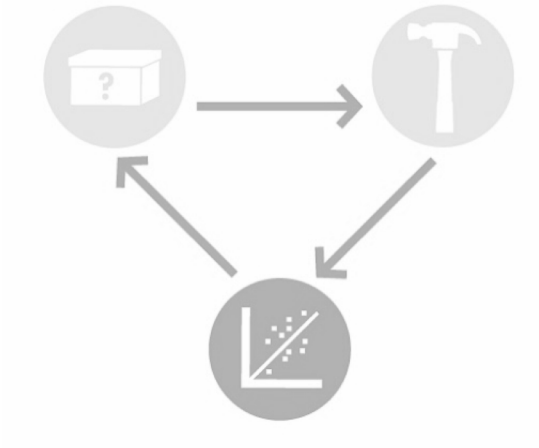
STUDENT MANUAL

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



Developed in collaboration with

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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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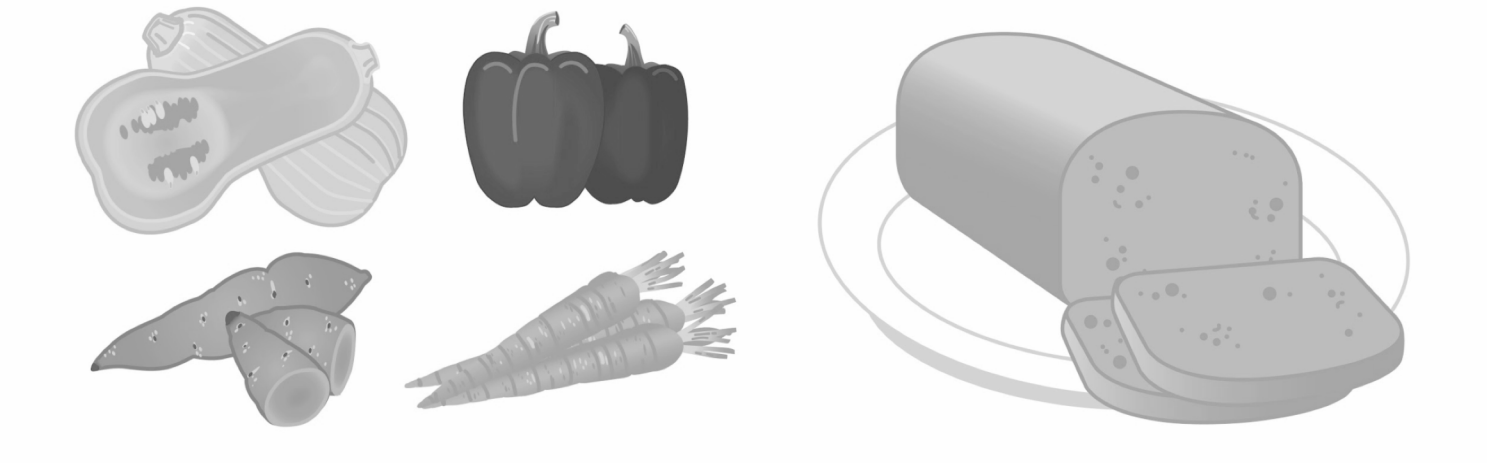
ABOUT GOLDEN YEAST

