DNA Sizing and Barcoding: Alternatives to Isoenzyme Analysis

Cell line identity testing is a critical regulatory requirement for recombinant cell lines in order to confirm the cell line's species of origin, as well as to assist in detecting contamination from other cell lines.

Biochemical analysis of isoenzyme polymorphism has historically been considered the standard test for mammalian and insect cell line identification; however, the methodology suffers several limitations, including:

- Limited species coverage
- Lack of sufficient sensitivity
- Difficulty in data interpretation, particularly when trying to differentiate closely related species
- Test kits provided by a single supplier
- Recent shortage of reagent supply

To overcome these limitations and challenges, Eurofins Lancaster Laboratories has developed DNA Sizing and Barcoding Methods for species identification. These methods utilize the mitochondrial genes Cytochrome Oxidase 1 (CO1) and Cytochrome B (Cyt B) as targets for the molecular identification of cell lines commonly used in biopharmaceutical production.

Why Choose Eurofins Lancaster Laboratories?

Our comprehensive cell line characterization services, along with our cell banking capabilities, provide you with a single-source solution for all of your cell line needs.

CO1 DNA Sizing and Barcoding Method

- For CO1/Cyt B Sizing, genomic DNA is isolated from the cells being tested and amplified using a set of species specific primers.
- Species identification and detection of cross contamination can be made based upon the presence/absence of PCR amplification by given sets of primers and the size of the PCR amplicon. Cell lines of cow, mouse, dog, rat, monkey, hamster, cat, human and pig origin can be tested using CO1/Cyt B Sizing.
- Identification of cell lines derived from a wider variety of animal and insect species can be made by CO1 DNA Barcoding. The comparison between the determined CO1 sequence of tested cell lines and the confirmed species specific sequences deposited in the Consortium for the Barcode of Life database allows for unambiguous genetic identification.
- The Sizing and Barcoding Methods are based on a more robust technology than that of the isoenzyme assay and can therefore provide more accurate speciation. They can also be easily implemented in a quality control environment.
- The PCR amplification and DNA sequencing techniques used for the Sizing and Barcoding Methods decrease dependency on a single supplier for test kits and reagents.
- The Sizing and Barcoding Methods are more sensitive with regard to the detection of cell line cross contamination than isoenzyme analysis.

Please contact us for a technical discussion prior to sample submission.
Unambiguous Species Identification Based on CO1/Cyt B Amplicon Size

**Lanes**

**M** = GeneRuler Low Range DNA Ladder
1 = DNA isolated from MDBK cells at a total of 5x10^6 cells amplified with primers specific for *Bos taurus* (cow) species
2 = DNA isolated from NIH3T3 cells at a total of 5x10^6 cells amplified with primers specific for *Mus musculus* (mouse) species
3 = DNA isolated from MDCK cells at a total of 5x10^6 cells amplified with primers specific for *Canis familiaris* (dog) species
4 = DNA isolated from XC cells at a total of 5x10^6 cells amplified with primers specific for *Rattus norvegicus* (rat) species
5 = DNA isolated from Vero cells at a total of 5x10^6 cells amplified with primers specific for *Cercopithecus aethiops* (Vervet monkey) species
6 = DNA isolated from CHO cells at a total of 5x10^6 cells amplified with primers specific for *Cricetulus griseus* (Chinese hamster) species
7 = DNA isolated from PG4 cells at a total of 5x10^6 cells amplified with primers specific for *Felis catus* (cat) species
8 = DNA isolated from MRC5 cells at a total of 5x10^6 cells amplified with primers specific for *Homo sapiens* (human) species
9 = DNA isolated from PT1 cells at a total of 5x10^6 cells amplified with primers specific for *Sus scrofa* (pig) species

**Internal Control**: Amplicon of the 18S rRNA gene region, which must be visible in all PCR amplifications