Auto-Annotation of Cell Types

Midterm Project – STAT 454 / 556

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Real-world problem and its importance

- In analyzing single-cell RNA sequencing data, it is crucial to discover what cell types exist within a particular sample. This process is often the first step in advancing our knowledge of cellular dynamics [1], however, manual annotation is partially subject to misinterpretation [2] or other biases, and it can be very time consuming.
- Various methods have been developed to associate gene expression data of single cells to a cell type; one can access curated marker gene databases [3] [4], correlate the gene expression profiles between samples [2], and more recently, transfer labels with supervised classification.
- This work evaluates the performance of several classification models for cell type annotation using gene expression profiles and the disease status of patients as predictors.

https://doi.org/10.1016/j.csbj.2021.01.015 [1]
https://doi.org/10.1165/rcmb.2017-0430TR [2]
https://doi.org/10.1093/nar/gky900 [3]
https://academic.oup.com/nar/article/47/D1/D721/5115823 [4]

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Exploratory data analysis

Summary of variables in our dataframe:

1. Predictors: column `condt` [4954 obs.], containing a binary status marker for 'control' or 'ILD'; column `count`, a table with entries of type double [18339 x 4954 obs.].
2. Labels: `celltype` [4954 obs.] which has 5 classes of type character, as denoted in the table below.
3. `count`: only 120 thousand unique values in `count` (of over 91 million cells); a 0.13% density of input types. Over 81 million zeroes, & over 9 million positive continuous values, a 10% input density.
4. `count` has 18391 genes as column labels, e.g. 'FAM87B', and as sample labels there are 4954 6-character labels with various nucleobase sequences appended to them, such as 'F00409_AAGACCTCAAAGGTGC'.

Cell Type	*Control*	*ILD(sample%)*	**#c.typ.**	*0's in 'count'*	*ILD 'count' 0's*
AT1	472	299 (39%)	771	710 (92%)	279 (93%)
B Cells	140	857 (86%)	997	890 (89%)	771 (90%)
Basal	65	1993 (97%)	2058	1844 (90%)	1785 (90%)
Fibroblasts	140	287 (67%)	427	385 (90%)	258 (90%)
Mast Cells	254	447 (64%)	701	630 (90%)	403 (90%)
Total	1071	3883 (78%)	**4954**	4459 (90%)	3496 (90%)

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More EDA & Feature Selection

1. We removed 1,031 all-zero columns from `count` before conducting Kruskal-Wallis (H) one way ANOVA, a non parametric method, the statistic is found via

$$H = \frac{12}{n(n+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(n+1)$$

   where R_i is the sum of ranks of sample i, n_i is the size of sample i, N is the sum of sample sizes, and k is the number of samples – tied observations are averaged.

2. We arbitrarily used a p-value cutoff of 0.005, leaving 1,645 predictors.
3. Then adopted a correlation cutoff of 0.25, to avoid collinearity within the 1,645 predictors, leaving 1,065 predictors.

