Supporting Information

Blocking the Thiol at Cysteine-322 Destabilizes Tau Protein and Prevents Its Oligomer Formation

Hui Chen[†,‡], Simu Liu[§,∥], Shuiming Li[∥], Jierui Chen[†], Jiazuan Ni[†], and Qiong Liu[†]

[†]Shenzhen Key Laboratory of Marine Bioresource and Eco-environmental Sciences, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China

[‡]Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, China

[§]Guangdong Provincial Key Laboratory for Plant Epigenetics, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China

[∥]Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, China

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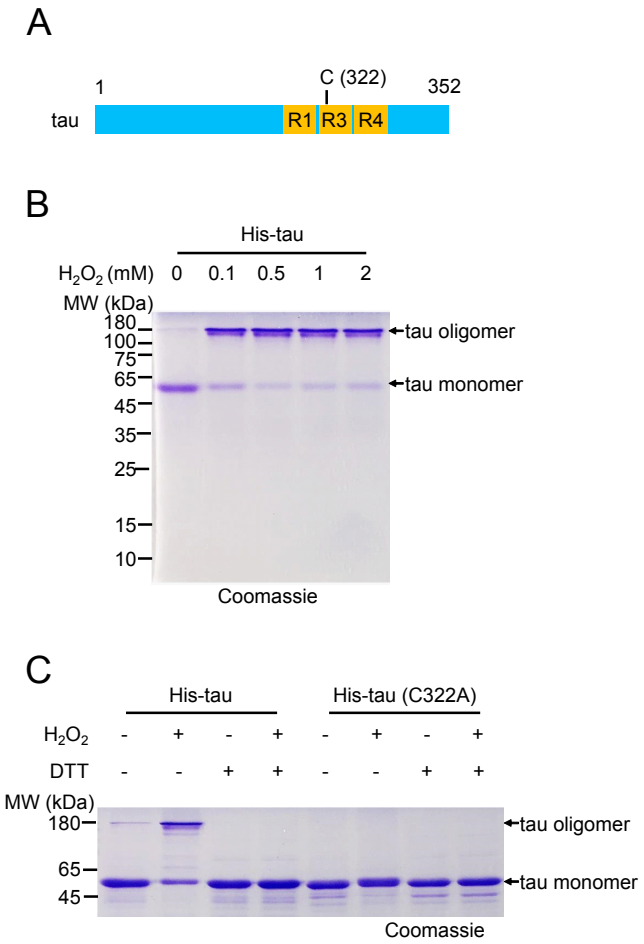

Figure S1. The Cys-322 is required for H_2O_2-induced tau oligomer formation. (A) Schematic illustration of the structures of human tau employed in this study. The cysteine number of tau is based on the longest tau isoform (isoform 2, amino acids 1-441). (B) H_2O_2 induces the formation of tau oligomer *in vitro*. (C) Substitution of Cys-322 with Ala inhibits H_2O_2-induced tau oligomer formation. The SDS-PAGE gel was stained using coomassie.

