Accelerated one-step screening and selection of PER.C6® clones using ClonePix technology

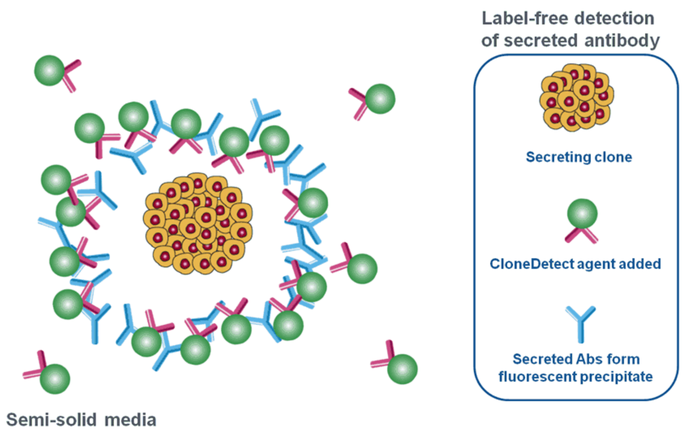
Nicola Latchem*, Natalie Smithers, Steve Game, & Alasdair Robertson
Genetix Ltd, Queensway, New Milton, Hampshire, BH25 5NN, UK
*Corresponding author: nicola.latchem@moldev.com

Introduction

The PER.C6® cell line represents an important vehicle for the expression of recombinant proteins in mammalian cells. The cell line displays a number of characteristics that make it well suited to protein expression: specifically, PER.C6 cells are able to replicate indefinitely with fast growth rates, are capable of expressing proteins at a very high level, and can grow to high densities.

ClonePix™ technology and associated reagents represent a well-established one-step solution for the identification and isolation of rare high-producer clones from large heterogeneous cell populations without the need for labour intensive cell screening techniques. In order to facilitate the application of the ClonePix workflow to PER.C6® cell line development, we have developed an animal-free PER.C6®specific semi-solid medium: CloneMedia-PER.C6. Here, we present data demonstrating that CloneMedia-PER.C6 supports the growth of cells into discrete colonies that can be imaged, ranked and picked using the ClonePix platform.

Overview of ClonePix technology



- Cells plated in viscous semi-solid medium with fluorescent detection agent
- Cells grow and divide to form clonal colonies
- Detection agent reacts with secreted target protein forming a fluorescent precipitate
- Colony fluorescence is proportional to colony productivity
- ClonePix system images colonies and ranks by fluorescence
- Clonal high-secreting colonies picked into 96 well plates

Materials

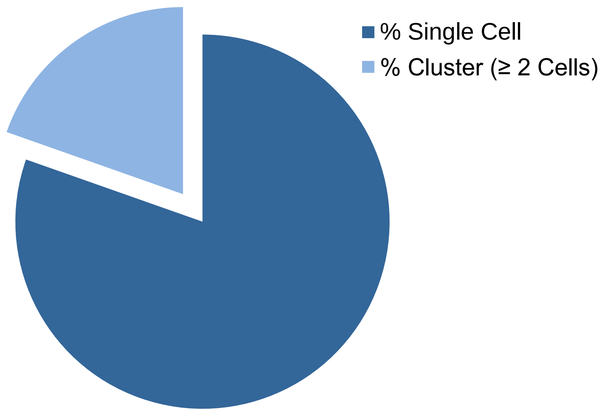
PER.C6® cells were generously provided by Dr Robert Lu, Percivia, Cambridge, MA, USA. Cells were routinely cultured in shaker flasks in HyClone CDM4PERMab (Thermo Scientific).

Reagents and Instrumentation:

- CloneMatrix (K8510)
- CloneMedia-PER.C6 (K8775)
- Recombinant CloneDetect (K8295)
- ClonePix
- CloneSelect Imager

1. Plating PER.C6® cells in semi-solid media

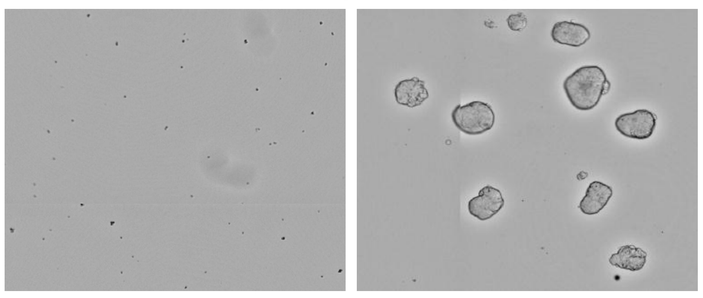
- Selection of monoclonal cell lines using ClonePix technology requires colonies to form from single cells
- Microscopic analysis of PER.C6® cells plated in semi-solid media revealed that the majority of cell foci are single cells (80% ± 2%, n=362) rather than clusters of >2 cells
- The PER.C6® cell line is therefore compatible with the ClonePix methodology



2. PER.C6® cell growth in semi solid media

- CloneSelect Imager was used to assess PER.C6® cell growth/colony formation in semi solid media 13 days post-plating
- PER.C6® cells did not grow in standard liquid culture medium + CloneMatrix
- PER.C6® cells grew well in PER.C6-specific semi solid medium: CloneMedia- PER.C6® (K8775)
- CloneMedia- PER.C6 is a complete animal component free formulation developed for optimum growth characteristics and optical clarity

