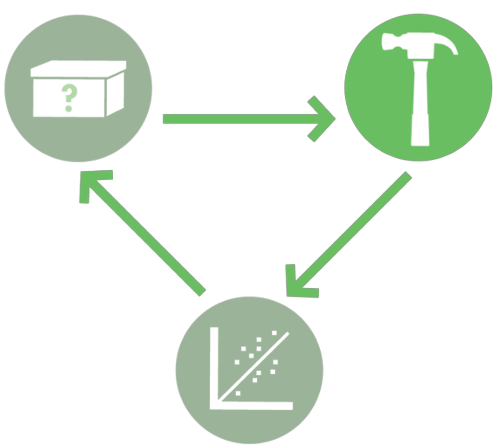
TEACHER MANUAL

BioBuilder

Synthetic Biology for Teachers

Golden Bread

Explores the science, engineering and bioethics of a yeast that's genetically modified to make a vitamin-enriched food. Lab activities include PCR, yeast transformation, codon shuffling and quantitative analysis of data



This teacher's booklet is meant to help support you and your students with the BioBuilder units. Let us know what you need and how it goes. Email us: **info@biobuilder.org**

Golden Bread

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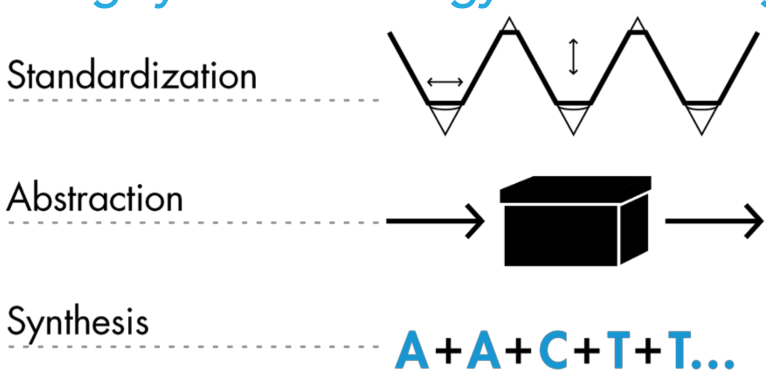
About Synthetic Biology

For the last decade, teachers have introduced genetic engineering techniques to students. It is becoming commonplace for students in Biology and AP Biology courses to conduct a standard set of "experiments" using gel electrophoresis and bacterial transformation techniques. Students who perform these experiments learn several basic techniques, but that is where the laboratory experience ends. There is little room for student inquiry or creativity. The students are more technicians than scientists.

A solution to this limitation comes not from biology but a relatively new field, Synthetic Biology. Synthetic biologists apply engineering principles and extend genetic engineering techniques to construct synthetic living systems. The synthetic biology approach familiarizes teachers and students with molecular biology, genetic engineering and microbiology methods in an engineering setting. The students learn designing, building or testing designs of engineered biological systems. In addition, this approach provides science teachers with a means of fulfilling state and national teaching standards that are hard to address in most biology classes.

Using synthetic biology to teach engineering



BioBuilder's engineering approach focuses on two important principles: abstraction and standardization, and relies on enabling technologies such as DNA synthesis. These principles and technologies extend the teaching of molecular techniques into real world, authentic applications. In the way that physics teachers can have students create functioning circuits and computer teachers can have students create 3-D animations, biology teachers can have students safely design, construct and analyze engineered biological systems.

The BioBuilder Curriculum

BioBuilder provides educational materials for students and teachers to explore the underpinnings of synthetic biology. All the material is modular and can be taught completely, in any order, or piecemeal, as individual exercises to supplement an existing program. BioBuilder's curriculum includes both classroom lessons and laboratory activities. Biodesign and Bioethics lessons can be carried out in any sized classroom and with many age groups. The laboratory investigations provide standard protocols as well as modifications to meet local situations and needs.

Biology teachers can use our materials to lead engineering challenges with students. Students gain first-hand experience with the engineering paradigm:

DESIGN BUILD TEST

Students are motivated to understand the underlying science within an authentic context of engineering challenges. BioBuilder students become more than technicians; they become engineers.

What A Colorful World

Examines the role of the cellular chassis in system performance. Students transform different strains of E. coli with DNA that turns the cells several bright colors. Students then observe how different the color intensity can be from strain to strain, despite being encoded by the same DNA sequence.

iTUNE Device

Examines the role of parts, such as promoters and ribosome binding sites, in predicting the output of a genetic device. The students measure β-galactosidase enzymatic activity as the device's output, thereby looking through the lens of molecular genetics to predict and then evaluate a device's behavior.

Picture This

Three activities to explore the role of modeling in circuit design. These activities include a downloadable program to computationally vary the parameters of a genetic circuit, an exercise to mimic a genetic circuit with electronic parts, and an opportunity to send a stencil that will be turned into a bacterial photograph.

