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Supporting Information

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Supporting Information

for

A New Approach for Reversible RNA Photocrosslinking Reaction: Application to Sequence-Specific RNA Selection

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Experimental Section

General method and materials: Mass spectra were recorded on a Voyager-DE PRO-SF, Applied Biosystems. Irradiation was performed by UV-LED (OMRON, ZUV, 366 nm, 1,600 mW/cm^2) or 15 W transilluminator (FUNAKOSHI, TR-312R/J, 312 nm). HPLC was performed on a Chemcobond 5-ODS-H column (10 $\times$ 150 mm, 4.6 $\times$ 150 mm) or a Chemcobond 5-ODS-H column (4.6 $\times$ 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 260 nm. The reagents for the DNA synthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite, CPG support, and Oligo-Affinity Support (PS) were purchased from Glen Research. Calf intestine alkaline phosphatase (AP) was purchased from Promega. Nuclease P1 was purchased from Yamasa.

Oligodeoxynucleotide synthesis: Oligodeoxynucleotide (ODN) sequences were synthesized by the conventional phosphoramidite method by using an Applied Biosystems 3400 DNA synthesizer. The coupling efficiency was monitored with a trityl monitor. The coupling efficiency of crude mixture of the phosphoramidite was 97% yield. The coupling time of crude mixture of phosphoramidite was 999 s. They were deprotected by incubation with 28% ammonia for 8 h at 55 ˚C and were purified on a Chemcobond 5-ODS-H column (10 $\times$ 150 mm) by reversed-phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% CH_3CN, linear gradient (30 min) at a flow rate of 3.0 mL/min. ODNs were fully digested with calf intestine alkaline phosphatase (50 U mL^{-1}) and P1 nuclease (50 U mL^{-1}) at 37 ℃ for 4 h. The digested samples were analyzed by using HPLC. The concentration of each ODN was determined

by comparing the peak areas with standard solutions that contained dA, dG, dC, and T at a concentration of 0.1 mM. Preparation of ODNs was confirmed by MALDI-TOF-MS analysis.

UV measurement: UV spectra of DNA (3.0 μM) were taken in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride using a Beckman Coulter DU800 UV/VIS spectrophotometer. In T_m measurements of the duplex, sigmoidal curves on the change of A_{260} were obtained, and the T_m value was calculated from the first part of the curve.

Photocrosslinking as monitored by HPLC: After irradiation, the progress of photoreaction was monitored by HPLC on a Chemcobond 5-ODS-H column (4.6 × 150 mm, elution with a gradient of 50 mM $HCOONH_4/CH_3CN$ (97:3 to 70:30 over 30 min) at a flow rate 1.0 mL/min).

Photocrosslinking as monitored by UPLC: UPLC was performed on a Waters Acquity UPLC system (Waters, Milford, MA) using an Acquity UPLC BEH Shield RP18 column (1.7 μm, 2.1 × 50 mm, elution with a gradient of 50 mM $HCOONH_4/CH_3CN$ (98:2 to 85:15 over 3.4 min) at a flow rate 0.6 mL/min). The temperature of the column was maintained at 30 ℃.

Quantum yield measurement: Quantum yields were measured by using a 300 W Xe lamp fitted to a monochromator set to 366 nm. The monochromator was calibrated by the chemical actinometer of valerophenone. The amount of photocrosslinked product was measured by HPLC analysis.

RNA selection as monitored by capillary gel electrophoresis: The experiments were carried out on a Beckman P/ACE System MDQ (Beckman Coulter, Fullerton, CA) equipped with an UV absorbance detector. Separations were performed at an applied voltage of 20 kV and at a temperature of 30 °C. RNAs were detected by monitoring their absorbance at 254 nm.

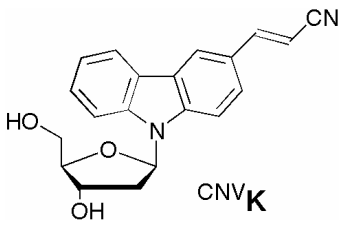
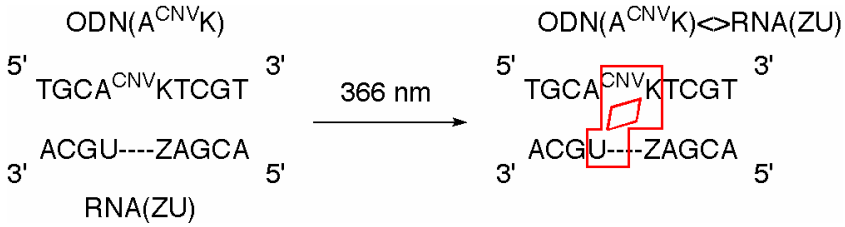


Figure S1. Structure of 3-cyanovinylcarbazole nucleoside (^{CNV}K).



Scheme S1. Photocrosslinking reaction of ODNs with ^{CNV}K.

