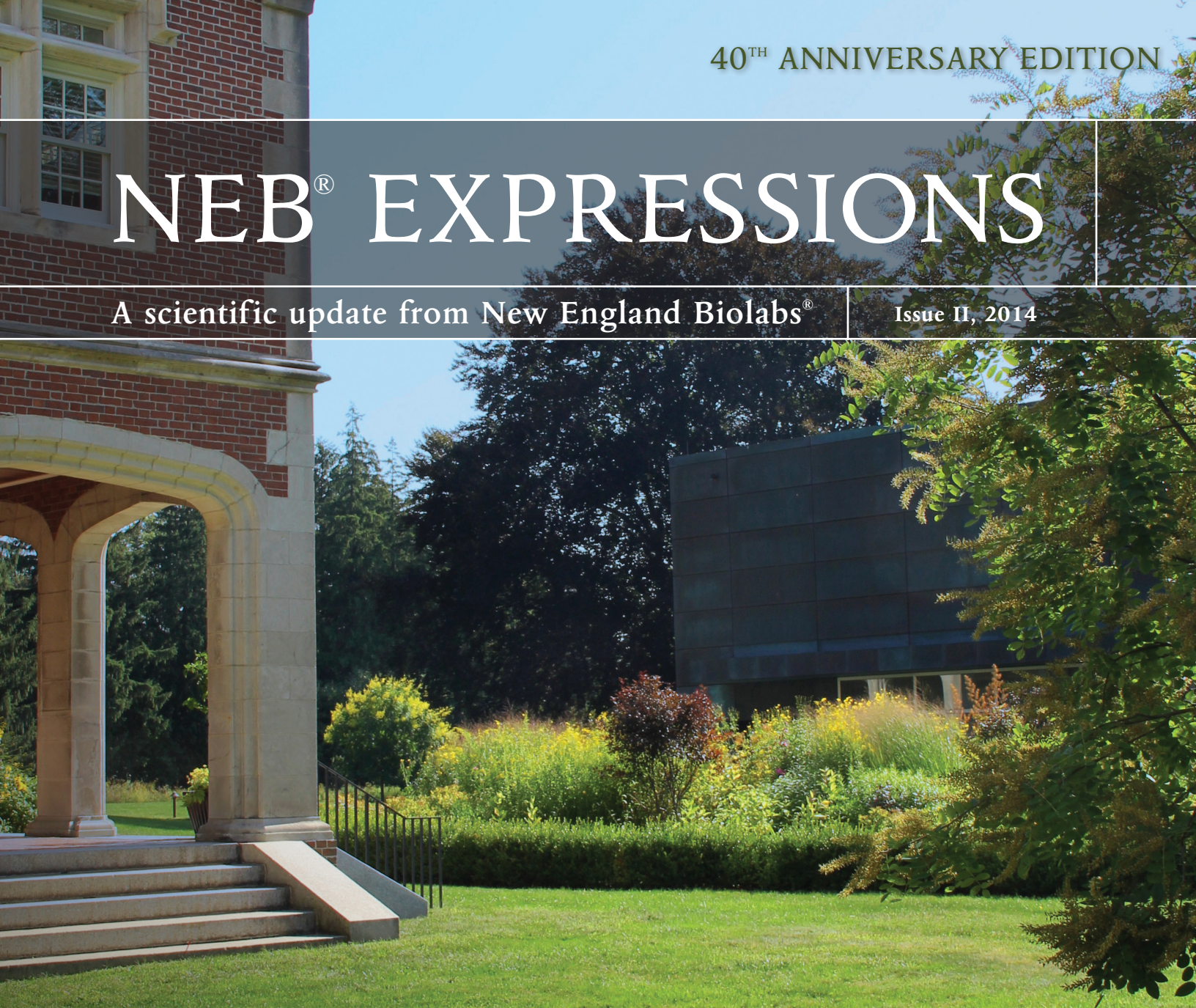
40TH ANNIVERSARY EDITION

NEB® EXPRESSIONS

A scientific update from New England Biolabs®

Issue II, 2014



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Issue II, 2014

COVER PHOTO

A peaceful corner of the New England Biolabs' campus, where tradition meets innovation.

A Letter from the CEO

Dear Researcher,

New England Biolabs is proud to be recognized as a world leader in the discovery, production and supply of reagents for the life science industry. For 40 years, we have been committed to meeting the needs of the scientific community. While our product portfolio and distribution network have expanded, our commitment to our customers remains the same:

- Set the standard for product quality and value
- Provide personalized technical and commercial support of the highest level
- Maintain a strong research program that contributes to the advancement of science
- Promote and pursue sound ecological practices and environmental sustainability

In this anniversary edition of NEB Expressions, we share some of the history and corporate philosophy that makes us a unique company, including our research efforts in parasitology and our unique approach to technical support.

In commemoration of our 40th anniversary, we are also pleased to introduce the Passion in Science Awards™, which recognize members of the scientific community who are committed to making a difference through their science, humanitarian service, environmental stewardship or artistic and creative spirit. We've always believed that science is more than a vocation – it embodies an ethos that inspires acts of compassion, brilliance and originality. We want to celebrate the many unsung heroes of the lab, not just for their discoveries, but for their passions that contribute to making the world a better place. Award winners will be announced in early October, and we invite you to visit NEBPassionInScience.com for more details.

