Molecular Characterization of the Gene Encoding *SJCHGC* 03921 Protein of the Lung Stage of Schistosoma Mansoni (7-Days Schistosomula)

Samir Mahgoub
Department of Biochemistry, Faculty of Medicine, Al-Minia University

ABSTRACT

*The parasitic helminth **Schistosoma mansoni (S. mansoni)** is a major public health concern in many developing countries. Over 200 million people have, and another 600 million are at risk of contracting schistosomiasis which is one of the major neglected tropical diseases. For this dangerous disease the development of long - lasting immunity through vaccination may be the real solution to control the spread of the disease. The molecules on the surface or associated with the tegument of S. **mansoni** are a major focus as potential vaccine candidates. In the present study, all surface and internal proteins of the lung stage of the parasite were screened to increase the chances for the discovery of a unique protein of the parasite to be targeted by the immune system of the host. Pooled sera were collected from **S. mansoni** chronically infected patients, then, purified over a column made of soluble extract of the lung stage (7-days schistosomula) of **S. mansoni**. The eluted antibodies were used to immunoscreen **λgt11** cDNA library of 7-days schistosomula. A number of cDNA clones were identified after three rounds of immunoscreening and plaques purification. The phage DNAs of the isolated clones were amplified by polymerase chain reaction (PCR) using **λgt11** forward and reverse primers, then, cloned in **PCR™II** plasmid vector. The isolated clone 4-65 was fully sequenced and was found encoding the gene of **SJCHGC 03921** protein of 7-days schistosomula of S. **mansoni**. Also, the 0.9 kb cDNA clone was found to have a single open reading frame (ORF) encoding 269 amino acids, which exhibited 94% homology with the gene of **SJCHGC 03921** protein of **Schistosoma japonicum.***

INTRODUCTION

Despite the mass chemotherapy programs, schistosome reinfection rates and prevalence continue to be unexpectedly high. Similarly rebound prevalence and morbidity will be high; an evitable consequence if ongoing interventions are not sustained[1]. Furthermore, there is increasing concern about the development of parasite resistance to chemotherapy. Consequently, vaccine strategies represent an essential component for the future control of schistosomiasis as an adjunct to chemotherapy[2].

Schistosomes are truly a formidable adversary that won't easily be beaten. It has been shown that hosts can develop an acquired immunity against challenge infection either after primary infection, immunization with irradiated larvae, or with defined antigens[3,4].

A vaccine would enhance attempts to control and eradicate the

disease that currently relies on treatment with a single drug [5].

An effective schistosome vaccine is a desirable control tool but progress towards that goal has been slow [6]. Attempts to develop a schistosome vaccine began half a century ago. By analogy with successful microbial and viral vaccines, they involved the vaccination of mice with crude worm extracts or purified components, followed by a cercarial challenge [7].

Perhaps there were a few key antigens that needed to be identified [6]. So, a particular attention was thus given to identify and characterize sensitive and specific ***S. mansoni*** antigens to obtain better diagnostic tool and vaccine development [8].

A few defined soluble antigens were separated to show high sensitivity and specificity in endemic areas [9]. Vaccines in combination with other control strategies, including the use of new drugs, are needed to make elimination of schistosomiasis possible [10].

Despite the discovery and publication of numerous potentially promising vaccine antigens from ***S. mansoni*** and, to a lesser extent, ***Schistosoma hematobium,*** only one vaccine, namely, ***BILHVAX***, or the 28-kDa GST from ***Schistosoma hematobium***, has entered clinical trials [11].

By reviewing the most recent and pertinent data on the major vaccine antigens for schistosomiasis; the available antigens and prototype vaccine formulations induce 40 to 50% protection in animals, at best as shown by reduced worm burden or egg production and viability [2].

The aim of the present research was the isolation of a cDNA clones from 7-days schistosomula **λgt11**cDNA library which could be a targets for immune attack and hopefully vaccine candidates for ***S. mansoni*** elimination.

