Development of a Cationic Reagent for Transient Transfection of Mammalian Cells

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Introduction:

For rapid recombinant protein production in small to medium size volumes, transient transfection of mammalian cells is still the method of choice in biotechnology. However, the available transfection reagents represent a bottleneck due to the high costs associated with the commercial use of lipofectamines and other polycationic transfection reagents such as the widely used polyethylenimine (PEI). While these reagents produce seemingly high transient transfection rates, there is still a strong desire for transfection reagents which allow more secure and easier handling and higher recombinant protein production. To maintain competitiveness, InVivo Biotech Services initiated a joint venture with emp Biotech to develop novel reagents (NB) for transient transfection and recombinant protein production in mammalian cells.

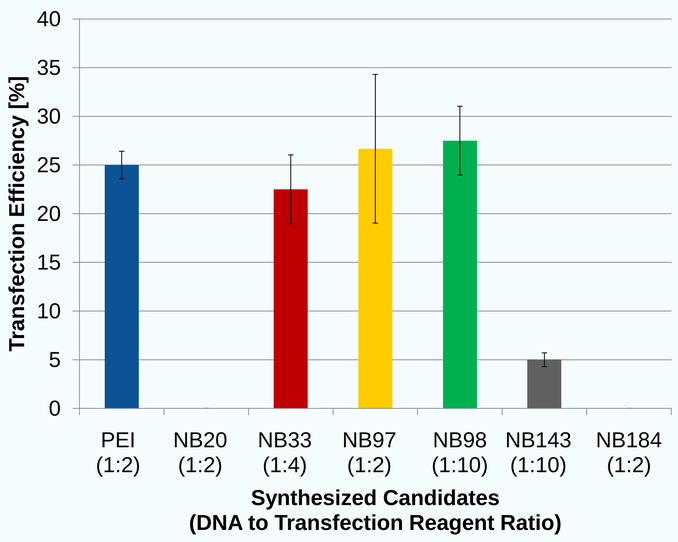
Biological Analysis:

Toxicity:

More than 20 different candidates were checked on toxicity from 0.4 to 40 pg potential transfection reagent per cell. Only one of those candidates showed toxic characteristics. When using two other candidates, cells showed morphological abnormities, but at concentrations far out of application range.

Transfection Efficiency:

Non toxic candidates were checked for transfection efficiency in a far-ranging DNA to transfection reagent ratio. Selected experiments are shown in Fig.1. Candidates NB 33, NB 97 and NB 98 turned out to work best. Most other candidates showed no or very low transfection efficiency. The optimum in DNA to transfection reagent ratio was tested for most convenient candidates.



