

ssPATHS: Single Sample PATHway Score (version 0.1.0)

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1 Introduction

Precision oncology requires that a single patient can be accurately and meaningfully characterized in order to tailor treatments. Using our method, ssPATHS, we are able to estimate pathway deviations for a single patient, by first learning a discriminative gene weighting from a reference cohort. In this vignette, we use TCGA gene expression data to learn a weighting on hypoxia-related genes then apply it to pancreatic cancer cell lines to estimate the level of hypoxia within each sample.

2 Formatting Data

First, we load the appropriate packages we will need.

```
> library("dml")
> library("data.table")
> library("ROCR")
> library("MESS")
> library("ggplot2")
> library("ssPATHS")
> library("plyr")
```

Next, we will read in the TCGA data and format it appropriately.

```
> data(tcga_expr_df)
```

Let's look at the format of our data. To learn our gene weights we will need a *Y* column and a *sample_id* column.

```
> tcga_expr_df[1:6,1:5]
```

	tcga_id	study	is_normal
1	TCGA-CQ-6224-01A-11R-1915-07	HNSC	FALSE
2	TCGA-TQ-A7RP-01A-21R-A34F-07	LGG	FALSE
3	TCGA-13-1510-01A-02R-1565-13	OV	FALSE
4	TCGA-HC-8265-01A-11R-2263-07	PRAD	FALSE
5	TCGA-HC-7079-01A-11R-1965-07	PRAD	FALSE
6	TCGA-4X-A9FC-01A-11R-A42C-07	THYM	FALSE

	libsize_75percent	ENSG00000078369
1	0.5201350	146190.89
2	0.4887488	105250.39
3	0.5159627	58773.63
4	0.3658994	101336.60
5	0.4617693	81393.47
6	0.3735174	101473.18

Since we are interested in hypoxia, we want to learn a weighting only on genes associated with hypoxia. In this package we have a helper function to retrieve these genes, but other gene sets can be used for different pathways of interest. Gene sets can be easily fetched using the package *msigdb*.

```
> hypoxia_gene_ids = get_hypoxia_genes()
> hypoxia_gene_ids = intersect(hypoxia_gene_ids, colnames(tcga_expr_df))
> hypoxia_df = tcga_expr_df[,c("tcga_id", "is_normal", hypoxia_gene_ids)]
```

Now we will need to identify how we want to discriminate our samples. Here, we use the assumption that normal samples are less hypoxic than tumor samples. Therefore, we will use the *is_normal* column as our *Y* column. We set normal samples to 0 and tumor samples to 1. This implies that a higher score indicates a more hypoxic sample.

```
> colnames(hypoxia_df)[1:2] = c("sample_id", "Y")
> hypoxia_df$Y = 0
> hypoxia_df$Y[tcga_expr_df$is_normal==TRUE] = 0
> hypoxia_df$Y[tcga_expr_df$is_normal==FALSE] = 1
```

3 Get Reference Gene Weightings

Now that our data is in the appropriate format, we can learn the weightings.

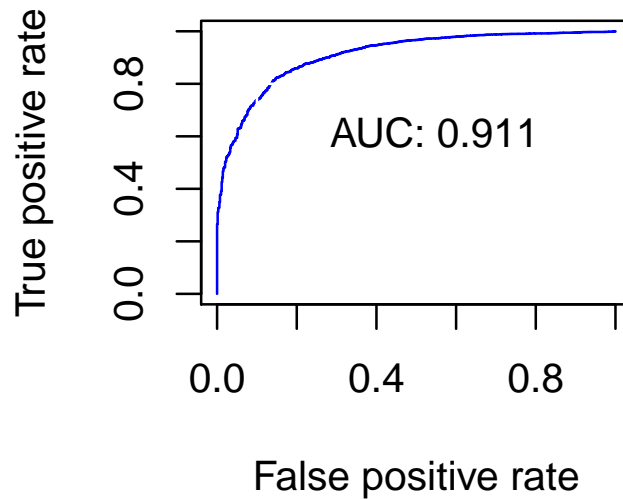
```
> res = get_gene_weights(hypoxia_df)
> gene_weights = res[[1]]
> sample_scores = res[[2]]
```

