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Optimization of yeGFP Expression as a Reporter for Prospective RNAi Analysis in *Pichia pastoris*

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Green fluorescent protein from the jellyfish *Aequorea victoria* is a widely used reporter gene in genetic engineering. Yeast codon optimized green fluorescent protein (yeGFP) has been found to have higher expression, and hence it is more reliable as a yeast reporter. *Pichia pastoris* can be used as a model organism to study gene regulation such as RNA interference (RNAi) because it does not have RNAi, by monitoring the yeGFP expression. This study focuses on the optimization of the expression of yeGFP in the *Pichia pastoris* GS115 strain, under the control of AOX1 promoter for prospective RNAi studies. yeGFP gene was cloned into the pPICZ A vector to create pPICZ A-yeGFP expression plasmid. *P. pastoris* GS115 strain was transformed with pPICZ A-yeGFP and positive transformants were selected on Zeocin plates and further confirmed by PCR. Five transgenic colonies were tested for the yeGFP expression by methanol induction in MMH (Minimum Methanol Histidine) media containing 0.5%, 1%, and 2% (v/v) methanol for six days. Fluorescence intensities were measured using SpectraMax M3 spectrophotometer at an excitation wavelength of 395 nm, and emission of 510 nm. According to the Relative fluorescence units of the induced cultures, expression increased with the time till the fifth day and decreased on the sixth day. 0.5% methanol and 2% methanol-induced cultures showed the least and highest fluorescence respectively. Transgenic lines 1 and 4 had the highest yeGFP expression in 2% methanol on the fifth day. For studies on gene regulation by RNAi, optimum yeGFP expression under the AOX1 promoter can be measured in 2% methanol on the fifth day of induction.

Keywords: *Pichia pastoris, yeGFP, methanol induction*