

SyRNaseE	331	NIGGVIIIDFIDMDSHKDQLQLLEHFNRCL	ETDKAR	PQIAQLTELGLVELTRKRQGNLYELFGQPCPEC	400
EcRNaseE	338	DLGGLIVIDFIDMTPVRHQRAVENRLREAVRQDRARIQISHISRFGLLEMSRQRLSPSLGESSHHVCPRC			407
EcRNaseG	339	NLGGIIIDFIDMNNEDHRRRVLHLSLEQALS	KDRVK	TSVNGFSALGLVEMTRKRRTRESIEHVL	CNECPTC 408
2bx2.pdb_chainL_s001	328	DLGGLIVIDFIDMTPVRHQRAVENRLREAVRQDRARIQISHISRFGLLEMSRQRLSPSLGESSHHVCPRC			397
Consensus aa:		sLGGILIDFIDM	ss.ccp..lbpphpptlppD+h+.pls.ho.hGLLEhoRpR...	sl.c...p.CPpC	
Consensus ss:		eeeee	hhhhhhhhhhhhhhhh	eee	eeeeee hhhh
<hr/>					
SyRNaseE	401	GGLGHLVELPGEKGFVLSPTAVNSSIPPRLVEKPILSPPVAKVNDLP	-----	KKEEAKISSPLDL	461
EcRNaseE	408	SGTGTVRDNESLSLSILRLIEEEALKENTQEVHAI	VPVPIASYLLNEKRS	SAVNAIETRQDGVRCVIVPND	477
EcRNaseG	409	HGRGTVKTVETVCYEIMREIVRVHHAYDSDRFLVYASPAVAEALKGEE	SHSLAEVE-IFV	GKQVKVQIEP	477
2bx2.pdb_chainL_s001	398	SGTGTVRDNESLSLSILRLIEEEALKENTQEVHAI	VPVPIASYLLNEKRS	SAVNAIETRQDGVRCVIVPND	467
Consensus aa:		.G.Ghl.p.s...l.....bssp.h...h.ss.h..l.sb.....b...ph...p.			
Consensus ss:		eeeeehhhhhhhhhhhhhhhhh	eeeeee	hhhhhhh	hhhhhhh hh eeeeee
<hr/>					
Zn Link					
SyRNaseE	462	LF-HPNYQEQGDRD	-----	-----	474
EcRNaseE	478	QMETPHYHVLVRVRKGEETPTLSYMLPKLHEEAM	ALPSEEEFAERKRPEQ	PALATFAMPDVPPAPTPAEPA	547
EcRNaseG	478	LYNQEQFDVVM	-----	-----	489
2bx2.pdb_chainL_s001	468	QMETPHYHVLVRVRKGEETPTLSYMLPKLHEEAM	-----	-----	500
Consensus aa:		bh.p.p@p.....			
Consensus ss:		eeeee			
<hr/>					
C1					
SyRNaseE	475	-----	-----	-----SNRR-----	478
EcRNaseE	548	APVVAPAPKAAPATPAAPAQPGLLSRFFGALKALFSGGEETKPTEQPAPKAEA	KPERQ	DRRKPRQNNRR	617
EcRNaseG		-----	-----	-----	
2bx2.pdb_chainL_s001		-----	-----	-----	
Consensus aa:				
Consensus ss:				hhh	
<hr/>					
SyRNaseE	479	-----	RRRRRGSEFS	EKENIKSVGISRSKGPSPTKEKVTGTAPPRRERPSR	526
EcRNaseE	618	DRNERDTRSERTEGSDNREENRRNRRAQQQTAE	TRESRQAE	----VTEKARTADEQQAPRRERSRR	682
EcRNaseG		-----	-----	-----	
2bx2.pdb_chainL_s001		-----	-----	-----	
Consensus aa:				
Consensus ss:			hhhhh		
<hr/>					
C2					
SyRNaseE	527	RVEKTLVPVDVAMTTLEQDIYARMGISPLIKTEYADQDPRSFMVSVVTAG	AALEGNTNGSGSLVNAVITT		596
EcRNaseE	683	RNDDKRQAQQEAKALNVE	-----EQSVQETEQEER	-----VRPVQPRRKQRQLNQKVRYEQSV	736
EcRNaseG		-----	-----	-----	
2bx2.pdb_chainL_s001		-----	-----	-----	
Consensus aa:				
Consensus ss:					

C3

SyRNaseE 597 VDNGDNGDNVPSDGLTIVSEVTAPTIVIEQPREETVEPEQVVLPLQLDDETPAAPVAEESAPIETKKRP-- 664
 EcRNaseE 737 **EEAVVAPVVEETVAA**--EPIVQEAPAPRTELVKVPLP**VVAQTAP****PEQQEENNADNRD**NGGMPPRRSRRSPRH 804
 EcRNaseG -----
 2bx2.pdb_chainL_s001 -----
Consensus aa:
Consensus ss:

SyRNaseE 665 ---**GRRRRRS**-----**SAE**----- 674
 EcRNaseE 805 LRVSG**QRRRRRYRDER**YPTQSPMPL**TVACASPEL**ASGKVIWIRYPIVRPQDVQ**VEEQREQE**EVHVQPM**VT**EV 874
 EcRNaseG -----
 2bx2.pdb_chainL_s001 -----
Consensus aa:
Consensus ss: hhh
 C4

