Video Article

Adapting 3' Rapid Amplification of CDNA Ends to Map Transcripts in Cancer

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Abstract

Maturation of eukaryotic mRNAs involves 3' end formation, which involves the addition of a poly(A) tail. In order to map the 3' end of a gene, the traditional method of choice is 3' rapid amplification of cDNA ends (3' RACE). Protocols for 3' RACE require the careful design and selection of nested primers within the 3' untranslated region (3' UTR) of the target gene of interest. However, with a few modifications the protocol can be used to include the entire 3' UTR and sequences within the open reading frame (ORF), providing a more comprehensive picture of the relationship between the ORF and the 3' UTR. This is in addition to identification of the polyadenylation signal (PAS), as well as the cleavage and polyadenylation site provided by conventional 3' RACE. Expanded 3' RACE can detect unusual 3' UTRs, including gene fusions within the 3' UTR, and the sequence information can be used to predict potential miRNA binding sites as well as AU rich destabilizing elements that may affect the stability of the transcript.

Video Link

The video component of this article can be found at https://www.jove.com/video/57318/

Introduction

The formation of the 3' end is a critical step in mRNA maturation that comprises the cleavage of the pre-mRNA downstream of a PAS followed by the addition of ~250 untemplated adenines, which make up the poly(A) tail[1,2]. The poly(A) binding protein (PABP) binds to the poly(A) tail, and this protects the mRNA transcript from degradation, and facilitates translation[1].

Current estimates suggest that 70% of human genes have multiple PASs, and thus undergo alternative polyadenylation, resulting in multiple 3' ends[3]. Thus, it is important to identify where the poly(A) tail attaches to the rest of the 3' UTR, as well as identify the PAS used by any given transcript. The advent of next-generation sequencing has resulted in the simultaneous identification of the 3' UTRs and the PASs of thousands of genes. This increase in sequencing capability has required the development of bioinformatic algorithms to analyze data involving alternative polyadenylation of the 3' end. For the *de novo* detection or validation of the PAS and hence mapping of the 3' end of individual genes from large scale sequencing data, 3' RACE remains the method of choice[4,5]. The sequences included in cDNA products of 3' RACE normally include only a portion of the 3' UTR that contains the poly(A) tail, the cleavage site, the PAS, and the sequences upstream of the PAS. Unlike PCR, which requires the design and use of gene specific forward and reverse primers, 3' RACE only requires two gene specific nested forward primers. Hence, PCR requires a more detailed knowledge of the nucleotide sequence of a large region of the gene being amplified[4,6]. Since 3' RACE uses the same reverse primer that targets the poly(A) tail for all polyadenylated RNA transcripts, only the forward primers need to be gene specific, thus, only requiring knowledge of a significantly smaller region of the mRNA. This enables the amplification of regions whose sequences are not fully characterized[4,7]. This has allowed 3' RACE to be used not only to determine the 3' end of a gene, but to also determine and characterize large regions upstream of the PAS that form a significant portion of the 3' UTR. By combining 5' RACE with the modified 3' RACE that includes larger portions of the 3' UTR and flanking regions, it is possible to fully sequence or clone an entire mRNA transcript from the 5' end to its 3' end[8].

An example of this application of modified 3' RACE is the recent identification of a novel *CCND1-MRCK* fusion gene transcript from Mantle Cell Lymphoma cell lines and cancer patients. The 3' UTR consisted of sequences from both the *CCND1* and *MRCK* genes and was recalcitrant to miRNA regulation[9]. The two nested *CCND1* specific forward primers were complementary to the region immediately adjacent and downstream of the *CCND1* stop codon. Although whole transcriptome sequencing together with specific bioinformatic tools can be used to detect gene fusions within the 3' UTR[10], many labs may lack the financial resources or bioinformatic expertise to make use of this technology. Hence, 3' RACE is an alternative for *de novo* identification and validation of novel fusion genes involving the 3' UTR. Considering the drastic increase in the number of reported fusion genes as well as read through transcripts, 3' RACE has become a powerful tool in characterizing gene sequences[11,12]. In addition, recent studies have shown that different sequences within the 3' UTR as well as the length of the 3' UTR can affect mRNA transcript stability, localization, translatability, and function[13]. Due in part to an increased interest in mapping the transcriptome, there has been an increase in the number of different DNA polymerases being developed for use in the lab. It is important to determine what types of modifications can be made to the 3' RACE protocol in order to utilize the available repertoire of DNA polymerases.

