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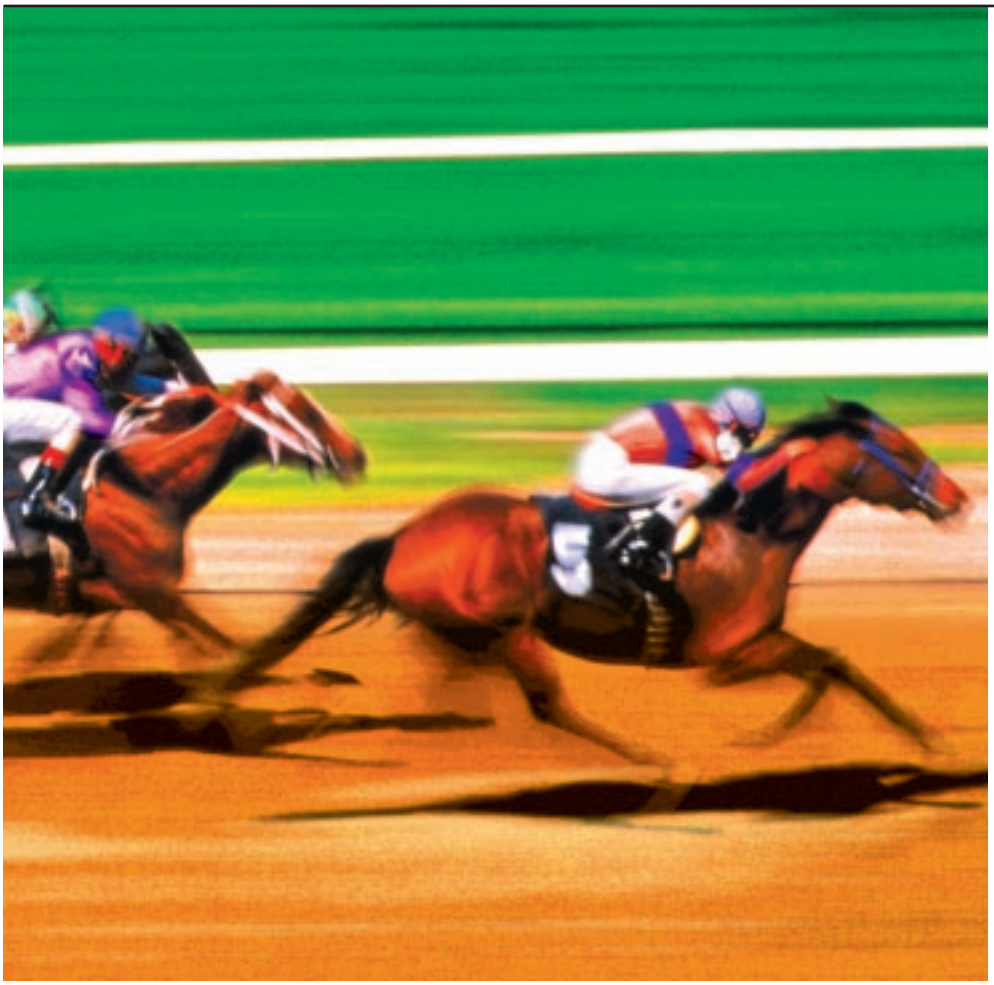
Now with SuperScript™ II RT

Accept Nothing Less than a Full-Length Finish



GeneRacer™ uses an advanced RLM-RACE technique to:

- **Capture** only full-length 5´ end sequence
- **Eliminate** amplification of truncated cDNA
- **Save time** in downstream analysis

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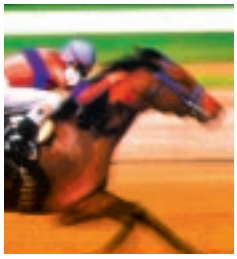
Advanced RACE Technique Saves Time in Downstream Analysis

