Video Article

Genetic Barcoding with Fluorescent Proteins for Multiplexed Applications

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Abstract

Fluorescent proteins, fluorescent dyes and fluorophores in general have revolutionized the field of molecular cell biology. In particular, the discovery of fluorescent proteins and their genes have enabled the engineering of protein fusions for localization, the analysis of transcriptional activation and translation of proteins of interest, or the general tracking of individual cells and cell populations. The use of fluorescent protein genes in combination with retroviral technology has further allowed the expression of these proteins in mammalian cells in a stable and reliable manner. Shown here is how one can utilize these genes to give cells within a population of cells their own biosignature. As the biosignature is achieved with retroviral technology, cells are barcoded 'indefinitely'. As such, they can be individually tracked within a mixture of barcoded cells and utilized in more complex biological applications. The tracking of distinct populations in a mixture of cells is ideal for multiplexed applications such as discovery of drugs against a multitude of targets or the activation profile of different promoters. The protocol describes how to elegantly develop and amplify barcoded mammalian cells with distinct genetic fluorescent markers, and how to use several markers at once or one marker at different intensities. Finally, the protocol describes how the cells can be further utilized in combination with cell-based assays to increase the power of analysis through multiplexing.

Video Link

The video component of this article can be found at <http://www.jove.com/video/52452/>

Introduction

Technologies such as fluorescence spectroscopy, fluorescence microscopy and flow cytometry, all rely on fluorescence, a property widely exploited in biochemical, biomedical, and chemical applications. Fluorescence, whether intrinsic or through labeling, has been exploited for the analysis of protein expression patterns and profiles, cell fate, protein interactions and biological functions¹⁻⁹, and through fluorescence/Förster resonance energy transfer for the detection of biomolecule interactions and conformational changes¹⁰⁻¹³. Since the isolation of the *Aequorea victoria* green fluorescent protein (GFP)¹⁴, the discovery of additional naturally occurring fluorescent proteins from other cnidarians, particularly corals, has largely increased the number of existing fluorescent proteins with distinguishable excitation/emission spectra. These, together with the introduction of mutations in their genes¹⁵⁻¹⁹, have further expanded the possibilities, obtaining a true palette of fluorescent proteins available to scientists that exploit microscopy, flow cytometry and other fluorescence-based technologies for their research.

In parallel, although independently, the development of retroviral technology has drastically facilitated the stable expression of ectopic genetic information in mammalian cells²⁰⁻²³. It is thus not surprising that this technology has been used to transfer genes of fluorescent proteins into a broad number of cell types and tissues²⁴⁻²⁸ or for production of transgenic animals²⁹⁻³¹. Following the nature of retroviruses, the genetic information of the ectopic fluorescent protein is introduced within the genome of the cell³² and the cell becomes fluorescent 'for ever'. This property has allowed tracking of cell fate, or of a single cell within a population of cells. The now fluorescent cell has thus acquired its own biosignature and can be defined as barcoded. Its unique biosignature identifies it from other cells, and importantly, distinguishes it from cells genetically manipulated to express different fluorescent proteins with distinguishable absorption/emission spectra. Biological applications such as the tracking of reprogramming factors toward pluripotency³³, the analysis of subnuclear factors for the elucidation of nucleolar localization³⁴, the construction of fluorescent reporter plasmids for transcriptional studies³⁵ or the genetic labeling of neurons for the study of neuronal network architecture³⁶, are just four examples of the many that have exploited different fluorescent protein genes for the same experimental setup.

Flow cytometry has been broadly utilized for the analysis of biological processes at the single cell level, such as gene expression, cell cycle, apoptosis, and signaling through phosphorylation³⁷⁻⁴³. The stable expression of fluorescent protein genes in mammalian cells has further enhanced the utility of flow cytometry for cell analysis^{38,44} and ligand-receptor interactions⁴⁵. Enhanced capabilities have allowed flow cytometry to become a widely utilized methodology for high-throughput and high-content screening⁴⁶. Despite the now expanded number of fluorometers and robotics technologies that can couple plate reader systems, imaging and flow cytometry, there seems to be a lack in experimental design that can exploit and fit these enhanced technological capabilities.

