

Poster Presentation

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Expression of genes encoding membrane proteins from the hyperthermophilic Archeon *Pyrococcus abyssi* in *Pichia pastoris* Cécile Labarre*, Karine Blondeau and Herman van Tilbeurgh

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Experimental strategies, first results and identified bottlenecks of a structural genomics initiative on proteins of hyperthermophilic Archaea *Pyrococcus abyssi* are discussed here. Five ORFs have been cloned by a standard protocol in the methylotrophic *Pichia pastoris* expression system, using two different constructs, with or without the signal sequence α mating factor of *S. cerevisiae*. The C-myc epitope and 6 His codons were added at the 3'-end of the targeted genes to allow immunodetection of recombinant proteins and to facilitate their further purification. We have selected at least one producer clone for each protein of interest and for almost each construction. All the membrane proteins of interest were produced in Erlenmeyer flasks culture and in fed-batch cultivation using for large scale cells preparation. Production efficiencies were relatively low in both cases but the quantities of biomass produced have allowed us to collect sufficient amount of membrane fractions. The five proteins were extracted, solubilized and partially purified. Further work should involve structural biology investigations.