Antibody-based tools and protocols for improving stem cell characterization workflows

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ABSTRACT

Stem cell biology constitutes one of the fastest growing areas in the life sciences. Accordingly, there is strong demand for improving the characterization tools and protocols available to stem cell researchers. We report here the development of a series of antibody-based tool sets and protocols that facilitate detection of important cellular markers of pluripotent stem cells and the differentiated cell types that can be derived from them (e.g., three germ layers, neural stem cells, cardiomyocytes). First, optimized immunocytochemistry reagent sets were identified by screening panels of validated primary antibodies against established stem cell markers and matching them up with appropriate dye-conjugated secondary antibodies and optimized fixative, permeabilization, blocking, and wash buffer systems. We demonstrate how these reagent sets can be applied to simplify traditional fixed-cell immunocytochemistry workflows and enable more information per sample via multiplex staining strategies that are compatible with a variety of imaging platforms. A second series of live-cell imaging reagents was generated to improve culture characterization and clone selection during cellular reprogramming workflows that are used to generate induced pluripotent stem cells. These tool sets are composed of dye-conjugated primary antibodies against select cell surface markers that are paired together with an imaging medium specifically designed to maximize fluorescence signal detection while maintaining cell health. We anticipate that this series of protocol and technology improvements will significantly augment the current characterization approaches available to stem cell researchers.

