STEM CELL SOCIETY
SINGAPORE

life technologies™

STEM CELL SOCIETY SEMINAR

Co-organised with Invitrogen, Life Technologies

4.30pm - 6.00pm

16 November 2011, Wednesday • Aspiration Theatrette, Matrix Building Level 2M,
30 Biopolis Street, Singapore 138671

Programme

4.30 - 5.30pm	Dr Uma Lakshmipathy Senior Staff Scientist, Primary & Stem Cell Systems, Life Technologies "A Novel Alkaline Phosphatase Dye for Live Staining of Pluripotent Stem Cells"
5.30pm onwards	Network Social Provided by Invitrogen, Life Technologies
Hosted by	Dr Vivek Tanavde Principal Investigator, Bioinformatics Institute
Registration required	Please register at www.invitrogen.com/scss2011

SPEAKER

Dr Uma Lakshmipathy

A Novel Alkaline Phosphatase Dye for Live Staining of Pluripotent Stem Cells

Abstract

A steep challenge in stem cell research is the identification and characterization of cells. Commonly, surface antibodies are utilized but this method is often expensive and sterility is a concern. An ideal solution would be the use of nontoxic small molecules that are substrates for differentially expressed enzymes. Alkaline Phosphatase is one such differentially expressed enzyme and its high activity is used as a measure of pluripotency. Alkaline phosphatase staining has been used to identify emerging pluripotent colonies during the process of somatic reprogramming. Current available alkaline phosphatase substrates are toxic to the cells and once stained cannot be propagated further.

We have developed a novel live alkaline phosphatase substrate using a modular synthesis platform. This dye can be applied to adherent embryonic stem cells and pluripotent stem cells in culture and robust expression observed within 20-30 minutes. Staining is specific to pluripotent stem cells with minimal background in MEF feeders and primary fibroblast cells. After removal of dye from the media, fluorescent labeled cells lose their signal within 60-90 minutes. The stained colonies can be further expanded without loss of proliferation or pluripotency. This novel live alkaline phosphatase dye was further used to identify emerging pluripotent colonies from BJ human fibroblasts transduced with CytoTuneTM; a Sendai-virus based non integrating reprogramming method. Clones picked based on live Alkaline phosphatase expressed other pluripotent markers such as SSEA4 and TRA-1-60. Clones propagated to over twelve passages were karyotypically normal and retained their pluripotency based on pluripotence marker expression and differentiation potential. Gene expression analysis of the iPSC lines showed expression patterns similar to H9 ESC line and distinct from parental fibroblast cells.

These results indicate that the novel live alkaline phosphatase substrate detects pluripotent stem cells without altering its survival, proliferation or pluripotency. This tool will provide an easy to use, live monitoring method to track cells during reprogramming or during routine culture of ESC and iPSCs.

Biography

Dr Uma Lakshmipathy has been involved in the field of stem cells for nearly a decade. Her doctoral degree in Molecular Biophysics and subsequent postdoctoral experience in DNA repair brought new perspective and led her to the area of stem cell research with focus on developing ex vivo gene repair systems. As a junior faculty at the Stem Cell Institute, University of Minnesota, she identified efficient gene delivery methods into stem cells to enable repair of adult stem cells from monogene disorders. She moved to Invitrogen, Life Technologies, in 2005 and was involved in the development of novel technology platforms for creating labeled stem cells. Her current research interests are regulation of stem cells maintenance, development of technologies for identification, characterization and differentiation of stem cells.