SyRNaseE -----
 EcRNaseE 875 **PVAAAIEPVVSA****PVVEEVAGVVE**APVQVAEPQPEVVETTHPE**VI**AAAVTEQ**QVI****TESDVAVAQEVAEQ**A 944
 EcRNaseG -----
 2bx2.pdb_chainL_s001 -----
Consensus aa:
Consensus ss:

SyRNaseE -----
 EcRNaseE 945 EPVVEP**Q**EE**TADIEEVVETAEVVVAE**PEVVAQPA**PVVAEVA**AEVETVA**AVEPEVTVE**HNHATAPMTRAP 1014
 EcRNaseG -----
 2bx2.pdb_chainL_s001 -----
Consensus aa:
Consensus ss:

SyRNaseE -----
 EcRNaseE 1015 APEYVPEAPRHSDWQRPTFAFEGKGAAGGHTATHHASAAPARPQV**E** 1061
 EcRNaseG -----
 2bx2.pdb_chainL_s001 -----
Consensus aa:
Consensus ss:

B

SyRNaseJ -----
 BsRNaseJ1 -----
 BsRNaseJ2 -----
 CrRNaseJ 1 MQGGQ**V**LAGVSPASTSPAP**AAQRARSL**STRN**VVA**QADNS**ERWR**TQGGRGGGGGGGGGGGGGGGGGGGG 70
 rnj_3BK1.pdb_chainA_s001 -----
Consensus aa:
Consensus ss:

SyRNaseJ	1	-----MAK-----NTQTQALKILPLGGLHEIGKNTCVFEYD	31
BsRNaseJ1	1	-----MK-----FVKNDQTAVFALGGLGEIGKNTYAVQFQ	30
BsRNaseJ2	1	-----MK-----KKNTENVRIIALGGVGEIGKNLYVIEID	30
CrRNaseJ	71	RRGKTYVSESGMTLKSYSVASDQQEHLGSAIQKRRYKFPFPPTNGGPPPLRILPIGGLGEIGMNCMLAGVG	140
rnj_3BK1.pdb_chainA_s001	1	-----GPQDHVEIIPLGGMGEIGKNITVFRFR	27
<u>Consensus aa:</u>	M.....s.s..lcIIsIGGLGEIGbhhhh.h.	
<u>Consensus ss:</u>		eeeeee eeeeee	
SyRNaseJ	32	DEILLDAGLAFPTDDMHGVNVVLPMTYLRENRKIKGMVVTGHGEDHIGGIAYHLKQFDI----PIIY	97
BsRNaseJ1	31	DEIVLIDAGIKFPEDELLGIDYVIPDYTYLVKNEDKIKGLFITGHGEDHIGGIYLLRQVN----IPVY	95
BsRNaseJ2	31	SDIFVVDAGLMHPENEMLGIDVVIPDISYLIERADRVKAIFLTGHGDENIIGGVFYLLNKLS----VPVY	95
CrRNaseJ	141	DRYVVIDAGLMFPDFSDLGMQKILPDTDFLAQWKDRIEALIIITGHGEDHIGALPWVVPALD---PATPIY	207
rnj_3BK1.pdb_chainA_s001	28	DEIFVLDGGLAFPEEGMPGVLLIIPRVYLIHRHKIKAWVLTGHGEDHIGGLPFLLPMTFGKESPVPIY	97
<u>Consensus aa:</u>		DchhllDAGLhFP-.p.hGhp.llPDhs@Lhp.c-+IcthhlTHGHEDHIGtIs@ll..Is....hPIY	
<u>Consensus ss:</u>		eeeeee hhhhhhhhhhhh eeeee hhhh hhhhhh eee	
		M1 M2	
SyRNaseJ	98	GPRLAMALLRDKLEEAGMLE-RTNLQTVSPREMVRLGKSFVVEFIRNTHSIADSYCLAHTPLGVMHSG	166
BsRNaseJ1	96	GGKLAIGLLRNKLEEHGLLR-QTKLNIIGEDDIVKFR-KTAVSFFRTTHSIPDSYGIIVKTPPGNIVHTG	163
BsRNaseJ2	96	GTKLTLALLREKRLKQYGHNR-KTDLREIHSKSVITFE-STKVSFFRTIHSIPDSVGVSFKTLGSLGIVCTG	163
CrRNaseJ	208	ASSFVMQLVKRRLTEYNLWD-EKRFITFDMRQRFQAG-PFEIEPVRVTHSIPDCCGLIMRSDEGTIVHTG	275
rnj_3BK1.pdb_chainA_s001	98	GARLTLGLLRGKLEEFGLRPGAFNLKEISPDRIQVGRYFTLDFRMTHSIPDNSGVVIRTPIGTIVHTG	167
<u>Consensus aa:</u>		tsphhh.Ll+p+LpE@sh.c.ppph.phs.cp.hph..h.lp.hrhTHSIPDthGllh+osbGsIVHTG	
<u>Consensus ss:</u>		hhhhhhhhhhhhh eeee eeee eeeeeee eeeeeeee eeeeeee	
		M3	
SyRNaseJ	167	DFKIDHTPIDGEFFDLQKVAEYGEKGVLCLLSDSTNAEVPGITPSEASVIPNLDRVFSQA--EGRLMVT	234
BsRNaseJ1	164	DFKFDFTPV-GEPANLTKMAEIGKEGVLCLLSDSTNSENPEFTMSERRVGESIHDI FRKV--DGRIIFAT	230
BsRNaseJ2	164	DFKFDQTPALNQTCDIGEIAKIGNSGVLALLSDSANAERPGYTPSEAAVSGEISDALYNS--QNRVIIAV	231
CrRNaseJ	276	DWKIDENPLDGEQFDRELFMALGSEPVALLMMSDSTNVLSPGRTLSEQVVHDSLVEKVKLEHNGRGRVICTQ	345
rnj_3BK1.pdb_chainA_s001	168	DFKLDPTPIDGKVSHLAKVAQGAEGVLLLIADATNAERPGYTPSEMEIAKELDRVIGRA--PGRVFTT	235
<u>Consensus aa:</u>		D@Khd.sPl.Gc..bh.hGpcsVhhhhSDSTNhbPG.T.SE..V..plsc.h.ph..pGRlhhhp	
<u>Consensus ss:</u>		hhhhhhh eeee hhhhhhhhhhhhhh eeeee	
		M4 A	
β-CASP			
SyRNaseJ	235	FASSVHRVNIILSLAQKHQRKVAVVGRSMLNVIAHARKLGVIKCPDNLFVPLKAARNLPDQQQLILTGS	304
BsRNaseJ1	231	FASNIHRLQQVIEAAVQNGRQVAVFGRSMESAIEIGQTLGYINCPKNTFIEHNEINRMPANKVTILCTGS	300
BsRNaseJ2	232	FASNIHRIQQVIAAAQNGRKIAVAGKNLQSVLQARKLGYIEADDELFIQVQVKKYPKREVVAIITAGS	301
CrRNaseJ	346	FASNLHRLYGVKRAADAAGRKICFVGASLNHYLEAAWRDGRAPFDPKELMPTQLRHANPNEVLIVTTGS	415
rnj_3BK1.pdb_chainA_s001	236	FASHIHRIQSVIWAAEKYGRKVAMEGRSMLKFSRIAELGVLKVKDR-LYTLLEVKDLDPHQVLILATGS	304
<u>Consensus aa:</u>		FASslHRlb.VbpaA...GRKlthhg.Sh.phlpha.c.G.h.hssp.hshppl+chsspVhIltGS	
<u>Consensus ss:</u>		hhhhhhhhhhhhh eeeee hhhhhhhhhhhh hhh hhhh eeeee	

