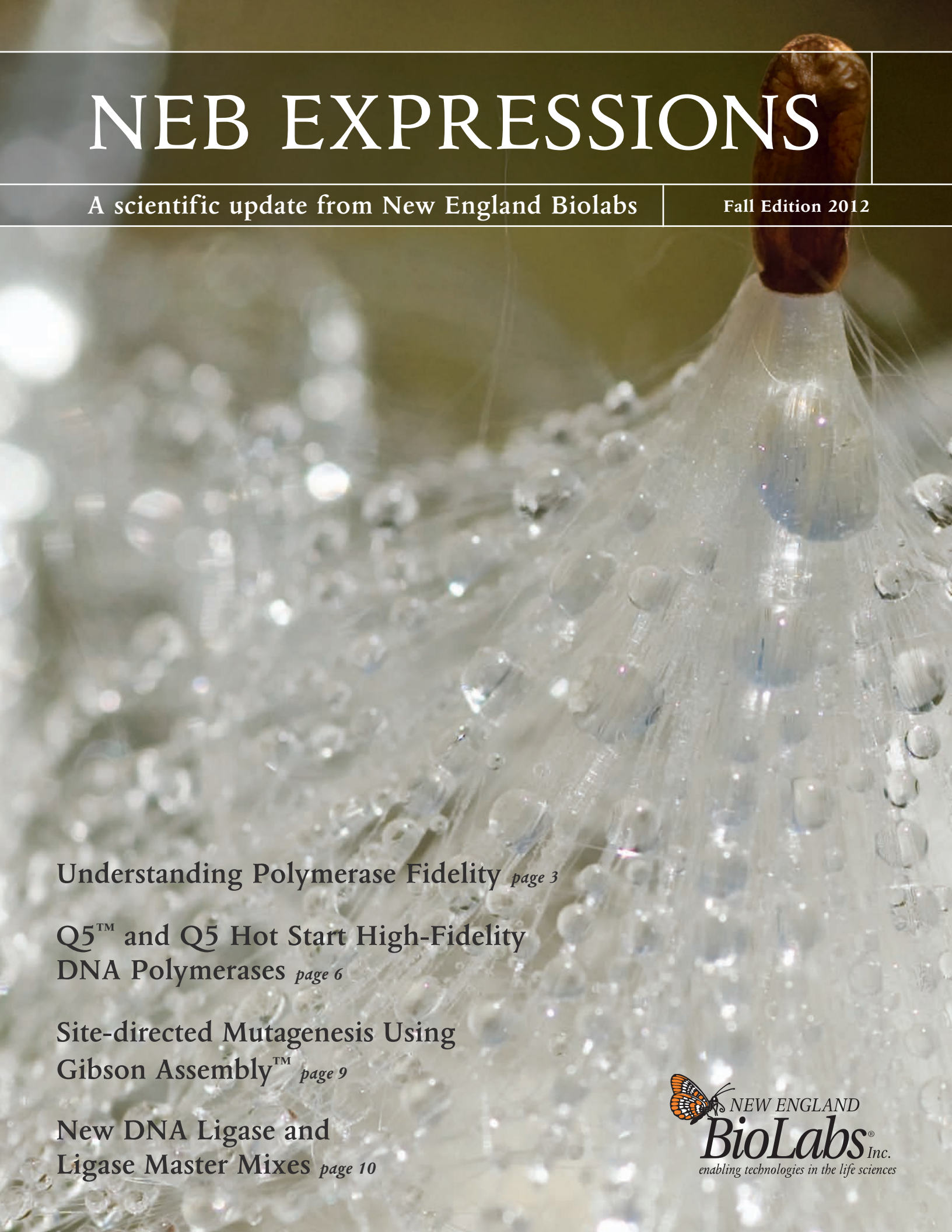
NEB EXPRESSIONS

A scientific update from New England Biolabs

Fall Edition 2012



NEW ENGLAND BioLabs® Inc.

enabling technologies in the life sciences

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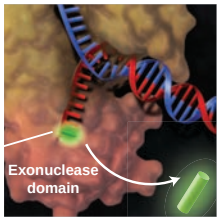
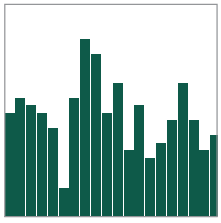
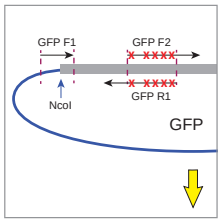
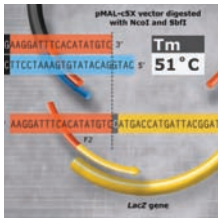
Fall Edition 2012









ON THE COVER: Milkweed seed (*Asclepias syriaca*) can be found exploding from its pod during the autumn months in New England. Milkweed is a host plant for the Monarch butterfly *(Danaus plexippus)*.

FEATURE ARTICLE

Polymerase Fidelity: What is it, and what does it mean for your PCR?

The discovery and development of high-fidelity polymerases has for many years been a key focus at New England Biolabs (NEB). High-fidelity amplification is essential for experiments whose outcome depends upon the correct DNA sequence (e.g., cloning, SNP analysis, NGS applications). Whereas traditional fidelity assays are sufficient for *Taq* and other moderately faithful enzymes, Q5, an ultra high-fidelity enzyme, pushes the limits of current methods used to assess this critical feature of DNA polymerases.

John A. Pezza, Ph.D., Rebecca Kucera, M.S., Luo Sun, Ph.D., New England Biolabs, Inc.

Introduction: What is fidelity?

