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FoldSeek screen

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James Marshall[1], Eleanor Warren[1], John Bergqvist[1]

[1]MRC Laboratory of Medical Sciences, Imperial College London

Behavioural Genomics

John Bergqvist
MRC Laboratory of Medical Sciences, Imperial College London

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We use this protocol and it's working

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Abstract

This protocol outlines the screening procedure of a library of transformed E. coli expressing proteins with insecticidal potential. The two outputs from this procedure is 1) the OD_{600} as a measurement of how much C. elegans have consumed the transformed E. coli, and 2) recording worms under bluelight stimulation to capture variations in motility.

Materials

100 mm Petri dishes
Round-bottom Nunclon 96-well plates

2 mL deep 96-well plates
Breathable film cover for 96-well plates

Lysogeny Broth medium
Nematode Growth Medium
M9 medium
S-medium
Kanamycin
Nystatin
IPTG
Bleach solution
MilliQ water

C. elegans (N2)
E. coli (OP50)

Day 1 - Preparing worms

30m

1 **Egg laying** 30m

Pick 20 adult worms onto six 100 mm plates seeded with 1.5 mL of OP50. Leave on plates for 24:00:00 h .

Day 2 - Removing adult worms & making S-medium

2h 20m

2 **Remove adult worms from the 100 mm plates if sufficient eggs have been laid** 20m

3 **Check if S-medium is available.** 2h

If not, make S-medium.

Note

1 L Pre-autoclave S-medium (S-basal)

NaCl	5.85 g	4th floor
K_2HPO_4	1 g	4th floor
KH_2PO_4	6 g	4th floor
MilliQ water	1 L	4th floor

Note

1 L Post-autoclave S-medium
**Kanamycin, Nystatin and IPTG do not last long and so should only be added to smaller aliquotes upon use.*

Cholesterol ([M] 5 mg/mL in ethanol)	1 mL	4th floor
1 M potassium citrate pH 6	10 mL	4th floor
trace metals solution	10 mL	4th floor
1 M CaCl2	3 mL	4th floor
1 M MgSO4	3 mL	4th floor

1000x Kanamycin*	1 mL	4th & 6th floor
5000x Nystatin*	200 µL	4th floor
1 M IPTG*	100 µL	4th & 6th floor

If needed, make more 1 M IPTG by mixing 2.38g IPTG in 10 mL sterile water, followed by filtering using a 0.22 µm filter.

Day 4 - Beginning of Bacterial Growth

30m

4 **Grow FoldSeek Library of plates**

30m

Fill the four pre-made FoldSeek transformed E. coli plates with 100 µL of LB broth+Kanamycin. Cover plates with breathable film cover.

Leave overnight at 37 °C and shaking at 200 rpm.

Note

Make LB+Kanamycin by pipetting 500 µL of 1000x Kanamycin into 500 mL LB.

Day 5 - Inducing bacteria & bleaching worms

5h 30m

5 **Dilute overnight cultures 1 in 100**

30m

Dilute 5 µL of overnight cultures in 450 µL LB broth+Kanamycin in 2 mL deep-well plates (LB+Kan can be filled by ViaFill using programme '16 DEEP WELL').

6 **Grow diluted cultures to OD_{600} 0.4 - 0.8** 3h

Incubate the plates at 37 °C 230 rpm for about 03:00:00 h until they have reached an OD_{600} between 0.4 - 0.8.

7 **Induce bacterial cultures with IPTG at OD_{600} 0.4 - 0.8** 30m

Make a 10x solution of 1 M IPTG (1000x) in LB+Kanamycin.

When cultures have grown to an OD_{600} of 0.4 - 0.8, use ViaFill (programme '16 DEEP WELL') to dispense 50 µL of LB+Kan+IPTG in each well. Rinse ViaFill tubes with heated MilliQ water.

Leave overnight at 37 °C and 250 rpm.

Note

4x 96-well plate:
50 µL x 384 wells = 19.2 mL

The ViaFill requires about 5 mL for priming. As such, to ensure there is enough dispensed without risking bubbles, *make up a solution of 40 mL*.

