

Small antisense DNA-based gene silencing enables cell-free bacteriophage manipulation and genome replication – Supporting Information

Kilian Vogele[†], Elisabeth Falgenhauer[†], Sophie von Schönberg[†], Friedrich C. Simmel[†], Tobias Pirzer^{*†}

† Physics of Synthetic Biological Systems–E14, Physics Department and ZNN, Technische Universität München, 85748 Garching, Germany

E-mail: pirzer@tum.de

Phone: +49 (0)89 289-11609. Fax: +49 (0)89 289-11612.

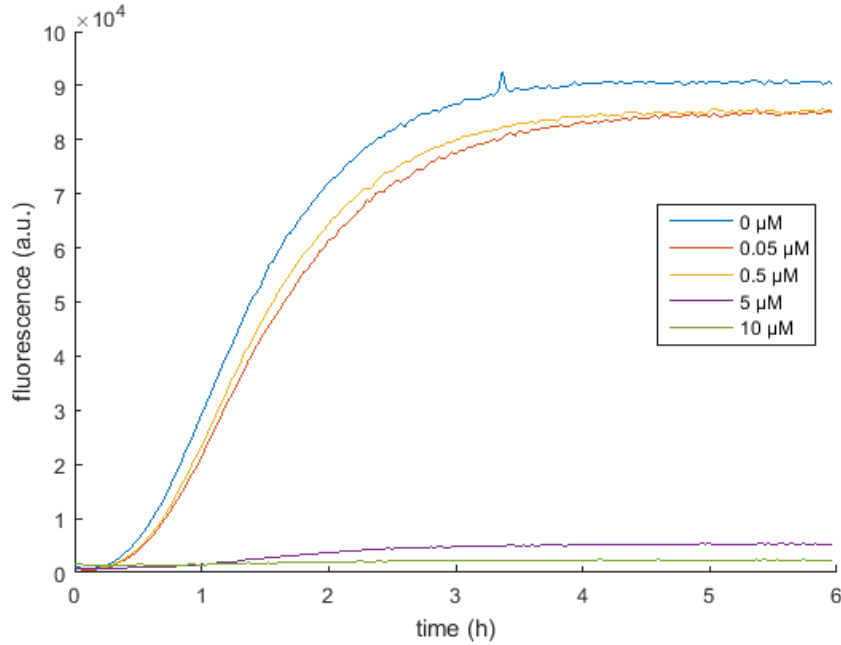


Figure S1. Cell-free expression of the fluorescence protein YPet in the presence of sDNA. Fluorescence measurement was carried out using a commercial plate reader. The concentrations used for the mRNA-complementary sDNA were 0 μM (cyan), 0.05 μM (red), 0.5 μM (yellow), 5 μM (purple), 10 μM (green). For the plasmid encoding YPet we used 5 nM and the used promotor was T7.

