Supplementary Materials for

Using gut microbiome metagenomic hypervariable features for diabetes screening and typing through supervised machine learning

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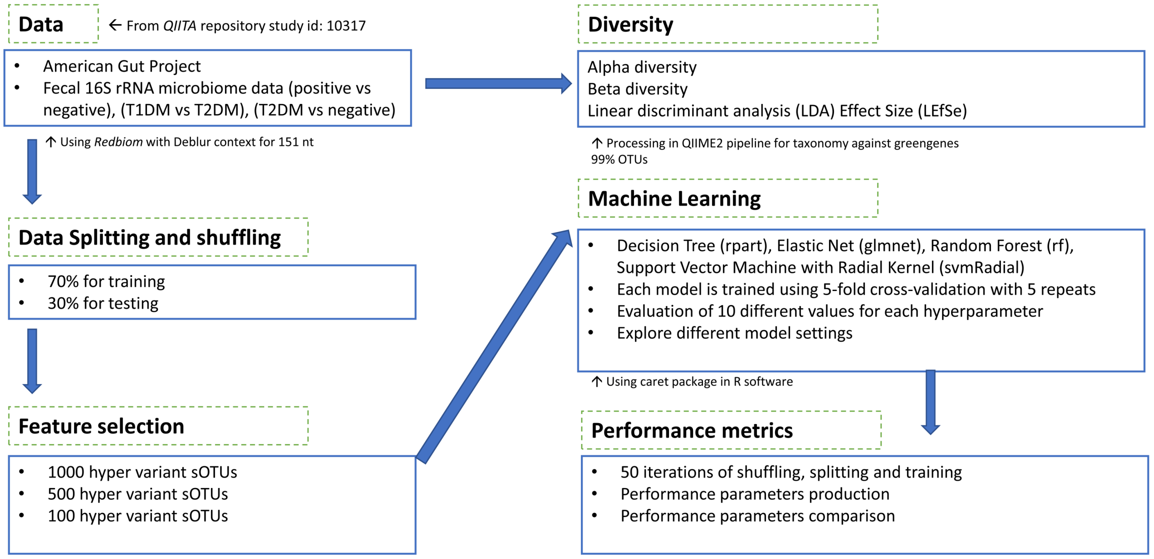


Data ← From *QIITA* repository study id: 10317

- American Gut Project
- Fecal 16S rRNA microbiome data (positive vs negative), (T1DM vs T2DM), (T2DM vs negative)

↑ Using *Redbiom* with Deblur context for 151 nt

Data Splitting and shuffling

- 70% for training
- 30% for testing

Feature selection

- 1000 hyper variant sOTUs
- 500 hyper variant sOTUs
- 100 hyper variant sOTUs

Diversity

Alpha diversity
Beta diversity
Linear discriminant analysis (LDA) Effect Size (LEfSe)

