# Schistosoma Mansoni: Partial Molecular Characterization of the Gene Encoding Zinc Finger Protein of the Lung Stage (7-Days Schistosomula)

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## ABSTRACT

*Schistosomiasis is a serious parasitic disease with world-wide distribution, causing an estimated 200 000 deaths per year. Despite the fact that the global distribution of schistosomiasis has changed significantly in the past 50 years, particularly in regions where control strategies have been successfully employed, the disease remains endemic in over 70 developing countries and more than 200 million people are estimated to be harboring the disease. Chemotherapy, although effective, it does not prevent re-infection, and in addition, partial drug resistance may occur. Hence, immunological intervention in the form of a vaccine would contribute to the success of the present efforts. Most of the trials in the development of anti-schistosomiasis vaccine were involving membrane-associated antigens contained in the adult* ***Schistosoma mansoni (S. mansoni)*** *tegument because they are capable of stimulating protective immunity. In the current study, a trial to obtain antigen varieties which could be vaccine candidates by incorporating internal antigens of the lung stage of* ***S. mansoni*** *(7-days schistosomula) in addition to the tegumental antigens was done. The soluble extract of the lung stage was obtained by sonicating the whole parasite, then, coupled to Sepharose-4B column for affinity purification of pooled sera collected from chronically infected patients. The purified sera were used to immunoscreen* ***λgt 11*** *cDNA library of 7-days schistosomula. The plaques purification after three rounds of immunoscreening gave a number of cDNA clones. one of the isolated clones (clone 2-4) was amplified by PCR using* ***λgt 11*** *forward and reverse primers, then ,cloned in a plasmid vector* ***(PCR™II)****. The cloned insert was partially sequenced 270 bp from the 5′- end using Sp6 primer as well as 187 bp from the 3′-end using T7 primer. The sequenced part of the clone showed it has two open reading frames (ORFs) with 31-36% homology to the gene that encodes* ***Zinc Finger*** *protein (the transcriptional regulatory protein) from a number of eukaryotic species including human , rat and mice.*

## INTRODUCTION

Schistosomiasis is a Neglected Tropical Disease that ranks with malaria and tuberculosis as a major source of morbidity affecting approximately 210 million people in 76 countries of the world, despite of strenuous control efforts[1], the illness is a more serious problem than it was previously thought to be[2].

In the last two decades great progress has been made in characterizing human responses to

schistosomes[3] and testing different antigens of the parasite and different vaccination strategies[4].

In recent years, considerable effort has been made to develop a protective vaccine against schistosome infection and several potential DNA constructs encoding several candidate molecules have been identified[5,6,7,8,9] that could be useful for diagnosis, or may act as targets of protective immunity and/or chemotherapy[10].

Transcription in eukaryotic cells is regulated by proteins that bind to specific regulatory sequences and modulate the activity of RNA polymerase[11].

Many different transcription factors have now been identified in eukaryotic cells, as DNA binding domains in eukaryotic transcription factors which exhibit a variety of structures. The most common structural motifs are the homeodomain, basic zipper (leucine zipper), helix-loop-helix and several types of ***zinc fingers***[12].

***Zinc finger*** domains contain repeats of cysteine and histidine that bind central $Z^{+2}$ ions and fold producing a compact domain from a relatively short length of the polypeptide chain. This structural motif is recognized in DNA- binding domains and also in proteins that do not bind to DNA. These domains were initially identified in the polymerase II factor TFIIIA but, are also common among transcription factors that regulate polymerase II promoters[12].

The first ***zinc finger*** structure is $C_2H_2$ ***zinc finger***, containing three or more repeating finger units that bind as monomers; it is the most common DNA binding motifs in eukaryotic transcription factors[13].The second type is $C_4$ ***zinc finger***, found in more than 100 transcription factors, contains only two finger units and binds to DNA as homodimers or heterodimers. $C_6$ ***zinc finger*** is the third type, binds to DNA as homodimer in which the monomers associate through hydrophobic interactions along one face of their α-helical regions[14],

The molecular characterization of these regulatory proteins in ***Schistosoma*** may contribute to a better understanding of the biology of the parasite as well as the evaluation of these proteins as targets for immunotherapy or drug therapy[15].

The main goal of the current study was the identification and molecular characterization of some unique antigens from the lung stage of ***S. mansoni*** and in the future studying their immunogenic potential as vaccine candidates for the protection against the parasite.

