

	30	40	50	60	70	130	
<i>Homo sapiens</i> ***	GN	GTSMISLIIPK	QISRVA	KMLADE	FGTASN	IKSRVNR	SVL // LYLCDNKFH
<i>Arabidopsis thaliana</i> ***		M.R.V..T..G..Y..			Q..		//
<i>Dictyostelium discoideum</i> ***		R.G..AK.N..E.Y..					//
<i>Thecamonas trahens</i> ***	S..	T.A.G..V.SL	TRL.T.A.S				// M.M..SR..
<i>Emiliana huxleyi</i> ***		L.GS..QAS..E.Y..					//
<i>Guttulinopsis vulgaris</i> ***		V..V..T.L.Q.SQ..IT.E..			Q..		// M..R..
<i>Giardia intestinalis</i> ***	S..	V..L..GE.L.S.TG..TN.Y..			KNA		// I..S..
<i>Roombia truncata</i> ***			R..		N..		//
<i>Trichomonas vaginalis</i> ***	D..	VTIL..G.AVH.MRQK.T..L..			N..Q..E		// I..RR..
<i>Reticulomyxa filosa</i> ***		VV..	Q..Q..H..				//
<i>Entamoeba histolytica</i> ***	D..	T..MK.ME..KMMQK.VE.Y..			Q..		// T..R..
<i>Nannochloropsis gaditana</i> ***			R..N..Y..T..T..				//
<i>Ostreococcus lucimarinus</i> ***		V..G.VNL.Q..IE.M..			T..Q..		// M..
<i>Reclinomonas americana</i> ***		L..GE..AN..SE.Q..T..			Q..		//
<i>Telonema subtilis</i> ***		L..D.A.A..G..Q..					//
<i>Symbiodinium minutum</i> ***		A.T..LNA.QD.NLTN..TE.Y..			G..		// M..S..
<i>Didinium nasutum</i> ***	S..	T..NF..NIH.R..E.V..K..			T.QN.T		// K..SV..
<i>Dileptus margaritifer</i> ***	S..		NY..NPFM..V..V..K..		T.QN.T		// R..SV..
<i>Litonotus pictus</i> ***	S..	T..NS..	PSMR..TE.V..K..T..		T.QN.T		// R..SV..
<i>Climacostomum virens</i> ***		L..SR..LNFAS.LVTE.Y..K..A..			V.QA.Q		//
<i>Protocruzia adhaerens</i> ***			A..VAKAN..VE..		TT.Q..		//
<i>Fabrea salina</i> ***		L..	LPMTN..ITE.Y..K..A..		V.NA.Q		// I..Q
<i>Blastocl. emersonii</i> ***		L..E.LP..N..Y..A..					// M.M..
<i>Naegleria gruberi</i> ***			A..T..QE.Y..		I..Q		//
<i>Trypanosoma brucei</i> ***	M..	V..IYMT..E..GMVTK.NN.Y..			HT.K..Q		//
<i>Euglena gracilis</i> ***		T.MLT..	ALMT.K.NE.Y..T..H..K..				// M.M..
<i>Holosticha</i> sp. QQ*		V..VV..	D.N..S.L.TM.LS..Q..QT.Q..I				// F.F.GG..Q
<i>Tetrahymena thermophila</i> QQ*	S..	V..	K..NDST.LIS..SK.T..D..		Q..Q		// F.F.S..
<i>Sterk. histriomuscorum</i> QQ*		V..	D.N.KYG.L.TG.MSA.Q..ITKQ..V				// F.F.GG..Q
<i>Paramecium tetraurelia</i> QQ*	TA..	VV.VY..	RI..DITNR.NTOYAE.AS..DKG..I..Q				// F.F.PQ..
<i>Loxodes striatus</i> QQ*		L..	E..LINQK.T.AH.R.Q..KAVQK.Q				// FF.E.T.Y
<i>Gonostomum</i> sp. QQ*		V..V..	DVM..N.L.TQ.ISS.G..SKT.Q..Q				// F.F.S..K
<i>Pseudoco. persalinus</i> QQ*	STT..	L..NK..DYT..T..ISQ.NS..D..QA.Q					// I.R..S..Y
<i>Eschaneustyla</i> sp. QQ*		T..V..	D.NIIT.KVTQ.LSS.Q..TT.Q..I				// A.Y..R..
<i>Urostyle</i> sp. QQ*		VT.VV..	ED.NM.S.K.TQ.LSS.QS..KTT.S..				// F.F.S..
