

Note S1 Tool availability and implementation

Smash++ is implemented in C++ language and is available at [1]. This tool is able to find and visualize rearrangements in sequences, FASTA and FASTQ files; although, it is highly recommended to use sequences as input. In the following sections, we describe installing and running the Smash++ tool.

S1.1 Install

To install Smash++ on various operating systems, follow the instructions below. Note that the precompiled executables are available for 64 bit operating systems in the “bin/” directory.

Linux

- Install “git” and “cmake”:

```
1 sudo apt update
2 sudo apt install git cmake
```

- Clone Smash++ and install it:

```
1 git clone https://github.com/smortezah/smashpp.git
2 cd smashpp
3 ./install.sh
```

macOS

- Install “Homebrew”, “git” and “cmake”:

```
1 /usr/bin/ruby -e "$(curl -fsSL https://raw.githubusercontent.com/Homebrew/
  install/master/install)"
2 brew install git cmake
```

- Clone Smash++ and install it:

```
1 git clone https://github.com/smortezah/smashpp.git
2 cd smashpp
3 ./install.sh
```

Windows

- Download and install “CMake”, e.g., from <https://github.com/Kitware/CMake/releases/download/v3.14.4/cmake-3.14.4-win64-x64.msi>. Make sure to add it to the system PATH. For example, if CMake is installed in “C:\Program Files”, add “C:\Program Files\CMake\bin” to the system PATH.
- Download and install “mingw-w64”, e.g., from <https://sourceforge.net/projects/mingw-w64/files/latest/download>. Make sure to add it to the system PATH. For example, if it is installed in “C:\mingw-w64”, add “C:\mingw-w64\mingw64\bin” to the system PATH.
- Download and install “git”, from <https://git-scm.com/download/win>.
- Clone Smash++ and install it:

```
1 git clone https://github.com/smortezah/smashpp.git
2 cd smashpp
3 .\install.bat
```

S1.2 Run

A reference file and a target file are clearly mandatory to run Smash++ (without visualization).
Running

```
1 ./smashpp
```

provides the following guide:

```
1 SYNOPSIS
2   ./smashpp [OPTIONS] -r <REF-FILE> -t <TAR-FILE>
3
4 OPTIONS
5   Required:
6   -r <FILE>           = reference file (Seq/FASTA/FASTQ)
7   -t <FILE>           = target file   (Seq/FASTA/FASTQ)
8
9   Optional:
10  -l <INT>             = level of compression: [0, 5]. Default -> 0
11  -m <INT>             = min segment size: [1, 4294967295]      -> 50
12  -e <FLOAT>           = entropy of 'N's: [0.0, 100.0]          -> 2.0
13  -n <INT>             = number of threads: [1, 8]              -> 4
14  -f <INT>             = filter size: [1, 4294967295]           -> 256
15  -ft <INT/STRING>     = filter type (windowing function):      -> hann
16                        {0|rectangular, 1|hamming, 2|hann,
17                        3|blackman, 4|triangular, 5|welch,
18                        6|sine, 7|nuttall}
19  -fs [S][M][L]        = filter scale:                          -> L
20                        {S|small, M|medium, L|large}
21  -d <INT>             = sampling steps                          -> 1
22  -th <FLOAT>           = threshold: [0.0, 20.0]                 -> 1.5
23  -rb <INT>            = ref beginning guard: [-32768, 32767]    -> 0
24  -re <INT>            = ref ending guard: [-32768, 32767]       -> 0
25  -tb <INT>            = tar beginning guard: [-32768, 32767]    -> 0
26  -te <INT>            = tar ending guard: [-32768, 32767]       -> 0
27  -dp                 = deep compression                        -> no
28  -nr                 = do NOT compute self complexity          -> no
29  -sb                 = save sequence (input: FASTA/FASTQ)      -> no
30  -sp                 = save profile (*.prf)                     -> no
31  -sf                 = save filtered file (*.fil)               -> no
32  -ss                 = save segmented files (*.s[i])            -> no
33  -sa                 = save profile, filetered and              -> no
34                        segmented files
35  -rm k,[w,d,]ir,a,g/t,ir,a,g:...
36  -tm k,[w,d,]ir,a,g/t,ir,a,g:...
37                        = parameters of models
38                        <INT> k: context size
39                        <INT> w: width of sketch in log2 form,
40                        e.g., set 10 for w=2^10=1024
41                        <INT> d: depth of sketch
42                        <INT> ir: inverted repeat: {0, 1, 2}
43                        0: regular (not inverted)
44                        1: inverted, solely
45                        2: both regular and inverted
46                        <FLOAT> a: estimator
47                        <FLOAT> g: forgetting factor: [0.0, 1.0)
48                        <INT> t: threshold (no. substitutions)
49  -ll                 = list of compression levels
50  -h                  = usage guide
51  -v                  = more information
52  --version            = show version
```

The arguments “-r” and “-t” are used to specify the reference and the target, respectively, which are highly recommended to have short names. Level of compression, that is an integer between 0 and 5, can be determined with “-l”. By setting “-m” to an integer value, only those regions in the reference file that are bigger than that value would be able to be considered for compression.

