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TITLE:
Light induced GFP expression in zebrafish embryos using the optogenetic TAEL/C120 system

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SUMMARY:
Optogenetics is a powerful tool with wide-ranging applications. In this protocol, we demonstrate how to achieve light-inducible gene expression in zebrafish embryos using the blue light-responsive TAEL/C120 system.

ABSTRACT:
Inducible gene expression systems are an invaluable tool for studying biological processes. By using light as the inducing agent, optogenetic expression systems can provide precise control over the timing, location, and amplitude of gene expression. In this protocol, we use an optogenetic expression system to achieve light-inducible gene expression in zebrafish embryos. This system relies on an engineered transcription factor called TAEL that is based on a naturally occurring light-activated transcription factor from the bacterium *E. litoralis*. When illuminated with blue light, TAEL dimerizes, binds to its cognate regulator element called C120, and activates transcription. This protocol uses transgenic zebrafish embryos that express the TAEL transcription factor under control of the ubiquitous *ubb* promoter while the C120 regulatory element drives expression of a fluorescent reporter gene (GFP). Using a simple LED panel to deliver activating blue light, induction of GFP expression can first be detected after 30 minutes of illumination and reaches a peak of more than 130-fold induction after 3 hours of light treatment. Expression induction can be assessed by quantitative real-time PCR (qRT-PCR) and by fluorescence microscopy. This method is a versatile and easy to use approach for optogenetic gene expression.

INTRODUCTION:
Inducible gene expression systems are useful for providing control over the location, amount, and timing of gene expression. However, achieving very precise spatial and temporal control in multicellular organisms has been challenging. Temporal control is most commonly achieved through addition of small molecule compounds [1] or activation of heat shock promoters [2], but both approaches are vulnerable to issues of timing, induction strength, and off-target stress responses. Spatial control is mostly achieved by use of tissue-specific promoters [3], but this approach requires a suitable promoter or regulatory element, which are not always available, and it is not conducive to sub-tissue level induction.

In contrast to such conventional approaches, light-activated optogenetic transcriptional activators have the potential for finer spatial and temporal control of gene expression [4]. The blue light-responsive TAEL/C120 system was developed and optimized for use in zebrafish embryos [5,6]. This system is based on an endogenous light-activated transcription factor from the bacterium *E. litoralis* [7,8]. The TAEL/C120 system consists of a transcriptional activator called TAEL that contains a Kal-TA4 transactivation domain, a blue light-responsive LOV (light-oxygen-voltage

sensing) domain, and a helix-turn-helix (HTH) DNA-binding domain [5]. When illuminated, the LOV domains undergo a conformational change that allow two TAEL molecules to dimerize, bind to a TAEL-responsive C120 promoter, and initiate transcription of a downstream gene of interest [5,8]. The TAEL/C120 system exhibits rapid and robust induction with minimal toxicity, and it can be activated by several different light delivery modalities. Recently, improvements to the TAEL/C120 system were made by adding a nuclear localization signal to TAEL (TAEL-N) and by coupling the C120 regulatory element to a cFos basal promoter (C120F) (Fig. 1A). These modifications improved induction levels by more than 15-fold [6].

In this protocol, a simple LED panel is used to activate the TAEL/C120 system and induce ubiquitous expression of a reporter gene, GFP. Expression induction can be monitored qualitatively by observing fluorescence intensity or quantitatively by measuring transcript levels using quantitative real-time PCR (qRT-PCR). This protocol will demonstrate the TAEL/C120 system as a versatile, easy to use tool that enables robust regulation of gene expression *in vivo*.

PROTOCOL:

This study was performed with the approval of the Institutional Animal Care and Use Committee (IACUC) of the University of California Merced.

1. Zebrafish crossing and embryo collection.

1.1 To minimize spurious activation, maintain separate transgenic zebrafish lines containing either the TAEL transcriptional activator or the C120-controled reporter gene.

1.2 Cross adult zebrafish from each line using standard methods [9] to produce double transgenic embryos that contain both the TAEL and C120 components (Fig. 1B). NOTE: Alternatively, both components can be expressed transiently through microinjection of mRNA or plasmid DNA using standard methods [10].

1.3 Collect embryos in petri dishes containing egg water (60 µg/mL Instant Ocean sea salt dissolved in distilled water).

1.4 To minimize unintended activation by ambient light (see Table 1), place dishes in a light-proof box or cover with aluminum foil.

2. Global light induction.

2.1 Use a blue-light (465 nm) LED panel to deliver activating blue light to several embryos at once.

2.2 Position the LED panel relative to the petri dishes containing embryos so that the actual power of light received by the embryos is approximately 1.5 mW/cm^2 as measured by a light power and energy meter (Fig. 2A).

