TransScript® 5'/3' RACE Kit

Please read the datasheet carefully prior to use.

Version No. Version 1.0

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Cat. No. AR101

Storage: 5' RACE TS Oligo is stored at -70°C for one year, others are stored at -20°C for one year.

Description

TransScript® 5'/3' RACE Kit can be used to efficiently reverse transcription the full-length cDNA using RNA as the template, and efficiently amplify its 5' or 3' end using the cDNA as the template.The kit contains a reverse transcriptase with high thermal stability, fast synthesis speed, and template switching activity and a high-fidelity rapid amplification Mix. 5' RACE or 3' RACE components can be selected according to the experimental requirements.

Features

• Short reaction time of each step, easy to operate and store.
• Transcript templates up to 20 kb can be reverse-transcribed.
• Suitable for transcript templates with expressions as low as 1.4 TPM (Transcripts Per kilobase Million).

Application

Amplification of gene-specific fragments of different species and different expression levels.

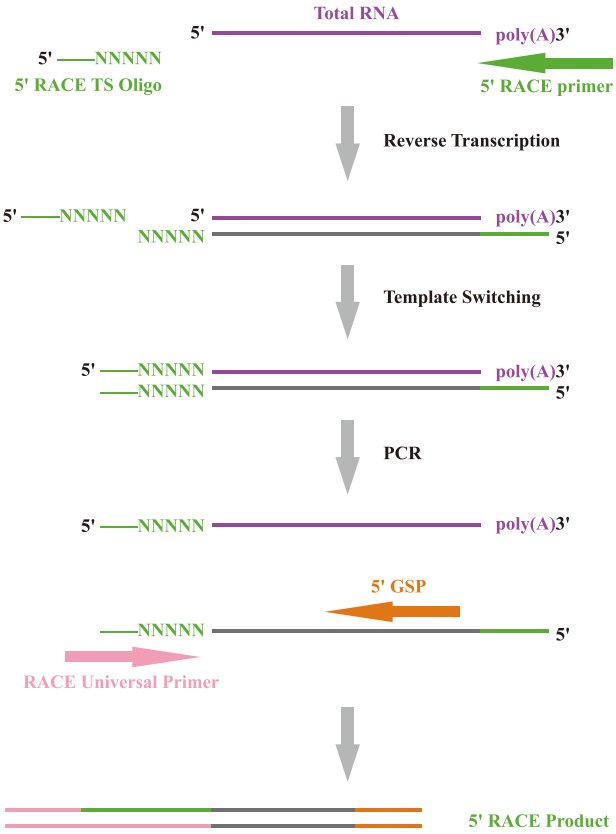
Kit Content

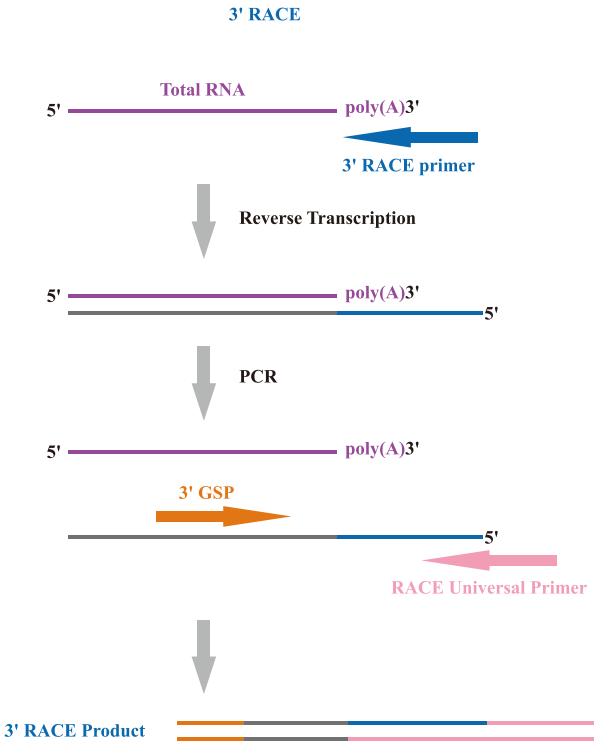
Component	AR101-01 (12 rxns)
5×RACE RT Buffer	48 µl
TransScript® RACE Reverse Transcriptase	24 µl
3' RACE Primer	24 µl
5' RACE Primer	24 µl
5' RACE TS Oligo	12 µl
RACE Universal Primer	600 µl
RACE Nested Primer	240 µl
TransStart® RACE Amplification SuperMix（2×）	2×1 ml
Dilution Buffer	1 ml
RNase-free Water	2×1 ml

Input Sample Recommendations

10 ng - 1 µg Total RNA is recommended for 5'/3' RACE amplification.

Principle Chart





Recommended self-prepared reagents

• 5'/3' gene-specific primers (5'/3' GSP).

• Gel recovery/purification reagent: *EasyPure*® Quick Gel Extraction Kit (TransGen, EG101).

• Cloning reagent: *pEASY*®-Blunt Cloning Kit (TransGen, CB101).

•Transformation reagents: Trans1-T1 Phage Resistant Chemically Competent Cell (TransGen, CD501); Ampicillin (TransGen, GG101); *TransCult*® LB Agar Plate (Ampicillin) (TransGen, CP111).

• Colony PCR reagent: 2×*EasyTaq*® PCR SuperMix (+dye) (TransGen, AS111).

Procedures

1. Guidelines for gene-specific primers design

(1) Primer length: 23 - 28 nucleotides. Longer primers will increase the specificity of the amplified product.

