

SUPPLEMENTAL FIGURES AND LEGENDS

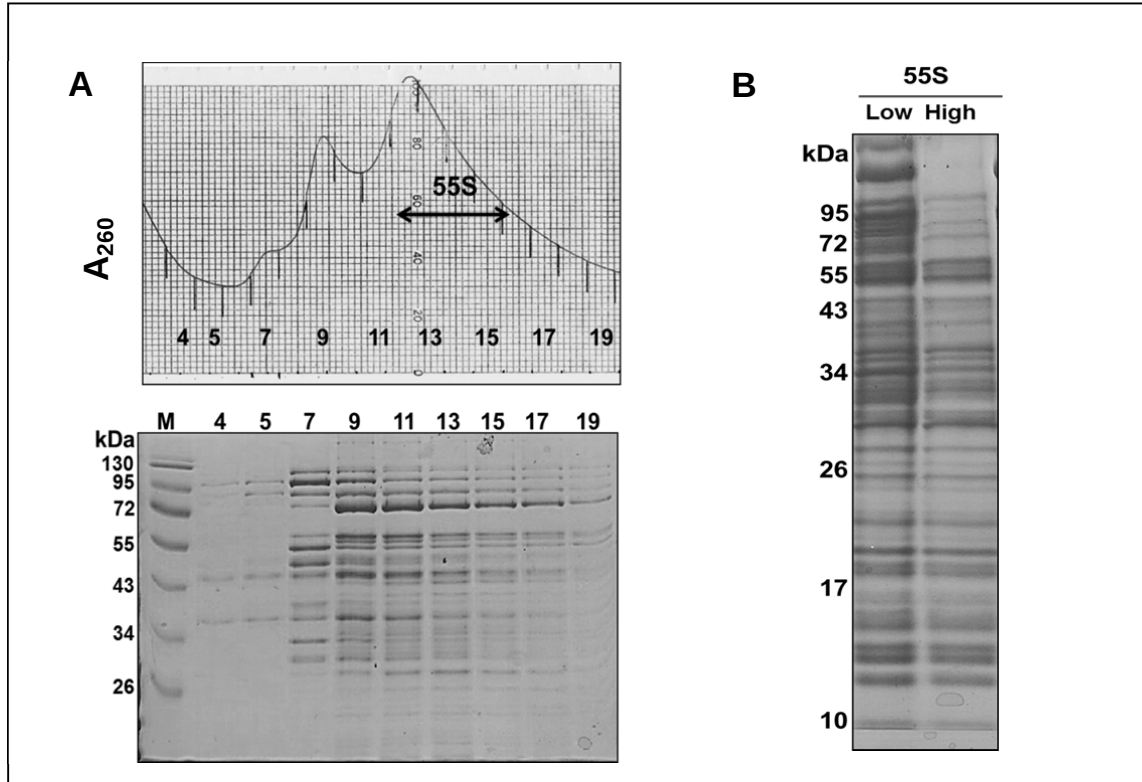


Figure S1. **A.** Approximately 50-60 A_{260} units of high salt and detergent preparations of crude ribosomes were separated on 10-30 % linear sucrose gradient to sediment purified 55S ribosomes (fractions containing purified 55S ribosomes are shown by an arrow). Different subsets of metabolic enzyme complexes were removed by sedimentations performed at high salt and detergent conditions; however, 55S fractions shown by the arrow mainly contained the components of the mitochondrial translation machinery. Sucrose gradient fractions were separated on 12 % SDS-PAGE. **B.** The gradient fractions (shown by the arrow) obtained from high and low salt and detergent preparations were collected as Low and High 55S subunits and 0.5 A_{260} units of each sample were separated on 14 % SDS-PAGE and stained with Coomassie Blue. Mitochondrial ribosomal proteins and ribosome-associated proteins were identified by analyses of in-gel tryptic digests of gel pieces by capLC-MS/MS (Tables S1 and S2). High salt 55S sample was fractionated further for the preparation of purified 28S and 39S subunits shown in Fig. 2A.

Figure S2- Alignment of primary sequences of new mitochondrial ribosomal proteins, MRPS37 (CHCHD1), MRPS38 (AURKAIP1), MRPS39 (PTCD3), MRPL58 (ICT1), and MRPL59 (CRIF1) among different species using ClustalW on Biology Workbench and BOXSHADE. The predicted signal peptide cleavage sites by MITOPROTII are shown by arrows (35).

