

Supplementary Information

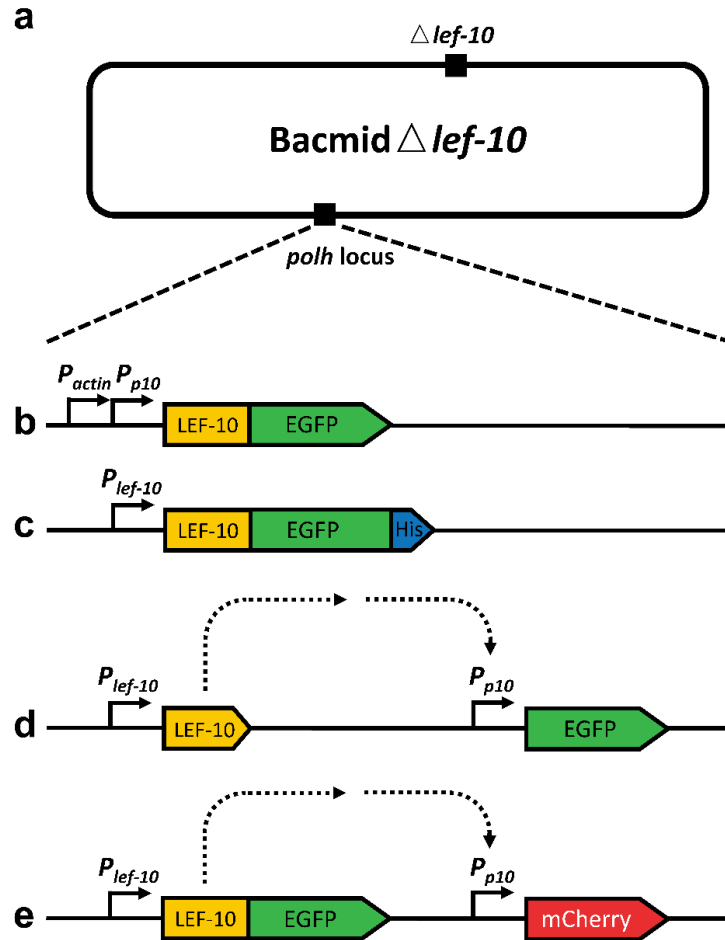
A viral expression factor behaves as a prion

Nan et al.

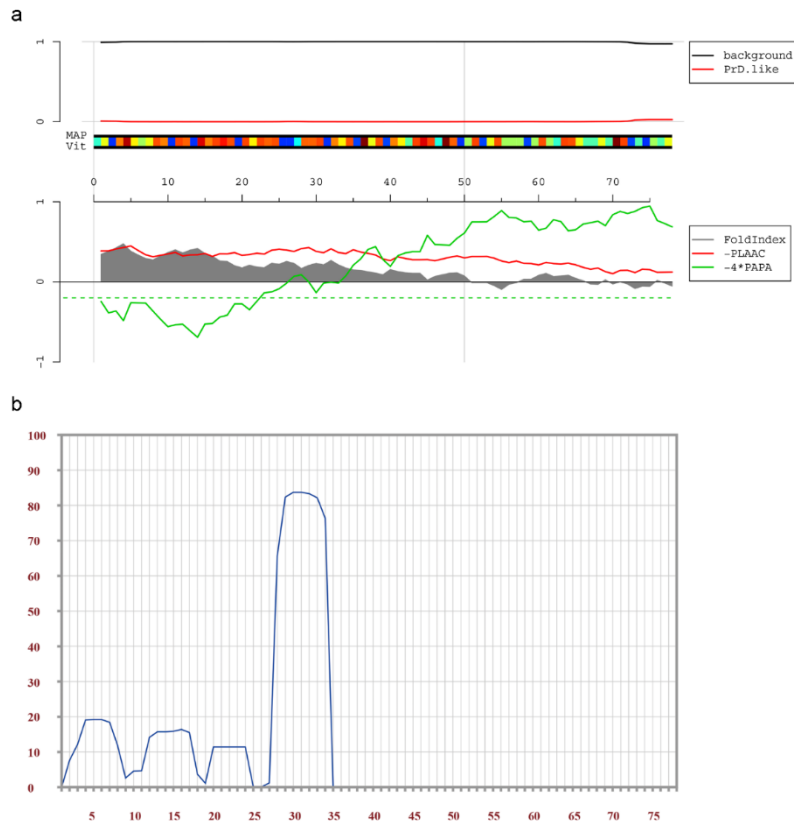
This file includes:

