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Product Information

Product Name: 3' RNA NEXTseq RACE Kit
Catalog #: KIT98
Size: 20 Reactions

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Product Description:

This kit is for RNA ligase-mediated (RLM) 3' RACE (Rapid Amplification of cDNA Ends). It is used to clone the 3' end of RNA. The multiple-step procedure is performed in a **single tube** except tube change for PCR reactions. It is convenient, fast and sensitive. Since the 3' linker is derived from Illumina HiSeq, a minor modification of this protocol can be used to make libraries compatible with Illumina HiSeq and NEXTseq platforms with build-in 8nt barcodes in the 3' linker (additional information is available upon request).

Components:

24 µl 10x ligation buffer (without ATP)
90 µl 50% PEG-8000 (for cloning 2'-O-modified RNAs)
24 µl Ligation enzyme mix A
24 µl Reverse transcriptase
24 µl 3' linker :
45 µl RT primer (10uM in inactivation buffer)
GTGACTGGAGTTCAGA CGTGTGCTCTTCCGATCT
15 µl 10 µM 3' PCR primer (Barcode #1, other primers with different barcodes are also available):
CAAGCAGAAGACGGCATACGAGATAG
TTCCACGTGACTGGAGTTCAGACGTGT
24 µl 10 mM dNTP
10µl 10 mM dTTP
45 µl 100 mM DTT
50 µl 10x RT dilution buffer
30 µl PCR enhancer
30 µl H_2O

Procedure:

NOTE: RNase inhibitor may be needed if samples have RNase contamination. However, do not use RNA inhibitor if you don't have to. Since PEG-8000 is used, please mix the sample well at each step using 20 µl tip by pipetting. Carefully watch it to make sure the sample is mixed well.

Step 1: 3' ligation

Denature the RNA at 95 °C for 2 min and then chill it on ice. And then mix the following components in a clean tube and incubate at 16 °C for 1 hour to overnight. Overnight is preferred for a higher ligation efficiency. However, shorter time is fine for abundant RNA, such as actin mRNA.

H_2O/RNA (4µg total RNA*	3 µl
10x ligation buffer (without ATP)	1 µl
PEG-8000	4 µl
3' linker	1 µl
Ligation Enzyme mixture A	1 µl

*Please keep PEG-8000 at room temperature since PEG-8000 is very viscous. If a dried RNA pellet is used as RNA substrate, please make a master mixture without PEG-8000 to dissolve the RNA pellet first and then add PEG-8000. Glycogen may precipitate in reactions containing PEG-8000. However, it does not affect the ligation and can be spun down (recommended). It is not necessary to remove glycogen precipitates.
* RNA isolated by column, phenol, or Trizol-based methods are suitable for this kit. RNA samples should be EDTA-free.

Step 2: RT Primer Annealing

Add 2µl RT primer and 2µl H_2O to each reaction and incubate at 65°C for 15 min and then chill on ice for 2 min.

Step 3: Reverse transcription (RT)

Prepare the following mixture in a clean tube and add 6.4µl into each reaction from step 2, and then incubate at 42 °C for 60 minutes.

RT dilution buffer	2 µl
dNTP	1 µl
dTTP	0.4 µl
DTT	2 µl
Reverse transcriptase	1 µl

Step 4 : 1st PCR (50 µl reaction)

Mix the following components and perform PCR: the following PCR settings are based on our testes and need to be optimized by customers. PCR enzyme and reagent are sold separately.

H_2O	39 µl
10x PCR buffer	5.0 µl
PCR enhancer	1.5 µl
dNTP	1 µl
5' PCR primer 1 (gene specific, outer primer) 10uM	1 µl
3' PCR primer (barcode #1)	1 µl
cDNA from RT reaction	1.0 µl
PCR polymerase	0.5 µl

1 denaturing cycle 94°C 1 min
15 PCR cycles { 94°C 20 sec; 53 °C 20 sec; 68°C 60 sec

Step 5: Nested PCR (50 µl reaction)

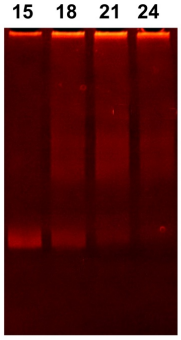
Mix the following components and perform Nested PCR

H2O	39 µl
10x PCR buffer	5.0 µl
PCR enhancer	1.5 µl
dNTP	1 µl
5' PCR primer 2 (gene specific nested or inner primer) 10uM	1 µl
3' PCR primer (barcode #1)	1 µl
PCR product from 1st PCR	1.0 µl
PCR polymerase	0.5 µl

1 denaturing cycle 94°C 1 min
15,18,21,24 PCR cycles { 94°C 20 sec; 53 °C 20 sec; 68°C 60 sec

The extension time should be dependent on the product size.

PCR gel picture:



Note: the Actin cDNA was obtained at cycle 15. However bulged Actin product was formed at cycle 18 and increase over more cycles. This bulged product was indicative of cDNA with a various size of poly (A) tail. When overcycled, these cDNAs were annealed to each other. The non-overcycled actin contained a poly (A) tail of size 150-250 nt, as sequenced.