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 in a cell without affect on its function, while the potential of expression of recombinant proteins that result in altered regulation and expression pattern. Thus, there exists a strong need for a method that can direct specific integration of a reporter into the genome to produce a cell expressing the corresponding fusion protein containing endogenous regulatory pathways. Two Finger nuclease (ZFN) can generate targeted DNA sequence specific to create a double stranded break (DSB). The cell then repairs the DSB with the integration of either homologous or non-homologous end joining (NHEJ) paths. We report the development of a novel technology that allows the development of fluorescently tagged high resolution cell lines with ZFNs. We first report a method for developing a high resolution cell line with expression of an endogenous GFP reporter. Then we describe the development of a cell line expressing an endogenous RFP-tubulin reporter. We finally introduce a novel technology that combines a double ZFN approach with two independent DSBs and a non-coding transgene to generate a novel technology for engineering cell line with spectrally diverse reporters. We believe this work will promote the development of cell lines for the research field.

Materials and Methods
1. U2OS (Cat. No. HTB-96) cells were obtained from ATCC and cultured according to the product manual.
2. Nucleofection was performed with the Nucleofector® device (Cat. No. A-100-011) and Nucleofector® HX Kit (Cat. No. VCA-1003) from Lonza AG according to the product manual.
3. Donor plasmids were designed and ordered from Sigma-Aldrich.
4. Fluorescent reporter genes were obtained from Evrogen (http://evrogen.com/products/Evrogen).
5. CompoZr® ZFNs were designed and manufactured by Sigma-Aldrich.
6. The cells were infected with an automated Nucleofector® device. 10^6 cells were treated with 8 µl of 4.5 VPM electroporation mix buffer (kit) and 2 µl of 100 µg/ml DNA, BFP-ACTB (β-actin, actin stress fibers), LMNB1 (lamin B1, nuclear envelope) and HMGA1 (high mobility group AT-hook 1, nucleus).

Results
Successfully Tagged Lines

Molecular Analysis

Introduction
Genetically modified mammalian cells are one of the most challenging and interestingly important fields that impact drug discovery and cellular studies. Genetic modification by homologous recombinational repair is the method of choice for investigational applications. In the present work, we describe the engineering of a novel technology that allows the development of cellular fluorescence expression of recombinant proteins. The ZFN technology has gained popularity in the gene targeting field due to its capability to both homologously and non-homologously induce a DSB. In the present work, we describe the engineering of a novel technology that allows the development of fluorescently tagged high resolution cell lines with ZFNs. We first report a method for developing a high resolution cell line with expression of an endogenous GFP reporter. Then we describe the development of a cell line expressing an endogenous RFP-tubulin reporter. We finally introduce a novel technology that combines a double ZFN approach with two independent DSBs and a non-coding transgene to generate a novel technology for engineering cell line with spectrally diverse reporters. We believe this work will promote the development of cell lines for the research field.

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Discussion
1. The cells were imaged with an automated Nikon TE2000-E inverted microscope. BFP: ex 395-410 / em 430-480,
2. ACTB gene has 3 isoform A (AT-hook) - a non-histone protein HMG-I/HMG-Y inner nuclear membrane meshwork that lies beneath the lamina, an intermediate filament structure that comprises the nuclear envelope.  The inner nuclear membrane is a protein meshwork that lies beneath the lamina, an intermediate filament structure that comprises the nuclear envelope.  The integration resulted in endogenous expression of the corresponding fusion protein. Four genes were tagged with TUBA1B (α-tubulin 1a - microtubule), ACTB (β-actin, actin stress fibers), LMNB1 (lamin B1, nuclear envelope) and HMGA1 (high mobility group AT-hook 1, nucleus). Single cell clones were isolated in U2OS cells with at least one copy of a double integration for development of various high content screening (HCS) assays for compound screening where target gene regulation and expression pattern can be monitored.
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4. We would like to thank Danhui Wang, David Briner, and JiaJian Liu along with the CompoZr® Operations Team for their discussion and assistance.

Conclusions
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