

Engineering of NEMO as calcium indicators with large dynamics and high sensitivity

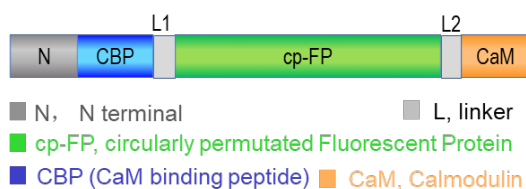
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Supplementary Table 1. *In cellulo* screening results of constructs with a GCaMP-like design^s

No	Name*	N terminal mutations	CBP**	L1***	cp-FP mutations	Mutations in CaM	F ₀ (afu)	Dynamic range	N #	Primer ###
1	GCaMP6m	/	M13	LE	Template	Template	609±44	24.7±1.2	137/48	
2	Kozak-GCaMP6m	Template	M13	LE	/	/	927±24	22.9±0.3	1058/304	66,67
3	jGCaMP8f	6×his-TR	NOSP-N10T-S15I-S17R	LKI	K18H	Q41L-G76M-S77K	692±29	7.5±0.1	298/100	
4	jGCaMP8s	6×his-TR	NOSP-N10T-S15I-S17M	LKI	K18H	F12Y-Q41H-G76M-S77K	872±41	6.1±0.1	362/128	
5	jGCaMP8m	6×his-TR	NOSP-N10T-S15I-A16G-S17R	LKI	K18H	F12Y-G76M-S77K	764±34	7.6±0.1	352/105	
6	NEMO-0.1	/	M13	LE	mNG(146-236)-GGTGGs-mNG(1-145)	/	5405±435	1.2±0.01	25/25	1~4,62~65
7	NEMO-0.2	/	M13	LE	mNG(148-236)-GGTGGs-mNG(1-147)	/	3143±697	1.1±0.02	17/17	1~8
8	NEMO-0.3	/	M13	LE	mNG(160-236)-GGTGGs-mNG(1-159)	/	0	0	30/30	1~4,9~12

Note:

§, diagram showing the design of GCaMP-like GECIs



/ , NA

* , Kozak: Kozak consensus sequence was used

^{**}, NOSP: Nitric Oxide Synthase Peptide CaM binding domain for jGCaMP8 variants.

***, L1, Linker between CBP and cp-FP

[#], N=A/B: A, number of cells used for measurements of the basal fluorescence, B, number of cells used for calculating the dynamic ranges.

##, Primer. See Supplementary Table 6 for primer sequences corresponding to numbering.

Supplementary Table 2. *In cellulo* screening results of NEMO constructs with a NCaMP7-like design^{\$}

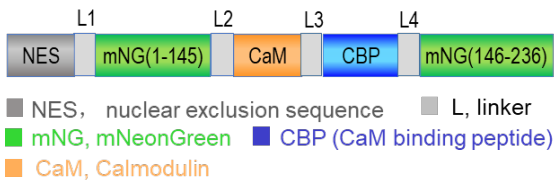
No	Name ⁺ (All contain Kozak)	NES- L1 [*]	Mutations in CaM	L3 ^{**}	CBP ^{***}	#L4	##FP mutations	F ₀ (afu)	Dynamic range	N ###	Primer ####
1	NCaMP7	/	Template	GGSGGGSSS	M13	MYF	Template	3499±194	15.8±0.5	205/86	
2	NCaMP7-with 6×his tag	/	/	GGSGGGSSS	M13	MYF	/	4925±372	17.9±0.4	149/56	68,24
3	Neo-linker-0	/	/	/	M13	MYF	/	717±51	56.1±4.1	47/20	13,14,15,24
4	Neo-linker-1	/	/	G	M13	MYF	/	365±72	150.0±4.9	38/35	13,14,16,24
5	Neo-linker-2	/	/	GG	M13	MYF	/	348±53	205.9±4.2	45/38	13,14,17,24
6	Neo-linker-3	/	/	GGG	M13	MYF	/	187±13	157.8±8.8	116/14	13,14,18,24
7	Neo-linker-4	/	/	GGGG	M13	MYF	/	586±58	80.6±9.4	82/20	13,14,19,24
8	Neo-linker-5	/	/	GGGGG	M13	MYF	/	526±35	154.5±4.8	175/59	13,14,20,24
9	Neo-linker-6	/	/	GGSGGG	M13	MYF	/	952±43	95.1±1.6	538/173	13,14,21,24
10	Neo-linker-7	/	/	GGGSGGG	M13	IYF	/	280±14	237.2±3.0	246/108	13,14,38,24
11	Neo-linker-8	/	/	GGGSGGGG	M13	MYF	/	625±53	48.1±4.1	68/43	13,14,23,24
12	5-Q3D	/	Q3D	GGGGG	M13	MYF	/	675±98	107.9±8.1	34/34	13,25,33,24
13	5-A15E	/	A15E	GGGGG	M13	MYF	/	272±46	134.1±9.8	32/32	13,26,34,24
14	5-A15L	/	A15L	GGGGG	M13	MYF	/	603±69	61.6±2.1	72/72	13,27,35,24
15	5-A15D	/	A15D	GGGGG	M13	MYF	/	546±71	44.9±1.7	44/44	13,28,36,24
16	5-A15Q	/	A15Q	GGGGG	M13	MYF	/	469±83	127.6±11.2	40/40	13,29,37,24

