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"Light-activated gene expression in zebrafish embryos with the TAEL 2.0 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 2.0 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 a blue light-activated expression system called TAEL 2.0 to achieve light-inducible gene expression in zebrafish embryos.

Introduction

Inducible gene expression systems are extremely 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

26 molecule compounds [1] or activation of heat shock promoters [2], but both approaches
27 are vulnerable to issues of timing, induction strength, and off-target stress
28 responses. Spatial control is mostly achieved by use of tissue-specific promoters [3],
29 but this approach requires a suitable promoter or regulatory element, which are not
30 always available, and it is not conducive to sub-tissue level induction.
31
32 In contrast to such conventional approaches, light-activated optogenetic
33 transcriptional activators have the potential for finer spatial and temporal control of
34 gene expression [4]. Here, we will use the blue light-responsive TAEL/C120 system
35 that was developed and optimized for use in zebrafish embryos [5,6]. This system is
36 based on an endogenous light-activated transcription factor from the bacterium *E.*
37 *litoralis* [7,8]. The TAEL/C120 system consists of a transcriptional activator called TAEL
38 that contains a Kal-TA4 transactivation domain coupled to a blue light-responsive
39 LOV (light-oxygen-voltage sensing) domain [5]. When illuminated, the LOV domains
40 undergo a conformational change that allow two TAEL molecules to dimerize and
41 activate transcription downstream of a TAEL-responsive C120 promoter [5,8]. We have
42 shown that the TAEL/C120 system exhibits rapid and robust induction with minimal
43 toxicity and can be activated by several different light delivery modalities. Recently,
44 we made improvements to the TAEL/C120 system by adding a nuclear localization
45 signal to TAEL (TAEL-N) and by coupling the C120 regulatory element to a cFos
46 basal promoter (C120F). These modifications, collectively referred to as TAEL 2.0,
47 improved induction levels by more than 15-fold [6].
48
49 In this protocol, we will use a simple LED panel to activate the TAEL 2.0 system and
50 induce ubiquitous expression of a reporter gene, GFP. Expression induction can be

51 monitored qualitatively by observing fluorescence intensity or quantitatively by
52 measuring transcript levels using qRT-PCR. This protocol will demonstrate the TAEL/
53 C120 system as a versatile, easy to use tool that enables robust regulation of gene
54 expression *in vivo*.
55
56 **Protocol**
57
58 *Step 1. Zebrafish crossing and embryo collection.* We maintain separate transgenic
59 zebrafish lines containing either the TAEL transcriptional activator or the C120-
60 controled reporter gene. Adult zebrafish from each line are crossed together
61 produce double transgenic embryos that contain both the TAEL and C120
62 components. Breeding is performed using standard methods [9]. For this protocol, we
63 crossed a C120-responsive GFP reporter line (*Tg(C120F:GFP)*^{ucm107}) with a transgenic
64 line that expresses TAEL-N ubiquitously (*Tg(ubb:TAEL-N)*^{ucm113}). Alternatively, both
65 components can be expressed transiently through microinjection of mRNA or
66 plasmid DNA using standard methods [10]
67
68 *Step 2. Global light induction*. A blue-light (465 nm) LED panel placed in an
69 incubator is used to deliver activating blue light (Fig. 1A). In this demonstration, we
70 used a MARS AQUA 165W dimmable LED aquarium light. Actual power of light
71 received is approximately 1.5 mW/cm2. We have found that pulsed light at intervals
72 of 1 hour on/1 hour off induced higher expression levels compared to constant
73 illumination, potentially because of reduced photodamage to the TAEL
74 transcriptional activator [5,8]. Light pulses are achieved by coupling the LED panel to a
75 timer relay. For this protocol, embryos are exposed to pulsed illumination for 6

76 hours starting at approximately 24 hours post-fertilization. The duration of
77 illumination may need to be optimized for specific applications. While illuminated,
78 petri dish lids are removed to minimize light scattering from condensation. Dark
79 controls for all conditions are placed in a lightproof box in the same incubator as
80 light-treated samples.

