AccelerRT® 5G Full Length cDNA Synthesis & Amplification Kit User Manual

——For Full-Length Gene Reverse Transcription and Amplification

Cat. No: PC030 (12 reactions)

PC031 (24 reactions)

PC032 (96 reactions)

User Manual

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AccelerRT® 5G Full Length cDNA Synthesis & Amplification Kit

I. Description

The AccelerRT® 5G Full Length cDNA Synthesis & Amplification Kit can synthesize cDNA ranging from 1-1000 cells or 10 pg - 100 ng of total RNA and an Oligo(dT)VN Primer as a reverse primer. Upon reaching the 5′ end of the RNA template, a specific adapter sequence is annealed and extended to the 3′ end of the cDNA by the terminal deoxynucleotidyl transferase (TdT) activity of the 5G Template Switching Reverse Transcriptase. The full-length cDNA is further amplified by PCR with the adapter sequence, which effectively avoids the 3' bias in the process of cDNA synthesis. The full-length cDNA amplification products can be used to analyze gene expression differences, variable splicing, fusion genes and other genetic regulatory information.

The kit contains reagents of optimizing cell lysis buffer, reverse transcription (RT), and PCR mix. It is not recommended to change the dosage and concentration of any reaction component or replace the component in this kit with another equivalent product.

■ **Advantages**

1. High sensitivity: Low abundance targets can be detected from a small number of cells or total RNA.
2. High quality cDNA: Double-ended primers amplify full-length cDNA, effectively avoiding 5' end and 3' end bias.
3. Time saving: Shorter cell lysis and reverse transcription time.
4. Wide compatibility: Pre-amplification compatible with downstream analysis of NGS or Real-time PCR.

II. Contents and Storage

Cat. No: PC030 (12 reactions)

Cat. No.	Contents	Part No.	Quantity	Store
PC030 (12 reactions)	Template Switching Oligo (TSO, 20 μM)	PC030-01	12 μl	-80℃
	10× Cell Lysis Buffer	PC030-02	12 μl	-20℃ (stable for at least 12 months)
	RNase Inhibitor (25 U/μl)	PC030-03	15 μl	
	3′ Oligo(dT) Primer (10 μM)	PC030-04	12 μl	
	5× First-Strand Synthesis Buffer	PC030-05	48 μl	
	5G Reverse Transcriptase Mix	PC030-06	12 μl	
	2× PCR Amplification Mix	PC030-07	300 μl	
	PCR Forward Primer (20 μM)	PC030-08	12 μl	
	PCR Reverse Primer (20 μM)	PC030-09	12 μl	
	ddH_2O (RNase/DNase free)	PC030-10	500 μl	

Cat. No: PC031 (24 reactions)

Cat. No.	Contents	Part No.	Quantity	Store
PC031 (24 reactions)	Template Switching Oligo (TSO, 20 μM)	PC030-01	24 μl	-80℃
	10× Cell Lysis Buffer	PC030-02	24 μl	-20℃ (stable for at least 12 months)
	RNase Inhibitor (25 U/μl)	PC030-03	30 μl	
	3′ Oligo(dT) Primer (10 μM)	PC030-04	24 μl	
	5× First-Strand Synthesis Buffer	PC030-05	96 μl	
	5G Reverse Transcriptase Mix	PC030-06	24 μl	
	2× PCR Amplification Mix	PC030-07	600 μl	
	PCR Forward Primer (20 μM)	PC030-08	24 μl	
	PCR Reverse Primer (20 μM)	PC030-09	24 μl	
	ddH_2O (RNase/DNase free)	PC030-10	500 μl	

Cat. No: PC032 (96 reactions) is assembled from 4 PC031.

III. Preparation

■ Contamination prevention requirements

1) The detection sensitivity of the kit is high, and it is necessary to avoid experimental cross-contamination. It is recommended to separate the experimental area of cDNA synthesis from that of the PCR reaction and clean the experimental area regularly.

2) As much as possible, solution reagents, pipetters, tubes, etc. employed in the experimental reaction process should be treated with DEPC water before being used, and then autoclaved to avoid the degradation of RNA. Throughout the experiment, put on disposable gloves and refrain from speaking.