## MATERIALS & METHODS

**Soluble extract of 7- days schistosomula**. Cercariae were kindly offered by the Biochemistry laboratory of Theodore Bilharz Research Institute. Schistosomula were obtained by mechanical transformation of cercariae, then, separated from tails by centrifugation for 15 min over 70% percoll gradient at 2000 rpm[16]. Schistosomula were recovered, washed three times, then, incubated in Modified MEM (Minimum Essential Medium containing 10% foetal calf protein) at

37°C in a humidified 5% $CO_2$ incubator for 7 days. Finally, the living schistosomula were separated by centrifugation for 15 min at 2000 rpm over 60% percoll gradient[17].The soluble extract was made by sonication of the parasites in a buffer containing 20 mM Tris, pH 7.2 and 2 mM phenyl methyl sulphonyl fluoride (PMSF), then , centrifuged at 6000 rpm for 20 min. The supernatant was collected and stored at -70°C.

**Affinity purification of sera.** Sera used in the library immunoscreening were pooled from ***S. mansoni*** chronically infected patients admitted to Department of Tropical Medicine, Zagazig University Hospitals. Cyanogen bromide – activated Sepharose 4B was used to purify sera according to manufacturer s instructions by coupling 6-8 mg of 7- days' schistosomula soluble extract to the column. Pooled sera were precipitated with 45% $(NH_4)_2SO_4$, the precipitate was redissolved in phosphate buffered saline (PBS) (0.4 g NaCl, 1.44g $Na_2HPO_4$ and 0.24 g $KH_2PO_4$/liter) and dialyzed against PBS overnight (O/N). The dialysate was, then, passed onto the column coupled to which schistosomula soluble extract. The column was washed with 30 ml PBS. Antibodies bound to the column were eluted by 0.1 M glycine–HCl, pH 2.6 and collected as 1 ml fractions. The pH of the elute was immediately adjusted to 7.0 with 100 µl 1M Tris-base., then, it was dialyzed against PBS over night to be ready for immunoscreening.

**Immunoscreening of schistosomula λgt 11 cDNA library[18].** To grow cells for transfection with the library , a single colony of ***E.Coli Y1090*** was incubated in 50 ml LB-ampicilline medium (LB-amp) (10 g Bacto-tryptone, 5 g Bacto-yeast extract, 10 g NaCl, and distilled $H_2O$ up to 1 liter, pH 7.0) containing 0.2% maltose and ampicilline 100 mg/ml allowed to grow overnight at 37°C, to be used as hosts for plating the library. For the primary screening of the library, 150 mm LB-amp plates were used and 90 mm plates were used for secondary and tertiary screenings. 0.6 ml/large plate and 0.2 ml/small one of the overnight bacterial culture was incubated with 0.1 ml of SM medium (5.8 g NaCl, 2.0 g $MgSO_4.7\ H_2O$, 50 ml 1M Tris ; pH 7.5 and 5 ml of 2% gelatin solution/ liter). The cell suspension was incubated at 37°C for 15 min to allow the adsorption of the phage to the bacterial cells. 7 ml/large plate and 3.5 ml/small plate of the molten top agar was cooled to 50°C and added to the infected cells, then, poured onto the LB-amp plates pre-warmed to 37°C. The plates were incubated at 40°C for 3-4 hours (hrs). Dry nitrocellulose (132 mm and 82 mm) circular filters were used for large and small plates, respectively. The filters were saturated in 10 mM IPTG and air dried, then, placed onto the plates. The plates were transferred to a 37°C incubator for another 3 hrs. then, the filters were removed from the plates and transferred to the Blotto buffer [5g non fat dry milk /100 ml TBST (37.5 ml 4M NaCl , 10 ml 1M Tris; pH 8.0, double distilled $H_2O$ up to 1liter and 0.05%Tween-20 )] to block the non-specific binding protein sites. The filters were ,then, washed 3 times in TBST for 10 min each,

followed by incubation for 3 hrs with the purified sera over schistosomula soluble extract column (primary antibody) , then, washed 4 times at room temperature (RT) in TBST for 20 min each. The anti-rabbit IgG alkaline phosphatase conjugate (secondary antibody) diluted in TBST was incubated with the primary antibody-antigen complex for 1 hr at RT. Then, the filters were washed 4 times in TBST for 10 min each, dried and transferred to the color development substrate solution [33 µl of 50 mg/ml Nitro Blue Tetrazolium (NBT) + 16.5 µl of 50 mg/ml BCIP per ml AP buffer (10 ml of 1 M Tris; pH 9.5, 2 ml of 5 M NaCl 0.5 ml of 1 M MgCl, distilled $H_2O$ up to 100 ml)]. The filters were incubated in dark until the desired color intensity had been developed, then, rinsed in distilled water. The developed filters were used to pick up agar plugs containing phage particles corresponding to the signals on the filters (the positive plaques) to be suspended into 0.5 ml of SM medium. The purified phage plaques were used for the next round of screening.

