Art plasmid transformations for high-school learning kit

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Abstract:

Genetic transformations are critical in genetic research and the treatment of some diseases. Transformations are when you express foreign DNA in a host organism. In order to educate students on transformations, kits can be created to give students hands-on experiences with this technique to learn genetics. However, currently available educational kits lack proper safety features for students. The yeast species *Saccharomyces cerevisiae* has the ability to take in simple circular DNA molecules (plasmids). These plasmids can be used to add specific genes to yeast cells. Specially engineered plasmids—dubbed "art plasmids"—which were designed to genetically modify the color of a yeast sample are used in this work. The safety concern is that current art plasmids use Kanamycin—a hazardous chemical—as a selective marker, making it unsafe to use in an educational setting. Additionally, plasmids currently being used are missing a key pigment: green. By developing and running transformation procedures, we can address and solve these problems, and the educational kit will be one big step closer to being ready for distribution throughout schools. Here, I amplify a non-toxic selector gene, CaUra3MX. Then, as the yeast sample is transformed with each art plasmid, I swap the CaUra3MX gene with the toxic KanMX gene through homologous recombination, then plate the transformed cells on their respective medium. The transformed yeast cells were then stored in Glycerol to be used for the kit. To achieve a green pigmentation, I transform a blue art plasmid with a Histidine resistance gene (His3MX) and co-transform it with an orange art plasmid with the KanMX gene. The results of the co-transformation did not show green but the procedure will be edited and redone. Although not successful, the results of the co-transformation provide a good first step towards achieving a green-appearing color in yeast. Additionally, given the successful results of the KanMX swap, this procedure will be used for every remaining art plasmid in the kit to make them safe for high school use.

Introduction:

Genetics is the research of genes, their structure, their function, and how organisms inherit and pass on those genes (National Institute of General Medical Sciences [NIH], 2022). Genes are made up of DNA, the primary building block of all life. Every living thing has genes, and those genes determine the characteristics of an organism by providing vast amounts of data to encode a variety of proteins (Hiyoshi, 2011). Researching genetics is essential to understanding how life works and how one's characteristics are passed down to the offspring. It also helps to understand how diseases work, and finds ways to cure diseases. Altogether, genetic research helps to improve medicine and saves many lives.

One major element in genetics research is genetic transformations. A genetic transformation is when DNA is extracted from a foreign organism and is inserted and expressed into a new organism (Broach, 1979). Transformations are commonly used to either isolate and study the function of a specific gene or to implement a new characteristic into an organism of choice and are most commonly done by using small, circular, double-stranded pieces of DNA called plasmids (Gietz, 2002). Plasmids are naturally occurring DNA structures in bacteria that are separate from bacteria's chromosomal DNA. Naturally occurring plasmids can carry resistance genes for the bacteria, which can then be implemented into other organisms to give the new organism the resistance as well (Fair, 2014). Millions of plasmids are cloned inside bacterial cells and they can be extracted and isolated for scientific purposes (Solar, 1998). The plasmid can then be engineered with a custom gene, meaning that whatever gene is needed to be studied can be implemented into the plasmid using digestion and ligase procedures (Gibson, 2009). Once the gene is implemented into the plasmid and the plasmid is properly transformed into a new organism, the compatible custom gene will be expressed in the new organism, thus successfully inputting a foreign piece of DNA into a different organism and consequently changing the characteristics of the transformed organism (See Figure 1).

