Where Have All The Monkeys Gone?: Evaluating SIV-Specific CTL in the Post-Mamu-A*01 Era

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Simian immunodeficiency virus (SIV) infected rhesus macaques are currently the most widely used animal model for evaluating different vaccine modalities. Most candidate vaccines now seek to engender cytotoxic T-lymphocyte (CTL) responses, either singly or in concert with other immune responses, as CTL are clearly important in the naturally occurring immune responses to HIV and SIV [1–4]. The lack of well-characterized CTL epitopes in SIV remains a serious bottleneck in vaccine research. Most vaccines evaluate the quality of the CTL response by determining the frequency of CTL directed against a single epitope, the Mamu-A*01 restricted $Gag_{181–189}$ CM9 epitope. The focus on this epitope is understandable in light of the facts that Mamu-A*01 positive animals are common in captive bred macaque populations [5] and that $Gag_{181–189}$ CM9 is conserved in several commonly used SIV challenge strains, including SIVmac239, SIVmac251, and SHIV89.6P. However, the intense selection of these animals for inclusion in vaccine studies has created an acute shortage of Mamu-A*01-positive animals [6]. Therefore, it remains important to identify and characterize SIV-specific CTL responses restricted by other common rhesus MHC class I alleles to expand the number of animals accessible to vaccine research.

Fortunately, Mamu-A*01 is not the only common MHC class I allele in captive bred macaques. Sequence-based genotyping of macaques has identified four additional alleles (Mamu-A*02, -A*08, -B*01, and -B*17) that are present in $>$10% of macaques (unpublished data). The peptide binding motif for Mamu-B*17 has already been determined, and this information is currently being used to identify putative CTL epitopes in commonly used SIV strains [7]. This work has been facilitated by the development of techniques such as intracellular cytokine staining (ICS) [8–10] and IFN-γ ELISPOT that allow rapid *ex vivo* detection of responding CTL without time-consuming *in vitro* restimulations. Within the next few years, rhesus with other common MHC alleles will likely supercede Mamu-A*01 animals as the preferred model for testing SIV vaccines.

Another avenue for increasing the number of animals available for vaccine studies is to utilize Chinese rhesus macaque in addition to the more commonly utilized Indian rhesus macaques. Both Chinese and Indian macaques are readily infected with common SIV challenge strains including SIV_{mac} 251 and SIV_{mac} 239 [13, 14]. However, the immunogenetics of Chinese macaques differ substantially from Indian macaques (unpublished data). In an analysis of over 30 Chinese macaques, no animals expressing Mamu-A*01 were detected [15], though this allele is present in over 25% of Indian rhesus macaques [5]. These differences at the MHC class I loci are supported by evidence of genetic and morphological differences between the groups [16, 17]. Therefore, using Chinese macaques for SIV research will likely necessitate a duplication of the immunogenetic work that has already been performed for Indian macaques; identifying common MHC class I alleles, defining the peptide binding motifs for these alleles, predicting CTL epitopes based on the peptide binding motifs, and finally verifying these CTL responses *ex vivo* from SIV-infected Chinese macaques.

Despite the need to expand the SIV-infected rhesus macaque model, the description of new CTL epitopes is impeded by financial and practical considerations. The value of ICS for epitope identification has been tempered by the cost of applying this technique to comprehensively monitor immune responses to whole viral genomes. For example, simply generating a set of overlapping 15-mers spanning each of the proteins in SIV_{mac} 239 costs approximately $80,000. This initial expenditure, plus access to flow cytometers and SIV-infected animals, places CTL epitope identification beyond the means of most non-specialist laboratories. The routine costs of CTL epitope mapping are a burden even to specialist labs that have the capacity for high-throughput ICS. A single analysis of the entire cellular immune response against SIV costs over $700 and identifies only peptide pools (approximately 10 15-mer peptides each) that are reactive. To deconvolute the pool and identify the minimal, optimal CTL epitope, another $600 in ICS tests is required, plus the synthesis of $3500 in 8-mers, 9-mers, and 10-mers that span the reactive 15-mer. Finally, the restricting element for a response can be determined by testing antigen presenting cells expressing well-defined MHC class I alleles with the reactive CTL. However, these specialized antigen presenting cell lines are time-consuming to generate and have limited utility beyond epitope mapping. In sum, the cost for mapping a single novel SIV CTL epitope is upwards of $5000 (excluding