No	Name [†] (All contain Kozak)	NES- L1*	Mutations in CaM	L3**	CBP****	#L4	##FP mutations	F ₀ (afu)	Dynamic range	N ###	Primer ####
17	5-M36F	/	M36F	GGGGS	M13	MYF	/	566±46	94.0±7.2	50/50	13,30,38,24
18	5-Q41L	/	Q41L	GGGGS	M13	MYF	/	533±40	202.1±8.1	186/186	13,31,39,24
19	5-Q3D-A15E	/	Q3D-A15E	GGGGS	M13	MYF	/	143±17	106.3±4.7	30/30	13,26,34,24
20	5-Q3D-M36F	/	Q3D-M36F	GGGGS	M13	MYF	/	725±65	76.1±1.9	75/75	13,30,38,24
21	5-E14A-A15E	/	E14A-A15E	GGGGS	M13	MYF	/	224±29	70.5±6.9	22/22	13,41,42,24
22	5-A15E-M36F	/	A15E-M36F	GGGGS	M13	MYF	/	312±48	91.1±5.5	34/34	13,30,38,24
23	5-A15L-M36F	/	A15L-M36F	GGGGS	M13	MYF	/	2102±168	69.5±1.4	185/67	13,30,38,24
24	5-Q3D-A15L	/	Q3D-A15L	GGGGS	M13	MYF	/	2023±191	59.8±2.1	194/77	13,27,35,24
25	5f	/	Q3D-A15E-M36F	GGGGS	M13	MYF	/	521±35	79.4±0.9	219/112	13,30,38,24
26	5-Q3D-A15L-M36F	/	Q3D-A15L-M36F	GGGGS	M13	MYF	/	1827±109	43.8±0.5	211/80	13,30,38,24
27	5f-Q41L	/	Q3D-A15E-M36F-Q41L	GGGGS	M13	MYF	/	445±24	96.7±1.8	187/58	51,52,53,24
28	5f-N42S	/	Q3D-A15E-M36F-N42S	GGGGS	M13	MYF	/	256±31	72.3±2.4	27/27	13,44,46,24
29	5-8f-2	/	/	GGGGS	NOSP-N10T-S15I-S17R	MYF	/	1849±220	12.6±0.35	56/56	13,47,48,24
30	5-jGCαMP8f	/	Q41L	GGGGS	NOSP-N10T-S15I-S17R	MYF	/	1448±161	15.0±0.5	48/48	13,31,39,24
31	5f-ckkap-LHWL	/	/	GGGGS	ckkap-LHWL	MYF	/	638±164	1.1±0.01	14/14	13,49,50,24
32	6f	/	Q3D-A15E-M36F	GGSGGS	M13	MYF	/	430±52	81.8±4.5	33/33	13,14,21,24
33	6f-Q41L	/	Q3D-A15E-M36F-Q41L	GGSGGS	M13	MYF	/	490±42	81.1±1.2	175/52	13,31,39,24
34	7f	/	Q3D-A15E-M36F	GGSGGS	M13	IYF	/	231±22	172.9±6.9	63/63	13,30,38,24
35	7f-Q41L	/	Q3D-A15E-M36F-Q41L	GGSGGS	M13	IYF	/	419±38	164.7±3.1	162/46	13,31,39,24
36	7f-N42S	/	Q3D-A15E-M36F-N42S	GGSGGS	M13	IYF	/	229±31	155.4±4.5	35/35	13,44,46,24
37	7-I324M	/	/	GGSGGS	M13	MYF	/	455±32	137.1±1.8	57/57	13,14,22,24
38	NCaMP7-MI	/	/	GGSGGGSSS	M13	IYF	/	1198±56	170.8±3.2	785/235	13,32,40,24
39	NCaMP7-MI-A15E	/	A15E	GGSGGGSSS	M13	IYF	/	246±16	172.7±4.6	142/52	13,26,34,24
40	NCaMP7-MI-A15D	/	A15D	GGSGGGSSS	M13	IYF	/	437±32	122.8±2.4	168/48	13,28,36,24
41	NCaMP7-MI-E14A-A15E	/	E14A-A15E	GGSGGGSSS	M13	IYF	/	129±9	176.8±3.3	159/54	13,41,42,24
42	NCaMP7-MI-Q3D-A15E	/	Q3D-A15E	GGSGGGSSS	M13	IYF	/	165±13	148.7±2.4	148/45	13,25,33,24
43	NCaMP7-MI-A15E-M36F	/	A15E-M36F	GGSGGGSSS	M13	IYF	/	184±11	192.4±6.1	174/52	13,30,38,24
44	NCaMP7-MI-Q3D-A15E-M36F	/	Q3D-A15E-M36F	GGSGGGSSS	M13	IYF	/	302±22	147.2±2.5	156/56	13,30,38,24
45	5-MI (NEMOe)	/	/	GGGGS	M13	IYF	/	445±25	422.2±15.3	195/57	13,32,40,24
46	5-A15L-Q3D-MI	/	Q3D-A15L	GGGGS	M13	IYF	/	603±39	236.9±6.4	257/65	13,32,40,24
47	5f-MI	/	Q3D-A15E-M36F	GGGGS	M13	IYF	/	178±11	266.2±8.2	144/52	13,32,40,24
48	5-A15L-Q3D-M36F-MI	/	Q3D-A15L-M36F	GGGGS	M13	IYF	/	557±32	279.8±7.4	268/65	13,32,40,24
49	6-MI	/	/	GGSGGS	M13	IYF	/	322±21	280.1±5.7	148/56	13,32,40,24