Eau That Smell

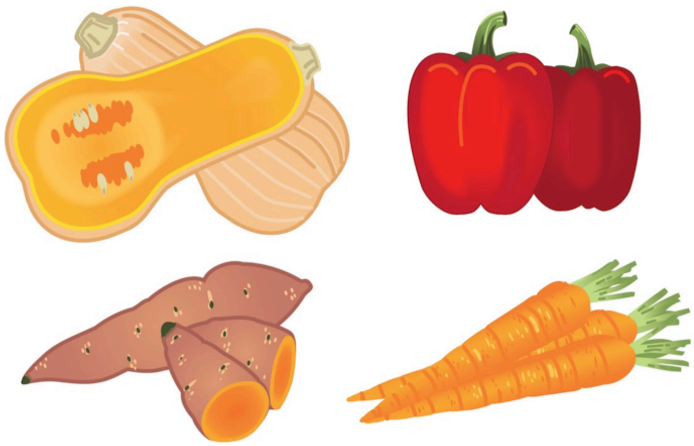
Compares two alternative genetic designs. Both programs should make the cells smell like ripe bananas as the cells grow.

Golden Bread

Explores the science, engineering and bioethics of a yeast that's genetically modified to make a vitamin-enriched food. Lab activities include PCR, yeast transformation, codon shuffling and quantitative analysis of data

ABOUT GOLDEN BREAD

This lab focuses on a strain of baker's yeast that has been modified to produce β-carotene, a nutrient we naturally obtain from eating foods such as carrots, sweet potatoes, and broccoli. In the body, β-carotene is converted to vitamin A, which is crucial for vision, the immune system, and other biological functions.

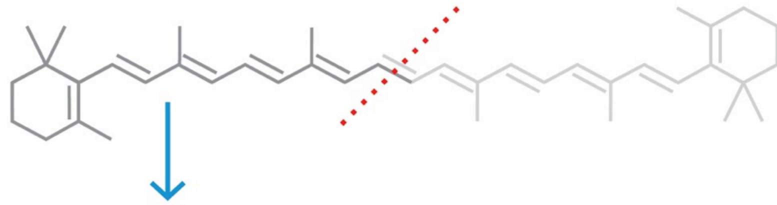


In some developing countries that struggle with malnutrition, vitamin A deficiency is a critical public-health issue. Researchers hope that an engineered strain of baker's yeast designed to generate β-carotene, like the one in this activity, could be used in bread to treat vitamin A deficiency. Such bread might appear a golden color from the added vitamin, hence the name, "Golden Bread."

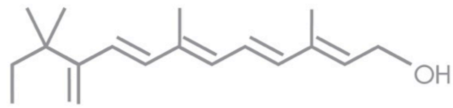
The Golden Yeast was developed as part of an iGEM Project called "VitaYeast." The iGEM team wanted this yeast to substitute for standard baker's yeast, making it possible to bake vitamin A-enriched loaves of bread. The iGEM team worked with an engineered version of baker's yeast, extending some work published in 2007 by researchers who genetically manipulated the strain known as Saccharomyces cerevisiae. *The modified yeast could express all their usual genes plus three β-carotene biosynthesis genes isolated from another fungus.*

ENGINEERING GOLDEN YEAST

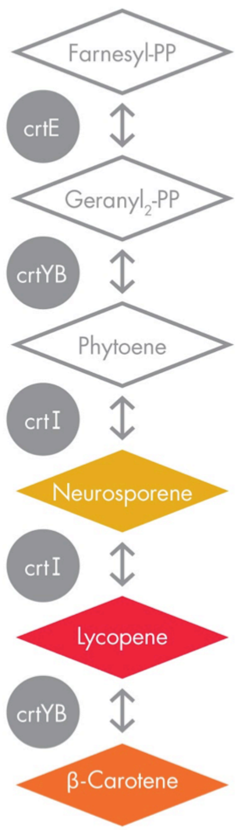
Beta carotene



Retinol (vitamin A)



The metabolic pathway for making vitamin A consists of three enzymes that convert farnesyl phosphate to β-carotene, which then spontaneously breaks in half to become vitamin A.



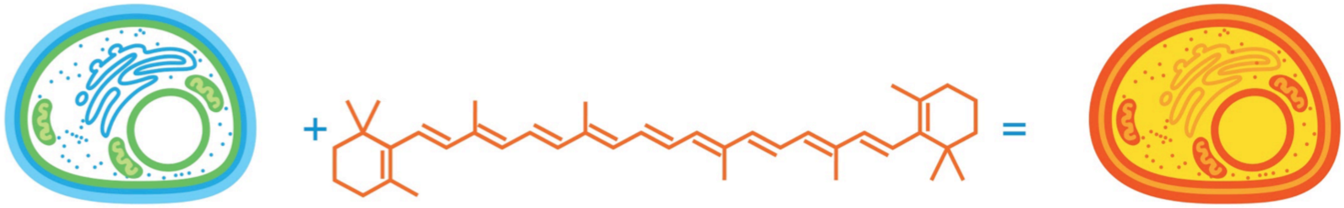
The baker's yeast strain, S. cerevisiae, naturally produces farnesyl diphosphate. The strain also expresses an enzyme encoded by the BTS1 gene that converts the farnesyl diphosphate to geranylgeranyl diphosphate. Other yeast use a similar gene called crtE for this process. Converting geranylgeranyl diphosphate into β-carotene requires the action of two more genes, crtYB and crtI, which are not naturally found in S. cerevisiae and so they were engineered into baker's yeast from a different, red-colored yeast called X. dendrorhous.

