

# Demo

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## Install

The `bbbq` package depends on the `tmhmm` package, which needs to be installed first:

```
devtools::install_github("richelbilderbeek/tmhmm")
#> Skipping install of 'tmhmm' from a github remote, the SHA1 (c7aeeabe) has not changed since last install
#> Use `force = TRUE` to force installation
```

The `tmhmm` package relies on TMHMM that needs to be installed:

```
tmhmm::install_tmhmm("http://www.cbs.dtu.dk/download/D3198788-0F1D-11E9-883C-84B3B9CD16B5/")
```

The URL can be obtained by requesting a download link at the TMHMM website at <http://www.cbs.dtu.dk/services/TMHMM>. As this URL expires after four hours, `tmhmm` cannot do this for you.

The installation of NetMHC2pan is checked, with the goal of giving a helpful error message:

```
tmhmm::check_tmhmm_installation()
```

Additionally, The `bbbq` package depends on the `netmhc2pan` package, which needs to be installed as well:

```
devtools::install_github("richelbilderbeek/netmhc2pan")
#> Skipping install of 'netmhc2pan' from a github remote, the SHA1 (2cb7ff90) has not changed since last install
#> Use `force = TRUE` to force installation
```

The `netmhc2pan` package relies on NetMHCIIpan that needs to be installed:

```
netmhc2pan::install_netmhc2pan("http://www.cbs.dtu.dk/download/33A6B0AC-0F2E-11E9-B4D1-8ABCB9CD16B5/")
```

The URL can be obtained by requesting a download link at the NetMHCIIpan website at <http://www.cbs.dtu.dk/services/NetMHCIIpan>. As this URL expires after four hours, `netmhc2pan` cannot do this for you.

The installation of NetMHC2pan is checked, with the goal of giving a helpful error message:

```
netmhc2pan::check_netmhc2pan_installation()
```

Another dependency is the `epitopeome` package. Installing the `epitopeome` package:

```
devtools::install_github("richelbilderbeek/epitopeome")
#> Skipping install of 'epitopeome' from a github remote, the SHA1 (72ff7c38) has not changed since last install
#> Use `force = TRUE` to force installation
```

```
devtools::install_github("richelbilderbeek/bbbq")
#> Skipping install of 'bbbq' from a github remote, the SHA1 (113b34f1) has not changed since last install
#> Use `force = TRUE` to force installation
```

## Usage

From a protein sequence, `bbbq` estimates where amino acids of transmembrane proteins are located (inside, outside, in the membrane) and which bind to an MHC2 allele with a certain strength.

We need a FASTA file with at least one protein sequence in it to work on:

```
fasta_filename <- system.file("extdata", "short.fasta", package = "bbbq")
```

This is how such a FASTA file looks like:

```
cat(head(readLines(fasta_filename, warn = FALSE)), sep = "\n")
#> >sp/A0A089QKZ7/Y155A_MYCTU Uncharacterized protein Rv1155A OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv)
#> MGESKSPQESSSEGETKRKFREALDRKMAQSSSGSDHKDGGGKQSRAGPVASRREFRRK
#> SG
#> >sp/A0A089QRB9/MSL3_MYCTU Mycolipanoate synthase OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv)
#> MRTATATSVAVIGMACRLPGGIDSPQRLWEALLRGDDLVEIPADRWANVYYDPEPGVP
#> >sp/E2FZM4/SOCA_MYCTU Uncharacterized protein SocA OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv)
```

Different MHC2 alleles bind differently to protein epitopes. By default, bbbq uses only the default MHC2 allele used by NetMHCIIpan. In this demo, we'll use the first two MHC2 alleles from the complete NetMHCIIpan set of more than 5000 alleles:

```
alleles <- netmhc2pan::get_netmhc2pan_alleles()[1:2]
testit::assert(all(alleles %in% netmhc2pan::get_netmhc2pan_alleles()))
```

