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Hypothesis

A gp63 based vaccine candidate against Visceral Leishmaniasis

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Abstract:

Visceral leishmaniasis is a macrophage associated disorder which leads to a profound decrease in the natural immunotherapeutic potential of the infected subjects to combat the disease. The major surface glycoprotein gp63 has been found to be a significant vaccine candidate against visceral leishmaniasis. The current study addresses the levels of similarity and identity in the gp63 obtained from different species of Leishmania viz donovoni, chagasi and infantum linked to the cause of visceral leishmaniasis. The results from BLAST, Phylogram and Cladogram studies indicate significant identity, similarity and conservation of important residues in the protein which lead us to conclude that a common gp63 based vaccine can be used as a therapeutical tool against visceral leishmaniasis caused by different species strains of leishmania.

Keywords: Visceral leishmaniasis (VL), Glycoprotein 63(gp63), Basic Local Alignment Search Tool (BLAST), Cladogram, Phylogram

Background:

Leishmaniasis is an infectious disease complex caused by several species that are members of the protozoan parasite genus Leishmania. In humans, disease manifestation ranges from self-healing cutaneous lesions to lifethreatening visceral leishmaniasis (VL). This disease complex affects 12 million people, and there are 1.5 million new cases annually [1]. VL. caused by Leishmania donovoni, Leishmania infantum and Leishmania chagasi, remains the main agent of morbidity and mortality in leishmaniasis. The parasite has a simple life cycle, and abundant clinical and experimental evidence indicates that of all the parasitic diseases, leishmaniasis in particular should be an appropriate target for effective control through vaccination. There are, however, no vaccines in routine use against any form of the disease [2, 1]. Currently available vaccines against a variety of infectious diseases mediate protection by a long-lived humoral response through the production of antibodies. For diseases such as tuberculosis, malaria, human immunodeficiency virus infection, and leishmaniasis, however, the cellular immune response comprising primarily Th1 and CD8 effector T cells has been shown to be critical for mediating protection against the infection [3].

Visceral leishmaniasis is fatal if not treated, and development of a vaccine with long-term immunity remains a challenge. The attachment of *Leishmania* promastigotes to macrophages, crucial for intracellular parasitism and for the outcome of the infection, has been demonstrated by many investigators to be a specific receptor-mediated event **[4-6]**. Emphasis has been placed on the critical role of two abundant surface molecules gp63 **[7-10]** and lipophosphoglycan **[11]** that independently mediate parasite attachment to macrophages. Both molecules, when reconstituted into liposomes, mediate protection against cutaneous leishmaniasis and are considered as good vaccine candidates **[12]**. The current study has been undertaken with an idea to determine the regions of identity in the gp63 protein which will help in the development of a vaccine against all forms of VL.

Methodology:

By Sequence alignment we arranged the sequences of protein obtained from NCBI to identify regions of similarity that may be a consequence of functional, structural, or evolutionary relationships between the sequences.

Basic Local Alignment Search Tool:

Basic Local Alignment Search Tool, or BLAST, has been applied for comparing primary biological sequence information, like the amino-acid sequences of gp63 obtained from different species of leishmania. A BLAST search enabled us to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold.

Conservation:

Changes at a specific position of an amino acid sequence that preserve the physico-chemical properties of the original residue.

Bit score:

The value S' is derived from the raw alignment score S in which the statistical properties of the scoring system used have been taken into account. Because bit scores have been normalized with respect to the scoring system, they have been used to compare alignment scores from different searches.

H:

H is the relative entropy of the target and background residue frequencies. H can be thought of as a measure of the average information (in bits) available per position that distinguishes an alignment from chance. At high values of H, short alignments can be distinguished by chance, whereas at lower H values, a longer alignment may be necessary.

HSP: High-scoring segment pair.

HSPs are local alignments with no gaps that achieve one of the top alignment scores in a given search.

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P value: The probability of an alignment occurring with the score in question or better.

The p value is calculated by relating the observed alignment score, S, to the expected distribution of HSP scores from comparisons of random sequences of the same length and composition as the query to the database. The most highly significant P values are close to 0.

PAM:

Percent Accepted Mutation has been used to quantify the amount of evolutionary change in a protein sequence. 1.0 PAM unit is the amount of evolution which will change, on average, 1% of amino acids in a protein sequence. A PAM(x) substitution matrix is a look-up table in which scores for each amino acid substitution have been calculated based on the frequency of that substitution in closely related proteins that have experienced a certain amount (x) of evolutionary divergence.

