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Molecular biomarker analysis — Vocabulary for molecular biomarker analytical methods in agriculture and food production

Analyse de biomarqueurs moléculaires — Vocabulaire pour les méthodes d'analyse de biomarqueurs moléculaires dans l'agriculture et la production agroalimentaire

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

This second edition cancels and replaces the first edition (ISO 16577:2016), which has been technically revised.

The main changes are as follows:

— definitions have been updated, and new definitions have been added;

— typographical errors have been corrected.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Molecular biomarker analytical testing methods in agriculture and food production cover a broad spectrum of molecular technologies including but not limited to the analysis of nucleic acids, proteins, lipids and glycosides for biomarker identification and quantification, variety identification and detection of plant pathogens. This document includes terminology for biomolecular methods and processes in the food chain from primary production to consumption, as well as animal and vegetable propagation materials, in particular, as applied to sampling, methods of test and analysis, product specifications, food and feed safety, quality management, and requirements for packaging, storage and transportation. It includes terms that are useful metrologically in biomarker analysis of food and food products such as those from Codex Alimentarius and those applied to genetically modified organism (GMO) testing. It is important that a harmonized compendium of terms is available so that terms are used accurately and consistently throughout this field of standardization.

The terms in this document conform to the foundational FAIR principles: findability, accessibility, interoperability and reusability. They serve as a basis for terminology applied to horizontal methods for molecular biomarker analysis of food products.

Molecular biomarker analysis — Vocabulary for molecular biomarker analytical methods in agriculture and food production

1 Scope

This document defines terms for horizontal methods for molecular biomarker analysis in agriculture and food production.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1 Bioinformatics

3.1.1
bioinformatic analysis
bioinformatics
multidisciplinary examination of life sciences data using information technology as part of the methodology, as well as a reference to specific analytical "pipelines" to understand and interpret these biological data

Note 1 to entry: Life sciences data include genomics (including sequencing, massively parallel sequencing, metagenomics, epigenomics and functional genomics), transcriptomics, translatomics, proteomics, metabolomics, lipidomics, glycomics, enzymology, immunochemistry, life science imaging, synthetic biology, systems biology, systems medicine and related fields.

3.1.2
FASTA format
text-based format for representing either nucleotide sequences or amino acid (protein) sequences, in which nucleotides or amino acids are represented using single-letter codes

Note 1 to entry: A sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line (defline) is distinguished from the sequence data by a greater-than (">") symbol at the beginning. It is recommended that all lines of text be shorter than 80 characters in length.

Note 2 to entry: An example sequence in FASTA format is:

>P01013 GENE X PROTEIN (OVALBUMIN-RELATED)
QIKDLLVSSSTDLDTTLVLVNAIYFKGMWKTAFNAEDTREMPFHVTKQESKPVQMMCMNNSFNVATLPAEKMKILELP-
FASGDLSMLVLLPDEVSDLERIEKTINFEKLTEWTNPNTMEKRRVKVYLPQMKIEEKYNLTSVLMALGMTDLFIPSANLT-
GISSAESLKISQAVHGAFMELSEDGIEMAGSTGVIEDIKHSPESEQFRADHPFLFLIKHNPTNTIVYFGRYWSP*

Note 3 to entry: Blank lines are not allowed in the middle of FASTA input. Sequences are represented in the standard IUB/IUPAC amino acid and nucleic acid codes, with these exceptions:

— lower-case letters are accepted and are mapped into upper-case;

— a single hyphen or dash can be used to represent a gap of indeterminate length;

— in amino acid sequences, U and * are acceptable letters.

It is common to end the sequence with an "*" (asterisk) character and to leave a blank line between the description and the sequence.

3.1.3
FASTQ format
FASTQ files
text based format for nucleic acid sequence files that includes the sequence and per base Phred or Q quality scores

Note 1 to entry: FASTQ files consist of a definition line that contains a read identifier and possibly other information, nucleotide base calls, a second description line (definition line), and per-base quality scores, all in text form.

Note 2 to entry: There are many variations of FASTQ formats.

Note 3 to entry: The following terms and formats are defined in general:

— Decimal-encoding, space-delimited: [0-9]+ | <quality>\s[0-9]+

— Phred-33 ASCII: [\!\"\#\$\%\&\'\(\)*\+,\-\.\/0-9:;<=>\?\@A-I]+

— Phred-64 ASCII: [\@A-Z\[\\\]\^_`a-h]+

Note 4 to entry: Quality string length should be equal to sequence length.

Note 5 to entry: In a limited set of cases, log odds or non-ASCII numerical quality values will succeed during a sequence read archive (SRA) submission. Files from various platforms employing this format are acceptable:

> @<identifier and expected information>; <sequence>; +<identifier and other information OR empty string>; <quality>

Note 6 to entry: Where each instance of Identifier, Bases and Qualities are newline-separated, extra information added beyond the < identifier and expected information > examples is likely to be discarded/ignored. As indicated above, the Qualities string can be space-separated numeric Phred scores or an ASCII string of the Phred scores with the ASCII character value = Phred score plus an offset constant used to place the ASCII characters in the printable character range. There are two predominant offsets: 33 (0 = !) and 64 (0=@).

3.1.4
metadata
data providing information about one or more aspects of the datasets ("data about data")

EXAMPLE Means of creation of the data, purpose of the data, file size, data quality, source of the data.

Note 1 to entry: Metadata are used to summarize basic information about data which can make tracking and working with specific data easier.

3.2 Immunology

3.2.1
antibody
Ab
host proteins produced in response to the presence of foreign molecules, organisms or other agents in the organism

Note 1 to entry: Antibodies are useful reagents that can bind with high affinity to chosen antigens.

Note 2 to entry: In animals, antibodies are synthesized predominantly by plasma cells, terminally differentiated cells of the B-lymphocyte lineage, and circulate throughout the blood and lymph where they bind to antigens.

3.2.2
antibody specificity
ability of an antibody to specifically bind to an antigenic determinant (epitope) but not to other similar structures on that or other antigens

3.2.3
antigen
Ag
molecule, macromolecule or molecular structure, containing epitopes that can be bound by an antibodies (Ab), B-cells or T-cells

Note 1 to entry: The presence of antigens in vertebrates can trigger an immune response.

Note 2 to entry: The antigen binding site of an antibody is formed by the variable regions of the heavy and light chains.

3.2.4
blocking reagent
compound used to saturate the residual unspecific binding sites

Note 1 to entry: Blocking agents are typically used in preparation of an ELISA plate: blocking the plate with a non-reactive protein is used to prevent non-specific adsorption of proteins added in subsequent steps and storage of the ELISA plates.

Note 2 to entry: A blocking reagent can be used to decrease the background in protein or nucleic acid hybridization methods.

3.2.5
conjugate
material produced by attaching two or more substances together by a covalent bond via chemical groups

Note 1 to entry: Conjugates of antibodies with fluorochromes (e.g. a chemical entity, such as a molecule or group that emits light in response to excitation by absorbed incident light, radiolabelled substances, gold or enzymes) are often used in immunoassays.

3.2.6
enzyme-linked immunosorbent assay
ELISA
in vitro assay used for qualitative, semi-quantitative or quantitative purposes that combines enzyme-linked antibodies and a substrate to form a coloured or a fluorescence emitting reaction product

3.2.7
epitope
antigenic determinant
spatially localized components of an antigen to which an antibody binds that can be formed by contiguous or non-contiguous amino acid sequences and haptens

Note 1 to entry: The association between an Ab and an Ag involves myriad of non-covalent interactions between the epitope (the binding site on the Ag) and the paratopes (the binding site on the Ab).

3.2.8
lateral flow device
LFD
lateral flow membrane assay
lateral flow strip
LFS
immunoassay in which antibodies are bound in specific zones on a porous membrane of one or more layers, and where a liquid sample is applied to one end of the membrane and drawn through the reagent zones by capillary action, usually assisted by an absorbent at the opposite end of the membrane

Note 1 to entry: Typically, a coloured "control line" furthest from the end of the strip that is inserted into the sample indicates whether the test performed successfully. Results of the test are indicated by the presence or absence of one or more additional test lines that are expected between the point of sample application and the "control line".

Note 2 to entry: Immunoassay is the most common form of LFD but other biorecognition systems, e.g. nucleic acid hybridization, are also used.

3.2.9
monoclonal antibody
mAb
population of antibody molecules that share the same amino acid sequence, bind the same epitope, and are produced by a cell line derived from a single clonal cell or are produced recombinantly

3.2.10
polyclonal antibody
population of antibody molecules secreted by different B-cell lineages that react against a specific antigen, each potentially identifying a different epitope

3.3 Metrology

3.3.1
absolute error
result of a measurement minus the true value of the measurand

3.3.2
accordance
similarity of consistent results from a qualitative method (i.e. both positive or both negative) from identical test items analysed in the same laboratory under repeatability conditions

3.3.3
accuracy
closeness of agreement between a test result or measurement result and a reference value

Note 1 to entry: The term "accuracy", when applied to a set of test results or measurement results, involves a combination of random components and a common systematic error or bias component.

Note 2 to entry: When applied to a test method, the term "accuracy" refers to a combination of trueness and precision.

3.3.4
analyte
component of a system to be analysed

Note 1 to entry: This definition can be applied to molecular biological analytical methods, e.g. protein, lipid, RNA or DNA.

3.3.5
applicability
fitness for purpose
scope of application of the method identifying the matrix, analyte or species being measured, its concentration range and the type of study or monitoring, or both, efforts for which the procedure is suited, as judged from its performance characteristics

Note 1 to entry: In addition to a statement of the range of capability of satisfactory performance for each factor, the statement of applicability (scope) may also include warnings as to known interference by other analytes, or inapplicability to certain matrices and situations.

3.3.6
applicability range
range of quantification
dynamic range
quantity interval within which the analytical procedure has been demonstrated by a collaborative trial or other appropriate validation (e.g. reference materials or dilutions) to have a suitable level of precision and accuracy

3.3.7
background
intrinsic level of signal resulting from the instruments, reagents and consumables used in the reaction

3.3.8
baseline
level of detection or the point at which a reaction reaches a signal intensity above the background level

Note 1 to entry: The baseline is used in quantitative polymerase chain reaction analyses and can be automatically set by the instrument or manually determined.

3.3.9
bias
measurement bias
difference between the expectation of the test result or measurement result and the true value or conventional quantity value

Note 1 to entry: Bias is the total systematic error as contrasted to random error. There can be one or more systematic error components contributing to bias. A larger systematic difference from the accepted reference value is reflected by a larger bias value.

Note 2 to entry: The bias of a measuring instrument is normally estimated by averaging the error of indication over the appropriate number of repeated measurements. The error of indication is the "indication of a measuring instrument minus a true value of the corresponding input quantity".

Note 3 to entry: Expectation is the expected value of a random variable, e.g. assigned value or long-term average estimate of a systematic measurement error.

3.3.10
binary result
qualitative result
result from a method of analysis where there are only two possible outcomes

3.3.11
calibration
operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties, and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

Note 1 to entry: A calibration may be expressed by a statement, calibration function, calibration diagram, calibration curve or calibration table. In some cases, it may consist of an additive or multiplicative correction of the indication with associated measurement uncertainty.

Note 2 to entry: Calibration should not be confused with adjustment of a measuring system, often mistakenly called "self-calibration" nor with verification of calibration.

Note 3 to entry: Often the first step in the above definition is perceived as being calibration.

3.3.12
certified reference material
CRM
reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceability, using valid procedures

Note 1 to entry: Documentation is given in the form of a "certificate".

Note 2 to entry: Procedures for the production and certification of CRMs are given in, for example, ISO 17034 and ISO Guide 35.

Note 3 to entry: In this definition, "uncertainty" covers both "measurement uncertainty" and "uncertainty associated with the value of the nominal property", such as for identity and sequence. "Traceability" covers both "metrological traceability of a value" and "traceability of a nominal property value".

