High-throughput image-based drug screening of *Caenorhabditis elegans* movement, development, and viability

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Abstract

*Caenorhabditis elegans* is an important model organism for identifying and studying druggable targets in a high-throughput manner [1–3]. We have previously described a modular pipeline for image processing and analysis for both parasitic and free-living worms [4]. Here, we detail the upstream assay set up for high throughput drug screens of the free-living nematode *C. elegans*.

Following a standard bleaching protocol, embryos are hatched overnight and synchronized L1s are added to a 96-well plate. Concentrated *Escherichia coli* strain HB101 bacterial food [5] is added to each well to sustain growth for 48 hours along with compounds of interest. Plates are incubated for 48 hours until worms reach the young adult stage. While this assay has been optimized for image acquisition with an ImageXpress high-content imager, it can be adapted for use on similar imaging platforms. Analysis of images from an ImageXpress is seamless using wrmXpress [4], but other analysis software can be used.

Introduction

Reagents

HB101 food stocks

*E. coli* HB101 frozen stock

LB agar 10 cm plate

Inoculation loop

LB media

Autoclaved water

50 mL conical tube

Flasks: 150 mL, 1 L

1.5 mL tubes

K-media

Bleaching and titering

Glass microscopy slide

15 mL conical tubes
K-media

Bleach solution (6 mL per strain)

M9

Plate set-up and imaging

*E. coli* HB101 food stocks

Kanamycin (stock 50 mg/ml)

96-well plate (Greiner-Bio One 655180)

25 and 50 mL troughs (DOT Scientific ER-025S-5, USA Scientific 1930-2520)

K-media

100x drug stocks

Breathable plate seals (Sigma-Aldrich Z380059-1PAK)

NaAz dissolved in M9 (125 mM; 1:8 dilution of 1M NaAz stock in M9)

Plastic storage box

CellTox Green kit (Promega G8741)

Parafilm

**Equipment**

Centrifuge (3,000 x *g*)

Plate rocker (Fisher)

Shaking incubator

Vortex

Plate vortex (Fisher)

Microscope
Automatic multichannel pipettes

ImageXpress Nano (Molecular Devices)

optional: VWR plate spinner, Molecular Devices AquaMax plate washer 2000, other suitable imaging system

**Procedure**

**Making HB101 food stocks:**

1. Streak HB101 from frozen stock onto LB agar plate (use plate within a week) and grow overnight at 37°C
2. Inoculate 1 colony in 50 mL LB for 16 hr at 37°C, with shaking (220 rpm)
3. Inoculate 500 µL of overnight culture in 500 mL of LB at 37°C, 220 rpm for 13 hours (OD ~ 0.8 -0.9 for HB101)
4. Transfer the bacterial overnight culture to 8 x 50 mL tubes
   1. Pre-weigh 2 of the 8 tubes and record weight on tube
5. Centrifuge for 10 min at 3,000 x g
6. Discard LB and repeat steps 4 & 5 until all bacteria is pelleted
7. Discard LB and add 40 mL of autoclaved H2O to 2 tubes (of 8)
   1. Vortex to resuspend bacterial pellet
   2. Sequentially transfer volume to 4 other tubes and vortex to resuspend
   3. This combines the pellets of 10 tubes into the 2 pre-weighed tubes
   1. **Note:** If the pellet is large, 20mL can be added initially to get the pellet into solution, add up to 40mL after pellet is resuspended
8. Centrifuge for 10 min at 3,000 x g
9. Discard H2O and resuspend in 40 mL of autoclaved H2O via vortexing
10. Centrifuge for 10 min at 3,000 x g
11. Discard H2O and weigh the tubes to calculate the bacterial pellet weight
12. Re-suspend and combine pellets to a final concentration of 50 mg/mL in K-medium
   1. Pellet total weight = X grams; Total resuspension volume = 20*X mL of K-medium

*Example: 1.8g pellet should be resuspended in 36 mLs (= 36 x 1 mL aliquots)*
1. Thoroughly vortex and then shake for a half hour at room temperature
2. Aliquot into 1 mL units and store at -80°C (label box with “HB101 HTA” and date)

Note: Aliquots are 20x stocks. 5 µL of these aliquots are added into wells with a total volume of 100 µL. Each 1 mL bacteria stock tube can be used to feed two entire 96-well plates. Plates are fed on day one of the assay. Expected Yield: This protocol typically yields ~200 mL of 50 mg/mL HB101 stock, aliquoted into 200 x 1 mL tubes, which can feed a total of approximately 400 96-well plates.

