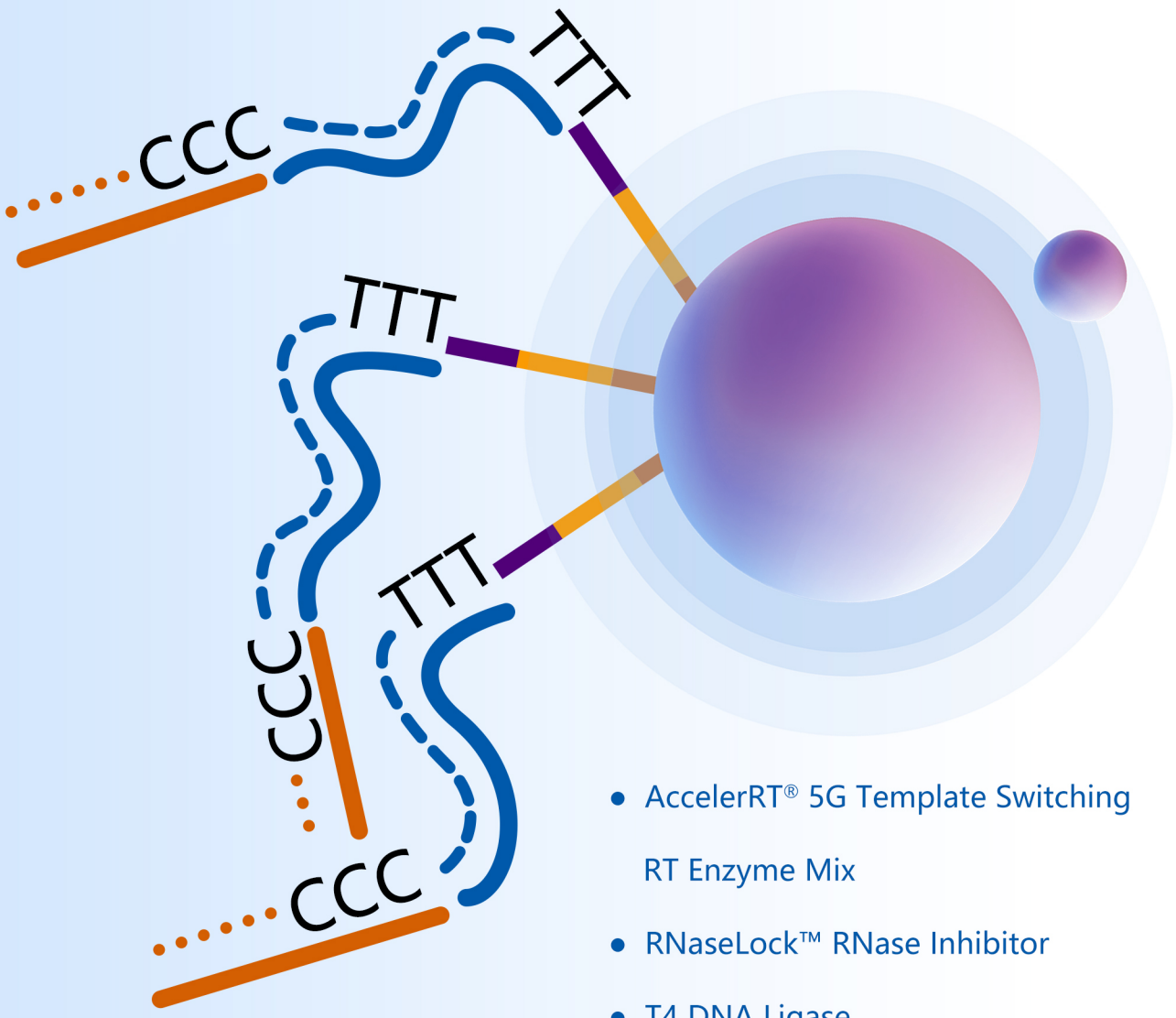
GeneCopoeia™
Expressway to Discovery



- AccelerRT® 5G Template Switching RT Enzyme Mix
- RNaseLock™ RNase Inhibitor
- T4 DNA Ligase

POPULAR ENZYMES FOR SINGLE CELL RNA-SEQ LIBRARIES

If you are interested in these products, please feel free to contact us.

