

Research Article

Luciferase NanoLuc as a reporter for gene expression and protein levels in *Saccharomyces cerevisiae*

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Abstract

Reporter proteins are essential tools in the study of biological processes and are employed to monitor changes in gene expression and protein levels. Luciferases are reporter proteins that enable rapid and highly sensitive detection with an outstanding dynamic range. Here we evaluated the usefulness of the 19 kDa luciferase NanoLuc (Nluc), derived from the deep sea shrimp *Oplophorus gracilirostris*, as a reporter protein in yeast. Cassettes with codon-optimized genes expressing yeast Nluc (yNluc) or its destabilized derivative yNlucPEST have been assembled in the context of the dominant drug resistance marker kanMX. The reporter proteins do not impair the growth of yeast cells and exhibit half-lives of 40 and 5 min, respectively. The commercial substrate Nano-Glo® is compatible with detection of yNluc bioluminescence in < 50 cells. Using the unstable yNlucPEST to report on the rapid and transient expression of a heat-shock promoter ($P_{CYC1-HSE}$), we found a close match between the intensity of the bioluminescent signal and mRNA levels during both induction and decay. We demonstrated that the bioluminescence of yNluc fused to the C-terminus of a temperature-sensitive protein reports on its protein levels. In conclusion, yNluc and yNlucPEST are valuable new reporter proteins suitable for experiments with yeast using standard commercial substrate. © 2016 The Authors. Yeast published by John Wiley & Sons Ltd.

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Introduction

Reporter proteins are essential tools for both *in vivo* and *in vitro* experiments of biological processes. In their most fundamental form, the *in vivo* reporter proteins reflect on the expression of promoters and the levels of mRNA and proteins. For example, fusion of promoters or genes to the bacterial *LacZ* gene enables the detection of the expressed β -galactosidase activity and has been essential in the study of many aspects of gene expression (Juers *et al.*, 2012). However, although apparently simple, β -galactosidase activity assays are time-consuming and detection suffers from a poor dynamic range. Moreover, the enzyme is a large and stable tetrameric 1023 amino acid residues protein that, in the eukaryotic setting, takes

time to translate and fold, making it a slow reporter for monitoring rapid changes of gene expression in yeast. Other examples of reporter proteins include fluorescent and bioluminescent proteins (Tsien, 1998; Wilson and Hastings, 1998). Both classes facilitate rapid detection by sensitive instruments, with bioluminescence outperforming fluorescent detection.

Bioluminescent luciferases have become popular as protein reporters, due to their high sensitivity and dynamic range and the ease and speed of using the detection instruments (Thorne *et al.*, 2010). Examples include firefly (*Photinus pyralis*) luciferase (FFluc), *Renilla reniformis* luciferase (Rluc) and bacterial luciferases (*lux* operons) (de Wet *et al.*, 1985; Lorenz *et al.*, 1991; Engebrecht *et al.*, 1983). FFluc is the most commonly used

luciferase and produces green light in an ATP-dependent reaction. In budding yeast (*Saccharomyces cerevisiae*), FFluc has been used successfully for gene fusions to report on transcriptional activity (Vieites *et al.*, 1994). However, certain features make FFluc less than ideal as a reporter protein in yeast. First, it is a large multidomain protein of 61 kDa that takes time to express and exhibits slow turnover. Second, the protein denatures and form aggregates *in vivo* when cells are subjected to heat shock or other stressors that perturb protein homeostasis (proteostasis) (Nathan *et al.*, 1997). Third, folding and reactivation of FFluc is highly dependent on the potentially limiting molecular chaperones Hsp40, Hsp70 and Hsp104 (Schröder *et al.*, 1993; Glover and Lindquist, 1998). The tendency to aggregate and the chaperone-dependent reactivation have made FFluc an important tool when studying proteostasis (Hartl and Hayer-Hartl, 2002), yet the very same characteristics call for the evaluation of new and improved luciferases as reporter proteins in yeast.

Originally from the deep sea shrimp, *Oplophorus gracilirostris* (Inouye *et al.*, 2000), NanoLuc (Nluc) is a novel engineered luciferase that is smaller (19 kDa) and more stable during denaturing conditions and changes in pH and temperature than FFluc and Rluc. It uses an imidazopyrazinone substrate (furimazine), commercialized as Nano-Glo®, to produce a signal around 150-fold brighter than FFluc in an ATP-independent manner (Hall *et al.*, 2012). Nluc was originally developed as a reporter protein for human cell lines (Hall *et al.*, 2012) but has also been shown to function in bacteria (Loh and Proft, 2014). Yet the use of Nluc and its substrate in yeast has not been evaluated.

In this study, we have generated stable and destabilized Nluc genes codon-optimized for yeast expression and report on the characteristics and usefulness of this novel and highly sensitive luciferase reporter in yeast.

