Lab Work 2 Report

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Abstract

In this project, we explore the potential to identify the types of organisms present in a metagenomic sample by comparing their similarity to multiple known reference sequences. To achieve this, we use **Normalized Relative Compression (NRC)** with a **finite-context model**. The implementation follows a methodology that compares one sample against multiple references in a database file. The tool begins by training a finite-context model using the sample, and then computes the NRC value for each sequence of DNA in the database. Based on these values, we rank the top candidate matches the sample.

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NRC is a measure of similarity or distance between two sequences (or strings) based on how well one sequence can be **compressed using information from the other**. This can be given by the following formula:

$$\mathcal{NRC}(x \parallel y) = \frac{C(x \parallel y)}{|x| \log_2(\mathcal{A})}$$
 (1)

Where C(x||y) represents the number of bits needed to lossless compress x given exclusively a model trained with y, |x| the size of the sequence x, and $\log_2(A)$ the alphabet of the sequence x, which in this case it will be 4 and, hence, $\log_2(4) = 2$.

To achieve the goals define for this assignment, the following workflow must be followed:

1. Train a finite-context mode (or a Markov Model) using only the

- **metagenomic sample** y. During this process, the model will learn the frequency of patterns present in the sample;
- 2. Freeze the model's counts, meaning that after training on y, the model counts will no longer be updated;
- 3. For each sequence x^i in the reference database of known organisms:
 - Estimate the amount of bits required to compress x^i using the model trained on y;
 - Apply the Normalized Relative Compression (NRC) to measure the relative compression of x^i to y.
- 4. Sort by NRC the names of the sequences and print a top 20.

2 Implementation

The implementation of the finite-context model used for this assignment was the same as the one presented in the last assignment, however a new function was included in this module that will be explained.

Our implementation is divided into multiple files:

- data_base_processor.rs
- model_saver_loader.rs
- metaClass.rs
- finite_context_model_image.rs
- image_processor.rs

2.1 Data Base Processor

Following the workflow described in 1 the first step is to read the **metagenomic sample** that's inside the file **meta.txt** and train a finite context model with it. For it we added a new function to our *file_reader.rs* called **read_line** to read a specific file line by line. With the sequence obtained, a new model is trained.

The next step is to obtain known DNA sequences that are inside a text file also provided by the class professors (**db.txt**). For this we created a new class called **DataBaseProcessor** with the following methods:

- new Creates a new instance of the DataBaseProcessor;
- **get_database** Retrieves the database containing all the samples;
- read_samples Reads all the samples that are in the db.txt file. It parses the name of each sample by identifying the character @ and stores the DNA sequence in a HashMap, where the key

is the name of the sample and the value is the sequence;

- get_sequence_by_name Retrieves a specific sequence that's inside the database:
- comparative_nrc_analysis Performs a comparative analysis of normalized relative compression (NRC) scores among a list of sequences that have a score bellow a threshold. For each sequence in this list, it constructs a finite context model using the context size and a smoothing factor, then calculates how well this model compresses every other sequence in the list. Each sequence is compared against the others, and the results are sorted and collected into a vector;
- export_nrc_comparisons_to_json
 Saves data obtain from the previous function (comparative_nrc_analysis) to a json file. To achieve we had to create to structures ComparisionResult and MatchScore, the first one contains two elements base_sequence, the name of the sequence used to train the model and matches, a vector with the NRC scores for the other samples, the second one contains also two elements, target_name, the name of the sequence that was used to calculate the NRC, and nrc_score the NRC score.

2.2 Save and Load a Model

The file **model_saver_loader.rs** contains two simple functions:

- save_model Serializes a FiniteContextModel instance and saves it in both JSON and BSON.
- load_model Reads a previously saved FiniteContextModel from a JSON file, deserializing its contents and reconstructing the model from the provided file path.

2.3 Meta Class

This file serve as the main entry point of the MetaClass pipeline. Its main goal is to analyze a metagenomic sample by building a probabilistic model (Finite Context Model) and using it to compare known DNA sequences from a reference database using the Normalized Relative Compression (NRC) metric.

It is divided into 6 main parts:

1. Argument Parsing:

- Uses an **argument parser** crate to allow command-line specification of:
 - -s: Path to the metagenomic sample (required);
 - d: Path to the reference database file (also required);
 - k: Context size for the finitecontext model (the default value is 3);
 - -a: Smoothing factor α (the default value is 0.01);
 - -t: Number of top sequences to display (the default value is 20);
 - -l: Threshold for low NRC scores (the default value is 0.5).
- Includes validation to ensure parameter ranges are within acceptable bounds.

2. Model Training

- Reads the metagenomic sample character-by-character using a custom file-reader that was explained in the previous report;
- Trains a Finite Context Model (FCM) on the same sample using

the specified k and α , recording the time taking for this phase.

3. NRC Score Computation

- Initializes a *DataBaseProcessor* with the given reference file;
- Computes the NRC score for each sequence in the database using the previously trained model;
- Sorts all sequences by NRC score and displays the top-N most similar ones.

