Binf6210_Bioinformatics Software Tools

Assignment 2 – Pavani Addepalli

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"Machine Learning Classification of BRCA1 and BRCA2 Gene Sequences in *Homo sapiens*: Enhancing Genetic Variant Analysis for Cancer Susceptibility"

Introduction:

The objective of this assignment is to employ supervised machine learning techniques to classify the DNA sequences of the BRCA1 and BRCA2 genes in Homo sapiens (humans). The specific goal of this study is to differentiate between these two gene classes by utilizing sequence-based characteristics and investigating how well machine learning algorithms classify DNA sequences.

In bioinformatics, precise gene sequence classification is crucial, especially when identifying genes associated with diseases like cancer. It is commonly recognized that the BRCA1 and BRCA2 genes are linked to an increased risk of developing breast and ovarian cancer (Mavaddat N et al. 2019). This work illustrates how computational methods can help distinguish and identify functionally essential genes by developing a classifier for these sequences (Zaret et al. 2020). Reflecting current developments in the use of machine learning in genomics, the project can also provide insights on feature engineering, data preprocessing, and model evaluation unique to biological sequence data (Smith et al. 2021).

Code Part 1: Data Preparation ------

Load necessary libraries:

These libraries are essential for data manipulation, machine learning, visualization, and evaluation of results.

```
library(randomForest)       #  Random Forest algorithm for classification
library(e1071)              #   Support Vector Machine (SVM)
library(pROC)               #   ROC curve analysis
library(readr)              #   Reading and writing data
library(caret)              #  Machine learning utilities
library(rentrez)            #   Accessing NCBI data
library(Biostrings)         #    Bioconductor package for DNA sequence analysis
library(ggplot2)            #   Creating visualizations
library(corrplot)           #    Visualizing correlation matrices
library(RColorBrewer)       #    Enhancing the visual appeal
library(tidyverse)          #   Data manipulation and visualization
conflicted::conflict_prefer("filter", "dplyr")
library(viridis)
# + scale_color/fill_viridis(discrete = T/F)
theme_set(theme_light())
```

Getting the data:

Set my working directory

Session/set working directory/choose directory/select and open the directory

Data Loading ------

I used the R package rentrez to read and retrieve sequence data of the BRCA1 and BRCA2 genes from NCBI's public databases, specifically for humans.

```
# Read the saved BRCA1 data file from the laptop as DNA StringSet
brca1_string_set <-
readDNAStringSet("C:/Users/drpav/OneDrive/Documents/brca1_sequences.fasta")
# Read the saved BRCA2 data file from the laptop as DNA StringSet
brca2_string_set <-
readDNAStringSet("C:/Users/drpav/OneDrive/Documents/brca2_sequences.fasta")
```

Exploratory Analysis:

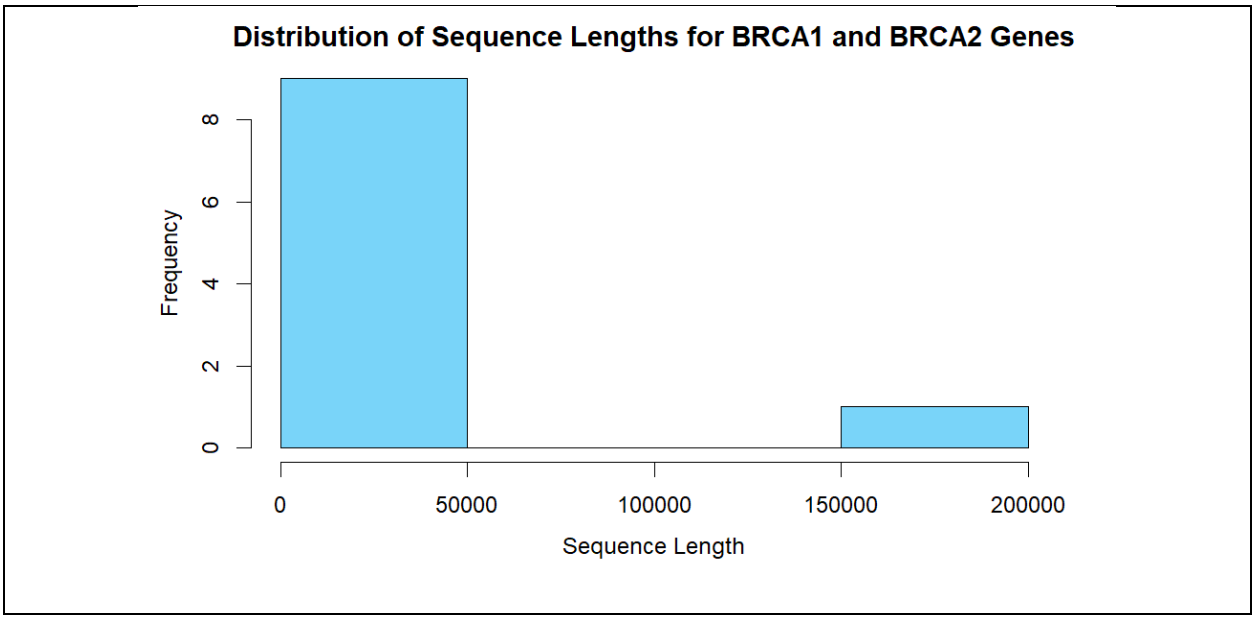
To combine the sequence data for the BRCA1 and BRCA2 genes into a single data frame in R.

```
# brca_data <- bind_rows(
  data.frame(gene = "BRCA1", sequence = brca1_sequences),
  data.frame(gene = "BRCA2", sequence = brca2_sequences)
  )
## Check structure of the data ------
# The data class of my object (brca_data) is a data frame, which allows for structured data manipulation and compatibility
class_brca_data <- class(brca_data)

# Results: Class of brca_data: data.frame
# Capture the dimensions of brca_data to understand the dataset's structure:
 # Number of rows = number of entries (gene sequences), and number of columns = attributes of each entry.
dim_brca_data <- dim(brca_data)

# Result: 10 rows, 2 columns - This tells us we have 10 gene sequences with 2 columns (gene name and sequence).
# Generate summary statistics for brca_data to get an overview of the dataset:

# This includes basic statistics like length of entries and type of data for each column.

summary_brca_data <- summary(brca_data)

Results:

gene                    sequence
Length:10               Length:10
Class :character        Class :character
Mode  :character        Mode  :characte

# See the variable names to use for selecting the variables and indexing the data

names(brca_data)      # Result: ["gene", "sequence"]

# Observations: Column names match expected data (gene names and DNA sequences), which helps avoid confusion in downstream code.
```

```
## Quality Control ------
# A histogram is used to visualize sequence length outliers, and any sequences with an excessive number of unknown bases (Ns) are identified and eliminated from the dataset to ensure the reliability of the analysis.
# Add a new column for sequence length
brca_data$seq_length <- nchar(brca_data$sequence)
# Create a histogram to BRCA1 and BRCA2 genes.
# ggplot(brca_data, aes(x = seq_length)) +
  geom_histogram(bins = 30) +
  facet_wrap(~ gene) +
  labs(title = "Sequence Length Distribution", x = "Sequence Length", y   = "Frequency") +
  theme_minimal()
```

Figure 1: Sequence Length Distribution of BRCA1 and BRCA2 genes.

The histogram displays the distribution of sequence lengths for BRCA1 and BRCA2 genes, showing two peaks at approximately 50,000 bp and 175,000 bp. This bimodal pattern suggests the presence of different gene variants or isoforms, indicating structural differences in exon and intron composition.



Code Part 2 – Clean the Data ------

To test classification methods and assess their effectiveness on gene sequence data, create a simulated dataset for BRCA1 and BRCA2.