RESULTS

Generating optimized immunocytochemistry reagent sets

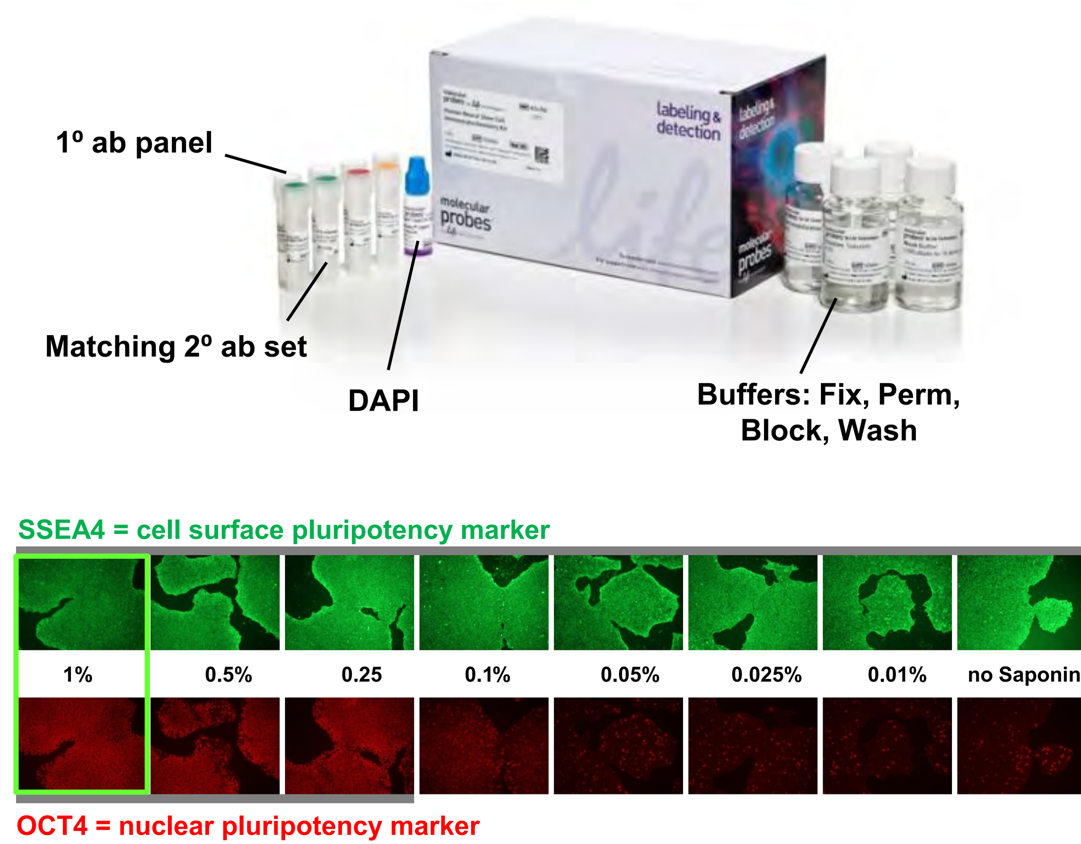

Figure 1. Example of an optimized reagent set design that contains all of the components necessary to perform immunocytochemistry (ICC). Antibodies were screened for optimal ICC performance, including the simultaneous detection of nuclear and cell surface markers via saponin-based permeabilization.

Streamlining traditional immunocytochemistry protocols

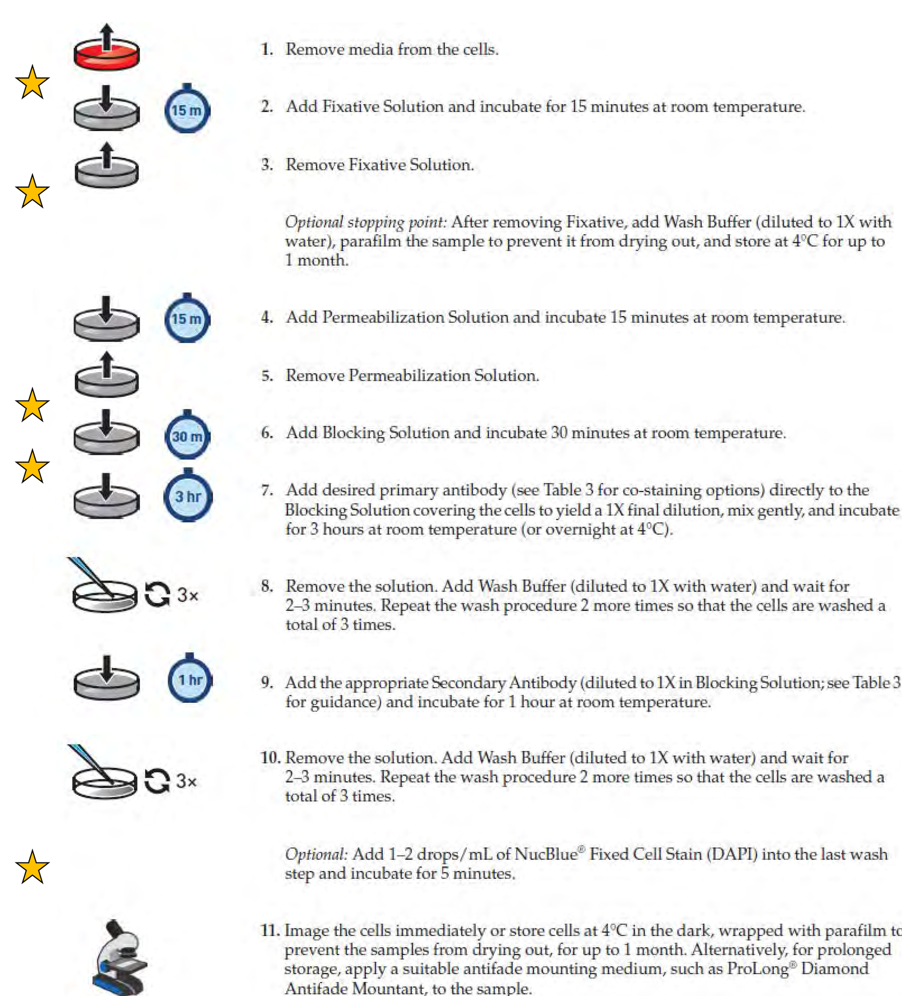

Figure 2. Example of a streamlined ICC protocol in which several unnecessary wash steps have been eliminated (yellow stars indicate where wash steps have been removed), which saves significant time and effort relative to traditional ICC workflows.

MATERIALS & METHODS

Unless noted otherwise, all materials were obtained from Life Technologies, a Thermo Fisher Scientific brand.

