High throughput screening and selection of stable high secreting clones

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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 4000 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 totally animal free conditions and has been adopted by many of the major pharmaceutical companies.

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 rapidly using both whitelight and fluorescent imaging. Figure 1 shows number of clones verses fluorescence from a single plate. 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 will contain a monoclonal population of cells; thus ensuring homogeneity as well as being positive for the desired fluorescent readouts.

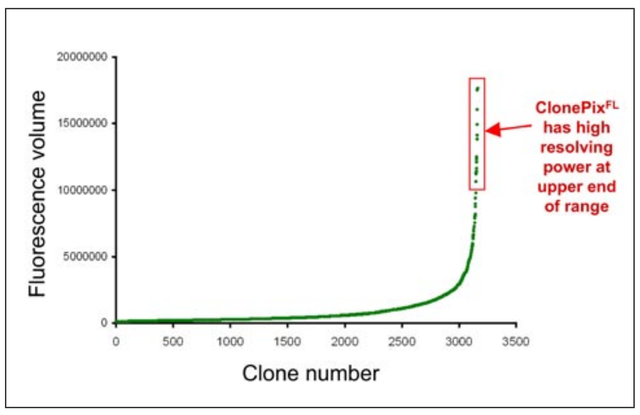

Figure 1. Genetix ClonePix FL can image over 3000 colonies in an hour. Distribution of fluorescence against clone number is shown.

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 and both suspension-adapted and adherent CHO cells.

The Fluorescent Based Assay

The fluorescent detection assay requires the secreted product to be immobilised around the colony using a detection antibody. The detection antibody can be either directly labelled with a fluorescent tag or a secondary detection agent can be utilised. Multiple detection agents labelled with different fluorophores may be used and detected (multiplexing). Figure 2 shows a cartoon representation of the detection reaction. Example images generated by the ClonePix FL are shown in Figure 3.

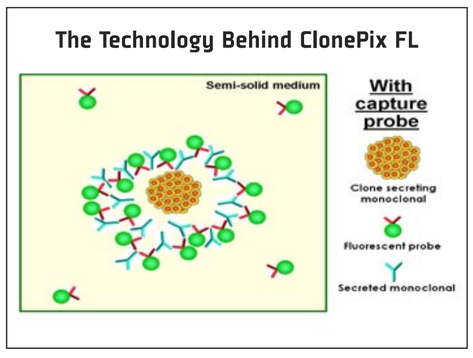

Figure 2. Formation of fluorescent precipitate around a clonal colony. Secreted IgG is cross-linked around a colony using a polyclonal FITC tagged anti IgG.

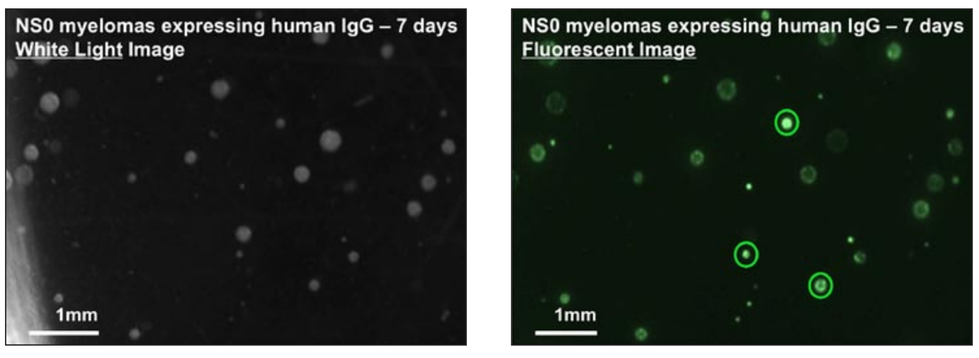

Figure 3. Fluorescent detection of a secreted IgG from an NSO myeloma cell line. An NS0 cell line expressing a human IgG against a bacterial protein was plated out and grown in CloneMatrix semi-solid media in the presence of FITC anti-IgG. After 7 days individual cells have grown into colonies (left 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.

Fluorescence Detection Correlates to Cell Line Productivity

Fluorescence intensities obtained for secreting clones are directly correlated to antibody secretion. Figures 4 and 5B 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 4). Use of the Genetix CloneSelect Imager enables rapid confluence measurement of a 96-well plate complementing the ClonePix FL.

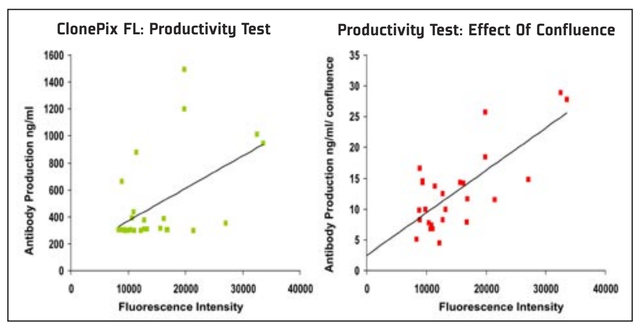

Figure 4. Protein secretion corresponds to fluorescence intensity. An IgG secreting NSO cell line was picked according to fluorescence. Productivity was measured by ELISA following 1 weeks growth (left panel). Taking into account confluence dramatically increases correlation between fluorescence and production (right panel).

