# Cutting-Edge Transient Gene Expression:

## FROM DNA PREP VIA MEDIA, REAGENT AND CELL LINE DEVELOPMENT TO HOLISTIC PROCESS OPTIMIZATION

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During the last years, InVivo BioTech Services has realized a novel technology for efficient transient transfection and expression in HEK and CHO cells. In the process of transient gene expression, introduction of the gene of interest into the host cell can be performed by various physical, chemical or biological methods. Because of the greater scalability compared to physical methods and no safety concerns or restrictions that are associated with the use of viral systems, a transfection using chemical methods is the method of choice. However, up to now up-scaling is limited by various scientific and economic bottlenecks regarding plasmid preparation and vector design, transfection reagents, host cell lines and cultivation media. To overcome these bottlenecks, InVivo BioTech Services developed in cooperation with emp Biotech, Berlin, and Xell AG, Bielefeld, a transfection reagent and a new culture medium that can be used for transfection and production. The in-house establishment of a TGE optimized HEK cell line and a method for large-scale plasmid preparation completed the production platform for multi-parallel approaches and large-scale transfection for the production of gram quantities IgG within days.

## Partners

InVivo BioTech Services GmbH

emp BIOTECH · CeBiTec Center for Biotechnology · xell

## Methods

### Cultivation and transfection

Mammalian cells were cultivated and transfected in Xellvivo TM medium (Xell AG) under conditions of 37 °C, 5 % $CO_2$ and 185 rpm agitation speed at 50 mm orbital diameter. For screening approaches 5x10E6 cells/mL were transfected with 2 pg DNA/cell and INVect transfection reagent (Cat. No. FK-0101-M001.0-001, emp Biotech GmbH) with INVect to DNA ratio of 6:1 (w/w) or 25 kDa L-PEI with PEI to DNA ration of 2:1 (w/w) in 8 mL culture volume in 50 mL bioreactor tubes. Expression of IgG1 was performed in 30 mL culture volume in 125 mL shake flasks, in 150 mL culture volume in 500 mL shake flasks or 1500 mL in 3000 mL shake flasks respectively.

### Directed evolution

Directed evolution was performed as described in the literature. In detail, an iterative process of evolution rounds followed by analysis of favorable attributes, cell selection and recovery was implemented. The corresponding flow cytometry analysis was performed using a Bio-Rad S3 cell sorter.

### Design of Experiments

To provide holistic process optimization we utilize a DoE based strategy uses surface response methodology. First, basic transfection parameter e.g. cell density, amount of DNA and INVect were optimized. Afterwards process parameter including chemical effectors and feeding were screened and synergistically combined.

### Plasmid preparation

Several *E.coli* strains and media were screened for high productivity, high quality and flexibility for DNA preparation in comparison to commercial kits in mini-preparation scale. A purification process was implemented using a reusable and scalable anion exchanger. For large scale plasmid preparation, 6 L suspension was lysed, clarified and purified using an Äkta chromatography system.

## Analytics

### Transfection efficiency

Transfection efficiency was determined 24 hours post transfection by counting green fluorescent positive cells using a FACSCalibur (BD).

### Productivity

SEAP expression was determined in cell culture supernatant on day 6 post transfection by a photometric pNPP turn-over assay. Quantification of IgG was performed by protein A affinity chromatography on day 6 post transfection.

### Viable cell counting

Viable cell counting was performend by trypan blue exclusion in a Neubauer chamber or propidium iodide assay for a flow cytometry-based method.

## Conclusions

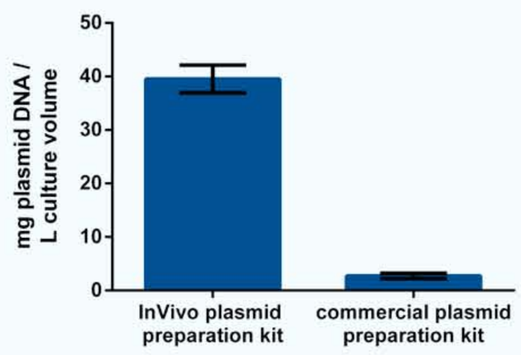
In conclusion, InVivo's TGE system includes an optimized and advanced cell line as well as vector system, a novel transfection reagent and a specially designed medium. With this system we offer multi-parallel production of recombinant proteins for early development and lead identification as well as gram-scale production for pre-clinical trials. In addition, our proprietary vector system enables switching to stable cell line generation for the production of larger amounts.

