

## *In Situ* purification of human embryonic stem cell colonies

### Introduction

There is a significant need for the purification of specific subpopulations of adherent cells in complex cultures. Often the unique property of the specific subpopulation is destroyed by conventional approaches to purification. To date, a robust system that can adequately address this problem has been lacking. Even when markers are available, most systems (e.g., flow cytometry, magnetic beads, etc.,) require that the desired cells be physically manipulated, often leading to changes in cell morphology and/or decreased cell survival, particularly with sensitive cells like human embryonic stem cell lines. The Laser-Enabled Analysis and Processing (LEAP™) system has been developed to address current limitations in cell purification. LEAP operates through laser-mediated *in situ* elimination of undesired cells without physically manipulating the cells that are preserved. LEAP has been used for high-throughput laser-mediated cell elimination for general cell purification (Koller et al. 2004), as well as purification of cells based on direct measurement of antibody secretion by individual cells (Hanania et al. 2005). Further, LEAP purification can be efficiently performed on small samples with very low cell numbers.

Embryonic stem cells represent one example of a high value cell type that is difficult to purify, due in part to their growth as tight colonies, low abundance, sensitivity to stress, and/or lack of highly specific identifying markers. There is intense interest in using engineered

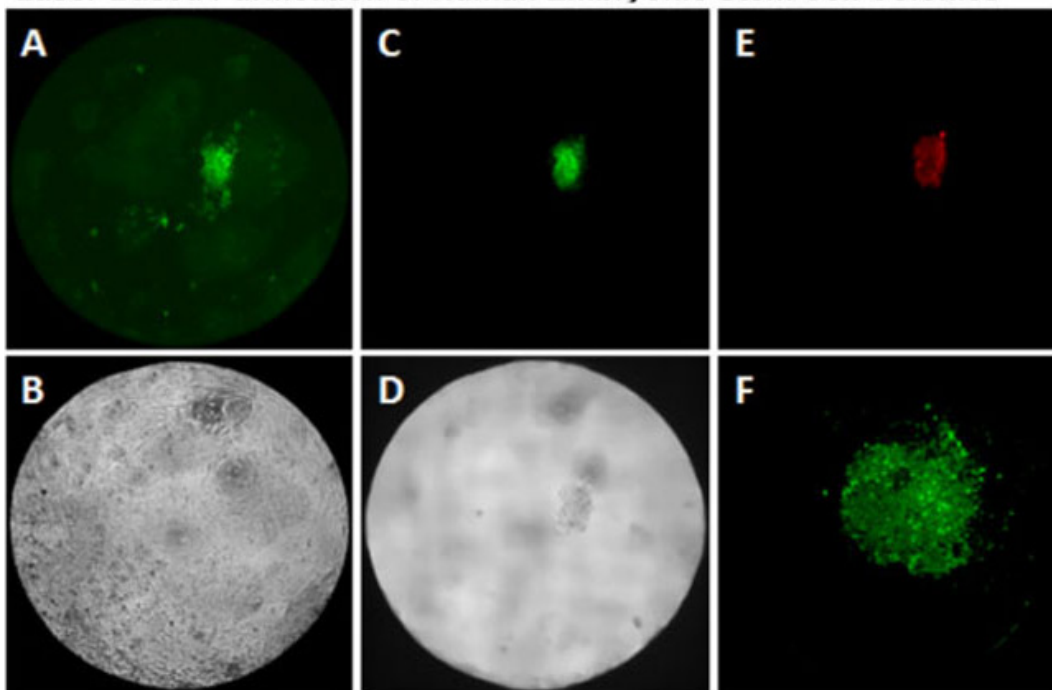
hESCs in high-throughput screening assays for identifying compounds involved in self-renewal or differentiation of these cells.

During the induced differentiation of stem cells, the signal-to-noise ratio of follow-on screening assays could additionally benefit from removal of cells differentiating toward undesirable lineages.

