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Trusted Answers

Automated Methods for Conducting Cell Culture Quality Control Studies and Assay Optimization

Application Compendium

Table of Contents

Introduction

Automated imaging tools can provide researchers with valuable information for improving routine cell culturing techniques and increasing the effectiveness and reproducibility of downstream cell-based assays. In this compendium, we describe quantitative imaging-based methods designed to provide accurate and objective evaluation of a number of important variables that contribute to experimental results.

Conventional methods for evaluating cell culturing techniques and assay optimization consist of manual inspection of a small subset of the cell population at random locations and time points. These methods rely on subjective measurements that are difficult to standardize and contribute to increased variability from round to round. In contrast, continuous monitoring of cell cultures using instruments capable of automated image capture and analysis provide a detailed record of a broad range of cell characteristics (Figure 1).

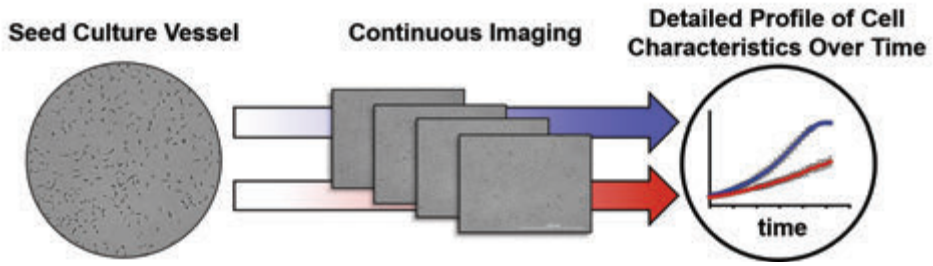

Figure 1. Kinetic monitoring of live cells using automated imaging and analysis provides detailed profiles of cell behavior over time while delivering increased assay reliability and walk-away time.

Agilent BioTek instruments, including the Agilent BioTek Lionheart FX and Agilent BioTek Cytation automated imaging systems, Agilent BioTek MultiFlo FX multimode dispenser, and the Agilent BioTek BioSpa live cell analysis system are designed to improve the accuracy and reproducibility of cell-based assays from culture to analysis (Figure 2).

Figure 2. The Agilent BioTek MultiFlo FX multimode dispenser, Agilent BioTek BioSpa automated incubator, and the Agilent BioTek Cytation cell imaging multimode reader support a diverse range of cell-based applications. The three instruments can be integrated into a single multi-platform workstation that provides complete workflow automation.

Cell Culture Quality Control and Assay Optimization Techniques

Robust image analysis method for quantifying cell density

Accurate quantitation of cell density, or confluence, is required for cell-based assays as well as routine tissue culture maintenance. Often, cell density measurements are assessed using highly subjective and imprecise methods, consisting of visual inspection of a small area of the culture vessel to approximate overall cell confluence. This process leads to inconsistent maintenance of cell lines and reduces the efficiency and reproducibility of downstream assays. In contrast, Ageilent BioTek Gen 5 automated image analysis tools provide accurate and reproducible percent confluence measurements for diverse cell types (Figure 3).