This work reports adapting 3' RACE to map the entire 3' UTR, the PAS, and the 3' end cleavage site of the *ANKHD1* transcript by using nested primers within the *ANKHD1* section of the transcript and two different DNA polymerases.

Protocol

Wear a lab coat, gloves, and safety glasses at all times while performing all procedures in this protocol. Ensure that containers/tubes containing the phenol and guanidine isothiocyanate reagent are only opened in a certified hood, and dispose of phenol waste in a designated container. Use DNAse/RNAse-free sterile tubes, tips, and reagents.

1. Cell Culture

1. Grow the HeLa cell line and two suspension mantle cell lymphoma cell lines, Granta-519 and Jeko-1, in DMEM containing 10% FBS and 100 U/mL Penicillin/Streptomycin in a humidified incubator with 5% CO_2 at 37 °C. Dilute the cells 1:10 and count using a particle counter[14].
2. For RNA extraction, plate 500,000 cells/well in a 6 well plate (2 mL media per well). After incubation for 24 h, collect RNA from the cells.

2. RNA Extraction

1. Harvesting cells
   NOTE: With the exception of the centrifugation step, perform all the listed steps in a tissue culture hood to maintain sterility.
   1. For suspension cells (Jeko-1 and Granta-519):
      1. Transfer the cells (together with 2 mL of media) to a 15 mL tube, and centrifuge at 1,725 x g for 5 min. Aspirate the media and leave the cell pellet in the bottom of the tube.
      2. Resuspend the cell pellet in 50 µL of 1x Phosphate Buffered Saline (PBS). Add 500 µL of monophasic phenol and guanidine isothiocyanate reagent to each sample in the 1.5 mL microcentrifuge tube. Mix well and leave at room temperature (RT) for 5 min while inverting the microcentrifuge tube every 1 min.
   2. For adherent cells (HeLa):
      1. Aspirate the cell culture media from each well. Add 1 mL of PBS to each well to wash off debris.
      2. Remove the PBS and add 500 µL of monophasic phenol and guanidine isothiocyanate reagent to each well.
      3. Incubate at RT for 5 min with gentle rocking. Transfer to a 1.5 mL microcentrifuge tube.
2. Cell lysis
   1. Freeze the phenol and guanidine isothiocyanate cell mixture at -20 °C for 1 h.
      NOTE: The mix can be left at -20 °C overnight or until needed.
   2. Thaw the phenol and guanidine isothiocyanate cell mixture on ice. Add 100 µL of chloroform to the microcentrifuge tube in a PCR hood. Vortex the sample for 10 - 15 s until the mixture is pink and opaque. Incubate the sample on ice (at 4 °C) for 15 min.
   3. Centrifuge the sample for 15 min at 20,800 x g and 4 °C. Carefully remove the upper colorless aqueous phase (~ 200 µL) and transfer to a new 1.5 mL microcentrifuge tube.
3. RNA precipitation
   1. Add an equal volume of isopropanol to each sample. Add 1 µL of coprecipitant (see **Table of Materials**) to each sample to act as a carrier for the RNA and help visualize the RNA in subsequent steps. Incubate the sample at -80 °C for at least 4 h. For best results, incubate overnight at -80 °C.
   2. Transfer the sample to a cooled centrifuge. Centrifuge for 35 min at 20,800 x g / 4 °C; the RNA will appear as a blue speck on the bottom of the tube. Carefully remove the isopropanol from the sample. Add 500 µL of 80% ethanol and resuspend the pellet by briefly vortexing for 3 s.
   3. Centrifuge the sample at 20,800 x g at RT for 5 min. Remove all the ethanol from the sample. Let the sample air-dry for about 10 min. Resuspend the sample in 35 µL of RNAse-free water. Place on a heat block (65 °C) for 5 min to ensure that the RNA is fully in solution, and immediately place the tube on ice.
   4. Determine the concentration of total RNA using a spectrophotometer as previously described, except use 1.5 µL of the RNA sample instead of 1 µL[15]. Optionally, run 1 µg of total RNA on a 1% agarose gel to determine the RNA integrity.