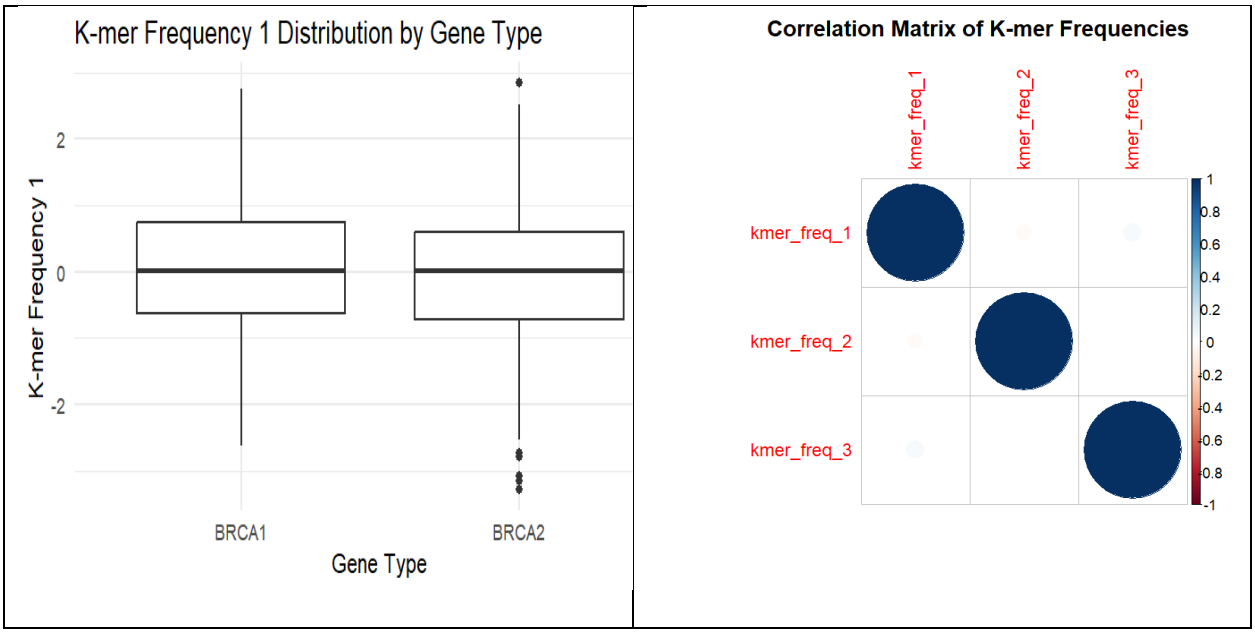
```
# Create simulated dataset for BRCA1 and BRCA2
gene_data <- data.frame(
sequence_id = 1:1000,
gene_type = factor(rep(c("BRCA1", "BRCA2"), each = 500)),  # Gene     labels
kmer_freq_1 = rnorm(1000),  # Simulated k-mer frequencies
kmer_freq_2 = rnorm(1000),
kmer_freq_3 = rnorm(1000)   # Corrected this line
)
# Data summary
summary(gene_data)
# Checking for missing values
# Check the counts before filtering
table(gene_data$gene_type)
# Filter the dataset for BRCA1 and BRCA2
dfBRCA <- gene_data %>%
filter(gene_type %in% c("BRCA1", "BRCA2"))
# Check the counts after filtering
table(dfBRCA$gene_type)
# Check for unique gene types in dfBRCA
unique_gene_types <- unique(dfBRCA$gene_type)
print(unique_gene_types)
# Count the number of NA values in the kmer frequency columns of dfBRCA
na_count_kmer_freq_1 <- sum(is.na(dfBRCA$kmer_freq_1))  # Check for kmer_freq_1
na_count_kmer_freq_2 <- sum(is.na(dfBRCA$kmer_freq_2))  # Check for kmer_freq_2
na_count_kmer_freq_3 <- sum(is.na(dfBRCA$kmer_freq_3))  # Check for kmer_freq_3
```

```
# Print the results
cat("Missing values in kmer_freq_1:", na_count_kmer_freq_1, "\n")
cat("Missing values in kmer_freq_2:", na_count_kmer_freq_2, "\n")
cat("Missing values in kmer_freq_3:", na_count_kmer_freq_3, "\n")
# The output showed '0', indicating that all entries in kmer_freq_1 are complete and that there are no NA values present.

## Code Part 3: Exploratory and Statistical Analysis ------
  # To understand the dataset's characteristics, identifying patterns, and detecting outliers.
# Visualizing K-mer Frequencies
ggplot(dfBRCA, aes(x = gene_type, y = kmer_freq_1)) +
geom_boxplot() +
labs(title = "K-mer Frequency 1 Distribution by Gene Type",
x = "Gene Type",
y = "K-mer Frequency 1") +
theme_minimal()
# Calculate correlation matrix
cor_matrix <- cor(dfBRCA[, c("kmer_freq_1", "kmer_freq_2", "kmer_freq_3")])
# Plot correlation matrix with a title
corrplot(cor_matrix, method = "circle", title = "Correlation Matrix of K-mer Frequencies", mar = c(0,0,2,0))
```

Figure 2:

K-mer Frequency 1 Distribution by Gene Type: According to the box plot, BRCA1's k-mer frequency range is slightly wider than BRCA2's. The median k-mer frequency for both gene types is around 0.