No	Name ⁺ (All contain Kozak)	NES- L1*	Mutations in CaM	L3**	CBP***	#L4	##FP mutations	F ₀ (afu)	Dynamic range	N ###	Primer ####
50	NCaMP7-MI-AC10	/	/	GGSGGGSSS	M13	IYF	AC10	1720±103	32.9±0.7	218/64	13,54
51	5-MI-AC10	/	/	GGGGS	M13	IYF	AC10	362±35	214.6±7.9	121/34	13,54
52	NES-Neo6	1	/	GGSGGS	M13	MYF	/	1078±88	49.2±1.7	83/29	51,52,53,24
53	NES-Neo7	1	/	GGSGSGS	M13	IYF	/	681±83	189.8±8.2	27/27	51,52,53,24
54	NES-Neo5f	1	Q3D-A15E- M36F	GGGGS	M13	MYF	/	639±40	73.1±1.3	166/60	51,52,53,24
55	NES-Neo7f	1	Q3D-A15E- M36F	GGSGSGS	M13	IYF	/	268±28	150.1±4.7	17/17	51,52,53,24
56	NES-6-MI (NEMOm)	1		GGSGGS	M13	IYF	/	1085±93	240.7±7.6	260/90	51,52,53,24
57	NES-7f-Q41L	1	Q3D-A15E- M36F-Q41L	GGSGSGS	M13	IYF	/	531±32	144.7±4.5	255/83	51,52,53,24
58	NES-5f-Q41L	1	Q3D-A15E- M36F-Q41L	GGGGS	M13	MYF	/	593±38	76.6±2.8	245/67	51,52,53,24
59	NES-5-Q41L	1	Q41L	GGGGS	M13	MYF	/	936±54	124.1±4.9	301/104	51,52,53,24
60	NES-5f-MI (NEMOf)	1	Q3D-A15E- M36F	GGGGS	M13	IYF	/	508±51	245.5±8.6	200/59	51,52,53,24
61	NES-NCaMP7-MI (NEMOb)	1	/	GGSGGGSSS	M13	IYF	/	2230±95	128.8±3.1	629/139	51,52,53,24
62	NES-5-MI	1	/	GGGGS	M13	IYF	/	220±20	384.1±16.7	158/49	51,52,53,24
63	NES-5-A15E-MI	1	A15E	GGGGS	M13	IYF	/	114±7	423.5±17.3	134/43	53,26,34,24
64	NES-5-E14A-A15E-MI	1	E14A-A15E	GGGGS	M13	IYF	/	143±13	304.8±11.8	166/50	53,41,42,24
65	NES-NeoCaMP5-A15L-Q3D	1	Q3D-A15L	GGGGS	M13	MYF	/	1562±117	36.6±0.9	169/64	51,52,53,24
66	NES-NeoCaMP5-A15L-Q3D- M36F	1	Q3D-A15L- M36F	GGGGS	M13	MYF	/	2025±96	39.4±1.2	199/55	51,52,53,24
67	NES-NCaMP7-MI-AC10	1	/	GGSGGGSSS	M13	IYF	AC10	4141±233	28.8±1.2	198/56	53,54
68	NES-5-A15E-MI-AC10	1	A15E	GGGGS	M13	IYF	AC10	152±12	212.3±6.2	91/43	53,54
69	NES-5f-MI-AC10	1	Q3D-A15E- M36F	GGGGS	M13	IYF	AC10	235±15	72.2±3.8	106/34	53,54
70	NES-6-MI-AC10	1	/	GGSGGS	M13	IYF	AC10	1807±178	90.6±3.8	183/55	13,54
71	NES2-NCaMP7-MI (NEMOs)	2	/	GGSGGGSSS	M13	IYF	/	2010±151	102.3±4.0	294/83	52,53,55,56,2 4
72	NES2-NCaMP7-MI-AC10	2	/	GGSGGGSSS	M13	IYF	AC10	2440±210	15.5±0.6	154/47	53,54

