Minimizing the Anticodon-Recognized Loop of *Methanococcus jannaschii* Tyrosyl-tRNA Synthetase to Improve the Efficiency of Incorporating Noncanonical Amino Acids

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Table S1. Primers used for this study.

Sequence	Usage
*Mj*Loop-1-s: accctgtgcggcTTGACAGTTAATAGCTATGAGGAG *Mj*Loop-1-as: caagccgcacagGGTTAAAGGATATTCAAGGAAGT	*Mj*TyrRS-Loop1
*Mj*Loop-2-s: accggaagtggtTTGACAGTTAATAGCTATGAGGAG *Mj*Loop-2-as: caaaccacttccGGTTAAAGGATATTCAAGGAAGT	*Mj*TyrRS-Loop2
*Mj*Loop-3-s: accggaggtagtggtTTGACAGTTAATAGCTATGAGGAG *Mj*Loop-3-as: caaaccactacctccGGTTAAAGGATATTCAAGGAAGT	*Mj*TyrRS-Loop3
eGFP150-TGA-s: CAGCCACtgaGTGTACATCATGGCGGACAAGC eGFP150-TGA-as: TGTACACtcaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TGA
*Mj*tRNA-TGA-s: CGGCGGACTtcaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TGA-as: GGATTtgaAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TGA
eGFP150-TAA-s:CAGCCACtaaGTGTACATCATGGCGGACAAGC eGFP150-TAA-as:TGTACACttaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TAA
*Mj*tRNA-TAA-s: CGGCGGACTttaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TAA-as: GGATTtaaAGTCCGCCGTTCTGCCCTGCTGAACT	MjtRNA-TAA
eGFP150-TAGA-s:CAGCCACtagaGTGTACATCATGGCGGACAAGC eGFP150-TAGA-as:TGTACACtctaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TAGA
*Mj*tRNA-TAGA-s: CGGCGGACTtctaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TAGA-as: GGATTtagaAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TAGA
eGFP150-TAGT-s:CAGCCACtagtGTGTACATCATGGCGGACAAGC eGFP150-TAGT-as:TGTACACactaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TAGT
*Mj*tRNA-TAGT-s: CGGCGGACTactaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TAGT-as: GGATTtagtAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TAGT
eGFP150-TAGC-s:CAGCCACtagcGTGTACATCATGGCGGACAAGC eGFP150-TAGC-as:TGTACACgctaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TAGC
*Mj*tRNA-TAGC-s: CGGCGGACTgctaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TAGC-as: GGATTtagcAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TAGC
eGFP150-TAGG-s:CAGCCACtaggGTGTACATCATGGCGGACAAGC eGFP150-TAGG-as:TGTACACcctaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TAGG
*Mj*tRNA-TAGG-s: CGGCGGACTcctaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TAGG-as: GGATTtaggAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TAGG
eGFP150-TGAA-s:CAGCCACtgaaGTGTACATCATGGCGGACAAGC eGFP150-TGAA-as:TGTACACttcaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TGAA
*Mj*tRNA-TGAA-s: CGGCGGACTttcaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TGAA-as: GGATTtgaaAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TGAA
eGFP150-TGAT-s:CAGCCACtgatGTGTACATCATGGCGGACAAGC eGFP150-TGAT-as:TGTACACatcaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TGAT
*Mj*tRNA-TGAT-s: CGGCGGACTatcaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TGAT-as: GGATTtgatAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TGAT
eGFP150-TGAC-s:CAGCCACtgacGTGTACATCATGGCGGACAAGC eGFP150-TGAC-as:TGTACACgtcaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TGAC
*Mj*tRNA-TGAC-s:CCGGCGGACTgtcaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TGAC-as: GGATTtgacAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TGAC
eGFP150-TGAG-s:CAGCCACtgagGTGTACATCATGGCGGACAAGC eGFP150-TGAG-as:TGTACACctcaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TGAG
*Mj*tRNA-TGAG-s: CGGCGGACTctcaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TGAG-as: GGATTtgagAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TGAG
eGFP150-TAAA-s:CAGCCACtaaaGTGTACATCATGGCGGACAAGC eGFP150-TAAA-as:TGTACACtttaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TAAA

*Mj*tRNA-TAAA-s: CGGCGGACTtttaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TAAA-as: GGATTtaaaAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TAAA
eGFP150-TAAT-s:CAGCCACtaatGTGTACATCATGGCGGACAAGC eGFP150-TAAT-as:TGTACACattaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TAAT
*Mj*tRNA-TAAT-s: CGGCGGACTattaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TAAT-as: GGATTtaatAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TAAT
eGFP150-TAAC-s:CAGCCACtaacGTGTACATCATGGCGGACAAGC eGFP150-TAAC-as:TGTACACgttaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TAAC
*Mj*tRNA-TAAC-s: CGGCGGACTgttaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TAAC-as: GGATTtaacAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TAAC
eGFP150-TAAG-s:CAGCCACtaagGTGTACATCATGGCGGACAAGC eGFP150-TAAG-as:TGTACACcttaGTGGCTGTTGTAGTTATACTCCAGCT	eGFP150-TAAG
*Mj*tRNA-TAAG-s: CGGCGGACTcttaAATCCGCATGGCAGGGGTTCAAATC *Mj*tRNA-TAAG-as: GGATTtaagAGTCCGCCGTTCTGCCCTGCTGAACT	*Mj*tRNA-TAAG

Table S2. The MjTyrRS mutants used in this study.

Substrate/position	32	65	70	107	108	109	158	159	162	167	286
MjTyrRS(WT)	Y	L	H	E	F	Q	D	I	L	A	D
O-Me-Tyr	Q	L	H	T	F	Q	A	I	P	A	D
4-N_3-Phe	T	L	H	N	F	Q	P	L	Q	A	D
4-I-Phe	L	L	H	S	F	Q	P	L	E	A	D
4-$B(OH)_2$-Phe	S	A	M	E	F	Q	S	I	E	A	D
3-NO_2-Tyr	H	L	C	E	F	Q	S	A	R	A	D
4-NO_2-Phe	L	V	H	E	W	M	G	A	L	A	D
AcFRA.1	L	L	H	E	F	Q	G	C	R	D	R
AzFRA.1	L	L	H	E	F	Q	V	M	D	Y	R
AzFRA.2	L	L	H	T	Y	M	G	C	R	A	R

Supplementary Sequence 1 The sequence of TyrRSs from giant viruses

Acanthamoeba castellanii mamavirus

MENTDYTNNEHRLNQLLSIAEECETLDRLKQLVDSGRIFTAYNGFEPSGRIHIAQALITVMNTNKIIECGGQMIIYIAD
WFAKMNLKMNGDINKIRELGRYFIEVFKACGINLDGTRFIWASEFIASNPSYIERMLDIAEFSTISRVKRCCQIMGRNE
SDCLKASQIFYPCMQAADVFELVPEGIDICQLGIDQRKVNMLAIEYANDRGLKIPISLSHHMLMSLSGPKKKMSKSD
PQGAIFMDDTEQEVSEKISRAYCTDEIFDNPIFEYIKYLLLRWFGTLNLCGKIYTDIESIQEDFSSMNKRELKTDVANYI
NTIIDLVREHFKKPELSELLSNVKSYQQPSK

Acanthamoeba castellanii mimivirus

MENTDHTNNEHRLTQLLSIAEECETLDRLKQLVDSGRIFTAYNGFEPSGRIHIAQALITVMNTNNIIECGGQMIIYIAD
WFAKMNLKMNGDINKIRELGRYFIEVFKACGINLDGTRFIWASEFIASNPSYIERMLDIAEFSTISRVKRCCQIMGRNE
SDCLKASQIFYPCMQAADVFELVPEGIDICQLGIDQRKVNMLAIEYANDRGLKIPISLSHHMLMSLSGPKKKMSKSD
PQGAIFMDDTEQEVSEKISRAYCTDETFDNPIFEYIKYLLLRWFGTLNLCGKIYTDIESIQEDFSSMNKRELKTDVANYI
NTIIDLVREHFKKPELSELLSNVKSYQQPSK

Acanthamoeba polyphaga lentillevirus

MENTDYTNNEHRLNQLLSIAEECETLDRLKQLVDSGRIFTAYNGFEPSGRIHIAQALITVMNTNKIIECGGQMIIYIAD
WFAKMNLKMNGDINKIRELGRYFIEVFKACGINLDGTRFIWASEFIASNPSYIERMLDIAEFSTISRVKRCCQIMGRNE

SDCLKASQIFYPCMQAADVFELVPEGIDICQLGIDQRKVNMLAIEYANDRGLKIPISLSHHMLMSLSGTKKKMSKSD
PQGAIFMDDTEQEVSEKISRAYCTDEIFDNPIFEYIKYLLLRWFGTLNLCGKIYTDIESIQEDFSSMNKRELKTDVANYI
NTIIDLVREHFKKPELSELLSNVKSYQQPSK

Acanthamoeba polyphaga mimivirus

MENTDHTNNEHRLTQLLSIAEECETLDRLKQLVDSGRIFTAYNGFEPSGRIHIAQALITVMNTNNIIECGGQMIIYIAD
WFAKMNLKMNGDINKIRELGRYFIEVFKACGINLDGTRFIWASEFIASNPSYIERMLDIAEFSTISRVKRCCQIMGRNE
SDCLKASQIFYPCMQAADVFELVPEGIDICQLGIDQRKVNMLAIEYANDRGLKIPISLSHHMLMSLSGPKKKMSKSD
PQGAIFMDDTEQEVSEKISRAYCTDETFDNPIFEYIKYLLLRWFGTLNLCGKIYTDIESIQEDFSSMNKRELKTDVANYI
NTIIDLVREHFKKPELSELLSNVKSYQQPSK

Acanthamoeba polyphaga moumouvirus

MEQQILSTEEKITRLISICEECETPDRLEELIKSGKKFTAYNGFEPSGRIHIAQALITVMNTNEIISCGGRMIIYIADLFAK
MNKKMDSDLDKIKDVGLYFIEVFKACGINMSETKFIWSSDLFNKNPEKYFTRLLDISEETSLSRSKRCCQIMGRKDGD
ELSVSQIIYPCMQVADIFELEPEGIDICQLGVDQRKVNMLALEYAKKNGLKVPVILSHHMLMGLGGPKNKMSKSDP
KSAIFMEDSYEEIKEKILRAFCTDDINENPIFEYIKHILFRWYGKLELCGKNYDNMSDIEKDFCDMNKRELKNNVAEY
INKIIEPVRIHFQQPHLKNLLDKVSSYRVTK

Cafeteria roenbergensis virus BV-PW1

MNQFKELLTNVENISLIYNDSDNRVMFTMKSHLLYFLTRFKNSDIVKIIEKDNLKYFMLDEISSDNYLDIYDKFIKEYQ
IYDLDYDNFIDQRVQNLLSISEECDTIDRLKELVESFKNFTAYNGFEPSGRIHIAQALITVMNTNTIIQNGGSMIIYIAD
WFAQMNHKMGGDLEKIRDVGKYFIEVFKACGINMSGTKFIWASEFFNDNATKYFERMLHIANQTSLARAKRCCQI
MGRREGDELSSSQIIYPCMQVADIFELNPNGIDICQLGVDQRKVNMLAIEYAKRNNLKTPIILSHHMLMGLKGSKN
KMSKSDPENAIFMEDSREDIFKKIHKAYCTDEIIDNPIYEYIKYILLRWFGTITLCGQKYNNIDDINKNFATMLKKDLK
NDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN

Catovirus CTV1

MNIQENIKTIKSIGEEIIGGDSLERLLTHKQRVYAYDGFEPSGRMHIAQGLLRTHNVNKFIDSGVHFKFWVADWFAL
MNLKLGGDIKKIQQAGKDMILMWKACDMNLDKVDKDGNKMIEFLWSSEEINKRPDEYWKLVLDIATKFSLNRIK
KCTQIMGRKEEDEIFNTILDLNDKIVDLFNNCDFEQKKKDDILGLLKKLLDTKLDELAASQIFYPVMQCADVFFLGI
DIASLGMDQRKVNTLALEYCDKIKRKNKPIIISHHMLKGLDGSDKMSKSNPDNTIFMDDSETEIIRKIKKSFCEPGNI
DKNPLLDWIEHLILPIKKTIVIKTKNDIGEEIDMSFDNANLIADKFKNGYIHPKCLKNAVTTVLVNTLIPIQQKYIALK
KNDN

Cotonvirus japonicus

MDNFTRFCAELSQNRDINVINISENEIIFEFVDKSLYFTNKFEEDRIIDITFIDNQKYLCVTREDVDSNFIPDSTYVIKCH
DSYVETFDVDVKKRVDQLVAISEECDDRDRLRDLIISGKKFVAYNGFEPSGRIHIAQAVITVLNTNTIIENGGRMIIYIA
DWFAQLNHKLGGDLEKIQNVGRYFIEVFKACGIDTSNTEFIWASEFFAKNPSRYFQRMMDVSINATLARTKRCCQI
MGRAEGDNLSSSQIIYPCMQTADIFELVEGGVDICQLGVDQRKVNMFALDYANSQKLKLPVILSHHMIMGLRGPKH
KMSKSDPLNAIFMEDTTQEIHDKISKAFCPDTHLDNPIFEYIKYILLRWFGNLTLCDNFYTNIDFIIQDFPHFDKKRLK
TDVANYIDRIIKPVRDHFAQDELKDLYDKVIN

Edafosvirus sp.

