Formative Assessment 2

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2025-02-13

Instruction

For the first set of questions, we will look again at the CyTOF data Download CyTOF data. Each row in the dataset represents a cell, and each column in the dataset represents a protein, and the value is element i, j of the dataset represents the amount of protein j in cell i.

1. Use pivot_longer to reshape the dataset into one that has two columns, the first giving the protein identity and the second giving the amount of the protein in one of the cells. The dataset you get should have 1750000 rows (50000 cells in the original dataset times 35 proteins).
2. Use group_by and summarise to find the median protein level and the median absolute deviation of the protein level for each marker. (Use the R functions median and mad).
3. Make a plot with mad on the x-axis and median on the y-axis. This is known as a spreadlocation (s-l) plot. What does it tell you about the relationship betwen the median and the mad?

```
data <- read.csv("C:\\Users\\spike\\Downloads\\cytof_one_experiment (2).csv")
head(data)
```

```
##        NKp30    KIR3DL1      NKp44    KIR2DL1 GranzymeB       CXCR6
CD161
## 1  0.1875955  3.6156932 -0.5605694 -0.2936654  2.477893 -0.14470053 -
0.3152872
## 2  1.0348518  1.7001820 -0.2889611 -0.4798280  3.261016 -0.03392447 -
0.4112129
## 3  2.9996398  6.1411419  1.9032606  0.4823102  4.277562  1.94654156 -
0.5022347
## 4  4.2998594 -0.2211586  0.2425707 -0.4831267  3.351808  0.92622195
3.8772370
## 5 -0.4386448 -0.5035892 -0.1526320  0.7506128  3.194145 -0.05893640
1.0907379
## 6  2.0883050 -0.3992646  3.4550676 -0.5200856  4.345102 -0.36434277 -
0.5705891
##       KIR2DS4     NKp46      NKG2D      NKG2C       X2B4     CD69
KIR3DL1.S1
## 1  1.94497046 4.0818316  2.6200784 -0.3573817 -0.2711557 3.849965 -
0.2554637
## 2  3.80251714 3.7339299 -0.4832788 -0.4675984 -0.5594752 2.910197 -
0.2909482
## 3 -0.32010171 4.5594631 -0.5069090  2.6193782 -0.4554785 3.113454
```

```
3.6613886
## 4 -0.16969487 4.4831486  1.9272290 -0.3110146  1.6350771 3.045998
0.2871241
## 5 -0.05033025 0.8379358 -0.4581674  0.9216947  1.2419054 2.644422
0.4218294
## 6 -0.45033591 4.0550848  3.4283565  0.6272837 -0.4157104 3.958158
0.7993406
##          CD2    KIR2DL5    DNAM.1         CD4        CD8       CD57
TRAIL
## 1  5.3529769 -0.5092906 0.8811347 -0.32347280 -0.2822405  3.3254704 -
0.6084228
## 2  4.3132510  3.7774776 1.5406568 -0.13208167  0.9161920  2.4946442 -
0.5034739
## 3  5.5969513  0.8128166 1.0005903 -0.59933641  1.8382744  3.9897914 -
0.2749380
## 4 -0.5002885  0.3612212 1.2663267 -0.12568567  0.7667204  1.9950916 -
0.5130930
## 5 -0.5479527  1.0638327 0.8722272 -0.07107408 -0.1059012  3.4291302 -
0.1433044
## 6  5.1028564  3.0918867 0.8717267 -0.47986180 -0.2577198 -0.5784575 -
0.5731323
##       KIR3DL2      MIP1b     CD107a      GM.CSF       CD16        TNFa
## 1 -0.30668543  1.2497120 -0.1295305 -0.43074102  3.9951417  0.90143498
## 2 -0.54320954  2.8693060 -0.1887180 -0.16283845  4.4082309  1.93590153
## 3  2.06488239  4.0955112 -0.1998480  3.18853825  6.0023244 -0.02336999
## 4  2.11247859  3.3726018 -0.5720339  0.91310694  5.8238698 -0.60793749
## 5 -0.02505141 -0.3099826 -0.1068511 -0.60370379  4.0122501 -0.61989100
## 6 -0.28337673 -0.4108283 -0.1797545 -0.06372458 -0.5832926  0.14311030
##           ILT2 Perforin KIR2DL2.L3.S2      KIR2DL3      NKG2A    NTB.A
CD56
## 1 -0.386027758 6.431983    1.22710292  2.660657999 -0.5220613 4.348923
2.897523
## 2  2.983874845 6.814827   -0.04141081  3.841304627  4.6771149 3.474335
3.782870
## 3 -0.521099944 5.099562   -0.16705075 -0.009694396 -0.4730573 5.634341
5.701186
## 4 -0.043783559 5.841797   -0.51753289 -0.592990887 -0.4059049 4.598021
6.065672
## 5  1.182703288 4.888777   -0.36251589 -0.398123704 -0.5440881 3.606101
1.966169
## 6 -0.003258955 3.952542   -0.20194392 -0.202592720  3.8882776 2.346275
6.473243
##         INFg
## 1 -0.3841108
## 2  2.7186296
## 3  2.5321763
## 4  2.4564582
## 5  3.1470092
## 6  2.8282987
```

