Data Management and Quality Control

By Thomas Lübberstedt, Walter Suza (ISU)

Introduction

You learned about promises of genomic technologies in plant breeding. In this module you will learn about limitations. Marker data are not perfect and do contain errors. Unlike phenotyping, genotyping is often not replicated to minimize costs. There are differences in error rates for different types of markers. Therefore, it is important to know factors that affect marker data quality and to employ quality control to minimize error. Whereas DNA is consistent across cells, RNA and cellular metabolites are not. Therefore, there is an even higher chance of variation between replications for non-DNA markers because of environmental effects. Also, if not exactly the same stages or cells are sampled when different tissues are considered (for example, seed vs. leaves), this may have a bearing on the marker data quality.

Objectives

- To understand sources of error in marker data development
- To understand approaches to minimize errors in marker data development
- To become familiar with marker data management systems

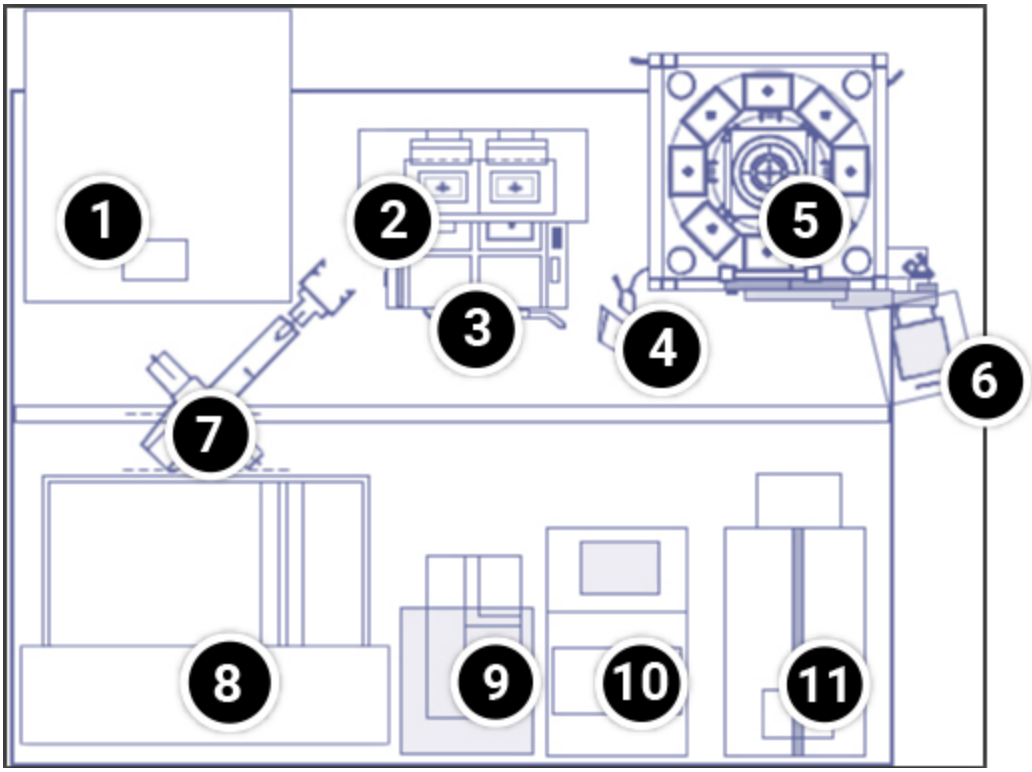
Marker Data Pipelines

Marker Data Information

A marker data pipeline is a system through which marker analysis is conducted as a means to supply marker data to inform research and cultivar development processes. In practicality, the analytical part of the system is tied to the data generation components. For example, robots used to handle samples for PCR analysis in thermo-cyclers. Thermo-cyclers have both internal computers and peripheral conduits for connection to external computers for data storage and analysis.

Select a location in the layout below to view more detailed information on each item. Current DNA marker laboratories in major breeding companies generate more than 1 million SNP datapoints per day.

1. Visualization of multiple PCR products is achieved at a single installation
2. Thermocycler PCR blocks and docking connectors
3. Thermocycler PCR blocks and docking connectors
4. PCR plates are barcoded for identification using computers
5. Refridgerators for storing PCR plates are strategically arranged to increase throughput
6. Careful disposal of potentially toxic waste is important
7. Transfer of sample plates between instruments by robotic fixtures
8. Robots are used to ensure accuracy and reproducibility in measuring and mixing small volumes
9. Robots are used to ensure accuracy and reproducibility in measuring and mixing small volumes
10. PCR plates must be sealed to prevent loss of samples due to evaporation during high temperature PCR cycles
11. Unsealing of PCR plates may be necessary to further evaluate the PCR products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Equipment for Marker Data Development

The type of equipment required for marker data development and service available impact the cost of genotyping. Table 1 illustrates the cost of various genotyping assays and companies that provide such services.