## Results

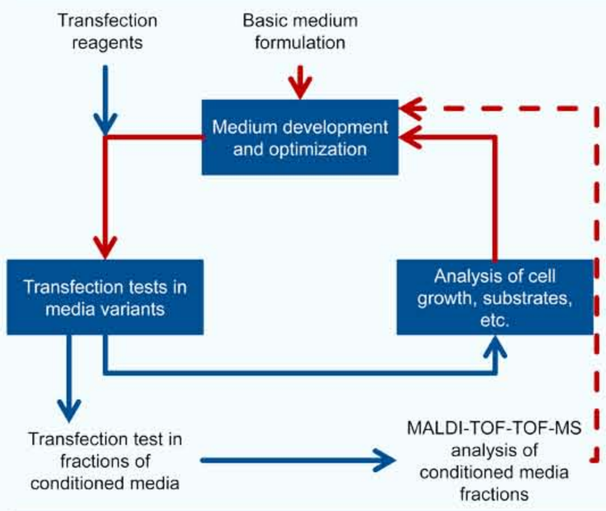
### Plasmid Preparation

In order to achieve a high yielding plasmid preparation process, production of a TGE optimized plasmid was compared by using different *E. coli* strains cultivated in three different media. Quality attributes highly depended on the host strains, whereas absolute yield was found to be a function of the cultivation medium. Additionally, a purification process was implemented using an anion exchanger. Four independent runs resulted on average in 40 mg plasmid DNA/L culture volume. Up-scaling this process results in approx. 250 mg purified plasmid DNA.

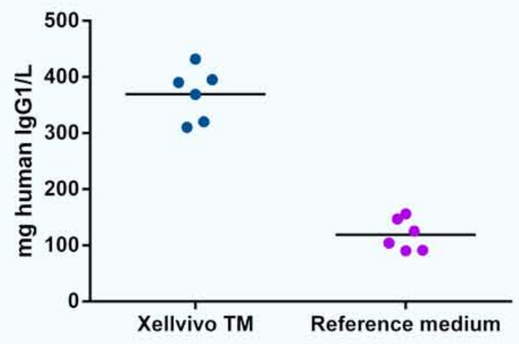
| Host Strain | Medium 1 | Medium 2 | Medium 3 |
|---|---|---|---|
| A | 11 mg/L | **32 mg/L** | 17 mg/L |
| B | 15 mg/L | **28 mg/L** | 11 mg/L |
| C | 17 mg/L | 17 mg/L | **27 mg/L** |
| D | 8 mg/L | **28 mg/L** | 12 mg/L |



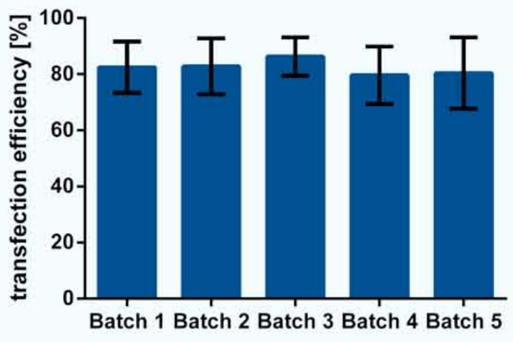

### Media Development

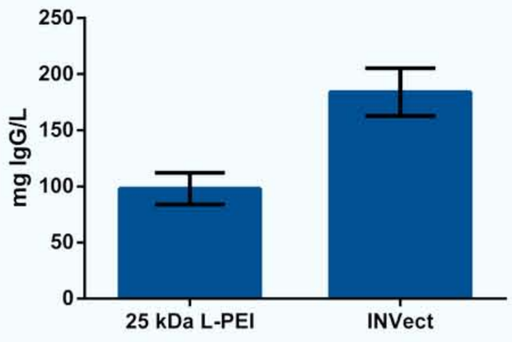



Starting from a basal medium we were able to generate a novel medium, which supports high titer transient gene expression. Improvements were achieved by stepwise screening and optimization of media ingredients with regards to higher cell growth, transfection efficiency and productivity, resulting in a 4-fold increase in comparison to a reference medium. Furthermore, the new medium supports cell growth and easy adaption to suspension of various parental and producer HEK cells.



