Purification of Human iPSC-derived Endodermal and Retinal Progenitors

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The use of human pluripotent ESCs and iPSCs as sources of specialized cells for use in drug toxicity screening and discovery will require efficient differentiation protocols combined with robust downstream methods for purification of differentiated cell types. Differentiated adherent cells derived from human ESCs and iPSCs are notorious for growing as tight cell clusters and colonies which is problematic for traditional cell purification methods, such as flow sorting and magnetic bead separation. We have developed a novel approach for automated *in situ* isolation of SC-derived progenitors and specialized adherent cell clusters by eliminating surrounding, contaminating cells on the LEAP Cell Processing Workstation. LEAP uses brightfield (BF) and fluorescent (FL) imaging combined with image analysis to target cells and colonies for laser-mediated isolation within a sterile, closed environment. Importantly, cells are purified directly within multi-well plates (384- to 6-well), enabling iterative elimination of immature cells in a step-wise manner during different days and stages of differentiation while still maintaining high yield and purity. To demonstrate the utility of LEAP for purification of cell clusters, we used live FL imaging in combination with laser-mediated colony isolation to enrich hESC-derived cardiomyocytes and hiPSC-derived pancreatic progenitors to ~100% detectable purity with high viability and yield. In addition laser-mediated colony isolation was used to purify a variety of differentiating cells based on BF imaging alone, including neural rosettes, retinal pigment epithelial (RPE) cells and hepatocyte-like cells. These data demonstrate a novel approach for the step-wise purification and isolation of SC-derived cell types, including pancreatic and neural progenitors, cardiomyocytes, hepatocyte-like cells and RPE cells, providing a robust method for the production of specialized cells in multi-well plates for drug discovery.