Fall/Winter **2015**

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in this issue

NEW ENGLAND BioLabs® Inc.

The Quantitation Question: How does accurate library quantitation influence sequencing?

The determination of the number of sequencing-ready molecules present after library preparation is an important step in the next generation sequencing workflow and has a strong influence on the success of both a sequencing run and a sequencing-based experiment. Before selecting the quantitation method you'll use, it can be helpful to understand what happens to a library during sequencing, and exactly what quantitation does and does not tell you.
A typical NGS workflow starts with library preparation and ends with sequencing and data analysis; each of these steps is of critical importance to the quality and reproducibility of the sequencing data. However, between library preparation and sequencing is, perhaps, one of the less-discussed steps in the NGS workflow: library quantitation.

WHY DO I NEED TO QUANTITATE MY LIBRARY?

There are two primary reasons that libraries must be quantitated.

1. The chemistries that underlie Illumina sequencing require an optimal amount of adaptor-ligated DNA fragments to be loaded into the cluster generation step, for example 6-10 pM for the MiSeq® instrument (v3 chemistry).
2. If multiple libraries are sequenced in one run, it is desirable for the sequence coverage to be equal for each library, and an equal amount of each library to be moved into the cluster generation step.

What happens to your library during sequencing?

To fully understand the importance of accurate library quantitation before sequencing, it is first necessary to understand sequencing chemistries and their interactions with the samples you'll be sequencing.

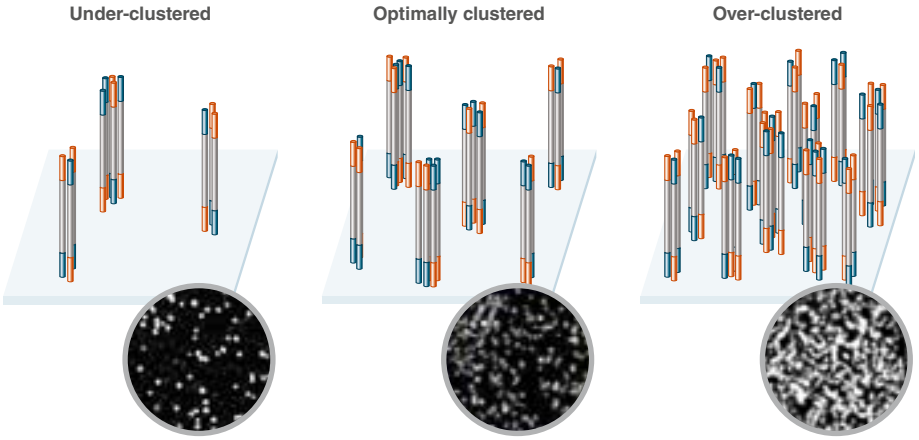
For the purposes of this article, we'll focus on the chemistries that underlie the popular (and market leading) Illumina sequencers, although library quantitation is an important step for sequencing on any platform.

Building bridges & counting clusters

Core components of Illumina's sequencing technology are its flow cells and their cluster-generating capabilities. Illumina's sequencers are based on optical detection of DNA clusters that form on the glass flow cell, a phenomenon enabled by a dense lawn of primers pre-immobilized to the flow cell channel. As you add your library to the flow cell, the single-stranded, adaptor-ligated fragments hybridize to the immobilized primers studded across the flow cell. This step is where the accuracy of your library quantitation is put to the test.

FIGURE 1:
Optimal cluster density enables efficient & accurate quantitation

The density of library clusters as they form on the flow cell prior to sequencing is a key factor in the success of a sequencing run. Low concentration libraries (Left) fail to make optimal use of the space, while high concentration libraries (Right) lead to densely packed clusters that are difficult to call. Optimal cluster density (Center) makes the best use of flow cell real estate, without over crowding. Representative optical data generated during sequencing depicts variation in cluster densities is shown in the insets.



Cluster generation then occurs: each hybridized molecule undergoes multiple rounds of amplification to produce up to 1,000 copies of the same molecule in the same location on the flow cell: a "cluster", whose diameter is 1 micron or less. For more details on cluster generation, visit Illumina.com.

The amount of DNA initially loaded onto the flow cell directly influences the density of the clusters that form. Too little DNA and the clusters are likely to sparsely populate the flow cell. Too much DNA and the clusters will be too close together, making it difficult to interpret the sequencing data due to poor resolution and require resequencing of libraries (Figure 1). Illumina's recommended input ranges, which differ depending on the specific Illumina instrument, help to ensure that the clusters forming on the flow cell have sufficient resolution, without wasting valuable flow cell space.

A deeper dive into equivalent representation

When you pool libraries, you increase the value of each sequencing run by increasing the number of samples that can be sequenced in a single run. However, if libraries are combined in unequal concentrations, this leads to biased representation of certain libraries over others. In cases where libraries are significantly under-represented, these libraries will need to be resequenced, costing time and money. Over-representation of libraries can result in generation of more sequence data than required, and the subsequent discarding of sequence reads, wasting sequence capacity.

Figure 2 (next page) is an example of uneven library pooling resulting in uneven sequence coverage and the need to resequence. With 16 libraries in this pool, each library should theoretically have 6.25% of the sequence reads. However, this is not the case and some of the libraries, such as libraries 5 and 15, would need to be resequenced.

Why do my library fragments need to be adaptor-ligated?

Sequences required downstream of library preparation, such as those for cluster generation and sequencing, must be added to the DNA fragments to be sequenced, and this is the primary goal of library preparation. In PCR-free library preparation workflows, all of the required sequences must be included in the adaptor sequence. In workflows including amplification, some of the sequences, including the sequences required for cluster generation (indicated by P5 and P7 in Figure 3, next page), can be added during PCR instead.

FIGURE 2: **Uneven pooling of libraries yields uneven sequence coverage**

Inadequate or uneven pooling of libraries can result in suboptimal data, and even lead to the need for library resequencing, as seen with library #5.