Note 4 to entry: Specified values of CRMs require metrological traceability with associated measurement uncertainty.

3.3.13
coefficient of variation
C_v
DEPRECATED: relative standard deviation
standard variation divided by the mean

Note 1 to entry: The coefficient of variation is commonly reported as a percentage.

3.3.14
colorimetric detection
method of detecting an analyte by measuring a colorimetric signal, usually by using a spectrophotometer

EXAMPLE A method for detecting hybridization using immobilized probe DNA measuring a colorimetric signal.

Note 1 to entry: A fluorophore such as SYBR® Green[1)] may also be used.

Note 2 to entry: An enzyme-linked detection system (conjugated with an enzyme) is often used to measure the signal in an ELISA assay colorimetrically.

Note 3 to entry: Colloidal gold is also used for this purpose in lateral flow devices.

Note 4 to entry: Colorimetry may be used qualitatively or quantitatively.

3.3.15
concordance
similarity or agreement of results (i.e. both positive or both negative) from identical test items that are analysed in two different laboratories in terms of qualitative analysis

3.3.16
conventional quantity value
DEPRECATED: conventional true quantity
number and reference together expressing magnitude of a quantity attributed by agreement to a quantity for a given purpose

Note 1 to entry: Sometimes a conventional quantity value is an estimate of a true quantity value.

1) SYBR® Green is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

Note 2 to entry: A conventional quantity value is generally accepted as being associated with a suitably small measurement uncertainty, which can be effectively considered to be zero.

3.3.17
critical value
value of the net concentration or amount, the exceeding of which leads, for a given error probability, α, to the decision that the concentration or amount of the analyte in the analysed material is larger than that in the blank material:

$$P_r\left(\hat{L} > L_C \,\middle|\, L = 0\right) \leq \alpha$$

where

P_r is the probability function

$\hat{L}$ is the estimated value

L_C is the critical value

L is the expectation or true value

Note 1 to entry: The definition of critical value is important for defining the limit of detection (LOD). The critical value L_C is estimated by:

$$L_C = t_{1-\alpha\nu} s_o$$

where $t_{1-\alpha\nu}$ is Student's-t, based on ν degrees of freedom for a one-sided confidence interval of 1–α, and s_o is the sample standard deviation.

Note 2 to entry: If L is normally distributed with known variance, i.e. $\nu = \infty$ with the default α of 0,05, then $L_C = 1{,}645 s_o$.

Note 3 to entry: A result falling below the L_C triggering the decision "not detected" should not be construed as demonstrating analyte absence. Reporting such a result as "zero" or as < LOD is not recommended.

Note 4 to entry: The estimated value and its uncertainty should always be reported.

3.3.18
defining method of analysis
empirical method of analysis
conventional method of analysis
method in which the quantity measured is defined by the result found upon following the stated procedure

Note 1 to entry: Defining methods of analysis are used for purposes that cannot be covered by rational methods.

Note 2 to entry: Bias in defining methods of analysis is conventionally zero.

3.3.19
error
measured quantity value minus a reference quantity value

Note 1 to entry: The concept of measurement "error" can be used: a) when there is a single reference quantity value to refer to, which occurs if a calibration is made by means of a measurement standard with a measured quantity value having a negligible measurement uncertainty or if a conventional quantity value is given, in which case the measurement error is known; and b) if a measurand is supposed to be represented by a unique true quantity value or a set of true quantity values of negligible range, in which case the measurement error is not known.

3.3.20
expanded measurement uncertainty
product of a combined standard measurement uncertainty and a factor larger than the number one

Note 1 to entry: The factor depends upon the type of probability distribution of the output quantity in a measurement model and on the selected coverage probability.

Note 2 to entry: The term "factor" in this definition refers to a coverage factor.

Note 3 to entry: Expanded measurement uncertainty is termed "expanded uncertainty".

3.3.21
false negative
error of failing to reject a null hypothesis when it is in fact not true

Note 1 to entry: A false negative is the result for a positive sample that has been classified as negative by the method/analysis.

3.3.22
false negative rate
probability that a known positive test sample has been classified as negative by the method

Note 1 to entry: The false negative rate is the number of misclassified known positives divided by the total number of positive test samples.

3.3.23
false positive
error of rejecting a null hypothesis when it is actually true

Note 1 to entry: A false positive is the result for a negative sample that has been classified as positive by the method/analysis.

3.3.24
false positive rate
probability that a known negative test sample has been classified as positive by the method

Note 1 to entry: The false positive rate is the number of misclassified known negatives divided by the total number of negative test samples.

3.3.25
good laboratory practice
GLP
set of rules and regulations issued by an authoritative body or standards organization, or generally agreed upon best practices for laboratory operation, that establishes broad methodological guidelines for laboratory procedures and record keeping

Note 1 to entry: GLP in the regulatory environment is a set of requirements that guide how laboratory studies are planned, performed, monitored, recorded, reported and archived in order to ensure the credibility and traceability of data submitted to regulatory bodies. Requirements can differ between countries.

3.3.26
HorRat
normalized performance parameter indicating the acceptability of methods of analysis with respect to interlaboratory precision (reproducibility)

Note 1 to entry: The HorRat is the ratio of the observed reproducibility coefficient of variation among laboratories calculated from the actual performance data ($C_{V,R}$) to the corresponding predicted $C_{V,R}$ calculated from the Horwitz equation:

$$C_{V,R\_\text{predicted}} = 2C^{-0,15}$$

where C is concentration expressed as a mass fraction (both numerator and denominator expressed in the same units).

Note 2 to entry: Normal values lie within 0,5 to 2. (To check the proper calculation of predicted $C_{V,R}$, a C of 10^{-6} should give a predicted $C_{V,R}$ of 16 %.)

Note 3 to entry: If applied to within-laboratory studies, the normal range of HorRat(r) is 0,30 to 1,30.

Note 4 to entry: For concentrations less than 0,12 mg/kg, the predicted standard deviation 22 % should be used.

3.3.27
identical test item
identical measurement item
prepared sample that is presumed to be identical for the intended purpose of measurement of the measurand

3.3.28
identity preservation
process or system of maintaining the segregation and documenting the identity of a product

3.3.29
intraclass correlation coefficient
ICC
measure of the reliability of measurements as performed by different groups

Note 1 to entry: Groups in this context equates to different laboratories with two or more results measured on identical test items (e.g. DNA copy number, % mass, seed count).

Note 2 to entry: This coefficient represents agreements between two or more results measured on identical test items.

3.3.30
item
entity
anything that can be described and considered separately

3.3.31
laboratory performance study
proficiency test
study consisting of one or more measurements performed independently by a group of laboratories on one or more homogeneous, stable, test samples by the method selected or used by each laboratory in the group where the reported results are compared with those from other laboratories or with the known or assigned reference value, usually with the objective of improving laboratory performance

Note 1 to entry: Laboratory performance studies can be used to support laboratory accreditation of laboratories or to audit performance. If a study is conducted by an organization with some type of management control over the participating laboratories (organizational, accreditation, regulatory or contractual), the method can be specified or the selection may be limited to a list of approved or equivalent methods. In such situations, a single test sample is insufficient to judge performance.

Note 2 to entry: A laboratory performance study can be used to select a method of analysis that will be used in a method performance study. If all laboratories or a sufficiently large subgroup of laboratories use the same method, the study may also be interpreted as a method performance study, provided that the test samples cover the range of concentration of the analyte.

Note 3 to entry: Laboratories of a single organization with independent facilities, instruments and calibration materials are treated as different laboratories.

3.3.32
limit of detection
LOD
detection limit
true net concentration or amount of the analyte in the material to be analysed that will lead, with probability (1-β), to the conclusion that the concentration or amount of the analyte in the analysed material is larger than that in the blank material

Note 1 to entry: The LOD is defined as:

$$P_r\left(\hat{L} \leq L_c \mid L = L_D\right) = \beta$$

where

P_r is the probability function;
$\hat{L}$ is the estimated value;
L_c is the critical value;
L is the expectation or true value;
L_D is the LOD.

Note 2 to entry: The LOD is estimated by:

$$L_D \approx 2t_{1-\alpha\nu}\sigma_o$$

where

L_D is the LOD;
$\alpha = \beta$;
$t_{1-\alpha\nu}$ is the Student's t-distribution value, based on ν degrees of freedom for a one-sided confidence interval of 1-α;
σ_o is the standard deviation of the true value (expectation).

Note 3 to entry: L_D = 3,29 σ_o, when the uncertainty in the mean (expected) value of the blank is negligible, $\alpha = \beta$ = 0,05 and L is normally distributed with known constant variance. However, L_D is not defined simply as a fixed coefficient (e.g. 3, 6) times the standard deviation of a pure solution background. To do so can be extremely misleading. The correct estimation of L_D can take into account degrees of freedom, α and β, and the distribution of L as influenced by factors such as analyte concentration, matrix effects and interference.

Note 4 to entry: This definition provides a basis for considering exceptions to the simple case that is described, i.e. involving non-normal distributions and heteroscedasticity (e.g. "counting" (Poisson) processes as those used for real-time polymerase chain reactions).

Note 5 to entry: It is essential to specify the measurement process under consideration, since distributions, standard deviations and blanks can be dramatically different for different measurement processes.

Note 6 to entry: At the L_D, a positive identification can be achieved with reasonable or previously determined confidence, or both, in a defined matrix using a specific analytical method.

Note 7 to entry: An empirically derived determination based on the results of a collaborative trial is called the "practical LOD". It is defined as the lowest relative quantity of the target DNA that can be detected, given a known (determined/estimated) number of target taxon copies. The practical LOD is related to the test portion, and the quality/quantity of the template DNA, and L_D = 3,29 σ_o, which has also been called the absolute LOD of the method with 95 % confidence.

Note 8 to entry: In qualitative testing, an estimate of the L_D is measured at the chosen POD. The L_D can only be discretely determined for a quantitative method.

3.3.33
limit of detection for microarray platform
LODP
lowest relative quantity of the external measurement standard (or reference material) that can be detected experimentally at a 95 % confidence level, given a known (determined/estimated) number of copies or concentration or both of the external measurement standard (or reference material)

3.3.34
limit of quantification
LOQ
quantification limit
method performance characteristic generally expressed in terms of the signal or measurement (true value) that will produce estimates having a specified reproducibility coefficient of variation ($C_{V,R}$), commonly less than 25 %

Note 1 to entry: The LOQ is estimated by:

$$L_Q = k_Q \sigma_Q,\ k_Q = 1/\ C_{V,R}$$

where

L_Q is the LOQ;
σ_Q is the standard deviation at that point;
k_Q is the multiplier whose reciprocal equals the selected $C_{V,R}$.

The approximate $C_{V,R}$ of an estimated σ, based on ν-degrees of freedom is $1/\sqrt{2\nu}$.

Note 2 to entry: If σ is known and constant, then $\sigma_Q = \sigma_o$, since the standard deviation of the estimated quantity is independent of concentration. Substituting 25 % in for k_Q gives:

$$L_Q = (25 * \sigma_Q) = 25\ \sigma_o$$

In this case, the L_Q is just 7,60 times the limit of detection, given normality and $\alpha = \beta = 0{,}05$.

Note 3 to entry: At the LOQ, a positive identification can be achieved with reasonable or previously determined confidence or both in a defined matrix using a specific analytical method.

Note 4 to entry: This definition provides a basis for taking into account exceptions to the simple case that is described, i.e. involving non-normal distributions and heteroscedasticity (e.g. "counting" (Poisson) processes as those used for real-time polymerase chain reactions).

Note 5 to entry: The practical or relative limit of quantification is lowest relative quantity of the target DNA that can reliably be quantified, given a known (determined/estimated) number of target taxon genome copies. The molecular mass of the respective species genome can be used to calculate the copy numbers.

3.3.35
linearity
ability of a method of analysis, within a certain range, to provide an instrumental response or results proportional to the quantity of analyte to be determined in the laboratory sample

Note 1 to entry: This proportionality is expressed by an *a priori* defined mathematical expression.

