Supplementary Information

Engineered Protein Nanocages for Concurrent RNA and Protein Packaging *In Vivo*

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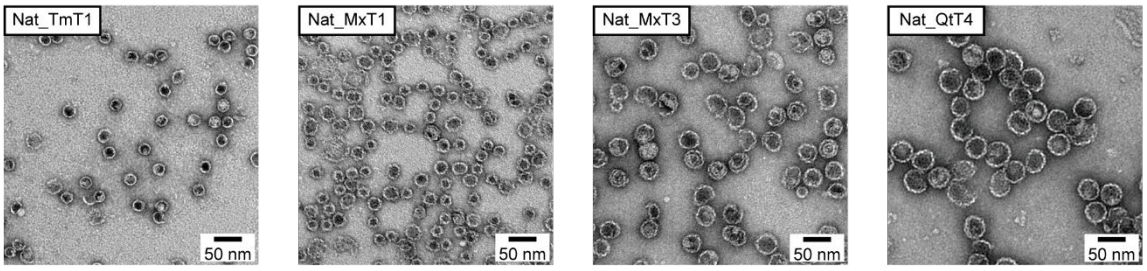
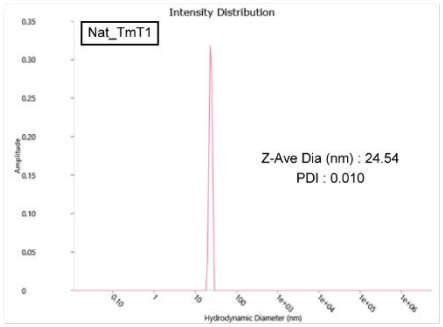
Content: Figures S1-S5, Tables S1-S2

