Supplemental Information (SI)

This material is available free of charge via the internet at http://pubs.acs.org.

1. Materials and methods

1.1. Isolation of mitochondria and nuclei from liver tissues

A mitochondria-enriched fraction was isolated from a homogenate by differential centrifugation, as described in a previous report [1-2]. A 500 μL of liver homogenate suspension was transferred to ice-cold tubes containing 500 μL of mitochondrial isolation buffer [250mM sucrose, 2 mM Tris–HCl, 1 mM EDTA, pH 7.4], and then centrifuged at 800*g* for 5 min at 4°C to obtain the pellet for nuclei isolation ($Pellet_{nu}$). A 500 μL aliquot of the supernatant was transferred to an ice-cold tube containing 500 μL of MIB, and then centrifuged at 800*g* for 5 min at 4°C. A 500 μL aliquot of the supernatant was transferred to an ice-cold tube containing 500 μL of MIB, and the suspensions were centrifuged at 7,500*g* for 10 min at 4°C. The pellets were washed with EDTA-free MIB, and the pellets were resuspended in EDTA-free mitochondrial isolation buffer [250mM sucrose, 2 mM Tris–HCl, pH 7.4] and the resulting suspensions were used as the mitochondria-enriched fraction.

A nuclei-enriched fraction was obtained from the $Pellet_{nu}$ by differential centrifugation, referred to the previous report [3-4]. A 1 mL aliquot of phosphate buffered saline (pH 7.4) was added to an ice-cold tube containing $Pellet_{nu}$, and then resuspended. The resulting suspension was centrifuged at 2,000*g* for 5 min at 4°C and the pellets were washed with nuclear isolation buffer (10 mM Tris-HCl, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% IGEPAL CA 630, pH7.4) twice, and the pellets were resuspended in nuclear isolation buffer and the resulting suspensions were used as the nuclei-enriched fraction.

1.2. Quantification of DNA by q-PCR

q-PCR was performed using a 7500 Real Time PCR system (Applied Biosystems) with the following parameters: an initial denaturation at 95ºC for 10 min, followed by 40 cycles at 95ºC for 15 sec and 60ºC for 1 min. SYBR Green qPCR Mastermix (TOYOBO, Osaka, Japan) was used to indicate target gene amplification and the specificity of amplification was examined by automated dissociation stage analysis. A portion of the extracted DNA was analyzed for pDNA3.1, mtDNA or nDNA, using primers pDNA3.1 (+) and pDNA3.1 (-); primers M D-loop (+) and M D-loop (-); primers M Gapdh (+) and M Gapdh (-) (**Table S1**). The copy number of pDNA, mtDNA or nDNA was absolutely quantified using real time PCR. To quantify the mtDNA and nDNA, a standard curve was prepared using mtDNA and nDNA obtained from liver.

1.3. In vitro transcription assay using nuclear extract

Transcription efficiencies of pHSP-ND4FLAG, pCMV-ND4FLAG and pND4FLAG in nuclear conditions were evaluated using HeLaScribe Nuclear Extract *in vitro* Transcription System (Promega). The sequence information for these plasmids is summarized in Figure S1 A. The pCMV-ND4FLAG contains the cytomegalovirus (CMV) promoter as a positive control, and pND4FLAG without a promoter was used as a negative control. It is well known that the CMV promoter achieves an effective transcription in HeLaScribe Nuclear Extract. A 10 ng of sample template DNA (pHSP-ND4FLAG, pCMV-ND4FLAG or pND4FLAG) and 10 ng of standard control template DNA containing CMV promoter and firefly luciferase gene (pDNA3.1) were incubated with Nuclear Extract (8 U) at 30°C for 1 hr, in a reaction mixture (25 μL) containing HeLa Nuclear Extract Buffer, 3.0 mM $MgCl_2$ and the four ribonucleoside 5′- triphosphates (1 mM each). The reaction was terminated by the addition of HeLa Nuclear Extract Stop Mix, and the transcribed RNA was purified by ethanol precipitation, combined with DNase I digestion for the degradation of DNA in crude RNA samples using TURBO DNase treatment and removal reagents (Ambion by Life Technologies, Carlsbad, CA, USA), according to the manufacture's protocol.

