RNA Editing Detection by Direct Sequencing

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[Abstract] RNA editing is a widespread post-transcriptional phenomenon through which primary RNA sequences are altered by nucleotide insertion/deletion or base conversion. It occurs in a variety of organisms and cooperates with alternative splicing in increasing both proteomic and transcriptomic complexity. We describe here a method allowing RNA editing events detection by performing direct sequencing of both genomic DNA and cDNA from the same source.

Materials and Reagents

1. DNeasy Plant mini kit (QIAGEN, catalog number: 69104)
2. RNeasy mini kit (QIAGEN, catalog number: 74104)
3. pJET PCR cloning system (Thermo Fisher Scientific, Fermentas, catalog number: K1231)
4. Recombinant DNase I (Life Technologies, Ambion®, catalog number: AM2235)
5. M-MLV reverse transcriptase (Promega corporation, catalog number: 28025-013)
6. Oligo(dT) 15 Primer (500 mg) (Promega corporation, catalog number: C1101)
7. KOD high fidelity polymerase (TOYOBO, catalog number: KOD-201 200U)
8. Zymoclean Gel DNA Recovery Kit (ZYMO RESEARC, catalog number: D4001)

Equipment

1. NanoDrop (Thermo Fisher Scientific)

Procedure

1. DNA and total RNA were extracted with respectively DNeasy Plant mini kit and RNeasy mini kit, according to the manufacturer's instructions.

2. Each RNA sample was DNase treated (Recombinant DNase I) and quantified with a Nanodrop.
3. cDNAs were generated by M-MLV reverse transcriptase using oligo (dT) according to the manufacturer's instructions.
4. The cDNA produced was diluted with four parts of sterile deionized water.
5. PCR amplification of the target (containing the putative editing region) was performed using KOD high fidelity polymerase and target specific oligonucleotide primers (it's better to design primers that spans introns to prevent the amplification of residual contaminant genomic DNA).
6. PCR product was purified using a Zymoclean Gel DNA Recovery Kit and cloned into a pJET vector using pJET cloning kit.
7. Direct sequencing was performed on plasmid containing the target cloned in pJET vector. For each sample, 2 independent RT-PCR reactions were performed.

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