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Amelogenin Evolution and Tetrapod Enamel Structure

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Abstract

Amelogenins are the major proteins involved in tooth enamel formation. In the present study we have cloned and sequenced four novel amelogenins from three amphibian species in order to analyze similarities and differences between mammalian and non-mammalian amelogenins. The newly sequenced amphibian amelogenin sequences were from a Red-eyed tree frog (*Litoria chloris*) and a Mexican axolotl (*Ambystoma mexicanum*). We identified two amelogenin isoforms in the Eastern Red-backed Salamander (*Plethodon cinereus*). Sequence comparisons confirmed that non-mammalian amelogenins are overall shorter than their mammalian counterparts, contain less proline and less glutamine, and feature shorter polyproline tripeptide repeat stretches than mammalian amelogenins. We propose that unique sequence parameters of mammalian amelogenins might be a pre-requisite for complex mammalian enamel prism architecture.

Introduction

Amelogenin is the major protein component (90%) of the mammalian enamel protein matrix (1–2). A series of genetic, antisense, knockout, and crystal growth studies of the recent decade have established amelogenin's pivotal role in the control of enamel crystal growth and enamel formation (3–6). While amelogenins are not the only proteins in the developing enamel matrix, they have nevertheless been attributed a major role in the growth of elongated enamel crystals (3–6). In previous studies from our laboratory, we have characterized the structure of the amelogenin-rich enamel protein matrix (4,7,8) and its functional changes related to enamel crystal growth (4). Our studies have established a functional relevance for the structured amelogenin matrix to control enamel crystal growth (4) and a close relationship between crystal nucleation and changes in matrix configuration during initial crystal formation (8).

In the recent years, our knowledge of the enamel protein composition and function of nonmammalian vertebrates has seen significant progress. Amelogenin sequences from reptilian and amphibian teeth have been published (9–10), and biochemical and immunohistochemical studies have enhanced our knowledge of enamel protein homologies between different vertebrate species (12–16). Immunohistochemical findings have confirmed earlier reports on a predominance of enamelins in shark enameloid, compared to a predominance of amelogenins in reptilian and mammalian enamel (16). Based on molecular phylogenetic studies, the amelogenin signal peptide in exon 2 has been linked to a similar region of the SPARC, and suggesting that exon 2 was duplicated to amelogenin approximately 630 MYA (11). According to homology analyses, intermediaries in the molecular evolution of amelogenins from SPARC were SPARCL1, enamelin, and ameloblastin (17).

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In the present study we have generated sequence data for four novel amphibian amelogenins to analyze and compare key parameters of mammalian and non-mammalian amelogenins. In addition, we have documented prismatic mammalian enamel with non-prismatic reptilian and amphibian microarchitecture.

Materials and Methods

Source and isolation of the genomic DNA

For the present analysis, three amphibian species were chosen, a Red-eyed tree frog, (*Litoria chloris*); a Mexican axolotl (*Ambystoma mexicanum*) and an Eastern Red-backed Salamander (*Plethodon cinereus*). Amphibians were euthanized according to approved guidelines by the UIC animal care committee. The genomic DNA was isolated using GenEluteTM Mammalian Genomic DNA Miniprep (Sigma-Aldrich Co., St. Louis, Mo) following the manufacturer's instruction. The isolated genomic DNA was kept at -80° C for future use.

RNA isolation

RNA isolation was performed as previously reported (10). Briefly, amphibian jaws were removed and immediately frozen in liquid nitrogen and homogenized. The homogenized teeth tissue in TRI AGENT[®] reagent (Sigma) was mixed with 0.2 ml of chloroform and shaken vigorously for 30 sec. The mixture was centrifuged at 12,000× g for 20 min at 4 °C after 10 min incubation at room temperature. The aqueous phase was mixed with equal volume of isopropanol, and then centrifuged at 12,000× g for 20 min at 4 °C. The pellet was washed with 70% ethanol and dissolved in DEPC-treated H₂O. The isolated RNA was kept at −80 °C for future use.

cDNA synthesis, cloning and sequencing

The sequencing strategy was as described by Wang et al. (10,18). Briefly, primers were selected based on sequence analysis of amelogenin genes from selected species with the aid of Lasergene software (DNASTAR Inc., Madison, WI). The consensus sequences among different species were chosen as primers for amplification of novel amelogenin genes. Reverse transcriptase reaction was performed using SuperSriptTM II Reverse Transcriptase (Invitrogen, Carlsbad, CA). The PCR products were purified with QIAquick[®] Gel Extraction Kit (Qiagen Inc., Valencia, CA), ligated to pGEM[®]-T Easy vector, and transformed into JM109 competent cells (Promega, Madison, WI). Transformants were picked up, and cultivated in LB medium containing ampicillin at a final concentration of $50\mu g/ml$. Recombinant plasmids were isolated with Wizard[®] *Plus* SV Minipreps DNA Purification System (Promega), identified by enzyme digestion with *EcoR* I, and sequenced using the ABI 377 sequencer (Northwoods DNA Inc, Becida, MN). Three colonies were selected and sequenced 2 times from both orientations with either T7 or SP6 primers.

Sequence analysis

The four newly discovered amelogenin amino acid sequences were deduced from the nucleotide sequence, and sequence analyses were performed to identify its features. Sequences were manually aligned to represent optimum interspecies homology.

Scanning electron microscopy

For scanning electron microscopy, frog, squamate, and mammalian teeth from the du Brul collection at the University of Illinois were longitudinally sectioned and etched using EDTA (ethylenediaminetetraacetic acid). Etched enamel surfaces were then analyzed using a Joel JSM-6320F at the UIC RRC laboratory.

Results

Higher complexity of tooth enamel microstructure in mammals versus amphibians/reptilians

Scanning electron micrographs illustrated prismatic organization of enamel microstructure in all mammals investigated (human, *Homo sapiens*; pig, *Sus scrofa*; steer, *Bos Taurus*; Virginia opossum, *Didelphis virginiana*; and La Plata river dolphin, *Pontoporia blainvillei*), while there was no prismatic enamel structure in most squamates and amphibians (e.g. Green Iguana, *Iguana iguana*; Leopard frog, *Rana pipiens*). The Spiny-tailed lizard (*Uromastyx maliensis*) was unique among squamates as its enamel is prismatic (Fig. 1).

