SMP: Protein

Project: Subcloning of Full-length ORFs into Expression Plasmids

Uwe Radelof - RZPD-Deutsches Ressourcenzentrum für Genomforschung GmbH, Berlin - radelof@rzpd.de
Erich Wanker – Max-Delbrück Center for Molecular Medicine, Berlin-Buch - ewanker@mdc-berlin.de

Introduction

In order to express and purify human proteins, cDNA sequences need to be inserted into expression vectors (Langlais *et al.,* 2003). Previous studies have shown that the host, the vector system and the growth conditions influence the expression of proteins. In all cases, the sequences coding for the proteins need to be inserted 'in frame' into the expression system in order to ensure the production of the recombinant protein of interest (Wiemann *et al.,* 2001). Shifting the frame or cloning artefacts lead to non-complete expression of proteins or to the translation of artifical peptides. Therefore, reliable and homogeneous source clones for protein expression are essential.

Aim of the subproject

The aim of RZPD's subproject is to generate a common resource of high-quality expression clones for protein production, protein-protein, protein-DNA and protein-drug interaction studies performed in the different subprojects of SMP-Protein. To achieve this goal in the most cost-effective way, RZPD applies the well-established Gateway® (Invitrogen) sub-cloning system for the transfer of cDNA fragments into expression plasmids. The Gateway® system is a recombination-based cloning system that allows for the high-throughput transfer of cDNA fragments into expression plasmids. Previous studies have shown that this technology is well suited for the sub-cloning of ORFs into yeast two-hybrid plasmids for systematic interaction studies (Reboul *et al.,* 2003). RZPD will make use of its proven strengths in high-throughput sub-cloning as well as its distribution logistics to ensure that the materials will be delivered to all SMP-Protein partners and beyond. Using the established pipelines of cloning and material handling, RZPD guarantees highest product quality, and aims for sub-cloning at a success rate exceeding 95%.
In addition, new Gateway®-compatible expression vectors (Destination Vectors) will be constructed and tested according to the requests of SMP-Protein project partners. *De novo* cloning of Full-length ORFs in Gateway®-compatible Entry Vectors will be carried out according to the needs of the SMP-Protein.

Project description

In order to provide high-quality and standardized protein expression vectors and expression clones to the SMP-Protein, RZPD will carry out the following work:

1) Provision of 2,200 Gateway®-compatible Full-ORF Entry-Clones:
    a. 1,500 Entry Clones out of a pool of already existing clones
    b. 700 Entry Clones will be cloned *de novo* according to the needs of SMP-Protein project partners.
2) Provision of up to 9,000 Full-ORF Expression Clones, which are derived of the above mentioned Entry Clones, in up to 10 different Gateway®-compatible Destination Vectors, according to the requests of SMP-Protein project partners (Table 1).
3) Construction of up to 6 different new Gateway®-compatible Destination Vectors according to the requests of SMP-Protein project partners.
4) Distribution of the constructed vectors and generated clones to the members of SMP-Protein as *E.coli* strains or plasmid DNA in single tubes or 96-well microtiter plates.

Destination Vectors	Fusion-protein / Tag
pBTM116-D9	LexA
pGAD426-D3	Gal4
pGEX-6p-D21	GST
pTLHA1-D48	HA
pcDNA3.1-PA-D57	ProteinA
pDESTco	HIS_6
pdEYFP-rfb	YPF
pdECFP-amp	CFP
pMAL-D41	MBP
pcDNA-N-TAP	ProteinA + CMB

***Tab 1**: Plasmids used for the generation of Full-ORF Expression Clones for the expression of human proteins in E. coli, yeast and mammalian cells.*

For all generated clones and newly constructed vectors a comprehensive quality control is accomplished, e.g. sequencing of all provided Entry Clones and newly constructed vectors, BsrGI digestion of all generated expression clones. The results of all quality control procedures are electronically documented and provided to the SMP-Protein project partners upon request. Moreover, a comprehensive annotation for all Entry Clones as well as Destination Clones will be provided.