Note 2 to entry: The linearity limits are the experimental limits of concentrations between which a linear calibration model can be applied with an acceptable uncertainty.

3.3.36
material certification study
interlaboratory study that assigns a reference value ("true value") to a quantity (concentration or property) in the test material, usually with a stated uncertainty

Note 1 to entry: A material certification study often utilizes selected reference laboratories to analyse a candidate reference material by a method(s) judged most likely to provide the least-biased estimates of concentration (or of a characteristic property) and the smallest associated uncertainty.

3.3.37
matrix
all relevant components of a sample inclusive of analyte

3.3.38
measurand
quantity intended to be measured

Note 1 to entry: The specification of a measurand requires knowledge of the kind of quantity, description of the state of the phenomenon, body or substance carrying the quantity, including any relevant component, and the chemical entities involved.

Note 2 to entry: In chemistry, "analyte" or the name of a substance or compound are terms sometimes used for measurand. This usage is erroneous because these terms do not refer to quantities.

3.3.39
measurement procedure
procedure
standard operating procedure
SOP
detailed description of a measurement according to one or more measurement principles and to a given measurement method, based on a measurement model and including any calculation to obtain a measurement result

Note 1 to entry: A procedure is usually documented in sufficient detail to enable an operator to perform a measurement.

Note 2 to entry: A procedure can include a statement concerning a target measurement uncertainty.

3.3.40
naturally incurred sample
sample that contains the measurand by virtue of its inherent characteristics rather than the measurand being intentionally added

3.3.41
negative process control
recognized reference sample lacking a target analyte, which is put through the same process steps as the test samples

3.3.42
non-laboratory field setting
workspace lacking conditions controlled for environmental aerosol contamination and sophisticated nucleic acid purification apparatus

3.3.43
outlier
member of a set of values which is inconsistent with other members of that set as determined by statistical analysis

Note 1 to entry: The following practice is recommended for dealing with outliers:

a) Tests such as Cochran's or Grubb's tests are applied to identify stragglers or outliers. If the test statistic is less than or equal to its 5 % critical value, the item tested is accepted as correct. If the test statistic is

greater than its 5 % critical value and less than or equal to its 1 % critical value, the item tested is called a "straggler" and is indicated by a single asterisk. If the test statistic is greater than its 1 % critical value, the item is called a "statistical outlier" and is indicated by a double asterisk.

b) Next, it is investigated whether the stragglers and/or statistical outliers can be explained by some technical error, e.g. a slip in performing the measurement, an error in computation, a simple clerical error in transcribing a test result or analysis of the wrong sample. Where the error was one of the computation or transcription type, the suspect result should be replaced by the correct value. Where the error was from analysing a wrong sample, the result should be placed in its correct cell. After such correction has been made, the examination for stragglers or outliers should be repeated. If the explanation of the technical error is such that it proves impossible to replace the suspect test result, then it should be discarded as a "genuine" outlier that does not belong to the experiment proper.

c) When any stragglers or statistical outliers, or both, remain that have not been explained or rejected as belonging to an outlying laboratory, the stragglers are retained as correct items and the statistical outliers are discarded unless the statistician for good reason decides to retain them.

3.3.44
per cent error
relative error expressed as a percentage

3.3.45
platform
device or combination of devices and technologies that supports an analytical procedure

3.3.46
positive process control
well-characterized reference sample containing a detectable amount of an analyte

Note 1 to entry: The positive process control goes through exactly the same process steps as the test samples.

3.3.47
practicability
ease of operations, in terms of sample throughput and costs, to achieve the required performance criteria and thereby meet the specified purpose

3.3.48
precision
closeness of agreement between independent test/measurement results obtained under stipulated conditions

Note 1 to entry: Precision depends only on the distribution of random errors and does not relate to the true value or to the specified value.

Note 2 to entry: The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. Less precision is reflected by a larger standard deviation.

Note 3 to entry: Quantitative measures of precision depend critically on the stipulated conditions. Repeatability and reproducibility conditions are particular sets of extreme conditions.

Note 4 to entry: Intermediate conditions between these two extreme conditions are also conceivable, when one or more factors within a laboratory (intralaboratory, e.g. the operator, the equipment used, the calibration of the equipment used, the environment, the batch of reagent and the elapsed time between measurements) are allowed to vary and are useful in specified circumstances.

Note 5 to entry: Precision is normally expressed in terms of standard deviation.

3.3.49
qualitative method
binary method
method of analysis with two possible outcomes

3.3.50
quality assurance
all planned and systematic actions necessary to provide adequate confidence that analytical results will satisfy given requirements for quality

3.3.51
quantitative analysis
quantitative method
analytical method or analysis where the amount or concentration of an analyte is determined and expressed as a numerical value in appropriate units

3.3.52
range of reliable signal
ability (within a given range) to provide results that are directly proportional to the concentration or copy number, or both, of the external measurement standard (or reference material)

3.3.53
rational method of analysis
method that determines identifiable chemical(s) or other analyte(s) for which there can be several equivalent methods of analysis available

3.3.54
recovery
recovery factor
proportion of the amount of analyte detected in a final measurement

Note 1 to entry: An analyte can be naturally present or added to (e.g. spiked control), or both, in the portion of test material measured.

Note 2 to entry: Recovery is assessed by the ratio $R = C_{obs} / C_{ref}$ of the observed concentration or amount C_{obs} obtained by the application of an analytical procedure to a material containing analyte at a reference level C_{ref}. C_{ref} is:

a) a reference material certified value;

b) measured by an alternative definitive method;

c) defined by a spike addition,

d) marginal recovery.

Note 3 to entry: Recovery is primarily intended for use in methods that rely on transferring the analyte from a complex matrix into a simpler solution, during which loss of analyte can be anticipated.

3.3.55
reference material
material, sufficiently homogeneous and stable with respect to one or more specified properties, that has been established to make the material fit for its intended use in a measurement process or in examination of nominal properties

Note 1 to entry: Examination of a nominal property provides a nominal property value and associated uncertainty. This uncertainty is not a measurement uncertainty.

Note 2 to entry: Reference materials with or without assigned values can be used for measurement precision control whereas only reference materials with assigned values can be used for calibration and measurement trueness control.

Note 3 to entry: Some reference materials have assigned values that are metrologically traceable to a measurement unit outside a system of units. In a given measurement, a given reference material can only be used for either calibration or quality assurance.

Note 4 to entry: The specification of a reference material should include its material traceability, indicating its origin and processing.

3.3.56
reference sample
material or substance, one or more of whose property values are sufficiently homogeneous and well-established to be used for the calibration of an apparatus, the assessment of a measurement method or for assigning values to materials

Note 1 to entry: The reference material may be provided by the customer, internal to the laboratory or an officially designated reference.

3.3.57
reference value
quantity value used as a basis for comparison with values of quantities of the same kind

Note 1 to entry: A reference quantity value can be a true quantity value of a measurand, in which case it is unknown, or a conventional quantity value, in which case it is known.

Note 2 to entry: A reference quantity value with associated measurement uncertainty is usually provided with reference to a material, e.g. a certified reference material, a device, a reference measurement procedure, a comparison of measurement standards.

3.3.58
relative error
absolute error (3.3.1) divided by the magnitude of the true (best accepted) value

3.3.59
repeatability
precision under repeatability conditions

3.3.60
repeatability coefficient of variation
$C_{V,r}$
DEPRECATED: repeatability relative standard deviation
DEPRECATED: RSD_r
repeatability standard deviation divided by the mean

Note 1 to entry: Coefficient of variation is a useful measure of precision in quantitative studies.

Note 2 to entry: This calculation is done so that a variability of sets with different means can be compared. Coefficient of variation values are independent of the amount of analyte over a reasonable range and facilitate comparison of variabilities at different concentrations.

Note 3 to entry: The result of a collaborative test may be summarized by giving the coefficient of variation for repeatability ($C_{V,r}$) and reproducibility ($C_{V,R}$).

3.3.61
repeatability conditions
observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in the same test or measuring facility by the same operator using the same equipment within short intervals of time

Note 1 to entry: Repeatability conditions include:

— the same measurement procedure or test procedure;

— the same operator; the same measuring or test equipment used under the same conditions;

— the same location and repetition over a short period of time.

3.3.62
repeatability limit
r
value less than or equal to which the absolute difference between two test results obtained under repeatability conditions can be expected to be within a probability of 95 %

Note 1 to entry: When examining two single test results obtained under repeatability conditions, the comparison should be made with the repeatability limit $r = 2{,}8\ s_r$.

Note 2 to entry: When groups of measurements are used for the calculation of the repeatability limits (referred to as the "critical difference"), more complicated formulae are required.

3.3.63
repeatability standard deviation
s_r
standard deviation of test results or measurement results obtained under repeatability conditions

Note 1 to entry: It is a measure of the dispersion of the distribution of test or measurement results under repeatability conditions.

3.3.64
replicate test sample
sample taken from a bulk sample such that the replicate test samples are as close to identical as achievable, in order to constitute identical test items

3.3.65
reproducibility
precision under reproducibility conditions of measurement

3.3.66
reproducibility coefficient of variation
$C_{V,R}$
DEPRECATED: reproducibility relative standard deviation
DEPRECATED: RSD_R
reproducibility standard deviation divided by the mean

Note 1 to entry: Coefficient of variation is a useful measure of precision in quantitative studies.

Note 2 to entry: This calculation is done so that a variability of sets with different means can be compared. Coefficient of variation values are independent of the amount of analyte over a reasonable range and facilitate comparison of variabilities at different concentrations.

Note 3 to entry: The result of a collaborative test may be summarized by giving the coefficient of variation for repeatability ($C_{V,r}$) and reproducibility ($C_{V,R}$).

3.3.67
reproducibility conditions
observation conditions where independent test/measurement results are obtained with the same method on identical test/measurement items in different test or measurement facilities with different operators using different equipment

3.3.68
reproducibility limit
R
value less than or equal to which the absolute difference between final values, each of them representing a series of test results or measurement results obtained under reproducibility conditions can be expected to be found within a probability of 95 %

Note 1 to entry: When examining two single test results obtained under reproducibility conditions, the comparison should be made with the reproducibility limit, $R = 2{,}8\sigma_R$.

Note 2 to entry: When groups of measurements are used as the basis for the calculation of the reproducibility limits (referred to as the "critical difference"), more complicated formulae are required.

3.3.69
reproducibility standard deviation
s_R
standard deviation of test results or measurement results obtained under reproducibility conditions

Note 1 to entry: It is a measure of the dispersion of the distribution of test or measurement results under reproducibility conditions.

3.3.70
result
set of quantity values being attributed to a measurand together with any other available relevant information

Note 1 to entry: A measurement result generally contains "relevant information" about the set of quantity values, such that some can be more representative of the measurand than others. This may be expressed in the form of a probability density function.

Note 2 to entry: A measurement result is generally expressed as a single measured quantity value and a measurement uncertainty. If the measurement uncertainty is considered to be negligible for some purpose, the measurement result may be expressed as a single measured quantity value. In many fields, this is the common way of expressing a measurement result.

Note 3 to entry: In the traditional literature and in Reference [3], "measurement result" was defined as information about the magnitude of a quantity, obtained experimentally, and noted to consist of a set of quantity values reasonably being attributed to the measurand, usually summarized as a single quantity value and a measurement uncertainty. The single quantity value is an estimate, often an average or the median of the set.

3.3.71
robustness
ruggedness
measure of the capacity of an analytical procedure to remain unaffected by small variations in method parameters and provides an indication of the method's reliability during normal usage

3.3.72
sample
small portion or quantity, taken from a population or lot that is ideally a representative selection of the whole

Note 1 to entry: A sample can be made up of one or more sampling units.

