FASTAptameR 2.0 - User Interface Tutorial

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1 Introduction

FASTAptameR 2.0 is an R-based update of FASTAptamer (Alam KK 2015). Like its predecessor, FASTAptameR 2.0 is an open-source toolkit designed to analyze populations of sequences resulting from combinatorial selections. This updated version features a user interface (UI), interactive graphics, more modules, and a faster implementation of the original clustering algorithm.

This user guide walks you through installation and each of the analytical modules, and it highlights what options you have when analyzing your data through the UI. Please see package documentation (**LINK**) for details on running these scripts through R.

1.1 Overview

- **FASTAptameR-Count**
  - *This module is the entry point into FASTAptameR 2.0*
  - Input: preprocessed FASTQ/A
  - Workflow:
    1. count all unique sequences (`Reads`)
    2. sort by counts (`Rank`)
    3. normalize counts as reads per million (`RPM`)
  - Plotting:
    1. line plot of reads-per-rank
    2. histograms of sequence lengths - one for the unique sequences and one for all reads
  - Output: FASTA or CSV
- **FASTAptameR-Translate**
  - Input: counted FASTA
  - Workflow: translate D/RNA sequences to amino acid sequences
  - Plotting:
    1. line plot of reads-per-rank
    2. histograms of sequence lengths - one for the unique sequences and one for all reads
  - Output: FASTA or CSV
- **FASTAptameR-Motif_Search**
  - Input: counted FASTA
  - Workflow: search for user-defined patterns in sequences
  - Output: FASTA or CSV
- **FASTAptameR-Motif_Tracker**
  - Input: two or three counted FASTAs
  - Workflow: track how user-defined patterns enrich across two or three populations
  - Output: CSV
- **FASTAptameR-Distance**
  - Input: counted FASTA
  - Workflow: compute the Levenshtein edit distance (LED) between a single query sequence and all other provided sequences
  - Plotting: histograms of edit distances - one for the unique sequences and one for all reads
  - Output: CSV
- **FASTAptameR-Enrich**
  - Input: two or three counted FASTAs

  - Workflow: calculate how each sequence enriches across two or three populations
  - Plotting:
    1. histogram(s) of `log2(Enrichment)`
    2. scatter plot(s) of RPM
    3. volcano plot of `log2(Enrichment)` and a term related to frequency
  - Output: CSV

- **FASTAptameR-Cluster**
  - Input: counted FASTA
  - Workflow:
    1. filter out low-read sequences based on user-defined input
    2. treat most abundant, non-clustered sequence as cluster seed
    3. add all sequences within a user-defined LED of the seed to the cluster
    4. Repeat until all sequences are clustered or a maximum number of clusters are created
  - Output: FASTA or CSV

- **FASTAptameR-Cluster_Diversity**
  - Input: clustered FASTA
  - Workflow: provide metadata for each cluster
  - Plotting:
    1. metaplots for count of unique sequences, count of total reads, and average LED per cluster
    2. k-mer PCA plot, colored by cluster identity
  - Output: CSV

- **FASTAptameR-Cluster_Enrich**
  - Input: two or three clustered FASTAs
  - Workflow: calculate how each cluster enriches across two or three populations
  - Output: CSV

A summary of input and output file types is given by **Table 1**. Please note that each module requires the user to upload a file or, in the case of **FASTAptameR-Count**, optionally provide a GitHub link to the data. At present, none of this data will "live" on the server to be passed between modules.

Table 1: Module Inputs and Outputs

Module	Input Files	Output Files
FASTAptameR-Count	Preprocessed FASTQ/A	FASTA or CSV
FASTAptameR-Translate	Counted FASTA	FASTA or CSV
FASTAptameR-Motif_Search	Counted FASTA	FASTA or CSV
FASTAptameR-Motif_Tracker	2 or 3 counted FASTAs	CSV
FASTAptameR-Distance	Counted FASTA	CSV
FASTAptameR-Enrich	2 or 3 counted FASTAs	CSV
FASTAptameR-Cluster	Counted FASTA	FASTA or CSV
FASTAptameR-Cluster_Diversity	Clustered FASTA	CSV
FASTAptameR-Cluster_Enrich	2 or 3 cluster-analysis CSVs	CSV

Many function inputs / outputs are simply FASTA files, so FASTAptameR 2.0 can be easily integrated into most analytical pipelines. Note that *counted* FASTA files are the minimum input (*e.g.*, FASTAptameR-Translate needs *at least* a counted FASTA but could also accept a searched or clustered FASTA file). See **Fig. 1** for a web of all possible module connections.

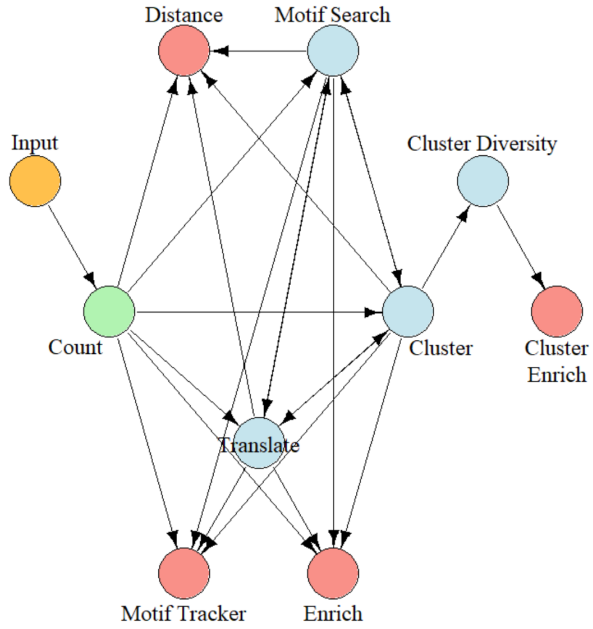
Importantly, FASTAptameR 2.0 does not provide any functions that are easily addressed by other software

(*e.g.*, merging paired-end reads, trimming constant regions, predicting structures, *etc.*). Rather, the focus of this application is to provide flexible downstream analyses for the selections field.

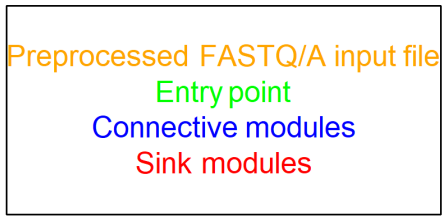
1.2 Example workflows

We show all module connections and two example workflows in **Fig. 1**. *Orange* represents the preprocessed FASTQ or FASTA formatted input file, which we abbreviate as 'FASTQ/A input file'. *Green* represents the entry point into the software and, as such, should be included in all pipelines. *Blue* represents modules that can feed into others. Finally, *red* represents modules that cannot feed into subsequent modules.

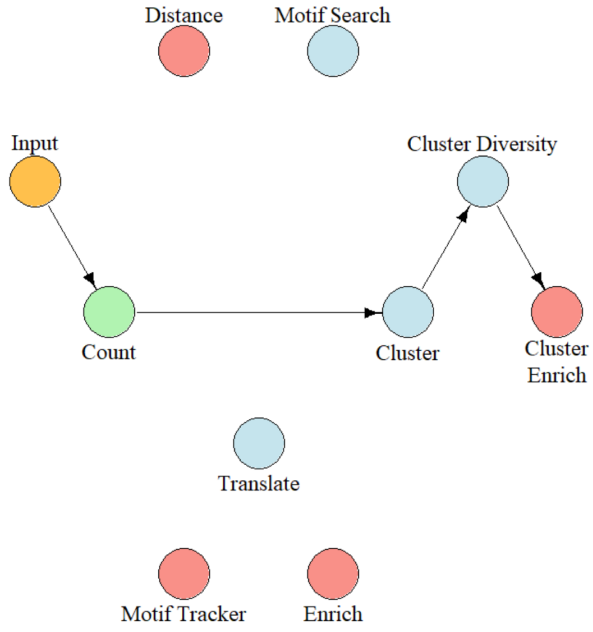
Note that all modules will always need a file upload (*i.e.*, data will not 'live' on the web server to be passed between modules).



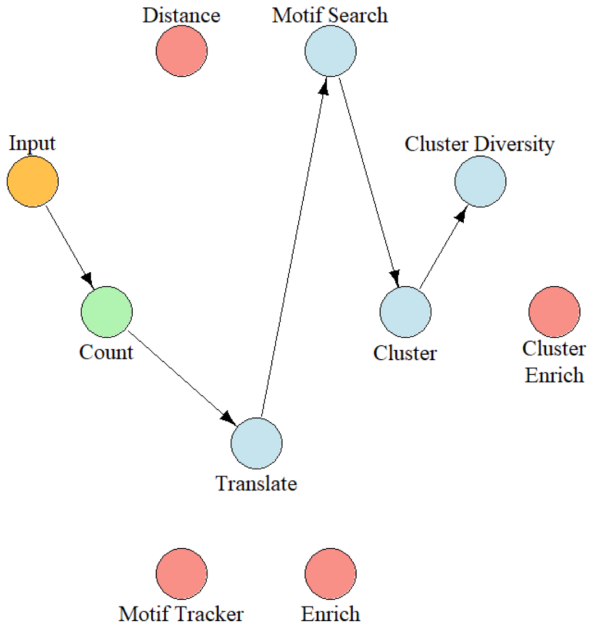
A) All module connections



B) Color legend



C) Example workflow to compare multiple clustered populations of aptamers



D) Example workflow to cluster translated sequences that have a user-defined motif

Figure 1: Example workflows

2 How to get started

Exactly like its predecessor, FASTAptameR 2.0 is designed to be **easy** to use. There are three main ways for users to interact with FASTAptameR 2.0. The *web server* is the easiest way to interact with this application because it only requires an internet connection and browser. However, you can run it locally as a *Docker container*. Finally, this application can be accessed through *R* if you are familiar with the language.

2.1 User interface

2.1.1 Web Server

This is the easiest way to use the FASTAptameR 2.0 UI. The web server can be accessed from **LINK**, which is hosted by the Digital Biology Laboratory under the direction of Dr. Dong Xu. However, this option only works if the files you need to upload are less than 2 GB.

2.1.2 Docker

The web server can also be run locally on your machine(s) via Docker. In brief, Docker is a convenient tool that may be used to construct *images* of software. The *image* essentially functions as the blueprint for an application. The *image* of FASTAptameR 2.0, for example, contains all relevant software (*e.g.*, `R`), files (*e.g.*, this PDF), and packages (*e.g.*, `Shiny`). For details on Docker or its installation, please see https://www.docker.com/ and https://docs.docker.com/get-docker/, respectively.

The FASTAptameR 2.0 *image* must be pulled from a repository (*i.e.*, Docker Hub) by running

```
docker pull skylerkramer/fastaptamer2:publicupload01
```

in a Docker-active terminal. Windows and Mac users will have a Docker-active terminal after proper installation of Docker Desktop. Linux users will have a Docker-active terminal after proper installation of Docker. Windows, Mac, or Linux users can check if their terminal is Docker-active by running

```
docker version
```

which should indicate which version of Docker has been installed.

Importantly, the FASTAptameR 2.0 *image* is built on Linux. Thus, it is necessary to run it from a Linux environment or virtual machine. For Mac or Windows users, the installation instructions for Docker Desktop (https://docs.docker.com/get-docker/) will show you how to do this.

Once you have this application's *image*, running

```
docker run -d --rm -p 3838:3838 skylerkramer/fastaptamer2:publicupload01
```

from a Docker-active terminal will launch a local instance - a *container* - of FASTAptameR 2.0. You will then interact with this *container* in the same fashion as the web server by navigating to `localhost:3838` in your web browser.

Explanation of flags from the previous command:

- `-d`: enable detached mode, which allows you to use your command line / terminal even with the active *container* (*i.e.*, *container* is detached from your terminal and runs in the background)
- `--rm`: automatically remove the container upon exit
- `-p 3838:3838`: publish `3838` host port (1st number) to the `3838` container port (2nd number)
- `skylerkramer/fastaptamer2:publicupload01`: the local path to the **FASTAptameR 2.0** Docker *image*
- `localhost:3838`: navigate here from your web browser to start interacting with **FASTAptameR 2.0**

To recap:

1. Install Docker on a Linux machine or install Docker Desktop on a Windows or Mac machine to establish a 'Docker-active terminal' on your local system.
2. Pull the FASTAptameR 2.0 image from the Docker Hub repository.
3. Execute the `docker run ...` command and navigate to `localhost:3838` in your web browser.

2.2 R

For experienced R users, all code (UI and modules) can be accessed through GitHub: **LINK**. `install_packages.R` will install all required packages and should be run first. `app.R` and `Functions.R` should be kept in the same directory. The former contains the UI and server code, whereas the latter contains all functions necessary to analyze your data.

The functions from `Functions.R` are available as a GitHub package and can be installed by **INSTALL COMMAND**. For details on usage, see the corresponding documentation: **LINK TO MANUAL**.

2.3 Software usage

If you use, adapt, or modify FASTAptameR 2.0, please cite: **CITATION**.

For any questions or concerns, please email burkelab@missouri.edu or stk7c9@umsystem.edu.

3 Tutorial

3.1 Data requirements

FASTAptameR 2.0 utilizes many string-based functions in its backend. Thus, this program can be used to analyze many types of biological populations. However, all libraries must be initially saved in a FASTA or FASTQ format and passed through **FASTAptameR-Count** prior to any subsequent analyses. Further, any data preprocessing steps must be made outside of this application.