MSEDKYNIITRNLQEIIGSDDLKELLKNKNPKIYWGTAPTGRIHIAYFLPLLKIADFLDAGCHVKILIADLHAYLDNM
KSSLDQLNSRCEYYTHMIKEILKVLNVNLSKLEFVKGTDFQLNKEYTMDVYKAHNMISYNEARHAGAEVVKQTDN
PNINSLLYPTLQALDEQYLDVDIQFGGNDQRKIFMHARKILPSIGYKKRIYLMNSIIPALSKVSLKDPQQESTITNKKM
SSSDNTSKIDVLEDEKSIKKKINSTYCLEGDISDNTLLTMSKLLIFPILNRLNQKLIIQRPEKYGGNIIYDNYTLLESDFET
KKLHPSDLKNGISLFIINLIKPIQIEFQKEELVKLLQKAYS

Fadolivirus 1

MELTVDQKYELITRNLQEKIVDEEIMKKILAARPLKIYWGTAPTSLCHLGYFAPMFKIIDFLQAGCEVTILIADLHAVL

DNMKSTFEQVDARTKVYTVLIQELLKSLNVDISRLKFVKGTDYQLSKEYTLDMYKAHTLISVSEAKHAGAEVVKQS
DNPKMTGLLYPTLQALDEQYLGSDAELGGIDQRKIFVHARAIMPALGYKKRFHLMNRMVPGLRFEKTTPLQSVQN
EQKESLKEKVLSLVNSEADEKVLADRLQSLLENNDQSNIQLEKMSSSNADSKISLLDTRNQIRSKINKAYCLPGDVD
DNCLLTMLDKIVFPVLKLKGLDFVINRKEEHGGKLVYTNIDDVTNDFKTEKLHPADLKLGMVDSLDLMMEPLRNA
FKSKESVQLLNKAYPQK

Harvfovirus sp.

MQSRYDSITAGVKEVINAKYLKALLEDASYSPKGYWGTAPTGRIHIGYLRAMFVIADLVNANCEVIILLADIHAFLDS
RKSPQDNQLRTQYYQIMITELLKLLNVDMSKIKFVIGSSFQETKEYMRELLAIANVTNCSNAKDAGTEVVMQDSDP
KLTSLLYPIMQVLDEKYLNADFELGGVDQRKIFTFGIDHVHRHTKHKITYLMNSMVPALSGISGDSGVKMSASGSVG
KLDLLDSPKELLKKIKKVYCLQGDIADNTLLQLCKHIVFPLLSRVNKIMIVTKKFDHVESEIKNYEDLENLFASGLHP
DDFKTSVALTIGELLKPIRDKFLETDMVTLLKKAYDSK

Hirudovirus strain Sangsue

MENTDYTNNEHRLNQLLSIAEECETLDRLKQLVDSGRIFTAYNGFEPSGRIHIAQALITVMNTNKIIECGGQMIIYIAD
WFAKMNLKMNGDINKIRELGRYFIEVFKACGINLDGTRFIWASEFIASNPSYIERMLDIAEFSTISRVKRCCQIMGRNE
SDCLKASQIFYPCMQAADVFELVPEGIDICQLGIDQRKVNMLAIEYANDRGLKIPISLSHHMLMSLSGPKKKMSKSD
PQGAIFMDDTEQEVSEKISRAYCTDEIFDNPIFEYIKYLLLRWFGTLNLCGKIYTDIESIQEDFSSMNKRELKTDVANYI
NTIIDLVREHFKKPELSELLSNVKSYQQPSK

Hokovirus HKV1

MNKLTIDEKRELITKNLQEILGKQELDNLLLQEEIKIYWGTAPTGRIHIGYFIQFLKIVDYIKAGCKVKILIADLHAVLD
NLKTSMELLNARTEYYITMIKEVLLSLNVDLDLIEFVKGSDYQLSQNYTLDVYKLNSKISYQEAKHAGAEVVKQTQ
NPTMTGLLYPSLQALDEHYLDVHIQSGGIDQRKIFTFARSNLNLLGYKKSIHLMTPMVQGLRFNKKEIKNVITKEEK
LKLLNIDFDLPDNYVDTLYKKYINNDTIKDDKMSSSNNDSKIDLLDTKNEIKKKINKSYCLPCDVEDNCLMDLLEKV
IFPVLSYKNQQFIINRKEKFGGIIIYDSFDNIKNDFALGKLHPQDFKLGITDSLNYILEPIRNSFETKERKLLLKKAYN

Homavirus sp.

MTNLSVDEKYNLITRNLQEVLGSEADIKSILAERPLNIYWGTAATGKIHIGYIFQMMKIADYLQAGCNVTILIADLHA
YLDNMKSSLELINYRTEYYTKMIQTILITMGVDISRLTFVKGTSFQLSEKYTMDMYRANSMISVKDAQHAGAEVVKQ
TDNPIMNGLLYPTLQALDEQYLNIDAETSGIDQRKLFAHAKMIMPKLGYKKRNYFMTEMISGLRFVKKQEQSHSQP
VKLNRDDIINLLNIESDDALINNLQEMIDKYNKEKELKNNIQLEKMSSSDNNSKIDLLDTKNQIKAKINKCYCLAGD
IKDNCLMNILEKLLFPMMKYKGLDFIINRPDKYGGPIVYTEFDNVKNDFEKELLHPSDFKMGITDGFDTIIKPIRDIFG
TPELQKLIKQAYP

Klosneuvirus KNV1

MELSVDEKFNLITRNLQETILDEVIMKKIIAARPLKVYWGTAPTSAPSVAYFVPMMKIRDLVMAGCEVTILIADLHAI
LDNLKSTFEQVGHRSEYYITMIKALLTSLNIDVNKIKFVKGTEFQLSKEYTLDMYKAHSLITVNEAKHAGAEVVKQA
EHPKMTGLMYPTLQALDEQYLGADVEIGGIDQRKLFMHARNIMPQLGYKKRMYLMNKMISGLRTQKQEPKEGEN
IQDQKMSSSNQDSKIDLLDSKNQLKAKINKAYCFPGDINDNCLLEMLENLVFPILHIKGLNFVIERKEEYGGNITYN
NIDNVKNDFKSEKLHPMDLKQGMINNLDLILDSIRKTFETPEMKKLIKMAYS

Marseillevirus LCMAC201

MSNFDLIVKGLHEIVGKDLLEKIAVERVVKVYWGTAPTGRIHIGYYIPLLKIAELVKAGCEVYILIADLHAMLDNLKS
TEKQVHNRSDYYTRTIKNMLTQLNVDLDKVTFILGSTYQTKPEYTMDMYRLNMLCKVSDARHAGSEVVKQSNNP
MMTGLLYPSLQALDEEYLHVDAQLGGIDQRKIFMFAREFLPKIGYRKRIHLMTPMVPGIRHCASSKSMDSQEKMSA
SDTKTKLDMLDTRNQIKKKIASAFCIPGDVTDNSILDILERVLFPLLKHLSKDFVIIRREEHGGPITFTNFNKVREAFVA
EKLHPADLKAGIVHTLDIFIEPVRKEFESREWRQVLKHSYAED

Marseillevirus LCMAC202

MSNYDLIVKDLHEIVGEDILKKIAAERTVKVYWGTAPTGRIHIGYYTPLLKIAELVQAGCEVTILIADLHAMLDNLKS

TEKQVHNRSDYYTRIIKNMLTQLNVDLDKVTFVRGSSFQFTPEYTMDMYRLNTVCSFHDAQHAGAEVVKQTSNPI
MTGLLYPSLQALDEEYLHVDAQLGGIDQRKIFMFAREFLPKIGYRKRVHLLTPMVPGLRCCASTQDNEDKMSTSNT
KTKLDMLDSRNEIKKKIAAAYCLPGDITDNSVLDILDRVLFPLLRHLSKDFIIFRREEHGGPIIFTNFDQVREAFSTQQL
HPADLKAGT

Megavirus chiliensis

MNQFKELLTNVENISLIYNDSDNRVMFTMKSHLLYFLTRFKNSDIVKIIEKDNLKYFMLDEISSDNYLDIYDKFIKEYQ
IYDLDYDNFIDQRVQNLLSISEECDTIDRLKELVESFKNFTAYNGFEPSGRIHIAQALITVMNTNTIIQNGGSMIIYIAD
WFAQMNHKMGGDLEKIRDVGKYFIEVFKACGINMSGTKFIWASEFFNDNATKYFERMLHIANQTSLARAKRCCQI
MGRREGDELSSSQIIYPCMQVADIFELNPNGIDICQLGVDQRKVNMLAIEYAKRNNLKTPIILSHHMLMGLKGSKN
KMSKSDPENAIFMEDSREDIFKKIHKAYCTDEIIDNPIYEYIKYILLRWFGTITLCGQKYNNIDDINKNFATMLKKDLK
NDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN

Megavirus courdo7-1

MEQQILSTEEKITRLISICEECETPDRLEELIKSGKKFTAYNGFEPSGRIHIAQALITVMNTNEIISCGGRMIIYIADLFAK
MNKKMDSDLDKIKDVGLYFIEVFKACGINMSETKFIWSSDLFNKNPEKYFTRLLDISEETSLSRSKRCCQIMGRKDGD
ELSVSQIIYPCMQVADIFELEPEGIDICQLGVDQRKVNMLALEYAKKNGLKVPVILSHHMLMGLGGPKNKMSKSDP
KSAIFMEDSYEEIKEKILRAFCTDDINENPIFEYIKHILFRWYGKLELCGKNYDNMSDIEKDFCDMNKRELKNNVAEY
INKIIEPVRIHFQQPHLKNLLDKVSSYRVTK

Megavirus courdo7-2

MNQFKELLTNVENISLIYNDSDNHVMFTMKSHLSYFLTRFKNSDVVKIIEKDNLKYFMLDEIASDNYLDVYDKFMK
EYQIYDLDYDNFIDQRVQNLLSISEECDTIDRLKELVESFKNFTAYNGFEPSGRIHIAQALITVMNTNTIIQNGGSMIIYI
ADWFAQMNHKMGGDLEKIRDVGKYFIEVFKACGINMSGTKFIWASEFFNDNATKYFERMLHIANQTSLARAKRC
CQIMGRREGDELSSSQIIYPCMQVADIFELNPNGIDICQLGVDQRKVNMLAIEYAKRNNLKTPIILSHHMLMGLKGS
KNKMSKSDPENAIFMEDSREDIFKKIYKAYCTDEIIDNPIYEYIKYILLRWFGTITLCGQKYNNIDDINKNFATMQKKD
LKNDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN

Megavirus courdo11

MNQFKELLTNVENISLIYNDSDNHVMFTMKSHLSYFLTRFKNSDVVKIIEKDNLKYFMLDEISSNNYLDIYDKFMKE
YQIYDLDYDNFIDQRVQNLLSISEECDTIDRLKELVESFKNFTAYNGFEPSGRIHIAQALITV
MNTNTIIQNGGSMIIYIADWFAQMNHKMGGDLEKIKDVGKYFIEVFKACGINMSGTKFIWASEFFNDNATKYFERM
LHIANKTSLARAKRCCQIMGRREGDELSSSQIIYPCMQVADIFELNPNGIDICQLGVDQRKVNMLAIEYAKRNNLKT
PIILSHHMLMGLKGSKNKMSKSDPENAIFMEDSREDIFKKIHKAYCTDEIIDNPIYEYIKYILLRWFGTITLCGQKYNNI
DDINKNFATMLKKDLKNDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN

Megavirus lba isolate LBA111

MNQFKELLTNVENISLIYNDSDNHVMFTMKSHLSYFLTRFKNSDVVKIIEKDNLKYFMLDEISSDNYLDIYDKFIKEY
QIYDLDYDNFIDQRVQNLLSISEECDTIDRLKELVESFKNFTAYNGFEPSGRIHIAQALITVMNTNTIIQNGGSMIIYIA
DWFAQMNHKMGGDLEKIKDVGKYFIEVFKACGINMSGTKFIWASEFFNDNATKYFERMLHIANQTSLARAKRCC
QIMGRREGDELSSSQIIYPCMQVADIFELNPNGIDICQLGVDQRKVNMLAIEYAKRNNLKTPIILSHHMLMGLKGSK
NKMSKSDPENAIFMEDSREDIFKKIYKAYCTDEIVDNPIYEYIKYILLRWFGTITLCGQKYNNIDDINKNFATMQKKD
LKNDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN

Megavirus vitis

MNQFKELLTNVENISLIYNDSDNHVMFTMKSHLSYFLTRFKNSDVVKIIEKDNLKYFMLDEIASDNYLDVYDKFIKE
YHIYDLDYDNFIDQRVQNLLSISEECDTIDRLKELVESFKNFTAYNGFEPSGRIHIAQALITVMNTNTIIQNGGSMIIYI
ADWFAQMNHKMGGDLEKIRDVGKYFIEVFKACGINMSGTKFIWASEFFNDNATKYFERMLHIANQTSLARAKRC
CQIMGRREGDELSSSQIIYPCMQVADIFELNPNGIDICQLGVDQRKVNMLAIEYAKRNNLKTPIILSHHMLMGLKGS
KNKMSKSDPENAIFMEDSREDIFKKIYKAYCTDEIIDNPIYEYIKYILLRWFGTITLCGQKYNNIDDINKNFATMQKKD

LKNDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN

Mimivirus Bombay

MENTDHTNNEHRLTQLLSIAEECETLDRLKQLVDSGRIFTAYNGFEPSGRIHIAQALITVMNTNNIIECGGQMIIYIAD
WFAKMNLKMNGDINKIRELGRYFIEVFKACGINLDGTRFIWASEFIASNPSYIERMLDIAEFSTISRVKRCCQIMGRNE
SDCLKASQIFYPCMQAADVFELVPEGIDICQLGIDQRKVNMLAIEYANDRGLKIPISLSHHMLMSLSGPKKKMSKSD
PQGAIFMDDTEQEVSEKISRAYCTDETFDNPIFEYIKYLLLRWFGTLNLCGKIYTDIESIQEDFSSMNKRELKTDVANYI
NTIIDLVREHFKKPELSELLSNVKSYQQPSK

Mimivirus LCMiAC01

MTELTTFDFITRNLEPLVGNDAVRLLEIIKDRPLKLYWGTAPTGAPHFGYLVPLRKIADFLKAGCEVTIMFADIHAML
DNLKTKPDLIQIRTTCYEMIIKRILQIFKVPLHKLRFIRGSEFQTSPEYINKFYELTAYTTIKQAIHAGAEVVKQTSSPKLS
SVIYPLLQALDEEFLGVDGQFGGIDQAKIFMFAKDQLPRIGISKRIHLMNALIPGLKKGGKMSSSIPYSKIDFRDTDEDI
NRKILGAFSIDGHVGNGKNKNCMLAMMEHVIYPTLNGESFMIKRDRLYGGNIKITSYKELEKMFQLNKLSSIDLKIS
MVPYIQKLIKPIRAMISEQSELFDSACWHAHHANVPIISSSLQSIQSTQLKKMTKKMTKKITKKTIELSLEDKIACELK
AIQKMEQHIIFKKKKVEELRRQMGEKE

Mimivirus reunion

MENTDYTNNEHRLNQLLSIAEECETLDRLKQLVDSGRIFTAYNGFEPSGRIHIAQALITVMNTNKIIECGGQMIIYIAD
WFAKMNLKMNGDINKIRELGRYFIEVFKACGINLDGTRFIWASEFIASNPSYIERMLDIAEFSTISRVKRCCQIMGRNE
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PQGAIFMDDTEQEVSEKISRAYCTDEIFDNPIFEYIKYLLLRWFGTLNLCGKIYTDIESIQEDFSSMNKRELKTDVANYI
NTIIDLVREHFKKPELSELLSNVKSYQQPSK

Mimivirus sp. SH

MNQFKELLTNVKNISLIYNDNDHHVMFTMKSHLSYFLTRFKNSDVVKIIEKDNLEYFIFDEITSDNYLDIYDKFIKQY
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DWFAQMNHKMGGDLEKIRDVGKYFIEVFKACGIDMSGTKFIWASEFFNDNSTKYFERMLHIANQTSLARAKRCCQ
IMGRREGDELSSSQIIYPCMQVADIFELNPNGIDICQLGVDQRKVNMLAIEYAKRNNLKTPIILSHHMLMGLKGSKN
KMSKSDPENAIFMEDSREDIFKKIYKAYCTDAITDNPIYEYIKYILLRWFDTVTLCGQKYNNIDDINKDFATMNKKDL
KNNVADYIDNIIEPVRRHFQDPQMAELYSRVRSYTN

Moumouvirus australiensis

MEQQTLSVEEKFTRLISISEECETPDRLVELINTGKKFTAYNGFEPSGRIHIAQALITVMNTNEIISCGGTMIIYIADLFA
KMNKKMDSDLDKIKDVGLYFIEVFKACGINMSETKFIWSSDLFNKNPEKYFTRLLDISEQTSLSRSKRCCQIMGRKEG
DELSVSQIIYPCMQVADIFELEPEGIDICQLGVDQRKVNMLALEYAKKNGLKVPIILSHHMLMGLGGPKNKMSKSDP
KSAIFMEDSYEEIKEKILRAFCTDDTKDNPIFEYIKYILFRWYGKLELCGKNYNNMDEIEKDFHDMNKRELKNNVAE
YINKIIEPVRIHFQQPHLKDLLEKVSSYRVTK

Niemeyer virus-1

MENTDHTNNEHRLTQLLSIAEECETLDRLKQLVDSGRIFTAYNGFEPSGRIHIAQALITVMNTNNIIECGGQMIIYIAD
WFAKMNLKMNGDINKIRELGRYFIEVFKACGINLDGTRFIWASEFIASNPSYIERMLDIAEFSTISRVKRCCQIMGRNE
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PQGAIFMDDTEQEVSEKISRAYCTDETFDNPIFEYIKYLLLRWFGTLNLCGKIYTDIESIQEDFSSMNKRELKTDVANYI
NTIIDLVREHFKKPELSELLSNVKSYQQPSK

Niemeyer virus-2

MENTDYSNNEHRLNQLLLIAEECETLDRLKQLVDSGKIFTAYNGFEPSGRIHIAQALITVMNTNKIIECGGQMIIYIA
DWFAKMNLKMNGDIEKIRELGRYFIEVFKACGINLGGTKFIWASEFIASNPSYIERMLDIAEFSTLSRVKRCCQIMGR
NESDCLKASQIFYPCMQAADVFELVPGGIDICQLGIDQRKVNMLAIEYANDRGLKIPISLSHHMLMSLNGPKKKMS
KSDPQSAIFMDDTEQEINEKISRAYCTDEIFDNPIFEYIKYLLLRWFGTLNLCGKVYTDIKSIEQDFSSMNKRELKTDVA

NYINIIIDLVREHFKKPELSDLLSNIKSYQQSSK

Orpheovirus IHUMI-LCC2

MNTYNLITRNLQEVVGNDELLKKIDNGEDIKVYWGTAITGNPHLGYFVPILKIADLLNAGCKVTILLADLHGYLDN
LKTDWDLLSLRIEWYELIIKEMLKRVGVSLENLKFVTGSTFQLSKEYTLDMYKLSALVTTTAMQHASAEVVKQSKNP
LLSGLLYPILQALDEKYLGVNVQLGGIDQRKIFMFAREILSEVDYPKSIHLMNPLVPGLGKPVKGNKGKNEGNDIEG
KMSSSDKNSKIDFSDSDEVIKDKMKKAYSVDGQVEGNCLMSMCKLILFPFLELEGRKFVVEREEKYGGDVHFDNYN
SIEETFGKKELASMDLKVAIAKEIIKLVNPIRKIVNDNVELMKSAYPTEKN

Pandoravirus dulcis

MISDDGDDNSGGKEAAHLPVGNIDPDQGDVEQRYALVVRDLDEVIGDPAEIKAIMAERPLIIYWGTAPTGNPHLGY
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LPFVVTRPSEYGGPQTFATYAEVEAAFVRAGVPLVEGGGERLFSVDLKPAVADLLCEFLAPLRGVLAARADLFAAAY
PV

Pandoravirus japonicus

MIDDDDDGGKETAHLPTDDGALDQDDVEQRYALVVRDLDEVIGEPAEIKAIMAERPLAVYWGTAPTGNPHLGYFV
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TLDLLRLTARVTVATAQKAAAQVVKMERNPLLSNVIYPLMQALDEEHLDVDAQFGGRDQRKIFAFARDHLPALGY
RKRVHLLNPLVPGLTKSGKMSASEAASKIGLDDPDEVIRAKIRQAYSVDGCAEGNGLLAILRFVLWRWLEPAGLPFV
VTRPPEYGGPQTFATYAEVEAAFVRAGVPPAEGDGERLFSVDLKPAVADLLCEFLAPLRGVLAARADLLHAAYPPPS

Pandoravirus macleodensis

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QFGGRDQRKIFAFARDHLPALGYRKRFHLLNPLVPGLTKDGKMSASEAASKIDLDDAEEAIRAKIRRAYSVDGRAEG
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PAIADLLCEFLAPLRGVLGARADLLRAAYPPMPH

Pandoravirus salinus

MMDDTDHQHREKTEKSVHSQGGNGSPTPIGVDSTARQQTDGDDVEHRYGLVVRDLDEVIGDPADIKAIMAERPLC
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LRGVLAARADLLATAYPSAP

Samba virus

MENTDHTNNEHRLTQLLSIAEECETLDRLKQLVDSGRIFTAYNGFEPSGRIHIAQALITVMNTNNIIECGGQMIIYIAD
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SDCLKASQIFYPCMQAADVFELVPEGIDICQLGIDQRKVNMLAIEYANDRGLKIPISLSHHMLMSLSGPKKKMSKSD
PQGAIFMDDTEQEVSEKISRAYCTDETFDNPIFEYIKYLLLRWFGTLNLCGKIYTDIESIQEDFSSMNKRELKTDVANYI
NTIIDLVREHFKKPELSELLSNVKSYQQPSK

Saudi moumouvirus

MEQQILSTEEKITRLISICEECETPDRLEELIKSGKKFTAYNGFEPSGRIHIAQALITVMNTNEIISCGGRMIIYIADLFAK
MNKKMDSDLDKIKDVGLYFIEVFKACGINMSETKFIWSSDLFNKNPEKYFTRLLDISEETSLSRSKRCCQIMGRKDGD

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KSAIFMEDSYEEIKEKILRAFCTDDINENPIFEYIKHILFRWYGKLELCGKNYDNMSDIEKDFCDMNKRELKNNVAEY
INKIIEPVRIHFQQPHLKNLLDKVSSYRVTK

Terrestrivirus sp.

MNPQEQYDLISHRISPDDIVGKSYLMEKLQAGKQLKGYVGFAPTGRCHIGYASLLLKVAQCLDAGCEIIILLADVHA
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AEDPLLSSLVYPLMQSLDETHLDPDLDFELSGTDQRHIFMFSVDYVNKSKNKKMTYIMNQLVPGLSKESLRNPETKV
VTSQKMSSSDSVGKLDLLDTPKEILNKLKKAYCLEGDAEDNTVLVMCKHIIFPVLERLNKQFVIARDEKYGGNLVIN
SHDELYALFADKSIHPSDLKKSVANIITDLFEPIRQHFSTNDMVKLLEQAYGK

Tupanvirus deep ocean

MEIPDDKLSLITSRLQEIIGLDELQKIIQSRPLKIYFGTAPTGRIHIGYLVPLLKIADFLKAGCEVKILFADLHAVLDNLKS
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SGLLYPILQALDEQYLDVDAQFGGVDQRKLFTFAADVLPLIGYKKRIHLMNPMLTAINAMPKDSNENTNISEIKMSS
SDLNSKIDMLDSKNEIKKKINRAYCLEGDLSFNPLMELMKHVTFPLLNNCGSSIFKINRKEIHGGPLSFSNYADLEKD
FVEKKLHPQDLKTGIIDCLNLFMEPIRAEFNDKESQSLIKQAYN

Tupanvirus soda lake

MEVVQDKISLITSRLQEVIGIDELQNIIKSRPLKIYWGTAPTGKVHIGYLVPLLKIADFLKSGCEVKILFADLHAVLDNL
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MSGLLYPILQALDEQYLDVDAQFGGIDQRKLFTFAADVLPLIGYNKRIHLMNPMLTAINAMPKNINEDTNLSEVKM
SSSDLNSKIDMMDSKNEVKKKINRAYCLEGDLSFNPLMELMKLVIFPLLDNCNINIFTINRHEKYGGPLNYSNFEELE
KDFVQKTLHPQDLKMGIIDCLNNFMDPIRQEFSDKETQQLIKKAYN

Supplementary Sequence 2 The plasmid sequences used in this work

pEvole-MjTyrRS(wt)

TCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTCATTATGGTGAAAGTTGGAACCTCTTAC
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pEvole-MjTyrRS(wt)-Loop1