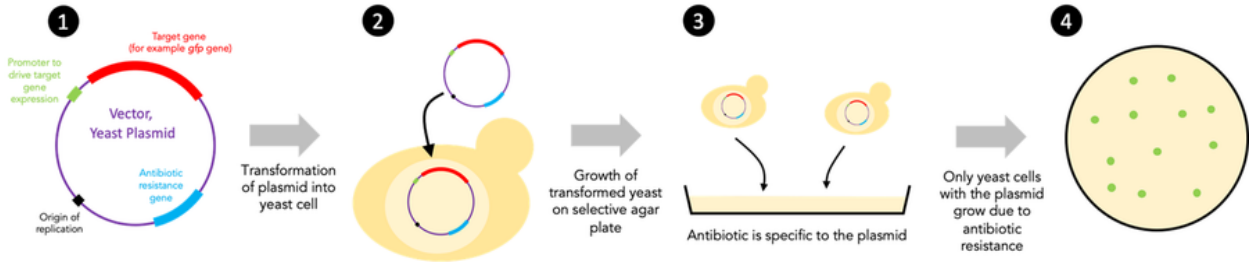

Figure 1. A plasmid with a specified target gene and antibiotic resistance is transformed into yeast cells. The yeast cells are then grown onto an agar plate with a selection that matches the antibiotic resistance gene in the plasmid. Ideally, only the yeast cells successfully transformed with the antibiotic resistance will be able to grow on the selective agar plate, thus making a successful selection of the transformed yeast and ending up with yeast that has the specified target gene. In the case of the art plasmids, the target gene would be one that encodes proteins that express a specific color, respective to the art plasmid being used (Lohner, 2020).

Here, Baker's Yeast - *Saccharomyces cerevisiae* - was used. Baker's yeast is great for biological research because it has eukaryotic cells, making it very similar to the structure of human cells. Using yeast cells gives an accurate representation of many genest that also exist in human cells, making yeast a widely used model organism. Another beneficial property of Baker's Yeast is that it reproduces very quickly, making it excellent for research involving the regrowth of cells. Baker's yeast is one of the best organisms for research on genetic transformations because it is very susceptible to taking in plasmids, making the transformation efficiency much greater than in other organisms, and yielding better results (Dujon, 1996). Understanding how genetic transformations work on a deeper level will lead to even more advanced research and medical discoveries in the genetic field that can lead to an even greater amount of lives improved or saved.

One way to aid the advancement of this research is to help raise interest in the field at a younger age. To do this, an affordable and safe learning kit for high-school students is being developed. This high-school genetic transformation kit will be based around art plasmids: a collection of plasmids that each have a custom gene that creates proteins that change the color of the yeast. Transforming each different art plasmid into yeast gives a different color (See Table 1). Seeing a change in color in the yeast gives an easy way of knowing if the transformation worked, making it perfect for high school use.

Colored Yeast packet #	Pigment	JT strain number	Original Strain number	Parent yeast strain	Mating	Yeast selectable marker	Bacterial Plasmid Selection	Genotype	Plasmid
1	Violacein Black	JTy0393	yLM1084	BY4741	MATa	URA3, KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJC105]	pJC105
2	Violacein Gray	JTy0394	yLM1085	BY4741	MATa	URA3, KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJC105]	pAW075
3	WT White	JTy0407	JTy0407	BY4741	MATa	KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pMS002]	pMS002
4	Sud's Bright White	JTy0190	JTy0190	BY4741	MATa	KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 rho0 [pMS002]	pMS002
5	Ade2 Red	JTy0188	JTy0188	VL6-48	MATalpha	KanMX	ampicillin	MATalpha his3-Δ200 trp1-Δ1 ura3-52 lys2 ade2-1 met14 cir0 [pMS002]	pMS002
6	Anenome Blue	JTy0184	JTy0184	BY4741	MATa	KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJT0177]	pJT0177
7	AmilCP Purple	JTy0230	JTy0230	BY4741	MATa	KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pMS003]	pMS003
8	RFP Pink	JTy0231	yLM1195	BY4741	MATa	KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pMS008]	pMS008
9	ShCP Pink	JTy0272	JTy0272	BY4741	MATa	KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pMS0313]	pJT0313
10	SHCP mut Pink	JTy0273	JTy0273	BY4741	MATa	KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pMS0314]	pJT0314
11	Beta Carotene Pink	JTy0408	yLM1082	BY4741	MATa	URA3, KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJC181]	pJC181
12	Beta Carotene Dark Orange	JTy0409	yLM1280	BY4741	MATa	URA3, KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJC137]	pJC137
13	Beta Carotene Light Orange	JTy0410	yLM1280	BY4741	MATa	URA3, KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJC137]	pJC137
14	Beta Carotene Dark Yellow	JTy0411	yJC001	BY4741	MATa	URA3, KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJC175]	pJC175
15	Beta Carotene Bright Yellow	JTy0412	yJC001	BY4741	MATa	URA3, KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJC178]]	pJC178
16	Beta Carotene Light Yellow	JTy0413	yJC001	BY4741	MATa	URA3, KanMX	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJC184]	pJC184
17	Dark Anenome blue	N/A	yLM1194	BY4741	MATa	KanMx	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pAW74]	pAW74
18	Light Anenome blue	JTy0182	JTy0182	BY4741	MATa	KanMx	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pJT178]	pJT178
19	Darker Viol Purple	N/A	yJC172	BY4741		URA3, KanMX	ampicillin		
20	Lighter Viol purple	N/A	yJC172	BY4741		URA3, KanMX	ampicillin		
21	beta carotene red-orange	N/A	yLM071			URA3	ampicillin		pAW079
22	Brown		yJC0175						
23	Anenome Blue	N/A		BY4741	MATa	URA3	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pAW088]	pAW088
24	AmilCP Purple	N/A		BY4741	MATa	URA3	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pAW081]	pAW081
25	RFP Pink	N/A		BY4741	MATa	URA3	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pAW083]	pAW083
26	ShCP Pink	N/A		BY4741	MATa	URA3	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pAW085]	pAW085
27	SHCP mut Pink	N/A		BY4741	MATa	URA3	ampicillin	MATa leu2Δ0 ura3Δ0 his3Δ1 met15Δ0 LYS2 [pAW086]	pAW086