Table 1 Equipment cost and service marker systems.

Assay	Equipment costs (Detection)	Service
SSRs	~$1,000	TraitGenetics
AFLPs	~$1,000	Keygene
Taqman	~$100,000	TraitGenetics
Sequenome-Massarray	~$500,000	Sequenom
Illumina-Beadarray		Illumina, TraitGenetics
Affymetrix		Affymetrix
Illumination-Infineon		Illumina

Steps in Marker Data Production

Steps in Marker Data Production - Step 1

Step 1: Plant Materials

Handling of a sample once it arrives at the laboratory is a critical step. It is customary to label samples and enter the data into a data management system. High throughput laboratories are computerized with databases that track samples to determine what to test for. Labeling mistakes will, therefore, have an impact on data interpretation. However, the use of barcode labels helps alleviate the problem of sample identification. For grain testing, the first step is usually inspection of the sample. However, commercial grain may be contaminated with other grain, which may lead to wrong conclusions.

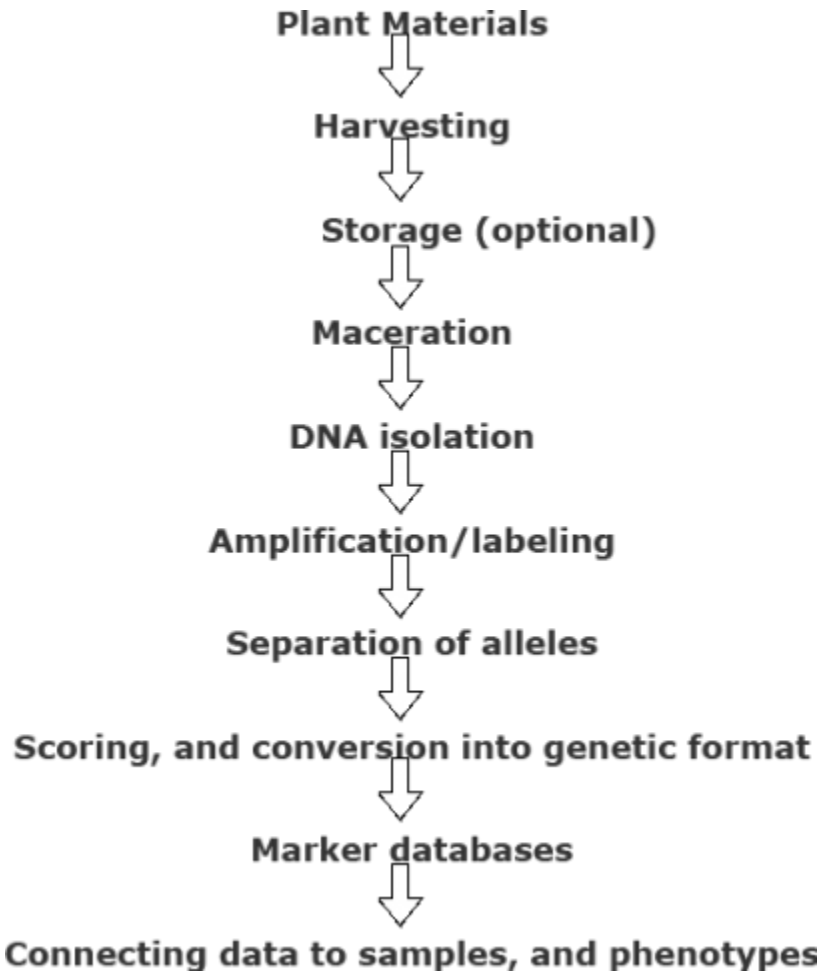

Fig. 1 Steps in marker data production.

Steps 2 to 4

Step 2: Harvesting

Sample deterioration after harvesting may cause degradation of target metabolite markers and impact the quality of DNA. To minimize deterioration of especially the vegetative tissues, samples must be quickly immersed into liquid nitrogen or placed in dry ice. DNA isolation and its quality may be compromised by plant metabolites such as tannins and phenolics. These metabolites increase in concentration during leaf development, thus reducing DNA quality extracted in mature compared to young leaves.

Step 3: Storage

Careful and organized storage of samples and extracted DNA is important in case of a possibility of repeating the analyses. Plant samples may be homogenized and aliquoted into small volumes for long-term storage at -80 ^{O}C. Extracted DNA may be stored below -15 ^{O}C for at least three months.

Step 4: Maceration

Maceration describes a procedure to grind and soften tissue by soaking into a liquid resulting in separation of consituents for subsequent analysis. During this process, compounds such as phenolics, tannins and anthocyanins are leached from the sample. Therefore, inefficient maceration may have a negative impact on the quality of DNA and affect subsequent analytical processes resulting in failure to detect an allele.

