

Stem Cell Colony Purification Powered by LEAP™

In situ Laser-Based Isolation of Specific Stem Cell Colonies

Introduction

Manual picking of stem cell colonies is the most common technique for isolating specific colonies of interest. This method is typically performed to derive new human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), genetically manipulate existing stem cell lines, and expand particular undifferentiated colonies. Colony picking is done using a brightfield dissecting microscope to visualize a small area of the culture, followed by removal of the plate lid and manual picking of a specific colony using a pipette tip or needle. There is a significant risk of contamination associated with this approach and it can be inconsistent due to varying expertise. Furthermore, manual picking is incompatible with immunostaining (e.g., for stem cell markers) to identify and pick the best colonies. Finally, this approach is very difficult to scale up. High-throughput, reproducible isolation of stem cell colonies will be essential for production of new high-quality and clinical-grade iPSC lines that can be banked and utilized reliably by researchers and clinicians around the world.

The LEAP Stem Cell Colony Purification application, one of the applications within the Stem Cell Manager on the LEAP cell processing workstation, allows users to select colonies of interest based on phenotype using live fluorescent and brightfield images and automatically isolates these colonies using *in situ* laser processing instead of a mechanical device, thereby enabling higher throughput production of stem cell lines with less risk of contamination.

Stem Cell Colony Purification Application Benefits

Automated, reproducible isolation of ESC and iPSC colonies
Cultures processed in sterile, closed environment
Standardizes stem cell colony purification
Whole-well imaging enables isolation of the “best” SC colony
Purifies SC colonies using fluorescent-marker expression
Provides high-throughput colony isolation in multi-well formats

Approach & Results

Early human iPSC lines (several passages after derivation) were cultured in normal iPSC growth medium on mitomycin-C-treated primary mouse embryonic fibroblasts (MEFs) for 5 days in 96-well plates [1]. Live cell staining was performed using stem cell-associated markers: SSEA4 (Fig 1), TRA1-60, or TRA1-81. These antibodies (conjugated to Alexa Fluor 555 or 488) were diluted to the recommended concentration in iPSC medium and incubated for 1 hour at 37 °C. Cultures were washed once with medium and imaged on a LEAP workstation. In some instances, antibodies were also used in combination with Hoechst staining. Live Hoechst staining was successfully

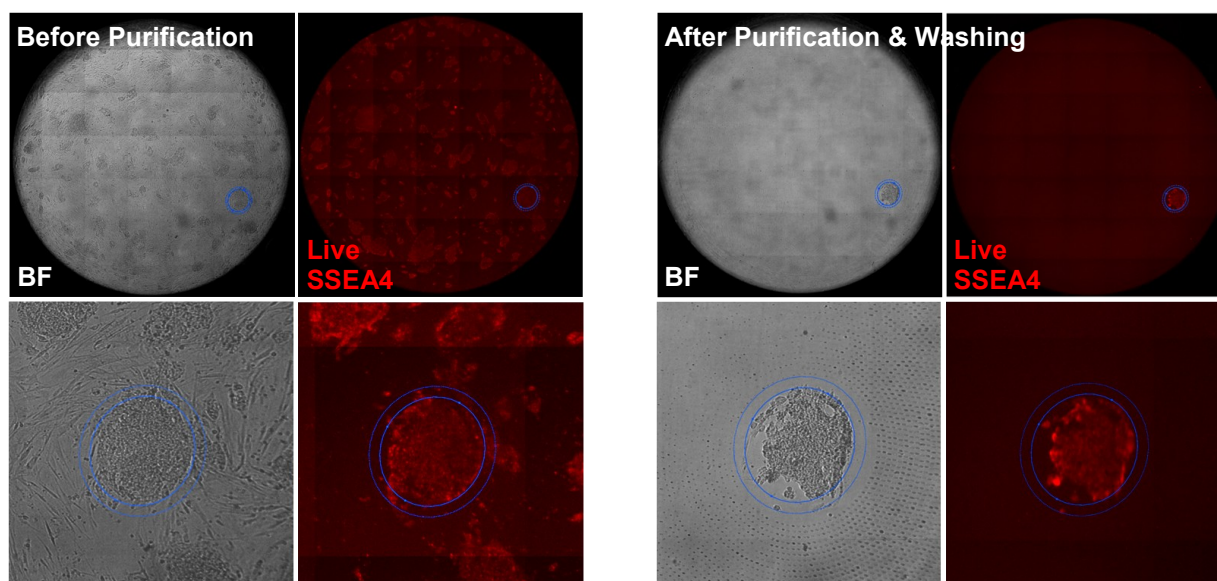


Fig 1. Isolation of a Single Stem Cell Colony Using the Stem Cell Colony Purification Application. Brightfield (BF) and fluorescent (live SSEA4 staining) images of human iPSC cultures before purification (left) and after purification and washing (right). Desired colony is shown by blue colony border with 50 µm safety zone.

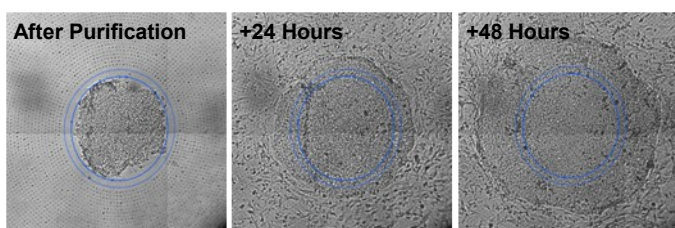
Approach & Results continued

performed on stem cells when Hoechst 33342 was diluted to a low concentration in medium (0.5 µg/ml for 1 hour) [2-4].