1. Use pivot_longer to reshape the dataset into one that has two columns, the first giving the protein identity and the second giving the amount of the protein in one of the cells. The dataset you get should have 1750000 rows (50000 cells in the original dataset times 35

```
library(tidyverse)
```

```
## ── Attaching core tidyverse packages ──────────────────────── tidyverse
2.0.0 ──
## ✓ dplyr     1.1.4     ✓ readr     2.1.5
## ✓ forcats   1.0.0     ✓ stringr   1.5.1
## ✓ ggplot2   3.5.1     ✓ tibble    3.2.1
## ✓ lubridate 1.9.4     ✓ tidyr     1.3.1
## ✓ purrr     1.0.2
## ── Conflicts ────────────────────────────────────────
tidyverse_conflicts() ──
## ✖ dplyr::filter() masks stats::filter()
## ✖ dplyr::lag()    masks stats::lag()
## ℹ Use the conflicted package (<http://conflicted.r-lib.org/>) to force all
conflicts to become errors
```

```
library(ggrepel)
```

```
# Reshape the dataset
long_data <- data %>%
  pivot_longer(
    cols = everything(),  # Select all columns to pivot
    names_to = "Protein",  # New column for protein identity
    values_to = "Amount"   # New column for protein amount
  )

# Check the structure
print(dim(long_data))  # Should be (1750000, 2)
```

```
## [1] 1750000       2
```

```
head(long_data)
```

```
## # A tibble: 6 × 2
##   Protein   Amount
##   <chr>      <dbl>
## 1 NKp30      0.188
## 2 KIR3DL1    3.62
## 3 NKp44     -0.561
## 4 KIR2DL1   -0.294
## 5 GranzymeB  2.48
## 6 CXCR6     -0.145
```

2. Use group_by and summarise to find the median protein level and the median absolute deviation of the protein level for each marker. (Use the R functions median and mad).

```
# Compute median and MAD for each protein marker
protein_stats <- long_data %>%
  group_by(Protein) %>%  # Group by protein marker
  summarise(
    Median_Level = median(Amount, na.rm = TRUE),  # Calculate median
    MAD_Level = mad(Amount, na.rm = TRUE)  # Calculate median absolute
deviation
  ) %>%
  arrange(desc(Median_Level))  # Optional: Sort by median value

# Print results
print(protein_stats)
```

```
## # A tibble: 35 × 3
##    Protein   Median_Level MAD_Level
##    <chr>            <dbl>     <dbl>
##  1 Perforin          7.14     0.760
##  2 CD56              5.71     0.998
##  3 CD16              5.12     0.874
##  4 CD69              4.59     1.02
##  5 NTB.A             4.44     0.821
##  6 CD2               3.95     1.68
##  7 NKp46             3.85     1.05
##  8 NKG2A             3.83     1.81
##  9 NKp30             3.78     1.30
## 10 GranzymeB         3.68     0.928
## # ℹ 25 more rows
```

