Supporting Information

A modular vector toolkit with a tailored set of thermosensors to regulate gene expression in *Thermus thermophilus*.

Carlos Verdú, Esther Sanchez, Carmen Ortega, Aurelio Hidalgo, José Berenguer, Mario Mencía*.*

AUTHOR ADDRESS. Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid – Consejo Superior de Investigaciones Científicas, 28049 Madrid, Spain

Table S1. Strain list

Strain	Genotype	Phenotype/use
E. coli DH5α	F^- *endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1* Δ(*argF-lacZYA*)*U169* Φ80d*lac*ΔM15	Cloning Promoter assay
Thermus thermophilus HB27	ATCC BAA-163/DSM7039	Wild type
Thermus thermophilus HB27ΔAgo	Deletion of *argonaute* [1]	Enhanced competence

Table S2. Plasmid list

Plasmid	Description	Use	Reference
pEM2S (pMK18)	plasmid with the Tth minimal replication origin from pMY1	Source of replication origin	[2]
pMKnqosIFP	pMK derivative with IFP expression driven by Pnqo	IFP expression test	[3]
pTT8	Endogenous plasmid of Tth HB8.	Source of the pTT8 replication origin	[4]
pSEVA1311	pSEVA plasmid with the broad host range pBBR1 replication origin	Source of the pBBR1 E.c. replication origin	[5]
pMoTK110	pMY Tth origin, Kanamycin, pUC *E.coli* origin, Pnqo driving IFP gene (NQO)	Basis for pMoT series	This work
pMoTK100	pMoTK110 with linker A cloned between NheI and HindIII	pMoTK cloning	This work

pMoTK111	pMoTK110 with linker 1 cloned between NdeI and HindIII	pMoTK Pnqo library cloning	This work
pMoTK112	pMoTK110 with linker 2 cloned between NdeI and HindIII	pMoTK Pnqo library cloning	This work
pMoTK113	pMoTK110 with linker 3 cloned between NdeI and HindIII	pMoTK Pnqo library cloning	This work
pMoTK115	pMoTK110, with lac promoter driving IFP (LAC)	Plac test	This work
pMoTK116	pMoTK110 cut with NheI and HindIII and filled-in and religated to eliminate Pnqo-IFP sequence (pMoTK)	⍰ Pnqo-IFP control	This work
pMoTK117	pMoTK110 cut with NheI and EcoRI filled-in and religated to delete Pnqo	⍰ Pnqo control	This work
pMoTK118	pMoTK110, with 16S rRNA promoter driving IFP (16S)	P16S test	This work
pMoTK119	pMoTK110, with *Haloferax volcanii* promoter driving IFP (3K)	3K test	This work
pMoTK1103A	pMoTK110, with 3A variant of Pnqo driving IFP (K3A)	Pnqo 3A test	This work
pMoTB1103A	pMoTK1103A, with bleomycin resistance casette cloned between PacI and AscI	pMoT with bleomycin expression test	This work
pMoTH1103A	pMoTK1103A, with hygromycin resistance casette cloned between PacI and AscI	pMoT with hygromycin expression test	This work
pMoTK2103A	pMoTK1103A, with pTT8 replication origin cloned between PstI and AatII (Ori2-3A)	Origin pTT8 expression test	This work
pMoTK120	pMoTK110 with nqo91 variant of Pnqo	Pnqo91assa y	This work
pMoTK121	pMoTK110 with nqo72 variant of Pnqo	Pnqo72 assay	This work
pMoTK122	pMoTK110 with nqo57 variant of Pnqo	Pnqo57 assay	This work

pMoTK123	pMoTK110 with nqo33 variant of Pnqo	Pnqo33 assay	This work
pMoTK124	pMoTK110 with nqo91-35 variant of Pnqo	nqo91-35 assay	This work
pMoTK125	pMoTK110 with nqo91 E-10 variant of Pnqo	nqo91 E-10 assay	This work
pMoTK126	pMoTK110 with nqo91-10 variant of Pnqo	nqo91-10 assay	This work
pMoTK127	pMoTK110 with nqo91-7 variant of Pnqo	nqo91-7 assay	This work
pMoTK128	pMoTK110 with nqo72 E-10 variant of Pnqo	nqo72 E-10 assay	This work
pMoTK129	pMoTK110 with nqo72 -10 variant of Pnqo	nqo72 -10 assay	This work
pMoTK130	pMoTK110 with nqo72-7 variant of Pnqo	nqo72-7 assay	This work
pMoTK140	pMoTK110 with 3Knqo72 variant of Pnqo	3Knqo72 assay	This work
pMoTH100	pMoTH1103A with linker A cloned between NheI and HindIII	pMoTH cloning	This work
pMoTH150	pMoTH with IFP expressed cotranscriptionally with the hygromycin resistance gene. DF, direct fusion, control construct without thermosensor. See Sequence File	DF construct IFP assay	This work
pMoTH151	pMoTH150 with polyA stretch cloned between XbaI and NdeI	polyA construct IFP assay	This work
pMoTH152	pMoTH150 with thermosensor TS0 cloned between XbaI and NdeI	thermosen-sor TS0 assay	This work
pMoTH153	pMoTH150 with thermosensor TS10 cloned between XbaI and NdeI	thermosen-sor TS10 assay	This work
pMoTH154	pMoTH150 with thermosensor TS8 cloned between XbaI and NdeI	thermosen-sor TS8 assay	This work

pMoTH155	pMoTH150 with thermosensor TSP cloned between XbaI and NdeI	thermosensor TSP assay	This work
pMoTH156	pMoTH150 with thermosensor TS1 cloned between XbaI and NdeI	thermosensor TS1 assay	This work
pMoTH157	pMoTH150 with thermosensor TS2 cloned between XbaI and NdeI	thermosensor TS2 assay	This work
pMoTH158	pMoTH150 with thermosensor TS4 cloned between XbaI and NdeI	thermosensor TS4 assay	This work
pMoTH159	pMoTH150 with thermosensor TS6 cloned between XbaI and NdeI	thermosensor TS6 assay	This work

Table S3. Oligonucleotides list

Name of oligonucleotide	Sequence	Utilization
EM2SRepAat	TGTTGGCGACGTCCTCAGTTGACC	Primer Fw to amplify TthRep1 from pEM2S with AatII site
EM2SRepPstRev	AAACTGCAGGTTATACTCCCCCCGGG	Primer Fw to amplify TthRep1 from pEM2S with PstI site
PslAPacPst	AAAACTGCAGTTAATTAAAGAAACCTTAAGGCCCGACCG	Primer Fw to amplify PslpA-kanamycin resistance cassette from pMK184 with PstI and PacI sites
KatAscNheRv	GTCGTGCTAGCTACAGGCGCGCCACGTCGTGACTGGGAAAAC	Primer Rev to amplify PslpA-kanamycin resistance cassette from PMK184 with AscI and NheI sites
ColENheHind	CACAGCTAGCTACGAAGCTTAGCCGGAAGCATAAAG	Primer Fw to amplify pUC Ec replication origin with NheI and HindIII sites
ColE1AatNot	CTGACGTCAAGCGGCCGCAGTTTACTCATATATACTTTAGA	Primer Rev to amplify pUC Ec replication origin with AatII and Not sites
TermSLNheFw	[Phos]CTAGTAAAGTTCTAACCCCTGGCGGTCCCGCCCCCGCCCCCTTTGGGGGGGCGGGGGGTTTTTGTTCTCCCG	Primer Fw to generate the SlpA terminator (to NheI site)
TermSLNheRv	[Phos]CTAGCGGGAGAACAAAAACCCCCCGCCCCCCCAAAGGGGGCGGGGGCGGGACCGCCAGGGGTTAGAACTTTA	Primer Rv to generate the SlpA terminator (to NheI site)
PnqoNhe	AAAAGCTAGCCTCCAGGGGCCTTCTTTCC	Primer Fw to amplify promoter nqo with NheI site
PnqoEcoRv	AAACATATGCCCCTCCTTTCGTGCACGAAAGAATTCTTCTTCACGAAAC	Primer Rv to amplify promoter nqo with sites EcoRI and NdeI
sIFPFw	GGGCATATGAGCAAAGGAGAAGAA	Primer Fw to amplify sIFP gene with site NdeI
sIFPRv	CCGCAAGCTTTTATTATTTGTAGAGCTC	Primer Rv to amplify sIFP gene with site HindIII