A


Nat_TmT1
50 nm
Nat_MxT1
50 nm
Nat_MxT3
50 nm
Nat_QtT4
50 nm

B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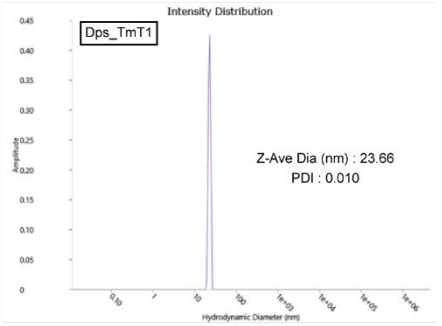
PDI : 0.010
Amplitude
Hydrodynamic Diameter (nm)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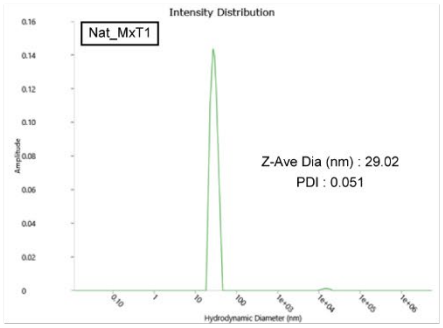
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Z-Ave Dia (nm) : 29.02
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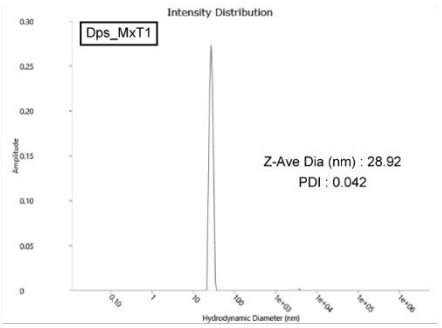
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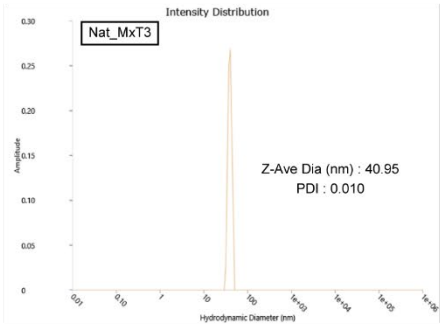
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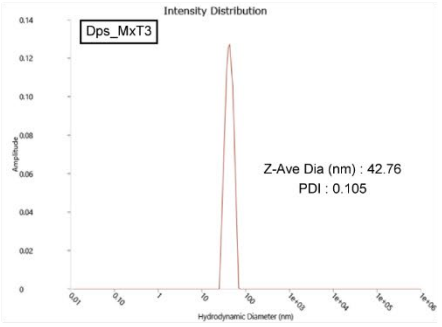
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Z-Ave Dia (nm) : 42.76
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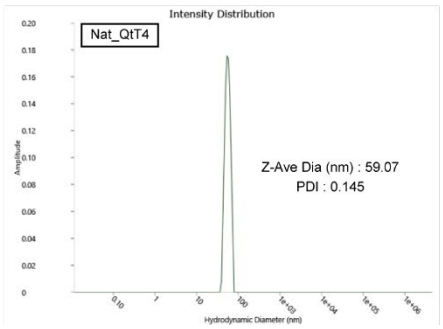
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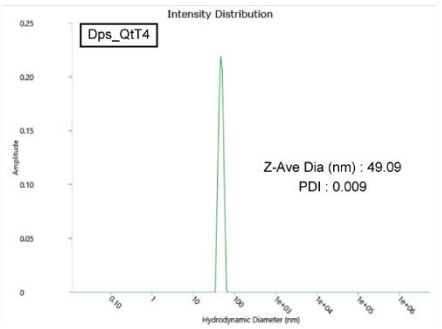
Nat_QtT4
Z-Ave Dia (nm) : 59.07
PDI : 0.145
Amplitude
Hydrodynamic Diameter (nm)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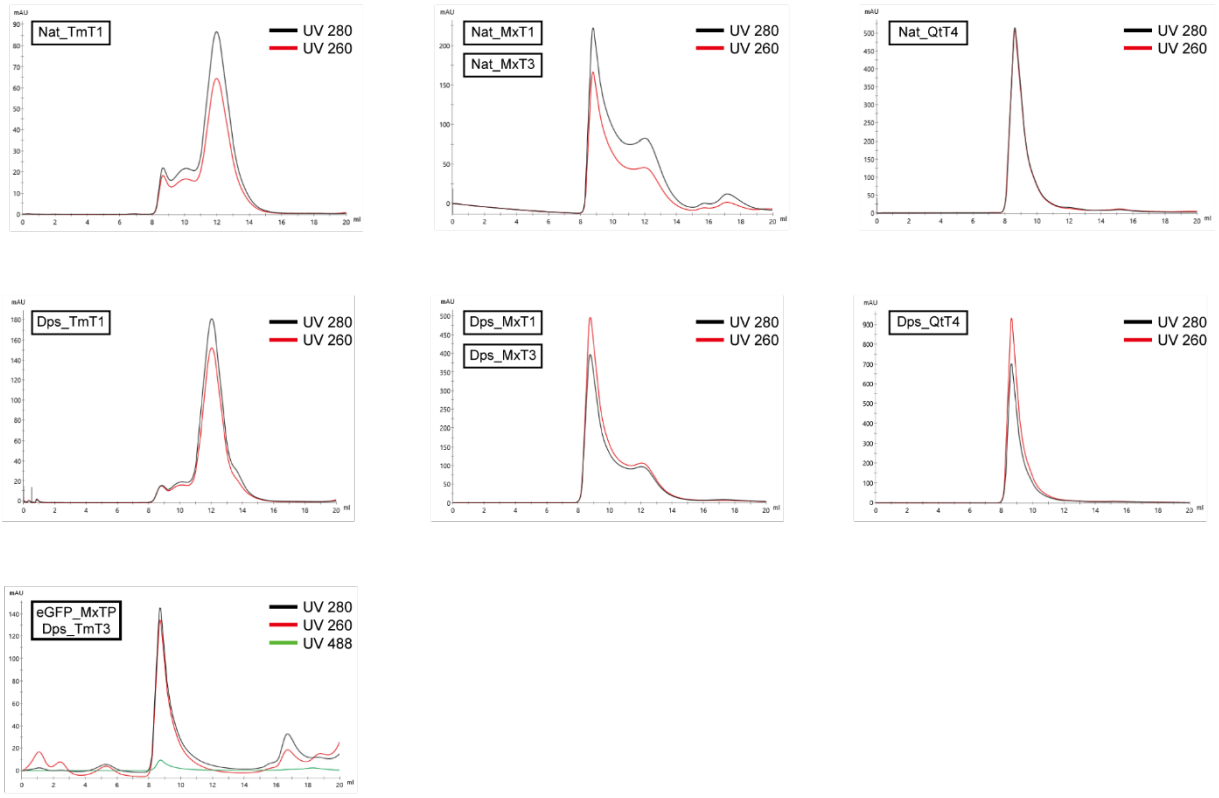
PDI : 0.009
Amplitude
Hydrodynamic Diameter (nm)