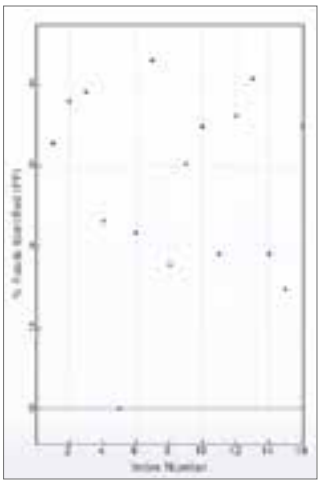
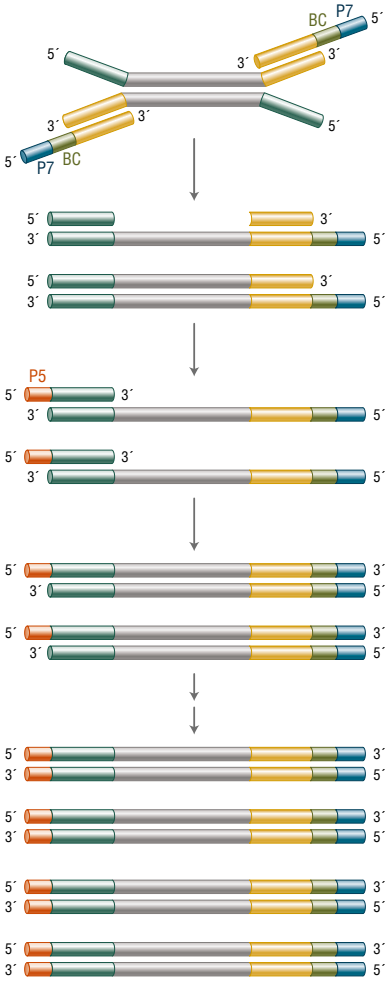


FIGURE 3:
Adaptor ligation workflow

The stepwise addition of the adaptor sequences P5 and P7 and the barcode (BC) can be achieved during PCR amplification of the library.



BC = Barcode

Only fragments that have a P5 sequence at one end and a P7 sequence at the other are capable of participating successfully in cluster generation. Therefore, ideally, only fragments to which both of these sequences have been attached should be counted during a quantitation step.

However, in addition to the desired fragments with an adaptor at both ends, libraries may also contain fragments that have no adaptors, one adaptor or adaptor-dimers. Fragments with no adaptors or one adaptor ligated will not form clusters. Adaptor-dimers will efficiently cluster, but contain no sequence data (Figure 4, page 4).

HOW SHOULD I QUANTITATE MY DNA LIBRARY?

Simply put, library quantitation refers to a variety of methods for determining the number of nucleic acid molecules present in a specific volume of your library. Unlike other molecular biology techniques, where the recommended input range is broad and forgiving, the basic chemistry of NGS requires that a narrow input range of library fragments be further prepared for sequencing. Therefore, quantitation must be precise. It's also important to consider whether you're quantitating productive library molecules – ones that will be (clustered and) sequenced – or if you're simply quantitating total DNA or even total nucleic acid. Accurate quantitation of a library is essential for optimal sequencing outcomes, so choosing the right quantitation method may mean the difference between a successful run and a sub-optimal, or even failed, run. The library will then need to be adjusted and resequenced – an expensive and time-consuming proposition.

When choosing a quantitation method, there are many important considerations, including accuracy, throughput and cost. Several common methods are compared (Table 1) and discussed below.

WHAT ABOUT SPECTROPHOTOMETRIC RATIOS AND FLUOROMETRIC QUANTITATION?

Due to their utility in multiple molecular biology applications, many labs already have spectrophotometers and fluorometers, and these enable relatively low cost quantitation.

continued on page 4...

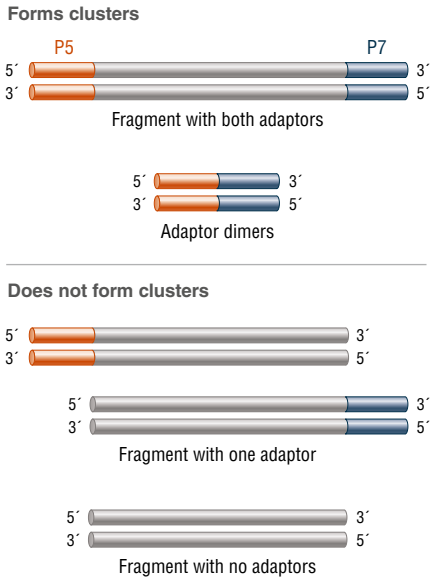
TABLE 1: **Comparison of common library quantitation methods**

When choosing a quantitation method there are many important considerations, including cost, throughput and accuracy.

METHOD	EXAMPLE	BRIEF DESCRIPTION	BENEFITS/LIMITATIONS
Spectrophotometry (260/280)	NanoDrop™	This method detects the absorption of UV light by the macromolecules in the sample.	✓ Low cost, as most laboratories already have access to UV/vis spectrophotometers ✗ Not specific for DNA ✗ Results can be skewed by RNA or protein contamination ✗ Cannot determine fragment sizes
Fluorimetry	Qubit®	This method measures the enhanced fluorescence of a dye upon binding to DNA/ macromolecules.	✓ Low cost, as most laboratories already have access to fluorimeters ✓ Can quantitate dsDNA, specifically ssDNA, RNA or protein ✗ Quantitates all nucleic acid present in sample, not just molecules to be sequenced ✗ Cannot determine fragment sizes
Electrophoretic	Bioanalyzer®, TapeStation®, Fragment Analyzer™	This method relies on capillary electrophoresis of DNA fragments for size estimation, as well as intercalating dyes for quantity determination.	✓ Accurate determination of fragment size distribution ✗ Less reliable quantitation ✗ Requires expensive equipment
Quantitative PCR (qPCR)	NEBNext	This method measures fluorescence of a dye bound to dsDNA at each PCR cycle, quantitating relative to included standards.	✓ Most accurate quantitation method ✗ More expensive ✗ Cannot determine fragment sizes

FIGURE 4: **Adaptors are the hallmark of productive molecules**

Only library fragments containing both a P5 and a P7 adaptor will result in a flow-cell cluster. Other molecules are insufficient for cluster formation or contain no sequence data, so efforts should be made to exclude them from quantitation.



