LITE Switch Light-Inducible Expression System User Manual
Cat. No. A001

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IMPORTANT INFORMATION

Shipping and storage
The LITE Switch System is shipped at room temperature. Store at –20°C.

Kit contents

pVEL, regulatory plasmid	20 µl (at 500 ng/µl)
pC120, inducible expression plasmid	20 µl (at 500 ng/µl)

INTRODUCTION

LITE Switch is a blue light-inducible gene expression system for eukaryotic cells. The system uses an engineered version of EL222, a bacterial photosensor protein that binds to a specific DNA sequence when illuminated with blue light (Figure 1A). EL222 contains a photosensory light-oxygen-voltage (LOV) domain and a helix-turn-helix (HTH) DNA-binding domain. In the dark, the LOV domain binds the HTH domain and prevents dimerization and DNA binding (Nash *et al.,* 2011). Blue light illumination (450-495 nm) triggers a photochemical reaction between the LOV domain and its flavin chromophore that disrupts the inhibitory LOV-HTH interactions and allows EL222 to dimerize and bind its cognate DNA sequence (Rivera-Cancel *et al*., 2012; Zoltowski *et al*., 2013). Once returned to the dark, these structural changes are spontaneously reversed and lead to the rapid inactivation of EL222 (τ ~11 s at 37 °C; Zoltowski *et al.* 2011).

The first generation engineered EL222 transactivator was described by Motta-Mena et al., 2014. Working in cultured mammalian cell lines, this VP-EL222 system demonstrated a relatively large (~100-fold) dynamic range of protein expression, rapid activation (<10 s) and deactivation kinetics (<50 s), and a linear response to light. Importantly, dark-state and red light controls showed changes of less than 2-fold, establishing minimal leakiness under non-inducing conditions.

A new second generation EL222 transactivator called VEL is now part of the LITE Switch System. The VEL protein consists of the transcriptional activation (AD) from the VP16 protein fused to an optimized EL222. Cells that express the VEL transactivator protein and contain a gene of interest under the control of the EL222-specific C120 promoter will express high levels of the gene of interest, but only when cells are illuminated with blue light. The VEL transactivator provides a higher fold induction than the first generation VP-EL222 protein from Motta-Mena *et al*., 2014 (Figure 1B; 102-fold VP-EL222 vs. 345-fold VEL).

Two components of LITE Switch System

- VEL transactivator protein

EL222, a small (222-residue) bacterial blue light sensing DNA-binding protein (Nash *et al.,* 2011), is the basis of the LITE Switch system. The VEL transactivator consists of the transcriptional AD from the VP16 protein fused to an optimized version of EL222.

- C120 inducible promoter

The inducible promoter C120 provides for low basal expression and high maximal expression after induction. It consists of 5 repeats of the 20 bp long, EL222-binding clone-1 sequence (Rivera-Cancel *et al*., 2012) upstream of a minimal TATA box promoter.

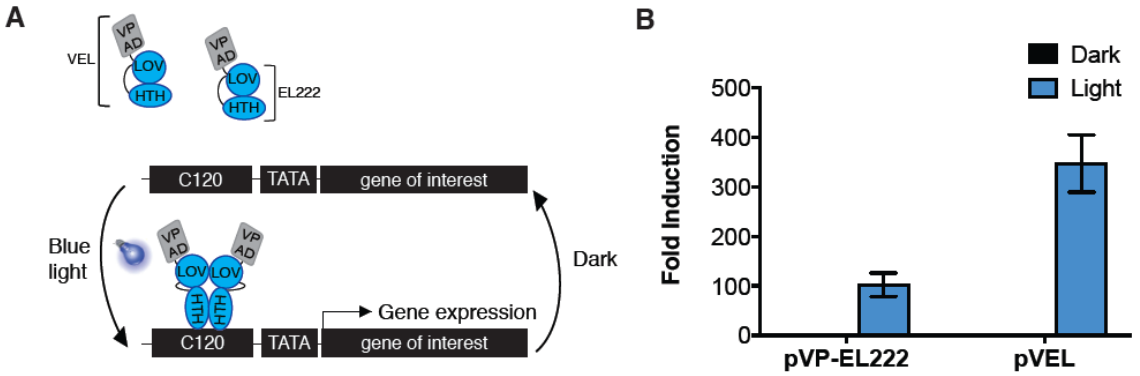

Figure 1. The LITE Switch System enables inducible gene expression upon stimulation with blue light. (A) The VEL protein consists of the transcriptional AD from the VP16 protein fused to EL222, which contains both LOV and HTH domains. In the dark, VEL is unable to bind its target C120 promoter; however, exposure to blue light triggers a photochemical reaction between the LOV domain and its flavin chromophore. This reaction causes a conformational change in the attached HTH domain that allows it bind the C120-TATA box promoter and turn on gene transcription. (B) The optimized VEL transactivator, now part of the LITE Switch System, provides a higher fold induction than the VP-EL222 protein previously described by Motta-Mena *et al.*, 2014. Following transfection with either pVP-EL222 or pVEL and pC120-Fluc, 293T cells were illuminated with pulsed blue light for 18 hr (465 nm; 20 s on, 60 s off; 2.9 mW/cm^2).

