Scop3D – Manual

Where to find Scop3D

Scop3D is now available through an intuitive web-interface: https://iomics.ugent.be/scop3d

Example: Kras.

Kras is part of the Ras-Raf-MEK-ERK pathway. This pathway was the first one to be elucidated and remains an important research topic because of its central role in cellular differentiation in development. Because of this central role, inappropriate and/or continuous activation of this pathway is very common in cancer. Hence, several SNP variants are available for Kras.

Conservation analysis

Step 1: sequence analysis

homo sapiens	p01116
rattus norvegicus	p08644
mus musculus	p32883
danio rerio	q6aza4
pan troglodytus	h2q5m0
macaca mulatta	f6sfg7
felis catus	m3xau6
gallus gallus	f5ant2
ovis aries	w5qfh7
oryctolagus cuniculus	g1t2m0
bos taurus	e1bmx0

Sequence variants can be presented in fasta-format but can originate from several resources such as UniProt (The UniProt Consortium 2014 – www.uniprot.org) or NCBI (www.ncbi.nlm.nih.gov/protein). User-specific sequences can be used as well.

Fasta-file of these sequences

```
>sp|P01116|RASK_HUMAN GTPase KRas OS=Homo sapiens OX=9606 GN=KRAS PE=1 SV=1
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQDLARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGC
VKIKKCIIM
>sp|P08644|RASK_RAT GTPase KRas OS=Rattus norvegicus OX=10116 GN=Kras PE=1
SV=3
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQELARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGC
VKIKKCVIM
>sp|P32883|RASK_MOUSE GTPase KRas OS=Mus musculus OX=10090 GN=Kras PE=1 SV=1
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQELARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGC
VKIKKCVIM
>tr|Q6AZA4|Q6AZA4_DANRE K-ras OS=Danio rerio OX=7955 GN=kras PE=1 SV=1
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
QSHNVDSKQAQDLARSYGIPFIETSAKTRQGVDDAFYTLVREIRKHKEKMSKEGKKKKKK
SKTKCALM
>tr|H2Q5M0|H2Q5M0_PANTR KRAS isoform 2 OS=Pan troglodytes OX=9598 GN=KRAS
PE=2 SV=1
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAFYTLVREIRKHKEKMSKDGKKKKKK
SKTKCVIM
>tr|F6SFG7|F6SFG7_MACMU KRAS proto-oncogene, GTPase OS=Macaca mulatta OX=9544
GN=KRAS PE=4 SV=1
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQDLARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGC
VKIKKCIIM
