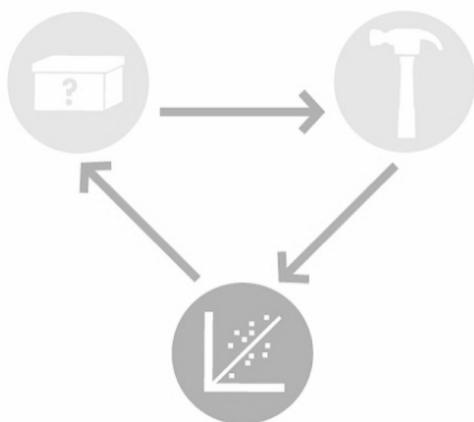


BioBuilder

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



Developed in collaboration with

minipcr bio™

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

BioBuilder

Golden Bread

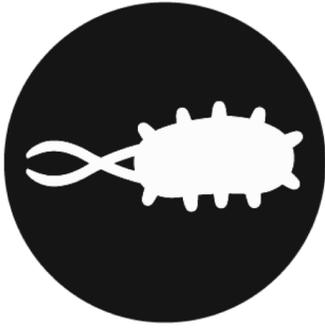
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About Synthetic Biology

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.

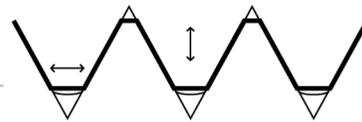


A solution to this limitation comes not from biology but a relatively new field, Synthetic Biology. Synthetic biologists apply engineering principles and extend genetic engineering techniques to construct synthetic living systems. The synthetic biology approach familiarizes teachers and students with 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

Standardization

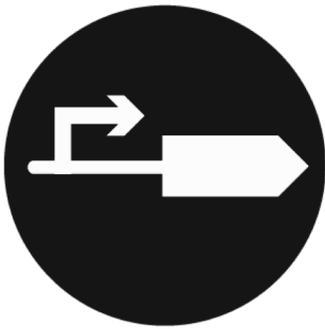


Abstraction



Synthesis

A+A+C+T+T...



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

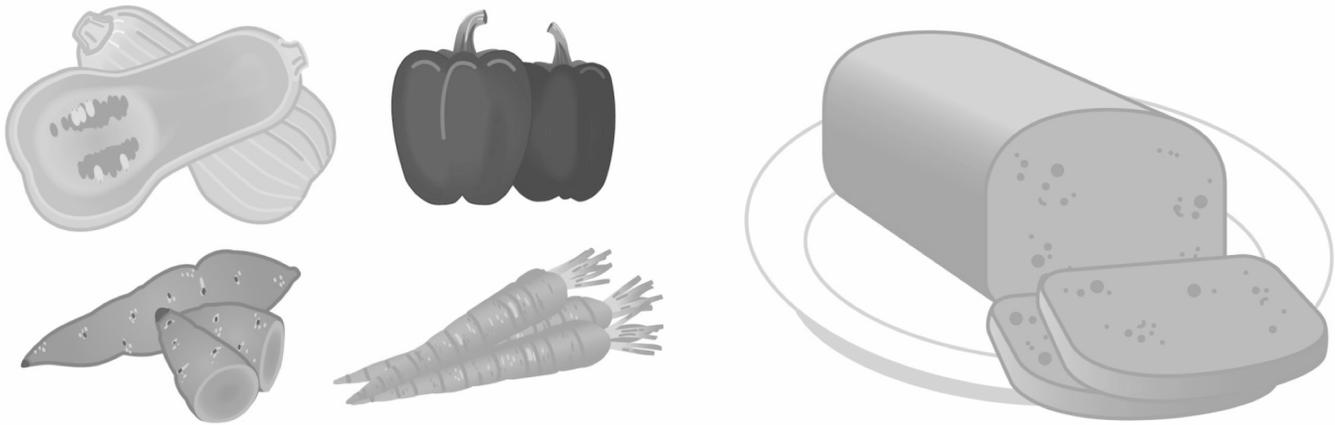
Compares two alternative genetic designs. Both programs should make the cells smell like ripe bananas as the cells grow.