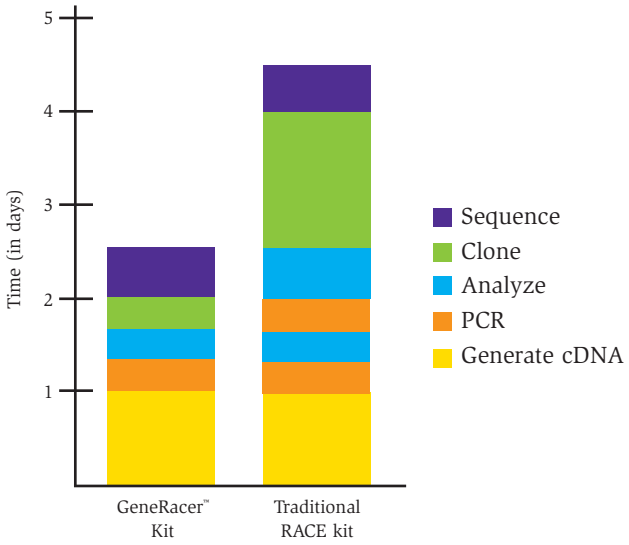


RACE, or rapid amplification of cDNA ends, was designed to amplify the full-length 5′ end sequence. Traditional RACE methods often produce a pool of both full-length and truncated products. This means spending hours of time analyzing sequences to identify the full-length 5′ end of your gene. GeneRacer™ is an advanced RACE technique that overcomes the disadvantages of traditional RACE and ensures that you capture only complete, full-length cDNA ends. You'll get better results and save days of time in downstream analysis.

Efficiently capture full-length 5′ ends

RACE is a PCR-based technique used to obtain full-length 5′ end sequence (1). Studying the 5′ end sequence allows you to find heterogeneous transcription start sites and generate full-length open reading frames (ORF) for protein expression. Although traditional RACE methods can produce the full-length 5′ end sequence, they typically amplify truncated cDNA (2). This results in multiple PCR products that must be characterized and screened in order to isolate the full-length sequence. The GeneRacer™ Kit uses an RNA ligase-mediated (RLM) method to efficiently target the 5′ end of full-length mRNA (3,4). Only these full-length targets will be amplified to eliminate screening through pools of truncated products, saving you days of time (Figure 1).

Figure 1 - Comparison of RACE methods



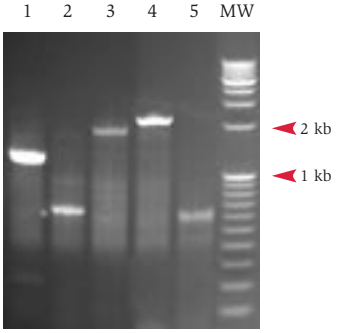
Eliminate amplification of truncated cDNA

GeneRacer™ provides you with a distinct advantage over other RACE protocols. The advanced GeneRacer™ method amplifies only 5´ capped mRNA. This advantage is two-fold–only full-length 5´ capped mRNA is targeted for amplification and truncated cDNA is not amplified. As a result, 5´ RACE PCR products represent only full-length mRNA that includes the transcription start site. There's often no need to perform nested PCR. You'll get results after just one round of PCR, saving you days of valuable research time.

SuperScript™ II RT enhances RACE results

High-quality RACE results depend on the abundance, length, and complexity of the target. GeneRacer™ includes the powerful SuperScript™ II reverse transcriptase (RT) to amplify full-length 5´ ends of complex and long mRNA. The RNase H portion of SuperScript™ II has been inactivated to prevent the cleavage of mRNA when hybridized to a DNA primer or growing cDNA strand. This increases the yield and size of the cDNA, allowing you to obtain the highest yields of full-length cDNA. In addition, SuperScript™ II allows you to perform RT reactions at higher temperatures than other RTs require. By incubating your reaction at 50°C rather than 37-42°C, you'll relax secondary structure of complex templates to allow complete transcription to occur. To demonstrate, we used the GeneRacer™ Kit with SuperScript™ II RT to amplify the 5´ end of a 9 kb gene, which has 90% GC content at the 5´ end, and a rare gene present at 30 copies per cell (Figure 2). Sequencing results of the 5´ ends revealed 44-57 additional bases beyond the data listed in GenBank.

