A sequenced library of human DNA fragments for molecular genetics teaching: linking bench experiments with informatics

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Aims:

The goal was to create a small well-characterised library of human DNAs cloned into bacterial plasmids to support the teaching of second-year Genetics and Medical Genetics students.

Methods:

The library was constructed as follows:

- Human genomic DNA was partially digested with the restriction enzyme *Sau*3AI
- The DNA was fractionated by agarose gel electrophoresis and fragments of 2–4 kb were recovered
- The fragments were ligated into the *Bam*HI site of the small high-copy-number plasmid vector pUC18Not and transformed into *E. coli*
- Colonies were picked, plasmid DNA was purified and the insert sequence was determined from each end by the standard Sanger di-deoxy method: a typical sequencing trace is shown in Figure 1

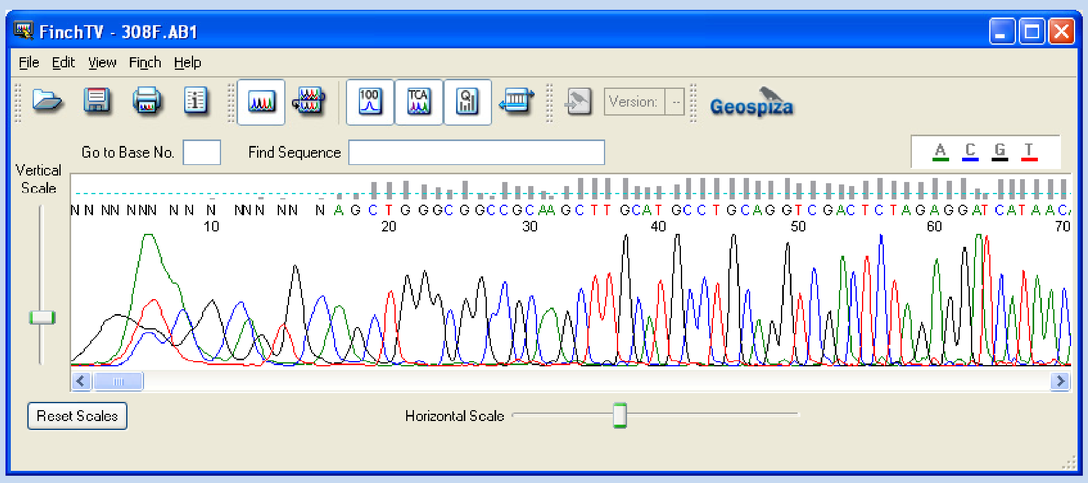

Figure 1:
Forward sequencing trace for clone 308 showing the boundary between the vector and the insert from base 61 to 66

Students activities:

Students are assigned individual clones from the mini library and are required to carry out a series of analysis tasks involving both wet-lab and informatics approaches:

- Preparation of plasmid DNA from an overnight bacterial culture
- Determination of the insert size by digestion of the DNA with restriction enzymes followed by agarose gel electrophoresis
- PCR amplification of the insert using the DNA sequencing primers followed by agarose gel electrophoresis
- Determination of the insert size and genomic location with the UCSC Human Genome Browser using the DNA sequence information from each end of the insert: see Figures 2 and 3
- Comparison of the insert length results using the three different approaches
- Southern blot analysis of the PCR amplification gel followed by hybridisation of the blot with a probe specific for the abundant human dispersed Alu repeat element
- Comparison of the blot results with determination of the number of Alu elements annotated in the human genome assembly for each cloned region