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[12]. Schistosomula were recovered, washed three times, and then, incubated in Modified MEM (Minimum Essential Medium containing 10% foetal calf protein) at 37^0C 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 [13]. 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^{o}C.

Affinity purification of sera. Sera used in immunoscreening experiment 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. The dialysate was, then, passed onto the column coupled to which schistosomular 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 λgt11 cDNA library [14]**.** To grow cells for transfection with the library, a single colony of ***E.Coli Y1090*** was incubated in 50 ml LB-ampicillin medium (LB-amp) (10 g Bacto-tryptone,5 g Bacto-yeast extract, 10 g NaCl and distilled H_2O up to 1L , pH 7.0) containing 0.2% maltose and ampicillin 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/L). 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 1L 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 colour development substrate solution [33 µl of 50 mg/ml Nitro Blue Tetrazolium (NBT) + 16.5 µl of 50 mg/ml BCIP/ml AP buffer (10 ml of 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 colour intensity had been developed, then, rinsed in

distilled H_2O. 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.

Polymerase Chain Reaction (PCR)[15]. The isolated phage DNA was amplified using a pair of primers, **λgt11** forward (5′GGT GGCCACGACTCCTGGAGGCGG-3′) and **λgt11** 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 plasmid vector[16]. 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 overnight 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 [17], using 1% agarose gel electrophoresis.

Small scale preparation of plasmid DNA [18]. 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 overnight shaking (O/N). 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 room temperature (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 for incubation 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 using *fmol* DNA System (Promega)[19]. In each one of four microfuges labeled (G, A, T, C), 2 µl of d/ddNTPs, 1µg DNA template, 25 µg primer (M13 at 5/-end and T7 a 3/-end) 1µl of **α-^{35}S**, 5µl sequencing buffer and deionized distilled (dd) H_2O up to 16 µl were mixed, then, to each tube 1µl of sequencing grade Taq DNA polymerase was added to the template/primer mix. The tubes were placed in a thermal cycler to follow this profile, 2 min at 95°C, 30 sec at 90°C, then, 1 min at 70°C for 30 cycle. The reaction was stopped by adding 3µl stop solution to each tube. 3µl of each tube were loaded onto the sequencing gel (8% Polyacrylamide, 8 M urea gel), the run was continued for 2.5 and 6.5 hrs.. After the electrophoresis, the gel was fixed in a solution of 10% acetic acid and 10% methanol for 30 min, dried by heating, then, exposed to an X-ray film, which was developed and the information obtained from DNA sequence was analyzed using the Genetics Computer Group Sequence analysis Software package.

RESULTS

Sera obtained from ***S. mansoni*** chronically infected patients were purified over an antigen coupled column made from soluble extract of 7-days schistosomula. The elute containing affinity purified antibodies was used to immunoscreen 7-days schistosomula **λgt11** cDNA library. One of the identified cDNA clones by affinity purified antibodies (clone 4-65) contained a 0.9 kb insert. The full DNA sequence of the clone showed a single open reading frame (ORF) of 269 amino acids with high identity (94%) to the gene of ***SJCHGC 03921*** protein of ***Schistosoma japonicum*.**

The 0.9 clone which was completely sequenced in both directions after being inserted into **PCR^{TM}II** vector, did not contain the entire coding region. The 5/- upstream region in the sequence obtained showing that the first initiation codon (ATG) is located -201 bp from the beginning of this region with neither transcription activation TATA nor CAAT boxes. There are three putative polyadenylation signals AAATAA, AATTA and ATAA located +114, +22 and +6 bp, respectively, from the 3/-downstream region, there is no polyadenylation site (poly A tail) (**Fig. 1**).Some of the isolated clones were checked for their sizes after being inserted in the plasmid vector using two restriction enzymes ***EcoR1 and BamH1*** (**Fig. 2**), all clones showed no ***BamH1*** site, while, being digested by ***EcoR1*** gave the actual size of each insert. The selected clone was sequenced using two primers (M13 from the 5/- end and T7 from the 3/-end) followed by another two pairs of primers to complete the sequence of the isolated clone, each sequence gel was exposed to an X- ray film for 24 hrs, then, developed and read from the bottom of the autoradiogram (**Fig. 3**).

