

Getting started with *SleepMat*

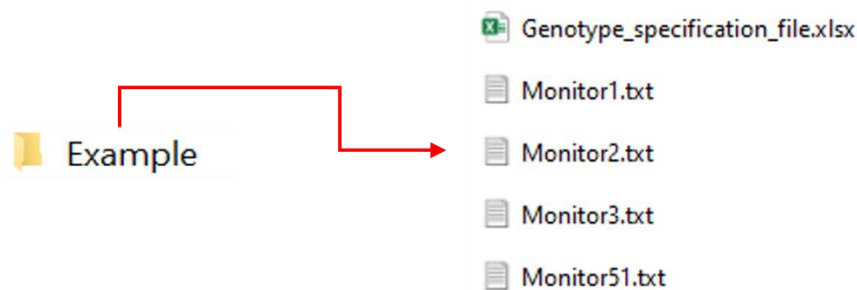
This walkthrough is an introduction to the use of SleepMat software to analyze the sleep and circadian behavior of *Drosophila*.

Walkthrough Example

This walkthrough is based on data submitted on the Github repository

<https://github.com/shijusisobhan/SleepMat2024.1>

Download the zip folder 'Example.zip', unzip it and extract the files. Open the folder, now the folder should look like this:



The folder contains monitor files and a genotype specification file. Monitor file number 1,2, and 3 have behavioral data starting from *1 Feb 24 10:38:00* and ending on *8 Feb 24 10:58:00*. Monitor file 51 have environment monitor data that collects temperature level, light level and humidity level data, which starting at *1 Feb 24 10:40:00* and ending on *8 Feb 24 10:59:00*.

Create a genotype specification Genotype specification file

Creating a genotype specification file is the most important, and time-consuming steps in the *SleepMat* analysis. Genotype specification file describes the experiment, genotype, monitor files,

channel number, and dates. Here already we provide a genotype specification file (*Genotype specification file.xlsx*) and explain below how it prepared.

- Open an excel file

- column A (**Mandatory**) : Here user have to specify the run number. First cell (A1) is the header and A2 onwards are the run numbers. If you don't know about the run number, then enter some arbitrary numeric/alpha numeric value there (don't leave it as blank).

- Column B (**Mandatory**): This is the monitor number and must be a numeric value. In this example if we want to analyze the files 'Monitor1.txt', then you should enter 1 in this column.

- Column C (**Mandatory**): This is the genotype name, and it can be either alphanumeric or plain text. (Do not use the characters /, \, <, or > when naming the genotype.) In this example, different genotypes are loaded into different channels on the same monitor board. For instance, R84H11-DBD x 70623 flies are loaded into channels 1 to 7 of Monitor1, while channels 8 to 16 are loaded with R84H11-DBD x 74486 flies.

- Column D, E (**Mandatory**): These are the range of channels you need to analyze. Column D is the start channel and column E is the end channel. These are numeric values and should be between 1 and 32.

- Column F (**Mandatory**): This is the start date of analysis, and its format is the same as that appears in the monitor file (eg: **3 Feb 24**). It must be plain text. Start date doesn't mean that it is the actual starting date of Monitor file. It is user's choice. However, there is some rules to select the start date.

- 1) The start date should be present in the Monitor file.
- 2) light on time specified in the GUI should be present in the start date.
- 3) Start date is a reference date to analyze the data. If the user specifies a start date in the column F, every other day specified in the GUI should be with respect to this date.
- 4) It must be plain text and the format is same as that appears in the monitor file (3 Feb 24), not in a date format (3/02/24)

In this walkthrough example, Montor1.txt have data starting from *1 Feb 14 10:38:00*. First two days of data is redundant, and we don't want those data. So here we interested only the data from *3 Feb 24* onwards. So, we specify the start date is *3 Feb 24* in the column F. Also, in this example analysis our light on time is 8 AM, which is available in the monitor file (Rule 1 & 2). So, when you analyze the Monitor file 1, Day-1 is 3 Feb 24. Here the date appeared in the monitor file 1 is, 3 Feb 24. So, you must enter the date in that format. If the date appeared in the Monitor file as 3-Feb-24, you should enter in that format.