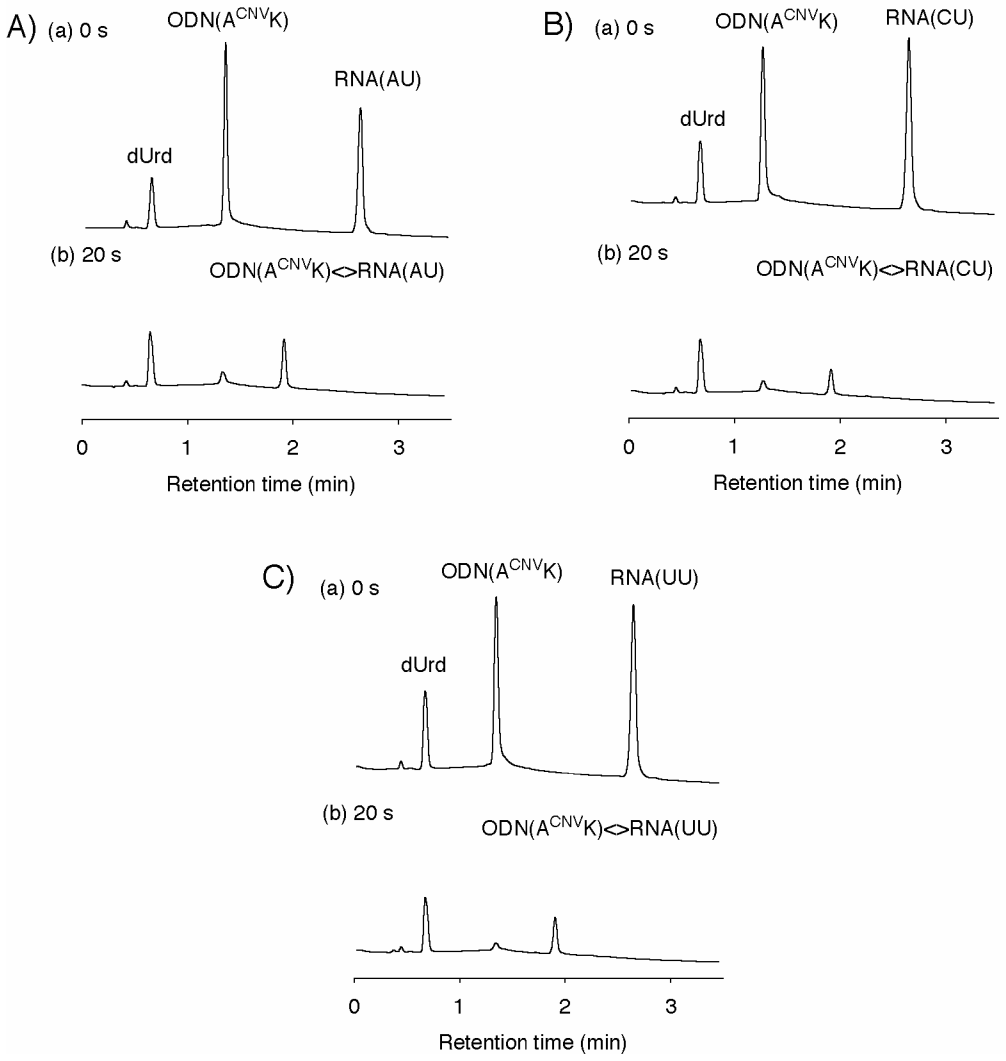
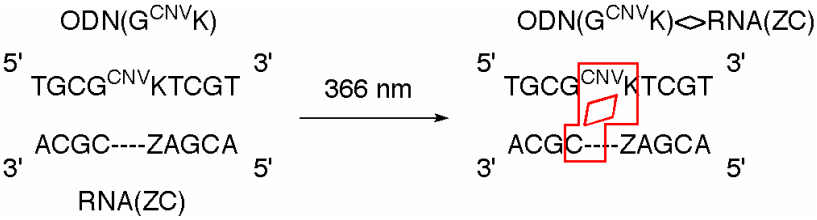


Figure S2. UPLC analysis of the irradiated ODN(A^{CNV}K) in the presence of RNA(ZU): A) base pair between ^{CNV}K and A; B) base pair between ^{CNV}K and C; C) base pair between ^{CNV}K and U. 2'-Deoxyuridine (dUrd) was used as an internal standard.



Scheme S2. Photocrosslinking reaction of ODNs with ^{CNV}K.

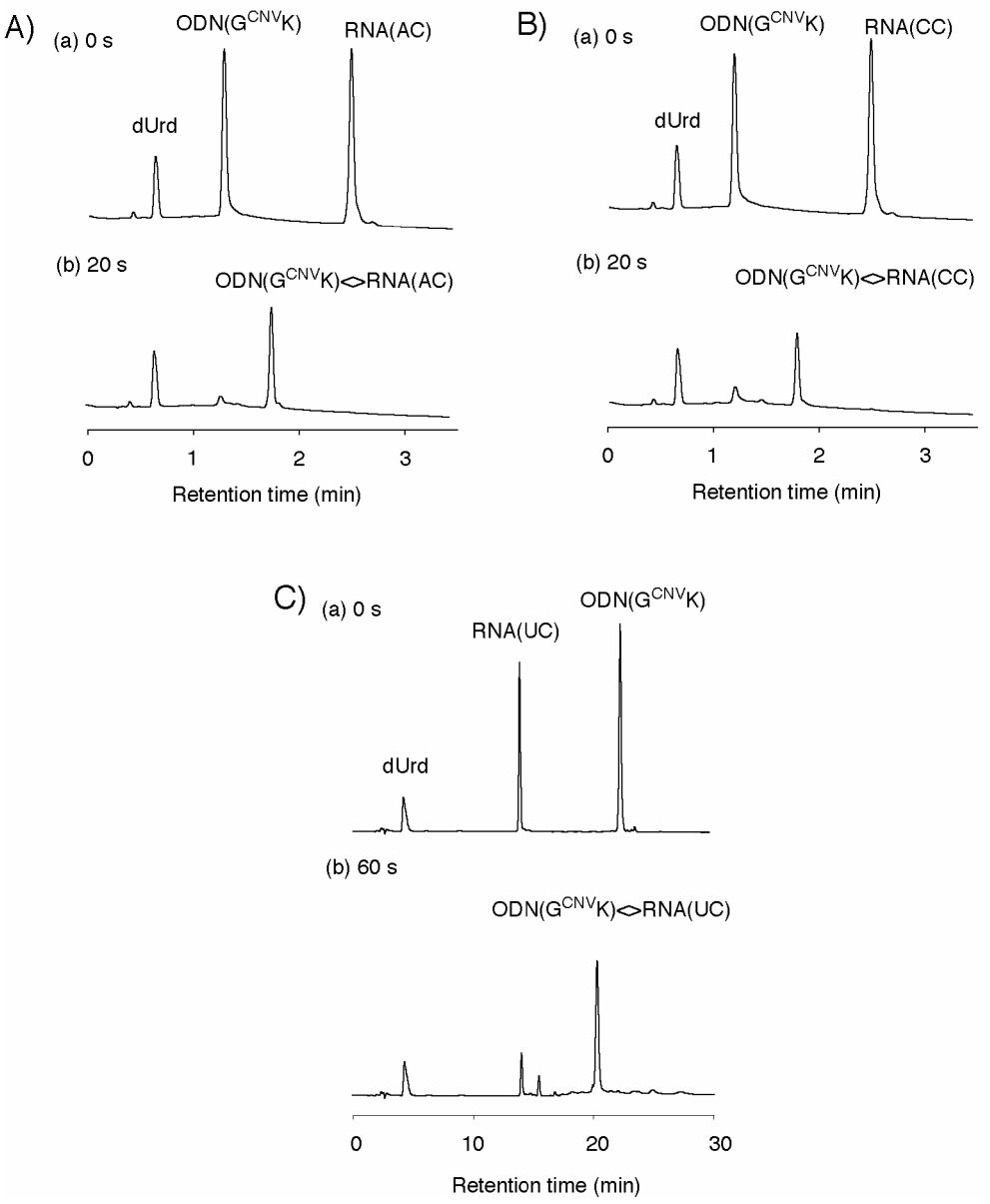


Figure S3. UPLC analysis of the irradiated ODN(G^{CNV}K) in the presence of RNA(ZC): A) base pair between ^{CNV}K and A; B) base pair between ^{CNV}K and C. C) HPLC analysis of the irradiated ODN(G^{CNV}K) in the presence of RNA(UC). 2'-Deoxyuridine (dUrd) was used as an internal standard.

