A MODERATELY REPEATED DNA SEQUENCE FROM WUCHERERIA BANCROFTI

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Highly repeated DNA sequences have been characterized and developed as diagnostic probes for Brugia malayi and Brugia pahangi (2). In order to undertake the development of such a probe for Wuchereia bancrofti a genomic library was established in EMBL3. The number of recombinants were approximately 80,000. The library was subjected to differential screening by in situ plaque hybridization technique (1) with ^{32}p-labelled nick-translated total genomic DNA of W.bancrofti and human (placental) DNA. An initial screening of approximately 15,000 recombinants resulted in the isolation of 150 putative clones, of which 8 gave very strong signals on autoradiography. These were further purified by several cycles of plaque purification and one clone designated EMBL3Wb34 was selected for further characterization.

Restriction endonuclease cleaved genomic DNA of W.bancrofti when resolved by agarose gel electrophoresis and Southern blotted, and probed with ^{32}p-labelled nick-translated clone (EMBL3Wb34) indicated the moderately repeated nature of the cloned fragment. The cloned fragment did not hybridize to genomic DNA of S.digitata and human (placental) DNA on dot-blot assay indicating the specificity of the cloned fragment for W.bancrofti.

Evaluation of the sensitivity of the cloned fragment showed that ^{32}p-labelling technique can detect 25ng of genomic DNA, while the biotin labelling procedure can detect 150pg of genomic DNA of W.bancrofti. Thus the cloned fragment which is moderately repeated in the genome of W.bancrofti, has the potential for development as a species-specific DNA probe for bancroftian filariasis.

References:

1) Benton, W.D. & Davis, R.W. (1977). Screening λgt recombinant clones by hybridization to single plaque in situ. Science 196, 180-182

2) McReynolds, L.A. DeSimone, S.M. & Williams, S.A.(1986). Cloning and comparison of repeated DNA sequences from the human filarial parasite Brugia malayi and the animal parasite Brugia pahangi Proc. Natl. Acad., USA 83: 797-801