The resulting RNA suspension was reverse transcribed using a High Capacity RNA-to-cDNA kit (Applied Biosystems) according to the manufacturer's protocol. A q-PCR was performed using a 7500 Real

Time PCR system and SYBR Green qPCR Mastermix as described above. The copy number of DNA derived from mRNAs coded in sample template DNA (pHSP-ND4FLAG, pCMV-ND4FLAG or pND4FLAG) and standard control template DNA (pDNA3.1) was absolutely quantified using real time PCR, using primers Nd4flagII (+) and Nd4flag (-); primers pDNA3.1 (+) and pDNA3.1 (-) (**Table S1**). Transcription efficiency value was calculated as follows;

$$\text{Transcription efficiency value} = \text{Copy of mRNA}_{\text{sample}} / \text{Copy of mRNA}_{\text{standard}}$$

where Copy of $\text{mRNA}_{\text{sample}}$ and Copy of $\text{mRNA}_{\text{standard}}$ represent copy number of DNA derived from mRNA coded in the sample template DNA (pHSP-ND4FLAG, pCMV-ND4FLAG or pND4FLAG) and the standard control template DNA (pDNA3.1)

Supplementary Table

Table S1 Primers used in this study.

Primers	Nucleotide sequence	Target gene
pDNA3.1 (+)	5'-GGTCCTATGATTATGTCCGGTTATG-3'	pDNA3.1 (exogenous gene)
pDNA3.1 (-)	5'-ATGTAGCCATCCATCCTTGTCAAT-3'	
M D-loop (+)	5'-CTGCCAATAGAACTTCCAATCCGTATAT-3'	D-loop region of mice mtDNA (endogenous gene)
M D-loop (-)	5'-AAGGTTAACGCTCTTAGCTTCATAGTGAAA-3'	
M Gapdh (+)	5'-TTGTGATGGGTGTGAACCAC-3'	Gapdh region of mice nDNA (endogenous gene)
M Gapdh (-)	5'-GTTGTCATGGATGACCTTGG-3'	
Nd4flag (+)	5'-TACCAAAAGCCCATGTTGAAGC-3'	Nd4flag region in pHSP-ND4FLAG, pCMV-ND4FLAG and pND4FLAG (exogenous gene)
Nd4flagII (+)	5'-AACCCTCACACACACGAGAACT-3'	
Nd4flag (-)	5'-CGTCGTCATCCTTGTAATCTATTG-3'	
M Nd6 (+)	5'-GGTTAGCATTAAAGCCTTCACCT-3'	Nd6 region of mice mtDNA (endogenous gene)
M Nd6 (-)	5'-CATCAACCAATCTCCCAAACCAT-3'	
Nluc (+)	5'-CCTTGAACAGGGAGGTGTGT-3'	Nluc luciferease region in pHSP-mtLuc (CGG) and pHSP-mtLuc (TAG) (exogenous gene)
Nluc (-)	5'-TTGATGGTTACTCGGAACAGC-3'	

Oligonucleotides were purchased from Sigma Genosys Japan (Ishikari, Japan) in purified form.