Table 1. Documentation of every existing art plasmid along with what color it expresses in yeast. Also documents the yeast strain number, the parent yeast strain, the mating type, the selectable marker in the plasmid, the bacterial selection, the genotype, and the name of the art plasmid (Wudzinska, 2018).

Here, we tackled the challenge of adding non-toxic selections to the existing art plasmids in the collection. The already developed art plasmids have only been used in labs and implemented the KanMX gene as the selector. Kanamycin is a toxic selector so, in order to make the art plasmids safe for high schoolers, the KanMX gene will be swapped out with a CaUra3MX gene using homologous recombination (See Figure 2). Then, we tackled the challenge of finding a way to add an art plasmid to the kit's collection that can express a green color in yeast. Research has been conducted to find a naturally occurring gene that expresses green but, so far, while there are many genes for green color, nothing ideal for yeast transformations has been found (Chuang et al., 2018). This research details the unique procedure of co-transforming an already developed orange-expressing art plasmid with a blue-expressing art plasmid in hopes of ending up with green-looking yeast. The blue plasmid will be transformed to have a different selector (His3MX) from that of the orange plasmid (KanMX). The two art plasmids will then be co-transformed into yeast and plated on the necessary dual medium with the hopes of expressing a green pigmentation.

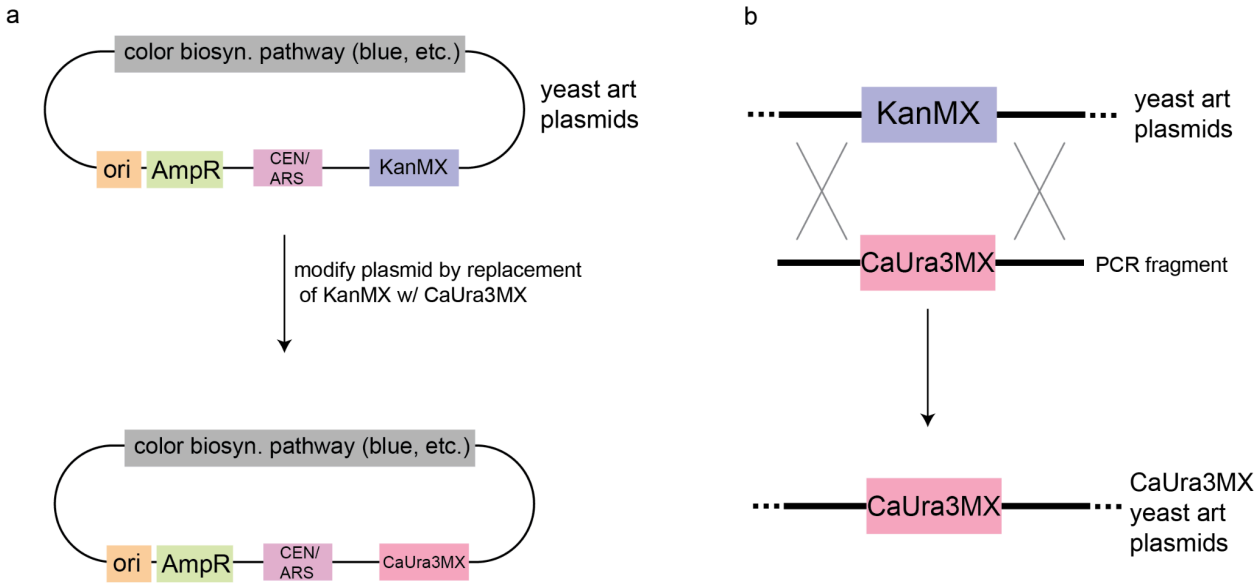

Figure 2. (a) This diagram details a yeast art plasmid's KanMX gene being swapped out by a CaUra3MX through homologous recombination. (b) The HR happens naturally in the yeast's reproduction when the base pairs around two genes are almost identical and can be swapped around. The coding sequence marked with an X is identical (Haase, 2021).

Materials and Methods:

Transforming Yeast with Art Plasmids and CaUra3MX

Using previous stocks, five art plasmids (pMS003, pMS008, JT313, JT314, and JT177) were transformed into yeast. To do this, all the art plasmids stocks were inoculated in a Kanamycin-based medium and grown overnight. The plasmids were extracted through mini-prep and transformed into yeast. During the transformation, a PCR product of CaUraMX3 was also added. Using homologous recombination, the CaUraMX3 gene swapped with the already present KanMX gene in the art plasmid. The transformed yeast was then selectively plated on Sc-Ura medium. The yeast samples of these five successful transformations were collected and stored in glycerol to be added to the kit.

