The Yeast Sex Detector: Visual Mating Type Determination System for S. cerevisiae

[The Johns Hopkins iGEM Team]

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Abstract

• Haploid S. cerevisiae is either mating type **a** or alpha.
• Necessary to track meiotic segregation of a heterozygous diploid for yeast trait determination
• Four haploid cells; two *MATa* and two *MATα*.
• **Problem**: 3 to 4 day assay to differentiate mating types of four haploid cells
• **Project**: Yeast vector with different fluorescent proteins expressed depending on mating type
• **Solution**: Determine mating type in seconds using fluorescence microscope

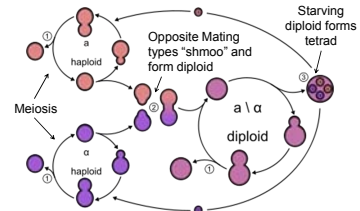Shmoo formation. No visual distinction between MATa and MATα.

• The convergent design, using a "two way transcriptional stop", normally found between two convergent ORFs in the yeast genome, allows each promoter to be isolated from the other, minimizing the chance of "cross talk".

Why Yeast?

• Yeast is a eukaryote, with fast reproduction, and few introns.
• Many homologous proteins found in humans and *S. cerevisiae*.
• Completely mapped genome and all ORF regions located.
• A simple and analogous method to study humans.
• Useful in many other ways:

Yeast Mating Pathway

Opposite Mating types "shmoo" and form diploid

Starving diploid forms tetrad

a haploid

Meiosis

α haploid

a \ α diploid

Mating type switching can occur in OH strain to increase chance of mating

Mating Pathway

• (A) Regulator proteins control MAT**a** and MATα specific gene expression. Even though both mating types contain genes from both α and **a**, they maintain regulated expression for their own mating type.
• For example: transcription as indicated by a squiggly line, of αSG and HSG results in MATα while of **a**SG and HSG results in MAT**a**.
• (B) While a-factor expressed in MAT**a** leads to shmoo formation in nearby Matα, α-factor found in MATα induces shmoo formation in MAT**a** cells
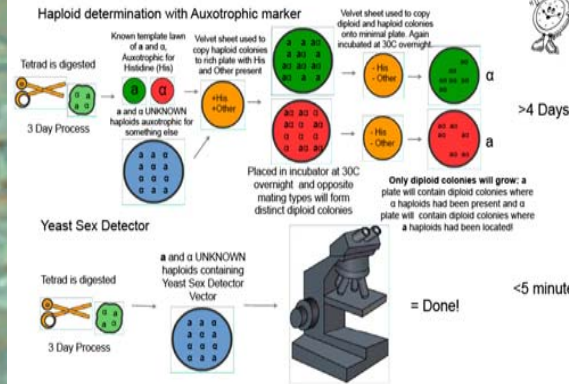
Yeast Vector

• Our Yeast vector will contain an α specific promoter which will initiate production of an mCherry fluorescent protein when in a MATalpha haploid, and an a-specific promoter which will initiate production of YFP when in a MAT**a**.
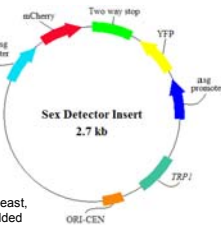
• To ensure the vector stays in yeast, a TRP1 selectable marker is added

Sex Detector Insert
2.7 kb

Determining Haploid Mating Type

Haploid determination with Auxotrophic marker

Tetrad is digested
3 Day Process

Yeast Sex Detector
Tetrad is digested
3 Day Process

a and α UNKNOWN haploids containing Yeast Sex Detector Vector

= Done!

<5 minutes

Tetrad digestion is always a three day process, however, our Yeast sex detector saves the four days normally used for plating and growing haploid colonies on rich and minimal plates for mating type determination.

Results
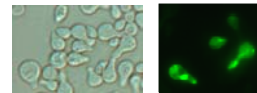
• H
• G
• k

Biobrick Status

	Description	Biobricks	Source	Sequence verified	Contain proper restriction sites	In Biobrick Vector	Confirmed function
FP's	YFP RtL	BBa_K110021		√	√		
	YFP LtR		iGEM Registry	√		√	
	yESapphire LTR	BBa_K110010					
	yESapphire RTL	BBa_K110017					
	mCherry		iGEM Registry	?		√	
			iGEM Registry				
a Promoters	MFA1 LtR	BBa_K11008		√			
	MFA1 RtL	BBa_K110015		√			
	Ste2 LtR	BBa_K11009		√			
	Ste2 RtL	BBa_K110016		√		√	
α Promoters	MFa2 LtR	BBa_K110017		√			
	MFa2 LtR	BBa_K110017		√		√	
Two Way Stop	Long	BBa_K110001	Yeast Bud27/FRS2				
	Long	BBa_K110003	Yeast SWP82/EMP47	√	√	√	
	Short	BBa_K110011	Yeast Bud27/FRS2	√			
	Short	BBa_K11012	Yeast STE2/BST1	√			

Pre-iGEM Conference: JHU, Virginia, Penn State

We held a pre-iGEM conference with Univ. of Virginia and Penn State hoping for mutual critique and advice on project execution, funding ideas, protocols and team dynamics.

Photos taken by Dan Tarjan from University of Virginia

The conference was both informative and inspiring

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