Now includes
Induro® Reverse Transcriptase

Reverse Transcriptases

PRODUCTS, KITS, AND MIXES FOR OPTIMAL REVERSE TRANSCRIPTION AND CDNA SYNTHESIS

NEW ENGLAND Biolabs®

Reverse Transcriptases for improved cDNA Synthesis

The synthesis of DNA from an RNA template, via reverse transcription, produces complementary DNA (cDNA). Reverse transcriptases (RTs) use an RNA template and a short primer complementary to the 3´end of the RNA to direct the synthesis of the first strand cDNA, which can be used directly as a template for applications such as PCR, qPCR, LAMP, or sequencing workflows.

NEB offers a selection of retroviral and intron-encoded RTs that are available standalone or in kits or mixes. Products have been optimized for increased thermostability, minimized RNase H activity, increased processivity, increased inhibitor tolerance, speed and flexibility. For guidance on what products we offer and their recommended use, please refer to the infographic below.

View the full product portfolio at
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

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 specialized 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

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

GROUP II INTRON RTs

NEW

Induro® RT
NEB #M0681

Recommended use:

Long cDNA synthesis (up to 20 kb)

Impure RNA samples

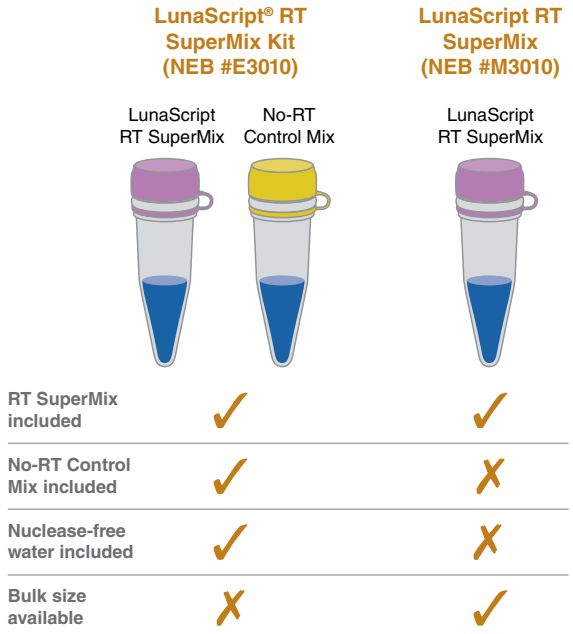
RNA-seq workflows

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.

