Rapid high–throughput screening and selection for clonal populations of therapeutic protein/antibody producer cells and reporter cell–lines, using fluorescence cell based assay techniques

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Introduction

Isolation of mammalian cell clones by limited dilution, ring cloning or simply manual collection of colonies is a time consuming, resource intensive and costly procedure that is prone to cross contamination of cells and user error. The Genetix ClonePix FL technology for automated screening of over 3000 clones per hour enables informed decisions prior to isolation of only the high value clones. It significantly shortens timescales, simplifies down stream culture and delivers a powerful return on investment. This is now a proven technology for working under animal free conditions and has been adopted by many of the major pharmaceutical companies. As such ClonePix FL can be used in many bio-manufacturing applications:

- Screening and selecting the cells secreting specific monoclonal antibody
- Automated selection of colonies of cells secreting the highest amount of therapeutic antibody (for scale up to bio-manufacturing levels)
- Selecting cells secreting the highest levels of recombinant protein or therapeutic peptide
- Screening and selecting cells based on the expression of various reporter genes
- Selecting colonies of cell-lines, primary cells or mES cells based on cell surface marker expression

Examples of each of these applications will be shown here.

ClonePix FL Technology

Genetix ClonePix FL technology is based on the culture of mammalian cell lines in semi-solid media such that cells form discrete clonal colonies, originating from one single parent cell. Thousands of heterogeneous colonies can be raised in a single 6-well plate and screened using both white-light and fluorescent imaging. All clones can be ranked by our specialist software and only the most desirable are selected for automated picking into 96-well destination plates. Plates of isolated colonies can then be grown to confluence in preparation for clone expansion and scale-up. Each well of the destination plate will contain a monoclonal population of cells; thus ensuring homogeneity as well as being positive for the desired fluorescent readouts.

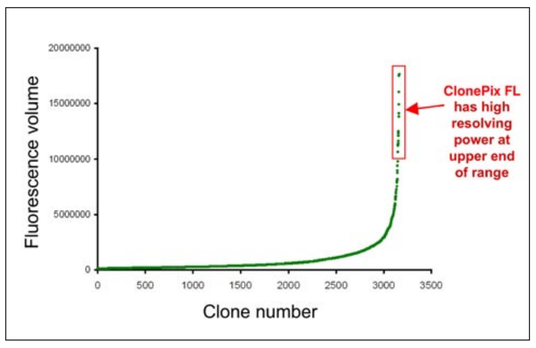

Figure 1. Genetix ClonePix FL can image over 3000 colonies in an hour.
Distribution of fluorescence against clone number is shown.

The application has been validated for the specific detection of secreted antibodies and monomeric proteins, cell surface proteins as well as fusion proteins and has been successfully adapted for a range of cell types including hybridoma, myeloma, HEK293, mES, Jurkat and both suspension-adapted and adherent CHO cells. Example images generated by the ClonePix FL are shown in Figure 2.

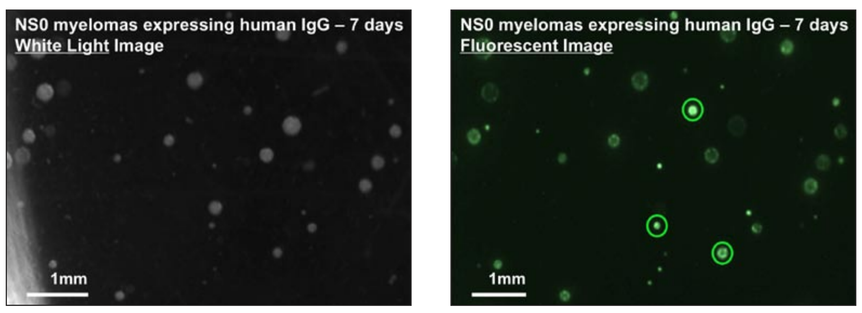

Figure 2. Fluorescent detection of a secreted IgG from an NS0 myeloma cell line.
An NS0 cell line expressing a human IgG against a bacterial protein was plated out and grown in CloneMatrix semi-solid media in the presence of FITC anti-IgG. After 7 days individual cells have grown into colonies (left panel), these are exposed to an in situ fluorescence based assay to detect and quantify IgG secretion (right panel). The image is colourised and a high, low and medium secretor are highlighted.

