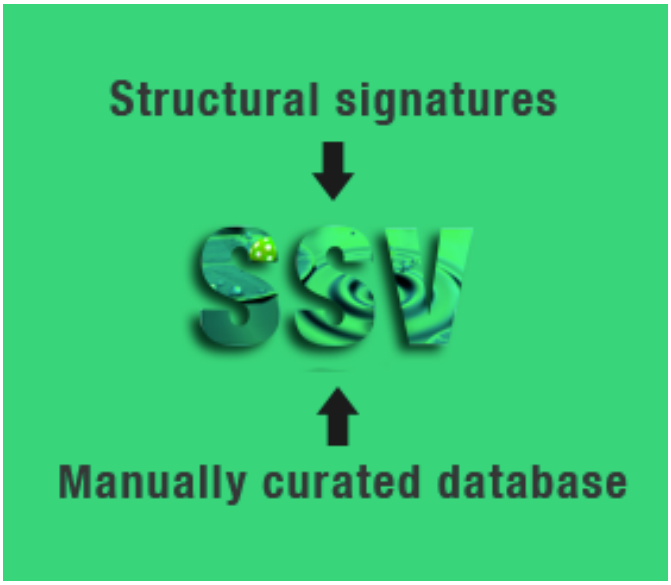
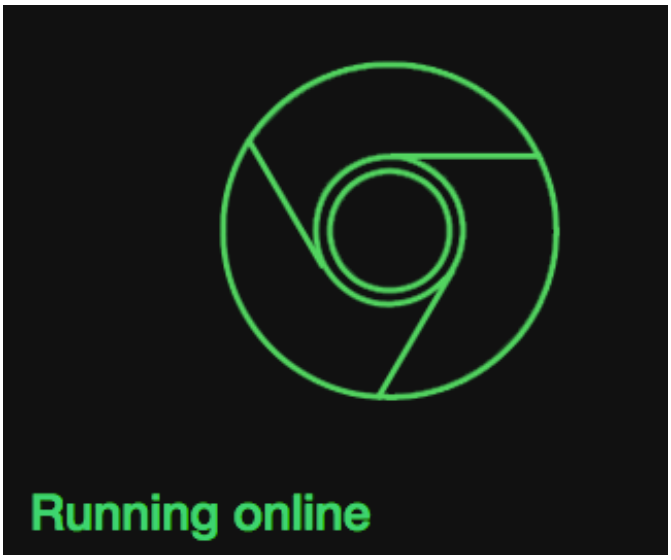
SSV docs





Structural Signature Variation

SSV is a method to calculate if a mutation could be beneficial or not for an enzyme.

Summary

SSV documentation

What is SSV?

SSV (Structural Signature Variation) is a method to propose mutations for enzymes used in industrial applications. **SSV** uses structural signatures to detect patterns, which can improve the activity of enzymes. A real and important application for SSV is the second-generation biofuel production.

Case study: Second-Generation Biofuel problem

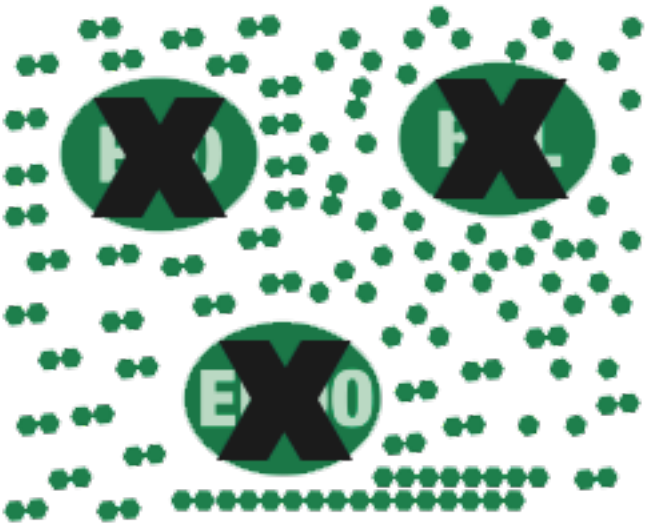
We used SSV as a method to propose mutations for β-glucosidase enzymes used in Second-Generation Biofuel production. SSV uses structural signatures to detect patterns, which can improve the activity of non-tolerant β-glucosidases, based on a manually curated database of glucose-tolerant β-glucosidases.

Second-Generation Biofuel is produced by residues obtained from the first generation biofuel production. They can be obtained from biomass, such as corn, algae, sugarcane, and so on. The production process is based on the extraction of fermentable sugars from cellulose.