Figure 2 - 5´ RACE results for long transcripts and rare messages



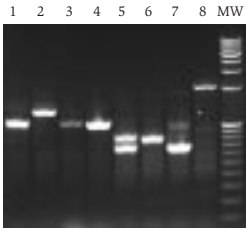
HeLa total RNA (3.5 Mg) was treated according to the GeneRacer™ protocol. cDNA generated with the GeneRacer™ Oligo dT Primer and SuperScript™ II RT was PCR amplified with the GeneRacer™ 5´ Primer and a gene-specific primer. Genes were amplified with one round of a 30-35 cycle PCR. Fifteen microliters of a 50 µl PCR reaction were run on a 1.2% agarose E-Gel® and visualized under UV light.

Lane 1: HPRT (hypoxanthine phosphoribosyltransferase), 1.3 kb, 30 copies/cell, sequence reached known cap site
Lane 2: SMAP (Thyroid hormone receptor coactivating protein), 3.1 kb, 52 additional bases found
Lane 3: Cleavage and polyadenylation specificity factor, 4.5 kb, 57 additional bases found
Lane 4: TFRC (transferrin receptor), 5.0 kb, sequence reached known cap site
Lane 5: IGFIIR (Insulin-like growth factor II receptor), 9 kb, 90% GC-rich at 5´ end, 44 additional bases found
MW: 100 bp ladder and 1 kb ladder

Great RACE results

GeneRacer™ is ideal for determining the 5´ end sequence of genes with unknown transcription start sites. We found additional 5´ end sequence, ranging from 16 to 116 bp, in eight different genes listed in GenBank with unknown start sites. Figure 3 presents the 5´ end amplification results and Table 1 indicates the number of additional bases discovered. With GeneRacer™, you'll get clear bands and the 5´ end sequence result you need.

Figure 3 - Determination of 5´ ends of genes with unknown start sites



Genes are listed in Table 1 below.

One microgram of HeLa total RNA was treated according to the GeneRacer™ protocol. cDNA generated with the GeneRacer™ Oligo dT Primer and AMV RT was PCR amplified with the GeneRacer™ 5´ Primer and a 5´gene-specific primer. Genes were amplified with one round of a 30-cycle (lanes 1-4) or a 35-cycle (lanes 5-8) PCR. Fifteen microliters of each 50 µl PCR reaction were run on a 1.2% agarose E-Gel® and visualized under UV light. Lanes are described in Table 1.
MW: Mixed Ladder (100 bp and 1 kb ladder).

Table 1 - Sequence beyond GenBank

Lane in Figure 4	Gene	GenBank Number (mRNA)	Size (kb)	GeneRacer™ Sequence Beyond GenBank
1	Heterogeneous nuclear ribonucleoprotein complex K (hnRNP K)	S74678	2.3	+ 62
2	Subunit for coatamer complex	X70476	3.1	+ 38
3	Muscle phosphofructokinase (PFKM)	M26066	2.8	+ 16
4	ADP-ribosylation factor 4 (ARF4)	M36341.1	1.5	+ 116
5	Isoleucyl-tRNA synthetase*	U04953	4.5	+ 49
6	Putative tumor suppressor (LUCA 15)	U23946.1	2.6	+ 81
7	Thyroid hormone receptor coactivating protein (SMAP)	NM_006696.1	3.1	+ 52
8	Cleavage and polyadenylation specificity factor	U37012.1	4.5	+ 57