C



D

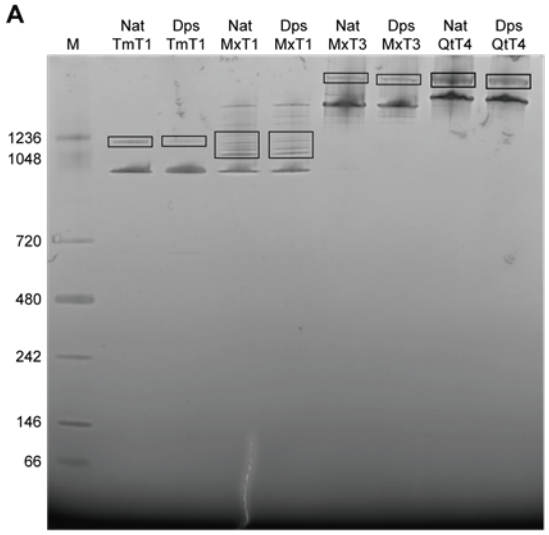
	Collected elution volume from SEC (ml)	A260/A280
Nat_TmT1	11 – 13	0.76
Dps_TmT1	11 – 13	0.86
Nat_MxT1	11 – 13	0.64
Dps_MxT1	11 – 13	1.11
Nat_MxT3	8 – 10	0.77
Dps_MxT3	8 – 10	1.24
Nat_QtT4	8 – 10	0.98
Dps_QtT4	8 – 10	1.33
eGFP_MxTP Dps_MxT3	8 – 10	0.96

E

	Amount of used protein for RNA extraction (ug)	Amount of extracted RNA (ng)	Amount of packaged RNA per 1 mol shell (kg/mol)
Dps_TmT1	350.4	1358	7.5
Dps_MxT1	66.69	1309	39.2
Dps_MXT3	360.875	5775	95.9
Dps_QtT4	356.8	5264	119.8
eGFP_MxTP _MxT3	170.4	1995	70.2

Figure S1. A) Negative-stain TEM micrographs of all four purified Nat_Encs. B) Dynamic light scattering (DLS) analysis of Nat_Encs (left) and Dps_Encs (right). Z-average diameter and polydispersity index (PDI) for each sample are shown next to the peak. C) Size-exclusion chromatography (SEC) analysis of Nat_Encs (top), Dps_Encs (middle), and eGFP_MxTP_Dps_MxT3 (bottom). UV 280 tracking the protein signal is shown in black line and UV 260 tracking the nucleic acid signal is shown in red line and UV 488 tracking the eGFP signal is shown in green line. D) Table showing the collected elution volume for each Nat_Enc, Dps_Enc, and eGFP_MxTP_Dps_MxT3 from the respective SEC runs (second column) and the direct A260/A280 measurement of the collected and concentrated Nat_Encs, Dps_Encs, and eGFP_MxTP_Dps_MxT3 (third column). E) Table showing the absolute amount of RNA extracted from given amount of each Nat_Enc, Dps_Enc, and eGFP_MxTP_Dps_MxT3. Amount of packaged RNA per shell is also shown in the last column. Since Dps_MxT1 sample partially contained Dps_MxT3 as shown in Figure 1D and Figure 4, the amount of extracted RNA accounting for sole Dps_MxT1 would be less than is shown here.