Fast, reliable, simple and robust cell-based methodologies are drastically needed for multiplexed applications that further enhance high-throughput capacity. This is especially true in the field of drug discovery where engineering cell-based assays in a multiplexed format can

enhance the power of high-throughput screening^{39,47-50}. Multiplexing, as it allows simultaneous analyses in one sample, further enhances high-throughput capabilities⁵¹⁻⁵⁴. Fluorescent genetic barcoding not only allows for elegant multiplexing, but also, once engineered, circumvents the need of time consuming protocols, reduces costs accompanied with antibodies, beads and stains^{39,52,55}, and can reduce the number of screens required for high-throughput applications. We have recently described how retroviral technology can enhance multiplexing through fluorescent genetic barcoding for biological applications, by expressing an assay previously developed to monitor HIV-1 protease activity^{56,57} with different clinically prevalent variants⁵⁸. The methodology is explained in a more descriptive manner focusing on how to select and amplify genetically fluorescent barcoded cells and how to produce panels of clonal populations expressing distinct fluorescent proteins and/or different fluorescence intensities. Panels of cell populations distinguishable based on their fluorescent characteristics enhance multiplexed capabilities, which can be further exploited in combination with cell-based assays that tackle different biological questions. The protocol also describes how to engineer a panel of barcoded cells bearing one of the cell-based assays previously developed in the laboratory, as example⁵⁹. This protocol is thus not intended to show the well-established retroviral/lentiviral technology for genetic transfer, the value of fluorescent proteins or the applications of flow cytometry^{60,48} but rather to show the enhancing power of combining the three for multiplexed applications.

Protocol

1. Preparation of Mammalian Cells, Viral Production and Transduction for Genetic Barcoding

1. Plate 2.5×10^6 adherent cells in a 10 cm plate (or around 50-60% confluency) one day prior to transfection in Dulbecco's modified eagle medium (DMEM) with 10% fetal calf serum (FCS). For retroviral production use packaging cell-line of choice such as Phoenix-GP (a kind gift from Gary Nolan, Stanford University).
NOTE: The packaging cell line stably expresses Gag and Pol proteins for retroviral particle formation *in trans*. Make sure cells are healthy and adhered to the plate in a monolayer, prior to transfection.
2. 24 hr later, prepare the DNA transfection mixture.
 1. To 125 μ l of serum-free DMEM add 3 μ g Vesicular Stomatitis Virus Envelope glycoprotein vector (pCI-VSVg) and 3 μ g of transfer vector carrying the fluorescent protein gene of interest. Mix and add 15 μ l of polyethylimine (2 mg/ml).
 2. Incubate mixture at room temperature for 15 min and add to cells drop-wise.
NOTE: Polyethylimine is added to the homogenous DNA mixture as transfection reagent for the formation of polyplexes. In order to obtain viral particles carrying different fluorescent protein markers, perform each transfection independently with the marker of choice. Remember that the retroviral transfer vector is around 7 kb in length and cannot be packaged if above 10 kb.
3. Incubate plates at 37 °C. In order to increase viral production place plates at 30 °C or 32 °C instead.
NOTE: 24 hr post transfection media can be replaced with 7 ml of fresh media, and although this may improve cell health it may decrease viral load in the supernatant.
4. 48 hr post transfection collect the supernatant-containing viral particles and filter with a 0.45 μ m polytetrafluoroethylene filter. Use freshly collected viral supernatant for transduction. Otherwise keep supernatant in aliquots at -80 °C and avoid freeze-thaw. Remember that viral titer will decrease probably up to 50% with each freezing-thawing cycle.
5. Plate cell line of choice 24 hr prior to transduction if adherent, or immediately before transduction if non-adherent. Seed 2.0×10^5 to 3.0×10^5 adherent cells (here Huh 7.5.1 and HEK 293T) or 5.0×10^5 to 1×10^6 non-adherent cells (here SupT1) per well in a 6-well plate.
NOTE: Make sure to calibrate the number of cells to be seeded prior to transduction, especially so for adherent cells, so cells achieve a confluency of around 60% at the time of transduction.
6. Add 5 μ g/ml Polybrene (hexadimethrene bromide) to seeded cells and then add previously collected viral supernatant to obtain around 20-40% infection (here 2 ml).
NOTE: The MOI or percentage of infection is mostly arbitrary, as sorting will allow to amplify any subpopulation of cells. Obviously, a higher rate of infection eases and speeds up the process of amplification.
7. Transduce cells by centrifugation in a hanging bucket rotor at 1,500 x g for 120 min at 32 °C. Resuspend non-adherent cells with fresh media prior to incubation. For adherent cells, replace with fresh media 24 hr post-transduction.
NOTE: It is recommended to seal the plates prior to centrifugation with parafilm to avoid spilling over. To increase genetically barcoded cell diversity, transduce cells with viral particles carrying different fluorescent proteins (obtained from step 1.4). When choosing fluorescent markers, make sure they are compatible with the instrumentation and they have distinguishable fluorescent parameters.