Stem cell culture and differentiation was performed in accordance with the manufacturer protocols for the indicated media systems. Reference protocols can also be found at the following link:
http://www.lifetechnologies.com/us/en/home/references/protocols/cell-culture/stem-cell-protocols.html

Microscopy and imaging was performed using an EVOS® FL Imaging System (Cat. No. AMF4300) or a Zeiss Axiovert 25 CFL.

Enabling more information per sample via multiplex staining

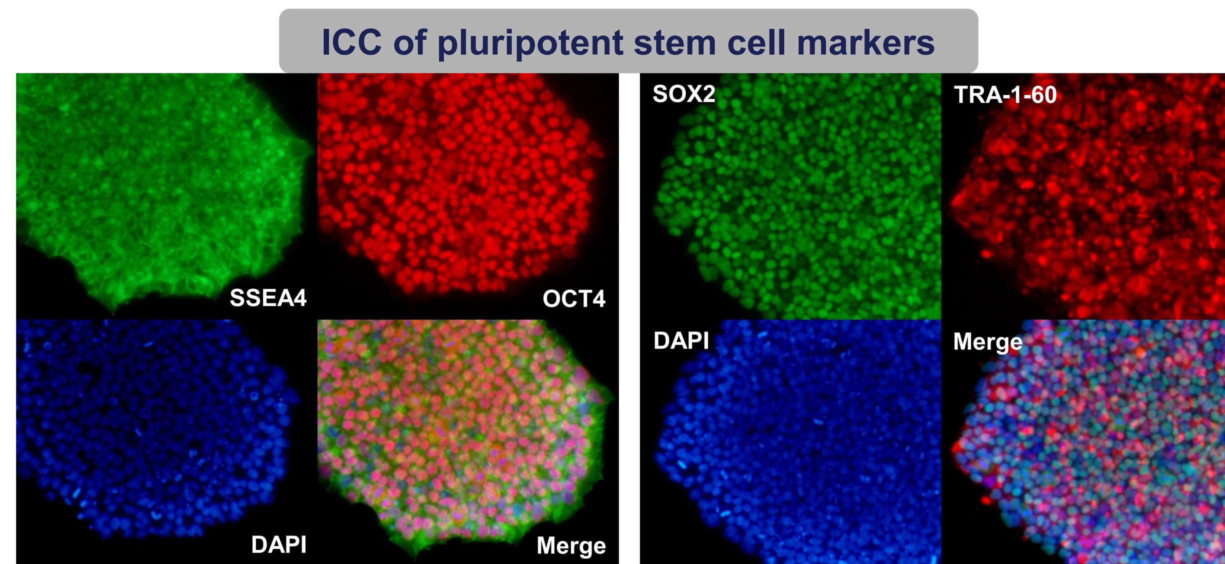

Figure 3. Induced pluripotent stem cells (iPSCs) derived from CD34+ cord blood (Gibco® Human Episomal iPSC Line, Cat. No. A18945) were grown under feeder-free conditions using Essential 8™ Medium (Cat. No. A1517001) in wells coated with vitronectin (Cat. No. A14700). The cells were stained for pluripotency markers using the PSC 4-Marker ICC Kit (Cat. No. A24881).

