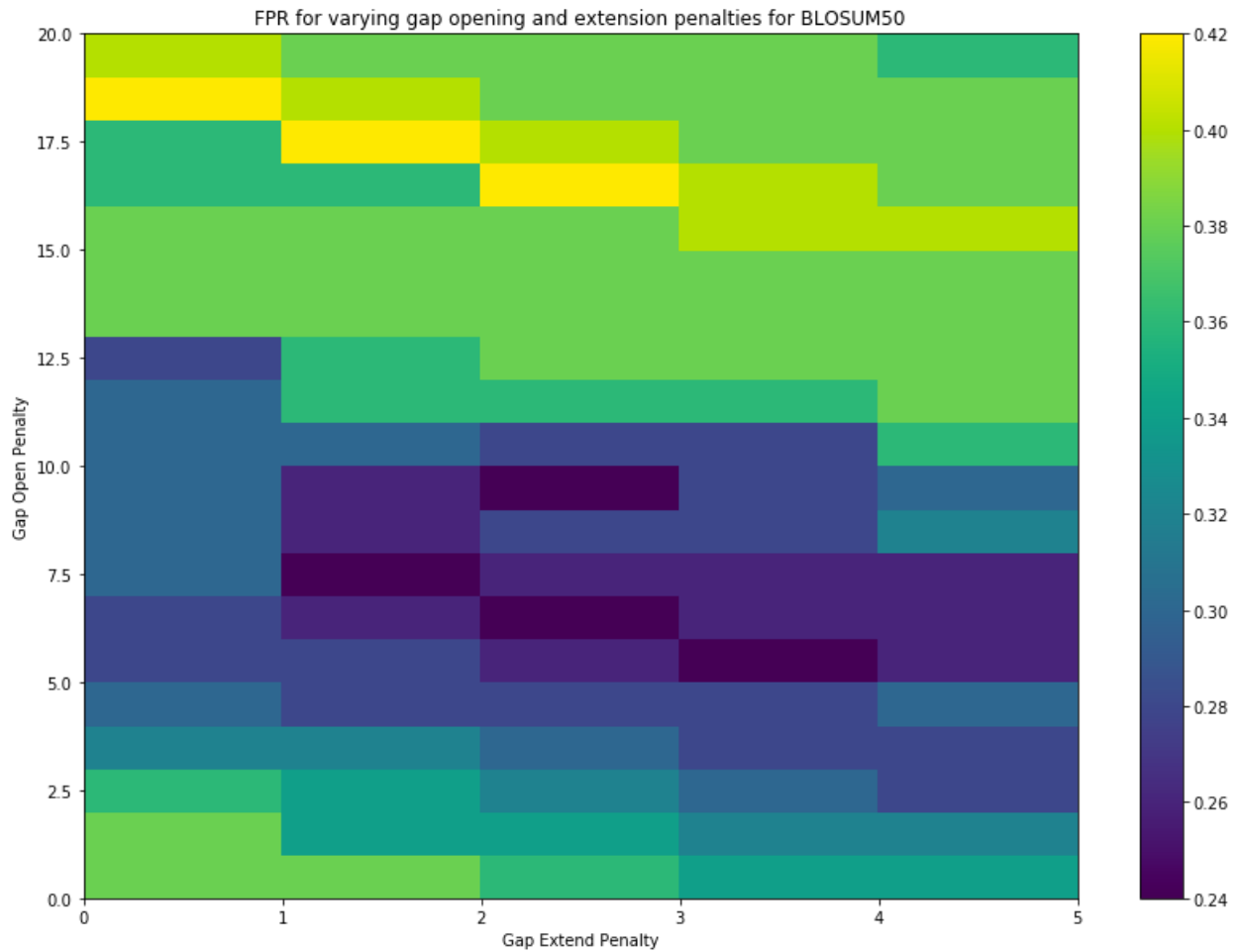


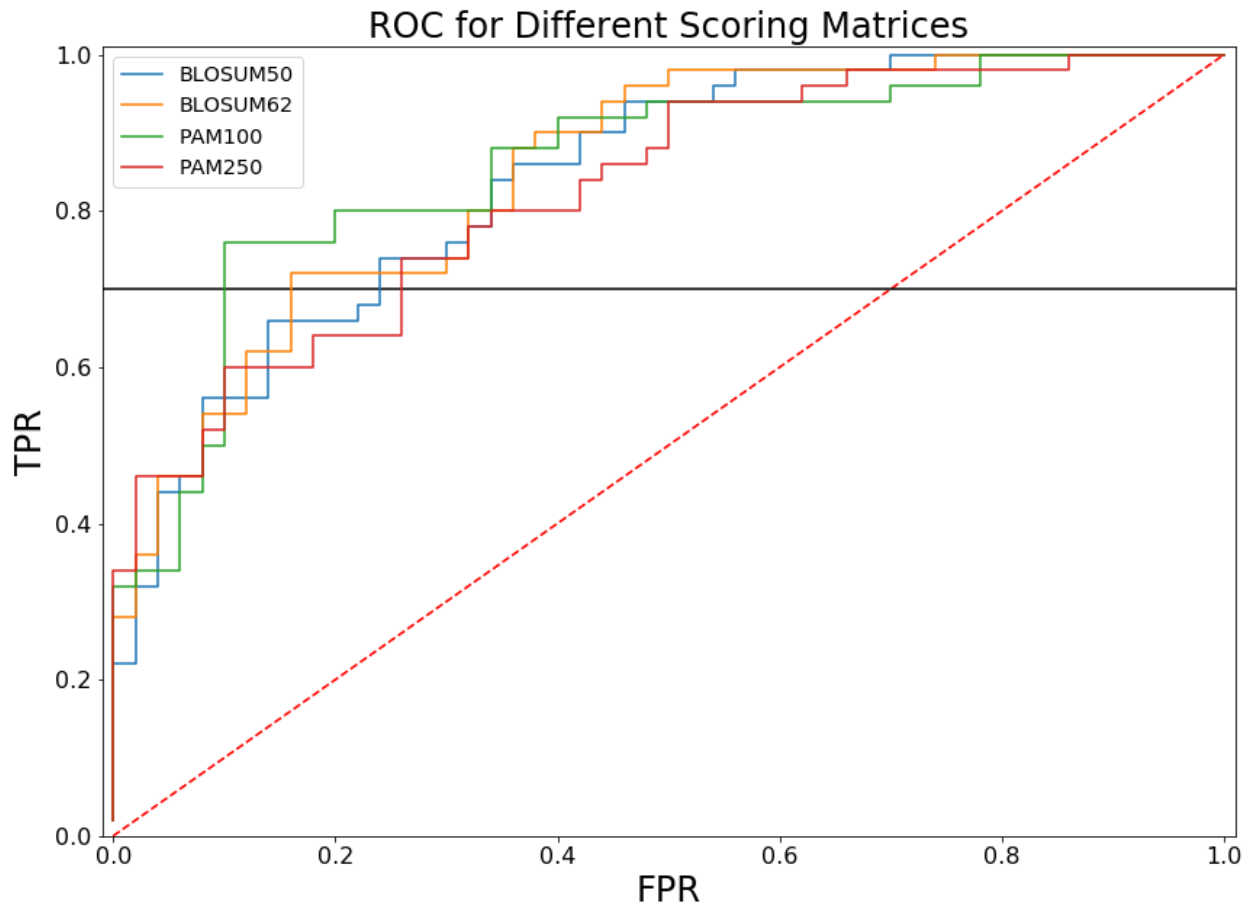
HW3
Greyson Lewis

Part 1

- 1) Implemented and uploaded onto github. The best false positive rate I can achieve with the BLOSUM50 matrix is 0.24 at gap open = 6 and gap extension = 4. However, there are other combinations which appear to give identical FPRs.