The fidelity of a DNA polymerase is the result of accurate replication of a desired template. Specifically, this involves multiple steps, including the ability to read a template strand, select the appropriate nucleoside triphosphate and insert the correct nucleotide at the 3′ primer terminus, such that Watson-Crick base pairing is maintained. In addition to effective discrimination of correct versus incorrect nucleotide incorporation, some DNA polymerases possess a 3′→5′ exonuclease activity. This activity, known as "proofreading", is used to excise incorrectly incorporated mononucleotides that are then replaced with the correct nucleotide. High-fidelity PCR utilizes DNA polymerases that couple low misincorporation rates with proof-reading activity to give faithful replication of the target DNA of interest.

When is fidelity important?

Fidelity is important for applications in which the DNA sequence must be correct after amplification. Common examples include cloning/subcloning DNA for protein expression, SNP analysis and next generation sequencing applications. Fidelity is less important for many diagnostic applications where the read-out is simply the presence or absence of a product.

How does a high-fidelity polymerase ensure that the correct base is inserted?

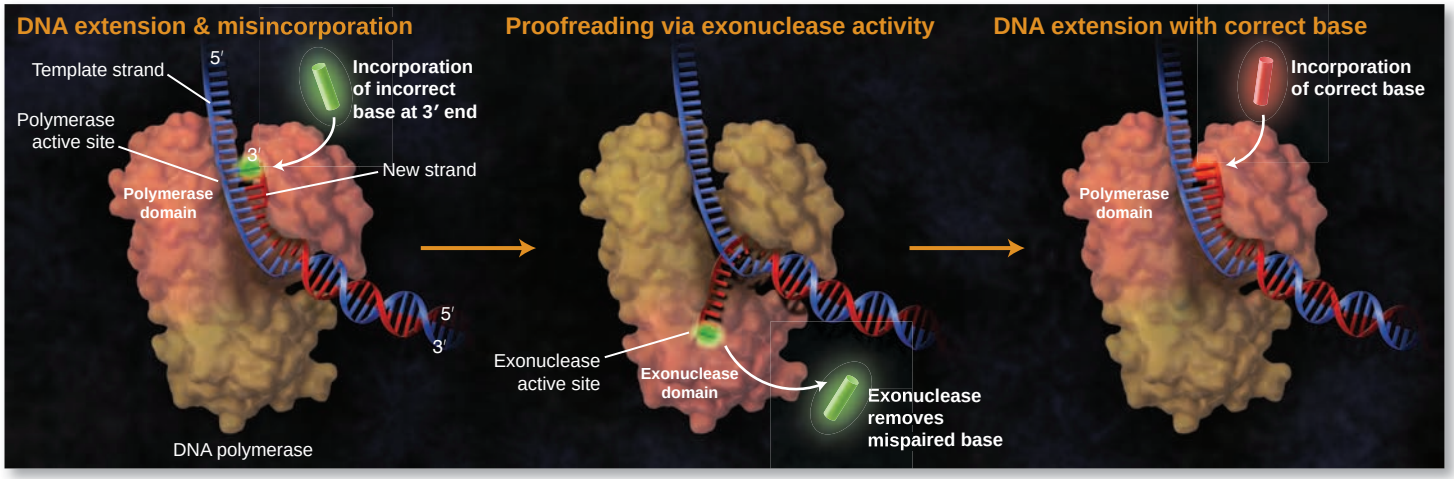
High-fidelity DNA polymerases have several safeguards to protect against both making and propagating mistakes while copying DNA. Such enzymes have a significant binding preference for the correct versus the incorrect nucleoside triphosphate during polymerization. If an incorrect nucleotide does bind in the polymerase active site, incorporation is slowed due to the sub-optimal architecture of the active site complex. This lag time increases the opportunity for the incorrect nucleotide to dissociate before polymerase progression, thereby allowing the process to start again, with a correct nucleoside triphosphate (1,2). If an incorrect nucleotide is inserted, proofreading DNA polymerases have an extra line of defense (Figure 1). The perturbation caused by the mispaired bases is detected, and the polymerase moves the 3′ end of the growing DNA chain into a proofreading 3′→5′ exonuclease domain. There, the incorrect nucleotide is removed by the 3′→5′ exonuclease activity, whereupon the chain is moved back into the polymerase domain, where polymerization can continue.

How is fidelity measured?

A variety of polymerase fidelity assays have been described in the literature over the years, perhaps the most famous being that of Thomas Kunkel (3). The Kunkel method uses portions of the *lacZ*α gene in M13 bacteriophage to correlate host bacterial colony color changes with errors in DNA synthesis. Wayne Barnes built upon this assay and utilized PCR to copy the entire *lacZ* gene and portions of two drug resistance genes with subsequent ligation, cloning, transformation and blue/white colony color determination (4). In both assays, errors incorporated in the *lacZ* gene cause a disruption in β-galactosidase activity leading to a white colony phenotype. With these *lacZ*-based experimental approaches, the percentage of white colonies must be converted to the number of errors per base incorporated. As a more direct read-out of fidelity, Sanger sequencing of individual cloned PCR products can also score DNA polymerase fidelity and offers the advantage that all mutations will be detected. Using this method, the entire mutational spectrum of a polymerase can be determined and there is no need to correct for non-phenotypic changes.

A modification of the *lacZ* Barnes assay is commonly used at NEB for determination of DNA polymerase fidelity, as the 1,000 amino acid open reading frame affords a reasonable sequence window for the scoring of DNA polymerase errors (Figure 2). In this study, results from the *lacZ* assay were compared to Sanger sequencing to assess the fidelity of Q5, a new NEB DNA polymerase (for more info on Q5, please see page 6).