<i>Oxytricha trifallax</i> QQ*		V..	D.NKYG.L.TG.MSA.Q..ITKQ..V				// F.F.GG..Q
<i>Uroleptus</i> sp. QQ*		V..V..	D.N.SG.L..S.LSA.N..IT.Q..				// M.F.GG..Q
<i>Paraurostylea weissaei</i> QQ*		V..	D.N.SG.L.VQ.LSA.N..IT.Q..				// M.F.GG..Q
<i>Stichotrichida</i> sp. QQ*		V..V..	DVN..G.L.TQ.LSQ.Q..ITKQ..I				// F.F.GG..Q
<i>Tetmemena pustulata</i> QQ*		V..V..	D.NKYN.LIVG.LSA.Q..KITKQ..T				// F.F.GG..Q
<i>Ichthyop. multifiliis</i> QQ*	Q..	V..L..K..	DAT.L.NE.Y.K.T..D..I..Q				// FIFIVN.M
<i>Strombidium inclinatum</i> QQ*		VT.V..	EAVAQ.TSF.NK.YAE.A..KQMT..Q				// M.K.E.R..
<i>Pseudokerenopsis</i> sp. QQ*		T.VV..	D.NIIT.K.NL.QSS.Q..N..QT.Q..M				// V.F..S..
<i>Flavella ehrenbergii</i> QQ*		FV..Y..	AG.PVTKHMQ.L.S.LAG.ES..QT.Q..				// A.K.Q.T.Y
<i>Aristerostoma</i> sp. QQ*		V..V..	K.L.DITQK.TE.I.A..NIV.K..				// Q..SS..
<i>Uronema</i> sp. QQ*	CS..	T.V..	NK.MAEFTRH.TT.YSE.NS..D..A..Q..Q				// F.R..S..Y
<i>Spironucleus salmonicida</i> QQ*	T.S..	VV..VM..	GED.NKMVQ..NQ.ATQ.DG..Q..QA.Q				// M.S..GV..
<i>Trentepohlia iolithus</i> QQ*		Y..	YM..VAK//VS..TE.Y..V..N..I..				// R.R.E..I
<i>Carchesium polypinum</i> EE*	IS..	VVTI.M..	RKNVAEIT..Q..K..AS..DKT.KNA.I				// I.Y..S..Y
<i>Mesodinium pulex</i> YY*	IQ..	C..I..	A..DTVTK.TE.Y..V..KLT.T..				// I.F..G..
<i>Iotanema spirale</i> *Q*	C..G..L..TA..	AG..	TMMSHR.VT.Y.A..				// I.H.GGQ.V
Rhizarian exLh *L*		V..VV..	VP.LCS..TN.IA..V..I..A..I				// R..R..
<i>Blepharisma americanum</i> **W		L..	RE.LPIIN..ITE.Y..KS..IV.QA.Q				// I..
<i>Blepharisma japonicum</i> **W		L..	RE.LPIIN..ITE.Y..KS..IV.QA.Q				// I..
<i>Euplotes focardii</i> **C		LLS.R.A..K.QG..G.M..T..KQA..					// K.I..R..C
<i>Euplotes crassus</i> **C		LLS.R.A..K.QG..T.M..T..QA..					// K.I..C
<i>Eupl. octocarinatus/B</i> **C	D..	LLS.R..K.QA..G.A..V..QA..					// K.I..C
<i>Eupl. aediculatus/B</i> **C		LLS.R..K.QG..G.A..V..QA..					// K.I..C
<i>Eupl. octocarinatus/A</i> **C		LLS.R.A..PK.QG..G.Y..ES..KI..QA.Q					// K.I..Y
<i>Eupl. aediculatus/A</i> **C	G..	LLS.R.A..K.QG..SS.S..ES..QA.T					// K.I..Y
<i>Nyctotherus ovalis</i> **-		GE..TKMQ.K.I..G..K..Q..E					// Y.Y.GER.D
<i>Nyctotherus ovalis/B</i> **-		GE..AKMQ.K.I..G..K..Q..E					// Y.Y.GER.D
<i>Parduczia</i> sp. QQ(W)	H..	L.T..	LWN.NINE..K..AQ..LV.Q..Q				// M..SR..N
<i>Condyllostoma magnum</i> (QQW)		L..	RE..NLIN.QMN..L..K..A..V..GA.Q				// M..SR..
<i>Blastocrithidia</i> sp. (EE)W	M..	V..AY.T..E.VAS.VTK.NN.Y..			HS.KIG.Q		// M..