Preparing the pAW74 Art Plasmid

For the co-transformation procedure, a blue plasmid (pAW74) and orange plasmid (pJC137) were selected. As both plasmids had a Kanamycin-based selector gene, pAW74 was transformed with a different selector. A swab of the blue plasmid from a glycerol stock was grown overnight in G418. The culture was then miniprepped and the plasmid DNA was transformed into bacteria. Cultures of the transformed bacteria were swabbed, inoculated in G418 again, and miniprepped. This purified plasmid DNA was then transformed into competent yeast cells along with a PCR product of His3MX - a resistance gene to Histidine - and plated on two Histidine-medium plates: one 10% plate and one 90% plate. Once the transformed yeast grew, a colony was picked and inoculated in the appropriate medium.

Co-Transformation

A swab of the orange plasmid (pJC137) was collected and grown overnight in G418 medium. The yeast culture was then shaked (using BeadBeater BioSpec) and miniprepped. The miniprepped plasmid DNA was then transformed into bacteria and grown overnight. Cultures of the transformed bacteria were then swabbed, inoculated in G418, and miniprepped again. The sample of pJC137 plasmid DNA was then transformed into competent yeast cells by a lithium acetate carrier. The transformed yeast was plated on a 10% plate and a 90% plate. Once the transformed yeast grew, a colony was picked and purified. Then, two co-transformations were done by putting both the orange and blue plasmids in competent yeast cells. One co-transformation sample got the orange plasmid as well as the blue plasmid with the Histidine selector while the other only got the orange plasmid with purified water as a control (See Figure 3). The two co-transformed yeast samples were plated on 10% and 90% plates with a Histidine-Kanamycin combination medium and grown overnight. The dual medium plates were made my taking already existing Histidine-medium plates and adding a layer of the Kanamycin medium on top.

