

Title	Artificial RNA editing by using ADAR1 deaminase in Ochre Stop codon for restoration of genetic code
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ABSTRACT

Artificial RNA Editing By Using ADAR1 Deaminase in Ochre Stop Codon for Restoration of Genetic Code

Editing of mutated gene can be a possible means of treatment for genetic diseases caused by stop codon Ochre (TAA). Here, I have tried to engineer deaminase domain of ADAR1 (Adenosine Deaminase Acting on RNA) and MS2 system to target specific Adenosine (A) to restore Guanosine (G) to Adenosine (A) mutations. For this ADAR1 deaminase domain has been fused with RNA binding protein MS2, which binds to MS2-RNA. Guide RNA was designed complementary to target RNAs. Thus ADAR1 deaminase domain was carried to desired editing site to convert A to I. As a target, Ochre (TAA) stop codon was mutated at 58th amino acid Trp (TGG) of EGFP. MS2 system has the ability to convert Stop codon (TAA) to normal codon (TGG) in cellular system (*e.g.*, HEK 293). The system converted TAA to TGG and turn on fluorescence (**Fig. 1: 1A, 1B and 1C**). cDNA was synthesized from positive cells followed by RNA extraction and PCR-RFLP was done by using BmgT120I restriction enzyme. Only wild type EGFP and edited were cut (**Fig. 2**).

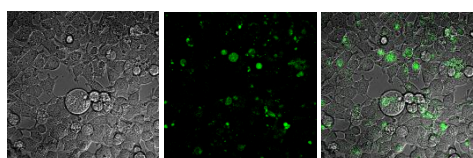


Fig 1A: Three factors (ADAR1 + Ochre + gRNA) producing fluorescence expression

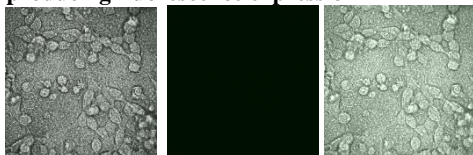


Fig 1B: Two factors (ADAR1 + Ochre) do not produce fluorescence expression

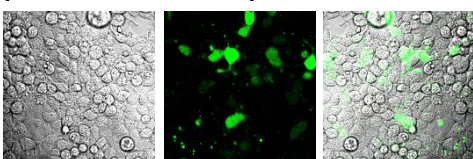


Fig 1C: Wild type of EGFP produces fluorescence expression

Fig 1: Fluorescent expression by transfection with the three factors, two factors and wild type of EGFP into the HEK 293 cell line (LSM confocal Microscopic images)

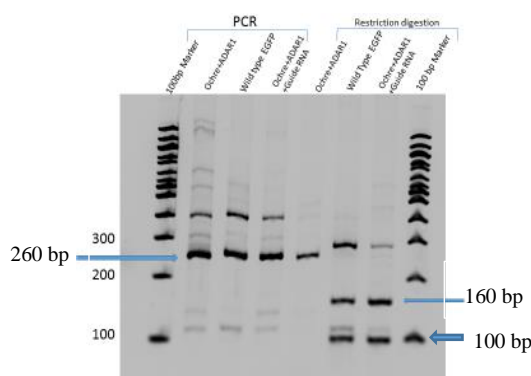


Fig 2: Restriction Digestion done by using BmgT120I restriction enzyme. For PCR without digestion the band was 260 bp and for uncut after digestion the 2 factors (ADAR1 and Ochre stop codon) the band was also at 260bp. For cut (Wild type EGFP and edited) the band was found at 160 bp and 100 bp

Successful artificial editing of RNA *in vivo* by MS2 system can pioneer genetic code restoration therapy including stop-codon read through therapy for various genetic diseases.

Key words: RNA editing, Ochre stop codon, ADAR 1, Guide RNA, Genetic code