3.2 Sample Data and Uploading User Data

All data shown in this tutorial come from the 14th and 15th rounds of an aptamer selection against HIV-1 reverse transcriptase (Burke DH 1996; Ditzler MA 2013; Whatley AS 2013). These data are preprocessed (trimmed and filtered) and available from http://burkelab.missouri.edu/fastapamer.html.

To start analyzing the sample data or your own data, please do one of two things. Either **1)** upload a local copy of the file via the file browser in **FASTAptameR-Count** or **2)** supply a link to the data via the text box labeled as `Online source` in **FASTAptameR-Count**. This module is the entry point to **FASTAptameR 2.0**, so each analysis should start here.

3.3 FASTAptameR-Count

3.3.1 Description

FASTAptameR-Count serves as the entry point into this suite of modules, and, thus, it should be run prior to any of the following modules. This function accepts either a FASTQ/A file chosen with the file browser or a link to such a file (*e.g.*, the default GitHub link in the text box labeled as `Online source`) and returns a *counted* data table as output that can be downloaded as a FASTA or CSV file.

Input FASTQ files should be properly formatted (4 lines per entry with the 2nd line of each entry being the sequence). Input FASTA files are not required to have sequence identifiers. No pre-existing sequence identifiers will be conserved by this module. Instead, output sequence identifiers are defined by the statistical representation of each sequence. Sample input files are shown in **Fig. 2**. A screenshot of the module interface is shown in **Fig. 3**.

```
AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC
AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC
AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT
```

(a) Sample FASTA input without sequence identifiers

```
>Description Line 1
AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC
>Description Line 2
AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC
>Description Line 3
AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC
>Description Line 4
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT
```

(b) Sample FASTA input with sequence identifiers

```
@HWI-700167R:511:C3N2DACXX:5:1101:4016:2214 1:N:0:GACGGCT
AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC
+
IIJJJIJJJJJJJJJJIIIJIJJJHHHFF<ADBDDDDDDDDDDCCDDDDDDDDDDCACCDCDDDDECDD
@HWI-700167R:511:C3N2DACXX:5:1101:4997:2191 1:N:0:GACGGCT
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT
+
IIJIJJGIJJJJIIJJJJIJIJJJJJJGHHIIJJJIJJJHHFFFEEEEDDDDDDDDDDDDDDDBB@DCDD
```

(c) Sample FASTQ input

Figure 2: Valid input types

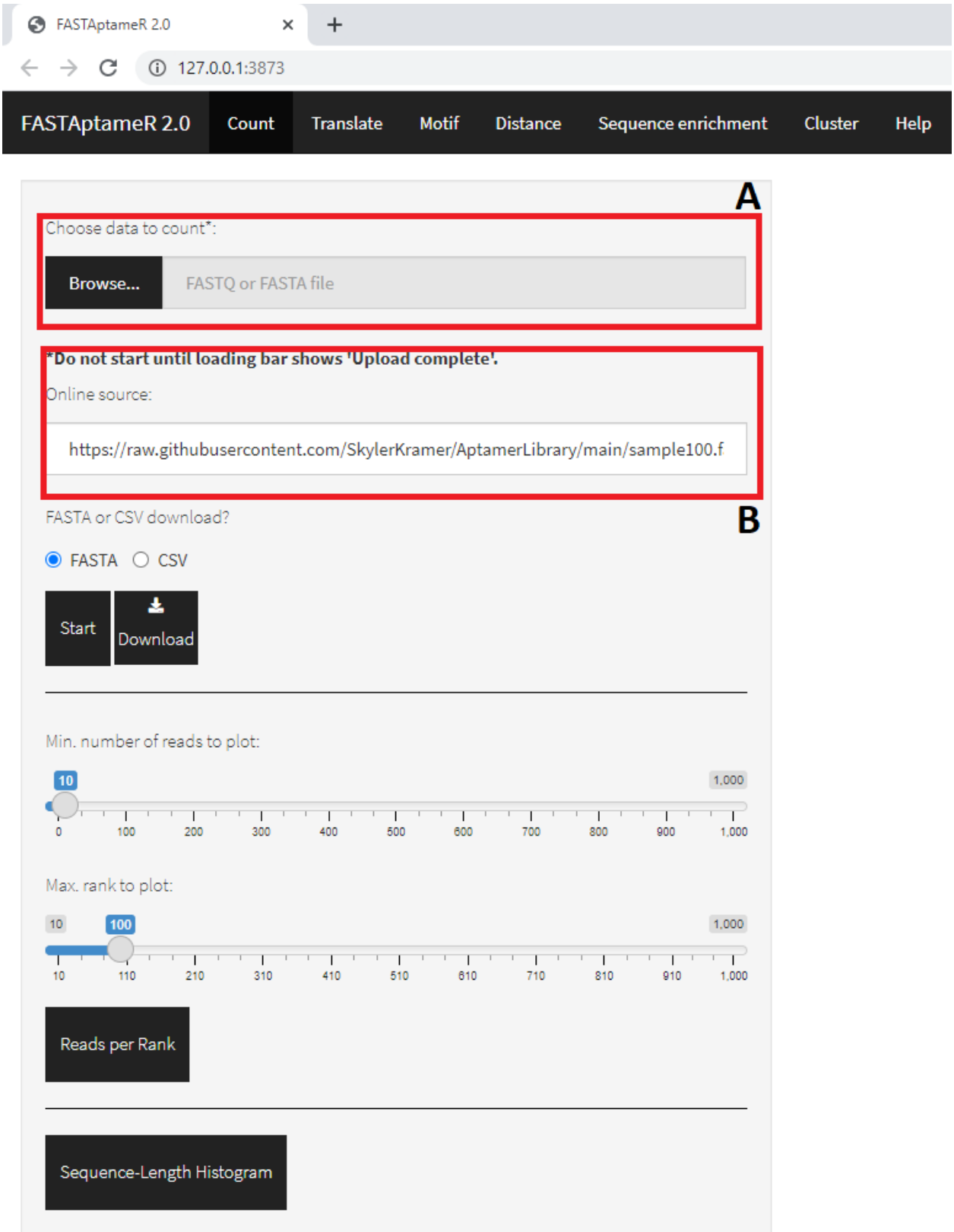


Figure 3: Screenshot of FASTAptameR-Count

3.3.2 Usage

The input FASTQ/A file must be chosen with the file browser (**Fig. 3a**) or linked in the text box (**Fig. 3b**). A sample link is already provided in the text box. Note, if a file is uploaded via the file browser **AND** a link is provided, the uploaded file (**NOT** the linked one) will be analyzed.

The `Start` button will begin the counting process. The results will be displayed as a data table on the right side of the screen. For file uploads, please wait for the loading bar to show *Upload complete* before using the `Start` button.

If you start the module before the upload is finished **AND** a file link is provided, then the file link will be analyzed. If you start the module before the upload is finished **AND** no link is provided, then you will get an error that says `No file or link provided!`. If you start the module before the upload is finished **AND** no file link is provided **AND** you have previously uploaded a file to this module, then the previously uploaded file will be reanalyzed. In any of these cases, reuploading the file, *waiting for it to finish uploading*, and then starting the module should correctly analyze your data. If an error persists (*e.g.*, one that says `Error in nchar()`), please refresh the page.

A sample output data table is shown in **Fig. 4**.

Show 10 entries Search:

id	Rank	Reads	RPM	Length	seqs
All	All	All	All	All	All
>1-417696-193358.44	1	417696	193358.44	70	ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT
>2-313312-145037.35	2	313312	145037.35	70	CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG
>3-174096-80591.94	3	174096	80591.94	70	AACCGCAAGCAACACCCAGCAAGAAACATCCGACGCACGACGGGAGAAAGTGCATTACCACGATGTCGAT
>4-94978-43966.9	4	94978	43966.9	70	CATAGCGACTGCCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG
>5-74389-34435.91	5	74389	34435.91	70	ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCGCGCTTGGTGT
>6-57625-26675.57	6	57625	26675.57	69	CCCTCCTTGTATGACGCTAACTGAGAATCCGAAGTCCAACGGGAGAAAGGACACTTATGACGTGGCGCG
>7-53608-24816.04	7	53608	24816.04	70	ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCATGCTTGGTGT
>8-39793-18420.84	8	39793	18420.84	69	AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC
>9-33800-15646.58	9	33800	15646.58	70	TTGACAATAACTCGAGAAGAACCGAGGTGCAAACGGGAGAACACAATGGATTACACCGAGCTCGGCTGAC
>10-29794-13792.14	10	29794	13792.14	70	GCGAACCAAACCCAGATTACTAACCGTGGGCCTGAAACACGGGACAAAACAGGCATCAATGGAGTGGTAC

Showing 1 to 10 of 72,921 entries Previous 1 2 3 4 5 … 7293 Next

Figure 4: FASTAptameR-Count Output

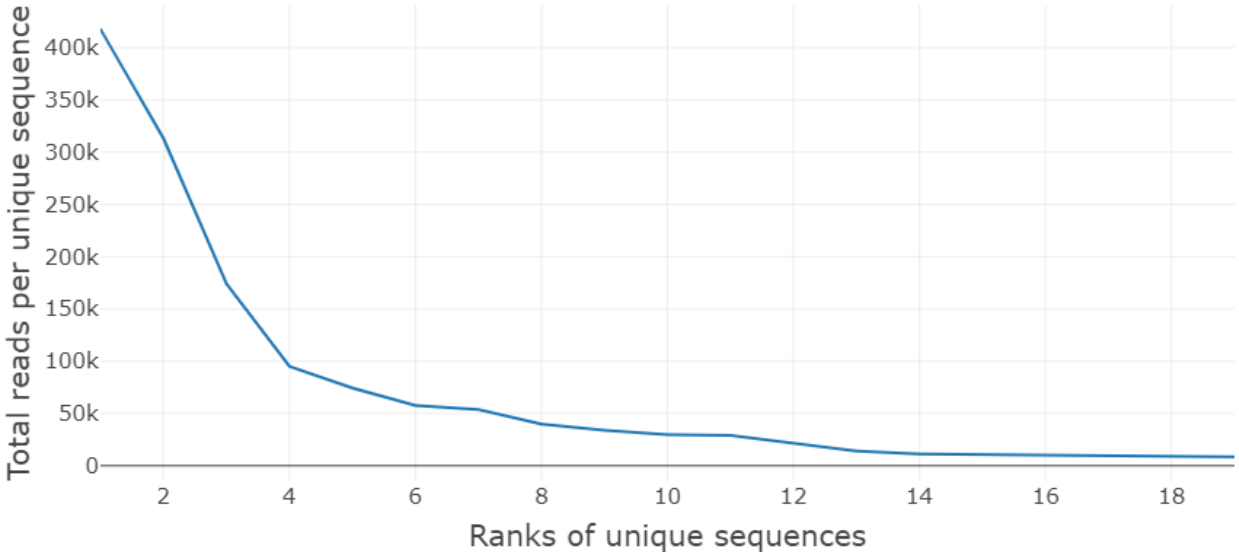
Note that the *id* column has the following format: `>Rank-Reads-RPM`, where `Rank` is the order of sequences after sorting by `Reads`, which is the raw abundance of each sequence. `RPM` - Reads per Million - is the value of `Reads`, normalized by the total population size: `RPM = Reads / (populationSize / 1e6)`.

The total number of sequences, number of unique sequences, and module runtime will be displayed below the `Start` and `Download` buttons after running is finished. The `Download` button opens a file browser prior to downloading the output as a FASTA or CSV file (`DEFAULT = FASTA`, which is required for subsequent modules). Keep the *count* file in an easily accessible folder, as this file will serve as input for many other FASTAptameR 2.0 modules.

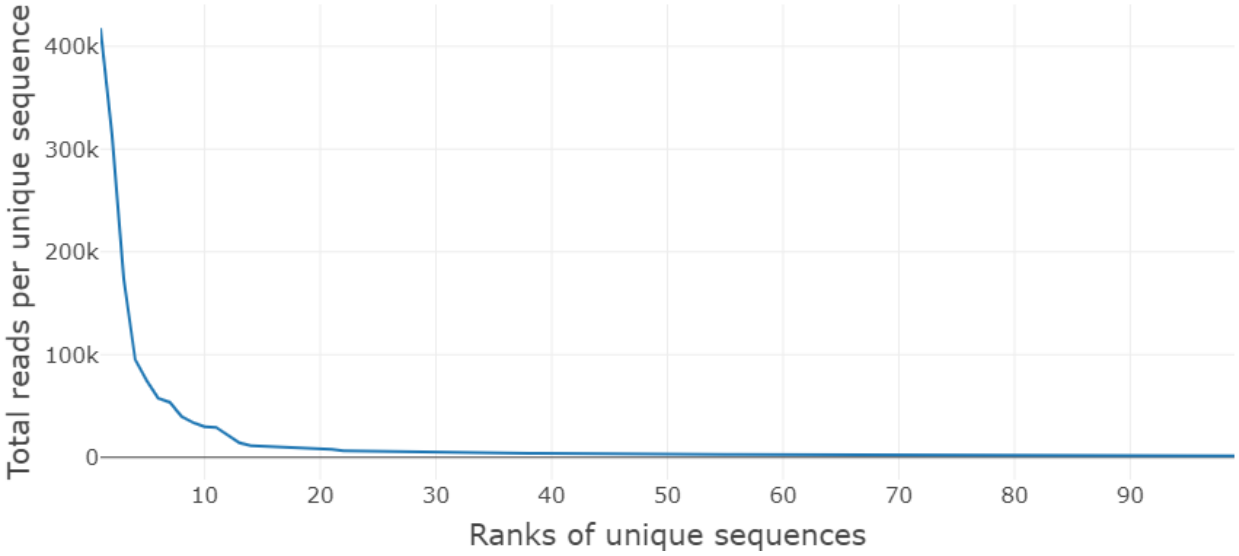
3.3.3 Plotting

This module can also generate two types of interactive plots based on the counted data: a line plot of reads-per-rank (**Fig. 5**) and two histograms of sequence lengths (**Fig. 6**). The line plot is filterable by 1) minimum number of reads to plot and 2) maximum rank to plot. Both values are chosen with a slider bar.