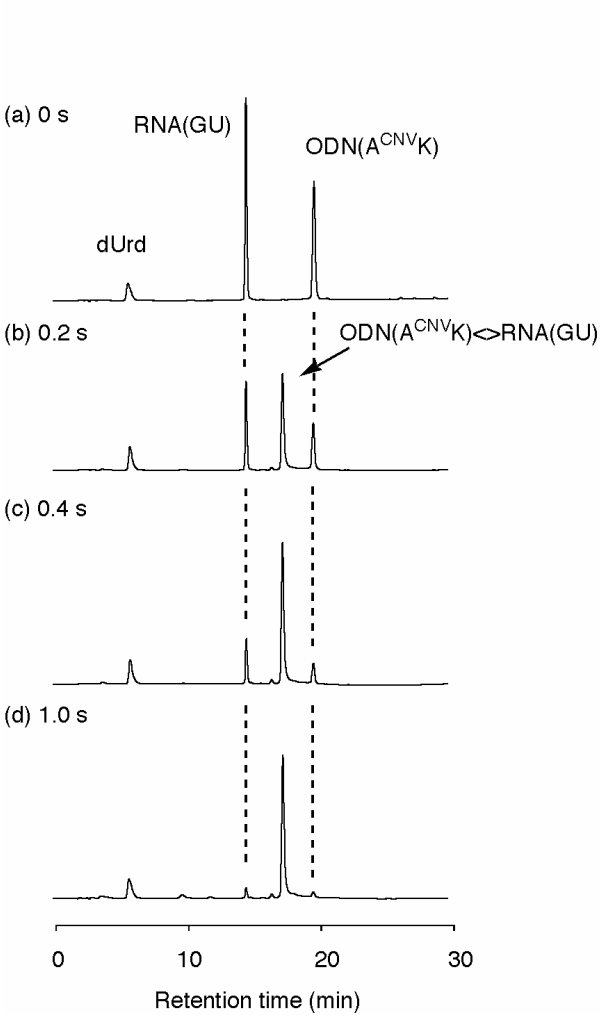


Figure S4. HPLC analysis of the irradiated ODN($A^{CNV}K$) in the presence of RNA(GU). 2'-Deoxyuridine (dUrd) was used as an internal standard.

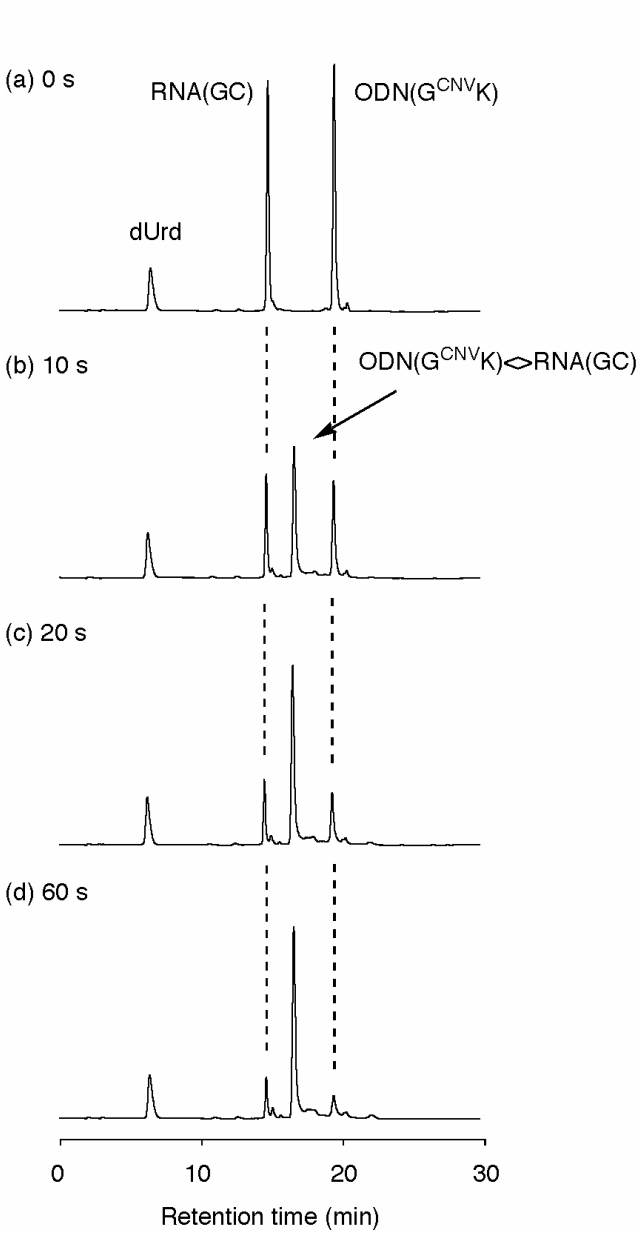


Figure S5. HPLC analysis of the irradiated ODN($G^{CNV}K$) in the presence of RNA(GC). 2'-Deoxyuridine (dUrd) was used as an internal standard.