↑ Processing in QIIME2 pipeline for taxonomy against greengenes 99% OTUs

Machine Learning

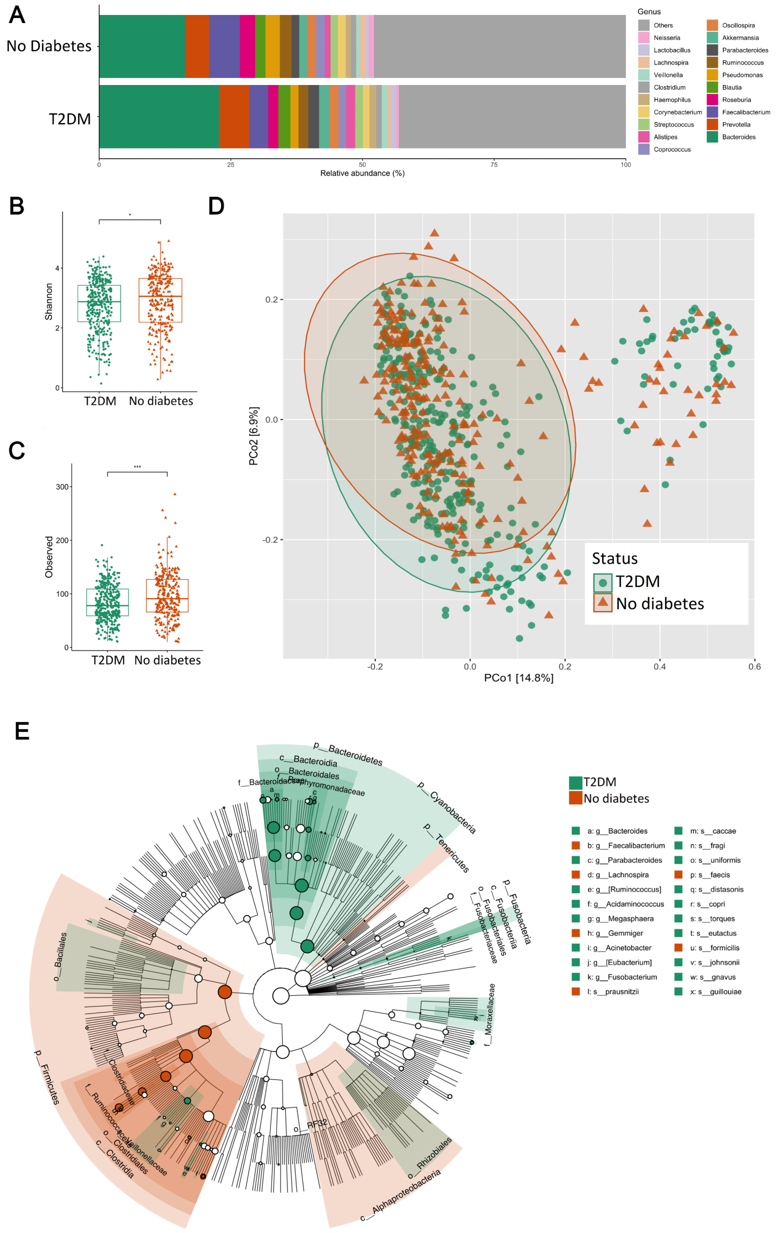
- Decision Tree (rpart), Elastic Net (glmnet), Random Forest (rf), Support Vector Machine with Radial Kernel (svmRadial)
- Each model is trained using 5-fold cross-validation with 5 repeats
- Evaluation of 10 different values for each hyperparameter
- Explore different model settings

