HiScript-TS 5'/3' RACE Kit

RA101

Vazyme

Instruction for Use

Version 26.1

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

The HiScript-TS 5'/3' RACE Kit enables efficient synthesis of full-length cDNA from RNA templates and rapid amplification of either the 5' or 3' cDNA ends. It includes all necessary components for both 5' and 3' RACE, allowing users to choose according to experimental needs. The kit features a directionally modified reverse transcriptase with Template-Switching (TS) activity, enabling adapter-free cDNA synthesis. The high-fidelity PCR Mix ensures accurate and reliable amplification. Reagents are supplied as convenient premixed formulations, simplifying the workflow and reducing contamination risk. All components are rigorously quality-controlled and functionally validated to ensure consistent and reproducible results.

02/Components

	Components	RA101-01 (10 rxns)
BOX 1	5' TS Oligo	10 μl
	Control 293 cell Total RNA (1 μg/μl)[a]	5 μl
BOX 2	3' CDS Primer	20 μl
	5' CDS Primer	20 μl
	5 × FS Buffer	40 μl
	10 × Enzyme Mix	20 μl
	dNTP Mix	20 μl
	2 × PCR Mix	2 × 650 μl
	10 × Universal Primer Mix (UPM)[b]	400 μl
	5' Control GSP[a]	10 μl
	3' Control GSP[a]	10 μl
	5' Random Primer	20 μl
	Nested Primer	50 μl
	RNase-free ddH_2O	1 ml
	Dilution Buffer	1 ml

▲ The color marked in the product components table represents the color of tube lid of each component.

a. Control 293 cell Total RNA was used as the positive control template, and 5' Control GSP and 3' Control GSP were used as amplification primers for the positive control group. The amplification products of 5' and 3' RACE are 580 bp and 1,380 bp, respectively.

b. 10 × Universal Primer Mix (UPM) contains Universal Primer-Long/Universal Primer-Short.

03/Storage

BOX 1: Store at -85 ~ -65°C and ship on dry ice;

BOX 2: Store at -30 ~ -15°C and ship at ≤0°C.

04/Applications

This product is suitable for 5'/3' RACE amplification of 10 ng - 1 μg of Total RNA or Poly A^+ RNA.

05/Self-prepared Materials

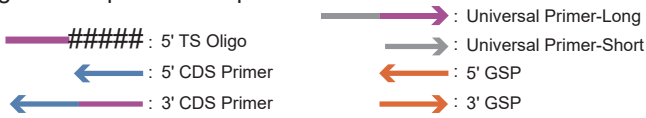
5′/3′ GSP: 5′/3′ Gene Specific Primer;
Purification reagents: FastPure Gel DNA Extraction Mini Kit (Vazyme #DC301) or other equivalent products;
Cloning reagents: Ultra-Universal TOPO Cloning Kit (Vazyme #C603) or other equivalent products;
PCR reagents for bacteriologic test: 2 × Rapid Taq Plus Master Mix (Dye Plus) (Vazyme #P223) or other equivalent products;
Transformation reagents: Chemically competent cells;
Other materials: RNase-free PCR tubes, low adsorption EP tubes, ddH_2O, PCR instruments, etc.