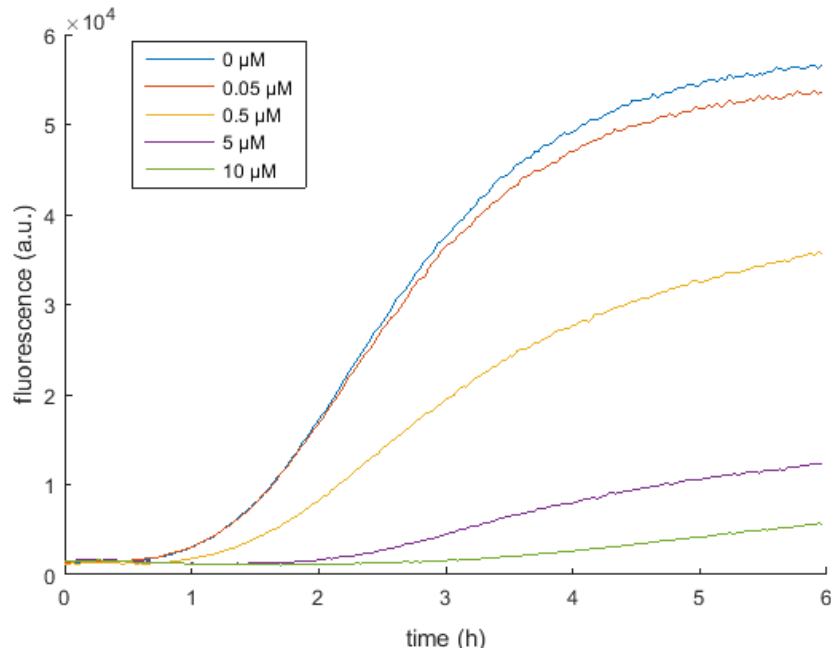


Figure S2. Cell-free expression of the fluorescence protein YPet in the presence of sDNA. Fluorescence measurement was carried out using a commercial plate reader. The concentrations used for the mRNA-complementary sDNA were 0 μM (cyan), 0.05 μM (red), 0.5 μM (yellow), 5 μM (purple), 10 μM (green). For the plasmid encoding YPet we used 5 nM and the used promotor was a constitutive promotor (J23106).

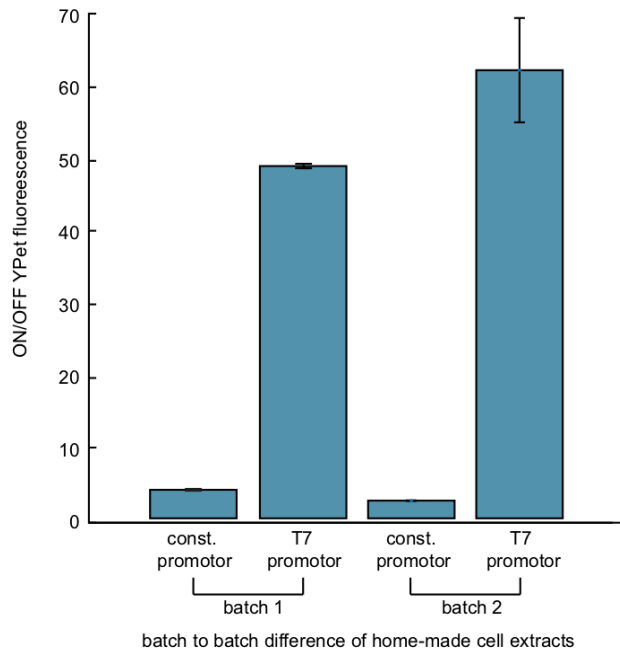


Figure S3. Comparison of ON/OFF YPet fluorescence for two different batches of home-made crude cell extracts. The lysis process was slightly different for both batches. The sDNA used had 60 nt and a concentration of 10 μ M. The given uncertainties are S.E.

