Chapter 10: Plant and Field Sampling

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Introduction

This chapter will focus on tissue sampling techniques used by the seed and crop production industries. Determining the genetic makeup of a growing plant has applications throughout the value-chain starting with plant breeding. By definition tissue sampling is the collection of cells from a specific part of an actively growing plant. Leaf punches, leaf discs, entire leaves or leaflets are the most common tissue sample types utilized in the industry. Unlike testing bulk seed, bulk grains and related commodities, fresh tissue has actively growing, metabolizing, and replicating cells. This has a direct impact on the techniques, handling requirements and application of the methods. Sampling for plant health, such as in nutrient analysis and plant pathogen identification, are not addressed in this book. The techniques described in this chapter lend themselves to both high-volume and low volume sampling. Tissue samples are used by plant breeders to accelerate their work and ensure a clean hand-off of varieties and lines to parent seed producers. Crop advisors use tissue samples to verify that a crop is tolerant, or has a trait to resist a non-selective herbicide, prior to application. Tissue samples are also used to verify the identity and purity of crops and outside contaminants in seed and specialty production. The following will cover breeding applications as well as seed and commercial crop testing.

Plant Breeding

Plant breeders can generate hundreds to hundreds of thousands of tissue samples as part of a project or breeding program. Plants are labeled by various means to maintain the identity of each plant, row or group. A tissue sample is then carefully collected for testing, typically for laboratory analysis. Data from the lab is returned to the breeder pre-pollination if plants will be selected prior to mating. Pre-pollination data is also the best way to remove undesired plants, those plants classified as contaminants, from a nursery or a small seed increase. Removing unwanted plants prior to flowering eliminates the chance of unintentional cross pollinations that would perpetuate unwanted genetics. Even in self-pollinated species where all means of

preventing unwanted cross pollinations are used, pre-flowering removal of plants is part of most stewardship programs.

Genetic markers for specific traits or entire genetic profiles can be run on the tissue samples collected. The use of molecular markers applies to any heritable characteristic. Breeders have embraced molecular markers for their ability to accelerate breeding work, as well as a means of addressing contamination. Historically plant breeders would fight the odds of limited information about the genotype by increasing the number of plants in the field as well as increasing the number of pollinations or crosses they made. The ability of molecular markers to eliminate the need to preform blind selections or blind backcrossing is a significant driver in modern plant breeding.

A blind backcross derives its name from using a segregating population of individuals for crossing. Disease resistance genes are a good example of heritable qualities that are not readily apparent by phenotype in a segregating population. Artificially inducing disease can be problematic or impossible to use as a consistent selection tool. Therefore, the chance of selecting an individual with the desired trait is unknown or blind.

With informative genetic markers, the chance of using desired plants in the breeding process is dramatically increased. Only the natural segregation in the gametes of desirable plants selected from a breeding population will affect the genetic makeup of next generation of seed. Breeders also accelerate the rate of genetic gain through the use of complete genetic profiles. A complete genetic profile can be used to rapidly identify backcross progeny that match the desired recurrent parent thereby eliminating another source of uncertainty and inefficiency in the selection process.

Seed & Commercial Crop Testing

Inspectors that are charged with determining crop identity and purity may also collect or test tissue samples. Tissue testing can be used to confirm the identity and check the purity of seed production and grain crops during the growing season. Inspectors may randomly test plants or test variants and off-types identified as potential contaminants in a seed crop. When permissible, nearby crops can be tested to determine if they will be an undesirable source of pollen for the seed crop. Tissue testing can also be used to meet requirements for regulated field trials. Post

season checks are required to determine if regulated materials persist at the trial site or if off-site movement of materials has occurred. Where crops with Plant Incorporated Protectants (PIPs) are prevalent, inspectors can also verify the crop trait(s) and its refuge. Integrated refuge products, such as "refuge in the bag" or "integrated refuge" products, can also be sampled and tested in the field to identify the typical 5-20% refuge component of the blend. Crop advisors, those tasked with crop protection and production services, can use tissue samples to manage several issues. By confirming the resistance trait prior to herbicide application, crop damage or crop destruction can be avoided. Crop advisors may also test samples for nutrient analysis, crop health, pathogen detection and phytosanitary purposes which will not be addressed here.