The 1st histogram corresponds to the sequence-length distribution of unique sequences, whereas the 2nd histogram corresponds to the sequence-length distribution of all reads. The histograms are not filterable.



A) Top 20−ranked sequences (min. reads = 10)



B) Top 100−ranked sequences (min. reads = 10)

Figure 5: Reads-per-Rank line plots. Two views of the 70HRT14 data, where panels (A) and (B) include the top 20 and top 100 ranked sequences, respectively. These values are chosen with the corresponding slider bars (shown in Fig. 3).

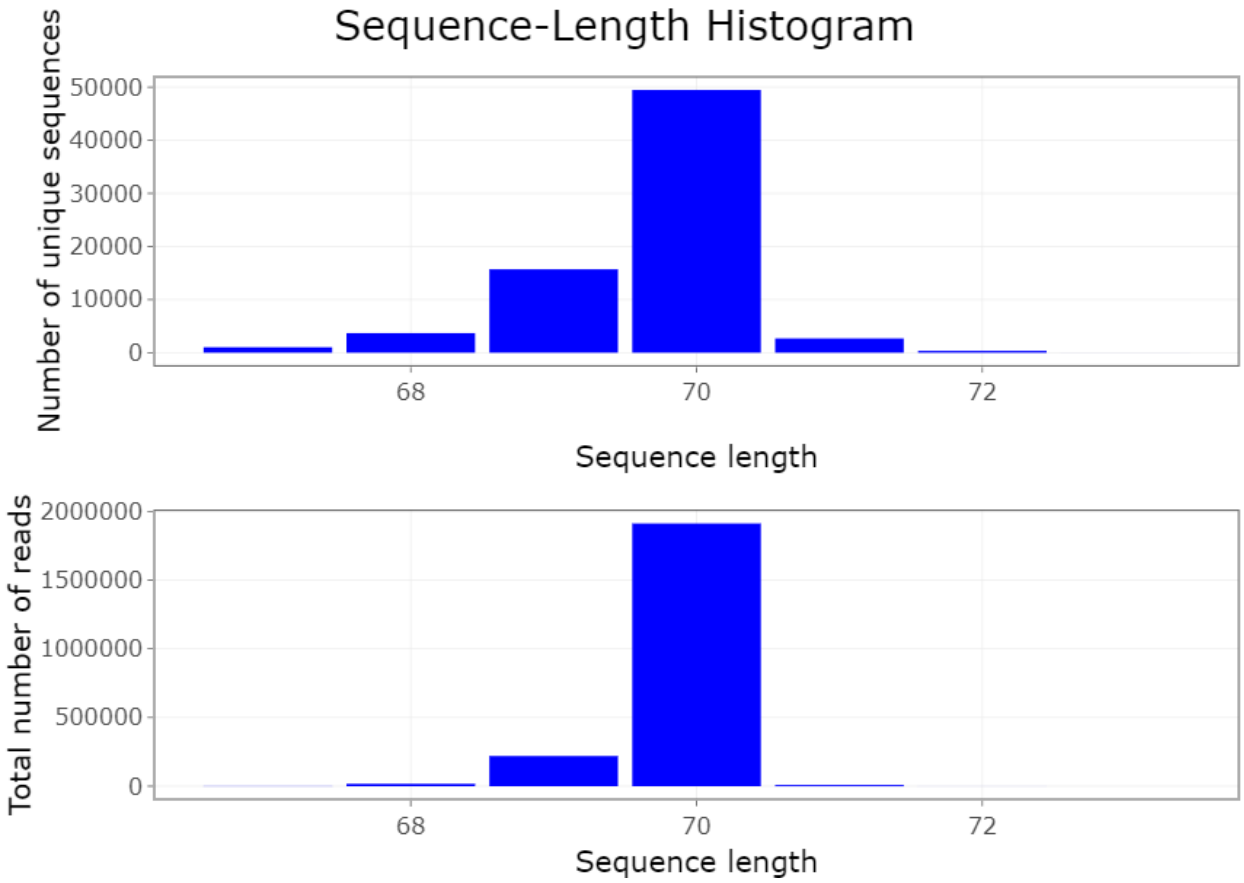


Figure 6: Sequence-Length Histogram. Two views of the 70HRT14 data, where the top and bottom panels correspond to unique sequences and total reads, respectively. Both plots are generated together in the UI.

3.4 FASTAptameR-Translate

3.4.1 Description

FASTAptameR-Translate translates input nucleotide sequences into amino acid sequences following the standard genetic code, treating the input nucleotide sequences as positive-sense mRNA. This module accepts a *counted* FASTA file and returns a *translated* data table that can be downloaded as a FASTA or CSV file. A screenshot of the module interface is shown in **Fig. 7**.

3.4.2 Usage

The input FASTA file must be chosen with the file browser (**Fig. 7a**). The open reading frame may be selected by the 1st set of radio buttons (`DEFAULT = 1`) (**Fig. 7b**). The 2nd set of radio buttons indicates whether nucleotide sequences that encode the same amino acid sequence should be merged (`DEFAULT = Yes`) (**Fig. 7c**). If `Yes`, then redundantly encoded amino acid sequences are converged, and a new column (`Unique.Nt.Count`) will specify how many non-unique nucleotide sequences from the *counted* input were merged into each amino acid sequence. If `No`, then each unique nucleotide sequence is treated separately, even if multiple sequences encode the same amino acid sequence.

The `Start` button begins the translation process. The *translated* data table will be shown on the right side of the screen. The `Download` button opens a file browser prior to downloading the output as a FASTA or CSV file (`DEFAULT = FASTA`, which is required for subsequent modules).

3.4.3 Plotting

This module generates the same two types of plots as FASTAptameR-Count: a line plot of reads-per-rank and two histograms of sequence lengths. See that section for more details.

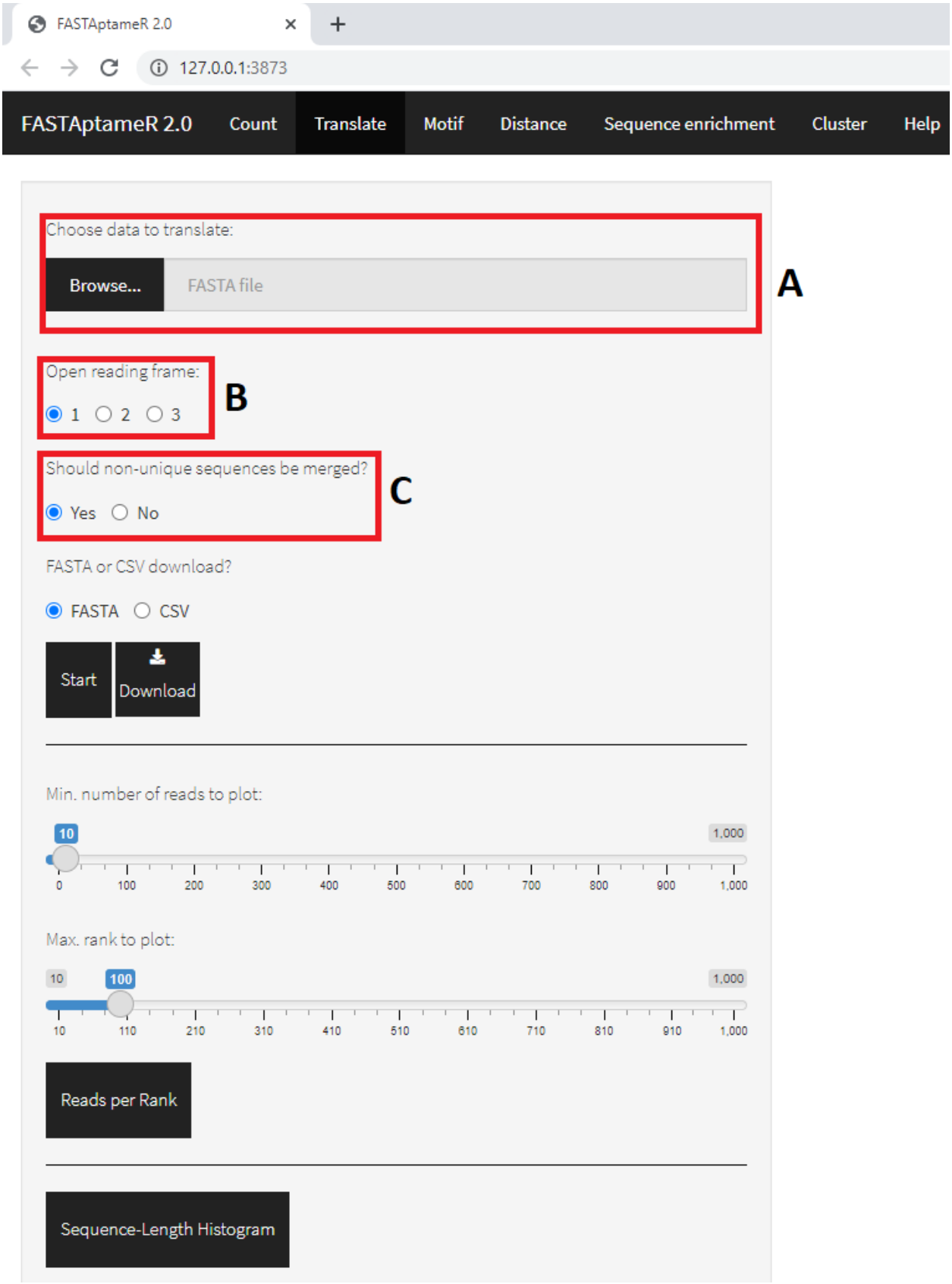


Figure 7: Screenshot of FASTAptameR-Translate

3.5 FASTAptameR-Motif_Search

3.5.1 Description

FASTAptameR-Motif_Search identifies sequences that contain one or more user-specified sequence motifs, or 'patterns.' The module accepts a *counted* FASTA file and returns a *searched* data table that can be downloaded as a FASTA or CSV file. Sequences in the output must have at least one occurrence of each pattern or at least one occurrence of at least one pattern (see details below for the `partial match` radio button). A screenshot of the module interface is shown in **Fig. 8**.

3.5.2 Usage

The input FASTA file must be chosen with the file browser. The following text box (**Fig. 8a**) must contain at least one pattern (*e.g.*, `AAA`). If the user wishes to search for multiple patterns, the patterns must be separated by commas (*e.g.*, `AAA,GTG`).

The 1st set of radio buttons (**Fig. 8b**) determines whether the output has parentheses set around identified patterns (`DEFAULT = No`). For example, when `pattern = GGC` and `sequence = AAAGGCT`, the output is `AAA(GGC)T`. Note, when two or more patterns overlap, output only displays parentheses around the first search term that is matched. For example, when `pattern = AGGC,GGCT` and `sequence = AAAGGCT`, the output is `AA(AGGC)T`. Note that parentheses will be treated as individual characters by subsequent modules and may alter downstream analyses.

The 2nd set of radio buttons (**Fig. 8c**) governs how the software deals with multiple search terms. When the query contains multiple patterns, the search can be carried out either as a Boolean `AND` function by requiring all parts of the query to be present within a given sequence (this is the `default`, with button set to `No`), or as a Boolean `OR` function to identify sequences that contain any part of the query (set button to `Yes`). If `Yes`, filtered sequences must have at least one occurrence of **at least one** of the listed patterns. If `No` (`DEFAULT`), filtered sequences must have at least one occurrence of **each** of the listed patterns.

The 3rd set of radio buttons (**Fig. 8d**) determines the type of pattern (`DEFAULT = Nucleotide`). If `Nucleotide`, then degenerate nucleotide codes are allowed, and T/U are interchangeable. Degenerate search patterns are **not** allowed for other sequence types. Importantly, all patterns are converted to uppercase and have white spaces removed regardless of the pattern type.

1. **A**/**T**/**G**/**C**/**U** - single bases
2. **R** - puRine (A/G)
3. **Y** - pYrimidine (C/T)
4. **W** - Weak (A/T)
5. **S** - Strong (G/C)
6. **M** - aMino (A/C)
7. **K** - Keto (G/T)
8. **B** - not A
9. **D** - not C
10. **H** - not G
11. **V** - not T/U
12. **N** - aNy base (not *gap*)

The `Start` button begins the search process. The *searched* data table will be shown on the right side of the screen. The `Download` button opens a file browser prior to downloading the output as a FASTA or CSV file (`DEFAULT = FASTA`, which is required for subsequent modules).

A sample output data table is shown in **Fig. 9** with the following parameters: `comma-separated patterns = UCCG,CGGGAnAA`, `parentheses = No`, `partial filtering = No`, and `pattern type = Nucleotide`.