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 YEAST

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.



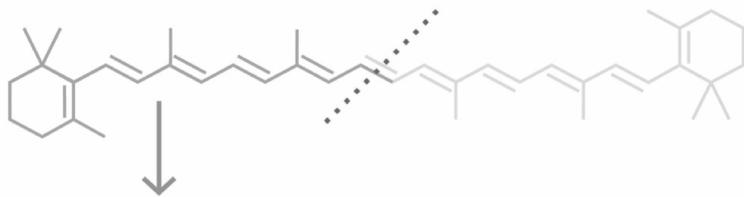
In some developing countries that struggle with malnutrition, vitamin A deficiency is a critical public-health issue. Researchers hope that an engineered strain of baker's yeast designed to generate β -carotene, like the one in this activity, could be used in bread to treat vitamin A deficiency. Such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

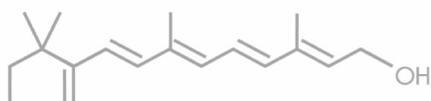


THE SCIENCE OF VITAMIN PRODUCTION

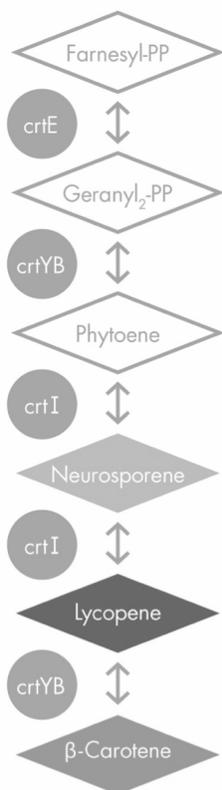
Beta carotene



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.



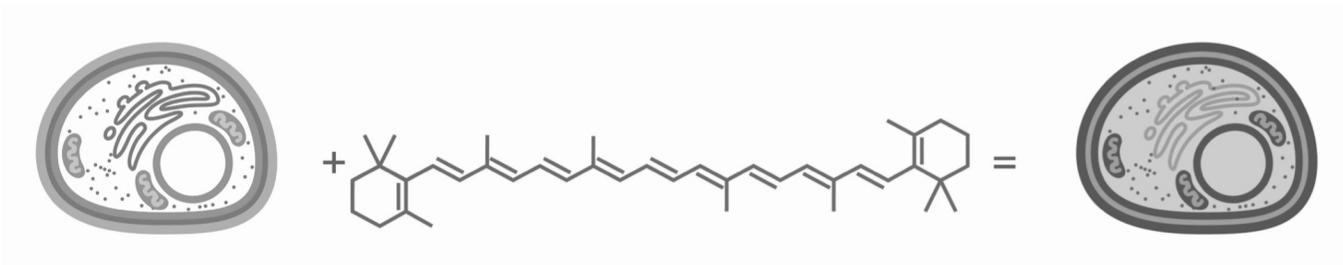
The baker's yeast, *S. cerevisiae*, naturally produces farnesyl diphosphate. The yeast also expresses an enzyme encoded by the *BTS1* gene that converts the farnesyl diphosphate to geranylgeranyl diphosphate. Other yeast use a similar gene called *crtE* for this process. Converting geranylgeranyl diphosphate 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.

GROWING GOLDEN YEAST

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 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 scientific challenge is to test different colored yeast colonies for the presence of *crtYB*, one of the genes that's needed for β -carotene production.

MOLECULAR BIOLOGY TOOLKIT

With modern lab techniques it is now possible to explore and perhaps diagnose cellular defects at the molecular level. Three of the most crucial and well-established techniques of molecular biology help

- Read the DNA code, aka “sequence DNA”
- Copy existing DNA sequences, a technique known as Polymerase Chain Reaction (PCR)
- Insert specific DNA sequences into existing DNA strands, creating recombinant DNA

These techniques weren’t invented from scratch. They exploit natural cellular processes in a way that can provide valuable information and raw materials for further study.