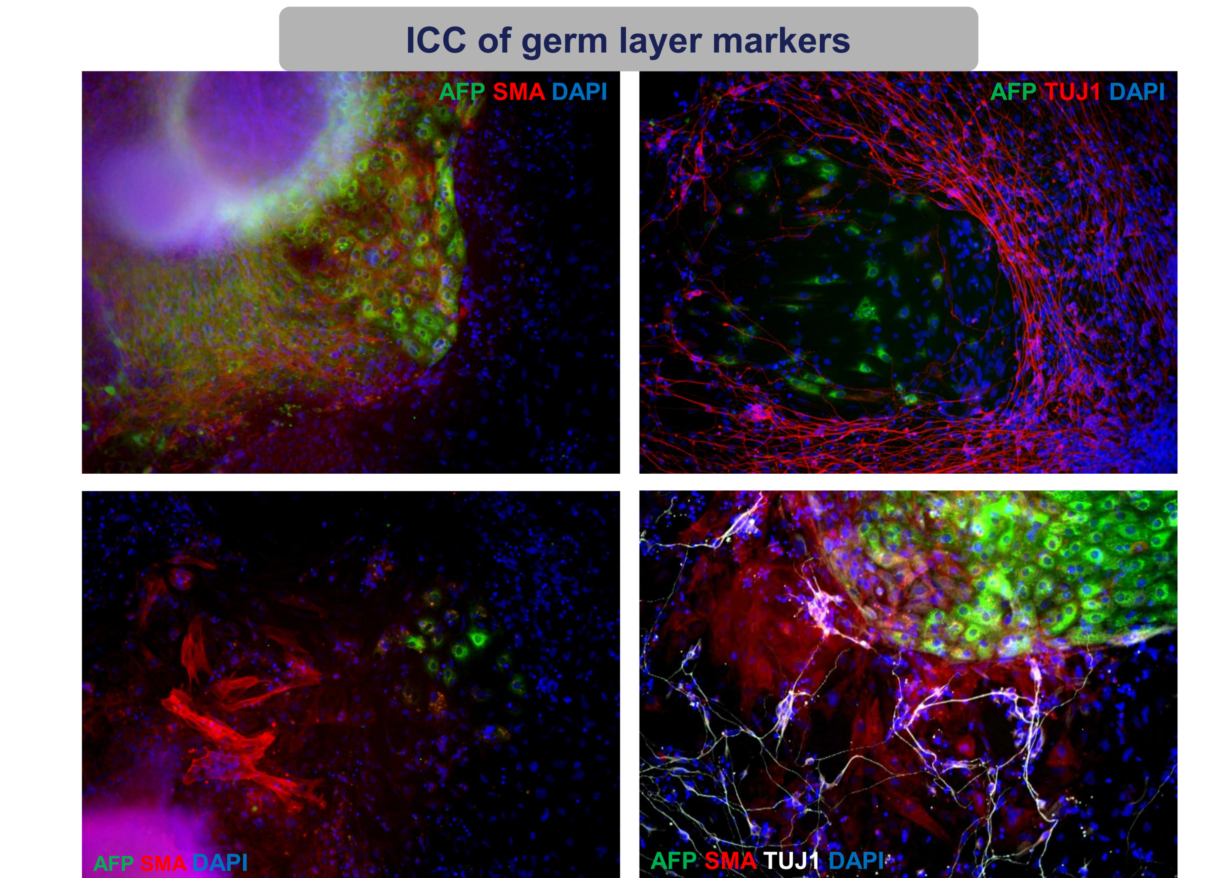

Figure 4. H9-derived embryoid bodies were cultured on Geltrex® (Cat. No. A1569601) and allowed to randomly differentiate for 17 – 20 days in DMEM/F12 medium with KnockOut™ Serum Replacement (Cat. No. 10828028). The cells were stained for the following markers using the 3-Germ Layer ICC Kit (Cat. No. A25538): endoderm marker alpha-fetoprotein (AFP), mesoderm marker smooth muscle actin (SMA), or ectoderm marker beta-III tubulin (TUJ1).