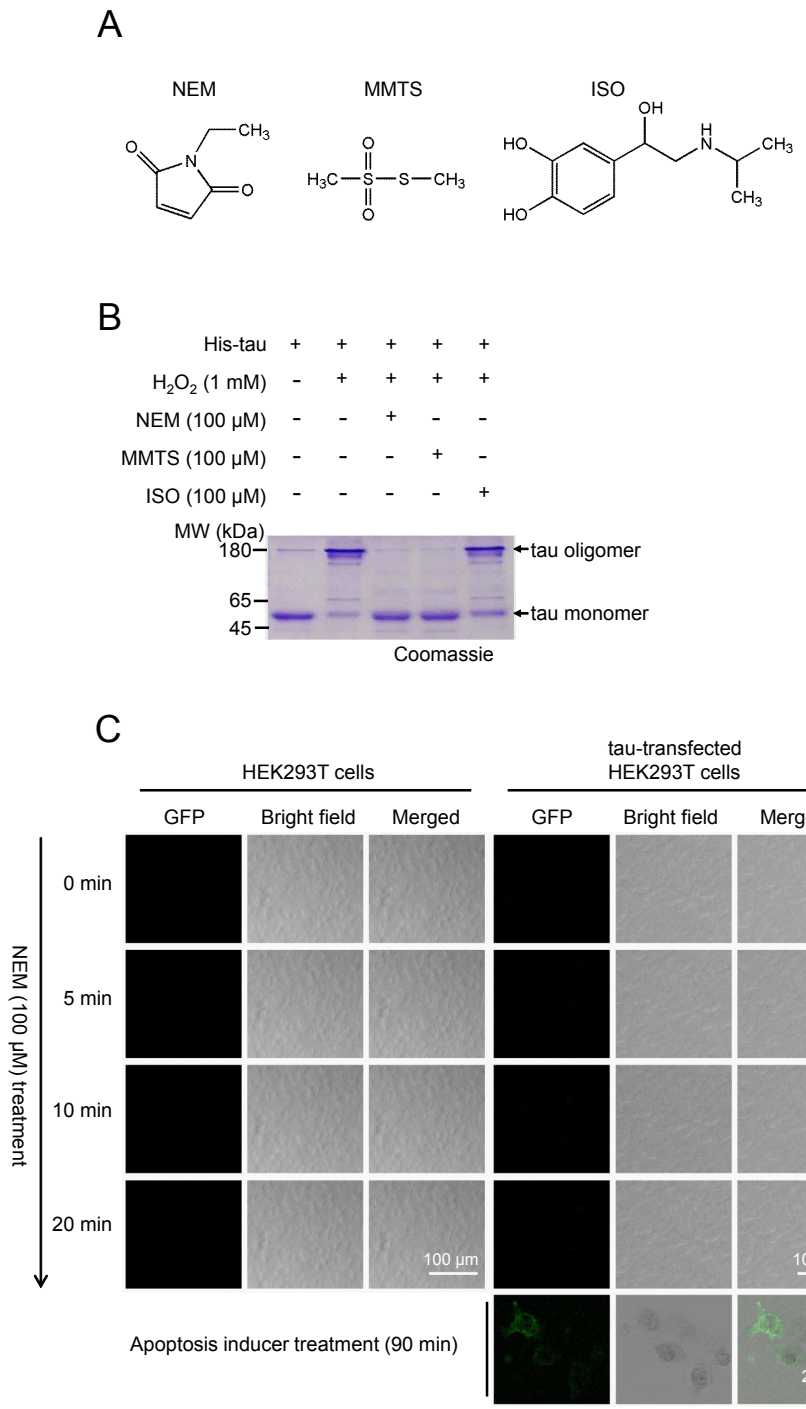

Figure S2. Effects of chemicals on tau aggregation and cell apoptosis. (A) *N*-ethylmaleimide (NEM), *S*-methylmethanelthiosulfonate (MMTS) and isoproterenol (ISO) were chose to perform these assays. (B) Effects of chemicals on H_2O_2-induced tau oligomer formation. The SDS-PAGE gel was stained using coomassie. (C) CLSM images of NEM-treated HEK293T cells with Annexin V-EGFP apoptosis detection. Green fluorescence indicates cell apoptosis. Cells treated with apoptosis inducer were used as a positive control.

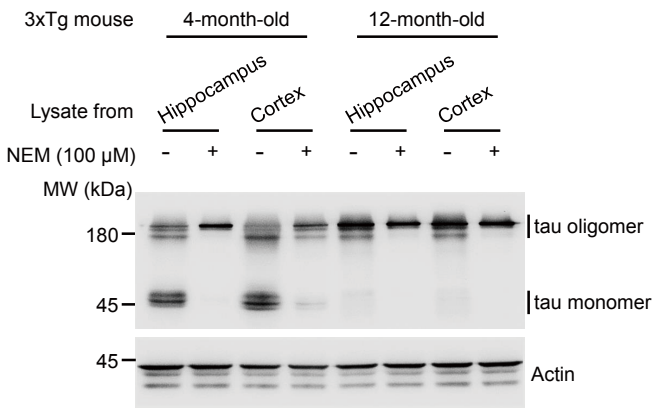

Figure S3. Effects of NEM on stability of tau proteins extracted from 3xTg mouse brain. The Flag-tau was detected using anti-tau (tau 5) antibody. Actin was used as a loading control.