Select a binding strength. For example, a value of 5.0 will select those epitopes that are in the top 5 percent.

```
binding_strength_threshold <- 5.0
```

Here, the BBBQ is answered:

```
df <- bbbq::answer_bbbq(
  fasta_filename = fasta_filename,
  alleles = alleles,
  binding_strength_threshold = binding_strength_threshold
)
```

Resulting in:

```
knitr::kable(df)
```

epitopium	n
i	276
m	27
o	774
I	69
M	19
O	148

Legend:

Location	Strong binder	Weak binder
outside	O	o
membrane	M	m
inside	I	i

## Appendix

This appendix shows the intermediate files created by bbbq.

- **tmhmm\_filename**: the TMHMM results file, containing the location of the amino acids (inside, outside, in membrane)
- **netmhc2pan\_filename**: the NetMHC2pan results file, containing the binding of all MHC2 alleles to all protein epitopes
- **epitopeome\_filename**: the epitopeome results file, containing the combined informationb

Answering the BBBQ again (and ignorening its result):

The TMHMM results file:

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```
knitr::kable(read.csv(netmhc2pan_filename))
```

Pos	Peptide	ID	Allele	one_minus_log50k	nM	Rank
13	HSRLATFALALAAAA	tr I6WZ30 I6WZ30_MYCTU	DRB1_0101	0.804	8.31	0.25
14	SRLATFALALAAAAAL	tr I6WZ30 I6WZ30_MYCTU	DRB1_0101	0.828	6.46	0.06
15	RLATFALALAAAAALP	tr I6WZ30 I6WZ30_MYCTU	DRB1_0101	0.837	5.84	0.04
16	LATFALALAAAAALPL	tr I6WZ30 I6WZ30_MYCTU	DRB1_0101	0.838	5.78	0.03
17	ATFALALAAAAALPLA	tr I6WZ30 I6WZ30_MYCTU	DRB1_0101	0.842	5.55	0.03
18	TFALALAAAAALPLAG	tr I6WZ30 I6WZ30_MYCTU	DRB1_0101	0.830	6.26	0.06
19	FALALAAAAALPLAGC	tr I6WZ30 I6WZ30_MYCTU	DRB1_0101	0.797	9.03	0.40
20	ALALAAAAALPLAGCS	tr I6WZ30 I6WZ30_MYCTU	DRB1_0101	0.728	19.01	3.00
107	VRGVFTYRVNKAGLI	tr I6WZD7 I6WZD7_MYCTU	DRB1_0101	0.727	19.08	3.00
108	RGVFTYRVNKAGLIT	tr I6WZD7 I6WZD7_MYCTU	DRB1_0101	0.751	14.78	1.60
109	GVFTYRVNKAGLITN	tr I6WZD7 I6WZD7_MYCTU	DRB1_0101	0.753	14.53	1.60
110	VFTYRVNKAGLITNM	tr I6WZD7 I6WZD7_MYCTU	DRB1_0101	0.758	13.70	1.40
111	FTYRVNKAGLITNMR	tr I6WZD7 I6WZD7_MYCTU	DRB1_0101	0.744	16.01	2.00
112	TYRVNKAGLITNMRG	tr I6WZD7 I6WZD7_MYCTU	DRB1_0101	0.701	25.42	5.00
24	MGTFALHGLTHRLPS	sp E2FZM4 SOCA_MYCTU	DRB1_0102	0.517	185.21	4.00
25	GTFALHGLTHRLPSA	sp E2FZM4 SOCA_MYCTU	DRB1_0102	0.526	169.36	3.50
26	TFALHGLTHRLPSAS	sp E2FZM4 SOCA_MYCTU	DRB1_0102	0.514	192.71	4.50
14	IEFFFRYLTWGLRGV	tr I6WXK8 I6WXK8_MYCTU	DRB1_0102	0.506	210.26	5.00
15	EFFFRYLTWGLRGVE	tr I6WXK8 I6WXK8_MYCTU	DRB1_0102	0.509	201.89	4.50
16	FFFRYLTWGLRGVET	tr I6WXK8 I6WXK8_MYCTU	DRB1_0102	0.509	203.20	4.50
6	ITGVVLAAGRSNRLG	tr I6WY86 I6WY86_MYCTU	DRB1_0102	0.513	193.38	4.50
7	TGVVLAAGRSNRLGT	tr I6WY86 I6WY86_MYCTU	DRB1_0102	0.510	200.04	4.50
45	GFDQLILTLGGAASA	tr I6WY86 I6WY86_MYCTU	DRB1_0102	0.519	182.44	4.00
46	FDQLILTLGGAASAV	tr I6WY86 I6WY86_MYCTU	DRB1_0102	0.546	135.28	2.50
1	MSTIFDIRSLRLPKL	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.551	129.29	1.90
2	STIFDIRSLRLPKLS	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.566	109.24	1.30
3	TIFDIRSLRLPKLSA	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.580	94.01	0.90
4	IFDIRSLRLPKLSAK	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.578	96.03	1.00
5	FDIRSLRLPKLSAKV	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.555	123.16	1.70
