Comparison of genetic and gene expression classifications in data generated by BostonGene and MD Anderson

Laura Hilton

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Background

This is an experiment to test and compare the performance of the gneSeqCOO model on RNAseq to that of NanoString. It will also evaluate our new gneSeqDZsig model. All of the comparisons will be performed in the validation cohort.

```
Normalizing count matrix...Done!
Predicting COO...Model not specified. Using the 21-gene GOYA model...
Predicting DZsig...Model not specified. Using the 29-gene GAMBL model...
Done!
```

gneSeqCOO vs DLBCL90 COO

```
         BG
BCC       GCB UNCLASS ABC
  GCB      38       3   0
  UNCLASS   5      18   2
  ABC       0       2  40
```

```
           gneSeqCOO_BCC
NanoString GCB UNCLASS ABC
   GCB      40       8   0
   UNCLASS   2      11   6
   ABC       0       1  34

           gneSeqCOO_BG
NanoString GCB UNCLASS ABC
   GCB      39       7   1
   UNCLASS   2      13   4
   ABC       0       0  35

           BGTP
NanoString GCB UNCLASS ABC
   GCB      48      10   0
   UNCLASS  10       9   1
   ABC       2       2  33

          BCC
MDA        GCB UNCLASS ABC
  GCB       20       1   0
  UNCLASS    1       4   0
  ABC        0       0   5
```

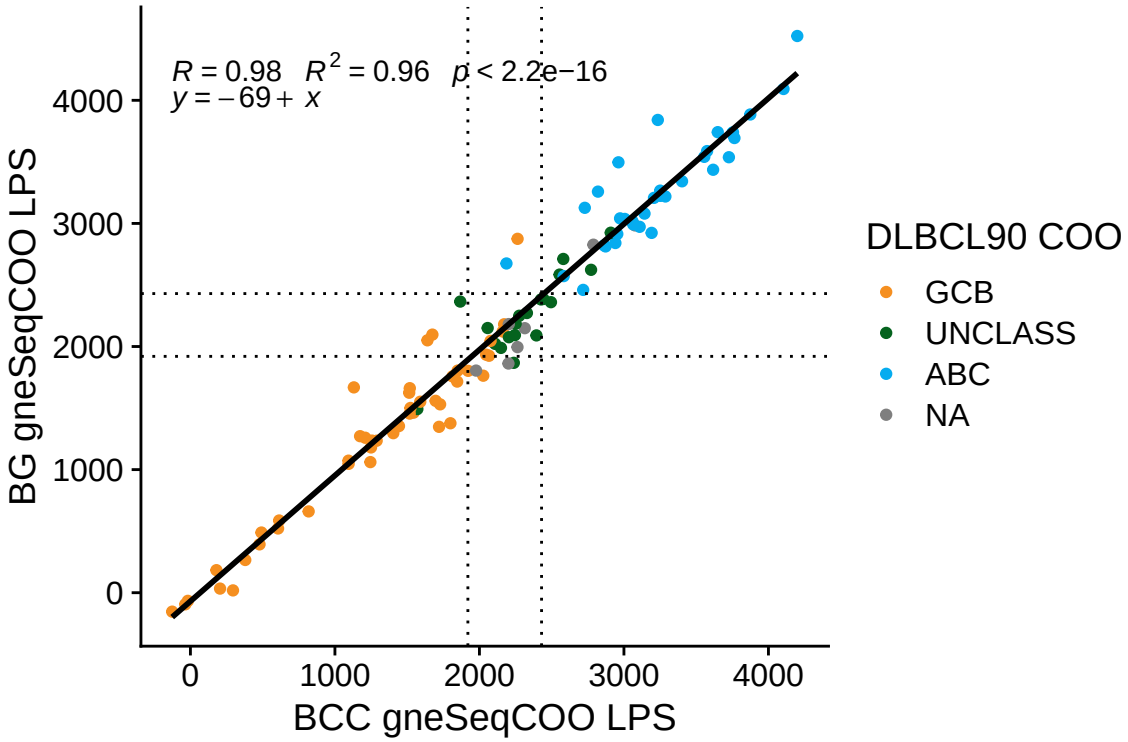

Figure 1: Comparison of COO classifications from gneSeqCOO at BCC and BG.

