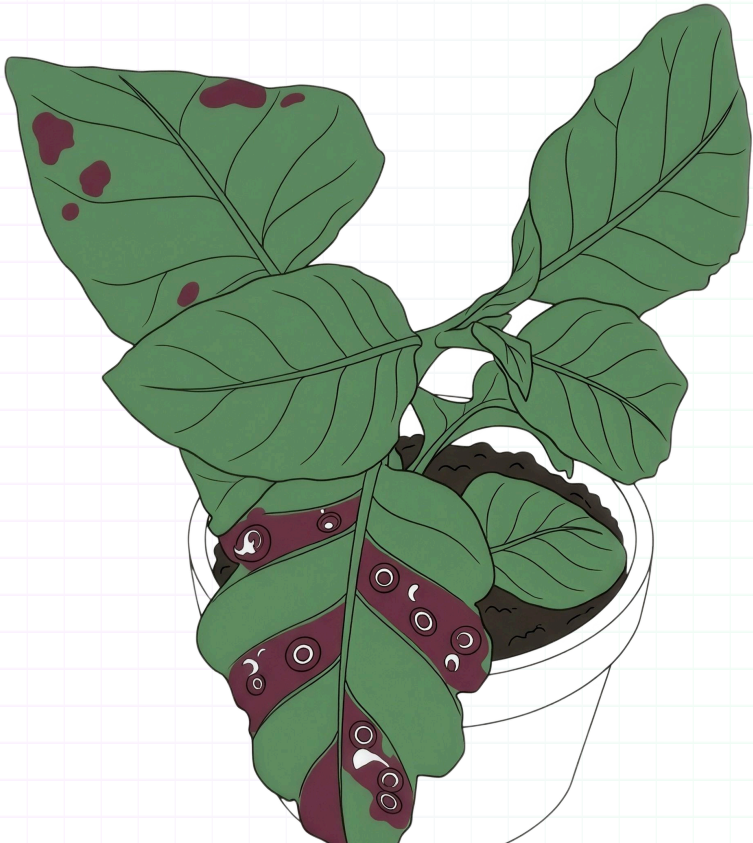




PROTOCOL

Plant Pigment Genetic Engineering Kit

Turn Tobacco Leaves Red with Agrobacterium.



What You'll Do

In this experiment, you'll use a bacterium called *Agrobacterium* to deliver new genes directly into plant cells.

The genes you'll insert — CYP76AD1, DODA, and Glucosyltransferase — work together to convert the amino acid tyrosine into betalain, a bright red pigment found in beets and other plants.

If the transformation is successful, the leaf tissue where you injected will turn a dark red or purple color within 3–7 days.

Safety Notes



Agrobacterium is generally regarded as safe but should be handled as a BSL-1 organism.



Wear gloves at all times when handling Agrobacterium cultures.



Do not eat any part of the plant during or after the experiment.



Wash hands after the experiment.



To dispose of bacteria: pour 5% bleach on any plates, wait 10 minutes, then trash.



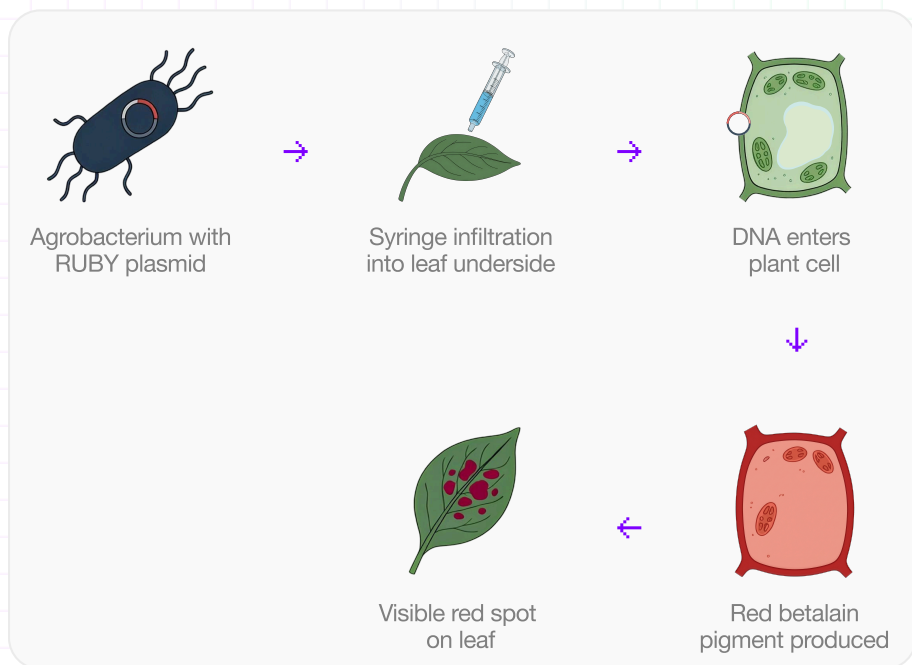
Keep your work area clean. Wipe surfaces with rubbing alcohol before you start.