Figure 1. DNA Replication with a Proofreading Polymerase



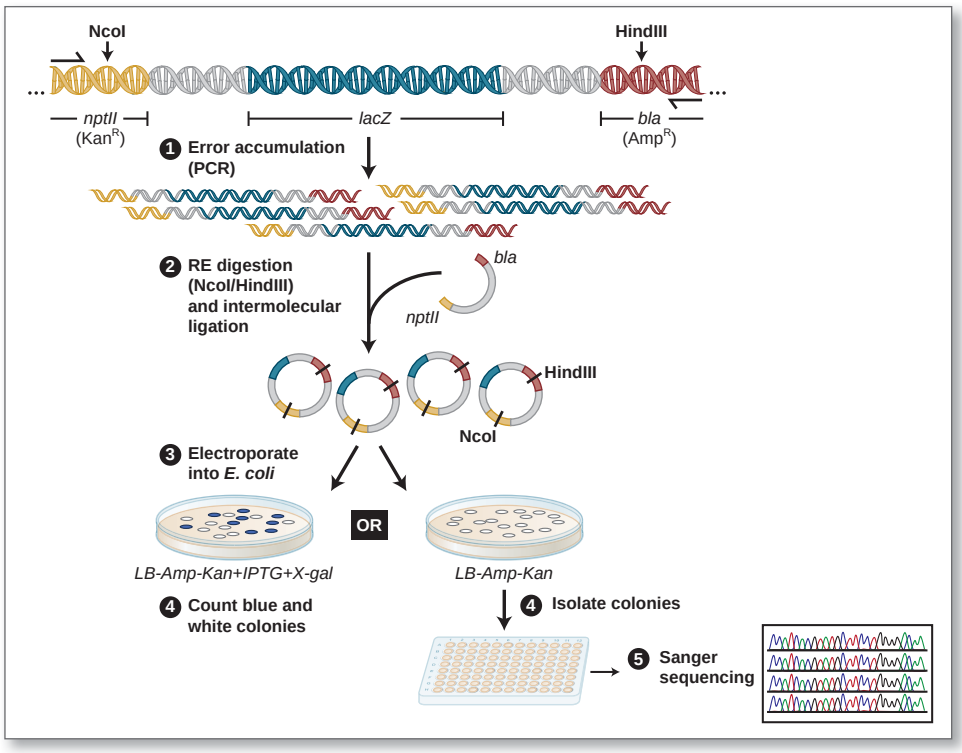
Extension proceeds along the template strand at the 3′ end of the newly synthesized strand. When the polymerase recognizes an error, the mismatched base is transferred to the exonuclease active site and the base is excised. The extended strand returns to the polymerase domain, re-anneals to the template strand, and replication continues.

Results

In this study, Q5 was examined to determine its fidelity compared to *Taq* DNA polymerase using the two methods described below (Figure 2). A 3,874 bp target was PCR amplified with either *Taq* (Thermopol Buffer), Q5 (Q5 Reaction Buffer with or without GC enhancer) or Phusion® (Phusion HF Buffer) DNA Polymerase. Observed mutation rates were determined using both the blue/white selection method after 16 PCR cycles (4) and by Sanger sequencing after 25 PCR cycles (Table 1). The error rate per base incorporated was determined after calculating the effective number of amplification cycles for each experiment as described previously (4, 5). Comparing the data sets from *Taq* indicates that the two methods generate similar results with error rates of ~1 in 3,500 bases. Q5, on the other hand, yielded a significantly lower number of errors than *Taq* in both assay systems, consistent with an error rate of ~10^{-6}. The side-by-side evaluation of *Taq* and Q5 using the blue/white method suggests that Q5 is ~200X more faithful at replicating DNA than *Taq*. Similar results were observed for Q5 when the GC enhancer was added to the reactions (data not shown). For Phusion, the error rate was determined to be 80±39 times better than *Taq* using the blue/white method and 84 times *Taq* using the sequencing method.

For Sanger sequencing, only two mutations were detected in the Q5 data set (despite sequencing over 440,000 nucleotides). Although this speaks directly to the strikingly high fidelity of the Q5 enzyme, it is more difficult to make statistically significant conclusions about either the absolute Q5 error rate or the comparative fidelity rate versus *Taq* using this particular data set.

Figure 2. Fidelity testing workflow



Conclusion

The ultra low error rate of Q5 is extremely beneficial for many applications. However, the low number of identified errors makes absolute error rate quantitation difficult for this enzyme, even with extensive experimentation and analysis. From blue/white screening, we have observed that Q5 is approximately 200X more faithful at replicating DNA than *Taq,* but results from Sanger sequencing hint that this value may actually be an underestimate.

Because the values generated from blue/white methods vary significantly between individual replicates and rely on a series of calculated extrapolations, we have chosen to conservatively represent the fidelity of Q5 High-Fidelity DNA Polymerase as >100X *Taq,* and Phusion High-Fidelity DNA Polymerase as >50X *Taq.* With ever-decreasing costs and extremely large datasets, next generation sequencing techniques may soon be able to provide direct, cost effective methods for more accurately quantitating error rates for an ultra high-fidelity polymerase like Q5.

Translating blue/white error rates into practical use, this data suggests that after using 25 PCR cycles to amplify a 400 bp fragment with *Taq*, several isolates should be screened since about half of the clones are predicted to have an error. For larger fragments of ~1000 bp, each clone amplified with *Taq* is likely to have an undesired mutation while the ultra low error rates of Q5 High-Fidelity DNA Polymerase predict that 199/200 clones amplified with this new enzyme will be correct.

Table 1. Calculations for fidelity testing of *Taq* and Q5 DNA Polymerases

METHOD		NOTES	*Taq*	Q5
Blue/white screening	Total colonies scored		30,192	22,296
	White colonies scored		17,589	119
	Error rate per base	Corrects for non-phenotypic changes and error propagation during PCR (4)	$2.7x10^{-4} \pm 0.8x10^{-4}$ (1 per 3,700 bases)	$1.4x10^{-6} \pm 0.6x10^{-6}$ (1 per 710,000 bases)
	Fold over *Taq*			193 ± 101
Sanger sequencing	Clones sequenced		340 (~215,000 nts)	710 (~440,000 nts)
	Mutations detected		279	2
	Error rate per base	Corrects for error propagation during PCR (5)	$\sim 3.0x10^{-4}$ (1 per 3,300 bases)	[$\sim 1.0x10^{-6}$] [~1 per 1,000,000 bases]
	Fold over *Taq*			[~300]

±= 95% confidence
Numbers in brackets have limited statistical significance as only 2 mutations were detected after sequencing 441,670 nucleotides.

