## udiFIGURES and TABLES



Figure 1. Experimental setup. The illustration shows the structure of the experimental setup for RNA-Seq & Protein data after cleaning out inconsistent data points in a general perspective. There are seven different sets of experiments; glucose time course experiment, glycerol time course experiment, gluconate experiment, lactate experiment, high sodium experiment, low magnesium experiment and high magnesium experiment. We have RNA-Seq data for all seven experiments and protein data for four of them. The four different carbon sources used for the experiments and varying MG and Na levels are also shown in figure. Number of test tubes represents the number of different batches for each experimental setup so basically there are 3 replicas for each experiment and there are 21 different batches in total. The colors of test tubes refer to the carbon sources used in experiments, glucose, glycerol, gluconate and lactate are represented with light purple to dark purple. The green tones in the background represent the phase of the experiment. Light green is exponential phase, darker green is stationary phase and dark green is late-stationary phase. The x-axis shows time and for glucose, glycerol, gluconate and lactate experiments we show the exact hours that the samples are collected. On the other hand for Na and Mg experiments the x-axis is divided into pieces representing different concentrations of Na and Mg. The asterisk “[\*]” near the concentrations of Mg and Na levels represent the base value. The circular points with an x on them, above the axis represents the time or phase that the RNA sample is collected and diamond shaped points, below the axis represents the time or phase that the protein sample is collected. Colors of points represent how many batches we have at that point. Red represents a single batch, yellow represent two batches and green represent three batches.