2.3 If illuminating for more than 3 hours, pulse the light at intervals of 1 hour on/1 hour off using a timer relay to reduce the risk of photodamage to the TAEL transcriptional activator [5,8]. NOTE: The exact duration of illumination may need to be optimized for specific applications.

2.4 Remove petri dish lids to minimize light scattering from condensation.

2.5 For dark controls, place petri dishes containing control embryos in a lightproof box or cover with aluminum foil.

3. Quantitative assessment of induction by qRT-PCR.

3.1 After the desired activation duration, remove embryos from illumination.

3.2 Extract total RNA from 30-50 light-activated and 30-50 dark embryos using an RNA isolation kit following the kit's instructions.

3.3 Use 1 µg total RNA for cDNA synthesis using a cDNA synthesis kit following the kit's instructions.

3.4 Prepare qPCR reactions containing SYBR green enzyme master mix, 5-fold diluted cDNA (step 3.3), and 325 nM each primers for GFP (GFP forward: 5'-ACGACGGCAACTACAAGCACC-3'; GFP reverse: 5'-GTCCTCCTTGAAGTCGATGC-3') and a reference gene such as *ef1a* (ef1a forward 5'-CACGGTGACAACATGCTGGAG-3'; ef1a reverse: 5'-CAAGAAGAGTAGTACCGCTAGCAT-3').

3.5 Carry out qPCR reactions in a real-time PCR machine using the following program: initial activation at 95°C for 10 min, followed by 40 cycles of 30 s at 95°C, 30 s at 60°C and 1 min at 72°C. Once the PCR is completed, perform a melt curve analysis to determine reaction specificity. Perform three technical replicates for each sample.

3.6 Calculate light-activated induction as fold change relative to embryos kept in the dark using the $2^{-\Delta\Delta Ct}$ method [11]. Statistical significance can be determined with a statistics software package.

4. Qualitative assessment of induction by fluorescence microscopy.

4.1 After the desired activation duration, remove embryos from illumination.

4.2. Immobilize embryos for imaging in 3% methylcellulose containing 0.01% tricaine in glass depression slides.

4.3 Acquire fluorescence and brightfield images on a fluorescent stereomicroscope connected to a digital camera using standard GFP filter settings. Use identical image acquisition settings for all samples.

4.4 Brightfield and fluorescence images can be merged after acquisition with image processing software.

REPRESENTATIVE RESULTS:

For this demonstration, we crossed a C120-responsive GFP reporter line (*Tg(C120F:GFP)^{ucm107}*) with a transgenic line that expresses TAEL-N ubiquitously from the *ubiquitin b* (*ubb*) promoter (*Tg(ubb:TAEL-N)^{ucm113}*) to produce double transgenic embryos containing both elements. Beginning at 24 hours post-fertilization, we exposed embryos to activating blue light pulsed at a frequency of 1 hour on/1 hour off. Induction of GFP expression was quantified by qRT-PCR at 30 minutes, 1 hour, 3 hours, and 6 hours post-activation (Fig. 2B and Table 1). Compared to control sibling embryos kept in the dark, we could detect induction of GFP expression as soon as 30 minutes after blue light exposure. Levels of GFP expression then continued to steadily increase up to 6 hours post-activation.

We also qualitatively assessed GFP induction by examining fluorescence intensity at the same time points post-activation (Fig. 2C-F). GFP fluorescence above background levels was first observed at 3 hours post-activation and became noticeably brighter at 6 hours post-activation. In contrast, sibling control embryos kept in the dark did not exhibit any appreciable GFP fluorescence at all time points (Fig. 2G-J).

FIGURE AND TABLE LEGENDS:

Figure 1. Schematic of TAEL/C120 function and experimental design. A. The TAEL/C120 system consists of a transcriptional activator called TAEL fused to a nuclear localization signal (TAEL-N) and a TAEL-responsive regulatory element called C120 coupled to a cFos basal promoter (C120F) driving expression of a gene of interest. TAEL-dependent transcription is active in the presence of blue light, but not in the dark. NLS, nuclear localization signal. **B.** In this protocol, a transgenic line the expresses TAEL-N ubiquitously (*Tg(ubb:TAEL-N)*) is crossed to a C120-driven GFP reporter line (*Tg(C120F:GFP)*) to produce double transgenic embryos. Starting at 24 hpf, embryos are exposed to activating blue light for various durations up to 6 hours. Illustrations created with BioRender.

