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Master of Science HES-SO in Life Sciences

Development of a transfection evaluation method for cell line construction using chinese hamster ovary

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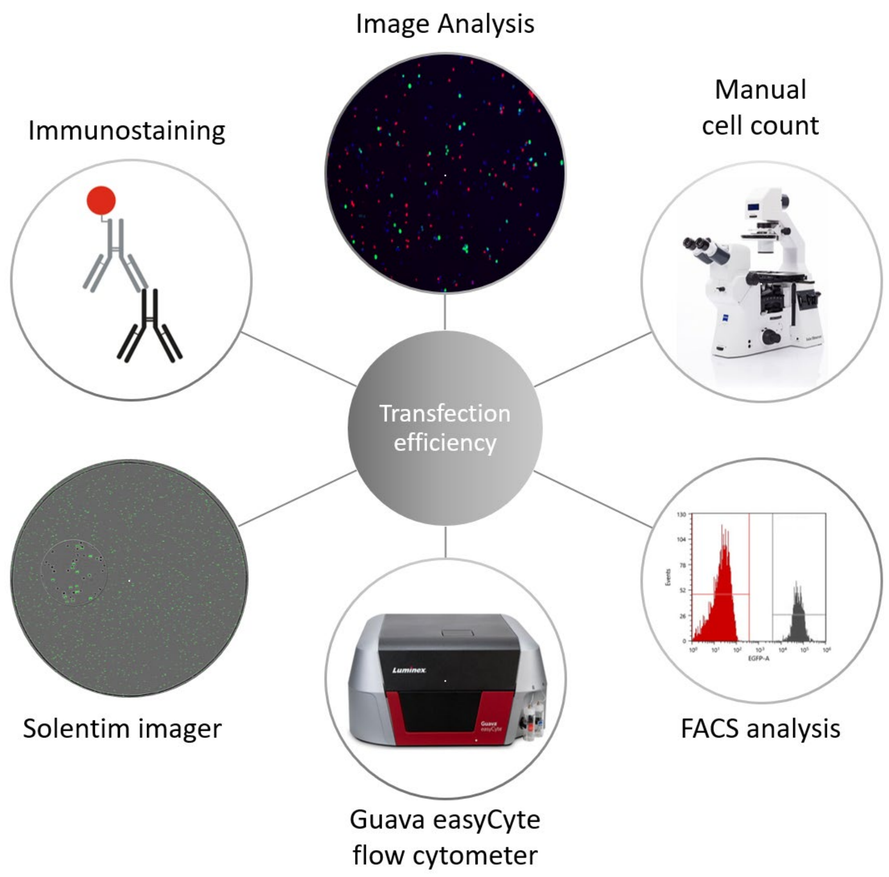
DESCRIPTION

This master thesis was focusing on the cell line construction process in cell culture department (CCD), Mammalian Development Services, at Lonza, Visp. It aimed to develop a simple, reliable and economic method to evaluate transfection efficiency in Chinese hamster ovary cells. This method was developed in order to control and optimize the transfection process. It could be implemented as a routine analysis as control of transfection performance and could be used to study transfection strategies and optimize transfection efficiency. Therefore, different methods to measure transfection efficiency were evaluated in this study.

OBJECTIFS

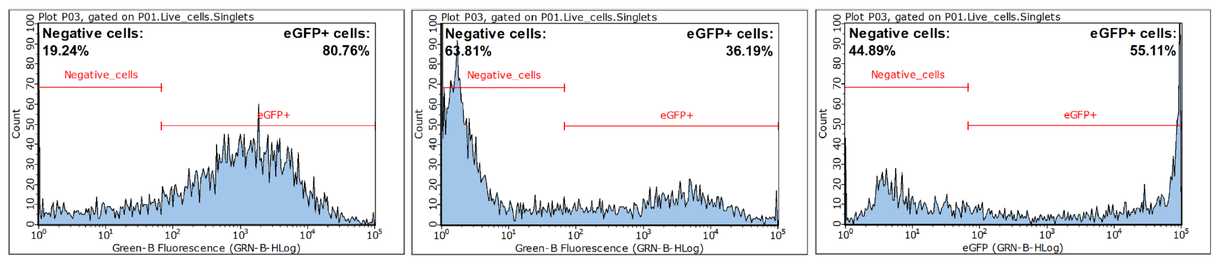


Manual cell count was used as a standard reference for the determination of transfection efficiency. The following methods were evaluated in this study:

1) Automated image analysis to calculate transfection efficiency based on acquired microscopic images
2) Solentim cell metric imager to obtain high-resolution whole well image for cell counting and fluorescent signal detection,
3) Flow cytometric analysis using fluorescence-activated cell sorter (FACS)
4) A second flow cytometric analysis using Guava® easyCyte™ flow cytometer
5) Fluorescent immunostaining of IgG expressing cells using an anti-IgG antibody conjugated to a fluorochrome

