PLANT GENETIC ENGINEERING KIT

GENETIC ENGINEERING OF PLANTS

This experiment uses *Agrobacterium* to genetically modify the leaves of the plant by injecting DNA into the cell. The *Agrobacterium* has been modified to contain the genes *CYP76AD1, DODA,* and *Glucosyltransferase*. These three genes code for enzymes that create a reaction that converts tyrosine into betalain and causes a red color.

TABLE OF CONTENTS

Kit contents.....p.3

Growing your own plant.....p.4

Timeline.....p.5

Protocol.....p.7

This document is continuously being updated with tips and contains embedded video links. You can find the most up-to-date version here:

KIT CONTENTS

Non-perishables:

1 - 100-1000uL pipette
1 - Pipette tips
1 - Glass bottle
1 - LB Rifampicin/Spectinomycin agar
1 - Injection media concentrate
1 - 7 petri plate sleeve
1 - 10 nitrile gloves
10 - Inoculation loops
5 - Blunt syringes
4 - 50mL tubes
1 - 50mL tube for measuring
5 - 15mL tubes
1 - Sterile water
5 - Sprouting discs
1 - Bag of soil
1 - Pot
1 - Plastic tub for sprouting plants
1 - *Nicotiana tabacum* seeds

Perishables (store in freezer):

1 - *Nicotiana tabacum* plant (if selected)
2 - ***Agrobacterium*** with RUBY Plasmid

IF STARTING FROM SEEDS

1. Expand sprouting disc by placing them in water for 1 minute. Tear a hole in the top after its expanded.

2. To germinate seeds, poke seed into top of expanded sprouting disc. Put expanded disc with seed in a container with shallow water. Place near a light source. Monitor periodically to make sure there is always water in the tub. After approximately 10 days, your seeds should sprout.

3. When roots begin to emerge from the sides of the expanded sprouting discs, they are ready to be transplanted into the pot with soil. Cover the bottom of the pot with soil, place expanded sprouting disc in the center and fill in with soil. Do not cover leaves.

4. Let plants grow until a leaf is about the length of your index finger. Depending on growth conditions, plants can take 1-3 months to get to this point.

TIMELINE FOR EXPERIMENT

Preparation:

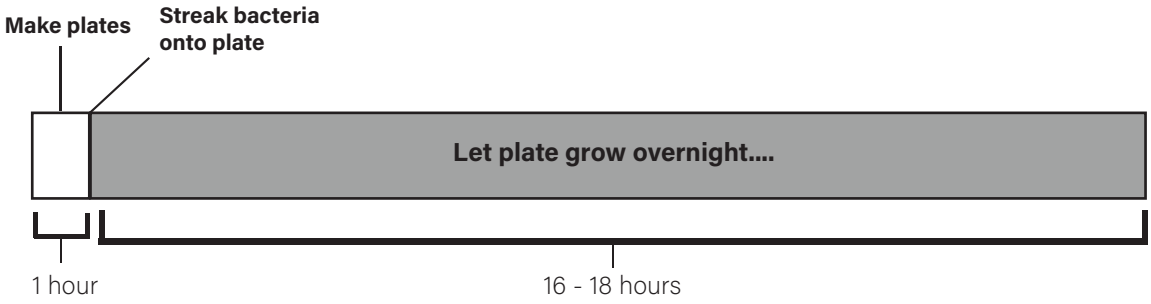
- **1 hour -** make agar plates. Set aside more time if this is your first time making plates.