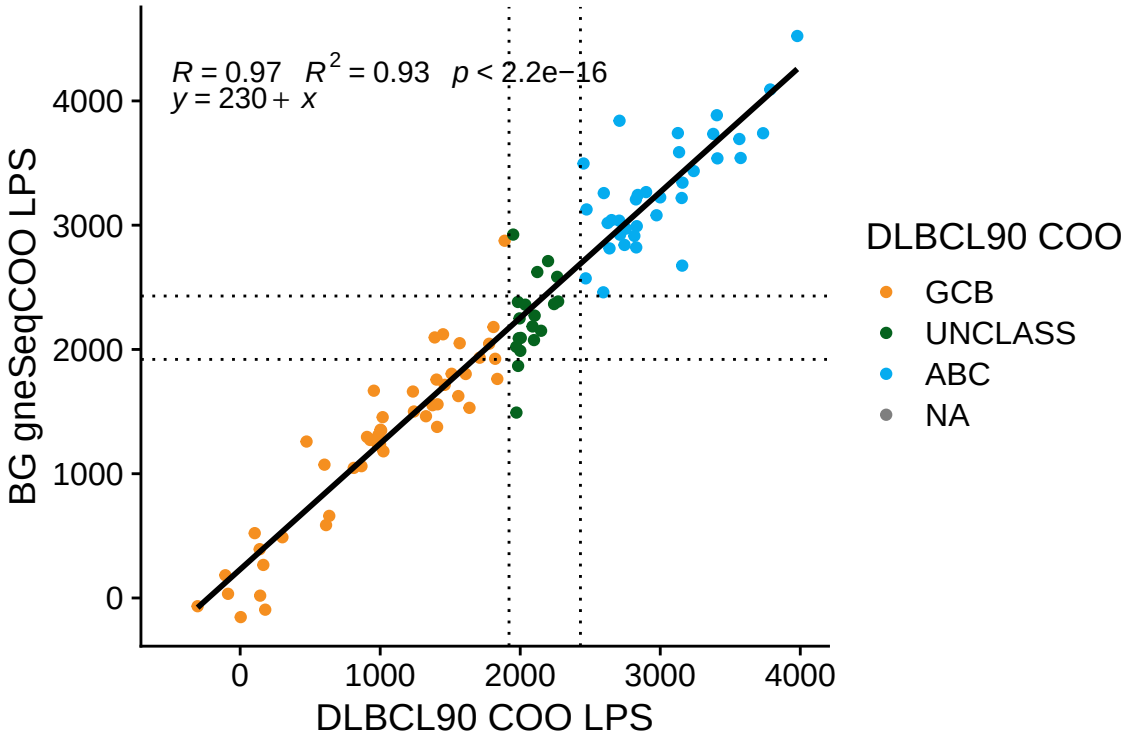

Figure 2: Comparison of COO classifications from gneSeqCOO and NanoString.