Direct Testing in the Field

The first step in sampling tissue is to understand the requirements of the assay or laboratory analytical method. Immunoassay test kits that utilize lateral flow test stick or strips are commonly used in the field for rapid trait checks and pathogen detection. Lateral flow devices or strips come in ready to use kits that include the reagents and materials necessary to conduct the test in the field or an on-site work area. Eppendorf tubes are common in many of these test kits. An "Eppendorf" is a small, 1.5-2.0 ml in volume, plastic tube with a hinged cap (photo 1). It is a single use disposable container that is suited for direct use in the field with lateral flow devices but can also be labeled and shipped to a laboratory for analysis. The hinged cap makes the Eppendorf tube a sampling tool and container all in one. The lip of the cap fits into the tube and when closed over the leaf tissue to be sampled, a leaf disc is created. For some protocols the leaf is folded over to achieve a double leaf disc. Analysis can be conducted in the field by using a disposable mini-pestle designed to grind the tissue for extraction and analysis. The tissue can be processed fresh and can stay with the plant during field analysis. Eppendorf tubes can also be submitted to a laboratory for analysis. Laboratory submission requires coordination with the lab regarding the method of submission. The labeling of plants, tubes and racks are all considerations for laboratory submission. The Eppendorf tube sampling method is not typically used for high volume sampling due to the time it takes to label individual tubes and collect the sample. For direct testing in the field be sure to use a kit that is specific to leaf and other fresh tissues. Grain and seed kits should be avoided as they may not have been developed or validated for fresh tissue. When large amounts of chlorophyll are present test lines may turn green

resulting in a false positive.Antibodies may be rendered ineffective resulting in a false negative. Once analysis is complete the results can be recorded and the plant can be removed if undesirable, labeled as desirable, etc..

Sampling for Laboratory Testing

The first step in sampling is to understand the requirements of the assay.The lab conducting the processing and testing should be your primary resource for sampling and submission requirements. Test results may be affected and resampling may be required to achieve usable results if sampling requirements and protocols are not followed. Good laboratories will have pre-defined sampling requirements that cover sampling technique, containers, handling, and shipping methods. In some cases laboratories will supply specific containers to use. Always consult and confirm the appropriate process with the laboratory to ensure success in sampling and testing. A discussion of containers or “plastics” should be part of the pre-sample planning.

The term “plastics” has been used by laboratories and field personnel as a general term that applies to any tube, honeycomb box or similar container used to house samples. Plastic ZipLoc™-type bags are an obvious choice for relatively few samples or whenever entire leaves or leaflets are collected. For high volume testing the subject of "plastics" can be brand and catalog-number specific to ensure that robotic handling equipment performs properly in the laboratory. Plates, blocks or racks typically have an 8x12 pattern of wells (holes) or individual tubes for a total of 96 wells per block or 96 tubes per rack. There are also 4x6 (48-well) and 16x24 (384-well) plastics that occupy a similar footprint as a 96-well block. The wells or holes of a 48-well block are significantly larger and more suited to individual seed testing. The 384-well block being significantly smaller and designed primarily for liquids and high-throughput laboratories. However, the 8x12 (96-well) system is the middle-ground and is almost universally used to collect fresh leaf tissue in the form of a leaf punch or leaf disc. The terms plate, block and tube-rack in the 8x12 pattern are also used interchangeably. For simplicity the term “block” will be used throughout the text for any of the 8x12 pattern plastics.

Marking and Labeling

Data is rendered useless when results cannot be traced directly back to plants from which samples were collected. This applies to fields, rows, individual plants and in some cases specific

tissues of an individual plant. Therefore the next step to successful tissue sampling is how to label and maintain the identity of the material being sampled.

Labeling for low volume sampling can be as simple as manually writing on the sample container with an indelible marker. As the volume of samples increases the procedures need to change to maintain accuracy within the time constrains of the work. Printed tags, labels and blocks that include barcodes for the laboratory's LIMS (Laboratory Information Management Systems) are common in high volume sampling programs. The 8x12 blocks and racks are typically labeled on the south or west side with Row A serving as north. Again the laboratory should clearly define the sampling procedure as well as the handling and shipping requirements. To be clear the laboratory requirements must be followed to avoid delays, imperfect data or re-sampling requests.