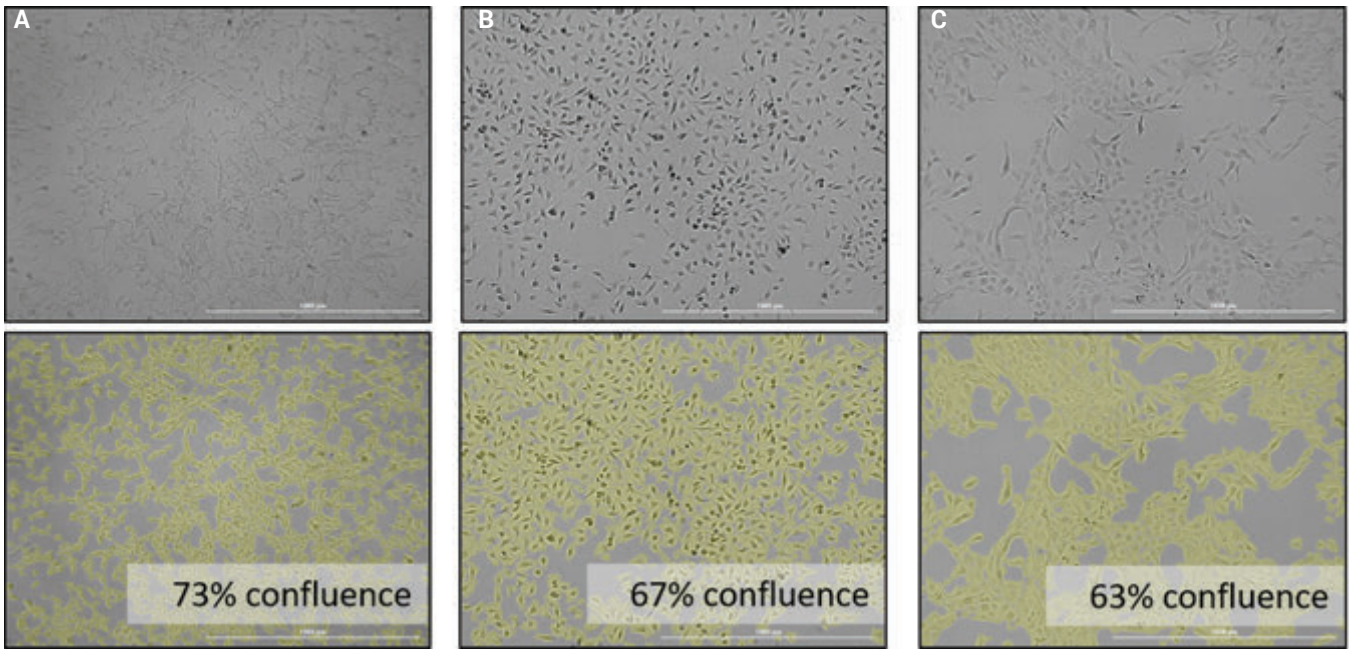

Figure 3. Agilent BioTek Gen 5 analysis tools enable accurate and reproducible quantitation of cell culture density for diverse cell morphologies. (A) NIH/3T3 (B) U-87 and (C) MCF-10A imaged using high contrast brightfield (HCBF) and percent confluence determined using Gen5 image analysis tools.

Measuring cell density in culture vessels

Reproducible cell-based assays require consistent maintenance of cell cultures and accurate evaluation of culture characteristics. Automated quantification of cell density within culture vessels enables routine passaging of cells at the optimal density and provides valuable information for conducting cell culture quality control studies. The entire area, or a representative subset of large culture vessels, including flasks and multiwell dishes, can be routinely imaged with confluence values automatically reported (Figure 4).