Supplementary Figure

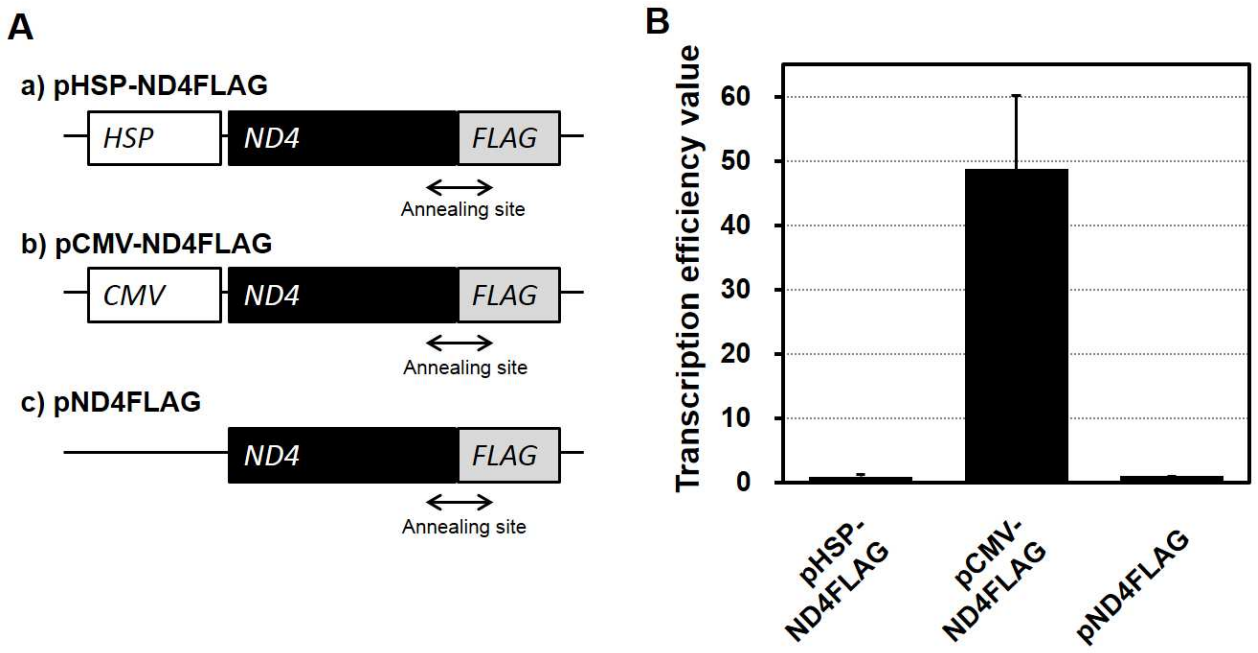

Figure S1 *In vitro* transcription assay in nuclear conditions. Transcription efficiency of pHSP-ND4FLAG, was evaluated using the nuclear extract. In this experiment, we also evaluated the transcription efficiencies of pCMV-ND4FLAG containing cytomegalovirus (CMV) promoter as a positive control and pND4FLAG without promoter as a negative control. It is well known that the CMV promoter is a strong promoter that is frequently used for achieving nuclear transgene expression in mammalian cells. The sequence information of plasmids used in this experiment are summarized in Figure S1 A. The transcription efficiency value are shown in Figure S1 B. Bars represent the mean±SD. (n=3).

Supplementary Vector sequences

The DNA vectors were designed by inserting the DNA fragment gene as shown below (**Sequences S1-S5**) into pBluescript SK (-) vector (Stratagene) between the multi cloning site (*EcoR* I and *Sma* I sites).

Sequence S1 for pHSP-ND4FLAG. The DNA sequence contains HSP promoter [highlighted in green], ND4 gene [highlighted in gray] and the sequence corresponding to FLAG [highlighted in yellow]. The HSP and ND4 were derived from mouse mtDNA corresponding to bases 16,103-16,300 and bases 10,168-11,544 (GenBank: JF286601.1), respectively.