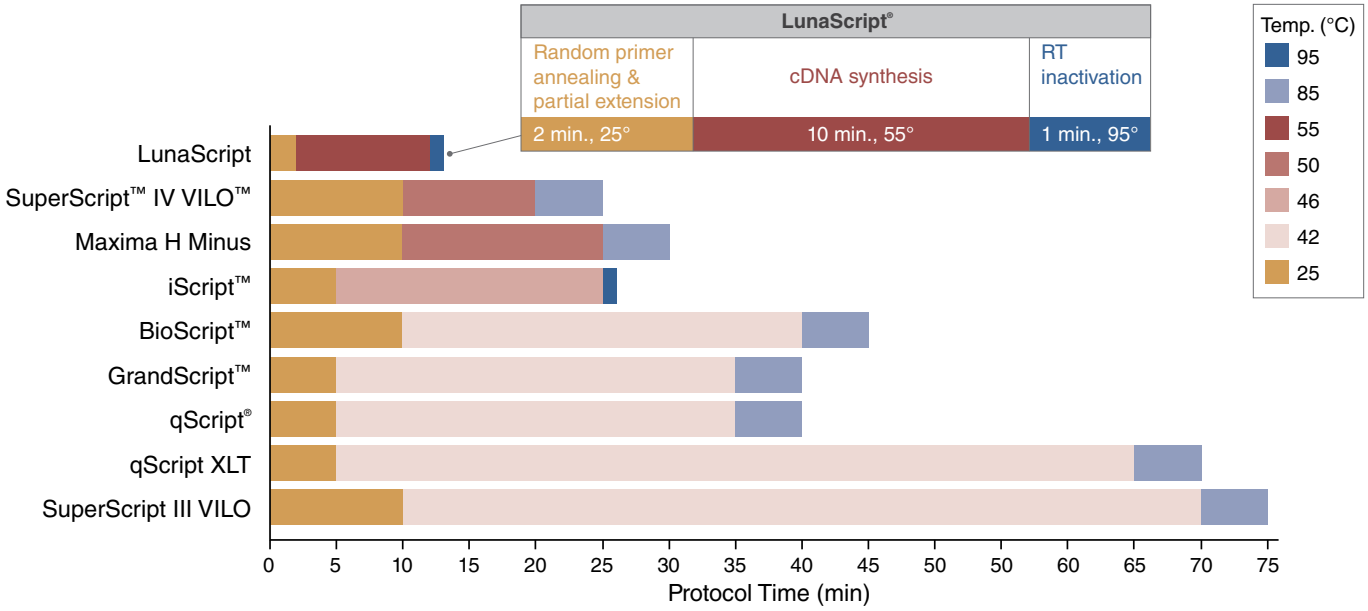
LunaScript RT SuperMix

LunaScript RT SuperMix is optimized for first strand cDNA synthesis and can be used for RNA targets up to 3 kb, such as in amplicon sequencing or a two-step RT-qPCR workflow. It employs a novel, *in silico*-designed Luna RT engineered for improved performance in a supermix format containing random hexamer and oligo-dT primers, dNTPs, and Murine RNase Inhibitor. This mix delivers unsurpassed features with a short reaction time (< 15 min) and tolerates elevated temperatures (55°–65°C) for working with templates with complex secondary structures. LunaScript RT SuperMix is offered in multiple formats (see right).

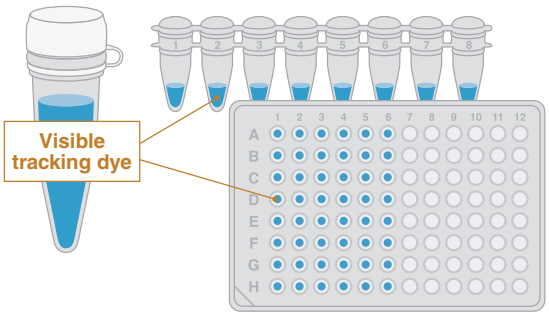
The cDNA products generated by LunaScript have been extensively evaluated in qPCR using the Luna qPCR Master Mixes (NEB #M3003/M3004). LunaScript RT SuperMix has been featured in the SARS-CoV-2 sequencing workflow from the ARTIC Network and is a component in the NEBNext® ARTIC SARS-CoV-2 Library Prep Kits.



At just 13 minutes, LunaScript RT SuperMix offers the shortest available first-strand cDNA synthesis protocol



Luna products feature a blue tracking dye



LunaScript forms a dark blue pellet at the bottom of the reaction vessel, easily discernible in clear or blue color mixes.

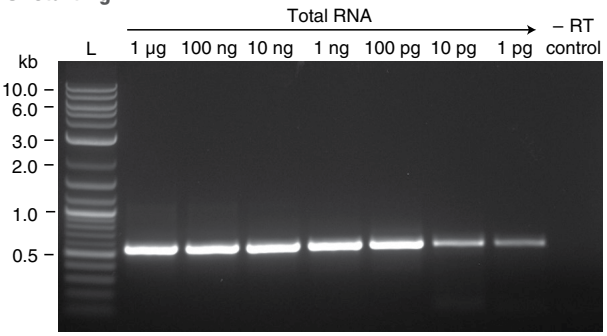
Learn more about the LunaScript RT SuperMix and request a sample at
www.neb.com/E3010

ProtoScript II Reverse Transcriptase

ProtoScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 48°C, providing higher specificity, higher yield of cDNA and more full-length cDNA product up to 10 kb. ProtoScript II RT is included in our Protoscript II First Strand cDNA Synthesis Kit (NEB #E6650).