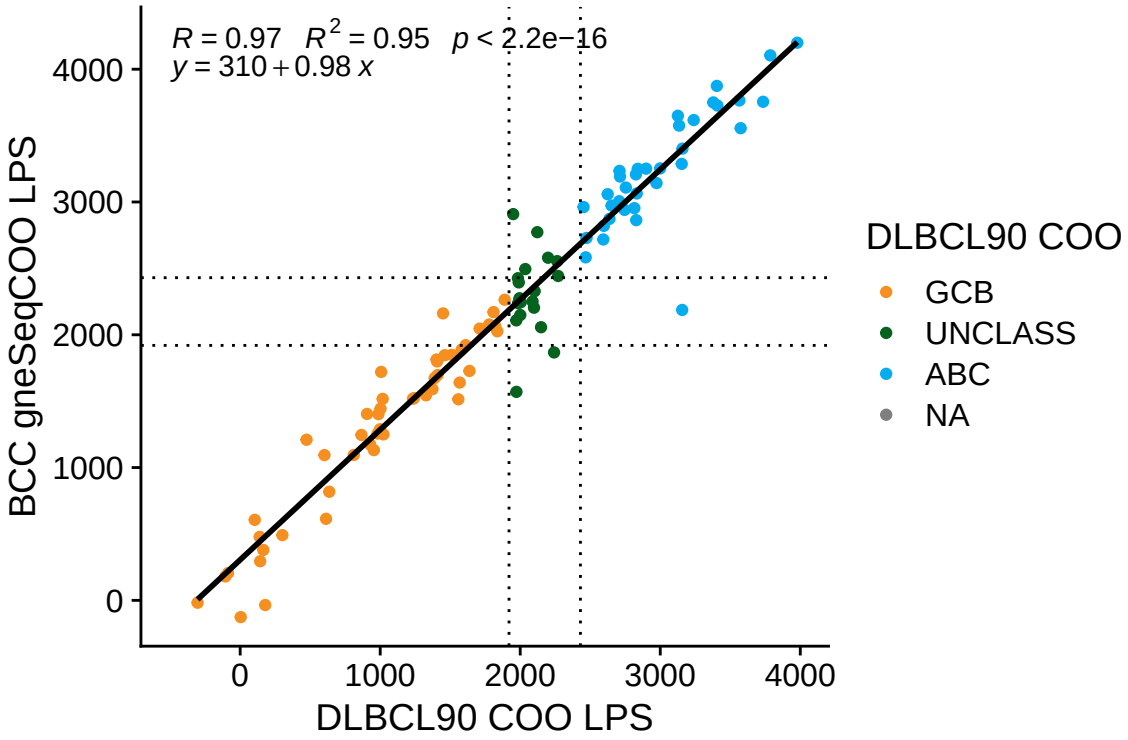

Figure 3: Comparison of COO classifications from gneSeqCOO (as run at BCC) and NanoString.

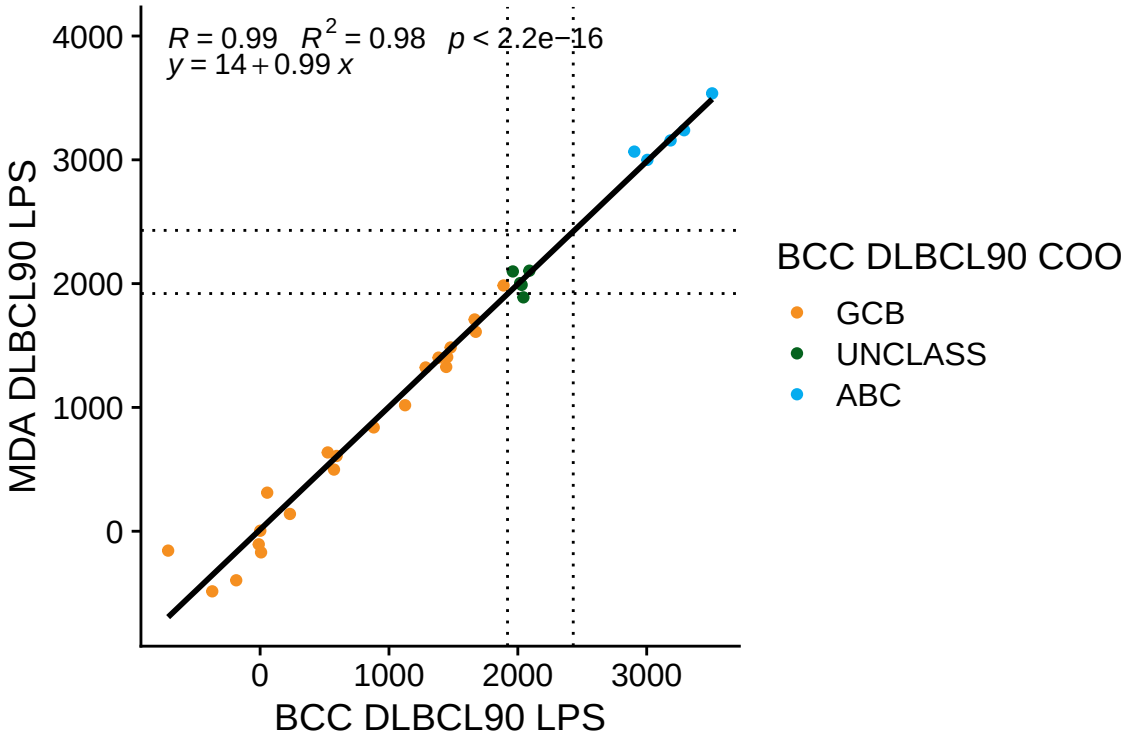

Figure 4: Comparison of COO classifications from DLBCL90 run at BCC and MDA.

Score correction

The gneSeqCOO LPS scores generated at BCC have a line of best fit with an intercept of 310 and a slope of 0.98. This is similar to the score shift observed for FFPE Ribodepletion samples from GAMBL, whereas fresh frozen samples have a smaller score shift (intercept of ~170-190). Here I will apply the same score shift correction determined from GAMBL (300) to see if this adequately corrects the score shift.