How It Works

Agrobacterium tumefaciens is a naturally occurring soil bacterium that evolved to inject DNA into plant cells. It's essentially a biological gene delivery system.

Scientists have repurposed this ability for plant genetic engineering by loading *Agrobacterium* with a plasmid (called a binary or RUBY plasmid) carrying the genes we want to express.

In this kit, the RUBY plasmid carries the three-gene betalain pathway. When injected into a leaf, the *Agrobacterium* delivers this DNA into plant cells. The cells then express the enzymes, which produce betalain, a red pigment visible to the naked eye.

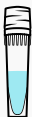


What's in the Kit

1. Equipment

 250mL glass bottle (for melting agar) 1x	 50mL tube (for measuring water) 1x	 50mL tube 1x
 blunt-tipped syringes (1mL) 5x	 inoculation loops 10x	 pairs of nitrile gloves 5x
 pasteur pipettes 5x	 petri plates 7x	 15mL tube 5x
 sprouting disks 5x	 bag of soil + pot 1x	 plastic sprouting tub 1x

2. Perishables 🌡️ (store in fridge on arrival)



1x

sterile water
tube



1x

LB Rif/Spec
powder tube



5x

injection media
concentrate



1x

nicotiana tabacum plant
(pre-grown, if selected
options)



1x

nicotiana tabacum seeds
(if growing from seeds)

3. Perishables ❄️ (store in freezer on arrival)



2x

tubes of *Agrobacterium*
with RUBY Plasmid

Two Ways to Start

OPTION: A TIME: Faster

Use the Pre-Grown Plant

If your kit included a pre-grown *Nicotiana tabacum* plant, you can skip straight to the *Agrobacterium* growth steps once the plant stabilizes. Look for at least one leaf about the length of your index finger.

OPTION: B TIME: 1–3 months

Grow from Seed

- 1 Soak a sprouting disc in water for 1 minute until expanded. Tear a small hole in the top. ☐
- 2 Poke a seed into the hole in the top of the disc. ☐
- 3 Place the disc in the plastic tub with a small amount of water at the bottom. Set near a light source. ☐
- 4 Check every few days and top up water as needed. Seeds sprout in approximately 10 days. ☐
- 5 When roots emerge from the sides of the sprouting disc, transplant into the pot with soil. Place the disc in the center, fill around it with soil, and do not cover any leaves. ☐
- 6 Let the plant grow until at least one leaf is approximately the length of your index finger. This typically takes 1–3 months. ☐



Timeline at a Glance



DAY 1, AFTERNOON (~1 HOUR)

Make agar plates. Streak *Agrobacterium* onto a plate.



DAY 1, EVENING

Let bacteria grow overnight (16-18 hours).



DAY 2, MORNING (~20 MINUTES)

Dilute media. Combine with *Agrobacterium*. Inject into leaves.



DAY 3-7

Check leaves for red spots.

Warning:



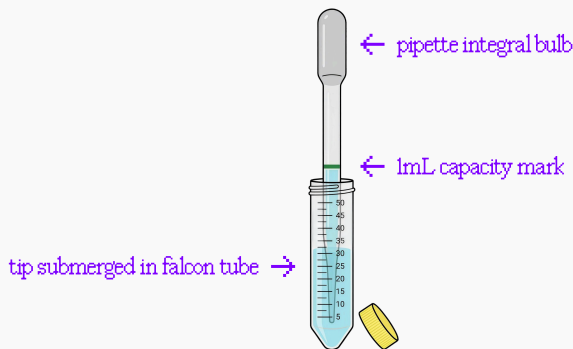
The freshness of your *Agrobacterium* colony is critical.



Make sure you can complete the injection on the day after streaking — do not let the plate sit more than 24 hours before use

Before You Start: Pipette Technique

This kit uses Pasteur pipettes instead of a mechanical pipette. Proper technique ensures you draw up exactly 1mL (1000 μ L) without air bubbles.



- 1 Squeeze the bulb at the top of the pipette to push all air out. ☐
- 2 While still squeezed, submerge the tip 2–3mm in liquid. ☐
- 3 Slowly release the bulb to draw up liquid until it reaches the 1mL mark. ☐
- 4 Check for bubbles. If any are visible, expel and repeat. ☐
- 5 Practice with water until you can consistently draw up exactly 1mL before proceeding. ☐

60-Second Video Guides

Before starting your experiment, check out these fast 60-second video protocols.

They walk you through every step of making agar plates, from melting and pouring to cooling and storage, so you can avoid common mistakes and set up your lab work for success.



