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Real-Time Reporter and Efficient Enzymes for DNA Editing

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Real-Time Reporter for DNA Editing by APOBEC-Cas9 or Cleavage by Cas9 in Living Cells

Base editing is an exciting new genome engineering technology. C-to-T mutations in genomic DNA have been achieved using ribonucleoprotein complexes comprised of rat APOBEC1 single-stranded DNA deaminase, Cas9 nickase (Cas9n), uracil DNA glycosylase inhibitor (UGI), and guide (g)RNA. Here, we report the first real-time reporter system for quantification of APOBEC-mediated base editing activity in living mammalian cells. The reporter expresses eGFP constitutively as a marker for transfection or transduction, and editing restores functionality of an upstream mCherry cassette through the simultaneous processing of two gRNA binding regions that each contain an APOBEC-preferred 5'TCA target site. Using this system as both an episomal and a chromosomal editing reporter, we show that human APOBEC3A and APOBEC3B base editing complexes are more efficient than the original rat APOBEC1 construct. We also demonstrate coincident enrichment of editing events at a heterologous chromosomal locus in reporter-edited, mCherry-positive cells. The mCherry reporter also quantifies the double-stranded DNA cleavage activity of Cas9, and may therefore be adaptable for use with many different CRISPR systems. The combination of a rapid, fluorescence-based editing reporter system and more efficient, structurally defined DNA editing enzymes broadens the versatility of the rapidly expanding toolbox of genome editing and engineering technologies.

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- $10,000 conversion fee (TRY to BUY)
- Royalty rate of 3% (2% for MN company)
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Advantages over Current Systems:

- Fluorescence-based reporter provides real-time quantification of base editing
- The reporter quantifies successful DNA editing events without sequencing
- Next-generation editing constructs have the highest reported efficiencies
- Reporter activation enables FACS enrichment of cells with editing events at heterologous sites (ex. Single copy chromosomal genes)
- Reporter enables screens for modifiers of editing activity
- Efficient, structurally known editing enzymes
- 13 days faster than standard methods to “Analyze Sequencing” step
- Reduces operating costs by increasing assessment throughput, editing efficiency

Applications:

- Molecular biology research
- Genome engineering
- Biotechnology
- Editing optimization for therapeutic applications
- Crop engineering
- Animal engineering

Phase of Development:

In vitro data/working prototype. Reporter and editing constructs have been built and validated in a range of mammalian cell types.

Researchers

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