DiveR

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Note: This project is under active development.

CHAPTER

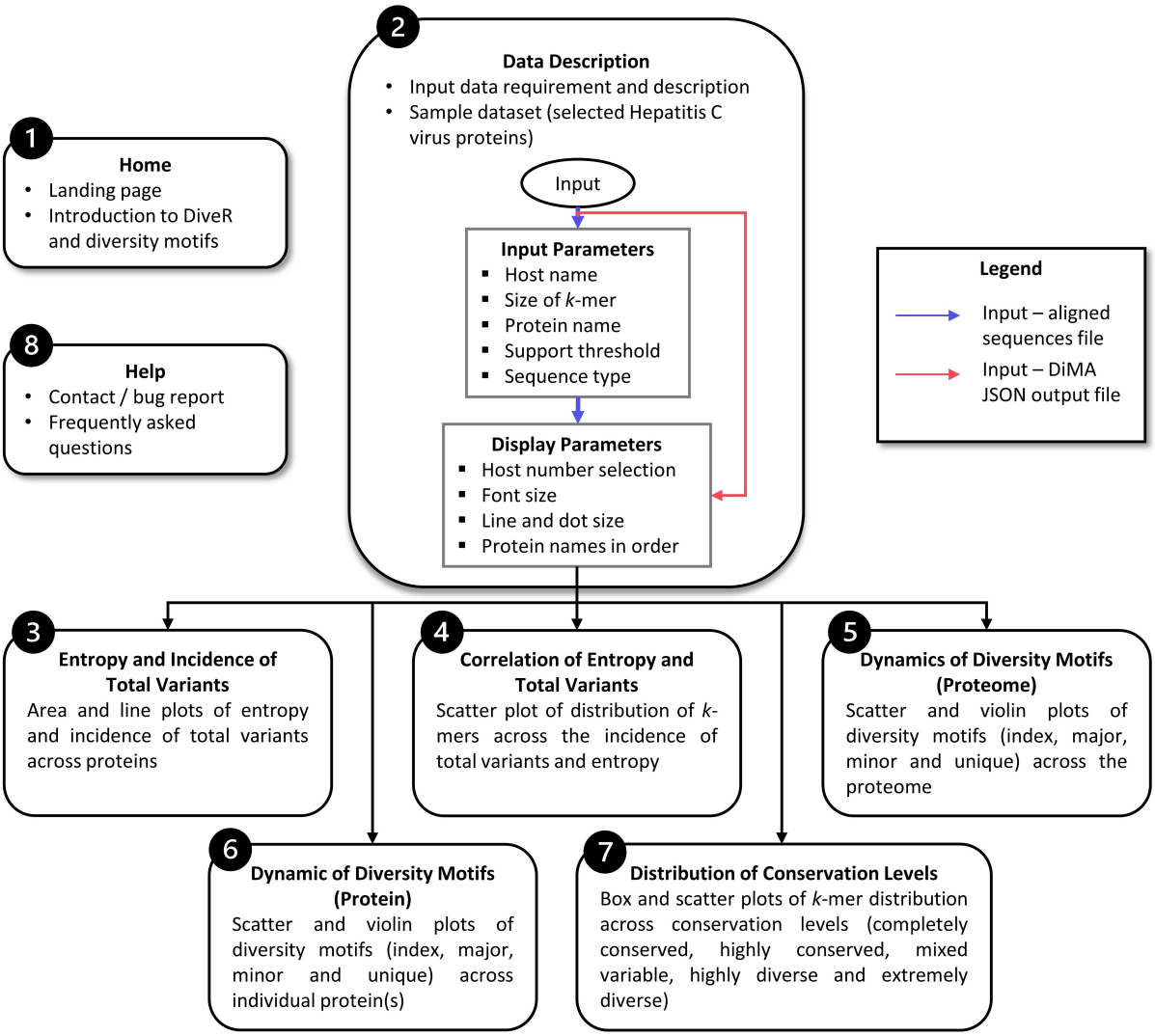
ONE

1. ABOUT

1.1 1.1. Viral Sequence Diversity Dynamics Visualization in R (DiveR)

Viruses are one of the main contributors to the global burden of infectious-related mortality and disability. Sequence diversity, as a result of various evolutionary forces, can expand host repertoire or enhance infective ability of viruses, resulting in immune escape. This poses a challenge to the design of diagnostic, prophylactic, and therapeutic interventions against viruses. Thus, it is crucial to understand the diversity and the dynamics of viral se-quence change to aid in the design of vaccines or development of therapeu-tics and diagnostics against a virus. The publicly available tool, Diversity Motif Analyser (DiMA; https://github.com/PU-SDS/DiMA) was developed to facilitate the dissection of sequence diversity dynamics for viruses. DiMA quantifies the sequence diversity using Shannon's entropy for each aligned overlapping *k*-mer positions, distributes the *k*-mers into four diversity motifs (index, major, minor and unique) and stores this information in JSON format. However, interpretation and analysis of data stored in JSON data might be a challenging task to biologists who have limited or no knowledge of bio-informatics or programming background.