Interestingly, each of these enzymes serves double duty when making β-carotene. The crtYB-encoded enzyme plays a role early in the synthesis, converting geranylgeranyl diphosphate into phytoene and then comes back into play for the last step of the synthesis, converting lycopene into β-carotene. Between the crtYB enzyme-catalyzed steps are two reactions that require the activity of the crtI enzyme, which was also imported into the baker's yeast strain from the red-colored yeast. The enzyme converts the phytoene first to neurosporene and then to lycopene.

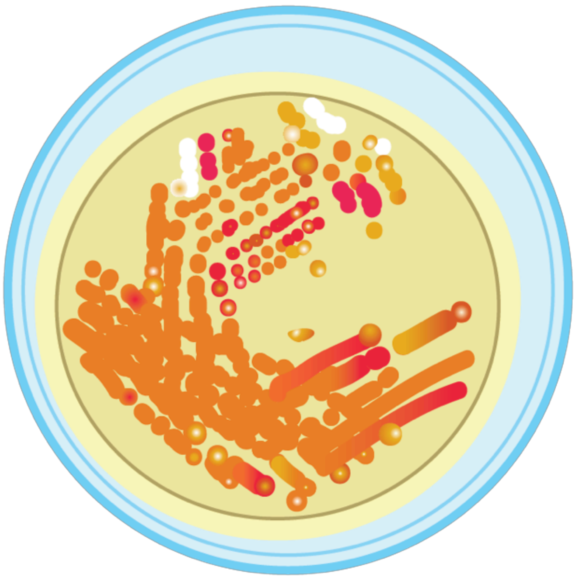
Nature has provided a simple way to detect pigments produced by this pathway, resulting in a convenient visual test for the design. The first three compounds in this pathway are colorless, but the last three are colored yellow, red, and orange, respectively. Unengineered yeast appear white, whereas yeast successfully making β-carotene turn bright orange. Yeast making mostly lycopene turn red like a tomato, which has a naturally high lycopene concentration, and yeast making mostly neurosporene appear yellow. Engineered cells that appear white may have lost one or more of the crt genes.

ENGINEERING CHALLENGE

Researchers knew they had successfully added the three β-carotene biosynthesis genes into baker's yeast, S. cerevisiae, because they saw that the normally white-colored cells were grew as orange colonies.



Much to the researcher's disappointment, though, the strain was not orange 100 percent of the time. When streaked out on a petri dish, the engineered yeast strain grew as orange colonies most of the time, but they could also see red, yellow, and white colonies, indicating that some of the steps in the pathway were not working.



Undaunted, they took two approaches to improving the reliability of the strain's β-carotene production.

First, they stopped using the easy-to-work-with plasmids and instead moved the crtYB and crtI genes into the chromosome of the baker's yeast they were building. These integrated copies of the genes were less likely to be lost from the yeast, and so the strains were expected to be more reliably orange-colored.

Second, they tried to improve the production of β-carotene by adding a second copy of the crtE gene and a second copy of the crtI gene. They hoped these extra copies would make more of the needed enzymes. The second copy would also provide a backup in case the first copy failed. This concept of redundancy is explored further in the engineering toolbox.

Much to their disappointment, however, the strain was still unstable, giving rise to orange, red, yellow and white colonies.

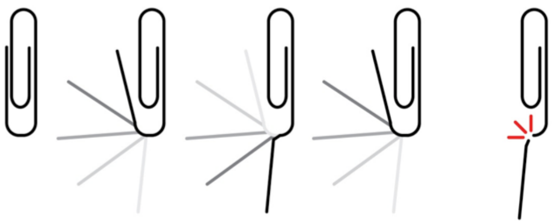
Your engineering challenge is to investigate this instability and to improve the strain's performance.

ENGINEERING TOOLBOX

For any new food or drug to become widely available for use, manufacturers must show that they can reliably produce the material. In fact, reliability is crucial for nearly all engineering endeavors. How do engineers think about and then build-in reliability?

Concept 1: Mean time to failure

The mean time to failure (MTF) helps designers predict when a system will break. It also guides the designer on when and how to intervene through regular maintenance of the system. Engineers include MTF calculations in their design process so that they can recommend when parts should be serviced and how to use them for greatest longevity.

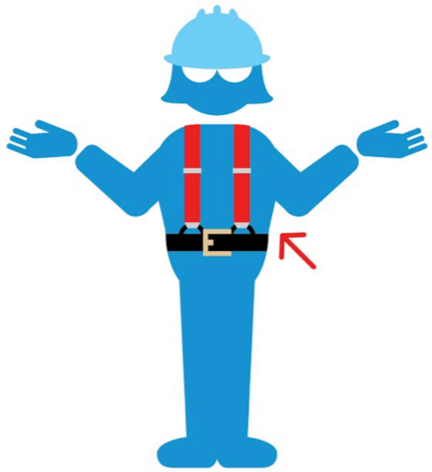


A paperclip's MTF: Bending a paperclip back and forth, as shown here, will eventually cause it to break. The number of bends before breaking can be used to calculate MTF.