Identity:

The extent to which two (nucleotide or amino acid) sequences are invariant.

Similarity:

The extent to which nucleotide or protein sequences are related. The extent of similarity between two sequences can be based on percent sequence identity and/or conservation. In BLAST similarity refers to a positive matrix score.

Expect value:

The Expect value (E) describes the number of hits one can "expect" to see by chance when searching a database of a particular size. It decreases exponentially as the Score (S) of the match increases. Essentially, the E value describes the random background noise. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

Gap:

A space introduced into an alignment to compensate for insertions and deletions in one sequence relative to another.

Clustal:

It is a widely used multiple sequence alignment computer program

ClustalW: command line interface.

A phylogenetic tree or evolutionary tree shows the evolutionary relationships among various biological species or other entities that are believed to have a common ancestor. In a phylogenetic tree, each node with descendants represents the most recent common ancestor of the descendants, and the edge lengths in some trees correspond to time estimates. Each node is called a taxonomic unit. Internal nodes are generally called hypothetical taxonomic units (HTUs) as they cannot be directly observed.

Dendrogram:

A dendrogram (from Greek dendron "tree", -gramma "drawing") is a tree diagram used to illustrate the arrangement of the clusters produced by a clustering algorithm.

Discussion:

BLAST (Table 1 see supplementary material) shows a high level of similarity and identity almost in the range of more than 90% and negligible percentage of gaps among the amino acid residues of the gp63 molecule. T-COFFEE (Figure 2) results show high level of identity, similarity and positives almost towards Good then towards Average and least towards Bad which again strongly advocate the high level of conservation with in aminoacid residues of gp3. CLADOGRAM and PHYLOGRAM (Figure 1) analysis also show tight vicinity among the gp63 residues during the process of evolution since the nodes are very close to each other. The Cladogram is lower in length, it has fewer homoplasies and it is more parsimonious. CAB51784.1 and CAB42815.1 are more closely related and both of them are close towards CAB42816.1. Moreover CAB51783.1 is related to all three. In the other branch CAB51797.2 and CAB51794.1 are closely related. They are further close to CAB51793.1. These gp63 proteins obtained from seven different species strains of Leishmania are more closely related amongst themselves as they arose from gene duplication. Moreover the sequence CAB06018.1 has altogether a different branch and it has relatively much less similarity with other gp3 proteins. The scale of Phylogram is 0.3 and it also strongly supports the results of Cladogram analysis.



Figure 1: Cladogram and Phylogram analysis of gp63 sequences of different species strains of Leishmania associated with Visceral Leishmaniasis