Cellulose is decomposed by the action of three enzymes: endoglucanases, exoglucanases, and β-glucosidases. Endoglucanases act first, cleaving cellulose in oligosaccharides of several lengths. Then, exoglucanases cleave the oligosaccharides in disaccharides, such as cellobiose. In the end, beta-glucosidases cleaves the cellobiose in two molecules of glucose, that will be used in the fermentation process to obtaining biofuel.

β-glucosidases are strongly inhibited by high glucose concentrations (same used for biofuel production).

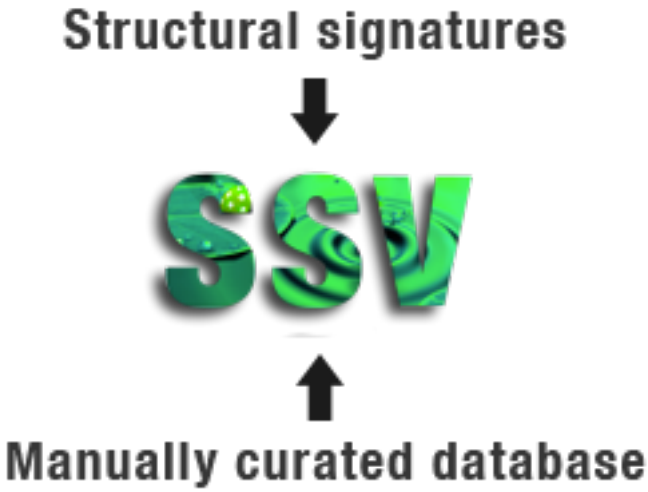
In addition, it increases cellobiose concentration that inhibits endoglucanases and exoglucanases.



Glucose-tolerant β-glucosidases: β-glucosidases of high resistance to glucose inhibition, also called glucose-tolerants, can help to improve the biofuel production. Also, some mutations can turn non-tolerant in glucose-tolerant β-glucosidases.

How does SSV method work?

SSV method constructs structural signatures for wild and mutant proteins and compares the signature's variation with a manually curated database. In the case study, we used a database of glucose-tolerant β-glucosidases, called **Betagdb** (available at http://bioinfo.dcc.ufmg.br/betagdb).



Inputs

SSV requires three proteins:

1. Wild protein

2. Mutant protein
3. Template (reference)

SSV steps for the case study

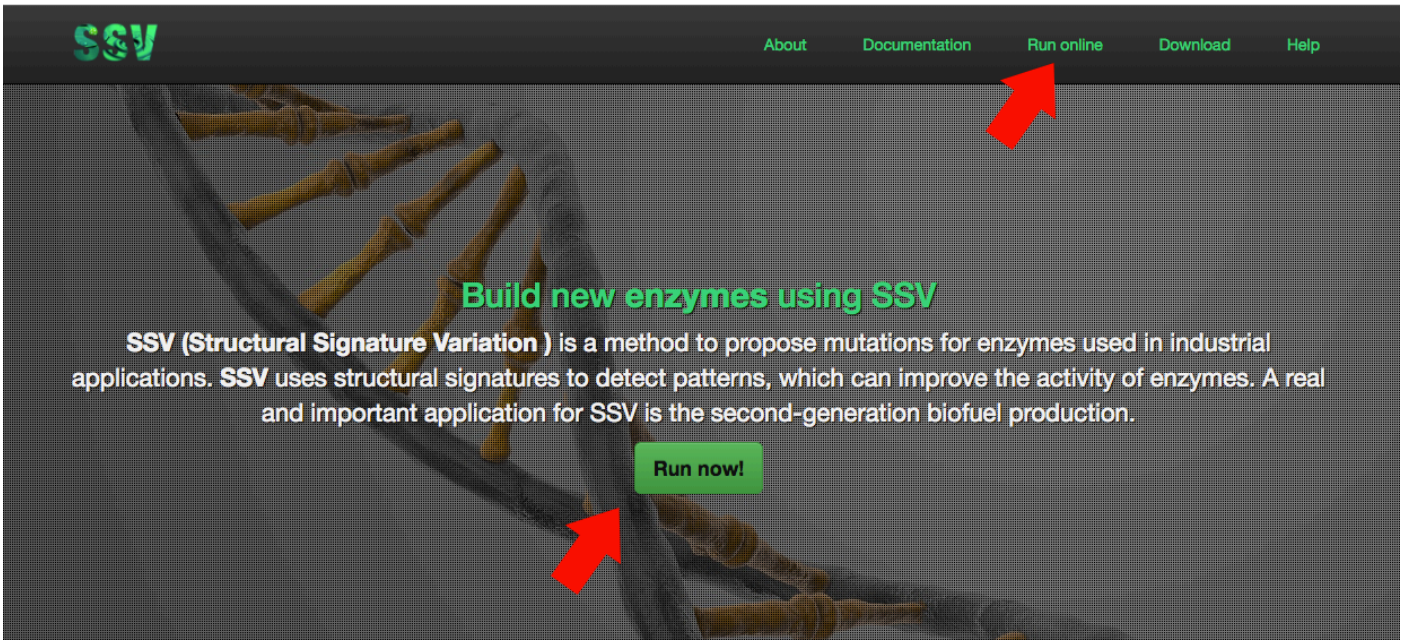
1. We constructed structural signatures using aCSM-ALL. We used cutoff ranging from 0Å until 10Å, and step distance of 0.1Å. However, we did not use the whole protein, only the region near to the active site. First, we performed the extraction of the catalytic pocket residues. This is not necessary for all projects run in SSV, however, if you use the whole protein, this increase the computational costs. The mutant and wild protein are aligned with 3VIK, the only beta-glucosidase in complex with cellobiose detected in the literature. The residues of the catalytic pocket of 3VIK are known: Q45, H148, W149, N192, S193, L195, T196, D199, M207, N253, I254, N255, Y273, N335, F336, Y337, T338, L340, W374, E402, W444, E451, W452, and F460. We performed alignments using MultiProt (http://bioinfo3d.cs.tau.ac.il/MultiProt);
2. We previously calculated the signature of 23 glucose-tolerant beta-glucosidases of betagdb. We calculated the distance among all glucose-tolerant beta-glucosidase and wild and mutant. The lowest values correspond to the templates;
3. We calculated the distance between wild and its template (ΔSSV_{Wt}), and mutant and template (ΔSSV_{Mt}). The difference between both distances is the variation of the signature variations ($\Delta\Delta SSV$).