MRPS37 (CHCHD1)

Bovine	1	--MATP-SLRGLAR-LGNPRKPI-LKPNKPLILANHVGE-RRREKGEATCITEMSIMMA
Human	1	--MATP-SLRGLAR-FGNPRKPV-LKPNKPLILANRVGE-RRREKGEATCITEMSVMA
Mouse	1	--MATP-SLRGLAR-FANPGKPI-LKPNKPLILANRVGN-RRREKGEATCITEMSMMMMA
Fly	1	--MRVPGALFAARGR-APQSEKDVPFQEILPLRLKNTVSG-KADSGS-VACLQEMGVLF
Worm	1	MMFSSPLLKEKALARGKSIYPKVAVFSEILPLASKNRVQAGQKPRASSCTQELQALFG
Yeast	1	-----MSGKPPV-YRLPPLPR-LKVKKPTI-----RQEAN--KCLVLMSNLLQ

Bovine	55	CWKQNEFRDEACRKEIRDFDCASR--AEARKMRSIQED-LGELGS-----LPPRKLNK
Human	55	CWKQNEFRDDACRKEIQGFLDCAAR--AQEARKMRSIQET-LGESGS-----LLPNKLNK
Mouse	55	CWKQNEFRDEACRKEIQDFDCSSR--AQEARKMRSIQES-LGQSES-----LSPHKMTK
Fly	57	CLKDNEFVEKYCHKEISQFQNCYKCYMDRKFEAKKTVNQG-IVQPGSN-----LNYKQLNK
Worm	61	CLKKWEFDDVPCSKOHTLYMDCVHKGAEFAAYRDATRKGT-LGESGAGGKQSM TSAQFNK
Yeast	40	CWSSYGHMSPKCAGLVTELKSCTS---ESALGKRNNVQKSNIN-----YHAARLYD

Bovine	107	LLQRFNKPPLS-----
Human	107	LLQRFNKPPLS-----
Mouse	107	LLQRFNKPPLS-----
Fly	112	YMRRYPNPV-----
Worm	121	TQKLFQPD LGKQPYRQMKRLPTQDYADDTFHRKHWGSKRS
Yeast	88	RINGKPHD-----

MRPS38 (AURKAIP1)

Bovine 1 MFLMRLTSQLLR-A--VPRAGCSGPWPVLGVLGGRHACRPCYSTKPTGPGSGVASLPGRRVH
 Human 1 MLLGRLTSQLLR-A--VPWAGGRPPWPVSGVLGSRVCGPLYSTSPAGPGRAASLPKGAQ
 Mouse 1 MFLARLTSRLARTV--VPWAGFSRSCPGSGVLGSAFRPLYSLOPASPSRAASLP GKRTQ
 COX24_YEAST 1 -----MLGRALR-----PGWLGITR-----TVVKKPSCGSYFN-----RTFQ
 Worm 1 --MSRAVSRLTEACGRLSITQRSQMHHFRPPPTSSILPPLVTPSTTIVEKSIELPSLENP

Bovine 58 MELEEMLVPRKMSISPLESWLTIRYLLPRLDTGAPGTVSPAQLYECPPSRVGEVEQGGK
 Human 58 LELEEMLVPRKMSVSPLESWLTARCFLLPRLDTGAGTVAPPQSYQCPPSQIGEGAEQGD
 Mouse 59 LELEEFVLPKMAISPLESWLTAQYLLPRRNVEVPVTLAPSQFYECPPRQGEIEAQGGVR
 COX24_YEAST 33 TAINTTMP-----MQEGMLSTMMMTATATRITGTVSEP-----LNGSN
 Worm 59 QQIYTFPTLHSPTIVAPGAILTEILEKLAPTAEGFLIAPGTPNKS-----MWLSP

Bovine 118 DVFDAPQMQCKNVLKIRRRKMNNHHKYRKLVKRTRFLRRKVREARLKQMKFERDLRRIW
 Human 118 GVADAPQIQCKNVLKIRRRKMNNHHKYRKLVKRTRFLRRKVQEGRLRRKQIKFEKDLRRIW
 Mouse 119 EAWDATPVQCKNVLKIRRRKMNNHHKYRKLVKRTRFLRRKVREGRLKMKQIKFEKDLKRIW
 COX24_YEAST 73 -----IVMQLDSVMRKRKKKMKKKHKLRRRKREKAERRKLSQGR-----
 Worm 111 RLTIIRKKMKKHKRRRRYDRDFFKYQKYHREKKLKSEREFQKRKMSLLTELEAFNPEKY
 Fly* 175 -----KMEARLIVIRRRKMKKHKLKLLRRKMKFEWAKVRQRREMRKEAFQAKLISQI

