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SUPPLEMENTARY INFORMATION
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The host-encoded RNase E endonuclease as the crRNA maturation enzyme in a CRISPR–Cas subtype III-Bv system

Juliane Behler[1], Kundan Sharma[2,3], Viktoria Reimann[1], Annegret Wilde[4,5], Henning Urlaub[2,3] and Wolfgang R. Hess[1,6]*

[1]Genetics and Experimental Bioinformatics, Faculty of Biology, University of Freiburg, Freiburg, Germany. [2]Bioanalytics Research Group, Department of Clinical Chemistry, University Medical Centre, Göttingen, Germany. [3]Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany. [4]Molecular Genetics, Faculty of Biology, University of Freiburg, Freiburg, Germany. [5]BIOSS Centre for Biological Signalling Studies, University of Freiburg, Freiburg, Germany. [6]Freiburg Institute for Advanced Studies, University of Freiburg, Freiburg, Germany.
*e-mail: wolfgang.hess@biologie.uni-freiburg.de

The host-encoded RNase E endonuclease as the crRNA maturation enzyme in a CRISPR-Cas subtype III-Bv system

Supplementary Information

Juliane Behler[1], Kundan Sharma[2,3], Viktoria Reimann[1], Annegret Wilde[4,5], Henning Urlaub[2,3] and Wolfgang R. Hess[1,6,*]

[1]Genetics and Experimental Bioinformatics, Faculty of Biology, University of Freiburg, Schänzlestr. 1, D-79104 Freiburg, Germany;

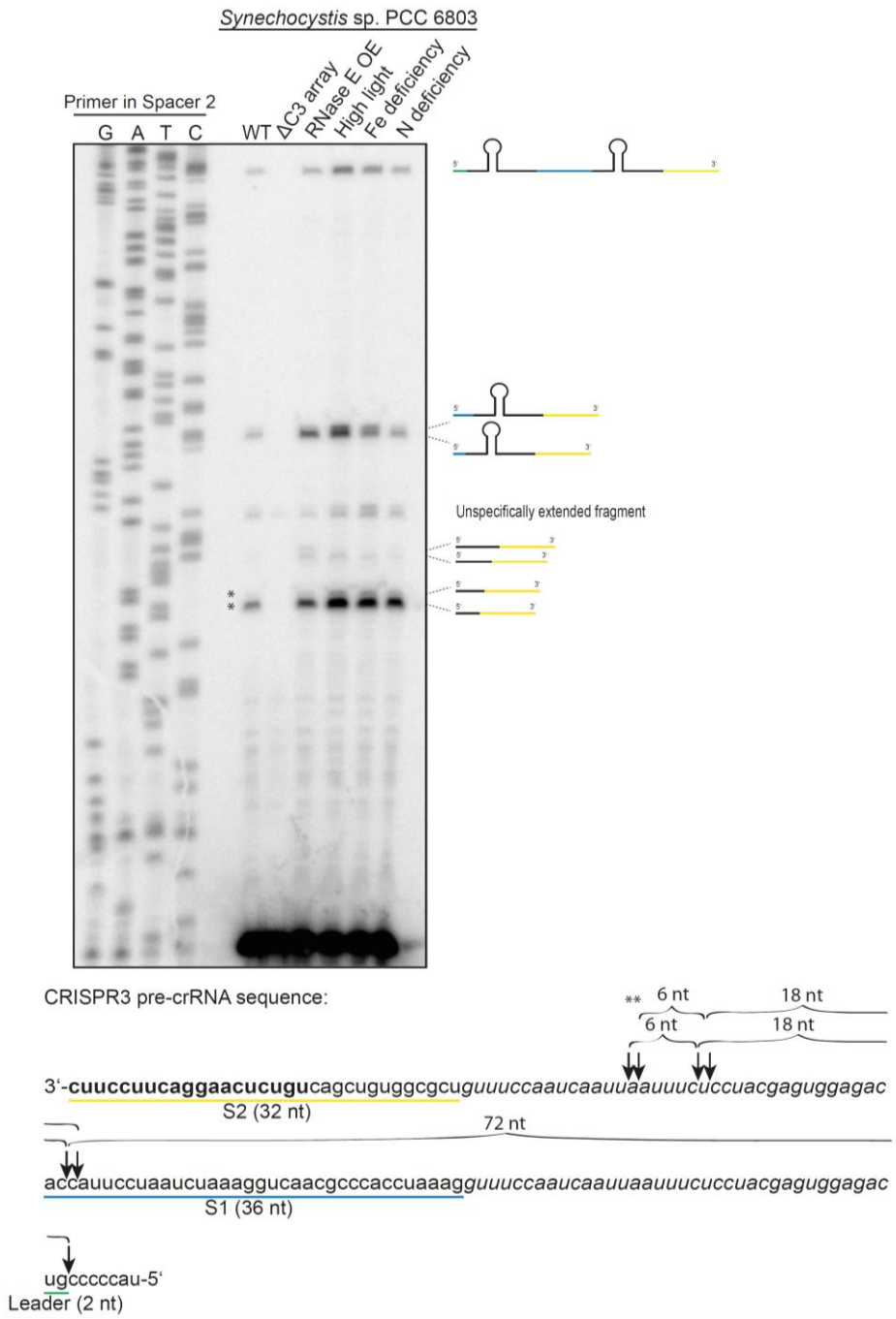
[2]Bioanalytics Research Group, Department of Clinical Chemistry, University Medical Centre, D-37075, Göttingen, Germany;

[3]Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry, D-37077 Göttingen, Germany;

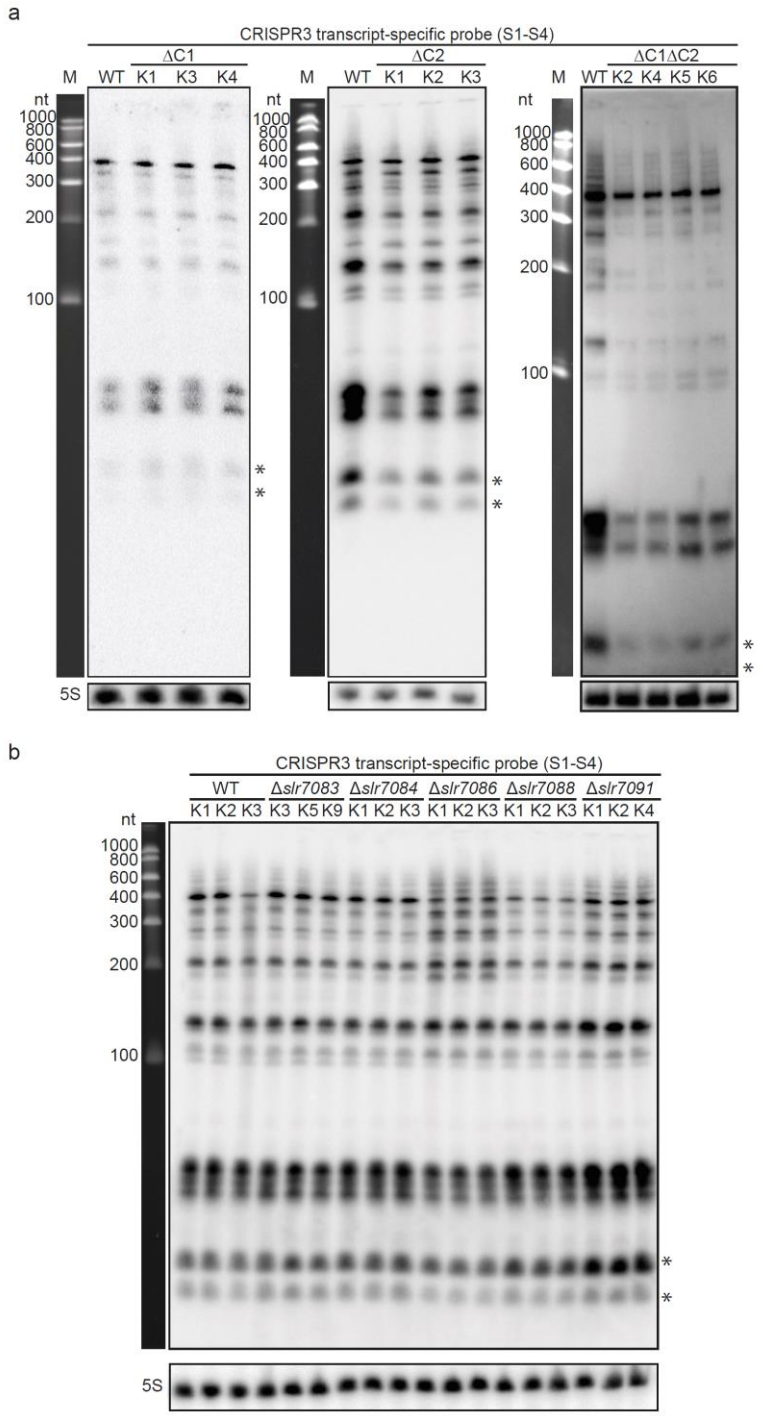
[4]Molecular Genetics, Faculty of Biology, University of Freiburg, Schänzlestr. 1, D-79104 Freiburg, Germany;

[5]BIOSS Centre for Biological Signalling Studies, University of Freiburg, Schänzlestr. 18, D-79104 Freiburg, Germany;