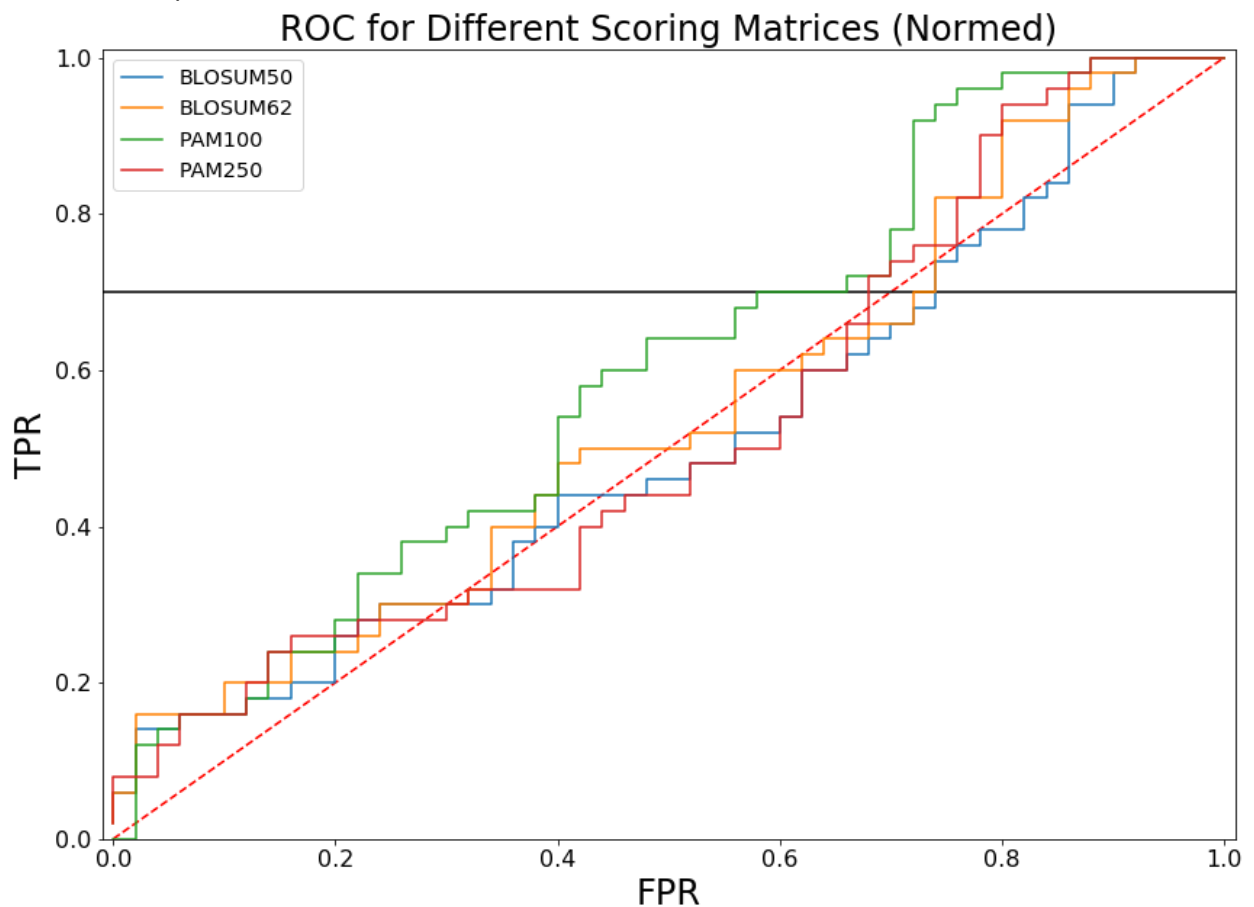


- 2) PAM100 scores the best, at an FPR of 0.1



- 3) If the scores are normed by the length of the shorter sequence, PAM100 remains the best-performing substitution matrix. However, PAM250 gets much better. The FPRs get worse for normed scores, because dividing score by the length of the sequence compresses the range of scores, making the positive and negative alignments more

difficult to separate.