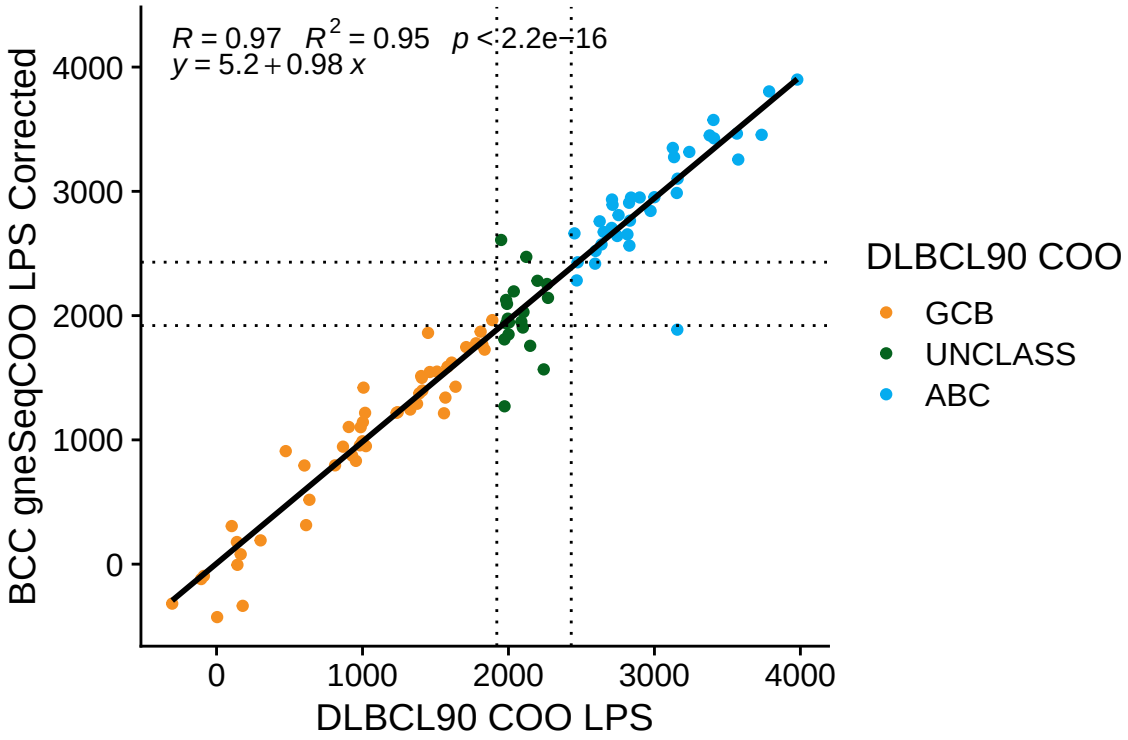

Figure 5: Comparison of COO classifications from score-corrected gneSeqCOO at BCC vs DLBCL90.

The accuracy of gneSeqCOO as run by BostonGene is 0.86 (95% CI 0.78 - 0.92), and as run by BCC (with score correction) is 0.87 (95% CI 0.79 - 0.93). Comparing the gneSeqCOO results generated at BCC vs BG, the accuracy is 0.81 (95% CI 0.72 - 0.88). A McNemar's test comparing whether the corrected gneSeqCOO scores from BCC are more accurate than the uncorrected scores shows no significant difference (P = 0.4795001). Therefore, while the score correction does align the scores better, it does not significantly alter the classification accuracy.