Materials and methods

Yeast strains and plasmids

All *S. cerevisiae* strains and plasmids used in this study are listed in Tables 1 and 2, respectively. The strains are derivatives of BY4741 (Brachmann *et al.*, 1998). CAY1015 is a meiotic segregant in the BY4741 background (Gowda *et al.*, 2013) and CAY1259 was constructed by inserting *mCherry* in the *HTB2* locus of CAY1015 (Keppler-Ross *et al.*, 2008). The *yNlucPEST* gene was synthesized codon-optimized for expression in *S. cerevisiae* and subcloned into plasmids using PCR and homologous recombination in yeast. Details regarding plasmid construction are available upon request.

Yeast media

Cells were grown in standard yeast peptone dextrose (YPD) or on ammonia-based synthetic complete dextrose (SC) medium supplemented to support the growth of auxotrophic strains. The cells were grown at 30°C.

Bioluminescence determination

Nano-Glo substrate (Promega GmbH, Germany) was diluted 1:100 with the supplied lysis buffer and mixed 1:10 with cells grown in SC in a white 96-well plate. Bioluminescence was determined immediately, using an Orion II Microplate Luminometer (Berthold Technologies GmbH & Co. KG, Germany). Bioluminescence light units (BLU) are defined as the relative light units (RLU)/s of 1 ml cells at OD₆₀₀=1.0. To monitor turnover, 1 mg/l cycloheximide (Sigma-Aldrich, St. Louis, MO, USA) or 5 µM lactimidomycin (Merck-Millipore) was added. For *in vitro* bioluminescence measurements, cells were subjected to glass bead lysis for 30 s in a bead beater and cell

Table 1. Yeast strains

Strain	Genotype	Reference/source
BY4741	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Brachmann et al., 1998
CAY1015	MATa <i>his3Δ1 leu2Δ0 ura3Δ0</i>	Gowda <i>et al.</i> (2013)
CAY1259	MATa <i>his3Δ1 leu2Δ0 ura3Δ0 HTB2::mCherry-kanMX</i>	This study

Table 2. Plasmids

Plasmid	Description	Type	Reference/source
pAM09	URA3 VC	CEN/ARS	This study
pAM10	URA3 P _{CYC1-HSE} -Nluc	CEN/ARS	This study
pCA873	URA3 P _{SSA4} -NlucPEST	CEN/ARS	This study
pCA955	URA3 P _{CYC1-HSE} -NlucPEST	CEN/ARS	This study
pGA1	URA3 P _{TEF1} -GFP- γ Nluc	CEN/ARS	This study
pGA2	URA3 P _{TEF1} -Z- γ Nluc	CEN/ARS	This study
pGA3	URA3 P _{TEF1} -EGFP-Ubc9 ^{ES} - γ Nluc	CEN/ARS	This study
pGA4	URA3 P _{TDH3} - γ Nluc-EGFP-Ubc9 ^{ES}	CEN/ARS	This study
pZJ-HSE2-26	URA3 P _{CYC1-HSE} -LacZ	2 μ	Slater and Craig (1987)

debris was removed by 10 min centrifugation at 1500 \times g. Cell-free lysates were diluted to a protein concentration of 0.05 mg/ml (Nanodrop, 280 nm) and mixed with cycloheximide before determining bioluminescence.

Determination of mRNA levels

RNA was extracted from cells grown in SC using a RiboPureTM RNA Purification Kit for Yeast (Ambion). cDNA was synthesized from DNase I-treated RNA using Superscript[®] III Reverse Transcriptase (Invitrogen). qPCR was performed using KAPA SYBR[®] Fast Universal qPCR Kit (KAPA Biosystems), with primers specific for the *yNlucPEST* (ATGGTGTACTGG TTGGCGTTTATG and GCACAAGCAGC AGGATGACGAT) and *TAF10* transcripts (ATATTCCAGGATCAGGTCTTCCGTAGC and GTAGTCTTCTCATTCTGTTGATGTTGTTGTTG). Quantification was performed using the 2^{- $\Delta\Delta$ CT} method (Livak and Schmittgen, 2001) and expression was normalized to *TAF10* (Teste *et al.*, 2009).

β -Galactosidase activity determination

β -Galactosidase activity was determined using *N*-lauroyl-sarcosine-permeabilized cells (Kippert, 1995; Andréasson and Ljungdahl, 2004). After pre-incubation of cells in 800 μ l 0.2% w/v sodium *N*-lauroyl-sarcosine Z buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, 50 mM β -mercaptoethanol, pH 7) at 30°C for 15 min, a 160 μ l aliquot of 4 g/l *o*-nitrophenyl- β -D-galactopyranoside (ONPG) solution was added and the tubes were mixed by inversion. The reaction was stopped by the addition of 400 μ l 1 M

Na₂CO₃ and cell debris was removed by centrifugation. The absorbance of the supernatant was measured at 420 nm.