↑ Using caret package in R software

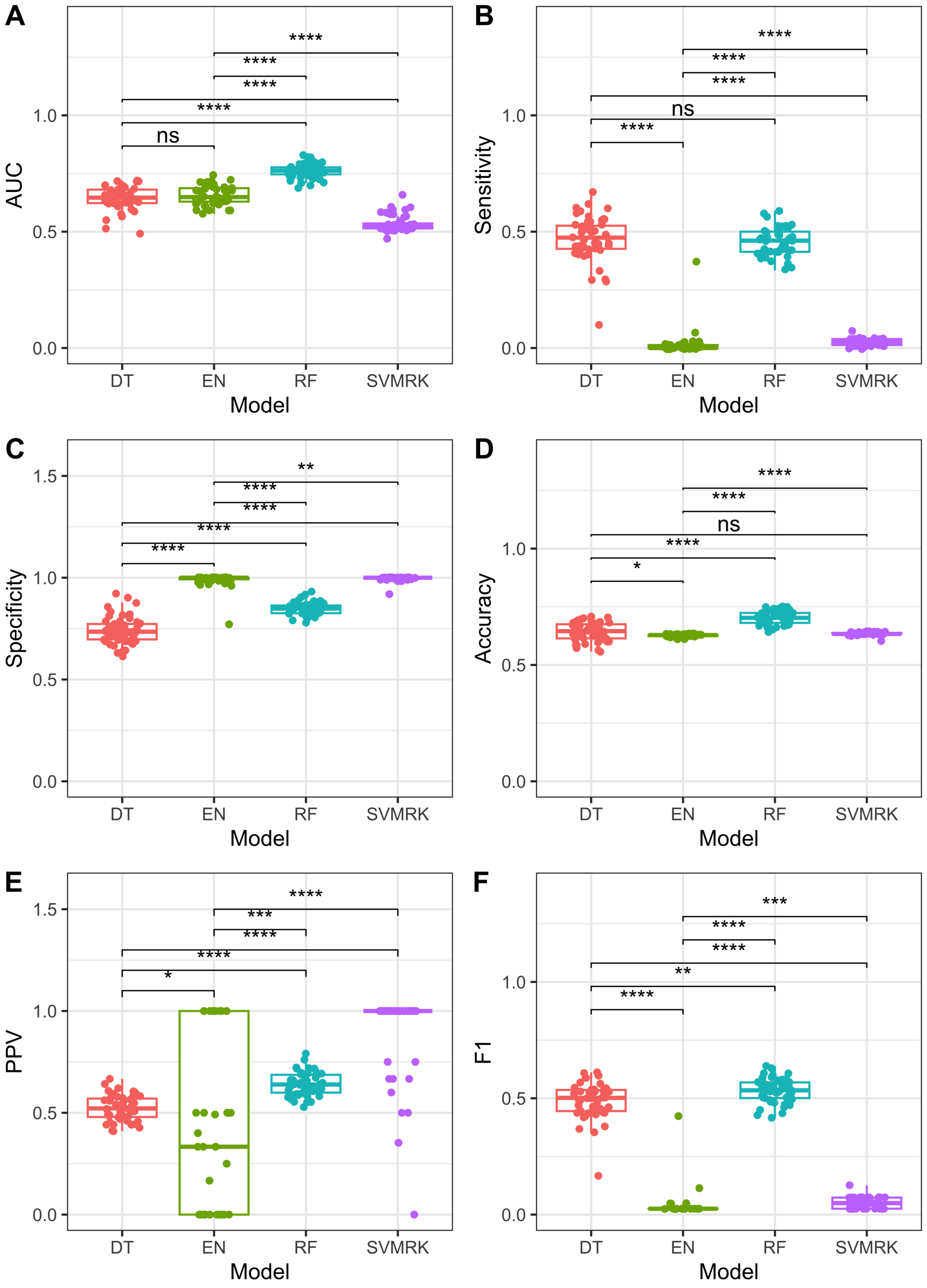
Performance metrics

- 50 iterations of shuffling, splitting and training
- Performance parameters production
- Performance parameters comparison

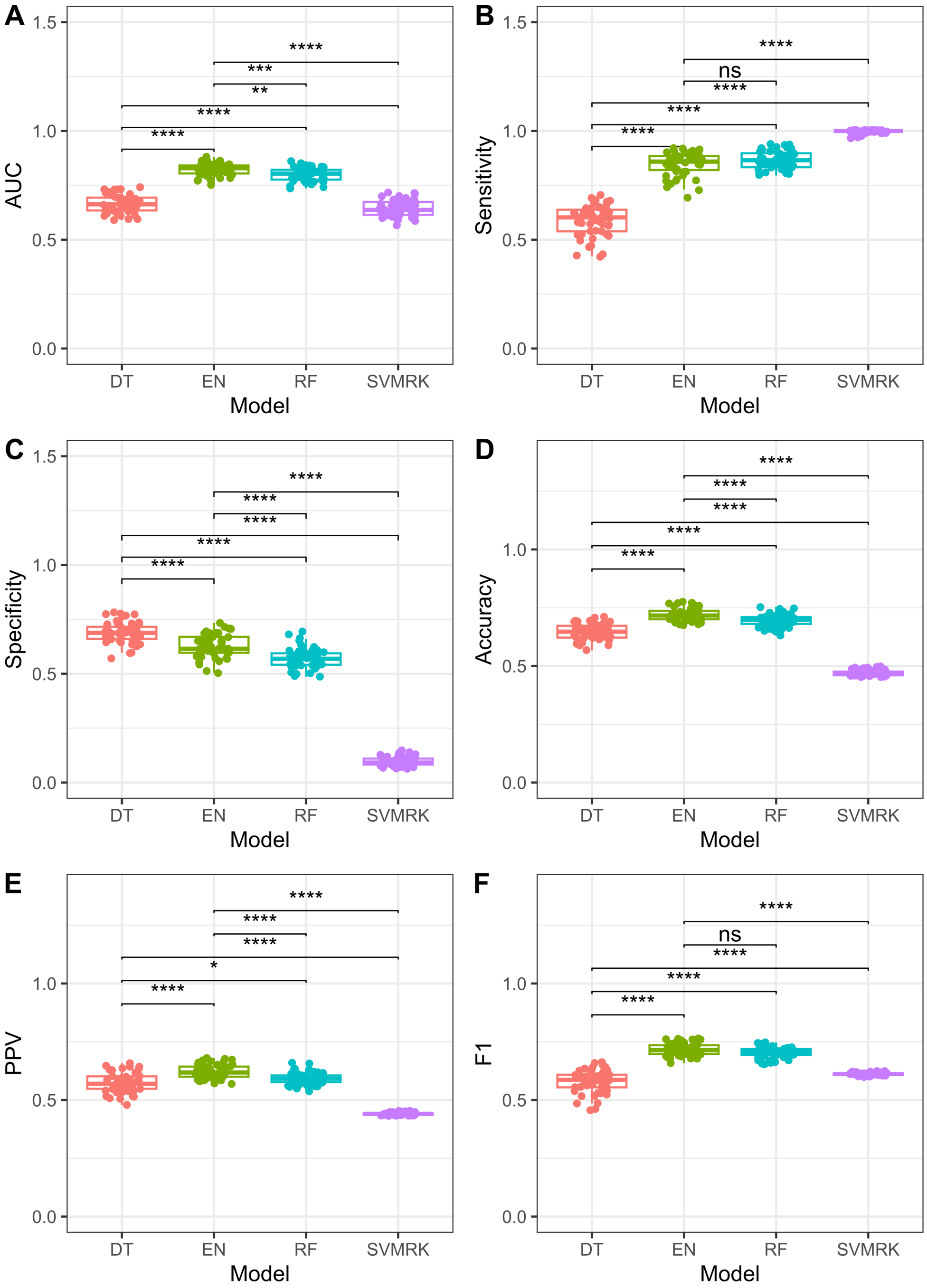
Supplementary Figure S1. Methodology workflow of the present study.