Four novel amphibian amelogenin genes confirm conserved elements of tetrapod amelogenins

For this study, RNA was isolated from the tooth-bearing elements of three amphibian species, a Red-eyed tree frog, *Litoria chloris*; a Mexican axolotl *Ambystoma mexicanum*; and an Eastern Red-backed Salamander *Plethodon cinereus*. Based on RNA extracts, four novel amphibian amelogenin cDNAs were cloned, sequenced, and deposited in Genbank (Red-eyed tree frog, *Litoria chloris*, accession number DQ069788; Mexican axolotl *Ambystoma mexicanum*, accession number DQ069791; and two amelogenin isoforms from the Eastern Red-backed Salamander *Plethodon cinereus*, accession numbers DQ069789 and DQ069789). Translated amino acid sequences are listed an aligned in Fig. 2. In this alignment, sequence elements encoded by exons 2, 3, and 5 were highly conserved, while portions of the exon 6 encoded domain were highly variable among species (Fig. 2). Nevertheless, also in the exon 6 encoded regions, unique polyproline tripeptide repeats were fairly conserved (Fig. 2).

Mammalian amelogenins are longer, contain more proline and glutamine, and have more proline-tripeptide repeats that their amphibian/reptilian counterparts

In order to determine differences between mammalian and non-mammalian amelogenin proteins, a number of key parameters such as length, amino acid composition, and the number of proline repeats were analyzed based on amelogenin sequences presented in Fig. 2. Comparing mammalian and non-mammalian amelogenins, the overall amelogenin length increased by 9%, the proline content increased by 21.5%, the glutamine content increased by 31.3%, and the number of proline-tripeptide repeats increased by 72.8% (Fig. 3).

Discussion

Already studies by Bonass et al. pointed toward a functional significance of the amelogenin center domain as an evolutionary "hotspot" by identifying seven tandem repeats of a section of nucleotides with the consensus sequence CTGCAGCCC (19). Further support for the importance of the central amelogenin domain for enamel crystal growth has been provided by transgenic studies documenting that LRAP (a small amelogenin-derived peptide containing most of the A- and B-domain) fails to rescue an amelogenin null phenotype (20). Recent studies have linked the emergence of genes with new functions to gene duplication and alternative splicing (21,22) providing theoretical support for a novel evolutionary mechanism by which mammalian amelogenin may have evolved through tandem exon duplication and substitution alternative splicing. According to evolutionary studies, the rapid evolution of the central amelogenin domain is primarily accomplished by insertions of PXX or PXQ tripeptide motifs (19), with both proline and glutamine causing structural rigidity of the newly added tripeptide complexes (23). These studies suggest that amelogenin evolution is associated with significant alterations in the physico-chemical properties of the amelogenin molecule.

Here we have confirmed that amphibian amelogenins are overall shorter than their mammalian counterparts (9), contain less proline and less glutamine, and most significantly, feature shorter

Front Oral Biol. Author manuscript; available in PMC 2010 January 1.

polyproline tripeptide repeat stretches than mammalian amelogenins. Moreover, there is ample evidence for a simple, prism-less enamel microarchitecture in most amphibians and reptilians, while mammalian enamel is often organized into prisms that frequently form a plywood structure (24). While direct evidence for a relationship between amelogenin gene structure and enamel prism architecture has not yet been established, we suggest that the increased length and changed composition of mammalian amelogenins provides a basis for an organized protein matrix that might promote increased enamel crystal length and prismatic architecture. Especially the presence of polyproline repeat motifs would provide a potential explanation for the increased rigidity of mammalian amelogenin protein assemblies and thus a mechanism for an orderly growth of long and parallel apatite crystals in complex prism patterns. In addition, the dolphin with its thin and radial enamel and its short polyproline amelogenin repeat motifs might provide one example of a "link" species, in which the lack of typical mammalian amelogenin characteristics are associated with a reduction in mammalian enamel features.

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Front Oral Biol. Author manuscript; available in PMC 2010 January 1.

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Diekwisch et al.



Figure 1. Enamel prisms and the non-mammalian/mammalian transition

Scanning electron micrographs resolve long and parallel enamel prisms in omnivores (human, *Homo sapiens*, and pig, *Sus scrofa*). Note the pronounced plywood structure in ruminants (steer, *Bos taurus*) and marsupials (Virginia opossum, *Didelphis virginiana*). The enamel layer of dolphins (La Plata river dolphin, *Pontoporia blainvillei*) is fairly thin for eutherians and consists mostly of radial enamel. The Spiny-tailed lizard (*Uromastyx maliensis*) is unique among squamates as its enamel is prismatic. In most squamates (e.g. Green Iguana, *Iguana iguana*) and amphibians (e.g. Leopard frog, *Rana pipiens*) the enamel is devoid of prisms.

Front Oral Biol. Author manuscript; available in PMC 2010 January 1.

Diekwisch et al.

