Imaging, screening and automated selection of mouse embryonic stem cells

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Introduction

The propagation of undifferentiated stem cells is of paramount importance to stem cell research, cell line development and drug discovery research that depends on stem cell culture. Here, we demonstrate a non-invasive method by which mouse Embrionic Stem (ES) cell cultures are monitored using the CloneSelect™ Imager system that provides a simple, affordable measure of stem cell colony number and growth rate. In addition we demonstrate that stem cell colonies can be identified using probes specific for pluripotency and selected for further propagation in an automated high throughput manner using the ClonePix™ 2 picking system.

Instrument Overview

CloneSelect Imager

- Label-free brightfield imaging of cells
- Objective, quantitative assessment of cell growth
- Image and analyse a 96-well plate in < 2.5 min.
- Simple, user-friendly software interface
- Multiple applications
  - Monitor cell growth
  - Verification of monoclonality
  - Assess viability following cytotoxic challenge
  - Analysis of Cell Migration

ClonePix2 Colony Picker

- Automated mammalian cell colony picking system
- Images colonies in white light and fluorescence
- Ranks colonies using user-definable criteria
- Target colonies identified and picked and picked into 96 well destination plates
- Multiple applications
  - Hybridoma generation
  - Generation of stable-transfectants

Materials

- CGR8 mouse ES cells (ECACC)
- CGR8 (with feeder) culture medium:
  - DMEM (Sigma) containing:
  - 15% fetal calf serum (Gibco)
  - 1% pen/strep (Gibco)
  - 1% MEM non-essential amino acids (Gibco)
  - 2mM L-glutamine (Gibco)
  - 1mM sodium pyruvate (Gibco)
- Gelatin in PBS (Gibco)
- Phycoerythrin conjugated anti-SSEA 1 (R&D systems)

Imaging and Analysis of Mouse ES Cells Colonies on Feeder Layers

- Stem cells grow as small compact colonies on a MEF feeder layer. This feeder secretes factors that help maintain stem cell pluripotency
- Stem cells were plated on a feeder monolayer of MEF
- Some stem cell colonies differentiated whilst in culture
- The CloneSelect Imager (CSI) was able to detect the stem cell colonies and distinguish them from the MEF feeder layer (Fig 1 A, B)
- Stem cell colony detection facilitated the calculation of colony number and growth rates (Fig 1 C, D)
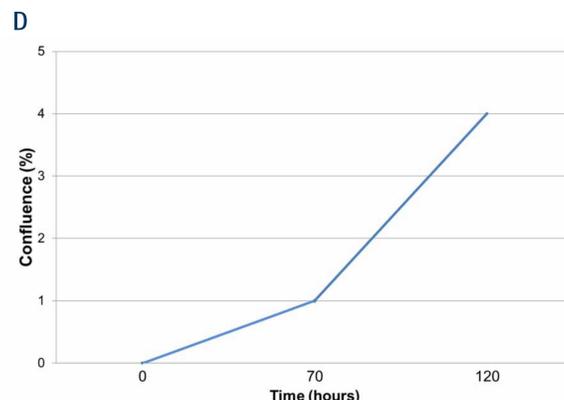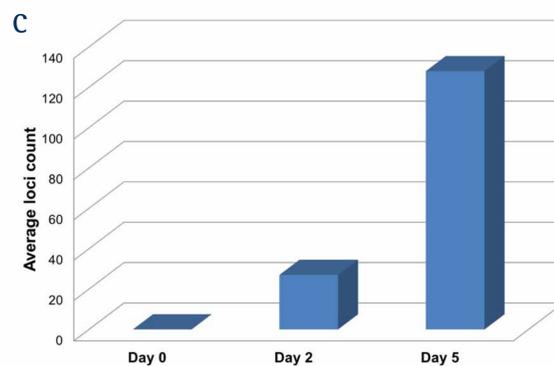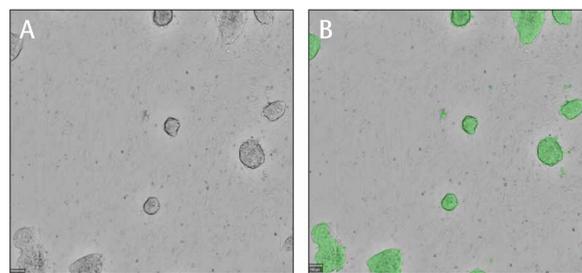

Figure 1 – (A) Raw image from CSI of stem cell colonies on MEF feeder. (B) Stem cell colonies identified by CSI software detection overlay (green). (C) Average loci count and (D) Average growth rate determined by CSI software detection overlay over a 5 day time period.

Detection and Automated Selection of Mouse ES Cells

- SSEA 1 is found on the surface of mouse embryonic stem cells
- SSEA 1 is not present on differentiated mouse cell membranes
- Stem cell colonies were distinguished from differentiated colonies using fluorescently-labelled anti-SSEA1 antibody
- Mouse embryonic stem cells were successfully detected in the fluorescence channel of the ClonePix 2 colony picking instrument (Fig 2 A–C)
- Stem cell colonies were picked from a mixed population into destination plates with a picking efficiency of 100%
- Picked colonies were observed microscopically under white light and fluorescence. Cells were fluorescent confirming their undifferentiated state (Fig 3D, E)
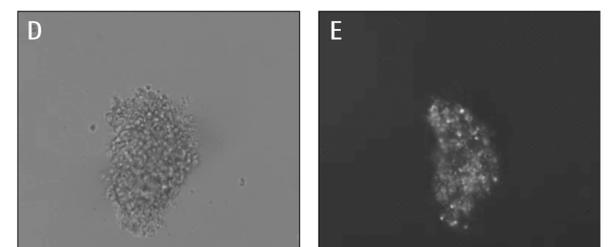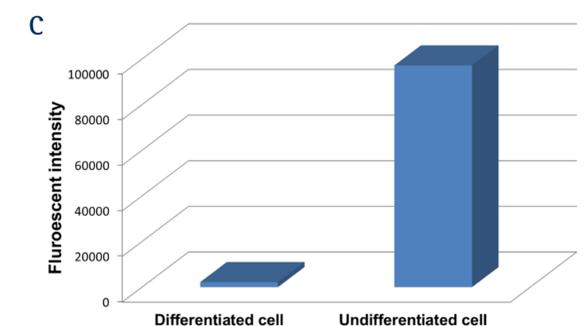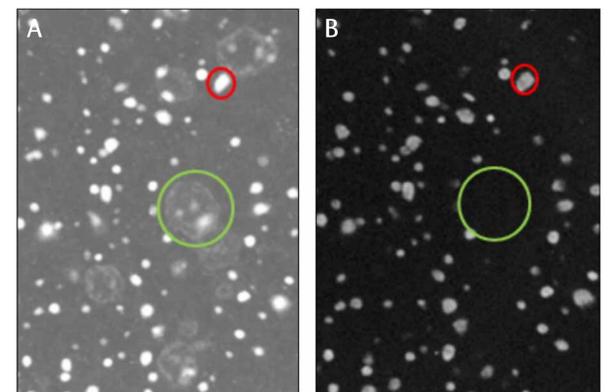

Figure 2 – ClonePix2 image in white light (A) and rhodamine fluorescence channel (B). Red annotation identifies undifferentiated stem cell. Green annotation shows differentiated stem cell colony. (C) Fluorescent intensity in rhodamine channel of differentiated and undifferentiated cells. Microscope images (10x lens) in white light (D) and with fluorescent filter (E) of a representative colony post-pick.

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