A



B

Identified Proteins (74)	Accession Number	Molecular Weight	SpC Sample 1
Nat_TmT1|SequenceA|SequenceA	Custom|SequenceA|Seque	30 kDa	42
50S ribosomal protein L20 OS=Escherichia coli (strain K12) OX=83333 GN=rplT PE=1	sp|P0A7L3|RL20_ECOLI	13 kDa	14
Elongation factor Tu 1 OS=Escherichia coli (strain K12) OX=83333 GN=tufA PE=1 SV	sp|P0CE47|EFTU1_ECOLI (+	43 kDa	13
50S ribosomal protein L2 OS=Escherichia coli (strain K12) OX=83333 GN=rplB PE=1	sp|P60422|RL2_ECOLI	30 kDa	12
Glyceraldehyde-3-phosphate dehydrogenase A OS=Escherichia coli (strain K12) OX	sp|P0A9B2|G3P1_ECOLI	36 kDa	10

Identified Proteins (51)	Accession Number	Molecular Weight	SpC Sample 2
Dps_TmT1|SequenceB|SequenceB	Custom|SequenceB|S	32 kDa	34
Glutamine synthetase OS=Escherichia coli (strain K12) OX=83333 GN=glnA PE=1 SV=2	sp|P0A9C5|GLN1B_E(	52 kDa	13
Elongation factor Tu 1 OS=Escherichia coli (strain K12) OX=83333 GN=tufA PE=1 SV=1	sp|P0CE47|EFTU1_EC	43 kDa	9
Biosynthetic arginine decarboxylase OS=Escherichia coli (strain K12) OX=83333 GN=speA	sp|P21170|SPEA_ECC	74 kDa	9
Chaperonin GroEL OS=Escherichia coli (strain K12) OX=83333 GN=groEL PE=1 SV=2	sp|P0A6F5|CH60_EC(	57 kDa	7

Identified Proteins (166)	Accession Number	Molecular Weight	SpC Sample 3 (Nat_MxT1)	Spc Sample 5 (Nat_MxT3)
Nat_MxT1/3|SequenceC|SequenceC	Custom|SequenceC|	32 kDa	253	224
Ribonucleoside-diphosphate reductase 1 subunit alpha OS=Escherichia coli (strain K12)	sp|P00452|RIR1_ECC	86 kDa	0	2
Maltodextrin phosphorylase OS=Escherichia coli (strain K12) OX=83333 GN=malP PE=1	sp|P00490|PHSM_E(	91 kDa	0	6
RNA polymerase sigma factor RpoD OS=Escherichia coli (strain K12) OX=83333 GN=rpoD	sp|P00579|RPOD_EC	70 kDa	0	8
Beta-galactosidase OS=Escherichia coli (strain K12) OX=83333 GN=lacZ PE=1 SV=2	sp|P00722|BGAL_EC	116 kDa	7	12

Identified Proteins (67	Accession Number	Molecular Weight	SpC Sample 4 (Dps_MxT1)	SpC Sample 6 (Dps_MxT3)
Dps_MxT1/3|SequenceD|SequenceD	Custom|SequenceD|S	33 kDa	122	135
30S ribosomal protein S6 OS=Escherichia coli (strain K12) OX=83333 GN=rpsF PE=1 SV=1	sp|P02358|RS6_ECOL	16 kDa	4	3
30S ribosomal protein S7 OS=Escherichia coli (strain K12) OX=83333 GN=rpsG PE=1 SV=3	sp|P02359|RS7_ECOL	20 kDa	6	5
50S ribosomal protein L15 OS=Escherichia coli (strain K12) OX=83333 GN=rplO PE=1 SV=1	sp|P02413|RL15_ECO	15 kDa	3	4
Outer membrane porin F OS=Escherichia coli (strain K12) OX=83333 GN=ompF PE=1 SV=1	sp|P02931|OMPF_EC(	39 kDa	3	2