3. DNase Treatment

1. After quantification of the RNA, perform DNAse treatment on the total RNA to degrade any genomic DNA. For each sample, add the following reagents from the RNAse-free DNAse kit to make a 20 µL total reaction mix in a separate microcentrifuge tube:
   1. Mix 2 µL of DNase 10X reaction buffer with 4.4 µg of total RNA. Bring to a total of 14 µL with water.
   2. Add 2 µL of RNase-free DNAse. Incubate at 37 °C for 30 min in the presence of 2 µL of RNase inhibitor (from the Reverse Transcription Kit).
   3. Add 2 µL of Stop Solution and heat on a heat block for 10 min at 70 °C to inactivate the DNAse enzyme.
      NOTE: DNAse treatment is optional.

4. cDNA Synthesis

For a final reaction volume of 50 µL:

1. For a final reaction volume of 50 µL: Transfer 22 µL of the DNase treated RNA to a new tube, or transfer 4 µg of total RNA with added water to a total volume of 22 µL to a new tube. Add 2 µL of the T7 oligo dT_{25} primer from 10 µM primer solution. The sequence for the T7 oligo dT_{25} primer is 5'-GCCGGTAATACGACTCACTATAGTTTTTTTTTTTTTTTTTTTTTTTTT-3'.
2. From the Reverse Transcription kit add 2 µL of the RNase inhibitor (20 U/µL), 8 µL of the 5x Reaction buffer, 4 µL of 10 mM dNTPs, and 2 µL of the Reverse transcriptase (200 U/µL).
   NOTE: Set up a reaction without reverse transcriptase to act as a negative control.
3. Incubate at 42 °C for 1 h. Transfer directly to ice. Heat the tube at 75 °C for 5 min and place the tube on ice.

5. Primer Search for Fusion Gene Transcript

1. **Primer design**
   1. Download the cDNA sequence from the Ensemble genome browser (ENST00000360839.6/NM_017747), and identify a region within the ORF of the target transcript (*ANKHD1*) upstream of the stop codon. Copy and paste the entire region (up to 700 nucleotides) containing sequences upstream of the stop codon into the sequence entry region of the primer design software (<https://www.idtdna.com/Primerquest/Home/Index>).
   2. Select the show custom design option and select 10 for the number of primers that the system should generate (results to return) and leave all the other parameters set to default.
   3. Choose five forward primers and two reverse primers that do not overlap. Order primers and reconstitute with RNAse/DNAse-free water to a final concentration of 10 µM. More details about general primer design for PCR are covered elsewhere[16].

2. **PCR to identify potential primers for use in subsequent 3' RACE**
   NOTE: To determine the final forward primers to use in the reaction, test the designed primers by setting up different combinations of forward and reverse primers for regular PCR in a PCR hood.
   1. Transfer the cDNA (2 µL) to a fresh 0.5 mL PCR tube. Add 1 µL of the forward primer and 1 µL of the reverse primer (from a 10 mM primer solution). Make up to 12.5 µL by adding 8.5 µL of nuclease-free water. Add 12.5 µL of the 2x PCR-to-Gel Master Mix.
   2. Use the following PCR thermal cycling conditions: 95 °C for 2 min followed by 29 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min. Set the final cycle at 68 °C for 7 min.
   3. After the PCR, run the products on a 1% agarose gel stained with ethidium bromide. Details of electrophoresis are as previously described with a few modifications[17].
      1. Dissolve 1 g of agarose in 100 mL of Tris-Acetate (TAE) buffer in a 250 mL flask. Boil in a microwave.
      2. Leave to cool for 1 min and add 7.5 µL of ethidium bromide solution (10 mg/mL stock) in a fume hood. Mix well by swirling the flask and pour into a horizontal gel apparatus. Leave at RT in the fume hood for at least 30 min to allow the gel to solidify.
      3. Cover the gel with 1x TAE buffer and load 10 µL of the PCR product onto the agarose gel together with 3 - 5 µL of the DNA molecular weight ladder. Run at 175 V for 10 min. Visualize the products using an imager, and if further separation of products is needed, run the gel for an additional 5 - 10 min.

