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Using aptamers to control enzyme activities: Hot Start *Taq* and beyond

by Nicole M. Nichols, Ph.D. and Nathan A. Tanner, Ph.D., New England Biolabs, Inc.

As molecular biology and diagnostic applications have become more demanding and sensitive, the ability to control enzymatic activities, and therefore reaction specificity, has naturally followed. Biotechnological innovations have led to several "hot start" approaches to control DNA polymerases, enabling the spread of PCR from the lab to the clinic with precise enzyme activation. Though powerful, these traditional methods typically require long activation times and very high temperature incubations that are incompatible with essential enzymes like reverse transcriptases (RTs). The aptamer-based hot start approach involves the selection of specific, modified aptamers for control of any enzymatic target of interest, even those that can't tolerate the extreme temperatures of PCR. NEB's selection of aptamers targeted to RTs and isothermal polymerases has enabled the first "warm start" enzymes that can be used in RT-qPCR and isothermal amplification. Control of enzyme activities remains a critical feature for demanding nucleic acid amplification methods, and the use of aptamer technology brings the benefits of hot start/warm start enzymes to the next generation of rapid, sensitive, and even isothermal molecular tests.

INTRODUCTION

The polymerase chain reaction (PCR) is a widely used technique, and the foundation of numerous diagnostic applications that seek to detect minute amounts of DNA via exponential amplification. Successful PCR requires a number of components, including a DNA polymerase capable of tolerating high temperature incubations (94°C or higher) that occur during a typical thermal cycling protocol. *Taq* DNA Polymerase, originally isolated from *Thermus aquaticus*, is most commonly used in PCR assays (1). In the early stages of PCR development, it became clear that reaction specificity impacted experimental success (2). Like many non-proofreading Family A DNA polymerases, *Taq* Polymerase possesses the ability to add bases onto the end of ssDNA in a non-template-dependent manner (i.e., terminal transferase activity). This activity is present, even at room temperature, and can result in the addition of non-specific bases onto the ends of DNA primers in the reaction, enabling them to bind to undesired locations on the DNA and reducing overall reaction specificity. In contrast, at higher temperatures, nonspecific binding is reduced as annealing becomes more stringent. As PCR applications became more complex and demanding, preventing this low temperature activity became critically important to increasing reaction specificity, and numerous techniques and methods have been employed to achieve this protection.

INCREASING REACTION SPECIFICITY

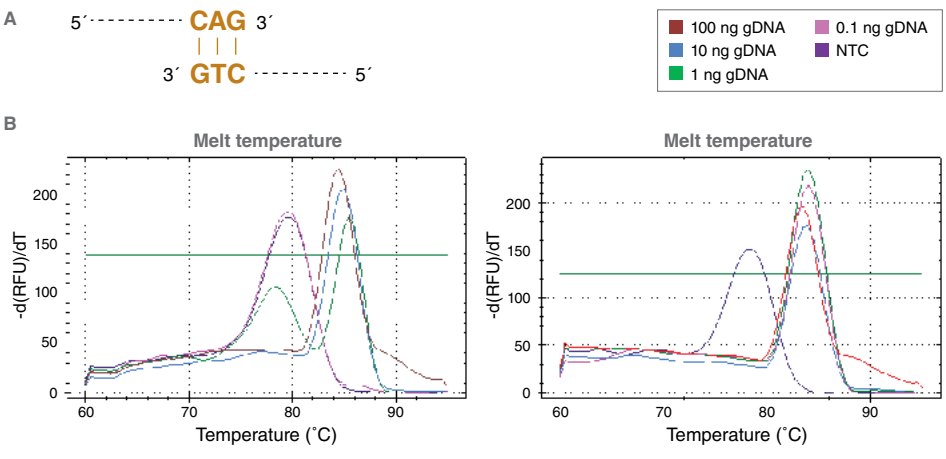
Early methods focused on the exclusion of key reaction components to mitigate undesired activity at low temperature. As the temperature was increased, any missing components (e.g., polymerase, cofactor) could be spiked into the mixture, triggering the reaction under a more restrictive, high temperature condition – a so-called "hot start" reaction. This method worked, but required the user to open reaction tubes and make very small volume additions, a labor-intensive and contamination-prone process. Subsequent methods aimed to improve upon this approach included sequestering reaction components with wax layers or beads (3). This was also effective, but was not widely adopted by the research community.