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ccg aag tct aag cac gca tgc aag cgc cat ctc cga cac acc gca cag atc cac tac agc cag tta gta
aca ctc acg gcc cat aaa ctg agc caa aga gcc acc tac cga ccc aac caa gac cga cag aaa tca gac

ctg atc agt ttg acc ata gca gac agg cag cag tcg tcg tgg tgc aca cct gag aaa gtg cac atg aca
                                                                          Met T

agc aga ccc tca cac caa cag tgc gcg cgt cgt aag caa acg att cac aca tac cac cca aaa agg aag
 S  R  P  S  H  Q  Q  C  A  R  R  K  Q  T  I  H  T  Y  P  P  K  R  K
 gca cct gcg gtt aaa cag aca ctg aag gcg gcc agt atg ccc aag tac aaa tca cac tca tca gct gaa
  A  P  A  V  K  Q  T  L  K  A  A  S  M  P  K  Y  K  S  H  S  S  A  E
ctc cca gag ctt gca gtt caa ctc cac ccg ttt acc ttt gag cgg ttt cac gca ctg ttt act ctc tct
 L  P  E  L  A  V  Q  L  H  P  F  T  F  E  R  F  H  A  L  F  T  L  S
tca aag tac ttt tca act ttc cct cac ggt act tgt ttg cta tcg gac tcg tgt aag tat tta gcc ttg
 S  K  Y  F  S  T  F  P  H  G  T  C  L  L  S  D  S  C  K  Y  L  A  L
gat gga gtt tac cac cca ctt tgg gct gca ttc aca aac aac ccg act cca agg gta gct cag agc aaa
 D  G  V  Y  H  P  L  W  A  A  F  T  N  N  P  T  P  R  V  A  Q  S  K
act gtc aca ctt gat ctc tgc ccc cac ggg cct ttc acc ctc ttt ggg cca gga tgg gaa gcc gta ctc
 T  V  T  L  D  L  C  P  H  G  P  F  T  L  F  G  P  G  W  E  A  V  L
att gct gga ctt ggg aca gag cag gta atg cct gaa gcc acc cta aac acc aca ttg ctt tac gat caa
 I  A  G  L  G  T  E  Q  V  Met  P  E  A  T  L  N  T  T  L  L  Y  D  Q
ata acg gca ggc ttc ggt gtt ggg cta atc cct gtt cac tcg cag tta cta ggg gaa tcc ttg tta gtt
 I  T  A  G  F  G  V  G  L  I  P  V  H  S  Q  L  L  G  E  S  L  L  V
tct ttt cct ccc ctg agt ata tgc tta agt tca gcg ggt aat cac gcc tga tcg agg tcg ggg tca att
 S  F  P  P  L  S  I  C  L  S  S  A  G  N  H  A Stop
aaa taa ttc gtg atc ata cac aca caa tcg gta caa acc ata gac caa acc aga gac aag atc aag tga

tta acg tag cat acg ata ggt gcg aat tat ccc gag gat gta taa tgt cag
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Fig. 1: The Complete nucleotide and deduced amino acids sequences of the gene encoding ***SJCHGC 03921*** protein of ***S. mansoni*** isolated from **λgt11**cDNA library of 7-days schistosomula, start codon (**ATG**), stop codon (**TGA**), three polyadenylation signals (**AAATAA**), (**AATTA**) and (**ATAA**) are underlined.