Learn more about ProtoScript II Reverse Transcriptase at **www.neb.com/M0368**

Generate high quality cDNA even with very low amounts of starting DNA

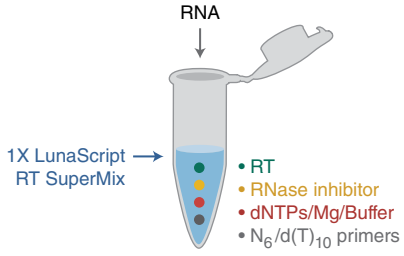
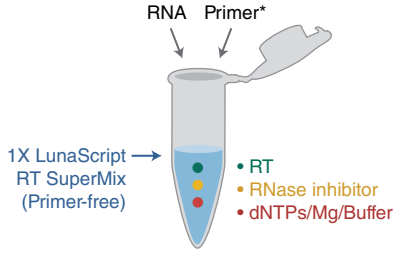
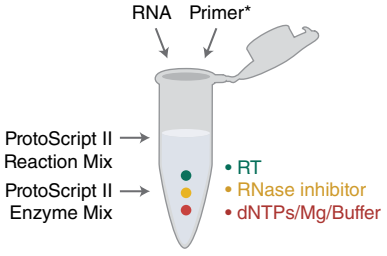


Decreasing amounts of Jurkat total RNA (1µg – 1pg) were used in 20 µl first strand cDNA synthesis with 200 units of NEB ProtoScript II Reverse Transcriptase. Reactions were incubated at 42°C for 50 minutes, followed by heat inactivation for 5 minutes at 80°C. 1 µl of cDNA was used in a 25 µl PCR using LongAmp Taq Master Mix (NEB #M0553) for 40 cycles. The target is a 0.6 kb fragment of GAPDH. Ladder L is the 2-Log DNA Ladder (NEB #N0469).

If you are looking for primers, we also offer:

PRODUCT	NEB #
Oligo d(T)23 VN	S1327S
Random Primer Mix	S1330S
Deoxynucleotide (dNTP) Solution Mix	N0447

How do LunaScript and ProtoScript kits compare?

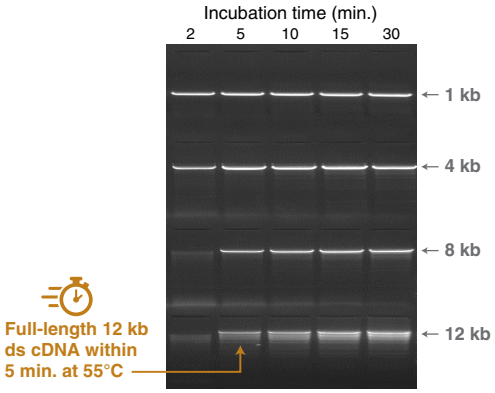
	LunaScript RT SuperMix (NEB #E3010/M3010)	LunaScript RT Master Mix (Primer-free) Kit (NEB #E3025)	ProtoScript II First Strand cDNA Synthesis Kit (NEB #E6560)
Kit Components	1. LunaScript RT SuperMix (5X) 2. No-RT Control Mix (5X)* 3. Nuclease-free Water* *Offered with E3010 kit	1. LunaScript RT Master Mix (Primer-free)(5X) 2. No-RT Control Mix (Primer-free)(5X) 3. Nuclease-free Water	1. ProtoScript II Enzyme Mix (10X) 2. ProtoScript II Reaction Mix (2X) 3. Oligo $d(T)_{23}$ VN (50 µM) 4. Random Primer Mix (60 µM) 5. Nuclease-free Water
RT	Luna Reverse Transcriptase	Luna Reverse Transcriptase	ProtoScript II Reverse Transcriptase
Component Details with User Additions		 ** Primer can be dT, random or gene-specific*	 ** Primer can be dT, random or gene-specific*
Protocol	(25°C 2 min;) **55°C 10 min**; 95°C 1 min	(25°C 2 min;) **55°C 10 min**; 95°C 1 min	(25°C 5 min;) **42°C 60 min**; 80°C 5 min
Features & Benefits	• SuperMix • Short Protocol • Tracking dye • Higher reaction temperature	• Flexible choice of primers • Short Protocol • Tracking dye • Higher reaction temperature	• Flexible choice of primers • Kit format includes all components