ADDITIONAL MATERIALS REQUIRED

1. Illumination setup

Figure 2 and the protocol below explain how to setup an LED panel to illuminate cultured cells from above inside of a conventional CO_2 incubator. This setup requires the following items:

- A panel of blue LEDs: 12'' blue LED Grow Light. Manufactured by HQRP: 250 blue LED, 14W/110 Volt, 465 nm. Available on Amazon.com and other retailers.
  Users might find LED panels made by other manufacturers that have the same specifications (14 W/110 Volt, etc.), these should be compatible and useful for this application.
- Electronic intervalometer: Model 451 from GraLab. Available on Amazon.com and other retailers.
- Velcro straps: VELCRO brand industrial strength tape 2 in X 4 ft. Available on Amazon.com and other retailers.

If the LED panel is setup as described in Figure 2 and in the Protocol below, the intensity of the light received by cells should measure 1-2.9 mW cm^{-2}. Although not completely necessary in the beginning, users might want to measure light intensity later on with a light meter. For example, using the LI-190 Quantum Sensor and the LI-250A light meter, both from LI-COR Biosciences.

As an alternative to using an LED panel, there is a Samsung Galaxy Tab based app developed for controlling light wavelength and intensity in a 96-well or petri plate format. The app is called E.Colight and it has been tested for use in bacteria to control light-driven transcription (E.Colight, 2012 & 2013). While the app has not been tested for use with eukaryotic or mammalian cell cultures, it is in the public domain and is freely available to users who would like to try it.

Note: The LED panel described above works best when illuminating cells grown in 24 well plates. The reason for this is that the distance between each LED bulb in the panel is the same distance as the well center to well center spacing. This enables each well to be illuminated by an individual LED bulb.

2. Phenol red-free cell culture media

Growing cells in media without phenol red will ensure maximum amount of light reaches the cells in the well. Several manufacturers offer phenol red-free media so there are many brands for users to choose from. Nevertheless, in every case the user should make sure to add the necessary supplements to the media to make it complete, e.g. fetal bovine serum, sodium pyruvate, L-glutamine, etc.

On a related point, it has been documented from experiments with cultured cells using fluorescent microscopy and other related techniques, that high irradiation intensities and prolonged exposure to light can cause some components of culture media and supplements to be converted to toxic free radicals by light (Magidson and Khodjakov, 2013). HEPES buffer is a supplement in particular that should not be added to media that will used to grow cells that will be illuminated. It has been shown that the addition of HEPES to media increases the production of cytotoxic products during exposure to visible light (Zigler *et al.*, 1985). The presence of sodium pyruvate in the media has been shown to counteract the negative effects of HEPES, however it is best to avoid HEPES addition all together.

Motta-Mena *et al.* 2014 previously showed that illumination of 293T cells or zebrafish embryos for 24 hr did not substantially affect viability. Other immortalized cell lines showed similar behavior. However, users who are working with neuronal and/or primary cultured cells, which are known to be more sensitive cell types, might find it beneficial to use specialized media with specific phototoxic components eliminated and replaced, e.g. LiveLight™ from Cell Guidance Systems.