Running online

Running online is the best way to execute GTS. It is available online at: <http://bioinfo.dcc.ufmg.br/gts>.

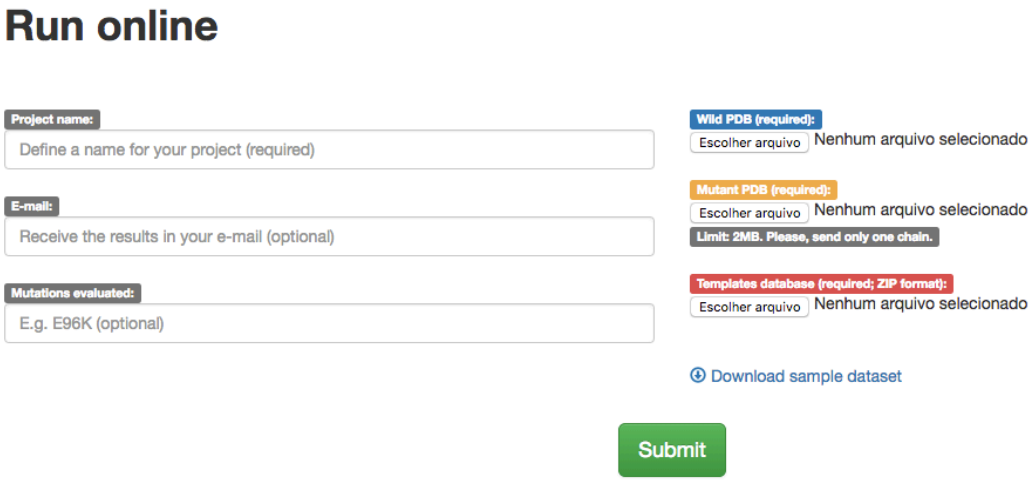
Accessing the "Run online" panel

Click on "Run online" or in the "Run now!" Button.