Induro Reverse Transcriptase

Induro Reverse Transcriptase is a group II intron-encoded RT that exhibits high processivity, increased thermostability, and increased tolerance of inhibitors in the synthesis of cDNA from RNA. It is an ideal enzyme for challenging cDNA synthesis from long transcripts, RNAs with strong secondary structures, and RNA samples with inhibitors. With improved 5´ sequencing coverage of long transcripts, Induro can enable RNA-seq applications such as direct RNA and long read cDNA sequencing workflows and is recommended by Oxford Nanopore Technologies® in their SQK-RNA004 protocols.

Learn more about Induro Reverse Transcriptase at
www.neb.com/M0681

Induro Reverse Transcriptase exhibits high processivity, permitting rapid cDNA synthesis

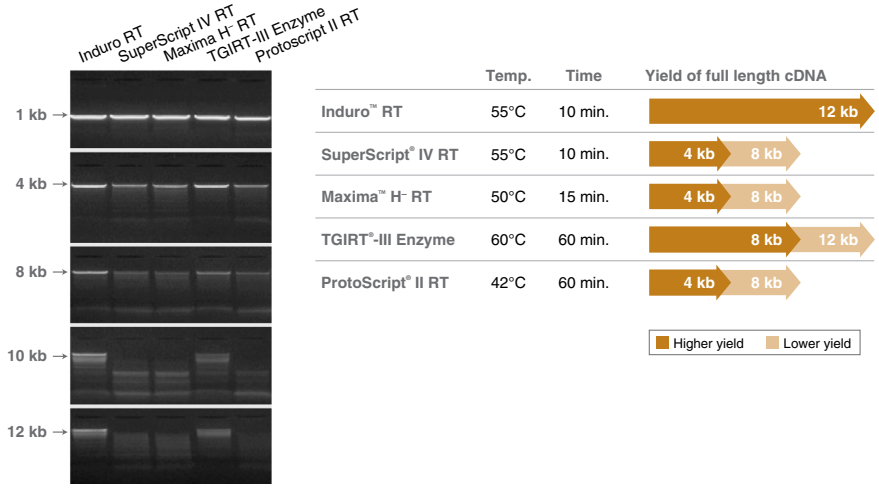


Induro Reverse Transcriptase can synthesize a full-length 12 kb cDNA product within 5 min. at 55°C. In vitro transcribed poly(A) RNA templates (1 kb, 4 kb, 8 kb or 12 kb) were used to investigate full-length cDNA synthesis. After first-strand cDNA synthesis, RNA was hydrolyzed immediately by NaOH. Subsequently, 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.

Benefits

- Rapidly generate high yields of long cDNA
- Strong inhibitor tolerance
- Recommended by Oxford Nanopore Technologies for direct RNA sequencing (SQK-RNA004) protocols
- Generate cDNA at higher temperatures
- Experience comparable fidelity to retroviral RTs

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.