Step 5

Step 5: DNA Isolation and Quality

Successful quantification of DNA depends on the quality of the sample DNA analyzed. Therefore, appropriate extraction methods for each sample type must be determined to attain accurate DNA quantification (Holden et al., 2003). Table 2 shows how different reagents kits for DNA isolation impact DNA quality and the associated cost for using a particular kit.

Table 2 Impact of various DNA extraction kits on sample quality. Data from Zetzsche et al., 2008.

Kit	Company	Relative Extraction Efficiency	OD Ratio 260/280 (Ø)	Fragment length (Ø)	Handling Time [in h, 20 preps]	Ma Co US (10 pre
Nucleospin	MACHERY & NAGEL	0.15	1.91		2.5	202
GeneElute Plant Genomic DNA	SIGMA	0.16	2.05		2.5	167
Mag DNA Isolation	AGOWA	0.19	1.77		1	105
Invisorb Spin Plant Mini	INVITEK	0.36	1.60		2.5	199
Power Plant DNA Isolation	MOBIO	0.28	2.08		2.5	377
DNeasy Plant Mini	QIAGEN	0.16	1.51		2.5	238
Plant DNAzol	INVITROGEN	0.64	1.66		3.5	120
Puregene DNA Tissue	GENTRA/QIAGEN	0.87	1.49		5	105
Genomic Tip 20/G (adapted)	QIAGEN	0.63	1.93		8	924
Laboratory protocol	(BGBM)	0.44	1.59		3	140

Kit	Company	Relative Extraction Efficiency	OD Ratio 260/280 (Ø)	Fragment length (Ø)	Handling Time [in h, 20 preps]	Ma Co US (10 pre
Genomic DNA Isolation Plants	NEXTTEC	*	*		1	21
good	acceptable	poor		* = could not be determined		

FYI: Click here to learn about the contribution of haploid genomes in maize and soybean.

Steps 6 and 7

Step 6: Amplification/Labeling

If DNA isolation is inefficient, the DNA may be degraded or contaminated with compounds that interfere with the PCR process. DNA degradation will reduce the sensitivity of PCR amplification. Certain contaminants may reduce the efficiency of PCR amplification, while some contaminants may inhibit the reaction or lead to artifact PCR products that may result in wrong interpretation of resluts. Usual good laboratory practices such as changing gloves and laboratory coats, using disposable pipette tips, separate reaction reagents and pipette sets, and so on for each room, significantly decrease the chance of contamination between differerent stages of the detection procedure.

https://www.youtube.com/watch?v=_YgXcJ4n-kQ

Step 7: Separation of Alleles

Electrophoresis artifacts can distort the allele size due to altered DNA migration through the gel resulting in incorrect interpretation of the results. **http://www.youtube.com/watch?v=vq759wKCCUQ**

Fig. 2 A research laboratory. Photo by Iowa State University.

Step 8

Step 8: Scoring and Conversation Into Genetic Format

In addition to errors, marker data development process may encounter other challenges such as missing data. As shown in Table 3, certain marker systems will produce more missing data than others.

Table 3 A comparison of marker systems in relation to missing data.

		Records	**Missing data**	**Average % missing data ± standard deviation**
SSRs	Replicate 1	5,520	652	11.8
	Replicate 2	5,520	868	15.7
	Average across replicates	5,520	760	13.8 ±2.77
SNP-MassARRAY	Replicate 1	8,142	154	1.9
	Replicate 2	8,142	187	2.3
	Avearge across replicates	8,142	170.5	2.1 ±0.28
SNP-Invader	Replicate 1	4,761	161	3.4
	Replicate 2	4,761	138	2.9
	Average across replicates	4,761	150	31.±0.34

Steps 9 and 10

Step 9: Marker Databases

A major challenge in genomics is how to both integrate and analyze rapidly increasing sequence information as a result of new technologies.

Step 10: Connecting Data to Samples and Phenotypes

As mentioned earlier, marker data are not free of errors. However, as illustrated in Table 4, certain marker systems may result in higher rates of error than others.

Table 4 Reliability of marker data among marker systems

Marker type	Polymorphism status of parents	Average % allele match to inbred parents ± S.D.	Average % partial mismatch ± S.D.
SSRs	Monomorphic	96.8±4.8	96.8±4.8
	Polymorphic	73.3 ± 1.6	73.3 ± 1.6
	All markers	81.9 ±1.4	81.9 ±1.4
SNP-MassARRAY	Monomorphic	98.3 ±2	98.3 ±2
	Polymorphic	95.4±5.5	95.4±5.5
	All markers	97.0 ±3.8	97.0 ±3.8
SNP-Invader	Monomorphic	98.3±1.6	98.3±1.6
	Polymorphic	94.2±6.3	94.2±6.3
	All markers	95.5±5.4	95.5±5.4

FYI: See more about Steps in Marker Data Production here.

