MICROSATELLITE INSTABILITY CLASSIFICATION BY NGS

Laila Sathe

Microsatellite Instability

- Characterized by the “spontaneous loss or gain of nucleotides from repetitive DNA tracts”
  - Caused by defective mismatch repair

- Is a diagnostic phenotype for certain cancer types with clinical implications
  - Actionable marker for immune-checkpoint-blockade therapy

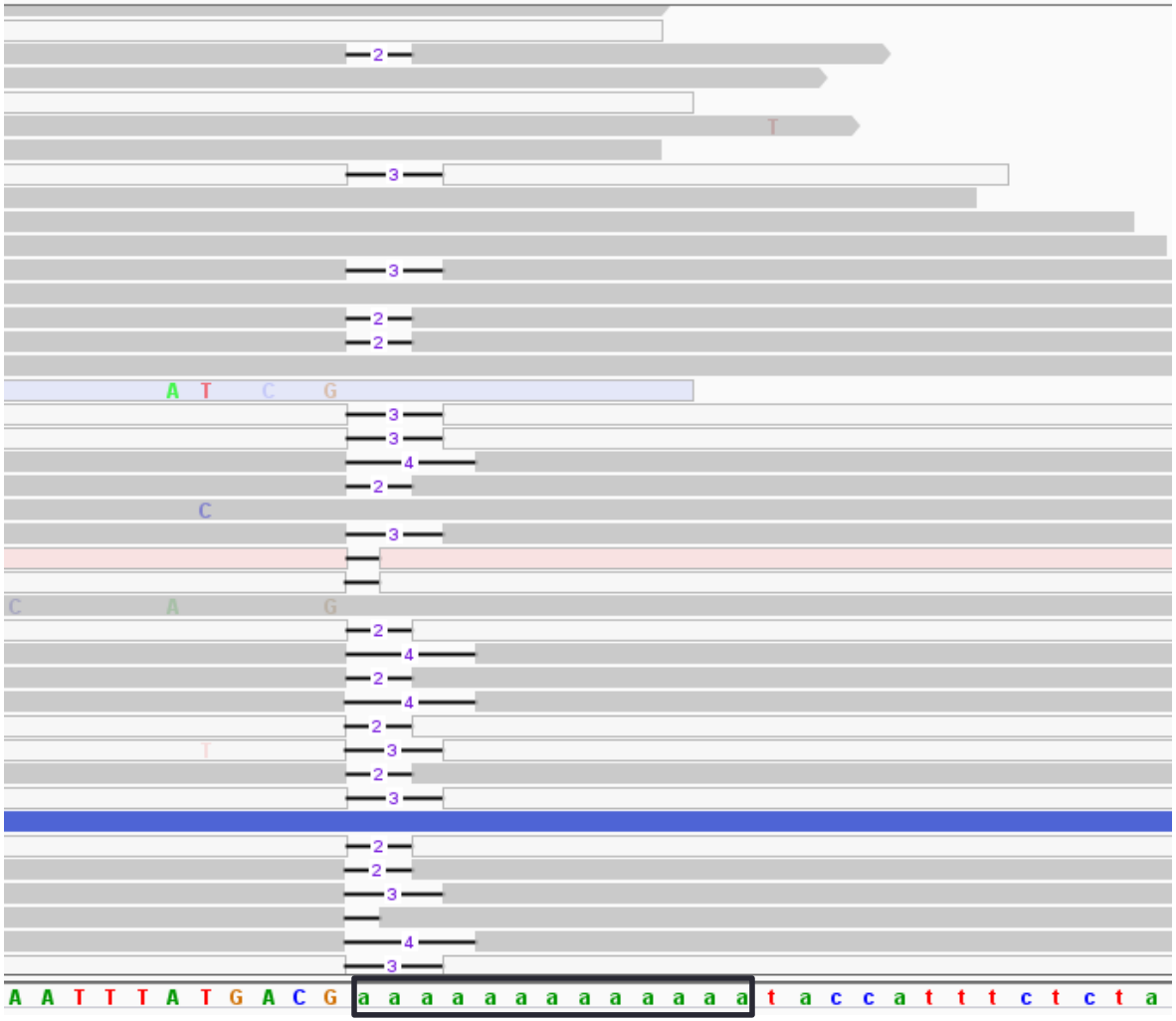
  (Hause et al. 2016)