81

82 *Step 3. Quantitative assessment of induction by qRT-PCR.* Light-activated induction
83 of gene expression can be quantitively assessed by measuring mRNA transcript
84 levels with quantitative real-time PCR (qRT-PCR). In this protocol, we used qRT-PCR
85 to quantify GFP expression at various time points after exposure to activating blue
86 light (Fig. 1B).

87 *Step 3.1 RNA extraction and cDNA synthesis.* RNA was extracted from embryos
88 exposed to 30 minutes, 1 hour, 3 hours, or 6 hours light treatment as well as
89 embryos kept in the dark for the same duration of time. 30-50 embryos were
90 used for each sample and RNA extraction was performed using the illustra™
91 RNAspin Mini kit (GE Healthcare). For each sample, 1 μg total RNA was then used
92 for cDNA synthesis with qScript XLT cDNA SuperMix (Quantabio).

93 *Step 3.2. qRT-PCR.* qPCR reactions were performed with PerfeCTa® SYBR green
94 fast mix (Quantabio) and carried out in a QuantStudio3 (Applied Biosystems) real
95 time PCR machine. The housekeeping gene *ef1a* was used as a reference. For
96 each sample, three technical replicates were performed. qPCR primers used in
97 this protocol are GFP forward: 5'-ACGACGGCAACTACAAGCACC-3'; GFP reverse:
98 5'-GTCCTCCTTGAAGTCGATGC-3'; ef1a forward 5'-CACGGTGACAACATGCTGGAG-
99 3'; ef1a reverse: 5'-CAAGAAGAGTAGTACCGCTAGCAT-3'

100 *Step 3.3. Analysis.* Light-activated induction of GFP expression was calculated as
101 fold change relative to GFP expression of embryos kept in the dark using the $2^{-\Delta\Delta Ct}$
102 method [11]. Statistical significance was determined with Prism software
103 (GraphPad).
104

105 *Step 4. Qualitative assessment of induction by fluorescence microscopy.* Because
106 we used a fluorescent reporter line to demonstrate this protocol, light-activated
107 expression can also be assessed by fluorescence microscopy. Here, we qualitatively
108 assessed GFP induction by observing fluorescence intensity at various time points
109 after exposure to activating blue light (Fig. 1C-J).

110 *Step 4.1.* After 30 minutes, 1 hour, 3 hours, or 6 hours of light treatment,
111 embryos were embedded in 3% methylcellulose (Sigma-Aldrich) containing
112 0.01% tricaine (Sigmal-Aldrich) in glass depression slides.

113 *Step 4.2.* Fluorescence and brightfield images were acquired on an Olympus
114 SZ51 stereomicroscope equipped with an X-Cite 120 LED light source, a 545 nm
115 excitation filter, and Lumenera Infinity color CCD digital camera.

116 *Step 4.3.* For each time point, we also imaged control sibling embryos that had
117 been kept in the dark. Identical image acquisition settings were used for all
118 samples.

119 *Step 4.4.* Brightfield and fluorescence images were merged after acquisition with
120 Adobe Photoshop software.
121

122 **Representative Results**

123

124 For this demonstration, we crossed a C120-responsive GFP reporter line
125 (*Tg(C120F:GFP)*) with a transgenic line that expresses TAEL-N ubiquitously
126 (Tg(ubb:TAEL-N) to produce double transgenic embryos containing both elements.
127 Beginning at 24 hours post-fertilization, we exposed embryos to activating blue light
128 pulsed at a frequency of 1 hour on/1 hour off. Induction of GFP expression was
129 quantified by qRT-PCR at 30 minutes, 1 hour, 3 hours, and 6 hours post-activation
130 (Fig. 1B and Table 1). Compared to control sibling embryos kept in the dark, we
131 could detect induction of GFP expression as soon as 30 minutes after blue light
132 exposure. Levels of GFP expression then continued to steadily increase up to 6
133 hours post-activation.
134
135 We also qualitatively assessed GFP induction by examining fluorescence intensity at
136 the same time points post-activation (Fig. 1C-F). GFP fluorescence above
137 background levels was first observed at 3 hours post-activation and became
138 noticeably brighter at 6 hours post-activation. In contrast, sibling control embryos
139 kept in the dark did not exhibit any appreciable GFP fluorescence at all time points
140 (Fig. 1G-J).
141