Causes of Errors in Marker Data Production

Errors in Marker Production

Errors in marker production processes can have a huge impact on biological conclusions and, therefore, should not be neglected. Errors are due to various causes, but their occurrence and impact on data quality can be minimized by considering these causes in the production and analysis of the data. However, increased effort to control errors increases the costs per data point. Certain applications may require the most sensitive procedures and may warrant the high cost associated with error control, for example, procedures to estimate the degree of “contamination” with transgenes. There are other applications, however, which are more robust. For example, procedures for fingerprinting germplasm tend to be more robust, and even with considerable error they may still allow classification of germplasm into various categories (for example, different heterotic groups).

We will use testing of genetically modified organisms (GMOs) as an example of how errors may occur during various steps of data development, and how such errors can be minimized. GMO detection is conducted by both private and public entities and may focus on seed, food and feed. Testing of GMO is based on the detection of recombinant DNA (rDNA) or recombinant protein in the GMO.

Part A: GMO Detection Methods

The detection of rDNA (recombinant DNA) by the polymerase chain reaction (PCR) is widely used. Therefore, this section is entirely focused on causes of error in detection of rDNA (Fig. 3 and Table 5).

DNA-associated causes of errors that can impact the overall decision regarding presence or absence of rDNA in food and feed are listed in Table 5.

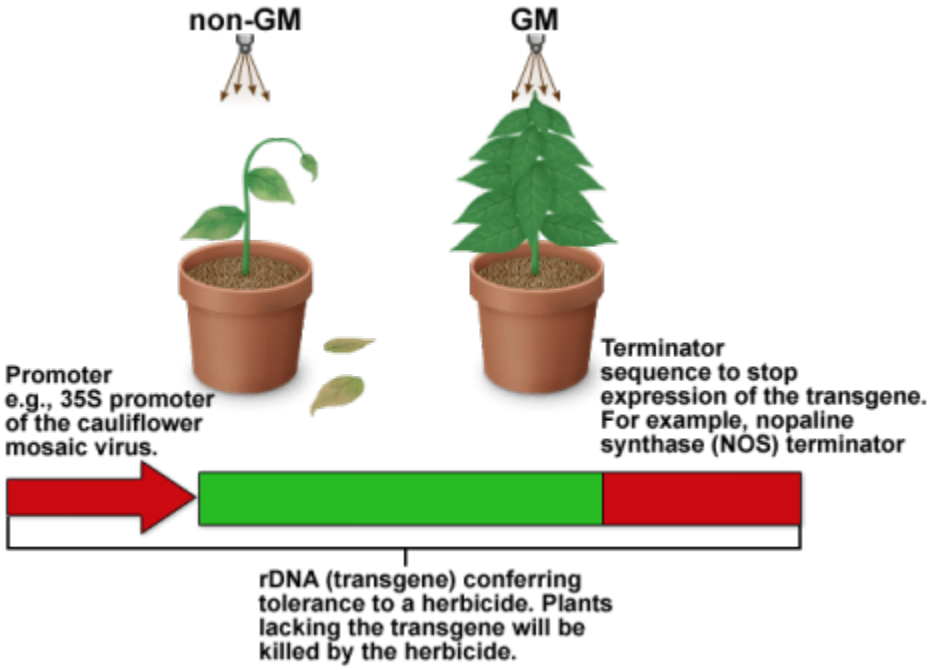

Fig. 3 The rDNA is the target for the DNA-based detection of GMOs. Plants are sprayed with a herbicide. GM plants contain a transgene conferring tolerance to the herbicide. Non GM plants are not tolerant to the herbicide.

DNA-Associated Causes of Errors

Table 5 DNA-associated causes of errors. Data from Pompanon et al., 2005.

Causes of error	How the error occurs	Effect of the error on data
DNA sequence flanking the marker	No amplification (or less efficient amplification) because of a mutation in the target primer sequence	Null product
DNA sequence flanking the marker	Insertion or deletion in the amplified fragment	Size homoplasmy of different targets
DNA sequence flanking the marker	In heterozygous individuals, preferential amplification of one allele when its denaturation is favored (because of low /GC content)	False-negative
Sample quality		
Contamination of the DNA by foreign DNA	Amplification of non-target sequence	Mistaken product
Presence of inhibitors in DNA solution	Inhibition of restriction enzymes and PCR failure	False-negative
Biochemical artifacts and equipment		
Low quality reagents	Poor fragment labeling and detection	False-negative, or mistaken product
Poor equipment precision or reliability	Uneven pipetting, evaporation during PCR, poor fluorescent label detection	False-negative, or mistaken product
Tag polymerase errors	Slippage in the steps of the PCR	False product
Tag polymerase errors	Incomplete addition of extra adenine residues at the 3' end of the amplified fragments	False product
Lack of specificity	Amplification of non-specific products that is due to annealing of the primer to another locus	Mistaken product
Lack of specificity	Non-specific restriction reaction	Mistaken product
Electrophoresis artifact	Inconsistency of allele size between different experiments	