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pEvole-MjTyrRS(wt)-Loop2

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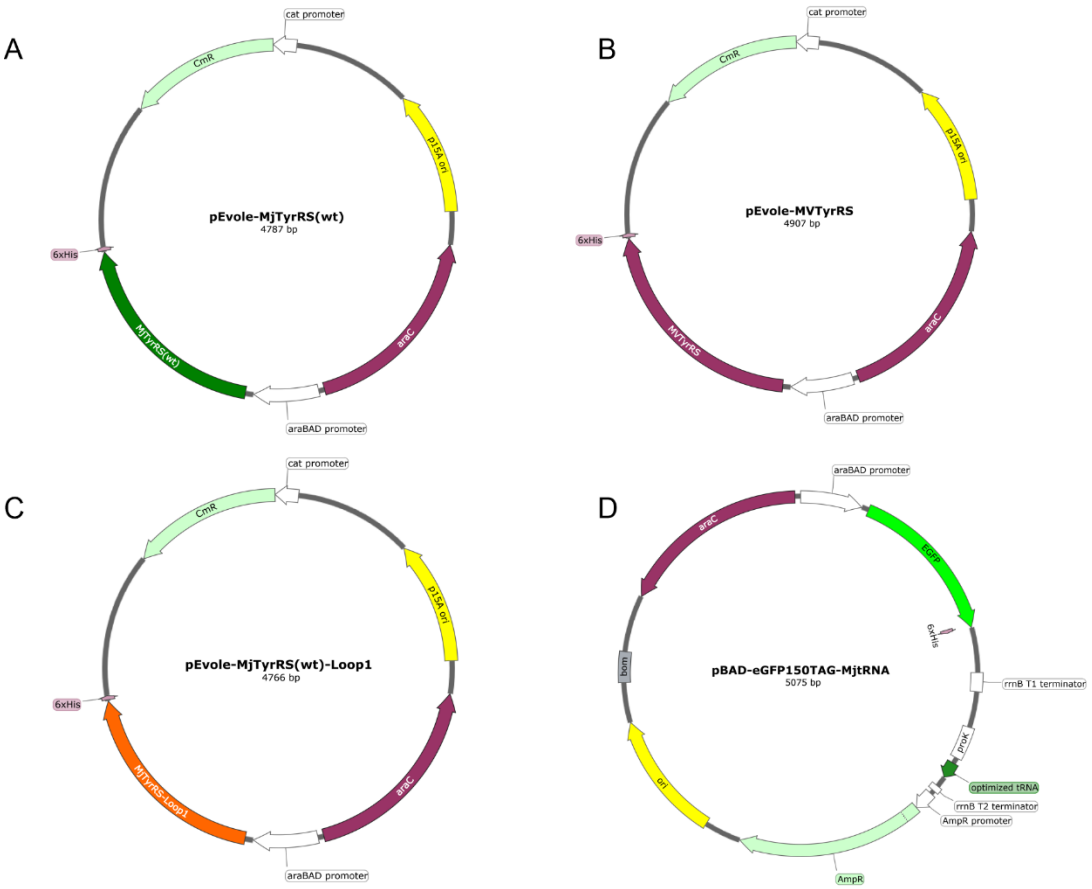
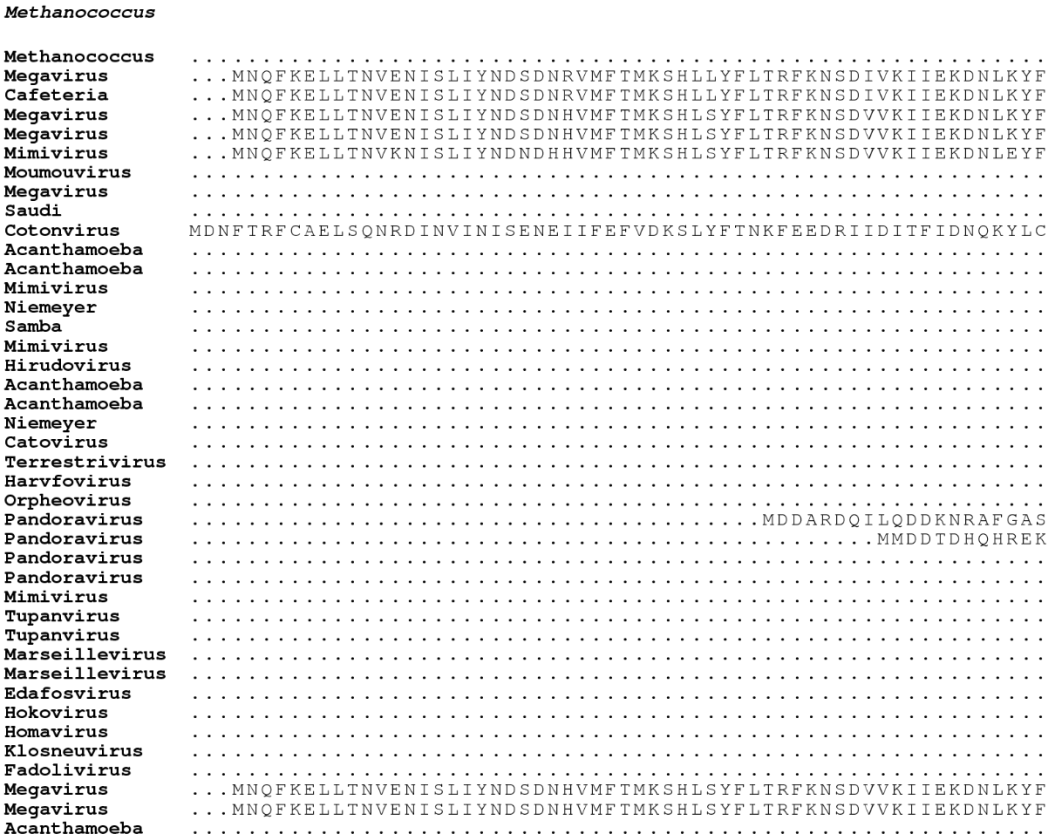


