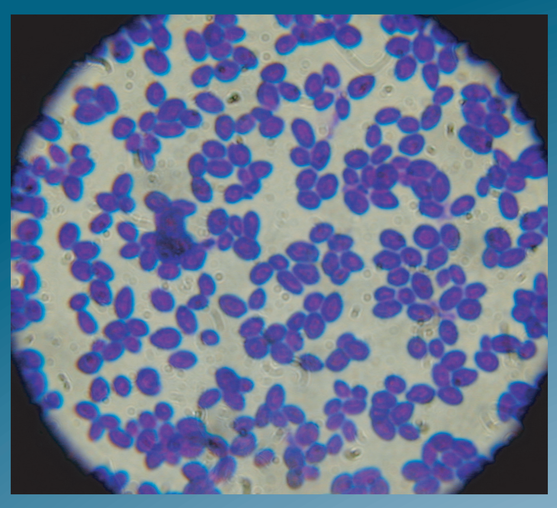

Development of the new *yeast expression* vector system for large scale protein production

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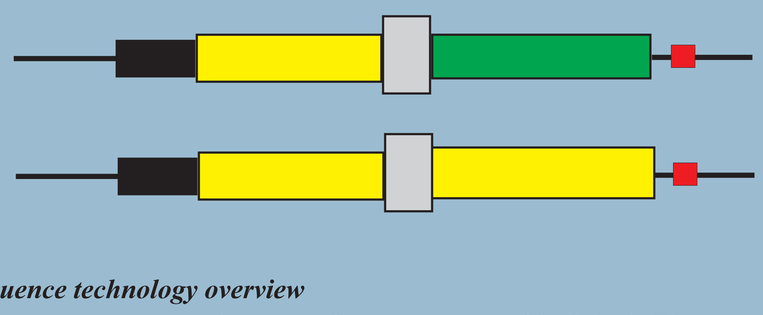
Large-scale protein production is traditionally accomplished by using large capacity growth vessels with host cell system of high productivity. Such systems are described to be bacterial, yeast or fungal expression platforms (1). However, if large expression capacities are not available could one be successful with system described as an IRES-mediated expression. The system allows also multiple gene expression from a single mRNA-strand simultaneously by using IRES-based alternative ribosome scanning method (2). The technology can be used for the expression of multiple copies of same gene or different genes in yeast cells allowing eucaryotic genes to be handled as a monocistronic ones.



IRES-sequence technology overview
The vector system is using galactose inducing promoter (black box). The genes to be expressed (yellow and green box) are divided with IRES element (gray box). Terminator is indicated as red box. IRES-based construct allows also simultaneous co-expression of two or more gene copies (indicated as yellow boxes in lower picture).

Materials and methods

Yeast transformation and induction of gene expression. The yeast strain wt303 was transformed by pYe vector (negative control), pYe-CP-IRES-GUS, pYe-CP-IRESemcv-GUS and pYe-CP-IRESu1-GUS plasmids. Transformation was performed as follows: 3 ml of yeast cultures were grown in YPD to an OD_{600} of 0.2 at 30°C. After harvesting by centrifugation and washing with sterile water the cells were suspended in 0.5 ml of LiAc/TE solution (1 M lithium acetate, 1 M Tris-HCl pH 7.5, 0.5 M EDTA). 2 µg of melted plasmid DNA were added to 0.1 ml of yeast cells, mixed and incubated for 10 minutes in the ice bath. 0.6 ml of PEG/LiAc solution (1M lithium acetate, 1M Tris-HCl pH 7.5, 0.5M EDTA, 50 % PEG_{4000}) was added with mixing. The yeast cells were grown for 30 minutes at 30°C. After adding DMSO and vortexing, the cells were incubated for 10 minutes at 42°C and then rapidly chilled on ice. The yeast cells were harvested by centrifugation, washed in sterile water, suspended in 0.3 ml of water and the aliquots of 0.1 ml were incubated for 3 days at 30°C on Petri-dishes with agar medium without histidine. Induction of gene expression from inserted plasmid constructs was performed by growing of transformed yeast clones for 48 hours at 30°C in the 3 ml of galactose-rich medium.

