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

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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. This protocol demonstrates 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. Optogenetic expression systems can provide precise control over gene expression timing, location, and amplitude using light as the inducing agent. In this protocol, an optogenetic expression system is used to achieve light-inducible gene expression in zebrafish embryos. This system relies on an engineered transcription factor called TAEL 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 the control of the ubiquitous *ubb* promoter. At the same time, the C120 regulatory element drives the 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 min of illumination and reaches a peak of more than 130-fold induction after 3 h 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 help control the amount, timing, and location of gene expression. However, achieving exact spatial and temporal control in multicellular organisms has been challenging. Temporal control is most commonly achieved by adding small-molecule compounds[1] or activation of heat shock promoters[2]. Still, both approaches are vulnerable to issues of timing, induction strength, and off-target stress responses. Spatial control is mainly achieved by the 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 allows 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) (**Figure 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 the 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 Maintain separate transgenic zebrafish lines containing either the TAEL transcriptional activator or the C120-controlled reporter gene to minimize spurious activation.

1.2 Cross 6–8 adult zebrafish from each line using standard methods[9] to produce double transgenic embryos that contain both the TAEL and C120 components (**Figure 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), with approximately 30 embryos per condition to be tested.

1.4 Place the dishes in a lightproof box or cover with aluminum foil to minimize unintended activation by ambient light (see **Table 1**).

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 (**Figure 2A**).

2.3 Pulse the light at intervals of 1 h on/1 h off using a timer relay if illuminating for more than 3 h 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. In this protocol, examples of illumination duration of 30 min, 1 h, 3 h, and 6 h are provided.

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

2.5 Place Petri dishes containing control embryos in a lightproof box or cover with aluminum foil for dark controls.

3 Quantitative assessment of induction by qRT-PCR

3.1 Remove embryos from illumination after the desired activation duration.

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.

3.2.1 Transfer embryos to a 1.5 mL microcentrifuge tube and remove excess egg water. Add lysis buffer and homogenize the embryos with a plastic pestle.

3.2.2 Transfer the lysate to a kit-provided column and continue with the kit's instructions. Immediately proceed to step 3.3 or store purified RNA at -20 °C to -80 °C.

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

3.3.1 Take a thin-walled 0.2 mL PCR tube, add 1 µg of RNA to 4 µL of 5x cDNA reaction master mix (containing optimized buffer, oligo-dT and random primers, and dNTPs), 1 µL of 20x reverse transcriptase solution, and nuclease-free water to a total volume of 20 µL.

3.3.2 Place the tube in a thermocycler programmed as follows: 22 °C for 10 min, 42 °C for 30 min, 85 °C for 5 min, and then hold at 4 °C. Immediately proceed to step 3.4 or store cDNA at -20 °C.

3.4 Prepare qPCR reactions containing SYBR green enzyme master mix, 5-fold diluted cDNA (step 3.3), and 325 nM of each primer for GFP.

NOTE: Primers used in this protocol as follows. GFP forward: 5'-ACGACGGCAACTACAAGCACC-3'; GFP reverse: 5'-GTCCTCCTTGAAGTCGATGC-3'; ef1a forward 5'-CACGGTGACAACATGCTGGAG-3'; ef1a reverse: 5'-CAAGAAGAGTAGTACCGCTAGCAT-3').

3.5 Carry out qPCR reactions in a real-time PCR machine.

NOTE: PCR 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.

3.6 Perform a melt curve analysis once the PCR is completed to determine the reaction specificity. Perform three technical replicates for each sample.

3.7 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 Remove the embryos from illumination after the desired duration of activation.

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 Merge brightfield and fluorescence images after the acquisition with image processing software.

REPRESENTATIVE RESULTS:

For this demonstration, a C120-responsive GFP reporter line (*Tg(C120F:GFP)^{ucm107}*) was crossed 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. 24 h post-fertilization, the embryos were exposed to activating the blue light, pulsed at a frequency of 1 h on/1 h off. Induction of GFP expression was quantified by qRT-PCR at 30 min, 1 h, 3 h, and 6 h post-activation (**Figure 2B** and **Table 1**). Compared to controlling the sibling embryos kept in the dark, induction of GFP expression was detected as soon as 30 min after the blue light exposure. Levels of GFP expression then continued to increase up to 6 h post-activation steadily.