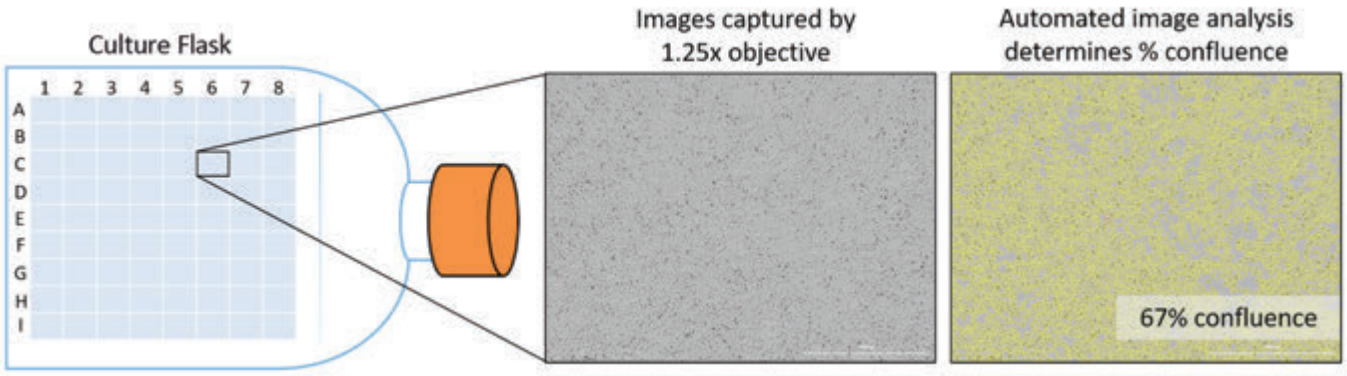

Figure 4. Automated quantitation of cell culture density within a large culture vessel.

Even distribution of cells within a culture chamber promotes predictable growth kinetics and uniform characteristics within the cell population. Gen5 generated cell density reports, including mean confluence and variation, can be employed to identify and address problems with cell culturing techniques that contribute to uneven cell density across the culture vessel (Figure 5). Additionally, this process can be used to standardize the process for identifying when to proceed with downstream applications and defining culture vessels exhibiting optimal properties.

Technical Tip

One common lab practice that contributes to uneven distribution of cells within culture vessels, especially microplates, is placing the vessel directly into the culture incubator immediately after seeding. The rapid change in temperature promotes a higher density of cells at the perimeter of the culture chamber compared to the center. To reduce this effect, culture vessels should be kept at room temperature for approximately 30 minutes after seeding, allowing adherent cells to evenly settle down and attach to the culture surface before transferring the vessel to a cell culture incubator.

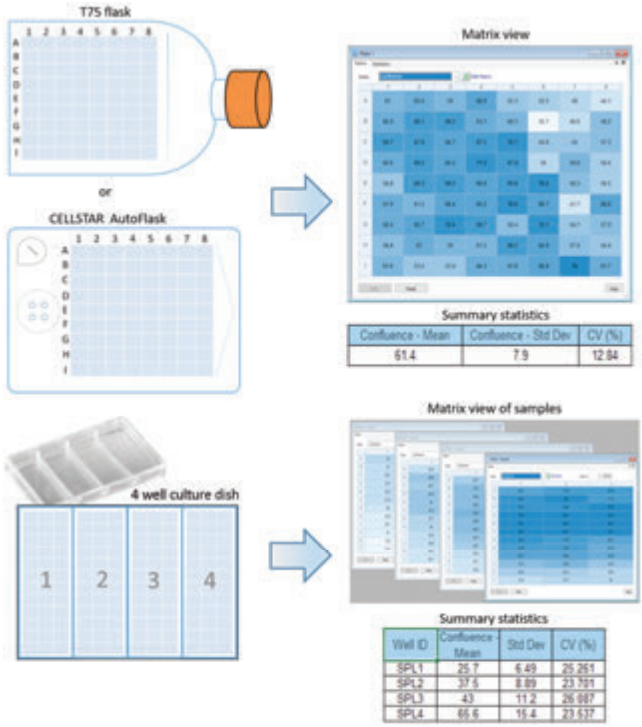

Figure 5. Cell culture confluence and density uniformity statistics can be determined within diverse culture vessels, including large culture flasks and multiwell dishes.

Monitoring cell culture growth kinetics

Cell proliferation studies for evaluating cell culture conditions and assay optimization

Kinetic growth profiles provide valuable information when designing live cell assays, including determining the appropriate number of cells to load per well based on doubling time and cell size, as well as desired experiment duration (Figure 6). This process can also be used to guide the optimization of cell culturing conditions, such as determining ideal serum and supplement concentrations.

