NEB EXPRESSIONS

A scientific update

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IN THIS ISSUE

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Advances in next generation sequencing: How researchers at NEB are working to improve our understanding of the genome, epigenome and transcriptome.

by Joanne Gibson, Ph.D. & Betsy Young, Ph.D.

Conducting innovative basic research has always been integral to New England Biolabs' philosophy; our scientists have contributed to the advancement of science and modern molecular biology technologies for almost 50 years and, as a result, have authored or co-authored over 1,500 publications.

Basic research at NEB has led to the development of new technologies, streamlined workflows, and has helped facilitate a deeper understanding of the scientific questions we seek to answer. This empowers not only our scientists but also the scientists we serve - scientists working for scientists.

Advances in NGS are a major area of focus for NEB researchers. As scientific questions evolve, our research team works to fill technological gaps to enable more insightful genomic, epigenomic and transcriptomic analysis. Most recently, our scientists have applied their expertise in enzymology and NGS to address unmet needs in the analysis of genome-wide methylation, transcriptional start sites, full-length transcriptomes, chromatin accessibility, and much more.

This article gives an overview of some of the exciting sequencing technologies developed at NEB and discusses the applications they enable.

Methylation analysis

The most abundant form of epigenetic modification in the genomes of both prokaryotes and eukaryotes is methylation, which plays a role in gene regulation and cell differentiation. Methylome analysis has traditionally been restricted to sodium bisulfite treatment, which causes extreme damage, followed by short-read sequencing using platforms like Illumina®. With the recent introduction of NEBNext® Enzymatic Methyl-seq (EM-seq™) (NEB #E7120) [1], it has become possible to analyze methylation across a genome without the challenges of conversion-induced DNA damage with sub-nanogram amounts of DNA.

Long-Read EM-seq (LR-EM-seq)

Long-read sequencing has steadily grown in popularity. Companies like Oxford Nanopore Technologies® (ONT) and Pacific Biosciences® (PacBio®) are facilitating ever-longer sequencing read lengths. LR-EM-seq, developed in the Ettwiller lab, preserves the integrity of DNA using a highly effective enzyme-based conversion that minimizes damage [2]. It allows long-range methylation profiling of 5mC and 5hmC within amplicons up to 5 kb using a long-read sequencing protocol. When applied to biologically relevant, differentially methylated genomic regions (DMR) with various methylation percentages and contexts, the result from LR-EM-seq is in accordance with previous studies.

Long-range phasing of methylated cytosines is essential for several applications, including studying DMRs of the genome, particularly where methylation status is a known disease biomarker. LR-EM-seq can also support haplotyping of methylation patterns and targeted methylation analysis.

This new sequencing technology is a comprehensive solution for analyzing 5mC and 5hmC methyl marks in various contexts (e.g., CpG, CHG, CHH). LR-EM-seq only requires a small amount of starting material – as low as a few ng of DNA. It uses the same analytical strategies developed for bisulfite sequencing.

Rapid Identification of Methylase Specificity (RIMS-seq)

Also developed in the Ettwiller Lab, RIMS-seq seamlessly combines shotgun sequencing of bacterial genomes with 5mC methylase detection in a single experiment using Illumina sequencing [3]. There are three types of methylation in bacteria: 5mC (5-methylcytosine), 4mC (N^4-methylcytosine), and m6A (N^6-methyladenine). While PacBio single molecule real-time (SMRT®) sequencing can easily detect m6A and 4mC, it is more challenging to detect 5mC. This is because the polymerase stalls at the methylation site for a certain amount of time; however, the pause of the polymerase is much shorter for 5mC, which makes it more challenging to identify.

Inspired by the success of SMRT sequencing in revealing epigenetic landscapes, researchers in the Ettwiller lab modified the Illumina library preparation protocol and added an alkaline treatment incubation step. This treatment induces a level of deamination large enough to reveal 5mC methylation specificity commonly found in bacterial-RM systems while still producing sequence quality comparable to standard Illumina DNA sequencing. RIMS-seq can identify the 5mC methylome of mixed communities of unknown bacteria.

Because of the simplicity of this approach and its broad applicability, RIMS-seq has the potential to replace standard DNA-seq for bacterial genome sequencing (Figure 1).

Identification of Transcription Start Sites (TSS)

Identifying transcription start sites (TSS) gives vital information relating to RNA transcripts, regulatory regions, promoters, and transcription factor binding sites in a sequence.

Cappable-seq

Developed in the Ettwiller and Schildkraut labs, Cappable-seq is a sensitive and robust method for directly enriching the 5′ end of primary transcripts from bacteria and microbiomes [4]. This method enables the determination of transcription start sites (TSS) at a single base resolution. Prokaryotes have a unique triphosphate at the beginning of the RNA transcript. One of the advantages of this technique is that it directly targets 5′ triphosphorylated RNA – the first nucleotide incorporated by the RNA polymerase upon initiation of transcription – in total RNA preparations. Cappable-seq uses this feature to capture the 5′ end of the molecule. The overwhelming majority of a total RNA sample is made up of processed RNA, such as ribosomal RNA, but by targeting 5′ triphosphorylated RNA, the rRNA population is reduced to just 3%, and the need to perform rRNA depletion beforehand is eliminated; therefore, it offers the ability to investigate the triphosphorylated RNA molecules that would otherwise be overwhelmed by processed RNA. This reduces the complexity of the transcriptome to a single quantifiable tag per transcript resulting in the ability to sequence the enriched 5′ triphosphorylated RNA population at a much deeper level at a lower cost, enabling the profiling of gene expression in a microbiome.

