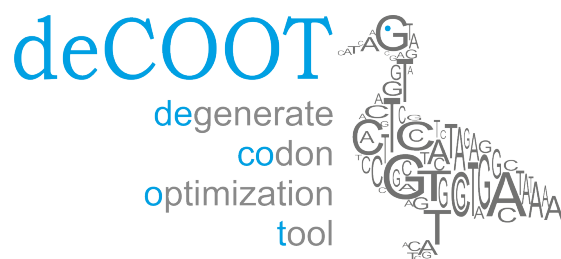


DeCOOT instruction manual

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1 Introduction

DeCOOT (degenerate codon optimization tool) serves for automatic design of randomized protein libraries with given amino acid distribution. Inputs of the program involve amino acid distribution, length of resulting proteins in the library and level of degeneracy. Optional parameters are organism codon bias, removed triplets and codon reassignment. Program outputs a set of degenerate or spiked codons which provide a protein library with desired characteristics. Principle of method lies in stochastic genetic algorithm, therefore solutions for approximations of a given distribution can differ between different runs.

The screenshot shows the deCOOT software interface. On the left, there are input fields for 'length of AA sequence' (set to 10), 'removed triplets' (set to 0.9), 'model distribution' (a list of organisms with 'none' selected), and 'reassign codons' (a list of amino acids with their corresponding reassignment rates). At the bottom left, there are buttons for 'clear', 'compute', and 'permute codons'. On the right, there is a table showing 'expected', 'reached', and 'difference' values for various amino acids. Below the table, there are fields for 'mean error of a single protein', 'variance of error of a single protein', 'mean GC content of the DNA template', and 'mean mass of a protein'. At the bottom right, there is an 'output string' field and a button for 'export to pdf & save imgs'.

	expected	reached	difference
F	0	0	0
L	0	0	0
I	0	0	0
M	0	0	0
V	0	0	0
A	0	0	0
G	0	0	0
S	0	0	0
T	0	0	0
Y	0	0	0
Q	0	0	0
N	0	0	0
C	0	0	0
D	0	0	0
E	0	0	0
H	0	0	0
K	0	0	0
R	0	0	0
STOP	0	0	0

mean error of a single protein
unknown
variance of error of a single protein
unknown
mean GC content of the DNA template
unknown
mean mass of a protein
unknown

output string

export to pdf & save imgs

Figure 1: Main window of degenerate codon optimization tool – DeCOOT

2 Installation

DeCOOT prerequisites are Python 3 and supplementary packages listed in requirements.txt. In case that you do not have Python installed we recommend to download Miniconda from <https://docs.conda.io/en/latest/miniconda.html> which will install Python 3 and few additional useful packages. After Miniconda installation:

1. open Anaconda prompt and run the command (without quotation marks) "cd xxx", where xxx is the path to the directory with the DeCOOT.
2. On first use run command (without quotation marks) "conda install --file requirements.txt"
3. run the command "python gui.py"

On a few MacOS machines script was not able to load. This is due to serious bugs with Apple supplied Python. In case that happens, it is recommended to install the newest version of python and start script with command "pythonw gui.py". (<https://www.python.org/download/mac/tcltk/>)

If Python 3 is already installed on your computer or/and you do not want to install Miniconda, use "pip install" command to install all prerequisite packages in requirements.txt file. After the installation change your working directory in terminal by command "cd xxx", where xxx is the path to the directory with DeCOOT and run DeCOOT with "python gui.py" command.

Although DeCOOT was tested on Linux distribution Ubuntu 18.04, other operating systems (Windows, MacOS) should be compatible with fully python-based script.

3 Setting up

3.1 Mandatory input

Obligatory inputs for DeCOOT are length of the library proteins and amino acid distribution. These attributes can be defined in “length of AA sequence” and specific amino acid brackets. Amino acid ratios do not need to be normalized to sum to 100 or 1. Ratios of amino acids (e.g A 1, T 2, S 2) will be normalized to fractions of 1 automatically (therefore A 0.2, T 0.4, S 0.4). An alternative way for input is provided by “input from file” (Section 3.2).

