Plant Biotechnology

Bayer Russia Plant Biotechnology Conference

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Molecular Assays

Leaf Sampling

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- PhD in Microbiology from North Carolina State University
- Over 25 years experience in Plant Biotechnology at Bayer Crop Science
- Project lead experience leading commercial development of Biotech crops
- Eight years of experience in developing and running molecular screening assays for Biotech crops
- Four years of experience as lead of the STL TaqMan Lab in Chesterfield, MO site

Leaf sampling methods for soy and corn plants

Soy plants ready for sampling

Corn plant ready for sampling

Additional considerations for leaf sampling for molecular analysis

- Sample size should be compatible with the DNA extraction method. Too much tissue may overload the extraction method and too little tissue may not produce sufficient DNA quantity
  - A variety of sizes of leaf punches are available (3 mm or 6 mm diameter, for example)
- Young tissue is preferred for high DNA quantity and high-quality grind
- Handling of tissue after sampling is important to preserve DNA quality and avoid PCR inhibitors
  - Lyophilization is preferred to preserve tissue quality
  - Freeze-thaw cycles in handling of leaf tissue should be avoided to minimize degradation of DNA and presence of inhibitors to downstream assays
  - Other methods may be used, but may require quality checks for DNA level, fragment size, and PCR amplification quality to ensure suitability for the method
- Some plant species may produce PCR inhibitors, so leaf sampling, DNA extraction method, and PCR reagents should be tested with the desired species

Soy leaf sampling: assemble materials for sampling

- Soy leaf can be routinely sampled with a standard six-millimeter leaf paper punch into a 96-well plate
- When doing many samples it is best to sample into a plate on dry ice.
- For small numbers of samples, leaf can be sampled into a 96-well plate at room temperature and then placed on to dry ice. Once frozen, samples should not be allowed to thaw.

Sampling three punches and adding to well

- Punch an upper leaf three times to create three leaf punches.
- Use tweezers to collect the leaf punches and place them into one well of a 96-well plate.
- Then proceed with sampling additional plants.

Soy leaf sampling with Rapid-Core sampling tool

- Next is an alternative way to sample soy plants using a six millimeter Rapid-Core sampling tool.
- This sampling utilizes a six millimeter Rapid-Core sampling tool.
- It is also important to use a rubber finger-tip cover or other cut-resistant glove on one hand to avoid cuts from the sampling tool.

- Six millimeter Rapid-Core sampling tool from Electron Microscopy Sciences in Hatfield, Pennsylvania.
- The current catalog number for this tool is 69039-60.

Sample soy plants

- Gently squeeze together a single leaf and use the Rapid core to sample twice using a small twist of the core to create two leaf punches with each action.
- Ensure that a cut-resistant rubber finger tip cover or cut-resistant glove is used on the opposite hand, as the sampling tool is extremely sharp.
- Then eject leaf punches from the core sampling tool into a single well of a 96-well plate.

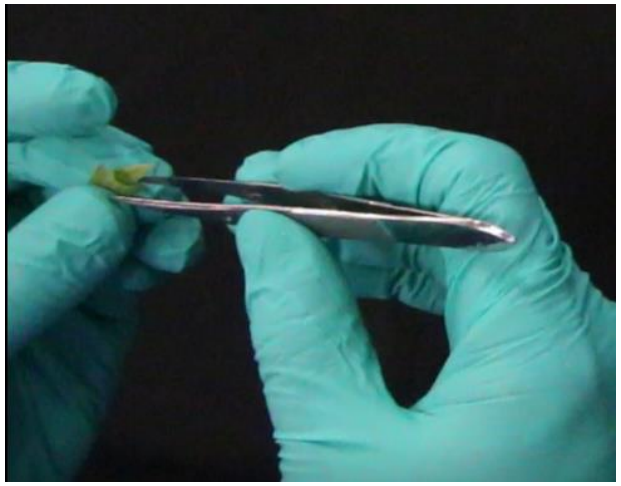
Leaf sampling from corn plants

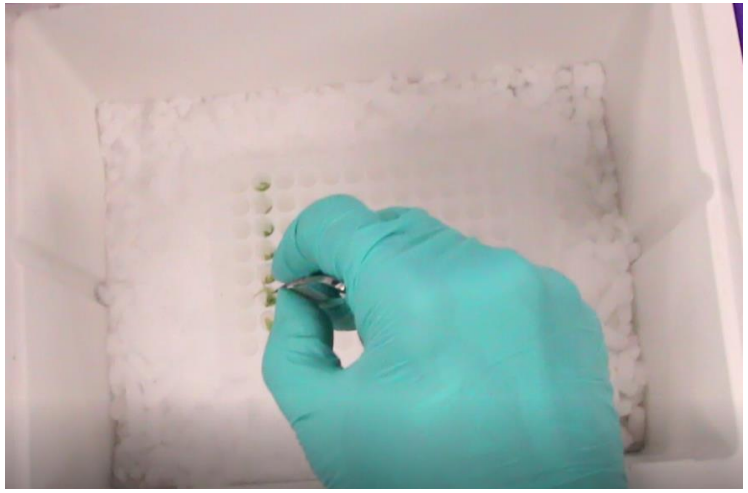
- The following describes sampling of corn plants for molecular analysis.
- Plants are sampled into a 96-well plate and frozen on dry ice.

