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RESEARCH SPOTLIGHT

Lessons from the sea: Sea urchins as models for aging and cancer research

Andrea Bodnar, Ph.D., Science Director,
Gloucester Marine Genomics Institute

NEB has long recognized the potential of the marine environment as a source for new discovery and the need to protect and preserve the ocean's vast biodiversity for the benefit of humankind. The unique adaptations of marine organisms have made them valuable models for biomedical research, provided novel therapeutics for human disease and uncovered new tools to advance biotechnology. Located just 12 miles from NEB's headquarters in Ipswich MA, a new marine biotechnology institute, Gloucester Marine Genomics Institute (GMGI), is applying innovative genomic technologies to marine science for discoveries that impact human health, biotechnology and fisheries. In February, GMGI's Science Director, Dr. Andrea Bodnar, visited NEB to provide an overview of the research programs at GMGI and to present some of her work using sea urchins as models to unlock the secrets of living a long and healthy life.

Understanding Extreme Longevity

The oceans are home to many of the Earth's longest-lived animals with several non-colonial marine invertebrates and vertebrates documented to live for more than 100 years (Table 1) (1–8). Many of these animals grow and reproduce throughout their lifespans with no apparent functional decline, no increased incidence of disease or increase in mortality rate with age. A better understanding of the mechanisms by which these animals achieve their extraordinary life histories may reveal exceptionally effective defenses against the destructive process of aging and suggest novel avenues to prevent or treat human age-related degenerative diseases.

The red sea urchin is among the Earth's longest-lived animals, estimated to live for more than 200 years without evidence of age-related decline and no reported cases of cancer (6,7,9). Sea urchins have served as model organisms for scientific research for more than a century and provide a unique opportunity to investigate the mechanisms underlying extreme longevity and negligible aging. Sea urchins have contributed to our understanding of important biological processes including fertilization, the role of chromosomes in inheritance and the gene regulatory networks that guide embryonic development. The fact that cell division is synchronized in early sea urchin development facilitated Tim Hunt's Nobel prize winning discovery of cyclins, proteins that play a key role in controlling cell cycle. Part of their value as a model organism is their close genetic relationship with humans; as non-chordate deuterostomes, sea urchins are one of our closest invertebrate relatives. This makes them ideal models to investigate the cellular pathways contributing to longevity and disease resistance with direct relevance to human health.

In addition, sea urchins are commercially fished and therefore considerable data are available regarding their growth, survival, longevity, susceptibility to disease and reproductive patterns as this information is essential for effective fisheries management (10). From these data it has been noted that different species of sea urchins exhibit very different natural lifespans in the wild. While the red sea urchin (*Mesocentrotus franciscanus*) is reported to be very long-lived, the purple sea urchin (*Strongylocentrotus purpuratus*) has an estimated maximum life expectancy of more than 50 years and the variegated sea urchin (*Lytechinus variegatus*) has an estimated life expectancy of about 4 years (6,7,11-13). Comparisons between long-, intermediate- and short-lived species provide an excellent model to understand mechanisms of lifespan determination and can provide insight into how these animals avoid the process of aging.

Aging is a complex and multifactorial process and there have been many theories proposed to explain this phenomenon at the molecular, cellular, systemic and evolutionary levels (14). Human aging is accompanied by the shortening of telomeres (caps that protect the ends of chromosomes), accumulation of cellular oxidative damage, and reduced ability to repair and replenish damaged tissues. In contrast, sea urchins maintain their telomeres(15,16), have little accumulation of oxidative damage (17) and maintain the ability to continually regenerate lost or damaged appendages throughout their lives (18). In most animals, there is a delicate balance between promoting cell renewal and regeneration for maintaining healthy tissues, and the danger of unchecked, abnormal cell growth that defines cancer. Notably, there are no documented cases of cancer in sea urchins (19,20). The ability of sea urchins to continually grow and regenerate while apparently resisting cancer holds great promise for discovering naturally occurring cancer prevention mechanisms.

Initial studies with sea urchins have demonstrated that the circulating cells (coelomocytes) are highly resistance to DNA damaging agents, invoke a robust DNA repair response and can effectively repair DNA damage (21,22). However, more study is required to determine if the high resistance to DNA damage in sea urchin cells contributes to the low incidence of cancer and to understand the cellular mechanisms protecting DNA.

At GMGI, our goal is to use sea urchins and other long-lived marine animals as models to identify the key genes and cellular pathways involved in long-term maintenance of tissues and resistance to cancer. Insight gained from studying exceptional longevity in sea urchins may reveal novel strategies to slow the destructive process of aging and identify new avenues for prevention or treatment of age-related diseases such as cancer.

Dr. Bodnar would like to thank Dr. Barton Slatko for the invitation to participate in NEB's seminar series.

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< The red sea urchin (*Mesocentrotus franciscanus*) is one of the earth's longest-lived animals, potentially living for more than 200 years with no signs of aging and no reported cases of cancer.

LONGEVITY IN NON-COLONIAL MARINE ANIMALS

Common Name	Species	Oldest Recorded Lifespan (Years)
Ocean quahog clam	*Arctica islandica*	507[1]
Greenland shark	*Somniosus microcephalus*	392[2]
Marine tubeworm	*Lamellibrachia sp.*	250[3]
Bowhead whale	*Balaena mysticetus*	211[4]
Rougheye rockfish	*Sebastes aleutianus*	205[5]
Red sea urchin	*Mesocentrotus franciscanus*	200[6,7]
Geoduck clam	*Panopea abrupta*	168[8]

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Founded in 2013, Gloucester Marine Genomics Institute is a 501(c)3 with a mission to conduct world class marine biotechnology research which expands the regional economy.

Breaking through the Limitations of Golden Gate Assembly – The Co-Evolution of Test Systems, Engineered Enzymes and Understanding Ligase Fidelity

by Rebecca Kucera, M.Sc. and Eric Cantor, Ph.D., New England Biolabs, Inc

INTRODUCTION

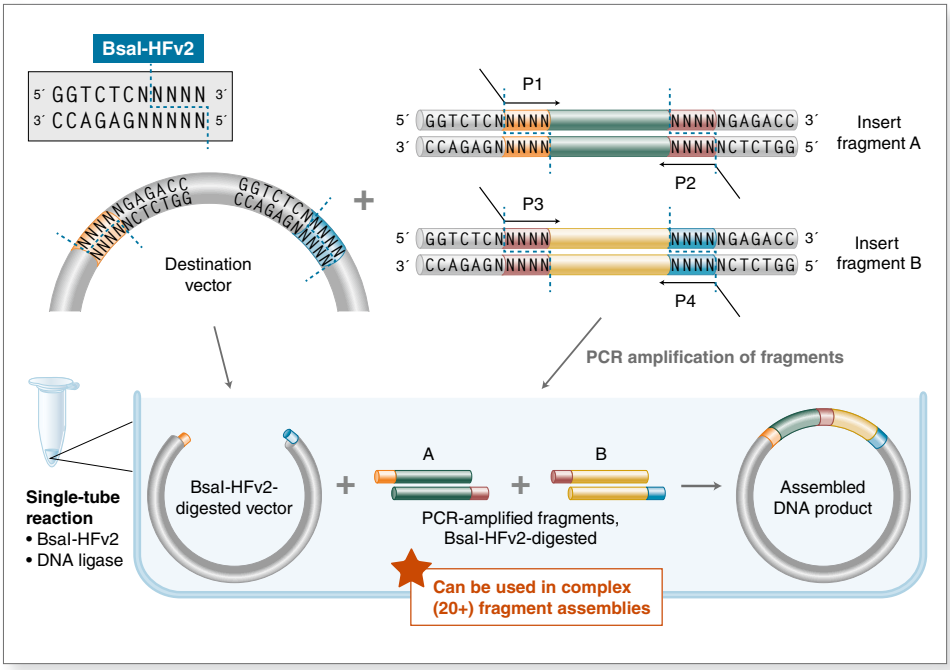
Golden Gate Assembly is a molecular cloning technique that utilizes simultaneous digestion with type IIS restriction enzymes and ligation by a DNA ligase to enable the scarless, ordered assembly of multiple fragments (1). Embraced by the synthetic biology community as well as the general molecular biology community, it is commonly used to assemble 2–10 inserts, or modules, in a single "one-pot" reaction to form complex, multi-insert modular assemblies that enable biosynthetic pathway engineering and optimization. However, Golden Gate is also useful for both single-insert cloning, and inserts from diverse populations that enable library creation.

