Rapid Screening and Selection of Stable High Producing Clones

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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 here the findings with a refined method for selecting high producers that are also stable secretors.

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 fluorescence-based measurement of IgG secretion in a single round of screening using ClonePix FL correlates directly with protein productivity (R² = 0.744, p<0.001). However, recently transfected cell lines such as suspension-adapted CHO under thermally defined conditions have a high ratio of unstable clones. Under these conditions, the subpopulation of clones selected by ClonePix FL, whilst being high secretors compared with the starting population, often show little or no correlation between fluorescence measured at the time of selection and their protein productivity rate measured subsequently by ELISA (Figure 2). We questioned whether clone instability is responsible for the lack of correlation.

Figure 2. 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 (FL1) intensity. Day 0 refers to the day the clones were picked, whereas Day 7 shows the production of the selected clones at the time of imaging. All production values were corrected for confluence and productivity (Genetix CloneSelect images). The top 6 clones isolated in the first screen were picked and re-plated. These 6 clones showed an average of 20 fold higher secretor production than the original transfected population. In the second round subcloning step, the six clones were found to secrete IgG at a rate 80% higher 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 the original transfected population. Under these conditions, the subpopulation of clones selected by ClonePix FL, whilst being high secretors compared with the starting population, often show little or no correlation between fluorescence measured at the time of selection and their protein productivity rate measured subsequently by ELISA (Figure 2). We questioned whether clone instability is responsible for the lack of correlation.

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 (FL1) intensity. Day 0 refers to the day the clones were picked, whereas Day 7 shows the production of the selected clones at the time of imaging. All production values were corrected for confluence and productivity (Genetix CloneSelect images). The top 6 clones isolated in the first screen were picked and re-plated. These 6 clones showed an average of 20 fold higher secretor production than the original transfected population. In the second round subcloning step, the six clones were found to secrete IgG at a rate 80% higher than the original transfected population. Under these conditions, the subpopulation of clones selected by ClonePix FL, whilst being high secretors compared with the starting population, often show little or no correlation between fluorescence measured at the time of selection and their protein productivity rate measured subsequently by ELISA (Figure 2). We questioned whether clone instability is responsible for the lack of correlation.

Figure 4. Correlation between secretion measured by fluorescence and productivity of recently isolated super-secretors. 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 images). 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 Optimize 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 IgG secretion obtained in the course of 4 rounds of screening of a heterogeneous cell line transfected to produce humanized IgG. The top 4 clones isolated in the first screen were picked and re-plated. These 4 clones showed an average of 20 fold higher secretor production than the original transfected population. In the second round subcloning step, the four clones were found to secrete IgG at a rate 80% higher than the original transfected population. Further rounds of screening showed a remarkable similar overall pattern of distribution to that observed in the second round. These results indicate that, within any clonal population, a small number of super-secretors 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 the duration of the production run.

Summary and Conclusions

The method described here reduces the timeline for generating stable high-producing cell lines from over 60 days to traditional techniques in 26 days with just 9 hours of labour. The low labour requirement means that new cell populations can be interrogated in parallel, thus increasing cell line throughput. Additional benefits are the ability to screen larger populations than by existing methods, and streamlining downstream procedures.