- Column G (**Optional**): This is the triage date, and it is optional. If you wish to evaluate triage conditions (condition for determining whether the fly dead or not) on a specific date, then you can enter the dates here. Date format is the same as that appears in the monitor file (eg: **7 Feb 24**). If this column is left blank, by default the software will evaluate triage conditions using the last day of analysis.

- Column H (**Optional**): This is the control genotype. Researchers may be interested in screening genotypes based on sleep and circadian parameters for further experiments. In such cases, the statistical significance of these parameters is crucial. *SleepMat* evaluates the statistical significance of a genotype compared to the control genotype by performing a t-test and calculating the p-value. In this example, R84H11-DBD x 36304 is treated as the control genotype.

- Column I (**Optional**): This is the environment monitor number and must be a numeric value. In this example if we want to analyze the environment monitor file 'Monitor51.txt', then you should enter 51 in this column.

Note : Columns G, H, and I are optional; they may or may not contain entries. However, each column is designated for specific information: G for the triage date, H for the control genotype, and I for the environment monitor file. For instance, if you are not setting a specific triage date but have a control genotype to evaluate the p-value, you should leave column G empty and enter the control genotype in column H.

After completing the genotype specification excel file, save it as .xlsx file with any file name.

Mandatory

Optional

	A	B	C	D	E	F	G	H	I
1	Run Name	Monitor	Geno type	start channel	end channel	Start date	Triage date	control genotype	Environment Monitor
2	M13	1	R84H11-DBD x 70623	1	7	3 Feb 24	7 Feb 24	R84H11-DBD x 36304	51
3	M13	1	R84H11-DBD x 74486	8	16	3 Feb 24	7 Feb 24		
4	M13	1	R84H11-DBD x 68872	17	22	3 Feb 24	7 Feb 24		
5	M13	1	R84H11-DBD x 70194	23	26	3 Feb 24	7 Feb 24		
6	M13	2	R84H11-DBD x 71645	1	14	3 Feb 24	7 Feb 24		
7	M13	2	R84H11-DBD x 36304	15	27	3 Feb 24	7 Feb 24		
8	M13	1	R84H11-DBD x 75744	27	29	3 Feb 24	7 Feb 24		
9	M13	3	R84H11-DBD x 74415	1	7	3 Feb 24	7 Feb 24		
10	M13	1	R84H11-DBD x 70969	30	32	3 Feb 24	7 Feb 24		
11	M13	2	R84H11-DBD x 71535	28	32	3 Feb 24	7 Feb 24		
12	M13	3	R84H11-DBD x 68585	8	18	3 Feb 24	7 Feb 24		
13	M13	3	R84H11-DBD x 70646	19	27	3 Feb 24	7 Feb 24		

Example genotype specification file format

GUI

Before opening the GUI, make sure that your genotype specification file and all the monitor files (including Environment monitor file, if any) are in the same folder.

- Open the *SleepMat* GUI

The screenshot shows the SleepMat GUI with three main panels:

- General input (Blue):**
 - Open file: C:\Users\shijun\OneDrive
 - Project Name: project-1
 - Light on time (Clock time, 24 hr format): 8
 - Light off time (Clock time, 24 hr format): 20
 - Lifespan: ☐ Yes ☒ No
 - Activity threshold: 2
 - Maximum days of lifespan: 11
 - Individual output files: ☐ Yes ☒ No
 - Start button
 - Status update
 - Version information: V2024.2 (Gamma), Date modified: 10/09/2024
- Sleep analysis (Orange):**
 - Sleep analysis: ☒ Yes ☐ No
 - Sleep Analysis/Interval: ☐ Yes ☒ No
 - Start day of sleep analysis: 1
 - Number of days of sleep analysis: 4
 - Per interval: 1
 - Sleep Deprivation: ☒ Yes ☐ No
 - Cumulative sleep figure: ☐ Yes ☒ No
 - SL interval: 12
 - Rebound interval: 12
 - SD day: 3
 - SD starting time (Clock time, 24 hr format): 20
 - SD duration: 12
 - Minimum SD% threshold: ☒ Yes ☐ No
 - Minimum SD %: 90
 - Specific date for triage: ☐ Yes ☒ No
 - Triage condition for sleep analysis:
 - ☒ Last day of activity < 5
 - ☐ Last day activity(Lon-6 to Lon+6) < 2
 - ☐ Activity per 30 min < 1
- Circadian analysis (Green):**
 - Circadian analysis: ☒ Yes ☐ No
 - Anticipation analysis: ☐ Yes ☒ No
 - Eduction analysis: ☒ Yes ☐ No
 - Specific date for eduction/anticipation triage: ☐ Yes ☒ No
 - Triage condition for sleep eduction analysis:
 - ☒ Last day of activity < 5
 - ☐ Last day activity(Lon-6 to Lon+6) < 2
 - ☐ Activity per 30 min < 1
 - Start day of eduction/anticipation: 1
 - Number of days of eduction / anticipation: 4
 - Periodogram analysis: ☒ Yes ☐ No
 - Periodogram figure: ☒ Yes ☐ No
 - Start day of periodogram: 1
 - Number of days of periodogram: 4
 - Significance level: 0.01
 - Specific date for periodogram triage: ☐ Yes ☒ No
 - Triage condition for periodogram:
 - ☒ Last day of activity < 5
 - ☐ Last day activity(Lon-6 to Lon+6) < 2
 - ☐ Activity per 30 min < 1
 - Minimum period: 14
 - Maximum period: 34
 - Actogram: ☒ Yes ☐ No
 - Start day of actogram: 1
 - Number of days of actogram: 4

General Input

1. **Open file:** Click the 'Open file' button to browse and select the genotype specification file for the analysis. In this walkthrough we are uploading the '*Genotype_specification_file.xlsx*' which is stored in the Example folder.

1. **Project Name:** Users can assign a project name for the current analysis. Here we are giving a project name as 'project-1'

2. **Light on time (Clock time, 24hr format):** This is the light onset time of the experiment. Here we are entering the default time 8

3. **Light off time (Clock time, 24hr format):** This is the light offset time of the experiment. We are entering the default time 20

4. **Lifespan (Yes/No):** Users can select to perform lifespan analysis by checking Yes.

1. **Activity threshold:** This is the minimum value of activity to determine the lifespan of a fly. Enter the default value 2

2. **Maximum days of lifespan:** Enter the default value 11 here. If the lifespan exceeds 11, *SleepMat* automatically assign lifespan as 11.

5. **Individual output file (Yes/No) :** SleepMat analysis produces different sleep and circadian parameters, which are stored in the excel files. By default, program do not produce separate files for each parameter, instead clubbed together in single excel files for each analysis. If the user wishes to generate separate files for sleep and circadian parameter, check Yes. In this example select the default No.

Sleep analysis

1. **Sleep analysis (Yes/No):** Users can select to perform sleep analysis by checking Yes.
2. **Start day of sleep analysis:** Enter the starting day of sleep analysis. Here we enter the default value 1
3. **The number of days of sleep analysis:** Enter the number of days to analyze. We are entering the default value 4.
4. **Sleep analysis/Interval (No/ Yes):** select Yes
5. **Per interval (hr):** Enter a value 1
6. **Sleep deprivation (Yes/No):** Sleep deprivation analysis can be performed by selecting Yes.
7. **Cumulative sleep figure (Yes/No):** This example you can select No
8. **SL interval** – It is the duration you need to evaluate sleep loss (default 12 hr)
9. **Rebound interval** – It is the duration you need to evaluate rebound (default 12 hr)
10. **SD day:** The day when the flies are sleep deprived is entered here. (default 3)
11. **SD starting time (Clock time, 24 hr format):** The time at which sleep deprivation experiment starts (default ZT 20)
12. **SD duration:** The duration of the sleep deprivation experiment in hr (Default is 12 hr)
13. **Minimum SD% threshold (Yes/No):** Select No. If you select yes, you should set a minimum sleep deprived percentage (Default is 90 %).
14. **Minimum SD%** (Default is 90 %): If you select No in the previous step, then this will be deactivated.
15. **Triage condition for sleep analysis:** Select the first condition, Last day of activity < 5

