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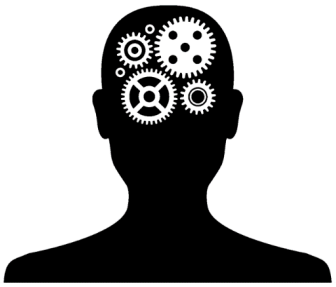
Volume 8 Issue 1 2023



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LETTER FROM THE EDITORS

NSURJ is excited to present:

Volume 8 Issue 1 Spring 2023

The Nevada State Undergraduate Research Journal (NSURJ) was established in 2014 by the Associated Students of the University of Nevada (ASUN) to offer undergraduate students in the higher education system of Nevada the opportunity to demonstrate their intellectual merit by publishing their outstanding work in a unified, scholarly, peer-reviewed journal. NSURJ strives to provide an accessible, peer-reviewed journal specifically for undergraduate students in all NSHE institutions, showcasing the quality of a Nevada education and professionalizing students' education and experiences through the important milestone of publication. The journal creates scholarly connection between peers and mentors creating an allowance for professional development in public speaking, research, and academic branching.

Volume 8 marks 9 years since the publication first volume of the journal, signaling continuous growth and support from Nevadan Undergraduates. Volume 8 marks another opportunity for undergraduate researchers to develop pre-professional academic careers while giving a voice to their original research.

In 2021, NSURJ officially became a unit of Undergraduate Research under Research and Innovation. Growth and communication between researchers of all disciplines and those who value and want to publish their work was greater than it has been in the past. Researchers were offered workshop experiences to further their success in publication writing, while also gaining experience in what will be expected of them in less pre-professional formats.

Students demonstrated excellence in their academic merit, shining a bright light at the end of the pandemic that threatened life on Earth just three years ago. The continuation of this journal could not have been possible without the amazing work brought on by those students who prevailed in research beyond impossible odds. The editors of this edition could not be more grateful to those who supported the undergraduate researchers and those who supported the journal itself.

The fate of NSURJ is in the hands of the students, faculty, and those who make undergraduate research possible. This is a journal led by the students for the students, and as the senior editors lay the journal in the hands of our predecessors we must remind our supporters the importance of that which is the growth of undergraduate research and the connections made between editors and researchers.