In the context of this experiment you can ask: how easily does this system "break," i.e. fail to produce β-carotene? Also, can we lengthen the MTF to improve the performance of the system?

Concept 2: Redundancy

Building redundancy into an engineered system is another technique used in many fields to ensure more reliable performance. For example, engineers working on automotive safety have chosen to deploy both seat belts and airbags to protect passengers in a car crash. Having two safety systems increases the likelihood that passengers will be unharmed in a crash, and so they are worth the added cost.



Redundancy in living cells is also important for their survival. DNA can be damaged by mutagens in the cell's environment, inducing changes in the DNA sequence and making some of the genetic instructions essentially unreadable. Having two copies of the genome is a natural form of genetic redundancy and provides the cell with some insurance.

By adding suspenders to a belt, you can be extra confident that there will be no wardrobe malfunctions.

You will apply these engineering tools to investigate the effect of a second copy of crtYB for restoring β-carotene production in isolated white colonies.

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RE-ENGINEERING GOLDEN BREAD

To fix the genetic instability seen in the Golden Yeast strain you will rely on the engineering strategy of redundancy.

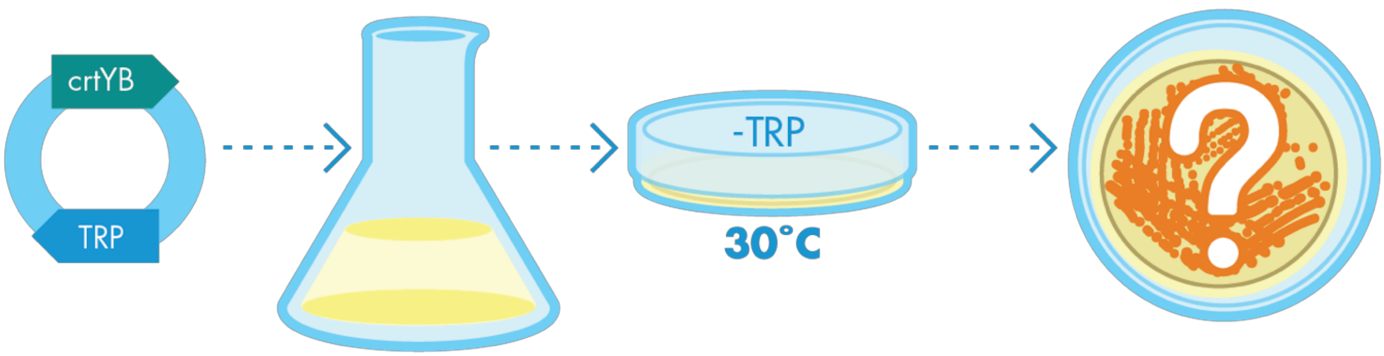
You will focus on the crtYB gene because it is the only gene of the system that does not have any redundancy already engineered into the strain. The second copy of the gene will be called crtYB to distinguish it from the copy that is already in the strain. To make crtYB*, the DNA sequence of crtYB was "***codon shuffled***" and then ordered from a DNA synthesis company. Codon shuffling substitutes synonymous codons for the ones in the natural gene, for example writing TTC in place of TTT in the gene since both DNA sequences encode the same amino acid, phenylalanine. The crtYB* gene sequence was inserted into a plasmid to more easily insert the gene in the engineered yeast.*

*Your experiment will also focus on the white colonies of engineered yeast, asking if the second copy of crtYB can restore their orange color. This kind of experiment is called a "***genetic complementation***" test or sometimes a "cis-trans test." If a component of a pathway can be added back to restore a function, then you have good evidence that the component was the broken part in the first place.*

Experimental Methods

TRANSFORMATION

The process of introducing new DNA into cell is called transformation. The Golden Yeast strain is not naturally ready to take new DNA in from the environment. To prepare the yeast for this experiment, they must be washed with water and then mixed with a salt solution that makes the strain porous. New DNA can be introduced into the strain when the cells are in this "competent" state.



In order to distinguish cells that have taken in the crtYB DNA from those that haven't, the experiment depends, again, on genetic complementation. Because of a defect in their genomic DNA, the Golden Yeast are not able to make their own tryptophan, a necessary amino acid for growth. The cells will grow on "complete" or "rich" media called YPD because the media provides tryptophan for the cells to use. If asked to grow on media that does not have tryptophan, the cells will die unless they have taken in the a plasmid that encodes the tryptophan synthesis enzyme. For this experiment, the crtYB* plasmid also carries the gene for tryptophan synthesis.*

RE-ENGINEERING GOLDEN BREAD Continued

Samples

NEGATIVE CONTROL

No DNA. This reaction is done to confirm that there will be no growth on media lacking tryptophan if there is no external DNA added to the cell.

