**Training materials for seminars**

Discipline “Safety of GMO and organic products

**Lab. work 1**  The molecular biotechnology used for n creation GMOs.

Main approaches and methods of molecular biotechnology

Whole blood samples are one of the main sources used to obtain DNA, and there are many different protocols available to perform nucleic acid extraction on such samples. These methods vary from very basic manual protocols to more sophisticated methods included in automated DNA extraction protocols.

Solution-based DNA extraction methods using organic solvents

Solution-based DNA extraction methods using salting out

Solid-phase DNA extraction methods

DNA extraction methods using silica and silica matrices

DNA extraction using anion exchange resins

DNA extraction methods using magnetic beads

Main types of DNA extraction methods from human whole blood samples

Methods for genomic DNA isolation

Three general methods for genomic DNA isolation are common, and each is based on a different biochemical principle. Selection of a method is based on the throughput required, equipment available in the lab, and how the purified genomic DNA will be used.  
*Organic extraction and precipitation*

Genomic DNA isolation by organic extraction involves the addition of phenol and quanidine isothiocynate to separate the DNA and proteins into different organic phases. Organic extraction is a low-cost method and, with advanced reagents such as [DNAzol](https://www.thermofisher.com/order/catalog/product/10503027), is a straightforward method requiring very little equipment.  
 *Silica*

DNA binds to silica (aka glass fibers) under high-salt conditions and can be released under low-salt conditions. Silica-containing columns provide an easy way to bind, wash, and elute purified genomic DNA from multiple clarified cell lysates in parallel.

Columns are designed to flow buffers through centrifugation, vacuum, or gravity. [PureLink Genomic DNA Mini Kits](https://www.thermofisher.com/order/catalog/product/K182002) use spin column technology and work in a microcentrifuge for easy prep of up to 18 samples at a time. Spin plates provide the same isolation technology in a high-throughput, automation-friendly format.

Paramagnetic beads

In this method, paramagnetic (attracted to magnet) beads are added to the sample, and genomic DNA binds to the beads. Using a strong magnet, the beads are held in place while removing unwanted material. After washing, the genomic DNA is eluted from the beads in water or a low-salt buffer. The bead-based method, used in [MagMAX multi-sample DNA isolation](https://www.thermofisher.com/order/catalog/product/4413020) and related kits, is scalable and automation compatible.

CTAB PROTOCOL FOR ISOLATING DNA FROM PLANT TISSUES

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Isolating Deoxyribonucleic Acid (DNA) from plant tissues can be challenging as the biochemistry between divergent plant species can be extremely different. Unlike animal tissues where the same tissue type from different species usually has similar characteristics, plant tissue can have variable levels of metabolites and structural biomolecules. Polysaccharides and polyphenols are two classes of plant biomolecules that vary significantly between species and are problematic when isolating DNA. Contaminating polysaccharides and polyphenols can interfere with manipulations of DNA following isolation.

The following protocols for isolating clean plant DNA, both start with a traditional approach using a cetyltrimethylammonium bromide (CTAB) buffer. At that point they diverge, the first protocol makes use of phenol and chloroform, and the second protocol uses a reverse solid phase extraction (i.e., capturing contaminants on a solid phase). Methods using phenol and chloroform are preferred for the isolation of high molecular weight DNA, however both chemicals are considered hazardous. Phenol can cause very serious chemical burns while chloroform is a known carcinogen. The CTAB method using solid phase extraction, avoids phenol and chloroform and is best used for assays where smaller DNA fragment sizes are acceptable.