Tel: 301-762-0888 or 1-888-860-4093 Email: Inquiry@genecopoeia.com

Product	Catalog#	Size
AccelerRT® 5G Template Switching RT Enzyme Mix	PC020	10 rxns
	PC021	50 rxns
	PC022	200 rxns
	PC023	1000 rxns
T4 DNA Ligase	A0101A	400 U/μl
T4 DNA Ligase(Without DTT)	Custom	1500 U/μl
RNaseLock™ RNase Inhibitor	PC005	40 U/μl, 50 μl

1、AccelerRT® 5G Template Switching RT Enzyme Mix

AccelerRT® 5G Template Switching RT Enzyme Mix (RNase H-) is a novel RT enzyme that was evolved in vitro from MMLV RT. The enzyme possesses RNA- and DNA-dependent polymerase activities but lacks RNase H activity.

Its efficient template-switching function allows it to be used for full length cDNA products. The engineered enzyme has greatly improved thermal stability, processability and synthesis rates compared to the wild type MMLV RT enzyme.

Applications

→ First-strand cDNA synthesis for full length cDNA products.

→ Construction of single cell sequencing libraries.

→ Discovery and detection of fusion genes.

→ Generation of labeled cDNA probes.

→ RNA analysis by primer extension.

■ Performance Data

Template Switching function

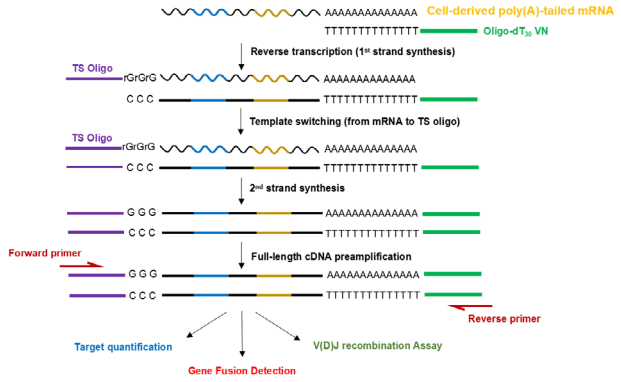
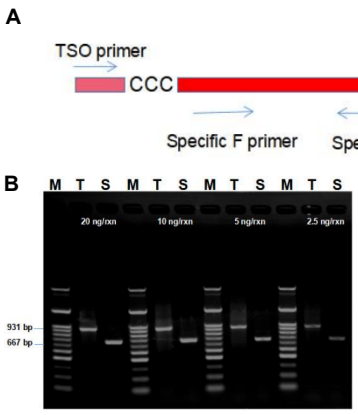


Figure 1. Introduction to the principle of the Template Switching RT Enzyme. The Oligo(dT) VN Primer is used to synthesize the first-strand cDNA. Upon reaching the 5′ end of the RNA template, a few non-template nucleotides are added to the 3′ end of cDNA (Template-switching) using the terminal deoxynucleotidyl transferase (TdT) activity of reverse transcriptase. The second-strand of the cDNA is synthesized using a template-switching oligo. Finally, the full-length cDNA product is obtained by PCR amplification using reverse gene-specific primers and forward Template-switching oligo (TSO)-specific primers.

High Efficiency of Template Switching Synthesis



M	3kb Marker
T	TSO primer 5'and specific R primer PCR products
S	Specific F primer and specific R primer PCR products

RNA(RT Xng/rxn)	Efficiency
20ng	75%
10ng	83%
5ng	82%
2.5ng	74%

Figure 2. AccelerRT® 5G Template Switching reverse transcription reaction was performed using the TSO primer, followed by PCR amplification using the 5 '-end portion of the TSO primer and the gene-specific primer(A). The PCR amplification products were subjected to agarose gel electrophoresis(B) and the efficiency of template switch was calculated by product density value and product length (table).