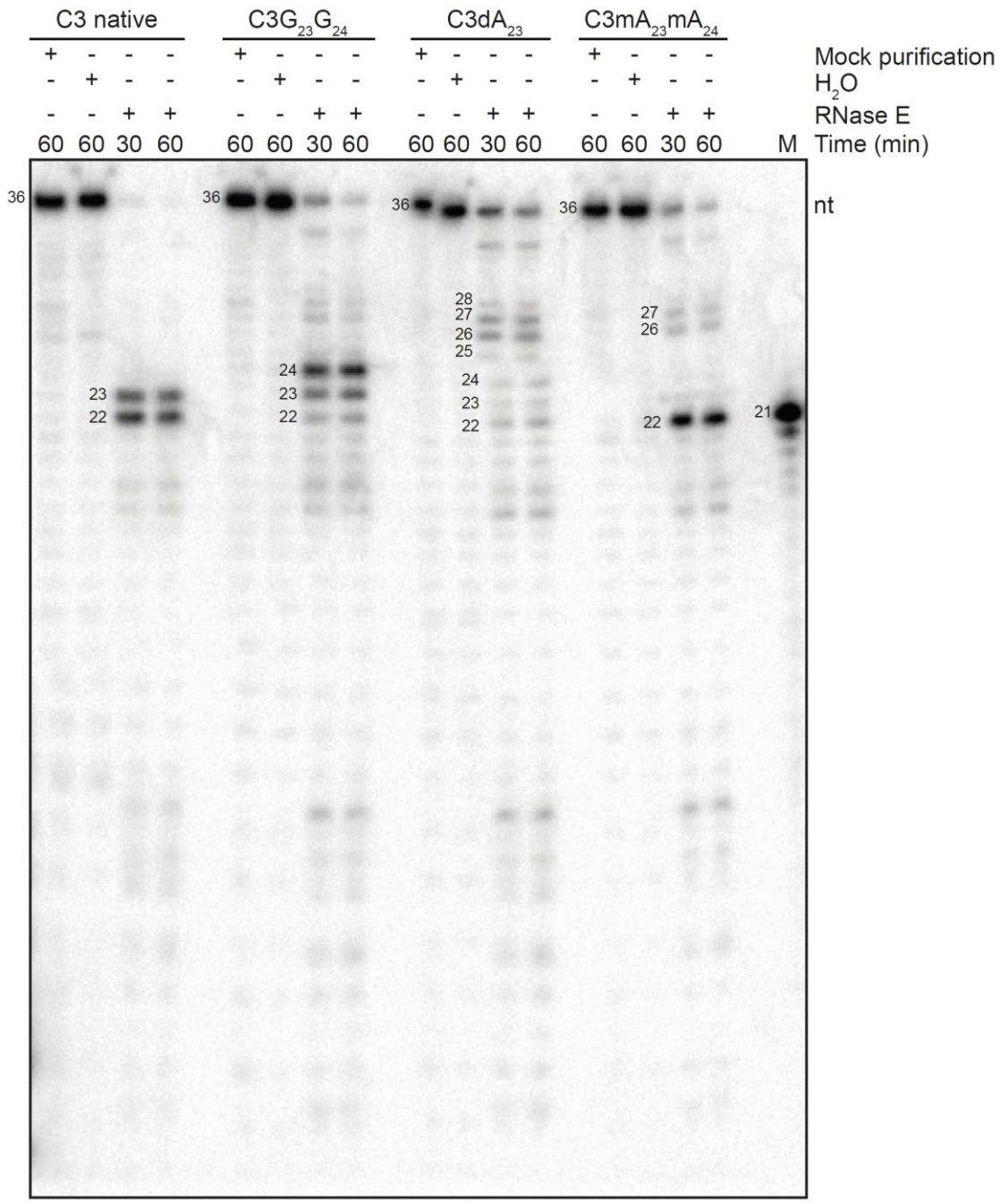
[6]Freiburg Institute for Advanced Studies, University of Freiburg, Albertstr. 19, D-79104 Freiburg, Germany



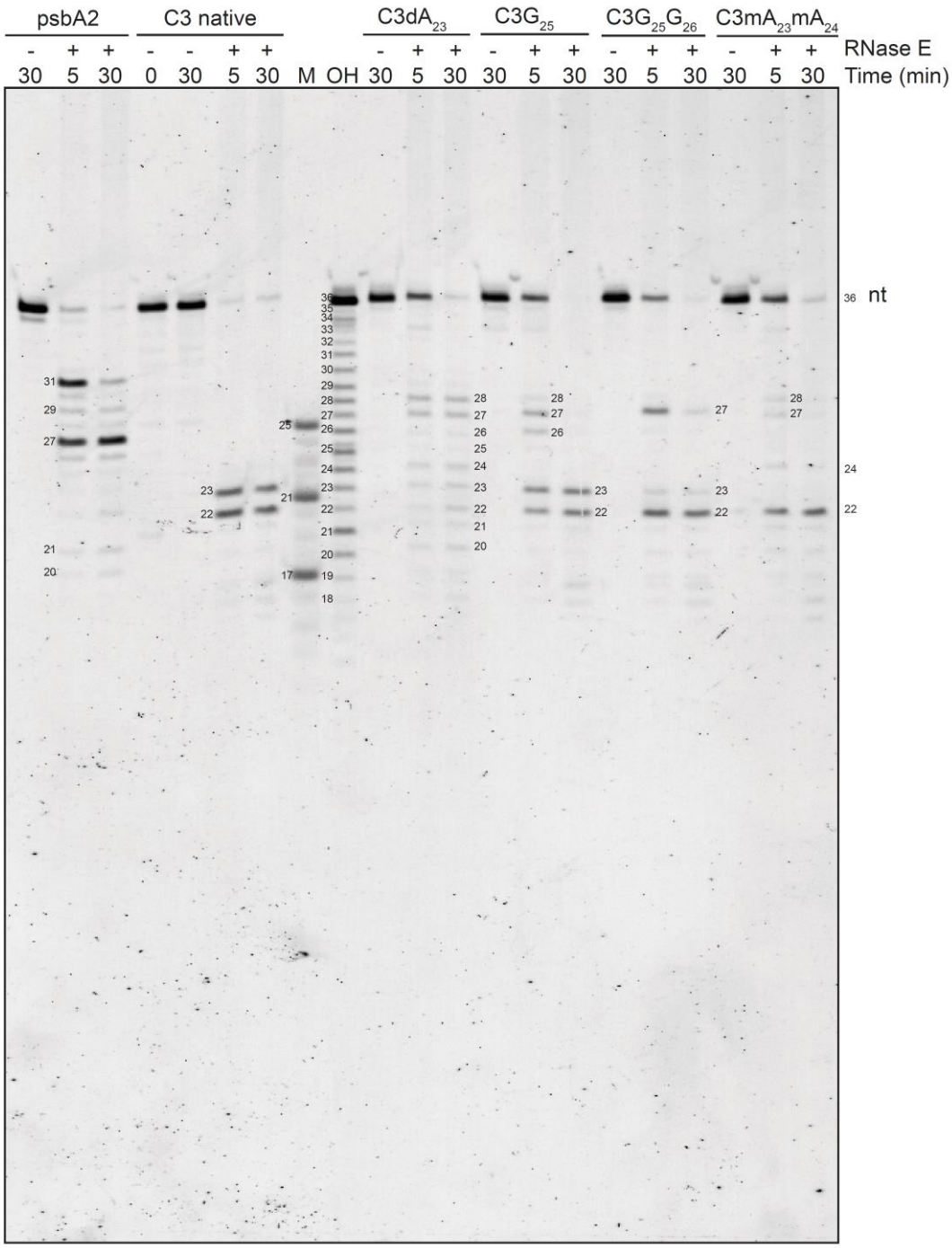
Supplementary Figure 1 │ Mapping of *in vivo* processing sites within CRISPR3 pre-crRNA by primer extension using a 5'-labeled primer located in spacer 2. Total RNA was isolated from wild-type *Synechocystis* 6803 (WT), the CRISPR3 repeat-spacer deletion mutant (ΔC3 array, negative control) and an RNase E overexpression mutant (RNase E OE) under standard growth conditions, as well as WT exposed to high light, iron or nitrogen deficiency and subjected to primer extension analysis using the 5'-labeled oligonucleotide primerExt_S2_rev (**Supplementary Table 1**) and 2 µg of total RNA per reaction. The detected sites are indicated by cartoons (upper part, repeat sequences in black, spacer and leader sequences in color) or arrows alongside the reverse complement pre-crRNA sequence (lower part, primer sequence in bold). Mapping of *in vivo* cleavage sites within CRISPR3 repeats revealed 13 and 14 nt 5'-handles (highlighted by asterisks) and a 2 nt long leader sequence. The majority of additional processing sites followed a 6-nt periodicity or multiples of it as marked by the curly braces. A representative of two independent experiments is shown.



Supplementary Figure 2 │ Northern hybridization of individual deletion mutants using a radioactively labeled transcript probe spanning spacers 1-4 of CRISPR3. a, CRISPR1, CRISPR2 and CRISPR1CRISPR2 full-length knock-outs (ΔC1, ΔC2 and ΔC1ΔC2, respectively) reveal Cas6-independent CRISPR3 maturation in *Synechocystis* 6803. Deletion mutants of CRISPR1, CRISPR2 and CRISPR1CRISPR2 *cas* genes and repeat-spacer arrays have no influence on the accumulation of CRISPR3-derived transcripts compared to wild-type (WT), indicating that CRISPR3 crRNA maturation is independent of these systems *in vivo*. Three biological replicates are shown. **b**, The knock-out mutants Δ*slr7083*, Δ*slr7084*, Δ*slr7086*, Δ*slr7088* and Δ*slr7091* do not differ in CRISPR3-derived crRNA maturation when compared to wild-type (WT). Three biological replicates are shown. Mature crRNAs are indicated by asterisks. The RiboRuler Low Range RNA Ladder served as size marker in both panels.



Supplementary Figure 3 │ *In vitro* cleavage assay of the native CRISPR3 repeat RNA oligo and various mutated versions by RNase E. Cleavage of the radioactively 5'-labeled native CRISPR3 repeat results in two cleavage fragments of 22 and 23 nt in length corresponding to the 5' fragment of the repeat sequence including the hairpin. Substitution of the ribose sugar to deoxyribose on the adenine at position 23 ($C3dA_{23}$) resulted in multiple secondary targets. Mutations located further downstream of the processing sites ($C3G_{23}G_{24}$, $C3mA_{23}mA_{24}$) never completely prevented cleavage by RNase E. Methylated adenines at positon 23 and 24 within the repeat ($C3mA_{23}mA_{24}$) hindered the formation of the longer cleavage product supposing a steric hindrance. Two negative controls were included in the cleavage assay, one was incubated with RNase-free water, the other was incubated with eluate from a mock purification. M, the 21-mer DNA oligonucleotide included as a probe against the microRNA Marker (NEB) was used as a marker. Shown is a representative of three independent experiments.



