Automated screening of high producer HEK293F clones and analysis of post-translational modifications of secreted proteins

Peter Söhlemann, Claudia Kollert, Jessica Thiems, Elisabeth Casademunt and Carola Schröder
Octagene GmbH, Martinsried/Germany, www.octagene.com, schroeder@octagene.com

Introduction

Production of best tolerated therapeutics is the core competence of Octagene. Haemophilia patients are treated lifelong with drugs to prevent bleeding. The daily risk of potential infections is reduced by production of recombinant clotting factors in human cells. Human-identical post-translational modifications are target for best treatment.
ClonePixFL (Genetix) is a robot to analyze protein secretion and to pick best producers only. We developed the technology to detect and analyze proteins secreted by human cells in serum- and protein-free medium.

Objective

Selection of cell lines with best productivity

- Production in human cell line
- Growth in serum- and protein-free, semi-solid medium
- Detection of monomeric proteins

Method

- **Seeding for picking**: Spherical clones developed within 7 days in conditioned serum- and protein-free FreeStyle semi-solid medium in non-treated black EquiGlass 6wp (Genetix). A different medium was used for adherent growth in TC-treated 6wp (Nunc).
- **Imaging**: Clones growing spherically or adherently were identified with ClonePixFL white light box. Secreted protein was quantified with FITC-labelled antibody after 3 days. Multiplexing (fluorescence per white light signal) determined cell-specific productivity.
- **Picking**: Preferred pin size was 700 µm inner diameter.
- **Growth post-picking**: Confluent cultures developed post-picking in conditioned FreeStyle medium in 96wp (Nunc).
- **Software**: ClonePixFL software QSoft ExCellerate identified clones and protein secretion. Selection was based on plate-by-plate comparison. Multiplexing (cell-specific productivity) currently is developed by Genetix. The Fusion software will substitute ExCellerate in 2007 with improved imaging, multiplexing and batch analysis.

Process

- Transfection
- Primary screening/subcloning (→ image 3000, pick 300 producers)
- Analyses → growth, productivity & activity (→ 50 clones)
- Fermentation → scalability & inducibility (→ 5 clones)
- Subcloning of 5 clones

Results

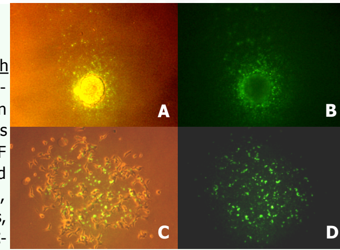
- Human HEK293F cells grow in semi-solid medium
- Serum- and protein-free FreeStyle is preferred for screening
- Suspension growth is superior to adherent growth
- Clone selection is automated (ClonePixFL, Genetix)
  - 90% viable clones grow post-picking
  - 90% of selected clones are high producers
  - Selected clones are >10x better than randomly picked clones
- Monomeric clotting factors are successfully detected
- ClonePixFL quantifies protein secretion and analyzes quality (**new**!)

Conclusions and outlook

1. Automated clone selection significantly lowers workload. Screening time is reduced and throughput is increased to thousands of imaged clones. Selected cells produce at least 10-fold more secreted protein compared to randomly picked cells.
2. For the ClonePixFL technology, we have developed assays for quantification and analysis of protein quality (post-translational modifications).

→We provide service for multiplexed selection of producers.

Figure 1



Clones for picking with ClonePixFL: The wild-type cDNA of a human coagulation factor was transfected into HEK293F cells. Cells were seeded in semi-solid medium, grown for ten days, stained with FITC-labelled antibody and imaged three days later. Clones were visualized by fluorescence and white light (**A** & **C**) or by fluorescence only (**B** & **D**). Spherical clones (**A** & **B**) allowed much better imaging than adherent clones (**C** & **D**).

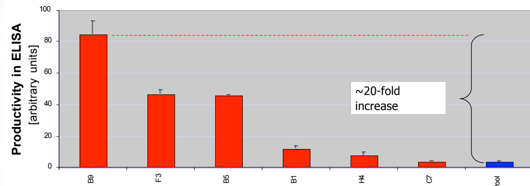


Figure 2

Major advantages of automated clone selection: Cells were grown as adherent cultures, stained and imaged. 15 clones were randomly picked (white light only). 243 clones were picked with respect to highest fluorescence by ClonePixFL. Protein levels were quantified by ELISA from supernatants of clones growing in 96wp. Clones were selected via fluorescence (red bars) and the best showed more than 10-fold higher productivity compared to randomly picked clones (pool, blue bar).

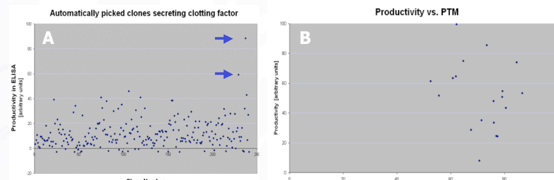
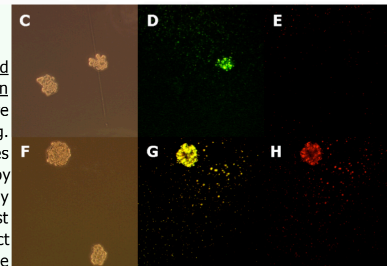


Figure 3



Quantification and analysis of protein quality: Cells were treated as in Fig. 2. 3000 clones were imaged by ClonePixFL. Only the very best clones with respect to fluorescence were picked. **A**, ELISA from supernatants of clones expanded to 96wp confirmed that the highest productivity corresponds to clones selected via multiplexing (arrows in the upper right corner). **B**, Post-translational modification of the clotting factor were monitored in a second ELISA. **C – H**, Proof-of-concept test to simultaneously monitor protein quantity and protein quality before picking. Cells were stained with a mixture of antibody 1-FITC (quantity) and antibody 2-PE (PTM, quality). Cells were visualized with white light (C & F), protein secretion with a filter for green fluorescence (D & G), and PTMs with a filter for red fluorescence (E & H). One clone (C – E) secretes the clotting factor devoid of the PTM, thus only green fluorescence is detected (D). In contrast, one clone (F – H) secretes the clotting factor with the PTM, which is seen in bright yellow (G) due to the overlay of green and red signals (H).

OCTAGENE
For Recombinant Proteins