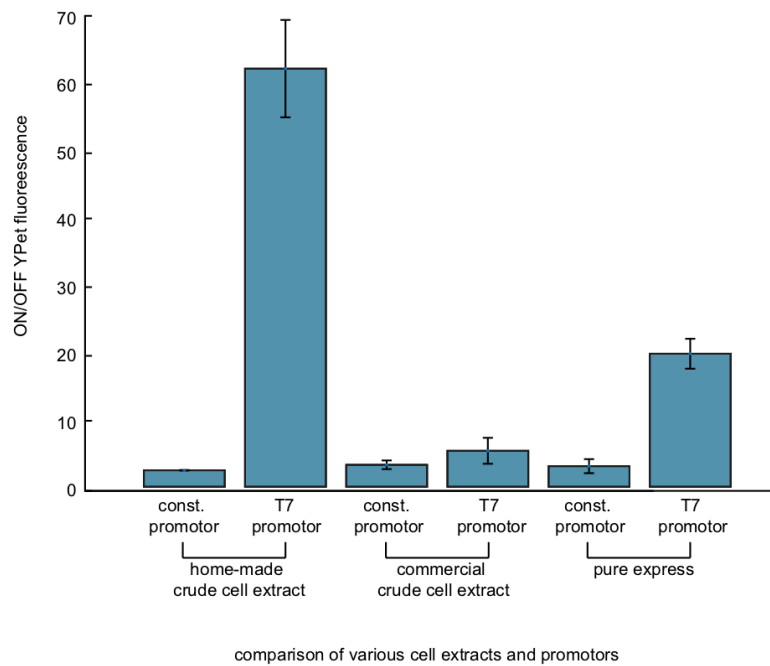


Figure S4. Comparison of ON/OFF YPet fluorescence for different cell free systems: a home-made crude cell extract, a commercially available crude cell extract (myTXTL from arbor biosciences) and the the PURExpress® system from NEB. The sDNA used had 60 nt and a concentration of 10 μ M. The given uncertainties are S.E.

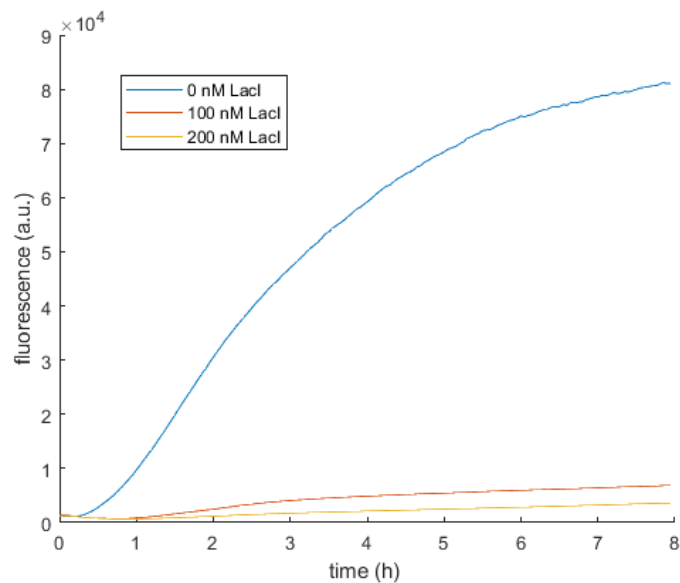


Figure S5. Cell-free expression of the fluorescence protein in the presence of Lacl. Fluorescence measurement was carried out using a commercial plate reader. The concentrations used for the Lacl were 0 nM (cyan), 100 nM (red), and 200 nM (yellow). The fluorescence protein was controlled by a lactose operon at 5 nM concentration. The ON/OFF ratio of the non-repressed signal to the repressed signal is for 100 nM Lacl approximately 16.9 and for 200 nM about 17.5.