Sincerely,

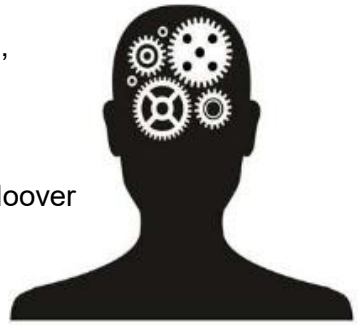
Co-Senior Editors

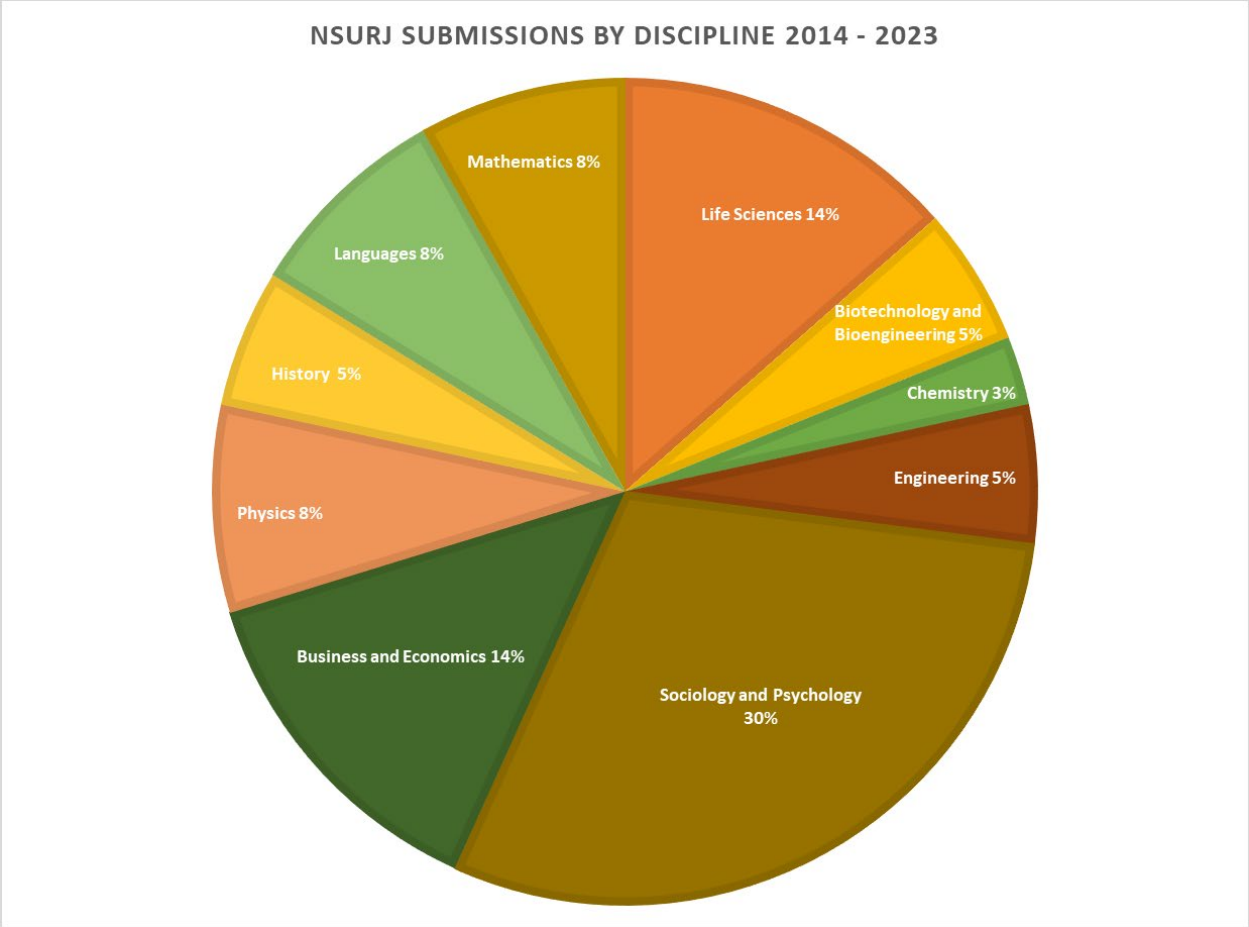
Pantera Kivisto, Lucy Burnham,

& Jeremy Guevin

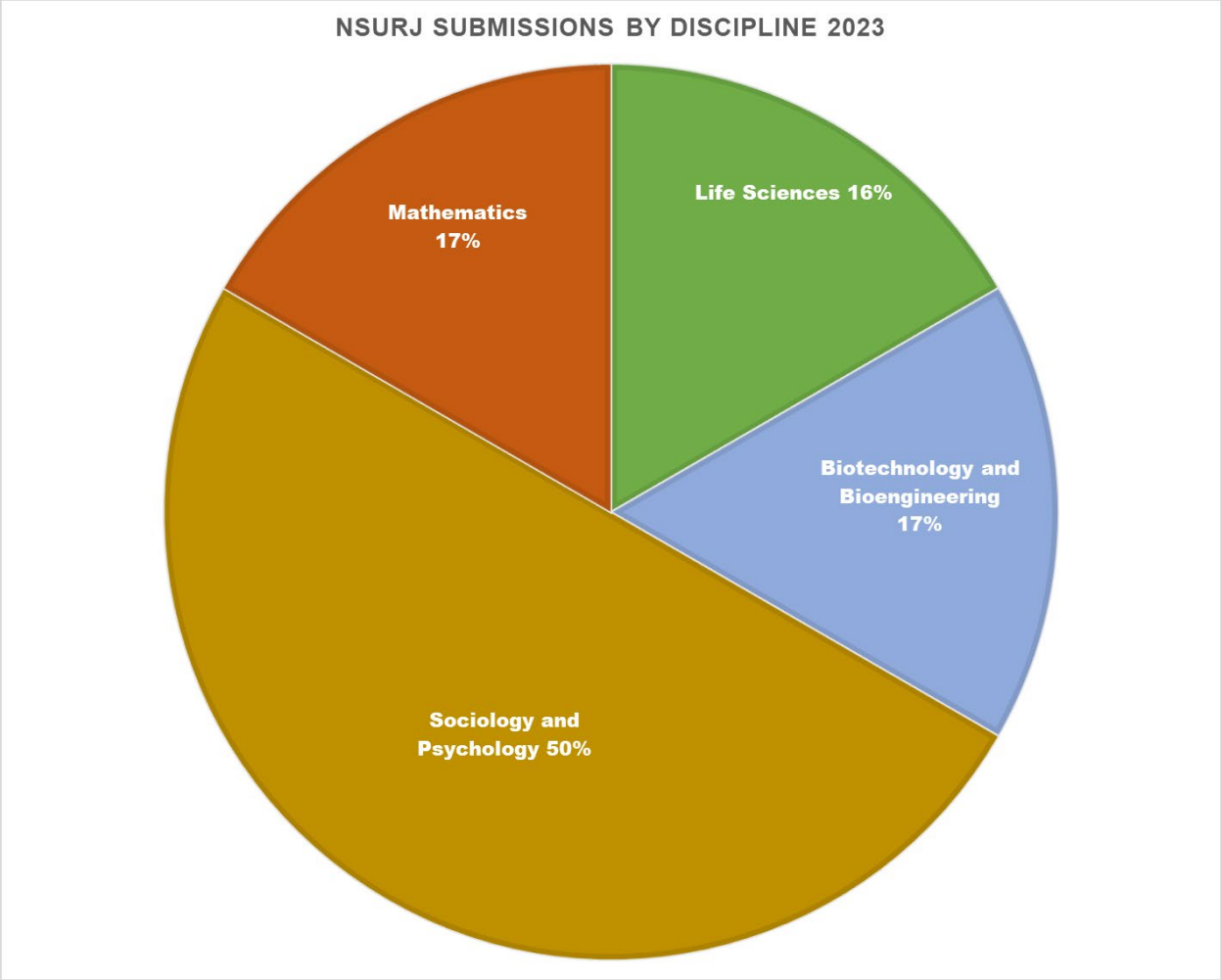
Co-Junior Editors

Jaiden Christopher & Anders Hoover





Statistics 1. NSURJ Submissions by Discipline 2014-2023. NSURJ initially published its first edition in 2014 featuring a wide range of interdisciplinary authors. Since then, editors have been keeping track of each discipline featured in every edition. NSURJ continues to fulfill its role as an interdisciplinary publication.



Statistics 2. NSURJ Submissions by Discipline 2023. NSURJ continues to fulfill its role as an interdisciplinary publication. Edition 8 successfully features at least four disciplines under umbrella identifications.

Application of Machine Learning in Clear Cell Renal Cell Carcinoma Prediction

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Abstract

Clear cell renal cell carcinoma (ccRCC), the most prevalent kidney cancer, is one of the leading causes of cancer-related deaths. Proteomics, which is the study of proteins, can provide biological insights about the development of ccRCC tumors that genomics alone cannot provide. Such insights may enhance our ability to effectively treat ccRCC patients. Here, the potential of proteomics in ccRCC was further explored. Specifically, machine learning models (e.g., Extreme Gradient Boosting, Random Forest, and two Support Vector machines) were built that classify tissue samples as ccRCC tumor tissue or normal tissue using proteomics data. The proposed machine learning models performed very well in differentiating between ccRCC tumor tissue vs. normal tissue with their accuracies of nearly 98%. Despite the limitations of our study (e.g., small sample size, correlation), we believe that the study demonstrated the potential of proteomics in ccRCC tumor predictions.