For quantitating nucleic acid, spectrophotometers assess the amount of UV light absorbed by the sample at two wavelengths, 260 nm and 280 nm. A ratio of the absorbance values can then be used to determine whether or not the sample has contaminating proteins. The 260/280 ratio of a purified DNA sample should be between 1.7 and 1.9. Spectrophotometers are great at estimating the total amount and relative purity of nucleic acid in solution, but they can't provide information about fragment size, and they can be confounded by an abundance of either RNA or protein in the sample.

Fluorometers, unlike UV-Vis spectrophotometers, rely on nucleic acid-specific dyes to assess the amount of nucleic acids in the sample. In this way, they avoid the pitfalls of spectrophotometry, and can specifically quantitate dsDNA, ssDNA, RNA, or protein, depending on the dye used. However, they too are limited to gathering data about the entire complement of dsDNA or ssDNA in the sample, and not just molecules that will be sequenced.

It is generally recommended not to use only spectrophotometry, fluorometery, or even a combination of the two as your sole quantitation method before sample loading.

What can electrophoretic methods/ instruments tell me?

Electrophoretic instruments, such as the Agilent Bioanalyzer, TapeStation, and AATI Fragment Analyzer, provide valuable data in a variety of forms. The output of these instruments is a visualization based upon laser excitation of an intercalating dye during the sample's passage through a chip matrix, and measurement of the time taken to travel through the matrix. The data can be formatted to look like the familiar banding pattern of electrophoresis or as a graph (a "trace"). On-chip electrophoresis enables faster, more standardized quantitation of nucleic acid samples than standard slab gel electrophoresis, with much smaller sample amounts.

Overall, electrophoretic instruments are exceptionally useful tools for library quantitation, and they are a part of many laboratories' NGS workflows. Electrophoretic methods are able to determine both the average library size and the size distribution of the library (important as a tight size range is generally more desirable than a broad size range). Still, electrophoresis-based quantitation of NGS libraries is not as accurate or consistent as qPCR-based methods, and is not specific for adaptor-ligated fragments. Additionally, electrophoresis-based methods are not sufficient for quantitating PCR-free library construction, as there is no PCR enrichment of adaptor-ligated molecules and they cannot discern between adaptor-ligated DNA molecules and unligated molecules.

qPCR: What's in a name?

As the name implies, qPCR (or quantitative PCR) can provide an additional level of information about your library. Beyond simply reporting the total amount of DNA in your sample, qPCR-based library quantitation uses specific primers that hybridize to the adaptor sequences and therefore measures only molecules with adaptor sequences at both ends. This added specificity ensures that the fraction of the library loaded onto the sequencer contains the expected number of adaptor-ligated molecules. As described above, exact titration of adaptor-ligated DNA molecules is important for NGS as only molecules with an adaptor on each end can be successfully processed through the sequencing workflow. The quantity of the library is determined by comparing to a standard curve generated from DNA standards (known concentrations of DNA of a known size), followed by a simple calculation to account for any difference in size between the library being measured and the DNA standards. Methods such as electrophoretic analysis, described above, are useful for library size determination.

qPCR-based methods, which quantitate DNA sequences that are attached to adaptors, will also quantitate adaptor-dimers (e.g., two ligated adaptors without any intervening library sequence). The presence of excessive adaptor-dimers in your library can skew your quantitation, but if this situation is suspected, running the sample on a Bioanalyzer or similar instrument will be informative. Frequently, qPCR-based quantitation methods and electrophoretic methods are used in parallel, to determine both the quantity and quality of your library.

Specific details on the use of qPCR-based library quantitation are available in the product manual for the NEBNext Library Quant Kit for Illumina which can be downloaded at www.neb.com/E7630. More information can also be found in our application note on page 11.

THE BIG PICTURE

So, which method for library quantitation is right for you? Your answer will depend on a number of factors that are specific to your situation, including your laboratory's preferred DNA quantitation method, the tools you have available, your source material, and the size and scope of your experiment. No matter which platform you'll be sequencing on, it is important to accurately determine the amount of sequence-ready DNA present. As we've described, accurate quantitation makes a meaningful difference in the quality of the data you'll create and the overall value of your experiment, by ensuring generation of optimal cluster densities and the equivalent representation of multiplexed libraries when pooling.

Using a qPCR-based approach, as we've just reviewed, ensures the most accurate quantitation, providing optimal conditions for Illumina's sequencing chemistries. To make NGS library quantitation more accurate and reproducible, New England Biolabs® (NEB®) offers the NEBNext Library Quant Kit for Illumina. This qPCR-based kit is compatible with a broad range of library insert sizes and GC content, and has a user-friendly workflow.

FEATURED PRODUCT

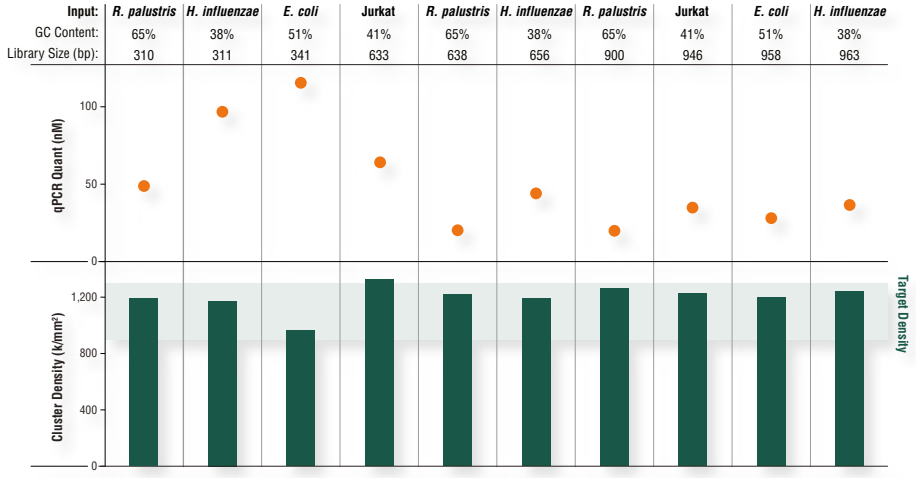
NEBNext Library Quant Kit for Illumina

The NEBNext Library Quant Kit delivers significant improvements to qPCR-based library quantitation for next gen sequencing. The NEBNext Library Quant Kit for Illumina contains components that are optimized for qPCR-based quantitation of libraries prepared for Illumina next generation sequencing platforms.