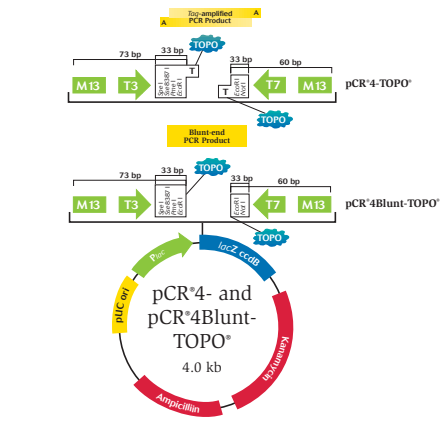
Genes were amplified using the GeneRacer™ protocol. PCR products were gel purified using S.N.A.P.™ gel purification columns and cloned into the pCR®4-TOPO® vector. For each gene, 12 clones were randomly picked and sequenced. Sequences were aligned with those in the GenBank database. The number of bases of previously unknown sequence is reported for each gene.

* Isoleucyl-tRNA synthetase had two strong 5´ GeneRacer™ PCR products (Figure 3, Lane 5). Sequencing results showed that this gene produced two mRNA populations, one having a deletion, possibly an alternatively spliced intron, at the 5´ end of the mRNA.

Streamlined cloning and sequencing

Once you've captured the full-length 5´ end, the next step is to analyze the sequence. For fast, efficient cloning and streamlined sequencing of your RACE PCR products, the GeneRacer™ Kit includes the TOPO Cloning® Kit for Sequencing(6). You'll get the pCR®4-TOPO® or pCR®4Blunt-TOPO® vector, provided linearized and covalently bound to topoisomerase I, for 5-minute, bench-top TOPO® Cloning (5) resulting in >95% recombinants. In addition, sequencing primer sites in pCR®4-TOPO® and pCR®4Blunt-TOPO® are located just 33 base pairs from the insert site (Figure 4) so you'll sequence more of your insert and less of the vector. With the GeneRacer™ Kit, cloning RACE PCR products is fast and sequencing is more efficient.