NdeItoVspIPsI	CATTATTAATCCTCACACCTCCTTAAGGGT	Primer Rv to amplify PlspA with site VspI
NdeHph17	GAGGTGTGAGGCATATGAAAAAGCC	Primer Fw to amplify Hph17 gene with site NdeI
EndHph17	CATAACCTGAAGGAGGCGCGCCATCTCTATTCCT	Primer Rv to amplify Hph17 gene with site AscI
NdeBleo	GGTGTGAGGCATATGGCCAAGT	Primer Fw to amplify Bleomycin resitance gene with site NdeI
EndBleo	CCCGGGGATGGCGCGCCTAGATTAGTC	Primer Rv to amplify Bleomycin resitance gene with site AscI
pTT8Fw	CGCTCACCCTGAGCG	Primer Fw to amplify pTT8 replication origin
pTT8PstRv	AAAAACTGCAGGCTATGGAGGGGTTCTCCCTG	Primer Rv to amplify pTT8 replication origin with site PstI
OripBBRFw	ATAGGTGAAGCTTAGGCCCACCCG	Primer Fw to amplify pBBR replication origin from pSEVA with HindIII site
OripBBRNot	AAAGCGGCCGCCCCCCTACGGGCTT	Primer Rv to amplify pBBR replication origin from pSEVA with Not site
MKsIFPFw	AGAAGAATTCTTTCGTGCACGAAAGGAGGGGC	Primer Fw to amplify the sIFP CDS from pMKnqosIFP with EcoRI site
MKsIFPRv	TGCGGCCGCAAGCTTTTATTATTTGTAG	Primer Rv to amplify the sIFP CDS from pMKnqosIFP with HindIII site
PlacNheFw	GCAGCTAGCACGACAGGTTTCC	Primer Rv to amplify lac promoter from pUC18 with NheI site
PlacEcoRv	TTGTTATGAATTCTCACAATTCCACAC	Primer Rv to amplify lac promoter from pUC18 with EcoRI site
P16SFw	AAAGCTAGCTCTACCGCGAGTTTGACTCGTGGAGCGCTTCATTGAGGAGATC CGCTGCCCTCGCAA	Primer Fw to generate 16S promoter with NheI site
P16SRv	TTTGAATTCAGAAAAGCCATGCTATCAATCCCCCTCCTTTTTGTCAAGGCTTG CGAGGGCAGCGGATCT	Primer Rv to generate 16S promoter with EcoRI site
MoTLinkerFw	[Phos]CTAGCAGATATCATCTAGAAAACATATGAGGTACCAGAATTCACTAG TGGATCCA	Oligonucleotide Fw to generate polylinker (to sites NheI and HindIII)
MoTLinkerRv	[Phos]AGCTTGGATCCACTAGTGAATTCTGGTACCTCATATGTTTTCTAGATG ATATCTGCTAG	Oligonucleotide Rv to generate polylinker (to sites NheI and HindIII)
Linker1Fw	[Phos]TATGGTCGACAAGATATCAGGATCCATCTAGAA	Oligonucleotide Fw to generate Linker1 (to sites NdeI and HindIII)
Linker1Rv	[Phos]AGCTTTCTAGATGGATCCTGATATCTTGTCGACCA	Oligonucleotide Rv to generate Linker1 (to sites NdeI and HindIII)
Linker2Fw	[Phos]TATGGGTCGACAAGATATCAGGATCCATCTAGAA	Oligonucleotide Fw to generate Linker2 (to sites NdeI and HindIII)
Linker2Rv	[Phos]AGCTTTCTAGATGGATCCTGATATCTTGTCGACCCA	Oligonucleotide Rv to generate Linker2 (to sites NdeI and HindIII)
Linker3Fw	[Phos]TATGGGGTCGACAAGATATCAGGATCCATCTAGAA	Oligonucleotide Fw to generate Linker3 (to sites NdeI and HindIII)
Linker3Rv	[Phos]AGCTTTCTAGATGGATCCTGATATCTTGTCGACCCCA	Oligonucleotide Rv to generate Linker3 (to sites NdeI and HindIII)
nqo91	GTAGGCTAGCCCCCTTGCGCC	Primer Fw to generate promoter nqo91
nqo72	TTGCGCGCTAGCCCGGGGTGAT	Primer Fw to generate promoter nqo72
nqo57	GGTGCTAGCATGGGCAGGAAATGA	Primer Fw to generate promoter nqo57

nqo33	AGGCCGGCTAGCTTTGGGGCTTTG	Primer Fw to generate promoter nqo33
3Knqo	CCAACGCAGCACTAGTGACGACGCTGGGT	Primer Rv to fuse promoter 3K to nqo 72 with site SpeI
siFP_Rv	AAAGAATTCTTATTTGTAGAGCTCATCCATGC	Primer Rv to amplify sIFP gene from pMoT with site EcoRI
TS_Parental	AAATCTAGACCTCCTTCAAAAAAGAAGGAGGATATACATATGAGCAAAGGA GAAGAACTTTTCA	Primer Fw to amplify sIFP gene adding thermosensor Parental (sites XbaI, EcoRI)
TS_Var_0	AAATCTAGAACTCCTTCAAAAAAGAAGGAGGATATACATATGAGCAAAGGA GAAGAACTTTTCA	Primer Fw to amplify sIFP gene adding thermosensor ts0
TS_Var_1	AAATCTAGACCTCCTTAACAAAAAAGTTAAGGAGGATATACATATGAGCAAA GGAGAAGAACTTTTCA	Primer Fw to amplify sIFP gene adding thermosensor ts1
TS_Var_2	AAATCTAGACCTCCTTAGCAAAAAAGCTAAGGAGGATATACATATGAGCAA AGGAGAAGAACTTTTCA	Primer Fw to amplify sIFP gene adding thermosensor ts2
TS_Var_4	AAATCTAGACCTCCTTACGCAAAAAAGCGTAAGGAGGATATACATATGAGC AAAGGAGAAGAACTTTTCA	Primer Fw to amplify sIFP gene adding thermosensor ts4
TS_Var_6	AAATCTAGACCTCCTTACGCGCAAAAAAGCGCGTAAGGAGGATATACATAT GAGCAAAGGAGAAGAACTTTTCA	Primer Fw to amplify sIFP gene adding thermosensor ts6
TS_Var_8	AAATCTAGACCTCCTTAAAGCAAAAAAGCTAAGGAGGATATACATATGAGC AAAGGAGAAGAACTTTTCA	Primer Fw to amplify sIFP gene adding thermosensor ts8
TS_Var_10	AAATCTAGACCTCCTTCAAAAAAAAAAAAAAAAAGAAGGAGGATATACATA TGAGCAAAGGAGAAGAACTTT	Primer Fw to amplify sIFP gene adding thermosensor ts10
TS_Var_PolyA	AAATCTAGAAAAAAAAACAAAAAAGAAGGAGGATATACATATGAGCAAAGG AGAAGAACTTTTCA	Primer Fw to amplify sIFP gene adding thermosensor PA
TS_Var_ContC	AAATCTAGAAAGGAGGATATACATATGAGCAAAGGAGAAGAACTTTTCA	Primer Fw to amplify sIFP gene adding thermosensor ContC
Bgal_TS8_Fw	AAATCTAGACCTCCTTAAAGCAAAAAAGCTAAGGAGGATATACATATGTCGA CCGTTTGTTACTACCC	Primer Fw to amplify beta-galactosidase gene adding thermosensor ts8
Bgal_EcoRI_Rv	TTTTGAATTCTCATGTCTCCTCCCACACGGC	Primer Rv to amplify beta-galactosidase gene with site EcoRI

Table S4. List of component sequences

Relevant restriction sites are in italics and underlined. Relevant ATGs and thermosensors in bold.

> Tth origin of replication derived from pMY1
*GCGGCCGC*TT*GACGTC*AGCGCAGAAGTGGTCAGCTTGCATGCCTGTTGGCATGCCTCAGTTGA
CCCCATTGACCCTTCTCTCTGGAGGTGGTAGCTAGAGGGGAATGGTCCCACCCTGGAGCCCCA
CCCCTTGCCAACCCTGGGCCCGGTATAATGCCGGGCAATGGGAAGACCGGGCCCCCTGGGGG
CCCAAGGCGGCAGGGCCGCGTGAGGACCAAAAAGAGAGGCTCCCGCTGGGAGGGAACCCAA
ATTCCAGCAACCTTTTTCTTCGCCTAAGGTTATCCCCTCCCAGCGGGAAAGGCAATAGGCGCCA
AGGCGGCGCGCGGGGGAGTGCCGTCTCCGACGCGCGAAACGGGAGGGGTTTATGCCCCAAG
AAGGAGTTGAAGGGAAGAGCTATAAAGAACAGCCGGAAGGACTCACCGAGCGGGGCCGGG
CCTTCCTCGAGGCCCTGGCCCGGAGGAAGCGGGCGAGGGGCGAGGAGCTTCCGCCCCTCTAC
CTGAAGCTCCTGGAGGGGGCCGCGCCCCCGGAGAAGGGGGTCCATGAGCCCCCGCCCGAGG
AGGCGCCTCCCCCGGAGGACCTCCGCCGGAAGCTCCGGGAGGCGCCGCCTTCCCCGGCCCTC
CCCAACCGACAGGAGCTCTCCGCCTCCCCGCCCCCGCCCGAGATGAGGCGGGACGCCTGGAG
CCTCGCCGACCGCCTCCTGGAGGAGGGGGAGCGGCGGGGCACCCTGCCAGGGCTTTCCGAG
CGGGAGCGGAGGGTGTACCGGACCCTCCTCGCCCTGGGCCTCGAGGTCCTGGCCCGGAGGCT
GGGCCCGGGGAGGCCCCTGCCCAGGAACCTGTCCCAGGTCTCCTTCTTCGCCGTGAACGACG

