Supporting Information

Sensitively Distinguish Intracellular Precursor and Mature MicroRNA Abundance

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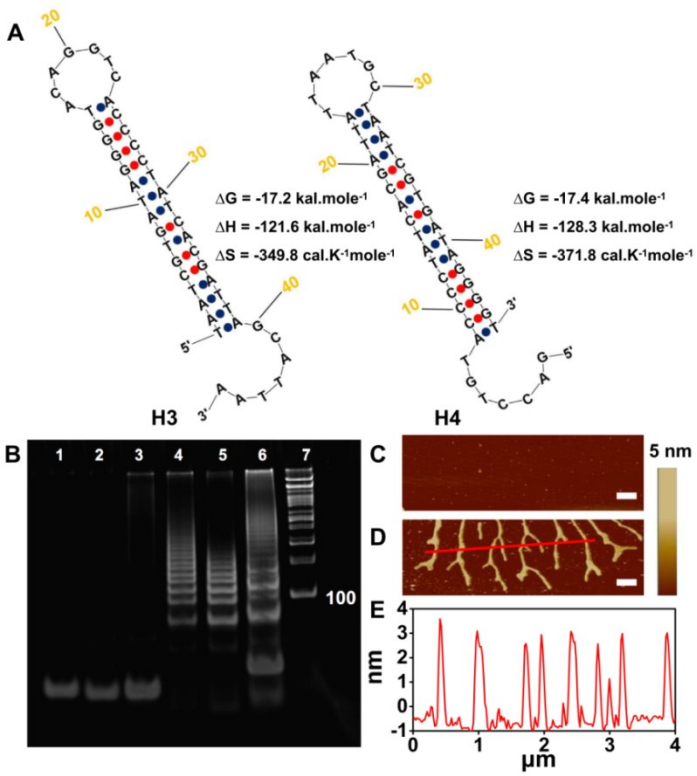

Fig. S1. (A) Ideal structure and thermal kinetic parameters of H3, H4. (B) PAGE analysis of HCR. Lanes 1−7 represent H3 (2 μM), H4 (2 μM), H3+H4 (2 μM and 2 μM), H1+H2+miRNA-155 (2 μM, 2 μM and 2 μM), H1+H2+2×miRNA-155 (2 μM, 2 μM and 4 μM), H3+H4+pre-miRNA-155 (2 μM, 2 μM and 6 μM), and DNA ladder marker, respectively. AFM phase images of HCR (C) without and (D) with miRNA-155. Scale bar: 500 nm. (E) Cross-section profile of the black line in (D).

Fig. S2. PAGE analysis. Lanes 1−7 represent H1+H2+H3+H4, H3+H4, H1+H2, H4, H3, H2, H1, respectively, each hairpin probe: 2 μM.

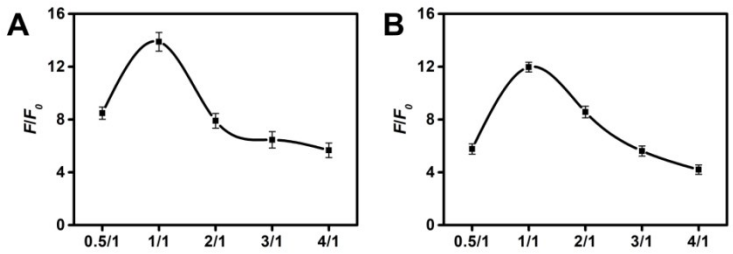

Fig. S3. Optimization of the concentration of H1 and H4, respectively. (A) The reaction containing different concentrations of H1 (50, 100, 200, 300, 400 and 500 nM) and H2 (100 nM). *F* and F_0 are the fluorescence intensities at (C) 523 nm in the presence and absence of pre-miRNA-155 (100 nM). (B) The reaction containing different concentrations of H3 (50, 100, 200, 300, 400 and 500 nM) and H4 (100 nM). F and F0 are the fluorescence intensities at (C) 565 nm in the presence and absence of miRNA-155 (100 nM). The data error bars indicate means ± SD (n=3).