Exceptional RNA template sensitivity

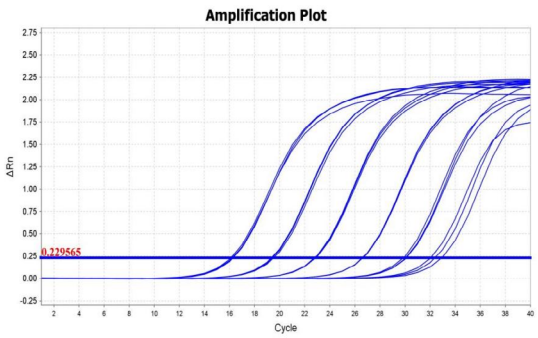


Figure 3. First-strand cDNA was generated from 100 ng to 1 pg of total RNA from Hela cells using AccelerRT® 5G Template Switching RT Enzyme Mix. The synthesized cDNA was used as a template for qPCR using BlazeTaq™ SYBR® Green qPCR mix 2.0(Cat.# QP031).

Broad thermostability

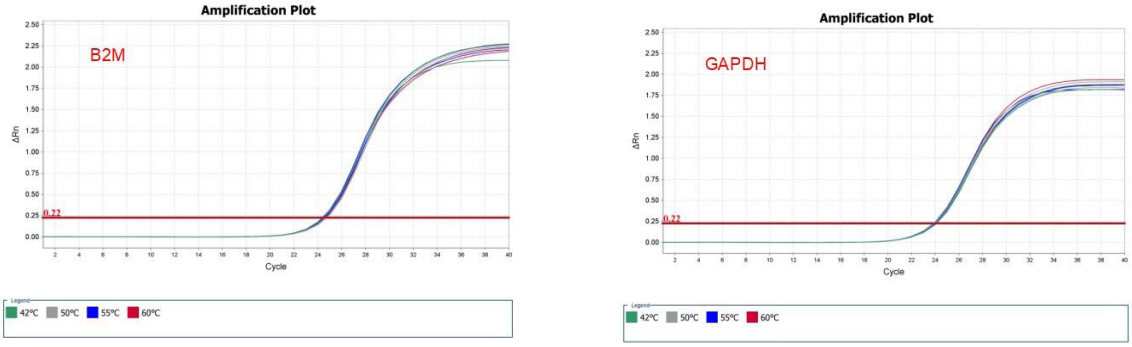


Figure 4. Broad thermal stability of AccelerRT® 5G Template Switching RT Enzyme Mix. 1ng Hela total RNA was reverse transcribed using AccelerRT® 5G Template Switching Reverse Transcriptase at different temperatures from 42 ℃ to 60℃. The GAPDH and B2M cDNAs were amplified with BlazeTaq™ SYBR® Green qPCR mix 2.0(Cat.# QP031).

High efficiency of reverse transcription

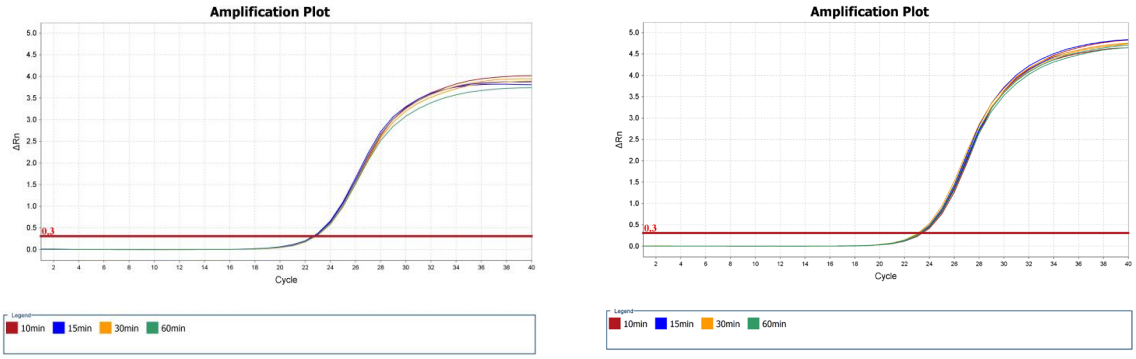


Figure 5. RT efficiency of AccelerRT® 5G Template Switching RT Enzyme Mix was demonstrated using 1ng Hela total RNA reverse transcribed for 10min, 15min, 30min and 60min. The GAPDH and B2M cDNAs were amplified using BlazeTaq™ SYBR® Green qPCR mix 2.0(Cat.# QP031).

