Transient Transfection of Human CAP-T Cells - Basics and Optimization

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Introduction:

As a contract manufacturer of recombinant proteins and antibodies InVivo Biotech employed transient expression technology for prompt production of small and medium size volumes. Therefore we have strong interest in continuously optimizing expression efficiency aiming on a reliable process capable for production of a broad variety of recombinant protein at maximum quality. For transient transfection HEK cells as well as variants thereof like HEK293-EBNA and HEK293-F are the most commonly used cell lines. Recently a new cell line, CAP-T™, developed by CEVEC Pharmaceuticals GmbH, Cologne, arose as new potent platform for transient gene expression. Here we report experiments leading to a solid process allowing cost effective and standardized production providing efficiencies beyond conventional expression systems.

CAP® cells:

CAP is a human amniocyte cell line, derived from amniotic fluid cells and immortalized using adenoviral functions. In addition, CAP-T cells stable express SV40 large T-antigen. The cell line is characterized by good transfection capabilities, easy cultivation in serum free suspension and high viability even in extensive expression processes. In addition these cells are specially promoted for most human like glycosylation pattern and are capable of attaching an terminal sialic acid. Therefore CAP-T cells are a promising tool for production of complex mammalian proteins for all kinds of purposes, particularly for preclinical related applications. In combination with expression plasmids harboring the SV40 ori a high long-term plasmid yield after transfection is achieved subsequently resulting in high titers of recombinant proteins. Consequently we started to use CAP-T cells for transient gene expression.

Development of a Transient Transfection Protocol for CAP-T Cells:

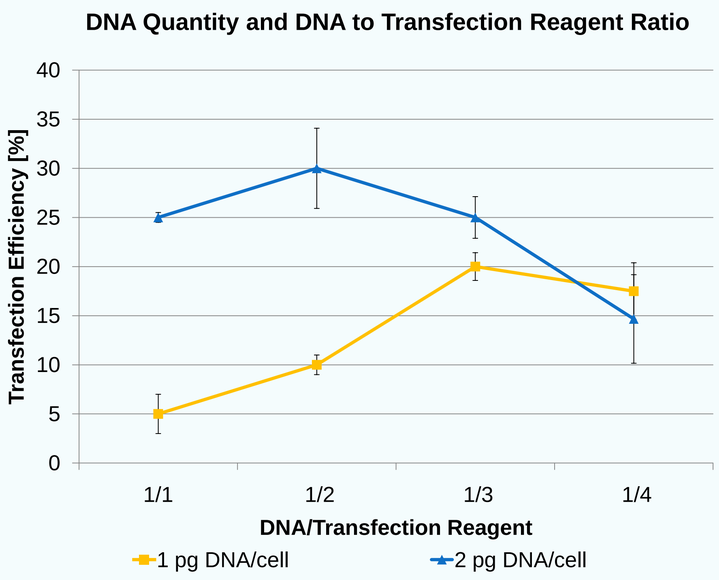

Figure 1: Impact of DNA quantity and DNA to transfection reagent ratio on transfection efficiency. CAP-T™ cells (CEVEC, Cologne) were transfected with 1 pg or 2 pg per cell of a GFP-expression plasmid at a cell density of 5 x 10^6 cells per mL in 4 mL FreeStyle™ 293 Expression Medium (Invitrogen) with 25 kDa linear polyethylenimine (PEI) from Polysciences as transfection reagent by varying DNA to transfection reagent ratio. Transfected cultures were supplemented 4 hours post transfection with same volume Protein Expression Medium (Invitrogen). Transfection efficiency was determined 24 hours post transfection by counting green fluorescent positive cells by fluorescence microscopy.

