Supporting Information

Ultrasensitive electrochemical detection of *Dicer1* 3'UTR for the fast analysis of alternative cleavage and polyadenylation

Hui Zhao[1,#], FengLiu[1,#], Shilian Wu[2], Long Yang[2,*], Ya-Ping Zhang [1,3,*], Can-Peng Li[2,*]

[1]Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming 650091, PR China.

[2]School of Chemical Science and Technology, Yunnan University, Kunming 650091, PR China.

[3]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, PR China.

[#]These authors contributed equally to this work.

[*]To whom should be correspondence. Fax or Tel: 86-871-65031119.

E-mail: long133109070@126.com (L. Yang); zhangyp@mail.kiz.ac.cn (Y.-P. Zhang); lcppp1974@sina.com (C.-P. Li).

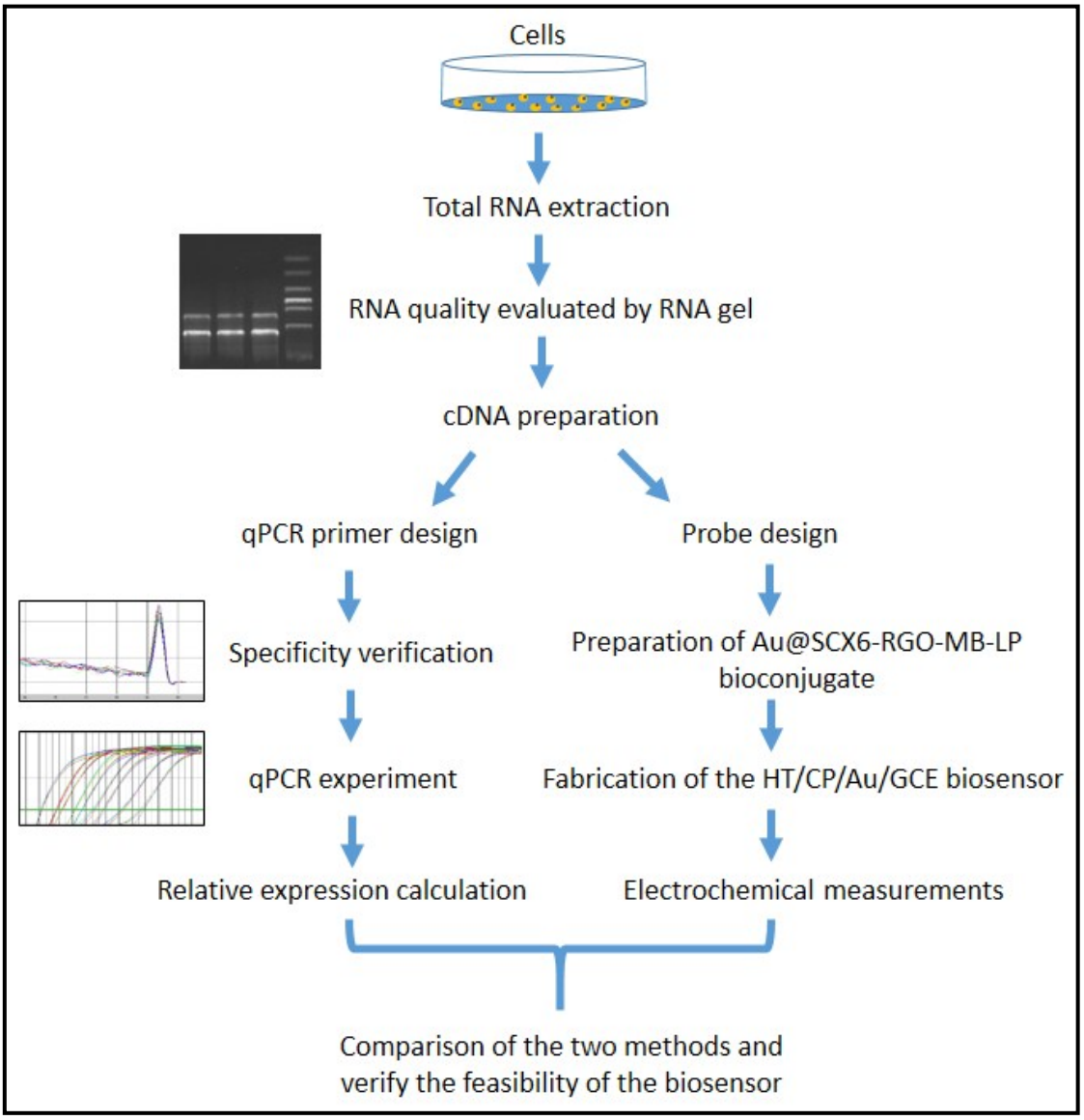

Figure S1. Schematic diagram of APA detections by electrochemical biosensor and by RT-qPCR method.