POSITIVE CONTROL

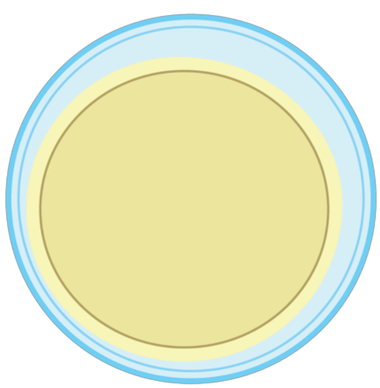
DNA encoding the TRP gene but no crtYB sequence. This reaction is done to confirm that the process of transformation is working. It also confirms that any changes seen with the experimental sample are due to the crtYB* gene and not to the process of transformation itself.*

EXPERIMENTAL SAMPLE

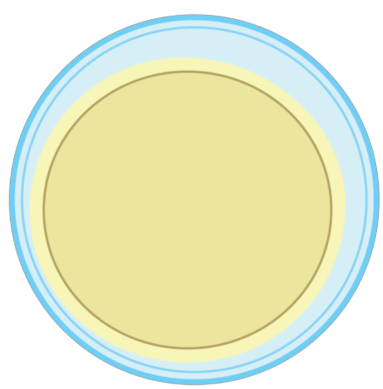
DNA encoding the TRP gene and the crtYB gene. If the white colonies have are defective in the crtYB enzyme but encode crtI and crtE, then the DNA will complete the* ***β-carotene*** *pathway and the cells should appear orange.*

Predictions

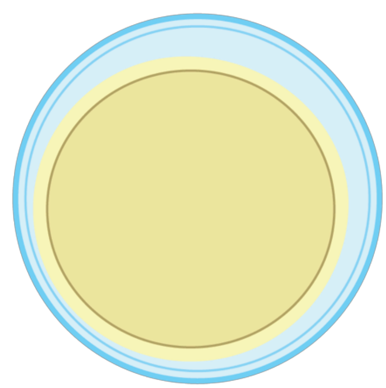
What are the expected outcomes for the three transformations if the media shown below has no tryptophan? It may not be possible to know how many colonies will grow on some of the plates, but if the white colonies have a defect in both the TRP gene and the crtYB gene, then you can predict the color of the colonies on the templates below



Negative Control (no DNA)



Positive Control DNA (+TRP gene)



Expt'l (+TRP + crtYB)*

PRE-LAB QUESTIONS

The genus and species name for commonplace baker's yeast is __ S. cerevisiae __.

*By engineering baker's yeast with three genes from another yeast species, the baker's yeast should express _ **β-carotene** __, and so should appear ___ orange __.*

An engineered yeast that appears red instead of orange might have a defective __ crtYB __ gene.

The addition of an airbag into a car that has seatbelts is an example of __ redundancy ___ which is expected to increase reliable passenger safety in the event of a car crash.

To increase the reliable production of **β-carotene by** *the engineered yeast, the researchers made two modifications to the strain. Name them.*

-- they integrated the crtI, crtE and crtYB genes into the chromosome rather express them from plasmids

-- they duplicated the crtI and crtE genes

Why was the crtYB gene chosen for the transformation experiment performed here?

It was the only gene in the pathway that had not already been duplicated

The gene design technique that replaces the triplet codons in a gene with a synonymous codons is called _ codon shuffling __. This technique maintains the amino acid sequence in a protein but adjusts the DNA sequence encoding it.

If the pathway for **β-carotene** *synthesis is broken so the cells grow as white colonies rather than orange, which genes might be defective? crtI, crtE, crtYB*

If transformation of the crtYB gene into the white cells turns them orange, what do you know about the genetic defect in the white ones? The defect that made the cells white had been in the crtYB gene*

Prior to transformation the engineered strain is expected to grow on "complete media" but not media that lacks __ tryptophan ___.

You are expecting the negative control for transformation __ to result in no colonies growing on media that lacks tryptophan ___.

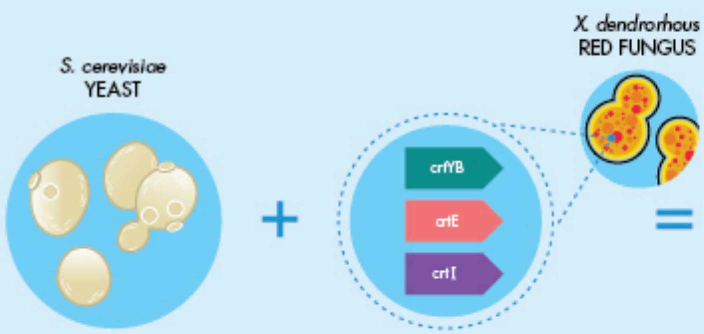
You are expecting the positive control for transformation __ to result in white colonies growing on media that lacks tryptophan ___.

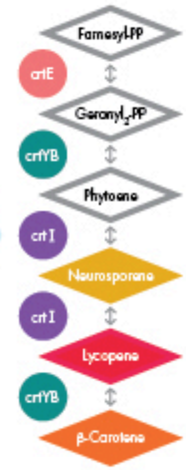
GOLDEN BREAD

THE SYSTEM

A NEW HYPOTHESIS

Genes from *X. dendrorhous* can be added to *S. cerevisiae*, resulting in colonies that produce beta carotene





WHITE colonies may have lost one or more of the crt genes

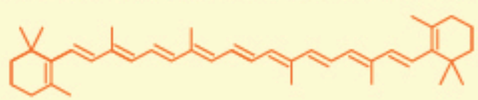
YELLOW colonies may be accumulating neurosporene.

RED colonies may be accumulating lycopene.