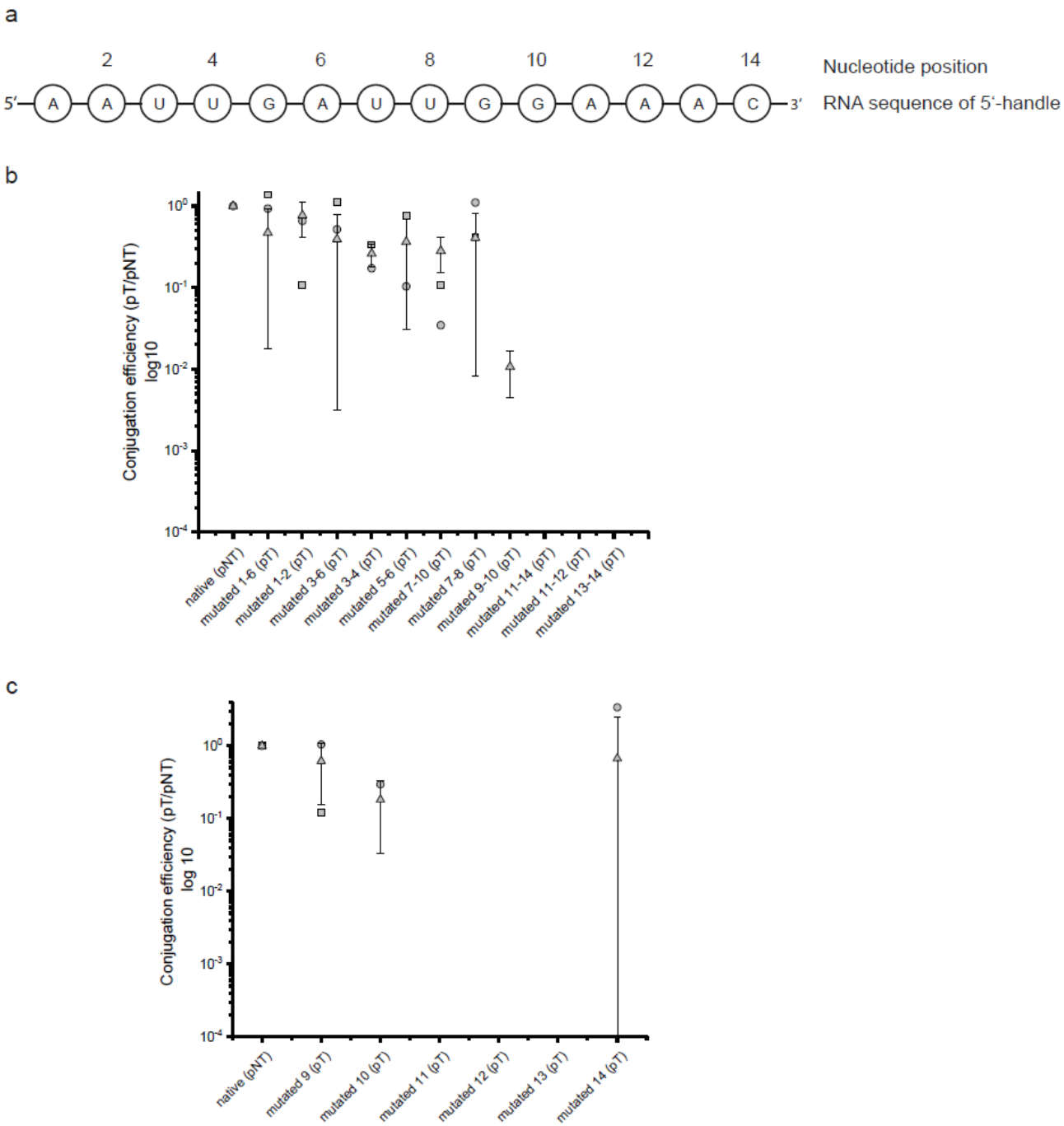
Supplementary Figure 4 │ *In vitro* cleavage assay of the native CRISPR3 repeat RNA oligo and various mutated versions by RNase E. Cleavage of the native CRISPR3 repeat results in two cleavage fragments of 22 and 23 nt in length corresponding to the 5' fragment of the repeat sequence including the hairpin. Substitution of the ribose sugar to deoxyribose on the adenine at position 23 ($C3dA_{23}$) resulted in multiple secondary targets. Mutations located further downstream of the processing sites ($C3G_{25}$, $C3G_{25}G_{26}$, $C3mA_{23}mA_{24}$) never completely prevented cleavage by RNase E. Methylated adenines at positon 23 and 24 within the repeat ($C3mA_{23}mA_{24}$) hindered the formation of the longer cleavage product supposing a sterical hindrance. The *psbA2* RNA oligo was used as a positive control for RNase E functionality. OH is the alkaline hydrolysis ladder derived from the CRISPR3 repeat and M is a microRNA Marker (NEB). A representative of three independent experiments is shown.

RNase E amino acid sequence

MPKQIVIAEKHQVAAVFWKDQIQELVVSTGSQQVGDIYLGLVDNILPSIDAAFINIGDTEKNGFIH
VSDLGPVRLRRTAGSISELLSPQQRVLVQVMKEPTGNKGPRLTGNISMPGRYMVLMPYGRGV
NLSRRINREEERSRLRALAVLIKPPGMGLLVRTEAEDVPEDAIIEDLENLQKQWELVQQQAMTR
SAPMLLDRDDDFIKRVLRDMYSSEVNRIVVDTPAGMKRIKQQLMNWDQGRLPEGVLIDCHRES
LSILEYFRVNATIREALKPRVDLPSGGYIIIEPTEALTVIDVNSGSFTHSANSRETVLWTNYEAATE
IARQLKLRNIGGVIIIDFIDMDSHKDQLQLLEHFNRCLETDKARPQIAQLTELGLVELTRKRQGQN
LYELFGQPCPECGGLGHLVELPGEKGFVSLSPTAVNSSIPPRLVEKPILSPPVAKVNDLPKKEEA
KISSPLDLLFHPNYQEQGDRDSNRRRRRRRGSEFSEKENIKSVGISRSKGPSPSPTKEKVTGT
APPRRERPSRRVEKTLVPVDVAMTTLEQDIYARMGISPLIKTEYADQDPRSFMVSVVTAGAALE
GNTNGSGSLVNAVITTVDNGDNGDNVPSDGLTIVSEVTAPTPVIEQPREETVEPEQVVLPQLDD
ETPAAPVAEESAPIETKKRPGRRRRRSSAERSHHHHHH

Cross-linked peptides and nucleotides:
NGFIHVSDLGPVR + U
VLVQVMKEPTGNK + U-H_2O
ENIKSVGISR + U-H_2O
EKVTGTAPPR + AU-H_2O

Supplementary Figure 5 | Mass-spectrometry analysis of cross-linked protein-RNA interactions between RNase E and the CRISPR3 repeat. Four identified RNase E peptides (red) directly cross-linked to the indicated nucleotides were subjected to tandem mass spectrometry fragmentation. This allowed the identification of four amino acids (green) that interact with the repeat sequence. The RNase E sequence is shown as used in the experiment, with the additional final RSH_6 residues.



Supplementary Figure 6 | Self and non-self discrimination in *Synechocystis* 6803. a, Schematic representation of the CRISPR3 5'-handle sequence with numbered nucleotide positions. **b**, Self- and non-self discrimination assay of CRISPR3 5'-handle repeat variants containing multiple mutations in *Synechocystis* 6803. **c**, Self- and non-self discrimination assay of CRISPR3 5'-handle repeat variants containing single mutations in *Synechocystis* 6803. Experimental procedure and statistical analysis for panels (**b**) and (**c**): The conjugation efficiency of the control plasmid maintaining the native repeat sequence (pNT) was set to 1 and the number of colonies for the tested repeat mutation variants pT was normalized to the control plasmid pNT. Hence, conjugation efficiencies were calculated by the ratio of pT/pNT. Numbers indicate the corresponding mutated nucleotides within the 5'-handle. Data points are shown as dot plots and standard deviations were calculated for three independent biological replicates. Data points with 0 values were not considered because log(0) is not defined.

Supplementary Table 1

DNA and RNA oligonucleotides used in this study. DNA oligonucleotides were ordered from SIGMA-ALDRICH®, RNA oligonucleotides were ordered PAGE-purified from PURIMEX. T7 promoter sequences are highlighted in bold.

DNA oligonucleotides (sequence 5'-3')		
Primer extension		
PrimerExt_Ladder_fwd	CTCGATGGCTTACTGTATAAG	DNA template for reference sequence ladder, primer extension
PrimerExt_S3_rev	CTACTAAGCTCGACAATTG	DNA template for reference sequence ladder, primer extension, annealing site in spacer 3
PrimerExt_S2_rev	CTTCCTTCAGGAACTCTG	DNA template for reference sequence ladder, primer extension annealing site in spacer 2
ΔC1 knock-out		
F1_up_1.8kb_fwd	ttgtaaaacgacggccagtgAAGTAAAATAGATTGTTGTAAATTCAG	Homologous region for ΔC1 *cas* gene array knock-out
F1_up_1.8kb(Km+)_rev	cgctggtgagTTAGGTCTGATGCAGAATC	
Km+_fwd	tcagacctaaCTCACCAGCGGCAACCGC	Antibiotic resistance marker (kanamycin) gene for ΔC1 *cas* gene array knock-out
Km+_rev	ctggcggcgtGCCGCCGTCCCGTCAAGT	
F2_down_1.2kb(+Km)_fwd	ggacggcggcACGCCGCCAGCCCACCAG	Homologous region for ΔC1 *cas* gene array knock-out
F2_down_1.2kb_rev	ctatgaccatgattacgccaTTCTCCGCTACACAAATGCGTACCCAG	
DC1total_Seg_rev	ACTTGGAGATGATGTTAACTGGCGCTGG	Verification of ΔC1 *cas* gene array knock-out
DC1tot_Seg_S14_fwd	TTGCTAGAGAAAGCGCAACAAAACAAGC	
DC1tot_Seq_S3_fwd	TTTGCTTCAGCTAGTACCAAAGGCTAGC	
DC1total_Seg_fwd	TCGTACATAAGTGCGATTCAACGATTCTGC	
ΔC2 knock-out		
pUC19_rev_shorterbackbone2	TCACCGTCATCACCGAAACG	pUC19 backbone for Gibson assembly

