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Nanoluciferase Assay in C. elegans

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Protocol status: Working
We use this protocol in our group and it is working.

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Abstract

A highly sensitive Nanoluciferase (NanoLuc)-based method in multi-well format to detect constitutive and inducible gene expression in whole *C. elegans*. Worms have to be lysed for this end-point assay, no spatial resolution of expression can be detected with this protocol.

Attachments

Nanoluciferase Assay...

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Materials

- Costar 96-well plates: white opaque (Corning, Cat #3912)
- Silicon Carbide beads: 1 mm diameter (BioSpec Products, #11079110sc)
- NanoGlo kit: Nano-glo Substrate (-20°C) and Buffer (fridge) (Promega, Cat# N1110)
- Vortexer with tube attachment: Disruptor Genie (Scientific Industries)
- 1X Lysis Buffer: 50 mM HEPES pH 7.4, 1 mM EGTA, 1 mM MgCl2, 100 mM KCl, 10% Glycerol, 0.05% NP40, 0.5 mM DTT, protease inhibitor (Millipore Sigma, cOmplete™ Cat# 11836170001). Good for 7 days at 4°C after protease inhibitor is added.
- Optional: Repeater pipette, Eppendorf Repeater plus with 0.5 ml tips (Cat# 89232-934)

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A.Grow up/Treat Worms and Prepare Lysate

Grow up and/or treat worms as required, usually on 3.5 or 6 cm NGM plates seeded with OP50-1. N2 can be included as no-Nanoluciferase control, ERT513 (*unc-119 (ed3) III; jySi35 [pET618 (vha-6p::NANOLUC::unc-54 3' UTR, unc-119 (+)]*)or ERT529 (*unc-119 (ed3) III; jySi40 [pET635 (vha-6p::NANOLUC::3XFLAG::unc-54 3' UTR, unc-119 (+)]*) can be used as Nanoluciferase (NanoLuc) expressing control. Well-fed adults give highest signal.

i.Prepare Lysate of 1 to <50 Worms

1. Prepare 1.7 ml microfuge tubes with 80 - 100 ul 1x Lysis Buffer
2. Pick worms into Lysis Buffer, making sure that they were released from the (eyelash) pick
3. Add approximately 5 - 10 Silicon Carbide beads to tube with worms
4. Place Disruptor Genie to 4°C and vortex samples for 4 minutes
5. Spin down sample for 1 minute at max speed with a table top centrifuge
   Note: Possible to freeze supernatant (worm lysate) to -80°C for later use. Transfer supernatant to new 1.7 ml tube and freeze.*
6. Transfer 50 ul of the supernatant (worm lysate) to well of white 96-well plate
7. Prepare 25 ul of NanoGlo Reagent per sample well (1 part NanoGlo Substrate **+** 50 parts NanoGlo Buffer)
   Note: Do not resuspend worm lysate and NanoGlo Reagent by pipetting up and down, bubbles might mess up reading. Shaking on plate shaker is sufficient to mix.
8. Add 25 ul of NanoGlo Reagent to every well with worm lysate (using repeater pipette is most sufficient to stay bubble free)
9. Agitate plate for 10 minutes at room temperature.

ii.Prepare Lysate of ≥ 50 to < 1,000 Worms

1. Wash worms from growth plate into 1.7 ml tubes with M9-T (M9 with 0.1 % Tween-20)
2. Spin worms down at 2,000xg for 1 minute, remove supernatant to 100 ul line
3. Add 300 ul of 1X Lysis Buffer
4. Spin worms down at 2,000xg for 1 minute, remove supernatant to 100 ul line
5. Add approximately 10-15 Silicon Carbide beads to tube with worms
6. Place Disruptor Genie to 4°C and vortex samples for 4 minutes

7. Spin down sample for 1 minute at max speed with a table top centrifuge
   Note: Possible to freeze supernatant (worm lysate) to -80°C for later use. Transfer supernatant to new 1.7 ml tube and freeze.*
8. Transfer 50 ul of the supernatant (worm lysate) to well of white 96-well plate
9. Prepare 25 ul of NanoGlo Reagent per sample well (1 part NanoGlo Substrate + 50 parts NanoGlo Buffer)
   Note: Do not resuspend worm lysate and NanoGlo Reagent by pipetting up and down, bubbles might mess up reading. Shaking on plate shaker is sufficient to mix.
10. Add 25 ul of NanoGlo Reagent to every well with worm lysate (using repeater pipette is most sufficient to stay bubble free)
11. Agitate plate for 10 minutes at room temperature.

iii. Prepare Lysate of ≥ 1,000 Worms

1. Wash worms from growth plate into 15 ml tubes with M9-T (M9 with 0.1 % Tween-20)
2. Spin worms down at 2,000xg for 1 minute, remove supernatant to 300 ul line
3. Transfer worms to 1.7 ml tubes with glass Pasteur pipette
4. Add 300 ul of 1X Lysis Buffer
5. Spin worms down at 2,000xg for 1 minute, remove supernatant to 100 ul line or above in case worm pallet is larger
6. Add approximately 15-20 Silicon Carbide beads to tube with worms
7. Place Disruptor Genie to 4°C and vortex samples for 4 minutes
8. Spin down sample for 1 minute at max speed with a table top centrifuge
   Note: Possible to freeze supernatant (worm lysate) to -80°C for later use. Transfer supernatant to new 1.7 ml tube and freeze.*
9. Transfer 50 ul of the supernatant (worm lysate) to well of white 96-well plate
10. Prepare 50 ul of NanoGlo Reagent per sample well (1 part NanoGlo Substrate + 50 parts NanoGlo Buffer)
    Note: Do not resuspend worm lysate and NanoGlo Reagent by pipetting up and down, bubbles might mess up reading. Shaking on plate shaker is sufficient to mix.
11. Add 50 ul of NanoGlo Reagent to every well with worm lysate (using repeater pipette is most sufficient to stay bubble free)
12. Agitate plate for 10 minutes at room temperature.

2 B. Measure Luminescent Signal with NovoStar Plate Reader

1. Use at least two wells with 1X Lysis Buffer with NanoGlo Reagent as blank (ideally, Lysis Buffer was vortexed with Silicon Carbide beads)

2. Switch on plate reader (switch located at right site of reader), open black sliding door on top of plate reader; Install optic fiber for luminescent measurement for top reading, close sliding door tightly
3. Open “NovoStar” software
4. Choose “Setup” > “Reader configuration” > “Luminescence”
5. Choose “Test setup” > “Test protocol” > “Bright Glo” (new window opens) > “Edit” > “Layout” > mark samples (and blank) according to your plate design (only those wells are measured)
   Bright Glo measures signal for 1 second per well without filters.
6. Choose “Measure” > “Plate out” > insert uncovered plate > “Plate in”
7. Choose “Measure” (new window opens) > “Measure”
   Note: Before pressing “Measure” in new window, shaking and optic settings can be adjusted:
   7.i) Shaking settings should be linear shaking 1mm: additional shaking time: 10s before plate reading; positioning delay 0.2 sec.
   7.ii) Gain should be adjusted on well with highest expected signal. If that’s unknown, click plate number “96” in upper left corner to determine best well for gain adjustment. Highest possible gain is 4095.
8. To open results, choose “Results” > “Open last test Run” (MARS software opens and displays results in plate or table format as well as a summary of program settings)
9. Export data from each tab with Excel button
10. To shut down, close MARS software, remove plate and close NovoStar software, shut down computer, switch off NovoStar plate reader