SyRNaseJ 305 QGEPLAAMTRISNGEHPQIKIRQGDTVVFSANPIPGNTIIVVNTIDRLMMQGANVIYGKHQGIHVSGHAS 374
 BsRNaseJ1 301 QGEPMAALSRIANGTHRQISINPGD TVVFS SSP I PGNTI SVSRTINQLYRAGAEVIHGPLNDIHTSGHGG 370
 BsRNaseJ2 302 QGEPLAALTRMANKAHQKQLNIEEGD TVVIA STPIPGQELIYSKTVDLLARAGA QVIFAQK-RVHVS GHGS 370
 CrRNaseJ 416 QGEPRAQLSMAARDQSNILKTLPGDLLLSAKVIPGNEGKVTKMLNALAGQGARVRQSRADNLHTSGHAY 485
 rnj_3BK1.pdb_chainA_s001 305 QGQPMSVLHRLAFEGHAKMAIKPGD TVILSSSPIPGNEEA VNRVINRLYALGAYVLYPPTYKVHASGHAS 374
 Consensus aa:
 Consensus ss:
 hhhhhhhh eee eeeee hhhhhhhhhhhhh eeeee eeeee

SyRNaseJ 375 QEEHKMLLALTRPKFFVPVHGEHRMLVKHSQMAQAQGIPISENIVIVNNGDVIELTGDRIRVAGQVP SGIE 444
 BsRNaseJ1 371 QEEQKLMRLIKPKFFMPIHGEYRMQKMHVKLATDCGIP EENCFIMDNGEVLALKGDEASVAGKIPSGSV 440
 BsRNaseJ2 371 QEELKLMINILKPKYLIPVNGEYRMQKAHSKIAEETGMKRSDIFLIEKGDVVEFRGQNVKIGDKVPYGN I 440
 CrRNaseJ 486 QEELVELLQSVRPQHFLPVHGEYAFLEHALLAKTR-AGVNF TDVIRNGEMLAVRERR--NRNTVSTGSM 552
 rnj_3BK1.pdb_chainA_s001 375 QEELKLILNLTPRFPLPVHGEVRHQMNFKWLAESMSRP PEKTLIGENGAVYRLTRET FEKVG E VPHGVL 444
 Consensus aa:
 Consensus ss:
 hhhhhhhhhh eeeee hhhhhhhhhhhhh hhhhee eeeee eeeeeeee e

SyRNaseJ 445 LVDQAGIVH-----ESTMAERQQMAEDGLVTVA A---ALSK-TGTL 482
 BsRNaseJ1 441 YIDGSGIGDIG-----NIVLRDRRILSEGLVIVVV---SIDMDDFKIS 481
 BsRNaseJ2 441 LIDGLGVGDIG-----NIVLRDRRLS QDGI LIVVI---TLDKQKHLV 481
 CrRNaseJ 553 AVAAARGGF EAPTMVKYDGE EPTYF FNDGGKGTGTRTEME IDMRGT MAMEG VVVGV D VIRR SAGDYGLG 622
 rnj_3BK1.pdb_chainA_s001 445 YVDGLGVGDIT-----EELADRRHMAEEGLVVITA---LAGEDP--- 481
 Consensus aa:
 Consensus ss:
 eee hhhhhhhhhhh eeeee eee ee