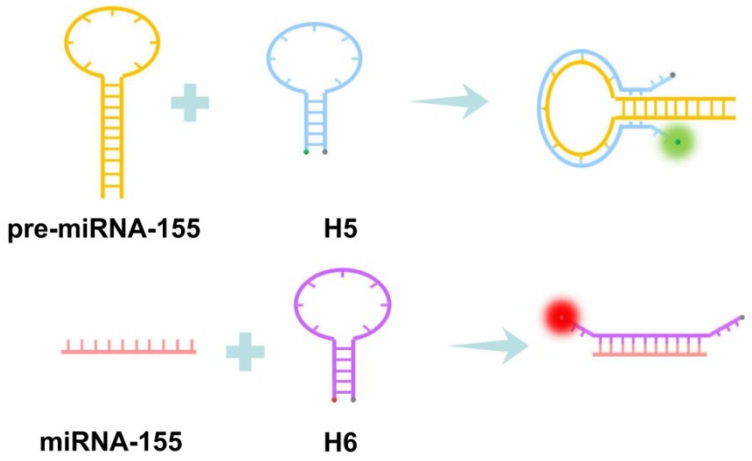

Fig. S4. Schematic illustration of pre-miRNA-155 and miRNA-155 hybridization to MB.

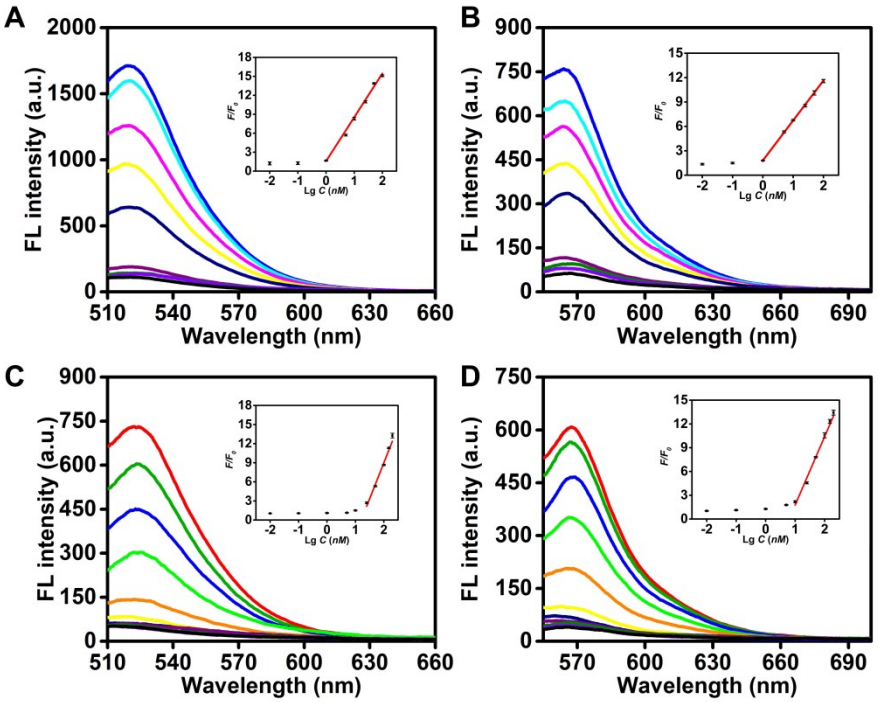

Fig. S5. Fluorescence intensity corresponding to HCR (each hairpin probe: 100 nM) response to different concentrations of (A) pre-miRNA-155 and (B) miRNA-155. Fluorescence intensity corresponding to MB (100 nM) response to different concentrations of (C) pre-miRNA-155 and (D) miRNA-155. Insert: linear relationship between the F/F_0 and the logarithm of target concentration.