initial peptide and animal husbandry costs). The reward for this expenditure can be uncertain, as peer-reviewed journals such as the *Journal of Virology* have stated that "[we] will not publish papers that simply. . . identify new immunodominant peptides representing T- or B- cell epitopes. . . Such information or reagents must instead be used in further experimentation to test an idea or relate a clear set of novel conclusions that derive from the data [18]." Though this guideline is intended to prevent the repetitive publication of manuscripts containing new CTL epitopes, it also actively discourages the identification of new CTL epitopes by favoring in-depth analysis of previously described epitopes.

Regardless, eight new SIV and SHIV CTL epitopes have been mapped in the two years since the last sequence compendium review on this topic [19]. Epitopes that have been fully characterized, including MHC class I restriction, are subdivided into those restricted by A-loci alleles (Table I) and B-loci alleles (Table II). Responses that have not been minimally mapped or whose restriction is uncertain are shown in Table III.

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Table I. Defined CTL Epitopes with Known Restricting MHC class I A Loci Molecules

Virus	Species	Protein	Epitope	Restricting Allele[a]	GenBank Acc. No.	Reference
SIVmac239	Rhesus	Gag 149-157	LSPRTLNAW	Mamu-A*01	U50836	11
SIVmac251	Rhesus	Gag 181-189	CTPYDINQM	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Gag 254-262	QNPIPVGNI	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Gag 340-349	VNPTLEEMLT	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Gag 372-379	LAPVPIPF	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Pol 10-20	EAPQFPHGSSA	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Pol 106-115	LGPHYTPKIV	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Pol 110-118	YTPKIVGGI	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Pol 322-331	GSPAIFQYTM	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Pol 437-446	IYPGIKTKHL	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Pol 551-559	QVPKFHLPV	Mamu-A*01	U50836	11
SIVmac251	Rhesus	Pol 584-592	STPPLVRLV	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Pol 655-663	SGPKTNIIV	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Env 233-240	CAPPGYAL(L)	Mamu-A*01	U50836	11
SHIV-89.6	Rhesus	Env 435-443	YAPPISGQI	Mamu-A*01	U50836	20
SIVmac239	Rhesus	Env 502-510	ITPIGLAPT	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Env 620-628	TVPWPNASL[b]	Mamu-A*01	U50836	11
SIVsmE660	Rhesus	Env 620-628	TVPWPNETL[b]	Mamu-A*01	U50836	21
SIVmac239	Rhesus	Env 726-734	SSPPSYFQT	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Env 727-737	SPPSYFQTHT	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Env 761-769	SWPWQIEYI	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Tat 28-35	STPESANL	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Vif 14-22	RIPERLERW	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Vif 144-152	QVPSLQYLA	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Vpx 8-18	IPPGNSGEETI	Mamu-A*01	U50836	11
SIVmac239	Rhesus	Vpx 39-48	HLPRELIFQV	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Vpx 102-111	GPPPPPPPGL	Mamu-A*01	U50836	Unpublished[c]
SIVmac239	Rhesus	Rev 86-95	DPPTNTPEAL	Mamu-A*01	U50836	Unpublished[c]
SIVmac251	Rhesus	Nef 159-167	YTSGPGIRY	Mamu-A*02	U50837	22
SHIVHXBc2	Rhesus	Env 99-106	KPCVKLTP	Mamu-A*08		23
SIVmac251	Rhesus	Env 305-312	YNLTMKCR	Mamu-A*02	U50837	24
SIVmac239	Rhesus	Env 495-502	GDYKLVEI	Mamu-A*11		25-27
SIVmac32H-J5	Cynomolgus	Gag 242-250	SVDEQIQWM	Mafa-A*02		28

[a] MHC class I molecule designations: Rhesus macaque (*Macaca mulatta*; Mamu); cynomolgus macaque (*Macaca fascicularis*; Mafa).