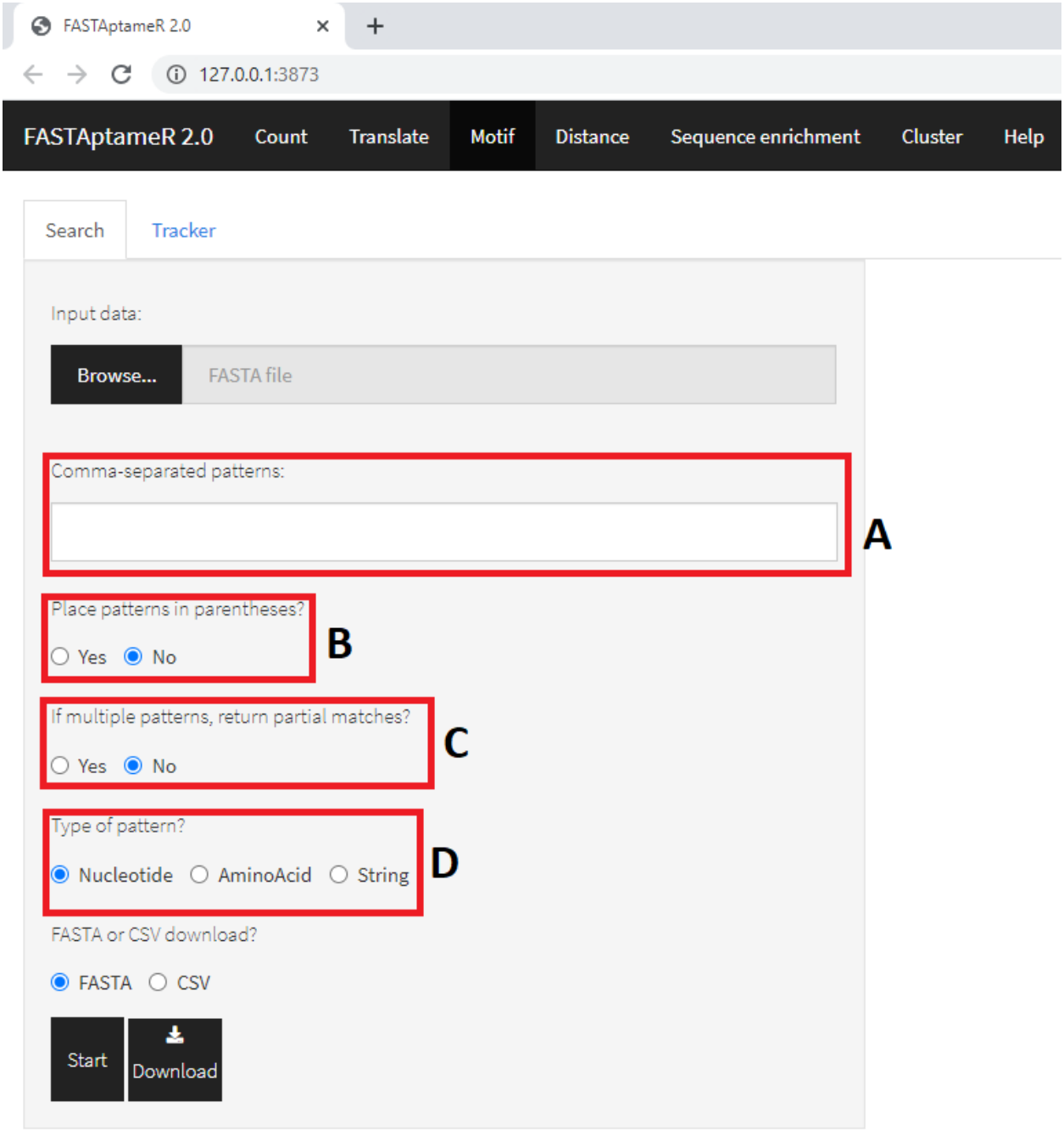
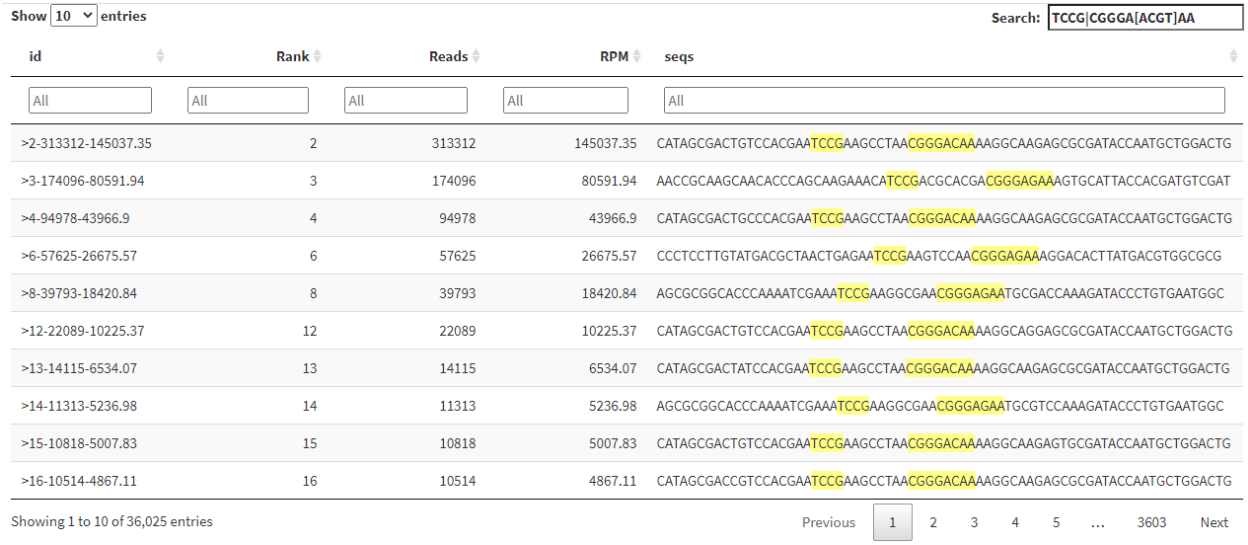


Figure 8: Screenshot of FASTAptameR-Motif_Search


Show 10 entries Search: TCCG|CGGGA[ACGT]AA

id	Rank	Reads	RPM	seqs
>2-313312-145037.35	2	313312	145037.35	CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG
>3-174096-80591.94	3	174096	80591.94	AACCGCAAGCAACACCCAGCAAGAAACATCCGACGCACGACGGGAGAAAGTGCATTACCACGATGTCGAT
>4-94978-43966.9	4	94978	43966.9	CATAGCGACTGCCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG
>6-57625-26675.57	6	57625	26675.57	CCCTCCTTGTATGACGCTAACTGAGAATCCGAAGTCCAACGGGAGAAAGGACACTTATGACGTGGCGCG
>8-39793-18420.84	8	39793	18420.84	AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC
>12-22089-10225.37	12	22089	10225.37	CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAGGAGCGCGATACCAATGCTGGACTG
>13-14115-6534.07	13	14115	6534.07	CATAGCGACTATCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG
>14-11313-5236.98	14	11313	5236.98	AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGTCCAAAGATACCCTGTGAATGGC
>15-10818-5007.83	15	10818	5007.83	CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGTGCGATACCAATGCTGGACTG
>16-10514-4867.11	16	10514	4867.11	CATAGCGACCGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG

Showing 1 to 10 of 36,025 entries Previous 1 2 3 4 5 … 3603 Next

Figure 9: FASTAptameR-Motif_Search Output

3.6 FASTAptameR-Motif_Tracker

3.6.1 Description

FASTAptameR-Motif_Tracker reports on the occurrence of one or more query patterns across multiple populations. The module accepts two or three *counted* FASTA files as input and returns a data table of metadata related to the enrichment of the query pattern(s) across multiple populations. Multiple FASTA files should be selected from the file browser at the same time. Columns of the output data table include the following:

1. Input file names
2. Number of unique reads in each population
3. Total number of reads in each population
4. Total number of reads that contain the query pattern(s)
5. RPM of matching sequences
6. Total number of occurrences of the query pattern(s)
7. Total motif RPM

This output can be downloaded as a CSV file and will include appended enrichment scores. A screenshot of the module interface is shown in **Fig. 10**.

3.6.2 Usage

The input FASTA files must be chosen with the file browser. The following text box must contain at least one pattern. If the user wishes to search for multiple patterns, the patterns must be separated by commas. The set of radio buttons determines the type of pattern (`DEFAULT = Nucleotide`). If `Nucleotide`, then degenerate nucleotide codes are allowed. Note, the pattern is converted to uppercase and white spaces are removed regardless of the pattern type.

The `Start` button begins the motif enrichment process. The resulting data table will be shown on the right side of the screen. The `Download` button opens a file browser prior to downloading the output as a CSV file. The enrichment scores (`RPM of 2nd file divided by RPM of 1st file, etc.`) are displayed below these buttons. These scores will be included in the downloaded CSV file.

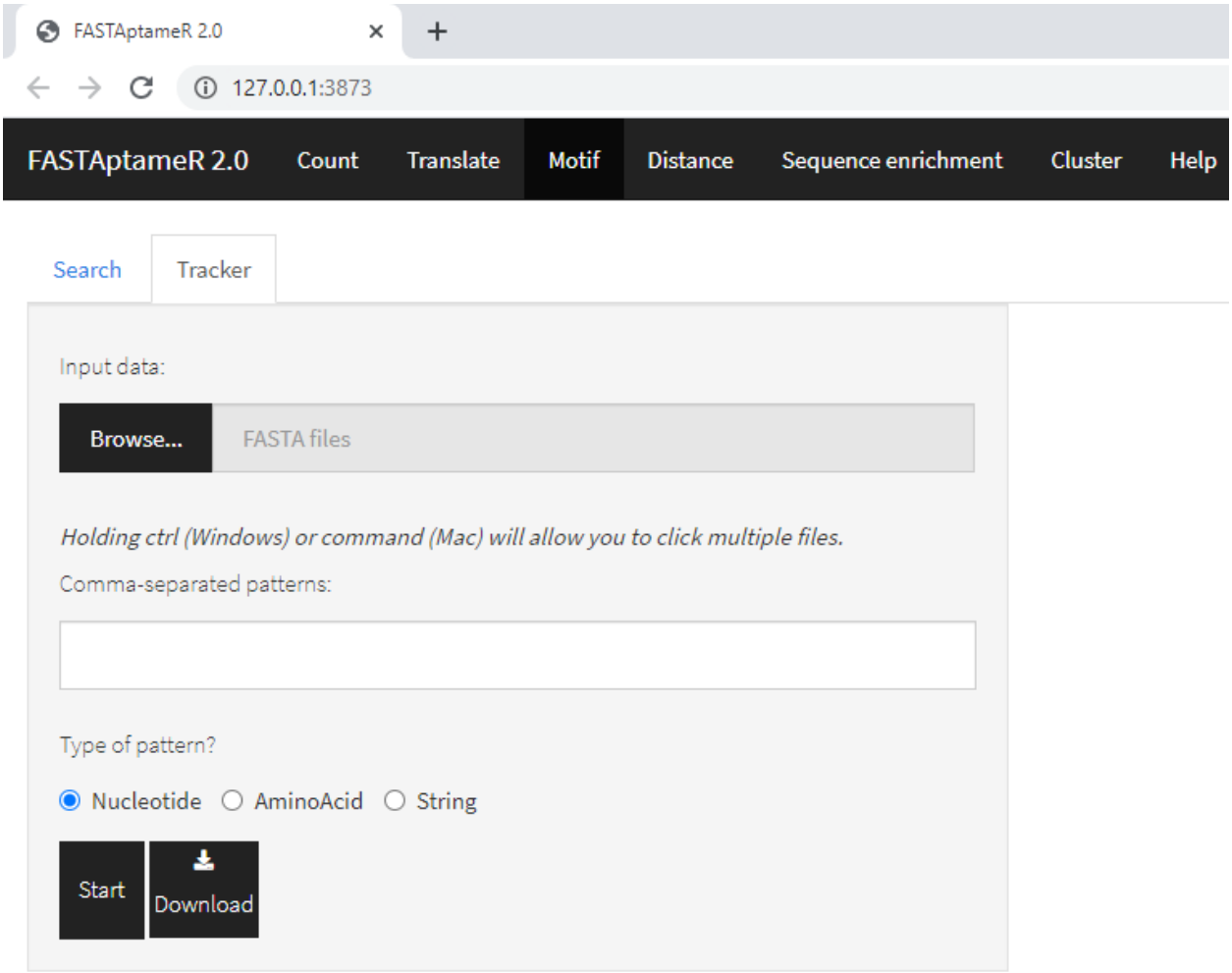


Figure 10: Screenshot of FASTAptameR-Motif_Tracker

A screenshot of a sample output data table is shown in **Fig. 11** with the following pattern: `UCCGnnnnnnnnCGGGAnAA` (a Family 1 Pseudoknot).

	A	B	C	D	E	F	G
1	File.Names	Unique.Reads	Total.Reads	Seqs.With.Motif	Seqs.RPM	Motif.Occurrences	Motif.RPM
2	70HRT14-count.fasta	72921	2160216	1138044	526819.54	1138044	526819.54
3	70HRT15-count.fasta	62444	1987867	998422	502257.95	998422	502257.95
4							
5	Enrichment (populations 2:1): 0.953						

Figure 11: FASTAptameR-Motif_Tracker Output

3.7 FASTAptameR-Distance

3.7.1 Description

FASTAptameR-Distance tabulates the distribution of distances from a user-defined reference sequence for all sequences in a population. The module accepts a *counted* FASTA file as input and returns a data table that contains a column for the Levenshtein edit distance (LED) between each input sequence and a query sequence. The output can be downloaded as a CSV. A screenshot of the module interface is shown in **Fig. 12**.