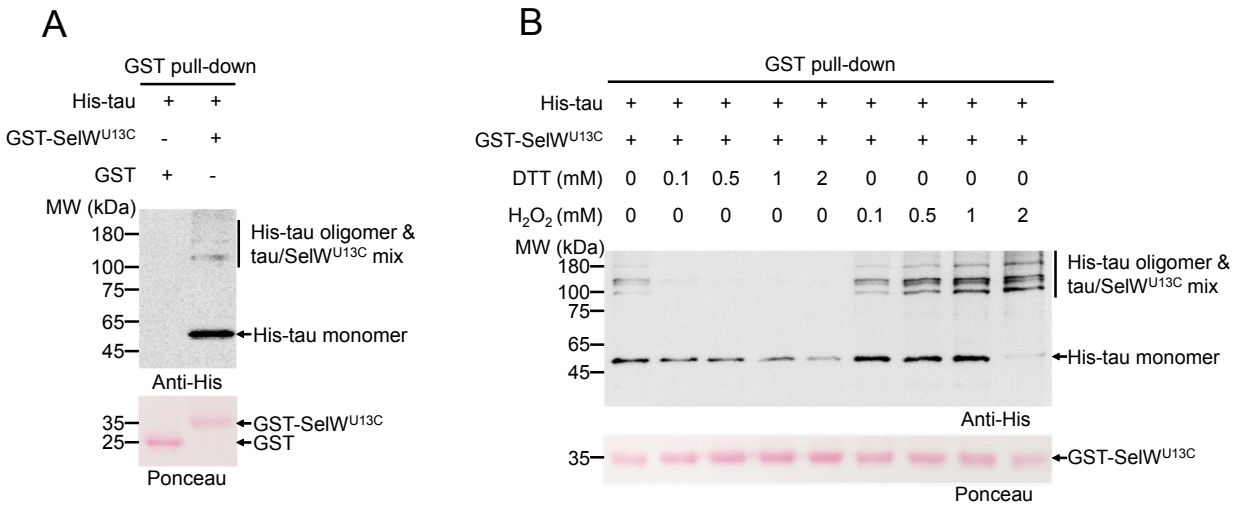

Figure S4. SelW^{U13C} interacts with tau. (A) GST pull-down assays performed with His-tau and GST-SelW^{U13C}. GST was used as a negative control. (B) The effects of DTT and H_2O_2 on interaction between GST-SelW^{U13C} and His-tau. DTT or H_2O_2 with indicated concentration was added into the pull-down buffer to perform these assays. His-tau was detected using anti-His antibody, and GSTE and GST-SelW^{U13C} proteins were detected by ponceau staining.

