MS-DIAL、MetaboAnalyst の利用方法

1. MS-DIAL をダウンロード。

https://systemsomicslab.github.io/compms/msdial/main.html Ver 4.9.221218 をダウンロード (Ver 4.9 のシリーズ)



- 適当な場所(マイトキュメントなど)にテータ解析用フォルターを作成 フォルダー名は、メタボローム等わかりやすい名前が良い。
- データ解析用フォルダーに、MS-DAIAL 用のライブラリをダウンロード。 https://systemsomicslab.github.io/compms/msdial/main.html#MSP All records with Kovats RI (9062 unique compounds) EI-MS

| All records with Kovats RI (9062 unique compounds) | FEI-MS | 28,220 records | • |
|---|--------|----------------|-------|
| Fiehn BinBase DB (Rtx5-Sil MS, predicted Kovats RI) | FEI-MS | 1,021 records | Ŀ |
| RIKEN DB (Rtx5-Sil MS, Kovats RI) | FEI-MS | 241 records | ÷ |

- netCDF データを直接読み込むため、下記サイトから netCDF-C 4.9.2 (以降最新版)を ダウンロードし、オプションのパスを追加にチェックを入れてインストールする。 https://docs.unidata.ucar.edu/netcdf-c/current/winbin.html
- 6. データ解析用フォルダーに、装置パラメータファイル T100GCv_35-600_5-32.med2 を コピー。

初回時、データと一緒に渡します。解析時に使用しますので、消去しないでください。

- データ解析用フォルダーに、測定データ(.cdf)と RI 用 Alkanes データ(Alkanes.cdf) および RI 用テキストデータ(Alkane-Dictionary.txt)を収納するフォルダーを作成し、 それらのデータを収納。フォルダー名は、測定日にしておくと、わかりやすい。
- 8. 解凍したフォルダー内の MSDIAL.EXE をダブルクリックし、MS-DIAL を起動する。



9. $\forall = \neg -$ File \rightarrow New project

(新規測定データの解析の場合)。

 MS-DIAL ver. 4.9.221218

 File
 Data processing

 New project
 ak :

 Open p
 Start a project.

 Save as....
 on

 Save parameter setting
 Display filter

ポップアップした Start up a project ウインドウ →Project file path の「Browse」をクリックし、 6.で作成したフォルダーを指定する。

以前解析したデータは、Open project で開く。

| 10. 次いで、Ionization type の Hard ioniz | ation(GC/MS)を選択し「Next」をクリック。 |
|---|---|
| 🤏 Start up a project | × |
| Project file path: | ① I Browse |
| Soft ionization (LC/MS, LC/MS/MS, or precursor-oriented) | GC/MS/MS) |
| Hard ionization (GC/MS) | |
| Chromatography (GC, LC, CE, or SFC) Ion mobility (now coupled with liquid chromatography) | |
| MS method type Conventional LC/MS or data dependent MS/MS SWATH-MS or conventional All-ions method All-ions Experiment file: | s with multiple CEs (cycled like 0V-10V-40V) Browse |
| Data type (MS1) | Data type (MS/MS) |
| Profile data Centroid data | Profile data Centroid data |
| lon mode | Target omics |
| Positive ion mode | Metabolomics |
| O Negative ion mode | Lipidomics |
| Network Advanced: add further meta data | |
| | 3 Next |

 Analysis file paths→Browse をクリックし、ファイルの拡張子を netCDF file(*.cdf) に変更して、ファイルを選択し「開く」をクリック。Alkanes.cdf も忘れずに。Ctrl+A で 全選択可能。

| New project wind Analysis file paths | Browse ne pan | nose the a | File name | Type | Class ID | Batch | Analytical order | Inject. volume (µL) | Included | × . | ABF file(*.abf) ABF file(*.abf) netCDF file(*.cdf) bF file(*.wiff) WIFF file(*.wiff) WIFF2 file(*.wiff2) Raw file(*.raw) LCD file(*.lcd) QGD file(*.lcd) QGD file(*.qgd) | |
|---|------------------|------------|-----------|------|----------|-------|------------------|---------------------|----------|-----|---|--|
| | | | | | | | | Next | Cance | 1 | | |

