# UC Davis - Y-Maze 🔗

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## ABSTRACT

**Summary:**

The y-maze is a modification of the T- maze which evaluates memory and special learning in rodents though quantification of spontaneous alternation (a measure of spatial cognition). This measure has previously been shown to be affected in mouse models of Alzheimer's.

**References:**

Ohno et al. Neuron 41:27-33, 2004. Oakley et al. J Neuroscience 26(40):10129-10140, 2006. Ohno et al. Neurobiol Dis 26:134-145, 2007. Holcomb et al. Nat Med 4:97-100, 1998.

## EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=279&docType=Protocol

## MATERIALS

| NAME ⌄ | CATALOG # ⌄ | VENDOR ⌄ |
|---|---|---|
| Black Tempura paint | | Jazz |
| Smart software | | SMART ( Simple Modular Architecture Research Tool) |

## MATERIALS TEXT

**Reagents and Materials:**

• Y maze
• Nolvasan
• Microsoft LifeCam Studio [TM]
• Wood step ladders
• Lab coat
• Thermometer
• Gloves

**Note:**

**SMART,** [RRID:SCR_005026](#)

1   **Study design:**

Time of day: For any experiment, testing should take place during the same 3-4 hour period on each day. Afternoon testing at least one hour prior to dark cycle onset is best. Testing after "lights out" in the vivarium is not appropriate.

Mixed sex test groups: Males and females should be tested in different test groups after a thorough maze cleaning, preferably on different days.

Test order: Test order strongly affects performance in the Y-maze. The experimental groups (as designated in the design) should be randomized in the test order. A confounded experiment results if all animals in one group are tested before all the animals in another group. If multiple cohorts are used, the randomization should extend across all cohorts. The number of animals run on a given day should be standardized as much as possible.

Blinding: To assure lack of bias, the experimental groups of the animals should not be displayed on the animal housing, the lab schedule, or the data sheets. Experimental group can be added to the datasets after completion of the study.

Group housing: The caging used for the study should be standardized and reported with the data. If caging changes (animals separated or dying) this should be reported with the data also. In order to randomize test order when 4-5 mice in the same experimental group are housed together, the mice need to be separated into individual cages prior to starting the study.

Test group size: Because of the short test interval, up to about 20 mice can be run in a 4 h period. Untested mice should be kept in the hall before test is initiated and left in the test room afterward.

Protocol deviations: A log of protocol deviations should accompany the data sets to help evaluate "outliers" in the dataset.

## 2    Protocol:

### Setting Up

1. Transport the mice to the testing room at least one hour prior to testing, record the time of transfer and when the recording starts on the log sheet.

2. Turn the white lights off and turn on the four red lights (60W indirect)and the power strip to the camera.
    a. Microsoft LifeCam Studio is used.

3. Open the Smart zone configuration file for the Y maze and place the maze so that the arms correspond (1, 2, 3). The maze should be 2 feet above the ground and in the same room location for all mice. The arm borders should not include the shadow of the vertical wall or the last few cm. at the end of the wall (mouse length) where there is shadow.

4. If testing white mice, paint a black stripe painted from head to rump with Jazz washable tempera paint. This will insure tracking works properly.
    a. If the experiment includes mice of various colors, all mice should go through the marking process even if they don't need the mark for tracking.

5. Use balanced test order for the experiment. If more than one mouse per cage is going to be tested, place the mice in a clean cage after testing until all are tested, then return to home cage.

### To Set Up For Recording

1. Open SMART program by clicking on the icon on the desktop.

2. Go to "Data Acquisition"> "Single Subject Tracking" then "Configuration"> "Timings", Setting should be as follows
    a. "Pre-Set" box checked
    b. "Acquisition Period" set at "00:08:00"
    c. "Stop Control" on the bottom, "When Programmed time is Over" is checked.
    d. Boxes that read after= 1 visits to target zone 1 and after 1.0 sec. visits to target should **NOT** be checked
    e. After reviewing the settings, select "**O.K**."

3. Prior to recording, select "File"> "Open"> "Zones"; click through error messages. Opening "Y Maze 112114.zon" (C:\Program Files (x86)\Panlab\Smart v3.0\Zones).