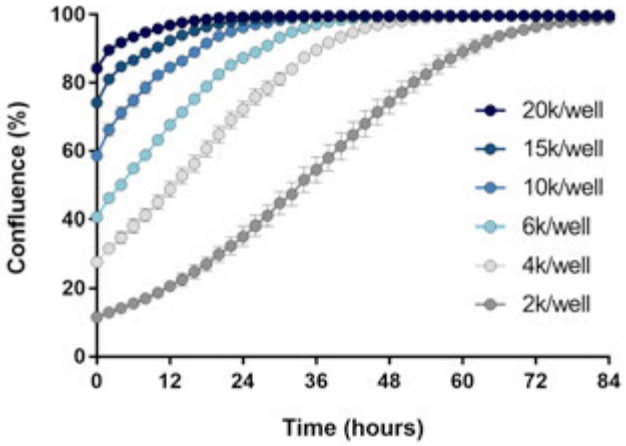

Figure 6. Kinetic growth profiles of HT1080 seeded at a range of cell densities. Images were acquired every two hours and percent confluence was determined using Agilent BioTek Gen5 image analysis tools.

Optimize staining protocols for kinetic live cell applications

A broad range of fluorescent stains are available for directly counting cells that are not amenable to label-free counting methods. However, the addition of these reagents can produce unintended cell type-dependent cytotoxic effects. Gen5 image analysis tools provide the ability to determine the appropriate concentrations of reagents to achieve efficient labeling through the experiment without significantly altering cellular behavior in the process. In the following example, HT1080 cells were stained with a range of Hoechst 33342 concentrations to determine an effective staining protocol to accurately measure treatment-induced changes in HT1080 proliferation rates. The minimal amount of stain necessary to effectively identify and count each cell was determined by imaging HT1080 stained with a range of Hoechst 33342 concentrations over a 48-hour time course and then calculating the signal-to-noise ratios over time (Figure 7).

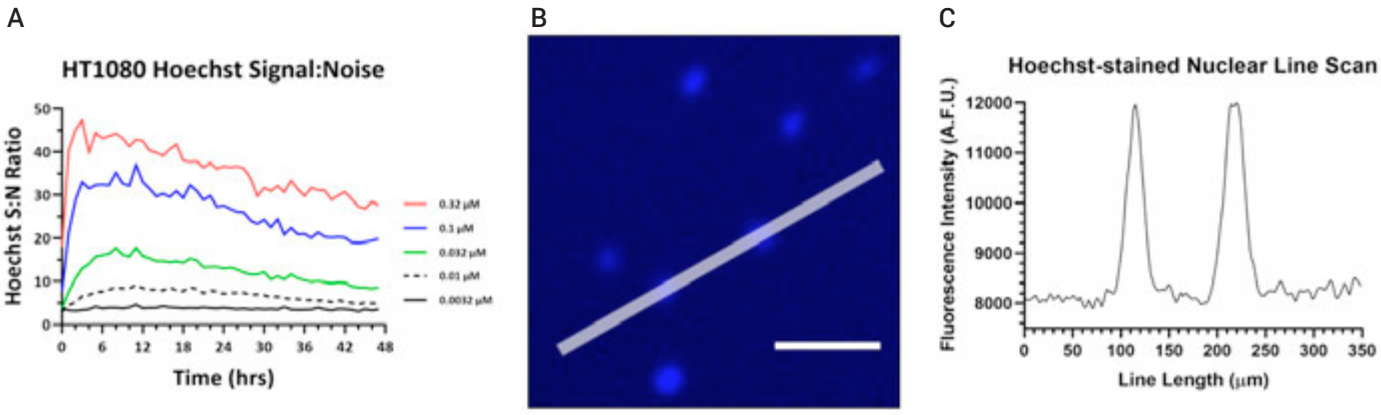

Figure 7. Procedure for determining minimal stain concentration for downstream assays using signal-to-noise ratio. (A) Signal-to-noise (S/N) ratio of Hoechst 33342 in proliferating HT1080 cells over time. Signal was derived by setting a primary mask around each nucleus, while background was measured using a secondary mask that extended 5 µm beyond the primary nuclear mask. The S/N value is the mean nuclear signal divided by the square of the standard deviation of the background signal. The S/N ratio at each concentration in the plot represents the mean SN ratio for all nuclei counted within a 4x image. (B) HT1080 nuclei imaged using an Agilent BioTek Cytation 5 cell imaging multimode reader after 12-hour incubation with 10 nM Hoechst 33342. The line represents a line scan to assess fluorescence intensity of signal above background. (C) A 350 µm line was drawn across two HT1080 nuclei and their fluorescence intensity profile was plotted to demonstrate sufficient signal of Hoechst 33342 at 10 nM.