Microsatellite Instability

- Develop a model that can accurately detect Microsatellite Instability using NGS data

- Add informative loci to the STAMP selector
  - Clinically relevant and actionable

Repeat Identification



Repeat Identification

- Flanking regions
  - 7 bases long
    - Long enough to be unique, identifiable
    - Short enough to maximize usable reads
  - Immediately adjacent to the mononucleotide repeat

Repeat Identification

- Pull all reads containing bases in the region of the locus
  - Psyam
- Scan and find flanking regions
  - Ordered search
    - First flank *must* be found before the second
  - Brute-force fuzzy matching
    - Mismatch allowance = 2
- Validation: filter
  - Baseline = 90%
  - Modified for some loci
    - Those that contain a non-homogenous run

MSI Calling

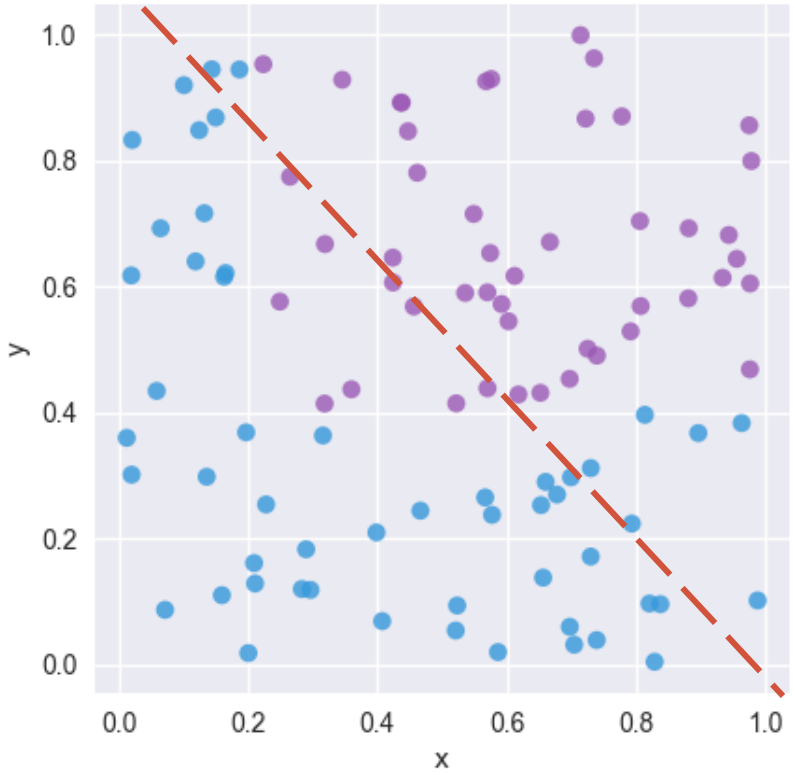
- Binary classifier
  - Simplification of MSI-H and MSI-L
- Optimization
  - Accuracy
    - Sensitivity
    - Specificity
- Locus-based calls
  - More common in literature
- Overall call based on the proportion of all 27 loci that are unstable

Machine Learning Approach

- TensorFlow
  - Python API developed by Google
- Minimize error in linear function, apply sigmoid curve to calculate p(MSI)
  - Supervised learning
  - Classification problem
- Simplification
  - 7 loci with high number of BAMs with reads available, good depth at the loci