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T-COFFEE (Version 6.85(Tue Sep 9 14:03:25 WEST 2008) Cedric Notredame CPU TIME:10 sec. SCORE=85 BAD AVG GOOD gi_CAB0601 73 85 89 gi_CAB5179 gi_CAB51 3 : 89 86 88 88 CAB 51 giCAB51794. qi CAD4 28 1! gi CAD4 28 16 87 cri CAB5 1793 gi CAB5 1794 86 85 gri_CAB0601 gri_CAB5179 1MSVI RC48 -1 1F gri_CAB51_3 gri_CAB51_4 1------0 1-----0 gi CAB51794.1 -----0 gi CAD4 28 15 1MSVDSSSTHRHRSVAARLVRLAAAGAAVTAAVGTAAAWAHAGAVOHRC48 gi CAD4 28 16 1M STHRHRSVAARLVRL AA AG AAVTA AVGT AA AWAH A /0HRC48 cri CAB5 1793 1------ - 0 gi CAB5 1794 1----cons 48 1 gi_CAB0601 gi_CAB5179 49<mark>1HD AMQ AR VR</mark>QS VARIHITA PG AV SA VGL PYVTLD TA AA AD RRP 2------1 gi_CAB51_3 gi_CAB51_4 1------1-----0 gi CAB51794.1 1-----0 gi CAD4 28 15 49<mark>1HD AMH PR VR OS VARHHTA PG AV SA VGL PY VT LD TA AA AD RRP GS</mark> 49IHD AMH PRVROSVARHHTA PGAV SAVGL PYVTLD TA AA AD RRPO qri CAD4 28 16 **APT**96 giCAB51793 giCAB51794 1--1------ 0 cons 49 96 gi_CAB0601 gi_CAB5179 gi_CAB51_3 97<mark>VVRA ANW</mark>GALR I AVS TEDLTDP AYHC ARVGQHIKRRL GGVD I CT AED<mark>I</mark> 144 2------1 1----cri CAB 51 4 1------0 giCAB51794.1 ------97 VVRA ANWGALR LAVS TEDLTDP AYHC ARVGQRVNNHAGA LATCT ADDI 144 gi CAD4 28 15 cri CAD4 28 16 IATCTADDI 144 971 giCAB51793 1-----giCAB51793 giCAB51794 cons gi_CAB0601 gi_CAB5179 -1-----KRD IL VKHL I PQALQLHTERLKVRQVQDKWK<mark>VTGMGDDVCS</mark>D 192 1 0 gri_CAB51_3 gri_CAB51_4 1----gi_CAB 51_4 gi CAB 51_4.1 gi CAB 48 15 gi CAD 428 15 gi CAD 428 16 gi CAB 51793 gi CAB 51794 cons gi CAB 51794 cons gi CAB 51794 gi CAB 513 gi CAB 513 gi CAB 514 gi CAB 514 gi CAB 514815 1-----C T DECRED ILVKYL I PORLOLHTERLKVROVODKVKVTDMVDEI CGDFK 192 SUTDERED ILVKYL I PORLOLHTERLKVROVODKVKVTDMVDEI CGDFK 192 1 0 0 2---------- 1 ----- 0 1----giCAB51794.1 giCAD42815 giCAD42816 giCAB51793 giCAB51794 cons gi_CAB51794 ciCAB51794 gi_CAB51794 gi_CAB51_3 gi_CAB51_4 giCAB5124.1 giCAB51794.1 _____ 193 VP PAHLTDGLSNTDF VMXVASV PSEE GVLAWAT TC QVFSDGHPAVG VI 24 0 193 24 0 ANT ASRYDOLVTRVVTHEMAHALGFSVGFFEGARILESISM TEXLLVTOPPINIR Z------TEXLLV 1------XSS0X1LL 1-------17 gri CAD4 28 15 241<mark>NI PAANIAS RYDOLV TRVVTHEMAHALGF SGTFFTEILVVTOR</mark> 241<mark>NI PAANIAS RYDOLV TRVVTHEMAHALGF SGTFFTEILVVTOR</mark> 288 giCAD42816 giCAD51793 giCAB51794 288 1------0 giCAB51794 cons gi_CAB0601 gi_CAB5179 gi_CAB51_3 gi_CAB51_4 giCAB51794.1 28 33 63 62 65 DFDVPV INSS TAVAK 28 16 15 18 gi CAD4 28 15 gi CAD4 28 16 289KDFNVSVINSSTAVAKAREQYGCGTLEYLEIED QGGAGS AG SHIKMRN 336 289 giCAB51793 giCAB51794 cons 1 0 289 336

