Introduction

Yeast cultures have been used throughout recorded history to ferment drinks and to leaven bread, but the usefulness of yeast extends outside of the kitchen. Yeast is a powerful tool for genetic research with many advantages including a short generation time, a well-defined mating pattern, and many distinct mutant phenotypes. The yeast we will be working with are non-pathogenic and they even smell nice, which is unusual for a lab organism.

The most widely studied species of yeast is Saccharomyces cerevisiae. This is also the species that is used for baking and brewing. It can grow in liquid or on solid media. The medium commonly used to grow yeast is called “YPD.” It contains yeast extract and peptone, which are the Y and the P in YPD as well as glucose, which is the “D” in YPD since glucose is another name for dextrose. In addition agar, a polysaccharide derived from seaweed, can be added to make the solid media for the petri plates. When grown in liquid media, yeast makes the solution appear cloudy. When grown in petri plates, each viable yeast cell gives rise to a colony.

Other media, less rich than YPD, can be used to grow yeast as long as the medium meets all of that yeast’s nutritional requirements. Wild type yeast can make their own amino acids, but many yeast mutants cannot. For example some mutant yeast strains are unable to make their own tryptophan, one of the 20 amino acids that assemble into proteins. Consequently, tryptophan must be added to a minimal media if those mutant yeast are to grow. This is a very handy tool for laboratory experiments since tryptophan can be left out (“dropped out” in yeast jargon) of a minimal medium, restricting growth on that medium to only yeast that can make tryptophan.

"Image of yeast life cycle removed due to copyright reasons."

In this experimental module you will use yeast to express and display antibody fragments. Antibodies are amazing proteins, capable of recognizing almost any foreign substance we encounter. The overall structure of every antibody is identical. They are made from four polypeptides: two “heavy” (i.e. longer) chains and two “light” chains. The four chains assemble into the hallmark “Y” shape, with a disulfide bond holding each light chain to a heavy chain and another disulfide bond linking the two heavy chains. Yet each antibody has a unique “variable” region, at the tip of each Y, allowing for a specific interaction with a particular antigen. The variable region, called Fv for “variable fragment,” is defined by the sequences at the tip of the heavy chain and the tip of the light chain. You will be working with genetically engineered yeast that can display Fv regions on their surface, no small feat for a yeast since evolution has never required that yeast learn to make antibodies! To ease the task a little, the variable region of the light and heavy chains have been linked into a single polypeptide chain. Remarkably, a yeast cell can cram up to 100,000 copies of the single chain variable fragment (scFv) onto its surface.

"Image removed due to copyright reasons."

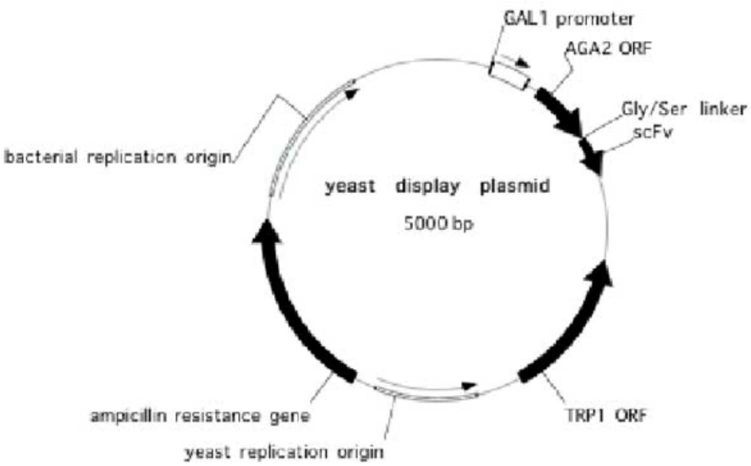
To display the scFv protein on the yeast surface, a protein fusion was made between the scFv sequence and Aga2, a part of the a-agglutinin receptor that yeast normally express to help them mate. Two polypeptides, Aga1 and Aga2, are disulfide bonded to form the a-agglutinin receptor, with Aga1 embedded in the yeast cell wall. The Aga2:scFv fusion is expressed from a plasmid and the product is exported to the cell surface. It is anchored and displayed through disulfide links to the Aga1 protein.

Two additional sequences were engineered into the fusion. A short sequence called the HA-epitope was placed between the Aga2 and the scFv proteins, and another short sequence called the c-myc epitope was engineered at the C-terminal side of the scFv. These epitopes can be detected with commercially-available antibodies, and so they are useful for quantifying the amount of fusion protein at the cell surface.

The yeast express the Aga2:HA:scFv:c-myc fusion from a plasmid called pCT-CON. Other features of this plasmid include

- A yeast centromere

    - This allows the plasmid to replicate prior to each cell division
- The TRP1 gene
    - This allows yeast carrying the plasmid to be selected using –trp media
- The GAL1 promoter
    - This allows the display to be controlled by addition of galactose to the media
- A bacterial origin of replication and an ampicillin resistance gene
    - This allows the plasmid to be copied and genetically manipulated in bacterial cells



Yeast display plasmid

The yeast surface display system has been used to study protein:ligand interactions. Screens of antibody libraries have identified variants of pCT-CON that bind interesting ligands. Some of the novel pCT-CON variants that have been isolated bind other proteins (e.g. lysozyme, or the tumor antigen c-erb), others bind small molecules (e.g. fluorescein), and others even bind metals, as you will see today. Once a novel binder has been isolated, it is possible to mutate it further to identify new variants that bind with even greater affinity. This "affinity maturation" approach has been used to increase the affinity of one scFv:ligand interaction four orders of magnitude, changing the time required for the complex to dissociate from seconds to days! (Boder, Midelfort and Wittrup. PNAS 2000 97(20):10701-10705 [1]).

Today you will work with a yeast strain bearing either the original pCT-CON plasmid, that expresses an Aga2:scFv fusion that does not bind gold, or a variant called pAu1 whose AGA2:scFv product was selected based on its gold-binding ability. You will also screen a library of 36 base pair randomized sequences that are fused to Aga2 to find ones that bind to gold (the chemistry and math behind this part of the experiment will be further explored on Day3).