Now let's see how well we did in separating the two classes defined by *Y*.

```

> training_res = get_classification_accuracy(sample_scores, positive_val=1)
> # plot the ROC curve
> plot(training_res[[4]], col="blue", ylim=c(0, 1))
> roc_text = paste("AUC:", round(training_res$auc_roc,3))
> legend(0.1,0.8, roc_text,
        border="white",cex=1,box.col = "white")

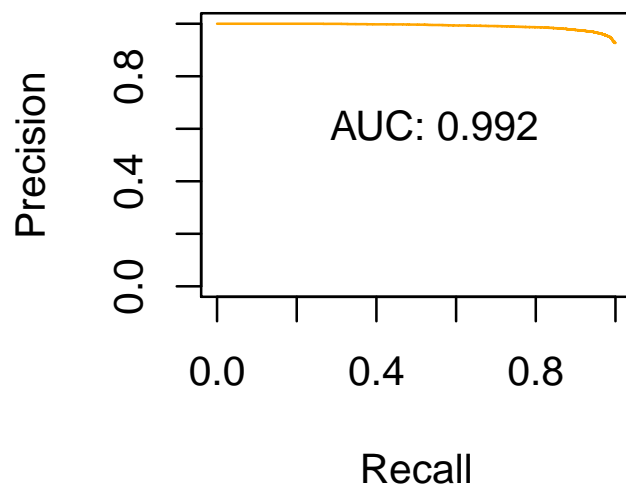
```



```

> # plot the PR curve
> plot(training_res[[3]], col="orange", ylim=c(0, 1))
> pr_text = paste("AUC:", round(training_res$auc_pr,3))
> legend(0.1,0.8, pr_text,
        border="white",cex=1,box.col = "white")

```



4 Apply Gene Weightings to New Samples

Now, using the gene weightings learned from the reference set, we can apply it to a new sample.

```
> data(new_samp_df)
> new_score_df = get_new_samp_score(gene_weights, new_samp_df)
> new_score_df
```

	sample_id	pathway_score
9	exp_norm_ctrl_C	-0.64176821
7	exp_norm_ctrl_A	-0.62781700
8	exp_norm_ctrl_B	-0.57268638
12	exp_norm_noHIF_C	-0.52251848
10	exp_norm_noHIF_A	-0.52041674
4	exp_hyp_noHIF_A	-0.50703636
5	exp_hyp_noHIF_B	-0.44738709
11	exp_norm_noHIF_B	-0.35926202
6	exp_hyp_noHIF_C	-0.31590814
3	exp_hyp_ctrl_C	-0.04498432
1	exp_hyp_ctrl_A	-0.02130225
2	exp_hyp_ctrl_B	0.12366948

Now lets see if the derived score match our experimental expectations. Samples with **hyp** or **norm** in the sample id are cell lines that were exposed to hypoxic or normoxic conditions respectively. Samples with **ctrl** or **noHIF** were samples that were able to produce a HIF-mediated hypoxic response or not, respectively.

```
> plot_scores <- function(hif_scores){

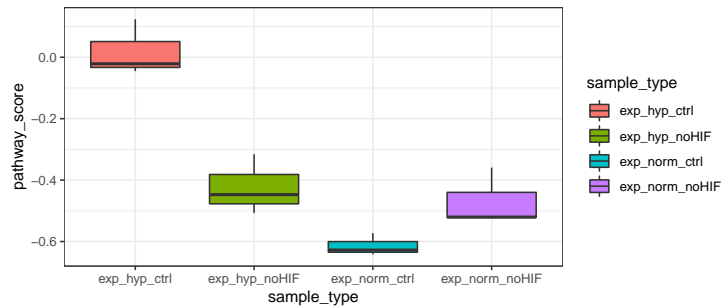
  # format the sample IDS
  hif_scores$sample_type = substr(hif_scores$sample_id, 1,
                                  nchar((hif_scores$sample_id))-2)
  colnames(hif_scores)[2] = "pathway_score"

  gg = ggplot(hif_scores, aes(x=sample_type, y=pathway_score,
                              fill=sample_type)) +

    geom_boxplot() +
    theme(axis.text.x = element_text(angle = 90, hjust = 1)) +
    theme_bw()

  return(gg)

}
> gg = plot_scores(new_score_df)
> print(gg)
```



Accordingly, we find the the **hyp_ctrl** samples have the highest pathway score. According to our labeling (tumor/hypoxic is 1 and normal/normoxic is 0), this implies that these samples are the most hypoxic. Furthermore, we see that the samples that were not able to produce a hypoxic response, even in the absence of oxygen (**hyp_noHIF**) are found to have similar score to the normoxic samples.