Figure 2. Representative results of light-activated gene expression with TAEL/C120. A. A typical light activation set up includes a blue LED light source placed in an incubator. Petri dishes containing zebrafish embryos are positioned relative to the light source so that the received power of light is approximately 1.5 mW/cm^2 (dotted line). Petri dish lids are removed during light activation to minimize light scattering. **B.** Quantification of GFP mRNA levels by qRT-PCR at the indicated time points after activation with blue light. Data is presented as GFP fold induction relative to sibling control embryos kept in the dark. Dots represent biological replicates (clutches). Solid horizontal bars represent mean. Error bars, standard deviation. *$p<0.05$, **$p<0.01$, ***$p<0.001$. p values were determined by one-way ANOVA. **C-J.** Representative images showing GFP fluorescence intensity of embryos exposed to blue light (C-F) or kept in the dark (G-J). Fluorescent images (green) have been merged with corresponding brightfield images (grayscale). Scale bars, 500 µm.

Table 1. Comparison of TAEL/C120-induced expression by blue and ambient light. Fold induction of GFP mRNA levels after exposure to activating blue light (465 nm) or ambient light for the indicated amount of time, normalized to control sibling embryos kept in the dark. mRNA levels were quantified by qRT-PCR. Data is reported as average fold induction +/- upper and lower limits. p values were determined by multiple t test. n=3 clutches for all time points.

DISCUSSION:

This protocol describes the use of the optogenetic TAEL/C120 system to achieve blue light-inducible gene expression. This system consists of a transcriptional activator, TAEL, that dimerizes upon illumination with blue light and activates transcription of a gene of interest downstream of a C120 regulatory element. Induced expression of a GFP reporter can be detected after as little as 30 minutes of light exposure, suggesting that this approach possesses relatively fast and responsive kinetics.

Several factors that can affect induction levels. Most critical are the wavelength and power of activating light. In this protocol, we used 465 nm LED lights delivered at 1.5 W/cm^2. Shorter and longer wavelengths (purple and green light, respectively) and lower light power do not activate expression effectively (data not shown). On the other hand, increasing the light power risks

photodamaging the embryos. Thus, for successful use of the TAEL system, activating light must be 1) in the blue range of the visible light spectrum and 2) at sufficient power to balance effective activation of TAEL with reduced photodamage risk. Effective light power may vary depending on experimental conditions and so may need to be empirically determined. Care should also be taken to protect embryos from ambient light, which may contain some amount of blue light, prior to activation. We have found that TAEL/C120-dependent expression can be induced by broad-spectrum ambient light albeit at much lower levels compared to blue light only (Table 1).

While expression of GFP can first be detected by qPCR after 30 minutes of illumination, expression levels are not steady but continue to rise until reaching a peak at 3 hours of light treatment, after which these high expression levels are maintained for up to 6 hours. These results suggest that, in addition to wavelength and light power, TAEL/C120-induced expression levels are also dependent on the duration of illumination, at least until the system reaches a maximum or saturation state. In contrast to these qPCR results, we do not qualitatively observe appreciable GFP fluorescence until after 3 hours of illumination, and fluorescence intensity continues to increase for up to 6 hours of illumination. The discrepancy between our qPCR and fluorescence intensity observations is likely explained by the additional time needed for GFP synthesis, folding, and maturation - factors that are likely to vary depending on the gene of interest. Therefore, some optimization of illumination duration may be needed depending on the application.

In this protocol, we presented the simplest method for activating the TAEL/C120 system using a blue light LED panel to globally illuminate zebrafish embryos. This approach has the advantages of both ease of use and cost effectiveness. However, light activation can also be spatially controlled if needed. We previously demonstrated that TAEL-induced expression can be spatially restricted using multiple modalities to deliver user-defined, spatially patterned blue light [5]. Additional spatial specificity can be achieved by using tissue-specific promoters to regulate expression of the TAEL transcriptional activator [6].

Compared to drug- or heat shock-inducible expression systems, optogenetic expression systems potentially offer better spatial and temporal control over expression by using light as the inducing agent. In addition to TAEL/C120, other light-activated transcriptional systems have been developed [12-15]. However, TAEL/C120 may be especially well-suited for use in zebrafish (and potentially other multicellular systems) for several reasons. First, the TAEL transcriptional activator functions as a homodimer, which simplifies the number of required components. In addition, LOV domain-containing proteins like TAEL require a flavin chromophore for light absorption [16]. This cofactor is endogenously present within animal cells, removing the need to add an exogenous chromophore as with other systems. Finally, activated TAEL is predicted to have a relatively short half-life of approximately 30 seconds in the absence of blue light [8], enabling more precise on/off control. However, this short half-life also means that long-term or chronic expression would require long-term illumination of embryos, which may or may not be desirable depending on the circumstances.

In summary, this protocol demonstrates that the TAEL/C120 system is a blue light-activated gene

expression system that is easy to use, possesses fast and responsive kinetics, and is particularly well-suited for *in vivo* applications.

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DISCLOSURES:
No conflicts of interest declared.