Fluorescence microscopy

Transformants of strain CAY1259 carrying plasmid pGA1 were grown to logarithmic phase and images were acquired using an Axiovert 200M inverted fluorescence microscope (Carl Zeiss, Germany), Plan-Apochromate \times 63/1.4 oil DIC, DG4 light source (Sutter Instrument Novato, CA) equipped with Roper Cascade 1K camera (Carl Zeiss) and SlideBook 6 software. Binning of 2 \times 2 was used to acquire images that were processed using SlideBook Reader.

Flow-cytometric determination of GFP signal

The growth medium of cells in logarithmic growth phase was removed and exchanged for phosphate-buffered saline (PBS). Data were acquired on a FACS Calibur instrument (BD Biosciences) and average green fluorescent protein (GFP) signal was determined using FlowJo software.

Sedimentation assays

The sedimentation assay was adopted from Theodoraki *et al.* (2012). Briefly, cells were subjected to glass bead lysis and cell debris was removed by centrifugation at 700 \times g in a table-top centrifuge. The cleared lysate was centrifuged at 12 800 \times g for 15 min to separate soluble and insoluble proteins.

Western blot analysis

Protein extracts were prepared from cells in logarithmic phase (Silve *et al.*, 1991). Briefly, NaOH was added directly to the cultures to a final concentration of 0.37 M and the cells were incubated for 10 min on ice before the addition of trichloroacetic acid to a final concentration of 8.3%. After centrifugation and removal of the supernatant, the pellet was rinsed with 1 M Tris base and equal amounts of SDS-solubilized protein were separated by SDS-PAGE and analysed by quantitative western blotting, using the Odyssey infrared imaging system (Li-COR Biosciences). GFP signal (anti-GFP, Sigma-Aldrich; 1:10000) or Nanoluc signal (anti-Nanoluc, Promega GmbH, Germany; 1:1000) was normalized to the Pgl1 signal (anti-Pgl1, Life Technologies; 1:10000).

Results and discussion

Nanoluc reporters in yeast

To create a new gene reporter system based on Nluc for *S. cerevisiae*, we codon-optimized its coding sequence for optimal expression in yeast (yNluc). The leucine codon CUG was omitted from the sequence to enable the future use of the reporter in *Candida albicans*, a yeast that non-canonically reads the CUG codon as serine (White *et al.*, 1995). Since turnover of the yNluc reporter in yeast cells was not known and this characteristic greatly impacts on the usefulness of the reporter to monitor rapid expression level changes, we also codon-optimized a version of yNluc with a potentially destabilizing PEST sequence (yNlucPEST) (Marchal *et al.*, 1998). The genes encoding yNluc and yNlucPEST were introduced into plasmids in the context of the dominant G418 resistance cassette kanMX to facilitate promoter fusion by direct genomic integration, using PCR and homologous recombination (Figure 1A).

To evaluate the usefulness of the reporters in yeast, we chose to drive the expression of yNluc and yNlucPEST from a widely used heat shock-responsive promoter built from the core *CYC1* promoter (P_{CYC1}) and a heat shock element (HSE) from the Hsp70 gene *SSA1* (Slater and Craig, 1987) (Figure 1A). While wild-type cells (BY4741)