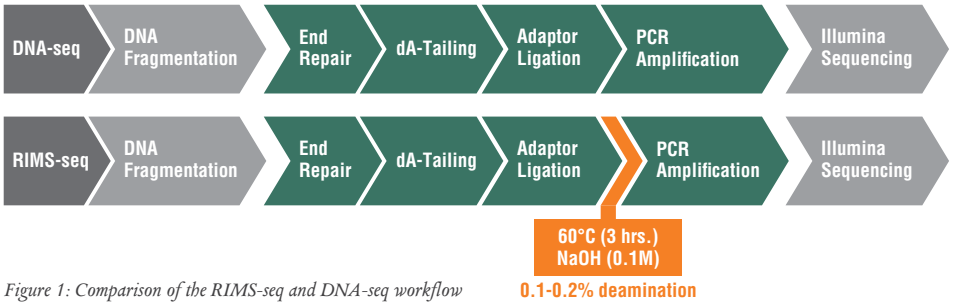

Figure 1: Comparison of the RIMS-seq and DNA-seq workflow

ReCappable-seq

The Ettwiller and Schildkraut labs built upon Cappable-seq with ReCappable-seq [5] to capture the TSS of non-RNA Polymerase II transcripts in addition to the TSS of 7-methyl G-capped transcripts derived from RNA Polymerase II. Therefore, ReCappable-seq overcomes the limitation of other methods that only determine RNA Polymerase II transcripts, which entirely exclude the TSS derived from eukaryotic RNA Polymerase I, RNA Polymerase III and mitochondrial RNA Polymerase that all produce uncapped non-coding RNA. To achieve this, they took advantage of the property of the yeast scavenger decapping enzyme (yDcpS) to convert capped RNA into di-phosphorylated RNA that can be "re-capped" by the Vaccinia Capping Enzyme, hence the name ReCappable-seq. Recappable-seq enriches both RNA Polymerase II- and non-RNA Polymerase II- derived transcripts and provides the ability to comprehensively evaluate both mRNA and non-capped primary transcripts all in one library, genome-wide, at single nucleotide resolution. It allows a unique opportunity to simultaneously interrogate the regulatory landscape of coding and non-coding RNA in biological processes and diseases.

Like Cappable-seq, ReCappable-seq produces sequencing libraries from total RNA without the need to deplete rRNA beforehand. Because it is species agnostic, it can be used with complex communities composed of both prokaryotic and eukaryotic organisms.

SMRT-Cappable-seq

This high-throughput technique was derived from Cappable-seq in the Ettwiller lab, but it differs in that it generates a snapshot of the full-length bacterial transcriptome at base resolution, whereas Cappable-seq identifies the TSS only [6]. To achieve this, the triphosphorylated 5′ ends of unfragmented transcripts are captured using an adapted Cappable-seq methodology, again removing the need to perform RNA depletion beforehand.

Because the transcripts do not need to be fragmented PacBio single-molecule long-read sequencing from TSS to the termination sites can be carried out. This is valuable when trying to gain information regarding bacterial operons, which are made up of a group of genes under the control of a common promoter – with short sequencing reads, much of the operon complexity is overlooked or hidden. Long-read sequencing keeps the information on the 5′ and 3′ ends of operons intact, facilitating their identification.

Additionally, SMRT-Cappable-seq can be used on complex microbiomes for which reference genomes are not readily available.

Analysis of chromatin accessibility

Within the nucleus, mammalian DNA is packaged as chromatin, along with essential proteins and RNA. The chromatin of the nuclear genome must be accessible to the transcriptional machinery to produce RNA for translation to cellular proteins. The dynamic nature of chromatin accessibility in cellular function is vital to gene expression and development.

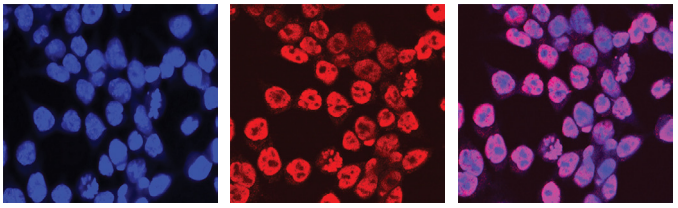

Figure 2: Confocal microscopic images of colon carcinoma cell line (HCT116)

Left: DAPI-stained nuclei, Middle: Texas Red-dNTP-stained accessible chromatin using NicE-viewSeq technology, Right: DAPI + Texas Red-dNTP

Universal Nicking Enzyme-assisted Sequencing (UniNicE-seq) and Nicking Enzyme-assisted Viewing and Sequencing (NiCE-view-seq)

Developed in the Pradhan lab, UniNicE-seq captures and reveals open chromatin sites (OCS) and transcription factor occupancy at single nucleotide resolution; it reveals the transcriptionally active genome [7,8].

UniNicE-seq utilizes a labeling mix containing a sequence-specific nicking enzyme (Nt.CviPII), DNA polymerase I and dNTPs (containing biotin-conjugated dATP, dCTP and 5-methyl-dCTP). This mix labels the open chromatin region in the nucleus; then, the DNA is extracted and sonicated/digested to ~200 bp. This DNA undergoes library preparation, and Streptavidin magnetic beads capture the biotinylated library components for further PCR library preparation and sequencing.

UniNicE-seq may be used with cell lines (formalin-fixed and unfixed), mammalian- or plant-tissue nuclei, frozen tissue sections, and formalin-fixed paraffin-embedded (FFPE) tissue sections. It can be used with high resolution and a broad range of cell number inputs (25-250,000). This technology is capable of automation and cell-to-library preparation in one tube.

Lending an added dimension for analysis, NicE-viewSeq (Figure 2) includes a biotinylated dCTP mix and a Texas Red® tagged dATP mix that enables visualization of regions of accessible chromatin and subsequent sequencing for genome analysis, enabling pharmacological studies of chromatin- modifying drug efficacy [9].

Protect-seq

At the periphery of the metazoan nucleus, the nuclear chromatin becomes less accessible to transcriptional machinery and, in some cases, in direct apposition to the nuclear lamina (in what are known as lamina-associated domains, or LADs). Understanding which sequences tend to become arrayed in these LADs is an essential step toward understanding their functions.

Using a familiar technique in a new way, the Pradhan lab developed Protect-seq [10]. It relies on a cocktail of nucleases targeted at degrading and removing the open and accessible chromatin, as in NicE-seq, but with the goal of leaving the less-accessible, sonication-resistant LADs for sequencing. It is an efficient way to identify constitutive heterochromatin around the nuclear periphery. Protect-seq is a simple, easy-to-use, cost-and-time-effective method that does not require actively dividing cells, specialized equipment, or reagents. The entire protocol can be performed in a day.

Techniques that strive to answer similar questions require particular constructs, genome modifications, the establishment of cell lines, actively dividing cells or highly specific antibodies. In contrast, Protect-seq requires only an enzyme cocktail and nuclei from fixed cells or tissues.