Figure S1. The maps of some plasmids used in this work.(A) pEvole-MjTyrRS(wt) (B) pEvole-MVTyrRS (C) pEvole-MjTyrRS(wt)-Loop1 (D) pBAD-eGFP150TAG-MjtRNA.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Acanthamoeba
Mimivirus
Niemeyer
Samba
Mimivirus
Hirudovirus
Acanthamoeba
Acanthamoeba
Niemeyer
Catovirus
Terrestrivirus
Harvfovirus
Orpheovirus
Pandoravirus
Pandoravirus
Pandoravirus
Pandoravirus
Mimivirus
Tupanvirus
Tupanvirus
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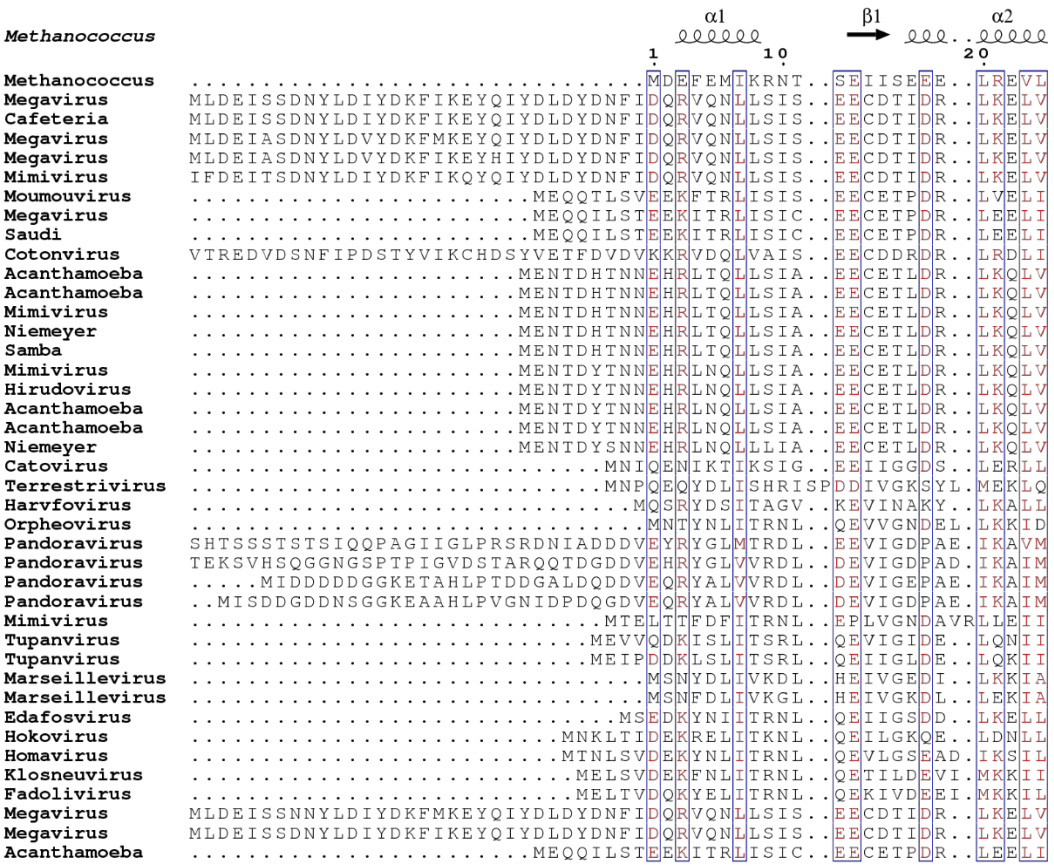
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Megavirus
Saudi
Cotonvirus
Acanthamoeba
Acanthamoeba
Mimivirus
Niemeyer
Samba
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Niemeyer
Catovirus
Terrestrivirus
Harvfovirus
Orpheovirus
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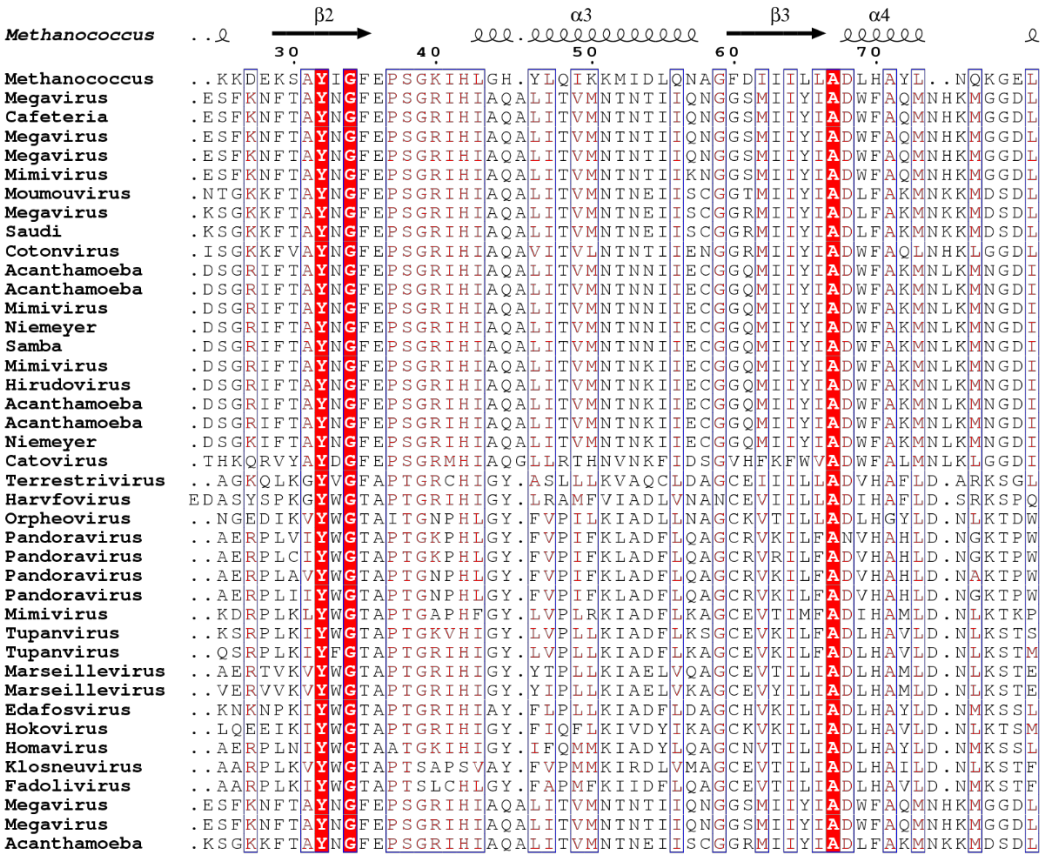
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Moumouvirus
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Cotonvirus
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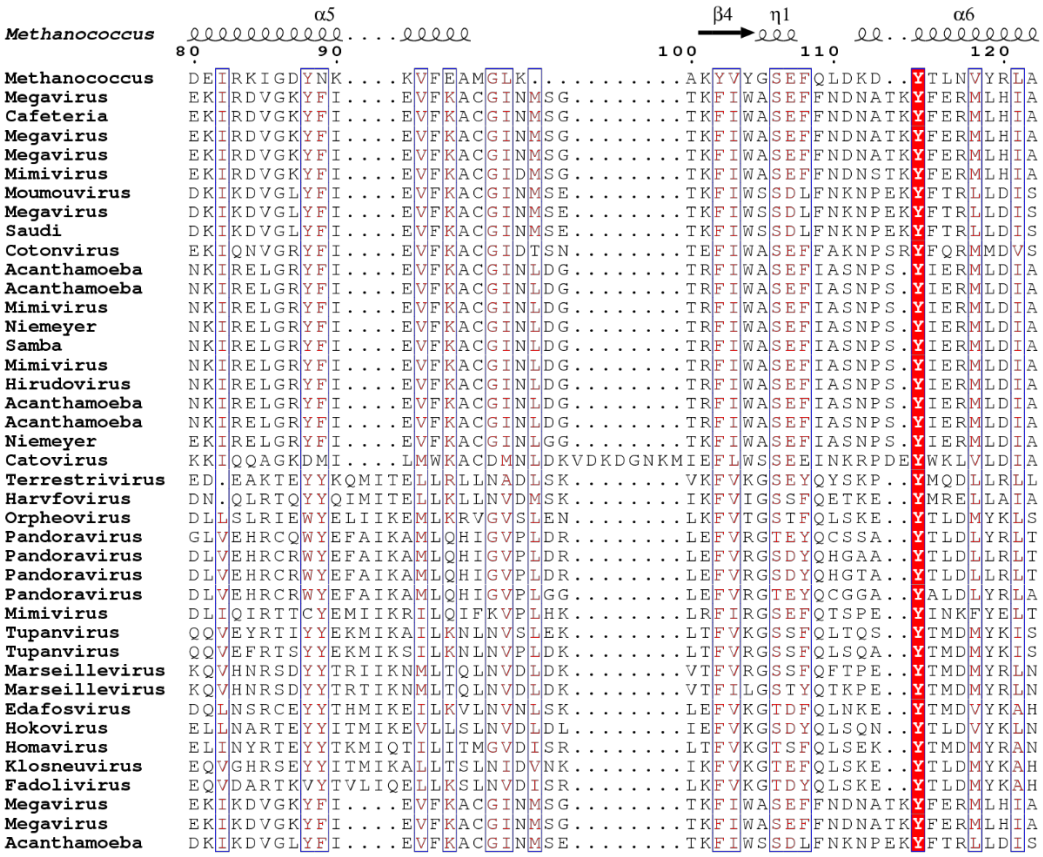
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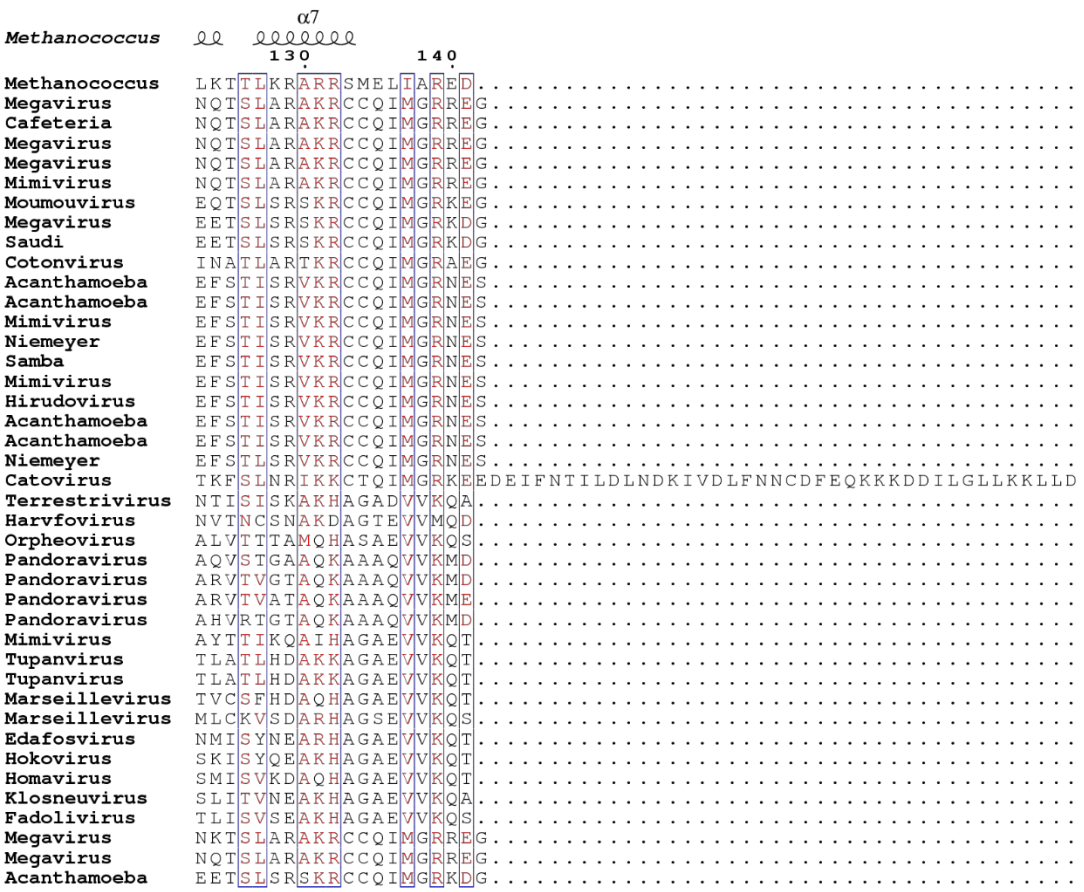
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Megavirus NQTSLARAKRCCQIMGRREG
Megavirus NQTSLARAKRCCQIMGRREG
Mimivirus NQTSLARAKRCCQIMGRREG
Moumouvirus EQTSLSRSKRCCQIMGRKEG
Megavirus EETSLSRSKRCCQIMGRKDG
Saudi EETSLSRSKRCCQIMGRKDG
Cotonvirus INATLARTKRCCQIMGRAEG
Acanthamoeba EFSTISRVKRCCQIMGRNES
Acanthamoeba EFSTISRVKRCCQIMGRNES
Mimivirus EFSTISRVKRCCQIMGRNES
Niemeyer EFSTISRVKRCCQIMGRNES
Samba EFSTISRVKRCCQIMGRNES
Mimivirus EFSTISRVKRCCQIMGRNES
Hirudovirus EFSTISRVKRCCQIMGRNES
Acanthamoeba EFSTISRVKRCCQIMGRNES
Acanthamoeba EFSTISRVKRCCQIMGRNES
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Catovirus TKFSLNRIKKCTQIMGRKEEDEIFNTILDLNDKIVDLFNNCDFEQKKKDDILGLLKKLLD
Terrestrivirus NTISISKAKHAGADVVKQA
Harvfovirus NVTNCSNAKDAGTEVVMQD
Orpheovirus ALVTTTAMQHASAEVVKQS
Pandoravirus AQVSTGAAQKAAAQVVKMD
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Mimivirus AYTTIKQAIHAGAEVVKQT
Tupanvirus TLATLHDAKKAGAEVVKQT
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Edafosvirus NMISYNEARHAGAEVVKQT
Hokovirus SKISYQEAKHAGAEVVKQT
Homavirus SMISVKDAQHAGAEVVKQT
Klosneuvirus SLITVNEAKHAGAEVVKQA
Fadolivirus TLISVSEAKHAGAEVVKQS
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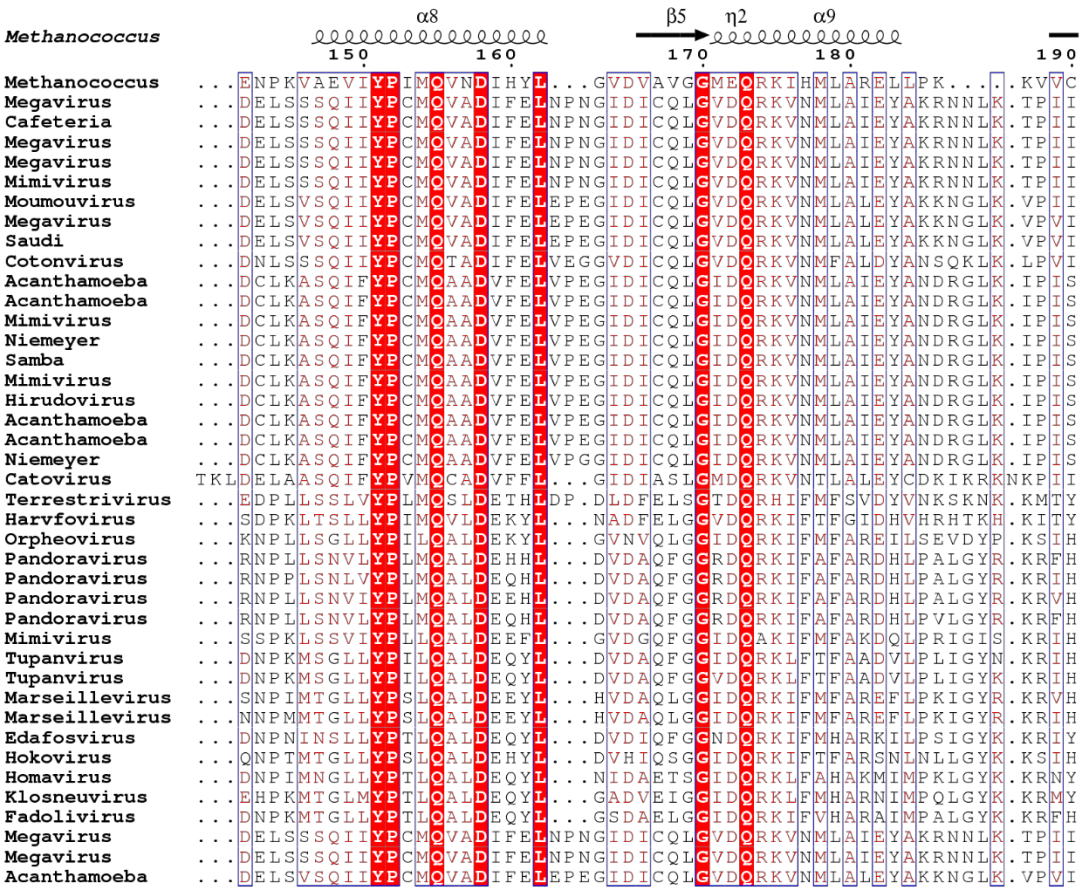
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α9
Methanococcus
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Saudi ...DELSVSQIIYPCMQVADIFELEPEGIDICQLGVDQRKVNMLALEYAKKNGLK.VPVI
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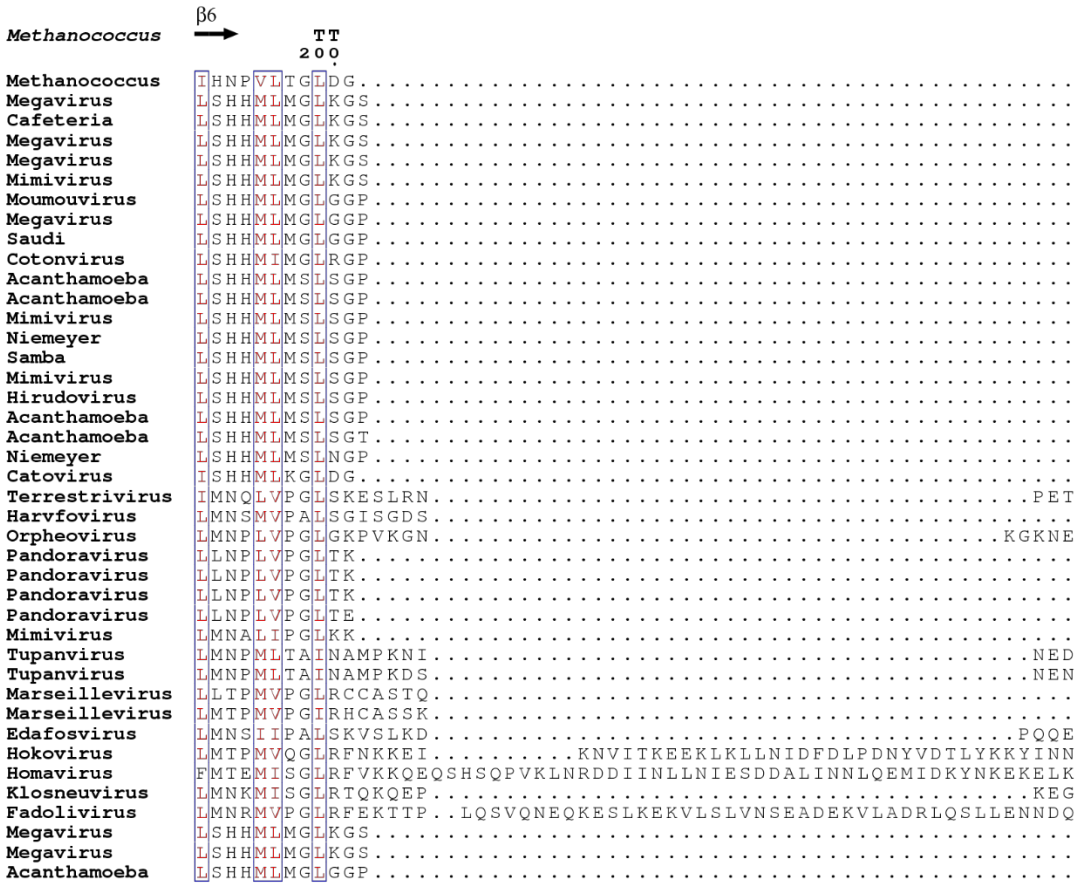
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Moumouvirus LSHHMLMGLGGP
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Saudi LSHHMLMGLGGP
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Mimivirus LSHHMLMSLSGP
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Catovirus ISHHMLKGLDG
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Pandoravirus LLNPLVPGLTK
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Edafosvirus LMNSIIPALSKVSLKD.....PQQE
Hokovirus LMTPMVQGLRFNKKEI.....KNVITKEEKLKLLNIDFDLPDNYVDTLYKKYINN
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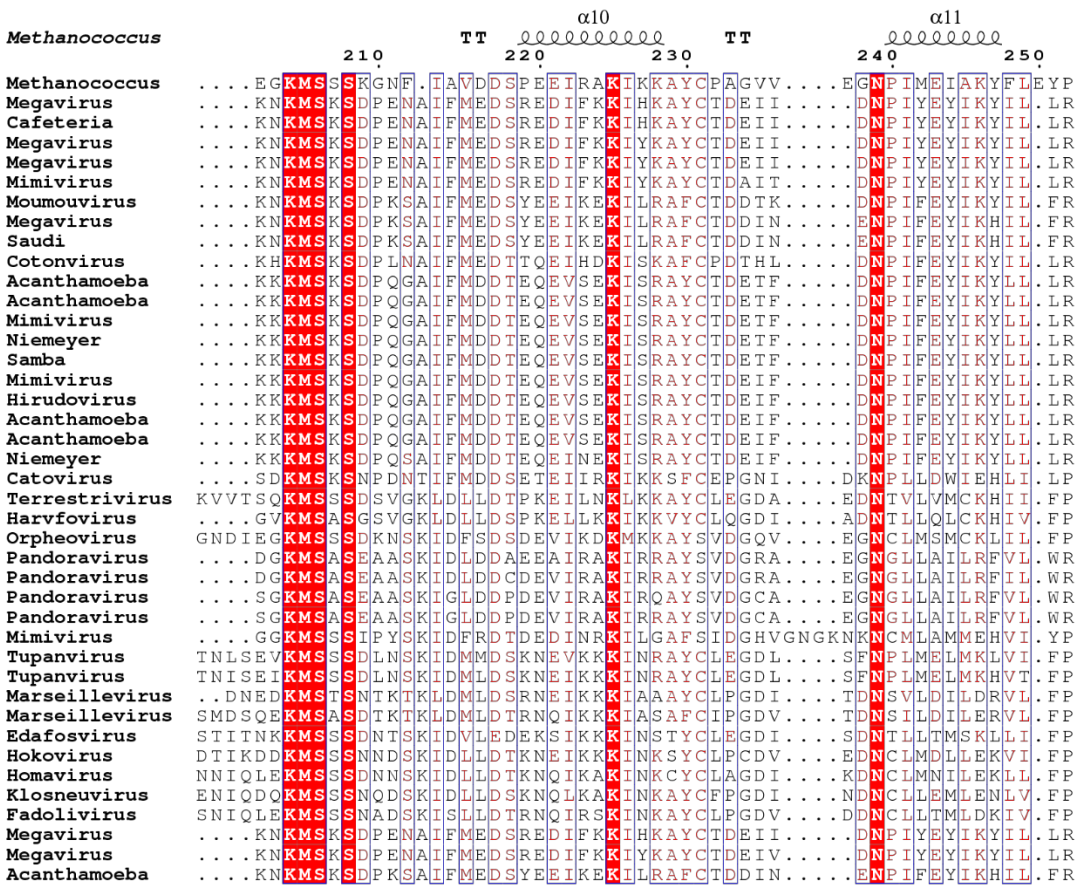
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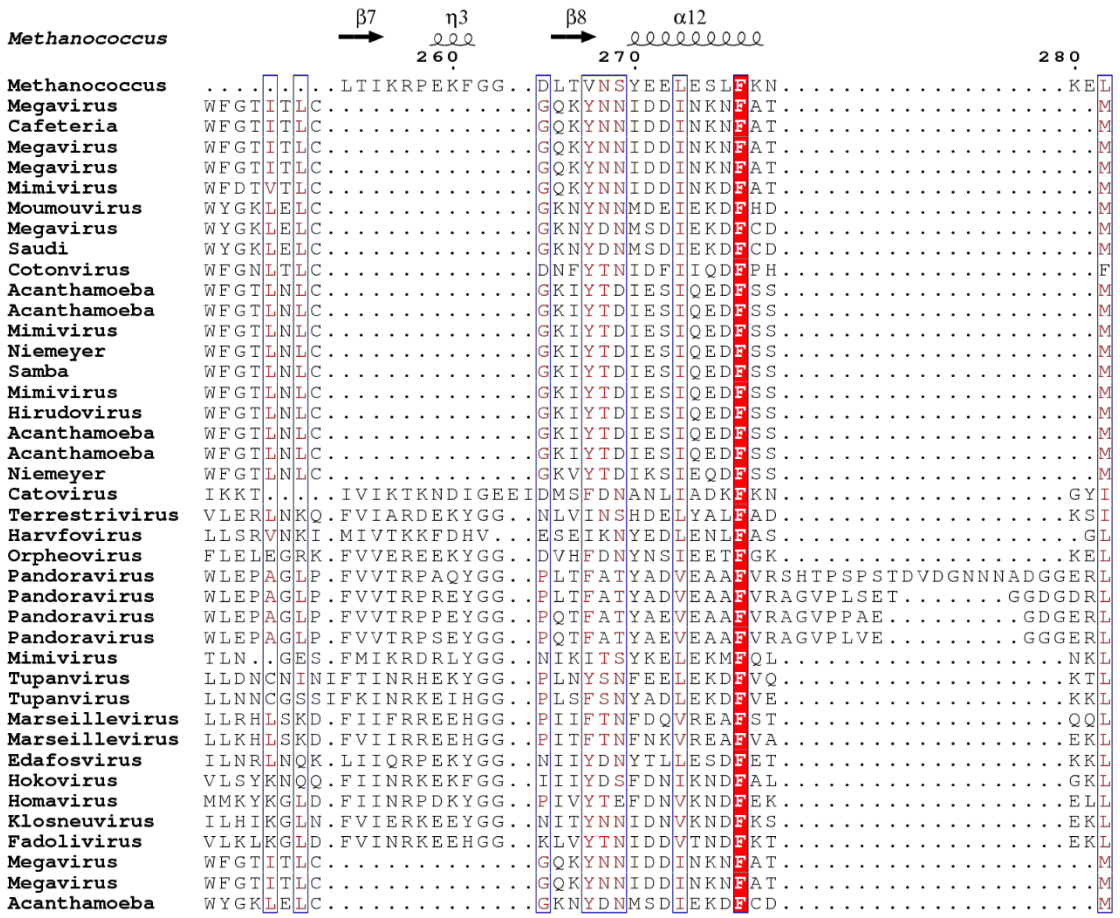
Edafosvirus STITNKKMSSSDNTSKIDVLEDEKSIKKKINSTYCLEGDI....SDNTLLTMSKLLI.FP
Hokovirus DTIKDDKMSSSNNDSKIDLLDTKNEIKKKINKSYCLPCDV....EDNCLMDLLEKVI.FP
Homavirus NNIQLEKMSSSDNNSKIDLLDTKNQIKAKINKCYCLAGDI....KDNCLMNILEKLL.FP
Klosneuvirus ENIQDQKMSSSNQDSKIDLLDSKNQLKAKINKAYCFPGDI....NDNCLLEMLENLV.FP
Fadolivirus SNIQLEKMSSSNADSKISLLDTRNQIRSKINKAYCLPGDV....DDNCLLTMLDKIV.FP
Megavirus ....KNKMSKSDPENAIFMEDSREDIFKKIHKAYCTDEII.....DNPIYEYIKYIL.LR
Megavirus ....KNKMSKSDPENAIFMEDSREDIFKKIYKAYCTDEIV.....DNPIYEYIKYIL.LR
Acanthamoeba ....KNKMSKSDPKSAIFMEDSYEEIKEKILRAFCTDDIN.....ENPIFEYIKHIL.FR