Plants can be labeled individually and breeding organizations generally have high speed printers that utilize rolls of plastic slip-lock plant labels. Stakes of various sizes can be used to mark individual rows or a series of plants in a row for bulk sampling. The proper labeling of plants and sample containers is key to preserving data integrity and preventing mix-ups. Samplers must ensure the right data comes back to the correct plant, the correct row or the correct field. Therefore the loading template or pattern must also be established with the laboratory when utilizing 8x12 blocks.

The 8x12 pattern plastics utilize an alpha numeric system of labeling that is typically molded into the block; rows A to H and columns one to twelve (photo 2). As indicated previously, the depth, style and manufacture of the blocks are often specific to the laboratory and substitutions should be avoided or approved by the laboratory. Samples can be numbered left to right, west to east, A1, A2, A3...H10, H11, H12 or top to bottom, north to south, A1, B1, C1, D1... F12, G12, H12. In addition to the loading template orientation, specific check wells may need to be left empty for the laboratory. Each lab varies with the number and placement of its check wells. Paper sampling templates are one of the most common ways of properly labeling and subsequently loading blocks from well A1 to H12 while in the field. An 8x12 paper template is created, printed and affixed to the block. The paper template is punctured as the sample tube is placed in the proper well. The sampler moves plant to plant in the field puncturing the template to place each new tissue sample. (Fig. 1).

Collecting the Sample

Young leaves are far and away the most common tissue type that is sampled for protein and nucleic acid (DNA, RNA) analysis. Good quality tissue ensures rapid and reliable results. Tissue should be actively growing and turgid (not wilted) when sampled. Sampling stressed tissue should be avoided due to the low quality and quantity of both proteins and nucleic acids that are targeted by most assays. Excessively damp or wet leaves can be blotted dry before collection. When using a lyophilizer to freeze dry tissue, avoid wet or unevenly wet leaves as they can produce uneven products for the lab to test or store. For tissue samples that are shipped fresh, degradation should be a concern. Samples that are heavily colonized with actively growing saprophytes should be avoided during sampling. Diseased tissue, infected with live plant pathogens, should also be avoided. Infected tissues can possess foreign proteins, DNA, and metabolites that may affect testing. The presence of plant pathogens may also prevent the shipment of samples due to plant protection and quarantine regulations. Shipping prohibited plant materials, live pests and soil is illegal in many cases. This is especially true when shipping between states, countries and regions that have established phytosanitary requirements.

Cleanliness is also important for successful sampling. Sampling tools should be kept clean of cell exudates that can create cross contamination. Cleaning of the sampling tool can be as simple as punching a clean piece of paper or wiping the cutting surfaces. For some applications a solvent such as alcohol may be required. A simple rubbing alcohol, such as 70% EtOH, is effective but results may vary across tissue type and plant species. The use of 70% percent of alcohol is recommended over stronger concentrations.Pure alcohol may cause protein to coagulate, effectively sealing single celled organisms such as bacteria or stray plant cells to the surface you are trying to clean. The higher osmotic pressure of 70% ethanol also allows the alcohol to cross cell membranes and destroy bacterial cells by denaturation. A 2% solution of NaClO is another cleaning solvent that can be found in the literature. Regardless of the method, it is important to validate the cleaning process. This can be achieved by subjecting equipment to known positive plant tissues followed by know non-positives. Testing the resulting samples will reveal the persistence or carry-over with and without cleaning.

Along with clean sampling it is also important to protect samples from sun, heat and other elements that can cause sample degradation. Collect samples as close as possible to the time you

will be shipping or lyophilizing them. All types of tissue samples should be free of moisture, soil, and insects. Blocks should also be protected from foreign material, moisture, and physical damage. Coolers and cold packs are often used to keep blocks cool until they can be brought into a controlled environment. However, even if a cooler is used, the time between collection and the next step in the process (test, ship, or lyophilize) must be minimized.