- **1 min -** streak out *Agrobacterium*

Incubate & wait for growth:

- Grow plate at room temperature overnight, 16-18 hours is best

  **Note: the freshness of your *Agrobacterium* is critical. Make sure you can complete the protocol in the allotted time



Day of experiment:

- Dilute infiltration media and combine *Agrobacterium* ~ 5 min

- Inject into plant leaves ~ 15 min

  Successful transformations will generally take 2-7 days to express

Total timeline: Approximately 4-9 days

IMPORTANT NOTES

Proper pipette technique is important! Please read through the pipetting tutorial before you start by scanning the QR code below or visiting this link: https://goo.gl/nrA8hT

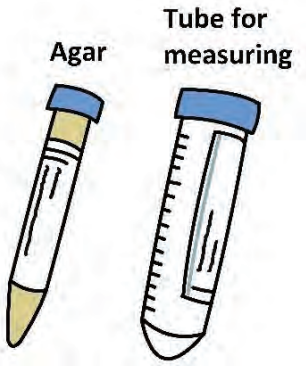
This is 1000µL (1mL) of liquid in a pipette tip.

The liquid should go up to the demarcation at the end of the tip, closest to the pipette. Make sure you can accurately draw up 1000µL before you proceed.

Pipette tutorial:

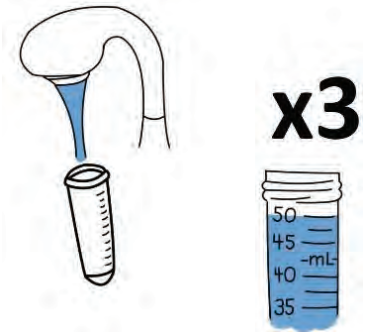
Making plates:

You can find a step by step walk-through for making plates with photos at: https://goo.g|/7zpA1

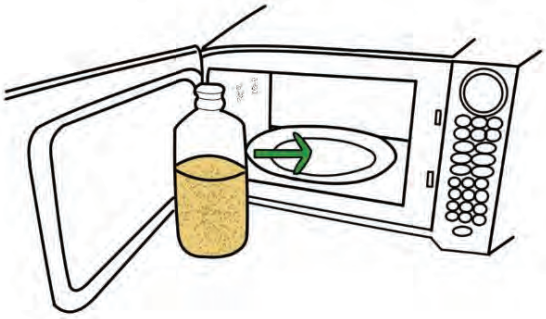


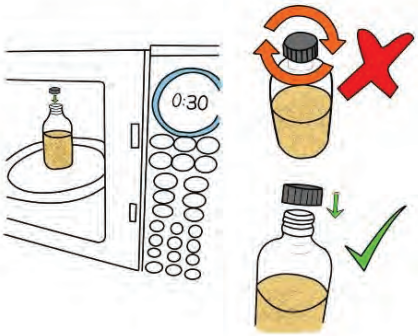
1. Take a tube labeled LB Rif/Spec Agar and dump its contents into the 250mL glass bottle

2. Using the 50mL conical tube labeled "Tube for Measuring", measure and add 150 mL of water to the glass bottle. **Shake vigorously.**



3. Heat the agar to dissolve it. Put the bottle in the microwave for a few seconds at a time, being careful not to let the bottle boil over. **DO NOT SCREW THE LID DOWN TIGHT!** (Just place it on top)