Supplementary Figure 1-9

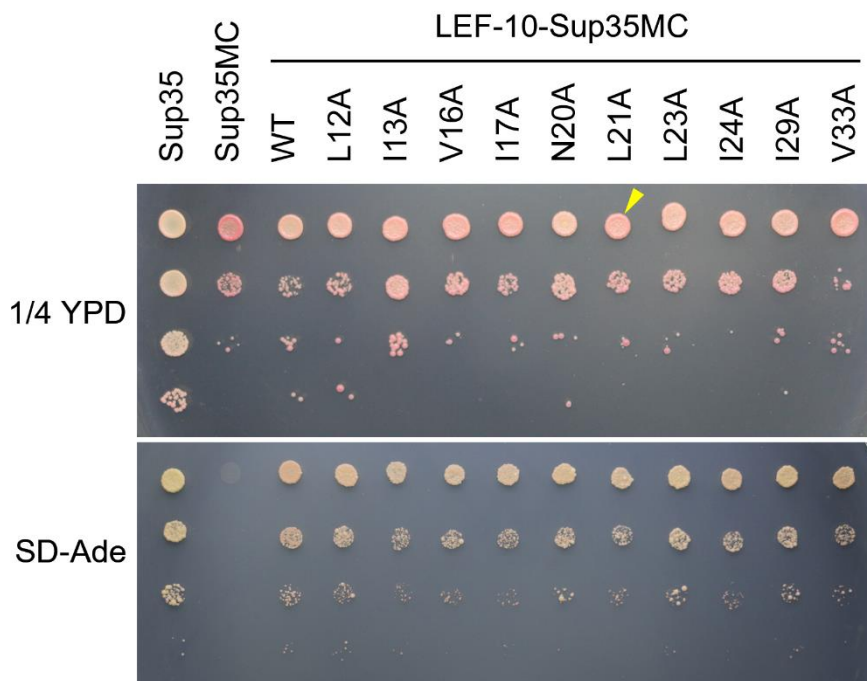
Supplementary Table 1-4



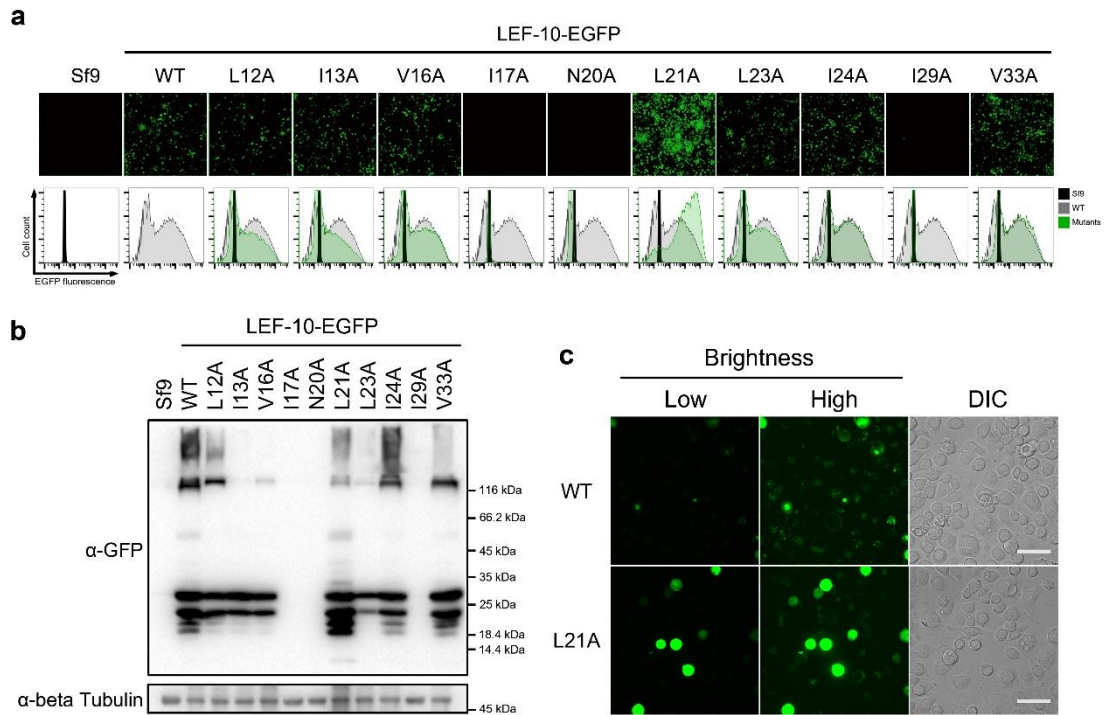
Supplementary Figure 1 Diagram of generation of recombinant baculoviruses based on the *lef-10* knock-out Bacmid via homologous recombination. Homologous recombination occurs between the Bacmid $\Delta lef-10$ (a) and cassette fragments shown in b, c, d and e. a Bacmid $\Delta lef-10$ comprises a *polh* locus as the recombination site²⁵. b LEF-10-EGFP was regulated by two tandem promoters. LEF-10-EGFP gene driven by *chicken actin* promoter could rescue Bacmid $\Delta lef-10$ as the promoter can drive the gene expression immediately after transfection or infection, and LEF-10-EGFP was over-expressed by the regulation of *p10* promoter (a strong promoter) in the late stage of infection. c LEF-10-EGFP gene driven by native *lef-10* promoter could rescue Bacmid $\Delta lef-10$. d LEF-10 gene driven by its native promoter could rescue Bacmid $\Delta lef-10$, and *p10* promoter driven EGFP could report the expression of baculovirus late gene. e LEF-10-EGFP gene driven by its native promoter could rescue Bacmid $\Delta lef-10$, and *p10* promoter driven mCherry could report the expression of baculovirus late gene.



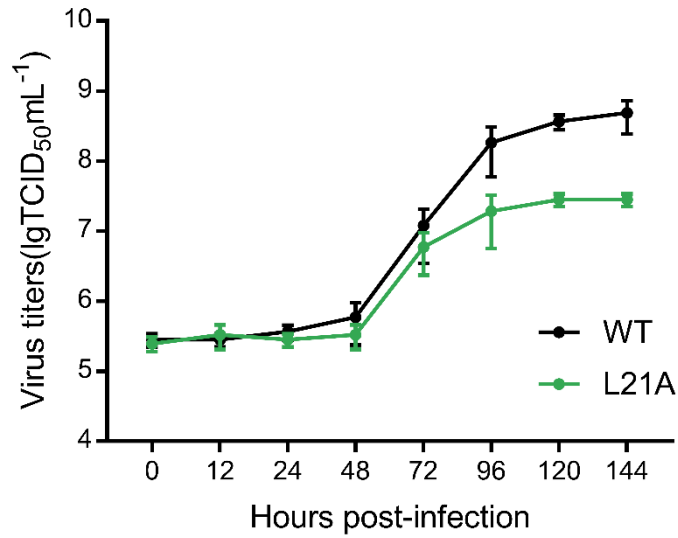
Supplementary Figure 2 Computational analysis of LEF-10. **a** PLAAC prediction tools show that LEF-10 does not contain a prion-like domain. **b** The TANGO algorithm predicts that LEF-10 contains four aggregating regions, including three low probability regions and one high probability region.