3) To avoid cross-contamination of experimental samples, it is recommended to use a nuclease free gun head with a filter element, absorb different components to replace the gun head, and wipe the pipette and desktop with 75% ethanol after the experiment.

4) For the first experiment, to avoid false positives caused by environmental pollution, it is recommended to design a negative control.

■ Sample requirements

1) Cell sample

Prepare fresh mammalian cells suspended in a PBS solution and the cells can be lysed directly using the lysate in this product. The product is not suitable for cells that have been fixed by formaldehyde, acetone, etc. Since this kit uses Oligo dT as a primer for reverse transcription, it is not suitable for samples without a Poly A tail.

2) RNA samples

For the best results, use high quality Poly(A) RNA and total RNA samples with high integrity and purity. Make sure the RNA does not contain contaminants such as residual proteins, organic solvents, and salts that can degrade the RNA or reduce enzyme activity and sensitivity. Before the experiment, the Agilent RNA 6000 Pico Kit could be used to evaluate RNA integrity, and it is recommended to use RNA with an RIN ≥ 8.

■ Required material not included

1) Purification reagent: Macherey-Nagel (Cat.No. 744970.50) or other equivalent products.

2) Library construction reagents: Suitable library construction kits can be selected according to the sequencing platform.

3) Other materials: 0.2mL PCR tube, low adsorption 1.5mL EP tube, anhydrous ethanol, iQuant™ NGS-HS dsDNA Assay Kit (Genecopoeia, Cat.No. N020-2).

4) Instruments: Agilent Technologies 2100 Bioanalyzer, PCR instrument, Qubit 4 Fluorometer, pipette, magnetic rack, scroll oscillator, centrifuge.

IV. Procedures

Template Switching Function

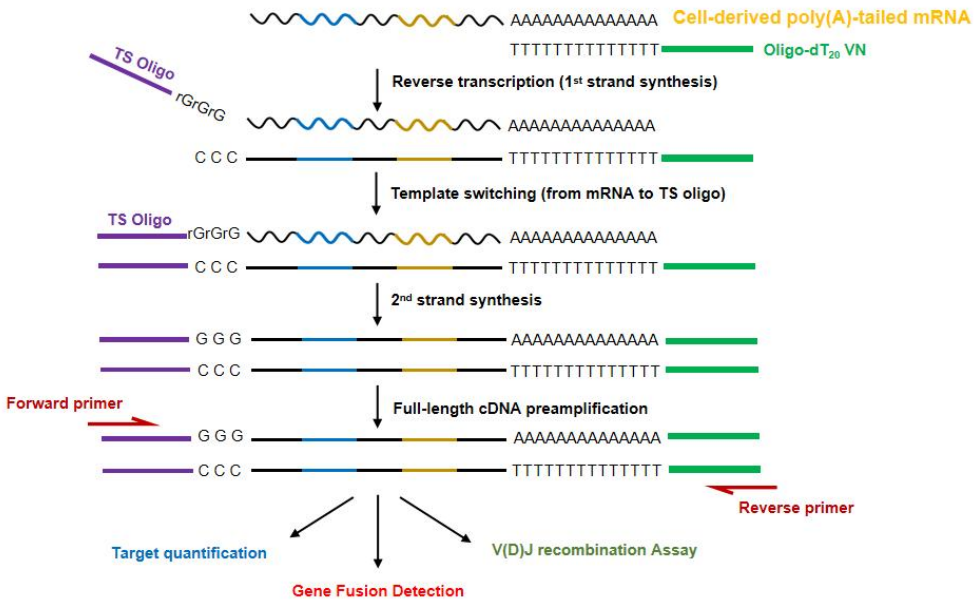

Figure 1. Introduction to the principle of the AccelerRT® 5G Full Length cDNA Synthesis & Amplification Kit. The Oligo(dT) VN Primer is used to synthesize the first-strand cDNA. Upon reaching the 5′ end of the RNA template, a few non-template nucleotides are added to the 3' end of cDNA (Template-switching) 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 5'-Template-switching oligo (TSO)-specific primer and 3'universal adapter primers.