Majority	MGTWILFACI_LGAAF.	AMP LPPHPGHPGYINF	SYE ·····	·V <mark>L</mark> TPLKWYOSMIRHP	YPS <mark>YGYEPMGG</mark> WLHHQ	9I1PVLSQQ	HPPS <mark>H</mark> TLQP	HHHI - PVV - PAQQ - PVV	PQQ-PMM-PVP-GQ	
Commo binous	NOTION DAGING AND		ave	UNTER VINCONTENIE				T DARL DAGO DURI	DOD DIAN DUD CO.	107
Capra nifcus	PIGIWIEFACELGAAF	SPIP SPPRFGRPGIINF	SIE	· VP IP BKWLQSMIKHP	1PS1G1EPMGGwLnnQ		TPONAADOP	THE PHU-PAQQ-PVV	POQ-PPM-PVP-GQ	107
Rog taurug (X)	MOTNEL FACIL GAAF	SMP L.PDHDGHDGYTNE	CVF	UT TEL KWYOSMIPHE	VPSVGVRPMGGWLHHO			T - DMV - PAQQ - FVV	POQ-PIM-DVP-GQ	107
Bos taurus (Y)	MGTWIL FACIL GAAF	SMP LPPHPGHPGYTNF	SYE	VITPLKWYOSMIRHP	YPSYGYEPMGGWLHHO	TTPVVS00	TPONHAIOP	HHUT-PMV-PAOO-PVV	POO-PMM-PVP-GO	107
Tursiops truncatus				VLTPLKWYONVIRHP	YPSYGYEPMGGWLRHO	DIIPVVSOO	AAP	HHF-PMV-PAOO-PVG	PCO-TMM-PIP-GO	69
Sus scrofa	MGTWIFFACLLGASL	AMP LPPHPGHPGYINE	SYE	VLTPLKWYONMIRHP	YTSYGYEPMGGWLHHO	DIIPVVS00	TPOSHAP	HHI-PMV-PAOO-PGI	PCO-PMM-PLP-GO	107
Loxodonta africana					YPSYGYEPMGGWLHHQ	IIPVLS	HPOSHTP	HHHF-PMV-AAQQ-PVV	PCO-PMM-PLP-GO	58
Equus caballus	MGTWILFASLLGAAF.	AMP LPPHPGHPAYINF	SYE	-VLTPLKWYOSLIROP	YTSYGYEPMGGWLHHO	IIPVLS00	NPSNHALQP	HHHI-PMV-SAOH-PVV	PCO-PMM-PLP-GO	
Pan troglodytes(Y)	MGTWILFACI, VGAAF.	AMP			YSSYGYEPMGGWLHHQ	IIPVVSQ	HPLTHTLQS	HHHI-PVV-PAQQ-PRV	PQQ-AMM-PVP-GQ	76
Homo sapiens(Y)	MGTWILFACLVGAAF.	AMP LPPHPGHPGYINF	SYE	·VLTPLKWYQSMIRPP	YSS <mark>YGYEPMGG</mark> WLHHQ	IIPVVSQQ	HPLT <mark>H</mark> TLQ <mark>S</mark>	HHHI-PVV-PAQQ-PRV	RCQ-ALM-PVP-GQ	107
Otolemur garnettii	MGTWILFACLLGAAF.	AMP LPPHPGHPGYINF	SYE -KSYSQAINIDRTAL	·V <mark>L</mark> TPVKWYQSMLRPP	YPS <mark>YGYEPMGG</mark> WLHHQ	IIPVLSQQ	HPPT <mark>H</mark> TLQP	HHHI-PVV-PAQQ-PVV	PQQ-PMM-PVP-GQ	121
Lemur catta	MGTWILFACLLGAAF.	AMP LPPHPGHPAYINF	SYE -NSHSQAISIYGTEL	·V <mark>L</mark> TPLKWYQSMIRPP	YPS <mark>YGYEPMGG</mark> WLHHQ	QII <mark>P</mark> VLSQQ	HPQT <mark>H</mark> TLQ <mark>P</mark>	HHHI-PVV-QAQQ-PVV	PQQ-PMM-PVP-GQ	121
Saimiri sciureus	MGTWILFACLLGAAF.	AMP LPPHPGHPGYINF	SYE ·NSHSQAINIDRTGL	·V <mark>L</mark> TPLKWYQSMIRPA	YPS <mark>YGYEPMGG</mark> WLHHQ	QII <mark>P</mark> VLSQQ	HPPT <mark>H</mark> TLQ <mark>P</mark>	HHHI-PVV-PAQQ-PVV	PQQ-PMM-PVP-GQ	121
Homo sapiens(X)	MGTWILFACLLGAAF.	AMP		·VLTPLKWYQS-IRPP	YPS <mark>YGYEPMGG</mark> WLHHQ	QII <mark>P</mark> VLS <mark>QQ</mark> I	HPPT <mark>H</mark> TLQP	HHHI-PVV-PAQQ-PVI	PQQ-PMM-PVP-GQ	90
Pan troglodytes(X)	MGTWILFACLLGAAF.	AMP LPPHPGHPGYINF	SYE ·NSHSQAINVDRTAL	·VLTPLKWYQS-IRPP	YPS <mark>YGYEPMGG</mark> WLHHQ	QII <mark>P</mark> VLS <mark>QQ</mark> I	HPPT <mark>H</mark> TLQ <mark>P</mark>	HHHI-PVV-PAQQ-PVI	PQQ-PMM-PVP-GQ	120
Rattus norvegicus	MGTWILFACILGAAF.	AMP LPPHPGSPGYINI	SYE ·KSHSQAINTDRTAL	·VLTPLKWYQSMIRQP	YPSYGYEPMGGWLHHQ	QIIPVLSQQ	HPPS <mark>H</mark> TLQP	HHHL-PVV-PAQQ-PVA	PQQ-PMM-PVP-GH	121
Mus musculus	MGTWILFACLLGAAF.	AMP LPPHPGSPGYINI	SYE	·VLTPLKWYQSMIRQP	YPSYGYEPMGGWLHHQ	9I1PVLS00	HPPSHTLQP	HHHL-PVV-PAQQ-PVA	PQQ-PMM-PVP-GH	107
Mesocricetus auratus	····	-MP LPPHPGSPGYINI	SYE ·KSHSQAINTDRTAL	-VLTPLKWYQSMIRQP	YPSYGYEPMGGWLHHQ	011PVLS00	HPQS <mark>H</mark> TLQP	HHHL-PVV-PAQQ-PVA	PCQ-PMM-PVP-GH	105
Cavia porcellus	MGTWILFACILGTAF.	AMP LPPHPGHPGYINF	SYE ·KSHSNAINIDRTAL	-VLTPLKWYQSMIRQP	YPSYGYESMGGWVHHQ	0VIPVLS00	HPPS <mark>H</mark> TTLPP	HHHI-PVG-PAQQ-PVV	PCQ-PLM-PVP-GH	122
Monodelphis domestica	••••••••••••••••••••••••••••••••••••••	- IP LPPHPGHPGY INF	SYE	-VLTPLKWYQSMMRHE	YPSYGYEPMGGWLHHQ	011PVLS00	HSPSHSP	OHHI-PIM-AAQQ-PAP	PCQ-PVM-PVP-GQ	91
Litoria chloris	MKSCIMLTSLIGAAF	SIP LPLHPQHPGYVNL	SYE ·····	· ILTPLKWYQSMLKHQ	YPSYGYEPMSGWLKNP	PVAPM-S-PMMPQQ	-PLS <mark>H</mark> PHAIPKLP <mark>L</mark>	HHPMLM	POP-PMV-SAH-GH	106