(5' EcoRI) -

ctgcagccctcctcttaatgccaaaccccaaaaacactaagaacttgaaagacatataatattaactatcaaaccctatgtcctgatcaattctagtagttcccaaa
atatgacttatattttagtacttgtaaaaattttacaaaatcatgttccgtgaaccaaaactctaatcatactctattacgcaataaacattaacaaatgctaaaaattat
tcttccctcactaatgctactaccactaacctgactatcaagccctaaaaaaacctgaacaaacgtaacctcatatagttttctaattagtttaaccagcctaacact
tctatgacaaaccgacgaaaattataaaaacttttcaaatatattctcctcagaccccctatccacaccattaattattttaacagcctgattactgccactaatattaa
tagctagccaaaaccacctaaaaaaagataataacgtactacaaaaactctacatctcaatactaatcagcttacaaattctcctaatcataaccttttcagcaact
gaactaattatattttatattttatttgaagcaaccttaatcccaacacttattattattacccgatgagggaaccaaactgaacgcctaaacgcagggatttatttcct
attttataccctaatcggttctattccactgctaattgccctcatcttaatccaaaaccatgtaggaaccctaaacctcataattttatcattcacaacacacaccttag
acgcttcatgatctaacaacttactatggttggcatgcataatagcatttcttattaaaataccattatatggagttcacctatgactaccaaaagcccatgttgaag
ctccaattgctgggtcaataattctagcagctattcttctaaaattaggtagttacggaataattcgcatctccattattctagacccactaacaaaatatatagcata
ccccttcatccttctctccctatgaggaataattataactagctcaatctgcttacgccaaacagatttaaaatcactaatcgcctactcctcagttagccacatagc
acttgttattgcatcaatcataatccaaactccatgaagcttcataggagcaacaatactaataatcgcacatggcctcacatcatcactcctattctgcctagcaa
actccaactacgaacggatccacagccgtactataatcatggcccgaggacttcaaatggtcttcccacttatagccacatgatgactgatagcaagtctagct
aatctagctctaccccccttcaatcaatctaataggagaattattcattaccatatcattattttcttgatcaaactttaccattattcttataggaattaacattattattaca
ggtatatactcaatatacataattattaccacccaacgcggcaaactaaccaaccatataattaacctccaaccctcacacacacgagaactaacactaatagcc
cttcacataattccacttattcttctaactaccaatccaaaactaattacaggcctgacaatagattacaaggatgacgacgataagtaactgcag - *(3' Sma I)*

Sequence S2 for pCMV-ND4FLAG. The DNA sequence contains CMV promoter [highlighted in green], ND4 gene [highlighted in gray] and the sequence corresponding to FLAG [highlighted in yellow]. The ND4 were derived from mouse mtDNA corresponding to bases 10,168-11,544 (GenBank: JF286601.1). The CMV promoter were derived from pTriEx-3 Neo Vector ((Madison, WI, USA) to bases 1,021-1,596.

(5' EcoRI) -

ctgcagattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcctggctgaccgccca
acgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgccc
acttggcagtacatcaagtgtatcatatgccaagtccgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttacgg
gactttcctacttggcagtacatctacgtattagtcatcgctattaccatgctgatgcggttttggcagtacaccaatgggcgtggatagcggtttgactcacggg
gatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaataaccccgccccgttgacgcaaa
tgggcggtaggcgtgtacggtgggaggtctatataagcagacgtcgtttagtgaaccatgctaaaaattattcttccctcactaatgctactaccactaacctga
ctatcaagccctaaaaaaacctgaacaaacgtaacctcatatagttttctaattagtttaaccagcctaacacttctatgacaaaccgacgaaaattataaaaactt
ttcaaatatattctcctcagaccccctatccacaccattaattattttaacagcctgattactgccactaatattaatagctagccaaaaccacctaaaaaaagataa
taacgtactacaaaaactctacatctcaatactaatcagcttacaaattctcctaatcataacctttcagcaactgaactaattatattttatattttatttgaagcaacc
ttaatcccaacacttattattattacccgatgagggaaccaaactgaacgcctaaacgcagggatttatttcctattttataccctaatcggttctattccactgctaa
ttgccctcatcttaatccaaaaccatgtaggaaccctaaacctcataattttatcattcacaacacacaccttagacgcttcatgatctaacaacttactatggttgg
catgcataatagcatttcttattaaaataccattatatggagttcacctatgactaccaaaagcccatgttgaagctccaattgctgggtcaataattctagcagcta
ttcttctaaaattaggtagttacggaataattcgcatctccattattctagacccactaacaaaatatatagcatacccccttcatccttctctccctatgaggaataatt
ataactagctcaatctgcttacgccaaacagatttaaaatcactaatcgcctactcctcagttagccacatagcacttgttattgcatcaatcataatccaaactcca
tgaagcttcataggagcaacaatactaataatcgcacatggcctcacatcatcactcctattctgcctagcaaactccaactacgaacggatccacagccgtac
tataatcatggcccgaggacttcaaatggtcttcccacttatagccacatgatgactgatagcaagtctagctaatctagctctaccccccttcaatcaatctaatag
gagaattattcattaccatatcattattttcttgatcaaactttaccattattcttataggaattaacattattattacaggtatatactcaatatacataattattaccaccc
aacgcggcaaactaaccaaccatataattaacctccaaccctcacacacacgagaactaacactaatagcccttcacataattccacttattcttctaactaccaa
tccaaaactaattacaggcctgacaatagattacaaggatgacgacgataagtaactgcag - *(3' Sma I)*

Sequence S3 for pND4FLAG. The DNA sequence contains ND4 gene [highlighted in gray] and the sequence corresponding to FLAG [highlighted in yellow]. The ND4 were derived from mouse mtDNA corresponding to bases 10,168-11,544 (GenBank: JF286601.1).