Skip ahead to the protocols:

[Plant DNA Isolation using Phenol/Chloroform Extraction](https://opsdiagnostics.com/notes/protocols/ctab_protocol_for_plants.htm#phenolpr)

[Plant DNA Isolation using Reverse Solid Phase Extraction](https://opsdiagnostics.com/notes/protocols/ctab_protocol_for_plants.htm#Synergy)

 ISOLATION CHEMISTRY

As mentioned, polysaccharides and polyphenols are problematic when isolating DNA from plant tissues. CTAB buffers are effective at removing polysaccharides and polyphenols from plant DNA preparations. CTAB (also called hexadecyltrimethylammonium bromide) is a cationic detergent that facilitates the separation of polysaccharides during purification while additives, such as polyvinylpyrrolidone, aid in inactivating polyphenols. CTAB based extraction buffers are widely used when purifying DNA from plant tissues. The hazard with traditional CTAB protocols is the protein component of plant lysates is usually removed using phenol and chloroform. These two solvents are generally considered hazardous. The solid phase protocol listed below is an alternative. CTAB is more than a surfactant and its properties can be used in several ways to purify DNA. One option for purifying DNA using CTAB exploits the different solubilities of polysaccharides and DNA in CTAB depending upon the concentration of sodium chloride. At higher salt concentrations (1.4 M), polysaccharides are insoluble, while at lower concentrations (600 mM) DNA is insoluble. Consequently, adjusting salt concentration in lysates with CTAB, polysaccharides and DNA can be differentially precipitated. Most methods use CTAB to remove polysaccharides, followed by protein removal and DNA separation using precipitation or spin columns.

Plant cells contain phenolic compounds, such as catechol, that are catalyzed by polyphenol oxidase to o-quinones. The o-quinones in turn can alkylate and inactivate proteins. Polyphenol oxidases are found in plastids (i.e., chloroplasts) while catechol is found in vacuoles. When plant cells and tissues are disrupted, the enzyme and substrate mix and generate the reactive o-quinones (which is associated with browning of damaged leaves and fruit). Therefore, homogenizing plant tissue yields reactive molecules that can potentially interfere with subsequent manipulation of the DNA. To avoid the production of o-quinones, phenolic precursors are captured by polyvinylpyrrolidone (PVP) that is present in the homogenization buffer. PVP binds strongly with aromatic compounds, such as catechol and subsequent polyphenols, and prevents the formation of reactive o-quinones. CTAB-based protocols tend to work very well, but with one significant disadvantage phenol/chloroform extractions are routinely used to separate protein from the DNA. As chloroform is carcinogenic, many institutions frown upon its use. Furthermore, phenol can cause serious chemical burns. The traditional protocol will be covered, as well as an alternative protocol that uses solid phase extraction. The solid phase extraction is the basis of the Synergy™ Plant DNA Extraction Kit.

**Lab. work 2** *Methods of extraction of nucleic acids from different biological materials*

DNA extraction protocols using organic solvents derived originally from a series of related RNA extraction methods. Some of the main steps used in these methods are:

1) cell lysis undertaken by adding a detergent/chaotropic-containing solution, including SDS or N-Lauroyl sarcosine;

2) inactivation of DNases and RNases, usually through the use of organic solvents;

3) purification of DNA and removal of RNA, lipids, and proteins;

4) resuspension of extracted nucleic acids.

PLANT DNA ISOLATION USING PHENOL/CHLOROFORM EXTRACTION

This method is best for isolating high molecular weight DNA. Caution is needed when working with liquid nitrogen, chloroform, and phenol. Consult your organization’s safety guidelines when working with these hazardous materials.

MATERIALS

* CTAB buffer: 2% cetyl trimethylammonium bromide, 1% polyvinylpyrrolidone, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, or CTAB Extraction Buffer
* Polypropylene tubes (don’t use polycarbonate tubes with phenol and chloroform)
* Centrifuge (at least 14,000 x g)
* RNase A Solution (10 mg/ml in water, DNase-free)
* Isopropanol
* 70% Ethanol
* 2 ml polypropylene centrifuge tubes
* Centrifugal Vacuum Concentrator (e.g., SpeedVac)
* TE Buffer (10 mM Tris, pH 8, 1 mM EDTA)
* Phenol/Chloroform/Isoamyl Alcohol (25:24:1 ratio) stored under TE buffer, pH 8

METHOD

Plant samples can be prepared by cryogenically grinding tissue in a mortar and pestle after chilling in liquid nitrogen. Freeze dried plants can be ground at room temperature. In either case, a fine powder is best for extracting DNA.

