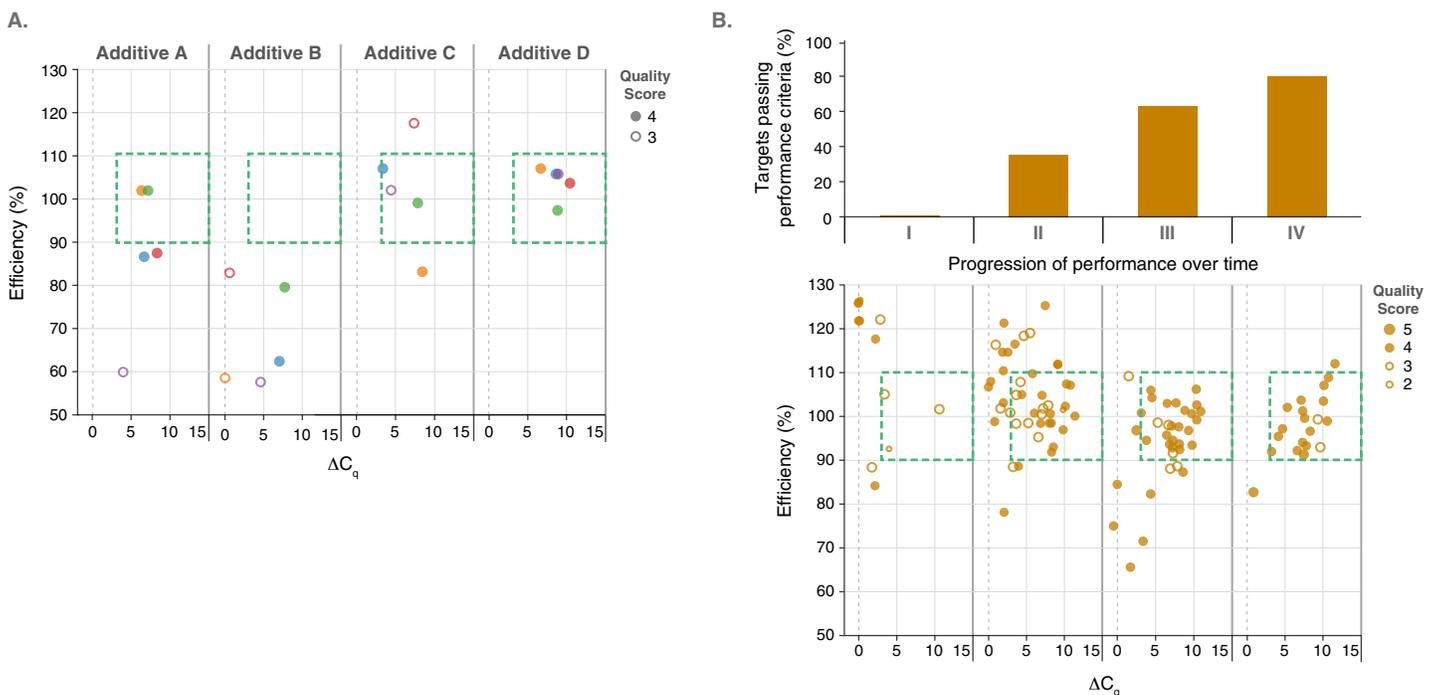
assessed on a development lot of the DNA dye-based master mix. Additive D resulted in the best performance on this particular panel of five amplicons. Unfortunately, improved performance on one particular qPCR panel did not necessarily translate to positive performance across all panels evaluated. Thus, 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  $\Delta C_q$  = average  $C_q$  of non-template control – average  $C_q$  of lowest input). In addition, consistency, reproducibility and overall curve quality were assessed (Quality Score, Table 1).