Circadian Analysis

1. **Circadian analysis (Yes/No):** Select Yes to perform circadian analysis.
2. **Anticipation analysis (Yes/No):** Select Yes to perform anticipation analysis.
3. **Eduction analysis (Yes/No):** Yes
4. **Start day of eduction/anticipation:** Enter the starting day of anticipation/eduction analysis.
Enter the default value 1.
5. **Number of days of eduction/anticipation analysis:** Enter the default value 4
6. **Traige condition for sleep eduction analysis:** Select the default condition *Last day of activity < 5*

Periodogram

1. **Periodogram (Yes/No):** Select Yes to perform chi square periodogram analysis for individual flies.
2. **Periodogram figure (Yes/No):** If the user would like to produce an average periodogram figure for each genotype, then select Yes.
3. **Start day of periodogram:** Enter the value 1.
4. **Number of days of periodogram:** Enter the number of days of periodogram analysis as 4.
5. **Significance level:** Enter the default value 0.01.
6. **Traige condition for periodogram:** Select the default condition *Last day of activity < 5*

Actogram

1. **Actogram (Yes/No):** To produce double-plotted actogram figures for individual flies, select Yes.
2. **Start day of actogram:** Enter the default is 1.

3. **Number of days of actogram:** Enter default value 4.

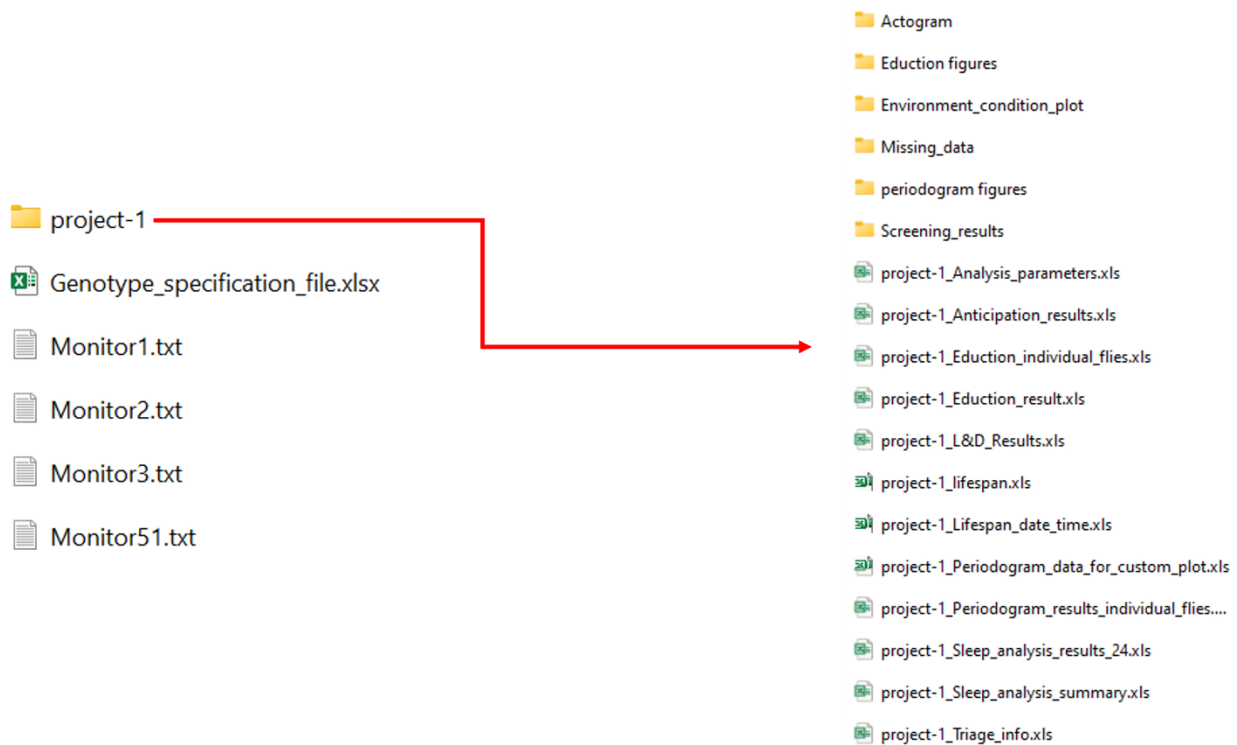
After entering the all values, click on the **Start** green button on the general input pannel. Analysis will start and the status will display on the **Status update** window. Now GUI should like this