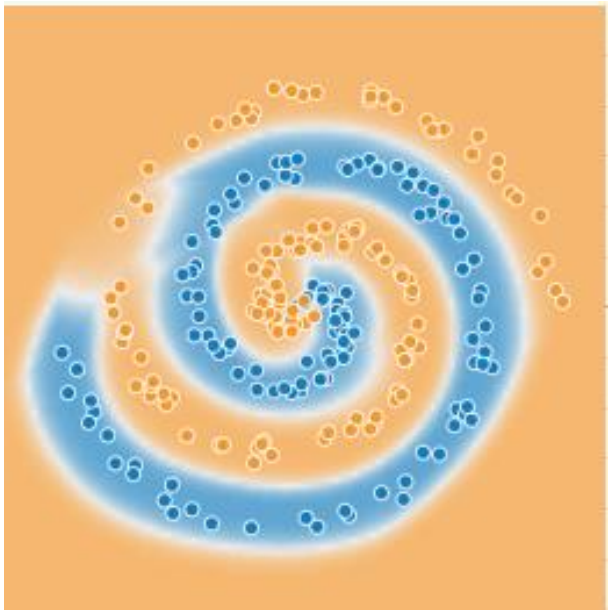
Google's 'ML Crash Course'

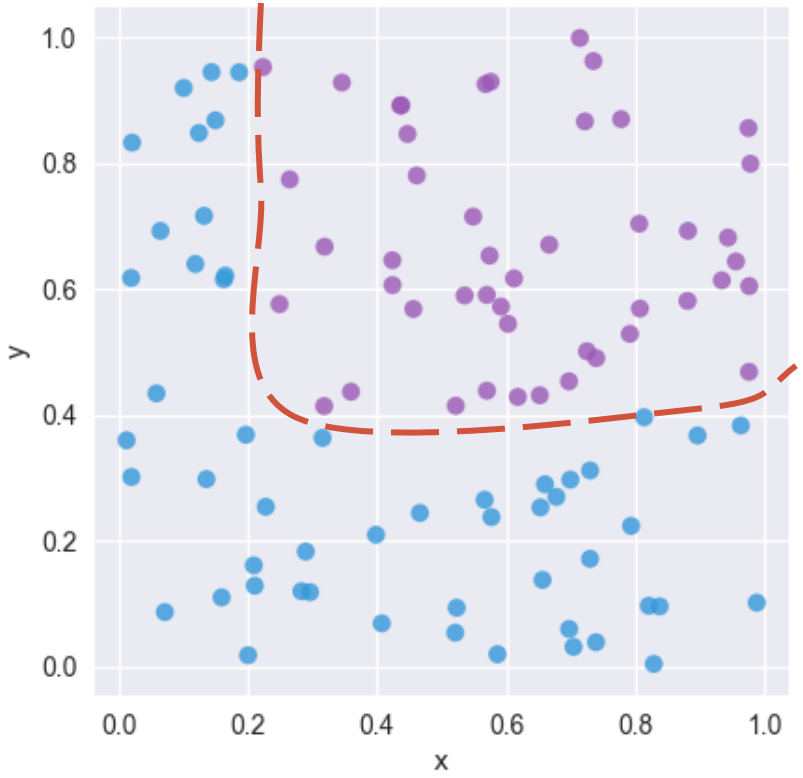
- Finding the best 'best-fit line' to distinguish two groups of data
  - Minimizing error



Google's 'ML Crash Course'

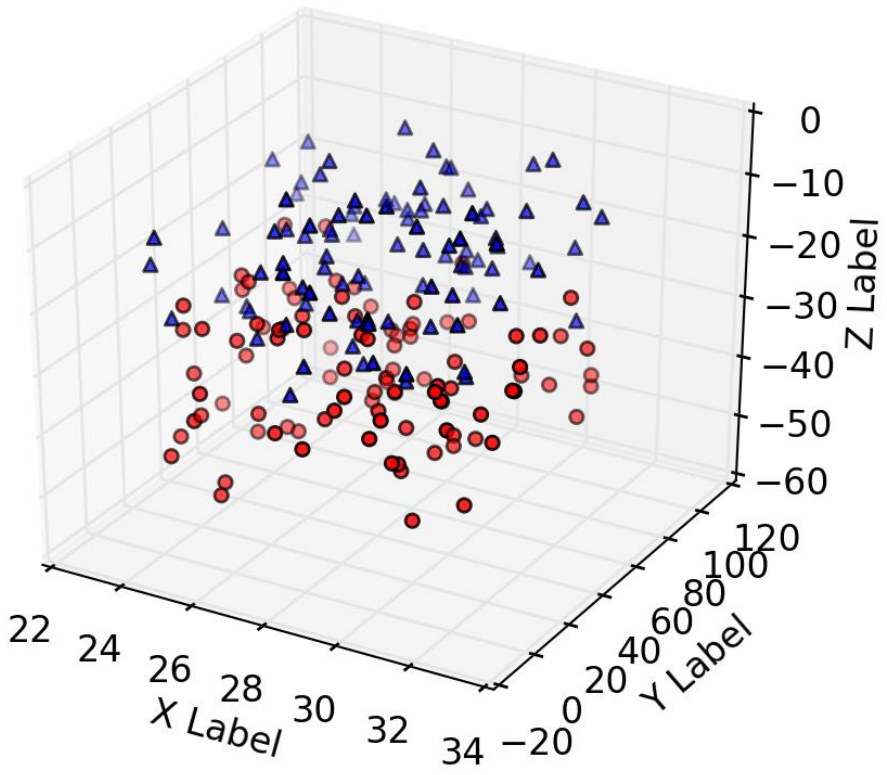
- Finding the best 'best-fit line' to distinguish two groups of data
  - Minimizing the error





