Molecular Devices

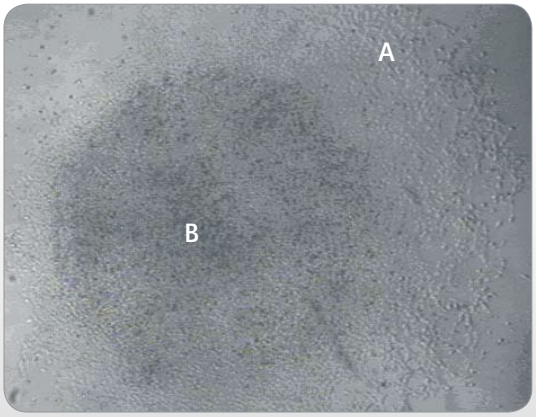
Selecting clones of mouse embryonic stem cells

The targeted propagation of undifferentiated cells is of particular interest in mouse embryonic stem cell (mESC) culture and cell line development. The technology in ClonePix Systems lends itself to the specific challenges of mESC culture, propagation and cell line development. This system enables the automated picking of mESC using either white light or fluorescence.

The latter may be a viability stain or an assay that detects stage-specific cell surface markers and thus discriminates differentiated from pluripotent mESC colonies. This application of ClonePix System technology offers benefits for stem cell research , in areas such as:

- Cell line maintenance: ensuring the propagation of clonal stem cells using markers for pluripotency
- Cell line development: screening against differentiated cells
- Genetic modification assays: detection of expression of inserted or deleted genes in developmental research and gene/stem cell therapy studies
- Cell based assays: drug development, toxicity effects, cell signalling and feedback pathways

Figure 1. Differentiated and undifferentiated CGR8 meS cells



Large, differentiated CGR8 mES cell colony showing differentiated **(A)** and undifferentiated **(B)** cell regions.

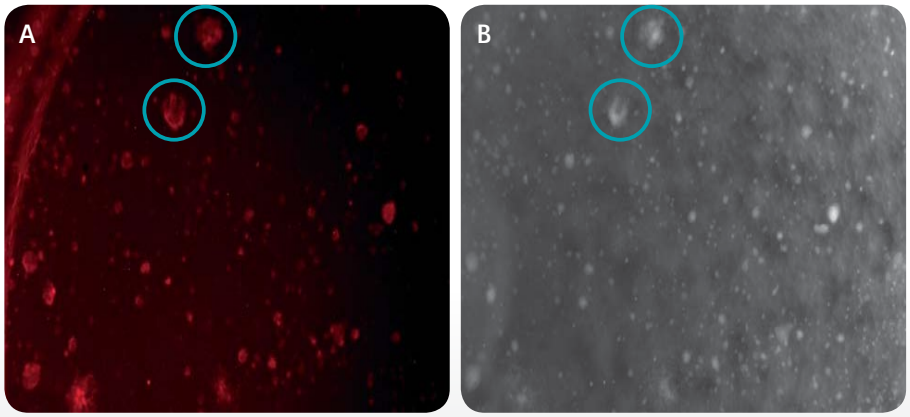
Fluorescent imaging and detection of mESC using cell surface receptor markers

Receptors expressed in pluripotent mESC which are down regulated upon differentiation can be targeted by a fluorescent assay and imaged using ClonePix to selectively pick colonies of cells that remain pluripotent. The resultant picked cells are not only clonal, but undifferentiated and thus suitable for further propagation.

Using the same fluorescent detection method, ClonePix may also be employed to isolate cells that are expressing a protein of interest such as a specific cell surface marker for a particular lineage or a heterologous receptor.

The imaging and detection of such cell surface markers (Figure 2) can be achieved using a fluorescently conjugated antibody directed against the marker of interest. The ClonePix imaging and software system allows a range of fluorophores to be imaged depending on the users' needs and availability of conjugated antibody. In addition, fluorescent images can be multiplexed with white-light imaging or up to four other fluorescent wavelengths to take additional factors into account such as colony morphology, viability or cell cycle stage.

Figure 2. Imaging and detection of surface markers



Adherently grown colonies of CGR8 mESC, imaged on a ClonePix System to detect pluripotency using rhodamine-conjugated anti-SSEA-1 (Stage Specific Embryonic Antigen 1)—only expressed during pluripotency; in fluorescent mode (colorized image, **A**), compared with all colonies present (white light image, **B**). Blue circles: large, yet pluripotent colonies to be picked and propagated.