3. **Selection of nested primers for 3' RACE**
   1. Analyze the results of the PCR agarose gel to select primers that have a distinct, strong, single PCR band. Use the 5'-most primer (*ANKHD15'*-CCTTCCCAGCGAGTTTCTAC-3') together with the T7 oligodT_{25} primer in the first PCR run.
   2. Select the nested primer that is located downstream of the first primer (*ANKHD1* 5'-CGTTGGACACAGTGGAATCT-3'), and use it with the T7 primer (5'-GGCCTAATACGACTCACTATAG-3') in the second set of the PCR reactions.

6. Optimizing 3' RACE to Map the 3' UTR Using Two Different Enzymes

NOTE: There has been an increase in the diversity of DNA polymerases used for PCR; therefore, we wanted to determine standard conditions that can be applied even when using different enzymes for 3' RACE PCR reactions. The reverse primers for any transcript are kept constant; the only changes are in the nested forward primers that are specific for the target transcript.

1. **Protocol 1: 3' RACE using a modified DNA polymerase from *Pyrococcus furiosus (Pfu)***
   1. First PCR
      1. Transfer 1 µL of cDNA to a PCR tube and add 5 µL of 10x *Pfu* reaction buffer. Add 1 µL of the first nested forward primer, 1 µL of the T7 oligodT_{25} primer, and 1 µL of dNTPs (from a 10 mM solution).
      2. Make up to 49 µL total volume by adding 40 µL of water. Add 1 µL of *Pfu* DNA polymerase and mix well. Run the PCR using the PCR profile in **Table 1**.
   2. Second PCR
      1. Transfer 2 µL of the products from the first PCR to a new PCR tube and mix with 5 µL of 10x *Pfu*Ultra II reaction buffer. Add 1 µL of the second nested PCR primer, 1 µL of the T7 primer, and 1 µL of dNTPs (10 mM solution).
      2. Make up to 49 µL total reaction volume by adding 39 µL of water. Add 1 µL of *Pfu* DNA polymerase. Run the PCR using the same PCR profile as the first PCR setup (**Table 1**).

2. **Protocol 2: 3' RACE using a chimeric DNA polymerase consisting of a DNA binding domain fused to a *Pyrococcus*-like proofreading polymerase PCR master mix**
    1. First PCR
        1. Transfer 1 µL of cDNA to a PCR tube. Add 1 µL of the first nested forward primer and 1 µL of the T7 oligodT_{25} primer. Make up to 25 µL by adding 22 µL of water.
        2. Add 25 µL of 2x PCR Master Mix and mix well. Use the PCR cycling conditions described in **Table 2**.
    2. Second PCR
        1. Transfer 2 µL of the products from the first PCR to a new PCR tube. Add 1 µL of the second nested PCR primer together with 1 µL of the T7 reverse primer.
        2. Make up to 25 µL by adding 22 µL of water. Add 25 µL of 2X PCR Master Mix. Run the PCR using the same PCR profile as the first PCR setup (**Table 2**).

7. Verify the second PCR product of 3' RACE.

1. Take 5 - 10 µL of the second PCR product (as well as product from the first round of PCRs if the transcript is abundantly expressed) and run on an agarose gel. Set up the gel and run the electrophoresis as previously described (steps 5.2.3.1 to 5.2.3.3). Visualize results on an imager.

8. Product Purification and Sequencing

1. Purify the gel PCR products from the second PCR using the Gel and PCR Fragment DNA Clean-Up System as per the manufacturer's protocol.
2. Perform Sanger sequencing (alternatively, send the gel purified PCR product samples and appropriate sequencing primers to a sequencing lab). Analyze the sequencing data after Sanger sequencing.
3. Download sequence-analyzing software (see **Table of Materials**) and import the trace files into the software. Use the individual PURE Base QVs (Quality Values) to obtain the Base call information[18]. Download the chromatogram with high quality traces for viewing.
   Optional: The gel purified product can be cloned using an appropriate cloning kit and the isolated clones sent for Sanger sequencing.

Representative Results

Nested Forward Primer Search:

The agarose gel from Figure 1 shows two distinct PCR gel products (Lanes 1 and 2) which use the same forward primer but different reverse primers. Lane 3 has a distinct PCR product and has a distinct forward and reverse primer. The ideal primers to use for the PCR based reaction are those that give one distinct PCR product (Lane 3). The forward primer used in Lanes 1 and 2 gives the strongest band and gives the largest PCR products (expected), and is used as the first forward primer. The second nested primer for the second set of PCR reactions in 3' RACE is the forward primer from Lane 3.

