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Oral Presentation

Use of a "universal" yeast vector (CoMed™) system for the production of proteins in Hansenula *polymorpha and Arxula adeninivorans*

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Background

A range of yeasts has been developed as attractive production systems for recombinant proteins. Some like *Hansenula polymorpha* [1] are already distinguished by an impressive track record as producers of valuable proteins that have already reached the market whereas other newly defined systems like *Arxula adeninivorans* [2] have yet to establish themselves but demonstrate a great potential for industrial applications. All yeast systems have special favorable characteristics, but also limitations and drawbacks – as is the case with all expression systems. As there is clearly no single system that is optimal for all possible proteins, it is advisable to assess several selected organisms in parallel for their capability to produce a particular protein in desired amounts and quality to avoid costly time- and resource-consuming failures. The availability of a vector that can be targeted to the various platform candidates would greatly facilitate such a comparison. As such a vector system (CoMed™) has been designed that is built up in a modular way [3]. Certain combinations of elements result in vectors that can be addressed to a wide range of yeast hosts.

Results

The basic design of the CoMed vector is depicted in figure 1. Several *ARS* sequences are available, a range of different *A. adeninivorans* and *H. polymorpha*-derived rDNA sequences, a variety of dominant and auxotrophic selection markers and of expression cassettes equipped with a great selection of yeast promoter elements. The final constructs can be linearized in a way that leaves behind all sequences of bacterial origin.

The design of vectors suited for a wide range of fungal organisms must meet several prerequisites. Such a plasmid must contain a targeting element suitable for all test species. The promoter that drives heterologous gene expression must be functional in all these organisms and the vector/host system must employ a dominant selection marker or a sequence that can complement the auxotrophy in all selected organisms. Certain combinations of vector elements presented before fulfill all requested criteria. The rDNA is highly conserved, the rDNA genes are present in high copy numbers and they are readily accessible for efficient transcription. The *A. adeninivorans*-derived *TEF1*-promoter was found to function in all yeast systems tested so far, for selection the *A. adeninivorans*-derived *LEU2* gene is available that can complement the leucine auxotrophy of all leu$^-$ yeast strains assessed so far.

A vector containing a combination of an rDNA integration sequence, the *LEU2* selection marker and an expresion cassette harbouring the *TEF1* promoter for expression control was therefore selected to address a range of respective auxotrophic yeast strains, among others *A. adeninivorans* and *H. polymorpha*.

In a first example we tested both species for the capability to produce IL-6. We observed a different extent of correctly processed precursor molecules.

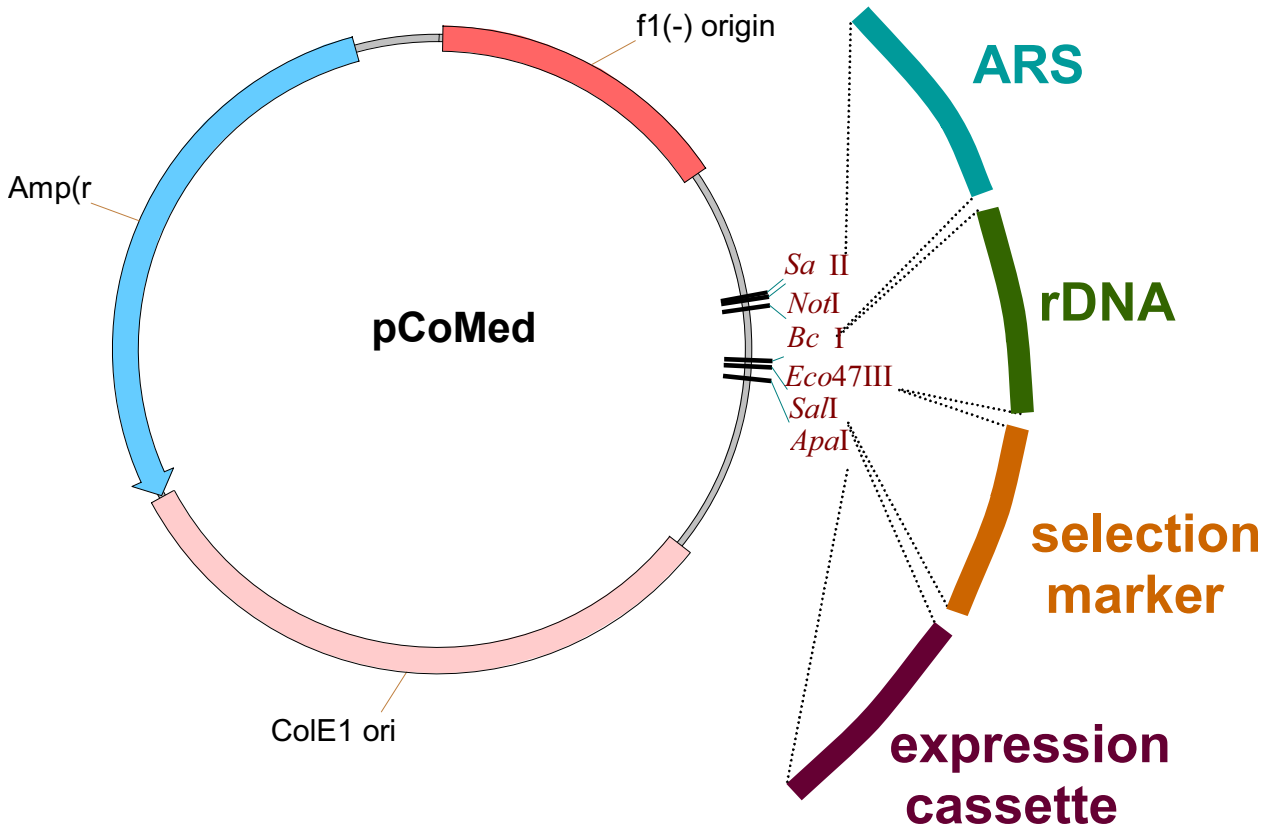

Figure 1
Basic design of a CoMed™ vector For further information see text.

The rDNA integration sequence provides a tool that is not only suited to address several platform candidates in parallel but also to co-integrate several vectors at the same time. The option was executed for the generation of recombinant IFNγ-secreting *H. polymorpha* strains.

In a rDNA co-integration approach several genes of the secretory pathway were assessed for their impact on the secretion of the cytokine. Upon co-integration and co-expression of *CNE1* secretion of the interferon was found be considerably improved, the glycosylated secretion product was of distinct size corresponding to core-glycosylated molecules instead of hyperglycosylated proteins present without co-expression of *CNE1* [4].

Conclusion

A "universal" yeast vector system based on rDNA integration has been developed which on one hand is able to address in parallel a range of selected expression organisms and by which on the other hand several expression plasmids can be co-integrated. The newly developed system constitutes an attractive novel tool for the application of yeast expression platforms to heterologous protein production.

References

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