This sequencing technique is compatible with short-read Illumina sequencing and long-read ONT sequencing, broadening its accessibility for researchers.

Analysis of DNA damage and modification

All living cells are exposed to DNA-damaging agents that are found exogenously, such as UV radiation, and endogenously, such as reactive oxygen species. These DNA-damaging agents can cause the formation of a wide variety of DNA lesions that can be mutagenic and cytotoxic to the cell. Cells have evolved several DNA repair pathways that recognize, remove and repair these DNA lesions. In higher eukaryotes, the formation of DNA lesions and faulty repair has been shown to cause cancer, neurological disorders and premature aging.

RAre DAmage and Repair sequencing (RADAR-seq)

To understand the formation, persistence and repair of DNA lesions, NEB scientists have developed RADAR-seq [11,12]. This technique replaces a DNA lesion with a patch of modified bases that PacBio SMRT sequencing can detect. RADAR-seq can measure the frequency and map the locations of various rare DNA lesions genome-wide without requiring DNA amplification or enrichment. To understand *in vivo* DNA damage and repair pathways in a particular organism, RADAR-seq can be used to determine the DNA damaging effects of a specific DNA damaging agent, locate DNA damage hotspots across a genome, or locate the specific genomic site of any DNA nicking enzyme.

Several currently used DNA damage detection techniques employ short-read next generation sequencing methods that amplify damaged DNA; the drawback is that only the enriched regions with damaged, and not undamaged, DNA are sequenced.

This gives a relative measure of DNA damage without information regarding absolute levels of damage in a genome. In contrast, RADAR-seq utilizes the PacBio long-read sequencing platform, which provides information on both damaged and undamaged DNA. In addition, most DNA damage detection techniques are tailored to locate a specific DNA lesion (i.e., ribonucleotides or cyclobutane pyrimidine dimers). RADAR-seq can locate any DNA lesion with an associated nicking enzyme or repair glycosylase. Furthermore, RADAR-seq can detect rare DNA damage, as low as one lesion per 1 Mb bases sequenced. Finally, RADAR-seq library construction is fast and can be completed in less than one day (Figure 3).

EcoWI-seq

This sequencing method was also developed in the Ettwiller lab [13] and can determine the pattern of phosphorothioate (PT) modifications in bacteria. PT modifications occur on the DNA sugar-phosphate backbone rather than on the nucleotide (a non-bridging oxygen is replaced by sulfur) and are maintained at a particular density in a genome. The PT modification is widespread in prokaryotes and is a horizontally transferred epigenetic system that makes the phosphorothioate oligonucleotide more resistant to degradation. The modification occurs on a small proportion of the EcoWI recognition sequences in the genome, and whether they occur stochastically or deterministically is an area of investigation.

The restriction enzyme EcoWI is a PT-dependent endonuclease with recognition sequence GAAC/GTTC. EcoWI creates a double-stranded break only when a PT modification is present on both DNA strands. Subsequent sonication generates short sequences compatible with Illumina sequencing. The ability to detect PT modifications relies on the mapping pattern: fragments generated from sonication will map to the genome at random positions and provide sequencing information that can be used for genome assembly; fragments generated by EcoWI digestion map to fixed pattern ends, which provide the location of the modification at base resolution. This methodology is scalable and requires little starting material.

Enzyme discovery

The discovery and characterization of new enzymes aids the development of new technologies. Microbiomes are an untapped resource for discovering enzymes that can be harnessed for industrial purposes. Metagenomic, epigenomic, and transcriptomic pipelines are being used to rapidly discover novel enzymes.

Metagenomics Genome-Phenome Association (Meta-GPA)

The field of microbiome research has evolved rapidly and is a topic of great scientific and public interest. Nonetheless, microbiome studies are often limited to shotgun sequencing providing detailed descriptions of species composition and gene content, but direct links to function are missing. In other words, while we now understand *who* (species) are out there, it remains very difficult to understand *what* they are doing.

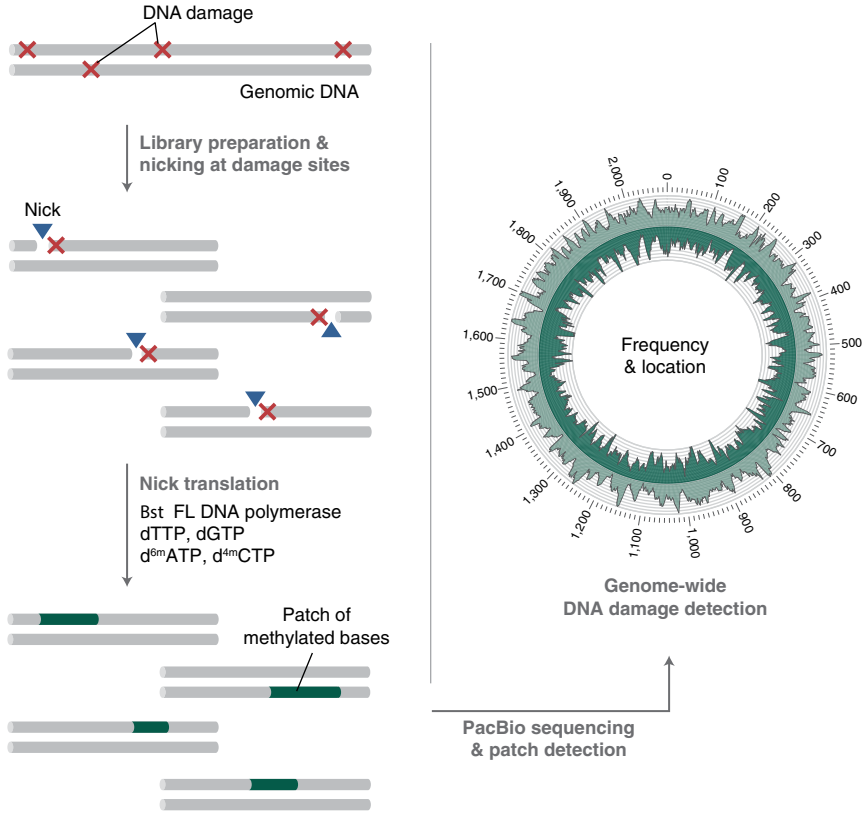

Figure 3: RADAR-seq overview. Details in Current Protocols of Molecular Biology *[12]*

MetaGPA bridges this fundamental gap between genetic information and functional phenotype using next generation shotgun metagenomic sequencing [14]. MetaGPA is conceptually close to Genome-Wide Association Studies (GWAS), where control and case cohorts are compared to identify associated variants in the case cohorts. Likewise, metaGPA associates genetic data with phenotypic traits at the level of an entire microbiome. The association analysis can be done at the pathway, protein, or even single amino acid resolution level, pinpointing the residues within a protein domain that underlie a respective phenotype within a microbiome, irrespective of whether the organisms within that microbiome are known or culturable.