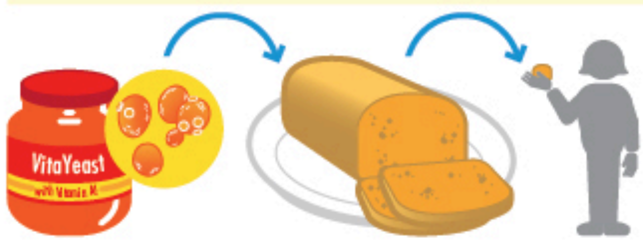
ORANGE colonies produce beta carotene, as designed.

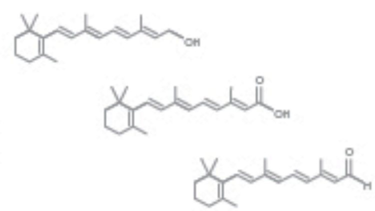
THE BIG PICTURE

Baking with beta-carotene producing yeast creates bread "biofortified" with vitamin A



Beta-carotene (left) is converted in the body to vitamin A and its derivatives (below), which are involved in vision, development, and maintaining a healthy immune system.





ENGINEERING WITH REDUNDANCY

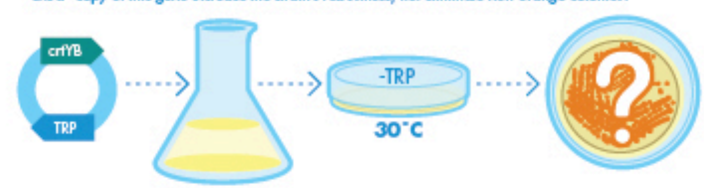
GENES in Vita YEAST

crtYB
crtE bts1
crtI crtI

ENZYMES in Vita YEAST

- Bifunctional enzyme: Phytoene Synthase/Lycopene B-Cyclase
- Geranylgeranyl Diphosphate Synthase
- Phytoene Desaturase

The VitaYeast strain has two copies of every enzyme in the pathway except for crtYB. Will adding an "extra" copy of this gene increase the strain's robustness, i.e. eliminate non-orange colonies?



BioBuilder

CHECKLIST FOR KIT CONTENTS

- ☐ Empty Petri Dishes (100x15)
- ☐ Eppendorf Tubes (18 tubes needed)
- ☐ Disposable spreaders (60 spreaders)
- ☐ Sterile inoculating loops (30 loops)
- ☐ Melt & Pour YED Media (aka "YPD") (1 bottle, 350 mL)
- ☐ Vita-Yeast (1 stab)
- ☐ Control Plasmid DNA (50 ul of 0.5 ug/ul)
- ☐ Experimental Plasmid DNA (crtYB*) (50 uL of 0.5 ug/uL)
- ☐ -trp petri dishes (CSM-trp or SC-trp) (1 sleeve, 25 plates)
- ☐ Zymo Brand EZ-Yeast Transformation Kit (1 kit, 200 reactions)

Unpacking your kit

- Store the YPD media on the shelf until ready to pour.
- -trp petri dishes and EZ-Yeast Transformation Kit to fridge.
- Plasmid DNA to fridge (4°C) or freezer (-20°) if you have one.
- Vita Yeast at room temperature or in fridge

Up to two weeks in advance of lab

▶ ***Prepare YPD media: LOOSEN THE CAP of the media bottle and then melt the contents in the microwave, heating for 60 seconds and then swirling the bottle while wearing a hot mitt. Alternatively melt the media in a hot water bath set at 100° or in an autoclave for 5 minutes. When the media is fully melted, the bottle will be VERY HOT. Take care when pouring the media into sterile petri dishes, filling each ~⅓ of the way. Leave on the bench to harden then store in the fridge.***

▶ ***Streak the Golden Yeast from the stab: Using a sterile loop, touch the yeast that will arrive growing in the stab or slant vial, picking up a small but noticeable amount of yeast. Touch the loop to an area on the YPD petri dishes you have prepared and streak the yeast across the media as shown in the video on the BioBulder.org website.***

Pre-lab preparation

Each lab group will need

- A white yeast colony. Several groups can work from one YPD plate that has the yeast streaked out, or each group can work with a plate of their own
- An aliquot of each plasmid: 5 ul of Control DNA and 5 ul of crtYB* DNA for each group in eppendorf tubes plus an empty eppendorf tube for the No DNA control
- Transformation Solutions: aliquots of 500 ul EZ Solution I, 150 ul EZ Solution II, 1.5 ml EZ Solution III for each group unless sharing reagent bottles
- Three CSM-trp petri dishes
- One loop or toothpick
- Three sterile spreaders

The lab will need:

- Microfuge
- Biohazardous waste disposal
- 30° incubator, though petri dishes can be incubated at room temperature for one day longer
- Micropipets and tips
- Sharpies
- Latex gloves

