Method for rapid regeneration of modifiable stable cell lines

Unmet Need

Characterizing the composition and function of protein complexes can facilitate the understanding of key cellular processes. Indeed, the global proteomics market was estimated to be USD 25.9 billion in 2021. However, the analysis of protein expression and interactions has been limited by the tools available, such as mass spectrometry and antibody-based protein detection, which are only useful for a certain range of targets and experimental contexts. In addition, plasmid-based overexpression of tagged target proteins may affect protein localization and folding, distort downstream signaling, and hinder the physiological function assessment, thus leading to false positives. There is a need for endogenous protein fusions in stable isogenic cell lines that can generate enough material for proteomic analysis.

Technology

Duke inventors have developed a method to generate stable and modifiable cell lines. This is intended to be used for the creation of isogenic cell lines that can be easily modified with a wide variety of gene/protein fusions for proximity labeling and proteomic analysis. Specifically, homology-independent universal genome engineering (HiUGE) is combined with recombination mediated cassette exchange (RMCE) to achieve this goal. First, HiUGE allows for rapid gene-fusion insertion through CRISPR-Cas9 technology and the selection of desired cells through the selectable fusion tag. Then, RMCE enables the swap of the fusion tag, which is flanked by FRT sites, into any other fusion tag by transfecting the cells with Flpase recombinase and a donor vector. This has been demonstrated by transfecting HEK 293T cells with a knock-in vector containing a puromycin selection gene tag. Cells that stably expressed the tag were selected and isolated, and rapid tag exchange was confirmed by immunostaining.

Advantages

- Novel method of combining two genetic modification approaches to generate and modify stable cell lines
- Easily and efficiently modify cell lines with any protein tag to enable a wide variety of experimental needs
- Demonstrated to successfully select and modify tagged HEK 293T cells through *in vitro* studies

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Publication(s)

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