Rapid Screening and Selection of Stable High Producing Clones

Steven Watters, Kerensa Jones, Gabe Longoria, Irene Bramke, Chris Mann and Julian F. Burke

Genetix Ltd, Queensway, New Milton, Hampshire, BH25 5NN, U.K. E-mail: steve.watters@genetix.com, Web: www.genetix.com

Introduction

- Screening cell populations for stable, high producing clones is a key rate-limiting step in the development and production of new protein therapeutics.
- We previously reported a novel technology, ClonePix FL, for isolating high-secreting clones in a rapid one-step process.
- We extend the findings here with a refined method for selecting high producers that are also stable secretors.

ClonePix FL Technology

Heterogeneous mammalian cell populations such as hybridoma fusions or transfected cell lines are plated into semi-solid medium such that cells form discrete clonal colonies. Thousands of colonies can be raised per 6-well plate and are screened by using a fluorescent probe that quantifies the *in situ* rate of protein secretion from each clone (Figure 1). The best clones are then selected for automated picking into pre-filled 96-well destination plates. The collection of clonal colonies rather than single cells, together with minimal handling, significantly enhances viability and facilitates expansion for scale-up.

The application has been validated for the specific detection of monoclonal antibodies, secreted monomeric proteins, cell surface proteins and intracellular GFP fusion proteins. The method is compatible with a range of cell types including hybridomas, myelomas, HEK293, and both suspension-adapted and adherent CHO cells.

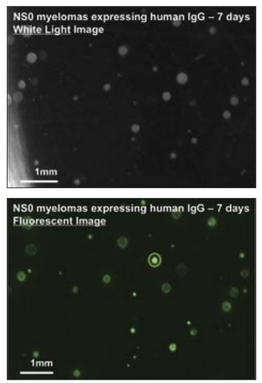

Figure 1. *In situ* detection of high-secreting clones. An NS0 cell line expressing a human IgG was plated out and grown in CloneMatrix-based semi-solid medium in the presence of CloneDetect-FITC. Individual cells were grown into colonies (upper panel), and were screened by the *in situ* fluorescence based assay to detect and quantify IgG secretion (lower panel). A typical high secretor is circled in green.

Second-Round Screening Eliminates Unstable Clones

A major consideration when selecting high producing clones for production is that they must continue to produce protein at a high rate for the duration of the production run.

We have previously shown that fluorescent-based measurement of clone secretion rate in a single round of screening by ClonePix FL correlates directly with protein productivity (R^2=0.744, $p<0.001$). However, recently transfected cell lines such as suspension-adapted CHO under chemically-defined conditions have a high ratio of unstable clones. Under these conditions, the subpopulation of clones selected by ClonePix FL, while being high secretors compared with the starting population, often show little or no correlation between fluorescence measured at the time of selection and their production rate measured subsequently by ELISA (Figure 2). We questioned whether clone instability is responsible for the lack of correlation.

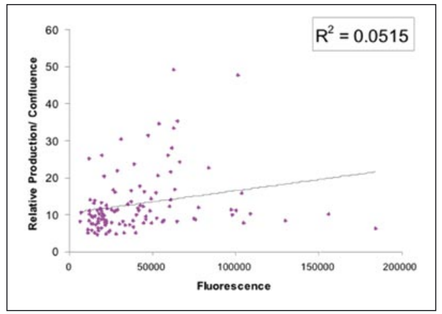

Figure 2. Lack of correlation observed between secretion measured by fluorescence and productivity of recently transfected cells. A heterogeneous population of IgG secreting CHO-S cells were screened under chemically-defined conditions using ClonePix FL, and the highest secretors (top 2%) were picked based on fluorescence intensity. After 14 days growth in 96-well plates, the clones were measured for productivity using an IgG-specific ELISA. The graph shows a significant number of clones that fluoresced strongly at the time of picking but showed low productivity at the time of measurement. All production values were corrected for confluence (Genetix CloneSelect Imager).

We have developed a protocol for testing the stability of clones selected by ClonePix FL, which requires minimal input and runs in parallel with clone expansion so that the overall timeline remains unchanged. The candidate high-secreting clones are subjected to a small-scale second round of plating into semi-solid medium and ClonePix FL screening to identify the stable clones. A clone with good stability will generate subclones with high and homogeneous secretion (see Figure 3A), whereas a non-stable clone leads to subclones with heterogeneous expression (see Figure 3B). This requires approximately four hours extra labour and provides results within one week.