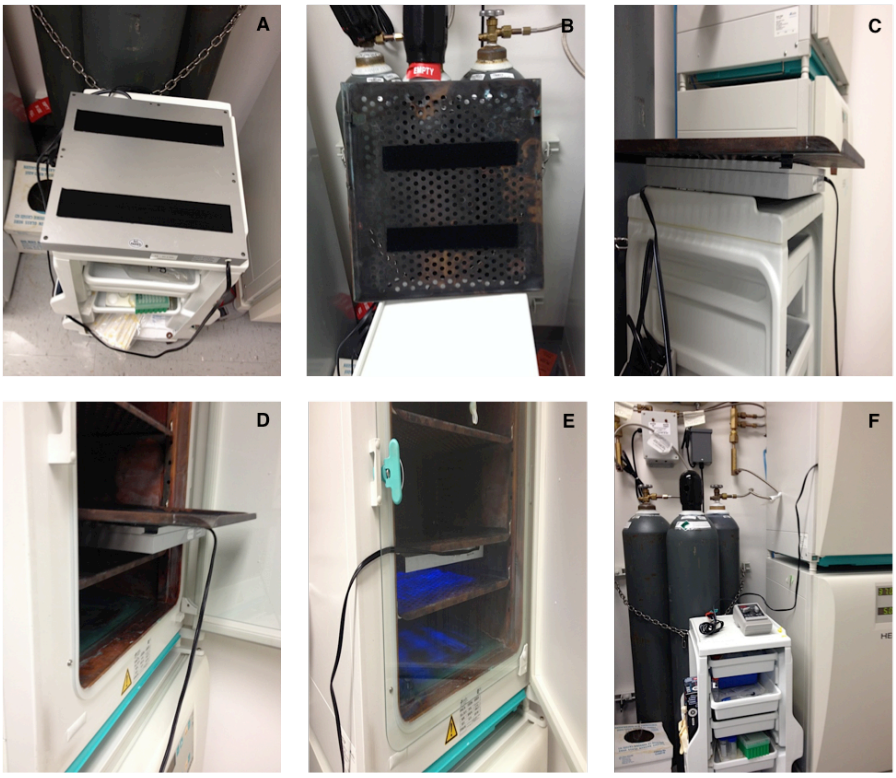

Figure 2. Setting up the LED panel inside a CO_2 incubator to illuminate cultured cells. First connect the two sides of the Velcro strap, the rough side is called hook and the softer mate is called loop. Remove the protective label to expose the adhesive side on the hook side and glue that to the LED panel. Next, remove the label on the soft mate. Take out the middle shelf from the incubator and line up the underside of the shelf with the LED panel so that it is centered. Attach the shelf to the soft mate, the shelf and LED panel are now attached and the shelf can be placed by in the incubator. The culture plate with cells that are to be illuminated can be placed in the bottom shelf so that the LED panel can illuminate the plate from the top. Once the illumination period is complete, the user can take out the LED panel from the incubator by pulling the LED panel to detach the two Velcro straps. This way the LED panel can be stored outside the incubator for later use. Panel A-F show how re-attach the LED panel to the shelf to use it again for an illumination experiment.

PROTOCOL

Testing the LITE Switch system in cultured mammalian cells using a Firefly Luciferase reporter

1. Clone a Firefly luciferase gene into the multiple cloning site of the pC120 plasmid.

2. Day 1: Plate 0.5-1.5 × 10^5 293T cells per well in two 24-well plates, one will be illuminated ("light") and the second will be kept in the dark and serve as a control ("dark").

3. Day 2: Transfect pC120-FLuc and pVPEL plasmids into pre-plated cells using a reporter:regulator plasmid ratio of 1:5. As a first pass one could try transfecting 0.5 µg of total DNA using 1-1.5 µl of Lipofectamine-3000. It is good practice to also transfect an internal vector control that contains Renilla luciferase under the control of a constitutive promoter. In this case the reporter:regulator:control plasmid ratio would be 1:5:0.02.

   Note: Follow the manufacturer's instructions for using Lipofectamine-3000 or other transfection reagent of choice.

4. Day 3: Following the instructions in Figure 2, attach the blue LED panel to the underside of middle shelf in the cell incubator. Place the bottom shelf two notches below the middle shelf. Place the "light" plate on the bottom shelf directly underneath the LED panel and centered. In this setup the LED panel should be approximately 8-10 inches away from the plate.

   The "dark" plate can be placed on the top shelf where it is still dark. One may cover the "dark" plate with foil to further protect it from the light coming from the LED panel. Alternatively if a second incubator is available one can place the "dark" plate in there.

   Connect the LED panel to an electronic timer and set to a cycle of 20s on/60s off. Max induction is achieved after 6-12 hr of illumination, however as a first pass, an overnight incubation (~18 hr) with light can also be done. Alternatively, users may initially try to illuminate cells continuously instead of doing a pulsed illumination cycle with a timer; however, in this case consider doing a shorter illumination period to reduce the possibility of "photo-bleaching" the transactivator by exposing to prolonged periods of light.

   Note: If the LED panel is setup as described above the intensity of the light received by cells should measure 1-2.9 mW cm^{-2}.

5. Day 3 or 4: After the predetermined period of illumination, cells are assayed for luciferase activity using a suitable luciferase assay kit according to the manufacturer's instructions.