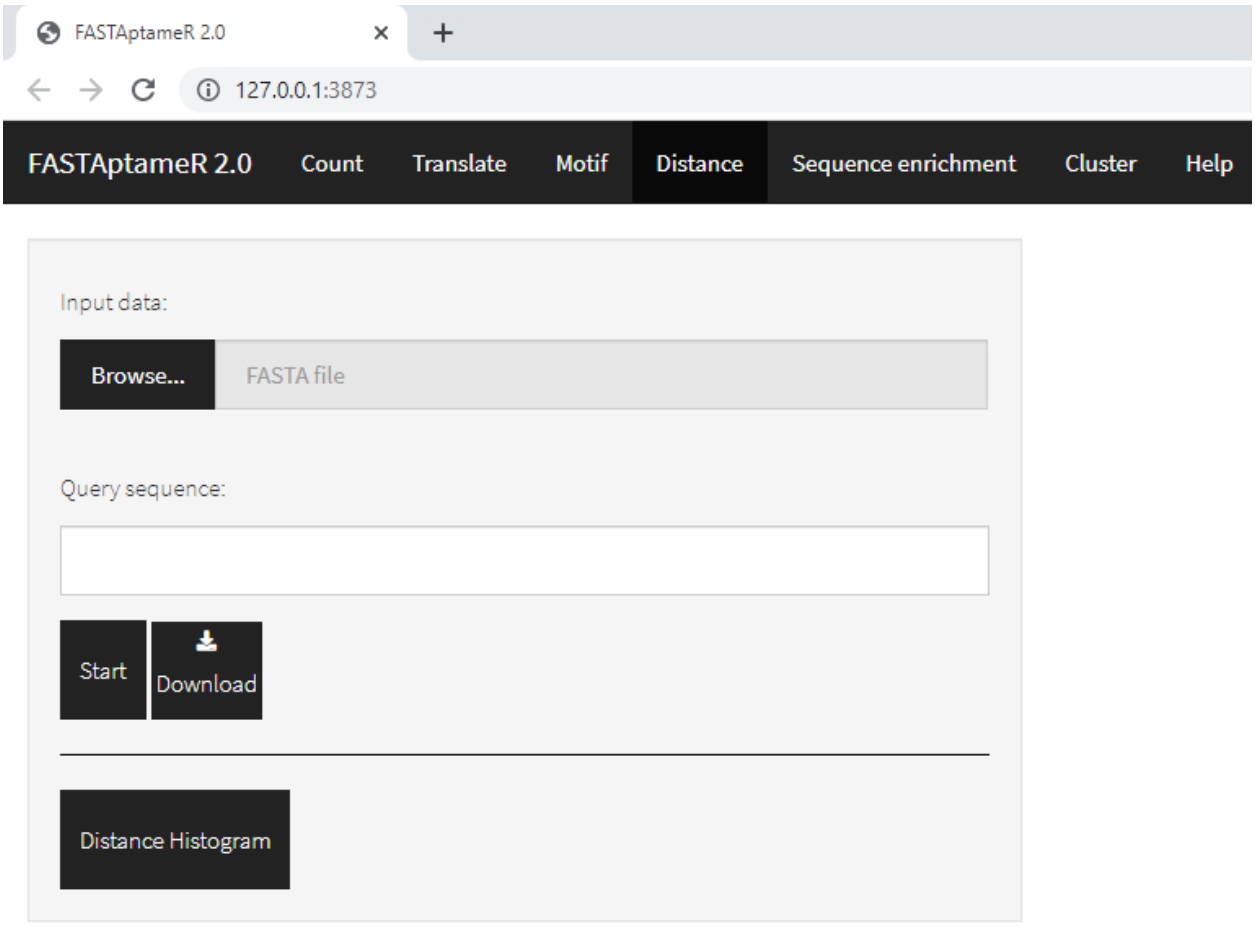


Figure 12: Screenshot of FASTAptameR-Distance

3.7.2 Usage

The input FASTA file must be chosen with the file browser, and the following text box must contain a single query sequence. Note, this query sequence may not have any degenerate nucleotide codes. The `Start` button begins the distance calculations. The resulting data table will be shown on the right side of the screen. The `Download` button opens a file browser prior to downloading the output as a CSV file.

A sample output data table is shown in **Fig. 13** with the following query sequence (the most abundant sequence from the 70HRT14 dataset):

`ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT`.

Show 10 entries Search:

seqs	Rank	Reads	RPM	Distance
All	All	All	All	All
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT	1	417696	193358.44	0
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCGCGCTTGGTGT	5	74389	34435.91	1
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCATGCTTGGTGT	7	53608	24816.04	1
ACGTTGTCGAAAGCCTATGCAAACTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT	20	8003	3704.72	1
ACGTTGTCGAAAGCCTATGCAAATCAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT	21	7815	3617.69	1
ACGTTGTCGAAAGCCTATGCAGATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT	23	6177	2859.44	1
ACGTTGTCGAAAGCCTATGCGAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT	26	5487	2540.02	1
ACGTTGTCGAAAGCCTGTGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT	28	5302	2454.38	1
ACGTTGTCGAAAGCCTATGCAAATTGAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT	29	5079	2351.15	1
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAGACCTTGCGTAGACTCGCCACGCTTGGTGT	31	4504	2084.98	1

Showing 1 to 10 of 72,921 entries Previous 1 2 3 4 5 … 7293 Next

Figure 13: FASTAptameR-Distance Output

3.7.3 Plotting

This module can also generate interactive histograms of distances (**Fig. 14**). The top plot corresponds to the distances between the query and unique sequences, whereas the bottom plot corresponds to the distances between the query and all sequences. In both cases, the query sequence is displayed at the top of the plot.

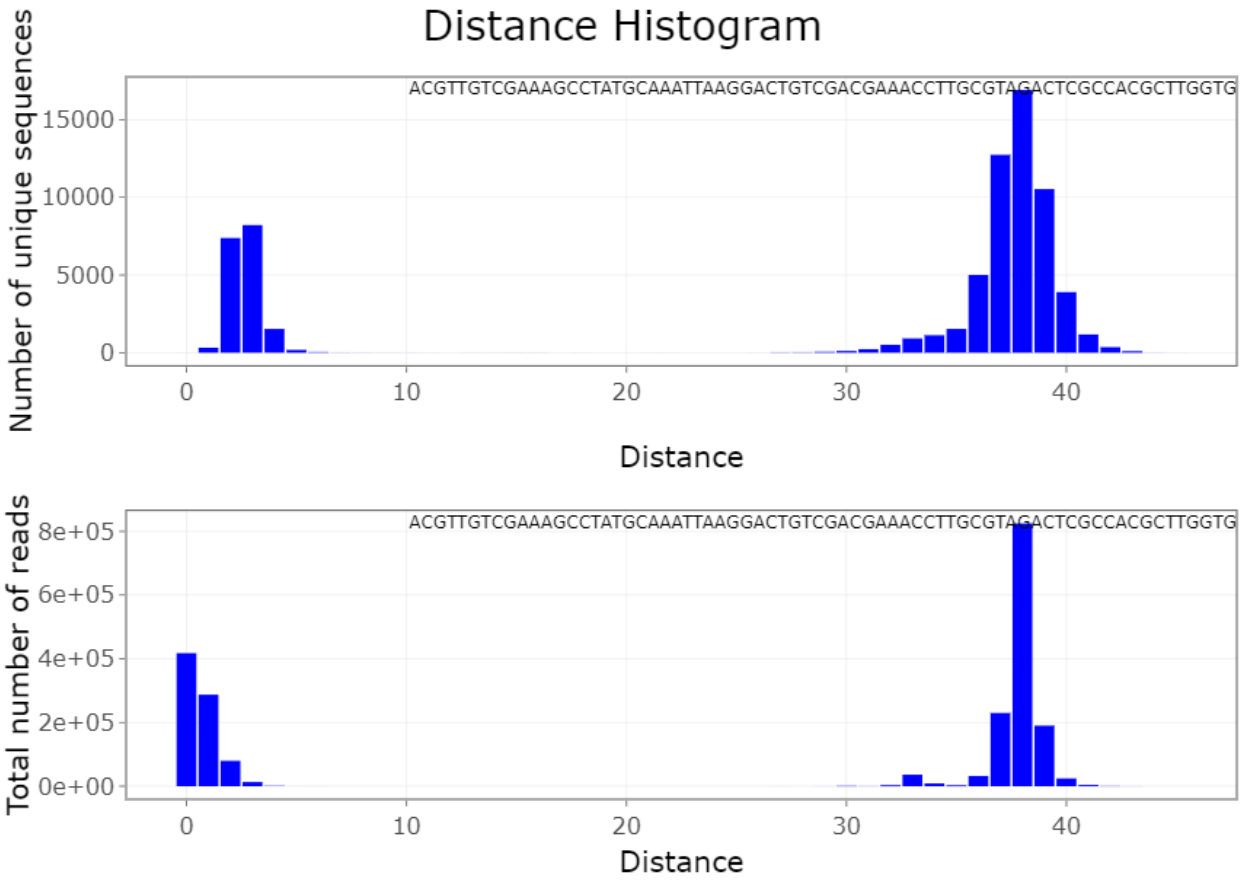


Figure 14: Distance Histogram. Two views of the 70HRT14 data, where the top and bottom panels correspond to unique sequences and total reads, respectively. Both plots are generated together in the UI.

3.8 FASTAptameR-Enrich

3.8.1 Description

FASTAptameR-Enrich calculates the enrichment (or depletion) of each sequence in one population relative to other populations. The module accepts two or three *counted* FASTA files as input and returns a single data table after merging by sequences. Column headers for output data correspond to sequences from the 1st, 2nd, and optional 3rd file and are appended with *.x*, *.y*, and *.z*, respectively. Additional columns include enrichment scores ("Enrichment" = `RPM2 / RPM1`) and the base-two logarithm of the Enrichment ("log2(E)" = `log2(Enrichment)`). The order of comparisons are `y:x`, `z:y`, and `z:x`. This output can be downloaded as a CSV.

A screenshot of the module interface is shown in **Fig. 15**.

3.8.2 Usage

The input FASTA files must be chosen with the file browser. The following set of radio buttons determine whether missing values are allowed in the output. Missing values result from sequences that are only present in a subset of the input files.

The `Start` button begins the enrichment calculations, and the resulting data table will be shown on the right side of the screen. All numeric columns in this data table are filterable by typing into the corresponding text box (*e.g.*, `1 ... 10` to keep values in the range `[1:10]`) or by using the slider bar that is displayed after clicking in the corresponding text box. Note, these filters apply the mask only to the displayed data, so calculations will **not** be repeated when the filters are altered. To display all data again, delete the filters from the text boxes.

The `Download` button opens a file browser prior to downloading the output as a CSV file. A sample filtered output data table is shown in **Fig. 16**.

3.8.3 Plotting

This module can generate three types of interactive plots: $\log_2(Enrichment)$ histograms (one per comparison - **Fig. 17**), RPM scatter plots (a 2D plot for two populations - **Fig. 18** - or a rotatable 3D plot for three populations - *not shown*), and volcano plots (one per comparison - **Fig. 19**).

The spread of the $\log_2(Enrichment)$ histogram relative to a vertical line at `x = 0` can indicate the magnitudes of enrichment (or depletion), while displacement of the centroid of the distribution from this line can indicate possible directionality of the population's evolution.

Similarly, the spread of the RPM scatter plot relative to the diagonal line at `y = x` can also indicate the magnitudes of enrichment and possible directionality.

The volcano plot is used to show the relationship between the respective $\log_2(Enrichment)$ and number of reads for each sequence, which is an indication of the statistical strength of the observed ratios. Specifically, for the purposes of this module, the y-axis is given by $y(seq) = \sqrt{\log_2(seq.Reads)/log_2(Total.Reads)}$, based on the fact that the variance of sample count for a species from a resampled population varies with the square root of the number of times a given species is resampled.