pUC19_fwd_shorterbackbone2	AGCTCACTCAAAGGCGGTAA	
DCRISPR2_FL1_fwd	cgtttcggtgatgacggtgaAAAGCCCAGTTCAAACGC	Homologous region for ∆C2 *cas* gene array knock-out
DCRISPR2_FL1_Km+_rev	cgctggtgagGTCATAAATAAATTCAATTAGTGAAGTTC	
DCRISPR2_Km+_fwd	tatttatgacCTCACCAGCGGCAACCGC	Antibiotic resistance marker (kanamycin) gene for ∆C2 *cas* gene array knock-out
DCRISPR2_Km+_rev	cctcaggcgcGCCGCCGTCCCGTCAAGT	
DCRISPR2_FL2_Km+_fwd	ggacggcggcGCGCCTGAGGTGGTCAAT	Homologous region for ∆C2 *cas* gene array knock-out
DCRISPR2_FL2_rev	ttaccgcctttgagtgagctAATCTAATGATGGTAAAAATCGTTTATTTGCC	
DCRISPR2_Segregation_fwd	GTTTACTGAAGAACTTCACTAATTG	Colony PCR during cloning in *E. coli* and segregation PCR in *Synechocystis* 6803
DCRISPR2_Segregation_rev	GTGTTGAACGAGCCATGG	
∆C1∆C2 knock-out		
pUC19_Rev	CACTGGCCGTCGTTTTACAACG	pUC19 backbone for Gibson assembly
pUC19_Fw	TGGCGTAATCATGGTCATAGC	
DDeltaC1C2_Fl1_Fw	ttgtaaaacgacggccagtgAAAGCCCAGTTCAAACGC	Homologous region for ∆C1C2 *cas* gene array knock-out
DDeltaC1C2_Fl1_Rev	tacgataataGTCATAAATAAATTCAATTAGTGAAGTTCTTC	
DDeltaC1C2_StrepR_Fw	tatttatgacTATTATCGTAGTTGCTCTCAG	Antibiotic resistance marker (streptomycin) gene for ∆C1C2 *cas* gene array knock-out
DDeltaC1C2_StrepR_Rev	cctcaggcgcGTACAGAGTGATGTCAAC	
DDeltaC1C2_Fl2_Fw	cactctgtacGCGCCTGAGGTGGTCAATAG	Homologous region for ∆C2 *cas* gene array knock-out
DDeltaC1C2_Fl2_Rev	ctatgaccatgattacgccaAATCTAATGATGGTAAAAATCGTTTATTTGCC	
Transcript-specific probe (S1-S4)		
antiC3S1_fwd	CTTTAGGTGGGCGTTGACCT	Northern hybridization
antiC3S4_rev	**TAATACGACTCACTATAGGG**taatagtaatgacaggcag	
sgRNA and dCas9		
sgRNA2_fwd	TCAGCAATGACAATTTGTTTGTTTTAGAGCTAGAAATAGCAAG	Inverse PCR to replace the protospacer region of Addgene plasmid #44251 by sgRNA2 targeting *slr1129*
sgRNA2_rev	AAACAAATTGTCATTGCTGAAGCTCCCAGTATCTCTATCAC	

sgRNA_Segregation_fwd	GATACTCGCTTCTTGGATG	Segregation PCR of sgRNA2 into *slr2030-slr2031* homology site
sgRNA_Segregation_rev	CCAGAAACTGATTCAGATTATG	
dCas9_Segregation_fwd	GGCTTGATCTGGCATTTAC	Segregation PCR of dCas9 into the *psbA1* neutral site
dCas9_Segregation_rev	GATTCATTACATTTCACCCTAG	
Partial *slr1129* knock-out		
RNaseEf	GTTGCAATTCCTTTTGGCCCAG	Deletion of *slr1129* encoding RNase E
RNaseEr	GTGGACCGGGCTACTCATCTG	
Heterologous overexpression of *slr1129*		
pQE70_Gibson_Codonopt_fwd	CGTTCTCATCACCATCACCATC	pQE70 for Gibson assembly, codon optimization
pQE70_Gibson_Codonopt_rev	GCTTAATTTCTCCTCTTTAATGAATTCTGTG	
slr1129_Subcloning_fwd	TTCGGGCTTTGTTACTCCGCTGAAGAACGGCGGCGGCGGCGGCCAGG	Codon optimization of *slr1129* and subcloning
slr1129_Subcloning_rev	AAATACAGGTTTTCCTCGAGCTCCGCTGAAGAACGGCGGCGGCGGCGGCCAGG	
slr1129_Codonopt_fwd	ttaaagaggagaaattaagcATGCCAAAACAAATTGTCATTG	Codon-optimized and TEV site-fused RNase E gene (*slr1129*) for Gibson assembly
slr1129_Codonopt_rev	tggtgatggtgatgagaacgCGCGCCCTGAAAATACAG	
Site-directed mutagenesis of *slr1129*		
F64_fwd	AAAAAATGGCgcgATCCACGTCAGTGAC	F64A of *slr1129*
F64_rev	TCGGTGTCCCCAATGTTA	
K98_fwd	GCAGGTGATGgcgGAACCCACCG	K98A of *slr1129*
K98_rev	ACCAACACTCTTTGTTGAG	
K494_fwd	GGAAAATATTgcgTCTGTGGGAATTTCCC	K494A of *slr1129*
K494_rev	TTTTCAGAAAACTCCGAG	
K512_fwd	CACTAAGGAGgcgGTGACGGGCACTGCTCCTCCCC	K512A of *slr1129*
K512_rev	GGGCTGGGGCTGGGACCC	
Overexpression of *slr1129*		
P4_RNase E_fw	ATGCCAAAACAAATTGTCATTGCTG	Overexpression of RNase E in *Synechocystis* 6803 (*Nde*I)
P5_RNase E_rev	GGAATAAAAAACGCCCGGCGGCAACCGAGCGTTCAGGCGATCGCCACCTAATC	Overexpression of RNase E in *Synechocystis* 6803 (with *oop* terminator)

Design of the interference assay		
GentaR_pUC19_fwd	cggtgatgacggtgaGATTCCATTTTTACACTGATGAATGTTCCGTTGCG	Gentamicin resistance cassette with overlaps to pUC19
GentaR_pUC19_rev	cgcctttgagtgagctCCCGGCATTCGCTGCGCT	
CRISPR3_S2sense_fwd	GtagcgccacagctgacagagttcctgaaggaagctaatTAACAATTCGTTCAAGCCGAGATC	Spacer 2 sense orientation
CRISPR3_S2sense_rev	attagcttccttcaggaactctgtcagctgtggcgctacGGTGGCGGTACTTGGGTC	
CRISPR3_S2as_fwd	attagcttccttcaggaactctgtcagctgtggcgctacTAACAATTCGTTCAAGCCGAGATC	Spacer 2 antisense orientation
CRISPR3_S2as_rev	gtagcgccacagctgacagagttcctgaaggaagctaatGGTGGCGGTACTTGGGTC	
GentaR_ColPCR_fwd	CTACGTGCAAGCAGATTACGGTGACG	Colony PCR for spacer integration
GentaR_ColPCR_rev	GGCGTTGTGACAATTTACCGAACAACT	
GentaR_pVZ322_fwd	tctgctcctgcaggtcgactGATTCCATTTTTACACTGATGAATGTTCCGTTGCGCTGCCC	Gentamicin resistance cassette with overlaps to pVZ322
GentaR_pVZ322_rev	CCCGGCATTCGCTGCGCTTATGGCAGAGCA	
GentaR_Seq_rev	GCTTATGGCAGAGCAGGG	Sequencing primer
C3S2_Ref_self_fwd	ttagcttccttcaggaactctgtcagctgtggcgctgtttccaatcaattaatttctcctacgagtggagacTAACAATTCGTTCAAGCCGAGATC	Inverse PCR, native repeat sequence
C3S2_Ref_self_rev	TTAgtctccactcgtaggagaaattaattgattggaaacagcgccacagctgacagagttcctgaaggaagctaaGGTGGCGGTACTTGGGTC	
C3S2_mut.35-36_fwd	tgttccaatcaattaatttctcctacgagtggagacTAACAATTCGTTCAAGCCGAGATC	Inverse PCR, integration of double mutation at position 13-14
C3S2_mut.35-36_rev	TTAgtctccactcgtaggagaaattaattgattggaacaagcgccacagctgacaga	
C3S2_mut.33-34_fwd	gtggccaatcaattaatttctcctacgagtggagacTAACAATTCGTTCAAGCCGAGATC	Inverse PCR, integration of double mutation at position 11-12
C3S2_mut.33-34_rev	TTAgtctccactcgtaggagaaattaattgattggccacagcgccacagctgacaga	
C3S2_mut.31-32_fwd	gtttttaatcaattaatttctcctacgagtggagacTAACAATTCGTTCAAGCCGAGATC	Inverse PCR, integration of double mutation at position 9-10