2. Selection and Amplification of Genetically Barcoded Cells

1. Analyze transduced mammalian cells by flow cytometry at least 72 hr post transduction to quantitate the percentage rate of transduction. Use a comparable non-transduced cell line as negative control.
NOTE: As sorting will be used to purify individual barcoded cell populations, a high percentage rate of initial transduction, while not necessary, should speed up the process of amplification.
2. Utilize the cell-sorter of choice to obtain the cells that express the protein of interest.
 1. For that purpose, ensure the gates are properly set in the right channels.
 1. To do so use the same naïve cell line as negative control and set the parameter/channel voltage so that the negative population is below the value of 10^3 on each fluorescent axis. Determine gating for positive fluorescence as events that appear above that of the negative control for each fluorescent channel.
 2. Be aware that each instrument may vary and the correct voltage for determining gating should be adjusted accordingly, making positive and negative controls imperative in every experimental setup.

1. For fluorescence detection in this experimental setup use the FITC channel (530/30 band pass filter preceded by a 502 long pass filter) for eGFP, the PE channel (585/42 band pass filter preceded by a 556 long pass filter) for td Tomato, and the APC channel (660/20 band pass filter) for E2 Crimson.

NOTE: The 488 nm laser excites eGFP and td Tomato while E2 Crimson is excited by the 633 nm laser.

3. Sort into 15 ml conical tubes with 1 ml of 100% FCS.
NOTE: The process of sorting fluorescent cell populations is not compulsory as one can directly proceed with the sort of individual clones (see below). Sorting tight populations based on chosen fluorescence ensures a backup population for future selection of individual clones. Keep in mind that individual cells are at times difficult to grow while a large population of cells can be easily frozen, and then thawed and amplified at a later stage.
4. Spin down the sorted populations in a hanging bucket rotor centrifuge at 524 g at 32 °C for 5 min to pellet cells. Re-suspend in 1 ml of fresh media, and re-plate cells so that cell confluency is below 60% to allow growth and division for at least two days.
 1. For example, plate adherent cells at around 3×10^5 cells in 2 ml of media per well in a 6-well plate and 2×10^6 cells in 2 ml of media in a 6 cm plate. Use DMEM for adherent cells and RPMI for non-adherent cells (or any specified medium if required). Place plated cells in an incubator at 37 °C for several days to allow amplification.

3. Obtain Clonal Populations of Genetically Barcoded Cells at Different Intensities

1. Obtain clonal populations from cells originally transduced (step 1.7) or from the sorted population (step 2.3).
NOTE: This will ensure equal or similar level of fluorescent protein expression among all the cells within the population (a tight population with a small CV in mean fluorescence intensity of up to 40%).
 1. For that purpose, sort single cells into 96-well plates with an automated cell deposition unit (ACDU). Set gates for sorting according to populations of interest. Ensure gates are set at least one log-scale apart to obtain distinguishable populations when choosing cells at different fluorescent intensities. For the procedure shown here, use the same flow cytometry experimental set up as the one described in step 2.2.
2. Amplify individual clones in an incubator at 37 °C for at least 2 weeks. Through the process of amplification analyze the cells under the microscope to ensure that growing populations arise from individual cells and not from more than one.
NOTE: Remember that the sorter will place one cell per well on average, and while some wells may not have any cell at all, others may have more than one.
 1. To ensure that a population arises from one cell only, be extremely gentle with the plate and never disturb the wells by pipetting. Observe the plate, well by well, under the microscope.
NOTE: While individual cells may at times be difficult to identify, once they start dividing they will be easily recognizable.
 2. Be aware that often cells 'clump' along the edges. Discard those wells where multiple clonal populations can be observed and keep those that have one tight population.
NOTE: It is advisable to transfer those into larger plates for amplification.
3. Screen the putative clonal populations by analyzing them by flow cytometry. Compare them to a negative control utilizing the same flow cytometry experimental setup.
 1. Analyze only a percentage of the sample and keep the rest as backup. Choose the most distinctly separate populations based on average intensity and tightness of the population. Amplify them as needed or freeze stocks in freezing media with 20% glycerol at -80 °C for short periods of time or liquid nitrogen for longer.
NOTE: Remember that a 'true' clonal population should have a very tight fluorescence pattern.