Rana pipiens	MKSWIILTSLIGAAF	CLP LPPHPQHPGYVNL	SYE ·····	· ILTPLKWYQSMMKHQ	YPSFGYEPMSGWLQNP	PMIPL-S-PMMP00	-QLP <mark>H</mark> PHAIPKLP <mark>P</mark>	HHSVLI	PCQ-PMI-PLS-PH	106
Xenopus laevis	MRPWLMLTALIGVAF	SVP LPPHPQHPGYVNF	SYE	· ILSPLKWYQSMMTHQ	YPNYGYEPVSGWLQNP	PIIPA-P-PMMP00	2-Q- <mark>-</mark> -NAVPKLP <mark>P</mark>	IIIPLLI	POH-PLV-PVP-VH	103
Ambystoma mexicanum	MGPWILLTCILSASC.	AMP LPPHPNHPGYINF	SYE	-VMTPLKWYQSMMRQQ	YPSYGYEPMGGWLQSQ	0MMPG-S-PMMP00	H-M- <mark>-</mark> -QPIPQMQ <mark>P</mark>	HHPMML	PCQ-PML-PH	100
Plethodon cinereus	MRTLILLTCLLSATF.	AMP LPPHPSHPGYINF	SYE	-VMTPLKWYQSMMRQQ	YPSYGYEPISGWLQSQ	VMPG-S-PMMPPQ	H-LPHLHAVPQMQP	HHPMML	PCQ-PML-PH	103
Chalcides viridanus	ILSATF.	AIP LPPHARHPGYVNF	SYE	-VITPLKWYQSLIGHQ	YPRYGYEPIGSWMRHH	IS-G-PMLPQ-	LPHQSIYQSLH-PMQTP	LHQLH-PLP-	PON-PSM-QQP-AH	98
Paleosuchus palpebrosus	MEGWMLITCHLGATF.	AIP LPPHPHPPGYVNF	SYE	-VLTPLKWYQSLMRQP	-YSSYGYEPMGGWLHQ-	PMLPIAQQ	H-PP <mark>I</mark> QTLTP	HHQIP-FLS-	POH-PLM-QMP-GP	100
Elaphe quadrivirgata	····			-VMTPLKWRQSIIGHQ	PRYGYEPMGGWMHHN	IAGPMM	H-PPHFHGVHA	H PAL-HQKQPQQL	PQN-PQL-QQP-GL	70
	EX011 Z	EXUITS	EX0114	EXUND			EXONO			
Majority	H-SMT-PTQ-HHQ-P	NL-PPP-AQQ-PFQ	PQ-PVQPH	IQ		PMQ - PQP - PVH - PMQ	- PLP - PQP - PLP - PMF - PM	2 - PLP - - PML - PDLPLEAW	PATDKTKREEV ·	D
Capra hircus	H-SMT-PTO-HHO-P	NT PI.P ACO - PEO	PO-STO	O-PLO-PLO-PMO-PLO-PLO		PLO-POP-PVH-PTO	PLP DOD PLL DIF PM	- HI.P PMT - PDVI.I.PDW	DATINTER PREV .	D 207
Hippopotamus amphibius	H-SMT-PTO-HHO-P	VE-DLP-MOO-DEO	PO-PTOPOPH	the stand many stand many stand	*	HLO-DKD-DMH-DTO	PLI - DOD - DIS - DMF - DM	-T.T.PT.MT P		124
Bos taurus(X)	H-SMT-PTO-HHO-P		PO-STOPOPH	IO - DI O - DHO - DI O - DMO - DM		DLO DOD DIN DTO		A PERSON AND A		
DOD COULOD (11)						Manual a local of a second a second sec	- DID - DOD - DID - DIF - DM	DID_ DIT.D_ DMI DT.DI.DI	DATDRTKPERV .	D 213
Bog taurug(V)	H-SMT-PTO-HHO-P	NL-PLP-AQQ-PFQ	PO-PTOPH	10-210-210-210-210-210	0- <u>MB0</u> - <u>HEQ</u>	PLO-POP-PVH-PIQ	- PLP - PQP - PLP - PIF - PM - PLP - POP - PLP - PIF - PM	- PLP PML - PDLPLEAW	PATDKTKREEV -	D 213
Bos taurus(Y) Tursions truncatus	H-SMT-PTQ-HHQ-P	NL-PLP-AQQ-FFQ NL-PLP-AQQ-SFQ	PQ-PIQPQPH	IQ	0- MDO -MDO	PLQ-PQP-PVH-PIQ	- PLP - PQP - PLP - PIF - PM - RLP - PQP - PLP - PIF - PM - PLL - POP - PLP - PMF - PM	0 - PLP PML - PDLPLEAW 0 - PLP PVL - PDLPLEAW 0 - PLP PML - PDLPLEAW	PATDKTKREEV · PATDKTKREEV · PATD	D 213 D 192
Bos taurus(Y) Tursiops truncatus Sus scrofa	H-SMT-PTQ-HHQ-P H-SMT-PSQ-PHQ-P H-SMT-PTO-HHQ-P	NL-PLP-AQQ-PFQ NL-PLP-AQQ-SFQ HL-PVP-AQQ-P	PQ-PIQPQPH	(Q	2-MBQ-PEG2	PLQ - PQP - PVH - PIQ	- PLP - PQP - PLP - PIF - PM - RLP - PQP - PLP - PIF - PM - PLL - PQP - PLP - PMF - PM - PLI - POP - PIP - PMF - SM	2-PLPPML-PDLPLEAW 2-PLPPVL-PDLPLEAW 2-PLPPML-PDLPLEAW 2-SLL-PDLPLEAW	PATDKTKREEV · PATDKTKREEV · PATD PATDKTKREEV ·	D 213 D 192 129 D 189
Bos taurus(Y) Tursiops truncatus Sus scrofa Loxodonta africana	H-SMT-PTQ-HHQ-P H-SMT-PSQ-PHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P	NL-BLP-AQQ-FFQ HL-PVP-AQQ-P NL-PLP-AQQ-PFQ NL-PLP-AQQ-PFQ	PQ-PIQ	IQ	0-800-800-	PLQ-PQP-PVH-PIQ PLQ-PQS-PMH-PIQ PLQ-PQS-PMH-PIQ PMQ-POP-PVH-PIQ	- PLP - PQP - PLP - PIF - PM - RLP - PQP - PLP - PIF - PM - PLL - PQP - PLP - PMF - PM - PLL - PQP - PLP - PMF - SM - PLP - POP - PLP - PMF - PM	D- PLP PML-PDLPLEAW D- PLP PVL-PDLPLEAW D- PLP PML-PDLPLEAW D- SL L-PDLPLEAW D- PLP PML-HDLPLEAW	PATDKTKREEV - PATDKTKREEV - PATD PATDKTKREEV - PATD	D 213 D 192 129 D 189 135