4. Threshold Filtering and Comparative Analysis

- Sequences with NRC scores **below the specified threshold** are considered significantly similar to the metagenomic sample;
- A comparative NRC analysis is conducted on these sequences, and the obtained results are exported to JSON file.

5. Complexity Profile Generation

- Computes the **complexity pro- file** of the metagenomic sequence
 and all sequences with a NRC score
 below the threshold;
- Uses an instance of the *Chart-Genereator* class to visualize these profiles and saves the results as an image.

6. Performance Logging

- Logs the time spent in each major phase:
 - Model training;
 - NRC score computation;
 - Total execution time.

2.4 NRC for Images

2.4.1 Introduction

To further explore the concept of **NRC**, we attempted to apply it in other domains such as images and audio. Due to time constraints and more promising results with images, we decided to focus more deeply on the image domain.

Initially, we used a dataset of grayscale images of human faces. However, the pictures were close-ups, showing only facial features without any context such as head shape or hair. We used an algorithm similar to the one used for DNA sequences, treating the image as a sequence of pixels and using the last pixels as context the pixels behind the current pixel. This approach did not yield good results, so we began researching alternatives and came across a paper on using Normalized Compression Distance (NCD) with finite context models^[2] and decided to test the same approach but using NRC.

2.4.2 Concept

As we explained in the introduction, we initially used the same algorithm as for the genomic sequences. This created an issue in which the context of each pixel was limited to the k preceding pixels, since the image was treated as a one-dimensional sequence (Figure 1).

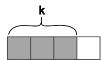


Figure 1: Context of a pixel with any k for first implementation.

This proves to be a limitation when working with images, as they are represented in a 2D space, not as a simple sequence. This means we can access context around a pixel. However, since we are using compression, we can only use as context pixels that are already uncompressed, specifically, the ones that appear in the rows above and in the same row in

columns preceding the pixel. To solve this, we started implementing the context presented in the paper^[2], but only for k=2, where the context pixels are the one directly above and the one directly behind the current pixel, as shown in the image(Figure 2).

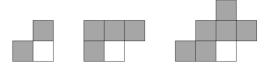


Figure 2: Context templates used by the encoder, corresponding to model orders of 2, 4 and $6.^{[2]}$

This significantly improved the results; however, it was still somewhat distant from the results presented in the paper. Therefore, we decided to fully implement the method used in the paper, where the NRC is calculated by a weighted average of three models with k=2,4,6. The context representation for this method can be seen in Figure 2. According to the following expressions from the paper^[2]:

$$P(X_{n+1} = s) = \sum_{k=2,4,6} P(X_{n+1} = s \mid c_{k,n}) w_{k,n}$$

$$w_{k,n} \propto w_{k,n-1}^{\gamma} P(X_n = x_n \mid c_{k,n-1})$$

$$\sum_{k=2.4.6} w_{k,n} = 1.$$

2.4.3 Implementation

2.4.3.1 finite context model image

The finite context model class was modified to comply with the image requirements. The functions that were altered or added are as follows:

- train_mat_image changed to use a Mat from OpenCV instead of a sequence.
- **get_context** a function that calculates the context based on the current pixel, the frame, and the desired k, where

 $k \in [2, 4, 6]$. It uses an auxiliary function **is_in_frame** to check if the context pixels are within the frame. If any pixel is out of bounds, the context pixel is replaced by the current pixel.

- compute_probability modified to support both the pixel and the context of the image.
- calculate_information_content updated to use a Mat and iterate over all pixels of the image.

2.4.3.2 image_processor

The class is responsible for loading the dataset, processing quantization, and computing the NRC for the saved images with the given models:

- compute_nrc_multi_model computes the NRC for multiple models, all using equal weights.
- compute_weighted_nrc computes the NRC using weighted average based on the behavior of each model.
- **compute_nrc** computes the NRC for a single model.
- quantize_image quantizes the image using the Lloyd-Max quantizer.

2.4.3.3 image

This is the main class to process the image and the database of images, the flow of the class processing is the following:

- Parse command-line arguments using argparse, including:
 - -i: path to the input image file (required)
 - **-d**: path to the database file (required)
 - -a: smoothing parameter α (default: 0.5)

- -t: number of top sequences to display (default: 10)
- -1: number of quantization levels for the image (default: 256)
- Load the dataset using the **ImageProcessor** class with the provided database path.
- Read the input image in grayscale using

- OpenCV and apply quantization.
- For each k, create a new **FiniteContextModelImage** with the smoothing parameter α , train it on the quantized image, and store it in the map.
- Compute the weighted NRC score for each image in the dataset using the trained models and a decay factor $\gamma = 0.99$.

3 Experiments

In this section we present a comparative analysis to evaluate our implementation, in terms of performance, accuracy, etc. For this we developed multiple experiments like see the affect of k and alpha values, compared complexity profiles between the samples that have a lower NRC score, evaluate the accuracy, apply our new finite context model for images and see the NRC results.