Tool	Molecular biology technique	Natural cellular process
Reading DNA	Sequencing	DNA replication
Copying DNA	PCR	DNA replication
Inserting DNA	rDNA with restriction enzymes and ligases	Defense from infection, DNA recombination and repair

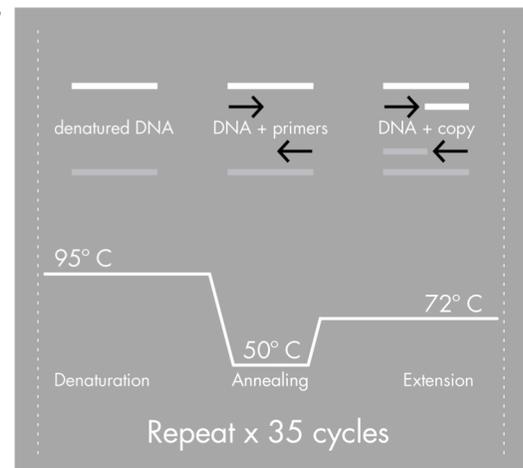
About PCR

Rare or unidentified biological samples can be studied and manipulated with PCR. The technique has been used in diverse and important ways such as pathogen detection, genetic modification, forensic analysis and DNA sequencing. In this experiment, PCR will be used to “find a needle in a haystack,” i.e. to detect and then copy one section of DNA from the long and complex yeast genome. This detection feature will help you clarify what is “broken” (genetically speaking) in the engineered yeast that are not growing as orange colonies.

The materials that go into a PCR experiment are remarkably simple. Reactions combine:

- **Template** = the DNA to be studied
- **Primers** = short snippets of DNA that specify where the replication should begin and end
- **DNA polymerase and nucleotides** = an enzyme and its substrate to copy the DNA template
- **Buffer** to maintain pH and provide ions for the reactions

The mixture is then cycled through different temperatures. In the “denaturation step” the mixture is raised to a high temperature so that all the DNA bases unpair. For the “annealing step,” the temperature is lowered, allowing the primers to bind to the template DNA. Finally, the “extension step” raises the temperature slightly to allow the DNA polymerase to work. This process is repeated dozens of times to create billions of copies of the desired DNA fragment.



You will conduct a PCR experiment to determine whether the crtYB gene is still present in the different colored colonies.

INVESTIGATING GOLDEN YEAST

To study the genetic instability seen in the Golden Yeast strain you will follow a simple protocol to

1. extract DNA from yeast of different colors
2. use PCR to amplify two genes from the yeast extracts: crtYB and actin1
3. analyze the PCR products. The two genes are expected to generate different lengths of PCR product and so will be distinguishable through agarose gel electrophoresis.



Your experiment tests the hypothesis that different colors of yeast arise from the presence or absence of a gene in the pathway for β -carotene production.

You will focus on the crtYB gene because it is the only part of the metabolic pathway that was engineered without redundancy. Only one copy of the crtYB gene is present in the Golden Yeast. And if crtYB is missing, then the yeast are not expected to make β -carotene. But it is important to remember that there could be other explanations for the unreliable production of β -carotene. The different colored yeast could arise from changes in the action of genes not primarily involved in the β -carotene pathway, or from point mutations in the pathway, or from completely nongenetic causes such as differences in the growth medium, temperature or humidity. Although you will not investigate these other potential sources of unreliability here, they represent excellent follow-up experiments.

Samples

NEGATIVE CONTROL (not included)

Though not specified in the protocol, it is relatively straightforward to include a “No Template” reaction to ensure that any DNA seen on the agarose gel was specifically amplified by PCR and did not arise from DNA contamination of stocks.

POSITIVE CONTROL

Plasmid DNA with the crtYB sequence is included to confirm that the PCR is working.

INTERNAL POSITIVE CONTROL

The extraction procedure might vary from strain to strain so amplification of the actin1 gene is included to confirm that PCR can amplify DNA from each lysate. Actin is an essential gene and so is expected to be present in all yeast cells, no matter what color.