Experimental Procedure

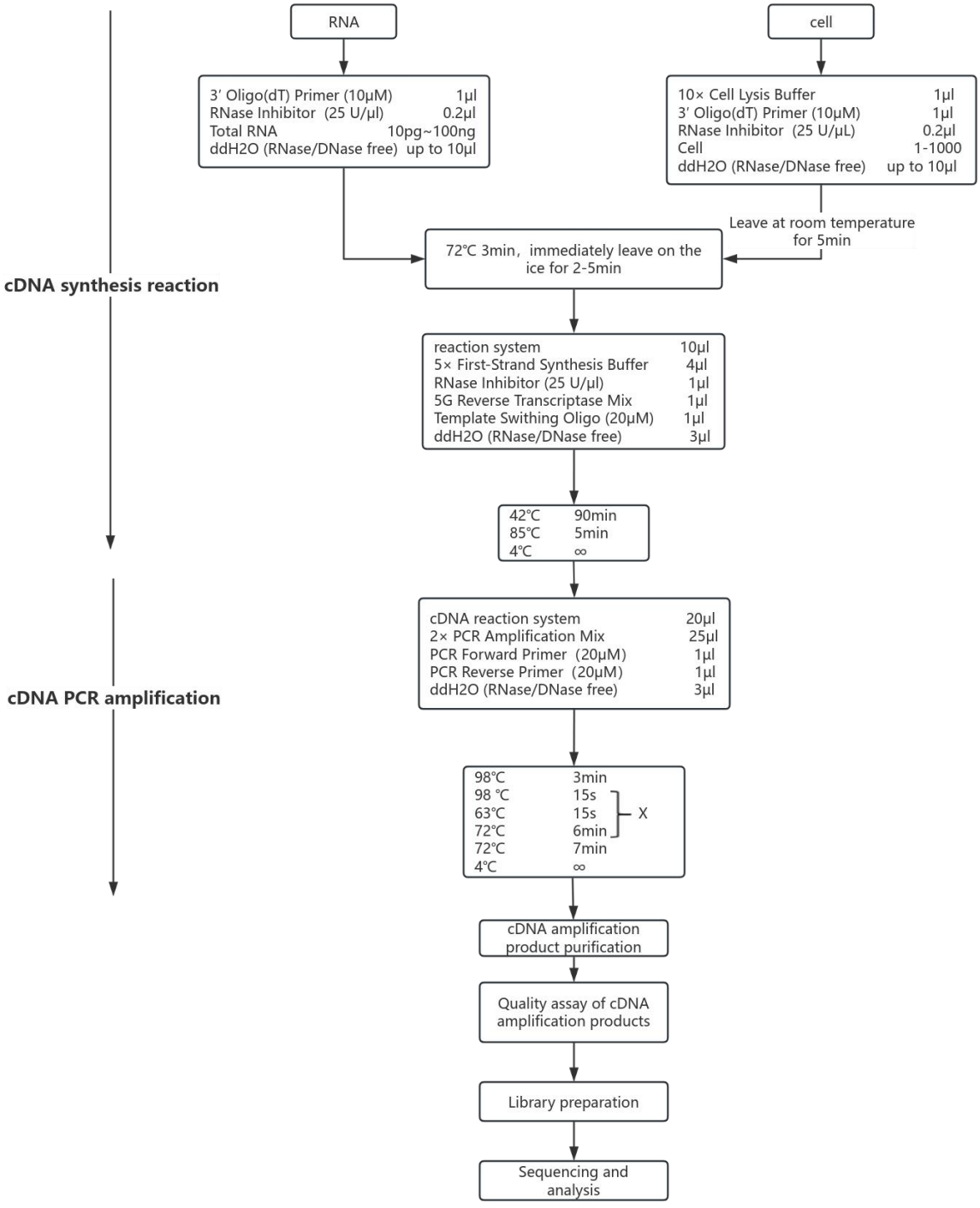

Figure 2. Full-length cDNA synthesis using AccelerRT® 5G Full Length cDNA Synthesis & Amplification Kit.

1. Cell lysis or RNA samples prepare

1) Configure **Mix1** according to the table below, adding the reagent components required for each reaction to the PCR tube.