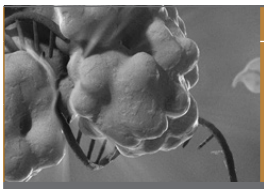
References

1. Johnson, K.A. (2010) *Biochemica et Biophysica Acta,* 1804, 1041–1048.
2. Joyce, C.M. and Benkovic, S.J. (2004) *Biochemistry,* 43, 14317–14324.
3. Kunkel, T.A. and Tindall, K.R. (1988) *Biochemistry,* 27, 6008–6013.
4. Barnes, W.M. (1992) *Gene,* 112, 29–35.
5. Eckert, K.A. and Kunkel, T.A. (1991) *PCR Methods and Applications,* 1, 17–24.

PCR Polymerase Selection Chart

For over 35 years, New England Biolabs, Inc. has been a world leader in the discovery and production of reagents for the life science industry. NEB offers a wide range of DNA polymerases and, through our commitment to research, ensures the development of innovative, high-quality tools for PCR.

When choosing a polymerase for PCR, we recommend starting with **One*Taq*** or **Q5 DNA Polymerase** for routine PCR applications, and **NEBNext High-Fidelity Master Mix** for next gen sequencing (NGS) library construction (shown below in gold). These all offer robust amplification, and can be used on a wide range of templates (routine, AT- and GC-rich). Q5 and NEBNext Master Mix (a Q5 formulation) provide the added benefit of maximum fidelity.



	STANDARD PCR		HIGH-FIDELITY PCR			SPECIALTY PCR		
			Highest Fidelity		Moderate Fidelity	Long Amplicons	Bisulfite Sequencing	Blood Direct PCR
	One*Taq*/ One*Taq* Hot Start	*Taq*	Q5/Q5 Hot Start	Phusion[4]/ Phusion[4] Flex	Vent$_R$/ Deep Vent$_R$™	LongAmp®/ LongAmp Hot Start *Taq*	Epimark® Hot Start *Taq*	Hemo Klen *Taq*™
DNA APPLICATIONS								
Fidelity vs. *Taq*	2X	1X	>100X	>50X	5–6X	2X	1X	ND
Amplicon Size	<6 kb	≤5 kb	≤20 kb	≤20 kb	≤6 kb	≤30 kb	≤1 kb	≤2 kb
Extension Time	1 kb/min	1 kb/min	6 kb/min	4 kb/min	1 kb/min	1.2 kb/min	1 kb/min	0.5 kb/min
Resulting Ends	3′ A/Blunt	3′ A	Blunt	Blunt	Blunt	3′ A/Blunt	3′ A	3′ A
3′→5′ exo	Yes	No	Yes	Yes	Yes	Yes	No	No
5′→3′ exo	Yes	Yes	No	No	No	Yes	Yes	No
Units/50 µl Reaction	1.25	1.25	1.0	1.0	0.5–1.0	5.0	1.25	N/A
APPLICATIONS								
Routine PCR	★	●	●	●	●	●		
Colony PCR	★	●						
Enhanced Fidelity	●		★	●	●	●		
High Fidelity			★	●				
High Yield	★	●	★	●				
Fast			★	●				
Long Amplicon			★	●		★		
GC-rich Targets	★		★		●	●		
AT-rich Targets	★	●	★	●		●	★	
High Throughput	●	●	●	●			●	
Multiplex PCR	●	★[1]	●	●				
Extraction-free PCR								★
DNA-labeling		★						
Site-directed Mutagenesis			★	●				
Bisulfite sequencing							★	
NGS APPLICATIONS								
NGS Library Amplification	●		★[3]	●				
FORMATS								
Hot Start Available	●	●	●	●		●	●	
Kit		●		●		●		
Master Mix Available	●	●	●	●		●		
Direct Gel Loading	●	●[2]						

(1) Use Multiplex PCR 5X Master Mix.
(2) Use Crimson Taq DNA Polymerase.
(3) Use NEBNext High-Fidelity 2X PCR Master Mix
(4) Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific.

★ indicates recommended choice for application
ND indicates not determined

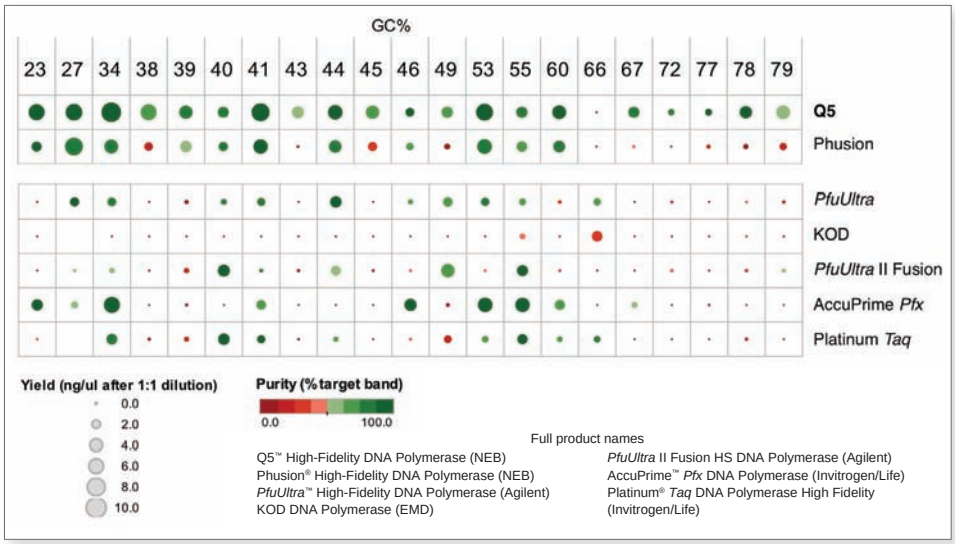
Q5 High-Fidelity DNA Polymerase

Q5 High-Fidelity DNA Polymerase sets a new standard for both fidelity and performance. With the highest-fidelity amplification available (>100 times higher than *Taq*), Q5 DNA Polymerase results in ultra-low error rates. Q5 DNA Polymerase is composed of a novel polymerase that is fused to the processivity-enhancing Sso7d DNA binding domain, improving speed, fidelity and reliability of performance.