GFP induction was also qualitatively assessed by examining the fluorescence intensity at the same time points post-activation (**Figure 2C–F**). GFP fluorescence above background levels was first observed at 3 h post-activation and became noticeably brighter at 6 h post-activation. In contrast, control embryos for all time points that were kept in the dark did not exhibit any appreciable GFP fluorescence (**Figure 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 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, the embryos are exposed to activating blue light for various durations up to 6 h—illustrations created with a web-based science illustration tool (see **Table of Materials**).

Figure 2: Representative results of light-activated gene expression with TAEL/C120. (**A**) A typical light activation setup 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 the mean. Error bars, standard deviation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. One-way ANOVA determined p values. (**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*-tests. 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 min of light exposure, suggesting that this approach possesses relatively fast and responsive kinetics.

Several factors can affect induction levels. Most critical are the wavelength and power of activating light. In this protocol, 465 nm LED lights delivered at 1.5 W/cm^2 were used. 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, more light power increases the risk of 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, containing some amount of blue light, before activation. It has been 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 GFP expression can first be detected by qPCR after 30 min of illumination, expression levels are not steady. Still, they continue to rise until reaching a peak at 3 h of light treatment, after which these high expression levels are maintained for up to 6 h. These results suggest that, in addition to wavelength and light power, TAEL/C120-induced expression levels are also dependent on illumination duration, 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 h of illumination, and fluorescence intensity continues to increase for up to 6 h of illumination. The discrepancy between the 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.

This protocol presented the most straightforward method for activating the TAEL/C120 system using a blue-light LED panel to illuminate zebrafish embryos globally. This approach has the advantages of both ease of use and cost-effectiveness. However, light activation can also be spatially controlled if needed. It was previously demonstrated that TAEL-induced expression could be spatially restricted using multiple modalities to deliver user-defined, spatially patterned blue light[5]. Additional spatial specificity can be achieved using tissue-specific promoters to regulate the 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 overexpression 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 such as 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 s 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.

ACKNOWLEDGMENTS:

We thank Stefan Materna and members of the Woo and Materna labs for helpful suggestions and comments on this protocol. We thank Anna Reade, Kevin Gardner, and Laura Motta-Mena for valuable discussion and insights while developing this protocol. This work was supported by grants from the National Institutes of Health (NIH; R03 DK106358) and the University of California Cancer Research Coordinating Committee (CRN-20-636896) to S.W.

DISCLOSURES:

No conflicts of interest were declared.