The next generation of hot start methods, still in use today, didn't involve physical removal or exclusion, but instead focused on covalent and non-covalent enzyme interactions or modifications to block activity. The most common of these methods involved the development of an antibody specific to *Taq* Polymerase, which renders *Taq* inactive at room temperature, but denatures and dissociates from the enzyme after the initial, high-temperature denaturation step (4,5). Antibody-based Hot Start *Taq* (Ab-*Taq*) addressed many of the concerns with prior approaches, and soon became a popular option for anyone looking to increase reaction specificity. However, original Ab-*Taq* offerings utilized animal-derived antibodies (increasing the possibility of reaction contamination) and were limited in commercial practice to those with a license; it was not surprising that other solutions continued to appear in the marketplace. The most successful of these alternatives was the use of chemicals to reversibly modify amino acid side chains of the polymerase (6). Initial work in this area yielded effective results, but came at a cost: the enzyme could clearly be inactivated, but required long activation times at very high temperatures to reactivate, and even then, only a fraction of the initial protein activity could be restored. Advances in this approach, namely changes to the covalent side chain modifications, have resulted in improved versions of chemically-based Hot Start *Taq* (Chem-*Taq*), with shorter activation times (4 minutes instead of 10–15 minutes). The increased potential of inducing DNA damage and the persistent need to add significant amounts of protein may have kept this approach from becoming more widely employed.

FIGURE 1:
Monitoring hot start effectiveness via reaction specificity

A. To assess reaction specificity, primers that create a stable, primer-dimer product via 3 complementary bases at their 3´ ends were used in PCR with *Taq* or Hot Start *Taq*.

B. Post reaction melt-temp analysis demonstrates the presence of the non-specific product only in the absence of template (NTC) for the Hot Start *Taq* reactions (purple curve, right) but reaction specificity is observed at all other inputs. However, in the absence of a hot start mechanism (left), primer-dimer product can be observed with two lower input concentrations, as well as in the NTC sample. Reactions (25 µl) contained 0.2 µM primers.



DEVELOPMENT OF APTAMER-BASED APPROACH DELIVERS ADDITIONAL IMPROVEMENTS

A number of years ago, New England Biolabs® (NEB®) investigated the use of aptamers to impart hot start activation of our enzymes. Generally, aptamers are engineered oligonucleotides that bind to a specific target molecule through non-covalent interactions. SOMAmers®, the aptamer-based technology developed by SomaLogic that we have further adapted for use in our products, include specific nucleobase modifications that can improve inhibition profiles and/or reduce unintended side effects (7,8). As with previously-designed *Taq* inhibitors, the *Taq* aptamer evolved and engineered by NEB also inhibited polymerase activity at room temperature. This function can be monitored by a variety of assays that include read-outs not just for polymerase activity, but also for reaction specificity. For example, one assay includes an excess of off-target DNA in the reaction and results in the production of multiple amplicons in the presence of non-Hot Start *Taq*, but only a single, target product in the presence of the aptamer-based Hot Start *Taq* (NEB-HS *Taq*). Another assay employed at NEB includes two primers that contain three complementary bases at the 3´ end of each primer (9). These "poor" primers can serve as a substrate for *Taq*, and, even in the presence of additional input DNA, can generate a clear primer-dimer product in the presence of *Taq* alone. However, in the presence of NEB-HS *Taq*, only the desired target-specific product is observed (Figure 1, page 2).

Although the main function of an aptamer-based inhibitor is similar to other hot start mechanisms, there are some key differences between NEB-HS *Taq* and either Chem-*Taq* or Ab-*Taq*. First, unlike other methods, the aptamer-based inhibition/activation process is fully reversible. At the end of thermal cycling, when the temperature of the reaction is decreased, the aptamer rebinds to *Taq* Polymerase, inhibiting any further activity in the sample. This has proven to be important in workflows where undesired polymerase activity after reaction completion can disturb baseline readings of negative samples, such as in workflows that include a significant delay between interrogation of the first and the last samples of a set (e.g., strip-tubes, 96-well plates, or droplet digital PCR). The second major difference lies in the release of inhibition. Whereas Ab-*Taq* and Chem-*Taq* are only activated once the reaction temperature is raised to 94–95°C, the aptamer in NEB-HS *Taq* dissociates from the polymerase at much lower temperatures (T_m = approximately 45°C), eliminating the need for a specific high temperature activation step, and enabling faster protocols (Table 1). Furthermore, reaction specificity is not impaired (Figure 2). The benefits of an aptamer-based hot start approach can be seen in the numerous NEB products that contain NEB-HS *Taq*, from the flexible One*Taq* family of routine PCR products, to the recently-released Luna® products that support best-in-class qPCR and RT-qPCR performance.