The screenshot shows the deCOOT software interface. The 'Degenerate codons' section is active, with 'length of AA sequence' set to 10. The 'model distribution' is set to 'none'. The 'reassign codons' section shows a list of amino acids with their corresponding ratios. The 'input from file' section is also visible.

amino acid	ratio
F	0.1208
L	0.0
I	0.0
M	0.0734
V	0.0
P	0.0
A	0.0
W	0.0
G	0.0
S	0.0905
T	0.0679
Y	0.118
Q	0.122
N	0.157
C	0.0
D	0.0
E	0.0388
H	0.0394
K	0.1722
R	0.0
STOP	0.0

	expected	reached	difference
L	0	0	0
F	0	0	0
I	0	0	0
M	0	0	0
V	0	0	0
P	0	0	0
A	0	0	0
W	0	0	0
G	0	0	0
S	0	0	0
T	0	0	0
Y	0	0	0
Q	0	0	0
N	0	0	0
C	0	0	0
D	0	0	0
E	0	0	0
H	0	0	0
K	0	0	0
R	0	0	0
STOP	0	0	0

mean error of a single protein
unknown
variance of error of a single protein
unknown
mean GC content of the DNA template
unknown
mean mass of a protein
unknown

output string

clear
compute
permute codons
export to pdf & save imgs

Figure 2: Most important variables for resultant degenerate libraries - length in amino acids and amino acid composition. Both variables can be entered manually or using file input

3.2 Input from file

Input file must be ".txt" file. It needs to contain amino acid distribution, which will be further normalized. If an amino acid is not present in input file, it will not appear in the output at all. Further items are optional, if they will not be present in the input file, they will be read from the GUI.

Here we provide an example content of such input file.

```
F=1
L=2
A=1
T=5
Q=2.5
N=0.3
D=0.12
H= 5
K=5
length=11
model_distribution=E.coli
maximum_rate=0.95
removed_triplets=AAA GGG
reassigned_codons=ACT 2, GTG 3
spiked
```

Frequency of amino acid is coded by its one-letter abbreviation, followed by the equality sign and a numerical value. First letter of the line has to be the amino-acid name and the second is the equality sign. The numerical value can be inserted in arbitrary form. Note that "." is used as decimal separator.

Further lines of the example input specify optional parameters for DeCOOT. Each parameter must be defined exactly and must be followed by a "=". Only exceptions are "spiked" and "degenerated" parameters, which enable/disable the usage of spiked codons without any other specification.

Other parameters are followed by the same format of input as in the GUI, with the exception of model_distribution, which contains a name of one of distributions in the GUI.

If a line differs in format from those described above (even a superfluous space counts!), then it is ignored and may be used for comments.

The order of lines does not matter.

3.3 Degenerate codon type

DeCOOT offers approximation via degenerate (default) and spiked codons. Optimization with spiked codons might take longer time to converge, but offer higher precision in shorter library lengths, difficult amino acid combinations or in cases where more solutions with higher degeneracy are required. In longer templates (> 15 amino acids) the difference in precision between degenerate and spiked solutions become negligible.

The screenshot shows the deCOOT application window. The 'Degenerate codons' tab is selected. The 'length of HH sequence' is set to 10. The 'removed triplets' field is empty. The 'maximum rate' is set to 0.9. The 'model distribution' is set to 'none'. The 'reassign codons' section shows a list of amino acids with their corresponding rates: F (0.1208), L (0.0), I (0.0), M (0.0734), V (0.0), P (0.0), A (0.0), W (0.0), G (0.0), S (0.0905), T (0.0679), Y (0.118), Q (0.122), N (0.157), C (0.0), D (0.0388), E (0.0394), H (0.1722), K (0.0), R (0.0), and STOP (0.0). The 'input from file' field is empty. The 'clear' and 'compute' buttons are visible. The 'permute codons' button is at the bottom. The 'output string' field is empty. The 'export to pdf & save imgs' button is at the bottom right.

	expected	reached	difference
L	0	0	0
I	0	0	0
M	0	0	0
V	0	0	0
P	0	0	0
A	0	0	0
W	0	0	0
G	0	0	0
S	0	0	0
T	0	0	0
Y	0	0	0
Q	0	0	0
N	0	0	0
C	0	0	0
D	0	0	0
E	0	0	0
H	0	0	0
K	0	0	0
R	0	0	0
STOP	0	0	0

mean error of a single protein
unknown
variance of error of a single protein
unknown
mean GC content of the DNA template
unknown
mean mass of a protein
unknown