3.3.73
selectivity
extent to which a method can determine particular analyte(s) in a mixture(s) or matrice(s) without interferences from other components of similar behaviour

Note 1 to entry: Selectivity is the recommended term in analytical chemistry to express the extent to which a particular method can determine analyte(s) in the presence other components. Selectivity can be graded. The use of the term "specificity" for the same concept is to be discouraged as this often leads to confusion.

Note 2 to entry: Sequence specificity in molecular biomarker analysis is differentiated from chemical analyte selectivity.

3.3.74
sensitivity
quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured

Note 1 to entry: The sensitivity of a measuring system can depend on the value of the quantity being measured.

Note 2 to entry: The change considered in the value of the quantity being measured shall be large compared with the resolution.

3.3.75
single-laboratory validation
validation process that evaluates functional characteristics of a method, including repeatability, when applying a specific method within a specific laboratory, by (a) specific operator(s) using specific equipment

3.3.76
specificity
analytical specificity
diagnostic specificity
ability of a detection method to distinguish the specific organism or pathogen from other organisms, whether related or not, and the extent to which the analysis can distinguish known or unknown variants of the organism

Note 1 to entry: Specificity is a term that describes the same phenomenon as selectivity but in a different way; while selectivity is applied to analytical chemistry and physics, specificity is applied to organisms and pathogens.

3.3.77
surrogate
pure compound or element added to the test material, the chemical and physical behaviour of which is taken to be representative of the native analyte

3.3.78
systematic error
component of measurement error that, in replicate measurements, remains constant or varies in a predictable manner

Note 1 to entry: A reference quantity value for a systematic measurement error is a true quantity value or a measured quantity value of a measurement standard of negligible measurement uncertainty or a conventional quantity value.

Note 2 to entry: Systematic measurement error and its causes can be known or unknown. A correction can be applied to compensate for a known systematic measurement error.

Note 3 to entry: Systematic measurement error equals measurement error minus random measurement error.

3.3.79
test control
control
one or more samples that have undergone all or part of the analytical procedure designed for test samples

Note 1 to entry: Both positive and negative test control samples can be used as a basis for evaluating performance characteristics of a method, aiding in identifying process errors and making it easier to interpret results.

3.3.80
test kit
set of chemicals, materials and instructions for use, packaged together and intended for use as specified by the manufacturer of the kit

3.3.81
true value
quantity value consistent with the definition of a quantity

Note 1 to entry: In the error approach to describing measurement, a true quantity value is considered unique and, in practice, unknowable. The uncertainty approach is to recognize that, owing to the inherently incomplete amount of detail in the definition of a quantity, there is not a single true quantity value but rather a set of true quantity values consistent with the definition. However, this set of values is, in principle and in practice, unknowable. Other approaches dispense altogether with the concept of true quantity value and rely on the concept of metrological compatibility of measurement results for assessing their validity.

Note 2 to entry: When the definitional uncertainty associated with the measurand is considered to be negligible compared to the other components of the measurement uncertainty, the measurand may be considered to have an "essentially unique" true quantity value.

3.3.82
trueness
freedom from bias
closeness of agreement between the average value of an infinite number of replicate measured quantity values and a reference quantity value

Note 1 to entry: Trueness is not a quantity and thus cannot be expressed numerically.

Note 2 to entry: Trueness is inversely related to systematic error but is not related to random error.

Note 3 to entry: Accuracy should not be used for trueness and vice versa.

Note 4 to entry: Trueness is the ability of a method of measurement to give indications without systematic errors.

3.3.83
uncertainty
measurement uncertainty
non-negative parameter characterizing the dispersion of the quantity values attributed to a measurand based on the information used

Note 1 to entry: Measurement uncertainty includes components arising from systematic effects, such as components associated with corrections and the assigned quantity values of measurement standards, as well as the definitional uncertainty. Sometimes, estimated systematic effects are not corrected for but, instead, associated measurement uncertainty components are incorporated.

Note 2 to entry: The parameter may be, for example, a standard deviation called "standard measurement uncertainty" (or a specified multiple of it), or the half-width of an interval, having a stated coverage probability.

Note 3 to entry: Measurement uncertainty comprises, in general, many components. Some of these may be evaluated by Type A evaluation of measurement uncertainty from the statistical distribution of the quantity values from series of measurements and can be characterized by standard deviations. The other components, which may be evaluated by Type B evaluation of measurement uncertainty, can also be characterized by standard deviations, evaluated from probability density functions based on experience or other information.

Note 4 to entry: In general, for a given set of information, it is understood that the measurement uncertainty is associated with a stated quality value attributed to the measurand. A modification of this value results in a modification of the associated uncertainty.

3.3.84
validated range
part of the concentration range of an analytical method which has been subjected to validation

3.3.85
validated test method
accepted test method for which validation studies have been completed to determine the accuracy and reliability of this method for a specific purpose

3.3.86
validation
verification where the specified requirements are adequate for an intended use

3.3.87
validation experiment
determination of method performance parameters from a series of test results reported by one or more usually a number of participating laboratories

3.3.88
verification
provision of objective evidence that a given item fulfils specified requirements

Note 1 to entry: When applicable, measurement uncertainty should be taken into consideration.

Note 2 to entry: The item may be, for example, a process, measurement procedure, material, compound or measuring system.

Note 3 to entry: The specified requirements may be that a manufacturer's specifications are met.

Note 4 to entry: Verification in legal metrology, and in conformity assessment in general, pertains to the examination and marking or issuing or both of a verification certificate for a measuring system.

Note 5 to entry: Verification should not be confused with calibration. Not every verification is a validation.

Note 6 to entry: In chemistry, verification of the identity of the entity involved, or of activity, requires a description of the structure or properties of that entity or activity.

3.4 Molecular biology

3.4.1
adapter sequence
synthetic oligonucleotide of a known sequence that can be added to the 3′ or 5′ end, or both ends, of a nucleic acid fragment

3.4.2
annealing
pairing of complementary single strands of nucleic acids to form a double-stranded molecule

3.4.3
biological tissue
group of similar cells, from the same origin, that together with their extracellular matrix perform a specific function

Note 1 to entry: In animals, it can be classified as connective, muscle, nervous and epithelial. Different tissues can have very different physical and biochemical characteristics.

3.4.4
complementary sequence
single-stranded nucleotide sequence able to anneal via hydrogen bonding to form a double-stranded double helical structure when aligned respectively in antiparallel orientation with its cognate nucleic acid sequence

EXAMPLE Adenine pairs with thymine or uracil, guanine pairs with cytosine.

Note 1 to entry: The complementary sequence of 5′-CATAGTCTATTG-3′ is 5′-CAATAGACTATG-3.

3.4.5
cross-hybridization
non-specific binding of probe DNA to non-targeted nucleic acid

3.4.6
cross-reactivity
reactivity of an observed agent which initiates reactions outside the main reaction expected

Note 1 to entry: For immunogenic methods, it is the degree to which binding occurs between an antibody and antigenic determinants which are not the analyte of primary interest.

3.4.7
denaturation
process of partial or total alteration of the native structure of a macromolecule resulting from the loss of tertiary or secondary structure, or both, that is a consequence of the disruption of stabilizing weak bonds

Note 1 to entry: Denaturation can occur when proteins and nucleic acids are subjected to elevated temperature, extremes of pH, non-physiological concentrations of salt, organic solvents, urea or other chemical agents.

3.4.8
denaturation of protein
physical or chemical treatments, or both, which destroy or modify the structural, functional, enzymatic or antigenic properties of the protein of interest

3.4.9
denatured deoxyribonucleic acid
denatured DNA
DNA that has been converted from double-stranded to single-stranded form by a denaturation process such as heating

3.4.10
deoxyribonuclease/ribonuclease
DNase/RNase
enzyme that catalyses the hydrolytic cleavage of deoxyribonucleic acid/ribonucleic acid that can produce a single nucleotide residue by cleavage at the end of the chain or a polynucleotide by cleavage at a position within the chain

3.4.11
deoxyribonuclease/ribonuclease inhibitor
substance that either fully or partially blocks deoxyribonuclease/ribonuclease activity

3.4.12
deoxyribonucleic acid
DNA
polymer of deoxyribonucleotides occurring in double-strand (dsDNA) or single-strand (ssDNA) form that is the carrier of genetic information, encoded in the sequence of purine and pyrimidine bases (nitrogen containing ring compounds that are either purines or pyrimidines), and is present in chromosomes and chromosomal material of cell organelles as well as in plasmids and in viruses

3.4.13
deoxyribonucleic acid microarray
DNA microarray
DNA chip
solid substrate where a collection of probe DNA arranged in a specific design is attached in a high-density fashion, directly or indirectly, that assays large amounts of biological material using high-throughput screening methods

3.4.14
deoxyribonucleic acid polymerase
DNA polymerase
enzyme that synthesizes DNA by catalysing the addition of deoxyribonucleotide residues to the free 3′-hydroxyl end of a DNA molecular chain, starting from a mixture of the appropriate nucleotide triphosphate bases

3.4.15
deoxyribonucleic acid quality
DNA quality
DNA template of sufficient length, chemical purity and structural integrity for any application

Note 1 to entry: Good quality DNA will have an A260/A280 ratio of 1,7 to 2,0, and an A260/A230 ratio > 2,0.

Note 2 to entry: Good quality DNA lacks polymerase chain reaction (PCR) inhibitors and can be PCR amplified.

3.4.16
deoxyribonucleotide triphosphate
dNTP
tri-phosphorylated deoxyribonucleoside that includes deoxyadenosine nucleotide triphosphate (dATP), deoxycytidine nucleotide triphosphate (dCTP), deoxyguanosine nucleotide triphosphate (dGTP), deoxythymidine nucleotide triphosphate (dTTP) and deoxyuridine nucleotide triphosphate (dUTP)

3.4.17
detection assay
identification assay
procedure or method that is used to identify the presence of traits, microorganisms, pests or other analytes in a biological sample

3.4.18
electrochemical detection
ECD
method of detecting hybridization by measuring electric currents of an electrode onto which probe DNA are immobilized

3.4.19
electrophoresis
method used for separating, identifying and purifying molecules (e.g. plasmid DNA, DNA fragments resulting from digestion, RNA, protein and polymerase chain reaction products) based upon the differential movement of charged particles through a matrix when subjected to an electric field

3.4.20
exonuclease
enzyme that hydrolyses (cleaves) terminal phosphodiester bonds of a nucleic acid

Note 1 to entry: Activity may be from either the 5′ or 3′ end of the nucleic acid strand.

Note 2 to entry: Not all enzymes possess exonuclease activity. For certain molecular techniques, the lack of activity is desirable, e.g. inclusion of an "A" overhang in TA cloning.

3.4.21
external measurement standard
material or substrate prepared for testing the compatibility of the microarray-based methods of analysis, whose property value is derived as a consensus value based on collaborative experimental work under the auspices of a scientific or engineering group

3.4.22
genome editing
gene editing
genetic engineering producing predictable changes to a gene or a genome

3.4.23
integration-border region
junction region where one element originates from the host organism and the other originates from the DNA introduced during transformation

3.4.24
junction region
DNA sequence spanning the interval between two consecutive juxtaposed sequences

Note 1 to entry: Event-specific genetically modified organism (GMO) detection methods target DNA sequences that are detected and identified with a specific transformation event, usually a DNA sequence spanning the junction region between the inserted DNA and the host genome (the integration border region).

Note 2 to entry: Construct-specific GMO detection methods target a combination of DNA sequences that are only found in a specific combination of DNA sequences, usually a DNA sequence spanning the junction region between the promoter or terminator and the gene.

3.4.25
library
collection of molecules in a stable form that represents some aspect of an organism

3.4.26
melting temperature
T_m
temperature at which 50 % of a specific double-stranded DNA helix is dissociated

Note 1 to entry: T_m is dependent upon A-T, G-C content.

Note 2 to entry: A DNA double helix melts in a temperature range rather than at one very specific temperature.