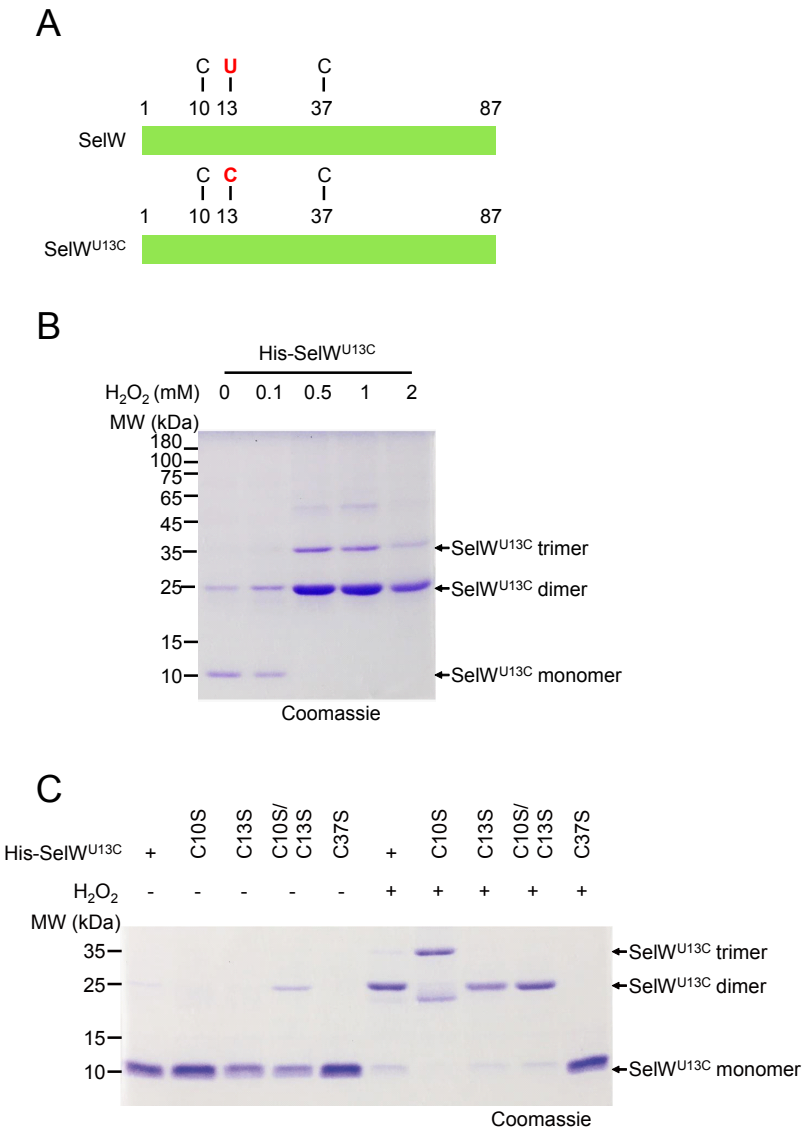

Figure S5. The Cys-37 is required for H_2O_2-induced SelW^{U13C} oligomer formation. (A) Schematic illustration of the structures of human SelW employed in this study. SelW^{U13C} harbors the substitution of selenocysteine (U) with cysteine (C). (B) H_2O_2 induces the formation of SelW^{U13C} oligomers *in vitro*. (C) Substitution of SelW^{U13C} Cys-37 with Ser inhibits H_2O_2-induced His-SelW^{U13C} oligomerization. The SDS-PAGE gel was stained using coomassie.

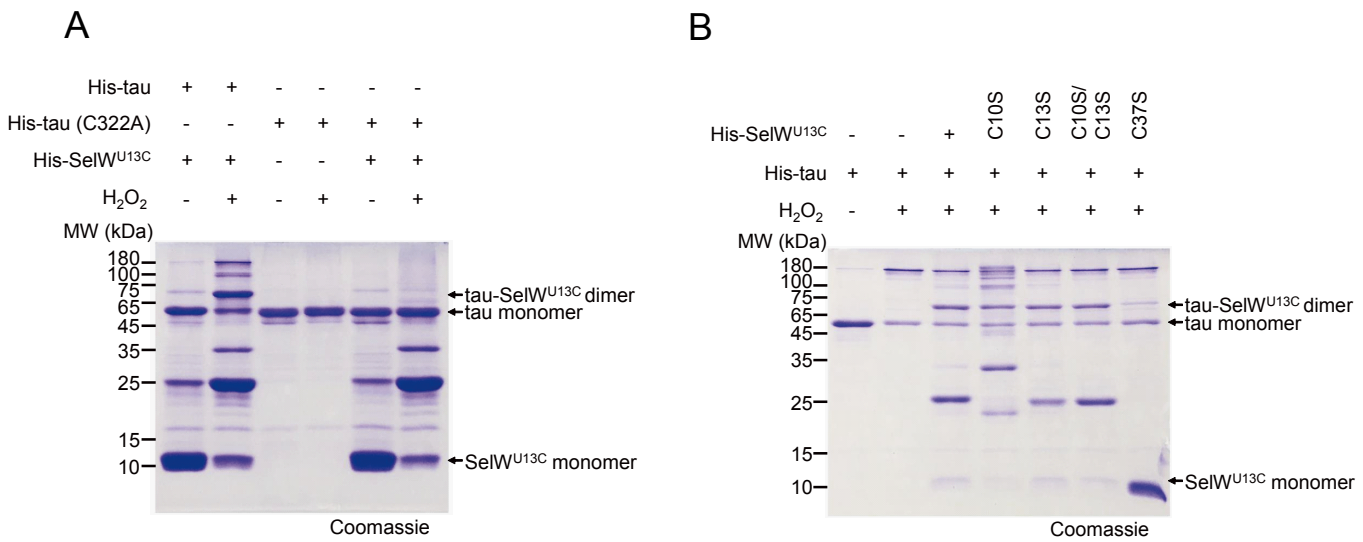

Figure S6. Identification of the cysteines involved in disulfide linkage. (A) Substitution of Cys-322 with Ala inhibits H_2O_2-induced tau-SelW^{U13C} dimer formation. (B) Substitution of SelW^{U13C} Cys-37 with Ser inhibits H_2O_2-induced tau-SelW^{U13C} dimer formation. The SDS-PAGE gel was stained using coomassie.