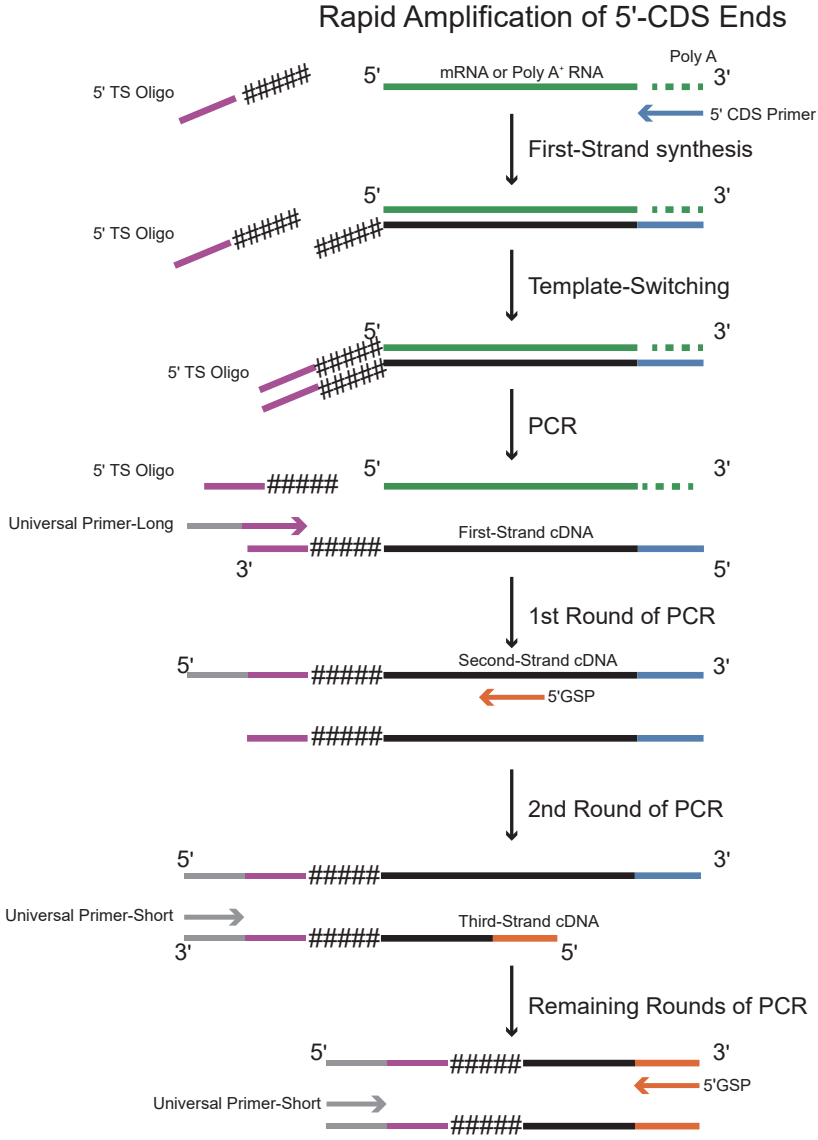
06/Notes

1. The success of RACE amplification is highly dependent on the quality of the RNA template. The integrity and purity of total RNA or poly A^+ RNA are critical factors. Degraded RNA can significantly reduce cDNA synthesis efficiency and negatively impact amplification performance, potentially leading to experiment failure. It is recommended to assess RNA integrity prior to the experiment using agarose gel electrophoresis or the Agilent RNA 6000 Pico Kit.
2. GSP/NGSP (Gene Specific Primer or Nested Gene Specific Primer) should be designed in strict accordance with the requirements described in 08-1/Preparation before experiment, otherwise the amplification products may be nonspecific, or the experiment may even fail.
3. For the same transcript to be tested, multiple GSPs can be designed for RACE amplification to increase the success rate.
4. The end length of the target transcript for amplification: it is recommended not to exceed 3 kb; if the transcript is large, GSP/NGSP should be designed as close to the end of the known sequence as possible.
5. If the abundance of the target transcript is low, the amount of RNA input can be increased, while it is recommended not to exceed 4 μg.
6. If the copy number of the target transcript is low, the First-Strand cDNA product can be directly used for subsequent experiments without dilution.
7. For transcripts lacking a poly(A) tail, the 5′ Random Primer can be used instead of the 5′ CDS Primer for 5′ end analysis. The usage and amount of the 5′ Random Primer should be the same as those of the 5′ CDS Primer.
8. If the initial PCR yields no distinct major band or the target product is nonspecific, a second round of amplification using Nested PCR is recommended to improve specificity and sensitivity.
9. To maintain RNA integrity and enzyme activity, all operations are recommended to be performed on ice throughout the entire experiment.