Note:

[§], diagram showing the design of NCaMP7-like GECIs



/: NA

⁺, Kozak: Kozak consensus sequence was used

^{*}, NES-L1 sequence:

1: MLQNELALKLAGLDINKTG-GGS

2: MLQNELALKLAGLDINKTG-GITLGMDELYK

^{**}, L3, Linker between CaM and CBP

^{***}, Nitric Oxide Synthase Peptide CaM binding domain (NOSP)-N10T-S15I-S17R

[#], L4, Linker between CBP and cpEGFP or mNG

##, ΔC10, 10 amino acids were truncated from the C-terminus of mNG-146-236

###, N=A/B: A, number of cells used for measurements of the basal fluorescence, B, number of cells used for calculating the dynamic ranges

####, Primer numbers, See Supplementary Table 6 for primer sequences with corresponding numbers.

Supplementary Table 3. *In vitro* Ca²⁺ titration results and kinetics of NEMO variants

Sensor	Mg ²⁺	K _d (nM)	Hill number	F _{min} (10 mM EGTA)	F _{rest} (100 nM Ca ²⁺)	F _{max} (38 μM Ca ²⁺)	Dynamic range (F _{max} /F _{min})	K _{off} (s ⁻¹)	K _{on} ¹ (*10 ⁶ , M ⁻¹ s ⁻¹)
GCaMP6m	-	174.3±6.1	2.5±0.2	155±6	700±49	4023±93	26.7±0.9	2.48±0.02	14.20
	+	206.4±5	2±0.1	176±4	680±34	4133±85	23.6±0.6	2.52±0.02	12.21
GCaMP6f	-	354.4±1.5	2±0.2	228±4	515.8±8.9	8019±252	35.2±1.3	3.72±0.004	10.49
	+	568.8±9.4	1.7±0.0	268±3	477.8±4.4	8250±212	30.7±0.6	4.35±0.03	7.65
NCaMP7	-	97.9±2	2.5±0.1	406±29	16870±1964	32753±2394	80±3.2	3.29±0.01	33.56
	+	98.3±1.5	2.4±0.1	677±45	16216±1325	30628±1927	45.5±0.9	2.48±0.01	25.23
NEMOc	-	492.8±15.9	3.4±0.3	98±4	152±4	15875±236	165.3±7.7	4.59±0.03	9.31
	+	557.3±15.5	3.8±0.4	89±7	137±6	15267±578	182.2±12.6	3.16±0.02	5.67
NEMOm	-	198.9±4.8	3.3±0.2	123±5	1735±150	19396±725	159.2±4.3	2.22±0.01	11.17
	+	248.4±3.7	3.3±0.1	134±6	1065±21	19706±520	149.1±3.5	1.46±0.01	5.88
NEMOf	-	440.1±19.4	2.9±0.3	118±1	205±13	16705±411	141.5±3.8	6.73±0.04	15.30
	+	528.4±17	3.2±0.3	118±2	304±53	15085±265	127.9±2.3	5.57±0.04	10.54
NEMOs	-	129.1±3.1	3.1±0.2	65±3	2572±254	8351±194	131.3±3.4	2.26±0.01	17.48
	+	155.8±3.8	2.7±0.2	87±5	1890±115	8489±311	99.7±2.9	1.46±0.01	9.37
NEMOb	-	198.7±2.6	2.5±0.1	340±1	3880±89	27829±713	81.9±2.1	1.96±0.003	9.85
	+	141±1.3	2.6±0.1	414±4	8019±54	27857±408	67.4±1.3	1.56±0.01	11.06

Note: ¹ K_{on} was calculated from K_{off} and K_d with this equation: K_{on} = K_{off}/K_d

Supplementary Table 4. *In vitro* biophysical properties of NEMO sensors

Sensor	*1Mg ²⁺	Ca ²⁺	ρ ^{*2}		ε _{max} (mM ⁻¹ *cm ⁻¹)		Φ		F ₁ ^{*3} (mM ⁻¹ *cm ⁻¹)		pKa
			Anionic	Neutral	Anionic	Neutral	Anionic	Neutral	Anionic	Neutral	
GCaMP6m	-	+	0.47±0.00	0.53±0.00	82.18±1.48	40.60±0.65	0.65	0.14	25.10±0.45	3.01±0.05	7.18±0.09
		-	0.05±0.05	0.95±0.01	60.89±12.06	40.10±1.35	0.38	0.09	1.15±0.05	3.42±0.13	/
	+	+	0.46±0.02	0.54±0.02	77.99±2.95	38.16±4.43	0.66	0.14	23.66±1.22	2.87±0.26	7.27±0.17
		-	0.04±0.01	0.96±0.01	85.96±13.50	35.89±1.56	0.38	0.09	1.11±0.13	3.11±0.11	/
NCaMP7	-	+	0.84±0.02	0.16±0.02	116.40±2.20	42.22±4.71	0.75	0.16	73.67±2.70	1.03±0.05	6.07±0.62
		-	0.21±0.01	0.79±0.01	14.99±0.33	51.92±2.50	0.35	0.08	1.12±0.07	3.26±0.10	/
	+	+	0.85±0.02	0.15±0.02	107.10±0.91	38.60±3.12	0.72	0.18	65.63±1.73	1.02±0.04	4.93±0.33
		-	0.22±0.01	0.78±0.01	15.29±1.26	52.16±1.95	0.40	0.08	1.34±0.06	3.26±0.16	/
NEMOc	-	+	0.85±0.02	0.15±0.02	113.20±3.44	40.31±1.14	0.67	0.18	64.54±3.02	1.08±0.08	5.07±0.33
		-	0.20±0.02	0.80±0.02	7.35±1.13	49.56±0.91	0.29	0.06	0.43±0.06	2.37±0.01	/
	+	+	0.86±0.01	0.14±0.01	111.40±0.91	41.81±1.67	0.67	0.17	64.26±2.67	0.99±0.05	4.97±0.43
		-	0.19±0.01	0.81±0.01	7.68±0.25	50.07±1.59	0.15	0.07	0.22±0.01	2.84±0.06	/