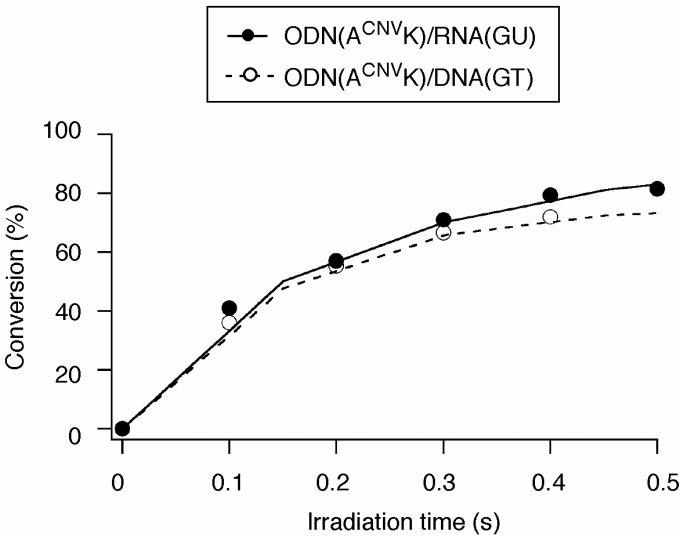


Figure S6. Time course of the photocrosslinking reaction with RNA(GU) (filled symbols) and DNA(GT) (open symbols). DNA(GT) = 5'-d(ACGAGTGCA)-3'.

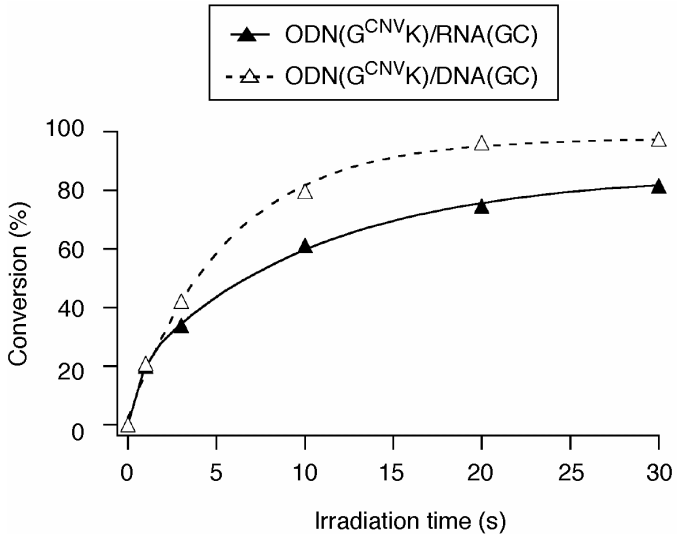


Figure S7. Time course of the photocrosslinking reaction with RNA(GC) (filled symbols) and DNA(GC) (open symbols). DNA(GC) = 5'-d(ACGAGCGCA)-3'.

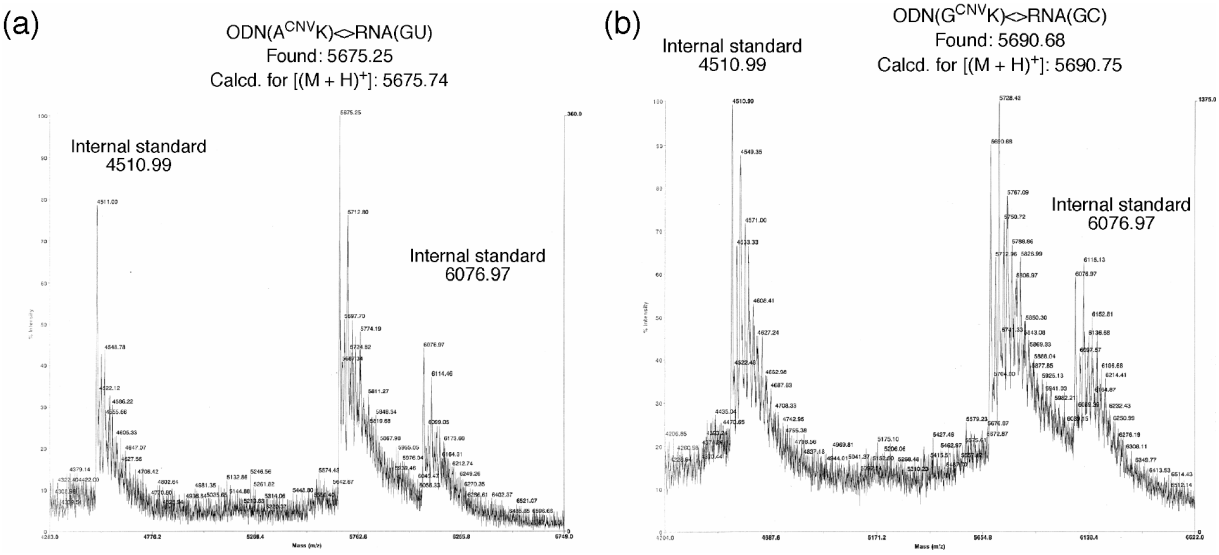


Figure S8. MALDI-TOF-MS analysis using a 3-hydroxypicolinic acid as matrix: (a) ODN(A^{CNV}K)<>RNA(GU); (b) ODN(G^{CNV}K)<>RNA(GC).

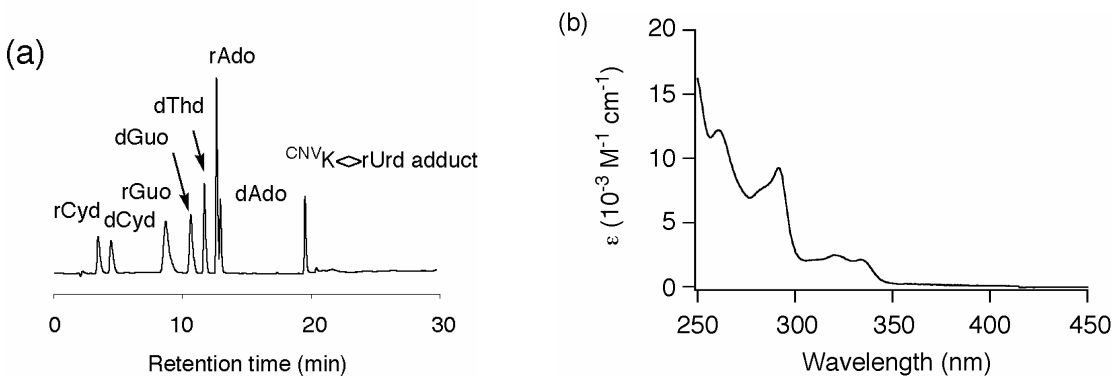


Figure S9. (a) HPLC analysis of products during enzymatic digestion process of ODN(A^{CNV}K)<>RNA(GU), (b) UV spectrum of ^{CNV}K<>rUrd photoadduct.