Safety Considerations

Safety of sampling personnel is an important consideration during field work. Basic exposure risks include sun, heat and other elements inherent to working outside. There are some additional issues specific to handling tissue that should also be addressed to protect workers. Pesticides are common under field and greenhouse conditions and Restricted Entry Intervals (REIs) must be respected or addressed through proper Personal Protective Equipment (PPE). PPE includes, but is not limited to, long sleeves/pants, socks, close-toed shoes, hat and sunscreen as well as a consideration for protective eyewear. Eyewear should be considered for UV protection as well as physical protection when working with taller plants/crops (e.g. maize). As all tissue sampling involves intimate contact with plants and soil, pesticide residues should be considered. Pesticide residues are especially a concern where hand sampling is employed. Depending on the height of the crop to be sampled the ergonomics of sampling can be an issue. Constant stooping or bending can be a concern along with any number of repetitive actions. Working with a low growing crop can be facilitated by working in teams of two. The sampler sits or kneels and moves along the row handing the sample or sampling tool to the loader. Two person teams can also be of benefit for all crops by allowing better focus on plant by plant sampling and loading samples into containers. Periodically the two should also switch roles to avoid mental and physical fatigue. When collecting samples from taller crops, PPE such as protective eyewear and long sleeve shirts should be considered to shield eyes and skin from abrasions. Sampling tools can also pinch or cut depending on the type of tool and its design. Some tissue sampling tools utilized for sampling plants were originally designed for mammalian histology, dissection and biopsy applications and are very sharp. Examples include the Harris and Whatman Unicore samplers. Worker protection is an important consideration when planning any type of tissue sampling program or project.

Specific Procedures

Sampling can be as simple as placing entire leaves or leaflets in plastic bags. It can be done entirely by hand or with more sophisticated leaf punch tools that create 4-6mm leaf discs, not unlike a paper punch. High volume sampling is most often used by breeders interested in sampling a large number of plants. The following sections describe some, but by no means all, of the common tissue sampling procedures.

Tube Sampling

Tube sampling utilizes the 8 x 12 pattern plastics that hold individual removable tubes in a rack system. Each individual tube is removed from the rack to collect a leaf disc cut from the plant with the open end of the tube. To perform the collection, the open end of the tube is pressed into a leaf that has been placed against a backer to cut the leaf disc. Simple card stock or Post-It® note pads can be used as a backer. The leaf disc typically remains in the mouth of the tube when the tube is returned to its rack. The next tube selected from the rack can be used to push the previous leaf disc down into its tube (Photo 2). The process is then repeated until the rack is filled. The mouth of the tube is not the ideal cutting tool and care should be taken to avoid cross contamination with leaf exudates. The backer should also be replaced as needed to avoid cross contamination. Advantages to this system include a uniform sample size with no equipment other than the backer and the tubes. Also, this system is capable of collecting a large number of samples rapidly. One of the disadvantages with tube sampling is the crushing of tissue on the lip of the tube. This can result in plant extracts that may cross contaminate other samples. Another disadvantage is the use of loose tubes in a rack. While most tube systems utilize a cover or lid to lock tubes in place when not in use, loose tubes can be misplaced in the rack or the entire rack can of tubes can be spilled.

Hand Sampling

Sampling by hand is not necessarily a low volume approach and can be used for high throughput automated laboratories. However, hand sampling requires samples of equal size to avoid clogging or overwhelming the liquid handling activities of an automated lab. To sample by hand, a small amount of leaf tissue is pinched off and rolled between the fingers prior to placing it in the sample container. The rapid nature of the technique lends itself to high volume sampling that utilizes 8 x 12 blocks. A pre-printed paper template should be used as described in the Marking

and Labeling section of this chapter. The paper template that is affixed to the 8 x 12 block is punctured prior to placing the tissue sample in the proper well in the block. A mini pestle or similar rod is then used to push the tissue sample down into the well. The advantage of this method is the rapid sample collection without special tools. As a disadvantage the technique can be subject to sample size variation that creates processing issues in the laboratory. Too much or too little sample can impede or obstruct the DNA extractions and subsequent reactions performed. A high degree of dexterity and practice is required for hand sampling and exposures to pesticide residues should be considered.