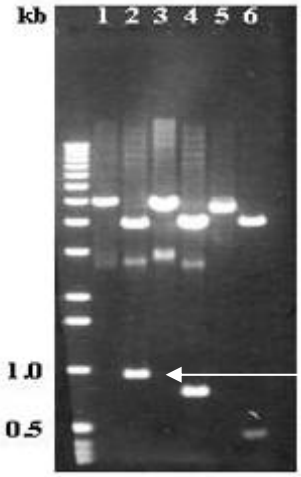

Fig. 2: 1% agarose gel showing the digestion pattern of three isolated clones from **λgt11** cDNA library of 7-day schistosomula, cloned in **PCR™II** vector , digested by two restriction enzymes ***Eco*R1** and ***BamH1***, the plasmid DNA samples were arranged in double, each represents from left to right, ***Eco*R1** digested and ***BamH1*** digested DNA. 1kb ladder was indicated on the left side of the gel. The selected clone (4-65) was run in lanes 1 and 2, its size is 0.9 kb.

Fig 3: An autoradiogram showing sequence of the gene encoding **SJCHGC 03921** protein of ***S. mansoni*** isolated from **λgt11** cDNA library of 7-days schistosomula cloned in **PCR^{TM}II** vector.

DISCUSSION

A world wide problem is that over 200 million people have and another 600 million are at risk of contracting schistosomiasis[20].

The recent studies on schistosomiasis have focused on identification and characterization of defined antigens that may have vaccine and/ or diagnostic potential. The development of vaccine against schistosomiasis would provide a powerful tool for the control of this important parasitic disease and it must be effective which should be confirmed by protection test [21].

Several vaccine strategies have been tried such as the use of synthetic peptides [22].

The tegument associated antigens expressed on newly transformed and developing schistosomules and involved in important host-parasite interactions are important candidates for vaccine development [23].

The current research was focusing on identification, characterization and study of the vaccine potential of tegumental and internal antigens of 7-days schistosomula. The technique used was not the extraction of surface proteins only, but all the parasite was sonicated for obtaining all antigens either they are tegumental or internal. After three rounds of immunoscreening of **λgt11** cDNA library of 7-days schistosomula by affinity purified antibodies obtained from the sera of patients, a number of cDNA clones were isolated, one of them (clone 4-65) was amplified by PCR using **λgt11** forward and reverse primers , then , cloned in **PCR^{TM}II** vector. The size of the selected clone was shown to be 0.9 kb by checking the pattern of restriction endonuclease digestion using ***EcoR1 and BamH1*** enzymes, the result of enzymatic digestion was run on 1% agarose gel, the digestion by ***EcoR1*** proved the size of the insert. The DNA sequence did not contain the entire coding region of the gene. The upstream region the transcription activation

boxes (TATA and CAAT) were not found, similarly, there is no polyadenylation site in the downstream region. The DNA sequence of the identified clone **showed** that it has (94%) homology with the gene that encodes ***SJCHGC 03921*** protein of ***Schistosoma hematobium.***

[24] reported some of the most promising ***S. mansoni*** vaccine antigens as well as those that were independently tested under the umbrella of the TDR/WHO committee in the mid-1990s**,** the group of isolated antigens include**s** tetraspanins, although their functions are unknown, but a family of them is expressed in the schistosome tegument and at least three of these show promise as vaccines.

Sm23 is a tetraspanin expressed in the tegument of S**.** ***mansoni*** and is one of the independently tested WHO/TDR vaccine candidates, it is the most efficacious when delivered as a DNA vaccine [25] and does not confer protection as a recombinant protein when formulated with alum.

Sm28-GST has GST properties and is expressed in subtegumental tissues of most developmental stages of the parasite. Vaccination of semipermissive rats and permissive hamsters with recombinant Sm28-GST resulted in significant reductions of worms [26].

Also, the group of the selected antigens includes Smp80 calpain [27] which is a calcium-activated neutral cysteine protease, superoxide dismutase (**SOD**) which inhibits granulocyte toxicity for egg metabolic activity and hatching [28] and paramyosin which is expressed on the surface tegument of lung-stage schistosomula in the penetration glands of cercariae [29].

