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Cupid's Arrow: A Tale of a Complex and Dynamic Protein

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Transformation Optimization

Introduction

The Pollard lab studies the impacts of genetic variation on protein expression dynamics in yeast. The impacts of genetic variants are compared by swapping specific alleles between the lab (s288c) and clinical (YJM145) strains and measuring the resulting protein expression for specific genes of interest.

Transformations are commonly used in yeast studies as a method of incorporating new DNA into a strains genome. This works because yeast undergo homologous recombination more readily than many other organisms. In genetic engineering, homologous recombination is used as a form of gene targeting, in which an engineered mutation is introduced into a specific gene as a means of investigating the gene's function. In our experiment, foreign DNA that is flanked by sequences identical to the ones upstream and downstream of the target gene's location is introduced into a cell. The cell recognizes the identical flanking sequences as homologues, causing target gene DNA to be swapped with the foreign DNA sequence during replication. The exchange replaces the existing piece of DNA with the foreign DNA.

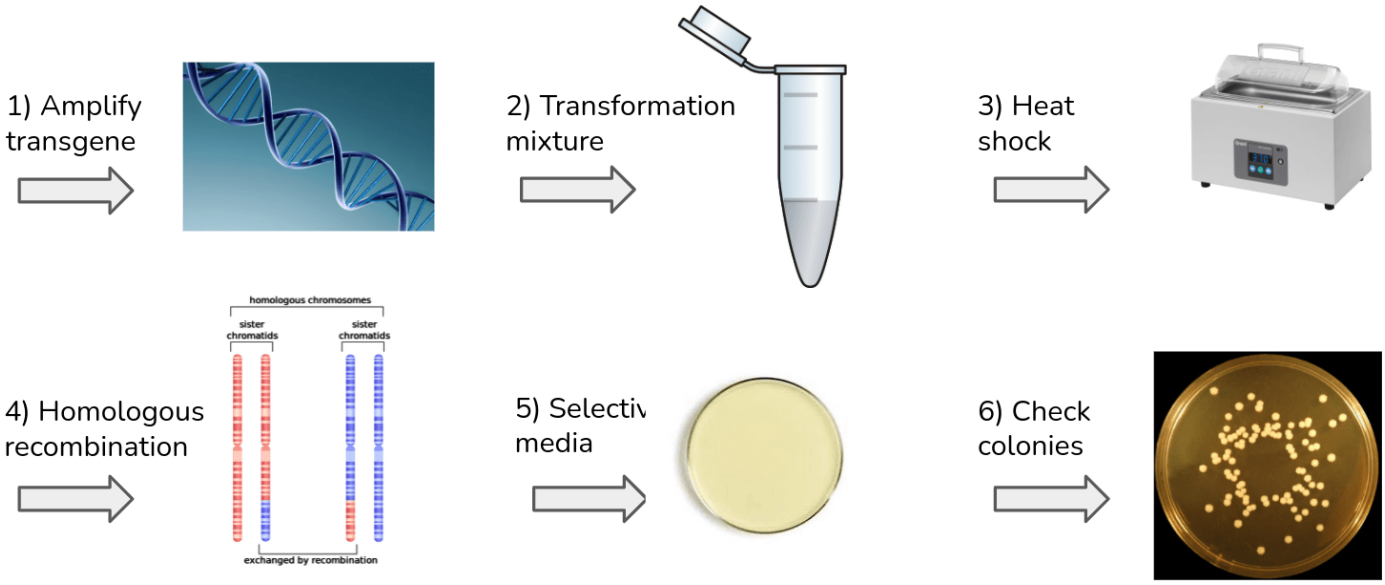


Figure 1: The Pollard lab often works with transformation that integrate linear PCR products into the genome. This begins with amplify transgene (with antibiotic resistance gene and homology tags), mixing transgene and yeast cells together with transformation mixture, and heatshock cells to take up transgene, with some of the cells integrate the transgene through homologous recombination. The transformation mixtures is then plated on selective media, and after a few days one looks for colonies.

Several methodologies for effective transformations have been developed. Our lab uses the Lithium Acetate-mediate method originally developed by the Geitz lab. However, several failed transformation attempts including false positives, have made modifying our transformation protocol to improve transformation efficiency necessary.

Through discussion held within the lab and with other yeast genetics labs, the following variable were identified as potential sources of optimization:

–**Protocol**: the Zymo Research transformation kit was suggested as an alternative transformation method

–**Competent Cell Optical Density (OD)**: Geitz and Zymo protocols recommended growing competent cells to ODs of 1 and 2, as opposed to current protocols using cells from a culture OD of 0.6.

–**Number of plated cells**: Plating fewer cells could help decrease false positives by reducing rates of layered growth, which is where too many cells are plated, cells may grow on top of each other, rather than be selected by the media.

–**Recovery media:** Other laboratories have found that transformations that recover in media that are less rich in nutrients, such as YNB+dextrose, also improves transformation efficiency.

