AORC Guidelines

For the Minimum Criteria for Identification of transgenes or vectors by Polymerase Chain Reaction (PCR) analysis

1. This document provides a set of internationally agreed recommendations for the comparison of polymerase chain reaction (PCR) data consistent with the relevant sections of ILAC-G7 Part B "Guide for Establishing the Presence of Prohibited Substances". The AORC recognises that these represent guidelines for analysts and laboratories, whose responsibility is to ensure the quality and integrity of the data is defensible and fit for purpose. Furthermore, AORC laboratories should have their own minimum criteria defined and documented.

2. Samples obtained and analysed to identify transgenes or vectors should be collected, received, identified, and receipted in accordance with ILAC-G7 Part B "FORENSIC INTEGRITY".

3. The following sections outline additional guidelines for establishing the presence of transgenes or vectors.

Sampling

4. Samples should be collected using appropriate methods for downstream molecular biology activities.

5. Adequate sample (e.g. number of tubes) should be collected to allow a split-sample analysis and independent extractions to be performed.

6. PCR-based methods involve the amplification of target DNA. Due to the method sensitivity, pre-PCR work should be performed in a separate clean location from PCR and post-PCR work. Laboratories should have a cleaning protocol to prevent the accumulation of genetic material.

7. A laboratory should determine which matrix is appropriate for the targeted identification. The matrix may include, but not be limited to, whole blood, plasma, buffy coat or urine.

PCR Analysis

8. Nuclease-free PCR reagents and plasticware should be procured from reputable suppliers and be appropriate for the analysis.

9. The reagent name, catalogue, lot number and expiry date should be recorded.

10. Water used in PCR reactions should be of an appropriate grade and nuclease-free.

11. The instrument type, model and software version should be documented.

12. The method should be appropriately validated. Instruments should be appropriately evaluated for the method used.

13. The cycling conditions used for the method should be recorded.

Reference Materials and Controls

14. Reference materials as described in ILAC-G7: 04/2021 Part B Clauses 16.2, 16.3 and 16.4 are suitable.

15. Reference materials can be in the form of a DNA plasmid(s) or synthetic DNA/RNA fragment containing the full, partial, identical, or modified transgene or vector sequence.

16. The same set of primers and hydrolysis probe (if applicable) must be used for the test sample and appropriate controls in each step of the analysis.

17. The testing procedure should include the appropriate controls:
    a. Positive control (PC),
    b. Negative control (NC),
    c. PCR reagents positive control (PTC) to verify the correct function of the PCR assay,
    d. PCR reagents negative control (NTC) to verify the absence of assay contamination.

Confirming a Finding

18. The number of sample replicates to be performed is determined by the laboratory based on its method validation.

19. For transgenes, the PCR assay will target an exon/exon junction sequence whenever applicable.

20. For real-time PCR:
    a. A sample is considered positive when its valid quantification cycle (Cq) value is significantly differentiated from the negative control within the same assay.
    b. An interpretation of the amplification curve (a plot of fluorescence signal against cycle number) is necessary to assess validity of the confirmation. Amplification of the target transgene will produce either a full or partial sigmoidal curve.

21. For digital PCR, a sample is considered positive when its positive droplets with significant amplitude (fluorescent intensity) are differentiated from the negative control within the same assay.