Product Insert
3' RACE Kit

Catalogue Number:
BIO-86032 20 Reactions

Features
- Rapid amplification of 3' cDNA ends
- Contains a highly efficient blend of reverse transcriptase and BIO-X-ACT™ Long DNA Polymerase

Applications
- Studying alternative splicing forms of mRNA
- Identification of translation termination codon
- Characterizing 3' untranslated sequence
- Obtaining the full sequence of a gene

Description
The 3' RACE Kit employs the rapid amplification of cDNA ends (RACE) PCR method for obtaining the full-length 3' cDNA sequence from a known partial cDNA sequence. Known sources of partial cDNA sequence include interaction trap assays (e.g. yeast 2 hybrid system), differential display, library screening, cDNA subtraction and microarray subtraction assays. This Kit is also suitable for the characterization of alternative splicing forms of eukaryotic mRNAs.

The Kit is ideal for the amplification and cloning of long sequences, owing to the inclusion of Bioline's reagents BIO-X-ACT™ Long DNA Polymerase and Reverse Transcriptase. RiboSafe RNase Inhibitor is also included, to prevent RNA degradation, which is especially important for the analysis of rare transcripts.

The 3'-RACE Kit contains the following components:

Component	20 Reactions
5x RT Buffer	60µl
(200u/µl) Reverse Transcriptase	20µl
(10u/µl) RNase Inhibitor	20µl
(100mM) dNTP Mix	40µl
(50mM) $MgCl_2$	50µl
(5u/µl) BIO-X-ACT Long	20µl
10x OptiBuffer	100µl
(1ug/µl) pUC19 cloning vector	50µl
DEPC-treated Water	1ml
3' Outer Reverse Primer (10µM) 5'CAGTCGGTCCTGCAGGGTTCAAGCGCATCTGAGG3' *Sbf* I	200µl
3' Nested Reverse Primer (10µM) 5'CAGTCGGTCCTGCAGGGCATCTGAGGTGAACCATGA 3' *Sbf* I	200µl
3' RACE Adaptor (50µM) 5'CCCTGTTCAAGCGCATCTGAGGTGAACCATGAACCG TGCTTTTTTTTTTTTTTTTT3'	100µl

Product Specifications

Batch details:
Batch No: See vial
Units per vial: See vial
Concentration: See vial

Storage Conditions:
The 3' RACE Kit components can be stored for 6 months at -20°C.

Shipping Conditions:
On Dry Ice or Blue Ice

Safety Precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for information regarding hazards and safe handling practice.

Xn: HARMFUL
Xi: IRRITANT

Associated Products:

Product Name	Pack Size	Cat No
X-GAL/IPTG Solution	10ml	BIO-37086
α-Select Gold Efficiency	1ml (20 x 50µl)	BIO-85027
α-Select **Bronze Competent Cells**	2ml (10 x 200µl	**BIO-85025**
CH_3-Blue **Competent Cells**	1ml (10 x 100µl)	BIO-85039

Notes
1. Purchase of this product does not convey a licence to perform any patented process.
2. This product insert is a declaration of analysis at the time of manufacture.
3. Research Use Only