3.4.27
modern biotechnology
in vitro nucleic acid techniques, including recombinant DNA technologies or fusion of cells, that overcome natural physiological, reproductive or recombination barriers and that are not techniques used in traditional breeding and selection

3.4.28
molecular biomarker
biomarker
molecular marker
detectable and/or quantifiable molecule or group of molecules used to indicate a biological condition, state, identity or characteristic of an organism

EXAMPLE Nucleic acid sequences, proteins, small molecules such as metabolites, other molecules such as lipids and polysaccharides.

3.4.29
non-specific deoxyribonucleic acid staining
non-specific DNA staining
method of staining DNA products, typically used following electrophoresis, that is independent of the DNA sequence

3.4.30
northern blotting
transfer of RNA fragments separated in electrophoretic gels to membrane filters for detection of specific sequences complementary to a labelled DNA or RNA probe

3.4.31
nucleic acid extraction
deoxyribonucleic acid extraction
DNA extraction
process to liberate nucleic acid from a sample such that further manipulations can be performed

Note 1 to entry: Nucleic acid extraction can be DNA or RNA extraction.

3.4.32
nucleic acid purification
deoxyribonucleic acid purification
DNA purification
process to separate nucleic acid from other components in a mixture such that further manipulations can be performed

Note 1 to entry: Nucleic acid purification can be performed on DNA or RNA.

Note 2 to entry: A highly purified DNA or RNA sample contains negligible observable or measurable effects attributable to inhibitors of any downstream process, e.g. polymerase chain reaction, massively parallel nucleotide sequencing.

3.4.33
nucleoside
glycosidic compound consisting of a purine or pyrimidine base and a pentose (ribose or deoxyribose)

3.4.34
nucleotide
subunit of DNA or RNA composed of a base (purine or pyrimidine), a pentose (ribose or deoxyribose) and a phosphate group

3.4.35
nucleotide sequence specificity
capacity to exclusively recognize a specific nucleic acid target sequence, distinguishing it from other nucleic acids and contaminants

3.4.36
oligonucleotide
short DNA or RNA molecule

3.4.37
plasmid
extrachromosomal DNA molecule in cells physically separated from the chromosome and capable of autonomous replication

Note 1 to entry: Both circular and linear plasmids have been identified.

Note 2 to entry: Plasmids have been observed in both prokaryotes and eukaryotes.

3.4.38
primer
oligonucleotide primer
DNA primer
oligonucleotide of defined length and sequence complementary to a segment of an analytically relevant DNA sequence

Note 1 to entry: Polymerase chain reaction primers are used to amplify a specific target sequence.

3.4.39
primer extension
enzymatic reaction that synthesizes a new DNA strand by adding a deoxyribonucleotide to the 3′ end of the primer sequence

3.4.40
probe
DNA probe
probe DNA
labelled single-strand nucleic acid molecule of known sequence defined by its property to target a specific nucleic acid sequence by base complementarity hybridization, where the stringency of the binding is linked with the length and nucleic acid composition of the probe, along with the reaction parameters

Note 1 to entry: A short sequence of DNA may be labelled isotopically or chemically for use in the detection of a complementary nucleotide sequence.

3.4.41
repeat region
genomic region in which a particular DNA or RNA sequence occurs in multiple copies or multiple times within a given sequence

3.4.42
reporter gene
gene used to facilitate detection of gene expression, as determined by specific regulatory sequences

3.4.43
restriction endonuclease
restriction enzyme
class of bacterial enzymes that cleaves (i.e. restricts) DNA into fragments at or near specific and unique recognition sites within deoxynucleotide sequences known as "restriction sites"

Note 1 to entry: Restriction endonucleases are classified as endonucleases.

Note 2 to entry: Restriction endonucleases are produced by bacteria and archaea to defend against bacteriophage viruses.

3.4.44
restriction fragment length polymorphism
RFLP
nucleotide difference in partially homologous DNA sequences that can be detected by the presence of restriction fragments of different lengths after digestion of the DNA samples with specific restriction endonucleases

Note 1 to entry: RFLP, as a molecular marker, is specific to a single probe/restriction enzyme combination.

3.4.45
reverse transcriptase
class of RNA-directed DNA polymerase enzymes that allow for the synthesis of DNA (complementary to the RNA) using suitable primers and reaction conditions

3.4.46
reverse transcription
process of making DNA from an RNA template, using the enzymatic activity of a reverse transcriptase associated with one or more oligonucleotide primers under a suitable set of conditions

3.4.47
ribonucleic acid
RNA
polymer of ribonucleotides that consists of a nucleobase (adenine, guanine, thymine or uracil), a ribose sugar and a phosphate group

Note 1 to entry: RNA is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes.

Note 2 to entry: RNA is usually single stranded in its biological roles; however, a single RNA molecule by complementary base pairing can form intra-stranded double helices, e.g. tRNA.

3.4.48
ribonucleic acid extraction
RNA extraction
separation of RNA from the other cellular components in a test sample

3.4.49
sequence homology
biological homology between DNA, RNA or protein sequences, defined in terms of shared ancestry in the evolutionary history of life

3.4.50
Southern blotting
transfer of DNA fragments separated in electrophoretic gels to membrane filters for detection of specific sequences by either radio-labelled or chemically labelled complementary probes

Note 1 to entry: The method is named after the British biologist Edwin Southern, who first published it in 1975.

3.4.51
specific staining
procedure of staining biomolecules based on use of a reporter molecule bound to a probe that is capable of binding specifically onto the target

3.4.52
target sequence
DNA target
target fragment
DNA sequence that is recognized in a biomolecular reaction such as annealing, restriction or polymerization

Note 1 to entry: In polymerase chain reaction (PCR) applications, this is the DNA sequence that becomes selectively amplified during PCR-based detection, identification or quantification.

Note 2 to entry: The PCR target sequence is characterized by being located between the primers, and in the case of real-time PCR, may include the probe hybridization site.

3.4.53
target taxon
taxon to which the organism belongs

Note 1 to entry: In this context, taxon usually means species but it can be of lower or higher taxonomic rank, e.g. kingdom, phylum, class, order, family, genus, species, sub-species, variety.

3.4.54
taxon
taxonomic group of any rank, such as a species, family or class

3.4.55
taxon-specific target sequence
endogenous target sequence
sequence known to be consistently present in the target taxon but absent in other taxa

Note 1 to entry: There are at least two types of target taxon-specific sequences:

— variable number or multicopy sequences that can be used, for example, to assess the presence of nucleic acid from the target taxon;

— low copy number or single copy sequences that can also be used, for example, as a reference sequence to establish the background of target taxon genome equivalents in a quantitative analysis.

3.4.56
tissue
group of similar cells, from the same origin, that together with their extracellular matrix perform a specific function

Note 1 to entry: In animals, tissues are classified as connective, muscle, nervous and epithelial. Different tissues can have very different physical and chemical characteristics.

3.4.57
transformation
process in which DNA is transferred and expressed in a competent recipient organism

3.4.58
vector
agent used to carry new genes into cells

Note 1 to entry: A vector can also be an organism that spreads pathogens.

3.4.59
western blotting
protein immunoblot transfer of protein(s) to a binding surface following separation by electrophoresis that can be visualized using a variety of methods, e.g. with a specific radio-labelled or enzyme-conjugated antibody followed by the addition of an enzyme-specific substrate to form a colour reaction product

3.5 Molecular genetics

3.5.1
allele
one of several alternate forms of a gene that occurs at the same locus on homologous chromosomes, is separated during meiosis and can undergo recombination following the fusion of gametes

3.5.2
allele frequency
proportion of the number of copies of a specific allele within a population relative to the total number of copies of all alleles at that locus in the same population

3.5.3
biotechnology-derived trait
bioengineered trait
biological characteristic derived from selective, deliberate alteration of genes (genetic material) by means of recombinant DNA technology

3.5.4
cisgenic organism
organism that contains genetic material, originally derived from the same or sexually compatible species, that has been inserted into the genome using recombinant DNA techniques

3.5.5
cisgenic plant
plant that possesses single or multiple genes derived from the same or sexually compatible species, transferred into the plant using recombinant DNA technology

3.5.6
clone
organism or cell, or groups of organisms or cells, produced asexually from one ancestor or stock to which they are genetically identical

3.5.7
cultivar
group of cultivated plants which can be clearly defined by morphological, physical, cytological, chemical or other characteristics and which, after sexual or asexual reproduction, keep their distinct character

Note 1 to entry: The concept of "cultivar" is essentially different from the concept of the botanical variety "varietas", in that "cultivar" is an infraspecific division resulting from controlled selection, even if empirical, whereas "varietas" is an infraspecific division resulting from natural selection. The terms "cultivar" and "variety" (in the sense of cultivated variety) are equivalent. In translations or adaptations of botanical nomenclature for particular uses, the terms "cultivar" or "variety" (or their equivalents in other languages) may be used in the text.

Note 2 to entry: Cultivars can be differentiated genetically.

3.5.8
event
transgene construct and its unique site of insertion into a genome

3.5.9
gene
sequence of DNA or RNA bases encoding a regulatory or structural function or the synthesis of a product, e.g. RNA or protein

Note 1 to entry: Each organism has a genome providing a complete set of genes (hereditary information) to support its biological activity.

Note 2 to entry: A gene can be discontinuous in eukaryotes, where RNA splicing activity occurs during mRNA production.

3.5.10
genetic engineering
bioengineering
selective, deliberate alteration of genes (genetic material) by means of recombinant DNA technology

Note 1 to entry: Modern biotechnology includes recombinant DNA technology.

3.5.11
genetic modification
alteration in the genetic composition of an organism (plant, animal or microorganism) that results from adding, deleting or changing hereditary traits, irrespective of the method

Note 1 to entry: Genetic modification encompasses a broad range of methods that can be used to alter the genetic composition of a plant or animal including minor modifications, such as a single mutation that affects one gene, or major alterations of genetic material derived from conventional breeding (e.g. selection, traditional hybridization or breeding techniques that affect many genes).

3.5.12
genetically engineered content
GE content
measured value that identifies and/or quantifies levels of genetic engineering, GE-derived material, GE traits or GE-derived material in a lot or product

Note 1 to entry: Generally, the GE content is estimated by analyte detection (identification and quantitation).

3.5.13
genetically engineered organism
GEO
bioengineered organism
organism and its propagules (e.g. seeds, spores, tubers, eggs, reproductive cells) containing genetic material that has been modified through *in vitro* recombinant DNA techniques

3.5.14
genetically modified content
GMO content
measured value of identification or quantification, or both, of levels of genetic modification, GM-derived material, GM traits or GM-derived material in a lot or product

Note 1 to entry: The term "GMO content" is often used to refer to genetically engineered content.

3.5.15
genetically modified organism
GMO
organism and its propagules (e.g. seeds, spores, tubers, eggs, reproductive cells) containing genetic material that has been modified through any alteration to its genetic composition (e.g. through traditional breeding)

Note 1 to entry: The term "GMO" can be used to refer to a genetically engineered organism (GEO).

3.5.16
genome
hereditary basis of every organism

Note 1 to entry: The genome comprises polymeric deoxyribonucleic acid.

3.5.17
living modified organism
LMO
any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology

Note 1 to entry: LMO is a term defined by the Convention on Biological Diversity and is equivalent in most cases to the term "genetically modified organism (GMO)".

Note 2 to entry: An LMO may consist of any taxa of living organism.

3.5.18
microsatellite
simple sequence repeat
simple sequence repeat marker
SSR marker
region of repetitive DNA elements, consisting of short in tandem repeat motifs of one to a few nucleotides in length that tend to occur in non-coding DNA of eukaryotic genomes and that are sometimes referred to as variable number of tandem repeats (VNTRs)

Note 1 to entry: The number of repeat units present at a specified microsatellite, and thus the overall length of the microsatellite, often varies among individuals (typically 5 to 50 times).