3.1 Top 20 sequences contained in Metagenomic Sample

In the first experiment, we aimed to identify which known genomic sequences are most similar to the metagenomic sample provided. To do this, we trained a Finite Context Model using the full metagenomic data and evaluated all sequences from the reference database using the Normalized Relative Compression (NRC) metric. The experiment was conducted with $\mathbf{k} = \mathbf{10}$ (context size) and $\alpha = \mathbf{0.01}$ (smoothing factor), as these parameters provided a good balance between context sensitivity and generalization.

Table 1: Top 20 Sequences with ordered by NRC score

Rank	Sample Name	NRC
1	@NC_001348.1 Human herpesvirus 3, complete genome	0.196636
2	@NC_005831.2 Human Coronavirus NL63, complete genome	0.241053
3	@Super ISS Si1240	0.262869
4	@OR353425.1 Octopus vulgaris mitochondrion, complete genome	0.310705
5	@Super MUL 720	0.397340
6	@gi 49169782 ref NC_005831.2 Human Coronavirus NL63, complete	0.735003
	genome	
7	@gi 14141972 ref NC_002786.1 Maize rayado fino virus isolate Costa	1.507647
	Rican, complete genome	
8	@gi 971748077 ref NC_028836.1 Pseudomonas phage DL62, complete	1.510804
	genome	
9	@gi 9629255 ref NC_001793.1 Oat blue dwarf virus complete genome	1.517540

4.0		1 700000
10	@gi 84662653 ref NC_007710.1 Xanthomonas oryzae phage OP2 DNA,	1.522362
	complete genome	
11	@gi 472339725 ref NC_020840.1 Tetraselmis viridis virus S20 genomic	1.547256
	sequence	
12	@gi 475994049 ref NC_020902.1 Equine Pegivirus 1 isolate C0035, com-	1.555544
	plete genome	
13	@gi 927594140 ref NC_027867.1 Mollivirus sibericum isolate P1084-T,	1.555690
	complete genome	
14	@gi 971752197 ref NC_028885.1 Pseudomonas phage DL64, complete	1.556349
	genome	
15	@gi 22855189 ref NC_004168.1 Yellowtail ascites virus segment A, com-	1.559760
	plete sequence	
16	@gi 33867953 ref NC_005075.1 Helicobasidium mompa No.17 dsRNA	1.560387
	virus small segment, complete sequence	
17	@gi 113478394 ref NC_008311.1 Norovirus GV, complete genome	1.567778
18	@gi 50253405 ref NC_005997.1 St Croix river virus segment 1 putative	1.573770
	RNA-dependent RNA polymerase VP1 gene, complete cds	
19	@gi 21427658 ref NC_004018.1 Gremmeniella abietina RNA virus MS1	1.574988
	RNA 1, complete genome	
20	@gi 33867950 ref NC_005074.1 Helicobasidium mompa No.17 dsRNA	1.577041
	virus large segment, complete sequence	

A few key observations can be drawn from these results:

- Human-associated viruses rank among the top, including Human herpesvirus 3 (NRC = 0.1966) and Human Coronavirus NL63 (NRC = 0.2411)

 These low values suggest a high degree of similarity with the patterns present in the metagenomic data, potentially indicating contamination, cohabitation, or shared genomic characteristics;
- Two entries labeled @Super ISS Si1240 and @Super MUL 720 also appear in the top 5. These might be synthetic constructs, assemblies, or sequences derived from previous space, related studies. Their presence hints at content in the metagenomic sample that aligns closely with known ISS-associated sequences;
- Starting from rank 6, the NRC values increase sharply. The duplication of the *Human Coronavirus NL63* entry in a

different identifier format (rank 6) reinforces the idea of consistent similarity detection across naming schemes and identifiers;

- Sequences with NRC values above 1.5, such as Maize rayado fino virus, Pseudomonas phage, and Xanthomonas oryzae phage, show considerably lower similarity. These results may reflect incidental or random matches due to limited pattern overlap;
- The diversity of organisms present in the top 20 includes phages, plant viruses, mollivirus, norovirus, and even RNA viruses. This diversity suggests the metagenomic sample could be a complex mixture of genetic material from different sources either real, synthetic, or environmental.

3.2 Experimenting with different values of k and alpha

To better understand the impact of the model parameters on the NRC computation, we designed a second experiment focusing on the influence of the **context size** k and the **smoothing factor** α . This experiment was divided into two parts:

- In the first part, we varied the value of k from 2 to 20 in increments of 2, while keeping α fixed at 0.01. This allowed to observe how increasing the context window size affects the model's ability to capture patterns and perform compression
- In the second part, we fixed the value of k = 10 and varied α over a range of values: 0.001, 0.005, 0.01, 0.05, 0.1, 0.2, 0.3, 0.5. These values were chosen to explore both very low and relatively high smoothing effects on the model's behavior.