QUICK GUIDE: GOLDEN BREAD

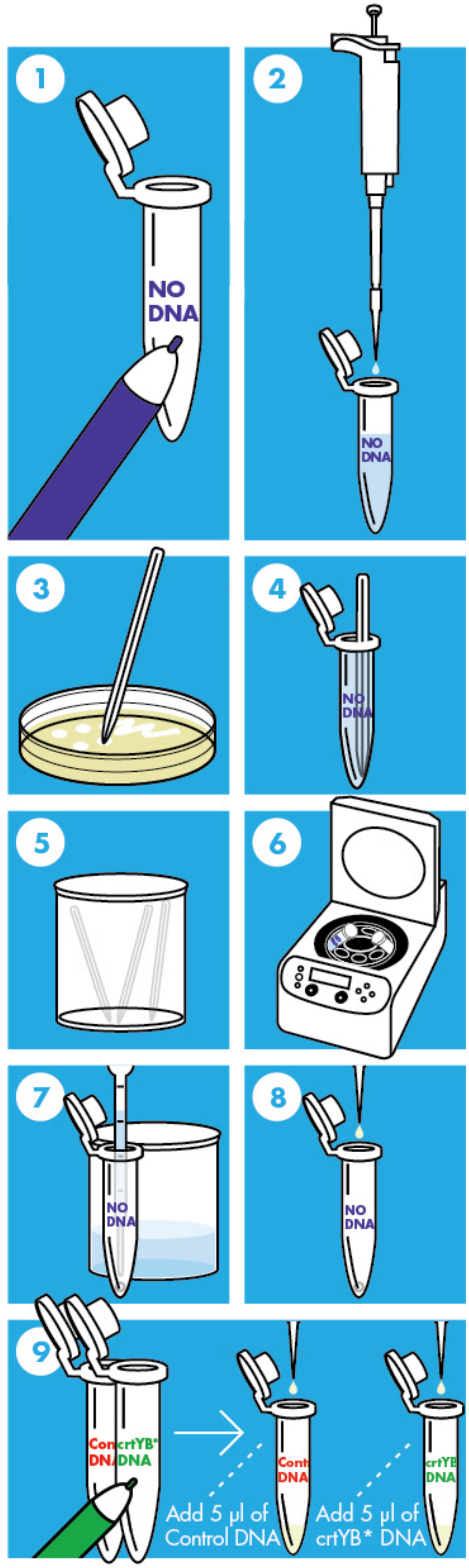
IN ADVANCE

Melt YPD in microwave and pour plates.

Restreak "Golden Yeast" onto YPD to isolate single white colonies**

DAY OF LAB

1. Label 1 microfuge tubes: **NO DNA**.
2. Add 500 µl of **EZ - Solution I** to the **NO DNA** tube.
3. Use a sterile pipet tip, toothpick or inoculating loop, scrape an isolated white colony of yeast off the petri dish.
4. Swirl the colony into the **EZ - Solution I** in the **NO DNA** tube.
5. Discard the pipet tip, toothpick or inoculating loop into a waste receptacle to be decontaminated.
6. Microfuge the tube you have prepared, coordinating with another group or setting up a "blank" microfuge tube with 500 µl of water. Spin the tubes for 30 seconds at full speed.
7. After microfuging the tubes, the cells will have collected as a white pellet in the bottom of the tube. Remove as much of the supernatant as you can, using a pipet and discarding the liquid into a waste receptacle to be decontaminated.
8. Resuspend the pellet in 150 µl of **EZ - Solution II**, pipetting up and down to make a homogeneous solution.
9. Label two more microfuge tubes: **Control DNA**, **crtYB* DNA**. Add 5 µl of the appropriate DNA to the tubes, changing tips between aliquots. Skip this step if the DNA has already been aliquoted for you.



10. Add 50 µl of cells from the **NO DNA** tube to the **Control DNA** tube. Pipet up and down to mix.

11. Add 50 µl of cells from the **NO DNA** tube to the **crtYB* DNA** tube. Pipet up and down to mix.

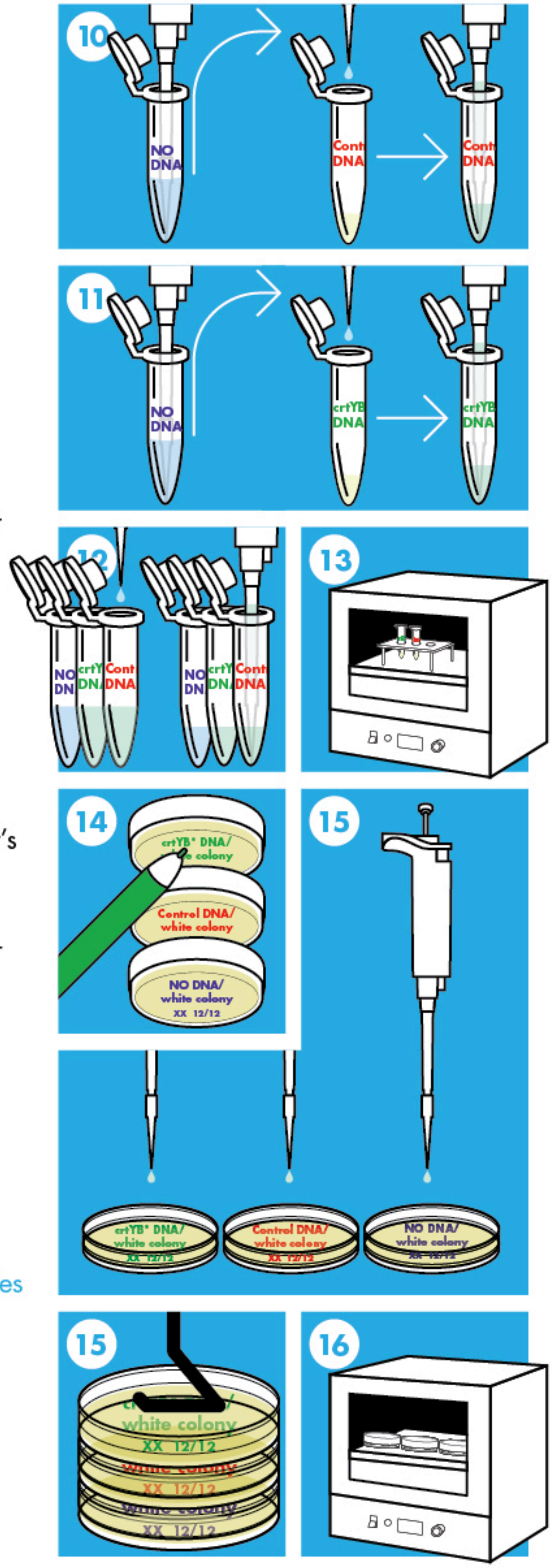
12. Add 500 µl of **EZ - Solution III** to each microfuge tube. Solution III will be goopy, but the amount you pipet does not need to be precise. Pipet up and down to mix, changing tips between tubes.