The Q5 buffer system is designed to provide superior performance with minimal optimization across a broad range of amplicons, regardless of GC content. For routine or complex amplicons up to ~65% GC content, the Q5 Reaction Buffer provides reliable and robust amplification. For amplicons with high GC content (>65% GC), addition of the Q5 High GC Enhancer ensures continued maximum performance. Q5 and Q5 Hot Start DNA Polymerases are available as standalone enzymes or in master mix format, for added convenience. Master mix formulations include dNTPs, Mg^{++} and all necessary buffer components to ensure robust performance, even on GC-rich templates.

In contrast to chemically-modified or antibody-based hot start polymerases, NEB's Q5 Hot Start DNA Polymerase utilizes a unique synthetic aptamer. This structure binds to the polymerase through non-covalent interactions, blocking activity during the reaction setup. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. Q5 Hot Start does not require a separate high temperature activation step, shortening reaction times and increasing ease-of-use. Q5 Hot Start is an ideal choice for high specificity amplification and provides robust amplification of a wide variety of amplicons, regardless of GC content.

Q5 provides superior performance across a wide range of genomic targets



PCR was performed with a variety of amplicons, with GC content ranging from high AT to high GC, using Q5 and several other commercially available polymerases. All polymerases were cycled according to manufacturers' recommendations, including the use of GC Buffers and enhancers, when recommended. Yield and purity of reaction products were quantitated and represented, as shown in the figure key, by dot color and size. A large dark green dot represents the most successful performance. Q5 provides superior performance across the range of GC content.

Ordering Information

PRODUCT	NEB #	SIZE
Q5 High-Fidelity DNA Polymerase	M0491S/L	100/500 units
Q5 Hot Start High-Fidelity DNA Polymerase	M0493S/L	100/500 units
Q5 High-Fidelity 2X Master Mix	M0492S/L	100/500 reactions
Q5 Hot Start High-Fidelity 2X Master Mix	M0494S/L	100/500 reactions

Five Quality Features of Q5 DNA Polymerase

- **Fidelity** – the highest-fidelity amplification available (>100X higher than *Taq*)
- **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)

Mandarin Ducks *(Aix galericulata)* are frequently featured in Chinese art and regarded as a symbol of fidelity.

NEBNext High-Fidelity 2X PCR Master Mix for Next Generation Sequencing Library Amplification

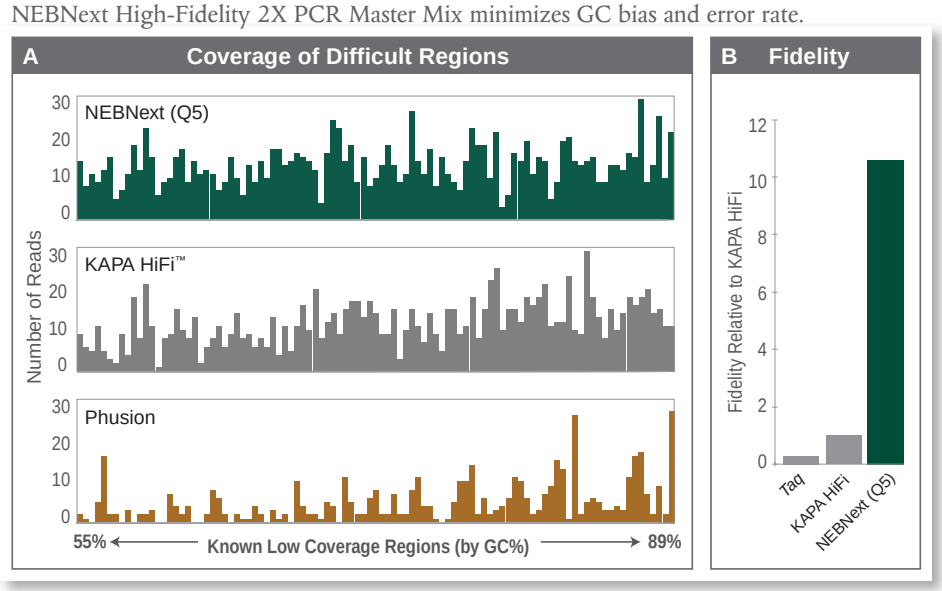
The NEBNext High-Fidelity 2X PCR Master Mix is specifically optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries, regardless of GC content. This unique formulation combines the ultra low error rates of Q5 High-Fidelity DNA Polymerase with specific buffers and reaction enhancers to support robust amplification of a broad range of templates, even those with high GC content. The buffer component of the master mix has been optimized for robust amplification, even with GC-rich amplicons.

This combination makes the NEBNext High-Fidelity 2X PCR Master Mix ideal for NGS library construction. This convenient 2X master mix contains dNTPs, Mg^{++} and a unique buffer formulation that eliminates the need for additional GC enhancers. The addition of primers and DNA templates are all that is required for robust amplification.