Lastly, we recognize that scientists depend on us to provide exceptional quality products and technical support. We are proud and humbled that our reagents and basic research advance science, help cure disease and bring hope and opportunity to the developing world.

Thank you for your support over the last four decades. We wish you continued success in your research.

Jim Ellard, CEO

NEW ENGLAND BioLabs® Inc. | PASSION IN SCIENCE AWARDS™

Science and art come together in the artwork displayed on the front of the NEB laboratory facility in Ipswich, MA, USA. *Artwork created by Nicole Gordon.*

FEATURE ARTICLE

Making a Difference: Research on Neglected Tropical Diseases at NEB

Parasitology research has been part of New England Biolabs' mission since its inception. Approximately 40 years ago, NEB's founder, Dr. Donald Comb, was travelling through Asia and witnessed first hand the suffering inflicted by infectious disease agents amongst some of the world's most impoverished populations. Several years later, Dr. Comb established a research group at NEB devoted to parasitology. The focus of this group was, and continues to be, the poorly funded and understudied/neglected tropical diseases that are prevalent in many developing countries, and which put approximately 25% of the world's population at risk of infection.

Jeremy Foster, Ph.D., Sanjay Kumar, Ph.D., Larry McReynolds, Ph.D., Francine Perler, Ph.D., Barton Slatko, Ph.D., George Tzertzinis, Ph.D., Yinhua Zhang, Ph.D. and Clotilde Carlow, Ph.D.

NEB parasitology research group – the early days

In 1980, Dr. Comb recruited two scientists to conduct basic research projects at NEB – one researcher focused on restriction enzymes and the other worked on malaria. Five years later, a Parasitology Group was formed which accounted for approximately 50% of the research staff. The group's focus soon shifted towards the poorly funded and understudied tropical diseases lymphatic filariasis and onchocerciasis. These diseases are caused by parasitic filarial nematodes and are transmitted by insect vectors. They are prevalent in over 80 countries, where over 150 million individuals are afflicted (Fig. 1). Currently, there are no vaccines for filarial diseases, but there are a few widely-used drugs (e.g., ivermectin, albendazole and diethylcarbamazine). However, these drugs only target larval stages and not the adult worms. Additionally, there are now signs of drug resistance developing to these commonly used drugs, causing great concern.

Overview of lymphatic filariasis and onchocerciasis

The World Health Organization (WHO) recognizes lymphatic filariasis as the third-leading cause of infectious disease-associated disability, after malaria and tuberculosis, while The Centers for Disease Control & Prevention (CDC) ranks onchocerciasis as the second-leading cause of infectious blindness after trachoma. These filarial diseases significantly impact the well-being of many individuals in the developing world, and contribute to the extreme poverty typical of endemic communities.

In lymphatic filariasis, adult worms, such as *Wuchereria bancrofti* and *Brugia malayi,* mature and reproduce in the host lymphatic system, where they may remain for up to 10 years. Millions of larval worms are released into the bloodstream where they may be acquired by a female mosquito during a blood meal to initiate the insect-vector phase of parasite development. Infected individuals often present with kidney and lymphatic damage, where blockage of lymphatic ducts leads to the characteristic swelling (lymphedema) of limbs and genitalia. In severe chronic disease, gross disfigurement known as elephantiasis ensues (Fig. 2, page 5), causing disability, social stigma and psychological trauma. The related filarial nematode, *Onchocerca volvulus*, is the causative agent of onchocerciasis (river blindness) and is transmitted to humans by blackflies of the genus *Simulium*, which breed in fast flowing rivers. Long-lived adults reside and reproduce in host subcutaneous tissues, while the larvae released by female worms migrate to the skin and eyes, causing intense skin pathologies and eye lesions (Fig. 3, page 6).