Introduction

Renal cell carcinomas (RCCs) encompass a diverse set of cancers, not all entirely related to one another, with differing genetic causes and widely varying treatment methods and outcomes. (Linehan et al., 2003; Jonasch et al., 2014) Some of the most common cancers classified as renal cell carcinomas include Papillary RCC, Chromophobe RCC, and Clear Cell RCC (ccRCC). (Jonasch et al., 2014) Of these diseases, ccRCC is the most prevalent, constituting 70% of cases of all kidney cancers, not exclusive to carcinomas. (American Cancer Society, 2013) Not only is ccRCC the most prevalent of all kidney cancers, but it is also one of the leading causes of cancer-related deaths. (Hsieh et al., 2017)

Common treatments for ccRCC include cytotoxic chemotherapies. However, these chemotherapies often face disease resistance, of which the underlying causes are still largely not understood, leading to poorer outcomes in many cases. (Diamond et al., 2015) In order to better understand the role of genetics in both the causes and relative successes of treatments of ccRCC, previous studies have sought to investigate the genetic causes through extensive genomic sequencing of ccRCC tumors and analyses. (Hsieh et al., 2018) Despite thorough efforts, a full understanding of genetic mechanisms leading to ccRCC is still not fully grasped. (Nargund et al., 2017) With the gap of understanding from a purely genomic point of view in ccRCC, other studies have proposed to include proteomic data to help bridge the gap. By combining proteomic, phosphoproteomic, genomic, epigenomic, and transcriptomic analyses, new understandings and observations into ccRCC have been made, furthering the understanding of the functioning of the disease and helping to provide a path for discovering more effective treatments. (Clark et al., 2019)

To further illustrate the unique and important role of proteomic data in the study of ccRCC, in this study we seek to identify tissue samples as ccRCC tumors or normal tissue through purely proteomic means. By demonstrating the ability of only proteomic data to identify the presence of ccRCC tumors, we further validate the importance

of the inclusion of proteomics data in the study of ccRCC.

Methods

Data

The dataset contains a total of 194 tissue samples from ccRCC patients. (Clark et al., 2019) Of those 194 samples, 110 were from ccRCC tumors and 84 were from normal tissues adjacent to the tumors. The collected ccRCC tumors had not been exposed to any treatment at the point when the samples were collected. From these samples, the relative abundances of 4,483 proteins were obtained. All these protein abundances were used as predictor variables in all of the models.

Models

Three different baseline models were constructed to predict whether the sample was sourced from a ccRCC tumor or from normal tissue. All three models were penalized linear regression models, specifically, Lasso, Ridge, and Elastic Net Regression.
Four new machine learning models were proposed to predict the presence of a tumor sample:

- Extreme Gradient Boosting
- Random Forest
- Support Vector Machine (Linear Kernal)
- Support Vector Machine (Radial Kernel)

Previous studies predicting other information relating to ccRCC, specifically the risk of death and the Fuhrman grade of ccRCC, using imaging, have found success using these types of models, suggesting potential applicability to their usage in other ccRCC prediction tasks. (Nazari et al., 2021; Lin et al., 2019)

Training

The data set was split into a training set (80%) and a testing set (20%). All decisions affecting the performance of the model were made with the training set, and the only usage of the testing set was purely for the evaluation of the completed models.

Lasso and Ridge Regression

Lasso and Ridge Regression are both forms of penalized regression, where the coefficients of a linear model are scaled to improve model performance. In Ridge Regression, none of the coefficients can ever be completely scaled to zero eliminating them from the model, as opposed to Lasso Regression where coefficients can be removed from the model by scaling them all the way to zero.
To train both Lasso and Ridge Regression models, the training set was used in a five-fold cross validation to tune the value of the parameter, responsible for controlling the amount of scaling to the coefficients in the model. After finding the optimal value of this parameter through tuning, the final model was constructed using the entire training set and subsequently evaluated using the testing set.

Elastic Net Regression

Elastic Net Regression combines the penalization properties of both Lasso and Ridge Regression and introduces an additional parameter that controls the mix between the two types of regression. To train the Elastic Net Regression model the training set was used in a five-fold cross validation to tune the parameters. After finding the optimal parameter values, the entire training set was used to create the final Elastic Net Regression model which was then evaluated with the testing set.

Random Forest

The Random Forest model was built by tuning both the number of variables to select from in each round and the number of trees in the model. To tune the model, the entire training set was used, and the Out of Bag (OOB) error was used to evaluate each potential model. After finding the optimal set of parameters within the search space for the parameters, the entire training set was

used to build the final model and then the model was evaluated using the testing set.

