

## Current Perspectives on DNA Sequence Analysis of Biological Therapeutics

a report by

**Carl W Balezentis, PhD**

*Lark Technologies, A Genaissance Company*

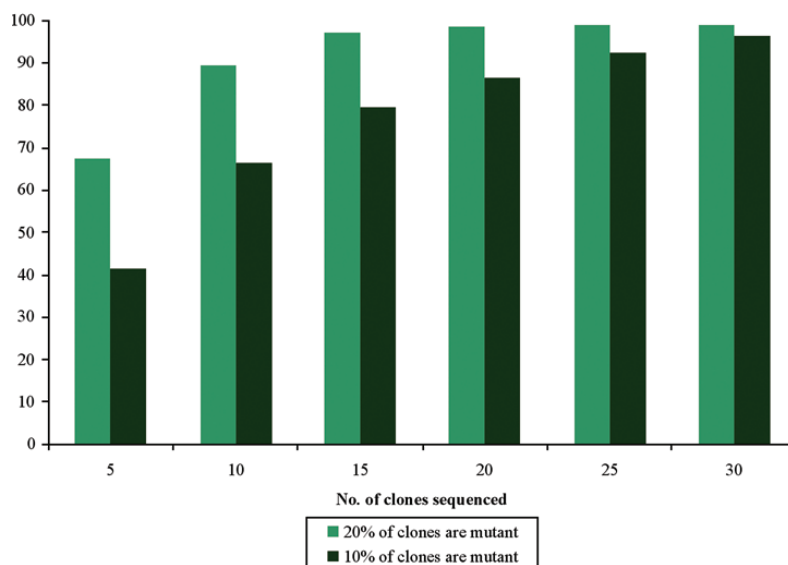
A key component of any bio-safety testing programme for manufacturers of biological therapeutics is the characterisation of the complete nucleotide sequence of the product. Since 1982, when recombinant production of insulin first began, the number and variation of biological therapeutics has exploded, resulting in the emergence of many new key products.

There are several important considerations to performing US Food and Drug Administration (FDA) submission DNA sequencing from the standpoint of the manufacturer. It is critical to determine the nature and extent of DNA sequencing that will be performed. There are several regulatory and guidance documents, published by such regulatory authorities as the FDA, the International Conference on Harmonization (ICH) and in the *Iyakuhin Kenkyu*. A certain degree of flexibility is inherent in these documents, and DNA sequence analysis of each product should be reviewed on a case-by-case basis. Important questions and determinations arise when characterising the DNA sequence of a viral vector. These questions cover issues such as when, in the development and production process, it is necessary to sequence the entire vector, and whether the entire vector should be sequenced, or if it is sufficient to sequence the genetic insert plus flanking regions and any significant modifications to the vector backbone.

In determining if mutations arose early on in the expansion of a cell line to become significant in the final product, DNA sequence analysis is a poor method for detecting and quantifying admixtures. Detection can be achieved by plating out cells from the cell bank and sequencing the plasmids from multiple independent colonies (see *Figure 1*). How many clones should be sequenced to identify the percentage of mutant variants? Genetic variation from product to product necessitates a custom approach to the characterisation of each product.

The strategy for verifying the nucleic acid sequence in the product of interest must also be considered. Once the nature and extent of the DNA sequence analysis to be performed has been determined, the strategy must be designed to effectively guarantee 100% accuracy of those nucleic acid regions. Often, as in the case of

**Figure 1: Relationship Between Number of Clones Sequenced and Probability of Detecting Mutations Present in a Cell Bank**



DNA sequence analysis of a plasmid from a cell bank, the amount of starting material available is limited, prohibiting direct sequencing and requiring alternate strategies to obtain the nucleic acid sequence without sacrificing a large number of valuable vials of cell banks. Resolution of every base pair is often further complicated by the DNA sequence itself, which can have areas of high guanine and cytosine (GC) content, difficult secondary structure or identical repeat structures. A custom approach to developing protocols that will account for the unique requirements of each product being characterised is often critical to resolving every base pair with 100% accuracy.

Finally, and probably most importantly, is the regulatory environment under which the DNA sequence analysis is performed, resulting in a quality approach to minimising the possibility of contamination, mixtures and errors. While good laboratory practice (GLP) regulations are considered adequate to perform FDA submission DNA sequence analysis, GLP compliance does not guarantee or sufficiently address issues of internal controls including:

- critical procedure training/qualifications;
- equipment qualification;



- method validation;
- raw material handling;
- change control; and
- a formal system for the handling of deviations and complaints governed by the quality assurance (QA) unit rather than the scientists performing the study.

The implementation of sufficient regulations is largely based on common sense practices and is a continuum, such that the GLP standard of

yesterday is perceived by manufacturers and regulatory agencies as inadequate for the testing of biological therapeutics, and that current good manufacturing practice (cGMP) regulations should supplement the GLP standards of performance for FDA submission DNA sequence analysis. To ensure that the work is being performed in a regulatory environment that will adequately meet performance standards, an audit of the facility providing the analysis must be performed. ■