DNA Sequence Information

The crtYB sequence can be retrieved from the Registry of Standard Biological Parts ([BBa_K530000](#)) or from the [BioBuilder site on Benchling](#). The forward and reverse PCR primers to amplify this gene are 5'-GCTCTCGCATATTACCAGATC and 5'-GGTGATGAGTAAGGAGAGTGC giving a 486 bp PCR product.

The actin1 gene sequence can be retrieved from the *Saccharomyces cerevisiae* database ([SGD](#)) or from the [BioBuilder site on Benchling](#). The forward and reverse PCR primers to amplify this gene are 5'-GAACACGGTATTGTCACCAAC and 5'-CAGCGTAAATTGGAACGACGT. Giving a 289 bp PCR product.

Exercises to predict the product size and the gel's appearance are included in the pre-lab questions.

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 white instead of orange might be missing the *crYB* gene. (*crE* is also a good answer).

Provide a different reason that an engineered yeast might appear white instead of orange:

Answers here will vary but could include: a point mutation in the *crYB* gene, environmental conditions that impact growth, other genetic defects that regulate the engineering pathway.

Polymerase Chain Reaction includes a denaturation step that unpairs the DNA template , an annealing step that allows the DNA primers to bind the template, and an extension step in which the DNA is copied by DNA polymerase .

Why was the *crYB* gene chosen for the PCR experiment performed here?

It was the only gene in the pathway that had not been duplicated, and so might be missing

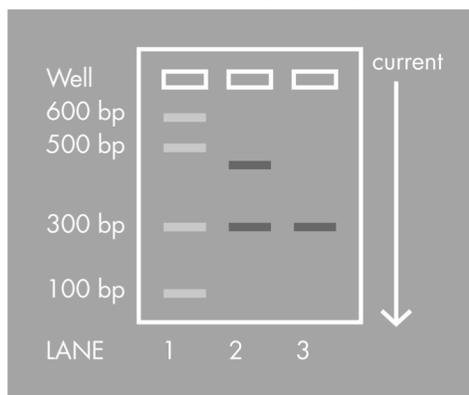
Many online tools are available for analyzing and manipulating DNA sequences. Use the sequence information on pg 9 and an online tool such as the [Bioinformatics Sequence Manipulation Suite](#) to predict the length of DNA that will be amplified with the *crYB* PCR.

486 bp

Using the same online tool, what do you predict for the length of DNA you expect to see from the actin gene PCR.

289 bp

Agarose gel electrophoresis uses current to separate DNA based its length. DNA samples are loaded into shallow wells on one end of the agarose slab, and they are "run" by passing a current through the gel. DNA of a given length appears as a "band" once the run is complete, with shorter pieces of DNA moving further through the gel than longer pieces. Based on the lengths for the DNA bands shown in lane 1 of the figure below, sketch in Lane 2 what you expect to see if BOTH the *crYB* gene and the actin gene is amplified. Sketch in Lane 3 what you expect to see if ONLY the actin gene is amplified.



Which lane shows a result you might expect for a white colony?

Lane 3

Which lane shows a result you might expect for an orange colony?

Lane 2



BETA-CAROTENE IN YEAST

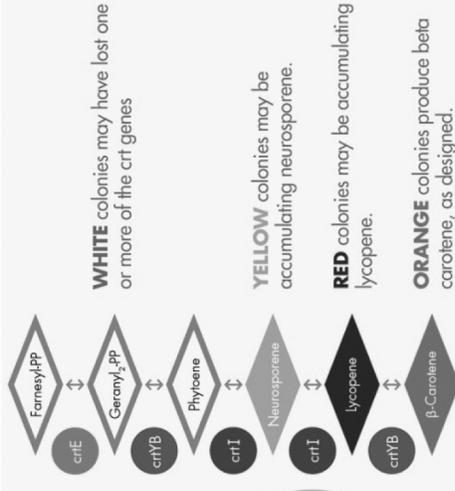
Many kinds of plants and fungi make beta-carotene naturally, but animals can't make their own from scratch. *Saccharomyces cerevisiae* can be engineered to produce beta-carotene.