The NEBNext Library Quant Kit contains primers which target the P5 and P7 Illumina adaptor sequences and a set of high-quality, pre-diluted DNA standards to enable reliable quantitation of diluted DNA libraries between 150–1000 bp.

NEBNext Library Quant Kit delivers accurate quantitation for a variety of sample types and sizes

Libraries of 310-963 bp from the indicated sources were quantitated using the NEBNext Library Quant Kit, then diluted to 8 pM and loaded onto a MiSeq (v2 chemistry; MCS v2.4.1.3). Library concentrations ranged from 7-120 nM, and resulting raw cluster density for all libraries was 965-1300 k/mm2 (ave. =1199). Optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit for all library sizes.



advantages

- Provides **more accurate** and **reproducible** quant values than alternative methods and kits
- Compatible with libraries with a **broad range of insert sizes** and GC content, made by a variety of methods
- Requires only 4 standards, allowing **more libraries to be quantitated per kit**
- The NEBNext Library Quant **Master Mix** requires only the addition of primers
- Utilizes a **single extension time for all libraries**, regardless of insert size
- **ROX is included** in the kit, for use with qPCR instruments that require a reference dye for normalization

ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
NEBNext Library Quant Kit for Illumina	E7630 S	100 rxns
NEBNext Library Quant Kit for Illumina	E7630 L	500 rxns

Even more from less.

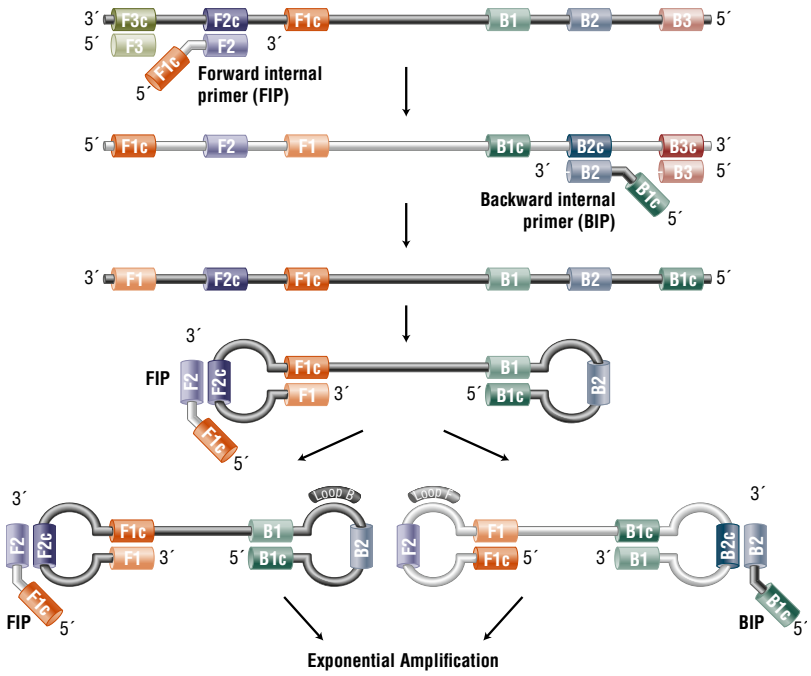
- **Highest library yields**
- **Inputs as low as 500 pg**
- **Fewer PCR cycles**

LOOP-MEDIATED
Isothermal Amplification

LAMP (loop-mediated isothermal amplification) is designed to detect a target nucleic acid without sophisticated equipment. LAMP uses 4-6 primers recognizing 6-8 distinct regions of the target DNA. A strand-displacing DNA polymerase initiates synthesis and two of the primers form loop structures to facilitate subsequent rounds of amplification. LAMP provides high sensitivity (fg levels or <10 copies of target), and reactions can be performed in as little as 5–10 minutes. Additionally, reactions can be performed with limited resources (e.g., using a water bath for incubation, and detection of results by eye), or with real-time measurement and high-throughput instruments.

Detection of RNA targets is accomplished by simple addition of a reverse transcriptase to the LAMP reaction (e.g., WarmStart® RTx Reverse Transcriptase, NEB #M0380), or by use of a DNA polymerase with RT activity (e.g., *Bst* 3.0 DNA Polymerase), with RT-LAMP performed as a true one-step, isothermal workflow.