1. Transfer the ground plant tissue to a polypropylene tube.
2. For every 100 mg of homogenized tissue add 500 µl of CTAB Buffer. Mix and thoroughly vortex.
3. Place the tube in a 60°C water bath for 30 minutes.
4. Centrifuge the homogenate for 5 minutes at 14,000 x g.
5. Transfer supernatant to a new tube.
6. Add 5 µl of RNase A solution and incubate at 37°C for 20 minutes.
7. Add an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1).
8. Vortex for 5 seconds then centrifuge the sample for 1 minute at 14,000 x g to separate the phases.
9. Transfer the aqueous upper phase to a new tube. Repeat this extraction until the upper phase is clear.
10. Transfer the upper aqueous phase to a new tube.
11. Add 0.7 volume cold isopropanol and incubate at -20°C for 15 minutes to precipitate the DNA.
12. Centrifuge the sample at 14,000 x g for 10 minutes.
13. Decant the supernatant without disturbing the pellet and subsequently wash with 500 µl ice cold 70% ethanol.
14. Decant the ethanol. Remove the residual ethanol by drying in a SpeedVac.
15. Dry the pellet long enough to remove alcohol, but without completely drying the DNA.
16. Dissolve the DNA pellet in 20 µl TE buffer (10 mM Tris, pH 8, 1 mM EDTA). The pellet may need to be warmed, in order to dissolve.

OPTIONAL PROTOCOL:

[Protocol](https://opsdiagnostics.com/notes/protocols/spin_column_plant_protocol.html)for higher quality DNA, using silica spin columns to further purify the DNA.

**DNA extraction methods using silica and silica matrices**

Silica matrices have unique properties for DNA binding. They are positively charged and have high affinity toward the negative charge of the DNA backbone. High salt conditions and pH are achieved using sodium cations, which bind tightly to the negatively charged oxygen in the phosphate backbone of DNA. Contaminants are removed with a series of washing steps, followed by DNA elution under low ionic strength (pH ≥7) using TE buffer or sterile distilled water.A substance that contains high amounts of silica (up to 94%) known as kieselguhr, diatomite, or diatomaceous earth has also been used for DNA purification.

**Lab. work 3** *Construction of recombinant DNA.*

Creation of GM DNA is a complex multi-step process. You should know that before

DNA will be constructed that carry the necessary for transfer genetic information, it is necessary to search for organisms, sources of target genes. Having decided on the donor organism, from it DNA carrying the target gene is recovered.

The researcher must be aware gene characteristics, specific recognition sites in its structure,

allowing "cut" it from DNA. "Cutting out" of genes is carried out

with the participation of enzymes of type II restriction endonucleases (restriction enzymes).

The student should be familiar with several basic types of restrictases used to create GMOs: Eco RI, HpaI, HpaII, Bam HI, PstI NotI and others.

DNA- T4 ligase is another important enzyme that provides stable DNA double strand structure and target sequence crosslinking nucleotides with the vector.

*Choice of cloning vector (gene transfer)* – next an important step in the creation of recombinant DNA. It should be emphasized that vectors serve both to transfer the target gene into the organism being exposed

transgenosis (host organism), and for replication in the cell of the organism -

recombinant DNA host.

Therefore, the cloning vector must contain elements of replicative systems. Currently for transgenesis uses different types of vectors, for example: bacterial plasmids (pBR 322, pUC 19, Ti and Ri plasmids of agrobacteria, etc.) viruses (bacteriophage ?), cosmids (vectors combining features of plasmids and phage ?),

chimeric constructs (plasmid vector based on P1 phage),

artificial bacterial chromosome (BAC) and others.