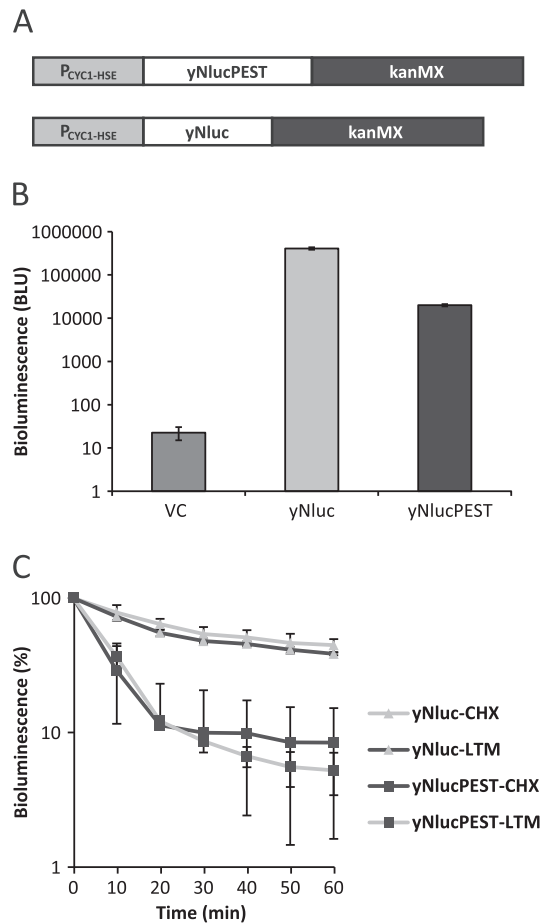


Figure 1. Expression of yNluc and yNlucPEST gene reporters in yeast. (A) Schematic representation of yNanoluc and yNlucPEST yeast codon-optimized gene reporter cassettes designed for PCR amplification and integration into the yeast genome: a dominant G418 selection marker is provided by kanMX; for the purposes of this study, the minimal *CYC1* promoter (P_{CYC1}) fused to a heat shock element (HSE) drives the expression of each reporter variant. (B) Bioluminescence (bioluminescence light units, BLU) in cells carrying vector control (VC) or expressing yNluc or yNlucPEST; error bars represent SD ($n=3$). (C) Turnover of yNluc and yNlucPEST bioluminescence following arrest of translation by cycloheximide (CHX) or lactimidomycin (LTM) addition; error bars represent SD ($n\geq 3$)

growing at 30°C and carrying empty vector control (pAM09) emitted 22 (± 7.64 SD) BLU upon addition of the Nano-Glo® substrate, expression of yNluc (pAM10) or yNlucPEST (pCA955) resulted in 18000- and 900-fold increases, respectively, of the bioluminescence (Figure 1B). Importantly, yNluc exhibited 20-fold higher bioluminescence compared to yNlucPEST, consistent with the

notion that the PEST sequence destabilizes the reporter protein in yeast.

We directly monitored the turnover of yNluc and yNlucPEST by arresting translation (cycloheximide/lactimidomycin) and followed the decay of bioluminescence. Consistent with the expression levels, the signal from yNluc was more stable over the time course than the signal from yNlucPEST, with half-lives of 40 and 5 min, respectively (Figure 1C). Apparently, a small fraction of yNlucPEST escapes inactivation and accumulates over time to represent approximately 20% of the protein population. The half-lives in yeast are generally shorter than what has been reported from expression in mammalian cells (Nluc did not exhibit turnover over 6 h and NlucPEST had a half-life of 20 min) (Hall *et al.*, 2012). The more stable yNluc accumulates to high levels and is potentially suitable for experiments that require sensitivity and a stable signal. In contrast, yNlucPEST facilitates the detection of rapid changes of reporter activity including its down-regulation. Moreover, during our experiments we found that cycloheximide did not interfere with the bioluminescent activity in cell-free lysates (see supporting information, Figure S1).

yNluc or yNlucPEST do not impair growth of yeast cells

We assessed whether yNluc and yNlucPEST expression is toxic to yeast cells. Growth measured in liquid medium over 24 h showed no difference in the growth rates of yNluc and yNlucPEST compared to VC (Figure 2A). Since $P_{CYC1-HSE}$ is a heat shock-responsive promoter, cells were grown in 10-fold dilution on solid medium at 25°C, 30°C and 37°C, to assess whether higher expression together with the additional proteotoxic stress that comes with an elevated temperatures impacts on growth. The reporters did not elicit growth inhibition at any temperature (Figure 2B). We conclude that yNluc and yNlucPEST do not impair the growth of yeast cells.

Sensitivity of yNluc or yNlucPEST bioluminescence

Sensitivity is crucial for a reporter system to obtain accurate measurements and to be able to monitor

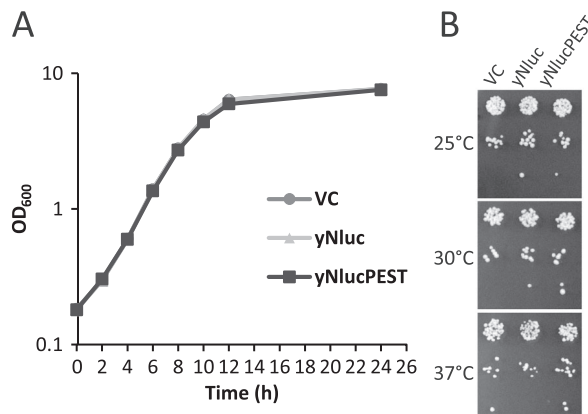


Figure 2. Growth characteristics of cells expressing yNluc or yNlucPEST. (A) Density (OD_{600}) of cells growing in liquid medium carrying vector control (VC) or expressing yNluc or yNlucPEST. (B) Ten-fold serial dilution of cells, as in (A), spotted onto solid growth medium and grown at the indicated temperatures

small changes with limited sample material. To test the sensitivity of yNluc and yNlucPEST, a serial dilution of Nano-Glo® substrate was added to 10^7 cells. For both reporters the signal decreased linearly upon dilution of the substrate until reaching levels < 100 BLU, a value similar to the background signal detected in cells without the reporter (Figure 3A).