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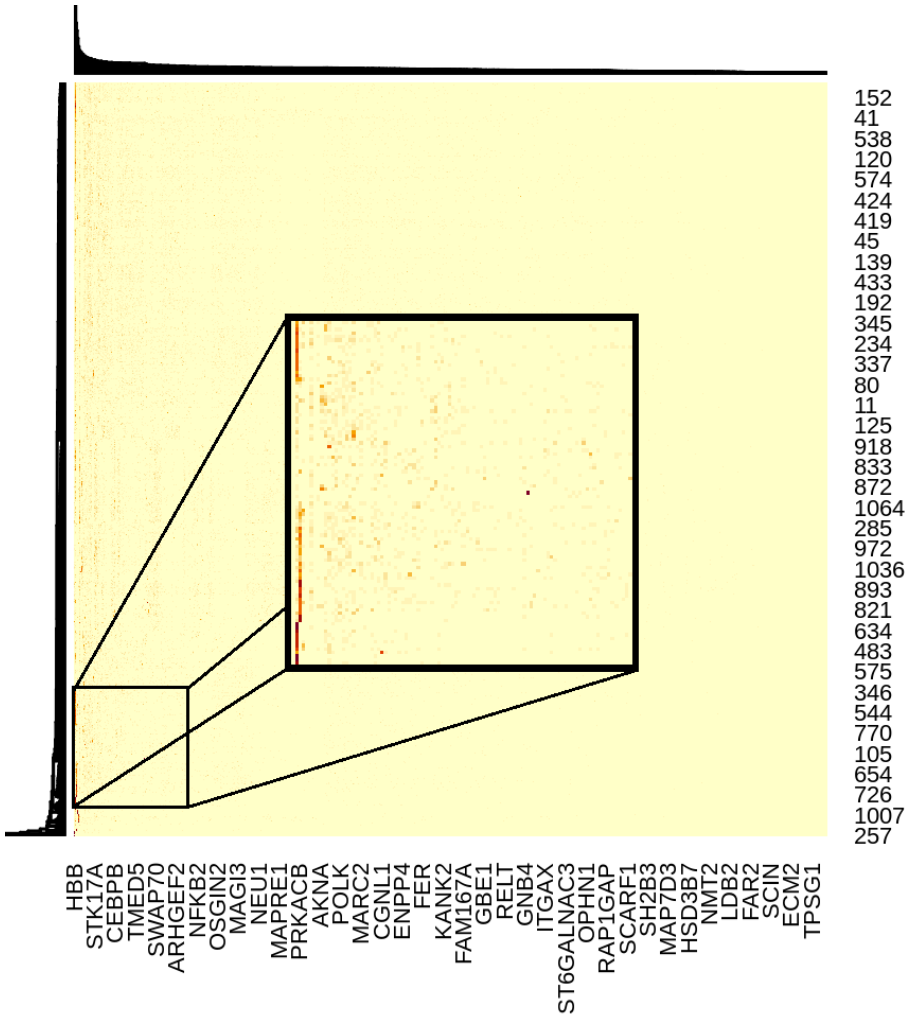
Heat map - Selected features

Due to high dimensions and correlation cutoff, we zoom in on a smaller portion to demonstrate internal behaviour.



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Methodology and Models

- Job submitted to Compute Canada for 10-fold Cross Validation 100 times.
- Time to run was 20.1 hours on 64 cores with 8Gb memory per cpu.
- In `R`, we ran the code in parallel using `foreach` and `doParallel` packages with file backed matrices from `bigstatsr` to fit the following models

1. Lasso (L1 penalized linear regression)
   `cv.glmnet(...,` α `= 1, family = 'multinomial')`
2. 5 Elastic Nets
   `cv.glmnet(...,` α `= c(0.1,0.3,0.5,0.7,0.9), family = 'multinomial')`
3. LDA (Linear Discriminant Analysis) – `MASS::lda()`
4. Classification Tree by `rpart()`, performing `rpart::prune()` to trim the tree with a complexity parameter chosen for parsimony, that is, for the simplest model (tied) within one standard error of the achieved minimum.