Advantages

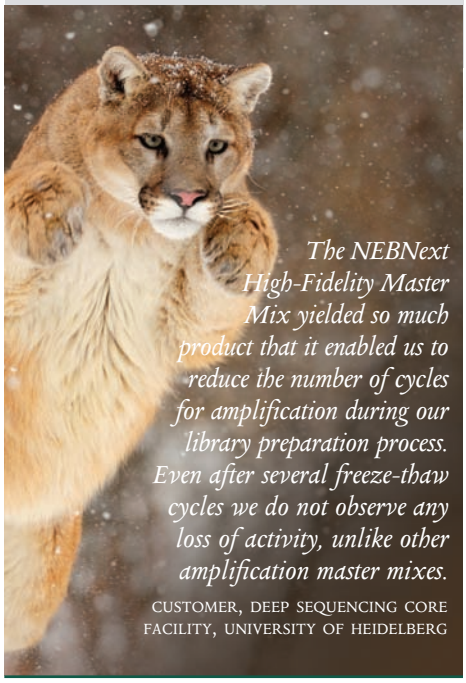
- Minimizes GC bias
- Ultra-high fidelity amplification
- Robust, even amplification
- Optimized for NGS library amplification
- Convenient 2X master mix contains dNTPs, Mg^{++} and a proprietary buffer, and requires only the addition of primers and DNA template for robust amplification

NEBNext High-Fidelity 2X PCR Master Mix minimizes GC bias and error rate.



A. Indexed human IMR90 DNA libraries were split and amplified using 8 cycles of PCR with Phusion High-Fidelity PCR Master Mix with HF Buffer, KAPA HiFi HotStart ReadyMix or NEBNext High-Fidelity 2X PCR Master Mix, followed by sequencing on an Illumina HiSeq™ 2000. 180 million reads were randomly extracted from each data set and the number of reads overlapping distinct low-coverage regions of the human genome (Aird et.al. Genome Biology, 2011) are shown for each library.

B. Fidelity measurements of Taq DNA Polymerase (in Standard Taq Buffer), KAPA HiFi HotStart ReadyMix and NEBNext High-Fidelity 2X PCR Master Mix were measured, side-by-side, in a PCR-based mutation screening assay using a lacZ method. Values ($n \geq 2$) are expressed relative to KAPA HiFi HotStart ReadyMix.



Ordering Information

PRODUCT	NEB #	SIZE
NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 reactions

APPLICATION NOTE

Multiple Base, Site-Directed Mutagenesis Using Gibson Assembly Master Mix

Ezra Schildkraut, Ph.D., New England Biolabs, Inc.

Introduction

Site-directed mutagenesis (SDM) is a commonly-used technique for introducing mutations into a gene of interest. Existing techniques for SDM, such as whole plasmid SDM, while effective, are time consuming and prone to off-target mutation incorporation. Further, verification of mutation incorporation can be difficult when the insertion site sequence lacks convenient restriction sites for analysis. This can be a serious impediment to the planning and execution of SDM experiments.

Gibson Assembly, developed by Daniel Gibson and his colleagues at the J. Craig Venter Institute, is a rapid and reliable method for the assembly of DNA fragments. The technique, which involves the design of complimentary flanking primers to align fragments, can be readily adapted for SDM applications. In addition, it is unnecessary to use phosphorylated primers for Gibson Assembly, reducing both cost and time. In one step, two or more PCR products with overlapping ends can be assembled into a pre-cut vector. An exonuclease creates single-stranded 3´ overhangs that promote annealing of complementary fragments at the overlap region. A polymerase then fills in the gaps which are sealed by the DNA ligase. By introducing multiple complementary mutations in the primers at the overlap region (Figure 1), the Gibson Assembly Master Mix forms a single, covalently bonded DNA molecule, containing the desired mutations, that is able to be directly transformed into competent cells and screened or sequenced.

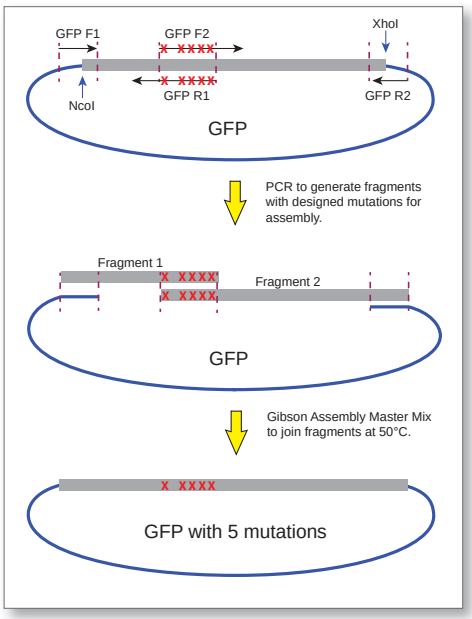
Results

In this experiment, Gibson Assembly Master Mix was used to alter 5 of the 6 nucleotides at positions 174-179 of enhanced Green Fluorescent Protein (eGFP), from CTGACC (LeuThr) to TTCTAT (PheTyr). Overlapping primers were designed to incorporate the appropriate mutations to alter the 2 amino acids (Table 1). PCR was performed using Q5 High-Fidelity DNA Polymerase (NEB# M0491) according to the recommended protocol. The vector DNA was digested with NcoI and XhoI. The Gibson Assembly reaction was then performed, and the resulting DNA was transformed into NEB T7 Express I^q Competent *E. coli* (NEB# C3016). Transformation resulted in several thousand colonies. Ten colonies were screened by sequencing, with 5 of the 10 containing the desired sequence changes.

Summary

The Gibson Assembly Master Mix was used to successfully incorporate 5 single base changes within a six base sequence of the GFP gene. Two PCR amplicons, overlapping at the location of altered bases, were assembled with a restriction enzyme-digested vector in one step. The resulting colonies were screened by sequence analysis, demonstrating that 50% of the colonies contained the desired sequence change. In addition to multiple base mutation, Gibson Assembly can be used to incorporate multiple mutations in different locations throughout a sequence by creating multiple fragments for assembly in one step. As opposed to traditional methods which involve creating mutations sequentially, this method offers significant time and cost savings.