Monoclonal Antibody Secretion: Fluorescence Correlates with Productivity

Fluorescence intensities obtained for secreting clones are directly correlated to antibody secretion. Figures 3 and 4B show data obtained for NSO and CHO-S antibody secreting cell lines, respectively. In most instances a single round of screening is sufficient to generate stable high secreting clones. When analysing productivity it is necessary to normalise for cell number (see Figure 3).

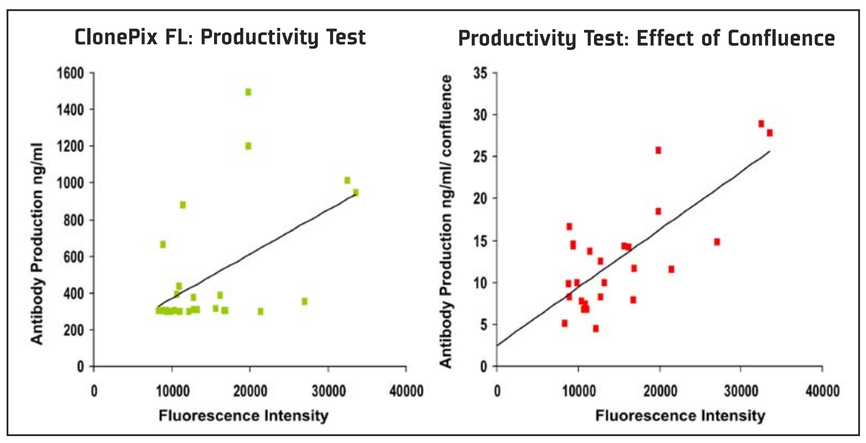

Figure 3. Protein secretion corresponds to fluorescence intensity.
An IgG secreting NSO cell line was picked according to fluorescence. Productivity was measured by ELISA following 1 weeks growth (left panel). Taking into account confluence dramatically increases correlation between fluorescence and production (right panel).

Recently transfected CHO cell lines are inherently unstable, which leads to a high drop-out rate of high producers that do not sustain productivity over time. To compensate for this, candidate clones can be subjected to a second round of plating and screening to identify stable high secreting clones. Example images of a stable high secreting clone and a non-stable clone which have been re-plated are shown in panels B and C of Fig. 4.

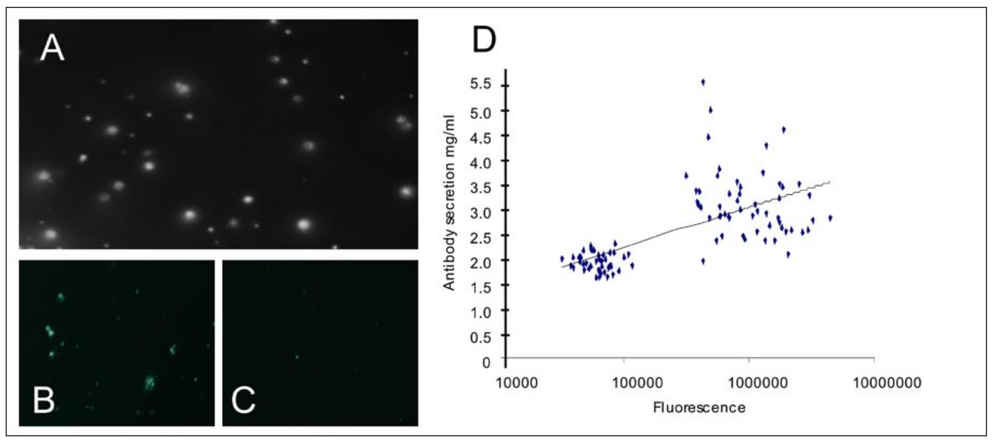

Figure 4. A second round of plating/selection increases stability of expression.
Panel A shows a FITC image from a heterogeneous population of an IgG secreting CHO-S cell line. High secretors were picked and re-plated following 7 days growth at the 96-well plate stage. A colourised image of a stable high secreting clone is shown in Panel B and an unstable clone identified as a high secretor in round 1 is shown in Panel C. Panel D shows productivity measurements of "high" and "low" secreting CHO colonies verses fluorescence as determined by ELISA.