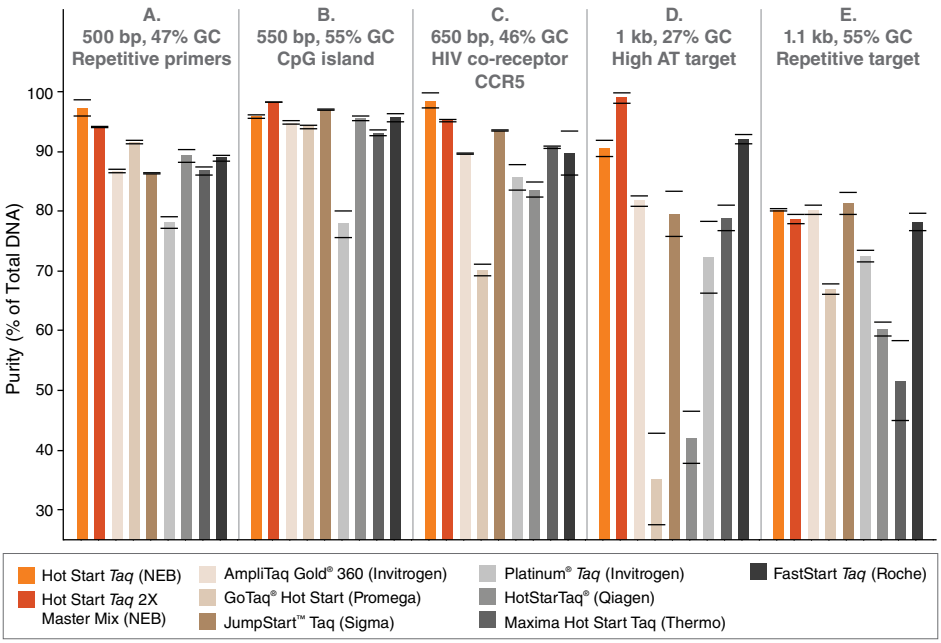
TABLE 1:
Polymerase activation times

DNA POLYMERASE	HOT START METHOD	ACTIVATION TIME*
AmpliTaq Gold 360 (Applied Biosystems)	Chem	10 min.
Platinum *Taq* (Invitrogen)	Ab	30 sec.
Hot Start *Taq* (NEB)	Aptamer	None
GoTaq Hot (Promega)	Ab	2 min.
Hot StarTaq (Qiagen)	Chem	15 min.
FastStart Taq (Roche)	Chem	4 min.
JumpStart *Taq* (Sigma)	Ab	1 min.
Maxima Hot Start *Taq* (Thermo Fisher Scientific)	Chem	4 min.

** May include initial denaturation step, if not specified by manufacturer.*

FIGURE 2:
Target specificity of commercially-available *Taq* products

Endpoint PCR analysis of various Hot Start *Taq* polymerases on human genomic DNA amplicons. Triplicate reactions were conducted according to manufacturer's recommendations in the buffer supplied with each enzyme. Input template concentration (20 ng), number of PCR cycles (30) and source of dNTPs (NEB) were held constant for all reactions. Products were quantitated by microfluidic LabChip® analysis and specificity of the final product was calculated as % purity of the expected amplicon.