In implementation of the reference-based compression, we have replaced ‘N’ bases in the references and the targets with ‘A’s and ‘T’s, respectively. On reference-free compression, they are replaced with ‘A’s, in both references and targets. If a user tends to replace ‘N’ bases in a sequence with a normal distribution of ‘A’, ‘C’, ‘G’ and ‘T’s, he/she can employ GOOSE toolkit [2]. Note that we have set by default the entropy of ‘N’s to 2.0, however, it can be changed to a value of interest using “-e” option.

Creating multiple finite-context models and substitutional-tolerant Markov models can be done in a multi-threaded fashion by setting “-n” to an integer.

In the process of finding similar regions in the reference and the target sequences, the information content that would be obtained by compression needs to be filtered. Size of the window and type of the windowing function can be set by “-f” and “-ft” options, respectively. Besides Hann window that is used by default to smooth the information content (profile), we have implemented several other windowing functions, including Blackman [3], Hamming [4], Nuttall [5], rectangular [6], sine [7], triangular [8] and Welch [9] windows. These functions are given by

$$\begin{aligned}
 w[n] &= 1, & (\text{rectangular}) \\
 w[n] &= 1 - \left| \frac{n-N/2}{L/2} \right|, \quad L = N, & (\text{triangular/Bartlett}) \\
 w[n] &= 1 - \left(\frac{n-N/2}{N/2} \right)^2, & (\text{Welch}) \\
 w[n] &= \sin \left(\frac{\pi n}{N} \right), & (\text{sine}) \\
 w[n] &= 0.54348 - 0.45652 \cos \left(\frac{2\pi n}{N} \right), & (\text{Hamming}) \\
 w[n] &= 0.42659 - 0.49656 \cos \left(\frac{2\pi n}{N} \right) + 0.07685 \cos \left(\frac{4\pi n}{N} \right), & (\text{Blackman}) \\
 w[n] &= 0.35577 - 0.48740 \cos \left(\frac{2\pi n}{N} \right) + 0.14423 \cos \left(\frac{4\pi n}{N} \right) - 0.01260 \cos \left(\frac{6\pi n}{N} \right), & (\text{Nuttall})
 \end{aligned}$$

(Eq. S1)

and are plotted in Fig. S1. Scale of the filter can be set as S (small), M (medium) or L (large), using “-fs”. Also, instead of considering the whole information content, the user is able to make samples of it by steps of which size can be determined by “-d”.

For the purpose of segmenting the filtered information content, the average entropy of reference-based compression is used by default as the threshold, but the threshold can be altered by “-th” option.

Smash++ is capable of finding even very small similar regions in two sequences. However, we have found experimentally that when it is running in a very sensitive mode, there might be some cases in which the size of similar regions in the reference and the target are not balanced. These cases can be handled by “-rb”, “-re”, “-tb” and “-te” options, that can resize the beginning and ending guards of reference and target regions, respectively. For example, if “-tb 10” is used, the first 10 bases in each target region will be ignored, which results in smaller regions. Note that when the guard sizes of target regions are increased, the models built from these regions would be slightly different than the original models; consequently, the sizes of reference regions that are detected as being similar to the ones from the target would be modified, as well. Therefore, changing the guard sizes of target regions will affect the sizes of reference regions.

In the case of activating deep compression, by “-dp”, changing the guard sizes of reference regions would affect the sizes of target regions, as well. Here, the deep compression means that similar regions in target and reference sequences are found in three phases instead of two, meaning that first, the model of the reference is built and the target is compressed based upon that model, second, the model of each detected region is built and the whole reference is compressed based on these models, and third, the model of each detected reference region is built and the corresponding target regions will be compressed based on that model.

Triggering “-nr” makes the tool not to perform the reference-free compression (self-complexity computation).