The results are presented in the following figures:

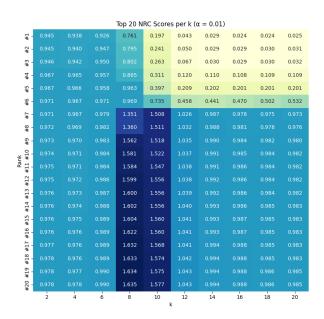


Figure 3: Results obtained by altering the values of k

1	Top 20 NRC Scores per α (k = 10)									
\$\begin{array}{cccccccccccccccccccccccccccccccccccc	₩-	0.198	0.196	0.197	0.208	0.225	0.256	0.283	0.331	
# - 0.312	- #2	0.244	0.241	0.241	0.251	0.266	0.294	0.319	0.364	- 1.8
\$\begin{array}{cccccccccccccccccccccccccccccccccccc	£ -	0.290	0.270	0.263	0.258	0.268	0.295	0.322	0.367	
\$\begin{array}{cccccccccccccccccccccccccccccccccccc	# -	0.312	0.310	0.311	0.320	0.333	0.357	0.379	0.417	- 1.6
1.4	£ -	0.460	0.415	0.397	0.368	0.367	0.382	0.400	0.437	
\$\begin{array}{cccccccccccccccccccccccccccccccccccc	9# -		0.781	0.735	0.638	0.608	0.590	0.589	0.600	
\$\begin{array}{cccccccccccccccccccccccccccccccccccc	4-	1.798	1.594	1.508	1.313	1.234	1.163	1.126	1.087	- 1.4
0 - 1.823	ee - #	1.806	1.599							
## 1.863	6# -	1.822	1.609							- 1.2
## 1.863	#10 #	1.823	1.612							
C1 - 1.874 1.651 1.556 1.340 1.254 1.178 1.137 1.095 C2 - 1.875 1.651 1.556 1.341 1.254 1.178 1.139 1.095 C3 - 1.880 1.653 1.556 1.342 1.257 1.178 1.140 1.097 C3 - 1.883 1.657 1.560 1.342 1.257 1.181 1.141 1.097 C4 - 1.887 1.658 1.560 1.344 1.258 1.181 1.141 1.097 - 0.6 C4 - 1.894 1.665 1.568 1.348 1.258 1.182 1.142 1.098 C5 - 1.900 1.671 1.574 1.349 1.262 1.183 1.143 1.098 - 0.4 C5 - 1.911 1.676 1.575 1.352 1.263 1.184 1.144 1.100 C8 - 1.913 1.677 1.577 1.353 1.264 1.185 1.145 1.100	#11 #	1.863	1.642	1.547						- 1.0
1.875 1.651 1.556 1.341 1.254 1.178 1.139 1.095 2. 1.880 1.653 1.556 1.342 1.257 1.178 1.140 1.097 2. 1.883 1.657 1.560 1.342 1.257 1.181 1.141 1.097 2. 1.887 1.658 1.560 1.344 1.258 1.181 1.141 1.097 -0.6 2. 1.894 1.665 1.568 1.348 1.258 1.182 1.142 1.098 3. 1.900 1.671 1.574 1.349 1.262 1.183 1.143 1.098 -0.4 3. 1.911 1.676 1.575 1.352 1.263 1.184 1.144 1.100 3. 1.913 1.677 1.577 1.353 1.264 1.185 1.145 1.100		1.874	1.651							
1 - 1880 1.653 1.556 1.342 1.257 1.178 1.140 1.097 1 - 1883 1.657 1.560 1.342 1.257 1.181 1.141 1.097 2 - 1887 1.658 1.560 1.344 1.258 1.181 1.141 1.097 -0.6 1 - 1894 1.665 1.568 1.348 1.258 1.182 1.142 1.098 2 - 1900 1.671 1.574 1.349 1.262 1.183 1.143 1.098 -0.4 3 - 1911 1.676 1.575 1.352 1.263 1.184 1.144 1.100 8 - 1913 1.677 1.577 1.353 1.264 1.185 1.145 1.100	#13	1.875	1.651	1.556						
1.883	#14	1.880	1.653							- 0.8
1887 1.658 1.560 1.344 1.258 1.181 1.141 1.097 -0.6 1		1.883	1.657	1.560						
1 - 1.894 1.665 1.568 1.348 1.258 1.182 1.142 1.098 2 - 1.900 1.671 1.574 1.349 1.262 1.183 1.143 1.098 -0.4 3 - 1.911 1.676 1.575 1.352 1.263 1.184 1.144 1.100 3 - 1.913 1.677 1.577 1.353 1.264 1.185 1.145 1.100	#16	1.887	1.658	1.560						- 0.6
- 1.900 1.671 1.574 1.349 1.262 1.183 1.143 1.098 - 0.4 - 1.911 1.676 1.575 1.352 1.263 1.184 1.144 1.100 - 1.913 1.677 1.577 1.353 1.264 1.185 1.145 1.100	#17	1.894	1.665	1.568						
9 - 1.911 1.676 1.575 1.352 1.263 1.184 1.144 1.100 2 - 1.913 1.677 1.577 1.353 1.264 1.185 1.145 1.100	#18	1.900	1.671							- 0.4
N- 1.913 1.677 1.577 1.353 1.264 1.185 1.145 1.100	#19	1.911	1.676							
	#20 #	1.913	1.677	1.577						
0.001 0.005 0.01 0.05 0.1 0.2 0.3 0.5 -0.2		0.001	0.005	0.01			0.2	0.3	0.5	- 0.2