3.5.19
mismatch
position at which non-complementary pairing of nucleotides occurs between otherwise complementary nucleotide sequences

3.5.20
monomorphic locus
genome region that presents only one form, i.e. is not polymorphic in a specific population or collection

3.5.21
multiple species product
product containing in its composition more than one species

3.5.22
null allele
mutant copy of a gene that completely lacks that gene's normal function

Note 1 to entry: In the context of a polymerase chain reaction (PCR), a null allele is a sequence variant that precludes PCR amplification of a particular target, resulting in the absence of detectable PCR product.

3.5.23
phylogenetic tree
branching diagram based on sequence data used to illustrate hypothetical cladistic relationships among various taxa based on mathematical algorithms that compare sequence similarity between highly conserved genes of several organisms, e.g. rRNA or housekeeping genes

3.5.24
plant molecular farming
PMF
use of plants in agriculture for the production of compounds such as pharmaceuticals, diagnostic products, vaccines, biologics, industrial chemicals and biodegradable plastics

3.5.25
plant with novel trait
PNT
plant to which a unique (novel) trait has been added by classical breeding or other means

3.5.26
polymorphic locus
genetic locus with two or more alleles within an individual or a population

Note 1 to entry: It is this genetic variation that is exploited by microsatellites and single nucleotide polymorphism (SNP) markers.

Note 2 to entry: Alleles are used in the description of genotypes.

3.5.27
species
group of organisms that have a high level of genetic (genomic) similarity and are capable of interbreeding (often containing subspecies, varieties or races)

Note 1 to entry: A species is designated in italics by the genus name followed by the specific name, e.g. *Ananas comonus*.

3.5.28
stacked events
two or more transformation events accumulated in the same organism as a result of traditional breeding and/or successive transformation steps

3.5.29
stacked genes
two or more genetic loci bred into a single organism

Note 1 to entry: Stacking is often used to describe two or more genetically modified (GM) insertions into the genome of an organism.

3.5.30
transgene
novel gene or genetic material, i.e. DNA that is artificially introduced into an organism

EXAMPLE Genes expressing *Bacillus thuringensis* genes, genes conferring herbicide tolerance, antisense sequences.

Note 1 to entry: An antisense gene used to regulate expression is a transgene.

3.5.31
transgenic organism
organism that contains novel genetic material, e.g. originally derived from different species or synthetic, that has been inserted into the genome using recombinant DNA techniques

3.5.32
transgenic plant
plant that possesses single or multiple genes, synthetic or derived from other species, transferred into the plant using recombinant DNA technology

3.5.33
variety
distinct, uniform and stable breeding member of a species of plant (except for hybrid species) that retains its characteristics from generation to generation through its natural mode of reproduction

Note 1 to entry: The concept of "cultivar" is essentially different from the concept of the botanical variety "varietas", in that "cultivar" is an infraspecific division resulting from controlled selection, even if empirical, whereas "varietas" is an infraspecific division resulting from natural selection. The terms "cultivar" and "variety" (in the sense of cultivated variety) are equivalent. In translations or adaptations of botanical nomenclature for particular uses, the terms "cultivar" or "variety" (or their equivalents in other languages) may be used in the text.

3.6 Nucleic acid amplification technology (NAAT)

3.6.1
absolute polymerase chain reaction
absolute PCR load
lowest nominal (average) number of target copies in the template volume distributed to individual PCR reactions that will allow for an acceptable probability of detecting the target

3.6.2
amplicon
DNA or RNA that is the source or product of amplification or replication events

Note 1 to entry: An amplicon may be natural or *in vitro*.

Note 2 to entry: A product may also be an amplicon.

3.6.3
clustered regularly interspaced short palindromic repeats-CRISPR associated system
CRISPR-Cas
recombinant DNA technology consisting of a protein and bioactive RNA complex originally sourced from microbial anti-bacteriophage defence systems and re-engineered to provide a sequence specific recognition, cleavage and excision system

Note 1 to entry: The combination of CRISPR-Cas and native recombination and repair mechanisms in any cell can enable many different types of genetic engineering.

Note 2 to entry: CRISPR-Cas9 was the first of the CRISPR-Cas recombinant technologies; however, Cas12a and Cas13 systems have also been developed.

Note 3 to entry: The bioactive RNA in Cas9 can be reengineered to recognize any cognate target sequence. It is called "single guide" or "sgRNA".

Note 4 to entry: The Cas9 complex contains a nickase.

3.6.4
clustered regularly interspaced short palindromic repeats (CRISPR) associated system protein 9 nicking amplification reaction
Cas9nAR
isothermal amplification technology for the detection of DNA only

Note 1 to entry: Cas9nAR uses a specific Cas9 nickase that creates single strand breaks at desired DNA sequences. A pair of sgRNAs designed to direct the Cas9 ribonucleoprotein to each border of the target DNA allows induction of a pair of nicks in the complementary strand, resulting in a ssDNA target sequence after strand displacement. The released ssDNA is then exponentially amplified in a second circuit

Note 2 to entry: See *CRISPR-Cas* (3.6.7).

3.6.5
competitive allele amplification
competitive phenomenon that results in the preferential amplification of one allelic sequence over another in a heterozygous or mixed sample during the application of nucleic acid amplification technologies such as the polymerase chain reaction

3.6.6
construct-specific detection method
method that targets a specific combination of inserted DNA sequences (such as genes, promoters, terminators or other genetic elements of interest) unique to genetically modified organisms

3.6.7
conventional polymerase chain reaction
conventional PCR
classical PCR
traditional PCR
PCR
in vitro enzymatic technique to increase the number of copies of a specific DNA fragment by several orders of magnitude using thermal cycling

Note 1 to entry: A conventional PCR method requires a post-PCR step such as gel electrophoresis for detection or visualization of amplification products to provide a qualitative result.

3.6.8
copy number
number of molecules (copies) of a DNA sequence

3.6.9
crossing point
C_p
cycle quantification
C_q
cycle threshold
C_t
threshold cycle
T_c
cycle in real-time polymerase chain reaction at which the fluorescence signal from the reaction crosses a threshold level at which the signal can be distinguished from background levels

Note 1 to entry: Crossing point, cycle quantification, cycle threshold and threshold cycle are used with the same meaning.

3.6.10
degenerate primers
mixture of oligonucleotide primers in which some nucleotide positions contain a number of different possible bases, giving a population of primers with similar sequences that cover multiple possible nucleotide combinations for a given genomic sequence

Note 1 to entry: The nucleoside inosine can pair with any of the four bases; however, it pairs preferentially to cytosine.

3.6.11
deoxyribonucleic acid species quantification
DNA species quantification
process whereby the amount of DNA within a larger DNA sample is quantified according to the organism taxon

Note 1 to entry: DNA species quantification for each organism is expressed as a percentage.

3.6.12
detection of polymerase chain reaction product
detection of PCR product
confirmation of PCR amplification
act of noting or discovering the existence of a PCR product

Note 1 to entry: The detection can be done directly by incorporating fluorescent dye into new amplicon products during PCR (e.g. SYBR® Green[2)]) or post-PCR by staining of the PCR products (e.g. ethidium bromide (EtBr) in agarose gels), using fluorescent probes to track PCR product amplification in real time (e.g. TaqMan®[2)]), or end-labelling products post-PCR (e.g. KASP®[2)]).

Note 2 to entry: Some isothermal PCR methods provide evidence of amplification by producing a precipitate.

3.6.13
digital polymerase chain reaction
digital PCR
dPCR
application of the PCR to directly quantify target DNA, cDNA or RNA in a sample by partitioning the sample nucleic acid to a limit dilution of approximately one copy per reaction in a microwell or droplet prior to amplification

Note 1 to entry: The dPCR approach can be used to amplify nucleic acid libraries.

Note 2 to entry: Droplet digital PCR (ddPCR) uses a water-oil emulsion containing mostly single target copy droplet reactions instead of a microwell plate.

Note 3 to entry: Linear quantitation is possible with dPCR while conventional and real-time PCR is best used for exponential quantitation.

3.6.14
endogenous amplification control
gene sequence naturally present in template DNA (e.g. housekeeping gene with known copy #/genome) that is amplified to check the quality and yield of a DNA extract

2) SYBR® Green, TaqMan® and KASP® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the products named.

3.6.15
endogenous deoxyribonucleic acid sequence
endogenous DNA sequence
defined reference DNA sequence native to a corresponding taxon

Note 1 to entry: The endogenous DNA sequence can be used to determine the quantity of genome equivalents of the target taxon if the sequence is present in a constant copy number and does not show allelic variation among cultivars of the target taxon.

3.6.16
end-point polymerase chain reaction
end-point PCR
method where amplicons are detected at the end of the PCR, typically by gel electrophoresis, and the amplified product is visualized with a fluorescent dye

3.6.17
environment control
control used to demonstrate that no contamination from the environment was mixed into test samples

3.6.18
event-specific method
method that detects a specific DNA insertion, deletion or modification at a particular genome location

Note 1 to entry: Typically, one assay primer anneals with the target gene construct while the second assay primer anneals with the host organism's genomic sequence.

Note 2 to entry: This type of method commonly targets a sequence at the integration-border region of the DNA insertion, deletion or modification.

Note 3 to entry: This type of method can also detect a specific DNA combination that is present only in the specific event, without the necessity to target the insert/plant border sequences.

3.6.19
external amplification control
spiked amplification control
DNA added to an aliquot of the extracted nucleic acid in a defined amount or copy number serving as a control for amplification in nucleic acid-based reactions

3.6.20
extraction control
extraction blank control
reagent blank
negative control reaction generated by performing all the required steps in an extraction procedure except for the addition of the test portion

Note 1 to entry: For example, not including the test sample in the test portion.

Note 2 to entry: This control is used to demonstrate the absence of contamination during extraction.

3.6.21
fluorescence detection
fluorescence detection system
method for disclosing the presence or absence of an analyte by measuring the emission of fluorescence from an excited fluorescence emitting moiety associated with the analyte by hybridization, or through an enzymatic reaction such as conjugation or hydrolysis

3.6.22
fluorescence resonance energy transfer
FRET
distance-dependent energy transfer from a donor molecule to an acceptor molecule resulting in enhanced fluorescence of the acceptor molecule after excitation with electromagnetic radiation of a defined wavelength

3.6.23
fluorescent probe
fluorescent labelled oligonucleotide
oligonucleotide or oligonucleotide analogue complementary to a defined target sequence labelled with one or more fluorescent moieties capable of emitting a fluorescent signal after excitation

Note 1 to entry: Hybridization of a fluorescent probe to the target nucleic acid sequence can be detected by a fluorescent detection system.

Note 2 to entry: Fluorescent probes have many uses in biomolecular analysis, e.g. polymerase chain reaction, nucleotide sequencing.

3.6.24
fluorophore
molecule with a functional group that absorbs energy of a specific wavelength and re-emits energy at a different (but equally specific) wavelength dependent on both the fluorophore and the chemical environment

3.6.25
forward flow
principle of material/sample handling applied to ensure that laboratory samples, and raw and processed test portions flow in a defined sequence through sampling and analytical processes so that they remain physically segregated during the entire procedure to ensure that contamination arising from later steps does not contaminate the previous steps

Note 1 to entry: Forward flow refers to the situation that the samples and operators move in one direction and are not allowed to contact prior steps in the process.

3.6.26
forward primer
positive sense oligonucleotide that anneals to the negative (–) sense strand of denatured DNA in a polymerase chain reaction

3.6.27
helicase-dependent amplification
HDA
isothermal nucleic acid amplification strategy that uses a DNA helicase to denature double-stranded DNA (dsDNA), providing single-stranded templates for primer hybridization and subsequent extension by a strand-displacing DNA polymerase

Note 1 to entry: Only two primers are required for HDA.

3.6.28
hot-start polymerase chain reaction
hot-start PCR
method that uses a thermostable DNA polymerase enzyme that becomes activated at a specific temperature through an initial heating step

Note 1 to entry: Hot-start PCR is used to reduce non-specific amplification.