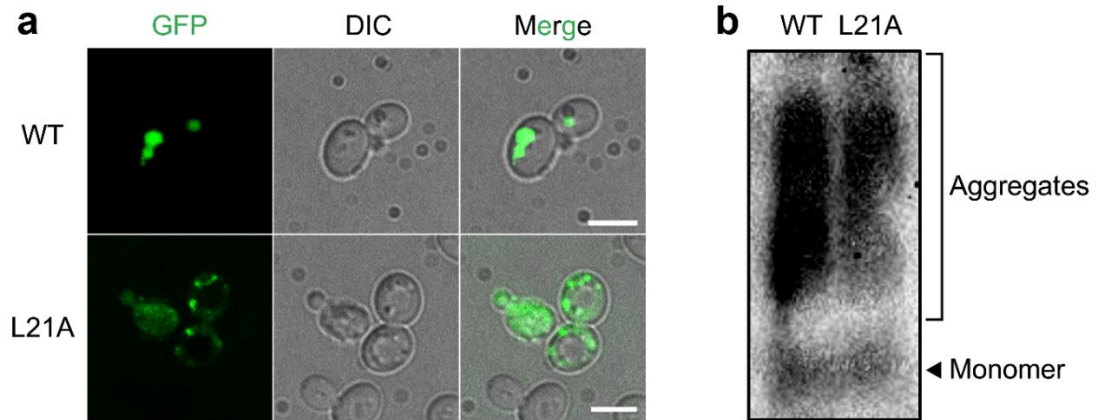
Supplementary Figure 3 Alanine substitution assays for ten highly conserved amino acid residues in LEF-10 C1 region. Alanine substitution assays were performed to assess the role of the ten highly conserved amino acid residues in maintaining the LEF-10 prion properties. The strains harboring LEF-10-Sup35MC mutants showed varying degree of color change, from white to faint red. All strains growing on SD-Ade medium indicated the read-through of *ade1-14* premature stop codon, and the result suggested that the single alanine substitutions of these conserved amino acid residues in LEF-10 could not fully abolish the prion prone of LEF-10.



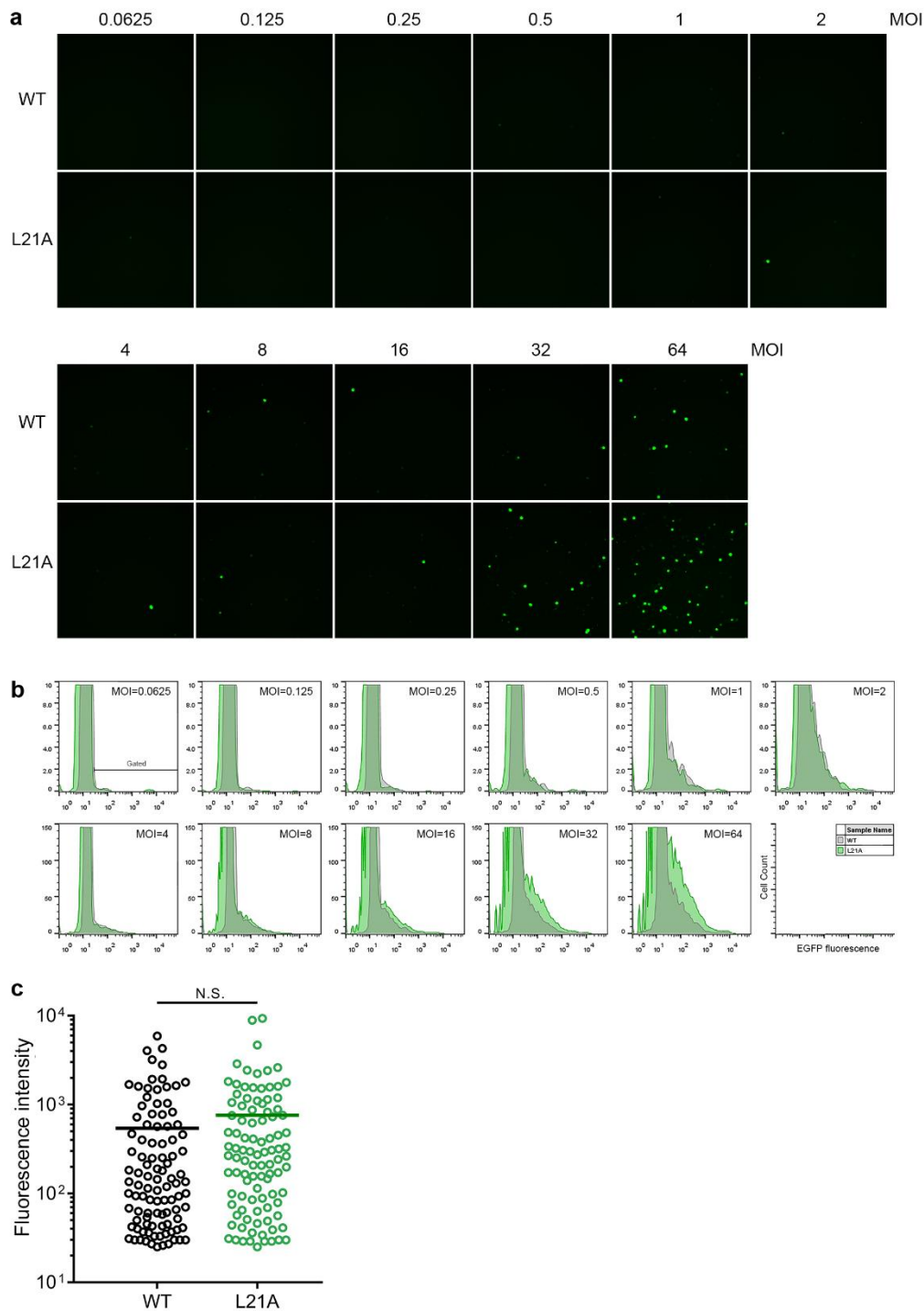
Supplementary Figure 4 Virus rescue assays using over-expressed wild-type LEF-10 and LEF-10 mutants. Wild-type LEF-10 and 10 LEF-10 mutants fused with EGFP were driven by a tandem *actin/p10* promoter (Supplementary Fig. 1b). **a** Baculovirus-infected *Sf9* cells were observed under a fluorescence microscope (top panel) and analyzed by flow cytometry (bottom panel) at 48 hpi. Among the LEF-10 mutants, LEF-10^{L21A} displayed much higher activity than wild-type LEF-10, while three LEF-10 variants (LEF-10^{I17A}, LEF-10^{N20A} and LEF-10^{I29A}) failed to rescue viruses. Other LEF-10 mutants showed similar activity with wild-type LEF-10 in the virus rescue assay. Uninfected *Sf9* cells were examined as control. **b** Western blot analysis of baculovirus-infected *Sf9* cells expressing LEF-10-EGFP chimeras. The protein levels detected by an anti-GFP antibody were consistent with their corresponding fluorescence intensity (**a**). The protein level of beta-tubulin in the cell lysates was determined as a loading control. **c** Laser confocal microscopy images of over-expressed LEF-10-EGFP and LEF-10^{L21A}-EGFP in infected *Sf9* cells at 48 hpi. Wild-type LEF-10-EGFP formed aggregates, whereas the LEF-10^{L21A}-EGFP mutant is evenly distributed in baculovirus-infected *Sf9* cells with low brightness. Non-diffuse fluorescence exhibited by LEF-10^{L21A}-EGFP could be observed in some cells with high brightness. Scale bar, 50 μ m.



