

Supplementary data

Ultrasensitive detection of mRNA extracted from cancerous cells achieved by DNA rotaxane-based cross rolling circle amplification

Sai Bi,^{*a,b} Yangyang Cui^b and Li Li^b

^aSchool of Chemistry and Chemical Engineering, Linyi University, Linyi 276005, P.R.China. Fax: 86 539 8766600; Tel: 86 539 8766600; E-mail: bisai11@126.com.

^bKey Laboratory of Biochemical Analysis, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P.R.China.

Table S1. Oligonucleotides used in the experiments^a

No.	Description	Sequences(5'-3')
1-1	cDNA specific for <i>ACTB</i>	CGGATCGGCAAAGGCGAGGCTCTGTGCTCGCGGGGCGGACGCGGTCTC GGCGGT
1-2	cDNA specific for <i>TERT</i>	CGTCCCAGGGCACGCACACCAGGCACTGGGCCACCAGCGCGCGGAAAG CCGCCG
1-3	cDNA specific for <i>MYC</i>	CTGCTATGGGCAAAGTTTCGTGGATGCGGCAAGGGTTGCGGACCGCTG GCTGGG
1-4	cDNA specific for <i>ERBB2</i>	TGCAGGAAGGACAGGCTGGCATTGGTGGGCAGGTAGGTGAGTTCCAGG TTTCCC
2	biotinylated primer-I	biotin-AGCACAGAGCCTCGCC
3	linear DNA rod-I	CAGGGATTTTTATCCCTGCGCGTCCGCCCGGAGCACAGAGCCTCGCC TTTGCCGATCCGGAATAATTTTTAGTTCTTT
4	circle template-I	TGTGCTCGGTTTCTCCTCATCTTTGACTTCCCCTTCCCAACCCGCCCTA CCCCTCCTCATCTTTGACTTCCCCTTTTTGGCGAGGCTC
5	release-DNA-I	AAGGGGAAGTCAAAGATGAGGAGTTT
6	biotinylated primer-II	biotin-CATCTTTGACTTCC
7	linear DNA rod-II	CAGGGATTTTTATCCCTGCTCCTCATCTTTGACTTCCCCTTGAATAATT TTTTAGTTCTTT
8	circle template-II	AAAGATGTTCCGCGAGCACAGAGCCTCGCCTTTGCCCAACCCGCCCT AACCCTCCGCGAGCACAGAGCCTCGCCTTTGCCCTTGGAAGTC
9	release-DNA-II	GGCAAAGGCGAGGCTCTGTGCTCGCGGTTT
10	DNA primer	ATCATCCATGGTGAAGTGGCGGCGG
^a Font colors correspond to the line colors in the schemes.		

Nondenaturing PAGE Results of RT-PCR. PCR products of standard cDNA samples and RT-PCR products of *ACTB* extracted from MCF-7 cells were detected by nondenaturing PAGE (15%) (Fig. S1), whose data were analyzed by VisionWorks[®]LS Image Acquisition and Analysis software (Ultra-Violet Products Ltd., Cambridge, UK). The calibration curve is shown in Fig. S2.

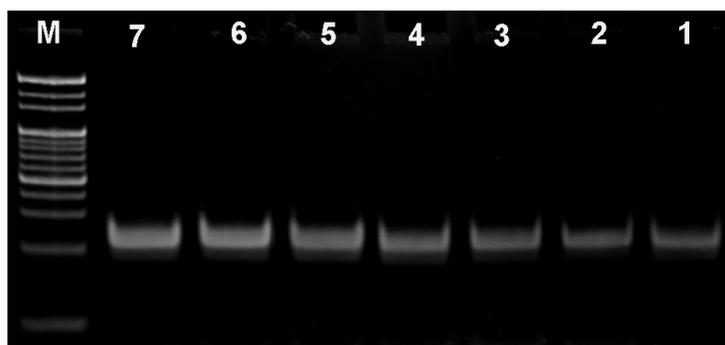


Fig. S1. The sensitivity of PCR for detection of cDNA that is specific for *ACTB*. M: Marker; lanes 1~7: differently diluted cDNA template (10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} copies per reaction).

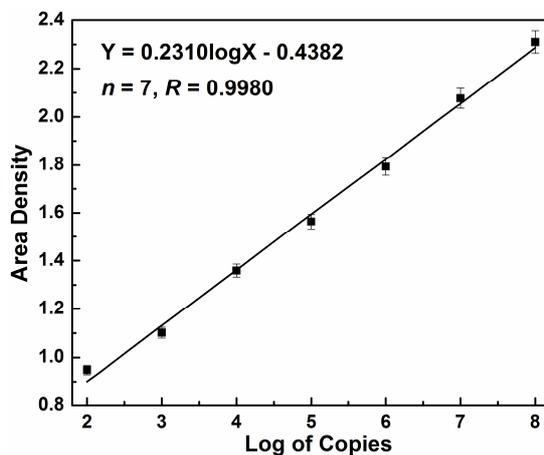


Fig. S2. Standard curve of the PCR products of cDNA. Synthetic cDNA input ranges from 10^2 copies per reaction to 10^8 copies per reaction in PCR.

Table S2. Comparison of the Proposed C-RCA-Based Method with the RT-PCR Method for the

Detection of *ACTB* in MCF-7 Cells

No ^[a]	The proposed C-RCA method (copies per cell)	RSD (%)	RT-PCR method (copies per cell)	RSD (%)
1	117	3.1	138	1.8
2	100	2.3	129	1.6
3	128	3.5	144	1.2

^[a] Each sample is analyzed in triplicate, and the results are the average values.