# Isolation of Strawberry DNA for PCR Analysis

PURPOSE

To determine if strawberries have been genetically modified. Protocols and reagents used in this lab are similar to those research labs use to purify DNA for genetic analysis and sequencing and testing for genetic modification.

**MATERIALS & EQUIPMENT Per Person**

- ☐ 10 ml DNA Extraction Buffer
- ☐ One fresh or frozen strawberry
- ☐ Kitchen knife to cut and core strawberry
- ☐ Distilled water
- ☐ 1 freezer bag
- ☐ 1 funnel
- ☐ 10% Chelex in $H_2O$
- ☐ 1 test tube rack
- ☐ 1 test tube
- ☐ Cheesecloth
- ☐ Wooden stir rod
- ☐ 5ml 95% ethanol
- ☐ 1 microfuge tube

**#1 DNA Extraction Protocol:**

1. Cut a small strawberry in half.
2. Core out the inner white core of the strawberry.
3. Place the cored half strawberry into a zip lock bag and seal the bag tightly again.
4. Mash the berry for one minute, kneading it between your fingers.
5. Add 10 ml of DNA extraction buffer and reseal the bag.
6. Mash the strawberry and DNA extraction buffer for 20-30 seconds.
7. Filter the liquid through 2 layers of cheesecloth-lined funnel into a collection test tube.
8. Collect about 4 ml of filtrate.
    a. If the liquid is not moving through the filter, you can use the bottom of the ethanol tube to press it through.
9. Slowly add 5 ml of cold ethanol along the side of the test tube.
10. Watch as the ethanol layer will separate the DNA from the buffer.
11. Use a wooden rod (or bacterial streaking loop) to spool the DNA by twirling and place it into a 1.5 µl microfuge tube.
12. Once DNA is in the tube, use the rod to gently press down to release excess alcohol from precipitate.
13. Carefully aspirate the excess alcohol with a micropipette.
14. Add 1 ml (1000 µl) TE pH 8.0 (10.1).
15. Invert until the DNA has completely dissolved in the buffer and **PUT ON ICE.**
16. Pour the excess liquid in the sink from your test tubes.
17. Discard the cheesecloth in the trash can and place the test tube into the wash bucket.
18. Clean up your area and wash your hands.

**#2 DNA Purification Protocol**

1. Obtain the Chelex tube from the stock station.
2. Be sure to resuspend the Chelex immediately before pipetting.
3. Place 500 µl Chelex into a sterile microtube.
4. Add 50 µl of your DNA extract dissolved in TE.
5. Mix the contents of the microtube gently by inverting several times.
6. Place your microtube containing Chelex and DNA into the 100°C heating block and incubate for 5 minutes.
7. Centrifuge at 12-14,000 rpm for 5 minutes.
8. VERY CAREFULLY….transfer the supernatant to a sterile microtube
9. Store in 4°C refrigerator or freezer until ready to do further testing.

**DNA Extraction Buffer**: Enough for 100 strawberries.

100 ml liquid dishwashing liquid (Palmolive or Dawn)
15 grams NaCl
900 ml water

Adapted by Cindy McIntyre, Bill Monahan, and Carole Tanner from Hutch High, Science Education Partnership, DNA Extraction from Strawberries, SEP Protocol, Version 11/12/07,Adapted from Biotechnology in the Classroom, UC Davis and Diane Sweeney Labs *Biology: Exploring Life*