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TRYPANOSOME SURFACE ANTIGEN GENES: ANALYSIS USING
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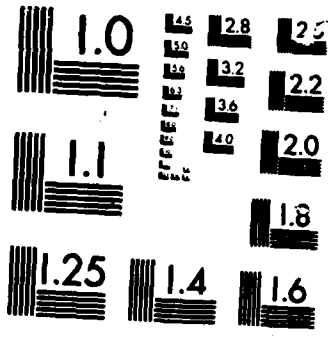
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TRYPANOSOME SURFACE ANTIGEN GENES:
ANALYSIS USING RECOMBINANT DNA

ANNUAL REPORT
12/1/81 - 12/1/82
KENNETH D. STUART, Ph.D.

June 15, 1984

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) The objective of this project is to identify and isolate early (frequently expressed) variant antigenic types (VATs), and clone and characterize their expressed variant surface glycoprotein (VSG) genes. Numerous syringe passaged and cyclically transmitted, frequently expressed VATs have been isolated, monoclonal antibodies prepared to their VSGs, and the expressed VSG genes have been cloned. We have shown that many diverse stocks express VSG epitopes related to the early IsTat epitopes. The VSG gene organization in the genome and sequence organization has been characterized. We have confirmed sequence homology at the 3' terminus of the VSG genes and have discovered additional homology near the 5' terminus of unrelated VSG genes. Numerous relapse VATs have also been isolated and characterized with respect to telomeric location of VSG genes and duplicative or non duplicative mode of VSG gene expression. We have found that two fundamental processes control the expression of VSG genes. One involves gene duplication; the			
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19. Abstract (Con't)

✓ other telomeric activation. In addition, the telomeric location of the gene affects its probability of expression and stability. DNA sequence analysis in and around flanking VSG genes has characterized some of the internal homology and identified flanking sequence homology blocks. The latter probably function in duplicative activation of VSG genes. *Keywords:*

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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SUMMARY

PURPOSE

The purpose was to isolate and characterize genes encoding the variant surface antigens (VSGs) expressed by early or frequently occurring variant antigenic types (VATs) of African trypanosomes

METHODS

Several VATs were isolated from a chronically infected mouse by single cell cloning. The VSG was isolated from each VAT using lectin affinity chromatography and used to immunize BALB/c mice. The spleens from these mice were used to produce VSG specific monoclonal antibodies. These antibodies were used to monitor the purity of VAT population and assess the expression of related VSGs in other stocks by indirect immunofluorescence. VSG genes were cloned from poly A(+) RNA as cDNAs in pBR322 plasmid and from cellular DNA as genomic clones in lambda 1059 after partial digestion by Bam HI and Mbo I. The cDNA clones were isolated by differential screening using cDNA probes from different VATs. These cDNAs were used to screen the genomic libraries. The cloned probes were examined by nucleotide sequence analysis and used to examine genomic DNA by Southern blot analysis.

RESULTS

Numerous cell clones expressing six related VATs were isolated and examined. Most other stocks examined expressed VSGs related to those expressed by our early VATs. The VATs occurred in a similar sequence in several independent infection from the same line. The size of each VSG gene family varied and the expressed VSG gene was always telomeric. Nucleotide sequences were conserved in the 3' end of the VSG coding sequence and flank and near the 5' end of the gene.

CONCLUSIONS

Two distinct mechanisms mediate antigenic variation. One employs gene conversion the other control of transcription. The early VATs of the IStAr 1 serodeme express VSGs related to those conserved by other stocks. One VAT is expressed much more frequently than others after a relapse. The genomic location of a gene affects its frequency of expression. Some sequences in and around VSG genes are conserved among these genes.

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(1) OBJECTIVES.

The antigenic variation program has substantial scope as necessitated by the complexity of the problem. In anticipation of the complexity of the molecular genetic events associated with antigenic variation, we embarked on an integrated biological/immunological/molecular genetic analysis of the problem. The wisdom of this decision has been confirmed by the complexity of the data collected to date and the inability to establish a molecular model for antigenic variation that is consistent with all these data. This view has recently been adopted by other investigators in the field (18).

