ImmunoYeast: Revolutionizing Antibody Discovery
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Our Idea
We have designed a biological machine capable of antibody discovery AND production. Our strain is capable of screening a library of antibodies for one that binds an antigen and secreting that antibody protein for simple purification.

We demonstrate the feasibility of using the yeast as a streamlined machine that can easily discover and produce new antibodies - all in one test tube!

Current Technology
Current in vivo antibody discovery platforms rely on either surface display of antibody fragments or two-hybrid systems such as our own.

However, after antigen-binding antibodies are found using these systems the antibody genes must undergo significant processing to produce a purified protein product.

Our project addresses this issue by enabling the immediate secretion of easily-purified protein from the very same yeast used for the screening.

Antibody Selection
Which antibody binds your antigen?
Using a modified form of the yeast two-hybrid screening system, the yeast cells are able to sense the amount of antibody:antigen interaction inside the cell.

When the antibody binds the target antigen, it anchors the VP16 transcriptional activator just upstream of Ura3. When grown on –uracil media, only the cells containing an antibody that binds your antigen will survive.

Experimental Methods
Because iGEM is such a short time period we decided to engineer a proof of principle for our two-hybrid screen and inducible recombination pathway.

For a positive control of our two-hybrid screen we physically linked the Lex DNA binding domain to VP16. Expression of this construct should result in high levels of Ura3 transcription.

BioBricks

Yeast Promoters (w/ RBS)
- pGAL:RBS
- pTEF:RBS
- lexO:pCYC:RBS

Yeast Functional Tags
- Aga2 Surface Display Protein
- Aga2:linker
- Secretion Tag

Universal Biobricks
- 36bp loxP site
- (Gly3)Ser4 Flexible Linker

Yeast Selection
- Ura3 Nutritional Marker

Useful Controls
- Aga2:linker: eCFP: Terminator
- pGAL:LexBD:NLS:VP16: Terminator

Overlap PCR

While 3A biobrick assembly is suited for stepwise building of devices, we also looked at faster construction methods. We decided to investigate using an Overlap Assembly Method adapted from the protocol from the JCVI (Gibson et al.).

Overlap PCR

By preparing the parts with overlapping sequences and using this procedure, all of the parts in a complex system can be assembled in one day.

References

