An Investigation on the Efficiency of Sequence Alignment Tools

Final Report

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Abstact

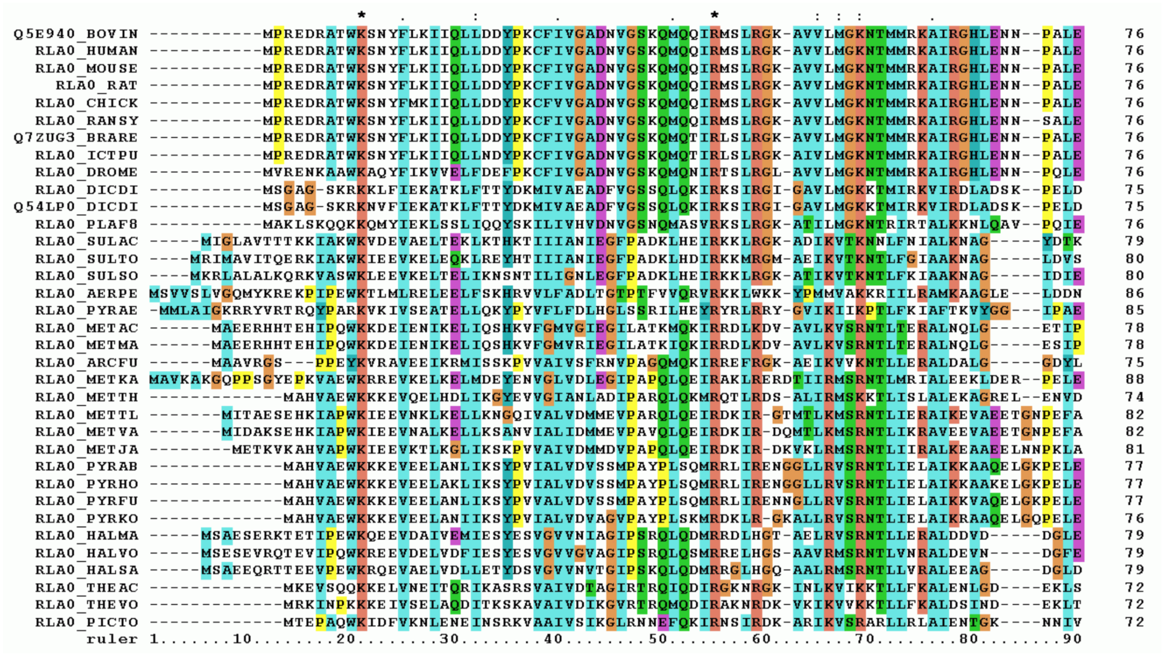
A Sequence Alignment program that utilizes a global alignment algorithm to optimize protein sequence alignments was developed and its computational efficiency was compared to other open source and industry standard programs.

Given mismatch, gap penalty, and match scoring parameters, a similarity matrix is dynamically generated using the Needleman-Wunsch algorithm between two user-provided sequences. The two sequences may then be scored according to user-provided scoring parameters.

Execution of the BSD time command reveals a real time of 0.024 seconds for our dynamically programmed code, as compared to a time of 0.026 seconds for another open source sequence alignment program.