Applications

- Challenging cDNA synthesis
  - long cDNA synthesis (>10 kb)
  - RNA with isoforms or secondary structures
  - 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)

VIEW OUR NEBTV WEBINAR

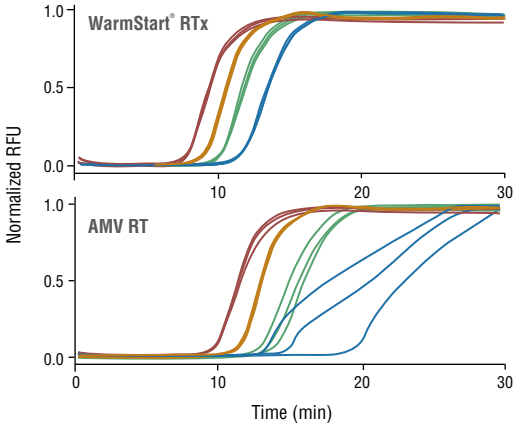
Induro Reverse Transcriptase: A Robust, Thermostable, Intron-Encoded RT for Full-Length cDNA Synthesis

WarmStart RTx Reverse Transcriptase

WarmStart RTx Reverse Transcriptase (NEB #M0380) is a unique *in silico*-designed, RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template. RTx is a robust enzyme for RNA detection in amplification reactions and is particularly well-suited for use in LAMP. The WarmStart property enables high throughput applications, room temperature setup, and increases the consistency and specificity of amplification reactions. RTx contains intact RNase H activity.

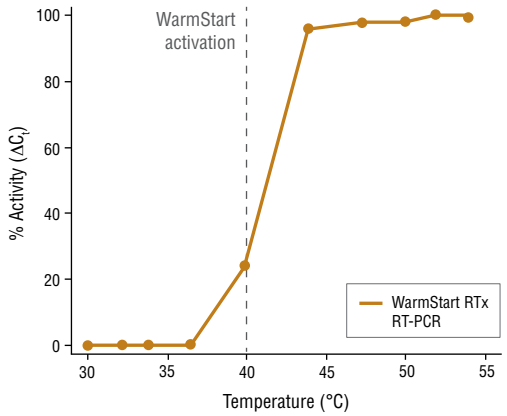
Learn more about WarmStart RTx Reverse Transcriptase at **www.neb.com/M0380**

WarmStart improves speed and sensitivity in RT-LAMP



RT-LAMP reactions with Bst 2.0 WarmStart DNA Polymerase and the indicated reverse transcriptase were incubated at 65°C with 1 pg – 100 ng of Jurkat total RNA. Reactions were monitored with real-time fluorescence, and resulting curves are shown. WarmStart RTx provides faster reaction threshold times for improved consistancy and sensitivity with lower input RNA amounts. RT-LAMP reactions performed with AMV Reverse Transcriptase resulted in inconsistent detection, as indicated by wide variation at lower RNA input concentrations (blue curves).

WarmStart control of WarmStart RTx



cDNA synthesis was performed for 10 minutes, followed by qPCR analysis. Resulting Cts were normalized to a "no RT" control for 0% activity and fastest Ct for 100% activity. WarmStart RTx is inhibited by a reversibly bound aptamer at temperatures below 40°C, and is fully active at temperatures 42°C and higher.

What products are recommended for molecular diagnostics?

NEB offers a selection of RTs, DNA polymerases, and master mixes for customers to use in the development of rapid and sensitive molecular diagnostics. To support the needs of this market, products are available in fully lyophilized, high concentration, or glycerol-free/lyo-compatible formats. All formats are available for customization.

WarmStart RTx is ideal for LAMP and isothermal amplification workflows and is a core component of a lyophilized LAMP/RT-LAMP mix (NEB #L4401). Luna RT supports RT-qPCR workflows and is available in a lyophilized format (NEB #L4001). These enzymes have been adopted in numerous diagnostic assays, including for COVID-19.

The NEB Lyophilization Sciences™, combines lyophilization expertise with our diverse product portfolio to offer formats and services designed for diagnostic assay and instrument developers. **Please email custom@neb.com to discuss your needs and visit www.neb.com/lyoprime and www.neb.com/mdx to learn more.**