読み込んだデータの各グループ(群)に対応した、Class ID に変更する。例えば、
 1, 2。あるいは WT、KO の様に。

Alkanes の Type を Sample から Blank へ変更。次いで、Class ID を Alkane に変更し、「Next」をクリックする。

| Witigger¥Documents¥MS測定デー9¥GCMS 10 Sample 1 1 1 1 ✓ Witigger¥Documents¥MS測定デー9¥GCMS 12 Sample 1 3 1 ✓ Witigger¥Documents¥MS測定デー9¥GCMS 13 Sample 1 4 1 ✓ Witigger¥Documents¥MS測定デー9¥GCMS 14 Sample 1 4 1 ✓ Witigger¥Documents¥MS測定デー9¥GCMS 15 Sample 1 5 1 ✓ Witigger¥Documents¥MS测定デー9¥GCMS 16 Sample 2 6 1 ✓ Witigger¥Documents¥MS测定デー9¥GCMS 16 Sample 2 8 1 ✓ Witigger¥Documents¥MS測定デー9¥GCMS 18 Sample 2 9 1 ✓ Witigger¥Documents¥MS測定デー9¥GCMS 18 Sample 2 10 1 ✓ Witigger¥Documents¥MS測定テー9¥GCMS 19 Sample 2 10 1 ✓ Witigger¥Documents¥MS測定テ9¥GCMS Alkanes Blank Alkane | File path | File name | Туре | Class ID | Batch | Analytical order | Inject. volume (µL) | Included |
|--|-------------------------------------|-----------|--------|----------|----------|------------------|---------------------|----------|
| Witigger¥Documents¥MS測定データ¥GCMS 12 Sample 11 3 1 1 2 Witigger¥Documents¥MS測定データ¥GCMS 13 Sample 11 4 4 1 2 Witigger¥Documents¥MS測定デー9¥GCMS 14 Sample 1 5 1 4 Witigger¥Documents¥MS測定デー9¥GCMS 15 Sample 2 1 6 1 Witigger¥Documents¥MS測定デー9¥GCMS 16 Sample 2 1 8 1 2 Witigger¥Documents¥MS測定デー9¥GCMS 18 Sample 2 1 9 1 2 Witigger¥Documents¥MS測定デー9¥GCMS 19 Sample 2 1 0 1 2 Witigger¥Documents¥MS測定デー9¥GCMS 10 Sample 2 1 0 1 2 Witigger¥Documents¥MS測定デー9¥GCMS 10 Sample 2 1 0 1 2 Witigger¥Documents¥MS測定デー9¥GCMS 10 Sample 2 1 0 1 2 Witigger¥Documents¥MS 11 1 1 2 Witigger¥Documents¥MS 11 1 1 2 Witigger¥Documents¥MS 11 1 1 2 Witigger¥Documents¥MS 11 1 1 1 Witigger¥Documents¥MS 11 1 1 1 1 Witigger¥Documents¥MS 11 1 1 1 1 Witigger¥Documents¥MS 11 1 1 1 1 Witigger¥Documents¥MS 11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 | : ¥tigger¥Documents¥MS測定データ¥GCMS | 10 | Sample | 1 | h | 1 | 1 | ~ |
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| ※tigger¥Documents¥MS測定データ¥GCMS 15 Sample 2 1 6 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | :¥tigger¥Documents¥MS測定データ¥GCMS | 14 | Sample | 1 | 1 | 5 | 1 | > |
| ¥tigger¥Documents¥MS測定データ¥GCMS 16 Sample 2 1 7 1 7 1 1 ✓ ¥tigger¥Documents¥MS測定データ¥GCMS 17 Sample 2 1 8 1 0 ✓ ¥tigger¥Documents¥MS測定データ¥GCMS 19 Sample 2 1 9 1 0 1 ✓ ¥tigger¥Documents¥MS測定データ¥GCMS Alkanes Blank Alkane 1 1 1 1 1 ✓ | :¥tigger¥Documents¥MS測定データ¥GCMS | 15 | Sample | 2 | 1 | 6 | 1 | > |
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| :¥tigger¥Documents¥MS測定デー9¥GCMS3 Alkanes Blank Alkane 1 1 I | :¥tigger¥Documents¥MS測定データ¥GCMS¥ | 19 | Sample | 2 | 1 | 10 | 1 | > |
| | | | | | | | | |
| |):¥tigger¥Documents¥MS測定データ¥GCMS | Alkanes | Blank | Alkane | J | 11 | 1 | V |
| | :¥tigger¥Documents¥MS測定データ¥GCMS | Alkanes | Blank | Alkane | <u>,</u> | 11 | 1 | V |
| | :¥tigger¥Documents¥MS測定デーダ¥GCMS | Alkanes | Blank | Alkane | <u> </u> | 11 | 1 | |

 ポップアップした Analysis parameter setting ウインドウ→Data collection タブの 最下部の「Load」をクリックし、T100GCv_35-600_5-32.med2 を選択。ひとつ上の階 層に保存してある。

| () Analysis par | rameter setting | | | | | _ | | × |
|-----------------|-----------------|-----------|------------------------------|-------------|-------------|-----------|--------|------|
| Data collection | Peak detection | MS1Dec | Identification | Alignment | Filtering | | | |
| Mass scan ran | ige | | | | | | | |
| Mass range | begin: | | | | | 0 Da | | |
| Mass range | end: | | | | 100 | Do Da | | |
| Advanced | | | | | | | | |
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| | | | | | | | | |
| Load | ✔ Together with | Alignmen | t | | | Finish | Ca | ncel |
| 14. Ident | ification タブの | の RI or F | RT:が Use ret | ention inde | ex(RI)が遅 | 選択され | ている | のを確 |
| 認し、Ind | ex file:の「Set | 」をクリ | ックし、Car | bon-RT(m | in) diction | nary file | path o | > 「」 |
| をクリック | クして、6.で指 | 定したフ | ォルダー内の | > Alkane-D | ictionary. | txt を選 | 択。フ | アイハ |
| 名の上で、 | マウスの右ボタ | ンをクリ | 「ックし、プ <i>」</i> 「C 」 ナ ケッ | レダウンメ | ニュー→ | Auto | fill」を | クリッ |
| り。すべて | このファイルに | 指正し「 | Set」をクリ | ック。 | | | | |

次に、MSP file:の「Browse」をクリックし、4.でダウンロードした GCMS DB-Public-Kovats RI-VS3.msp を選択。ひとつ上の階層に保存してある。

| () Analysis parameter sett | ing | | | _ | |
|--|---|---|--|--|--|
| Data collection Peak deter | tion MS1Dec Identified | cation Alignment | Filtering | | |
| Retention time setting | (1) | | | | |
| RI or RT: 💿 Use rete | ention index (RI) O Us | e retention time (m | in) | | |
| Index file: Set | (2) Status: empty | | | | |
| Index type: 💿 Alkanes | G FAMEs | | | | |
| MSP file and identification | n setting | | | 6 | |
| MSP file: | | | | | Browse |
| | | | | | |
| (Retention index dictionary setting | 9 | | | - | |
| File path | File name | Carl | oon-RT(min) die | tionary file path | |
| | | | | | ± |
| D:¥tigger¥Documents¥MS測定デー? | 10 | D:¥tigger¥Documents | ¥MS測定データ | ссмехотимы | ******************** |
| D:¥tigger¥Documents¥MS測定デー D:¥tigger¥Documents¥MS測定デー | 10 11 | D:¥tigger¥Documents D:¥tigger¥Documents | ¥MS測定データ ¥MS測定データ | Auto fill | 805¥/ |
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5 Set