Figure 4: Results obtained by altering the values of alpha

From the first heatmap Figure 3, we observe that the NRC scores tend to increase as the value of k grows, especially from k=8 onward. This trend is particularly evident from rank 6 to 20, where the scores stabilize and reach higher values with increasing k. In contrast, the top five ranks show relatively lower scores at higher k, with noticeable spikes at lower k values (especially k=8 for ranks 6–20). This suggests that smaller k values may provide higher sensitivity for identifying top-ranked sequences, while larger k values yield more consistent and elevated scores for the broader $\frac{1}{2}$ set of relevant sequences.

In the second heatmap Figure 4, increasing the α value results in a general increase in NRC scores, especially for ranks 6 through 20. The scores for the top five ranks remain relatively low across all α values, with only gradual increases. However, for lower-ranked sequences (e.g., 6–0), the effect of α is much more pronounced, showing a steady rise in NRC values with higher α . This indicates that higher α values may help in amplifying the contribution of weaker signals, thereby enhancing the detection of less dominant sequences in the dataset.

3.3 Execution Time

In this experiment, we analyzed the computational cost of our method by measuring the **training time**, **NRC computation time**, and **total execution time** across multiple combinations of k (ranging from 1 to 20) and three values of α (0.01, 0.1 and 1). For each pair (k, α) . For this we compiled our program using the flag -release and obtained the following results:

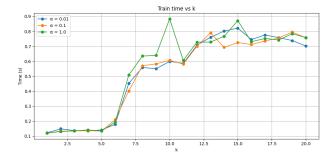


Figure 5: Training time variation

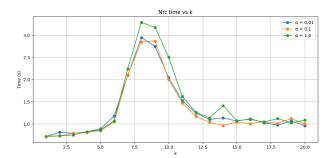


Figure 6: NRC computation time variation

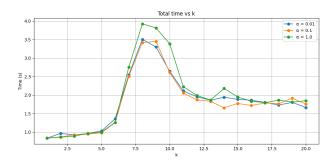


Figure 7: Total execution time variation

By observing this visualizations it is possible to conclude that:

• In Figure 5, the time taken to train a model **increases** with **k** and does **not peak sharply** like NRC time. Training

is generally less sensitive to α , but with $\alpha = 1.0$ is again slower, especially at mid to high k. From k = 10 to k020, the training time is more stable but still slightly increasing.

- In Figure 6, for all α values, NRC time rises steeply until k=8, then drops off. $\alpha=1.0$ shows the highest peak, followed by 0.01 and 0.1. This may indicate that **higher** α **values slow down the NRC process** when k is small to mid-range. After k=10, times converge and stabilize near 1s, with minor fluctuations
- In Figure 7, total time closely follow the NRC time trend, which implies NRC is the **dominant contributor** to overall time. The difference between α values becomes more noticeable at peak (again, $\alpha = 1.0$ is slowest), but less so at higher k. After k = 10, all α values result in more consistent and lower total times.

With all of this we can conclude that mid range k values (around 7-9) should be avoided. Use α within a range of 0.01 and 0.1 for better performance and higher k values (ξ 10) seems to bring more consistent and manageable times for all phases.

3.4 Accuracy of the solution

One of the most important experiments we conducted was to evaluate the **accuracy** of our solution—specifically, its ability to assign **lower NRC scores** to sequences that are truly part of a metagenomic sample.

To perform this experiment, we used the tool GTO to generate random sequences. These sequences allowed us to build a new test database and simulate different mutation levels on sequences embedded within metagenomic samples.

We generated a test database containing 50 random samples. The length of each sequence was randomly chosen between 1,000

and 10,000 bases. For reproducibility, the sample identifier served as the seed for each sequence generation.

With the test database ready, we proceeded to construct **7 metagenomic samples**. In each of these, **5 random sequences** from the test database were included. These same 5 sequences appeared in all 7 metagenomic samples but with **varying mutation rates**: **0%**, **1%**, **5%**, **10%**, **15%**, **20%**, **and 25%** respectively.

To introduce additional randomness, each metagenomic sample was also supplemented with **30 new random sequences**. These were generated by randomly selecting se-

quences from the database and creating a new **151-base-long subsequence** using each sequence that was randomly selected. These random sequences were all included in each metagenomic sample.

The sequences which suffered mutation were @seq_48, @_43, @seq_19, @seq_14, and @seq_23. The values of k and alpha were set to 10 and 0.01, respectively.