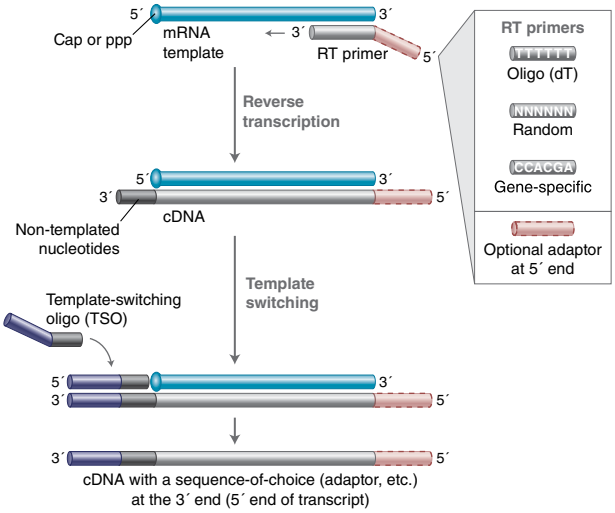
Products can be lyophilized in different form factors.

Template Switching RT Enzyme Mix

The Template Switching RT Enzyme Mix and accompanying reaction buffer enable efficient template switching activity in a reverse transcription reaction. The mix contains a unique RT and Murine RNase Inhibitor. Unlike competitor RT products, no additives (such as PEG or betaine) are required for optimal performance, simplifying reaction setup. In conjunction with a template switching oligo (TSO), cDNA is synthesized with a known sequence of choice attached to the 3´ end.

Learn more about Template Switching RT Enzyme Mix at **www.neb.com/M0466**

Template switching overview



Upon reaching the 5´ end of the RNA template, the reverse transcriptase adds a few non-templated nucleotides to the 3´ end of the cDNA. These non-templated nucleotides can anneal to a TSO with a known sequence handle of choice, prompting the reverse transcriptase to switch from the RNA template to the TSO. The resulting cDNA contains a universal sequence (complementary to the TSO sequence) at the 3´ end.

Advantages

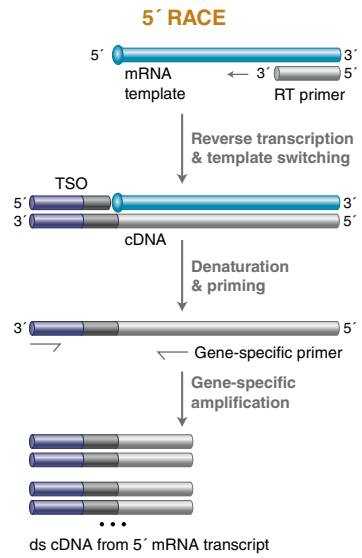
- Prepare RNA-seq libraries from extremely low input: single cells/nuclei or 2 pg total RNA
- Low background for RNA-seq or 5´ RACE
- Use with various TSOs, RT primers and DNA polymerases for full-length cDNA amplification
- Enjoy faster protocols as compared to alternative RNA-seq methods (i.e., Smart-Seq®)

Applications

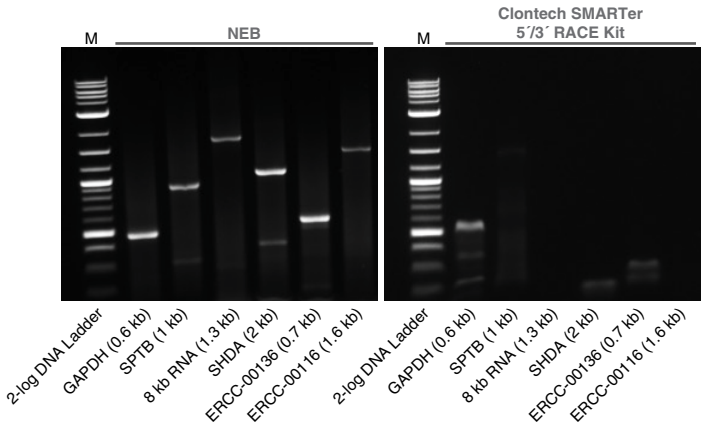
- cDNA amplification
- 5´ RACE
- 2nd strand cDNA synthesis (full coverage of the 5´ end of the transcript)

Template Switching RT Enzyme Mix offers a simple workflow and superior performance for 5´ RACE

A.