Time Post-activation	**GFP Fold Induction (mean ± S.D.)**
30 minutes	$5.36^{+2.8}_{-1.84}$
1 hour	$23.44^{+22.91}_{-11.59}$
3 hours	$48.09^{+23.9}_{-15.97}$
6 hours	$131.46^{+32.02}_{-25.75}$

Table 1. Fold induction of GFP mRNA levels quantified by qRT-PCR after exposure to activating blue light for the indicated amount of time, normalized to control sibling embryos kept in the dark for the same duration. S.D., standard deviation.

Figure 1. Schematic of TAEL 2.0 function and experimental design. A. The TAEL 2.0 system consists of a transcriptional activator called TAEL fused to a nuclear localization signal (NLS) and a TAEL-responsive regulatory element called C120 coupled to a cFos basal promoter driving expression of a gene of interest. TAEL-dependent transcription is active in the presence of blue light, but not in the dark. 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 2.0. A. Our typical light activation set up includes a blue LED light source placed in an incubator. Note, petri dish lids are removed during light activation to minimize light scattering. **B.** Quantification of GFP mRNA levels induced at the indicated time points post-light activation by qRT-PCR. Data is presented as GFP fold induction relative to control embryos kept in the dark. Gray dots represent biological replicates (clutches). Solid horizontal bars represent mean. Error bars, standard deviation. *$p<0.05$. **$p<0.01$. ***$p<0.001$. **C-J.** Representative images showing GFP fluorescence intensity of embryos exposed to light treatment (C-F) or

166 kept in the dark (G-J). Fluorescent images (green) have been merged with
167 corresponding brightfield images (grayscale). Scale bar, 500 µm.
168
169 **Discussion**
170
171 This protocol describes the use of the TAEL 2.0 system to achieve blue light-
172 inducible gene expression. This system consists of a transcriptional activator, TAEL-
173 N, that dimerizes upon illumination and activates transcription of a gene of interest
174 downstream of a C120 regulatory element. Compared to drug- or heat shock-
175 inducible expression systems, optogenetic expression systems like TAEL 2.0
176 potentially offer better spatial and temporal control over expression by using light
177 as the inducing agent. By qPCR, we can detect induced expression of a GFP reporter
178 after as little as 30 minutes of light exposure, suggesting that TAEL 2.0 possesses
179 relatively fast and responsive kinetics. Although we do not observe appreciable GFP
180 fluorescence until 3 hours post-activation, this discrepancy is likely explained by the
181 additional time needed for GFP synthesis, folding, and maturation - factors that are
182 likely to vary depending on the gene of interest. Therefore, some optimization of
183 illumination times may be needed depending on the application.
184
185 In this protocol, we presented the simplest method for activating the TAEL 2.0
186 system using a blue light LED panel to globally illuminate zebrafish embryos. This
187 approach has the advantages of both ease of use and cost effectiveness. However,
188 activation of the TAEL 2.0 system can also be spatially controlled if needed. We
189 previously demonstrated that TAEL-induced expression can be spatially restricted
190 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-N transcriptional activator [6].

In addition to TAEL 2.0, other light-activated transcriptional systems have been developed [12-15]. Compared to these systems, TAEL 2.0 is especially well-suited for use in zebrafish (and potentially other multicellular systems). First, the TAEL-N transcriptional activator functions as a homodimer, which simplifies the number of components that must be used. In addition, LOV domain-containing proteins like TAEL-N 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.

In summary, this protocol demonstrates that TAEL 2.0 is a blue light-activated gene expression system that is easy to use, possesses fast and responsive kinetics, and particularly well-suited for *in vivo* applications.

Disclosures

No conflicts of interest declared.