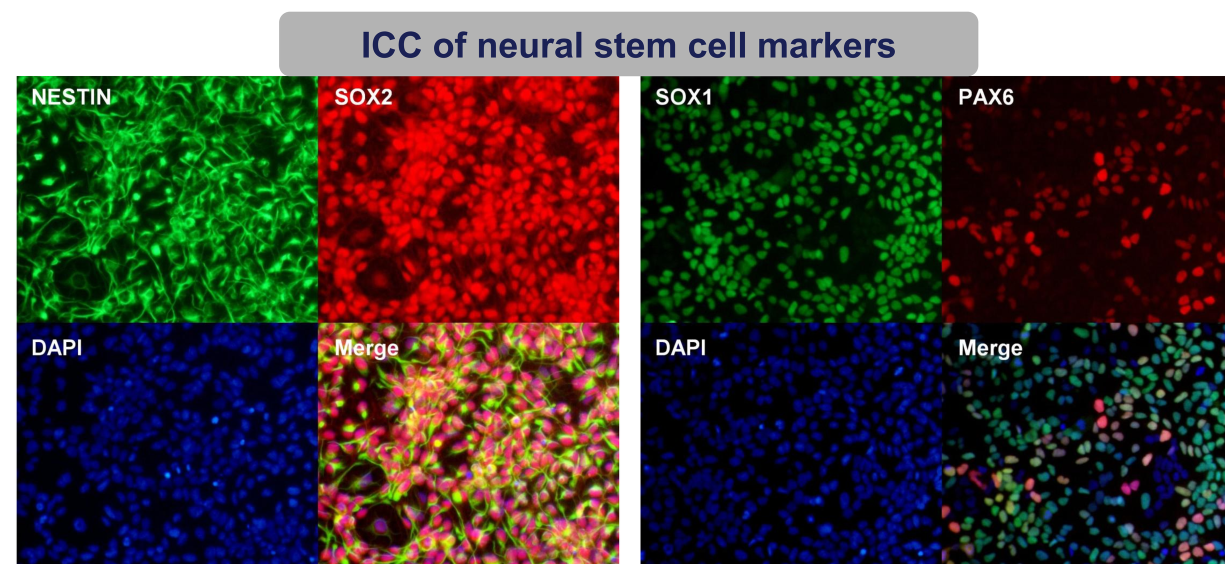

Figure 5. Neural stem cells (NSCs) were generated from an iPSC line using Gibco® PSC Neural Induction Medium (Cat. No. A1647801). Cells were cultured on a Geltrex® (Cat. No. A1413301) coated plate and stained for NSC markers using the Human Neural Stem Cell ICC Kit (Cat. No. A24354).

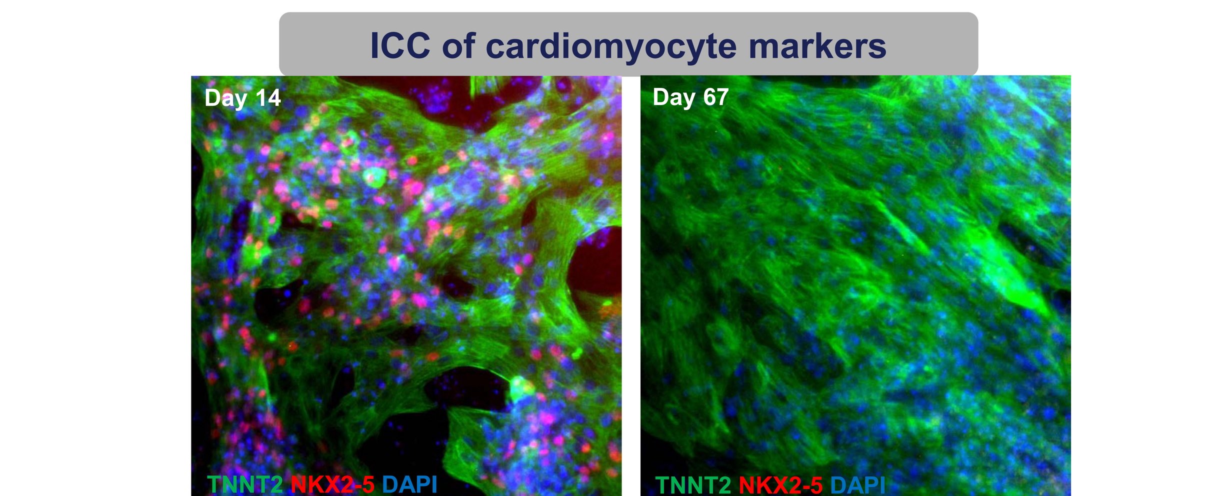

Figure 6. Induced pluripotent stem cells (iPSCs) were differentiated using the PSC Cardiomyocyte Differentiation Kit (Cat. No. A25042SA) for 14 or 67 days prior to staining for the following markers with the Human Cardiomyocyte ICC Kit (Cat. No. A25973): NKX2-5 for early cardiac mesoderm and TNNT2/cTNT for cardiomyocytes.