Cell surface markers and downstream differentiation

The experiment described above (Figure 2, assay based on SSEA-1, pluripotency marker) resulted in excellent out-growth of cells post picking. The picked material did not show increased levels of differentiation following the mechanical action of picking the colonies, nor as an effect of binding of antibody to the SSEA-1 receptor. As can be seen in Figure 3, undifferentiated stem cells were still present in the clonal population of cells picked from the original colony (dark center of main colony). Figure 3, also shows good cell growth after picking into 96-well destination plates. This demonstrates the feasibility of an antibody-based assay without any impact on cell lineage. However, the effect of picking cells assayed with a fluorescently labelled antibody should be validated for any new target receptor.

Figure 3. CGR8 growth over 4 days post-picking

Depicting CGR8 growth by day 3 (**A**) and day 4 (**B**) post-picking.

Methods

Growing and picking adherent colonies of mESC

Cells should be plated at a final density of 500 cells per well of a 6-well tissue culture plate in 2 mL of suitable liquid media.

Colonies should be grown to a size of ~1-2 mm diameter over approximately 7–10 days.

Change media every 48 hours to ensure optimal growth and avoid differentiation.

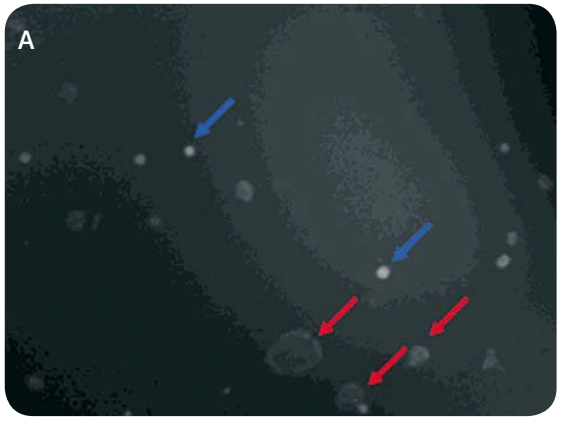
If using a fluorescent assay, media should be removed 2–6 hours prior to imaging/picking and replaced with ~300 µL of detection antibody (diluted in PBS/media) and incubated for 5 minutes to allow binding. Cells should then be overlaid (without removing antibody) in a further 2 mL of media and incubated for ~2 hours.

Colonies can be picked with the ClonePix System following standard adherent cell picking procedures.

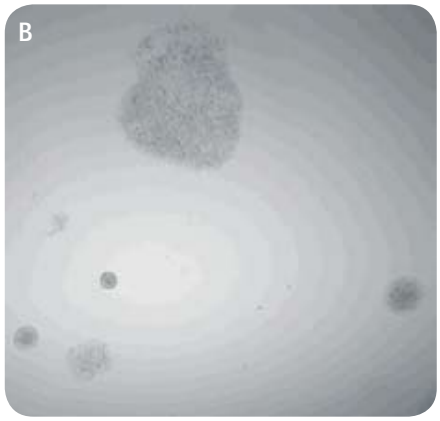
Growing and picking suspended colonies of mESC in CloneMatrix-based media

CGR8 cells may also be grown in CloneMatrix-based semi-solid media (K8510), as shown in Figure 4. Best growth and survival rates were achieved in IMDM medium containing 20% FCS and 0.1M LIF. Cells were seeded at 500 cells/mL in the semi-solid media and grown for 3–4 days to give the colonies shown. Using this method results in less differentiation than growing the cells as adherent colonies. These suspended colonies were successfully marked with anti-SSEA1 and picked on a ClonePix System.

Figure 4. CGR8 cell growth pre- and post-picking



Growth of mES (CGR8) cell colonies in CloneMatrix-based medium probed with anti-SSEA-1 stain (as in Figure 2) and post-pick image showing colony integrity. **A:** ClonePix System image of CGR8 cells labelled with anti-SSEA-1 antibody showing small, round undifferentiated colonies with bright fluorescence (blue arrows) and larger, more irregular, faint, differentiated colonies (red arrows). **B:** CGR8 growth in CloneMatrix/GMEM semi-solid media. **C:** CGR8 colony post pick showing clonal and undifferentiated morphology.



Conclusions

Applying the unique technology in ClonePix Systems to the selection and propagation of mouse embryonic stem cells removes the laborious bottlenecks associated with mES cell propagation, and ensures that only the most undifferentiated colonies are propagated further. The use of our semi-solid media actually increases the number of undifferentiated colonies (presumably due to the cells not adhering to anything), thus increasing the efficiency further. ClonePix Systems therefore provide a powerful tool for mES cell research, either in the propagation of pluripotent stem cell lines (with or without the use of fluorescent markers for pluripotency), or in the downstream selection of differentiation pathways and cell-based assay using our unique fluorescent imaging processes.

Reagents and consumables

To support this application, Molecular Devices has an extensive range of optimized media. Visit **www.moleculardevices.com/genetix** for more information.

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