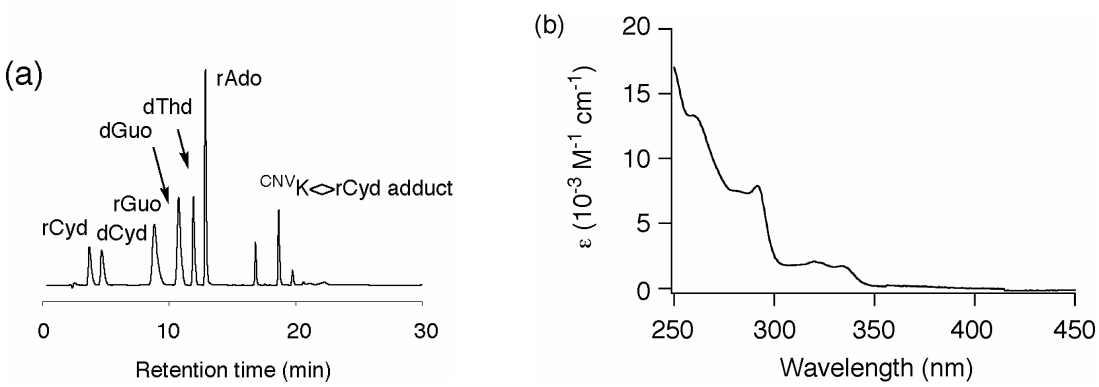
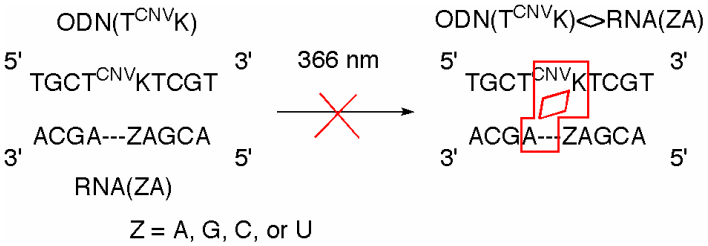


Figure S10. (a) HPLC analysis of products during enzymatic digestion process of ODN(G^{CNV}K)<>RNA(GC), (b) UV spectrum of ^{CNV}K<>rCyd photoadduct.



Scheme S3. Photocrosslinking reaction of ODNs with ^{CNV}K.

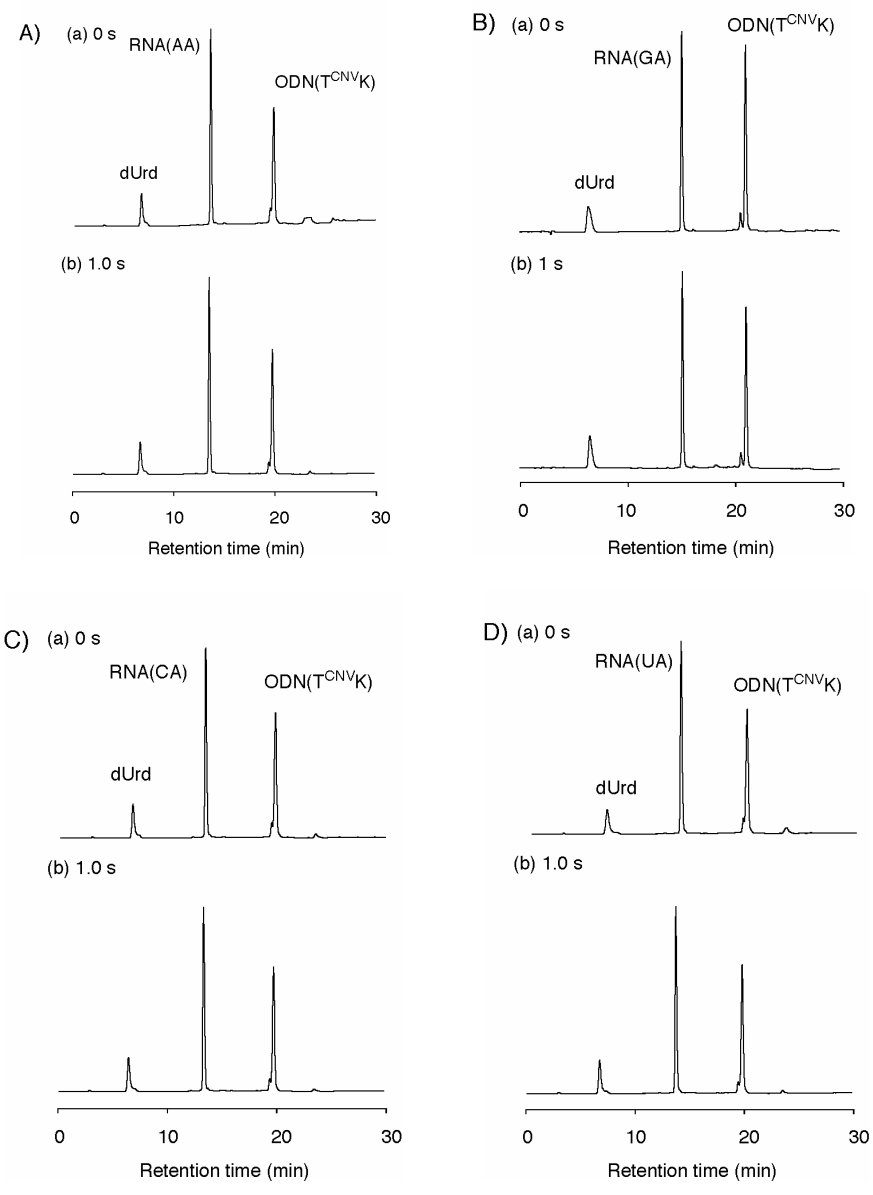
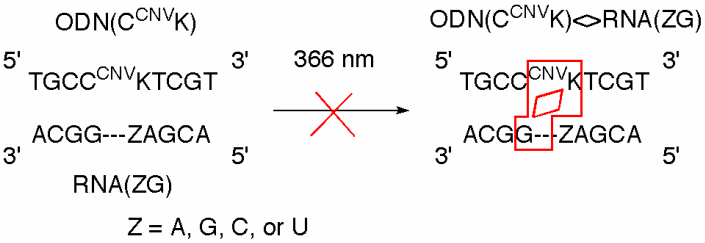


Figure S11. HPLC analysis of the irradiated ODN(T^{CNV}K) in the presence of RNA(ZA): A) the photocrosslinking reaction between ODN(T^{CNV}K) and RNA(AA); B) the photocrosslinking reaction between ODN(T^{CNV}K) and RNA(GA); C) the photocrosslinking reaction between ODN(T^{CNV}K) and RNA(CA); D) the photocrosslinking reaction between ODN(T^{CNV}K) and RNA(UA). 2'-Deoxyuridine (dUrd) was used as an internal standard.