Improving live-cell staining workflows

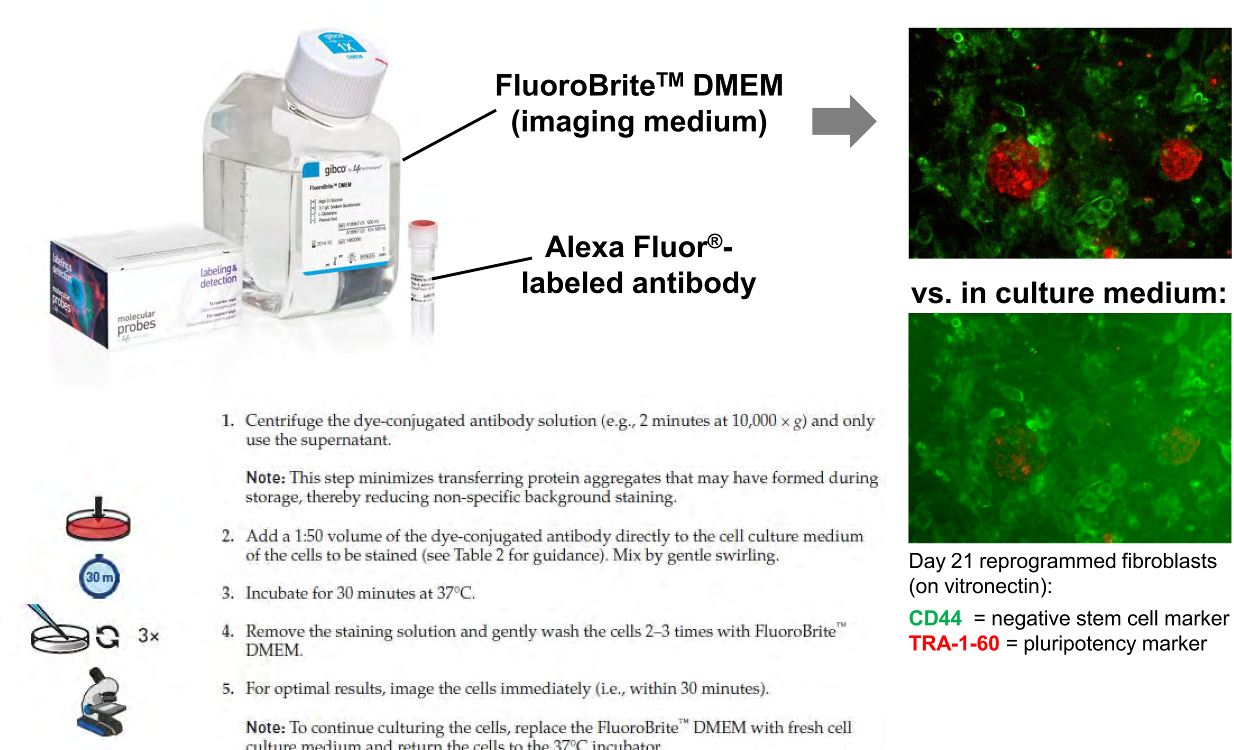

Figure 7. Example of an optimized live-cell imaging tool set that enables rapid cell staining, maximizes fluorescence signal detection and maintains cell health.

