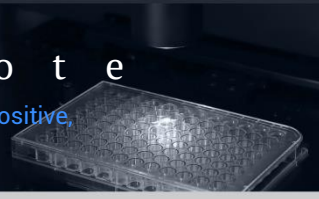

# Evaluating transfection efficiency using the CELENA® X

## 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 $1 \times 10^4$ 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 μg, 0.1 μg, and 0.2 μg) using Lipofectamine 2000 (Invitrogen, 11668) according to the manufacturer's protocol. 24-hours post-transfection, cells were fixed in 100 μL 4% PFA for 15 minutes at room temperature. To count total cell numbers, nuclei were counterstained with Hoechst 3342 (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 autofocusing 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 3342 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 | *IdentifyPrimaryObjects* | To find Hoechst-stained nuclei |
| 2 | *IdentifySecondaryObjects* | To find GFP-expressing cells |
| 3 | *MeasureObjectIntensity** | To measure GFP intensity |
| 4 | *FilterObjects* | To filter out non-transfected objects based on GFP intensity |
| 5 | *GrayToColor* | To produce color images from grayscale images |
| 6 | *OverlayOutlines* | 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).

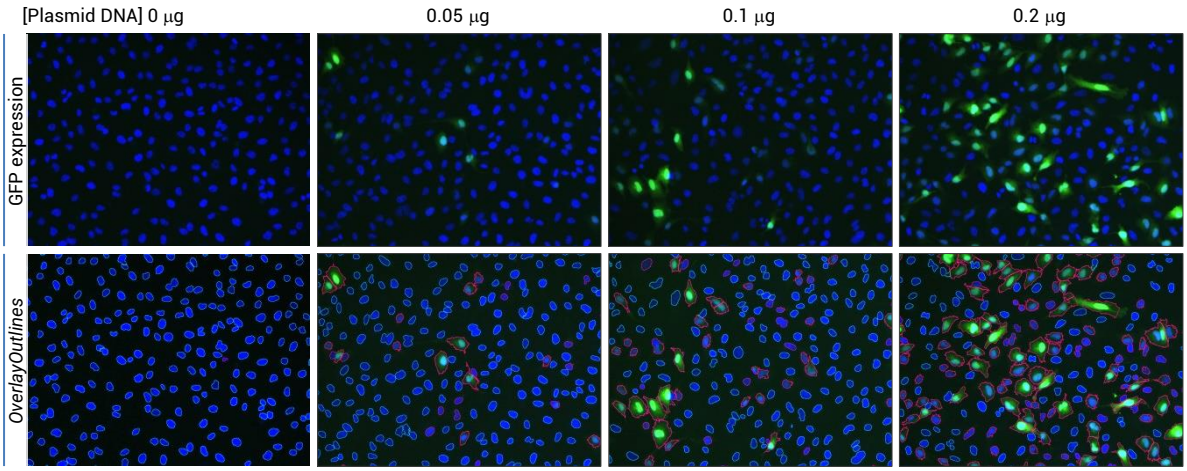


***Figure 1.*** *Image analysis of GFP expression in HeLa cells 24 h post-transfection. Higher plasmid concentrations correlated with increased numbers of GFP-positive cells (green). Blue lines indicate control Hoechst-stained nuclei (blue) and magenta lines outline GFP-positive cells.*

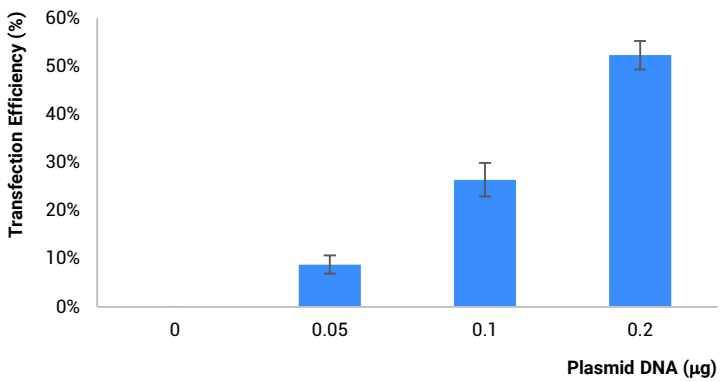


***Figure 2.*** *Quantification of transfection efficiency. The number of GFP-positive cells were compared to the number of Hoechst-stained nuclei. Transfection efficiency improved with increasing concentrations of the GFP-expressing pCG-HttQ103 plasmid.*

## 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.

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