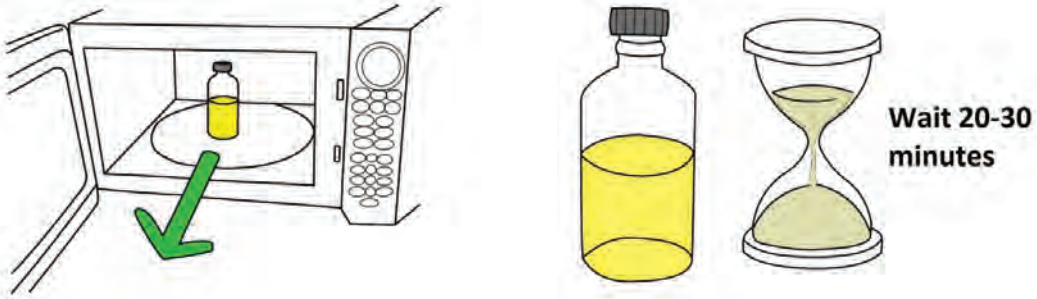




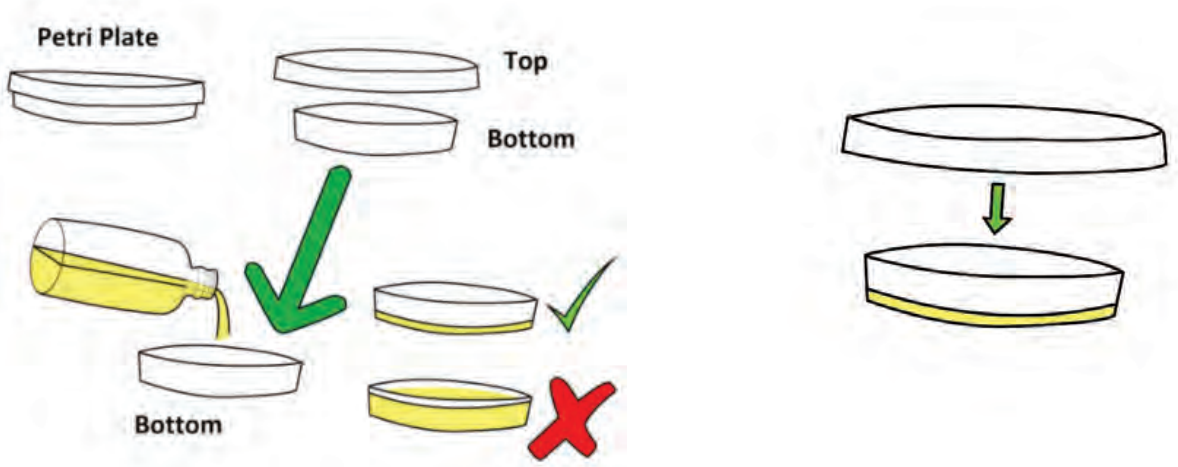
4. If you see the liquid start to boil, stop the microwave and let the agar cool for a few seconds. Keep going until the agar is translucent and there are no visible suspended particles.

Boiling

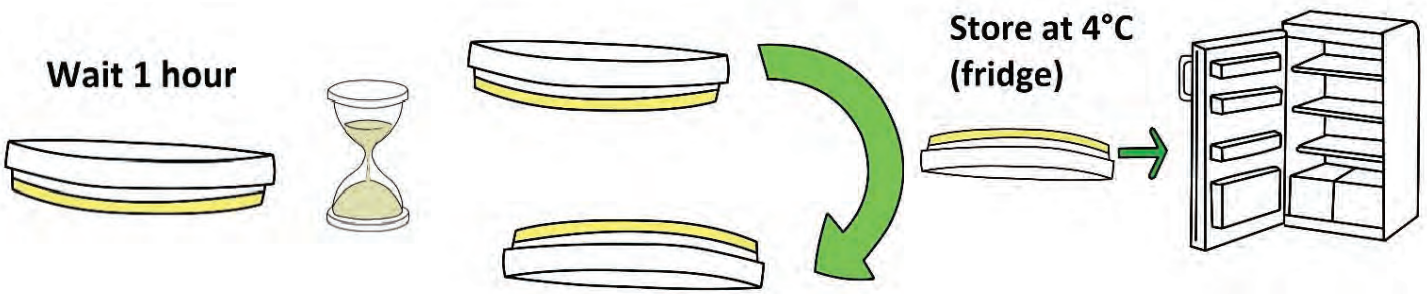
5. You will know it's done when the liquid looks clear yellow after microwaving. Take the bottle out (caution contents hot) and let it cool until you are able to touch it without much discomfort. It will take 20-30 minutes to cool.



6. While the bottle remains somewhat warm, pour the plates. One at a time, remove the lid of 7 plates and pour just enough of the LB Rif/Spec agar from the bottle to cover the bottom half of the plate. Put the lid back on. It will solidify when it cools.