Because sequencing is conducted on environmental matter, there is no reference genome. Therefore, once the DNA is isolated and sequenced at a deep level, a *de novo* reference meta-genome must be created using a *de novo* assembler. The case and control genomes are plotted to reveal those with a high frequency in the test group. An enrichment score calculation associates the contigs with the likelihood of being a feature of the case group. These contigs are then analyzed for functional units.

Conclusion

At NEB, our scientists are passionate about developing sequencing technologies that can keep pace with their imaginations. As they continue developing new and exciting methodologies for diverse applications to analyze the genome, epigenome and transcriptome, they also continue to foster NEB's ongoing commitment to sharing knowledge and collaborating with the broader scientific community.

Learn more about the exciting research performed in the Ettwiller, Pradhan, Gardner and Schildkraut labs at www.neb.com/research.

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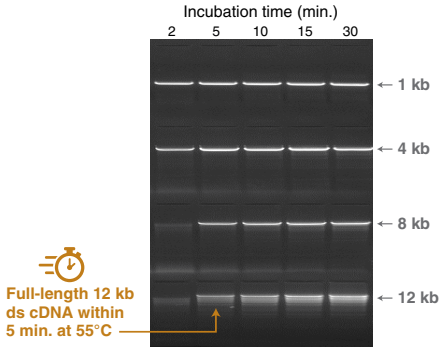
A new class of performance

Induro™ Reverse Transcriptase

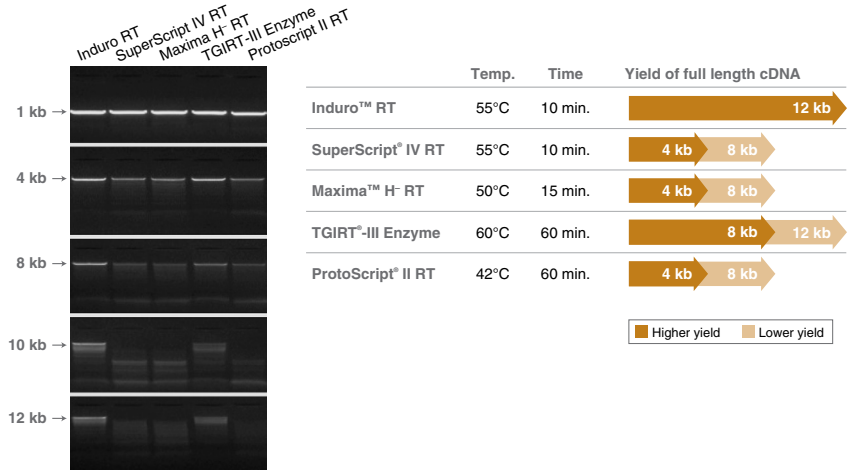
Induro Reverse Transcriptase is a group II intron-encoded RT that exhibits high processivity, increased thermostability, and increased tolerance of inhibitors for the synthesis of cDNA from RNA. It is an ideal enzyme for challenging cDNA synthesis from long transcripts (>8 kb), RNAs with strong secondary structures, and RNA samples with inhibitors. With improved 5´ sequencing coverage of long transcripts, Induro Reverse Transcriptase can enable RNA-seq applications such as direct RNA and long read cDNA sequencing workflows.

Induro Reverse Transcriptase exhibits high processivity, permitting rapid cDNA synthesis



Induro Reverse Transcriptase can synthesize a full-length 12 kb cDNA product within 5 min. at 55°C. In vitro transcribed poly(A) RNA templates (1 kb, 4 kb, 8 kb or 12 kb) were used to investigate full-length cDNA synthesis. After first-strand cDNA synthesis, RNA was hydrolyzed immediately by NaOH. Subsequently, an aliquot of the cDNA products was used to make full-length ds cDNA in the presence of a 5´ specific primer. Equal volume of ds cDNA was analyzed on an agarose gel.

Induro Reverse Transcriptase generates highest yields of long cDNA



Induro Reverse Transcriptase generates the highest product yields for cDNA ≥ 8 kb. RNA templates were in vitro transcribed poly(A) RNA (1 kb, 4 kb, 8 kb, 10 kb or 12 kb). After first strand cDNA synthesis, RNA was degraded and the second strand cDNA synthesis was performed in the presence of a 5´ specific primer.

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Induro Reverse Transcriptase: A Robust, Thermostable, Intron-Encoded RT for Full-Length cDNA Synthesis

Benefits

- Rapidly generate high yields of long cDNA with our unique group II intron-encoded RT
- Strong inhibitor tolerance enables robust cDNA synthesis performance
- Support direct RNA sequencing and long read cDNA sequencing workflows
- Generate cDNA at higher temperatures, which is ideal for challenging sample types
- Experience comparable fidelity to retroviral RTs

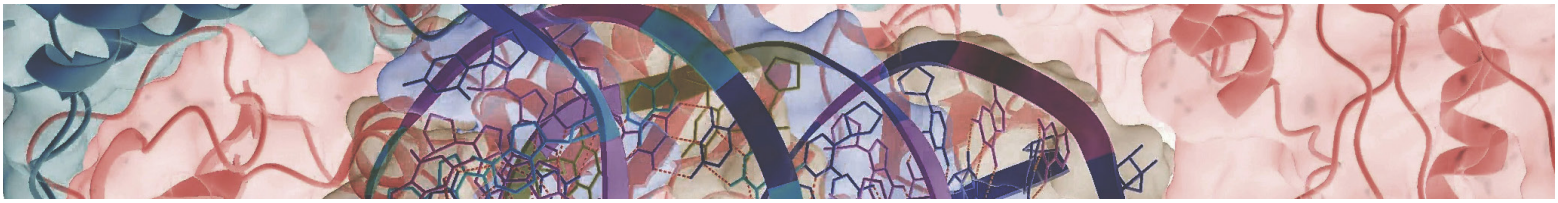
Applications

- Challenging cDNA synthesis or when other RTs fail, including long cDNA synthesis (>8 kb), RNA with many isoforms or difficult structures, and RNA samples with impurities/inhibitors
- RNA-seq workflows, including direct RNA sequencing on the ONT platform, direct long read cDNA sequencing on the ONT platform, and RNA-seq studies of modifications and structures
- Applications where intron RTs are used (e.g., 3´ template switching)