**Small scale preparation of bacteriophage DNA[19].** A bacteriophage suspension in ***E.Coli Y1090*** culture ( O/N ) culture , incubated at 37°C for 15 min, then, to which 4 ml of NZCY-ampicilline medium ( 10 g NZ amine, 5 g Bacto-yeast extract, 5 g NaCl, 2 g $MgSo_4.7$ $H_2O$ and distilled $H_2O$ up to 1 liter ,then , autoclaved at 121°C and ampicilline was added 100 mg/ml). The culture was agitated at 37°C for 9 hours (hrs), followed by adding 0.1 ml chloroform. The lysate was, then, centrifuged at 8000 rpm for 10 min. Ribonuclease A and DNase I (Sigma) were added to supernatant to final concentration 1 µg/ml of each. An equal volume of ice cold solution containing 20% PEG-8000 and 2 M NaCl in SM medium were added to lysate and chilled on ice for 1 hr, followed by centrifugation at 10000 rpm at 4°C to pellet the phage particles which were suspended in 0.5 ml SM medium. To the suspension 5 µl of each 10% SDS and 0.5 M EDTA, pH 8 were added and incubated at 68°C for 15 min. The solution was, then, extracted with phenol, phenol-chiasm and chiasm respectively, each extraction was done once, then, precipitated by adding 1/10 volume of 3M Sodium acetate and 2.5 volume of ice-cold absolute ethanol and stored at -20°C O/N, then, dissolved in 100 µl distilled $H_2O$ ,then, checked by using 0.7% agarose gel electrophoresis.

**Polymerase Chain Reaction (PCR)[20].** The isolated phage DNA from plaques was amplified using a pair of primers, **λgt 11** forward (5′-GGTGGCCACGACTCCTGGAGGCGG-3′) and **λgt 11** reverse (5′-TTGACA CCAGACCAACTGGTAATC-3′). A typical PCR reaction was done (10 µl 10 X Taq DNA polymerase buffer, 16 µl of 1.25 mM dNTP, 5 µl forward primer, 5 µl reverse primer, 2 µl (100 ng) phage DNA template, 0.5 µl Taq DNA polymerase (Perkin-Elmer Cetus and Stratagene), sterile distilled $H_2O$ up to 100 µl) .The reaction components were mixed in a microfuge and a drop of mineral oil was added. In the thermal cycler (Gene Amp 9600, Perkin-Elmer), a 3-file program was used to amplify samples, where they were denatured

in the first file at 94°C for 1 min, then, the primers were annealed to the denatured templates at 55°C for 2 min and finally extended at 72°C for 10 min. The amplicons were withdrawn from underneath the oil and 10 µl aliquots were separated on 1% agarose gel.

**Subcloning of the recombinant gene in PCR™II vector[19].** The original TA cloning Kit (Invitrogen) was used for direct insertion of the amplicon into **PCR™II** vector at ***EcoR1*** site. A ligation reaction was prepared (1 µl PCR product , 1 µl of 10X ligation buffer, 2 µl plasmid vector, sterile $H_2O$ up to 9 µl, 1 µl DNA ligase),then, incubated O/N at 15°C till ready for transformation. The vial containing the ligation reaction was placed on ice. Two µl of 0.5M β-mercaptoethanol and 2 µl of ligation reaction were added to each vial of the INV competent cells and mixed gently , then, the vial was incubated on ice for 30 min followed by 30 sec in 42°C water bath, then , on ice for 2 min, finally shaked at 37°C for 1hr with 450 µl of SOC medium . An aliquot of 50 µl was spread onto LB-amp plate and the plate was placed inverted at 37°C for at least 18 hrs. Positive transformants can be selected by using Cracking gel procedure[21], then, checked by 1% agarose gel electrophoresis.