[b] This CTL epitope, with amino acid substitutions at positions 6 and 7, has been identified in both SIVmac239 and SIVsmE660 infected macaques.

[c] These epitopes were mapped as part of reference but were omitted from the manuscript because of limited reproducibility.

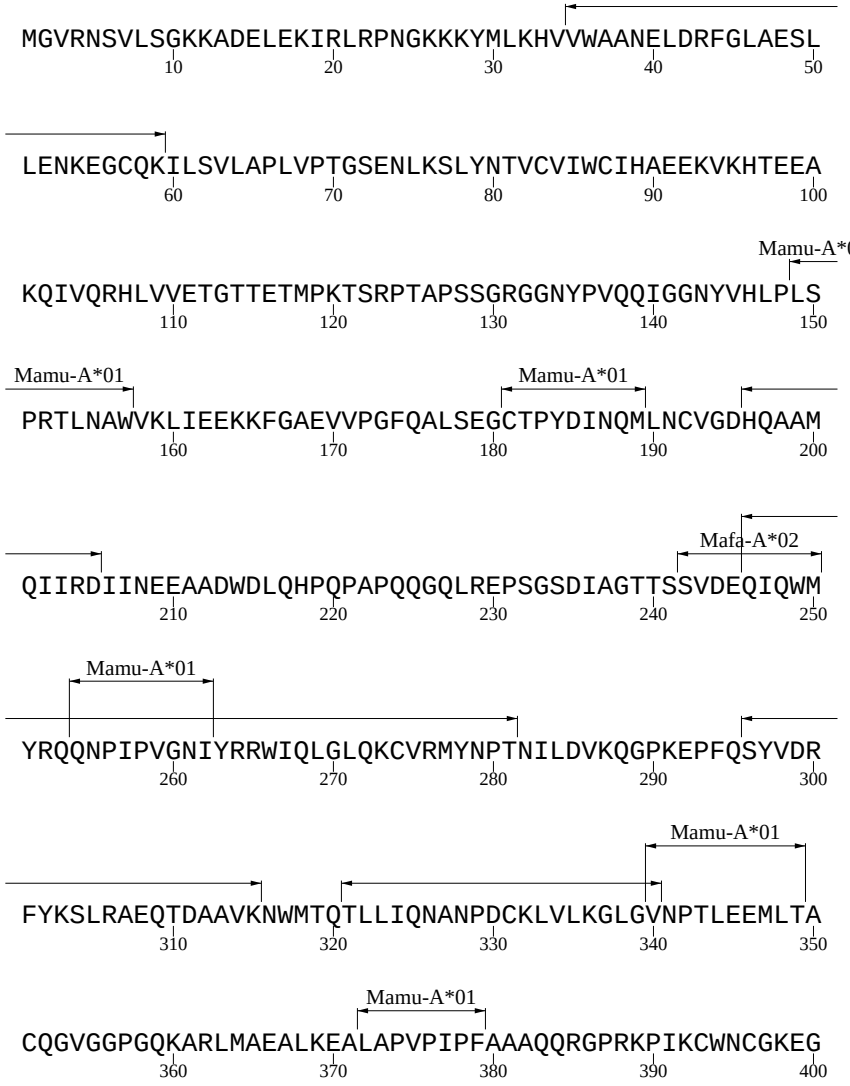
Table II. Defined CTL Epitopes with Known Restricting MHC class I B Loci Molecules

Virus	Species	Protein	Epitope	Restricting Allelea	GenBank Acc. No.	Reference
SIVmac251	Rhesus	Env 501-509	EITPIGLAP	Mamu-B*01	U42837	29
SIVmac239	Rhesus	Nef 136-146	ARRHRILDMYL	Mamu-B*03	U41825	25-27
SIVmac239	Rhesus	Env 573-581	KRQQELLRL	Mamu-B*03	U41825	25-27
SIVmac239	Rhesus	Nef 62-69	QGQYMNTP	Mamu-B*04	U41826	25-27
SHIVHXBc2	Rhesus	Env 553-561	NNLLRAIEA	Mamu-B*12		23
SIVmac239	Rhesus	Nef 165-173	IRYPKTFGW	Mamu-B*17		25-27

