ASSAY FOR CHEMOTAXIS TOWARD SOLUBLE ATTRACTANTS AND REPELLENTS.

Phylum TECH | Drug Discovery Solutions

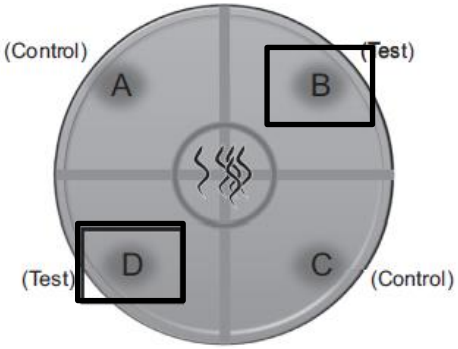
What you need:

Chemotaxis plates (6 well-plate or 35mm plates).
Chemotaxis (CTX) buffer.
Test solution: ammonium chloride 100 mM in H_20d. Control solution: H_20d.
Synchronized populations of well-fed adult worms in seeding NGM plates (OP50).
Arena device.

Procedure:

1. Preparing the Test Plates

-Mark five spots on the back of each chemotaxis plate. Place the marks A, B, C and D about 0.4 cm from the edge, and the worm spot in the middle of the well.



- Spot the plates with 10 µl of the test solution onto test spot and 10 µl of the control solution onto control spot. When the liquid has dried the plates are placed at 23ºC for an hour to let the gradient develop.

2. Preparing/washing the Worms

-Remove adult worms from plates using CTX buffer and transfer them in a sterile 1.5 ml Eppendorf.
-Let the worms settle by decantation. Throw out the supernatant with a micropipette taking care not to disturb the pellet.
-Repeat the wash step 2 times more with 1 ml of CTX buffer.
-Add 1 ml of CTX buffer. Briefly shake or invert the tube and count #worms in 10 µl in triplicate and calculate the average.
-Prepare a suspension to get [50 worms/5 µl]. Adjust volume in CTX buffer.

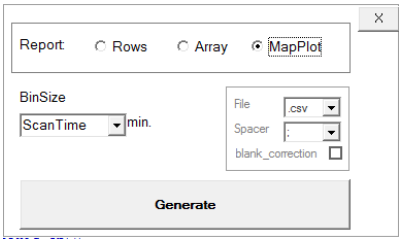
3. Running the Assay

- Use a micropipette to transfer about 50 worms to the worm spot in the assay plate.

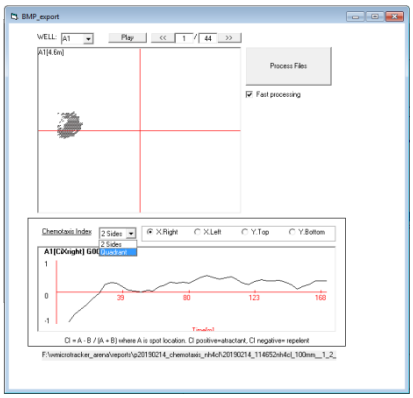
- Immediately, register worm activity using Arena device at 23ºC. N2 worms usually reach the attractant spot in the first hour.

4. Generate the report of the assay

- To generate the report of the chemotaxis assay choose MapPlot option.



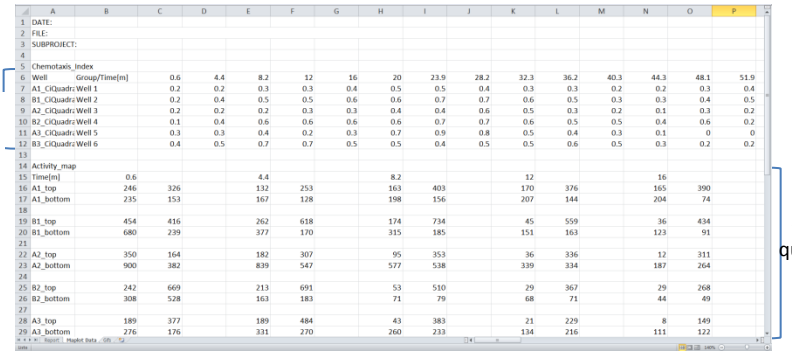
- The following window will appear:



Choose the correct design of the plate for the analysis (2 sides/quadrant) and the test spot location.