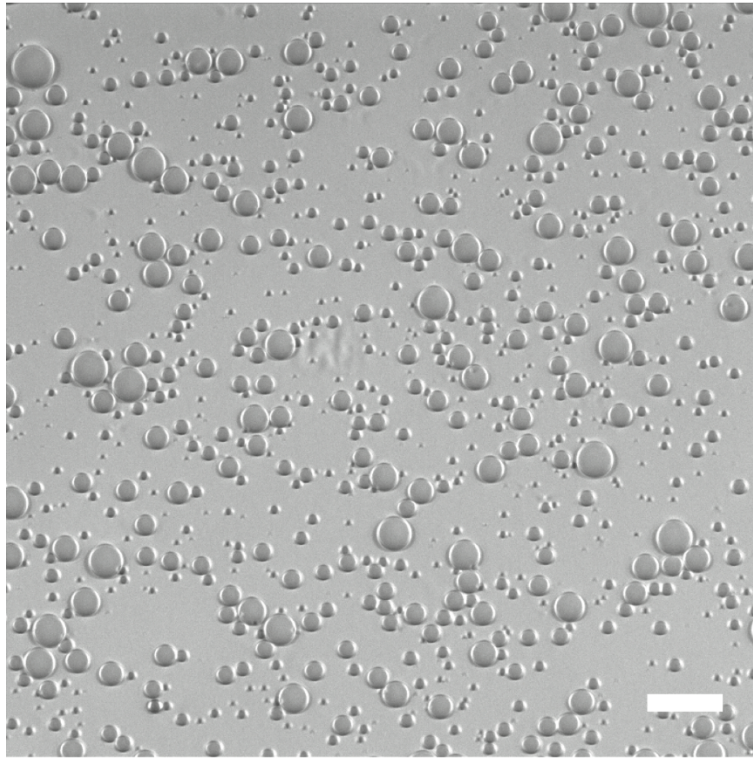


Figure S6. A typical micrograph of droplets created by shaking. Scale bar: 200 μm .

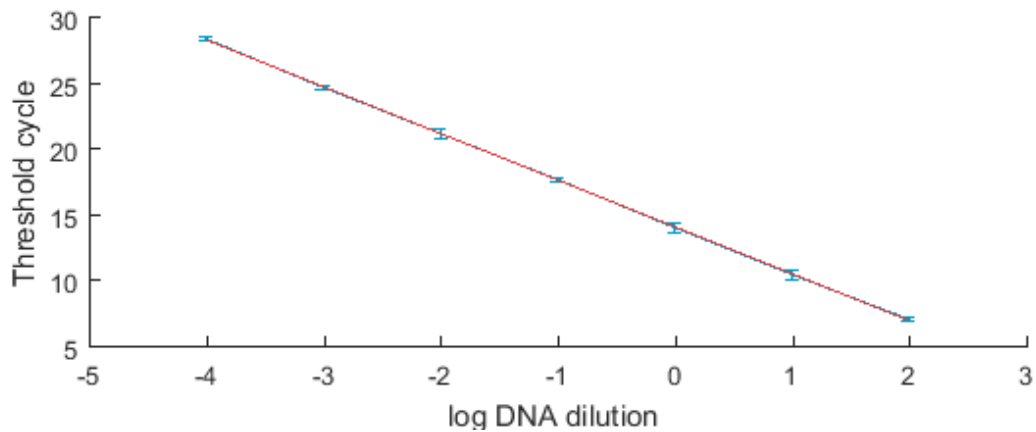


Figure S7. qPCR calibration curve to calculate the fold change of T7 DNA. The primer sequences used were ggaccacgtaaaccacatc and cattcgctaactccagagc. The linear fit of the threshold cycle vs. the log dilution of the T7 DNA resulted in an intersection with the y axis at 14.1613 ct and a slope of -3.5507 ct. The R^2 coefficient was 0.9999 and the efficacy of the PCR reaction was 91.26%.