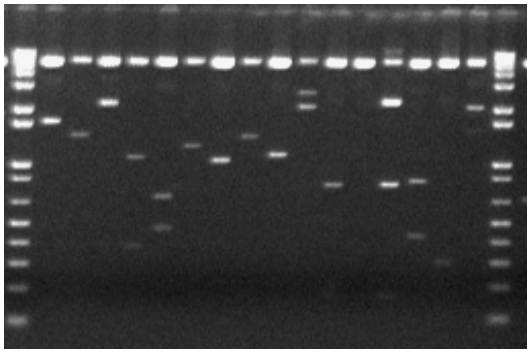

***Fig 1:** Quality control of each and every Expression Clone after sub-cloning. This gel-image depicts 16 BsrGI digestion results of Full-ORF Destination Vector Constructs (vector:* pcDNA-N-TAP). Lane 1 and 18: *1Kb Plus DNA ladder from Invitrogen.*

Project Status

According to the project plan, the following work has already been carried out:

1) Provision of 1,546 Gateway®-compatible Full-ORF Entry-Clones.
2) Provision of 3,818 Full-ORF Expression Clones in 9 different Gateway®-compatible Destination Vectors, according to the requests of project partners of the SMP-Protein (Table 2).
3) Construction of 3 different new Gateway®-compatible Destination Vectors according to the requests of project partners of the SMP-Protein: pMAL-D41, pc-myc-CMV-D12 and pFLAG-CMV-D11.
4) All generated material has been provided to the members of SMP-Protein as *E.coli* strains or plasmid DNA in single tubes or microtiter plates along with accompanying information.

Destination Vectors	Number of clones
pGAD426-D3	803
pGEX-6p-D21	20
pTLHA1-D48	1121
pDESTco	539
pdEYFP-rfb	20
pcDNA3.1-PAD57	1181
pMAL-D41	20
pcDNA-N-TAP	94
pdECFP-amp	20

***Tab 2**: Type of Destination Vector and number of destination clones that have been generated, quality controlled and delivered along with accompanying information to the project partners of SMP-Protein.*

Outlook

Remaining work will be carried out according to the project plan. All generated material will be made available as a public-domain resource via RZPD's distribution channels.

Lit.*: **1.** Langlais, C., Schatten, R., Henze, S., Gernold, N., Scheuermann, T. Ebert; L., Maurer, J. and Korn, B. (2003), Funktionsbiologie benötigt Zugang zu rekombinanten Proteinen: Klonierung, Validierung und Expression, Trancript 3, 6. **2.** Reboul J, Vaglio P, Rual JF, Lamesch P, Martinez M, Armstrong CM, Li S, Jacotot L, Bertin N, Janky R, Moore T, Hudson JR Jr, Hartley JL, Brasch MA, Vandenhaute J, Boulton S, Endress GA, Jenna S, Chevet E, Papasotiropoulos V, Tolias PP, Ptacek J, Snyder M, Huang R, Chance MR, Lee H, Doucette-Stamm L, Hill DE, Vidal M. (2003), C. elegans ORFeome version 1.1: experimental verification of the genome annotation and resource for proteome-scale protein expression. Nat Genet. 34, 35-41. **3.** Wiemann, S., Weil, B., Wellenreuther, R., Gassenhuber, J., Glassl, S., Ansorge, W., Bocher, M., Blocker, H., Bauersachs, S., Blum, H., Lauber, J., Dusterhoft, A., Beyer, A., Kohrer, K., Strack, N., Mewes, H. W., Ottenwalder, B., Obermaier, B., Tampe, J., Heubner, D., Wambutt, R., Korn, B., Klein, M. and Poustka, A. (2001) Toward a catalog of human genes and proteins: sequencing and analysis of 500 novel complete protein coding human cDNAs, Genome Res. 11, 422.*