Supplementary Figure 5 One-step growth curve of two recombinant viruses expressing LEF-10 and LEF-10^{L21A}. *Sf9* cells were infected with recombinant viruses expressing LEF-10 or LEF-10^{L21A} at an MOI of 0.5. The virus titers were determined by a TCID₅₀ endpoint dilution assay. The virus titers were converted to log base 10. TCID₅₀, 50% tissue culture infective dose. Data are represented as mean±SD from three replicates. Source data are provided as a Source Data file.

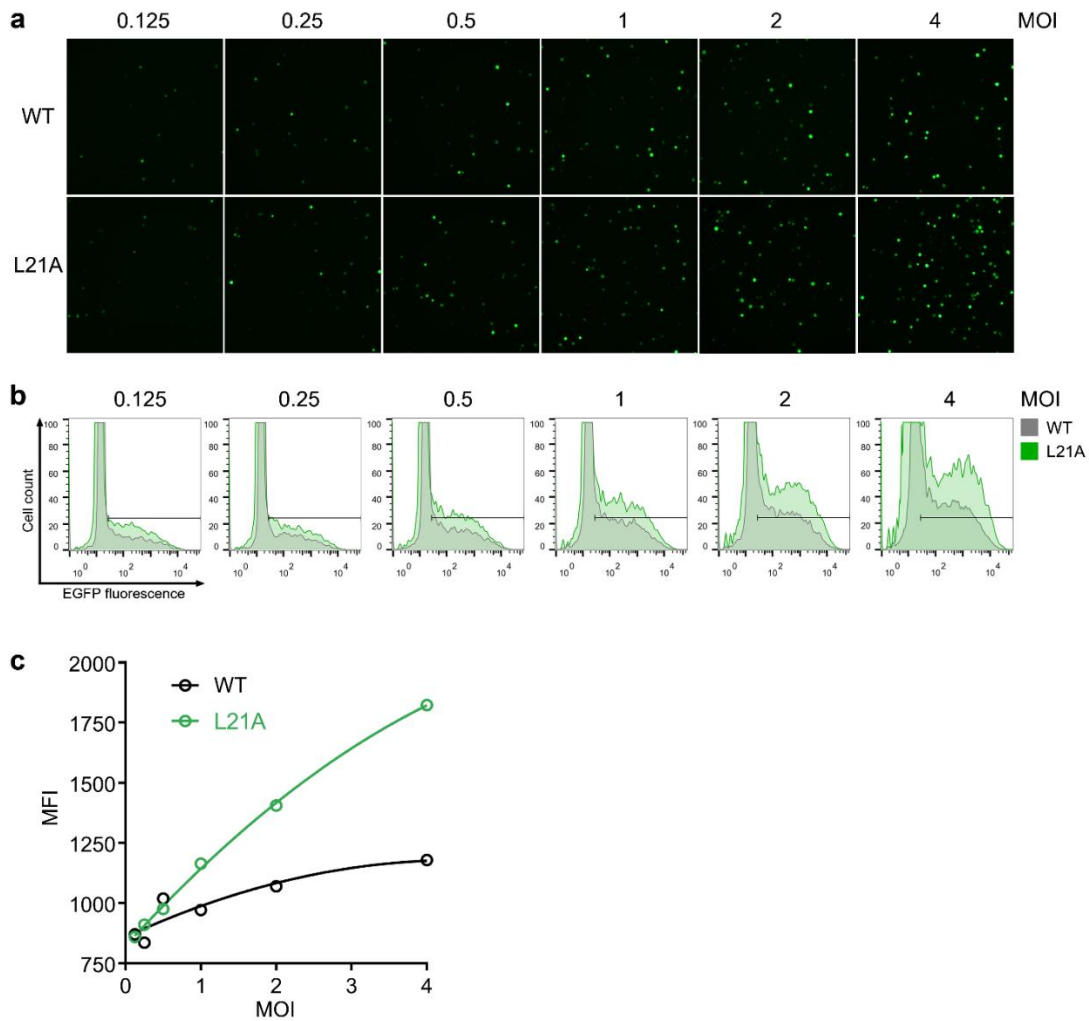


Supplementary Figure 6 Characterization of GFP-tagged LEF-10 and GFP-tagged LEF-10^{L21A} in yeast cells. **a** Laser confocal microscopy images displayed that LEF-10-GFP and LEF-10^{L21A}-GFP fusion proteins exhibit different non-diffuse fluorescence patterns in yeast cell after 4 hours expression induced by 25 μ M CuSO₄. LEF-10-GFP could form foci, whereas the LEF-10^{L21A}-GFP formed punctate fluorescence pattern. Scale bar, 5 μ m. **b** SDD-AGE analysis of LEF-10-GFP and LEF-10^{L21A}-GFP fusion proteins. The SDS-resistant aggregates could be detected in cell lysates of yeast strains containing LEF-10-GFP and LEF-10^{L21A}-GFP fusion proteins using α -GFP antibody.



Supplementary Figure 7 Comparison of LEF-10-regulated late gene expression in infected *Sf9* cells. *Sf9* cells were infected with recombinant baculovirus encoding LEF-10 or LEF-10^{L21A} under the control of native *lef-10* promoter. EGFP driven by *p10* promoter was detected as a reporter of late gene expression (Supplementary Fig. 1d). The sensor system functions by transmitting the aggregation state of LEF-10 to downstream gene reporter

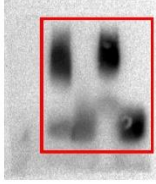
EGFP outputs. Two-fold dilutions of viruses at MOI of 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 were used for the *Sf9* cell infection. Infected cells were imaged and collected at 36 hpi. **a** Fluorescence images showed that the baculovirus late gene expression regulated by LEF-10 was at comparable levels with that regulated by LEF-10^{L21A} at low MOIs (MOI≤8). However, following the protein expression increase at high MOIs, the late gene expression regulated by LEF-10 was significantly