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# Phylogenetic Characterization and Threading Based-Epitope Mapping of Leptospiral Outer Membrane Lipoprotein LipL41

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#### Abstract

Leptospiral outer membrane Lipoprotein LipL41 is one of the key virulence factor expressed during leptospiral infection on mammalian host. Phylogenetic analysis of LipL41 from 87 pathogenic species has shown the comprising lineages of LipL41 with largely varying rates of evolution. The species of *L. borgpetersenii* are clustered together and form a group with highest bootstrap value. Relationships between the species Lai and Copenhageni are resolved by 20 and 8 sequences separately with highest and lowest bootstrap values of 99 and 50, respectively. Molecular model of LipL41 was predicted in Raptor X server to map eight sequential and conformational B cell epitopes of LipL41 in Ellipro server. All these epitopes were found to be conserved among *Leptospira* and these could be used for the development of vaccine and diagnostic kit to detect *Leptospira*.

Keywords: Leptospira; LipL41; Phylogenetic analysis; B-cell epitope

**Abbreviations:** SAVES: Structural Analysis and Verification Server; MEGA: Molecular Evolutionary Genetic Analysis; LBP: Local Bootstrap values; ACC: Auto Cross Covariance; PI: Protrusion Index; NEFF: Number of Effective sequence homologs

#### Introduction

Leptospirosis is one of the most widespread zoonotic diseases in the world and is caused by the pathogen *Leptospira* [1]. Susceptible animals, including humans are infected by direct contact with urine from rodents, or indirectly through contaminated water. Transmission occurs via dermal abrasions or inoculation of the mucous or conjunctival membranes [2]. It is also known as Weil's syndrome and the clinical manifestations of leptospirosis include high fever, bleeding, and renal failure. The major target of *Leptospira* is the renal proximal tubular cells of kidney. Mortality ranges from 10-15% in cases of the traditional Weil's disease and can be more than 70% in cases of severe pulmonary haemorrhage syndrome (SPHS) [3-5]. Leptospirosis is endemic in most of the southern states of India like Kerala, Tamil Nadu and certain parts of Andhra Pradesh [6-8].

The *Leptospira* genus is sub-classified into 18 genomospecies that included both saprophytic and pathogeneic species [1,9]. Based on serologic methods, approximately 300 serovars have been identified; of which more than 200 are pathogenic [1,2,10]. The availability of genome sequence data for different *Leptospira* strains drives the discovery of new diagnostic tools and vaccines for Leptospirosis [11]. The major problem associated with Leptospirosis is the diagnosis of the disease, as it shows multiple symptoms; very often it has been confused with other diseases. The diagnosis of the leptospiral infection is very much complicated when compared with other ailments.

A number of leptospiral outer membrane proteins (OMPs) have been characterized including OmpL1 [12], LipL41 [13], LipL36 [14], LipL32 [14], LipL21 [15], LipL46 [16], LenA [17], Loa22 [16] and Omp52 [12]. However their performance in diagnostic assays for acute leptospirosis or as vaccine candidates has been problematic [12,18]. Among these OMPs, LipL41 appears to be a great interest since it is one of the key virulence determinants involved in host-pathogen interactions and it is being formed only in the host during the infection [13,15,18]. LipL41 is essential for virulence of *L. interrogans* in the animals [19]. Recent studies have revealed that LipL41 may play an important role in the infection and produces immunological responses in the host during the infection of *Leptospira* [12].

Considering the large number of pathogenic leptospiral serovars and broad distribution of leptospiral host reservoirs, the potential effect of selective pressure on the evolutionary mapping of the LipL41 proteins was not studied so far. The availability of genomic sequences of various serovars and strains opened up opportunities to identify evolutionary relationships among different pathogenic strains of *L. interrogans* and others representing various kinds of serotypes (serogroups and serovars). Given the potential of the LipL41 proteins as diagnostic antigens and vaccine candidates, we examined the evolutionary relationship of LipL41 with 87 sequences from various serovars and strains followed by protein threading and mapping of B-cell epitopes from the conserved region of the alignment to develop vaccine for Leptospirosis.

#### Materials and Methods

# Sequence retrieval, sequence alignment and dendrogram construction

Amino acid sequences used in this study were retrieved from protein knowledgebase (UniProt KB) (http://www.uniprot.org/ uniprot/). A total of 87 sequences of LipL41 from different serovars and strains (Table 1) were collected from UniProtKB. Sequences with

