Title: Multiple and directed integration of recombinant protein expression cassettes into the genome of *Pichia pastoris*

Supervisor / Institute: Apl. Prof. Karl Friehs, AG Fermentationstechnik and Dr. Jörn Kalinowski, Center for Biotechnology, Bielefeld University

Background:
*Pichia pastoris* (renamed as *Komagataella pastoris*) is an established producer for recombinant proteins. The expression cassette, together with e.g. the resistance against Zeocin for selection, is integrated via recombination into the genome. The locus of integration is determined through the flanking 5'UTR and 3'UTR of the cassette. An often used locus is the AOX1 site. After successful integration, the AOX1 gene should be replaced, leading to a very low methanol consumption (mut^S^-type). But often clones show the selection phenotype and still have a functional AOX1 (mut^+^-type) (Itzcoatl et al., 2006). That means, the integration happened in a random manner. Sometimes the cassette is integrated more than once, leading to a higher copy number of cassettes and occasionally to a higher productivity of recombinant proteins.

Aims of the project:
The aim of the project is to analyze the random integration and to enhance the copy number of cassettes in the genome. The integration locus and the copy number of a sufficient number of mut^+^- and mut^S^-types are analyzed via inside-out-PCR cloning and sequencing and qRT-PCR. Are there favored loci? Does the length of the flanking 5'UTR and 3'UTR has an influence on the loci? The copy number of expression cassettes in the genome could be increased by multiple transformations. To use the same selection marker and expression cassette, the integrated markers have to be eliminated. For such a knock out, the lox/Cre function (Pan et al., 2011; Gueldener et al., 2002) can be used with the Zeocin cassette, flanked by the lox sequences. After the knock out, other specific distinct loci can be chosen, like the first enzyme in the glycosylation pathway or a protease. This should allow getting clones with a very high copy number and hopefully high productivity of recombinant proteins.
Sequencing the genome of real high producers clones could answer the questions of loci and copy numbers at once.

**Requirements:**
Applicants should have finished their academic studies with an MSc or equivalent degree in molecular biological sciences. Experience in molecular biology and in genomics and transcriptomics of microorganisms is very helpful. A good background in genetic engineering and microbial cultivation is highly welcome.

**References:**
Pan et al. (2011) Sequential deletion of *Pichia pastoris* genes by a self-excisable cassette. FEMS Yeast Res. 11(3):292-8