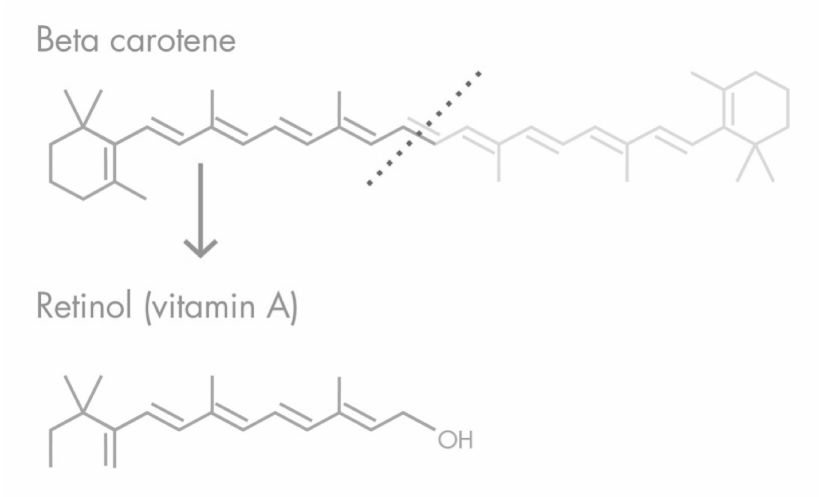
This lab focuses on a strain of baker's yeast that has been modified to produce **β**-carotene, a nutrient we naturally obtain from eating foods such as carrots, sweet potatoes, and broccoli. In the body, **β**-carotene is converted to vitamin A, which is crucial for vision, the immune system, and other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



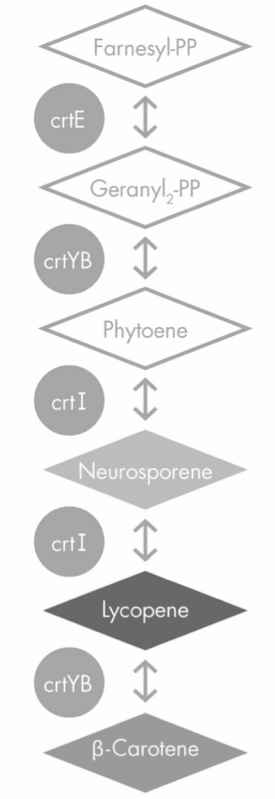
In some developing countries that struggle with malnutrition, vitamin A deficiency is a critical public-health issue. Researchers hope that an engineered strain of baker's yeast designed to generate **β**-carotene, like the one in this activity, could be used in bread to treat vitamin A deficiency. Such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



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.



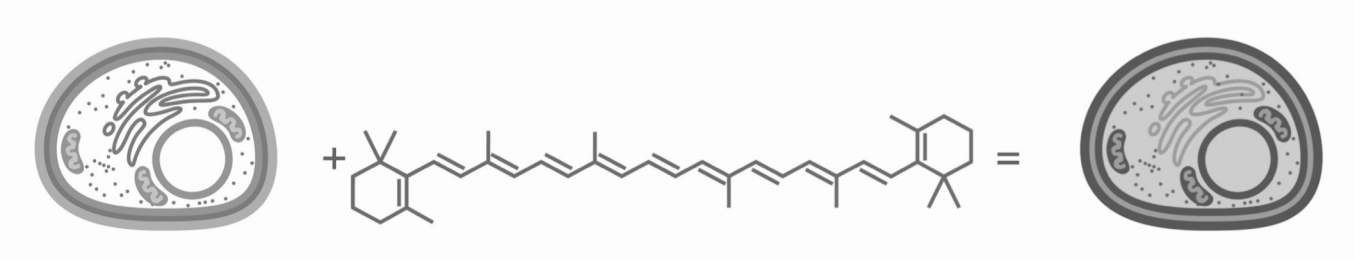
The baker's yeast, *S. cerevisiae,* naturally produces farnesyl diphosphate. The yeast also expresses an enzyme encoded by the BTS1 gene that converts the farnesyl diphosphate to geranylgeranyl diphosphate. Other yeast use a similar gene called crtE for this process. Converting geranylgeranyl diphosphate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