RESULTS

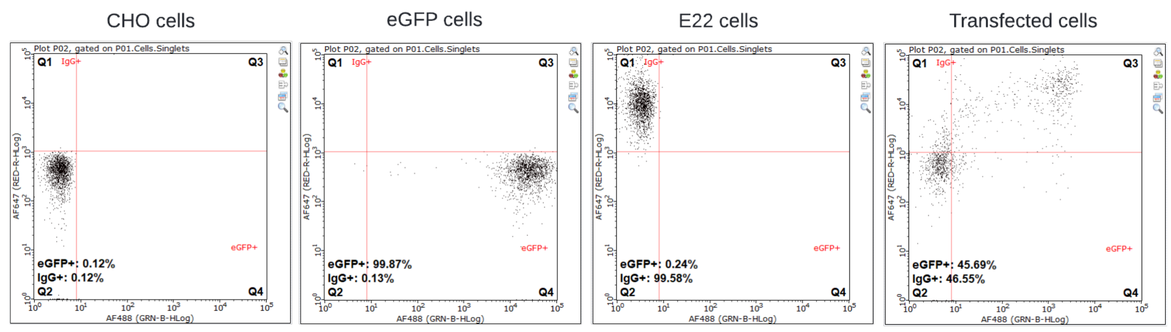
Guava® easyCyte™ is a simple bench sized flow cytometer. Its special feature relies on a microcapillary flow cell for direct sampling rather than hydrodynamic focusing overcoming the need of sheath fluid. This fluidics simplicity facilitate the maintenance as no laser alignment nor calibration is required. Moreover, this allows an absolute cell count while drastically reducing the waste. This device is also adapted for high throughput acquisition. Indeed, robust walkaway automation composed of the capillary and a mixing paddle allow rapid and automatic mixing and acquisition in 96-well plates or tubes. Guava instrument showed accurate and reliable results for the determination of transfection efficiency in different type of samples transfected with an eGFP vector.



Flow cytometric analysis of transfected samples with the Guava easyCyte instrument

Moreover, its 3 lasers enabled the measurement of multiple fluorescent signals simultaneously. This device was used for the dual measurement of transfection efficiency.

A direct method for transfection efficiency determination was developed by quantifying IgG expressing cells after transfection via fluorescent immunostaining. This method is illustrated on cells transfected with both IgG and eGFP and uses dual measurements of transfection efficiency using two fluorescent channels. The red fluorescence is measured on the y-axis in a logarithmic scale and the green fluorescence on the x-axis. Fluorescence signals comes from either expressed eGFP or from anti-IgG secondary antibody conjugated with Alexa Fluor 647 fluorochrome (emitting red fluorescence) that bound to expressed IgG. Therefore, CHO cells correspond to the negative signal after staining for both fluorescence signals, eGFP expressing cells are the positive signal for green fluorescence and finally, E22 cells (stably expressing IgG1) are the positive control for the immunostaining procedure (red fluorescence).



Dual measurements of transfection efficiency via immunostaining

With dual measurement, transfection efficiency can be interpreted in two ways, either using eGFP expression or using IgG expression. Quadrants Q1 and Q3 represent the proportions of IgG expressing cells and quadrants Q3 and Q4 represent the eGFP positive cells. Both can be used as readouts of transfection efficiency.

CONCLUSION

Direct and indirect ways to measure transfection efficiency were assessed. Indirect approach was applied by performing an additional transfection with vector expressing fluorescent marker protein for evaluating transfection efficiency, while direct approach used immunostaining with an anti-IgG antibody conjugated with a fluorochrome for the measurement of expressed exogenous IgG in the cytoplasma as direct control of transfection efficiency. For the indirect measurement, eGFP was selected as marker of transfection and cell population was analyzed with different methods. It has been demonstrated that each method has their specific characteristics and limitations that fits to different purposes. Moreover, experimental outcomes of different transfection conditions highlighted the importance to standardize transfection efficiency determination methods in order to obtain a fair and unbiased assessment. The developed method using the Guava instrument for the measurement of eGFP transfected cells showed to be rapid, cost-effective and accurate. The direct measurement of expressed IgG using immunostaining demonstrated a high efficiency and reliability during dual measurement of co-expressed genes. In conclusion, two reliable methods were developed for evaluating transfection efficiency in this study with their specific applications. The indirect measurement of eGFP transfected cells with the Guava instrument is recommended to be applied as a routine analysis for the control of transfection efficiency.

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