Supplementary Material (ESI) for The Analyst

Highly Multiplexed Detection of Labeled Oligonucleotides Using Surface Enhanced Resonance Raman Scattering (SERRS)

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Supplementary Information

The full set of results from bootstrapped PLS1 calibration (200 iterations) for the six oligonucleotides labeled with the six different dyes (ROX, HEX, FAM, TET, Cy3, and TAMRA) is provided within this supplementary information. For each figure there are six components that are labeled as:

A - Scatter plot depicting the mean training and test predictions for each sample across all 200 bootstrapped models.

B - Boxplot to complement the scatter plot. The boxes have lines at the lower quartile, median, and upper quartile values; the whiskers are lines extending from each end of the boxes to show the extent of the rest of the data, and outliers are marked by crosses.

C - Histogram showing the distribution of training and test predictions across all 200 bootstrapped models.

D - Histogram showing the distribution of training and test prediction *errors* across all 200 bootstrapped models.

E - Receiver operating characteristic (ROC) & ROC convex hull (ROCCH) give the true-positive (TP) rate and false positive (FP) rate of classification; ROC curves are beneficial because they avoid having to apply a numerical threshold which defines the class boundary. A ROC curve with an area of 1 shows 100% classification accuracy. For this study the ROC has been calculated using the full complement of *test predictions only* across all 200 bootstrapped models.

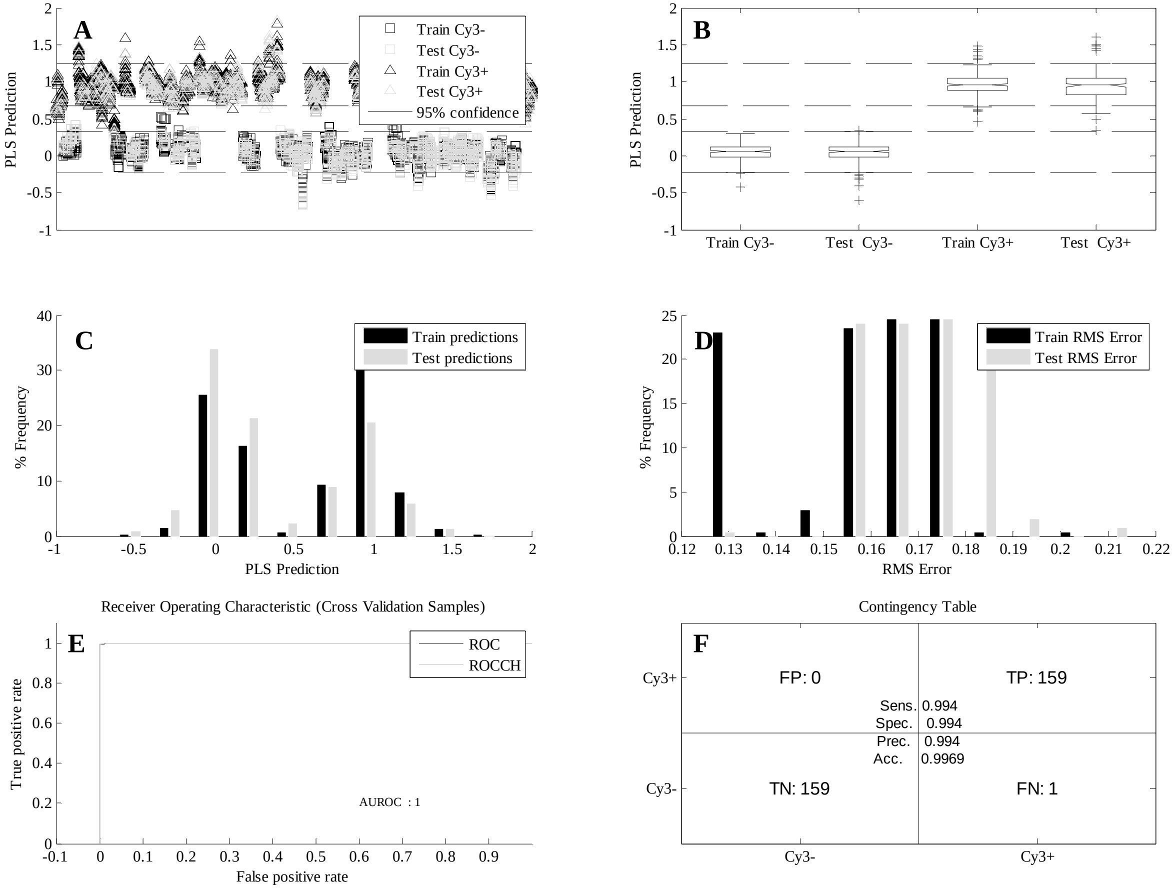
F – A confusion matrix giving the number of true positive (TP), false positive (FP), true negative (TN), false negative (FN) classifications based upon the mean of PLS1 test predictions for each sample across the 200 bootstrapped models. This is based upon an arbitrary classification boundary of >=0.5 (positive), <0.5 (negative). Some common metrics that can be derived from this calculation (sensitivity, specificity, precision and accuracy) are also provided. Where:

$$sensitivity = \frac{TP}{TP + TN}$$

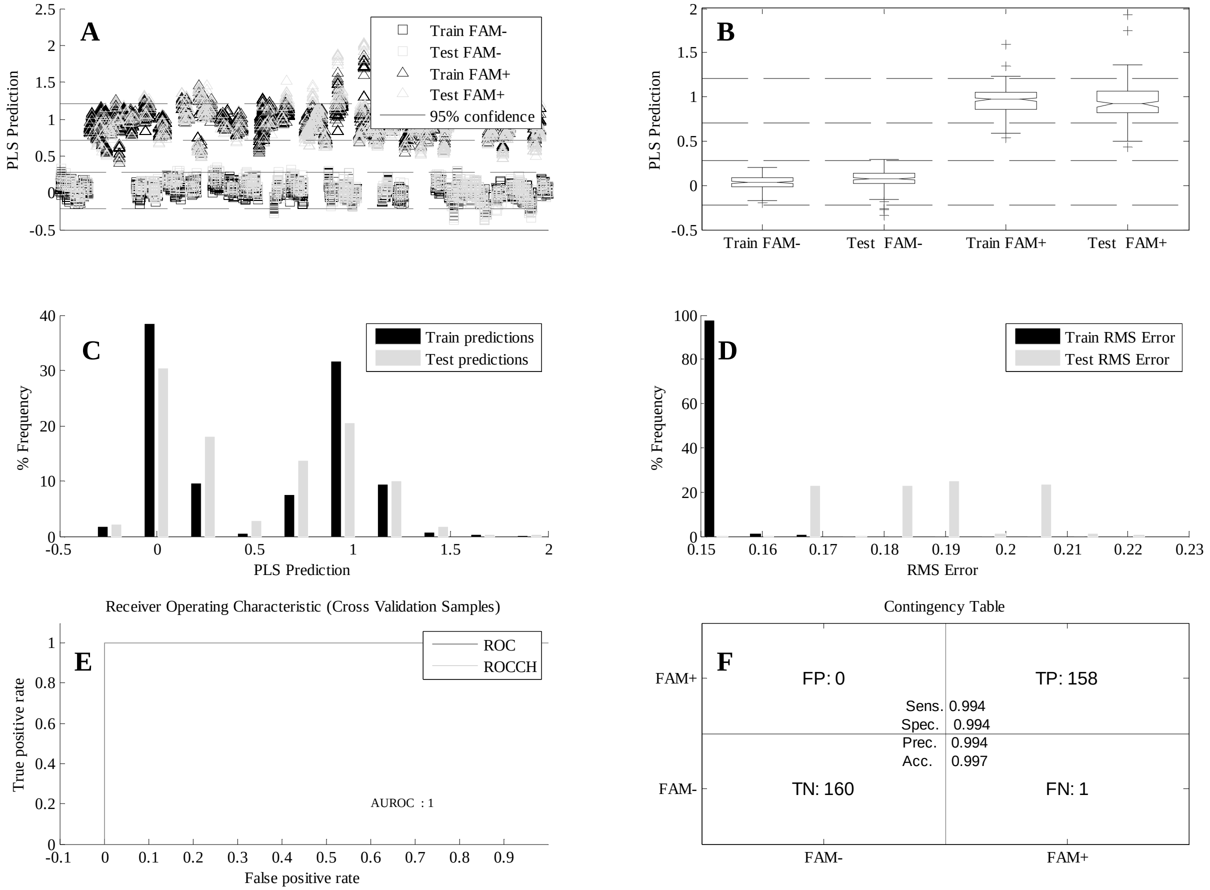
$$specificity = \frac{TN}{TN + FP}$$