Figure 3: Standard degenerate codons with equimolar base ratios and spiked codons with variable base ratios can be chosen for distribution approximation

3.4 Codon removal and reassignment

Specific triplets can be removed by specifying triplets in space separated format into the “removed triplets” bracket (for example ”AAA ACT GTG”). DeCOOT will output solutions containing only those degenerate codons which do not code removed triplets. Another option is codon reassignment. If defined in the “reassign codons” in format ”XXX number” (e.g. ATG 0.3), codon will be treated as another amino acid (and no longer as methionine) with appropriate ratio (0.3) and will be included into the degenerate output. There may be multiple instances of such reassigned codons. In the input, they are separated by commas (for instance ”ACT 0.074, GTG 0.111”)

deCOOT

☐ Spiked codons
☒ Degenerate codons

length of AA sequence 10

removed triplets AAA ACT GTG

maximum rate 0.9

model distribution
☐ E.coli
☐ S.cerevisiae
☐ H.sapiens
☐ M.musculus
☐ S.frugiperda
☐ P.pastoris
☒ none

reassign codons ATG 0.3

F 0.1208
L 0.0
I 0.0
M 0.0734
V 0.0
P 0.0
A 0.0
W 0.0
G 0.0
S 0.0905
T 0.0679
Y 0.118
Q 0.122
N 0.157
C 0.0
D 0.0
E 0.0388
H 0.0394
K 0.1722
R 0.0
STOP 0.0

input from file example.txt

clear
compute
permute codons

	expected	reached	difference
F	0	0	0
L	0	0	0
I	0	0	0
M	0	0	0
V	0	0	0
P	0	0	0
A	0	0	0
W	0	0	0
G	0	0	0
S	0	0	0
T	0	0	0
Y	0	0	0
Q	0	0	0
N	0	0	0
C	0	0	0
D	0	0	0
E	0	0	0
H	0	0	0
K	0	0	0
R	0	0	0
STOP	0	0	0

mean error of a single protein
unknown
variance of error of a single protein
unknown
mean GC content of the DNA template
unknown
mean mass of a protein
unknown

output string

export to pdf & save imgs

Figure 4: Certain triplets can be removed from final degenerate solution or reassigned to encode a different amino acid

3.5 Organism codon bias

Output can be optimized according to organism codon bias, solution will be enriched in codons preferred by the selected model organism.

The screenshot shows the deCOOT software interface. On the left, under 'Degenerate codons', the 'model distribution' is set to 'E.coli'. Below this, a vertical bar shows the frequency of each amino acid (F, L, I, M, V, P, A, W, G, S, T, Y, Q, N, C, D, E, H, K, R, STOP) with corresponding numerical values. On the right, a table shows the 'expected', 'reached', and 'difference' for each amino acid. The 'expected' column is mostly 0, while the 'reached' column shows values for F, L, I, M, V, P, A, W, G, S, T, Y, Q, N, C, D, E, H, K, R, and STOP.

	expected	reached	difference
F	0	0	0
L	0	0	0
I	0	0	0
M	0	0	0
V	0	0	0
P	0	0	0
A	0	0	0
W	0	0	0
G	0	0	0
S	0	0	0
T	0	0	0
Y	0	0	0
Q	0	0	0
N	0	0	0
C	0	0	0
D	0	0	0
E	0	0	0
H	0	0	0
K	0	0	0
R	0	0	0
STOP	0	0	0

Figure 5: DeCOOT can start approximation from codon optimized seed template. This option increases the probability of organism specific triplets enrichment in final degenerate solution

3.6 Example output

With default settings and distribution, solution can be generated by "compute" button. Calculation can take several minutes depending on template library length, type of codons and specific amino acid alphabet.

Output provides essential statistics on amino acid approximation and differences from input, variance protein amino acid content in the designed library, GC content of designed DNA library and mean molecular weight of protein product. Degenerate codons are represented by pie charts where slices are contents of hydrophobic (shades of yellow), polar (reds), negatively (greens) and positively charged (blues) amino acids. Numbers above the pie chart represent positions of degenerate codons in the template. Codon positions are arbitrary and can be shuffled by the "permute codons" button.