B.



A. Overview of template switching-mediated 5´ RACE. After the template switching reverse transcription reaction, 5´ RACE PCR is performed with a reverse gene-specific primer and a forward TSO-specific primer.

B. Agarose gel analysis of 5´ RACE products for various RNA targets using the NEB Template Switching RT Enzyme Mix 5´ RACE protocol (left) or Clontech SMARTer 5´/3´ RACE Kit (right). Input included 1µg of Jurkat total RNA,10 pg of 8 kb synthetic RNA and 10 ng of ERCC RNA Mix 1 to evaluate the performance as a function of transcript length and copy number. For the NEB reaction, oligo (dT)40 VN was used as an RT primer and GCTAATCATTGCAAGCAGTGGTATCAACGCAGAGTACATrGrGrG as the TSO-specific PCR primer underlined. The same internal gene-specific PCR primer was used for both methods. Target names and expected sizes are as indicated.

Product	NEB #	Size
LunaScript RT SuperMix Kit	E3010S/L	25/100 reactions
LunaScript RT SuperMix	M3010L/X/E	100/500/2,500 reactions
LunaScript RT Master Mix Kit (Primer-free)	E3025S/L	25/100 reactions
LunaScript Multiplex One-Step RT-PCR Kit	E1555S/L	50/250 reactions
Induro Reverse Transcriptase	M0681S/L	4,000/10,000 units
ProtoScript II Reverse Transcriptase	M0368S/L/X	4,000/10,000/40,000 reactions
ProtoScript II First Strand cDNA Synthesis Kit	E6560S/L	30/150 reactions
ProtoScript First Strand cDNA Synthesis Kit	E6300S/L	30/150 reactions
M-MuLV Reverse Transcriptase	M0253S/L	10,000/50,000 units
AMV Reverse Transcriptase	M0277S/L	200/1,000 units
WarmStart® RTx Reverse Transcriptase	M0380S/L	400/2,000 units
WarmStart RTx Reverse Transcriptase (Glycerol-free)	M0439L	2,000 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

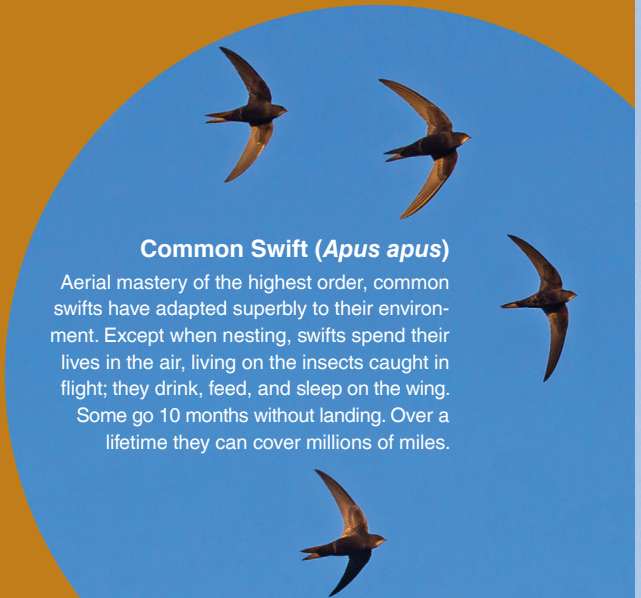
FEATURED ONLINE TOOLS

PCR Selector

For help with choosing the best polymerase for your PCR, try our PCR selector at **PCRselector.neb.com**.

Tm Calculator

For help with calculating annealing temperatures, try our Tm Calculator at **TmCalculator.neb.com**.



Common Swift (*Apus apus*)

Aerial mastery of the highest order, common swifts have adapted superbly to their environment. Except when nesting, swifts spend their lives in the air, living on the insects caught in flight; they drink, feed, and sleep on the wing. Some go 10 months without landing. Over a lifetime they can cover millions of miles.

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