Quantification of GFP Transfection using a Cost-effective Microplate Reader

Introduction

Transfection, the introduction of exogenous DNA into a eukaryotic cell, is an important tool for studying the regulation of gene expression as well as protein expression and function[1]. In stable transfection, the foreign DNA becomes integrated into the genomic DNA of the cell so that it is passed on in the cell lineage and continues to express the encoded gene of interest. More commonly used is transient transfection, in which the foreign DNA are present in the cell for only a limited period of time. There are several methods available for cell transfection, including the formation of DNA - DEAE dextran or DNA - calcium phosphate complexes to facilitate endocytosis and electroporation, which use high voltage pulses to form transient pores in the cell membrane through which the DNA can enter. Currently, the most common method for transfecting cells uses cationic lipids that result in very high transfection efficiencies with low cytotoxicity[3].

It is often difficult to identify a successful transfection in the gene of interest, so a reporter gene is frequently used to determine the percentage of cells transfected. The reporter gene can be present on the same vector as the gene of interest or located on a separate plasmid and introduced at the same time. Likewise, the reporter can be constructed as a fusion protein with the gene of interest. Fluorescent proteins, such as GFP, are the most widely used type of reporter gene. Here we use GFP expressing HEK293 cells to demonstrate the ability of the cost-effective Synergy™ LX Multi-Mode Reader to measure fluorescence and determine transfection efficiency.

Materials and Methods

Human embryonic kidney cells (HEK293) and HEK293-GFP cells expressing a GFP fluorescent protein were cultured in Advanced DMEM supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37 °C in 5% CO_2. Cultures were routinely trypsinized (0.05% Trypsin-EDTA) at 80% confluence. For experiments, mixtures of the two cell lines were plated into Corning 3904 black sided clear bottom 96-well microplates such that there were 50,000 total cells per well using phenol red free media. The ratio of the two cells was varied such that there was a range of HEK293-GFP cells from 0 to 100% of the total. After 24 hours to allow for attachment, Hoechst 33342 was added to the cultures at a final concentration of 1 µg/mL to stain the nuclei of both cell lines and provide a way to quantify the total number of cells by monitoring blue fluorescence.

PMT based Fluorescence Determination

The fluorescence of each well was determined using a Synergy LX Multi-Mode Reader (BioTek Instruments, Winooski, VT) using either a blue or a green fluorescence cube. The blue fluorescence cube is configured with a 360/40 nm excitation filter, a 460/40 nm emission filter, and a 400 nm cut off dichroic mirror, while the green fluorescence cube is configured with a 485/20 excitation filter, a 528/20 emission filter, and a 510 nm cut off dichroic mirror.

Imaging

GFP transfected cells were imaged using a Cytation™ 5 Cell Imaging Multi-Mode Reader (BioTek Instruments, Winooski, VT) configured with a GFP light cube and a 2x objective. The imager uses a combination of LED light sources in conjunction with band pass filters and dichroic mirrors to provide appropriate wavelength light. The GFP light cube uses a 469/35 excitation filter and a 525/39 emission filter. After image preprocessing to subtract background fluorescence from captured digital images, object analysis was used to determine the number of GFP positive cells. Object analysis of the GFP channel identified individual cells by their fluorescence using a threshold of 5000 and a minimum and maximum size selection of 15 µm and 100 µm respectively.

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Results

Increasing percentages of GFP positive cells results in an increase in green fluorescence. Figure 1 demonstrates the green and blue fluorescence of samples of Hoechst 33342 stained HEK293 cells with the same number of total cells, but with various percentages of GFP positive cells. The increase in green fluorescence is linear with respect to the number of GFP positive cells, whereas blue fluorescence remains relatively constant reflecting equivalent cell numbers.

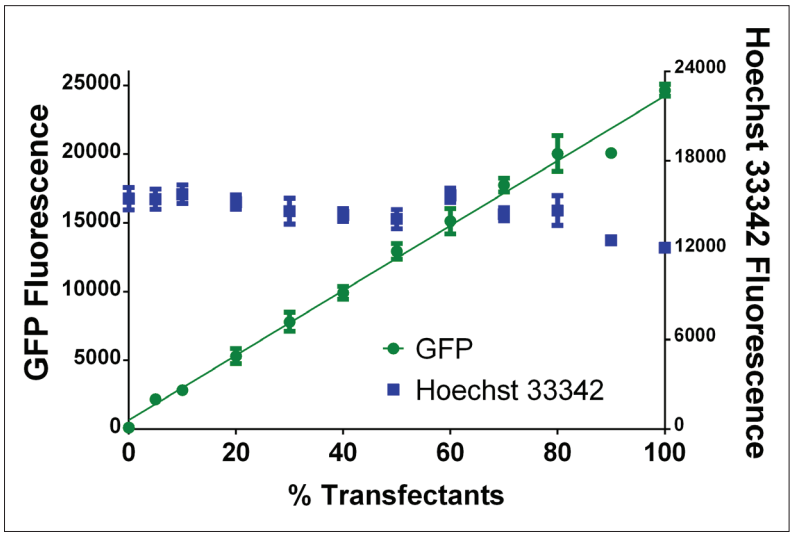

Figure 1. **Comparison of GFP and Hoechst 33342 fluorescence in HEK293 cells.** Mixtures of increasing percentages of HEK293-GFP cells along with non-fluorescent HEK293 cells were seeded such that a total of 50,000 cells were in each well. After staining live cells with Hoechst 33342 dye, the blue and green fluorescence was measured using a Synergy™ LX and plotted. Data points represent the mean and standard deviation of eight determinations.