OVERVIEW OF LAMP



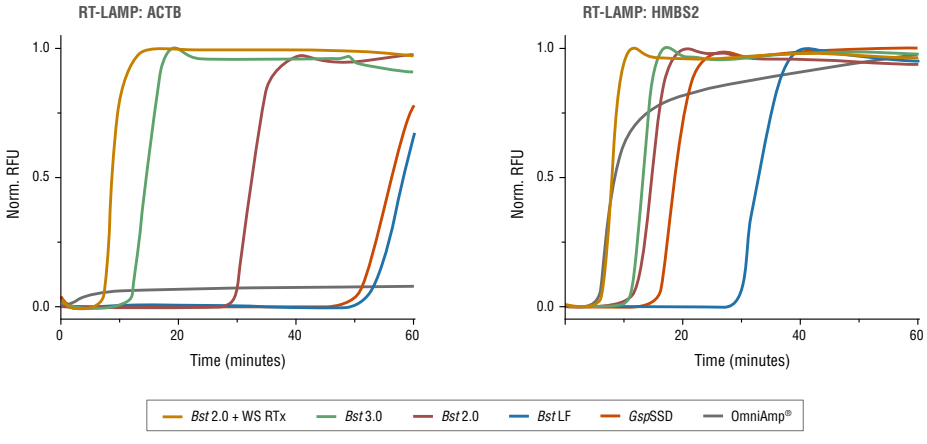
Validated for LAMP

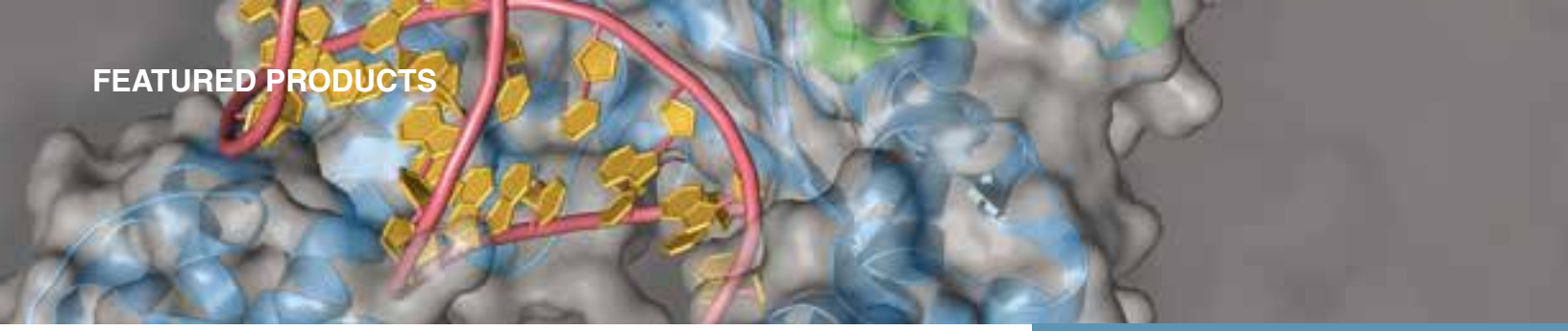
Bst 3.0 DNA Polymerase

Bst 3.0 DNA Polymerase (NEB #M0374) is an *in silico*-designed homolog of *Bacillus stearothermophilus* DNA Polymerase I, Large Fragment (NEB #M0275), engineered for improved isothermal amplification performance and increased reverse transcription activity. *Bst* 3.0 contains 5′→ 3′ DNA polymerase activity with either DNA or RNA templates and strong strand displacement activity, but lacks 5′→ 3′ and 3′ → 5′ exonuclease activity. *Bst* 3.0 demonstrates robust performance even in high concentrations of amplification inhibitors and features significantly increased reverse transcriptase activity compared to *Bst* DNA Polymerase.

Fast, single-enzyme RT-LAMP can be performed using *Bst* 3.0

RT-LAMP (reverse-transcriptase LAMP) was performed using indicated DNA polymerase and Jurkat total RNA and primers for two genes (ACTB, left; HMBS2, right). Fastest results were observed with a 2-enzyme system, *Bst* 2.0 DNA Polymerase and WarmStart RTx Reverse Transcriptase (WS RTx), but robust amplification was also observed using *Bst* 3.0 without additional RT. *Bst* DNA Polymerase, Large Fragment (*Bst* LF), *Bst* 2.0 DNA Polymerase and competitor enzymes showed highly variable performance, with slow threshold times or reaction failure on one of the two targets.





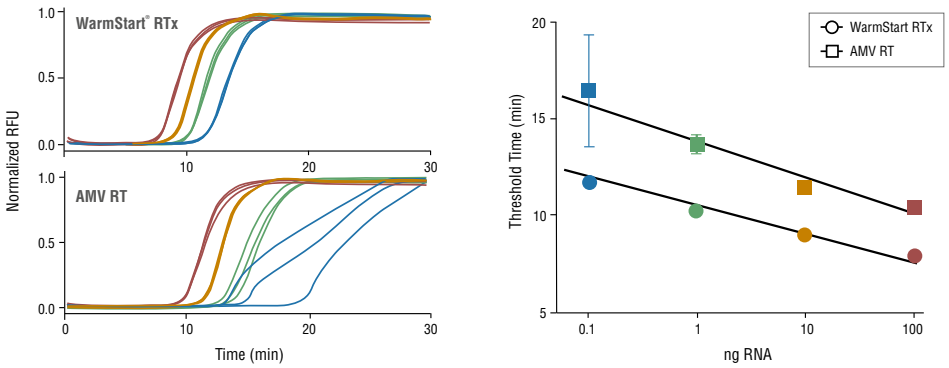
Validated for RT-LAMP

WarmStart RTx Reverse Transcriptase

WarmStart RTx Reverse Transcriptase (NEB #M0380) is a unique *in silico*-designed, RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template. RTx is a robust enzyme for RNA detection in amplification reactions and is particularly well-suited for use in loop-mediated isothermal amplification. The WarmStart property enables high throughput applications, room temperature setup, and increases the consistency and specificity of amplification reactions. RTx contains intact RNase H activity.

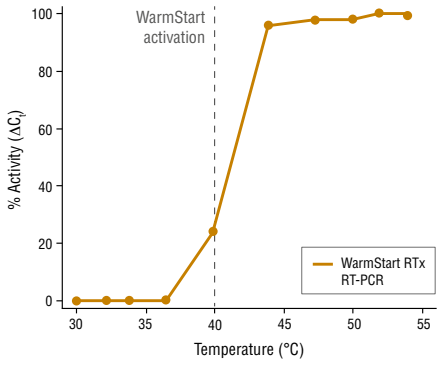
WarmStart improves speed and sensitivity in RT-LAMP

RT-LAMP reactions with *Bst* 2.0 WarmStart DNA Polymerase and the indicated reverse transcriptase were incubated at 65°C with 1 pg – 100 ng of Jurkat total RNA. Reactions were monitored with real-time fluorescence, and resulting curves are shown (left), with corresponding threshold times (right). WarmStart RTx provides faster reaction threshold times for improved consistency and sensitivity with lower input RNA amounts. RT-LAMP reactions performed with AMV Reverse Transcriptase resulted in inconsistent detection, as indicated by wide variation at lower RNA input concentrations (blue curves).