(1.1) Total Program Objectives

The long term objective of the total program is to determine the molecular basis for antigenic variation. As indicated in the background section, the molecular mechanisms must account for several characteristics of antigenic variation in addition to the control of VSG gene expression. The more basic aspects of the program are being conducted with NIH support. These include the isolation and characterization of VSG genes and especially their flanking sequences. These studies are directed at determining if transposition is a prerequisite for VSG gene expression, if the structure of VSG genes and their flanking sequences is consistent with conventional transposition mechanisms, if transcription of VSG genes is under positive or negative control, if VSG gene expression requires gene duplication, and characterization of the genomic alterations of VSG genes that are not associated with antigenic variation or changes in VSG gene expression.

(1.2) Total Project Objectives

The objectives of the project supported by the USAMRDC while not divorced from these important considerations focus on questions that are less basic in nature and more supportive of USAMRDC programs. The overall objective of the project supported by the USAMRDC is to determine if there exist restricted numbers of VSG genes or gene segments that encode for VSGs or VSG segments that are expressed early in the natural infection. The presence and early expression of these genes or gene segments in natural populations and specifics of African trypanosomes especially human pathogens will be tested.

The objectives for the period for which support is requested are:

1. To identify, isolate and characterize VATs which initiate or occur early in infections. These include metacyclic, predominant and early VATs.

2. To characterize the VSGs and the corresponding VSG gene sequences of these VATs and determine if they contain regions of homology that could result in immunological cross reaction among the VSGs. These data will be compared to those derived by analysis of mid and late VATs.

3. To determine if the VATs, VSGs and regions of VSG homology are shared by various stocks of African trypanosomes especially the human pathogens.

These objectives are designed to critically assess:

1. If syringe passaged and cyclically transmitted members of the same clone express VSGs in a consistent sequence at least in the early stages of the infections.

2. If the sequence relationships among VSG genes reflect the time at which they are expressed during the infection.

3. If VSG genes contain regions of homology that are shared by VATs from different serodemes and species.

4. If VSG coding sequences remain unaltered during antigenic variation and during cyclic transmission.

(2). PROGRESS REPORT.

Much of the progress on this project since initiation of support in December 1981 was presented during a visit in June 1982 and described in the preprints submitted with this application. Hence, progress will be briefly summarized in this section except where data not contained in the preprints is concerned.

(2.1) Biological

The cloning and characterization of the VATs of the IStat 1 serodeme is near completion. Seven early, two middle and two late VATs from the syringe passaged line have been isolated. These are the 1.A, 1.D, 1.1a, 1.3a, 1.5a, 1.7a, and 1.11a early VATs; 1.27a and 1.28a mid VATs and the 1.42a and 1.44a late VATs. Clones have been established from 15 sequential isolations from the cyclically transmitted line and designated cl.1a to cl.15a. The terminal a will not be used henceforth for simplicity.

The antigenic relationships among these VATs is being determined. The 1.1 and 1.D VATs are very similar as reported in preprints 1 and 2. The similarity among their VSGs is best illustrated by the observation that 20 monoclonal antibodies produced after immunizations with 1.1 VSG reacted with both the 1.1 and 1.D VSGs by an ELISA assay (preprint 2, figure 2). Despite the probable identity between the 1.1 and 1.D VSGs, these VATs differed at the genomic level. The 1.A and 1.27 VATs were also found to be similar. 5 1.A VSG specific monoclonal antibodies (Mabs) also reacted with 1.27 cells by the immunofluorescence assay (IFA) using fixed cells. Antiserum specific for 1.A by IFA using living cells also reacted with the 1.27 cells.

We have recently begun to examine the cyclically transmitted clones for recurrence of the early VATs. We have recovered a

1.A-like VAT. We refer to the VATs as early, mid and late VATs because they were isolated early, mid and late in the infection. Although we are interested in the sequence in which these VATs are expressed, we cannot yet be sure that the VATs were clonally expressed in the sequence isolated. We have reinfected deer mice with some of the early IStat 1 VATs and have been following the infection by IFA. From the limited data we have collected the occurrence of the VATs has been in the sequence in which they were originally collected. However, VATs for which we lack antisera intervened. One such experiment is illustrated in figure 1. We will assay the frozen isolates of the blood collected from the deer mice during the original infection with the monoclonal antibodies as soon as time permits. All early VATs have been grown as procyclic forms and DNA has been extracted for Southern blots to determine the genomic consequence of cessation of VSG production. Production of the IStat 1 serodeme and characterization of the early VATs is detailed in preprints 1 and 2.

