

## Optimized large-scale transient gene expression with a serum-free suspension-growing HEK293 cell line

### SUMMARY

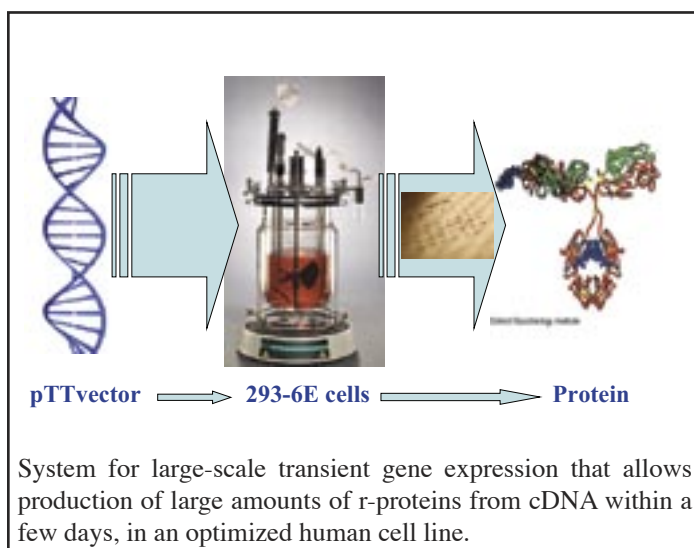
Large-scale transient gene expression in mammalian cells is becoming an established technology for the fast production of large amounts of recombinant proteins (r-proteins). However, efforts are still needed to optimize production parameters in order to maximize productivity, while maintaining product quality. This technology presents key improvements in expression vectors, serum-free media and process of a system for transient gene expression in human cells that have significantly increased the ease and yield of r-protein production.

### APPLICATIONS

- Large-scale transient gene expression in serum-free medium.
- High yield, low cost production of r-proteins for various applications within a few days.
- Production of recombinant proteins with high enzymatic activity.

### CONCEPT

Transient gene expression by transfection of mammalian cells is commonly known as a technique employed at small scale for obtaining micrograms of r-proteins; however, this technique has recently begun spreading to the large scale bioreactors as a powerful technology to generate large amounts of r-proteins within a few days. Despite the successful use of HEK293 cells in gene expression by large scale transfection, the high cost / low yield



characteristic of mammalian cell culture processes still remains an important drawback. Therefore, there is room for optimization of various production parameters that would enhance cell density and/or specific productivity. Major increase in the expression yields of r-proteins have been obtained following key improvements in the expression vector, cell line, culture medium and process. A modified serum-free medium amended by the addition of various additives was shown to increase r-protein production and in some cases to stabilize their enzymatic activity. Furthermore, various truncated but functional forms of EBNA1 were obtained and one of these (EBNA1t) was shown to significantly enhance r-protein expression when stably expressed in HEK293 cells (293-6E) or transiently co-expressed in *cis* or in *trans*.

### FEATURES AND BENEFITS

#### Highly stable 293EBNA1 clone

Shorter, functional forms of EBNA1 reduced the difficulty of obtaining stable clones allowing the isolation and characterization of a new 293EBNA1 cell line, 293-6E that stably expresses a truncated EBNA1 protein.

#### Increase yields or r-proteins through co-expression

Further enhancement of r-protein expression was achieved by co-expression of selected genes in *cis* or *trans*. Use of an expression cassette for transient co-expression of truncated forms of EBNA1 in *cis* or in *trans* increased r-protein production in 293EBNA1 and non-EBNA1 cells as well as in the new 293-6E cell line.

#### Increased enzymatic stability and higher expression yields with a modified serum-free medium

Feeding with peptone significantly increases yields while preserving enzyme activity. Supplementation of a commercially available defined, serum-free medium with various additives such as TN1 peptone was shown to positively impact expression of various proteins in transfected 293-6E cells.

#### Rapid and high yield r-protein production

This combination of vectors, cell lines, serum-free media and process provides a convenient and flexible transient gene expression system that allows the production of large amounts of r-protein in human cells within a few days.

### PROTECTION STATUS

Expression vectors for enhanced transient gene expression and mammalian cells expressing them (NRC no. 11565).

### CONTACTS

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