

# rvd\_gen User Manual

## Background Information

rvd\_gen is a command line tool that can be used to generate a designer TAL effector RVD sequence from a given nucleotide sequence. Because rvd\_gen is written in C, it should run on any modern UNIX-like operating system with little to no modification. It has been tested and verified as functional on GNU/Linux, macOS, FreeBSD, and Windows (via Cygwin). For the rvd\_gen software license and terms, see the end of this document.

## Installation Instructions

### GNU/Linux, macOS, or similar command line operating systems

rvd\_gen can be installed by cloning from github using the **git** command line tool (for installation of git, see the developer website at <https://git-scm.com/downloads>). To install rvd\_gen, use **git** to clone the distribution, then use **make** to compile, as below:

```
~$ git clone https://github.com/stephen-cohen/rvd_gen
Cloning into 'rvd_gen'...
Checking connectivity... done.
~$ cd rvd_gen
~/rvd_gen$ make
cc -Os -Wall -o rvd_gen rvd_gen.c
```

The output binary file is **rvd\_gen**, which can be run directly or copied to a directory in \$PATH (e.g. ~/bin for a single user, or /usr/local/bin in Linux for multiple users) for easy access.

### Windows instructions

rvd\_gen can be installed in Windows using GNU tools in Cygwin (downloadable at <https://www.cygwin.com/>). When installing Cygwin, install the development tools **make**, **gcc-core**, and **git**. Using the **git** and **make** commands as described above will produce **rvd\_gen.exe**.

## Usage Instructions

To use rvd\_gen, you must supply a nucleotide sequence. The sequence may be supplied as a command line argument or as input, as below:

```
~/rvd_gen$ ./rvd_gen ACGTACGTACGTACGT
NI HD NK NG NI HD NK NG NI HD NK NG NI HD NK NG
~/rvd_gen$ ./rvd_gen
ACGTACGTACGTACGT
NI HD NK NG NI HD NK NG NI HD NK NG NI HD NK NG
```

By default, the maximum sequence length allowed is 41, because most available dTALE construction kits do not allow target sequences to be larger than ~30. Source code (rvd\_gen.c, line 41) may be altered to raise or lower this limit. There is no minimum input for the program. Input may be upper- and/or lowercase.

## About the Program

This program generates an optimal TAL effector RVD sequence given a nucleotide sequence. Table 1 shows the nucleotide-RVD pairs used by `rvd_gen`, which are available in modules from the dTALE construction kit made available by Cermak et al. (2011)<sup>1</sup>.

**Table 1. Nucleotide-RVD pairs used by `rvd_gen` and the relative strength of each corresponding interaction.**

Nucleotide(s)	RVD	Strength <sup>2</sup>
A	NI	weak
C	HD	strong
G	NH / NK	weak
T	NG	weak
A / G	NN	strong

In the case of sequences that would lead to stretches of 6 or more weak RVDs, `rvd_gen` substitutes NN for binding to A or G to break up the weak stretches, based on recommendations from Streubel et al. (2012)<sup>2</sup>. Input sequences with stretches of 6 or more Ts cannot be strengthened and should be avoided as EBEs. `rvd_gen` outputs NK for G specificity, but NH may be substituted.

A user may force usage of the RVD NN in a specific location by using the nucleotide symbol R, as below:

```
~/rvd_gen$ ./rvd_gen AAAAAA
NI NI NI NI NI NN
~/rvd_gen$ ./rvd_gen ARAAAA
NI NN NI NI NI NI
```

Uninterpretable input returns “??” in place of valid RVDs.

## References

1. Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF. *Nucleic Acids Res.* 2011; 39(12):e82.
2. Streubel J, Blücher C, Landgraf A, Boch J. *Nat Biotechnol.* 2012;30:593-595.

## Citing `rvd_gen`

To cite `rvd_gen` in publications, use:

Cohen SP. 2018. `rvd_gen`: a command line tool for generating designer TAL effector RVD sequences. Available from: [https://github.com/stephen-cohen/rvd\\_gen](https://github.com/stephen-cohen/rvd_gen).

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