07/Mechanism & Workflow

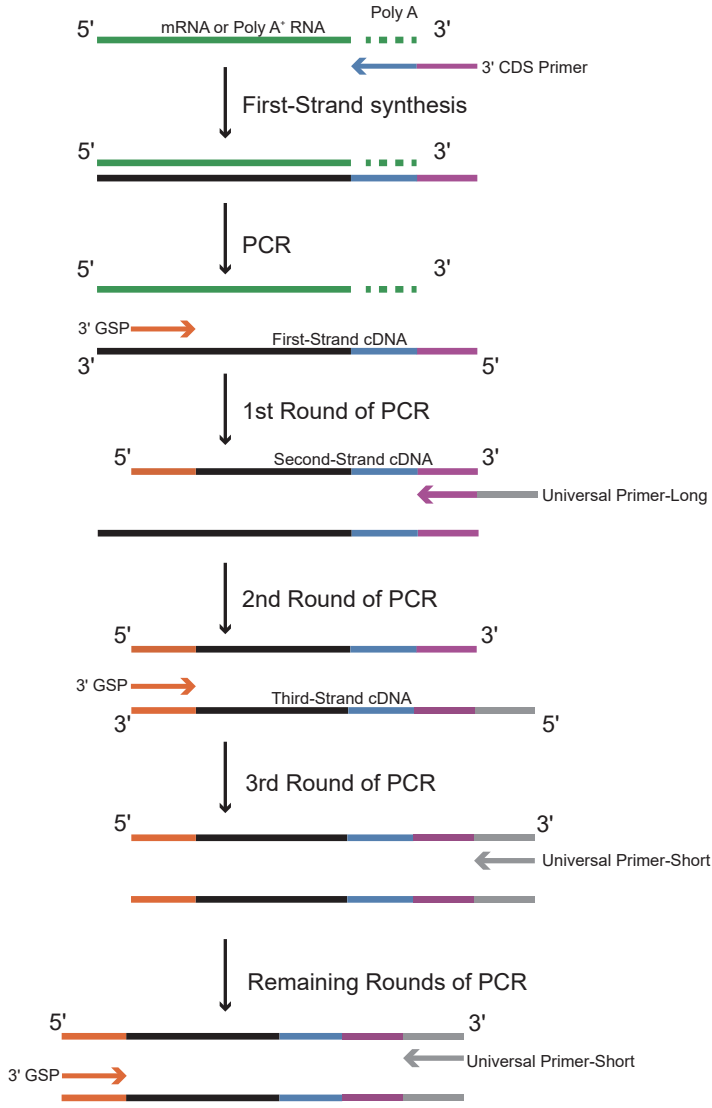
Legends for primers/adapters:





Vazyme

Rapid Amplification of 3'-CDS Ends



08/Experiment Process

08-1/Preparation before experiment

1. GSP (Gene Specific Primer) design principle:

a. Primer length: 23 - 28 nt. It is recommended not to exceed 30 nt;

b. GC content: 50% - 70%;

c. Tm value: Tm ≥65°C. In the case of Tm >70°C, the use of Touch Down PCR is helpful to improve the product specificity;

d. For long (>10 kb) or low abundance transcripts to be tested, it is recommended that the GSP should be designed as close to the end of the cDNA as possible (≤3 kb);

e. Recommendation: For the same transcript to be tested, multiple GSPs can be designed for RACE amplification to increase the success rate.

2. NGSP (Nested Gene Specific Primer) design principle:

a. Primer length, GC content and Tm value: Should follow the same guidelines as those used for GSP design.

b. Design position: NGSP should be designed downstream (3' end) of the corresponding GSP. It is recommended that the 5' end of the NGSP overlaps the 3' end of the GSP by 5 - 15 nucleotides. This overlap enhances specificity during the second round of nested PCR amplification.

3. Integrity test of Total RNA template:

a. Agarose gel electrophoresis: Evaluate RNA integrity by checking for clear and intact ribosomal RNA (rRNA) bands.

b. Bioanalyzer assay: Use the Agilent RNA 6000 Pico Kit and Agilent 2100 Bioanalyzer to assess RNA quality and integrity.

