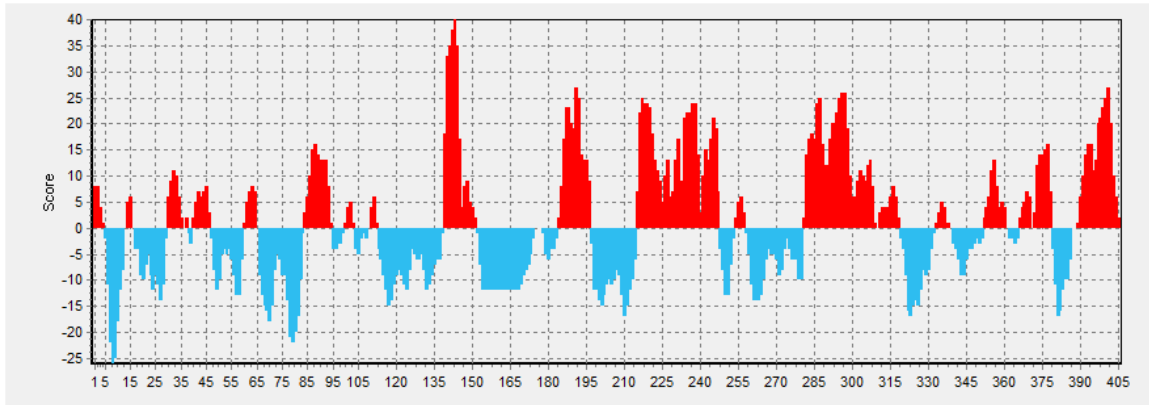


Predicted Immunodominant Epitopes

Whole Sequence overview:



Positions and relative "weight" of Epitopes

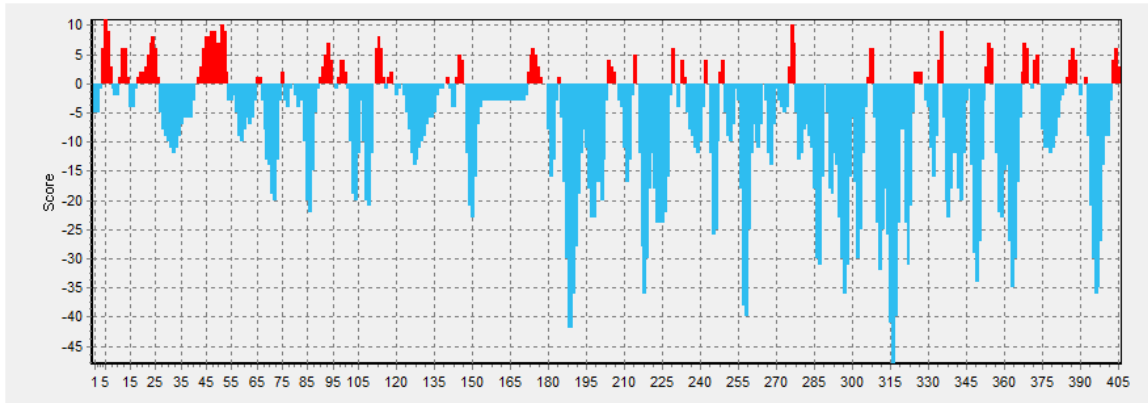
Host B7.1
 Frame 6
 Gap 0
 Treshold 0
 Peptide size 8 and above

Start	End	Length	Sequence	AGI	APB
139	151	13	AALASQFFKSGNN	248	19
184	196	13	FMNSNNNNQQGQN	213	16
215	247	33	MHSNNNQNSNNSQQGYNQS YQNGNQNSQGYNNQ	497	15
281	318	38	GQTQSNQQQYNQQGQNNQQQYQQQGQNYQHQQQQGQQQQ	464	12
387	405	19	FNFSGNFSQQNNGNQNR Y	241	12
84	94	11	KGSSQTQLGKL	112	10
366	378	13	SNEYGRPQYGGNQ	105	8
30	37	8	AQSNPNDE	46	5
352	361	10	ANEYGRPQQN	57	5

With respect to immunogenicity, there are not excellent immunodominant sequences (ABP>20). Another problem is presence of repeats or low complexity sequences in parts that may be immunogenic.