Google's 'ML Crash Course'

- Finding the best 'best-fit line' to distinguish two groups of data
  - Minimizing the error
- ML allows expansion of this idea to *n*-dimensions



Machine Learning Approach

- TensorFlow
  - Python API developed by Google
- Supervised learning
  - Labeled data
- Classification problem
  - Binary: MSI = ‘1’, MSS = ‘0’
- Simplification
  - 7 loci with high number of BAMs with reads available, good depth at the loci

MSI Calling - Features

- Number of lengths
  - [11, 12, 11, 13, 14] = 4
- Distance from mode
  - Average distance from MSS sample mode of all reads
- Standard deviation
- Average Length

Datasets

- All files
  - 602 BAM files with MSS, MSI-L or MSI-H annotation
    - 241 COAD-READ, 361 UCEC
    - 212 MSI, 390 MSS
- Mode training set
  - Randomly generated set of 100 BAM files with MSS status to generate mode length
    - 43 COAD-READ, 57 UCEC
    - 0 MSI, 100 MSS

Datasets (continued)

- Training Set
  - 300 BAM files of mixed status to train the model
    - 118 COAD-READ, 182 UCEC
    - 130 MSI, 170 MSS
- Validation Set
  - 100 BAM files of mixed status to validate the model
    - 44 COAD-READ, 56 UCEC
    - 40 MSI, 60 MSS
- Test Set
  - 102 remaining BAM files of mixed status to test the model
    - 36 COAD-READ, 66 UCEC
    - 42 MSI, 60 MSS

Datasets (continued)

- Randomly generated sets
  - Representative of both cancer types

- Low coverage problem
  - Low coverage at certain loci excludes some files from some locus analyses
    - Actual size of datasets vary based on individual BAM coverage at any given locus

Locus-Based Calling

- Generate ML model for each locus individually
  - Weight for each feature, bias
  - Sigmoid activation function to generate p(MSI) in range (0, 1)
- Choose loci with highest AUC
  - Exclude those that have no reads in many BAM files
  - Try different combinations of loci
- Determine MSI status based on number of loci with ‘MSI’ call

Locus-Based Calling (continued)

- Loci examined
  - BAT-26
  - MSI-07
  - MSI-09
  - H-06
  - MSI-06
  - MSI-04
  - HSPH1-T17

- Threshold: 0.500000
- Min no. loci: 4
- Total files: 93
- Correct predictions: 78
  - True pos: 24
  - True neg: 54
  - False pos: 2
  - False neg: 13
- **Accuracy: 0.838710**
- **Sensitivity: 0.648649**
- **Specificity: 0.964286**

Probability-Based Calling

- Generate ML model for each locus individually
  - As before
- Choose loci with highest AUC
  - As before
- Determine MSI status based on average p(MSI) across loci examined

Probability-Based Calling (example)

- BAM file: TCGA-00-0000
  - BAT-26
    - p(MSI) = 0.998
  - MSI-07
    - p(MSI) = 0.488
  - MSI-09
    - p(MSI) = 0.478
  - H-06
    - p(MSI) = 0.499
  - MSI-06
    - p(MSI) = 0.898
  - MSI-04
    - p(MSI) = 0.978
  - HSPH1-T17
    - p(MSI) = 0.408