EXTENDING APTAMER UTILITY TO OTHER APPLICATIONS

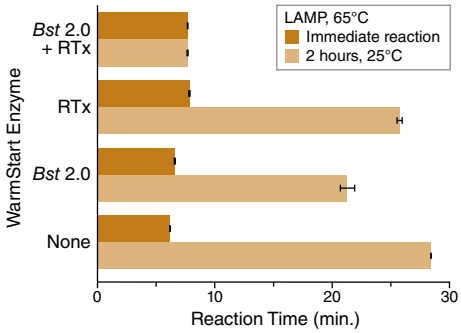
Dissociation at lower temperatures has enabled the use of aptamers for a broader range of polymerases and enzymes, including those that cannot tolerate the high temperatures employed in PCR. For example, aptamers have been particularly useful for isothermal amplification applications, where mesophilic and moderately thermophilic enzymes that catalyze these reactions cannot survive an initial high-temperature denaturation step (and instead use a strong strand displacement activity to separate the DNA duplex). In addition to developing aptamers for an enhanced version of *Bst* DNA Polymerase (WarmStart® *Bst* 2.0 DNA Polymerase) to increase specificity in these types of workflows, in 2014, NEB launched the first warm start reverse transcriptase, WarmStart RTx Reverse Transcriptase, specifically for RT-LAMP. Similar to the nonspecific primer extension described above, enzymes utilized in isothermal applications can also give rise to undesired products that affect reaction performance. Reaction conditions, such as very high primer and Mg^{2+} concentrations, as well as lower optimal temperatures for the enzymes, make isothermal methods especially prone to effects from any off-target activity that occur during reaction set up. As an example, Figure 3 (page 4) shows a LAMP reaction where room-temperature incubation results in a significant increase in reaction time (from ~6 minutes to nearly 30 minutes); by utilizing the dual-warm start formulation (WarmStart RTx plus WarmStart *Bst* 2.0) the time increase is prevented and consistent reaction performance is maintained. Critically, this protection requires the activities of both enzymes to be controlled.

Having observed the utility of a warm start RT in RT-LAMP applications, NEB next focused its efforts on the development and launch of Luna WarmStart Reverse Transcriptase, to support RT-qPCR applications. As with our previous aptamer development for the DNA-dependent DNA polymerases, these warm start RNA-dependent

on page 4...

FIGURE 3:
RT-LAMP specificity

Duplicate RT-LAMP reactions were prepared on ice and either immediately placed at 65°C with real-time fluorescence detection (dark gold), or incubated for 2 hours at 25°C before heating to 65°C (light gold). A 2-enzyme combination of *Bst* 2.0 and RTx was used for the reactions, with neither, one, or both enzymes used with their specific WarmStart aptamer as indicated on the y-axis. With 0 or 1 of the enzymes in WarmStart form, a dramatic increase in LAMP detection time resulted from the 2 hr room-temperature incubation due to nonspecific activity one or both polymerases. With both WarmStart aptamers used, no change in LAMP time was seen, indicating efficient protection from nonspecific activity by the dual-WarmStart format.



DNA polymerases (i.e., reverse transcriptases) are inhibited at room temperature, but the aptamer is still released as the temperature is increased, enabling full activity despite the use of moderate reaction temperatures (50–60°C). As with RT-LAMP, it has been through the use of these aptamers that we have been able to identify and prevent RT-mediated non-specific amplification that can occur in certain settings, such as challenging assays or workflows that include a delay between reaction set up and intended initiation. A one-step RT-qPCR example is shown in Figure 4, where a number of reactions were set up simultaneously and half were immediately transferred to a thermocycler and half were left at room temperature for 24 hours. No non-specific amplification can be detected in any of the samples that contained the Luna WarmStart RT. In contrast, clear evidence of non-specific amplification was detected when a more typical RT-qPCR reagent (containing only a Hot Start *Taq*) was used (Figure 4).

In addition to polymerase activity, aptamers can also be selected to inhibit or moderate other enzymatic activities. For example, the hot start aptamers that were developed for use with Q5® High Fidelity DNA Polymerase were not created to inhibit the polymerase activity, but instead to inhibit its exonuclease activity. Q5 is an engineered polymerase that most closely resembles an archaeal Family B polymerase, and as such, it possesses little-to-no detectable 5′→3′ polymerase activity at room temperature. However, Q5 does have a robust 3′→5′ exonuclease (proofreading) activity that enables high-fidelity replication, and this activity remains even at room temperature. This difference in activity temperature profiles compared to *Taq* (which possesses measurable polymerase activity at room temperature but has no 3′→5′ exonuclease activity at any temperature) can lead to alternate modes of non-specific amplification, thus requiring different solutions (Table 2). Whereas the mechanism of non-specific amplification by *Taq* is generally via non-templated addition at the 3′ end of the primers, reducing the probability of a Watson-Crick base pair at the desired annealing site, non-specific amplification with a Family B polymerase that possesses proofreading activity appears to occur via exonucleolytic primer degradation at the 3′ end, again reducing specificity at the desired annealing site and increasing the probability of off-target amplification.