Serial dilution of cells expressing either reporter and bioluminescent detection using 10^{-3} dilution of the Nano-Glo® substrate demonstrated that with the higher expression level of yNluc, 50 cells were sufficient to produce a signal higher than background (Figure 3B). The cell density was verified by plating. By increasing the amount of Nano-Glo® substrate, this sensitivity can be increased further to monitor even fewer cells or reporters with lower expression levels.

yNlucPEST as a reporter for transient changes of gene expression

The study of rapid and transient change of gene expression depends on reporters that recapitulate both the induction and repression of transcript levels. We investigated how transcriptional variation translated to changes in yNlucPEST expression by simultaneously monitoring bioluminescence and mRNA levels by qPCR before, during and after transient heat shock. Strikingly,

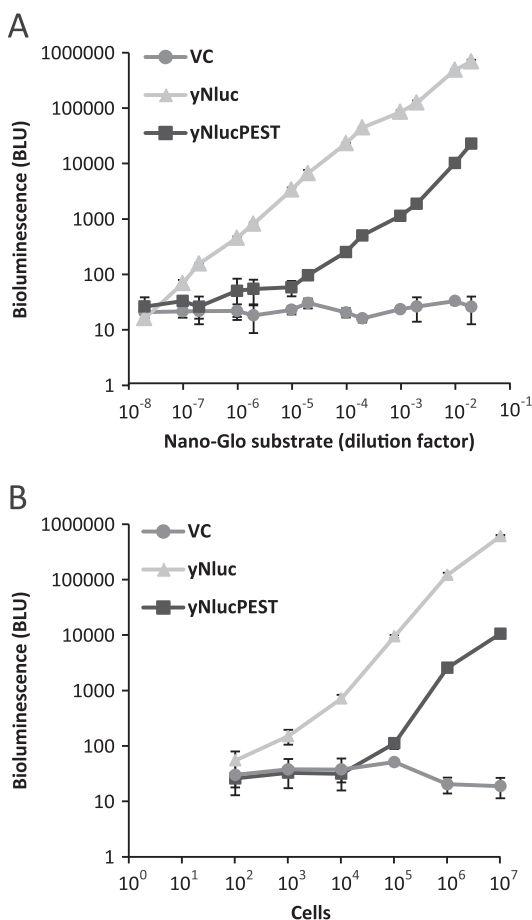


Figure 3. Sensitivity of the yNluc and yNlucPEST reporter. (A) Bioluminescence in 10^7 cells carrying VC or expressing yNluc or yNlucPEST with dilute Nano-Glo® substrate. (B) Bioluminescence detected in 10^2 – 10^7 cells expressing yNluc or yNlucPEST. Error bars represent SD ($n=3$) in both experiments

the bioluminescent signal faithfully followed the transiently induced mRNA levels of the reporter over the entire heat-shock and recovery phases (Figure 4A). Arrest of translation by cycloheximide demonstrated that the rapid turnover of the reporter was not inhibited by the heat-shock response (Figure 4B).

LacZ has commonly been used to observe changes in gene regulation, including studies of the heat-shock response (Ellwood and Craig, 1984; Nussbaum *et al.*, 2014). However, the expressed β -galactosidase is a tetrameric enzyme of 1024 amino acids that takes time to express and exhibits a half-life of >20 h (Bachmair *et al.*, 1986). The low turnover of the reporter introduces

an obstacle when measuring rapid downregulation of promoter activity. Using identical heat shock promoters to drive the expression of yNlucPEST (pCA955) and *LacZ* (pZJ–HSE2-26), we directly compared the performance of the reporter genes before, during and after transient heat shock. While yNlucPEST in real-time reported on the accumulation and decay of transcripts, the *LacZ* reporter signal accumulated slowly over 60 min to reach a stable plateau (Figure 4C). The data indicate that while *LacZ* is not a suitable reporter for studies of rapid and transient changes of gene expression, yNlucPEST is well-suited for the purpose.

Finally, we tested yNlucPEST performance in the context of the endogenous promoter *SSA4*, a gene that expresses stress-inducible Hsp70 (Werner-Washburne *et al.*, 1987). We fused the yNlucPEST to the *SSA4* promoter (pCA873) and subjected transformed cells to a transient heat stress while measuring bioluminescence. The bioluminescent signal peaked 20 min following shift to elevated temperature and then returned to pre-heat shock baseline values (Figure 4D). The behaviour of the reporter reproduces the transient induction characteristics following heat shock that have been established for *SSA4* (Saavedra *et al.*, 1996).