Smash++ accepts FASTA and FASTQ files as input, in addition to sequences. In these cases,

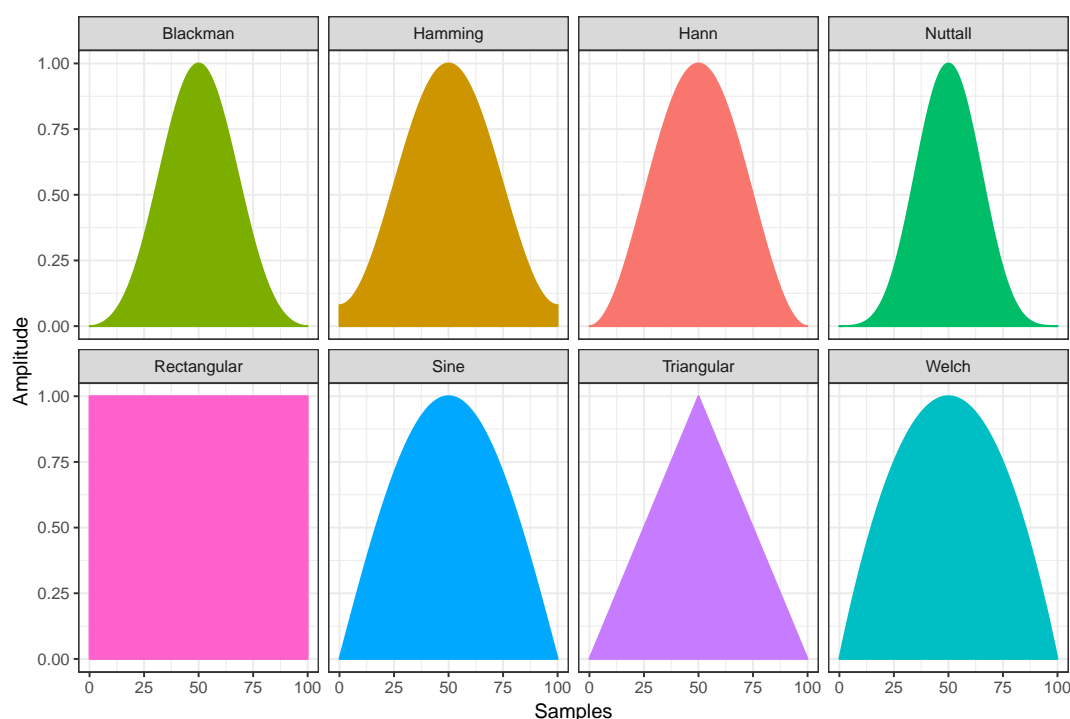


Fig. S1. Window functions.

the input files are first converted to sequences and then processed further. It is possible to save these sequences by “-sb” option. When the information profile is obtained, Smash++ smoothens then removes it by default. However, it can be preserved by “-sp” option. The same thing happens to the smoothed file, i.e., it is segmented then removed. But, the user can use “-sf” to save the filtered file. Also, the segmented files can be saved using “-ss”. The user can save all the information profile (content), filtered and segmented files, by triggering “-sa” option.

For the purpose of compression, it is recommended to use “-l” option, since it configures the models automatically. However, using “-rm” and “-tm”, the user would be able to manually configure the reference model, for reference-based compression, and the target model, for reference-free compression, respectively. Parameters of the models are described in detail in section ?? . Note that using “-ll” option, the list of parameters that would be chosen for each model automatically, will be shown.

Running Smash++ (without visualization), positions of the similar regions in the reference and the target, and also complexity of the regions is saved in a “*.pos” file. This file can be visualized by

```
1 ./smashpp -viz
```

which gives

```
1 SYNOPSIS
2 ./smashpp -viz [OPTIONS] -o <SVG-FILE> <POS-FILE>
3
4 OPTIONS
5 Required:
6 <POS-FILE>           = position file, generated by
7                      Smash++ tool (*.pos)
8
9 Optional:
10 -o <SVG-FILE>        = output image name (*.svg)           -> map.svg
11 -rn <STRING>         = reference name shown on output. If it
12                      has space, use double quotes, e.g.
13                      "Seq label". Default: name in header
14                      of position file
```

```

15  -tn <STRING>      = target name shown on output
16  -l <INT>          = type of the link between maps: [1, 6] -> 1
17  -c <INT>          = color mode: [0, 1] -> 0
18  -p <FLOAT>        = opacity: [0.0, 1.0] -> 0.9
19  -w <INT>          = width of the sequence: [15, 100] -> 16
20  -s <INT>          = space between sequences: [15, 200] -> 62
21  -f <INT>          = multiplication factor for
22                    color ID: [1, 255]
23  -b <INT>          = beginning of color ID: [0, 255] -> 0
24  -rt <INT>          = reference tick: [1, 4294967295]
25  -tt <INT>          = target tick: [1, 4294967295]
26  -th [0][1]        = tick human readable: 0=false, 1=true -> 1
27  -m <INT>          = minimum block size: [1, 4294967295] -> 1
28  -vv              = vertical view -> no
29  -nn              = do NOT show normalized relative
30                    compression (NRC) -> no
31  -nr              = do NOT show self complexity -> no
32  -ni              = do NOT show inverse maps -> no
33  -ng              = do NOT show regular maps -> no
34  -h              = usage guide
35  -v              = more information
36  --version        = show version