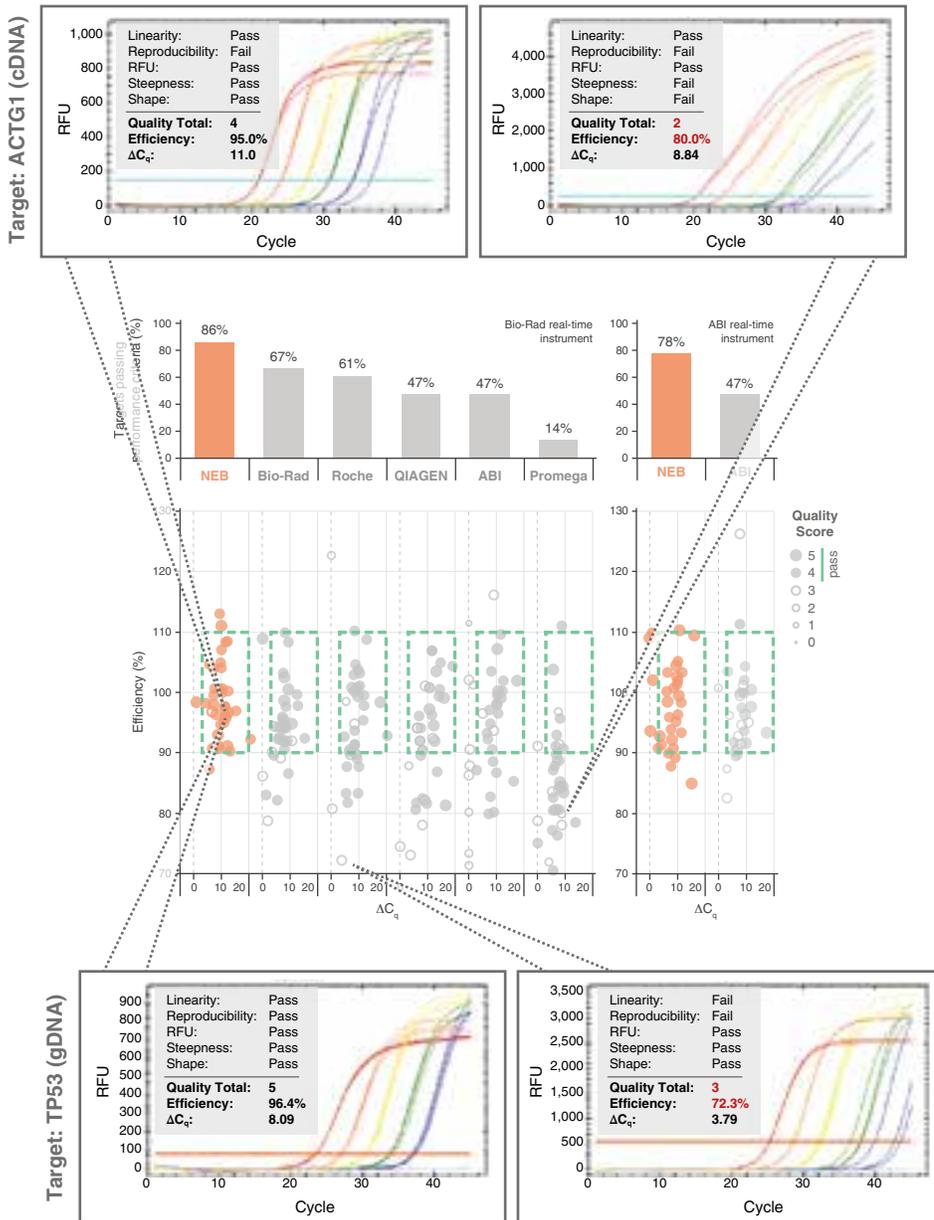
A) In this example, additives A through D were screened on five amplicons, each represented by a colored dot, to examine their effect on qPCR performance. Additive D resulted in successful amplification of all targets while Additive B was detrimental to amplification, resulting in low PCR efficiencies. 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](http://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

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Go to page 6-7 and visit [LUNAqPCR.fr](http://LUNAqPCR.fr) for further information, videos & sample requests.

# Use "MIQE guideline validated" reagents: Experience excellent Luna Universal qPCR & RT-qPCR performance in all your experiments!



New England Biolabs offers a bright new choice of uniquely designed enzyme formulations for your qPCR and RT-qPCR assays. Luna Universal qPCR Kits have been optimized and extensively tested for excellent performance and robustness on diverse sample sources and target types following the MIQE guidelines for best qPCR practice (see page 2-5). Luna products can be used on all instrument platforms, including those that require a ROX reference signal. So, no excuses left for not using "MIQE-qualified" reagents.