Cancel

15. Alignment タブの RI or RT:の Use retention index(RI)を選択し、「Finish」をクリ ックすると処理が始まる。

| | ameter setting | | | | | | - | | × |
|---------------------------|----------------|------------|-------------|-------|---------------|------------|--------|-----|---|
| Data collection | Peak detection | MS1Dec | Identificat | tion | Alignment | Filtering | | | |
| Parameter setti | ing | | | | | | | | |
| Result name: | | | | | nmentResult | _2024_1_9_ | 13_54_ | | |
| Reference fil | e: | | 10 | | | Ŷ | | | |
| RI or RT: | Ose retention | index (RI) | 🔿 Use r | eten | tion time (mi | in) | | | |
| Retention in | dex tolerance: | | | | | | 20 | | |
| Retention time tolerance: | | | | 0.075 | | | | min | |
| El similarity tolerance: | | | | | 70 | | | | |
| Advanced | | | | | | | | | |

| Load 🗹 Together with Alignment | Finish | Cancel |
|--------------------------------|--------|--------|
| | | |

16. Alignment navigator に表示されているファイルをダブルクリックし、ポップアップしたウインドウの「OK」をクリックする。



 Peak spot navigator→Display filter の Ref. matched にチェックを入れ、同定された ファイルのみを表示させる。「Show ion table」をクリックし、Alignment Table を開き、 Metabolite Name Filter に 2-iso と入力すると、内部標準として加えた 2-Isopropylmalic acid のみが表示されるので、ID を控えておく。Alignment Table を閉じる。



| () Aligi | nment Table | | | • • | | | | | | - | | × |
|----------|---|--------|------------|--------|--------------------------------|---------|-------|------------------|--------------------------|----------|----------|---|
| Num of | Num of rows: 1 Metabolite Name Filter Comment Filter 38.02 Mz Range 558.34 5.1 RT Image: Comment Filter Image: Comment Filter | | | | | | | 1 RT F | Range | 31.6 | | |
| | RT(min) | RI | Quant mas: | Fill % | Metabolite name | Comment | S/N | ANOVA P-value | Fold change (Max/Min) | E | larChart | |
| 211 | 13.59 | 1568.2 | 147.0 | 0.91 | 2-Isopropylmalic acid; GC-EI-T | | 101.1 | 2.60E-01 | 1.21 | — | <u> </u> | |

 Option→file property setting を選択し、Alkanes の Included のチェックを外して 「Finish」をクリック。

| | | | | | | | - 🗆 X |
|-----------|-----------|---------------------------------------|-------|------------------|-----------------------|------------|---|
| File name | File type | Class ID | Batch | Analytical order | Injection volume (µL) | Y variable | Included |
| 10 | Sample | 1 | 1 | 1 | 1 | 0 | Image: A start of the start of |
| 11 | Sample | 1 | 1 | 2 | 1 | 0 | Image: A start of the start of |
| 12 | Sample | 1 | 1 | 3 | 1 | 0 | ✓ |
| 13 | Sample | 1 | 1 | 4 | 1 | 0 | ✓ |
| 14 | Sample | 1 | 1 | 5 | 1 | 0 | Image: A start of the start of |
| 15 | Sample | 2 | 1 | 6 | 1 | 0 | Image: A start of the start of |
| 16 | Sample | 2 | 1 | 7 | 1 | 0 | Image: A start of the start of |
| 17 | Sample | 2 | 1 | 8 | 1 | 0 | Image: A start of the start of |
| 18 | Sample | 2 | 1 | 9 | 1 | 0 | Image: A start of the start of |
| 19 | Sample | 2 | 1 | 10 | 1 | 0 | |
| Alkanes | Blank | Alkane | 1 | 11 | 1 | 0 | |
| | • | · · · · · · · · · · · · · · · · · · · | | | | | |

Option→Alignment result property setting を選択し、Target ID の「-1」を内部標準の ID に変更し、その上でマウスの右ボタンをクリックしプルダウンメニューの「Auto fill」をクリック。すべてのファイルに指定し「Finish」をクリック。

| () Normalizatio | on property setting for | alignmentResult_2024_ | 1_9_13_54_36 | - | | × 5.068 |
|-----------------|-------------------------|-----------------------|--------------|-------------------------------|-----------|-----------|
| Alignment ID | Retention index | RT [min] | Quant mass | Metabolite name | Target ID | 976.4 |
| 0 | 976.4218 | 5.068 | 77.02301 | | 211 | ↑ 77 02 |
| 1 | 980.5114 | 5.134 | 281.0531 | | -1 | Auto fill |
| 2 | 984.4051 | 5.197 | 221.0849 | | -1 | Сору |
| 3 | 985.0146 | 5.207 | 155.0938 | | -1 | Paste |
| 4 | 985.1142 | 5.209 | 147.0659 | | -1 | INA |
| 5 | 988.8133 | 5.269 | 58 | ETHYL-N-PROPYLAMINE; EI-B; MS | -1 | |
| 6 | 994.4907 | 5.361 | 41.0406 | | -1 | Ray |
| 7 | 995.3861 | 5.375 | 57.08001 | decane | -1 | |
| 8 | 997.572 | 5.411 | 136.0365 | | -1 | BinV |
| ٩ | 999 2935 | 5 439 | 117 | Propylepealycol | -1 | |

^{20.} Data visualization→Normalization を選択し、Internal standard を選択し「Done」

| 🍓 Normalization for alignmentR 🛛 — | | × |
|--|---|-----|
| Options | | |
| ○ None | | |
| Internal standard | | |
| ○ LOWESS | | |
| Internal standard + LOWESS | | |
| O SPLASH lipidomix | | |
| Total ion chromatogram (TIC) | | |
| \bigcirc mTIC: TIC of identified metabolites | | |
| | | |
| | D | one |

をクリック。

「Show ion table」をクリックすると、簡易定量の結果が表示される。 21.