Causes of error	How the error occurs	Effect of the error on data
Electrophoresis artifact	Distortion of the allele size by factors that alter DNA migration through a gel (for example, temperature or high concentration of PCR products)	Size homoplasmy of different products; mistaken product
Human error		
Sample handling	Confusion between samples (for example, mislabeling or tube mixing)	Mistaken product
Experimental error	Contamination with foreign DNA or cross-contamination between samples	Mistaken product
Experimental error	Use of wrong protocols (for example, omission of reagents, incorrect primers, or concentration of reactants)	False-negative; mistaken product
Data handling	Misreading of the profile or misidentification of florescence peak	Mistaken product
Data handling	Miscopying or confusion of the genotypes in the database	Mistaken product
Data handling	Data computation and analysis	Mistaken product

PCR in GMO Testing

PCR is used to determine presence (end-point PCR) or amount based on quantitative PCR (qPCR) amplification of rDNA in a sample. Therefore, many factors that affect the PCR method will also have a bearing on application of this method in GMO detection.

The predominant use of PCR in GMO testing stems from the following reasons:

- PCR allows the detection of the smallest amounts of DNA.
- The entire PCR reaction can be completed within hours.
- Automation of the PCR process allows processing of hundreds of samples in parallel.

The success in detecting small quantities of rDNA depends on PCR sensitivity. The sensitivity of PCR is affected by various factors (**Table 5**). Another important aspect is specificity of PCR, which determines whether a specific target or multiple targets will be amplified by the reaction. Before preparing samples for PCR analysis two important questions arise (1) How much sample should be analyzed and (2) How does sample size affect **limit of detection** and **limit of quantification**.

Limit of Detection

The first challenge in GMO detection is defining the limit of detection. Limit of detection (LOD) is the smallest amount of GMO which can be detected quantitatively with a sufficient degree of precision. The limits of GMO detection: **http://www.nature.com/nbt/journal/v19/n5/pdf/nbt0501_405.pdf**

The size of the genome of a species influences LOD of GM seeds in a ground sample. Using maize as an example, there is 0.0027% (wt/wt) of a single copy of the haploid maize genome in 100 ng DNA sample. Thus, levels of DNA below 0.0027% cannot be detected reliably in a 100 ng sample (Kay and Van dem Eede, 2001).

Limit of Quantification

Limit of quantification (LOQ) is the smallest amount of GMO for which a percentage can be determined with a sufficient degree of precision.

Reference Materials

Different kinds of reference materials are used as positive controls for qualitative and quantitative purposes in PCR-based detection. Certified powdered reference materials derived from GM and non-GM samples, or plasmids used for transformation can be used to validate PCR methods.

Part B: Sources of Error in GMO Testing

Sources of error in GMO testing can be classified into two groups, (1) Pre-laboratory sources of error, and (2) Laboratory sources of error.

1. Pre-Laboratory sources of errors

a. GMO introgression into fields

The possible source of GMO in conventional fields is surrounding authorized trial or commercial cultivation of GM varieties. For example, the chance of pollen from a GM plant fertilizing a non-GM plant is high for open-pollinated plants than for self-pollinated plants, and increases in cases of wind pollination than insect pollination.

b. GMO introgression into fields

Minute quantities of GMO in seeds can be carried over to GM free seed lots during transport, especially when the same containers are used for transportation of both GM and non GM products. Moreover, the PCR method is highly sensitive such that small amount of rDNA in dust may result in false-positive results. Therefore, one of the most critical considerations in GMO testing is prevention of cross-contamination of the samples.

c. Sampling

In order to identify seed lots with detectable amounts of GM seed before marketing, sampling must be made immediately after harvesting at the processing facility. Importantly, seed lot testing plans must establish (a) the number of individual seeds to sample and test, and (b) the maximum number of unacceptable seed that can be tolerated in the sample before a decision is made to reject the seed lot. Official sampling procedures are available (**http://www.seedtest.org/en/stats-tool-box-_content---1--1143.html**).

GM Testing Plan

For example, a testing plan is designed such that less than 5 out of 500 individuals testing positive for rDNA is acceptable, but results above this threshold warrants rejection. Whether such a plan is “good” or “bad” cannot be ascertained until certain parameters are established using statistical models. The models help define the following:

i. **Lower quality limit (LQL)** is the lowest level of purity in the seed lot than can be regarded as acceptable to the consumer.

ii. **Acceptable quality level (AQL)** is the lowest level of purity in a seed lot that current production practices can support.

iii. **Producer’s risk** is regarded as the chance of rejecting a seed lot that is nearly pure.

iv. **Consumer’s risk** is the chance of accepting a seed lot that contains a small amount of GM seed. In ideal situations, the consumer may prefer complete purity, that means LQL = AQL = 100%. However, due to practical limitations complete purity of seed lots may not be achieved.