## This is how your research benefits from unparalleled performance:

### OPTIMIZE YOUR RT-QPCR WITH LUNA WARMSTART REVERSE TRANSCRIPTASE OR LUNASCRIPT RT SUPERMIX KIT

- Employs novel, thermostable Reverse Transcriptases (RT) for fast protocols at elevated temperatures, exceptional robustness and sensitivity
- One-Step Kits: the unique WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness
- The convenient LunaScript RT SuperMix Kit optimized for best-in-class two-step protocols includes dNTPs, primers and RNase Inhibitor

### EXPERIENCE BEST-IN-CLASS PERFORMANCE

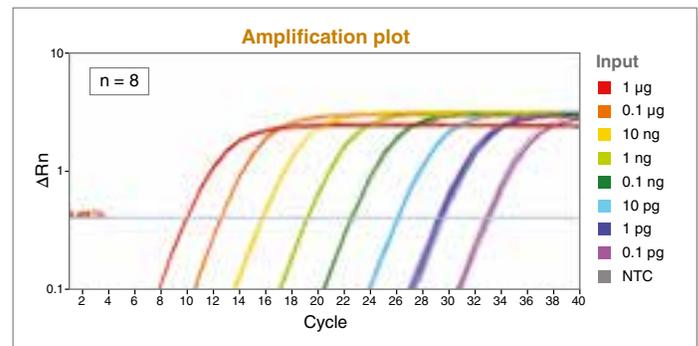
- All Luna products have undergone rigorous testing following the MIQE guidelines to optimize specificity, sensitivity, accuracy and reproducibility.
- A comprehensive evaluation of commercially-available qPCR and RT-qPCR reagents demonstrates superior performance & robustness of Luna products.

### MAKE A SIMPLE CHOICE

- Convenient master mix formats and user-friendly protocols simplify reaction setup.
- Non-interfering, visible tracking dye helps to eliminate pipetting errors.

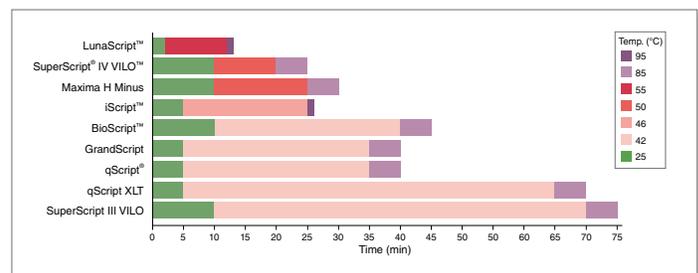
		2 Select your detection method	
		Dye-based	Probe-based
1 Select your target	Genomic DNA or cDNA	Luna Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)
	RNA	One-Step: Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	One-Step: Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006)
		Two-Step: LunaScript Supermix Kit (NEB #E3010) & Luna Universal qPCR Master Mix (NEB #M3003)	Two-Step: LunaScript Supermix Kit (NEB #E3010) & Luna Universal Probe qPCR Master Mix (NEB #M3004)

## Luna Universal One-Step RT-qPCR Kit offers exceptional sensitivity, reproducibility & RT-qPCR performance

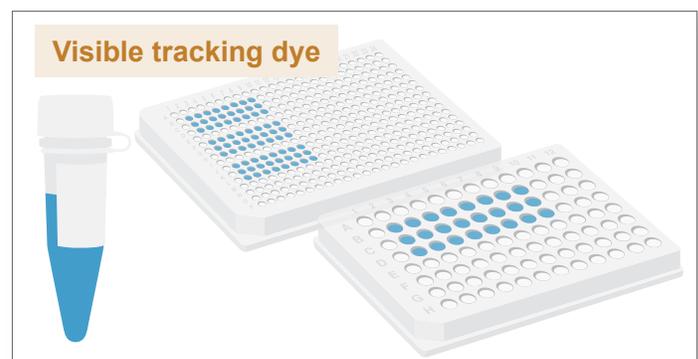


RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit (Input: 1 μg – 0.1 pg Jurkat total RNA); NTC = non-template control

## The LunaScript RT SuperMix Kit offers the shortest available first-strand cDNA synthesis protocol



Comparison of recommended protocols for cDNA synthesis. The LunaScript RT SuperMix Kit requires the shortest reaction time and tolerates elevated temperatures.

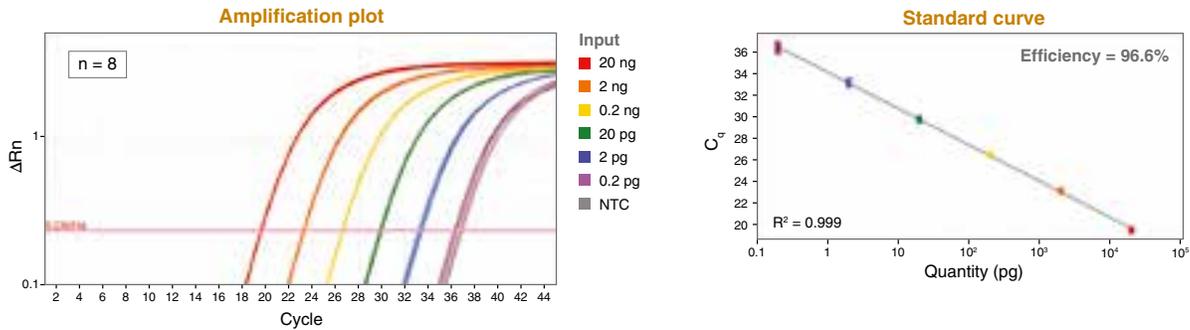


A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates.