Scheme S4. Photocrosslinking reaction of ODNs with ^{CNV}K.

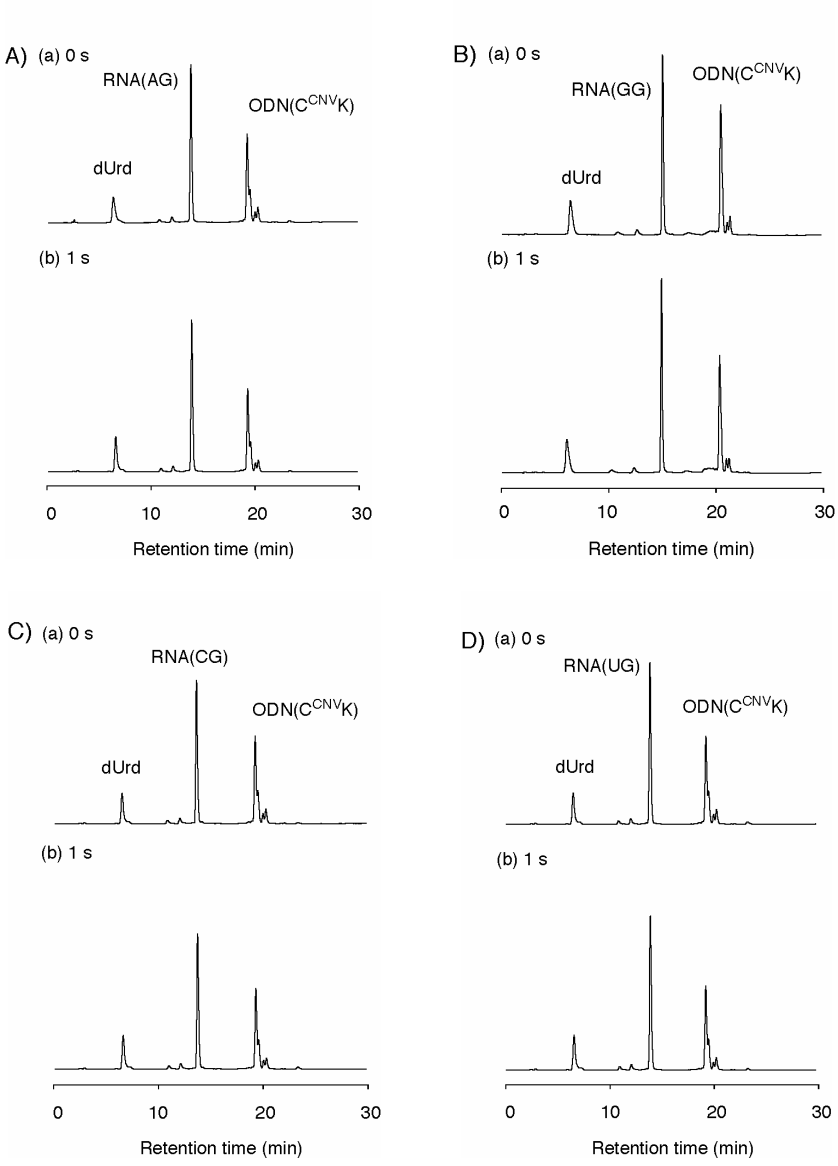


Figure S12. HPLC analysis of the irradiated ODN(C^{CNV}K) in the presence of RNA(ZG): A) the photocrosslinking reaction between ODN(C^{CNV}K) and RNA(AG); B) the photocrosslinking reaction between ODN(C^{CNV}K) and RNA(GG); C) the photocrosslinking reaction between ODN(C^{CNV}K) and RNA(CG); D) the photocrosslinking reaction between ODN(C^{CNV}K) and RNA(UG). 2'-Deoxyuridine (dUrd) was used as an internal standard.

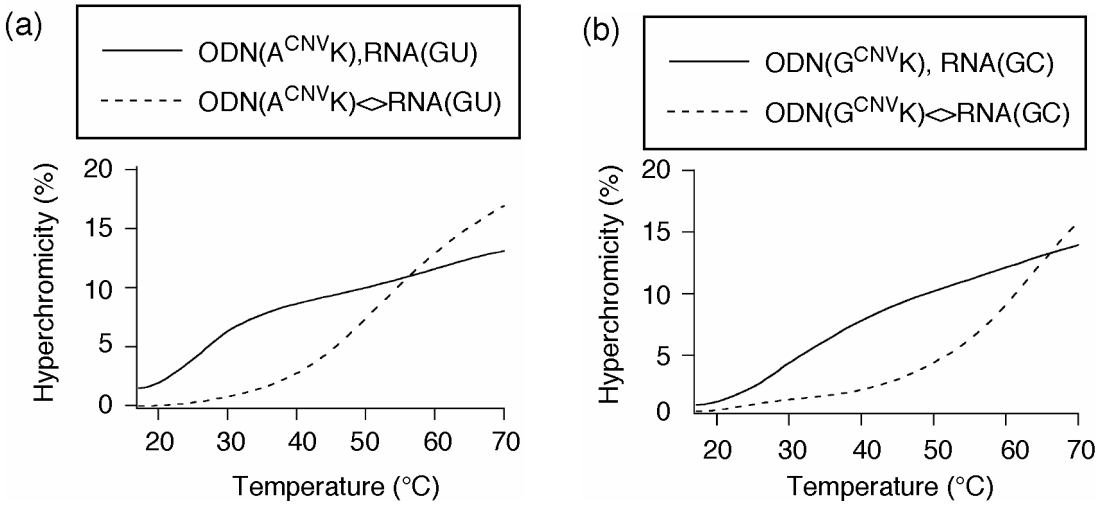


Figure S13. Melting curves: (a) the duplex ODN(A^{CNV}K)/RNA(GU) and the crosslinked ODN(A^{CNV}K)<>RNA(GU); (b) the duplex ODN(G^{CNV}K)/RNA(GC) and the crosslinked ODN(G^{CNV}K)<>RNA(GC).