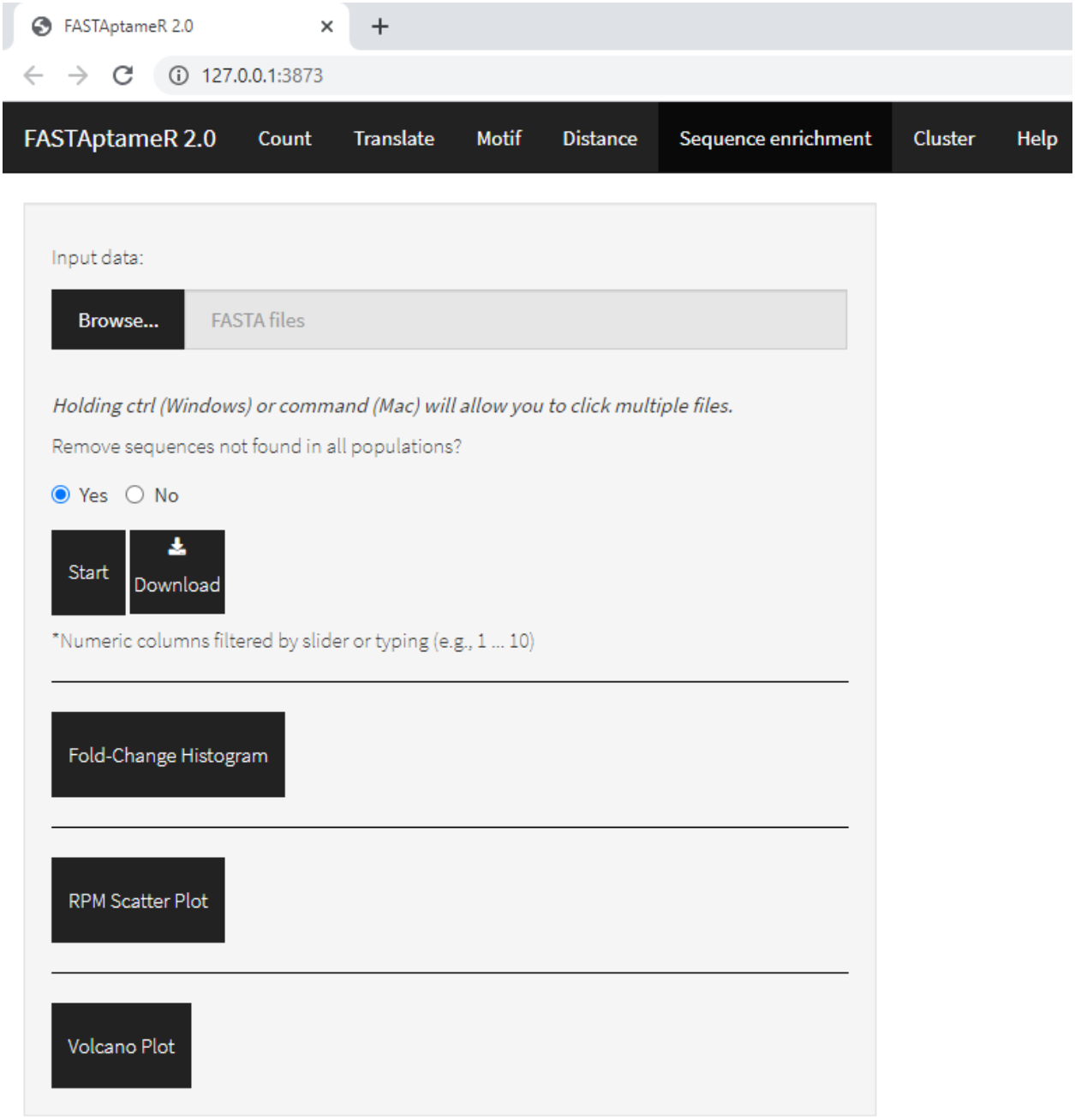


Figure 15: Screenshot of FASTAptameR-Enrich

Show 10 entries Search:

seqs	Rank.x	Reads.x	RPM.x	Rank.y	Reads.y	RPM.y	enrichment_yx
All	All	All	All	All	All	All	1.000 ... 10.000
CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG	2	313312	145037.35	1	382391	192362.47	1.326
TTGACAATAACTCGAGAAGAACCGAGGTGCAAACGGGAGAACACAATGGATTACACCGAGCTCGGCTGAC	9	33800	15646.58	4	136505	68669.08	4.389
ACGTTGTGCACGGATGCCCACGGTCGCACGAAACCTTGTGTGGGATAGCGCGAATACTGACGAGTGTGCC	11	29062	13453.28	11	30391	15288.25	1.136
CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAGGAGCGCGATACCAATGCTGGACTG	12	22089	10225.37	12	21927	11030.42	1.079
CATAGCGACTATCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG	13	14115	6534.07	13	17601	8854.21	1.355
CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGTGCGATACCAATGCTGGACTG	15	10818	5007.83	14	17071	8587.6	1.715
CATAGCGACCGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG	16	10514	4867.11	16	14496	7292.24	1.498
TTGGCAATAACTCGAGAAGAACCGAGGTGCAAACGGGAGAACACAATGGATTACACCGAGCTCGGCTGAC	17	10172	4708.79	8	35996	18107.85	3.846
CCCTCCTTGTATGACGCTAACTGAGAATCCGAAGTCCAACGGGAGAATGGACACTTATGACGTGGCGCG	18	9120	4221.8	22	8591	4321.72	1.024
CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCGATGCTGGACTG	19	8498	3933.87	17	11970	6021.53	1.531

Showing 1 to 10 of 12,081 entries (filtered from 21,924 total entries) Previous 1 2 3 4 5 ... 1209 Next

Figure 16: Filtered FASTAptameR-Enrich Output

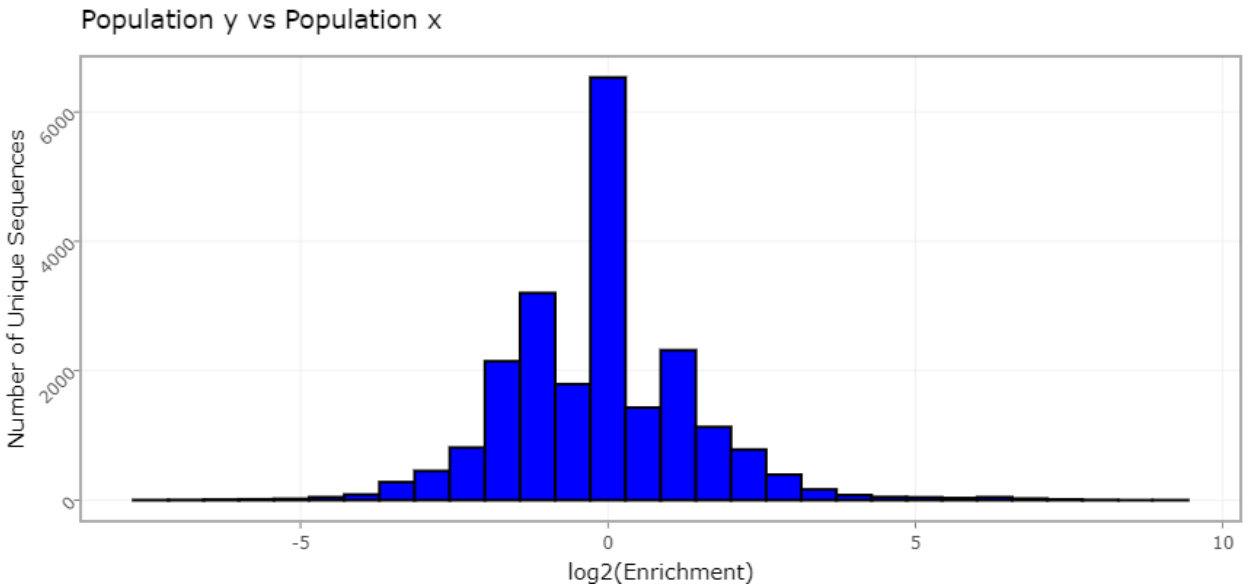


Figure 17: $\log_2(Enrichment)$ Histogram

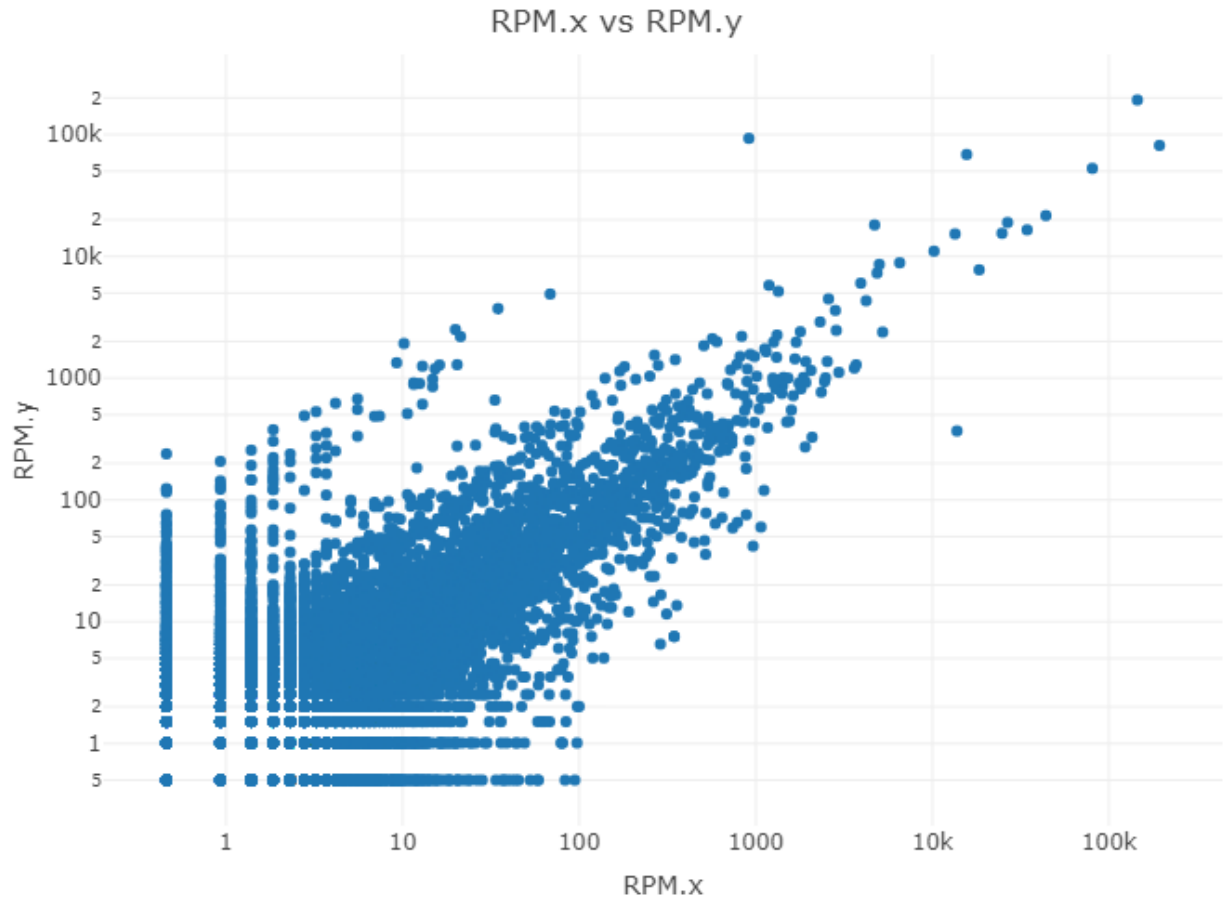


Figure 18: RPM Scatter Plot

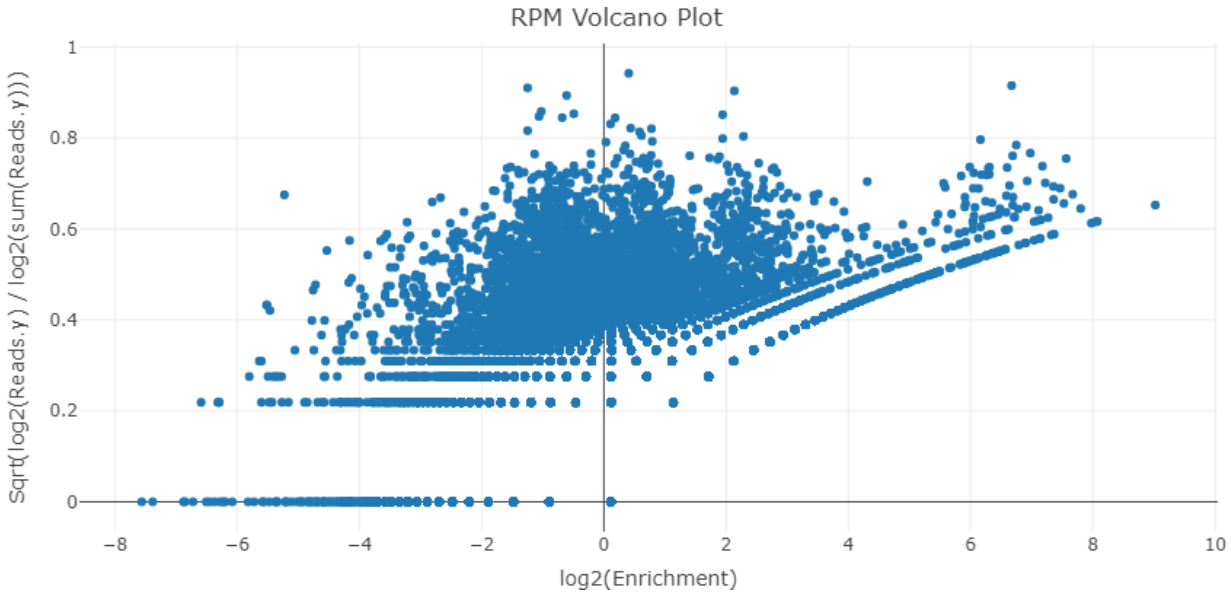


Figure 19: Volcano Plot

3.9 FASTAptameR-Cluster

3.9.1 Description

FASTAptameR-Cluster groups sequences according to sequence relatedness for all sequences in the population within a user-defined threshold of similarity. The module accepts a *counted* FASTA file as input. If no output directory is specified (the default setting), the module returns a *clustered* data table to the screen. This data table contains all sequences and clusters and can be downloaded as a single FASTA or CSV file. If an output directory is specified, then no data table will be created, and one FASTA file per cluster will be written to the output directory.

Briefly, the module identifies clusters in an iterative manner. During each iteration, the most abundant sequence that has not yet been clustered becomes a cluster "seed" for that iteration. Any other sequences that have not yet been clustered and that are within a user-defined edit distance of this seed sequence are added to this cluster. This process repeats until all sequences are clustered or a predefined number of clusters is created.