β7
η3
β8
α12
Methanococcus
260
270
280
Methanococcus .....LTIKRPEKFGG..DLTVNSYEELESLFKN.....KEL
Megavirus WFGTITLC.....GQKYNNIDDINKNFAT.....M
Cafeteria WFGTITLC.....GQKYNNIDDINKNFAT.....M
Megavirus WFGTITLC.....GQKYNNIDDINKNFAT.....M
Megavirus WFGTITLC.....GQKYNNIDDINKNFAT.....M
Mimivirus WFDTVTLC.....GQKYNNIDDINKDFAT.....M
Moumouvirus WYGKLELC.....GKNYNNMDEIEKDFHD.....M
Megavirus WYGKLELC.....GKNYDNMSDIEKDFCD.....M
Saudi WYGKLELC.....GKNYDNMSDIEKDFCD.....M
Cotonvirus WFGNLTLC.....DNFYTNIDFIIQDFPH.....F
Acanthamoeba WFGTLNLC.....GKIYTDIESIQEDFSS.....M
Acanthamoeba WFGTLNLC.....GKIYTDIESIQEDFSS.....M
Mimivirus WFGTLNLC.....GKIYTDIESIQEDFSS.....M
Niemeyer WFGTLNLC.....GKIYTDIESIQEDFSS.....M
Samba WFGTLNLC.....GKIYTDIESIQEDFSS.....M
Mimivirus WFGTLNLC.....GKIYTDIESIQEDFSS.....M
Hirudovirus WFGTLNLC.....GKIYTDIESIQEDFSS.....M
Acanthamoeba WFGTLNLC.....GKIYTDIESIQEDFSS.....M
Acanthamoeba WFGTLNLC.....GKIYTDIESIQEDFSS.....M
Niemeyer WFGTLNLC.....GKVYTDIKSIEQDFSS.....M
Catovirus IKKT.....IVIKTKNDIGEEIDMSFDNANLIADKFKN.....GYI
Terrestrivirus VLERLNKQ.FVIARDEKYGG..NLVINSHDELYALFAD.....KSI
Harvfovirus LLSRVNKI.MIVTKKFDHV...ESEIKNYEDLENLFAS.....GL
Orpheovirus FLELEGRK.FVVEREEKYGG..DVHFDNYNSIEETFGK.....KEL
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Mimivirus TLN..GES.FMIKRDRLYGG..NIKITSYKELEKMFQL.....NKL
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Marseillevirus LLKHLSKD.FVIIRREEHGG..PITFTNFNKVREAFVA.....EKL
Edafosvirus ILNRLNQK.LIIQRPEKYGG..NIIYDNYTLLESDFET.....KKL
Hokovirus VLSYKNQQ.FIINRKEKFGG..IIIYDSFDNIKNDFAL.....GKL
Homavirus MMKYKGLD.FIINRPDKYGG..PIVYTEFDNVKNDFEK.....ELL
Klosneuvirus ILHIKGLN.FVIERKEEYGG..NITYNNIDNVKNDFKS.....EKL
Fadolivirus VLKLKGLD.FVINRKEEHGG..KLVYTNIDDVTNDFKT.....EKL
Megavirus WFGTITLC.....GQKYNNIDDINKNFAT.....M
Megavirus WFGTITLC.....GQKYNNIDDINKNFAT.....M
Acanthamoeba WYGKLELC.....GKNYDNMSDIEKDFCD.....M
α13
Methanococcus
290
300
Methanococcus HPMDLKNAVAEELIKILEPIRKRL.....
Megavirus LKKDLKNDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN.....
Cafeteria LKKDLKNDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN.....
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Megavirus QKKDLKNDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN.....
Mimivirus NKKDLKNNVADYIDNIIEPVRRHFQDPQMAELYSRVRSYTN.....
Moumouvirus NKRELKNNVAEYINKIIEPVRIHFQQPHLKDLLEKVSSYRVTK.....
Megavirus NKRELKNNVAEYINKIIEPVRIHFQQPHLKNLLDKVSSYRVTK.....
Saudi NKRELKNNVAEYINKIIEPVRIHFQQPHLKNLLDKVSSYRVTK.....
Cotonvirus DKKRLKTDVANYIDRIIKPVRDHFAQDELKDLYDKVIN.....
Acanthamoeba NKRELKTDVANYINTIIDLVREHFKKPELSELLSNVKSYQQPSK.....
Acanthamoeba NKRELKTDVANYINTIIDLVREHFKKPELSELLSNVKSYQQPSK.....
Mimivirus NKRELKTDVANYINTIIDLVREHFKKPELSELLSNVKSYQQPSK.....
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Samba NKRELKTDVANYINTIIDLVREHFKKPELSELLSNVKSYQQPSK.....
Mimivirus NKRELKTDVANYINTIIDLVREHFKKPELSELLSNVKSYQQPSK.....
Hirudovirus NKRELKTDVANYINTIIDLVREHFKKPELSELLSNVKSYQQPSK.....
Acanthamoeba NKRELKTDVANYINTIIDLVREHFKKPELSELLSNVKSYQQPSK.....
Acanthamoeba NKRELKTDVANYINTIIDLVREHFKKPELSELLSNVKSYQQPSK.....
Niemeyer NKRELKTDVANYINIIIDLVREHFKKPELSDLLSNIKSYQQSSK.....
Catovirus HPKCLKNAVTTVLVNTLIPIQQKYIALKKNDN.....
Terrestrivirus HPSDLKKSVANIITDLFEPIRQHFSTNDMVKLLEQAYGK.....
Harvfovirus HPDDFKTSVALTIGELLKPIRDKFLETDMVTLLKKAYDSK.....
Orpheovirus ASMDLKVAIAKEIIKLVNPIRKIVNDN..VELMKSAYPTEKN.....
Pandoravirus CSADLKPAIADLLCEFLAPLRGVLGAR..ADLLRAAYPPMPH.....
Pandoravirus FSVDLKPAVADLLCEFLAPLRGVLAAR..ADLLATAYPSAP.....
Pandoravirus FSVDLKPAVADLLCEFLAPLRGVLAAR..ADLLHAAYPPPS.....
Pandoravirus FSVDLKPAVADLLCEFLAPLRGVLAAR..ADLFAAAYPV.....
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Marseillevirus HPADLKAGIVHTLDIFIEPVRKEFESREWRQVLKHSYAED.....
Edafosvirus HPSDLKNGISLFIINLIKPIQIEFQKEELVKLLQKAYS.....
Hokovirus HPQDFKLGITDSLNYILEPIRNSFETKERKLLLKKAYN.....
Homavirus HPSDFKMGITDGFDTIIKPIRDIFGTPELQKLIKQAYP.....
Klosneuvirus HPMDLKQGMINNLDLILDSIRKTFETPEMKKLIKMAYS.....
Fadolivirus HPADLKLGMVDSLDLMMEPLRNAFKSKESVQLLNKAYPQK.....
Megavirus LKKDLKNDVADYIDYILEPVRRHFQDPQMAELYSRVRSYTN.....
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Acanthamoeba NKRELKNNVAEYINKIIEPVRIHFQQPHLKNLLDKVSSYRVTK.....