4. Verify that that the zone map is mapping the Y maze apparatus perfectly. If the Y maze is not centered then carefully adjust it. **Do NOT** change the mapping.

5. Then, on the bottom right hand corner, adjust "Threshold" to read "70" and "Erosion" to be "0".
    a. The erosion setting may need to be changed if the mice are not solid black.

6. On the bottom left of screen, press the "Dark Subject" button.

**To Start Recording Tracks**

1. Go to "File> "New", then input a name or label for the data (mouse# and date:"copy" to use later to paste into file id).

2. Retrieve mouse from home cage, manually click once on "Start" and immediately put the mouse on the center of the Y maze, facing a random corner (between arms).
   a. The tracking does not start until the mouse is in the maze, so timing is not critical.

3. Monitor the recording session patiently and quietly from the same location (in front of computer).
If the mouse jumps out of the maze cease the session and mark it down in the comments section of the test worksheet.

4. The program should stop after 8 minutes as a pre-set automatic function.

5. When the recording ends automatically, a screen will show up to acquire data input.
   a. Under "Subject ID", paste in the mouse# and date as copied earlier.
   b. Under "Experimenter", enter your own name. then select "O.K."

6. Go to "File"> "New"

7. When asked to save or not, select "Yes". Then save the track to "M:\"> "MBAL" > "Golub" > "Golub" > "Projects" > project folder, pasting in the mouse# and date as copied earlier.

8. Remove mouse from maze and return to home cage. Be sure to clean the Y maze in between animals using Nolvasan and paper towels to dry.

9. Repeat steps 1 to 8 for each mouse.

**To Retrieve Saved Data Files**

1. Open SMART program by clicking on the icon on desktop

2. Select "Analysis" on the top menu bar.

3. Under the "Zone Definition" menu, select the "Y Maze 112114.zon" (which is located in C:\Program Files (x86)\Panlab\Smart v3.0\Zones). This zone file has been updated and refined.

4. Under the "Report Definition" menu, select the summary report and include the following parameters for the report:
   a) Trial Name
   b) Trial Date
   c) Exp. File Name
   d) Exp. File Date
   e) Subject Name
   f) Trial Time
   g) Trial Duration (HH:MM:SS:00)
   h) Distance in Zone
   i) Distance in Zone (%)
   j) Entries in Zone
   k) Resting Time in Zone (%)
   l) Resting Time in Zone (seconds)
   m) Alternation Triplet
   n) Alternation Triplet (%)
   o) Max. Alternation Triplet
   p) Total Arm Entries

5. Under the "Time Interval" menu, select "Full Trial"

6. Click the "Analyze" box and save the data report to the study folder.

7. The test order, treatment group and cohort information need to be hand entered into the raw data file.
   a. In the raw data file, insert 2 new columns: D and E. This will be between the columns labeled "Exp. File Name" and "Subject Name"
   b. Hand enter the treatment group information (column D) and cohort ID (column E) for each mouse.
   c. Label the column D header as "Treatment Group"
   d. Label the column E header as "Cohort."

e. Column A is automatically labeled "Trial Name" by the software. This is the order in which the mice were tested. Rename this header "Test Order."

8. Once all additional columns/data have been entered, add this excel spreadsheet to the master dataset for the study.

**Computing results manually**

1. Delete reentries (choosing same arm two or more times in a row) must be deleted (leave the first entry, delete reentries).

2. Delete arm "4" entries which is the zone in the middle off the arms.

3. To score the Y-maze data, an **alternation index** is computed as follow
   # triads with all 3 zones
   #arms entered -2

4. Triads are overlapping sequences of three choices (e.g. arm entries 1-2-3, 2-3-4, 3-4-5 etc.)
   a. Score as "1" if it contains all three arms, and "0"if it does not contain all three arms.
   i. Remember that the denominator "arms entered" does not include zone 4 and arm reentries. This scoring method is described in Oakley et al. paper.

*SUPPLEMENTARY NOTE:*
The camera is mounted at an angle of approximately 90 degrees and centered to facilitate the best viewing conditions
Arm 1: red
Arm 2: yellow
Arm 3: green
Arm 4: blue (center)