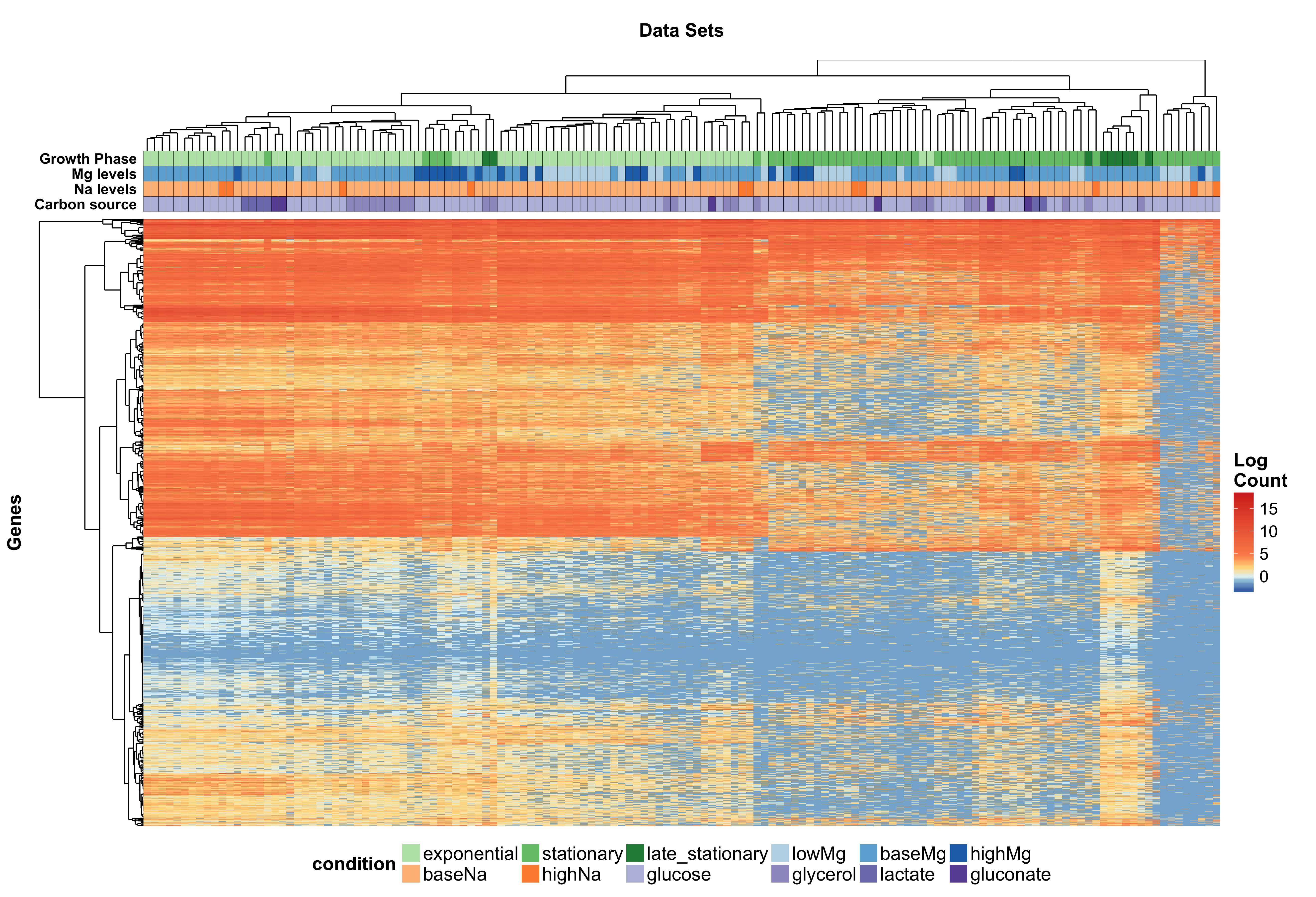


Figure 2: Heatmap of all mRNAs data. The figure represents the heatmap of all 143 samples with 3698 different normalized mRNAs levels. The x-axis shows different samples and the dendogram of x-axis calculates the clustering of the data using Euclidian distance. A similar approach is used to cluster different mRNAs also and generated dendogram is shown on y-axis. The reddish area represents the highly abundant mRNAs and bluish area represents less abundant mRNAs. The clustered data sets are color coded with 12 different conditions with in 4 different categories. The four different variables are represented in a color table at top. The variables are ordered with respect to quality of clustering. The order of categories is growth time, Mg level, Na level and carbon source from best clustered to least clustered category.