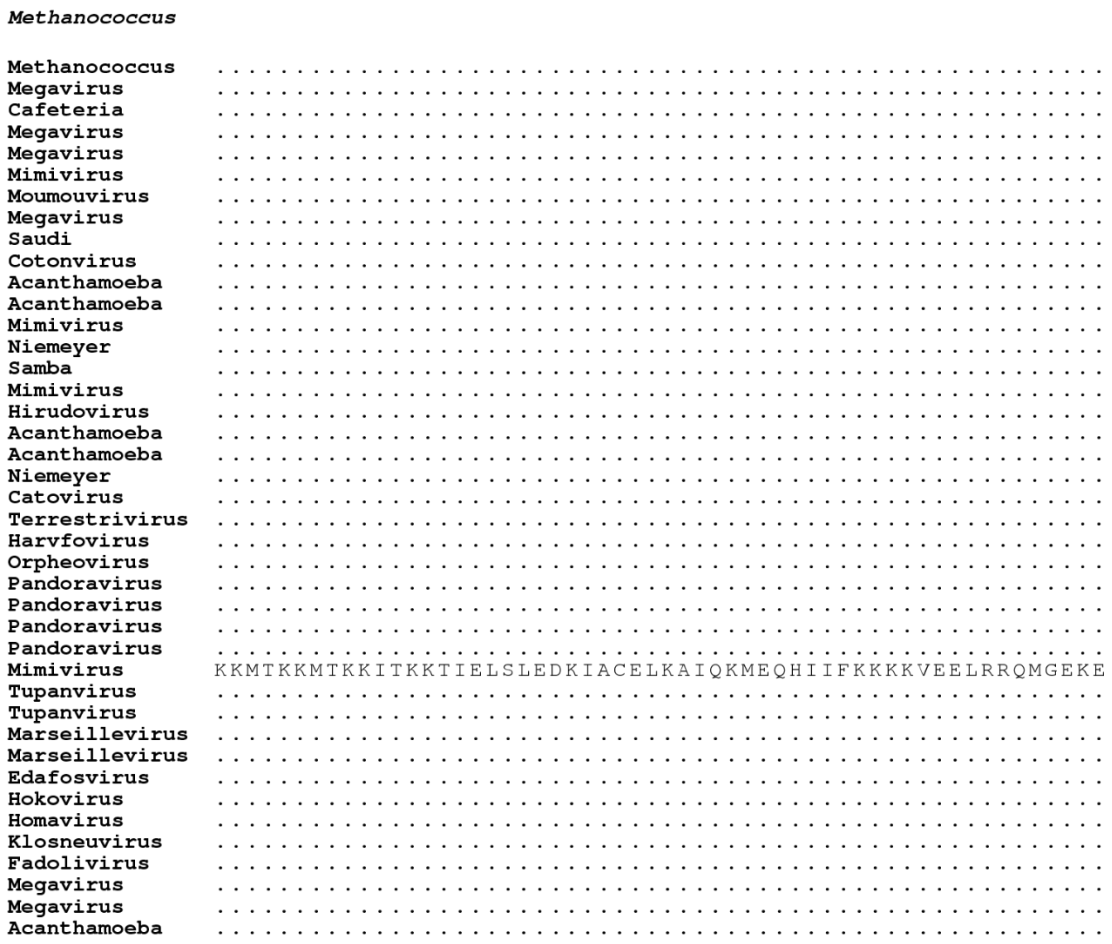

Figure S2. Sequence alignment of MjTyrRS and 40 giant virus derived TyrRSs. The TyrRSs are from *Methanococcus jannaschii*; *Megavirus chiliensis*; *Cafeteria roenbergensis virus BV-PW1*; *Megavirus courdo7-2*; *Megavirus vitis*; *Mimivirus sp. SH*; *Moumouvirus australiensis*; *egavirus courdo 7-1*; *Saudi moumouvirus*; *Cotonvirus japonicus*; *Acanthamoeba castellanii mimivirus*; *Acanthamoeba polyphaga mimivirus*; *Mimivirus Bombay*; *Niemeyer virus-1*; *Samba virus*; *Mimivirus reunion*; *Hirudovirus strain Sangsue*; *Acanthamoeba castellanii mamavirus*; *Acanthamoeba polyphaga lentillevirus*; *Niemeyer virus-2*; *Catovirus CTV1*; *Terrestrivirus sp.*; *Harvfovirus sp.*; *Orpheovirus IHUMI-LCC2*; *Pandoravirus macleodensis*; *Pandoravirus salinus*; *Pandoravirus japonicus*; *Pandoravirus dulcis*; *Mimivirus LCMiAC01*; *Tupanvirus soda lake*; *Tupanvirus deep ocean*; *Marseillevirus LCMAC202*; *Marseillevirus LCMAC201*; *Edafosvirus sp.*; *Hokovirus HKV1*; *Homavirus sp.*; *Klosneuvirus KNV1*; *Fadolivirus 1*, *Megavirus courdo11,Megavirus lba isolate LBA111,Acanthamoeba polyphaga moumouvirus*. The secondary structural elements of the *M. jannaschii* are indicated above the alignment as coils for α-helices and arrows for β-strands. The residues that are conserved among the *M. jannaschii* and giant viruses are marked in red, and slightly less conservative residues are in red font and framed by blue boxes. Alignments were done with ClustalX2. The figure was drawn with ESPript.

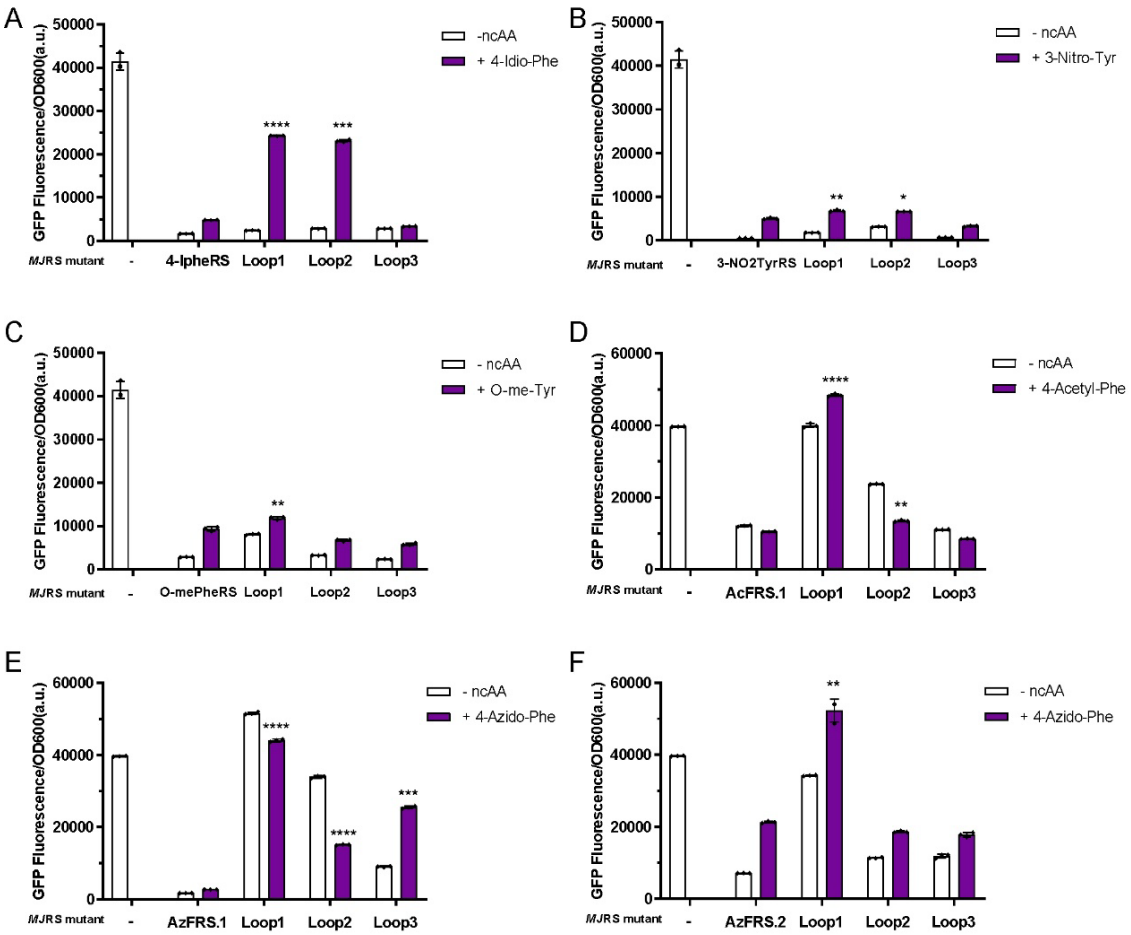

Figure S3. The fluorescence assays of the MjTyrRS loop-minimized mutants to incorporate ncAAs. For the protein expression, pEvolve bears MjTyrRS and pBAD plasmid bears tRNA and GFP reporter. (A) 4-I-Phe MjTyrRS mutant (B)3-NO_2-Tyr MjTyrRS mutant (C) O-Me-Tyr MjTyrRS mutant (D) AcFRS.1 (E)AzFRS.1 (F) AzFRS.2 Error bars represented the ±standard error of the mean from three biologically independent experiments. Statistical significance is quantified with ordinary one-way ANOVA.

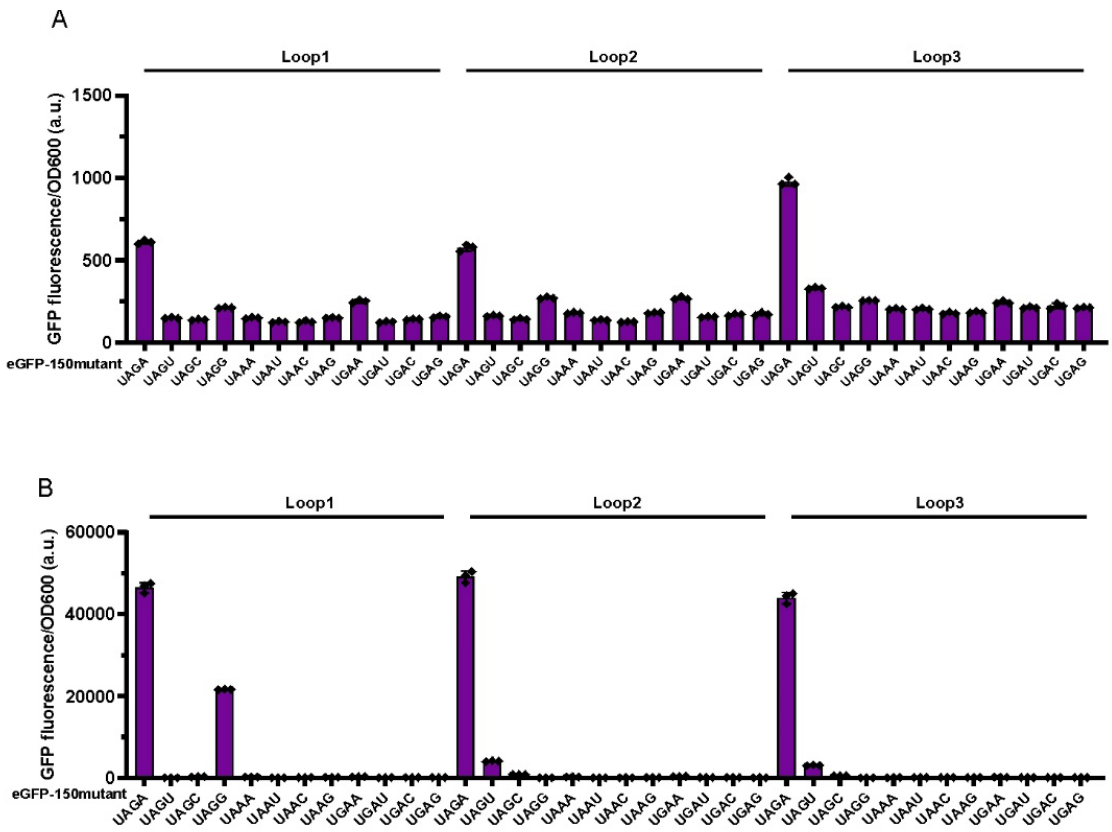

Figure S4. Analysis of quadruplet codons suppression activity of the loop-minimized MjTyrRS. The suppression efficiency is characterized by eGFP reporter assay in *E. coli* (A) DH10B and (B) C321.ΔA.expΔPBAD strain respectively. Error bars represented ± standard error of the mean from three biologically independent experiments.