Herein, we present DiveR, a DiMA wrapper implemented as a web-based application, hosted on R Shiny server https://protocol-viral-diversity.shinyapps.io/DiveR, to ease the visualization of outputs from DiMA. DiveR allows visualization of the diversity motifs (index, major, minor and unique) for elucidation of the underlying inherent dynamics. The sequence with the highest incidence at a given *k*-mer position in a protein alignment is the index, while all the others at the position are variants to the index. Major variant is the predominant sequence amongst the variants, while minor variants are distinct sequences with frequency lesser than the major variant, but occur more than once. Unique variants are distinct sequences that occur only once. DiveR presents a total of eight tabs: 1) homepage, 2) data description, with tabs 3) to 7) presenting five plots depicting sequence variability dynamics and lastly 8) help page tab (Figure 1). DiveR generates five plots for k-mer positions of a viral protein/proteome: (i) entropy and in-cidence of total variants, (ii) relationship between entropy and total variants, (iii) dynamics of diversity motifs for the collective proteome and individual proteins (iv), and (v) distribution of conservation levels (completely conserved, highly conserved, mixed variable, highly diverse, and extremely diverse). In summary, the simplicity of DiveR makes the study of viral protein sequence diversity dynamics more accessible to a wider community of researchers. This should help better understand the dynamics of sequence change among viruses and further explore its effects on intervention strategies. Besides being available as a web server, DiveR can also be downloaded as a standalone R Shiny App (https://github.com/pendy05/DiveR).

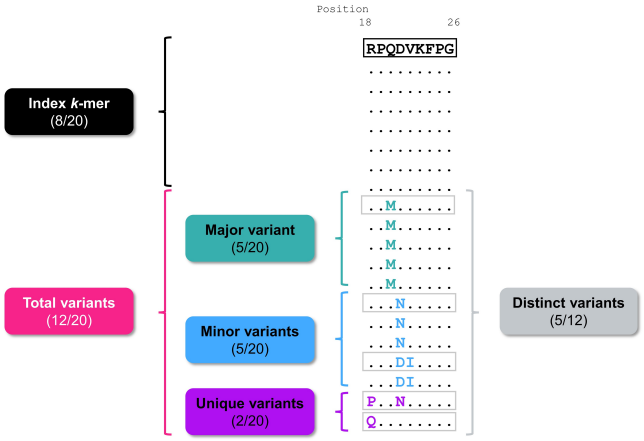


1.2 1.2. Availability

DiveR is publicly available at https://protocol-viral-diversity.shinyapps.io/DiveR/ and the R source code is released under the MIT License and openly available from the GitHub repository at https://github.com/pendy05/DiveR.

1.3 1.3. Diversity Motifs

Viral sequence diversity dynamics quantified as four motifs



Glossary:

(i) Diversity motifs:

- Index: the most prevalent sequence
- Major variant: the most common variant
- Minor variants: multiple different sequences that are observed more than once and of less frequency than the major variant
- Unique variants: sequences that are observed only once

(ii) Total variants:
All sequences that are at least one base different from index

(iii) Distinct variants:
Distinct sequences for a given k-mer position

Figure 1. Definitions of diversity motifs.

CHAPTER

TWO

2. INPUT FILE AND PARAMETERS

2.1 2.1. Input file

DiveR requires either aligned sequence file(s) or DiMA output file(s) (JSON format) as input file(s), where DiveR will convert and concatenate them (the inputs) into a single CSV file (Figure 2), which will act as the source for subsequent data visualisation. Each aligned sequence / DiMA output file is treated as one viral protein. Currently, DiveR accepts aligned FASTA or JSON files generated using multiple sequence alignment (MSA) tools and DiMA, respectively.