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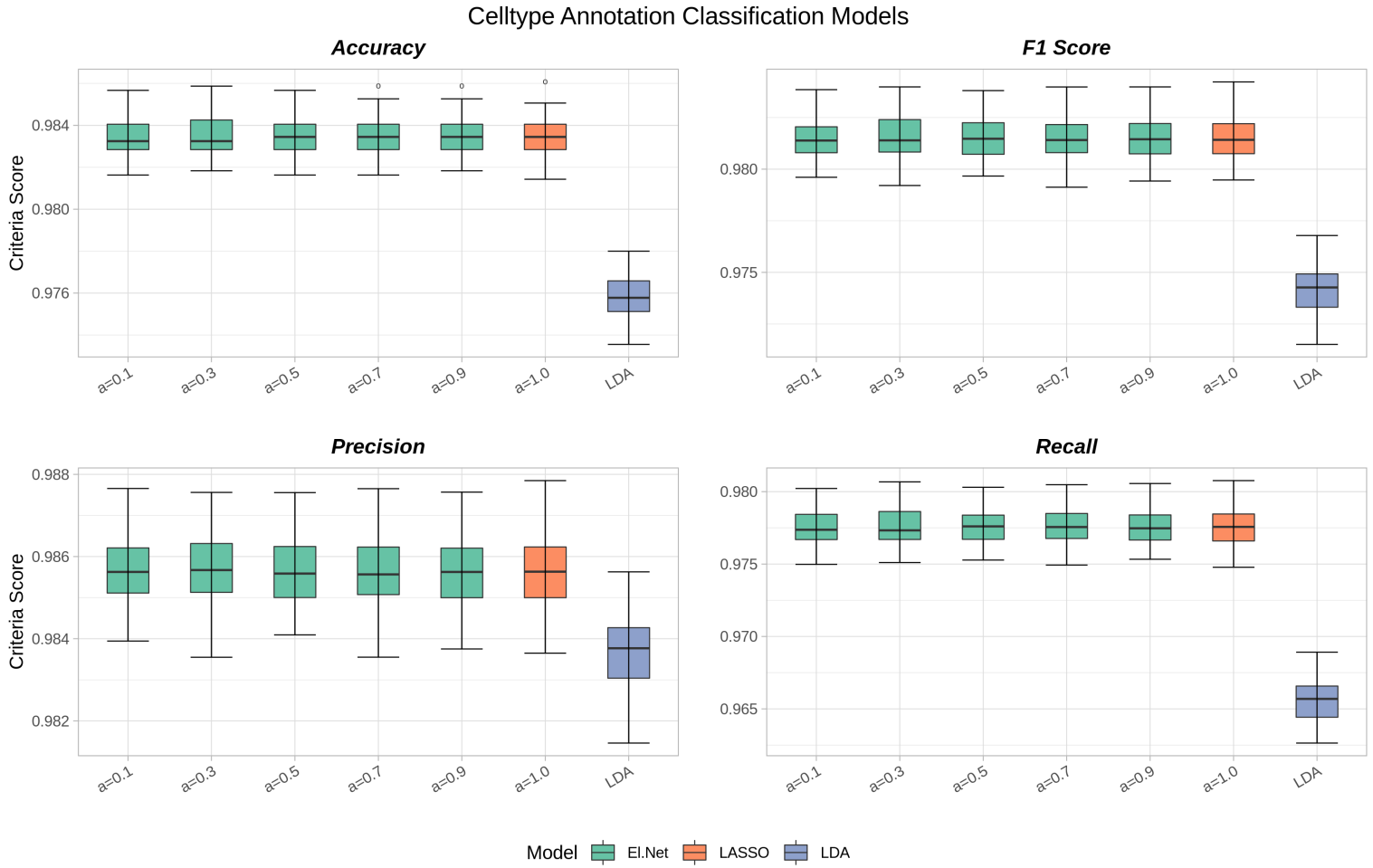
Visualization of Results



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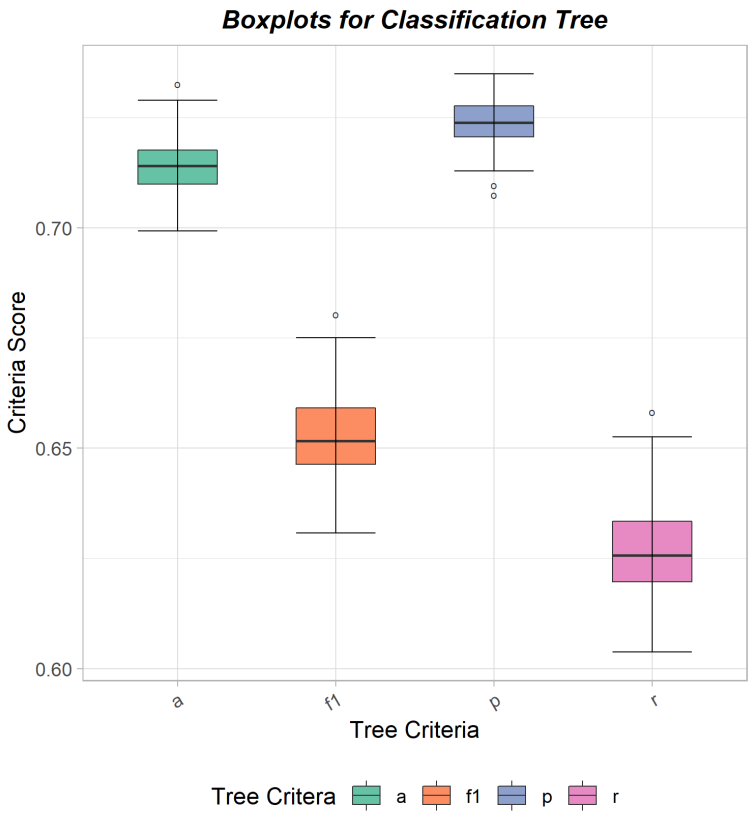
CLTree left off due to poor performance, see next slide.