Component	Volume
10× Cell Lysis Buffer	1 μL
3′ Oligo(dT) Primer (10 μM)	1 μL
RNase Inhibitor (25 U/μL)	0.2 μL
Template	10pg~100ng total RNA, 1~1,000 cells
ddH_2O (RNase/DNase free)	add to 10 μL

Note: If the template is RNA, replace the 10× Cell Lysis Buffer with ddH_2O.

2) Mix with pipette 5-10 times, then centrifuge and leave at room temperature for 5 min (If the template is RNA, then proceed directly to the next step after mixing).
3) Incubate at 72℃ for 3 min and place on ice immediately after reaction.

2. Prepare RT reaction mix

1) Thaw the reagents on ice and mix thoroughly. Centrifuge the tubes and store on ice until use.
2) Configure **Mix2** according to the table below, adding the reagent components required for each reaction to the PCR tube.

Component	Volume
5× First-Strand Synthesis Buffer	4 μL
RNase Inhibitor (25 U/μL)	1 μL
5G Reverse Transcriptase Mix	1 μL
Template Switching Oligo (TSO, 20 μM)	1 μL
ddH_2O (RNase/DNase free)	3 μL
Total	10 μL

3) Mix the reaction solution well, then briefly centrifuge to collect the contents.

3. RT reaction

1) Add **Mix2** to **Mix1**, mix the solution well, then briefly centrifuge to collect the contents.
2) Incubate according to the following procedure:

Temperature	Time
42℃	90 min
85℃	5 min
4℃	∞

◎ **Stopping point: Samples can be stored at -20℃.**

4. cDNA Pre-Amplification

1) Thaw the reagents on ice and mix thoroughly. Centrifuge the tubes and store on ice until use.
2) Add the reagent components required for each reaction to the PCR tube on ice according to the table below.

Component	Volume
2× PCR Amplification Mix	25 μL
PCR Forward Primer (20 μM)	1 μL
PCR Reverse Primer (20 μM)	1 μL
cDNA	20 μL
ddH_2O (RNase/DNase free)	Up to 50 μl

3) Mix the reaction solution well, briefly centrifuge to collect the contents. Make sure there are no bubbles in the solution and that it is placed at the bottom of the PCR tube or plate.
4) Set up the PCR procedure according to the following table.

Temperature	Time	cycle
98℃	3 min	1
98℃	15 sec	X*a
63℃	15 sec	
72℃	6min	
72℃	7 min	1
4℃ ∞		

*a: The following table is the number of PCR amplification cycles based on Hela cell and Hela total RNA, for reference only. The expression of different cells or RNA is different, so the optimal number of cycles needs to be further explored. The high number of amplification cycles will result in the bias of PCR amplification, resulting in the decrease of DNA library volume. The low number of amplification cycles will lead to insufficient DNA amount, which will affect the library analysis.

Total RNA	Cell	PCR cycle
10pg	1	17~18
100pg	10	14~15
1ng	100	11~12
10ng	1000	8~9
100ng	—	7~8

◎ **Stopping point: Samples can be stored at -20℃.**

5. Purify the cDNA amplification product

Using the DNA purification kit-NucleoMag kit for clean up and size selection of NGS library prep reactions (Macherey-Nagel, Cat.No. 744970.50) as an example:

Preparation before experiment:

For the first time using the DNA purification kit, the beads can be divided into 1.5ml centrifuge tubes according to the experimental conditions and stored at 4℃. Before each experiment, fresh 80% ethanol can be prepared according to the experimental amount, and each sample needs 400 μl.