It is important that AQL >LQL to establish a reasonable testing plan that takes into account both producer’s and consumer’s risks. If AQL<LQL, it would be impossible to produce seed that is pure enough to be acceptable to teh consumer. For example, AQL and LQL of 99.9% and 99% respectively may require a testing plan that rejects at least 95% of the samples with purity levels at or below the 99% LQL and accept at least 90% of the samples with purity of 99% or greater. ,>

Operation Characteristic Curve

Producer's and consumer's risk probabilities are based on binomial distribution probabilities. The formulas used in the calculations are described in a report by Remund et al., 2001. A statistical program called Seedcalc is used to evaluate testing plans against established criteria. Figure 4 is an example of an operation characteristic (OC) curve generated by the Seedcalc tool.

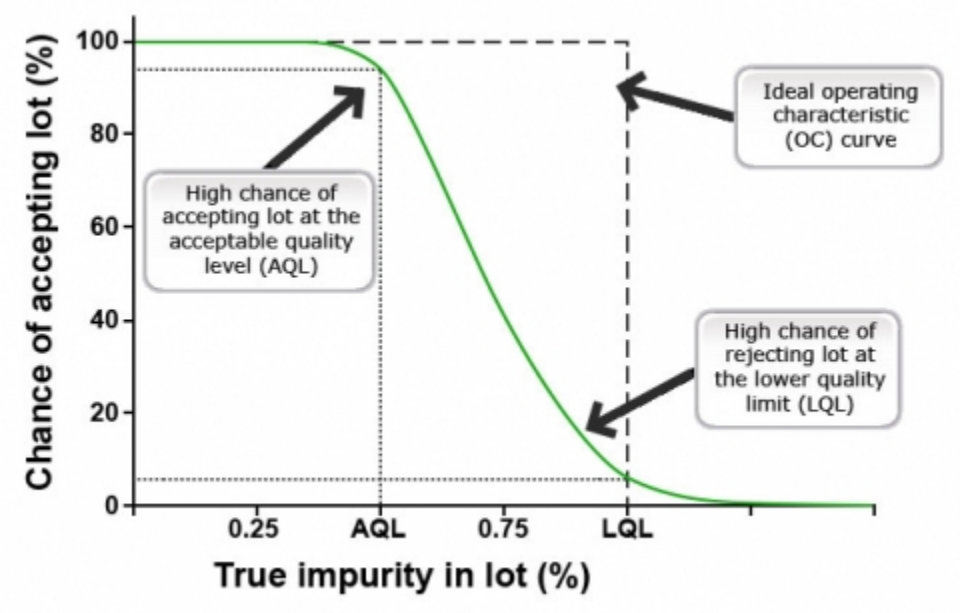

Fig. 4 An operation characteristic curve is a tool used to evaluate producer's and consumer's risks. The ideal OC curve can only be achieved by testing the entire seed lot. Adapted from Remund et al., 2001.

2. Laboratory sources of error

a. Sample preparation

Reducing the laboratory sample by grinding can affect both LOD and LOQ of GMO in a sample. It is important to ensure that samples are ground to sufficiently fine particles. More particles are present in a sample of finely ground mix. Importantly, different particle sizes affect DNA recovery the performance of qPCR performance (Holden et al. 2003). Care must also be taken to prevent cross-contamination by dust during sample preparation. Dust suction systems may be installed to control contamination.

b. DNA extraction

As discussed earlier, PCR is an enzymatic reaction and can be affected by the presence of inhibitors and other substances that can impair specificity. Assessment of DNA purity is necessary and must be done prior to running a PCR reaction. Also, DNA extraction methods must be optimized for the relevant rDNA target and a species.

c. PCR detection

Ironically, the weakness of the PCR in the context of GMO detection is the high sensitivity of the reaction. This means, even minute copies of rDNA in a PCR mix may result in a false positive outcome. Importantly, the source of contamination is often the previous PCR products that may have spilled over, or dried up and spread in aerosols. Another important source of contamination is dust generated during grinding of materials containing rDNA. The risk of false positives is also high in laboratories that handle reference materials for verification of specific transgenic events.

d. False-positives and false-negative results

In GMO testing if the test result is positive (genetic modification target is detected) when the actual condition is negative (GMO target is absent), this is referred to as a false positive. False positives occur due to carryover contamination with non-target DNA. The most significant source of contamination in PCR analysis is aerosols from previously performed PCR reactions and new samples. A false negative occurs if the test result is negative when the actual condition is positive. False negatives may occur due to certain causes of errors listed in **Table 4**, for example, human error. Information is available about ways to prevent false positive in PCR analysis:

- **http://www.ncbi.nlm.nih.gov/pubmed/1571142**
- **http://products.invitrogen.com/ivgn/product/AM9890**

e. Unexpected reactions

Unexpected reactions may occur as a result of both human and mechanical errors. Failure to design primers may render the process of rDNA quantification unreliable due to lack or primer specificity. Technologies such as TaqMan (the module on Markers and Sequencing) require the design of a primer and a probe for each GMO. However, there are no standardized procedures for developing such TaqMan primers and probes. In addition, the polymerase and other PCR reagents may become defective through cycles of freezing and thawing and need to be tested whenever new supplies are purchased. Uncalibrated instruments such as spectrophotometers and pipettes may result in incorrect DNA concentrations.

f. Method validation

The goal of method validation is to evaluate the performance characteristics and limitations of GMO testing methods. Parameters used for method validation are described in the **Parameters for GMO activity** page**.**

Data Management and Quality Control

Parameters for GMO Detection

An example of a system for handling and managing marker data (Fig. 5) is provided by the International Center for Agricultural Research in the Dry Areas (ICARDA) Generation Genomic LIMS & GEMS. The ICARDA system consists of four main components offering users and researchers means to manage and share research information. The ICARDA LIMS & GEMS components are (1) LIMS Laboratory Information Management System, (2) GEMS Gene Management System, (3) Storage Management, and (4) Extra tools and services.

- **Accuracy**
- **Precision**
- **Sensitivity**
- **Specificity**
- **Ruggedness (roughness)**
- **Applicability**
- **Repeatability**
- **Reproducibility**
- **Limit of detection (LOD)**
- **Limit of quantitation (LOQ)**
- **Range of quantification (ROQ)**

Parameters for GMO detection method validation.

Steps in Genotyping Process

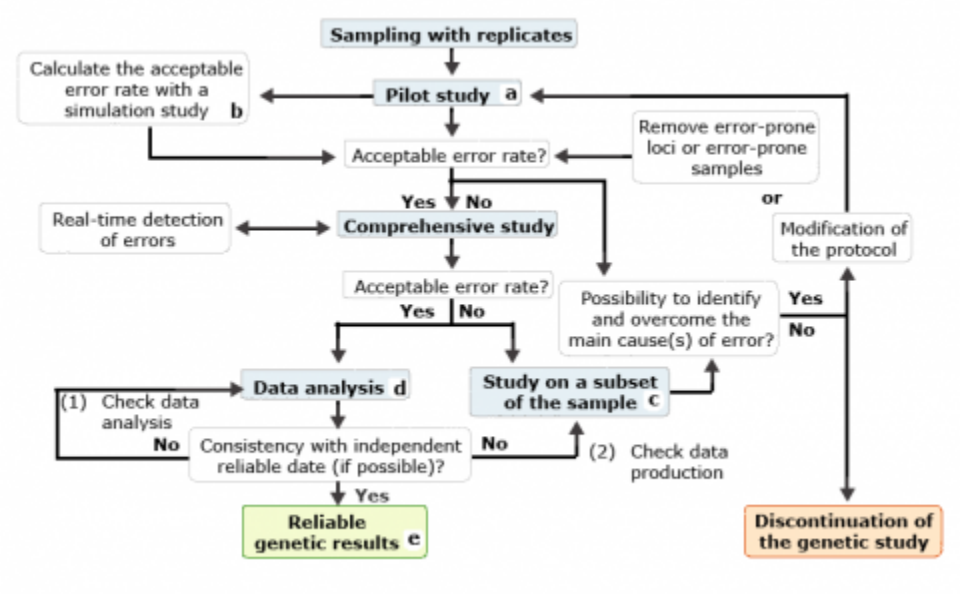

Fig. 5 Example of steps in genotyping process for minimizing generation and impact of errors in the ICARDA quality control system. Steps that end with a superscript letter (a-e) are defined as follows: a. Objective is to estimate the error rate associated with the samples, the method and the protocol used. This may be done by replicating a sufficient number of samples. b. Deciding on an acceptable error rate depends on the error rate, the purpose of the genetic study, the genotyping method used, the ability to detect eventual errors and the cost in terms of money and time. c. The control study aims to find the cause of errors that did not exist in the pilot study. d. The calculated error rate must be considered in the data analysis. e. The results with a reliability index that is based on the error rate measured are used for breeding purpose.

Status of Marker Technology in Breeding Companies

In 2000, Monsanto® switched to SNP-based genotyping at the Ankeny, IA, facility with gel-free detection systems and a fully automated genotyping process. From 2000 to 2006, total molecular marker data point production grew over 40-fold, while cost per data point decreased over six fold. More than 1 million SNP datapoints are handled per day by mostly automated pipelines in laboratories of major breeding companies.

Fig. 6 A genetic research laboratory. Photo by Iowa State University.