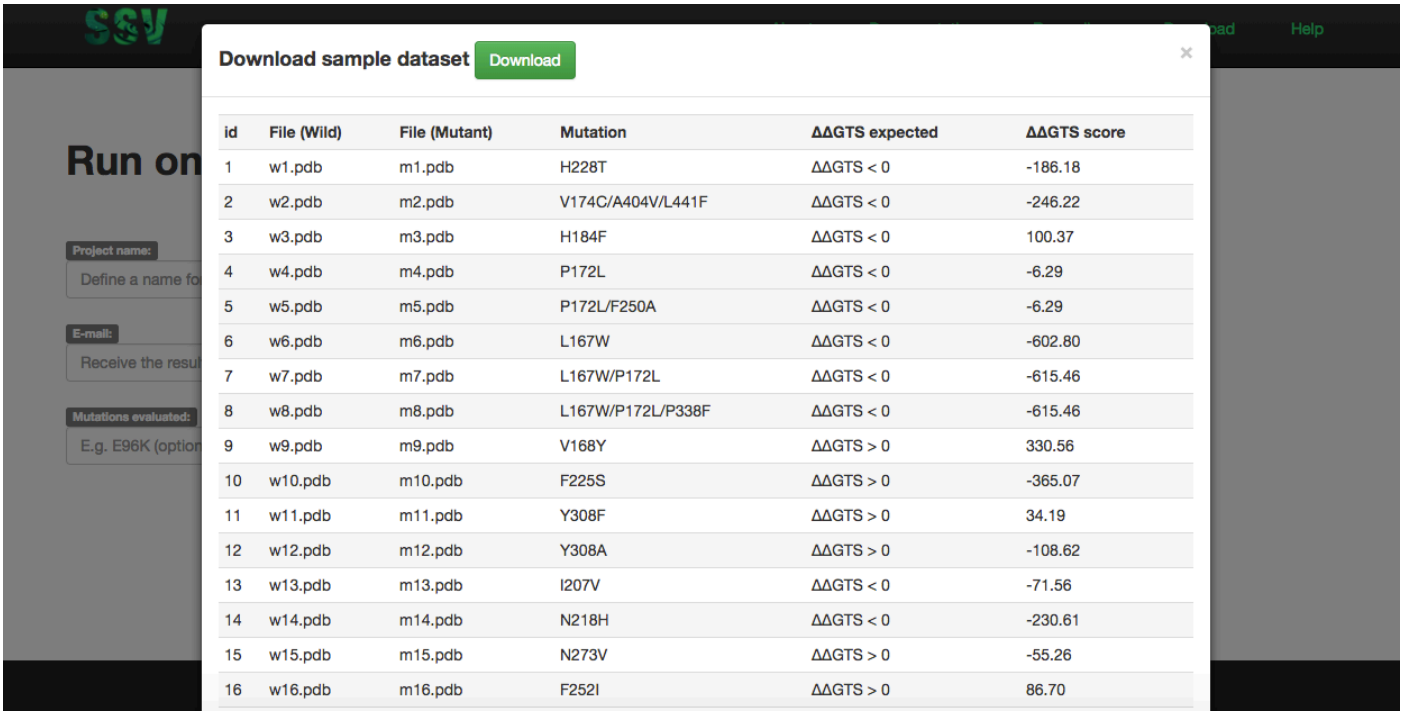


The "Run online" panel requires some information to execute:

- **Project name**: define a name for your project. You can use any name (the system will create a unique ID for each project);
- **E-mail**: declare your e-mail (optional);
- **Mutation evaluated**: insert the point mutation or multiple mutations evaluated (optional);
- **Wild PDB:** input the wild PDB file;
- **Mutant PDB:** input the mutant PDB file;
- **Templates database:** input a set of proteins that it will be used as templates (send a zip file).



We made available a database with 27 mutations evaluated in the paper and the 23 proteins of betagdb (the templates for the case study). We can download the database and perform analysis using SSV online. We also made available a table with expected values and the values found.