| () Aligr | nment Table | | | | | | | | | – 🗆 X |
|----------|-------------|--------|---------------|--------|-------------------------------|---------|-------|------------------|--------------------------|-------------------|
| Num of | rows: 150 | Me | tabolite Name | Filter | Comment | Filter | 38.0 |)2 Mz Range | 558.34 | 5.1 RT Range 31.6 |
| CR | 🔒 💽 | Set | | | | | | | | |
| ID | RT(min) | RI | Quant mas: | Fill % | Metabolite name | Comment | S/N | ANOVA P-value | Fold change (Max/Min) | BarChart |
| 5 | 5.27 | 988.8 | 58.0 | 0.91 | ETHYL-N-PROPYLAMINE; EI-E | | 103.8 | 3.87E-02 | 2.67 | ^ |
| 7 | 5.38 | 995.4 | 57.1 | 0.82 | decane | | 11.2 | 5.54E-01 | 1.09 | |
| 9 | 5.44 | 999.3 | 117.0 | 0.91 | Propyleneglycol | | 19.6 | 8.39E-01 | 1.12 | |
| 17 | 6.01 | 1034.2 | 152.0 | 0.91 | 2-Hydroxypyridine; GC-EI-TO | | 632.8 | 8.76E-02 | 1.31 | |
| 19 | 6.14 | 1042.4 | 93.0 | 0.91 | BIS(2-CHLOROETHOXY)METH | | 173.2 | 6.71E-01 | 1.07 | |
| 20 | 6.24 | 1048.7 | 174.0 | 0.91 | pyruvic acid | | 17.2 | 1.34E-02 | 2.13 | |
| 23 | 6.35 | 1055.0 | 147.0 | 0.91 | propane-1,3-diol NIST | | 11.7 | 8.09E-01 | 1.04 | |
| 24 | 6.40 | 1058.5 | 73.0 | 0.91 | 2-Hydroxyisobutyric acid; GC· | | 93.9 | 1.66E-01 | 1.43 | |
| 25 | 6.40 | 1058.5 | 147.0 | 0.91 | lactic acid | | 410.2 | 1.70E-01 | 1.43 | |
| 29 | 6.67 | 1074.9 | 73.0 | 0.91 | Glycolic acid; GC-EI-TOF; MS; | | 5.7 | 5.44E-01 | 1.27 | |
| 32 | 6.88 | 1088.0 | 72.0 | 0.82 | DI-N-PROPYLAMINE; EI-B; MS | | 39.9 | 2.79E-01 | 1.43 | |
| 33 | 6.93 | 1090.7 | 154.0 | 0.91 | maleimide | | 31.2 | 7.20E-01 | 1.32 | |
| 34 | 7.08 | 1100.0 | 131.0 | 0.91 | 2-hydroxybutanoic acid | | 46.2 | 4.23E-02 | 1.69 | |
| 35 | 7.08 | 1100.0 | 57.1 | 0.55 | undecane | | 11.9 | 7.93E-01 | 1.05 | |
| 36 | 7.09 | 1100.4 | 116.0 | 0.91 | L-Alanine; GC-EI-TOF; MS; 3 T | | 804.3 | 6.02E-01 | 1.29 | |
| 1 .a | | | 400.0 | 0.04 | 1 1 71404 | | 270.0 | 5 07E 04 | 4.00 | U |

22. MetaboAnalyst を用いた解析のためのデータエクスポート。

Export→Alignment result を選択 し、ポップアップした Alignment result export の「Browse」をクリ ック。エクスポート先のフォルダ ーを選択する。

Raw data matrix(Height) と Normalized data matrix にチェッ クを入れ「Export」をクリックし、 テキストファイルを出力する。

| ion | Ехро | rt | Help | |
|------|------|----|-------------------|----------------------|
| ot | | Pe | eak list result | |
| ſmir | | A | ignment result | |
| | | Μ | olecular spectru | m networking export |
| - | | C | opy screenshot to | o clipboard (emf) |
| | | Pa | arameter export (| (Tab-delimited text) |
| | | Ex | port as lipoquali | ty database format |
| | | Ex | port normalizati | on result |

MetaboAnalyst で読み込ませるための、データフォーマット修正。 23. エクスポートしたテキストファイル(Normalized)を、Excel に読み込ませる。

Metabolite、Sample 以外の column を消す (A-D、F-AB、AM-AS)。

| A1 | L | ▼ I × ∨ | fx | | | | | | | | | | | | | | | | | | | | | | | ` |
|----|---------|--------------|-------------|----------|-------------|---------|-----------|-------------|-----------|-----------|---------|-----------|------------|----------|------------|-----------|-----------|----------|-----------|-----------|--------------|--------------|------------|-------------|----------|-----------|
| 1 | A | В | С | D | E | F | G | н | 1.1 | J | К | L | М | N | 0 | Р | Q | R | S | т | U | v | W | х | Y | Z |
| 1 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | Alignme | nt Average R | R Average R | Quant ma | Metabolit | eFill % | Reference | e Reference | e Formula | Ontology | INCHIKE | Y SMILES | Annotation | RT/RI ma | t EI-MS ma | a Comment | Manually | Manually | Total sco | RT simila | ır RI simila | ri Total spe | c Dot prod | u Reverse o | Fragment | S/N avera |
| 6 | | 0 5.068 | 976.42 | 77.02301 | Unknown | 0.909 |) null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 31.08 |
| 7 | | 1 5.134 | 980.51 | 281.0531 | l Unknown | 0.545 | 5 null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 5.25 |
| 8 | | 2 5.197 | 984.41 | 221.0849 | 0 Unknown | 0.909 |) null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 122.02 |
| 9 | | 3 5.207 | 985.01 | 155.0938 | 8 Unknown | 0.818 | 3 null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 6.65 |
| 10 | | 4 5.209 | 985.11 | 147.0659 | 0 Unknown | 0.909 |) null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 9.35 |
| 11 | | 5 5.269 | 988.81 | 58 | BETHYL-N | 0.909 | 1 | 1 988.18 | C5H13N | Dialkylam | XCVNDB | IL CCCNCC | 440 | FALSE | FALSE | | FALSE | FALSE | 85.8 | 3 null | 10 | 0 76. | 4 74. | 3 67.6 | 5 100 | 103.77 |
| 12 | | 6 5.361 | 994.49 | 41.0406 | 6 Unknown | 0.182 | 2 null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 5.2 |
| 13 | | 7 5.375 | 995.39 | 57.08001 | l decane | 0.818 | 3 - | 1 987 | C10H22 | Alkanes | DIOQZVS | 0000000 | 440 | FALSE | FALSE | | FALSE | FALSE | 88.6 | 5 null | 81 | 4 93. | 5 9 | 4 89.4 | 100 | 11.21 |
| 14 | | 8 5.411 | 997.57 | 136.0365 | 5 Unknown | 0.364 | l null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 9.28 |
| 15 | | 9 5.439 | 999.29 | 117 | 7 Propylene | 0.909 | 4.28 | 8 1002.01 | C3H8O2 | 1,2-diols | DNIAPM | SCC(0)CO | 440 | FALSE | FALSE | | FALSE | FALSE | 88.4 | 7.3 | 3 98. | 8 81. | 5 70. | 6 88.5 | 5 100 | 19.6 |
| 20 | | o | | 070.0000 | | 0.000 | | | | | | - | | | C 11 OC | | E + 1 O E | E41.05 | | | | | | | | 5.40 |

