



Genetics of transcriptional regulation of *AOX1* promoter in *Pichia pastoris*

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Over the past ten years the use of *Pichia pastoris* as an expression system for a variety of heterologous proteins has grown exponentially. One essential characteristic that has greatly contributed to *Pichia's* success as an expression system is the presence of a strong, tightly regulated, and easily manipulated promoter –the *AOX1* promoter derived from the alcohol oxidase (*AOX*) gene. While it is known that the *AOX1* promoter generally controls gene expression through a repression/derepression mechanism and an induction mechanism little is known about the molecular details of these mechanisms. *Restriction Enzyme-Mediated Integration* (REMI) was used for the generation of mutant strains to obtain disruptions in and analyze genes involved in the transcriptional regulation of the *AOX1* promoter. A *Bam*HI linearized pREMI-Z vector containing a zeocin antibiotic resistance cassette was randomly integrated into the *P. pastoris* genome. Mutants were selected for phenotype analysis on various selective media and genomic DNA from selected mutants was then isolated and digested with a restriction enzyme which does not cut the vector (*Hind*III) yielding DNA fragments, at least one of which contained the pREMI-Z vector with flanking portions of genomic DNA. The vector+genomic DNA was ligated and then transformed into *E. coli* for amplification and finally sequenced to allow us to determine the identity of the disrupted genes through searches in the *P. pastoris* genomic database (ERGO, Integrated Genomics Inc., Chicago, IL). In our study we were able to analyze mutations in 7 transformants; this allowed us to identify several genes which may be linked to the transcriptional regulation of the promoter. In addition, our sequencing data also holds the potential to refine and improve the annotated *Pichia pastoris* genome which is still in development.