Different DNA Polymerases Used in 3' RACE Produce Similar Results:

Two different DNA polymerases produced the same sized PCR products despite having different PCR cycling conditions for the 3' RACE (**Figure 2**). There is only one distinct band for the expected PCR product. All of the minus reverse transcriptase negative controls (-) do not have a band, showing no genomic contamination of the RNA used in cDNA synthesis as well as in subsequent downstream reactions.

Sanger Sequencing Results:

The PCR products were gel purified and sent for Sanger sequencing. The results shown in **Figure 3A** show a portion of a sequence chromatograph from Sanger sequencing. A representative sequence identifies the location of the stop codon, putative polyadenylation signal, and the cleavage site, as well as the poly (A) tail in the 3' RACE product (**Figure 3B**).

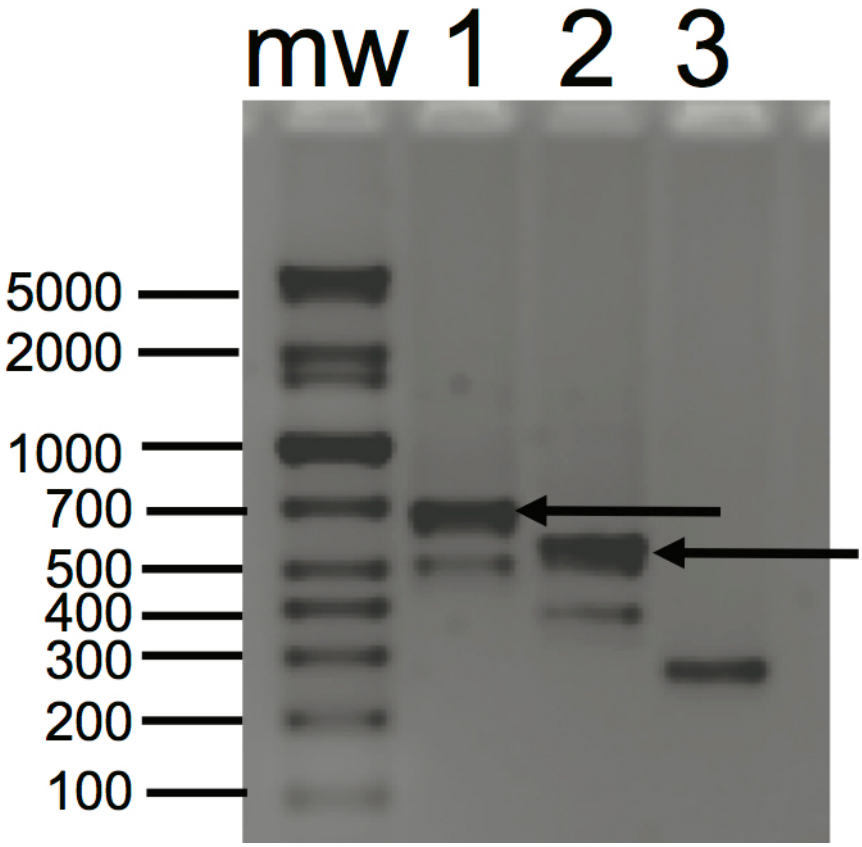

Figure 1: Identification of two nested gene specific forward primers to use in 3' RACE. Shown is an example of PCR products from different combinations of gene specific forward and reverse primers using HeLa cDNA run on an ethidium bromide stained agarose gel with the molecular weight (mw) ladder. Lanes 1 and 2 are PCR products from the same gene specific forward primer but different reverse primers. The arrows point to the expected PCR product for each lane. In addition to the band for the expected PCR product, there is an additional band. The single PCR product in Lane 3 is from a different gene specific nested forward and reverse primer and shows the single expected band.