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1.         Lafetquere internogenar         Lafe         totorbasemorthagiae         96001         0976E1           3.         Laptquere internogenar         Copenhageni (tetorbasemorthagiae         Proziz L1-32         0724Y1           4.         Laptquere internogenar         Clerohamemorthagiae         Unchanceforzad probin         Proziz L1-32         0724Y1           5.         Laptquere internogenar         Internote internote probin         Doyster         NSXII 4           7.         Laptquere internogenar         Valluzzi         Unchanceforzad probin         Doyster         NSXII 4           8.         Laptquere internogenar         Canctolin         Mago Cuerre menthame protein         Construction         OGSXC2           9.         Laptquere internogenar         Lai         Mago Cuerre menthame protein         Construction         OGSXC2           9.         Laptquere internogenar         Lai         Mago Cuerre menthame protein         Construction         OGSXC2           11.         Laptquere internogenar         Aulumatis         Mago Cuerre menthame protein         Construction         OGSXC3           12.         Laptquere internogenar         Aulumatis         Mago Cuerre menthame protein         OGSXC3           13.         Laptquere internogenar         Aulumatis		Organism	Serovar	Serogroup/Name/ uncharacterized protein	Strain	Uniprot ID
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4 Laptoppin wells Advances (Construction) (Constructions) (Con	3.	Leptospira interrogans	Icterohaemorrhagiae	Uncharacterized protein	Verdun	M6R807
5.         Laptaspin interragans         -         Onder Marntarag protein         -         PT1435           6.         Laptaspin interragans         Poincol         Uncharacterized protein         Kennewick LC2:25         JAT583           7.         Laptaspin interragans         Poincol         Uncharacterized protein         Kennewick LC2:25         JAT583           9.         Laptaspin interragans         Austall         Major outer membrane protein         CGCXC2         GGCXC2           10.         Laptaspin interragans         Austall         Major outer membrane protein         CGCXC2         GGCXC2           11.         Laptaspin interragans         Austall         Major outer membrane protein         GGCXC2         GGCXC2           12.         Laptaspin interragans         Multice         Formation         GGCXC2         GGCXC2           13.         Laptaspin interragans         Wolfinder         Major outer membrane protein         GGCXC2         GGCXC2           14.         Laptaspin interragans         Wolfinder         Major outer membrane protein         GGCXC2         GGCXC2           15.         Laptaspin interragans         Wolfinder         Major outer membrane protein         GGCXC2         GGCXC2           16.         Laptaspin interacterized protein	4.	Leptospira weilii	Manhao	Major Outer Membrane protein	-	Q6GXB6
6.         Leptospin Interrogens         Valbuci         Uncharacterized protein         Dysfer         NAXE14           8.         Leptospin Interrogens         Canicola         Major outer menthome protein         Control         OGSXCB           9.         Leptospin Interrogens         Auturnalis         Major outer menthome protein         Control         OGSXCB           10.         Leptospin Interrogens         Auturnalis         Major outer menthome protein         Control         OGSXCB           12.         Leptospin Interrogens         Multice         Control         OGSXCB           12.         Leptospin Interrogens         Multice         OGSXCB         OGSXCB           13.         Leptospin Interrogens         Multice         OGSXCB         OGSXCB           14.         Leptospin Interrogens         Major outer methoms protein         Holewoo         MERGAD           15.         Leptospin Interrogens         Wolff         Major outer methoms protein         OGSXCB           16.         Leptospin Interrogens         Wolff         Major outer methoms protein         OGSXCB           16.         Leptospin Interrogens         Wolff         Major outer methoms protein         SIG2_CT         SUB/ST           17.         Leptospin Interrogens         M	5.	Leptospira interrogans	-	Outer Membrane protein	-	P71435
7.         Leptogum Interrogens         Promos         Uncharacterized protein         Kennecki, LC2-25         J41538           9.         Leptogum Interrogens         Lai         Major outer merbrane protein         OGSX03           9.         Leptogum Interrogens         Australis         Major outer merbrane protein         OGSX03           11.         Leptogum Interrogens         Australis         Major outer merbrane protein         OGSX03           12.         Leptogum Interrogens         Maniae         OGSX03         OGSX03           13.         Leptogum Interrogens         Maniae         OGSX03         OGSX03           14.         Leptogum Interrogens         Malor outer merbrane protein         H1         KerC1.0           16.         Leptogum Interrogens         Auturnalis         Major outer merbrane protein         S22_CT         S3UFS2           16.         Leptogum Interrogens         Auturnalis         Uncharacterized protein         R1M         MIX21           17.         Leptogum Interrogens         Auturnalis         Uncharacterized protein         S22_CT         S3UFS2           16.         Leptogum Krachnen         Gynopoten         Uncharacterized protein         S1M         MIX21           12.         Leptogum Krachnen         Bin	6.	Leptospira interrogans	Valbuzzi	Uncharacterized protein	Duyster	N6XR14
8.         Leptoppin Interrogens         Cencola         Major outer membrane protein         —         OGGXCB           10.         Leptoppin Interrogens         Auturnalis         Major outer membrane protein         —         OGGXCB           12.         Leptoppin Interrogens         Auturnalis         —         OGGXCB         OGGXCB           12.         Leptoppin Interrogens         Hobbanadis         —         OGGXCB         OGGXCB           12.         Leptoppin Interrogens         Manilee         —         OGGXCB         OGGXCB           13.         Leptoppin Vincement         Bugins on         —         OGGXCB         OGGXCB           14.         Leptoppin Vincement         Bugins on         —         OGGXCB         OGGXCB           14.         Leptoppin Vincement         OgGXCB         OGGXCB         OGGXCB         OGGXCB           15.         Leptoppin Vincement         OgGXCB         OGGXCB         OGGXCB         OGGXCB           16.         Leptoppin Vincement         OgGXCB         OGGXCB         OGGXCB         OGGXCB           16.         Leptoppin Vincement         Onthinaceterized protein         Staturnalis         Major outer membrane protein         OGGXCB           17.         Leptoppin Vincem	7.	Leptospira interrogans	Pomona	Uncharacterized protein	Kennewicki LC82-25	J4T638
9.         Lappospire interroganse         Lai         Major outer membrane protein         OGSXC2           11.         Lappospire interroganse         Australis         Major outer membrane protein         OGSXC2           12.         Lappospire interroganse         Australis         OGSXC3         OGSXC3           12.         Lappospire interroganse         Manilas         OGSXC3         OGSXC3           13.         Laptospire interroganse         Manilas         OGSXC3         OGSXC3           14.         Laptospire interroganse         Autumalis         Major outer membrane protein         OGSXC3           16.         Laptospire interroganse         Autumalis         Major outer membrane protein         OGSXC3           17.         Laptospire interrodanse         Wordfi         Major outer membrane protein         OGSXC3           16.         Laptospire interrodanse         Wordfi         Major outer membrane protein         OGSXC3           17.         Laptospire interrodanse         Wordfi         Major outer membrane protein         OGSXC3           18.         Laptospire interrodanse         Wordfi         Major outer membrane protein         OGSXC3           12.         Laptospire interrodanse         Skoinee         Uncharacteread protein         Skoinee         Ma	8.	Leptospira interrogans	Canicola	Maior outer membrane protein	_	Q6GXC6
10.         Leptospire interrogane         Autumalia         Major outer membrane protein         005XV2           12.         Leptospire interroganes         Madmalia         0338N/1           13.         Leptospire interroganes         Manilae         0338N/1           13.         Leptospire interroganes         Manilae         0338N/1           14.         Leptospire interroganes         Manilae         0338N/1           15.         Leptospire interroganes         Autumalia         Major outer membrane protein         0458X3           16.         Leptospire interroganes         Wdfl         Major outer membrane protein         046857           17.         Leptospire interroganes         Wdfl         Major outer membrane protein         3522_0T         SSUFG2           18.         Leptospire interromenes         Cynopteri         Uncharacterezed protein         3522_0T         SSUFG2           19.         Leptospire interromenes         Southered         Uncharacterezed protein         RMT         MMS221           11.         Leptospire interromenes         Bim         Uncharacterezed protein         #r. MMS14         MMS224           23.         Leptospire inscrittered         Grippotyphosa         MST         MS2400         #r. 1207         MSVS14	9.	Leptospira interrogans	Lai	Maior outer membrane protein		Q6GXC8
11         Leptospire interrogene         Autumalis         0.338N1           12         Leptospire interrogene         Hexkonwalis         0.338N0           13         Leptospire interrogene         Manilae         0.338N0           14         Leptospire interrogenes         Manilae         0.338N0           15         Leptospire interrogenes         Autumalia         Major outer membrane protein         H1         KSFCL0           16         Leptospire interrogenes         Autumalia         Major outer membrane protein         0.66XS3           17         Leptospire interrogenes         Wolffi         Major outer membrane protein         0.66XS5           18         Leptospire interrodenes         Sokoine         Uncharacterized protein         2.0517.4           18         Leptospire interrodenes         Sokoine         Uncharacterized protein         1.051         MBBV4           22.         Leptospire interrodenes         BEm         Uncharacterized protein         8tr. JPUO_1274         MEXED1           23.         Leptospire interrodenes         Grippotphosa         1.051         MBBV4           24.         Leptospire interrodenes         Grippotphosa         1.051         MBV143         MBOUL6           25.         Leptospire interrodenes <td>10.</td> <td>Leptospira interrogans</td> <td>Australis</td> <td>Maior outer membrane protein</td> <td></td> <td>Q6GXC2</td>	10.	Leptospira interrogans	Australis	Maior outer membrane protein		Q6GXC2
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13.         Leptospira interrogans         Maniae         0.338/M7           14.         Leptospira interrogans         Auturnalis         Major outer membrane protein         H1         K6FCL0           15.         Leptospira interrogans         Auturnalis         Major outer membrane protein         0.265XC3           16.         Leptospira interrogans         Wotfi         Major outer membrane protein         0.265XC3           17.         Leptospira kirschneri         Cycopteri         Uncharacterized protein         0.265XC3           17.         Leptospira kirschneri         Sokone         Uncharacterized protein         0.80801774         M62521           12.         Leptospira kirschneri         Bim         Uncharacterized protein         81, M01433         M62042           22.         Leptospira kirschneri         Bim         Uncharacterized protein         strM01433         M62042           23.         Leptospira kirschneri         Grippotyphosa         strAM52         A84709         strAM52         A84709           27.         Leptospira kirschneri         Grippotyphosa         strLaptospira kirschneri         Grippotyphosa         strLaptospira kirschneri         Grippotyphosa         strLaptospira kirschneri         J45XV5           27.         Leptospira kirschneri </td <td>12.</td> <td>Leptospira interrogans</td> <td>Hebdomadis</td> <td></td> <td></td> <td>Q33BN0</td>	12.	Leptospira interrogans	Hebdomadis			Q33BN0
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15.     Leptospira kirschneri     H1     KBFCL0       16.     Leptospira interrogans     Auturnalis     Mejor outer membrane protein     G6GXG3       17.     Leptospira kirschneri     Cynopteri     Uncharacterized protein     S52_CT     S3UFG2       17.     Leptospira kirschneri     Uncharacterized protein     200801774     M6XE01       17.     Leptospira kirschneri     Uncharacterized protein     200801774     M6XE01       17.     Leptospira kirschneri     Bim     Uncharacterized protein     RM1     M6IZ21       18.     Leptospira kirschneri     Bim     Uncharacterized protein     strPUO_1247     M6ETW2       17.     Laptospira kirschneri     Bim     Uncharacterized protein     strPUO_1247     M6ETW2       17.     Laptospira kirschneri     Grippotyphosa     strMOX KK840     M6BUL6       17.     Laptospira kirschneri     Grippotyphosa     strMOX KK840     M6BU16       17.     Leptospira noguchi     Parama     strLOR3K82     J4SXV5       18.     Leptospira interrogans     Uncharacterized protein     strMOX H8817       19.     Leptospira interrogans     Uncharacterized protein     strLOR3K84       11.     Leptospira interrogans     Uncharacterized protein     strLOR3K84       12.	14.	Leptospira kirschneri	Bulgarica		Nikolaevo	M6F5M3
16         Leptoppin interrogens         Autumatis         Major outer membrane protein         0667XC3           17.         Leptoppina interrogens         Wolffi         Major outer membrane protein         0667XC3           17.         Leptoppina interrogens         Wolffi         Uncharacterized protein         3522_CT         S3UFG2           18.         Leptospina kirschneri         Ornoptein         Uncharacterized protein         20080/174         MeSKC1           17.         Leptospina kirschneri         Solone         Uncharacterized protein         RM1         MeSIZ           21.         Leptospina kirschneri         Bim         Uncharacterized protein         str_MASA         MeSIVa           22.         Leptospina kirschneri         Vilbuzzi         Uncharacterized protein         str_MASA         K8HDP3           23.         Leptospina kirschneri         Gripotyphosa         str_MASA         K8HDP3         str_MASA         K8HDP3           24.         Leptospina kirschneri         Gripotyphosa         str_MASA         K8HDP3         str_MASA         K8HDP3         str_MASA         K8HDP3         str_MASA         K8HDP3         str_MASA         K8HDP3         str_MASA         K8HDP3         str_MASA         Str_MASA         Str_MASA         Str_MASA         St	15.	Leptospira kirschneri			H1	K6FCL0
17.         Leptospire interrogans         Wolffi         Major outer membrane protein         0682X85           18.         Leptospire kirschnen         Cynopteri         Uncharacterized protein         3522_CT         SSUFG2           19.         Leptospire kirschnen         Uncharacterized protein         200801774         M&XE01           20.         Leptospire kirschnen         Sokoine         Uncharacterized protein         RM1         M&IZ21           21.         Leptospire kirschnen         Bim         Uncharacterized protein         str. PUO_1247         MBETV42           22.         Leptospire kirschnen         Bim         Uncharacterized protein         str. PUO_1247         MBETV42           23.         Leptospire kirschnen         Grippotyphosa         strUnosava (K&HDP3         MBOUL6           24.         Leptospire kirschnen         Grippotyphosa         strUnosava (K&HDP3         MBVT15           23.         Leptospire noguchi         Panama         strUnosava (K&HDP3         MBVT15           24.         Leptospire noguchi         Autumnalis         Uncharacterized protein         strUN03005666         SSUH03           24.         Leptospire noguchi         Autumnalis         Uncharacterized protein         strUN142         MBUJ70	16.	Leptospira interrogans	Autumnalis	Maior outer membrane protein		Q6GXC3
18.         Leptospire Kirschneri         Cynopleri         Uncharacterized protein         3522_CT         S3UFG2           19.         Leptospire Kirschneri         Uncharacterized protein         200801774         MKXED1           21.         Leptospire Kirschneri         Sokolne         Uncharacterized protein         200801774         MKXED1           21.         Leptospire Kirschneri         Bim         Uncharacterized protein         1051         M6IBV4           22.         Leptospire Kirschneri         Bim         Uncharacterized protein         strPUO_1247         M6ETV02           23.         Leptospire Kirschneri         Grippotyphosa         uncharacterized protein         strMKS2         MSXV5           24.         Leptospire Kirschneri         Grippotyphosa         uncharacterized protein         strL1207         M6M715           25.         Leptospire moguchi         Parama         strL1207         M6M715         StrL02074         MGV811           21.         Leptospire noguchi         Parama         strL1207         M6W711         StrL02074         MGV811           21.         Leptospire noguchi         Automalis         Uncharacterized protein         strL02074         MGV811           23.         Leptospire noguchi         Automali	17	l eptospira interrogans	Wolffi	Major outer membrane protein		Q6GXB5
Image: Provide and the second secon	18.	Leptospira kirschneri	Cynopteri	Uncharacterized protein	3522 CT	S3UFG2
20.         Leptospira kirschneri         Sokoine         Uncharacterized protein         RN1         M6XED1           21.         Leptospira kirschneri         Bim         Uncharacterized protein         1051         M68Y4           23.         Leptospira kirschneri         Bim         Uncharacterized protein         str. PUO_1247         M6ETVV2           24.         Leptospira kirschneri         Vincharacterized protein         str. M01493         M6DUL6           25.         Leptospira kirschneri         Vincharacterized protein         str. M01493         M6DUL6           25.         Leptospira kirschneri         Grippotyphosa         str. Moskva         K8HDP3           26.         Leptospira kirschneri         Grippotyphosa         str. Moskva         K8HDP3           28.         Leptospira inferrogans         Uncharacterized protein         str1207         M6M715           30.         Leptospira noguchii         Panma         str201412         M6U370           31.         Leptospira noguchii         Autumalis         Uncharacterized protein         str201412         M6U370           32.         Leptospira inferrogans         Autumalis         Uncharacterized protein         str200001578         M6U17           33.         Leptospira inferrogans	19.	Leptospira kirschneri	2 TE	Uncharacterized protein		Q48587
1         Leptopira kirscheri         Sokolne         Uncharacterized protein         FM1         M6JZ21           21.         Leptospira kirscheri         Bim         Uncharacterized protein         615         M68/94           22.         Leptospira kirscheri         Bim         Uncharacterized protein         strPUO_1247         MBETW2           24.         Leptospira kirscheri         Valuzzi         Uncharacterized protein         strMoskva         K8HDP3           25.         Leptospira kirscheri         Grippotyphosa         strMoskva         K8HDP3           27.         Leptospira kirscheri         Grippotyphosa         strRMS2         J4SXV5           28.         Leptospira kirscheri         Grippotyphosa         strCZ214         T0F.119           30.         Leptospira noguchi         Panama         strCZ214         T0F.119           31.         Leptospira noguchi         Uncharacterized protein         strZUN142         M6U310           31.         Leptospira noguchi         Uncharacterized protein         strZUN142         M6U310           32.         Leptospira interrogans         Autumalis         Uncharacterized protein         str200001870         KKKY78           33.         Leptospira interrogans         Icterohaemorrhagia	20.	Leptospira kirschneri		Uncharacterized protein	200801774	M6XE01
22.         Leptopira kirschneri         Bim         Uncharacterized protein         1051         M68Y4           23.         Leptospira kirschneri         Bim         Uncharacterized protein         str. PUO_1247         M6ETVv2           24.         Leptospira kirschneri         Grippotyphosa         strM01493         M6DUL6           25.         Leptospira kirschneri         Grippotyphosa         strRMS2         J4SV5           25.         Leptospira kirschneri         Grippotyphosa         strRMS2         J4SV5           26.         Leptospira kirschneri         Grippotyphosa         strRMS2         J4SV5           28.         Leptospira kirschneri         Grippotyphosa         strRMS2         J4SV5           29.         Leptospira noguchi         Panama         strC2214         T0FJ19           30.         Leptospira noguchi         Autumnalis         Uncharacterized protein         strAUN142         M6U370           31.         Leptospira noguchi         Uncharacterized protein         str2007001578         M6U17           32.         Leptospira interogans         Australis         Lipoprotein         G338M8           33.         Leptospira kirschneri         Uncharacterized protein         str200801787         K4KY78	21.	Leptospira kirschneri	Sokoine	Uncharacterized protein	RM1	M6JZZ1
Intervention         Intervention         Intervention         Intervention           23.         Leptospira kirschneri         Uncharacterized protein         strMUD1493         M6D'U42           24.         Leptospira kirschneri         Valuzzi         Uncharacterized protein         strMONAva         K8H/DP3           25.         Leptospira kirschneri         Grippotyphosa         strMoskva         K8H/DP3           27.         Leptospira kirschneri         Grippotyphosa         strC2214         T0FJ19           28.         Leptospira kirschneri         Grippotyphosa         uncharacterized protein         strL1207         M6M'T15           28.         Leptospira interrogans         Uncharacterized protein         strL1207         M6M'T15           29.         Leptospira interrogans         Uncharacterized protein         strZUN142         M6U37           31.         Leptospira interrogans         Uncharacterized protein         str2006001870         K8KY78           32.         Leptospira interrogans         Loncharacterized protein         str_200801870         K8KY7           33.         Leptospira kirschneri         Uncharacterized protein         str_200801870         K8KY7           34.         Leptospira kirschneri         Uncharacterized protein         str_	22.	Leptospira kirschneri	Bim	Uncharacterized protein	1051	M6I8Y4
Leptospira kirschneri         Valbuzzi         Uncharacterized protein         str_MND1493         M6DUL6           25.         Leptospira kirschneri         Grippotyphosa         str_Z00702274         K812W0           26.         Leptospira kirschneri         Grippotyphosa         str_RN42         J4SXV6           27.         Leptospira kirschneri         Grippotyphosa         str_L1207         M6M715           28.         Leptospira kirschneri         Grippotyphosa         str_L1207         M6M715           29.         Leptospira noguchii         Panana         str_L21207         M6M715           30.         Leptospira noguchii         Uncharacterized protein         str_L930005006         S3HUG3           31.         Leptospira noguchii         Uncharacterized protein         str_2000701578         M6U370           32.         Leptospira noguchii         Uncharacterized protein         str_200601870         Q33BM9           33.         Leptospira interrogans         Australis         Lipoprotein         G33BM8           34.         Leptospira kirschneri         Uncharacterized protein         str_200801825         M6XK47           35.         Leptospira kirschneri         Uncharacterized protein         str_200801825         M6XK47           36.	23	l eptospira kirschneri	Bim	Uncharacterized protein	str PUO 1247	M6FTW2