Bos taurus(Y) Tursiops truncatus Sus scrofa Loxodonta africana Ecuus caballus	H-SMT-PTQ-HHO-P H-SMT-PSQ-PHO-P H-SMT-PTQ-HHO-P H-SMT-PTQ-HHO-P H-SMT-PTQ-HHO-P	NL-PLP-AQQ-PFQ HL-PVP-AQQ-SFQ HL-PLP-AQQ-P NL-PPP-IQQ-PFQ NL-PPP-IQQ-PYQ	PQ - PIQ PQ PI VQ PQ PI PQ - PVQ PQ PI PQ - PVQ PQ PI PQ - PVQ PQ PQ	10	- HID - PILO -	PLO - POP - PVH - PIO PLO - POP - PVH - PIO PLO - POP - PVH - PIO PMO - POP - PVH - PIO PIO - POP - PLH - PIO	- PLP - PQP - PLP - PIF - PM - RLP - PQP - PLP - PIF - PM - PLL - PQP - PLP - PMF - PM - PLP - PQP - PLP - PMF - SM - PLP - PQP - PLP - PMF - PM - PLP - POP - PLP - PIF - PL	0 - PLP PML - PDLPLEAW 0 - PLP PVL - PDLPLEAW 0 - PLP - PML - PDLPLEAW 0 - SL L - PDLPLEAW 0 - PLP - PML - HDLPLEAW 0 - PLP - PML - PDLPLEAW	PATDKTKREEV - PATDKTKREEV - PATD PATDKTKREEV - PATD PATDKTKREEV -	D 213 D 192 129 D 189 135 D 192
Bos taurus(Y) Tursiops truncatus Sus scrofa Loxodonta africana Equus caballus Pan troolodytes(Y)	H - SMT - PTQ - HHQ - P H - SMT - PSQ - PHQ - P H - SMT - PTQ - HHQ - P H - SMT - PTQ - HHQ - P H - SMT - PTQ - HHQ - P O - SMT - PTQ - HHQ - P	NL-PLP-AQ-FFQ HL-PVP-AQ-FQ NL-PLP-AQ-FQ NL-PLP-AQ-FQ NL-PPP-IQ-FYQ NL-PPP-VQ-PFH	PQ-PIQ	10	- HID - PILO -	PLQ - PQP - PVH - PIQ PLQ - PQS - PVH - PIQ PMQ - PQS - PVH - PIQ PMQ - PQP - PVH - PIQ PIQ - PQP - PLH - PIQ PMO - POP - PVO - PMO	- FLP - PQP - PLP - PIF - PM - RLP - PQP - PLP - PIF - PM - PLL - PQP - PLP - PMF - PM - PLL - PQP - PLP - PMF - SM - PLP - PQP - PLP - PMF - PM - PLP - PQP - PLP - PMF - PM - PLL - POP - PLP - PMF - PM	0 - PLP PML - PDLPLEAW 0 - PLP PVL - PDLPLEAW 0 - PLP PML - PDLPLEAW 0 - PLP L - PDLPLEAW 0 - PLP - PML - HDLPLEAW 0 - PLP - PML - PDLPLEAW R - PLP - PTL - PDLHLEAW	PATDKTKREEV - PATDKTKREEV - PATD PATDKTKREEV - PATD PATDKTKREEV - PATDKTKREEV - PATDKTKREEV -	D 213 D 192 129 D 189 135 D 192 VSTP 164
Bos taurus(Y) Tursiops truncatus Sus scrofa Loxodonta africana Equus caballus Pan troglodytes(Y) Homo sapiens(Y)	H-SMT-PTQ-HHQ-P H-SMT-PSQ-PHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P Q-SMT-PTQ-HHQ-P Q-SMT-PTQ-HHQ-P O-SMT-PTQ-HHQ-P	NL = LP - AQ - FYQ NL = PLP - AQ - SFQ	PQ-PIQ PQ PI PQ-PIQ PQ PQ PQ-PVQ PQ PQ	10	- HIND - HILD	PLQ - PQP - PVH - PIQ PLQ - PQS - PMH - PIQ PMQ - PQP - PVH - PIQ PMQ - PQP - PVH - PIQ PMQ - PQP - PVQ - PMQ PMQ - PQP - PVQ - PMQ	PLP PQP PDP PLP PIF PM RLP PQP PLP PIF PM PLL PQP PLP PMF PM PLP PQP PLP PMF PM PLP PQP PLP PMF PM PLP PQP PLP PMF PM PLP PQP PLP PMF PM	0-PLP-PML-PDLPLEAW 0-PLP-PML-PDLPLEAW 0-PLP-PML-PDLPLEAW 0-SLL-PDLPLEAW 0-PLP-PML-PDLPLEAW 0-PLP-PML-PDLPLEAW R-PLP-PIL-PDLPLEAW R-PLP-PIL-PDLPLEAW	PATDKTKREEV - PATDKTKREEV - PATD PATDKTKREEV - PATDKTKREEV - PATDKTKREEV - PATDKTKOBEV - PATDKTKOBEV -	D 213 D 192 L29 D 189 135 D 192 VSTP 164 D 192
Bos taurus (Y) Tursiops truncatus Sus scrofa Loxodonta africana Equus caballus Pan troglodytes (Y) Homo sapiens (Y) Otolemur qarnettii	H-SMT-PTQ-HHQ-P H-SMT-PSQ-PHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMV-PTQ-HHQ-P Q-SMT-PTQ-HHQ-P Q-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P	NL = UP - AQ Q = FPQ +L = PVP - AQ Q = SPQ - VL = PUP - AQ Q = PPQ - VL = PUP - AQ Q = PPQ - VL = UP - AQ Q = PPQ - VL = UP - AQ Q = PPQ - VL = UP - AQ Q = PPQ 	PQ-PIQ PQ PQ PQ PQ-PVQ PQ PQ PQ	00-2000-2000-2000-2000-2000-2000-2000-	- HILD	PLO - PQP - PVH - PIQ PLO - PQS - PMH - PIQ PLO - PQP - PVH - PIQ PLO - PQP - PVH - PIQ PLO - PQP - PVQ - PMQ PMQ - PQP - PVQ - PMQ PMO - PQP - PVH - PMO	PLP = PQP = PLP = PLF = PM PLL = PQP = PLP = PLP = PMF = PM PLL = PQP = PLP = PMF = PM PLP = PQP = PLP = PMF = PM PLL = PQP = PLP = PMF = PM PLL = PQP = PLP = PMF = PM PLL = PQP = PLP = PMF = PM PLL = PQP = PLP = PMF = PM	0 - PLP PML - PDLPIE RAW 0 - PLP - PML - PDLPIE RAW R - PLP - PIL - PDLHIE RAW 0 - PLP - PML - PDLHIE RAW 0 - PLP - PML - PDLHIE RAW	PATDKTKREEV - PATDKTKREEV - PATD PATDKTKREEV - PATDKTKREEV - PATDKTKGEEV - PATDKTKGEEV - PATDKTKGEEV - PAT	D 213 D 192 129 D 189 135 D 192 VSTP 164 D 192 209