Table III. Regions of SIV Recognized By CTL Without Optimally Defined CTL Epitopes or Known MHC Class I Restriction

Virus	Species	Protein	Epitope	Reference
SIVmac251	Rhesus	Gag 35-59	VWAANELDRFGLAESLLENKEGCQK	30
SIVmac251	Rhesus	Gag 246-281	QIQWMYRQQNPIPVGNIYRRWIQLGLQKCVRMYNPT	31-34
SIVmac251	Cynomolgus	Gag 296-315	SYVDRFYKSLRAEQTDAAYK	35
SIVmac251	Rhesus	Env 21-30	YCTLYVTVFY	Unpublished
SIVmac239	Rhesus	Env 113-121	CNKSETDRW	36
SIVmac251	Rhesus	Env 262-281	SCTRMMETQTSTWFGFNGTR	Unpublished
SIVmac251	Rhesus	Env 292-301	GRDNRTIISL	Unpublished
SIVmac251	Rhesus	Env 312-331	RRPGNKTVLPVTIMSGLVFH	Unpublished
SIVmac251	Rhesus	Nef 108-123	LRAMTYKLAIDMSHFI	31-34, 42
SIVmac251	Rhesus	Nef 128-137	GLEGIYYSAR	31-34
SIVmac251	Rhesus	Nef 155-169	DWQDYTSGPGIRYPK	31-34
SIVmac251	Rhesus	Nef 164-178	GIRYPKTFGWLWKLV[d]	26, 31-34
SIVmac251	Rhesus	Nef 171-179	FGWLWKLVP	27
SIVmac251	Rhesus	Nef 201-225	SKWDDPWGEVLAWKFDPTLAYTYEA	31-34
SIVmac239	Rhesus	Nef 157-167	QDYTSGPGIRY[e]	37
SIVmac239	Sooty mangabey	Nef 20-28	LLRARGETY	37
SIVmac239	Rhesus	Vpr 74-81	RGGCIHSR[f]	Unpublished
SIVmac239	Rhesus	Nef 45-53	GLDKGLSSL[f]	Unpublished
SIVmac251	Rhesus	Nef 169-178	KTFGWLWKLV	38
SIVmac251	Rhesus	Nef 211-225	LAWKFDPTLAYTYEA	38
SIVmac251	Rhesus	Nef 112-119	SYKLAIDM	38, 42
SIVmac251	Rhesus	Nef 120-135	SHFKEKGGLEGIYYS	42
SIVmac251	Rhesus	Nef 125-138	EKGGLELIYYSARR	42
SHIV-HXBc2	Rhesus	Gag 321-340	TLLIQNANPDCKLVLKGLGV	39
SHIV-HXBc2	Rhesus	Gag 421-440	DHVMAKCPDRQAGFLGLGPW	39
SIVNef 239	Rhesus	Env 484-492	AEVAELYRL	40
SIVmac239	Sooty mangabey	Gag 196-205	HQAAMQIIRD	41
SIVmac239	Sooty mangabey	Env 429-437	YVPCHIRQI	41
Naturally-infected	Sooty mangabey	Env 428-437	NYVPCHIRQI	41
SIVmac239	Sooty mangabey	Env 339-363	PKQAWCWFGGKWKDAIKEVKQTIVK	41
SIVmac239	Sooty mangabey	Nef 21-30	LRARGETYGR	41
SIVmac239	Sooty mangabey	Nef 20-30	LLRARGETYGR	41
Naturally-infected	Sooty mangabey	Nef 21-32	LRARGETYGRLL	41

gag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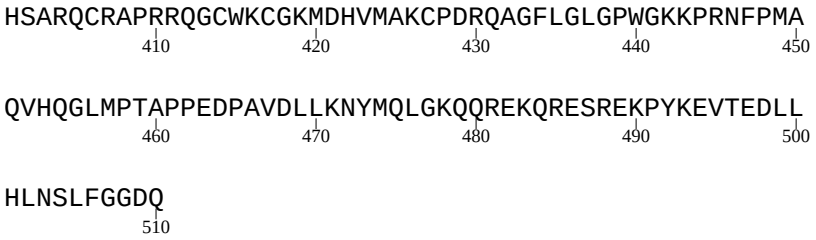
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pol