(5' EcoRI) -

ctgcagatgctaaaaattattcttccctcactaatgctactaccactaacctgactatcaagccctaaaaaaacctgaacaaacgtaacctcatatagttttctaatt
agtttaaccagcctaacacttctatgacaaaccgacgaaaattataaaaacttttcaaatatattctcctcagacccccctatccacaccattaattattttaacagcct
gattactgccactaatattaatagctagccaaaaccacctaaaaaaagataataacgtactacaaaaactctacatctcaatactaatcagcttacaaattctccta
atcataaccttttcagcaactgaactaattatattttatattttatttgaagcaaccttaatcccaacacttattattattacccgatgagggaaccaaactgaacgcct
aaacgcagggatttatttcctattttataccctaatcggttctattccactgctaattgccctcatcttaatccaaaaccatgtaggaaccctaaacctcataattttatc
attcacaacacacaccttagacgcttcatgatctaacaacttactatggttggcatgcataatagcatttcttattaaaataccattatatggagttcacctatgacta
ccaaaagcccatgttgaagctccaattgctgggtcaataattctagcagctattcttctaaaattaggtagttacggaataattcgcatctccattattctagaccca
ctaacaaaatatatagcatacccccttcatccttctctccctatgaggaataattataactagctcaatctgcttacgccaaacagatttaaaatcactaatcgcctact
cctcagttagccacatagcacttgttattgcatcaatcataatccaaactccatgaagcttcataggagcaacaatactaataatcgcacatggcctcacatcatc
actcctattctgcctagcaaactccaactacgaacggatccacagccgtactataatcatggcccgaggacttcaaatggtcttcccacttatagccacatgatg
actgatagcaagtctagctaatctagctctaccccccttcaatcaatctaataggagaattattcattaccatatcattattttcttgatcaaactttaccattattcttata
ggaattaacattattattacaggtatatactcaatatacataattattaccacccaacgcggcaaactaaccaaccatataattaacctccaaccctcacacacac
gagaactaacactaatagcccttcacataattccacttattcttctaactaccaatccaaaactaattacaggcctgacaatagattacaaggatgacgacgataa
gtaactgcag - *(3' Sma I)*

Sequence S4 for pHSP-mtLuc(CGG). The DNA sequence contains HSP promoter [highlighted in green], ND4 gene [highlighted in gray], the sequence corresponding to FLAG [highlighted in yellow], mtLuc (CGG) gene [highlighted in aqua] and $tRNA^{Asp}$ gene with the 5'UTR [highlighted in pink]. The HSP, ND4 gene, $tRNA^{Asp}$ gene and the 5'UTR of the $tRNA^{Asp}$ gene were derived from mouse mtDNA corresponding to bases 16,103-16,300, bases 10,168-11,544, bases 6,942-7,011 and bases 6,873-6,941 (GenBank: JF286601.1), respectively. The mtLuc (CGG) gene was synthesized by insertion of point mutation by UPPER CASE into Nanoluc luciferase gene of pNL1.1[Nluc] corresponding to bases 100-615 (GenBank: JQ437370.1).