>tr|M3XAU6|M3XAU6_FELCA KRAS proto-oncogene, GTPase OS=Felis catus OX=9685
GN=KRAS PE=4 SV=2
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAFYTLVREIRKHKEKMSKDGKKKKKK
SKTKCIIIFIAALDISVKASHQVTLVRPNII
>tr|F5ANT2|F5ANT2_CHICK K-Ras protein OS=Gallus gallus OX=9031 GN=K-RAS PE=2
SV=1
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAFYTLVREIRKHKEKMSKDGKKKKKE
TKTKCIIM
>tr|W5QFH7|W5QFH7_SHEEP KRAS proto-oncogene, GTPase OS=Ovis aries OX=9940
GN=KRAS PE=4 SV=1
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQDLARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGC
VKIKKCIVM
>tr|G1T2M0|G1T2M0_RABIT KRAS proto-oncogene, GTPase OS=Oryctolagus cuniculus
OX=9986 GN=KRAS PE=4 SV=1
GLPSPSPPARRGPGSRTPLPRSPALAGRPPAASPGHAAALAPGAQAGAGESLLKMTEYKL
VVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSA
MRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDLPSRTVD
TKQAQDLARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGCVKIKKC
NVM
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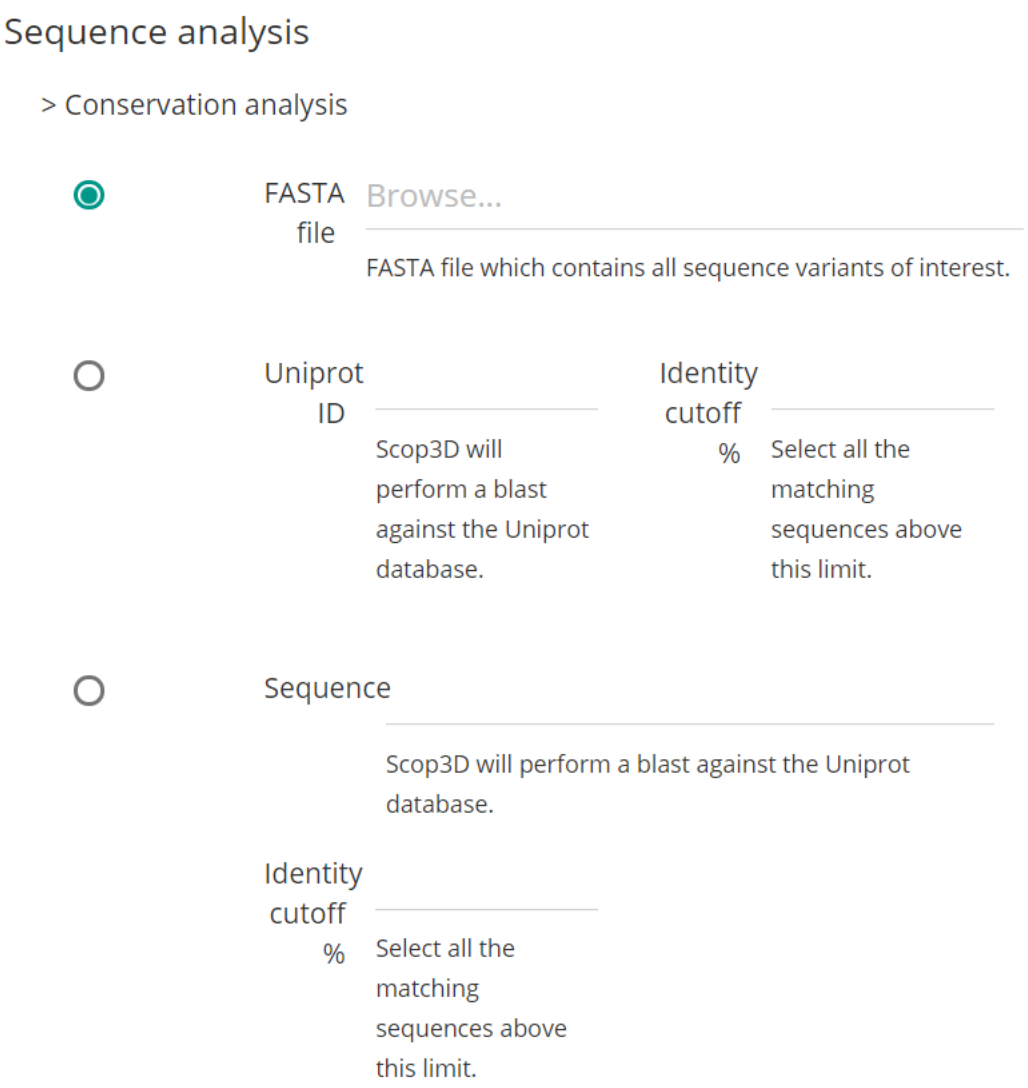
>tr|E1BMX0|E1BMX0_BOVIN KRAS proto-oncogene, GTPase OS=Bos taurus OX=9913 GN=KRAS PE=4 SV=2
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAFYTLVREIRKHKEKMSKDGKKKKKK
SKTKCIIM

To perform a conservation analysis of a protein or several sequence variants of protein, you have to fill the following section:



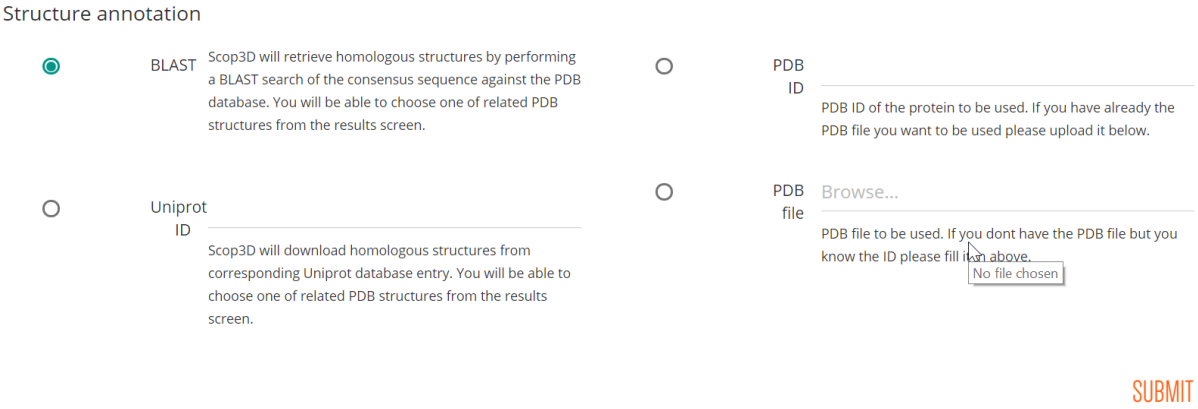
In the **first option** '***FASTA-file***', you can upload a fasta-file with sequence variants of the protein of interest. An example of such a fasta-file is given on page 2.

In the **second option** '***Uniprot ID***', you can provide the uniprot ID of the protein you are interested in. For example: P01116.
This ***identity cutoff*** is of importance for the calculation of the consensus sequence which is calculated with the aid of BioPython. It specifies how common a particular residue has to be at a position before it is added to the consensus sequence. The default of this threshold is 0.7 (70%) as defined by BioPython. The usage of a lower value is advised when there are a low number of sequence variants.

In the **third option** '***sequence***', you can provide the protein sequence of the protein you are interested in. The ***identity cutoff*** has the same functionality as in the second option.
For example:

```
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAG
QEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDL
PSRTVDTKQAQDLARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGC
VKIKKCIIM
```

Step 2: structure annotation



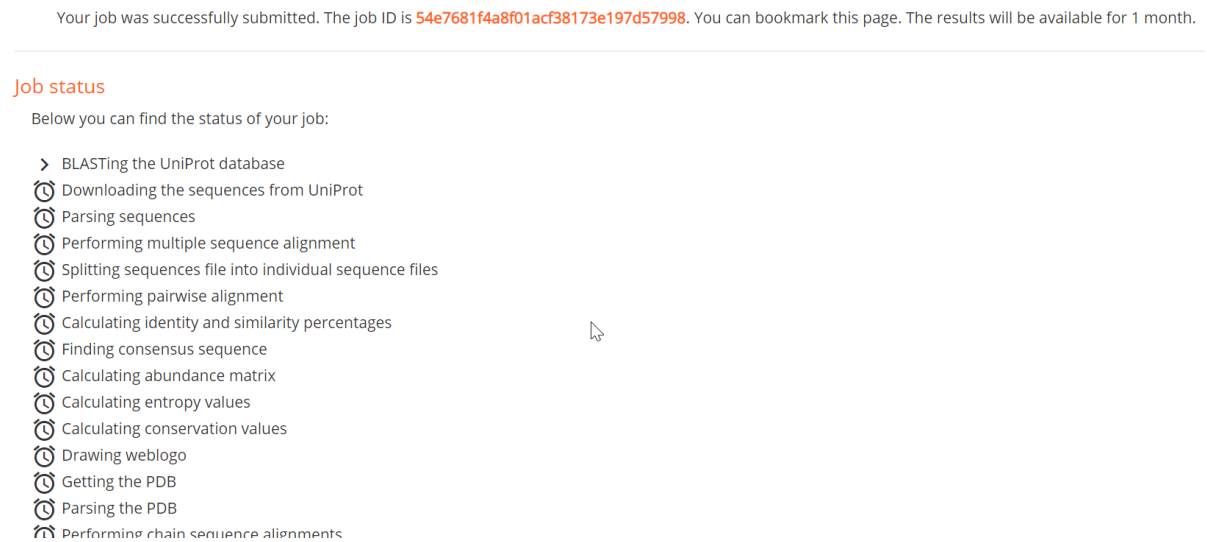
Here again, there are different options to add structural information.
The **first option** '***blast***' can be used when you have no prior structural information. A BLAST (Johnson et al. 2008) against the PDB (Berman et al. 2000) using Biopython (Cock et al. 2009) with the consensus sequence as query sequence to identify the closest homologous structure is performed in this option.

In the **second option** '***Uniprot ID***', the user can provide a uniprot accession of the protein of interest. Scop3D then goes to uniprot and retrieves all structures available for that uniprot accession. The user can subsequently select a structure from this list to continue the analysis. For example: p01116

The **third option** '***PDB ID***' can be used if the user knows which PDB-file (protein structure) her or she wants to work with. In our example, PDB-entry 4TQA can be used.

In the **last option** '***PDB file***', the user is able to submit his or her own PDB-file for the analysis.

If all the information needed is given, you can press the submit button and scop3D starts its calculations.
After you have submitted your job, you can follow the status of your job:



When the BLAST search is performed to determine the best matching structure, the user can choose which structure he wishes to continue with. The five best hits are given.

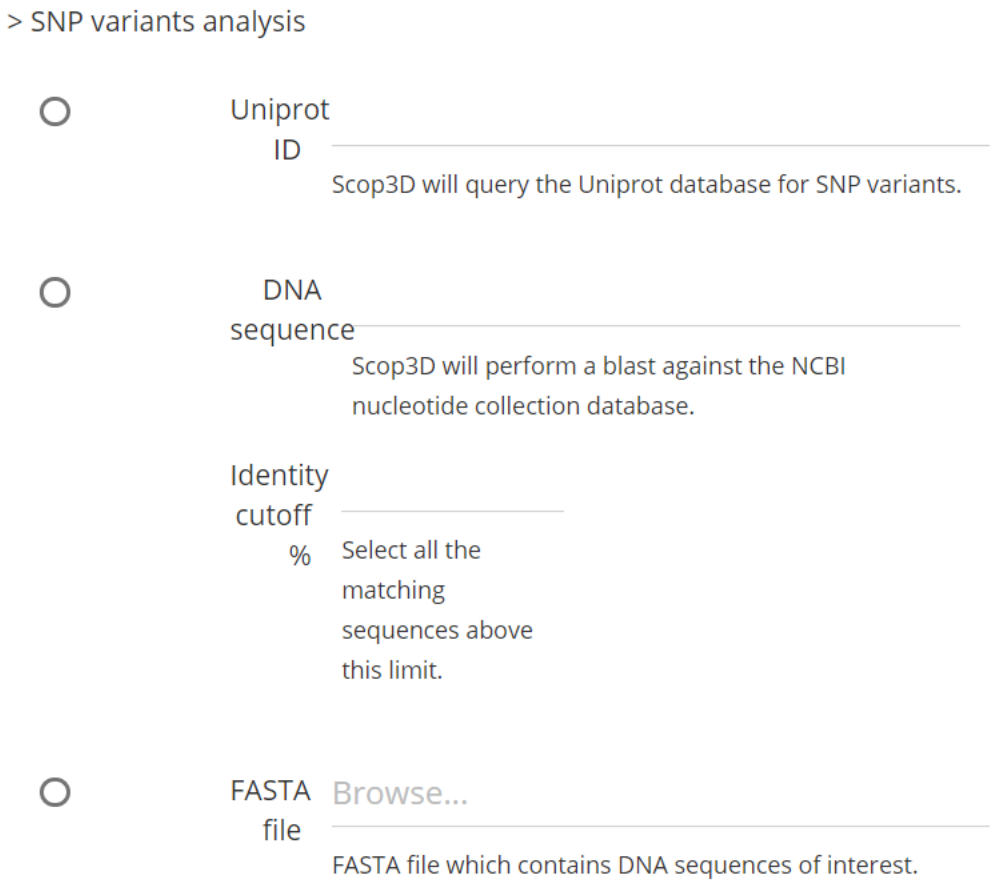