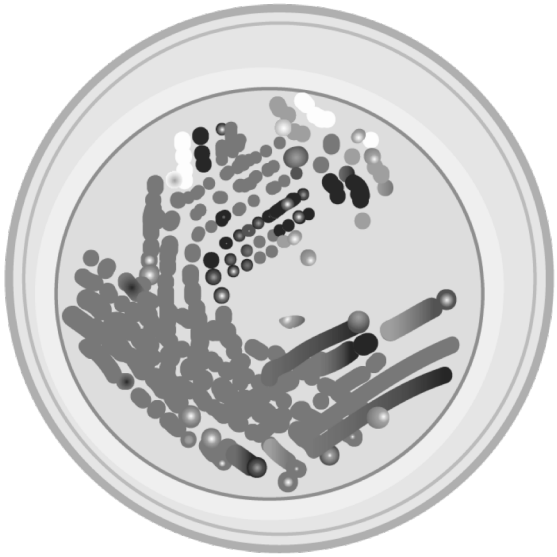
Nature has provided a simple way to detect pigments produced by this pathway, resulting in a convenient visual test for the design. The first three compounds in this pathway are colorless, but the last three are colored yellow, red, and orange, respectively. Unengineered yeast appear white, whereas yeast 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

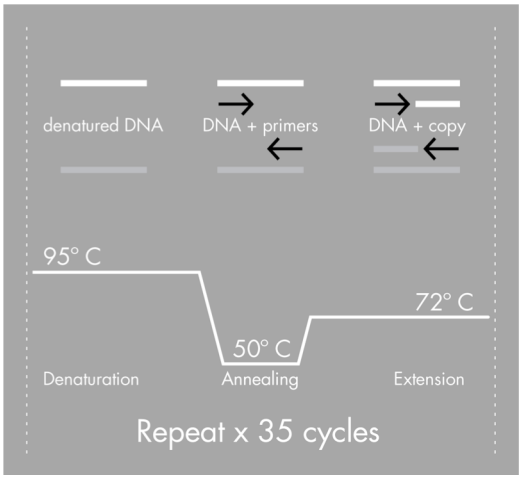
About PCR

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 ____.

By engineering baker's yeast with three genes from another yeast species, the baker's yeast should express ___, and so should appear _____.

An engineered yeast that appears white instead of orange might be missing the ____ gene.

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

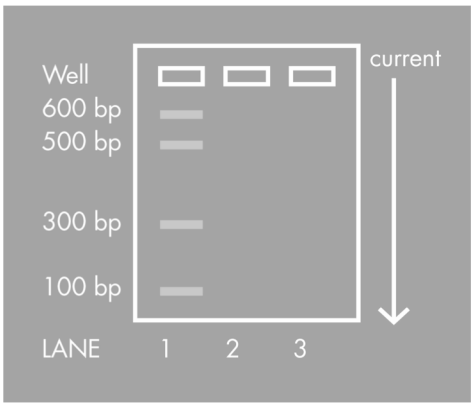
Polymerase Chain Reaction includes a _____ step that unpairs the _____, an _____ step that allows the _____ to bind the template, and an _____ step in which the DNA is copied by _____ .

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

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 crtYB PCR.

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

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 crtYB gene and the actin gene is amplified. Sketch in Lane 3 what you expect to see if ONLY the actin gene is amplified.



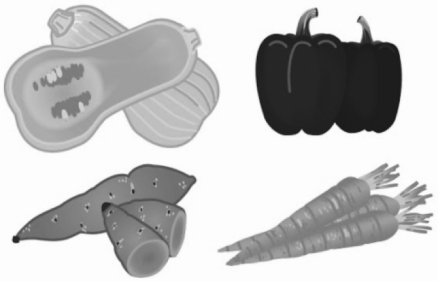
Which lane shows a result you might expect for a white colony?

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

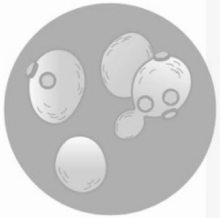
GOLDEN BREAD

BETA-CAROTENE IN YEAST

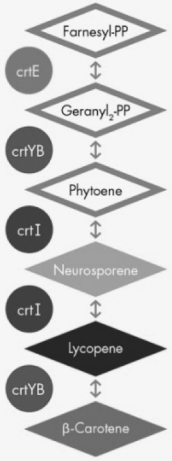
Many kinds of plants and fungi make beta-carotene naturally, but animals can't make their own from scratch. *Saccharomyces cerevisiae* can be engineered to produce beta-carotene.