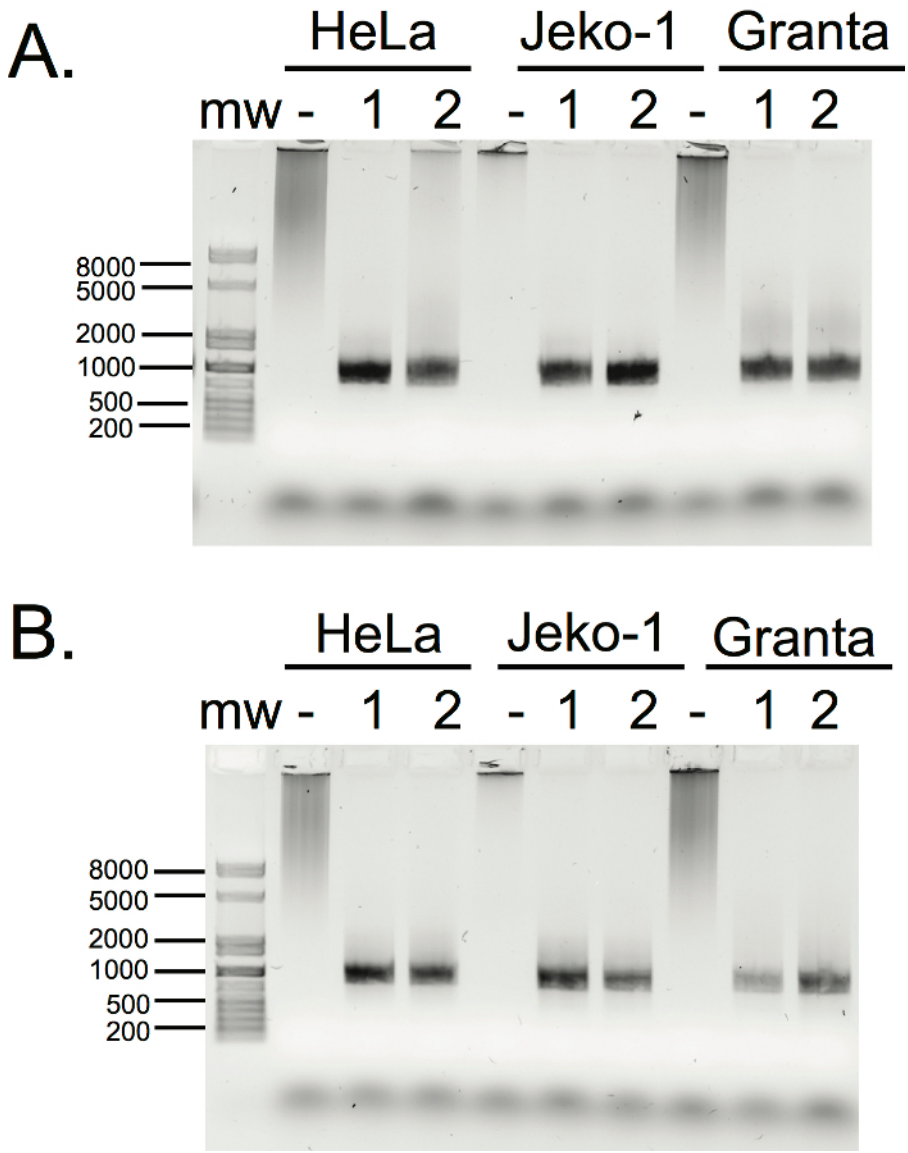

Figure 2: Verification of 3' RACE products from the second set of PCR reactions. Products from the second PCR reaction with (**A**) *Pfu* DNA Polymerase and (**B**) Chimeric DNA polymerase were run on an agarose gel with ethidium bromide. Lanes depicted by (-) are from the negative control sample for each cell line when cDNA synthesis from RNA was carried out in the absence of the reverse transcriptase enzyme. Lanes 1 and 2 are PCR products from biological replicates of each cell line. Please click here to view a larger version of this figure.