Important to notice is that the information about which chain that is homologous to your protein variants is given within the BLAST results.

If the PDB-entry is composed of multiple chains, the user has the option to choose which chain has to be adjusted. The output given in the BLAST results can be used by the user to guide the chain selection.

SNP variant analysis

Step 1: sequence analysis

In this example, we focus on the SNP variant analysis of Kras from *homo sapiens*.
To perform an SNP variant analysis, the following section has to be filled:



The user has again several input options.

In the **first option** '***Uniprot ID***', the user has to provide the uniprot accession of the protein of interest; for example P01116 (for human Kras). Scop3D will subsequently retrieve the SNP variants available in UniProt and ensembl for this protein.

In the **second option** '***DNA sequence***', a DNA sequence can be provided. This sequence is subsequently used in BLAST search against the NCBI nucleotide collection database to identify homologous sequences. The identity cutoff allows you to retrieve sequences with a certain percentage of sequence identity.

While the **third option** '***FASTA file***' allows the user to upload a fasta-file with his or her own DNA sequences that need to be analyzed.

Step 2: structure annotation

In this step, the user can follow the same input strategy as described in the conservation analysis.

Further help

In case you encounter problems with the usage of Scop3D or the interpretation of your results, you can contact the developers by email:
scop3d.compomics@vib-ugent.be