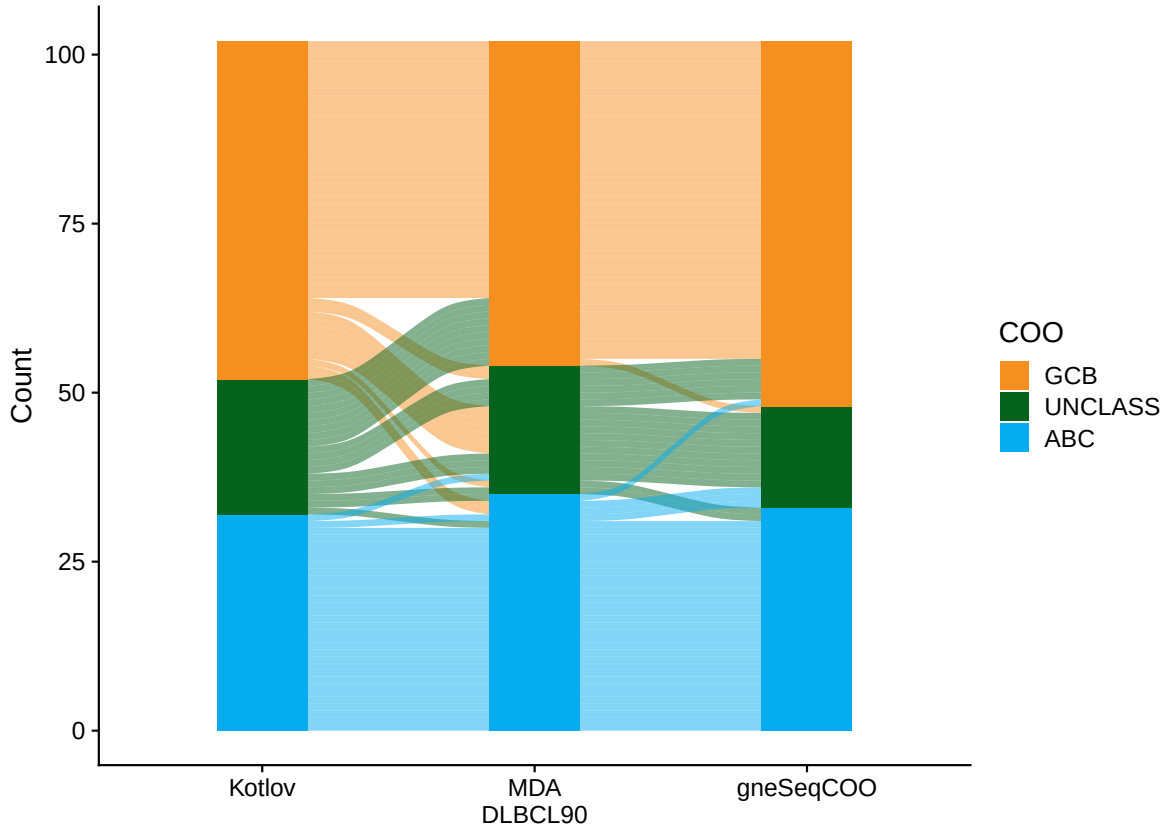

Figure 6: Alluvial plot comparing BGTP COO and NanoString classifications.

Table 1: Frank misclassification by gneSeqCOO relative to NanoString.

misclassified	n	percent
FALSE	78	98.734177
TRUE	1	1.265823

The frank misclassification rate (switching from ABC to GCB) is 1/75 (1.3%) when comparing gneSeqCOO (BG) to NanoString, or 0% when comparing gneSeqCOO (BCC) to NanoString or gneSeqCOO results run at BG vs at BCC.

gneSeqDZsig vs DLBCL90 DZsig

```
          gneSeqCOO_BCC
NanoString DZsig- UNCLASS DZsig+
   DZsig-      43       0      0
```

```
UNCLASS       0       18       0
DZsig+        0        0      23
```

```
                 BCC DLBCL90 DZsig
MDA DLBCL90 DZsig DZsig- UNCLASS DZsig+
          DZsig-      81       0      0
          UNCLASS      0      21      0
          DZsig+       0       0     23
```