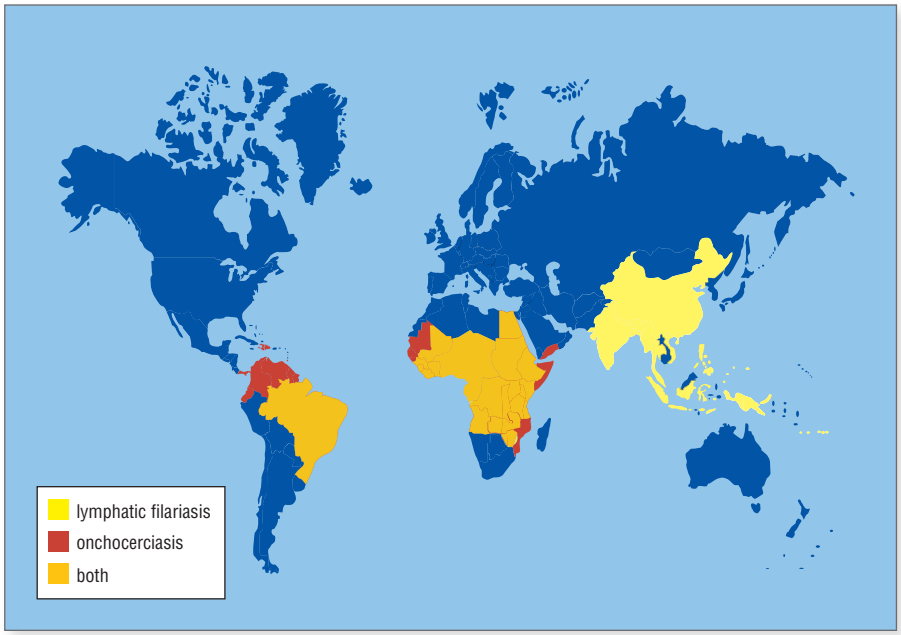

Figure 1: *Global distribution of lymphatic filariasis and onchocerciasis.*

Detection of filarial parasites: antibodies, antigens and DNA probes

NEB researchers in the Parasitology Group strive to apply the latest techniques to better understand the basic biology of filarial nematodes. In the early days, one such technique involved the construction of expression libraries for screening with various sera (1,2,3). Another approach involved radiolabelling parasite surface molecules to identify and characterize potentially protective antigens (4,5) and other molecules expressed at the host-parasite interface as candidate vaccine components and diagnostic tools. Vaccine studies were eventually carried out (6), and a protective nematode polyprotein allergen was identified (2). Immunoparasitological studies led to a method to identify mosquitoes transmitting *B. malayi* infection (7) and a biomarker for heartworm infection in animals (8,9). Molecular biology research resulted in the identification of a highly-repeated DNA sequence in *B. malayi*, which formed the basis of a genus-specific DNA probe to detect infection in humans (10,11).

The genomics revolution: *Wolbachia* symbiont, drug targets and lateral gene transfer

The 1990s ushered in the era of modern molecular approaches to analyze parasite genomes. Fully sequencing metazoan parasite genomes of

Figure 2: *Elephantiasis of the lower leg, one of the visible symptoms of lymphatic filariasis. (Provided by WHO/TDR).*

~100 Mb was considered unrealistic at that time, so NEB joined the WHO/TDR-funded Filarial Genome Project, with a focus on parasite stage-specific gene discovery. cDNA libraries were constructed from different life cycle stages of *B. malayi,* and clones were partially sequenced to generate expressed sequence tags (ESTs). Through bioinformatic comparison to genes sequenced from other organisms, these ESTs shed light on biological processes in *B. malayi* (12). This was an early approach to transcriptome analysis that generated >25,000 ESTs from 25 cDNA libraries, and resulted in partial sequence information for nearly 10,000 clusters (genes) (13).

During this analysis, bacterial sequences with similarity to Rickettsial bacteria were consistently observed and eventually attributed to the endosymbiotic bacterium *Wolbachia*, first suggested by EM studies in the 1960s, and confirmed by molecular means in 1995 (14,15). This maternally-inherited bacterium is an obligate mutualist in almost all filarial nematodes that infect humans, and has now been validated as a new drug target for filarial disease control in numerous human clinical trials using the antibiotic doxycycline (see (16) for review). Anti-*Wolbachia* treatments cause a long lasting sterility of female worms but, more importantly, lead to adult worm death without any serious adverse effects in humans (16). However, due to difficulties of administering a protracted doxycycline treatment to large populations in endemic countries, alternative anti-*Wolbachia* therapies are being sought. To facilitate this approach, NEB scientists reported the full genome sequence of the *Wolbachia* endosymbiont from *B. malayi* in 2005 (17). A few years later, with advances in sequencing technology and throughput, members of the Parasitology Group also contributed to the full genome sequence of the nematode *B. malayi* (18). More recently, they published the first comprehensive identification of microRNAs (miRNA) in various developmental stages of *B. malayi*. This study forms the background for understanding miRNA function and the regulatory networks in the complex filarial life cycle (19). The availability of these and other genome sequences has enabled NEB researchers to develop rational drug target selection methodologies to generate a pipeline of potential drug targets (20,21). The rapid release of this data has provided scientists around the world with valuable resources that can highlight new approaches for attacking filarial parasites.

Several potential targets are being further investigated at NEB (22–30), and programs for new drug discovery are underway. We have incorporated forward and reverse genetic approaches using the genetically tractable free-living nematode *Caenorhabditis elegans*. This has enabled the validation of selected nematode targets and experimental characterization that would be impossible in parasitic species (26,31,32,33).

Comparison of the *B. malayi* and *Wolbachia* genome sequences identified metabolites that might be sourced from one organism to the other and thereby underlie the mutualistic symbiosis. Among these are nucleotides, riboflavin and heme. *B. malayi* is deficient in *de novo* synthesis of these molecules, while *Wolbachia* has retained biosynthetic capabilities despite having a streamlined genome typical of most endosymbionts (17,18).