proteinName	position	count	lowSupport	entropy	indexSequence	index. incidence	major. incidence	minor. incidence	unique. incidence	totalVariants. incidence	distinctVariant. incidence	multiIndex	host	highestEntropy. position	highestEntropy	averageEntropy
Core	1	4214	FALSE	1.9723	MSTNPKPQR	60.6075	27.71713	9.990508	1.68486	39.3925	7.8915663	FALSE	human	66	5.159295108	1.815266029
Core	2	4218	FALSE	2.6073	STNPKPQRK	55.3106	21.83499	20.27027	2.584163	44.689426	9.761273	FALSE	human	66	5.159295108	1.815266029
Core	3	4289	FALSE	2.6219	TNPKPQRKT	55.4908	21.52017	20.33108	2.657962	44.50921	10.162389	FALSE	human	66	5.159295108	1.815266029
Core	4	4292	FALSE	2.9487	NPKPQRKTK	52.5629	20.66636	23.69525	3.075489	47.43709	11.100196	FALSE	human	66	5.159295108	1.815266029
Core	5	4424	FALSE	1.9136	PKPQRKTKR	76.2432	7.617541	13.74322	2.396022	23.75678	16.745956	FALSE	human	66	5.159295108	1.815266029
Core	6	4441	FALSE	1.8925	KPQRKTKRN	76.4468	7.746003	13.44292	2.364332	23.553253	16.347992	FALSE	human	66	5.159295108	1.815266029
Core	7	4440	FALSE	1.9515	PQRKTKRNT	75.7433	7.792792	14.32432	2.13964	24.256758	15.413184	FALSE	human	66	5.159295108	1.815266029
Core	8	4506	FALSE	2.9709	QRKTKRNTN	57.5455	14.40302	25.21083	2.840657	42.454506	12.232097	FALSE	human	66	5.159295108	1.815266029
Core	9	4564	FALSE	2.933	RKTKRNTNR	57.6906	14.63628	24.8028	2.870289	42.309376	12.0145	FALSE	human	66	5.159295108	1.815266029
Core	10	4621	FALSE	2.931	KTKRNTNRR	57.3685	14.88855	24.90803	2.834884	42.631466	11.624365	FALSE	human	66	5.159295108	1.815266029
Core	11	4682	FALSE	2.3725	TKRNTNRRP	63.4771	16.14695	18.41093	1.964972	36.522854	10	FALSE	human	66	5.159295108	1.815266029
Core	12	4748	FALSE	2.8811	KRNTNRRPQ	55.1179	15.33277	27.4642	2.085088	44.882057	9.103707	FALSE	human	66	5.159295108	1.815266029
Core	13	4772	FALSE	2.6451	RNTNRRPQD	58.1727	14.60604	25.54485	1.676446	41.827328	8.316633	FALSE	human	66	5.159295108	1.815266029
Core	14	4878	FALSE	2.6588	NTNRRPQDV	57.9336	13.71464	26.67077	1.681017	42.06642	7.94347	FALSE	human	66	5.159295108	1.815266029
Core	15	5103	FALSE	2.7021	TNRRPQDVK	57.8875	13.03155	27.31726	1.763668	42.11248	7.910656	FALSE	human	66	5.159295108	1.815266029
Core	16	5127	FALSE	2.5627	NRRPQDVKF	58.8648	13.71172	25.92159	1.501853	41.13517	6.9701276	FALSE	human	66	5.159295108	1.815266029
Core	17	5207	FALSE	1.5631	RRPQDVKFP	77.0117	9.141541	12.80968	1.037065	22.988285	8.103592	FALSE	human	66	5.159295108	1.815266029
Core	18	5421	FALSE	1.5407	RPQDVKFPG	77.1075	8.891349	13.07877	0.922339	22.892456	7.8968577	FALSE	human	66	5.159295108	1.815266029
Core	19	5440	FALSE	1.5524	PQDVKFPGG	77.0588	8.860293	13.16176	0.919118	22.941177	7.852564	FALSE	human	66	5.159295108	1.815266029
Core	20	5442	FALSE	1.597	QDVKFPGGG	76.9019	8.857038	13.1018	1.139287	23.098125	8.67144	FALSE	human	66	5.159295108	1.815266029
Core	21	5457	FALSE	0.9726	DVKFPGGGQ	87.9971	5.2593	5.515851	1.227781	12.002933	16.183207	FALSE	human	66	5.159295108	1.815266029
Core	22	5465	FALSE	0.7102	VKFPGGGQI	92.882	1.591949	4.336688	1.189387	7.118024	26.73522	FALSE	human	66	5.159295108	1.815266029
Core	23	5474	FALSE	0.608	KFPGGGQIV	94.4282	0.858604	3.544026	1.169163	5.5717936	34.42623	FALSE	human	66	5.159295108	1.815266029
Core	24	5564	FALSE	0.5325	FPGGGQIVG	95.3451	0.305536	3.199137	1.150252	4.6549244	39.76834	FALSE	human	66	5.159295108	1.815266029

Figure 2. DiMA JSON-Converted CSV Output Format.

1. proteinName: name of the protein
2. position: starting position of the aligned, overlapping *k*-mer window
3. count: number of *k*-mer sequences at the given position
4. lowSupport: *k*-mer position with sequences lesser than the minimum support threshold (TRUE) are considered of low support, in terms of sample size
5. entropy: level of variability at the *k*-mer position, with zero representing completely conserved
6. indexSequence: the predominant sequence (index motif) at the given *k*-mer position
7. index.incidence: the fraction (in percentage) of the index sequences at the *k*-mer position
8. major.incidence: the fraction (in percentage) of the major sequence (the predominant variant to the index) at the *k*-mer position

9. minor.incidence: the fraction (in percentage) of minor sequences (of frequency lesser than the major variant, but not singletons) at the *k*-mer position
10. unique.incidence: the fraction (in percentage) of unique sequences (singletons, observed only once) at the *k*-mer position
11. totalVariants.incidence: the fraction (in percentage) of sequences at the *k*-mer position that are variants to the index (includes: major, minor and unique variants)
12. distinctVariant.incidence: incidence of the distinct *k*-mer peptides at the *k*-mer position
13. multiIndex: presence of more than one index sequence of equal incidence
14. host: species name of the organism host to the virus
15. highestEntropy.position: *k*-mer position that has the highest entropy value
16. highestEntropy: highest entropy values observed in the studied protein
17. averageEntropy: average entropy values across all the *k*-mer positions

2.2 2.2. Parameters

2.2.1 2.2.1. Input Parameters

2.2.1.1. Host Name

Species name of the organism host to the studied virus.

2.2.1.2. Size of *k*-mer

k-mer, a window with size of *k*, gives us the overview, overall diversity of that particular window. By default, DiMA uses *k*-mer size of nine to evaluate the viral diversity, with respect to cellular immune response.