yNluc as a reporter to monitor protein levels

Fusion of activity tags to proteins facilitates the rapid determination of protein levels by directly monitoring the activity of the tag. This experimental approach potentially enables high-throughput screening and reduces the variability associated with quantitative western blot analysis. To assess the usefulness of yNluc as an activity fusion tag in yeast (CAY1015), we initially tested an N-terminal fusion of GFP to yNluc (GFP–yNluc; pGA1). Unexpectedly, the bioluminescence was reduced to 55% of the initial activity within 10 min following arrest of translation with cycloheximide (Figure 5A). The kinetics of loss of bioluminescence at later time points was slower, suggesting that the fusion protein populated at least two species. Importantly, loss of bioluminescence was not the result of protein degradation, since both GFP fluorescence (flow-cytometry measurements) and protein levels (Western blotting) remained stable over the entire time-course. A

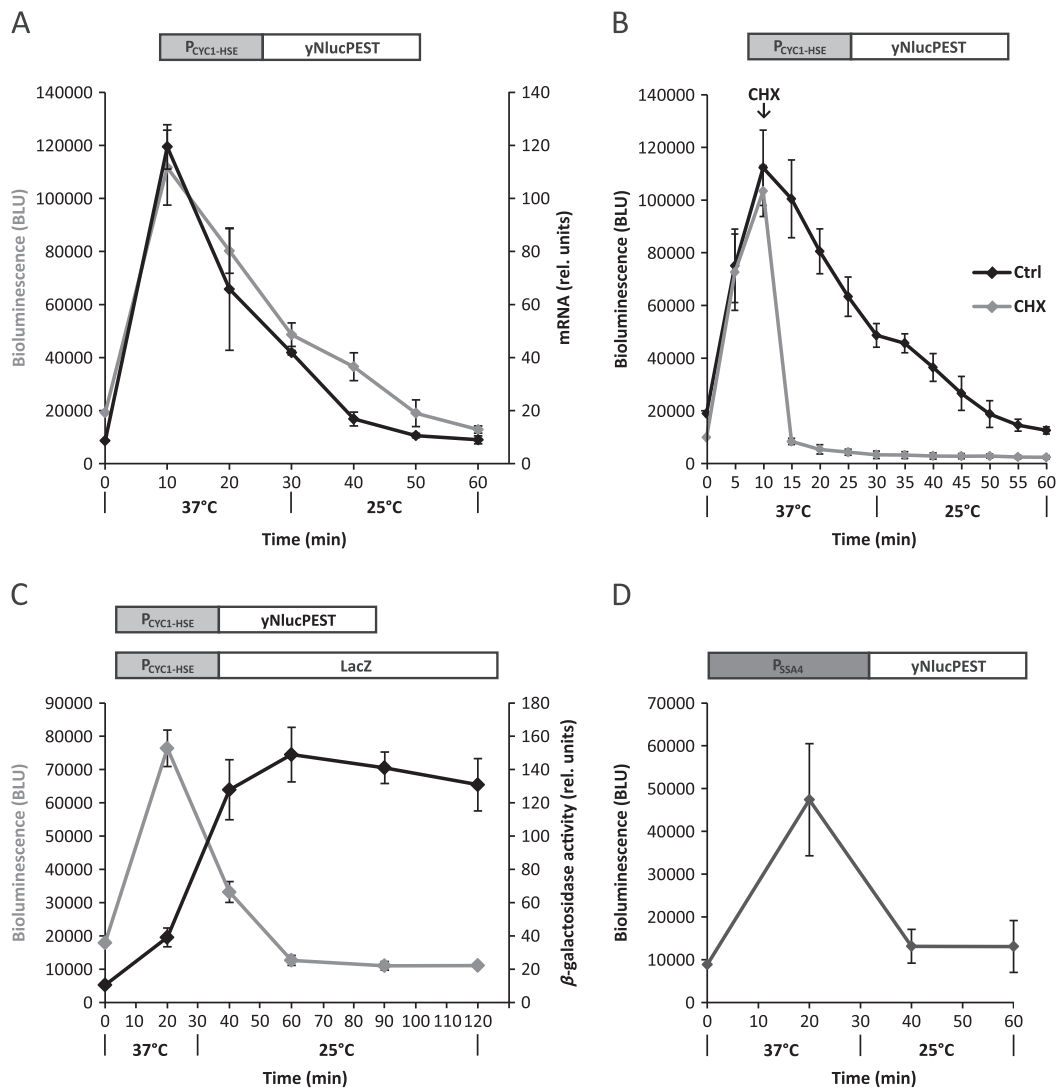


Figure 4. yNlucPEST as a reporter of rapid and transient changes of heat shock-regulated gene expression. (A) yNlucPEST expression from a heat-shock response promoter (P_{CYC1} -HSE) upon acute heat shock (37°C) followed by recovery (25°C); bioluminescence (grey line) and mRNA levels (black line) were monitored simultaneously. (B) Bioluminescence measurements, as in (A), but cycloheximide (CHX, grey line) was added to arrest translation after 10 min of heat shock. (C) Comparison of expression from the heat-shock response promoter (P_{CYC1} -HSE) using yNlucPEST and LacZ as reporter genes. (D) Bioluminescence of yNlucPEST expressed from the endogenous Hsp70 (*SSA4*) promoter during transient heat shock; cells were grown at 25°C prior to heat shock and error bars represent SD ($n = 9$) in all experiments