A role for *Wolbachia* in meeting its nematode host's requirement for riboflavin and heme has been confirmed through experimentation at NEB (29,34), validating the biosynthetic pathways leading to these metabolites as potential drug targets. The riboflavin pathway is fully functional in *Wolbachia*, and implicated a nutritional role for the endosymbiont by showing supplementation with this vitamin partially rescued filarial parasites treated with doxycycline to remove their *Wolbachia*. With respect to the heme biosynthetic pathway, *B. malayi* contains only ferrochelatase, the enzyme that catalyzes the last step in the pathway. We discovered that the gene encoding ferrochelatase is the result of lateral gene transfer (LGT) from a *Rhizobiale* bacterium. The protein product is both functional and essential for *B. malayi* (31). Further genome comparisons have revealed a surprisingly high level of trans-kingdom LGT, with about 15% of the *Wolbachia* genome also being present in the *B. malayi* genome (35,36). The LGTs include over 30 full-length genes, some of which appear to be transcribed at high levels in a life-cycle stage-specific manner.

New approaches, new technologies

Ongoing areas of parasitology research at NEB are exploiting the post-genomics revolution and associated emerging technologies to address new questions and re-visit old problems with contemporary approaches. For example, an oligo capture method has been adapted to enable targeted sequencing of additional *Wolbachia* genomes, without the need to simultaneously sequence the 100-fold larger nematode host genome (37). This allows quick and efficient sequencing of *Wolbachia* from different clinical isolates and from other filarial nematodes. Various other 'omics studies are being pursued at NEB. These include transcriptomics (RNA-seq) and microRNA analysis (19) of multiple life cycle stages of different filarial nematodes and their *Wolbachia* endobacteria, as well as tissue-specific transcriptomics. Proteomic studies are also being conducted with a focus on the nematode surface glycoproteome – a research area that featured in the early days of parasitology at NEB, as well as the feeding apparatus of mosquito vectors.

Filarial diagnostics also featured prominently some 30 years ago, and is now benefiting from state

Parasitology Research at NEB

1985
NEB's parasitology group was founded

>100 NEB researchers
have contributed to the parasitology program since 1985

NEB Parasitology Publications by Category

Drug Discovery	52
Symbiosis	28
Diagnostics	25
Vaccine Studies	25
Genome Biology	19
Glycobiology	8

For the full list of NEB publications, visit **www.neb.com/ParasitologyPublications**

Figure 3: *Ocular lesions in an onchocerciasis (river blindness) victim blinded from sclerosing keratitis. (Provided by WHO/TDR).*

of the art, modern DNA-detection technologies. Loop-mediated isothermal amplification (LAMP) of filarial-specific sequence results in a visual read-out of amplification with high sensitivity and specificity without the need for sophisticated instrumentation (38), making the approach well-suited to field use in endemic countries.

Filarial nematodes have previously been intractable to genetic manipulation, but recent advances in this area by NEB scientists are opening the door to studies not possible a few years ago. These include a more efficient method for silencing genes by RNA interference (RNAi) (39). This involves soaking worms in a heterogeneous mix of short interfering RNAs, where the molecules in the mixture are produced enzymatically from a longer dsRNA with the correct size and end modifications for efficient silencing (40,41). Methods for whole-mount immunofluorescence have also been developed for examining, in detail, the cell biology of *Wolbachia* and filarial nematodes in both wild-type and RNAi-treated worms (39,42). A system for transfecting *B. malayi* larvae has also been developed, in which the transfected parasites maintain transgene expression around the life cycle and into the subsequent generation (43). It is hoped that these studies will pave the way for performing future genetic analyses directly in filarial nematodes.

Perspective

For nearly 35 years, parasitologists at NEB have applied the latest biochemical and molecular techniques to the study of malaria, filarial nematodes and their *Wolbachia* endosymbionts in the areas of genomics, vaccines, diagnostics and drug targeting. The aim throughout has been to uncover and freely disseminate details concerning the basic biology of these organisms to help the research community achieve the goal of eliminating these neglected parasitic diseases.

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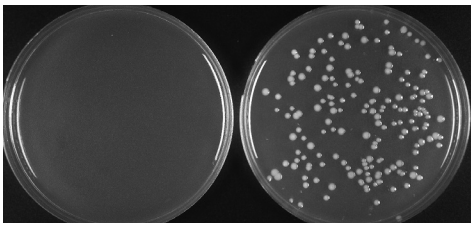
NEB PCR Cloning Kit

The NEB PCR Cloning Kit allows quick and simple cloning of all your PCR amplicons, regardless of the PCR polymerase used. This kit utilizes a novel mechanism for background colony suppression – a toxic mini gene is generated when the vector closes upon itself – and allows for direct cloning from your reaction with no purification step. Enjoy faster cloning with more flexible conditions.

Advantages:

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

PCR cloning with low/no background



A 500 bp PCR product incubated with the linearized vector in a 3:1 ratio according to recommended protocol. 2 µl of reaction was transformed into the provided NEB 10-beta Competent E. coli and 1/20th of the outgrowth was plated. The left plate serves as the control, with vector backbone only. The right plate shows colonies containing the PCR insert.

Ordering information

PRODUCT	NEB #	SIZE
NEB PCR Cloning Kit	E1202S	20 reactions

Easy Tips for Success with the NEB PCR Cloning Kit

Improve the success of your PCR cloning experiments by following these simple tips:

1 Did you know the most critical step in PCR cloning is stopping your ligation?
If you wish to store your ligations to allow transformations at a later time, make sure your freezer is cold enough (-20°C) to freeze the ligations. Or, you may quick freeze with a dry ice/alcohol bath, before transferring the samples to -20°C. If you find your freezer-stored ligations have remained in liquid form, this may have allowed further low level ligation of the vector backbone to occur. In this circumstance, plate less outgrowth medium (< 50 µl).