2.2.1.3. Protein Name

Name of the protein.

2.2.1.4. Support Threshold

Support is defined as the number of sequences at a given k-mer position that are free of gaps, unknown or ambiguous nucleotide bases, and amino acid residues. Positions with less than 30 sequences (default) are defined as of low support.

2.2.1.5. Sequence Type

Nucleotide or amino acid sequence.

2.2.2 2.2.2. Display Parameters

2.2.2.1. Host Number Selection

Select the number of host studied (one (default) or two hosts). DiveR supports co-visualization of viral diversity dynamics between two hosts.

2.2.2.2. Font Size

Font size displayed on the plots.

2.2.2.3. Line and Dot Size

Line and dot size displayed on the plots.

2.2.2.4. Protein Names in Order

Determine the order of proteins displayed on plot (Please ensure the protein names provided are the same as the one used in input run!).

CHAPTER

THREE

3. STANDALONE DIVER

3.1 3.1. Installation

.. note:: These instructions assume you have Python (3.7 <= version < 3.11>), RStudio and R (version 3.3.0+ as requested by RStudio) on your computer.

.. code-block:: bash

```
git clone https://github.com/pendy05/DiveR.git
pip install dima-cli==4.1.1
```

3.2 3.2. Usage

At R Studio, open either *server.R* or *ui.R* in the "DiveR/" folder and click on the *Run App* button on the top right corner of R Studio. DiveR will run just like the DiveR R Shiny App on web server, the only difference is, DiveR is run locally.

.. note:: If you would like to customize the R plots as shown in DiveR, you may access those corresponding R scripts in "R-individual-scripts/" subfolder.

CHAPTER

FOUR

4. SAMPLE RESULTS

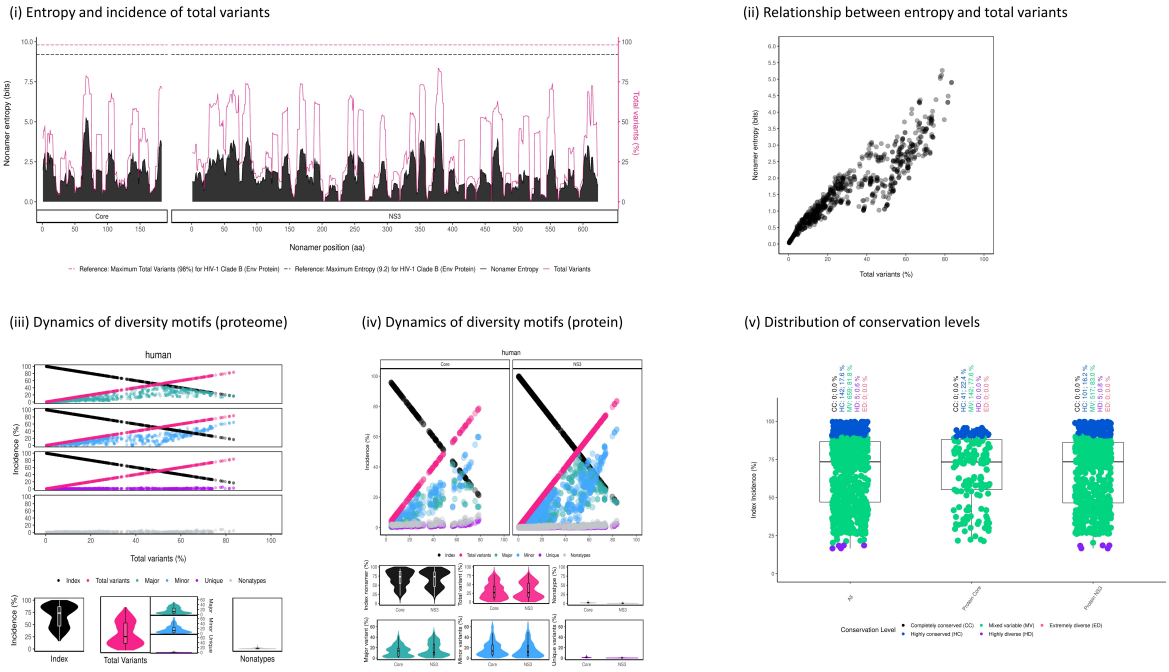


Figure 3. An example of DiveR output, comprising of five plots for sample HCV proteins (Core and NS3)

4.1 4.1. Test Data

To demonstrate the functionality of DiveR, the core and NS3 proteins of Hepatitis C virus (HCV) were selected and used as sample datasets. The hu-man host HCV viral protein sequences were retrieved from the publicly available database, National Center for Biotechnology Information (NCBI) Virus (Hatcher et al., 2017). Subsequently, the data was deduplicated using Cluster Database at High Identity with Tolerance (CD-HIT) (Li & Godzik, 2006) and aligned using Multiple Alignment using Fast Fourier Transform (MAFFT) (Katoh et al., 2002). The HCV sample datasets are provided for users to download and run the visualization of sequence change dynamics in DiveR.

.. note:: Sample result is accessible on DiveR R Shiny App via the “Load Sample Dataset” and “Download Sample Dataset” buttons on its side panel.