13. Incubate the tubes at 30° Celcius (C) for one hour. Periodically flick the tubes to mix during the incubation.

14. Label the media side of three SC-trp petri dishes as **NO DNA/white colony**, **Control DNA/white colony**, and **crtYB* DNA/white colony**. Add your initials and today's date to each.

15. Pipet 200 µl of each sample onto the media of the appropriate petri dish. Spread evenly across the dish with a sterile spreader.** Discard spreader and microfuge tubes into the waste receptacle to be decontaminated.

16. Incubate petri dishes, media side up, for 2 days at 30° C.



After the petri dishes have incubated for 2 days, count the colonies of each color in every dish.

** VIDEO OF PROCEDURE AVAILABLE ONLINE

INTERPRETING THE RESULTS

If the white colonies of engineered yeast you used in this experiment have all the pathway components ***except*** *a functional crtYB enzyme, then the addition of the crtYB* plasmid should restore the full pathway and return the cells to orange.*

The control reactions will strengthen the conclusions that can be drawn.

NEGATIVE CONTROL	POSITIVE CONTROL	EXPERIMENTAL
- Cells require plasmid DNA to grow in the absence of externally provided tryptophan - No growth indicates that cells and reagents were not contaminated	- Cells with plasmid DNA can grow in the absence of externally provided tryptophan - Growth indicates that cells were not killed by transformation reagents or the process of transformation	- Cells with plasmid DNA can express the full pathway for **β-carotene production** **- Growth of orange colonies is consistent with our hypothesis that the white mutant cells carried a defect in the crtYB gene and that the codon shuffled crtYB* can express the necessary enzyme**

The negative and positive control can also help to interpret unexpected data. For example, if cells are growing on the negative control then there is a contaminated reagent (cells, media, plates, reagents).

In our experience, most but not all the colonies transformed with crtYB are orange. There are many sensible interpretations of this data, all of which would require more experiments to investigate.*

POST-LAB QUESTIONS

The negative control (no DNA) you ran was expected to have no colonies because the yeast cannot make their own tryptophan and the cells were grown on media that ____lacked tryptophan________.

If you DO see colonies of yeast growing on the negative control -trp plate, you might think that
one of the samples or reagents was contaminated with yeast that can make tryptophan

If you accidentally plated the negative control (no DNA) on YPD, what would you expect to see
a lawn of yeast growth

If you see white colonies growing on the positive control, then you know that
the transformation procedure was successful
the transformation procedure is not sufficient to change the color of the colonies
the cells will grow on media that lacks tryptophan when the TRP gene is provided on the plasmid DNA

If you see orange colonies growing on the experimental petri dish, then you know that
the crtYB plasmid is expressing both the TRP gene and the crtYB gene*
the crtYB gene was defective in the engineered cells that had been growing as white colonies

If you see orange and red and yellow and white colonies growing on the experimental petri dish, what can you conclude?
That the crtYB gene can restore the full biosynthetic pathway, but genetic redundancy alone does not completely remove failures.*

Can you think of other ways to improve the reliable performance of the system?
Many possible answers but these could include: kill switch if cells don't produce b-carotene, exploring alternative growth conditions to assess impact of temperature etc on MTF, codon shuffled genes in crtI and crtE, regulated production of each enzyme, ….

If you wanted to build a business around the health benefits of Golden Yeast, what would you need to do?
Many possible answers but these could include: improved reliability, safety testing, market analysis, socializing GMO in communities that might benefit from its application.

Would you eat bread made with Golden Yeast? Why or why not?
Many possible answers

Ideally, the interpretation of these results should encourage more experimentation, provide ideas for improved designs, and build excitement to explore and do more.

TEACHER MANUAL

Do you have an idea for improving and extending the units? Please email us: **info@biobuilder.org**