Current best practices for assemblies of more than 10 modules often rely on two-step hierarchical approaches using different type IIS restriction enzyme specificities at each step. In the past, variables such as enzyme efficiency, stability and compatibility in a common buffer have placed practical limits on single- or two-step assemblies. We have greatly reduced those limitations through ongoing engineering efforts of type IIS restriction enzymes and the use of experimentally-derived T4 DNA Ligase fidelity data to guide the selection of four base overhangs. This work demonstrates that it is now possible to achieve 20+ fragment assemblies with both robust efficiency and accuracy.

DEVELOPMENT OF GOLDEN GATE ASSEMBLY TEST SYSTEMS

New England Biolabs has been committed to further developing Golden Gate protocols and enzymes for a number of years, a commitment that has enabled the development of a variety of test systems with increasing complexities. These more difficult assemblies have allowed us to identify and implement improvements in Golden Gate assembly that could not be detected with simpler 1–10 insert test systems.

FIGURE 1:
Golden Gate Assembly workflow for complex assemblies



Our research focused on three different levels of assembly:

- high efficiency and accurate single-insert assembly
- intermediate 5- or 12-fragment assembly, mirroring the commonly perceived "upper limit" for assembly
- more complex 24-fragment assembly

Table 1 (page 4) illustrates the breadth of testing systems used by NEB to address this range of usage for Golden Gate as assembly approaches have evolved.

Before assembly optimization, each test system was evaluated in a variety of ways. Single insert cloning based on the acquisition of a selectable antibiotic marker allowed fast throughput testing of efficiencies. This cloning was also compared to a similar-sized lambda amplicon to indicate any possible bias towards suppression of background by antibiotic selection. Screening of transformants by colony PCR confirmed the insertion of the lambda insert at the same high frequencies. The 5-, 12- and 24-assembly systems are based on the ability to correctly assemble a *lacI*/*lacZ* cassette (designed by NEB for use in many of our cloning systems) to produce a blue color phenotype upon growth on LB/Cam/X-gal/IPTG agar plates, indicating successful reconstruction of the coding sequence for beta-galactosidase in the *lacI*/*lacZ* cassette. Additional validation of these assembly systems was performed by the sequencing of plasmids isolated from blue or white colonies. Sequencing of blue colonies showed the expected complete sequence for the *lacI*/*lacZ* genes (2), while sequencing of white colonies were a mixture of mis-assemblies and very occasional uncut or cut/re-ligated pGGA destination plasmids. A final validation of the 5-, 12- or 24-fragment test systems was performed by setting up assembly reactions in which a single component was purposefully omitted. Since any assembly is dependent on the presence of every module, destination construct and functioning type IIS restriction enzyme and DNA ligase, any single omission should completely block the formation of a complete assembly that would result in a blue phenotype. Indeed, this was seen in all *lacI*/*lacZ* assembly test systems; no blue colonies were obtained if any single component was omitted.

TABLE 1:
Assembly test systems of increasing complexities designed at New England Biolabs

All inserts self-assemble into the pGGA destination construct. Reactions were incubated at 37°C for 5-min. or 60-min. for single inserts, and 30 cycles of (5 min. 37°C → 5 min. 16°C) for the 5, 12 and 24 fragment assemblies. In both cases, a terminal soak of 5 min. at 55°C was used to complete the assemblies at a temperature that favors cutting of any uncut/re-formed pGGA to reduce background. Single-insert assemblies used purified amplicon inserts at a 2:1 insert:vector ratio, while the *lacI/lacZ* cassettes used precloned inserts at equimolar levels to pGGA. Full details for complex assemblies using BsaI-HFv2 and T4 DNA Ligase are included at the end of this paper.

NUMBER OF INSERTS	GOLDEN GATE ASSEMBLY TEST SYSTEM	SIZE OF INDIVIDUAL INSERTS	SIZE OF ASSEMBLED INSERTS	INDICATION OF CORRECT ASSEMBLIES INTO pGGA (Cam^R) DESTINATION PLASMID
1	Amp^R, Kn^R, or Lambda amplicon	~1 kb	~1 kb	Growth on Cam/Amp, Cam/Kn or Cam/colony PCR
5	*lacI/lacZ* Cassette	~1 kb	~5 kb	Blue colony on Cam/X-gal/IPTG plates
12	*lacI/lacZ* Cassette	~300–600 bp	~5 kb	Blue colony on Cam/X-gal/IPTG plates
24	*lacI/lacZ* Cassette	~100–300 bp	~5 kb	Blue colony on Cam/X-gal/IPTG plates

All Golden Gate assemblies feature an inverse proportionality between the complexity of the assembly (number of inserts or modules) and the resulting efficiency of assembly (number of transformants); the greater the number of inserts, the lower the number of transformants. This is often compensated for by plating greater volumes of the outgrowth on the selection plate to achieve enough transformants for downstream screening. Figure 2 shows representative transformation plates obtained from 1-, 12- and 24-fragment assemblies of the *lacI/lacZ* cassette, and illustrates how the volume of the 1 ml outgrowth spread on each transformation plate can be manipulated to result in appropriate levels of colony plating densities.

BREAKING THROUGH THE LIMITS OF GOLDEN GATE ASSEMBLY

Five fragment *lacI/lacZ* cassette assembly was easily achievable with high levels of transformants and low backgrounds – so much so that there was little range for detectable improvements in the methodology. The decision was made to re-design the test system for 12 and 24 fragments. This was guided by both advances in the re-engineering of the original BsaI-HF® type IIS restriction enzyme and the completion of T4 DNA Ligase fidelity studies conducted by Potapov et al. at NEB (1,2). T4 DNA Ligase, the mainstay of most biotechnological cloning efforts for over 50 years, greatly prefers to ligate only correct Watson-Crick base pair annealed overhangs. However, a lower level of mismatch ligation events can be detected. Using 256 synthetic substrates that represent all possible 4 bp overhangs, NEB researchers profiled the fidelity of cohesive end ligation by this enzyme under standard reaction conditions. This data set allows quantitation of ligation efficiency and identification of mismatch-prone junctions. These observations can be used to predict highly accurate sets of connections for Golden Gate cloning.

Accurate "high-fidelity" junction sets for both the 12- and 24-fragment versions of the *lacI/lacZ* cassette were designed and synthesized. In conjunction with BsaI-HFv2 (NEB #R3733), re-engineered to provide improved Golden Gate performance, a series of optimization experiments for these more complex assemblies were performed. It was found that high efficiencies and accurate assembly levels were indeed possible, with correct, in-frame assembly proceeding in 99% of 12-fragment assemblies and over 90% for 24-fragment assemblies (Figure 3, Table 2). Additionally, the stability of the enzymes allowed use of a greater number of thermocycles, pushing the efficiency levels even higher than afforded by the standard 30 cycles for those wishing maximal transformation levels. Extended reaction cycling is only successful when using highly stable enzymes that maintain activity beyond the standard 5 hours required for 30 cycles, utilizing 5-minute stages at temperatures favoring digestion (37°C) and ligation (16°C).

LOOKING TO THE FUTURE

DNA assembly methods are important tools for many areas of science, and researchers continue to test the limits of DNA assembly approaches with increasingly complex experimental conditions. The ability to construct more complex, multi-fragment assemblies, as shown in this work, will fuel additional efforts to push the technique forward. Our research and development efforts continue to focus on providing optimized reaction components for a wider number of type IIS specificities and substantial improvements to the methodology, with the goal of enabling routine, efficient and accurate assembly of 50 fragments in a single tube in the not-so-distant future.

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FIGURE 2:
Representative Transformation Plates of Golden Gate Assemblies Featuring Increasing Complexities

Assembly reactions were transformed into competent *E. coli* cell strains NEB 10-beta (#C3019) (1 fragment) and T7 Express (#C2566) (12 and 24 fragments) and incubated for 16 hours at 37°C. While many cell strains support assembly protocols and #C3019 10-beta is routinely recommended due to its ability to stably maintain large construct plasmid sizes, the non-alpha complementing #C2566 T7 Express cell strain was used for the *lacI/lacZ* cassette testing to avoid any possibility of alpha-fragment LacZ complementation.