Ordering information

Product	NEB #	Size
Induro Reverse Transcriptase	M0681S/L	4,000/10,000 units
Companion Products		
Oligo d(T)23 VN	S1327S	1 A_{260} units
Random Primer Mix	S1330S	100 µl
Deoxynucleotide (dNTP) Solution Mix	N0447S/L	8/40 µmol

View more technical data and recommended protocol at www.neb.com/m0681



Retroviral vs Group II Intron-encoded Reverse Transcriptases – Similarities, Differences & Applications

by Joanne Gibson, Ph.D.

At the time reverse transcriptases (RTs) were discovered in the 1970s, the central dogma of molecular biology stated that information only flows from DNA to RNA to protein.

The idea that researchers investigating and theorizing the existence of an enzyme that could copy RNA into DNA, contrary to the central dogma, was initially met with skepticism. However, in the 50 years since the Nobel Prize-winning discovery of RTs, the ability to synthesize DNA from an RNA template has enabled researchers to study RNA using the same molecular approaches as DNA investigations. Typically, reverse transcription is coupled with methods such as PCR, qPCR, or loop-mediated isothermal amplification (LAMP) for further downstream analysis.

The most common and well-characterized RTs are derived from retroviruses. Many commercially-available retroviral RTs have been genetically engineered with mutations that confer specific attributes, such as reduced RNase H activity, increased half-life and increased thermostability. A second family of RTs, distinct in sequence and domain organization, is found within the intron-encoded proteins of group II introns. Group II intron-encoded RTs are commonly found in bacteria, archaea and eukaryotic organelles.

Both retroviral RTs and group II intron-encoded RTs can make complementary DNA (cDNA). They share some conserved domains; both have RNA-directed DNA polymerase activity and DNA-directed DNA polymerase activity. A typical retroviral RT also contains an RNase H domain at the C terminus, and therefore has RNase H activity and can degrade the parental RNA strand in the DNA-RNA hybrid. An intron-encoded RT lacks this domain and activity.

NEB's current RT portfolio includes several retroviral RTs, including the thermostable LunaScript® RT SuperMix (NEB # M3010) and WarmStart® RTx, Reverse Transcriptase (NEB #M0380) as well as AMV Reverse Transcriptase (NEB #M0277), M-MuLV Reverse Transcriptase (NEB #M0253), and ProtoScript® II Reverse Transcriptase (NEB #E6560). Retroviral RTs are widely available and have been incorporated into many established protocols.

We have recently added our first group II intron-encoded RT to the portfolio, Induro™ Reverse Transcriptase (Induro stands for Intron-Encoded Endurance RT). While group II intron-encoded RTs do not replace retroviral RTs, they do offer some advantages, specifically when it comes to challenging RT reactions such as full-length cDNA synthesis from long transcripts. Induro RT demonstrates high processivity, is thermostable, tolerates a wide range of common inhibitors found in RNA samples, and can bypass modified nucleotides. For more details on Induro, see page 5.

1. High processivity is required for long cDNA synthesis

Long transcripts can be challenging to reverse transcribe, but the high processivity of group II intron-encoded RTs ensures success in preserving the complete information present in the RNA molecule. A non-processive RT will produce more truncated cDNA transcripts. This makes it difficult to get an accurate picture of isoform abundance in a long transcript.

Processivity refers to the ability to incorporate many nucleotides into the cDNA molecule during a single template binding event. It also applies to how tightly bound the RT is to its template, aiding in its ability to overcome inhibitors that may be present in the RNA sample.

Intron-encoded RTs have higher processivity than retroviral RTs. Induro can synthesize cDNA at least 14 kb in length in as little as 10 minutes. Retroviral RTs have a very high turnover rate, so long DNA can be made if there is sufficient enzyme and adequate incubation time. Still, their ability to synthesize challenging full-length cDNAs from long transcripts is limited. However, for general cDNA synthesis (up to roughly 8 kb), retroviral RTs are still the recommended option.

2. A thermostable RT helps with difficult secondary structures

Long transcripts typically have more complex, folded secondary or tertiary structures. A higher reaction temperature can facilitate opening up these structures. A thermostable RT can maintain enzymatic activity at elevated temperatures. For example, Induro RT can generate cDNA at temperatures as high as 60°C, with an optimal temperature of 55°C.

3. Better inhibitor tolerance gives a higher cDNA yield

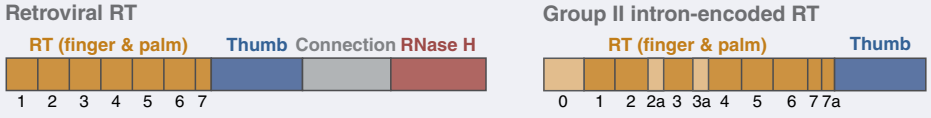
Inhibitors in RNA samples can carry over from the extraction and purification. Some common inhibitors include KCl, NaCl, $MgCl_2$, formalin, paraffin, Tween20, Igepal, ethanol, isopropanol, DMSO, and denaturing chemicals such as urea and GnCl. Induro RT is more tolerant of all these common inhibitors and can still synthesize full-length cDNA in a reaction where they are present.

4. Bypass activity prevents stalling at modified nucleotides

Cellular RNAs have many different modified ribonucleotides (psU, m6A, 5mC, 2'-O-meU, mIA), which can cause the premature termination of cDNA synthesis. Bypass activity refers to the ability of the RT to perform continuous cDNA synthesis across these modified ribonucleotides without the RT stalling and resulting in a truncated cDNA product. A truncated cDNA can give biased coverage of the 5´ end of the sequence. An RT with good bypass activity will navigate the modification and generate full-length cDNA. Induro has the ability to bypass modified ribonucleotides more frequently than retroviral RTs.