Table 2

MDA_DZsig	DH_BCL2	n
DZsig-	FALSE	27
UNCLASS	FALSE	14
UNCLASS	TRUE	2
DZsig+	FALSE	7
DZsig+	TRUE	15

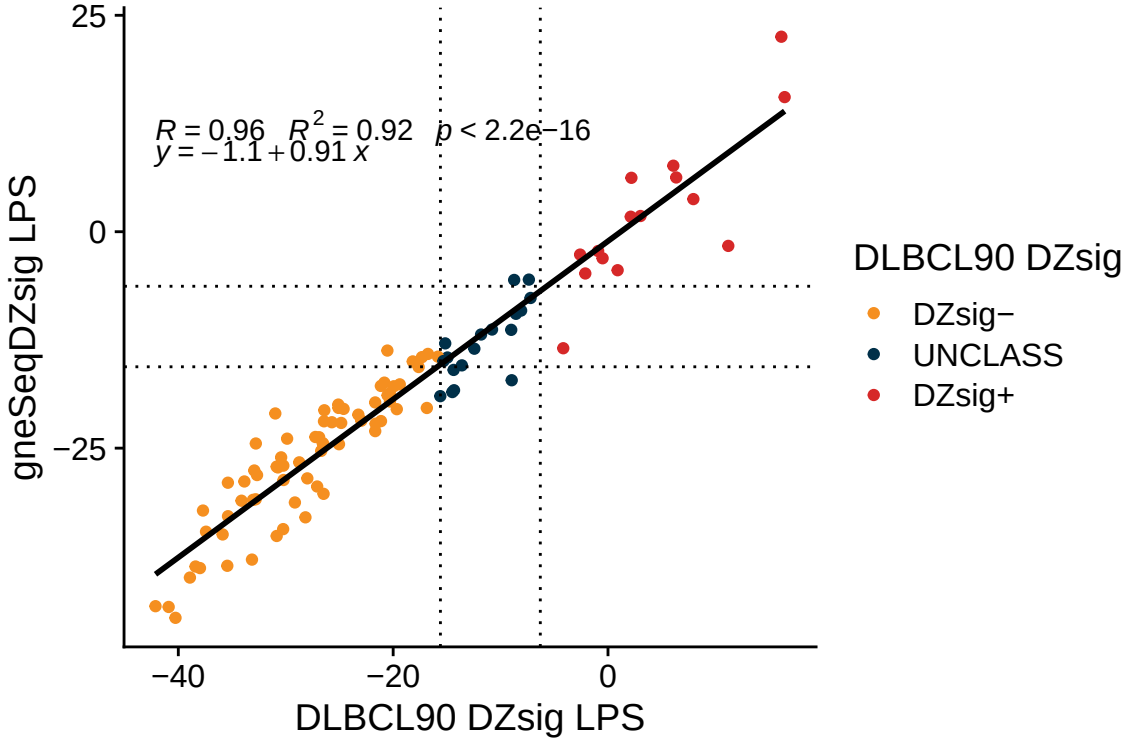

Figure 7: Comparison of DZsig classifications from gneSeqCOO and NanoString.

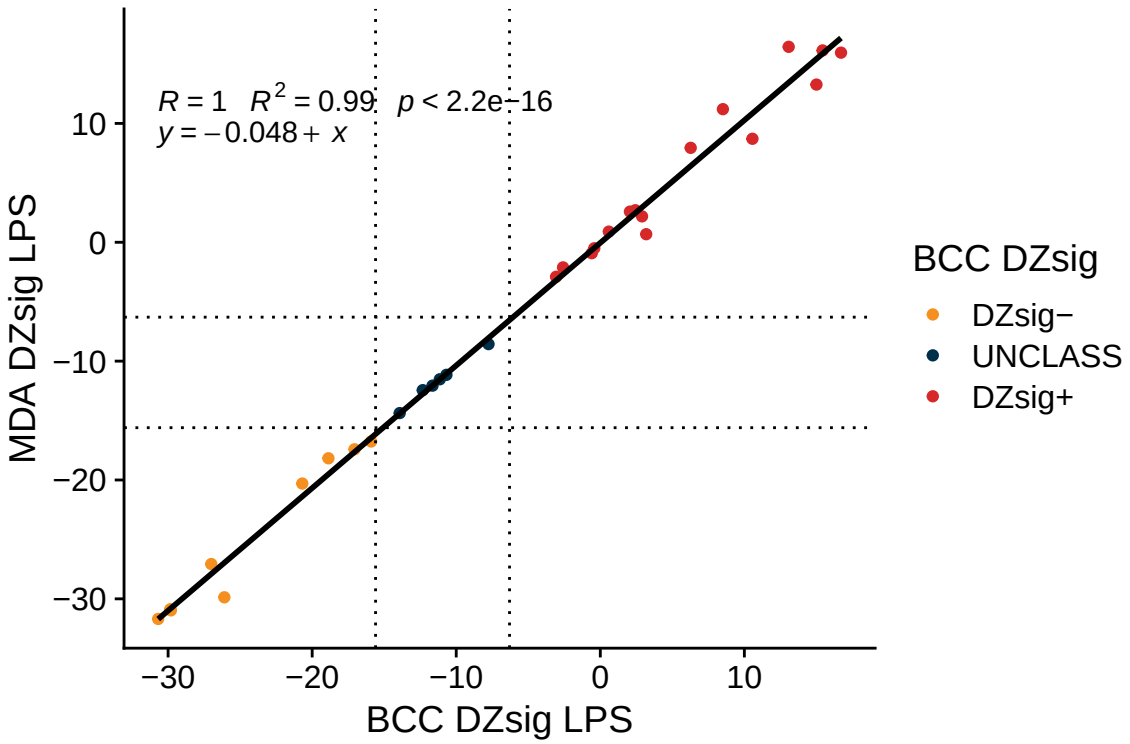

Figure 8: Comparison of DZsig classifications NanoString performed at BCC and MDA.