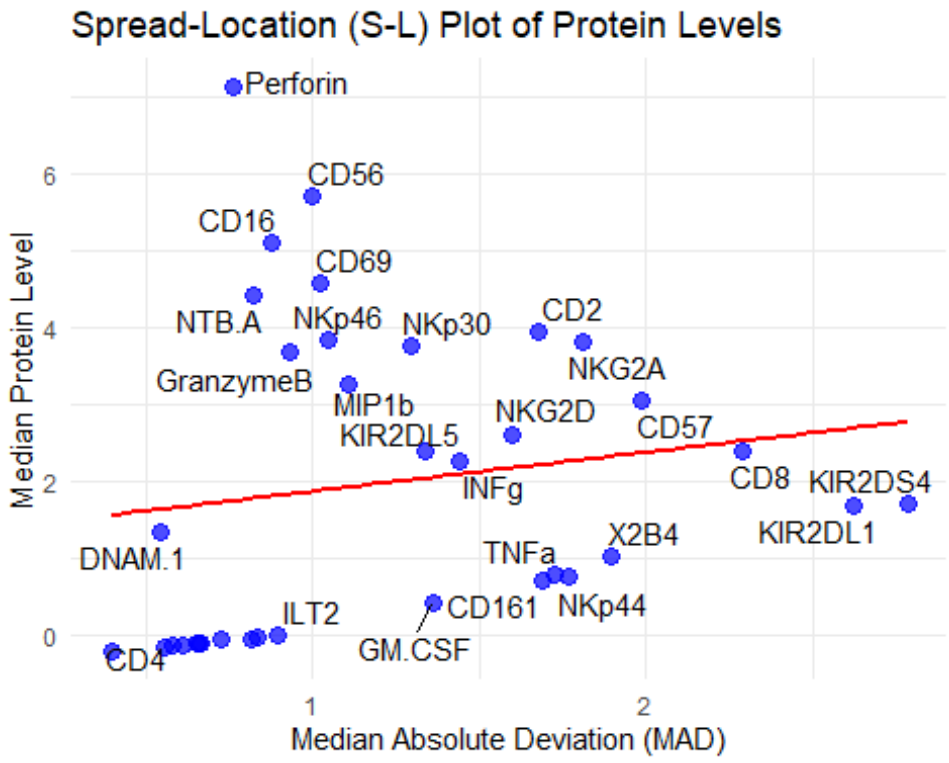
3. Make a plot with mad on the x-axis and median on the y-axis. This is known as a spreadlocation (s-l) plot. What does it tell you about the relationship betwen the median and the mad?

```
ggplot(protein_stats, aes(x = MAD_Level, y = Median_Level, label = Protein))
+
  geom_point(color = "blue", size = 3, alpha = 0.7) +  # Scatter plot points
  geom_smooth(method = "lm", color = "red", se = FALSE) +  # Trend line
  geom_text_repel(size = 4, max.overlaps = 15) +  # Smart text labels
  labs(
    title = "Spread-Location (S-L) Plot of Protein Levels",
    x = "Median Absolute Deviation (MAD)",
    y = "Median Protein Level"
  ) +
  theme_minimal()
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

```
## Warning: The following aesthetics were dropped during statistical
transformation: label.
## ℹ This can happen when ggplot fails to infer the correct grouping
structure in
##   the data.
## ℹ Did you forget to specify a `group` aesthetic or to convert a numerical
##   variable into a factor?