Introduction

Sequence alignment (SA) tools are used by computational biologists to track evolutionary relationships, mutations, and predict structure vs. function relationships among living systems. These tools can be used to assist in the identification of patterns in protein families, prove homology, and assess conservation in genetic characteristics across species. Figure 1 illustrates a multiple sequence alignment analysis of different protein sequences.



```
                                  *   .      :       .          .   *         : : :      .
 Q5E940_BOVIN -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE   76
   RLA0_HUMAN -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE   76
   RLA0_MOUSE -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE   76
     RLA0_RAT -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE   76
   RLA0_CHICK -----------MPREDRATWKSNYFMKIIQLLDDYPKCFVVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE   76
   RLA0_RANSY -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQQIRMSLRGK-AVVLMGKNTMMRKAIRGHLENN--SALE   76
 Q7ZUG3_BRARE -----------MPREDRATWKSNYFLKIIQLLDDYPKCFIVGADNVGSKQMQTIRLSLRGK-AVVLMGKNTMMRKAIRGHLENN--PALE   76
   RLA0_ICTPU -----------MPREDRATWKSNYFLKIIQLLNDYPKCFIVGADNVGSKQMQTIRLSLRGK-AIVLMGKNTMMRKAIRGHLENN--PALE   76
   RLA0_DROME -----------MVRENKAAWKAQYFIKVVELFDEFPKCFIVGADNVGSKQMQNIRTSLRGL-AVVLMGKNTMMRKAIRGHLENN--PQLE   76
   RLA0_DICDI -----------MSGAG-SKRKKLFIEKATKLFTTYDKMIVAEADFVGSSQLQKIRKSIRGI-GAVLMGKKTMIRKVIRDLADSK--PELD   75
 Q54LP0_DICDI -----------MSGAG-SKRKNVFIEKATKLFTTYDKMIVAEADFVGSSQLQKIRKSIRGI-GAVLMGKKTMIRKVIRDLADSK--PELD   75
   RLA0_PLAF8 -----------MAKLSKQQKKQMYIEKLSSLIQQYSKILIVHVDNVGSNQMASVRKSLRGK-ATILMGKNTRIRTALKKNLQAV--PQIE   76
   RLA0_SULAC -----MIGLAVTTTKKIAKWKVDEVAELTEKLKTHKTIIIANIEGFPADKLHEIRKKLRGK-ADIKVTKNNLFNIALKNAG-----YDTK   79
   RLA0_SULTO ----MRIMAVITQERKIAKWKIEEVKELEQKLREYHTIIIANIEGFPADKLHDIRKKMRGM-AEIKVTKNTLFGIAAKNAG-----LDVS   80
   RLA0_SULSO ----MKRLALALKQRKVASWKLEEVKELTELIKNSNTILIGNLEGFPADKLHEIRKKLRGK-ATIKVTKNTLFKIAAKNAG-----IDIE   80
   RLA0_AERPE MSVVSLVGQMYKREKPIPEWKTLMLRELEELFSKHRVVLFADLTGTPTFVVQRVRKKLWKK-YPMMVAKKRIILRAMKAAGLE---LDDN   86
   RLA0_PYRAE -MMLAIGKRRYVRTRQYPARKVKIVSEATELLQKYPYVFLFDLHGLSSRILHEYRYRLRRY-GVIKIIKPTLFKIAFTKVYGG---IPAE   85
   RLA0_METAC ------MAEERHHTEHIPQWKKDEIENIKELIQSHKVFGMVGIEGILATKMQKIRRDLKDV-AVLKVSRNTLTERALNQLG-----ETIP   78
   RLA0_METMA ------MAEERHHTEHIPQWKKDEIENIKELIQSHKVFGMVRIEGILATKIQKIRRDLKDV-AVLKVSRNTLTERALNQLG-----ESIP   78
   RLA0_ARCFU ------MAAVRGS---PPEYKVRAVEEIKRMISSKPVVAIVSFRNVPAGQMQKIRREFRGK-AEIKVVKNTLLERALDALG-----GDYL   75
   RLA0_METKA MAVKAKGQPPSGYEPKVAEWKRREVKELKELMDEYENVGLVDLEGIPAPQLQEIRAKLRERDTIIRMSRNTLMRIALEEKLDER--PELE   88
   RLA0_METTH -------------MAHVAEWKKKEVQELHDLIKGYEVVGIANLADIPARQLQKMRQTLRDS-ALIRMSKKTLISLALEKAGREL--ENVD   74
   RLA0_METTL -------MITAESEHKIAPWKIEEVNKLKELLKNGQIVALVDMMEVPARQLQEIRDKIR-GTMTLKMSRNTLIERAIKEVAEETGNPEFA   82
   RLA0_METVA -------MIDAKSEHKIAPWKIEEVNALKELLKSANVIALIDMMEVPAVQLQEIRDKIR-DQMTLKMSRNTLIKRAVEEVAEETGNPEFA   82
   RLA0_METJA --------METKVKAHVAPWKIEEVKTLKGLIKSKPVVAIVDMMDVPAPQLQEIRDKIR-DKVKLRMSRNTLIIRALKEAAEELNNPKLA   81
   RLA0_PYRAB -------------MAHVAEWKKKEVEELANLIKSYPVIALVDVSSMPAYPLSQMRRLIRENGGLLRVSRNTLIELAIKKAAQELGKPELE   77
   RLA0_PYRHO -------------MAHVAEWKKKEVEELAKLIKSYPVIALVDVSSMPAYPLSQMRRLIRENGGLLRVSRNTLIELAIKKAAKELGKPELE   77
   RLA0_PYRFU -------------MAHVAEWKKKEVEELANLIKSYPVVALVDVSSMPAYPLSQMRRLIRENNGLLRVSRNTLIELAIKKVAQELGKPELE   77
   RLA0_PYRKO -------------MAHVAEWKKKEVEELANIIKSYPVIALVDVAGVPAYPLSKMRDKLR-GKALLRVSRNTLIELAIKRAAQELGQPELE   76
   RLA0_HALMA -----MSAESERKTETIPEWKQEEVDAIVEMIESYESVGVVNIAGIPSRQLQDMRRDLHGT-AELRVSRNTLLERALDDVD-----DGLE   79
   RLA0_HALVO -----MSESEVRQTEVIPQWKREEVDELVDFIESYESVGVVGVAGIPSRQLQSMRRELHGS-AAVRMSRNTLVNRALDEVN-----DGFE   79
   RLA0_HALSA -----MSAEEQRTTEEVPEWKRQEVAELVDLLETYDSVGVVNVTGIPSKQLQDMRRGLHGQ-AALRMSRNTLLVRALEEAG-----DGLD   79
   RLA0_THEAC -------------MKEVSQQKKELVNEITQRIKASRSVAIVDTAGIRTRQIQDIRGKNRGK-INLKVIKKTLLFKALENLGD----EKLS   72
   RLA0_THEVO -------------MRKINPKKKEIVSELAQDITKSKAVAIVDIKGVRTRQMQDIRAKNRDK-VKIKVVKKTLLFKALDSIND----EKLT   72
   RLA0_PICTO -------------MTEPAQWKIDFVKNLENEINSRKVAAIVSIKGLRNNEFQKIRNSIRDK-ARIKVSRARLLRLAIENTGK----NNIV   72
        ruler 1.......10........20........30........40........50........60........70........80.......90
```