(5' EcoRI) -

ctgcagccctcctcttaatgccaaaccccaaaaacactaagaacttgaaagacatataatattaactatcaaaccctatgtcctgatcaattctagtagttcccaaa
atatgacttatattttagtacttgtaaaaattttacaaaatcatgttccgtgaaccaaaactctaatcatactctattacgcaataaacattaacaaatgctaaaaattat
tcttccctcactaatgctactaccactaacctgactatcaagccctaaaaaaacctgaacaaacgtaacctcatatagttttctaattagtttaaccagcctaacact
tctatgacaaaccgacgaaaattataaaaacttttcaaatatattctcctcagaccccctatccacaccattaattattttaacagcctgattactgccactaatattaa
tagctagccaaaaccacctaaaaaaagataataacgtactacaaaaactctacatctcaatactaatcagcttacaaattctcctaatcataaccttttcagcaact
gaactaattatattttatattttatttgaagcaaccttaatcccaacacttattattattacccgatgagggaaccaaactgaacgcctaaacgcagggatttatttcct
attttataccctaatcggttctattccactgctaattgccctcatcttaatccaaaaccatgtaggaaccctaaacctcataattttatcattcacaacacacaccttag
acgcttcatgatctaacaacttactatggttggcatgcataatagcatttcttattaaaataccattatatggagttcacctatgactaccaaaagcccatgttgaag
ctccaattgctgggtcaataattctagcagctattcttctaaaattaggtagttacggaataattcgcatctccattattctagacccactaacaaaatatatagcata
ccccttcatccttctctccctatgaggaataattataactagctcaatctgcttacgccaaacagatttaaaatcactaatcgcctactcctcagttagccacatagc
acttgttattgcatcaatcataatccaaactccatgaagcttcataggagcaacaatactaataatcgcacatggcctcacatcatcactcctattctgcctagcaa
actccaactacgaacggatccacagccgtactataatcatggcccgaggacttcaaatggtcttcccacttatagccacatgatgactgatagcaagtctagct
aatctagctctaccccccttcaatcaatctaataggagaattattcattaccatatcattattttcttgatcaaactttaccattattcttataggaattaacattattattaca
ggtatatactcaatatacataattattaccacccaacgcggcaaactaaccaaccatataattaacctccaaccctcacacacacgagaactaacactaatagcc
cttcacataattccacttattcttctaactaccaatccaaaactaattacaggcctgacaatagattacaaggatgacgacgataagatggtcttcacactcgaag
atttcgttggggactg*A*cgacagacagccggctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgtttcagaatctcggggtgtccgtaac
tccgatccaa*C*ggattgtcctgagcggtgaaaatgggctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcggcgaccaaatgggccaga
tcgaaaaaatttttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgcactatggcacactggtaatcgacggggttacgccgaacatgatcg
actatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgc
ctgatcaaccccgacggctccctgctgttccgagtaaccatcaacggagtgaccggctggcggctgtgcgaacgcattctggcgtaagaaaggaaggaatc
gaaccccctaaaattggtttcaagccaatctcatatcctatatgtctttctcaataagatattagtaaaatcaattacataactttgtcaaagttaaattatagatcaata
atctatatatcttatctgcag - *(3' Sma I)*

Sequence S5 for pHSP-mtLuc(TAG). The DNA sequence contains HSP promoter [highlighted in green], ND4 gene [highlighted in gray], the sequence corresponding to FLAG [highlighted in yellow], mtLuc (TAG) gene [highlighted in aqua] and $tRNA^{Asp}$ gene with the 5'UTR [highlighted in pink]. The HSP, ND4 gene, $tRNA^{Asp}$ gene and the 5'UTR of the $tRNA^{Asp}$ gene were derived from mouse mtDNA corresponding to bases 16,103-16,300, bases 10,168-11,544, bases 6,942-7,011 and bases 6,873-6,941 (GenBank: JF286601.1), respectively. The mtLuc (TAG) gene was synthesized by insertion of point mutation indicated by UPPER CASE into Nanoluc luciferase gene of pNL1.1[Nluc] corresponding to bases 100-615 (GenBank: JQ437370.1). The sequences (**bold**) indicate muted DNA fragment of pHSP-mtLuc (CGG) amplified by PCR as described in Experimental section of main text.

