Development of Novel Knock-In Cell Lines with Target Genes Endogenously Tagged by Fluorescent Reporters Utilizing Zinc Finger Nuclease Technology

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Abstract

Fluorescent protein (FP) tagging is extensively used to provide a visual readout on the protein of interest at the cell level. However, many methods of expression rely on heterologous promoters that result in altered regulation and expression pattern. To address these issues, we report a method that can direct specific integration of a reporter into the endogenous locus by using the corresponding loss of function for developing various high content screening (HCS) assays for compound screening where target gene regulation and corresponding protein function are preserved.

Introduction

Genomic modification of mammalian cells is one of the most challenging and therapeutically important fields that impacts drug discovery and personalized medicine. Reporters tagging by transcriptional regulation of target genes are moving closer to clinical application. Using transcription factors (TFs) to direct targeted integration is a strategy to generate genetically modified cells with targeted gene expression. The development of engineered TFs, such as zinc-finger nucleases (ZFNs), has shown promise in the generation of genesplicing with the ability to target DNA at precise locations. However, current methods of expression often rely on heterologous promoters that result in distorted regulation and corresponding protein function.

Materials and Methods

1. U2OS (Cat. No. HTB-46) cells were obtained from ATCC and cultured according to the product manual.
2. NucléoDetects were performed with the Katana NucléoDetect™ (GeneCat No. AAD-1001) and NucléoDetect® (GeneCat No. AAD-1006) according to the product manual.
3. Donor plasmids were designed and constructed by Sigma-Aldrich GmbH.
4. The cells were transfected with an a-haKU vector (TUBA1B) or a-haKU vector (ACTB) at 80 µM for a-actin and 100 µM for TUBA1B.
5. All experiments were performed on cells from Sigma-Aldrich GmbH.
6. The cells were exposed to 21 µM Cytochalasin B. Over time, shortening of actin filaments can be observed. By blocking monomer addition actin filaments are shortened. RFP tagged cytoskeletal structures are affected.

Results

Successfully Tagged Loci

<table>
<thead>
<tr>
<th>Chromosome (gene name, encoded protein)</th>
<th>Organism</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
<th>Successful Tagging</th>
<th>Undetermined Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTB (α-actin)</td>
<td>Human</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>TUBA1B (α-tubulin 1b)</td>
<td>Human</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>LAMB1 (lamin B1)</td>
<td>Human</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>LMNA (lamin A/C)</td>
<td>Human</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>HIST1H3A (H1 histone family, group A, member A)</td>
<td>Human</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Molecular Analysis

Double integration was confirmed by Southern hybridization and PCR analysis. The double integration results were consistent with the expected sizes of the DNA fragments. The Southern blot analysis confirmed the presence of the integrated DNA fragments by hybridization with probes specific for the targeted genes. The PCR analysis was performed using genomic DNA from the transfected cells as template. The PCR products were visualized on a gel and compared with the expected sizes.

Conclusions

This approach provides a novel method for generating genetically modified cell lines with targeted gene expression. The integration of engineered TFs into the genome allows for precise control of gene expression, which can be used for various applications including drug discovery and personalized medicine. The use of ZFNs for gene targeting can be further optimized to increase the efficiency and specificity of gene integration, leading to improved generation of genetically modified cell lines.

Acknowledgments

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