Figure 1: Representation of a protein multiple sequence alignment produced with ClustalW. The sequences are instances of the acidic ribosomal protein P0 homolog (L10E) encoded by the Rplp0 gene from multiple organisms. Only the first 90 positions of the alignment are displayed. The colors represent the amino acid conservation according to the properties and distribution of amino acid frequencies in each column. Note the two completely conserved residues arginine (R) and lysine (K) marked with an asterisk at the top of the alignment. [1]

Sequences of interest are usually protein, DNA, or RNA chains. For different sequence types, different analytical methods must be employed for comparison. Therefore, SA tools are often found in a variety of forms, tailored to address specific biological problems. [2]

Depending on the desired level of accuracy and computational efficiency, there are many different types of alignment methods. Global alignments attempt to align and match every residue or nucleotide in each sequence, making them better suited for more similar and equal-sized sequences. Local alignments are better suited for locating smaller subsequences that match between two sequences. For proteins, this could indicate a shared protein family and similar domains of interest and function. However, analyzing local alignments requires additional levels of complexity and constraint parameters, which can drastically increase computational costs. [3]

Due to the complexity and length of most sequences of interest, the computational efficiency of SA tools is of particular importance. This project will analyze the Needleman-Wunsch algorithm for assessing the global alignment of two sequences, and compare the efficiency of the algorithm with other open source, global alignment SA tools.

Needleman-Wunsch Algorithm

The Needleman-Wunsch algorithm is a global alignment method used in many sequence alignment tools. The algorithm compares two sequences by dynamically generating a similarity matrix with scoring values calculated based user-provided parameters.

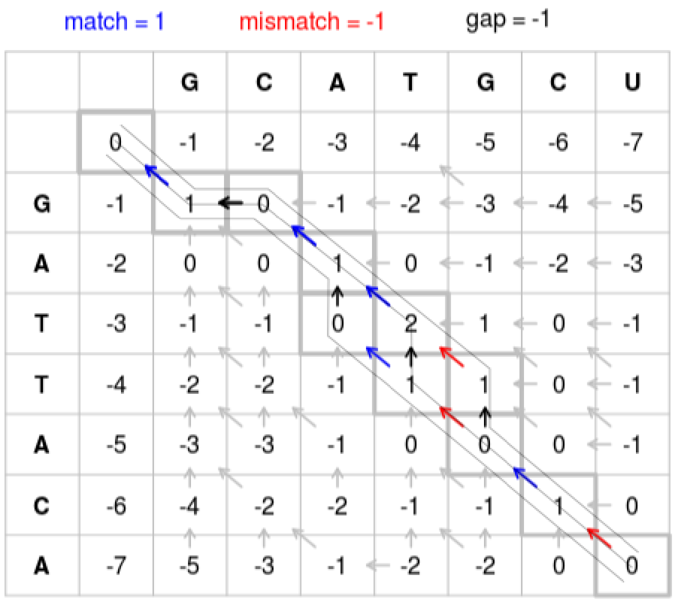
Equation 1 defines the Needleman-Wunsch algorithm.

$$\begin{aligned} M(0,j) &= j \times p && \text{for first row, where p is the gap penalty} \\ M(i,0) &= i \times p && \text{for first column} \end{aligned}$$

$$M(0,j) = max \begin{cases} M(i-1,j)+p & \text{top} \\ M(i,j-1)+p & \text{left} \\ M(i-1,j-1)+s(a_j,b_i) & \text{diagonal} \end{cases} \tag{1}$$

Where $s(a_j, b_i)$ = match/mismatch score for sites j and i in sequences a and b. [4]

Figure 2 provides a graphical illustration of how the Needleman-Wunsch algorithm populates a similarity matrix of scoring values.