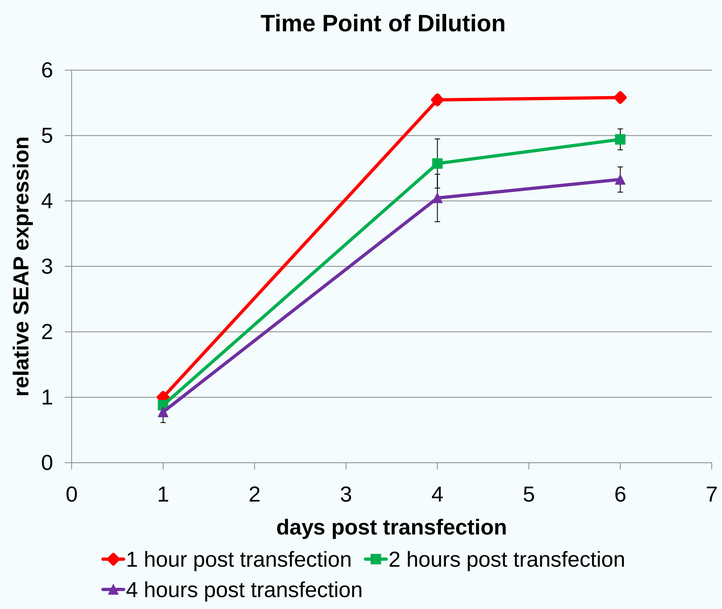

Figure 2: Variation of dilution time point. CAP-T cells were transfected with 2 pg per cell of a *secreted alkaline phosphatase* (*SEAP*) gene harboring plasmid at a cell density of 5 x 10^6 cells per mL in 4 mL FreeStyle Medium with PEI at a DNA to transfection reagent ratio of 1 to 2. Transfected cultures were supplemented at different time points post transfection with same volume Protein Expression Medium. SEAP expression was determined in cell culture supernatant of day 1, 4 and 6 post transfection by a photometric pNPP turn-over assay. SEAP expression was normalized to sample “1 day post transfection – diluted 1 hour post transfection”.

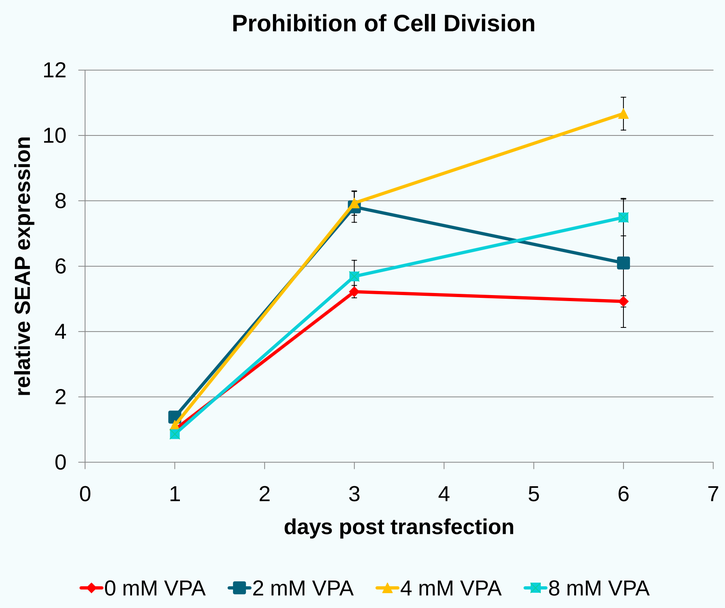

Figure 3: Increased productivity by prohibition of cell division. CAP-T cells were transfected with a *SEAP* gene harboring plasmid (2 pg / cell, cell density: 5 x 10^6 cells / mL in 4 mL FreeStyle Medium, with DNA / PEI ratio 1/2). Transfected cultures were supplemented1 h post transfection with same volume Protein Expression Medium and a 400 mM solution of valproic acid (VPA – Sigma-Aldrich) was added to indicated final concentration. SEAP expression was determined in cell culture supernatant of day 1, 3 and 6 post transfection by a photometric pNPP turn-over assay. SEAP expression was normalized to sample “1 day post transfection – 0 mM VPA”.

