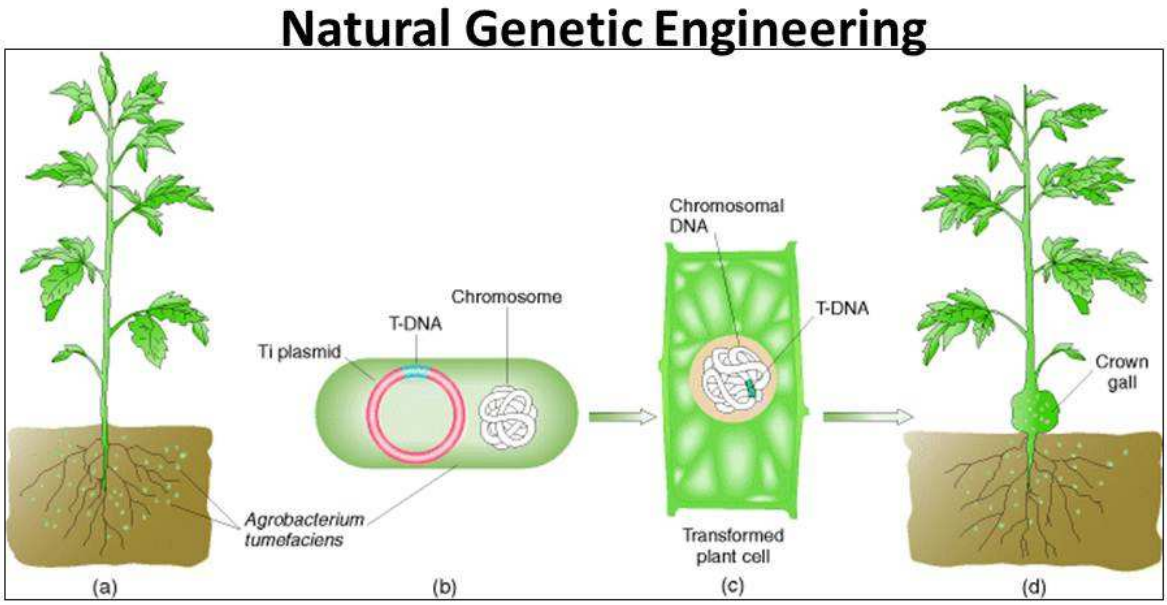
Inoculation of Plants to Observe Tumors (Galls) Due to *Agrobacterium tumefaciens*

The technique described below is adapted from the *Agrobacterium* section (authored by L.W. Moore, H. Bouzar and T. Burr) of the *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 3rd edition, edited by N.W. Schaad, J.B. Jones and W. Chun and published by APS Press, St. Paul, MN. in 2001

For background information, see https://en.wikipedia.org/wiki/Agrobacterium_tumefaciens

Quick overview:
The bacterium *Agrobacterium tumefaciens* is a plant pathogen that causes tumors or galls to form on certain plant species. The bacterium does this because it contains a plasmid containing a specific piece of DNA, called T-DNA, that will be inserted into the plant's DNA. The T-DNA then forces the plant cells to produce a deformed growth that is called a gall. See the cartoon below. This is why the bacterium is considered the "natural genetic engineer". Scientists have taken advantage of this natural characteristic to transform plants with specific foreign DNA to yield new plant characteristics, often referred to as genetic engineering.



(a) *Agrobacterium tumefaciens* cells in the soil come in contact with the plant roots.

(b) Individual cell of the bacterium. The reddish circle is a plasmid (extrachromosal DNA) and contains a specific piece of DNA (in blue-green, the T-DNA) that will be inserted into the plant's DNA

(c) T-DNA from the bacterium inserted into the chromosomal DNA of the plant cell.

(d) The T-DNA forces the plant cells to produce the deformed growth.