| 24 | 次に | Row1をRow2へ | Row5 を Row1 へ移動後 | Row3-Row5 を消去。 |
|--------------|-----|----------------|--------------------|----------------|
| 2 4 . | クル、 | ROWI & ROWL So | NOWJ で NOWI 「少野」反、 | NUWJ-NUWJ を旧ム。 |

| A | S1 | • I × \ | / fx NA | | | | | | | | | | | | | | | | | | | | | | | |
|----|--------|---------------|-------------|----------|-----------|--------|-----------|-------------|---------|-----------|----------|---------|------------|----------|------------|---------|----------|----------|-----------|-----------|--------------|--------------|-------------|---------|----------|-----------|
| | A | В | С | D | E | F | G | н | 1.1 | J | К | L | М | N | 0 | P | Q | R | S | Т | U | V | W | Х | Y | Z |
| 1 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | Alignm | ent Average I | R Average R | Quant ma | Metabolit | Fill % | Reference | e Reference | Formula | Ontology | INCHIKEY | SMILES | Annotation | RT/RI ma | t EI-MS ma | Comment | Manually | Manually | Total sco | RT simila | ar RI simila | ri Total spe | c Dot produ | Reverse | Fragment | S/N avera |
| 6 | | 0 5.06 | 976.42 | 77.02301 | Unknown | 0.909 |) null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 31.08 |
| 7 | | 1 5.13 | 4 980.51 | 281.0531 | Unknown | 0.549 | 5 null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 5.25 |
| 8 | | 2 5.19 | 7 984.41 | 221.0849 | Unknown | 0.909 |) null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 122.02 |
| 9 | | 3 5.20 | 7 985.01 | 155.0938 | Unknown | 0.818 | 3 null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 6.65 |
| 10 | | 4 5.20 | 985.11 | 147.0659 | Unknown | 0.909 |) null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 9.35 |
| 11 | | 5 5.26 | 988.81 | 58 | ETHYL-N | 0.909 | - 1 | 1 988.18 | C5H13N | Dialkylam | XCVNDB | CCCNCC | 440 | FALSE | FALSE | | FALSE | FALSE | 85. | 3 null | 10 | 0 76. | 4 74.3 | 67. | 5 100 | 103.77 |
| 12 | | 6 5.36 | 994.49 | 41.0406 | Unknown | 0.182 | 2 null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 5.2 |
| 13 | | 7 5.37 | 5 995.39 | 57.08001 | decane | 0.818 | 3 -1 | 1 987 | C10H22 | Alkanes | DIOQZVS | 0000000 | 440 | FALSE | FALSE | | FALSE | FALSE | 88. | 5 null | 81. | 4 93. | 5 94 | 89. | 4 100 | 11.21 |
| 14 | | 8 5.41 | 1 997.57 | 136.0365 | Unknown | 0.364 | 1 null | null | null | null | null | null | 4 | FALSE | FALSE | | FALSE | FALSE | null | null | null | null | null | null | null | 9.28 |
| 15 | | 9 5.43 | 999.29 | 117 | Propylene | 0.909 | 4.28 | 8 1002.01 | C3H8O2 | 1.2-diols | DNIAPMS | | 440 | FALSE | FALSE | | FALSE | FALSE | 88. | 7. | 3 98. | 8 81. | 5 70.6 | 88. | 5 100 | 19.6 |

| N | 9 \checkmark : $\times \checkmark f_x$ | | | | | | | | | | | |
|----|--|------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| | А | В | С | D | E | F | G | н | 1 | J | К | L |
| 1 | | File type | Sample |
| 2 | Metabolite name | El spectru | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 3 | Unknown | 34.98378:2 | 0.018658 | 0.013129 | 0.019063 | 0.028697 | 0.016449 | 0.016978 | 0.012538 | 0.015727 | 0.015581 | 0.012057 |
| 4 | Unknown | 35.05195:1 | 0.002149 | 0.000379 | 0.001322 | 0.000404 | 0.001283 | 0.001655 | 0.001236 | 0.000352 | 0.000336 | 0.000942 |
| 5 | Unknown | 38.01551:0 | 0.016454 | 0.015969 | 0.048452 | 0.058963 | 0.041979 | 0.04919 | 0.022185 | 0.208684 | 0.033714 | 0.033337 |
| 6 | Unknown | 35.97857:1 | 0.001855 | 0.001013 | 0.001575 | 0.002323 | 0.001864 | 0.000264 | 0.002649 | 0.003034 | 0.002746 | 0.002752 |
| 7 | Unknown | 35.4156:42 | 0.037343 | 0.03591 | 0.050803 | 0.074896 | 0.05276 | 0.051874 | 0.038892 | 0.048756 | 0.038974 | 0.040205 |
| 8 | ETHYL-N-PROPYLAMINE; EI-B; MS | 35.978:170 | 0.123493 | 0.075759 | 0.064891 | 0.0786 | 0.156312 | 0.027657 | 0.02057 | 0.02922 | 0.043682 | 0.038189 |
| 9 | Unknown | 35.06587:1 | 0.002481 | 0.002396 | 0.003454 | 0.004923 | 0.002796 | 0.003216 | 0.002799 | 0.002594 | 0.002097 | 0.002297 |
| 10 | decane | 35.2275:15 | 0.00196 | 0.001509 | 0.002646 | 0.003369 | 0.002124 | 0.00249 | 0.001851 | 0.002309 | 0.001586 | 0.001474 |
| 11 | Unknown | 35.97847:1 | 0.002025 | 0.001985 | 0.004248 | 0.005085 | 0.003038 | 0.003105 | 0.002433 | 0.002492 | 0.002467 | 0.002493 |
| 12 | Propyleneglycol | 37.00885:3 | 0.008302 | 0.00696 | 0.060664 | 0.017433 | 0.012164 | 0.009002 | 0.007599 | 0.037635 | 0.009511 | 0.010752 |