The proliferation profiles generated for each population of stained cells were compared to test for any unintentional effect on HT1080 viability and growth kinetics (Figure 8). From these results, it was determined that 32 nM Hoechst 33342 delivered efficient staining throughout the specified timecourse without altering proliferation rates compared to unstained cells.

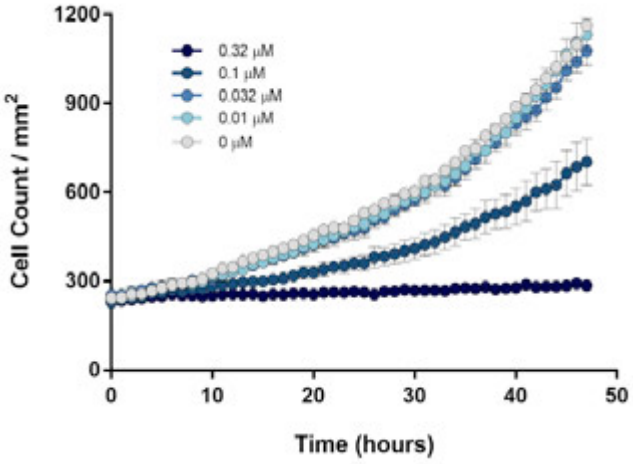

Figure 8. Effect of Hoechst 33342 concentrations on HT1080 proliferation kinetics. Cell counts of HT1080 stained with a range of Hoechst 33342 concentrations were determined using Agilent BioTek Gen 5 analysis tools over a 48-hour time course to reveal effects on proliferation rates. Label-free high contrast cell counting was used to measure negative control conditions (0 µM).

Automated culture techniques for long-term maintenance of 3D structures

Culturing cells in 3D provides a more *in vivo*-like environment, allowing cells to maintain high viability when cultured for extended time periods. To maintain the highest levels of viability within untreated cells and to ensure accurate evaluation of treatment-induced effects, media exchanges and redosing are necessary throughout the experiment, particularly those lasting days to weeks. Media exchanges with cell models that do not rely on adherence to labware can be daunting when relying on manual methods. Multichannel pipettes must remove and dispense media at an extremely slow rate, and care must be taken to keep pipette tips away from the actual spheroids to avoid accidentally damaging or removing the spheroids.

The Agilent BioTek MultiFlo FX multimode dispenser with the AMX automated media exchange module provides an automated method for effectively performing media exchanges without disturbing unattached 3D spheroids in 96- and 384-well formats. The AMX system delivers more consistent results compared to manual maintenance of 3D cell structures (Figure 9) and when coupled with Agilent BioTek automation, the media exchange tool provides a walk-away solution for monitoring and maintaining long-term 3D experiments. (Figure 10).

