

## Introduction

Last time you varied one experimental condition that may affect the behavior of a given gold binder (pAu1). The change you made to the binding protocol may increase or decrease the yeast's apparent affinity for its substrate or it may change the amount of non-specific binding, measured by its effect on the pCT-CON sample. Every yeast in the negative and positive control samples bear the pCT-CON or the pAu1 plasmid, respectively and should react identically to the new experimental condition.

By contrast it's unlikely that all the candidates you identified from your initial library screen will bind gold equally well. The affinity will depend on the sequence of the Aga2 fusion protein and each yeast colony from the library could have a different sequence. In fact, some of the candidates may not really bind gold at all. For example cells that were trapped on the glass behind the gold slide would appear to have bound the gold in your initial screen. Today you will further evaluate four library candidates, re-examining their gold-binding ability and determining their relative affinity. Based on the results of your optimization experiment you may want to change the panning protocol. Alternatively, you can rescreen your four candidates under precisely the same conditions as they were first isolated. It is your choice to make (with your lab partner, of course), but be sure to keep notes on the protocol you decide to follow. Based on the results of today's experiment you will choose two of the four candidates to send for sequence analysis of the Aga2 fusion.