Bos taurus(Y) Tursiops truncatus Sus scrofa Loxodonta africana Equus caballus Pan troglodytes(Y) Homo sapiens(Y) Otolemur garnettii Lemur catta	H-SMT-PTO-HHO -P H-SMT-PTO-HHO -P H-SMT-PTO-HHO -P H-SMT-PTO-HHO -P H-SMT-PTO-HHO -P Q-SMT-PTO-HHO -P Q-SMT-PTO-HHO -P H-SMT-PTO-HHO -P H-SMT-PTO-HHO -P H-SMT-PTH-HHO -P	NI = 11 - AUQ - FPQ III = 11 - AQQ - SPQ VIL = 11 - AQQ - PPQ VIL = 11 - AQQ - PPQ VIL = 11 - PP - IQQ - PPQ VIL = 11 - QQ - PPQ VIL = 11 - AQQ - PPQ - VIL = 11 - AQQ - PP	PQ-PIQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PVQ- PQ-PQ- PQ-PVQ- PQ-PQ- PQ-PV	0 0 0 0 0 0 0 0 0 0 0 0 0 0	- MINU - MINU 	PLO - PQP - PVH - PIO PLO - PQP - PVH - PIO PMO - PQP - PVH - PIO PMO - PQP - PVH - PIO PMO - PQP - PVO - PMO PMO - PQP - PVO - PMO PMO - PQP - PVH - PMO PIO - POP - PUH - PMO	$\begin{array}{l} \text{PLE} = \text{POP} = \text{PLP} = \text{PLP} = \text{PN} \\ \text{RLE} = \text{POP} = \text{PLP} = \text{PLP} = \text{PN} \\ \text{PLL} = \text{POP} = \text{PLP} = \text{PNP} = \text{PN} \\ \text{PLL} = \text{POP} = \text{PLP} = \text{PNP} = \text{PNP} = \text{PN} \\ \text{PLL} = \text{POP} = \text{PLP} = \text{PNP} = \text{PNP} = \text{PNP} \\ \text{PLL} = \text{POP} = \text{PLP} = \text{PNP} = $	0 - P.D PML - PDLPI EAN 0 - PLP - PML - PDLPI EAN 1 - PLP - PML - PDLPI EAN 2 - PLP - PIL - PDLHI EAN 2 - PLP - PIL - PDLHI EAN 0 - PLP - PML - PDLPIEAN 0 - PLP - PML - PDLPIEAN 0 - PLP - PML - PDLPIEAN	PATDKTKREEV - PATDKTKREEV - PATD PATDKTKREEV - PATD PATDKTKREEV - PATDKTKGEEV - PATDKTKGEEV - PATT PAT	D 213 D 192 D 189 D 189 U 192 VSTP 164 D 192 209 215
Bos taurus(Y) Tursiops truncatus Sus scrofa Loxodonta africana Equus caballus Pan troglodytes(Y) Homo sapiens(Y) Otolemur garnettii Lemur catta Saimiri sciureus	H-SMT-PTO-HHC-P H-SMT-PSO-PHC-P H-SMT-PSO-PHC-P H-SMT-PTO-HHC-P H-SMT-PTO-HHC-P G-SMT-PTO-HHC-P G-SMT-PTO-HHC-P H-SMT-PTO-HHC-P H-SMT-PTO-HHC-P H-SMT-PTO-HHC-P	NL LP AQ PFQ NL PL AQ SFQ NL PL AQ PFQ NL PF IQ PFQ NL PP IQ PFQ NL PP IQ PFQ NL PP AQ PFQ	PQ-PIQ PQ-PIQ PQ-PIQ PQ-PVQ PQ-PIQ PQ-PIQ	C	- MAR - MAR - PTO	PLC = PQF = PVH = PIQ PLC = PQF = PVH = PIQ PMC = PQF = PVH = PIQ PIQ = PQF = PVH = PIQ PMC = PQF = PVQ = PMQ PIQ = PQF = PVH = PMQ PIQ = PQF = PVH = PMQ	PLD = PQP = PLD = PLF = NN FLD = PQP = PLD = PLF = NN PLL = PQP = PLD = PLF = NN PLL = PQP = PLD = PLF = NN PLD = PQP = PLD = PLF = PL PLL = PQP = PLD = PLF = NN PLL = PQP = PLD = PLF = NN PLD = PQP = PLF = PLF = PLF = PLF = NN PLD = PQP = PLF = PLF = NN PLD = PQP = PLF = PLF = NN PLD = PQP = PLF = PLF = PLF = NN PLD = PQP = PLF = PLF = PLF = NN PLD = PQP = PLF = PLF = PLF = NN PLD = PQP = PLF = PLF = PLF = NN PLD = PQP = PLF	0 - DLP - PML - PDLPI SAN 0 - PLP - PML - PDLPI SAN	PATDKTKREEV - PATDKTKREV - PATD PATDKTKREEV - PATDKTKREEV - PATDKTKGEV - PATDKTKGEV - PATDKTKGEV - PAT	D 213 D 192 129 D 189 135 D 192 VSTP 164 D 192 209 215 197