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gri_CAB0601	337 <mark>AQDELMAPAAAAGYYSALTMAIFQDLGFYQADFSKAEVMPWGRNAGCA</mark> 384
gri_CAB 5179	64 AKDELMAPAAAAGYYSALTMAIFQDLGFYQADFSKAEVMPWGRNAGCA111
gi_CAB51_3	63 AKDELMA PA AA AGYY SALTMAIFQDL GFYQADF SKAEEMPWGRN AGCA 110
g1_CAB51_4 ciCAP51384_1	66 AKDELMAPAAAAGYYSALTMALFUDLGFYUADFSKAEEMPWGRNAGCA11
gi CAD4 28 15	3 37 AKDEL MA PA AA AGVV SALTMAT FODI. GEVOADESKAEEM PVGRN AGCA 384
qiCAD42816	3 37 AKDELMA PA AA AGYY SALTMAIF QDLGF YQADF SKAEEM PWGRN AGCA 384
gi CAB5 1793	10
gi CAB5 1794	10
cons	337 384
gri_CAB0601	385 <mark>FLSEK CMERN IT KWPAMF CNENE VTMR CPTSRLSLGKC GVTRH</mark> -PDLP431
gi_CAB5179	112FL SEKCMERNI TKWP AMFCNENEV TMRC PTSRLMVGT CG IRGYS TPFS 15
g1_CAB51_5 cri_CAB51_4	1 14 FL SEKCHEUNT TKWP MECHVS VD VVRCPTSPLMLGTCGTRGTSTPFS 15 6
gi_CAB51794.1	1XXVGTXGXRGYSTPFS16
qi CAD4 28 15	385FL SEKCMEQNI TKWP AMF CNVS VD VVRC PTSRLML GT CG IR GYS TPFS 432
gi CAD4 28 16	385 <mark>FL SEKCMEQNI TKWP AMF CNVS VD VVRC PTSRLML GT CG IR GYS TPFS</mark> 432
gi CAB5 1793	1 <mark>MVGTCGIRGYSTPFS</mark> 15
gi CAB5 1794	1XVGTXGXRGYSTPFS16
cons	385 · · · · · · · · · · · · · · · · · · ·
GI_CABUSUI	4 32 PY WUYFTDPSLAGIS AF MDCCP VVEP YGDGS CAURASEA GA PFKGFNV 4 7 9
gi_CAB513	159 PVW)VETNI SL CCVS PELDYCPEVICYCDCS CNOD ASL A TOFFGAENU 20.6
gi_CAB51_5 cri CAB51_4	162 PYWDYFTNI SLGGYS PFLDY CPFV IGYGDGS CNOD ASLATGFFG AFNV 20
giCAB51794.1	17 LYNUYFTNASL GGYS PFLDY CPFVIGYSDGS CNUD ASLA AGFFS AFNV 64
gi CAD4 28 15	433 PYWUYFTNI SLGGYS PFLDY CPFVIGYGDGS CNUD ASLA TGFFG AFNV48
gri CAD4 28 16	433 PYNUYFTNI SLGGYS PFLDY CPFVIGYGDGS CNOD ASLATGFFG AFNV480
giCAB51793	16 LYNDYFTNASL GGYS PFLDY CPFVIGYSDGS CNOD ASLAAGFFS AFNV 63
gi CAB5 1794	17 LYWUYFTNASLGGYSPFLDYCPFVIGYSDGSCNUD ASLAAGFFSAFNV64
cons ci CAB0601	4 5 3 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
gi_CAB5179	200199
gri CAB 51 3	207 FSDR ARC ID GAFR PKNR TRADGYY AGLC ANVRCDT ATRTYS VQV CGSM 254
gi_CAB51_4	2 10 FSDA ARC ID GAFR PKNR TAADGYY AGLC ANVRCDT ATRTYS VQV CGSM 25 7
giCAB51794.1	65 <mark>FSDAARCID GAFR PKNR TAANGYY AGLC ANVRCD</mark> T ATRTYS VQVRG SM <mark>112</mark>
gi CAD4 28 15	481FSDAARCID GAFR PKNR TAADGYY AGLC ANVRCDT ATRTYS VQVCGSM528
giCAD42816	4 8 1FSDA ARC 1D GAFR PKNR TAADG YY AGLC ANVRCDT ATRTYS VQV CGSM 52 8
g1CRD51795 cri CBB51794	64 FSDAARCIDGEFFFKRKTAARGITAGLCARVRCDTATRTSVUVRGSM111
CONS	481 528
qri CAB0601	528GYANCTPGLRVELSTVSSAFEEGGYITCPPYVEVCQGNVQAAKD-GG-573
gi_CAB 5179	200199
gi_CAB51_3	255 DYVNCTPGLRVELSTVSSAFEEGGYITCPPYVEVCQANVKGAKDFAGD 302
gri_CAB51_4	2 58 DYVNCTP GLRVEL STVS SAFEE GGYI TC PPYVE VCQANVKG AKD FAGD 30
g1CAB51794.1	113DY VNCTPGLRVELSTVS SAFEEGGY1 TCPPY VEVCUANVKG AKDFAGD 160
eri (73.D.4.9.9.15	5 90 DUBLOWD CT DUET COVIC CAREFORCEVET WODDULE MODAMINE AND FACD 53 4
g1CHD42015 cri CAD42816	5 2 9 DI VINCI POLICI DI UNI STUSSI E E GOLI I CIPI VE V CURIVINO INDI RODI 7 0 5 9 DI VINCI DI UNI STUSSI E E GOLI I CIPI VE V CURIVINO INDI RODI 7 0
g1CAD42010	1 12 DYVNCTPGL RVEL STVS SAFFE GGYT TC PPYVEVCOANVKG AKDFA GD 15 9
gri CAB5 1794	113DYVNCTPGLRVELSTVSSAFEEGGYITCPPYVEVCOANVKGAKDFAGD160
cons	529 576
gri CAB0601	5 74 <mark>NAAAGRRG PRAA</mark> <mark>AT ALL VAALLA VA</mark> 598
gri_CAB 5179	200199
gri_CAB51_3	3 0 3 <mark>SD SS SSAGD AADR AAMQRWNDRMAGL AT AAMVLLGMVLSLMALVVVWL</mark> 35 0
gi_CAB51_4	3 0 6 <mark>SD SS SSAGD AADR AAMQRWNDRMAGL AT AAMVLLGMVLSLMALVVVML</mark> 35 3
giCAB51794.1	161 <mark>SD SS SSAGD AADR AAMQRWNDRMAGL AT AAMVLLGMVLSLMALVVVWL</mark> 208
gi CAD4 28 15	577SD SS SSAGD AADR AAMQRWNDRMAGL AT AAMVLLGMVLSLMALVVVWL 624
gi CAD4 28 16	577SD SS SSAGD AADR AAMORWNDRMAGL AT AAMVLLGMVLSLMALVVVML 624
g1CAB51793	160SD SS SSAGD AADR AAMURWINDRMAGE AT AAMVELGMVESEMAEVVVVIL 207
G1CHD31794	1015D 55 55RGD ANDK ARMUK MADKMAGL AT ARM VLLGM VLSLMALV VV ML20 0
CUIIS	311 <u>024</u>
	F
gi_CAB0601	599
g1_CAB5179	200 199 251 111 Terretore of the 263
GI_СНВЭІ_3 cri_СЛРБ1_4	331 HELICENNICORECCEPT 307 354 111 TODAWCORECCEPT 370
gr_CAB51794 1	209 III.T.C. PWW.CKEGGT.PT 225
gri CAD4 28 15	625 LLLTCPWWCCKFGGLPT 641
gi CAD4 28 16	625 ILLTC PWWC CKFGGL PT 641
giCAB51793	208 LLLTC PWWC CKFGGL PT 224
gi CAB5 1794	209 LLLTC PWWC CKFGGL PT 225
cons	625 641