Recombinant Therapeutic Protein Production: Monomeric Protein/Peptides

As shown in figure 5, our unique technology can also be applied to screening and selecting cell lines secreting other, non-antibody, proteins such as recombinant therapeutic proteins and peptides. This allows the highest producer cells of the therapeutic protein of interest to be scaled up to bio-manufacturing levels; making the selection and propagation of high secreting cell lines far more efficient and cost effective.

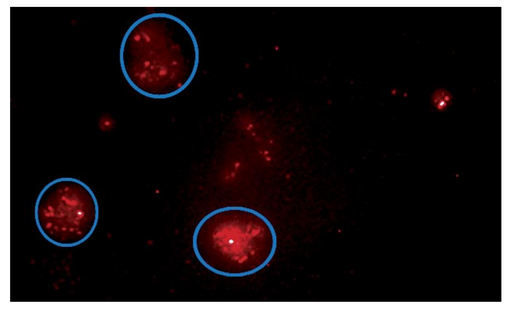

Figure 5. Detection of therapeutic protein secretion.
Adherent HEK 293 cells stably transfected with a monomeric human protein of therapeutic interest: Cells were seeded to form adherent clonal colonies and imaged with ClonePix FL (with Rhodamine excitation/emission spectra). Image shows secreted protein detected with Conjugated polyclonal antibody to the protein of interest. Blue circles indicate the highest producing colonies, with the associated secreted protein clearly visible around the cells.

Reporter Cell-Line Development

Screens using reporter genes to provide fluorescent readouts, such as CCF-2/4 ß-lactamase assays, require a sensitive homogeneous population of reporter cells. We have tested ß-lactamase cell based assays on our high-throughput cell imaging system to show that we can image individual cells loaded with CCF-4 substrate and can clearly see the associated increase in fluorescence as the CCF-4 is metabolised from its negative (green fluorescent) to its positive (blue fluorescent) form over time, by stably expressed ß-lactamase.

Developing this technology further on ClonePix FL will allow reporter gene cell lines to be engineered that are monoclonal and have more specific, sensitive or brighter response to positive control compounds for the pathway under investigation. Thus the sensitivity and homogeneity of the screen will be dramatically increased, making such campaigns more efficient.

Cell Surface Marker Expression and mES Cell Picking

The ability of ClonePix FL to image colonies of cells using fluorescence allows the instrument to be used to select specific cell types, from a mixed population, based on their cell surface expression. As with flow cytometry applications, fluorescently conjugated antibody to cell surface receptors can be used to get ClonePix FL to select cells expressing the highest levels of the receptor (or combination of receptors, using the multiplex facility) of interest. It is possible to image endogenous levels of cell surface marker expression as well as recombinant/over-expressed receptors for cell-line engineering and development.

The advantages of using ClonePix FL for such an application are that the resulting populations will be monoclonal cell populations and that the multiplexing ability of ClonePix FL allows receptor expression to be combined with other criteria such as antibody and/or cytokine secretion, viability, etc. Examples of different cell lines and receptors imaged with ClonePix FL are shown in figure 6.

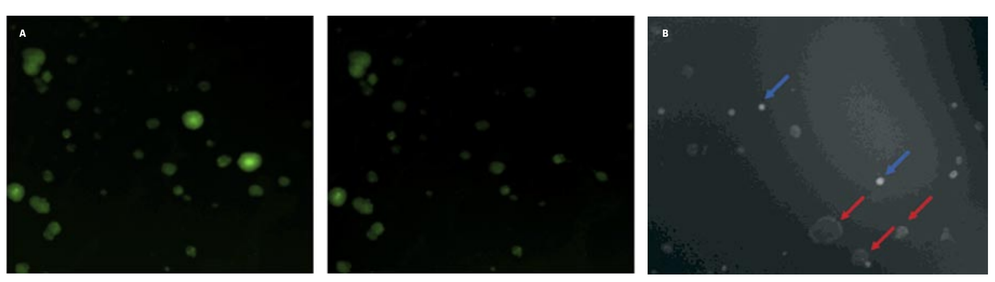

Figure 6: Selecting clonal populations of cells by cell surface marker expression.
ClonePix FL image of colonies of A) HEK293-F cells grown for 14 days in CloneMatrix based semi-solid media and detected/picked with FITC labelled antibody to the endogenous adhesion molecule ICAM-1. Left panel; pre-pick, right panel; post-pick, showing the removal of the two brightest colonies. B) CGR8 mouse Embryonic Stem cells grown for 10 days in CloneMatrix based semi-solid media and detected/picked with FITC labelled antibody to the pluripotancy marker, SSEA-1: blue arrows indicate undifferentiated colonies with high expression levels of SSEA-1; Red arrows show highly differentiated colonies (low SSEA-1 expressers).