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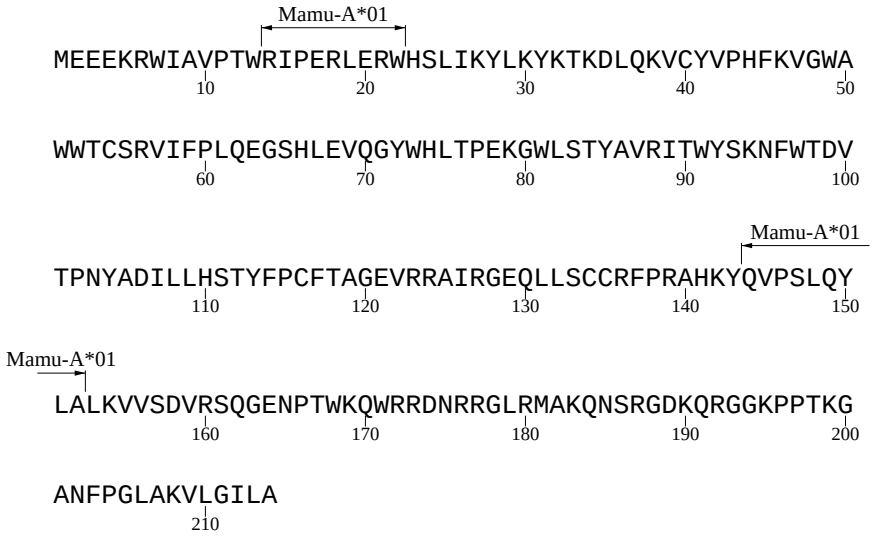
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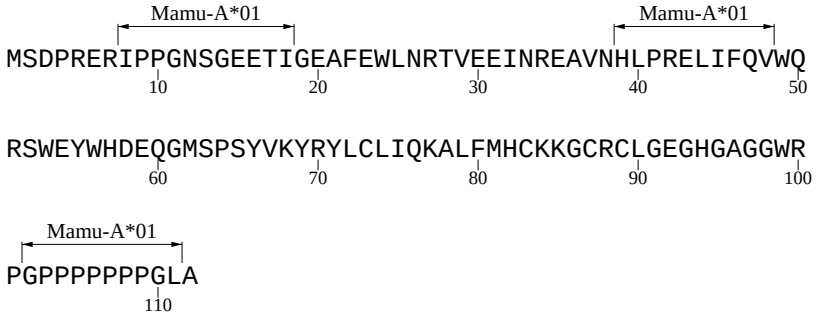
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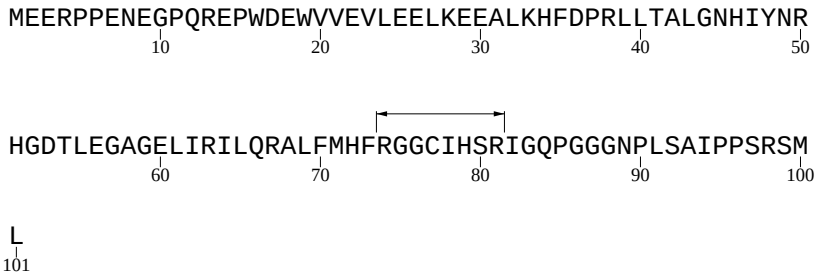
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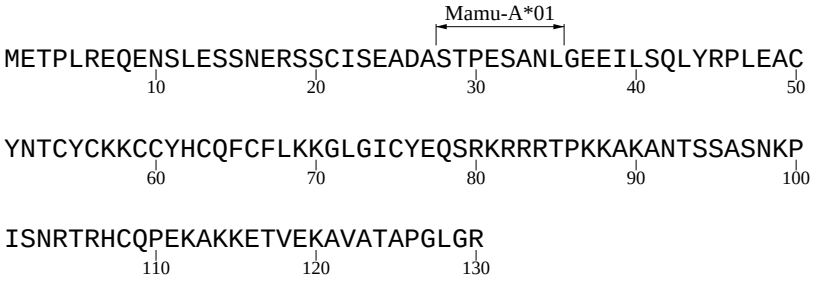