FABP (Sm 14), the ***S. mansoni*** fatty acid binding protein, despite a high efficacy of recombinant Sm14 protein in mouse vaccine trials [30], Sm14 failed to induce protection levels of >40% when tested in different laboratories [31].

The current ***Schistosoma*** vaccine candidates may prove not to be the most effective. It is important to identify new target antigens and to explore alternative vaccination strategies to improve vaccine efficacy [32].

There is an abundance of reports on schistosome antigens obtained from different anatomic locations and stages of the developing parasite. The tegument antigens of a live worm are those that researchers focus efforts are truly exposed to the host immune system, so, the tegument plasma membrane proteins should be a major focus for future vaccinology efforts[33], because a focus on identification of new intracellular antigens show moderate protection at best[34].

The deficits in lipid metabolism that makes schistosomes dependent on the host are revealed and the identification of membrane receptors, ion channels and more than 300 proteases provide new insights into the biology of the life cycle and novel targets [35].

There is shortage of informations about the isolated gene in this study which advocate continuing the efforts to perform further researches for picking up the full length gene, identifying its localization, its function and its vaccine potential.

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التوصيف الجزيئي للجين المنتج لبروتين *SJCHGC 03921* من الطور الصدري لطفيل الشستوسوما مانسونای

سمير محجوب
قسم الكيمياء الحيوية- كلية الطب- جامعة المنيا

طفيل الشستوسوما مانسونای يعد من الاهتمامات الرئيسية في العديد من الدول النامية وهناك أكثر من ٢٠٠ مليون مصاب بمرض البلهارسيا المعوية والتي يسببها هذا الطفيل و٦٠٠ مليون آخرون عرضة للإصابة بة. ولذا فان الحل الأمثل لإيقاف انتشار هذا المرض الخطير يكون بالحصول على مناعة طويلة المدى باستخدام أمصال للتطعيم ضده. معظم الأبحاث التي أجريت فى هذا المجال كانت تركز على بروتينات السطح الخارجي للطفيل وذلك للقيام بعذلها ليكونوا مرشحوا اللقاح المحتملون. ولكن في هذه الدراسة تم مسح كل بروتينات الطور الصدري للطفيل مناعيا سواء كانت البروتينات على السطح الخارجي للطفيل أو داخلية و ذلك لزيادة فرص اكتشاف احد البروتينات الفريدة ليكون هدفا لجهاز المناعة للعائل و يستخدم كلقاح محتمل ضد هذا الطفيل. ولإجراء هذا البحث تم جمع عينات من مرضى مصابون بمرض البلهارسيا المعوية المزمن ونقيت على عامود من المقتطف الذائب للطور الصدري للطفيل. الأجسام المضادة المنقاة و المزاحة من عامود المقتطف الذائب للطور الصدري للطفيل استخدمت لعمل المسح المناعي لمكتبةλgt11 للطور الصدري. بعد ثلاثة دورات من المسح المناعي و تنقية plaques تم عزل بعض النسخ. باستخدام تقنية تفاعل البلمرة المتسلسل PCR تم تكبير هذه النسخ لتتم كلونتها في عائل بلازميدي $PCR^{TM}II$. بمعرفة التتابعات النيكلوتيدية لأحد هذه النسخ المعزولة وجد أن طوله 0.9 kb وله إطار قراءة مفتوح واحد. وجد أن هذا الجين يتطابق بنسبة (٩٤%) مع الجين المشفر لبروتين *SJCHGC 03921* لطفيل الشستوسوما هيماتوبيم مما يدل على أن هذا الجين الذي تم عزلة هو الجين المشفر لبروتين *SJCHGC 03921* لطفيل الشستوسوما مانسونای. سيتم إكمال الدراسة في المستقبل للتعرف أكثر على هذا الجين للحصول علية كاملا ثم استنساخه في عائل expression لإنتاج بروتينه لدراسة ما إذا كان من الممكن استخدامه كأحد اللقاحات المرشحة للاستخدام ضد طفيل الشستوسوما مانسونای.