2 Be sure to follow the protocol
The protocol has been optimized to have a low background; if you have inadvertently deviated from the optimized protocol (e.g., extended ligation incubation, overly-concentrated outgrowth), compensate by plating less outgrowth medium (< 50 µl).

3 Add the cloning master mix to the reaction last
Some people try to save time by preparing a mix of water, ligation master mix and pMiniT, aliquoting this to tubes and adding the insert DNA. This allows pMiniT to recircularize, since ligation can begin before the amplicon is added. This may result in lower cloning efficiency.

4 Use an insert:vector ratio of 3:1
A higher insert:vector ratio can actually result in fewer colonies. This is due to the fact that inserts may ligate to both ends of the vector, obscuring the phosphorylated 5' ends, preventing circularization.

5 Plate 50 µl or less of the 1 ml outgrowth
Plating too much of the outgrowth can increase background, and cause problems with colony PCR. If you need more colonies, spread 50 µl of outgrowth each onto multiple plates.

6 Follow the transformation protocol carefully
The number of colonies will decrease significantly if you incubate the ligated DNA with the competent cells for less than 20 minutes, or if the outgrowth with SOC is less than 60 minutes.

7 Do not incubate the transformation plates at room temperature
The slow growth rate of the cells at room temperature will increase the number of background colonies.

8 Use with blunt or single-base overhangs:
You can use the NEB PCR Cloning Kit to clone any fragment that has a blunt end or a single-base overhang.

9 Check compatibility with other strains:
You can use other strains of high-efficiency competent *E. coli*, as long as they grow quickly. NEB Stable Competent *E. coli* (NEB #C3040) is an excellent choice for cloning direct and indirect repeats, and works well with the NEB PCR Cloning Kit. NEB Turbo Competent *E. coli* (NEB #C2984) and NEB Express Competent *E. coli* (NEB #C2523) are also good choices. NEB 5-alpha Competent *E. coli* (NEB #C2987) grows slowly, so the number of background colonies is higher than with NEB 10-beta Competent *E. coli* (NEB #C3019).

Inside NEB's Technical Support Program

If you've ever had a question about one of NEB's products, and called for technical support, chances are that you spoke with a scientist who uses that product in his or her own research. In fact, when available, questions are routed to the scientist who either developed or manages the product, ensuring that you get the best, most relevant answers. Our international customers always have the option of contacting the technical support teams at their local NEB subsidiary for immediate technical support. The technical teams from our network of international distributors and subsidiaries work closely with scientists at NEB headquarters to provide the same level of support that customers receive when contacting us directly.

To help us explore NEB's Technical Support Program, Ana Egana, Ph.D., Technical Support Manager and Product Manager, and Jeremy Foster, Ph.D., Staff Scientist answer a few questions:

Q. What is NEB's model for Technical Support, and how does it differ from other companies'?

Ana. *From the beginning, NEB has operated under the model of "scientists helping scientists". This means that NEB is committed to having active bench scientists answering customer questions. Scientists from our Research, Applications Development and Production groups speak directly with our customers. Technical questions that come to NEB via e-mail or neb.com are addressed directly by our scientists that have expertise on that specific product, application or area of research. This approach differs from other companies, where a dedicated technical support group is designated to deal with customer questions.*

Q. What types of questions are customers asking?

Ana. *We get all types of questions from customers with varying levels of experience. Some questions are specific to a product and its use in an application. Sometimes they can be very general such as, "How would you recommend that I approach this experiment?". We have spoken to high school students interested in the history and use of restriction enzymes, as well as scientists carrying out cutting-edge research, asking whether we can provide advice on how to push the limits of an enzyme to do a new and very specific job.*

Q. How many Technical Support calls does NEB receive each year?

Ana. *In the U.S., we receive over 20,000 calls annually. In addition to those, several thousand questions are addressed by our seven subsidiary offices and many distributors throughout the world.*

Q. Do you also answer questions via email and social media?

Ana. *We will address any technical question that comes to us from any source. However, the most efficient way for us to help our customers is if they contact us directly by phone, email or by filling out the tech support form on our website.*

Q. Ana, why do you have such a vested interest in providing exceptional Technical Support?

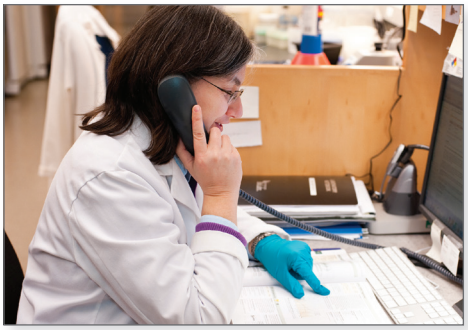
Ana. *People who have been trained in the lab believe that there is an unwritten rule of "paying it forward". Somebody had to take the time to train us, so we feel obligated to train the people who join a lab after us. Everyone in science has done this. We know that there is an educational component to the work we do in a lab. This same spirit is what drives our technical support team.*

Q. Jeremy, can you give an example of one call that stands out in your mind as demonstrating the depth of knowledge and breadth of resources that are available to our customers?