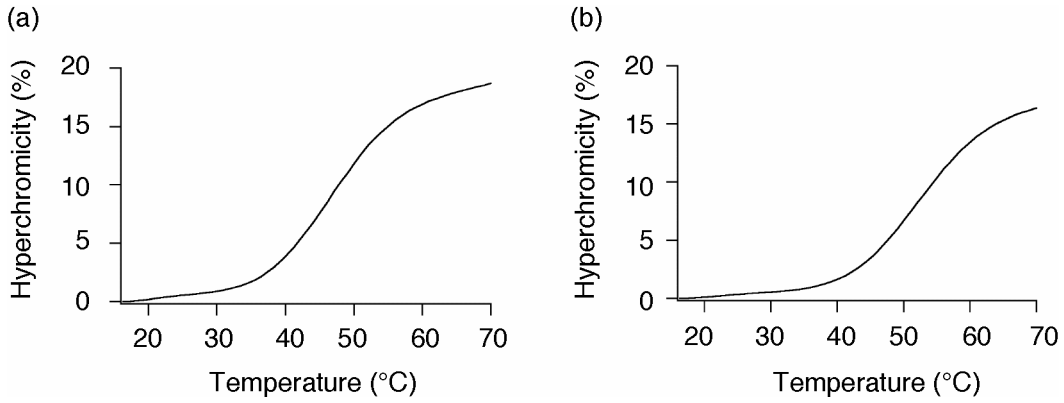
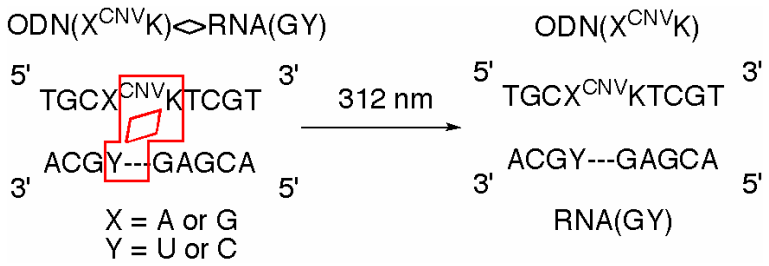


Figure S14. Melting curves: (a) the duplex ODN(AC)/RNA(GU); (b) the duplex ODN(GC)/ RNA(GC). ODN(AC) = 5'-TGCACTCGT-3', ODN(GC) = 5'-TGCGCTCGT-3'.



Scheme S5. Photosplitting reaction of photocrosslinked ODNs.

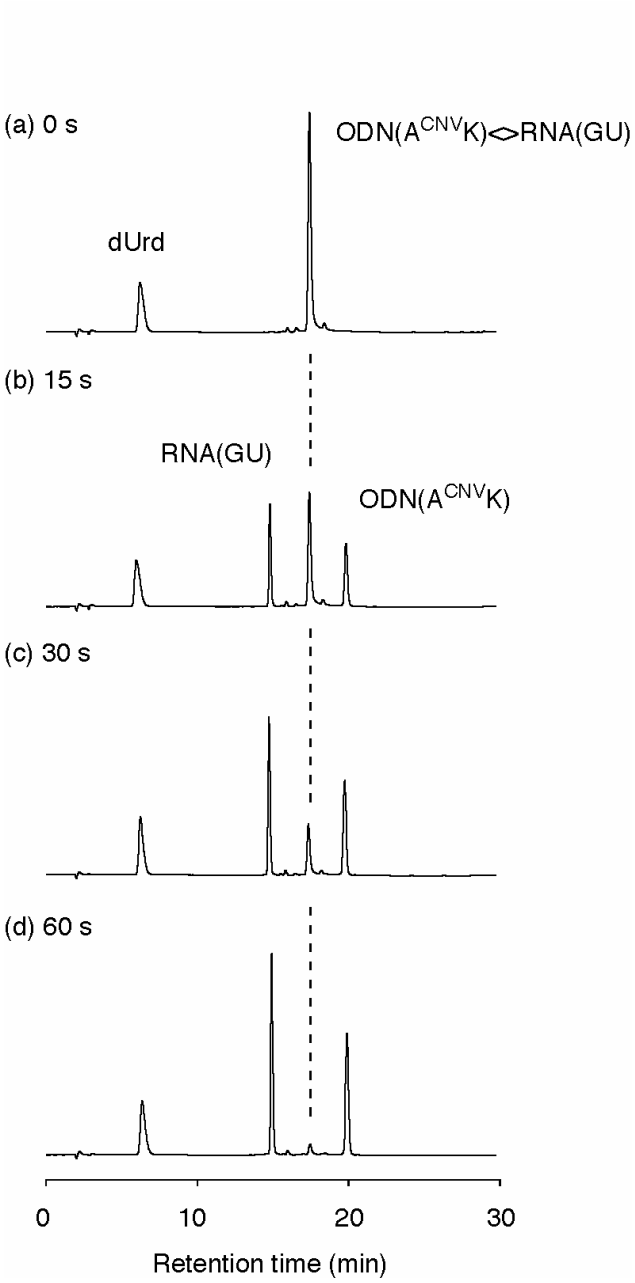


Figure S15. HPLC analysis of the photosplitting reaction of the photocrosslinked ODN(A^{CNV}K)<>RNA(GU). 2'-Deoxyuridine (dUrd) was used as an internal standard.