(2.2) Immunological

Monoclonal antibodies have been produced after immunization of BALB/c mice with purified VSGs. 83 Mabs have been characterized and we have isolated 57 Mabs which are specific for early IStat 1 VSGs. All hybridomas were selected on the basis of production of antibody that reacted with the immunizing antigen using ELISA assay. Ascites fluids prepared from the hybridomas were titred against each of the VSG preparations. The ascites fluids were also tested against acetone fixed cells by IFA, against bloodstream form and procyclic cell lysates to demonstrate stage specific reaction with the bloodstream antigen and against purified Con A which contaminates the antigen preparations as a result of affinity purification.

As shown in Table 1 in the appendix, 57 of the Mabs were specific for VSGs by the criteria of IFA assay and ELISA assays using both purified VSGs and total cell lysates. 5 Mabs cross reacted and 20 have unknown specificity. The results of these assays are presented in Tables 2 - 7 in the appendix. One of the 1.A specific Mabs (1H3-8-2) was derived from an immunization with 1.3 VSG. The ELISA assay reveals 1.A antigen in the 1.3 preparation probably since the 1.3 VAT is less stable than others and all VATs tend to switch to 1.A. One of the 1.11 specific Mabs (11+9-11-1) reacted with 1.1 and 1.D VSGs by ELISA and IFA but at 2 logs lower titer. This implies similarity but not identity between epitopes of these two VSGs.

Five Mabs, designated by underlines in Tables 2-7, cross reacted with the VSGs and whole cell lysates from the VATs. The cross reaction by IFA was ambiguous but this may reflect the fact that all these Mabs were of the IgM subclass. All the cross reacting Mabs also reacted with Con A implying recognition of glycosylated epitopes. Two of the cross reacting Mabs reacted with procyclic cell lysates by the ELISA assay. The titers of the cross reacting Mabs were generally low which may reflect the

relative instability of IgMs.

The specificity of 20 Mabs, which are designated with an * in Tables 2-7, have not been established. Many of these had low titers and IgMs. They shared the general the general properties of the IgM Mabs described above and may belong to the same general class but have a lower titer. However, 9 of the Mabs with unknown specificity were IgGs and had modest to high titers against the VSG preparations in the ELISA assay. These Mabs may be specific for other VSGs that were present at low concentration in the VSG preparations much as the 1.A specific Mab (1H3-8-2) was isolated after immunization with a 1.3 VSG preparation. These Mabs may be very useful since they are likely to be directed against other trypanosome components that co-purified with the VSG or may be directed against non trypanosome materials that contaminated the immunizing antigen preparations such as non Con A material from the affinity column.

As indicated in previous progress reports, conventional VAT specific antisera have been prepared against the early IsTat Vats (see preprint 1). Similar antisera have also been prepared against the mid and late IsTat VATs and are being prepared against the cyclically transmitted IsTat VATs.

(2.3) Molecular Genetic

cDNA clones have now been prepared from all of the early IsTat 1 VATs. These cDNA clones have been shown to contain VSG gene sequences by hybridization to RNA extracted from the homologous VAT but not with RNA extracted from heterologous VATs. These experiments also demonstrate that VSG gene expression is regulated at the level of transcription. The fact that the cDNA clones encode VSGs was also demonstrated by using the cDNA clones to select mRNA from the homologous VATs and translating that mRNA in vitro. The polypeptide translated in vitro from this hybridization selected mRNA had the same molecular weight as in vitro translated VSG and was immunoprecipitated by homologous VAT specific antisera but not immunoprecipitated by heterologous VAT specific antisera (preprint 2, figure 3; preprint 3, figure 1). These data presented in preprints 2 and .