Eppendorf Cap Method

The Eppendorf cap method of sampling uses a specific tube that is common in laboratories and test kits. The Eppendorf tube has a hinged cap making it a sampling tool and container all in one. The lip of the cap fits into the tube and when closed over a leaf, a leaf punch or disc is created. For some protocols the leaf is folded over to achieve double leaf discs (Photo 5). Analysis can be conducted in the field by macerating the leaf disc(s) using a mini pestle. Depending on the kit protocol a buffer or similar solvent is added and the extract can be tested with a lateral flow device directly in the tube. The tube can either stay with the plant during field analysis or can be placed in a rack to be tested elsewhere. Once analysis is complete the results can be recorded and the plant can be labeled or removed if undesirable, within minutes of sample collection. Eppendorf tubes can also be submitted to a laboratory for analysis. Laboratory submission requires coordination with the lab regarding the method of submission. The labeling of plants, tubes, and racks are all considerations for laboratory submission. Advantages for the Eppendorf sampling technique are the lack of tools to clean, uniform sample size, immediate sealing of the sample container and the tube can stay with the plant to be analyzed in the field. The tubes are often included in test kits simplifying purchasing. One disadvantage is that cap may not cleanly cut the leaf tissue during the closure process, making for an untidy sampling process. The method requires good dexterity and is not typically used for high volume sampling due to the time it takes to label individual tubes and collect the sample. When testing leaf tissue, be sure to use a kit that is specific to leaf tissue testing. Grain kits should be avoided as they may not have been developed or validated for fresh tissue. As mentioned previously, leaf tissue contains large

amounts of chlorophyll, therefore test material may turn green resulting in a false positive or antibodies may be rendered ineffective by the matrix resulting in a false negative.

Core Samplers

The Harris™ Uni-core and similar sampling tools can be used for any volume of sampling where a high degree of precision in leaf disc size is desired. The open end of the tool is pressed against a backer and twisted to cut the leaf disc. Simple card stock or Post-It® note pads may be used as a backer; however, a specialized self-healing cutting mat is recommended due to the razor sharp cutting edge of the Harris™ Uni-core tool (Photo 4). This technique can be used for high volume sampling that utilizes 8 x 12 blocks. A pre-printed paper template is used as described in the Marking and Labeling section of this chapter. The paper template is punctured prior to placing the tissue sample in the proper well on the block. The Harris Uni-Core design includes a plunger that pushes or ejects the leaf disc out of the cutting tool. This tool can also be used to rapidly collect multiple leaf punches, seven to ten depending of leaf thickness, which can be tested in bulk as a more economical and higher throughput screening process. With bulk sampling, positive results may condemn the entire subset of plants or a second round of sampling may be applied after the initial results are returned. The tool should be kept clean of cell exudates that can create cross contamination. As mentioned in the Collecting the Sample section of this chapter, a simple wiping of the cutting surfaces by twisting the tool on a paper towel may be sufficient. However, for some applications a solvent such as alcohol or 2% NaClO may also be required. Advantages of the Harris™ Uni-core is uniformity and speed. Disadvantages range from cleaning to safety. The tool is razor sharp for the collection of muscle tissue samples. A degree of dexterity and proficiency with a clear understanding of safety is required when utilizing razor sharp cutting tools. As the tool dulls cleaning may need to be intensified and staff need to be aware of when to discard a dull tool. Additionally, there are tools similar to the Harris™ Uni-core tool, which can be sharpened with a specialized sharpener rather than discarding when the tool becomes dull.