6	DIRSLRLPKLSAKVV	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.532	157.56	3.00
7	IRSLRLPKLSAKVVV	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.534	155.43	3.00
8	RSLRLPKLSAKVVVV	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.509	202.61	4.50
27	VVLAVVAAAAGARLY	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.509	202.91	4.50
28	VLAHVAAAAGARLYR	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.543	140.74	2.50
29	LAVVAAAAGARLYRK	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.553	125.96	1.80
30	AVVAAAAGARLYRKL	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.521	177.41	4.00
36	AGARLYRKLTTTTTVV	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.519	182.64	4.00
37	GARLYRKLTTTTTVVA	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.543	141.03	2.50
38	ARLYRKLTTTTTVVAY	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.543	140.97	2.50
39	RLYRKLTTTTTVVAYF	tr I6WYT7 I6WYT7_MYCTU	DRB1_0102	0.526	169.10	3.50
7	RSVVLVLLGAHPAW	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.509	202.25	4.50
8	SVVLSVLLGAHPAWA	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.539	146.78	2.50
9	VVLSVLLGAHPAWAT	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.548	132.41	2.00
10	VLSVLLGAHPAWATA	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.547	133.80	2.50
11	LSVLLGAHPAWATAS	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.525	170.27	3.50
36	IKETTLRVALTRMVG	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.538	147.68	2.50
37	KETTLRVALTRMVGGA	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.571	104.04	1.20
38	ETTLRVALTRMVGAG	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.565	111.27	1.40
39	TTLRVALTRMVGAGD	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.557	120.08	1.70
40	TLRVALTRMVGAGDL	tr I6WZ26 I6WZ26_MYCTU	DRB1_0102	0.532	157.81	3.00
13	HSRLATFALALAAAA	tr I6WZ30 I6WZ30_MYCTU	DRB1_0102	0.566	109.00	1.30
14	SRLATFALALAAAAAL	tr I6WZ30 I6WZ30_MYCTU	DRB1_0102	0.604	72.33	0.50

Pos	Peptide	ID	Allele	one_minus_log50k	nM	Rank
15	RLATFALALAAAALP	tr I6WZ30 I6WZ30_MYCTU	DRB1_0102	0.615	64.46	0.40
16	LATFALALAAAALPL	tr I6WZ30 I6WZ30_MYCTU	DRB1_0102	0.619	61.66	0.30
17	ATFALALAAAALPLA	tr I6WZ30 I6WZ30_MYCTU	DRB1_0102	0.624	58.48	0.25
18	TFALALAAAALPLAG	tr I6WZ30 I6WZ30_MYCTU	DRB1_0102	0.606	71.31	0.50
19	FALALAAAALPLAGC	tr I6WZ30 I6WZ30_MYCTU	DRB1_0102	0.554	124.77	1.80
1	MTRQQLAHLRRACA	tr I6WZ83 I6WZ83_MYCTU	DRB1_0102	0.538	147.71	2.50
2	TRQQLAHLRRACAV	tr I6WZ83 I6WZ83_MYCTU	DRB1_0102	0.553	126.56	1.80
3	RQQLAHLRRACAVV	tr I6WZ83 I6WZ83_MYCTU	DRB1_0102	0.562	114.54	1.50
4	QQLAHLRRACAVVG	tr I6WZ83 I6WZ83_MYCTU	DRB1_0102	0.545	137.78	2.50
5	QLAHLRRACAVVGD	tr I6WZ83 I6WZ83_MYCTU	DRB1_0102	0.526	169.03	3.50
107	VRGVFTYRVNKAGLI	tr I6WZD7 I6WZD7_MYCTU	DRB1_0102	0.509	202.53	4.50
108	RGVFTYRVNKAGLIT	tr I6WZD7 I6WZD7_MYCTU	DRB1_0102	0.511	199.31	4.50
30	LAGRGFPVALGARRM	tr I6WZD9 I6WZD9_MYCTU	DRB1_0102	0.518	184.00	4.00
31	AGRGFPVALGARRMD	tr I6WZD9 I6WZD9_MYCTU	DRB1_0102	0.517	186.44	4.00
32	GRGFPVALGARRMDK	tr I6WZD9 I6WZD9_MYCTU	DRB1_0102	0.539	146.12	2.50
33	RGFPVALGARRMDKL	tr I6WZD9 I6WZD9_MYCTU	DRB1_0102	0.553	125.79	1.80
34	GFPVALGARRMDKLA	tr I6WZD9 I6WZD9_MYCTU	DRB1_0102	0.517	186.30	4.00

The epitopeome results file:

```
cat(readLines(epitopeome_filename), sep = "\n")
#> >sp|A0A089QKZ7|Y155A_MYCTU
#> oooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooo
#> >sp|A0A089QRB9|MSL3_MYCTU
#> iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii
#> >sp|E2FZM4|SOCA_MYCTU
#> iiiiiiiiiiiiiiiiiiiiiiIIIIIIIIIIIIIIIIIIIIiiiiiiiiiiiiiiii
#> >sp|E2FZM5|SOCB_MYCTU
#> iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii
#> >sp|I6WXS6|VPB51_MYCTU
#> oooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooo
#> >tr|I6WX95|I6WX95_MYCTU
#> iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii
#> >tr|I6WXX4|I6WXX4_MYCTU
#> oooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooo
#> >tr|I6WXX8|I6WXX8_MYCTU
#> oooooooooooooo0000000000000000oooooooooooo
#> >tr|I6WY86|I6WY86_MYCTU
#> iiiIIIIIIIIIIIIIIIIIIIIiiiiiiiiiiiiiiiiiiiiIIIIIIIIIIIIIIII
#> >tr|I6WYT7|I6WYT7_MYCTU
#> IIIIIIIIIIIIIIIIIIIIMMMmmmmMMMMMMMMMMMMMM000000000000ooooo
#> >tr|I6WYU2|I6WYU2_MYCTU
#> oooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooo
#> >tr|I6WYY7|I6WYY7_MYCTU
#> oooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooo
#> >tr|I6WZ26|I6WZ26_MYCTU
#> oooooo00000000000000000000oooooooooooo0000000000000000ooooo
#> >tr|I6WZ30|I6WZ30_MYCTU
#> oooooooooooooo00000000000000000000ooooooooooooooooooooooooo
#> >tr|I6WZ39|I6WZ39_MYCTU
#> oooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooo
#> >tr|I6WZ58|I6WZ58_MYCTU
```

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