The screenshot shows the 'sleepmat' application window with three main panels:

- General input (Cyan):** Contains fields for 'Project Name' (project-1), 'Light on time' (8), 'Light off time' (20), 'Lifespan' (Yes/No), 'Activity threshold' (2), 'Maximum days of lifespan' (11), and 'Individual output files' (Yes/No). A green 'Start' button is at the bottom.
- Sleep analysis (Orange):** Contains 'Sleep analysis' (Yes/No), 'Sleep Analysis/Interval' (Yes/No), 'Start day of sleep analysis' (1), 'Number of days of sleep analysis' (4), 'Per interval' (1), 'Sleep Deprivation' (Yes/No), 'Cumulative sleep figure' (Yes/No), 'SL interval' (12), 'Rebound interval' (12), 'SD day' (3), 'SD starting time' (20), 'SD duration' (12), 'Minimum SD% threshold' (Yes/No), 'Minimum SD %' (90), 'Specific date for triage' (Yes/No), and 'Triage condition for sleep analysis' (Last day of activity < 5, Last day activity(Lon-6 to Lon+6) < 2, Activity per 30 min < 1).
- Circadian analysis (Green):** Contains 'Circadian analysis' (Yes/No), 'Anticipation analysis' (Yes/No), 'Eduction analysis' (Yes/No), 'Specific date for eduction/anticipation triage' (Yes/No), 'Triage condition for sleep eduction analysis' (Last day of activity < 5, Last day activity(Lon-6 to Lon+6) < 2, Activity per 30 min < 1), 'Periodogram analysis' (Yes/No), 'Periodogram figure' (Yes/No), 'Start day of periodogram' (1), 'Number of days of periodogram' (4), 'Significance level' (0.01), 'Specific date for periodogram triage' (Yes/No), 'Triage condition for periodogram' (Last day of activity < 5, Last day activity(Lon-6 to Lon+6) < 2, Activity per 30 min < 1), 'Actogram' (Yes/No), 'Start day of actogram' (1), and 'Number of days of actogram' (4).

A 'Status update' window at the bottom left shows 'Sleep analysis started...'. Version information at the bottom left indicates 'V2024.2 (Gamma)' and 'Date modified : 10/09/2024'.

If everything goes well, at the end you will get a message “Analysis completed!” on the Status Update window. All the results will be stored in a folder project-1 (or the project name that you give). The folder should like this



Error and troubleshooting

Error message	Possible reason
ERROR! Please enter valid dates (See monitor file and genotype specification file)	1) Date format in the genotype specification file does not match with Monitor file 2) Date entered in the GT file is not present in the Monitor file
ERROR! start day of sleep or Number of days of sleep analysis is out of range	1)The start day exceed the number of days of data present in the data file. 2)Number of days of analysis extend past the end of the data available
Error! check sleep deprivation entries	Combination of SD day, SD starting time, SD duration exceed the number of days of data present in the data file
ERROR! starting day or number of days of education/anticipation is out of range	1)The start day exceed the number of days of data present in the data file 2) Number of days of anticipation extend past the end of the data available
Error! start day OR number of days of periodogram is out of range	1.The start day exceed the number of days of data present in the data file 2) Number of days of anticipation extend past the end of the data available
'Error ! check triage date'	1)Triage date entered in the GT file is not in the correct format 2) Triage date is not present in the data available
Unknown Error! Please check the input (eg: genotype specification file, days...)	1)GT file format is not correct (eg: columns are interchanged) 2)Monitor files are not available 3)Input are not in the reasonable range 4) start time (light ON time) is not available in the monitor file 5) problem with name of the

	genotype used in the genotype specification file (don't use >, <, , /, :, ?, ",for genotype naming or project naming) 6) Other unknown errors (Missing toolbox when running on MATLAB)
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