These variables were tested through variations on a central transformation, with transformation efficiency measured by the number of cell colonies that formed on selective media culture plates.

First Optimization Experiment

Variables: The protocol, competent cell ODs, number of plated cells

Zymo vs. **Geitz Protocol Differences**:

-Zymo-specific solutions for competent cells and transformations

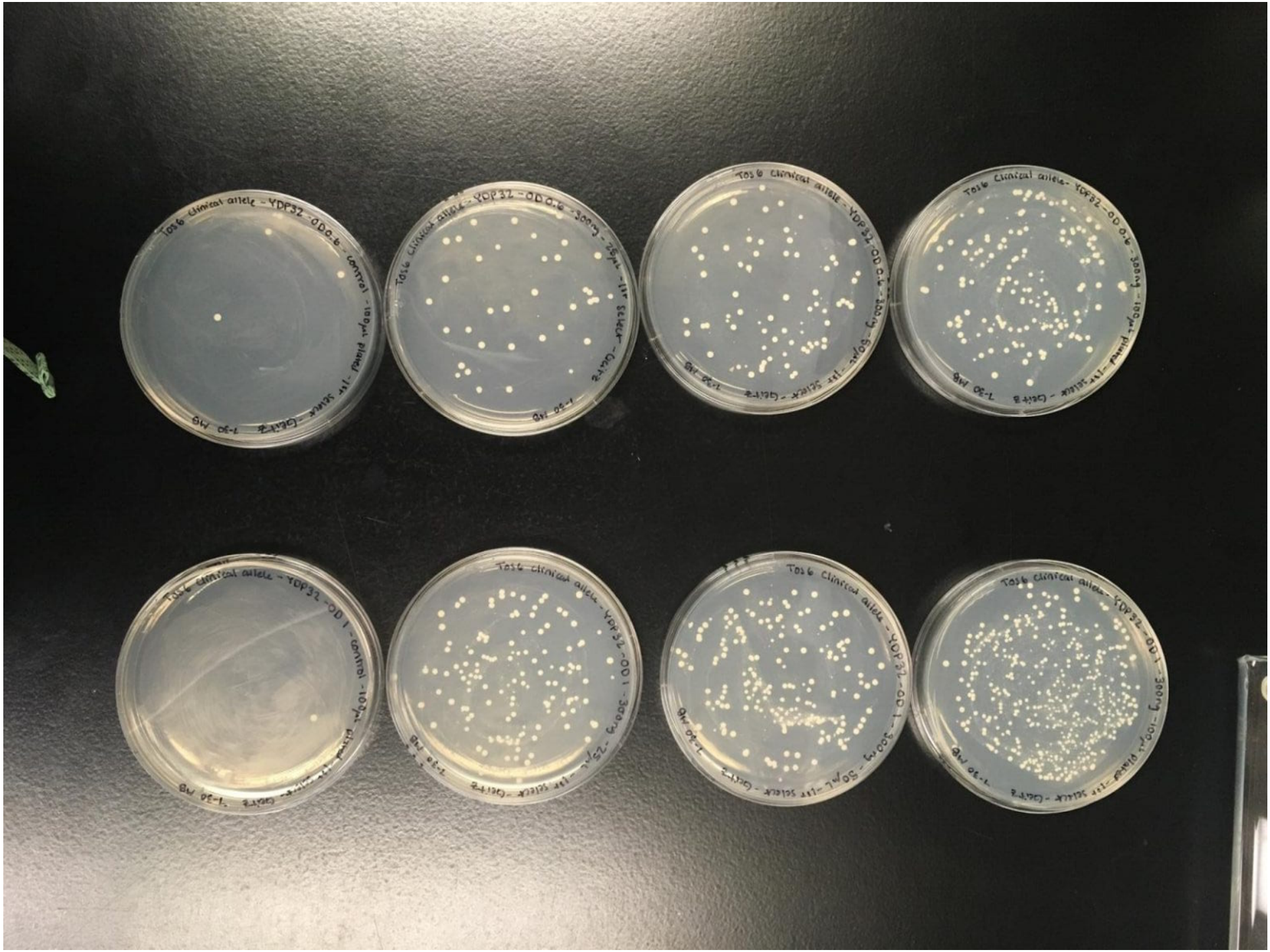
-Higher competent cell ODs, fewer cells per competent cell tube and lower recommended percentage cells plated

First Optimization Experiment Results

-Zymo protocol had significantly fewer colonies than Geitz (Table 1).

-Increasing OD and number of plated cells increased the number of colonies formed for both protocols (Figure 2, Table 1).

-Within the Geitz protocol, higher percentage of cells plated and higher OD yielded more colonies but decreased single colony resolution (Figure 2).