WarmStart control of WarmStart RTx

cDNA synthesis was performed for 10 minutes, followed by qPCR analysis. Resulting Cts were normalized to a "no RT" control for 0% activity and fastest Ct for 100% activity. WarmStart RTx is inhibited by a reversibly bound aptamer at temperatures below 40°C, and is fully active at temperatures 42°C and higher.



ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
Bst 3.0 DNA Polymerase	M0374S/L/M	1,600/8,000/8,000 units
Bst 2.0 WarmStart DNA Polymerase	M0538S/M/L	1,600/8,000 units
Bst 2.0 DNA Polymerase	M0537S/M/L	1,600/8,000 units
Bst DNA Polymerase, Large Fragment	M0275S/M/L	1,600/8,000 units
Bst DNA Polymerase, Full Length	M0328S/L	500/2,500 units
WarmStart RTx Reverse Transcriptase	M0380S/L	50/250 rxns

The purchase of NEB RTx products conveys to the purchaser the limited, nontransferable right to use the purchased products to perform reverse transcription loop-mediated isothermal amplification ("RT-LAMP") for research use only. LAMP is a patented technology belonging to Eiken Chemical Co., Ltd., and any use other than research may require a license from Eiken Chemical Co., Ltd. A patent is pending for NEB's RTx product.

LEARN MORE AT NEB.COM

Interested in learning how NEB scientists are using isothermal amplification in their research?

Visit **www.neb.com/IsothermalAmplification** to find videos, protocols and recent articles, including a recent publication from NEB scientists, describing a pH-sensitive isothermal detection method.

Request your new technical brochure from your local distributor!

CloneWithNEB.com

What you need to be successful with your cloning experiments

NEW PRODUCT

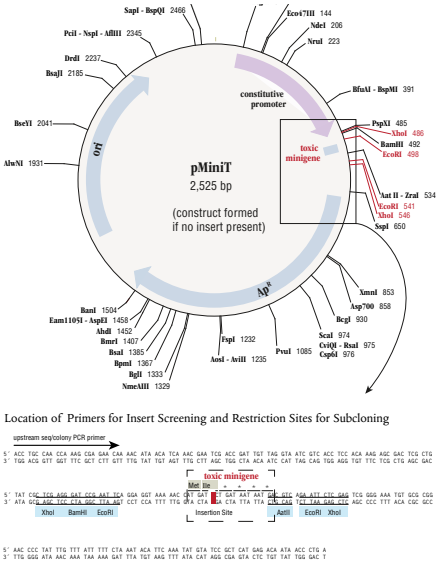
SPECIAL PRICE*

NEB PCR Cloning Kit: With or without competent cells

NEB's PCR Cloning Kit includes an optimized *Cloning Mix* containing a proprietary ligation enhancer and a linearized vector that uses a novel mechanism for background colony suppression to give a low background. It allows simple and quick cloning of any PCR amplicon, whether the amplification reactions produce blunt ends or single base overhangs. This is possible due to "in-visible" end polishing components in the master mix that are active during the ligation step only if needed. The kit also allows direct cloning from amplification reactions without purification, and works well whether the primers used in the PCR possess 5´-phosphate groups or not.

The kit is available with the optimal NEB10-beta competent *E. coli* cells for excellent results. However, the kit is now also available without competent cells to allow for the use of your preferred *E. coli* strain.

PCR cloning has never been easier!



Location of Primers for Insert Screening and Restriction Sites for Subcloning

Figure: Map shown above displays the construct formed if no insert is present. Unique restriction sites are shown in black. Additional restriction sites that can be used for subcloning are shown in red. Expanded box below shows location of sequencing primers, restriction sites for subcloning, and placement of insertion site within the toxic minigene.

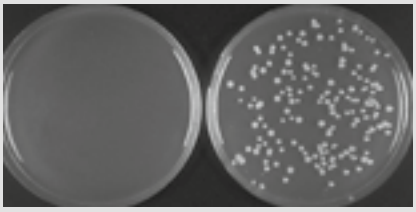
ORDERING INFORMATION – INTRODUCTORY PRICE:

PRODUCT	NEB #	SIZE	Promo code PCRCLO15*
NEB PCR Cloning Kit (without competent cells)	E1203 S	20 rxns	99 €
NEB PCR Cloning Kit (incl. NEB10-beta competent cells)	E1202 S	20 rxns	259 €

** Valid till 31/01/16 only with promocode PCRCLO15 & not cumulative with other discounts or special prices.*

advantages

- Works with both **blunt** and **TA ends**
- Clone faster, with **low/ no background**
- Get the colonies you need, with **high transformation efficiency**
- **No** need for **end-modification steps**
- **No blue/white screening** required



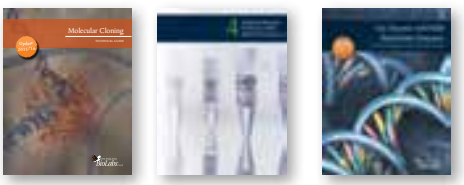
PCR cloning with low/no background

A 500 bp PCR product was cloned using the NEB PCR Cloning kit (with competent *E. coli*). The left plate serves as the control, with vector backbone only. The right plate shows colonies containing the PCR insert.

NEB's Restriction Enzymes: CutSmart = CloneSmart!

Did you know?:

- Over **200 NEB Restriction Enzymes** exhibit 100% activity in the same CutSmart reaction buffer.
- You can **choose the incubation time** (15 min or over night) according to your needs.
- Most "downstream" **cloning enzymes** (incl. Ligase, Klenow, Alkaline Phosphatase) are also **100% active** in CutSmart reaction buffer.
- NEB offers **dedicated technical support** incl. video tutorials, smart phone apps and online tools to aid your research.



Find more tips & tricks in the new corresponding NEB brochures, available free of charge from your local distributor!

Which DNA Ligase works best for you? Choose the right product for your needs.