Now with the metagenomic files and the test database created, we created a simple **Python** script, that indicates the top 20 sequences that are present in the metagenomic sample for each sample created, And obtained the following results:

	NRC Scores vs Mutation Rate for Top 20 Sequences						
#1	0.041	0.151	0.513	0.801	0.958	1.032	1.069
	@seq_14	@seq_23	@seq_48	@seq_48	@seq_19	@seq_48	@seq_2
- #5	0.041	0.173	0.538	0.808	0.963	1.039	1.070
	@seq_23	@seq_43	@seq_14	@seq_43	@seq_23	@seq_43	@seq_6
- #3	0.042	0.178	0.543	0.823	0.970	1.039	1.071
	@seq_43	@seq_14	@seq_43	@seq_23	@seq_43	@seq_19	@seq_49
#4	0.042	0.184	0.557	0.833	0.984	1.041	1.071
	@seq_19	@seq_19	@seq_19	@seq_19	@seq_14	@seq_23	@seq_14
42	0.044	0.199	0.582	0.859	0.989	1.061	1.072
	@seq_48	@seq_48	@seq_23	@seq_14	@seq_48	@seq_14	@seq_11
9#	1.066	1.063	1.072	1.070	1.070	1.068	1.073
	@seq_6	@seq_6	@seq_5	@seq_4	@seq_15	@seq_5	@seq_23
4,	1.071	1.068	1.073	1.071	1.074	1.070	1.074
	@seq_5	@seq_5	@seq_2	@seq_6	@seq_9	@seq_6	@seq_19
8 -	1.074	1.072	1.073	1.073	1.076	1.075	1.076
	@seq_21	@seq_21	@seq_4	@seq_45	@seq_40	@seq_28	@seq_43
6#	1.075	1.073	1.074	1.073	1.076	1.076	1.076
	@seq_45	@seq_4	@seq_6	@seq_11	@seq_21	@seq_40	@seq_41
Rank (1-20)	1.075	1.077	1.074	1.073	1.077	1.076	1.077
#11 #10	@seq_2	@seq_9	@seq_9	@seq_21	@seq_24	@seq_15	@seq_46
Rank	1.075	1.077	1.074	1.075	1.078	1.077	1.078
#11	@seq_4	@seq_45	@seq_24	@seq_36	@seq_50	@seq_21	@seq_31
#12	1.076	1.077	1.076	1.077	1.078	1.078	1.078
	@seq_9	@seq_41	@seq_40	@seq_9	@seq_16	@seq_49	@seq_5
#13	1.077	1.077	1.076	1.078	1.078	1.078	1.078
	@seq_41	@seq_2	@seq_41	@seq_50	@seq_35	@seq_26	@seq_15
#14	1.078	1.078	1.077	1.078	1.078	1.078	1.079
	@seq_24	@seq_24	@seq_17	@seq_10	@seq_26	@seq_31	@seq_27
#15	1.080	1.078	1.079	1.078	1.079	1.078	1.079
	@seq_44	@seq_49	@seq_21	@seq_5	@seq_10	@seq_2	@seq_50
#16	1.080	1.079	1.080	1.079	1.080	1.078	1.080
	@seq_46	@seq_10	@seq_27	@seq_46	@seq_44	@seq_50	@seq_39
#17	1.080	1.079	1.081	1.079	1.080	1.079	1.080
	@seq_10	@seq_46	@seq_35	@seq_24	@seq_4	@seq_42	@seq_4
#18	1.080	1.080	1.081	1.080	1.081	1.079	1.080
	@seq_50	@seq_27	@seq_50	@seq_35	@seq_6	@seq_44	@seq_24
#19	1.080	1.080	1.081	1.080	1.081	1.079	1.080
	@seq_27	@seq_3	@seq_39	@seq_3	@seq_3	@seq_29	@seq_48
#50	1.081	1.081	1.081	1.081	1.081	1.079	1.080
	@seq_49	@seq_18	@seq_34	@seq_44	@seq_46	@seq_10	@seq_13
	0% 1% 5% 10% 15% 20% 25% Mutation Rate						

Figure 8: Results for the accuracy experiment

Figure 8 demonstrates that:

- The sequences known to be part of the metagenomic samples (even with increasing mutation rates) generally exhibit lower NRC scores compared to the background random sequences;
- The heatmap shows that the five target sequences (@seq_48, @_43, @seq_19, @seq_14, and @seq_23) consistently appear within the top 20 ranked sequences across all of the mutation rates;
- As the mutation rate increases from 0% to 25%, there is a **noticeable trend of increasing NRC scores** for the target sequences. This is expected, as higher mutation levels make the sequence more dissimilar to the original database sequences;
- As expected, at 0% mutation, the original sequences likely have the lowest NRC scores and are ranked very high.

3.5 Complexity Profiles of the top sequences

Another experiment that we conducted was, to evaluate the complexity profile of sequences that had a NRC score below a threshold defined previously.