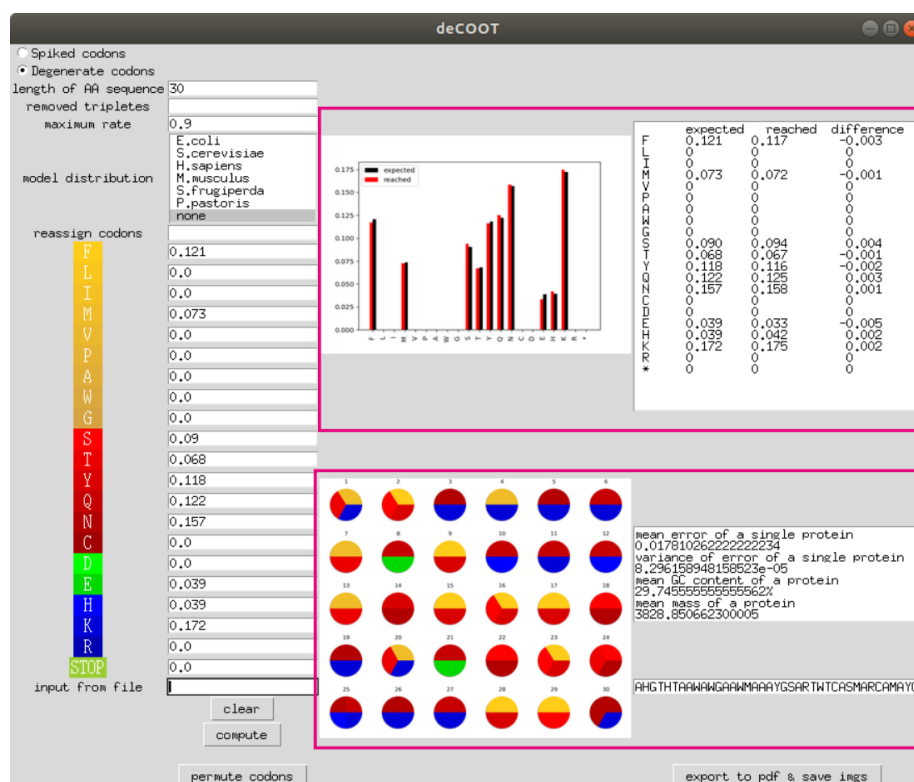


Figure 6: Example output of default settings and amino acid distribution on 30 amino acid library template

3.7 Degeneracy of solution

Degeneracy of solution can be tuned by “maximum rate” parameter. Rate represents maximum ratio of amino acid representable by degenerate codon (e.g. codon RGN codes for two arginines, two serines and four glycines, so ratios of R, S and G are 0.25, 0.25 and 0.5 respectively - thus codon RGN will never be included in solution if ”maximum rate” was set on 0.2).

Here is the example solution where the maximum ratio was limited to 0.4 (e.g. maximum representation of a single amino acid by degenerate codon is 40 %). Pie charts provide a visual proof of increased degeneracy compared to previous example output with default rate of 0.9. In some cases, higher degeneracy comes with a price of lower precision as shown here. This occurs when input amino acid codons are not very similar and increased degeneracy significantly limits the codon pool for approximation. If precision is important, we recommend to increase maximum amino acid rates or use spiked codons.

Default ratio 0.9 ensures that only degenerate codons are included in the solution, maximum ratio 1 will include non-degenerate codons and lower ratios will use more degenerate codons accordingly.