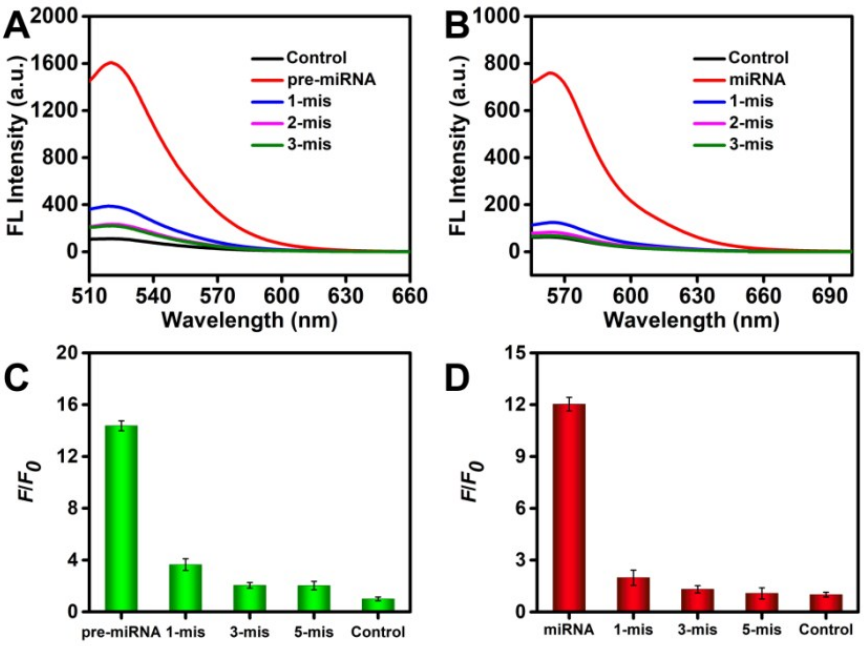

Fig. S6. (A) Fluorescence emission spectra of mixture H1 (100 nM) and H2 (100 nM) after incubation with (a) complementary target pre-miRNA-155 (CM) (100 nM), (b) single-base-mismatched strand (mis-1) (100 nM), three-base-mismatched strand (mis-3) (100 nM), five-base-mismatched strand (mis-5) (100 nM) and control; (B) Fluorescence emission spectra of mixture H3 (100 nM) and H4 (100 nM) after incubation with (a) complementary target miRNA-155 (CM) (100 nM), (b) single-base-mismatched strand (mis-1) (100 nM), three-base-mismatched strand (mis-3) (100 nM), five-base-mismatched strand (mis-5) (100 nM) and control; (C) and (D) fluorescence intensity ratio F/F_0 for (A) and (B), respectively.

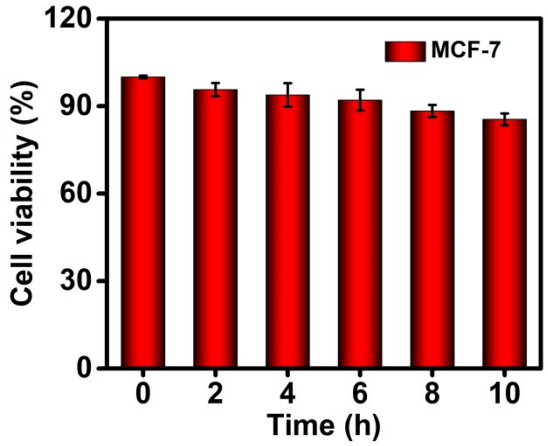

Fig. S7. Cell viability assay: MCF-7 cells treated with the H1, H2, H3 and H4 (100 nM) for 2h, 4h, 6h, 8h and 10 h at 37 ^{o}C.