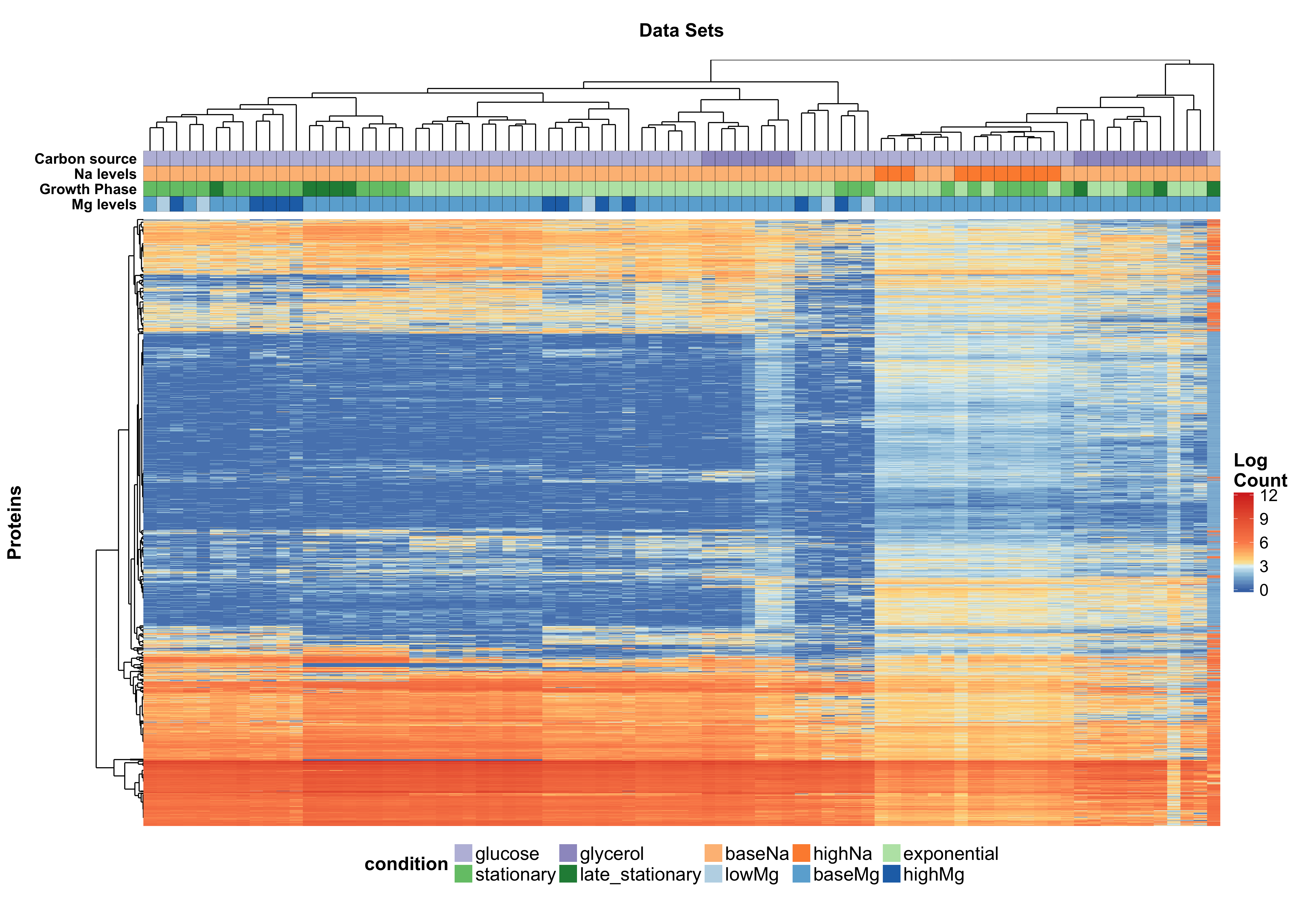


Figure 3. Heatmap of all protein data. The figure represents the heatmap of all 81 samples with 3117 different normalized protein levels. The x-axis shows different samples and the dendogram of x-axis calculates the clustering of the data using Euclidian distance. A similar approach is used to cluster different proteins also and generated dendogram is shown on y-axis. The reddish area represents the highly abundant proteins and bluish area represents less abundant proteins. The clustered data sets are color coded with 10 different conditions with in 4 different categories. The four different variables are represented in a color table at top. The variables are ordered with respect to quality of clustering. The order of categories is carbon source, Na-level, growth phase and Mg-level from best clustered, to least clustered category.