Figure 1. Site-directed mutagenesis of eGFP using Gibson Assembly



In this example, 5 nucleotides were changed by introducing the mutations into the overlapping primer, followed by PCR. Gibson Assembly was then used to join the fragments with restriction enzyme-digested vector.

Table 1. Overlapping primers used for SDM of eGFP*

PRIMER	SEQUENCE 5´→ 3´
GFP-F1	TTAAGAAGGAGATATACCATGGAGCTTTTCACT
GFP-R1	CACGCCGTAATAGAAGGTGGTCACGAGGGTGGG
GFP-F2	GTGACCACCTTCTATTACGGCGTGCAGTGCTTC
GFP-R2	GATCTCCTAAGGCTCGAGTTAGATCCCGGCGGCGGTCAC

* *red indicates changed nucleotides*

Ordering Information

PRODUCT	NEB #	SIZE
Gibson Assembly Master Mix	E2611S/L	10/50 reactions
COMPANION PRODUCTS	**NEB #**	**SIZE**
T7 Express I^q Competent *E. Coli*	C3016I/H	0.2/0.05 ml/tube
Q5 High-Fidelity DNA Polymerase	M0491S/L	100/500 units
NcoI	R0193T/S/M/L	1,000/5,000 units
XhoI	R0146S/M/L	5,000/25,000 units
Deoxynucleotide Solution Mix	N0447S/L	8/40 µmol of each

NEW PRODUCTS

Gibson Assembly Cloning Kit

New England Biolabs has revolutionized your laboratory's standard cloning methodology. The Gibson Assembly Cloning Kit combines the power of the Gibson Assembly Master Mix with NEB 5-alpha Competent *E. coli,* enabling fragment assembly and cloning in just under two hours. Save time, without sacrificing efficiency.

Gibson Assembly was developed by Dr. Daniel Gibson and his colleagues at the J. Craig Venter Institute and licensed to NEB by Synthetic Genomics, Inc., and allows for successful assembly of multiple DNA fragments, regardless of fragment length or end compatibility.

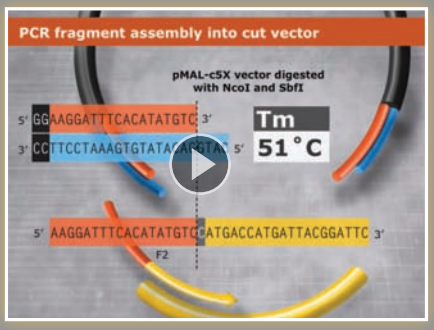
Gibson Assembly efficiently joins multiple overlapping DNA fragments in a single-tube isothermal reaction. The Gibson Assembly Master Mix includes three different enzymatic activities that perform in a single buffer: 1) The exonuclease creates a single-stranded 3´ overhang that facilitates the annealing of fragments that share complementarity at one end, 2) The polymerase fills in gaps within each annealed fragment, and 3) The DNA ligase seals nicks in the assembled DNA. Resulting DNA is ready to be transformed.

Making ends meet is now quicker and easier than ever before, with the Gibson Assembly Cloning Kit from NEB.

Advantages

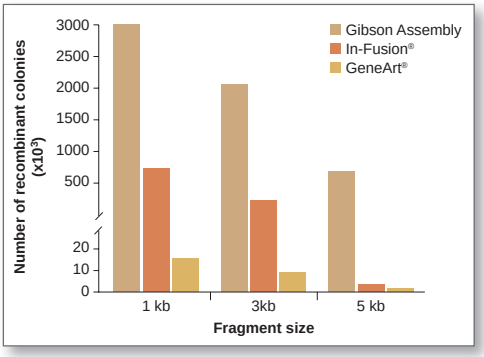
- Rapid cloning into any vector with no additional sequence added
- Easy-to-use protocols enable cloning and transformation in just under two hours
- No PCR clean-up step required
- High efficiencies, even with assembled fragments up to 20 kb
- Includes competent cells

Find out more…



Learn how simple it really is to use the Gibson Assembly Cloning Kit! Visit NEBGibson.com to view our latest tutorials.

Gibson Assembly Cloning Kit provides robust transformation efficiencies



Assembly reactions containing 25 ng of linear pUC19 vector and 0.04 pmol of each fragment were performed following individual suppliers' recommended protocols and using their competent cells. The total number of recombinant colonies was calculated per 25 ng of pUC19 vector added to the assembly reaction.

Ordering Information

PRODUCT	NEB #	SIZE
Gibson Assembly Cloning Kit	E5510S	10 reactions

Some components of this product are manufactured by New England Biolabs, Inc. under license from Synthetic Genomics, Inc.

SYNTHETIC GENOMICS®

NEBuilder™ for Gibson Assembly

Speed up your experimental design with our new primer design tool at **NEBGibson.com**.

Choose the Right DNA Ligase Product for Your Needs

While more than one ligase may work for your application, the following selection chart presents our recommendations for optimal performance.