Helper Plasmids:

The addition of helper plasmids expressing different growth factors did not significantly improve expression rate during transient transfection (data not shown).

Protein Expression: CAP-T versus HEK293-F cells

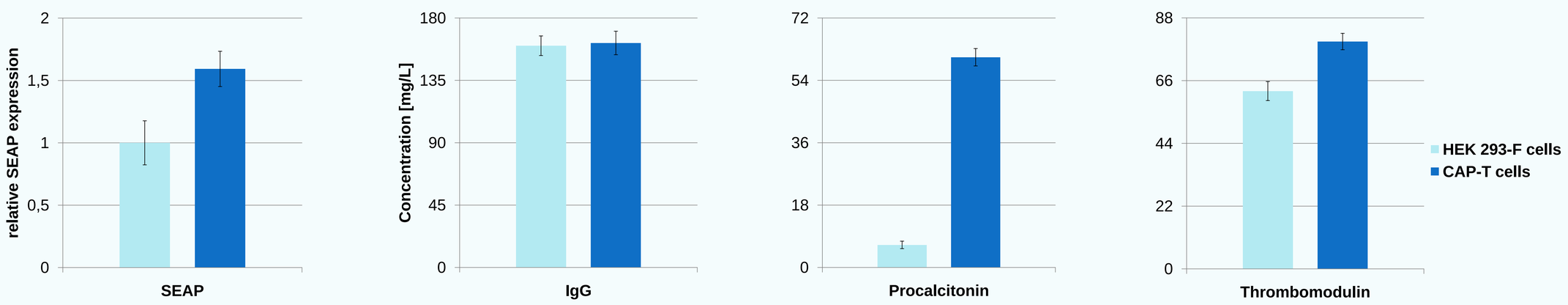

Figure 4: Evaluation of protein expression from CAP-T and HEK293-F cells. CAP-T and HEK-293-F cells (Invitrogen) were transfected with respective cDNA containing expression plasmids (2 pg / cell, cell density: 5 x 10^6 cells / mL in 4 mL FreeStyle Medium, with DNA / PEI ratio 1/2, supplementation 1 h p. t. with same volume PEM, 4 mM VPA). Protein concentrations were determined in cell culture supernatant 7 days post transfection. SEAP expression was measured as described previously, IgG was quantified by analytical protein-G quantification, Procalcitonin concentration was measured using Cryptor assay (Thermo Fisher Scientific) and Thrombomodulin was quantified by ELISA (american diagnostica).

Transient Transfection Protocol for CAP-T Cells:

- Medium: FreeStyle™ 293 Expression Medium (Invitrogen)
- DNA per cell: 2 pg
- Cell density: 5 x 10^6 cells / mL
- Supplementation: 1 hour post transfection
  - Same volume Protein Expression Medium (Invitrogen)
  - 4 mM Valproic Acid (Sigma-Aldrich)

Future work to be done:

- Based on previously performed experiments we expect to increase productivity significantly by additional feeding during cultivation.
- Protein expression can be enhanced on transcriptional level by optimizing expression plasmids.

More than 20 different recombinant proteins have been produced successfully using different sized batches up to 2,0 L total transfection volume.

Rec. Protein	Yield / L [mg]
IgG-r1	136
Thrombomodulin *	80
Vaspin *	71
IgG-i75-3	68
Procalcitonin	60

Table 1: Selected medium size batch productions of recombinant proteins. * = highly glycosylated protein

All contract manufacturing transfections have been performed with NB33, a novel developed transfection reagent, compare to poster # **4.140** (Levison, D. *et al.* “Development of a Cationic Reagent for Transient Transfection of Mammalian Cells”). Medium sized test transfections were also performed using 25 kDa linear PEI (Polysciences) with significantly lower yields.

Conclusion:

We optimized transfection parameters for CAP-T cells and increased protein production based on the determination of optimal dilution time point and prohibition of cell division by adding VPA.

We showed that recombinant protein expression is up to ten times higher when using CAP-T cells instead of HEK293-F.