4. Ensure Multiplexing Capabilities for High Throughput Screening (HTS)

1. Combine as many distinct clonal populations as needed that can be further utilized in a multiplexed format. Choose clones with distinguishable absorption/emission spectra and/or different intensities. If a 96-well plate format is used, which is suitable for HTS, seed 50,000 cells in 200 µl per well.
NOTE: Keep in mind that the number of cells is only an estimate and may change based on cell-type and whether it is adherent or not.
2. Analyze the sample using flow cytometry to ensure that each of the independent established fluorescent cell lines can be distinguished from each other. Prior to the analysis prepare negative and single color controls to set parameters and compensation values.
NOTE: Setting the parameters to clearly distinguish the mixed populations will enable them to be further utilized in a multiplexed format.

5. Adapt Genetically Barcoded Cell Lines to the Biological Application of Choice

1. Transduce established genetically barcoded cell lines with viral particles that contain the assay elements of choice, as described here in Stolp *et al.*, 2013⁵⁹. Remember that the assay of choice should occupy an additional and distinguishable fluorescent channel and thus it is to be transferred to the appropriate barcoded cell line.
NOTE: Each barcoded cell line can bear a different assay.
2. Sort genetically fluorescent barcoded cells for those that contain assay elements.
NOTE: Assay elements can be engineered coupled to a tag or fluorescent protein (as a fusion or through an internal ribosome entry site) for straightforward detection through western blotting, flow cytometry or microscopy. The system utilized in this protocol relies on HA epitope surface expression alone, or with additional FLAG epitope expression.
 1. Stain the clonal populations containing the assay with HA and APC-fluorescently coupled antibodies. Then analyze the clonal populations with anti-FLAG and FITC-conjugated antibody staining.

2. To track back the assay, analyze or 'decode' the populations in the appropriate channel where the distinct genetically barcoded cell lines can be distinguished. Here, decode the nature of the substrate by analyzing in the PE channel for td Tomato detection.

Representative Results

Multiplexing fluorescent genetically barcoded cells for the purpose of biological applications can only be achieved once individual clonal populations have been generated. Multiplexing is most effective when barcoded populations have clear distinct fluorescent characteristics with minimal spectral overlap. The example shown in **Figure 1** with clonal populations of mammalian SupT1 cells illustrates that barcoded cells with mCherry and cyano fluorescent protein (CFP) can be easily analyzed simultaneously without losing their individual fluorescent characteristics. This matrix thus exemplifies a panel of fluorescent genetically barcoded cells that is usable for multiplexing biological applications with a readout in yet an additional available channel. In order to obtain such a panel it is important to remember the nature of retroviral technology, which will lead to variable ranges of fluorescent intensities within the population due to insertional effects and/or MOI. **Figure 2** illustrates that initial transduction of mammalian cells with viral particles containing either td Tomato or E2 Crimson results in cells that express either one or both of the fluorescent proteins at a wide range of intensities (left panel). Selection and sorting of single cells into 96-well plates as shown by the gated boxes (mid panel), allows one to obtain tight clonal populations following expansion (right panel). A tight population should be defined as having a small CV which may range according to cell type, normally 30-40% in mean fluorescence intensity. **Figure 2** also illustrates that to further increase the number of genetically barcoded populations with a matrix of only two fluorescent proteins, one can also exploit fluorescence intensity. Generally, one log deviation in the mean fluorescence intensity between populations is suitable for achieving appropriate separation from each other following sorting and amplification. Td Tomato was chosen in the shown example to illustrate this feature, where two populations of differing intensities, mid and high, were obtained for multiplexing with E2 Crimson at a single intensity.