- Locus-based call: MSS
  - 3 MSI, 4 MSS
- Probability-based call: MSI
  - Avg p(MSI): 0.678

Probability-Based Calling (continued)

- Loci examined
  - BAT-26
  - MSI-07
  - MSI-09
  - H-06
  - MSI-06
  - MSI-04
  - HSPH1-T17

- Threshold: 0.500000
- Total files: 93
- Correct predictions: 78
  - True pos: 23
  - True neg: 55
  - False pos: 1
  - False neg: 14
- **Accuracy: 0.838710**
- **Sensitivity: 0.621622**
- **Specificity: 0.982143**

Probability-Based Calling (continued)

- Problem: some loci may be more indicative of MSI status than others
  - Need weights assigned to each measure at each locus individually

A Bigger ML Problem

- Solution: instead of individually looking at one locus with *m* features, consider all *n* loci with all *m* features at once
  - Machine learning problem in *n* x *m* dimensions

- Exclude loci with too few usable files

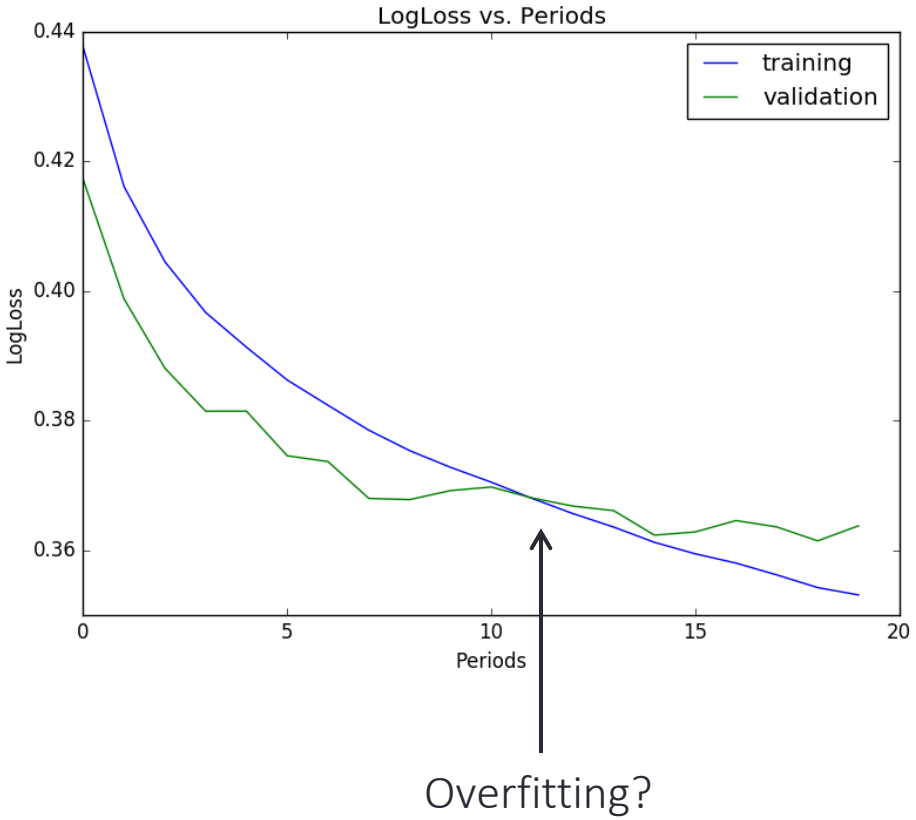
Benefits

- Can report a probability instead of a binary call
- Easy to add new loci
  - Will be considered relative to other loci
- Easy to choose the most relevant loci
  - Defining a selector for STAMP