Bos taurus(Y) Tursiops truncatus Sus scrofa Loxodonta africana Equus caballus Pan troglodytes(Y) Homo sapiens(Y) Otolemur garnettii Lemur catta Saimiri sciureus Homo sapiens(X)	H-SMT-PTO-HHG -P H-SMT-PTO-HHG -P H-SMT-PTO-HHG -P H-SMT-PTO-HHG -P H-SMT-PTO-HHG -P Q-SMT-PTO-HHG -P Q-SMT-PTO-HHG -P H-SMT-PTO-HHG -P H-SMT-PTO-HHG -P H-SMT-PTH-HHG -P H-SMT-PTO-HHG -P H-SMT-PTO-HHG -P	NL LP AQ PFQ NL LP AQ SPQ NL PV AQ P NL PV AQ P NL PV AQ P NL PV AQ PFQ NL PV AQ PQ	PQ-PIQ PQ <		- MAR - MAR 	PIC - POP - PVH - PIC PIC - POP - PVO - PVO PIC - POP - PVH - PVO	$\begin{array}{c} \mathbf{L} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} = \left\{ \mathbf{C} \mathbf{C} + \mathbf{C} \mathbf{L} \mathbf{C} = \mathbf{C} \mathbf{L} \mathbf{C} = \mathbf{C} \mathbf{L} \mathbf{C} \right\} \\ \mathbf{L} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} = \mathbf{C} \mathbf{L} \mathbf{C} = \mathbf{C} \mathbf{L} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} = \mathbf{C} \mathbf{L} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} = \mathbf{L} \mathbf{L} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{D} \mathbf{L} = \left\{ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{C} \mathbf{L} \mathbf{C} \right\} \\ \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C} \right\} \\ \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C} \\ \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C} \mathbf{C}$	> PLP - PMI - PDLP: SA	PATDKTKREEV - PATD PATDKTKREEV - PATD PATDKTKREEV - PATDKTKREEV - PATDKTKREEV - PAT PAT PAT PAT PAT	D 213 D 192 129 D 189 135 D 192 VSTP 164 D 192 209 215 197 D 175
Bos taurus(Y) Tursiops truncatus Sus scrofa Loxodonta africana Equus caballus Pan troglodytes(Y) Homo sapiens(Y) Otolemur garnettii Lemur catta Saimiri sciureus Homo sapiens(X) Pan troglodytes(X)	H-SMT-PTQ-HHQ-P H-SMT-PSQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P Q-SMT-PTQ-HHQ-P Q-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P	LL LP AQ FPQ LL LP AQ SPQ LL LP AQ PP LL LP AQ PP LL LP AQ PP LL LP AQ PP LL PP IQ PP	PQ-PIQ PQ <	0 - PMO - PM) - <u>BIO</u>	PEG - POP - PMI - PIO PEG - POP - PMI - PIO PMG - POP - PMI - PIO PTG - POP - PMI - PIO PTG - POP - PMO - PMO PMG - POP - PMO - PMO PTG - POP - PMI - PMO PMG - POP - PMI - PMI - PMO PMG - POP - PMI - PMI - PMO PMG - POP - PMI - PMI - PMI - PMI - PMO PMG - PMI -	$\begin{array}{c} LLB = 0(D-PLLB = 0 LB =$	PIP-POINT PDIP-POINT PDIP-POI	PATDKTKREEV - PATD PATDKTKREEV - PATD PATDKTKREEV - PATDKTKREEV - PATDKTKQEEV - PATDKTKQEEV - PAT PATTKKREEV - PAT	D 213 D 192 129 D 189 135 D 192 VSTP 164 D 192 209 215 197 D 175 200
Bos taurus(Y) Tursiops truncatus Sus scrofa Loxodonta africana Equus caballus Pan troglodytes(Y) Homo sapiens(X) Otolemur garnettii Lemur catte Saimiri sciureus Homo sapiens(X) Pan troglodytes(X) Rattus norvegicus	H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P Q-SMT-PTQ-HHQ-P Q-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P H-SMT-PTQ-HHQ-P	$N_{L} = L_{P} - A_{Q} - PFQ$ $N_{L} = L_{P} - A_{Q} - SFQ$ $N_{L} = L_{P} - A_{Q} - P$ $N_{L} = L_{P} - A_{Q} - PFQ$ $N_{L} = PP - I - Q - PFQ$ $N_{L} = PP - I - Q - PFQ$ $N_{L} = LP - A_{Q} - PFQ$ $N_{L} = PP - A_{Q} - PFQ$	PQ-PIQ PQ-PIQ PQ-PIQ PQ-PIQ PQ-PVQ PQ-PIQ		2 - MAND - MANU- 5 - PITO -	PRG - POP - POM - PIO PRG - POP - PMM - PMO PRG - PMO	0.00 0.00 <td< td=""><td>0 - 01.0 - MM - PDLP: BAN - 91.0 - VM - PDLP: BAN - 91.0 - VM - PDLP: BAN - 91.0 - MM - PDLP: BAN - 91.0 - MM - PDLP: BAN - 91.0 - NM - PDLP: BAN - 91.0 - NM - PDLH: BAN - 91.0 - NM - PDLH: BAN - 91.0 - MM - PDLP: BAN</td><td>PATDKTKREEV - PATD PATD PATD PATD PATD PATD PATD PATD</td><td>D 213 D 192 129 D 189 D 189 USTP 164 D 192 209 215 D 175 D 175 200 D 210</td></td<>	0 - 01.0 - MM - PDLP: BAN - 91.0 - VM - PDLP: BAN - 91.0 - VM - PDLP: BAN - 91.0 - MM - PDLP: BAN - 91.0 - MM - PDLP: BAN - 91.0 - NM - PDLP: BAN - 91.0 - NM - PDLH: BAN - 91.0 - NM - PDLH: BAN - 91.0 - MM - PDLP: BAN	PATDKTKREEV - PATD PATD PATD PATD PATD PATD PATD PATD	D 213 D 192 129 D 189 D 189 USTP 164 D 192 209 215 D 175 D 175 200 D 210