次に、「unknown」と RI 算出に用いた「Alkane」を消す。更に、MetaboAnalyst では、Metabolite nameの重複はエラーになるため、重複がある場合は Metabolite nameの末尾に数字などを付けて重複を回避する。

| A144 \checkmark : $\times \checkmark f_x$ undecane | | | | | | | | | | | | |
|--|------------|-------------|-----------|-----------|------------|------------|-----------|-----------|-----------|------------|-----------|---------|
| A | В | С | D | E | F | G | Н | 1 | J | K | L | М |
| 11 tyrosine minor | 35.00985:4 | 0.033987 | 0.033471 | 0.04513 | 0.023128 | 0.032629 | 0.035439 | 0.050696 | 0.04181 | 2 0.039573 | 0.04999 | 3 |
| 42 UDP-glucuronic acid | 35.97789:5 | 0.062555 | 0.044383 | 0.036959 | 0.069351 | 0.033347 | 0.062143 | 0.041519 | 0.03928 | 2 0.044115 | 0.0388 | 1 |
| 13 undeesne | 25.07015-1 | 0.007205 | 0.004602 | 0.007226 | 0.012967 | 0.005055 | 0.006007 | 0.005002 | 0.00644 | 0.005210 | 0.00515 | |
| Unknown | 34.98378:2 | 0.018658 | 0.013129 | 0.019063 | 0.028697 | 0.016449 | 0.016978 | 0.012538 | 0.01572 | 7 0.01558 | 0.01205 | 7 |
| 5 Unknown | 35.05195:1 | 0.002149 | 0.000379 | 0.001322 | 0.000404 | 0.001283 | 0.001655 | 0.001236 | 0.000352 | 2 0.000330 | 6 0.00094 | 2 |
| 5 Unknown | 38.01551:6 | 0.016454 | 0.015969 | 0.048452 | 0.058963 | 0.041979 | 0.04919 | 0.022185 | 0.208684 | 4 0.033714 | 0.03333 | 7 |
| ^{Unknown} Unknown の削除 | 35.97857:1 | 0.001855 | 0.001013 | 0.001575 | 0.002323 | 0.001864 | 0.000264 | 0.002649 | 0.003034 | 4 0.002740 | 6 0.00275 | 2 |
| Unknown | 35.4156:42 | 0.037343 | 0.03591 | 0.050803 | 0.074896 | 0.05276 | 0.051874 | 0.038892 | 0.048750 | 6 0.038974 | 0.04020 | 5 |
| Unknown | 35.06587:1 | 0.002481 | 0.002396 | 0.003454 | 0.004923 | 0.002796 | 0.003216 | 0.002799 | 0.002594 | 4 0.00209 | 0.00229 | 7 |
| Unknown | 35.97847:1 | 0.002025 | 0.001985 | 0.004248 | 0.005085 | 0.003038 | 0.003105 | 0.002433 | 0.002492 | 2 0.00246 | 0.00249 | 3 |
| Unknown | 35.97845:1 | 0.000779 | 0.000687 | 0.00109 | 0.001669 | 0.000163 | 0.001001 | 0.000884 | 0.001010 | 6 0.000976 | 6 0.00096 | 9 |
| 20 sknown | 25.61254-5 | 0.004477 | 0.002256 | 0.005624 | 0.00067 | 0.004061 | 0.0047 | 0.004456 | 0.00527 | 2 0.004251 | 0.00411 | 2 |
| | 01.0003 | | | 0.00- | | | | | | | | 100002 |
| 12 D-(+)-Maltose: GC-EL-TOE: MS: n TMS: RT | 35 1757 | 74-1 0 0008 | 885 0.001 | 085 0.00 | 141 0.00 | 0.00 | 6763 0.00 | 1059 0.0 | 01688_0 | 001678_0 | 001127_0 | 001562 |
| 4 decane | 35.2275 | 5:15 0.001 | 196 0.001 | 509 0.002 | 646 0.003 | 3369 0.00 | 2124 0.0 | 0249 0.0 | 01851 0. | 002309 0. | 001586 0 | .001474 |
| 45 denyaroascorbic acid | 55.511- | +2.1 0.130 | 150 0.155 | 145 0.155 | | | 1250 0.25 | 10124 0.2 | .05154 0. | 190090 0. | 202005 0 | 105401 |
| 46 dehydroascorbic acid 2 | 38.0169 | 95:3 0.020 | 054 0.02 | 154 0.019 | 272 0.01 | 5863 0.01 | 1079 0.02 | 9915 0.0 | 35755 0. | 032522 (| 0.03035 0 | .030221 |
| 47 D-Glucose 6-phosphate; GC-EI-TOF; MS; 6 TMS; 1 MEOX; BP | 35.9768 | 34:2 0.0275 | 558 0.093 | 414 0.039 | 157 0.02 | 3457 0.04 | 7442 0.06 | 3644 0.0 | 82633 0. | 062161 0. | 075801 | 0.05157 |
| 48 DI-N-PROPYLAMINE; EI- | 34.9825 | 53:7 0.0073 | 388 0.012 | 483 0.034 | 538 0.0 | 0.01 0.01 | 6414 0.01 | .3659 0.0 | 24899 0. | 011271 0. | 018728 0 | .016871 |
| ⁴⁹ DL-alpha-Hydroxybutyric Alkanesの削除 | 35.9785 | 54:3 0.069 | 942 0.03 | 011 0.042 | 883 0.05 | 5097 0.04 | 1757 0.02 | 0853 0.0 | 15763 0 | 0.01183 0. | 018653 0 | .016915 |
| 50 Die bete Hudeswitzubeite | 25.0705 | 1.4 0.670 | 42 0 640 | 252 0.610 | 000 0.0 | 100 0.00 | 0210 0.40 | 0542 0.2 | 51105 0 | 020700 0 | 507261 0 | 204260 |
| docosane | 35.9784 | 14:8 0.0012 | 217 0.00 | 142 0.001 | .536 0.002 | 2255 0.0 | 0079 0.00 | 1533 0.0 | 01262 0. | 001178 0. | 001049 0 | .000893 |
| DOCOSANE; EI-B; MS | 35.9786 | 62:1 0.0005 | 532 0.000 | 699 0.00 | 0131 0.003 | 1764 0.00 | 0958 0.00 | 0719 0.0 | 00733 0. | 000864 0. | 000746 0 | .000887 |
| 55 DOCOGANOIC ACID, EI B, WO | 51.0051 | 0.0000 | 0.00 | 0.00. | | | 1402 0.00 | 100- | | | | |
| 54 D-Ribose 5-phosphate: GC-EL-TOE: MS: 5 TMS: 1 MEOX: BP | 35 2463 | 22.1 0.052 | 724 0.071 | 944 0.05 | 826 0.05 | \$413 0.03 | 7821 0.04 | 0342 0.0 | 47103 0 | 037535_0 | 041124_0 | 035145 |
| eicosane | 35.1977 | 78:2 0.001 | 161 0.001 | 569 0.001 | 794 0.00 | 1772 0.00 | 1388 0.0 | 0176 0.0 | 01614 0. | 001925 0. | 001118 0 | .000567 |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | 17 1000 | | | | | | | | | | | |

