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Spectral Recording of Gene Expression History by Fluorescent Timer Protein

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.8jvhun6

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ABSTRACT

Monitoring spatio-temporal patterns of gene expression by fluorescent proteins requires longitudinal observation, which is often difficult to implement. Here, we fuse a fluorescent timer (FT) protein with an immediate early gene (IEG) promoter to track live gene expression in single cells. This results in a stimulus- and time-dependent spectral shift from blue to red for subsequent monitoring with fluorescence activated cell sorting (FACS) and live cell imaging. This spectral shift enables imputing the time point of activity *post-hoc* to dissociate early and late responders from a single snapshot in time. Thus, we provide a tool for tracking stimulus-driven IEG expression and demonstrate proof of concept exploiting promoter::FT fusions, adding new dimensions to experiments that require reconstructing spatio-temporal patterns of gene expression in cells, tissues or living organisms.

EXTERNAL LINK

https://www.future-science.com/doi/10.2144/btn-2019-0050

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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Spectral Recording of Gene Expression History by Fluorescent Timer Protein.pdf

GUIDELINES

We do not go into details regarding the plasmid cloning process, as we welcome everyone who is interested in using it, to contact us and we will provide it. Therefore, we will only describe the cell culture experiments.

Mathematical extrapolation of cellular activity

For the mathematical differentiation of induced and noninduced cells, z-scores of the fluorescent intensity for each color were calculated from a total of 20 cells. The mean and SD for both colors were calculated separately, including all cells and time points of interest. Z-scores were then calculated by subtracting the value of interest by the mean and dividing through the SD of the respective color. To determine the time point of cellular activity of individual cells during the live cell imaging, we established a mathematical model that allows extrapolating gene activity depending on the ratio of blue to red fluorescence. Thus, the change of the fluorescent signal of blue and red over time was calculated. To this end, the logarithm of the ratio of the fluorescent intensities of five randomly chosen cells was plotted over time. In a next step, the linear curve function was used to extrapolate the time point of cellular activity of five cells (again randomly chosen) from the same experiment with unknown induction time points. To verify this approach, we repeated these calculations with a larger dataset with a total of 18 randomly chosen cells from an independent experiment. For the mathematical differentiation of early and late responders, the median response rate to the stimulus was used as a cut-off. If the calculated response was before the median, cells were grouped as early responders, whereas cells, for which the calculated response time was more or equal than the median, were allocated to the late responder group.

$$y = log10\left(\frac{blue_n - blue^{t1}}{red_n - red^{t1}}\right)$$

Troubleshooting

If you see a positive signal in your - TPA cells, try handling your cells with even more care, as the FT expression can be activated very easily. Make sure medium is really on 37 °C and not too cool before you add it. Avoid any type of stress for your cells as good as possible.

MATERIALS

NAME	CATALOG #	VENDOR
6-well cell culture plates		Eppendorf
DMEM		Life Technologies
FCS (Fetal Calf Serum)		Life Technologies
Pen/Strep (Penicillin/Streptomycin)		Life Technologies
L-Glutamine		Life Technologies
TransIT®-LT1 Transfection Reagent	MIR 2304	Mirus Bio
Opti-MEM I reduced serum medium		Life Technologies
Phorbol-12-myristate 13-acetate	P8139	Sigma Aldrich
PBS		Life Technologies
Trypsin/EDTA		Life Technologies

MATERIALS TEXT

- The FT-ESARE/ArcMin plasmid can be obtained from the authors directly.
- HeLa cells (ATCC, CCL2)

Recipes

standard medium: DMEM + [M]10 undefined **FCS** + [M]1 undefined **Pen/Strep** + [M]1 undefined **L-Glutamine**

Required Equipment

- Cell Culture working space (incubator, hood, centrifuge)
- FACS (BD LSR Fortessa2, BD Biosciences)
- Chambered cell culture slides (8-well Cell Culture Slides, CCS-8, MatTek Corporation)
- Spinning Disk microscope (Spinning Disk Confocal UltraView Vox, controlled by software Volocity)

SAFETY WARNINGS

Please refer to the Safety Data Sheets associated with each reagent for safety information.

Cell Culture and Transfection

1

Grow HeLa cells overnight in standard incubator conditions (**37.5 °C** , 5 % CO_2) on 6-well plate in standard medium (DMEM + 10 % FCS + 1 % Pen/Strep + 1 % L-Glutamine) to a confluency of about 80 %.

2 Transfection with the TransIT-LT1 transfection reagent according to manufacturer: To **1 µg/µl plasmid in Opti-MEM I reduced serum medium** , add TransIT-LT1 reagent.

3

Incubate for **00:30:00** at **Room temperature** .

4 Add reagent to cells for **24:00:00** .

5

Remove medium containing transfection reagent, wash with PBS and add fresh standard medium.

6 There are alternative options for the following steps:

1. Fluorescence-activated cell sorting (FACS) (alternative I)
2. Life-cell imaging (LCI) (alternative II)

step case

Fluorescence activated cell sorting

alternative I

FACS

7 Leave cells undisturbed until induction (**48:00:00** are recommended).

8 Baseline measurement: cells dislodged with trypsin and fluorescence and cell number determined by FACS.

9

Induction with TPA.

TPA is highly cancerogenic so wear respective protective gear!

TPA is very volatile: make sure you only add TPA to the wells, in which you want to induce the cells!

10 Grow + and - TPA cells in separate 6-well plates to guarantee no TPA spills over into - TPA wells, as also the smallest amount of TPA induce IEG expression.

11 Incubate for **00:40:00** with **100 ng/ml TPA diluted in standard medium** .

12 Carefully remove all TPA and also remove medium of - TPA cells.

13 Wash with PBS.

14 Add fresh medium **or** add about **2 ml trypsin** until cells are detached from the plate.

15 Add **2 ml standard medium** to the cells for deactivation.

16 Pellet cells by centrifugation.

17 Resuspend in PBS.

18 Perform FACS measurements at the time points of your choice.

We recommend every two hours for the first 9h and again at 24h post-induction.

19 You can analyze FACS data with any standard FACS analysis program (e.g. FlowJo).

Life-cell imaging

step case

Live-cell imaging

alternative II

7 Transfer cells into chambered cell culture slides **24:00:00** after transfection.

8 Take cell growth into account when determining your cell density seeded onto the plates as they will stay on the culture slides for another **48:00:00** .