Leptospira kirschneri         Valbuzzi         Uncharacterized probin         str200702274         K812V/0           25.         Leptospira kirschneri         Grippotybnosa         strMosiva         K812P3           26.         Leptospira kirschneri         Grippotybnosa         strMosiva         K812P3           26.         Leptospira interrogans         Uncharacterized protein         strL1207         M6M715           28.         Leptospira interrogans         Uncharacterized protein         str1993005606         S31U63           31.         Leptospira interrogans         Uncharacterized protein         str201070578         M6I0.7           32.         Leptospira interrogans         Uncharacterized protein         str200601870         K8KY78           33.         Leptospira interrogans         Autmanalis         Uncharacterized protein         str200601870         K8KY78           34.         Leptospira interrogans         Cleptospira kirschneri         Q33BM9         Q33BM9         Q33BM9         Q33BM9         Q33BM9         Q33BM9         Q33BM8         Leptospira kirschneri         Q33BM8         K2447         K8GUX5         M6XX47         Q3444         Leptospira kirschneri         Q04VM6         Q32BM9         Q32BM9         Q32BM8         Q32BM9         Q32BM8         <	24	Leptospira kirschneri		Uncharacterized protein	str_MMD1493	M6DUL6
Explosion         Explosion         Explosion         Explosion         Explosion         Explosion           22.         Leptospira kirschneri         Grippotyphosa         strMoskva         K8HDP3           27.         Leptospira kirschneri         Grippotyphosa         strLTO7         M6W715           23.         Leptospira noguchi         Panama         strLTO7         M6W715           29.         Leptospira noguchi         Panama         strLTO7         M6W715           30.         Leptospira noguchi         Autumnalis         Uncharacterized protein         strLTA156         M6V9T1           31.         Leptospira noguchi         Autumnalis         Uncharacterized protein         strZ00700178         M6I0J7           32.         Leptospira noguchi         Autumnalis         Uncharacterized protein         strZ00801870         K8KY78           33.         Leptospira interrogans         Luporotein         G33BM8         G32BM8         G32BM8 <td< td=""><td>2<del>.</del> 25</td><td>Leptospira kirschneri</td><td>Valbuzzi</td><td>Uncharacterized protein</td><td>str00702274</td><td>K8I2W0</td></td<>	2 <del>.</del> 25	Leptospira kirschneri	Valbuzzi	Uncharacterized protein	str00702274	K8I2W0
Explosipia kirschneri         Grippotybiosa         Intervention         Str. RMS2         J4SXV5           28.         Leptospira interrogans         Uncharacterized protein         Str. L1207         M6M715           29.         Leptospira noguchi         Panama         StrCX214         T0F.19           30.         Leptospira noguchi         Autumnalis         Uncharacterized protein         StrHA1556         M6V6T1           31.         Leptospira noguchi         Autumnalis         Uncharacterized protein         StrVA142         M6U370           32.         Leptospira noguchi         Autumnalis         Uncharacterized protein         Str200701578         M6I0J7           33.         Leptospira interrogans         Australis         Lipoprotein         Str200801870         K8KY78           34.         Leptospira kirschneri         Uncharacterized protein         Str200801825         M6XK47           37.         Leptospira kirschneri         Uncharacterized protein         str200801303         M6W9C5           38.         Leptospira kirschneri         Uncharacterized protein         str200801303         M6W4C5           41.         Leptospira kirschneri         Uncharacterized protein         str200801303         M6W4C5           42.         Leptospira kirs	26	Leptospira kirschneri	Grinnotynhosa		str. Moskva	K8HDP3
Leptospir interrogans         Uncharacterized protein         str1207         M6M715           22.         Leptospir anguchi         Panama         str0207         M6M715           23.         Leptospir anguchi         Panama         str03005606         S3HU63           30.         Leptospir anguchi         Autumalis         Uncharacterized protein         str193006506         S3HU63           31.         Leptospir anguchi         Autumalis         Uncharacterized protein         str2007001578         M6I0J7           33.         Leptospir anguchi         Uncharacterized protein         str2007001578         M6I0J7           34.         Leptospira interrogans         Australis         Lipoprotein         str200801870         M6I0J7           35.         Leptospira kirschneri         Uncharacterized protein         str000801870         M6KX47           36.         Leptospira kirschneri         Uncharacterized protein         str000802841         M6W25           37.         Leptospira kirschneri         Uncharacterized protein         str000802841         M6W25           38.         Leptospira kirschneri         Uncharacterized protein         str0090128         M6W25           41.         Leptospira borgpetersenii         Hardjo-bovis         Lipoprotein<	20.	Leptospira kirschneri	Grippotyphosa		strM05/2	14SXV5
Explosizina noguchi         Panama         Inclustration point         Inclustration point           30.         Leptospira noguchi         Panama         Inclustration point         Itr[193005006)         S3HU63           31.         Leptospira noguchi         Autumalis         Uncharacterized protein         strHAI1536         M6V6T1           32.         Leptospira noguchi         Autumalis         Uncharacterized protein         str2007001578         M6I03T0           33.         Leptospira noguchi         Uncharacterized protein         str2007001578         M6I03T0           34.         Leptospira interrogans         Australis         Lipoprotein         str2008018270         K8KY78           35.         Leptospira interrogans         Icterohaemorrhagiae         Uncharacterized protein         str200801925         M6X447           36.         Leptospira kirschneri         Uncharacterized protein         str200802841         K6GUX5           37.         Leptospira kirschneri         Uncharacterized protein         str200802841         K6GUX5           38.         Leptospira kirschneri         Uncharacterized protein         str200802841         K6GUX5           39.         Leptospira kirschneri         Uncharacterized protein         strU009931         S3URD2	28	L'entospira interrogans	Chippotyphosa	Incharacterized protein	str.   1207	M6M715
Leptospira noguchii       Str. 1993005606       S3HU63         31.       Leptospira noguchii       Str. 1993005606       S3HU63         31.       Leptospira noguchii       Autumnalis       Uncharacterized protein       str. 201701578       M6V6T1         32.       Leptospira noguchii       Uncharacterized protein       str. 200701578       M6I017         34.       Leptospira noguchii       Uncharacterized protein       str. 200701578       M6I017         34.       Leptospira interrogans       Lucaracterized protein       str. 200801870       K8KY78         35.       Leptospira interrogans       Icterohaemorrhagiae       Lipoprotein       Q33BM9         37.       Leptospira kirschneri       Uncharacterized protein       str. 2008013703       M6W9C5         38.       Leptospira kirschneri       Uncharacterized protein       str. 200802811       K6OUX5         40.       Leptospira interrogans       Citerohaemorrhagiae       Outer membrane liporotein       strU.9931       S3UR02         41.       Leptospira borgpetersenii       Javanica       Uncharacterized protein       strU.9931       S3UR02         42.       Leptospira borgpetersenii       Balum       Major outer membrane protein       str200901865       M6W4K3         43.	29	Leptospira monogano	Panama		str C7214	T0F.I19
Deptospira intergrans       Uncharacterized protein       strHA1536       M6/0611         31.       Leptospira noguchii       Autumalis       Uncharacterized protein       strZUN142       M6/0370         32.       Leptospira noguchii       Uncharacterized protein       strZ007001578       M6/037         33.       Leptospira interrogans       Australis       Lipoprotein       str200601870       K8KY78         34.       Leptospira interrogans       Australis       Lipoprotein       str200801920       Q33BM9         36.       Leptospira interrogans       Icterohaemorrhagiae       Lipoprotein       str200801925       M6KK47         37.       Leptospira interrogans       Icterohaemorrhagiae       Uncharacterized protein       str200802841       K6GUX5         38.       Leptospira interrogans       Grippotyphosa       Uncharacterized protein       str200802841       K6GUX5         40.       Leptospira interrogans       Icterohaemorrhagiae       Outer membrane lipoprotein       strain_JB197       Q04VM6         41.       Leptospira borgpetersenii       Hardjo-ovis       Lipoprotein       strain_JB197       Q04VM6         42.       Leptospira borgpetersenii       Juncharacterized protein       str_20010861       M6W4K3         44.	30	Leptospira noguchii			str 1993005606	S3HLI63
Deptospira noguchii       Autumnalis       Uncharacterized protein       str_ZUN142       M6U310         33.       Leptospira noguchii       Uncharacterized protein       str_ZUN142       M6U310         34.       Leptospira noguchii       Uncharacterized protein       str_ZUN142       M6U310         34.       Leptospira interrogans       Australis       Lipoprotein       Q33BM8         36.       Leptospira interrogans       Australis       Lipoprotein       Q33BM8         37.       Leptospira kirschneri       Uncharacterized protein       str_200801925       M6XK47         38.       Leptospira kirschneri       Uncharacterized protein       str_200802841       K6GUX5         39.       Leptospira interrogans       Grippotyphosa       Uncharacterized protein       str_200802841       K6GUX5         40.       Leptospira interrogans       Icterohaemorrhagiae       Outer membrane lipoprotein       str_200802841       K6GUX5         41.       Leptospira borgpetersenii       Hardjo-bovis       Lipoprotein       str_u_0931       S3URD2         42.       Leptospira borgpetersenii       Ballum       Major outer membrane protein       str_U_09931       S3URD2         43.       Leptospira borgpetersenii       Duncharacterized protein       str_U009818	31	L'entospira interrogans		Incharacterized protein	str. HAI1536	M6V6T1
Deptospira         Industrial         Uncharacterized protein         Str_2007001578         MR017           33.         Leptospira noguchii         Uncharacterized protein         str_2007001578         MR017           34.         Leptospira interrogans         Leterona         Q33BM9           35.         Leptospira interrogans         Icteronaemorrhagiae         Lipoprotein         Q33BM8           37.         Leptospira interrogans         Icteronaemorrhagiae         Uncharacterized protein         str_200801925         M6XK47           38.         Leptospira interrogans         Icteronaemorrhagiae         Uncharacterized protein         str_200803703         M6W9C5           39.         Leptospira interrogans         Icteronaemorrhagiae         Uncharacterized protein         str_200802841         K6GUX5           41.         Leptospira interrogans         Icteronaemorrhagiae         Outer membrane lipoprotein         strain_JB197         Q04VM6           42.         Leptospira borgpetersenii         Javanica         Uncharacterized protein         str_20100851         M6W4K3           44.         Leptospira borgpetersenii         Ballum         Major outer membrane protein         str_20100851         M6W4K3           45.         Leptospira borgpetersenii         Duncharacterized protein         str_2010	32	Leptospira menogans	Autumnalis		str. 71 IN142	MOVOTI
Captospira noguchii       Oncharacterized protein       Str_200601870       MRKY78         34.       Leptospira interrogans       Australis       Lipoprotein       Q33BM9         35.       Leptospira interrogans       Australis       Lipoprotein       Q33BM9         36.       Leptospira interrogans       Icterohaemorrhagiae       Lipoprotein       Q33BM8         37.       Leptospira kirschneri       Uncharacterized protein       str_200801925       M6K477         38.       Leptospira kirschneri       Uncharacterized protein       str_200803703       M6W9C5         39.       Leptospira interrogans       Gripotyphosa       IOncharacterized protein       str_200803703       M6W9C5         41.       Leptospira interrogans       Icterohaemorrhagiae       Uncharacterized protein       strain_JB197       Q04VM6         42.       Leptospira borgpetersenii       Javanica       Uncharacterized protein       str_200901868       M6W4K3         44.       Leptospira borgpetersenii       Pomona       Uncharacterized protein       str_201000851       N6XF19         45.       Leptospira borgpetersenii       Mini       Uncharacterized protein       str_200001868       M6W4K3         46.       Leptospira borgpetersenii       Mona       Uncharacterized protein	33	Leptospira noguchii			str 2007001578	M6I0 I7
St.       Leptospira interrogans       Australis       Lipportolin       Control       Castalis         36.       Leptospira interrogans       Icterohaemorrhagiae       Lipportolin       Castalis       Castalis         36.       Leptospira interrogans       Icterohaemorrhagiae       Lipportolin       Str_200801925       MKXK47         37.       Leptospira kirschneri       Uncharacterized protein       str_200803703       MKW9C5         39.       Leptospira kirschneri       Uncharacterized protein       str_200802841       K6GUX5         40.       Leptospira interrogans       Grippotyphosa       Incharacterized protein       str_200802841       K6GUX5         41.       Leptospira interrogans       Icterohaemorrhagiae       Outer membrane lipoprotein       strain_JB197       Q04VM6         43.       Leptospira borgpetersenii       Javanica       Uncharacterized protein       strain_JB197       Q04VM6         44.       Leptospira borgpetersenii       Ballum       Major outer membrane protein       str_200901868       MWK43         45.       Leptospira borgpetersenii       Mini       Uncharacterized protein       str_200901868       MWK43         46.       Leptospira borgpetersenii       Mini       Uncharacterized protein       str_200801910       K81509	34	Leptospira noguchii			str2006001870	K8KY78
One         Leptospira         Advance         Lipoprotein         Construction           36.         Leptospira interrogans         Icterohaemorrhagiae         Lipoprotein         Str_200801925         M6XK47           37.         Leptospira kirschneri         Uncharacterized protein         str_200803703         M6W9C5           39.         Leptospira kirschneri         Uncharacterized protein         str_200803703         M6W9C5           39.         Leptospira kirschneri         Uncharacterized protein         str_200802841         K6GUX5           41.         Leptospira interrogans         Icterohaemorrhagiae         Outer membrane lipoprotein         str_1U_09931         S3URD2           42.         Leptospira borgpetersenii         Javanica         Uncharacterized protein         str_1U_09931         S3URD2           43.         Leptospira borgpetersenii         Ballum         Major outer membrane protein         str_200901868         M6W4K3           44.         Leptospira borgpetersenii         Pomona         Uncharacterized protein         str_201000851         NSKFT9           47.         Leptospira borgpetersenii         Mini         Uncharacterized protein         str_200701203         M3HTU4           48.         Leptospira borgpetersenii         Uncharacterized protein         str_20080	35	L'entospira interrogans	Australis		302000001070	033BM9
OneDeproductionSecond Second37.Leptospira kirschneriUncharacterized proteinstr_200801925M6XK4738.Leptospira kirschneriUncharacterized proteinstr_200803703M6W9C539.Leptospira kirschneriUncharacterized proteinstr_200802841K6GUX540.Leptospira interrogansGrippotyphosaIDAYM441.Leptospira interrogansIcterohaemorrhagiaeOuter membrane lipoproteinC9EH9042.Leptospira borgpeterseniiJavanicaUncharacterized proteinstrU_09931S3URD243.Leptospira borgpeterseniiBallumMajor outer membrane proteinC6GXC545.Leptospira borgpeterseniiBallumMajor outer membrane proteinstr200901868M6W4K346.Leptospira borgpeterseniiMiniUncharacterized proteinstr201000851N6KFT947.Leptospira borgpeterseniiMiniUncharacterized proteinstr201000851N6KFT948.Leptospira borgpeterseniiUncharacterized proteinstr200701203M3HTU450.Leptospira borgpeterseniiCastellonisUncharacterized proteinstr200801926K6IQU551.Leptospira borgpeterseniiCastellonisUncharacterized proteinstr200801926K6IQU552.Leptospira borgpeterseniiUncharacterized proteinstr200801926K6IQU553.Leptospira borgpeterseniiUncharacterized proteinstrBrem_307M62BK554.Leptospira borgpetersenii <td>36</td> <td>L'entospira interrogans</td> <td>Icterohaemorrhagiae</td> <td></td> <td></td> <td>Q33BM8</td>	36	L'entospira interrogans	Icterohaemorrhagiae			Q33BM8
Site       Leptospira kirschneri       Uncharacterized protein       str200803703       M6W9C5         39.       Leptospira kirschneri       Uncharacterized protein       str200803703       M6W9C5         39.       Leptospira kirschneri       Uncharacterized protein       str200803703       M6W9C5         39.       Leptospira kirschneri       Uncharacterized protein       str200803703       M6W9C5         41.       Leptospira borgpetersenii       Hardjo-bovis       Lipoprotein       strain_JB197       Q04VM6         42.       Leptospira borgpetersenii       Javanica       Uncharacterized protein       strUl_09931       S3URD2         44.       Leptospira borgpetersenii       Ballum       Major outer membrane protein       str201000851       N6W4K3         46.       Leptospira borgpetersenii       Mini       Uncharacterized protein       str200011203       M6B0C6         47.       Leptospira borgpetersenii       Mini       Uncharacterized protein       strNoumea       M6S0C6         48.       Leptospira borgpetersenii       Uncharacterized protein       str200801910       K8150         49.       Leptospira borgpetersenii       Castellonis       Uncharacterized protein       str0L09149       K8HKM7         51.       Leptospira borgp	37	Leptospira kirschneri	leteronaemormagiae		str 200801925	M6XK47
Oct.Expression instructionStructure proteinStructure proteinStructure protein39.Leptospira interrogansGrippotyphosaIOArM441.Leptospira interrogansIcterohaemorrhagiaeOuter membrane lipoproteinIOAYM441.Leptospira interrogansIcterohaemorrhagiaeOuter membrane lipoproteinStructure operation42.Leptospira borgpeterseniiHardjo-bovisLipoproteinStructure operationSJURD243.Leptospira borgpeterseniiBallumMajor outer membrane proteinGGCXC545.Leptospira borgpeterseniiPomonaUncharacterized proteinstructure operationGGCXC546.Leptospira borgpeterseniiPomonaUncharacterized proteinstructure operationMGSOC647.Leptospira borgpeterseniiMiniUncharacterized proteinstructure operationMGSOC648.Leptospira borgpeterseniiCastellonisUncharacterized proteinstructure operationMHTV450.Leptospira borgpeterseniiCastellonisUncharacterized proteinstructure operationKGUU551.Leptospira borgpeterseniiCastellonisUncharacterized proteinstructure operationKGUU552.Leptospira borgpeterseniiCastellonisUncharacterized proteinstructure operationstructure operation53.Leptospira borgpeterseniiJavanicaUncharacterized proteinstructure operationstructure operation53.Leptospira borgpeterseniiJavanicaUncharacte	38	Leptospira kirschneri			str200803703	M6W9C5
ConstructionDistructionDistructionDistructionDistructionDistructionDistructionDistruction40.Leptospira interrogansIcterohaemorrhagiaeOuter membrane lipoproteinIDA/W441.Leptospira borgpeterseniiHardjo-bovisLipoproteinstrain_JB197Q04VM642.Leptospira borgpeterseniiJavanicaUncharacterized proteinstr_U_09931S3URD244.Leptospira borgpeterseniiBallumMajor outer membrane proteinstr_20091868M6W4K345.Leptospira borgpeterseniiPomonaUncharacterized proteinstr_20100851N6XFT947.Leptospira borgpeterseniiMiniUncharacterized proteinstr_20100851N6XFT948.Leptospira borgpeterseniiUncharacterized proteinstr_20100851N6EBK549.Leptospira borgpeterseniiCastellonisUncharacterized proteinstr_200701203M3HTU450.Leptospira borgpeterseniiCastellonisUncharacterized proteinstr_200801910K8I5J951.Leptospira borgpeterseniiQatoricaUncharacterized proteinstr_200801910K8I5J952.Leptospira borgpeterseniiCastellonisUncharacterized proteinstr_200801910K8I5J953.Leptospira borgpeterseniiJavanicaUncharacterized proteinstr_200801926K6IQU554.Leptospira borgpeterseniiJavanicaUncharacterized proteinstr_MK146M6MSW754.Leptospira borgpeterseniiJavanicaMajor	39	Leptospira kirschneri		Uncharacterized protein	str200802841	K6GUX5
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52.Leptospira borgpeterseniiJavanicaUncharacterized proteinstr200801926K6IQU553.Leptospira borgpeterseniiJavanicaUncharacterized proteinstrMK146M6MSW754.Leptospira borgpeterseniiUncharacterized proteinstrBrem_328M6J8U855.Leptospira borgpeterseniiUncharacterized proteinstrBrem_307M6J29656.Leptospira borgpeterseniiJavanicaMajor outer membrane proteing6GXC757.Leptospira borgpeterseniiTarassoviMajor outer membrane proteing6GXB758.Leptospira borgpeterseniiHardjo-bovisLipoproteinstrin_L550Q04XU759.Leptospira borgpeterseniiHardjo-bovisUncharacterized proteinstrSponseleeM6BLU360.Leptospira santarosaiShermaniUncharacterized proteinstrSonseleeM6BLU3	51	Leptospira borgpetersenii		Uncharacterized protein	str UI 09149	K8HKM7
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55.Leptospira borgpeterseniiJavanicaUncharacterized proteinstrBrem_307M6000056.Leptospira borgpeterseniiJavanicaMajor outer membrane proteinQ6GXC757.Leptospira borgpeterseniiTarassoviMajor outer membrane proteinQ6GXB758.Leptospira borgpeterseniiHardjo-bovisLipoproteinstrain_L550Q04XU759.Leptospira borgpeterseniiHardjo-bovisUncharacterized proteinstrSponseleeM6BLU360.Leptospira santarosaiShermaniUncharacterized proteinstr. 1342KTS3WCD7	54	Leptospira borgpetersenii		Uncharacterized protein	str Brem 328	M6.18U8
Sc.Leptospira borgpeterseniiJavanicaMajor outer membrane proteinStDrefit_SoftMod25057.Leptospira borgpeterseniiTarassoviMajor outer membrane proteinQ6GXC758.Leptospira borgpeterseniiHardjo-bovisLipoproteinstrain_L550Q04XU759.Leptospira borgpeterseniiHardjo-bovisUncharacterized proteinstrSponseleeM6BLU360.Leptospira santarosaiShermaniUncharacterized proteinstr. 1342KTS3WCD7	55	Lentospira borgpetersenii		Uncharacterized protein	str Brem 307	M6.1296
Solution     Solution     Solution     Solution     Solution     Solution     Solution     Solution     Solution       57.     Leptospira borgpetersenii     Tarassovi     Major outer membrane protein     Q6CXB7       58.     Leptospira borgpetersenii     Hardjo-bovis     Lipoprotein     strain_L550     Q04XU7       59.     Leptospira borgpetersenii     Hardjo-bovis     Uncharacterized protein     strSponselee     M6BLU3       60.     Leptospira santarosai     Shermani     Uncharacterized protein     str. 1342KT     S3WCD7	56	Lentospira borgpetersenii	Javanica	Major outer membrane protein		066X07
St.     Leptospira borgpetersenii     Hardjo-bovis     Lipoprotein     strain_L550     Q04XU7       59.     Leptospira borgpetersenii     Hardjo-bovis     Uncharacterized protein     strSponselee     M6BLU3       60.     Leptospira santarosai     Shermani     Uncharacterized protein     str. 1342KT     S3WCD7	57	Leptospira borgpetersenii	Tarassovi	Major outer membrane protein		06GXB7
Solution     Solution     Solution     Solution     Solution     Solution       59.     Leptospira borgpetersenii     Hardjo-bovis     Uncharacterized protein     strSponselee     M6BLU3       60.     Leptospira santarosai     Shermani     Uncharacterized protein     str. 1342KT     S3WCD7	58	Lentospira borgpetersenii	Hardio-bovis	l inoprotein	strain 1.550	004XU7
Construction         Francesco         Construction         Stat_oponecicie         Modeloo           60.         Leptospira santarosai         Shermani         Uncharacterized protein         str 1342KT         S3WCD7	59	Lentospira borgpetersenii	Hardio-bovis	Lincharacterized protein	str Sponselee	M6BLU3
	60	Leptospira santarosai	Shermani	Uncharacterized protein	str 1342KT	S3WCD7