centrifugation-based solubility assay was performed to investigate whether the loss of bioluminescence was due to aggregation of GFP-yNluc. The protein did not populate the pellet fraction before and 10 min after cycloheximide addition (Figure 5B). Consistent with this observation, GFP-yNluc localized evenly throughout the cytoplasm of yeast (Figure 5C). Summarizing the data

for GFP-yNluc, the fusion protein is expressed as a soluble protein but a large fraction of the population rapidly loses its bioluminescence, presumably due to local unfolding that does not drive protein aggregation.

We considered the possibility that N-terminal fusions to yNluc triggered the loss of specific bioluminescence. Indeed, when fusing the well-folded

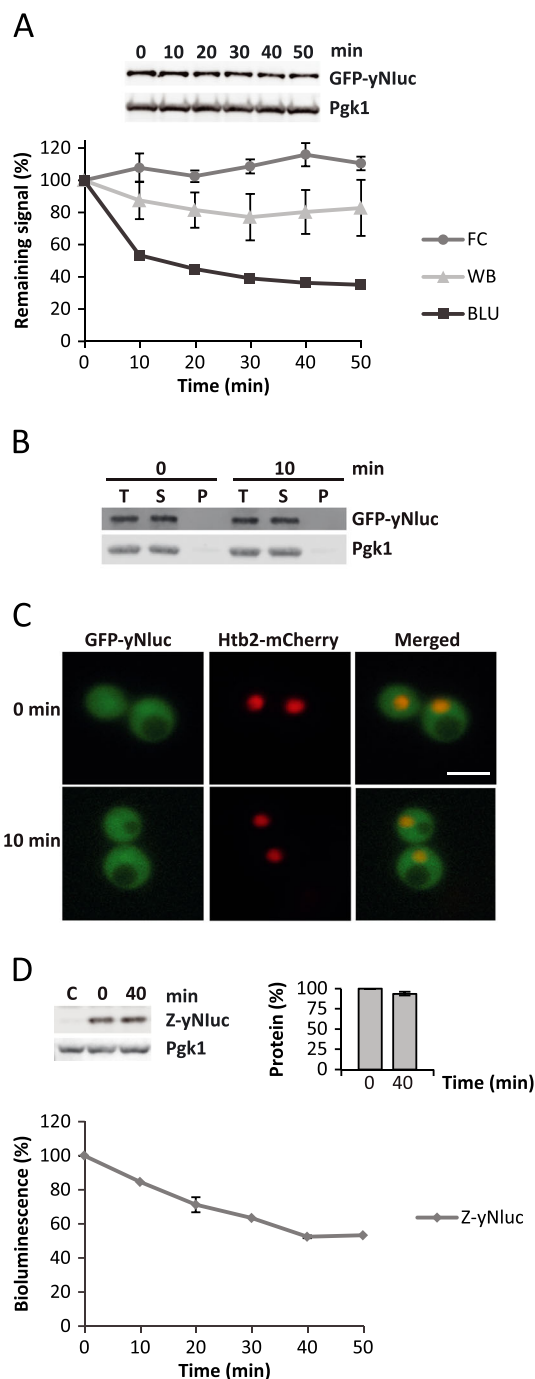


Figure 5. N-terminal fusions to yNluc triggers rapid loss of specific bioluminescence. (A) Decay of GFP-yNluc bioluminescence (BLU), protein levels (Western blot, WB) and GFP fluorescence (flow cytometry, FC) following translation arrest by cycloheximide. (B) Solubility assay showing the total (T), soluble (S) and pellet (P) fractions of centrifuged samples (12 800×g, 15 min) taken before and 10 min after cycloheximide addition. (C) Fluorescence microscopy of

triple α -helical Z domain of Protein A (Samuelsson *et al.*, 1994) to the N-terminus of yNluc (Z-yNluc; pGA2), we again observed loss of bioluminescence without loss of protein (Figure 5D). Our data suggest that N-terminal fusions to yNluc should be avoided when designing bioluminescent fusions that report on protein levels.