$$precision = \frac{TP}{TP + FP}$$

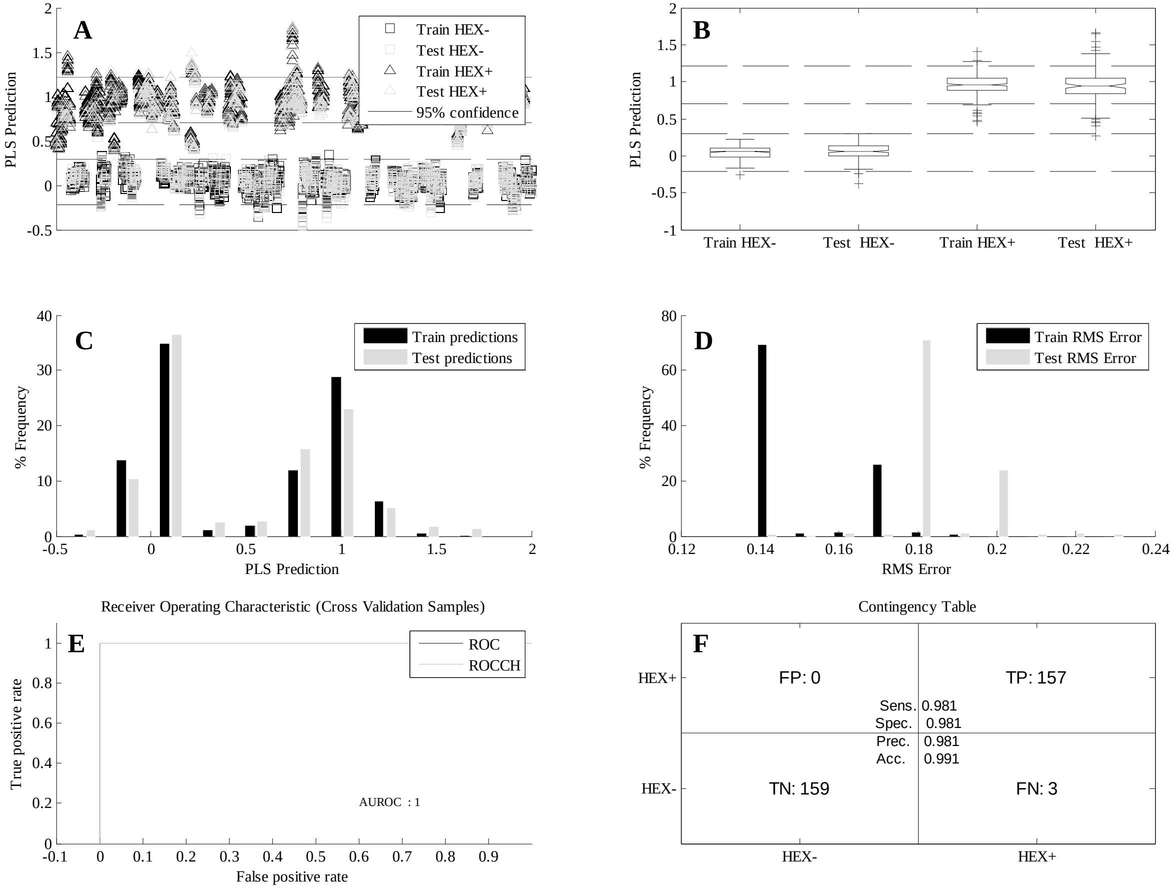
$$accuracy = \frac{TP + TN}{TP + FP + TN + FN}$$