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Figure 1

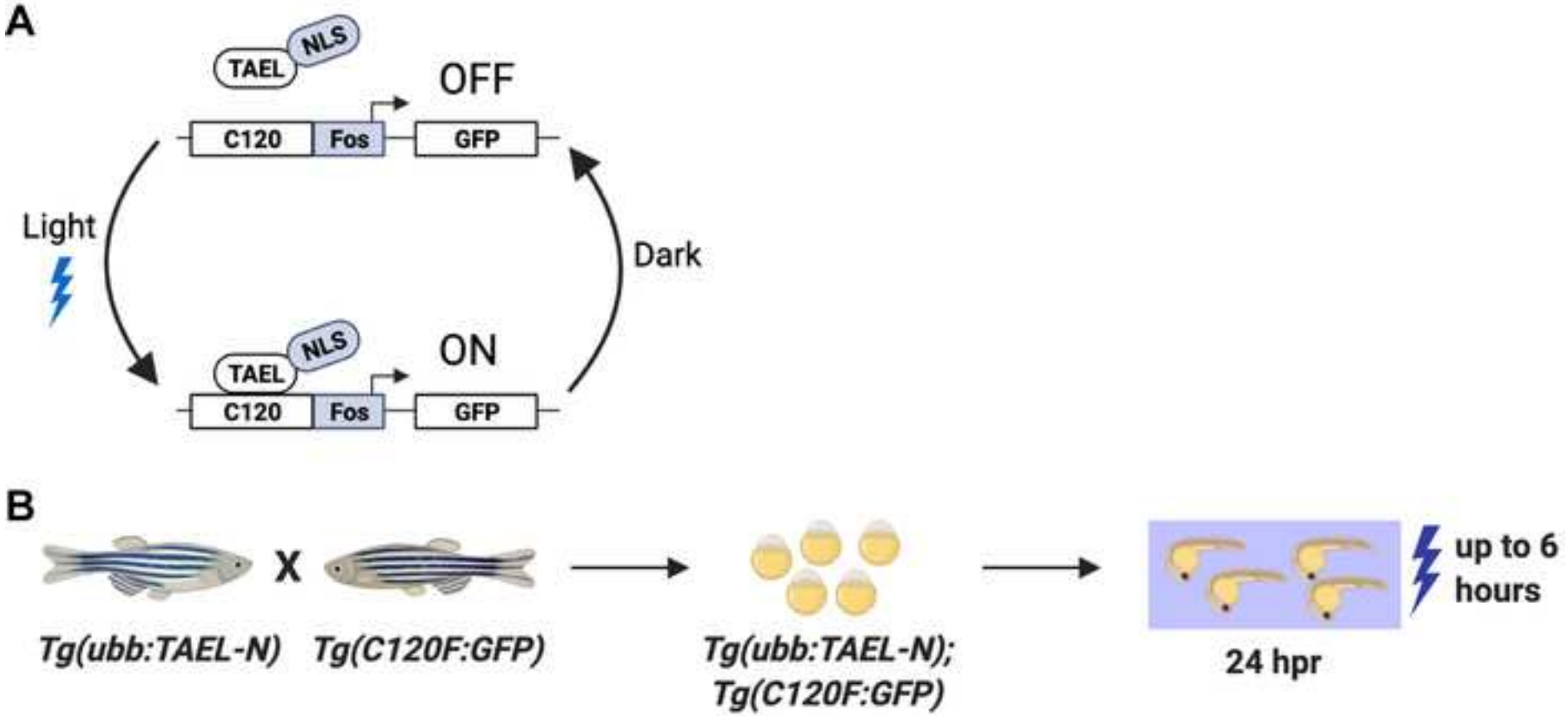

Figure 2

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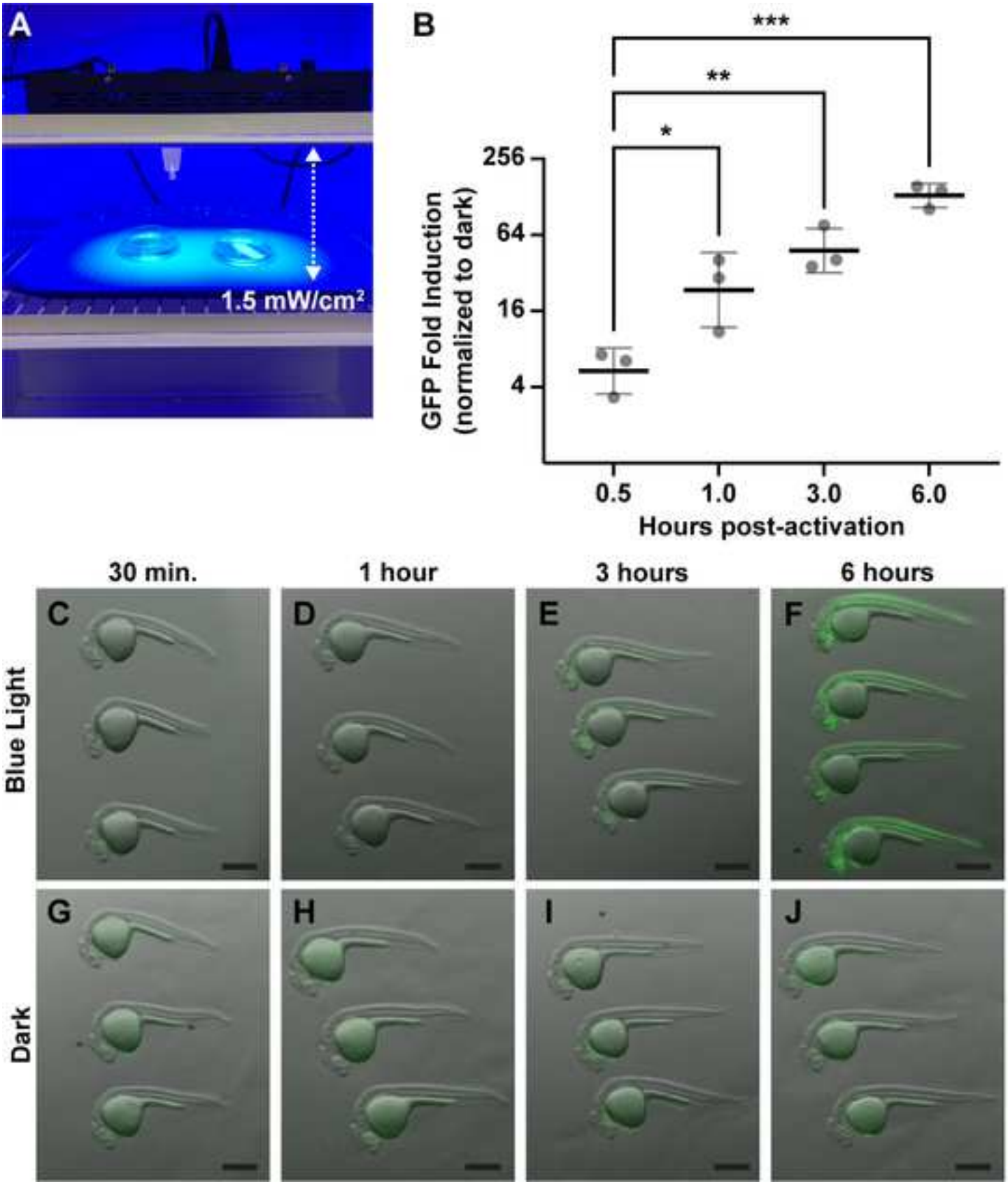