Bovine 178 QKAGLKEAPPGWQTPKIYLLGK-----
 Human 178 LKAGLKEAPEGWQTPKIYLRGK-----
 Mouse 179 LKAGLKEAPENWQTPKIYLLKNK-----
 COX24_YEAST -----
 Worm 171 VKDTIRMANKEWQDELAPTGRKLYPHWSRFMSLEQLYGLPKSEYIDKRAGLPTPEAEQI

Bovine -----
 Human -----
 Mouse -----
 COX24_YEAST -----
 Worm 231 KALKEKYAKLYRKK

(*) Only residues 177-238 were aligned for the Fly homolog (Q8IML6) of AURKAIP1.

MRPS39 (PTCD3)

Bovine	1	-----MASVASARWLRVSCGL
Human	1	-----MAVVS AVRWLGLRSRL
Mouse	1	-----MAAAVAARRLSFRSGL
Fly	1	-----MYLSR
Worm	1	-----



Bovine	17	CYPLTARRAGPCGRTPSSRFYSGSAVPKDEGADTAEVVIPKKKTWDKVAVLQALAS
Human	17	GQPLTGRRAGLCEQARSCRFYSGSATLSKVEGIDVTGIEEVVIPKKKTWDKVAVLQALAS
Mouse	18	VLLQTTTRGTGVCEPKVCCRFYAGTESLPKVEGSDITGIEETVIPKKKTWDKVAVLQALAS
Fly	6	QLRLPRANIACSLSSGAHYTTAAPADAP-----TEIPNRIERSPTDLQALAS
Worm	1	-----MGLGRVIRSLSSVEPALSQK-----LTIQPAIERSPTDLNALSE

Bovine	77	TVHRDTTAAPYAFQDDPYLIPITSSVESHSFLLAKKSGENAAKFIINSYPKYFQKDIAEPH
Human	77	TVNRDTTAVPYVFQDDPYLIPASSLESRSFLLAKKSGENAAKFIINSYPKYFQKDIAEPH
Mouse	78	TVNRDPTAAPYVFHDDPYLIPITSALESRSFLLAKKSGETAAKFIINSYPKYFQKDIAEPH
Fly	57	TVARDYTAPHYKYHDDPILIPMSNAAKRTYAMSKESGRKAAKWIKEEHRELFMHQEAQPA
Worm	42	TVGPDTTAPHFAYIDDPITIPSTQSTKKTIFYMAKEFGKRAARELATEPTLFAFDRDQPQ

Bovine	137	IPCLMP-EYFEPQIEISEAALQERIKLKVKASVDIFDQLLQAGTTVSLETTNSLLDLL
Human	137	IPCLMP-EYFEPQIKDISEAALKERIELRKVKASVDMFDQLLQAGTTVSLETTNSLLDLL
Mouse	138	IPCLMP-EYFEPQIEDVSEAALERIRLRKVASVDMFDQLLQAGTTVSLETTNSLLDLL
Fly	117	IEKFAP-SMVTEDSVDETSLAQLISQGEIKDAVLVYNLLEQKGNPISPELKQSLELV
Worm	102	LPVFRPRHLADPLQVEPTENNLLKMISSREVKDSCLYERMSENVDVSEKVQLELFRLLV

Bovine	196	CYYGNQEPSTNYNFQQHEQTEEELEEAEGDNMKSKKKAGHQLGVTWRARNHAERIFALMP
Human	196	CYYGDQEPSTDYHFQQTGQSEALEEE---NDETSRRKAGHQFGVTWRANKNAERIFSLMP
Mouse	197	CYYGDQEPAPADYFQQTTEHLENLEEAEEENQTSKMESG-----PWKAQNNARIFALMP
Fly	176	CHNNQEPPIPEYIEERWFLQ-----NNKRERESGK-----TWKGDGLAEKLYSEITE
Worm	162	TYYNSSNVFPAEWEFVGMR-----NFGEDSTS-----SWKSGAVADLLFETLP