Analysis of Immunologic Complexity

Whole Sequence overview:



Positions of sequences with sufficient complexity

Host C1.3
 Frame 3
 Gap 1
 Treshold 0
 Peptide size 7 and above

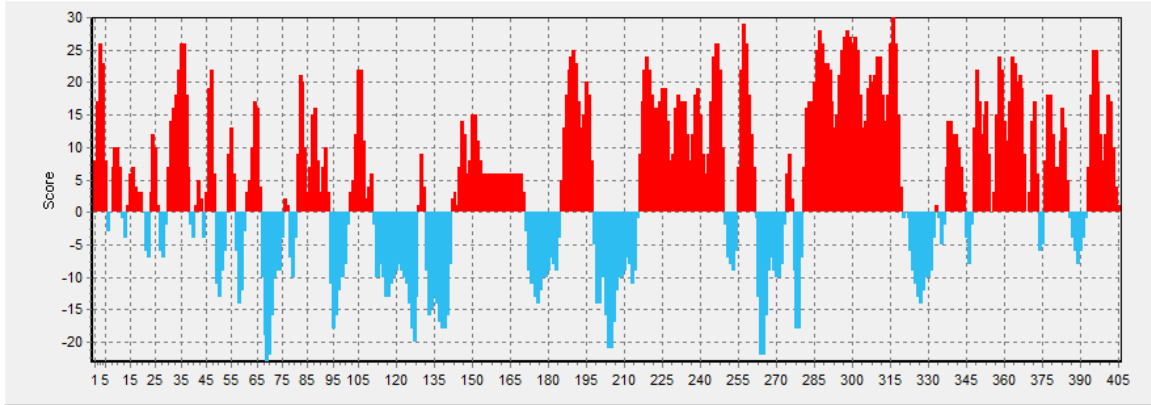
Start	End	Length	Sequence	AGI	APB
18	26	9	NHAEAVAKL	35	3
41	53	13	TIESLIQKIAGYV	79	6
90	100	11	QLGKLALLATV	30	2
111	119	9	NRGFDVGTV	23	2
172	179	8	GSFTALAS	22	2
367	374	8	NEYGRPQY	23	2
385	392	8	ESFNFSGN	14	1

Our previous conclusion is confined by complexity analysis. Therefore we have to aim epitopes that have highest complexity level.

This is clearly epitope 366-378 SNEYGRPQYGGNQ which overlaps with sequce of good complexity 367-374.

Predicted probability of surface exposure

Whole Sequence overview:



Positions of best sequences

Host EM3.2
 Frame 3
 Gap 1
 Treshold 0
 Peptide size 7 and above

Start	End	Length	Sequence	AGI	APB
1	11	11	MDTDKLISEAE	113	10
14	20	7	FSQGNHA	24	3
30	38	9	AQSNPNDEQ	154	17
41	48	8	TIESLIQK	54	6
81	93	13	ADSKGSSQTQLGK	132	10
102	110	9	THSSNKGSS	87	9
142	170	29	ASQFFKSGNNSQGQGQGQGQGQGQGQGQG	201	6
185	197	13	MNSNNNNQQGQNQ	221	17
216	249	34	HSNNNQNSNNSQQGYNQSYQNGNQNSQGYNNQQY	547	16
255	261	7	GYQQQQG	121	17
281	321	41	GQTQSNQQQYNQQGQNNQQQYQQQGQNYQHQQQGQQQQQGH	799	19
337	344	8	GNNSNSNS	79	9
348	373	26	GQQQANEYGRPQQNGQQQSNEYGRPQ	367	14
376	385	10	GNQNSNGQHE	115	11
393	405	13	FSQQNNNGNQNR	177	13

Most of the chosen epitope falls within sequence with high surface probability.