(5' EcoRI) -

ctgcagccctcctcttaatgccaaaccccaaaaacactaagaacttgaaagacatataatattaactatcaaaccctatgtcctgatcaattctagtagttcccaaa
atatgacttatattttagtacttgtaaaaattttacaaaatcatgttccgtgaaccaaaactctaatcatactctattacgcaataaacattaacaaatgctaaaaattat
tcttccctcactaatgctactaccactaacctgactatcaagccctaaaaaaacctgaacaaacgtaacctcatatagttttctaattagtttaaccagcctaacact
tctatgacaaaccgacgaaaattataaaaacttttcaaatatattctcctcagaccccctatccacaccattaattattttaacagcctgattactgccactaatattaa
tagctagccaaaaccacctaaaaaaagataataacgtactacaaaaactctacatctcaatactaatcagcttacaaattctcctaatcataaccttttcagcaact
gaactaattatattttatattttatttgaagcaaccttaatcccaacacttattattattacccgatgagggaaccaaactgaacgcctaaacgcagggatttatttcct
attttataccctaatcggttctattccactgctaattgccctcatcttaatccaaaaccatgtaggaaccctaaacctcataattttatcattcacaacacacaccttag
acgcttcatgatctaacaacttactatggttggcatgcataatagcatttcttattaaaataccattatatggagttcacctatgactaccaaaagcccatgttgaag
ctccaattgctgggtcaataattctagcagctattcttctaaaattaggtagttacggaataattcgcatctccattattctagacccactaacaaaatatatagcata
ccccttcatccttctctccctatgaggaataattataactagctcaatctgcttacgccaaacagatttaaaatcactaatcgcctactcctcagttagccacatagc
acttgttattgcatcaatcataatccaaactccatgaagcttcataggagcaacaatactaataatcgcacatggcctcacatcatcactcctattctgcctagcaa
actccaactacgaacggatccacagccgtactataatcatggcccgaggacttcaaatggtcttcccacttatagccacatgatgactgatagcaagtctagct
aatctagctctaccccttcaatcaatctaataggagaattattcattaccatatcattattttcttgatcaaactttaccattattcttataggaattaacattattattaca
ggtatatactcaatatacataattattaccacccaacgcggcaaactaaccaaccatataattaacctccaaccctcacacacacgagaactaacactaatagcc
cttcacataattccacttattcttctaactaccaatccaaaactaattacaggcctgacaatagattacaaggatgacgacgataagatggtcttcacactcgaag
atttcgttggggactgAcgacagacagccggctacaacctggacc**aagtccttgaacagggaggtgtgtccagtttgtttcagaatctcggggtgtccg**
taactccgatccaaTAgattgtcctgagcggtgaaaatgggctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcggcgaccaaatggg
ccagatcgaaaaaatttttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgcactatggcacactggtaatcgacggggttacgccgaacat
gatcgactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacg
agcgcctgatcaaccccgacggctccctgctgttccgagtaaccatcaacggagtgaccggctggcggctgtgcgaacgcattctggcgtaagaaaggaa
ggaatcgaaccccctaaaattggtttcaagccaatctcatatcctatatgtctttctcaataagatattagtaaaatcaattacataactttgtcaaagttaaattatag
atcaataatctatatatcttatctgcag - *(3' Sma I)*

References

(1) Shinohara, Y.; Almofti, M. R.; Yamamoto, T.; Ishida, T.; Kita, F.; Kanzaki, H.; Ohnishi, M.; Yamashita, K.; Shimizu, S.; Terada, H. Permeability transition-independent release of mitochondrial cytochrome c induced by valinomycin. *Eur J Biochem* **2002,** *269*, 5224-30.

(2) Yamada, Y.; Akita, H.; Kamiya, H.; Kogure, K.; Yamamoto, T.; Shinohara, Y.; Yamashita, K.; Kobayashi, H.; Kikuchi, H.; Harashima, H. MITO-Porter: A liposome-based carrier system for delivery of macromolecules into mitochondria via membrane fusion. *Biochimica et biophysica acta* **2008,** *1778*, 423-32.

(3) Yamada, Y.; Nomura, T.; Harashima, H.; Yamashita, A.; Yui, N. Post-nuclear gene delivery events for transgene expression by biocleavable polyrotaxanes. *Biomaterials* **2012,** *33*, 3952-8.

(4) Hama, S.; Akita, H.; Ito, R.; Mizuguchi, H.; Hayakawa, T.; Harashima, H. Quantitative comparison of intracellular trafficking and nuclear transcription between adenoviral and lipoplex systems. *Mol Ther* **2006,** *13*, 786-94.