The accuracy of gneSeqDZsig among validation cohort tumors classified as ABC or UNCLASS by the DLBCL90 COO assay is 1 (95% CI 0.96 - 1). There were no frank mis-classifications (i.e. switching from DZsig+ to DZsig- or vice versa) when comparing the gneSeqDZsig results to the NanoString results.

LymphGen vs COO

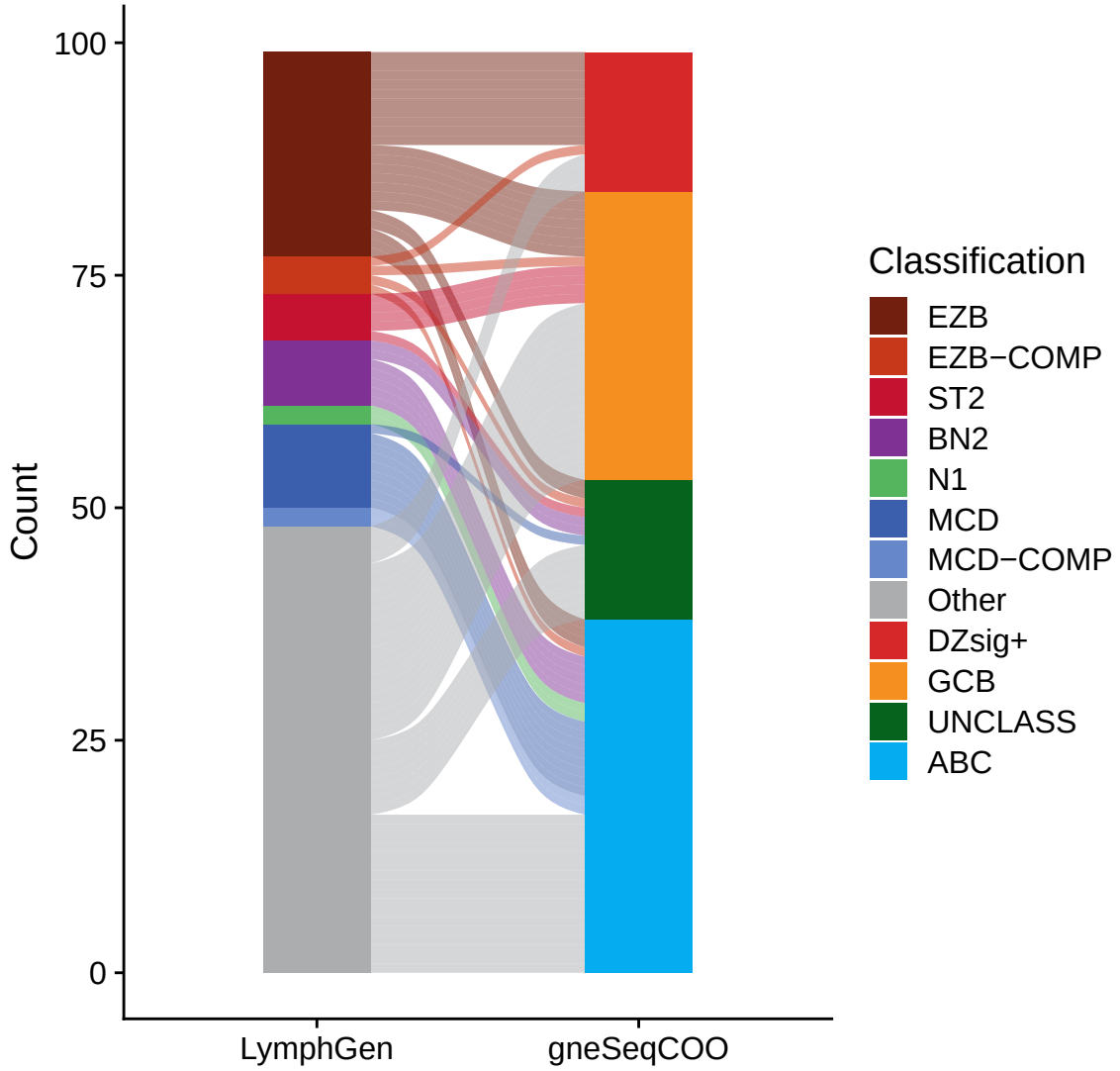

Figure 9: Alluvial plot comparing LymphGen and refined COO classifications from gneSeqCOO.