CONCLUSION

NEB's selection of aptamers enables the ability to inhibit activity at room temperature, and offers unique features that other hot-start approaches cannot. Aptamer-based inhibition is reversible, allowing for an additional level of reaction specificity as the reaction temperature is decreased. Aptamers dissociate rapidly at lower temperatures than traditional hot start methods, eliminating the need for specific activation steps. Additionally, this lower release temperature and the ability to tune release during the aptamer selection process has enabled the creation of "WarmStart" enzymes to bring the benefits of specificity and consistent reaction performance to enzymes outside of typical PCR workflows, such as reverse transcriptases and enzymes used in isothermal amplification methods. Modulating enzyme activity to reduce unwanted side activities remains an important consideration in numerous assays and technologies, and NEB continues to apply its expertise in aptamer chemistry to develop warm and hot start forms of any enzyme, where such control is beneficial. But as researchers and developers continue to increase the complexity of these workflows, it is likely that no single hot-start technology will serve all needs. As such, NEB continues to evaluate various methods to ensure that our products enable new and existing applications. With a full understanding of each method's benefits and limitations, we aim to provide comprehensive hot start reagents for a wide variety of demanding biotechnology applications.

TABLE 2:
***Taq*/Q5 polymerase properties**

	TAQ DNA POLYMERASE	Q5 HIGH-FIDELITY DNA POLYMERASE
Polymerase family	A	B
3′→5′ exonuclease*	No	Yes
5′→3′ flap exonuclease**	Yes	No
5′→3′ polymerase (room temperature)	Yes	No
Aptamer function	Inhibit polymerase activity at room temperature	Inhibit 3′→5′ exonuclease activity at room temperature
Fidelity (relative to *Taq*)***	1	280

* *Also referred to as proofreading activity, this is a key contributor to high fidelity DNA replication in vitro.*

** *Also referred to as 5′→3′ exonuclease activity, this activity enables the use of hydrolysis (e.g., TaqMan®) probes.*

*** *Potapov, V. and Ong, J.L. (2017) PLoS ONE. 12(1): e0169774.*

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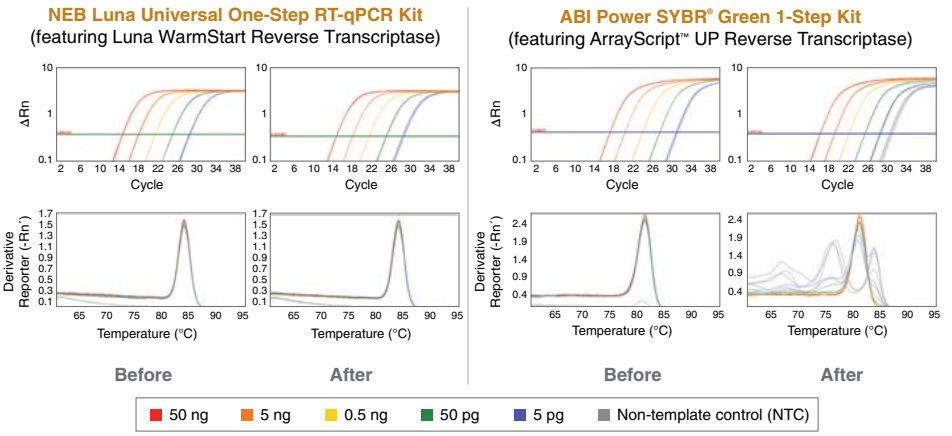
FIGURE 4:
WarmStart Luna RT prevents spurious amplification resulting from room-temperature pre-incubation

RT-qPCR targeting human ribosomal protein L32 was performed before and after a 24-hour incubation at room temperature, with triplicate reactions for a 5-log range of input human (Jurkat) total RNA and a non-template control. The Luna Universal One-Step RT-qPCR Kit featuring Luna WarmStart Reverse Transcriptase exhibited robust performance and no detectible non-template amplification, either with or without a 24 hour 25°C pre-incubation, while the ABI 1-Step Kit, featuring a non-WarmStart reverse transcriptase, exhibited significant non-template amplification.