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219 **References**

220 1. Knopf, F., Schnabel, K., Haase, C., Pfeifer, K., Anastassiadis, K. & Weidinger, G.
221 Dually inducible TetON systems for tissue-specific conditional gene expression
222 in zebrafish. *Proceedings of the National Academy of Sciences of the United*
223 *States of America* **107** (46), 19933–19938, doi:10.1073/pnas.1007799107
224 (2010).
225 2. Halloran, M. C., Sato-Maeda, M., *et al.* Laser-induced gene expression in
226 specific cells of transgenic zebrafish. *Development (Cambridge, England)* **127**
227 (9), 1953–1960 (2000).
228 3. Hesselson, D., Anderson, R. M., Beinat, M. & Stainier, D. Y. R. Distinct
229 populations of quiescent and proliferative pancreatic beta-cells identified by
230 HOTcre mediated labeling. *Proceedings of the National Academy of Sciences*
231 *of the United States of America* **106** (35), 14896–14901,
232 doi:10.1073/pnas.0906348106 (2009).
233 4. Tischer, D. & Weiner, O. D. Illuminating cell signalling with optogenetic tools.
234 *Nature reviews. Molecular cell biology* **15** (8), 551–558, doi:10.1038/nrm3837
235 (2014).
236 5. Reade, A., Motta-Mena, L. B., Gardner, K. H., Stainier, D. Y., Weiner, O. D. &
237 Woo, S. TAEL: a zebrafish-optimized optogenetic gene expression system with
238 fine spatial and temporal control. *Development (Cambridge, England)* **144** (2),
239 345–355, doi:10.1242/dev.139238 (2017).
240 6. LaBelle, J., Ramos-Martinez, A., *et al.* TAEL 2.0: An Improved Optogenetic
241 Expression System for Zebrafish. *Zebrafish* **18** (1), 20–28,
242 doi:10.1089/zeb.2020.1951 (2021).
243 7. Rivera-Cancel, G., Motta-Mena, L. B. & Gardner, K. H. Identification of natural
244 and artificial DNA substrates for light-activated LOV-HTH transcription factor
245 EL222. *Biochemistry* **51** (50), 10024–10034, doi:10.1021/bi301306t (2012).
246 8. Motta-Mena, L. B., Reade, A., *et al.* An optogenetic gene expression system
247 with rapid activation and deactivation kinetics. *Nature chemical biology* **10**
248 (3), 196–202, doi:10.1038/nchembio.1430 (2014).
249 9. Avdesh, A., Chen, M., *et al.* Regular care and maintenance of a zebrafish
250 (Danio rerio) laboratory: an introduction. *Journal of visualized experiments :*
251 *JoVE* (69), e4196, doi:10.3791/4196 (2012).
252 10. Holder, N. & Xu, Q. Microinjection of DNA, RNA, and Protein into the Fertilized
253 Zebrafish Egg for Analysis of Gene Function. *Molecular Embryology*, 487–490,
254 doi:10.1385/1-59259-270-8:487 (1999).
255 11. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using
256 real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San*
257 *Diego, Calif.)* **25** (4), 402–408, doi:10.1006/meth.2001.1262 (2001).
258 12. Wang, X., Chen, X. & Yang, Y. Spatiotemporal control of gene expression by a
259 light-switchable transgene system. *Nature methods* **9** (3), 266–269,
260 doi:10.1038/nmeth.1892 (2012).

261 13. Mruk, K., Ciepla, P., Piza, P. A., Alnaqib, M. A. & Chen, J. K. Targeted cell
262 ablation in zebrafish using optogenetic transcriptional control. *Development*
263 *(Cambridge, England)* **147** (12), dev183640, doi:10.1242/dev.183640 (2020).
264 14. Liu, H., Gomez, G., Lin, S., Lin, S. & Lin, C. Optogenetic control of transcription
265 in zebrafish. *PloS one* **7** (11), e50738, doi:10.1371/journal.pone.0050738
266 (2012).
267 15. Shimizu-Sato, S., Huq, E., Tepperman, J. M. & Quail, P. H. A light-switchable
268 gene promoter system. *Nature biotechnology* **20** (10), 1041–1044,
269 doi:10.1038/nbt734 (2002).
270 16. Krueger, D., Izquierdo, E., Viswanathan, R., Hartmann, J., Pallares Cartes, C. &
271 De Renzis, S. Principles and applications of optogenetics in developmental
272 biology. *Development (Cambridge, England)* **146** (20),
273 doi:10.1242/dev.175067 (2019).
274