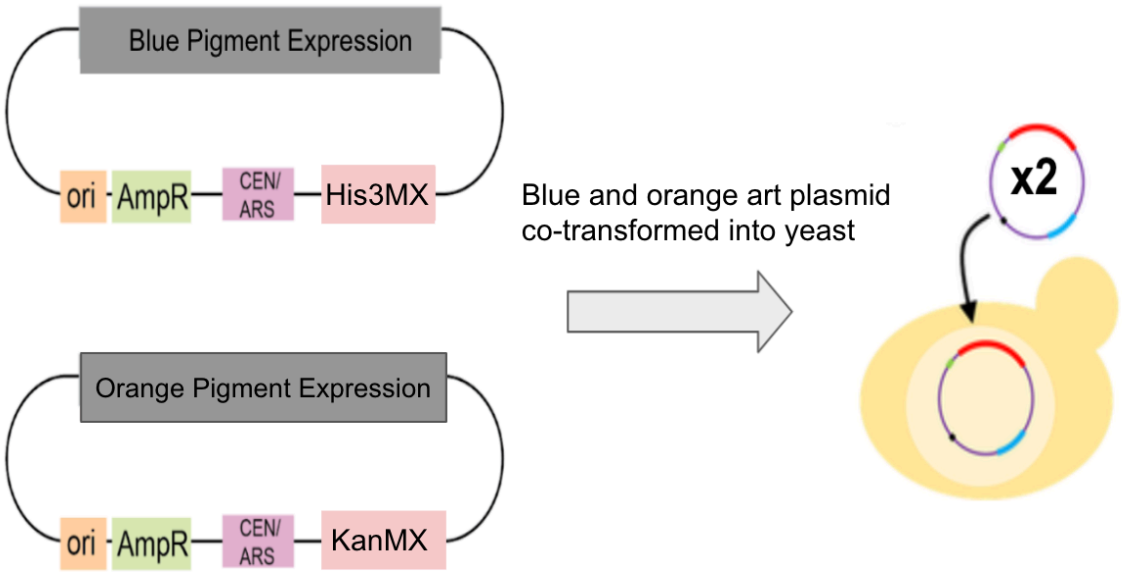

Figure 3. Simple diagram of the co-transformation in order to achieve green. The orange art plasmid has the KanMX selector while the blue art plasmid has the His3MX selector so that they are both expressed. The two art plasmids are then co-transformed into competent yeast cells (Excoffon, 2022).

Results:

Art Plasmids with CaUra3MX

After the art plasmids had the CaUraMX3 gene swapped in and the yeast was selectively plated on Sc-Ura medium, yeast samples of the successful transformations were collected and stored in glycerol to be added to the kit. The transformed yeast colonies were then all re-streaked on a new Sc-Ura plate. One of the art plasmids underwent a mutation during the transformation and expressed two different shades of pink pigmentation. One colony of each shade was re-streaked (See Figure 4).

A sample of each shade from the mutation was collected and sent for sequencing. Strangely, no change in base pairs was found anywhere on the CaUra3MX gene or the color biosynthetic pathway.

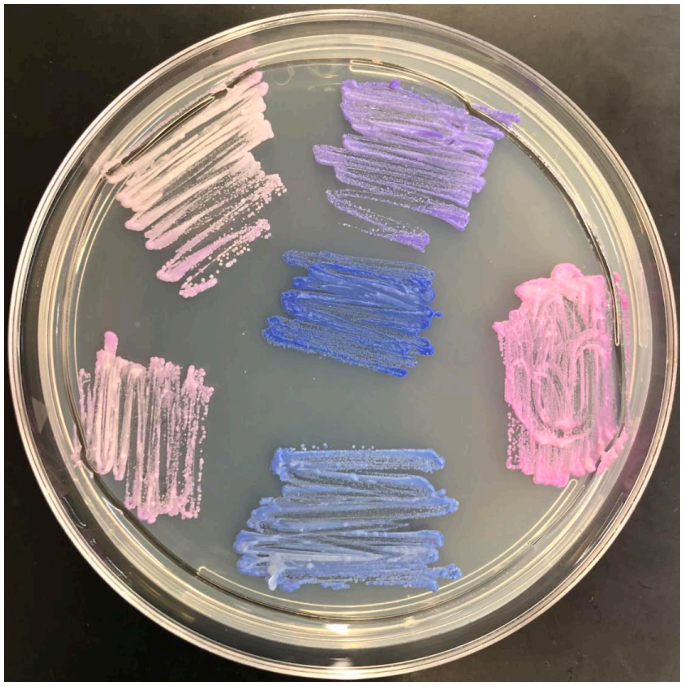

Figure 4. An agar plate with Sc-Ura medium and yeast samples with 5 different transformed art plasmids. The two pink lawns on the left are the transformation that underwent a mutation.