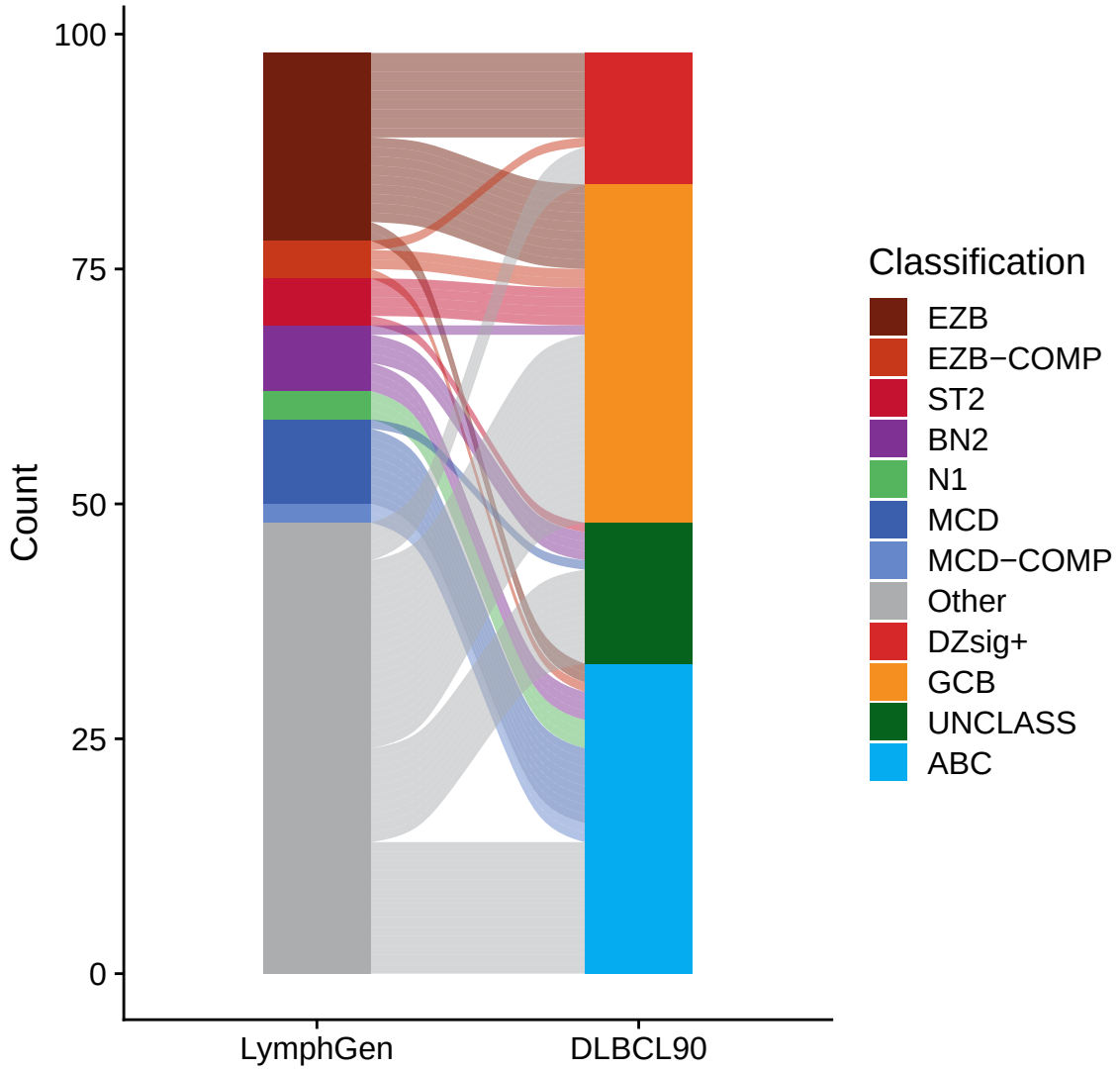

Figure 10: Alluvial plot comparing LymphGen and refined COO classifications from DLBCL90.