lower than the gene expression regulated by LEF-10^{L21A}. **b** Flow cytometry analysis confirmed that baculovirus late gene expression regulated by LEF-10 was close to the gene expression levels regulated by LEF-10^{L21A} at low MOIs (MOI≤8), but was much lower than that regulated by LEF-10^{L21A} at high MOIs. **c** Fluorescence intensity of *p10* promoter-driven EGFP in 100 infected cells. 100 infected *Sf9* cells were gated (MOI≤1) and their EGFP fluorescence intensity was measured at 36 hpi using FlowJo[®] software. Bars represented mean. Two-tailed unpaired Student's *t*-test was performed (N.S., not significant). *P* value =0.2094.



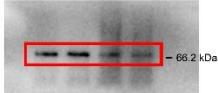
Supplementary Figure 8 Comparison of mean fluorescence intensity (MFI) in infected *Sf9* cells. *Sf9* cells were infected with recombinant baculovirus encoding LEF-10 or LEF-10^{L21A} under the control of the native *lef-10* promoter. EGFP driven by *p10* promoter was detected as a reporter of late gene expression (Supplementary Fig. 1d). Two-fold dilutions of viruses at MOI of 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 were used for the *Sf9* cell infection. Images were captured by fluorescence microscope (**a**) and cell samples were collected and analyzed by flow cytometry (**b**, **c**), at 60 hpi. **a** Fluorescence images showed that the baculovirus late gene expression regulated by LEF-10 was at comparable levels with that regulated by LEF-10^{L21A} at low MOIs (MOI≤0.5). However, at high MOIs, the late gene expression regulated by LEF-10 was significantly lower than the gene expression regulated by

LEF-10^{L21A}. **b, c** Flow cytometry analysis confirmed that baculovirus late gene expression regulated by LEF-10 was close to the gene expression levels regulated by LEF-10^{L21A} at low MOIs, but was much lower than that regulated by LEF-10^{L21A} at high MOIs. Cells were infected in parallel with a serial dilutions of viruses expressing wild-type LEF-10 or the LEF-10^{L21A} mutant. In all samples, infected *Sf9* cells were gated and the mean fluorescence intensity (MFI) was calculated by FlowJo[®] software. Source data are provided as a Source Data file.