S. cerevisiae
 YEAST

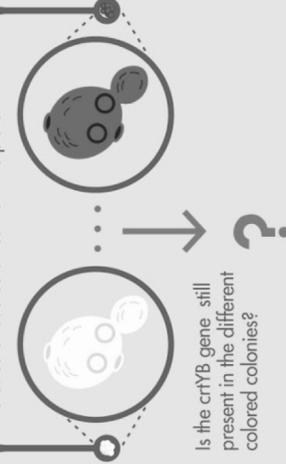


THE BIOSYNTHETIC PATHWAY



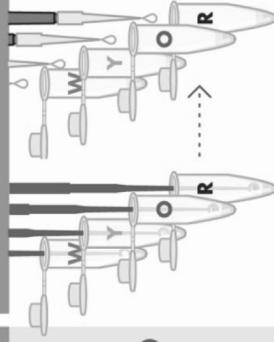
EXPERIMENTAL QUESTION

The *S. cerevisiae* colonies from the plate



Is the crtYB gene still present in the different colored colonies?

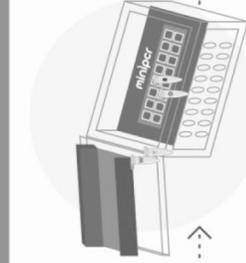
THE DETAILS



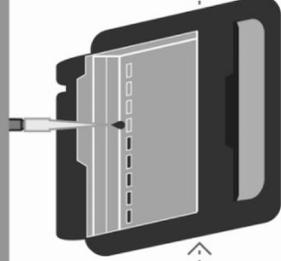
EXTRACT
 Extract DNA from white, yellow, orange, and red yeast

MIX
 Mix DNA, primers and Master Mix

AMPLIFY
 PCR to amplify the crtYB gene



VIZUALIZE
 Gel electrophoresis to visualize PCR products



INTERPRET
 Analyze and interpret data



CHECKLIST FOR KIT CONTENTS

- Empty Petri Dishes (1 sleeve, 100x15)
- Empty PCR Tubes (4 tubes per team)
- PCR tubes with Illumina Beads (5 per team)
- Sterile inoculating loops (4 per team)
-
- Melt & Pour YED Media (aka "YPD") (1 bottle, 350 mL)
- Vita-Yeast (1 stab)
- X-Tract buffer (200 ul per team minimum)
- PCR grade water
- DNA Primer mix
- Agarose gel electrophoresis (available as an add-on kit)

Unpacking your kit

Open the kit and remove the plasmid DNA (CRISPR+) (50 ul of 10 pg/ul TE)

- Store the YPD media on the shelf until ready to pour
- Plasmid DNA and primers to fridge (4°C) or freezer (-20°) if you have one
- X-Tract Buffer and Vita Yeast at room temperature or in fridge
- Store the PCR tubes with Illumina beads at room temperature in a cool, dry place

LAB PREPARATION

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 ~1/3 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.

Day of lab preparation

EACH STUDENT STATION WILL NEED:

- A streak of Golden Yeast. 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. Ideally there should be at least one isolated colony that is white, another that is yellow, a third that is red and a fourth that is orange.
- Four sterile loops or toothpicks
- An aliquot of nuclease free, PCR grade water
- An aliquot of PRIMER MIX (108 ul if they are setting up 5 reactions)
- An aliquot of positive control plasmid DNA (2 ul/ group, best left with instructor to give when needed)
- Empty PCR tubes for extracting the DNA from cells
- 6 lanes on a 2% agarose gel, loading dye and DNA ladder (available as an add-on kit)

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
- PCR machine
- Gel Electrophoresis Equipment



IN ADVANCE

- Melt YPD in microwave and pour plates
- Restreak "Golden Yeast" onto YPD to isolate single colonies of different colors**