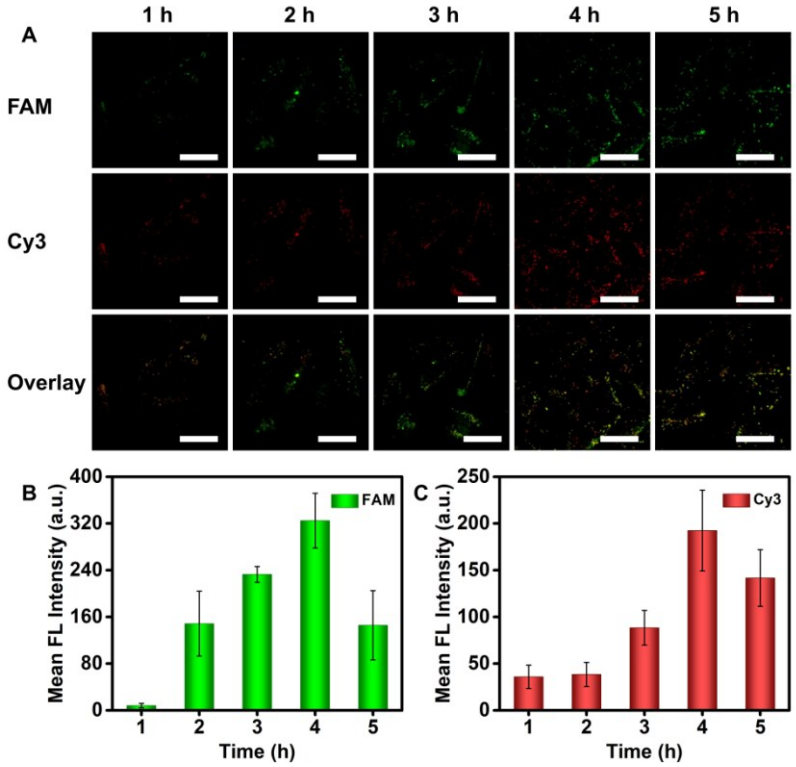

Fig. S8. (A) Time course of confocal images of A549 cells incubated with H1 (100 nM), H2 (100 nM), H3 (100 nM) and H4 (100 nM). The scale bar was 40 μm. Histogram of (B) corresponding FAM fluorescence intensity related to pre-miRNA-155 and (C) Cy3 fluorescence intensity associated with miRNA-155 of A549 cells in (A).

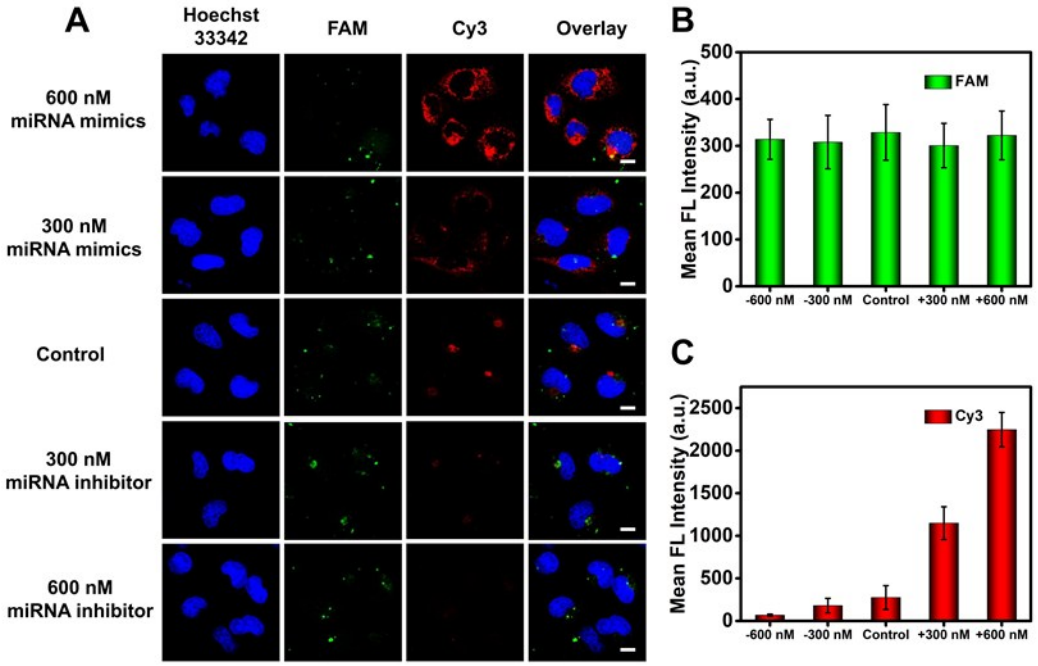

Fig. S9. (A) CLSM imaging for MCF-7 cells and A549 cells treated with miRNA-155 mimics (600 nM and 300 nM) and inhibitors (600 nM and 300 nM). The scale bar indicates 40 μm. Histogram of (B) corresponding FAM fluorescence intensity related to pre-miRNA-155 and (C) Cy3 fluorescence intensity associated with miRNA-155 of A549 cells in (A).