Extraction of total protein from yeasts. The yeast cells were harvested by centrifugation. The spheroplasts were done by suspending the cell pellet in the lysis solution I (1 M sorbitol, 0.5M EDTA, lyticase (10 u/µl)) with further incubation for 40 minutes at 37°C. Yeast spheroplasts were harvested by centrifugation and suspended in 100 µl of lysis solution II (50 mM sodium phosphate pH 7.0, 10 mM EDTA, 0.1% sarkosyl, 0.1% Triton X-100), freezed in liquid nitrogen and rapidly warmed up to 42°C. Freeze/thaw procedure was performed three times. After centrifugation the supernatant was taken and the total protein quantity was detected (3).

Results and discussion

The IRES sequences derived from the genome of tobamoviruses (crTMV and TMV UI) as well as $IRES_{EMCV}$ derived from animal virus (EMCV) are capable to promote the 3'-proximal GUS gene expression from bicistronic constructs in transformed yeast cells. Interestingly, the 148-nt sequence upstream of the CP gene of TMV UI, which is nonfunctional as IRES in plant cells *in vivo* and in WGE (*in vitro*), exhibited a moderate IRES activity in yeast cells. The efficiency of different IRES elements derived from genome of tobamoviruses varied in yeast cells, but CP-IRES-GUS was clearly the most efficient (Figure 2).

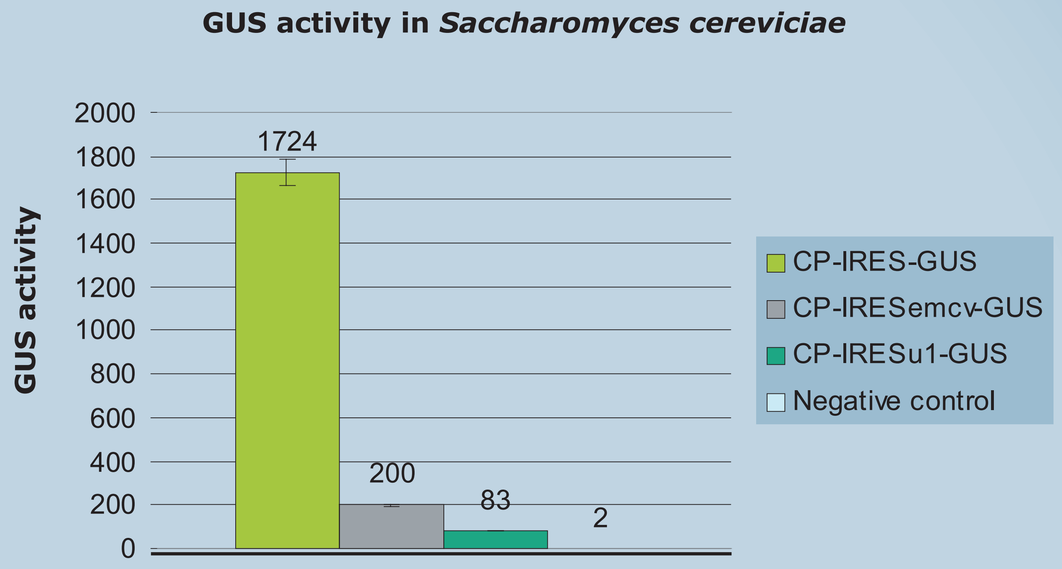


Figure 2. *GUS activity in Saccharomyces cerevisiae cells expressing various bicistronic DNAs. It can be evidently seen that CP-IRES-GUS construction is effectively translated in yeast cells.*

The results of 3-proximal GUS expression indicate that IRES sequence is capable to launch leaky scanning of the ribosomes and, therefore co-expression of given genes is possible. This approach may be used to maximize expression of any gene in yeast cells. This method have been shown to be functional also other species than *Saccharomyces*, such as *Hansenula* sp., *Nicotiana* sp. and HeLa cells (2). We are currently building this system for large-scale expression of protease gene family.

References

(1) Michael, K. et al. 2003. Trends in Biochem. 21:9, 371-414.
(2) Atabekov, J. et al. 2002. Patent, US2005014150
(3) Bradford, M.M. (1976) Anal. Biochem. 72, 248-254.

Planning

Together with the client, we design primers etc. for enhanced expression.

Optimisation

We optimise the expression with our rts-system giving the client information for selecting the most efficient expression system for the target protein.

Production

We produce proteins with different expression systems in compliance with GLP regulations. Production is carried out with a system selected together with the client.

Purification

We carry out protein purification with standard methods. When needed, we can also design unique purification procedures for selected target proteins.

Product

The client recieves the ready-to-use final product.

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