Jeremy. *A customer called technical support looking for a method to digest DNA into single nucleotides for subsequent mass spec analysis. I was able to provide them the details of our recommended cocktail of enzymes for doing exactly that – but, actually, digesting to nucleosides, instead of nucleotides, because that allows for a much more sensitive mass spec analysis at the end.*

When they realized that we had experience in the area, as well as the necessary enzymes and state-of-the-art mass spec instrumentation, they asked us to collaborate with them in analyzing their samples. We were happy to help, and it turned out to be a very fruitful collaboration, because they had engineered E. coli bacteria to take up unnatural nucleotide triphosphates, and incorporate them into this DNA during replication inside the cells. Our mass spec analysis provided definitive proof that the DNA recovered from the E. coli had the unnatural bases incorporated into it. This was later reported in the journal Nature, describing the first semi-synthetic organism containing an expanded genetic alphabet, going beyond the traditional A, G, C & T bases. (Malyshev, D.A. et al. (2014) Nature, 509, 385–388.)

For the full interview with Ana and Jeremy, visit **www.neb.com/TechnicalSupport**.



NEB scientists answer technical support questions directly from customers.

HOW TO PREPARE FOR YOUR TECHNICAL SUPPORT CALL:

1. Gather your protocols, reagent information (including NEB catalog and lot numbers), and data. You'll want to be able to tell the NEB scientist how you set up the reactions and what controls you've run. If it is determined that the product is not performing as expected, the scientist will ask you for the product lot number, so any issues can be investigated internally.
2. While talking to someone in technical support, have easy access to our website. Many times the answer can be found by using a tool or by looking at a chart on the web that the person on the call may be able to direct you to.
3. Be patient and try to answer all the questions that we may ask as accurately as possible. The NEB scientist is approaching your case from their past experience and, in many cases, the issue may be a small step that was omitted or a difference in protocol that you may have thought was insignificant.
4. Make the call – US customers can call 1-800-632-7799 (Monday - Friday 9:00 AM - 5:00 PM ET) or fill out a Technical Support Request Form **https://www.neb.com/support/technical-support-form** (anytime). International customers should contact their local subsidiary or distributor.

Online Tools & Databases to Support Your Research

Providing educational tools and resources that aid in experimental design is a priority at NEB. As technologies advance, so too must our methods of sharing and teaching science. To support researchers who prefer to turn to the web, rather than a catalog, we have developed accessible and comprehensive databases as well as web tools for bench scientists. By developing and sharing the information we have gathered over the past four decades, our goals are to support our customers and help expand the collective scientific knowledgebase.

Online Tools:

Known for our comprehensive Catalog & Technical Reference, we place a premium on communicating our scientific expertise with our customers. In order to keep apace with the latest developments in bench-side technology, NEB offers a series of interactive technical tools, which are available through the **Tools & Resources** tab of **www.neb.com**. From selection charts to laboratory calculators, these tools address some of the most common laboratory pain points. Examples of our tools include:

NEBioCalculator™

NEBioCalculator delivers scientific calculations and conversions, including molar ratios and molarity, via a straightforward user interface.

NEBcloner™

Use NEBCloner to find the right products and protocols for each step (digestion, end modification, ligation and transformation) of your next traditional cloning experiment. Also, find other relevant tools and resources to enable protocol optimization.

NEBcutter®

NEBcutter is used to identify the restriction sites within your DNA sequence. Choose between Type II and commercially available Type III restriction enzymes to digest your DNA. NEBcutter V2.0 will indicate cut frequency and methylation state sensitivity.

Double Digest Finder

Use Double Digest Finder to guide your reaction buffer selection when setting up double-digests, a common timesaving procedure. Choosing the right buffers will help you to avoid star activity and loss of product.

Tm Calculator

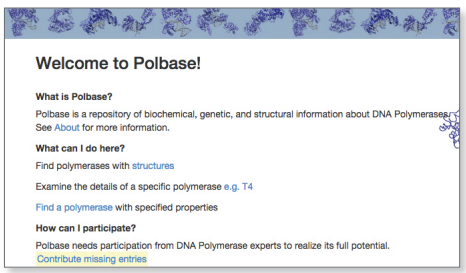
Use our Tm Calculator when designing PCR reaction protocols to help determine the optimal annealing temperature for your amplicon.

NEBuilder®

NEBuilder can be used to design primers for your Gibson Assembly reaction, based on the entered fragment sequences and the polymerase being used for amplification.

Databases:

NEB scientists have also worked on the development of several databases, including REBASE®, PolBase® and InBase. These open repositories offer comprehensive information on enzymes and proteins we're passionate about. They are updated frequently to capture recently published information. Our most popular database, REBASE, was developed over 40 years ago, and is a dynamic, curated database of restriction enzymes and related proteins. PolBase is a repository of biochemical, genetic and structural information about DNA Polymerases. InBase describes how inteins work and is home to the "Intein Registry", that includes a list of inteins and their properties. All of these databases are also accessible through the **Tools & Resources** link at **www.neb.com**.