Figure 4 - The pCR®4-TOPO® and pCR®4Blunt-TOPO® vectors

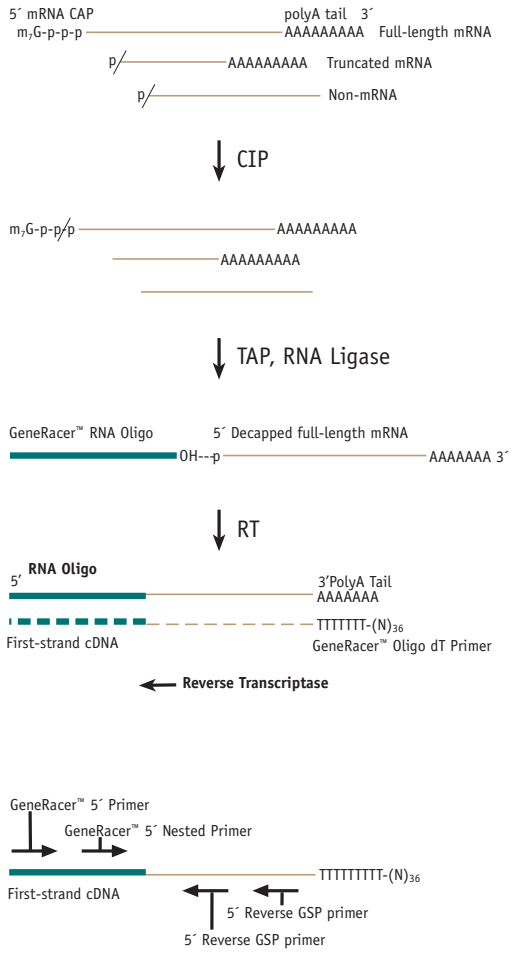


TOPO Represents covalently bound topoisomerase I

Advanced protocol targets full-length 5´ ends

Using the GeneRacer™ RNA ligase-mediated (RLM) RACE method, you'll capture the full-length 5´ end in just five steps. The advanced protocol starts at the RNA level by specifically targeting only 5´ capped mRNA (Figure 5). In subsequent steps, the cap is removed and replaced with the GeneRacer™ RNA Oligo using RNA ligase. During reverse transcription, the GeneRacer™ RNA Oligo sequence is incorporated into the cDNA. Only cDNA that is completely reverse transcribed will contain this common sequence. 5´ RACE PCR is then performed using a gene-specific primer and the GeneRacer™ 5´ Primer specific to the GeneRacer™ RNA Oligo sequence. The result is amplified DNA that contains only the full-length 5´ end sequence.

Figure 5 - The GeneRacer™ protocol



1. RNA (mRNA or total) is treated with calf intestinal phosphatase (CIP). CIP removes the 5´ phosphate from partial transcripts, preventing the GeneRacer™ RNA Oligo from ligating. CIP does not affect capped mRNA.

2. RNA is treated with tobacco acid pyrophosphatase (TAP), which removes the cap from capped mRNA and exposes the 5´ phosphate, permitting ligation of the GeneRacer™ RNA Oligo.

3. The GeneRacer™ RNA Oligo is ligated to the TAP-treated mRNA with T4 RNA ligase.

4. A cDNA template is generated by reverse transcription using SuperScript™ II RT or AMV RT and either the GeneRacer™ Oligo dT Primer, your gene-specific primer, or random primers.

5. 5´ ends are PCR amplified from these cDNA templates with a primer for the GeneRacer™ RNA Oligo (GeneRacer™ 5´ Primer) and your gene-specific primer. Only cDNA containing the GeneRacer™ RNA Oligo sequence will be amplified.

Quality-tested to ensure results

Each lot of GeneRacer™ Kits is functionally tested to ensure you get the best RACE results. To test the kit, 1 µg of HeLa total RNA is treated according to the protocol described in the GeneRacer™ manual. PCR with control primers must yield a single band of the expected size in one round of PCR. In addition, the enzymes supplied are tested to ensure the absence of RNase activity. These extensive testing procedures guarantee the performance of the GeneRacer™ Kit with your gene of interest.

Complete package

Each GeneRacer™ Kit includes everything you need to perform five RACE reactions (Table 2). S.N.A.P.™ gel purification columns are included for easy gel purification of your PCR products. The TOPO® Cloning Kit for Sequencing is added to the kit to allow fast cloning and streamlined sequencing of your RACE PCR products.

Table 2 - GeneRacer™ Kit components

CIP
TAP
RNA Ligase
SuperScript™ II RT or AMV RT
GeneRacer™ RNA Oligo
GeneRacer™ Oligo dT Primer
GeneRacer™ 5´ and 3´ PCR Primers
GeneRacer™ 5´ and 3´ PCR Nested Primers
Random Primers
RNaseOUT™ Recombinant Ribonuclease Inhibitor

Get the 5´ end results you need

The GeneRacer™ Kit ensures your success in capturing only the full-length 5´ end sequence of mRNA transcripts. With SuperScript™ II RT you'll generate even more full-length cDNA. Get the 5´ end results you need with GeneRacer™ and accept nothing less than a full-length finish. Call and order today.

Description	Quantity	Cat. no.
GeneRacer™ Kit		
with SuperScript™ II RT and TOPO TA Cloning® Kit for Sequencing	1 kit*	L1502-01
with SuperScript™ II RT and Zero Blunt® TOPO® PCR Cloning Kit for Sequencing	1 kit*	L1502-02
with AMV RT and TOPO TA Cloning® Kit for Sequencing	1 kit*	L1500-01
with AMV RT and Zero Blunt® TOPO® PCR Cloning Kit for Sequencing	1 kit*	L1500-02

*Includes sufficient reagents for the following reactions: 5 cDNA, 50 PCR, 10 TOPO® Cloning and transformation, and 1 control. PCR enzyme is available separately.

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