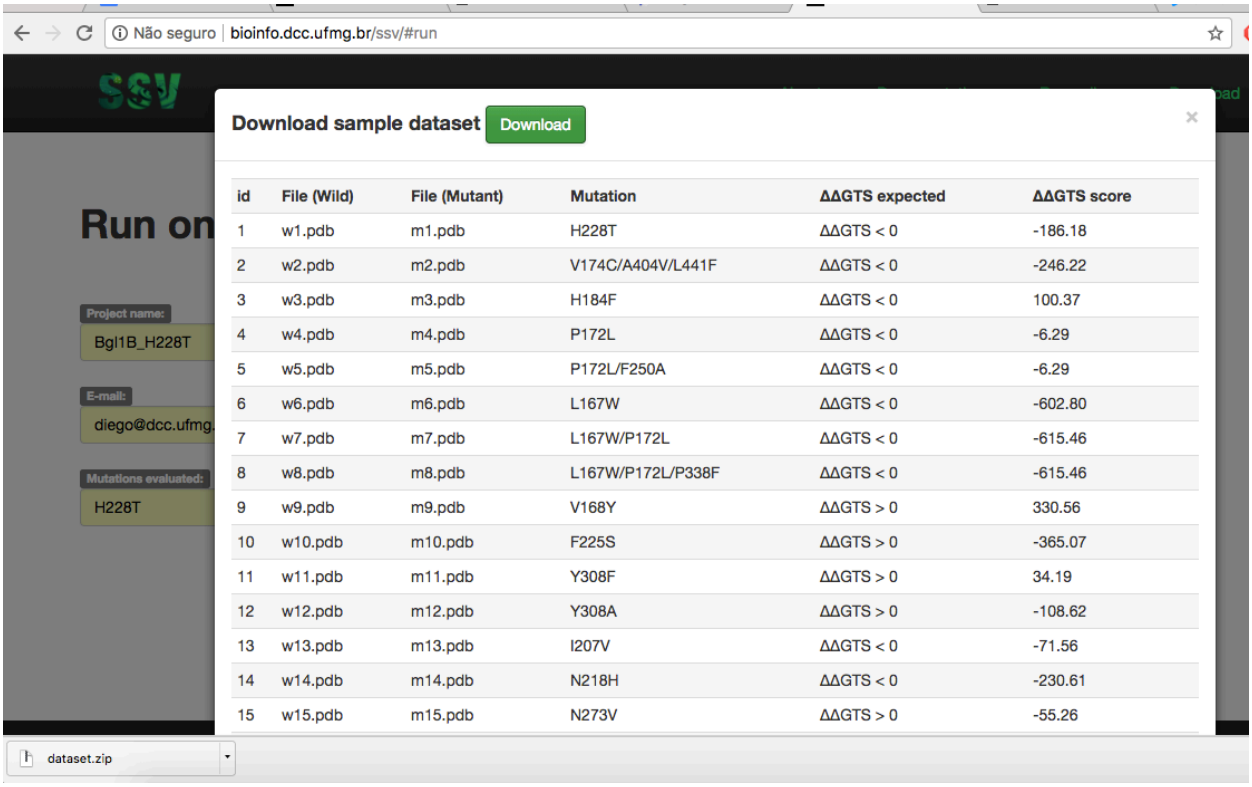
Download sample dataset Download

id	File (Wild)	File (Mutant)	Mutation	ΔΔGTS expected	ΔΔGTS score
1	w1.pdb	m1.pdb	H228T	ΔΔGTS < 0	-186.18
2	w2.pdb	m2.pdb	V174C/A404V/L441F	ΔΔGTS < 0	-246.22
3	w3.pdb	m3.pdb	H184F	ΔΔGTS < 0	100.37
4	w4.pdb	m4.pdb	P172L	ΔΔGTS < 0	-6.29
5	w5.pdb	m5.pdb	P172L/F250A	ΔΔGTS < 0	-6.29
6	w6.pdb	m6.pdb	L167W	ΔΔGTS < 0	-602.80
7	w7.pdb	m7.pdb	L167W/P172L	ΔΔGTS < 0	-615.46
8	w8.pdb	m8.pdb	L167W/P172L/P338F	ΔΔGTS < 0	-615.46
9	w9.pdb	m9.pdb	V168Y	ΔΔGTS > 0	330.56
10	w10.pdb	m10.pdb	F225S	ΔΔGTS > 0	-365.07
11	w11.pdb	m11.pdb	Y308F	ΔΔGTS > 0	34.19
12	w12.pdb	m12.pdb	Y308A	ΔΔGTS > 0	-108.62
13	w13.pdb	m13.pdb	I207V	ΔΔGTS < 0	-71.56
14	w14.pdb	m14.pdb	N218H	ΔΔGTS < 0	-230.61
15	w15.pdb	m15.pdb	N273V	ΔΔGTS > 0	-55.26
16	w16.pdb	m16.pdb	F252I	ΔΔGTS > 0	86.70

Running an example (H228T)

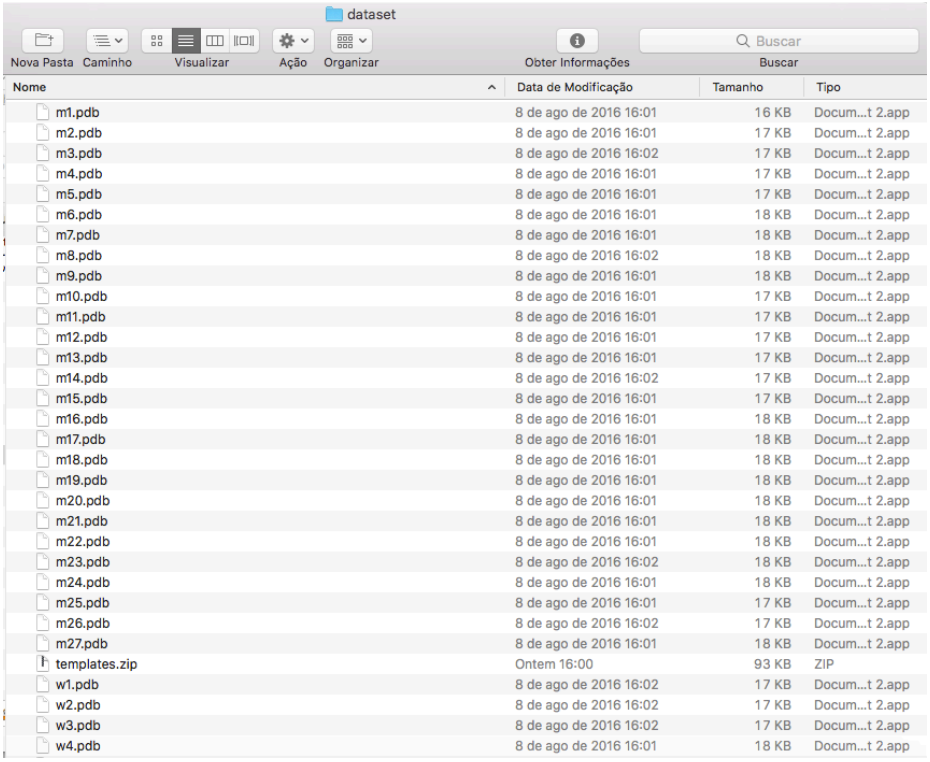
Now, we will run the first example of that database. The mutation H228T, where detected for a non-tolerant beta-glucosidase and improved its glucose tolerance. For this reason, we expected a ΔΔSSV negative.