Bleach synchronization:

1. Generate 10 cm NGM plates of gravid adults via picking 50 L4s to NGM plates 96 hours before bleaching, selecting transgenic worms if necessary. Alternatively, chunk to 10 cm NGM plates 72 hours before bleach.
2. Bleach (1 strain per 15 mL tube) when most adult worms are gravid (bleach protocol)
3. Resuspend embryos in nal volume of 2 mL M9
4. Titer embryos
   1. Briefly mix by inverting tube
   2. Count 1 x 5 µL aliquot
   3. Add K media such that there are 5 embryos/µL
   4. Count 3 x 5 µL aliquot
   5. Add K media such that there are 3 embryos/µL
   1. If the volume is >7.5 mL transfer to a 50 mL conical tube or split among multiple tubes
   6. Count embryos using 1 x 5 µL aliquots on a glass slide to ensure 3 embryos/µL
5. Hatch embryos for 12 - 20 hours at room temperature by nutating at setting speed 13 on the plate rocker or shaking at 180 RPM
6. After hatching count 10 x 5 µL aliquots on a glass slide
   1. Note: if you see too many carcasses, let tubes settle before drawing off L1s from the top.
7. Titer to 1 L1/µL in K media

Long-term growth assay (LTA):
1. Transfer titered L1s to a 25 mL trough
2. Using a multichannel pipette add 50 µL of titered L1s per well (50 L1s/well)
3. Using a multichannel pipette add 50 µL of HKM per well (final HB101 [2.5 mg/mL])
   
   1. To make HKM add 500 µL HB101 stock + 4.5 mL K media + 5 µL KAN stock (50 mg/mL, final 25 µg/mL) into a 50 mL trough
4. Add 1 µL of 100x drug or control per well

   1. Alternatively, worms and HKM can be added directly to assay ready drug plates
5. Seal drug plates with breathable film
6. Assemble and place drug plates in humid chamber:

   1. Clean (rinse thoroughly with ethanol and distilled water) plastic storage box
   2. Place damp paper towels at the bottom of the box
   3. Stack sealed drug plates into box
   4. Close box and seal with parafilm
7. Place box into incubator at 20°C, shaking at 180 rpm
8. Incubate for 48 hours

**Phenotyping:**

1. Motility:

   1. Remove plates from shaking incubator and remove breathable strips
   2. Wipe bottom of the plate with ethanol and kimwipe followed by a dry kimwipe
   3. Take transmitted light videos using the ImageXpress or equivalent

       1. 4x, 1 site, 5 timepoints (frames per well) at ~3.3 FPS
2. CellTox (live/dead staining):

   1. Remove plates from shaking incubator and remove breathable strips if not already removed for other phenotypic endpoints
   2. Prepare a 1:200 dilution of the CellTox stock into the provided buffer
   3. Add 10 µL of CellTox to each well
   4. Replace breathable strips and place lids on plates
   5. Incubate for 30 minutes at 20°C, shaking at 180 rpm, protected from light exposure
6. Wash three times using a multichannel pipette
   1. Add 200 µL of M9 to each well
   2. Gently shake for ~10 seconds
   3. Remove 200 µL
   4. Wash 2 additional times
7. Add 250 µL of M9-NaAz stock (NaAz final = 50 mM)
   1. Alternatively, use an AquaMax plate washer to dispense 200 µL M9 to each plate
   2. Spin the plates for 30 seconds using the VWR plate spinner
   3. After the spin has completed, carefully remove the plates
   4. Use the plate washer to aspirate to 5 mm
   5. Repeat once more
   6. Add 280 µL with M9-NaAz stock (NaAz final = 50 mM)
8. Top-fill wells with M9 to reduce shading during imaging
9. Wipe bottom of the plate with ethanol and kimwipe followed by a dry kimwipe
10. Image using an ImageXpress
    1. 2x, 1 site, 1 time point, GFP (other wavelengths as needed)
    2. Note: Transmitted light filter can be used during image acquisition if worm size is being measured.

3. Size:
   1. Remove plates from shaking incubator and remove breathable strips
   2. Wipe bottom of the plate with ethanol and kimwipe followed by a dry kimwipe.
   3. Add 250 µL of M9-NaAz stock (NaAz final = 50 mM)
   4. Top-fill wells with M9 to reduce shading during imaging
   5. Image using an ImageXpress
      1. 1 site at 2x with appropriate filters

*ImageXpress can be used to analyze motility, viability, and size data.*

**Troubleshooting**

**Time Taken**

**Anticipated Results**
References