Identified Proteins (170)	Accession Number	Molecular Weight	SpC Sample 7
Nat_QtT4|SequenceE|SequenceE	Custom|SequenceE	32 kDa	273
Type II NADH:quinone oxidoreductase OS=Escherichia coli (strain K12) OX=83333 GN=ndh PE=1 S	sp|P00393|NDH_E(	47 kDa	2
Maltodextrin phosphorylase OS=Escherichia coli (strain K12) OX=83333 GN=malP PE=1 SV=7	sp|P00490|PHSM_	91 kDa	9
Beta-galactosidase OS=Escherichia coli (strain K12) OX=83333 GN=lacZ PE=1 SV=2	sp|P00722|BGAL_E	116 kDa	10
Alanine--tRNA ligase OS=Escherichia coli (strain K12) OX=83333 GN=alaS PE=1 SV=2	sp|P00957|SYA_EC	96 kDa	4

Identified Proteins (113)	Accession Number	Molecular Weight	SpC Sample 8
Dps_QtT4|SequenceF|SequenceF	Custom|SequenceF|S	34 kDa	263
Maltodextrin phosphorylase OS=Escherichia coli (strain K12) OX=83333 GN=malP PE=1 SV=7	sp|P00490|PHSM_EC(	91 kDa	13
RNA polymerase sigma factor RpoD OS=Escherichia coli (strain K12) OX=83333 GN=rpoD PE=	sp|P00579|RPOD_EC(	70 kDa	5
Beta-galactosidase OS=Escherichia coli (strain K12) OX=83333 GN=lacZ PE=1 SV=2	sp|P00722|BGAL_ECC	116 kDa	5
30S ribosomal protein S6 OS=Escherichia coli (strain K12) OX=83333 GN=rpsF PE=1 SV=1	sp|P02358|RS6_ECOL	16 kDa	9

Figure S2. A) Native PAGE gel analysis of Nat_Encs and Dps_Encs stained with Coomassie Blue to visualize protein. Minor higher molecular weight bands for each Nat_Enc and Dps_Enc that were subjected to mass spectrometry identification are indicated by black boxes. B) Mass spectrometry results showing that all higher molecular weight bands represent the respective Nat_Encs or Dps_Encs. Only the top 5 most prevalent protein species are shown. Nat_Encs and Dps_Encs sequences are highlighted in grey.

	Dps_TmT1	Dps_MxT1	Dps_MxT3	Dps_QtT4
Change in luminal charges upon Dps_N fusion	+180	+180	+540	+720
Change in luminal surface charge density upon Dps_N fusion(nm^{-2})	+0.177	+0.398	+0.254	+0.177

Figure S3. Table showing the change in luminal charge and approximate luminal surface charge density upon Dps-N fusion in engineered Dps_Encs. Encapsulin shell thickness was assumed to be 3 nm. Interior surface area was calculated as follows: $4\pi r^2$ with r = shell radius – 3 nm, e.g., Tm: r = 12 nm – 3 nm = 9 nm. To obtain approximate luminal charge densities, we assumed the native luminal charge to be approximately neutral (or at least negligible, compared to the charge increase caused by Dps-N). Approximate luminal charge was calculated as follows: 3 x # of protomers / interior surface area.