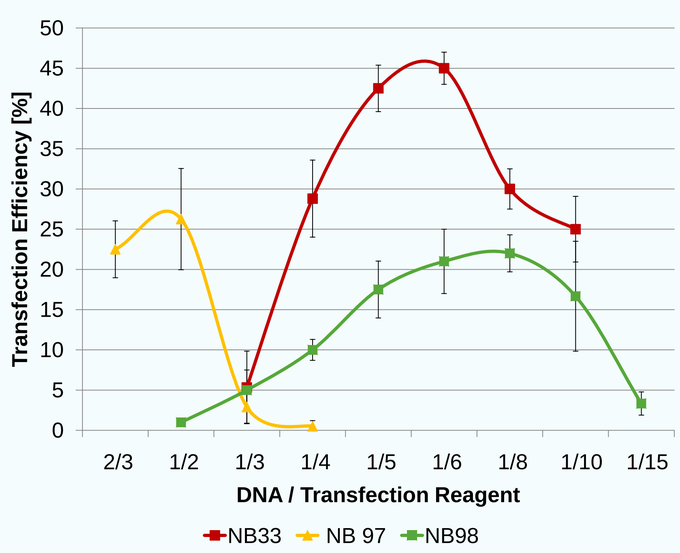


Figure 1: (a) Transfection efficiency using selected non toxic synthesized candidates at best DNA to transfection reagent ratios. CAP-T™ cells (CEVEC, Cologne) were transfected with 2 pg per cell of a GFP-gene harboring plasmid at a cell density of 5 x 10^6 cells per mL in 4mL FreeStyle™ 293 Expression Medium (Invitrogen) with indicated transfection reagent. DNA to transfection reagent ratio is shown beneath. Cultures were supplemented 1 hour post transfection with same volume Protein Expression Medium (Invitrogen). Transfection efficiency was determined 24 hours post transfection by counting green fluorescent positive cells using fluorescence microscopy. Further we used pure 25 kDa linear polyethylenimine (PEI) from Polysciences as reference material.
(b) Variation of DNA to transfection reagent ratio of selected candidates. Transfection efficiency of candidates with best results previously were optimized at an broader range of DNA to transfection reagent ratios.

Productivity:

Cell culture supernatant of transient transfected cells using new synthesized reagents being most efficient in transfection efficiency named NB 33 and NB 97 were checked on production of secreted alkaline phosphatase (SEAP). We compared those transfections where jetPEI™ (PolyPlus) or PEI were used.

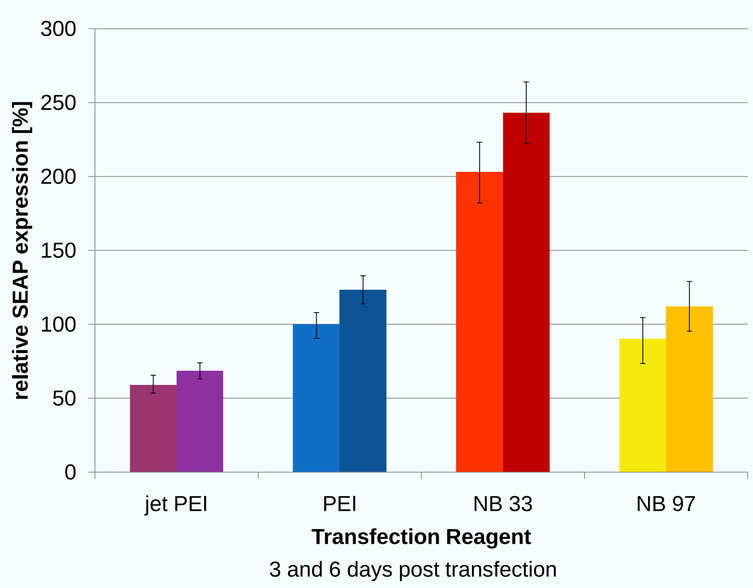


Figure 2: Relative SEAP expression following transient transfection using new synthesized reagents being most efficient in transfection. CAP-T cells were transfected with a *SEAP* gene harboring plasmid combined with most efficient transfection reagents in comparison to PEI and jetPEI™. PEI, NB33 and NB97 were used in previously optimized DNA to transfection reagent ratios. jetPEI™ was used according to manufacturers instructions, adapted to used cell density. SEAP expression was determined in cell culture supernatant of day 3 and day 6 post transfection by a photometric pNPP turn-over assay. SEAP expression was normalized to sample "PEI – day 4 post transfection".

Synthesis:

Required properties:

The task at hand for emp Biotech was the design and synthesis of various functionalized cationic and hydrophilic co-polymers that can potentially mediate between polyanionic plasmid DNA and the negatively charged cell surface, thereby facilitating uptake of DNA into the cells. Two improvements for transfection reagents were of primary interest, namely that of increased solubility and lower cytotoxicity.

Synthesis strategy:

We focused on the introduction of hydrophilic functional groups into the polymer structure to enhance solubility as well as on potential structures which allow rapid intracellular degradation and decrease overall cytotoxicity.

Results:

Several different cationic polymer candidates were chemically synthesized and provided to InVivo Biotech. All products are chemically defined and animal component free and consequently highly suitable for use in cell culture systems.

Increased yields of recombinant proteins using NB33:

We wanted to verify the profitability using NB33 as transfection reagent, shown at SEAP expression levels, under manufacturing conditions. We chose to express transiently thrombomodulin, a highly glycosylated protein, with an molecular weight of around 60 kDa.

