CELL LINE CHARACTERIZATION
DNA BARCODE ASSAY

For cells that are to be used in GMP biomanufacturing processes, confirmation of cell identity in a Master Cell Bank (MCB) is required\(^1,2\). The identification of species through genotypic testing is an acceptable confirmatory test, with species identification by “DNA analysis to detect a genomic polymorphism pattern” being specifically called out in the applicable regulations\(^1\). The desire to rapidly identify and discriminate cellular species \textit{de novo} by genetic markers originated in the 1980’s with the development of DNA fingerprinting\(^3\). However, it was not until the technology and scientific understanding had progressed sufficiently that a standardized approach to animal species classification was proposed in 2003\(^4\).

The cytochrome c oxidase subunit 1 (CO1) gene is an ideal target for species discrimination as this gene is conserved across higher eukaryotes, including insects, fish, birds and mammals. CO1 is located in the mitochondrial DNA, meaning it has a relatively fast mutation rate that aligns with speciation timescales. The absence of intronic regions on mitochondrial DNA also makes this a favorable target for near universal species identification. BioReliance offers two cell identity test services based on the CO1 barcode methodology. To perform this assay, a \(-650\text{bp}\) region of CO1, known as the Folmer (or barcode) region\(^5\), is amplified with degenerate primers, followed by DNA sequence analysis of the amplification product. This sequence is then compared with known (and validated), species-specific CO1, reference sequences to identify the sample’s origin. For insect species, a smaller region of the CO1 gene (~450bp) is targeted. The CO1 barcode assay workflow is outlined in Figure 1.

- CO1 barcoding is a well documented state-of-the-art technique for cell line identification.

- The use of CO1 barcode technique for species identification is well understood by the regulators and used widely by the large cell repositories.

- Availability of this assay in both the UK and USA reduces the burden of obtaining shipping permits.

- GMP assays enable successful regulatory submission.
DNA BARCODE ASSAY

CO1 Barcode Assay for Cell Line Identification

Degenerate primers with adaptor sections (see Figure 1) were designed, optimized and validated to target the ~650bp barcode region of the CO1 gene. After amplification of the extracted DNA, the sample is sequenced (Sanger method) prior to being compared to a known reference sequence. Current validated reference sequences are shown below. Additional species coverage will follow:

- Human (e.g., HEK293)
- Mouse (Mus musculus e.g., SP2/0)
- Chinese Hamster (e.g., CHO)
- Syrian Hamster (e.g., BHK)
- African Green Monkey (e.g., Vero)

CO1 Barcode Assay for Insect Cell Line Identification

Similar to the above assay, the CO1 barcode assay for insect cell identification uses degenerate primers with adaptor sections designed, optimized and validated to target the ~450bp barcode region for insect species identification. Sanger sequencing followed by comparison to the validated reference sequence provides confirmation. The current validated species coverage is shown below, with additional insect species to follow:

- Spodoptera frugiperda (e.g., Sf9)

Conclusion

The CO1 Barcode assay is ideal for cell line identification, as it is able to confirm the identity of either mammalian or insect species. This methodology is known to the regulators and is already widely adopted by the major cell repositories as the method of choice for species confirmation.

These assays are available from BioReliance's Glasgow, Scotland and Rockville, MD facilities.

References


For more information about BioReliance services, visit bioreliance.com

Ordering Information

<table>
<thead>
<tr>
<th>Assay Number</th>
<th>Assay Description</th>
<th>Sample Requirements</th>
<th>Regulatory Compliance</th>
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<td>CO1 Barcode Assay for Cell Line Identification</td>
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Contact Us

North America  Toll Free: 800 553 5372  Tel: +1 301 738 1000
Europe & International  Tel: +44 (0)141 946 9999
Japan  Tel: +81 (0)3 5796 7430
Email: info@bioreliance.com

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