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Integration of automated Colony Picking into fast track Cell Line Development Process

Introduction:

The generation of highly productive mammalian cell lines is a multistep process requiring optimization of each individual step.
This includes careful selection of promoters and stabilizing elements as well as fine-tuning of the vector, transfection and drug selection procedures to assure reproducible generation of clone pools that contain a substantial degree of high producer cells. Single cell cloning and screening is the second step with major impact on the outcome of cell line development. Due to its substantial time and labour requirements this step does greatly benefit from automation. Highly efficient screening, however, does not eliminate the need for detailed clone analysis and comparison of clones under conditions of a miniaturized process (batch and fed-batch).

Starting Point:

To compare automated versus manual clone generation we use a model antibody. An expression vector with two independent strong promoters for heavy- and light chain and two selection markers was transfected into into pre-selected CHO-DG44 cells. During a two week selection pools of stable highly productive clones were selected and used for further evaluation (see flow chart).

Automated Colony Picking:

The **ClonePixFL** from Genetix offers expression analysis, ranking and automated picking of mammalian cell colonies from a semi-solid medium. Colonies are imaged, selected and picked based on a number of parameters such as size, roundness, proximity to neighbours and assessment of target protein secretion. (fig.1)

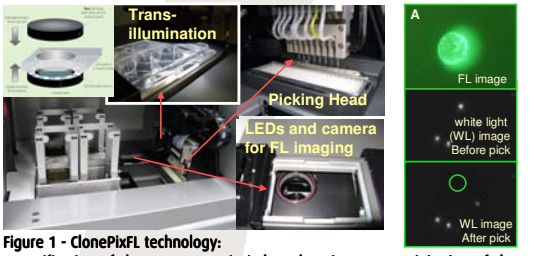
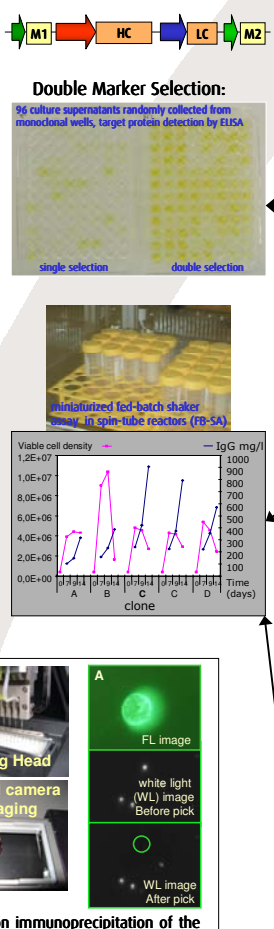


Figure 1 - ClonePixFL technology:
Quantification of the target protein is based on immunoprecipitation of the secreted protein with a specific fluorescently labeled detection antibody in semi-solid medium. The ClonePixFL can measure and integrate the fluorescent signal surrounding each colony (A). This integrated fluorescent (FL-) volume reflects the amount of secreted target protein.



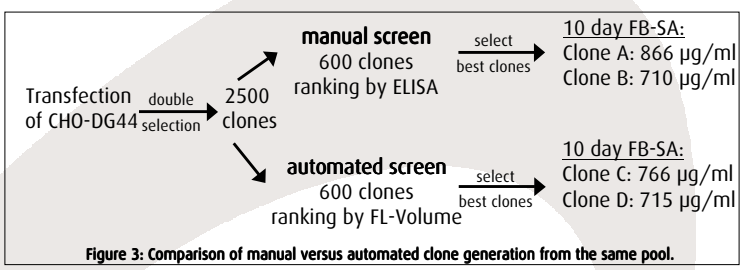


Figure 3: Comparison of manual versus automated clone generation from the same pool.

viable cells grow up to form colonies. This low survival rate raises the question whether the conditions in the semi-solid medium really are suitable for the best clones or if instead high- and very high producer clones are unwittingly eliminated. Therefore, we have generated clones from the same cell pool either manually or by the automated method. The best clones of each development were analysed in a 10 day fed batch process and show similar IgG production (fig.3).

Can we find very rare clones?

The ClonePixFL technology can easily screen several thousands of colonies and provides the opportunity to find even a very rare high producer. This should reduce the requirement for a generation of highly productive clone pools.

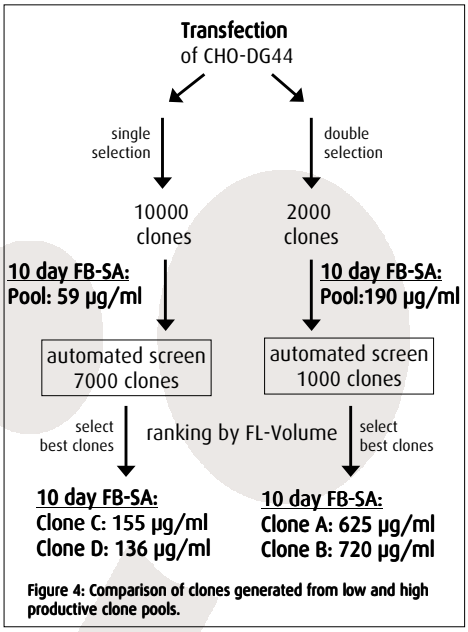
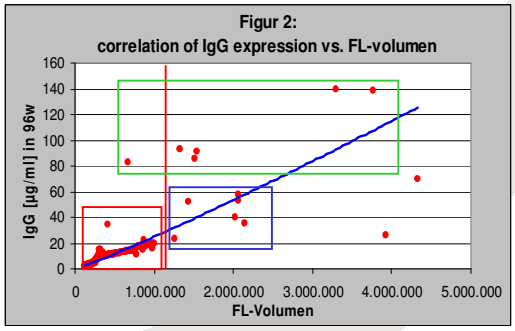


Figure 4: Comparison of clones generated from low and high productive clone pools.

The comparison of best clones from extensive screening of clone pools with significantly different productivity demonstrates that great clones can only be generated from high quality clone pools (fig.4).

Are we picking the best clones ?

At first we assessed the degree of correlation between fluorescent signal and the productivity of the picked colonies (fig.2) and found an excellent discrimination between low (red rectangle) and high producers (blue rectangle), whereas separation of very high (green rectangle) from high producers remains difficult. This requires selection of a higher number of clones for further clone analysis.
Secondly, we observed that after seeding CHO cells into semi-solid medium only 10-20% of the



Conclusions:

- **ClonePixFL can efficiently eliminate low producers and offers an excellent primary selection step with substantial time benefit.**
- **Automated analysis and picking compared with an extensive manual approach results in clones with comparable productivity.**
- **In summary, cell line development can substantially benefit from automated clone analysis and picking with ClonePixFL when it is integrated into a fully optimized process.**