61	Lentospira santarosai		Uncharacterized protein	etr AIM	
01. 62					MEV/0D7
02.		Ohannaai		FIDUIUZ LV4133	
63.	Leptospira santarosai	Shermani	Lipoprotein	strL1_821	K8Y7P9
64.	Leptospira santarosai		Uncharacterized protein	strJET	K8MNX0
65.	Leptospira sp		Uncharacterized protein	Fiocruz_LV3954	K6HER9
66.	Leptospira borgpetersenii	Mini	Uncharacterized protein	str200901116	M6UV01
67.	Leptospira santarosai		Uncharacterized protein	str2000027870	M6GU93
68.	Leptospira borgpetersenii		Uncharacterized protein	str200901122	K8LWU9
69.	Leptospira santarosai	Arenal	Uncharacterized protein	strMAVJ_401	M6JWP5
70.	Leptospira santarosai		Uncharacterized protein	strMOR084	K6FVF0
71.	Leptospira santarosai		Uncharacterized protein	str200403458	M6X740
72.	Leptospira interrogans	Hebdomadis	Uncharacterized protein	strR499	K8JJ55
73.	Leptospira weilii		Uncharacterized protein	strUI_13098	M6Q9M2
74.	Leptospira sp		Uncharacterized protein	P2653	M6A0W1
75.	Leptospira kirschneri		Uncharacterized protein	strH2	K6GP23
76.	Leptospira weilii		Uncharacterized protein	Topaz_strLT2116	M3GXL7
77.	Leptospira borgpetersenii	Mini	Major outer membrane protein		Q6GXB4
78.	Leptospira alstoni	Pingchang	Uncharacterized protein	str80-412	T0FYD6
79.	Leptospira alstoni	Sichuan	Uncharacterized protein	str79601	M6CS57
80.	Leptospira weilii		Uncharacterized protein	strLNT_1234	M6LQ40
81.	Leptospira interrogans	Paidjan	Outer_membrane_lipoprotein_LipL41_(Fragment)		Q5MJS6
82.	Leptospira weilii	Ecochallenge	Uncharacterized protein		N1U2R2
83.	Leptospira interrogans	Pomona	Outer_membrane_lipoprotein_LipL41_(Fragment)		Q5MJS8
84.	Leptospira interrogans	Bataviae	Uncharacterized protein	strHAI135	M6TFS7
85.	Leptospira weilii		Uncharacterized protein	str2006001855	M6FKQ9
86.	Leptospira interrogans	Pomona	Uncharacterized protein	strCSL4002	M5ZV00
87.	Leptospira interrogans	Valbuzzi	Uncharacterized protein	strDuyster	M5ZDN6

