



This document was written in response to the feedback from ISTA GMO Proficiency Test (PT) participants. It is intended to provide guidance with respect to the set up of calibration curves, quantification units, and the relationship between them:

Real-time PCR quantitative results of ISTA GMO Proficiency Tests: Standards and Units.

The absolute quantification of a gene of interest in Real-time PCR is derived from a standard curve (also referred to as a calibration curve). The standard curve can be prepared in a number of ways where each method will consequently define the quantity by a different unit. The two most common methods of preparing standard/calibration curves are by mass fraction, or, by haploid genome copy number.

Standard curve by mass fraction (w/w): The standards are produced from Certified Reference Material (CRM), typically flour. These certified samples consist of conventional seed flour fortified with genetically modified seed flour at a given w/w proportion; an example will be the CRMs produced and supplied by the Institute for Reference Materials and Measurements (IRMM).

There are generally two ways to prepare a calibration/standard curve by mass fraction:

- Extract DNA from a series of CRMs. A common series used is; 0.1%, 0.5%, 1%, 2%, 5%. Each standard DNA is isolated independently from the corresponding CRM and a set quantity of DNA of each standard (e.g. 100 ng) is used for the analysis.
- Extract DNA from a CRM of high percentage GM (e.g. 10%) and subsequently make a serial dilution from this DNA sample. For example; DNA isolation from a 10% CRM and a -2 X dilution series of it that will yield 5%, 2.5%, 1.25%, and 0.625% standards.

Regardless of the method by which the standards were produced they are referred to as mass %. This is true even if the laboratories obtain the copy number by multiplying the DNA quantity by the C value of the corresponding crop species (for example, for maize $1C = 2.6 \text{ pg}$ or $1 \text{ ng} = 385$ haploid genomes, see Arumuganathan and Earle, 1991). The quantitative results obtained using any of the two types of standard curve by mass fraction shall be reported as mass%.

Please note that when preparing standard curve by mass fraction, the genetic structure (zygosity, tissue ploidy, and origin of the transgenic allele) of the seeds used for the production of the CRM is not

taken into account and corrected for in the definition of the standards, and thus, in the quantifications obtained.

Standard curve by haploid genome copy number: The standards are made of DNA extracted from genetically modified plant tissue (leaf or seeds). Typically, the plant tissue used will be homozygous for the modified trait. It is however possible to use hemizygous tissue for the preparation of the standard curve; in which case, the GM haploid genome copy number shall be calculated while considering the genetic structure of the hemizygous tissue used. These calculations are obviously species specific. For example, in maize hemizygous seed, the GM haploid genome copy number can be calculated as follows: 100 ng of genomic DNA consists of approximately 38500 haploid genomes; hence, a 1% standard contains about 160 GM haploid genomes when the transgenic gene is introduced by the male parent or, 225 GM haploid genomes when the GM DNA sequence is introduced by the female parent (see Zhang et al, 2008).

Regardless of the initial source (tissue or zygosity) of the standards; the quantitative results obtained using this standard curve shall be always reported as % DNA copies. This term: % DNA copies should be understood as: *“the percentage of specified trait/s DNA copy numbers in relation to target taxon specific DNA copy numbers calculated in terms of haploid genomes”* (EC Recommendation, 2004/787/EC). Therefore, reporting results as % DNA copies requires by definition the preparation of the standard curve by haploid genome copy number for both GM and taxon PCR targets as described above.

References:

D. Zhang, A. Corlet, S. Fouilloux: Impact of genetic structures on haploid genome-based quantification of genetically modified DNA: theoretical considerations, experimental data in MON 810 maize kernels (*Zea mays* L.) and some practical applications. *Transgenic Res* (2008), 17:393-402. (DOI10.1007/s11248-007-9114-y).

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Arumuganathan K. and Earle E.D. Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.*, 1991, 9, 208-218

EC Recommendation 2004/787/EC