Tos6 clinical allele - YDP32 - OD 0.6 - control - 100µL plated - 1hr select - Cat+R - 1-30 MB
Tos6 clinical allele - YDP32 - OD 0.6 - 300mg - 25µL - 1hr select - Cat+R - 1-30 MB
Tos6 clinical allele - YDP32 - OD 0.6 - 300mg - 50µL - 1hr select - Cat+R - 1-30 MB
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Tos6 clinical allele - YDP32 - OD 1 - control - 100µL plated - 1hr select - Cat+R - 1-30 MB
Tos6 clinical allele - YDP32 - OD 1 - 300mg - 25µL - 1hr select - Cat+R - 1-30 MB
Tos6 clinical allele - YDP32 - OD 1 - 300mg - 50µL - 1hr select - Cat+R - 1-30 MB
Tos6 clinical allele - YDP32 - OD 1 - 300mg - 100µL plated - 1hr select - Cat+R - 1-30 MB

Figure 2: First selection plates of the first Geitz protocol transformations. The columns on the left are OD 1 transformation plates, and the right column are OD 0.6 plates. Top to bottom is the percentage of cells plated: 50%, 25%, 12.5%, and control (50%)

	OD				
Amount of Cells Plated	0.6	1	0.6	1	2
Low	37	144	0	1	6
Medium	72	183	0	1	8
Recommended	158	365	2	3	12
Control	5	2	0	0	0

Table 1: Estimated amount of yeast colonies counted on first selection plates of the transformation optimization experiment using Geitz (white) and Zymo (grey) protocol with varying competent cell ODs and amounts of cells plated

Second Optimization Experiment

Variables: Competent cell OD, number of plated cells, recovery media

Only using Geitz lab protocol

Added recovery step

Second Optimization Experiment Results

-YDP recovery yielded more colonies than YNB+Dextrose at both ODs (Figure 3, Table 2)

-OD 1 yielded higher colony counts than OD 0.6 (Table 2)

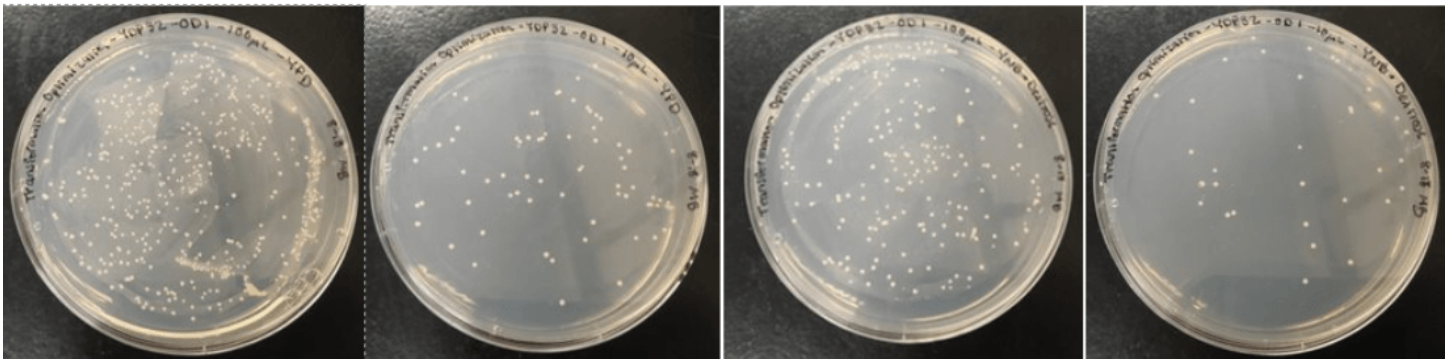

Figure 3: First selection plated for OD 1 of the second optimization experiments. From left to right: Recover in YDP with 50% of cells plated, YDP with 5% cells plated, YNB+Dextrose with 50% cells plated, and YNB+Dextrose with 5% cells plated

	OD			
% Cells Plated	0.6		1	
5	11	2	75	34
50	89	40	502	252

Table 2: Estimated amount of yeast colonies on first selection plates with recovery in YDP (white) and YNB+Dextrose (grey) at varying competent cell ODs and amounts of cells plated. All control plates had zero colonies.

Conclusion

Using the Geitz protocol, but growing competent cells to an OD of 1 rather than 0.6 yielded the highest colony counts of all the conditions tested, so in the future we will grow competent cells to an OD of 1. Plating fewer cells led to fewer but more distinct colonies. Therefore, plating both a low and a high number of cells may increase the

chances of getting a true positive colony. If a recovery step is needed for a transformation, our results indicated using YDP rather than YNB+Dextrose will yield more transformation colonies.

Further study:

-Track false positives/off target insertion rate

-Test if findings also apply to transformation with antibiotic selection

-Optimize with clinical strain

LAB NEWS

We've launched our first website! (https://wp.wwu.edu/pollardlab/2022/02/04/weve-launched-our-first-website/) February 4, 2022