

The use of plasmids as reference materials

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Introduction to Reference Materials

The use of reference materials is essential to ensure accuracy of results obtained from analytical methods. Among these methods is the Polymerase Chain Reaction (PCR), which is often used for the detection of biotechnology-derived traits. A reference material (RM) is any material sufficiently homogenous and with known characteristics such that it can be used for calibrating analytical methods, in assigning values to unknown materials, or as a control material in assessing the performance of a method (ISO 2016). A single RM cannot be used for both calibration and validation of results in the same measurement procedure (NIST, 2012).

Types of Reference Materials

Two distinct types of reference materials have been used in PCR-based methods when applied to the detection of biotechnology-derived products. 1) Plant-based reference materials may be either plant tissue, from which genomic DNA is extracted (e.g. seed or leaf) or, alternatively, purified plant genomic DNA. These plant-based reference materials include the target DNA sequences and the endogenous reference sequence (a native DNA target), both within the context of the full plant genome. 2) Plasmid reference materials are circular vectors or other DNA that carry short segments of the target sequence from the introduced trait and/or of the endogenous reference system. These target sequences are typically contained in a bacterial vector multiplied in bacteria to produce large quantities. Plasmid sequences, by definition, are not in the context of the full plant genome.

Plant based reference materials: Advantages and Challenges

Plant based reference materials (RMs) are ideal because they most closely represent the characteristics of the test material. RM derived from plant material is critical to ensure a representative control for establishing accuracy of a method and serve as calibrants in sample analysis. The conformational integrity of the target sequence is maintained in plant-derived RMs (e.g., it is in the full genomic context), a feature which contributes to their effectiveness as representative controls (Lin *et al.*, 2011). Plant material or DNA has remained the ‘gold standard’ of Certified Reference Material (CRM) for this purpose. However, plant-based reference materials are costly and challenging to produce in large quantities at the level of purity and characterization required.

Plasmid based reference materials: Advantages and Challenges

Plasmid reference materials are being used as calibrators for PCR detection methods, particularly for screening methods that use genetic elements common in multiple products. Plasmids are easy to produce in large quantities with high purity. They can be used to produce linear dilution series in calibration curves that give equal or better precision of the standard curve than equivalent genomic DNA derived from plant-based reference materials. However, errors can be introduced due to the need for very high dilution factors required to obtain

desirable plasmid standards from solutions with measurable DNA content (Allnutt *et al.*, 2005). Additional concerns regarding the use of plasmids are related to the loss of the plant derivation, including matrix and genomic context. Finally, plasmids can be a troublesome source of contamination in laboratories.

Use of Plasmid Reference Materials

Several laboratories have demonstrated that plasmid DNAs are appropriate for use as calibrators (Burns *et al.*, 2006; Tavernier *et al.*, 2004; Charels *et al.*, 2007; Shindo *et al.*, 2002; Caprioara-Buda *et al.*, 2012). However, use of plasmid reference materials require validations in which quantification values generated using plasmid reference material are compared to data generated using plant-derived genomic DNA reference materials. Differences between amplification of the plasmid DNA and the plant DNA may occur, requiring a correction factor for accurate quantification. (Allnut *et al.*, 2005 ; Meng *et al.*, 2011; Charels *et al.*, 2007). The use of spiked plasmid DNA in non-target plant-derived genomic DNA can minimize these issues; however, each quantification scenario (i.e. specific plasmid used for a specific GM event) must be evaluated on a plasmid-specific basis.

Availability of Plasmid Reference Materials

Today, several organizations are producing plasmid RM or CRM to be used as independent calibrants in conjunction with plant-based RM (Corbisier *et al.*, 2008; IRMM 2020). In Japan and China, plasmid calibrants have been developed that contain multiple event-specific sequences (Kuribara *et al.*, 2002).

CLI Recommendation

CLI member companies recommend the use of plant derived CRMs where available. These materials most accurately reflect the test substance under evaluation and are most likely to deliver highly accurate results that are required to validate a method or as proficiency test samples. However, when availability of plant-derived RM is limited, plasmid reference materials can be a useful option, but validation to demonstrate equivalency of the plasmid RM to plant-derived RM is a prerequisite for their use. Plasmid DNA may only be used as a calibrant in these circumstances.

Intellectual property protection

While event-specific sequences may be publicly disclosed, these sequences are often the intellectual property (IP) of the trait developer and are typically the subject of patent applications. The CLI member companies' position on the manufacture and distribution of plasmids containing proprietary sequences is consistent with that for plant-based reference materials. Reference materials that contain IP may not be produced, distributed, or analyzed without written approval or license from the patent owner.

Summary:

- Plant derived RMs are recognized as the preferred reference material for detection of biotechnology derived traits because they are most representative of the sample under evaluation
- Plasmid-based reference material can be useful as a calibrant if sufficient validation is conducted to demonstrate equivalency to results obtained using plant-derived RMs

Because of IP considerations, patent owners must be contacted and grant approval prior to production of plasmid-based reference materials.

Glossary

Accuracy: closeness of agreement between a measured quantity value and a true quantity value of a measurand (ISO 2016)

Calibrant/Calibrator: Material that is used to adjust instrumentation that is based on or traceable to a reference preparation or material, and whose values are determined by acceptable reference methods. R&D Systems. 2010.

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 . (ISO 2016).

Control material: Material used for the purposes of internal quality control or external quality assessment (proficiency testing), and subjected to measurement according to the same or part of the same measurement procedure as that used for unknown samples in order to monitor analytical performance. Dybkaer, R. 1997.

Precision: The expression of the variability of analysis, or an indication of the amount of random error that exists in an analytical process. R&D Systems. 2010.

Reference material (RM): 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 ISO. 2016.

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