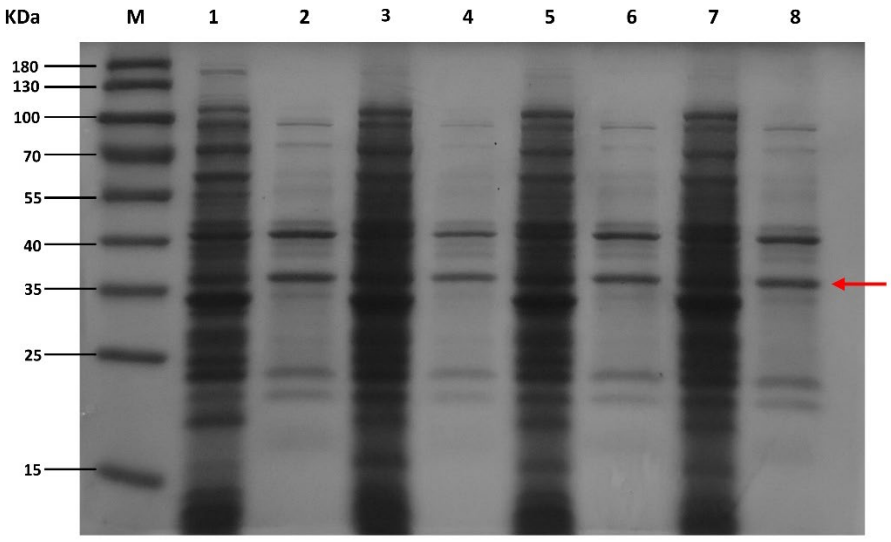

Figure S5. The SDS-PAGE result of the supernatant and precipitate fractions of the MjTyrRS and its loop-minimized mutants. After overnight expression, the bacteria were harvested and lysed by Bugbuster. The supernatant and precipitate fractions were subsequently separated by centrifugation for SDS-PAGE separately. The lanes in the gel are: M: 180 kDa Prestained

Protein Marker; Lane 1: MjTyrRS precipitate fraction; Lane 2: MjTyrRS supernatant fraction; Lane 3: MjTyrRS Loop1 mutant precipitate fraction; Lane 4: MjTyrRS Loop1 mutant supernatant fraction; Lane 5: MjTyrRS Loop2 mutant precipitate fraction; Lane 6: MjTyrRS Loop2 mutant supernatant fraction; Lane 7: MjTyrRS Loop3 mutant precipitate fraction; Lane 8: MjTyrRS Loop3 mutant supernatant fraction. The red arrow points to the position corresponding to the size of MjTyrRS.

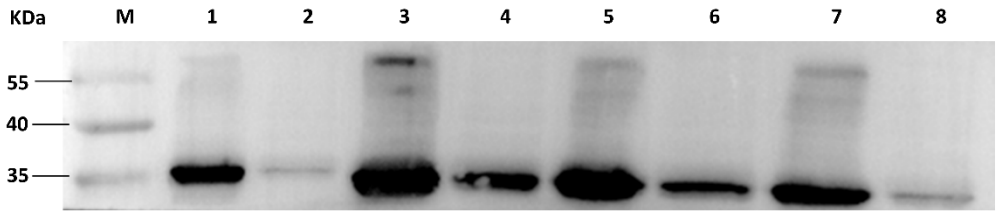

Figure S6. The Western Blot result of the supernatant and precipitate fractions of the MjTyrRS and its loop-minimized mutants. After the expression of MjTyrRS and its loop-minimized mutants fused with the HA tag, the cell fragmentation supernatants and precipitates were carried out for SDS-PAGE, followed by the Western Blot test. The lanes on the cellulose-nitrate membrane are: M: partial bands of the 180 kDa Prestained Protein Marker; Lane 1: MjTyrRS precipitate fraction; Lane 2: MjTyrRS supernatant fraction; Lane 3: MjTyrRS Loop1 mutant precipitate fraction; Lane 4: MjTyrRS Loop1 mutant supernatant fraction; Lane 5: MjTyrRS Loop2 mutant precipitate fraction; Lane 6: MjTyrRS Loop2 mutant supernatant fraction; Lane 7: MjTyrRS Loop3 mutant precipitate fraction; Lane 8: MjTyrRS Loop3 mutant supernatant fraction.

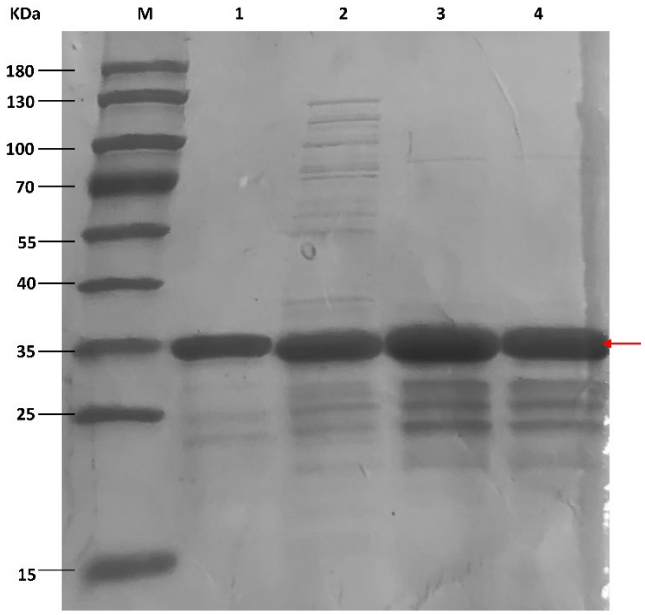

Figure S7. The SDS-PAGE result of purification of the MjTyrRS and its loop-minimized mutants by Co^{2+} affinity chromatography. The lanes in the gel are: M: 180 kDa Prestained

Protein Marker r; Lane 1: wild-type MjTyrRS; Lane 2: MjTyrRS Loop1; Lane 3: MjTyrRS Loop2; Lane 4: MjTyrRS Loop3. The red arrow points to the position corresponding to the size of MjTyrRS.

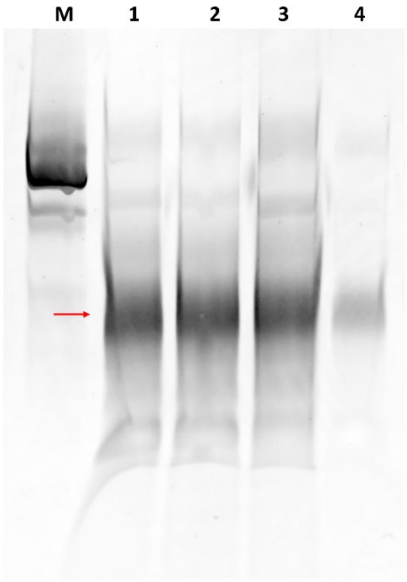

Figure S8. The denaturing polyacrylamide–urea gels result of the purified MjtRNA. The MjRNAs were resolved by electrophoresis on 7 M urea/12% acrylamide gels. The bands on the gel are: M: DNA template used for transcription as marker(139bp) 1~2: purified MjtRNA$_{CUA}$(77nt) 3~4: purified MjtRNA$_{UCUA}$(78nt). The red arrow indicates the position of the corresponding band of mjtRNA.

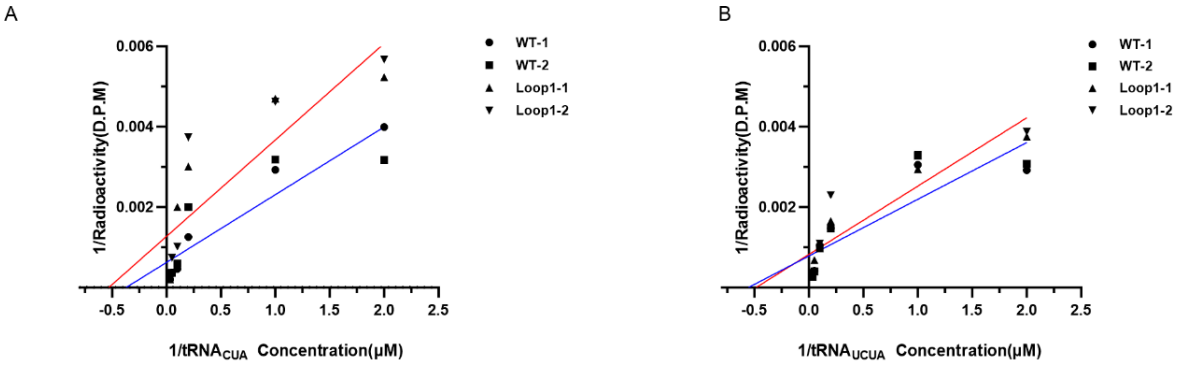

Figure S9. The aminoacylation assays of wild-type MjTyrRS and MjTyrRS Loop1 in response to (A) MjtRNA$_{CUA}$ and (B) MjtRNA$_{UCUA}$. The assay method was described in the **Aminoacylation assays in vitro**. The substrates for the reactions were triplet MjtRNA$_{CUA}$ and quadruplet MjtRNA$_{UCUA}$, respectively. The horizontal coordinates in the graph above represent the reciprocal of the substrate concentration, and the vertical coordinates represent the reciprocal of the measured radioactivity. The fitting curves of wild-type MjTyrRS and loop1 are shown in blue and red, respectively. Each set of experiments was repeated twice, distinguished by 1 and 2 after the group name.

Table S3. Kinetic parameter for tyrosylation by wild-type MjTyrRS and MjTyrRS Loop1.

	K_m(μM) for wt-MjTyrRS	K_m(μM) for MjTyrRS Loop-1
$MjtRNA_{CUA}$	4.74	1.88
$MjtRNA_{UCUA}$	1.77	1.82

tRNA preparation：

The MjtRNA used in this work, whose corresponding DNA sequences begin with an unfavorable nucleotide for transcription by T7 polymerase, was prepared by the 'transzyme' method. After transzyme transcription, the reaction was incubated at 60 °C for autocatalytic cleavage to produce the desired tRNA. The transcripts were then purified using a DEAE sepharose column, and the purified MjtRNA was obtained by elution using a buffer containing NaCl. The tRNA was then precipitated overnight by adding double-volume isopropanol. The precipitated MjtRNA was washed twice with ice-ethanol the following day and then dissolved in aminoacylation assay buffer.

Aminoacylation assays in vitro:

The MjtRNA transcripts were obtained by in vitro transcription, and the purified MjTyrRS and its loop-minimized mutants were obtained by affinity chromatography methods. Before the aminoacylation assay, the MjtRNA transcripts were renatured by heating for 2 minutes at 80 °C, followed by slow cooling to room temperature. Tyrosylation of RNAs was performed in 150 mM Hepes/KOH pH 7.5, 15 mM $MgCl_2$, 10 mM ATP,.5 mg/mL BSA, and 20 M dithioerythritol. ^{14}C isotope-labeled tyrosine was diluted to a radioactivity of 18.75 μci/mL. Kinetic parameters were determined in the presence of 2μM of MjTyrRS or its loop-minimized mutants and transcripts concentrations varying from 0.5μM to 30μM. After incubation at 37 °C for 5 min, aliquots were removed, spotted on Whatman 3mm paper, and precipitated by trichloroacetic acid. The incorporation of radioactive tyrosine was measured by liquid scintillation. Radioactivity values were obtained by a Hitachi Aloka LSC-8000. The kinetic constants K_m were derived from Lineweaver–Burk plots, and the data shown represent an average of at least two independent experiments.