Download the dataset:

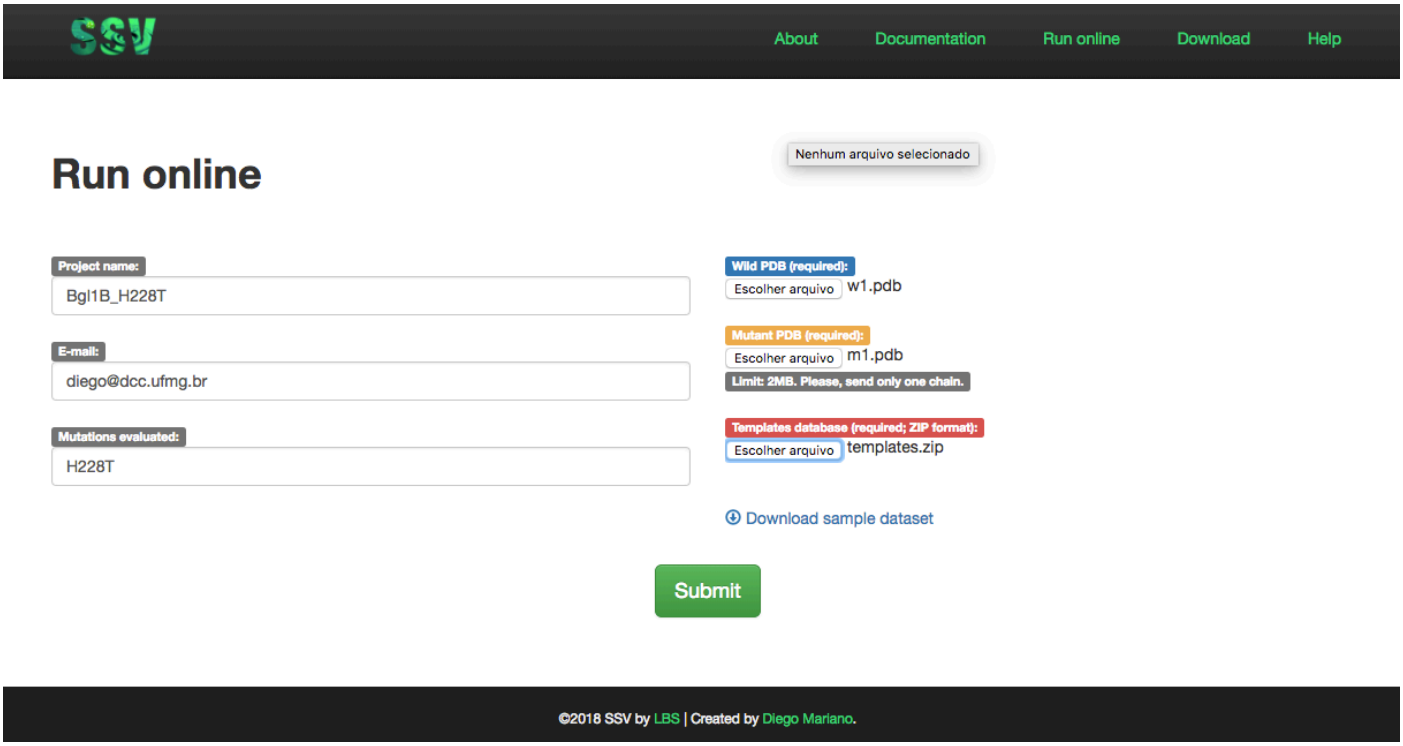


id	File (Wild)	File (Mutant)	Mutation	ΔΔGTS expected	ΔΔGTS score
1	w1.pdb	m1.pdb	H228T	ΔΔGTS < 0	-186.18
2	w2.pdb	m2.pdb	V174C/A404V/L441F	ΔΔGTS < 0	-246.22
3	w3.pdb	m3.pdb	H184F	ΔΔGTS < 0	100.37
4	w4.pdb	m4.pdb	P172L	ΔΔGTS < 0	-6.29
5	w5.pdb	m5.pdb	P172L/F250A	ΔΔGTS < 0	-6.29
6	w6.pdb	m6.pdb	L167W	ΔΔGTS < 0	-602.80
7	w7.pdb	m7.pdb	L167W/P172L	ΔΔGTS < 0	-615.46
8	w8.pdb	m8.pdb	L167W/P172L/P338F	ΔΔGTS < 0	-615.46
9	w9.pdb	m9.pdb	V168Y	ΔΔGTS > 0	330.56
10	w10.pdb	m10.pdb	F225S	ΔΔGTS > 0	-365.07