Improve sensitivity and specificity with aptamer-containing products from NEB

Aptamers have been incorporated into several NEB products that target either a DNA polymerase, reverse transcriptase, or both. More information on these products, including recommended applications and performance data, can be found on the corresponding product pages at neb.com.

PRODUCTS WITH APTAMERS TARGETING A SINGLE ENZYME

PRODUCT	NEB #	FEATURE
Q5 Hot Start High-Fidelity Polymerase	M0493	High-fidelity amplification
Q5 Hot Start High-Fidelity 2X Master Mix	M0494	Just add primers and DNA template
NEBNext® Ultra™ II Q5 Master Mix	M0544	Optimized for high yields in NGS amplification
One*Taq*® Hot Start DNA Polymerase	M0481	Robust yields with minimal optimization
One*Taq* Hot Start 2X Master Mix with Standard or GC Buffer	M0484, M0485	Just add primers and DNA template
One*Taq* Hot Start Quick-Load® 2X Master Mix with Standard or GC Buffer	M0488, M0489	Quick-load format for direct loading onto gels
Hot Start *Taq* DNA Polymerase	M0495	5´ → 3´ polymerase activity and 5´ flap endonuclease activity
Hot Start *Taq* 2X Master Mix	M0496	Just add primers and DNA template
LongAmp® Hot Start *Taq* DNA Polymerase	M0534	For PCR products up to 30 kb
LongAmp Hot Start *Taq* 2X Master Mix	M0533	Master mix for PCR products up to 30 kb
EpiMark® Hot Start *Taq* DNA Polymerase	M0490	For amplification from bisulfite-converted DNA
Bst 2.0 WarmStart DNA Polymerase	M0538	Improved speed, yield and salt tolerance
WarmStart RTx Reverse Transcriptase	M0380	Temperature-activated reverse transcription
Luna Universal qPCR Master Mix or Luna Universal Probe qPCR Master Mix	M3003, M3004	Best-in-class performance. Includes inert tracking dye for ease-of-use.
One*Taq* One-Step RT-PCR Kit	E5315	Sensitive and robust endpoint detection of RNA templates
One*Taq* RT-PCR Kit	E5310	Two-step RT-PCR amplification

Luna Universal qPCR Master Mix

PRODUCTS WITH APTAMERS TARGETING MULTIPLE ENZYMES

PRODUCT	NEB #	ENZYME-CONTAINING APTAMER/FEATURE
WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)	M1800	*Bst* 2.0 DNA Polymerase, WarmStart RTx/ Isothermal amplification
WarmStart LAMP Kit (DNA & RNA)	E1700	*Bst* 2.0 DNA Polymerase, WarmStart RTx/ Isothermal amplification
Luna Universal One-Step RT-qPCR Kit or Luna Universal Probe One-Step RT-qPCR Kit	E3005, E3006	Hot Start *Taq* DNA Polymerase, Luna WarmStart Reverse Transcriptase/ Best-in-class RT-qPCR

FEATURED ENZYME

Luna WarmStart Reverse Transcriptase

Luna WarmStart Reverse Transcriptase (RT) paired with Hot Start *Taq* enables room temperature setup and offers robust performance in multiplex applications.

Luna WarmStart RT is included in the Luna Universal One-Step RT-qPCR Kit (NEB #E3005) and the Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006).

To request a sample, visit **LUNAqPCR.com**

Built for efficiency.

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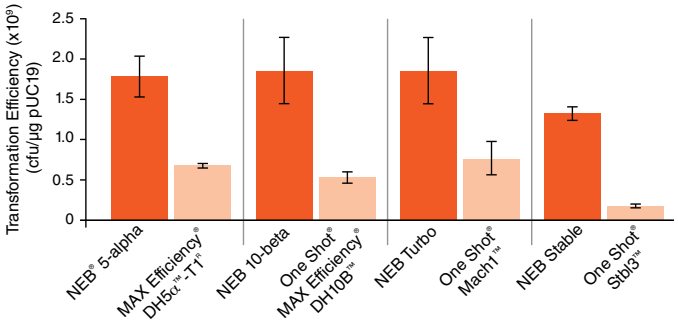
Ensure successful transformations with NEB competent *E. coli* for cloning. Choose from several high efficiency competent cell strains in a variety of formats that deliver performance, convenience and value.

