

Bioc471a/571a Homework 9 - Due at the start of class November 13

1. (2 pts) What is the primary difference between the method used to clone Dolly the sheep, and that used by the group in Hawaii to generate Cumilina the mouse? Cite two reasons why the development of Pharm animals for commercial use may be years away despite proof of principle experiments showing it is possible?

The key difference between the two methods is that Wilmut and colleagues used whole cell fusion by electroporation, whereas, the Honolulu group injected enucleated cells with a single nucleus taken from a diploid somatic cell.

The two main reasons why the use of Pharm animals is still years away for commercial use is the low frequency of success using nuclear cloning and societal concerns about the use of genetically modified organisms.

2. (3 pts.) Describe how an Affymetrix type DNA chip can be used to create identification profiles based on DNA sequence differences, i.e., how is an Affymetrix chip used to sequence DNA based on hybridization? What are the two essential limiting parameters in designing an instantaneous "finger prick" assay as depicted in the movie "GATTACA" that must be worked out before this type of fingerprinting could be a reality? (hint: PCR solved these two problems with regard to the limited use of Southern blotting for DNA forensics).

An Affymetrix type chip can be used for DNA sequencing by designing clustered oligonucleotides that differ by only one base at the center, thereby creating the opportunity to "sequence" the DNA based on the differential intensity of the hybridization signal (intense signal denotes the correct Watson-Crick base pair, whereas, faint signals indicate a mismatch at the center nucleotide).

The two limitations to developing the "GATTACA" assay for genotypic fingerprinting are sensitivity and specificity, the same problems that plagued Southern blotting - but were solved by PCR.

3. (2 pts.) Using the amino acid sequence in the Lecture 23 notes, what *protein function* would you predict the "AMG Test Protein" to have based on your own GenBank tblastn and Psi blast query results? Justify your answer. How would you test this prediction?

The best matches to a known function are DNA binding domain proteins, such as transcription factors, specifically HMG box proteins. This could be tested by expressing an AMG-GFP fusion protein in cells and finding out if it localized to the nucleus. Another test would be to express the AMG protein in bacteria and see if the purified protein has high binding affinity for DNA.

4. (3 pts.) What has contributed to the application of mass spectrometry to proteomics, i.e., what are the two major breakthroughs that permitted the field of proteomics to expand so rapidly? Explain how random fragmentation at peptide bonds by tandem mass spectrometry can be used in conjunction with genomic databases to predict the sequence of a peptide.

The two major breakthroughs that permitted mass spectrometry to proteomics were development of ionization methods (MALDI and ESI), and the use of bioinformatics to match mass spectrometry data to predicted proteins in the genomic databases.

Random peptide fragments differ by the exact mass of individual amino acids. The key to this method is knowing which spectral peaks are derived from the y ions (C-terminus) and which are coming from the b ions (N-terminus). This mass information is then blasted against the appropriate genomic databases which predict the protein sequence based on theoretical masses.