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Forked from Open field test for mice

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Protocol status: Working

We use this protocol and it's working

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### Abstract

The locomotion test is an open field test in darkness, so it does not aim to measure anxiety, only locomotor activity.

### **Materials**

- A 50×50 cm arena which is open in the bottom.
- An IR-sensitive camera (1280×1024 pixels Basler camera and a Basler 2200000178 objective).
- Infrared lights.

## **Troubleshooting**



Behaviour		1h 4m 30s
1	Transport the mice to the behaviour room and put the mice in individual cages.	5m
2	Leave them in the lobby in darkness or with red light for at least 30 minutes.	30m
3	Turn on the red lights in the room.	
4	Place a 50×50 cm arena on the table.	
5	Clean the arena and the table with 70% ethanol.	1m
6	Turn on the infrared lights below the arena. Open Pylon Viewer (see settings below), and make sure that you get a good image quality.	2m
7	Bring a mouse to the behaviour room. Open the cage and gently get the animal out. Do not chase it around in the cage.	2m
8	Place the animal in the center of the arena. Depending on your handling protocol, you can put it in by the tail or in the palm of your hand.	30s
9	Make sure to start the recording at the moment when you place the mouse in the arena.	
10	Make a recording of 30 min. The recording will be automatically saved; make sure to rename it.	30m
11	Pick the mouse out of the arena and put it back in the cage.	2m
12	Count poop pellets and take a note of them.	1m



13 Clean the arena and the table with 70% ethanol. If you feel the necessity, you can rinse the area with distilled water.

1m

## **Pylon Viewer**

- 14 Connect the camera to the computer and to a power source.
- 15 Open the Pylon Viewer and select the camera.
- 16 At AOI controls, crop the image, so that you only see the setup. You can only change the width and the height of the image when there is no continuous shot or recording.
- 17 With the help of a test mouse, you can set the image quality. On the camera, you can set the aperture and the focus. In Pylon Viewer, you can play around with Analog Controls > Gain (Raw), Black level (Raw), and Acquisition Controls > Exposure time (Raw).
- 18 Go to Window and select Recording Settings. Set the Output format to .mp4, the fixed playback speed to 67 fps, and the Recording buffer size to 100 frames. Record a frame every 15 milliseconds. Stop the recording after 30 min. Set the output folder.

# Data analysis

19 Tracking of animal kinematics most easily done with DLC (link)

To identify individual behavior motifs we use VAME (<u>link</u>) or A-Soid (<u>link</u>)