Fig. S2. Sequence comparison of the conserved N-terminal domain of the eukaryotic release factor 1 (eRF1) from various eukaryotes with different types of the nuclear genetic code. Only two parts of the alignment containing motifs that are thought to be important for stop codon recognition are shown (separated with a double slash). The type of the genetic code is indicated to the right of the species name by indicating the meaning of UAA, UAG, and UGA codons (in this order) as follows: asterisk – termination codon, one-letter amino acid abbreviation – sense codon with the respective specificity. Codons with context-dependent dual meaning (sense when in-frame, stop when at the end of the coding sequence) are

indicated with the respective amino acid abbreviation in brackets. The human eRF1 sequence (on the top) is used as a reference (with the numbers above indicating the actual position within the full-length sequence), identity with the reference is indicated as a dot in the other sequences. Functionally significant motifs and positions are printed in bold in the reference sequence and the respective regions are highlighted with a grey background throughout the alignment. Amino acid residues in these regions deviating from the canonical sequence are highlighted with a light blue background.

Accession numbers of used eRF1 gene sequences (GenBank or MMETSP database): *Homo sapiens* NP_004721, *Arabidopsis thaliana* AT1G12920.1, *Dictyostelium discoideum* EAL63131.1, *Thecamonas trahens* XP_013755399.1, *Emiliana huxleyi* XP_005786825.1, *Guttulinopsis vulgaris* JT844977.1, *Giardia intestinalis* ESU42869.1, *Roombia truncata* JP445973, *Trichomonas vaginalis* XP_001317480.1, *Reticulomyxa filosa* ETO12436.1; *Entamoeba histolytica* XP_655687.1, *Nannochloropsis gaditana* EWM25096.1, *Ostreococcus lucimarinus* XP_001417750.1, *Reclinomonas americana* EC793298.1, *Telonema subtilis* FN392508.1, *Symbiodinium minutum* BASF01007484.1, *Didinium nasutum* AB086368.1, *Dileptus margaritifer* AB086369, *Litonotus pictus* CAMNT_0010815823, *Climacostomum virens* CAMNT_0052066353, *Protocruzia adhaerens* CAMNT_0002413411 (MMETSP), *Fabrea salina* CAMNT_0049035673 (MMETSP), *Blastocladiella emersonii* CO974532.1, *Naegleria gruberi* XP_002682100.1, *Trypanosoma brucei gambiense* DAL972 XP_011779402, *Euglena gracilis* EC680368.1, *Holosticha* sp. AAT39329.1, *Tetrahymena thermophila* XP_001018735, *Sterkiella histriomuscorum* Q9BMX3.1, *Paramecium tetraurelia* XP_001425245, *Loxodes striatus* Q5CD84.1, *Gonostomum* sp. AAT39330.1, *Pseudocohnilembus persalinus* KRX05899.1, *Eschaneustyla* sp. AAT39331.1, *Urostyla* sp. AAT39328.1, *Oxytricha trifallax* AAK12090.1, *Uroleptus* sp. AAT39327.1, *Paraurostyla weissei* AAT39326.1, *Stichotrichida* sp. Alaska AAN62563.1, *Tetmemena pustulata* AAN62568.1, *Ichthyophthirius multifiliis* XP_004032541, *Strombidium inclinatum* CAMNT_0010754279 (MMETSP), *Pseudokerenopsis* sp. CAMNT_0010847507 (MMETSP), *Flavella ehrenbergii* CAMNT_0010732437, *Aristerostruma* sp. CAMNT_0001789297, *Uronema* sp. CAMNT_0038737683 (MMETSP), *Spironucleus salmonicida* EST45466.1, *Trentepohlia iolithus* (newly assembled contig, SRR1044982), *Carchesium polypinum* (c22299_g1_i1, SRX849478), *Mesodinium pulex* CAMNT_0004595873, *Iotanema spirale* a12404c01, Rhizarian exLh GBHO01004851, *Blepharisma americanum* AF317831.1, *Blepharisma japonicum* CAC16186.1, *Euplotes focardii* CAMNT_0053081483.1, *Euplotes crassus* (MMETSP), *Euplotes octocarinatus*/B AAG25924.1, *Euplotes aediculatus*/B AAK07830.1, *Euplotes octocarinatus*/A CAC14170, *Euplotes aediculatus*/A AAK07829.1, *Nyctotherus ovalis* AAX19092.1, *Nyctotherus ovalis*/B AAX19093.1, *Parduczia* sp. CAMNT_0047593165 (MMETSP), *Condyllostoma magnum* CAMNT_0008277929 (MMETSP), *Blastocrithidia* sp. GDHC01021218.1.