C3S2_mut.31-32_rev	TTAgtctccactcgtaggagaaattaattg attaaaaacagcgccacagctgacaga	
C3S2_mut.29-30_fwd	gtttcccctcaattaatttctcctacgagtgg agacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of double mutation at position 7-8
C3S2_mut.29-30_rev	TTAgtctccactcgtaggagaaattaattg aggggaaacagcgccacagctgacaga	
C3S2_mut.27-28_fwd	gtttccaagaaattaatttctcctacgagtgg agacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of double mutation at position 5-6
C3S2_mut.27-28_rev	TTAgtctccactcgtaggagaaattaattt cttggaaacagcgccacagctgacaga	
C3S2_mut.25-26_fwd	gtttccaatcccttaatttctcctacgagtgg agacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of double mutation at position 3-4
C3S2_mut.25-26_rev	TTAgtctccactcgtaggagaaattaagg gattggaaacagcgccacagctgacaga	
C3S2_mut.23-24_fwd	gtttccaatcaaggaatttctcctacgagtg gagacTAACAATTCGTTCAAGC CGAGATC	Inverse PCR, integration of double mutation at position 1-2
C3S2_mut.23-24_rev	TTAgtctccactcgtaggagaaattccttg attggaaacagcgccacagctgacaga	
C3S2_mut.33-36_fwd	tgggccaatcaattaatttctcctacgagtg gagacTAACAATTCGTTCAAGC CGAGATC	Inverse PCR, integration of quadruple mutation at position 11-14
C3S2_mut.33-36_rev	TTAgtctccactcgtaggagaaattaattg attggcccaagcgccacagctgacaga	
C3S2_mut.29-32_fwd	gtttttcctcaattaatttctcctacgagtgga gacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of quadruple mutation at position 7-10
C3S2_mut.29-32_rev	TTAgtctccactcgtaggagaaattaattg aggaaaaacagcgccacagctgacaga	
C3S2_mut.25-28_fwd	gtttccaagaccttaatttctcctacgagtgg agacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of quadruple mutation at position 3-6
C3S2_mut.25-28_rev	TTAgtctccactcgtaggagaaattaagg tcttggaaacagcgccacagctgacaga	
C3S2_mut.23-28_fwd	gtttccaagaccggaatttctcctacgagtg gagacTAACAATTCGTTCAAGC CGAGATC	Inverse PCR, integration of sixtuple mutation at position 1-6
C3S2_mut.23-28_rev	TTAgtctccactcgtaggagaaattccgg tcttggaaacagcgccacagctgacaga	
C3S2_mut.36_fwd	ttttccaatcaattaatttctcctacgagtgga gacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of single mutation at position 14
C3S2_mut.36_rev	TTAgtctccactcgtaggagaaattaattg attggaaaaagcgccacagctgacaga	
C3S2_mut.35_fwd	ggttccaatcaattaatttctcctacgagtgg agacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of single mutation at position 13

C3S2_mut.35_rev	TTAgtctccactcgtaggagaaattaattg attggaaccagcgccacagctgacaga	
C3S2_mut.34_fwd	gtgtccaatcaattaatttctcctacgagtgg agacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of single mutation at position 12
C3S2_mut.34_rev	TTAgtctccactcgtaggagaaattaattg attggacacagcgccacagctgacaga	
C3S2_mut.33_fwd	gttgccaatcaattaatttctcctacgagtgg agacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of single mutation at position 11
C3S2_mut.33_rev	TTAgtctccactcgtaggagaaattaattg attggcaacagcgccacagctgacaga	
C3S2_mut.32_fwd	gttttcaatcaattaatttctcctacgagtgga gacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of single mutation at position 10
C3S2_mut.32_rev	TTAgtctccactcgtaggagaaattaattg attgaaaacagcgccacagctgacaga	
C3S2_mut.31_fwd	gtttctaatcaattaatttctcctacgagtgga gacTAACAATTCGTTCAAGCC GAGATC	Inverse PCR, integration of single mutation at position 9
C3S2_mut.31_rev	TTAgtctccactcgtaggagaaattaattg attagaaacagcgccacagctgacaga	
RNA oligonucleotides (sequence 5'-3')		
C3	GUCUCCACUCGUAGGAGAAA UUAAUUGAUUGGAAAC	Native CRISPR3 repeat sequence
C3dA_{23}	GUCUCCACUCGUAGGAGAAA UUdAAUUGAUUGGAAAC	Modified CRISPR3 repeat, 2-deoxyribose adenine at position 23
C3$G_{23}G_{24}$	GUCUCCACUCGUAGGAGAAA UUGGUUGAUUGGAAAC	Modified CRISPR3 repeat, two guanine nucleotides at position 23 and 24
C3mA_{23}mA_{24}	GUCUCCACUCGUAGGAGAAA UUmAmAUUGAUUGGAAAC	Modified CRISPR3 repeat, two methylated adenine nucleotides at position 23 and 24
C3dU_{22}	GUCUCCACUCGUAGGAGAAA UdUAAUUGAUUGGAAAC	Modified CRISPR3 repeat, 2-deoxyribose uridine at position 23
C3G_{25}	GUCUCCACUCGUAGGAGAAA UUAAGUGAUUGGAAAC	Modified CRISPR3 repeat, guanine nucleotide at position 25
C3$G_{25}G_{26}$	GUCUCCACUCGUAGGAGAAA UUAAGGGAUUGGAAAC	Modified CRISPR3 repeat, two guanine nucleotides at position 25 and 26
C3$C_{15}C_{17}$	GUCUCCACUCGUAGCACAAA UUAAUUGAUUGGAAAC	Modified CRISPR3 repeat, two cytosine nucleotides at position 15 and 17
C3$A_{21}A_{22}$	GUCUCCACUCGUAGGAGAAA AAAAUUGAUUGGAAAC	Modified CRISPR3 repeat, two adenine nucleotides at position 21 and 22
psbA2	AGUCAGUUCCAAUCUGAACA UCGACAAAUACAUAAG	Positive control for RNase E *in vitro* cleavage activity

