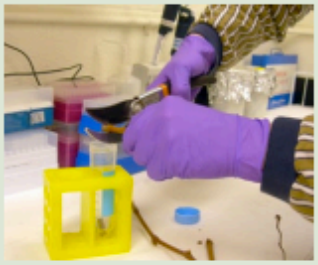
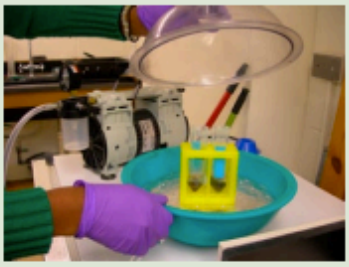
More Efficient Crown Gall Testing

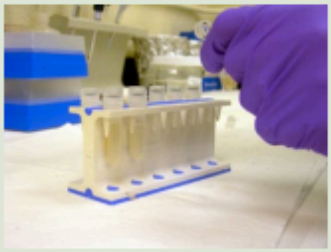
A new method for testing for crown gall, using a technique called *magnetic capture hybridization* is under development in the Burr laboratory. It can detect as few as 10 *A. vitis* cells in a grape tissue sample, and greatly improves the sensitivity of tests available for *A. vitis*. Previous tests involved callusing cane tissue (6 wk), incubating on selective media (1-3 wk), and amplifying and testing DNA from colonies to verify identity of *A. vitis*. Detection limit was around 1,000,000 (10^6) bacteria. This new method should improve testing accuracy, and results are available in 3-4 days.



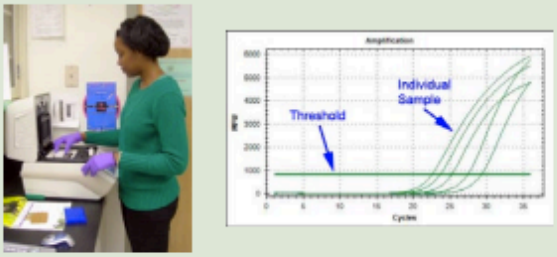
1. Nodes from test canes are cut and put in vials containing buffer solution to soak tissue.



2. Vials placed in vacuum to extract Agrobacterium vitis from vine tissue. Nutrient broth is inoculated with PBS containing extracted Agrobacterium and incubated for 3-4 days.



3. DNA is extracted and placed in small tubes with small beads coated with a matching DNA probe that selects for A. vitis DNA and leaves behind DNA from the plant and other microorganisms that could interfere with the test.



4. Real-time PCR: Samples are placed in a DNA Polymerase Chain Reaction (PCR) cycler (left), which also provides a read out of the amount of crown gall DNA produced (right). Each green line represents a single sample; a positive result is recorded when the amount exceeds a threshold (horizontal green line).

Excerpt from Appellation Cornell Research Focus 2012-1. "How Close are We to Crown Gall-Free Nursery Stock?" By Tim Martinson and Tom Burr.

Link: [Appellation Cornell Research Focus March 2012]