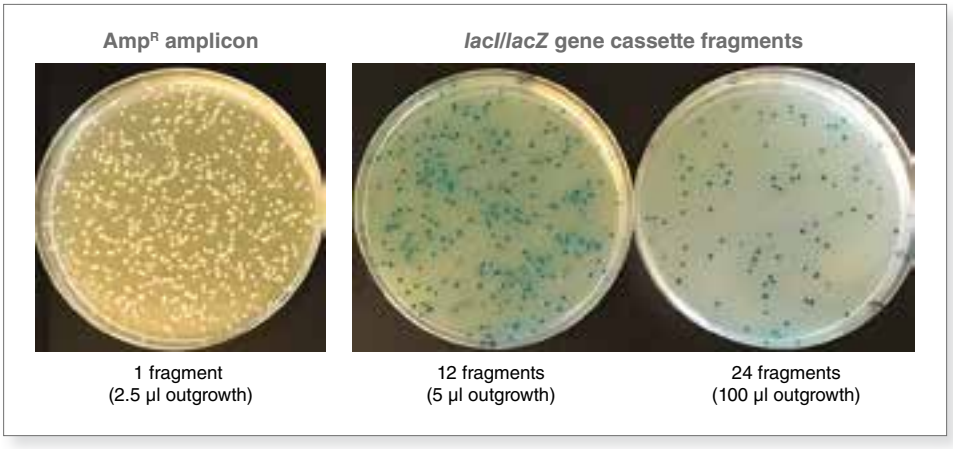

FIGURE 3:

Golden Gate Assembly of 24 fragments can be achieved with high efficiency and accuracy

Twenty-four fragment assemblies of the *lacI/lacZ* cassette were performed using the protocol available at www.neb.com/GoldenGate. While 30 cycles is sufficient to achieve 24 fragment assemblies, the stability of the BsaI-HFv2 and T4 DNA Ligase allows continued assembly through 45 and 60 cycles with a low background. (a) Efficiency of assembly and (b) accuracy of assembly versus cycle number. This continued functionality past the traditional 30 cycles of assembly indicates a high level of enzyme stability.

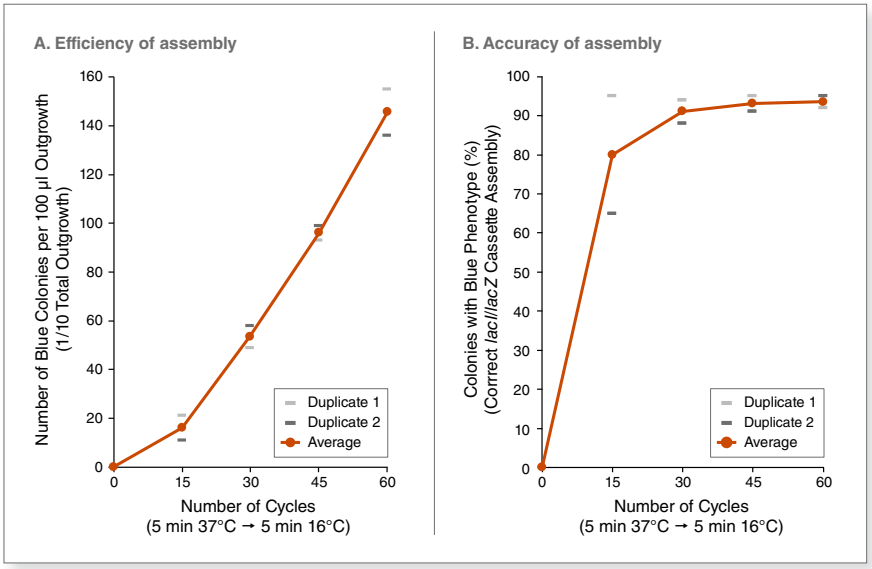

TABLE 2:

Yields and fidelities for Golden Gate Assemblies with BsaI-HFv2 and T4 DNA Ligase

Efficiency of assemblies per plate using outgrowth volumes described in Figure 3, with calculated yields from entire outgrowth built from 2 µl of the assembly reaction, and from the entire assembly reaction. All assembly protocols had a 5 min, 55°C terminal soak before transformation.

NUMBER OF FRAGMENTS ASSEMBLED	GOLDEN GATE ASSEMBLY PROTOCOL* (VOLUME OF 1 ml OUTGROWTH PLATED)	CORRECT ASSEMBLIES PER PLATE	FIDELITY OF ASSEMBLY (PERCENT CORRECT)	CALCULATED COLONY TOTALS	
				PER 2 µl ASSEMBLY REACTION	PER FULL ASSEMBLY REACTION**
1	5 min., 37°C (2.5 µl)	687	100%	274,200	2,742,000
1	60 min., 37°C (2.5 µl)	1,623	100%	649,200	6,492,000
12	(5 min., 37°C → 5 min. 16°C) x 30 (5 µl)	245	99.5%	48,900	489,000
24	(5 min., 37°C → 5 min. 16°C) x 30 (100 µl)	78	90.7%	783	9,792

** All assembly reactions had a 5 minute terminal soak after either 37°C incubations or cycling.*
*** Assembly reaction volumes were 20 µl (1, 12 fragments) or 25 µl (24 fragments).*

PDF

To access the protocol for 20+ fragment assembly using BsaI-HFv2 and T4 DNA Ligase visit:
www.neb.com/GoldenGate

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NEB MEDIA RESOURCES

Improved alternate cloning technique – Golden Gate Assembly

Pushing the Limits: 20+ fragment assembly now achievable with high efficiency and accuracy!

With constant advances both in the development of new enzymes (e.g., BsaI-HFv2) and research on maximizing enzyme functionality (e.g., ligase fidelity), NEB has become the industry leader in pushing the limits of Golden Gate Assembly and related methods such as MoClo, GoldenBraid, Mobius Assembly and Loop Assembly. NEB has all the products and information you need to perform complex assemblies, as demonstrated with 20+ fragment assemblies exhibiting high efficiencies, high accuracy (> 90%) and low backgrounds. **(Read feature article on pages 3-5)**

Advances in Ligase Fidelity

Research at NEB has led to increased understanding of ligase fidelity, including the development of a comprehensive method for profiling end-joining ligation fidelity in order to predict which overhangs have improved fidelity.
This research has enabled complex fragment assemblies with high efficiency and > 90% accuracy.

Advantages

- Seamless cloning – no scar remains following assembly
- Fast (5 min.) protocols for routine, single-insert cloning
- High efficiencies for cloning during library creation
- Ordered assembly of multiple fragments (20+) in a single reaction
- Efficient with regions with high GC content and areas of repeats
- Compatible with a broad range of fragment sizes (< 100 bp to > 15 kb)
- Free assembly tool available at **GoldenGate.neb.com**

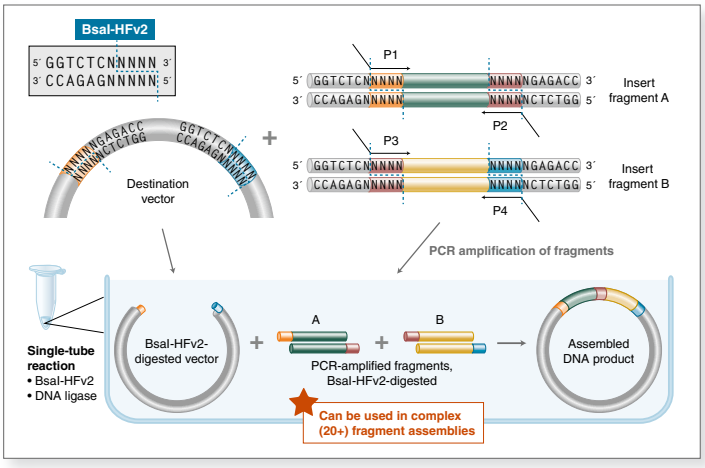
Type IIS Restriction Enzymes

NEB offers more Type IIS (i.e., recognize asymmetric DNA sequences and cleave outside of their recognition sequence) restriction enzymes than any other supplier, many of which are used in Golden Gate Assembly. NEB is pleased to introduce two new restriction enzymes for use in Golden Gate: Esp3I, an isoschizomer of BsmBI that is recommended for use at 37°C, stored at -20°C, and is supplied with CutSmart® Buffer, and the improved BsaI-HFv2, which is optimized for Golden Gate Assembly. **Free samples of BsaI-HFv2 are available** upon request.

NEB Golden Gate Assembly Mix

The NEB Golden Gate Assembly Mix incorporates digestion with BsaI and ligation with T4 DNA Ligase into a single reaction, and can be used to assemble up to 12 fragments in a single step.