Multiplexing is a powerful tool to facilitate the analysis of many samples at the same time and for the ability to decode masked populations. Enhancing multiplexing can be achieved with yet a third fluorescent protein such as eGFP, as long as spectral properties do not interfere with each other. In the experimental procedure illustrated in **Figure 3** the panel of six populations obtained with td Tomato and E2 Crimson was exploited to increase multiplexing with eGFP. Retroviral technology was used to transfer eGFP to the populations represented in one of the matrices. When observed in the eGFP channel, the non-green naïve six-population matrix (upper left panel in **Figure 3**) is indistinguishable from the eGFP-transduced population matrix (lower left panel in **Figure 3**). Importantly, the panels can be analyzed in the channels occupied by the original genetic barcode (td Tomato and E2 Crimson). While indistinguishable in these channels (compare populations 1-6 with populations 7-12 in mid panels, **Figure 3**), the individual populations can be analyzed in the eGFP channel, and decoded or tracked back, as shown in the histograms (right panels in **Figure 3**). After repeating the process of transduction, selection, sorting, and amplification, taking advantage of the unoccupied channels is useless if right compensation is not applied to adjust for possible spectral overlap. To prove this, when populations 1, 2, 3 (non-green) and 7, 8, 9 (green) are analyzed in the eGFP and td Tomato channels, populations 7 and 8 are difficult to distinguish (left panel in **Figure 4**). It is thus necessary to choose naïve cells (population 1) as a negative control so that the parameters of the instrumentation can be set. When analyzing any matrix, especially with fluorophores that spectrally overlap, it is imperative that single color controls are used to determine the correct compensation values. In the analysis of **Figure 4** populations 2 or 3, and 7 serve this purpose, allowing to better define populations that are truly double positive (populations 8 and 9).

Genetically barcoded cells with fluorescent markers retain their own identity as defined by their biosignature, when analyzed in the right fluorescent channel with the right instrument. However, the biosignature becomes just an additional individual property unless exploited for biological applications. In order to prove the power of fluorescent genetic barcoding for multiplexing we decided to introduce one of our previously developed assays for drug discovery into some of the fluorescent barcoded mammalian cell lines. By doing so, fluorescent genetic barcoding was exploited to achieve three assays in one sample. In this procedure a scaffold protein containing one of three putative viral substrate for proteolysis was transferred into the genetically barcoded cells. In the assay, cleavage is revealed by the loss of the FLAG epitope on the cell surface (**Figure 5B**). In contrast, FLAG surface staining represents lack of cleavage. **Figure 5** illustrates the result of the analysis of three different substrates; HIV-1 Envelope wild type (Env wt), HIV-1 Envelope mutant (Env mut), and Dengue Virus (Denv) prM. Each of them was introduced into 1 of 3 barcoded cell lines, naïve, and 2 others utilizing td Tomato at different intensities (mid and high; **Figure 5A**). Staining for FLAG surface expression reveals which of the substrates was cleaved based on the respective barcode. In the example only HIV Env mut retains the FLAG tag (positive following staining), as seen in the green-FITC channel (**Figure 5C**, right bottom panel). The analysis of the assay can thus be performed independently of the original barcoding, and be further exploited to decode and track back the distinct populations. This method of multiplexing thus relies on an additional channel reserved for the biological readout of interest.

Figure 1: Barcoding for multiplexing. Individual populations genetically barcoded using distinct fluorescent proteins such as mCherry, CFP or both (left panel) can be mixed, analyzed, and decoded (right panel) in their respective channels via flow cytometry. Adapted from Smurthwaite, C. *et al.*, 2014⁵⁸. [Please click here to view a larger version of this figure.](#)

Figure 2: Sorting and amplification of barcoded cells. Mammalian cells were analyzed 48 hr following transduction with viral particles containing either td Tomato or E2-Crimson (left panel). Gates are then set for sorting to include cells expressing td Tomato at different intensities, with/out E2-Crimson (middle panel). Clones from the sorts were amplified and re-analyzed to generate a matrix of six distinguishable populations (right panel). [Please click here to view a larger version of this figure.](#)

Figure 3: Decoding reveals masked populations. The matrix of six populations obtained in **Figure 2** (td Tomato and/or E2-Crimson) can be further engineered to express an additional fluorescent protein such as eGFP. **(A)** The original matrix, when analyzed in the eGFP channel (left panel) is negative and the six populations are indistinguishable from each other. Each of the six populations can be independently analyzed in the eGFP channel as shown in the histograms (right panels). **(B)** The same matrix, now expressing also eGFP, was analyzed as in **A**, revealing now their green fluorescent characteristic. Adapted from Smurthwaite, C. *et al.*, 2014⁵⁸. [Please click here to view a larger version of this figure.](#)

Figure 4: Compensation ensures correct separation. Some of the populations originally chosen based on td Tomato and/or eGFP expression (populations 7 and 8) cannot be properly separated when analyzed together (left panel) unless appropriate compensation for these channels is adjusted (right panel). [Please click here to view a larger version of this figure.](#)