FIGURE 1:
Benefit from high transformation efficiencies

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



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- High transformation efficiencies
- Compatible with NEBuilder® HiFi DNA Assembly and Gibson Assembly® reactions, as well as ligation reactions. No dilution required!
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	NEB 5-ALPHA COMPETENT *E. coli* (#C2987)	NEB TURBO COMPETENT *E. coli* (#C2984)	NEB 5-ALPHA F´ I^q COMPETENT *E. coli* (#C2992)	NEB 10-BETA COMPETENT *E. coli* (#C3019)	dam-/dcm- COMPETENT *E. coli* (#C2925)	NEB STABLE COMPETENT *E. coli* (#C3040)
FEATURES						
Versatile	●					●
Fast growth (< 8 hours)		●				
Toxic gene cloning		●	●			●
Large plasmid/BAC cloning				●		
Dam/Dcm-free plasmid growth					●	
Retroviral/lentiviral vector cloning						●
RecA$^-$	●		●	●		●
endA$^-$	●	●	●	●	●	●
FORMATS						
Chemically competent	●	●	●	●	●	●
Electrocompetent	●	●		●		
Subcloning	●					
96-well format*	●					
384-well format*	●					
12 x 8-tube strips*	●					

** Other strains are available in these formats upon request. For more information, contact custom@neb.com.*

NEW PRODUCT

Cloning Competent *E. coli* Sampler

Still not sure which cloning strain to choose? The NEB Cloning Competent *E.coli* Sampler allows you to try four of our popular chemically competent strains.

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

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

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

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

NEB Turbo
Features fast colony growth (6.5 hours) and tight expression control (*lacI*^{q}). Isolate DNA after only 4 hours of growth.

ORDERING INFORMATION

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

Visit www.neb.com/CloningCompCells to find:

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

FAQs

Electrocompetent vs. chemically competent cells

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

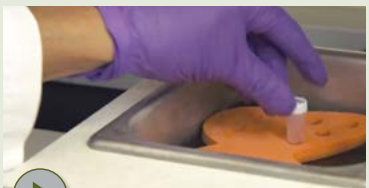
A: Electrocompetent and chemically competent cells differ in the way uptake of DNA is achieved. Chemically competent cells are treated with a salt solution, which promotes membrane binding of DNA and subsequent passage into the cell. Heat shock further enhances DNA passage. With electrocompetent cells,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

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

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

FEATURED VIDEOS
Find these and other helpful tips at **www.neb.com/CloningCompCells**


How to perform a transformation with NEB competent cells


Tips for successful transformations with NEB competent cells

UnBelizeable People Power

Janelle Chanona,
Vice President, Oceana

It's easy to be overwhelmed by the daily barrage of stories about extinctions, effluence and environmental disasters that are forever altering the planet, our home and our lives. That's why the story of how Belizeans rallied to write a new chapter in conservation leadership is so sensational – because it brings hope for the future.

Home to the second longest barrier reef in the world and an impressive collection of biodiversity, Belize is also one of less than fifty places in the world recognized as a World Heritage Site, a place of outstanding and universal value, by the United Nation's Educational, Scientific and Cultural Organization (UNESCO). The most iconic scene associated with Belize is a collapsed submarine cave, the Great Blue Hole.

Until recently, this marine treasure chest was threatened by the inherently dirty and dangerous reality of offshore oil. In the aftermath of the BP oil spill in 2010, Belizeans started asking, "What if that happened here?" So, when it became public knowledge that the Government of Belize had already sold off everything in offshore oil concessions to the highest bidders, Belizeans took the insult to heart. More than sixty percent of Belizeans depend daily on coastal and marine resources. Fifteen thousand people directly benefit from fishing. One in every four Belizeans works in the tourism sector, where marine destinations are the leading attraction. This explains why as much as 30% of the country's economy depends on the health and integrity of the marine resources.

