MiniSOG-mediated Photoablation in *Caenorhabdtis elegans*

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[Abstract] This protocol describes a method for light-inducible cell ablation in live worms. miniSOG (mini Singlet Oxygen Generator) generates singlet oxygen upon blue light illumination (Shu *et al.,* 2011). Mitochondrially membrane targeted miniSOG (the first 55 a. a. of *C. e.* tomm-20 fused at the N'-terminus of miniSOG, termed as mito-miniSOG in the following) is transgenically expressed in specific cells/tissues (Qi *et al*., 2012). Groups of transgenic animals are illuminated under open field fluorescence light on a compound microscope or LED light setup for photo-ablation.

Materials and Reagents

1. Mito-miniSOG construct* driven by your chosen promoter, *e.g.*, *Pnmr-1-mito-miniSOG* (interneuron) (http://www.wormbase.org provides general information for *C. e.* specific promoters. http://chinook.uoregon.edu/neuron.html provides a list of neuronal promoters)
   *Requests for miniSOG construct should be addressed to Roger Y. Tsien (UC San Diego).
2. (*optional*) non-green fluorescence marker to label your target cells or tissues, *e.g*., *Pnmr-1-mCherry*
3. Control construct that is free miniSOG driven by specific promoters, *e.g*., *Pnmr-1-miniSOG*.
4. 100 mM $CuCl_2$
5. Filter paper
6. NGM (nematode growth media) plates (seeded and unseeded)
7. NGM (nematode growth media) (see Recipes)

Equipment

1. Fluorescence stereomicroscope (dissecting microscope, *e.g.,* Leica, model: M165)
2. Upright epi-fluorescecne microscope (compound microscope, *e.g.,* Zeiss Axio Imager 2)

3. Prizmatix LED (UHP-Mic-LED-460), and Digital Function Generator (PASCO Scientific, catalog number: PI-9587C)
4. Microinjection rig to generate transgenic worms

Procedure

1. Inject your mito-miniSOG construct together with non-green fluorescence marker to produce transgenic worms.
   (*Tips*: Optimize the injection concentration empirically based on the promoter strength. 20-50 ng/µl would be a good starting range; for weak promoters the concentration may need to be increased up to 100 ng/µl. Screen for transgenic lines with moderate mito-miniSOG expression, *i.e.,* the green fluorescence from mito-miniSOG should be visually detectable under a 63x objective on an epi-fluorescence microscope. If the green fluorescence can be easily detected under a 10x objective, the expression of mito-miniSOG may be too high. Mito-miniSOG transgenic animals can be kept in standard incubators under the cover of aluminum foil, and caution should be taken in keeping animals away from continuous room light exposure).
2. L4 (or any developmental stages) transgenic animals are isolated using behaviorally visible markers or non-GFP co-injection markers under fluorescence dissecting microscope; avoid exposure to blue light.
3. If you intend to use compound microscope for blue light illumination, precede to steps 4-10. If a LED light is used, go to steps 11-13.
4. Cut a filter paper in a ring shape with the outer diameter of 50 mm (to fit into the 60 mm dish) and the inner diameter of 15 mm, about the size of the illumination spot. Place the filter paper on an unseeded 60 mm NGM plate.
5. Drop ample amount of 100 mM $CuCl_2$ solution (about 0.5 ml) onto the filter paper until the paper is adequately wet; the Cu^{2+} is to restrict worms inside the illumination spot, as worms show aversion to Cu^{2+}.
6. Add OP50 paste in the ring opening by pressing a chunk of seeded agar (from a seeded plate) upside down then flipping it off. Addition of OP50 is to keep animals well fed during illumination.
7. Transfer transgenic worms inside the copper ring.
8. Place the plate without cover on the compound microscope stage right under one empty position of the turret (or remove one objective lens), such that blue light passes down directly onto the plate.
9. Turn on the blue fluorescence light (475 ± 20 nm). An example of the light intensity worms received was 57 mW/cm^2 on compound microscope.

(*Tips*: Animals can be exposed to either continuous blue light, or pulse blue light (0.5 sec on, 1.5 sec off). The pulse light is provided by an electrical shutter, *e.g.,* 30 mm W/HS, LUDL Electronics and a signal device, *e.g.*, Uniblitz unit (VMM-D1)).

10. Illuminate animals for 30 min to 1 h; optimal length should be determined empirically with respect to the specific microscopes and light sources and the transgenes. After light illumination, worms were transferred to freshly seeded plates prior to any analysis.
11. (To use LED for illumination) Drop about 0.5-1 ml of 100 mM $CuCl_2$ solution by the edge of an 40 mm NGM plate (40 mm is about the size of the illumination spot under our LED setup) and swirl the liquid around the dish wall for a couple of times, then remove excessive liquid.
12. Transfer transgenic worms onto the center of the 40 mm NGM plate (we prefer to use seeded plates unless other specific conditions are demanded otherwise). Place the plates under the LED light source. The distance between worms and the LED light plate is about 12 cm. Turn on the LED light. The frequency of LED light is set to 4 Hz. The intensity of blue light (460 nm) from LED set up is around 800 mW/cm^2.
13. Illuminate the worms for any desired length of time. Then transfer worms onto a new seeded plate.

    (*Tips*: Optimal exposure time should be determined empirically, depending on cell type and expression levels of mito-miniSOG. For example, flashes 4 times per sec for 2 min can sufficiently damage hypodermis expressing mito-miniSOG, but much longer time illumination would be needed to kill neurons.)

Recipes

1. NGM (nematode growth media)

Acknowledgments

This protocol is adapted from Qi *et al*. (2012) and Shu *et al*. (2011).

References

1. Qi, Y. B., Garren, E. J., Shu, X., Tsien, R. Y. and Jin, Y. (2012). Photo-inducible cell ablation in *Caenorhabditis elegans* using the genetically encoded singlet oxygen generating protein miniSOG. *Proc Natl Acad Sci U S A* 109(19): 7499-7504.

2. Shu, X., Lev-Ram, V., Deerinck, T. J., Qi, Y., Ramko, E. B., Davidson, M. W., Jin, Y., Ellisman, M. H. and Tsien, R. Y. (2011). A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. *PLoS Biol* 9(4): e1001041.