Table 1

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

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June 22, 2021

Dear Dr. Iyer,

Thank you very much for your review of our manuscript “Light induced GFP expression in zebrafish embryos using the optogenetic TAEL/C120 system" (manuscript ID JoVE62818). Please find attached a revised version of this manuscript for publication in JoVE. Revised portions are highlighted in blue text.

We thank the editors and reviewers for their thoughtful and constructive evaluation of our work. We have revised the manuscript as completely as possible to address their concerns. A point-by-point response to their comments is appended to this letter.

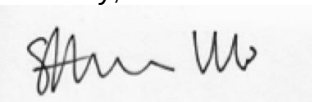
As suggested by the editor and reviewers, we have:

- Replaced references to “TAEL 2.0” with the more general “TAEL/C120 system.”
- Revised the Protocol section to use the imperative tense and number each step
- Revised the Discussion section to include discussion of critical steps, troubleshooting, limitations of the technique, significance with respect to existing methods, and future applications.
- Added data to Table 1 to show low-level activation of the TAEl/C120 system by ambient room light.
- Revised the Protocol, Discussion, Fig. 2A, and Materials Table to emphasize the importance of light power and add details for measuring light power.

We believe that these revisions have improved and strengthened our manuscript, which we now hope is suitable for publication in Zebrafish.

Thank you again for your time and consideration.

Sincerely,



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Point-by Point Response to editorial and reviewer comments (comments are italicized, reponses are nonitalicized).

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

We have made every effort to ensure there are no spelling or grammar issues and to define all abbreviations.

2. Please replace "with the TAEL 2.0 system" in your title with something sounding less commercial.

We have revised the title to make it more accessible to a general audience. The revised title is "Light induced GFP expression in zebrafish embryos using the optogenetic TAEL/C120 system."

3. Please provide an email address for each author.

We have provided email addresses for all authors

4. Increase the word count of your abstract to150-300 words.

We have revised the abstract and increased the word count to approximately 180 words.

5. JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. To this end, we ask that you please reduce the number of instances of "TAEL 2.0" within your text. The term may be introduced but please use it infrequently and when directly relevant. Otherwise, please refer to the term using generic language.

Our optogenetic expression system consists of two major components: 1) a light-sensitive transcription factor called TAEL and 2) a TAEL-responsive regulatory DNA sequence called C120. "TAEL 2.0" refers to collective modifications we have made to both the transcription factor and regulatory elements that improve performance (LaBelle et al., 2021). The term "TAEL 2.0" is used to distinguish these new constructs from the unmodified versions described in our initial publication (Reade et al., 2017) and does not refer to any commercial product. However, to minimize confusion, we have replaced instances of "TAEL 2.0" with a more general "TAEL/C120" (similar to "Gal4/UAS"). Unfortunately, we do not feel that a more generic term would be appropriate. Although there are other optogenetic expression systems in addition to TAEL/C120 (Shimizu-Sato et al., 2002; Liu et al., 2012; Wang et al., 2012), each system is unique in their activating light wavelengths, activation half-life, and other properties. Thus, the specific system used should be specified when describing optogenetic gene expression experiments.