Multiplexing for Viability in Cell Based/Productivity Assays

The ability to simultaneously measure the viability of cells used in cell based assays, be they the secretion/production assays demonstrated previously, or reporter cell assays for screening, is of great benefit to the drug discovery process and to cell line engineering.

We have developed a fluorescent dye that stains only viable (living) cells for use in a range of cell based assays – LiveDetect Green.

LiveDetect Green is an acetoxymethyl based cytoplasmic dye that only fluoresces when absorbed into the cytoplasm of living cells, where it is metabolised to its fluorescent form. Cells can be imaged, in the FITC excitation/emission spectra, either as individual cells (in cell imaging technologies or epi-fluorescent microscopy) or as viable colonies of cells (for ClonePix FL colony screening, as shown in figure 7).

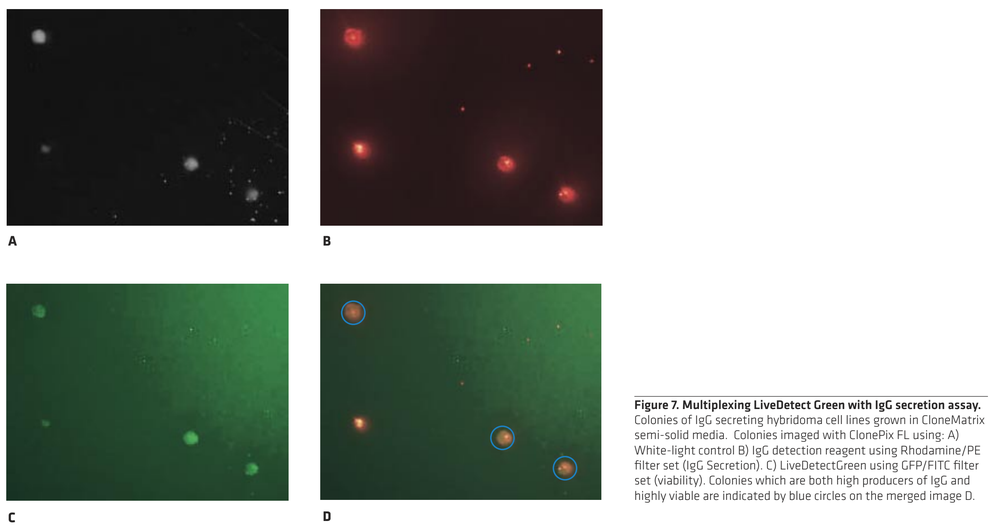

Figure 7. Multiplexing LiveDetect Green with IgG secretion assay.
Colonies of IgG secreting hybridoma cell lines grown in CloneMatrix semi-solid media. Colonies imaged with ClonePix FL using: A) White-light control B) IgG detection reagent using Rhodamine/PE filter set (IgG Secretion). C) LiveDetectGreen using GFP/FITC filter set (viability). Colonies which are both high producers of IgG and highly viable are indicated by blue circles on the merged image D.

The advantages of multiplexing viability with other fluorescent readouts give much more power to a single assay or screen:

- Functional screening: hit compounds simultaneously screened for cytotoxicity, allowing them to fail early if cytotoxic
- Cell line development: allows clones to be selected based on viability as well as production – figure 7
- Cell based assays: provides quality control of cells used in the assay, to ensure comparable viabilities across samples

Summary

Our unique ClonePix FL technology has been proven to significantly improve efficiency during the selection and optimisation of cell lines for bio-manufacturing, by shortening timelines and simplifying the downstream cell culture processes. This powerful technology has also proven to be highly effective in selecting not only the highest secreting, stable clones of cells producing specific monoclonal antibody, but also in selecting the highest secreting colonies of cells producing any therapeutic protein and cells expressing a specific receptor, combination of receptors or any other cell surface protein of interest. ClonePix FL is also being developed to image, analyse and select cells which contain fluorescent reporter dyes, etc; making it a powerful tool in many cell line development and selection applications in the biopharmaceutical sector.

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