CCCTGGCCGTGGCCCTGGGAATCCCCCGGCCTCCCTCTACCGGGTCCTGGCCTCCCTGGAGG
CCAAGGGGCTCATCCGCCGGAGGGCCTGGCGCACCCCGGCCACCCTCAAGGGCCGGACGGG
GGTCTACGCCGGCGGGACCCTCTACGCCGTGCGCCTGCCCCACCGGGAGGCCCGCCCCCGCC
TGGACCCGGAGGACTTCCGCCACCCCTGGCGGGACCTGGAGGGGGACGCCCGTCAGGGGCG
CACCGCCTGGAGCTTGAGAGAGTCATATACAAGTCCTCCTAAGGAGGACTCCGGGGTCCTCCA
GCTCCTCCTTCGGTTTTCGTTATCCCCTGGCGAAGCCGAAACTCCGTTAGCTTTAGACTCTCTCA
CCGCCCTCCTCCGGGCCCGCCCCGCCGAGCGCCGGGCCCTGGTGGAGGCCCTCGCCCTCTCCC
TGGCCCGGGAGTTCCGGGATCGCGGGAGCGTGCGCTTCTACGCCTGGGTCCTCTGGAACGCC
CTCCGGGCCGAGCTCTACGGCCTGATGGAGGGGGCCCTCGAGGCCGTCGCCTGGGCGGTCC
GGCGGGCGCGGGAGGCCGCGGCCAAGGTCCTCTGGAGCCCGAGGGGCGAGGGGGTCCGGC
GCCCCGGGGCCCTCCTCGCCCACCTCCTCCGGGAGCGGGGCCTCCTGGAGCTCTTCCGCCAGG
CCCCTCAGTGGCGGGTGGCGTAGGGCTCCCGCCGGGCTAAGCTGGAGGTAGCCCATCCAGG
GCCTCTGGGGGGTGGCTTGTGTATCAAGACTGGCTGTTCAAGGACTGCCCAGGGGGCTGGCG
CCTCCGCTACCGCCGGAACGGGGGCGGCTACGAGGCCTCCCGGGACGGTGAGAGGTGGGAG
AAGGTCCCCTCGGTCACGGAGGTTACGGGGCGCCTGAACAAGAACCTTCAGGACTGGGCCGT
GCGCCAGGTGGTGGAGTACCTCCAGGGGGAGCTGGTCCCCCGGAGATCCCCGGGGGGAGTA
TAACCTG*CTGCAGTTAATTAA*

> Tth origin of replication derived from pTT8
*GCGGCCGC*TT*GACGTC*TACCGCTAGGGACCCCCGGGGGGTGAGCGCCCGCTCAGCGCCCGGG
GTGAGCGGTGAGCGCTCACCCTGAGCGCCTGGGTAGACTGGGGTCGTGGAGCACCCGGAAG
GGGAAGACCCGACCCTCCTGCCCCCGGCCTTGGCCGCCCGGGCCCTGGGGGTCTCCCCGGCC
ACCCTCCGGCGCTACGCCGCCCTGTGGGAGCGGTTGGTAGGTCCCCTGCCCCGGGATCCCCG
GGGGGGACGGCTCTGGCCCAAGGAGGCCCTGGCCCGCCTCCGGGCGGCCCGGGAGGCCCAC
CTGCGGGAAGGCCTCCCCCTGGAGGAGGCCCTGGCCCGGGTCCAGGGAGACTTCCCGGCCCT
CGCCCTCCCCTCCGAGGGGGAGGCCCTGGCCCTCCTCCGGGCCCTGGCCGAGCGGCTGGAGC
GGGTGGAGGGGGAGCTAAGGGCCCTGCGGGAGGAGAACGCCCGCCTCCGGGAGGCCCTGA
AGGCCCTGGAGCCCCCGCGGAGGCGGCCCTGGTGGCGGTTCTGGGGGCATTGACCTGGGGA
CTACCGCTTGGTCCCGGGGCTTGTCGCTAGAATGGGGGGCATGAATGAAACAGACCTCGGCC
GGGTTAACCGCCGAGGCCTCTCCTCCCCAGGAGGCCTCCATCCTAACCCGGCCGGGGGGAAG
GAGGCAAGAGATGGAGCACCACACCCGGCCGGAAGCGCAAATCCCGGACACCCTCGCTAAA
ATCGCAGGACTCTTCCAGATAAACCCCGACCTGGGGGAGGTGGTTCTTCGCGCCTACGCCGCC
CTGCGCGGCCTCTCCCCGGAGGCCCTCCGCGCCCACCTCCTGGCCCCTCCCCTCCGCCCGGAG
CGGGCCCGGGAGGCCTTCCAGCGGCCCTACCTCGCCCACTTCGCCCAGACTCTCCCCCGCTAC
CCCTACGCCACGGACGACCCCAAGGAGGGGGTGCGCATCTACAAGCGGGAGAACGCCCTGA
AGCGGGTCCACGTCCAGGTGGGCCACTACCCCCACGCCGTCTTGCGGCTGGTGGTGGACGTG
GACCTCCCCTGGCCCCAGGTGGAGGAGCGGATCCACGCCCTCCCCCCCTCCCTGGTCCTGGTC
AACCCGAGATCGGGCCACTTCCACGCCTGGTACGAGCTGGACCCCATCCCCCTCACGCCCCCG
CCCGGGCGGGAGGGGAGCCTGAAGGGGGCCCTGGCCCTTCTCGCGGAGGTGGAGGCCCTGC
TGGAGGCCTACTACGGGGCGGACCCGGGCTACAACGGTCTCCTCTCCCGAAACCCCTTCCTCC
ACCCCCCGGAGTGGACCTGGGGCGGGGGGAAGCGGTGGAGCCTGCGGGACCTCCACCGGG
AGCTCCGGGGGCTCCTTCCCTCCGGGACCCGGAGGCGGGTGGACCCGGGCCTGGCCTCCTAC
GGGCGGAACAACGCCCTGTTTGACCGCCTGCGGGCGGAGGCCTACGCCCACGTGGCCCTCTT
CCGGGGCGTCCCCGGGGGGGAGGAGGCCTTCCGGGCCTGGGTGGAGCAGAGGGCCCACGC
CCTGAACCAGTCCCTCTTCCGGGACCACCCCAAGGGGCCCCTTGACCCCCGGGAGGTCCACCA
CACGGCGAAGAGCGTGGCCAAGTGGACCTACCGGAACTACCGGGGGGCGAGGGTCTACCCG
GTCTCCTCCACGGGGAGGCCGGACCGGAGCCGCCTCTCTCCCCAGGCCCGGGCCCTGATCCC

GCCCCTCCAGGGCCAGGAGCTCCAGGAGGCGGTGCGGGAGGGCGGAAGGCGGCGCGGATC
CCGGCGCAGGCAGGAGGCCGAGGAGAAGCTCACGGAGGCCCTGAAGCGCCTCCAGGCCCGG
GGGGAGCGGGTCACGGCCAGGGCCCTGGCCCGGGAGGCGGGGGTCAAGCCCCATACCGCCT
CCAAGTGGTTGAAGAGGATGCGGGAGTAGCGTCCAGGACGGCACAAACCCCAAAAATGTGC
CCCAAGCTGGTGGCTATCAGGGTATACAGGCGGGGGTGCGGGGGTGCAACCCCCGCCAGCC
CCGAAGGGGCTGTATTGGAGAACCTAGAGAGTCTCCTTAGCGTAGTTGACGGCTAAAGTCCC
CCTTCTTTTTCCTGGAAGCCGCAAAAATTTTGTTTTTCTTGGGGTAGACACCTTGAGATCCGAG
AGAAAGTGAGATAACTCACATATTTACCCCGCCCGCACGCAATTTTCATCGTCCAGGACGGCA
AAAACACTGATTTTCCCCCTCGAGGACCGCCCCTAAAACGGCTTTTAGGCGGGGGGTGGGCG
GCGGGCACCAATCCACCCTCCCCCCGCCCCCCGGCCTTCTAGGGGCCTTAGGACGGGTGAGA
GGGTCCTTCTGGGGGCAGGGTCCGGAGCCCGCTCTGGCCCGGGGGCTGGACCGGCCCTCGG
GCGCTCAAGCGTAGACAGGGAGACCCCTCCATAGC*CTGCAGTTAATTAA*

> Ec replication origin derived from pUC
*AAGCTT*AGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACAT
TAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATG
AATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCAC
TGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAA
TACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCA
AAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGA
CGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGAT
ACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGG
ATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAAAGCTCACGCTGTAGGTAT
CTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCC
GACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCG
CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG
AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTC
TGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG
CTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAG
AAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGA
TTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTT
TAAATCAATCTAAAGTATATATGAGTAAACT*GCGGCCGC*TT*GACGTC*

> E.c. replication origin derived from pBBR (pSEVA1311)
AAGCTTAGGCCCACCCGCGAGCGGGTGTTCCTTCTTCACTGTCCCTTATTCGCACCTGGCGGTG
CTCAACGGGAATCCTGCTCTGCGAGGCTGGCCGTAGGCCGGCCCTACCGGCGCGGCAGCGTT
ACCCGTGTCGGCGGCTCCAACGGCTCGCCATCGTCCAGAAAACACGGCTCATCGGGCATCGG
CAGGCGCTGCTGCCCGCGCCGTTCCCATTCCTCCGTTTCGGTCAAGGCTGGCAGGTCTGGTTC
CATGCCCGGAATGCCGGGCTGGCTGGGCGGCTCCTCGCCGGGGCCGGTCGGTAGTTGCTGCT
CGCCCGGATACAGGGTCGGGATGCGGCGCAGGTCGCCATGCCCCAACAGCGATTCGTCCTGG
TCGTCGTGATCAACCACCACGGCGGCACTGAACACCGACAGGCGCAACTGGTCGCGGGGCTG
GCCCCACGCCACGCGGTCATTGACCACGTAGGCCGACACGGTGCCGGGGCCGTTGAGCTTCA
CGACGGAGATCCAGCGCTCGGCCACCAAGTCCTTGACTGCGTATTGGACCGTCCGCAAAGAA
CGTCCGATGAGCTTGGAAAGTGTCTTCTGGCTGACCACCACGGCGTTCTGGTGGCCCATCTGC
GCCACGAGGTGATGCAGCAGCATTGCCGCCGTGGGTTTCCTCGCAATAAGCCCGGCCCACGC
CTCATGCGCTTTGCGTTCCGTTTGCACCCAGTGACCGGGCTTGTTCTTGGCTTGAATGCCGATT
TCTCTGGACTGCGTGGCCATGCTTATCTCCATGCGGTAGGGGTGCCGCACGGTTGCGGCACCA