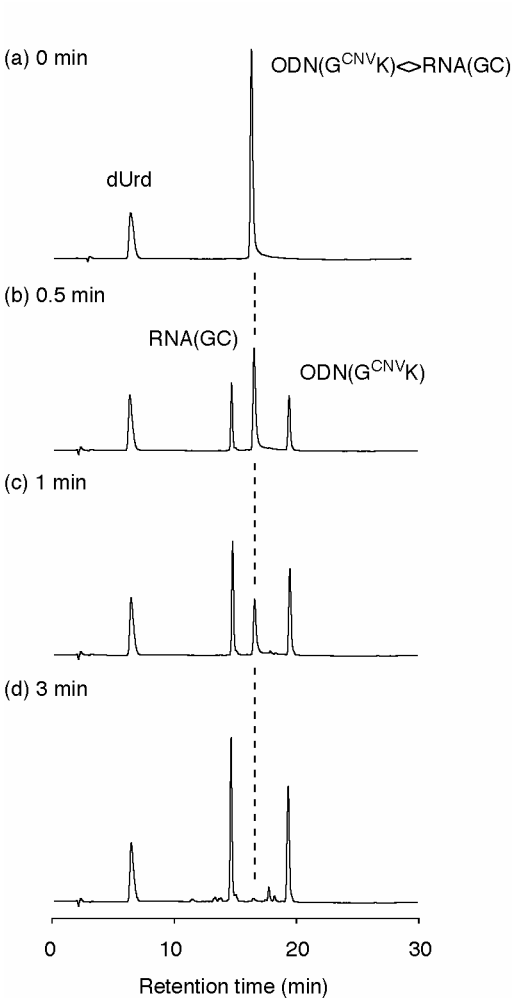


Figure S16. HPLC analysis of the photosplitting reaction of the photocrosslinked ODN(A^{CNV}K)<>RNA(GC). 2'-Deoxyuridine (dUrd) was used as an internal standard.

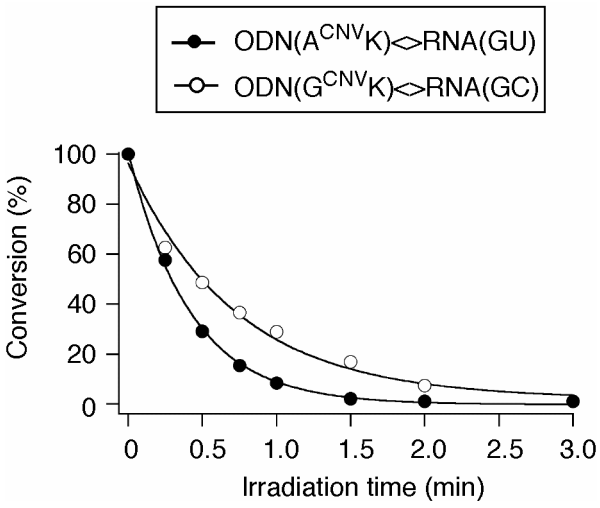


Figure S17. Time course of the photosplitting reaction with ODN(A^{CNV}K)<>RNA(GU) (filled symbols) and ODN(G^{CNV}K)<>RNA(GC) (open symbols).

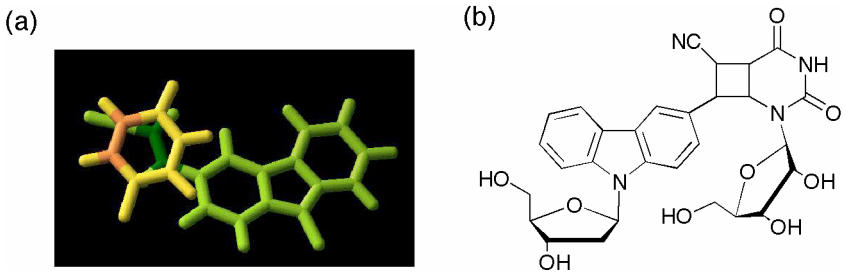


Figure S18. (a) Molecular modeling of stacked geometry in A-form of the hybrid duplex between DNA and RNA. The model was optimized by AMBER* force field in water by using MacroModel version 8.1. Yellow, and green molecules are rUrd, and ^{CNV}K, respectively. (b) Proposed structure of ^{CNV}K<>rUrd photoadduct.

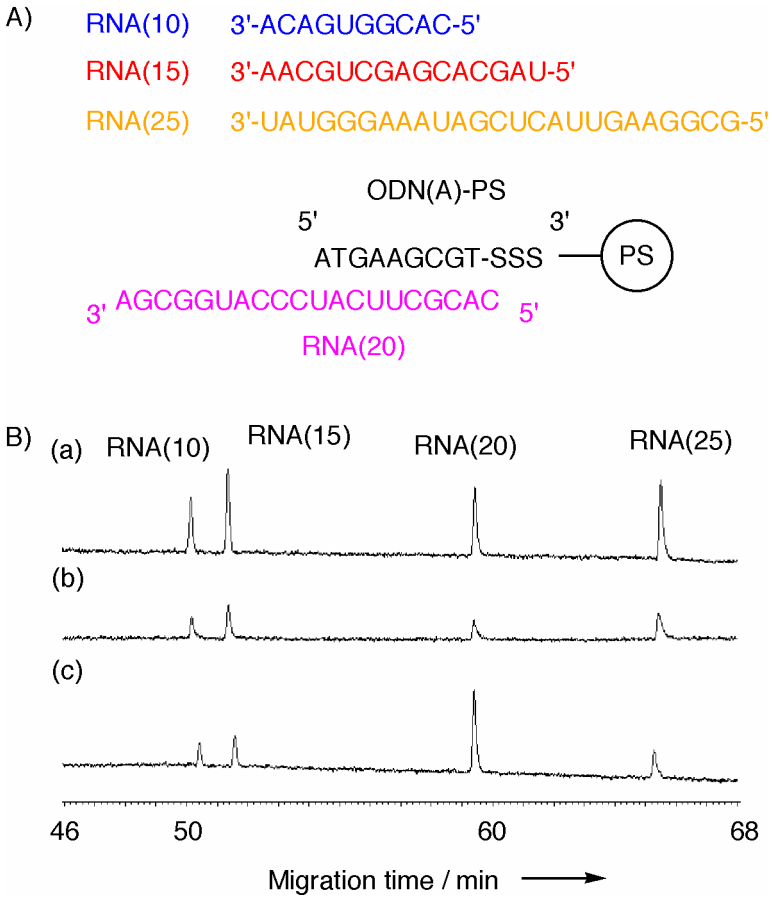


Figure S19. A) Capture and target RNA sequences used in RNA selection by using hybridization method. B) CGE analysis for each operation: (a) before RNA selection; (b) the washed solution after hybridization at 4 ℃; (c) after RNA selection.