Co-Transformation

The co-transformation to attain a green pigmentation did not give favorable results. Every colony that grew was orange with a few outliers that had a gray-ish coloring (See Figure 5).

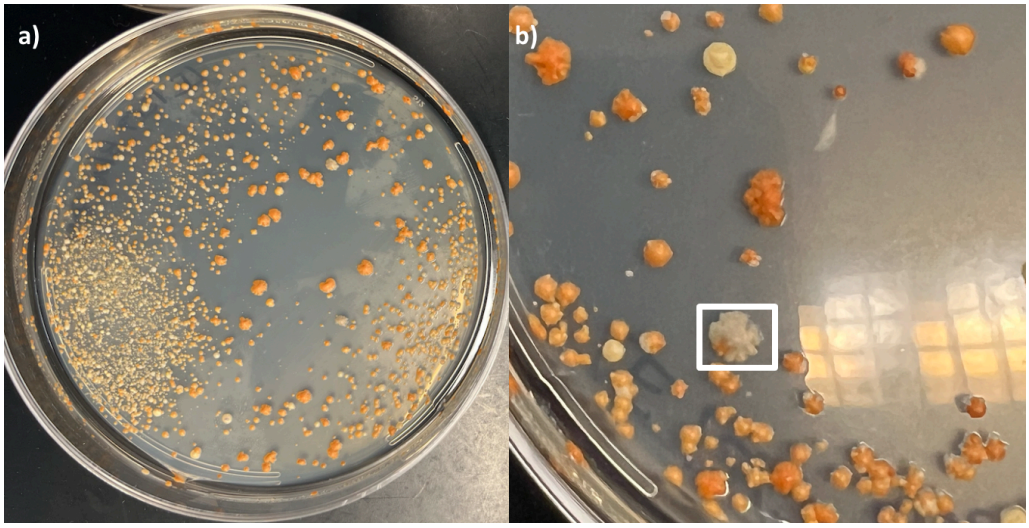

Figure 5. (a) Full view of agar plate with Histidine-Kanamycin medium and co-transformed yeast colonies. (b) Zoomed in view of co-transformed yeast colonies. The white square shows one of the colonies with a gray-ish coloring opposed to the normal orange pigmentation.

Discussion:

In this work, we aimed to improve the educational kit that is being developed by removing the toxic Kanamycin-based selector from art plasmids and replacing it with a safer Uracil-based selector. As the swap of KanMX with CaUra3MX worked, we are hopeful that much progress will be made with the educational kit. The homologous recombination procedure will have to be repeated for all the other plasmids in the educational kit. As the procedure is not too long and can be done with multiple plasmids at the same time, the entire collection can be converted to Uracil selection in only a few days. Once all the art plasmids in the kit are swapped to Uracil-based selection, the kit will be one large step closer to being ready for student use.

It was also very interesting to notice a change in yeast pigmentation through one of the five art plasmid transformations. More research will be done to explore the cause for the change in pigmentation as well as to see if new colors can be controlled for or developed through mutations. As the current sequencing analysis does not show any clear signs of impactful base pair changes, the future research will include lots of additional analyzing of sequencing as well as repeated tests with the art plasmid that underwent the mutation.

Regarding the co-transformation, we believe to know why all the transformed yeast colonies expressed an orange pigmentation. During the creation of the dual medium agar plates, the Kanamycin medium was poured on top of the layer of Histidine medium so it is possible that only the Kanamycin medium affected the yeast cells plated on top. This would explain why the yeast cells were orange (plasmid with the Kanamycin selector) and not blue (plasmid with the Histidine selector). The procedure will be modified to address this potential issue and the experiment will be redone in hopes of activating both plasmids inside the yeast cells.

