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Week 2 Paper Questions

BIFX 504-1

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**What techniques are used to discover genetic variants?**

The techniques explained in the paper were GTG banding, FISH, and aCGH, Sanger and NGS.

**What sequence analysis steps does the paper describe for use when sequencing is used to detect variants? What can you learn by using those techniques?**

The paper explained that PCR is used to catalyze DNA with fluorescently labeled dNTPs. The PCR consists of several sequential DNA replication cycles. Each with a cycle labeled the DNA fragment with a new fluorescent color. Each color corresponds to a particular nucleotide. The end result is a DNA fragment with color-coded nucleotides. This makes it easy to spot variations.

**Why is it important to take epigenetic modifications into account when considering the impact of genetic variants? Give an example.**

Epigenetic modifications can result in changes to gene expression without changing DNA. It is important to understand which changes are due to epigenetics versus DNA. An example of this would be DNA methylation, which often correlates with transcriptional suppression.

**What methods are used to detect DNA methylation? Histone modifications?**

Bisulfite conversion and MDRE are often used to detect DNA methylation. ChIP, ChIP-chip, and ChIP-seq are used to detect histone modifications.

**What part of the RNA transcription process is most tightly regulated?**

The most regulated part of the process is transcriptional initiation.

**How do scientists identify transcription start sites and transcription factor binding sites?**

Usually, a comparative bioinformatics approach is used to construct alignments between orthologous sequences. But the paper states that ChIP could possibly be used too. It also mentions that epigenetic markers could be helpful in tracking down the start and bind sites.

**Why is it beneficial to study mRNA (this paper calls it mRNR for some reason) or cDNA in addition to genomic DNA? What can you learn?**

Studying mRNA or cDNA allows us to study a DNA sequence without its exons. This is helpful because it can reveal abnormal splicing of exons.

**What techniques are used to study mRNA? Explain very briefly how each one works.**

The traditional methods are Northern Blot, SAGE, and qPCR. However more modern techniques like cDNA microarray and RNA-seq are replacing them. Northern Blot involves hybridization with a radioactive probe. SAGE converts RNA to a short unique tag. qPCR uses fluorescence intensity to track the transcription of target cDNA in real-time. cDNA microarray is based on hybridization of fluorescently labeled cDNA with probes on a specific microarray. RNA-seq produces a transcription map by aligning short reads with a reference genome.

**Why is it important to look at proteomics and interactomics in order to assign function to DNA sequences in the genome?**

Gene expression may not physically change DNA or mRNA, but still alter the proteins produced. This can subsequently affect their function. If we know which proteins are connected to which genes, when the function of said protein is altered, we know where to look.

**What techniques are used to study proteins? Explain very briefly how each one works.**

The techniques include ELISA, 2-DE, MS, and Y2H. ELISA uses an immunoassay and is therefore reliant on antibodies. 2-DE separates protein by two properties via gel electrophoresis. MS measures mass-to-charge ratio of ions. Y2H involves measuring protein interaction in yeast.

**How are model systems used to study functional genomics? Give a few examples.**

There are two main approaches: Knock out and knock in. Knock out suppresses the gene of interest. Knock up incorporates mutations seen in humans. These can be performed in various animal models. Examples of these model systems include mice, fruit flies, and zebrafish. This allows us to explore gene function without the ethical complications of human experimentation.