All NEB products undergo rigorous testing to ensure optimal performance, and Luna is no exception. We took into consideration numerous important traits when evaluating qPCR, including specificity, sensitivity, accuracy and reproducibility, to develop best-in-class qPCR reagents. Furthermore, we did a

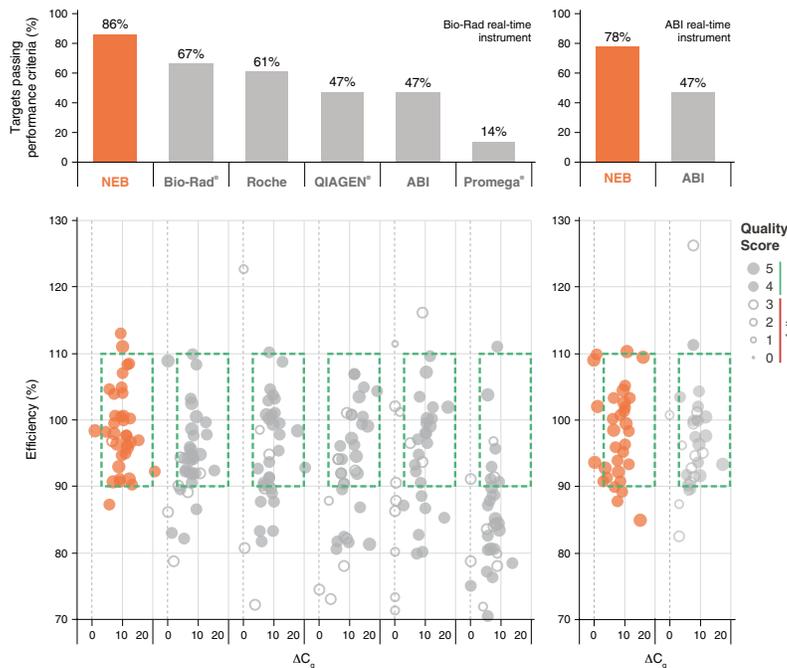
comprehensive evaluation of commercially-available qPCR and RT-qPCR reagents, and developed a method of analysis that allows you to quickly compare and evaluate the performance of these products (see page 2-4). We wanted to be sure that Luna products will perform to your expectations for all your targets.

### Luna products offer exceptional sensitivity, reproducibility and qPCR performance



qPCR targeting human GAPDH was performed using the Luna Universal Probe qPCR Master Mix over a 6-log range of input template concentrations (20 ng – 0.2 pg Jurkat-derived cDNA) with 8 replicates at each concentration. cDNA was generated from Jurkat total RNA using the NEB Protoscript® II First Strand cDNA Synthesis Kit (NEB #E6560). NTC = non-template control

### Evaluation of commercially-available dye-based qPCR reagents demonstrates higher performance, robustness and specificity of Luna reagents



qPCR reagents from NEB and other manufacturers were tested across 16–18 qPCR targets varying in abundance, length and %GC, using either Jurkat genomic DNA or Jurkat-derived cDNA as input (10 genomic DNA targets and 8 cDNA targets on a Bio-Rad real-time instrument, 9 genomic and 7 cDNA targets on an ABI instrument). For each testing condition, data was collected by 2 users and according to manufacturer's specifications. Results were evaluated for efficiency, low input detection and lack of non-template amplification (where  $\Delta C_q$  = average  $C_q$  of lowest input – average  $C_q$  of non-template control). In addition, consistency, reproducibility and overall curve quality were assessed (Quality Score). Bar graph indicates % of targets that met acceptable performance criteria (indicated by green box on dot plot and Quality Score > 3). 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. NEB's Luna Universal qPCR Master Mix outperformed all other reagents tested.



Request a free sample at [LUNAqPCR.fr](http://LUNAqPCR.fr)

Get Free Seedballs with your purchase

see page 12



Order your Luna Universal (RT)-qPCR Reagent now!