| 43 | D-(+)-Maltose: GC-EL-TOE: MS: n TMC- | DT | DE 17674.1 | 0.000005 | 0.001005 | 0.00141 | 0.001696 | 0.006763 | 0.001059 | 0.001688 | 0.001678 | 0.001127 | 0.001562 |
|----|--------------------------------------|-------------------|-------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| 4 | dehydroascorbic acid | N. I. 1. | の手 | 「「」 | ki ete | 133796 | 0.133497 | 0.071296 | 0.236724 | 0.269194 | 0.198658 | 0.202663 | 0.185461 |
| 5 | dehydroascorbic acid 2 | Metabolite name | の里 | 笈の) | 可心 | 019272 | 0.015863 | 0.011079 | 0.029915 | 0.035755 | 0.032522 | 0.03035 | 0.030221 |
| 46 | D-Glucose 6-phosphate; GC-EI-TOF; I | | | | | 039157 | 0.023457 | 0.047442 | 0.063644 | 0.082633 | 0.062161 | 0.075801 | 0.05157 |
| 47 | DI-N-PROPYLAMINE; EI-B; MS | | 34.98253:7 | 0.007388 | 0.012483 | 0.034538 | 0.00911 | 0.016414 | 0.013659 | 0.024899 | 0.011271 | 0.018728 | 0.016871 |
| 40 | DE LISES HURLINGS STATE STATION FLIT | 05. MO. 0 TMO. 00 | DE 070E 4-5 | 0.00040 | 0.00011 | 0.040000 | 0.000007 | 0.041757 | 0.000050 | 0.015700 | 0.01100 | 0.010050 | 0.010015 |

26. データフォーマット修正終了後、「.csv 形式」で保存する。

Web ブラウザーで、MetaboAnalyst を開く。

URL https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml

トップページの「Click here start」をクリックして「Module Overview」の「Statistical 28. Analysis [one factor]」をクリック。

| soboAney, | MetaboAnalyst 👀 - from raw spectra to biomarkers, patterns, functions and systems biology |
|--|--|
| | 2 News & Updates |
| Home | Releasing MetaboAnalyst 6.0! Your comments and feedback are welcome (12/20/2023): 159- |
| Data Formate | • The Causal Analysis modules is now available for evaluating causal relationships based on metabolomics GWAS studies (12/18/2023); not studies (12/ |
| Data Formats | The MS2 Peak Annotation module is now available for annotation of both DDA and SWATH-DIA data (12/16/2023); |
| Tutorials | • Updated metabolic pathways (supporting > 120 species), metabolite set libraries and metabolic networks based on the latest releases (12/15/2023); 👯 ; |
| User Forum | Updated the master compound libraries to the latest HMDB 5.0 release (12/14/2023); 12(1) |
| | Added support for interactive heatmaps with more control for visual exploration in the two statistics modules! (12/12/2023). The state of the s |
| MetaboAnalystR | Added a new module Uses kesponse Analysis to support metabolomic-based next as assessment for chemical exposure (12/10/2023); ****; Liver can providentimis the ford field erisin (PA/2012, A expression) (11/15/2023); ****; |
| Publications | Added support for enrichment analysis on ~3700 metabolomics pathways from RoMP in Enrichment Analysis module (11/09/2023):394 ; |
| Design Distance | Read more |
| Update History | |
| APIs | |
| Llear State | Click here to start |
| 0301 51015 | |
| About | |
| Contact | |
| | |
| • | Overview |
| NSERC CRSNG | MataboAnalyst is a wab-based platform dedicated for comprehensive metabologics data analysis interpretation and |
| | a set of the set of |
| | analysis for targeted metabolomics data, towards more streamlined analysis for untargeted LC-MS data. In addition to |
| * | many feature enhancements, the current MetaboAnalyst (V6.0) contain three new modules - tandem MS spectral |
| Canada Research Chairs | processing and compound annotation, dose response analysis for chemical risk assessment application, and |
| | leveraging metabolomics-genome wide association analysis (mGWAS) and Mendelian randomization (MR) for |
| INNOVATION | integration with genomics. |
| Conada Poundation for Innovation pour l'Innovation | |
| | |
| 🚯 Manage Cookies | |
| | |