Tissue Punch

Tissue punches include any of a number of designs that mimic a single-hole hand-held paper punch. While a paper punch can be used as a sampling tool, most are not suited to cutting soft,

moist materials cleanly or uniformly into a complete disc. Midco Global, a supply company, offers a punch designed specifically for leaf tissue sampling (Photo 6). This device can hold a tube for the leaf disc to fall into upon punching. There is also a rod extending from the bottom of the tool which can be used to push leaf discs into the sample tube prior to returning it to the sample rack. This tool can also be used to rapidly collect multiple leaf punches which can be tested in bulk as a more economical screening process. With bulk analysis, positive results may condemn the entire subset of plants or a second round of sampling may be applied after the initial results are returned, therefore the tool should be kept clean of cell exudates that can create cross contamination. Cleaning may be achieved by punching clean paper or wiping down the cutting parts of the tool, but for some applications a solvent such as alcohol may also be required. Most designs allow for the tool to be disassembled for regular cleaning. Simple rubbing alcohol such as 70% EtOH is effective but results may vary across tissue type and plant species. Pure alcohol may cause protein to coagulate effectively sealing single celled organisms such as bacteria or stray plant cells to the surface you are trying to clean, thus the use of 70% percent of alcohol is recommended over stronger concentrations. The higher osmotic pressure of 70% ethanol also allows the alcohol to cross cell membranes and destroy bacterial cells by denaturation. A 2% NaClO solution is another option that can be found in the literature. The advantages of using a tissue punch include the rapidity of sample collection and the precise size of the leaf discs generated. Disadvantages range from cleaning to possible limitations in sample containers. This type of tool is primarily designed to hold single tubes or vials (see the tube sampling process detailed in this chapter). With some high throughput labs requiring solid blocks for samples the purchase of a tissue punch should be considered only after determining what plastics will be required.

Shipping Samples

Prior to shipping, blocks are typically sealed with a plastic film, gas-permeable membrane, sealing mat, or cap system. Some types of sealing mats may be designed for easy piercing with a pipette tip or the shaft of a syringe. Solid sealing mats are designed for removal by the lab prior to processing the sample block. Again, the type of sealing mat or film may also be prescribed by the laboratory. Laboratories may also have specific packing requirements for shipping. For example, fresh leaf samples may need to be double bagged and shipped with no heavy objects

such as ice packs that may crush tissue or puncture bags during shipping. The method for sealing blocks should be specified by the lab prior to sampling and the materials must be on-hand for immediate shipment. In certain instances, caps or sealing mats can separate from the block during shipment due to improper sealing or the pressure changes that occur during shipping. Letting blocks warm to room temperature after lyophilization helps ensure that caps are not displaced due to the expansion of warming air. The use of a large ink roller, cap press, or similar tool that helps apply the sealing mat or caps securely can ensure delivery without spillage. Samples should be sealed and prepared for shipping in a clean, preferrably air conditioned, environment. For lyophilized samples, static electricity may cause dried tissue samples to move unexpectedly and cling to surfaces. Consult with the laboratory if the issue of static electricity persists and ask if they have a recommended way to remedy it. The remedy may range from a household anti-static product to a benchtop air ionization unit.

Samples shipped internationally or between territories that have phytosanitary requirements will require additional preparation, inspection and/or permits. Samples that are incorrectly shipped can be stopped by inspectors at the port of entry and refused entry or can even be destroyed. Laboratories with permits to receive such shipments may not be able to receive unsolicited samples. Improperly shipped samples that do happen to make it past a port of entry may be destroyed as a condition of the laboratory's permit. In all cases, communication between those shipping samples and those receiving them must be clear. Package samples in sturdy containers that will survive the type of mishandling that can occur in commercial shipping. Always use a reputable carrier capable of tracking packages.

Summary

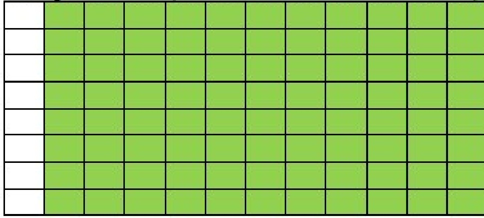
This chapter has outlined several tissue sampling techniques, how they are used, and for what purpose. Unlike testing bulk seed and bulk commodities, plant and field analysis involves taking a sample from a growing plant. Testing the tissue sample establishes the identity of a crop or the identity of a specific plant within in it (e.g. transgenic and conventional). Plant breeders have eliminated much of the guess work in selecting parent materials and progeny through the use of tissue sampling and testing. The high volume of samples generated, along with the turnaround times necessary to make selections of actively growing plants, has necessitated the development of highly automated laboratories. These automated laboratories require specific sampling