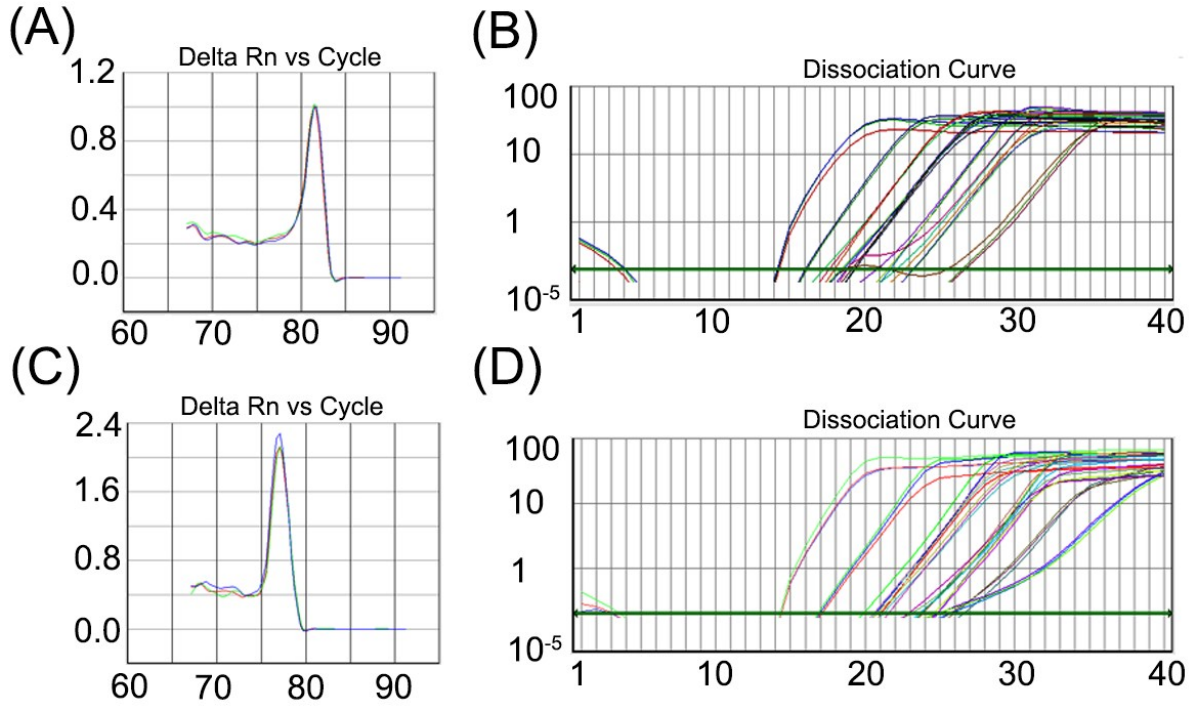

Figure S2. The detection of *Dicer1*-S and *Dicer1*-L using RT-qPCR method.

In this study, the tradition RT-qPCR method was performed to obtain the relative expression levels of *Dicer1*-S and *Dicer1*-L. The specificity of the two primer pairs to amplify *Dicer1*-S and *Dicer1*-L was confirmed by a single peak in the melting curve (A and B). The relative expression levels of *Dicer1*-S and *Dicer1*-L were presented in **Fig. S2** using RT-qPCR analysis. (A) and (B) show the melting peaks of the primer pairs to amplify *Dicer1*-S and *Dicer1*-L, respectively. (C) and (D) indicate amplification curve of RT-qPCR for *Dicer1*-S and *Dicer1*-L detection, respectively.