- Click on Process Files button to run the analysis, and a file with the data will be generated:

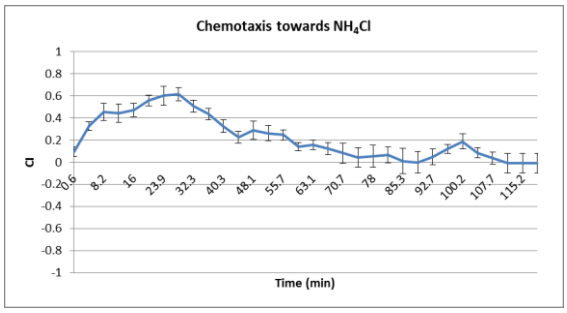
The analysis software calculates well at di



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	DATE:															
2	FILE:															
3	SUBPROJECT:															
4																
5	Chemotaxis_Index															
6	Well	Group/Time[m]	0.6	4.4	8.2	12	16	20	23.9	28.2	32.3	36.2	40.3	44.3	48.1	51.9
7	A1_CiQuadr:Well 1		0.2	0.2	0.3	0.3	0.4	0.5	0.5	0.4	0.3	0.3	0.2	0.2	0.3	0.4
8	B1_CiQuadr:Well 2		0.2	0.4	0.5	0.5	0.6	0.6	0.7	0.7	0.6	0.5	0.3	0.3	0.4	0.5
9	A2_CiQuadr:Well 3		0.2	0.2	0.2	0.3	0.3	0.4	0.4	0.6	0.5	0.3	0.2	0.1	0.3	0.2
10	B2_CiQuadr:Well 4		0.1	0.4	0.6	0.6	0.6	0.6	0.7	0.7	0.6	0.5	0.5	0.4	0.6	0.2
11	A3_CiQuadr:Well 5		0.3	0.3	0.4	0.2	0.3	0.7	0.9	0.8	0.5	0.4	0.3	0.1	0	0
12	B3_CiQuadr:Well 6		0.4	0.5	0.7	0.7	0.5	0.5	0.4	0.5	0.5	0.6	0.5	0.3	0.2	0.2
13																
14	Activity_map															
15	Time[m]	0.6			4.4			8.2			12			16		
16	A1_top	246	326		132	253		163	403		170	376		165	390	
17	A1_bottom	235	153		167	128		198	156		207	144		204	74	
18																
19	B1_top	454	416		262	618		174	734		45	559		36	434	
20	B1_bottom	680	239		377	170		315	185		151	163		123	91	
21																
22	A2_top	350	164		182	307		95	353		36	336		12	311	
23	A2_bottom	900	382		839	547		577	538		339	334		187	264	
24																
25	B2_top	242	669		213	691		53	510		29	367		29	268	
26	B2_bottom	308	528		163	183		71	79		68	71		44	49	
27																
28	A3_top	189	377		189	484		43	383		21	229		8	149	
29	A3_bottom	276	176		331	270		260	233		134	216		111	122	

Activity data for each quadrant of each well at different times.

- You can plot CI vs. time.



4. Interpreting the Chemotaxis Index (CI)

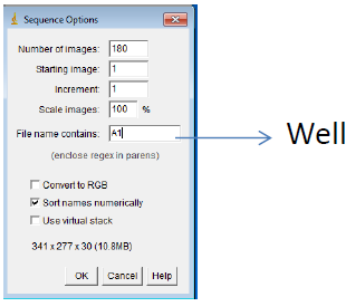
CI = (# activity in test spots (B+D) - # activity in control spots (A+C)) / Total # activitiy in the well.

CI_{max} = maximum value of CI.
T_{max} = time when CI = CI_{max}
A +1.0 CI score indicates maximal attraction towards the target and represents 100% of the worms arriving to the spots containing the chemical target. An index of -1.0 is evidence of maximal repulsion.

5. How to obtain the GIF?

Images are saved in:
wmicrotracker_arena\reports\proyect_name\assay_name\3scans_images

-Open Image J
-File \ Import \ Image Sequence \ choose the images-assay's folder



-Image \ Type \ 8-bit
-File \ Save as \ Gif

NOTES:

- For this experiment the test/control solution need to be added to chemotaxis plates ne hour before adding the worms.
- We recommend perform at least four technical replicates and at least two biological replicates.
- Do not add azide to the attractant spot.
- When adding the worms, be careful not to scratch the agar with the pipette – worms tend to crawl into any break in the agar surface.

<u>Medium and solutions needed:</u>

Chemotaxis plates			
Final volume (ml)	100	200	Final Concentration
Agar (g)	2	4	2%
Add after sterilizing:			
1M KPO_4 buffer (pH6) (ml)	0.5	1.0	5mM
1M $CaCl_2$ (ml)	0.1	0.2	1 mM
1M $MgSO_4$ (ml)	0.1	0.2	1 mM

For mw6 or p35 plates, add 4ml per well.

Chemotaxis buffer			
Final volume (ml)	100	200	Final Concentration
Add to sterile H20d:			
1M KPO_4 buffer (pH6) (ml)	0.5	1.0	5mM

1M $CaCl_2$ (ml)	0.1	0.2	1 mM
1M $CaCl_2$ (ml)	0.1	0.2	1 mM

KPO_4 buffer, $CaCl_2$ and $CaCl_2$ solutions must be sterilized by autoclaving.