A

B

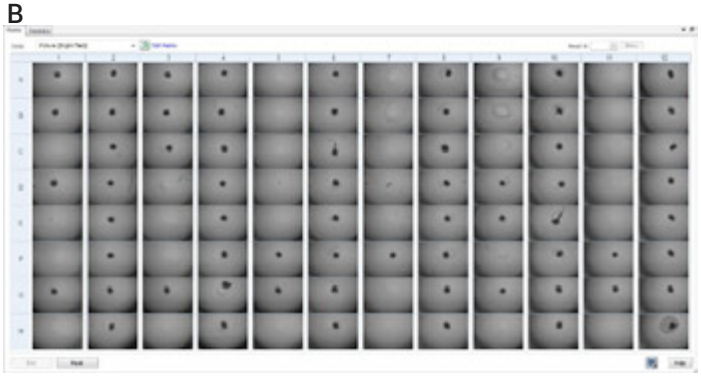

Figure 9. Automated maintenance of spheroid cultures conducted with the Agilent BioTek MultiFlo FX multimode dispenser. (A) The MultiFlo FX with AMX automated media exchange module performs reliable automated maintenance of spheroid cultures. (B) The 4x brightfield images were acquired of spheroid cultures in 96-well ULA microplates after two rounds of 3x 100 µL media exchanges. All spheroids remained undisturbed using the AMX module (even columns), while manual washes (odd columns) resulted in the loss of approximately 50% of spheroids after one round, and 70% after two rounds.

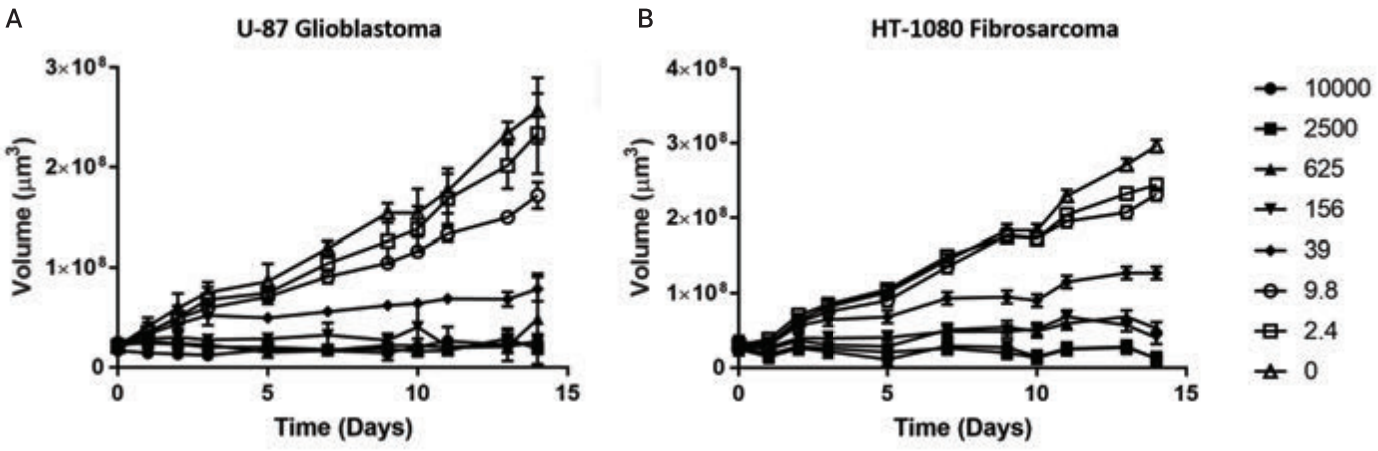

Figure 10. Integrated AMX module-driven media exchanges enable long-term automated 3D proliferation assays. Spheroids cultured within the Agilent BioTek BioSpa live cell analysis system over a 14-day time course received media exchanges every three days using the integrated Agilent BioTek MultiFlo FX AMX module. Effect of chronic exposure to camptothecin concentrations on spheroid growth was evaluated using Agilent BioTek Gen5 image analysis tools and calculated volume measurements. (A) U-87 glioblastoma spheroids in Greiner 96-well spheroid plates; (B) HT1080 spheroids in Corning 96-well spheroid plates.

Measuring cellular transfection and transduction procedures

Introduction of novel genetic elements into cultured cells is routinely conducted for a variety of applications. While there are numerous methods to introduce foreign DNA into tissue culture cells, all require cell-type dependent optimization to achieve desired results. Important variables include the amount of plasmid or virus that is delivered, culture conditions, and expression kinetics of the construct. Automated imaging and analysis tools provide an efficient and robust method for measuring transfection and transduction efficiency and characterizing transient gene expression kinetics.

