

I'm happy to guide you through how significance works for your data set. My main concern is that many of your seemingly significant gene expressions at the 0.05 level are falsely significant.

Hypothesis testing starts with a null hypothesis, that the gene expression values are not different between DLBCL and FL patients, and an alternative hypothesis, that the gene expression values are different between DLBCL and FL patients. The p-value from your t-tests measures how likely the value is assuming that the null hypothesis is true. A p-value of 0.05 means that the value has a 0.05 chance of happening. Since there is a very low chance of the null hypothesis being true at a p-value of 0.05 or lower, we are confident enough that the null hypothesis is not true, so we reject the null hypothesis and accept the alternative. This cut off point is called the significance level  $\alpha$ , which is  $\alpha=0.05$  in your case.

The significance level  $\alpha=0.05$ , even if it's sufficient to declare a difference between DLBCL and FL patients, still means that there is 0.05 chance (or less) that the null hypothesis is correct. If you're only testing 10 gene expressions,  $\alpha=0.05*10$  expressions might not be significant even if they are past the cut off point (falsely significant). This becomes an issue for the your data set where around 356 ( $\alpha=0.05*7129$  expressions) may be falsely significant.

I would suggest two methods to deal with this issue: the (1) Bonferroni method or the (2) Benjamini Hochberg method. Both will provide new cut off points and reduce the number of significant gene expressions. The (1) Bonferroni method reduces the cut off point so that fewer gene expressions can be significant at the chosen significance level. We would divide the significance level  $\alpha$  by the number of independent variables we're testing for, so  $\alpha=0.05/7129$  gene expressions would be the new cut off point and any p-values at or below can be considered significant. This method reduces the number of significant gene expressions by imposing a stricter cut off point. The (2) Benjamini Hochberg method reduces the ratio of false null rejections to all false null rejections so there would be less false positives in general. We order the p-values from smallest to largest and calculate a new BH p-value for each that is  $(\text{position}*\alpha=0.05)/7129$  gene expressions. We then see the highest number position where the BH p-value is greater than the original p-value and use that position's BH p-value as the new cut off point. Any BH p-values at or below the BH p-value cut off would be significant at that significance level.

The (1) Bonferroni method tends to be stricter than the (2) Benjamini Hochberg method so (1) will return less significant gene expression than (2). This might be convenient if you're only interested in a few of thousands of gene expressions. I hope this was helpful.