3.6.29
hybridization
non-covalent sequence-specific interaction of two molecules with complementary nucleic acid sequences (either RNA and/or DNA) under an appropriate set of reaction conditions to give a double-stranded molecule

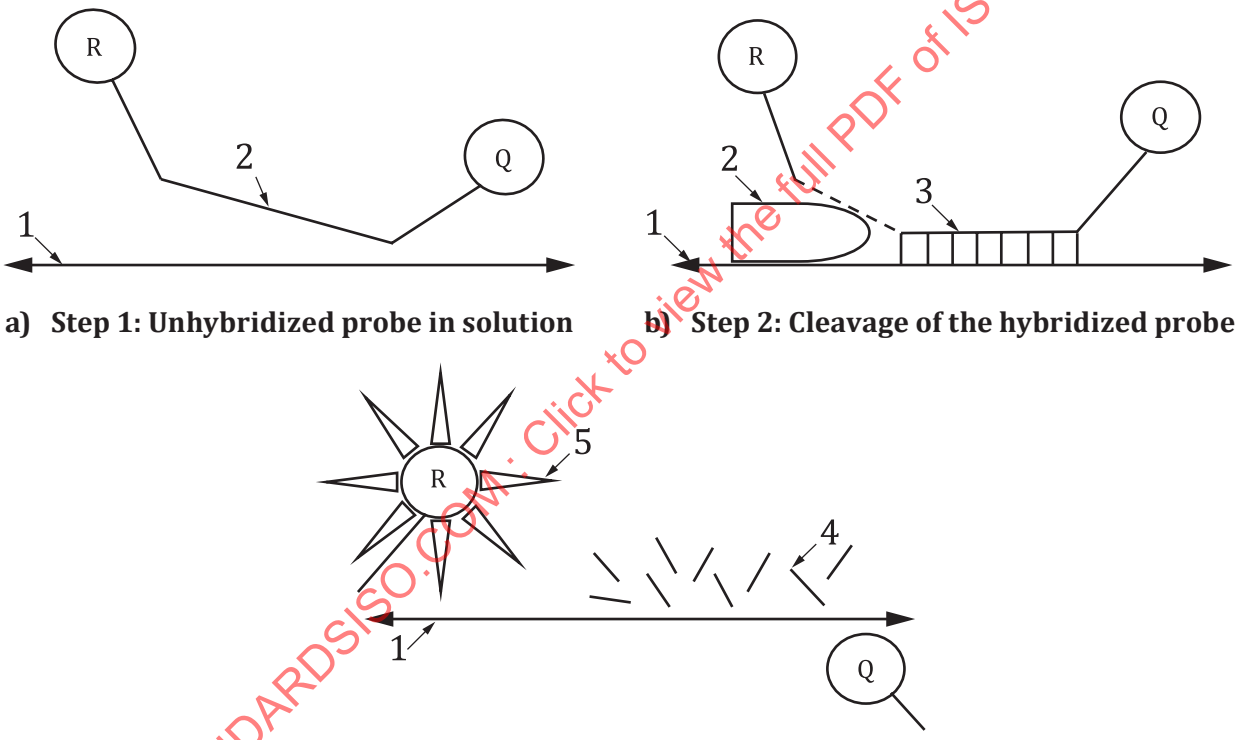
3.6.30
hybridization probe
fragment of DNA/RNA of variable length which is used to detect the presence of nucleotide sequences (the target) that are complementary to the nucleotide sequence in the probe

Note 1 to entry: Hybridization probes are most often end-labelled with a colorimetric- or fluorescence-emitting moiety.

3.6.31
hydrolysis probe
oligonucleotide with fluorescent and quencher moieties attached respectively at the 5′ and 3′ ends where no significant fluorescent signal is emitted after excitation only if the two moieties are linked by the oligonucleotide

Note 1 to entry: See Figure 1.

Note 2 to entry: In use, degradation of the hybridized oligonucleotide during polymerase chain reaction amplification separates the fluorescent and quencher moieties allowing a detectable fluorescent signal to be emitted. This emission can be measured in real time.



a) Step 1: Unhybridized probe in solution

b) Step 2: Cleavage of the hybridized probe

c) Step 3: Cleaved probe resulting in reporter fluorescence after excitation

Key

1	single-stranded nucleic acid	Q	quenching molecule
2	enzyme	R	fluorescent molecule
3	double-stranded nucleic acid		
4	enzymatically degraded nucleic acid		
5	emitted fluorescent light		

Figure 1 — Hydrolysis probe schematic

3.6.32
identification of nucleic acid sequences
identity of nucleic acid sequences
establishment of identity by comparison with a reference nucleic acid fragment/sequence

EXAMPLE Specific hybridization with a probe, matching restriction digest profiles or matching nucleic acid sequences.

3.6.33
inhibition control
polymerase chain reaction inhibition control
PCR inhibition control
sample that enables the analyst to check that there has been no inhibition affecting the results of a nucleic acid amplification assay

Note 1 to entry: This amplicon may or may not be different from the target fragment. An inhibition control makes it possible to unambiguously interpret a negative result (highlighting the false negatives obtained in the presence of inhibitors).

Note 2 to entry: Duplex (internal) inhibition controls consist of the addition of the control primers and templates producing a significantly different amplicon from the target to the sample reaction. Alternatively, a singleplex (external) reaction containing the inhibition control template and primers is run side by side in the same thermocycler with the same reagents as the samples.

3.6.34
isothermal polymerase chain reaction
isoPCR
isothermal nucleic acid amplification
isothermal amplification
PCR amplification of nucleic acids where the reaction is carried out without thermal cycling, i.e. at a constant temperature

Note 1 to entry: In some isoPCR applications, nucleic acids are denatured at a high temperature (≧ 95 °C) prior to the start of the amplification reaction.

3.6.35
loop mediated isothermal amplification
LAMP
LMIA
isothermal nucleic acid amplification strategy using 4 to 6 primers recognizing 6 to 8 distinct regions of the target DNA or RNA where a strand-displacing DNA polymerase initiates synthesis and 2 to 4 of the primers form loop structures to facilitate subsequent rounds of amplification

3.6.36
mass fraction
ratio of the mass of an analyte relative to the total mass of the mixture

Note 1 to entry: In genetically modified organism (GMO) seed analysis, this refers to the ratio of GM seeds/grains relative to the total seeds/grains corresponding to mass ratio.

3.6.37
master mix
polymerase chain reaction master mix
PCR master mix
mixture of the reagents required for PCR amplification but excluding oligonucleotide primers and DNA template

3.6.38
melting curve
temperature of dissociation
analysis describing the dissociation characteristics of double-stranded DNA observed during heating

Note 1 to entry: The information gathered can be used to infer the presence and identity of single-nucleotide polymorphisms. Temperatures of dissociation (defined as 50 % dissociation) can be revealed at peaks in a plot of the negative first derivative of the melting-curve.

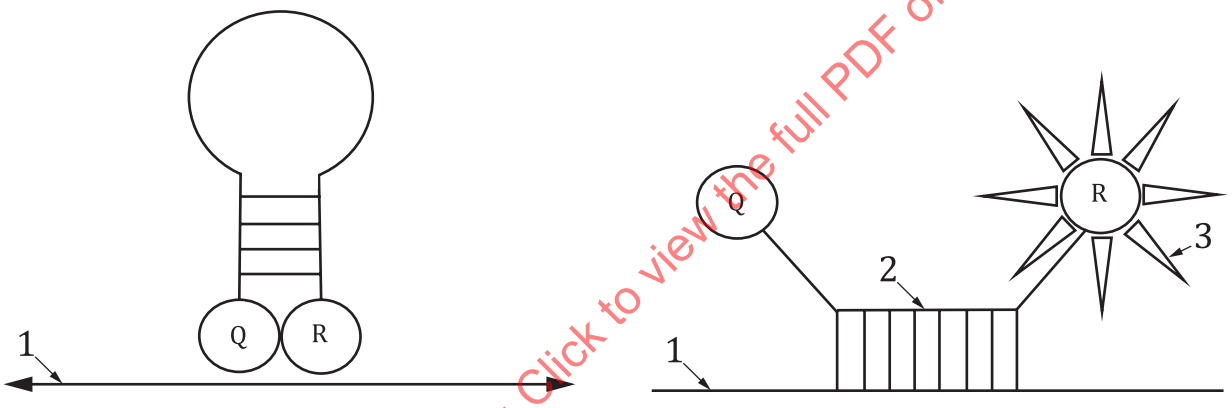
3.6.39
molecular beacon
fluorescent probe that consists of three different parts:

— the central component is complementary to the target nucleic acid sequence, whereas the 5′- and the 3′- components are complementary to each other

— the reporter is attached to one arm of the molecule

— the end of the other arm carries a quencher

Note 1 to entry: See Figure 2.



a) Unhybridized molecular beacon in solution **b) Hybridized molecular beacon resulting in reporter fluorescence**

Key

1	single-stranded nucleic acid	Q	quenching molecule
2	double-stranded nucleic acid	R	fluorescent molecule
3	emitted fluorescent light		

Figure 2 — Molecular beacon schematic

3.6.40
multiplex polymerase chain reaction
multiplex PCR
PCR technique that employs multiple pairs of primers combined simultaneously within a single reaction mixture to produce multiple amplicons

3.6.41
negative deoxyribonucleic acid target control
negative DNA target control
well-characterized DNA preparation material that does not contain target nucleic acid

3.6.42
nested polymerase chain reaction
nested PCR
specific PCR technique in which a second PCR is used to amplify a DNA sequence within an amplicon produced during a first PCR run

3.6.43
nicking enzyme amplification reaction
NEAR
isothermal method for exponential *in vitro* DNA amplification

3.6.44
nucleic acid sequence-based amplification
NASBA
single step isothermal amplification for the detection of RNA only

Note 1 to entry: NASBA utilizes avian myeloblastosis virus, reverse transcriptase, T7 RNA polymerase, RNase H and two target primers.

3.6.45
optical detection system
photosensitive element necessary for detecting fluorescence signals

Note 1 to entry: Two examples of optical detection systems are those typically used on real-time thermocyclers to detect a fluorescence signal, or those to measure absorbance at a specific wavelength, e.g. when reading an ELISA plate.

3.6.46
passive reference dye
fluorescent molecules present in the reaction mix used to normalize the signal, which can be coupled with nucleic acid sequences or other molecules not taking part in the reaction

3.6.47
polymerase chain reaction
PCR
in vitro enzymatic technique to increase the number of copies of a specific DNA fragment by several orders of magnitude

3.6.48
polymerase chain reaction enzyme-linked immunosorbent assay
PCR ELISA
immunoenzymatic method for detecting amplicons in liquid phase after they have been captured on a solid surface, such as in microplate wells

3.6.49
polymerase chain reaction inhibitor
PCR inhibitor
factor that negatively affects the amplification of nucleic acids through the PCR

Note 1 to entry: In a multiplex PCR, it is possible for the different sequences to have asymmetric inhibition effects, leading to disparity in their relative amplifications.

3.6.50
positive deoxyribonucleic acid target control
positive DNA target control
positive polymerase chain reaction control
positive PCR control
any reliable source of well-characterized positive sample material, containing intact target nucleic acid sequences for PCR

Note 1 to entry: Reference DNA or DNA extracted from a certified reference material is generally used to demonstrate that PCR reagents are working as intended.

3.6.51
product
polymerase chain reaction product
PCR product
DNA molecule/fragment produced by PCR amplification

Note 1 to entry: A PCR product is an *in vitro* amplicon.

3.6.52
quantitative polymerase chain reaction
quantitative PCR
qPCR
real-time PCR applied to quantification of the template DNA

Note 1 to entry: In this application, a calibration curve is required.

3.6.53
quencher
conjugated heterocyclic molecule located near a fluorescence emitting dye that following excitation of the fluorophore absorbs the emitted energy thereby quenching the dye

Note 1 to entry: Quenchers are useful in real-time polymerase chain reaction applications.