		G	C	A	T	G	C	U
	0	-1	-2	-3	-4	-5	-6	-7
G	-1	1	0	-1	-2	-3	-4	-5
A	-2	0	0	1	0	-1	-2	-3
T	-3	-1	-1	0	2	1	0	-1
T	-4	-2	-2	-1	1	1	0	-1
A	-5	-3	-3	-1	0	0	0	-1
C	-6	-4	-2	-2	-1	-1	1	0
A	-7	-5	-3	-1	-2	-2	0	0

Figure 2: Needleman-Wunsch pairwise sequence alignment. [5]

The gray arrows represent the filling of each position in the matrix by the match. mismatch, or gap scores. This algorithm starts at zero in one corner of the matrix (second row, second column). In order to determine the score of a particular position in the matrix, the corresponding residue in its column (sequence 1) and its row (sequence 2) are compared. Each of the positions beside the position is scored and the highest existing score calculated goes in the current position. For the second row, we add 0, -1, -2, -3, -4, -5, -6, -7 due to the lack of top and top-left cells. Then, in order to find the best alignment, we backtrack to the origin by following the arrows. The alignment is constructed by allowing the diagonal arrows to represent match or mismatches, horizontal arrows to represent gaps after the letter, and vertical arrows to represent a gap after the letter in the top sequence. [5]

Methodology and Results

All code was run on the TACC Lonestar5 system, with TP53 protein sequences for Mouse and Humans as comparison sequences. The GNU GDB debugger was used in the debugging and analysis of the code, and the Intel ICC compiler was used to compile the code.

Table 1 compares execution time under the BSD time command of our code with other SA programs.

Table 1: Comparison of Execution Times to Other SA Tools

	Open Source Code - NW	Ours - NW	MAFFT	MUSCLE	Clustal Omega
Real Time (s)	0.026	0.024	0.004	0.014	0.005
User Time (s)	0.016	0.016	0.000	0.004	0.000
System Time (s)	0.006	0.004	0.000	0.004	0.000

Dynamic generation of the similarity matrix allows for a slight increase in computational efficiency for our code, as compared with other open source codes.

Figures 3 and 4 show the flat profile and call graph as generated from GNU gprof for our code.

```
Flat profile:

Each sample counts as 0.01 seconds.
 no time accumulated

  %   cumulative   self              self     total
 time   seconds   seconds    calls  Ts/call  Ts/call  name
  0.00      0.00     0.00   154073     0.00     0.00  match_score
  0.00      0.00     0.00   153272     0.00     0.00  max_array
  0.00      0.00     0.00        2     0.00     0.00  reverseSeq
  0.00      0.00     0.00        1     0.00     0.00  finalize
  0.00      0.00     0.00        1     0.00     0.00  printMatrix
```

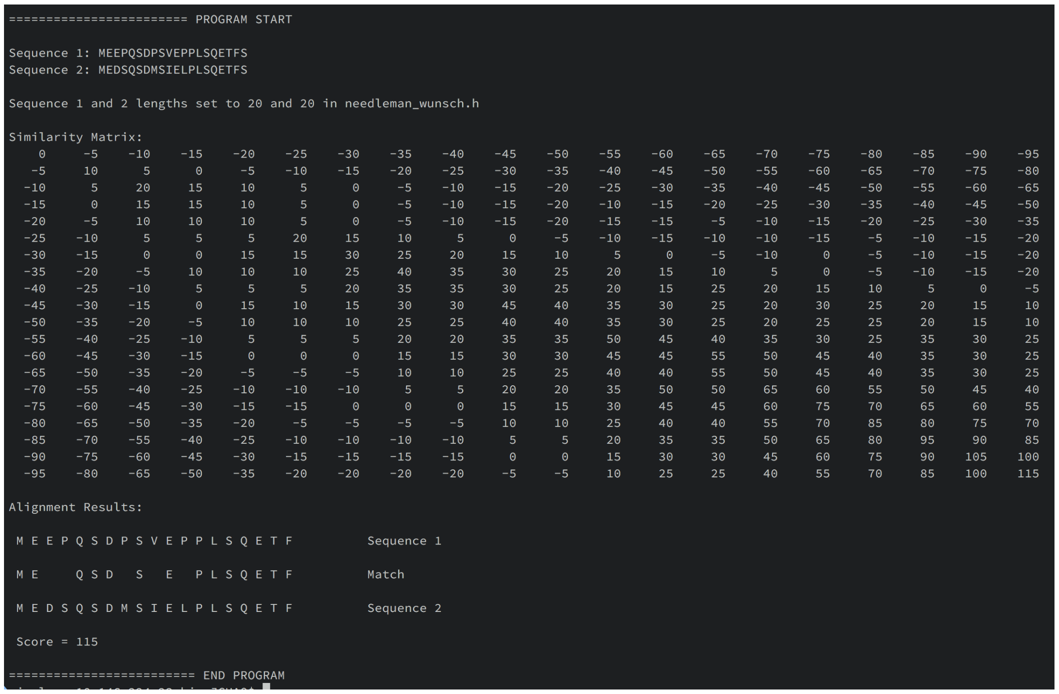
Figure 3: Flat profile of needleman_wunsch code.

```
                     Call graph


granularity: each sample hit covers 2 byte(s) no time propagated

index % time    self  children    called     name
                0.00    0.00     393/154073      finalize [4]
                0.00    0.00  153680/154073      main [12]
[1]      0.0    0.00    0.00  154073         match_score [1]
-----------------------------------------------
                0.00    0.00  153272/153272      main [12]
[2]      0.0    0.00    0.00  153272         max_array [2]
-----------------------------------------------
                0.00    0.00       2/2           finalize [4]
[3]      0.0    0.00    0.00       2         reverseSeq [3]
-----------------------------------------------
                0.00    0.00       1/1           main [12]
[4]      0.0    0.00    0.00       1         finalize [4]
                0.00    0.00     393/154073      match_score [1]
                0.00    0.00       2/2           reverseSeq [3]
-----------------------------------------------
                0.00    0.00       1/1           main [12]
[5]      0.0    0.00    0.00       1         printMatrix [5]
-----------------------------------------------
```