*Special attention* should be given to Ti and Ri plasmids of agrobacteria, which arethe main type of vectors for creating GM plants. The student mustrepresent the structure of nopaline and octopine agrobacterial

plasmid, T-DNA region device, characterize agrobacterial oncogenes, virulence region, conjugative transfer genes, region replication.

The target gene must not only be transferred into the host cell, but also be expressed in it. To achieve efficient expression vector constructs must contain additional genetic elements that control transcription and translation, such as strong a promoter (eg, lac, trp, tac, 35S, etc.); and a transcription terminator.

In addition, it is important to ensure the stability of proteins and their secretion in host organism. Expression systems must also provide the strength of mRNA binding to the ribosome, a sufficient number of copies cloned gene, efficient translation and product stability in host cell.

*Main types of expression systems*:

*Yeast Saccharomyces cerevisiae,*

*Kluyveromyces lactis,*

*Pichia pastoris* and other;

cell cultures of insects, mammals, baculoviruses, etc.

For subsequent identification of transgenic cells into cloning vectors introduce additional genetic inserts - reporter or marker, selective genes.

At present, ***selective genes*** of the first, second and third generations.

The student must know main marker genes:

*NPT gene* (neomycin phosphotransferase),

*GUS gene* (glucuronidase),

*GFP* (green fluorescent protein) gene, virtues anddeficiencies of these genes as markers.

**Lab. work 4.** *Methods for introducing recombinant DNA into a cell.*

Finished genetic constructs are transferred to target cells (organisms). For this is used as physical (electroporation, ballistic methods, vacuum infiltration, DNA microinjection), and biological

methods (viral transduction, bacterial transformation, transplastomic and mitochondrial transformations). The student must show how these procedures are carried out, in what cases use certain methods of transfer, their effectiveness.

In more detail, the student should know and characterize the transfer

transgene into plants with the participation of *Agrobacterium tumefaciens*.

Necessary understand how plant cells are infected agrobacteria, which is a signal for bacteria to chemotaxis in direction of plant cells, how embedding occurs agrobacterial plasmids in plant DNA.

**Lab 5.** Theme methods of analyses of nucleic acids

**Lab. work 6.** *Methods of determination of vitamins A, C, E, plant pigments, indispensable unsaturated fatty acids (IUFA), alimentary cellulose, and pre- and probiotics*

**Lab 6.** *Pre and pos-marketing issues to be solve before GM crops commercialisation*

**Lab 7.** Theme Molecular Characterization of GMOs. Southern blot analysis and polymerase chain reaction (PCR) Cell cultures or transgenic organisms are mosaic in terms of the genome. Only a part of the cells contains a built-in transgene, but it is these cells that are of interest for gene engineers. They need to be selected for further cloning.

For this use different approaches, for example, cell selection on media with antibiotics, detection of reporter gene expression products and/or their activities, sexing techniques for selection of transgenic insects and

etc.

Direct evidence of the integration and operation of the transgene is its expression and synthesis of the target protein or other product. Selected transgenic cells or individuals must be propagated.

Building on earlier acquired knowledge of the courses "Plant Physiology" and "Biotechnology"

students independently master issues such as cloning genetically modified organism, gemma- and embryoidogenesis in tissue culture, micrografting of regenerants, etc.

**Lab 8.** Theme Molecular Characterization of GMOs. Polymerase chain reaction (PCR) in its various formats such as real-time PCR (qPCR).

Molecular characterization of GM crops is a full description of the structural information of the transgene and stability of the trait. It is the foundation of all GM product safety assessments before commercialization and also serves as a baseline for the development of detection and identification tools to satisfy traceability and labeling requirements ([European Parliament, 2003](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B55)).

Stakeholders of both GM food and feed, must provide information on the genomic locus/loci modified, copy number of the inserted transgene, insertion site, and flanking regions.

Selection of low insertion copy number DNA transformants is preferred for the subsequent safety assessment process as it facilitates risk and hazard characterization.