SyRNaseJ 483 AYP EVHCRGVMTIQPKLLEELIVRTIENFLTERWSEFTHGSNGSTEVSWNALQKELESSLQRLIKRELQ 552
 BsRNaseJ1 482 AGPDLISRGFVYMRRESGDLINDAQELISNHLQKVMERK-----TTQWSEIKNEITDTLAPFLYEKTK 543
 BsRNaseJ2 482 SGPEIITRGFVYVRESEGLIVQATELVRSIVTEATETS-----NVEWSTLKQAMRDALNQFLYEKTK 543
 CrRNaseJ 623 CGVRVTTRGMWTD E--GKLP AELTA AVENAVARLSGT-----CSLVEVERAAIDAVKRRCIAFNN 680
 rnj_3BK1.pdb_chainA_s001 482 -VVEVVS RGFVK--AGERLLGEVRRMALEALKNGVRE-----KKPLERIRDDIYYPVKKFLKKTG 539
 Consensus aa:
 Consensus ss:
 eeeee eee hhhhhhhhhhhhhhhhhhh hhhhhhhhhhhhhhhhhhh

C-terminal domain

SyRNaseJ 553 SSPMVLMLQTDTPIELDQVPQNV-----STVSA----- 581
 BsRNaseJ1 544 RRP MILPIIMEV----- 555
 BsRNaseJ2 544 RKP MI IPIIMEV----- 555
 CrRNaseJ 681 KRPEVIAIAYEH DPRDAHVAEVAEARADAAARANRARGE PEDAGGPASVLT PRRKAQNPWESGAAGSSIR 750
 rnj_3BK1.pdb_chainA_s001 540 RDP MILPVVIEG----- 551
 Consensus aa:
 Consensus ss:
 +cPbllslhHE.....
 eeeeeeee

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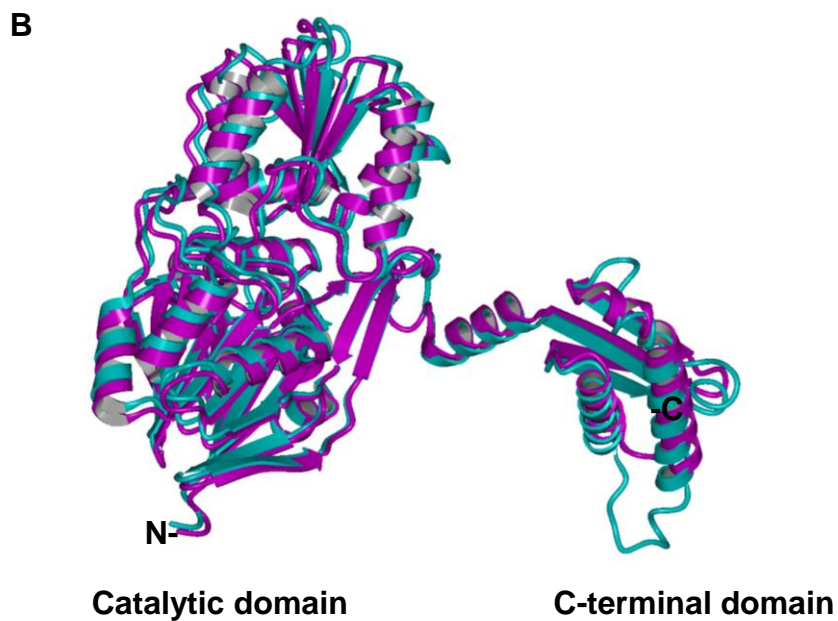
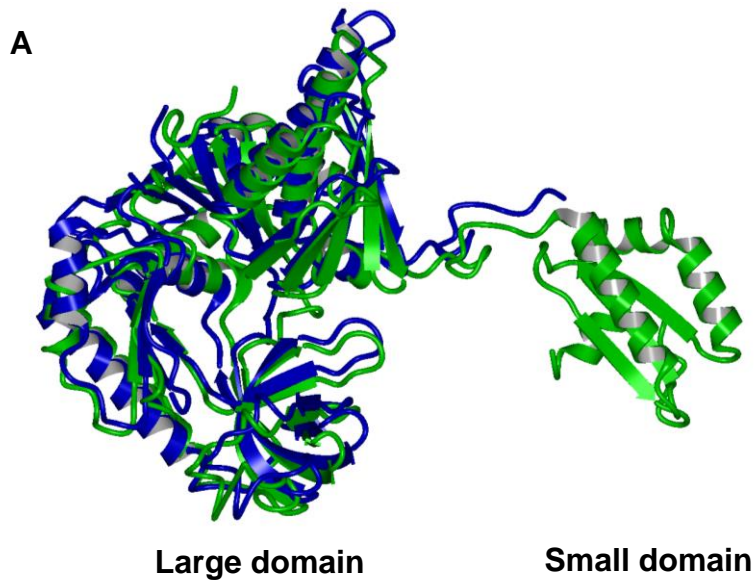
SyRNaseJ          582  -----TSATPAPRKKVVLTK-----TPEPKVKAKPEKVVVTTAEPSAQ-PVSTTTK 625
BsRNaseJ1
BsRNaseJ2
CrRNaseJ
rnj_3BK1.pdb_chainA_s001
Consensus_aa:
Consensus_ss:

SyRNaseJ          626  VYRRSRKRSTT-----SVSS----- 640
BsRNaseJ1
BsRNaseJ2
CrRNaseJ          814  KVRRSR--TTTAPAPDSGIISISGSTDDDEDAAAAAGPASGSVAADEDSKLSRMLRARKAAASTRRTRA 881
rnj_3BK1.pdb_chainA_s001
Consensus_aa:
Consensus_ss:

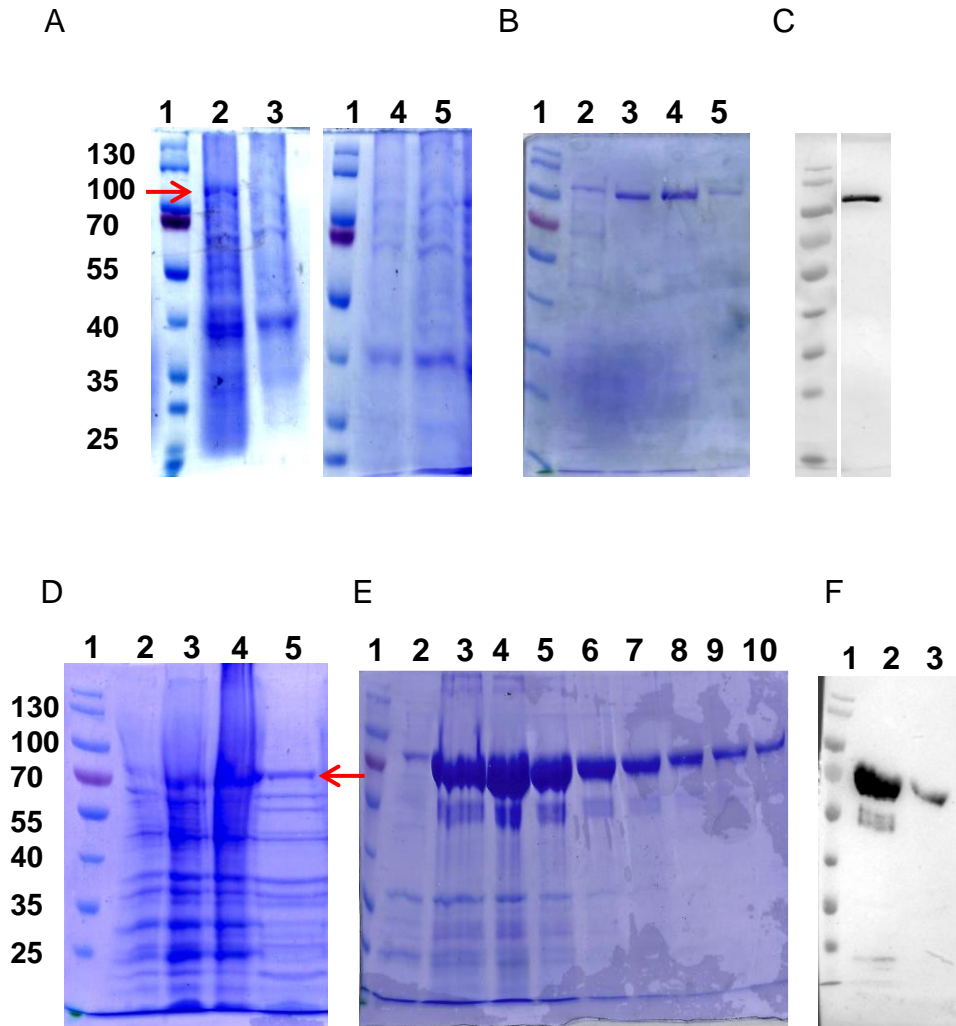
SyRNaseJ
BsRNaseJ1
BsRNaseJ2
CrRNaseJ          882  DKGEEGAGTTLTPLDPKVIADRRQRNPRDRPHSIEDPDYG 920
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Consensus_aa:
Consensus_ss:

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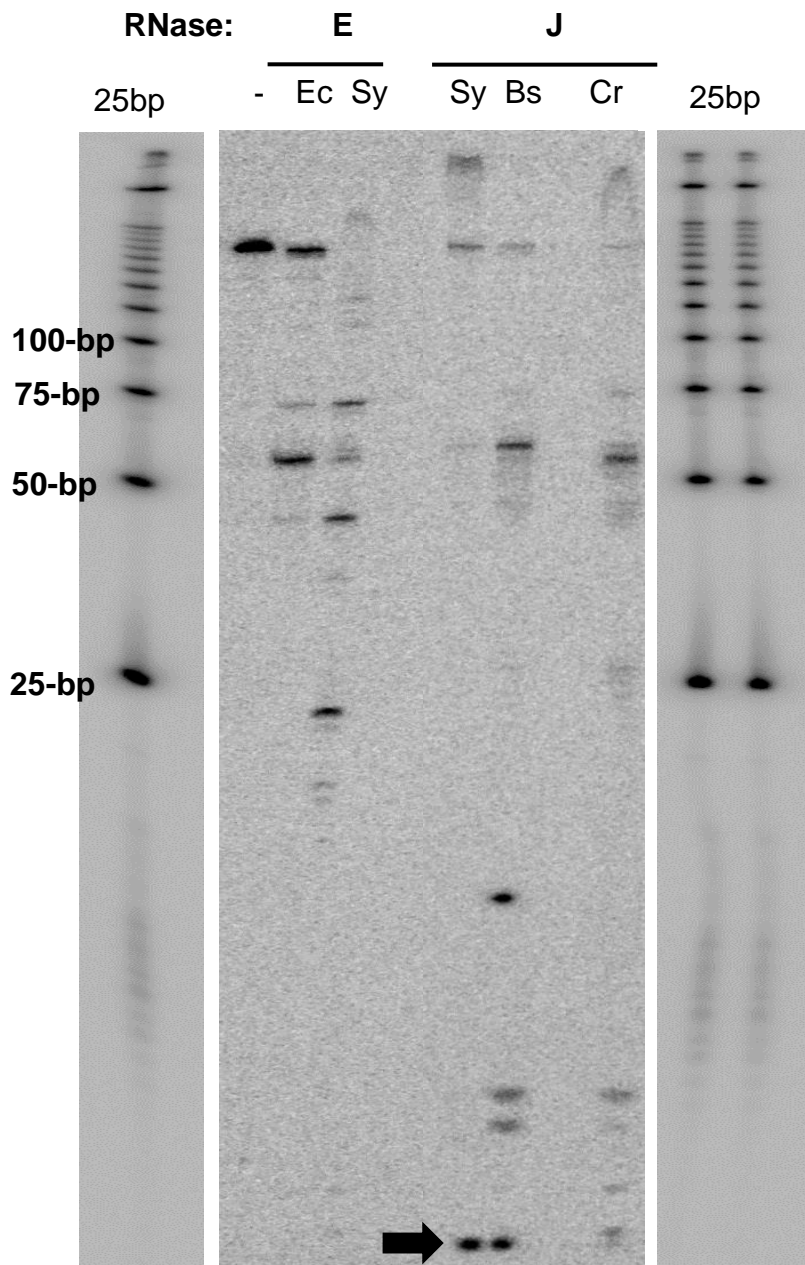
Supplementary Figure 1. Primary and secondary structure of Sy RNase E and Sy RNase J (PROMALS3D). A) Multiple protein sequence alignment of RNase E/G proteins from *Synechocystis* and *E. coli* using the structure template of *Ec* RNase E (PDB ID: 2bx2, chain L). Conserved domains are underlined: RNase H in green, S1 in blue, 5'sensor in pink and DNase I in red; the C1 to C4 motifs are highlighted in cyano. α -helix predictions are colored in red and β -strand in blue. B) Multiple protein sequences alignment of *Sy* RNase J, *Bs* RNase J1/J2 and *Cr* RNase J using the structure template of *Tt* RNaseJ1 (PDB ID: 3BK1 chain A). The β -lactamase motifs are in yellow, the β -CASP motifs in green. The β -CASP domain is underlined in blue and the C-terminal domain in pink. In A and B the consensus secondary structure (Consensus_ss) is indicated by h (α -helix) and e (β -strand). In the consensus amino acid sequence (Consensus_aa) conserved amino acids are in bold and uppercase letters; aliphatic: l; aromatic: @; hydrophobic: h; alcohol: o; polar residues: p; tiny: t; small: s; bulky residues: b; positively charged: +; negatively charged: -; charged: c.



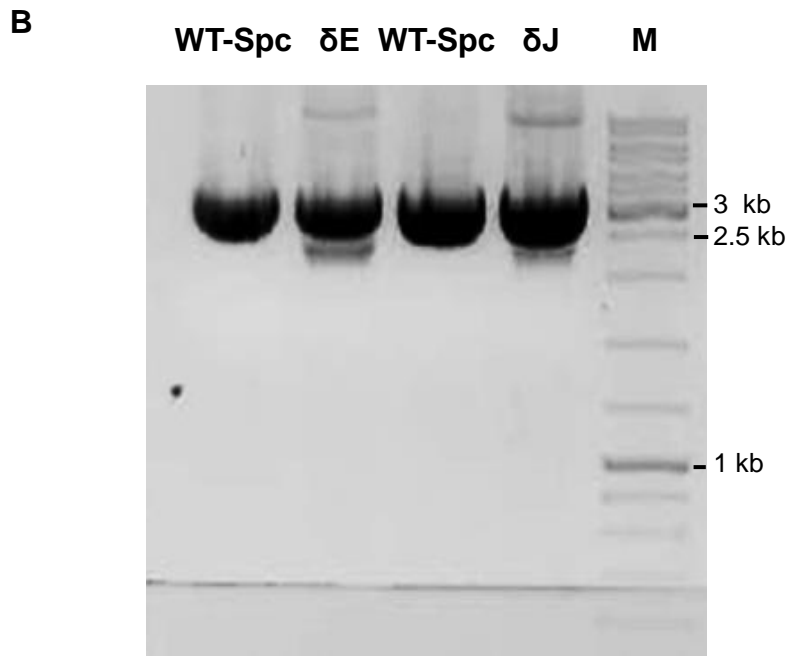
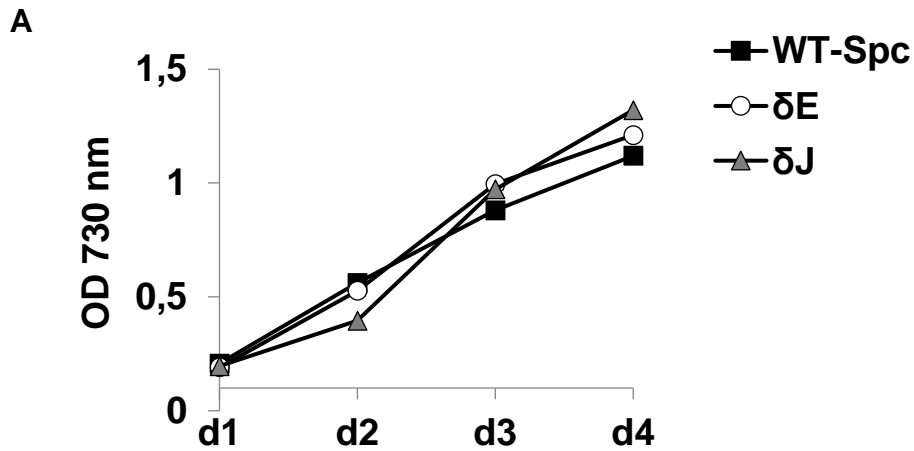
Supplementary Figure 2. Homology-based model of the *Sy* RNase and *Sy* RNase J structures. (A) The modeled catalytic region of *Sy* RNase E (blue) superimposed on the *E. coli* crystal structure PDB 2bx2, chain L (green). (B) The modeled structure of *Sy* RNase J (cyano) superimposed on the *T. thermophilus* crystal structure PDB 3BK1 (magenta).