11	w11.pdb	m11.pdb	Y308F	ΔΔGTS > 0	34.19
12	w12.pdb	m12.pdb	Y308A	ΔΔGTS > 0	-108.62
13	w13.pdb	m13.pdb	I207V	ΔΔGTS < 0	-71.56
14	w14.pdb	m14.pdb	N218H	ΔΔGTS < 0	-230.61
15	w15.pdb	m15.pdb	N273V	ΔΔGTS > 0	-55.26

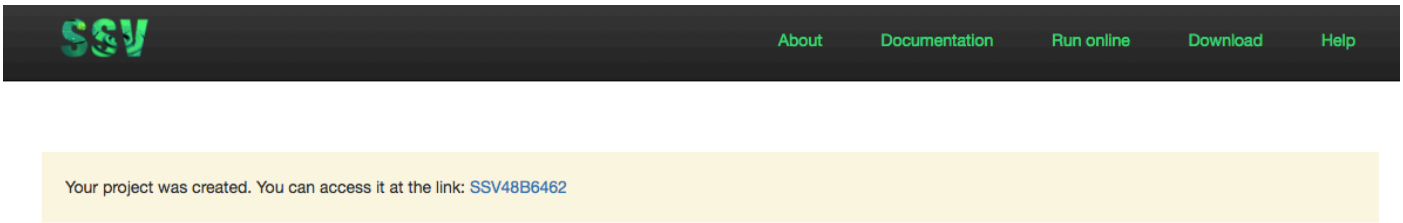
Extract the files:



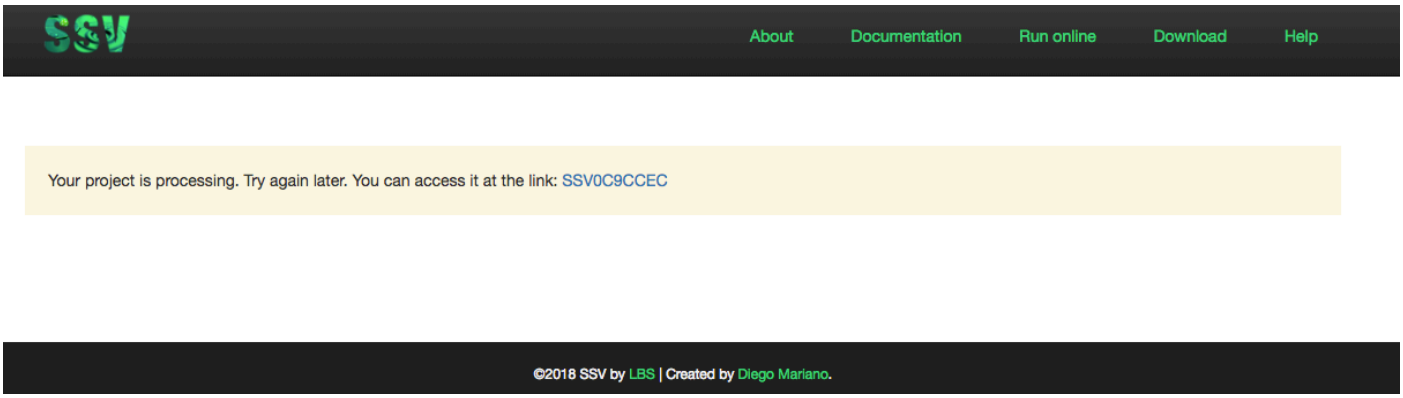
Run SSV using w1.pdb (wild), m1.pdb (mutant), and templates.zip (23 templates):



After submitting the data, SSV online will process your requisition. A unique ID will be created for your project. When the process to finish, you can click at the link.