The methods most commonly used to determinate the number of transgenes integrated have been Southern blot analysis and polymerase chain reaction (PCR), in its various formats such as real-time PCR (qPCR).

The Southern blot analysis involves a careful selection and broad screening of restriction enzymes and designing of probes, which in some cases dependent on prior sequence information of the transgene insertion.

However, the approach is relatively time-consuming and laborious, and also includes a manual interpretation process. In addition, the result may not accurately reflect the copy number of a transgene, if sequence rearrangements have occurred, which have affected the position(s) of the restriction enzyme recognition site(s) in the inserted transgene(s)).

**Lab 9.** *Molecular Characterization of GMOs.* *A qPCR-based assay*

A qPCR-based assay can more accurately quantify the copy number of transgenes by comparing to an endogenous reference sequence (endogene), which has provided a simplified alternative to Southern blot analysis ([Li et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B117)). However, identification of a single copy reference gene is occasionally difficult in crop species, due to ancestral whole genome duplications or due to polyploidy, causing complex structures and genetic redundancy ([Ren et al., 2018](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B154)). To overcome the identification of a reference gene and dependency on DNA calibrations, droplet digital PCR (ddPCR), a method that identifies the absolute DNA copy number in a sample, has been proposed for determination of GM copy number. .

Following identification of low-copy number transformants, the precise location(s) of the transgene(s) in the crop genome is required to be identified.

DNA sequencing approaches have been used for this purpose, and this process may also identify backbone sequence(s), which were not intended to be introduced from the transformation vector into the host genome ([Kononov et al., 1997](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B108)). The method traditionally used for this purpose was based on Sanger sequencing ([Guttikonda et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B84)). However, the second-generation sequencing (SGS) technologies have been proposed as a new tool for molecular characterization of GM crops, due to a larger sequencing capacity and potentially higher accuracy of the resulting assembled sequence (Kovalic et al., 2012; [Yang et al., 2013](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B205); [Pauwels et al., 2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B145); [Arulandhu et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B5)).

The SGS approaches can increase speed, scalability, and automation in the selection of potential valuable events on the basis of their molecular profile, facilitating post-transformation screening ([Kovalic et al., 2012](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B109); Pauwels et al., 2015; [Guttikonda et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B84)). However, these technologies do not directly provide information about the position of the insertion(s) in native DNA, due to short read lengths (50–400 base pairs) ([Goodwin et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B81)), while transgenic constructs are typically thousands of base pairs. A computational process for alignment and/or assembly of the short sequencing reads is essential for the molecular characterization purposes, and repetitive elements commonly found in plant genomes can generate problems for the alignment/assembly procedure ([Liang et al., 2014](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B118)).

Recently, single-molecule sequencing, also termed third-generation sequencing (TGS) platforms, have been commercialized allowing a large increase in read length up to tens of thousands of bases per read ([Loose et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B121)). Read length is limited by the input DNA fragment size, but over 300 kb have been reported ([Jain et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B102)). The increment in read lengths up to tens of thousands can facilitate a more reliable GM characterization process, by extending the sequence reads of the flanking regions present in the captured fragments and potentially solving alignment problems.

The most common TGS platforms are products from Pacific Biosciences (PacBio, Menlo Park, CA, US) and Oxford Nanopore Technologies (ONT Oxford Science Park, Oxford). PacBio uses a sequencing-by-synthesis method to capture a single DNA molecule and a circular consensus sequence (CCS) to increase accuracy. The CCS uses a circular DNA template by ligating hairpin adaptors to both ends of target double-stranded DNA, so the DNA template is sequenced multiple times to generate a continuous long read ([Weirather et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B197)). Nanopore sequencing, uses nanopores to sequence native single-stranded DNA, by measuring the changes in an electric current passed across the pore as the DNA bases pass through, disrupting the current to different levels with different nucleotides ([Giordano et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B76)).

