UCR
Stem Cell Core

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Lonza L7 kit:
Lonza L7 Human Pluripotent Stem Cell Media
Lonza L7 Human Pluripotent Stem Cell Passaging Solution
Lonza L7 Human Pluripotent Stem Cell Matrix

INTRODUCTION:

Lonza L7 kit contains L7-media, L7-passaging solution and L7-matrix designed for expansion and maintenance of human pluripotent stem cells in completely defined xeno-free conditions. Such products are important for the development of human pluripotent stem cell lines in completely defined xeno-free conditions for clinical use.

RESULTS:

During the last three months the UCR Stem Cell Core tested Lonza L7 kit on human pluripotent stem cells. RIV9 induced pluripotent stem cells (iPSC) were derived at the UCR Stem Cell Core using the classical Yamanaka system for generation of iPS cells. RIV9 is a normal 46XY iPS cell line that has been derived and maintained for several years in mTeSR media (Stem Cell Technologies) and passaged on Matrigel or Geltrex from BD Biosciences or Life Technologies, respectively.

RIV9 cells were first adapted during the course of three weeks by gradual transition from 100% mTESR to 100% L7-media. When the cells were exposed to mTeSR:L7-media (50%:50% ratio) they were passaged using L7-passaging solution instead of Accutase (Innovative Cell Technologies) and the cells were plated on L7-matrix, instead of Matrigel. From that point on RIV9 cells were cultured in the L7 complete kit for another two months (total of 14 passages).

Although we did observe some colony differentiation during the adaptation period, overall RIV9 iPSC line performed very well during the transition from mTeSR (Stem Cell Technologies) to L7 kit (Lonza). Sporadic differentiation during the adaptation period was observed in the edge of the colonies especially when the cells were passaged at low-density 1:10 to 1:12 (see figure 4). When cells were passaged at high-density 1:5, according Lonza recommendations, the differentiation was close to zero on any passaging day (see videos #1, 2 &3) and images (figures 1, 2, and 3) on the next page).

- L7 matrix did not show difference in terms of colony plating efficiency in comparison to Matrigel. L7 matrix did also not show difference in terms of colony growth or cell spreading.

- L7 passaging solution is very gentle on the cells during passaging. We are currently using L7-passaging solution to passage all human pluripotent cells at the Core.

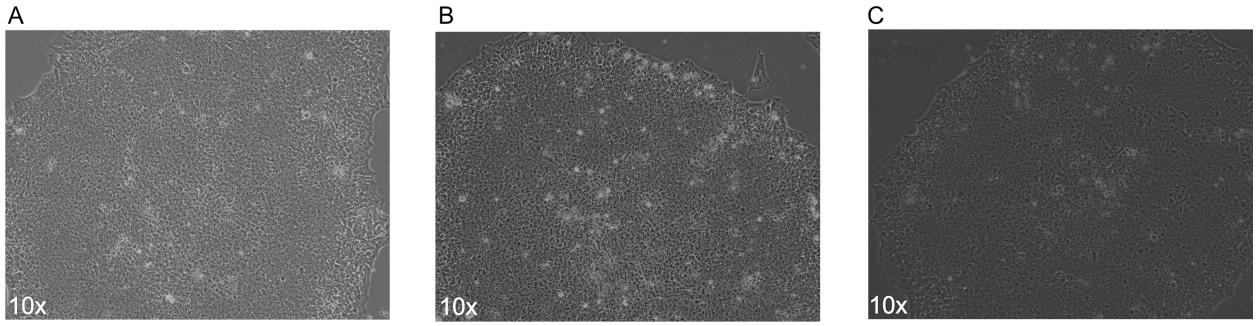

FIGURE 1: A. RIV9 cells grown on Matrigel and mTeSR (control); B. RIV9 cells grown under L7-conditions for one month; C. RIV9 cells grown under L7-conditions for two months. The images were taken at 10x magnification on Nikon Eclipse TS100. For better image presentation, please see ppt.file at the UCR Stem Cell Core web site listed here on the bottom of this page.