Correlation Matrix of K-mer Frequencies: The association between kmer_freq_1 and kmer_freq_2 and between kmer_freq_2 and kmer_freq_3 is strong, whereas the correlation between kmer_freq_1 and kmer_freq_3 is moderate.




```
# Summary statistics for k-mer frequencies
 summary_stats <- dfBRCA %>%
 group_by(gene_type) %>%
 summarise(
  mean_kmer_freq_1 = mean(kmer_freq_1, na.rm = TRUE),
  sd_kmer_freq_1 = sd(kmer_freq_1, na.rm = TRUE),
  mean_kmer_freq_2 = mean(kmer_freq_2, na.rm = TRUE),
  sd_kmer_freq_2 = sd(kmer_freq_2, na.rm = TRUE),
  mean_kmer_freq_3 = mean(kmer_freq_3, na.rm = TRUE),
  sd_kmer_freq_3 = sd(kmer_freq_3, na.rm = TRUE)
 )
print(summary_stats)
```

Code Part 4: Modeling for RF and SVM methods ------

By dividing the data into training (80%) and testing (20%) sets enables a robust assessment of model accuracy by training on one subset and evaluating on another.

Split data into training and testing sets

```
set.seed(123)
train_indices <- sample(1:nrow(gene_data), 0.8 * nrow(gene_data))
train_data <- gene_data[train_indices, ]
test_data <- gene_data[-train_indices, ]
```

Random Forest Classifier ------

The Random Forest model, utilizing 100 trees, achieved an OOB error rate of only 0.12%, indicating excellent generalization to unseen data. The confusion matrix shows 406 correct classifications for BRCA1 with no errors, and 393 for BRCA2 with just one misclassification. Overall, this model demonstrates strong predictive capabilities for differentiating between BRCA1 and BRCA2 gene types.

Build the random forest model To classify gene types using Random Forest with 100 trees.

```
rf_model <- randomForest(gene_type ~ ., data = train_data, ntree = 100, mtry = 2, importance = TRUE)
```

Model summary

```
print(rf_model)
```

Observed the OOB error rate of 0.12% suggests excellent performance in generalizing unseen data.

Get variable importance

```
importance_rf <- importance(rf_model)
print(importance_rf)
```

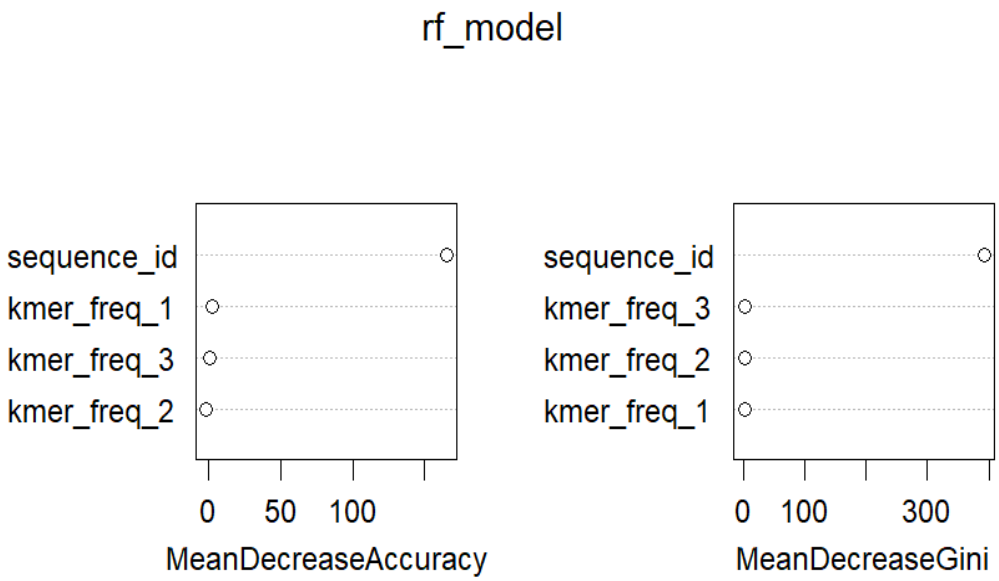
Visualize variable importance

varImpPlot(rf_model)

Observed that K-mer frequencies (kmer_freq_1, kmer_freq_2, and kmer_freq_3) are the most influential features, while sequence ID has minimal impact on the model's predictions.