Fig. 2b upper
SDD-AGE: Anti-Sup35C



middle
Western Blot: Anti-Sup35C



bottom
Western Blot: Anti-PGK1

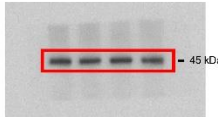
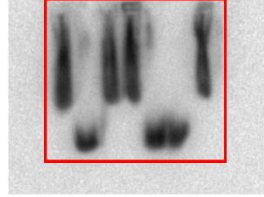
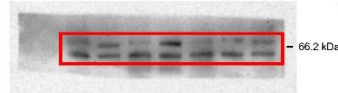


Fig. 4b upper
SDD-AGE: Anti-Sup35C



middle
Western Blot: Anti-Sup35C



bottom
Western Blot: Anti-PGK1

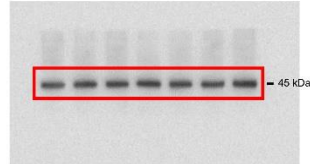


Fig. 5g SDD-AGE: Anti-His

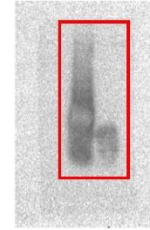
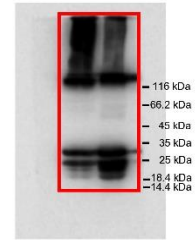
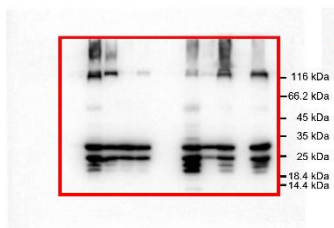


Fig. 5h Western Blot: Anti-His



Supplementary Figure 4b

upper
Western Blot: Anti-GFP

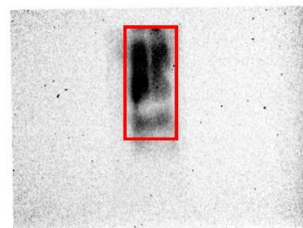


bottom
Western Blot: Anti-β-tubulin



Supplementary Figure 6b

SDD-AGE: Anti-GFP



Supplementary Figure 9 Uncropped scans of immunoblotting results. Red boxes highlight lanes used in figures.

Supplementary Table 1 Plasmids used in this study.

Vectors Name	Plasmid Description	Source
pUKC1620	pRS313- <i>P_{sup35}</i> :Sup35	50
pUKC1620-ΔN	pRS313- <i>P_{sup35}</i> :Sup35MC	this study
pUKC1620-LEF-10	pRS313- <i>P_{sup35}</i> :LEF-10-Sup35MC	this study
pUKC1620-LEF-10 ₁₋₄₁	pRS313- <i>P_{sup35}</i> :LEF-10 ₁₋₄₁ -Sup35MC	this study
pUKC1620-LEF-10 ₃₅₋₆₂	pRS313- <i>P_{sup35}</i> :LEF-10 ₃₅₋₆₂ -Sup35MC	this study
pUKC1620-LEF-10 ₅₄₋₇₈	pRS313- <i>P_{sup35}</i> :LEF-10 ₅₄₋₇₈ -Sup35MC	this study
pUKC1620-LEF-10 ₁₂₋₃₄	pRS313- <i>P_{sup35}</i> :LEF-10 ₁₂₋₃₄ -Sup35MC	this study
pUKC1620-LEF-10 ^{L12A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{L12A} -Sup35MC	this study
pUKC1620-LEF-10 ^{L13A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{L13A} -Sup35MC	this study
pUKC1620-LEF-10 ^{V16A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{V16A} -Sup35MC	this study
pUKC1620-LEF-10 ^{I17A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{I17A} -Sup35MC	this study
pUKC1620-LEF-10 ^{N20A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{N20A} -Sup35MC	this study
pUKC1620-LEF-10 ^{L21A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{L21A} -Sup35MC	this study
pUKC1620-LEF-10 ^{L23A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{L23A} -Sup35MC	this study
pUKC1620-LEF-10 ^{L24A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{L24A} -Sup35MC	this study
pUKC1620-LEF-10 ^{I29A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{I29A} -Sup35MC	this study
pUKC1620-LEF-10 ^{V33A}	pRS313- <i>P_{sup35}</i> :LEF-10 ^{V33A} -Sup35MC	this study
p6431	pRS313- <i>P_{CUP1}</i> :GFP	S. L. L.
p6431-LEF-10	pRS313- <i>P_{CUP1}</i> :LEF-10-GFP	this study
p6431-LEF-10 ^{L21A}	pRS313- <i>P_{CUP1}</i> :LEF-10 ^{L21A} -GFP	this study
pTriEx-LEF-10-EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10-EGFP	this study
pTriEx-LEF-10 ^{L12A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{L12A} -EGFP	this study
pTriEx-LEF-10 ^{L13A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{L13A} -EGFP	this study
pTriEx-LEF-10 ^{V16A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{V16A} -EGFP	this study
pTriEx-LEF-10 ^{I17A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{I17A} -EGFP	this study
pTriEx-LEF-10 ^{N20A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{N20A} -EGFP	this study
pTriEx-LEF-10 ^{L21A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{L21A} -EGFP	this study
pTriEx-LEF-10 ^{L23A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{L23A} -EGFP	this study
pTriEx-LEF-10 ^{L24A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{L24A} -EGFP	this study
pTriEx-LEF-10 ^{I29A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{I29A} -EGFP	this study
pTriEx-LEF-10 ^{V33A} -EGFP	pTriEx- <i>P_{actin-p10}</i> :LEF-10 ^{V33A} -EGFP	this study
pTriEx- <i>P_{lef-10}</i> -LEF-10-EGFP	pTriEx- <i>P_{lef-10}</i> -LEF-10-EGFP-His	25
pTriEx- <i>P_{lef-10}</i> -LEF-10 ^{L21A} -EGFP	pTriEx- <i>P_{lef-10}</i> -LEF-10 ^{L21A} -EGFP-His	this study
pTriEx-EGFP	pTriEx- <i>P_{p10}</i> :EGFP	this study
pTriEx-LEF-10/EGFP	pTriEx- <i>P_{lef-10}</i> :LEF-10/ <i>P_{p10}</i> :EGFP	this study
pTriEx-LEF-10 ^{L21A} /EGFP	pTriEx- <i>P_{lef-10}</i> :LEF-10 ^{L21A} / <i>P_{p10}</i> :EGFP	this study
pTriEx-LEF-10-EGFP/mCherry	pTriEx- <i>P_{lef-10}</i> :LEF-10-EGFP/ <i>P_{p10}</i> :mCherry	25
pTriEx-LEF-10 ^{L21A} -EGFP/mCherry	pTriEx- <i>P_{lef-10}</i> :LEF-10 ^{L21A} -EGFP/ <i>P_{p10}</i> :mCherry	this study

