Abstract No: BO-34

Fluorescence-activated cell sorting analysis of yEGFP expression in transgenic *Pichia pastoris* with reconstituted RNAi

C. S. Dharmarathna*[1], R. S. Dassanayake[1], Y. I. N. S. Gunawardena[2] and C. Hettiarachchi [1]

[1]Department of Chemistry, Faculty of Science, University of Colombo, Colombo 03, Sri Lanka
[2]Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka
chethanasithumini@gmail.com*

Pichia pastoris lacks an RNA interference (RNAi) mechanism, which is a way of post-transcriptional gene regulation present in almost all eukaryotes including humans. The absence of RNAi makes *P. pastoris* a model organism that can be used to study foreign RNAi mechanisms by introducing the essential genes involved in the RNAi pathway of a particular organism. Yeast-enhanced green fluorescence protein (yEGFP) can be used as a reporter protein to study the reconstituted RNAi in *P. pastoris*. The objective of this study was to analyze yEGFP expression in transgenic *P. pastoris* with reconstituted human RNAi. yEGFP expressing *P. pastoris* reporter strain was produced by integrating yEGFP gene into *Pichia* genome at the alcohol oxidase (*AOX*) locus with the aid of pPICZ A vector. RNAi was reconstituted by transforming human Argonaute, Dicer, and TRBP genes (RNAi genes) to yEGFP expressing *P. pastoris* reporter strain to create the ADT strain. Human RNAi genes were cloned with Gateway cloning. Galactose induction was carried out to activate RNAi genes followed by methanol induction to express yEGFP. After both inductions, the expression of yEGFP was analyzed by fluorescence-activated cell sorting (FACS). The FACS results were further analyzed with Flowjo™ software. Wild-type *P. pastoris* (WT) was used as the no-fluorescence control. WT strain showed 134 median FITC-A signal, ADT strain had 833 median FITC-A signal, and yEGFP expressing reporter strain elicited 153 median FITC-A signal. The genes were cloned under the control of *Gal1* promoter in Gateway cloning. When galactose induction was carried out first, the Gateway vectors will be activated and RNAi genes will be expressed. Now, the *Pichia* cells have Argonaute, Dicer, and TRBP proteins in their cells. Secondly, the methanol induction induces the yEGFP gene which was cloned under the influence of *AOX1* promoter. The reporter strain had a higher median fluorescence value indicating the amount of fluorescence produced by yEGFP in *Pichia*. However, the ADT strain having yEGFP, and also RNAi genes, showed a fluorescence value similar to the wild-type that did not have yEGFP. This is evidence for the inhibition of yEGFP expression by reconstituted human RNAi in *P. pastoris*. In conclusion, the reconstituted human RNAi in *P. pastoris* successfully ceases the yEGFP expression. This model system can be used to study the effect of RNAi for another gene by replacing the yEGFP.

Keywords: FACS, RNAi, *Pichia pastoris*

Acknowledgment
This work was supported by the Arturo Falaschi fellowship (Fellowship number F/LKA21-01) awarded by International Centre for Genetic Engineering and Biotechnology (ICGEB).