Note: *1 The effect of Mg²⁺ was tested because the free cytosolic Mg²⁺ concentration is in the range of mM and the presence of Mg²⁺ might affect the dynamic range of NCaMP7 (Table 1 in Subach et al, 2020), the parental template of NEMO sensors. *2 ρ is the relative concentration of chromophore; *3 F₁ is one-photon brightness defined as the product of ρ, ε and Φ.

Supplementary Table 5. Two-photon biophysical properties of NEMO sensors *in vitro*.

Sensor	Mg ²⁺	Ca ²⁺	σ ₂ ^{*1} (GM)		F ₂ ^{*2} (GM)	
			Anionic	Neutral	Anionic	Neutral
GCaMP6m	+	+	18.18±1.2	24.41±1.55	5.52±0.37	1.85±0.12
NCaMP7	+	+	42.37±4.13	16.9±3.69	25.93±2.53	0.46±0.10
NEMOc	+	+	51.94±6.07	21.59±5.01	29.93±3.50	0.51±0.12

Note: *1 σ₂ is two-photon cross-sections. *2 F₂ is two-photon brightness defined as the product of ρ, Φ and σ₂; ρ, Φ are from Supplementary table 4.

Supplementary Table 6. Sequences of primers used to generate GECI constructs listed in Supplementary Table 1&2.