Specific colonies were isolated using the Stem Cell Passage Kit and Stem Cell Colony Purification application on a LEAP workstation. This application images the entire well (without edge effects) in brightfield and/or fluorescence and allows the user to interactively identify colonies of interest based on image inspection. After desired colonies are identified, cultures are automatically purified by laser elimination of all other cells throughout the well, leaving only the desired colonies remaining [5]. Importantly, the plate lid remains in place during colony purification, as all laser processing occurs through the bottom of the plate. In an effort to create more stable iPSC lines, one colony per well was selected for purification from a population of early iPSC colonies, based on morphology and highest expression of stem cell-associated markers (Fig 1 Before Purification). After purification, the wells were washed twice with fresh medium, new MEFs were added to the culture, and the isolated colonies were allowed to grow for several days (Fig 1 After Purification & Washing).

Isolated colonies increased in size progressively for 4 days after purification (Fig 2). These colonies exhibited normal growth rates and characteristic stem cell morphology, with clear, discernable colony borders and cells with high nuclear-to-cytoplasmic ratio, indicating maintained health of laser-purified colonies.

Human iPSC colonies were purified and expanded with high efficiency using the Stem Cell Colony Purification application. After purification, ~89% of wells contained a single isolated colony and of these, >96% expanded normally (Fig 2 table). Less than 5% of the expanded colonies showed visible signs of differentiation and all stained for alkaline phosphatase, Oct4, Sox2, Nanog, SSEA4, TRA1-60, and TRA1-81 (Fig 3). Notably, over 5,000 wells have been processed without any contamination.



Colony Identification Method	Single Colonies	Colony Outgrowth
BF only	89%	96%
BF + TRA1-60	87%	99%
BF + SSEA4	90%	94%

Fig 2. Health of Laser-Purified Colonies. Brightfield images of a human iPSC colony after purification (left image) and outgrowth for 24-48 hours (right images). Purified colony area is shown by blue colony border with a 50 µm safety zone. The table summarizes purification and outgrowth results using varying methods of colony identification.

Purification of human iPSC colonies from several different lines, derived from both fetal and adult fibroblasts, resulted in similar outcomes (~82-97% of wells contained a single colony). In addition, human iPSC colonies have been purified in both KODMEM and DMEM/F-12 based medium growing on either feeder cells or Matrigel™. All colonies expanded normally and maintained typical stem cell morphology.

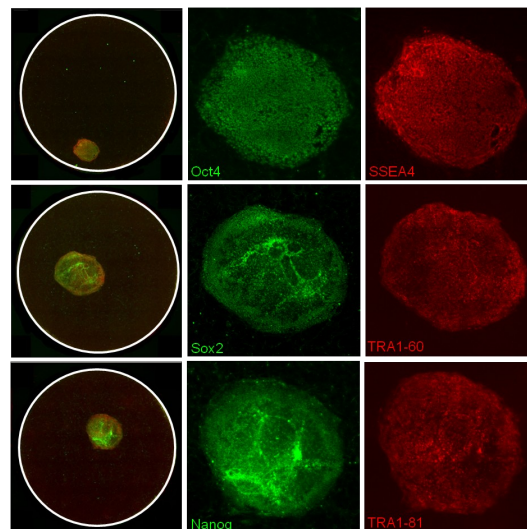


Fig 3. Pluripotency of Laser-Purified Colonies. Fluorescent images of human iPSC colonies after purification and outgrowth for 72 hours as analyzed by immunostaining for characteristic stem cell-associated transcription factors, Oct4, Sox2, and Nanog (green, middle) and surface antigens, SSEA4, TRA1-60, and TRA 1-81 (red, right). Whole images are shown on the left.

Conclusion

The Stem Cell Colony Purification application on the LEAP workstation combines live fluorescent and brightfield imaging to purify individual colonies in an aseptic and high-throughput manner, facilitating the generation of new iPSC lines and genetically modified lines. In addition, the LEAP workstation may be used to purify specialized differentiated cell types derived from stem cells (e.g., cardiomyocytes, hepatocytes, and retinal pigment epithelial cells). Human iPSC colonies were successfully isolated and expanded with higher efficiency using this application. These colonies expanded normally, maintained typical stem cell morphology, and expressed characteristic stem cell markers. The use of laser-mediated colony purification significantly reduces the contamination risk and inconsistency associated with colony picking methods and provides a standardized, reproducible method for efficient, high-throughput derivation of stem cell lines.

References

- 1 Kan, N and Mercola, M. iPSC lines generated by Sanford-Burnham Medical Research Institute.
- 2 Lowry, et al. PNAS 2008; 105: 2883-2888.
- 3 Chan, et al. Nat Biotechnol 2009; 27: 1033-1037.
- 4 Prashant et al. Stem Cells 2010; 4:713-720.
- 5 Koller, et al. Cytometry A 2004; 61:153-161.

Copyright © 2010 by Cyntellect, Inc. All rights reserved. Cyntellect® is a registered trademark, and LEAP™ is a trademark, of Cyntellect. Other products or company names mentioned in this document might be trademarks or registered trademarks of their respective owners, and are treated as such.
03-LEAP-APPNOTE-010-R00. July 2010.