Golden Gate Workflow



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

ORDERING INFORMATION

PRODUCT	NEB #	SIZE
NEB Golden Gate Assembly Mix	E1600S	15 reactions
BsaI-HF v2	R3733S/L	1,000/5,000 units
BsaI	R0535S/L	1,000/5,000 units
BbsI	R0539S/L	300/1,500 units
BbsI-HF	R3539S/L	300/1,500 units
BsmBI	R0580S/L	200/1,000 units
Esp3I	R0734S/L	300/1,500 units
T4 DNA Ligase	M0202S/L/T/M	20,000/100,000 units
T7 DNA Ligase	M0318S/L	1,000/5,000 units
NEB 5-alpha Competent *E. coli* (High Efficiency)	C2987H/I/P	20 x 0.05 ml/6 x 0.2 ml/ 1 x 96 well plate
NEB 10-beta Competent *E. coli* (High Efficiency)	C3019H/I	20 x 0.05 ml/6 x 0.2 ml

To request a free BsaI-HFv2 sample, contact us via info.fr@neb.com

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High-Fidelity (HF) restriction enzymes
HF enzymes offer reduced star activity, rapid digestion and 100% activity in CutSmart Buffer

Time-Saver restriction enzymes
>195 enzymes are Time-Saver qualified and will digest DNA in 5–15 minutes; digest overnight with no unwanted star activity

SEAMLESS CLONING

NEBuilder HiFi DNA Assembly Master Mix (#E2621), Cloning Kit (#E5520)
Rapid, seamless assembly/cloning of multiple fragments in as little as 15 minutes

NEB Golden Gate Assembly Mix (#E1600)
Efficient, ordered assembly of multiple fragments in a single reaction

TRANSFORMATION

NEB Turbo Competent *E. coli* (High-Efficiency) (#C2984)
Generate colonies in as little as 6.5 hours with a 5-minute transformation protocol

END MODIFICATION

Quick Dephosphorylation Kit (#M0508)
Complete dephosphorylation in 10 minutes with rapid, irreversible heat inactivation

Quick Blunting Kit (#E2621)
Blunt restriction enzyme-digested DNA in less than 30 minutes at room temperature

LIGATION

Instant Sticky-end Ligase Master Mix (#M0370)
Specially formulated to instantly ligate 2–4 basepair overhangs! Just add DNA, flick the tube, and ligation occurs instantly! No need for incubation.

Blunt/TA Ligase Master Mix (#M0367)
Specially formulated to ligate single-base and blunt ends in just 15 minutes

Quick Ligation Kit (#M2200)
Ligates both cohesive and blunt ends in just 5 minutes at room temperature

PURIFICATION

Monarch DNA Purification Kits
Purify high-quality DNA in minutes (5 minutes for the PCR & DNA Cleanup Kit!). Designed with sustainability in mind, Monarch kits use less plastic and responsibly-sourced packaging

AMPLIFICATION

Q5 High-Fidelity DNA Polymerases
With the lowest error rate of all known commercially-available PCR polymerases, Q5 enables extension times as low as 10 seconds per kb on templates up to 20 kb

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- Quick Dephosphorylation Kit
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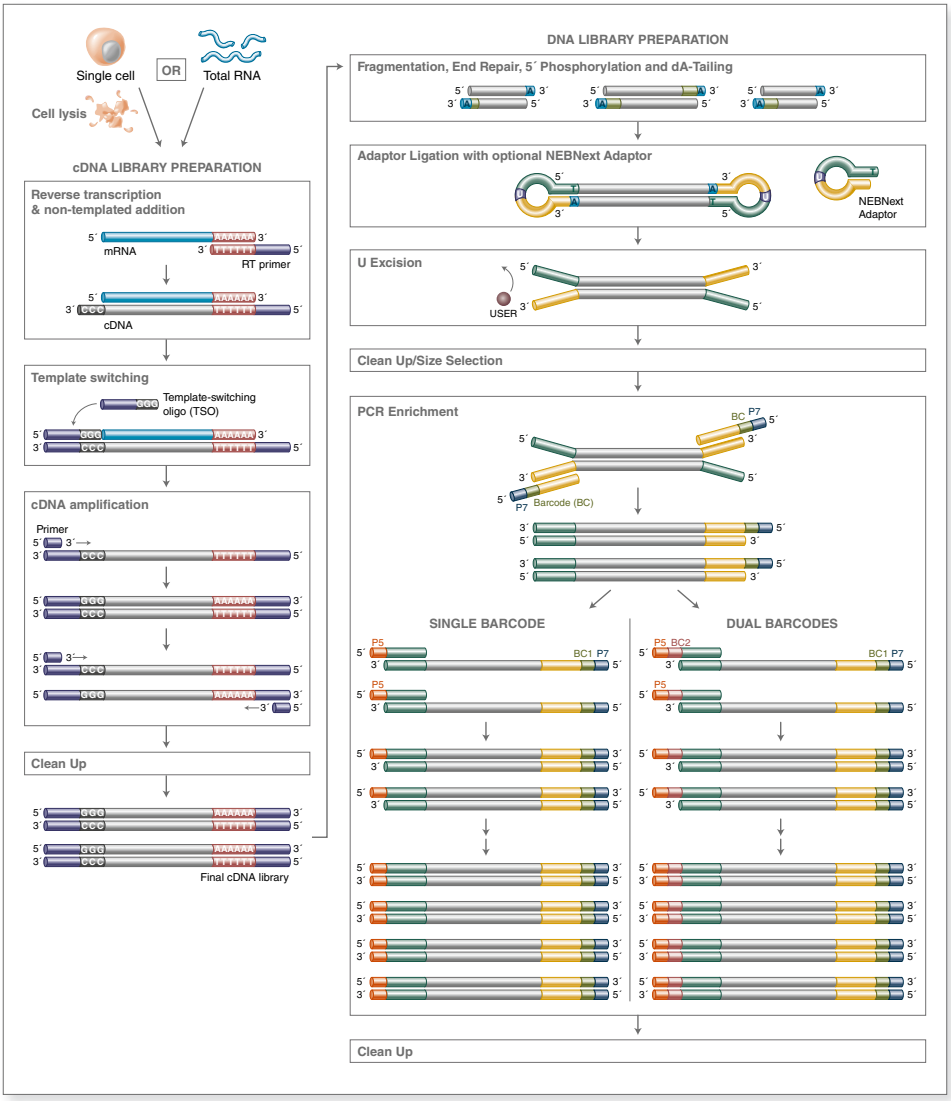
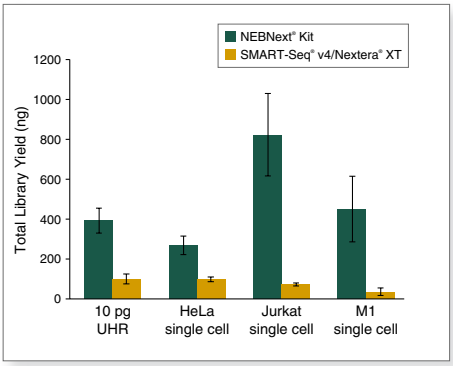
NEBNext Single Cell/Low Input RNA Library Prep workflow



Advantages

- Generate the highest yields of high-quality full-length transcript sequencing libraries from single cells, or as little as 2 pg – 200 ng total RNA
- Experience unmatched detection of low abundance transcripts
- Rely on consistent transcript detection for a wide range of input amounts and sample types
- Obtain full length, uniform transcript coverage, regardless of input amount or sample type
- Use with cultured or primary cells, or total RNA
- Save time with a fast, streamlined workflow, minimal handling steps and hands-on time
  - – Single-tube protocol from cell lysis to cDNA
  - – Enzymatic DNA fragmentation, end repair and dA-tailing reagents in a single enzyme mix, with a single protocol, regardless of GC content
- Available with or without library construction reagents

Generate higher library yields with the NEBNext Single Cell/Low Input RNA Library Prep Kit