4.2 4.2. Output Summary

In DiveR R Shiny App, after providing either aligned sequence file(s) or DiMA JSON output file(s) in tab 2, visualization of dynamics in sequence change in the form of plots will be presented in tabs 3 to 7, with a brief description of the implemented functionalities (Figure 1).

- Tab 3: Entropy and Incidence of Total Variants
- Tab 4: Correlation of Entropy and Total Variants
- Tab 5: Dynamics of Diversity Motifs (Proteome)
- Tab 6: Dynamics of Diversity Motifs (Proteins)
- Tab 7: Distribution of COnservation Levels

.. note:: If there is only one protein input, no plot is shown in Tab 5.

4.3 4.3. Output (Plots and Tables)

4.3.1 4.3.1. Entropy and Incidence of Total Variants

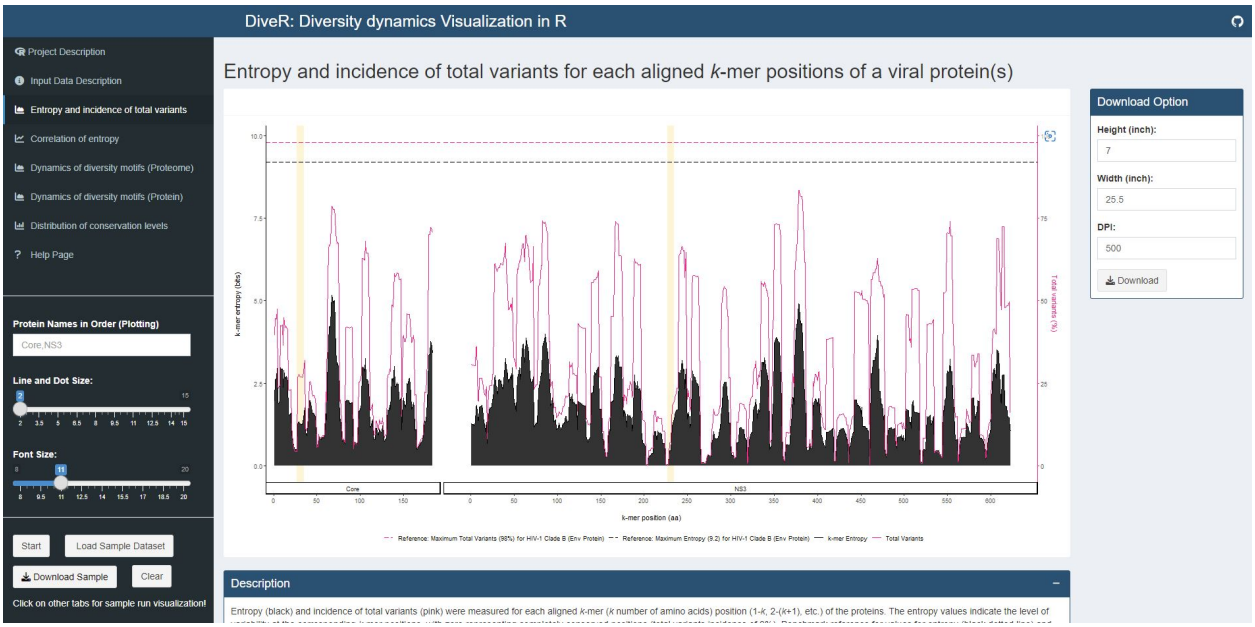


Figure 4.1. Entropy and Incidence of Total Variants Plot for sample HCV proteins (Core and NS3)

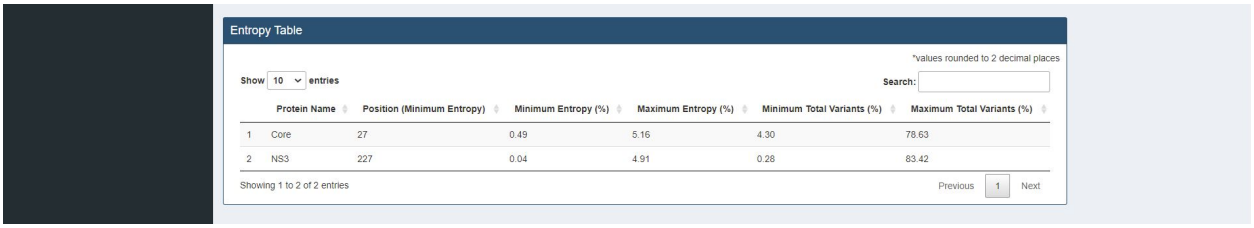


Figure 4.2. Entropy Table for sample HCV proteins (Core and NS3)

Description Entropy (black) and incidence of total variants (pink) were measured for each aligned k-mer position (1-*k*, 2-*k*+1, etc.) of the proteins. The entropy values indicate the level of variability at the corresponding *k*-mer positions, with zero representing completely conserved positions (total variants incidence of 0%). Benchmark reference for values for entropy (black dotted line; 9.2) and total variants (pink dotted line; 98%) that from HIV-1 clade B envelope protein (Hu et al., 2013) are provided. For both individual protein and across proteome, the minimum entropy value is zero. The region highlighted in yellow are *k*-mer positions with zero entropy value.