08-2/First strand cDNA synthesis (please operate in clean bench)

1. Take out the components required for the first strand synthesis and put them on ice for thawing. After all components are fully thawed, mix them gently and put them on ice after brief centrifugation.

▲5 × FS Buffer may precipitate. Before use, re-warm it to thaw, mix well, and place it on ice;

▲ Before using 5' TS Oligo and 10 × Enzyme Mix, flick the tube wall to mix well, do not vortex.

▲ It is recommended to use Control 293 cell Total RNA (1 μg/μl) provided in the kit as positive control.

2. Sample preparation

Prepare the reaction solution according to the table below:

5' RACE amplification

Components	Volume	
RNA	10 ng - 1 μg	
5' CDS Primer	2 μl	■
dNTP Mix	2 μl	■
RNase-free ddH_2O	up to 13 μl	■

Gently pipette up and down several times to mix thoroughly, then centrifuge it briefly to the bottom of the tube.

3' RACE amplification

Components	Volume	
RNA	10 ng - 1 μg	
3' CDS Primer	2 μl	■
dNTP Mix	2 μl	■
RNase-free ddH_2O	up to 14 μl	■

Gently pipette up and down several times to mix thoroughly, then centrifuge it briefly to the bottom of the tube.

3. Perform the following program using the PCR instrument:

Temperature	Time
72°C	3 min
Place the tube on ice immediately	2 min

4. Prepare the reverse transcription reaction system as follows:

Components	5' RACE amplification	3' RACE amplification	
Products of Step 3	13 μl	14 μl	
5 × FS Buffer	4 μl	4 μl	■
10 × Enzyme Mix	2 μl	2 μl	■
5' TS Oligo	1 μl	0 μl	■
Total	20 μl	20 μl	

▲Flick the tube wall of 5' TS Oligo and 10 × Enzyme Mix to mix well, do not vortex.

Gently pipette up and down several times to mix thoroughly, then centrifuge it briefly to the bottom of the tube.

5. Perform the following program using the PCR instrument:

Temperature	Time
42°C	90 min
70°C	15 min
4°C	Hold

▲The reaction product can be temporarily stored at 4°C for no more than 12 h; while at -20°C, the reaction products can be stored for 3 months.

6. Product dilution: dilute with Dilution Buffer.

Due to the different abundances of the transcripts to be measured and the large differences in the contents of each transcript in Total RNA of different samples, the dilution multiple will be directly affected. Take 10 μl of the reaction product for the first dilution according to the reference dilution multiple in the table below, while the remaining 10 μl of the product can be stored at -20°C for 3 months.

Dilution multiple for reference:

Starting template	Volume of product from the previous step	Dilution Buffer
≤200 ng Total RNA	10 μl	0 μl
>200 ng Total RNA	10 μl	10 μl
Poly A^+ RNA	10 μl	60 μl

▲ The subsequent dilution multiple can be adjusted according to the abundance of the target transcript and the results of the first experiment.
If the initial template is Total RNA, it is recommended that the ratio of Dilution Buffer to product should be no more than 5:1, i.e. 10 μl product can be diluted by adding 50 μl Dilution Buffer at most.

The obtained product is 5'/3' RACE-Ready cDNA, which can be stored at -20°C for 3 months.

08-3/Rapid amplification of cDNA ends (RACE)

1. Prepare the reaction solution according to the table below:

5' RACE amplification:

Components	5' RACE	Single primer control for UPM[a] (optional)	Single primer control for GSP[a] (optional)	
5' RACE-Ready cDNA	2.5 μl	2.5 μl	2.5 μl	
5' GSP (10 μM)[b]	1 μl	0 μl	1 μl	
10 × Universal Primer Mix (UPM)	5 μl	5 μl	0 μl	■
2 × PCR Mix	25 μl	25 μl	25 μl	■
ddH_2O (self-prepared)	16.5 μl	17.5 μl	21.5 μl	
Total	50 μl	50 μl	50 μl	

a. The use of single primer control for UPM/GSP is helpful to distinguish nonspecific amplification background products.

b. Positive control using 3' Control GSP.