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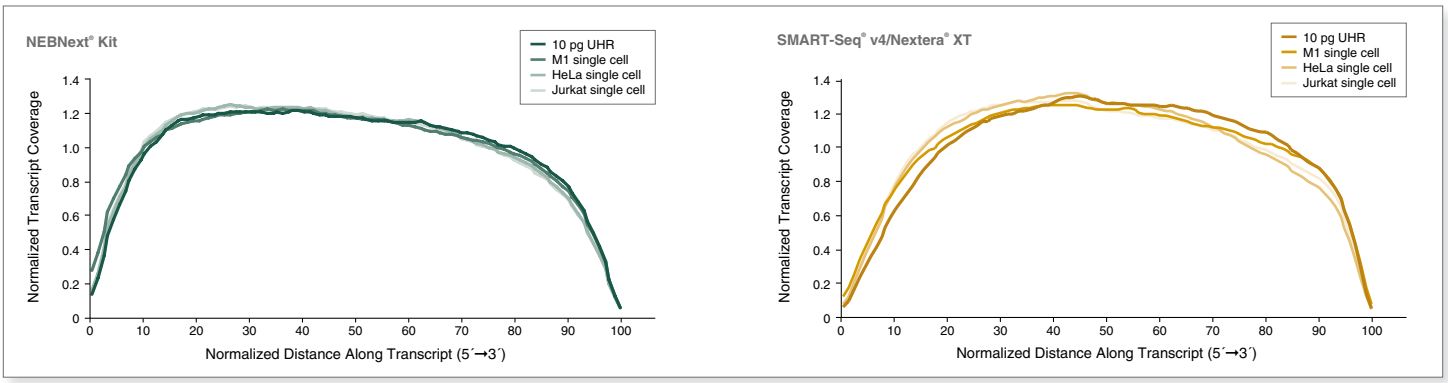
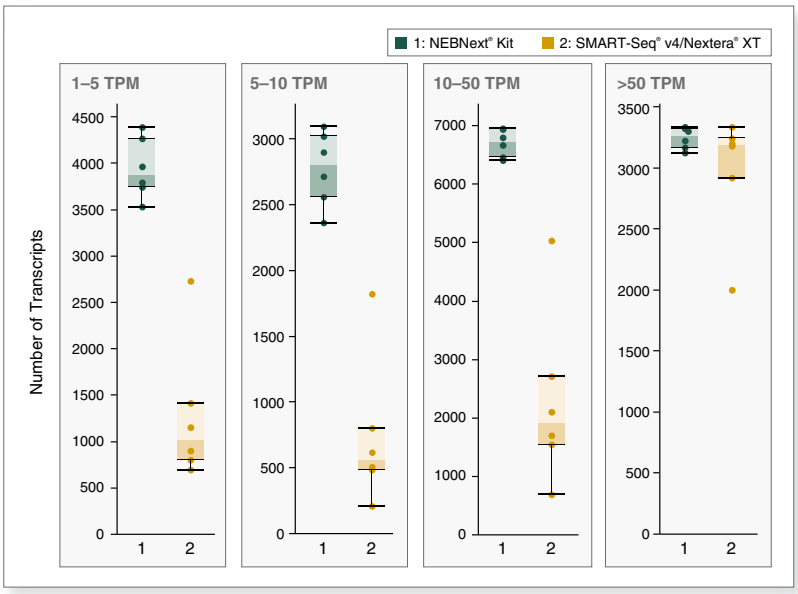
The NEBNext Single Cell/Low Input RNA Library Prep Kit provides uniform coverage across the length of transcripts



Sequencing libraries were generated from HeLa, Jurkat and M1 single cells, or 10 pg of Universal Human Reference (UHR) RNA (Agilent® #740000) with recommended amounts of ERCC RNA Spike-In Mix I (Thermo Fisher Scientific® #4456740). The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra® Low Input RNA Kit for Sequencing (Clontech® # 634891) plus the Nextera XT DNA Library Prep Kit (Illumina® #FC-131-1096) were used. Libraries were sequenced on an Illumina NextSeq® 500 using paired-end mode (2x76 bp). Gene body coverage shown is an average of four replicates and was calculated using Picard tools. The global view of the 5´ to 3´ coverage of the RefSeq transcripts reveals both consistency across different sample types and uniformity across the transcript length in the NEBNext libraries.



The NEBNext Single Cell/Low Input RNA Library Prep Kit increases transcript detection

Sequencing libraries were generated from Jurkat single cells (6 replicates) using the NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra® Low Input RNA Kit for Sequencing (Clontech® # 634891) plus the Nextera XT DNA Library Prep Kit (Illumina® #FC-131-1096). Libraries were sequenced on an Illumina NextSeq® 500 using paired-end mode (2x76 bp). TPM = Transcripts per Kilobase Million. Each dot represents the number of transcripts identified at the given TPM range, and each box represents the median, first and third quartiles per replicate. Salmon 0.6 was used for read mapping and quantification of all GENCODE v25 transcripts. Panels show the number of transcripts detected within the following TPM ranges: 1-5, 5-10, 10-50 and >50 TPM. Increased identification of low abundance transcripts is observed with the NEBNext libraries.

Customer Feedback:

„The implementation of this new single cell RNA-seq pipeline is an important addition to our core Scientific Operations and will enable our scientists to resolve at scale the transcriptional variation within a wide variety of single cells."

Sarah Teichmann, PhD, head of cellular genetics at the Wellcome Sanger Institute

To learn more about the technology, visit:
www.neb.com/nebnextsinglecell

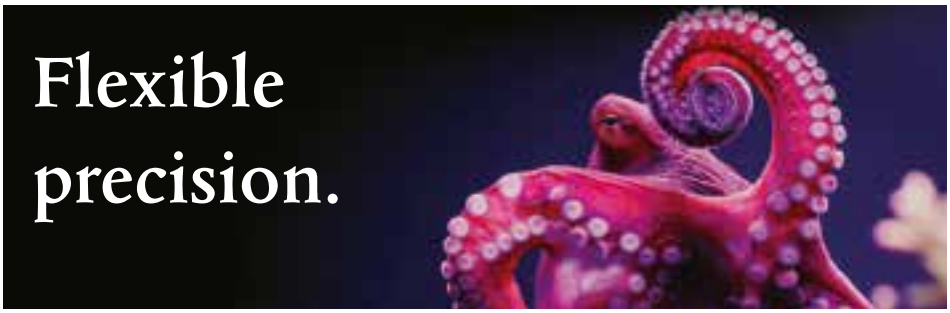
ORDERING INFORMATION

PRODUCTS	NEB #	SIZE
NEW NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24/96 rxns
NEW NEBNext Single Cell/ Low Input cDNA Synthesis & Amplification Module	E6421S/L	24/96 rxns

ALSO AVAILABLE	NEB #	SIZE
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1, 2, 3, 4)	E7335, E7500, E7710, E7730S/L	24/96 rxns
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 rxns

Request a free sample from your local distributor or visit www.neb.com/nebnextsinglecell

NEBNext Direct® Custom Ready Panels for NGS target enrichment



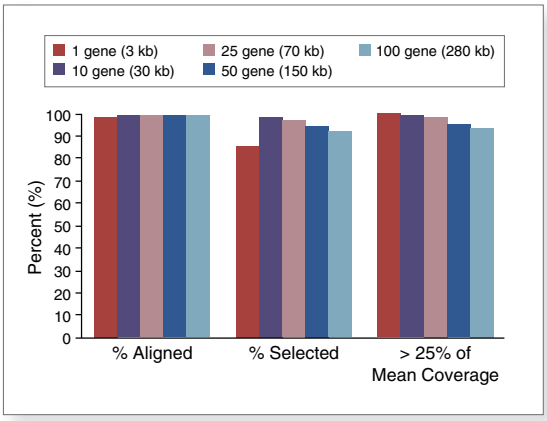
NEBNext Direct employs a unique technology that enables highly specific target enrichment of genomic regions of interest. The result of a partnership between New England Biolabs and Directed Genomics™, this innovative approach to target enrichment balances the speed and precision of multiplexed PCR-based approaches with the content scalability typical of hybridization-based methods. This flexibility allows a single workflow for assays ranging from single gene tests to comprehensive panels including several hundred genes. Regardless of sample type or assay content, NEBNext Direct allows you to enrich your targets with precision.

Employing the unique NEBNext Direct hybridization-based enrichment method, NEBNext Direct Custom Ready Panels allow rapid customization of targeted gene panels for Illumina® sequencing. Select from a list of genes for which baits have been carefully designed and optimized to produce complete coverage of the full coding regions. High quality panels can be designed by you and rapidly delivered from any combination of genes. NEBNext Direct Custom Ready Panels provide the content you want with the performance you need.