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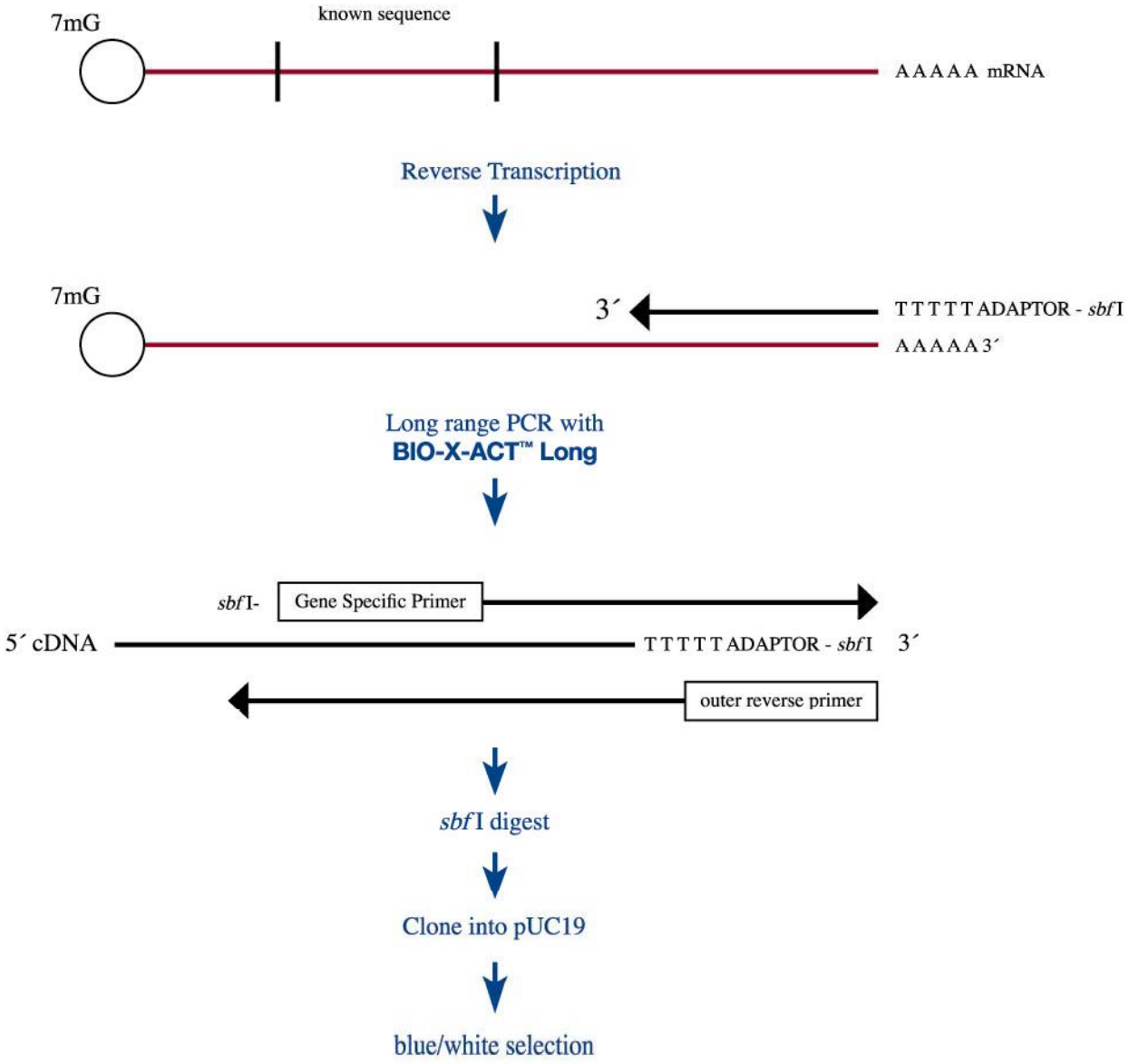
3’ RACE Kit Overview

3’ RACE Overview:
Rapid Amplification of cDNA Ends (RACE) is a technique for the amplification of cDNA sequences from an internal site of an mRNA template and either the 3’ or 5’ end of the mRNA.

The 3’RACE procedure is demonstrated in the schematic diagram below. First strand cDNA synthesis is carried out with either total RNA or poly(A)-selected RNA, using the supplied 3’ RACE Adaptor and Reverse Transcriptase.

PCR is then performed on the cDNA template using the supplied 3’ RACE Outer Reverse Primer, BIO-X-ACT Long, Bioline’s high fidelity polymerase for long templates, and a forward primer (user supplied). A single amplification is usually sufficient; however, should the 3’ RACE reaction require further amplification, a 3’ RACE Nested Reverse Primer is provided.

PCR products can then be cloned into the supplied pUC19 cloning vector using the *Sbf* I sites included in the 3’ RACE Reverse Primers and a forward primer (user supplied, see bellow for design). *Sbf* I is an 8-base nucleotide recognition site, minimizing the chance of the cDNA being digested. For optimal ligation conditions we recommend using Quick-Stick Ligase (Cat Nos.BIO-27027, BIO-27028).



3' RACE Reaction Protocol:

1) RNA Isolation
Ultra-pure total RNA is required to prevent inhibition of RACE reactions and to ensure representation of weakly expressed mRNA species. Use of TRIsure (Cat. No. BIO-38032 or BIO-38033) RNA extraction reagent will maximize success rates of downstream reactions and provide total RNA more representative of weakly expressed genes.

Total RNA should be analyzed by agarose gel electrophoresis. The 28S and 18S RNA bands should be discrete bands. The 28S RNA should be approximately twice the intensity of the 18S RNA. Human 28S RNA runs at approximately 5Kb and 18S RNA runs at approximately 1.9Kb. for more accurate determination of RNA integrity an automated microflow system such as the Experion (BioRad Laboratories) should be used.

The 3' RACE Kit is compatible with total and poly(A) RNA. If the gene of interest is thought to be weakly expressed it is advisable that poly(A) RNA is extracted from TRIsure purified total RNA. Several poly(A) purification kits are commercially available. Poly(A) RNA will give approximately a 20 to 50 fold enrichment of target.

Resuspend total/poly(A) RNA in DEPC-treated Water at approximately (1µg/µl). For general handling of RNA and prevention of RNase please refer to the RNA Guide (www.bioline.com).

2) Design of Gene Specific Primer(s)
From the region of known sequence a forward gene specific primer (gsp) must be designed. The Outer Reverse primer has a melting temperature of 60°C. The user designed gene specific primer must have approximately the same melting temperature (60°C +/- 3°C). For cloning the 5' of the primer must include the *Sbf* I restriction site.