## Warning: ggrepel: 9 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```



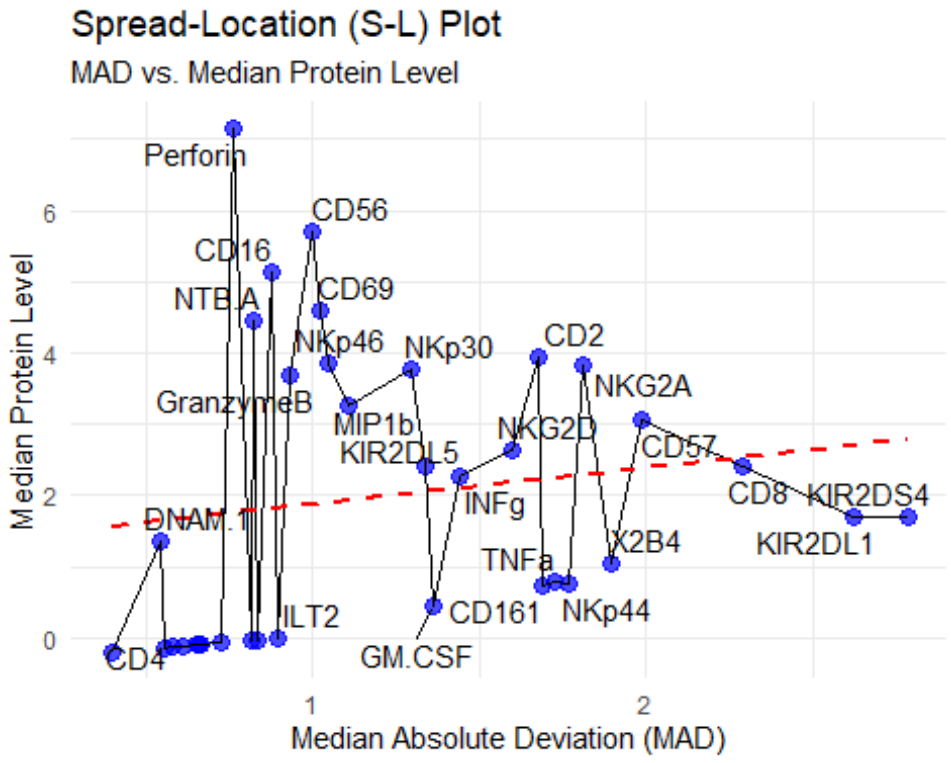
To better visualize this:

```
ggplot(protein_stats, aes(x = MAD_Level, y = Median_Level, label = Protein))
+
  geom_point(color = "blue", size = 3, alpha = 0.7) +  # Scatter plot points
  geom_line(aes(group = 1), color = "black") +  # Line connecting points
  geom_smooth(method = "lm", color = "red", linetype = "dashed", se = FALSE)
+  # Trend line
  geom_text_repel(size = 4, max.overlaps = 15) +  # Labels to avoid overlap
  labs(
    title = "Spread-Location (S-L) Plot",
    subtitle = "MAD vs. Median Protein Level",
    x = "Median Absolute Deviation (MAD)",
    y = "Median Protein Level"
  ) +
  theme_minimal()
```

```
## `geom_smooth()` using formula = 'y ~ x'

## Warning: The following aesthetics were dropped during statistical
transformation: label.
## ℹ This can happen when ggplot fails to infer the correct grouping
structure in
##   the data.
## ℹ Did you forget to specify a `group` aesthetic or to convert a numerical
##   variable into a factor?

## Warning: ggrepel: 9 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```



Insights:

a. Weak Positive Relationship The red dashed line (trend line) suggests a slight upward trend, meaning higher median protein levels tend to have slightly higher MAD values. However, the trend is not strong, indicating that median protein levels and MAD do not have a perfectly linear relationship.

b. High Variability at Low MAD Many proteins with low MAD values (left side of the plot) have high or very low median levels. This suggests that some proteins have stable expression (low MAD) but varying median levels.

c. Large MAD ≠ Large Median Some proteins with high MAD values do not necessarily have high median levels. This means that some proteins show high variability in expression even if their overall median level is not that high.

d. Outliers Perforin and CD56 have the highest median values but differ in MAD, meaning one is more stable than the other. TRAIL and KIR markers have very low median levels, but some still show variability.

4. Using either pivot_longer on its own or pivot_longer in combination with separate, reshape the dataset so that it has columns for country, event, year, and score.

```
remotes::install_github("dcl-docs/dcldata")
```

```
## Skipping install of 'dcldata' from a github remote, the SHA1 (0a08cbba)
has not changed since last install.
##   Use `force = TRUE` to force installation
```

```
library(dcldata)
```

```
gymnastics_long <- example_gymnastics_2 %>%
  pivot_longer(
    cols = -country,  # Keep 'country' column, reshape the rest
    names_to = "event_year",  # Create a new column with event-year info
    values_to = "score"  # Store the values in 'score'
  ) %>%
  separate(event_year, into = c("event", "year"), sep = "_")  # Split event
and year

# View the reshaped data
head(gymnastics_long)
```

```
## # A tibble: 6 × 4
##   country       event year  score
##   <chr>         <chr> <chr> <dbl>
## 1 United States vault 2012   48.1
## 2 United States floor 2012   45.4
## 3 United States vault 2016   46.9
## 4 United States floor 2016   46.0
## 5 Russia        vault 2012   46.4
## 6 Russia        floor 2012   41.6
```