Nanopore sequencing offers potential benefits in molecular characterization of GM products compared with PacBio, since it delivers raw data in real-time, is relatively easy to manipulate, and has low setup costs. The MinION from ONT is a portable device that has been successfully assessed for detection of unauthorized GM products ([Fraiture et al., 2018](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B67)). Further assessment demonstrated the capability of the MinION to determine the full molecular characterization of three transgenic crops (ryegrass, canola and clover) within 48 hours.

Although, new guidelines are emerging from regulatory bodies to generate pre-market submission of data using whole genome sequencing ([Health Canada, 2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B89)) and NGS ([UCD Centre for Food Safety et al., 2018](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B185)). Sequencing approaches are typically being used in tandem, for example Sanger sequencing with SGS or SGS with TGS, for verification and validation purposes during risk assessment of GM crops GM Traceability

GM traceability describes a system that enables tracking of GM food/feed products at all stages of the supply chain ([Giraldo et al., 2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B78)). Detection methods for GM products in different matrixes or substrates, such as grain, flour and forage, are not only important to ensure legality and traceability, but also to comply with GM labeling regulations ([European Parliament, 2003](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B55)).

Methods for GM detection and identification usually rely on certified reference materials that are in powdered form, however, routine detection must be performed in different agricultural and food products ([Cankar et al., 2006](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B26)). The selection of DNA extraction protocols is of crucial importance, since the DNA can be present in low amounts, carrying inhibitors or degraded ([SanJuan-Badillo et al., 2014](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B164)). Therefore, the extraction method should be evaluated for each agricultural product, guaranteeing high DNA yield and purity ([Turkec et al., 2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B183)).

The method chosen to comply to traceability and labeling requirements, should be sensitive enough to detect the transgene(s) at levels below the corresponding jurisdiction tolerance threshold (e.g., 5% in US, 1% AU, and 0.9% in EU) ([Ramessar et al., 2010](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B152)). Additionally, it should be able to detect the transgene(s) from raw agricultural commodities entering the feed production chain. For instance, fresh leaves, dry leaves (hay), pollen, seeds, tillers or stems, and forage that could enter the feed chain as unprocessed material ([Ardizzone et al., 2018](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B11); [Giraldo et al., 2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B78)).

Currently, qPCR is the standard method used in national reference laboratories for detection and quantification of GM events ([Dalmira et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B35)). The requirement for reference material to be used as calibrants, which sometimes are not commercially available, limits its effectiveness ([Dobnik et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B44); [Dalmira et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B35)). The GM product detection process followed by national reference laboratories consist of two consecutive steps; first, a qPCR screening of vectors commonly found in GM products, such as the 35S promoter from cauliflower mosaic virus, *Agrobacterium tumefaciens* (tNOS) and selectable markers. Then, the samples with a potential presence of GM materials, are tested using the corresponding GM event-specific method ([Fraiture et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B66)).

**Lab 10.** Theme: Molecular characterization of GM crops. Techniques for determination of alimentary cellulose, and pre- and probiotics.

is a full description of the structural information of the transgene and stability of the trait ([Li et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B117)). It is the foundation of all GM product safety assessments before commercialization and also serves as a baseline for the development of detection and identification tools to satisfy traceability and labeling requirements ([European Parliament, 2003](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B55)).

Stakeholders of both GM food and feed, must provide information on the genomic locus/loci modified, copy number of the inserted transgene, insertion site, and flanking regions ([Guttikonda et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B84)).

` Selection of low insertion copy number DNA transformants is preferred for the subsequent safety assessment process as it facilitates risk and hazard characterization ([Tiwari and Singh, 2018](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B180)). The methods most commonly used to determinate the number of transgenes integrated have been Southern blot analysis and polymerase chain reaction (PCR), in its various formats such as real-time PCR (qPCR) ([Li et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B117)).