Supplementary Figure S2. Diversity metrics of the gut microbiome from volunteers that reported T2DM vs. volunteers that did not report diabetes. Mean relative abundance of the top 20 gut microbiome sOTUs of volunteers that reported T2DM vs. volunteers that did not report diabetes at the genus level (A). Shannon index of the gut microbiome sOTUs of volunteers that reported T2DM vs. volunteers that did not report diabetes compared by Wilcoxon rank-sum test (B). Richness of the gut microbiome sOTUs of volunteers that reported diabetes vs. volunteers that did not report diabetes compared by Wilcoxon rank-sum test (C). Unweighted UniFrac distances PCoA of the gut microbiome of volunteers that reported diabetes vs. volunteers that did not reported diabetes tested with PERMANOVA (D). Cladogram of the top 50 more representative taxa based on LEfSe analysis from the gut microbiome of volunteers that reported diabetes vs. volunteers that did not report diabetes showing their phylogenetic relationship with the top 1000 most abundant taxa, labels in the cladogram show sOTUs (LDA > 2, *p* < 0.05) of superior taxonomic levels, labels outside the cladogram represent enriched sOTUs at the genus and species level (E).



Supplementary Figure S3. Performance metrics of four supervised machine learning algorithms for the screening of diabetes trained on the top 1000 hyper-variable OTUs of the gut microbiome across 50 iterations. Diabetes status was screened with the Decision Tree (DT), Elastic Net (EN), Random Forest (RF), and Support Vector Machine with Radial Kernel (SVMRK) models. Area under the receiver operating characteristic curve (A), sensitivity (B), specificity (C), accuracy (D), Positive predictive value (E), F-score (F). The models use a predicted probability classification threshold of 0.5 with default iterations and tree maxit levels.



Supplementary Figure S4. Performance metrics of four supervised machine learning algorithms for the screening of diabetes classifying negative status vs T2DM trained on the top 500 hyper-variable OTUs of the gut microbiome across 50 iterations. Diabetes status was screened with the Decision Tree (DT), Elastic Net (EN), Random Forest (RF), and Support Vector Machine with Radial Kernel (SVMRK) models. Area under the receiver operating characteristic curve (A), sensitivity (B), specificity (C), accuracy (D), Positive predictive value (E), F-score (F). The models use a predicted probability classification threshold of 0.65 with 10000 maximum iterations for the Elastic Net (EN) and Support Vector Machine with Radial Kernel (SVMRK) models, maximal depth of 30 for the Decision Tree (DT), 1000 maximum trees for the Random Forest (RF) models.