10x IPTG in LB+Kanamycin (1/100 dilution):
400 µL of 1 M IPTG (1000x IPTG) in 40 mL of LB+Kanamycin.

8 **Bleach worms and place in diapause** 1h 30m

Need around 50-100,000 L1s. To be used on Day 6, so leave spinning overnight.

Day 6. Add worms to bacteria & measure 3h 15m

9 **Complete S-meduim** 10m

Use a 500 mL measuring cylinder aliquote 400 mL of S-medium into a new autoclaved glass container. Add the following components to complete the S-medium:

Note

400 mL complete S-medium:

1000x Kanamycin	400 µL	4th & 6th floor
5000x Nystatin	80 µL	4th floor
1 M IPTG	40 µL	4th & 6th floor

Kanamycin, Nystatin and IPTG available on 4th floor 'Toxic' -20°C freezer in box labelled 'Spare antibiotics/antifungals' or in yellow boxes in a -20°C freezer on 6th floor.

Make more 1 M IPTG by mixing 2.38g IPTG in 10 mL sterile water, followed by filtering using a 0.22 µm filter.

10 **Pellet & re-suspend bacteria in S-medium** 30m

Pellet plates for 00:10:00 min at 500x g. Flick out the LB and replace the medium with 500 µL of S-medium using ViaFill.

Note

1630x rpm for centrifuge 5810R to get 500x g.

11 **Centrifuge again and refill with S-medium** 5m

Fill the wells with 300 µL of S-medium using ViaFill. Rinse the ViaFill tubes with MilliQ water to ensure no clogging occurs.

12 **Resuspend & make triplicates plates** 1h

Using a multichannel pipette, resuspend the cultures thoroughly.
Pipette 80 µL into three separate Nunclon round-bottom 96-well plates.

13 **Add worms to the plates** 30m

Add 20 µL of L1 worms to each well (between 50 - 100 worms).

14 **Measure OD** 30m

Measure OD_{600} of the plates using a plate reader (with 'James_OD_600' protocol).

File naming format: **FS21_1_JB1_Day1**
- **FS**: FoldSeek
- **21**: plate 2.1
- **1**: plate replicate 1 out of 3 of FS plate 2.1
- **JB1**: experiment repeat for *initals*
- **Day1**: day of the measurement out of 14.

15 **Record plates using Hydras** 30m

Plates recorded without lids using only one hydra for the entire screen.

Script: **python3 run_Short_bluelight.py -r 02 -f ToxAss_2024_*MM_DD*_JB1_d1_FS21_1**
- ***MM*** : month for when the first recording was made
- ***DD*** : day for when the first recording was made
- **JB1**: experiment repeat for *intials*
- **d1**: day of the measurement out of 14
- **FS21**: FoldSeek plate number 2.1
- **1**: plate replicate 1 out of 3 of FS plate 2.1

The script runs for 30s. When the blue light has stopped, the recording is done. Take out the plate and put in the new one. ***Make sure to click 'clear' under 'configurations' before starting to record the new plate.***

16 **Store plates**

Place plates in a box with a damp paper towel and store at 20 °C

Day 9 to Day 19 - Measuring OD_{600} and motility 1h

17 **Measure OD_{600} using the plate reader** 30m

Use the protocol 'James_OD_600'.

18 **Record plates using Hydras** 30m

Run the script 'run_Short_bluelight.py' to record the plates under bluelight.

Note

14 days in total for measurements (Day 6 - Day 19). Measurements done on Day 6 and from Day 9 to Day 19 of Experiment Days. If the first measurement starts on Monday, then the second day of measurement is on Thursday.

	Exp. Day	(Measurement day)	Day of week
1.	Day 6	(1)	Mon
2.	Day 9	(4)	Thurs
3.	Day 10	(5)	Fri
4.	Day 11	(6)	Sat
5.	Day 12	(7)	Sun
6.	Day 13	(8)	Mon
7.	Day 14	(9)	Tues
8.	Day 15	(10)	Wed
9.	Day 16	(11)	Thurs
10.	Day 17	(12)	Fri
11.	Day 18	(13)	Sat
12.	Day 19	(14)	Sun