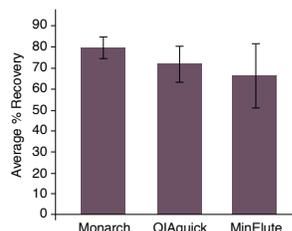
#### ORDERING INFORMATION

PRODUCT	NEB #	SIZE
Luna Universal qPCR Master Mix	M3003S/L/X/E	200/500/1.000/2.500 rxns
Luna Universal Probe qPCR Master Mix	M3004S/L/X/E	200/500/1.000/2.500 rxns
Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/1.000/2.500 rxns
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/1.000/2.500 rxns
LunaScript RT SuperMix Kit	E3010S/L	25/100 rxns

# Transform your DNA/RNA purification experience! Monarch Nucleic Acid Purification Kits

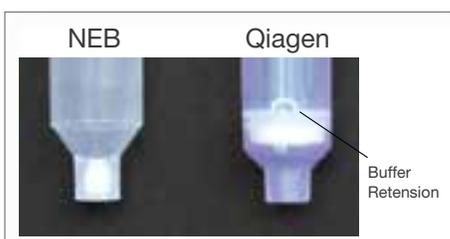
Designed with sustainability in mind, Monarch Nucleic Acid Purification Kits are the perfect complement to many molecular biology workflows. Available for DNA & RNA purification, with buffers and columns available separately, Monarch kits are optimized for excellent performance, convenience and value. Quickly and easily recover highly pure, intact DNA and RNA in minutes. Our expanding selection also includes kits for Total RNA Extraction and RNA Cleanup following enzymatic reactions.

## DNA purified from the Monarch DNA Gel Extraction Kit #T1020 is more highly concentrated, facilitating downstream applications

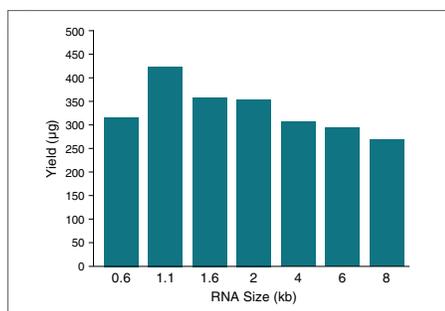


	Monarch	QIAquick®	MinElute®
$A_{260/280}$	1.9+/-0.08	1.9+/-0.08	1.9+/-0.04
Yield (µg)	0.80+/-0.05	0.72+/-0.09	0.66+/-0.13
Conc. (ng/µl)	144.9+/-9.6	25.7+/-3.1	73.8+/-14.2

## Optimized Monarch DNA column design



## The Monarch RNA Cleanup Kit (500 µg) #T2050 is suitable for cleaning up large quantities (>250 µg) of RNA from *in vitro* transcription reactions



## Monarch Total RNA Miniprep Kit (#T2010) has been validated for the following sample types:

- HeLa Cells
- HEK 293 Cells
- NIH 3T3 Cells
- Human Blood
- Rat Blood
- PBMC's
- Rat Liver
- Rat Spleen
- Rat Kidney
- Rat Brain
- Rat Muscle
- Mouse Muscle
- Mouse Heart
- Mouse Kidney
- *S. cerevisiae*
- *E. coli*
- *B. cereus*
- Corn Leaf
- Tomato Leaf
- Plasma
- Serum
- Saliva
- Buccal Cells
- *D. Melanogaster*
- Zebrafish larvae
- Nucleated Blood

For information on input amounts, yield, RIN values, please visit [neb.com/MonarchRNAinputs](http://neb.com/MonarchRNAinputs).

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see page 12



## ORDERING INFORMATION

PRODUCT	NEB #	SIZE	PRODUCT	NEB #	SIZE
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps	Monarch Total RNA Miniprep Kit	T2010S	50 preps
Monarch DNA Gel Extraction Kit	T1020S/L	50/250 preps	Monarch RNA Cleanup Kit (10 µg)	T2030S/L	10/100 preps
Monarch PCR & DNA Cleanup Kit (5µg)	T1030S/L	50/250 preps	Monarch RNA Cleanup Kit (50 µg)	T2040S/L	10/100 preps
			Monarch RNA Cleanup Kit (500 µg)	T2050S/L	10/100 preps



Try one of our free Monarch Nucleic Acid Purification Kit samples! Visit [NEBMonarch.fr](http://NEBMonarch.fr)

## Advantages DNA Kits

#T1010, T1020, T1030

- Designed with sustainability in mind
- Elute in as little as **6 µl**
- Prevent buffer retention and salt carryover with optimized column design
- Purify oligos and other small DNA fragments with simple protocol modification
- Save time with fast, user-friendly protocols