*The Southern blot analysis* involves a careful selection and broad screening of restriction enzymes and designing of probes, which in some cases dependent on prior sequence information of the transgene insertion ([Urquiza and Silva, 2014](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B187)). However, the approach is relatively time-consuming and laborious, and also includes a manual interpretation process. In addition, the result may not accurately reflect the copy number of a transgene, if sequence rearrangements have occurred, which have affected the position(s) of the restriction enzyme recognition site(s) in the inserted transgene(s) ([Yang et al., 2005](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B204)).

**Lab 11.** Theme: A qPCR-based assay to quantify the copy number of transgenes. Methods of determination of vitamins A, C, E, plant pigments, indispensable unsaturated fatty acids (IUFA).

A qPCR-based assay can more accurately quantify the copy number of transgenes by comparing to an endogenous reference sequence (endogene), which has provided a simplified alternative to Southern blot analysis ([Li et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B117)). However, identification of a single copy reference gene is occasionally difficult in crop species, due to ancestral whole genome duplications or due to polyploidy, causing complex structures and genetic redundancy ([Ren et al., 2018](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B154)). To overcome the identification of a reference gene and dependency on DNA calibrations, droplet digital PCR (ddPCR), a method that identifies the absolute DNA copy number in a sample, has been proposed for determination of GM copy number ([Głowacka et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B70); [Dalmira et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B35)).

Following identification of low-copy number transformants, the precise location(s) of the transgene(s) in the crop genome is required to be identified ([Park et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B144)). DNA sequencing approaches have been used for this purpose, and this process may also identify backbone sequence(s), which were not intended to be introduced from the transformation vector into the host genome ([Kononov et al., 1997](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B108)). The method traditionally used for this purpose was based on Sanger sequencing ([Guttikonda et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B84)). However, the second-generation sequencing (SGS) technologies have been proposed as a new tool for molecular characterization of GM crops, due to a larger sequencing capacity and potentially higher accuracy of the resulting assembled sequence ([Kovalic et al., 2012](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B109); [Yang et al., 2013](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B205); [Pauwels et al., 2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B145); [Arulandhu et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B5)).

The SGS approaches can increase speed, scalability, and automation in the selection of potential valuable events on the basis of their molecular profile, facilitating post-transformation screening ([Kovalic et al., 2012](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B109); [Pauwels et al., 2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B145); [Guttikonda et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B84)). However, these technologies do not directly provide information about the position of the insertion(s) in native DNA, due to short read lengths (50–400 base pairs) ([Goodwin et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B81)), while transgenic constructs are typically thousands of base pairs. A computational process for alignment and/or assembly of the short sequencing reads is essential for the molecular characterization purposes, and repetitive elements commonly found in plant genomes can generate problems for the alignment/assembly procedure ([Liang et al., 2014](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B118)).

Recently, single-molecule sequencing, also termed third-generation sequencing (TGS) platforms, have been commercialized allowing a large increase in read length up to tens of thousands of bases per read ([Loose et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B121)). Read length is limited by the input DNA fragment size, but over 300 kb have been reported ([Jain et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B102)). The increment in read lengths up to tens of thousands can facilitate a more reliable GM characterization process, by extending the sequence reads of the flanking regions present in the captured fragments and potentially solving alignment problems.

The most common TGS platforms are products from Pacific Biosciences (PacBio, Menlo Park, CA, US) and Oxford Nanopore Technologies (ONT Oxford Science Park, Oxford). PacBio uses a sequencing-by-synthesis method to capture a single DNA molecule and a circular consensus sequence (CCS) to increase accuracy. The CCS uses a circular DNA template by ligating hairpin adaptors to both ends of target double-stranded DNA, so the DNA template is sequenced multiple times to generate a continuous long read ([Weirather et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B197)). Nanopore sequencing, uses nanopores to sequence native single-stranded DNA, by measuring the changes in an electric current passed across the pore as the DNA bases pass through, disrupting the current to different levels with different nucleotides ([Giordano et al., 2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6918800/#B76)).

