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Drug tracking on hydra

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ABSTRACT

Protocol for preparing worms, preparing drug plates, dispensing worms with wormsorter and tracking on Hydra rigs

Prepare tracking plates and worms (-2 days)

1   Pour low peptone NGM into 96 well plates using VIAFILL dispenser. Dispense 🥛**200 μl** per well. Weigh 3 plates after they have cooled and store at 🌡 **4 °C** lidside down.

2   Refeed bleach synchronised worms onto 4 x 150mm plates by pipette 4 small droplets around the plate 2.5 days prior to tracking (eg 5pm on Monday for Thursday tracking)   60h

Prepare drugs onto plates (-1 days)

3   Get low peptone 96WP out of the 🌡 **4 °C** fridge, weigh three plates and then place the rest of the plates in the flow hood to dry for 3 hours. Weigh plates after drying, they should have lost at least 3% of their original mass before proceeding   3h

4   Label each (destination) plate with the source plate used and the location in the opentrons robot.

5   Prepare drug source plates in skirted 96 well plate so that each column corresponds to a single drug at a single concentration. The final concentration of DMSO should be 0.1%.

> 📄 Per well:
>
> 🥛**0.2 μl** **1000X drug in DMSO**
>
> 🥛**2.8 μl** **water**
>
> This needs to be scaled up to account for the number of replicates along each column (Eg.total_master_mix_volume = volume_per_well x 8 x n, where n=number of destination plates and 8 is number of wells per column.)

6

Run opentrons robot using prepared protocol.

> 📄 NB.
> - One drug source plate with each column corresponding to a drug for use with a multichannel pipette.
> - Destination and source plate locations are specified.
> - The opentrons robot will randomise the column locations from each source plate to a destination plate
> - The date is used as the random seed

6.1 Protocol parameters:

```
# multichannel pipette parameters and tipracks
multi_pipette_type = 'p10-Multi'
multi_pipette_mount = 'left'

tiprackdrugs_slots = ['3']
tiprackdrugs_type = 'opentrons-tiprack-10ul'
tiprackdrugs_startfrom = '1'

tiprackH2O_slot = '6'
tiprackH2O_type = 'opentrons-tiprack-10ul'
# tiprackH2O_startfrom = '1'

# water trough
H2O_source_slot = '9'
H2O_source_type = 'trough-12row'
H2O_source_well = 'A1'
H2O_volume = 5

# drugs source
drugs_source_slots = '2'#,'5','8','11']
drugs_source_type = '96-well-plate-pcr-thermofisher'
frombottom_off = +0.3 # mm from bottom of src wells
drugs_volume = 3

# destination plates
agar_thickness = +3.7 # mm from the bottom of the well for 200ul agar per well
destination_slots = ['1','4','7', '5']
destination_type = '96-well-plate-sqfb-whatman'

n_columns = 12

# create mapping from sources to destination.
seed = 20191031 # for reproducibility. Let's use the experimental date for the actual experiment, something else for debugging
np.random.seed(seed)
```

6.2 Make sure all labware is loaded correctly

6.3 Run protocol and monitor robot to ensure all the tips are removed

6.4　Tip racks have to replaced after each destination plate is filled

7　Allow the plates to dry for 30 minutes

Seed plates

8　Use VIAFILL dispenser to dispense 🖵5 µl 1:10 diluted OP50 into each well of each drug plate

9　Allow to dry for 30 minutes under the flow hood

10　Keep at room temperature over night (covered in the dark as some drugs are light-sensitive)

Disepensing worms

11　Wash worms off the 150mm plates with M9 using a pasteur pipette into 15ml falcons

12　Spin at ⊛1500 rpm **ascending 9, descending 7** for 2 minutes to pellet the worms

13　Remove supernatent and fill M9

14　Repeats steps 12-13 two more times

15　After final wash fill falcon with M9, transfer contents of 15mL falcons to 50mL and fill up to 45mL with M9.

16　Use wormsorter to dispense 2 worms per well

17　Allow liquid to dry off the plates for 30 mins under the flow hood

18　Incubate in drug for 4 hours

19　Hydra tracking : 15 mins 25fps, exposure 25000msec