For this a new function was added to the *finite_context_model.rs* to compute the complexity profile for a given sequence, based in a model trained with a metagenomic sequence, called *complexity_profile*. It takes a string as input and calculates the complexity profile as a vector of floating-point numbers.

For each character in the text (considering a sliding window of size $\mathbf{k}+\mathbf{1}$), it computes the **bit cost** of predicting that character based on the preceding \mathbf{k} characters (the context). This bit cost is derived from the logarithmic probability of the character appearing given its context, as learned by the model.

Some other functions where added $chart_generator.rs$:

- group_by_identifier: Organizes a list of names and their associated data into groups based on a common identifier found within the names;
- extract_identifier: Takes a name os a sequence and tries to pull out the sequence identifier from it using pattern matching. If no specific pattern is found, it tries to get the first word of the sequence name;
- smooth_profile: Takes a list of numbers and calculates a smoothed version of it by averaging values within a sliding window. This helps to reduce noise in the complexity profile of a sequence.
- draw_complexity_profiles: Represents the complexity profiles calculated previously in a line chart. If multiple sequences have the same identifier, then the complexity profile will appear in the same chart window with different colors. It also applies smoothing to the complexity profiles by using the smooth_profile function.

For this experiment the threshold was set to 0.8, the model was trained with 10 and 0.01 for k and alpha, respectively. The results can be seen in the following figure:

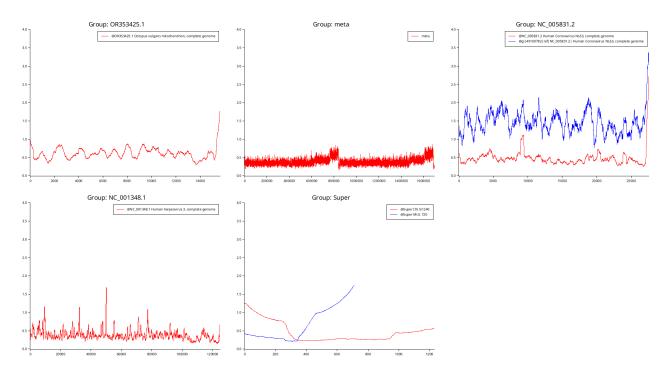


Figure 9: Complexity profiles of sequences with a NRC score lower than 0.8

By observing Figure 9 we can conclude that:

- When visualizing the **Group** NC_005831.2, we can see that the sample with the red line is more similar to the meta one, when compared with the blue line. This means that the the sample represented with the red line has a higher probability of being present in the metagenomic sequence provided by the class professors;
- For the **Group: Super** we can see that the blue line has more similarity with the metagenomic sequence at the beginning, and the red line is more similar after colliding with the blue line. This could indicate that both of these samples are present in the metagenomic sample.

3.6 Compare NRC Scores between top sequences

Another experiment that we performed was to evaluate the similarity between all the sequences that have a NRC score below a custom threshold.

For this two new functions where added to the data_base_processor.rs file called comparative_nrc_analysis and export_nrc_comparisons_to_json that were previously explain in subsection 2.1.

A finite context model was trained with 10 and 0.01 for the values of k and alpha respectively, and the threshold was set to 0.8.

The results can be seen in the following figure:

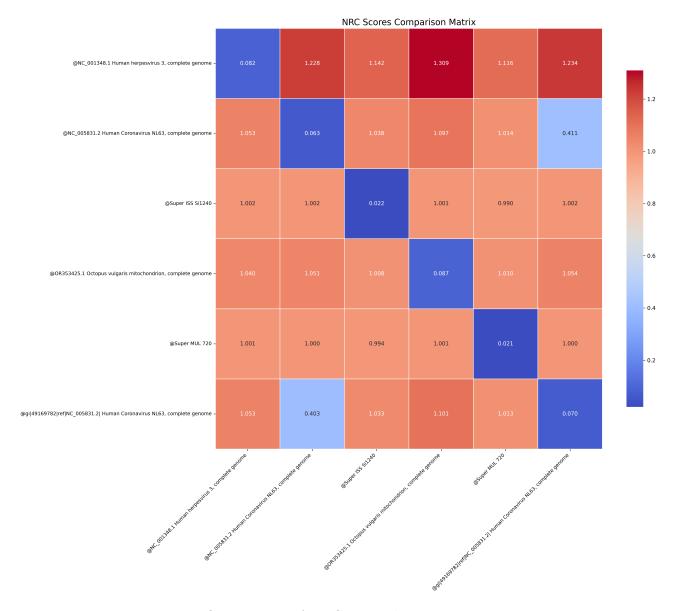


Figure 10: Comparison of NRC scores between top sequences

- Each base sequence has its lowest NRC score when compared to itself, as expected (scores close to 0);
- Sequences from the same or similar organisms (like the tow *Human Coronavirus NL63 samples*) show relatively low mutual NRC scores, indicating high similarity;
- Comparisons with unrelated sequences consistently yielded **higher NRC** scores (1.0 or above)

3.7 Image NRC results

The dataset used was the Olivetti Research Laboratory (ORL) face dataset ^[1]. It contains 400 images of 40 subjects, with 10 images per subject. To test our solution, we used the first image of each subject and compared it to all other images in the dataset. The goal was to identify the best results, expecting that the top 10 **NRC** values for each image would contain 10 images of the corresponding person.