Figure 4: Call graph of needleman_wunsch code.

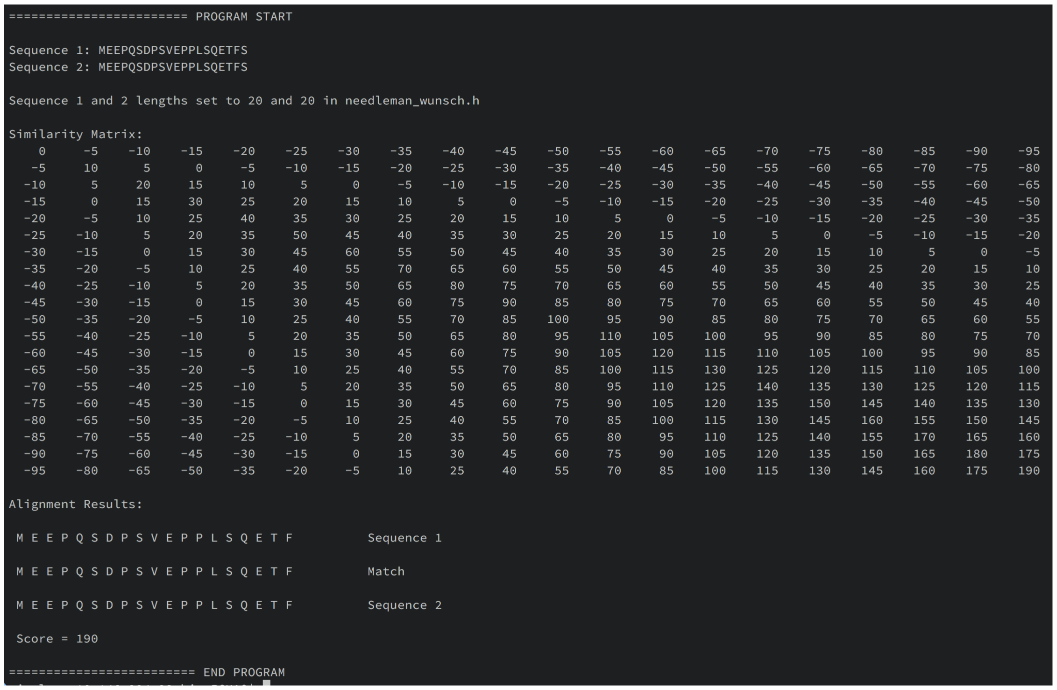
Our code was continuously profiled and restructured to allow for better efficiency between function calls and code execution, thereby decreasing computational costs.