5' ATTACCTGCAGG – KNOWN SEQ 3'
Sbf I

A second nested forward primer may also be synthesized. This second primer should be the same sense as the first forward primer and contain common sequence with the 3' end of the gene specific primer. For example if the user was interested in the gene β-actin:

1st gene specific β-actin primer:
5' ATTACCTGCAGG –CGGTCGAGTCGCGTCCACCCG 3'
Sbf I β-actin sequence

2nd gene specific β-actin primer (internal):
5' ATTACCTGCAGG –TCCACCCGCGAGCACAGCT 3'
Sbf I β-actin sequence

…CCGGTCGAGTCGCGTCCACCCGCGAGCACAGCTTC… β-actin cDNA sequence
CGGTCGAGTCGCGTCCACCCG 1st gene specific β-actin
TCCACCCGCGAGCACAGCT 2nd gene specific β-actin (internal)

The primer should be between 22 and 30 nucleotides in length, with a GC content of between 50 to 60%. Avoid self-complimentary primers. We recommend using freely available software such as Primer3 to design primers (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). It is advisable to design a pair or PCR primers within your known sequence in order to verify that the mRNA of interest is present in the total or poly(A) RNA.

3) Reverse Transcription of RNA (generation of single-strand cDNA with RACE Adaptor)

Prepare a mix containing the following:

Total RNA	0.5 to 5.0µg
or Poly(A) RNA	0.1 to 0.5µg
dNTPs (100mM)	2µl
3' RACE Adaptor	4µl
DEPC-treated Water	to 20µl final volume

Incubate at 65°C for 5 minutes to denature secondary structure, incubate on ice for 2 minutes.

Add the following to the 20µl denatured RNA:

RNase Inhibitor (10u/µl)	1µl
Reverse Transcriptase (200u/µl)	1µl
5x RT buffer	3µl
DEPC-treated Water	6µl

Incubate at 42°C for 60 minutes

4) Ensure gene is present in RNA (optional)
Verify that the gene of interest is present in the cDNA using forward and reverse primers designed against known sequence.

5) Amplification of unknown 3' sequence using RACE adapted cDNA

Add the following to a PCR reaction tube:

1µl	RACE adapted cDNA (from step 3)
1µl	User designed gsp (10µM)
1µl	3' Outer Reverse Primer (10µM)
1µl	dNTPs
5µl	10x OptiBuffer
4µl	$MgCl_2$ (50mM)
1µl	BIO-X-ACT Long
38µl	Water (ddH_2O)

Recommended Parameters for PCR of a 5Kb fragment with either BIO-X-ACT Long:

Temperature	Duration	Cycles
95°C (initial denaturation)	5 minutes	1
95°C (denaturation)	30 sec	30
(annealing temperature)*	1 min	
68°C (elongation)	5 min	
68°C (final elongation)	10 minutes	1

Note*: Annealing temperature is primer dependent

6) Cloning of RACE products into pUC19 cloning vector

Digest the pUC19 vector using *Sbf* I restriction endonuclease.

The RACE PCR products contain *Sbf* I sites incorporated in the forward and reverse 3'RACE primers.

QUICK-STICK™ LIGATION PROTOCOL:

1) Assemble the reaction in a microcentrifuge tube at room temperature in the order outlined below.
   a) Combine the vector and the insert in the appropriate ratio to make up no more than 100ng of DNA.
   b) Adjust volume to 14µl with ddH_2O.
   c) Add 1µl of QS Ligase.
   d) Add 5µl of 4x QS Buffer (Vortex before use).
2) Mix thoroughly with pipetting.
3) Incubate at room temperature for 5 minutes
4) *Optional:* run 2.5-5µl of ligation mixture on to an agarose gel to check ligation efficiency before subjecting the DNA to transformation reactions.

Ratio of Vector DNA:Insert DNA: The ideal vector:insert or phage:insert ratio, may be determined empirically, but is generally in the molar range of 1:3 for vector:insert ligations. For the first cloning of PCR-product, the ratio recommended for vector-insert ligation is 1/10 to 1/100.

3'RACE Troubleshooting Guide:

Observation	Possible Cause	Recommended solution(s)
No amplification products	RNA Degraded	Analyze RNA on a denaturing gel to verify integrity. Ensure that all reagents are RNase-free.
	RNA contained an RT inhibitor:	The presence of inhibitors can be determined by mixing a control RNA with some of the sample and comparing the yield with that of the original amplification. Remove inhibitors such as SDS, EDTA, formamide and pyrophosphate, by ethanol precipitation of RNA, including a 70% ethanol wash step.
	Too much first strand reaction used in the PCR	Dilute the RACE adapted cDNA preparation (from step 3)
Multiple amplification products	Genomic DNA contamination	Treat RNA samples with DNase I prior to the first strand cDNA synthesis
Poor cloning efficiency	Inefficient Ligation	Incorrect ratio of vector to insert. Verify the concentration of the vector and the insert. Perform the ligation using a different ratio of vector to insert.
		Vector not fully digested. Analyze digest by electrophoresis, repeat digest for a longer time period or add more restriction enzyme.
	Inactive competent cells	Verify that the competent cells are active, e.g. transform the undigested pUC19 plasmid DNA. We recommend using the Bioline Gold efficiency α-Select Competent Cells (BIO-85027).
	Poor restriction Digest due to residual bound DNA polymerase	Treat the PCR products with Proteinase K (BIO-37039).