Bovine	256	EKNAHSYCTMIRGMVKH---RAHTQALSYTELLNNRLRADVHTFNSLIEATALVNAKF
Human	253	EKNEHSYCTMIRGMVKH---RAYEQALNLYTELLNNRLHADVYTFNALIEATVCAINEKF
Mouse	252	EKNARSYCTMIRGMVKH---RAYAQALNLYTELLNNRLSADVYTFNALIEAKTFILNEKF
Fly	223	PKTPQSYASLIRGMAKY---LQGERAYALLQAGEKQVQLDTNTFNSVIEIVSFLKDT-A
Worm	206	-KTDETVSIMIAGLCKFSDHSSLERARELYKEHRAAKGKVYREAFNGLIGASSYSVG---

Bovine	313	EEKWNNDLLDKQMVAKVKNPLQTFNTILKCLRRF--YAFGKLPAQTFREMKAIGIEP
Human	310	EEKWSKILELLRMVMAQVKVKNPLQTFNTILKCLRRF--HVFARSPALQVLRMKAIGIEP
Mouse	309	EEKWNDLLDKHMVMAQVKVKNPLQTFNTILKCLRRF--YSLGRIPALQILREMKHIGIEP
Fly	279	EQRWQLCKDLLNEMSQQLRPNLGLTNAVLQCLSTFGNFKVARAAALQALPEFKQLGVNP
Worm	262	-----KKLVAEMISLRMTPTDIFTFNALSSAAKAGKFEDRVKAFTEIIGEMKEIGVEP

Bovine	371	SLATYHHIIQ-----LFY-QHESPSKGSSL-----IIYDIMDETGTGTFSPKDP
Human	368	SLATYHHIIR-----LFD-QPGDPLKRSSF-----IIYDIMNELMGKRFSPKDP
Mouse	367	SLATYHHIIH-----LFYPRDLSAIKMPSL-----IIYDIMNELGRTFSPQDL
Fly	339	SLGSYYYLLI-----IFCRERGP-----VSH-----VIVDINDISGKEFQIQHP
Worm	315	ALSSPHLIINKNIIDFKLLDNEKRESDEQKKTYNHQLTVAISWLNELIONSITGKTLKPITS

Bovine	414	DDDMFFQSAMRVCS-SLRDLELAYQVHGLLNTGDNRKFIGPDPRRNFYYSKFFSLCLME
Human	411	DDDKFFQSAMSICS-SLRDLELAYQVHGLLKTGDNKFIGPDQHRNFYYSKFFDLCLME
Mouse	411	DDGRFFQLAMSVCS-SLRDLELAYQVHRLNTGDNRKLVGHDPRLKVYYSKFFSLICSL
Fly	379	KDTYFFATAMDVCRNHLHDKSLAKKVDLELHTGKNYDLVGDSFKESIYYRNLALLCQTE
Worm	375	TCNLFFFEAMGTHYRAANENLAENLVSIYESKANEVKMPAFTIESMFYNRQLQLAVEQSA

Bovine	473	QIDVTLKWKDLPSPVFFPHSQTLLDLLQALDVAN--RLEMIPQIWKD---SKEYGHTFR
Human	470	QIDVTLKWEEDLPSPAFPHSQTMIHLLQALDVAN--RLEVIPKIWKD---SKEYGHTFR
Mouse	470	QIDVTLKWKDLPSPVLPHYQIFIGLLQALDVAN--RLELVPIWKD---SKEYSHTFR
Fly	439	STEDFMLSYDLLVPNIYIPEPGIMEEILRAIEING--AVEYVPRLWSD---MVF-DHTHR
Worm	435	SLNRIYDLYTTMVPRVGVNNYLSSLVFRKLAASSDRHWPLLRRVIIDGIAAGQMNGVIG

MRPS39 (PTCD3) cont'd

Bovine	528	SDLKEEILMLMARDQHPP-----ELQAAFADCAADIKSTYESQDARQTASEWPANSLNY
Human	525	SDLREEILMLMARDKHPP-----ELQVAFADCAADIKSAYESQPIRQTAQDWPATSLNC
Mouse	525	DALREEVLMMLMARDKHPP-----ELQVAFADCAADIKSTYEDQSARQPAFDWPANPLQY
Fly	494	ESLLLYVLRIILVDNKPNDSPAQAQLPEQGAQVADMFERVEEAIKRLRKVSETGQMLGD
Worm	495	EEMRKQLCNVQLHTLGTSEREQFTSLVQKLVAVWVEFSQFTIERMRLQRKLSPSQ-ISE