	T4 DNA LIGASE #M0202	QUICK LIGATION™ KIT #M2200	BLUNT/TA LIGASE MASTER MIX #M0367	INSTANT STICKY-END LIGASE MASTER MIX #M0370	ELECTRO-LIGASE® #M0369	T3 DNA LIGASE #M0317	T7 DNA LIGASE #M0318	*E. coli* DNA LIGASE #M0205	*TAQ* DNA LIGASE #M0208	9°N™ DNA LIGASE #M0238	NEBNEXT® QUICK LIGATION MODULE #E6056	SPLINTR® LIGASE #M0375
DNA APPLICATIONS												
Ligation of sticky ends	★★	★★★	★★	★★★	★★	★★	★★	★	★	★		
Ligation of blunt ends	★★	★★★	★★★	★	★★	★★						
T/A cloning	★★	★★	★★★	★	★★	★	★					
Electroporation	★★				★★★							
Ligation of sticky ends only							★★★					
Repair of nicks in dsDNA	★★★	★★★	★★★	★★★	★★★	★★★	★★★	★★★	★★★	★★★		
High complexity library cloning	★★★	★★	★★	★★	★★							
Ligase Detection Reaction & Ligase Chain Reaction									★★	★★★		
Ligation of DNA hybridized to RNA												★★★
DNA APPLICATIONS												
NGS Library Prep dsDNA-dsDNA (ligation)	▲		▲			▲					▲	
DNA APPLICATIONS												
Salt tolerance (> 2X that of T4 DNA Ligase)						✔						
Ligation in 15 min. or less	✔	✔	✔	✔		✔	✔		✔	✔	✔	✔
Master Mix Formulation			✔	✔							✔	
Thermostable									✔	✔		
Recombinant	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔

★★★ Optimal, recommended ligase for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
▲ Please consult the specific NGS protocol to determine the optimal enzyme for your needs

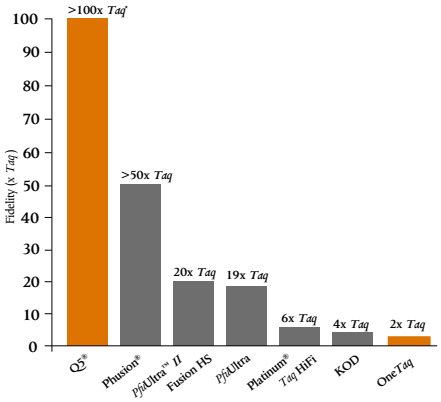
ORDERING INFORMATION

PRODUCT	NEB #	SIZE
T4 DNA Ligase	M0202 S/L	20,000/100,000 units
T4 DNA Ligase, conc.	M0202 T/M	20,000/100,000 units
Quick Ligation Kit	M2200 S/L	30/150 rxns
Blunt/TA Ligase Master Mix	M0367 S/L	50/250 rxns
Instant Sticky-end Ligase Master Mix	M0370 S/L	50/250 rxns
ElectroLigase	M0369 S	50 rxns

Find informative videos on Ligase and other topics on NEB's YouTube channel: www.youtube.com/nebiolabs

Q5 High-Fidelity DNA Polymerase – Ultra fidelity PCR

Q5 High-Fidelity DNA Polymerase is a high-fidelity, thermostable DNA polymerase with 3´-> 5´ exonuclease activity, fused to a processivity-enhancing Sso7d domain to support robust DNA amplification. With an error rate > 100-fold lower than that of *Taq* DNA Polymerase and 12-fold lower than that of Pyrococcus furiosus (Pfu) DNA Polymerase, Q5 High-Fidelity DNA Polymerase is ideal for cloning and can be used for long or difficult amplicons. Q5 High-Fidelity DNA Polymerase is supplied with an optimized buffer system that allows robust amplification regardless of GC content.



Highest fidelity DNA amplification available. Fidelity of various PCR Polymerases as compared to *Taq* DNA Polymerase.

ORDERING INFORMATION – SPECIAL DISCOUNT

PRODUCT	NEB #	SIZE
Q5 High-Fidelity DNA Polymerase	M0491 S/L	100/500 units
Q5 Hot Start High-Fidelity DNA Polymerase	M0493 S/L	100/500 units

advantages

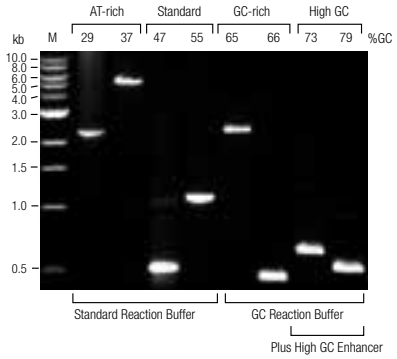
- **Fidelity** – the highest fidelity amplification available (> 100x higher than *Taq* and 2x higher than Phusion)
- **Robustness** – high specificity and yield with minimal optimization
- **Coverage** – superior performance for a broad range of amplicons (from high AT to high GC)
- **Speed** – short extension times
- **Amplicon length** – robust amplifications up to 20 kb for simple templates & 10 kb for complex

Request a **sample*** of **Q5 HiFi DNA Polymerase** at info.fr@neb.com

* Valid till 31/01/16 for one sample per person & per lab as long as stock lasts

One*Taq* DNA Polymerase – Robust PCR on nearly any template

One*Taq* DNA Polymerase is an optimized blend of *Taq* and Deep Vent DNA polymerases for use with routine and difficult PCR experiments. The 3´-> 5´ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robust amplification of *Taq* DNA Polymerase. The One*Taq* Reaction Buffers and High GC Enhancer have been formulated for robust yields with minimal optimization, regardless of a template's GC content.



Amplification of a selection of sequences with varying GC content.