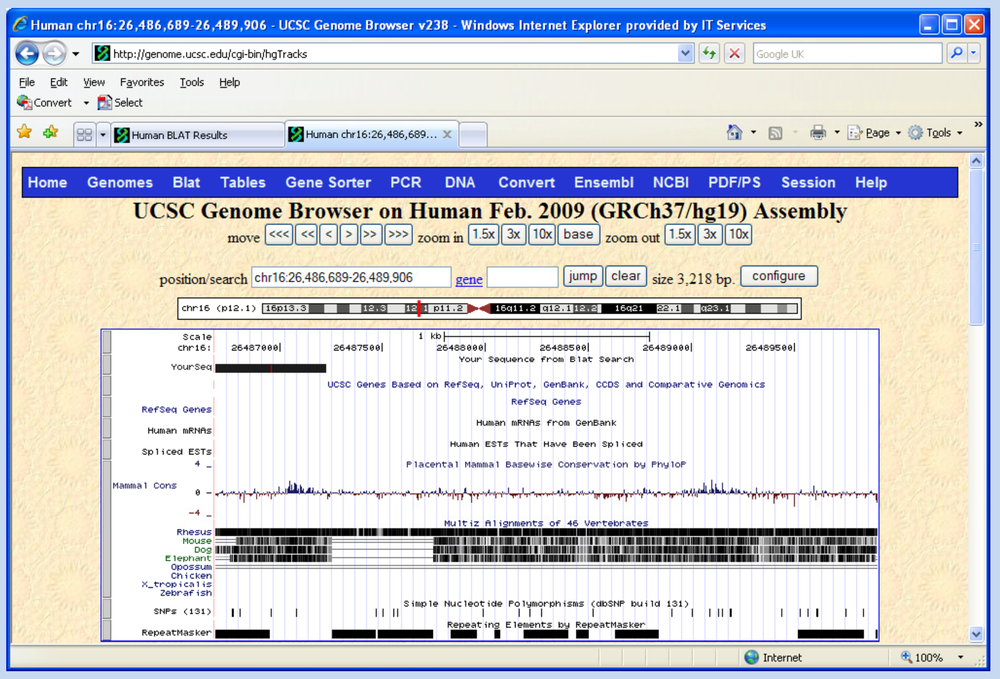

Figure 2:
Analysis of clone 308 using the UCSC Genome Browser. The insert size is 3218 bp and the sequence localises to chromosome 16p12.1

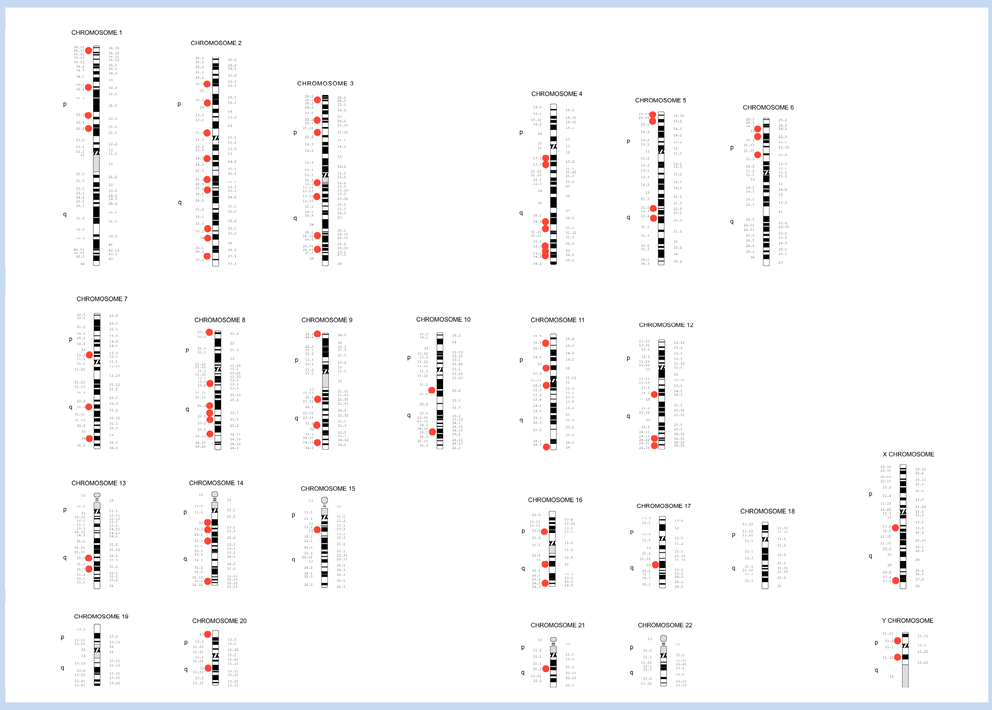

Figure 3:
The locations of the cloned human DNA are fragments are indicated by red dots on the chromosome ideograms

Summary:

The practicals based on this resource have been designed to integrate with lecture material covering both plasmid biology and human genome structure.

Anonymised feedback from students indicates that they enjoy being assigned their "*own*" individual plasmids, making the practicals "*more interesting and challenging*".

Requests are invited for access to the resource.

For more information, contact raymond.dalgleish@le.ac.uk or use the QR code
Find out more about GENIE at http://www.le.ac.uk/genie