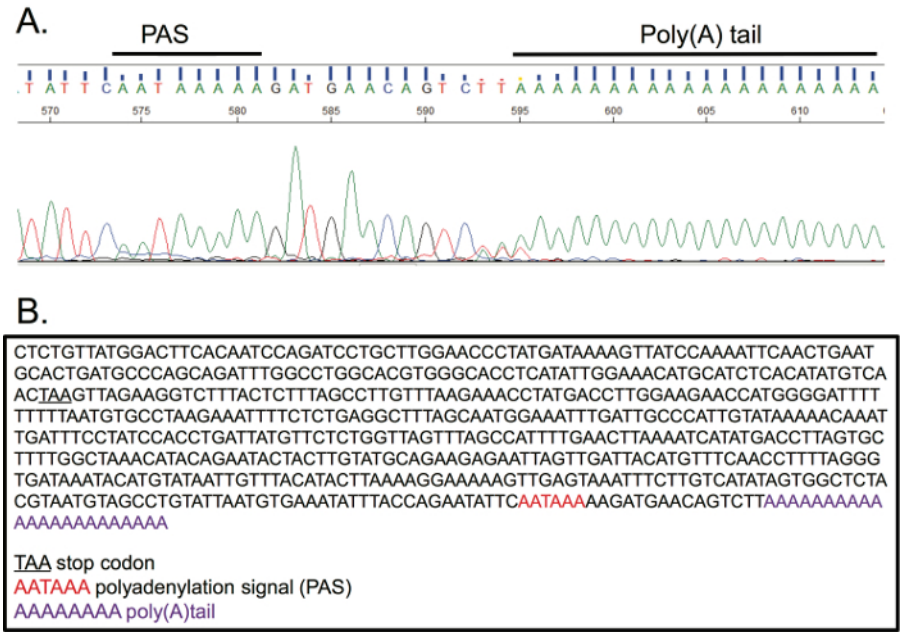

Figure 3: Mapping the PCR products of 3' RACE. (**A**) A portion of a sequence chromatogram of a gel purified 3' RACE product showing the predicted polyadenylation signal and the location of the poly(A) tail. (**B**) Representative sequences from Sanger sequencing data showing the stop codon, the polyadenylation signal (PAS), the cleavage and attachment site of the poly(A) tail as well as a section of the poly (A) tail sequences. Please click here to view a larger version of this figure.

Cycle step	Temperature and Duration	Number of cycles
Initial denaturation	95 °C for 3 min	1
Initial annealing and extension	50 °C for 5 min and 72 °C for 10 min	1
Subcyles (denaturation, annealing and extension)	95 °C for 40 s, 50 °C for 1 min, and 72 °C for 3 min	20
Final cycle (denaturation, annealing and extension)	95 °C for 40 s, 50 °C for 1 min, and 72 °C for 15 min	1
Hold	4 °C, ∞	

Table 1: *Pfu* DNA Polymerase PCR cycling conditions for 3' RACE.

Cycle step	Temperature and Duration	Number of cycles
Initial denaturation	98 °C for 3 min	1
Initial annealing and extension	50 °C for 5 min and 72 °C for 10 min	1
Subcyles (denaturation, annealing and extension)	98 °C for 30 s, 50 °C for 1 min, and 72 °C for 4 min	20
Final cycle (denaturation, annealing and extension)	98 °C for 30 s, 50 °C for 1 min, and 72 °C for 15 min	1
Hold	4 °C, ∞	

Table 2: Chimeric DNA polymerase PCR cycling conditions.

Discussion

Despite the advent of massive parallel sequencing technologies, on a gene-by-gene basis, 3' RACE still remains the easiest and most economical method to identify the PAS and nucleotides adjacent to the poly(A) tail. The adaptation described here expands using 3' RACE to both amplify and map sequences that include a portion of the ORF, the stop codon, and the entire 3' UTR of the *ANKHD1* mRNA transcript. A major advantage of 3' RACE is that with a few minor adaptations, products from 3' RACE can be cloned into other vectors to facilitate downstream interrogation of 3' UTR function including miRNA targeting, stability assays as well as other mechanistic assays. This can be done by including restriction enzyme sites within the nested primer sequences[9,10]. Adjustments can be made to include only the 3' UTR without any sequences from the ORF for cloning for 3' UTR functional assays[5].

A major determinant of the success of 3' RACE is the development of nested primers that target the gene of interest. The cDNA sequences from the Ensembl genome browser are recommended to best identify regions without polymorphisms in order to develop optimal primers.