Summary

In summary, while retroviral RTs are widely available and work well in many well-established protocols, intron-encoded RTs offer some advantages over retroviral RTs, and can be a better choice for cDNA synthesis from long transcripts, RNAs with strong secondary structures, and RNA samples with inhibitors. To learn more about RTs available from NEB, see page 7.



A typical retroviral RT contains a polymerase domain with several conserved motifs at the N-terminus and the RNase H domain at the C-terminus. Group II intron-encoded RTs, similar to retroviral RTs, contain several conserved motifs within the RT (motifs 1-7, brown). The N-terminal extension (0) and insertions between the conserved sequence blocks (motifs 2a, 3a, and 7a, tan) are observed in group II intron RT but not in retroviral RTs. The intron-encoded RTs often do not have the RNase H domain and therefore do not have the RNase H activity.

Adapted from Pyle, A.M., et al. (2018) RNA 24(2):183-195.

Reverse Transcriptases (RTs)
from New England Biolabs®

NEB has an extensive portfolio of RTs, which are available as standalone products or have been incorporated into convenient master mixes or kits. For more details and access to the full product listing, visit **www.neb.com/rt**.

RETROVIRAL RTs

For general cDNA synthesis

LunaScript® RT SuperMix
NEB #E3010/M3010

Product notes:
Single-tube supermix, fast 13-minute protocol. Contains random primers, ideal for RNA targets up to 3 kb.

LunaScript RT Master Mix Kit (Primer-free)
NEB #E3025

Product notes:
First strand cDNA synthesis with user-supplied primers

ProtoScript® II RT
NEB #M0368

Product notes:
RNase H⁻ mutant of M-MuLV RT with increased thermostability and reduced RNase H activity. Ideal for RNA targets up to 12 kb.

ProtoScript II First Strand cDNA Synthesis Kit
NEB #E6560

Product notes:
Kit contains ProtoScript II, Murine RNase Inhibitor, primers and dNTPs

M-MuLV RT **NEB #M0253** | **AMV RT** **NEB #M0277**

Product notes:
Robust RTs for a variety of templates

ProtoScript First Strand cDNA Synthesis Kit
NEB #E6300

Product notes:
Kit contains M-MuLV RT, Murine RNase Inhibitor, primers and dNTPs

For niche cDNA synthesis

LunaScript RT SuperMix
NEB #E3010/M3010

Recommended use:
Two-step RT-qPCR, amplicon sequencing

Luna® WarmStart RT
NEB #M3001*

Recommended use:
One-step RT-qPCR, One-step RT-PCR

Luna One-Step RT-qPCR reagents

- Luna Universal One-Step RT-qPCR Kit
  NEB #E3005
- Luna Universal Probe One-Step RT-qPCR Kit
  ROX: NEB #E3006
  No ROX: NEB #E3007
- Luna Probe One-Step RT-qPCR 4X Mix with UDG
  ROX: NEB #M3019
  No ROX: NEB #M3029
- LyoPrime Luna™ Probe One-Step RT-qPCR Mix with UDG
  NEB #L4001

LunaScript Multiplex One-Step RT-PCR Kit
NEB #E1555

Product notes:
Features Luna WarmStart RT and Q5® Hot Start High-Fidelity DNA Polymerase

Template Switching RT Enzyme Mix
NEB #M0466

Recommended use:
cDNA amplification with a template switching oligo, 5´ RACE, second strand cDNA synthesis

WarmStart® RTx
NEB #M0380

Recommended use:
Optimized for RT-LAMP

LAMP Master Mixes

- WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)
  NEB #M1800
- WarmStart Colorimetric LAMP 2X Master Mix with UDG
  NEB #M1804
- WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)
  NEB #M1708
- WarmStart LAMP Kit (DNA & RNA)
  NEB #E1700
- WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)
  NEB #E1708

* Available through OEM & Customized Solutions

GROUP II INTRON RTs

NEW

Induro™ RT
NEB #M0681

Recommended use:

Long cDNA synthesis (up to 20 kb)

Impure RNA samples

RNA-seq workflows

Product notes:
Group II Intron-encoded RTs are distinct in sequence and domain organization from common retroviral RTs. They exhibit unique features such as high processivity, increased thermostability, and strong inhibitor tolerance.

NGS library prep? We've got you covered.

For 13 years, NEB® has addressed library prep challenges by offering solutions that streamline workflows, minimize inputs and improve library yield and quality. In fact, use of NEBNext® reagents has been cited in >20,000 publications.

With over 90 products in the NEBNext portfolio and direct access to experts in NGS and enzymology, NEB can support library preparation for all of your sample types, for a wide range of sequencing platforms and throughputs.

The NEBNext Ultra™ II workflow, which lies at the heart of the NEBNext library prep solution, is available in a variety of streamlined and convenient kit formats. For added flexibility, NEBNext kits are easily scalable for automation on liquid handling instrumentation. And as a reagent manufacturer, NEB is ideally positioned to support your large volume and custom needs, through our OEM & Customized Solutions group.

Our expertise, proven track record and depth of portfolio make NEBNext a preferred choice for high-quality sample preparation. We're ready to show you why it should be yours.

Interested in giving NEBNext a try? Learn more and request a sample at NEBNext.com.

NEBNext® Ultra™ II DNA Sample Prep Solutions – now featuring PCR-free workflows

The NEBNext portfolio of Ultra II DNA library prep reagents has expanded to include PCR-free workflows, powering high performance without the need for amplification. By eliminating the risk of PCR bias, libraries are a clearer reflection of biology.

The NEBNext Ultra II workflow lies at the heart of NEB's portfolio for next generation sequencing library preparation, with kits and modules for optimal flexibility. You can be assured your DNA libraries will be of the highest quality and yield, even when starting from extremely low input amounts.