Figures 5 and 6 show output examples from our code comparing TP53 protein subsequences from mouse and human specimens.



```
====================== PROGRAM START

Sequence 1: MEEPQSDPSVEPPLSQETFS
Sequence 2: MEDSQSDMSIELPLSQETFS

Sequence 1 and 2 lengths set to 20 and 20 in needleman_wunsch.h

Similarity Matrix:
     0     -5    -10    -15    -20    -25    -30    -35    -40    -45    -50    -55    -60    -65    -70    -75    -80    -85    -90    -95
    -5     10      5      0     -5    -10    -15    -20    -25    -30    -35    -40    -45    -50    -55    -60    -65    -70    -75    -80
   -10      5     20     15     10      5      0     -5    -10    -15    -20    -25    -30    -35    -40    -45    -50    -55    -60    -65
   -15      0     15     15     10      5      0     -5    -10    -15    -20    -10    -15    -20    -25    -30    -35    -40    -45    -50
   -20     -5     10     10     10      5      0     -5    -10    -15    -20    -15    -15     -5    -10    -15    -20    -25    -30    -35
   -25    -10      5      5      5     20     15     10      5      0     -5    -10    -15    -10    -10    -15     -5    -10    -15    -20
   -30    -15      0      0     15     15     30     25     20     15     10      5      0     -5    -10      0     -5    -10    -15    -20
   -35    -20     -5     10     10     10     25     40     35     30     25     20     15     10      5      0     -5    -10    -15    -20
   -40    -25    -10      5      5      5     20     35     35     30     25     20     15     25     20     15     10      5      0     -5
   -45    -30    -15      0     15     10     15     30     30     45     40     35     30     25     20     30     25     20     15     10
   -50    -35    -20     -5     10     10     10     25     25     40     40     35     30     25     20     25     25     20     15     10
   -55    -40    -25    -10      5      5      5     20     20     35     35     50     45     40     35     30     25     35     30     25
   -60    -45    -30    -15      0      0      0     15     15     30     30     45     45     55     50     45     40     35     30     25
   -65    -50    -35    -20     -5     -5     -5     10     10     25     25     40     40     55     50     45     40     35     30     25
   -70    -55    -40    -25    -10    -10    -10      5      5     20     20     35     50     50     65     60     55     50     45     40
   -75    -60    -45    -30    -15    -15      0      0      0     15     15     30     45     45     60     75     70     65     60     55
   -80    -65    -50    -35    -20     -5     -5     -5     -5     10     10     25     40     40     55     70     85     80     75     70
   -85    -70    -55    -40    -25    -10    -10    -10    -10      5      5     20     35     35     50     65     80     95     90     85
   -90    -75    -60    -45    -30    -15    -15    -15    -15      0      0     15     30     30     45     60     75     90    105    100
   -95    -80    -65    -50    -35    -20    -20    -20    -20     -5     -5     10     25     25     40     55     70     85    100    115

Alignment Results:

 M E E P Q S D P S V E P P L S Q E T F          Sequence 1

 M E     Q S D   S   E   P L S Q E T F          Match

 M E D S Q S D M S I E L P L S Q E T F          Sequence 2

 Score = 115

======================== END PROGRAM
```

Figure 5: Sample output comparing a subsequence of TP53 protein in human vs. rat.



```
====================== PROGRAM START

Sequence 1: MEEPQSDPSVEPPLSQETFS
Sequence 2: MEEPQSDPSVEPPLSQETFS

Sequence 1 and 2 lengths set to 20 and 20 in needleman_wunsch.h

Similarity Matrix:
     0     -5    -10    -15    -20    -25    -30    -35    -40    -45    -50    -55    -60    -65    -70    -75    -80    -85    -90    -95
    -5     10      5      0     -5    -10    -15    -20    -25    -30    -35    -40    -45    -50    -55    -60    -65    -70    -75    -80
   -10      5     20     15     10      5      0     -5    -10    -15    -20    -25    -30    -35    -40    -45    -50    -55    -60    -65
   -15      0     15     30     25     20     15     10      5      0     -5    -10    -15    -20    -25    -30    -35    -40    -45    -50
   -20     -5     10     25     40     35     30     25     20     15     10      5      0     -5    -10    -15    -20    -25    -30    -35
   -25    -10      5     20     35     50     45     40     35     30     25     20     15     10      5      0     -5    -10    -15    -20
   -30    -15      0     15     30     45     60     55     50     45     40     35     30     25     20     15     10      5      0     -5
   -35    -20     -5     10     25     40     55     70     65     60     55     50     45     40     35     30     25     20     15     10
   -40    -25    -10      5     20     35     50     65     80     75     70     65     60     55     50     45     40     35     30     25
   -45    -30    -15      0     15     30     45     60     75     90     85     80     75     70     65     60     55     50     45     40
   -50    -35    -20     -5     10     25     40     55     70     85    100     95     90     85     80     75     70     65     60     55
   -55    -40    -25    -10      5     20     35     50     65     80     95    110    105    100     95     90     85     80     75     70
   -60    -45    -30    -15      0     15     30     45     60     75     90    105    120    115    110    105    100     95     90     85
   -65    -50    -35    -20     -5     10     25     40     55     70     85    100    115    130    125    120    115    110    105    100
   -70    -55    -40    -25    -10      5     20     35     50     65     80     95    110    125    140    135    130    125    120    115
   -75    -60    -45    -30    -15      0     15     30     45     60     75     90    105    120    135    150    145    140    135    130
   -80    -65    -50    -35    -20     -5     10     25     40     55     70     85    100    115    130    145    160    155    150    145
   -85    -70    -55    -40    -25    -10      5     20     35     50     65     80     95    110    125    140    155    170    165    160
   -90    -75    -60    -45    -30    -15      0     15     30     45     60     75     90    105    120    135    150    165    180    175
   -95    -80    -65    -50    -35    -20     -5     10     25     40     55     70     85    100    115    130    145    160    175    190

Alignment Results:

 M E E P Q S D P S V E P P L S Q E T F          Sequence 1

 M E E P Q S D P S V E P P L S Q E T F          Match

 M E E P Q S D P S V E P P L S Q E T F          Sequence 2

 Score = 190

======================== END PROGRAM
```