4.3.2 4.3.2. Correlation of Entropy

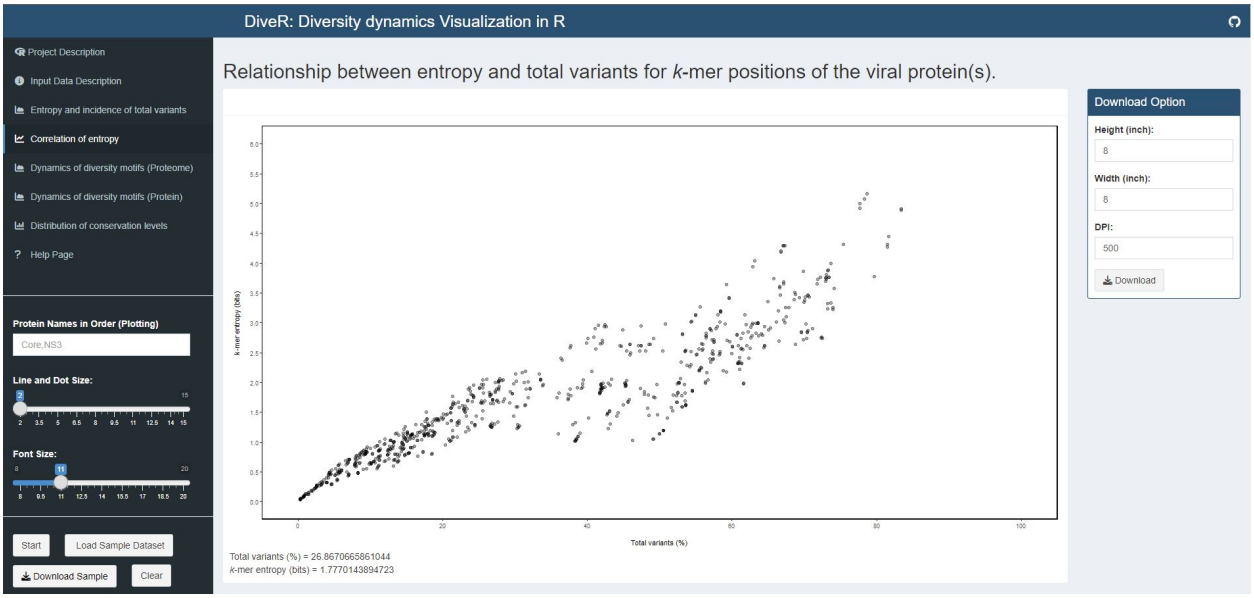


Figure 4.3. Correlation of Entropy and Total Variants Scatter Plot for sample HCV proteins (Core and NS3)

Description Relationship between incidence of total variants and entropy for viral proteome nonamer positions. At y-axis, the minimum entropy value is zero while the maximum entropy value is obtained by rounding the highest entropy encountered up to integer.

4.3.3 4.3.3. Dynamics of Diversity Motifs (Proteome)

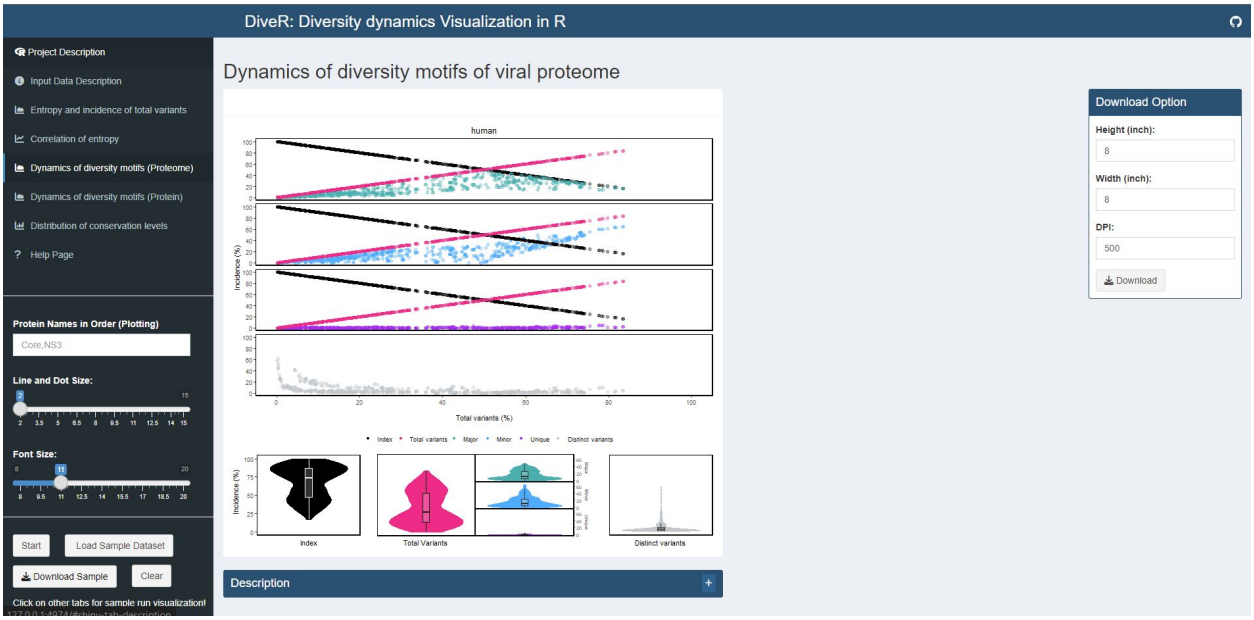


Figure 4.4. Dynamics of Diversity Motifs (Proteome) Plot for sample HCV proteins (Core and NS3)