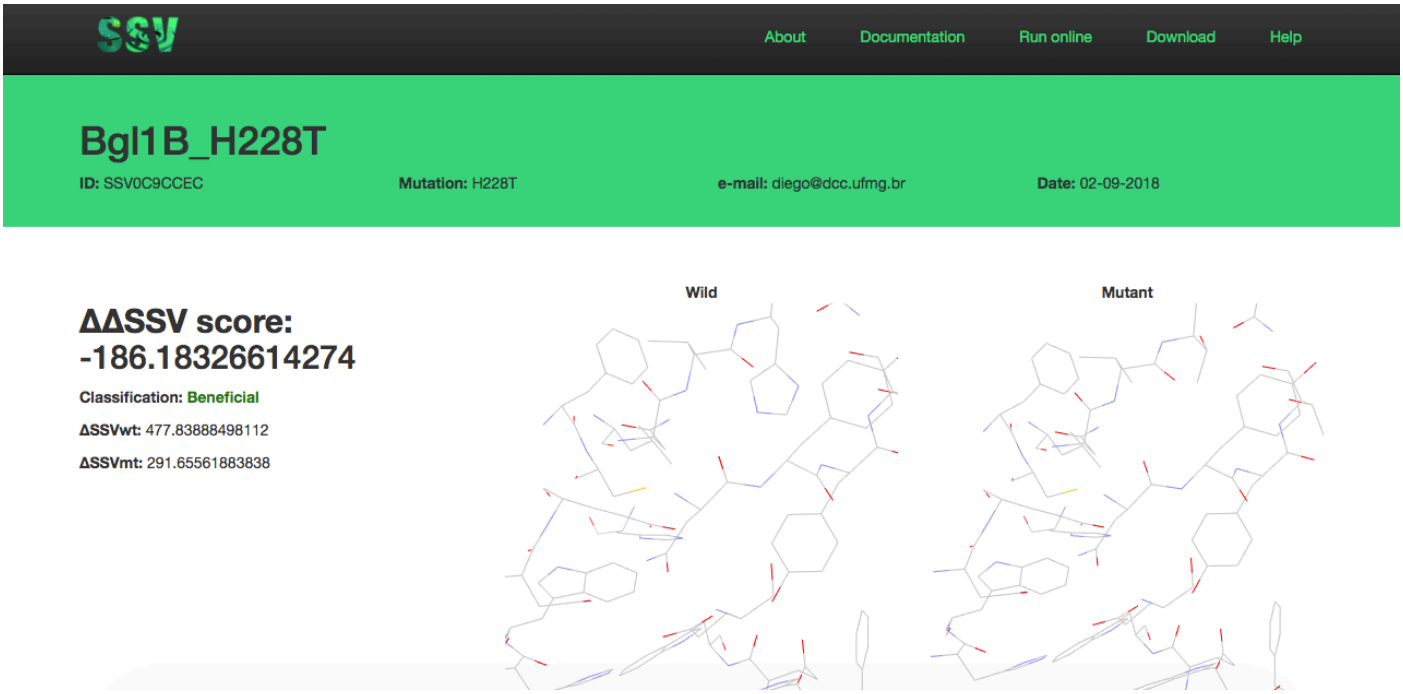
While SSV runs your project, you will receive this message:



In the end, you will be redirected to the individual page of the project. This page is identified by the unique ID, described under the project name (in the green sector).

Below the green sector, there are three important sectors:

1. The results of the ΔΔSSV calculation;
2. Wild visualization;
3. Mutant visualization.



In the main panel, it is shown:

- ΔΔSSV score;
- Classification;
- ΔSSV_{Wt};
- ΔSSV_{Mt}.

Run by scripts

We made available the scripts to run SSV. However, we cannot have sure that you will be able to reproduce the software environment. If you have problems, run the online version.

We strongly recommend running SSV online.

Requirements

- O.S. Linux 64bit (recommended Ubuntu 16.04)
- Python (version 2)
- Library Numpy
- Perl
- aCSM
- MultiProt

Scripts

Script	Input	Output
extractSite.py	PDBs files inserted into the folder "pdb".	PDBs files with the catalytic pocket extracted.
aCSM.pl	List with the addresses of PDBs files with the catalytic pocket extracted.	CSV file with the total of atoms in the cutoff distances.
dgts3.py	CSV file obtained from aCSM. The first 23 lines must represent the beta-glucosidases from betagdb in alphabetical order.	ΔΔSSV and templates for wild and mutant save in the file "result.txt".