Support Vector Regression

A Support Vector model seeks to find a hyperplane in a p-dimensional space which can best divide the data into outcome categories where p is the number of predictor variables. Support Vector models were trained with either a linear kernel (also known as a Support Vector classifier) or a radial basis kernel. For a Support Vector model with a radial basis kernel, the parameter that determines non-linearity was set to be 1 divided by p by default where p is the number of parameters. For both models, five-fold cross validation was used to tune the cost parameter based upon model accuracy. After finding the optimal value for the cost parameter, the entire training set was used to build the final model and then evaluated on the testing set.

Extreme Gradient Boosting

Extreme Gradient Boosting models have a large number of parameters that can be tuned. The following six parameters in the xgboost package were tuned:

- Gamma
- Child_weight
- Max_depth
- Subsample
- Col_subsample
- ETA

The training set and five-fold cross validation were used to tune these parameters. After completion of tuning, the entire training set was used to build the final model which was then evaluated with the testing set.

Software and Reproducibility

All of the discussed models were produced in R (R Core Team, 2021) using the following packages:

- glmnet (Friedman et al., 2010)
- caret (Kuhn, 2021)
- ROCR (Sing et al., 2005)
- e1071 (Meyer et al., 2021)
- xgboost (Chen et al., 2021)
- ranger (Wright & Ziegler, 2017)

Additionally, in any process that involved randomness, the seed value was fixed at 37 to ensure the ability to reproduce results found in this study. The code can be found at https://github.com/soyoungryu/TumorPrediction.

Model Evaluation

In order to be able to fairly and accurately evaluate all of the models, all decisions regarding the construction and choice of parameters for all models were based solely upon observations of the training set. Consequently, all model evaluation was then performed using the same testing set. Given that the models were predicting a binary outcome, evaluation metrics for classification were used. First, a confusion matrix was defined for the data. A confusion matrix allows for classification of different types of errors possible in a model with a binary outcome, such as incorrectly identifying a tumor sample as normal tissue as compared to incorrectly identifying normal tissue as a tumor sample. In the confusion matrix, a tumor sample was defined as a “positive” outcome and a normal tissue sample as a “negative” outcome, resulting in the following construction of a confusion matrix:

Table 1
Definition of Confusion Matrix

	Actual Positive	Actual Negative
Positive Prediction	True Positive (TP)	False Positive (FP)
Negative Prediction	False Negative (FN)	True Negative (TN)

With the confusion matrix defined, definitions for model evaluation based upon the definitions provided in the confusion matrix could be defined. Accuracy (ACC) was defined as the total number of true positives and true negatives divided by the total number of observations, as follows:

$$ACC = \frac{TP + TN}{TP + TN + FP + FN}$$

Sensitivity, also called True Positive Rate (TPR) was defined by the total number of true positive predictions divided by the sum of true positive predictions and false negative predictions. Conversely, specificity, also called True Negative Rate (TNR) was defined by the total number of true negative predictions divided by the sum of true negative predictions and false positive predictions. Both are given as follows:

$$TPR = \frac{TP}{TP+FN} \qquad TNR = \frac{TN}{TN + FP}$$

Additionally, the positive predictive value (PPV) and the negative predictive value (NPV) were calculated for all the models. Positive predictive value was defined by the number of true positive predictions divided by the sum of true positive predictions and false positive predictions. Negative predictive value was defined as the number of true negative predictions divided by the sum of true negative predictions and false negative predictions. The definitions for positive predictive value and negative predictive value are shown below, respectively:

$$PPV = \frac{TP}{TP + FP} \qquad NPV = \frac{TN}{TN + FN}$$

The false positive rate (FPR) as well as the false negative rate (FNR) were also calculated. False positive rate was defined by the number of false positive predictions divided by the sum of false positive predictions and true negative predictions. False negative rate was defined by the number of false negative predictions divided by the sum of false negative predictions and true positive predictions. Both were defined as shown below, respectively:

$$FPR = \frac{FP}{FP + TN} \qquad FNR = \frac{FN}{FN + TP}$$

The F_β scores for the models were also calculated. This score was used to measure the accuracy of the model, where the value of β can be altered to stress the importance of positive predictive value over true positive rate and vice versa. The F_β score was calculated as follows:

$$F_\beta = (1 + \beta^2) \cdot \frac{TPR \cdot PPV}{(\beta^2 \cdot PPV) + TPR}$$

The values of 1 and 2 as β were used in calculating the F_β scores for the models. Using the value of 1 calculates the harmonic mean of PPV and TPR, while the increase in β to 2 allowed for calculating the performance with a stronger emphasis on the importance of the true positive rate of the models.