**Lab 12.** Theme Food additives in organic products.

**10 Common Food Additives Found in Natural or Organic Foods**

Carrageenan. ...

Xanthan gum. ...

Guar gum. ...

Ascorbic acid. ...

Agar. ...

Gelatin. ...

Natural Flavors. ...

Lecithin.

**Lab 13.** Theme Nutritional quality of organic food

The major points are:

1/ organic plant products contain more dry matter and minerals (Fe, Mg); and contain more anti-oxidant micronutrients such as phenols and salicylic acid,

2/ organic animal products contain more polyunsaturated fatty acids,

3/ data on carbohydrate, protein and vitamin levels are insufficiently documented,

4/ 94–100% of organic food does not contain any pesticide residues,

5/ organic vegetables contain far less nitrates, about 50% less; and 6/ organic cereals contain overall similar levels of mycotoxins as conventional ones.

Thus, organic agricultural systems have already proved able to produce food with high quality standards. I propose also improvements of organic production to achieve sustainable food production for humans in the near future.

**Lab 14.** Theme Methods of determination of pesticides.

Pesticides are applied widely to protect plants from disease, weeds and insect damage, and usually come into contact with soil, where they undergo a variety of transformations that provide a complex pattern of metabolites. This article reviews the most relevant analytical methods for determining pesticides and their transformation products in soils. We address some recent advances in sampling and sample-preparation technologies for soil analysis. We discuss and critically evaluate procedures, such as liquid extraction methods (pressurized liquid extraction or microwave-assisted extraction) and solid-phase based methods (headspace solid-phase microextraction, solid-phase microextraction or matrix-solid-phase dispersion). Analysis of pesticides is generally carried out by;

gas chromatography (GC) or liquid chromatography (LC) coupled to different detectors, especially to mass spectrometers (MSs).

However, alternative and/or complementary methods, using capillary electrophoresis (CE), biosensors and bioassays have emerged recently.

Pesticides are widely applied to prevent unwanted pests from attacking crops and livestock which led to their access into the environment. Overuses of pesticides in environment are presence of pesticide residues and their metabolites that are causing serious detrimental effects on human health and all other living organisms. Several severe diseases (Cancer, chronic obstructive pulmonary disease, birth defects, infertility) and more damages of human health are associated with the exposure of pesticides. The maximum residue limits for pesticides have been regulated by the Codex Alimentarius Commission and European Union to protect human health. Thus, monitoring these compounds is extremely important to ensure that only permitted levels of pesticide are consumed. To date, several techniques have been developed for pesticide detection, from conventional analytical to advanced detection techniques.

The conventional analytical methods are [gas chromatography](https://www.sciencedirect.com/topics/food-science/gas-chromatography) and [high performance liquid chromatography](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/high-performance-liquid-chromatography) coupled with various detectors involved a sample preparation step prior to further analysis. Advanced detection methods refer to the sensors development such as electrochemical, optical, piezoelectric and molecular imprinted polymer. In this review, we summarized and explained the available analytical and advanced methods for determination of pesticides compound in environment and foodstuffs. Also, pesticides classification and its toxicity, and available extraction methods are briefly discussed.

**Lab 15.** Theme Contamination by microorganisms and mycotoxins

The contamination of food and feeds with mycotoxins poses a global health risk to humans and animals, with major economic consequences. Good agricultural and manufacturing practices can help control mycotoxin contamination. Since these actions are not always effective, several methods of decontamination have also been developed, including physical, chemical, and biological methods. Biological decontamination using microorganisms has revealed new opportunities. However, these biological methods require legal regulations and more research before they can be used in food production. Currently, only selected biological methods are acceptable for the decontamination of feed. This review discusses the literature on the use of microorganisms to remove mycotoxins and presents their possible mechanisms of action. Special attention is given to Saccharomyces cerevisiae yeast and lactic acid bacteria, and the use of yeast cell wall derivatives.