In the example shown in Figure 11, increasing amounts of GFP fusion tag lentiviral vector were added to NIH/3T3 cells in a multi-well microplate. After 24 hours cells were imaged and Gen5 enabled cell counting and subpopulation analysis was then used to report the relative number of cells successfully transduced for each condition. Identification of total cell number can be achieved using a nuclear marker, such as Hoechst 33342 shown here, or using Gen5 label free cell counting. A dose response curve was then generated to identify the optimal amount of vector for maximum transduction efficiency (Figure 12).

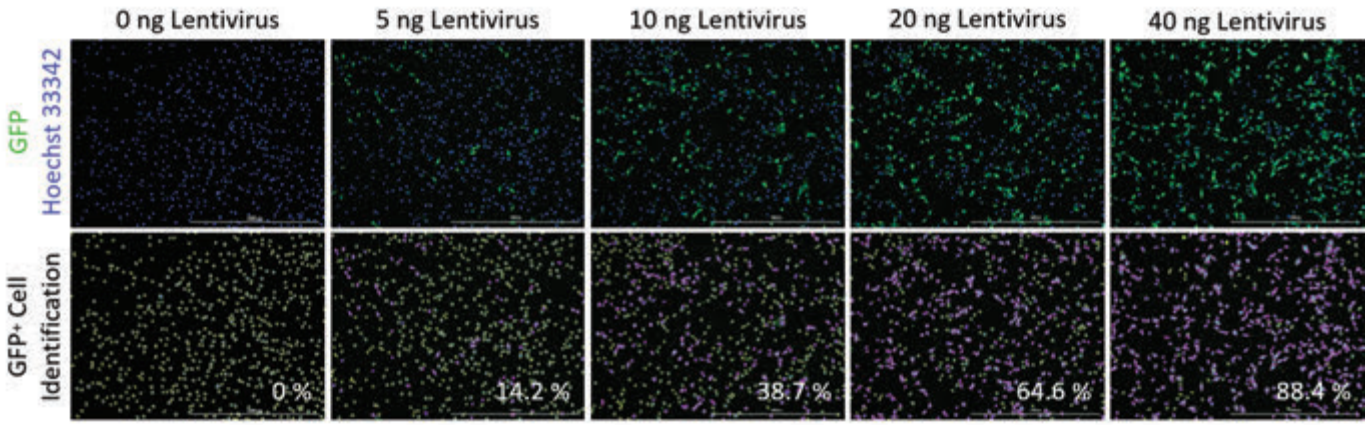

Figure 11. Transduction efficiency determined using automated imaging and subpopulation analysis. NIH/3T3 cells were seeded in a 96-well microplate with increasing amounts of GFP fusion tag lentiviral vector and 1 µM Hoechst 33342. After 24 hours, automated imaging and analysis was conducted to identify cells not expressing GFP (yellow mask) and cells expressing GFP above a defined threshold (violet mask), with the number of transduced cells relative to the total number of cells automatically reported per well.

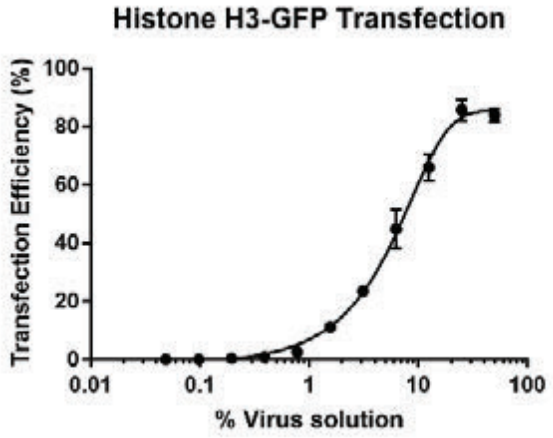

Figure 12. Agilent BioTek Gen 5 generated dose response curve for determining transfection efficiency as a function of viral titer.