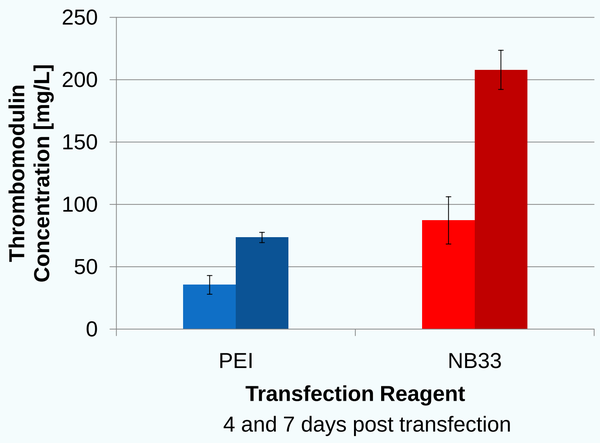


Figure 3: Thrombomodulin expression following transient transfection using PEI and NB33. CAP-T cells were transfected with a *thrombomodulin* cDNA harboring plasmid combined with the transfection reagent NB33 in comparison to PEI. Thrombomodulin concentration was calculated from cell culture supernatant at day 4 and day 7 post transfection by IMUBIND® Thrombomodulin ELISA Kit (american diagnostica).

These results led InVivo to perform contract transfections with NB33 instead of PEI. Since then more than 20 different recombinant proteins have been produced successfully using medium size batches up to 2,0 L total transfection volume.

Application of NB33 in different cell lines:

Transfection reagent NB33 was checked for applicability on transient transfections of different cell lines. Relative SEAP expression increased in each cell line when we used NB33 instead of PEI as transfection reagent.

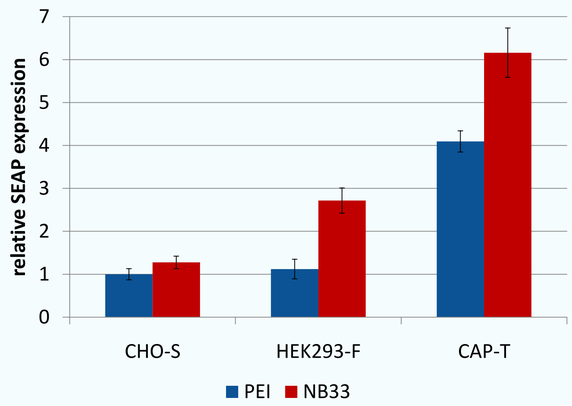


Figure 4: Transfection of different cell lines with NB33. HEK293-F, CHO-S and CAP-T cells were transfected with a *SEAP* gene harboring plasmid with transfection reagent NB33 in comparison to PEI. SEAP expression was determined in cell culture supernatant of day 6 post transfection, SEAP expression in CHO-S supernatant from PEI transfection was set to 1.0. All cell lines showed similar transfection efficiencies 24 hours post transfection when transfected with a GFP plasmid.

Improvement of Synthesis:

Experiments have been performed to reproduce synthesis of NB33 and prove long term storage of the product, which have been proceeded successfully.

Critical in-process parameters like temperature and duration of the significant reaction step were tested. These variations did not implicate any improvement of transfection efficiency.

Future works to be done:

We want to optimize synthesis parameter to yield a transfection reagent with even better capabilities than NB33. Furthermore there is a need to develop optimized protocols for transient transfection of different mammalian cell lines with NB33.

Conclusion:

We synthesized several candidates as novel cationic reagents for transient transfection of mammalian cells. When most promising candidate NB33 was used in productivity experiments, yield of recombinant proteins was increased up to 3 times, when compared to conventional transfection reagent PEI. NB33 is also suitable to be routinely used for cell lines HEK293F, CHO-S, and CAP-T cells as well.

Take home message:

- New developed transfection reagent
- Chemically defined and animal component free
- More productivity than using PEI or jetPEI™
- Up to 3 times increased productivity as compared to conventional PEI
- Tested for transfection of CHO, HEK and CAP-T cells