TGCGCAATCAGCTGCAACTTTTCGGCAGCGCGACAACAATTATGCGTTGCGTAAAAGTGGCAG
TCAATTACAGATTTTCTTTAACCTACGCAATGAGCTATTGCGGGGGGTGCCGCAATGAGCTGT
TGCGTACCCCCCTTTTTTAAGTTGTTGATTTTTAAGTCTTTCGCATTTCGCCCTATATCTAGTTCT
TTGGTGCCCAAAGAAGGGCACCCCTGCGGGGTTCCCCCACGCCTTCGGCGCGGCTCCCCCTCC
GGCAAAAAGTGGCCCCTCCGGGGCTTGTTGATCGACTGCGCGGCCTTCGGCCTTGCCCAAGG
TGGCGCTGCCCCCTTGGAACCCCCGCACTCGCCGCCGTGAGGCTCGGGGGGCAGGCGGGCG
GGCTTCGCCCTTCGACTGCCCCCACTCGCATAGGCTTGGGTCGTTCCAGGCGCGTCAAGGCCA
AGCCGCTGCGCGGTCGCTGCGCGAGCCTTGACCCGCCTTCCACTTGGTGTCCAACCGGCAAGC
GAAGCGCGCAGGCCGCAGGCCGGAGGCTTTTCCCCAGAGAAAATTAAAAAAATTGATGGGG
CAAGGCCGCAGGCCGCGCAGTTGGAGCCGGTGGGTATGTGGTCGAAGGCTGGGTAGCCGGT
GGGCAATCCCTGTGGTCAAGCTCGTGGGCAGGCGCAGCCTGTCCATCAGCTTGTCCAGCAGG
GTTGTCCACGGGCCGAGCGAAGCGAGCCAGCCGGTGGCCGCTCGCGGCCATCGTCCACATAT
CCACGGGCTGGCAAGGGAGCGCAGCGACCGCGCAGGGCGAAGCCCGGAGAGCAAGCCCGT
AGGGGG*GGCGGCCGC*TT*GACGTC*

> SlpA promoter - Kanamycin resistance gene - SlpA terminator
*CTGCAGTTAATTAA*AGAAACCTTAAGGCCCGACCGTTTGACAAGGGCGCGTGAGGTTTTTACG
ATAGCGCCGGATGCGGGGAAAAAGGGCTCCTTTTGGGGGGTTTTCCCCGCACCGGGCGGACC
TGGGCGGAGAGGAAACGCGGCAACTCGCCCGTCTCGGGTTCCCGCCCACGACCCTTAAGGAG
GTGTGAGGATT**ATG**AATGGACCAATAATAATGACTAGAGAAGAAAGAATGAAGATTGTTCAT
GAAATTAAGGAACGAATATTGGATAAATATGGGGATGATGTTAAGGCTATTGGTGTTTATGG
CTCTCTTGGTCGTCAGACTGATGGGCCCTATTCGGATATTGAGATGATGTGTGTCATGTCAAC
AGAGGAAGCAGAGTTCAGCCATGAATGGACAACCGGTGAGTGGAAGGTGGAAGTGAATTTT
TATAGCGAAGAGATTCTACTAGATTATGCATCTCAGGTGGAATCAGATTGGCCGCTTACACAT
GGTCAATTTTTCTCTATTTTGCCGATTTATGATTCAGGTGGATACTTAGAGAAAGTGTATCAAA
CTGCTAAATCGGTAGAAGCCCAAAAGTTCCACGATGCGATTTGTGCCCTTATCGTAGAAGAGC
TGTTTGAATATGCAGGCAAATGGCGTAATATTCGTGTGCAAGGACCGACAACATTTCTACCAT
CCTTGACTGTACAGGTAGCAATGGCAGGTGCCATGTTGATTGGTCTGCATCATCGCATCTGTT
ATACGACGAGCGCTTCGGTCTTAACTGAAGCAGTTAAGCAATCAGATCTTCCTTCAGGTTATG
ACCATCTGTGCCAGTTCGTAATGTCTGGTCAACTTTCCGACTCTGAGAAACTTCTGGAATCGCT
AGAGAATTTCTGGAATGGGATTCAGGAGTGGACAGAACGACACGGATATATAGTGGATGTGT
CAAAACGCATACCATTTTGAACGGAATTTATGCGGTGTGAAATACCGCACAGATGCGTAAGG
AGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATC
GGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAA
GTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGT*GGCGCGCC*TGTAGCTAGTAAAGTTCTAA
CCCCTGGCGGTCCCGCCCCCGCCCCCTTTGGGGGGGCGGGGGTTTTTGTTCTCCC*GCTAGC*

> SlpA promoter - Hygromycin resistance gene - SlpA terminator
*CTGCAGTTAATTAA*AGAAACCTTAAGGCCCGACCGTTTGACAAGGGCGCGTGAGGTTTTTACG
ATAGCGCCGGATGCGGGGAAAAAGGGCTCCTTTTGGGGGGTTTTCCCCGCACCGGGCGGACC
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GTGTGAGGATT**ATG**AAAAAGCCTGAACTCACCGCGACGTCTGTTGAGAAGTTTCTGATCGAA
AAGTTCGGCAGCGTCTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTCGTGCTTTCAGC
TTCGATGTAGGAGGGCGTGGATATGTCCTGCGGGTAAATAGCTGCGCCGATGGTTTCTACAA
AGATCATTATGTTTATCGGCACTTTGCATCGGCCGCGCTCCCGATTCCGGAAGTGCTTGACATT
GGGGAATTTAGCGAGGGCCTGACCTATTGCATCTCCCGCCGTGCACCGGGTGTCACGTTGCAA
GACCTGCCTGAAACCGAACTGCCCGCTGTTCTGCAACCGGTCGCGGAGGTTATGGATGCGATC

GCTGCGGCCGATCTTAGCCAGACGAGCGGGTTCGGCCCATTCGGACCGCAAGGAATCGGTCA
ATACACTACATGGCGTGATTTCATCTGCGCGATTGCTGATCCCCATGTGTATCACTGGCAAACT
GTGATGGACGACACCGTCAGTGCGTCCGTCGCGCAGGCTCTCGATGAGCTGATGCTTTGGGT
CGAGGACTGCCCCGAAGTCCGGCACCTCGTGCACGCGGATTTCGGCTCCAACAATGTCCTGAC
GGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGGATCCCCTAT
ACGAGGTCGCCAACATCTTCTTCTGGAGGCCGTGGTTGGCTTGTATGGAGCAGCAGGCGCGC
TACTTCGAGCGGAGGCATCCGGAGCTTGCAGGATCGCCGCGGCTCCGGGCGTATATGCTCCG
CATTGGTCTTGACCAACTCTATCAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCATGGGC
GCAGGGTCGATGCGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGGCGTACACAAATCG
CCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGTGAAGAAGTACTCGCCGATAGTGGAAAC
CGACGCCCCAGCACTCGTCCGAGGGCAAAGGAATAGAGAT*GGCGCGCC*TGTAGCTAGTAAAG
TTCTAACCCCTGGCGGTCCCGCCCCCGCCCCCTTTGGGGGGGCGGGGGGTTTTTGTTCTCCC*G
CTAGCC*

> SlpA promoter - Bleomycin resistance gene - SlpA terminator
*CTGCAGTTAATTAA*AGAAACCTTAAGGCCCGACCGTTTGACAAGGGCGCGTGAGGTTTTTACG
ATAGCGCCGGATGCGGGGAAAAAGGGCTCCTTTTGGGGGGTTTTCCCCGCACCGGGCGGACC
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GTGTGAGGATT**ATG**GCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCGCGCGCGACGTCGCC
GGAGCGGTCGAGTTCTGGACCGACCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGACTT
CGCCGGTGTGGTCCGGGACGACGTGACCCTGTTCATCAGCGCGGTCCAGGACCAGGTGGTGC
CGGACAACACCCAGGCCTGGGTGTGGGTGCGCGGCCTGGACGAGCTGTACGCCGAGTGGTC
GGAGGTCGTGTCCACGAACTTCCGGGACGCCTCCGGGCCGGCCATGACCGAGATCGTCGAGC
AGCCGTGGGGGCGGGAGTTCGCCCTGCGCGACCCGGCCGGCAACTGCGTGCACTTCGTGGCC
GAGGAGCAGGACTAATCTA*GGCGCGCC*TGTAGCTAGTAAAGTTCTAACCCCTGGCGGTCCCG
CCCCCGCCCCCTTTGGGGGGGCGGGGGGTTTTTGTTCTCCC*GCTAGCT*

> SlpA promoter - RBS - ATG
*CTGCAGTTAATTAA*AGAAACCTTAAGGCCCGACCGTTTGACAAGGGCGCGTGAGGTTTTTACG
ATAGCGCCGGATGCGGGGAAAAAGGGCTCCTTTTGGGGGGTTTTCCCCGCACCGGGCGGACC
TGGGCGGAGAGGAAACGCGGCAACTCGCCCGTCTCGGGTTCCCGCCCACGACCCTTAAGGAG
GTGTGAGGATT**ATG**