Profile transient gene expression kinetics

Kinetic live-cell analysis can provide valuable information about how cell types of interest express transgenic constructs over time. This information can be used to identify not only the most effective method for transducing or transfecting cells, but also for determining the optimal time to conduct an experiment, as well as defining the window of time in which an experiment is feasible. As shown in Figure 13, HEK293 cells were transduced with increasing concentrations of a BacMam vector carrying a fluorescent biosensor. After seeding within a microplate, the cells were continuously monitored to measure the amount of cells within the population expressing the biosensor over time. Kinetic profiles of biosensor expression for each condition reveals the optimal amount of vector to maximize transduction efficiency, as well as define the period of time post-

transduction in which expression levels stabilize (Figure 13A). Transduction and transfection protocols often require optimization for each cell type. HEK293 and HT1080 cells were transduced using the same protocol, however the HT1080 exhibit lower transduction efficiency compared to HEK293 with considerably different expression kinetics (Figure 13B).

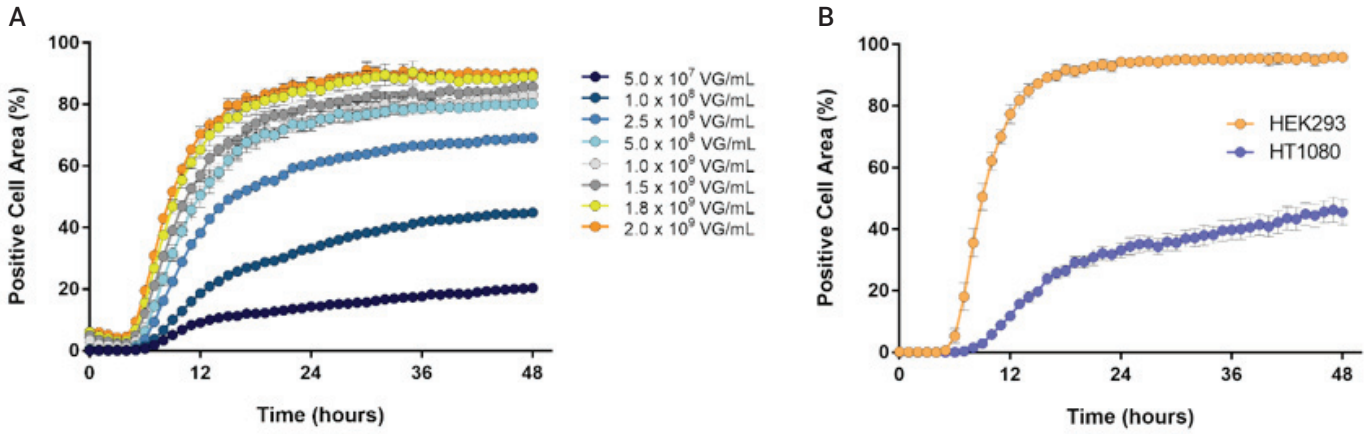

Figure 13. Characterizing expression kinetics over time. HEK293 cells were transduced with increasing concentrations of BacMam vector carrying a fluorescent biosensor. Brightfield and fluorescence time-lapse images were acquired at 10x. (A) Biosensor expression profiles were generated from the ratio of cell area with a fluorescent intensity above a set threshold to the total cell area defined using the brightfield channel. (B) Comparison of the relative transduction efficiency and biosensor expression profiles for HEK293 and HT1080 cells.

Conclusion

Agilent BioTek automated imaging systems and liquid handlers provide diverse tools for conducting cell culture quality control studies and optimizing cell-based assays. Automated workflows improve the efficiency and reproducibility of routine cell culture tasks and assay setup, improving the accuracy and reliability of downstream results. Image-based analysis provides accurate and objective quantitation of cell culture characteristics, including culture density, growth kinetics, and gene expression profiles. Automated liquid handling routines enable automated cellseeding and maintenance of cell cultures, including 3D formats that are easily disrupted using manual methods.

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