Genomic libraries have been prepared from all the early IsTat 1 VATs and from the WRATat 1 VAT. The IsTat 1 libraries are currently being screened for cloned sequences containing the VSG genes using the cDNA clones. The presence of the appropriate VSG gene sequences in these libraries has been demonstrated by extracting total library DNA and probing for VSG gene sequences by the Southern blot technique. VSG genes with the same size as in the genomic DNA has been confirmed. The screening will require primary secondary and tertiary screening to ensure the recovery of a single phase progeny. All seven libraries have been screened with the 1.1, 1.D and 1.11 cDNA clones to the secondary level and has resulted in the production identification of hundreds of recombinant phase carrying VSG genes. An example of a plaque lift is shown in Figure 2. Analysis of the gDNA

clones will permit detailed examination of the consequence of antigenic variation on the structure of the 1.1, 1.D and 1.11 VSG gene sequences. In addition, all genomic libraries have been probed with a few cDNA clones representing the early IsTat 1 VSGs. In all cases the altered (preprint 3, figure 2). This looks very much like what would be expected according to the ELC model. However, no additional DNA copies are observed in some expressors and the basic copies of the VSG genes (those not expressed) undergo numerous alterations in the genome. IsTat 1 VATs 1.A, 1.5 and 1.11 have an additional copy of VSG DNA in expressors (preprint 3, figures 2, 3, 4). However, VATs 1.3 and 1.7 do not (preprint 3, figures 5 and 6). Although the basic copy genes appear unaltered in VAT 1.11 rearrangements or expressed and unexpressed genes are seen in VATs 1.A, 1.1 and 1.5 (preprint 3, figures 3, 4, and 7). The changes in the number of VSG gene sequences, additional copy in expressors and genomic rearrangements not associated with expression are all observed simultaneously using the 1.1 cDNA probe. In addition, several genomic fragments which differ in size are replaced by multiple copies of fragments of the same size. These genomic fragments undergo identical changes in size in the genome (preprint 3, figure 7). The simplest explanation is that these genes are linked. It is important to remember that all the genomic alterations that are illustrated are all occurring in the same genomic DNAs. Therefore, many VSG genes undergo many alterations in the genome and many genomic alterations are not correlated with changes in the expression of the corresponding VSG gene.