Advantages

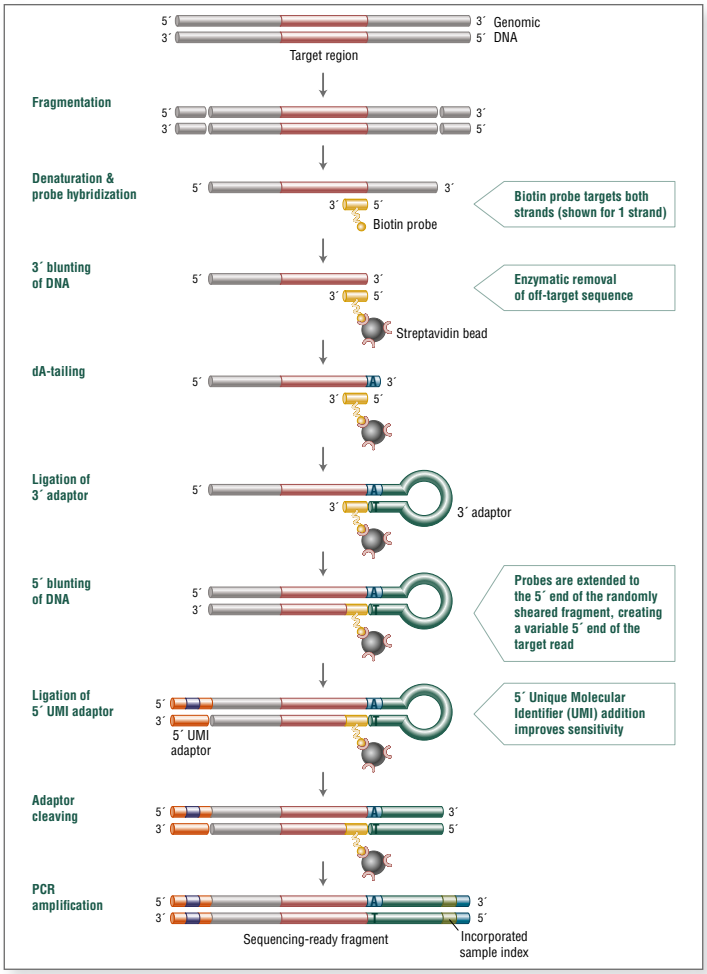
- Choose from a single gene to hundreds of genes
- Experience unmatched specificity and coverage uniformity
- Eliminate synthesis and optimization steps for faster turnaround
- Improve sensitivity with Unique Molecule Indexes (UMI)
- Generate results in one day with our automation-friendly workflow

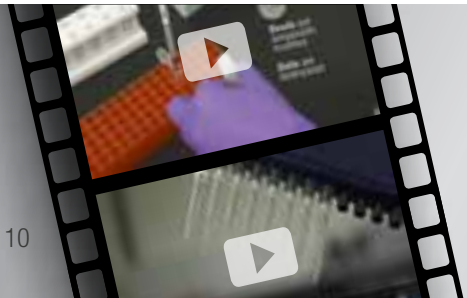
NEBNext Direct Custom Ready Panels demonstrate optimum performance across a wide range of panel sizes



Key target enrichment metrics demonstrate consistent performance across a range of panel sizes. 100 ng of DNA was tested against panels of 1, 10, 25, 50 and 100 genes, and sequenced using Illumina paired-end 150 bp sequencing. Larger panels included all genes present in smaller panels.

NEBNext Direct target enrichment workflow





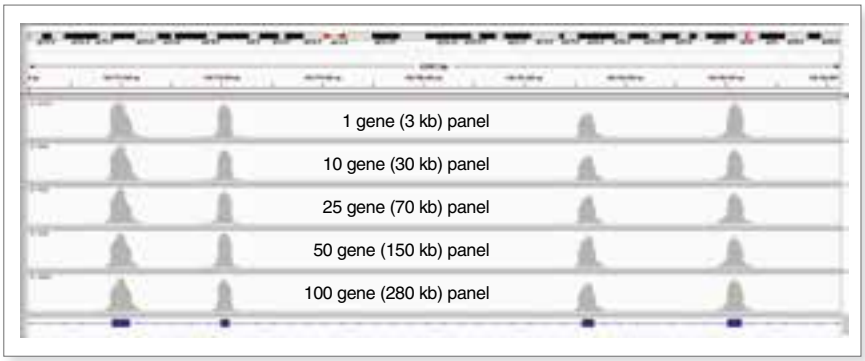
New to NGS Target Enrichment?

Watch our tutorial videos on NEBNextDirect.com incl.:

- Webinar "*Challenges and Opportunities for NGS target enrichment*"
- NEBNext Direct Workflow overview
- NEB TV – Episode 11 about target enrichment in clinical applications

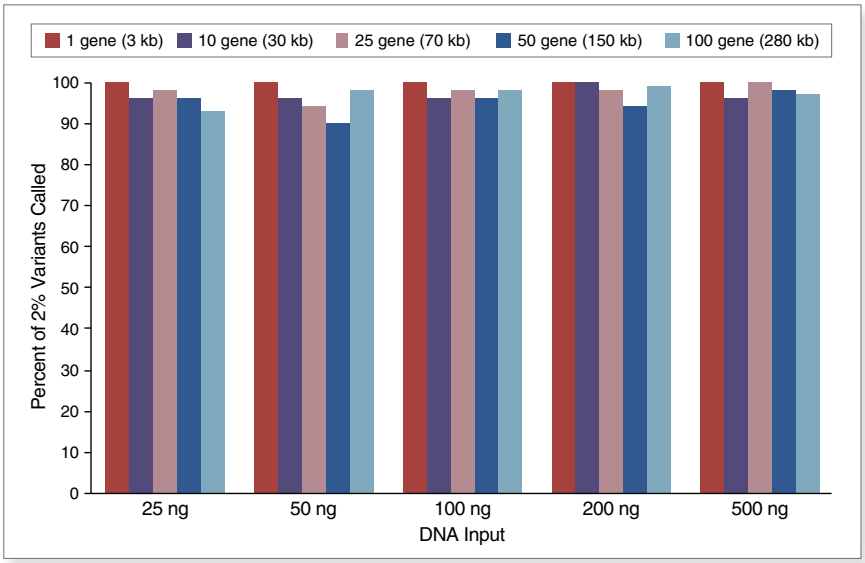
Sequence only what you need!

NEBNext Direct Custom Ready Panels demonstrate retention of target behavior across panel sizes



IGV image of coverage profile for 4 BRAF exons included in panels of 1, 10, 25, 50 and 100 genes, demonstrate consistent target behavior with the addition of gene targets. 100 ng of DNA was used as input for NEBNext Direct enrichment using the 5 panels, including the BRAF gene. Libraries were sequenced using Illumina 2 x 150 basepair sequencing.

Sensitivity in detection of variants across panel size and DNA input amount



24 HapMap samples were blended to create a range of variant allele frequencies (VAF) down to 2%. 25, 50, 100, 200 and 500 ng of this blended DNA was enriched using NEBNext Direct Custom Ready Panels of 1, 10, 50, and 100 genes. Larger panels were inclusive of the genes in smaller panels. Resulting libraries were sequenced using 2 x 150 bp Illumina sequencing and variants were called using Mutect and Vardict variant calling algorithms.

Customer Feedback:

„The kit and its technology are easy to use and easy to automate, allowing us to get up and running quickly. The protocol itself is fast and efficient to obtain deep coverage of targets, giving homogeneous results for FFPE and frozen tumors, therefore opening doors for customized panels."

Francis Rousseau, Ph.D., Director of Genomics for IntegraGen SA

"NEBNext Direct enrichment technology is by far the fastest and most automation friendly protocol available today. I can have samples on the sequencer in 6 hours starting from genomic DNA.(...)"

Eric C. Olivares, Founder, SEQanswers.com

"NEB was fantastic while developing our panel or updating an existing one. The protocol is simple and fast and the results have been superb."

Luca Magnani, Ph.D, CRUK Fellow, Imperial Centre for Translational and Experimental Medicine

See more testimonials on www.NEBNextDirect.com

To view our library of genes available for **NEBNext Direct Custom Ready Panels** as well as to learn more about the technology, please visit:

www.NEBNextDirect.com

ORDERING INFORMATION

PRODUCTS	NEB #	SIZE
NEW NEBNext Direct Custom Ready Panel	E6631S/L/X	8/24/96 rxns

ALSO AVAILABLE	NEB #	SIZE
NEBNext Direct Cancer HotSpot Panel	E7000S/L/X	8/24/96 rxns
NEBNext Direct BRCA1/BRCA2 Panel	E6627S/L/X	8/24/96 rxns

Get Belinda!