Mix well by gently pipetting up and down, briefly centrifuge to collect the solution and place on ice.

3' RACE amplification:

Components	3' RACE	Single primer control for UPM[a] (optional)	Single primer control for GSP[a] (optional)	
3' RACE-Ready cDNA	2.5 μl	2.5 μl	2.5 μl	
3' GSP (10 μM)[b]	1 μl	0 μl	1 μl	
10 × Universal Primer Mix (UPM)	5 μl	5 μl	0 μl	■
2 × PCR Mix	25 μl	25 μl	25 μl	■
ddH_2O (self-prepared)	16.5 μl	17.5 μl	21.5 μl	
Total	50 μl	50 μl	50 μl	

a. The use of single primer control for UPM/GSP is helpful to distinguish nonspecific amplification background products.

b. Positive control using 3' Control GSP.

Gently pipette up and down several times to mix thoroughly, then centrifuge it briefly to the bottom of the tube.

2. Perform the following program using the PCR instrument:

▲ If the Tm of GSP is >70°C or conducting a positive control test, use the following PCR program I:

Temperature	Time	Number of cycles
98°C	1 min	
98°C	10 sec	5
72°C	3 min[a]	
98°C	10 sec	5
70°C	15 sec	
72°C	3 min[a]	
98°C	10 sec	20 (Poly A$^+$ RNA) or 25 (Total RNA)[b]
68°C	15 sec	
72°C	3 min[a]	
72°C	5 min	
4°C	Hold	

a. If the fragment to be amplified is ≤3 kb, the extension time can be set to 3 min; If the fragment to be amplified is >3 kb, the extension time can be extended by 30 sec for each additional 1 kb.

b. If the amplification product bands is weak, the number of cycles in the final amplification stage can be appropriately increased.

▲ If the Tm of GSP is between 60 ~ 70°C, use the following PCR program II:

Temperature	Time	Number of cycles
98°C	1 min	
98°C	10 sec	20 (Poly A$^+$ RNA) or 25 (Total RNA)[b]
68°C	15 sec	
72°C	3 min[a]	
72°C	5 min	
4°C	Hold	

a. If the fragment to be amplified is ≤3 kb, the extension time can be set to 3 min; If the fragment to be amplified is >3 kb, the extension time will be extended by 30 sec for each additional 1 kb.

b. If the amplification product bands is weak, the number of cycles in the final amplification stage can be appropriately increased.

08-4/[Optional] Nested PCR amplification

If no distinct specific product is obtained in the initial PCR, or if multiple nonspecific bands are observed, it is recommended to perform a second-round Nested PCR using the Nested Primer provided in the kit along with a self-designed Nested GSP. This approach is particularly effective in cases where the initial amplification yields weak target bands accompanied by high background, as it can significantly enhance both specificity and yield.

1. Take 5 μl of the PCR products from the previous round, add it to 245 μl of ddH_2O, shake and mix well.

2. Prepare the reaction solution for Nested PCR according to the table below:

Components	Nested PCR	Single primer control for Nested Primer (optional)	Single primer control for Nested GSP (optional)	
Dilute PCR product of step 1	5 μl	5 μl	5 μl	
Nested GSP (10 μM)	1 μl	0 μl	1 μl	
Nested Primer	1 μl	1 μl	0 μl	■
2 × PCR Mix	25 μl	25 μl	25 μl	■
ddH_2O (self-prepared)	18 μl	19 μl	19 μl	
Total	50 μl	50 μl	50 μl	

▲ The use of single primer control for Nested Primer/Nested GSP is helpful to distinguish nonspecific amplification background products.

3. For the amplification program, refer to 08-3/Rapid amplification of cDNA ends (RACE) PCR program Ⅱ .

08-5/Purification and detection of RACE amplified products

It is recommended to use FastPure Gel DNA Extraction Mini Kit (Vazyme #DC301) or other equivalent products with the detailed procedure in the respective instructions for use.