3.6.54
reagent control
control that contains all reagents except the analyte

Note 1 to entry: The polymerase chain reaction reagent control is used to demonstrate the absence of contaminating nucleic acids in the reagents. Instead of the template DNA, for example, a corresponding volume of nucleic acid free water is added to the reaction.

3.6.55
real-time polymerase chain reaction
real-time PCR
enzymatic procedure that combines the *in vitro* amplification of specific DNA segments with the detection of specific PCR products during the amplification process, and that can be applied to both qualitative and quantitative methods

Note 1 to entry: The intercalating fluorescent dye SYBR® Green[3)] is often used to track the amplification signal in real-time PCR applications.

Note 2 to entry: As the TaqMan®[3)] PCR application amplifies the relevant DNA sequence, degradation of the probe releases the fluorescent marker which is simultaneously unquenched producing fluorescence in direct proportion to the amount of DNA present (which can theoretically be back-calculated to infer the original amount of that particular DNA present in a sample prior to initiation of PCR).

3) SYBR® Green and TaqMan® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the products named.

Note 3 to entry: Using appropriate calibration curves and reference values, real-time information about reaction rates and times translates into information about relative and absolute amounts of DNA present.

3.6.56
recombinase polymerase amplification
RPA
isothermal technology that utilizes the enzymatic activities of a recombinase and DNA polymerases to amplify target DNA

Note 1 to entry: If the target amplicon is RNA, a suitable reverse transcriptase is used to create DNA analogues for amplification.

3.6.57
reporter molecule
fluorescent molecule used to detect the hybridization of specific probes by excitation with electromagnetic radiation of an appropriate wavelength

3.6.58
reverse primer
negative sense oligonucleotide that anneals to the (+) sense strand of denatured DNA in a polymerase chain reaction

3.6.59
reverse transcription polymerase chain reaction
RT-PCR
process by which an RNA strand is first reverse transcribed into its DNA complement (complementary DNA or cDNA) using reverse transcriptase and the resulting cDNA is amplified using PCR

3.6.60
rolling circle amplification
RCA
isothermal enzymatic process where a short DNA or RNA primer is elongated to form a single-stranded DNA or RNA product using a circular DNA template and specialized DNA or RNA polymerases

Note 1 to entry: The RCA product is a concatemer containing tens to hundreds of tandem repeats that are complementary to the circular template.

3.6.61
screening method
method that rapidly and reliably identifies a biomarker(s) permitting the assessment of large numbers of test samples for a particular characteristic, trait or condition, the precise identity of which can require the application of a more rigorous and specific method on the now restricted sample set

Note 1 to entry: Screening tests are frequently applied to rapidly assess whether or not a sample under investigation is likely to contain materials derived from genetically modified (GM) plants.

3.6.62
specific primers
primers that are known to anneal to and produce polymerase chain reaction amplification from a specific nucleic acid sequence

3.6.63
strand displacement amplification
SDA
isothermal nucleic amplification strategy that relies on a strand-displacing DNA polymerase, typically Bst DNA polymerase, large fragment or Klenow fragment (3′-5′ exo–), to initiate at nicks created by a strand-limited restriction endonuclease or nicking enzyme at a site contained in a primer

3.6.64
SYBR® Green[4)]
asymmetrical cyanine dye used as a nucleic acid stain in molecular biology

Note 1 to entry: The IUPAC name for SYBR® Green is N′,N′-dimethyl-N-[4-[(E)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-N-propylpropane-1,3-diamine.

Note 2 to entry: SYBR® Green binds to DNA. The resulting DNA-dye-complex absorbs best 497 nanometer blue light (λmax = 497 nm) and emits green light (λmax = 520 nm).

Note 3 to entry: The dye preferentially binds to double-stranded DNA but will stain single-stranded (ss) DNA with lower performance. SYBR® Green can also stain RNA with a lower performance than ssDNA.

Note 4 to entry: Other dyes used in nucleic acid staining include ethidium bromide (EtBr), SYBR® Safe[4)] and Gel Red®[4)].

Note 5 to entry: The most common type of SYBR® Green used is SYBR® Green I[4)]. However, different manufacturers have designated their SYBR® Green products with additional nomenclature, e.g. SYBR® Green II[4)], Brilliant III[4)].

Note 6 to entry: SYBR® Green is used in real-time and quantitative polymerase chain reactions.

3.6.65
Taq DNA polymerase
thermostable DNA polymerase derived from the thermophilic bacterium *Thermus aquaticus* commonly utilized to catalyse polymerase chain reactions (PCRs), which are needed for thermal cycles utilized in the PCR technique

Note 1 to entry: Taq polymerase was one of the first thermostable DNA polymerases used for PCR. A number of thermostable DNA polymerases have now been introduced commercially for use in PCR from different microorganism sources and with different properties that can be preferred in certain PCR applications, e.g. high fidelity.

3.6.66
thermal cycling
thermocycling
process including numerous heating and cooling steps of a pre-determined temperature regime used to denature, anneal and elongate nucleic acids in a polymerase chain reaction

3.6.67
thermocycler
thermal cycler
automated laboratory apparatus used to raise and lower the temperature of a sample in preprogramed steps

3.6.68
universal primer
consensus primer
oligonucleotide known to anneal and produce polymerase chain reaction amplification in the majority of the species of a given taxonomic group or of a given repetitive sequence within an organism

Note 1 to entry: Adaptor or tail sequence is added to the ends of an amplicon to facilitate capture or identification.

3.6.69
uracil N-glycosylase
UNG
enzyme which removes uracil from deoxyuridine-containing nucleic acid sequences of either double- or single-stranded DNA, leaving apyrimidinic sites

4) SYBR® Green, SYBR® Safe, Gel Red®, SYBR® Green I, SYBR® Green II and Brilliant III are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the products named.

3.7 Nucleic acid sequencing

3.7.1
coverage
read depth
number of times that a given base position is read in a nucleotide sequencing run or stretch of nucleotide sequence

3.7.2
CRAM
compressed columnar file format for storing biological sequences aligned to a reference sequence

3.7.3
deoxyribonucleic acid barcoding
DNA barcoding
method of identifying organisms based on short, standardized DNA fragments containing both conserved and variable sequences from a specific region or regions of the genome

Note 1 to entry: The principle of DNA barcoding is that by comparison with a reference database the sequence from these DNA fragments can be used to uniquely identify an organism or link it to a specific taxon.

3.7.4
deoxyribonucleic acid library
DNA library
sequencing library
collection of genomic DNA fragments or polymerase chain reaction amplicons, or both, intended to be sequenced in a sequencing run

3.7.5
deoxyribonucleic acid metabarcoding
DNA metabarcoding
special case of DNA barcoding that is applied to samples which contain more than one organism using the same reference databases as barcoding

Note 1 to entry: DNA metabarcoding permits identification of taxa from mixed samples with high throughput sequencing methods.

3.7.6
deoxyribonucleic acid sequence database
DNA sequence database
genetic sequence database
nucleic acid sequence database
biological database that is composed of a collection of nucleic acid sequences stored electronically

Note 1 to entry: A DNA sequence database can contain any number of entries.

Note 2 to entry: A DNA sequence database may also contain protein sequences.

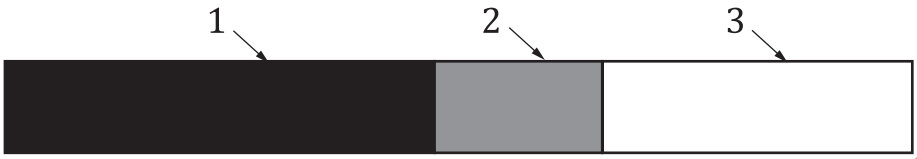
3.7.7
deoxyribonucleic acid sequencer
DNA sequencer
gene sequencer
genetic analyser
nucleic acid sequencer
sequencer
scientific instrument used to automate the DNA sequencing process

Note 1 to entry: A DNA sequencer is used to determine the order of the four bases, G (guanine), C (cytosine), A (adenine) and T (thymine), of a DNA sample which is then reported as a text string, called a “read”.

Note 2 to entry: Some DNA sequencers can be also considered optical instruments as they analyse light signals originating from fluorochromes attached to nucleotides.

3.7.8
fusion primer
polymerase chain reaction primer containing a sequencing platform-specific adapter sequence, a sample index and a target specific priming site producing amplification products that can be sequenced without additional adapters

Note 1 to entry: See Figure 3.



Key

1 sequencing platform-specific adapter sequence
2 sample index
3 specific priming site

Figure 3 — Fusion primer example

3.7.9
gap
nucleotide(s) missing in the same DNA position between alignments of two or more DNA sequences

3.7.10
massively parallel nucleotide sequencing
next generation sequencing
NGS
whole genome sequencing
WGS
high throughput nucleotide sequencing method capable of determining multiple DNA sequences simultaneously and in parallel

Note 1 to entry: The data from a single massively parallel sequencing analysis comprises of millions of sequences and the output is a file containing all sequences.

3.7.11
metagenomics
study of genetic material, i.e. DNA and RNA recovered directly from an environment, e.g. microbiome

3.7.12
molecular barcoding
common approach to labelling samples for multiplex sequencing and analysis where each molecule in a sample is labelled with a unique sequence prior to polymerase chain reaction (PCR) amplification

Note 1 to entry: With each nucleic acid in the starting material tagged with a unique molecular barcode (MBC), sequence analysis software can filter out duplicate reads and PCR errors to report unique reads.

3.7.13
MPEG-G
International Standard for the representation of genomic information based on the concept of the Genomic Record, a data structure consisting of either a single sequence read or a paired sequence read, and its associated sequencing and alignment information

Note 1 to entry: It may contain detailed mapping and alignment data, a single or paired read identifier (read name) and quality values.

3.7.14
Phred quality score
Q score
measure of the probability (P_r) that a nucleotide sequence base determined in a nucleotide sequencing method is incorrectly assigned at a given position in the sequence expressed as:

$$Q = -10_{\log} P_r$$

Note 1 to entry: A score of Q30 indicates that there is a 1 in 1 000 chance that a base is incorrectly assigned (i.e. the base call is 99,9 % accurate).

3.7.15
read
nucleotide sequence inferred from a fragment of DNA or RNA

3.7.16
sample indexing
sample barcoding
common approach to labelling samples for multiplex sequencing and analysis where all of the nucleic acids in a single sample are labelled with the same unique sample indexing sequence tag, and the resulting library is pooled with other libraries and sequenced in parallel in a single run

Note 1 to entry: During analysis, the sample-specific indexes enable bioinformatics pipeline software to separate the multiplexed sequence data into individual sample-specific data sets.

3.7.17
Sanger sequencing
method of DNA sequencing based on the selective incorporation of chain-terminating dideoxynucleotides by a DNA polymerase during *in vitro* DNA amplification

Note 1 to entry: This method was developed by Frederick Sanger in 1977.

3.7.18
sequence alignment
arrangement of nucleic acid sequences or protein sequences according to regions of similarity

3.7.19
sequence alignment map
SAM
sequence alignment map format
SAM format
text-based TAB-delimited format for storing biological sequences aligned to a reference sequence consisting of a header section, which is optional, and an alignment section

Note 1 to entry: SAM is widely used for storing data, such as nucleotide sequences, generated by massively parallel nucleotide sequencing technologies, and the standard has been broadened to include unmapped sequences. The format supports short and long reads (up to 128 Mbp) produced by different sequencing platforms and is used to hold mapped data within the Genome Analysis Toolkit (GATK) and across the Broad Institute, the Wellcome Sanger Institute, and throughout the 1000 Genomes Project. It may contain base-call and alignment qualities and other data.

3.7.20
sequence read archive
SRA
internet cloud based raw sequencing data repository

Note 1 to entry: The SRA accepts genetic data and the associated quality scores produced by massively parallel nucleotide sequencing technologies.

Note 2 to entry: SRA accepts binary files such as binary alignment map (BAM), standard flowgram files (SFF) and HDF5 formats, and text formats such as FASTQ.