Youden's J statistic was also calculated for all the models. False positive and false negative predictions are weighted equally in Youden's J statistic making it a good method for briefly understanding overall model performance. Youden's J statistic was defined as follows:

$$Youden's\ J\ Statistic = TPR + TNR - 1$$

Finally, the area under the receiver operating characteristic (ROC) curve was calculated. The area under the curve (AUC) allowed for understanding how well the model is able to separate the data into respective classes (positive and negative). A value of 1 represented a model that made no errors, the model was able to perfectly separate data into the correct divisions. A value of 0.5 means that the model had no ability to predict data as one class or another, predictions were essentially equivalent to a random decision. A value of 0 means that the model was able to distinguish data into separate classes, but it did it backwards. In other words, the model predicted positive values as negative values and negative values as positive values. The AUC values were calculated using the ROCR package in R.

Results and Discussion

A table summarizing the evaluation metrics provided in the previous section for all the baseline models is shown below in table 2.

Table 2

Evaluation of Baseline Models

Evaluation Metric	Lasso Regression	Ridge Regression	Elastic Net Regression
Accuracy	0.947	0.947	0.974
Sensitivity	1	1	1
Specificity	0.889	0.889	0.944
False Positive Rate	0.111	0.111	0.056
False Negative Rate	0	0	0
Positive Predictive Value	0.909	0.909	0.952
Negative Predictive Value	1	1	1
Area Under Curve	0.967	0.986	0.987
$\boldsymbol{F_1}$Score	0.952	0.952	0.976
$\boldsymbol{F_2}$Score	0.980	0.980	0.990
Youden's J Statistic	0.889	0.889	0.944

All of the baseline models showed similar performance, with identical sensitivities as well as identical false negative rates. This means that none of the baseline models falsely identified a tumor sample as a normal tissue sample. While the Lasso and Ridge Regression models shared very similar performance metrics, the elastic net model produced slightly better results, with an increased accuracy of 0.974, increased specificity of 0.955, as well as an increased area under the ROC curve, F_1 score, F_2 score, and Youden's J statistic. This is due to the Elastic Net Regression model identifying a lesser number of normal tissue samples as tumor samples, resulting in less false positives.

A table summarizing the evaluation metrics for all the proposed models is shown in table 3.

Table 3
Evaluation of Proposed Models

Evaluation Metric	Linear Support Vector Machine	Radial Support Vector Machine	Random Forest	Extreme Gradient Boosting
Accuracy	0.974	0.921	0.974	0.974
Sensitivity	1	1	1	1
Specificity	0.944	0.833	0.944	0.944
False Positive Rate	0.056	0.167	0.056	0.056
False Negative Rate	0	0	0	0
Positive Predictive Value	0.952	0.870	0.952	0.952
Negative Predictive Value	1	1	1	1
Area Under Curve	0.989	0.969	0.986	0.989
$\boldsymbol{F_1}$Score	0.976	0.930	0.976	0.976
$\boldsymbol{F_2}$Score	0.990	0.971	0.990	0.990
Youden's J Statistic	0.944	0.833	0.944	0.944

The proposed models showed similar results to the Elastic Net Regression model from the baseline models, with the exception of the radial Support Vector machine. The Support Vector machine with a radial basis kernel performed worse than all other models across every performance metric with the exception of area under the ROC curve, incorrectly identifying more samples than any other model. This may be due to using a default nonlinearity parameter. Tuning of this parameter may improve the performance of the Support Vector machine with a radial basis kernel. Overall, the Elastic Net Regression, linear Support Vector machine, Random Forest, and Extreme Gradient Boosting models produced the highest performances in terms of their accuracy and sensitivity/specificity. All four models shared identical measures for:

- Accuracy (0.974)
- Sensitivity (1)
- Specificity (0.944)
- False positive rate (0.056)
- False negative rate (0)
- Positive predictive value (0.952)
- Negative predictive value (1)
- F_1 score (0.976)
- F_2 score (0.990)
- Youden's J statistic (0.944).