No.	Sequence (5' to 3')
1	ACCGAGCTCGGATCCGCCACCATGGGTTCTCATCATCATC
2	GCTCCCTCCGGTACCGCCCTTGACAGCTCGTCCATGC
3	GGCGGTACCGGAGGGAGCATGGTGTCCAAGGGCGAAGA
4	TGGATATCTGCAGAATTCTCACTTCGCTGTCATCAT
5	TTCTTGCTTCTGCACCACTCGAGTGAGCTCAGCCGACCTATAG
6	CTATAGGTCGGCTGAGCTCACTCGAGTGGTGCAGAAGCAAGAA
7	TCAGTCAGTTGGTCCGGCAGGTCGGCGGCTGTGAGGCT
8	GACAGCCGCCGACCTGCCGGACCAACTGACTGAAGAGC
9	AAGGTGCTGATGATGGTCTCGAGTGAGCTCAGCCGACCTATAG
10	CTATAGGTCGGCTGAGCTCACTCGAGACCATCATCAGCACCTT
11	GCTCTTCAGTCAGTTGGTCCGGCAGCTTGTATTGGGGTAGGT
12	CCCCAATGACAAGCTGCCGGACCAACTGACTGAAGAGC
13	GGTACCGAGCTCGGATCCGCCACCATGCATCATCATCATCATGTGAGCAAGGGCGA
14	CTTGGCGGTCATCATCTG
15	CAGATGATGACCGCCAAGAGAAGAAAATGGAAT
16	CAGATGATGACCGCCAAGGGCAGAAGAAAATGGAAT
17	CAGATGATGACCGCCAAGGGCGGCAGAAGAAAATGGAAT
18	CAGATGATGACCGCCAAGGGCGGCAGCAGAAGAAAATGGAAT
19	CAGATGATGACCGCCAAGGGCGGCGGAAGCAGAAGAAAATGGAAT
20	CAGATGATGACCGCCAAGGGCGGCGGAGGCAGCAGAAGAAAATGGAAT
21	CAGATGATGACCGCCAAGGGCGGCAGCGAGGAAGCAGAAGAAAATGGAAT
22	CAGATGATGACCGCCAAGGGCGGCGGCAGCGAGGAAGCAGAAGAAAATGGAAT
23	CAGATGATGACCGCCAAGGGCGGCGGCAGCGAGGAGGAAGCAGAAGAAAATGGAAT
24	CTGGATATCTGCAGAATTCTTACTTGACAGCTCGTCCAT
25	TCTTCTGTCAGATCGTCGTGGGCTT
26	AGGCTAAACTCCTCCTTGAA
27	AGGCTAAACAGCTCCTTGAAT
28	TGTCGAACAGGCTAAAGTCCTCCTTGAATTCGGC
29	TGTCGAACAGGCTAAATTGCTCCTTGAATTCGGC
30	GGCCCAGGCTTCTGAACACTGTGCCCAGC
31	TCTGTGGGATTAAGGCCAGGCTTCT
32	TCGGCGAAGTAGATGCTAGAAAGTC
33	AAGCCACGACGATCTGACAGAAGA
34	TTCAAGGAGGAGTTTAGCCTG
35	TTCAAGGAGCTGTTTAGCCT