Use the selection chart below to determine which NEBNext Ultra II products best suit your needs:

FEATURES	ULTRA II DNA LIBRARY PREP	ULTRA II FS DNA LIBRARY PREP
	Unprecedented performance, enabling lower inputs and fewer PCR cycles	Perform fragmentation, end repair, and dA-tailing with a single enzyme mix
ULTRA II INPUT AMOUNTS	500 pg – 1 µg of sheared DNA	100 pg – 500 ng of intact DNA
Available with or without beads?	• With beads: NEB #E7103 • Without beads: NEB #E7645	• With beads: NEB #E7435 • Without beads: NEB #E7805
Modules available?	• Ultra II End Repair/dA-tailing Module (NEB #E7546) • Ultra II Ligation Module (NEB #E7595)	• Ultra II FS DNA Module (NEB #E7810)
ULTRA II PCR-FREE INPUT AMOUNTS	250 ng – 1,000 ng of sheared DNA	50 ng – 500 ng of intact DNA
Available with or without beads?	• With beads: NEB #E7415 • Without beads: NEB #E7410	• With beads: NEB #E7435 • Without beads: NEB #E7430
Modules available?	• Ultra II End Repair/dA-tailing Module (NEB #E7546) • Ultra II Ligation Module (NEB #E7595)	• Ultra II FS DNA Module (NEB #E7810)
COMPATIBLE WITH FFPE DNA?	Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)	Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)
COMPATIBLE WITH METHYLOME ANALYSIS?	Yes – Methylome analysis is supported; however, we recommend NEBNext EM-seq™ (NEB #E7120)	No – not compatible due to potential for loss of methyl marks
COMPATIBLE WITH OXFORD NANOPORE TECHNOLOGIES?	Yes. We recommend the NEBNext Companion Module for Oxford Nanopore Technologies® Ligation Sequencing (NEB #E7180).	

For more information on our NEBNext portfolio for DNA and RNA library prep, and to request a sample, visit NEBNext.com

Ordering information

Product	NEB #	Size
NEBNext Ultra™ II DNA Library Prep Kit for Illumina®	E7645S/L	24/96 reactions
NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S/L	24/96 reactions
NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 reactions
NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads	E7435S/L	24/96 reactions
NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 reactions
NEBNext Ultra II Ligation Module	E7595S/L	24/96 reactions
NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina	E7410S/L	24/96 reactions

Product	NEB #	Size
NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads	E7415S/L	24/96 reactions
NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina	E7430S/L	24/96 reactions
NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads	E7435S/L	24/96 reactions
NEBNext Ultra II FS DNA Module	E7810S/L	24/96 reactions
NEBNext FFPE DNA Repair Mix	M6630S/L	24/96 reactions
NEBNext Enzymatic Methyl-seq Kit	E7120S/L	24/96 reactions
NEBNext Enzymatic Methyl-seq Conversion Module	E7125S/L	24/96 reactions
NEBNext Companion Module for Oxford Nanopore Technologies® Ligation Sequencing	E7180S	24 reactions

NEB's Course Support Program

In this Q&A with Penny Devoe, an Associate Director in the Marketing Department at NEB, Penny shares insight on NEB's efforts to support educational outreach through our course support program.

Q: What is NEB's philosophy on supporting science education in the life sciences?

A: NEB has a strong commitment to support education of the life sciences. We are dedicated to inspiring young scientists and improving the quality of science education for students by providing reagents, educational materials and employee knowledge and time. In fact, many of our employees are involved in educational outreach and will present at local schools or volunteer to participate in local science fairs.

Q: What support does NEB offer to teaching labs?

In addition to the educational content available on neb.com, NEB offers a list of our most popular products for free to support teaching labs in the U.S. We donate many NEB products to high schools, colleges and teaching programs throughout the country.

Q: How long has NEB been offering free reagents to teaching labs?

NEB has been providing free reagents to institutions for over 40 years. Advancing science has always been a priority at NEB, and what better way to achieve this than supporting schools who are training our young scientists. We support hundreds of schools every year through this program.

Q: Can you share some examples of groups that have taken advantage of this program?

We have supported the Gloucester Marine Genomics Institute, and BioTeach, a life sciences education program offered through the Massachusetts Biotechnology Education. Other programs include Internationally Genetically Engineered Machines Competition (iGEM), BioBuilder®, International Directed Evolution Competition (iDEC), HHMI®, and the Amgen® Biotech Experience – a science education program to help teachers bring biotech to the classroom.

Q: How can an educator apply for course support?

Anyone who is interested can visit our Course Support and Reagent Donation page www.neb.com/reagentdonation to learn more. There, you can submit an inquiry for information, and we will quickly get back to you with more details. It is important that all the information needed is provided – we have detailed instructions available on our website for a successful submission.

Q: Can an educator from outside of the U.S. participate?

An international educator can contact their local subsidiary or distributor, which again, can be accessed from the Course Support page.

Q: What else does NEB do to promote life science education?

In addition to course support throughout the world, NEB employees also volunteer their time to offer Science Days for both local teachers and students. Please visit our webpage neb.com/promoting-science-education for additional information on other programs and initiatives that NEB is involved with in promoting the life sciences.

nebtv Learn more about NEB's course support program.

View Episode 26 of NEB TV at **www.neb.com/NEBTV**

Right Whales and Lobster boats: Saving icons of New England

by Beth McLeod

Stretching up to 52 feet long and weighing up to 62 tons, the North Atlantic right whale is one of the world's largest animals—and one of the most endangered whales.

Scientists estimate that there are fewer than 350 right whales remaining. For generations, the right whale was hunted for oil and baleen to the brink of extinction. Today, the leading cause of trauma to large whales around the world is entanglement in ropes used in commercial fixed gear fisheries.

Whales are critical to the health of the marine ecosystem and play an important role in mitigating climate change. Feeding at depth and then rising to the surface of the ocean they circulate vital nutrients to marine life in a process known as the "whale pump". In addition, their fecal plumes released at the surface of the ocean provide sustenance to phytoplankton, which in turn sequester an enormous amount of carbon dioxide from the atmosphere. Lastly, due to their size and longevity, whales accumulate massive quantities of carbon, which they take with them to the bottom of the ocean when they die.

Fixed gear fisheries, including those fishing for lobster and crab, use unattended traps, pots or gillnets on the sea floor that are often set to fish for days or weeks before being retrieved. In the U.S., these traps or nets are required to be marked at the surface with a buoy system. A vertical retrieval line connects the surface buoy to gear on the sea floor. Whales that encounter this vertical line often react by rolling into the line, exacerbating the entanglement. Restricting ropes wrapped on whales cut into tissue and sometimes into bone leaving whales physically compromised at best or killing them through starvation or infections at worst.