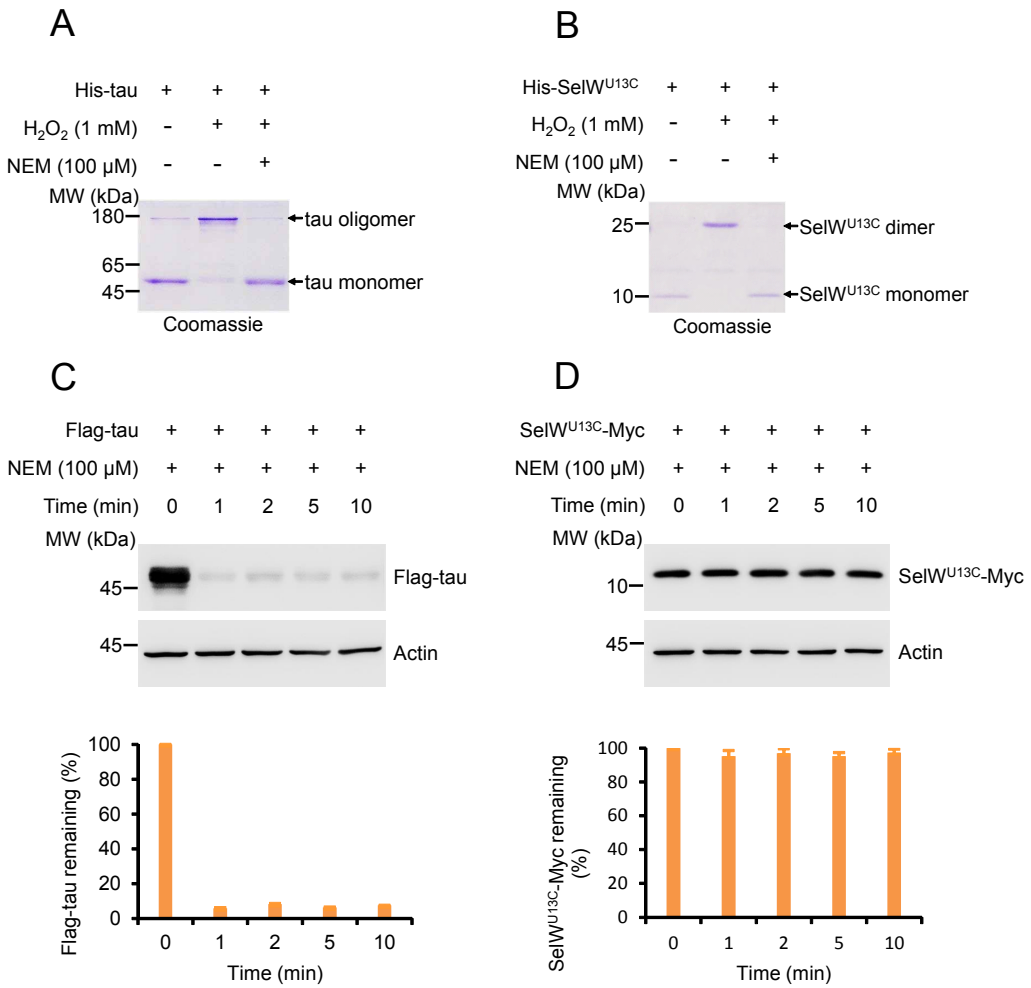

Figure S7. Application of NEM reduces tau but not SelW^{U13C} level. Effects of NEM on H_2O_2-induced tau (A) and SelW^{U13C} (B) oligomer formation. The SDS-PAGE gel was stained using coomassie. Effects of NEM on stability of Flag-tau (C) and SelW^{U13C}-Myc proteins (D). Protein crude extracts from HEK293T cells expressing Flag-tau or SelW^{U13C}-Myc were used in these assays. The Flag-tau and SelW^{U13C}-Myc were detected using anti-tau (tau 5) and anti-Myc antibodies, respectively. The protein level in the absence of chemical was set as 100%. Actin was used as a loading control. Error bars represent SD. Three biological repeats were performed and analyzed.

