A-03: Cloning and partial sequencing of a heat shock 70 gene sequence from the filarial parasite *Setaria digitata*

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Infection of *Setaria* species causes cerebrospinal nematodiasis in sheep, goats and horses. Infected animals develop locomotive defects, resulting in significant economic losses in husbandry of these animals.

Heat shock proteins are dominant antigens and have been cited as mediators of parasite differentiation. In addition, they perform a spectrum of vital cellular functions under both stressed and normal conditions. Cloning and characterization of the *S.digitata* heat shock 70 gene will therefore contribute immensely to the understanding of the molecular genetics of this parasite.

The isolation and partial sequencing of a heat shock 70 gene sequence from *S.digitata* is reported.

The insert of a hsp 70 cDNA clone (MRC3) from the free living nematode *Caenorhabditis elegans* was amplified by PCR under standard conditions using primers derived from the sequences flanking the cloning site.

Dot blots of *S.digitata* DNA were probed with the MRC3 insert labelled with [α -^{32}P]dCTP by random priming.

A *S.digitata* genomic library was constructed in the λ vector EMBL-3, amplified and titred. The library was screened using labelled MRC3 insert.

Three putative clones giving strong signals were plaque purified. Of these, a clone designated LESH 1 with an insert size of ~13.4kb was selected for further analysis. A Southern blot of LESH 1 digested with a few selected restriction enzymes was probed with labelled MRC3 insert to identify hsp70 related sequences. Strongly hybridizing restriction fragments were then subcloned into either pBluescript or pUC plasmid vectors. A subclone containing a ~1.8kb *Eco* RI fragment was isolated and partially sequenced. Using the above partial nucleotide and deduced amino acid sequence, a search was made of the Swiss-Prot-28, EMBL-37 and GenBank-81 databases (Geneworks CD-ROM release, 12th March 1994 Intelligenics Inc. USA).

Dot blots of *S.digitata* DNA probed with MRC3 insert gave positive signals under high stringent conditions (1xSSC, 1% SDS at 60°C) indicating the presence of sequences homologous to *C.elegans* hsp 70.

Thereafter, a genomic library of *S.digitata* in λEMBL-3 was screened with labelled MRC3 and this resulted in the isolation of 3 clones, one of which (LESH 1) was selected for further analysis. Two *Eco*RI fragments of LESH 1 of ~1.8kb and ~1.0kb were subcloned into a pUC plasmid to derive pHS 1 and pHS 3. A ~4.4kb *Bam*HI fragment of LESH 1, containing among others, the above two *Eco*RI fragments, was also subcloned into pBluescript. The ~1.8 fragment cloned in pUC and designated pHS 1 was partially sequenced from both ends. A search of the Swiss-Prot-28, EMBL-37 and GenBank-81 databases with the nucleotide and deduced amino acid sequence revealed a high degree of homology with heat shock genes isolated from a number of organisms from a wide range of taxonomic groups. The highest homology at amino acid level was obtained with heat shock cognate 71 (Hsc71) sequences of Human, Rat, Mouse and Chinese hamster.

A *S.digitata* heat shock 70 gene sequence has been isolated and partially sequenced. The sequence isolated from *S.digitata* displays a high degree of homology to heat shock cognate 71 (Hsc) sequences which are a subset of heat shock proteins expressed constitutively. Sequencing of the other subclans are in progress to determine the entire sequence coding for the *S.digitata* heat shock 70 gene.

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