S. cerevisiae
YEAST



THE BIOSYNTHETIC PATHWAY



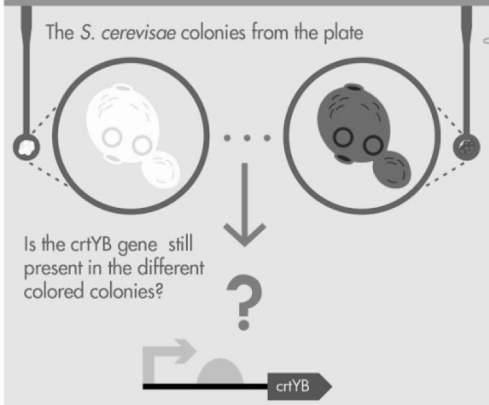
WHITE colonies may have lost one or more of the crt genes

YELLOW colonies may be accumulating neurosporene.

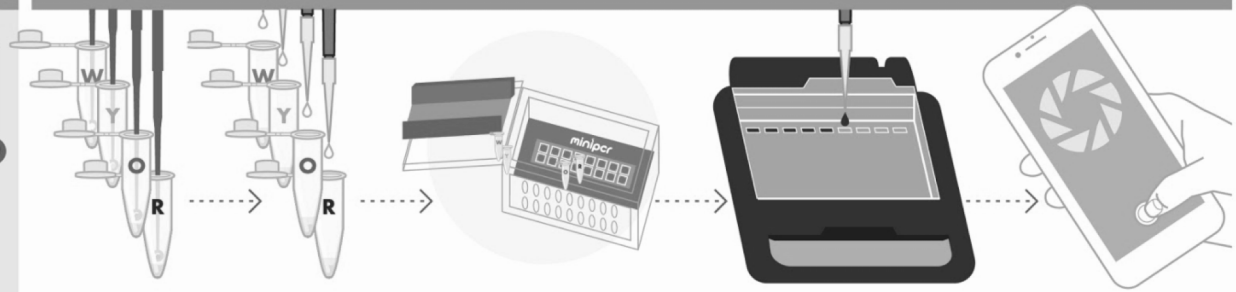
RED colonies may be accumulating lycopene.

ORANGE colonies produce beta carotene, as designed.