Table 1: List of LipL41 sequences of Leptospira species used for phylogenetic analysis in figure 1.

	Organism	Serovar	Serogroup/Name/ uncharacterized protein	Strain	Uniprot ID
1.	Leptospira interrogans	Hebdomadis			Q33BN0
2.	Leptospira interrogans	Manilae			Q33BM7
3.	Leptospira interrogans	Autumnalis			Q33BN1
4.	Leptospira interrogans	Copenhageni	Icterohaemorrhagiae	Fiocruz L1-130	Q72N71
5.	Leptospira interrogans	Lai	Icterohaemorrhagiae	56601	Q8F8E1
6.	Leptospira interrogans	Australis	Major outer membrane protein		Q6GXC2
7.	Leptospira interrogans	Wolffi	Major outer membrane protein		Q6GXB5
8.	Leptospira interrogans	Canicola	Major outer membrane protein		Q6GXC6
9.	Leptospira_kirschneri	Bulgarica		Nikolaevo	M6F5M3
10.	Leptospira_kirschneri			H1	K6FCL0
11.	Leptospira interrogans	Lai	Major outer membrane protein		Q6GXC8
12.	Leptospira interrogans	Autumnalis	Major outer membrane protein		Q6GXC3
13.	Leptospira_weilii	Manhao	Major outer membrane protein	-	Q6GXB6
14.	Leptospira interrogans	Australis	Lipoprotein		Q33BM9
15.	Leptospira interrogans	Icterohaemorrhagiae	Lipoprotein		Q33BM8
16.	Leptospira_interrogans		Uncharacterized protein	strL1207	M6M715
17.	Leptospira_interrogans	Icterohaemorrhagiae	Outer membrane lipoprotein		C9EH90
18.	Leptospira_interrogans	Icterohaemorrhagiae	Uncharacterized protein	Verdun	M6R807
19.	Leptospira_interrogans	Hebdomadis	Uncharacterized protein	strR499	K8JJ55
20.	Leptospira_kirschneri		Uncharacterized protein	strH2	K6GP23