Bovine	582	IATLFLRAGRTQEAWKMLGLFRKHNK-----IPRNELLNEFMDSAKASSSPA
Human	579	IATLFLRAGRTQEAWKMLGLFRKHNK-----IPRSELLNELMDSAKVSNSPS
Mouse	579	IAMFLLRGGRSQEAWKMLELFKKHKK-----IPRNELLEEFMDTAKASGSTA
Fly	554	ILTLLVRGGSYEKATEVFAHIDKNQHRIPG-----TPSLNALIEFDASVQEKSPS
Worm	554	CALLLTRIGEQQKAYELLDLLDETASSGDEATVYPRGHARPWMAELFEDALRKRDITYA

Bovine	629	QAEVVKLANSFSLPICEGLTQRLTADFTLSQEKEALGDLTALTSDSE----SDSDSDT
Human	626	QAEVVEELASAFSLPICEGLTQRMVSDFAINQEKEALSNLTALTSDSDTDSSSDSDSDT
Mouse	626	LAIEVVKLASAFSLPIGESLAQRVVMDFVDPEQEKEALGNLTENSSDG---ESSSDSDS
Fly	605	QALFALQYAVENNFDRE-LAKRIHEGFTLNETHLSKLKSLVGESFLDK-----
Worm	614	AATCLQIMSLTANRAKLEPLANRIFEKCNVNQEQVRITQGFIRLRPQ-----

Bovine	685	SKDK
Dog	728	SEGI
Human	686	SEGK
Mouse	683	DK-

MRPL58 (ICT1)



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Mouse 1  ----MATAWGLRWGLSRTGTL L L A P P A R C A R R A L H R Q E H ----
Human 1  ----MAATRCLRWGLSRAGVWLLPPPARCPRRALH K Q K D G T E F K S I Y S L D K L Y P E S Q G S
Bovine 1  ----MAAARCLRWGLSRAEAWLLPPPTSCCHRALHRQVEGTEFRSAYS L D K L Y P E S R G A
Fly 1  MNKITS A F I R V L R Q S S S G T G N L L G R Q L S Y K S D L S L D K I Y P G ----ARLQI
Worm 1  -----MLRN L S S-----FVVKSTRH I Q A S-----

Mouse 36 -----AKQASSY I P L D R L S I S Y C R S S G P G G Q N V N K V N S K A E V R F H L A S A D W I E E P V
Human 56 D T A W R V P N G A K Q A D S D I P L D R L T I S Y C R S S G P G G Q N V N K V N S K A E V R F H L A T A E W I A E P V
Bovine 56 D T A W R V P G D A K Q G N D D I P V D R L T I S Y C R S S G P G G Q N V N K V N S K A E V R F H L A S A D W I A E P V
Fly 49 Y T P P P P S G S D K F S G F I P N D R L E I T Y S R S S G P G G Q H V N T V N T K V D V R F K V A Q A D W I P E Q T
Worm 20 -----SSATFNGV I P T E K I E K R Y T L S S G P G G Q N V Q K N A T K V E I R F K V S E A E W L S E S L

Mouse 87 R Q K I A L T H K N K I N K A G E L V L T S E S S R Y Q F R N L A E C L Q K I R D M I - A E A S Q V P K - E P S K E D A
Human 116 R Q K I A I T H K N K I N R L G E L I L T S E S S R Y Q F R N L A D C L Q K I R D M I - T E A S Q T P K - E P T K E D V
Bovine 116 R Q K M A L T H K N K I N R A G E L I L T S E S S R Y Q F R N L A D C L Q K I R D M I - A E A S Q P A T - E P S K E D A
Fly 109 R Q K L L K V L A N R I T K D G Y F Y I K S D L T R S Q Q M N L A D A L E K L R T I I R S Q E A V V P A - P P S E E T L
Worm 72 R D L V E E K L S H R I N T A G E L I I D S D R T R E R H L N V A D C F D K L R S A I Y A I E N E Q G K R E M T E K D E

Mouse 145 R L Q R L R I E K M N R E R L R Q K R L N S A L K T S R R - - M T M D -
Human 174 K L H R I R I E N M N R E R L R Q K R I H S A V K T S R R - - V D M D -
Bovine 174 A L Q L R I E N M N R E R L R K K R I N S A I K T S R R - - V G T D -
Fly 168 E K L R R R Q E R A V R E R L Q L K R G R A Q V K A D R Q G P S G L D L
Worm 132 K I L R E R A A I A T Q H R L Q E K R R T S E K K A S R R A A V E F - -
  