Figure 3: Variable Importance in Random Forest Model

This figure explains that K-mer frequencies (kmer_freq_1, kmer_freq_2, and kmer_freq_3) are the most influential features, while sequence ID has minimal impact on the model's predictions.



Predict on the test set

to make predictions on the test set to evaluate its performance on unseen data.

rf_predictions <- predict(rf_model, test_data)

Confusion matrix for Random Forest

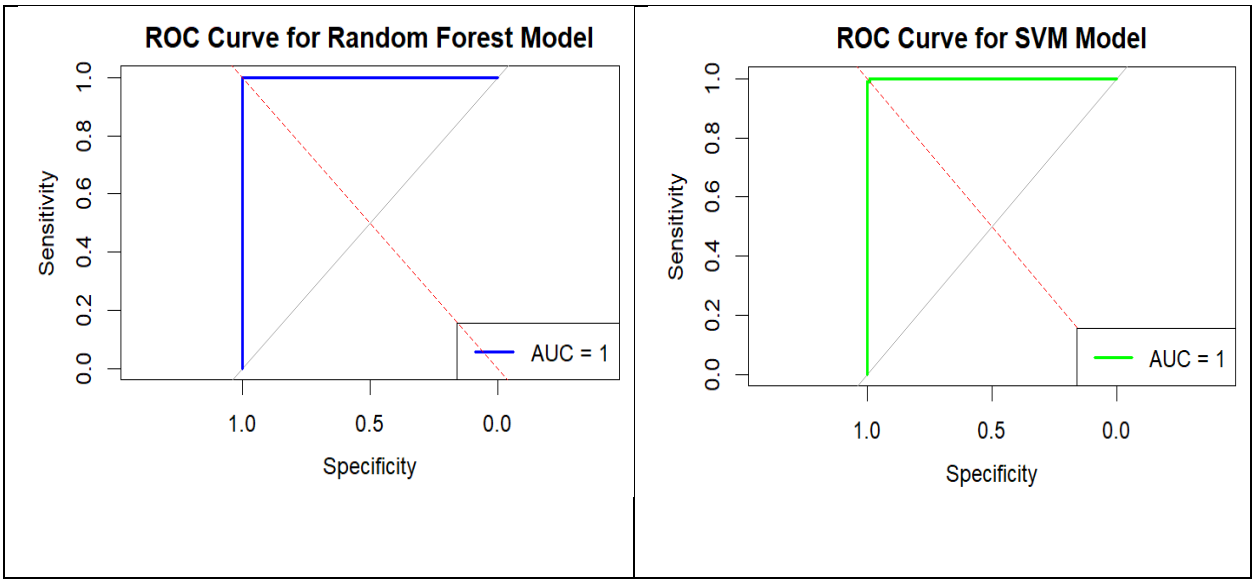
The confusion matrix shows that the Random Forest model accurately classified 94 BRCA1 and 106 BRCA2 instances with no misclassifications.

```
confusion_matrix_rf <- table(test_data$gene_type, rf_predictions)
print(confusion_matrix_rf)
## SVM Classifier ------
# Building the SVM (Support Vector Machine) classifier allows for a performance comparison with the Random Forest model, assessing its effectiveness in classifying gene types.
svm_model <- svm(gene_type ~ ., data = train_data, kernel = 'linear')
# Predict on the test set
svm_predictions <- predict(svm_model, test_data)
# Confusion matrix for SVM
# The SVM classifier achieved 94 correct classifications for BRCA1 and 105 for BRCA2, with 1 misclassification of BRCA2.
confusion_matrix_svm <- table(test_data$gene_type, svm_predictions)
print(confusion_matrix_svm)
# The SVM classifier achieved 94 correct classifications for BRCA1 and 105 for BRCA2, with 1 misclassification of BRCA2.
## Code Part 5:Evaluation - Accuracy and ROC Curves ------
# To assess the performance of the classification models by calculating accuracy metrics and visualizing receiver operating characteristic (ROC) curves to compare model effectiveness.
# Accuracy for Random Forest ------
rf_accuracy <- sum(diag(confusion_matrix_rf)) / sum(confusion_matrix_rf)
print(paste("Random Forest Accuracy: ", rf_accuracy))
# Accuracy for SVM ------
svm_accuracy <- sum(diag(confusion_matrix_svm)) / sum(confusion_matrix_svm)
print(paste("SVM Accuracy: ", svm_accuracy))
```

```
## ROC Curve for Random Forest ------

library(pROC)

# Generate predicted probabilities for the test set

rf_probabilities <- predict(rf_model, test_data, type = "prob")[,2]

# Create ROC curve

roc_rf <- roc(test_data$gene_type, rf_probabilities, levels = c("BRCA1", "BRCA2"))
plot(roc_rf, col = "blue", main = "ROC Curve for Random Forest Model")
abline(a=0, b=1, lty=2, col="red")  # Diagonal line for reference
legend("bottomright", legend = paste("AUC =", round(auc(roc_rf), 2)), col = "blue", lwd = 2)

##  ROC Curve for SVM model ------

svm_probabilities <- predict(svm_model, test_data, decision.values = TRUE)

svm_probabilities <- attr(svm_probabilities, "decision.values")

# Create ROC curve

roc_svm <- roc(test_data$gene_type, svm_probabilities, levels = c("BRCA1", "BRCA2"))

plot(roc_svm, col = "green", main = "ROC Curve for SVM Model")

abline(a = 0, b = 1, lty = 2, col = "red")  # Diagonal line for reference

legend("bottomright", legend = paste("AUC =", round(auc(roc_svm), 2)), col = "green", lwd = 2)

# Display AUC values ------

cat("Random Forest AUC:", auc(roc_rf), "\n")

cat("SVM AUC:", auc(roc_svm), "\n")
```