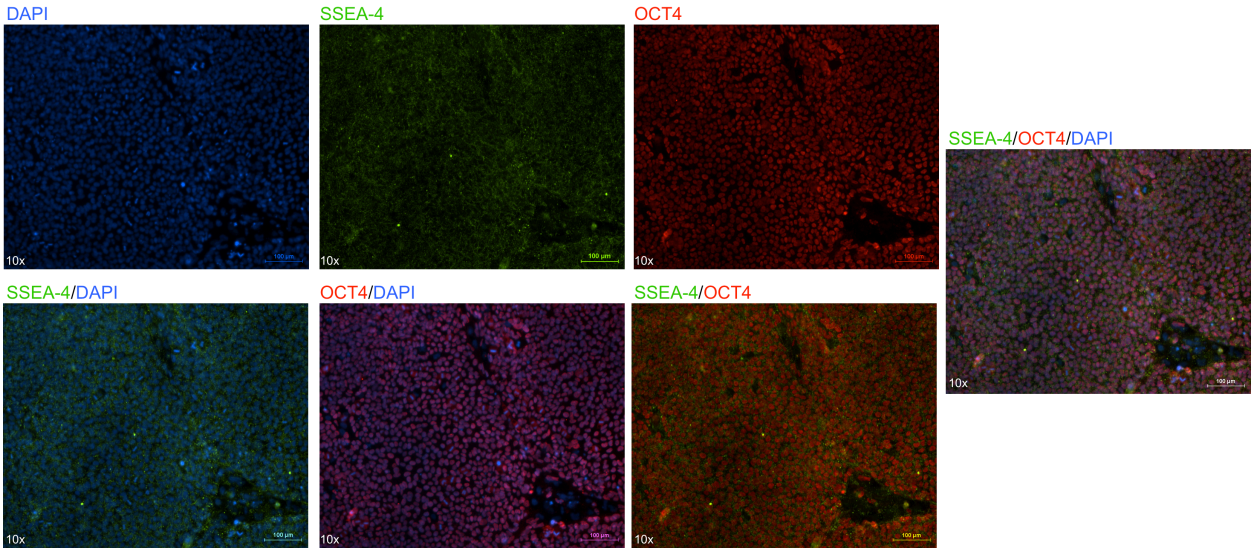

FIGURE 2: RIV9 (p14 in Lonza L7 media) iPS cells were maintained on L7 hPSC Matrix with L7 Media in a Nikon Biostation CT imaging system where time-lapse images were acquired every six hours for four days (please see videos #1, 2&3). RIV9 cells were then fixed and stained for pluripotency markers SSEA-4 and OCT4. The empty area in the images it is between three colonies touching each other. The images were taken at 10x magnification on Nikon Eclipse Ti. The scale bar is displayed on the right corner of each image. For better image presentation, please see ppt.file at the UCR Stem Cell Core web site listed here on the bottom of this page.

http://stemcellcore.ucr.edu/protocols.html
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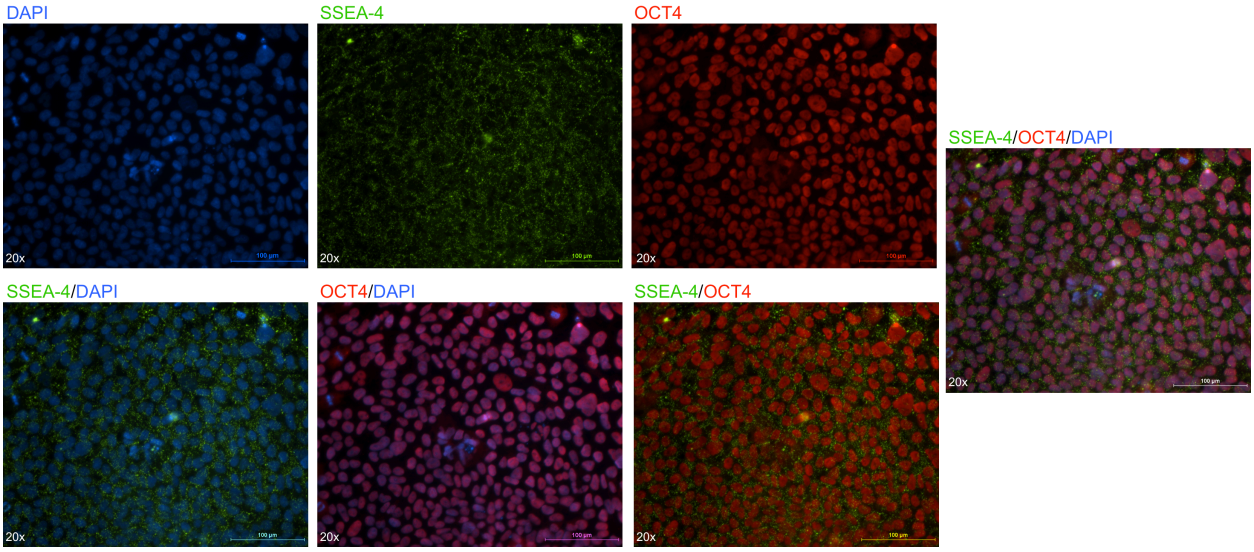

FIGURE 3: RIV9 (p14 in Lonza L7 media) iPS cells were maintained on L7 hPSC Matrix with L7 Media in a Nikon Biostation CT imaging system where time-lapse images were acquired every six hours for four days (please see videos #1, 2&3). RIV9 cells were then fixed and stained for pluripotency markers SSEA-4 and OCT4. The empty area in the images it is between three colonies touching each other. The images were taken at 20x magnification on Nikon Eclipse Ti. The scale bar is displayed on the right corner of each image. For better image presentation, please see ppt.file at the UCR Stem Cell Core web site listed here on the bottom of this page.

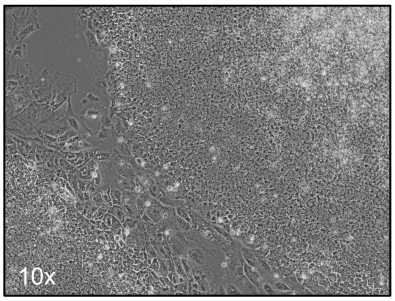

FIGURE 4: This image shows cell morphology in the edge of the colonies during growth when the cells were plated at low-density 1:10 to 1:12. Images of RIV9 iPS cells were taken with magnification 10x on Nikon Eclipse TS100.

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CONCLUSIONS:

We conclude that Lonza L7 HPSC media kit could be used successfully to maintain human pluripotent stem cells in defined xeno-free conditions.

The cells need a small interval for adaptation during the transition from mTeSR media growth to Lonza 7 HPSC media kit.

Human stem cell differentiation is minimal when the cells are passaged at higher density, for example 1:5 in comparison to 1:10.

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