procedures and materials to perform the necessary tests properly. Coordination with the testing laboratory is key to achieving actionable data in a timely fashion. Sampling details need to be established well ahead of any sampling in the field. A wide range of techniques, containers, and processes exist within the broader subject of tissue sampling. While plant breeders are by far the biggest users of tissue sampling techniques, seed inspectors, and crop advisors can also use tissue sampling and testing techniques. Inspectors and advisors are more likely to use test kits in the field to determine if a specific trait is present or absent. Lateral flow devices are available for many of the traits currently on the market allowing for rapid identification in the field. While purity testing can be done with these test kits, the industry focuses on testing tissue during the breeding and parent seed phases of crop development. The number of plants is relatively small, more manageable, and under the strict control of the breeder or breeding organization. Any errors or unwanted genetics that contaminate the breeders work will be perpetuated during the commercial production process. With the number of plants involved in commercial production, testing bulk seed and grain becomes the most effective method of addressing undesired traits in crops. It is crucial to understand how different sampling techniques and testing plans can be implemented in the crop improvement and production process.

Sampling for the sake of sampling serves no real purpose and may return misleading results. Be sure to evaluate your objectives and determine if tissue sampling is even necessary. What is your role? Are you enforcing a regulation or performing a quality assurance service? Are you looking to detect low level contamination of a crop or gross contamination and misidentification? The amount of resources expended to find a minor issue, such as adventitious presence, is typically used by breeders to ensure the purity of new varieties and hybrids. Census testing of plants in the nursery followed by bulk seed sample testing provides a critical control point in the development and commercialization of new seed products. Parent and commercial seed producers may use tissue sampling to a lesser degree. Looking for GMO contamination in a commercial field by leaf tissue analysis may be uninformative and economically prohibitive. Consumer, environmental, and economic considerations motivate sampling in many parts of the industry. It is important to determine what can be effectively achieved or resolved at the field level. Be sure to sample and test when and where it will be most effective for your goals or program.

ii. Examples:

Sample Block #1(and all other odd numbered racks)(samples in green)



Sample Block #2(and all other even numbered racks)(samples in green)

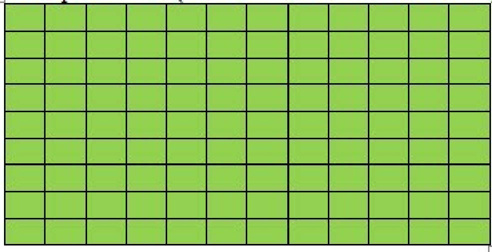


Figure 1. Empty or Check Wells left unfilled for the laboratory. The number and position of check wells varies necessitating good communication between the sampler and the laboratory.

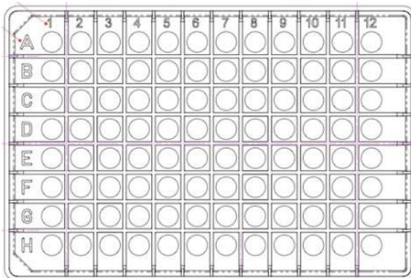


Figure 2. Standard 96-well layout.

Photo 1. Eppendorf sampling tube.

Tube Sampling

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Photo 2. Tube sampling

Hand Sample

Slide 12

Photo 3. Hand sampling.

Harris Uni-Core™ Tool

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Photo 4. Core Samplers

Eppendorf Cap Sampling

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Photo 5. Eppendorf Cap Sampling.

Photo 6. Leaf Punch Sampling

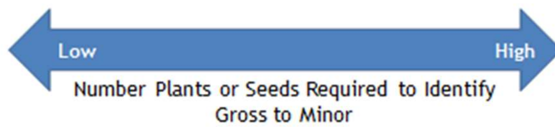
Realization

Gross

- Lack of Change Over
- Erroneous Field/Variety Identification
- Isolation Intrusions

Minor

- Unclean Equipment
- Parent Seed Impurities
- Hybrid Outcrossing
- Varietal Impurities



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