Get a free plush octopus – Belinda – with every qualified price quote or order of any NEBNext Direct Panel.

Please contact us via info.fr@neb.com for details!

As long as supply lasts.

PCR & Amplification at Special Prices!

NEB has a long history of developing reliable and convenient PCR tools, as NEB was the first company to sell *Taq* DNA Polymerase to the research market, the first to discover a PCR-stable, high-fidelity DNA polymerase, and the first to provide reagents for PCR performed in space. This commitment has continued with the development of One*Taq* DNA Polymerase for robust routine PCR and Q5 High-Fidelity DNA Polymerase for robust, ultra high-fidelity PCR.

NEB's Luna product line has been developed to simplify your qPCR reagent selection while accomplishing best-in-class performance.

Despite the ubiquitous nature of PCR and qPCR, it may not be the best option for all amplification needs. For point of care and other diagnostic applications, sequence-specific isothermal amplification methods, that eliminate the need for thermocycling, have been particularly useful. Instead of heat, these methods typically employ a strand-displacing DNA polymerases to separate duplex DNA e.g. in LAMP-Assays. NEB offers best-in-class isothermal LAMP Kits for DNA and RNA detection – proven performance even in Space!

Please be invited – Special reduced prices on select PCR & Amplification Products!

Benefit from NEB's unrivaled PCR and amplification products and save your research budget during our year's end campaign! Get the most prominent and most outstanding reagents at a special price you can't afford to miss.

*All eligible discounted products are marked with the "25% DISCOUNT" icon within this newsletter (pages 13-15). Campaign ends December 31st, 2018.

See conditions on **www.neb-online.fr**

PCR Polymerase Selection Chart

★ **indicates recommended choice for application**

	STANDARD PCR	HIGHEST FIDELITY PCR
	ONE*Taq*®/ ONE*Taq* HOT START	Q5®/Q5 HOT START
PROPERTIES		
Fidelity vs. *Taq*	2X	~280X(1)
Amplicon Size	< 6 kb	≤ 20 kb
Extension Time	1 kb/min	6 kb/min
Resulting Ends	3´ A/Blunt	Blunt
3´→5´ exo	Yes	Yes
5´→3´ exo	Yes	No
Units/50 µl Reaction	1.25	1.0
Annealing Temperature	$Tm^{-}5$	$Tm^{+}3$
APPLICATIONS		
Routine PCR	★	•
Colony PCR	★	
Enhanced Fidelity	•	★
High Fidelity		★
High Yield	★	★
Fast		★
Long Amplicon		★
GC-rich Targets	★	★
AT-rich Targets	★	★
High Throughput	•	•
Multiplex PCR	•	•
Site-directed Mutagenesis		★(2)
NGS APPLICATIONS		
NGS Library Amplification		★(3)
FORMATS		
Hot Start Available	•	•
Kit		•
Master Mix Available	•	•
Direct Gel Loading	•	

(1) Learn how PacBio sequencing was used to better understand sources of error introduced by PCR: Potapov, V. and Ong, J.L. (2017) PLOS One, 12(1): e 0169774

(2) Use Q5 Site-Directed Mutagenesis Kit (#E0554).

(3) Use NEBNext Ultra II Q5 Master Mix (#M0544).

TOOLS & RESOURCES

Visit **www.neb.com/tools-and-resources** to find the latest PCR tools, tips, protocols and videos from NEB Scientists, including:

- Tm Calculator Tool
- PCR Fidelity Estimator
- Choosing the right polymerase for your PCR
- Important tips for Q5 High-Fidelity DNA Polymerase
- Tips for setting up PCR
- How to amplify GC-rich DNA
- Tips for amplifying large amplicons
- Amplification of GC-rich regions
- etc. etc.

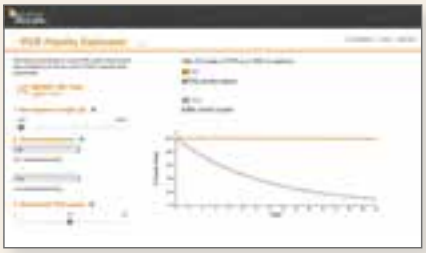
Did you know? PCR Fidelity at a glance

After 30 cycles of PCR on a 1500 nt amplicon using *Taq* DNA Polymerase, only less than 10% of all copies are correct – while using Q5 HiFi DNA Polymerase instead results in 99,1% correct copies!

Have you ever thought about the consequences of this on your research?

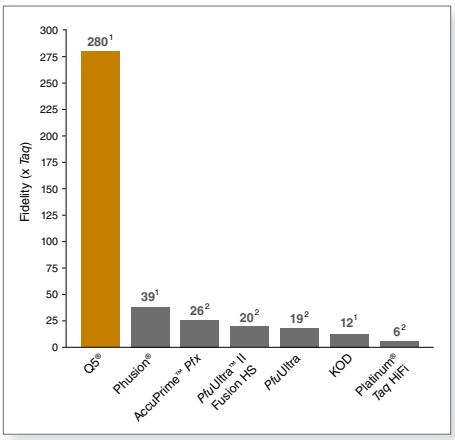
More screenings needed, more tests, more re-sequencing, more time & hassle...

Check out the NEB webtool "PCR Fidelity Estimator" to learn more.



Q5 High-Fidelity DNA Polymerase – highest fidelity PCR available

Q5 High-Fidelity DNA Polymerase is an ultra-fidelity, thermostable DNA polymerase with 3´-> 5´ exonuclease activity, fused to a processivity-enhancing Sso7d domain to support robust DNA amplification. With proofreading activity ~280-fold higher than *Taq* DNA Polymerase and ~15-fold higher than *Pyrococcus furiosus* (*Pfu*) DNA Polymerase, Q5 High-Fidelity DNA Polymerase is ideal for cloning and other application where sequence fidelity is important. It can also 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

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

25% DISCOUNT* PROMO CODE: PCRAMP18

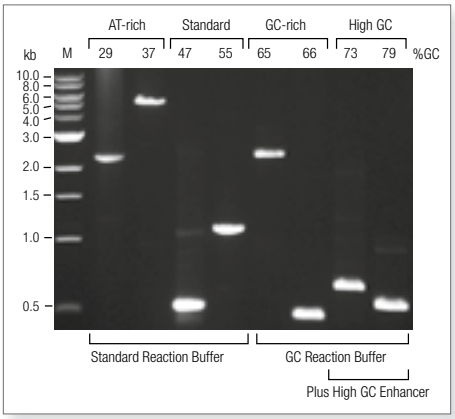
Advantages

- **Highest Fidelity** – virtually "error-free" PCR products for cloning, expressions, sequence analysis etc. due to extreme fidelity: ~280x higher than *Taq* and ~7 x 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 and 10 kb for complex templates

Q5 HiFi DNA polymerases benefit from a Tm+3 annealing temperature. Use the NEB Tm Calculator to ensure successful PCR at **TmCalculator.neb.com**.

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

One*Taq* DNA Polymerase is an optimized blend of *Taq* and Deep Vent DNA polymerases for use with standard, routine and difficult PCR experiments. The 3´-> 5´ exonuclease activity ("proofreading") 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

PRODUCT	NEB #	SIZE
One*Taq* DNA Polymerase	M0480S/L/X	200 / 1.000 / 5.000 units
One*Taq* Quick-Load DNA Polymerase	M0509S/L	100 / 500 units
One*Taq* Quick-Load 2X Master Mix with Standard Buffer	M0486S/L	100 / 500 rxns
One*Taq* Hot Start DNA Polymerase	M0481S/L/X	200 / 1.000 units
One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer	M0488S/L	100 / 500 rxns
One*Taq* Hot Start Quick-Load 2X Master Mix with GC Buffer	M0489S/L	100 / 500 rxns

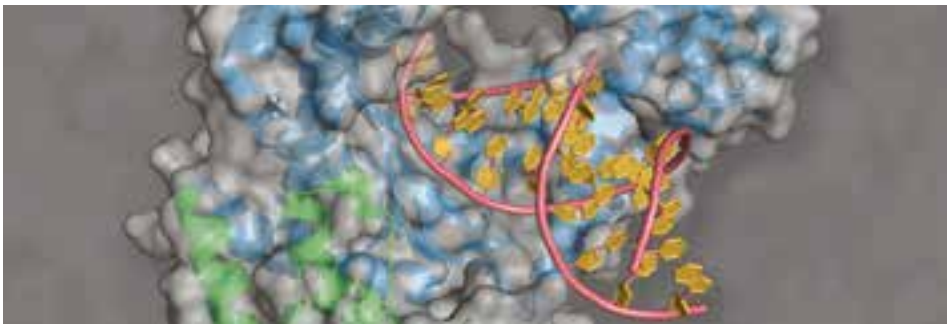
25% DISCOUNT* PROMO CODE: PCRAMP18

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, however **best product performance at 68°C Extention Temperature**

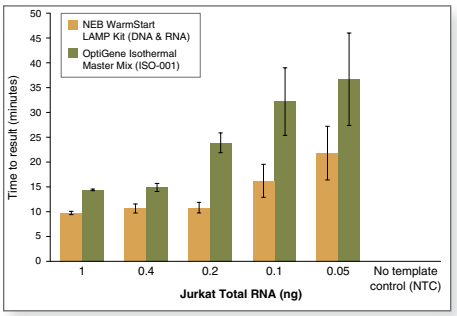
Request a free OneTaq sample via www.neb-online.fr/OneTaq!