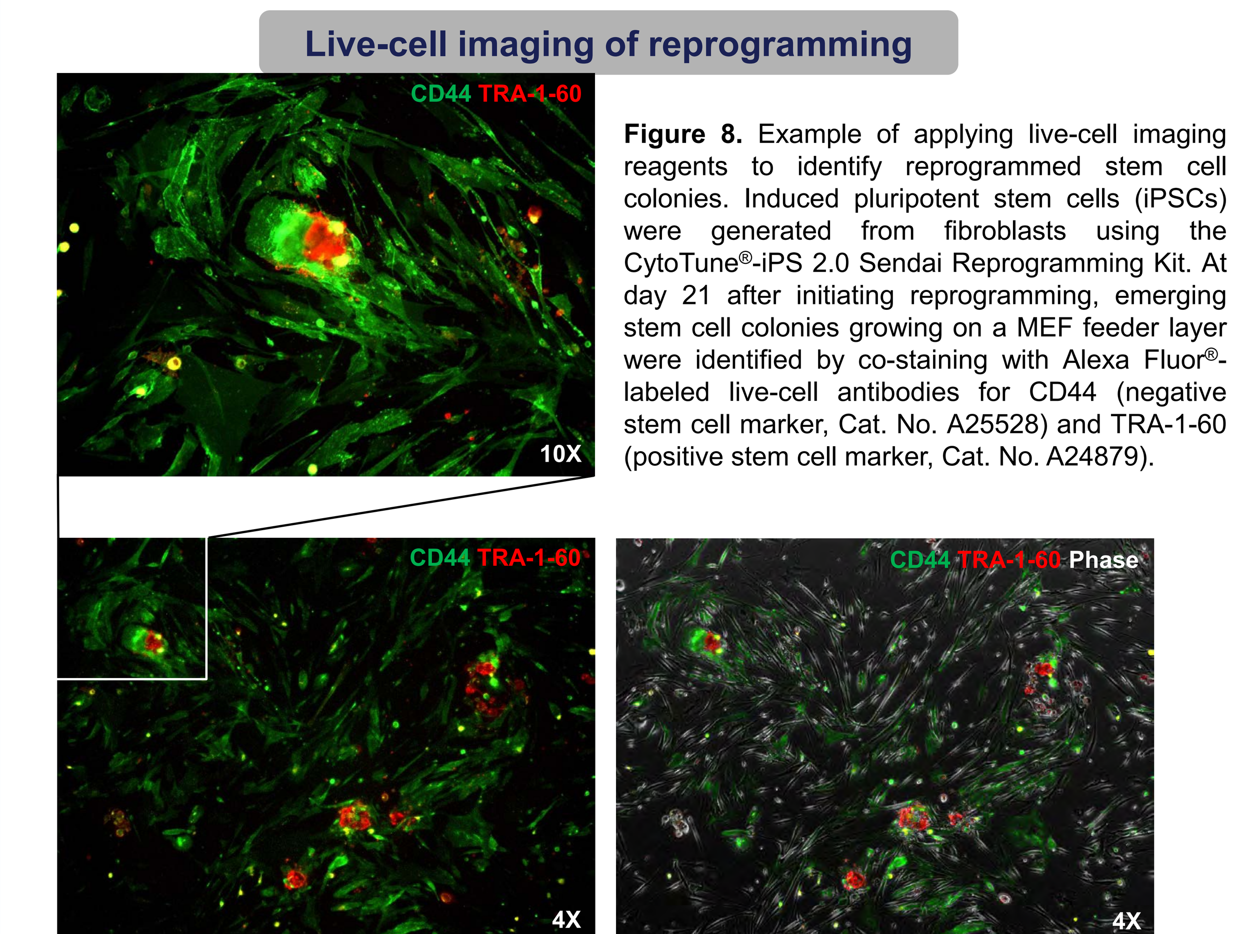

Figure 8. Example of applying live-cell imaging reagents to identify reprogrammed stem cell colonies. Induced pluripotent stem cells (iPSCs) were generated from fibroblasts using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit. At day 21 after initiating reprogramming, emerging stem cell colonies growing on a MEF feeder layer were identified by co-staining with Alexa Fluor®-labeled live-cell antibodies for CD44 (negative stem cell marker, Cat. No. A25528) and TRA-1-60 (positive stem cell marker, Cat. No. A24879).

CONCLUSIONS

The process of generating stem cells and/or differentiating them into cell types of interest involves multiple characterization steps and markers to confirm cell identity. We demonstrated here several improvements to the protocols and tool sets available for stem cell characterization workflows involving antibody-based detection methods for live-cell staining and fixed-cell immunocytochemistry.

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TRADEMARKS

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