Items needed for the exercise are listed below:

- Pathogenic strain of *Agrobacterium tumefaciens*
- Potato dextrose agar plate or tryptic soy agar plate
- Small bottle of purified or distilled water
- Sterile blade for dissecting scalpel or disposable sterile scalpel
- Sterile test tubes with caps or sterile vials with caps; the size is not super critical, but it is suggested to use a tube or vial that holds less than 10 ml
- Round, wooden toothpicks
- Tomato or sunflower plants – minimum of 6 plants
- Parafilm® or clear plastic wrap that sticks to itself (example: Saran™ Wrap)
- Gloves
- Safety goggles
- Self-closing plastic bag (to collect used toothpicks)

To obtain a pathogenic *Agrobacterium tumefaciens* strain, contact the Plant Pathology Department at the land-grant university in your state. As long as the pathogen is being shipped within the same state and NOT across state lines, you will probably not need a USDA permit. This link provides a list: http://www.apsnet.org/members/directories/Pages/PPPrograms.aspx. You may also purchase a culture of *Agrobacterium tumefaciens* from a laboratory supply company, such as Carolina Biological Supply, but this will normally require obtaining a USDA permit to move plant pests.

The best plants to use for stem inoculation are ones with sturdy stems. Tomato and sunflower are good choices. If buying tomato plants in the garden shop, select plants with thick stems and select all the same cultivar. Both tomato plants and sunflowers can be easily grown from seeds or small seedlings. Sturdy stems are needed as the student will be poking the stem with a toothpick.

Wounding is required for infection of the plant by the bacterium to occur. This is the purpose of the toothpicks. It is not necessary to sterilize the toothpicks prior to inoculation – after all, they are supposed to be used in your mouth! Look for round, wooden toothpicks in closed plastic containers that allow you to obtain just one toothpick at a time. This will help to keep the toothpicks clean.

If tomato plants are used and you keep the plants long enough for them to fruit, it is acceptable to eat the fruit (an added bonus!), as the fruit is pathogen free.

You will need a minimum of 6 plants. Four plants should be inoculated with the bacterial pathogen to provide 4 replicates. Two plants will serve as non-inoculated controls for comparison with the inoculated plants.

Steps:

1. Streak the *Agrobacterium tumefaciens* strain on the potato dextrose agar plate. Incubate at room temperature for 5 to 7 days.

2. Add water to the sterile tube or vial. The size of tube and amount of water is not critical – use whatever you have on hand or is cheapest to obtain. Scrape small amount of the bacterial growth off the plate with the scalpel blade and add to the sterile tube/vial containing the sterile water. Shake very well to disperse the bacterial cells in the water. The water should look slightly cloudy. The goal is to have at least 1 x 10^6 bacterial cells per ml of water. Start with a small amount of bacterial growth, as you can always add more bacteria if needed.

3. Dip a toothpick into this bacterial suspension and insert the dipped end of the toothpick into the plant stem BUT just a mm or two into the stem. Do not insert toothpick all the way through the stem! It is best to carefully hold the plant stem as you do this, so as not to break the stem. This step is called "inoculation" of the plant with the pathogen. Place the used toothpick into the plastic bag. For the 2 control plants, dip a clean toothpick into sterile water and then insert a mm or two into the stem.

4. Wrap the pricked portion of the stem with a small piece of Parafilm® or plastic wrap to keep the area from drying out. Even the control plants should be wrapped to fully mimic the inoculated plants.

5. It is acceptable to inoculate the stem in two places, but use a clean toothpick to dip into the bacterial suspension. Place the inoculation points at least 3 to 4 inches apart on the stem. It is suggested to inoculate the stem about 2 inches above the soil line of the potting mix. If a second inoculation point is made, it should be at least 3 to 4 inches above this point.

6. Properly dispose of all items.

7. The plants should be placed where they will receive normal light for growth, either indoors or outdoors. Water and fertilize as needed. Note that temperatures above 90°F (32°C) will inhibit the bacterium.

8. Remove the Parafilm® or plastic warp about 1 week after inoculating the plants.

9. As the galls develop, carefully measure the size on a weekly basis or obtain a weekly photograph. It usually takes at least 3 weeks before any tumors (galls) start to develop, so be patient!

10. When the experiment is finished, throw the plants and associated soil in the garbage or in a compost pile.