Machine Learning v2 – Setup

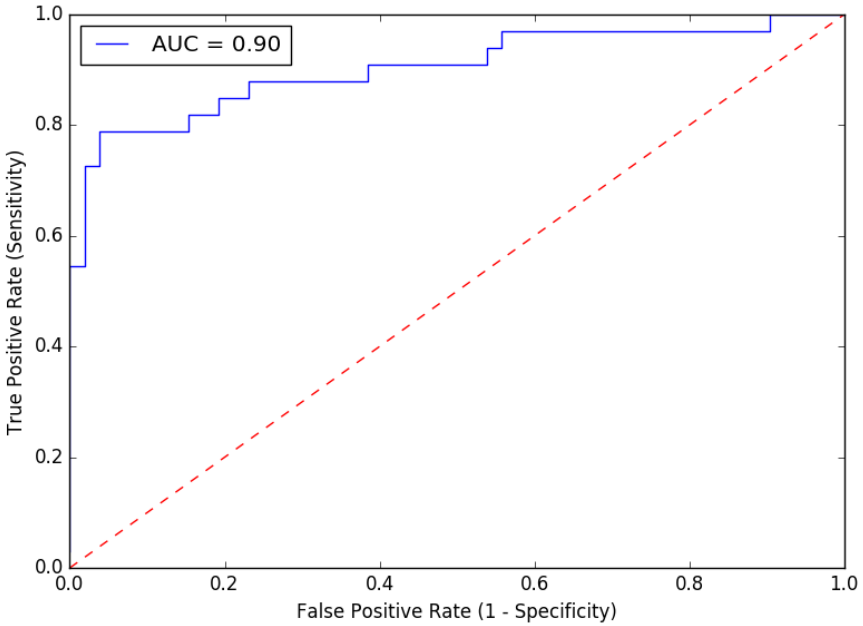
- 21 loci x 4 features each
  - 84-dimensional model
- Training Set
  - 266 cases
    - 124 MSI, 142 MSS
- Validation Set
  - 87 cases
    - 33 MSI, 52 MSS
- Test Set
  - 93 cases
    - 38 MSI, 55 MSS

Machine Learning v2 - Results

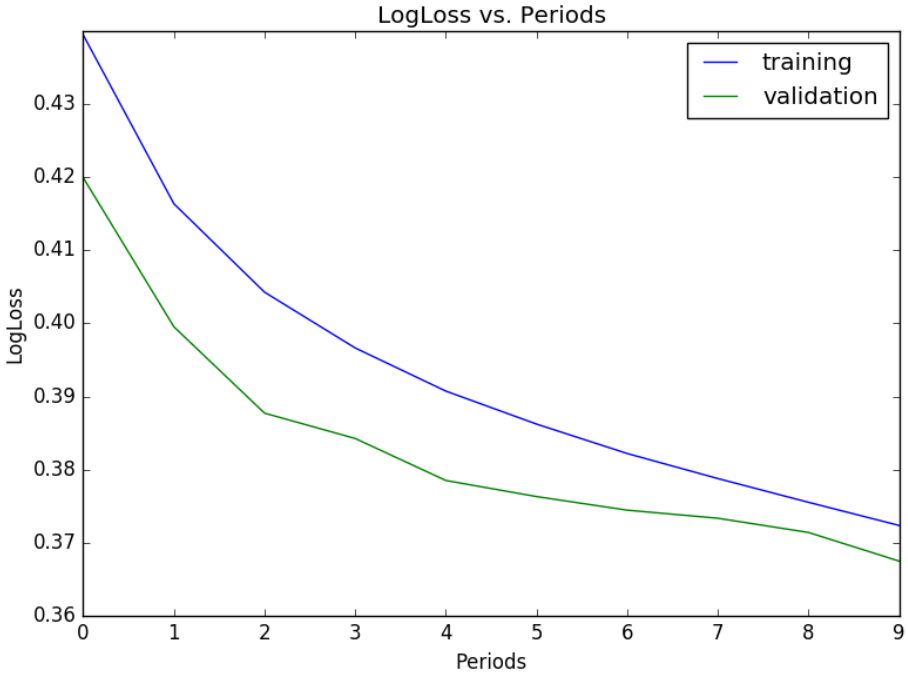


Overfitting?