The table shows reasonable results, but they are not as promising as those reported in the paper. This discrepancy may be due to the fact that the **NRC** performs worse than

the Normalized Compression Distance (NCD) when comparing images.

Table 2: Image Count per Person

Person	# Images
1	6
2	10
3	4
4	5
5	6
6	3
7	7
8	8
9	3
10	3
11	6
12	5
13	6
14	6
15	1
16	1
17	2
18	6
19	8
20	3
21	4
22	8
23	4
24	6
25	5
26	3
27	8
28	3
29	5
30	7
31	3
32	1
33	2
34	8
35	2
36	2
37	$\frac{2}{5}$
38	5
39	1
40	1
41	10
Sum	192

3.8 Image Quantization

From the tests we conducted, changing the quantization levels has a significant impact on the NRC algorithm, making its performance worse when the quantization levels are low. For example, in the image below (Figure 11), we can see that the best results occur when there is no quantization or when the quantization level is set to 256.

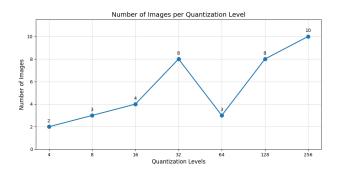


Figure 11: Results for different quantization levels

4 Instructions

How to install dependencies, compile, and run the programs

4.1 Dependencies

Before running the programs, *Rust*, *Cargo* and **OpenCV** need to be installed. Additionally we used the following packages from rust:

- argparse, version 0.2.2
- plotters, version 0.3.7
- rand, version 0.9.0
- *serde*, version 1.0 and an additional feature called **derive**
- $serde_json$, version 1.0.139
- bson, version 2.0
- opencv, version 0.94.4
- *regex*, version **1.11.1**

To install Rust and Cargo, run the following command (if on Linux or macOS systems):

curl https://sh.rustup.rs -sSf | sh

On Windows download and run this file rust-up-init.exe.

To install OpenCV, follow the tutorial through this link.

4.2 Compiling the Project

To compile the project, navigate to the root directory and run:

cargo build

After compilation, executables will be available in the 'target/debug' folder.

4.3 Running Meta Class

To execute the Meta Class executable run:

target/debug/metaClass

- -s {metagenomic_sample_path}
- -d {db_path}
- -k {k_value}
- -a {alpha_value}
- -t {top_sequences_display}
- -1 {threshold}

Where:

- metagenomic_sample_path: Path to the text file that contains the metagenomic sample;
- **db_path**: Path to the text file that contains a database with known samples;
- **k**: Context size, i.e., the number of characters considered before the current character;

- alpha: Smoothing parameter to avoid zero probabilities;
- top_sequences_display: Number of sequences to be displayed;
- threshold: A value between 0 and 1.0 for experimental analysis.

4.4 Examples

A predefined bash script is available in the 'scripts/bash' directory to execute the Meta Class. To execute the **generate_meta.sh** it is required to have gto installed. To do so follow this tutorial

To execute the bash script for the Meta Class

follow the following commands:

chmod u+x ./run_meta.sh
./run_meta.sh

Also some python scripts are also provided to generate visualizations, they can be found in the 'scripts/python' directory. All the visualizations created are saved to the 'visualizations' directory. To execute the python files its required to first create a virtual environment if none exists

python3 -m venv venv
source venv/bin/activate
pip install -r requirements.txt
python3 <name of the file to execute>

5 Conclusions

The goal of project was to develop a system capable of identifying the most similar organisms to a given metagenomic sample using NRC, built upon Finite Context Models. Throughout our experiments, we observed the impact of model parameters on the system's performance:

- Increasing k improves the model's ability to capture sequence structure, leading to more precise matches, but at the cost of increased computation time.
- Lower alpha values tends to improve specificity in data. In contrast, higher alpha improve robustness, i.e, the results are more generics.

Furthermore, we also implemented a feature that applies NCR concepts to image data, using an approach for face familiarity detection. With this approach, we obtained some interesting values, although they were not the best. However, we were able to understand how this could be implemented, even using NCD.

This project demonstrates the potential of algorithmic information theory as a practical tool for biological data analysis and we can conclude that with the integration of NRC and FCMs, it is possible to build models capable of identifying similarities in metagenomic data, even in the presence of mutations, noise, or unknown sequences.

References

- [1] marlon tavares. The olivetti research laboratory (orl) face dataset. https://www.kaggle.com/datasets/tavarez/the-orl-database-for-training-and-testing.
- [2] Armando J. Pinho and Paulo J. S. G. Ferreira. Image similarity using the normalized compression distance based on finite context models. https://ieeexplore.ieee.org/document/6115866.