APPLICATION NOTE

Selective depletion of abundant RNAs to enable transcriptome analysis of low-input and highly-degraded RNA from FFPE samples

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Introduction

Deep sequencing of cDNA prepared from total RNA (RNA-seq) has become the method of choice for transcript profiling and discovery. However, the standard whole-transcriptome approach faces a significant challenge, as the vast majority of reads map to ribosomal RNA (rRNA). One solution is to enrich the sample for polyadenylated transcripts using oligo (dT)-based affinity matrices; although, this also eliminates other biologically relevant RNA species, such as microRNAs and noncoding RNAs, and relies on having a high-quality and high-quantity RNA sample. Here, we present a method to eliminate abundant rRNAs from total RNA with different degradation levels, from intact RNA in highly degraded formalin-fixed paraffin-embedded (FFPE) samples.

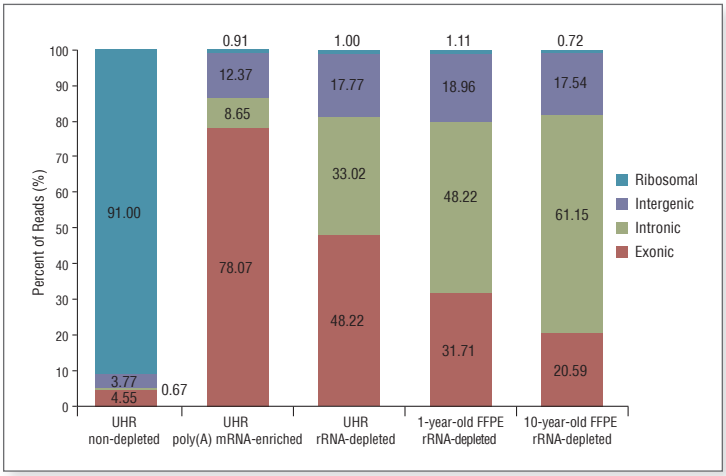
This method is based on the hybridization of probes to the targeted abundant rRNA, followed by subsequent enzymatic degradation. We applied this method to remove cytoplasmic and mitochondrial rRNA from different eukaryotic total RNA samples (human, mouse and rat), as well as degraded (archive age of 1 year) and highly degraded (archive age of 10 years) FFPE breast tumor biopsy RNA samples. We evaluated the depletion efficiency and off-target effects of this method using strand-specific RNA high-throughput sequencing.

Results

Sample input details

The NEBNext rRNA removal method was tested on different input amounts of RNA samples and with different RNA degradation levels. "Universal Human Reference RNA (UHR)" is a commercially available non-degraded RNA (Agilent). "1-year-FFPE RNA" was a pool of equal portions of RNA extracted from > 100 breast tumor biopsy samples with an archive age of one year. "10-year-old FFPE RNA" was a pool of equal portions of RNA extracted from eight FFPE breast cancer samples with an approximate archive age of 10 years. Efficiency of depletion of specific cytoplasmic and mitochondrial human rRNAs, as well as mouse and rat rRNA, was assigned.

Figure 1. Read distribution across transcripts.



RNA-seq libraries were generated from Universal Human Reference Total RNA (UHR, Agilent) or Breast Cancer FFPE RNA (with an archive age of 1 year and 10 years). RNA was either untreated or treated with the NEBNext poly(A) mRNA Magnetic Isolation Module (NEB #E7490) or the NEBNext rRNA Depletion Kit. RNA-seq libraries were made using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB #E7420). Reads were mapped to the hg19 genome and read distributions were determined using Picard RNA-Seq Metrics.

To investigate any effects on non-ribosomal RNA, correlation of transcript expression was determined between rRNA-depleted and non-depleted samples, for UHR and FFPE samples. Details of all of the above can be found in the full application note (see below).

Transcript composition

The composition of transcripts after rRNA depletion was assessed by determination of the proportion of reads mapping to annotated exons, introns and intergenic regions, and this was compared to the composition of non-depleted total RNA and of poly(A) mRNA-enriched RNA.

Libraries generated from rRNA-depleted RNA resulted in low rRNA reads, comparable to poly(A) mRNA-enriched RNA, while also retaining more noncoding reads. Effective rRNA depletion efficiency was achieved even with FFPE RNA. The exonic ratio was constant between total RNA input amounts of 100 ng (Fig. 1) and 1 µg (data not shown). FFPE RNA contained a higher percentage of intronic reads than fresh RNA, as previously reported.