**Small scale preparation of plasmid DNA[22].** A single bacterial colony that contains the desired plasmid was used to inoculate 100 ml of LB-amp medium incubated at 37°C with vigorous O/N shaking. The bacterial cells were centrifuged at 10000 rpm for 10 min. For cells lysis solution I was used (50 mM glucose, 25 mM Tris HCl, pH 8, 10 mM EDTA, pH 8) and freshly prepared lysozyme was added, then, followed by solution II [0.2 M NaOH, 1% sodium dodecyl sulphate (SDS)], the suspension was incubated at RT for 10 min followed by adding 20 ml of solution III (3 M potassium acetate, 2 M glacial acetic acid). DNA was recovered by adding an equal volume of isopropanol and precipitated by centrifugation at 10000 rpm for 10 min at RT. The pelleted DNA was dissolved in 100 µl distilled $H_2O$ to which RNase (10 mg/ml) was added, then, left at 37°C for 2 hrs. The DNA solution was, extracted with phenol-chiasm, then, precipitated by ethanol 2.5 volumes and 0.1 volume of 3 M sodium acetate. DNA pellet was dissolved in 50 µl distilled $H_2O$. **$O.D_{260}$** was used to quantitate the DNA, then, stored at –20°C.

**DNA sequencing[23].** 5 µl of the plasmid DNA were denatured by 20 µl of 0.2 M NaOH for 5 min , neutralized by 8 µl of 5 M ammonium acetate pH 7.4 , incubated at -70°C for 30 min after precipitation with 100 µl ice-cold absolute ethanol. The DNA was pelleted and dried , then, dissolved in 4 µl distilled $H_2O$ , 2 µl of 5X sequanase reaction buffer, 2 µl of the primer ( Sp6 and T7 for sequencing from $5^{/-}$ end and $3^{/}$-end, respectively) were added to the denatured template , this mixture was boiled , then , cooled gradually to RT. 1 µl of 0.1 M DTT , 1 µl **α-$^{35}$S-dATP** and 2 µl 1:4 diluted sequanase enzyme were added to the reaction mix and the tube was incubated at RT for 5 min . 3.5 µl aliquots were added to 4 different tubes each containing 2.5 µl of each of the ddNTPs

termination mixes. The reaction was stopped by adding 4 µl stop dye. The 4-tube set were labeled G,A,T,C was heated for 5 min , then , chilled on ice and loaded onto sequencing gel (8% Polyacrylamide, 8 M urea gel ), the run was continued for 2.5 and 6.5 hrs. After electrophoresis, the gel was fixed in a solution of 10% acetic acid and 10% methanol for 30 min, dried and exposed to an X-ray film. After 24 hrs exposure, the film was developed and read from the bottom. The informations obtained from DNA sequence were analyzed using the Genetics Computer Group Sequence analysis Software package.

## RESULTS

Sera obtained from *S. mansoni* chronically infected patients were purified over an antigen column made from soluble extract of 7-days schistosomula coupled to Sepharose-4B beads. The affinity purified eluted antibodies were, then, used to immunoscreen 7-days schistosomula λgt11 cDNA library. After the three rounds of immunoscreening. Phage DNA was prepared and checked using 0.7 % agarose gel (Fig. 2), then, cloned into PCR™II vector. Some of the isolated clones were checked for size after being inserted into the vector using two restriction enzymes *EcoR1* and *BamH1* (Fig. 3), which showed no *BamH1* site in the inserts , while, the enzymatic digestion of plasmid DNA by *EcoR1* gave the actual size of each insert (Fig. 3). The partial DNA sequence (270 bp from 5/-end using Sp6 and 187 bp from 3/-end using T7) (Fig. 4) of the insert showed that it has two open reading frames (ORFs) of 90 amino acids. It has 31-36 % homology with *zinc finger* protein from a number of eukaryotic species (including rat, mice and human). Fig. 1 shows the nucleotides and amino acids sequence.

**Sp6:** gct atg att caa gct tgg tac cga gct cgg atc gca cta gta acg gcc gcc agt gtg ctg gaa ttc ggc ttg gtg ggc agg act cct gga gcc cgt cag tat cgg cgg aat tcc tca cac caa cag tgc ggc gtc gta agc aaa caa ttc acc cat acc cac caa aaa gga agg cac ctt cgg tta aac aga ccc tgg agt ttt cca gta tgc cca ggt aca aat cac atc tca tca gcc gtg aat tcc cag agt ctt tca gtt caa tct

A **Met** I Q A W Y R A R I A L V T A A S V L E F G L V G R T P G A R Q Y R R N S S H Q Q C G V
V S K Q F T H T H Q K G R H L R L N R P W S F P V C P G T N H I S S A V N S Q S L S V Q S

**T7:** gcc gcc agt gtg atg gat atc tgc aga att ccg gct ttt cgc acc aga cca act ggt aat ggt agc gac cag ttt cag ctg gaa ttc cag cgg agg aaa aga aac gta aca agg att ccc cta gta act gcg agt gaa cag gga tta gcc caa ctc cga agc ctg cgt tat ttg atc gta agg caa t

**Fig. 1:** The partial nucleotides and deduced amino acids sequences of the gene encoding ***zinc finger*** protein isolated from **λgt 11** cDNA library of 7-days schistosomula.