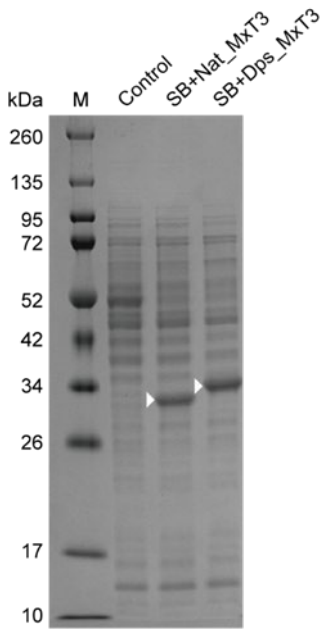
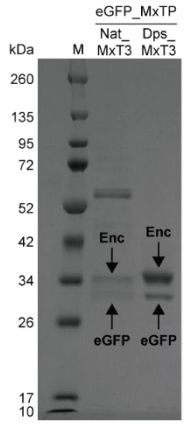


Figure S4. SDS-PAGE analysis of cell lysates from the control *E.coli* BL21(DE3) strain without any transformed plasmid, SB+Nat_MxT3, and SB+Dps_MxT3, showing that Nat_MxT3 and Dps_MxT3 are properly expressed at comparable levels. Bands corresponding to Nat_MxT3 and Dps_MxT3 are indicated by white arrows.

A



B

	eGFP_MxTP_Nat_MxT3	eGFP_MxTP_Dps_MxT3
eGFP_MxTP to capsid protomer band intensity ratio	0.22	0.20

Figure S5. Comparison of cargo-loading capacity of Nat_MxT3 and Dps_MxT3. A) SDS-PAGE analysis of purified eGFP-loaded Nat_MxT3 and Dps_MxT3 stained with Coomassie. B) Table showing the eGFP_MxTP to shell protomer band intensity ratio based on gel densitometry.

Table S1. Protein sequences of all proteins used in this study.

Construct	Protein sequence
Nat_TmT1	MEFLKRSFAPLTEKQWQEIDNRAREIFKTQLYGRKFVDVEGPYGWEYAAHPLGEVEVLSDENEV VKWGLRKSLPLIELRATFTLDLWELDNLERGKPNVDLSSLEETVRKVAEFEDEVIFRGCEKSGVK GLLSFEERKIECGSTPKDLLEAIVRALSIFSKDGIEGPYTLVINTDRWINFLKEEAGHYPLEKRVEE CLRGGKIITTPRIEDALVVSERGGDFKLILGQDLSIGYEDREKDAVRLFITETFTFQVVNPEALILLK F
Nat_MxT1/3	MPDFLGHAENPLREEEWARLNETVIQVARRSLVGRRILDIYGPLGAGVQTVPYDEFQGVSPGAV DIVGEQETAMVFTDARKFKTIPIIYKDFLLHWRDIEAARTHNMPLDVSAAAGAAALCAQQEDELIF YGDARLGYEGLMTANGRLTVPLGDWTSPGGGFQAIVEATRKLNEQGHFGPYAVVLSPRLYSQL HRIYEKTGVLEIETIRQLASDGVYQSNRLRGESGVVVSTGRENMDLAVSMDMVAAYLGASRMN HPFRVLEALLLRIKHPDAICTLEGAGATERR
Nat_QtT4	MNKSQLYPDSPLTDQDFNQLDQTVIEAARRQLVGRRFIELYGPLGRGMQSVFNDIFMESHEAKM DFQGSFDTEVESSRRVNYTIPMLYKDFVLYWRDLEQSKALDIPIDFSVAANAARDVAFLEDQMIF HGSKEFDIPGLMNVKGRLTHLIGNWYESGNAFQDIVEARNKLLEMNHNGPYALVLSPELYSLLH RVHKDTNVLEIEHVRELITAGVFQSPVLKGKSGVIVNTGRNNLDLAISEDFETAYLGEEGMNHPF RVYETVVLRIKRPAAICTLIDPEE
Dps_TmT1	MSTAKLVKSKATNGGSGGSEFLKRSFAPLTEKQWQEIDNRAREIFKTQLYGRKFVDVEGPYGW EYAAHPLGEVEVLSDENEVVKWGLRKSLPLIELRATFTLDLWELDNLERGKPNVDLSSLEETVRK VAEFEDEVIFRGCEKSGVKGLLSFEERKIECGSTPKDLLEAIVRALSIFSKDGIEGPYTLVINTDRW INFLKEEAGHYPLEKRVEECLRGGKIITTPRIEDALVVSERGGDFKLILGQDLSIGYEDREKDAVRL FITETFTFQVVNPEALILLKF
Dps_MxT1/3	MSTAKLVKSKATNGGSGGSPDFLGHAENPLREEEWARLNETVIQVARRSLVGRRILDIYGPLGA GVQTVPYDEFQGVSPGAVDIVGEQETAMVFTDARKFKTIPIIYKDFLLHWRDIEAARTHNMPLDV SAAAGAAALCAQQEDELIFYGDARLGYEGLMTANGRLTVPLGDWTSPGGGFQAIVEATRKLNE QGHFGPYAVVLSPRLYSQLHRIYEKTGVLEIETIRQLASDGVYQSNRLRGESGVVVSTGRENMD LAVSMDMVAAYLGASRMNHPFRVLEALLLRIKHPDAICTLEGAGATERR
Dps_QtT4	MSTAKLVKSKATNGGSGGSNKSQLYPDSPLTDQDFNQLDQTVIEAARRQLVGRRFIELYGPLGR GMQSVFNDIFMESHEAKMDFQGSFDTEVESSRRVNYTIPMLYKDFVLYWRDLEQSKALDIPIDF SVAANAARDVAFLEDQMIFHGSKEFDIPGLMNVKGRLTHLIGNWYESGNAFQDIVEARNKLLEM NHNGPYALVLSPELYSLLHRVHKDTNVLEIEHVRELITAGVFQSPVLKGKSGVIVNTGRNNLDLAI SEDFETAYLGEEGMNHPFRVYETVVLRIKRPAAICTLIDPEE
eGFP_MxTP	MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTL TYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGP VLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKGGSGGSPEKRLTVGSLRR

Table S2. DNA sequences of primer and Split_Broccoli used in this study.

	DNA sequence
Dps_TmT1 overhang primer_F	AAGTATAAGAAGGAGATATACAATGTCTACCGCCAAGCTCGTAAAAAGTAAAGCAACGAACG GTGGTAGCGGTGGCTCCGAATTCTTAAAGCGCAGTTTTGC
Dps_TmT1 primer_R	GCAGCAGCCTAGGTTAATTC
Dps_MxT1/3 overhang primer_F	AAGTATAAGAAGGAGATATACAATGAGCACGGCAAAATTGGTGAAATCGAAAGCAACGAACG GTGGTAGTGGTGGCAGCCCGGACTTTCTGGGGCATG
Dps_MxT1/3 primer_R	GCAGCAGCCTAGGTTAATTCAG
Dps_QtT4 overhang primer_F	AAGTATAAGAAGGAGATATACAATGTCAACCGCGAAGTTGGTCAAGTCAAAAGCTACTAACG GTGGGTCAGGGGGCTCAAATAAGAGTCAACTGTATCCGG
Dps_QtT4 primer_R	GCAGCAGCCTAGGTTAATTCAC
Split_Broccoli	TAATACGACTCACTATAGGATGATGGAGACGGTCGGGTCCAGGATCATTCATGGCAAGAGA CGGTCGGGTCCAGATGATGCGGATCAAAGCCCCCGAAAGGCGGGCTTTTTTTTAAGCTTGC ATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACTGGCCGTCGT TTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATC CCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTT GCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGT ATTTCACACCGCATATGTAATACGACTCACTATAGGATCCGCATCATCTGTCGAGTAGAGTGT GGGCTCTTGCCATGTGTATGTGGGTCAACCCACATACTCTGATGATCCTGTCGAGTAGAGTG TGGGCTCCATCATCCTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG Green : T7 promoter Blue : Split_Broccoli_TOP Red : T500 terminator Orange : spacer Purple : Split_Broccoli_Bottom Pink : T7 terminator