Table 2: List of LipL41 sequences of Leptospira species used for phylogenetic analysis in figure 2.

significant identity were aligned with ClustalW algorithm implemented in Molecular Evolutionary Genetic Analysis (MEGA 5.2.2) (http:// www.megasoftware.net) by using distance matrix and then it was trimmed to consensus. Neighbour Joining (NJ) trees were constructed with 1000 bootstraps at uniform divergence rates with distance 'p' as the evolutionary model and with a data subset to use with gaps/ missing data treatment as complete deletion [20]. Posterior probability and conserved regions among the closely related sequences were done with MEGA 5.2.2.

### Modelling, energy minimization and validation of the model

The amino acid sequence of LipL41 of *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Lai (strain 56601) was retrieved from UniProt KB (Uniprot Id: Q8F8E1). The sequence was submitted into the RaptorX server [21] (http://raptorx.uchicago.edu/) to derive the 3-dimensional structure. The modelled protein structures were viewed in Swiss-PdbViewer (http://www.expasy.org/spdbv/) and the individual residues were collected from 100 cycles of steepest

descent algorithm carried out in GROMOS96 [22] until the side chain interactions in the vicinity is readjusted and brings up lower potential energy and becomes more stable. Energy minimized models were assessed by PROCHECK [23] to analyse the stereo chemical quality and residual geometry of the model by submitting the co-ordinate file in Structural Analysis and Verification Server (SAVES) (http:// nihserver.mbi.ucla.edu/SAVES/). The value of the predicted LipL41 model was analyzed by using PYMOL [24,25].

### Computational mapping of epitopes

Linear and discontinuous B-Cell epitopes of LipL41 were mapped from the generated three dimensional structure of LipL41. Linear B-cell epitopes were chosen with two different algorithms: ABCPred and BepiPred. ABCPred uses a recurrent neural network to predict B-cell epitopes (http://www.imtech.res.in/raghava/abcpred/) [26,27]. The amino acid length of 16 and the scoring threshold of 0.8 were set to predict B-cell epitopes in ABCPred. The epitope prediction in BepiPred is based on hidden Markov model (http://www.cbs.dtu.dk/services/ BepiPred/) and propensity scale method [28,29]. The value, 0.35 was set as the threshold value, because at this value, the sensitivity/ specificity of predictions are maximized in BepiPred. BepiPred analyzes each amino acid independently and does not have a minimum or maximum number of amino acids to predict an epitope.

Discontinuous epitopes were predicted using Ellipro which is an Antibody Epitope Prediction server (http://tools.immuneepitope.org/ tools/ElliPro/iedbinput) [30,31]. Ellipro, with the best algorithm to predict discontinuous epitopes from 3-D structures when compared to six other software programs that predict discontinuous epitopes [30]. The default threshold value was set at 0.8. The predicted epitopes were additionally verified in VaxiJen server to predict the probability of an antigen (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen. html/) [32], with a threshold of 0.4. VaxiJen uses an alignment free approach for antigen prediction and works on an auto cross covariance (ACC) transformation of protein sequences into uniform vectors of principal amino acid properties. Sixteen sequences of LipL41 were taken randomly in T-coffee programme to identify the conserved amino acids residues.

