

Evaluating transfection efficiency using the CELENA[®] X

INTRODUCTION

Transfection is a common technique used to introduce nucleic acids into cells and is important to the study of the function and regulation of genes and their products. Establishing a reproducible way to assess transfection efficiency in a high-content capacity accurately is critical. In this study, the effects of plasmid concentration on transfection efficiency were investigated using the CELENA[®] X High Content Imaging System.

APPLICATION

Cell preparation

HeLa cells were counted with the LUNA-II™ Automated Cell Counter, seeded at a density of 1×10^4 HeLa cells/well on a 96-well plate, and cultured overnight. To evaluate transfection efficiency, we transfected HeLa cells with different concentrations of the GFP-expressing pCG-HttQ103 plasmid (control, 0.05 μ g, 0.1 μ g, and 0.2 μ g) using Lipofectamine 2000 (Invitrogen, 11668) according to the manufacturer's protocol. 24-hours post-transfection, cells were fixed in 100 μ L 4% PFA for 15 minutes at room temperature. To count total cell numbers, nuclei were counterstained with Hoechst 33342 (Life Technologies, H3570).

Imaging and analysis

GFP expression and cell nuclei were visualized using the CELENA[®] X High Content Imaging System. Images were acquired using image-based autofocus and a 10X LWD high NA objective in combination with filters for Hoechst 33342 (DAPI filter cube: Ex375/28, Em460/50) and GFP (EGFP filter cube: Ex470/30, Em530/50). One image field was acquired per well from 12 wells.

For quantitative analysis of transfection efficiency, the integrated CELENA[®] X Cell Analyzer software was used to batch process and analyze images automatically. Individual cells were segmented based on Hoechst 33342 nuclear staining using the *IdentifyPrimaryObjects* module, while GFP-expressing cells were identified using the *IdentifySecondaryObjects* module. GFP intensity was quantified within the Hoechst-defined boundaries for each cell with the *MeasureObjectIntensity* module. A fluorescence intensity threshold was set using the *FilterObjects* module to eliminate false-positives, identify the transfected cells, and determine transfection efficiency. The threshold was defined as the maximum value of the median intensity of the GFP-channel images of the negative control group of cells.

Table 1. Description of the pipeline used to assess transfection efficiency with CELENA[®] X Cell Analyzer

Order	Module	Use
1	<i>IdentifyPrimaryObjects</i>	To find Hoechst-stained nuclei
2	<i>IdentifySecondaryObjects</i>	To find GFP-expressing cells
3	<i>MeasureObjectIntensity*</i>	To measure GFP intensity
4	<i>FilterObjects</i>	To filter out non-transfected objects based on GFP intensity
5	<i>GrayToColor</i>	To produce color images from grayscale images
6	<i>OverlayOutlines</i>	To place outlines around identified nuclei and transfected areas

To visualize the data, the *GraytoColor* module was used to apply colors to the grayscale images of each channel, and the *OverlayOutlines* module was used to overlay the images with the outlines of the identified cells and transfected areas to confirm segmentation precision (Figure 1).

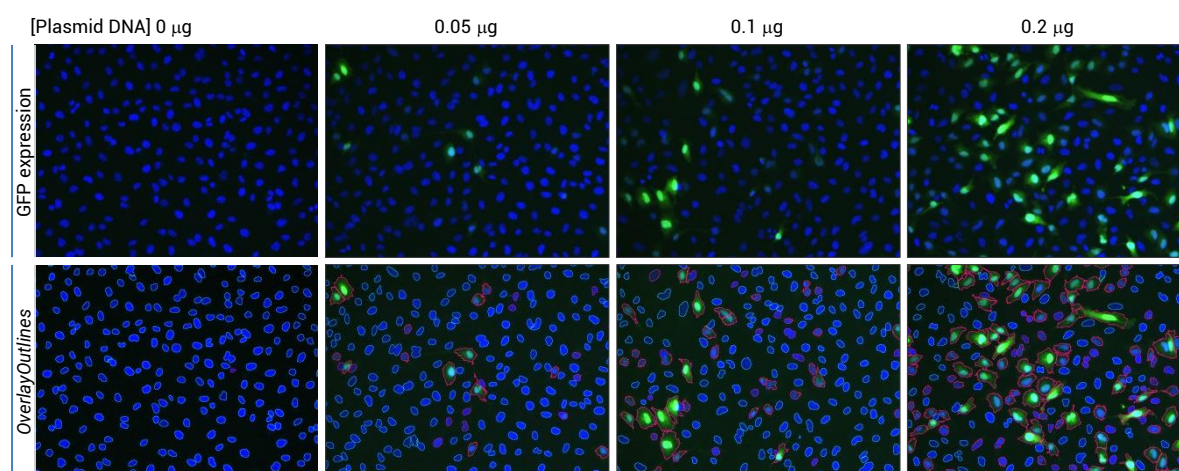


Figure 1. Image analysis of GFP expression in HeLa cells 24 h post-transfection. Higher plasmid concentrations correlated with increased numbers of GFP-positive cells (green). Blue lines indicate control Hoechst-stained nuclei (blue) and magenta lines outline GFP-positive cells.

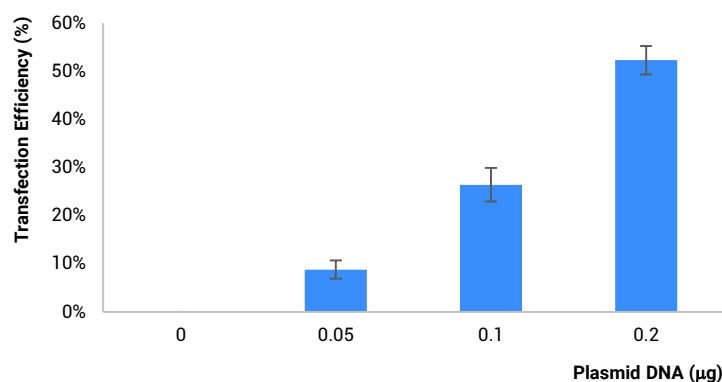


Figure 2. Quantification of transfection efficiency. The number of GFP-positive cells were compared to the number of Hoechst-stained nuclei. Transfection efficiency improved with increasing concentrations of the GFP-expressing pCG-HttQ103 plasmid.

CONCLUSION

In this study, we analyzed the effects of plasmid concentration on transfection efficiency using the CELENA® X High Content Imaging System. Images of the adherent HeLa cells in a multi-well plate were captured automatically and batch processed to identify objects, measure fluorescence intensity, apply a fluorescence intensity threshold, and even label images to help visualize data. As shown in Figure 1 and 2, transfection efficiency increased with plasmid concentration.

Using the CELENA® X for fluorescence cell imaging and to quantitatively analyze multiple cellular features from each image automatically gives researchers a simple way to monitor transfection-based cell assays. The same analysis pipeline can easily be reused to verify or compare results from subsequent experiments.

REFERENCES

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