**Fig. 2:** 0.7% agarose gel representing the preparation of phage DNA isolated from **λgt 11** cDNA library of the lung stage, the arrow points at the selected clone.

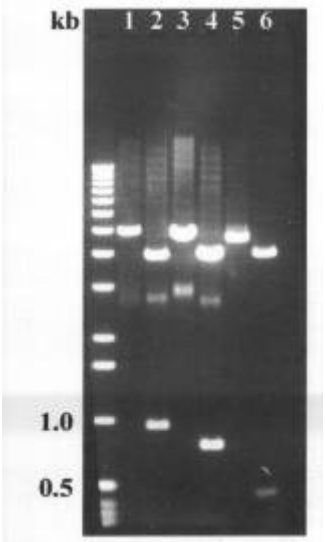


**Fig. 3:** 1% agarose gel showing the digestion pattern of three isolated clones from **λgt11** cDNA library of 7-day schistosomula cloned in **PCR™II** vector , using two restriction enzymes ***EcoR1*** and ***BamH1***, the plasmid DNA samples were arranged in double, each represents from left to right, ***BamH1*** digested and ***EcoR1***digested DNA. The selected clone (2-4) was run in lanes 5 and 6, its size is 0.4 kb and the 1kb ladder is indicated on the left side of the gel.

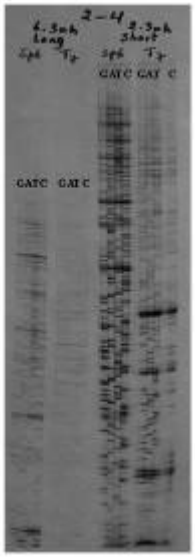

**Fig. 4:** An autoradiogram showing the partial sequence of the gene encoding ***zinc finger*** protein of ***S. mansoni*** isolated from **λgt 11** cDNA library of 7-days schistosomula.

## DISCUSSION

A useful approach to fight ***S. mansoni*** should include the infrastructure, the educational components, as well as the development of vaccines and new drugs[24], as recommended by ***WHO***[25].

Current advances in post-genomic techniques are providing new avenues to identify the secreted and surface exposed antigens that mediate protection[26,27,28].

The sequencing of the ***S. mansoni*** transcriptome and genome has opened up exciting new possibilities for antigen discovery[29].

As stated in the recent and high quality review on schistosome genomics by[30], some potential drug targets should include proteins involved in DNA replication, transcription and repair systems of the parasite.

During its development, the parasite ***S. mansoni*** is exposed to different environments and undergoes many morphological and physiological transformations as a result of profound changes in gene expression and characterization of proteins involved in the regulation of these processes.

***S. mansoni*** transcriptome project showed that 2.4% of the categorized Expressed Sequence Tags under the Molecular Function in Gene Ontology encode transcriptional regulators. A search for conserved domains using the Pfam database in a subset of those transcripts showed that 5% of them consist of ***zinc fingers*** of the $C_2H_2$ group[29]. These findings reinforce the importance of this class of regulatory proteins for ***S. mansoni*** biology.

In addition, using the SAGE approach, [31]found that 9.7% of the most abundant genes (genes containing more than 500 tags) from ***S. mansoni*** adult worms comprise those from the nucleic acid binding functional categories.

Proteins containing ***zinc finger*** motifs usually participate in regulatory processes and are considered the major class of transcription factors in eukaryotes[32] more over they are considered the most popular DNA binding proteins in mammals[33], involved in protein-DNA interactions and in the binding of RNA, lipids and proteins[34].

***SmZF1***, a ***S. mansoni zinc finger*** (***ZF***) protein, 19 kDa containing three $C_2H_2$ type zinc finger motifs, binds both DNA and RNA oligonucleotides, with a higher affinity to DNA molecules. A single zinc-finger domain by itself is not sufficient for high-affinity binding to a specific DNA target sequence[35].

This suggests that this protein might act as a transcription factor in the parasite and was detected in the nucleus of adult male worms **λ gt11** expression library and by PCR amplification in egg, cercariae and schistosomula cells cDNA libraries, but was not observed in female cells, suggesting it to be gender specific[32].