> SlpA terminator
*GGCGCGCC*TGTAGCTAGTAAAGTTCTAACCCCTGGCGGTCCCGCCCCCGCCCCCTTTGGGGGG
GCGGGGGGTTTTTGTTCTCCC*GCTAGC*

> Pnqo
*GCTAGC*CTCCAGGGGCCTTCTTTCCGCCCCCCAAGGTGTGGAGCAGCCTGGTGCGCCTCACCC
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CGGAAGACCCTCTTAAACGCCCTGGCCGCGGCCGGCTACCCCAAGGCGCGGGTGGAGGAGG
CCCTGAGGGCCTTGGGCCTTCCCCCTAGGGTGCGGGCCGAGGAGCTGGACCTCGAGGCCTTC
CGCCGGCTGAGGGAGGGCCTCGAGGGGGCGGTGTAGGCCCGCCTCCCTTCCCTACACCGTTC
CTTCTCCTTCTGTGTAGCCCACCCCCCTTGCGCCCCACCCCGGGGTGATAAGATGGGCAGGAA
ATGAGGCCGGCCCCTTTGGGGCTTTGGGGACCGGTTTCGTGAAGAA*GAATTC*TTTCGTGCACG
AAAGGAGGGG*CAT****ATG***

> Pnqo3A, deleted region indicated
*GCTAGC*CTCCAGGGGCCTTCTTTCCGCCCCCCAAGGTGTGGAGCAGCCTGGTGCGCCTCACCC
CCACGGGCGCCCCGGACGACCCCGGCCTCTTCCGCCTCGTTGAGGCCGCCTTCGGAAAGCGG
CGGAAGACCCTCTTAAACGCCCTGGCCGCGGCCGG~~CTACCCCAAGGCGCGGGTGGAGGAGG~~
~~CCCTGAGGGCCTTGGGCCTTCCCCCTAGGGTGCGGGCCGAGGAGCTGGACCTCGAGGCCTTC~~
~~CGCCGGCTGAGGGAGGGCCTCGAG~~GGGGCGGTGTAGGCCCGCCTCCCTTCCCTACACCGTTC
CTTCTCCTTCTGTGTAGCCCACCCCCCTTGCGCCCCACCCCGGGGTGATAAGATGGGCAGGAA
ATGAGGCCGGCCCCTTTGGGGCTTTGGGGACCGGTTTCGTGAAGAA*GAATTC*TTTCGTGCACG
AAAGGAGGGG*CATATG*

> IFP gene
CATATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGT
GATGTTAATGGGCACAAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAAACGGAAA
ACTCACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTGTCACTA
CTCTGACCTATGGTCTTATGTGCTTTTCCCGTTATCCGGATCACATGAAACGGCATGACTTTTTC
AAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGAC
CTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAA
GGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAACTCGAGTACAACTTTAACTC
ACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCG
CCACAACGTTGAAGATGGTTCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGG
CGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGTCCTTTCGAAAGAT
CCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACAT
GGCATGGATGAGCTCTACAAATAATAA*AAGCTT*

> Plac
GCTAGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGT
TAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAA
TTGTGA*GAATTC*TTTCGTGCACGAAAGGAGGGG*CAT**ATG***

> P3K
*GCTAGC*GTTTAAACTCTTACAGCTTGACGGCGTTTTTGTCCTTGAGCGATTCAGGGACGTTCGC
CCAGTAGGTGGTTTTCGAGCCGTACTGCGTGGTCACCGTCAACTGCACCTCGTCACCAGCGCC
GAGCTTGGAGCTGACGCCGCCGGCGTACATGATGACCTTGATGCGGTCGGtCTGCTGGACCA
GCACGTTGTCGTTGTTCCCCTTGATCGAGGTGGTATTGAACTCCTCACCCAGCGTCGTCTTGTC
AGCTGCGTTGGCGTGCGTTAGGGTGGTGGCTTTGTCCGGGCCGATCCACTGGATCGTGGATT
TGCTGAGGTTGATGTTGTCGGCTCCGGCGGCCTGGCGCACGGTGAGGTTCACGTAGTCGACT
CTAGA*GAATTC*TTTCGTGCACGAAAGGAGGGG*CAT**ATG***

> P16S
*GCTAGC*TCTACCGCGAGTTTGACTCGTGGAGCGCTTCATTGAGGAGATCCGCTGCCCTCGCAA
GCCTTGACAAAAAGGAGGGGGATTGATAGCATGGCTTTTCT*GAATTC*TTTCGTGCACGAAAG
GAGGGG*CAT**ATG***

> Pnar
*TCTAGA*GGATCCCCGGGTACCCCAGGGCCTTGACCCGCTCCCTGAGGTAGCGGGGGTCTTGCT
CCAGGGCGTGGGTGAAAAGGGGCTCGCGCCTCAGGGCCACGATCCCCGGGTAGCCCTCGCCC
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AGAGGTGCCGTCCCTCAGGGTCGGCGAGGAAGAGCTCCGCCGCCTCCATGCATGGCGAGGG
CCTCCCGGAGGGCCCGGAGGAAGTCCTGGACTCCTTAGGCGAAGGCCTCCGGGTGCCGGAG
CAGGTGCTGGGTGAGGTAGGAGGCTTCCCGCAGGAGGTCGGGCCCCTTGGTCCGCTCGGGG
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ACCACGAGGGGCGTGCTGGCGCGGTAGGCGCCCCGCTTCAGCTCCCGGAGCGCCGGGCACC
GGGCGCAAAGCGGCCGGCCGAAGGGGTCCTTTCCCTGGACCAGGTGGGCGCAGGGCACGCC
CGCGGCGCGAAACCCCAGGGAGGCTTCGGCCTCTACCTCCCGCACGAGGCCTTGACCGTCAA
GGCGGATCCGCGCCAGGGCTTCCATCCGCCCTCAGCCTAGGGGGGTTCCGGTAGGACAGATG
TCCCGGGGCTTGCCAGTGGGCGCGAGGAGGCCCGGGCACACTGGGGCCGGAGGTGACCATG
GGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGC*CAT**ATG***

> LinkerA
*GCTAGC*GA*GATATC*A*TCTAGA*AAA*CATATG*A*GGTACC*A*GAATTCACTAGTGGATCCAAGCTT*

> Linker1
*CAT**ATG***GTCGACAAGATATCA*GGATCC*A*TCTAGAAAGCTT*

> Linker2
*CAT**ATG***G*GTCGAC*AA*GATATC*A*GGATCC*A*TCTAGAAAGCTT*

> Linker3
*CAT**ATG***GG*GTCGAC*AA*GATATC*A*GGATCC*A*TCTAGAAAGCTT*

Pnqo variants

> Pnqo209
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CCCTTGCGCCCCACCCCGGGGTGATAAGATGGGCAGGAAATGAGGCCGGCCCCTTTGGGGCT
TTGGGGACCGGTTTCGTGAAGAA*GAATTC*TTTCGTGCACGAAAGGAGGGG*CAT**ATG***

> Pnqo91
*GCTAGC*CCCCTTGCGCCCCACCCCGGGGTGATAAGATGGGCAGGAAATGAGGCCGGCCCCTT
TGGGGCTTTGGGGACCGGTTTCGTGAAGAA*GAATTC*TTTCGTGCACGAAAGGAGGGGCAT**AT
G**

> Pnqo72
*GCTAGC*ACCCCGGGGTGATAAGATGGGCAGGAAATGAGGCCGGCCCCTTTGGGGCTTTGGG
GACCGGTTTCGTGAAGAA*GAATTC*TTTCGTGCACGAAAGGAGGGG*CAT**ATG***

> Pnqo57
*GCTAGC*ATGGGCAGGAAATGAGGCCGGCCCCTTTGGGGCTTTGGGGACCGGTTTCGTGAAGA
A*GAATTC*TTTCGTGCACGAAAGGAGGGG*CAT**ATG***

> Pnqo33

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GGG*CAT**ATG***

>3Knqo72
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CCAGTAGGTGGTTTTCGAGCCGTACTGCGTGGTCACCGTCAACTGCACCTCGTCACCAGCGCC
GAGCTTGGAGCTGACGCCGCCGGCGTACATGATGACCTTGATGCGGTCGGtCTGCTGGACCA
GCACGTTGTCGTTGTTCCCCTTGATCGAGGTGGTATTGAACTCCTCACCCAGCGTCGTCactagC
CCGGGGTGATAAGATGGGCAGGAAATGAGGCCGGCCCCTTTGGGGCTTTGGGGACCGGTTT
CGTGAAGAA*GAATTC*TTTCGTGCACGAAAGGAGGGG*CAT**ATG***