A linear increase in fluorescence is also evident in Figure 2 when the percentage of GFP transfected cells are titrated up to 50k cells/well. When this result is compared to image-based cell counting of the GFP transfected cells, an equivalent response is evident.

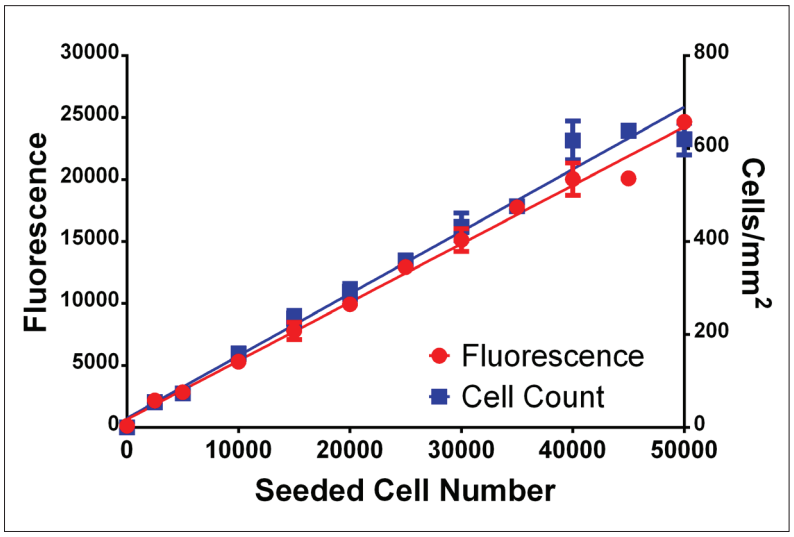

Figure 2. **Green fluorescence and imaged based cell counts of HEK293-GFP cells titrated from 0 to 50k cells/well.** Mixtures of increasing percentages of HEK293-GFP cells along with non-fluorescent HEK293 cells were seeded such that 50,000 cells were in each well. X-axis represents the estimated number of GFP expressing cells seeded. Cell count data are normalized to the field of view obtained with the 2x microscope objective. Data points reflect the mean and standard deviation of eight wells.

In Figure 3, the data from Figure 2 was replotted as a direct comparison between image-based counted cells and fluorescence detected by the Synergy™ LX. It is evident that a strong linear correlation exists between these two readouts.

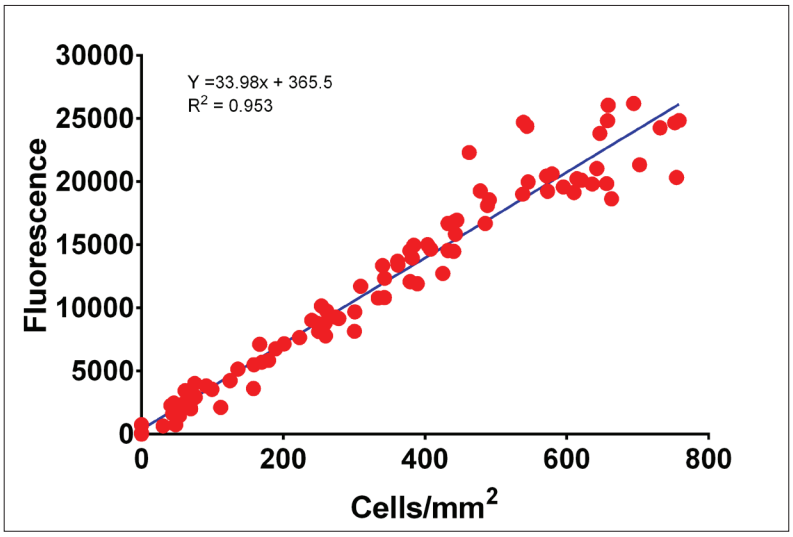

Figure 3. **Comparison of imaged-based cell counting and PMT-based fluorescence detection.** Fluorescence and GFP positive cell count data from each of the individual wells was plotted against each other along with a liner regression analysis of the data.

Discussion

These data demonstrate the ability of the Synergy LX to determine multiple color fluorescence in the same sample. When increasing percentages of cells constitutively expressing GFP are seeded into wells, an increase in mean green fluorescence is returned in a constant background of blue Hoechst 33342 stained nuclei. The whole-well mean fluorescence signal determined in the Synergy LX agrees closely with the number of GFP positive cells determined by image-based analysis. Likewise, when the fluorescence signal and cell count are compared directly a strong linear correlation exists.

References

1. "Transfection". Protocols and Applications Guide. Promega https://www.promega.com/resources/product-guides-and-selectors/protocols-and-applications-guide/transfection/

2. Fanelli, A. (2016) http://www.stablecellline.com/the-science-of-stable-cell-line-generation/

3. Felgner P.L., T.R. Gadek, M. Holm, R. Roman, H.W. Chan, M. Wenz, J.P. Northrop, G.M. Ringold, and M. Danielsen (1987). "Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure". PNAS USA 84 (21): 7413–7. doi:10.1073/pnas.84.21.7413. PMC 299306. PMID 2823261