

SAMPLE COLLECTION



RNA PURIFICATION



cDNA SYNTHESIS



LOAD TLDA PLATES & RT-PCR



CONVERT  $C_T$  TO LINEAR EXPRESSION VALUES,  $=2^{(40-CT)}$



IDENTIFY DISCRIMINATORY SINGLE EXPRESSION RATIOS; eg. GENE A/GENE B, WHERE THE GREATEST NUMBER OF SUBJECT RATIO SCORES ARE HIGHER THAN THE HIGHEST OR SECOND HIGHEST RATIO SCORE IN THE COMPARATOR SUBJECT GROUP



ESTABLISH A POINT SYSTEM: ONE POINT IS AWARDED TO A SUBJECT FOR EACH EXPRESSION RATIO WHERE IT ACHIEVES A HIGHER SCORE THAN THE HIGHEST OR SECOND HIGHEST RATIO SCORE IN THE COMPARATOR SUBJECT GROUP



PERMUTATION TESTING TO IDENTIFY COMBINATION OF RATIOS, RATIOSCORE, THAT BEST DISCRIMINATES ALL SUBJECTS IN THE TEST GROUP FROM ALL SUBJECTS IN THE COMPARATOR GROUP



TRAIN AND VALIDATE SVM.



EVALUATE PREDICTIVE ACCURACY WITH INDEPENDENT CASE COHORT

**Supplementary Figure 1.** Flow chart describing sample collection and processing, data generation, and methods of data analysis.