## Advantages Total RNA Kit

#T2010

- Use with a wide variety of sample types
- Purify RNA of all sizes, incl. miRNA & small RNAs >20 nucleotides
- Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent
- Protocols available for RNA fractionation and RNA cleanup
- Save money with value pricing for an all-in-one kit

## Advantages RNA Cleanup Kits

#T2030, T2040, T2050

- Isolate highly pure RNA in minutes
- Clean up RNA with simple protocol utilizing a single wash buffer
- Elute in as little as **6 µl** (NEB #T2030) or **20 µl** (NEB #T2040)
- Bind up to 500 µg of RNA (NEB #T2050)
- Adjust cutoff size down to 15 nt with a slight protocol modification



# Accelerate your research. Cloning in just one day!

With NEB, cloning in just one day has become a reality! For your restriction digest/ligation workflow choose from NEB's restriction enzymes: 195 of our restriction enzymes are **Time-Saver Qualified**  i.e. able to digest DNA in 5-15 minutes with no loss or degradation of product.

Ligate using one of our quick Ligase formulations in 5-15 min ligations or simply use **Instant Sticky-End Ligase Master Mix** that needs **no incubation time** at all!

NEB also offers Kits for modern "Cloning 2.0" methods e.g. Golden Gate or NEBuilder Assemblies, which even allow for cloning of multiple inserts in one reaction.

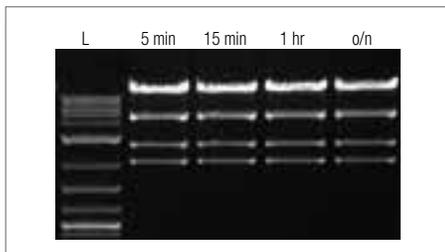
## When it comes to cloning, think NEB!

### A Small Selection chosen from >190 Time-Saver Qualified NEB Restriction Enzymes

ENZYME	Digest in 5 min	ENZYME	Digest in 5 min
BamHI-HF	 ✓	SacI-HF	 ✓
BglII	 ✓	Sall-HF	 ✓
EcoRI-HF	 ✓	SmaI	 ✓
HindIII-HF	 ✓	SpeI-HF	 ✓
NcoI-HF	 ✓	SphI-HF	 ✓
NotI-HF	 ✓	XbaI	 ✓
PstI-HF	 ✓	XhoI	 ✓

Time-Saver enzymes () will digest 1 µg of the specified DNA in 5 - 15 minutes with 1 µl of enzyme under recommended reaction conditions. All NEB High Fidelity (HF) Restriction Enzymes are also Time-Saver qualified.

### Time-Saver digest



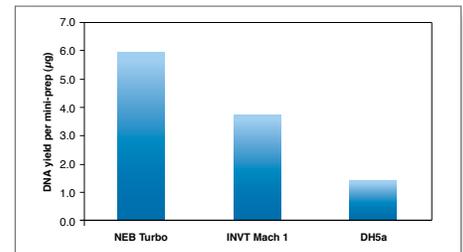
Over 190 of our regular restriction enzymes are "Time-Saver Qualified"  i.e. able to digest DNA in 5-15 minutes with no loss or degradation of product.

### Select the Ligase product that works best for you

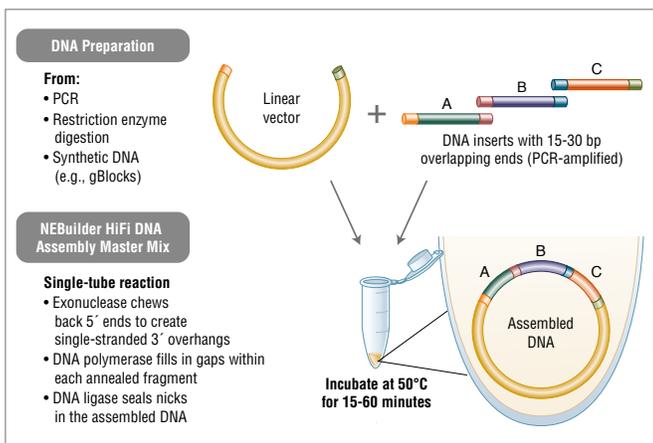
DNA APPLICATIONS	QUICK LIGATION™ KIT	BLUNT/T/A LIGASE MASTER MIX	INSTANT STICKY-END LIGASE MASTER MIX
LIGATION OF STICKY ENDS	★★★	★★	★★★
LIGATION OF BLUNT ENDS	★★★	★★★	★
T/A CLONING	★★	★★★	★