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Figure 1

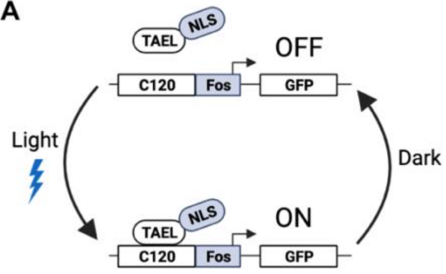

Figure 2

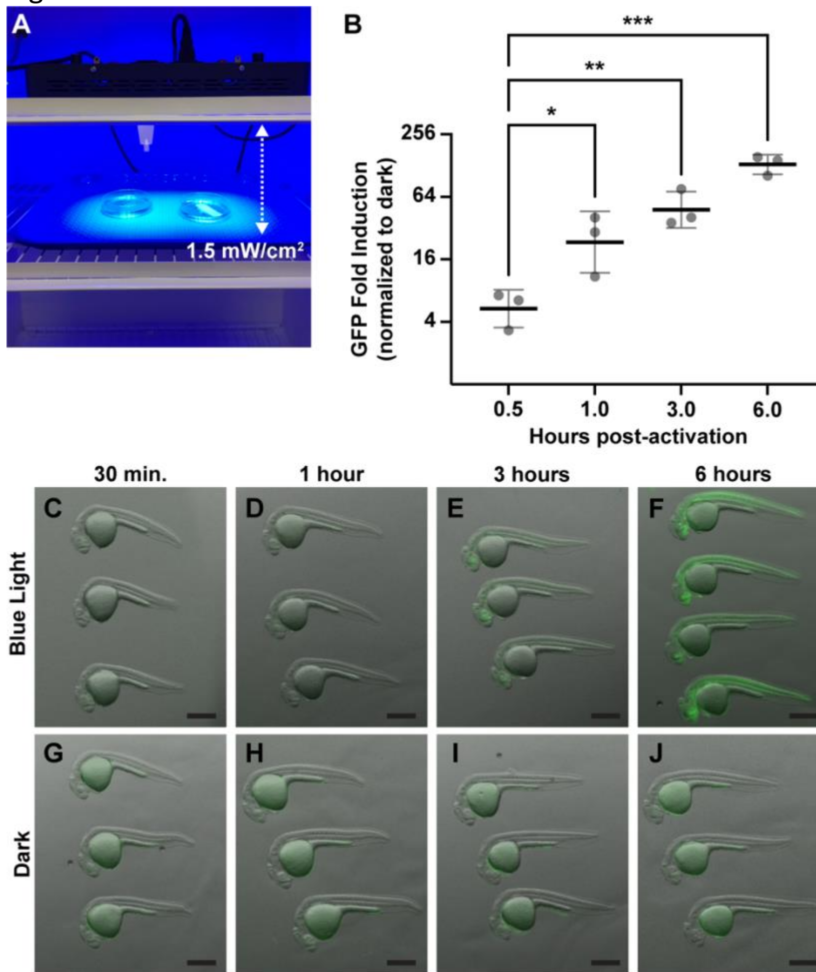

Table 1

Time Post-illumination	GFP Fold Induction Blue light (465 nm)			GFP Fold Induction Ambient light			
	Mean	Upper limit	Lower limit	Mean	Upper limit	Lower limit	p value
30 minutes	5.363121044	8.15857193	3.525502696	0.661534683	1.097728244	0.398667102	0.005291
1 hour	23.44	46.35044081	11.85160592	2.638682529	4.368971424	1.593657823	0.011145
3 hours	48.09177693	71.99347359	32.12539822	8.280376038	24.86850106	2.757087255	0.059959
6 hours	131.4637117	163.4891638	105.7116392	16.66536842	27.94334716	9.939199585	0.003102

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Color CCD digital camera	Lumenara	755-107	
Compact Power and Energy Meter Console, Digital 4" LCD	Thorlabs	PM100D	
Excitation filter, 545 nm	Olympus	ET545/25x	
illustra RNAspin Mini kit	GE Healthcare	95017-491	
MARS AQUA Dimmable 165W LED Aquarium light (blue and white)	Amazon	B017GWDF7E	
Methylcellulose	Sigma-Aldrich	M7140	
NEARPOW Programmable digital timer switch	Amazon	B01G6O28NA	
PerfeCTa SYBR green fast mix	Quantabio	101414-286	
Photoshop image procesing software	Adobe		
Prism graphing and statistics software	GraphPad		
qScript XLT cDNA SuperMix	Quantabio	10142-786	
QuantStudio 3 Real-Time PCR System	Applied Biosystems	A28137	
Stereomicroscope	Olympus	SZX16	
Tricaine (Ethyl 3-aminobenzoate methanesulfonate)	Sigma-Aldrich	E10521	
X-Cite 120 Fluorescence LED light source	Excelitas	010-00326R	Discontinued. It has been rep

laced with the X-Cite mini+