Figure 2: T-COFFEE showing the conservation of aminoacid residues of gp63 in different species strains of *Leishmania* associated with Visceral Leishmaniasis

Conclusion:

Previous findings support the major Leishmania surface glycoprotein (gp63) as a proteinase [4] with acidic pH optimum [6]. Specifically, evidences also show that it is a metalloenzyme, possibly with Zn in its active site and that it is present on the surface of both leishmanial stages with certain molecular changes during leismanial differentiation. The studies also suggest that its catalytic activity as a proteinase may protect Leishmania to parasitize macrophages successfully [15]. Immunization of

susceptible BALB/c mice with gp63 entrapped in these vesicles protected virtually all of the mice against progressive nonhealing infections with L. donovoni [17]. Moreover data from other studies demonstrated that gp63 encapsulated in stable cationic liposomes provides long-term protection [17] in contrast to previous studies of protein-based vaccines [16]. Our results of BLAST Cladogram and Phylogenetic tree analysis throw light on the fact that a high level of conservation and identity amongst gp63 residues may help in the designing of a common vaccine against visceral

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leishmaniasis caused by different species strains of *Leishmania*. T-COFFEE (Version_6.85) also showed that the level of similarity ranged from average to good in the strains we selected. The results reinstate our claim that a common gp63 based vaccine can be designed against all forms of visceral leishmaniasis.

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Supplementary material:

 Table 1: BLAST between gp63 of different species strains of Leishmania associated with Visceral Leishmaniasis