A screenshot of the module interface is shown in **Fig. 20**.

3.9.2 Usage

The input FASTA file must be chosen with the file browser. The 1st slider bar (**Fig. 20a**) sets the minimum number of reads a sequence must have to be clustered (`DEFAULT = 10`). Sequences with fewer reads are removed prior to clustering. The 2nd slider bar (**Fig. 20b**) sets the maximum Levenshtein edit distance to consider between a seed sequence and all other sequences (`DEFAULT = 7`). The 3rd slider bar (**Fig. 20c**) sets the total number of desired clusters (`DEFAULT = 20`). Note, any remaining sequences will be grouped as `NC` ("not clustered").

The 1st set of radio buttons (**Fig. 20d**) indicates whether non-clustered sequences should be kept (`DEFAULT = No`). If `Yes` then the sequence IDs of non-clustered sequences will be appended with `NC`.

The 2nd set of radio buttons (**Fig. 20e**) indicate whether each cluster should be written to a different FASTA file (`DEFAULT = No`). If `No`, then all clusters are grouped together and downloaded in a single file. If `Yes`, then each cluster will be written to its own FASTA file, and no data table will be displayed. Note, a directory path **must be copied or typed** into the corresponding text box (**Fig. 20f**) if this option is `Yes`.

The `Start` button will begin the clustering process. The results will be displayed as a data table on the right side of the screen. The `Download` button opens a file browser prior to downloading the output as a FASTA or CSV file (`DEFAULT = FASTA`, which is required for subsequent modules).

Algorithm progress will be shown below these buttons and will update after each cluster finishes. These notifications occur regardless of whether the module is writing to one or many files.

A sample output data table is shown in **Fig. 21**.

Note that the new *id* column is the old *id* with three new values appended to the end: `Cluster Number`, `Rank in Cluster`, and `Distance to Cluster Seed`.

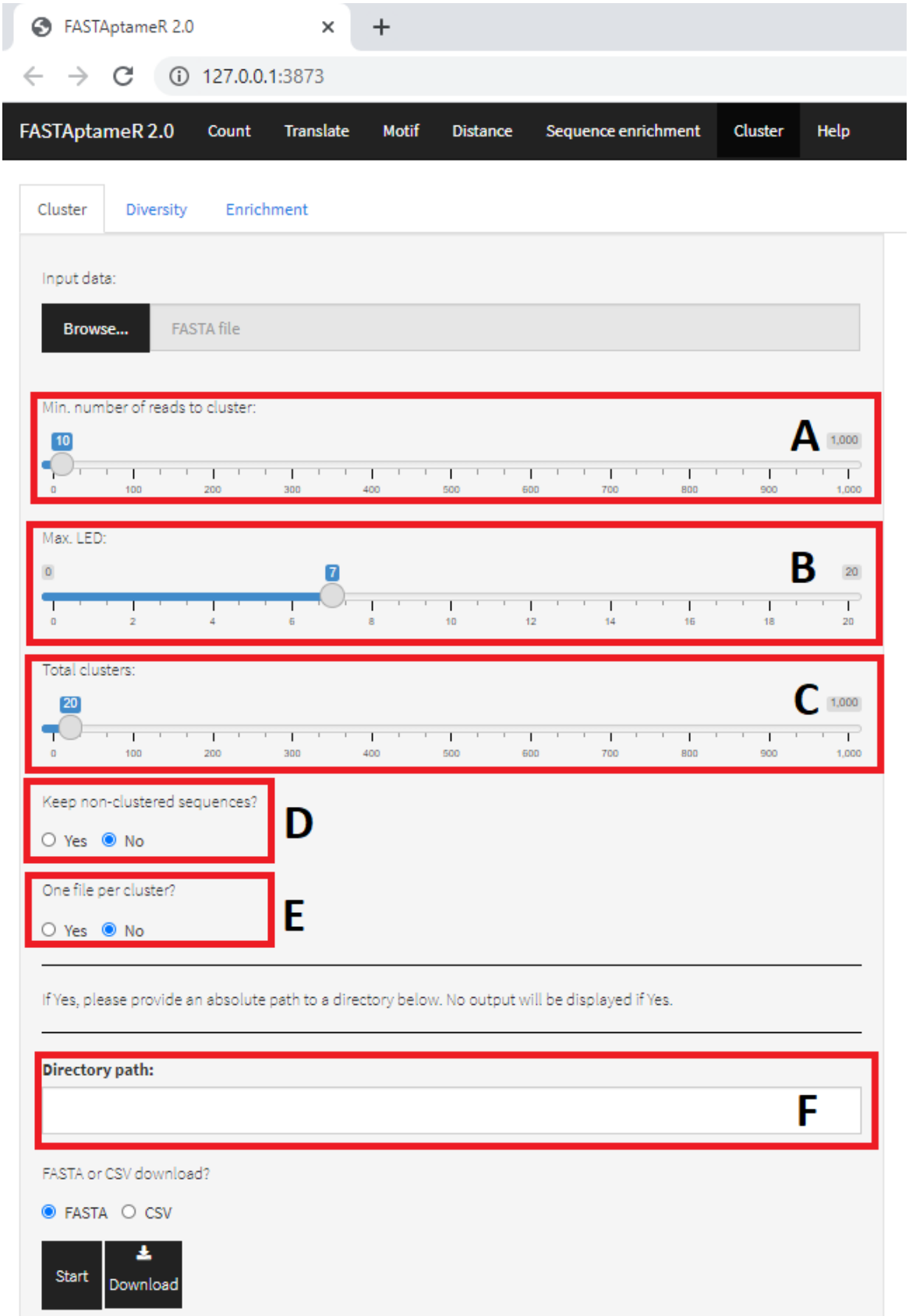


Figure 20: Screenshot of FASTAptameR-Cluster

Show 10 entries Search:

id	Rank	Reads	RPM	cluster	rankInCluster	LED	seqs
All	All	All	All	All	All	All	All
>1-417696-193358.44-1-1-0	1	417696	193358.44	1	1	0	ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT
>2-313312-145037.35-2-1-0	2	313312	145037.35	2	1	0	CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG
>3-174096-80591.94-3-1-0	3	174096	80591.94	3	1	0	AACCGCAAGCAACACCCAGCAAGAAACATCCGACGCACGACGGGAGAAAGTGCATTACCACGATGTCGAT
>4-94978-43966.9-2-2-1	4	94978	43966.9	2	2	1	CATAGCGACTGCCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG
>5-74389-34435.91-1-2-1	5	74389	34435.91	1	2	1	ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCGCGCTTGGTGT

Figure 21: FASTAptameR-Cluster Output

3.10 FASTAptameR-Cluster_Diversity

3.10.1 Description

FASTAptameR-Cluster_Diversity evaluates diversity across the *clustered* population and sequence relationships within and between clusters. The module accepts a *clustered* FASTA file as input and returns a data table with metadata for each cluster. This data table can be downloaded as a CSV file.

A screenshot of the module interface is shown in **Fig. 22**.

3.10.2 Usage

The input FASTA file must be chosen with the file browser. The `Start` button begins the analysis. The results will be displayed as a data table on the right side of the screen and include the following columns: `Cluster Number`, `Seed Sequence`, `Total Sequences`, `Total Reads`, and `Total RPM`. The `Download` button opens a file browser prior to downloading the output as a CSV file, which can be used by FASTAptameR-Cluster_Enrich. A sample output data table is shown in **Fig. 23**.

3.10.3 Plotting

This module can generate metaplots of the analyzed data (**Fig. 22a**). These line plots correponds to the number of unique sequences per cluster, total reads per cluster, and average LED to seed sequence per cluster (**Fig. 24**).

This module is also able to analyze clusters by converting all sequences into k-mer vectors and rendering an interactive 2D PCA plot (**Fig. 22b**), colored by cluster (**Fig. 25**). The value of `k` can be chosen with the 1st set of radio buttons (**Fig. 22c**) (`DEFAULT = 3`). The slider bar (**Fig. 22d**) indicates how many of the top clusters should be plotted (max = 21 clusters due to graphics limitations). The 2nd set of radio buttons (**Fig. 22e**) indicates whether non-clustered (`NC`) sequences should be plotted (`DEFAULT = Yes`). Note that non-clustered (`NC`) sequences in the output are marked as `NA` in this plot.

Importantly, only nucleotide sequences without ambiguities should be plotted in this module. The large k-mer matrix needed for peptide sequences may return errors related to memory usage. Further, this module will reject any set of sequences with characters outside of `[A, C, G, T/U]`. The resulting k-mer PCA plot will not display anything.

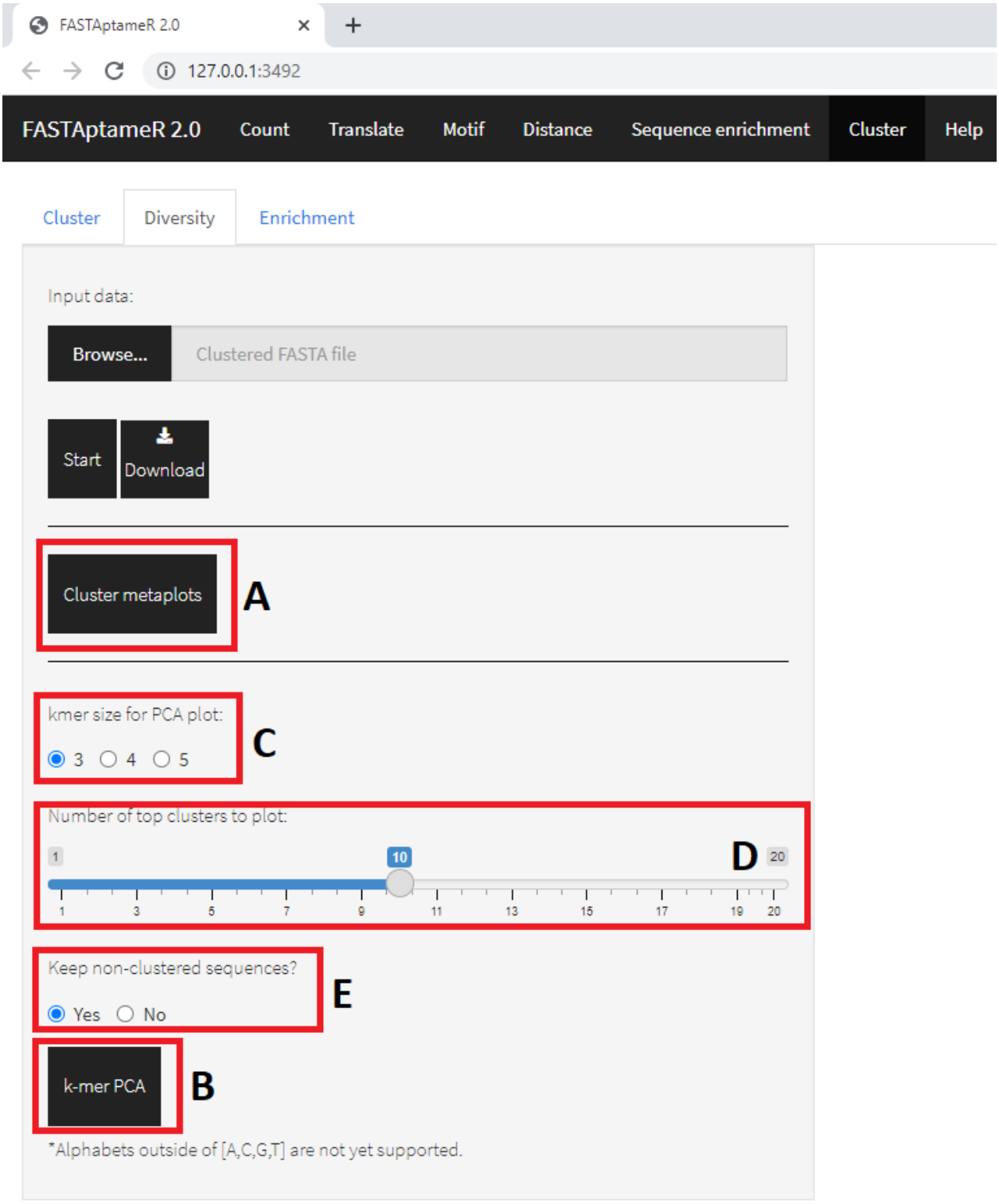


Figure 22: Screenshot of FASTAptameR-Cluster_Diversity

Show 10 entries Search:

Cluster	Seeds	TotalSequences	TotalReads	TotalRPM	AverageLED
All	All	All	All	All	All
1	ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT	1259	770383	356623.54	1.86
2	CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG	1295	652468	302038.75	1.97
3	AACCGCAAGCAACACCCAGCAAGAAACATCCGACGCACGACGGGAGAAAGTGCATTACCACGATGTCGAT	528	257675	119282.23	1.63
4	CCCTCCTTGTATGACGCTAACTGAGAATCCGAAGTCCAACGGGAGAAAGGACACTTATGACGTGGCGCG	324	101448	46962.14	1.49
5	AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC	262	73809	34167.47	1.47
6	TTGACAATAACTCGAGAAGAACCGAGGTGCAAACGGGAGAACACAATGGATTACACCGAGCTCGGCTGAC	275	67908	31435.87	1.51
7	GCGAACCAAACCCAGATTACTAACCGTGGGCCTGAAACACGGGACAAAACAGGCATCAATGGAGTGGTAC	148	41566	19241.67	1.16
8	ACGTTGTGCACGGATGCCCACGGTCGCACGAAACCTTGTGTGGGATAGCGCGAATACTGACGAGTGTGCC	163	44693	20689.16	1.26
9	ACCAAATCCCGAACTACAAATCCGAACGCTAACGGGACAATTGCGAAATGGAACATACGGGCCTGGTTGA	67	9114	4219.07	1.1
10	GTGCGCTACCACATGATCCGAGGCAAAACGGGAAAAGATAGCATCGATTACGGAACCGGCCACGCACA	54	7292	3375.58	0.98