Supplemental information figure
Raw full length gels and blots

Figure 2
Primer extension with primer in spacer 2

Figure S1
Primer extension with primer in spacer 3

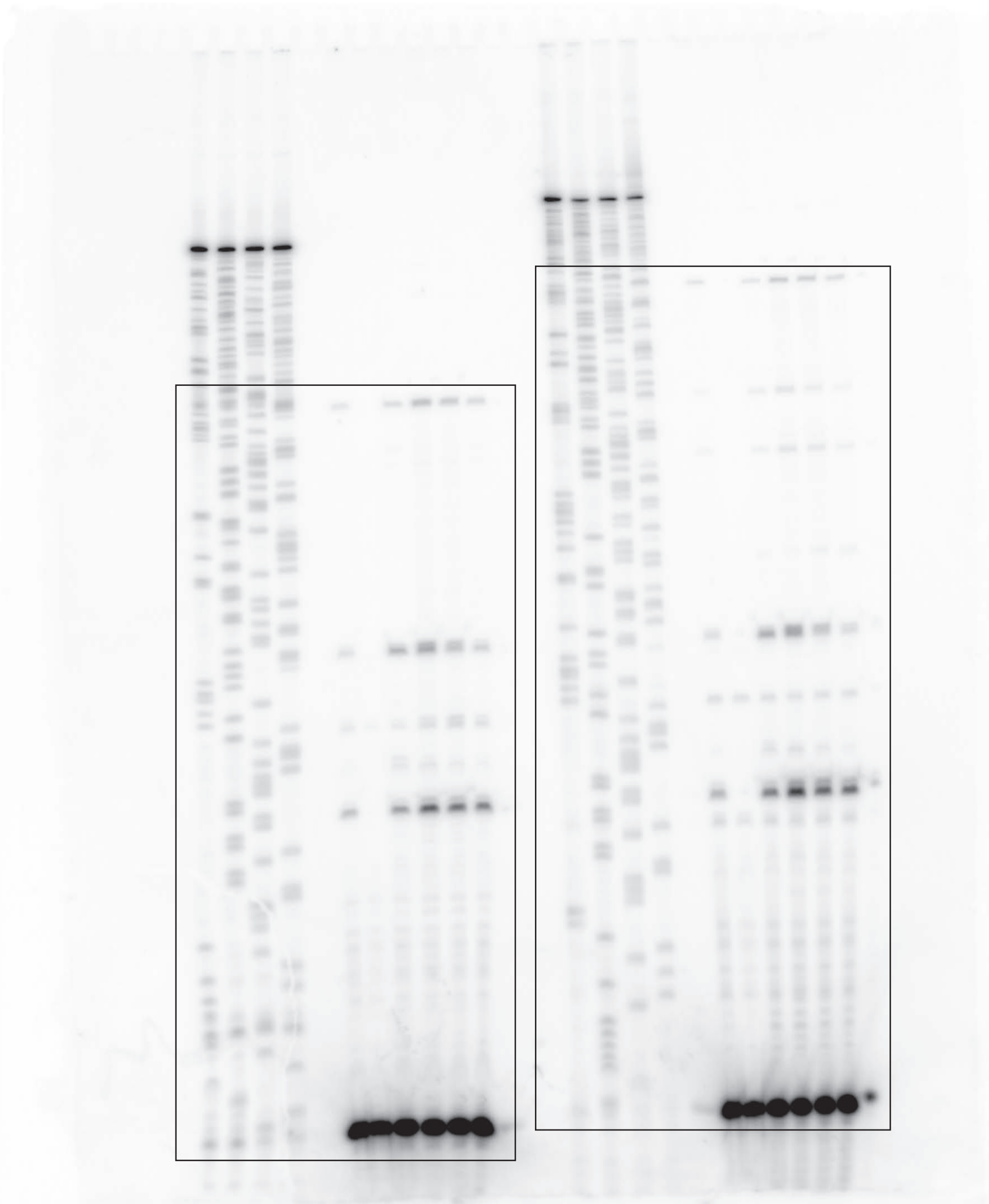


Figure 3

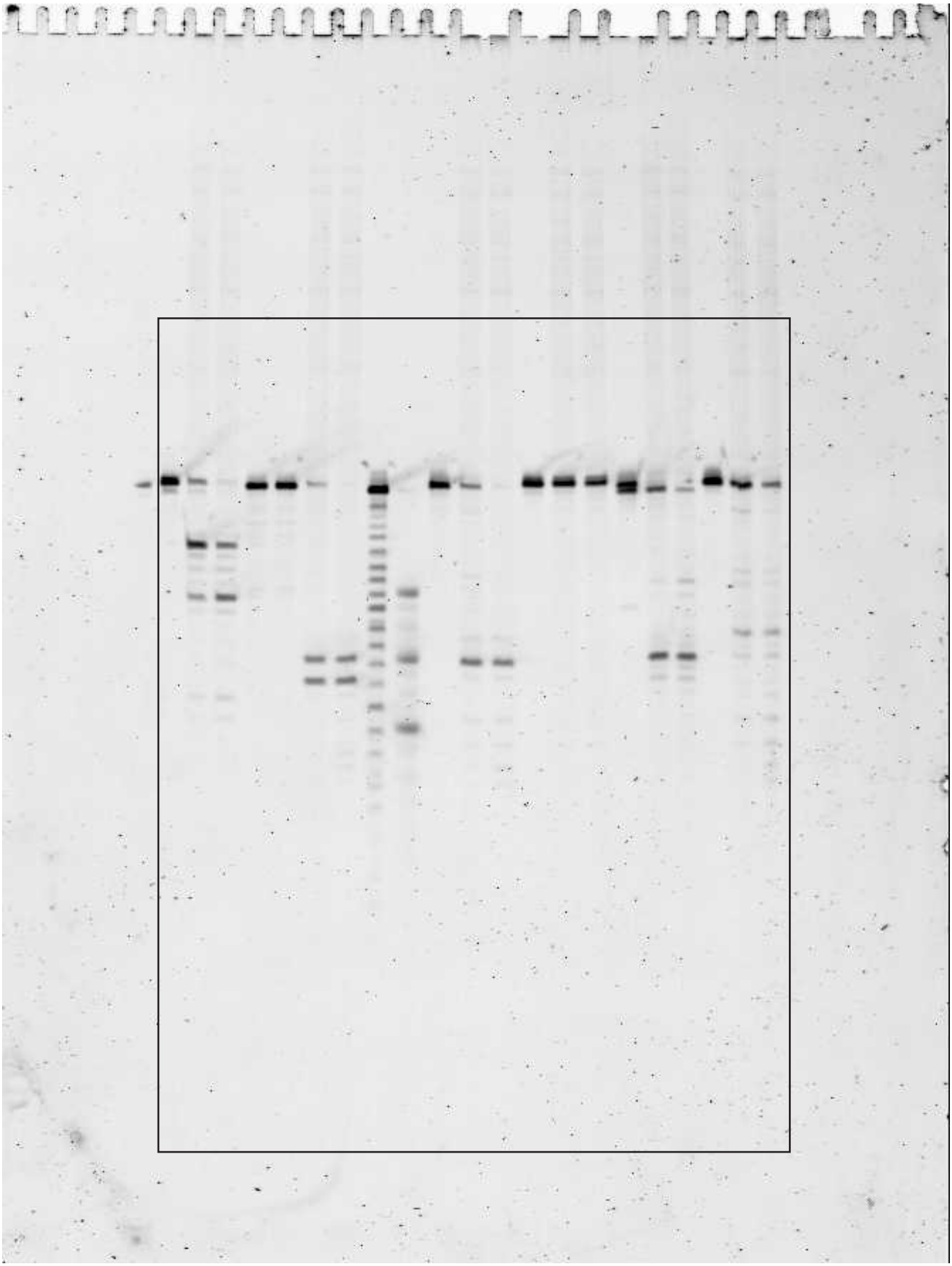
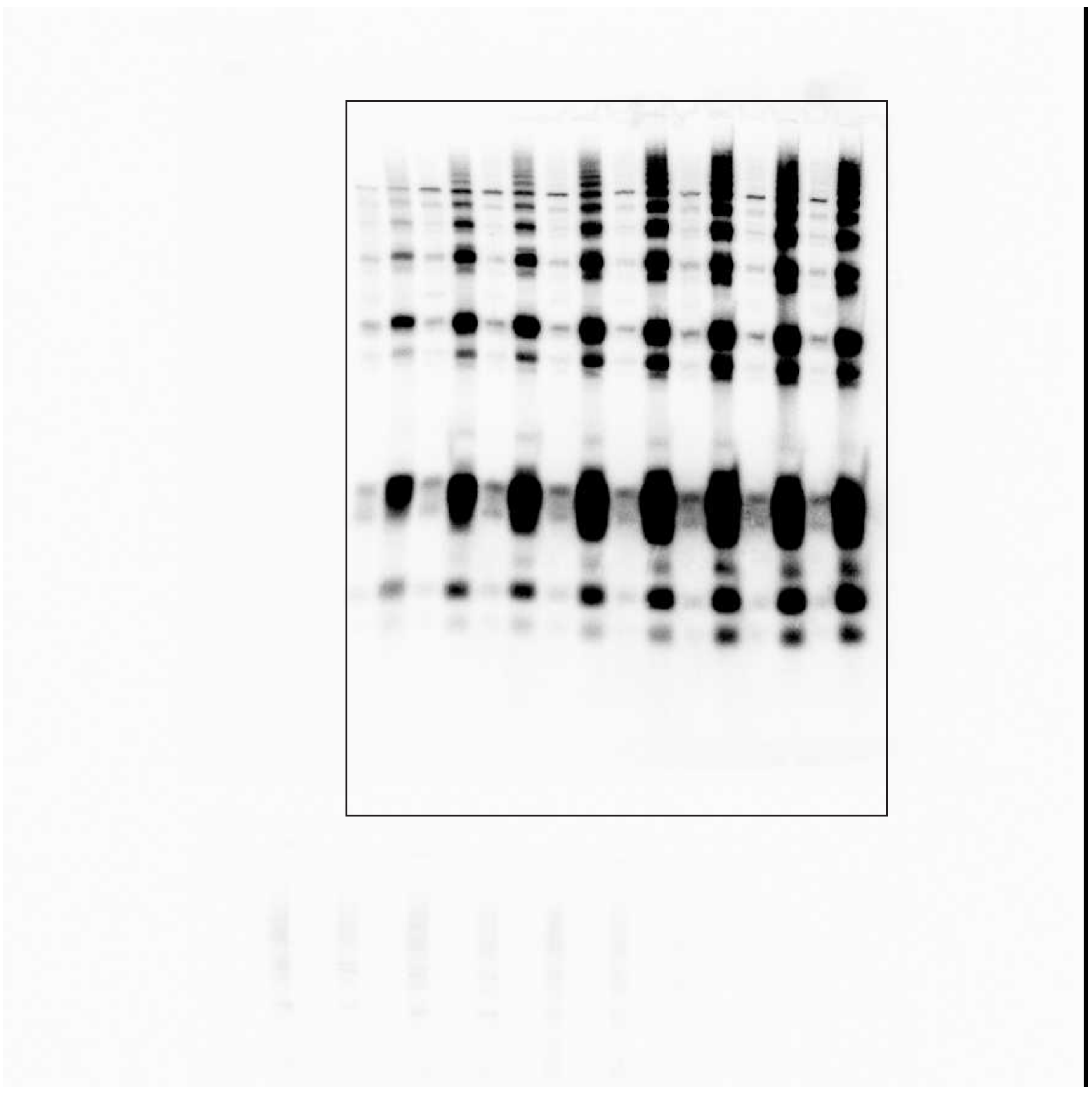
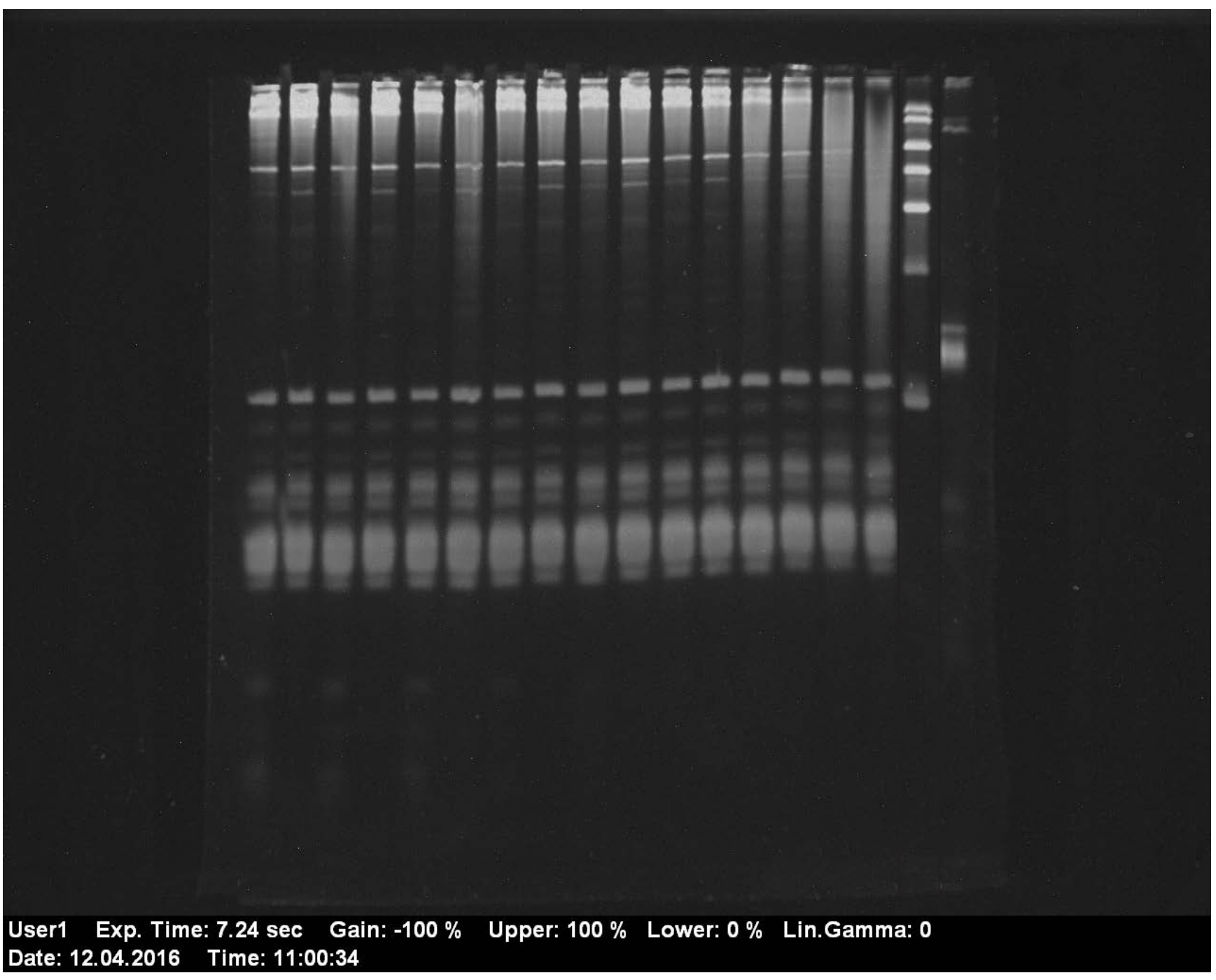