> Casette Hygromycin resistance gene - Thermosensor PA-sIFP- terminator
*CTGCAGTTAATTAA*AGAAACCTTAAGGCCCGACCGTTTGACAAGGGCGCGTGAGGTTTTTACG
ATAGCGCCGGATGCGGGGAAAAAGGGCTCCTTTTGGGGGGTTTTCCCCGCACCGGGCGGACC
TGGGCGGAGAGGAAACGCGGCAACTCGCCCGTCTCGGGTTCCCGCCCACGACCCTTAAGGAG
GTGTGAGGATT**ATG**AAAAAGCCTGAACTCACCGCGACGTCTGTTGAGAAGTTTCTGATCGAA
AAGTTCGGCAGCGTCTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTCGTGCTTTCAGC
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AGATCATTATGTTTATCGGCACTTTGCATCGGCCGCGCTCCCGATTCCGGAAGTGCTTGACATT
GGGGAATTTAGCGAGGGCCTGACCTATTGCATCTCCCGCCGTGCACCGGGTGTCACGTTGCAA
GACCTGCCTGAAACCGAACTGCCCGCTGTTCTGCAACCGGTCGCGGAGGTTATGGATCGCTGC
GGCCGATCTTAGCCAGACGAGCGGGTTCGGCCCATTCGGACCGCAAGGAATCGGTCAATACA
CTACATGGCGTGATTTCATCTGCGCGATTGCTGATCCCCATGTGTATCACTGGCAAACTGTGAT
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ACTGCCCCGAAGTCCGGCACCTCGTGCACGCGGATTTCGGCTCCAACAATGTCCTGACGGACA
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GTCGCCAACATCTTCTTCTGGAGGCCGTGGTTGGCTTGTATGGAGCAGCAGGCGCGCTACTTC
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GAAGCGCGGCCGTCTGGACCGATGGCTGTGAAGAAGTACTCGCCGATAGTGGAAACCGACG
CCCCAGCACTCGTCCGAGGGCAAAGGAATAGAGATCAGCTTGCATGCCTGCAGGTCGAC*TCT*
*AGA***AAAAAAACAAAAAAGAAGGAGGATATA***CAT**ATG*AGCAAAGGAGAAGAACTTTTCACT
GGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCCGTG
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GAACGCACTATATCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAA
GGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAACATT
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ACCTGTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCT
TGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAATAA*GAATTC*AG
GGTTTTCCCAGTCACGACGT*GGCGCGCC*TGTAGCTAGTAAAGTTCTAACCCCTGGCGGTCCCG
CCCCCGCCCCCTTTGGGGGGGCGGGGGGTTTTTGTTCTCCC*GCTAGC*

Thermosensors

> DF

*TCTAGA***CCTCCTTCAAAAAAGAAGGAGG**ATATA*CAT**ATG***

> PA

*TCTAGA***AAAAAAACAAAAAAGAAGGAGG**ATATA*CAT**ATG***

> TS0

*TCTAGA***ACTCCTTCAAAAAAGAAGGAGG**ATATA*CAT**ATG***

> TS1

*TCTAGA***CCTCCTTAACAAAAAAGTTAAGGAGG**ATATA*CAT**ATG***

> TS2

*TCTAGA***CCTCCTTAGCAAAAAAGCTAAGGAGG**ATATA*CAT**ATG***

> TS4

*TCTAGA***CCTCCTTACGCAAAAAAGCGTAAGGAGG**ATATA*CAT**ATG***

> TS6

*TCTAGA***CCTCCTTACGCGCAAAAAAGCGCGTAAGGAGG**ATATA*CAT**ATG***

> TS8

*TCTAGA***CCTCCTTAAAGCAAAAAAGCTAAGGAGG**ATATA*CAT**ATG***

> TS10

*TCTAGA***CCTCCTTCAAAAAAAAAAAAAAAAAAGAAGGAGG**ATATA*CAT**ATG***

> TSP

*TCTAGA***CCTCCTTCAAAAAAGAAGGA*****GG***TACC*AG**ATG**

> beta-galactosidase gene

*CAT**ATG***TCGACCGTTTGTTACTACCCGGAGCATTGGCCTGAAGAACGTTGGGAAGAGGACTTT
AAGGCCATGCGGGCTCTGGGGCTCCGCTATGTGCGCCTGGGGGAGTTTGCCTGGAGCGCCCT
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GGTACCCGGAGATCCTCCCTGTGGACCGGGAGGGGCGGAGGCGGAACTTTGGTGGGCGGCG
GCACTACTGCTTCTCCAGCCCCGCCTACCGGGAAGAGACTGCCCGCATCGTGGCCCTTCTAGC
GGAGCGTTACGGCCGCCACCCCGCGGTGGTAGGGTTCCAGGTGGACAACGAGTTTGGCTGTC
ACGGCACCGTGCGCTGCTACTGTCCCAACTGTCGTGAGGCCTTCCGGGGCTGGTTGAGGGCT
AAGTACGGGACGATTGACGCGCTGAACGCCGCCTGGGGAACGGTCTTTTGGAGCCAAACGTA
CCGGGATTTCGGTGAGGTGGAGCTTCCTCACCTCACCGTGGCCGAGGCCAACCCCAGCCACCT
TCTGGACTACTACCGTTTCGCATCAGACCAGGTGCGGGCCTACAATCGTTTCCAAGTGGACCT
CCTTCGGGATAATGCTCCTGGCCGGTTCATTACCCATAACTTCATGGGGTTTTTCACCGATCTG
GACCCCTTTGCCTTGGCCGAGGACCTGGATTTTGCCGCCTGGGACAGCTACCCCTTGGGCTTC
ACCGACCTGATGCCCCTTCCCCAGGAGGAGAAGGTTCAGTGGGCCCGCACGGGCCACCCCGA
TGTGGCCGCCTTCCACCACGACCTTTACCGGGGGGTAGGGCGGGGGCGGTTTTGGGTCATGG
AGCAGCAGCCGGGTCCCGTGAACTGGGCCCCCCACAATCCAAGCCCGGCGCCCGGGATGGTA
CGCCTTTGGACTTGGGAGGCCATAGCCCATGGGGCAGAGGTGGTTTCCTACTTCCGCTGGCG
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GTAATTGTCGGACCCCGTTCGGGGAGCAAAACGGAGAAATTCCAGATCCCCCCCGAAATCCCT
CCGGGCGCGCTCCAAGCCCTCCTTCCCCTTAAGGTAGTGCGGGTGGAGAGCCTGCCCCCGGG
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ATGAGGCTTGTACTGGGCGGCTTGGAGGTGCCCCTGAAGGCCCAAGGGGTGGAGGTCTTGG
AAGACGGGGCCCTCCTATGGGGCTCAGAGGTACGGGTCCACGCTCCTTTTCGGGCAGAGGGA
CCTGCAGCC*AAGCTT*

> CrtB, phytoene synthase
*CAT**ATG***AAAATGCCCGCAAGTATGGAGCCCGACTGGAAAGCCCTCCTCCGCGTCCTCCGCGCC
CACTCCGCCACCTTCTACCTGGGAAGCCTCCTCTTCCCCAAGGAGGCCCGCAAGGGGGCCTGG
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GAGTGGGAGAAGGGCCTCGCCTGGGCCCTGGAGCGCTGGGACATCCCCTTTGAGGCCTTCCT
CCACATGCGGGAGGGCTTCCTGACCGACCTCGGCCCCGTGCGGCTCGGGACGGAGGCGGAG
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GGGCGGCAAGGAGGCGGAAGCCCGGGCGGTGAAGCTGGGGCAGGCCATGCAGCTCACCAA
CATCCTCCGGGACGTGGGGGAGGACCTGGAGCGGGACCGGGTCTACCTGCCCTTGGACCTCC
TCCGGGCCCACGGGGTGGAGGTGGAGGACCTGCGGGCGGGGCGCGTCACCCCAGGGTACCG
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ct*AAGCTT*

> Alkaline phosphatase
*CAT**ATG***AAGCGAAGGGACATCCTGAAAGGTGGCCTGGCTGCGGGGGCCCTGGCCCTCCTGCC
CCGGGGCCATACCCAGGGGGCTCTGCAGAACCAGCCTTCCTTGGGAAGGCGGTACCGCAACC
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ACCCCGCCAGGCGCAAGGACGGGAAGGACCTCTACGCCGCCTTCGCCGCCAAGGGGTACGG
GGTGGTGCGCACCCTCGAGGAGCTCGCCCGTTCCAACGCCACCCGGCTCCTGGGCGTCTTCGC
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CGTGCTGGCGGCGGACGAGGTCCTGGAGCTCCTCACCGCCTTCGTGGACCGGAACCCGGACA

CCCTCCTCATCGTGGTCTCGGACCACGCCACCGGGGTAGGGGGGCTTTACGGGGCCGGGCGG
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ACATGCTCCGCGTCCTCGGCCAGGCCCCGGAGGCCTCCCAGGTCAAGGAAGCCTTCCGGGCC
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CTCGTGGACAACACCCACGTCTTCCGCCTCATGGGGGAGGCCCTTGGCCTCCGCTACCAGAAC
CCGGTGATGAGCGAGGAGGAGGCCCTGGAGATCCTCAAGGCCAGGCCCCAGGGGATGCGCC
ACCCCGAGGACGTCTGGGCCTAAgtcgacgcgt*AAGCTT*

> *Pseudomonas fluorescens* esterase I variant 34
*CAT**ATG***AGCACATTTGTTGCAAAAGACGGTACCCTGATCTATTTCAAGGACTGGGGCAGCGGT
AAACCGGTGTTGTTCAGCCACGGTTGGCTACTGGATGCCGACATGTGGGAATACCAGATGGA
GTACCTCAGCAGCCGCGGCTATCGCACCATCGCCTTTGACCGCCGCGGCTTTGGCCGCTCGGA
CCAACCCTGGACCGGCAACGACTACGACACCTTCGCCGACGACATCGCCCAGTTGATCGAACA
CCTGGACCTCAAGGAGGTGACCCTGGTGGGCTTCTCCATGGGCGGCGGCGATGTGGCCCGCT
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CTGTTCGGCCAGAAGCCCGACTATCCGCAGGGTGTCCCGCTCGATGTGTTCGCAAGGTTCAAG
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GGCCAAGATCGACGTACCCACCCTGGTGATCCATGGCGATGGCGACCAGATCGTGCCGTTCG
AGACCACCGGCAAAGTGGCGGCGGAGTTGATCAAGGGCGCCGAACTGAAGGTGTACAAGGA
CGCGCCCCACGGGTTCGCGGTGACCCACGCCCAGCAGTTGAACGAAGACCTGTTGGCGTTCTT
GAAACGCGGATCCCATCATCATCATCATCATTGACTGCAGCC*AAGCTT*

Table S5. References for component parts

pEM2S (pMY1) [2], pTT8 [4], kanamycin resistance gene [6], pUC18 , pSEVA131 [5], hygromycin resistance gene [7](and Bosch, Berenguer and Hidalgo, submitted), bleomycin B resistance gene [8], Pnqo[9], PslpA [10], Pnar [11], GFP from pMKnqosGFP [3], IFP (Berenguer and Hidalgo unpublished), beta-galactosidase [12], CrtB (phytoene synthase) [13], alkaline phosphatase [14], Pseudomonas fluorescenens esterase I variety 34 (Mate, Berenguer, Hidalgo, submitted). The sIFP gene combines the mutations of the superfolder GFP (sGFP) [15] with those of the Citrine version of GFP[16].