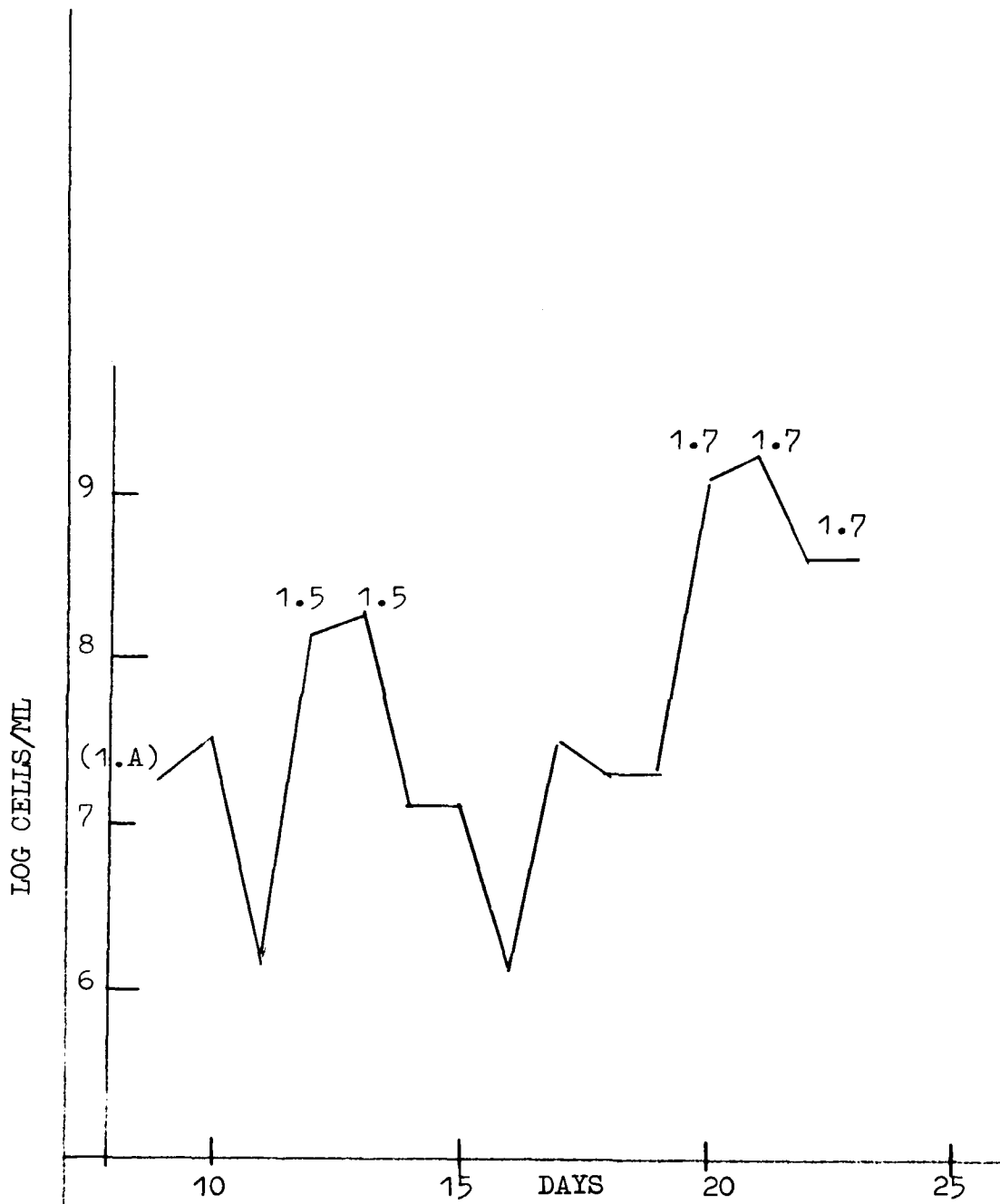


FIGURE 1. Infection of a deer mouse with VAT 1.A showing the sequence of VAT appearance.