- ★★★ Optimal, recommended ligase for selected application
- ★★ Works well for selected application
- ★ Will perform selected application, but is not recommended

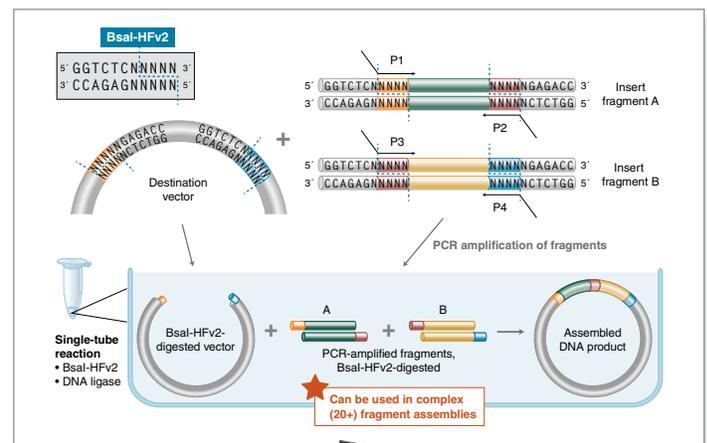
### NEBTurbo – the fastest growing strain. Comparison of DNA yield after 4h of growth



### NEBuilder HiFi DNA Assembly cloning method



### Golden Gate Assembly workflow



Please visit [www.neb.com/newtocloning](http://www.neb.com/newtocloning) for:

- video tutorials and product information
- technical tips & tricks,
- online resources & web tools

**that accelerate your cloning and research!**



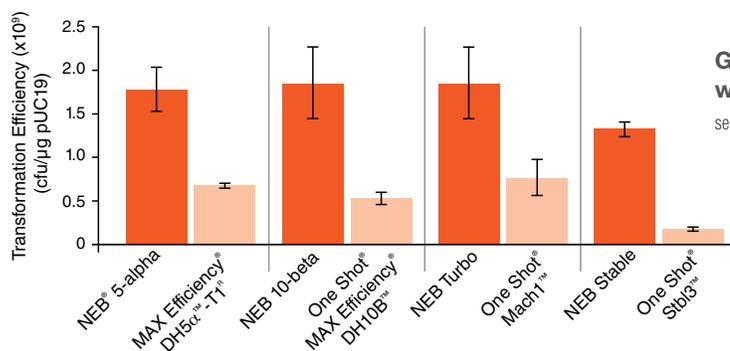


# Try NEB's competent cells for higher efficiency cloning

Ensure successful transformations with NEB competent *E. coli* for cloning. Choose from several high efficiency competent cell strains in a variety of formats that deliver performance, convenience and value.

## Benefit from high transformation efficiencies

Transformation efficiencies were compared using manufacturers' recommended protocols. Values shown are the average of triplicate experiments.



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see page 12



## Advantages

- High transformation efficiencies
- Compatible with NEBuilder HiFi DNA Assembly and Gibson Assembly reactions, as well as ligation reactions. No dilution required!
- Strains also available for cloning toxic genes
- All strains are free of animal products and are T1 phage resistant
- Outgrowth medium and control plasmid are included
- Choose from a variety of convenient formats, including single-use tubes
- Bulk formats and custom packaging are available

## Not sure which cloning strain suits you best?

Use the selection chart below to find the right competent cells for your cloning experiment.

	NEB 5-ALPHA COMPETENT <i>E. coli</i> (#C2987)	NEB TURBO COMPETENT <i>E. coli</i> (#C2984)	NEB 5-ALPHA F' I <sup>+</sup> COMPETENT <i>E. coli</i> (#C2992)	NEB 10-BETA COMPETENT <i>E. coli</i> (#C3019)	<i>dam</i> <sup>-</sup> / <i>dcm</i> <sup>-</sup> COMPETENT <i>E. coli</i> (#C2925)	NEB STABLE COMPETENT <i>E. coli</i> (#C3040)
<b>FEATURES</b>						
Versatile	•					•
Fast growth (< 8 hours)		•				
Toxic gene cloning		•	•			•
Large plasmid/BAC cloning				•		
Dam/Dcm-free plasmid growth					•	
Retroviral/lentiviral vector cloning						•
RecA <sup>-</sup>	•		•	•		•
endA <sup>-</sup>	•	•	•	•	•	•
<b>FORMATS</b>						
Chemically competent	•	•	•	•	•	•
Electrocompetent	•	•		•		
Subcloning	•					
96-well format*	•					
384-well format*	•					
12 x 8-tube strips*	•					

\* Other strains are available in these formats upon request. For more information, contact [bulks.fr@neb.com](mailto:bulks.fr@neb.com).