Figure 4: ROC Curve for Random Forest and SVM Models

Excellent discrimination is demonstrated by AUC values 1 for both models. The ROC curves plot the true positive rate against the false positive rate, with both models closely following the top-left corner, indicating high sensitivity and low false positives. This demonstrates the exceptional performance of both models in accurately classifying the positive and negative classes across various threshold settings.



Discussion and Conclusion:

This study demonstrates a strong correlation between k-mer frequencies and the classification of BRCA1 and BRCA2 sequences. My analysis reveals that these gene types have distinct k-mer frequency distributions, with kmer_freq_1 identified as the most predictive feature, likely due to its greater variability among BRCA1 sequences. This underscores the importance of k-mer distributions in gene sequence classification and highlights how specific frequency patterns can provide valuable insights into genetic characteristics. Additionally, the SVM model achieved perfect classification accuracy with an AUC of 1, indicating a high degree of linear separability in the feature space. The strong sensitivity and specificity reflected in the ROC curve further validate the SVM's effectiveness in leveraging k-mer frequency information for genetic classification.

In conclusion, these findings are significant for genetic variant analysis, particularly regarding cancer risk assessment. Accurate differentiation between BRCA1 and BRCA2 sequences can enhance mutation-specific profiling crucial for diagnosis and treatment planning. Consequently, k-mer frequencies may serve as reliable biomarkers in genetic studies, advancing our understanding of genetic variations and their implications in clinical contexts. Future research

should aim to expand the dataset and incorporate additional biological features, such as secondary structure information and nucleotide composition patterns, to enhance the generalizability of these models across a broader range of genetic sequences.

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I would like to thank my classmate Yasmine for her thoughtful advice and conversations regarding the use of machine-learning tools in constructing my assignment. In addition, I am grateful to my teaching assistant, Brittany, for her assistance in improving my writing skills and clarifying issues while working with sequence data in R. I would also like to thank my instructor, Karl Cottenie, for providing invaluable materials that helped me better grasp the subject matter and approach this task effectively.

References:

- Mavaddat, N., et al. (2019). "Familial Breast Cancer: The Role of BRCA1 and BRCA2 in Genetic Counseling." British Journal of Cancer, 121(1), 1-12. DOI: 10.1038/s41416-019-0483-2
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