Supplementary Table 2 *Saccharomyces cerevisiae* strains used in this study.

Strains Name	Genotype	Construction
LJ14	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; p[SUP35-URA3]; [PS ⁺]	50
LJ14-SUP1	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620; [PS ⁺]	[PS ⁺]; plasmid shuffle using LJ14
LJ14-SUP2	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620; [ps ⁱ]	[ps ⁱ]
LJ14-SUP3	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-ΔN;	plasmid shuffle using LJ14
LJ14-LEF1	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10; [PS ⁺]-like	[PS ⁺]-like; plasmid shuffle using LJ14
LJ14-LEF2	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10; [ps ⁱ]-like	[ps ⁱ]-like; plasmid shuffle using LJ14
LJ14-LEF3	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ₁₋₄₁	plasmid shuffle using LJ14
LJ14-LEF4	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ₃₅₋₆₂	plasmid shuffle using LJ14
LJ14-LEF5	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ₅₄₋₇₈	plasmid shuffle using LJ14
LJ14-LEF6	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ₁₂₋₃₄	plasmid shuffle using LJ14
LJ14-LEF7	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{L12A}	plasmid shuffle using LJ14
LJ14-LEF8	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{I13A}	plasmid shuffle using LJ14
LJ14-LEF9	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{V16A}	plasmid shuffle using LJ14
LJ14-LEF10	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{I17A}	plasmid shuffle using LJ14
LJ14-LEF11	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{N20A}	plasmid shuffle using LJ14
LJ14-LEF12	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{L21A}	plasmid shuffle using LJ14
LJ14-LEF13	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{L23A}	plasmid shuffle using LJ14
LJ14-LEF14	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{I24A}	plasmid shuffle using LJ14
LJ14-LEF15	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{I29A}	plasmid shuffle using LJ14
LJ14-LEF16	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620-LEF-10 ^{V33A}	plasmid shuffle using LJ14

Supplementary Table 2 (count.)

Strains Name	Genotype	Construction
LJ14-LEF17	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; hsp104::ura3; pUKC1620	LJ14-SUP1 <i>hsp104::Ura3</i>
LJ14-LEF18	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; hsp104::ura3; pUKC1620-LEF-10	LJ14-LEF1 <i>hsp104::Ura3</i>
LJ14-LEF19	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; hsp104::ura3; pUKC1620-LEF-10 ₁₋₄₁	LJ14-LEF3 <i>hsp104::Ura3</i>
LJ14-LEF20	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; hsp104::ura3; pUKC1620-LEF-10 ₁₂₋₃₄	LJ14-LEF6 <i>hsp104::Ura3</i>
LJ14-LEF-10-GF P	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620; [PSI+]; p6431-LEF-10	LJ14-SUP1 transformed with p6431-LEF-10
LJ14-LEF-10 ^{L21A} - GFP	<i>MATa ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112;</i> <i>SUP35::loxP</i> ; pUKC1620; [PSI+]; p6431-LEF-10 ^{L21A}	LJ14-SUP1 transformed with p6431-LEF-10 ^{L21A}

Supplementary Table 3 Viruses used in this study.

Virus Name	Target Encoding Proteins	Source
vAc/ <i>P</i> _{lef-10} :LEF-10-EGFP- <i>P</i> _{p10} :mCherry	LEF-10-EGFP and mCherry as the late gene reporter	25
vAc/ <i>P</i> _{lef-10} :LEF-10 ^{L21A} -EGFP- <i>P</i> _{p10} :mCherry	LEF-10 ^{L21A} -EGFP and mCherry as the late gene reporter	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10-EGFP	over-expressed LEF-10-EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{L12A} -EGFP	over-expressed LEF-10 ^{L12A} -EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{I13A} -EGFP	over-expressed LEF-10 ^{I13A} -EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{V16A} -EGFP	over-expressed LEF-10 ^{V16A} -EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{I17A} -EGFP	over-expressed LEF-10 ^{I17A} -EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{N20A} -EGFP	over-expressed LEF-10 ^{N20A} -EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{L21A} -EGFP	over-expressed LEF-10 ^{L21A} -EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{L23A} -EGFP	over-expressed LEF-10 ^{L23A} -EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{I24A} -EGFP	over-expressed LEF-10 ^{I24A} -EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{I29A} -EGFP	over-expressed LEF-10 ^{I29A} -EGFP	this study
vAc/ <i>P</i> _{actin-p10} :LEF-10 ^{V33A} -EGFP	over-expressed LEF-10 ^{V33A} -EGFP	this study
vAc/ <i>P</i> _{lef-10} :LEF-10-EGFP	LEF-10-EGFP-His	25
vAc/ <i>P</i> _{lef-10} :LEF-10 ^{L21A} -EGFP	LEF-10 ^{L21A} -EGFP-His	this study
vAc/ <i>P</i> _{lef-10} :LEF-10- <i>P</i> _{p10} :EGFP	LEF-10 and EGFP as the late gene reporter	this study
vAc/ <i>P</i> _{lef-10} :LEF-10 ^{L21A} - <i>P</i> _{p10} :EGFP	LEF-10 ^{L21A} and EGFP as the late gene reporter	this study

Supplementary Table 4 Primers used in this study.