Operational procedures:

1) The beads were mixed with sufficient vortices and placed at room temperature for equilibrium for 30 min. Then, scroll and mix again.
2) Add 50 μl balanced beads to 50 μl PCR amplification products (the ratio of beads:sample is 1 to 1). Vortices are thoroughly mixed and spin down briefly.
3) Incubate the beads with the DNA mixture at room temperature for 10 minutes to allow the DNA binding to the beads.
4) Place the sample on the magnetic rack for at least 5 minutes until the liquid is completely clear. Carefully remove the supernatant, do not scatter the beads.
5) Keep the PCR tube on the magnetic rack and add 200μl freshly prepared 80% ethanol (be careful not to disturb the magnetic bead when adding ethanol). Incubate at room temperature for 30s and carefully remove the supernatant.
6) Repeat Step 5.
7) Keep the PCR tube on the magnetic rack, air-dry the magnetic bead for 5-10 min until there is no ethanol residue.
8) Remove the tube from the magnetic rack and add 17-50 μl of Nuclease free water to the tube to resuspend the beads. Use pipette to blow mixed beads and incubate at room temperature for 2 minutes.
9) Centrifuge the PCR tube briefly, place it on a magnetic rack, and separate the beads and liquid until the solution clears (about 5 minutes).
10) Carefully absorb the supernatant and transfer it to the new low adsorption EP tube (do not attract beads). Store at -20℃ (To avoid DNA degradation, library construction is recommended as soon as possible).

◎ **Stopping point: Samples can be stored at -20℃.**

6. Quality assay of cDNA amplification products

1) The concentration of purified DNA amplification products were quantified using Qubit 4 Fluorometer and iQuant™ NGS-HS dsDNA Assay Kit (Genecopoeia, Cat. N020-2). Please refer to the instructions of the above products for specific operation.
2) Depending on the amount of template added, it is expected that it will produce 2-20 ng amplification products.

7. Library preparation

The appropriate library construction kit can be selected according to the sequencing platform.

V. Frequently Asked Questions

qPCR

Question	Possible cause	Recommended action
Low amplified product yield	The quality of RNA input was poor	Use high-quality RNA with intact poly(A) sequences at the 3' ends.
	The number of amplification cycles was insufficient.	The quantity of RNA in cells and other biological samples can vary significantly. Perform screening experiments to estimate the amount of RNA input, then adjust the number of amplification cycles accordingly.
Low-expression targets are not detected	The sample DNA concentration was too low.	Perform the purification procedure to concentrate the cDNA sample, then proceed directly to qPCR with undiluted sample.
Amplification curve shows no amplification for synthetic RNA spikes	The RNA spikes did not have poly(A) tails.	Use RNA spikes that contain high-quality poly(A) tails.
High base fluorescence signal	Excess preamplification primers in the PCR.	Purify the cDNA sample. Increase the sample dilution before qPCR.
Non-uniform amplification	The quality of RNA input was poor. Degraded 5' ends of RNA resulted in a bias towards amplification at the 3' end.	Use high-quality RNA with intact poly(A) sequences at the 3' ends.
	The RNA input was too high.	Do not use more than 10 ng of total RNA.
	Too many amplification cycles were used.	Do not use more cycles than recommended.

NGS

Question	Possible cause	Recommended action
High primer-dimer content in sequencing results	The purification procedure was not performed or was performed incorrectly.	Perform capillary electrophoresis to determine the primer concentration.
High primer-dimer content in the purified, amplified product	The purification procedure was not performed or was performed incorrectly.	Bring the volume of the sample up to 50 µL with nuclease-free water, then repeat the second stage of the purification procedure.
Low amplified product yield	The quality of RNA input was poor.	Use high-quality RNA with intact poly(A) sequences at the 3' ends.
	The number of amplification cycles was insufficient.	The quantity of RNA in cells and other biological samples can vary significantly. Perform screening experiments to estimate the amount of RNA input, then adjust the number of amplification cycles accordingly.
Extra peak(s) (>300 bp) are present in capillary electrophoresis data	The concentration of internal control spikes, such as ERCC RNA, was too high.	Reduce the concentration of internal control spikes. Optimization experiments may be needed to determine the appropriate concentration for each input amount and/or type.
	The amount of amplified rRNA products was too high.	Reduce the RT reaction time.
Extra peak(s) (<300 bp) are present in capillary electrophoresis data	The purification procedure was performed incorrectly.	Bring the volume of the sample up to 50 µL with nuclease-free water, then repeat the purification procedure again.

VI. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to the use of AccelerRT® 5G Full Length cDNA Synthesis & Amplification Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged, or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product, or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at 301-762-0888.

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