DAY OF LAB

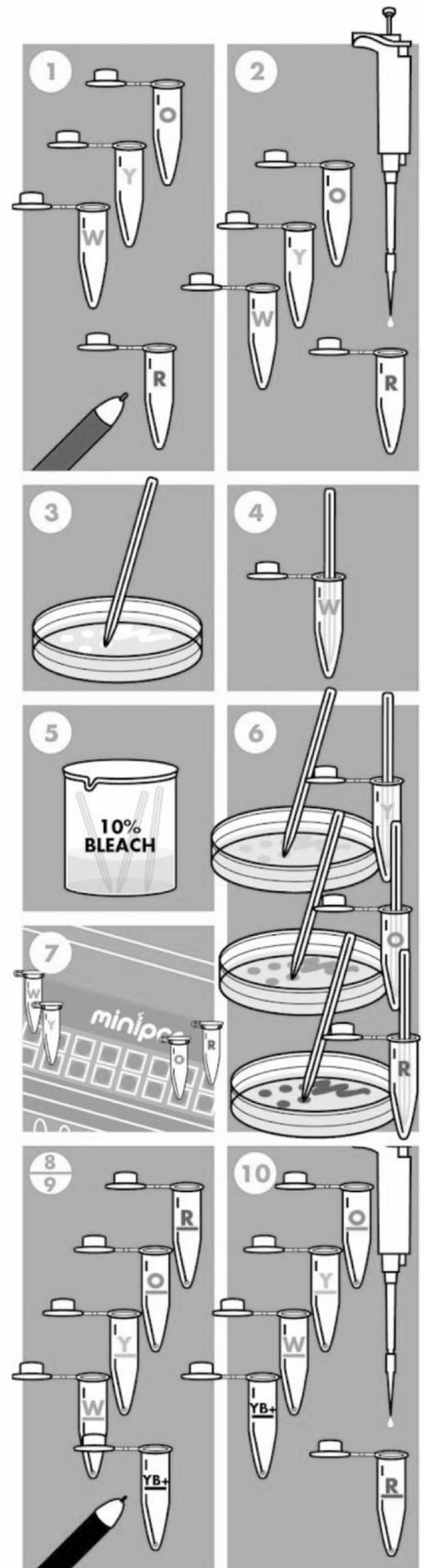
PART 1: DNA EXTRACTION

1. Label four 0.2 mL PCR tubes: W (for white), Y (for yellow), O (for orange), and R (for red)
2. Add 50 μ L of miniPCR X-tract buffer to each PCR tube
3. Using a sterile pipet tip, toothpick or inoculating loop, collect a small white colony.
4. Swirl the colony into the X-tract buffer in the PCR tube labeled "W" until there is a uniform suspension of cells.
5. Discard the pipet tip, toothpick or inoculating loop into the waste receptacle to be decontaminated.
6. Repeat steps 3-5 for the yellow, orange and red colonies, choosing a colony of the appropriate color each time.
7. Place the PCR tubes in the thermal cycler and heat them to 95° for 10 minutes.

PART 2: PCR

8. While the DNA is extracting, collect five Illumina PCR beads in 0.2 mL tubes.
9. Label the tubes: "YB+" "W" "Y" "O" "R"
10. To each tube, add 18 μ L of PRIMER MIX that includes forward and reverse primers for both the crtYB and actin1 genes.
11. Add 2 μ L of the crtYB+ DNA to the tube labelled YB+
12. Add 2 μ L of DNA extract from Part 1 to the corresponding tubes, e.g. DNA extracted from a white colony to the "W" tube.
13. Add 5 μ L of PCR-grade water to each tube, bringing the volume of all tubes to 25 μ L.

We recommend crossing off items on the following reaction checklist as the reagents get added.