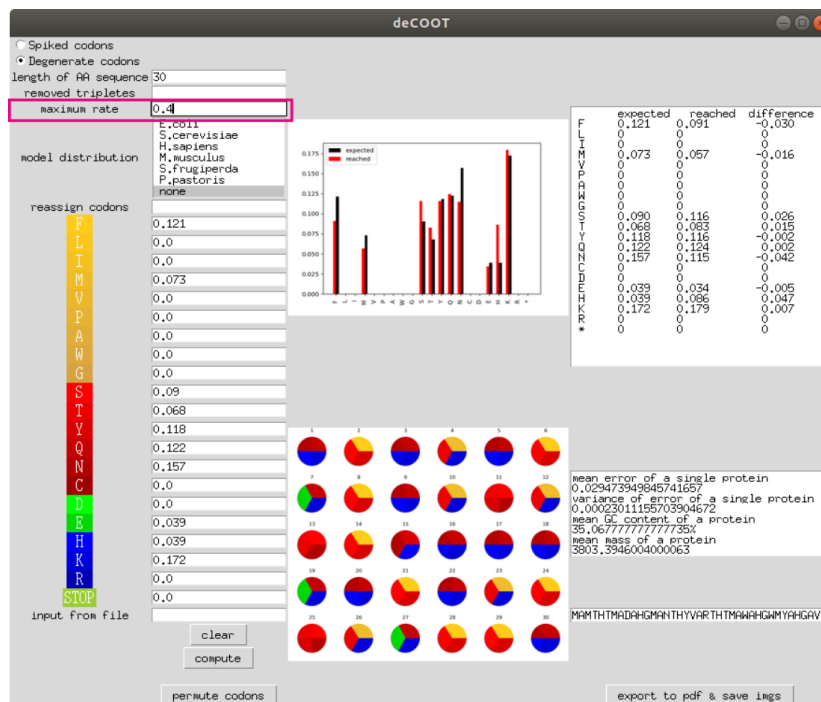


Figure 7: Degeneracy of solution can be fine-tuned, using lower values of rate parameter for higher degeneracy up to 1 for lowest degeneracy where algorithm is allowed to use non-degenerate codons for approximation

3.8 Spiked codons

Precision of solution can be improved by inclusion of spiked codons (e.g. codons with variable nucleotide ratios) into the design scheme. Here we present how spiked codons improve the precision of previously shown solution with increased degeneracy. However, not all commercial oligonucleotide synthesis providers are able to follow the spiked codon format and feasibility of the solution as a synthesis template should be consulted individually.

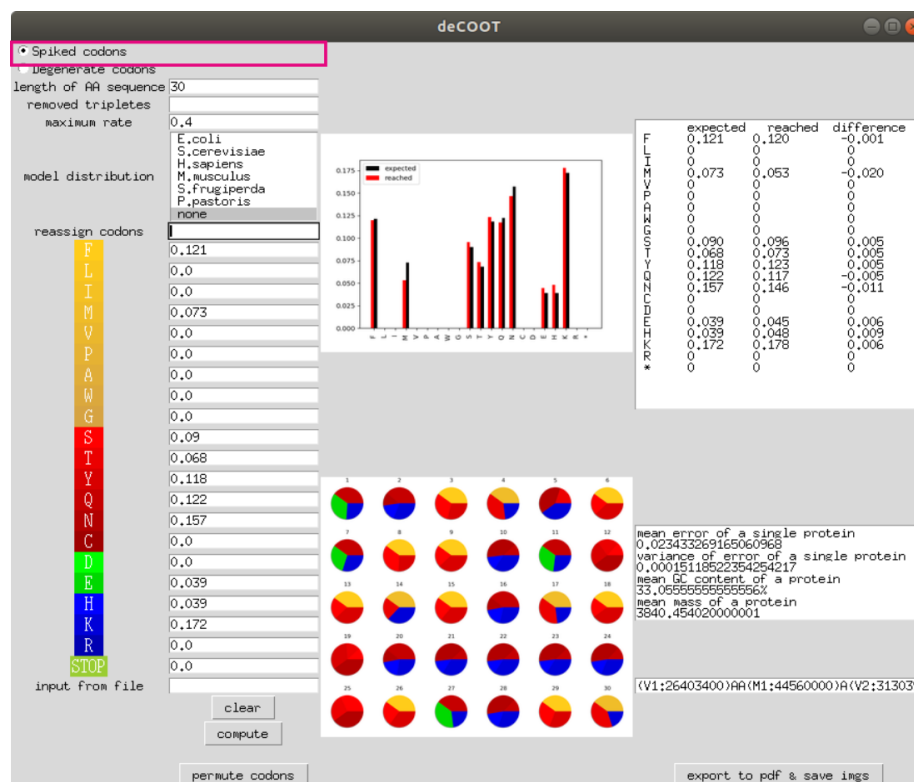


Figure 8: Utilization of spiked codons can bring higher precision. Disadvantage is necessity of individual consultation with oligonucleotide synthesis company

3.9 PDF report

All statistics shown in the main window are exported to pdf file in the DeCOOT directory.