Primer Name	Sequences (5'-3')
SUPMC-FA	tcaactctagatctgaattcatgtctttgaacgact
SUPMC-FB	aacatctatatctgccactagcaacaatgtcggattcaactctagatctgaattc
SUPMC-FC	aacaggatcctctcatcgacttctcgggaataacatctatatctgccactag
SUPMC-RABC	gcgggagctctatattgagagggaag
LEF-10-1F	agcctctagaacgaacgtatggtc
LEF-10-35F	gccgtctagagaccaagaaaccgatcaag
LEF-10-54F	attatctagaaccgatgcggccccaag
LEF-10-41R	cggcgaattcaactgatcggttcttg
LEF-10-62R	tacggaattctgcatcggcttggcggc
LEF-10-78R	tagagaattccgtggacgcgttacttg
LEF-10-L12A_Fm	gcgacggacgtcaacgctatcaattgttactg
LEF-10-L12A_Rm	cagtacacaattgatagcgttgacgtccgtcg
LEF-10-I13A_Fm	acggacgtcaacctggctaattgttactgaa
LEF-10-I13A_Rm	ttcagtacacaattagccaggttgacgtccg
LEF-10-V16A_Fm	aacctgatcaattgtctctgaaagataattat
LEF-10-V16A_Rm	taaattatcttcagagcacaattgatcaggtt
LEF-10-L17A_Fm	ctgatcaattgtgtactaaagataatttatt
LEF-10-L17A_Rm	aaataaattatcttagctacacaattgatcag
LEF-10-N20A_Fm	tgtgtactgaaagatgctttattttgatagat
LEF-10-N20A_Rm	atctatcaaaaaataaagcatcttcagtacaca
LEF-10-L21A_Fm	gtactgaaagataatgctttttgatagataat
LEF-10-L21A_Rm	attatctatcaaaaaagcattatcttcagtacac
LEF-10-L23A_Fm	aaagataatttttgctatagataataattac
LEF-10-L23A_Rm	gtaattattatctatagcaataaattatcttcag
LEF-10-I24A_Fm	gataattttttggctgataataattacatt
LEF-10-I24A_Rm	aatgtaattattatcagccaaaaataaattatc
LEF-10-I29A_Fm	atagataataattacgctattttaaatgtgttc
LEF-10-I29A_Rm	gaacacattaaaaatagcgttaattatctca
LEF-10-V33A_Fm	tacatttttaaatgcttcgaccaagaacc
LEF-10-V33A_Rm	ggttcttggtcgaagcatttaaataatgta
LEF-10-C1F	agcctctagaacgaacgtatggtc
LEF-10-C3R	tagagaattccgtggacgcgttacttg
Del-test-F	attgaacctccatcggttag
hsp104-test-R	ggaacaagtgacaaaggaacg
URA3-test-R	gaaaagctgtggtatggtgcac
DeIHSP104-F	ggcaaaagggcgcaacttatgcaacctgccagattatataaaggcgagcagattgtactgagagt gcacc
DeIHSP104-R	caattccatactgtcctcattatcgtcatcacctaacgtgcagccttagtttgctggccgcatctctc
LEF10-F (y)	ccgccaccgcggtatggctagcacgaacgtatggctcgcgac
LEF10-R (y)	gagttcttctcttggctagccgtggacgcgttactttgc
Sup35-F	ccgccaccgcggtatggctagctcggattcaaccaaggcaa

Supplementary Table 4 (count.)

Primer Name	Sequences (5'-3')
Sup35MC-F	ccgccaccgcgatggctagcatgtcttgaacgactttcaaaagc
Sup35-R	gagttcttctccttgctagcctcggcaatttaacaatttacc
LEF10-TEST-F	aacgatggttcgacgga
8XHIS-TEST-R	gtgatggtgatggtgatggtg
P(lcf10)-LEF10-F	agagagatctggagtgaacgtgatgcttc
P(lcf10)-LEF10-R	attactcgagcgtggacgcttacttgc
Lef10p-F	tatatagttgctgatgcaacgtgatgcttcgag
Lef10-R	cccggattaactccgttacgtggacgcttact
pTGFP-R	atcagcaactatatattgatag
pTGFP-F	cggagttaatccgggacct
P(lcf10)-LEF-10-GFP-HIS-F	agagagatctggagtgaacgtgatgcttc
P(lcf10)-LEF-10-GFP-HIS-R	taatgaattcttagtgatggtgatggtgatggtgcttgtagcagctcgatccatgccg
GFP-TEST-F	tctcgcatggacgagctgtacaag
mCh-F	agtaacgcgtccacgggatccgactacaaagacgatgacgacaagg
mCh-R	gtggtggtggtgctcgagctgtacagctcgatccatgcc
Lef-10-Muts-F	ctgtacaagggcgggatccacgaacgtatggttcgac
Lef-10-Muts-R	cagctgtcggccgaagctttacgtggacgcttacttgc