Description *k*-mers are classified into four different motifs, namely index, major, minor and unique, based on their incidences. *k*-merTypes defines as distinct sequence for a given *k*-mer position. The above dot plot showcases the relationshop between the distribution of four distinct motifs and mutations. The diversity of the position is depicted by the decline of the index incidences (black), the increase of total variant incidences (pink) and corresponding individual patterns of the major, minor, unique and *k*-merTypes motifs. The below violin plot demonstrates the frequency distribution of the motifs. The width of the plot (x-axis) represents the frequency distribution of a given incidence of the indicated motif. The black thick horizontal line of box plot in the middle represents the median incidence value.

4.3.4 4.3.4. Dynamics of Diversity Motifs (Protein(s))

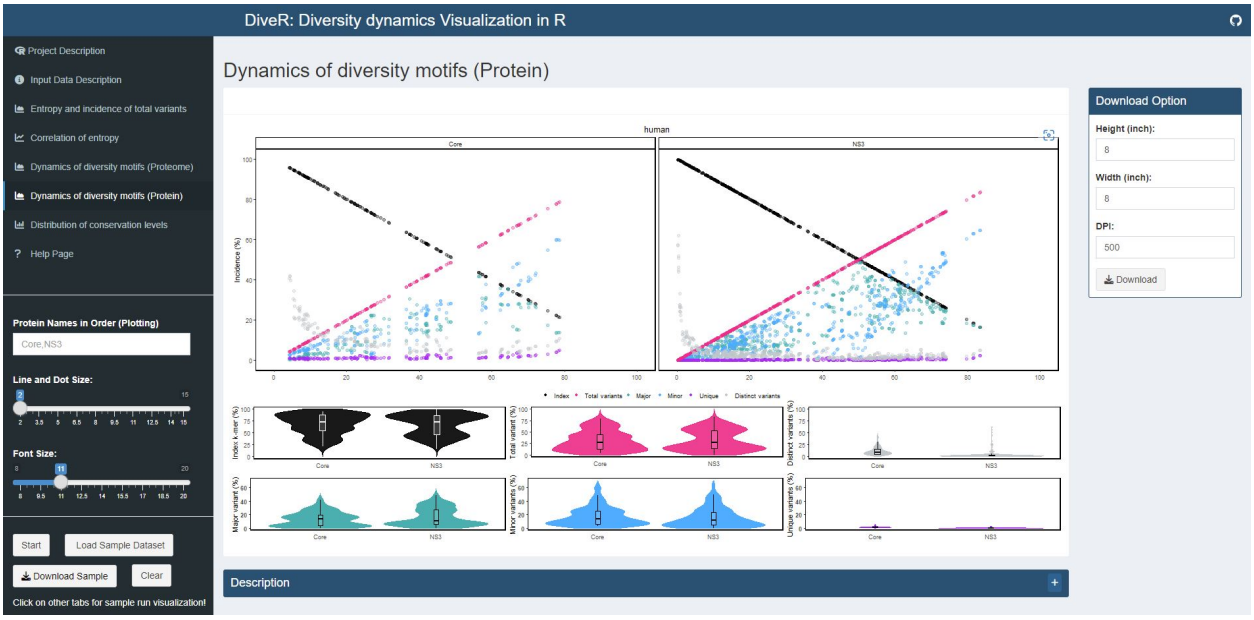


Figure 4.5. Dynamics of Diversity Motifs (Proteins) Plot for sample HCV proteins (Core and NS3)

Description *k*-mers are classified into four different motifs, namely index, major, minor and unique, based on their incidences. *k*-merTypes defines as distinct sequence for a given *k*-mer position. The above dot plot showcases the relationshop between the distribution of four distinct motifs and mutations. The diversity of the position is depicted by the decline of the index incidences (black), the increase of total variant incidences (pink) and corresponding individual patterns of the major, minor, unique and *k*-merTypes motifs. The below violin plot demonstrates the frequency distribution of the motifs. The width of the plot (x-axis) represents the frequency distribution of a given incidence of the indicated motif. The black thick horizontal line of box plot in the middle represents the median incidence value.