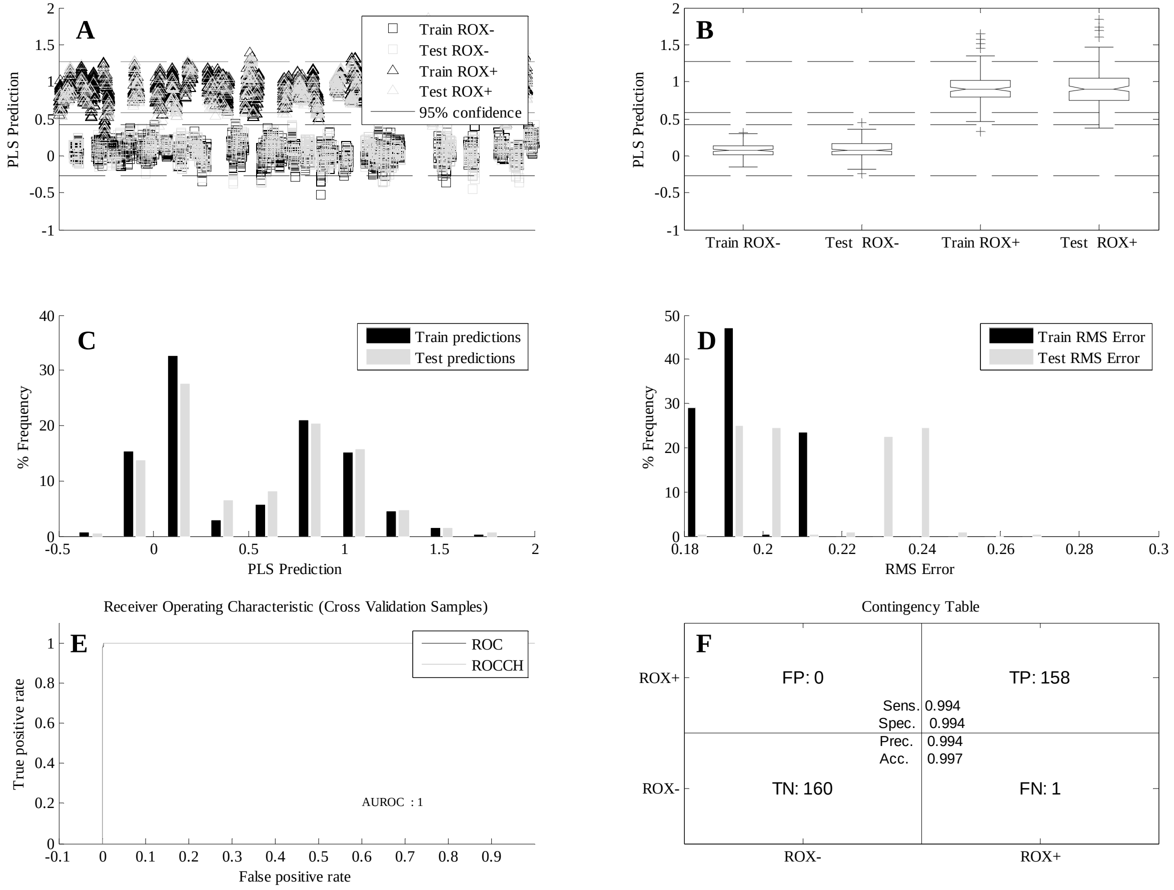
Supplementary Figure 1. PLS1 bootstrap results for Cy3.



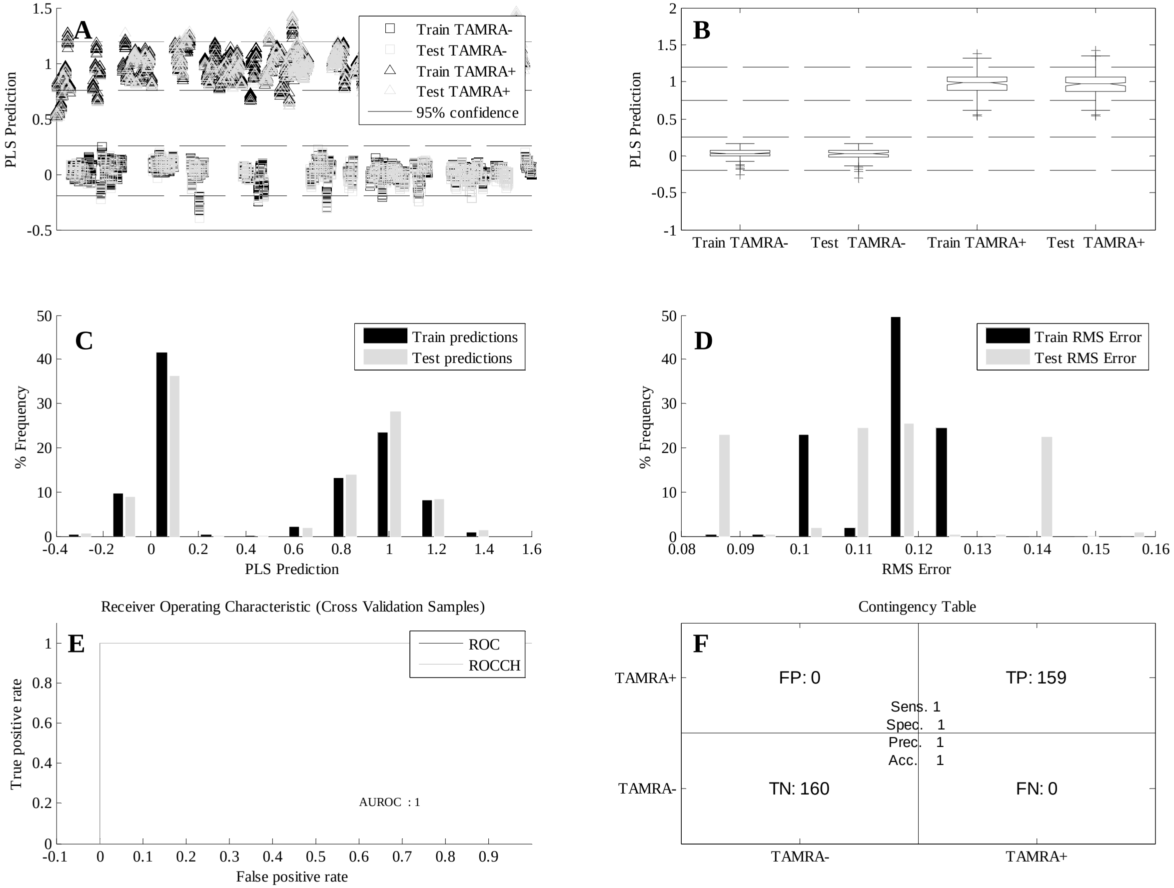
Supplementary Figure 2. PLS1 bootstrap results for FAM.