Consistent performance in the presence of a variety of inhibitors

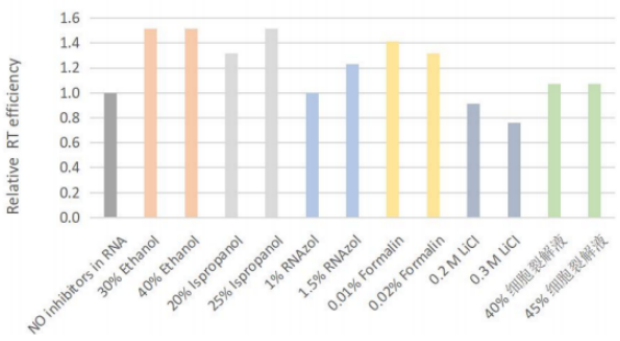


Figure 6. Various inhibitors were added to total HeLa RNA, then was used in a reverse transcription reaction with AccelerRT® 5G Template Switching RT Enzyme Mix, The synthesized cDNA was used as a template in subsequent qPCR using BlazeTaq™ SYBR® Green qPCR mix 2.0(Cat.# QP031).

Effective amplification of cDNA Synthesis

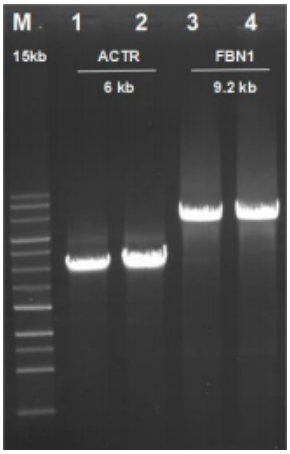


Figure 7. Total RNA from Hela cells was used in a reverse transcription reaction with AccelerRT® 5G Template Switching RT Enzyme Mix. The synthesized cDNA was used as a template in subsequent PCR using the UltraHiPF® DNA Polymerase Kit (Cat.# PC018).

Discovery and detection of fusion genes

Figure 8. Total RNA from H2228 cells was used in a reverse transcription reaction with AccelerRT® 5G Template Switching RT Enzyme Mix.
The synthesized cDNA was used as a template in subsequent PCR using the 5 '-end portion of the TSO primer and the gene-specific primer ALK gene(A). The amplification products in Figure A was verified by amplification with the EML4 forward primer and the ALK reverse primer(B).

2、RNaseLock™ RNase Inhibitor

Applications:

Effectively inhibits the activity of RNase A, RNase B and RNase C in eukaryotes.

In experiments with potential RNase contamination:

→ cDNA synthesis

→ RT-PCR

→ In vitro transcription

→ Isolation and purification of mRNA

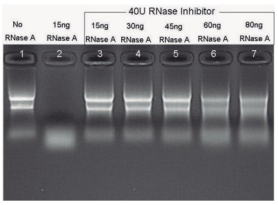


Figure 9. Effectively inhibits the activity of RNase A.

1. Total RNA from mouse liver tissue
2. Total RNA and 15 ng RNase A
3~7. Total RNA and 40 U RNase Inhibitor with different concentrations of RNase A

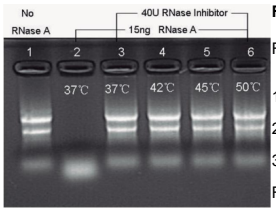


Figure 10. Broad thermal stability of of RNase Inhibitor.

1. Total RNA from mouse liver tissue
2. Total RNA and 15 ng RNase A
3~6. Total RNA and 15 ng RNase A with 40 U RNase Inhibitor at different temperatures

3、T4 DNA ligase

Applications:

→ Cloning of fragments cutting by restriction endonuclease

→ Connect linkers or adapters to the blunt ends of DNA fragments

→ Site-directed mutagenesis

Contact us:

GeneCopoeia, Inc.

9620 Medical Center Dr. Suite 101,
Rockville, MD 20850 USA

Email	inquiry@genecopoeia.com
Tel	+1 (301) 762-0888
Fax	+1 (301) 762-3888
Website	www.genecopoeia.com