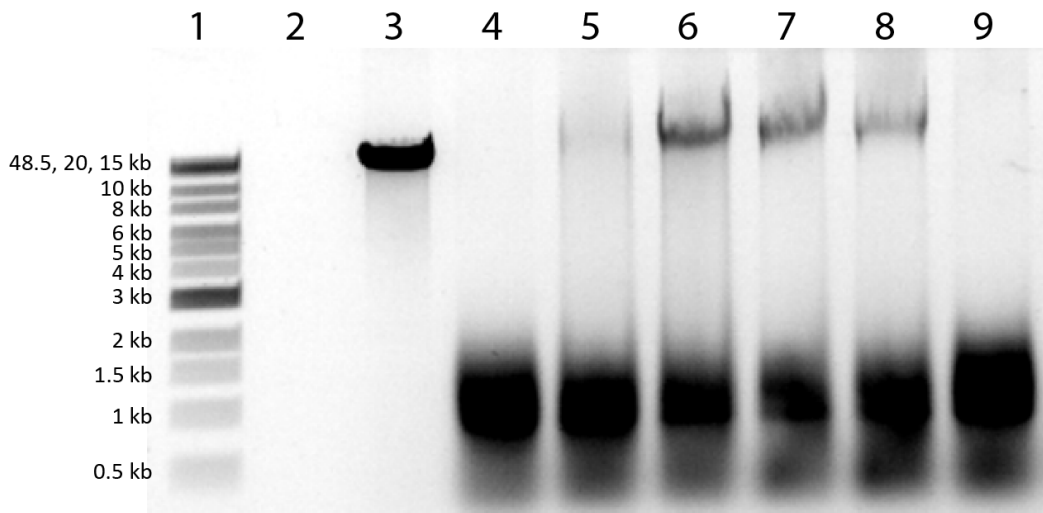


Figure S8. Agarose gel of the T7 DNA after several steps of dilution during the serial dilution experiments. From left to right: line 1, 1 kb extended ladder from NEB, lane 2, empty, lane 3, purified T7 DNA, lane 4, cell extract as a control, lane 5, cell extract and T7 DNA after 4th incubation and dilution, lane 6, cell extract and T7 DNA after 3rd incubation and dilution, lane 7, cell extract and T7 DNA after 2nd incubation and dilution, lane 8, cell extract and T7 DNA after 1st incubation and dilution, lane 9, cell extract and T7 DNA before 1st incubation.

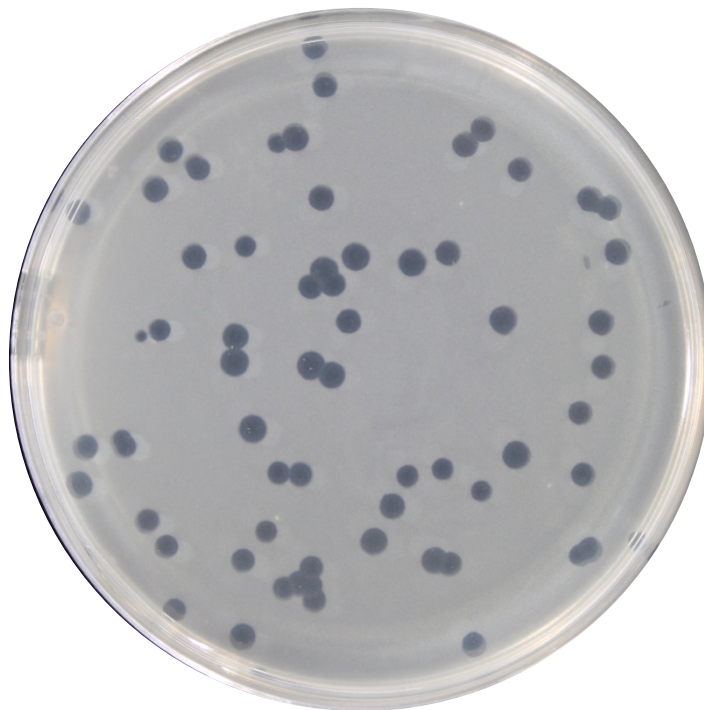


Figure S9. Example of a plaque assay of the cell-free produced T7 phage with *E. coli* to determine the phage titer of the sample.

DNA sequences

Plasmid sequences:

pSB1C3 T7 YPet:

gTAATACGACTCACTATAGGGTAGCGCAGCGCTCAACGGGTGTGCTTCCCGTTCTGATGAGTCCG
TGAGGACGAAAGCGCCTCTACAAATAATTTTGTAAATCATGAGaaagaggagaaaACTAGATGTCTA
AAGGTGAAGAACTGTTTACGGGTGTCGTGCCGATTCTGGTCGAGTTGGACGGCGACGTGAACGG
TCACAAATTCAGCGTGAGCGGCGAGGGCGAGGGTGACGCGACGTACGGTAAGCTGACTCTGAA
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c

pSB1C3 YPet:

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sDNA sequences:

40nt YPet:
AGACATCTAGTtttctcctcttCTCATGATTAACAAAA

50nt YPet:
CTTCACCTTTAGACATCTAGTtttctcctcttCTCATGATTAACAAAA

60nt YPet:
GTAAACAGTTCTTCACCTTTAGACATCTAGTtttctcctcttCTCATGATTAACAAAA

T7_phi10:
AGCCATATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGGGAAACCGTTG

qPCR Primer:

ggaccacgtaaacacatc
cattcggctaactccagagc

DNA and RNA structure analysis

We used the NUPACK Web Application to determine the secondary structure of the sDNA used. For the RNA structure analysis and for the calculation of the minimum free energy (MFE) of RNA-DNA conjugation we used the forna Server and the RNAcifold Server from the ViennaRNA Web Services (rna.tbi.univie.ac.at). In order to compare the stability of different sequences we altered the mRNA sequences for the calculations by poly-G resp. poly-U (Table S1). Table S2 shows the frequency of the MFE structures, DG and the single free energies of the monomers, homodimers and heterodimer of various ensembles.

shorthand naming	Description/name	sequence
Ypet	mRNA Ypet short	GAAAGCGCCUCUACAAAUAAUUUUUUUUUUAAU CAUGAGaaagaggagaaaACUAGAUGUCUAAAG GUGAAGAACUGUUUACGGGUGUCGUGCCG
YPet G	mRNA YPet short Poly-G	GAAAGCGCCUCUACAAAUAAUUUUU <u>GGGGGG</u> <u>GGGGGG</u> aaagaggagaaaACUAGAUGUCUAAA GGUGAAGAACUGUUUACGGGUGUCGUGCCG
YPet U	mRNA YPet short Poly-U	GAAAGCGCCUCUACAAAUAAUUUUU <u>UUUUUUU</u> <u>UUUUUU</u> aaagaggagaaaACUAGAUGUCUAAAG GUGAAGAACUGUUUACGGGUGUCGUGCCG
40 Ypet	sDNA 40 nt Ypet	AGACATCTAGTtttcctctttCTCATGATTAACAA AA
40 Ypet C	sDNA 40 nt Ypet Poly-C	AGACATCTAGTtttcctcttt <u>CCCCCCCCCCCCCA</u> AAA
40 Ypet A	sDNA 40 nt Ypet Poly-A	AGACATCTAGTtttcctcttt <u>AAAAAAAAAAAAAA</u> AA
50 Ypet	sDNA 50 nt Ypet	CTTCACCTTTAGACATCTAGTtttcctctttCTCAT GATTAACA AAAA
50 Ypet C	sDNA 50 nt Ypet Poly-C	CTTCACCTTTAGACATCTAGTtttcctcttt <u>CCCCC</u> <u>CCCCCCCC</u> AAAA
50 Ypet A	sDNA 50 nt Ypet Poly-A	CTTCACCTTTAGACATCTAGTtttcctcttt <u>AAAAA</u> <u>AAAAAAAA</u> AAAA
60 Ypet	sDNA 60 nt Ypet	GTAAACAGTTCTTCACCTTTAGACATCTAGTtttc tctctttCTCATGATTAACA AAAA
60 Ypet C	sDNA 60 nt Ypet Poly-C	GTAAACAGTTCTTCACCTTTAGACATCTAGTtttc tctcttt <u>CCCCCCCCCCCCC</u> AAAA
60 Ypet A	sDNA 60 nt Ypet Poly-A	GTAAACAGTTCTTCACCTTTAGACATCTAGTtttc tctcttt <u>AAAAAAAAAAAAA</u> AAAA
mcp	mRNA major capsid protein short	gggagaccacaacggUUUcccUcUagaaaUaaUUUUg UUUaacUUUaagaaggagaUaUacaUaUggcUagca UgacUggUggacagcaaaUg
T7_phi10	sDNA T7_phi10	AGCCATATGTATATCTCCTTCTTAAAGTTAAAC AAAATTATTTCTAGAGGGAAACCGTTG

Table S1. Overview of DNA and RNA sequences used for calculations.