Supporting Methods

Bacterial strains and growth conditions

Growth of *T. thermophilus* strains was carried out at the indicated temperatures (55 to 65ºC) with rotational shaking (150 rpm) in TB media. *T. thermophilus* colonies were grown aerobically on TB agar (1.5 % w/v agar) and the plates were supplemented with the relevant antibiotics. For liquid or solid selection, kanamycin (final concentration, 30 mg L^{-1}) hygromycin B (100 mg L^{-1}) and/or bleomycin (15 mg L^{-1}) were included as indicated.

Escherichia coli strain DH5α was the host for plasmid construction and promoter testing. *E coli* was grown at 37 ºC in liquid or solid LB media, with kanamycin (30 mg L^{-1}), ampicillin (100 mg L^{-1}), hygromycin B (100 mg L^{-1}) or bleomycin (3 mg L^{-1}) added when required.

E. coli competence was induced following Inoue's method [17]. Transformation of *T. thermophilus* was achieved by natural competence as described [18].

Plasmid Construction

The DNA sequence of the Promoter 3K was synthesized by Genscript. The nqo promoter mutants were constructed using the oligonucleotides designed in Oligonucleotides List as Fw primers and the sIFPRv as Rv primer and cloned between sites NheI and HindIII in pMoTK110. The linkers were constructed by hibridization of the corresponding pair of oligonucleotides and cloning between sites NheI and HindIII of pMoTK110 for linker A and between sites NdeI and HindIII for linkers 1, 2, and 3, and the 16S promoter was also generated by hybridization and cloning of the corresponding phosphorylated oligonucleotides between sites NheI and EcoRI of pMoTK110 plasmid. All the thermosensor bearing genes were cloned into vector pMoTH150 (see sequence and map below, Scheme S1) as XbaI-EcoRI fragments, that include the IFP gene, obtained by PCR performed with primers indicated in Oligonucleotides List.

> pMoTH150
CTGCTGCAGTTAATTAAAGAAACCTTAAGGCCCGACCGTTTGACAAGGGCGCGTGAGGTTTTT
ACGATAGCGCCGGATGCGGGGAAAAAGGGCTCCTTTTGGGGGGTTTTCCCCGCACCGGGCG
GACCTGGGCGGAGAGGAAACGCGGCAACTCGCCCGTCTCGGGTTCCCGCCCACGACCCTTAA
GGAGGTGTGAGGATTATGAAAAAGCCTGAACTCACCGCGACGTCTGTTGAGAAGTTTCTGAT
CGAAAAGTTCGGCAGCGTCTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTCGTGCTTT
CAGCTTCGATGTAGGAGGGCGTGGATATGTCCTGCGGGTAAATAGCTGCGCCGATGGTTTCT
ACAAAGATCATTATGTTTATCGGCACTTTGCATCGGCCGCGCTCCCGATTCCGGAAGTGCTTGA
CATTGGGGAATTTAGCGAGGGCCTGACCTATTGCATCTCCCGCCGTGCACCGGGTGTCACGTT
GCAAGACCTGCCTGAAACCGAACTGCCCGCTGTTCTGCAACCGGTCGCGGAGGTTATGGATC
GCTGCGGCCGATCTTAGCCAGACGAGCGGGTTCGGCCCATTCGGACCGCAAGGAATCGGTCA
ATACACTACATGGCGTGATTTCATCTGCGCGATTGCTGATCCCCATGTGTATCACTGGCAAACT
GTGATGGACGACACCGTCAGTGCGTCCGTCGCGCAGGCTCTCGATGAGCTGATGCTTTGGGT
CGAGGACTGCCCCGAAGTCCGGCACCTCGTGCACGCGGATTTCGGCTCCAACAATGTCCTGAC
GGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGGACCCCCTAT
ACGAGGTCGCCAACATCTTCTTCTGGAGGCCGTGGTTGGCTTGTATGGAGCAGCAGGCGCGC
TACTTCGAGCGGAGGCATCCGGAGCTTGCAGGATCGCCGCGGCTCCGGGCGTATATGCTCCG
CATTGGTCTTGACCAACTCTATCAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCATGGGC
GCAGGGTCGATGCGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGGCGTACACAAATCG
CCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGTGAAGAAGTACTCGCCGATAGTGGAAAC
CGACGCCCCAGCACTCGTCCGAGGGCAAAGGAATAGAGATCAGCTTGCATGCCTGCAGGTCG
ACTCTAGACCTCCTTCAAAAAAGAAGGAGGATATACATATGGGCAAAGGAGAAGAACTTTTC
ACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCC
GTGGAGAGGGTGAAGGTGATGCTACAAACGGAAAACTCACCCTTAAATTTATTTGCACTACTG
GAAAACTACCTGTTCCGTGGCCAACACTTGTCACTACTCTGACCTATGGTCTTATGTGCTTTTCC
CGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTA
CAGGAACGCACTATATCTTTCAAAGATGACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTT
GAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGATGGAAAC
ATTCTTGGACACAAACTCGAGTACAACTTTAACTCACACAATGTATACATCACGGCAGACAAAC
AAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCCACAACGTTGAAGATGGTTCCGTTCAAC
TAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCA
TTACCTGTCGACACAATCTGTCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTT
CTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAATAAGAATTC
AGGGTTTTCCCAGTCACGACGTGGCGCGCCTGTAGCTAGTAAAGTTCTAACCCCTGGCGGTCC
CGCCCCCGCCCCCTTTGGGGGGGCGGGGGGTTTTTGTTCTCCCGCTAGCCTCCAGGGGCCTTC
TTTCCGCCCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGG
GCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGT
ATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGA
ACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTT
TTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCG

AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCC
TGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTT
TCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGT
GTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA
ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCG
AGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAG
AACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTC
TTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTAC
GCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTG
GAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGAT
CCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTGCGGCCGCT
TGACGTCAGCGCAGAAGTGGTCAGCTTGCATGCCTGTTGGCATGCCTCAGTTGACCCCATTGA
CCCTTCTCTCTGGAGGTGGTAGCTAGAGGGGAATGGTCCCACCCTGGAGCCCCACCCCTTGCC
AACCCTGGGCCCGGTATAATGCCGGGCAATGGGAAGACCGGGCCCCCTGGGGGCCCAAGGC
GGCAGGGCCGCGTGAGGACCAAAAAGAGAGGCTCCCGCTGGGAGGGAACCCAAATTCCAGC
AACCTTTTTCTTCGCCTAAGGTTATCCCCTCCCAGCGGGAAAGGCAATAGGCGCCAAGGCGGC
GCGCGGGGGAGTGCCGTCTCCGACGCGCGAAACGGGAGGGGTTTATGCCCCAAGAAGGAGT
TGAAGGGAAGAGCTATAAAGAACAGCCGGAAGGACTCACCGAGCGGGGCCGGGCCTTCCTC
GAGGCCCTGGCCCGGAGGAAGCGGGCGAGGGGCGAGGAGCTTCCGCCCCTCTACCTGAAGC
TCCTGGAGGGGGCCGCGCCCCCGGAGAAGGGGGTCCATGAGCCCCCGCCCGAGGAGGCGCC
TCCCCCGGAGGACCTCCGCCGGAAGCTCCGGGAGGCGCCGCCTTCCCCGGCCCTCCCCAACCG
ACAGGAGCTCTCCGCCTCCCCGCCCCCGCCCGAGATGAGGCGGGACGCCTGGAGCCTCGCCG
ACCGCCTCCTGGAGGAGGGGGAGCGGCGGGGCACCCTGCCAGGGCTTTCCGAGCGGGAGCG
GAGGGTGTACCGGACCCTCCTCGCCCTGGGCCTCGAGGTCCTGGCCCGGAGGCTGGGCCCGG
GGAGGCCCCTGCCCAGGAACCTGTCCCAGGTCTCCTTCTTCGCCGTGAACGACGCCCTGGCCG
TGGCCCTGGGAATCCCCCCGGCCTCCCTCTACCGGGTCCTGGCCTCCCTGGAGGCCAAGGGGC
TCATCCGCCGGAGGGCCTGGCGCACCCCGGCCACCCTCAAGGGCCGGACGGGGGTCTACGCC
GGCGGGACCCTCTACGCCGTGCGCCTGCCCCACCGGGAGGCCCGCCCCCGCCTGGACCCGGA
GGACTTCCGCCACCCCTGGCGGGACCTGGAGGGGGACGCCCGTCAGGGGCGCACCGCCTGG
AGCTTGAGAGAGTCATATACAAGTCCTCCTAAGGAGGACTCCGGGGTCCTCCAGCTCCTCCTT
CGGTTTTCGTTATCCCCTGGCGAAGCCGAAACTCCGTTAGCTTTAGACTCTCTCACCGCCCTCC
TCCGGGCCCGCCCCGCCGAGCGCCGGGCCCTGGTGGAGGCCCTCGCCCTCTCCCTGGCCCGG
GAGTTCCGGGATCGCGGGAGCGTGCGCTTCTACGCCTGGGTCCTCTGGAACGCCCTCCGGGC
CGAGCTCTACGGCCTGATGGAGGGGGCCCTCGAGGCCGTCGCCTGGGCGGTCCGGCGGGCG
CGGGAGGCCGCGGCCAAGGTCCTCTGGAGCCCGAGGGGCGAGGGGGTCCGGCGCCCCGGG
GCCCTCCTCGCCCACCTCCTCCGGGAGCGGGGCCTCCTGGAGCTCTTCCGCCAGGCCCCTCAG
TGGCGGGTGGCGTAGGGCTCCCGCCGGGCTAAGCTGGAGGTAGCCCATCCAGGGCCTCTGG
GGGGTGGCTTGTGTATCAAGACTGGCTGTTCAAGGACTGCCCAGGGGGCTGGCGCCTCCGCT
ACCGCCGGAACGGGGGCGGCTACGAGGCCTCCCGGGACGGTGAGAGGTGGGAGAAGGTCC
CCTCGGTCACGGAGGTTACGGGGCGCCTGAACAAGAACCTTCAGGACTGGGCCGTGCGCCAG
GTGGTGGAGTACCTCCAGGGGGAGCTGGTCCCGGGGCTGGTCCTCACGGAGGAGGAGGTGA
ACCGCCTCCTGGACGGTGCGTCCAAAGCCCACCGCCTGGCCGCGCAGAGGGCCGCCGGGCGA
GGCGCCGACCTCCACGCCTGGGCGGAGGCCTACCTCAAAGGGCAAAGGCCTCCCTTCCCCGA
GGAGGAGCCCCTTCGGGGGATGGCCCTCGCCCTGGCGGACTGGTGGGACGGGAACGGGGG
CGAGGCCCTGCGCTCCGAGGAGGCGGTCTTCCACCCGGAGCACCGCTACGCCGGGCGGGTG
GACCTGGTGGCCCGGCTGGGGGGGAGGGTGGTGGTGGTGGACCTGAAGACCTCCCCCCGGG
CCTACCCGGAGCACCTCCTCCAGGTGGGGGCCTACCCCCTGGCCCTCCGGGCGGAGGGGGTG

