NemaMetrix

wMicroTracker Protocol

HIGH-THROUGHPUT SCREENING OF SMALL MOLECULES USING 96-WELL MICROPLATES

Goal:
To analyze the potentiality of drugs to suppress motility defects through automated drug screen.

What you need:
- wMicroTracker device
- Sterile 96-well flat-bottom microplates (Greiner)
- Stock solutions (i.e., from drug library) and appropriate solvent (see effect of solvents)
- 45-50 adult *C. elegans* worms

Protocol:

Grow adult synchronized populations in seeding NGM plates (OP50).
Remove worms from plates using M9 buffer and transfer them into a sterile 15 ml tube.
Let the worms settle by decantation.
Discard the supernatant taking care not to disturb the pellet.
Wash he worm pellet with 5 ml of M9. Briefly shake or gently invert the tube.
Repeat the decantation step.
Discard the supernatant and add 3 ml of M9.
Count worms in 10 µL in triplicate and calculate the average number of worms per 10 µL.
Prepare supplemented M9 with food at OD600 = 0.1 (OD600 up to 1.0 can be used).
Prepare a suspension of 5 worms/10 µl. Adjust volume in food-supplemented M9 up to desired final volume (see Notes 1 & 2).
Transfer 90 ul of worm solution to a 96-well microplate using a multichannel pipette (see Notes 1 & 2).
Let worms rest for 1h and measure basal activity for at least 30 minutes using WMicrotracker.
Add 10 µl of a 100 µM working solution of small molecules (10 µM to final concentration).
Record worm activity in triplicate for each condition (see Note 3).
Seal each plate with appropriate lid or PCR tape to prevent evaporation.
Register worm activity using WMicrotracker for at least 4 hours (see Note 4).
Compare the average movement score of each sample against the no-compound control. For drugs that rescue motor-deficient phenotypes (“hits”), a second screen could be performed to establish a concentration-response curve.

Notes and recommendations:

1- We recommend performing at least 3 technical replicates and at least 2 biological replicates per condition.
2- Include a no-compound control (solvent only). If the solvent is DMSO, do not exceed 1% v/v DMSO final concentration.
3- We recommend the use M9 buffer supplemented with bacteria OP50 to prevent worms from adhering to the plastic of the well.
4- For tests longer than 12 hours, consider using food to prevent starvation, as this could affect worm locomotion.

References:

A rapid chemical-genetic screen utilizing impaired movement phenotypes in C. elegans: Input into genetics of neurodevelopmental disorders. Schmeisser K, Fardghassemi Y, Parker JA. Exp Neurol. 2017 Jul;293:101-114. doi: 10.1016.

This protocol is adapted from Parker JA et al, 2017