ViennaRNA Web Services: RNAfold Server
free energy values are given in kcal/mol.

A	B	Frequ. MFE Struct.	ΔG hetero- dimer	AB hetero- dimer	AA homo- dimer	BB homo- dimer	A mono- mer	B mono- mer
Ypet	40 Ypet	8.25%	-54.78	-70.14	-37.14	-7.92	-14.04	-1.32
Ypet	50 Ypet	25.12%	-70.94	-86.75	-37.14	-9.12	-14.04	-1.77
Ypet	60 Ypet	24.98%	-86.73	-103.65	-37.14	-11.22	-14.04	-2.88
YPet G	40 Ypet C	8.34%	-72.74	-90.23	-42.16	-5.55	-17.00	-0.49
YPet G	50 Ypet C	25.40%	-88.88	-106.84	-42.16	-7.12	-17.00	-0.96
YPet G	60 Ypet C	25.26%	-104.42	-123.75	-42.16	-7.45	-17.00	-2.33
YPet U	40 Ypet A	7.86%	-40.38	-59.17	-43.53	-5.68	-18.22	-0.57
YPet U	50 Ypet A	23.94%	-56.44	-75.78	-43.53	-7.32	-18.22	-1.13
YPet U	60 Ypet A	23.80%	-72.06	-92.68	-43.53	-7.78	-18.22	-2.04
YPet G	40 Ypet C	8.34%	-72.74	-90.23	-42.16	-5.55	-17.00	-0.49
Ypet	40 Ypet	8.25%	-54.78	-70.14	-37.14	-7.92	-14.04	-1.32
YPet U	40 Ypet A	7.86%	-40.38	-59.17	-43.53	-5.68	-18.22	-0.57
YPet G	50 Ypet C	25.40%	-88.88	-106.84	-42.16	-7.12	-17.00	-0.96
Ypet	50 Ypet	25.12%	-70.94	-86.75	-37.14	-9.12	-14.04	-1.77
YPet U	50 Ypet A	23.94%	-56.44	-75.78	-43.53	-7.32	-18.22	-1.13
YPet G	60 Ypet C	25.26%	-104.42	-123.75	-42.16	-7.45	-17.00	-2.33
Ypet	60 Ypet	24.98%	-86.73	-103.65	-37.14	-11.22	-14.04	-2.88
YPet U	60 Ypet A	23.80%	-72.06	-92.68	-43.53	-7.78	-18.22	-2.04
Ypet	60 Ypet	24.98%	-86.73	-103.65	-37.14	-11.22	-14.04	-2.88
Ypet	60 Ypet C	26.54%	-65.85	-82.22	-37.14	-7.45	-14.04	-2.33
Ypet	60 Ypet A	10.95%	-63.52	-79.96	-37.14	-7.78	-14.04	-2.4
Ypet	40 Ypet	8.25%	-54.78	-70.14	-37.14	-7.92	-14.04	-1.32
Ypet	40 Ypet C	8.76%	-34.17	-48.7	-37.14	-5.55	-14.04	-0.49
Ypet	40 Ypet A	3.62%	-31.84	-46.45	-37.14	-5.68	-14.04	-0.57
Ypet	60 Ypet	24.98%	-86.73	-103.65	-37.14	-11.22	-14.04	-2.88
mcp	T7_phi10	39.55%	-71.8	-105.07	-52.54	-24.69	-23.63	-9.64

Table S2. Overview of Results from calculations using ViennaRNA Web Services RNAfold server.