36	GCCGAATTCAAGGAGGACTTTAGCCTGTTCGACA
37	GCCGAATTCAAGGAGCAATTTAGCCTGTTCGACA
38	TGGGCACAGTGTTTACAAGCCTGGGCCA
39	AGAAGCCTGGGCCTTAATCCCACAGA
40	TTCTAGCATCTACTTCGCCGA
41	ACAGGCTAAACTCGGCCTTGAATTCGGC
42	AATTCAAGGCCGAGTTTAGCCTGTTCGACA
43	TCTGTGGGATTAAGGCCAGGCTTCT
44	TCTGTGGGACTTTGGCCAGG
45	AGAAGCCTGGGCCTTAATCCCACAGA
46	CCTGGGCCAAAGTCCCACAGA
47	GGCGGTGGCCACCTCCTTGAAGGTCTTCTTGC GGCTGCCTCCGCCGCCCTTGGCGGTCA
48	GGAGGTGGCCACCGCGTGAAGATCATCGCCGCTGATGGGCATGTAATTCGCCGACT
49	TGACCAGGATCACAGTGGTCAGGCTGGGGATGTGCTTGACGCTGCCTCCGCCGCCCTTG
50	CTGTGATCCTGGTCAAGTCTATGCTGAGAAAGCGTTCCTTTGGAAACCATTTATGTACTTCGCCGACTG
51	GGACTTGATATTAACAAGACTGGAGGAGGTTCTCATCATCATCATCATGTGAGCAA
52	ATGTCGAGAACGAGCTTGCTCTTAAGTTGGCTGGACTTGATATTAACAAG

53	GGTACCGAGCTCGGATCCGCCACCATGCTGCAGAACGAGC
54	CTGTGCTGGATATCTGCAGAATTCTTAGGTGAAGGCCTTCTGCCACTC
55	TGGACGAGCTTTACAAGATGCATCATCATCATCATGTGAGCAAGGGC
56	CTTGATATTAACAAGACTGGAGGGATCACTCTCGGCATGGACGAGCTTTACAAG
57	GTGGTGGTGGTGGTGGTGTCTCGAGTTACTTGTACAGCTCGTCCATGCCCAT
58	TGGAGGAGAACCCAGGCCCAATGCATCATCATCATCATCATGTGAGCAAGGGCGA
59	ATGGTGTCTAAGGGCGAAGAGCTGA
60	TGGCCTGGGTTCTCCTCCACGTCGCCGGC
61	CTTTAAGAAGGAGATATACCATGCATCATCATCATCATGTGAGCAAGGGCGA
62	TTCTTGCTTCTGCACCAAGTCGGCCTCGAGTGAGCTCAGCCGACCTATAG
63	CTATAGGTCGGCTGAGCTCACTCGAGGCCGACTGGTGCAGAAGCAAGA
64	TCAGTCAGTTGGTCCGGCAGGGCTGTCAGGCTATTGGT
65	ACCAATAGCCTGACAGCCCTGCCGGACCAACTGACTGA
66	GCTGGATATCTGCAGAATTCTTACTTCGCTGTCATCAT
67	ACCGAGCTCGGATCCGCCACCATGGGTTCTCATCATCATC
68	GAGTTCGTGACCGCCGCCGAGGAGGAGAACATGG