Learning rate = 0.01
Steps = 200,000
Batch size = 10



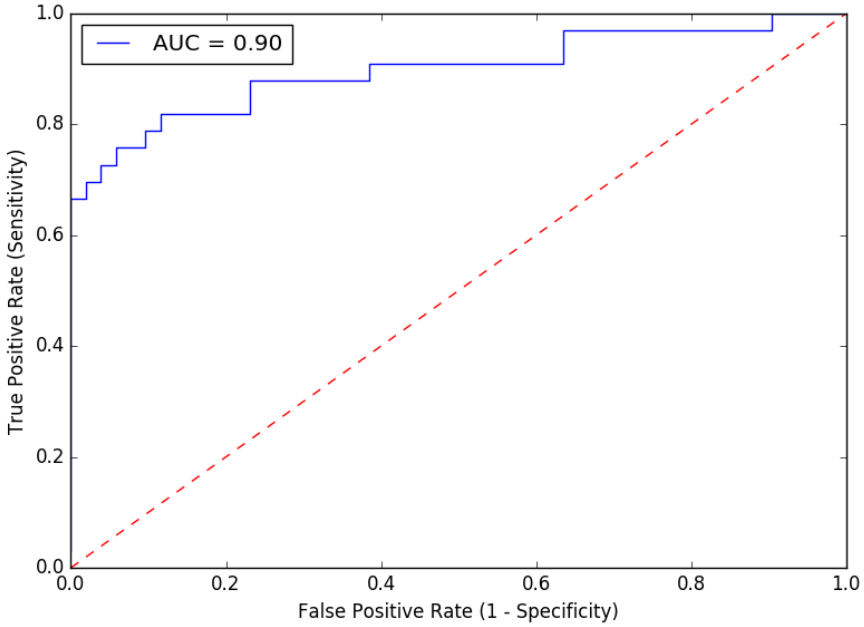
Machine Learning v2 - Results



Learning rate = 0.01

Steps = 100,000

Batch size = 10

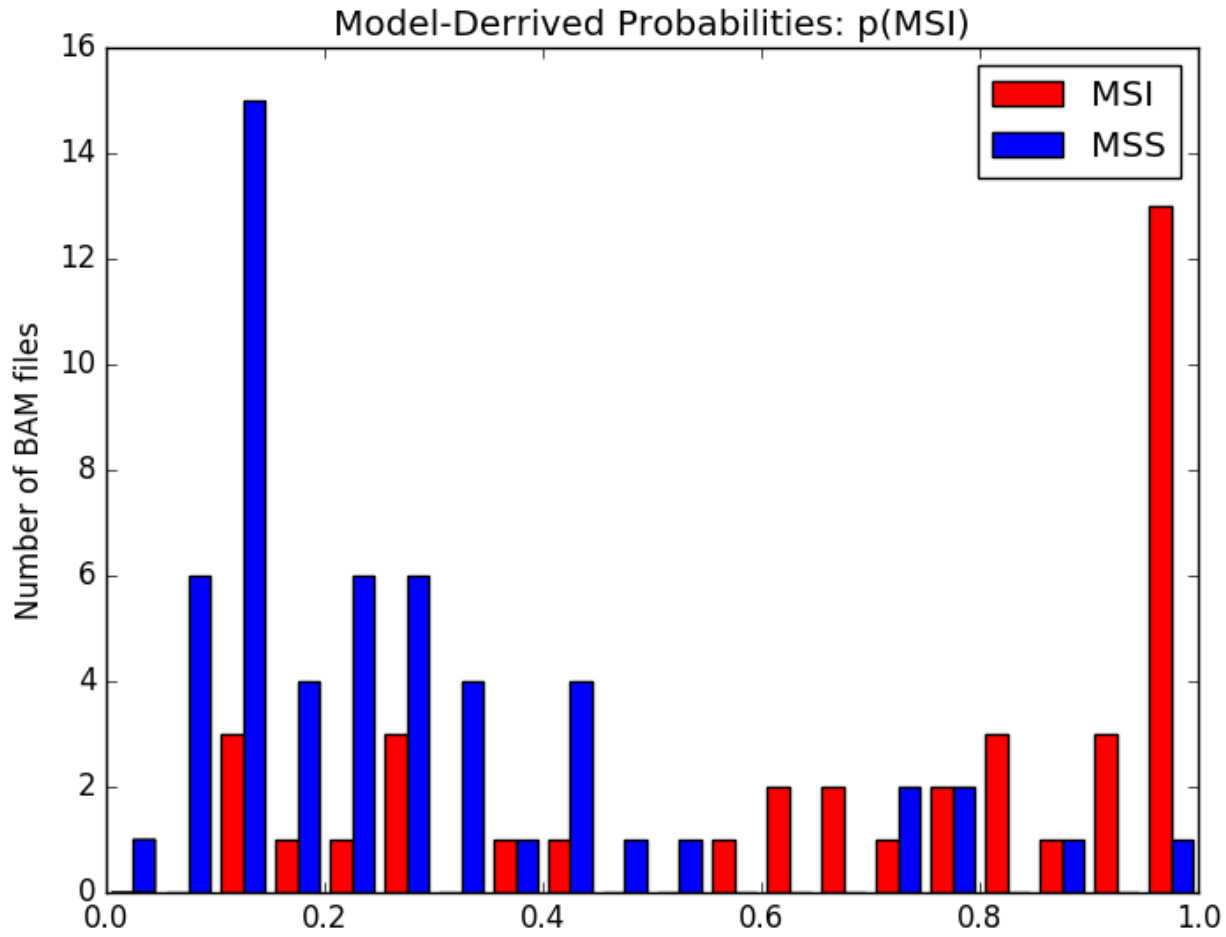


Machine Learning v2 – Test Set

- Loci examined
  - MSI-11
  - MSI-14
  - H-10
  - HSPH1-T17
  - BAT-26
  - BAT-25
  - MSI-04
  - MSI-06
  - MSI-07
  - MSI-01
  - MSI-03
  - MSI-09
  - H-09
  - H-08
  - H-01
  - H-03
  - H-02
  - H-04
  - H-07
  - H-06
  - H-05

- Threshold: 0.500000
- Total files: 93
- Correct predictions: 76
  - True pos: 28
  - True neg: 48
  - False pos: 7
  - False neg: 10
- **Accuracy: 0.817204**
- **Sensitivity: 0.736842**
- **Specificity: 0.872727**

Machine Learning v2 – Test Set



Machine Learning v2 – Test Set

- Loci examined
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  - MSI-01
  - MSI-03
  - MSI-09
  - H-09
  - H-08
  - H-01
  - H-03
  - H-02
  - H-04
  - H-07
  - H-06
  - H-05

- Upper threshold: 0.550000
- Lower threshold: 0.450000
- Total files: 93
- Indeterminate files: 2
- Predictions: 91
- Correct predictions: 75
  - True pos: 28
  - True neg: 47
  - False pos: 6
  - False neg: 10
- **Accuracy: 0.824176**
- **Sensitivity: 0.736842**
- **Specificity: 0.886792**
- **Indeterminate: 2%**

Summary

- Best AUC of ≈90%
  - Measures the predictive ability of the model
- Maximum accuracy of ≈82% on test set
  - Slightly more accurate on called cases if ‘indeterminate’ status is allowed
- Machine learning using all loci and all features is likely the best option
  - More samples with better depth to train on

Further Investigations

Improve Existing Model

- Get more BAM files
  - Training, validation and test sets are stale
  - Get BAM files with better coverage for more accurate results, better prediction
- Research clinical relevance of MSI diagnosis
  - Fine-tune parameters to favor either false positives or false negatives
- Regularization
  - L2 regularization
    - Penalizes any weight that is too large, drives weights asymptotically to 0
  - L1 regularization
    - Penalizes total weight of all features, drives non-informative weights to 0

Further Investigations

Expand Existing Model

- Add features
  - Earth mover distance
- Add a label for ‘MSI-L’ and ‘MSI-H’
- Investigate more loci
  - Hause et al. used 223,082 microsatellite loci to develop a MSI calling method with 96.6% accuracy
- Train using matched normal samples
  - Detect low, baseline level of MSI in tumor samples

Further Investigations

Investigate New Models

- Linear classifier models using different features
  - Feature crosses
- Neural nets
  - Multi-class neural nets
    - Require more training, validation and test data, overfitting concern