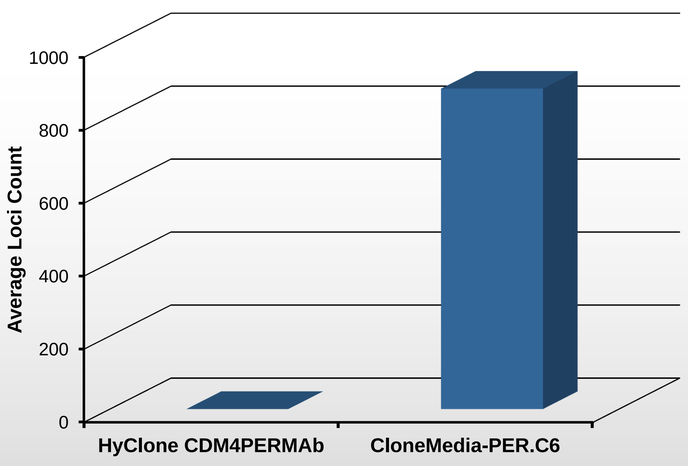
A. CloneSelect Imager brightfield images: Day 13



Standard Media + CloneMatrix

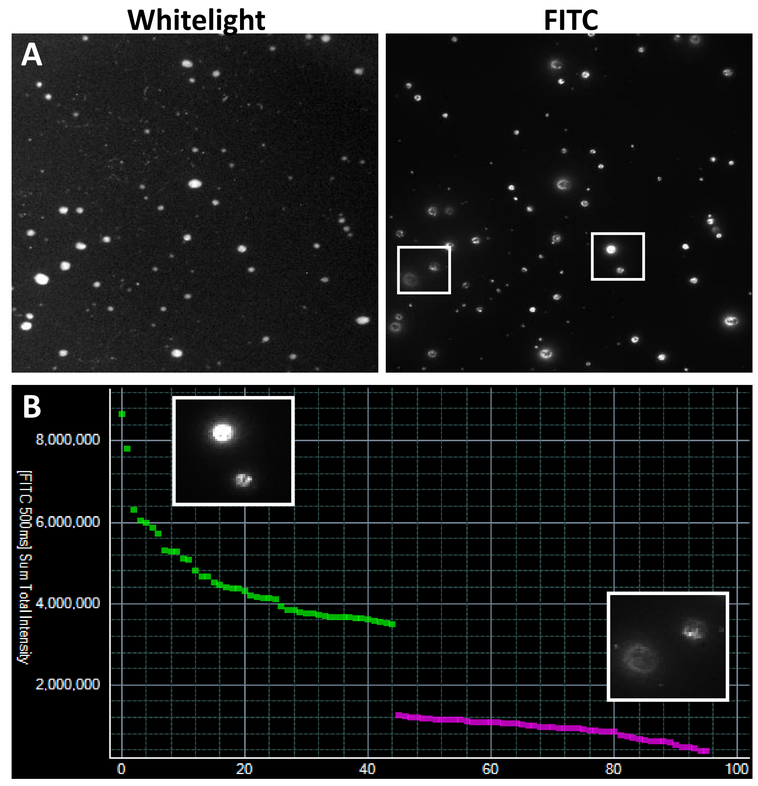
CloneMedia-PER.C6

B. CloneSelect Imager quantitation of colony formation



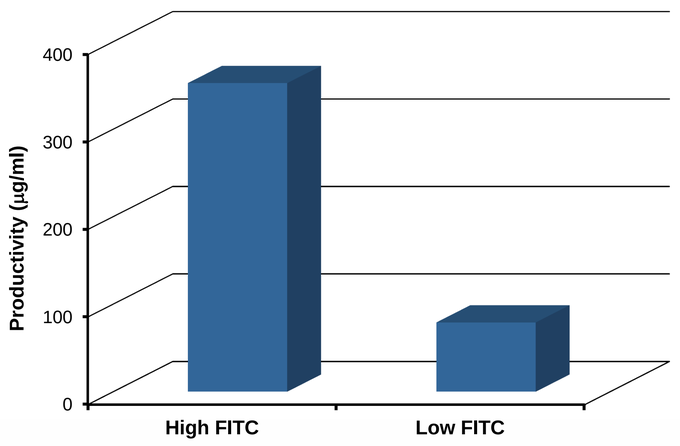
3. Detection and ranking of secreting clones

- IgG-secreting cells plated in CloneMedia-PER.C6 with an optimized detection reagent consisting of FITC-conjugated Recombinant CloneDetect (K8295) and an experimental "complex initiating catalyst"
- Fluorescent colonies are readily detectable (A) and can be ranked according to levels of fluorescence (B & inset) when plates are imaged and processed on ClonePix 13 days post plating
- Two groups were selected: colonies displaying high levels of fluorescence (green & inset), and colonies displaying low levels of fluorescence (pink & inset).



4. Productivity of ClonePix-selected cell lines

- Two populations selected: High & Low FITC
- Average productivity (normalized for cell growth) was determined by ELISA for each cohort
- Fluorescence shown to be an indicator of productivity



Summary

- When used in conjunction with CloneMedia-PER.C6 and optimized detection reagents, PER.C6 cells are well suited to ClonePix's accelerated cell line screening and selection workflow
- None of the raw materials comprising media or detection agent are of animal origin, making this workflow suitable for applications with stringent regulatory requirements

Together through life sciences.

Molecular Devices