```

The output of Smash++ visualizer is an “SVG” file, which its name is determined by “-o” option. By default, it is named “map.svg”. Names of the reference and the target, which are going to be printed on the output image, can be altered by “-rn” and “-tn”, respectively. They are by default the names written in the positions file. To have a vertical view of the image, instead of the default horizontal view, one can use “-vv” trigger.

Smash++ performs reference-based and reference-free compressions to calculate the normalized relative compression (NRC) and redundancy (self complexity), respectively. If the user is not interested in showing them, he/she can turn them off by “-nn” and “-nr” triggers. In addition, Smash++ considers both regular and reverse complement maps by default in its calculations. Triggering “-ni” and “-ng” will stop showing inverted and regular maps, respectively.

Options “-l”, “-c”, “-p”, “-w”, “-s”, “-f” and “-b” can be used to change the appearance of the image. Assigning integers to “-rt” and “-tt” options will change the tick sizes of the reference and the target, respectively. Smash++ prints the sizes on axes in human readable format, e.g., 1K, 2M, etc. However, it can be triggered by “-th” option. Note that, here, 1K is equivalent to 1000 and not 1024. Finally, by setting “-m” to an integer value, only the regions that are bigger than that value will be illustrated.

S1.3 Example

This section guides, step-by-step, employing Smash++ to find and visualize rearrangements in a sample genomic data.

Install Smash++ and provide the required files

First, we install Smash++:

```

1  git clone https://github.com/smortezah/smashpp.git
2  cd smashpp
3  ./install.sh

```

Then, we copy **smashpp** binary file into **example/** directory and go to that directory:

```

1  cp smashpp example/
2  cd example/

```

In this directory, a 1000 byte reference sequence, **ref**, and a 1000 byte target sequence, **tar**, are provided. Running

```

1  ./smashpp -r refs -t tars -w 45 -l 3
2  ./smashpp -viz refs.tars.pos

```

results in Fig. S2, which is saved as “map.svg”.

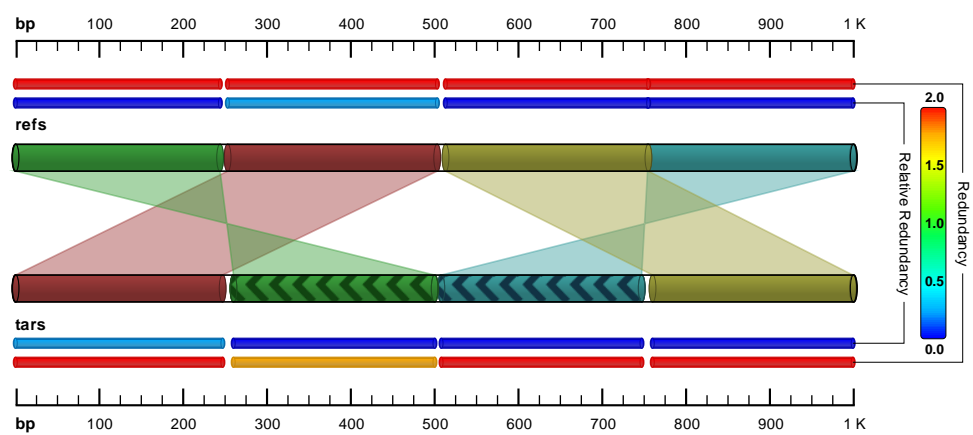


Fig. S2. The result of running Smash++ on ...

References

- [1] M. Hosseini, D. Pratas, and A. J. Pinho. Smash++. [Online]. Available: <https://github.com/smortezah/smashpp>
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- [3] R. Blackman and J. Tukey, "Particular pairs of windows," *The measurement of power spectra, from the point of view of communications engineering*, pp. 95–101, 1959.
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- [8] M. S. Bartlett, "Periodogram analysis and continuous spectra," *Biometrika*, vol. 37, no. 1/2, pp. 1–16, 1950.
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