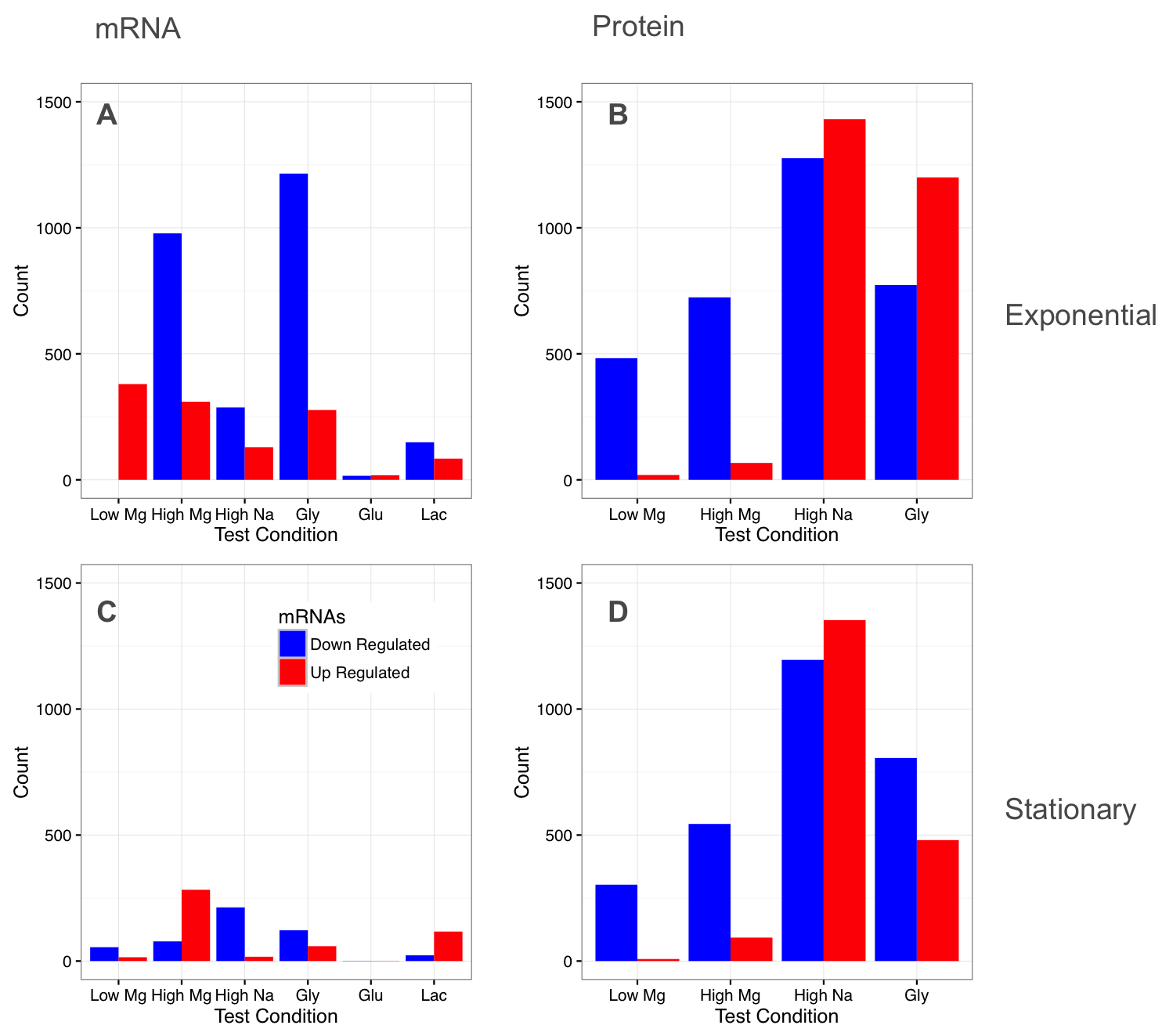


Figure 4. Cell response comparison between different categories of variables. We count the number of significantly differentiated mRNAs and proteins with respect to different variables by using DeSeq2 algorithm, by using a threshold of *P’<0.05*. We do the same analysis for both proteins and mRNAs and for both stationary and exponential phases. No log like normalization is applied to data. For exponential phase the base data is exponential glucose time course and for stationary phase base data is stationary glucose time course all other changes are calculated with respect to them. (A) Amount of significantly responding mRNAs in exponential phase. Figure represents the number of significantly up and down regulated mRNAs with respect to three different carbon sources (glycerol, gluconate, and lactate), high, low Mg levels and high Na levels. Most of the change occurred with increasing Mg levels and by switching the carbon source to glycerol. All mRNAs are up regulated with low Mg levels and globally down-regulation is dominant compared to up-regulation. (B) Amount of significantly responding proteins in exponential phase. Figure represents the number of significantly up and significantly down regulated proteins with respect to change carbon source from glucose to glycerol. High, low Mg levels and high Na levels are the other variables that we measure the number of responding proteins. With changing mg levels most of the proteins are down regulated and with changing Na levels almost two thirds of the proteins are either up or down regulated. (C) Amount of significantly responding mRNAs in stationary phase. Figure represents the number of significantly up and down regulated mRNAs with respect to three different carbon sources (glycerol, gluconate, and lactate), high, low Mg levels and high Na levels. Number of significantly responding mRNAs are fewer compared to exponential phase. There is almost no significantly responding mRNA as a response to change of the carbon source from glucose to gluconate. (D) Amount of significantly responding proteins in stationary phase. Figure represents the number of significantly up and significantly down regulated proteins with respect to change of carbon source from glucose to glycerol. High, low Mg levels and high Na levels are the other variables that we measure the number of responding proteins. The pattern of protein changes is similar to exponential phase except the switch to glycerol as carbon source.