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MRPL59 (CRIF1)



Bovine	1	MAAPV	RQTR	SLLG	WVT	LG	PGSR	GYR	APPP	RRS	---	RE	PWW	DP	DDP	---	LT	PRW	Q---		
Human	1	MAASV	RQAR	SLLG	VA	ATL	PGSR	GYR	ARPP	RRR	---	PG	PRW	DP	EDL	---	LT	PRW	Q---		
Mouse	1	MAALAM	RS	GYLL	RL	SVA	LG	PRSR	SYR	APPP	RRR	---	PG	PHSP	DP	ENL	---	LT	PRW	Q---	
Fly	1	MNVGK	ISV	VTRL	PALR	CA	QYSS	AAKA	EL	PAS	LVGD	VDVE	PTY	PQT	VD	RSGL	Q	PH	KNVL		
Worm	1	MLRNW	RL	LVRS	FATNS	EEI	AP	KV	DVS	FLR	PRHR	-----	II	AAG	MP	PV	QF	D	SERE	---	
Bovine	52	-----	LG	PRYA	AAKQ	FAR	HGA	ASG	VD	PG	SLW	PS	RE	QL	LE	EA	EER	E	WYPSLA		
Human	52	-----	LG	PRYA	AAKQ	FARY	GA	ASG	V	PG	SLW	PS	PE	QL	RE	EA	EER	E	WYPSLA		
Mouse	52	-----	LT	PRY	VAKQ	FGR	HGA	ISG	VPP	AS	SLW	PT	PE	QL	RE	EA	EER	E	WYPSLA		
Fly	61	LNKLP	YQEP	HSWI	HL	TE	KYQ	RAF	GRY	GAQ	SNV	PKIC	FD	SHG	---	EK	SR	QV	MQLE	TL	
Worm	52	-----	RAS	RRER	F	GKY	GLK	SGV	PVEE	L	FPT	AE	E	TE	EE	E	AIG	---	L	REFN	
Bovine	98	VMQES	LRVQ	QLA	EEQ	KRQ	ARE	QL	TE	EC	MAK	MPQ	MIEN	WR	QQ	QAR	RE	KAQ	ADKERRARLQ		
Human	98	TMQES	LRVK	QLA	EEQ	KRRE	REQ	HIA	EC	MAK	MPQ	MI	VNW	QQQ	QREN	WE	KAQ	ADKERRARLQ			
Mouse	98	TMQES	LR	QQQ	AL	EAR	QARE	QRIA	EC	MAK	MPQ	MIEN	WR	KQ	KRER	WE	KI	QADKERRARLQ			
Fly	118	KMLE	KNRA	QKAE	EL	ART	NARE	EDI	AKK	MEK	L	TQW	KADL	HAKI	AK	READ	AAAA	IQK	ERLV		
Worm	94	NAKKE	YKEL	Q	KKK	RESE	L	ARIAE	L	EKN	L	KKY	PAT	L	AKY	EAS	LVQ	EQEK	DEKEVALEKRI		
Bovine	158	AEAQER	LG	YHVD	PR	SARF	QELL	QD	LEKQ	HR	KRL	KEE	KQ	KK	KEA	RAAA	AAAA	AQD	PADS		
Human	158	AEAQE	L	LG	YQVD	PR	SARF	QELL	QD	LEK	KR	KRL	KEE	KQ	KK	KEA	RAAA	AAVA	AQD	PAAS	
Mouse	158	AEAQER	LG	YHVD	PR	SARF	QELL	QD	L	KQ	Q	RKRL	KEE	Q	Q	OK	KEA	RTAA	ASAE	QDSAVS	
Fly	178	EEVRR	HFG	FKVD	TR	DER	FK	EM	LE	QKE	KED	KK	QKE	AKR	KA	KE	KMM	AKL	VEKASV	-----	
Worm	154	R	IQEY	FGY	W	MDP	KD	PR	FEV	M	LQ	QKE	AEE	KK	A	KMA	KR	DEI	QKKR	FAENVQ	-----
Bovine	218	ET	PDS																		
Human	218	CA	PSS																		
Mouse	218	CE	PSS																		
Fly		-----																			
Worm		-----																			