SCIENCE OF GOLDEN BREAD



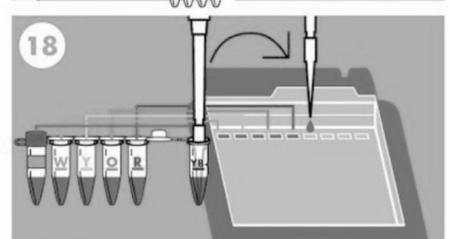
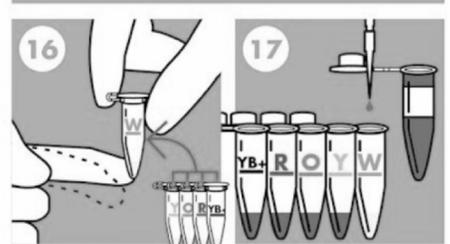
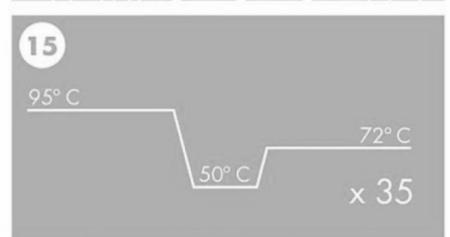
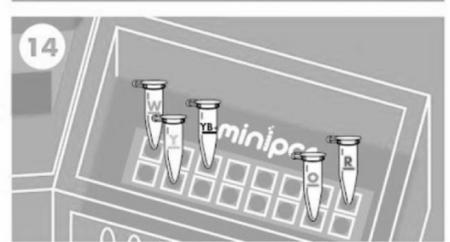
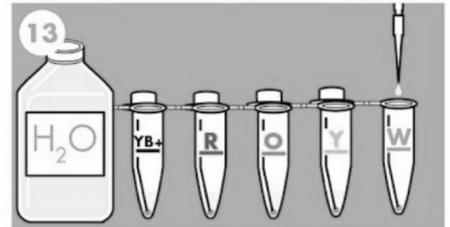
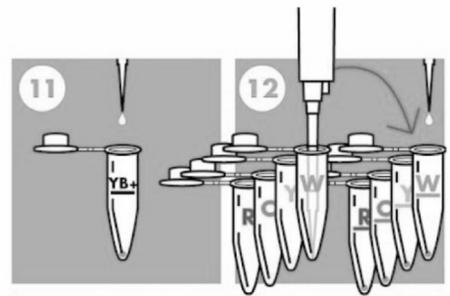
- Reaction checklist for each tube
- PCR bead
 - Primer Mix
 - Template DNA
 - crtYB+ positive control
 - from White yeast colony
 - from Yellow yeast colony
 - from Orange yeast colony
 - from Red yeast colony
 - PCR-grade water

14. Place the tubes in the thermal cycler.
15. Run PCR

Initial denaturation	95 degrees C, 1min
Denaturation	95 degrees C, 10sec
Annealing	50 degrees C, 10sec
Extension	72 degrees C, 15sec
Number of cycles	35
Final extension	72 degrees C, 1min

PART 3: ANALYSIS

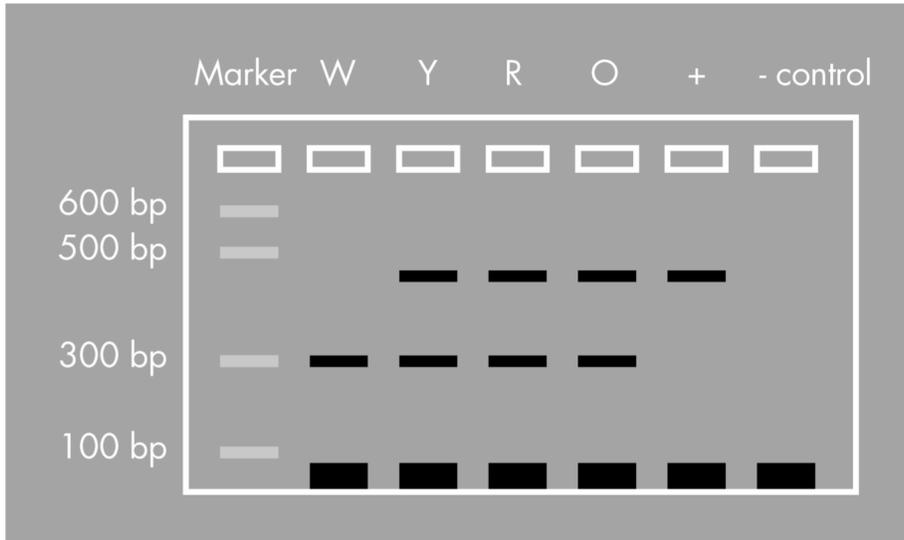
16. Remove PCR tubes from the machine and flick the contents to the bottom of each tube.
17. Add 5 µL of 6X loading dye to each PCR tube.
18. Load a 2% agarose gel with:
 - 12 µL of DNA Ladder
 - 12 µL of YB+ product
 - 12 µL of "W" PCR product
 - 12 µL of "Y" PCR product
 - 12 µL of "O" PCR product
 - 12 µL of "R" PCR product
19. Run gel for 10 minutes and photograph your result.