According to[36], investigating proteins differentially associated with each sex, they could reveal important clues concerning the formation of sexually mature schistosomes led to the description of novel chemotherapeutic targets acting in the maturation process, more over the protein is essential for the metabolism during different stages of the parasite life cycle[37].

In the present study, the gene encoding ***zinc finger*** protein of 7-days schistosomula was identified by its isolation from cDNA library of the lung stage of ***S. mansoni*** using sera obtained from the chronically infected patients, after purification over Sepharose-4B column made from sonicated soluble extract of 7-days schistosomula. The whole lung stage sonication was done to increase the chances for the isolation of a great number of ***S. mansoni*** vaccine candidates; in fact what was obtained in this study is not a surface associated protein.

Recently, there has been a great deal of progress in the development of modular protein domains that recognize specific DNA triplets. The $C_2H_2$ zinc finger motif is the ideal structural scaffold on which a sequence specific protein may be constructed[38]. DNA structural domain of zinc finger proteins usually consists of 3 or 6 zinc fingers. Artificial zinc finger proteins technology allows DNA sequences to be selected directionally and a DNA binding domain to be constructed[39].

Various screening procedure and artificial design strategies have also been attempted to make zinc finger proteins to bind to desired sequences[40,41]. Such artificial zinc finger proteins are expected to be artificial transcriptional factors and nucleases[42].

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# التوصيف الجزيئي الجزئي للجين المنتج لبروتين *Zinc finger* أحد البروتينات المهيمنة على عملية النسخ من طفيل الشستوسوما مانسونای

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يعد مرض البلهارسيا من الأمراض الطفيلية الخطيرة والمنتشرة في أنحاء العالم ويتسبب هذا المرض في ما يقارب 200000 حالة وفاة في العالم. على الرغم من إن انتشار المرض عالميا قد تغير الخمسين عاما الأخيرة تغيرا ملحوظا وخاصة في الأماكن التي طبقت فيها استراتيجيات السيطرة على المرض إلا أنة مازال مستوطنا في أكثر من ٧٠ دولة نامية وهناك ما يقارب ال ٢٠٠ مليون مصاب بالمرض . العلاج الكيماوي لم يوفر حلا مرضيا للقضاء على هذا المرض على الرغم من فاعليته إلا أنة لا يمنع تكرار العدوى ولذا كان ضروريا أن يساهم التداخل المناعي في صورة لقاح ضد المرض لإنجاح الجهود الحالية لو أضيف لاستراتيجيات السيطرة على هذا المرض . معظم محاولات تطوير اللقاح ضد البلهارسيا كانت تضمن المستضدات المرتبطة بالسطح الخارجي للطفيل لان لها القدرة على تحفيز المناعة الوقائية . ولكن في هذه الدراسة تم مسح كل البروتينات الطور الصدري للطفيل مناعيا سواء كانت على السطح الخارجي للطفيل أو داخلية و ولذلك لزيادة فرص الاكتشاف احد البروتينات الفريدة ليكون هدفا لجهاز المناعة للعائل ويستخدم كلقاح محتمل ضد هذا الطفيل . ولإجراء هذا البحث تم جمع عينات من مرضى مصابون من مرض البلهارسيا المعوية المزمن ونقيت على عمود من المقتطف الذائب من الطور الصدري للطفيل . الأجسام المناعية المزاحة من العامود استخدمت لعمل المسح المناعي لمكتبةλgt11 للطور الصدري . بعد ثلاثة دورات من المسح المناعي وتنقية اللوحات plaques تم عزل عدد من النسخ والتى تم تكبيرها باستخدام تفاعل البلمرة المتسلسل PCR باستخدام البادئات الأمامية والعكسية ثم استنسخت في عائل بلازميدي . احد هذه النسخ تم عمل تتابع جزئي لنيكلوتيداتة ووجد أنة يشفر للجين المنتج لبروتين zinc finger من الطور الصدري كما وجد أن طول هذه النسخة 0.4 kb وقد تم التعرف جزئيا على نيكلوتيداتة 270 من 5/ باستخدام البادئ Sp6 والبادئ T7 من 3/ ووجد أنة يشفر للجين المنتج لبروتين *zinc finger* من طفيل الشستوسوما مانسونای وهو احد أنواع البروتينات المهيمنة على عملية النسخ وله إطارين للقراءة المفتوحة . يماثل هذا الجين بنسبة 31- 36% الجينات المشفرة لنفس البروتين فى عدد من أنواع الكائنات الراقية مثل الإنسان والفار .