Part 2

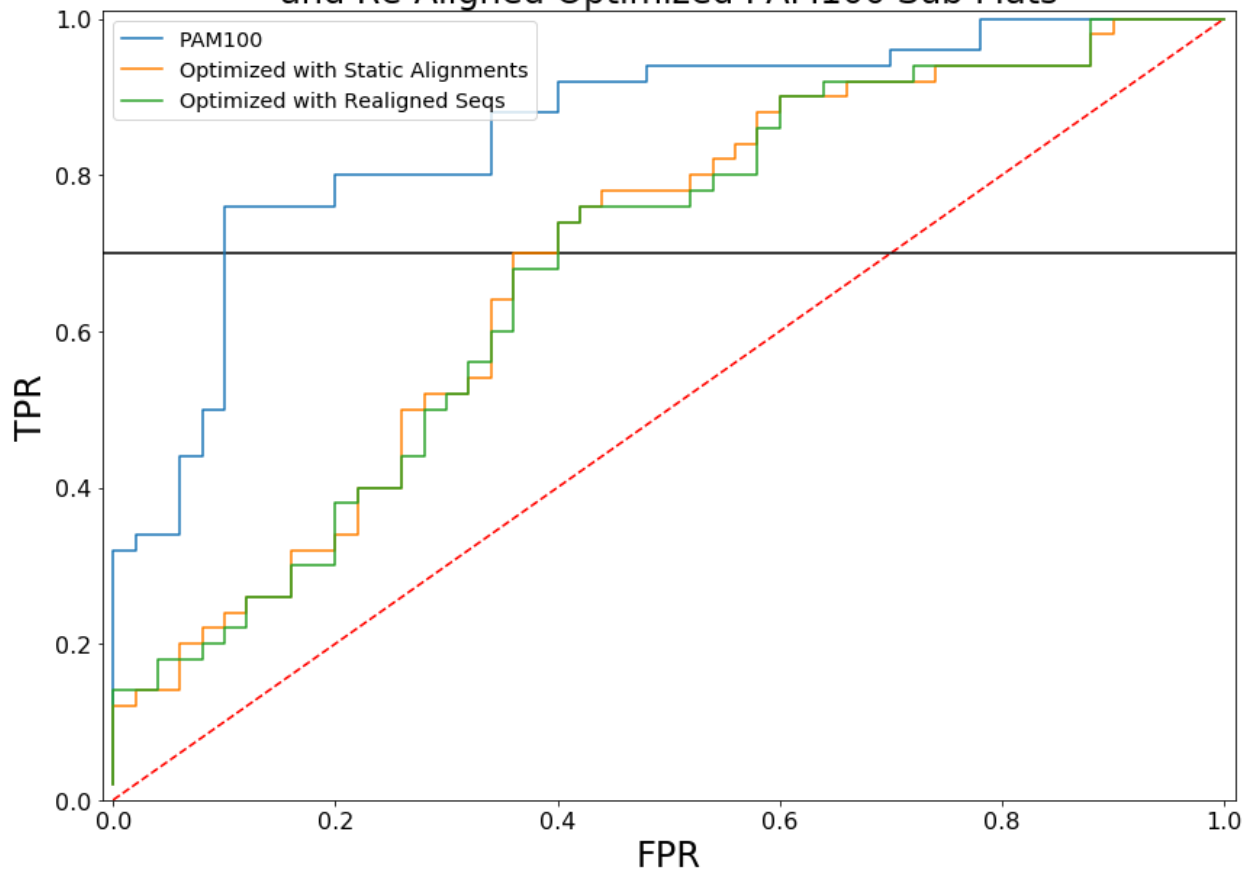
- 1) I implemented a simple version of simulated annealing. This works as follows. First, generate a rule for determining which new substitution matrices are accessible in a single step from the old substitution matrix. Next, create an annealing schedule which starts out at a high 'temperature' and gradually decreases. Iterate over the schedule. At each step, propose a candidate new substitution matrix. Compute f = the sum of TPRs for FPRs of 0, 0.1, 0.2, 0.3 for both the old substitution matrix and the candidate. If $f_{new} > f_{old}$, set $f_{old} = f_{new}$ and iterate again. Otherwise, if $\exp((f_{new} - f_{old}) / \text{temperature}) > \text{random}(0, 1)$, set $f_{old} = f_{new}$ and iterate again. If not, keep the same old matrix and iterate again.

In order to increase the probability that a new substitution matrix would do a better job of separating positive pairs from negative pairs, I computed the amino acid substitution frequencies across all of the positive pairs and negative pairs. If the frequency of a particular substitution was greater among the positive pairs than the negative pairs, a candidate substitution matrix would have a random value between 0 and 1 added to its current value.

- 2) I do not see improvement in fitness after running my optimization. This likely is either because my method of generating candidate substitution matrices is bad, or because I

did not run simulated annealing for long enough (1000 iterations).