ORDERING INFORMATION – SPECIAL DISCOUNT

PRODUCT	NEB #	SIZE
One*Taq* DNA Polymerase	M0480 S/L/X	200/1,000/5x1,000 units
One*Taq* Hot Start DNA Polymerase	M0481 S/L/X	200/1,000/5x1,000 units
One*Taq* Quick-Load 2x Master Mix with Standard Buffer	M0486 S/L	100/500 rxns
One*Taq* Quick-Load 2x Master Mix with GC Buffer	M0487 S/L	100/500 rxns
One*Taq* Hot Start Quick-Load 2x Master Mix with Standard Buffer	M0488 S/L	100/500 rxns
One*Taq* Hot Start Quick-Load 2x Master Mix with GC Buffer	M0489 S/L	100/500 rxns

advantages

- **Exceptional performance** in endpoint PCR across a wide range of templates (from AT to GC-rich)
- **Robust yields** with **minimal optimization**
- **2x higher fidelity** ("proof-reading") compared to standard *Taq* DNA Polymerase
- **Compatible** with standard *Taq* protocols

Request a **sample*** of **One*Taq* HS DNA Polymerase** at info.fr@neb.com

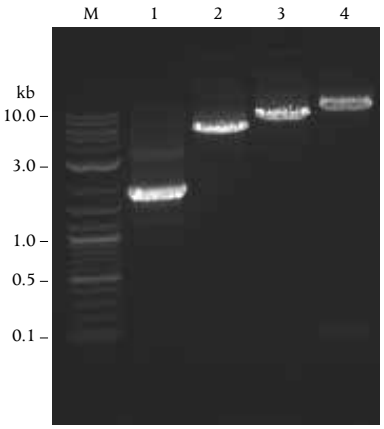
* Valid till 31/01/16 for one sample per person & per lab as long as stock lasts

SPECIAL DISCOUNT

ProtoScript II First Strand cDNA Synthesis Kit

ProtoScript II is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active at up to 48°C, providing higher specificity and higher yield of cDNA. ProtoScript II is available as stand-alone enzyme or as convenient ProtoScript II First Strand cDNA Synthesis Kit (including all reagents needed in reliable master mixes as well Oligo-d(T) and optimized Random Primer). The first strand cDNA product generated is up to 10 kb making ProtoScript II the best choice for any cDNA synthesis.

TechTip: ProtoScript II First Strand cDNA Synthesis Kit employs an unrivaled **RNase Inhibitor** to protect the precious RNA sample from unwanted degradation. The recombinant murine RNase Inhibitor has significantly improved resistance to oxidation compared to the human/porcine RNase inhibitors, and is stable at low DTT concentrations. Recombinant murine RNase Inhibitor is also available as a separate product.



First strand cDNA synthesis was carried out using the ProtoScript II First Strand cDNA Synthesis Kit at 42°C using 250 ng of Jurkat total RNA. Marker M is 2-Log DNA Ladder (NEB #N3200).

advantages

- **High yield** – more full length cDNA with reduced RNase H– activity
- **Convenience** – includes all necessary components for first strand cDNA synthesis from RNA
- **Streamlined protocols** – master mix formulation speeds reaction setup
- **Increased thermostability** – ProtoScript II Reverse Transcriptase exhibits improved thermostability over standard M-MuLV Reverse Transcriptase
- **Competitive pricing** – increased performance and value

-20%*

* Valid till 31/01/16 only with promocode **PROTO2015** & not cumulative with other discounts or special prices.

ORDERING INFORMATION – SPECIAL DISCOUNT

PRODUCT	NEB #	SIZE
ProtoScript II Reverse Transcriptase	M0368 S/L/X	4,000/10,000/40,000 units
ProtoScript II First Strand cDNA Synthesis Kit	E6560 S/L	30/150 rxns
Murine RNase Inhibitor	M0314 S/L	3,000/15,000 units

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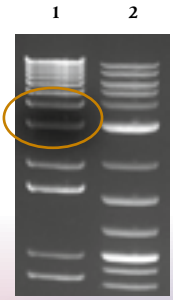
Purple is the new Black...

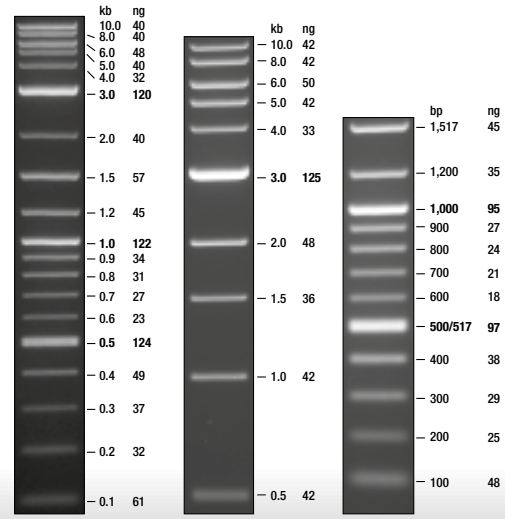
Now FREE Purple Gel-Loading Dye with every NEB DNA Ladder.

Nobody likes a UV shadow on their gels! With NEB's DNA Ladders, you will see sharper bands and no UV shadow at the dye front thanks to the new purple gel loading dye that is now included with NEB's DNA ladders.



UV shadow with conventional loading dye

Lane 1. Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder; Lane 2. NEB Quick-Load Purple 2-log DNA Ladder; 1 µg per gel lane



Please request your free NEB DNA Ladder magnet with your next order!

Ask for a **sample*** of **2-Log DNA ladder** at info.fr@neb.com

* Valid till 31/01/16 for one sample per person & per lab as long as stock lasts

ORDERING INFORMATION

PRODUCT	NEB #	SIZE
2-Log DNA Ladder (0.1-10.0 kb)	N3200 S/L	200/1,000 gel lanes
DNA Ladder 100 bp	N3231 S/L	100/500 gel lanes
DNA Ladder 1kb	N3232 S/L	200/1,000 gel lanes
Low Molecular Weight DNA Ladder	N3233 S/L	100/500 gel lanes
PCR Marker	N3234 S/L	100/500 gel lanes
50 bp DNA Ladder	N3236 S/L	200/1,000 gel lanes

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info@neb-china.com

Japan
New England Biolabs Japan, Inc.
Telephone: +81 (0)3 5669 6191
info@neb-japan.com

Singapore
New England Biolabs Pte. Ltd.
Telephone +65 6776 0903
sales.sg@neb.com