INTERPRETING THE RESULTS

What are the expected outcomes for the PCR products from the strains of various colors?

Sample data we have generated looks like:



The control reactions will strengthen the conclusions that can be drawn as well as troubleshoot any technical issues with the experiment:

NO TEMPLATE NEGATIVE CONTROL	crtYB + PLASMID POSITIVE CONTROL	EXPERIMENTAL
<ul style="list-style-type: none"> - Not specifically included in the protocol but easy to run if there are extra materials. - No band for either crtYB or actin suggests other PCR products are specific and not arising from contamination of reagents or equipment - Particularly heavy "primer smear" at the bottom of the gel is expected since none have been incorporated into products 	<ul style="list-style-type: none"> - DNA band from crtYB+ amplification is expected just under the 500 bp marker on the ladder. - No band is expected for the 300 bp actin product since the reaction only had crtYB+ plasmid DNA -This result allows you to conclude that the PCR reactions are working -A result different from this suggests a technical issue with the PCR, e.g. PCR beads not working, machine not cycling, primers either wrong or degraded 	<ul style="list-style-type: none"> - All the yeast have the gene for actin so are expected to generate a 300 bp product from PCR, no matter what colored colony was used. - The white colony could be missing the 500 bp crtYB product. This is consistent with the defect in b-carotene between the first and second step of the pathway. - The yellow, red, and orange colonies could have the 500 bp crtYB product since the activity of the enzyme is needed early in the biosynthetic pathway that leads to products of these colors. - The reason for the yellow and red colonies is not knowable from this experiment.

POST-LAB QUESTIONS

There were two kinds of positive controls you ran in this experiment. Describe them and in one sentence say what you can conclude if NO PRODUCT is seen in each case.

1. Plasmid DNA with the crtYB gene. No product from this reaction suggests something is not working with the PCR reagents or equipment
2. Actin primers in the yeast lysates. No product from this reaction suggests something about the lysates is causing the PCR to fail (assuming the other positive control is working).

If the PCR machine was malfunctioning and could only raise the temperature of the samples to 80°, what result would you expect for the crtYB plasmid positive control sample and why?

The positive control would not give rise to a band on the gel because the DNA in the PCR machine could not denature and would not be amplified

What can you conclude if the white colonies show a PCR product for the actin gene but no product for the crtYB gene?

The white colonies have lost the DNA to make the crtYB enzyme

How many bands do you expect PCR of the orange colony to generate?

Two

If you wanted to amplify a third gene in the biosynthetic pathway, what would you choose and what would you need to know to carry out that experiment?

To amplify either the crtE or the crtI genes, you would need to know their sequence to design primers. A well designed experiment would yield a product that is neither 500 nor 300 bp.

Can you think of other ways to investigate the unreliable performance of the engineered beta-carotene system?

Many possible answers but these could include: PCR of other genes, exploring changes in expression rather than the presence or absence of the genes, sequencing the genome, growing the yeast in different ways, or genetic approaches such as complementation (which can be done with the Engineering of Golden Yeast from BioBuilder).

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

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