   Note: At this point it is still safe to work under standard ceiling lights because a luciferase assay measures translational output (i.e. luciferase protein levels). It should be noted that while the VEL protein is blue light sensitive, it is safe to work with under red light. This might be most useful if the user is measuring transcriptional output (mRNA levels) or when even minimal levels of non-specific activation are a major concern.

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APPENDIX: Plasmid Information

The LITE Switch Inducible Expression System contains an inducible expression plasmid pC120 and a regulatory plasmid pVEL (Figure 3). The plasmid sequence is shown below.

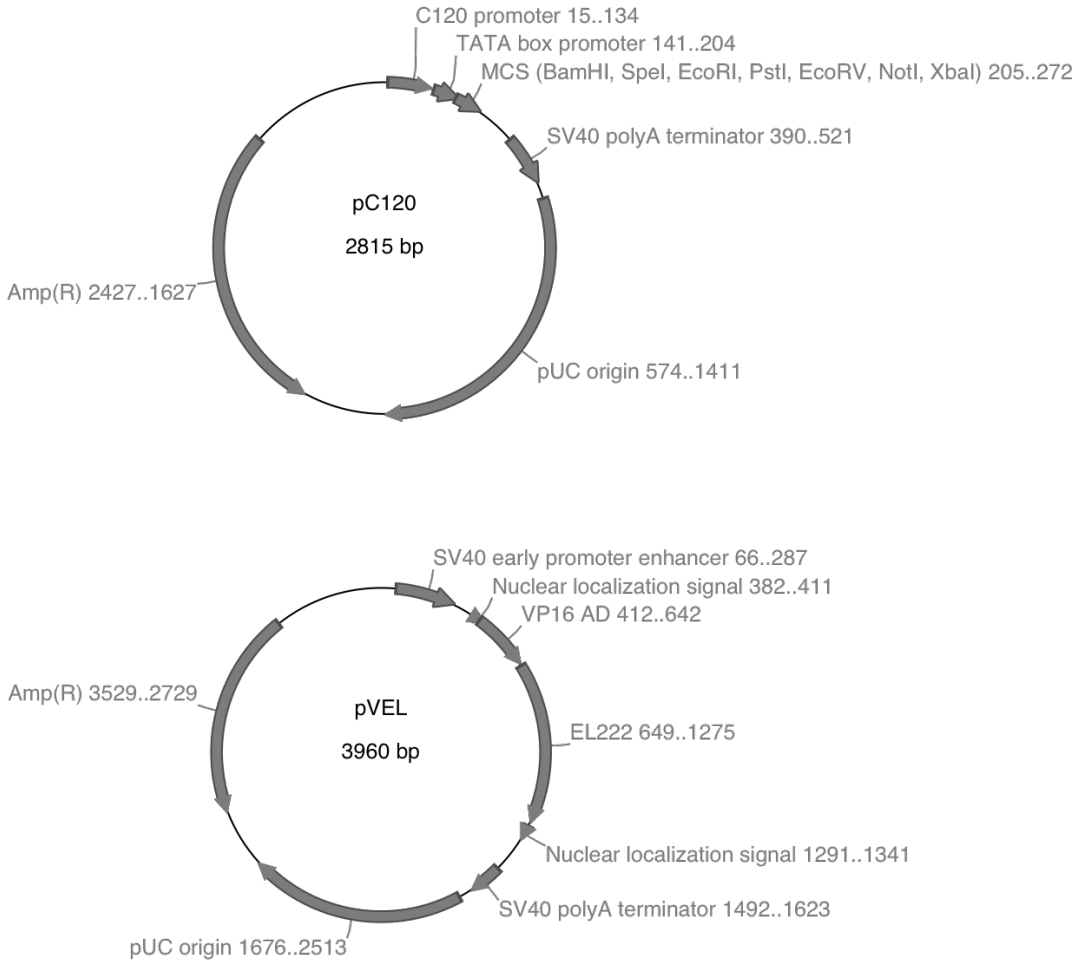

Figure 3. Plasmid maps for pC120 and pVEL constructs.

pVEL plasmid

SV40 Early Promoter Enhancer	bases 66—287
Nuclear Localization Signal	bases 382—411
VP16 Activation Domain	bases 412—642
Optimized EL222	bases 649—1275
Nuclear Localization Signal	bases 1291—1341
SV40 PolyA Terminator	bases 1492—1623
pUC Origin	bases 1676—2513
Amp(R)	bases 2729—3529

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pC120 plasmid

C120 Promoter	bases 15—134
TATA Box Promoter	bases 141—204
Multiple Cloning Site	bases 205—272
SV40 PolyA Terminator	bases 390—521
pUC Origin	bases 574—1411
Amp(R)	bases 1627—2427

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