**NEW PRODUCT**  
**Cloning Competent**  
***E. coli* Sampler**

The NEB Cloning Competent *E. coli* Sampler allows you to try four of our popular chemically competent strains.

**The NEB Cloning Competent *E. coli* Sampler includes:**

**NEB 5-alpha**

A derivative of the popular DH5α, NEB 5-alpha is our most popular strain for cloning.

**NEB 10-beta**

A derivative of DH10B, NEB 10-beta is ideal for cloning of large plasmids.

**NEB Stable**

Ideal for isolation of plasmid clones containing repeat elements and unstable inserts. Useful for isolating and propagating retroviral/lentiviral clones.

**NEB Turbo (*RecA*<sup>+</sup>)**

Features fast colony growth (6.5 hours) and tight expression control (*lacI*<sup>r</sup>). Isolate DNA after only 4 hours of growth.



**Visit [www.neb.com/](http://www.neb.com/)  
 CloningCompCells to find:**

- Selection charts and troubleshooting guides
- Videos containing protocols and tips for optimization
- Protocol video for transformation with glass beads

**ORDERING INFORMATION**

PRODUCT	NEB #	SIZE
Cloning Competent <i>E. coli</i> Sampler	C1010S	8 tubes
NEB 5-alpha Competent <i>E. coli</i> (High Efficiency)	C2987H/I/P/R/U	20 x 0.05 ml / 6 x 0.2 ml / 1 x 96 well plate/ 1 x 384 well plate/96 x 50 µl
NEB 10-beta Competent <i>E. coli</i> (High Efficiency)	C3019H/I	20 x 0.05 ml / 6 x 0.2 ml
NEB Stable Competent <i>E. coli</i> (High Efficiency)	C3040H/I	20 x 0.05 ml / 6 x 0.2 ml
NEB Turbo Competent <i>E. coli</i> (High Efficiency)	C2984H/I	20 x 0.05 ml / 6 x 0.2 ml

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see page 12



**FAQs**

**Electrocompetent vs. chemically competent cells**

**Q:** What are the differences between electrocompetent and chemically competent cells?

**A:** Electrocompetent and chemically competent cells differ in the way uptake of DNA is achieved. Chemically competent cells are treated with a salt solution, which promotes membrane binding of DNA and subsequent passage into the cell. Heat shock further enhances DNA passage. With electrocompetent cells, DNA binding is not required. Instead, an electrical field allows the DNA to move through the membrane.

**Q:** How do I decide which competent cells will work best for me?

**A:** In general, electrocompetent cells have a higher transformation efficiency (typically >1x10<sup>10</sup> versus 1-3x10<sup>9</sup> for chemically competent cells). However, with electrocompetent cells, an electroporator is required; no special equipment is required for chemically competent cells. With electroporation, the DNA sample must be free of ionic compounds and polyethylene glycol (PEG). This is best achieved by spin column clean-up. For transformation of large plasmids (>15 kb) electroporation is much more effective.

**FEATURED VIDEOS**

Find these and other helpful tips at [www.neb.com/CloningCompCells](http://www.neb.com/CloningCompCells)





be INSPIRED | drive DISCOVERY | stay GENUINE

## Let's build new habitats for bees & butterflies!

Being environmentally friendly in the lab, where sterility and convenience are of utmost importance, can be difficult. However, we can all make small changes. To help move toward the goal of a greener lab, we designed our Monarch Nucleic Acid Purification Kits (see page 8), installed an eco-friendly wastewater facility and recycle and compost as much as possible.

### Want to help? Use NEBs' Seedballs!

Seedballs contain seeds, soil and clay, and burst into life with just water and sunlight. These local wildflowers are meant to suit bees and butterflies as habitats!

Just order one or more of the NEB products marked with the seed ball icon in this magazine and indicate the **Promocode "Seedballs"** on your purchase order to receive a linen bag of Seedballs\*.



### Let's raise and release butterflies.

Share with us an image of your grown wildflowers on social media or by email to win one of the 10 free butterfly raising kits. Enjoy the raising of living caterpillars to adult butterflies until it's time to release them!

More information on [www.NEBSeedballs.eu](http://www.NEBSeedballs.eu)



Non-contractual image



*\*Offer closes June 30th 2019, or as long as stocks last. Limited to one bag of Seedballs per purchase.*



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