Currently, efforts to reduce entanglements focus on closing lucrative fishing grounds when and where right whales are predicted to be present. This is not only costly to economically and culturally important fishing communities but it does not fully protect right whales that are increasingly searching new areas for food in a changing climate. In the U.S., 14 fisheries have lost their Monterey Bay Seafood Watch sustainability ratings due to the impact that fixed fishing gear has on the recovery of right whales. In September, the Marine Stewardship Council announced an expedited audit of its sustainable certification of the Gulf of Maine lobster fishery due to its impact on right whales. This loss of sustainable market certifications can have devastating economic impacts on fishing communities.

Since 2018, there has been remarkable progress in the development of on-demand "ropeless"or "buoyless" retrieval systems that operate without the use of vertical lines, eliminating entanglement risk. This system digitally marks the location of the gear on the sea floor (Figure 1). Acoustic signals then release a retrieval rope or airbag, lifting the gear to the surface only when the fishing vessel is on site. Advancement of this technology has been expedited through testing by commercial fishermen whose input has significantly improved the design and performance.

In the Northeast, a collaborative trial was begun in 2019 by Conservation Law Foundation, the Northeast Fisheries Science Center, Woods Hole Oceanographic Institution and the International Fund for Animal Welfare and Whale and Dolphin Conservation. With support from New England Biolabs in 2021, the trial was expanded from 3 vessels to 27 vessels, from Massachusetts to the entire Northeast, and from 16 units to more than 180 units in testing.

This gear has now been used successfully in many commercial fisheries. In several U.S. and Canadian east coast fisheries, crab and lobster have been harvested using on-demand systems; with approximately 750,000 lbs of snow crab landed in Canada in whale conservation areas that are closed to traditional buoy lines. The expansion of this program to commercial use alone is incredibly exciting. However, if ropeless fishing is to be truly successful, there are a few issues that need to be resolved.

Traditional buoys and end-lines enable the fishermen to locate their gear and also provide a visual cue to other fishermen as to where gear is set. Without this marker other fisherman may accidentally overlay their gear on someone else's, or drag their bottom net through the gear to another location where it quickly becomes ghost gear, with no hope of ever being retrieved. In these high-density areas, more accurate acoustic methods are needed to locate gear, and this type of solution is taking longer to develop.

Ropeless gear requires a robust location marking solution that collects location information and transmits this information from the vessel at sea to a cloud database in real time. Ideally, gear location information from this cloud database can then be automatically received and transmitted to vessels at sea in real time and displayed on commercial chart plotters, standard equipment typically owned by commercial fishermen. This will enable other fixed bottom gear fishers and mobile draggers and scallopers to "see" and avoid bottom gear with no surface marker.

For areas where gear density is low, there is less risk of losing gear and marking the gear upon deployment with a global positioning system (GPS) receiver is simple and sufficient. This type of solution has been developed and demonstrated on the water. Both gear location methods (GPS and acoustic) will be critical to implement, and both can use the cloud database to appropriately share gear locations with other fishermen, enforcement officers, and regulators. Initial work has been done by several organizations to demonstrate components of this system, but investment is needed to integrate, coordinate, and quickly advance these efforts to fully realize this vision.

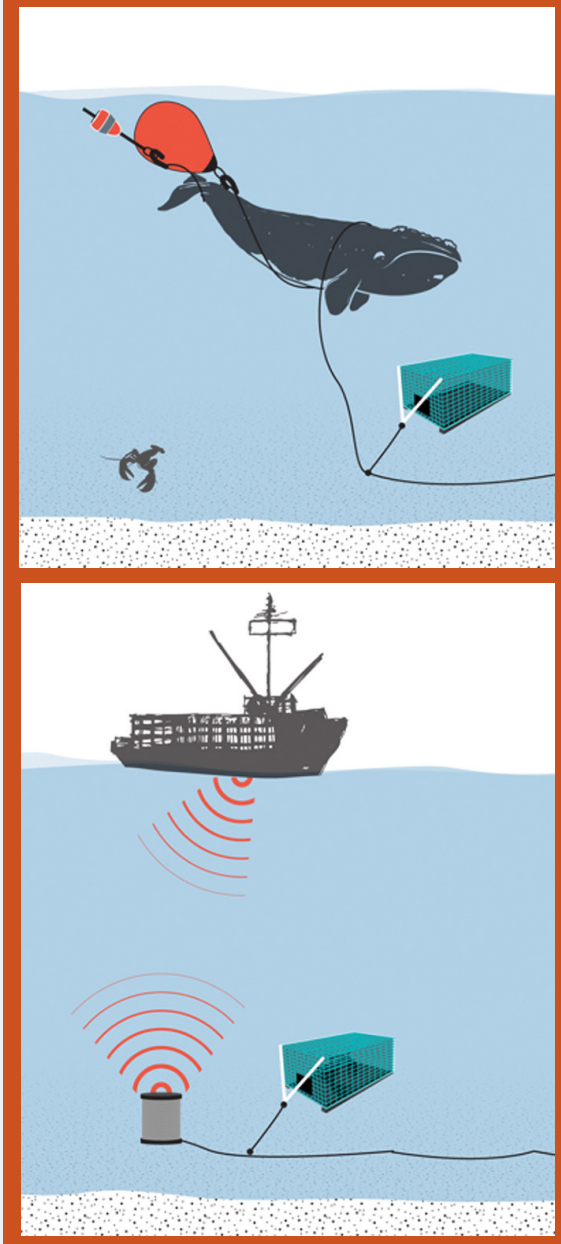


Figure 1. On-demand fishing: top panel shows entanglement risk in traditional vertical lines; bottom panel shows the use of an acoustic trigger to retrieve traps with no persistent vertical line, but instead a bottom stowed buoyant device for trap retrieval (© WHOI Creative).

With numbers dwindling and female whales calving much less frequently due to the stress of entanglement and warmer ocean temperatures, the situation is dire. The pressure is mounting on these magnificent creatures, and if we fail them, there will be another large marine mammal that will follow. Like so many other industries that are having catastrophic effects on our climate, it's time to utilize our sophisticated engineering capabilities and modernize the traditional methods and systems used in the fishing industry. It's time to make the North Atlantic right whale a priority.

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