Module Overview

| Input Data Type | Available Modules (click | on a module to proceed, or | scroll down to explore a tota | al of 18 modules including ut | tilities) |
|---|--------------------------------------|--|---|--------------------------------------|------------------------|
| LC-MS Spectra (mzML, mzXML or mzData) | | | Spectra Processing [LC-MS1 w/wo MS2] | | |
| MS Peaks (peak list or intensity table) | | Peak Annotation [MS2-DDA/DIA] | Functional Analysis [LC-MS1] | Functional Meta-analysis [LC-MS1] | |
| Generic Format (.csv or .txt table files) | Statistical Analysis [one factor] | Statistical Analysis [metadata table] | Biomarker Analysis | Statistical Meta-analysis | Dose Response Analysis |
| Annotated Features (metabolite list or table) | | | | | |
| Link to Genomics & Diseases (metabolite list) | | | Causal Analysis via mGWAS | | |

27.

 「A plain text file (.txt or .csv)の「Data Type:」を Concentrations、「Format:」を Samples in columns (unpaired)を選択。「Data File:」の「+ Choose」をクリックし、 保存した.csvを選択すし、「Submit」をクリックし処理を進める。

Please upload your data

| A plain text file (.txt o | or .csv): 😮 | |
|---------------------------|---|------------|
| Data Type: | (1) O Concentrations O Spectral bins O Peak intensities | |
| Format: | 2 Samples in columns (unpaired) V | (4) Submit |
| Data File: | (3) + Choose Normalized_0_20240105.csv 18.3 KB | |

30. 「Data Integrity Check:」の画面でエラーが表示されなければ、「Proceed」をクリ ックし、先に進む。

エラーが表示された場合は、エラーの対応を行う。

Data Integrity Check:

- 1. Checking the class labels at least three replicates are required in each class.
- 2. If the samples are paired, the pair labels must conform to the specified format.
- 3. The data (except class labels) must not contain non-numeric values.

Edit Groups

4. The presence of missing values or features with constant values (i.e. all zeros).

| | Data processing information: |
|-----------|---|
| Che | ecking data contentpassed. |
| San | nples are in columns and features in rows. |
| The | e uploaded file is in comma separated values (.csv) format. |
| The | e uploaded data file contains 10 (samples) by 135 (compounds) data matrix. |
| San | nples are not paired. |
| 2 g | roups were detected in samples. |
| On | ly English letters, numbers, underscore, hyphen and forward slash (/) are allowed. |
| Oth | her special characters or punctuations (if any) will be stripped off. |
| All | data values are numeric. |
| 2 fe | eatures with a constant or single value across samples were found and deleted. |
| A to | otal of 0 (0%) missing values were detected. |
| <u>By</u> | default, missing values will be replaced by 1/5 of min positive values of their corresponding variables |
| Clic | ck the Proceed button if you accept the default practice; |
| Or | click the Missing Values button to use other methods. |

Proceed

31. 「Normalization Overview:」の画面の「Data transformation」の Log transformation (base 10)を選択。「Data scaling」の Auto scaling を選択し、「Normalize」をクリック。

| Quantile normalization (suggested only for > 1000 features) | |
|--|--|
| Data transformation | |
| None | |
| O Log transformation (base 10) | |
| Square root transformation (square root of data values) | |
| Cube root transformation (cube root of data values) | |
| Data scaling | |
| ○ None | |
| Mean centering (mean-centered only) | |
| O Auto scaling (mean-centered and divided by the standard deviation of | f each variable) |
| Pareto scaling (mean-centered and divided by the square root of the sta | andard deviation of each variable) |
| Range scaling (mean-centered and divided by the range of each variable | e) |
| | |
| Normalize View Result | Proceed |
| OK の表示が出たら、「Proceed」をクリック | |
| し、結果画面に移る。 | () ок |
| | You can click View Result button to view the effect, or Proceed |
| | button to analysis page! |

 Select an analysis path to explore:」の T-tests、Volcano plot や PCA などをクリ ックして結果を表示させる。あるいは、左のメニューの「> Statistics」の「>」をクリ ックするとメニューが展開するので、確認したい項目をクリックして表示する。

| | Select an analysis path to explore : |
|---------------|---|
| Upload | · · · |
| V Processing | Univariate Analysis |
| Data check | Fold Change Analysis T-tests Volcano plot |
| Missing value | One-way Analysis of Variance (ANOVA) |
| Data filter | Correlation Heatmaps Pattern Search Correlation Networks (DSPC) |
| Data editor | A downed film (finger a Andrei |
| Normalization | Advanced Significance Analysis |
| ✓ Statistics | Significance Analysis of Microarray (and Metabolites) (SAM) |
| Fold change | Empirical Bayesian Analysis of Microarray (and Metabolites) (EBAM) |
| T-test | Chemometrics Analysis |
| Volcano plot | Principal Component Analysis (PCA) |
| ANOVA | Partial Least Squares - Discriminant Analysis (PLS-DA) |
| Correlations | Sparse Partial Least Squares - Discriminant Analysis (sPLS-DA) |
| DSPC network | <u>Orthogonal Partial Least Squares - Discriminant Analysis (orthopLS-DA)</u> |
| PatternHunter | Cluster Analysis |
| PCA | Hierarchical Clustering: Dendrogram Heatmaps |
| PLSDA | Partitional Clustering: K-means Self Organizing Map (SOM) |
| sPLSDA | Classification & Feature Selection |
| OrthoPLSDA | Random Forest |
| SAM | Support Vector Machine (SVM) |
| EBAM | |
| Dendrogram | |
| Heatmap | |
| SOM | |
| K-means 🗸 | |