Figure 4
a

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Date: 12.04.2016 Time: 11:00:34

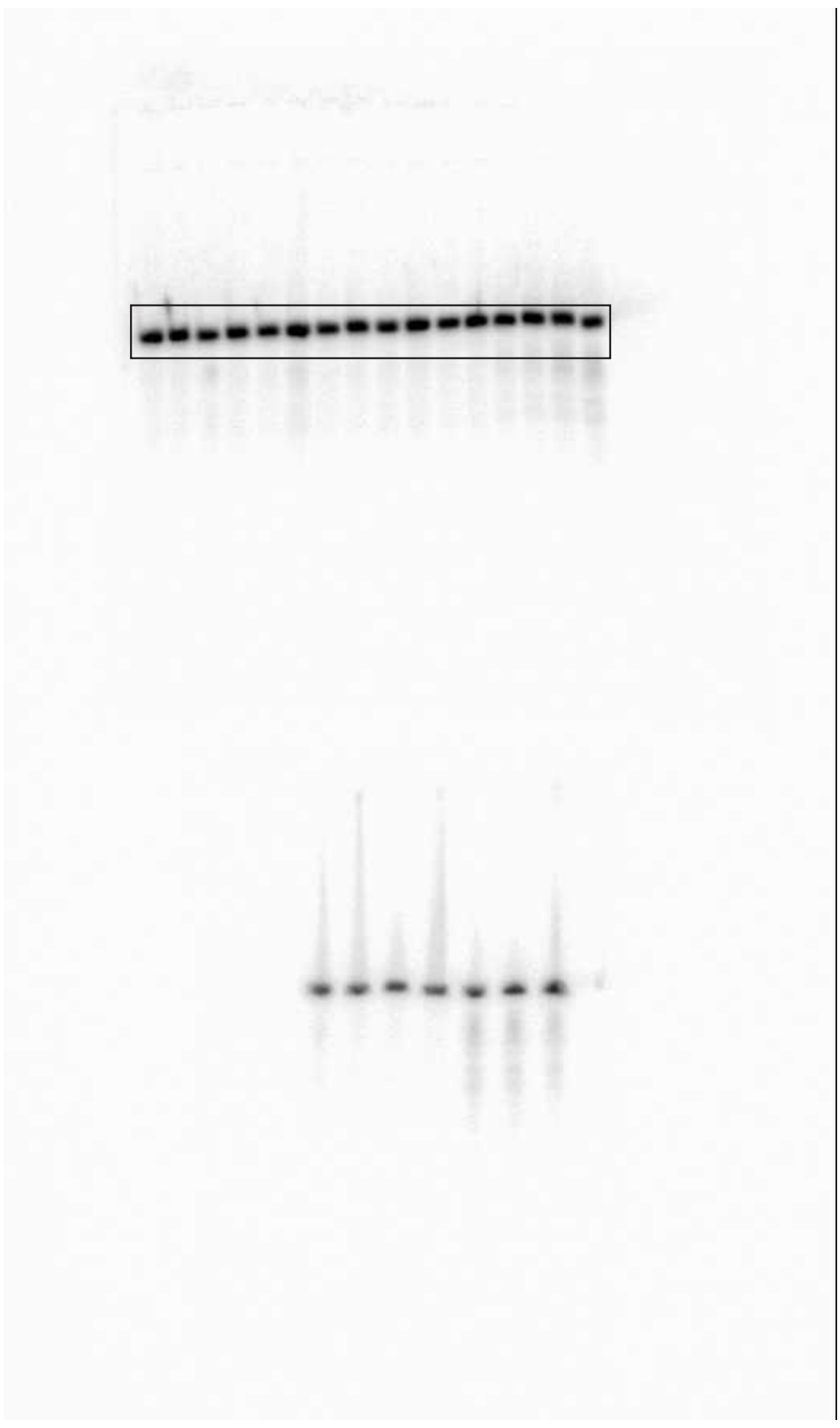
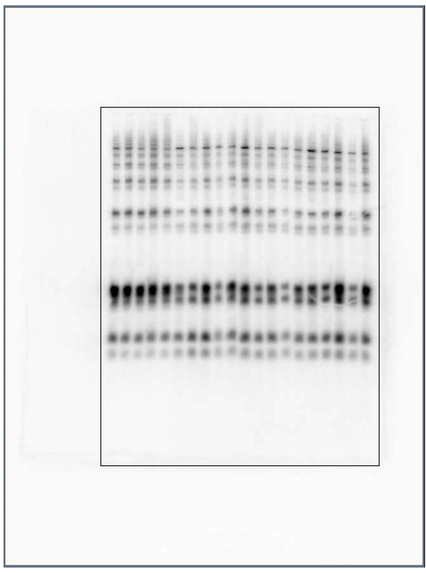
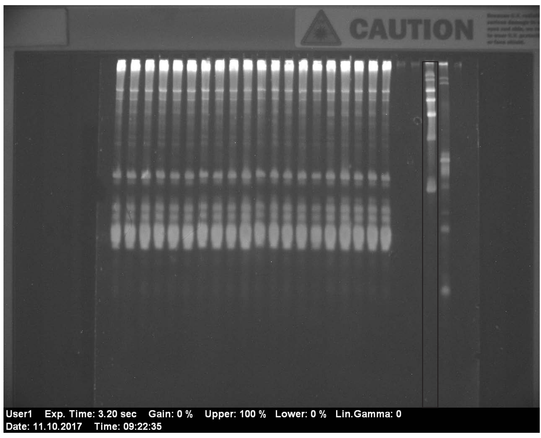


Figure 4
b




CAUTION
User1 Exp. Time: 3.20 sec Gain: 0 % Upper: 100 % Lower: 0 % Lin.Gamma: 0
Date: 11.10.2017 Time: 09:22:35

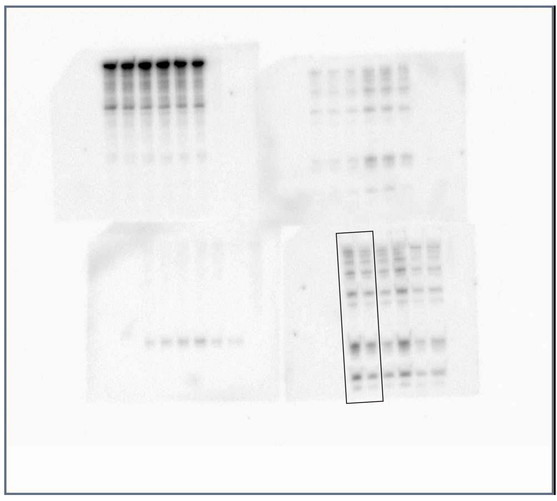


Figure 5 c
c

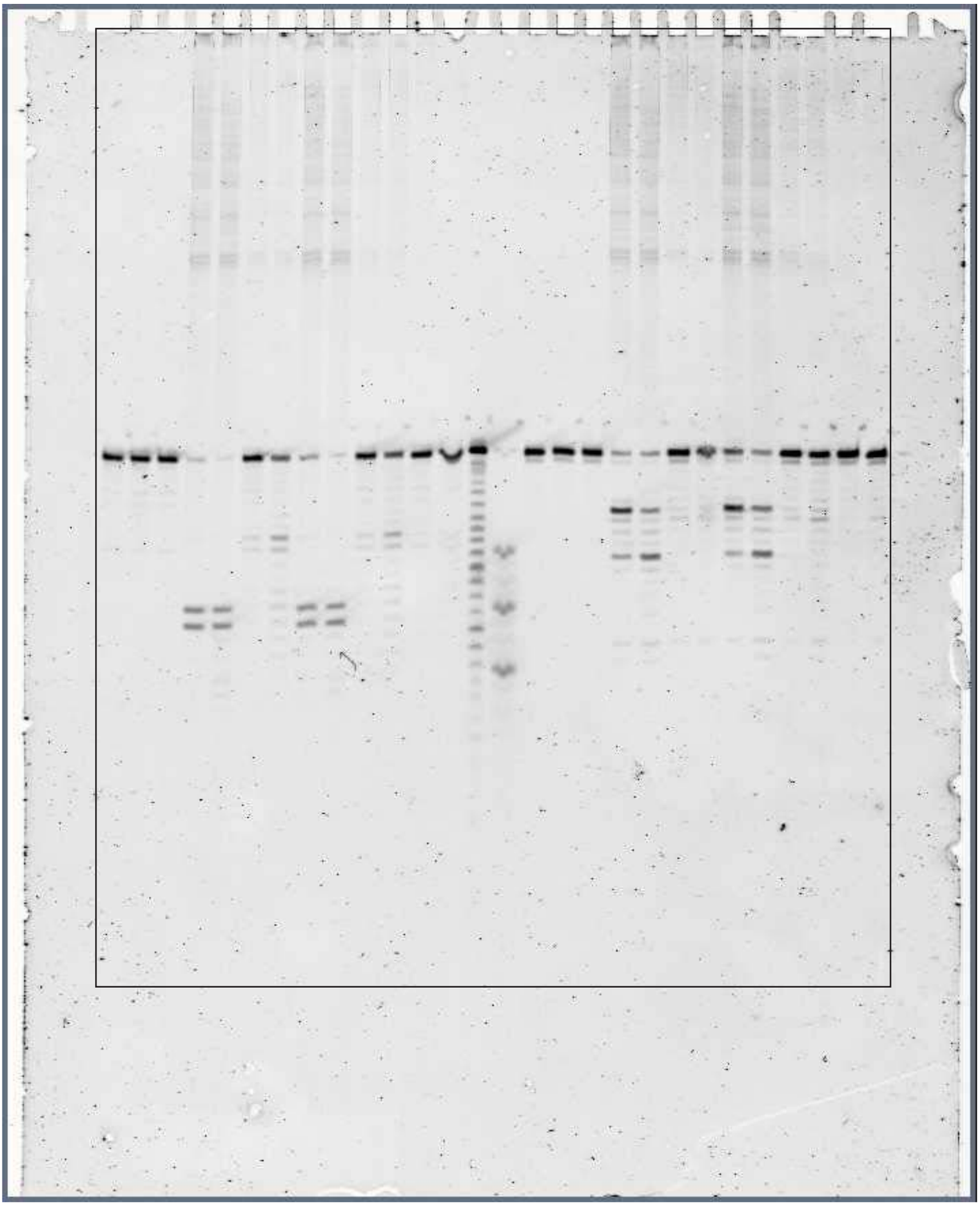
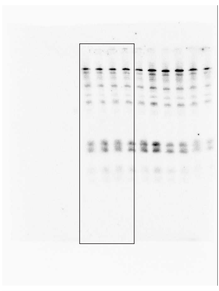
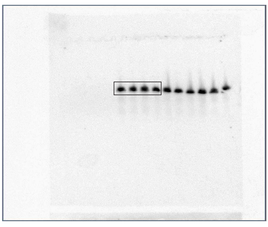
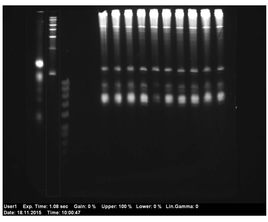