Figure 6: Sample output comparing a subsequence of TP53 protein in human vs. human.

Discussion

We initially proposed to compare our sequence alignment tool with other industry standard SA tools used by bioinformaticians. However, further investigation into how industry standard tools operate reveal the use of multiple alignment algorithms, as well as machine learning software and protein family prediciton tools for further optimization.

Since our alignment tool only utilizes a global alignment algorithm, an open source code also written in C was found for comparison purposes. A comparison of execution and run times between our code and the open source code revealed increases in computational efficiency and speed.

Conclusion

A sequence alignment program that utilizes the Needleman-Wunsch algorithm to globally align protein sequences was developed, and its computational efficiency was compared to other industry standard programs.

Tools such as GNU GDB and gprof were used to debug and profile our code. Execution of the BSD time command reveals increases in computational efficiency as compared to other open source programs.

Future studies may include analysis of optimization differences accross different compilers.

Appendix

The following contains source code taken from our program.

main.c

```
// main.c file for needleman_wunsch alignment program
#include "needleman_wunsch.h"
#include <stdio.h>
#include <stdlib.h>

// define scoring parameters
#define gap_penalty -5

#define A(i,j) score[(i) + (j)*n]

// variable declarations
int i, j, k, l, count = 0;
int score_current = 0, score_diagonal = 0, score_up = 0, score_left = 0;
int sizeAlign1 = 0;
int sizeAlign2 = 0;

// main.c takes two sequences as command line arguments
int main(int argc, char *argv[]) {

    // allocating plenty of memory to store each of the sequences
    char *align1 = malloc(500*sizeof(char));
    char *align2 = malloc(500*sizeof(char));

    if (argc != 3) {
        printf ("\nUsage: \n./Needleman_Wunsch <sequence1> <sequence2>\n\n");
        return 0;
    }

    printf("\n======================= PROGRAM START \n\n");

    int values[3];
    char seq1[m], seq2[n];

    // print sequences and user-defined lengths
    for (i=1; i<argc; i++)
        printf("Sequence %d: %s\n", i, argv[i]);
    printf("\nSequence 1 and 2 lengths set to %d and %d in needleman_wunsch.h\n", m, n);

    // assign command line arguments to array of chars for each sequence
    for (i=0; i<=m; i++)
        seq1[i] = argv[1][i];
    for (i=0; i<=n; i++)
        seq2[i] = argv[2][i];

    // allocate memory for scoring matrix
    int *score = malloc ((m + 1)*(n + 1)*sizeof(int));

    // initially set all values to 0
    for (i=0; i< m; i++)
        for (j=0; j< n; j++)
            A(i,j) = 0;

    // fill in first row
    for (k=0; k<n; k++)
        A(0,k) = gap_penalty * k;

    // fill in first column
    for (l=0; l<m; l++)
        A(l,0) = gap_penalty * l;

    // fill in other values
    for (i=1; i<m; i++) {
        for (j=1; j<n; j++) {
            values[0] = A(i-1, j) + gap_penalty;
            values[1] = A(i, j-1) + gap_penalty;
            values[2] = A(i-1, j-1) + match_score (seq1[i-1], seq2[j-1]);
            A(i,j) = max_array(values, 3);
        }
    }

    // print Similarity Matrix
    printf ("\nSimilarity Matrix: ");
    printMatrix(score);

    // begin traceback
    i = m - 1;
    j = n - 1;

    while (i>0 && j>0) {
        score_current = A(i,j);
        score_diagonal = A((i-1),(j-1));
        score_up = A((i),(j-1));
        score_left = A((i-1),(j));

        if (score_current == score_diagonal + match_score(seq1[i-1], seq2[j-1])) {
            align1[count] = seq1[i-1];
            align2[count] = seq2[i-1];
            i --;
            j --;
            sizeAlign1 ++;
            sizeAlign2 ++;
            count ++;
        }
        else if (score_current == score_left + gap_penalty) {
            align1[count] = seq1[i-1];
            align2[count] = '-';
            i --;
            sizeAlign1 ++;
            sizeAlign2 ++;
            count ++;
        }
        else if (score_current == score_up + gap_penalty) {
            align1[count] = '-';
            align2[count] = seq2[j-1];
            j --;
```

```
            sizeAlign1 ++;
            sizeAlign2 ++;
            count ++;
        }
    }

    // finish tracing up to top left cell
    while (i > 0) {
        align1[count] = seq1[i-1];
        align2[count] = '-';
        i --;
        sizeAlign1 ++;
        sizeAlign2 ++;
        count ++;
    }
    while (j > 0) {
        align1[count] = '-';
        align2[count] = seq2[j-1];
        j --;
        sizeAlign1 ++;
        sizeAlign2 ++;
        count ++;
    }

    finalize (align1, align2, sizeAlign1, sizeAlign2);

    printf("\n========================= END PROGRAM \n");
    return 0;
}
```