EXPERIMENTAL QUESTION



THE DETAILS



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

MIX
Mix DNA, primers and PCR Master Mix

AMPLIFY
PCR to amplify the ctrYB gene

VIZUALIZE
Gel electrophoresis to visualize PCR products

INTERPRET
Analyze and interpret data

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SCIENCE OF GOLDEN BREAD

IN ADVANCE

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

DAY OF LAB

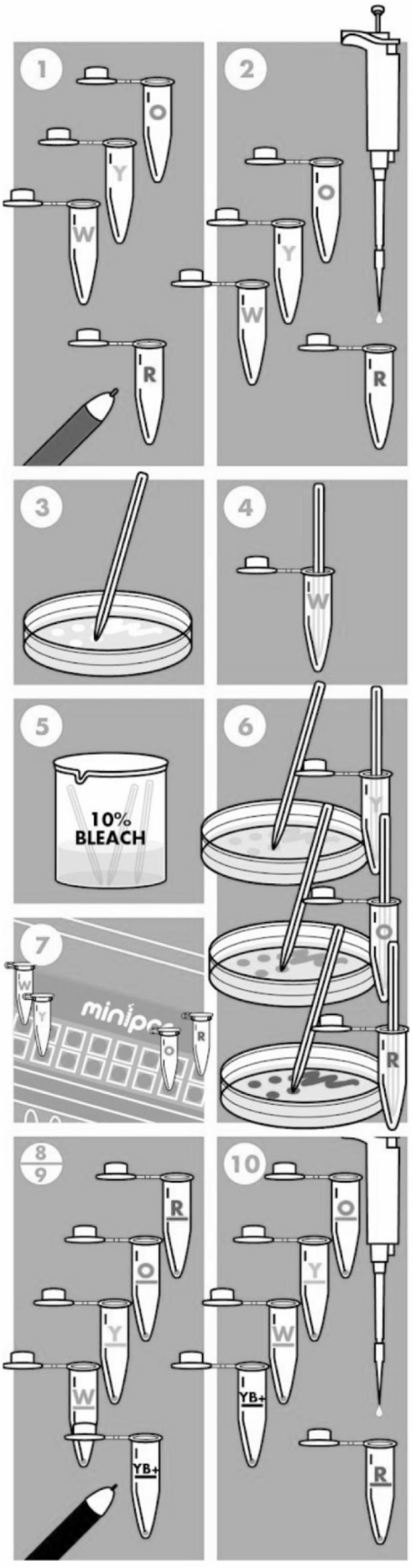
PART 1: DNA EXTRACTION

1. Label four 0.2 mL PCR tubes: W (for white), Y (for yellow), O (for orange), and R (for red)
2. Add 50 µL 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 µL of PRIMER MIX that includes forward and reverse primers for both the crtYB and actin1 genes.
11. Add 2 µL of the crtYB+ DNA to the tube labelled YB+
12. Add 2 µL 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 µL of PCR-grade water to each tube, bringing the volume of all tubes to 25 µL.

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



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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*

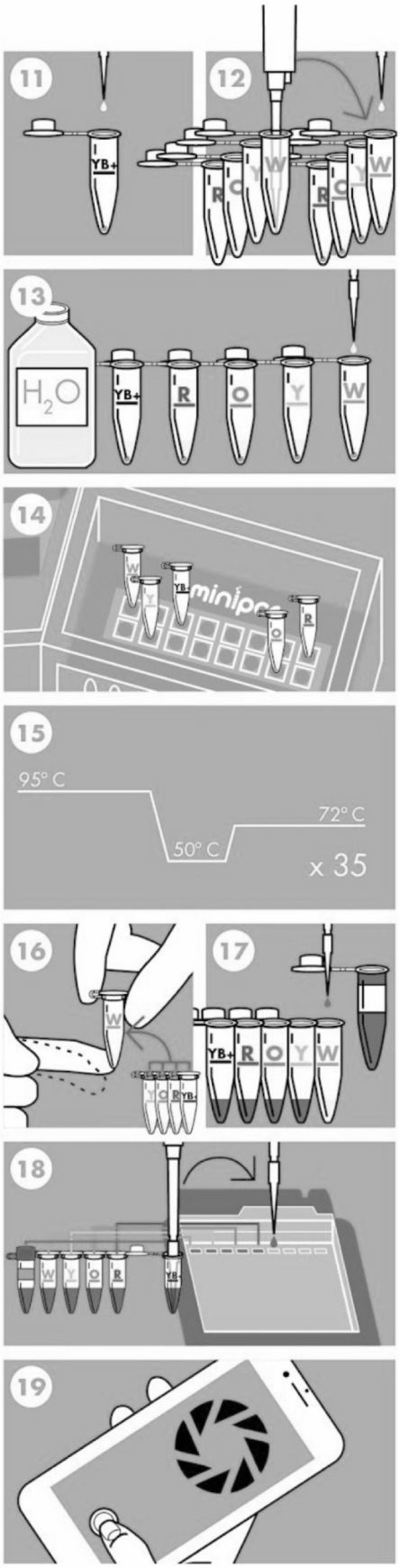
PART 3: ANALYSIS

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 µLl of "O" PCR product
    - 12 µL of "R" PCR product

19. Run gel for 10 minutes and photograph your result.



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** VIDEO OF PROCEDURE AVAILABLE ONLINE

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.

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?

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

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

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?

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

If you wanted to build a business around the health benefits of Golden Yeast, what would you need to do?

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

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

STUDENT MANUAL

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Developed in collaboration with

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