Showing 1 to 10 of 21 entries Previous 1 2 3 Next

Figure 23: FASTAptameR-Cluster_Diversity Output

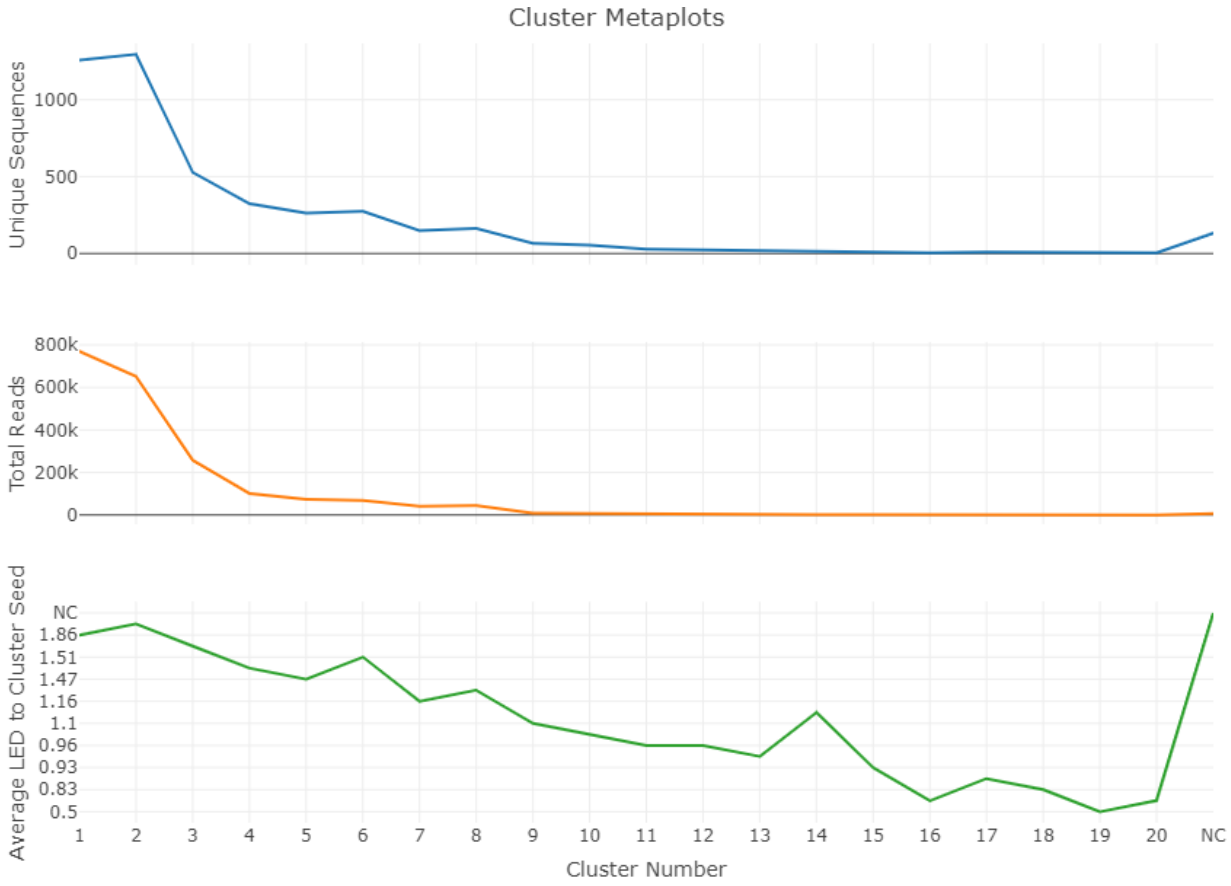


Figure 24: Cluster metaplots

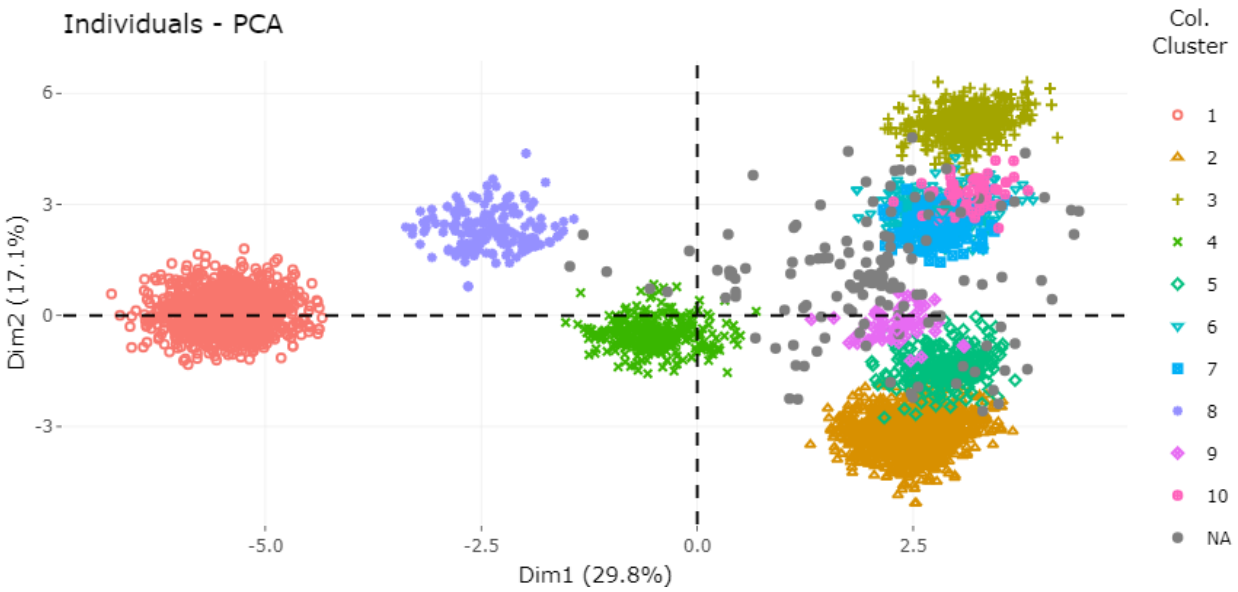


Figure 25: 4-mer PCA of top 20 clusters

3.11 FASTAptameR-Cluster_Enrich

3.11.1 Description

FASTAptameR-Cluster_Enrich calculates the enrichment (or depletion) of each cluster in one population relative to other populations. The module accepts two or three *cluster-analysis* CSV files as input and returns a data table after merging by `Seed Sequence`. Thus, this module assumes that cluster seeds are consistent across populations, though this may not always be a valid assumption.

The output data table includes a column for `Enrichment` that can be downloaded as a CSV file. A screenshot of the module interface is shown in **Fig. 26**.

3.11.2 Usage

The input FASTA files must be chosen with the file browser. The `Start` button begins the enrichment calculation. The results will be displayed as a data table on the right side of the screen and include a column for `Enrichment`. The `Download` button opens a file browser prior to downloading the output as a CSV file.

A sample output data table is shown in **Fig. 27**.

Note that columns 3-5 and 7-9 refer to *total* values in the given cluster.

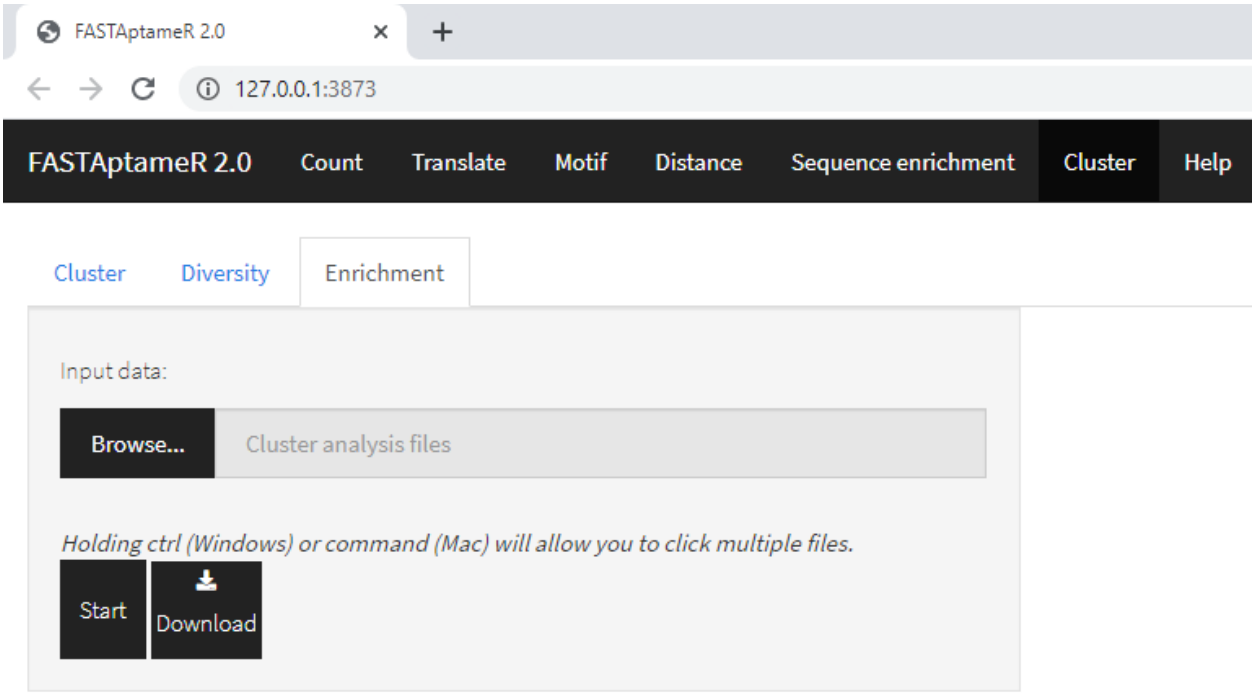


Figure 26: Screenshot of FASTAptameR-Cluster_Enrich

Show 10 entries Search:

Seeds	Cluster.x	TotalSequences.x	TotalReads.x	TotalRPM.x	Cluster.y	TotalSequences.y	TotalReads.y	TotalRPM.y	Enrichment_yx
All	All	All	All	All	All	All	All	All	All
ACGTTGTCGAAAGCCTATGCAAATTAAGGACTGTCGACGAAACCTTGCGTAGACTCGCCACGCTTGGTGT	1	1259	770383	356623.54	3	668	315492	158708.77	0.44503167121273
CATAGCGACTGTCCACGAATCCGAAGCCTAACGGGACAAAAGGCAAGAGCGCGATACCAATGCTGGACTG	2	1295	652468	302038.75	1	1261	685328	344755.34	1.14142751550919
AACCGCAAGCAACACCCAGCAAGAAACATCCGACGCACGACGGGAGAAAGTGCATTACCACGATGTCGAT	3	528	257675	119282.23	5	326	152680	76805.85	0.643900185300023
CCCTCCTTGTATGACGCTAACTGAGAATCCGAAGTCCAACGGGAGAAAGGACACTTATGACGTGGCGCG	4	324	101448	46962.14	6	167	60085	30225.89	0.643622501018906
AGCGCGGCACCCAAAATCGAAATCCGAAGGCGAACGGGAGAATGCGACCAAAGATACCCTGTGAATGGC	5	262	73809	34167.47	8	139	27584	13876.15	0.40612167070023
TTGACAATAACTCGAGAAGAACCGAGGTGCAAACGGGAGAACACAATGGATTACACCGAGCTCGGCTGAC	6	275	67908	31435.87	4	646	276605	139146.49	4.42636039657881
GCGAACCAAACCCAGATTACTAACCGTGGGCCTGAAACACGGGACAAAACAGGCATCAATGGAGTGGTAC	7	148	41566	19241.67	18	5	866	435.64	0.022640446489312
ACGTTGTGCACGGATGCCCACGGTCGCACGAAACCTTGTGTGGGATAGCGCGAATACTGACGAGTGTGCC	8	163	44693	20689.16	7	121	41469	20860.99	1.00830531544055
ACCAAATCCCGAACTACAAATCCGAACGCTAACGGGACAATTGCGAAATGGAACATACGGGCCTGGTTGA	9	67	9114	4219.07	9	90	14993	7542.27	1.78766173588018
GTGCGCTACCACATGATCCGAGGCAAAACGGGAAAAGATAGCATCGATTACGGAACCGGCCACGCACA	10	54	7292	3375.58	13	20	2567	1291.32	0.382547591821257

Showing 1 to 10 of 15 entries Previous 1 2 Next

Figure 27: FASTAptameR-Cluster_Enrich Output

4 Version history

References

Alam KK, Burke DH, Chang JL. 2015. "FASTAptamer: A Bioinformatic Toolkit for High-throughput Sequence Analysis of Combinatorial Selections." *Mol Ther Nucleic Acids* 4. https://doi.org/10.1038/mtna.2015.4.

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