4.3.5 4.3.5. Distribution of Conservation Levels

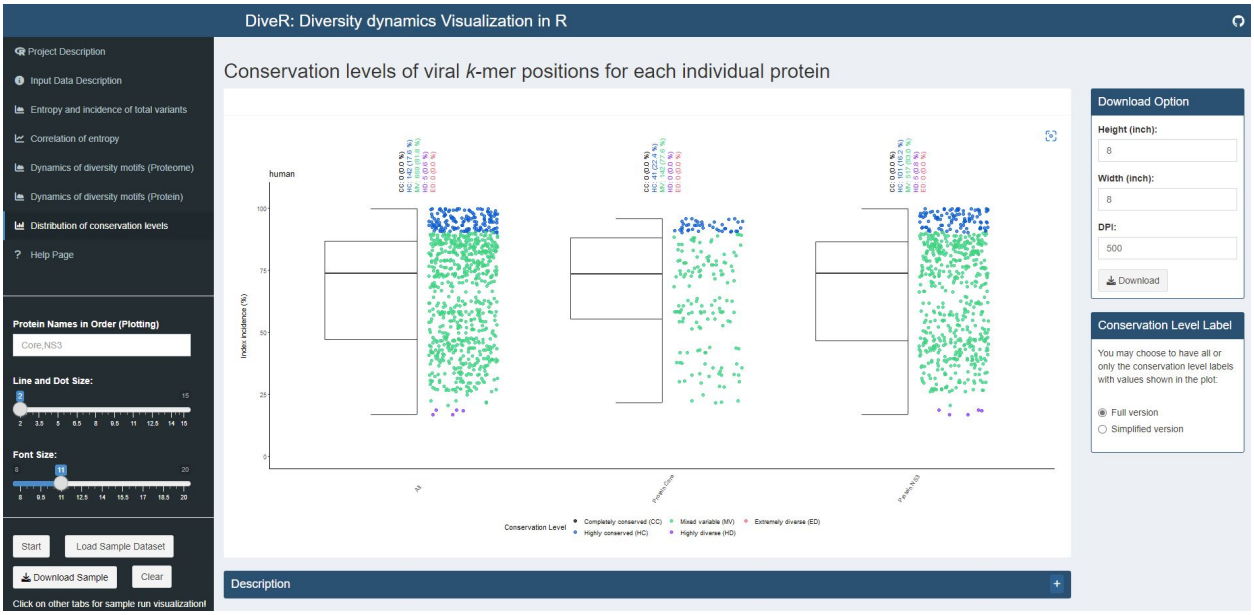


Figure 4.6. Distribution of Conservation Levels Plot for sample HCV proteins (Core and NS3)

Description The *k*-mer positions of the proteome and the individual proteins were defined as completely conserved (black) (= 100%), highly conserved (blue) (90% < 100%), mixed variable (green) (20% < 90%), highly diverse (purple) (10% < 20%) and extremely diverse (pink) (10%).

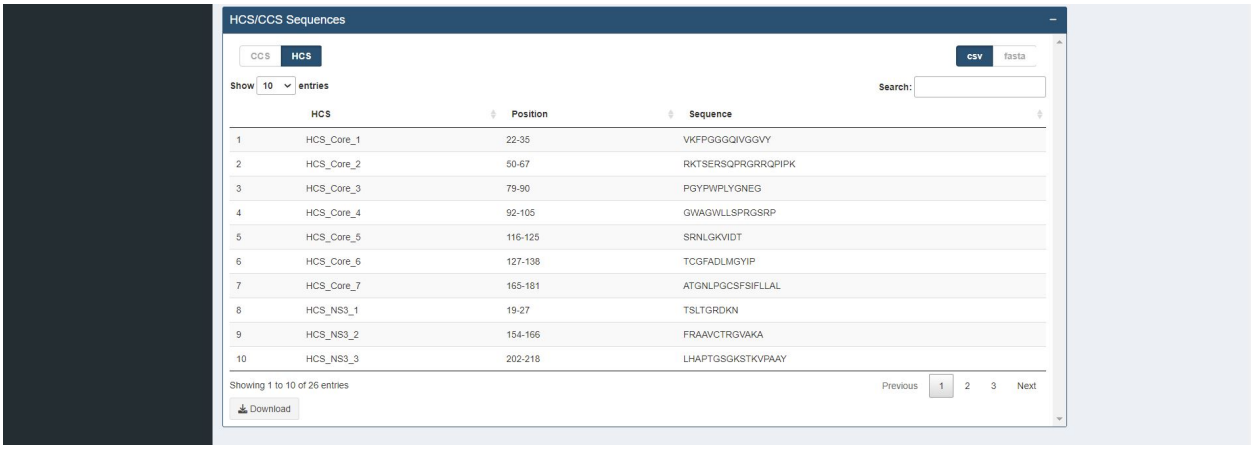


Figure 4.7. Identification of Completely (CCS) / Highly Conserved (HCS) Sequences Table for sample HCV proteins (Core and NS3)

Description The *k*-mer positions that overlapped at least one *k*-mer position or are adjacent to each other are concatenated and displayed in table format. The concatenated sequences can be used for further immune relevance analysis via the usage of the Immune Epitope Database and Analysis Resource (IEDB) (Vita et al., 2019).

CHAPTER

FIVE

5. FAQS AND SUPPORT

5.1 5.1. Support

For technical assistance or bug report, please reach us out via GitHub https://github.com/pendy05/DiveR. For the general correspondence, please email Dr. Asif M. Khan (*asif@perdanauniversity.edu.my*, *makhan@bezmialem.edu.tr*).

5.2 5.2. Team

- Pendy Tok
- Li Chuin Chong
- Evgenia Chikina
- Mohammad Asif Khan