UK Dementia Research Institute

# Codon optimisation of Cas proteins

CRISPR-Cas has emerged as one of the key techniques for gene editing. Although CRISPR-Cas is expected to transform medicine, enable new therapeutic modalities and improve research tools for drug screening, it presents some implementation challenges. One of these challenges is its expression efficiency in human cells, including iPSCs and differentiated cell lines derived from iPSCs.

## The Solution

Improved codon optimisation methods that enable the efficient expression of target nucleic acid sequences in a host cell

### The Method

Identify host/cell level codon biases to overcome gene silencing

Benefits over:

- codon optimisation alternative methods: looking at genome-level (species) generic codon usage
- vector design: multiple Cas copies, promoters engineering

### Codon Optimised Cas

Replacement of non-preferred codons (low frequency) with ones at a higher frequency at a cell level

- Higher level of expression: mRNA and protein level
- Sustained expression in mature iPSC-derived cells
- Higher nuclease activity
- Faster cutting efficiency
- Tuned expression levels

### Applications

- CRISPR-Cas therapeutic approaches: Cas9, Cas12a and Cas13Rx
- Cell-differentiated iPSCs research (neurons, hepatocytes, macrophages, cardiomyocytes...)
- Codon optimisation of non-human proteins for human health applications

*UK Priority application 2117583.1 (Filed on 6th Dec 2021)*

## Codon Optimisation - Scientific Rationale

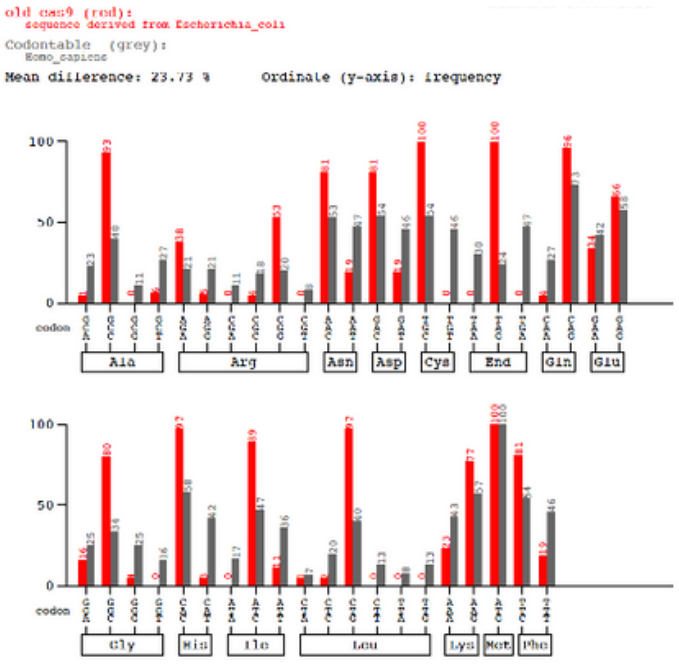
### Codon usage variation between species

- Each specie displays "bias" towards certain codons, displaying rare or common usage frequencies.
- When a gene of interest contains codons that are rarely used by a host, that leads to stalled translation and either reduced efficiency of expression or prevention of expression entirely within a cell from the host.
- Codon usage is a key determinant of translation elongation rates and co-translation protein folding.
- Host "preferred" codons present enhanced translational efficiency and folding fidelity.

### Secondary code within the specie-generic code

- There is an unequal usage of synonymous codons across different cell lines within the same specie or host, from yeast to humans.
- The secondary code usage frequency is a major regulator of translational speed and co-translational protein folding, and it is installed at a cellular level for specific proteins.

***Example of codon usage differences in Cas9 derived from E. coli for human cell optimisation:***




Founding funders:
UKRI Medical Research Council
Alzheimer's Society
Alzheimer's Research UK