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 all analytical methods. Among these methods is 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 having known characteristics such that it can be used for calibrating a method, in assigning values to unknown materials, or as a control material in assessing the performance of a method (ISO, 1993). 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 genomic complement. 2) Plasmid reference materials are circular vectors that carry short segments of the target sequence from the introduced trait and/or of the endogenous reference system. These target sequences are introduced into a bacterial vector by standard cloning procedures and multiplied in bacteria to produce large quantities. Plasmid sequences, by definition, are not in the context of the full plant genomic complement.

Plant based reference materials advantages/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. 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,C.H., et. al, 2011). However, plant based reference materials are costly and challenging to produce in large quantities. Ensuring the availability of a homogenous and sustainable source of reference materials can be problematic (Kuribara, H., et. al, 2002).

Plasmid based reference materials: Advantages and Challenges

There is a growing interest in using plasmid reference materials as calibrators for PCR detection methods. Plasmids are relatively 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 matrix reference materials. However, errors can be introduced due to the need for very high dilution factors required to obtain useful plasmid standards from solutions with measurable DNA content (Allnutt, T.R., 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 investigated the use of plasmid RM in PCR and demonstrated that they are appropriate for use as calibrators (Burns, et. al, 2006, Tavernier, I., et. al., 2004, Charels, D., et. al., 2007, Shindo, Y., et. al., 2002, Caprioara-Buda, M., et al., 2012) . However, these studies required exhaustive validations in which quantification values generated using plasmid reference material were compared to data generated using plant-derived genomic reference materials. Differences between amplification of the plasmid DNA and the plant DNA may occur, requiring a correction factor for accurate quantification. (Allnut, T.R., et. al., 2005, Meng, Y., et. al., 2011, Charels, D., et. al., 2007). The use of dual or multi-target plasmids help minimize these issues; however each plasmid/event quantification scenario must be evaluated on a plasmid-specific basis.

Availability of Plasmid Reference Materials

Today, several organizations are producing plasmid reference material (RM), or certified reference material (CRM) to be used as independent calibrants in conjunction with plant-based RM (Corbisier, P., et. al., 2008). In Japan, plasmid calibrants have been developed that contain multiple event-specific sequences (Kuribara, H., et. al., 2002).

CLI Recommendation

CLI member companies recommend the use of plant derived reference materials where available. These materials most accurately reflect the test substance under evaluation and are most likely to deliver highly accurate results. However, when availability of plant-derived RM is limited, plasmid reference materials can be a useful option. Under these circumstances, 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 but cannot be used to validate the method or as proficiency test samples.

Intellectual property protection

While event-related 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 to the true value, or the measure of truth of a result. R&D Systems. 2010.

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 a certificate, one or more of whose property values are certified by a procedure which established traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence. ISO. 1993.

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. 1993.

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