



r-PEx protein production technology

At U-Protein Express BV fully post-translational modified mammalian recombinant proteins or recombinant antibodies are produced via its r-PEx transient expression platform (patent applications pending). The r-PEx expression platform is based on Human Embryonic Kidney cells (HEK293E) and yields 1-100 mg amount of recombinant protein in 6-8 weeks. Using dedicated protocols the purified recombinant protein contains very low endotoxin levels.

The r-PEx pipeline from gene to purified protein comprises the following steps (see box):

1. Generation of coding cDNA, either via
 - i) synthetic gene design, including codon optimization and addition of standard restriction sites, or
 - ii) PCR using a cDNA template and gene-specific oligo's containing standard restriction sites.
2. Ligation of sequence confirmed coding sequence in several different expression vectors.
3. (Optional) small scale transient transfection to suspension growing HEK293E cells. Three to 6 days post transfection recombinant protein production is assayed and the most optimal expression vector is selected.
4. Large scale transient protein production in suspension growing HEK293E cells (1-30 L scale).
5. Purification and characterization of recombinant proteins.

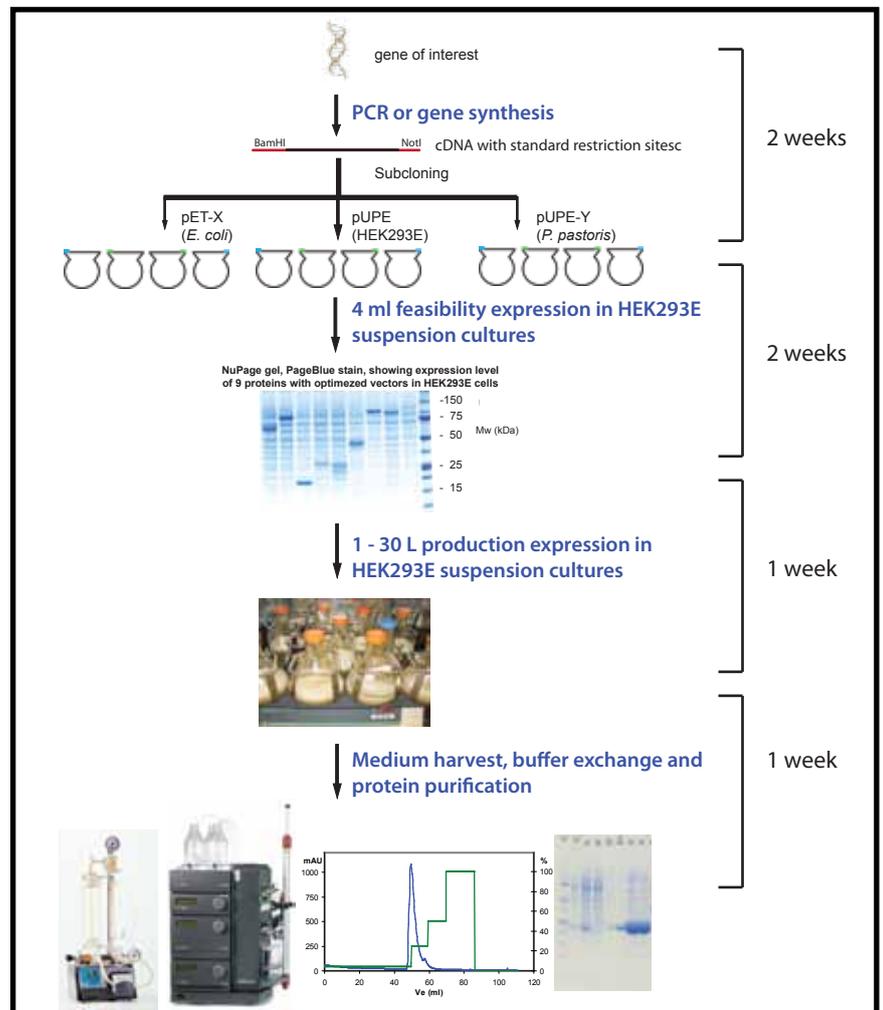
The advantage of the r-PEx transient expression platform is that the recombinant protein production can easily be switched to lower expression platforms like the yeast *Pichia pastoris* or the bacterium *Escherichia coli*, which both are available at U-Protein Express BV.

Examples of obtained expression levels in HEK293E (mg/l)

Protein	expression level (mg/l)
SEAP	100
rigG1	25 - 60
Complement B*(1)	12
Complement C2a (2)	15 - 20
Complement C3	2 - 3
Glycoprotein Ib α	4
TAFI* (3)	3 - 4
β 2-Glycoprotein I**	10 - 15

* Complement B has been produced in HEK293ES, a HEK293E cell line that produces proteins with small Man5-GlcNAc2 N-linked glycans
 ** β 2-Glycoprotein I has been produced in HEK293E and HEK293ES

(1) Nature Structural and molecular biology, 2007, 14, 224 - 228
 (2) Structure, 2006, 14, 1587 - 1597
 (3) Blood, 2008, 112, 2803 - 2809



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The r-PEX technology

Successful in recombinant protein production

Full mammalian post translational modification