# **Results and Discussion**

# Phylogenetic analysis for genetic relatedness

The phylogenetic tree was performed by using 87 sequences of LipL41 from various serovars and strains which were retrieved from UniProt Knowledgebase (Uniprot KB) (Table 1). The phylogenetic tree of LipL41 is evidenced that the isolates are clustered with different serovars and strains (Figure 1) and diverged to form different branches in the phylogenetic tree. The following mutations are observed for Borgpetersenii strains: 33S->T, 39M->I, 40F->Y, 125A->I, 126I->L, 130S->T, 139N->S, 191D->E, 336T->V; Weilii strains: 33S->A, 247I->V; Fiocruz strains: 80A->P, 177L->I, 183A->V, 186M->A, 197E->D; Santorasai strains: 125A->I, 126I->L, 130S->T, 139N->S, 336T->I; Krishneri strains: 176I->V, 336T->A; Noguchi strains: 269I->M, 274R->K, 336T->A (data not shown). The closest neighbouring clusters include strains of L. weilli, L. kirschneri and L. interrogans with 100% bootstrap confidence values. Based on the phylogenetic analysis, the cluster of LipL41 of L. borgpetersenii together forms a clade showing the evolutionary relationship of same serovars and strains with the highest bootstrap value of 98 which indicates that it has uniform support. The reliability of a branch length in MEGA 5 is based on confidence probability (CP). The branch length is high when the CP is high, thus

the branch length is considered to be statistically significant. MEGA 5 inferred the evolutionary tree by a Neighbour-Joining (NJ) algorithm by using a matrix of pairwise distances. In order to resolve the relationships of the sequences within each group of the constructed phylogenetic tree (Figure 1), a separate phylogenetic tree was constructed with 20 sequences from different serovars (Table 2) (Figure 2). It shows the highest bootstrap value of 99, indicate that the clade is close to 100%, which reveals that all the characters in a group believed to comprise all the evolutionary descendants of a common ancestor which is rooted with different serovars and strains as the ancestral group. In order to resolve the polytomies and to make the evolutionary relationship into dichotomies, 8 sequences of different serovars and strains (Table 3) (Figure 3) was used to make a separate branch of tree reflected Polytomies with Local bootstrap probability (LBP) values below 50% in L. interrogans Icterohaemorrhagiae serovar Lai and Copenhageni serovar (Figure 3). The phylogenetic tree evidenced that L. interrogans Icterohaemorrhagiae serovar Lai and Copenhageni serovar were closely clustered with other different serovars. The neighbouring groups also include serovars of L. autumnalis and L. hebdomadis which clustered with different bootstrap confidence values. Even though LipL41 is highly analogous protein present in all pathogenic Leptospira but the phylogenetic pattern of the present study exhibited the clonality of the sequences of the serovar Lai and Copenhageni used to analyze species separation. The evolutionary relationship was confirmed among the 87 sequences and on further confirmation with serovar Lai can be used for serodiagnosis of pathogenic leptospiral species.

# Structure prediction of LipL41 by threading method

Protein threading method is a fold recognition method of protein modelling which is based on the predicted structure properties, such as predicted secondary structures and predicted residue burial status [21]. Threading based prediction for LipL41 was done in RaptorX server which uses a non-linear scoring function to combine homologous information with structural information for the given templatesequence alignment [21] . The amino acid sequence of LipL41 of L. interrogans Icterohaemorrhagiae serovar Lai (Q8F8E1) was taken for modelling by protein threading. Given an input sequence, RaptorX predicted its tertiary structure as well as solvent accessibility and disordered regions (Figure 4A). The RaptorX assigned the confidence score which is based on P-value and uGDT (unnormalized Global Distance Test). P-value measures the relative quality and uGDT measures the absolute quality of protein model. uGDT has greater value of 50 is a indicator for a good model. The input of 355 amino acid residues of LipL41 were completely modelled with 100% and showed 2 domains (Figure 4A). In the model, 7 positions among 355 residues were predicted as disordered regions which are 1%. The model shows P-value with 3.52 e-03 and uGDT (GDT) with 137(38). The modelled LipL41 has two domains (Figure 4A); Domain 1 (254 to 355) showed p value of 5.32 e-3 and Domain 2 (1 to 253) showed p value 3.52 e-3.

LipL41 is a haem binding protein with Cys-Ser (CS) and Cys-Pro (CP) domains [9] (Figure 4B). The CS and CP are conserved domains of pathogenic *Leptospira* which are responsible for immunoprotection [9]. Mutation of these domains fails to cause immunoprotection in mice [12]. The motifs Cys-Pro or Cys-Ser has been determined in diverse proteins binding to heme (Fe2+)/hemin (Fe3+) [33,34]. It has been reported that the cysteine containing dipeptide: CS or CP is necessary for heme binding in HRM [35-37]. These conserved residues are found in LipL41 at 140 Cys-Ser and 220 Cys-Pro are located on the surface of the predicted structure (Figure 4B), and the thiol of cysteine may be a ligand for iron on heme [36,37].





Figure 2: A phylogram of 20 selected sequences of LipL41. The bootstrap values calculate the frequency for each taxon bipartition during replication and boot strapping denotes measures how consistently the data support given taxon bipartitions. The scale bar also represents branch length (number of amino acid substitutions/100 residues). The high bootstrap value 99 shows the uniform support and bootstrap values close to 100% which indicate that the clade is a group which means that all the characters in a group believed to comprise all the evolutionary descendants of a common ancestor which is rooted with different servoras and strains as the ancestral group.



Figure 3: A phylogram of 8 selected sequences of LipL 41. Polytomies reflect nodes with LBP values below 50% for Lai and Copenhageni serovar of *L. interrogans* Icterohaemorrhagiae. This tree resolves the relationship among different LipL 41 groups and the sequences within each group and serovars.

	Organism	Serovar	Serogroup/Name/ uncharacterized protein	Strain	Uniprot ID
1.	Leptospira interrogans	Wolffi	Major outer membrane protein		Q6GXB5
2	Leptospira interrogans	Canicola	Major outer membrane protein		Q6GXC6
3.	Leptospira interrogans	Australis	Major outer membrane protein		Q6GXC2
4.	Leptospira interrogans	Lai	Icterohaemorrhagiae	56601	Q8F8E1
5.	Leptospira interrogans	Copenhageni	Icterohaemorrhagiae	Fiocruz L1-130	Q72N71
6.	Leptospira interrogans	Autumnalis			Q33BN1
7.	Leptospira interrogans	Hebdomadis			Q33BN0
8.	Leptospira interrogans	Manilae			Q33BM7

**Table 3:** List of LipL41 sequences of Leptospira interrogans used for phylogenetic analysis in figure 3.

No.	Start Position	End Position	Peptide	Number of Residues	Score
1	22	42	ATVDVEYPVFPKDKEGRALQK	21	0.758
2	68	97	EGSSFIDQMPSKVFEAFDKESYYKLTDLSK	30	0.821
3	132	150	GYQKPYTECSTENKIDAVA	19	0.726
4	167	182	DVNTGNEPVSKPTGVR	16	0.608
5	185	189	LIPLD	5	0.521
6	195	213	VETGEVKKAVVSSPAKIFN	19	0.606
7	267	284	QEGYEEIVGETPSFKKAK	18	0.712
8	303	355	${\sf ANLATYYFSTGDFEKSIKLYEEAMKLKDADKSYLRELRKRVEATFAVDESNAK}$	53	0.774

 Table 4: List of epitopes predicted based on amino acid sequence and structure.

### Validation and evaluation of LipL41 model

The NEFF (Number of Effective sequence homologs) score which was ranging from 1 to 20 for the predicted structure was estimated by PROCHECK. The results obtained from PROCHECK [23,38] was evaluated for protein backbone conformations by Ramachandran Plot [39,40]. The phi-psi torsion angle for 92.7% of residues of LipL41 are in the most favourable region (A, B and L); 6.6%, 0.3% and 0.2% in additionally allowed, generously allowed and disallowed regions, respectively (a, b, l, p), indicate that LipL41 model is stereo chemically good (Figure 5) and the model derived from RaptorX was of higher quality in terms of protein folding.



Figure 4: A: Three dimensional structure of LipL41 of *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Lai (strain 56601 (Uniprot Id: Q8F8E1). B: LipL41 model showing haem binding regions (CS and CP) marked in red.



Figure 5: Ramachandran plot of the LipL41 model. The most favoured regions are colored red, additional allowed, generously allowed and disallowed regions are indicated as yellow, light yellow and white fields, respectively.

# Prediction and immunoinformatic analysis of antigenic peptides

Two different epitope prediction software programs (ABCPred and BepiPred) were utilized to predict the most immunogenic linear B-cell epitopes on the surface of the leptospiral OMP LipL41. ABCPred and BepiPred predicted 9 different overlapping and potentially immunogenic regions within LipL41, respectively (data not shown). ABCPred is able to predict epitopes with approximately 66% accuracy using the recurrent neural network [26]. ABCPred assigns scores between 0 and 1 for each epitope it predicts. If prediction shows score closer to 1, the particular prediction can be taken as epitope and prediction closer to 0 is not suitable for an epitope. Eight B- cell discontinuous epitopes of LipL41 were mapped from the predicted 3-D structure of LipL41 by using Ellipro (Figure 6). These epitopes spans (EP1: 22-42), (EP2: 68-97), (EP3: 132-150), (EP4: 167-182), (EP5: 185-189), (EP6: 195-213), (EP7: 267-284) and (EP8: 303-355) positions of LipL41 (Table 4). Ellipro predicts epitope with Protrusion Index (PI) value which is percentage of the protein atoms enclosed in the ellipsoid, at which the residue first becomes lying outside the ellipsoid; whereas all the residues which were lying 90% outside the ellipsoid had the PI value 9, i.e., 0.9 in Ellipro. This gives information of amino acids lying outside the ellipsoid.

The prediction of peptides is vital not only for diagnostics but also for vaccines. It became clear that the small segments of protein called the antigenic determinants or the epitopes are sufficient for eliciting the desired immune response. Based on the threshold value, all the predicted epitopes are antigenic nature. All these epitopes predicted can be used for the development of Monoclonal antibody or epitope based diagnostic kit for the Leptospirosis.

