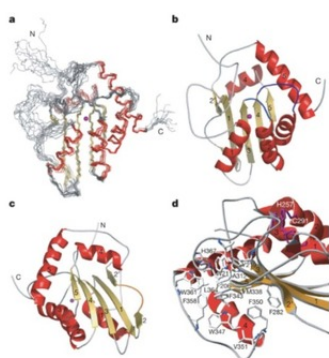




# Soluble, active DNA cytosine deaminase for use as a research tool

A method to produce soluble DNA cytosine deaminase for assays of potential inhibitors of HIV-1 and as a tool for biotech applications



**Figure 2 | NMR structure of A3G-2K3A.** a, Superimposition of ten NMR structures showing  $\alpha$ -helices in red,  $\beta$ -sheets in yellow and  $Zn^{2+}$  in purple. b, c, Ribbon diagrams of the NMR structure shown in a from the same (b) and 180° (c) angles, respectively. The  $\beta$ 3-to- $\beta$ 2 and  $\beta$ 4-to- $\beta$ 3 loops are coloured blue in b, and the  $\beta$ 2-bulge- $\beta$ 2' is coloured orange in c. d, Hydrophobic contacts between  $\alpha$ 4 and the  $\beta$ -strands and loops of the indicated regions ( $\beta$ 1,  $\beta$ 3,  $\beta$ 4, N-terminal-loop,  $\beta$ 2-loop and  $\beta$ 4-to- $\beta$ 3-loop). Amino acid side chain atoms are coloured yellow (sulphur), red (oxygen), blue (nitrogen) and white (carbon).  $Zn^{2+}$ -binding side chains are coloured purple.

Technology ID

Z08093

## Category

All Technologies

Life Sciences/Biochemicals &

Small Molecules

Life Sciences/Industrial Biotech

Life Sciences/Research Tools

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**IP Status:** US Patent Issued; Patent #: US7981655B2

## Applications

- Screening for new HIV drugs
- Cytosine-specific single strand DNA mutagenesis
- dC-to-dU conversion as a biomarker
- Approach for making other DNA deaminase family members soluble
- Replacing bisulfite sequencing
- Semi-random mutagenesis
- Map DNA in-vivo

## Key Benefits & Differentiators

- **Functional:** Solubility of the enzyme makes it broadly applicable to implementation in screens and within biotech kits
- **Versatile:** Decreased aggregation allows this enzyme to be prepared at a broader range of concentrations

## Technology Overview

Human APOBEC family members are important mediators of adaptive and innate immune responses. One family member, apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (APOBEC3G (A3G)), was identified as a cellular protein capable of blocking the replication of virion infectivity factor (Vif)-defective HIV-1. A3G inhibits the replication of HIV-1 and other retroviruses by deaminating viral cDNA cytosines to uracils during reverse transcription.

Naturally occurring cytosine deaminases such as human APOBEC3G and APOBEC3F are prone to aggregation and precipitation, especially at high concentrations. This prevents researchers from utilizing cytosine deaminases as a tool in screens for potential inhibitors of HIV-1, which is responsible for 95% of HIV cases worldwide. The HIV therapy market is expected to grow to \$45 billion in 2028 at a CAGR of 5.9%. Additionally, current approaches to modify DNA such as CRISPR/Cas9 need to cleave double-stranded DNA, while cytosine deaminase works on single-stranded DNA, opening the door for more versatile gene editing in biotechnology applications.

Researchers at the University of Minnesota have developed a method to produce soluble and functional DNA cytosine deaminase. This method incorporates key mutations to naturally occurring cytosine deaminase that improves solubility and may be used similarly with other DNA deaminase family members. The increased solubility provided by this method has enabled the use of APOBEC3G (A3G) in screening campaigns to identify inhibitors as potential HIV-1 therapeutics. Additionally, this method enables functional cytosine deaminase for biotechnology tool kits which may be directed to applications including dC-to-dU conversion as a biomarker, mapping DNA in-vivo, or semi-random mutagenesis.

## **Phase of Development**

### **TRL: 3-4**

Plasmid DNA constructs and the resulting protein are readily available. It has been utilized to develop high-throughput screens for testing anti-A3G inhibitors.

## **Desired Partnerships**

This technology is now available for:

- License
- Sponsored research
- Co-development

Please contact our office to share your business' needs and learn more.

## **Researchers**

- [Reuben Harris, PhD](#) Professor, Molecular Biology

## **References**

1. Kuan-Ming Chen, Elena Harjes, Phillip J. Gross, Amr Fahmy, Yongjian Lu, Keisuke Shindo, Reuben S. Harris & Hiroshi Matsuo(2008) , <https://www.nature.com/articles/nature06638>, <https://www.nature.com/>, 452, 116–119