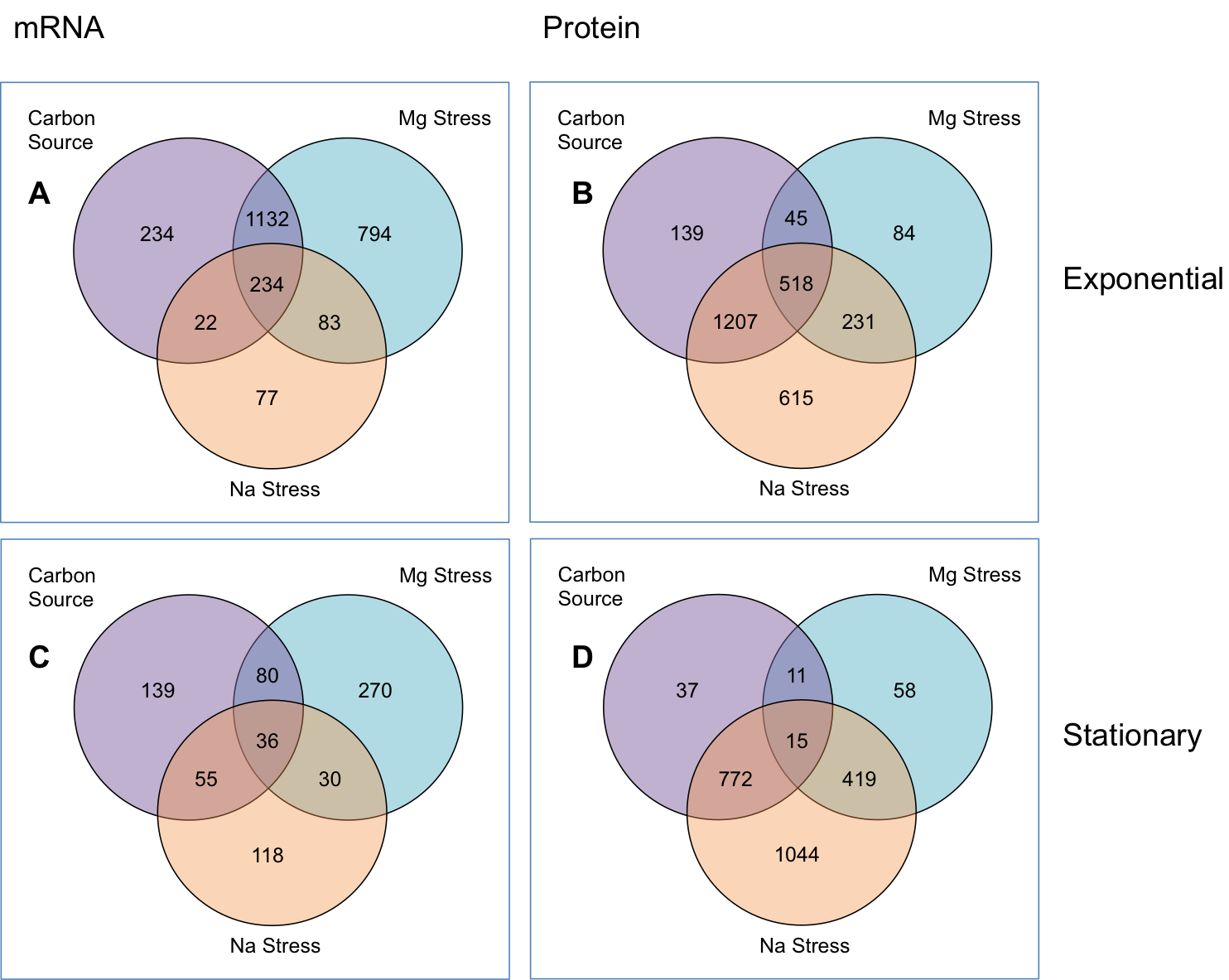


Figure 5. Co responding mRNAs and proteins between with different variables. Figures represent how the significantly responding (*P’<0.05*) mRNAs and proteins are distributed under three different categories. The categories are carbon source, Mg stress and Na stress. Carbon source includes all up or down-regulated mRNAs or proteins that are changing by the change of carbon source. Mg and Na stresses include all up-regulated or down-regulated mRNAs or proteins that are changing by the change of the Mg or Na levels. The numbers in the Venn diagrams represents the number of significantly altered mRNAs and proteins. We do the same analysis for both stationary and exponential phases. (A) Co-altered mRNAs in exponential growth phase. Most of the altered mRNAs are shared between Na stress and carbon source. (B) Co-altered proteins in exponential growth phase. Most of the altered proteins are shared between Mg stress and carbon source. (C) Co-altered mRNAs in exponential growth phase. There are few mRNAs altered compared to exponential phase. (D) Co-altered proteins in exponential growth

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **Overall z-score** | **Condition** | **Z-score** | **# elements** |
|  |  | exponential | -14.15 | 77 |
| Growth Phase | -2.82 | stationary | 3.14 | 57 |
|  |  | Late-stationary | -2.34 | 9 |
|  |  | lowMg | 1.52 | 35 |
| Mg Levels | -0.70 | baseMg | -2.33 | 85 |
|  |  | highMg | -0.80 | 23 |
| Batch number | -0.14 |  |  |  |
| Na Levels | -0.05 | baseNa | -0.76 | 132 |
|  |  | highNa | 0.83 | 11 |
|  |  | glucose | 1.68 | 107 |
| Carbon Source | 0.19 | glycerol | -1.39 | 24 |
|  |  | lactate | -2.49 | 6 |
|  |  | gluconate | -0.28 | 6 |

Table : Clustering quality of different categories. Table shows different categories of parameters and their z-scores in terms of clustering quality. To calculate the clustering quality with respect to a category, we calculate cophenetic distance between all pairs and calculate the mean value for all categories and conditions. We repeat the same calculation thousand times with mixed data and calculate the z-score of original mean value with respect to mean values of randomly mixed runs.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **RNA** | |  | **Proteins** | |
| **Variable** | **Z-score** |  | **Variable** | **Z-score** |
| Growth Phase | -3.34 |  | Carbon Source | -1.55 |
| Mg Levels | -0.79 |  | Na Levels | -1.22 |
| Na Levels | -0.18 |  | Growth Phase | -0.67 |
| Carbon Source | 0.13 |  | Mg Levels | -0.07 |
|  |  |  |  |  |
| Batch Number | -0.16 |  | Batch Number | -4.62 |