(2) The GC content of the primer is 50% - 70%; The Tm value is ≥65°C, if it is >70°C, use Touch Down PCR.

(3) Multiple GSPs can be designed for the same transcript to improve the success rate of amplification.

(4) It is recommended that the RACE amplification product should not exceed 2kb, especially for longer (>10 kb) or lower-expressed transcripts, and the GSP should be as close as possible to the end of the cDNA.
(5) When there are multiple GSPs in the same transcript, the GSP closer to the end of the cDNA can be selected as NGSP (nested GSP). If the amplification effect is still poor, allow ~10 nt overlap between the 5' end of NGSP and the 3' end of GSP to improve the specificity during the second round of nested PCR.
(6) The designed GSP/NGSP cannot match other gene sequences through Blast alignment.

2. Detection of RNA integrity

Use agarose gel electrophoresis, Agilent 2100 Bioanalyzer or other methods to detect the integrity of total RNA.

3. Full-length cDNA synthesis

(1) Take out all cDNA synthesis components in advance, thaw them completely and place them on ice temporarily.

Note: After 5' RACE TS Oligo is completely thawed, flick the tube wall to mix, do not vortex.

(2) Prepare the reaction as follows (on ice).

Reverse transcription system of **5' RACE:**

Component	Volume
Total RNA	10 ng - 1 μg
5' RACE Primer	2 μl
5' RACE TS Oligo	1 μl
5×RACE RT Buffer	4 μl
TransScript RACE Reverse Transcriptase	2 μl
RNase-free Water	up to 20 μl
Total volume	20 μl

Reverse transcription system of **3' RACE:**

Component	Volume
Total RNA	10 ng - 1 μg
3' RACE Primer	2 μl
5×RACE RT Buffer	4 μl
TransScript RACE Reverse Transcriptase	2 μl
RNase-free Water	up to 20 μl
Total volume	20 μl

(3) Pipet to mix, and spin briefly.
(4) Incubate the reaction in a thermocycler with the following steps.

50°C 60 min
85°C 5 sec
4°C Hold

(5) Dilute the product. According to the needs (such as the amplified gene expression level is high, etc.), use the Dilution Buffer in the kit to dilute the cDNA product. The dilution gradient can be adjusted. For example, a 10-fold dilution of the cDNA（input: 1 μg Total RNA）is recommended as a template for the next step.

4. Rapid amplification of cDNA ends (RACE)

（1）Prepare the reaction as follows (on ice)*.

Component	Volume
5' RACE-cDNA / 3' RACE-cDNA（the product of the previous step）	2.5 μl
5' GSP / 3' GSP（10 μM）**	1 μl
RACE Universal Primer	5 μl
TransStart RACE Amplification SuperMix（2×）	25 μl
RNase-free Water	16.5 μl
Total volume	50 μl

*The GSP/RACE Universal Primer single primer control groups can be set to distinguish non-specific amplification products.

**Be careful to distinguish the source of cDNA. 5' RACE-cDNA corresponds to 5' GSP; 3' RACE-cDNA corresponds to 3' GSP.

（2）Pipet to mix, and spin briefly.

（3）Perform PCR with the following cycling condition.

The Tm value of GSP is **60-70°C:**

98°C	1 min	
98°C	10 sec	25 cycles**
68°C	15 sec	
72°C	30 sec*	
72°C	5 min	
4°C	Hold	

The Tm value of GSP **≥70°C**:

98°C	1 min	
98°C	10 sec	5 cycles
72°C	30 sec*	
98°C	10 sec	5 cycles
70°C	15 sec	
72°C	30 sec*	
98°C	10 sec	5 cycles**
68°C	15 sec	
72°C	30 sec*	
72°C	5 min	
4°C	Hold	

*When amplifying products is >3kb, the 72°C extension time can be adjusted to 1 minute.

**If the target band is weak, the number of cycles can be increased appropriately.

（4）**Nested PCR** (optional): If the one-time amplification effect is not good, NGSP can be used for nested PCR. Prepare the reaction as follows (on ice). Note that nested PCR is best done once and not more than twice.

Component	Volume
Product of the previous round of amplification	1μl
5' NGSP / 3' NGSP（10 μM）	1 μl
RACE Nested Primer	1 μl
TransStart RACE Amplification SuperMix（2×）	25 μl
RNase-free Water	22 μl
Total volume	50 μl

Mix well by pipetting and briefly spin. Perform PCR with the same cycling condition of previous round.

5. Purification and identification of target fragments

It is recommended to use *EasyPure*® Quick Gel Extraction Kit (TransGen, EG101) or other equivalent products to perform gel recovery and purification of the target fragment. Perform first-generation sequencing identification of the purified product. Refer to the recommended self-prepared reagents for specific product information, and refer to the manual for specific steps.

Notes

- Primers must be designed according to primer design guidelines, otherwise it will be difficult to amplify ideal bands.
- Blast alignment is required for the designed GSP/NGSP. If it matches other gene sequences, it may cause non-specific amplification.
- It is recommended to try with 100 ng total RNA at first.
- The integrity of the RNA must be tested before operation, and total RNA with high integrity must be used as the starting template.
- The primers used in the reverse transcription of 5' RACE and 3' RACE are completely different and are colored differently. Please do not get them by mistake.
- 5' RACE TS Oligo should be closed immediately after use and stored at -70°C as soon as possible.

For research use only, not for clinical diagnosis.

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