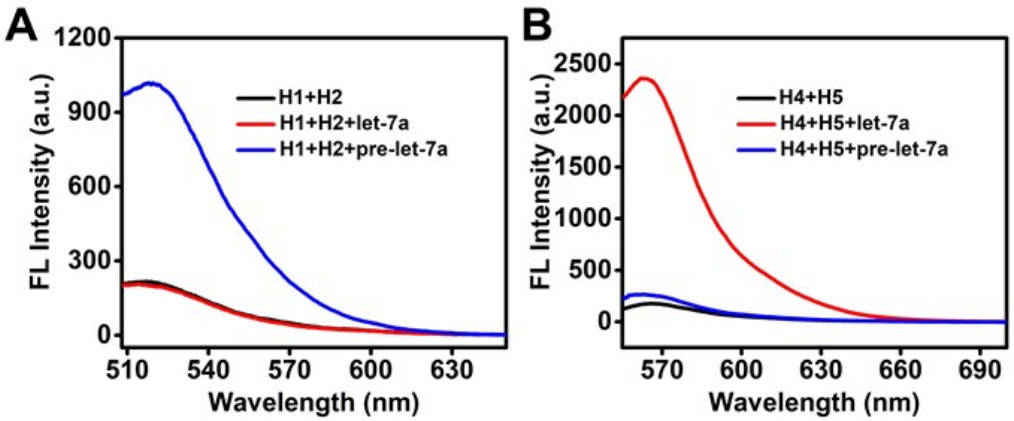

Fig. S10. The feasibility of HCR system for mature miRNA let-7a and its precursor. The fluorescence intensity of (A) H1 and H2, (B) H3 and H4 response to response to miRNA let-7a and pre-miRNA let-7a.

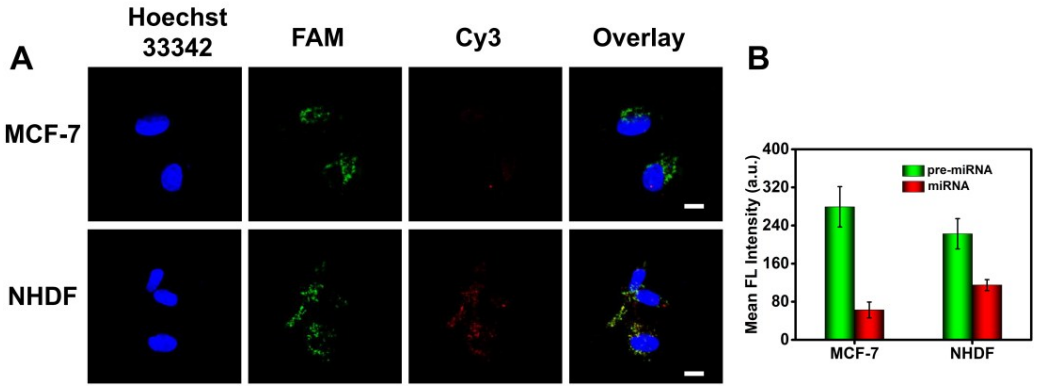

Fig.S11. (A) CLSM imaging for MCF-7 cells and NHDF cells incubated with HCR (100 nM). The scale bar indicates 40 μm. Histogram of (B) corresponding FAM fluorescence intensity related to pre-miRNA let-7a and (C) Cy3 fluorescence intensity associated with miRNA let-7a in (A).