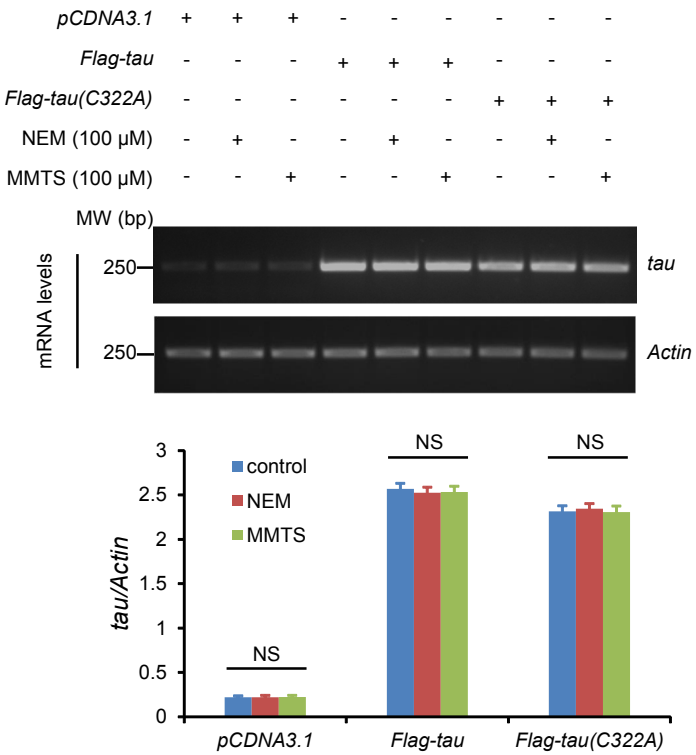

Figure S8. Effects of NEM and MMTS on transcription of *tau* gene. The data show mean ± SD. Three biological repeats were performed and analyzed. The mRNA levels of *tau* were normalized to *actin*. NS no statistically significant difference.

Table S1. Primers used for plasmid construction.

Primer	Sequence (5’-3’)
tau-PF	TAGAATTCATGGCTGAGCCCCGCCAGGAGTTC
tau-PR	GACTCGAGTCACAAACCCTGCTTGGCCAGGG
C322A-F	GACCTCCAAGGCTGGCTCATTAGG
C322A-R	CCTAATGAGCCAGCCTTGGAGGTC
SelW-PF	GTGGATCCCCGGAATTCATGGCTCTCGCCGTCCGAGTC
SelW-PR	CGATGCGGCCGCTCGAGTTAGCCCTGAGCCAAGGCGGC
SelW-3.1R	CGCTCGAGGCCCTGAGCCAAGGCGGCTTTG
U13C-F	CGGAATTC ATGGCTCTCGCCGTCCGAGTCGTTTATTGTGGCGCTTGCGGCTACAAG
C10S-F	CGGAATTCATGGCTCTCGCCGTCCGAGTCGTTTATTCTGGCGC
C13S-F	CGGAATTC ATGGCTCTCGCCGTCCGAGTCGTTTATTGTGGCGCTTCAGGCTACAAG
C1013F	CGGAATTC ATGGCTCTCGCCGTCCGAGTCGTTTATTCTGGCGCTTCAGGCTACAAG
C37S-F	CTGGACATCTCCGGCGAGGGA
C37S-R	TCCCTCGCCGGAGATGTCCAG

Table S2. Primers used for RT-PCR.

Primer	Sequence (5’-3’)
TAU-F	GAGCAAGGTGACCTCCAAGTG
TAU-R	GGAGACATTGCTGAGATGCCG
ACTIN-F	CAGAGCAAGAGAGGCATCCTC
ACTIN-R	GAGTCCATCACGATGCCAGTG