08-6/Cloning and transformation

1. It is recommended to use Ultra-Universal TOPO Cloning Kit (Vazyme #C603) or other equivalent products for cloning, with the detailed procedure in the respective instructions for use.
   ▲ If the band of the target amplified product is weak, it is recommended to perform multi-tube PCR amplification to enrich the product, and clone after purification which will help to improve the positive clone rate.

2. Transformation
   This product is compatible with variety of competent cells.

3. Identification of positive clones
   Bacterial colony/bacterial solution PCR identification: It is recommended to use 2 × Rapid Taq Plus Master Mix (Dye Plus) (Vazyme #P223) or other equivalent instructions, with the detailed procedure in the respective instructions for use.

4. Sequencing analysis
   Select the identified positive single clones for identification by the first-generation sequencing. Sequencing primers can be either universal vector primers or self-designed primers (it is recommended to select at least 8 - 10 single clones for sequencing).
   3' RACE recognition site: ATCAACGCAGTAC+TT... TNNN (target product sequence);
   5' RACE recognition site: ATCAACGCAGAGTACGGGNNN (target product sequence).
   ▲ If Vazyme # C603 is used for Topo clone sequencing identification, the target fragment may be connected to the vector in a forward or reverse direction. If the sequencing analysis software on Vazyme's official website is used for sequence alignment, there may be a situation where the measured sequence cannot be aligned with the GSP sequence. The measured sequence can be reverse complemented and aligned again.

09/FAQ & Troubleshooting

FAQ	Possible Cause	Solutions
No amplification product or smear bands	No target transcript in RNA sample	Design qPCR or conventional PCR primers targeting known regions of the transcript to confirm its presence in the RNA extract before proceeding with RACE.
	RNA degradation	Evaluate RNA integrity prior to cDNA synthesis. Use agarose gel electrophoresis or the Agilent RNA 6000 Pico Kit to assess rRNA band patterns or RIN values, ensuring the RNA is intact and of high quality.
	Low expression level of target transcript	Increase RNA input (up to 4 μg maximum); increase cDNA input and minimize dilution (for a 50 μl PCR reaction, undiluted cDNA input should not exceed 10 μl). Adjust GSP input according to the transcript abundance (e.g., ≤5 μl of 10 μM GSP in a 50 μl PCR). For low-copy targets, design and use Nested GSPs for two to three rounds of nested PCR to improve specificity and product enrichment
	Target fragment is too long	Extend the PCR elongation time by an additional 30 seconds for every 1 kb of target length beyond standard conditions to ensure complete amplification of long fragments.
	Low Tm of the designed GSP	Ensure that the melting temperature (Tm) of the GSP is ≥ 65 °C. If Tm exceeds 70 °C, a Touchdown PCR strategy is recommended to enhance amplification specificity and reduce background.
Complicated by gel extraction	Variable splicing forms of RNA precursors	Perform database comparison and design GSP for specific segment sequence.
	The target gene belongs to a polygenic family	
	Nonspecific amplification	Design additional NGSP to carry out multiple rounds of Nested PCR (no more than three rounds); Recover target fragment products by gel extraction and amplify by PCR again.
5' RACE without poly A^+ RNA	5' CDS Primer cannot be used for first strand cDNA synthesis	Use 5' Random Primer for first strand cDNA synthesis (provided in the kit).
		Use GSP for first strand cDNA synthesis. It is recommended that to use Nested PCR for amplification in the next step of PCR amplification, and the specificity and positive rate will be improved.
3' RACE without poly A^+ RNA	3' CDS Primer cannot be used for first strand cDNA synthesis	The experiment can be carried out after addition of A-tailing with Poly (A) Polymerase or other 3' terminal transferases.
No positive clone	Insufficient input of target fragments	Purify the amplification products enriched from PCR in multiple tubes and then clone; Expand the cloning system and increase the input of target fragments.

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