

Akadia Kachaochana's Abstract

Our laboratory is working towards completing a map of the short arm of human chromosome 21 (HC21p), which is a heterochromatic genome region primarily composed of repetitive elements. These elements are unstable and difficult to clone and sequence, and so were not studied by the Human Genome Project. In order to eliminate gaps in our map, I screened a phage lambda HC21-specific genomic library with SSW9, a low copy number repeat (LCNR) known to be on HC21p. I was not able to identify any positive plaques with SSW9, possibly because not enough plaques were screened. I then screened the library with $\beta 2$, a high copy number beta satellite sequence. Since our map also has a gap near this sequence, $\beta 2$ is a good marker to use both for a positive control and to help fill gaps in our map. After screening with $\beta 2$, I identified two positive phage plaques from the library, and went on to purify each plaque to 100% purity. I purified the DNA from these two plaques using the QIAGEN Lambda Midi kit. I designed PCR primers to amplify the inserts from lambda Charon 21A's *Hind*III site, and then did PCR reactions to isolate the $\beta 2$ inserts. I obtained a total of three different fragments. I digested the fragments with *Hind*III, and am ligating them into the pUC18 plasmid vector. Transformation with blue-white colony screening will permit isolation of the recombinant plasmids in bacterial cells. I will then purify the plasmids and send them out for sequencing. I will then analyse the sequences (using such programs as NCBI's BLAST and DNASTAR's Lasergene) in order to fill the gaps present in our map.