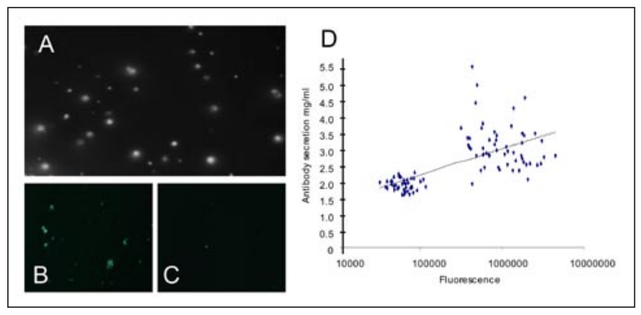

Figure 5. Protein secretion corresponds to fluorescence intensity in CHO-S cell lines. Panel A shows a FITC image from a heterogeneous population of an IgG secreting CHO-S cell line. High secretors were picked and re-plated following 7 days growth at the 96-well plate stage. A colourised image of a stable 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.

Recently transfected CHO cell lines are inherently unstable, which leads to a high drop out rate of high producers which 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 Figure 5.

Workflow for Generation of Stable Cell Lines

Based on the finding that some recent transfectants are inherently unstable, we have carried out multiple rounds of screening and picking for several cell lines. Figure 6 demonstrates the distribution of fluorescence obtained in the course of 4 rounds of screening of a transfected myeloma cell line. The top 6 clones isolated in the first round plating were picked and re-plated. These 6 clones showed an average of 20 fold higher protein production than the original population. Clones isolated in the second round showed productivity levels of up to 80% higher than those from the first round. However the population as a whole reflected an average of the 6 originally picked clones. Further rounds of selection did not alter the overall pattern. As a result of these studies we have devised the workflow shown in Figure 7 which utilises two rounds of screening.

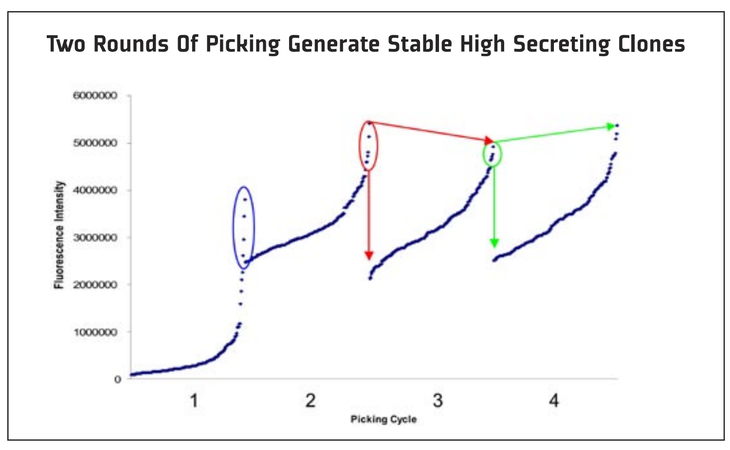

Figure 6. Multiple round screening of a stable mixed NSO cell line expressing a mouse IgG. Clones circled in blue were sub-cloned. The second round (red) gave a further 80 % increase in productivity. Subsequent rounds (green) gave no further increase in productivity.

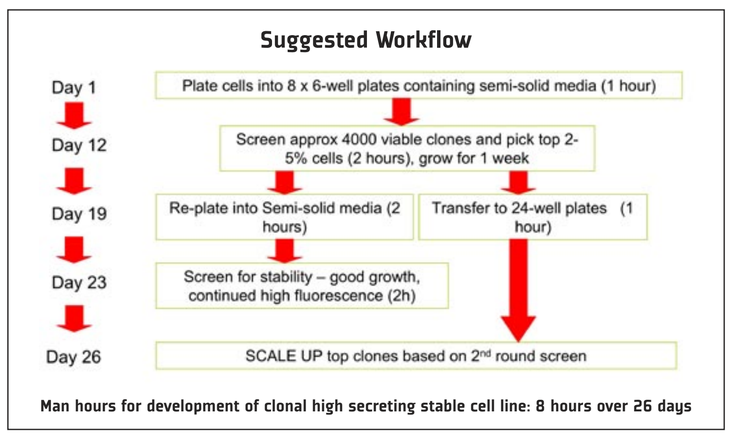

Figure 7. Generation of a stable high secreting cell line in 26 days.

Summary and Conclusions

Using Genetix ClonePix FL we have demonstrated the screening and selection of stable clonal populations of cells for use in the production of therapeutic antibodies, monomeric proteins, fusion proteins and cell surface receptors. Using the second-round screening workflow suggested here, reduces the timelines for generating a stable, high secreting clonal cell line from over 60 days when employing traditional techniques to just 26 days, with man hours of just 3 days.