	NEW Blunt/TA Ligase Master Mix	NEW Instant Sticky-end Master Mix	NEW ElectroLigase™	T4 DNA Ligase	Quick Ligation Kit	NEW T3 DNA Ligase	NEW T7 DNA Ligase	*E. coli* DNA Ligase	*Taq* DNA Ligase	9°N DNA Ligase	Quick Ligation Module
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									★★★	★★★	
NGS APPLICATIONS											
NGS Library Prep dsDNA-dsDNA (ligation)	▲			▲		▲					▲
FEATURES											
Salt tolerance (>2X that of T4 DNA Ligase)						✓					
Ligation in 15 min. or less	✓	✓		✓	✓	✓			✓	✓	✓
Master Mix Formulation	✓	✓									✓
Thermostable									✓	✓	
Recombinant	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ORDERING INFORMATION											
NEB #	M0367S/L	M0370S/L	M0369S	M0202S L/M/T	M2200S/L	M0317S/L	M0318S/L	M0205S/L	M0208S/L	M0238S/L	E6056S/L
Unit size	50/250 rxns	50/250 rxns	50 rxns	20,000 / 10,000 units	30/150 rxns	100,000 / 750,000 units	100,000 / 750,000 units	200/1,000 units	2,000 / 10,000 units	2,500 / 12,500 units	20/150 rxns

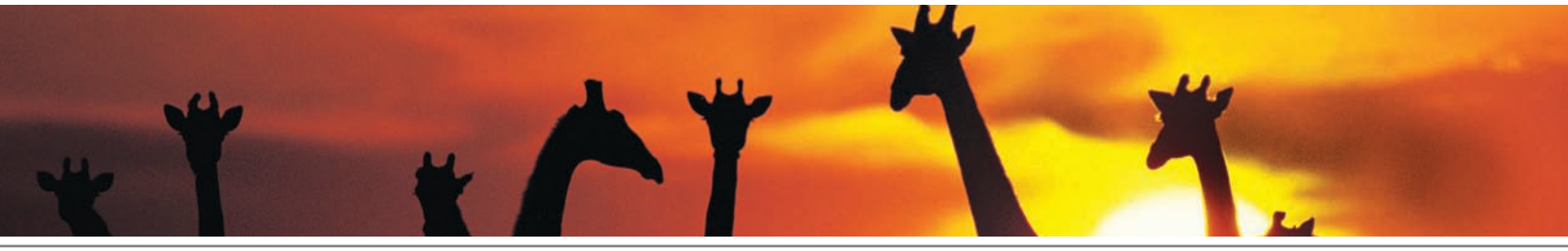
KEY

★★★ 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



Five Reasons to Choose DNA Ligases from NEB

1. Save time with faster ligations

If speed is essential, don't delay your cloning experiment for another moment! NEB's entire selection of DNA ligases is able to ligate your DNA completely in 30 minutes or less. Try our new **Instant Sticky-end Ligase Master Mix** for ligations without incubation!

2. Ensure successful ligations with formulations specific for blunt-, T/A or sticky-end substrates

NEB offers ligases specifically optimized for either blunt or sticky ends, including single-base overhangs. This increased specificity gives you confidence that your cloning experiment will succeed the first time, every time. New products include the **Blunt/TA Ligase Master Mix**.

3. Maximize your transformation efficiencies

NEB's highly pure DNA ligases ensure high specificity ligation, an absolute prerequisite for successful cloning. If electroporation is your preferred transformation method, use NEB's new specifically formulated **ElectroLigase**.

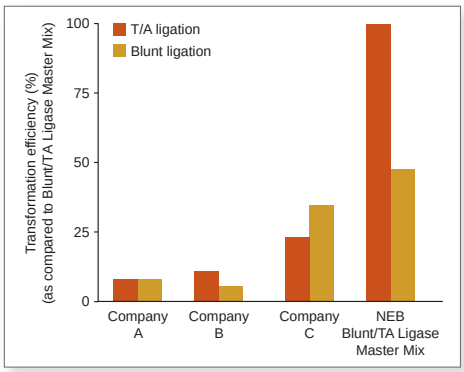
4. Experience extreme purity

NEB ligases and ligase master mixes are manufactured to the highest level of purity, and then rigorously tested for optimal performance. NEB's **T4 DNA Ligase** has been referenced in peer-reviewed publications for over 20 years, and is renowned for its consistent quality.

5. Choose from the widest selection of ligases commercially available

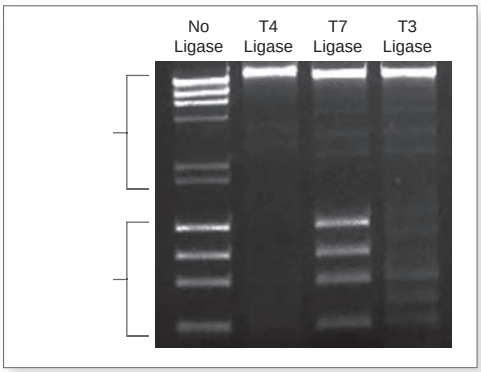
NEB offers 10 different DNA ligase products, more than any other supplier. Our expanded line of products now includes **T7 DNA Ligase**, specific for sticky-end ligation.

Blunt/TA Ligase Master Mix outperforms the competition



Duplicate ligation reactions of blunt or T/A vector/insert pairs were set up according to the master mix vendors' suggestions. Equal amounts of ligated DNA were used to transform NEB 10-beta Competent E. coli (NEB #C3019) and triplicate plating was performed. Transformation results were averaged and graphed as a percentage of the highest performing reaction, T/A ligation, using the Blunt/TA Ligase Master Mix.

Sticky-end specific ligation with T7 DNA Ligase



Ligation reactions containing blunt- (ΦX174 DNA-HaeIII Digest, NEB #N3026) and sticky-end (λ - HindIII Digest, NEB #N3012) fragments were set up with 200 ng of each substrate and 1 µl of each ligase, and incubated for 30 minutes at 25°C in their corresponding reaction buffers. Reactions were immediately stopped with 6X loading dye and resolved by electrophoresis on a 1% agarose gel and stained with ethidium bromide.

Visit NEBSticktogether.com

Learn how to optimize your ligation experiments in the latest videos from NEB scientists. Topics include:

- Why use PEG in a ligation?
- What is a difficult ligation?
- What are the best reaction times and temperatures?
- What are the best ratios of reactants?
- Which ligase should I choose?

Request a **free sample** of our Blunt/TA and Instant Sticky-end Ligase Master Mixes, give us your feedback and be entered to win an iPad®2.

Offer valid while supplies last. Limit one sample per customer. Winner will be notified by 11/30/12.

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