Summary

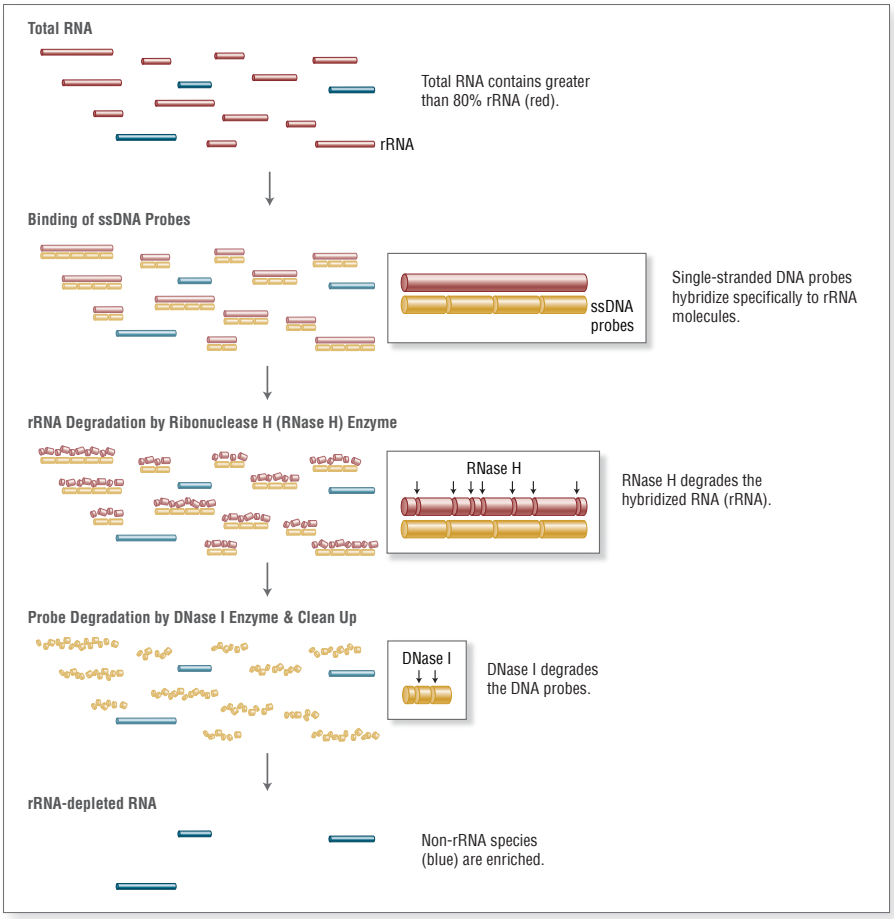
This method offers a robust and simple solution for transcriptome analysis of a variety of samples, including low quality and low quantity clinical samples such as FFPE RNA. Ribosomal RNA depletion resulted in a minimal percentage of total reads mapping to rRNA sequences, regardless of the species, input amount (1 µg or 100 ng), or degradation level. Additionally, there was very good transcript expression (FPKM) correlation (R > 0.93) between rRNA depleted and non-depleted libraries. Moreover, it is amenable to high-throughput sample preparation and robotic automation. This method is also sensitive, specific, and produces increased coverage of less abundant transcripts in RNA-Seq studies.

Visit **www.neb.com/E6310** to download the full application note.

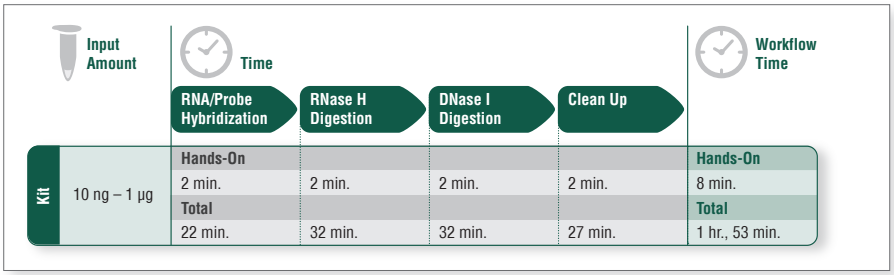
NEBNext rRNA Depletion Kit

The NEBNext rRNA Depletion Kit enables efficient removal of ribosomal RNA (rRNA) from total RNA from human, mouse and rat RNA samples. The kit works well for both low-quality, degraded RNA (including FFPE RNA) and high-quality, intact RNA. rRNA depletion with a broad range of input amounts (10 ng to 1 µg) is completed in approximately 2 hours, with less than 10 minutes of hands-on time.

NEBNext rRNA Depletion Kit Workflow



NEBNext rRNA Depletion Kit Workflow Times



Ordering Information

PRODUCTS	NEB #	SIZE
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns

ADVANTAGES

- A single kit that performs reliably well for all of your RNA samples:
  - FFPE (degraded) or high-quality (intact) RNA
  - 10 ng to 1 µg input amounts
- Remove > 95% of rRNA, and obtain more relevant sequence reads from your sample
- Obtain a more complete transcriptome picture through retention of noncoding & incomplete RNAs that are lost with oligo d(T) poly(A) mRNA enrichment methods
- Suitable for use with human, mouse or rat samples
- Easily integrated upstream of any downstream random-primed cDNA synthesis protocol
- No more need to homebrew: A reliable & convenient NGS-validated kit for the "RNase H protocol"[1,2] for rRNA depletion
- Visit **NEBNext.com** to find the full list of NEBNext reagents available, including kits for DNA, RNA and Small RNA for the Illumina®, Ion Torrent™, SOLiD™ and 454™ platforms.

1. Adiconis, X. et al. (2013). Nature Methods 10; 623-629.
2. Morlan, J.D. et al. (2012). PLoS One 7, e42882.

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