Ideally, several nested forward primers as well as at least two reverse primers within the gene of interest can be screened using standard PCR to identify the best two nested gene specific primers to use. In this study the first gene specific forward primer selected for 3' RACE initially gave two bands with standard PCR routinely used in the lab. Unfortunately, the primer design was limited by the significant number of SNPs in the transcript of interest. SNPs in the target transcript lead to mismatch between the primers and the target, which can result in inefficient annealing and amplification, and should be reduced to a minimum[19]. The presence of at least four SNPs in one primer, or the occurrence of a combination of five mismatches (three in one primer and two in the other) may result in complete inhibition of the PCR reaction, and hence the only option is to design more primers[20]. Instead of designing more primers to get one single band for the standard PCR used for the primer search in experiments reported here, the PCR cycling conditions and the DNA polymerases subsequently used in 3' RACE were altered in order to optimize the probability of getting one specific band. The denaturation temperature was increased to 98 °C for one of the DNA polymerases (an increase from 95 °C used in the primer search). For both DNA polymerases used in the 3' RACE PCR, the initial denaturation duration was increased to 3 min instead of the recommended 30 s to 2 min in order to fully denature the cDNA template. The T7 oligo dT_{25} primer for example is predicted to potentially form weak secondary structures, which may reduce the availability of primers[21]. Increasing the initial denaturation length and temperature breaks any secondary structures and helps yield the single specific band after the final cycle of 3' RACE. In addition, the non-specific band was lighter than the expected bands, suggesting that it appeared at higher PCR cycles (up to 35 cycles); limiting the PCR cycles to only 20 reduces the amplification of the non-specific band.

Another potential of 3' RACE, like other PCR based reactions, is incomplete amplification of the target region[22]. Hence, for both thermophilic enzymes the initial annealing temperature was set at 50 °C for 5 min to optimize annealing of the primers to the target and this was followed by an initial extension temperature at 72 °C for 10 min. The final PCR cycle for each enzyme had a final extension time of 15 min at 72 °C. These extension steps were longer than those recommended by the manufacturers of the DNA polymerases. The long extension (elongation) step allows complete synthesis of incomplete amplicons, enabling full extension of the initial and final amplification products[16].

PCR steps that occur in 3' RACE are highly sensitive, so they may detect genomic DNA (or other DNA contamination) instead of the target mRNA transcript, resulting in several unexpected bands on the gel[22]. One way to prevent contamination is to perform the PCR work in a dedicated PCR hood, wear gloves, and use clean reagents and autoclaved sterile tubes and pipette tips. In regular PCR, forward and reverse primers can be designed so they are located on different exons (across intron primers); in this way, an abnormally large PCR product would signify that a region containing an intron has been amplified. However, this may not always be feasible in the event that only the 3' UTR sequence, located within the same terminal exon, is the target for 3' RACE amplification. Alternatively, the RNA can be pre-treated with DNAse enzyme before cDNA synthesis. The use of the T7 oligo dT_{25} primer to prime the reverse transcriptase reaction instead of a random hexanucleotide for first strand cDNA synthesis from RNA also decreases amplification of the genomic products. Last but not least, setting up a negative control (the "–" lanes in **Figure 2**), where cDNA synthesis is set up in absence of reverse transcriptase is highly recommended. The control undergoes identical steps to the other samples in the subsequent 3' RACE procedures. The presence of a band in this control signifies potential genomic DNA contamination.

Despite the aforementioned challenges, 3' RACE is a powerful tool in mapping 3' ends on an individual gene basis. The advent of next-generation technology has led to an increase in the repertoire of transcripts that have alternative 3' ends resulting from alternative polyadenylation, which may or may not involve alternative splicing. To add to the complexity, in cancer cells, chromosomal rearrangements are frequent and can result in oncogenic gene fusion products[23]. The fusion between the two genes may involve the ORF or, in some cases, involve only sequences within the 3' UTR[9,10]. Furthermore, there are also conjoined/co-transcribed genes, which are transcribed simultaneously but may be translated into different proteins[11]. 3' RACE can be tailored to map all these unique, abnormal transcripts as well as normal transcripts. This is important because these abnormal transcripts may end up serving as disease specific biomarkers or specific drug targets, *e.g.*, the drug Imatinib targets the *BCR-ABL1* fusion gene in cancer.

Hence, 3' RACE is a highly versatile technique which can be used to amplify normal transcripts, identify novel 3' UTRs, and novel gene fusions within the 3' UTR[5,9]. In this report, 3' RACE was used to amplify and map the stop codon, the PAS, and the entire 3' UTR of the *ANKHD1* transcript using different DNA polymerases. The described approach can be used to map the 3' end of any polyadenylated transcript.

Disclosures

The authors have nothing to disclose.

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