Belize had multiple reasons to say 'no' to offshore oil, but someone in the corridors of power kept saying 'yes'. They were ignoring the economics, the science, the inevitable reality that awaited. That's why with the support of its funders, for more than seven years, Oceana in Belize empowered Belizeans to demand a meaningful role the in decision making processes. The first step was an unofficial, but national, referendum that showed more than 96% of participants were against offshore oil. Bolstered by those results, science, a dynamic communications plan and grassroots tactics, our efforts were eventually successful.

In December 2017, Belize made history by legislating an indefinite moratorium against offshore oil. Prime Minister Rt. Hon. Dean Barrow subsequently stated, "I quote Mahatma Gandhi, who famously said 'If the people lead, the leaders will follow.' It is in that context that I pay full tribute to Oceana in Belize's mobilization of citizens to persuade and to push Government to go further, I immediately concede, than perhaps might have been our original contemplation. That multifaceted campaign included a particularly comprehensive and instructive effort at education, and it helped raise awareness in a way that, I think, has enshrined now a widespread public consciousness of the great value of our marine resources and the corresponding need to always nurture and protect same."

Because the Belizean ocean constituency will ensure the longevity of the moratorium, it's been dubbed "The People's Law". While Belizeans feel empowered by the success of their efforts to protect their own environment, there are other threats that face their reef, such as hotter and acidifying oceans resulting from rising atmospheric CO_2, released worldwide by the unmanaged burning of fossil fuels. Belizeans are counting on other communities to feel inspired by their efforts, and join the rising tide of change to protect jobs, homes and way of life.

The mission of Oceana is to protect the world's oceans and make them rich, healthy and abundant as they once were. To learn more, visit oceana.org.

Photo of Great Blue Hole, courtesy of Oceana

Introducing the 2019 Passion in Science Awards

As NEB kicks off its 2019 Passion in Science Awards, Andy Bertera, NEB's Executive Director of Marketing & Sales, answers some questions about how these awards came about and what they mean to NEB and the scientific community.

Q: Why did NEB decide to launch the Passion in Science Awards?

A: When NEB turned 40, we put a lot of thought into whether this was a milestone that we should celebrate and, if we were to celebrate it, how we could do it in a meaningful way. We wanted to do something that reflected the values that NEB embraces, and that influence how we think and operate. With this in mind, we decided to hold an awards ceremony that celebrated our many customers who share the same values as NEB – people who are working to make the world a better place. This evolved into what we now call the "Passion in Science Awards", which proved to be an inspiring event for everyone – customers and NEB staff, alike.

Q: How did you decide on the categories for the Passion in Science Awards?

A: The Passion in Science categories reflect NEB's core values: the knowledge that there is overlap between art and science, that we all have a duty to help fellow humans, as well as to care for and protect our environment, and finally to inspire people by making scientific ideas and concepts accessible to everyone, not just our fellow scientists.

Q: What is the application process like?

A: You can start by visiting our website, **NEBPassionInScience.com**. From there, customers can apply directly, or nominate a colleague. The application process is relatively straightforward, but does require that the project(s) be explained in some detail. Applications are then reviewed by a panel of NEB scientists. The Winner(s) will be selected on the basis of how well they have impacted and enhanced the value of the category for which they have submitted their Award entry.

Q: What does it mean for NEB and the scientific community to continue to hold the Passion in Science Awards in the future?

A: By celebrating the scientists who embrace the same fundamental principles that guide NEB, we hope to be able to highlight how the research community can make a real difference in the world; differences that go well beyond their contributions to science – acts of kindness, solving social problems, and reducing our environmental footprint, to name but a few.

Q: What do the winners receive?

A: We invite the winners to our Ipswich, MA, USA campus, where they are involved in two days of presentations and discussions related to their inspiring work. The winners also receive $1,000 to donate to a charity of their choice or use towards travel to a scientific conference. We also try to spread the word about the great work the winners are doing through our website, social media, and our catalog.

Q: How do I nominate someone who has a passion for science?

A: To nominate someone you know who has a passion for science, visit **NEBPassionInScience.com**. Here, you can also learn more about the four award categories and check out our FAQs for more details.

Applications for the Passion in Science Awards are being accepted through January 31, 2019. Apply or nominate a colleague today!

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