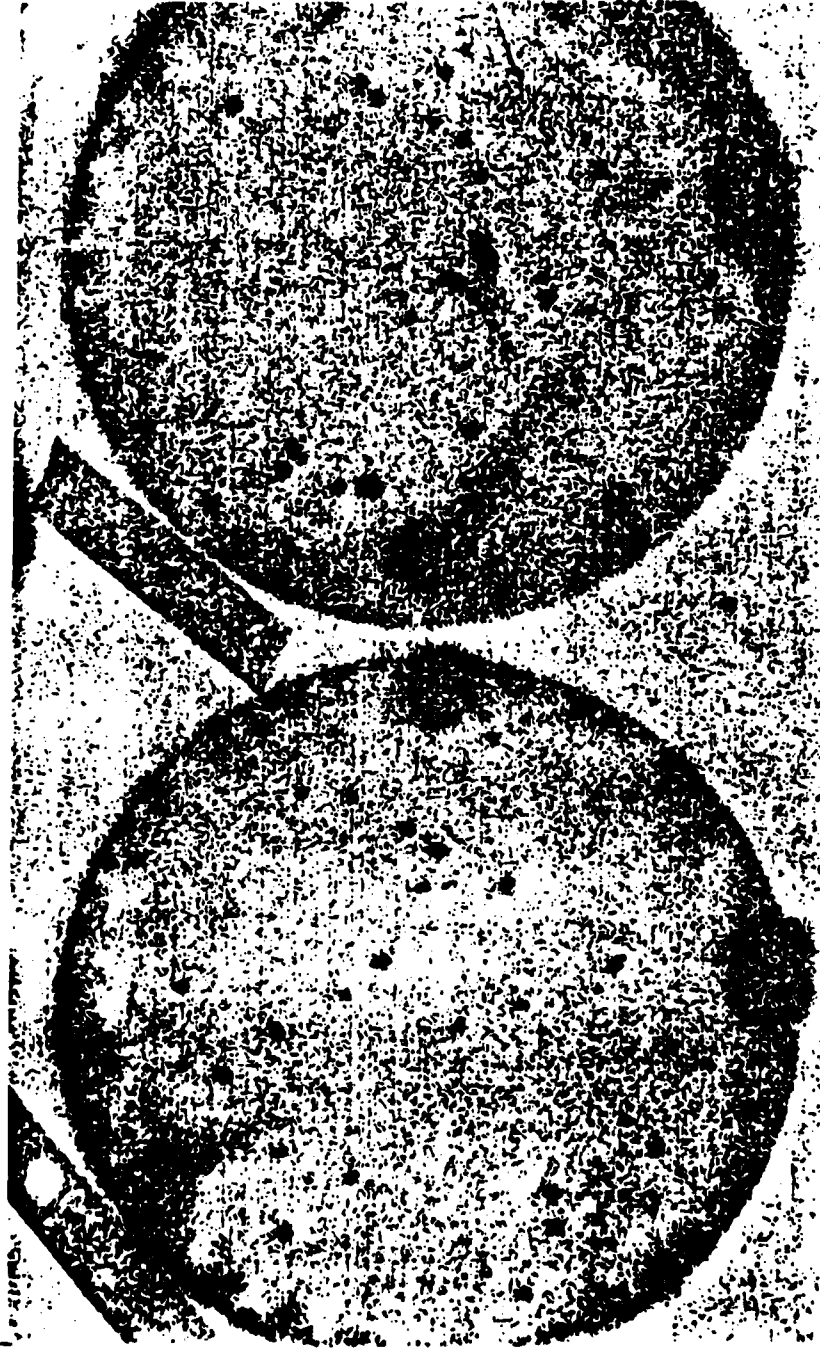


FIGURE 2. Phage plaque lift showing hybridization of phage recombinant DNA with cloned parasite DNA probe

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GGGGGGGGGGGGGGGGGGGATATACGACGGCAAAGCGAGGAAATCAAACA
 CAACAAGCAACGGCCGGTTGCAACCAGCAAGGAGCTGATCAAATCGGTGT
 TGTACTACCGAAACAAGAGACAGACAAATTTTTACGCATTCAAACAGGC
 CTCGAGCAGACAAAAGCGACTCAACAAGGCGGAAACACCAAATGCGAA
 GGAAGCAAATCTGAATGAGCCACCCTAAGGGTTGCGAATGGAATACGA
 CAGAAGAAAGTGTAACTCACAGAAGACGCACAAAAGATGCAGAAAGCGA
 ACACGAATAGGAAGCAGCAACCAACCACAGGACACATTCTTTGTCATTAC
 AGGCCTTTTGCTGCTTTOCTGTTTATTTTCTTTTCTAGTTGCTCTGCTCT
 GTTTCCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAGGGGGGGGGGGGGGGG

1.D
cDNA Sequence

Poly A tail (3')

GGGGGGGGGGGGGCTTTCTTTCTTTGGTCTTTCTTTGGTCTAATTCGG
 TTTGGAAAGATTTGGAGTTTGGTTTGGTCAAATTACACCCGTTTTTCCG
 TGCAATCATTTTTGTTACATGGTTTTTGCACACTGTCTGAGGTCCTTT
 GTTTGCTAACTTGTGGTGGACAATGATTGTTTGGCATAATTTGTTAAT
 TAAACCCCAAGTATGGCGCCTTTAGTGGTAAACTTTAATTTGTTATT
 AACCCAAGGGTTATGTGATTGTGTA!

1.11 cDNA
Partial Sequence

Poly A tail 14 mer seq Octomer

TTTTTTTTAAAGGAATTAATAATATCAGAAGTTTTCAAGTAGCAAAT
 TTTAAAAAGCGAGGGAAAAAGGTTTAAAAAGAGGGGGAAAATGTTGT
 TTCAAAAGGCAAGGCACAAATGCAGCAGAAACCATGCTGAGGGCGAATT
 TCTTGTTAGGAGATAGAGGAATCTTTGCAGCATTATTCTCCATTGCAA
 CCATTTGCCGATTCTTTTGTCTTTCTGTGCAGTGAGTCAGTTTCCATC
 TTCTTTCTT

Inverse Complement

1.11 cDNA
Partial Sequence

Figure 3. Nucleotide sequence of the 1.D VSG cDNA clone and partial sequence of the 1.11 VSG cDNA clone

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