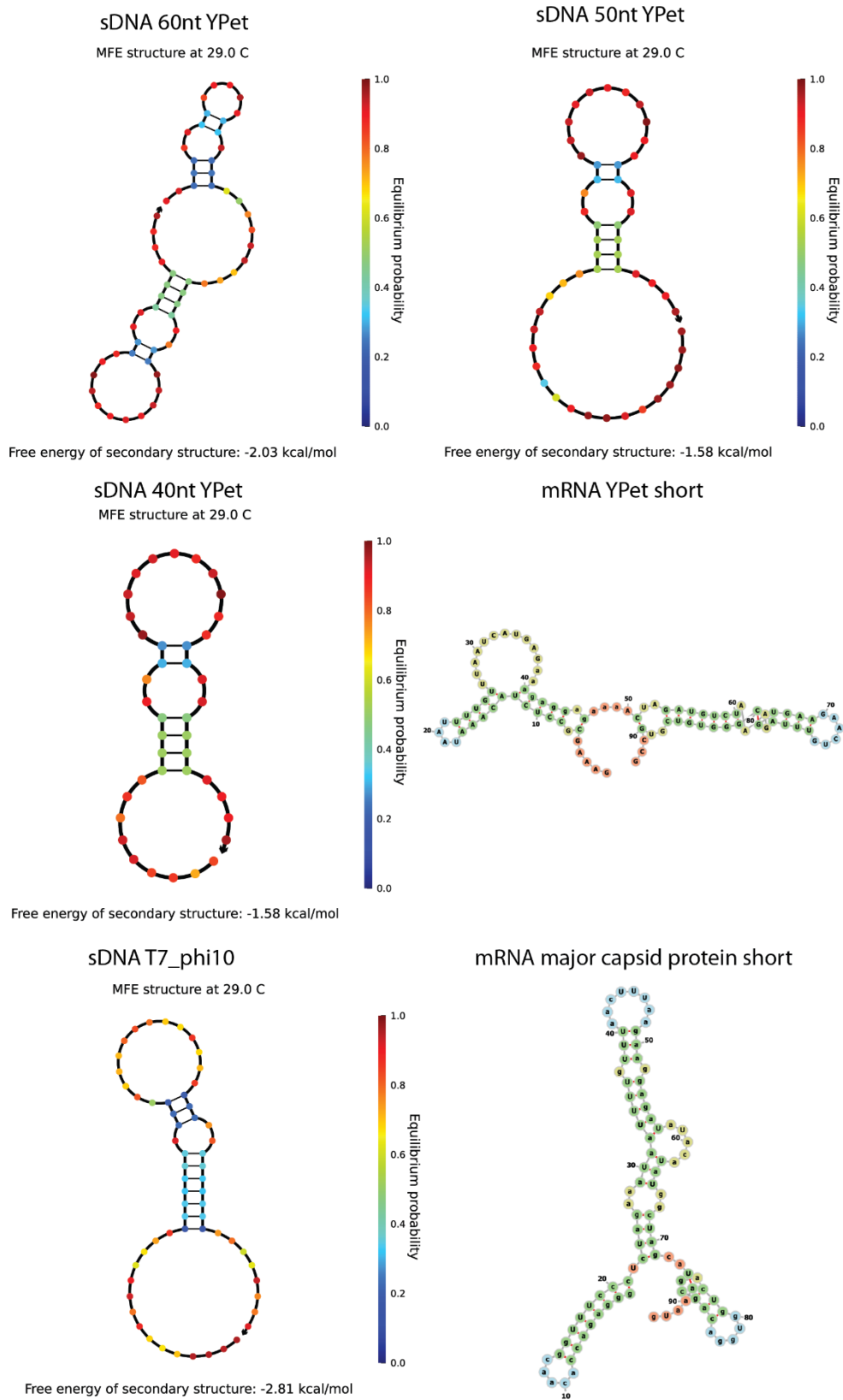


Figure S10. Overview of sDNA (NUPACK) and mRNA (ViennaRNA Web Services forna Server) secondary structures.