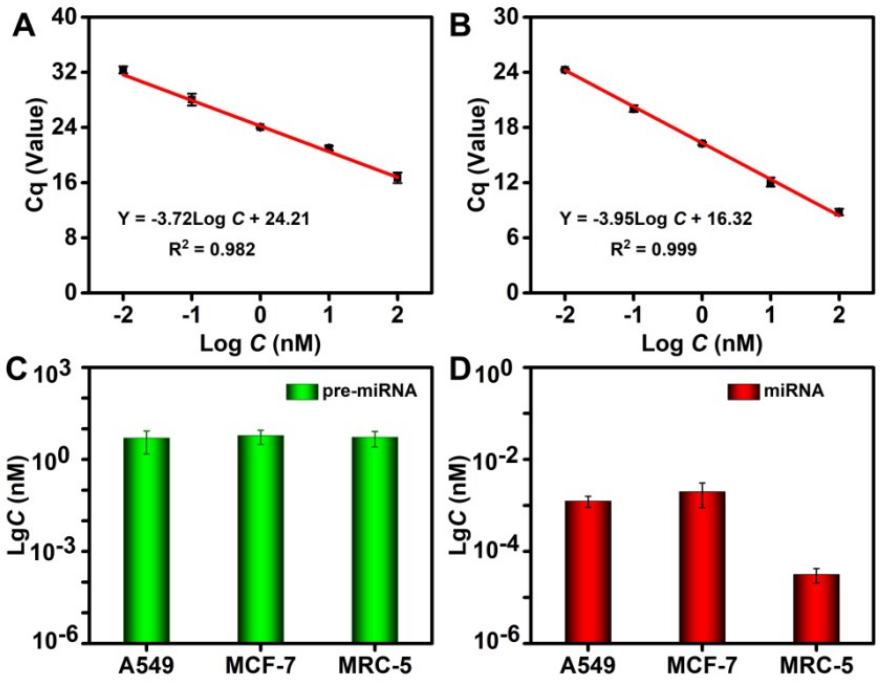

Fig. S12. Generation of calibration curve for (A) pre-miRNA-155 and (B) miRNA-155 quantification by qRT-PCR. The qRT-PCR analysis of (C) pre-miRNA-155 and (D) miRNA-155 in A549, MCF-7 and MRC-5 cells.

Table S1. DNA and RNA sequences used for miRNA-155 detection.

Name	Oligonucleotide sequences (5'—3')
H1	TCCAACTGACTCCTAATGTCGATAGGAGTCAGTTGGAGG CAAAA
H2	TCGACATTAGGAGT-FAMCAGTTGGATTTTGCCTCCAACT-BHQ1-GACTCCTA
H3	TAATCGTGATAGGGGTACAGGTCACCCCTATCACGATTAG CATTAA
H4	GACCTGTACCCCTAT-Cy3-CACGATTATTAATGCTAATCGT-BHQ2-GATAGGGGT
H5	FAM-ATCGGGTCAGTTGGAGGCAAAAACCCGAT-BHQ1
H6	Cy3-CCGGGTACCCCTATCACGATTAGCATTAAACCCGG-BHQ2
T1	CTGTTAATGCTAATCGTGATAGGGGTTTTTGCCTCCAACT GACTCCTACATATTAGCATTAACAG
T1mis-1	CTGTTAATGCTAATCGTGATAGGGGTTTTCGCCTCCAACT GACTCCTACATATTAGCATTAACAG
T1mis-3	CTGTTAATGCTAATCGTGATAGGGGTTGTCGACTCCAACT GACTCCTACATATTAGCATTAACAG
T1mis-5	CTGTTAATGCTAATCGTGATAGGGGTGGTCAACTCCAACT GACTCCTACATATTAGCATTAACAG
T2	TTAATGCTAATCGTGATAGGGGT
T2mis-1	TTACTGCTAATCGTGATAGGGGT
T2mis-3	TCACTGGTAATCGTGATAGGGGT
T2mis-5	TCGCTAGTAATCGTGATAGGGGT
pre-miRNA-155	CUGUUAAUGCUAAUCGUGAUAGGGGUUUUUGCCUCCA ACUGACUCCUACAUAUUAGCAUUAACAG
miRNA-155	UUAAUGCUAAUCGUGAUAGGGGU

The hairpin structures for H1, H2, H3 and H4 are labelled in the same color as it illustrated in the Figure 1. T1, T1mis-1, T1mis-3, T1mis-5 are the DNA analog of the pre-miRNA-155, single-base-mismatched strand, three-base-mismatched strand, and five-base-mismatched strand, respectively. T2, T2mis-1, T2mis-3, T2mis-5 are the DNA analog of the miRNA-155, single-base-mismatched strand, three-base-mismatched strand, and five-base-mismatched strand, respectively.

Table S2. DNA sequences used for miRNA let-7a detection.

Name	Oligonucleotide sequences (5'－3')
H1	TGCCCTGCTATGGGATAATGTCGATATCCCATAGCAGGGC AGAGCCCC
H2	TCGACATTATCCCATAGC/i6FAMdT/GGGCAGGGGCTCTGC CCTGC/iBHQ1dT/ATGGGATA
H3	GTAGGTTGTATAGTTACAGGTCAACTATACAACCTACTAC CTCA
H4	GACCTGTAACTATACA/iCy3/ACCTACTGAGGTAGTAGGT/i BHQ2dT/GTATAGTT
Let-7a (DNA)	TGAGGTAGTAGGTTGTATAGTT
Pre-let-7a (DNA)	GGGTGAGGTAGTAGGTTGTATAGTTTGGGGCTCTGCCCT GCTATGGGATAAAACTATACAATCTACTGTCTTTCCT