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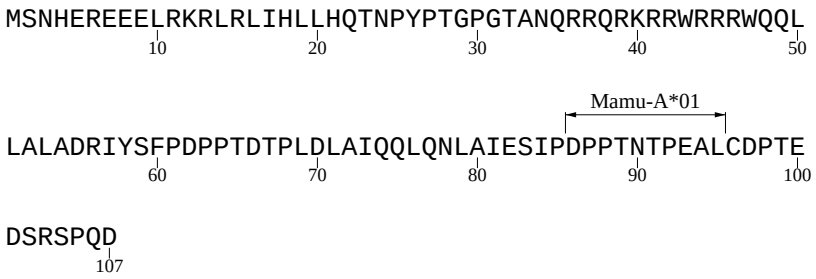
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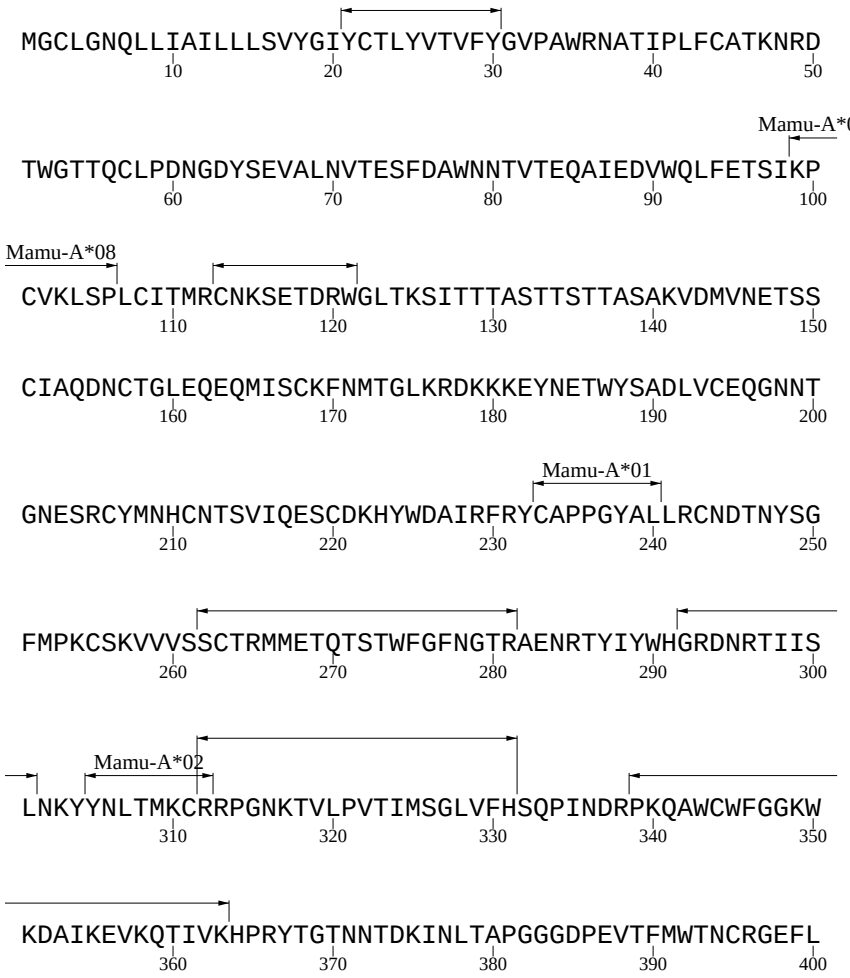
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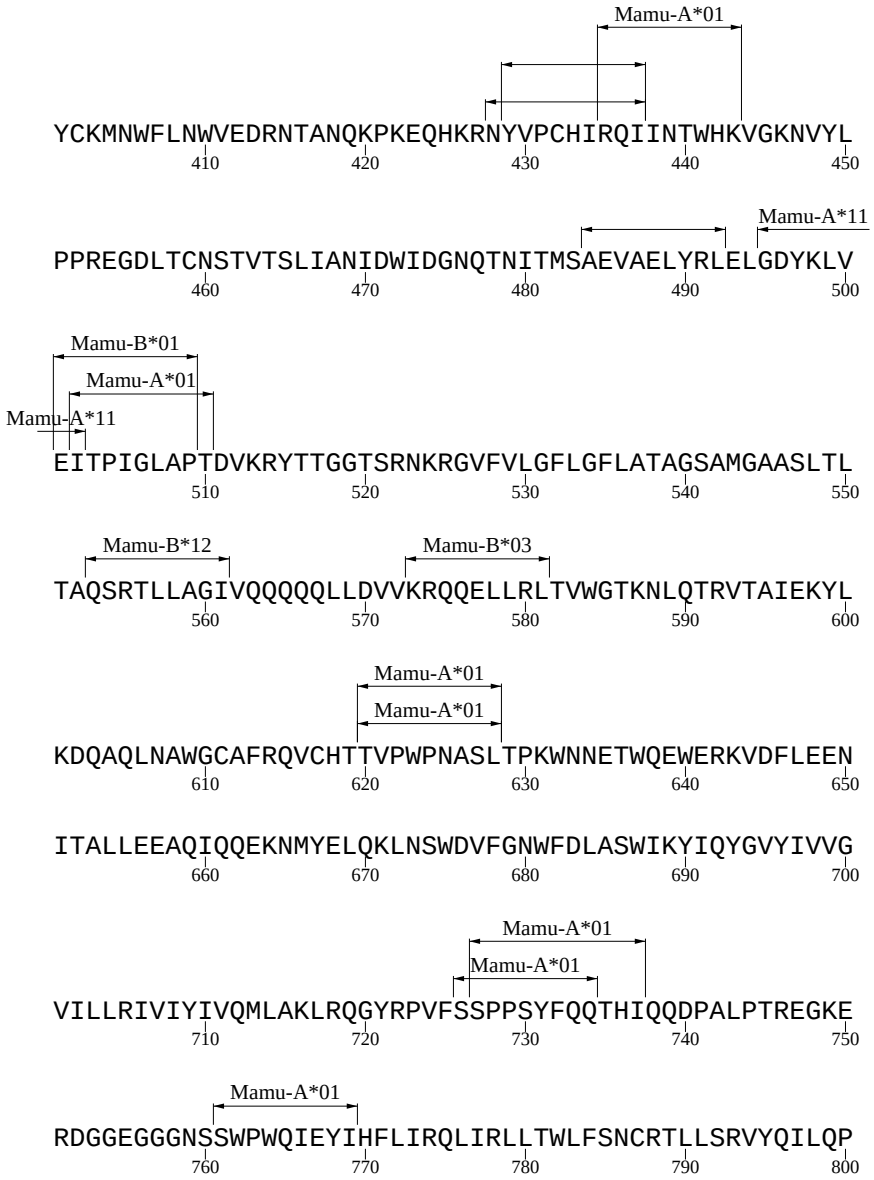
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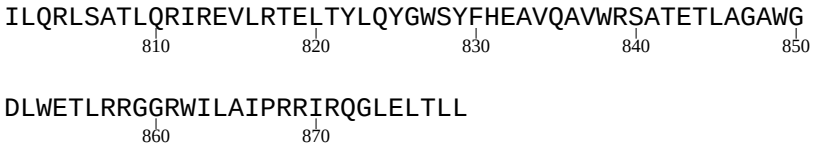
KDQAQLNAWGCAFRQVCHTTVPWPNASLTPKWNNETWQEWERKVDFLEEN
610 620 630 640 650