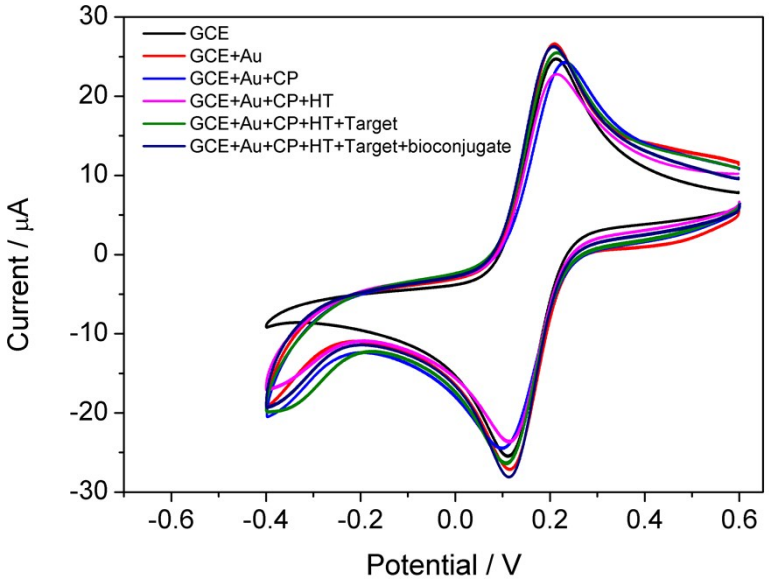

Figure S3. The CV characterizations of different modified electrode.

Figure S3 shows the CV characterizations upon the stepwise modification process. After electrode position of Au NPs, it was observed that the current of Au modified electrode increased compared with the bare GCE, indicating that the high conductivity of Au NPs can facilitate electron transfer to magnify the response. However, upon immobilization of CP-S on the modified electrode, the current decreased obviously, which suggested that the CP-S was successfully immobilized on the surface and formed a barrier. Non-conductive HT as the blocking agent made the current decreased again. Then, the incubation of *Dicer1*-S (10^{-10} M) led to further decrease of the current, attributing to the formation of CP-S-*Dicer1*-S complex on electrode surface. At last, when the Au@SCX6-RGO-MB-LP-S bioconjugate and AP-S hybridized with *Dicer1*-S (curve g), the current increased again, indicating that the synthesized Au@SCX6-RGO-MB-LP-S bioconjugate possess high conductivity and good electron transfer efficiency, although the nucleotide sequence adsorption layer acted as barrier to the interfacial electron transfer.

Table S1. The sequences of primers and probes used in this study.

For *Dicer1*-S 3'-UTR detection	**5' → 3'**
Short target DNA	ACACCTTTAAATTTCCCCTTTCCTACTACTTCCACAGT
Capture probe (CP-S)	AAGGGGAAATTTAAAGGTGT-$(CH2)_6$-SH
Labeled signal probe (SP-S)	TACTCCCCCAGGTGCACTGTGGAAGTAGTAGG-$(CH2)_6$-SH
Auxiliary probe (AP-S)	GCACCTGGGGGAGTATCCTACTACTTCCACAGTTGT
Single-base mismatch target (1MT-S)	ACACCTTTAAATGTCCCCTTTCCTACTACTTCCACAGT
Two-base mismatch target (2MT-S)	ACACCTTTAAATGGCCCCTTTCCTACTACTTCCACAGT
For *Dicer1*-L 3'-UTR detection	**5' → 3'**
Long target DNA	TTGCTATGTAACCTGCTGCTGCAGTGAATTCTTAGTGC
Capture probe (CP-L)	AGCAGCAGGTTACATAGCAA-$(CH2)_6$-SH
Labeled signal probe (SP-L)	TACTCCCCCAGGTGCGCACTAAGAATTCACTGC-$(CH2)_6$-SH
Auxiliary probe (AP-L)	GCACCTGGGGGAGTAGCAGTGAATTCTTAGTGC
Two-base mismatch target (2MT-L)	TTGCTAGATAACCTGCTGCTGCAGTGAATTCTTAGTGC
Non-complementary sequence (NC)	CCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGC
qPCR primers	**5' → 3'**
Dicer1_S_F	ACTGTGGAAGTAGTAGGAAAGGGGA
Dicer1_S_R	GGTTGATTAGCTTTGAGGCTTC
Dicer1_L_F	TTGTATTTTCCACTAGCAGTGAAA
Dicer1_L_R	TTGCTATGTAACCTGCTGCTGC