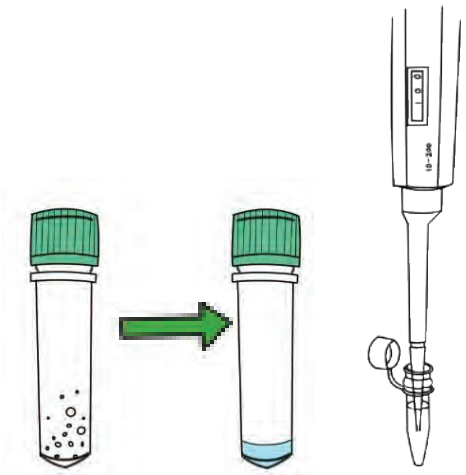


7. Let the plates cool for at least one hour before use. You can cool faster by putting them in the fridge but don't freeze them. If possible let the plates sit out for a couple of hours or overnight to let the condensation evaporate. Then store in your fridge at 4 °C, flipping the plates upside down so condensation doesn't drip on the plates.



Growing agrobacterium:

1. Take the freeze dried *Agrobacterium* tube. Add 100µL of sterile water to the tube and shake for one minute to ensure the bacteria is dissolved. Then add 50µL of the bacteria to a new LB Rif/Spec plate. Your 100µL - 1000µL pipette can dial down to 50µL— you do no need another pipete.



2. Using an inoculation loop, spread bacteria. See the following link or scan the QR code for a walk-through of how to streak out bacteria: https://goo.gl/GR8IOf

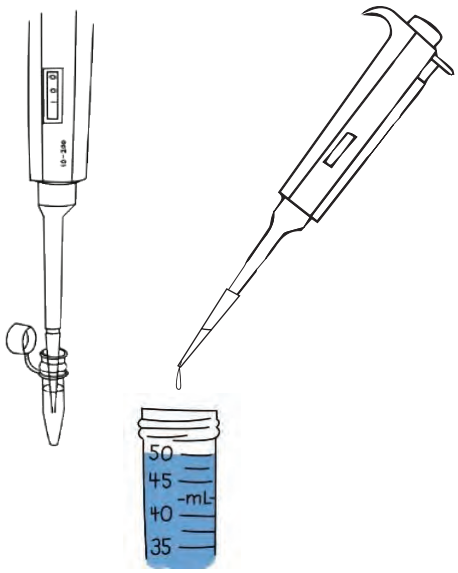
3. Put the lid on and let bacteria grow overnight for ~16-18 hours. Do not grow at temperatures exceeding 30°C.

Notice: Bacteria can take longer to grow depending on the temperature of your room. Colonies are ready for transformation when they look this picture:

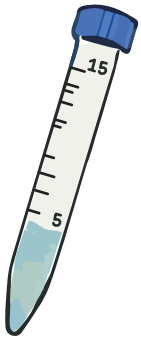
Transformation:

1. Prepare injection media by adding water.

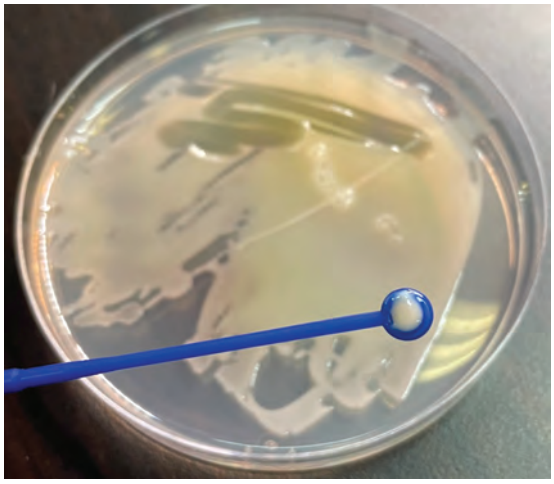
Grab a 50mL tube and fill it to the 50mL line with tap water. Then, pipette 1000µL of media into the 50mL. Screw on cap, and gently shake the media.