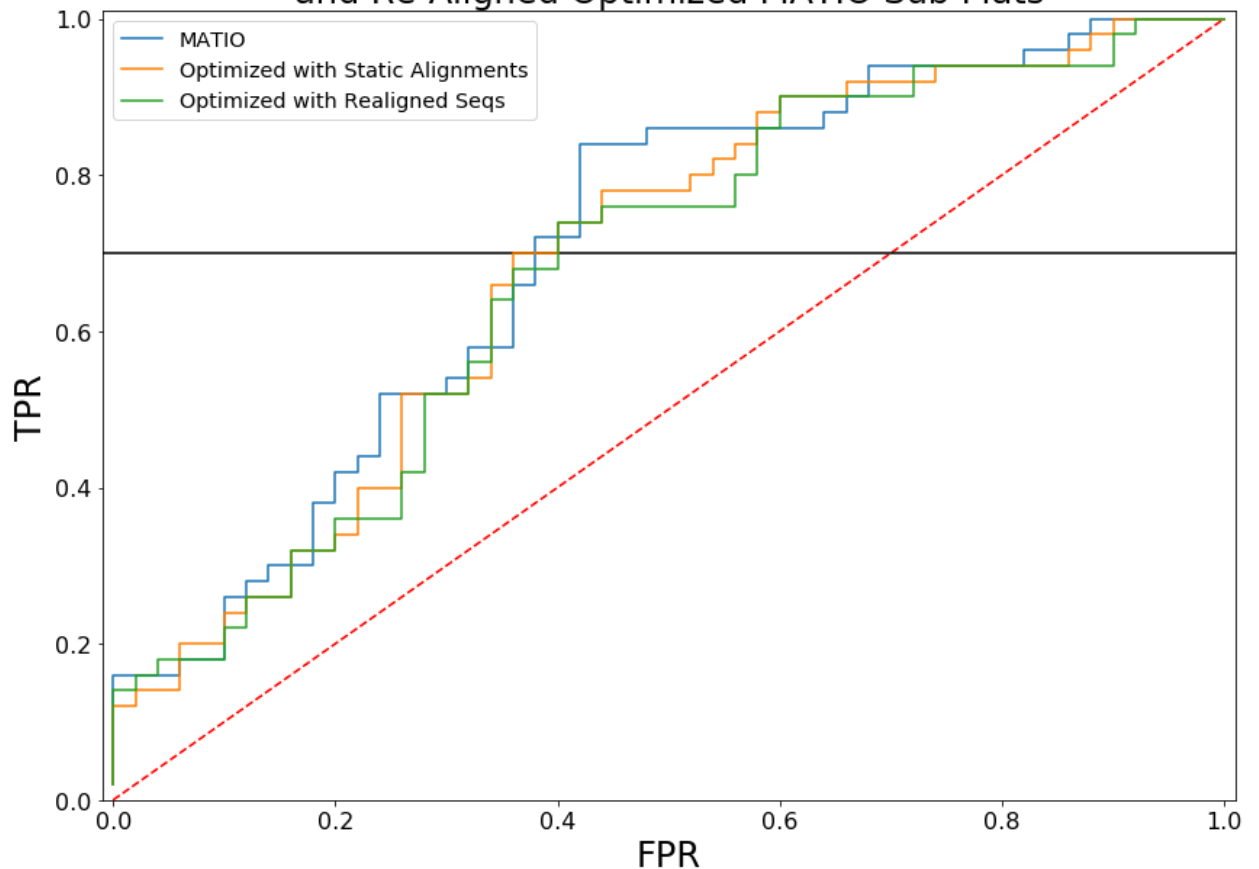
ROC for Original, Static-Alignment Optimized,
and Re-Aligned Optimized PAM100 Sub Mats



- 3) Optimization slightly improved the TPR, though it's difficult to say whether the change was random or actually directed. Re-optimization based on sequenced aligned after the first set of optimization resulted in a TPR slightly worse than the original MATIO matrix. Again, it is difficult to say whether the changes are due to a poor neighbor-generation

strategy for candidate matrices or if the number of iterations needs to be increased.

ROC for Original, Static-Alignment Optimized, and Re-Aligned Optimized MATIO Sub Mats



- 4) See 1). Improvement could occur through a better method of generating candidate substitution matrices, or by running the optimization for long/in parallel.
- 5) To demonstrate general utility, a substitution matrix should generate alignments that correspond to regions of functional and/or structural equivalence. Additionally, it should score unlikely/poor alignments as such, to convey to the user that the produced alignment, while optimal, is not good.

Matrices:

Pam 100 after optimization:

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Pam 100 after optimization with sequence realigned by optimized matrix

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MATIO after optimization

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MATIO after optimization with sequence realigned by optimized matrix

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