This means that all these models identified the same number of true positive, true negative, false positive, and false negative samples. Among these four models, the linear Support Vector machine and the Extreme Gradient Boosting model had a slightly better AUC value of 0.989, making the linear Support Vector machine and the Extreme Gradient Boosting models the highest performing models in this study. While many of the models shared identical performance metrics, the variance in AUC is explained in how AUC is calculated. All the other performance metrics besides AUC only use the counts of the true positives, true negatives, false positives, and false negatives for calculations. However, AUC also uses the probabilities of each identification. The probabilities of each identification can be viewed as the confidence in each identification by the model. Thus, AUC delivers higher performance metrics for a model that

correctly identifies a sample with a higher confidence as opposed to a model with correctly identifies a sample with a lower confidence.

None of the models predicted any false negatives, which leads to all the models sharing the same sensitivity (1), false negative rate (0), and negative predictive value (1). This means that none of the models falsely identified a tumor sample as being a normal tissue sample. While a lack of false negatives showed impressive model performance, it is also possible that a larger sample size would have produced false negatives. Limitations to this study result from the usage of a single data set with a limited number of observations.

Besides the sample size limitation, another limitation of the study is that the proposed models did not consider correlations between tumor and normal tissues from the same individuals. Thus, the performances of the models may be more optimistic than reality. Noting that the pair-matched information between tumor and normal tissues is known in this dataset, developing prediction models that incorporates the correlations between observations will be necessary in the future.

Conclusion

In this study, high model performance was defined by performance metrics as close to the value 1 as possible, except for false positive rate and false negative rate, where high performance was defined by a value as close to 0 as possible. By maximizing and minimizing these quantities we attempted to find models that correctly identified the greatest number of samples. The AUC value was used in the case of identical performance measures to reward models with higher confidences in correct identifications with a higher performance measure.

All the models have shown excellent performances with the Extreme Gradient Boosting and Linear Support Vector Machine models showing the highest performances, indicating that these models both correctly identified the greatest number of samples and did so with the highest confidences compared to the other models. The results showed that proteomics data is indeed a very important factor in the study of ccRCC, as nearly 98% of samples were correctly identified using only proteomics data in the best models. Furthermore, this was done without any false negative identifications and with high AUC metrics, showing that most identifications were made with relatively high confidence, further verifying proteomics as an effective tool in ccRCC identification. The further development of ccRCC tumor prediction model may lead to an alternative proteomic based approach to identify ccRCC tumors in the future.

Acknowledgments

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ACKNOWLEDGEMENTS

Undergraduate Research

NSURJ would like to acknowledge the Undergraduate Research Department for providing admirable opportunities for students to conduct research as undergraduates. Without the NURA, PREP, and Community-based award programs, NSURJ would not have been able to connect with as many intellectually bright undergraduate researchers.

The Undergraduate Research department provides many opportunities for students to grow professionally and financially from doing research at the University of Nevada, Reno. Students and faculty are in great benefit from having a program like this.

Undergraduate Research has always supported NSURJ in our mission to provide students the opportunity to professionally grow while showcasing their superb education.

Wolf Pack Discoveries

NSURJ would like to extend our gratitude to the premier undergraduate research symposium, held at the University of Nevada, Reno. Wolf Pack Discoveries helps early researchers gain experience presenting their research. This event supports the undergraduate academic community in the Nevada Higher Education System and supports the growth of research in an undergraduate system.

Thank you to all who host the event and to the event for providing the opportunity for NSURJ to connect with young researchers.

Associated Students of the University of Nevada (ASUN)

NSURJ would like to extend thanks towards the Associated Students of the University of Nevada (ASUN) for providing support towards the journal's first publication, as well as continuing publications such as this one. ASUN's continued support has allowed NSURJ to grow into the academic stepping stone it is, and has continually made it possible for NSURJ to continue its mission.

ASUN provides needed support for the journal to grow into an outlet for academic development and success for undergraduate researchers to partake in.