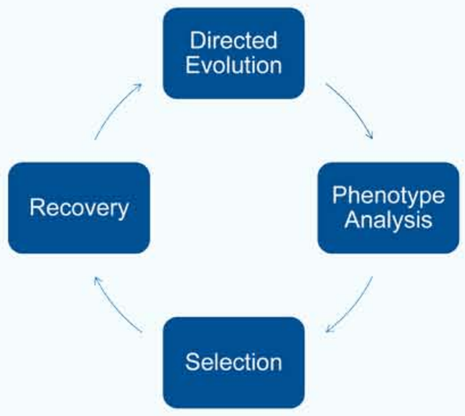

### Transfection Reagent Development



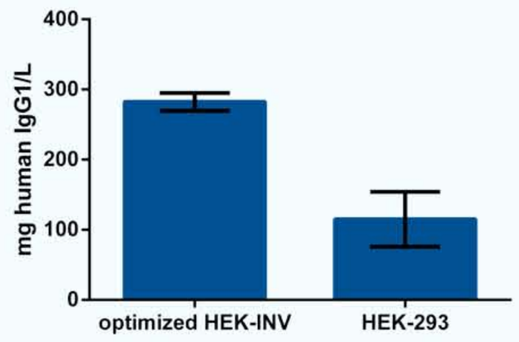




Commonly used transfection reagents such as polyethyleneimine (PEI) are quite cytotoxic when used in high concentrations. INVect is a transfection reagent which demonstrates low cell toxicity for transient transfection of mammalian cells and delivers extremely high transfection efficiencies up to 90%, 24h post transfection. The use of INVect for transfection under TGE conditions leads to exceptionally high levels of protein expression and outperforms 25kDa linear PEI by 2-fold.

### Cell Line Development




To generate an optimized host cell line for TGE processes we utilized a directed evolution approach, which results in a threefold increase in IgG productivity. For this purpose, an iterative process of evolution rounds followed by metabolomic phenotype analysis and selection of cells was performed. In detail, we mainly focused on productivity and growth characteristics. By this, the new cell line showed a 3-fold increase in IgG1 productivity in comparison to the parental host cell line.

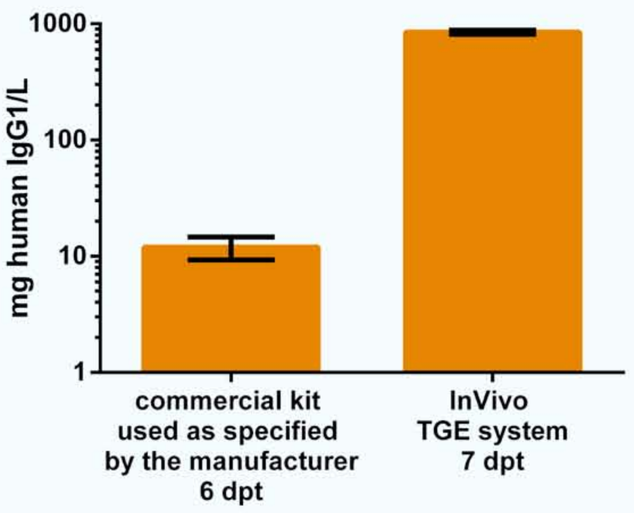



## Holistic Process Optimization

A broad range of various effector chemicals were screened including but not limited to histone deacetylase inhibitors, cell cycle arresting reagents and stimulating chemicals. At least two candidates turned out to improve either cell viability or productivity in a particular way and were chosen for further process development.

Adding a specialized feed supplement post transfection ensured that essential components remained at a non-limiting level throughout the fed-batch process. Early high volume feeding demonstrates great positive impact on cell density and viability and minimizes contamination risk due to minimal invasive intervention.

By combining all set screws, it was possible to generate a high yielding TGE production process. Using a holistic DoE-based optimization of all relevant parameters resulted in about 80-fold increase in human IgG1 production in comparison to a commercial available TGE system.




| Effector | Yield | Viability |
|---|---|---|
| A | + 16 % | ± 0 % |
| B | + 26,5 % | - 3 % |
| C | + 13 % | + 25 % |
| D | - 30 % | - 18,5 % |
| E | + 18 % | + 7,5 % |
| F | - 5,5 % | - 11 % |
| G | + 287 % | - 55 % |