Potential Cross-Reactivity for antibodies

With mouse proteins:

<u>Mus musculus</u> (mouse) [<u>rodents</u>] taxid 10090		
gb EDL30555.1 RIKEN cDNA 4931406I20, isoform CRA_c [Mus m...	<u>24</u>	4.6
gb AAH34875.1 Rnf220 protein [Mus musculus]	<u>24</u>	4.6
dbj BAE42996.1 unnamed protein product [Mus musculus]	<u>24</u>	4.6
dbj BAB30235.2 unnamed protein product [Mus musculus]	<u>24</u>	4.6
gb EDL30554.1 RIKEN cDNA 4931406I20, isoform CRA_b [Mus m...	<u>24</u>	4.6
ref NP_080015.3 E3 ubiquitin-protein ligase Rnf220 [Mus m...	<u>24</u>	4.6
sp Q6PDX6.1 RN220 MOUSE RecName: Full=E3 ubiquitin-protein...	<u>24</u>	4.6
gb AAH56359.1 Ring finger protein 220 [Mus musculus]	<u>24</u>	4.6
gb AAH58415.1 Ring finger protein 220 [Mus musculus]	<u>24</u>	4.6
dbj BAC27461.1 unnamed protein product [Mus musculus]	<u>22</u>	17
dbj BAC32507.1 unnamed protein product [Mus musculus]	<u>22</u>	17
ref NP_001021385.1 ectonucleoside triphosphate diphosphoh...	<u>22</u>	17
sp Q9WUZ9.1 ENTP5 MOUSE RecName: Full=Ectonucleoside triph...	<u>22</u>	17
gb AAK82949.1 AF136571_1 Pcph proto-oncogene protein [Mus ...	<u>22</u>	17
emb CAB45533.1 nucleoside diphosphatase (ER-UDPase) [Mus ...	<u>22</u>	17
dbj BAB22234.1 unnamed protein product [Mus musculus]	<u>22</u>	17
gb AAH15247.1 Ectonucleoside triphosphate diphosphohydroly...	<u>22</u>	17
dbj BAC29515.1 unnamed protein product [Mus musculus]	<u>22</u>	17
dbj BAC29861.1 unnamed protein product [Mus musculus]	<u>22</u>	17
dbj BAC37592.1 unnamed protein product [Mus musculus]	<u>22</u>	17
dbj BAC37862.1 unnamed protein product [Mus musculus]	<u>22</u>	17
dbj BAC38219.1 unnamed protein product [Mus musculus]	<u>22</u>	17
dbj BAC40362.1 unnamed protein product [Mus musculus]	<u>22</u>	17
dbj BAE36110.1 unnamed protein product [Mus musculus]	<u>22</u>	17
gb EDL02793.1 ectonucleoside triphosphate diphosphohydroly...	<u>22</u>	17
gb EDL02796.1 ectonucleoside triphosphate diphosphohydroly...	<u>22</u>	17
gb EDL02797.1 ectonucleoside triphosphate diphosphohydroly...	<u>22</u>	17
gb EDL02792.1 ectonucleoside triphosphate diphosphohydroly...	<u>22</u>	17
ref NP_031673.2 ectonucleoside triphosphate diphosphohydr...	<u>22</u>	17
dbj BAE37457.1 unnamed protein product [Mus musculus]	<u>22</u>	17
gb EDL02794.1 ectonucleoside triphosphate diphosphohydroly...	<u>22</u>	17
gb AAC05181.1 nucleoside triphosphatase [Mus musculus]	<u>22</u>	17
gb EDL02795.1 ectonucleoside triphosphate diphosphohydroly...	<u>22</u>	17
ref NP_001019891.2 hemicentin 1 precursor [Mus musculus]	<u>22</u>	17
gb AAM18538.1 fibrous sheath-interacting protein 2 [Mus m...	<u>22</u>	23
ref XP_918762.4 PREDICTED: fibrous sheath-interacting pro...	<u>22</u>	23
ref XP_141020.7 PREDICTED: fibrous sheath-interacting pro...	<u>22</u>	23

Final Selection:

According to our criteria there is one good candidate for epitope:

366-378 SNEYGRPQYGGNQ

It is relatively immunogenic, with a good level of complexity and likely to be exposed at the surface of the native molecule.

This epitope has a low risk to generate antibodies cross-reactive to other proteins. There is no risk of epitope being non-immunogenic in mice due to close similarity to one of mouse proteins.

However, this is not a typical strong immunodominant epitope, which means that not every mouse will be able to produce high affinity antibodies to it. Therefore we recommend using at least 4 mice of 2 different strains (8 in total) or hybrid mouse to improve the chance of getting a good responder.