ITALLEEAQIQQEKNMYELQKLNSWDVFGNWFDLASWIKYIQYGVYIVVG
660 670 680 690 700
Mamu-A*01
Mamu-A*01
VILLRIVIYIVQMLAKLRQGYRPVFSSPPSYFQQTHIQQDPALPTREGKE
710 720 730 740 750
Mamu-A*01
RDGGEGGGNSSWPWQIEYIHFLIRQLIRLLTWLFSNCRTLLSRVYQILQP
760 770 780 790 800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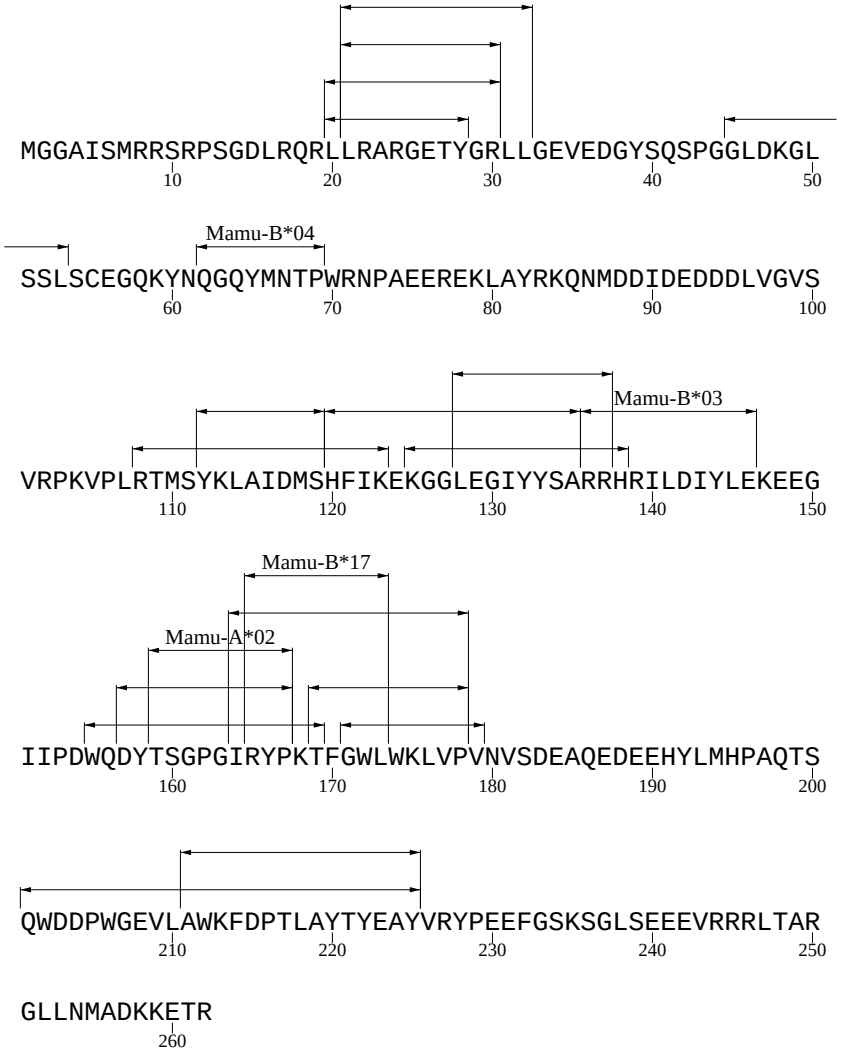
DLWETLRRGGRWILAIPRRIRQGLELTLL
860 870

nef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10
20
30
40
50
Mamu-B*04
SSLSCEGQKYNQGQYMNTPWRNPAEEREKLAYRKQNMDDIDEDDDLVGVS
60
70
80
90
100
Mamu-B*03
VRPKVPLRTMSYKLAIDMSHFIKEKGGLEGIYYSARRHRILDIYLEKEEG
110
120
130
140
150
Mamu-B*17
Mamu-A*02
IIPDWQDYTSGPGIRYPKTFGWLWKLVPVNVSDEAQEDEEHYLMHPAQTS
160
170
180
190
200
QWDDPWGEVLAWKFDPTLAYTYEAYVRYPEEFGSKSGLSEEEVRRRLTAR
210
220
230
240
250
GLLNMADKKETR
260

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