Analysis of Yeast TIMP-3 Library

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Abstract

The extracellular matrix (ECM) is composed of a complex network of components and allows the stabilization of the cell and tissue structure. Matrix metalloproteinases (MMPs) are proteases that are involved in the breakdown of the extracellular matrix and play an important role in cell structure regulation. Alteration of the MMP gene expression can lead to diseases. MMP activity is regulated in part by tissue inhibitors of metalloproteinases (TIMPs) (Dewing et al., 2020). The aim of this study is to investigate genetic mutations that would optimize TIMP-3 activity. A yeast library (containing TIMP-3 mutations) and bacteria (containing TIMP-3 wild-types) samples were obtained from the Dr. Maryam Raesszadeh-Sarmazdeh laboratory at the University of Nevada, Reno. Synthetic dextrose plus casein amino acids (SDCAA) media plates were made to culture the yeast and to evaluate the integrity of the samples. A miniprep was used to extract the wild-type TIMP-3 DNA from bacteria, PCR was performed to amplify the DNA. Gel electrophoresis was used to verify the wild-type TIMP-3 gene. The wild-type TIMP-3 gene from the bacteria will be used as a positive control. The TIMP-3 mutation was extracted by Zymoprep in yeast. The TIMP-3 mutation was PCR'd and a gel was used to verify the gene. The next step of this study is to sequence the TIMP-3 gene in yeast with possible mutations of interest.