Bos taurus(Y) Tursiops truncatus Sus sorofa Loxodonta africana Equus caballus Pan troglodytes(Y) Homo sapiens(Y) Otolemur garnettii Lemur catta Saimiri sciureus Homo sapiens(X) Pan troglodytes(X) Rattus norvegicus Mus musculus	H - SKT - PTO - HIO - P H - SKT - PTO - HIO - P O - SKT - PTO - HIO - P H - SKT - PTO - HIO - P	LL LP. AQ. FFQ LL P. AQ. FFQ LL VP. AQ. PFQ LL VP. AQ. PFQ LL VP. AQ. PFQ LL VP. YQ. PFQ LL VP. YQ. PFQ LL VP. AQ. PFQ	PQ-FIQ -O VQ -O PQ-FIQ -O Q-FIQ-FIQ-AIP -O Q-FIQ-FIQ-AIP -O Q-FIQ-FIQ-AIP -O		2 - 2010	PUC = PUC = PUC = 200 PUC = PUC = PUC = PUC = PUC = 200 PUC = PUC = PUC = PUC = 200 PUC = PUC = PU	1000 0.000		PATDKTKREV PATD PATDKTKREV PATD PATD PATD PATDKTKREV PATDKTKREV PATDKTKREV PAT PAT PAT PATKREV PSTDKTKREV PATDKTKREV	D 213 D 192 129 D 189 D 192 VSTP 164 D 192 VSTP 209 215 197 D 175 200 D 175 200 D 196
Bos taurus(Y) Tursiops truncatus Sus sorofa Loxodonta africana Equus caballus Pan troglodytes(Y) Homo aspiens(Y) Ocolemur garnettii Lainerta Homo aspiens(X) Pan troglodytes(X) Pan troglodytes(X) Rattus norvegicus Mus musculus	H - SMT - PTQ - HHQ - P. H - SMT - PSQ - PHQ - PH H - SMT - PTQ - HHQ - P. H - SMT - PTQ - HHQ - P. H - SMT - PTQ - HHQ - P. Q - SMT - PTQ - HHQ - P. H - SMT - PTQ - HHQ - P.	LL DF AQ SFQ LL VF AQ SFQ LL SFF AQ SFG LL SFF AQ SFG	PQ-PIQ PQ-PIQ PQ-PIQ PQ-PVQ PQ-PIQ PQ-PIQ PQ-PVQ-AIP-PQ-SIP PQ-PIQ PQ-PIQ Q-PPQ-PQ-AIP-PQ-SIP PQ-PIQ PQ-PIQ		2- BID	BUC BUC <td></td> <td>The second secon</td> <td>PATDKTKREV PATDKTKREV PATD PATDKTKREV PATD PATDKTKREV PATDKTKREV PATDKTKREV PATDKTKREV PAT PATKTKREV PATDKTKREV PATDKTKREV</td> <td>D 213 192 129 D 189 D 189 USTP 164 209 215 197 D 175 D 197 D 200 D 200 D 210 D 196 D 194</td>		The second secon	PATDKTKREV PATDKTKREV PATD PATDKTKREV PATD PATDKTKREV PATDKTKREV PATDKTKREV PATDKTKREV PAT PATKTKREV PATDKTKREV PATDKTKREV	D 213 192 129 D 189 D 189 USTP 164 209 215 197 D 175 D 197 D 200 D 200 D 210 D 196 D 194
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Figure 2. Conserved regions and evolutionary "hotspots" among tetrapod amelogenins

Note the highly conserved sequence elements in encoded by exons 2, 3, and 5 (marked in red and green). In contrast, the region encoded by exon 6 reveals significant differences among species. Conserved areas of polyproline tripeptide repeats are labeled in gray. This alignment features four newly discovered amphibian amelogenins (Red-eyed tree frog, *Litoria chloris*, accession number DQ069788; Mexican axolotl *Ambystoma mexicanum*, accession number DQ069791; Eastern Red-backed Salamander *Plethodon cinereus*, accession numbers DQ069789 and DQ069789). The Leopard frog (*Rana pipiens*) amelogenin sequence was reported earlier by our group (#AY695795).

Percent Increase of Amelogenin Amino Acid Sequence Parameters in Mammalian versus Amphibians/Reptilians



Figure 3. Differences between mammalian and non-mammalian amelogenin sequence parameters A numerical comparison of key parameters of the translated amelogenin protein sequence between mammalian and non-mammalian species revealed that the overall amelogenin length was increased by 9%, the proline content increased by 21.5%, the glutamine content increased by 31.3%, and the number of proline-tripeptide repeats increased by 72.8%.