PCR-free DNA & RNA Detection in just 15 – 45 min: NEB's WarmStart LAMP Kits



Loop Mediated Isothermal Amplification (LAMP) is a commonly-used technique for rapid nucleic acid detection. NEB's WarmStart LAMP products provide a simple, one-step solution for DNA or RNA targets. The master mix supplied with the WarmStart LAMP Kit contains the robust and rapid *Bst 2.0* WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase, both *in silico*-designed enzymes for improved performance in LAMP reactions. Available in two formats to reflect the preferred read-out/detection method of choice i.e. colorimetric visual detection or real-time fluorescence, turbidity and gel detection, NEB's WarmStart LAMP kits offer an extremely reliable and easy means for fast DNA or RNA detection within just 15 - 45 min. This is beneficial away from the bench without the need for Thermocyclers or detection devices: at the point of care, at bedside, in field tests – or even in space!

NEB's WarmStart LAMP Kit (DNA & RNA) offers speed and robust sensitivity



A RNA target (HMBS2) was amplified from Jurkat total RNA using the WarmStart LAMP Kit and OptiGene Master Mix (ISO-001). Reactions were performed at 65°C for 74 minutes on a real-time thermocycler in triplicate. NEB's WarmStart LAMP Kit resulted in faster and more sensitive detection as compared to the OptiGene Master Mix.

The WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA) allows for best-in-class visual detection:

The colorimetric variant of NEB's WarmStart LAMP Kit (DNA & RNA) #M1800 is designed to provide a fast, clear visual detection of amplification by a change in the solution color. This is based on the production of protons and subsequent drop in pH that occurs from the extensive DNA polymerase activity in a LAMP reaction, producing a change in solution color from pink to yellow.

Watch the newest episode of NEB TV and learn the basics of Colorimetric LAMP, and how this isothermal amplification method is used in field tests and point-of-care diagnostics.

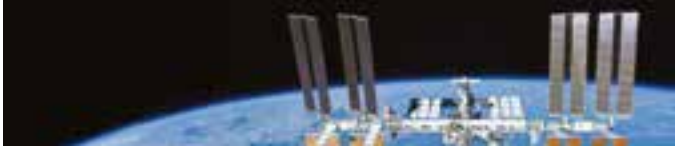
www.neb.com/nebtv

Did you know?

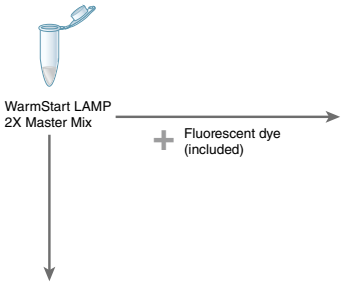
GENES IN SPACE

NEB's colorimetric LAMP Kit has already been used in Zika virus monitoring in Uganda, in the World Mosquito Program – as well as in Space!

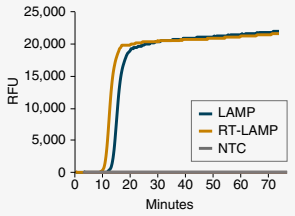
- To learn more about Zika virus detection from urine and serum, please read the paper at *https://doi.org/10.1371/journal.pone.0185340*
- To learn more about the World Mosquito Program, please watch NEB TV Episode #23 or visit *www.worldmosquitoprogram.org*
- For information on the first LAMP in space, please visit *https://www.genesinspace.org/blog/colorful-genetic-assay-space/*



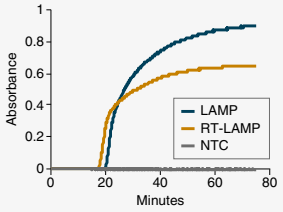
NEB's WarmStart LAMP Kit (DNA & RNA) #E1700 is compatible with multiple detection methods:



Real-time fluorescence measurement

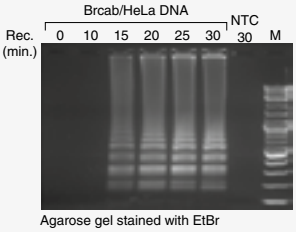


Turbidity detection

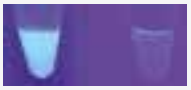


LAMP product is visible by eye due to the precipitation of magnesium pyrophosphate

Endpoint visualization



Agarose gel stained with EtBr



Visualization using a fluorescent intercalating dye (e.g., ethidium bromide, SYBR® green or calcein/HNBlue)

25% DISCOUNT* PROMO CODE: PCRAMP18

ORDERING INFORMATION

PRODUCT	NEB #	SIZE
WarmStart LAMP KIT (DNA & RNA)	E1700S/L	100/500 rxns
WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)	M1800S/L	100/500 rxns

Unrivaled performance in DNA/RNA Quantitation: LUNA Universal qPCR & RT-qPCR Reagents

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

New England Biolabs offers a bright, new choice for your qPCR and RT-qPCR. Luna products have been optimized for robust performance on diverse sample sources and target types. Available for dye-based or probe-based detection, Luna products can be used across a wide variety of instrument platforms.

EXPERIENCE BEST-IN-CLASS PERFORMANCE

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

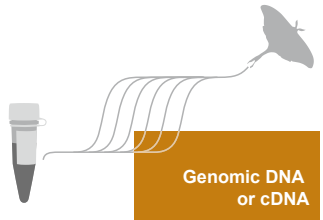
OPTIMIZE YOUR RT-QPCR: LUNA WARMSTART® REVERSE TRANSCRIPTASE OR LUNASCRIPT™ RT SUPERMIX KIT

- 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
- **NEW:** the convenient LunaScript RT SuperMix Kit optimized for best-in-class two-step protocols includes dNTPs, primers and RNase Inhibitor

MAKE A SIMPLER CHOICE

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

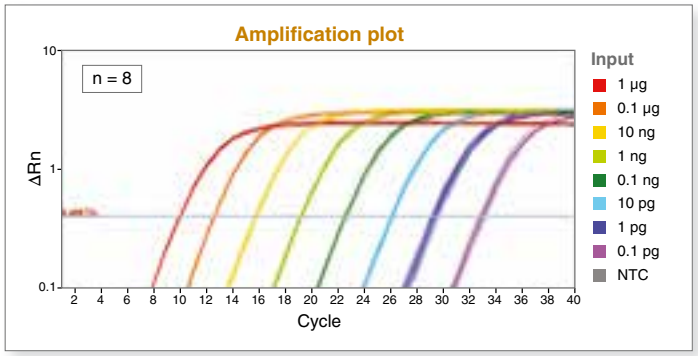
Learn more on **LUNAqPCR.com**



1 Select your target

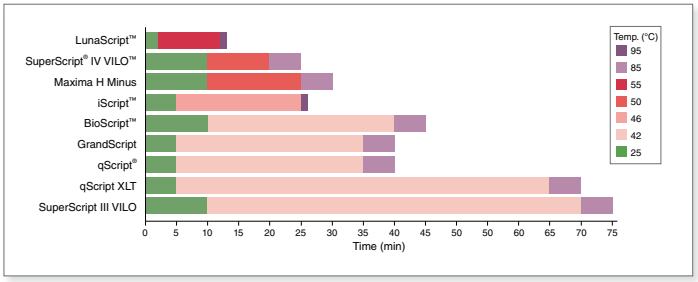
	2 Select your detection method: Dye-based	Probe-based
Genomic DNA or cDNA	Luna Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)
RNA	One-Step: Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	One-Step: Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006)
RNA	Two-Step: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

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Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/1.000/2.500 rxns
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