Figure 5: Selection of barcoded cells for further adaptation to chosen assay. (A) Selection and adaptation to assay of choice. Populations genetically barcoded with td Tomato at different intensities (top left panel) were used for biological applications. Each of the populations was further engineered to contain an assay that monitors cleavage, but each one of a different substrate (top right panel). (B) Depiction of the assay. Positive FLAG stain indicates lack of cleavage while negative FLAG stain indicates cleavage. (C) Analysis of the assay following FLAG staining. Mixed populations are distinguishable based on the td Tomato barcode but indistinguishable in the FITC channel (left panels). When stained with FLAG-FITC antibody, only one population is positive for FLAG. Decoding reveals, based on genetic barcoding, that this population bears the HIV Env mut substrate (right panel). [Please click here to view a larger version of this figure.](#)

Discussion

Here two well-established procedures have been combined; genetic engineering through retroviral technology and detection of fluorescent proteins utilizing flow cytometry. Fluorescent protein-based genetic barcoding for the production of unique cell lines provides a robust and simple way for multiplexed applications. Generating genetically engineered barcoded cells through retroviral technology, is initially a lengthy process, but allows one to obtain, once established, a reliable and stable source of cell material. The nature of this technology consistently produces genetically engineered cell lines with steady fluorescent characteristics that become their own true distinguishable biosignature.

Both adherent and non-adherent cell types can be barcoded utilizing fluorescent proteins that are easily distinguishable based on their physical absorbance/emission spectra. These include, but are not restricted to proteins such as CFP, mCherry, E2 Crimson and td Tomato. Once genetically barcoded with their distinguishable fluorescent protein, they can be combined to produce a panel of cell populations. Importantly, the panel contains cell populations with the exact genotypic make-up but differentiated only by their fluorescence trait. As such, these panels are perfectly suited for multiplexed applications, greatly enhancing high-throughput capabilities.

Due to the different insertional preferences of the retroviral particle into the host genome, coupled with differences in MOI, variable fluorescence intensities from the carried particular fluorescent protein gene are expected. Analysis of a genetically engineered population carrying a unique fluorescent gene will thus detect a range of expression profiles that can be defined based on fluorescence intensity. This differential expression profile can be exploited to create populations that are spectrally distinct from one another. One can thus combine choice of fluorescent markers with fluorescence intensity to fit the experimental design. Here, matrices of 4, 6, and 12 populations, which include two 6 population panels of E2 Crimson and td Tomato, with or without eGFP, are shown. In theory, this can be further expanded with different intensities of eGFP as well to create populations of 3 different fluorescent proteins; each one with different intensities associated with it. As stated previously, consideration should be taken when choosing the fluorescent proteins in order to ensure that separation can indeed be achieved. Chosen proteins should have distinct absorbance and emission spectra; however, when two spectrally distinguishable but similar fluorescent proteins are used, compensation should be applied. Importantly, proper compensation, which allows for physical separation of emission/absorbance spectra among different fluorophores or fluorescent proteins, is accomplished with the use of appropriate single color controls for detection via flow cytometry.

Multiplexing with fewer fluorescent proteins but exploiting a variety of intensities frees additional channels that can be then further exploited for the biological application of interest. This can increase flexibility of the experimental set-up and simplify the choice of the combination of fluorescent markers to be used. For example, the multiplexed assay shown in **Figure 5** relies on antibody staining, in this case coupled to FITC. As only the PE channel is occupied by genetic barcoding, one can utilize FITC-conjugated antibodies, or conversely, APC-coupled, a decision that can be made based on the appropriate instrumentation and/or antibody availability.

While the technological achievements in the field of flow cytometry, microscopy or combined methodologies are impressive, the bottleneck seems to be the available cell technology for biological applications and HTS. Retroviral technology coupled with the expanding number of fluorescent proteins can be merged to answer this query. Fluorescent genetic barcoding drastically facilitates multiplexing, which in turn, satisfies

the increasing need for expanding HTS capabilities of cell-based assays. Genetic barcoding leads to a robust, cost effective, and simple way to couple cell-based assays with high-throughput methodologies for biological applications and drug screening. The power of performing one rather than three screens using a 3 population panel has beneficial cost and time related implications. With the growing versatility in flow cytometry, high-content imaging and flow-cytometry-coupled microscopy, fluorescent genetic barcoding will be a consistently reliable tool for assay development.

Disclosures

The authors have nothing to disclose.

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