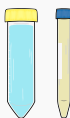
60 Second Video Protocols
“How to Make Agar Plates”.

STEP: 1 TIME: ~1h

Make Agar Plates

Agar plates are solid nutrient surfaces for bacteria to grow on.

Agrobacterium is grown on LB agar containing two antibiotics: Rifampicin and Spectinomycin. These select for Agrobacterium that carries the RUBY plasmid.



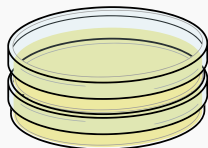
- 1 Pour the contents of the LB Rif/Spec Agar tube into the 250mL glass bottle. ☐
- 2 Use the 50mL measuring tube to add 150mL of tap water to the bottle. Shake vigorously. ☐
- 3 Loosely place the lid on top – do **NOT** screw it down tight. Place the bottle in the microwave. ☐
- 4 Microwave in 20–30 second bursts, swirling between each burst. Stop if you see boiling. ☐
- 5 Continue until the liquid looks completely clear and yellow with no visible grains or cloudiness. Total time: about 2–3 minutes of microwaving. ☐
- 6 Remove carefully (bottle is hot). Let cool 20–30 minutes until you can hold it comfortably. ☐
- 7 While still warm, pour into 7 petri plates: lift each lid, pour just enough to cover the bottom half, replace the lid. Don't move them until solid. ☐



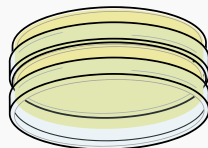
8 Let cool at least 1 hour.



9 Store plates upside down in the fridge (4°C). They'll last 1–2 months.



Petri plate columns with labels:
“LB agar”

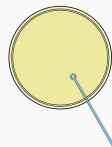


Correct upside-down storage in fridge

STEP: 2 TIME: ~10min

Prepare the Bacteria

Your *A. tumefaciens* arrives freeze-dried. You'll rehydrate it and streak it onto a plain LB agar plate to grow a fresh colony overnight.



10 Add 4 drops (~100 μ L) of sterile water to the freeze-dried bacteria tube using the disposable transfer pipette.



11 Shake for 1 minute until the bacteria are fully dissolved.



12 Pipette all 100 μ L of the bacteria suspension onto a LB Rif/Spec agar plate.



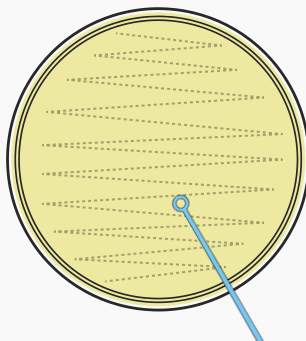
13

Use an inoculation loop to gently streak the bacteria across the plate in a zigzag pattern.



14

Place the lid back on and let the plate grow at room temperature overnight (16–18 hours). **DO NOT REFRIGERATE**. Colonies are ready to use when they appear as visible white or cream-colored dots spread across the plate.



Zigzag pattern across plate

Warning:



If your room is cold (below 18°C), bacteria may take longer – up to 24 hours – to grow.



Do not grow bacteria above 28°C. It damages the *Agrobacterium* and the experiment will fail.



If agar solidifies in the bottle before you finish, briefly re-microwave to liquefy it again.

STEP: 3 TIME: ~20m

Transfection:

Adding the RUBY Genes

This is the main event. You'll mix *Agrobacterium* with the injection media (which allows *Agrobacterium* to enter the plant cells) and inject transfection media into the leaves using blunt-tipped syringe.

3.1 Prepare the Injection Media

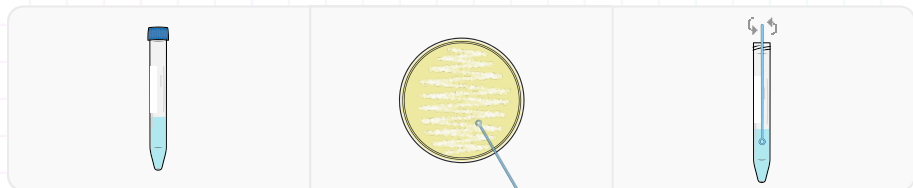
15 Fill a 50mL tube to the 50mL line with tap water. Using your Pasteur pipette, draw up 1000 μ L (1mL) of injection media concentrate and add it to the 50mL tube.



16 Screw on the cap and gently shake to mix. Transfer 4mL of the diluted media into a 15mL tube. Store the rest in the fridge.



3.2 Add Bacteria to the Media



17 Using a fresh inoculation loop, gently scrape bacteria from your overnight plate until the loop is visibly full.



18 Place the loop into the 15mL tube with 4mL of injection media. Swirl to release the bacteria. Shake the tube or gently pipette up and down to break up any clumps. The solution should look evenly cloudy.