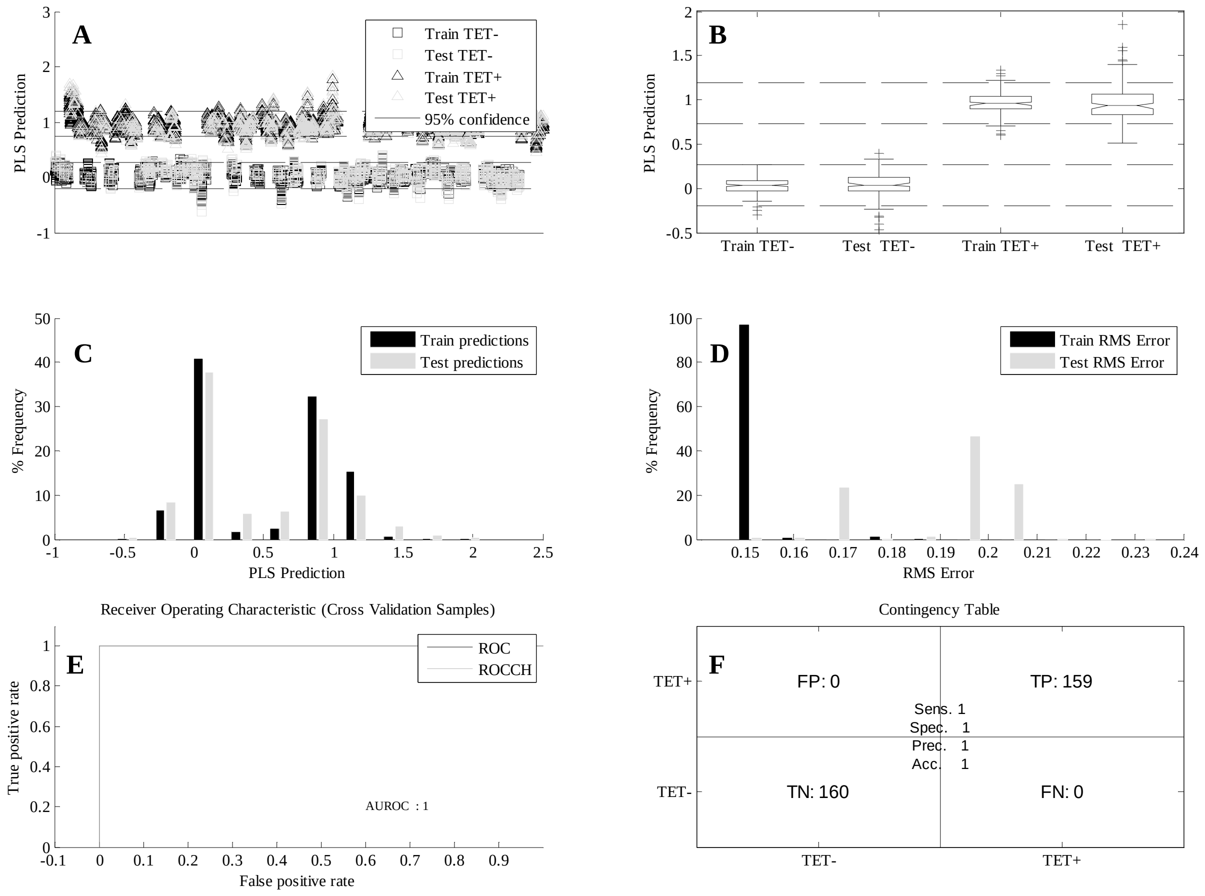
Supplementary Figure 3. PLS1 bootstrap results for HEX.



Supplementary Figure 4. PLS1 bootstrap results for ROX.



Supplementary Figure 5. PLS1 bootstrap results for TAMRA.



Supplementary Figure 6. PLS1 bootstrap results for TET.