needleman_wunsch.h

```
// needleman_wunsch.h header file for needleman_wunsch alignment program

#ifndef needleman_wunch_h // Include guard
#define needleman_wunch_h

#define m 20  // m = length of Sequence 1
#define n 20  // n = length of Sequence 2

int match_score(char a, char b);
int max_array (int a[], int num_elements);
void printMatrix (int matrix[m*n]);
void reverseSeq (char arr[], int start, int end);
void finalize (char align1[], char align2[], int sizeAlign1, int sizeAlign2);

#endif
```

needleman_wunsch.c

```
// needleman_wunsch.c file for needleman_wunsch alignment program
#include "needleman_wunsch.h"
#include <stdio.h>
#include <stdlib.h>

// define scoring parameters
#define gap_penalty -5
#define matchScore 10
#define mismatch -5

int match_score(char a, char b) {
    int m_score, i, a_num, b_num;
    if (a == b)
      return matchScore;
    else if (a == '-' || b == '-')
      return gap_penalty;
    else // there is a mismatch
      return mismatch;
}

int max_array (int a[], int num_elements) {
    int i, max=-5000;
    for (i=0; i<num_elements; i++)
        if (a[i]>max) max = a[i];
    return max;
}

void printMatrix (int matrix[m*n]) {
    int i, j;
    printf ("\n");
    for (i=0; i<m; i++){
        for (j=0; j<n; j++)
            printf ("%5.1d ", matrix[i+j*m]);
        printf ("\n");
   }
}

void reverseSeq(char arr[], int start, int end) {
    int temp;
    while (start < end) {
        temp = arr[start];
        arr[start] = arr[end];
        arr[end] = temp;
        start ++;
        end --;
    }
}

void finalize (char align1[], char align2[], int sizeAlign1, int sizeAlign2) {
    int i;
    // reverse sequences
    reverseSeq (align1, 0, sizeAlign1);
    reverseSeq (align2, 0, sizeAlign2);
```

```c
    char *symbol = malloc(500*sizeof(char));

    int scoreNum = 0, count = 1;

    for (i=1; i<=sizeAlign1; i++){
        // if two AAs are the same, output the letter
        if (align1[i] == align2[i]) {
            symbol[count] = align1[i];
            scoreNum = scoreNum + match_score(align1[i], align2[i]);
            count ++;
        }
        // if they are not identical and none of them is gap
        else if (align1[i] != align2[i] && align1[i] != '-' && align2[i] != '-') {
            scoreNum = scoreNum + match_score(align1[i], align2[i]);
            symbol[count] = ' ';
            count ++;
        }
        // if one of them is a gap, output a space
        else if (align1[i] == '-' || align2[i] == '-') {
            symbol[count] = ' ';
            scoreNum = scoreNum + gap_penalty;
            count ++;
        }
    }

    printf ("\nAlignment Results:\n\n");
    for (i=0; i<=sizeAlign1; i++) printf ("%c ", align1[i]);

    printf ("\t\tSequence 1\n\n");
    for (i=0; i<=count; i++) printf ("%c ", symbol[i]);

    printf ("\tMatch\n\n");
    for (i=0; i<=sizeAlign2; i++) printf ("%c ", align2[i]);

    printf ("\t\tSequence 2\n");

    printf ("\n Score = %d\n", scoreNum);
}
```

References

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[3] Ken Nguyen. Multiple biological sequence alignment: Scoring functions, algorithms and evaluation. *John Wiley and Sons*, 2016.

[4] Yechiam Yemini. Blosum scoring matrices. *Computational Genomics*, 2007.

[5] Zhongneng Xu. A teaching approach from the exhaustive search method to the needleman-wunsch algorithm. *Biochem. Mol. Biol. Educ.*, 2016.