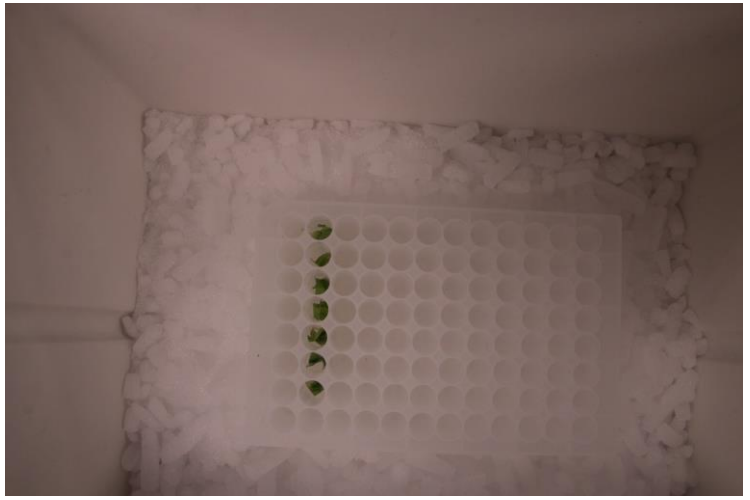
Sample corn plants

- Remove approximately 1 cm of tissue from the end of the upper leaf by hand.

Transfer corn samples to 96-well plates

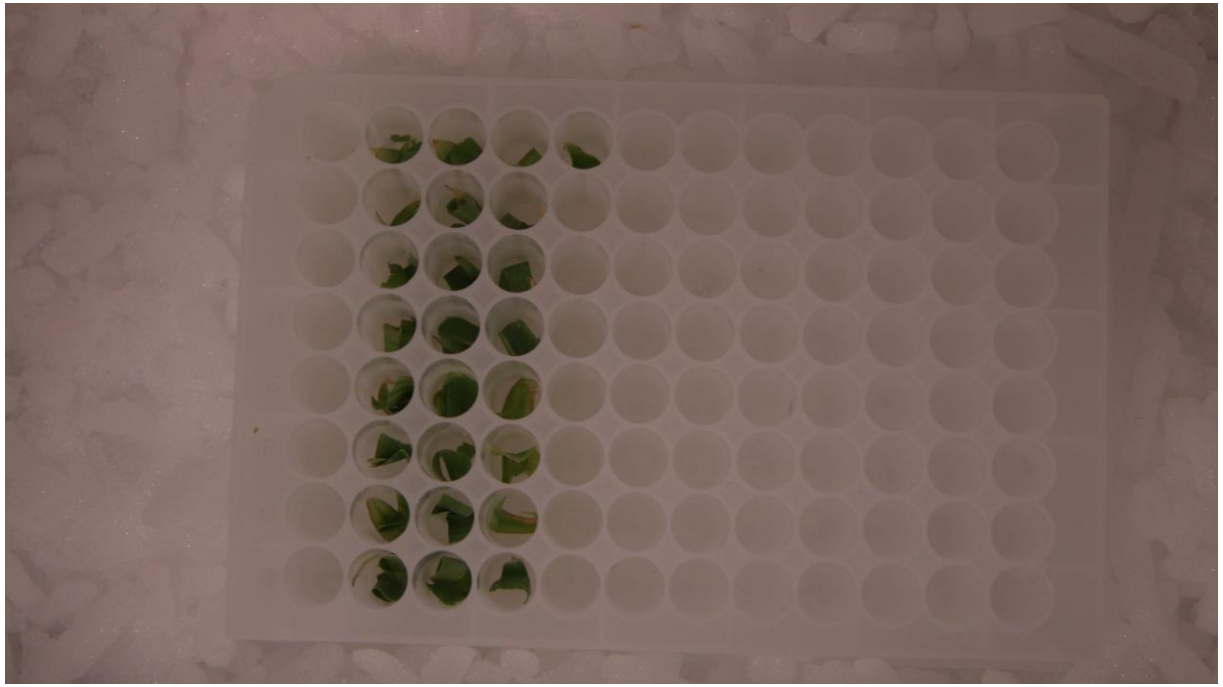






- Use tweezers to fold leaf, and place folded leaf into well of the 96-well plate.
- When doing many samples, it is best to sample into a plate on dry ice.
- For small numbers of samples, leaf can be sampled into a 96-well plate at room temperature and then placed on to dry ice. Once frozen, samples should not be allowed to thaw.

Complete corn leaf sampling



- After sampling is complete, place a cap mat over the samples for transfer to either a minus 20 degree Celsius or minus 80 degree Celsius freezer until ready for DNA extraction.

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Thank you!

Any questions?