3.3 Inject the Leaves



Read this entire section before starting.
The injection technique takes practice.

19

Draw 0.5mL of the *Agrobacterium* suspension into a blunt syringe. Push out any air from the syringe.



20

Turn the leaf over to expose the underside. Find a flat, smooth area between the veins. Press the open end of the syringe firmly against the underside of the leaf.



21

Place one finger on the opposite side of the leaf to create a seal and support the tissue.



22

Very slowly press the plunger to push the liquid into the leaf. You should see the tissue darken as the liquid spreads inside — this means it's working.



23

If the liquid leaks around the edge, press slightly harder and reposition the syringe so the end is flush with the leaf.



24

Repeat in as many spots as you want, more injections means more colored area if the experiment succeeds.



Tip:



Thicker leaves are easier to inject. If liquid keeps leaking, try a younger, slightly thicker leaf.



Avoid injecting over large veins. Flat tissue between veins works best.

STEP: 4 TIME: 3-7d

Check Your Results

Successful transformations will show dark red or purple patches in the injected areas within 3–7 days. The color comes from betalain pigment being produced by the plant cells that took up the RUBY gene.

- 25 Leave the plant in normal light at room temperature. Water as usual. ☐
- 26 Check the injected areas daily starting on day 3. ☐
- 27 Successful spots will appear as dark red or purple patches. The rest of the leaf will stay green. ☐



Leaf 2 days after injection
(no visible change)



Leaf 5 days after injection
(dark red spots visible at injection sites)

Success:



Dark red or purple spots at injection sites within 3–7 days.



No color change:

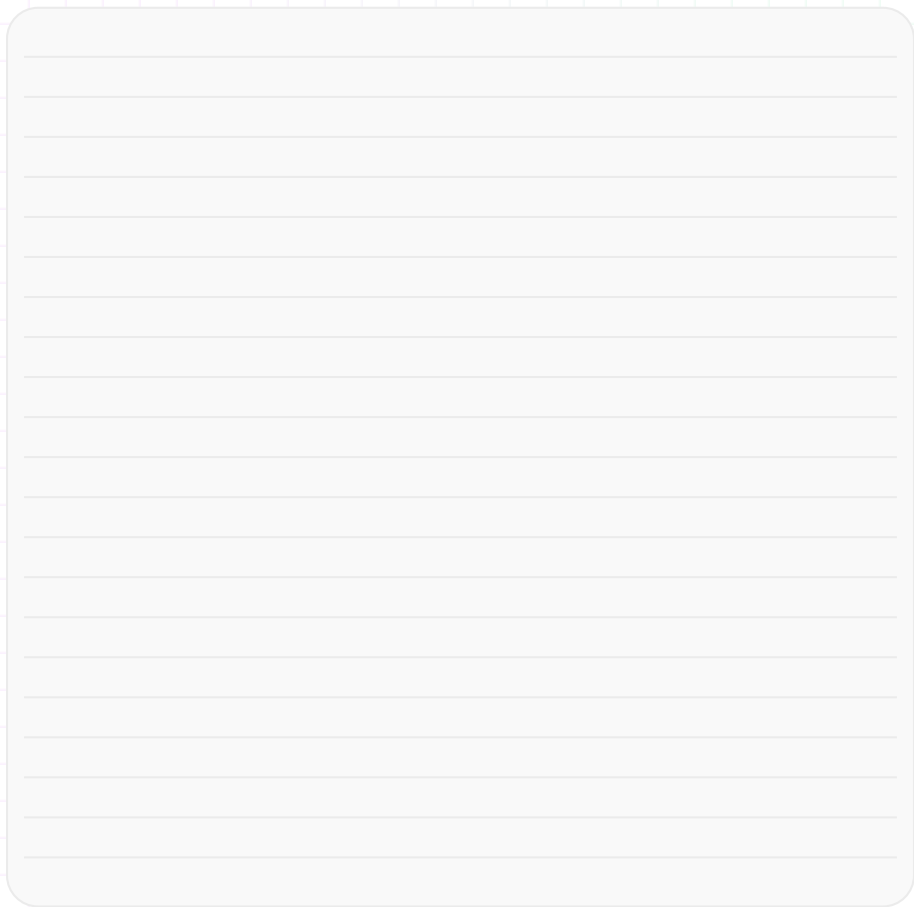
The injection may not have penetrated the tissue properly. Repeat on fresh leaves.



Pale color:

Try injecting more of the suspension into each spot.

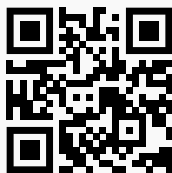
Record Your Results Here

A large, light gray rectangular box with rounded corners, containing horizontal lines for writing. The box is intended for recording results.



This Is Just the Beginning

Explore more experiments at the-odin.com



Need Help?

Contact us at odin@the-odin.com