Reflection

The **Module Reflection** appears as the last "task" in each module. The purpose of the Reflection is to enhance your learning and information retention. The questions are designed to help you reflect on the module and obtain instructor feedback on your learning. Submit your answers to the following questions to your instructor.

1. In your own words, write a short summary (< 150 words) for this module.
2. What is the most valuable concept that you learned from the module? Why is this concept valuable to you?
3. What concepts in the module are still unclear/the least clear to you?

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Molecular Plant Breeding Data Management and Quality Control Author: Thomas Lübberstedt, and Walter Suza (ISU)

Multimedia Developers: Gretchen Anderson, Todd Hartnell, and Andy Rohrback (ISU)

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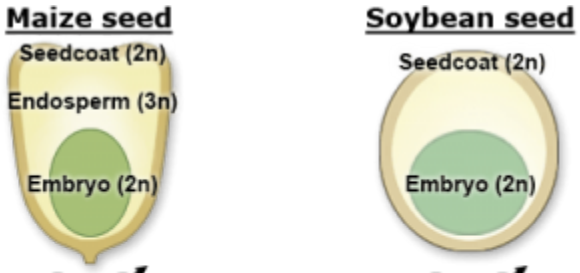
For Your Information

Steps in Marker Data Production

To learn more about bar code labeling for agriculture follow this link: **http://www.advanced-automationinc.com /industries/agriculture/**

Seed selection (chipping) technologies (**http://www.popsci.com/science/article/2011-01/ life-cycle-genetically-modified-seed**; **http://www.businessweek.com/news/2012-06-** 19/monsanto-files-suit-against-dupont-over-seed-development; **http://www.youtube .com/watch?v=gCb9TSpuxUU**) have helped to increase the throughput for genotyping without destroying the seed itself. Based on the genomic composition of seed (shown on the next slide), sampling the seed coat will only provide genomic information for the female parent, the endosperm (monocots) will provide information for both the male and the female parent (at unequal proportions). Although the embryo would provide equal proportions of the maternal and paternal genomes, it is not sampled to ensure seed viability.

Contribution of Haploid Genomes



SOURCE	♀	♂	♀	♂
EMBRYO	1	1	1	1
ENDOSPERM	2	1	NONE	
SEEDCOAT	2	0	2	0

Fig. 7 Contribution of haploid genomes from the parental gametes in seeds and tissues of maize and soybean. Adapted from Holst-Jensen et al., 2006.

DNA Isolation and Quality

More information:

- Effects of DNA extraction method and sample matrix on quantification of genetically modified organisms:
    - **http://www.biomedcentral.com/content/pdf/1472-6750-6-37.pdf**
- Evaluation of extraction methodologies for corn kernel DNA:
    - **http://pubs.acs.org/doi/pdf/10.1021/jf0211130**

Amplification/Labelling

More information:

- Avoiding false positives with PCR:
  - **http://www.nature.com/nature/journal/v339/n6221/pdf/339237a0.pdf**
  - Microarray-technology-based approaches are used to detect selected targets by hybridization of labeled PCR-amplified products. For example, Xu et al. (2007) developed event-specific oligonucleotide array for soybean. Also, a low density-DNA chip for the identification of transgenic events in maize is available (Leimanis et al., 2006). It is important to note that failure in PCR amplification of a target or labeling of targets will lead to failure to detect a transgenic event in a sample.
- Event-specific detection of GM targets in soybean by microarrays: **http://pubs.acs.org/doi/pdfplus/10.1021/jf070433m**
- A microarray-based detection system for GM foods: **http://www.ncbi.nlm.nih.gov/pubmed/16786296**

False Positive and Negative Results

Lamb and Booker (2011) describe a statistical approach based on simulation modeling to quantify low levels of GMO contamination to account for false positive and negative results in GMO testing.

The detection and quantification of the prevalence of genetically modified organism (GM) contamination in seed exports is a critical element of regulatory compliance. While the procedures to reliably detect high levels of GM contamination are well understood, no comparable statistical approaches are available for the quantification of levels of GM prevalence below the established detection rate of standard tests. We present a simple statistical approach based on simulation modelling for the quantification of low levels of GM contamination. The approach can be modified to match any sampling regime and can account for rates of false positive and negative assay results. The application of this method is demonstrated using the low level of contamination in Canadian flax breeder seed lots by the GM flax variety 'Triffid'. We show that GM contamination is likely present in seed lots at rates between two GM seeds per million and six seeds per hundred thousand. We also show that this low level of presumed contamination is indistinguishable from the number of positive tests expected from a clean seed lot given the potential rates of false positive tests.

Eric G. Lamb and Helen M. Booker (2011). Quantification of low-level genetically modified (GM) seed presence in large seed lots: a case study of GM seed in Canadian flax breeder seed lots. Seed Science Research, 21, pp 315-321. doi:10.1017/S0960258511000213.