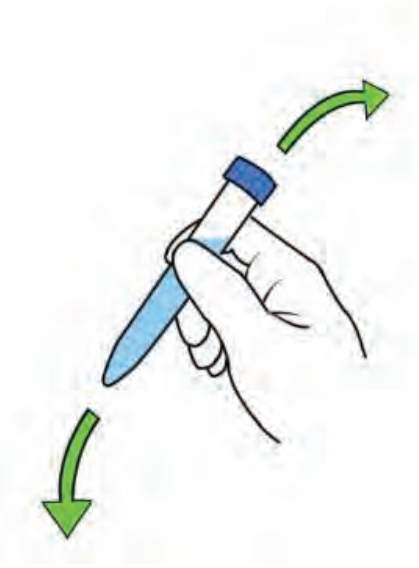


2. Add 4mL (4000ul) of diluted injection media to a 15mL tube. Store the remainder in the fridge.



3. Using an incoculation loop, gently scrape bacteria off a fresh plate until the loop is filled. Place loop into the tube with 4mL infiltration media and swirl to release bacteria. Shake or gently pipette up & down to break up any clumps.





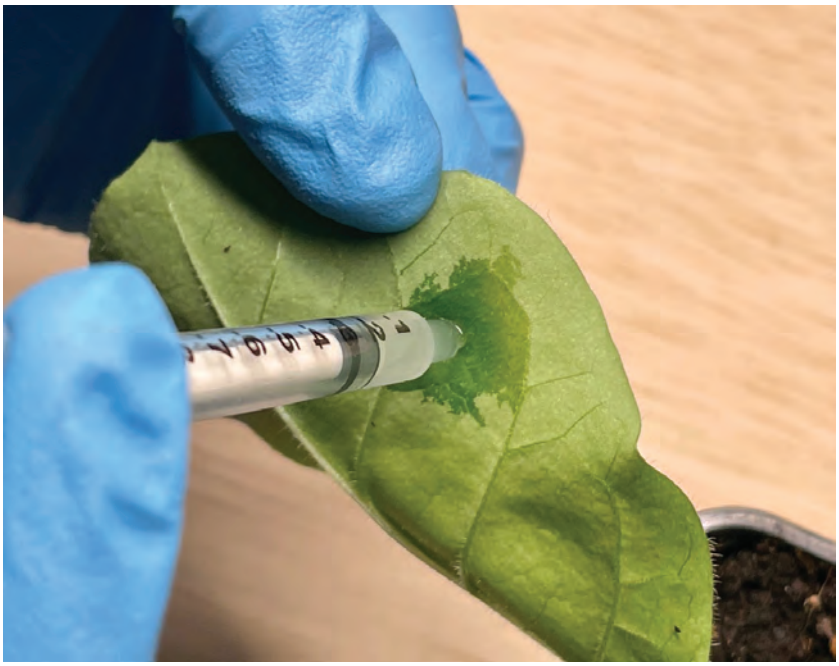
4. Use a 1mL blunt syringe to draw up 0.5mL of the media with bacteria. Push out any air from the syringe and place the end firmly against the underside of a leaf on a flat smooth spot avoiding veins.

5. Place a finger gently but firmly against the other side to support the leaf.

Very slowly depress plunger to push the media & bacteria inside the leaf.

If the media leaks around the end push a bit harder and make sure the end is flush against the leaf. You should see the liquid saturate **inside** leaf tissue as is spreads.

This may take practice, scan the QR code or follow this link for a video demonstration.



Repeat the injection as many times as you want to create color as you see fit.

7. If the experiment was sucessful, the places you injected the *Agrobacterium* into leaf should have a dark red spot.

MORE KITS TO TRY

DIY Bacterial Gene Engineering CRISPR Kit

https://www.the-odin.com/diy-crispr-kit/

Bioengineering 101 Kit

https://www.the-odin.com/bioe101/

Home Lab Kit

https://www.the-odin.com/genetic-engineering-home-lab-kit/