Human embryonic stem cells represent a high value cell type that investigators often genetically modify for use in further studies. This critical initial step is hindered by a number of challenges. hESCs are difficult to transfect, and therefore only a small percentage of the cell population may be expressing the desired gene of interest. These cells also grow as large clusters and are typically seeded onto additional cell types used for feeders. When hESCs are removed from these conditions and separated into single cells, they tend to lose their stem cell nature and begin to differentiate. These properties make hESC purification by flow-based cell sorting or magnetic beads undesirable. The typical method for isolating stem cell colonies involves scraping off an individual colony and removing it with a pipette. It is very difficult to avoid surrounding cells by this method. Finally, a majority of cells expressing the desired gene of interest reside in colonies that also contain non-expressing cells.

The results shown here establish that LEAP can be used for the purification of hESC colonies, demonstrating the capability of LEAP to address purification of unique adherent cell structures.

### Laser-Based Purification of Human Embryonic Stem Cell Colonies



*Fig. 1. A human embryonic stem cell line engineered to express nuclear GFP was seeded onto MEF feeder cells in a 384-well C-lect™ plate. GFP fluorescence was only detected in a small percentage of the stem cell colonies (compare GFP, image A with bright field, image B). LEAP was used to isolate only the GFP-positive hESC colony by eliminating all other cells in the well (C and D). Calcein-red viability stain (E) verified that cells in the GFP-positive hESC colony were the only cells that survived the process. Additional MEF feeder cells were added to the well and the GFP-positive hESC colony was allowed to expand for 96 hours (F).*

### LEAP System Features & Benefits

Whole well imaging – All cells in the well can be analyzed and processed

Purify specific subpopulations of cells *in situ*

Limited plate movements – Work effectively with attached and non substrate-attached cells

Image magnifications of 3X, 5X, 10X, or 20X

Highly efficient purification from very small samples with low cell numbers

### Stem Cell Purification Application Features and Benefits

Purification of specific colonies from a complex starting cell population

Isolation of hESC colonies without removal from screening wells or other perturbations

Re-iterative process allows downstream clearing of cells that spontaneously differentiate or lose engineered expression

Easy tracking of desired colonies from initial purification to final outgrowth

### Validation & Results

As a model system, cultured H9 hESC colonies that express GFP localized to the cell nucleus were purified from <10% to near 100% using LEAP. This result validates the use of LEAP for generation of pure genetically engineered populations of hESCs for downstream high-throughput screening applications. Pure populations of GFP-expressing hESCs are also of high value for tracking the fates of cells after transplantation experiments.

An established hESC line was transfected to express a nuclear localized GFP molecule. The frequency of GFP expressing hESCs was very low (< 10%), and most of the GFP-expressing cells were scattered throughout the hESC colonies in the cultures. When these transfected hESC cultures were seeded into 384-well plates for colony purification, only 2% of the wells contained desirable colonies, defined as colonies that were 100% positive for GFP expression or containing a large cluster of GFP-expressing cells within the colony.

The 384-well plates were processed on LEAP by photothermal laser purification of GFP-expressing colonies and clusters to near 100% purity (Fig. 1). LEAP achieved purification of a single viable hESC colony in each well with a success rate of 83.3%. Immediately after purification, growth medium was replenished and new MEF feeder cells were added to the wells. Outgrowth of purified colonies was monitored on LEAP for several days until the colonies were passaged to 96-well plates for further expansion.

### Conclusion

The LEAP system enables *in situ* isolation of hESC colonies without breaking them up into individual cells or even removing them from the culture well. Clusters of GFP-expressing cells were successfully isolated, even when they were very low in number, and/or residing in colonies that contained non-GFP-expressing cells. Expansion of these purified GFP-expressing hESCs will be useful for tracking cell engraftment and differentiation fate during stem cell therapy studies. These results also demonstrate that LEAP can facilitate the generation of new recombinant hESC lines. LEAP purification of stem cells in a 384-well plate format also enables easy transfer to other high-throughput studies, particularly the screening of small molecule libraries that modulate stem cell renewal and differentiation.

### References

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