6. Please revise the text, especially in the protocol, to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

We have made every effort to minimize the use of personal pronouns in the text.

7. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

We have revised the Protocol section to use the imperative tense.

8. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.
We have revised the Protocol section accordingly and moved any discussion of the steps to the Discussion section.

9. Please number the steps in the protocol under sections: 1., 1.1., 1.1.1., 1.1.1.1.
We have revised the Protocol section to number each step as suggested.

10. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action (e.g., step 3.1: how is RNA extracted; step 3.2: what were the conditions for qRT-PCR?). There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.
We have revised the Protocol section to add more detail and/or references as needed.

11. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique
We have revised the discussion section to address these issues.

12. Please include a scale bar for all images taken with a microscope to provide context to the magnification used. Define the scale in the appropriate Figure Legend.
We have added scale bars to all images and defined them in the figure legend.

13. Please sort the Materials Table alphabetically by the name of the material.
We have sorted the Materials Table alphabetically.

Reviewers' comments:
Reviewer #1:
Manuscript Summary:
This article details the basic experimental approaches needed to induce global gene expression using the blue-light activated TAEL 2.0 optogenetics system

Minor Concerns:
The protocol is for "a simple LED panel to activate 45 the TAEL 2.0 system and induce ubiquitous expression of a reporter gene, GFP". Some of the main challenges of using optogenetic systems in zebrafish are related to achieving specific light patterning. Is there scope within this Jove article to describe how the authors best achieve patterned light illumination? I

think the article is useful without this extra part - this is a suggestion for improvement only since the authors do already reference their publications that use patterned light.
We thank the reviewer for their suggestion. In fact, we have previously demonstrated several methods for achieving specific light patterning (Reade et al., 2017). However, because these approaches are subject to many variables that depend on lab's exact setup, we felt it was beyond the scope of this particular JoVE protocol.

Figure 2A. the figure included doesn't give much information. A schematic and/or specs of the LED panel would allow other users to better reproduce the set up.
We have revised Fig. 2A to emphasize that the distance between the LED panel and petri dishes should be adjusted so that the received power of light is approximately 1.5 mW/cm^2. We thank the reviewer for this helpful comment.

Reviewer #2:
Manuscript Summary:
This study by LaBelle and Woo describes the use of optogenetically-induced transcription using TAEL 2.0 which is well0suited for in vivo applications. This study nicely presents how to do these experiments in a living animal; however, the authors need to add a bit more detail to make it more useful to a new audience. With additional details, the manuscript is suitable for publication. Concerns are listed below:

Major Concerns:
Given the contribution of blue light to white light sources, how sensitive is TAEL 2.0 to standard room light or microscopy brightfield? How careful will someone have to be with room light exposure if utilizing TAEL 2.0 for their experiments? Controls for the experiments showing room light compared to dark would be useful but not necessary.
We thank the reviewer for bringing this issue to our attention. We measured GFP induction in embryos exposed to ambient room light and found that indeed, the TAEL/C120 system can be activated by ambient light but to a smaller extent compared to using the blue light LED panel (approximately 16-fold in room light versus 130-fold under blue light). These results have been added to Table 1, and we have included a note of caution to minimize ambient light exposure to the Discussions section.

The authors talk about how light is advantageous for spatial control in the intro and discussion but do not demonstrate how to carry out those experiments. Given the fluorescent reporter, it would be an easy experiment to visualize. This would significantly increase the utility of this protocol to other scientists.
We thank the reviewer for their suggestion. As noted above in response to Reviewer #1, we have previously demonstrated multiple methods to deliver spatially patterned light to achieve spatial control of TAEL activation (Reade et al., 2017). However, we felt this was beyond the scope of this particular JoVE protocol as these light patterning methods are subject to many variables that depend on lab's exact setup.

How is light intensity measured/calculated? For first-time users, they would need to know this process so they can optimize their illuminations accordingly.
Light power can be measured with a power and energy meter. We used a Thorlabs PM100D Laser and Power and Energy Meter Console. We have added this information the Protocol section (Step 2.2) and the Materials Table.

Minor Concerns:
None

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