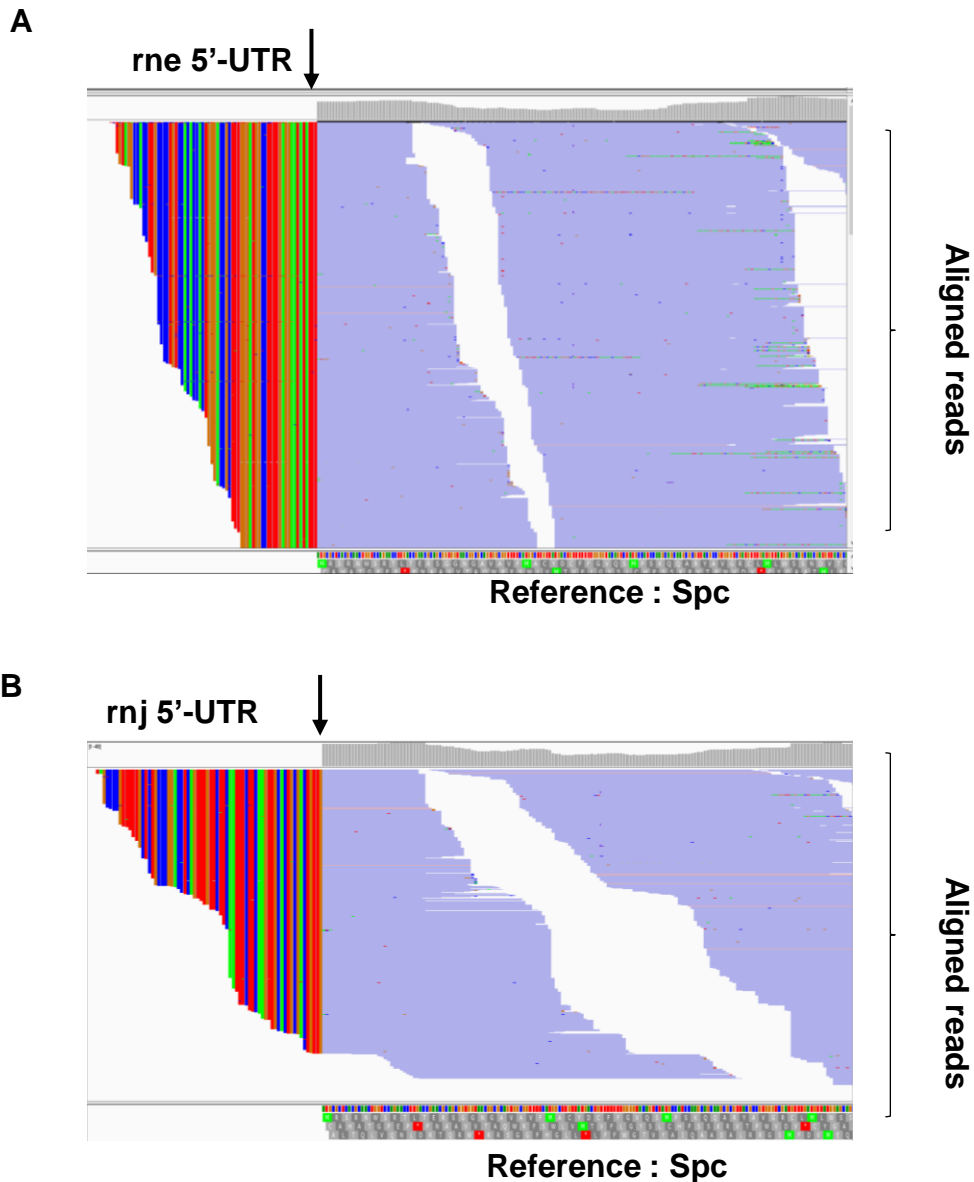
Supplementary Figure 3. Expression and purification of recombinant Sy RNase E (A-C) and Sy RNase J (D-F) in *E. coli*. SDS-PAGE was carried out on a 10% polyacrylamide gel and proteins were detected by Coomassie Brilliant Blue R-250 stain. A) Induction of Sy RNase E: Lane 1, MW standards. Lanes 2 and 3, insoluble fractions after and before induction with 1 mM IPTG, respectively. Lanes 4 and 5, soluble fraction after and before induction, respectively. B) Purification of Sy RNase J His-tagged recombinant protein. Lane 1, MW standards. Lanes 2 to 5, purified RNase E. C) Immunoblot with anti-His tag antibody on collected pooled dialyzed proteins from lanes 3-5 of the gel in B). D) Induction of Sy RNase J. Lane 1, MW standards; Lanes 2 and 3, whole cell lysates before and after induction, respectively. Lanes 4 and 5, insoluble fractions and soluble fraction after induction. E) Purification of Sy RNase J His-tagged recombinant protein. Lane 1, MW standards. Lanes 2 to 8, purified RNase J. F) Immunoblot with anti-His tag antibody. Pooled purified dialyzed proteins from lanes 1 to 4 (Lane 1) and from lanes 5 to 9, (Lane 2), respectively.