On the other hand, no matter the result of the blue-orange co-transformation, a future issue will be finding a way to have the green pigmentation without the use of non-toxic selectors for the eventual implementation to the kit for student use. As the proposed co-transformed yeast uses two selectors, both of which are toxic (Kanamycin and Histidine), it will take more planning and testing to find a way to swap the two selectors for other non-toxic selectors, one of which could be the CaUra3MX gene.

Additionally, the idea of the co-transformation could be redone with other art plasmid combinations. Common color knowledge states that green is made out of blue and yellow, so we have already tested to see if this rule would still apply to yeast. A blue art plasmid, pAW74 was paired with three different types of yellow-expressing plasmids: pJC175, pJC178, and pJC184. A plate was made with 18 different color combinations of the blue and yellow plasmids where the cultures were mixed and plated. The plate featured a normal version of each of the three yellow art plasmids along with 5 ratios for each of the combinations (See Figure 4). The greenest looking results were seen through the pAW74 - pJC178 combination. Therefore, this combination could be used in

the future to see if blue and yellow make more green-looking yeast than the blue and orange combination used in this study.

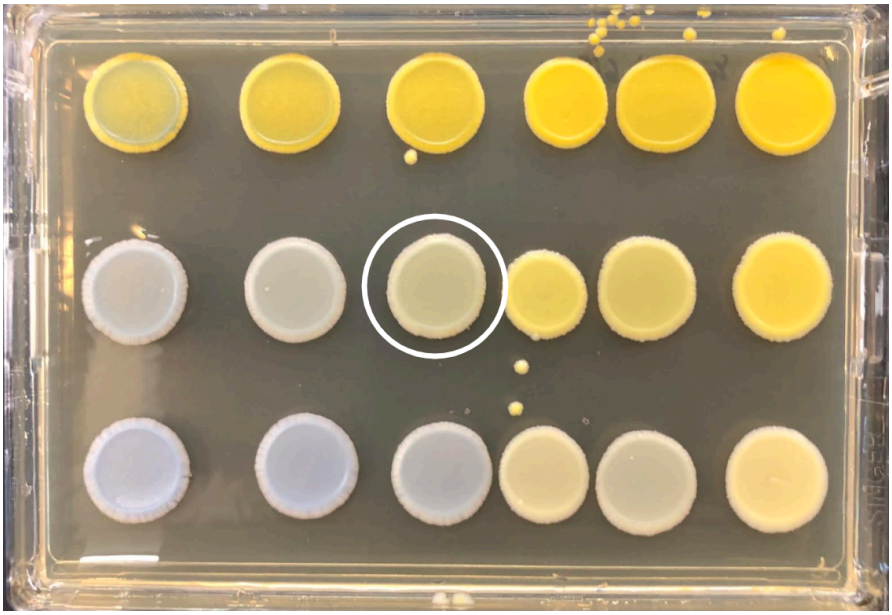

Figure 4. This shows the layout of all the possible blue-yellow plasmid combinations. Five ratios of blue-yellow plasmids were tested: 90%-10%, 70%-30%, 50%-50%, 30%-70%, and 10%-90% (from left to right) along with the three types of yellow plasmids (far right). The yeast circled in white was the blue-yellow combination chosen for the co-transformation because it gave the greenest appearance.

Conclusion:

The work done in this study provides great evidence that the collection of art plasmids for the educational kit can efficiently be transformed to having a non-toxic selector. The switch from Kanamycin to Uracil will greatly help the development of the kit and will overall improve the quality of the kit for teachers and students who use it. As the kit will be safer, more schools and teachers will want to buy it, which will lead to greater education of genetic transformations throughout schools.

Apart from the educational kit, the switch to a Uracil-based selector will be helpful to the development of yeast art. Yeast color is generally brighter on Sc-Ura plating than on G418 (Kanamycin-based) plating, so the swap to Uracil will improve the quality of the yeast art created in the future. Also, the addition of a green-expressing art plasmid in the collection will expand the options offered throughout yeast art. Currently, the co-transformation plan will need to be altered quite a bit as the yeast art only works if all the art plasmids being used have the same selector. Nonetheless, the results of the co-transformation provide a good first step towards achieving a green-appearing color in yeast.

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