SPECIES/SPECIES	ACCESSION NO/ACCESSION NO	SCORE	EXPEC T VALUE	IDENTITIES	POSITIVES	GAPS
L.INFANTUM/L.INFANTUM	CAB06018/CAB51797	326 BITS(835)	6e-94	155/198(78%)	166/198(83%)	1/198(0%)
L.INFANTUM/L.INFANTUM	CAB06018/ CAB51794	213 BITS(543)	5e-60	104/139(74%)	107/139(76%)	0/139(0%)
L.INFANTUM/L.INFANTUM	CAB06018/ CAB51793	216 BITS(549)	1e-60	107/154(69%)	113/154(73%)	1/154 (0%)
L.INFANTUM/L.INFANTUM	CAB51797/ CAB51794	108 BITS(270)	8e-29	50/53(94%)	50/53(94%)	0/53(0%)
L.INFANTUM/L.INFANTUM	CAB51797/ CAB51793	116 BITS(291)	2e-31	53/54(98%)	53/54(98%)	0/54(0%)
L.INFANTUM/L.INFANTUM	CAB51793/ CAB51794	450 BITS(1157)	1e-131	221/223(93%)	221/223(93%)	0/223(0%)
L.DONOVANI/L.DONOVANI	CAD42816/CAD42815	1330 BITS(3441)	0.0	641/641(100%)	641/641(100%	0/641(0%)
L.DONOVANI/L.DONOVANI	CAD42816/CAB51784	762 BITS(1968)	0.0	363/365(99%)) 364/365(99%)	0/365(0%)
L.DONOVANI/L.DONOVANI	CAD42816/CAB51783	766BITS(1978)	0.0	365/367(99%)	366/367(99%)	0/367(0%)
L.DONOVANI/L.DONOVANI	CAD42815/CAB51784	762 BITS(1968)	0.0	363/365(99%)	364/365(99%)	0/365(0%)
L.DONOVANI/L.DONOVANI	CAD42815/CAB51783	766 BITS(1978)	0.0	365/367(99%)	366/367(99%)	0/367(0%)
L.DONOVANI/L.DONOVANI	CAB51784/CAB51783	756 BITS(1952)	0.0	365/365(100%)	365/365(100%	0/365(0%)
L.DONOVANI/ L.INFANTUM	CAD42816/CAB06018	917 BITS(2370)	0.0	482/572(84%)) 502/572(87%)	1/572(0%)
L.DONOVANI/ L.INFANTUM	CAD42816/ CAB51797	386 BITS(992)	4e-112	182/198(91%)	187/198(94%)	0/198(0%)
L.DONOVANI/ L.INFANTUM	CAD42816/ CAB51794	390 BITS(1001)	4e-113	213/223(95%)	215/223(96%)	0/223(0%)
L.DONOVANI/ L.INFANTUM	CAD42816/ CAB51793	398 BITS(1022)	2e-115	216/224(96%)	218/224(97%)	0/224(0%)
L.DONOVANI/ L.INFANTUM	CAD42815/CAB06018	917 BITS(2370)	0.0	482/572(84%)	502/572(87%)	1/572(0%)
L.DONOVANI/ L.INFANTUM	CAD42815/ CAB51797	386 BITS(992)	4e-112	182/198(91%)	187/198(94%)	0/198(0%)
L.DONOVANI/ L.INFANTUM	CAD42815/ CAB51794	390BITS(1001)	4e-113	213/223(95%)	215/223(96%)	0/223(0%)
L.DONOVANI/ L.INFANTUM	CAD42815/ CAB51793	398 BITS(1022)	2e-115	216/224(96%)	218/224(82%)	0/224(0%)
L.DONOVANI/ L.INFANTUM	CAB51784/CAB06018	473BITS(1217)	7e-138	230/294(78%)	244/294(82%)	1/294(0%)
L.DONOVANI/ L.INFANTUM	CAB51784/ CAB51797	379BITS(974)	3e-110	181/194(93%)	185/194(95%)	0/194(0%)
L.DONOVANI/ L.INFANTUM	CAB51784/ CAB51794	384BITS(987)	1e-111	213/223(95%)	215/223(96%)	0/223(0%)
L.DONOVANI/ L.INFANTUM	CAB51784/ CAB51793	392BITS(1008)	4e-114	216/224(96%)	218/224(97%)	0/224(0%)
L.DONOVANI/ L.INFANTUM	CAB51783/CAB06018	473BITS(1217)	6e-138	230/294(78%)	244/294(82%)	1/294(0%)
L.DONOVANI/ L.INFANTUM	CAB51783/ CAB51797	382BITS(982)	3e-111	183/197(92%)	187/197(94%)	0/197(0%)
L.DONOVANI/ L.INFANTUM	CAB51783/ CAB51794	384BITS(986)	1e-111	213/223(95%)	215/223(96%)	0/223(0%)
L.DONOVANI/ L.INFANTUM	CAB51783/ CAB51793	392BITS(1007)	4e-114	216/224(96%)	218/224(97%)	0/224(0%)