# Conservancy of LipL41 epitopes

Universal epitope vaccine development requires conserved amino acids of a protein among the various pathogenic strains [41]. Thus 16 different strains and serovars of *Leptospira* were taken randomly. Multiple sequence alignment of LipL41 for 16 different strains and





serovars of Leptospira interrogans: Leptospira interrogans serovar Wolffi (Q6GXB5), Leptospira weilii serovar Manhao II (Q6GXB6), Leptospira interrogans serovar Canicola (Q6GXC6), Leptospira interrogans serovar Australis (Q6GXC2), Leptospira interrogans serovar Autumnalis (Q6GXC3), Leptospira kirschneri serovar Bulgarica str. Nikolaevo (M6F5M3), Leptospira interrogans str.L1207(M6M715), Leptospira interrogans serogroup Icterohaemorrhagiae serovar Lai (strain\_56601) (Q8F8E1), Leptospira interrogans serogroup Icterohaemorrhagiae serovar copenhageni (strain\_Fiocruz\_L1-130) (Q72N71), Leptospira interrogans serovar Autumnalis (Q33BN1), Leptospira interrogans serovar Hebdomadis (Q33BN0), Leptospira interrogans serovar Manilae (Q33BM7), Leptospira interrogans serovar Australis (Q33BM9), Leptospira interrogans serovar Icterohaemorrhagiae (Q33BM8), Leptospira kirschneri str. (K6FCL0), Leptospira interrogans serovar Icterohaemorrhagiae (C9EH90) were analyzed by using T-coffee program (http://www.tcoffee.org/) [42] (Figure 7) and found that all these epitopes are conserved among all these strains and serovars.

# Prediction of transmembrane domain, signal sequence and topology of LipL41

In order to ensure that the epitopic region should not overlap with signal peptide or transmembrane domain of LipL41, amino acid sequence of LipL41 of *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Lai (strain\_56601) was analyzed by TMHMM 2.0 server (http://www.cbs.dtu.dk/services/TMHMM/) [43]. It was found that amino acids from 1-6, 7-29, and 30-355 are located inside the plasma membrane, inside the transmembrane (TM) helix, and outside the plasma membrane of the cell, respectively. A combined trans-membrane topology and signal peptides were predicted using Phobius online server (http://phobius.sbc.su.se/) [44]. Based on the probability of occurrences of specific amino acids, the sequence from 1 to 22 was predicted to be a signal peptide and between 23 and 355 is non-cytoplasmic region of LipL41. This analysis confirms that all the eight epitopes are topologically surface exposed and do not have any signal sequences in them.

#### Conclusion

In this present study, we have characterized LipL41 for its genetic diversity among the *Leptospira* species. The phylogenetic relationship of *Leptospira* with LipL41 from 87 sequences of different serovars and serogroup have shown that the comprising lineages with largely varying rates of evolution. The alignment also has shown the presence of haem binding motifs are conserved in all the LipL41. The three dimensional structure of LipL41 was predicted by using RAPTOR X and was validated by Ramachandran plot. Eight B-cell epitopes were predicted from LipL41. Antibody developed against these conserved epitopic regions could be used to develop a detection kit or as a vaccine candidate for Leptospirosis.

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#### References

- Levett PN, Branch SL, Whittington CU, Edwards CN, Paxton H (2001) Two methods for rapid serological diagnosis of acute leptospirosis. Clin Diagn Lab Immunol 8: 349-351.
- 2. Faine S, Adler B, Bolin C, Perolat P (1999) Leptospira and leptospirosis. (2nd Edn0 MediSci, Australia.
- 3. Gouveia EL, Metcalfe J, de Carvalho AL, Aires TS, Villasboas-Bisneto JC, et

al. (2008) Leptospirosis-associated severe pulmonary hemorrhagic syndrome, Salvador, Brazil. Emerg Infect Dis 14: 505-508.

- McBride AJ, Athanazio DA, Reis MG, Ko AI (2005) Leptospirosis. Curr Opin Infect Dis 18: 376-386.
- Segura ER, Ganoza CA, Campos K, Ricaldi JN, Torres S, et al. (2005) Clinical spectrum of pulmonary involvement in leptospirosis in a region of endemicity, with quantification of leptospiral burden. Clin Infect Dis 40: 343-351.
- Koteeswaran AS (2006) Seroprevalence of leptospirosis in man and animals analysis of the gene encoding LipL32 of Leptospira in Tamil Nadu. Indian J Med Microbiol 24: 329-331.
- Balakrishnan G, Govindarajan R, Meenambigai TV, Jayakumar V, Manohar MB (2008) Diagnosis of leptospirosis by recombinant antigen: Seroprevalence of animal based single serum dilution ELISA. Indian Vet J 85: 227-228
- Abhinay G, Joseph S, Ambily R (2012) Sero prevalence of canine leptospirosis. Indian Vet J 89: 72-73.
- Lin MH, Chang YC2, Hsiao CD3, Huang SH, Wang MS, et al. (2013) LipL4, a hemin binding protein from Leptospira santarosai serovar Shermani. PLoS One 8: e83246.
- Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, et al. (2003) Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis 3: 757-771.
- 11. Adler B, de la Peña Moctezuma A (2010) Leptospira and leptospirosis. Vet Microbiol 140: 287-296.
- Haake DA, Mazel MK, McCoy AM, Milward F, Chao G, et al. (1999) Leptospiral outer membrane proteins OmpL1 and LipL41 exhibit synergistic immunoprotection. Infect Immun 67: 6572-6582.
- Shang ES, Summers TA, Haake DA (1996) Molecular cloning and sequence analysis of the gene encoding LipL4, a surface-exposed lipoprotein of pathogenic Leptospira species. Infect Immun 64: 2322-2330.
- Haake DA, Chao G, Zuerner RL, Barnett JK, Barnett D, et al. (2000) The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. Infect Immun 68: 2276-2285.
- Cullen PA, Xu X, Matsunaga J, Sanchez Y, Ko AI, et al. (2005) Surfaceome of Leptospira spp. Infect Immun 73: 4853-4863.
- Matsunaga J, Barocchi MA, Croda J, Young TA, Sanchez Y, et al. (2003) Pathogenic Leptospira species express surface-exposed proteins belonging to the bacterial immunoglobulin superfamily. Mol Microbiol 49: 929-945.
- Verma A, Brissette CA, Bowman AA, Shah ST, Zipfel PF, et al. (2010) Leptospiral endostatin-like protein A is a bacterial cell surface receptor for human plasminogen. Infect Immun 78: 2053-2059.
- Guerreiro H, Croda J, Flannery B, Mazel M, Matsunaga J, et al. (2001) Leptospiral proteins recognized during the humoral immune response to leptospirosis in humans. Infect Immun 69: 4958-4968.
- Monahan AM, Callanan JJ, Nally JE (2008) Proteomic analysis of Leptospira interrogans shed in urine of chronically infected hosts. Infect Immun 76: 4952-4958.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol 28: 2731-2739.
- Källberg M, Wang H, Wang S, Peng J, Wang Z, et al. (2012) Template-based protein structure modeling using the RaptorX web server. Nat Protoc 7: 1511-1522.
- Bonvin AMJJ, Mark AE, Van Gunsteren WF (2000) The GROMOS96 benchmarks for molecular simulation. Comput Phys Commun 128: 550-557.
- Unit M, Street G (1993) PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallogr 26: 283-291.
- 24. Schrodinger LLC (2010) The PyMOL Molecular graphics system, version~1.3r1.
- 25. DeLano WL (2002) The PyMOL Molecular graphics system.
- Pirovano W, Heringa J (2010) Protein secondary structure prediction. Methods Mol Biol 609: 327-348.

- 27. Saha S, Raghava GP (2007) Prediction of neurotoxins based on their function and source. In Silico Biol 7: 369-387.
- 28. Larsen JE, Lund O, Nielsen M (2006) Improved method for predicting linear B-cell epitopes. Immunome Res 2: 2.
- 29. Kringelum JV, Lundegaard C, Lund O, Nielsen M (2012) Reliable B cell epitope predictions: impacts of method development and improved benchmarking. PLoS Comput Biol 8: e1002829.
- 30. Ponomarenko J, Bui HH, Li W, Fusseder N, Bourne PE, et al. (2008) ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinformatics 9: 514.
- Ponomarenko JV, Regenmortel Marc HV (2009) B-cell epitope prediction. Struct Bioinfo 35: 849-881.
- Doytchinova IA, Flower DR (2007) VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics 8: 4.
- Lathrop JT, Timko MP (1993) Regulation by heme of mitochondrial protein transport through a conserved amino acid motif. Science 259: 522-525.
- 34. Zhang L, Guarente L (1995) Heme binds to a short sequence that serves a regulatory function in diverse proteins. EMBO J 14: 313-320.
- Huang TJ, McCoubrey WK Jr, Maines MD (2001) Heme oxygenase-2 interaction with metalloporphyrins: function of heme regulatory motifs. Antioxid Redox Signal 3: 685-696.
- 36. Yang F, Xia X, Lei HY, Wang ED (2010) Hemin binds to human cytoplasmic

arginyl-tRNA synthetase and inhibits its catalytic activity. J Biol Chem 285: 39437-39446.

- Westberg JA, Jiang J, Andersson LC (2011) Stanniocalcin 1 binds hemin through a partially conserved heme regulatory motif. Biochem Biophys Res Commun 409: 266-269.
- Laskoswki RA, MacArthur MW, Moss DS, Thorton JM (1993) PROCHECK: A program to check the stereochemical quality of protein structures. J Appl Crystallogr 26: 283-291.
- Ramachandran GN, Ramakrishnan C, Sasisekharan V (1963) Stereochemistry of polypeptide chain configurations. J Mol Biol 7: 95-99.
- Ramachandran GN, Sasisekharan V (1968) Conformation of polypeptides and proteins. Adv Protein Chem 23: 283-438.
- Sirskyj D, Diaz-Mitoma F, Golshani A, Kumar A, Azizi A (2011) Innovative bioinformatic approaches for developing peptide-based vaccines against hypervariable viruses. Immunol Cell Biol 89: 81-89.
- 42. Notredame C, Higgins DG, Heringa J (2000) T-Coffee: A novel method for fast and accurate multiple sequence alignment. J Mol Biol 302: 205-217.
- 43. Krogh A, Larsson B, von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305: 567-580.
- Käll L, Krogh A, Sonnhammer EL (2004) A combined transmembrane topology and signal peptide prediction method. J Mol Biol 338: 1027-1036.