Results (*cont.*)



Boxplot Recap:

- Elastic net with $\alpha = 0.3$ generally performed best (on 3/4 metrics), scoring near 0.98 or above in all metrics.
- LDA underperformed Elastic Net and LASSO (the latter on par with Elastic Nets).
- Pruned classification tree significantly the worst fit accross all models and scores (0.6-0.7 on criteria).

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Performance metrics of the models

	Model	$\overline{Accuracy}$	p-value	Model	$\overline{Precision}$	p-value	Model	$\overline{Recall}$	p-value	Model	$\overline{F_1}$ score	p-value
1.	a=0.3	0.983496	–	a=0.3	0.985696	–	a=0.5	0.977623	NA	a=0.3	0.981525	–
2.	a=0.5	0.983488	9.19075e-01	a=0.9	0.985670	5.45509e-01	a=0.9	0.977597	6.41415e-01	a=0.5	0.981521	8.51871e-01
3.	a=0.9	0.983482	8.81943e-01	a=0.1	0.985669	7.05568e-01	a=0.3	0.977591	4.82887e-01	a=0.9	0.981516	9.14037e-01
4.	a=0.7	0.983474	4.53733e-01	a=0.5	0.985654	3.61394e-01	a=0.7	0.977577	4.79488e-01	a=0.7	0.981492	3.72198e-01
5.	a=0.1	0.983449	5.25935e-01	a=0.7	0.985643	1.29808e-01	a=1.0	0.977537	2.58360e-01	a=0.1	0.981482	5.17190e-01
6.	a=1.0	0.983447	4.71160e-01	a=1.0	0.985637	1.51089e-01	a=0.1	0.977532	1.73686e-01	a=1.0	0.981468	3.10247e-01
7.	LDA	0.975801	3.89281e-18	LDA	0.983682	3.58860e-17	LDA	0.965502	3.95591e-18	LDA	0.974115	3.95591e-18
8.	TREE	0.714184	3.94877e-18	TREE	0.724120	3.95591e-18	TREE	0.626545	3.95591e-18	TREE	0.652554	3.95591e-18

- The p-values were found between best model and other models, i.e., all compared to best model.
- Lasso and some Elastic Nets are not significantly better among themselves, but there is statistical evidence that they were better them LDA and Classification Tree.
- Tree has poor recall, bringing down its F_1 score.

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Conclusions

- The analysis of data from genetic cell expressions is currently a ‘hot’ research topic, with cell type annotation the starting point for many data analyses.
- We performed best subset selection of features by Kruskal-Wallis (1645) and low correlation-cutoff of 0.25 left 1065 predictors in this study.
- Then examined the performance of LASSO, Elastic Net, LDA and Classification Tree models, using genetic profiles and patients health status as predictors.
- Our application of a pruned Classification Tree did not net comparable results on this data. LDA was consistently the penultimate.
- The best model was the Elastic Net with $\alpha = 0.3$, having accuracy of 0.983 and F_1 of 0.982.
- The Elastic Nets with different parameters have comparable performance (including LASSO).

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