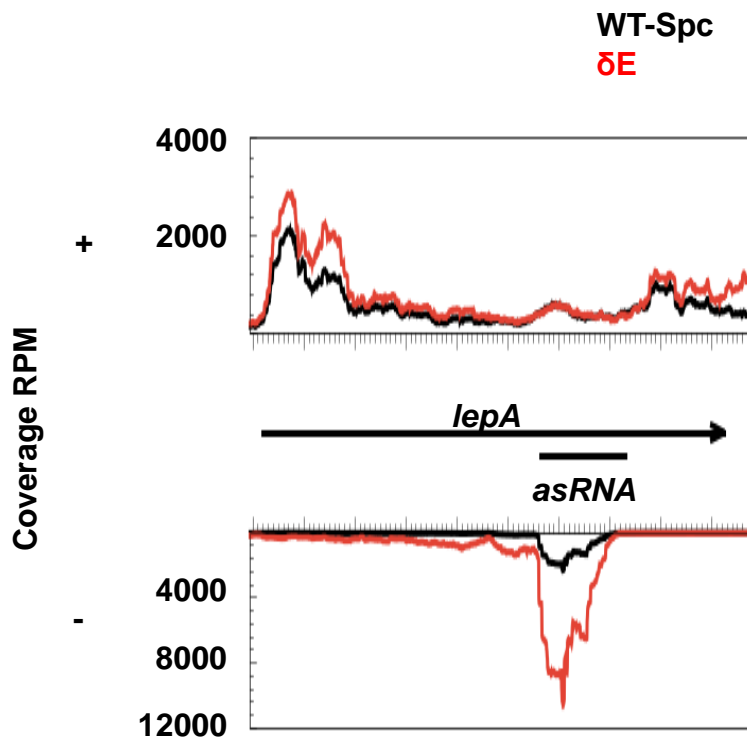
Supplementary Figure 4. *In vitro* activity of *Sy* RNase E and *Sy* RNase J. A 5'-monophosphorilated (P) - 32 P end-labeled *yitJ* was incubated with RNase E (E) from *E. coli* (*Ec*) and *Synechocystis* (*Sy*) and RNase J (J) from *Synechocystis* (*Sy*), *B. subtilis* (*Bs*), and *C.reinhardtii* (*Cr*). As control (lane 1) the transcript was incubated with the reaction buffer only (-). The reactions were separated on a 20% urea PAGE gel. The mononucleotides corresponding to liberated 5'-terminal nucleotide are at the bottom of the gel (depicted by an arrow) and are indicative of a 5'-3' exoribonuclease activity. L= 32 P-labeled 25 bp DNA ladder.



Supplementary Figure 5. Genetic instability of *rne* and *rnj* deletion. A) Growth curves of *Synechocystis* mutants and WT strains in the absence of antibiotic in BG11 medium. B) PCR analysis of wild-type (WT-Spc) and mutant strains (δE and δJ) grown in the absence of spectinomycin. The almost exclusive presence of the WT gene copy (~3.5 kb band) over the mutant gene copy (~2.5 kb and ~2.4 kb) in the mutant strains highlights the rapid loss of the partial gene deletions in the absence of antibiotic pressure.



Supplementary Figure 6. Browser view of RNA-Seq soft-clipped reads showing the integration of the Spc cassette at the intended locus. Reads from replicates S4 (A) and S7 (B) are shown as representative examples. In these snapshots, the first 160-nt of Spc sequence is shown at the bottom. The soft-clipped portion at the 5' end of the reads that do not match to the Spc coding sequence are shown as multicolour “rainbow” (C: blue, U: red; A: green; G: brown). The breakpoint of soft-clipped reads from reference is indicated by the arrow. The soft-clipped sequence corresponds to the sequence of 5'-UTR of *Sy rne* (A) and *Sy rnj* (B).



Supplementary Figure 7. Antisense coverage over *lepA*. RNA-Seq coverage over the *lepA* gene in the WT (black) and in the δE mutant (red), on the sense (+) strand (on the top) and on the antisense (-) strand (on the bottom). Arrow represents the *lepA* ORF and its orientation on the genome; the line represents the *asRNA* identified by (Georg et al., 2009). RPM: reads per million