Targeted Integration of Fluorescent Reporter Genes Utilizing Zinc Finger Nucleases

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Introduction

Engineering of the mammalian genome is an area of active research that also impacts drug discovery and cell-based assays. This can be accomplished using a variety of nucleases (ZFNs) that can introduce specific genomic modifications to the cell’s natural machinery. With the development of CRISPR-Cas9 technology, the ability to edit the genome on a large scale has become a reality.

The cell’s natural machinery repairs the break in one of two ways: non-homologous end joining (NHEJ) or homologous recombination (HR). In the case of HR, the repair template is introduced into the target site using a donor construct. The donor construct typically contains a fluorescent reporter gene, which allows for easy visualization of the targeted integration event.

Methods

U-2 OS (Cat. No. HTB-96®) cells were obtained from ATCC and cultured according to the product manual. Nucleofections were performed with the Amaxa® Nucleofector® device (Cat. No. A1001) and Nucleofector® U-2 OS (Cat. No. HTB-96TM) cells were obtained from ATCC and cultured according to the product manual. The ZFNs were designed and constructed in house. Fluorescent reporter genes were obtained from Evrogen (http://evrogen.com/products/). U-2 OS cells were transfected with a donor construct containing a fluorescent reporter gene. The expression of the reporter gene was analyzed using fluorescent microscopy.

Results

Successful integration of fluorescent reporter genes into U-2 OS cells was performed with a Nikon Eclipse TE2000-E inverted research microscope and Metamorph® software. The expression of the reporter gene was analyzed using fluorescent microscopy.

Discussion/Conclusion

U-2 OS cells were exposed to 20 µM vincristine for sixty minutes. As time progresses, tubulin isomerizes and filaments can be observed. With ZFNs it is now possible to create stable integration of reporter genes into a genome. Unlike fusion proteins, the reporter gene is integrated into the genome in a way that does not alter the function of the gene.

References/Endnotes


Acknowledgments

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Product Offerings

- Osteosarcoma Cell Line with GFP tagged stably (G2) (3510)
- Osteosarcoma Cell Line with GFP tagged stably (G2) (3510)
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