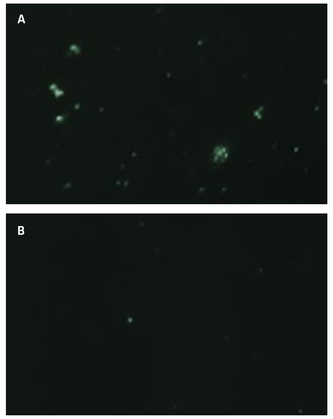

Figure 3. Second round screening rapidly identifies stable and unstable clones. A heterogeneous population of IgG secreting CHO-S cells were screened under chemically-defined conditions using ClonePix FL, and the highest secretors were picked based on fluorescence intensity. After 7 days growth in 96-well plates, the clones were re-plated into CloneMatrix-based semi-solid medium and re-imaged at day 7. A stable high secreting clone is shown in Panel A and an unstable clone identified as a high secretor in round 1 is shown in Panel B.

The efficiency of this second-round process to screen out unstable clones is confirmed by a strong direct correlation between fluorescence and productivity of the selected clones (Figure 4).

Two Rounds of Screening Optimise Cell Line Productivity

The ease with which ClonePix FL is able to rapidly screen large cell populations has allowed the possibility to observe cell populations through multiple rounds of screening. Figure 5 shows the distribution of clone secretion obtained in the course of 4 rounds of screening of a myeloma cell line transfected to produce humanised IgG.

The top 6 clones isolated in the first screen were picked and re-plated. These 6 clones showed an average of 20 fold higher protein production than the original transfected population. In the second-round subcloning step, the best clones were found to secrete IgG at a rate 80% higher than their parent clones. Further rounds of selection showed a remarkably similar overall pattern of distribution to that observed in the second round. These results indicate that, within any clonal population, a small number of subclones are responsible for much of the production, but that these super-secretors cannot generate a line of stable super-secretors. The data also indicate that two rounds of screening are ideal for cell line productivity optimisation.

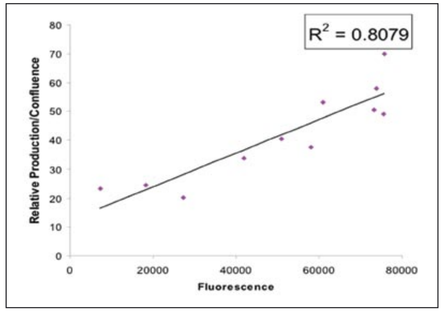

Figure 4. Correlation between secretion measured by fluorescence and productivity of recently transfected cells after second-round screening. The highest secretors of a heterogeneous population of IgG secreting CHO-S cells were re-screened as described in Figs 2 & 3. The graph shows a strong direct correlation after exclusion of unstable clones. All production values were corrected for confluence (Genetix CloneSelect Imager).

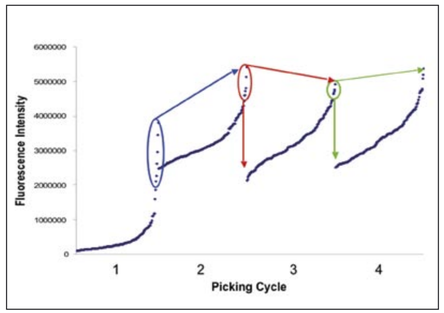

Figure 5. Two rounds of screening generate the highest stable secreting clones. An NS0 cell line transfected to express a human IgG was subjected to multiple rounds of screening on ClonePix FL. At each round, the 6 clones with highest fluorescent intensity were selected for re-screening. The second round (shown in blue) indicated clones with a further 80 % increase in productivity compared with clones from the first round. Subsequent rounds (red and green) gave no further increase in productivity. Note, however, that the fluorescent intensity of the poorest secretors remained constant.

Based on the results of these studies, a workflow is proposed which utilises two rounds of screening (Figure 6).

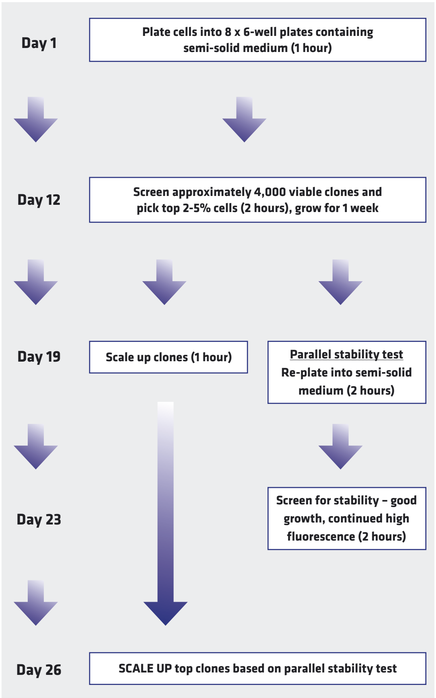

Figure 6. Suggested workflow for rapid generation of a stable high secreting cell line.

Summary and Conclusions

The method described here reduces the timeline for generating stable high producing clonal cell lines from over 60 days by traditional techniques to 26 days with just 8 hours of labour. The low labour requirement means that more cell populations can be interrogated in parallel increasing cell line throughput. Additional benefits are the ability to screen larger populations than by existing methods, and minimising downstream procedures.