Next we decided to use the conditionally destabilized temperature-sensitive protein EGFP-Ubc9^{ts} to assess the applicability of yNluc fusions to monitor the protein levels. EGFP-Ubc9^{ts} is a temperature-sensitive protein that is stable and well-folded at the permissive temperature of 30°C, but that misfolds and is degraded at the non-permissive temperature of 37°C (Kaganovich *et al.*, 2008). Again we observed that the N-terminal fusion of EGFP-Ubc9^{ts} to yNluc (EGFP-Ubc9^{ts}-yNluc; pGA3) resulted in rapid loss of bioluminescence following translation arrest, even at the permissive temperature of 30°C (Figure 6A). In contrast, a C-terminal fusion of EGFP-Ubc9^{ts} to yNluc (yNluc-EGFP-Ubc9^{ts}; pGA4) was well behaved and retained 80% of the bioluminescence over the entire time course of 40 min (Figure 6B). Upon temperature shift to the non-permissive 37°C, the bioluminescence of yNluc-EGFP-Ubc9^{ts} was reduced to 50% after 40 min of translation arrest. Importantly, assessment of yNluc-EGFP-Ubc9^{ts} protein levels at time points 0 and 40 min after cycloheximide addition confirmed that loss of bioluminescence was the result of loss of protein (Figure 6C). To summarize the data, yNluc is a useful fusion reporter to monitor protein levels, but N-terminal fusions display rapid loss of specific bioluminescence.

Conclusion

The two luciferase variants yNluc and yNlucPEST, together with its commercially available substrate

GFP-yNluc and nuclear histone 2B fused to mCherry (Htb2-mCherry) before and 10 min after cycloheximide addition; scale bar = 5 μ m. (D) Decay of Z-yNluc protein levels by Western blotting (WB) and bioluminescence (BLU) following arrest of translation using cycloheximide; error bars indicate SD; $n \geq 3$.

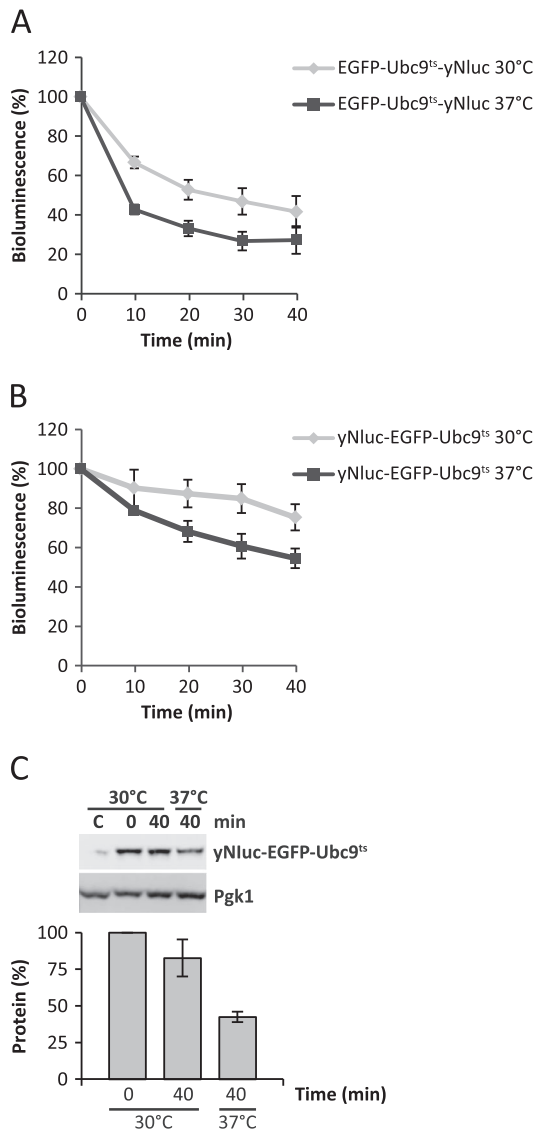


Figure 6. Bioluminescence of yNluc reports on the protein levels of C-terminally fused EGFP-Ubc9^{ts}. (A) Decay of EGFP-Ubc9^{ts}-yNluc bioluminescence (BLU) at 30°C (permissive for Ubc9^{ts}) and 37°C (non-permissive for Ubc9^{ts}) following translation arrest by cycloheximide. (B) Decay of yNluc-EGFP-Ubc9^{ts} bioluminescence, as in (A). (C) Western blot analysis of yNluc-EGFP-Ubc9^{ts} protein levels, as in (B); error bars indicate SD; $n \geq 3$

Nano-Glo®, function as sensitive reporter systems in budding yeast. The destabilized yNlucPEST facilitates the construction of reporter fusions that faithfully reproduce rapid and transient changes of gene expression. Higher expression levels, sensitivity and the option of an activity fusion tag

are offered by C-terminal fusions to the more stable yNluc. Both yNluc and yNlucPEST are available fused to kanMX for PCR amplification and direct gene targeting by homologous recombination.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Figure S1. Relative bioluminescence (RLU) in cell-free lysates prepared from yeasts that express yNluc or yNlucPEST.