Figure S2
a



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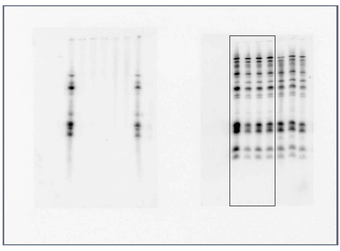
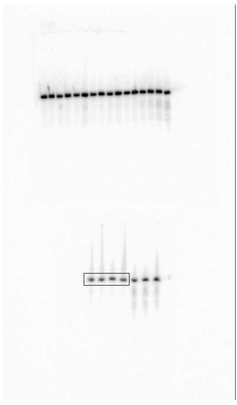
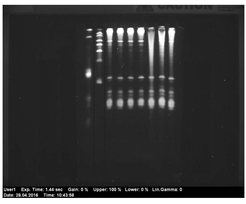
Date: 18.11.2016 Time: 10:00:47



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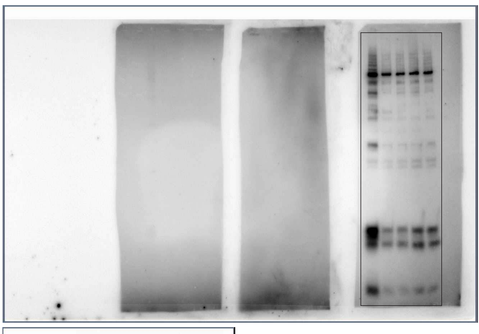
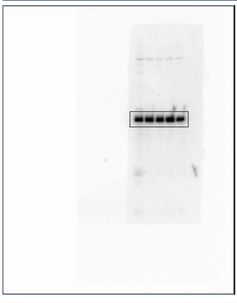
Date: 28.04.2016 Time: 10:45:58





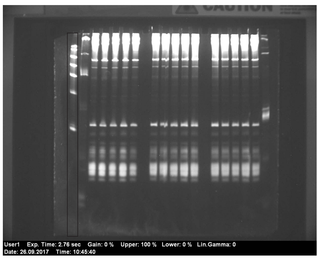

User1 Exp. Time: 2.78 sec Gain: 0 % Upper: 100 % Lower: 0 % Lin.Gamma: 0
Date: 26.09.2017 Time: 10:43:40

Figure S2
b

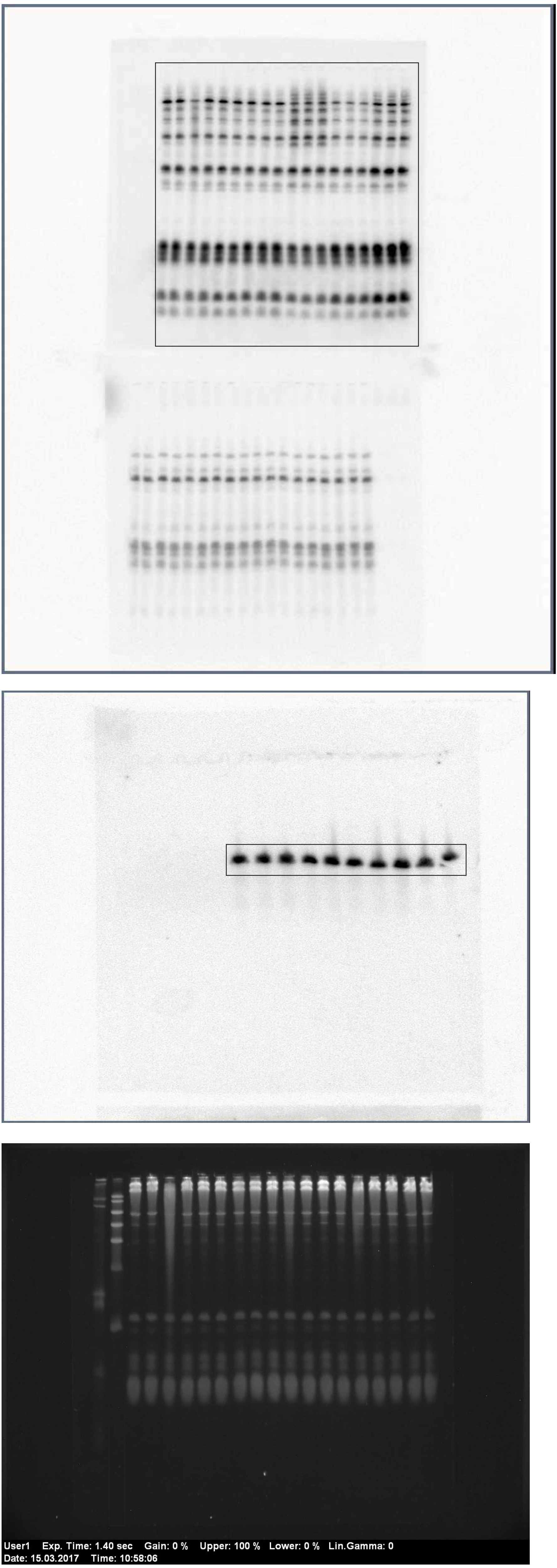

User1 Exp. Time: 1.40 sec Gain: 0 % Upper: 100 % Lower: 0 % Lin.Gamma: 0
Date: 15.03.2017 Time: 10:58:06

Figure S3

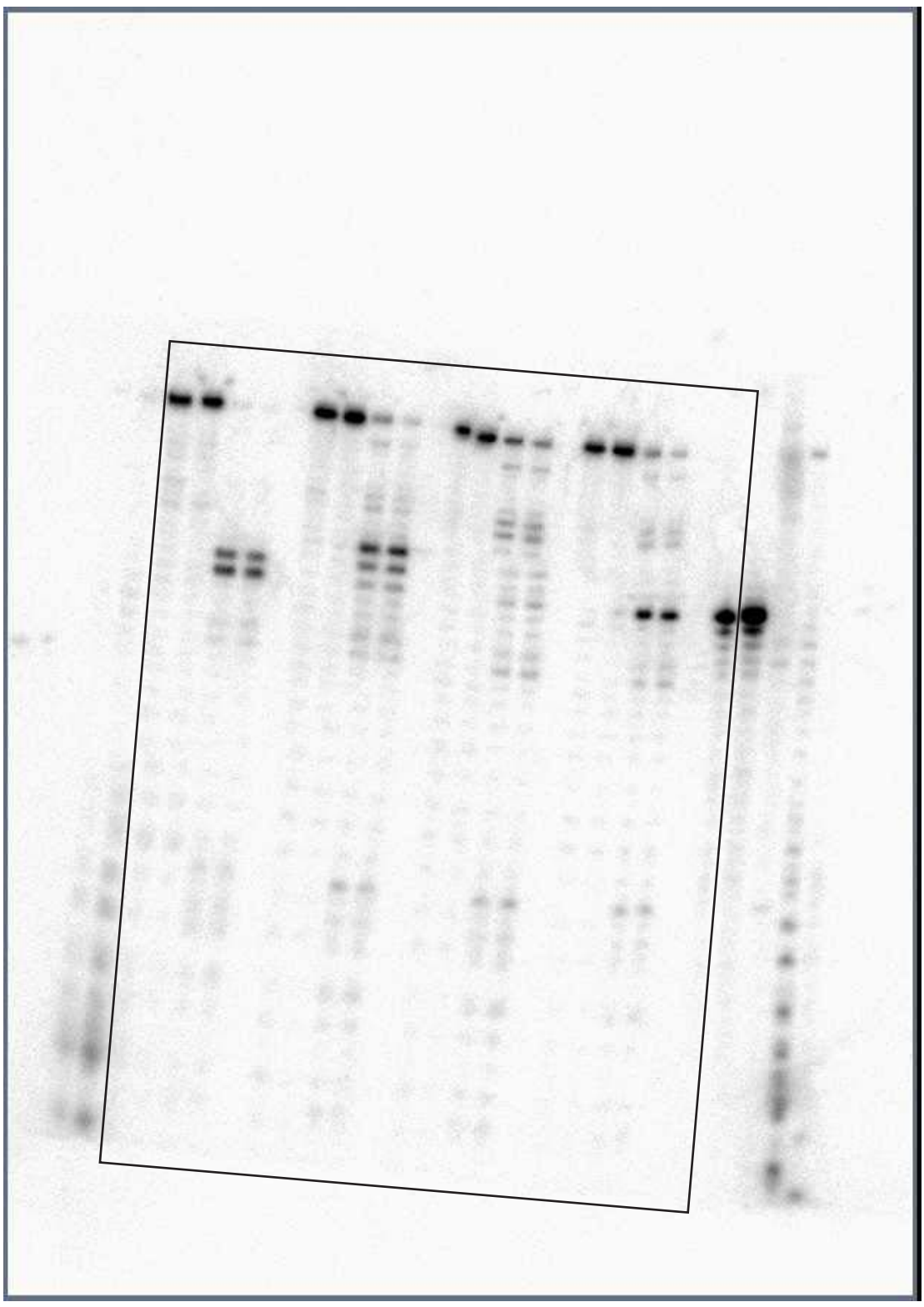


Figure S4