GCGGTGGAGGGGGGCTTCGTGCTGGCCCTGAGGGAAGGCTTAGCCTCCAGGAGGTCCCCCT
GGAGGAGGCGGCGCAGGCCTTCCTGGGGCTCCTGGCCGTCCACCGCTTTCTGAAGGTCCTCG
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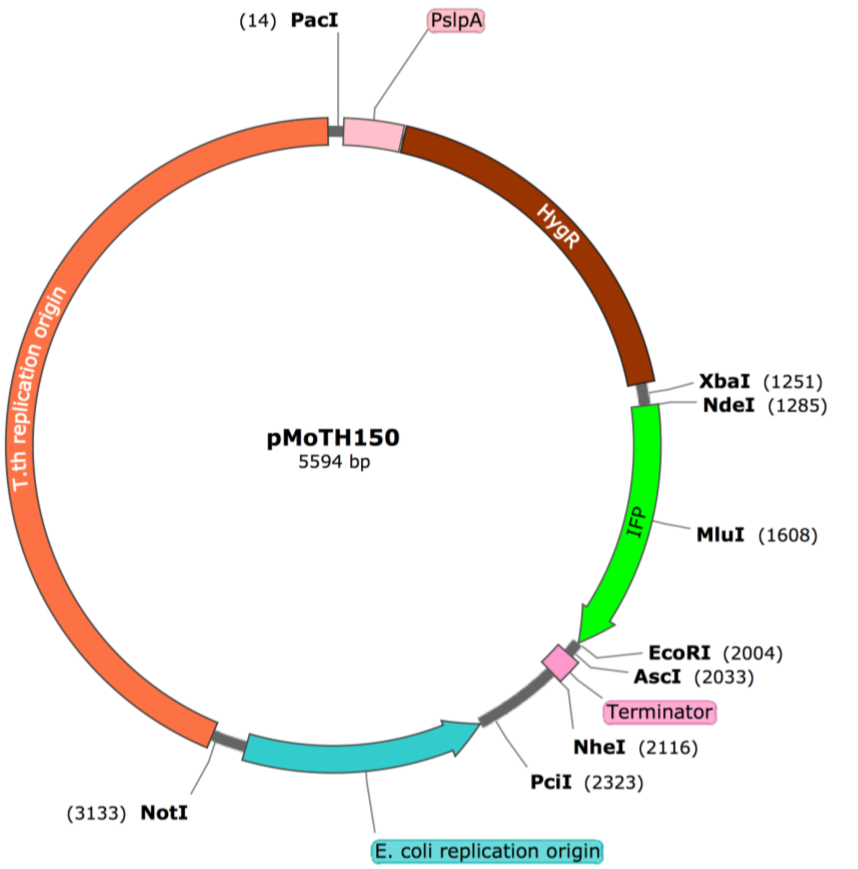
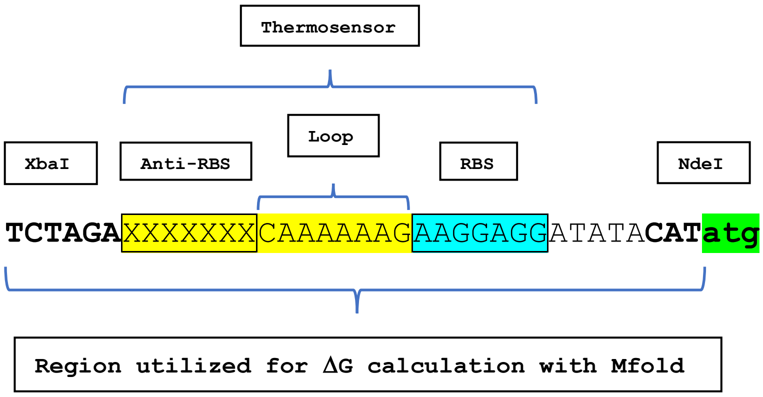


Scheme S1. Map of plasmid pMoTH150



TS0 (ΔG = -11.10 kcal/mol**)**
TCTAGAACTCCTTCAAAAAAGAAGGAGGATATA**CATatg**

TS10 (ΔG = -12.50 kcal/mol**)**
TCTAGACCTCCTTCAAAAAAAAAAAAAAAAAAGAAGGAGGATATA**CATatg**

TS8 (ΔG = -13.50 kcal/mol**)**
TCTAGACCTCCTTAAAGCAAAAAAGCTAAGGAGGATATA**CATatg**

TSP (ΔG = -14.20 kcal/mol**)**
TCTAGACCTCCTTCAAAAAAGAAGGAGGATATA**CATatg**

TS1 (ΔG = -16.20 kcal/mol**)**
TCTAGACCTCCTTAACAAAAAAGTTAAGGAGGATATA**CATatg**

TS2 (ΔG = -18.60 kcal/mol**)**
TCTAGACCTCCTTAGCAAAAAAGCTAAGGAGGATATA**CATatg**

TS4 (ΔG = -21.10 kcal/mol**)**
TCTAGACCTCCTTACGCAAAAAAGCGTAAGGAGGATATA**CATatg**

TS6 (ΔG = -26.90 kcal/mol**)**
TCTAGACCTCCTTACGCGCAAAAAAGCGCGTAAGGAGGATATA**CATatg**

PA
TCTAGAAAAAAAACAAAAAAGAAGGAGGATATA**CATatg**

DF
TCTAGAAAGGAGGATATA**CATatg**

Figure S1. Scheme of thermosensors. Different regions of the thermosensors are color-coded. ΔG values and RNA folding calculations were performed with the Mfold software [19].

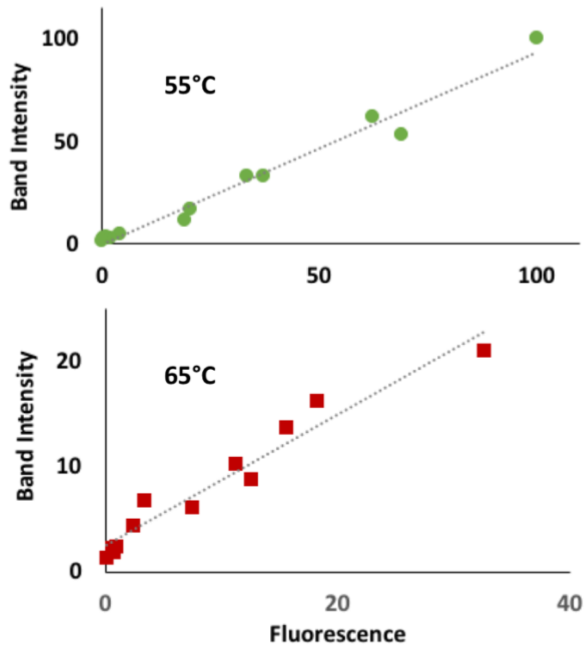


Figure S2. Correlation between sIFP fluorescence measurements and Western detection of the band corresponding to sIFP. Experiments performed at 55°C (green circles) and at 65°C (red squares) have been analyzed. The values of fluorescence intensity are those presented in Fig. 4A, quantification of sIFP band intensities as detected by Western blotting are from Fig. 4D. Dotted line represents a linear regression data fit.

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